

Applications of ^{15}N Hyperpolarisation in Nuclear Magnetic Resonance

Callum A. Gater

Doctor of Philosophy

University of York

Chemistry

September 2024

Abstract

Signal Amplification By Reversible Exchange (SABRE) is a hyperpolarisation technique that utilises an iridium based catalyst to transfer magnetisation from *parahydrogen* derived hydride ligands to substrate molecules through a *J*-coupling network on the catalyst. SABRE relies on ligand exchange for efficient polarisation transfer. Optimising this exchange has led to examples of >35,000-fold improved detectability of ^{15}N nuclei on molecules such as sodium nitrite, nicotine and anastrozole.

Facile methods of sample purification and polarisation storage have been exemplified, with the ^{15}N signal lifetime of nitrite reaching 6 minutes through storage in the solid phase. This storage also acts to remove the Ir catalyst, cleaning the sample for MRI applications. Alternative biphasic methods have also been successfully demonstrated. The ^{15}N signal lifetime ($3 \times T_1$) has also been shown to extend at lower magnetic storage fields reaching 12 minutes for acetonitrile and 2 minutes for anastrozole, when stored at 7 T.

Hyperpolarised reaction monitoring through ^{15}N NMR spectroscopy revealed mechanistic insights into the reaction of phenyl diazonium chloride with sodium azide showing evidence for a pentacyclic nitrogen intermediate. All reaction products and intermediates were detected starting from just one hyperpolarised $[\text{NO}_2]^-$ molecule.

Applications of hyperpolarised ^{15}N NMR spectroscopy for mixture analysis were exemplified through the development of ^{15}N SABRE-DOSY and detection of complex mixtures of nitrile compounds at natural ^{15}N abundance demonstrating single scan mM detection limits.

Improvements in ^{15}N hyperpolarisation were made more generally through the implementation of perfluorocarbon solvents. This demonstrated the high hydrogen solubility of these solvents can lead to >60,000-fold improved detectability for nicotine compared with only 18,000 in the more widely used solvent methanol- d_4 .

The optimised polarisation transfer catalysts presented herein allow the application of ^{15}N hyperpolarised systems to such applications as MRI, reaction monitoring, intermediate detection, diffusion spectroscopy and mixture analysis. Improved levels of ^{15}N polarisation are achieved for all substrates tested in perfluorous solvents.

Declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as references. X ray diffraction data were collected and solved by Dr. Adrian Whitwood, Dr. Richard Gammons or Dr. Theo Tanner. The iridium pre-catalysts used in this work were synthesised by Dr. Victoria Annis, or Dr. Peter J. Rayner and this is acknowledged. MRI data was collected and processed by Dr. Aneurin J. Kennerley. Characterisation data for the product **XZ** of Chapter 2 was collected and processed by Dr. Claire Condon. The kinetic fit used in Chapter 2 was developed in conjunction with by Dr. Ben J. Tickner. Solid state ^{15}N NMR spectroscopy data was collected alongside Dr. Matthew R. Gyton. Hyperpolarisation data for pyridine in dichloromethane- d_2 reported in Chapter 5, page 166, was collected by Dr. Kieren J. Evans. Characterisation data for complexes **11A**, **12A** and **13** were collected and processed by Prof. Simon B. Duckett. Characterisation data for the product shown in Figure 3.17 was carried out and processed by Dr. Peter J. Rayner. The python script for ‘jackknife’ processing of EXSY the data was produced with Benjamin G. Collins. This replaced the long-hand approach traditionally used; there is no difference in the output. Some of the work detailed in this thesis has been presented in the publications and patents listed below.

- Peter J. Rayner, Marianna Fekete, Callum A. Gater, Fadi Ahwal, Norman Turner, Aneurin J. Kennerley and Simon B. Duckett, Real-Time High-Sensitivity Reaction Monitoring of Important Nitrogen-Cycle Synthons by ^{15}N Hyperpolarized Nuclear Magnetic Resonance, *JACS*, 2022, **144**, 8756-8769.
- Ben. J. Tickner, Marcus Dennington, Benjamin G. Collins, Callum A. Gater, Theo F. N. Tanner, Adrian C. Whitwood, Peter J. Rayner, Daniel P. Watts, Simon B. Duckett, Metal-Mediated Catalytic Polarization Transfer from para Hydrogen to 3,5-Dihalogenated Pyridines, *ACS Catal.*, 2024, **14**, 994-1004.
- “Using hyperpolarised ^{15}N derived synthons to create hyperpharmaceuticals through SABRE” UK Patent Application No. GB2200964.1



Acknowledgements

I would firstly like to thank my supervisor Simon Duckett and my funder Norman Turner for their constant support and advice throughout my PhD. This journey would have been impossible without them and I am extremely grateful for all they have done for me over the years.

I whole heartedly thank Heather Fish and Matthew Davy for their helpful advice over the years and for always coming to the rescue when a sample gets stuck in a probe. Thanks go to Robert Evans for his help in implementing the DOSY work and to Kieren Evans for the kind use of some of his data. I would not have gotten far without the help of Matthew Gyton, Alastair Robson and Peter Rayner. Thank-you all for helping me throughout my PhD and being there to answer any silly questions I had. Thank-you to Aneurin Kennerley for his kind help with the imaging studies and helping me improve on my writing in the early days of my project. A huge thank-you to Victoria Annis for keeping the building from falling down and always being a welcome laugh when things are crazy.

I thank the Duckett and Halse groups past and present for their company and friendship over the last four years and for making CHyM such an enjoyable place to work. I would like to give thanks to my procrastination buddies Aminata and Pav for always providing me a distraction when needed. I thank my fellow northerner Helena for always stressing on my behalf and ensuring that I never missed a deadline. Thank-you to my office mate Vicky for allowing me to rant for the past six months and always being a kind voice in some tough days. I also thank the Ben's; Collins and Tickner for being good friends and for some very useful conversations. My biggest thanks go to my editor-in-chief Claire for the endless support while writing this thesis and for always being there to help when things got tough. To my friend Ana I thank you for the constant interesting science chats and for being as interested in all this nonsense as I am. Your constant support and encouragement is forever appreciated and I'll be forever indebted to you for teaching me how to make a half decent figure.

To my family, I thank you all for your constant support throughout the years and for always being there when I need you. I will always appreciate the support you have all given more than you could know. To my family that has passed, I hope this work does you proud. Thank-you to my family also for not understanding anything that I actually do, providing a welcome escape from the lab and a chance to talk about normal things. To my friends, the same message. Thank-you for being a welcome escape and for always being there.

Lastly, I would like to thank my darling fiancée, Carla. Thank-you for your constant support over the years and for putting up with my eternal days in the lab. Thank-you for always making sure I ate, keeping our house together and being my teammate. Without your love and care I'm not sure I would have made it through this journey and I am eternally grateful for the unwavering support you have given.

Table of Contents

Abstract	iii
Declaration	v
Acknowledgements	vi
Table of Contents	viii
List of Figures	xiii
List of Schemes	xxii
List of Tables	xxiii
Chapter 1. Introduction	1
1.1 Principles of Magnetic Resonance	3
1.1.1 Fundamentals of Nuclear Magnetic Resonance (NMR).....	3
1.1.2 Fundamentals of Magnetic Resonance Imaging (MRI).....	6
1.1.3 Improved Polarisation Using Brute Force Approach	8
1.1.4 Improved Signal to Noise Using Pulse Sequences	9
1.2 Hyperpolarisation Techniques	9
1.2.1 Principles of Hyperpolarisation	9
1.2.2 Parahydrogen Induced Polarisation (PHIP).....	10
1.2.3 Signal Amplification by Reversible Exchange (SABRE)	14
1.2.4 Dynamic Nuclear Polarisation (DNP)	18
1.2.5 Spin Exchange Optical Pumping (SEOP).....	18
1.3 Metal Carbenes.....	19
1.3.1 Carbenes	19
1.3.2 Fischer Carbene	20
1.3.3 Schrock Carbene.....	21
1.3.4 <i>N</i> -Heterocyclic carbenes.....	22
1.3.5 Measuring steric properties of NHC ligands	23
1.3.6 Measuring electronic properties of NHC ligands	23
1.4 Thesis Aims	25
Chapter 2. Understanding SABRE catalytic behaviour of multi N- donor 1,2,4-triazines. ..	29
2.1 Introduction	31
2.2 Understanding the mechanism of formation of SABRE active species with 3-methylthio-1,2,4-triazine in methanol- <i>d</i> ₄ and dichloromethane- <i>d</i> ₂	32
2.2.1 Probing the formation of SABRE active species in methanol- <i>d</i> ₄	32
2.2.2 Probing the formation of SABRE active species in dichloromethane- <i>d</i> ₂	38
2.3 Exploring ¹ H polarisation of 1,2,4-triazine-3-methylthio as a function of iridium pre-catalyst.....	52

2.4	^1H polarisation of 1,2,4-triazine-3-methylthio as a function of polarisation transfer field	57
2.5	^1H polarisation of 1,2,4-triazine-3-methylthio as a function of co-ligand used.	59
2.6	^1H polarisation level as a function of substrate concentration and shake time	62
2.7	Probing the ^{15}N polarisation and reactivity of 1,2,4-triazine-3-methylthio	63
2.8	Expansion to Toxoflavin.....	66
2.9	Conclusion	68
Chapter 3.	Optimising the hyperpolarisation of $[\text{}^{15}\text{NO}_2]^-$ for use in reaction monitoring and diffusion spectroscopy	73
3.1	Introduction.....	75
3.2	Optimising ^{15}N polarisation of $[\text{NO}_2]^-$	77
3.2.1	Understanding the formation of SABRE active species	77
3.2.2	Co-ligand approach to form a stable SABRE active complex for $[\text{}^{15}\text{NO}_2]^-$ (Y)..	78
3.2.3	^{15}N Polarisation of free Y as a function of polarisation transfer field with pyridine as a co-ligand.	81
3.2.4	^{15}N Polarisation as a function of co-ligand used	82
3.2.5	^{15}N Polarisation as a function of iridium pre-catalyst used.....	87
3.2.6	^{15}N Polarisation as a function of $[\text{NO}_2]^-$ concentration	90
3.2.7	^{15}N Polarisation as a function of $p\text{-H}_2$ pressure.....	92
3.2.8	Detection of unlabelled NaNO_2	95
3.2.9	Detection of ^{17}O satellites in the ^{15}N NMR spectrum of Y	96
3.3	Reaction Monitoring Using ^{15}N NMR Spectroscopy.....	97
3.3.1	Conversion of hyperpolarised $\text{Na}^{15}\text{NO}_2$ to a diazonium salt <i>via</i> $[\text{}^{15}\text{NO}]^+$	97
3.3.2	Azide formation <i>via</i> N-penta-cyclic intermediate.....	102
3.3.3	Copper free click chemistry – formation of a 1,2,3-triazole	107
3.3.4	Formation of gaseous $^{15}\text{N}_2$	110
3.4	Implementation of an automated flow system for hyperpolarisation of $[\text{}^{15}\text{NO}_2]^-$ and hyperpolarised ^{15}N diffusion spectroscopy.	111
3.4.1	Hyperpolarisation of Y using a flow system.....	111
3.4.2	Polarisation transfer field determination.....	112
3.4.3	Diffusion spectroscopy - control study using pyridine	114
3.4.4	Application for ^{15}N diffusion spectroscopy.....	118
3.5	Conclusions.....	119
Chapter 4.	Evaluation of sodium nitrite as an MRI contrast agent.....	125
4.1	Introduction.....	127
4.2	Hyperpolarisation of $[\text{}^{15}\text{NO}_2]^-$ in different solvents	128
4.2.1	^{15}N Polarisation in dichloromethane- d_2	129
4.2.2	Use of a combination of dichloromethane- d_2 and D_2O	130

4.2.3	Magnetic resonance imaging of $[^{15}\text{NO}_2]^-$ across a biphasic solvent system.	132
4.3	Evaluation of T_1 lifetime for $[^{15}\text{NO}_2]^-$	134
4.3.1	Assessment of hyperpolarised ^{15}N NMR signal lifetime of $[^{15}\text{NO}_2]^-$	134
4.3.2	Impact of solvent on the T_1 value of $[^{15}\text{NO}_2]^-$	137
4.4	Extension of T_1 lifetime	138
4.4.1	T_1 lifetime as a function of $[^{15}\text{NO}_2]^-$ concentration	138
4.4.2	T_1 lifetime of $[^{15}\text{NO}_2]^-$ as a function of magnetic storage field	140
4.4.3	T_1 lifetime of $[^{15}\text{NO}_2]^-$ as a function of temperature	146
4.4.4	T_1 lifetime of $[^{15}\text{NO}_2]^-$ in the solid state	147
4.4.5	Assessment of T_1 lifetime of $^{15}\text{N}_2$ triplet state in solution	155
4.5	Conclusion	159
Chapter 5.	Exploring the efficiency of SABRE in perfluorinated solvents	163
5.1	Introduction	165
5.2	Gas solubility measurements	166
5.2.1	Method development	166
5.2.2	Comparison between methanol and methylperfluorobutyl ether	167
5.2.3	Extension to other solvents	168
5.3	Exploring SABRE utilising pyridine as a substrate	169
5.3.1	Catalyst solubility in perfluorinated solvents	169
5.3.2	SABRE of pyridine in solvents J, N and Q	170
5.3.3	T_1 measurements of pyridine	174
5.4	Extension to S-nicotine	175
5.4.1	Comparison of SABRE of S-nicotine in methanol- d_4 and J	176
5.4.2	Optimising the polarisation transfer field for SABRE S-nicotine in J	179
5.5	Conclusions	181
Chapter 6.	Analysis and quantification of Nitrile functionalised compounds using SABRE. ...	185
6.1	Introduction	187
6.2	Effect of co-ligand on nitrile ^{15}N polarisation	188
6.2.1	Utilising $[\text{Ir}(\text{H})_2(\text{C})_3(\text{IMes})]\text{Cl}$ to hyperpolarise acetonitrile.	188
6.2.2	Screening the ^{15}N hyperpolarisation of acetonitrile- d_3 as a function of co-ligand. .	190
6.3	Exploring acetonitrile- d_3 ^{15}N polarisation as a function of the iridium pre-catalyst utilised.	193
6.4	Catalyst speciation as a function of acetonitrile- d_3 and 1,2,4-triazine-3-methyl concentration.	196
6.5	Effect of ^{15}N polarisation of acetonitrile- d_3 as a function of polarisation transfer field. .	202

6.6	Expansion of optimisation to other nitrile containing compounds	204
6.7	Analysis of Mixtures of Nitriles.....	206
6.8	Work towards nitriles as potential MRI contrast agents	213
6.9	Conclusions.....	218
Chapter 7.	Experimental	223
7.1	Equipment and materials.....	225
7.1.1	NMR spectrometers	225
7.1.2	<i>Para</i> -hydrogen generation	226
7.1.3	Solvents and substrates	226
7.1.4	X-ray Crystallography.....	226
7.1.5	Flow measurements.....	227
7.1.6	MRI Measurements.....	228
7.2	General experimental procedures.....	229
7.2.1	Shake and drop method.....	229
7.2.2	SABRE measurements under flow conditions.....	230
7.2.3	T_1 inversion recovery	230
7.2.4	T_1 saturation recovery	231
7.2.5	Hyperpolarised T_1 decay	231
7.2.6	EXSY measurements	232
7.2.7	Determination of rate constant values.....	233
7.2.8	DOSY measurements.....	234
7.2.9	Hydrogen solubility measurements.....	234
7.3	General data processing	236
7.3.1	Signal enhancement and polarisation calculations.....	236
7.3.2	Error calculations	237
7.3.3	Software	237
7.4	Characterisation of complexes	238
7.4.1	General characterisation procedures	238
7.4.2	Characterisation of 1,2,4-triazine-3-methylthio (X)	238
7.4.3	Characterisation for complex $[\text{IrCl}(\text{COD})(\text{IMes})]$ (1)	239
7.4.4	Characterisation for complex $[\text{Ir}(\text{COD})(\text{IMes})(\text{X})]\text{Cl}$ (2)	240
7.4.5	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{COD})(\text{IMes})(\text{X})]\text{Cl}$ (3)	241
7.4.6	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IMes})(\text{X})_3]\text{Cl}$ (4a)	242
7.4.7	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IMes})(\text{X})_3]\text{Cl}$ (4b)	243
7.4.8	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IM}^{p\text{-Cl}})(\text{X})_3]\text{Cl}$ (4a – $\text{IM}^{p\text{-Cl}}$).....	244
7.4.9	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IPr}^{\text{NMe}_2})(\text{X})_3]\text{Cl}$ (4a – $\text{IPr}^{\text{NMe}_2}$).....	245
7.4.10	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IM}^{p\text{-tBu}})(\text{X})_3]\text{Cl}$ (4a – $\text{IM}^{p\text{-tBu}}$)	246

7.4.11	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IM}^{p\text{-OMe}})(\text{X})_3]\text{Cl}$ (4a – $\text{IM}^{p\text{-OMe}}$).....	247
7.4.12	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{SIMes})(\text{X})_3]\text{Cl}$ (4a – SIMes)	248
7.4.13	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IMes}^{\text{NMe}_2})(\text{X})_3]\text{Cl}$ (4a – $\text{IMes}^{\text{NMe}_2}$).....	249
7.4.14	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IPr}^{\text{Cl}})(\text{X})_3]\text{Cl}$ (4a – IPr^{Cl})	250
7.4.15	Characterisation for complex $[\text{IrCl}(\text{H})_2(\text{COD})(\text{IMes})]$ (5)	251
7.4.16	Characterisation for complex $[\text{IrCl}(\text{H})_2(\text{IMes})(\text{X})_2]$ (6)	252
7.4.17	Characterisation for complex $[\text{Ir}_2\text{Cl}_2(\text{H})_4(\text{IMes})_2(\text{X})_2]$ (7).....	253
7.4.18	Characterisation for the product XZ ((6aS,7S,7aR)-3-(methylthio)-6,6a,7,7a,8,9-hexahydro-5H-cyclopropa[5,6]cycloocta[1,2-c]pyridin-7-yl)methanol.).....	255
7.4.19	Characterisation for the complex $[\text{Ir}(\text{COD})(\text{IMes})(\text{Y})]$ (8)	256
7.4.20	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{COD})(\text{IMes})(\text{Y})]$ (9)	257
7.4.21	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{COD})(\text{D})(\text{IMes})]\text{Cl}$ (10).....	258
7.4.22	Characterisation for complex $\text{Na}[\text{Ir}(\text{H})_2(\text{D})(\text{IMes})(\text{Y})_2]$ (11)	259
7.4.23	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{D})_2(\text{IMes})(\text{Y})]$ (12).....	260
7.4.24	Characterisation for complex $\text{Na}[\text{Ir}(\text{H})_2(\text{A})(\text{IMes})(\text{Y})_2]$ (11 _A)	261
7.4.25	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{A})_2(\text{IMes})(\text{Y})]$ (12 _A).....	262
7.4.26	Characterisation for complex $\text{Na}[\text{Ir}(\text{H})_2(\text{IMes})(\text{pyridine})(\text{Y})_2]$ (13) – Both Y ligand equatorial	263
7.4.27	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IMes})(\text{G})_3]\text{Cl}$ (14)	264
7.4.28	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{G})_2(\text{IMes})(\text{Y})]$ (12 _G).....	265
7.4.29	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{G})_2(\text{IPr}^{\text{NMe}_2})(\text{Y})]$ (12 _G - $\text{IPr}^{\text{NMe}_2}$)	266
7.4.30	Characterisation for ((5aR,6R,6aS)-1,4,5,5a,6,6a,7,8-Octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol6-yl)methanol	268
7.4.31	Characterisation for 1,2,4-triazine-3-methyl (V).....	268
7.4.32	Characterisation for acetonitrile (C)	269
7.4.33	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{C})_3(\text{IMes})]\text{Cl}$ (17).....	269
7.4.34	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{C})(\text{IMes})(\text{V})_2]\text{Cl}$ (18)	270
7.4.35	Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IMes})(\text{V})_3]\text{Cl}$ (19)	271
Chapter 8.	Conclusions	273
References		278
List of Compounds		288
List of Abbreviations		290
Appendix		292
A.1	‘T ₁ HyperP’ Pulse sequence utilised for reaction monitoring studies and hyperpolarised T ₁ measurements.	292
A.2	Crystallography data for Figure 3.13.....	293
A.3	Python Script for EXSY data processing.....	300

List of Figures

Figure 1.1 a) The random alignment of nuclear spins in the absence of a magnetic field. b) Energy level diagram showing nuclear spins now either aligned parallel or antiparallel with the applied magnetic field (B_0). The energy difference between these two states is small but the parallel arrangement is slightly lower energy and therefore preferred.	4
Figure 1.2 Applying a magnetic field gradient across the sample causes a difference in local precessional frequency and so NMR peaks appear at different chemical shifts representing the different magnetic fields across the sample.	7
Figure 1.3 Applying a magnetic field gradient across a sample causes a difference in local precessional frequency and therefore chemical shift as in Figure 1.2. Now, as the sample has a non-uniform number of spins in each section, the intensity of the NMR peak changes accordingly.....	7
Figure 1.4 An example hydrogenation reaction undertaken by Bowers and Weitekamp first used to demonstrate PHIP of acrylonitrile. The catalyst utilised was of the form $\text{RhCl}(\text{PPh}_3)_3$	10
Figure 1.5 The four possible nuclear spin alignments of nuclei in a molecule of hydrogen in the absence of an applied magnetic field.	11
Figure 1.6 The temperature dependence on the p- H_2 purity when H_2 is cooled in the presence of a suitable catalyst. As shown, cooling to 77 K produces circa 50% p- H_2	12
Figure 1.7: (Left) The four possible nuclear spin alignments of the H_2 derived nuclei in the product propionitrile from Figure 1.4 within a homogeneous high magnetic field (only the spins originating from H_2 are taken into account). The allowed NMR transitions are shown as blue arrows. (Right) The corresponding ^1H NMR spectrum produced from analysis of these specific spins in the product.	13
Figure 1.8 (Left) The two possible resulting nuclear spin alignments of the p- H_2 derived nuclei in the product propionitrile from Figure 1.4 under PASADENA conditions (only the spins originating from p- H_2 are taken into account). (Right) The corresponding ^1H NMR spectrum produced from analysis of these specific spins in product displaying PASADENA enhancements.	13
Figure 1.9 (Left) The resulting nuclear spin alignment of the p- H_2 derived nuclei in the product propionitrile from Figure 1.4 under ALTADENA conditions (only the spins originating from p- H_2 are taken into account). (Right) The corresponding ^1H NMR spectrum produced from analysis of these specific spins in product displaying ALTADENA enhancements.	14
Figure 1.10 An example SABRE catalytic transfer of magnetisation from p- H_2 derived hydride ligands to a substrate when both are in reversible exchange with an iridium catalyst (where NHC is an N-heterocyclic carbene) at low (0-140 mT) magnetic field.	15
Figure 1.11 A diagram showing the non-spherical charge distribution present in quadrupolar nuclei such as ^{14}N	18
Figure 1.12 A diagram showing the lone pair electron arrangement in a singlet and triplet carbene.	19
Figure 1.13 A diagram showing the binding of a Fischer style carbene as an L type ligand.....	20
Figure 1.14 A diagram showing the stabilising π -donation from neighbouring groups on the carbene carbon.	21
Figure 1.15 A diagram showing the binding of a Schrock style carbene as an X_2 type ligand...	21
Figure 1.16 A reaction showing the formation of the first NHC ligand as observed by Öfele...	22
Figure 1.17 Methods for the safe storage of NHCs. (Left) The imadazolium salt form of an NHC. (Right) The CO_2 -adduct form of an NHC.	23
Figure 1.18 A diagram showing the π back-donation from the metal to the empty π^* orbital of the carbon monoxide.....	24

Figure 1.19 A generic scheme detailing the SABRE-SHEATH method. A sample is pressurised with p-H ₂ , shaken in a microtesla magnetic field and then transferred to the NMR spectrometer for ¹⁵ N signal acquisition.	26
Figure 2.1 a) Structure of 1,2,4-triazine-3-methylthio (X); b) Structure of the antibiotic Toxoflavin (W); IUPAC name 1,6-Dimethylpyrimido[5,4-e][1,2,4]triazine-5,7(1H,6H)-dione.	32
Figure 2.2 ¹ H- ¹⁵ N HMQC NMR spectrum acquired at 9.4 T and a temperature of 243 K with a coupling constant of 12 Hz. The sample consisted of 10 mg of 1 with 5 equivalents of ligand X. Boxes show peaks for the free X and circles for the bound resonances.	33
Figure 2.3 ¹ H- ¹⁵ N HMQC 2D NMR spectrum showing correlation between the ¹ H NMR signal of the hydride ligand at δ -17.34 and the ¹⁵ N NMR signal of the trans X ligand at δ 318. The data was acquired using a coupling constant of 20 Hz without proton decoupling and hence a ¹ H- ¹⁵ N splitting of 24.5 Hz is seen.	34
Figure 2.4 Typical series of ¹ H NMR spectra acquired from a sample of 1 (2 mg) with 5 equivalents of X in 0.6 mL of methanol-d ₄ reacted with p-H ₂ under a pressure of 4 bar. Sample was stored at a field of 9.4 T at 243 K and a single scan spectra acquired using a 90° pulse every 68 s. For ease of viewing an offset of 0.1 ppm is applied to each spectrum to avoid overlap. Only the bottom spectrum displays the true ¹ H chemical shift.	35
Figure 2.5 Top: A typical ¹ H NMR spectrum consisting of 4 signal averages undertaken on a sample containing 1 (2 mg) with 5 equivalents of X in 0.6 mL of methanol-d ₄ . A period of 300 s was left between subsequent scans to ensure full relaxation of the signal. Bottom: A typical SABRE hyperpolarised ¹ H NMR spectrum collected in a one scan acquisition from a sample containing 1 (2 mg) with 5 equivalents of X in 0.6 mL of methanol-d ₄ shaken for 10 s at a field of 6 mT.	38
Figure 2.6 Comparison of the ¹ H NMR signal enhancement of free X for both ¹ H resonances determined at 9.4 T after a 10 s shake at 6 mT with 4 bar pressure of p-H ₂ alongside the raw ¹ H NMR signal intensity for complex 6. This is reflective of the amount of 6 present during each SABRE experiment.	41
Figure 2.7 Hydride region of a ¹ H NMR spectrum of a sample containing 1 (2 mg) with 5 equivalents of X in dichloromethane-d ₂ recorded at a temperature 298 K with a field strength of 7 T. Sample was pressurised to 4 bar with H ₂ gas and left to react for 5 days. Peaks of 7 are shown at δ -19.42 (H _a), -20.10 (H _b), -20.59 (H _c) and -23.65(H _d). Peaks for 6 shown at δ -21.80 and -23.00.	42
Figure 2.8 ¹ H NMR TOCSY data collected on a sample of 7. Data was collected at 263 K and an acquisition field of 11.7 T.	43
Figure 2.9 ¹ H- ¹⁵ N HMQC 2D NMR spectrum showing correlation between the ¹ H NMR signal of the hydride ligands at δ -19.42 and -20.10 and the corresponding ¹⁵ N NMR signal of the bridging trans X ligand at δ 275.70 and 318.80. These data were acquired using a coupling constant of 20 Hz, without proton decoupling and hence a ¹ H- ¹⁵ N splitting is seen of 22.5 and 29.2 Hz respectively.	44
Figure 2.10 Two dimensional ¹ H NMR NOESY data collected at 263 K and at a field of 9.4 T. Data shows nOe connections between the bound X ligand ¹ H NMR signal at δ 9.16 and the carbene ligands ¹ H NMR signals at δ 1.916 and 6.25 indicative of a cis arrangement.	45
Figure 2.11 Top: DOSY spectrum collected at 253 K. Bottom: DOSY spectrum collected at 298 K. Both datasets were acquired at a field of 11.7 T and processed as outlined in the experimental.	46
Figure 2.12 Left: Structure of Ir dimeric species presented by Oro et. al. Labels on atoms are reflective of those presented in Tables 2.1 and 2.2 Right: Structure of 7 the Ir dimeric species presented in this thesis. Labels on atoms are reflective of those presented in Scheme 2.3.	47

Figure 2.13 A typical reaction monitoring experiment of the reaction of 1 to 6 then to 7 at 298 K. The ‘measured’ (data points) represent the normalised signal intensity of each species in the spectra and the ‘predicted’ (solid line) representing the fit according to the model described...	50
Figure 2.14 Top the Eyring plot gained from rate constants determined for the conversion of 1 to 6. Bottom the Eyring plot gained from rate constants determined for the conversion of 6 to 7. Both graphs were plotted from data collected at temperatures ranging from 288 to 308 K	51
Figure 2.15 NHC ligands used in the pre-catalysts [IrCl(COD)(NHC)] for the optimisation of 1,2,4-triazine-3-methylthio	52
Figure 2.16 ¹ H NMR signal enhancements at 9.4 T as a function of pre-catalyst, data taken from Table 2.4. All samples consisted of 2 mg of pre-catalyst with 20 equivalents of X. Samples were shaken at a field of 6 mT for 10 s under a pressure of 4 bar p-H ₂	55
Figure 2.17 ¹ H NMR signal enhancements found for X at 9.4 T as a function of the polarisation transfer field (G). The sample contained 2 mg of the pre-catalyst [IrCl(COD)(IPr ^{NMe2})] and 15 equivalents of X. All data points were collected from a 10 s shake in each PTF under 4 bar p-H ₂	58
Figure 2.18 ¹ H NMR signal enhancement of X at 9.4 T as a function of polarisation transfer field. Sample contained 2 mg of pre-catalyst 1 and 15 equivalents of X. All data points were collected from a 10 s shake in each PTF with 4 bar p-H ₂	58
Figure 2.19 Chemical structures of co-ligands utilised in this study.	59
Figure 2.20 ¹ H NMR signal enhancements determined at 9.4 T for the δ 9.01 signal of free X as a function of the ratio of X to iridium. All samples contained 2 mg of the pre-catalyst [IrCl(COD)(IPr ^{NMe2})] and were shaken at a 60 G transfer field for 10 s under 4 bar of p-H ₂	62
Figure 2.21 ¹⁵ N polarisation level determined for the N ₄ site of free X as a function of the ratio of X to iridium. All samples consisted of 2 mg of the pre-catalyst [IrCl(COD)(IPr ^{NMe2})] and were shaken in a field of 60 G for 10 s under a pressure of 4 bar p-H ₂	64
Figure 2.22 A typical SABRE hyperpolarised ¹⁵ N NMR spectrum. The spectrum was collected from a sample containing the IPr ^{NMe2} pre-catalyst (2 mg) with 15 equivalents of X at a 9.4 T acquisition field shaken for 10 s at a PTF of 6 mT under a pressure of 4 bar p-H ₂	65
Figure 2.23 Typical SABRE hyperpolarised ¹ H NMR spectrum collected in a one scan acquisition from a sample containing 1 (0.3 mg) with 7 equivalents of W in 0.6 mL of methanol-d ₄ . The sample was shaken for 10 s at a field of 6 mT under 4 bar p-H ₂ prior to ¹ H NMR signal acquisition to gain the hyperpolarised response.	67
Figure 3.1 ¹⁵ N NMR signal acquired at 9.4 T as a function of polarisation transfer field used. The sample probed consisted of 2 mg of 1 with 25 equivalents of Y and 4 equivalents of pyridine as co-ligand. At each PTF the sample was shaken with 4 bar of fresh p-H ₂ for 10 s. The Figure shown overlaid ¹⁵ N NMR spectra and thus the y axis here is an arbitrary signal intensity.	82
Figure 3.2 Chemical structures of co-ligands used. DMAP stands for dimethylaminopyridine and DMSO for dimethylsulfoxide.....	83
Figure 3.3 ¹⁵ N NMR signal enhancement at 9.4 T (fold) as a function of co-ligand used from Figure 3.2. All samples consisted of 2 mg of 1 with 25 equivalents of Y and 4 equivalents of the corresponding co-ligand used. Samples were shaken for 10 s in a -0.35 μT PTF under a pressure of 4 bar p-H ₂	83
Figure 3.4 NHC ligands used in the pre-catalysts [IrCl(COD)(NHC)] for the optimisation of ¹⁵ N hyperpolarisation for Y. a) Increasing steric bulk shown from top to bottom. b) Increasing electron donation from top to bottom. c) Combination of increased steric bulk and increased electron donation. Note that iAm refers to the substituent chain CH(CH ₂ CH ₃) ₂	88
Figure 3.5 Effect on ¹⁵ N signal enhancement at 9.4 T caused by changing the steric and electronic properties of the SABRE pre-catalyst used. The samples all contained 2 mg of the	

respective pre-catalyst (Figure 3.4), 25 equivalents of Y and 6 equivalents of G-d ₂ . The samples were shaken at a field of -0.35 μ T for 10 s with 4 bar pressure of p-H ₂	89
Figure 3.6 Effect of increasing substrate excess compared to Ir on the detected ¹⁵ N response of Y shown as the resulting ¹⁵ N signal enhancement at 9.4 T in fold gain. All samples were shaken for 10 s at a field of -0.35 μ T with a pressure of 4 bar p-H ₂	91
Figure 3.7 Effect of increasing p-H ₂ pressure on the detected ¹⁵ N NMR response of Y shown as % polarisation for the ¹⁵ N nuclei. The sample was shaken for 10 s at a field of -0.35 μ T for each pressure. This sample contained 2 mg of the pre-catalyst IPr ^{NMe₂} with 20 equivalents of Y and 6 equivalents of G.....	93
Figure 3.8 Effect of increasing p-H ₂ pressure on the detected ¹⁵ N NMR response of Y shown as % polarisation for the ¹⁵ N nuclei. The sample was shaken for 10 s at a field of -0.35 μ T for each pressure. This sample contained 1 mg of the pre-catalyst IPr ^{NMe₂} with 20 equivalents of Y and 6 equivalents of G.....	94
Figure 3.9 Typical ¹⁵ N NMR spectrum from a hyperpolarised sample of Y (50 equivalents relative to Ir pre-catalyst) showing satellites with a 320 Hz coupling likely due to the presence of ¹⁷ O.	96
Figure 3.10 Possible reactions to monitor starting from hyperpolarised [NO ₂] ⁻ (Y). Note that some reactants required are omitted from the figure for simplicity. If the reaction is looked at in detail in a later section all details of the reaction will be included.....	97
Figure 3.11 a) A typical hyperpolarised ¹⁵ N NMR response of Y before the addition of any acid or aniline. The spectrum was acquired at 11.7 T using a 90° pulse acquire sequence after exposing the sample to 4 bar of p-H ₂ and shaking in the appropriate PTF. b) A series of ¹⁵ N NMR spectra obtained from a hyperpolarised sample of Y mixed with an excess of alanine and HCl. The spectra were all acquired at 11.7 T using a 9° pulse angle with a delay of 5 seconds between each acquisition. For ease of viewing an offset of 10 ppm is applied to each spectrum so only the bottom spectrum displays the true ¹⁵ N chemical shift.....	99
Figure 3.12 ¹⁵ N NMR spectrum obtained from a hyperpolarised sample of Y mixed with an excess of ¹⁵ N-aniline and HCl. The spectrum was acquired at 11.7 T using a 9° pulse angle. .	100
Figure 3.13 Structure of a nitrosyl complex formed determined by X-ray crystallography. Note any solvent of crystallisation and all hydrogen atoms have been omitted for clarity. The X-ray diffraction data was collected and solved by Dr. Adrian C. Whitwood. The numbers in the figure are the same as those given in the X-ray data found in Appendix A.2.	101
Figure 3.14 a) Series of ¹⁵ N NMR spectra obtained at different time points after a hyperpolarised sample of Y was mixed with excess ¹⁵ N-aniline, HCl and sodium azide. These NMR spectra were acquired consecutively at 11.7 T using a 9° degree pulse with a delay of 5 seconds between them. For ease of viewing an offset of 10 ppm is applied to each spectrum so only the bottom spectrum displays the true ¹⁵ N chemical shift. b) Expansion of the ¹⁵ N NMR signals at δ 356.8 and 298.2 to show their doublet splitting. c) Expansion of the ¹⁵ N NMR signals at δ 242.2 and 90.1 to show their doublet splitting.	104
Figure 3.15 a) Reaction scheme showing where the ¹⁵ N labels are located during the formation of phenyl azide from Y, ¹⁵ N-phenyl aniline and 1- ¹⁵ N sodium azide. b) A typical ¹⁵ N NMR spectrum showing the product signals seen during this reaction. This NMR spectrum was collected using a 9° pulse at an 11.7 T field. c) An expansion showing the fine structure associated with the ¹⁵ N NMR signal at δ 386.5 d) An expansion showing the fine structure associated with the ¹⁵ N NMR signal at δ 356.8 e) An expansion showing the fine structure associated with the ¹⁵ N NMR signal at δ 298.2 f) An expansion showing the fine structure associated with the ¹⁵ N NMR signal at δ 242.2 g) An expansion showing the fine structure associated with the ¹⁵ N NMR signals at δ 233.0 and δ 232.0 h) An expansion showing the fine structure associated with the ¹⁵ N NMR signal at δ 90.1	106

Figure 3.16 a) Reaction scheme for ^{15}N -Y (150 mM) reacting in 0.6 mL methanol- d_4 with an excess of ^{15}N -aniline and HCl followed by the addition of an excess of mono ^{15}N labelled sodium azide. The addition of a stoichiometric amount of the reactive alkyne the followed this step, and formed the isotopologues the 1,2,3-triazole shown. b) A typical ^{15}N NMR spectrum recorded directly after the addition of these reactants at 11.7 T is shown. This NMR spectrum was collected circa 12 s after the hyperpolarisation of Y took place and involved a 38° r.f. pulse.	108
Figure 3.17 a) Reaction scheme between a hyperpolarised 1- ^{15}N -sodium azide (150 mM) in 0.6 mL methanol- d_4 with a stoichiometric amount of the reactive alkyne to form the 1,2,3-triazole product shown. b) Hyperpolarised ^{15}N NMR signal from sodium azide collected from a 90° pulse acquisition sequence at 9.4 T. The sample was shaken for 10 s at $-0.35\ \mu\text{T}$ under 3 bar of p- H_2 . c) Hyperpolarised ^{15}N NMR signal of the 1,2,3-triazole product, note this is an average signal across all tautomers of the product.	110
Figure 3.18 Two alternate routes for the production of $^{15}\text{N}_2$ from hyperpolarised Y; either via a Sandmeyer reaction with ^{15}N -aniline and HCl followed by CuI or a spontaneous creation reacting with ^{15}N ammonium chloride.	111
Figure 3.19 ^{15}N NMR signal acquired at 9.4 T as a function of polarisation transfer field. The sample consisted of 10 mg of 1 with 25 equivalents of Y and 4 equivalents of D in 3 mL of methanol- d_4 . At each PTF the sample was bubbled with 3 bar of fresh p- H_2 for 10 s.	113
Figure 3.20 Series of ^{15}N NMR spectra acquired on a sample containing 1 (10 mg) with 25 equivalents of Y and 4 equivalents of D in 3 mL of methanol. For each spectrum the sample was bubbled for 10 s with 3 bar of p- H_2 before immediate pneumatic shuttling to a 9.4 T spectrometer for ^{15}N NMR signal acquisition. Note that the ^{15}N NMR spectra are stacked with an offset on the ppm axis. Note that time increases from bottom to top in these spectra. For ease of viewing an artificial shift of 10 ppm is applied to each subsequent spectrum and so only the bottom spectrum shows the true ^{15}N chemical shift value.	114
Figure 3.21 Diffusion coefficient D as a function of the relative excess of D (iridium complex at 5 mM) determined by ^1H measurement. The data point at 0 had no iridium present. All measurements presented here were conducted at 298 K on a 9.4 T NMR spectrometer.	115
Figure 3.22 A typical ^1H DOSY plot of a sample containing 2 mg of 1 with 50 equivalents of D. The spectrum was acquired in the flow probe after bubbling with 3 bar of N_2 for 10 s and pneumatic shuttling into a 9.4 T spectrometer prior to initiating the DOSY pulse sequence.	116
Figure 3.23 A plot showing how the diffusion coefficient of D, via ^1H data acquired using a flow system changes with settling time allowed in the spectrometer before application of the DOSY pulse sequence.	117
Figure 3.24 Typical ^{15}N DOSY spectrum gained from a sample containing 5 mM of 1 with 50 equivalents of Y and 6 equivalents of G in methanol- d_4 . The spectrum was acquired using a flow system bubbling the sample with 3 bar of p- H_2 for 10 s before pneumatic shuttling of the sample to a 9.4 T spectrometer where it was allowed to settle for a period of 5 s before application of the DOSY pulse sequence to acquire one gradient slice.	119
Figure 4.1 a) Setup for hyperpolarised imaging at 7 T of a reaction cell used for the biphasic preparation of $[\text{}^{15}\text{NO}_2]^-$ top solvent layer here is D_2O and bottom layer dichloromethane- d_2 ; b) high-resolution three-dimensional axial acquisition using spiral (out) encoding of $6\ \text{cm}^3$ over a $64 \times 64 \times 8$ matrix with a 6 s acquisition time; c) hybrid MRS(I) data collected using an EPSI pulse sequence and a 64×64 matrix (512 spectral points).	133
Figure 4.2 A series of ^{15}N NMR spectra, obtained from a hyperpolarised sample of Y to encode T_1 . These NMR spectra were acquired consecutively at 9.4 T using a 10° degree pulse with a delay of 5 seconds between each acquisition.	134

Figure 4.3 Exponential decay curve, fitted to the experimental ^{15}N NMR data to estimate T_1 relaxation. The sample was made up in methanol- d_4 stored at a field of 9.4 T and a temperature of 298 K. It contained 50 equivalents of Y relative to the $\text{IPr}^{\text{NMe}_2}$ pre-catalyst used.	135
Figure 4.4 T_1 of Y as a function of ratio of Y to iridium. For all samples, the concentration of 1 was 5 mM and the concentration of co-ligand G 30 mM. T_1 data was collected at a field of 9.4 T using the hyperpolarised signal decay method already outlined.	139
Figure 4.5 T_1 of Y given as a function of concentration of 1 present in the sample. All samples contained 250 mM of Y and 30 mM of G. All T_1 values were collected at 9.4 T and calculated using the hyperpolarised signal decay method outlined previously apart from that with 0 mM Ir, which was calculated by inversion recovery.	140
Figure 4.6 Typical T_1 inversion recovery curve gained from a 250 mM sample of Y in methanol- d_4 at a field of 16.4 T.	141
Figure 4.7 Average ^{15}N NMR signal integral area for Y plotted against time at a field of 1 T (40 MHz ^1H precession frequency). The sample consisted of 0.5 mg of 1 with 200 equivalents of Y and 24 equivalents of G. Each data point consisted of three repeat shakes of 10 s at a field of -0.35 μT with a pressure of 4 bar p- H_2	143
Figure 4.8 Average ^{15}N NMR signal integral area for Y plotted against time at a field of 50 μT (Earth's Field). The sample consisted of 0.5 mg of 1 with 200 equivalents of Y and 24 equivalents of G. Each data point consisted of three repeat shakes of 10 s at a field of -0.35 μT with a pressure of 4 bar p- H_2	144
Figure 4.9 Average ^{15}N NMR signal integral area for Y plotted against time at a field of a) 0.10 μT and b) 0.35 μT . The sample consisted of 0.5 mg of 1 with 200 equivalents of Y and 24 equivalents of G. Each data point consisted of three repeats of bubbling 10 s of 3 bar p- H_2 at a field of -0.35 μT followed by storage in specified field for the set time.	145
Figure 4.10 ^{15}N T_1 values for Y shown as a function of the absolute magnetic field used to store the sample. The methods used to collect each T_1 value did differ and so directly comparing the exact values of data points at the different fields should be treated with caution.	146
Figure 4.11 ^{15}N T_1 lifetime of Y presented as a function of temperature. These data were collected on a 250 mM sample of Y in methanol- d_4 at a field of 9.4 T.	147
Figure 4.12 Typical ^{15}N T_1 saturation recovery plot from a sample containing Y. The experiment utilised a 3 mm rotor with 20 kHz MAS and was conducted at a field of 9.4 T	148
Figure 4.13 ^{15}N NMR signal acquired at 9.4 T as a function of polarisation transfer field used. The sample probed consisted of 2 mg of 1 with 25 equivalents of Y and 6 equivalents of G used as a co-ligand. At each PTF the sample was shaken with 3 bar of fresh p- H_2 for 10 s.	150
Figure 4.14 Method outline for the hyperpolarisation of Y followed by precipitation through addition of more J. The solution is filtered through a syringe filter to separate the precipitate before redissolving in water and transferring to the NMR spectrometer for signal acquisition. The red outline represents the location of hyperpolarised Y.	152
Figure 4.15 A typical ^{15}N NMR spectrum collected at a field of 9.4 T in a single scan acquisition on a sample initially containing 2 mg of 1 with 50 equivalents of Y and 6 equivalents of G dissolved in 0.6 mL of a 1 : 1 mixture of methanol- d_4 and J. The sample was treated as described in the text before acquiring the spectrum.	153
Figure 4.16 a) A typical ^1H NMR spectrum of a sample containing 2 mg of 1 with 50 equivalents of Y and 6 equivalents of G in 0.3 mL 1 : 1 mixture of methanol- d_4 and J. b) The ^1H NMR spectrum resulting from the same sample now in D_2O after the process in Figure 4.14 had been carried out. The spectrum now shows a lack of ^1H resonances typical of those for the Ir catalyst. Both spectra were recorded with 16 signal averages at 9.4 T.	154
Figure 4.17 Formation of N_2 gas from the reaction of $[\text{NO}_2]^-$ and $[\text{NH}_4]^+$ as shown in Figure 3.18.	155

Figure 4.18 A typical ^{15}N NMR spectrum of a sample containing 0.6 mL of J and 0.1 mL of D_2O under a pressure of around 3 bar $^{15}\text{N}_2$. The signal at δ 308 is from $^{15}\text{N}_2$ dissolved in the mixture. The spectrum was acquired using 16 signal averages at 9.4 T.	156
Figure 4.19 T_1 inversion recovery curve for the ^{15}N NMR signal of $^{15}\text{N}_2$ gained from a 0.6 mL of J and 0.1 mL of D_2O under a pressure of around 3 bar $^{15}\text{N}_2$. The study was carried out at a field of 9.4 T.	157
Figure 4.20 ^{15}N T_1 values for $^{15}\text{N}_2$ dissolved in 0.6 mL of J and 0.1 mL of D_2O shown as a function of the magnetic field used to store the sample. All T_1 values were acquired using a T_1 inversion recovery pulse sequence.	158
Figure 4.21 Structure of selectively deuterated biomolecule sodium fumarate-1- ^{13}C -2,3- d_2 used by Levvit et al. for precipitation studies.	161
Figure 5.1 Chemical structures of solvents utilised for H_2 dissolution studies.	165
Figure 5.2 Plot of dissolved H_2 concentration versus external H_2 pressure for methanol- d_4 as measured by ^1H NMR spectroscopy. The sample contained 5 μL of DMSO as an internal standard. All the ^1H NMR spectra were all recorded on an 11.7 T spectrometer at 298 K.	167
Figure 5.3 Plot of dissolved H_2 concentration versus external H_2 pressure for J as measured by ^1H NMR spectroscopy. The sample contained 5 μL of DMSO as an internal standard. ^1H NMR spectra were all gained on an 11.7 T spectrometer at 298 K.	168
Figure 5.4 a) ^1H NMR signal enhancements at 9.4 T for ortho meta and para positions of pyridine hyperpolarised in three different samples a b and c, when they were shaken in a 6 mT field. a contained 2 mg of 1, 1.6 μL of D (would be 6 equivalents if all of 1 dissolved in J) and 0.6 mL of J. b contained 0.5 mg of 1, 20 equivalents of D and 0.6 mL of J. c contained 0.5 mg of 1, 20 equivalents of D and 0.6 mL of methanol- d_4 . b) ^{15}N NMR polarisation levels achieved for these samples when shaken in a 4 μT field. For both a) and b) the samples were shaken in the indicated fields for 10 s under 4 bar of p- H_2	173
Figure 5.5 Structure of nicotine (T) with relevant positions of the nuclei labelled to link to the corresponding data presented in Table 5.5, and in the text.	177
Figure 5.6 Typical hyperpolarised ^{15}N NMR spectra for nicotine in a) A sample containing 2 mg of 1 with 10 equivalents of S-nicotine dissolved in 0.6 mL of methanol- d_4 b) A sample containing 2 mg of 1 with 10 equivalents of nicotine in 0.6 mL of the solvent J (note that not all of 1 dissolved in this case so the concentration is lower than expected). Both samples were shaken for 10 s at a field of 0.3 μT under a pressure of 4 bar p- H_2	179
Figure 5.7 Both of these plots show ^{15}N NMR signal acquired at 9.4 T as a function of polarisation transfer field for S-nicotine. Top shows the resulting data for a sample containing 2 mg of 1, 5 μL of T in solvent J. Bottom shows the resulting data for a sample containing 2 mg of 1, 5 μL of T in methanol- d_4 . Both samples were shaken in the various fields each for 10 s with 4 bar p- H_2	180
Figure 6.1 Chemical structures of co-ligands used in this study.	188
Figure 6.2 ^1H NMR spectra acquired at 9.4 T after shaking for 10 s at a field of 6 mT with a pressure of 3 bar p- H_2	189
Figure 6.3 ^{15}N NMR spectrum, at 9.4 T, acquired from a sample containing 2 mg of 1 with 5 equivalents of C- d_3 and 5 equivalents of D. The sample was shaken in a field of -0.3 μT for 10 s with a pressure of 4 bar p- H_2 . The ^{15}N NMR peak for D was observed at δ 299.7 ppm while C is at δ 240.8 ppm.	191
Figure 6.4 A typical hyperpolarised ^{13}C NMR spectrum at 9.4 T acquired from a sample containing 2 mg of 1 with 5 equivalents of C- d_3 and 5 equivalents of X. The sample was shaken in a field of -0.3 μT for 10 s with a pressure of 4 bar p- H_2	192
Figure 6.5 NHC ligands used in the pre-catalysts $[\text{IrCl}(\text{COD})(\text{NHC})]$ for the optimisation of ^{15}N hyperpolarisation for C. a) Broad changes in steric bulk utilised to identify optimum ligand exchange window. Increasing steric bulk shown from top to bottom. b) Fine tuning associated	

with change in electron donation to more precisely match SABRE conditions to outcome.	
Electron donating ability increases top to bottom.	194
Figure 6.6 Effect of changing steric and electronic properties of the SABRE catalyst on the ^{15}N polarisation of acetonitrile. Samples all contained 2 mg of the respective pre-catalyst with 5 equivalents of C-d_3 and V shaken at a field of $-0.3\ \mu\text{T}$ for 10 s with 4 bar pressure of p-H_2	195
Figure 6.7 Hydride section of two ^1H NMR spectra obtained from a sample containing 2 mg of IPr^{Cl} with 5 equivalents of V and 5 equivalents of C-d_3 . a) Thermally equilibrated acquisition collected for 32 signal averages. b) Hyperpolarised single scan NMR spectrum showing PHIP signal complexity; the sample was shaken for 10 s at a 6 mT field with a pressure of 4 bar p-H_2 before acquisition.	197
Figure 6.8 ^{15}N polarisation displayed as a function of the excess of V on a series of samples containing 2 mg of the IPr^{Cl} pre-catalyst and 5 equivalents of C-d_3 . All samples were shaken for 10 s at a field of $-0.3\ \mu\text{T}$ with a pressure of 4 bar p-H_2	198
Figure 6.9 shows the effect on catalyst speciation caused by the increasing co-ligand concentration compared to catalyst.	199
Figure 6.10 ^{15}N polarisation and raw ^{15}N NMR integral area of C-d_3 displayed as a function of the excess of C-d_3 itself. The series of samples each contained 2 mg of the IPr^{Cl} pre-catalyst and 5 equivalents of V. All samples were shaken for 10 s at a field of $-0.3\ \mu\text{T}$ with a pressure of 4 bar p-H_2	200
Figure 6.11 A series of ^1H NMR spectra showing the effect on catalyst speciation caused by the increasing substrate concentration compared to the Ir catalyst.	201
Figure 6.12 ^{15}N Polarisation (%) of free C-d_3 as a function of polarisation transfer field (μT); the sample consisted of 2 mg of IPr^{Cl} with 5 equivalents of C-d_3 and 5 equivalents of V in methanol- d_4 shaken for 10 s with a pressure of 4 bar p-H_2 at the corresponding PTF.	202
Figure 6.13 ^{15}N Polarisation (%) of free C-d_3 as a function of polarisation transfer field (μT); the sample consisted of 2 mg of 1 with 5 equivalents of C-d_3 and 5 equivalents of V in methanol- d_4 shaken for 10 s with a pressure of 4 bar p-H_2 at the corresponding PTF.	203
Figure 6.14 shows the structures of the nitrile containing compounds selected for this study..	204
Figure 6.15 Polarisation as a function of polarisation transfer field. All samples consisted of 2 mg of IPr^{Cl} with 5 equivalents of V and 5 equivalents of the corresponding nitrile compound. Top left – acetonitrile- d_3 . Top Right – 4-cyanopyridine. Bottom left – 3-cyanopyridine. Bottom right – 2-cyanopyridine.	206
Figure 6.16 Typical SABRE hyperpolarised ^{15}N spectrum of a mixture containing 2 mg IPr^{Cl} with 5 equivalents of V and of both 3- and 4-cyanopyridine. Sample was shaken at a field of $-0.3\ \mu\text{T}$ for 10 s with 4 bar of p-H_2 and acquired at a field of 9.4 T.	207
Figure 6.17 (Top) ^1H NMR spectrum of 2-, 3- and 4-cyanopyridine shown to overlap at 9.4 T. (Bottom) ^{15}N NMR spectrum, gained after shaking for 10 s at $-0.3\ \mu\text{T}$ with a pressure of 4 bar p-H_2 . The spectrum was also collected at a field of 9.4 T.	209
Figure 6.18 Typical SABRE hyperpolarised ^{15}N NMR spectra gained from a sample containing 2 mg of the pre-catalyst IPr^{Cl} , 5 equivalents of all nitrile compounds in Table 6.5 and 5 equivalents of V also. The sample was shaken at a PTF of either -0.3 or $-1.0\ \mu\text{T}$ with a shake time of 10 s and a pressure of 4 bar p-H_2	211
Figure 6.19 Raw signal of the detected ^{15}N acetonitrile response as a function of acetonitrile excess. All samples were shaken for 10 s at a field of $-0.3\ \mu\text{T}$ with a pressure of 4 bar p-H_2 . .	212
Figure 6.20 a) A series of hyperpolarised ^{15}N NMR intensity data acquired to show the impact of ^{15}N signal relaxation. b) Typical plot showing the resulting ^{15}N signal intensity corrected for magnetisation loss against time for to an exponential decay linked to T_1 . All data was collected at 9.4 T. Each slice was collected using a pulse length equating to an angle of 14 degrees. The sample was shaken for 10 s in a field of $-0.3\ \mu\text{T}$ with a pressure of 4 bar p-H_2 before transfer to the magnet for data acquisition.....	213

Figure 6.21 Skeletal structure of the drug molecule anastrozole; IUPAC name 2,2'-[5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]bis(2-methylpropanenitrile).....	215
Figure 6.22 ¹⁵ N polarisation of anastrozole measured as a function of polarisation transfer field. The sample consisted of 2 mg of IPr ^{Cl} and 5 equivalents of anastrozole and V and was shaken for 10 s with 4 bar of p-H ₂ at each field.....	216
Figure 6.23 ¹⁵ N polarisation for anastrozole plotted as a function of the time the sample was shaken before signal acquisition. The sample consisted of 2 mg of IPr ^{Cl} with 5 equivalents of V and 5 equivalents of anastrozole.	217
Figure 7.1 Graph showing the magnetic field outcome (μT) as a function of the voltage applied (V) to the coil.	228
Figure 7.2 Structure of X determined from data given in Table 7.2.	238
Figure 7.3 Structure of 1 determined from data given in Table 7.3.	239
Figure 7.4 Structure of 2 determined from data given in Table 7.4.	240
Figure 7.5 Structure of 3 determined from data given in Table 7.5.	241
Figure 7.6 Structure of 4a determined from data given in Table 7.6.	242
Figure 7.7 Structure of 4b determined from data given in Table 7.7.	243
Figure 7.8 Structure of 4a – IM ^{p-Cl} determined from data given in Table 7.8.	244
Figure 7.9 Structure of 4a – IPr ^{NMe2} determined from data given in Table 7.9.	245
Figure 7.10 Structure of 4a – IM ^{p-tBu} determined from data given in Table 7.10.	246
Figure 7.11 Structure of 4a – IM ^{p-OMe} determined from data given in Table 7.11.	247
Figure 7.12 Structure of 4a – SIMes determined from data given in Table 7.12.	248
Figure 7.13 Structure of 4a – IMes ^{NMe2} determined from data given in Table 7.13.	249
Figure 7.14 Structure of 4a – IPr ^{Cl} determined from data given in Table 7.14.	250
Figure 7.15 Structure of 5 determined from data given in Table 7.15.	251
Figure 7.16 Structure of 6 determined from data given in Table 7.16.	252
Figure 7.17 Structure of 7 determined from data given in Table 7.17.	253
Figure 7.18 Structure of XZ determined from data given in Table 7.18.	255
Figure 7.19 Structure of 8 determined from data given in Table 7.19.	256
Figure 7.20 Structure of 9 determined from data given in Table 7.20.	257
Figure 7.21 Structure of 10 determined from data given in Table 7.21.	258
Figure 7.22 Structure of 11 determined from data given in Table 7.22.	259
Figure 7.23 Structure of 12 determined from data given in Table 7.23.	260
Figure 7.24 Structure of 11 _A determined from data given in Table 7.24.	261
Figure 7.25 Structure of 12 _A determined from data given in Table 7.25.	262
Figure 7.26 Structure of 13 determined from data given in Table 7.26.	263
Figure 7.27 Structure of 14 determined from data given in Table 7.27.	264
Figure 7.28 Structure of 12 _G determined from data given in Table 7.28.	265
Figure 7.29 Structure of 12 _G -IPr ^{NMe2} determined from data given in Table 7.29.	266
Figure 7.30 Structure of the compound ((5aR,6R,6aS)-1,4,5,5a,6,6a,7,8-Octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol6-yl)methanol.....	268
Figure 7.31 Structure of V determined from data given in Table 7.31.	268
Figure 7.32 Structure of C determined from data given in Table 7.32.	269
Figure 7.33 Structure of 17 determined from data given in Table 7.33.	269
Figure 7.34 Structure of 18 determined from data given in Table 7.34.	270
Figure 7.35 Structure of 19 determined from data given in Table 7.35.	271

List of Schemes

Scheme 2.1 Example reaction scheme detailing the conversion of a 1,2,4-triazine through a Diels Alder type reaction with a strained alkyne to produce a bicyclic product and nitrogen gas.	31
Scheme 2.2 Reaction products detected during the conversion of IrCl(COD)(IMes) (1) into [Ir(H) ₂ (IMes)(X) ₃]Cl (4a and 4b) in methanol-d ₄ solution, where X ₁ = 3-methylthio-1,2,4-triazine bound through N ₁ and X ₂ = 3-methylthio-1,2,4-triazine bound through N ₂ .	32
Scheme 2.3 Chemical changes associated with the conversion of 1 to 7 in dichloromethane-d ₂ through reaction with X and H ₂ .	39
Scheme 2.4 Reaction products detected during the reaction between X and Z to form the product XZ in methanol-d ₄ solution, where X = 3-methylthio-1,2,4-triazine Z = (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethanol and XZ = ((6aS,7S,7aR)-3-(methylthio)-6,6a,7,7a,8,9-hexahydro-5H-cyclopropa[5,6]cycloocta[1,2-c]pyridin-7-yl)methanol. *After a total of 14 days the yield had improved to around 50%.	66
Scheme 3.1 Reaction of IrCl(COD(IMes) (1) with NaNO ₂ (Y) and H ₂ in methanol-d ₄ leads to 9.	78
Scheme 3.2 Reaction of IrCl(COD(IMes) (1) with NaNO ₂ (NaY), pyridine (D) and H ₂ in methanol-d ₄ leads to several dihydride derived reaction products.	79
Scheme 3.3 Generic SABRE mechanism where NHC represents any carbene ligands from Figure 3.4, coL represents a co-ligand from Figure 3.2 and Y is still [NO ₂] ⁻ .	95
Scheme 3.4 Formation of phenyl diazonium chloride through the reaction of acidified Y with aniline.	98
Scheme 3.5 Possible reaction routes to the formation of phenyl azide and N ₂ from phenyl diazonium chloride and azide.	103
Scheme 6.1 Reaction of IrCl(COD(IMes) (1) with C, V, H ₂ in methanol-d ₄ leads to several dihydride derived reaction products.	192

List of Tables

Table 2.1 Some of the reported bond lengths from the structure reported by Oro et. al. as shown in the left of Figure 2.12	47
Table 2.2 Some of the reported bond angles from the structure reported by Oro et. al. as shown in the left of Figure 2.12	48
Table 2.3 Signal to noise ratio of the PHIP enhanced hydride ligand ^1H NMR signals for 3-NHC and their respective chemical shifts. NMR spectra were recorded at 243 K after addition of 4 bar p- H_2 at 77 K and shaking for 5 s. All samples utilised methanol- d_4 as a solvent.	53
Table 2.4 NMR signal gain for the two ^1H NMR resonances of X, both free and equatorially bound, (chemical shifts for the free X resonance are shown; A and B correspond to the bound versions of the resonances δ 9.01 and δ 8.55 signals in free X respectively). All the samples were shaken at 6 mT for 10 s with a pressure of 4 bar p- H_2 before signal acquisition at a field of 9.4 T.....	54
Table 2.5 ^1H NMR signal enhancements measured for the free ^1H NMR signals of X at δ 9.01 and 8.55 using precatalyst $[\text{IrCl}(\text{COD})(\text{IPr}^{\text{NMe}_2})]$ with a 5-fold excess of X and a 5-fold excess of the specified co-ligand in methanol- d_4 . These data utilised 4 bar of p- H_2 , a 60 G transfer field and a shake time of 10 s.....	60
Table 2.6 Rate of dissociation of X and H_2 at 298 K for the complexes $[\text{Ir}(\text{H})_2(\text{co-ligand})(\text{IPr}^{\text{NMe}_2})(\text{X})_2]\text{Cl}$ formed with the specified co-ligands (co-ligand axial to NHC). The samples all contained 2 mg of $\text{IPr}^{\text{NMe}_2}$ with 5 equivalents of X and 5 equivalents of the respective co-ligand, and were activated under a pressure of 4 bar H_2	60
Table 2.7 Rate of dissociation for X and H_2 at 298 K for the complexes $[\text{Ir}(\text{H})_2(\text{co-ligand})(\text{IMes})(\text{X})_2]\text{Cl}$ formed with the specified co-ligands (co-ligand axial to NHC). The samples all contained 2 mg of IMes with 5 equivalents of X and 5 equivalents of the respective co-ligand and were activated under a pressure of 4 bar H_2	61
Table 3.1 ^{15}N NMR signal enhancement at 9.4 T and ^{15}N polarisation of the free Y for each co-ligand used (leading to Figure 3.2).	84
Table 3.2 The relative proportion of each complex species present in the mixture for each corresponding co-ligand shown as a percentage of the overall amount of iridium in solution... ..	85
Table 3.3 ^{15}N NMR signal enhancement at 9.4 T and ^{15}N polarisation of the free Y for varying equivalents of G compared to Ir shown alongside the relative proportions of 11G and 12G in solution.....	86
Table 3.4 Rate of ligand dissociation for G- d_2 at 298 K for the series of NHC ligands from Figure 3.4.	90
Table 3.5 Key bond lengths for the structure presented in Figure 3.13.	102
Table 3.6 Key bond angles for the structure presented in Figure 3.13.	102
Table 4.1 Calculated T_1 values at 11.7 T for various reaction products from Chapter 3.	136
Table 4.2 T_1 values for Y at 9.4 T in the indicated solvent. Each sample contained the same amount of Y, with all apart from D_2O initially containing the pre-catalyst 1 (2 mg), 6 equivalents of G and 50 equivalents of 15-crown-5.	137
Table 4.3 T_1 values for Y gathered at various magnetic fields using a T_1 inversion recovery pulse sequence. All data was gathered on a sample of Y (250 mM) in methanol- d_4 under a hydrogen atmosphere of 4 bar.....	141
Table 5.1 Henry constants (k_{H}) determined at 298 K for the solvents presented in Figure 5.1 alongside the literature values where they are known.	169
Table 5.2 Solubility of $[\text{IrCl}(\text{COD})(\text{IMes})]$ (1) in 0.6 mL in each of the perfluorinated solvents J-Q of Figure 5.1.....	170

Table 5.3 NMR signal enhancement data presented for pyridine (D) from 1.6 μ L samples in solvents J, N, Q and methanol- d_4 with 2 mg of 1. NMR spectra were recorded at 9.4 T after shaking with 4 bar p- H_2 as described in the text.	171
Table 5.4 Calculated T_1 values for ^{15}N -D in different solvent systems. Each sample contained the same amount of ^{15}N -D (50 μ L) and was under a pressure of 4 bar H_2	175
Table 5.5 ^1H NMR signal enhancements at 9.4 T resulting for the indicated ^1H nuclei as detailed in Figure 5.5 after SABRE.	177
Table 5.6 T_1 values for the ^1H NMR signals in the labelled positions on Figure 5.5 all measured at 9.4 T.	178
Table 6.1 ^{15}N NMR signal with enhancement and % polarisation for the free ^{15}N signal of C at δ 240.8 in methanol- d_4 ; a typical sample contained 1 (5 mM), a 5-fold excess of C and a 5-fold excess of the co-ligand stated. These data utilised 4 bar of p- H_2 and a shake time of 10 s.	190
Table 6.2 Relative amount of 20, 21 and 22 in solution as a % of the overall signal from all hydride ligands. Note that 22 _A is an isomeric form of 22 with similar behaviour to 4a and 4b described in Chapter 2.	201
Table 6.3 % polarisation values for the ^{15}N responses of the nitriles shown in Figure 6.14; all samples were shaken at a field of -3 μ T with a pressure of 4 bar p- H_2 for 10 s with either ‘un-optimised’ or ‘optimised’ conditions as explained in the text above.	205
Table 6.4 ^{15}N polarisation and enhancement values at 9.4 T for the ^{15}N resonances present in 3- and 4-cyanopyridine.	208
Table 6.5 ^{15}N polarisation and enhancement values at 9.4 T for the ^{15}N resonances present in the complicated mixture.	210
Table 6.6 T_1 lifetime data collected at 11.7 T, 9.4 T and 7 T for a series of nitrile compounds for which the SABRE hyperpolarisation has been optimised.	214
Table 7.1 A summary of the NMR spectrometers utilised showing the magnetic field strength, ^1H NMR resonance frequency and the model of spectrometer	225
Table 7.2 NMR data collected in methanol- d_4 at 298 K for X shown in Figure 7.2. Resonance labels are stated according to Figure 7.2	238
Table 7.3 NMR data collected in methanol- d_4 at 263 K for 1 shown in Figure 7.3. Resonance labels are stated according to Figure 7.3	239
Table 7.4 NMR data collected in methanol- d_4 at 263 K for 2 shown in Figure 7.4. Resonance labels are stated according to Figure 7.4.	240
Table 7.5 NMR data collected in methanol- d_4 at 243 K for 3 shown in Figure 7.5. Resonance labels are stated according to Figure 7.5.	241
Table 7.6 NMR data collected in methanol- d_4 at 263 K for 4a shown in Figure 7.6. Resonance labels are stated according to Figure 7.6.	242
Table 7.7 NMR data collected in methanol- d_4 at 263 K for 4b shown in Figure 7.7. Resonance labels are stated according to Figure 7.7.	243
Table 7.8 NMR data collected in methanol- d_4 at 263 K for 4a – IM ^{p-Cl} shown in Figure 7.8. Resonance labels are stated according to Figure 7.8.	244
Table 7.9 NMR data collected in methanol- d_4 at 263 K for 4a – IPr ^{NMe2} shown in Figure 7.9. Resonance labels are stated according to Figure 7.9.	245
Table 7.10 NMR data collected in methanol- d_4 at 263 K for 4a – IM ^{p-tBu} shown in Figure 7.10. Resonance labels are stated according to Figure 7.10.	246
Table 7.11 NMR data collected in methanol- d_4 at 263 K for 4a – IM ^{p-OMe} shown in Figure 7.11. Resonance labels are stated according to Figure 7.11.	247
Table 7.12 NMR data collected in methanol- d_4 at 263 K for 4a – SIMes shown in Figure 7.12. Resonance labels are stated according to Figure 7.12.	248
Table 7.13 NMR data collected in methanol- d_4 at 263 K for 4a – IMes ^{NMe2} shown in Figure 7.13. Resonance labels are stated according to Figure 7.13.	249

Table 7.14 NMR data collected in methanol-d ₄ at 263 K for 4a – IPr ^{Cl} shown in Figure 7.14. Resonance labels are stated according to Figure 7.14.....	250
Table 7.15 NMR data collected in dichloromethane-d ₂ at 243 K for 5 shown in Figure 7.15. Resonance labels are stated according to Figure 7.15.....	251
Table 7.16 NMR data collected in dichloromethane-d ₂ at 243 K for 6 shown in Figure 7.16. Resonance labels are stated according to Figure 7.16.....	252
Table 7.17 NMR data collected in dichloromethane-d ₂ at 243 K for 7 shown in Figure 7.17. Resonance labels are stated according to Figure 7.17.....	253
Table 7.18 NMR data collected in methanol-d ₄ at 243 K for XZ shown in Figure 7.18. Resonance labels are stated according to Figure 7.18.....	255
Table 7.19 NMR data collected in methanol-d ₄ at 263 K for 8 shown in Figure 7.19. Resonance labels are stated according to Figure 7.19.....	256
Table 7.20 NMR data collected in methanol-d ₄ at 243 K for 9 shown in Figure 7.20. Resonance labels are stated according to Figure 7.20.....	257
Table 7.21 NMR data collected in methanol-d ₄ at 243 K for 10 shown in Figure 7.21. Resonance labels are stated according to Figure 7.21.....	258
Table 7.22 NMR data collected in methanol-d ₄ at 263 K for 11 shown in Figure 7.22. Resonance labels are stated according to Figure 7.22.....	259
Table 7.23 NMR data collected in methanol-d ₄ at 263 K for 12 shown in Figure 7.23. Resonance labels are stated according to Figure 7.23.....	260
Table 7.24 NMR data collected in methanol-d ₄ at 263 K for 11 _A shown in Figure 7.24. Resonance labels are stated according to Figure 7.24.....	261
Table 7.25 NMR data collected in methanol-d ₄ at 263 K for 12 _A shown in Figure 7.25. Resonance labels are stated according to Figure 7.25.....	262
Table 7.26 NMR data collected in methanol-d ₄ at 263 K for 13 shown in Figure 7.26. Resonance labels are stated according to Figure 7.26.....	263
Table 7.27 NMR data collected in methanol-d ₄ at 263 K for 14 shown in Figure 7.27. Resonance labels are stated according to Figure 7.27.....	264
Table 7.28 NMR data collected in methanol-d ₄ at 263 K for 12 _G shown in Figure 7.28. Resonance labels are stated according to Figure 7.28.....	265
Table 7.29 NMR data collected in methanol-d ₄ at 263 K for 12 _G -IPr ^{NMe₂} shown in Figure 7.29. Resonance labels are stated according to Figure 7.29.....	267
Table 7.30 NMR data collected in methanol-d ₄ at 263 K for V shown in Figure 7.31. Resonance labels are stated according to Figure 7.31.....	268
Table 7.31 NMR data collected in methanol-d ₄ at 263 K for C shown in Figure 7.32. Resonance labels are stated according to Figure 7.32.....	269
Table 7.32 NMR data collected in methanol-d ₄ at 263 K for 17 shown in Figure 7.33. Resonance labels are stated according to Figure 7.33.....	269
Table 7.33 NMR data collected in methanol-d ₄ at 263 K for 18 shown in Figure 7.34. Resonance labels are stated according to Figure 7.34.....	270
Table 7.34 NMR data collected in methanol-d ₄ at 263 K for 19 shown in Figure 7.35. Resonance labels are stated according to Figure 7.35.....	271
Table A.2.1 Fractional Atomic Coordinates (×104) and Equivalent Isotropic Displacement Parameters (Å ² ×103) for the structure presented in Figure 3.13. U _{eq} is defined as 1/3 of the trace of the orthogonalised U _{ij} tensor.	294

Chapter 1. Introduction

1.1 Principles of Magnetic Resonance

1.1.1 Fundamentals of Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) is one of the most powerful tools at the modern chemist's disposal. This technique can provide detailed information about the chemical environments of nuclei within a molecule, and allows qualitative and quantitative observations of reactions to be recorded whilst the reaction is taking place. This 'on demand' data is unrivalled in its simplicity to collect, with many NMR services having pre-set 'walk-up' experiment lists. This allows this complex and expensive technology to be used by non-scientists with ease, whilst still providing robust data for analysis.

The underlying principles of NMR require slightly more understanding than is associated with walking up and pressing go on a pulse sequence. It requires understanding of where magnetism arises from in an atom and how the associated magnetic nuclei behave when an external magnetic field is applied to them. Nuclear spin, described by the quantum number I , is an inherent property of nuclei which have an overall angular momentum. This spin can take values such as; $0, \frac{1}{2}, 1, \frac{3}{2}, 2$ etc. However, to be NMR active and classed as magnetic, the spin value must be greater than 0. When a non-zero spin nucleus is placed in a magnetic field the nucleus can interact with the magnetic field. Taking a spin $-\frac{1}{2}$ nucleus such as ^1H as a simple example, the spin of the ^1H could either interact in a positive or negative manner with the external field leading to an energy difference between the two states (Figure 1.1). This quantised energy gap, like in other spectroscopic techniques, is exploited to observe transitions between the ground and excited states. The frequency of electromagnetic radiation required to probe this gap can be calculated as shown in Equation 1.1 where ΔE is the energy gap in J , h is Planck's constant in $J\text{s}$ and ν is the frequency of light in s^{-1} .

$$\Delta E = h\nu \quad (1.1)$$

The two orientations shown in Figure 1 are determined by the angular momentum vector I which has $2I+1$ different permutations in an axis (for example the z component of I would be denoted as I_z) giving the different values shown for the magnetic quantum number, m , of $+\frac{1}{2}$ and $-\frac{1}{2}$ as m must have values from $+I$ to $-I$ changing value in steps of 1. The reasoning for the spin taking one of these two orientations derives from its link with the magnetic moment of the nucleus through a proportionality constant known as

the gyromagnetic ratio, given the symbol γ . This relationship is shown in Equation 1.2, where μ is the magnetic moment vector, γ is the gyromagnetic ratio of the nucleus and I is the angular momentum vector.¹

$$\mu = \gamma I \quad (1.2)$$

In the absence of a magnetic field all the spins would point in random directions and would have degeneracy in terms of energy. However, when a magnetic field is applied (vector B) then the degeneracy is broken and an energy difference is created between the spin states projected on the magnetic field (z direction) as shown in Figure 1.1 and described by Equation 1.3 where E is the energy between the spin states, μ_z is μ projected onto B and B is the applied magnetic field strength.

$$E = -\mu_z B \quad (1.3)$$

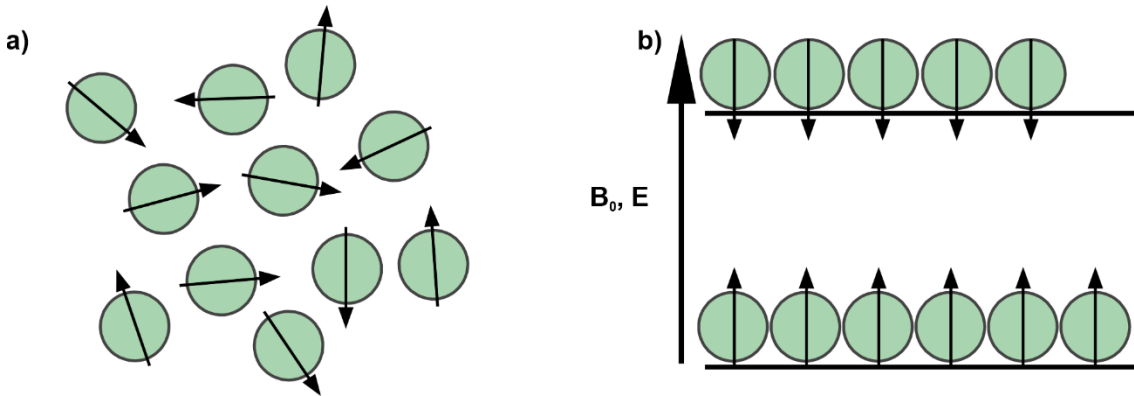


Figure 1.1 a) The random alignment of nuclear spins in the absence of a magnetic field. b) Energy level diagram showing nuclear spins now either aligned parallel or antiparallel with the applied magnetic field (B_0). The energy difference between these two states is small but the parallel arrangement is slightly lower energy and therefore preferred.

As this energy gap is quantised we can derive that the frequency of radiation needed to excite the nuclei is also dependent on the applied magnetic field. Thus as the magnetic field applied increases so will the energy gap between the two spin states. This idea is explored in more detail in Section 1.3 Improved Polarisation Using a Brute Force Approach where the idea of improving net magnetisation is discussed in detail. As the energy difference increases so does the frequency required to excite the spins; this frequency is known as the Larmor frequency. However, for NMR, this frequency always falls in radiofrequency (r.f.) showing just how low this energy match is for resonance. Equations 1.4 to 1.6 show the development of the energy gap to required frequency and then finally to angular frequency, ω . In these equations ΔE is the difference in energy between spin states, h is Planck's constant, ν is frequency, γ is the gyromagnetic ratio, B

is the applied magnetic field and ω is the angular frequency (the frequency, ν , multiplied by 2π).¹

$$\Delta E = h\nu = \frac{h\gamma B}{2\pi} \quad (1.4)$$

$$\nu = \frac{\gamma B}{2\pi} \quad (1.5)$$

$$\omega = -\gamma B \quad (1.6)$$

Using Equation 1.6 it becomes clear that with a magnetic field of 1.4 T vs 14.1 T the corresponding frequencies required to excite ^1H ($\gamma = 267.52 \times 10^6 \text{ rads}^{-1} \text{ T}^{-1}$) are ~60 MHz and ~600 MHz respectively proving the linear relationship between frequency and field strength. These frequencies are also known as the Larmor precession frequency. This precession frequency remains true if the magnetic field is completely homogeneous around the sample, a task that is attempted by shimming, a process by which current is applied through ‘shim coils’ to create localised fields to homogenise the applied field around the sample.

In reality a sample containing two chemically different ^1H protons can never be homogeneous. It can have a homogeneous field applied to it. However, the field will differ at the two ^1H sites due to the local magnetic properties of that environment. As the nuclei observed by NMR spectroscopy have electrons surrounding them the electron movement generates a local field that can counter the applied magnetic field leading to a distortion, making the two environments have a different ‘experienced field’. If the local field is strong, less of the applied field is felt (the nucleus is shielded from the applied field), this means the experienced field is lower than expected and so the local frequency will be lower than the Larmor frequency. If the local field is weak then the opposite occurs and the nucleus will precess faster than the Larmor frequency.

This idea of ‘faster’ or ‘slower’ is easy to comprehend qualitatively but in reality trying to observe a difference in the speed of precession for two nuclei, one at 400 MHz and the other just 1 Hz faster is impossible to imagine. The difference here is just 1 in 10^6 (400,000,000 vs. 400,000,001) a completely negligible difference in most fields of research. However, in NMR this is the difference we are interested in exploiting to identify our chemical species of interest within a mixture. In order to achieve this we deploy a tactic known as a rotating frame of reference. We allow our axes through which

we are watching this precession to rotate at a set frequency (say the Larmor frequency). Now as we are travelling at 400 MHz we see everything relative to this speed so a 1 in 10^6 difference now appears as 1 cycle per second around the axis of precession quicker than ourselves, a difference that is easy to comprehend, and observe. A good way to think of this rotating frame of reference is by thinking about a car on the motorway. Stood on a footbridge the cars appear to be travelling very fast. However, when you are in the car travelling at say 60 mph, you only notice those going past you, at say 70 mph, who appear to be going much quicker, and those at 50 mph whom you are overtaking, appearing to be going much slower. Those in front and behind you also travelling at 60 mph stay the same distance away from you. They appear stationary, relative to yourself.

1.1.2 Fundamentals of Magnetic Resonance Imaging (MRI)

The underlying principles of MRI are the same as that of NMR as they are both magnetic resonance techniques. However, one of the major differences between the two techniques relies on how we acquire and process data. To understand MRI is first important to understand the use of gradients. We will start by looking at imaging in a one-dimensional plane and then build up to 2D and even 3D images.

As discussed previously, the magnetic field strength has a direct effect on the precession frequency of nuclei in a sample. If the magnetic field differs across a sample then so will the frequency required to excite the spins and so different signals could be observed for the same species. Taking a sample of water in an imaginary spectrometer, capable of having fields at 1.4 T and 14.1 T split across the sample, and acquiring ^1H spectra at both fields simultaneously, one would expect ^1H NMR peaks to appear at 60 and 600 MHz for the fields respectively. If this were done across a wide range of fields (setting up a gradient of increasing field) then even more ^1H NMR peaks would appear for the water sample at the corresponding frequencies given by Equation 1.6.² This can be shown visually by Figure 1.2 and is known as frequency encoding.

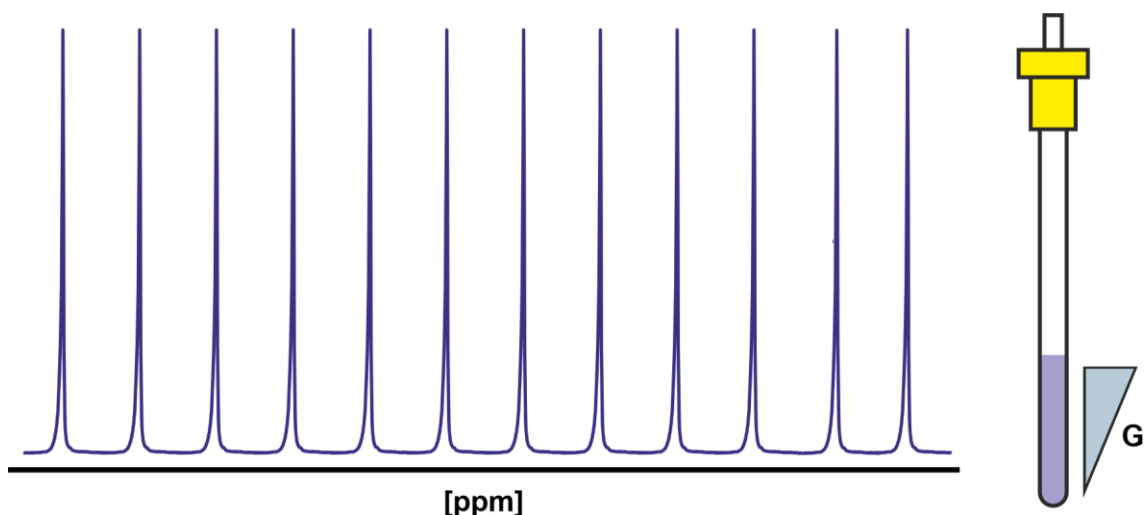


Figure 1.2 Applying a magnetic field gradient across the sample causes a difference in local precessional frequency and so NMR peaks appear at different chemical shifts representing the different magnetic fields across the sample.

If the water samples contain different amounts of water then the signal intensities gained from the samples will also be different as the number of active spins in the region is now more or less. This builds up a ‘picture’ of the intensity of spins across the axis that the gradient is applied on. Putting this into context of a more uniform sample, a bottle of water with a specific shape. If placed in a probe and a gradient is applied then a spectrum can be collected of each section by ‘slicing’ the bottle. These slices can then be sandwiched back together to give us the picture of the water bottle in one dimension as shown in Figure 1.3.

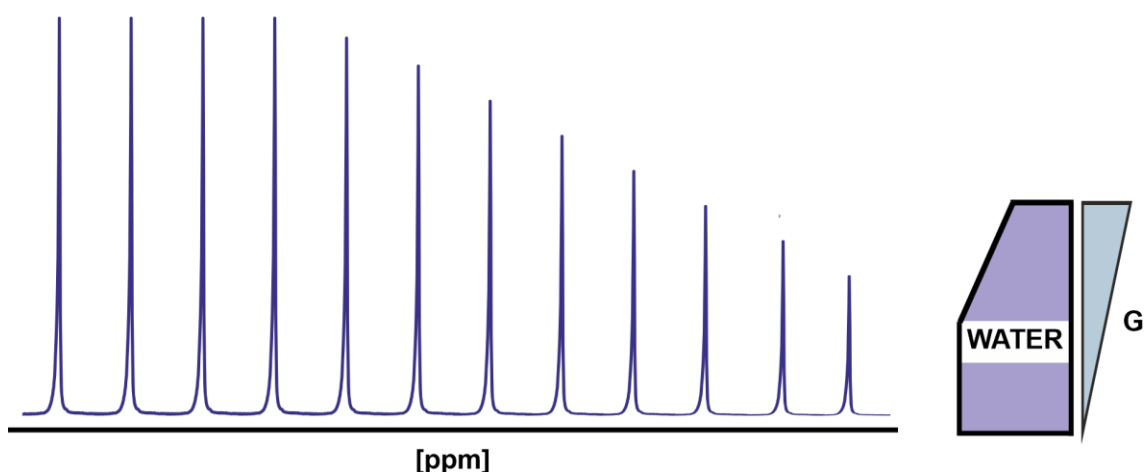


Figure 1.3 Applying a magnetic field gradient across a sample causes a difference in local precessional frequency and therefore chemical shift as in Figure 1.2. Now, as the sample has a non-uniform number of spins in each section, the intensity of the NMR peak changes accordingly.

This one-dimensional image of proton intensity tells us nothing about the other axes of the bottle and so give us no indication of the actual shape. There are an infinite number of three-dimensional shapes that would give the same ‘outline’ via this method.

It is therefore important to look in more than one dimension by applying gradients on more than one axis to transverse the 2D plane of the bottle. We can now build up an actual image of the bottle.

1.1.3 Improved Polarisation Using Brute Force Approach

The weak interaction between the nuclei and external magnetic field gives rise to a small energy gap between spin aligned states as discussed in section 1.1. This small energy gap means there is only a small preference for the lower energy state where the nuclei is aligned with the direction of the applied magnetic field. In fact at 9.4 T only 1 in every 32,000 ^1H nuclear spins are in excess in the α spin state.³ For other nuclei such as ^{15}N where the γ is 10 times lower than ^1H and the natural abundance of ^{15}N present is 0.4% vs. the ^1H natural abundance of near 100% leads to ^{15}N being 260,000 times harder to detect than ^1H .⁴ The difference between the two spin states can be described using Boltzmann statistics using Equation 1.7, where N_α and N_β are the populations of the α and β spin states observed in Figure 1.1, k is the Boltzmann constant, B_0 is the applied magnetic field and T is temperature.

$$\frac{N_\alpha}{N_\beta} = e^{\frac{\gamma h B_0}{4\pi k T}} \quad (1.7)$$

This polarisation is responsible for the observable signal of the nuclei and thus is the reason that NMR spectroscopy is classed as such an insensitive technique, with a several orders of magnitude higher limit of detection than other commonly available techniques. The polarisation itself refers to the extent to which nuclei align with, or against, the magnetic field applied to the sample vs. the total number of spins in the system. This relationship is given in Equation 1.8.⁵

$$P = \frac{N_\alpha - N_\beta}{N_\alpha + N_\beta} = \tanh\left(\frac{\gamma h B_0}{4\pi k T}\right) \quad (1.8)$$

Improving polarisation can be done relatively simply by exploiting variables in this equation, a method known as the brute force approach. By increasing the applied magnetic field or decreasing the temperature it is possible to increase the polarisation and thus get more observable signal from the same sample. In 2015 Kempf *et al.* demonstrated a 1600-fold signal enhancement utilising a 14 T magnet and a temperature of *circa* 2 K to establish a higher polarisation level prior to acquisition.⁶ They further demonstrated this polarisation could be transferred to ^{13}C by passing the sample through a low magnetic

field.⁷ Many developments have been made to aid this approach and ease detection of nuclear spins, including magnets of 28 T and through the use of liquid nitrogen cooling units capable of reaching much lower temperatures. Although in solution state NMR spectroscopy, one would be limited by the temperature range of the solvent.

1.1.4 Improved Signal to Noise Using Pulse Sequences

The result of improved polarisation is ultimately an improved signal to noise in the subsequent NMR experiment. This process can also be done by clever use of *r.f.* pulses to either use the polarisation of a more favourable nucleus or to collapse multiplet peaks in to a singlet peak, much larger in intensity than the original peaks. The most sensitive nucleus to be probed by NMR is that of ^1H . With its high gyromagnetic ratio ($\gamma = 42.6 \text{ MHz T}^{-1}$) and natural abundance of >99.9%, it can be detected with ease compared to other less sensitive nuclei like ^{13}C and ^{15}N . A method to overcome the poor sensitivity is to use the polarisation of the ^1H nucleus but detect on the ^{15}N , for example. This can be done through an “Insensitive Nuclei Enhanced by Polarisation Transfer” (INEPT) type pulse sequence.⁸ The INEPT type transfer sequence is widely utilised in many other common NMR experiments such as “Heteronuclear Single-Quantum Correlation” HSQC, widely used for routine characterisation. The other method involves collapsing multiplet signals, which could be achieved through decoupling, again routinely done for HSQC when characterisation is being undertaken. The process involves applying a repeat *r.f.* pulse on ^1H during signal acquisition. The rapid succession of the pulses causes heating due to the absorption of the *r.f.* power and so short acquisition times must be used to avoid damage to the hardware of the spectrometer. More complex methods exist to collapse all NMR peaks of a set resonance into a higher intensity singlet. This can serve to boost signal to noise but also make overlapping peaks easier to identify by observing their absolute chemical shift only and so are named “pure shift” methods.⁹⁻¹²

1.2 Hyperpolarisation Techniques

1.2.1 Principles of Hyperpolarisation

Hyperpolarisation refers to techniques that allow spin population of one of the Zeeman energy levels to rise above that of a thermal population, as determined by Boltzmann statistics, without changing parameters in equation 1.7 such as temperature (T), and the acquisition magnetic field strength (B_0). These techniques vary greatly in

their approach and so have various pros and cons associated with them which will be discussed in their corresponding section. The main hyperpolarisation technique utilised in this thesis is the Signal Amplification By Reversible Exchange (SABRE),¹³ which is a variation of the more classic *Para*Hydrogen Induced Polarisation (PHIP).¹⁴

1.2.2 Parahydrogen Induced Polarisation (PHIP)

Initially predicted by Bowers and Weitekamp in 1986,¹⁴ PHIP utilises the high spin order of *parahydrogen* ($p\text{-H}_2$), the nuclear singlet state of hydrogen. This can dramatically enhance the nuclear spin-polarisation of ^1H nuclei resulting from the reaction of a suitable substrate with the $p\text{-H}_2$. An example reaction for this is shown in Figure 1.4.^{15, 16}

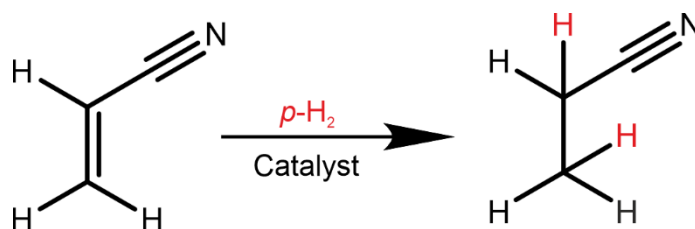


Figure 1.4 An example hydrogenation reaction undertaken by Bowers and Weitekamp first used to demonstrate PHIP of acrylonitrile. The catalyst utilised was of the form $\text{RhCl}(\text{PPh}_3)_3$.

This effect was actually observed before the initial proposition by Bowers and Weitekamp by Bergman et al. who proposed the enhanced signals observed during the hydrogenation were down to the Chemically Induced Dynamic Nuclear Polarisation (CIDNP) effect (another phenomenon within the field of hyperpolarisation).^{17, 18} The authors reported the magnitude of the enhancements increased when the sample was cooled in the presence of H_2 , a process that can generate $p\text{-H}_2$.¹⁹ Hydrogen takes the form of one of four spin states, three of which yield the higher energy *ortho*hydrogen $o\text{-H}_2$ and one yielding lower energy $p\text{-H}_2$ as detailed in Figure 1.5. Here $p\text{-H}_2$ lies lower in energy than the *ortho* states due to the antisymmetric nature of the total wavefunction, Ψ_{tot} , of H_2 . The Ψ_{tot} can be split into its independent contributions according to the Born-Oppenheimer approximation given in Equation 1.9.^{20, 21}

$$\Psi_{\text{tot}} = \Psi_{\text{el}} \times \Psi_{\text{vib}} \times \Psi_{\text{rot}} \times \Psi_{\text{trs}} \times \Psi_{\text{ns}} \quad (1.9)$$

Where Ψ_{el} , Ψ_{vib} , Ψ_{rot} , Ψ_{trs} and Ψ_{ns} are the electronic, vibrational, rotational, translational and nuclear spin contributions respectively. Within the Ψ_{tot} ; Ψ_{el} , Ψ_{vib} and Ψ_{trs} are all symmetric contributions and so the product of $\Psi_{\text{rot}}\Psi_{\text{ns}}$ has to be antisymmetric for Ψ_{tot} to be antisymmetric overall. The Ψ_{ns} contribution is symmetric for $o\text{-H}_2$ and

antisymmetric in $p\text{-H}_2$. Therefore the contribution Ψ_{rot} has to be antisymmetric for $o\text{-H}_2$ but symmetric in $p\text{-H}_2$ meaning that the lowest rotational energy levels, represented by the quantum number J , allowed for $o\text{-H}_2$ and $p\text{-H}_2$ is 1 and 0 respectively. The *para* form of H_2 can therefore occupy the lower rotational energy level, $J=0$, and therefore is lower energy.²⁰

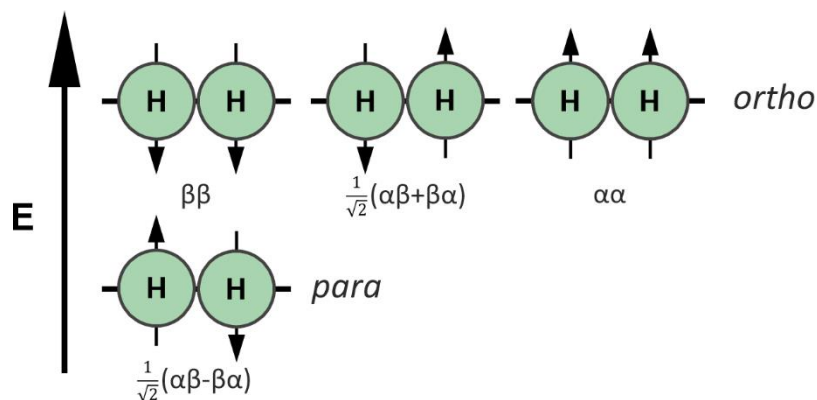


Figure 1.5 The four possible nuclear spin alignments of nuclei in a molecule of hydrogen in the absence of an applied magnetic field.

When hydrogen gas is generated at 298 K the energy gap between these spin states is very small and so it contains an equal mix of all spin isomers of H_2 and so only forms 25% $p\text{-H}_2$ purity. The conversion from o to $p\text{-H}_2$ is quantum mechanically forbidden and so occurs very slowly when attempted without the presence of a catalyst.¹⁹ The $p\text{-H}_2$ form can however be generated very easily by simply cooling the gas in the presence of a suitable *paramagnetic* catalyst such as iron (III) oxide (Fe_2O_3).²² The temperature dependence of this conversion has been well studied and has shown that 50% $p\text{-H}_2$ purity can be achieved by cooling to 77 K (Figure 1.6), achieved with liquid nitrogen.²³ Alternatively, cooling down to *circa* 23 K can achieve $p\text{-H}_2$ purity of >99%.²⁴

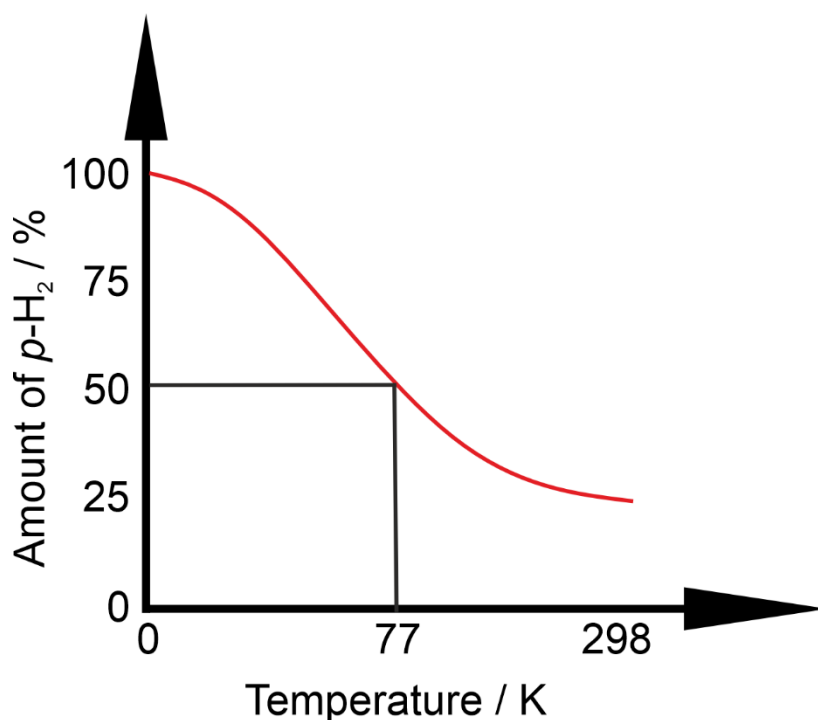


Figure 1.6 The temperature dependence on the $p\text{-H}_2$ purity when H_2 is cooled in the presence of a suitable catalyst. As shown, cooling to 77 K produces circa 50% $p\text{-H}_2$.

The lower energy $p\text{-H}_2$ is itself NMR silent due to its overall spin of 0. Therefore the $p\text{-H}_2$ singlet must be unlocked in order to realise its polarisation potential. This can be done through oxidative addition with a suitable metal complex or through addition into an organic substrate as already shown in Figure 1.4. The simplicity in utilising this method has seen wide uptake in its use and therefore its applications. The enhanced NMR signals provided have mainly been applied to work in elucidation of catalytic mechanisms.²⁵⁻²⁷ However, PHIP has seen applications in monitoring hydrogenation reactions catalysed by homogeneous catalysts,^{25, 28, 29} frustrated Lewis pairs,^{30, 31} heterogeneous catalysis³² and even enzymatically.³³ Utilising the $p\text{-H}_2$ can lead to different effects being observed, dependent upon the conditions of the experimental procedure. When placed in a strong magnetic field, typical of that utilised in an NMR spectrometer, the energy levels of $o\text{-H}_2$ displayed in Figure 1.5 lose their degeneracy and so acquire an energy ordering. When the H_2 is added to a structure as in the reaction shown in Figure 1.4, the two ^1H spins become inequivalent. If all other spins in the structure are ignored, addition of the H_2 under Boltzmann conditions means the four energy levels are equally populated and so lead to four possible transitions, yielding the spectrum in Figure 1.7.

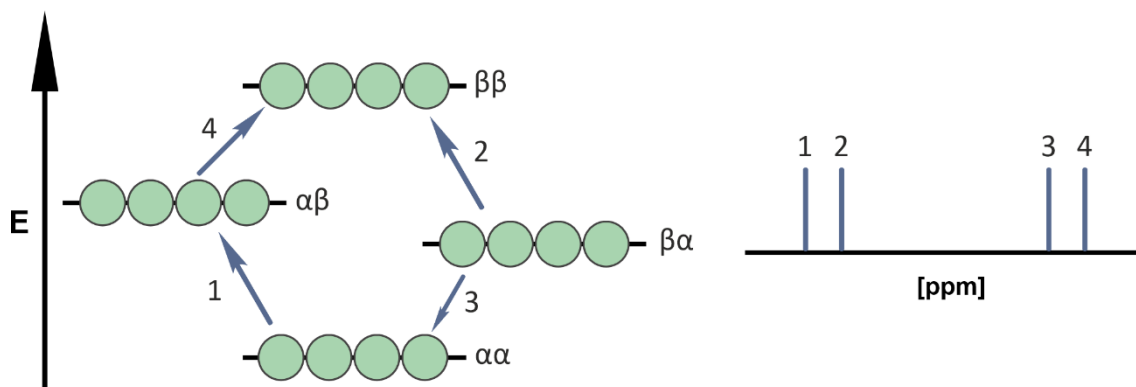


Figure 1.7: (Left) The four possible nuclear spin alignments of the H_2 derived nuclei in the product propionitrile from Figure 1.4 within a homogeneous high magnetic field (only the spins originating from H_2 are taken into account). The allowed NMR transitions are shown as blue arrows. (Right) The corresponding ^1H NMR spectrum produced from analysis of these specific spins in the product.

When enriched $p\text{-H}_2$ is used (above its normal 25% thermal distribution) the $\alpha\beta$ and $\beta\alpha$ energy levels will be overpopulated and so lead to a greater number of transitions from these levels leading to an NMR spectrum as shown in Figure 1.8. This kind of effect is known as ‘*parahydrogen* and synthesis allow dramatically enhanced nuclear alignment’ or PASADENA.¹⁵

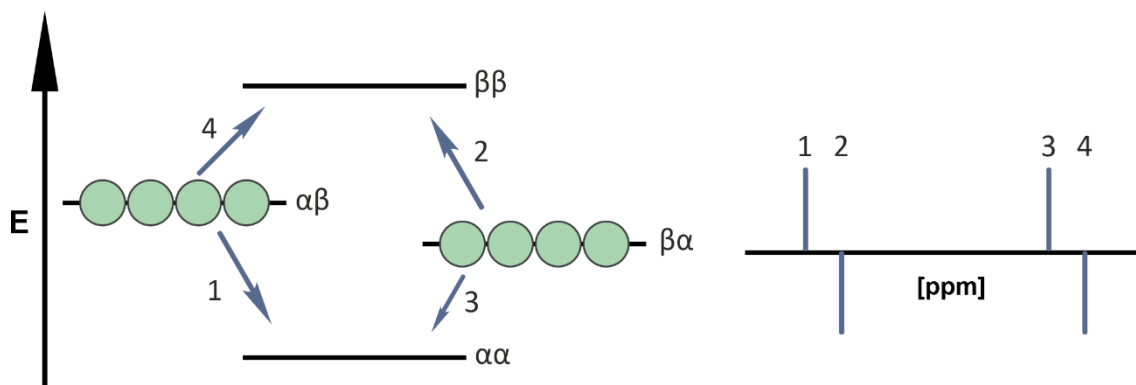


Figure 1.8 (Left) The two possible resulting nuclear spin alignments of the $p\text{-H}_2$ derived nuclei in the product propionitrile from Figure 1.4 under PASADENA conditions (only the spins originating from $p\text{-H}_2$ are taken into account). (Right) The corresponding ^1H NMR spectrum produced from analysis of these specific spins in product displaying PASADENA enhancements.

If the reaction takes place in the absence of a strong magnetic field followed by adiabatic transfer to a strong field for NMR signal acquisition then only the $\beta\alpha$ state is occupied. This in turn reduces the allowed transitions and so leads to an NMR spectrum typical of the case shown in Figure 1.9. This effect is known as ‘*adiabatic longitudinal transport after dissociation engenders nuclear alignment*’ or ALTADENA.³⁴

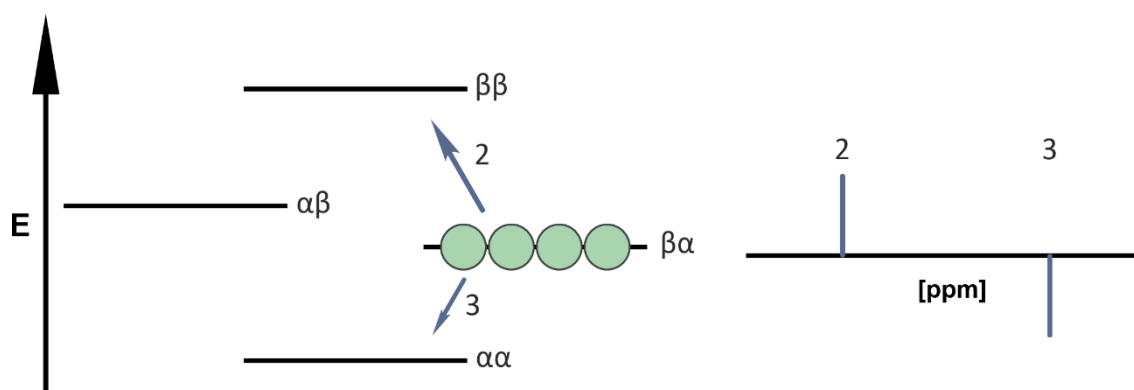


Figure 1.9 (Left) The resulting nuclear spin alignment of the $p\text{-H}_2$ derived nuclei in the product propionitrile from Figure 1.4 under ALTADENA conditions (only the spins originating from $p\text{-H}_2$ are taken into account). **(Right)** The corresponding ^1H NMR spectrum produced from analysis of these specific spins in product displaying ALTADENA enhancements.

The hyperpolarisation potential of PHIP is not limited to ^1H nuclei and many examples utilising INEPT type pulse sequences have been reported for ^{13}C , ^{14}N , ^{15}N and ^{31}P among many other NMR active nuclei.³⁵⁻³⁹

1.2.3 Signal Amplification by Reversible Exchange (SABRE)

Building on the work of PHIP, SABRE was discovered and pioneered by Duckett *et al.* (2009).^{13, 40} The technique has been described as a non-hydrogenative version of PHIP as the $p\text{-H}_2$ is not directly incorporated into the substrate being investigated, and so no chemical change of the substrate is undertaken. The technique most commonly utilises an iridium catalyst and allows $p\text{-H}_2$ and substrate ligands to exchange reversibly as shown in Figure 1.10. Allowing this process to occur in a low magnetic field of 0-140 mT allows spontaneous polarisation transfer to occur through the J -coupling network established on the catalyst between the two $p\text{-H}_2$ derived hydrides and the nuclear spins of the substrate molecule. This means that the substrate must be able to act as a Lewis base in order to ligate to the metal catalyst. Examples of species hyperpolarised by SABRE include amines,⁴¹⁻⁴³ nitriles,^{44, 45} N-heterocycles,⁴⁵ diazirines,^{46, 47} Schiff bases⁴⁸ and oxygen donors such as pyruvate⁴⁹ and acetate⁵⁰ also. Much like PHIP, SABRE is also not limited to ^1H nuclei and many studies have been reported for the enhancement of NMR signals of ^{13}C ,^(51, 52) ^{15}N ,^(4, 53, 54) ^{19}F ,^(11, 55) ^{31}P ,⁽⁵⁶⁾ ^{29}Si and ^{119}Sn .⁽³⁾ The fact that this process is reversible means the substrate in solution can be hyperpolarised many times, simply by introducing fresh $p\text{-H}_2$, thus giving an advantage over PHIP that is, instead, a ‘one-shot’ process. This in turn means that once the substrate has reacted, the hyperpolarised signals will cease to be observed.

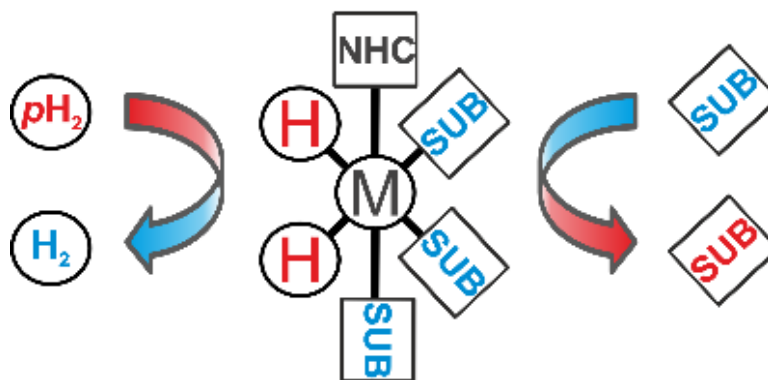


Figure 1.10 An example SABRE catalytic transfer of magnetisation from $p\text{-H}_2$ derived hydride ligands to a substrate when both are in reversible exchange with an iridium catalyst (where NHC is an N -heterocyclic carbene) at low (0-140 mT) magnetic field.

As shown in Figure 1.10 catalysts used in SABRE usually take on the active form $[\text{Ir}(\text{H})_2(\text{NHC})(\text{substrate})_3]\text{Cl}$, where NHC stands for N -heterocyclic carbene, in order to generate a complex of a stable pre-catalyst of the nature $[\text{IrCl}(\text{COD})(\text{NHC})]$ (COD = *cis-cis*-1,5-cyclooctadiene), which can be stored in air at room temperature. Upon the addition of H_2 , the COD ligand is hydrogenated and replaced by two substrate molecules forming the active SABRE catalyst.

As described by Barskiy *et al.* (2016),⁵⁷ it is essential to optimise the rate of exchange of the substrate ligand to match the time period needed for polarisation transfer.⁵⁸ In a polarisation transfer catalyst like that shown in Figure 1.10, it is the difference between the couplings from the *parahydrogen* derived hydride ligands that is a key factor in the optimum dissociation rate. If the hydride ligands are given the labels A and A' and the target nucleus B then this is described by $J_{AB} - J_{A'B}$, where J_{AB} and $J_{A'B}$ are *cis* and *trans* relationships respectively. In a system where $J_{AB} - J_{A'B} = 1$ Hz Barskiy describes the optimum rate as being 4.5 s^{-1} . For polarisation transfer to heteronuclei such as ^{15}N the difference between these couplings will be much larger and therefore a faster rate will be required.

It has been described by Rayner *et al.* that the rate of ligand dissociation can be easily optimised by varying the NHC ligand on the catalyst such as that in Figure 1.10.⁵⁹ By changing the electronic and steric properties of the NHC it is possible to force ligands to dissociate quicker (or stay bound longer). Dr. Peter Rayner has synthesised a library of these NHC containing pre-catalysts in the Duckett group that will be used for studies in this thesis. These display a broad range of steric and electronic effects as described by the

literature.⁵⁹ The methods typically used to measure the steric and electronic effects of these NHC ligands will be discussed in a later section in this introduction. Another tool to optimise exchange rate and form a favourable SABRE active catalyst is the use of co-ligands in place of one or two of the substrate ligands in Figure 1.10. Many studies have shown that the use of co-ligands can allow the binding and hyperpolarisation of weak ligands such as pyruvate.⁴⁹ It has also been shown by Tessari *et al.* that the use of a strongly binding triazole ligand can aid in the quantification of low quantification substrates by SABRE also.⁶⁰

Another requirement that must be met for optimum polarisation transfer in SABRE is that of the Level Anti-Crossing (LAC), the magnetic field at which polarisation transfer (allowed by strong coupling) can occur most efficiently. In the typical SABRE experiment spin mixing is carried out in a low magnetic field (0-140 mT for ¹H). In this environment the spins will be strongly coupled meaning that the difference in precessional frequency between the *parahydrogen* derived hydride ligands and the ¹H nuclei on the substrate is approximately equal to the combined *J* couplings of the system as given in Equation 1.10.

$$\delta\omega = \Delta\delta\gamma_{\text{H}}B_0 \quad (1.10)$$

Where $\delta\omega$ is the difference in Larmor frequency, $\Delta\delta$ is the difference in chemical shift, γ_{H} is the gyromagnetic ratio of ¹H and B_0 is the applied magnetic field. For transfer to heteronuclei such as ¹⁵N, the difference in Larmor frequency ($\delta\omega$) is given by $(\gamma_{\text{H}} - \gamma_{\text{X}})B_0$ where γ_{X} is the gyromagnetic ratio of the heteronucleus of interest. Hence in this instance the strong coupling occurs at even lower magnetic fields (μT). The specific field at which the LAC condition is met is quite specific for different systems and so usually a ‘field sweep’ experiment is undertaken to find the optimum polarisation transfer field (PTF).

The first example for this for ¹⁵N polarisation transfer came from Chekmenev *et al.* (2015), exemplifying >10% ¹⁵N polarisation utilising this μT transfer field. This method became known as “Signal Amplification by Reversible Exchange in SHield Enables Alignment Transfer to Heteronuclei” or SABRE-SHEATH.^{53, 61} Since then many studies have focussed on the hyperpolarisation of ¹⁵N achieving very high levels of ¹⁵N polarisation. Notably, Fekete *et al.* have demonstrated >50% ¹⁵N polarisation for the substrate pyrazine, this is >150,000-fold improved detectability at a 9.4 T detection field.⁴³ The μT field can be achieved by using a μ -metal shield to cancel the Earth’s magnetic field. The low μT field can then be achieved through supplying a current to a

copper wire coiled in a handheld shaker. Details of the shaker utilised in this thesis are outlined in the literature.⁴³

The detection of ^{15}N nuclei has become popular in recent years due to its potential application in MRI.^{4, 46, 47, 62-64} It has a low natural abundance of only 0.4%, with the rest being ^{14}N . This, combined with its low gyromagnetic ratio ($\gamma = -4.32 \text{ MHz T}^{-1}$) makes ^{15}N very hard to detect under Boltzmann conditions. However, the low γ of ^{15}N also means it typically benefits from a longer T_1 . A longer T_1 means a longer time to treat the sample, removing any toxic impurities, before imaging takes place. It also translates to longer signal observation times, allowing biological processes to be monitored as has recently been shown with ^{13}C MRI for the biomolecule pyruvate.⁶⁵⁻⁶⁷ As SABRE has been exemplified more broadly with N-donor type ligands, high levels of ^{15}N hyperpolarisation of a wider range of substrates are now starting to be achieved.

The spin lattice relaxation, or T_1 , represents the amount of time needed for roughly 63% of the magnetisation available to decay back to equilibrium. Applied to SABRE it is a measure of how long the hyperpolarised signal will stay visible for before returning to the Boltzmann distribution. Typically there is around a $3 \times T_1$ time period for this to occur. In traditional MRI, T_1 is a means by which contrast is achieved between tissues in the body. In hyperpolarised MRI, it is important the T_1 of any substrate injected is as long as possible to observe transport around the bloodstream to the site of interest within the patient.⁶⁸ There are many mechanisms that cause T_1 relaxation and therefore many factors to be considered when carrying out SABRE hyperpolarisation experiments.⁶⁹

Nuclei in close proximity can interact with one another through dipolar interactions known as couplings. It is these couplings that can cause relaxation of the net magnetisation in the sample by causing fluctuations in local magnetic fields of the nuclear spins.¹ This magnetic field fluctuation is actually caused by a number of factors, of which scalar coupling of this kind is just one factor. Another factor that can cause fluctuations is known as Chemical Shift Anisotropy (CSA), which describes the non-spherical nature of some atoms causing an uneven charge distribution on the atom as shown in Figure 1.11.

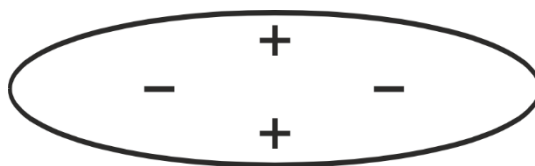


Figure 1.11 A diagram showing the non-spherical charge distribution present in quadrupolar nuclei such as ^{14}N .

In solution state NMR this causes them to act like a dipolar coupling when they rotate, affecting the local magnetic field of neighbouring nuclei. The CSA is very large for nuclei like ^{14}N .⁷⁰ The last factor discussed here is the motion of other molecules in solution. The random movement and rotation of these molecules also causes local magnetic field fluctuations for the nuclei being observed. This effect is most prominent when the motion of the surrounding molecules more closely matches the precessional frequency of the nuclei being observed and so the T_1 can vary with temperature because of this. As shown in Equation 1.6 the precessional frequency, ω , also varies with the applied magnetic field and so storing the sample in a suitable field where ω is far from the speed of molecular motion can also increase the T_1 .

1.2.4 Dynamic Nuclear Polarisation (DNP)

Dynamic nuclear polarisation or DNP is currently the most clinically developed method in hyperpolarised metabolic imaging.⁷¹ DNP works by utilising the higher level of polarisation of electron spins as the source of polarisation instead of *parahydrogen*. Interestingly the electron spins reach almost unity polarisation at lower temperatures (below 1 K).⁷² The method transfers polarisation by utilising microwave irradiation near the Electron *Paramagnetic* Resonance (EPR) transitions to stimulate transitions of polarisation through to the nearby nuclear spins.⁷² A typical DNP sample therefore usually consists of a suitable radical to ensure the presence of unpaired electrons nearby. The most widely used radical agents utilised are a class known as the trityls (triphenylmethyl radicals derived from triphenylmethyl chloride),⁷³ although nitroxides are common also.⁷⁴

1.2.5 Spin Exchange Optical Pumping (SEOP)

Spin Exchange Optical Pumping (SEOP) is another clinically relevant hyperpolarisation technique that is currently approved for use in human MRI. It is utilised to produce hyperpolarised ^3He and ^{129}Xe gas that are subsequently used for lung imaging by breath-hold type experiments. Patients breathe in the hyperpolarised noble gas and an

image of the lung cavity can be acquired. This area of the body is not normally accessible *via* MRI due to the low ^1H nuclei density in the lung space.^{75, 76} SEOP utilises a circularly polarised laser to form spin polarised alkali metal atoms which transfer polarisation to ^{129}Xe or ^3He gas through collisions. ^{129}Xe provides a cheaper alternative to the ^3He that has become more expensive in recent years. This, however, is at the cost of signal. ^{129}Xe provides less signal than ^3He due to its lower gyromagnetic ratio where the two are -11.8 MHz T^{-1} and -34.4 MHz T^{-1} respectively.^{77, 78} A benefit to using xenon is that it is partially soluble in blood making it possible to also gain images of the cardiovascular system as well as the lung space. This does however have a drawback. Once in the blood system, dissolved Xe can bind to proteins. This process of binding, specifically to N-methyl-D-aspartate receptors, causes xenon to act as an anaesthetic.^{79, 80}

1.3 Metal Carbenes

1.3.1 Carbenes

A carbene consists of a central carbon atom with a lone pair of electrons capable of forming a double bond with a metal centre. The carbon atom can have two substituent groups attached which can be varied to help stabilise the carbene formed.⁸¹ There are many methods to generate carbene ligands in solution with some common methods including nitrogen evolution of diazo compounds,⁸² or α -elimination involving a suitable leaving group.⁸³ Carbenes can be singlet or triplet in nature which refers to their arrangement of the electron pair situated on the carbon as shown in Figure 1.12.

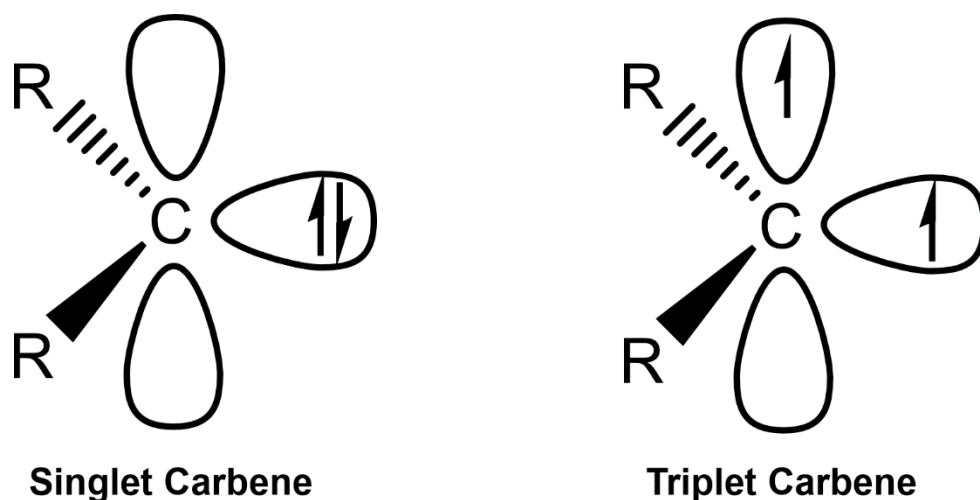


Figure 1.12 A diagram showing the lone pair electron arrangement in a singlet and triplet carbene.

When carbenes bind to a metal centre to form a metal carbene complex they can be organised into one of two categories, Fischer or Schrock carbenes, based on their singlet or triplet nature on the metal complex respectively.

1.3.2 Fischer Carbene

First published by Fischer and Maasböl (1964),⁸⁴ Fischer carbenes tend to form with low oxidation state, late transition metals.⁸⁵ They represent the singlet form of the carbene. When binding to the metal they donate 2 e^- and have an empty p-orbital on the carbene carbon that is able to accept π -back-bonding from the metal as shown in Figure 1.13.

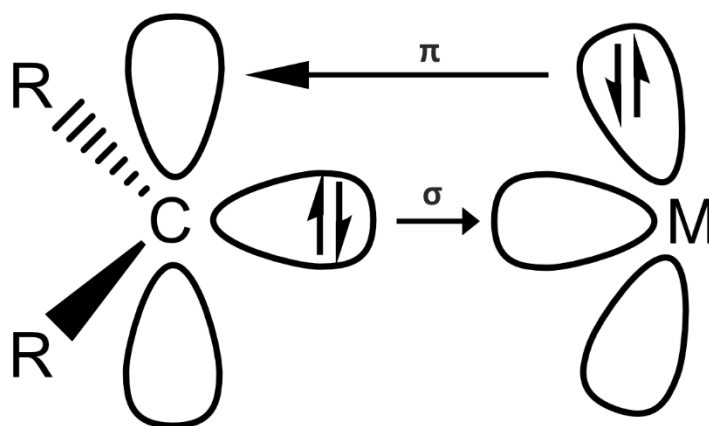


Figure 1.13 A diagram showing the binding of a Fischer style carbene as an L type ligand.

In Fischer carbenes the π back-bonding from the metal centre tends to be quite low as other ligands on the metal are typically π -accepting also, leaving the carbene electrophilic in nature. Due to this the Fischer style carbene ligands also tend to possess electron donating substituents on the carbene carbon to aid in stabilising the carbene through π -donation as shown in Figure 1.14.⁸¹

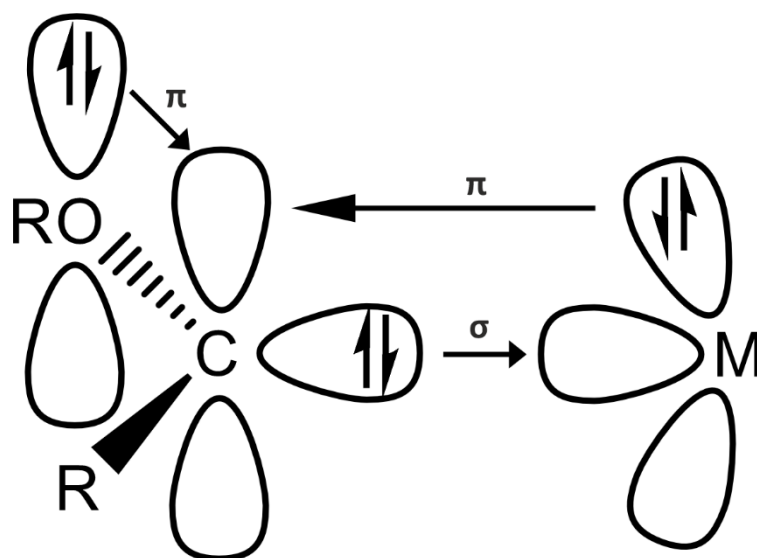


Figure 1.14 A diagram showing the stabilising π -donation from neighbouring groups on the carbene carbon.

These carbenes have been highlighted for their varied use in synthesis being able to functionalise the carbene ligand and form a number of different cyclic products through cycloaddition reactions.^{86, 87}

1.3.3 Schrock Carbene

Awarded the Nobel Prize in 2005 for the development of the metathesis method for exchanging substituent groups on alkenes,⁸⁸ Schrock (1974) reported the first carbene of this nature and pioneered like complexes in this field.⁸⁹⁻⁹¹ Schrock style carbene ligands tend to be associated with earlier, high oxidation state transition metals. They represent the situation where the carbene binds as a diyl ligand, due to back bonding a situation arises associated with a formal double bond. Therefore there is essentially no rotation about the resultant $M=C$ bond. As shown in Figure 1.15, this leads to a complex with electrons paired. Schrock carbenes count as an X_2 ligand and therefore are counted as raising the oxidation state of the metal by two units.⁸¹

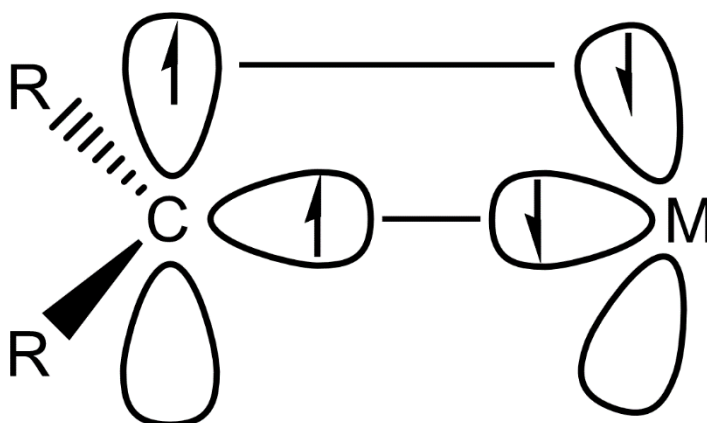


Figure 1.15 A diagram showing the binding of a Schrock style carbene as an X_2 type ligand.

Ligands on the metal centre are now generally weak π -acceptors and typically the R groups on the carbene carbon are weak π -donors. This means the metal is electrophilic, and the carbene nucleophilic in nature. Consequently, the exemplified reactions of Schrock carbenes illustrate this nucleophilic nature and, as mentioned, are widely used for their metathesis abilities.⁹²⁻⁹⁴

1.3.4 N-Heterocyclic carbenes

N-heterocyclic carbenes are a class of stable singlet carbenes with a neutral carbon with a lone pair of electrons directly bonded to at least one nitrogen atom in a cyclic scaffold. They were originally described independently by Wanzlick *et al.*⁹⁵ (1968), and Öfele (1968).⁹⁶ While trying to purify an imidazolium salt Öfele found that heating the salt in the presence of a chromium complex expelled a molecule of H₂ and formed the first NHC complex as shown in Figure 1.16.

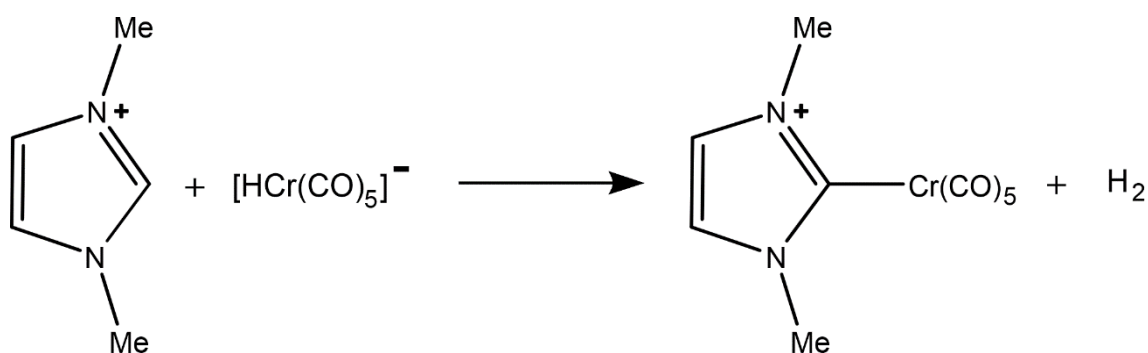


Figure 1.16 A reaction showing the formation of the first NHC ligand as observed by Öfele.

These complexes did not receive much notice when first reported until the isolation and full characterisation was carried out on the free NHC, 1,3-di-1-adamantyl-imidazol-2-ylidene by Kline *et al.* (1990).⁹⁷ It was then noticed that these carbene ligands surpassed the thermal stability of traditional carbenes and their utility as ligands for homogeneous catalysts was shortly up taken as a major area of research.⁹⁸ A major selling point for carbenes is the ability to store them as imidazolium salts or CO₂-adducts as shown in Figure 1.17. Storing NHCs in this form allows them to be stored at room temperature under air.

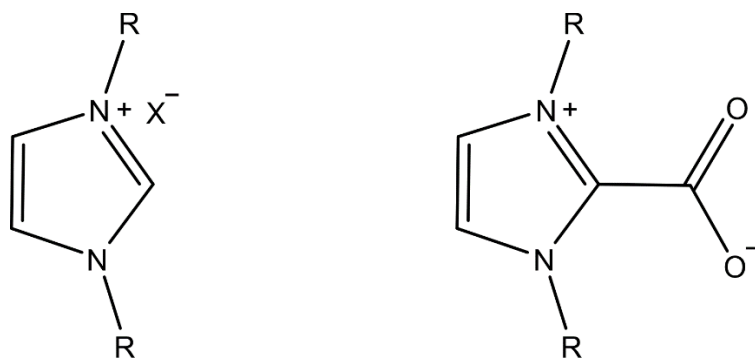


Figure 1.17 Methods for the safe storage of NHCs. (Left) The imidazolium salt form of an NHC. (Right) The CO₂-adduct form of an NHC.

To generate the carbene in solution one can either heat the CO₂-adduct to decarboxylate or treat the imidazolium salt with a base such as potassium *tert*-butoxide. Generally, NHC ligands have seen many applications across a wide range of fields including use in homogeneous catalysis for cross-coupling reactions and the olefin metathesis mentioned previously.⁹⁹ They have also seen applications in organometallic materials such as metal organic frameworks.¹⁰⁰

1.3.5 Measuring steric properties of NHC ligands

Traditionally the steric bulk of NHC ligands was determined using the Tolman cone angle. This is a method developed for phosphines and proposes measuring the size of a ligand from the cone angle, θ , defined with the metal at the vertex of the angle and the outer atoms of the ligand as the perimeter of the cone.¹⁰¹ As the library of these NHC ligands progressed it became apparent that the cone angle would not suffice as a measure of ligand size and often gave meaningless results for NHC ligands. Nolan *et al.* (1999) suggested using crystallographic data to measure the percentage volume of a sphere the ligand occupied.¹⁰² This became known as the percent buried volume (%V_{bur}).¹⁰³ In 2009 Cavallo *et al.* released “SAlerno Molecular Buried Volume Calculation” SAMVCA.¹⁰⁴ This free to use online tool for calculating %V_{Bur} became widely adopted and has now been shown in many studies for different metals and ligand types.¹⁰⁵

1.3.6 Measuring electronic properties of NHC ligands

Traditionally electronic properties of carbenes have been determined through the Tolman Electronic Parameter (TEP). This is determined by measuring the CO stretch of a bound CO ligand in the corresponding infrared spectrum. It has been shown by Rayner

et al. that a suitable complex can be formed by bubbling CO gas through a solution of the pre-catalyst $[\text{IrCl}(\text{COD})(\text{NHC})]$ forming the complex $[\text{Ir}(\text{CO})(\text{COD})(\text{NHC})]\text{Cl}$. The CO ligand binds here through a σ -bond but is a strong π -acceptor and so can accept electron density through back-bonding from the metal as shown in Figure 1.18.

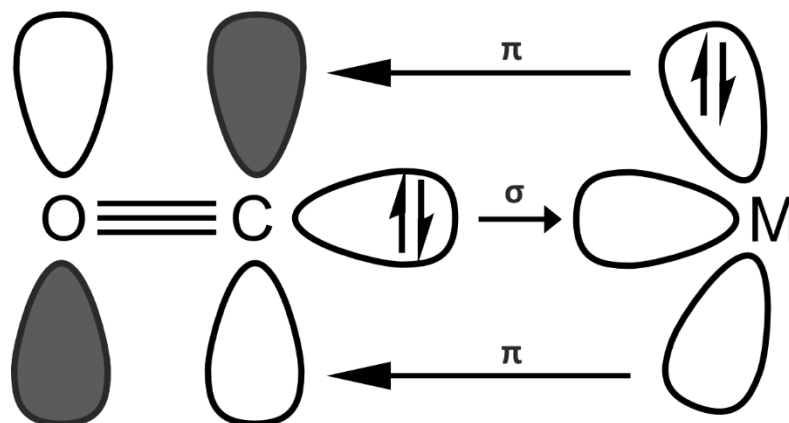


Figure 1.18 A diagram showing the π back-donation from the metal to the empty π^* orbital of the carbon monoxide.

In this situation the electron density from the back-bonding fills the π^* orbital of the CO, which, in turn causes the metal carbon bond to strengthen and the carbon oxygen bond to weaken. The higher the electron donation in the back-bonding the greater the degree the carbon oxygen bond weakens. Looking at the infrared stretch gives information about the strength of the CO bond also. If a lower wavenumber value is observed, then a higher degree of back-bonding can be expected and *vice versa*. As the electron density on the metal is directly influenced by its electronic interactions with the NHC ligand, the CO infrared stretch is also a measure of the electron donating or withdrawing ability of the NHC.

Traditionally TEP values were determined from a $[\text{Ni}(\text{CO})_4]$ complex and was developed for phosphine ligands whose σ -donation and π -acceptor behaviour is nearly inversely proportional.¹⁰⁶ Due to the toxicity issues associated with $[\text{Ni}(\text{CO})_4]$ the use of other metals has been reported, such as rhodium or the iridium example stated above.¹⁰⁷ Interestingly, there has been debate in the literature about the accuracy of TEP values for NHC ligands with Meyer *et al.* reporting on the π -back-donation from the metal itself skewing a result that is heavily reliant on only the NHC ligand affecting resulting CO infrared stretches.¹⁰⁸ This has led to works such as that published by Yang *et al.* instead utilising the ^{13}C NMR chemical shift of the bound carbene carbon to detect changes in the electronics.¹⁰⁹

1.4 Thesis Aims

This work is primarily focussed in developing efficient ^{15}N hyperpolarisation *via* SABRE for various families of nitrogen donor ligand species. These include, triazines, nitrite and nitriles. Currently, most of the literature surrounds the understanding of optimum conditions for efficient polarisation transfer to ^1H and ^{13}C nuclei. This limits the scope of SABRE, as other nuclei, such as ^{15}N , are not utilised to their full potential. This thesis will utilise current understanding of ^1H , ^{13}C and ^{15}N polarisation transfer conditions to optimise the polarisation transfer to ^{15}N in various substrates. As ^{15}N has a low natural abundance (0.365%) and has a low gyromagnetic ratio (-4.32 MHzT^{-1}) it has a low background signal in the body. Therefore this improved detectability gives a promise of ^{15}N contrast agents being possible in the near future as discussed in Chapters 4, 5 and 6. When measured by NMR spectroscopy ^{15}N also has a large chemical shift window which can be used to identify very similar chemical species when reaction monitoring as exemplified in Chapter 3. It is also useful as a probe into complex mixtures of N-containing substrates which is focussed on in Chapter 6.

In 2015 Chekmenev *et. al.* made significant progress in the optimisation of polarisation transfer to ^{15}N nuclei by developing the SABRE-SHEATH (already discussed above) method shown in Figure 1.19.⁵³ Since this discovery, further developments have been made that has seen ^{15}N polarisation of greater than 50% being achieved by the Duckett group.⁴³ This thesis goes beyond the current understanding of ^{15}N SABRE by exploring key factors in the polarisation transfer process such as; catalyst speciation, relaxation factors and polarisation transfer field. Understanding, how the use of co-ligands and different NHC ligands effect the ^{15}N transfer efficiency helps to determine catalytic species that will be optimum for each family of substrates.

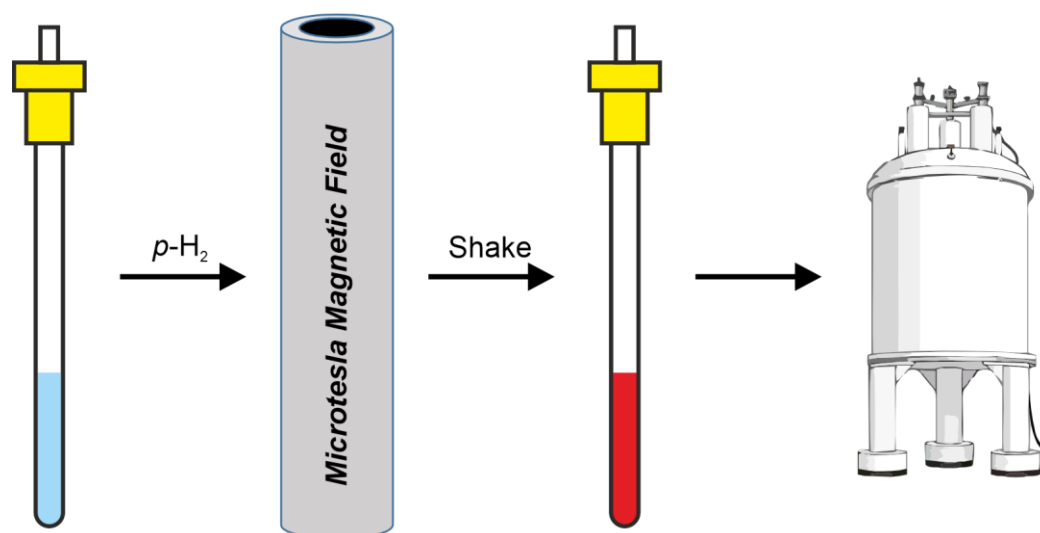


Figure 1.19 A generic scheme detailing the SABRE-SHEATH method. A sample is pressurised with $p\text{-H}_2$, shaken in a microtesla magnetic field and then transferred to the NMR spectrometer for ^{15}N signal acquisition.

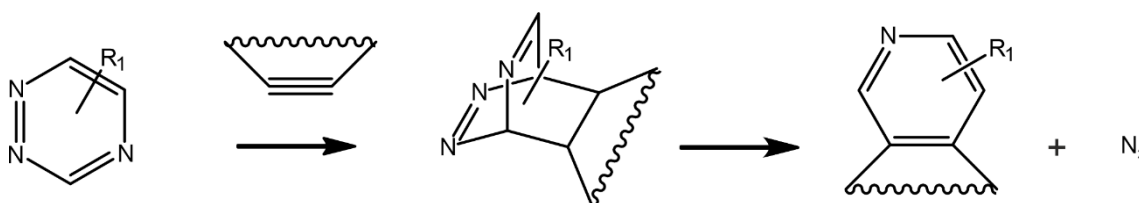
The thesis also aims to utilise these optimised polarisation transfer systems to a variety of applications. This involved simple substrate detection, reaction monitoring, diffusion spectroscopy, and mixture analysis. The wide variety of novel applications utilised in the thesis highlight the progress made in the field of ^{15}N polarisation transfer.

More generally, the thesis aims to improve SABRE as a whole through the use of solvents with a high hydrogen gas solubility. It is shown that the ^{15}N signal achieved can be dramatically improved by increasing the amount of $p\text{-H}_2$ in solution. The use of perfluorocarbons is investigated in Chapter 5 and easy substrate separation methods are highlighted, again progressing SABRE towards use in biomedical applications

Chapter 2. Understanding SABRE catalytic behaviour of multi N- donor 1,2,4-triazines.

2.1 Introduction

The substrate selected in this study is a 1,2,4-triazine, a six membered heterocycle with three nitrogen centres. Triazine derivatives play important roles in medicine and agriculture as anti-cancer,^{110, 111} antiviral,¹¹² bactericidal,¹¹³ herbicidal,¹¹⁴ antimalarial¹¹⁵ and antimicrobial agents.¹¹⁶ It is therefore of interest to be able to increase the ability to detect these compounds. Triazine compounds actually come in three forms; 1,2,3-, 1,2,4- and 1,3,5- triazines, where the numbers represent the positions of the nitrogen atoms in the six membered heterocycle. The 1,2,4-triazine variety was selected for this study due to their increased thermal stability over their 1,2,3-triazine counterparts which will act to increase viable sample lifetime. They are, however, more reactive than the 1,3,5-triazine moiety, making them more interesting for reaction monitoring purposes. An example reaction of interest is the ability of the 1,2,4-triazines to take part in an inverse electron demand Diels Alder type cycloaddition reaction as detailed in Scheme 2.1.^{117, 118}



Scheme 2.1 Example reaction scheme detailing the conversion of a 1,2,4-triazine through a Diels Alder type reaction with a strained alkyne to produce a bicyclic product and nitrogen gas.

The aim of this chapter is to explore and optimise the SABRE process for 1,2,4-triazines by reference to the model ligand 1,2,4-triazine-3-methylthio of Figure 2.1. The purpose of this study is to better understand the catalytic species involved in any SABRE polarisation experiments with this substrate, which will be complicated by the existence of three potential N-donor sites. This chapter starts by characterising these complexes, focussing on examining the effect that a change in solvent from methanol to dichloromethane can have on the species that form. The chapter then explores the optimisation of the SABRE process for 1,2,4-triazines by reference to ¹H and ¹⁵N signal intensity enhancements for the purposes of reaction monitoring and attempting to produce *paranitrogen*. The chapter finishes by applying these optimised systems to a real-world example of a 1,2,4-triazine in the form of toxoflavin,¹¹⁹ which is an antibiotic produced by *Burkholderia gladioli* bacteria.¹²⁰

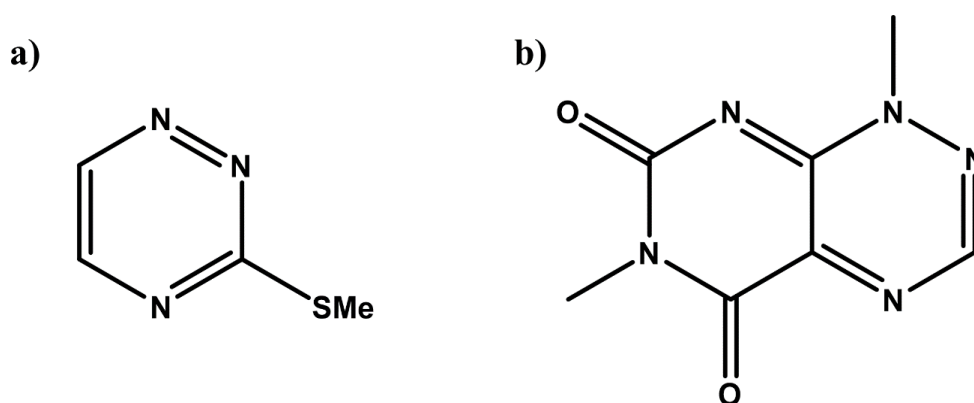
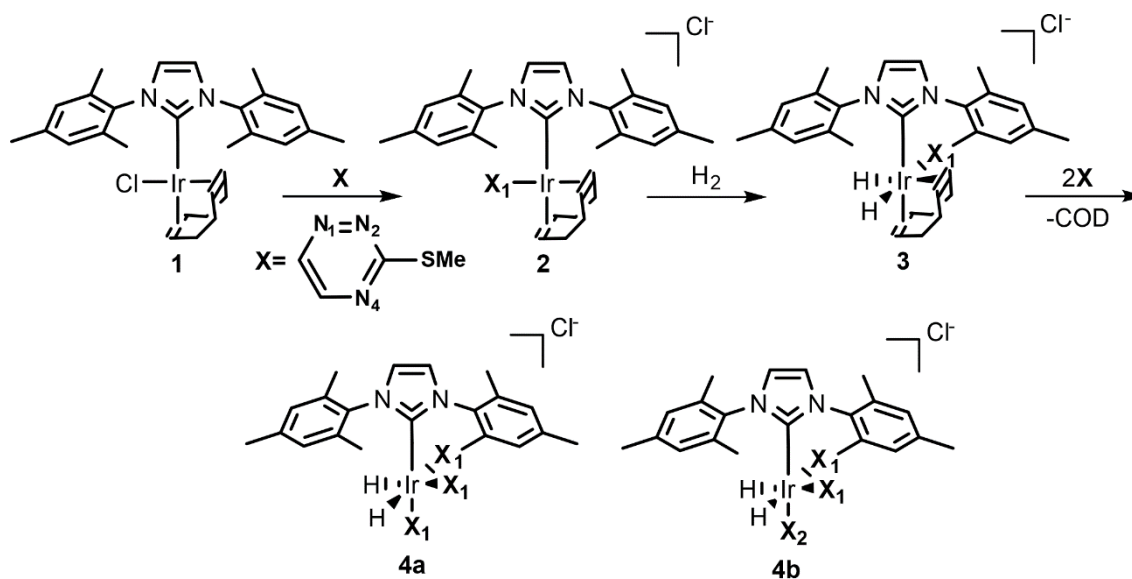


Figure 2.1 a) Structure of 1,2,4-triazine-3-methylthio (X); b) Structure of the antibiotic Toxoflavin (W); IUPAC name 1,6-Dimethylpyrimido[5,4-e][1,2,4]triazine-5,7(1H,6H)-dione.

2.2 Understanding the mechanism of formation of SABRE active species with 3-methylthio-1,2,4-triazine in methanol- d_4 and dichloromethane- d_2

2.2.1 Probing the formation of SABRE active species in methanol- d_4 .

This work started by examining the reaction of $\text{IrCl}(\text{COD})(\text{IMes})$ (**1**) (IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene; COD = cyclooctadiene) (2 mg) with 3-methylthio-1,2,4-triazine (**X**, 5-fold excess relative to (**1**)) in methanol- d_4 by multinuclear NMR spectroscopy. An assessment of the data gathered revealed the complete conversion of **1** into a single linkage isomer of $[\text{Ir}(\text{COD})(\text{IMes})(\text{X})]\text{Cl}$ (**2**) as detailed in Scheme 2.2.



Scheme 2.2 Reaction products detected during the conversion of $\text{IrCl}(\text{COD})(\text{IMes})$ (**1**) into $[\text{Ir}(\text{H})_2(\text{IMes})(\text{X})_3]\text{Cl}$ (**4a** and **4b**) in methanol- d_4 solution, where X_1 = 3-methylthio-1,2,4-triazine bound through N_1 and X_2 = 3-methylthio-1,2,4-triazine bound through N_2 .

Hence, despite **X** potentially binding through one of three nitrogen centres, only one linkage isomer forms cleanly. This suggests a high thermodynamic preference for one binding site. The identification of this site was achieved by low temperature ^1H - ^{15}N 2D NMR spectroscopy as N_1 . This deduction was based on the fact that the free signals for N_1 , N_2 , and N_4 of free **X** appear at δ 415.2, 355.6 and 292.2 respectively at 243 K in the corresponding ^{15}N NMR spectrum, whilst those in product **2** resonate at δ 332.3 (N_1), 341.7 (N_2) and 291.8 (N_4) at this temperature, as shown in Figure 2.2. It is therefore clear that the resonance position for N_1 moves dramatically upfield on binding, whilst the others signals are much less affected.

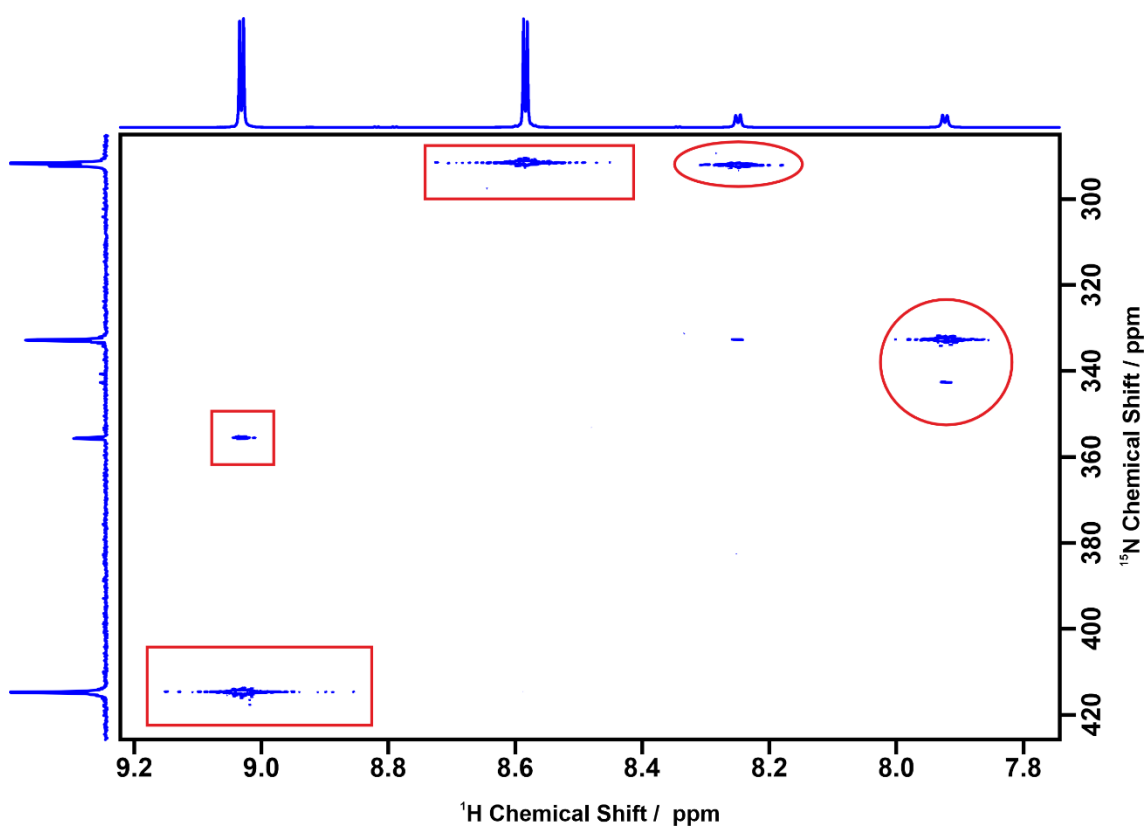


Figure 2.2 ^1H - ^{15}N HMQC NMR spectrum acquired at 9.4 T and a temperature of 243 K with a coupling constant of 12 Hz. The sample consisted of 10 mg of **1** with 5 equivalents of ligand **X**. Boxes show peaks for the free **X** and circles for the bound resonances.

Distinct alkene proton resonances for the η^2 - η^2 COD ligand of **2** were located at δ 3.77 and 3.50 in the corresponding ^1H NMR spectrum, which have moved relative to those of **1** where they appear at δ 4.04 and 3.11. Full characterisation details are available in Section 7.4.4.

This sample was then exposed to a 4 bar pressure of H_2 gas at 243 K. A slow reaction was observed to take place to form $[\text{Ir}(\text{H})_2(\eta^2\text{-}\eta^2\text{-COD})_2(\text{IMes})(\text{X})]\text{Cl}$ (**3**) of

Scheme 2.2 that was also identified on the basis of its NMR characteristics. These detail **3** as yielding a pair of hydride resonances at δ -13.47 and -17.34, which arise from ligands *trans* to alkene and **X** respectively. The binding of H_2 lowers the symmetry of the product relative to **2**, and this results in **3** yielding four inequivalent η^2 - η^2 -alkene CH resonances at δ 5.05, 4.97, 4.73 and 3.75, in addition to a carbene ^{13}C NMR signal at δ 154.06. ^{15}N NMR data again confirmed that **X** was bound *via* N_1 in **3** through the detection of a ^{15}N signal at δ 318.60, which proved to couple to the hydride ligand yielding the ^1H δ -17.34 signal *via* a *trans* J_{15NH} coupling of 24.5 Hz (Figure 2.3). Further investigation by ^1H - ^{15}N HMQC analysis revealed two further ^{15}N resonances for bound **X** in **3**, associated with N_2 and N_4 signals at 356.30 and 292.54 respectively that are again at very similar positions to those of free **X**. These six points fully specify the octahedral coordination sphere at iridium in **3** thereby ensuring a high level of confidence in the assignment; full NMR characterisation data for **3** can be found in Section 7.4.5.

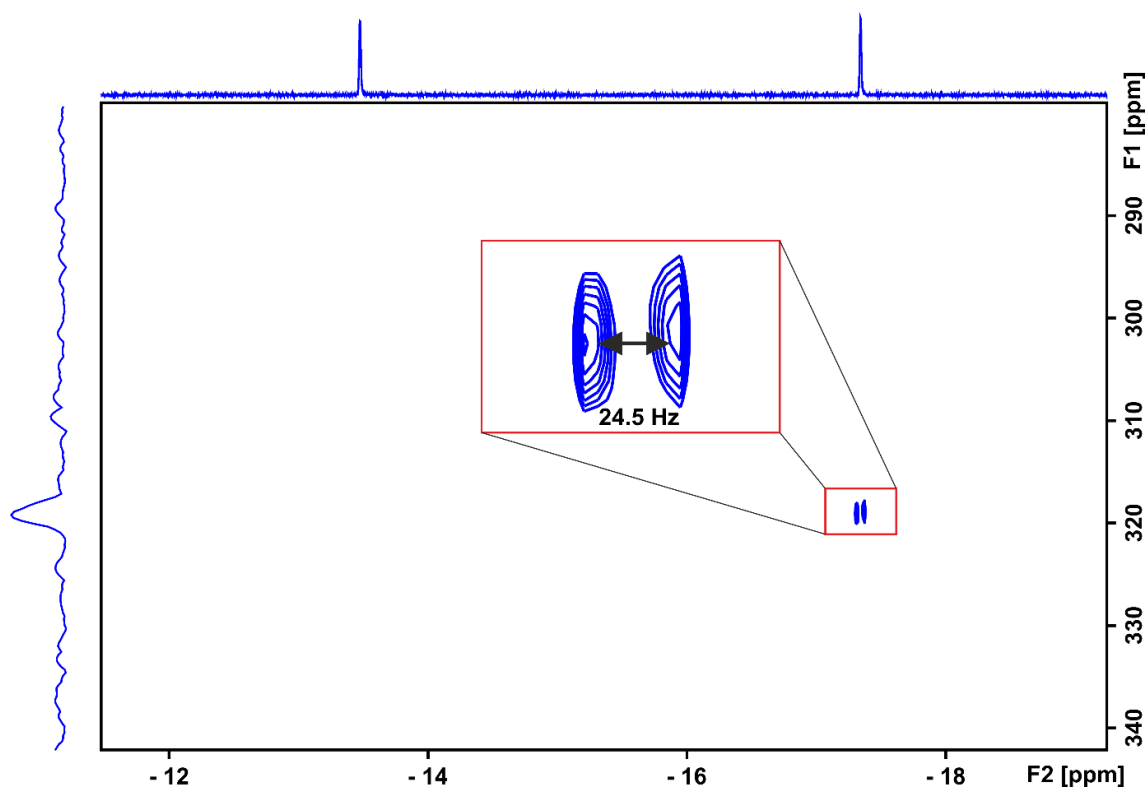


Figure 2.3 ^1H - ^{15}N HMQC 2D NMR spectrum showing correlation between the ^1H NMR signal of the hydride ligand at δ -17.34 and the ^{15}N NMR signal of the *trans* **X** ligand at δ 318. The data was acquired using a coupling constant of 20 Hz without proton decoupling and hence a ^1H - ^{15}N splitting of 24.5 Hz is seen.

The use of *p*- H_2 to create the PHIP effect is widely used to allow the detection of low concentration species through the increased signal strengths of protons located within the structure the *parahydrogen* has been chemically bonded to. When the reaction of a fresh sample of **2** with **X** and *p*- H_2 is monitored on a sample of **X** and **1** at ~243 K, signal

amplification for the two hydride ligand signals of **3**, associated with PHIP, is seen in the early measurements. These PHIP signals are, however, soon swamped by those of the thermally equilibrated hydride ligand responses, which results in the detection of a pair of distorted doublets rather than the pair of antiphase hydride ligand signals as seen initially. No SABRE enhancement was observed for the alkene protons of **3**, nor the carbene methyl groups at this point. Figure 2.4 shows a series of typical ^1H NMR spectra to illustrate these PHIP observations.

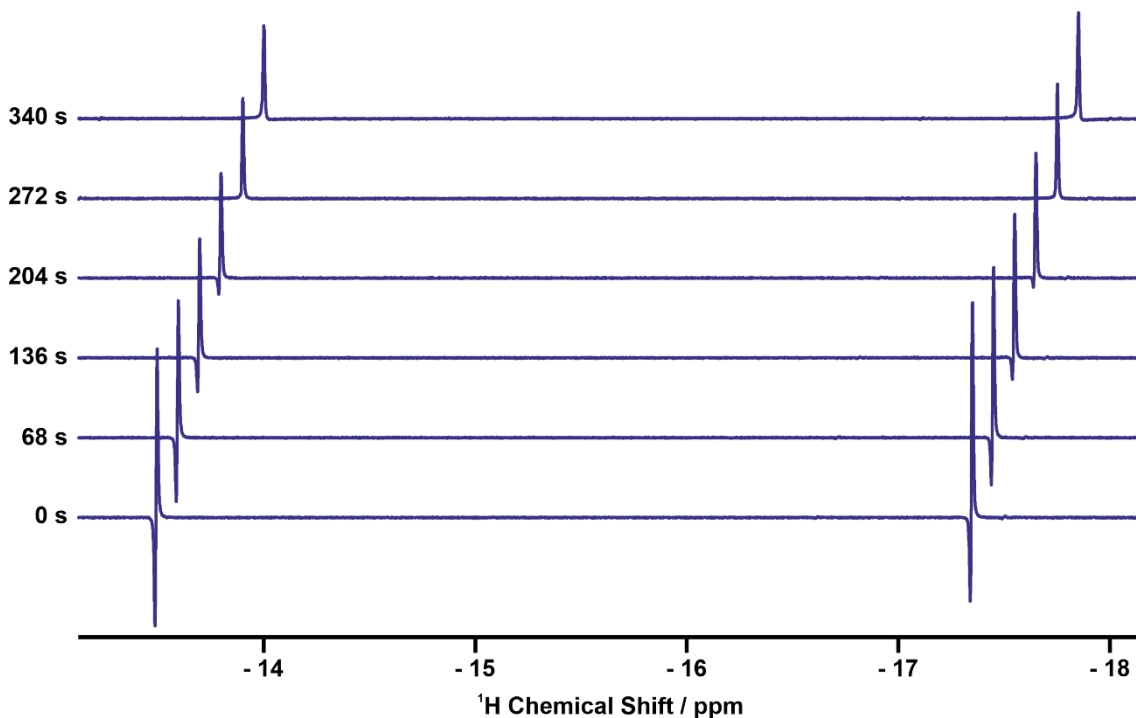


Figure 2.4 Typical series of ^1H NMR spectra acquired from a sample of **1** (2 mg) with 5 equivalents of **X** in 0.6 mL of methanol- d_4 reacted with $p\text{-H}_2$ under a pressure of 4 bar. Sample was stored at a field of 9.4 T at 243 K and a single scan spectra acquired using a 90° pulse every 68 s. For ease of viewing an offset of 0.1 ppm is applied to each spectrum to avoid overlap. Only the bottom spectrum displays the true ^1H chemical shift.

The sample was left to equilibrate at 243 K for 45 minutes, at this point the measured ratio of **2** : **3** proved to be 1 : 30, and at this time point the sample was warmed to 273 K to allow further reaction to take place. Hydrogenation of COD was now observed, with a singlet for cyclooctane (COA) becoming evident in the corresponding ^1H NMR spectrum at δ 1.58. The conversion of COD into COA led to a change in iridium complex speciation which resulted in the region around δ -21.00 now showing several hydride ligand resonances due to the formation of the expected $[\text{Ir}(\text{H})_2(\text{IMes})(\text{X})_3]\text{Cl}$ type products. A number of inequivalent hydride ligand signals were seen in this region and the region appeared very complex, which suggests that the binding of **X** is however no longer site selective. Consequently, this sample was warmed to 298 K for ~20 minutes to

fully consume **3** before cooling back to 243 K for more precise monitoring. Fortunately, the temperature cycling step simplified the hydride region of the resulting ^1H NMR spectra such that only two major products remained, **4a** and **4b** of Scheme 2.2.

Using ^1H NMR signal integration, the ratio of **4a** : **4b** at 243 K after about 1 hr of reaction was 2 : 1. At this point, a series of multinuclear NMR spectra were set to record for a time period of around 15 hrs for the purpose of characterising both **4a** and **4b**. Analysing these data revealed the two species yield single hydride ligand signals at δ -20.19 and δ -20.53, respectively, that exhibit *trans* couplings of 22.6 and 22.1 Hz to the respective ^{15}N centre of their **X** ligands. The corresponding ^1H - ^{15}N HMQC data at this temperature revealed the **X** ligand to be once again selectively bound through N_1 , with the ^{15}N NMR signals appearing at δ 344.50 and δ 346.73 respectively for **4a** and **4b**. The N_1 signal in **4a** proved to couple to a ^1H resonance at δ 8.69 that couples to a further ^1H NMR signal at δ 8.33, both of which are shifted from the corresponding δ 9.01 and 8.55 positions seen for free **X**. Two further triazine ^1H NMR resonances were also located at δ 9.35 and 8.32 in **4a**, for a triazine lying *trans* to the NHC, which integrate in a 1 : 2 ratio with respect to the corresponding equatorial **X** ligands ^1H NMR signals. This structural arrangement was further confirmed by visible nOe connections from the hydride ligand signal at δ -20.19 to bound triazine peaks at δ 9.35 and 8.69 for axially and equatorially bound positions respectively. A $^{15}\text{N}_1$ NMR signal for this additional ligand was located at δ 321.70, and shifted from the free materials response at δ 415.2 by 93.5 ppm upon binding. Hence, in dominant **4a** all three triazine moieties can be confirmed to bind through N_1 .

In contrast, the corresponding ^1H - ^{15}N HMQC analysis for **4b** revealed both hydride ligands lie *trans* to an N_1 centre that presents a ^{15}N chemical shift of δ 346.82. The axial ligand, however, is bound through N_2 , with a chemical shift of δ 291.84. This, as expected from previous studies, is dramatically shifted away from that of the free material; the difference in chemical shift here between the bound and the free **X** ^{15}N NMR signals was ~ 63 ppm. The N_1 and N_4 signals for this ligand now appear at δ 411.5 and 298.5 respectively, again close to those of the free ligand. This information confirms the binding *via* N_2 in this complex, something not previously observed. Complete NMR assignments for **4a** and **4b** are detailed in Sections 7.4.6 and 7.4.7 respectively.

At the end of this 15 hour recording period, a new ^1H NMR spectrum was recorded. This revealed further equilibration had taken place between the two hydride

ligand containing species such that **4a** was now 95% of the sample. Upon repeating this reaction at 298 K with a 20 fold excess of **X** relative to iridium a similar 95% ratio for **4a** : **4b** resulted after only 30 minutes. Hence, it can be concluded that although there is a clear thermodynamic preference for the binding of N₁, binding through N₂ is possible under kinetic control and low temperatures can be used to partially stabilise this isomer such that characterisation is possible. When considering the +M effect of the SMe substituent of **X** the resonance forms indicate that the N₂ and N₄ sites are more basic in nature than N₁, implying their lone pair of electrons should be more readily available for ligation. Therefore it is surprising that the binding of N₁ is shown to be so preferential, this suggests binding must be under steric control instead.

At this point, the sample was tested for SABRE efficacy by conducting a ‘shake and drop’ experiment as outlined in the experimental. This was done using 4 bar of *p*-H₂ of >99% purity, and shaking in a field of 6 mT for a period of 10 s before transfer to a 9.4 T magnetic field where ¹H data acquisition took place. These conditions resulted in the two ¹H NMR resonances for free **X** at δ 9.01 and 8.55 giving signals that were 107 ± 4 and 103 ± 4 larger than those normally returned at this acquisition field. Hence, the isomers of **4** are SABRE active for the hyperpolarisation of free **X**. At this time the hyperpolarisation of ¹⁵N was also attempted by shaking in both 6 mT and 0.30 μ T fields, but no ¹⁵N NMR signal was observable in one scan under these conditions. A typical SABRE hyperpolarised ¹H NMR spectrum is displayed with the corresponding thermal signal amplified 4-fold for ease of comparison in Figure 2.5.

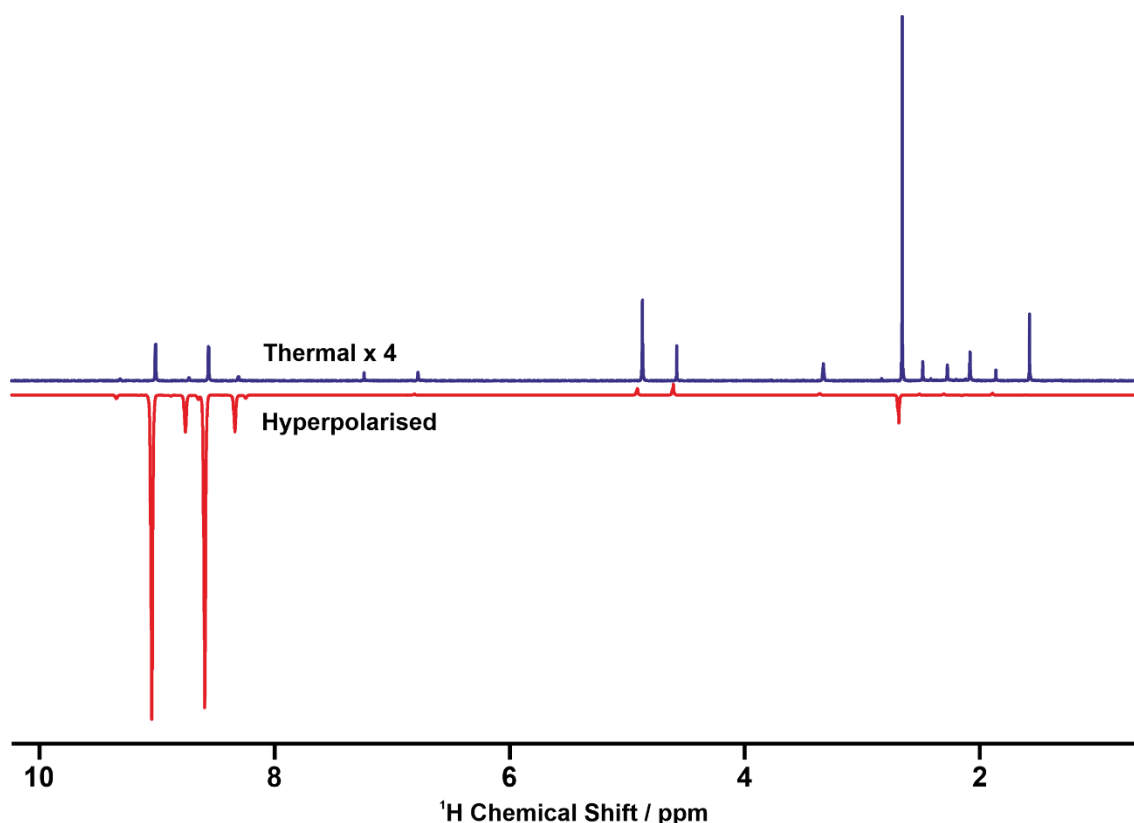


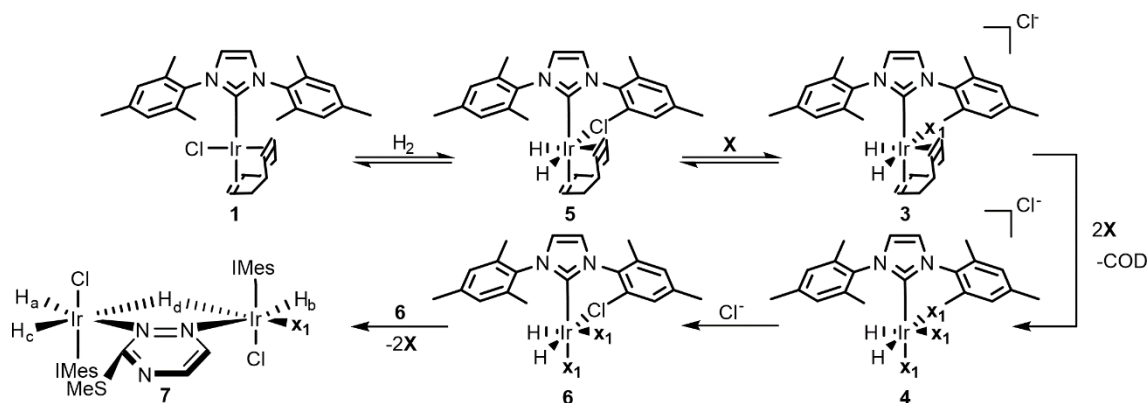
Figure 2.5 Top: A typical ^1H NMR spectrum consisting of 4 signal averages undertaken on a sample containing **1** (2 mg) with 5 equivalents of **X** in 0.6 mL of methanol- d_4 . A period of 300 s was left between subsequent scans to ensure full relaxation of the signal. Bottom: A typical SABRE hyperpolarised ^1H NMR spectrum collected in a one scan acquisition from a sample containing **1** (2 mg) with 5 equivalents of **X** in 0.6 mL of methanol- d_4 shaken for 10 s at a field of 6 mT.

Notably, the hyperpolarised peaks now appear negative in character which is a feature very commonly seen in SABRE if the higher nuclear spin energy level is polarised in the process. The peaks that remain positive here, such as those of the residual solvent and the H_2 are noticeably lower in intensity than in the thermal response. This is due to the ‘shake and drop method’ utilised, outlined in the experimental, acquiring a spectrum just seconds after insertion into the magnet. This does not give the thermally polarised signals time to equilibrate with the external magnetic field and build up polarisation, a process that would take $5 \times T_1$ of the spin being observed. As SABRE artificially increases polarisation of the protons of **X**, these signals appear larger than even the thermal signal that had ample time to equilibrate fully displayed in the top spectrum.

2.2.2 Probing the formation of SABRE active species in dichloromethane- d_2

The reactivity of **1**, **X** and H_2 was then monitored for comparative purposes in dichloromethane- d_2 , the reasoning for this being that the formation of species **4** should be

disfavoured and so it is of interest to understand which catalytic species will form instead. The formation of **4** first requires the dissociation of a Cl^- ligand. This process will be easier in methanol- d_4 compared to dichloromethane- d_2 due to the hydrogen bonding network that is established between the methanol- d_4 solvent molecules and the free chloride. The polar methanol- d_4 molecules will also aid in stabilising the resulting cationic metal complex, the ion pair of chloride. In contrast, dichloromethane- d_2 can only stabilise the resulting charges through weaker ion-dipole interactions, due to it being less polar in nature than the methanol- d_4 , such effects will be much smaller. It was therefore hypothesised that the neutral *bis* substituted $[\text{IrCl}(\text{H})_2(\text{IMes})(\text{X})_2]$ (**6**) would instead be formed preferably, with the chloride ligand remaining bound in the complex. It was hoped that, due to the asymmetry of the equatorial plane that this species may yield higher ^1H signal enhancements for the free **X** ligand. Using ^1H NMR analysis to monitor the resulting solution confirmed this to be the case, with the species $[\text{IrCl}(\text{H})_2(\text{IMes})(\text{X})_2]$ (**6** of Scheme 2.3) forming over 60 minutes at 298 K.



Scheme 2.3 Chemical changes associated with the conversion of **1** to **7** in dichloromethane- d_2 through reaction with **X** and H_2 .

The two hydride ligands in **6** are now inequivalent with ^1H NMR signals appearing at δ -21.78 and -23.10 at a temperature of 243 K. They also share a common J_{HH} of 8.0 Hz. The hydride ligand resonating at δ -21.78 was confirmed to lie *trans* to N_1 of **X** by ^1H - ^{15}N HMQC spectroscopy as it possesses a strong ^1H - ^{15}N coupling of 27.1 Hz to a ^{15}N signal at δ 352.4 which couples further to a ^1H resonance at δ 9.59. Both hydride ligands in **6** showed nOe connections to a ^1H NMR signal at δ 9.28 signal. As both hydride ligand signals showed nOe connections to the same **X** ligand it was deduced that this **X** must be *cis* to the hydride ligands in the axial position *trans* to the NHC ligand. This **X** ligand was confirmed to bind through N_1 as ^1H - ^{15}N HMQC data revealed the corresponding chemical shift for the ^{15}N NMR signal of N_1 at δ 333.1, again considerably shifted from that of the free material, whereas the other ^{15}N NMR resonances for N_2 and N_4 appear at δ 367.7 and

283.0 respectively, very close to the free values for **X**. The hydride ligand at δ -23.10 also show an nOe connection to the equatorially bound **X** ligand with a ^1H NMR signal at δ 9.59 confirming its *trans* arrangement to the hydride ligand yielding a ^1H NMR signal at δ -21.78. Collectively these data confirm the structure of **6** to be $[\text{IrCl}(\text{H})_2(\text{IMes})(\text{X})_2]$. Full NMR characterisation data for **6** are presented in Section 7.4.16.

It takes around 60 min to convert **1** into **6** in dichloromethane- d_2 under these conditions, in contrast to the reaction in methanol- d_4 which takes only 15 minutes to form **4**. This difference in behaviour reflects the change in reactivity of **1** and **2** towards H_2 . In methanol, **2** adds H_2 rapidly to form **3**, which reacts further to form **4**. In contrast, no evidence for the substitution of chloride by **X** to form **2** is seen in dichloromethane- d_2 and instead low temperature studies with **X** and H_2 reveal the slow formation of $[\text{IrCl}(\text{H})_2(\text{COD})(\text{IMes})]$ (**5**). **5** then proves to equilibrate with **3**, and so it would therefore appear that a hydride *trans* labilising effect operates in **5** to access the unfavoured, charged species **3**. The reaction of **3** with H_2 and **X** then leads to **4** as described earlier in methanol, but now the displacement of **X** by chloride acts to form neutral **6**. The ratio of **4** : **6** at the point where **6** achieved a maximum concentration proved to be 1 : 64 at 298 K. **6** proved unstable, and was shown to react further to form **7** as will be discussed shortly.

The longer term instability of **6** was first revealed through a SABRE study on **X** using **6** as the catalyst. Excitingly, **6** proved to exhibit a higher SABRE efficacy than **4** in methanol- d_4 . This was revealed through the corresponding ^1H NMR signal gains seen for free **X** now being 467 ± 21 and 455 ± 20 -fold for the resonances at δ 8.95 and 8.40 (note a slight difference in the ^1H NMR signals of free **X** due to the switch in solvent) respectively. To conduct the study the sample was shaken for 10 s in a field of 6 mT with a pressure of 4 bar *p*- H_2 before immediate transfer to a 9.4 T NMR spectrometer for ^1H NMR signal acquisition. This sample was shaken around 1 hr after first exposing the 2 mg of **1**, with 5 equivalents of **X** relative to **1**, to H_2 . As the concentration of **4** was so much lower than **6** in dichloromethane- d_2 this effect was believed to be due to the catalyst speciation change. The hyperpolarisation of ^{15}N was also reattempted, but again no observable ^{15}N signal was detected when using a polarisation transfer field (PTF) of 6 mT or 0.30 μT .

The subsequent SABRE experiments, carried out over the next 5 hours, showed a clear decrease in the enhancement of the free signals of **X** at 9.4 T. This is displayed in

Figure 2.6, and there is therefore a clear link between the level of observed ^1H NMR signal gain seen for free **X** and the concentration of **6** in solution.

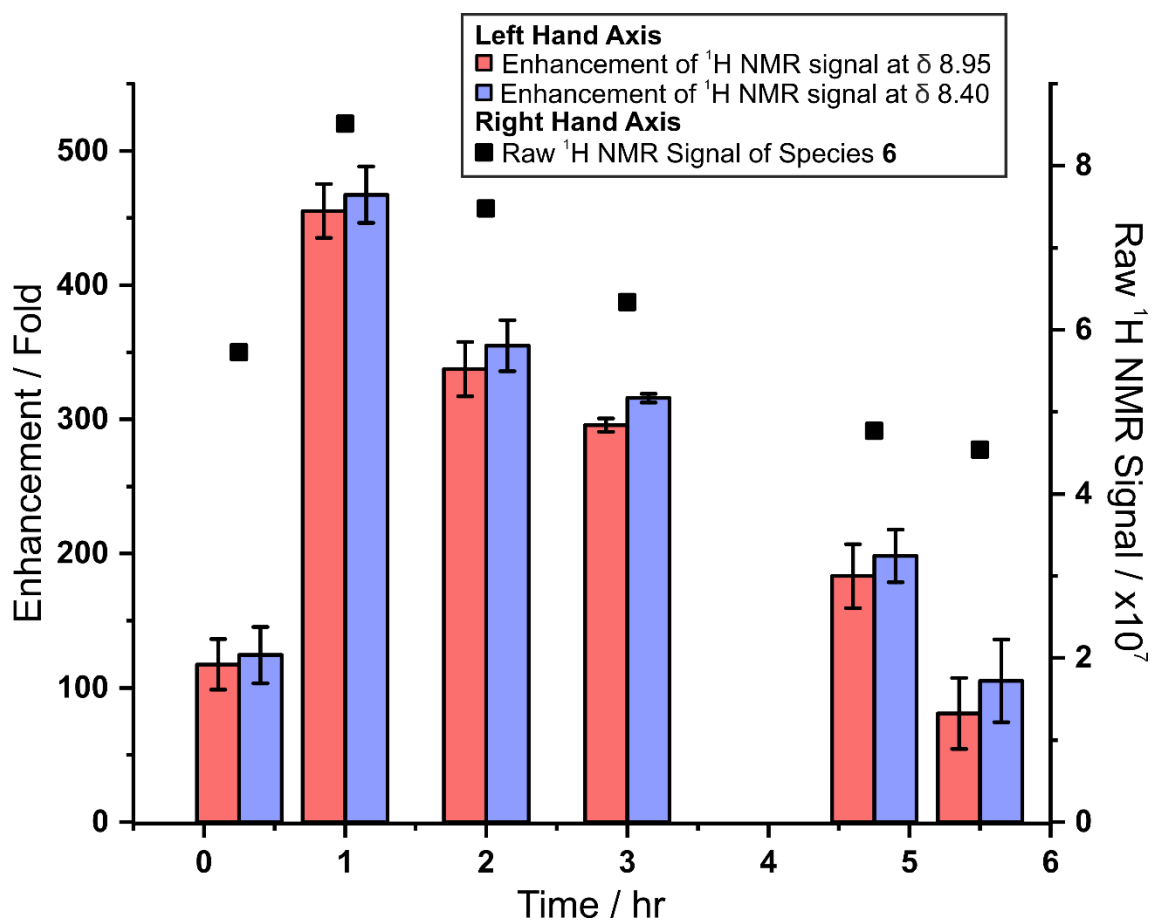


Figure 2.6 Comparison of the ^1H NMR signal enhancement of free **X** for both ^1H resonances determined at 9.4 T after a 10 s shake at 6 mT with 4 bar pressure of $p\text{-H}_2$ alongside the raw ^1H NMR signal intensity for complex **6**. This is reflective of the amount of **6** present during each SABRE experiment.

Notably, these ^1H NMR spectra proved to contain four further hydride ligand signals at δ -19.42 (H_a), -20.10 (H_b), -20.59 (H_c) and -23.65 (H_d) whose growth in intensity parallels the fall in signals for **6**. This species was assigned to **7**, and proved to be SABRE inactive as no enhancement was seen in its hydride ligand signals under PHIP. The conversion of **6** into **7** proceeds slowly, and reached ~92% after five days at a temperature of 298 K. Figure 2.7 shows a section of a ^1H NMR spectrum of the sample after five days to illustrate its hydride ligand signals.

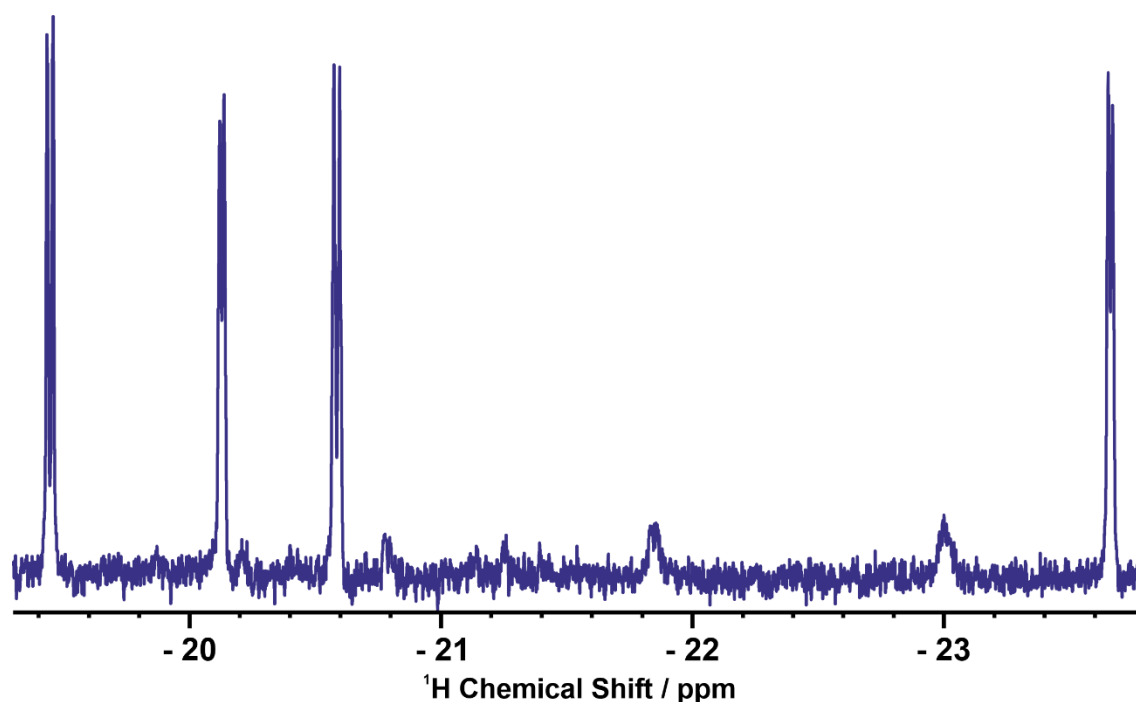


Figure 2.7 Hydride region of a ^1H NMR spectrum of a sample containing **1** (2 mg) with 5 equivalents of **X** in dichloromethane- d_2 recorded at a temperature 298 K with a field strength of 7 T. Sample was pressurised to 4 bar with H_2 gas and left to react for 5 days. Peaks of **7** are shown at δ -19.42 (H_a), -20.10 (H_b), -20.59 (H_c) and -23.65 (H_d). Peaks for **6** shown at δ -21.80 and -23.00.

Species **7** was then characterised by numerous complex low temperature multinuclear NMR experiments. Firstly, its four new hydride ligand signals were found to have equal intensity, and couple together in the corresponding ^1H - ^1H TOCSY NMR spectrum as shown in Figure 2.8. This necessitates **7** to be a multinuclear species, it was confirmed to be dinuclear by further analysis and integration of the ^1H NMR spectrum. The locations of the four hydride ligands in **7** were then assigned using COSY, nOe and TOCSY NMR measurements to confirm the positions detailed in Scheme 2.3.

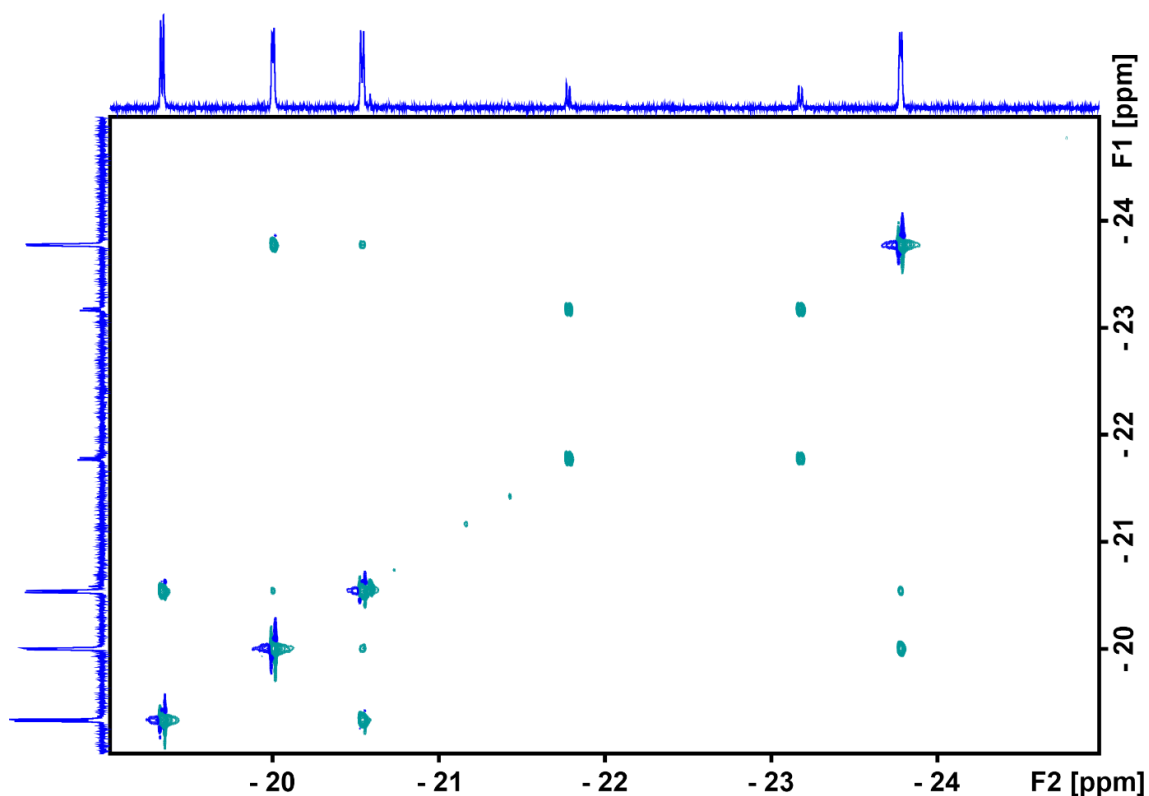


Figure 2.8 ^1H NMR TOCSY data collected on a sample of **7**. Data was collected at 263 K and an acquisition field of 11.7 T.

These data also revealed two sets of resonances for two distinct IMes ligands in **7**, whose symmetry was such that each yielded twelve distinct ^1H NMR resonances; nOe data confirmed the two NHC's adopt a mutual *trans* arrangement. Further resonances were located for two distinct **X** ligands in **7**, the first of which bridges the two iridium centres by binding through N_1 and N_2 , with these sites being *trans* to H_a and H_b on the respective iridium centres. Consequently, each of these two hydride ligands possess a strong ^1H – ^{15}N coupling, of 22.5 and 29.2 Hz respectively, illustrated in Figure 2.9.

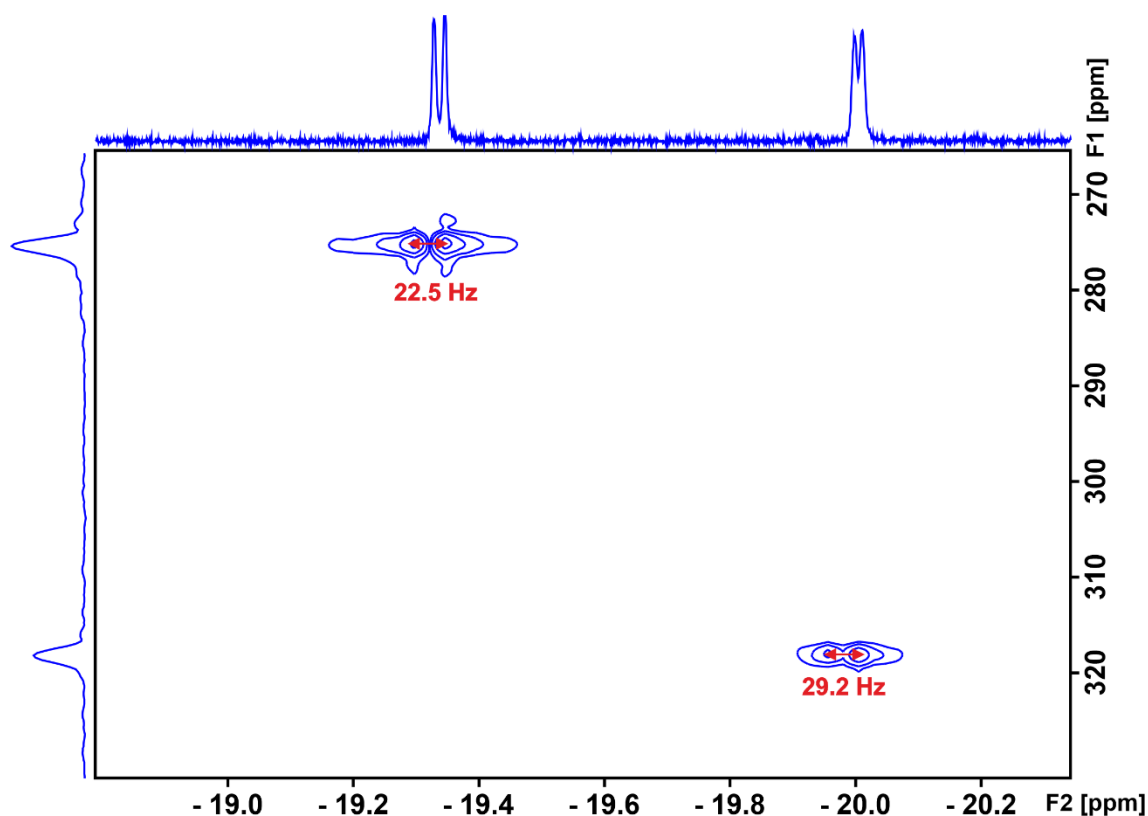


Figure 2.9 ^1H - ^{15}N HMQC 2D NMR spectrum showing correlation between the ^1H NMR signal of the hydride ligands at δ -19.42 and -20.10 and the corresponding ^{15}N NMR signal of the bridging trans **X** ligand at δ 275.70 and 318.80. These data were acquired using a coupling constant of 20 Hz, without proton decoupling and hence a ^1H - ^{15}N splitting is seen of 22.5 and 29.2 Hz respectively.

The hydride ligand ^1H NMR resonance for H_a , at δ -19.42, connects to a ^{15}N NMR resonance at δ 275.70 for N_2 of bound **X**. H_b proved to yield a ^1H NMR signal at δ -20.10 and connect to a ^{15}N NMR resonance at δ 318.80 for a bound N_1 of **X**. The latter of these ^{15}N resonances, due to bound N_1 , also exhibits a 10.6 Hz coupling to a ^1H resonance at δ 8.33, which couples to partner in the TOCSY measurement to a further signal that resonates at δ 8.08. This resonance further connects to a ^{15}N resonance at δ 292.20 for N_4 . This completes the three nitrogen assignments for the bridging **X** ligand and hence confirms binding through N_1 and N_2 due to the characteristic shift in the ^{15}N NMR signals. The hydride ligand resonance for H_b , at δ -20.10, showed an nOe connection to a further ^1H signal at δ 9.16 due to a second **X** ligand in **7** which proved to be bound through N_1 as its ^{15}N chemical shift was shifted from the free material to δ 295.80. The remaining ^{15}N signals in this second **X** ligand proved to appear close to those of the free **X** ligand at δ 355.50 and 295.40 for N_2 and N_4 respectively. Due to its nOe connection to H_b , this **X** ligand must be in a mutual *cis* arrangement. It was determined that this **X** ligand was in the equatorial plane as the ^1H signal at δ 9.16 belonging to **X** also exhibits an nOe connection to a carbene ligand (Figure 2.10) that was found to be on the same iridium due

to its further nOe connections to the hydride ligands present. This **X** ligand was therefore determined to be in a *trans* arrangement to the bridging hydride ligand H_d. However, no ¹H-¹⁵N coupling was observed to it in the HMQC NMR measurements. Full assignments for **7** can be found in Section 7.4.17.

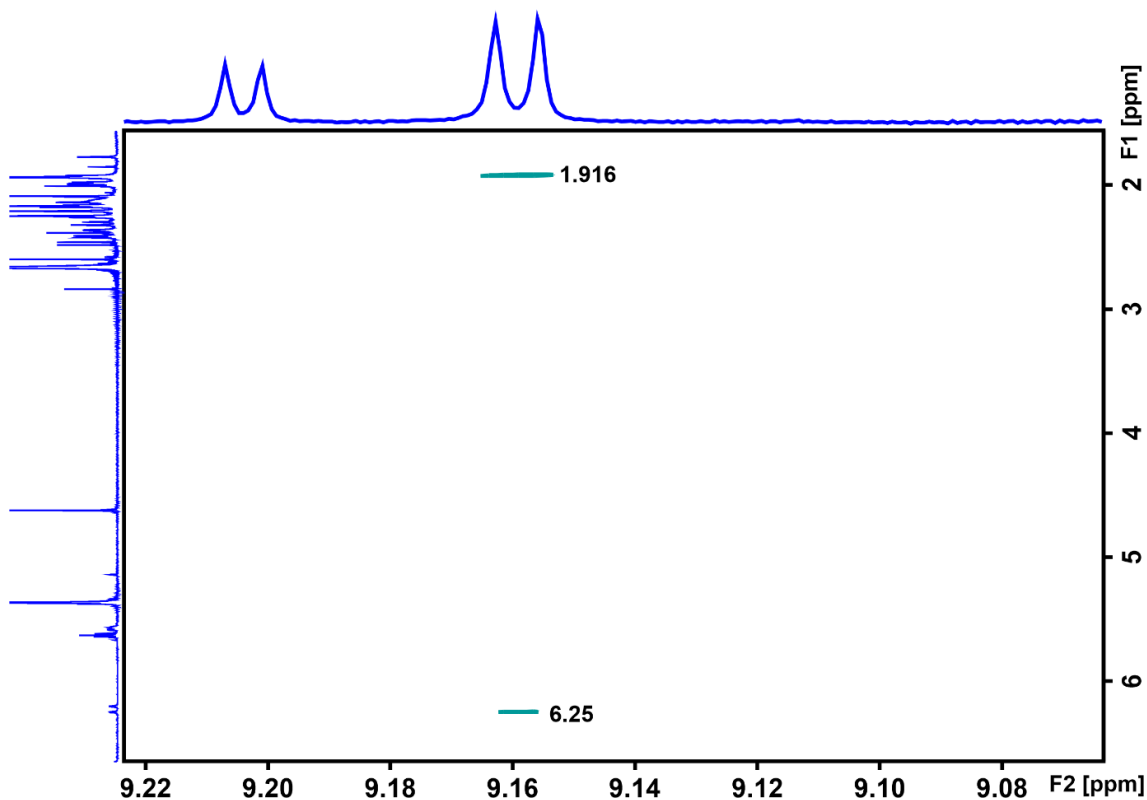


Figure 2.10 Two dimensional ¹H NMR NOESY data collected at 263 K and at a field of 9.4 T. Data shows nOe connections between the bound **X** ligand ¹H NMR signal at δ 9.16 and the carbene ligands ¹H NMR signals at δ 1.916 and 6.25 indicative of a *cis* arrangement.

The assignment of **7** as a binuclear product was supported by diffusion spectroscopy which confirmed that all four of its hydride ligand resonances map to the same $3.98 \times 10^{-10} \pm 0.18 \times 10^{-10} \text{ m}^2\text{s}^{-1}$ diffusion coefficient. Furthermore, this diffusion coefficient proved to be slower than that of mononuclear **6**, which was $4.6 \times 10^{-10} \pm 0.21 \times 10^{-10} \text{ m}^2\text{s}^{-1}$. It should be noted, that these diffusion coefficients were measured at 253 K in order to suppress hydrogen ligand exchange; the corresponding data at 298 K yielded diffusion coefficients of $7.44 \pm 0.15 \times 10^{-10} \text{ m}^2\text{s}^{-1}$ and $26.14 \pm 4.28 \times 10^{-10} \text{ m}^2\text{s}^{-1}$ for **6** and **7** respectively. This shows a much bigger difference in diffusion coefficient between the two species and a much larger error for **6**. This large difference was attributed to a contribution from free H₂, due to the hydride ligands of **6** undergoing exchange with dihydrogen. The corresponding DOSY spectrum for each species is shown for both temperatures for comparison in Figure 2.11.

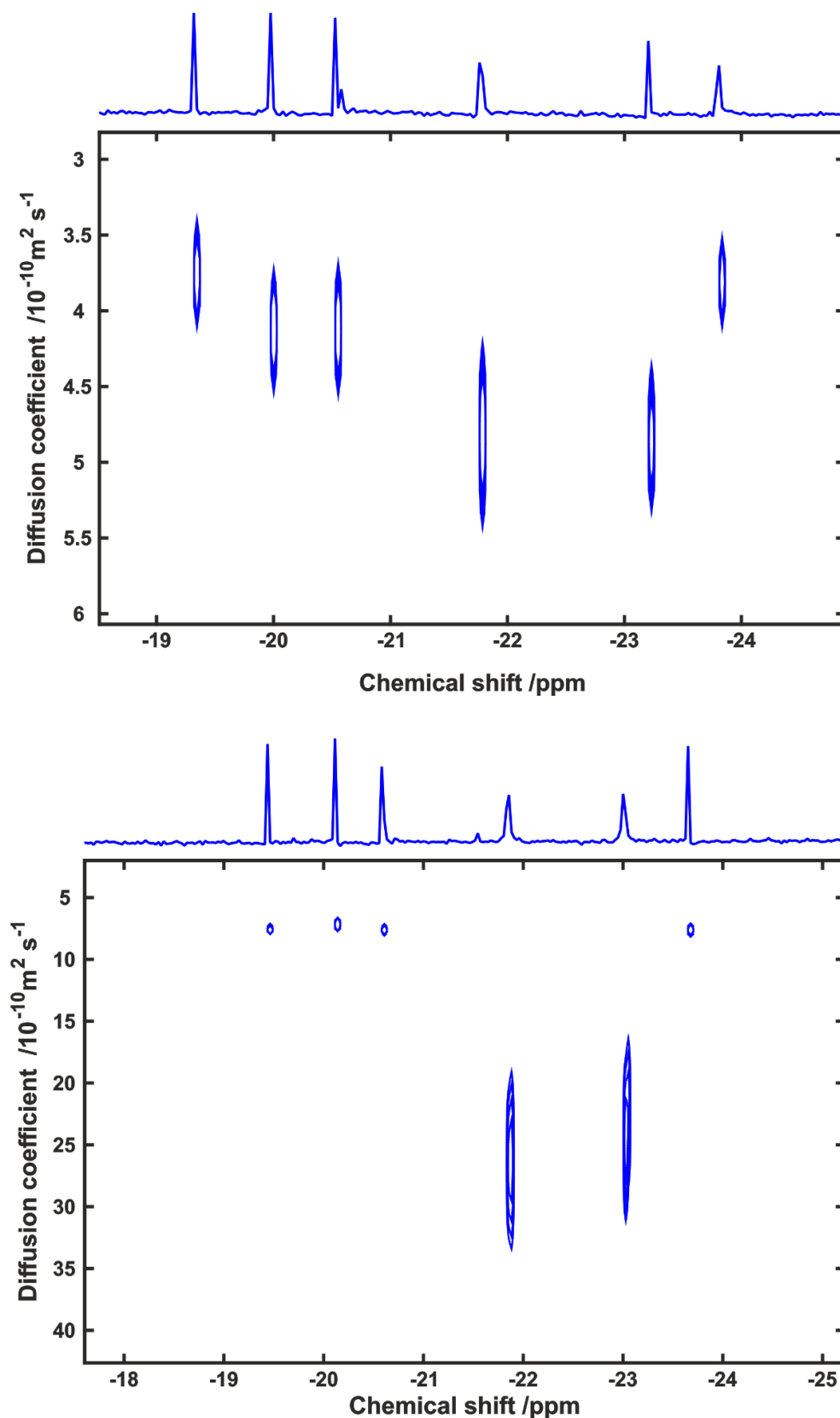


Figure 2.11 Top: DOSY spectrum collected at 253 K. Bottom: DOSY spectrum collected at 298 K. Both datasets were acquired at a field of 11.7 T and processed as outlined in the experimental.

Further confidence for the structural assignment in 7 stems from the fact that Oro *et al.* secured an X-ray structure for an iridium tetrahydride complex shown in Figure

2.12 alongside the structure of **7** for ease of comparison.¹²¹ Tables 2.1 and 2.2 shows some of the reported bond lengths and angles respectively, for the structure presented by Oro. In the structure there are four hydride ligands present but as these are placed to satisfy the coordination sphere they do not have errors associated with them and so are not discussed in detail.

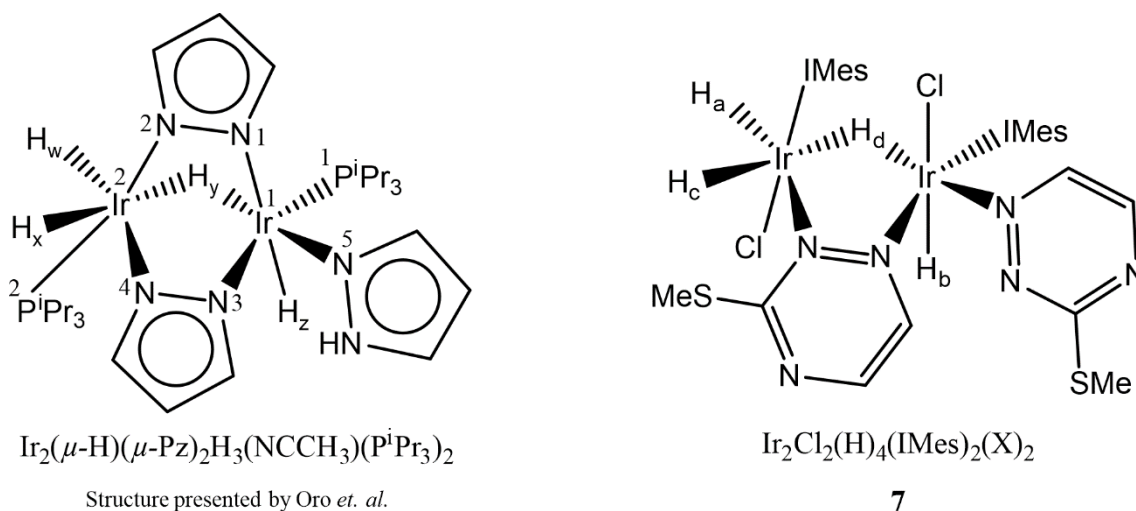


Figure 2.12 Left: Structure of Ir dimeric species presented by Oro *et. al.* Labels on atoms are reflective of those presented in Tables 2.1 and 2.2 **Right:** Structure of **7** the Ir dimeric species presented in this thesis. Labels on atoms are reflective of those presented in Scheme 2.3.

Table 2.1 Some of the reported bond lengths from the structure reported by Oro *et. al.* as shown in the left of Figure 2.12

Atom Labels	Bond Length / nm
Ir(1)-Ir(2)	29.817(14)
Ir(1)-P(1)	22.52(6)
Ir(1)-N(1)	21.2(2)
Ir(2)-P(2)	22.16(6)
Ir(2)-N(2)	20.8(2)
Ir(2)-N(4)	21.0(2)

Table 2.2 Some of the reported bond angles from the structure reported by Oro *et. al.* as shown in the left of Figure 2.12

Atom Labels	Bond Angle / deg	Atom Labels	Bond Angle /deg
P(1)-Ir(1)-N(1)	102.0(5)	P(2)-Ir(2)-N(4)	95.9(5)
P(1)-Ir(1)-N(3)	173.1(5)	P(2)-Ir(2)-N(2)	178.1(5)
P(1)-Ir(1)-N(5)	96.0(5)	N(2)-Ir(2)-N(4)	85.3(7)
N(1)-Ir(1)-N(3)	84.2(6)	H _y -Ir(2)-H _x	168
N(3)-Ir(1)-N(5)	86.7(7)	N(5)-Ir(1)-H _y	176
N(4)-Ir(2)-H _y	88	N(4)-Ir(2)-H _w	167

From these data it is clear that an almost regular octahedral arrangement around each iridium centre is adopted. The Ir-Ir bond length of 29.817(14) nm presented is quite long compared to typical intermetallic distances, implying no bond is present, although it is noted that the existence of longer Ir-Ir bonding has been proposed. Although the Ir-Ir bond is quite long compared to typical values, the presence of a single Ir-Ir bond is predicted theoretically to bring the overall electron count for the binuclear complex (34 electrons) to a more common 36 electrons. This would make both metal centres Ir³⁺, 18 electron. Surprisingly, despite this deduction, the diagrams drawn by Oro do not depict this Ir-Ir bond and so diagrams in this thesis also omit them as to be reflective of the literature description.

This description presented by Oro therefore gives support for the placement of the hydride ligands in **7**. In the paper Oro *et. al.* also presented hydride chemical shift data, they reported that the trans hydride ligands H_y and H_x gave rise to ¹H NMR signals at δ -24.26 and -23.70 respectively. This closely matches those presented as *trans* to each other in **7** yielding ¹H NMR signals at δ -20.59 (H_c) and -23.65(H_d). As the hydride chemical shift is reflective of the ligand *trans* to itself this strongly supports the placement of these hydride ligands.

To understand the formation of **7** further a series of samples containing 2 mg of **1** were made with 20 equivalents of **X** in dichloromethane-*d*₂ and reacted with hydrogen (4

bar) before immediate transition to the spectrometer where the sample was stored at different temperatures to monitor the rate of reaction. It was found that at 298 K the conversion of **1** to **6** was easy to monitor over a period of around an hour. **6** then converted slowly to form **7**. Hence, these two reaction steps were probed kinetically with the pseudo rate constants extracted for the conversion of **1** to **6**, and **6** to **7**. These data were obtained from a series of ^1H NMR measurements by integrating relevant peaks and accounting for the number of protons present in each integration region selected. The ^1H spectra were recorded every 140 s.

These data were then fitted to a kinetic model to extract the rates of conversion **1** into **6** and then **6** to **7**. Figure 2.13 shows a typical kinetic run at 298 K with both experimental and simulated points indicated. This process was repeated four times at different specified temperatures in order to assess the activation parameters associated with these changes. These were determined using the Eyring equation, as displayed in Figure 2.14. The corresponding activation parameters for the steps up to and including the rate determining step for the first change were $\Delta H^\ddagger = 119 \pm 32 \text{ kJmol}^{-1}$, $\Delta S^\ddagger = 31 \pm 108 \text{ JK}^{-1}\text{mol}^{-1}$ $\Delta G_{298}^\ddagger = 110 \pm 6.0 \text{ kJmol}^{-1}$. In contrast, the corresponding values for the second process were $\Delta H^\ddagger = 35 \pm 19 \text{ kJmol}^{-1}$ $\Delta S^\ddagger = -191 \pm 63 \text{ JK}^{-1}\text{mol}^{-1}$ and $\Delta G_{298}^\ddagger = 92.5 \pm 2 \text{ kJmol}^{-1}$. The entropy change of the second process where two species become three is clearly consistent with these data. For the first process, the high value of $\Delta H^\ddagger$ is certainly consistent with significant bond breakage leading to the transition state of the rate determining step. Based on literature data,¹²² this would be consistent with the loss of **X** from **6** to form **7**.

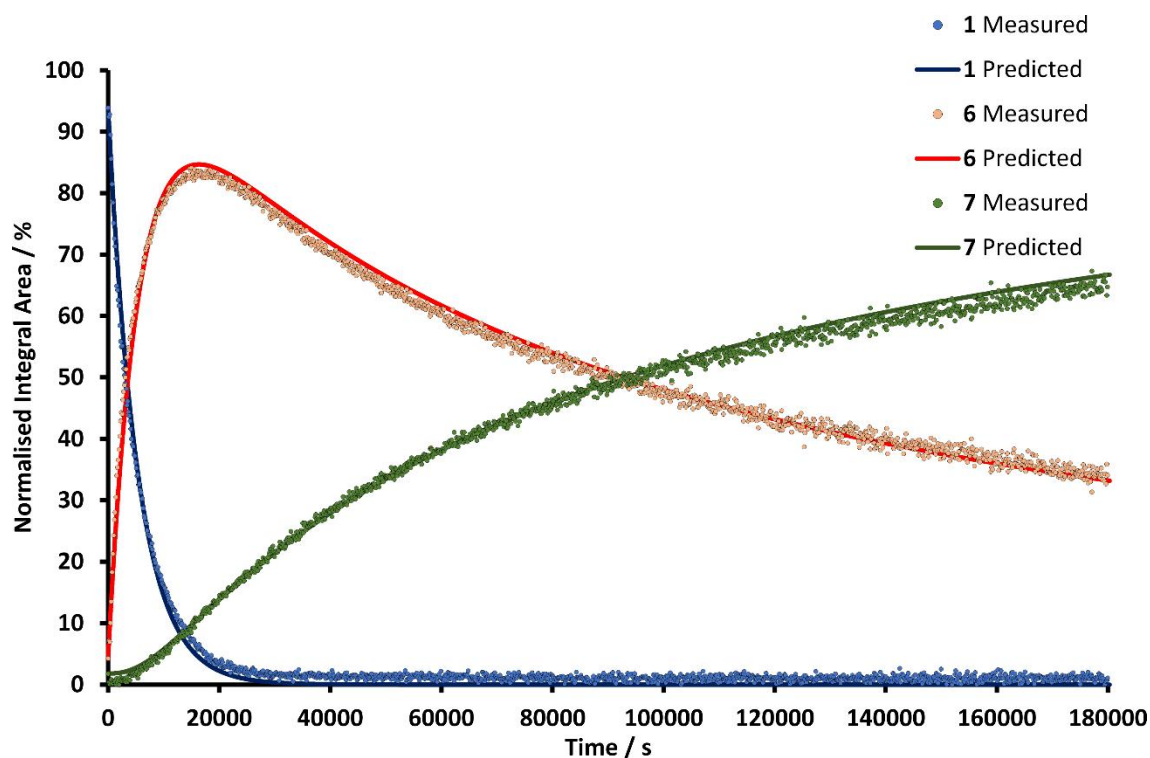


Figure 2.13 A typical reaction monitoring experiment of the reaction of 1 to 6 then to 7 at 298 K. The ‘measured’ (data points) represent the normalised signal intensity of each species in the spectra and the ‘predicted’ (solid line) representing the fit according to the model described.

Details of the kinetic model used to fit the data in this study are included in experimental Section 7.2.7. The rate constants determined were fit to the Eyring equation as already outlined in the text which are presented in Figure 2.14.

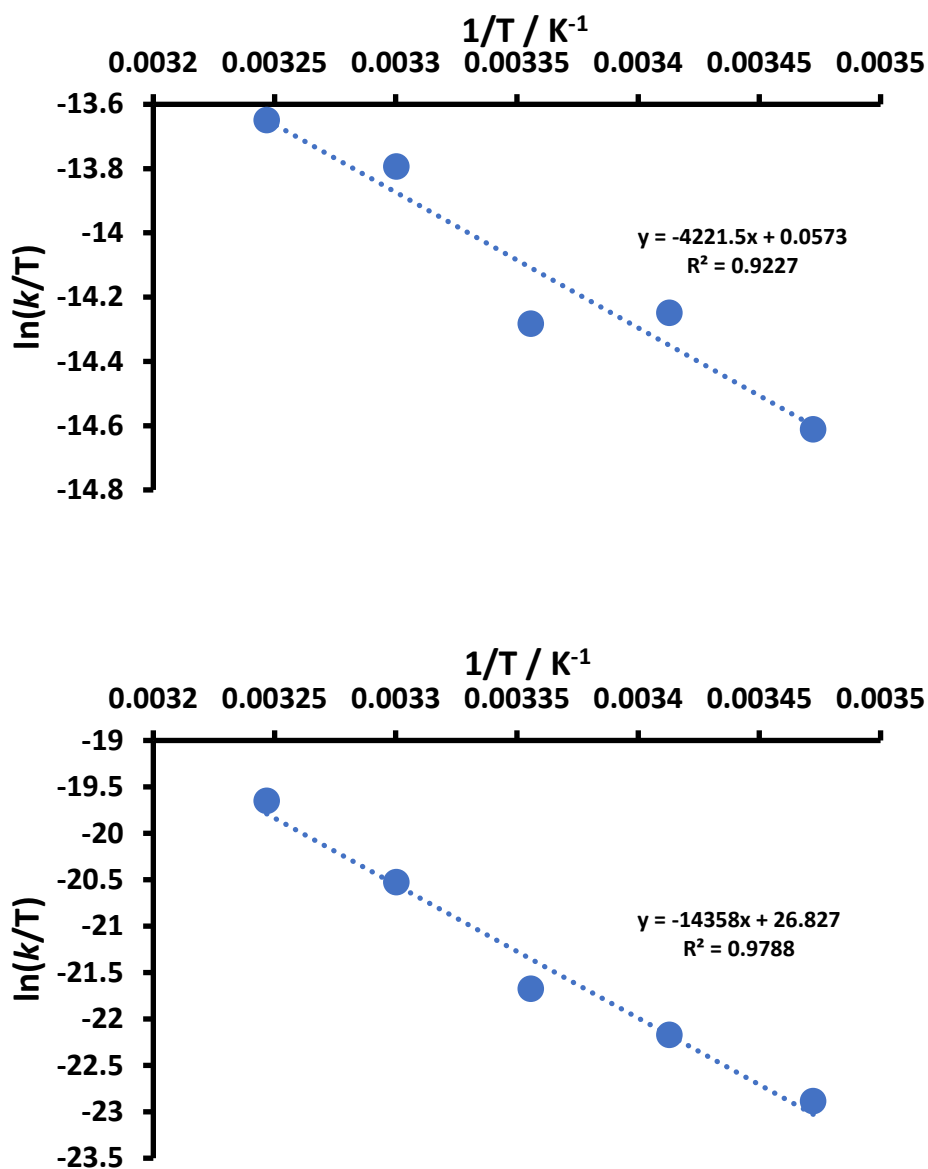


Figure 2.14 Top the Eyring plot gained from rate constants determined for the conversion of **1** to **6**. Bottom the Eyring plot gained from rate constants determined for the conversion of **6** to **7**. Both graphs were plotted from data collected at temperatures ranging from 288 to 308 K

As complex **7** stopped any SABRE activity it was decided to continue with the SABRE optimisation in methanol- d_4 where it has already been shown that **6** does not form, and **4a** forms as a major product.

2.3 Exploring ^1H polarisation of 1,2,4-triazine-3-methylthio as a function of iridium pre-catalyst

As the species **6** was shown to be a better SABRE catalyst than **4a** it was clear that major improvements could be made to **4a** to improve SABRE catalytic activity. Therefore it was decided to change the steric and electronic properties of the carbene ligand following a similar procedure to that undertaken by Rayner *et al.* which involved examining over 30 catalysts when nicotinate was the target.⁵⁹ The ligands of Figure 2.15 were selected for this study as they were felt to span the necessary steric and electronic properties needed to vary the ligand exchange rates appropriately.

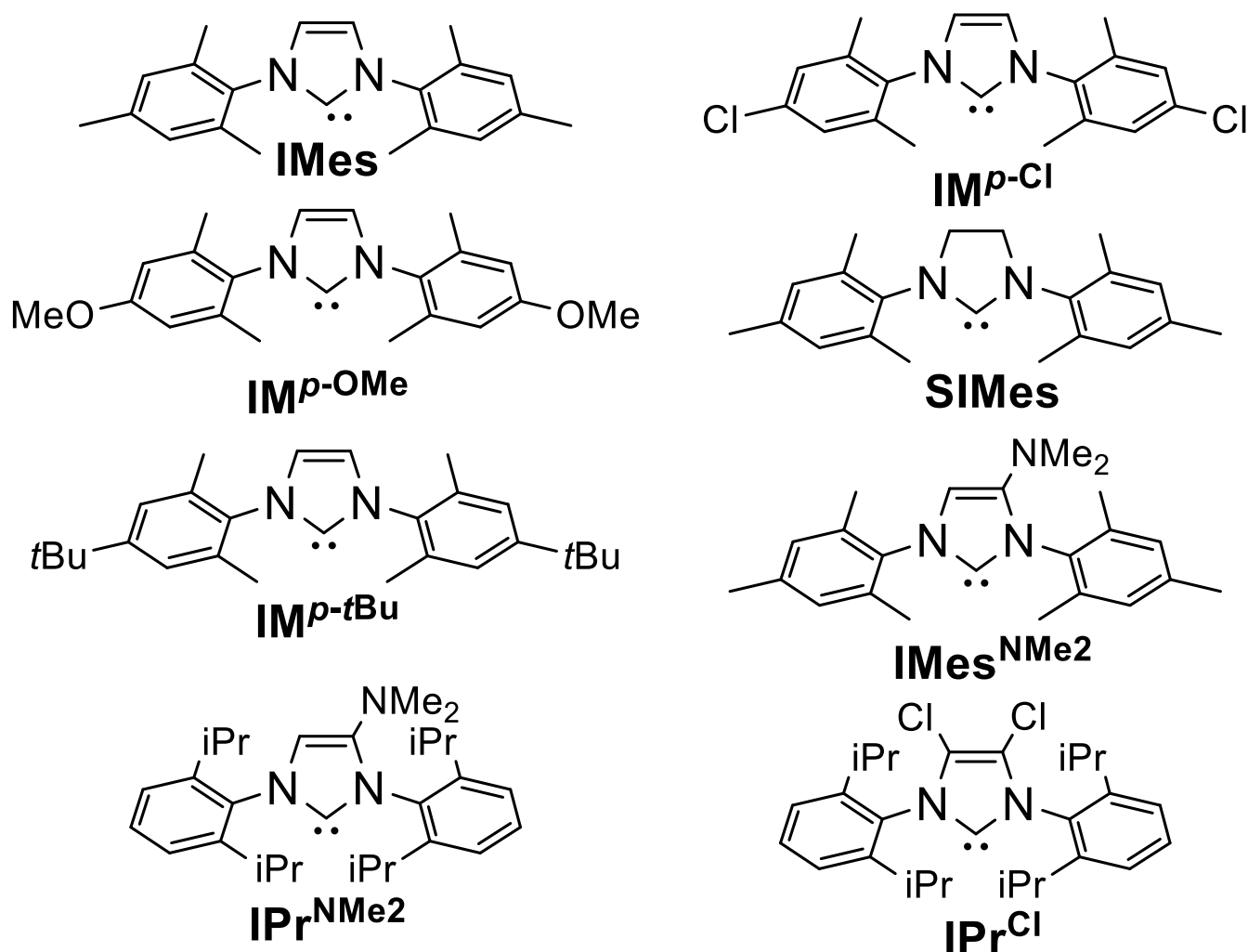


Figure 2.15 NHC ligands used in the pre-catalysts $[\text{IrCl}(\text{COD})(\text{NHC})]$ for the optimisation of 1,2,4-triazine-3-methylthio

The ability of the pre-catalysts $[\text{IrCl}(\text{COD})(\text{NHC})]$ to add H_2 and form a stable SABRE active species was first probed. This involved examining a series of samples containing 2 mg of each pre-catalyst and a 10 fold excess of **X** relative to the specified

[IrCl(COD)(NHC)], dissolved in 0.6 mL of methanol- d_4 under a 4 bar pressure of $p\text{-H}_2$. A PHIP study was first completed at 243 K to assess the formation of complexes analogous to **3**, evidenced by their inequivalent hydride ligands ^1H NMR signals. The resulting ^1H NMR spectra were acquired with a 45° pulse to initiate PHIP activity. In each case, a pair of dominant hydride ligand signals were seen with PHIP character that was indicative of the formation of a single isomer of **3**, with N_1 binding. In all cases, these hydride ligand signals exhibited similar chemical ligand signal intensities for these species immediately after $p\text{-H}_2$ addition, and their chemical shifts are detailed in Table 2.3.

Table 2.3 Signal to noise ratio of the PHIP enhanced hydride ligand ^1H NMR signals for 3-NHC and their respective chemical shifts. NMR spectra were recorded at 243 K after addition of 4 bar $p\text{-H}_2$ at 77 K and shaking for 5 s. All samples utilised methanol- d_4 as a solvent.

NHC	Hydride Ligand Chemical Shift / ppm	Signal : Noise
IMes	-13.51	338
	-17.35	430
IM^{p-Cl}	-13.87	10
	-18.14	11
IM^{p-OMe}	-13.45	343
	-17.30	567
SIMes	-13.21	205
	-17.26	423
IM^{p-tBu}	-13.93	537
	-17.45	811
IMes^{NMe2}	-13.70	618
	-17.37	932
IPr^{NMe2}	-13.62	6
	-17.94	5
IPr^{Cl}	-13.46	710
	-17.70	902

Comparison of the hydride ligand signal strengths revealed that electron rich **IMes^{NMe2}** yields the strongest response, and that increasing the steric bulk around the metal centre by replacing the 2,6 methyl groups of **IMes** with $i\text{Pr}$ in **iPr^{NMe2}** led to a

dramatic reduction in the corresponding hydride ligand signal intensities. Consequently, it is possible to deduce that the H_2 addition rate to the corresponding $[\text{Ir}(\text{COD})(\text{NHC})(\text{X})]\text{Cl}$ is substantially slowed as these signal intensities are direct probes of the chemical flux associated with H_2 addition.

Upon warming these samples to 298 K, complete conversion into $[\text{Ir}(\text{H})_2(\text{NHC})(\text{X})_3]\text{Cl}$, analogous to **4a**, occurs in each case. All these complexes could easily be easily identified according to their characteristic singlet hydride ligand resonance at around δ -20. This indicates high symmetry in the equatorial plane. These complexes were subsequently characterised by NMR as detailed in Sections 7.4.6 through to 7.4.14.

The ability of these complexes to catalyse SABRE, when **X** was initially present in a 20 : 1 ratio relative to the iridium pre-catalyst was then assessed. These measurements involved using 4 bar of $p\text{-H}_2$ and a 10 s shake time for the hyperpolarisation transfer step which took place at 6 mT. The resulting ^1H NMR signal enhancements for the resonances of free **X**, and those for the equatorially bound **X** ligand in **4a**, or its analogue, are detailed in Table 2.4.

Table 2.4 NMR signal gain for the two ^1H NMR resonances of **X, both free and equatorially bound, (chemical shifts for the free **X** resonance are shown; A and B correspond to the bound versions of the resonances δ 9.01 and δ 8.55 signals in free **X** respectively). All the samples were shaken at 6 mT for 10 s with a pressure of 4 bar $p\text{-H}_2$ before signal acquisition at a field of 9.4 T.**

NHC	Hydride Chemical Shift (ppm) $[\text{Ir}(\text{H})_2(\text{NHC})(\text{X})_3]\text{Cl}$	Free X Signal Gain		Bound X Signal Gain	
		δ 9.01	δ 8.55	A	B
IMes	-20.19	107 ± 3	103 ± 4	89 ± 2	52 ± 3
IM^p-Cl	-20.17	46 ± 1	44 ± 1	54 ± 6	42 ± 5
IM^p-OMe	-20.30	126 ± 3	122 ± 3	97 ± 5	87 ± 5
SIMes	-20.37	164 ± 13	159 ± 12	128 ± 14	145 ± 14
IM^p-tBu	-20.09	179 ± 6	176 ± 6	122 ± 6	76 ± 4
IMes^{NMe2}	-20.40	266 ± 10	253 ± 10	156 ± 5	193 ± 6
IPr^{NMe2}	-20.05	548 ± 13	532 ± 13	193 ± 23	374 ± 61

IPr^{Cl}	-20.12	419 ± 29	414 ± 24	309 ± 43	363 ± 18

It can be noted from these data that species analogous to **4a** form in all cases. Furthermore, all but **IMP^{Cl}** show an improvement in SABRE activity when compared to **IMes**. Rationalising this involved collecting kinetic data on the rate of ligand exchange for both the **IMes** and **IMP^{Cl}** catalysts. These exchange spectroscopy (EXSY) measurements involved sets of samples containing 2 mg of pre-catalyst and 20 equivalents of **X** with 4 bar of H₂ in 0.6 mL of methanol-*d*₄. The rate of **X** ligand loss to free **X** was $0.304 \pm 0.002 \text{ s}^{-1}$ for the **IMes** containing system at 298 K. This reduced to $0.191 \pm 0.001 \text{ s}^{-1}$ for the **IMP^{Cl}** in accordance with its poor SABRE performance. Values for the electronic and steric properties of these carbene ligands can be found in the study by Rayner *et al.* already discussed.⁵⁹ The **IMP^{Cl}** example here has similar steric properties to **IMes**, but is electron poor and therefore exhibits slower **X** ligand loss. For ease of comparison Figure 2.16 shows the order of increasing ¹H signal enhancement for free **X** at a 9.4 T acquisition field.

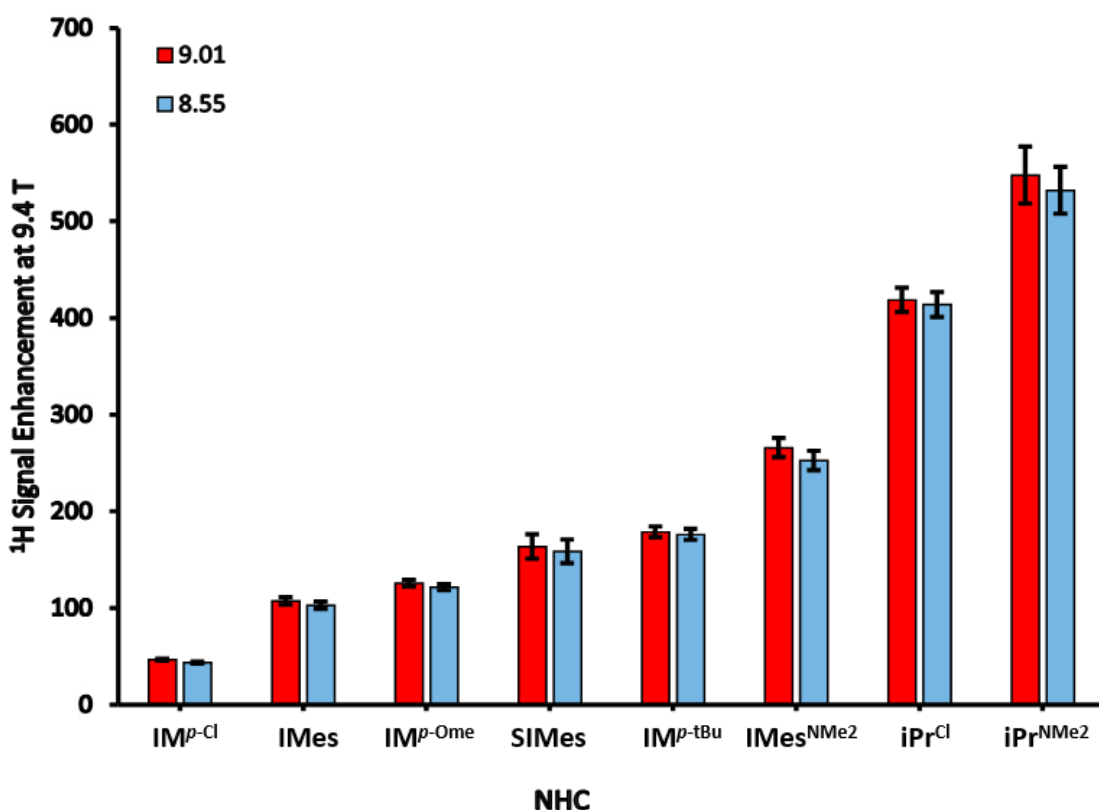


Figure 2.16 ¹H NMR signal enhancements at 9.4 T as a function of pre-catalyst, data taken from Table 2.4. All samples consisted of 2 mg of pre-catalyst with 20 equivalents of **X**. Samples were shaken at a field of 6 mT for 10 s under a pressure of 4 bar *p*-H₂.

Several trends are evident in these data. The first of these trends appears for the four catalysts containing *ortho* Me substituents and different *para* groups. It sees an

increase in delivered polarisation level with increase in electron donating ability of the NHC; values change from ~100-fold gain with **IMes** to ~179 for **IMPⁱ-tBu**. This, in turn would reflect an increase in the ligand exchange rate. The steric bulk of the NHC was then increased by moving from **IMes** to **SIMes**, then to **IPr**, with differing functionality; **IPr^{NMe2}** and **IPr^{Cl}**. This change could further promote ligand exchange by weakening the Ir-X bond as shown by Rayner *et al.* for Ir-CO bond lengths. As expected, the resulting catalysts yield yet further improvements in SABRE performance with the most electron rich and sterically bulky NHC **IPr^{NMe2}** delivering 548 ± 13 fold signal gain for the δ 9.01 resonance at 9.4 T and a 20 fold excess of **X**. Consequently, the performance of the **IPr^{NMe2}** based SABRE catalyst is ~5 times better than the corresponding **IMes** version.

At this point the **X** ligand dissociation rate for the **IPr^{NMe2}** based SABRE catalyst was probed to ensure the increasing exchange rate hypothesis was still maintained. A new sample was therefore prepared containing 2 mg of pre-catalyst **IPr^{NMe2}** and 20 equivalents of **X** activated with 4 bar of H₂ in 0.6 mL of methanol-*d*₄ and a series of EXSY measurements were undertaken. These data revealed a dissociation rate constant of $5.65 \pm 0.03 \text{ s}^{-1}$ much closer to the optimum exchange ascribed by Barskiy *et al.* who predicted a rate of 4.5 s^{-1} for transfer to a pyridine derived *ortho* proton to be optimal based on a 1 Hz transfer coupling.^{57, 123}

Notably, when the best performing **IPr^{NMe2}** was probed in dichloromethane-*d*₂ the steric bulk associated with employing the ⁱPr derivatives of the NHC suppresses the formation of **7** and therefore provides for longer-term sample stability in this solvent. However, for **iPr^{NMe2}** in dichloromethane-*d*₂, very inefficient SABRE is observed *via* the ¹H NMR spectrum with the results yielding just half the intensity of the original thermal signals of free **X**. This measurement was performed with a sample containing 2 mg of the [IrCl(COD)(**IPr^{NMe2}**)] pre-catalyst with 20 equivalents of **X** shaken at a field of 6 mT for 10 s with a pressure of 4 bar *p*-H₂.

This poor SABRE performance was due to the formation of [IrCl(H)₂(**IPr^{NMe2}**)(**X**)₂], which was found to exchange **X** with a rate constant of $10.62 \pm 0.09 \text{ s}^{-1}$ at a temperature of 243 K. Note that the ligand dissociation rate was too fast to measure at 298 K and hence the lower temperature of 243 K was used. This dissociation rate would be even faster at 298 K and thereby shows that this catalytic species exchanges **X** too quickly for efficient ¹H polarisation transfer. It has been shown that direct ¹⁵N polarisation transfer benefits from faster exchange and so shake and drop experiments

were undertaken on this sample shaking at a field of 0.3 μ T. Unfortunately no ^{15}N signal was observed in a one scan acquisition when shaken at 298 K or at 273 K by using an ice bath to store the sample for 2 min prior to shaking. Further EXSY measurements on complexes in methanol- d_4 will be discussed in a later section to further refine this work. Due to the poor SABRE performance in dichloromethane- d_2 in comparison to that in methanol- d_4 , it was decided to continue with methanol- d_4 as the only solvent for this study.

2.4 ^1H polarisation of 1,2,4-triazine-3-methylthio as a function of polarisation transfer field

For the optimum SABRE catalyst system of $[\text{Ir}(\text{H})_2(\text{iPr}^{\text{NMe}_2})(\text{X})_3]\text{Cl}$ a polarisation transfer field sweep was undertaken on a sample containing 2 mg of the pre-catalyst with 15 equivalents of **X**. This allows testing of the sensitivity of SABRE transfer to the magnetic field experienced by the sample during the catalysis step. The samples were shaken for 10 s under a pressure of 4 bar $p\text{-H}_2$ before transfer to a 9.4 T field for acquisition. Figure 2.17 shows the resulting ^1H NMR signal enhancements at 9.4 T in fold of signal gain as a function of the polarisation transfer field.

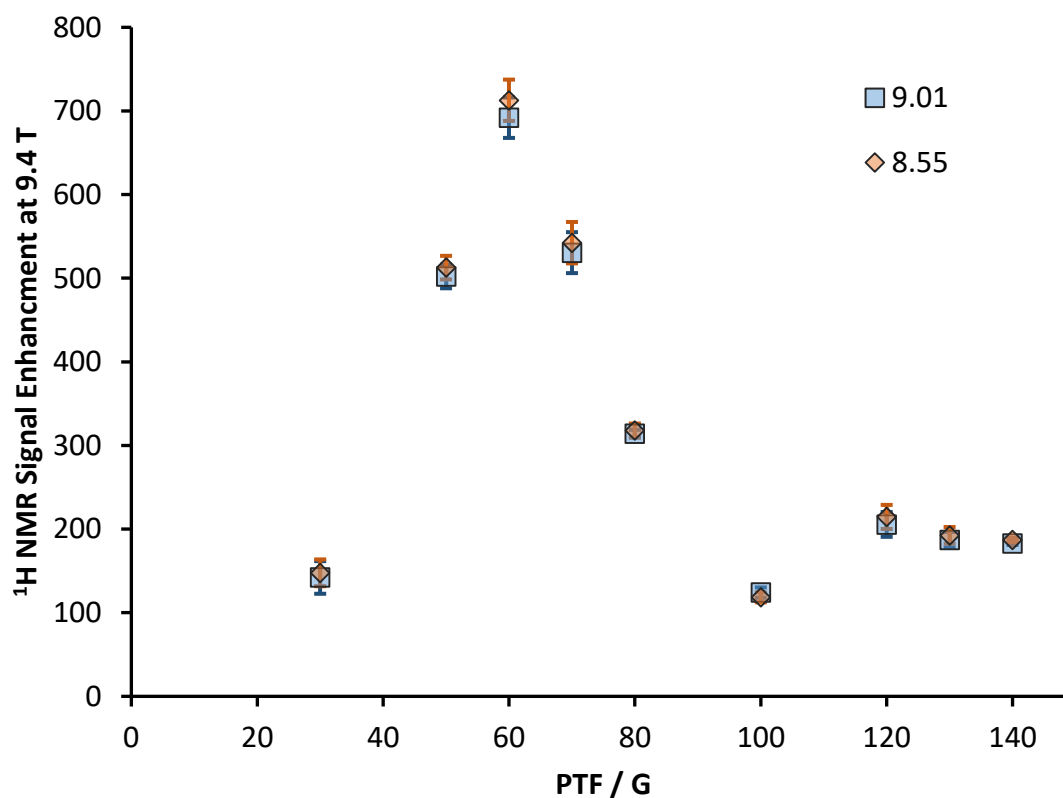


Figure 2.17 ^1H NMR signal enhancements found for *X* at 9.4 T as a function of the polarisation transfer field (G). The sample contained 2 mg of the pre-catalyst $[\text{IrCl}(\text{COD})(\text{IPr}^{\text{NMe}_2})]$ and 15 equivalents of *X*. All data points were collected from a 10 s shake in each PTF under 4 bar *p*- H_2 .

The PTF sweep data revealed a clear maximum in the enhancement plot at a field of 60 G (the 6 mT field already implemented in previous experiments). The profile is very sharp, showing a steep drop in delivered ^1H signal enhancement upon moving 10 G to either side of the 60 G maximum. As outlined in the experimental, the field strengths shown here were achieved by deploying permanent magnet field shakers, and so collecting data between these field values was not achievable. This study was repeated using the catalyst **1** to ensure the improvement in ^1H NMR signal enhancement at 9.4 T observed was definitely due to the change in exchange rate as described and not due to a change in optimum polarisation transfer field for the two catalytic systems. These data are shown in Figure 2.18.

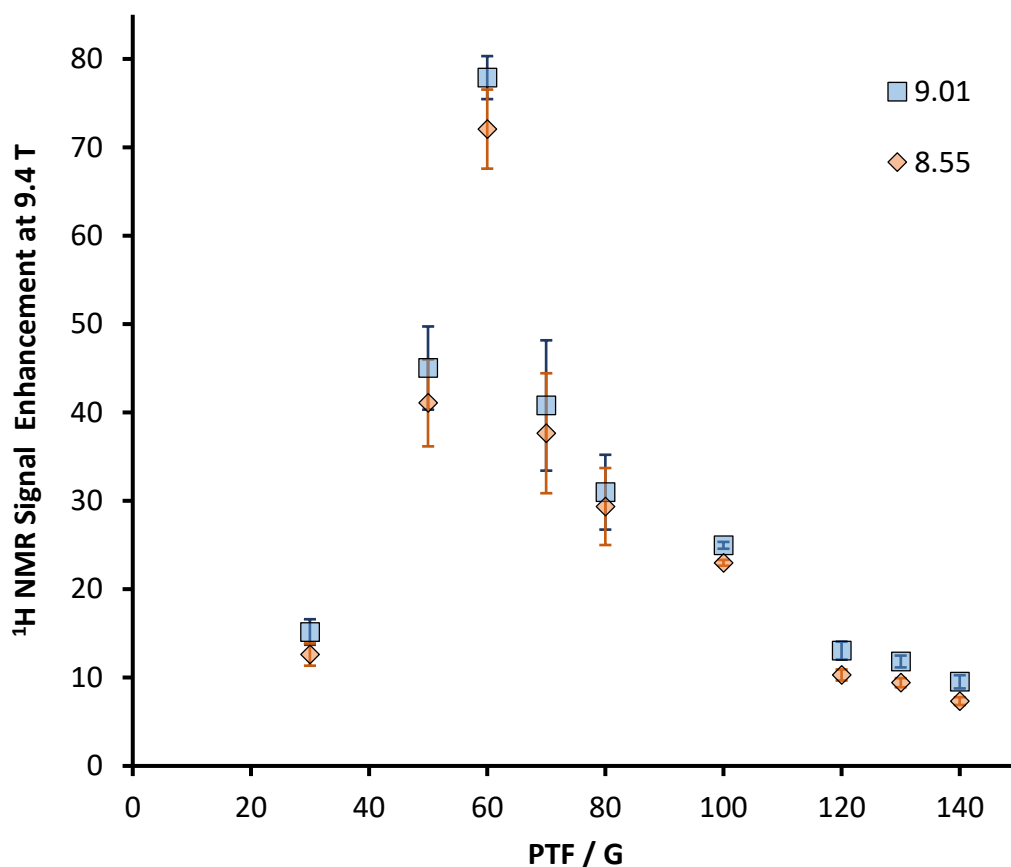


Figure 2.18 ^1H NMR signal enhancement of *X* at 9.4 T as a function of polarisation transfer field. Sample contained 2 mg of pre-catalyst **1** and 15 equivalents of *X*. All data points were collected from a 10 s shake in each PTF with 4 bar *p*- H_2 .

Although the enhancement at 9.4 T is much lower here as already discussed, the trend in enhancement at 9.4 T as a function of polarisation transfer field is very similar

for the two catalytic systems. Both show a very sharp maximum enhancement at 60 G, confirming the best PTF to use is the same and therefore the difference in enhancement level is principally due to a difference in ligand exchange rate.

2.5 ^1H polarisation of 1,2,4-triazine-3-methylthio as a function of co-ligand used.

Several recent studies have shown that introducing a co-ligand into the coordination sphere of the metal centre can improve the SABRE outcomes by creating catalysts of the form $[\text{Ir}(\text{H})_2(\text{co-ligand})(\text{NHC})(\text{X})_2]\text{Cl}$.⁵⁴ A series of studies were therefore undertaken in the presence of the co-ligands shown in Figure 2.19. The resulting ^1H NMR % polarisation obtained for these samples, employing a 5-fold excess of **X** relative to iridium, are listed in Table 2.5. Hence, it can be concluded that no real improvement in triazine polarisation level results with this variation. Interesting, though the co-ligand acetonitrile- d_3 (**C- d_3**) was created with >5% ^{15}N polarisation in these experiments which will be explored further in Chapter 6.

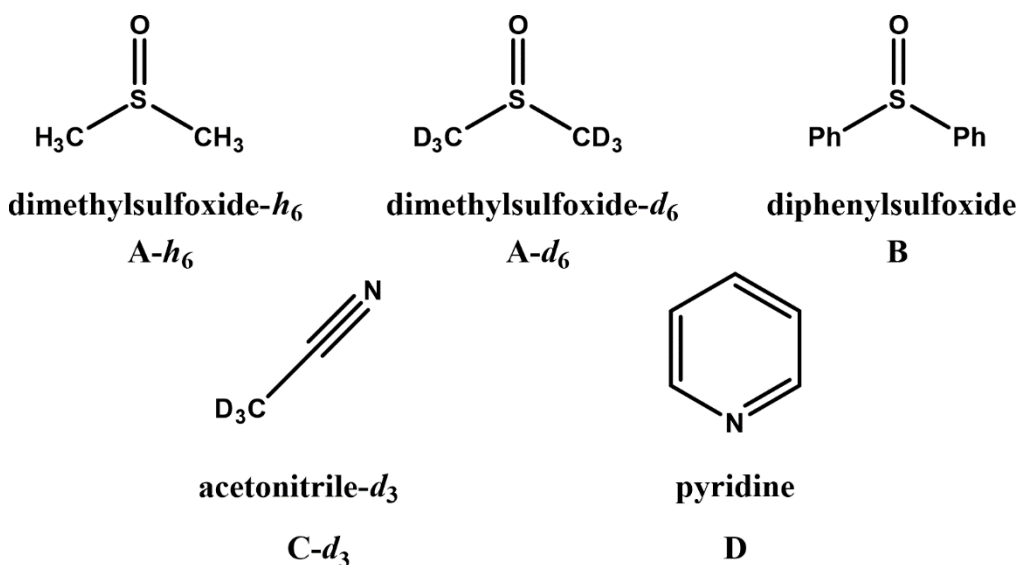


Figure 2.19 Chemical structures of co-ligands utilised in this study.

Table 2.5 ^1H NMR signal enhancements measured for the free ^1H NMR signals of **X** at δ 9.01 and 8.55 using precatalyst $[\text{IrCl}(\text{COD})(\text{IPr}^{\text{NMe}_2})]$ with a 5-fold excess of **X** and a 5-fold excess of the specified co-ligand in methanol- d_4 . These data utilised 4 bar of $p\text{-H}_2$, a 60 G transfer field and a shake time of 10 s.

Co-ligand	^1H Polarisation / %	
	δ 9.01	δ 8.55
N/A	6.9 ± 0.3	7.8 ± 0.4
DMSO- h_6 (A-h_6)	5.5 ± 0.1	6.9 ± 0.1
DMSO- d_6 (A-d_6)	6.0 ± 0.2	7.0 ± 0.3
DPSO (B)	4.7 ± 0.1	5.3 ± 0.1
Acetonitrile- d_3 (C-d_3)	1.3 ± 0.2	1.5 ± 0.1
Pyridine (D)	1.2 ± 0.3	N/A ^a

^a Signal enhancement could not be determined due to peak overlap.

As this method has been successfully implemented in the past it is essential to understand the reason for it not working here. This substrate is the only current example where co-ligands have not been successful at improving the level of polarisation transfer.

In order to probe this behaviour, the four best performing samples from Table 2.5 had their ligand exchange rates quantified. In order to do this, ^1H EXSY measurements were carried out on the four samples each containing 2 mg of the pre-catalyst $\text{IPr}^{\text{NMe}_2}$ with 5 equivalents of **X** and 5 equivalents of the corresponding co-ligand. The ligand exchange rate data for these samples is presented in Table 2.6.

Table 2.6 Rate of dissociation of **X** and H_2 at 298 K for the complexes $[\text{Ir}(\text{H})_2(\text{co-ligand})(\text{IPr}^{\text{NMe}_2})(\text{X})_2]\text{Cl}$ formed with the specified co-ligands (co-ligand axial to NHC). The samples all contained 2 mg of $\text{IPr}^{\text{NMe}_2}$ with 5 equivalents of **X** and 5 equivalents of the respective co-ligand, and were activated under a pressure of 4 bar H_2 .

Ligand axial to the NHC	Ligand Dissociation Rate / s^{-1}	
	X	H_2
X	5.65 ± 0.03	3.36 ± 0.03
DMSO- h_6 (A-h_6)	0.36 ± 0.01	0.21 ± 0.04

DMSO- <i>d</i> ₆ (A-d₆)	0.33 ± 0.01	0.20 ± 0.03
DPSO (B)	0.12 ± 0.01	0.10 ± 0.01

Interestingly these EXSY data show that the introduction of the co-ligand dramatically reduces both the ligand dissociation rate of **X** and the rate of H₂ formation. While this change should therefore lead to a reduction in SABRE efficiency, the reductions are not as severe as the ligand dissociation rates might first suggest. This reduced impact stems from the continued presence of the **IPr^{NMe2}** analogue of **4a** in these samples. This complex competes with the *bis*-substituted complex [Ir(H)₂(co-ligand)(**IPr^{NMe2}**)(**X**)₂]Cl for SABRE in all cases. Unfortunately, as the ratio of **4a** to [Ir(H)₂(co-ligand)(**IPr^{NMe2}**)(**X**)₂]Cl is essentially 4 : 1 in all cases its activity will dominate. It was therefore appropriate to repeat the study with a different NHC ligand to see how these ligand dissociation rates changed with NHC. This study was repeated with the same co-ligands but on a series of samples containing 2 mg of **1** with 5 equivalents of **X** and 5 equivalents of the respective co-ligands. The new ligand exchanges are presented in Table 2.7.

Table 2.7 Rate of dissociation for X and H₂ at 298 K for the complexes [Ir(H)₂(co-ligand)(IMes)(X)₂]Cl formed with the specified co-ligands (co-ligand axial to NHC). The samples all contained 2 mg of IMes with 5 equivalents of X and 5 equivalents of the respective co-ligand and were activated under a pressure of 4 bar H₂.

Ligand axial to the NHC	Ligand Dissociation Rate / s ⁻¹	
	X	H ₂
X	0.30 ± 0.02	0.15 ± 0.01
DMSO- <i>h</i> ₆ (A-h₆)	0.06 ± 0.01	0.04 ± 0.01
DMSO- <i>d</i> ₆ (A-d₆)	0.06 ± 0.01	0.04 ± 0.01
DPSO (B)	0.08 ± 0.01	0.06 ± 0.01

It is clear from these data that again a reduction in the ligand loss rate is observed for the respective $[\text{Ir}(\text{H})_2(\text{co-ligand})(\text{IMes})(\text{X})_2]\text{Cl}$ complexes. However, it was noted that now the relative proportion of **4a** : $[\text{Ir}(\text{H})_2(\text{co-ligand})(\text{IMes})(\text{X})_2]\text{Cl}$ was 3 : 7, suggesting the co-ligand substituted species is favoured with this less bulky carbene. After these studies it was deemed appropriate to explore further improvements in SABRE efficiency without using the co-ligand approach.

2.6 ^1H polarisation level as a function of substrate concentration and shake time

To further increase the ^1H polarisation on **X** it was hypothesised that the amount of **X** present could be reduced in order to reduce the spin dilution by focusing polarisation transfer into a smaller number of **X** molecules. The effect of **X** ligand excess for the better performing $\text{IPr}^{\text{NMe}_2}$ derived **4a** was therefore probed. This was done by creating a series of samples each containing 2 mg of the $\text{IPr}^{\text{NMe}_2}$ pre-catalyst with varying amounts of **X**, ranging from 5 to 30-fold equivalency in comparison to the catalyst. The samples were then activated under a pressure of 4 bar H_2 before shaking for 10 s in a 6 mT field under a pressure of 4 bar $p\text{-H}_2$. The results from these shake and drop experiments are displayed in Figure 2.20.

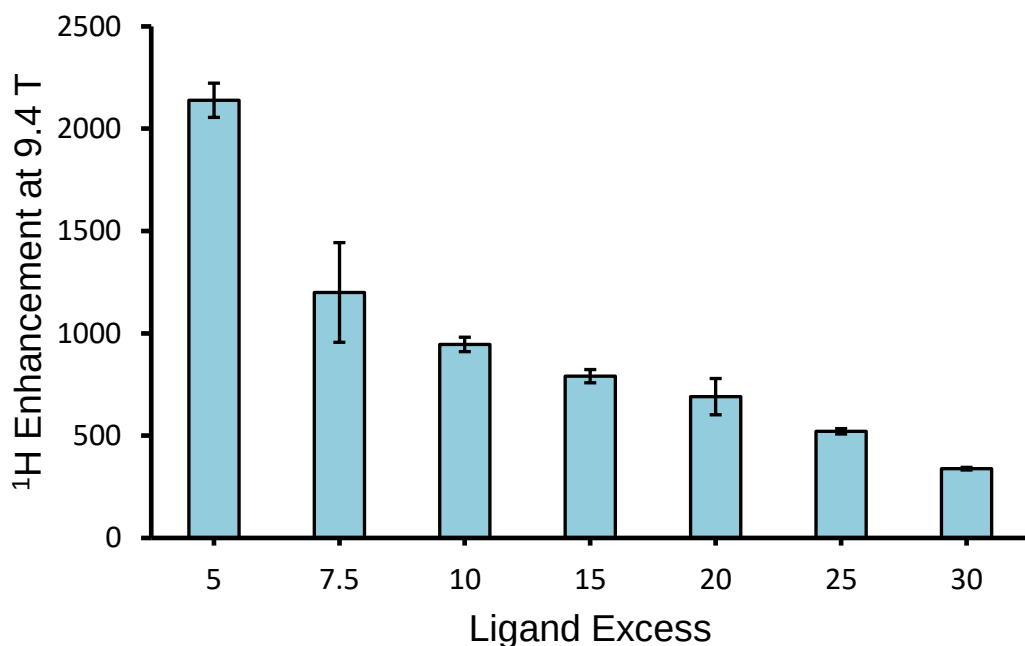


Figure 2.20 ^1H NMR signal enhancements determined at 9.4 T for the δ 9.01 signal of free **X** as a function of the ratio of **X** to iridium. All samples contained 2 mg of the pre-catalyst $[\text{IrCl}(\text{COD})(\text{IPr}^{\text{NMe}_2})]$ and were shaken at a 60 G transfer field for 10 s under 4 bar of $p\text{-H}_2$.

The resulting signal gains improved as the ligand excess reduced to the point that with an initial 5-fold excess relative to iridium a 2139 ± 84 fold signal gain for the ^1H NMR response at δ 9.01 was quantified. This value equates to 6.89% ^1H polarisation, as presented in Table 2.5. As the ^1H polarisation level achieved here was so high, it was natural to now test ^{15}N SABRE efficiency.

2.7 Probing the ^{15}N polarisation and reactivity of 1,2,4-triazine-3-methylthio

If optimal ^{15}N NMR signal enhancement could be achieved for this sample, it would create the opportunity to use it in reaction monitoring and the creation of hypothetical *paranitrogen*. In order to use the *trans* hydride $^{15}\text{N}_1$ coupling of ~ 25 Hz for SABRE the polarisation transfer step must now be completed in a μ -metal shield due to a μT field being required to satisfy the level anti crossing (LAC) requirement for direct polarisation transfer to ^{15}N .⁵³ Weaker indirect transfer *via* hyperpolarised ring protons is still possible from a PTF of 6 mT.

A sample was therefore prepared containing 2 mg of **1**, with 5 equivalents of **X**, the sample was degassed and activated under a pressure of 4 bar of H_2 and then probed for ^{15}N SABRE activity using a series of shake and drop experiments. The first of these experiments utilised a PTF of 6 mT. It therefore probed the activity of complex **4a** for **X** where the ^{15}N was present at natural abundance levels. After shaking for 10 s at this field under a pressure of 4 bar *p*- H_2 , the sample was transferred to a 9.4 T spectrometer for immediate ^{15}N NMR signal acquisition. A weak ^{15}N NMR peak, with antiphase character was visible for the N_4 site of free **X** that is characteristic of a heteronuclear $S_z I_z$ term. This suggests that the polarisation seen here is relayed from the ^1H nuclei as described previously, and that direct polarisation to nitrogen does not occur under these conditions. Interestingly, no ^{15}N NMR signal was seen for the expected N_1 site through which **X** binds to the catalyst. The enhancement value for this N_4 resonance was estimated to be 62 ± 34 -fold, relative to the more usual S_z starting term, which is reflective of creating a 0.02% polarisation level. When the experiment was repeated using a PTF of around 0.30 μT , typical of that needed for direct ^{15}N polarisation transfer, no ^{15}N NMR signals were observable in the corresponding one scan measurement.

A separate sample was then tested to compare these data with those obtained from the much faster exchanging **IPr^{NMe2}** derived **4a** with the hypothesis that the improved ^1H polarisation achieved would translate into improved ^{15}N polarisation also. The sample

contained 2 mg of the $\text{IPr}^{\text{NMe}_2}$ pre-catalyst with 5 equivalents of **X**. Analogous shake and drop experiments were then repeated, shaking at 6 mT and 0.30 μT for 10 s with 4 bar $p\text{-H}_2$. The 6 mT result revealed a huge improvement on the data seen for sample **1** with a 14300 ± 700 -fold signal enhancement determined for the N_4 ^{15}N NMR resonance at 9.4 T, which corresponds to $4.7 \pm 0.2\%$ ^{15}N polarisation. Unfortunately, even with this boost in ^{15}N polarisation, only the ^{15}N NMR signal for N_4 of **X** was observable. For the experiment conducted at a PTF of 0.30 μT there was still no observable ^{15}N NMR signals implying no direct polarisation transfer occurs. A similar study to that conducted in Figure 2.20 was then undertaken by conducting shake and drop experiments on a series of samples each containing 2 mg of the $\text{IPr}^{\text{NMe}_2}$ pre-catalyst with varying amounts of **X** ranging from 5 to 30-fold equivalency in comparison to the catalyst. The samples were each shaken for 10 s at a 6 mT PTF with a pressure of 4 bar $p\text{-H}_2$. Data for this study are presented in Figure 2.21.

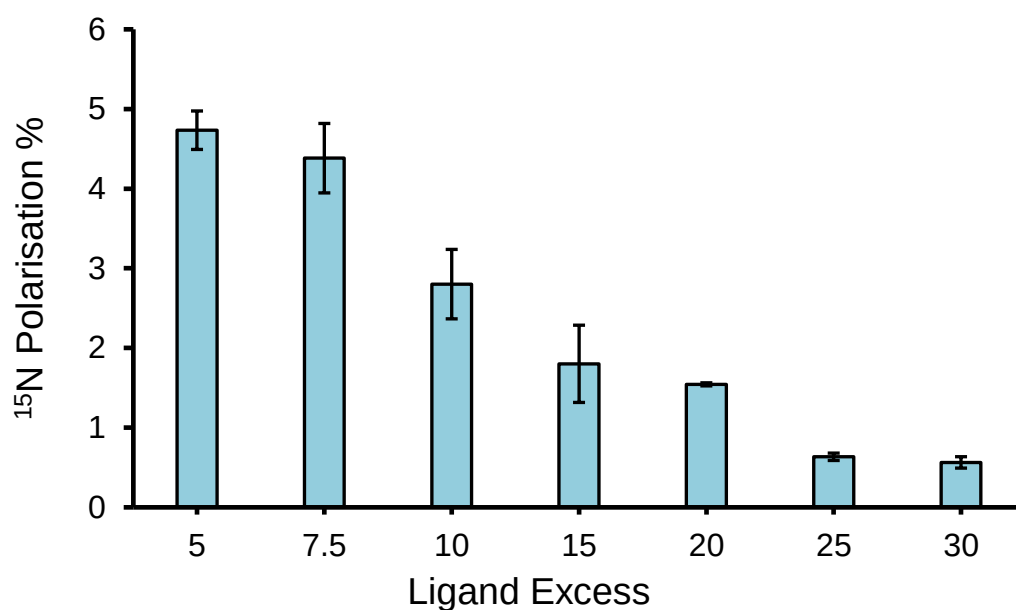


Figure 2.21 ^{15}N polarisation level determined for the N_4 site of free **X** as a function of the ratio of **X** to iridium. All samples consisted of 2 mg of the pre-catalyst $[\text{IrCl}(\text{COD})(\text{IPr}^{\text{NMe}_2})]$ and were shaken in a field of 60 G for 10 s under a pressure of 4 bar $p\text{-H}_2$.

Interestingly, the ^{15}N polarisation levels achieved follow the same trend as those in the study of Figure 2.20, further confirming that the ^{15}N polarisation resulting in this system is heavily reliant on the ^1H polarisation achieved. While a lower ^{15}N polarisation level is achieved with the 15-fold excess of **X** ($1.47 \pm 0.51\%$, which is a 4500 ± 1600 -fold enhancement when measured at 9.4 T), a better ^{15}N NMR signal to noise ratio results. This in turn enables both the N_1 and N_2 sites to be detected as antiphase peaks in a single

scan as shown in Figure 2.22. This is due to the fact that signal intensity is proportional to the product of concentration and polarisation level. The polarisation levels on N_1 and N_2 were 2000 ± 700 and 1000 ± 400 -fold, respectively. At this stage, direct ^{15}N polarisation by SABRE-SHEATH was reassessed. Unfortunately, again no visible ^{15}N signal resulted for any ligand excess utilised. We hypothesized that these naturally occurring samples present ^{15}N next to ^{14}N and rapid relaxation precludes resonance detection.¹²⁴

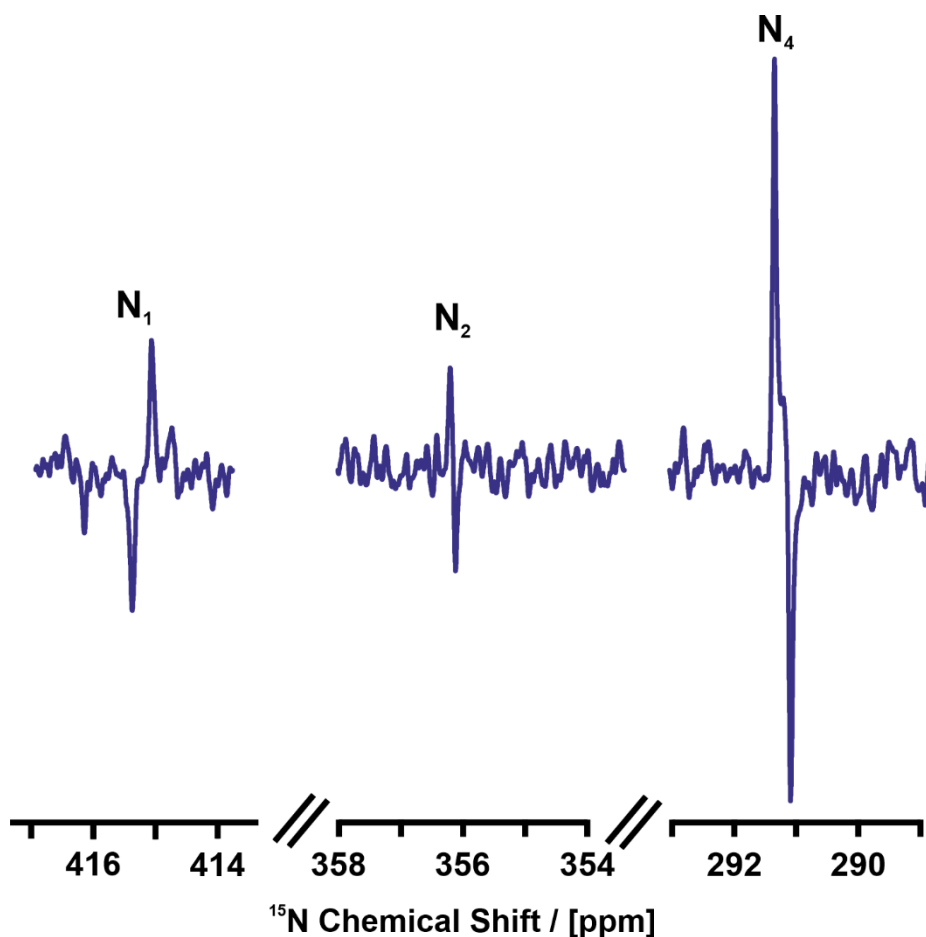
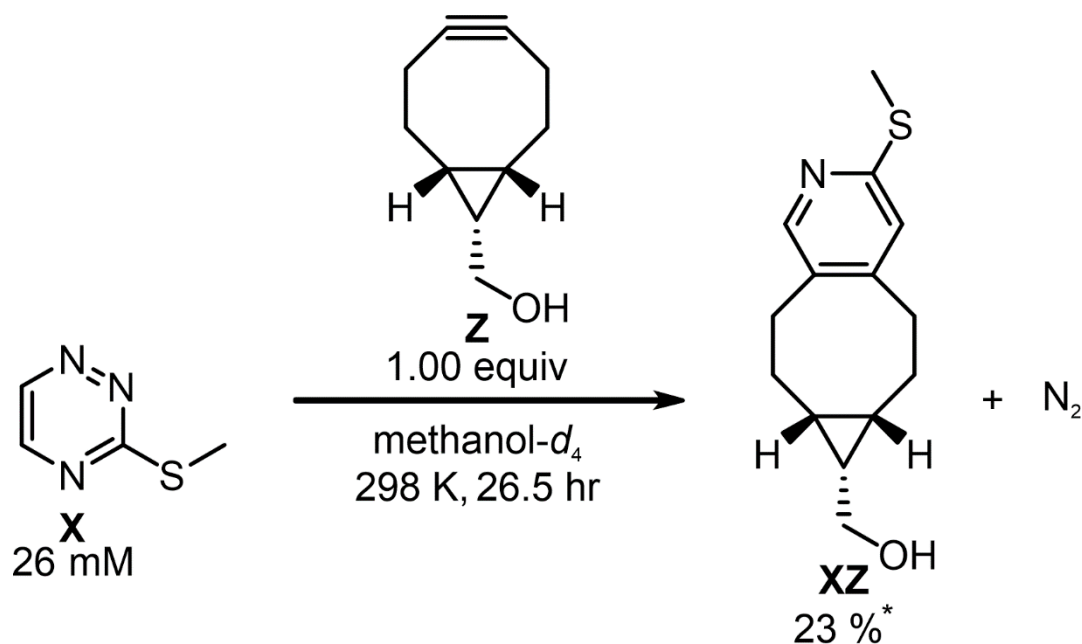


Figure 2.22 A typical SABRE hyperpolarised ^{15}N NMR spectrum. The spectrum was collected from a sample containing the $\text{IPr}^{\text{NMe}_2}$ pre-catalyst (2 mg) with 15 equivalents of **X** at a 9.4 T acquisition field shaken for 10 s at a PTF of 6 mT under a pressure of 4 bar $p\text{-H}_2$.

At this point the reaction of **X** with the strained alkyne (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethanol (**Z** of Scheme 2.4) was investigated by preparing a sample of **X** (26 mM) with a stoichiometric amount of **Z** in 0.6 mL of methanol- d_4 . The sample was then transferred to a 9.4 T magnet and the reaction, as detailed in Scheme 2.4, was probed by ^1H NMR spectroscopy.



Scheme 2.4 Reaction products detected during the reaction between **X** and **Z** to form the product **XZ** in methanol- d_4 solution, where **X** = 3-methylthio-1,2,4-triazine **Z** = (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethanol and **XZ** = ((6aS,7S,7aR)-3-(methylthio)-6,6a,7,7a,8,9-hexahydro-5H-cyclopropa[5,6]cycloocta[1,2-c]pyridin-7-yl)methanol. *After a total of 14 days the yield had improved to around 50%.

Unfortunately, the reaction progressed very slowly and after just over a day of reaction monitoring the reaction had only proceeded to 23% completion. Leaving the sample for a further 14 days at 298 K proved to be beneficial as the yield of **XZ** then improved to ~50% at which point full characterisation of the product was undertaken. Notably, new aromatic ^1H NMR resonances had appeared at δ 6.88 and 8.14 that shared a common coupling of 4.85 Hz. Further analysis by ^1H - ^{15}N HMQC NMR measurements revealed a cross peak from the ^1H NMR resonance at δ 8.14 to a ^{15}N NMR resonance at δ 289.8. These data reveal that **X** had definitively undergone reaction and lost N_2 as no other ^{15}N resonances were detected apart from those of free **X**. Full characterisation data are available in Section 7.4.18. Obviously, this reaction is too slow to monitor through the hyperpolarised signals as these are only present for around $3 \times T_1$ which is in the range of seconds rather than days. If reactivity is to be monitored by SABRE hyperpolarised NMR, a much more reactive species must be chosen. Vrabel *et al.* have produced work on a class of more reactive 1,2,4-triazines which could prove useful in progressing this work further.¹¹⁸

2.8 Expansion to Toxoflavin

As mentioned in the introduction to this chapter, 1,2,4-triazines have a wide variety of uses. Improved detection of these species has already been highlighted for its importance and so the antibiotic Toxoflavin (1,6-Dimethylpyrimido[5,4-

e][1,2,4]triazine-5,7(1H,6H)-dione), **W** of Figure 2.1b, was selected as an application for this work to test the ‘real world’ applicability of the research. Therefore a sample was prepared containing 0.3 mg of **1** with 7 equivalents of **W**. The sample was degassed and filled to a pressure of 4 bar H₂ where it was left to activate for approximately 30 min. The sample was then transferred to a 9.4 T magnet and probed by ¹H NMR spectroscopy. Interestingly, the ¹H NMR spectrum revealed no hydride ligand resonances in 16 signal averages and so no evidence for the binding of **W** was evident. At this point shake and drop studies were conducted on the sample by shaking the tube for 10 s under a pressure of 4 bar *p*-H₂ and a PTF of 6 mT. This revealed a hyperpolarised aromatic resonance for **W** reflecting 6.5-fold ¹H NMR signal gain as shown in Figure 2.23. It is noted that the hydride ligand ¹H NMR region now showed hydride ligands at around δ -31.00 implying that **W** actually binds through the carbonyl oxygen donors present in the molecule.¹²⁵ Unfortunately, due to the low concentration of these species, characterisation could not be undertaken.

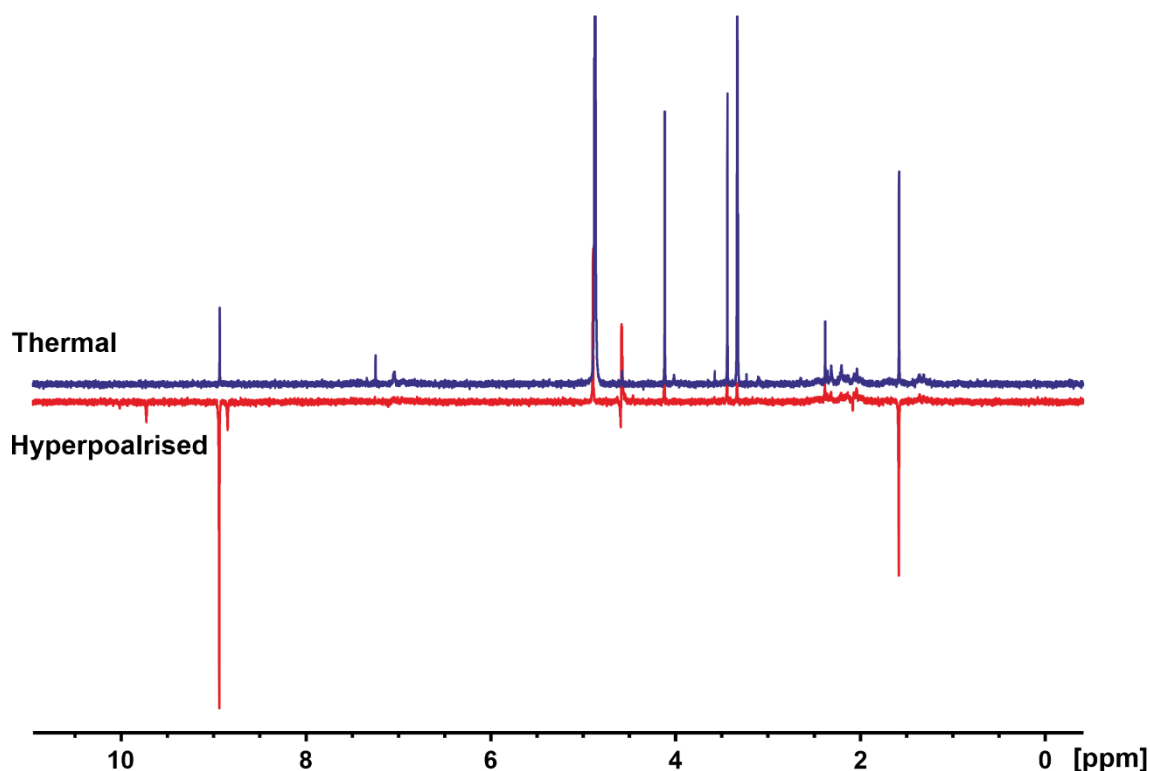


Figure 2.23 Typical SABRE hyperpolarised ¹H NMR spectrum collected in a one scan acquisition from a sample containing **1** (0.3 mg) with 7 equivalents of **W** in 0.6 mL of methanol-*d*₄. The sample was shaken for 10 s at a field of 6 mT under 4 bar *p*-H₂ prior to ¹H NMR signal acquisition to gain the hyperpolarised response.

Although the enhancement here is not as impressive as that represented for **X**, it does show that hyperpolarisation of these drug molecules is possible. In order to acquire a ^1H NMR spectrum of the same signal to noise ratio as that represented by the hyperpolarised spectrum displayed in Figure 2.23, approximately six times the number of scans would have to be undertaken and so use of this system yields a reasonable time saving. At this point polarisation transfer to ^{15}N was attempted shaking the sample under 4 bar of $p\text{-H}_2$ at both 6 mT and 0.3 μT for 10 s before transfer to the 9.4 T field for ^{15}N NMR signal acquisition. However, no ^{15}N NMR signal was observed in either case.

2.9 Conclusion

In this chapter a wide body of results related to the SABRE of 1,2,4-triazine-3-methylthio (**X**) have been outlined. This material was selected for study due to the important pharmaceutical role of related materials with this functionality. For its NMR analysis SABRE should deliver an improved detectability of its ^1H , ^{13}C and ^{15}N nuclei, which for MRI applications must in turn possess long relaxation times to enable detection in the body after a time period to allow movement from the site of injection to the measurement site of interest. The reaction of **X** with **1** in methanol- d_4 was found to yield the square planar **2**, wherein the successful displacement of the Cl^- ligand resulted in N_1 binding. This was established through appropriate ^{15}N detection and rationalised through nOe and other related analysis. Subsequently, the reaction of H_2 with **2** was examined. This proved to take place, even at a temperature of 243 K, leading to the dihydride product **3**. This octahedral complex was again fully characterised, and shown to bind selectively through N_1 and exhibit PHIP activity.

This complex was then allowed to warm to a temperature of 273 K which allowed the hydrogenation of the COD ligand and facilitated its conversion into isomers **4a** and **4b** in a reaction that proceeded under kinetic control, initially presenting in a 2 : 1 ratio respectively. Now, NMR characterisation demonstrated that whilst the dominant form of $[\text{Ir}(\text{H})_2(\text{IMes})(\text{X})_3]\text{Cl}$, **4a**, contained three **X** ligands bound *via* N_1 , the minor isomer **4b** exhibited one N_2 bound ligand that was *trans* in relation to the NHC ligand. The slow conversion of **4b** to **4a** confirmed that **4a** was the thermodynamically favoured product and the initial formation of **4b** was indeed under kinetic control. The barrier to exchange of the axial **X** ligand enables the demonstration that binding through N_2 is feasible. The basicity of the nitrogen sites involved are reflected in the pK_a of the conjugate acid.

When the same reaction was examined in dichloromethane- d_2 , the formation of **4a** was found to now proceed differently. Now the addition of H_2 occurs directly to **1**, albeit slowly. It is only upon the addition of H_2 to form **5** that Cl^- ligand substitution is observed by **X** to form **3**. The dichloromethane- d_2 solvent is relatively less polar than the methanol- d_4 used previously, and so neutral species are favoured evidenced as by the rapid succession of reactions that convert **3** to **4a** and then to neutral **6** at 298 K. Leaving the sample to react further then saw the slow formation of the SABRE inactive dimer **7**. All these species were characterised in full by NMR measurements including a series of novel DOSY measurements to demonstrate the effect of exchange on the diffusion coefficient, proving again that **7** does not exchange H_2 .

For SABRE to operate successfully a complex must undergo exchange of H_2 and the substrate, in this case **X**. Previous literature reports have established that the loss of **X** from species like **4** or **6** is dissociative,¹²³ with H_2 exchange proceeding *via* the resulting 16-electron intermediate. Our hypothesis that these would act as SABRE catalysts for **X** polarisation proved true. When exposed to $p\text{-}H_2$ the hyperpolarisation of the ^1H resonances in **X** were clearly seen. The degree of signal amplification achieved was found to be linked to the amount of these species in solution, with **6** proving superior to **4**. However, the ^1H NMR enhancement values achieved of 450-fold were lower than hoped.

The efficiency of the SABRE process has been shown to link to ligand exchange rate, which can be controlled by changing the electronic and steric behaviour of the NHC ligand, as outlined in the introduction. Consequently seven NHC ligands (Figure 2.15) spanning a variety of electronic and steric effects were screened for their ability to improve the SABRE of **X**. As predicted, these resulted in significant changes in the degree of ^1H NMR signal enhancement achieved for **X**. The electron rich and sterically encumbered **IPr^{NMe2}** ligand proved optimal, as expected on the basis of its determined ligand dissociation rate of **X** being $5.65 \pm 0.03 \text{ s}^{-1}$ for the complex $[Ir(H)_2(IPr^{NMe2})(X)_3]Cl$. A rate constant of *circa* 4.5 s^{-1} was predicted optimal when the transfer coupling is 1 Hz by Barskiy *et al.* (2016).⁵⁷ It was therefore decided to investigate this improved system more robustly and a ^1H NMR signal enhancement of 2000-fold was observed. Remarkably, this carried forward to allow the detection of a hyperpolarised ^{15}N NMR signal for N_4 that was 14300 ± 700 -fold larger than the thermally equilibrated signal would be, equating to $4.7 \pm 0.2\%$ polarisation.

When this optimum system **IPr**^{NMe₂} was tested in dichloromethane-*d*₂ it stopped the formation of the binuclear **7**. It instead formed a species of the form IrCl(H)₂(**IPr**^{NMe₂})(**X**)₂ which was found to rapidly exchange **X** with a rate constant of $10.62 \pm 0.09 \text{ s}^{-1}$ at a temperature of 243 K. This is far from the optimum value for ¹H mentioned previously but it was hoped that the faster exchange may benefit the direct hyperpolarisation of ¹⁵N. Unfortunately, this was not successful and no direct polarisation transfer to ¹⁵N was observed for this system. To try and vary the exchange rate of the system in methanol-*d*₄ the series of co-ligands; DMSO-*h*₆ (**A-h₆**), DMSO-*d*₆ (**A-d₆**), DPSO (**B**), acetonitrile-*d*₃ (**C-d₃**) and pyridine (**D**) were examined with [IrCl(COD)(**IPr**^{NMe₂})] and **X**. This led to the formation of the corresponding [IrCl(H)₂(co-ligand)(**IPr**^{NMe₂})(**X**)] complexes. None of these species yielded improved ¹H SABRE performance, due to their now slow rates of ligand exchange.

The hyperpolarised ¹⁵N NMR response achieved for **X** by the optimum **IPr**^{NMe₂} system was found to relax very rapidly. This was concluded to be due to interactions with its neighbouring quadrupolar ¹⁴N, therefore, to study the lifetimes and ¹⁵N hyperpolarisation of **X** in more detail, it is recommended to carry out ¹⁵N labelling of the substrate.

Chapter 3. Optimising the hyperpolarisation of $[\text{}^{15}\text{NO}_2]^-$ for use in reaction monitoring and diffusion spectroscopy

3.1 Introduction

The chemical shift window of ^{15}N in NMR spectroscopy is rather large in comparison to that of proton, sitting at around 1400 ppm.⁶⁴ This makes the identification of chemically similar species a lot easier as there is less chance of NMR peak overlap. ^{15}N also benefits from a low gyromagnetic ratio and so typically has much longer T_1 values.^{63, 64} Unfortunately, this property also makes ^{15}N harder to detect by NMR. Combined with a low natural abundance (0.36%) this makes ^{15}N very difficult to detect by NMR spectroscopy.⁴ SABRE has been shown to achieve >50% ^{15}N polarisation and so eliminates this issue. These points combined therefore make ^{15}N NMR spectroscopy an ideal candidate for reaction monitoring, utilising SABRE to artificially increase the ^{15}N NMR signal intensity. The long T_1 values are likely to last long enough for reaction products to be observed, with the species made being easily identifiable by ^{15}N NMR spectroscopy thanks to the wide chemical shift window.

The substrate of interest in this study is nitrite ($[\text{NO}_2]^-$) that will be known as **Y**. Plants utilise nitrite to form essential nitrogen-containing molecules such as amino acids.¹²⁶ Nitrite is also widely used as a food additive for cured meats and approximately 7% of ingested nitrite in the human diet is ingested *via* this route,¹²⁷ while the remainder comes from oral bacteria that reduce nitrate to nitrite.¹²⁸ Nitrites themselves are fairly benign and are classed as non-carcinogenic. However, their ability to form nitrosamines can lead to toxicity issues.¹²⁹ Nitrite is usually used in industry through the compound sodium nitrite, the form used in this study, and finds a wide variety of uses due to its ability to act as an oxidant and routine use in organic synthesis where it can be found in the Sandmeyer reaction,¹³⁰ or formation of azo-dyes.¹³¹

As outlined in the introduction, an essential part of SABRE is to form a suitable metal complex where the scalar coupling network created between the $p\text{-H}_2$ derived hydride ligands and the target nuclei (in this case the ^{15}N of **Y**) can facilitate transfer of magnetisation. The substrate **Y** is known to ligate in complexes with examples of Ni,^{132, 133} and Pt^{134, 135} being prevalent in the literature.¹³⁶ It is therefore hypothesised that a suitable SABRE complex should form and therefore hyperpolarisation of **Y** should be feasible.

This chapter sets out to assess the feasibility of polarisation transfer to **Y** *via* SABRE. First an appropriate stable complex is created with the use of co-ligands, a method widely outlined in the literature.⁵⁴ The steric and electronic properties of the

complex are then altered to optimise the exchange rate of the SABRE active species. This is done through modification of the NHC ligand,^{59, 137} as done in the previous chapter, and also through the use of co-ligands to form preferential SABRE species.⁴ The optimised system is then taken forward to utilise the polarisation gains in reaction monitoring by ¹⁵N NMR. The chapter then proceeds to also apply these novel techniques to look at reactivity and reaction monitoring of azide.

To diversify the applications of this ¹⁵N hyperpolarisation the chapter then ends by exploring the development of ¹⁵N Diffusion Spectroscopy (DOSY).¹³⁸ DOSY is a widely used NMR technique developed to measure the diffusion coefficients of molecules in solution. It has seen a broad set of applications including drug analysis,¹³⁹ battery performance¹⁴⁰ and protein NMR,¹⁴¹ to give a few examples.

DOSY works by applying gradients as outlined in the introduction Section 1.1.2 combined with a spin echo type experiment. This kind of *r.f.* pulse sequence applies a 90° pulse to transfer the magnetisation into the xy plane. A time period is then waited to allow evolution of the spins under transverse relaxation (T₂), before applying a 180° to refocus the spins. By applying the gradient pulses it is possible to ‘de-phase’ and therefore not detect any spins which have travelled away from their original position in the sample. Therefore the degree of the NMR signal attenuation corresponds to the movement of the particle in solution.¹⁴² Repeating the experiment with different gradient strengths or different time periods then builds an exponential decay which can be fitted to the Stejskal-Tanner shown in Equation 2.1 and the diffusion coefficient extracted.^{143, 144}

$$S(g) = S_0 e^{-D\gamma^2 \delta^2 g^2 \Delta'} \quad (2.1)$$

Where $S(g)$ is the NMR signal strength in a pulse sequence with a pair of balanced diffusion-sensitizing gradients of strength g , S_0 is the resulting signal from the same experiment without the gradient pulses, D is the diffusion coefficient, γ is the gyromagnetic ratio of the nuclei being observed, δ is the duration the gradient is applied for, g is the gradient strength applied, and Δ' is the time allowed for the spins to diffuse.

While this research was being undertaken, Clark *et al.* showed that ¹⁵N DOSY is possible through ¹⁵N labelling of the sample and running for multiple signal acquisitions.¹⁴⁵ SABRE hyperpolarised ¹⁵N DOSY offers an opportunity for cheaper experiments using unlabelled samples.

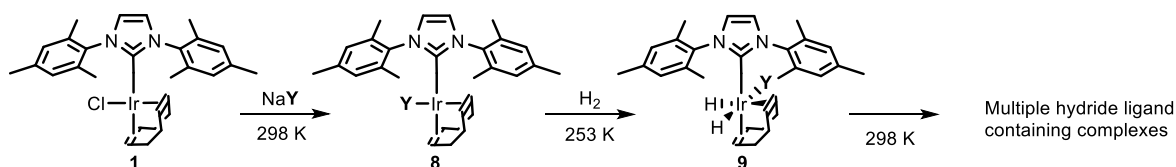
3.2 Optimising ^{15}N polarisation of $[\text{NO}_2]^-$

In order to be useful as a probe for either reaction monitoring or MRI, the maximum signal intensity must be delivered by SABRE for $[\text{NO}_2]^-$. This will ensure optimum detectability of the signal, minimising the amount of labelled material needed. This in turn lowers the cost of the experiments and makes the technique more accessible and useful. The optimisation techniques used here have been outlined earlier, but they must be repeated for every substrate to ensure maximum efficiency in polarisation transfer in each case.

3.2.1 Understanding the formation of SABRE active species

Firstly, the reaction of **1** with $[\text{NO}_2]^-$ (**Y**) was examined by NMR spectroscopy. An excess of 25 equivalents of **Y** in the form of $\text{Na}^{15}\text{NO}_2$ (>98% labelling purity, see experimental) compared to **1** was added to a 5 mM solution of **1** in methanol- d_4 and the sample degassed as outlined in the experimental. This saw the complete conversion of **1** into a new species $[\text{Ir}(\text{COD})(\text{IMes})(\text{Y})]$ (**8**) at 298 K. This conversion was evidenced by the detection of a ^{15}N NMR signal for the bound **Y** ligand at δ 490.70 at 253 K. The corresponding chemical shift for the ^{15}N NMR signal of free **Y** appeared in this NMR spectrum at δ 611.80, showing the large difference in chemical shift that results from ligation. Further evidence for the formation of **8** was gained through several complex 2D ^1H and ^{13}C NMR measurements. These revealed a ^{13}C NMR peak for the carbene carbon of the NHC ligand in **8** at δ 174.2, shifted from the corresponding peak in **1** which appears at δ 179.10. Furthermore, ^1H NMR signals were detected and assigned to its COD ligand at δ 3.79 and 3.50, which are again shifted from those of **1**, where they appear at δ 4.03 and 3.11. These new resonances satisfy the necessary four ligands around iridium, full characterisation data for **8** is presented in Section 7.4.19. Complex **8** was then exposed to a 4-bar pressure of H_2 in a dry ice / acetone bath at around 195 K; this prevented any reaction taking place until the sample thawed to 253 K in the spectrometer. Once at 253 K, the oxidative addition of H_2 was observed to take place and form the product $[\text{Ir}(\text{H})_2(\text{COD})(\text{IMes})(\text{Y})]$ (**9**) (Scheme 3.1). **9** proved to exist in equilibrium with **8**, with the ratio being 1 : 0.6 (**8** : **9**) after 20 min at 253 K. Product **9** was identified by NMR spectroscopy. Firstly, it was found to exhibit two ^1H NMR signals for its two hydride ligands at δ -18.77 and -14.17. The hydride ligand with a ^1H NMR signal at δ -18.77 showed a strong *trans* coupling of 23.1 Hz to a **Y** ligand which yielded a ^{15}N NMR signal

at δ 476.00 and this is indicative of N-binding. **9** yielded four inequivalent η^2 - η^2 -alkene CH resonances at δ 4.95, 4.60, 4.28 and 4.21. Finally, its NHC ligand was identified by a ^{13}C NMR signal located by ^1H - ^{13}C HSQC measurements with a chemical shift of δ 154.78. These six sets of NMR signals fully satisfy the coordination sphere in **9**. Full characterisation data for **9** is presented in Section 7.4.20.



Scheme 3.1 Reaction of $\text{IrCl}(\text{COD}(\text{IMes}))$ (**1**) with NaNO_2 (**Y**) and H_2 in methanol- d_4 leads to **9**.

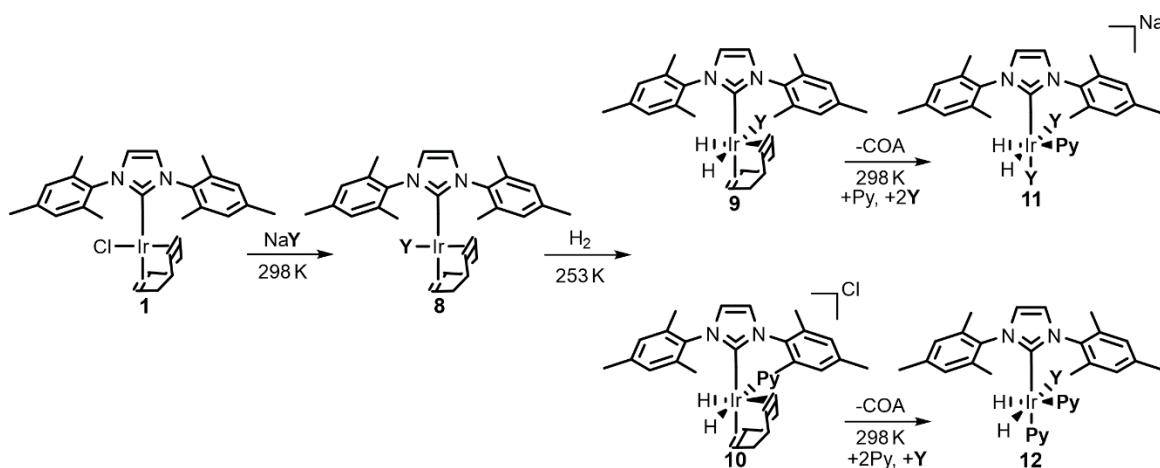
Upon warming this sample to 298 K for 20 mins, the formation of multiple hydride containing products was evident, which led to a very complicated ^1H NMR spectrum in the hydride region. Shaking the sample in the fringe field of the magnet with 4 bar of p - H_2 revealed PHIP activity for these hydride ligands thereby showing these complexes exchange hydrogen and so should therefore be SABRE active. Excitingly, shaking the sample in a $-0.50\ \mu\text{T}$ field for 10 s with 4 bar of fresh p - H_2 gave a hyperpolarised response for the free **Y** ^{15}N NMR signal at δ 611.8 equating to a 134 ± 18 -fold enhancement at 9.4 T ($\sim 0.04\%$ polarisation).

In order to simplify the spectrum, and identify any major SABRE active species the sample was left overnight to give time for any thermodynamically preferred products to form. Unfortunately, this method was unsuccessful and led to the formation of SABRE inactive complexes only, which were not characterised. Co-ligand addition, although unsuccessful in the last chapter due to slowing the exchange rate, was then used to form stable SABRE active complexes in conjunction with a weakly binding ligand species.

3.2.2 Co-ligand approach to form a stable SABRE active complex for $^{15}\text{NO}_2^-$ (**Y**)

This method was first implemented using pyridine (**D**) as the co-ligand. A sample was therefore prepared using 2 mg of **1**, with 5 equivalents of **Y** and 3 equivalents of **D** in 0.6 mL of methanol- d_4 and analysed using NMR spectroscopy. This again showed the formation of **8** thereby demonstrating that **Y** must outcompete pyridine for binding to the iridium centre. Upon exposure of this sample to a pressure of 4 bar H_2 at a temperature of 253 K further reaction occurs which led to a mixture of two dihydride species. The first

of these was identified as being the neutral **9**. The second species was found to be $[\text{Ir}(\text{H})_2(\text{COD})(\text{IMes})(\text{D})]\text{Cl}$ (**10**) of Scheme 3.2, and it exhibited hydride ligand ^1H NMR signals at δ -12.07 and -17.30. Alongside the hydride ligand signals for **10**, four unique COD resonances are located at δ 4.98, 4.69, 4.25 and 3.68 that proved to be located in the same C_8 ligand through complex ^1H TOCSY, COSY AND NOSEY measurements. Low temperature $^1\text{H} - ^{15}\text{N}$ HMQC measurements revealed a bound signal for a pyridine ligand, with a ^{15}N NMR signal at δ 220.63. This ^{15}N NMR resonance revealed a further coupling to a ^1H NMR resonance at 8.41 hence confirming this ligand was in fact pyridine and not **Y**. In using a coupling constant of around 20 Hz for a $^1\text{H} - ^{13}\text{C}$ HMQC experiment it proved possible to see a coupling between the hydride ligand ^1H NMR signals and the carbene ^{13}C NMR resonance at δ 153.80 hence again satisfying the coordination sphere around the iridium centre. Species **10** is known,¹⁴⁶ and its formation suggests that $[\text{NO}_2]^-$ binding to the Ir (III) centre is now weaker than that to the precursor complex.



Scheme 3.2 Reaction of $\text{IrCl}(\text{COD}(\text{IMes}))$ (**1**) with NaNO_2 (NaY), pyridine (**D**) and H_2 in methanol- d_4 leads to several dihydride derived reaction products.

This NMR sample was then allowed to warm to room temperature, which saw the hydrogenation of COD and the formation of two dihydride containing products; $\text{Na}[\text{Ir}(\text{H})_2(\text{IMes})(\text{D})(\text{Y})_2]$ (**11**) and $[\text{Ir}(\text{H})_2(\text{IMes})(\text{D})_2(\text{Y})]$ (**12**). Clearly, the co-ligand approach was successful and these products proved to be thermally stable as after 24 hrs at room temperature they retained their initial 15 : 85 ratio respectively. No signals from any other species were observed, suggesting the catalyst degradation that occurred before is prevented. When analysed by low temperature multinuclear NMR the minor product **11** showed a very high intensity cross-peak in a $^1\text{H} - ^{15}\text{N}$ HMQC experiment revealing a ^{15}N resonance for bound **Y** at δ 509.20 coupled to a hydride ligand ^1H NMR resonance at δ -23.27 with a J_{NH} coupling constant of 29.00 Hz. In the same measurement, a bound pyridine was located through a strong *trans* J_{NH} coupling of 20.00 Hz between the ^1H

NMR peak for the hydride ligand at δ -22.05 and a bound ^{15}N NMR peak at δ 254.50. When 2D ^1H NMR NOESY measurements were carried out on this sample a second pyridine ligand could not be located, however, nOe connections were seen from the hydride ligands to a ^1H NMR signal at δ 2.11 located to be the methyl protons of the mesityl arms of the carbene ligand. This resonance showed a further nOe connection to a ^1H NMR signal to an imidazole CH resonance at δ 7.00. ^1H - ^{13}C HMQC measurements were carried out which revealed a cross peak to a ^{13}C NMR signal at δ 154.16. A direct acquisition ^{15}N NMR experiment was carried out on the sample, which revealed a signal at δ 483.20 assigned to an axial **Y**.

Complex **12** showed hydride ligand signals at δ -22.53 and -21.30, which proved to share a common coupling constant of 8.00 Hz for the J_{HH} splitting. Low temperature ^1H - ^{15}N HMQC measurements at 253 K revealed a strong *trans* coupling to a ^{15}N NMR signal for each hydride confirming the presence of two N-donor ligands in the equatorial plane. For complex **12**, one of these ^{15}N NMR signals exhibits very high intensity due to the labelling in **Y**, and so it was deduced that the hydride at δ -21.30 is *trans* to **Y** whose ^{15}N NMR signal appeared at δ 511.10 and showed a J_{NH} coupling of 29.00 Hz. The other hydride ligand, with its ^1H NMR signal at δ -22.53, showed a weak cross-peak to the ^{15}N of its *trans* ligand in comparison. This was found exhibit a *trans* coupling of 20 Hz to a pyridine ligand with its ^{15}N NMR signal at δ 258.30. This ^{15}N NMR signal coupled further to another ^1H NMR signal at δ 8.62 confirming its origin in the bound pyridine ligand. In the axial plane, a ^{13}C NMR signal was located for a carbene carbon at δ 155.66. Two dimensional ^1H NMR NOESY measurements revealed a second pyridine ligand, located to be in the axial plane also due to nOe connections from a ^1H NMR signal at δ 8.24 to both hydride ligands on the complex. The ^1H NMR signal at δ 8.24 revealed a coupling to a ^{15}N NMR signal at δ 237.20 showing again the characteristic change in chemical shift associated with ligation and fully completing the coordination sphere around the iridium centre. Full characterisation data are shown in Sections 7.4.22 and 7.4.23. It is noted that no evidence for the formation of the known *tris* pyridine complex $[\text{Ir}(\text{H})_2(\text{IMes})(\text{D})_3]\text{Cl}$ was observed showing again the ability of **Y** to outcompete the pyridine ligand.

This sample was then shaken in a field of -0.50 μT with 4 bar pressure $p\text{-H}_2$ and then transferred to a 9.4 T magnet for ^{15}N NMR signal acquisition. A large signal enhancement of 880 ± 36 -fold for the free **Y** ^{15}N NMR signal was observed, which corresponds to $\sim 0.29\%$ polarisation. SABRE was also observed for the ^{15}N NMR signal of free pyridine showing a 172 ± 8 -fold signal gain equating to $\sim 0.06\%$ polarisation. This

spectrum also readily showed all bound **Y** resonances in species **11** and **12** in just a single scan acquisition. Repeating the experiment using a 6 mT PTF and acquiring a ^1H NMR spectrum instead revealed PHIP enhanced hydride signals for the species **11** and **12**, but did not show any evidence of an enhanced signal at δ -22.7 for the previously mentioned $[\text{Ir}(\text{H})_2(\text{IMes})(\text{pyridine})_3]\text{Cl}$. Hence if it does form, it must be in very minor quantities. SABRE hyperpolarisation was also quantified for the ^1H NMR resonances of free pyridine as $\sim$ 230, 60, and 150-fold for its *ortho*, *meta*, and *para* positions respectively. As hyperpolarising pyridine was not the aim here, these measurements were not repeated in triplicate and therefore no error value is given for the enhancements. These data therefore show the success of the co-ligand approach in forming a stable SABRE catalyst containing **Y**.

3.2.3 ^{15}N Polarisation of free **Y** as a function of polarisation transfer field with pyridine as a co-ligand.

As demonstrated in the previous chapter the PTF can be essential in achieving optimum polarisation transfer to the substrate. To ensure optimum polarisation transfer was being achieved using a mixture of catalyst species **11** and **12** a field sweep was carried out on a sample initially containing 2 mg of the pre-catalyst **1** with 25 equivalents of **Y** and 4 equivalents of the co-ligand pyridine. This mixture formed in the same ratio of 15 : 85 seen previously. Increasing the concentration of **Y** did though lower the enhancement achieved when shaking at the previous field of $-0.50\ \mu\text{T}$. This is likely due to the increased competition for the catalyst meaning a smaller proportion of the available substrate can be hyperpolarised in a given time with a set amount of $p\text{-H}_2$ and catalyst before relaxation starts to take effect. This effect is explored for **Y** later in this chapter, but has already been exemplified for **X** in the previous chapter. The sample was shaken for 10 s at a field ranging from -1.00 to $1.00\ \mu\text{T}$ (10 to -10 mG) before transfer to a 9.4 T spectrometer for ^{15}N NMR signal acquisition. The data for this study are presented in Figure 3.1 and show a clear maximum signal enhancement for the free **Y** ^{15}N resonance is achieved at $+0.50$ and $-0.30\ \mu\text{T}$ (5 and -3 mG in Figure 3.1).

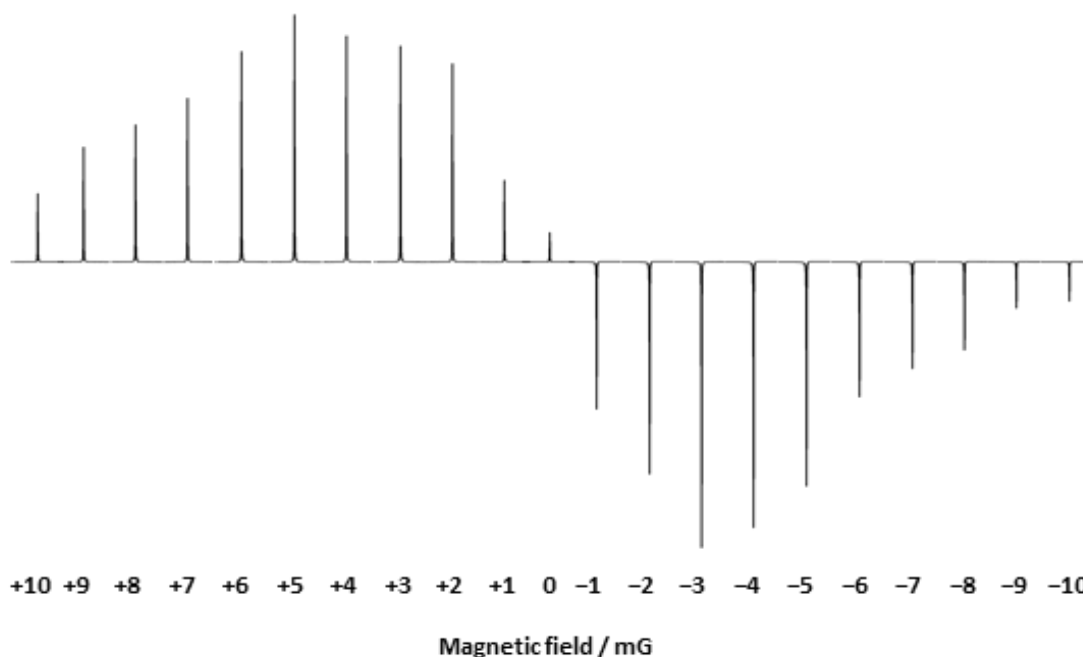


Figure 3.1 ^{15}N NMR signal acquired at 9.4 T as a function of polarisation transfer field used. The sample probed consisted of 2 mg of **1** with 25 equivalents of **Y** and 4 equivalents of pyridine as co-ligand. At each PTF the sample was shaken with 4 bar of fresh $p\text{-H}_2$ for 10 s. The Figure shown overlaid ^{15}N NMR spectra and thus the y axis here is an arbitrary signal intensity.

The signal enhancement data shown here at $+0.50$ and -0.30 μT equate to a 1948 ± 118 and 2054 ± 87 -fold signal gain, respectively. Further probing around the field of -0.3 μT revealed a slight increase again at -0.35 μT to 2329 ± 103 -fold enhancement equating to $0.77 \pm 0.03\%$ polarisation of the sample. Higher accuracy than this could not be achieved using the *mu*-metal shield set-up described in the experimental, so this field was taken as the best conditions achievable for the sample even though it may not actually be the best PTF.

3.2.4 ^{15}N Polarisation as a function of co-ligand used

In order to form complexes **11** and **12** efficiently **Y** must be able to outcompete the co-ligand being used in binding to the iridium centre. It was therefore hypothesised that the co-ligand species being used would dramatically affect the efficiency of the SABRE process. A series of samples was made up consisting of 2 mg of **1** with 25 equivalents of **Y** and 4 equivalents of a co-ligand from Figure 3.2.

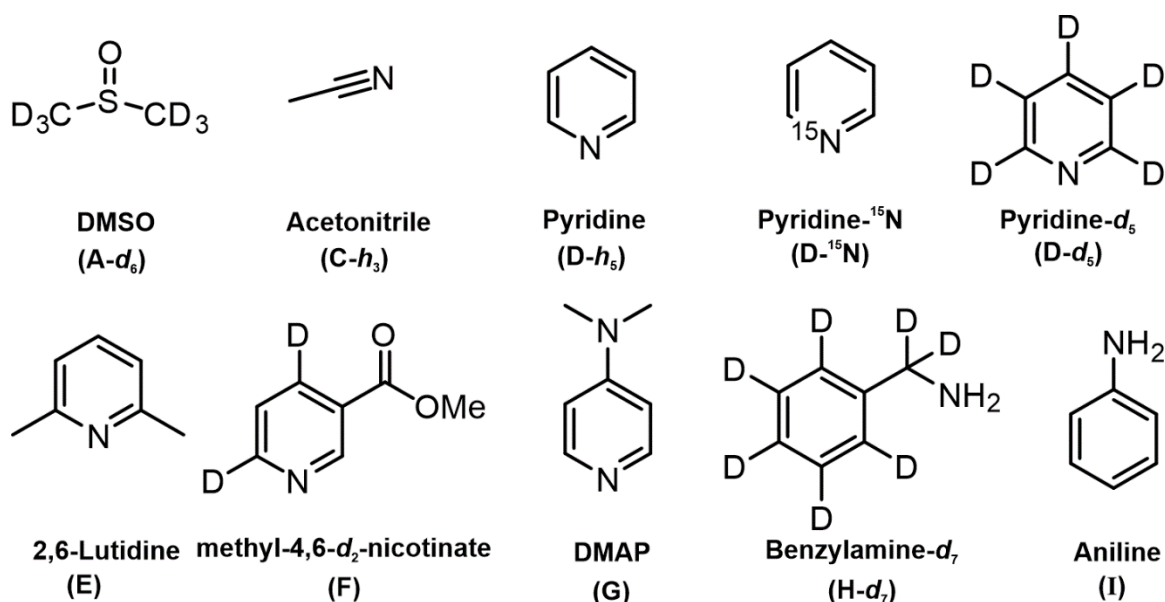


Figure 3.2 Chemical structures of co-ligands used. DMAP stands for dimethylaminopyridine and DMSO for dimethylsulfoxide.

The samples were then shaken in a field of $-0.35\ \mu\text{T}$ with a pressure of 4 bar of $p\text{-H}_2$ before transfer to a 9.4 T spectrometer for ^{15}N NMR signal acquisition. As expected, the speciation of the SABRE active complex did change with each co-ligand species used. This in turn had a major impact on the signal enhancement obtained. Figure 3.3 shows the ^{15}N NMR signal enhancement at 9.4 T as a function of co-ligand used from the selection in Figure 3.2. These data are also displayed in Table 3.1 as polarisation values.

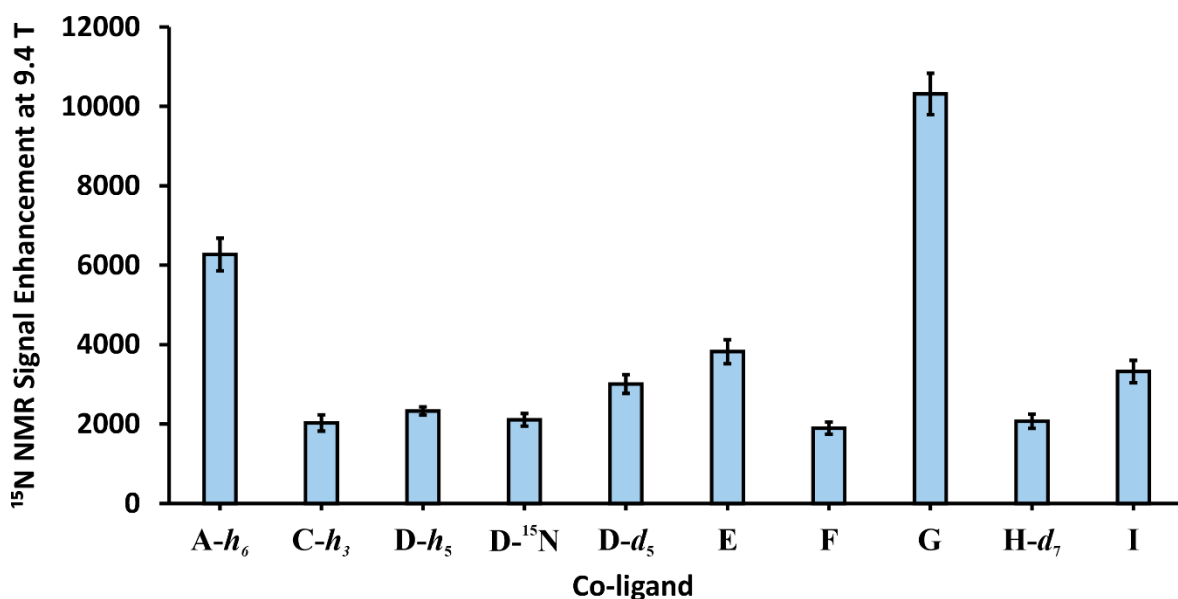


Figure 3.3 ^{15}N NMR signal enhancement at 9.4 T (fold) as a function of co-ligand used from Figure 3.2. All samples consisted of 2 mg of **1** with 25 equivalents of **Y** and 4 equivalents of the corresponding co-ligand used. Samples were shaken for 10 s in a $-0.35\ \mu\text{T}$ PTF under a pressure of 4 bar $p\text{-H}_2$.

Table 3.1 ^{15}N NMR signal enhancement at 9.4 T and ^{15}N polarisation of the free **Y** for each co-ligand used (leading to Figure 3.2).

Co-ligand	Enhancement / fold	Polarisation / %
A-d₆	6270 $\pm$ 410	2.05 $\pm$ 0.13
C-h₃	2030 $\pm$ 210	0.66 $\pm$ 0.07
D-h₅	2330 $\pm$ 100	0.76 $\pm$ 0.03
D-^{15}N	2110 $\pm$ 160	0.69 $\pm$ 0.05
D-d₅	3010 $\pm$ 240	0.98 $\pm$ 0.08
E	3820 $\pm$ 300	1.25 $\pm$ 0.10
F	1890 $\pm$ 150	0.62 $\pm$ 0.05
G	10310 $\pm$ 520	3.37 $\pm$ 0.17
H-d₇	2070 $\pm$ 180	0.68 $\pm$ 0.06
I	3320 $\pm$ 280	1.09 $\pm$ 0.09

First comparing the isotopologues of pyridine (**D**), it was noted that all three samples formed the same ratio of 15 : 85 for complexes **11** and **12** as expected. This confirmed that the change in behaviour observed is not due to catalyst speciation but due to the different isotopic labelling of **D**. The ^{15}N -labelled pyridine (**D- ^{15}N**) co-ligand yields a lower ^{15}N NMR signal enhancement for free **Y**, at 2110 $\pm$ 160-fold. This fall in enhancement was attributed to spin dilution caused by having more ^{15}N nuclei available to accept polarisation during the transfer process. This was confirmed by the ^{15}N NMR resonance for free pyridine at δ 301 now exhibiting a 1560 $\pm$ 120-fold signal gain, an improvement over the unlabelled sample. In contrast, utilising pyridine-*d*₅ (**D-d₅**) improved the polarisation transfer to **Y** yielding a ^{15}N NMR signal enhancement of 3010 $\pm$ 240-fold at 9.4 T. This improvement is likely a result of decreased relaxation due to the lower γ of deuterium now reducing the spin-spin dipole interaction. This improvement could also be due to reduced polarisation loss to the ^1H nuclei of **D**, thereby reflecting a decrease in spin dilution, although it is noted that the polarisation transfer efficiency to ^1H will be very low at this PTF.

While testing the other samples it was observed that they all formed a mixture of complexes, with the majority only forming analogues of **11** and **12**, where pyridine is replaced by the corresponding co-ligand of Figure 3.2. The two exceptions to this behaviour were DMSO-*d*₆ (**A-d₆**) and 2,6-lutidine (**E**). The sample containing **E** was very slow to activate with 48% of **9** still being present after 2 hours, all other samples showed no **9** present at this point. This observation suggests that the formation of an analogue of

10 is essential in transitioning to SABRE active **11** and **12**. Due to the hindered binding of **E** it may be more difficult to form **10_E** and therefore explains the slow conversion. For the sample containing **A-*d*₆**, a new hydride containing complex was detected alongside **11A-*d*₆** and **12A-*d*₆** with a hydride ligand ¹H NMR signal at δ -22.32. Through low temperature ¹H-¹⁵N HMQC measurements this hydride ligand was found to couple to a ¹⁵N NMR signal at δ 502 for bound **Y** with a J_{HN} coupling constant of 27.6 Hz. This complex was assigned to an isomer of **12A-*d*₆** with both bound **Y** ligands in the equatorial plane; this will be known as **13**. Complexes **11A-*d*₆** and **12A-*d*₆** and **13** are fully characterised in Sections 7.4.24 to 7.4.26. Note that characterisation measurements were undertaken with high concentration samples using *protio-A*. Table 3.2 shows the % proportions of each complex in the mixture for all co-ligands utilised.

Table 3.2 *The relative proportion of each complex species present in the mixture for each corresponding co-ligand shown as a percentage of the overall amount of iridium in solution.*

Co-ligand	Complex Proportion / %			
	9	11	12	13
A-<i>d</i>₆	-	60	7	33
C-<i>h</i>₃	-	-	100	-
D-<i>h</i>₅	-	15	85	-
¹⁵N-D	-	15	85	-
D-<i>d</i>₅	-	15	85	-
E	48	2	48	-
F	-	66	34	-
G	-	5	95	-
H-<i>d</i>₇	-	50	50	-
I	-	3	97	-

From these data, no direct trend is observed between catalyst speciation and ¹⁵N polarisation level achieved for free **Y**. The highest ¹⁵N polarisation of **Y** achieved was for that of the sample containing the co-ligand **G** at $3.37 \pm 0.17\%$, this delivered a ¹⁵N NMR signal enhancement of 10313 ± 522 fold at 9.4 T. This sample showed a 5 : 95 ratio for complex **11_G** : **12_G**. The data here is more complex than indicated, and so it should be noted that a multitude of other factors will feed into the SABRE success of these samples. This includes; the exchange rate of the substrate and hydride ligands, the J_{HN} coupling between the hydride ligand and the ¹⁵N of **Y**, and the chemical shifts of both the hydride ligand ¹H NMR signal and the ¹⁵N NMR signal of **Y**, and so catalyst speciation is not an isolated factor. Hence no clear trend is observed.

As the use of **G** proved optimal, this co-ligand was taken forward for further study. It was hypothesised that the equilibrium between complexes **11_G** and **12_G** should depend on the amount of amount of **G** in solution and this behaviour can be probed to see if further ^{15}N polarisation gain can be made by forming **12_G** in greater proportions as this will give information as to whether **11_G** or **12_G** is the better catalyst. To test this hypothesis a series of samples containing 2 mg of **1** with 25 equivalents of **Y** and varying equivalents of **G** from 3 to 20-fold compared to the Ir pre-catalyst were made. These were then activated and shake and drop experiments carried out using a PTF of $-0.35\ \mu\text{T}$ with a 4 bar pressure of *p*-H₂ before transfer to a 9.4 T magnet for ^{15}N NMR signal acquisition. Table 3.3 shows the equivalents of **G** used, relative proportions of **11_G** and **12_G** and the ^{15}N NMR signal enhancement and polarisation achieved for each sample.

Table 3.3 ^{15}N NMR signal enhancement at 9.4 T and ^{15}N polarisation of the free **Y** for varying equivalents of **G** compared to Ir shown alongside the relative proportions of **11_G** and **12_G** in solution.

Equivalents of G	11_G : 12_G	Enhancement / fold	Polarisation / %
3	1 : 8	9090 $\pm$ 420	2.97 $\pm$ 0.14
4	1 : 17	10310 $\pm$ 520	3.37 $\pm$ 0.17
6	1 : 24	11020 $\pm$ 510	3.60 $\pm$ 0.17
8	1 : 24	12040 $\pm$ 520	3.94 $\pm$ 0.17
10	1 : 24	12080 $\pm$ 570	3.95 $\pm$ 0.19
15	1 : 24	11890 $\pm$ 490	3.89 $\pm$ 0.16
20	1 : 24	9110 $\pm$ 420	2.98 $\pm$ 0.14
25	1 : 24	8950 $\pm$ 390	2.95 $\pm$ 0.13

From these data it is clear that increasing the concentration of **G** favours the formation of **12_G**, with the proportion of the complex increasing to 96% when using six equivalents of **G**; higher concentrations of **G** do not affect the equilibrium further with the proportions of **11_G** and **12_G** plateauing at 1 : 24, respectively. Increasing concentrations of **G** past 10 equivalents also saw the formation of a new complex, assigned to be $[\text{Ir}(\text{H})_2(\text{IMes})(\text{G})_3]\text{Cl}$ (**14**), which gave rise to a single hydride ligand ^1H NMR resonance at δ -22.76 that coupled to a ^{15}N NMR resonance at δ 212.80. Full characterisation for **14** can be found in Section 7.4.27. To test if **14** was present at smaller equivalents of **G** a shake and drop experiment was carried out shaking in the fringe field of the magnet with 4 bar of *p*-H₂ for each sample. This revealed a low intensity hyperpolarised signal for the hydride ligand signal of **14** when six or eight equivalents of **G** were used, but could not be observed when three or four equivalents were used, suggesting that **14** is now in too low a proportion to detect under these conditions. It is obvious that **14** cannot polarise **Y** and so is detrimental to the SABRE of **Y** as it will

consume $p\text{-H}_2$ with no useful effect. This is evidenced by the fall in polarisation of **Y** as higher equivalents of **G** are used and more of inactive **14** is formed. Therefore, from these data it is evident the optimum amount of **G** to be used is somewhere between six and ten equivalents.

As the method of using deuterated **D-d₅** was effective in improving the ^{15}N polarisation of **Y** it was hypothesised the same method could be used here using a deuterated version of **G**. To test this, a novel synthesis was carried for a selectively deuterated **G-d₂**. It was hypothesised that it is only necessary to deuterate the *ortho* protons of **G** as these are the protons that will couple strongest to **Y** and so increase the relaxation rate at the catalyst. As this ligand is not commercially available it was synthesised *via* H/D exchange using D_2O under microwave irradiation as outlined in literature.¹⁴⁷ To test the **G-d₂** co-ligand, a sample containing 2 mg **1**, 25 equivalents of **Y** and 6 equivalents of **G-d₂** in methanol- d_4 was shaken under a pressure of 4 bar $p\text{-H}_2$ for 10 s in a $-0.35\ \mu\text{T}$ PTF before ^{15}N NMR signal acquisition at a 9.4 T field. This yielded an enhanced ^{15}N NMR signal for free **Y** with a 13811 ± 483 fold signal gain over a thermally acquired response. This signal enhancement corresponds to a $4.56 \pm 0.16\%$ ^{15}N polarisation. This shows an improvement over the non-deuterated **G** showing that deuterium labelling of the *ortho* protons to make **G-d₂** was successful in this application.

3.2.5 ^{15}N Polarisation as a function of iridium pre-catalyst used

As previously exemplified, the efficiency of polarisation transfer can be dramatically improved by altering the steric and electronic properties of the carbene ligand on the pre-catalyst used.⁵⁹ To demonstrate the applicability of this method to the system used to hyperpolarise **Y** here, a series of samples were made using various pre-catalysts analogous to **1** where IMes was replaced with NHCs with a variety of steric and electronic properties. The pre-catalysts utilised are presented in Figure 3.4.

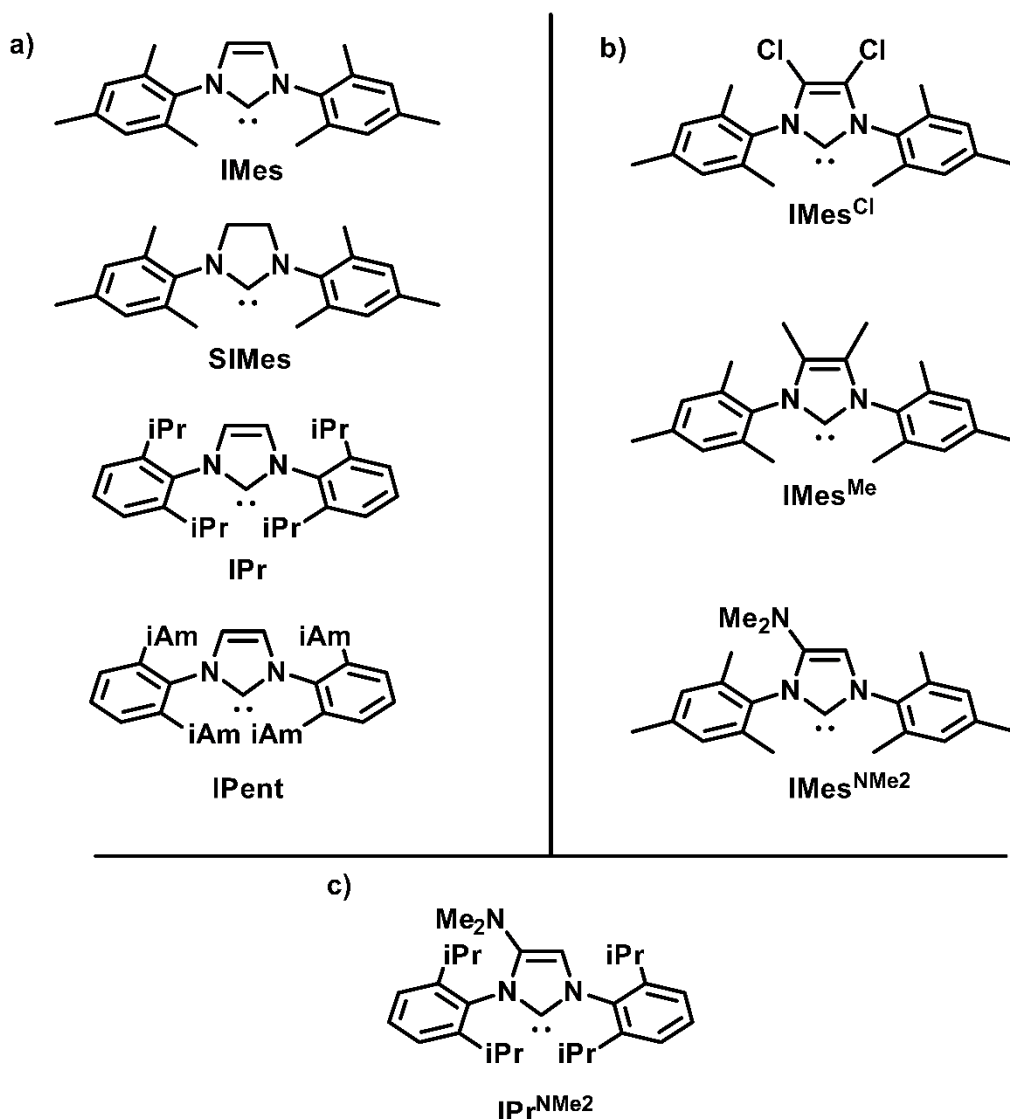


Figure 3.4 NHC ligands used in the pre-catalysts $[\text{IrCl}(\text{COD})(\text{NHC})]$ for the optimisation of ^{15}N hyperpolarisation for **Y**. a) Increasing steric bulk shown from top to bottom. b) Increasing electron donation from top to bottom. c) Combination of increased steric bulk and increased electron donation. Note that *iAm* refers to the substituent chain $\text{CH}(\text{CH}_2\text{CH}_3)_2$.

In order to test the efficiency of these pre-catalysts, samples were made containing 2 mg of each pre-catalyst 25 equivalents of **Y** and 6 equivalents of **G-d₂**. The samples were then exposed to a pressure of 4 bar H_2 and examined using ^1H NMR spectroscopy. All pre-catalysts used gave rise to a complex analogous to that of **12G-d₂** as evidenced by the characteristic hydride ligand ^1H NMR signals being observed in each case; integrating to >95% of all hydride ligand containing complexes for each pre-catalyst used. To test the SABRE efficiency the samples were then shaken under a 4 bar atmosphere of *p*- H_2 for 10 s and in a $-0.35\ \mu\text{T}$ PTF. The samples were then transferred to a 9.4 T magnet for ^{15}N NMR signal acquisition. Figure 3.5 shows the ^{15}N NMR signal enhancement of free **Y** at 9.4 T as a function of the pre-catalyst utilised.

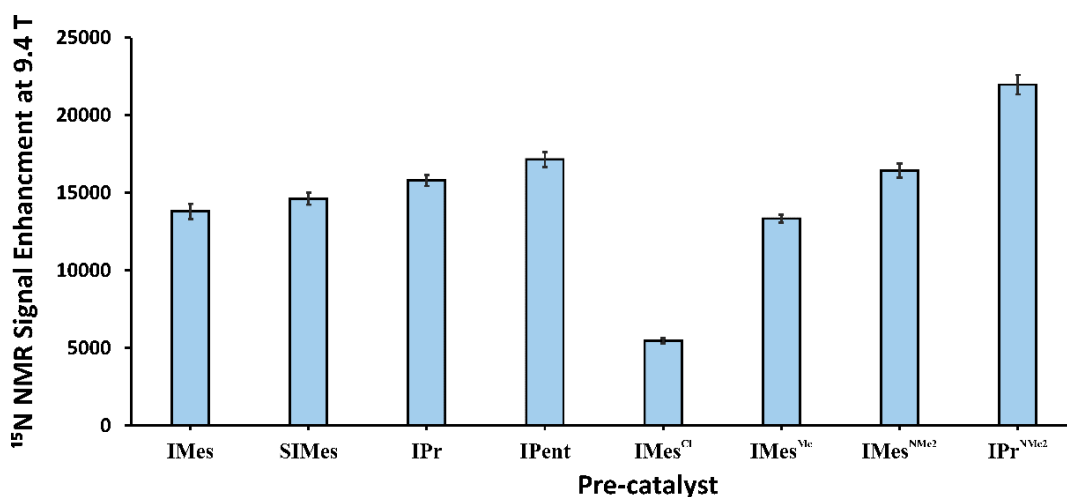


Figure 3.5 Effect on ¹⁵N signal enhancement at 9.4 T caused by changing the steric and electronic properties of the SABRE pre-catalyst used. The samples all contained 2 mg of the respective pre-catalyst (Figure 3.4), 25 equivalents of **Y** and 6 equivalents of **G-d₂**. The samples were shaken at a field of -0.35 μT for 10 s with 4 bar pressure of *p*-H₂.

These data show a clear trend of increased ¹⁵N NMR signal enhancement with increased steric bulk. The steric bulk of each pre-catalyst was quantified by the magnitude of the % buried volume (%BurV) as described in the literature.^{104, 105, 148} Here, the maximum ¹⁵N NMR signal enhancement of 20340 ± 630 was observed using the **IPent** pre-catalyst shown in Figure 3.4. This corresponds to a 6.71 ± 0.21% polarisation. This increased steric bulk should promote ligand exchange and so infers the polarisation transfer efficiency increases with a faster exchange rate in this case. A similar deduction was then made by changing electronic parameters of the carbene ligand. It was found that the electron withdrawing **IMes^{Cl}**, that would have a slower exchange rate, lowered the polarisation transfer efficiency only achieving 5471 ± 184 fold enhancement on the ¹⁵N NMR signal for free **Y**. As expected, the electron donating **IMes^{NMe2}** achieved a better response of 16427 ± 439 fold enhancement. Combining the optimum conditions observed of increased steric bulk and electron donation to increase the rate of ligand exchange by deploying the **IPr^{NMe2}** catalyst was found to result in the highest ¹⁵N signal enhancement, at 21967 ± 628 fold corresponding to 7.18 ± 0.21% polarisation.

In order to aid in explaining these trends, the ligand exchange rates of **G-d₂** were determined using ¹H NMR exchange spectroscopy measurements as displayed in Table 3.4. All NMR spectra were collected at 298 K, probing the equatorially bound **G-d₂** ligand. Although this is not the ligand of interest, the EXSY measurements are much more easily undertaken on the more sensitive ¹H nuclei present here. Therefore, the information needed on trends in the ligand dissociation rate from **12** can be obtained much quicker than probing **Y** by ¹⁵N NMR spectroscopy.

Table 3.4 Rate of ligand dissociation for **G-d₂** at 298 K for the series of NHC ligands from Figure 3.4.

NHC	Rate of dissociation of G-d₂ at 298 K / s ⁻¹
IMes	0.13 ± 0.01
SIMes	0.43 ± 0.01
IPr	0.99 ± 0.01
IPent	3.42 ± 0.02
IMes^{Cl}	0.05 ± 0.01
IMes^{Me}	0.27 ± 0.01
IMes^{NMe2}	0.83 ± 0.01
IPr^{NMe2}	4.24 ± 0.01

These data satisfy the hypothesis that a faster exchange rate is preferred for maximum SABRE efficiency. The slowest exchange rate observed was for the worst performing pre-catalyst **IMes^{Cl}** and the fastest exchange rate for the best performing **IPr^{NMe2}**.

3.2.6 ¹⁵N Polarisation as a function of [NO₂]⁻ concentration

As discussed previously, the polarisation of **Y** can be improved by reducing spin dilution around the iridium centre. This can be achieved further by changing the concentration of **Y** used for the sample. It was hypothesised that if the concentration of **Y** did not dramatically affect the catalyst speciation then reducing the concentration of **Y** would lead to a higher level of ¹⁵N polarisation. This theory was put to the test by making a series of samples each containing 2 mg of **IPr^{NMe2}** with 6 equivalents of **G-d₂** and varying equivalents of **Y**. The data from this study is presented in Figure 3.6.

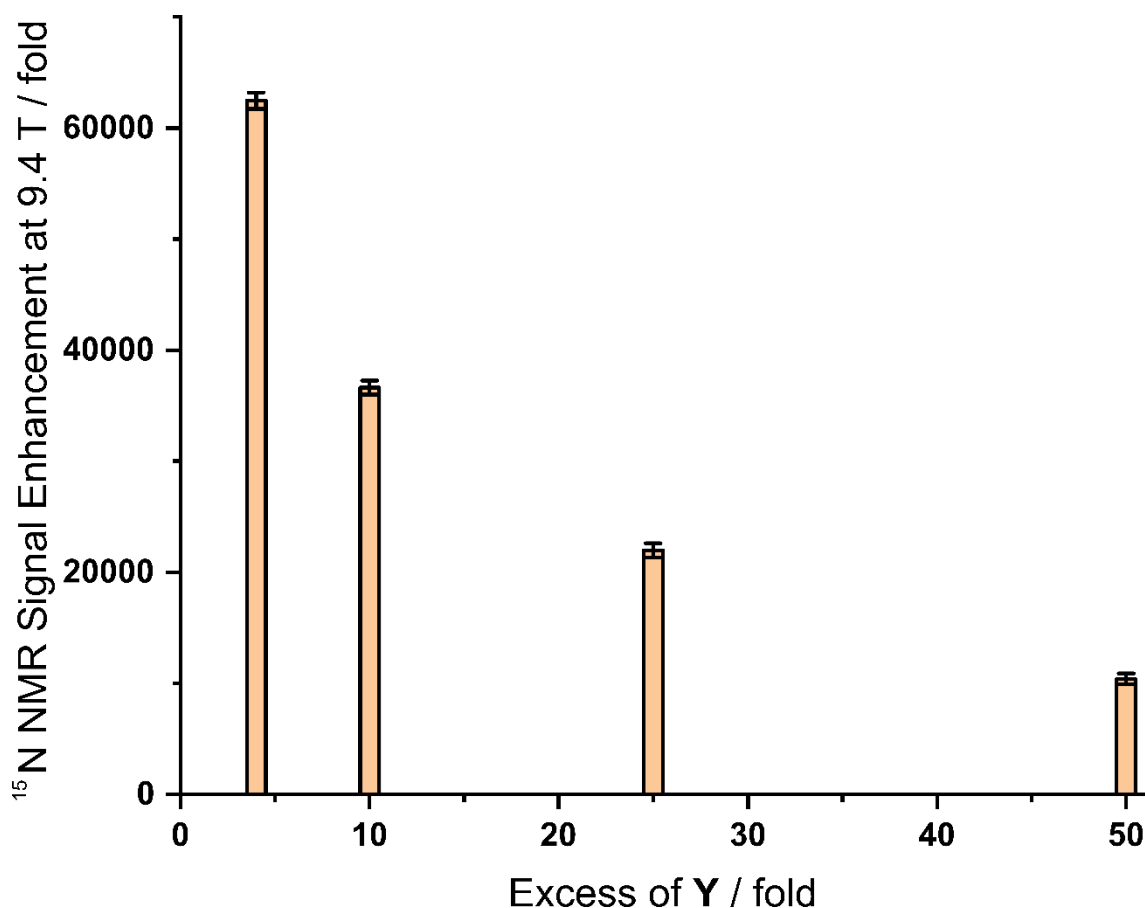


Figure 3.6 Effect of increasing substrate excess compared to Ir on the detected ¹⁵N response of Y shown as the resulting ¹⁵N signal enhancement at 9.4 T in fold gain. All samples were shaken for 10 s at a field of -0.35 μT with a pressure of 4 bar p-H₂.

As expected, at lower concentrations of Y the ¹⁵N NMR signal enhancement increases dramatically to 62470 ± 730 -fold due to the reduced spin dilution in the sample. This corresponds to a polarisation of $20.41 \pm 0.23\%$. Although the ¹⁵N polarisation achieved is now much higher, the overall ¹⁵N NMR signal intensity recorded is less than that achieved at the higher excesses of Y. Interestingly, all samples maintained a >95% proportion of **12G-d2** and so no catalyst speciation effects are taking place. It was hypothesised that decreasing the spin dilution even more could give yet another rise to higher ¹⁵N polarisation in the sample.¹⁴⁹⁻¹⁵¹ The issue with this method is Y needs to be present in sufficient proportion to occupy the necessary binding sites on the catalyst and also be in sufficient excess in solution to exchange also.

To overcome this, an unlabelled sample of Y can be used as the natural abundance of ¹⁵N is only 0.4%. This will decrease the spin dilution in the sample by a factor of 250 however the likelihood of a ¹⁵N binding will now be much lower. To try improve the ¹⁵N polarisation of Y further still, a sample was made containing only 0.1 mg of the pre-catalyst **IPr**^{NMe2} with the same 4 equivalents of Y and 6 equivalents of **G-d2**. When this

sample was shaken under 4 bar of *p*-H₂ for 10 s at a PTF of -0.35 μ T a ¹⁵N response was measured giving an 86100 ± 900 -fold enhancement measured at 9.4 T. This corresponds to a $28.42 \pm 0.31\%$ ¹⁵N polarisation. This phenomenon is likely due to increasing the excess of *p*-H₂ relative to the catalyst. As the *p*-H₂ is usually the limiting reagent it is hypothesised if the amount of *p*-H₂ can be increased in solution then the hyperpolarised response achieved will also increase in a linear relationship. These ideas of unlabelled samples and *p*-H₂ pressure will be explored in the next two sections.

3.2.7 ¹⁵N Polarisation as a function of *p*-H₂ pressure

In order to explore the effect of increasing *p*-H₂ pressure, a further sample containing 2 mg of the pre-catalyst **IPr**^{NMe₂} was prepared with 20 equivalents of **Y** and 6 equivalents of **G**. The sample was degassed (as outlined in the experimental Section 7.2.1), before being exposed to a pressure of 4 bar *p*-H₂ and left for 2 hours for sample activation. The sample was then degassed again and pressurised to only 1 bar *p*-H₂ before being shaken in the optimum PTF of -0.35 μ T for 10 s and transferred to a 9.4 T spectrometer for ¹⁵N NMR signal acquisition. After this the sample was then vented into a vacuum chamber while cooling using a slurry of dry ice and acetone to reduce solvent evaporation. The sample was then thawed before refilling with *p*-H₂. This process was repeated for three repeat shake and drop experiments before moving to the next pressure, increasing in 0.5 bar increments each time. Figure 3.7 shows the ¹⁵N NMR signal enhancement achieved for the sample up to 4 bar absolute pressure of *p*-H₂ (3 bar gauge pressure).

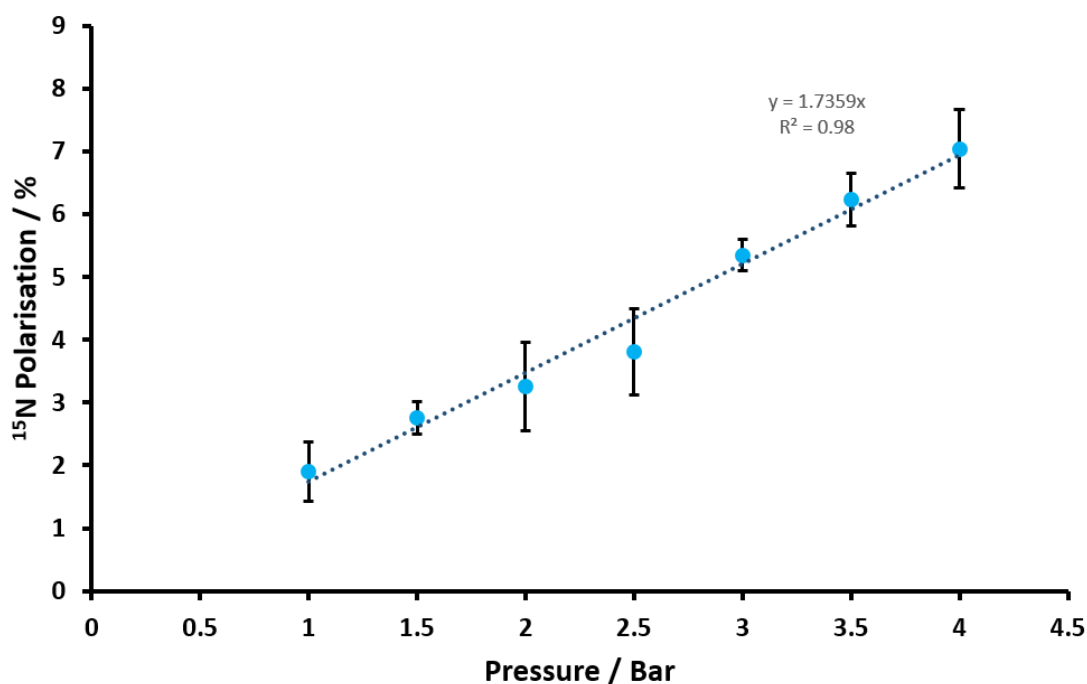


Figure 3.7 Effect of increasing $p\text{-H}_2$ pressure on the detected ^{15}N NMR response of **Y** shown as % polarisation for the ^{15}N nuclei. The sample was shaken for 10 s at a field of $-0.35\ \mu\text{T}$ for each pressure. This sample contained 2 mg of the pre-catalyst $\text{IPr}^{\text{NMe}_2}$ with 20 equivalents of **Y** and 6 equivalents of **G**.

These data show a clear linear trend between the pressure of $p\text{-H}_2$ utilised and the ^{15}N NMR response obtained. It is known that the concentration of the $p\text{-H}_2$ in solution relates to the pressure of $p\text{-H}_2$ in the headspace of the tube as described by Henry's law shown in Equation 3.1 where C is the concentration of dissolved gas, k is the Henry's Law constant and P is the partial pressure of the gas.

$$C = kP \quad (3.1)$$

This equation therefore shows a linear dependence between $p\text{-H}_2$ concentration in solution and the pressure in the tube. In order to improve the polarisation further it was necessary to utilise even higher pressures than this to maximise the amount of $p\text{-H}_2$ in solution. As the J. Youngs capped NMR tubes utilised for this study were only regulated to approximately 6 bar it was deemed unsafe to increase the pressure any further than the 4 bar already measured. To overcome this difficulty, a sample was made with half the concentration of catalyst, substrate and co-ligand, as lowering the catalyst and substrate concentration had already been confirmed to work in the previous section in improving ^{15}N polarisation yield by lowering the catalyst concentration to 0.25 mM. This method works by increasing the amount of $p\text{-H}_2$ in solution relative to the catalyst. This sample was then shaken for the same length of time and at the same PTF, under all pressures

tested previously to show a further increase in polarisation with an increased amount of $p\text{-H}_2$ relative to the catalyst, the data for which is shown in Figure 3.8.

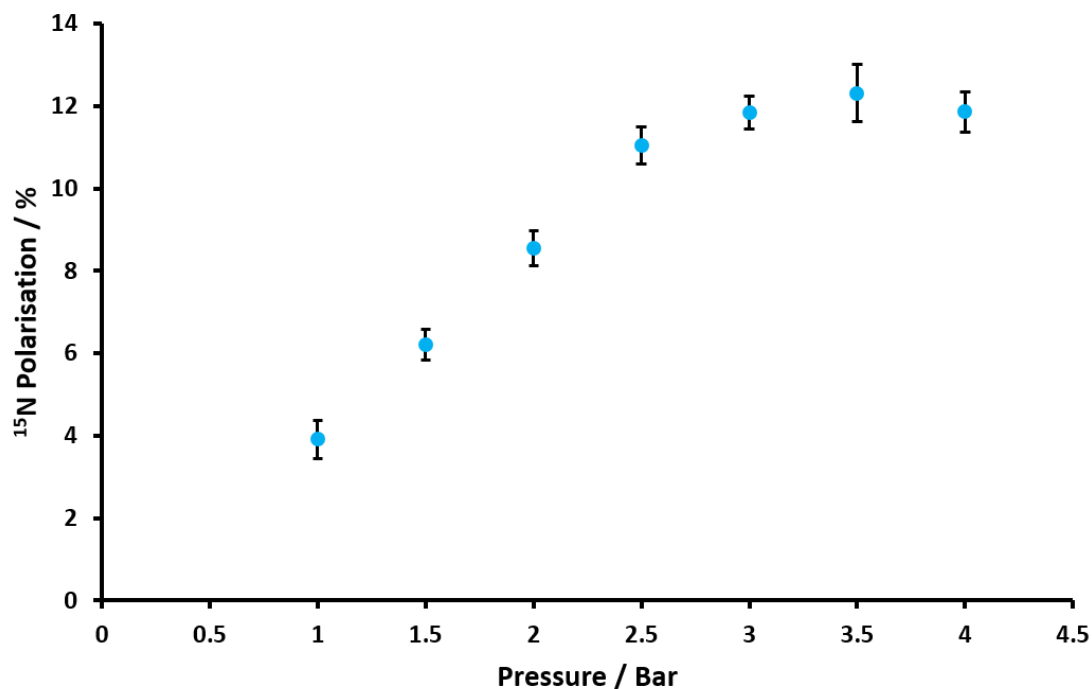
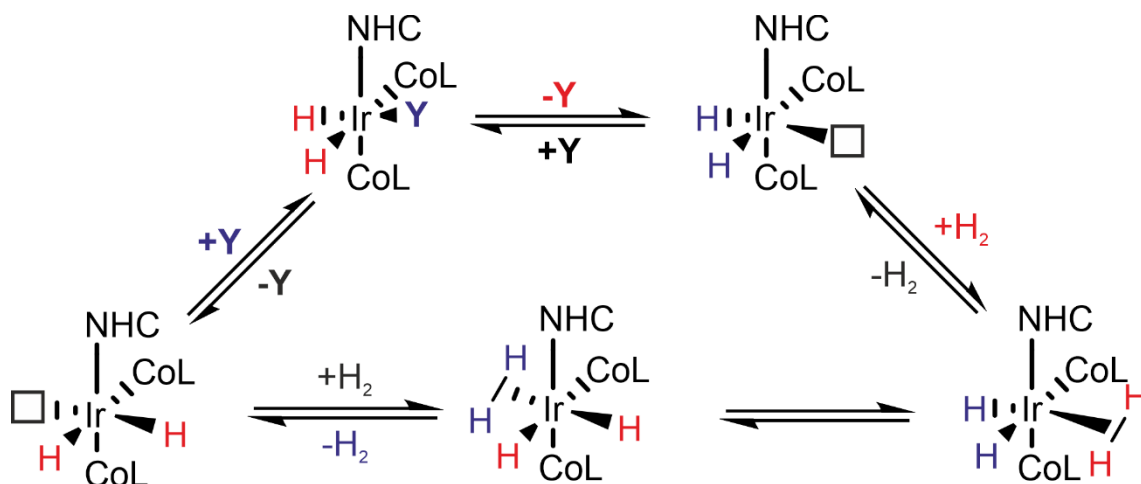


Figure 3.8 Effect of increasing $p\text{-H}_2$ pressure on the detected ^{15}N NMR response of **Y** shown as % polarisation for the ^{15}N nuclei. The sample was shaken for 10 s at a field of $-0.35\ \mu\text{T}$ for each pressure. This sample contained 1 mg of the pre-catalyst $\text{IPr}^{\text{NMe}_2}$ with 20 equivalents of **Y** and 6 equivalents of **G**.

By reducing the concentration of the catalyst and substrates by half it is now possible to observe the same linear region as observed in Figure 3.7, but now also a plateau in ^{15}N polarisation achieved at higher pressures. This behaviour has previously been reported in the literature for proton polarisation at much higher $p\text{-H}_2$ pressures by Adams *et al.*,¹⁵² but has not been observed before for ^{15}N . This plateau in ^{15}N polarisation at around 12 % represents a point in which the rebinding of $p\text{-H}_2$ in the SABRE mechanism is no longer limiting and a steady state of polarisation is reached under these conditions. Scheme 3.3 shows an example SABRE mechanism to help illustrate this step.^{123, 153}



Scheme 3.3 Generic SABRE mechanism where NHC represents any carbene ligands from Figure 3.4, coL represents a co-ligand from Figure 3.2 and Y is still $[\text{NO}_2]$.

In the scheme, vacant sites on the complex are presented as a box. In reality these vacant sites are rare and it is likely a methanol molecule will weakly coordinate to stabilise the complex.¹⁵⁴ The polarisation will not improve beyond this point unless the T_1 of the substrate is extended so a higher level of polarisation can be achieved before relaxation takes over. The alternative to this is to have more catalyst available, evidenced by the polarisation continuing to increase with the higher concentration sample in Figure 3.7. It is hypothesised that if a high-pressure tube were to be used the same plateauing trend would be observed for the sample in Figure 3.8 at higher pressures and lead to a higher overall ^{15}N polarisation.

3.2.8 Detection of unlabelled NaNO_2

As the optimisation of SABRE for Y had been so successful it was now deemed appropriate to test a sample at natural ^{15}N abundance. Currently ^{15}N isotopic labelling is very expensive and so running studies with the labelled material could be inaccessible to most. A sample was therefore made with the optimum ratio of substrates determined thus far but with natural abundance Y instead of ^{15}N labelled. This sample, containing 2 mg of the $\text{IPr}^{\text{NMe}_2}$ pre-catalyst, 4 equivalents of unlabelled Y and 6 equivalents of G-d₂ was shaken for 10 s in a field of $-0.35 \mu\text{T}$ under a pressure of 4 bar $p\text{-H}_2$. The ^{15}N response was then compared with an external ^{15}N labelled sample to calculate a signal enhancement. The enhancement was revealed to be a staggering 115592 ± 1282 -fold ^{15}N NMR signal gain corresponding to a $38.1 \pm 0.42\%$ polarisation. This signal gain achieved confirms the hypothesis outlined previously that reduced spin dilution boosts the ^{15}N polarisation even higher. It is noted here that this sample, although having a higher degree

of its ^{15}N nuclei hyperpolarised, is less useful than a labelled sample as there is less ‘bulk polarisation’ and so less hyperpolarised nuclei to observe. It does, however, show that these high levels of polarisation for **Y** are achievable if SABRE can be scaled into a larger sample volume to allow this increase in polarisation to be utilised as a greater overall ^{15}N NMR response by an increase in the concentration of ^{15}N nuclei whilst still maintaining the same $^{15}\text{N}/^{14}\text{N}$ ratio.

3.2.9 Detection of ^{17}O satellites in the ^{15}N NMR spectrum of **Y**

Using the optimisation carried out in this chapter a sample was made to try maximise ^{15}N NMR signal rather than polarisation. This is the amount of signal detected by the spectrometer. To do this a sample containing 2 mg of the **IPr**^{NMe₂} pre-catalyst, 50 equivalents of **Y** and 6 equivalents of **G-d₂** was taken and a shake and drop study carried out shaking for 10 s at a field of -0.35 μT for 10 s. When transferred to a 9.4 T spectrometer for ^{15}N NMR signal acquisition, the spectrum displayed in Figure 3.9 was recorded.

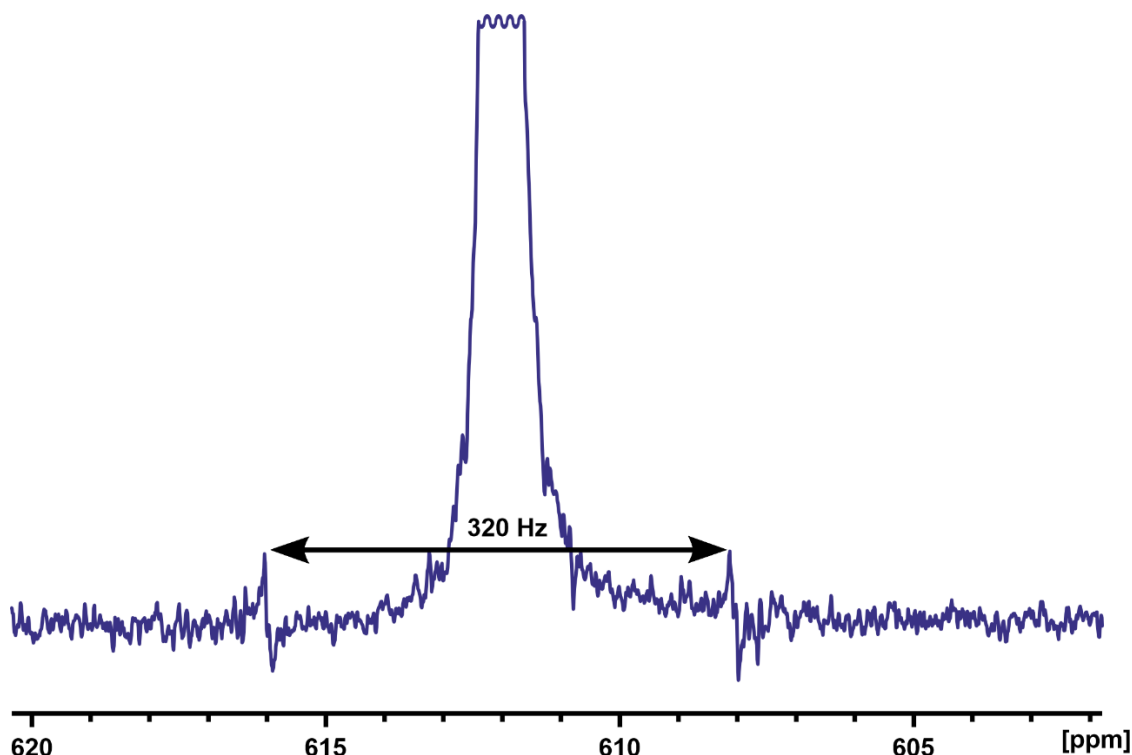


Figure 3.9 Typical ^{15}N NMR spectrum from a hyperpolarised sample of **Y** (50 equivalents relative to Ir pre-catalyst) showing satellites with a 320 Hz coupling likely due to the presence of ^{17}O .

The satellite peaks are very low intensity fitting with a very low natural abundance of ^{17}O of 0.04%. The coupling is also very large fitting a single bond coupling with the

^{17}O as the single bond couplings to ^{15}N and ^{17}O have been reported to be very large,¹⁵⁵ although a single bond coupling between these two nuclei has not been reported until now.

3.3 Reaction Monitoring Using ^{15}N NMR Spectroscopy

As the ^{15}N SABRE of **Y** was now sufficiently optimised, it was hypothesised that the reactive nature of **Y** could be probed in order to monitor some reactions and even use the high levels of ^{15}N NMR signal to detect and identify reactive intermediates to give additional mechanistic insight into the reactions. Figure 3.10 shows some example reactions that can be observed using **Y** as a starting point.¹³¹

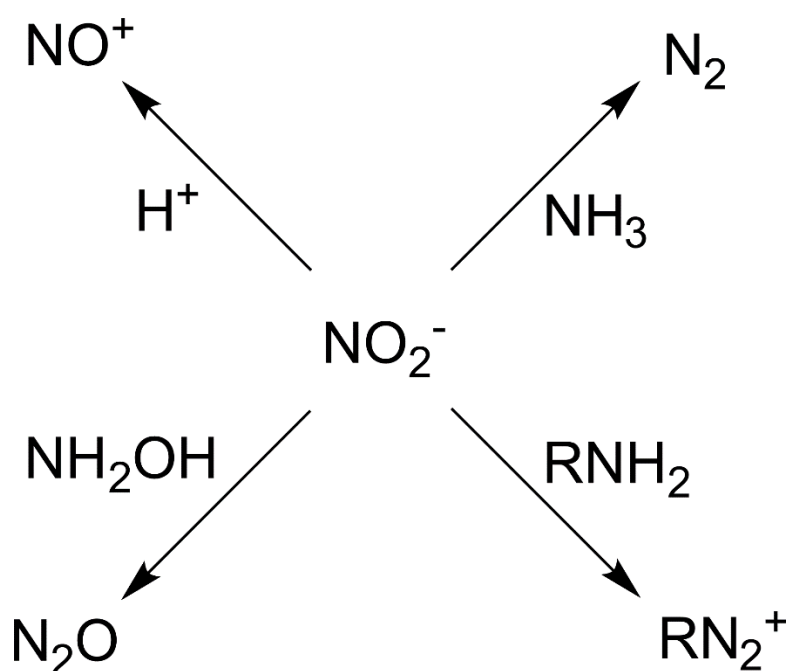
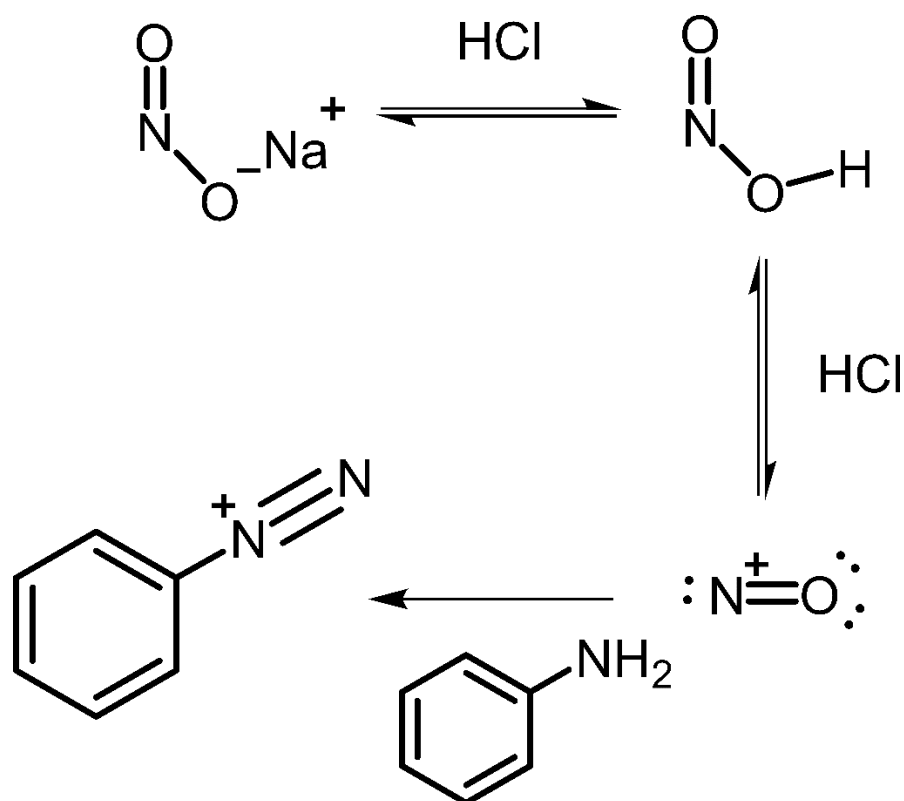


Figure 3.10 Possible reactions to monitor starting from hyperpolarised $[\text{NO}_2]^-$ (**Y**). Note that some reactants required are omitted from the figure for simplicity. If the reaction is looked at in detail in a later section all details of the reaction will be included.

3.3.1 Conversion of hyperpolarised $\text{Na}^{15}\text{NO}_2$ to a diazonium salt via $[\text{NO}]^+$

The first reaction focussed on in this chapter is that between nitrous acid (HNO_2) with aniline to form phenyl diazonium chloride. The formation of diazonium salts is of great importance in organic synthesis as they are responsible for the formation of azo dyes, cross coupling products, or aryl halides through a Sandmeyer reaction.^{130, 131, 156} To form nitrous acid, **Y** must be mixed with a strong acid such as HCl . Scheme 3.4 shows the formation of HNO_2 and the continued reaction to phenyl diazonium chloride upon addition of aniline.



Scheme 3.4 Formation of phenyl diazonium chloride through the reaction of acidified **Y** with aniline.

In order to probe this reaction using ^{15}N NMR spectroscopy, a sample was made up containing 2 mg of the pre-catalyst $\text{IPr}^{\text{NMe}_2}$ with 25 equivalents of **Y** and 6 equivalents of **G**. Although it is noted the ratio of substrates in this sample does not give the highest level of ^{15}N polarisation, it is important to have a higher raw ^{15}N NMR signal, which is achieved under these conditions. This sample was then activated and hyperpolarised using the previously optimised conditions. The sample was then opened to the air and 200 μL of a solution containing aniline (30 equivalents compared to Ir) dissolved in a 1 : 1 mixture of HCl (37% aqueous solution) and methanol- d_4 added. The sample was then quickly transferred to an 11.7 T spectrometer for ^{15}N NMR signal acquisition. A series of ^{15}N NMR spectra were then acquired using a 9° tip angle pulse for each. These were acquired every 5 seconds to monitor the reaction. The pulse sequence used for this data acquisition can be found in the appendix Section A.1. As the ‘shake and drop’ method was utilised here, (outlined in the experimental section 7.2.1) a delay of around five seconds is unavoidable due to sample transport. Due to this, the start of the reaction cannot be observed. The resulting series of ^{15}N spectra obtained from this experiment are displayed in Figure 3.11.

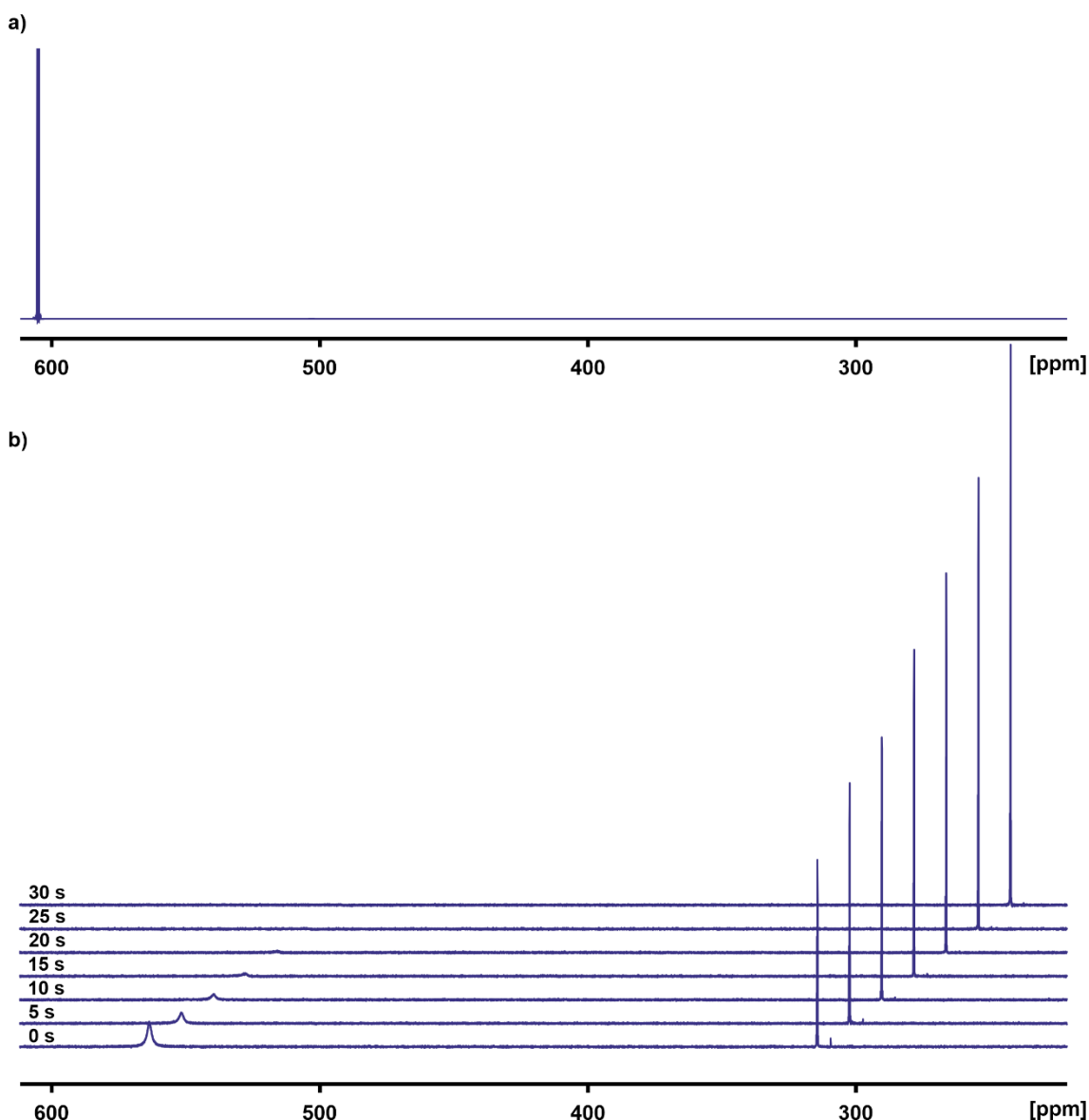


Figure 3.11 a) A typical hyperpolarised ^{15}N NMR response of **Y** before the addition of any acid or aniline. The spectrum was acquired at 11.7 T using a 90° pulse acquire sequence after exposing the sample to 4 bar of $p\text{-H}_2$ and shaking in the appropriate PTF. b) A series of ^{15}N NMR spectra obtained from a hyperpolarised sample of **Y** mixed with an excess of alanine and HCl. The spectra were all acquired at 11.7 T using a 9° pulse angle with a delay of 5 seconds between each acquisition. For ease of viewing an offset of 10 ppm is applied to each spectrum so only the bottom spectrum displays the true ^{15}N chemical shift.

In this experiment, new ^{15}N NMR signals are observed in addition to that of **Y**, indicating a reaction had indeed occurred. The resulting hyperpolarised ^{15}N NMR signals were observed at δ 563, indicative of nitrous acid (H^{15}NO_2); at δ 314, showing the formation of the phenyl diazonium chloride salt and at δ 308, showing the formation of *mono*- ^{15}N labelled N_2 . To confirm the identity of these species an independent synthesis without any catalyst present was conducted and all the resonances confirmed by ^{15}N NMR spectroscopy and comparison with literature data. These data clearly show during the reaction H^{15}NO_2 is consumed to make the other products.

When this process was repeated with ^{15}N labelled aniline, the reaction monitoring experiment revealed hyperpolarised ^{15}N NMR signals for both ^{15}N nuclei in the diazonium salt product at δ 314.0 and 233.0 as shown in Figure 3.12. These assignments once again agree with literature values for this species.¹⁵⁷ Amazingly, the hyperpolarisation of both ^{15}N sites is observed, even though aniline itself had not been exposed to $p\text{-H}_2$ and the catalyst by shaking in a suitable PTF; any interaction will be from the limited amount of $p\text{-H}_2$ left in solution. A control experiment was carried out here by making a sample containing 2 mg of the $\text{IPr}^{\text{NMe}_2}$ pre-catalyst with 25 equivalents of **Y** and aniline both at a natural ^{15}N abundance. Shaking the sample at $-0.3\ \mu\text{T}$ showed only a hyperpolarised response for **Y** suggesting that **Y** outcompetes aniline for binding in the mixture as aniline is known to be SABRE active.

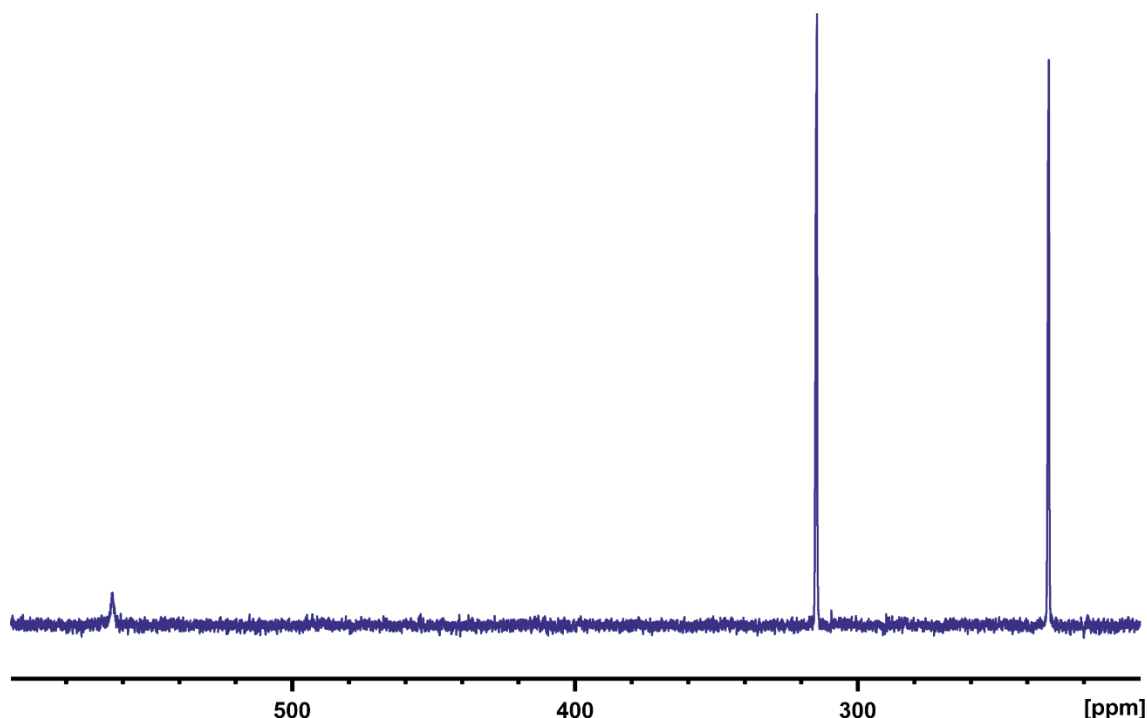


Figure 3.12 ^{15}N NMR spectrum obtained from a hyperpolarised sample of **Y** mixed with an excess of ^{15}N -aniline and HCl. The spectrum was acquired at 11.7 T using a 9° pulse angle.

Another control experiment was conducted in which a sample containing $\text{IPr}^{\text{NMe}_2}$ (2 mg) with 5 equivalents of phenyl diazonium chloride and 5 equivalents of **G** was exposed to a pressure of 4 bar $p\text{-H}_2$ and subsequently shaken in a PTF of $-0.35\ \mu\text{T}$ for 10 s before transfer to an 11.7 T magnet for ^{15}N NMR signal acquisition. The resulting NMR spectra only revealed a relatively weak hyperpolarised ^{15}N NMR signal for that of free **G** at δ 254.39 as the material was not labelled. This peak, however, was reflective of an enhancement of 3317-fold at 9.4 T, corresponding to $\sim 1.1\%$ polarisation. This presumably results from the presence of $[\text{Ir}(\text{H})_2(\text{G})_3(\text{IPr}^{\text{NMe}_2})]\text{Cl}$, which shows a ^1H NMR

ligand signal at $\delta -22.8$ that proved to be the dominant product when analysed by ^1H NMR spectroscopy. Therefore, it is confirmed that the efficient polarisation transfer occurring to reveal both hyperpolarised ^{15}N NMR resonances of the diazonium salt is not occurring due to SABRE *via* $p\text{-H}_2$, but instead a relay effect at low field that takes place in the μ -metal shield where the ^{15}N resonances are a coupled spin pair. It is therefore deduced that the hyperpolarised resonances seen in the reaction products and intermediates occur *via* hyperpolarised **Y**. These observations offered the possibility of being able to monitor even more complex reactions in a similar way.

In order to detect the formation of the nitrosonium ($[\text{NO}]^+$) intermediate, a separate sample containing 2 mg of **1**, 25 equivalents of **Y** and 6 equivalents of **G** was dissolved in 0.6 mL of methanol- d_4 . The sample was hyperpolarised by shaking in a -0.35 μT field for 10 s under a pressure of 4 bar $p\text{-H}_2$ before addition of 0.1 mL mixture of 37% HCl (aq) and methanol- d_4 followed by immediate transfer to a 9.4 T spectrometer for ^{15}N NMR signal acquisition. Unfortunately, no new ^{15}N NMR peaks were detected for the nitrosonium species. However, after leaving the sample overnight crystals had formed which were then analysed by X-Ray diffraction as outlined in experimental Section 7.1.4. This analysis revealed the following structure presented in Figure 3.13.

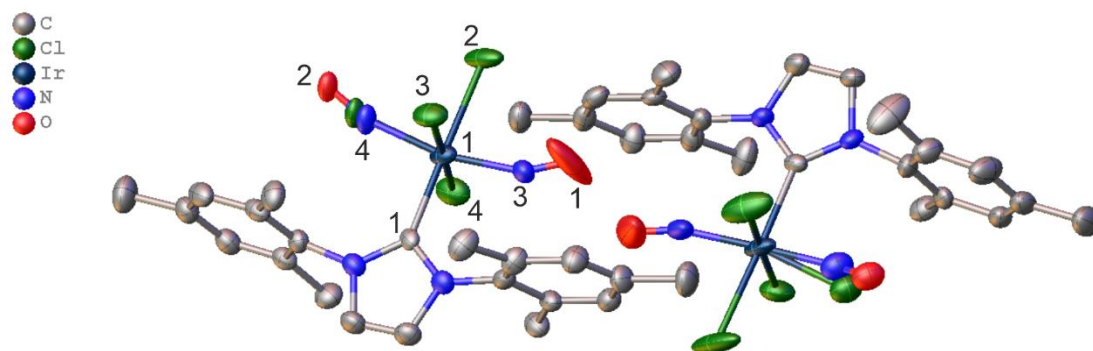


Figure 3.13 Structure of a nitrosyl complex formed determined by X-ray crystallography. Note any solvent of crystallisation and all hydrogen atoms have been omitted for clarity. The X-ray diffraction data was collected and solved by Dr. Adrian C. Whitwood. The numbers in the figure are the same as those given in the X-ray data found in Appendix A.2.

For ease of viewing some of the key bond lengths and bond angles are presented in Table 3.5 and 3.6 respectively. A full list of all bond lengths and bond angles as determined by the crystallography data can be found in Appendix A.2.

Table 3.5 Key bond lengths for the structure presented in Figure 3.13.

Atom Labels	Bond Length / nm	Atom Labels	Bond Length / nm
Ir(1)-C(1)	20.74(9)	Ir(1)-N(3)	18.41(10)
Ir(1)-Cl(2)	24.11(3)	Ir(1)-N(4)	18.89(17)
Ir(1)-Cl(3)	23.34(4)	N(4)-O(2)	11.15(18)
Ir(1)-Cl(4)	23.36(3)	N(3)-O(1)	10.79(12)

Table 3.6 Key bond angles for the structure presented in Figure 3.13.

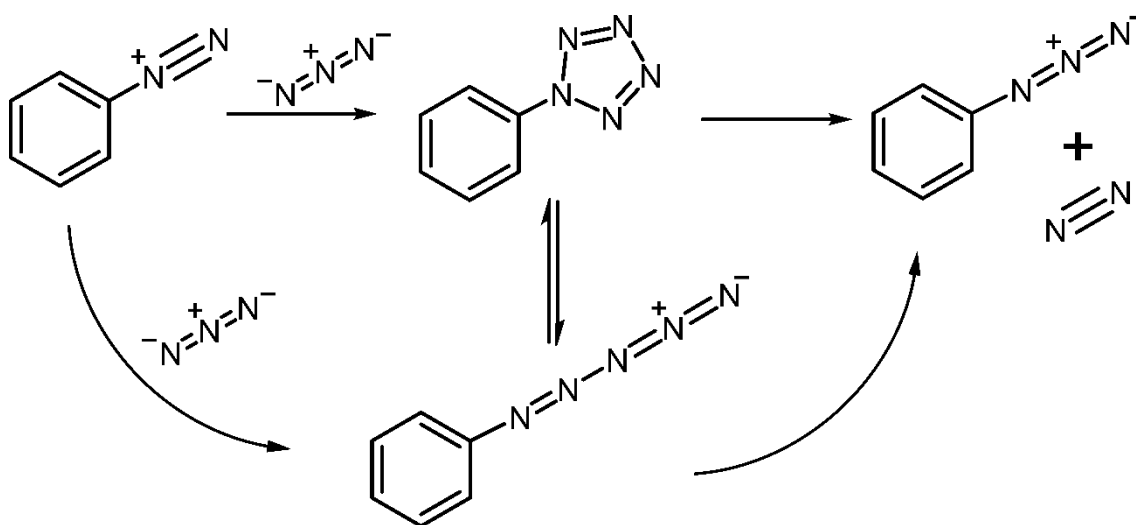
Atom Labels	Bond Angle / deg	Atom Labels	Bond Angle / deg
C(1)-Ir(1)-Cl(2)	177.9(3)	C(1)-Ir(1)-N(3)	96.7(4)
C(1)-Ir(1)-Cl(3)	88.0(3)	C(1)-Ir(1)-N(4)	87.9(16)
C(1)-Ir(1)-Cl(4)	93.2(3)	Cl(4)-Ir(1)-Cl(3)	176.71(13)
N(3)-Ir(1)-N(4)	169.2(16)	Cl(4)-Ir(1)-N(3)	92.6(4)
Ir(1)-N(3)-O(1)	163.4(11)	Ir(1)-N(4)-O(2)	153(4)

From these data it is clear that the structure is close to an octahedral geometry around the Ir centre. Interestingly, the two nitrosyl groups appear in different conformations. The first appears in a linear arrangement (Ir(1)-N(3)-O(1)) with a bond angle of 163.4(11)°. The other appears in a bent arrangement with a bond angle of 153(4)°. These values match closely with reported literature of similar arrangements.¹⁵⁸ These data therefore suggest an Ir³⁺ centre with one anionic (bent geometry) and one cationic (linear geometry) NO ligand. As expected the NO bond lengths for the ligands were 11.15(18) and 10.79(12) nm which are typical for that of the nitrosyl group.^{159, 160} These data therefore show the formation of an 18e⁻ complex containing two bound nitrosyl groups and hence confirming the formation of nitrosonium in solution. It is therefore likely the equilibrium between nitrosonium and nitrous acid lies heavily towards the previously detected nitrous acid.

3.3.2 Azide formation via N-penta-cyclic intermediate

The next reaction tested involved reacting phenyl diazonium chloride with sodium azide to produce phenyl azide. This reaction was selected as literature analysis revealed the existence of an ambiguity in the form of the predicted reaction intermediate.¹⁶¹⁻¹⁶³ It

could proceed via a cyclic and/or an acyclic species that would deliver either three or five unique ^{15}N resonances respectively. Scheme 3.5 shows the two intermediates that rearrange to phenyl azide on loss of N_2 .



Scheme 3.5 Possible reaction routes to the formation of phenyl azide and N_2 from phenyl diazonium chloride and azide.

In order to undertake this study, a sample of **Y** was first hyperpolarised as described in the previous section. A sample of hyperpolarised phenyl diazonium chloride was then generated by adding ^{15}N -aniline and HCl as described previously. This mixture was then treated with 25 equivalents of sodium azide (NaN_3), relative to iridium. The NMR sample was then immediately transferred into an 11.7 T magnet, and a series of low tip angle ^{15}N NMR spectra recorded. These data are presented in Figure 3.14.

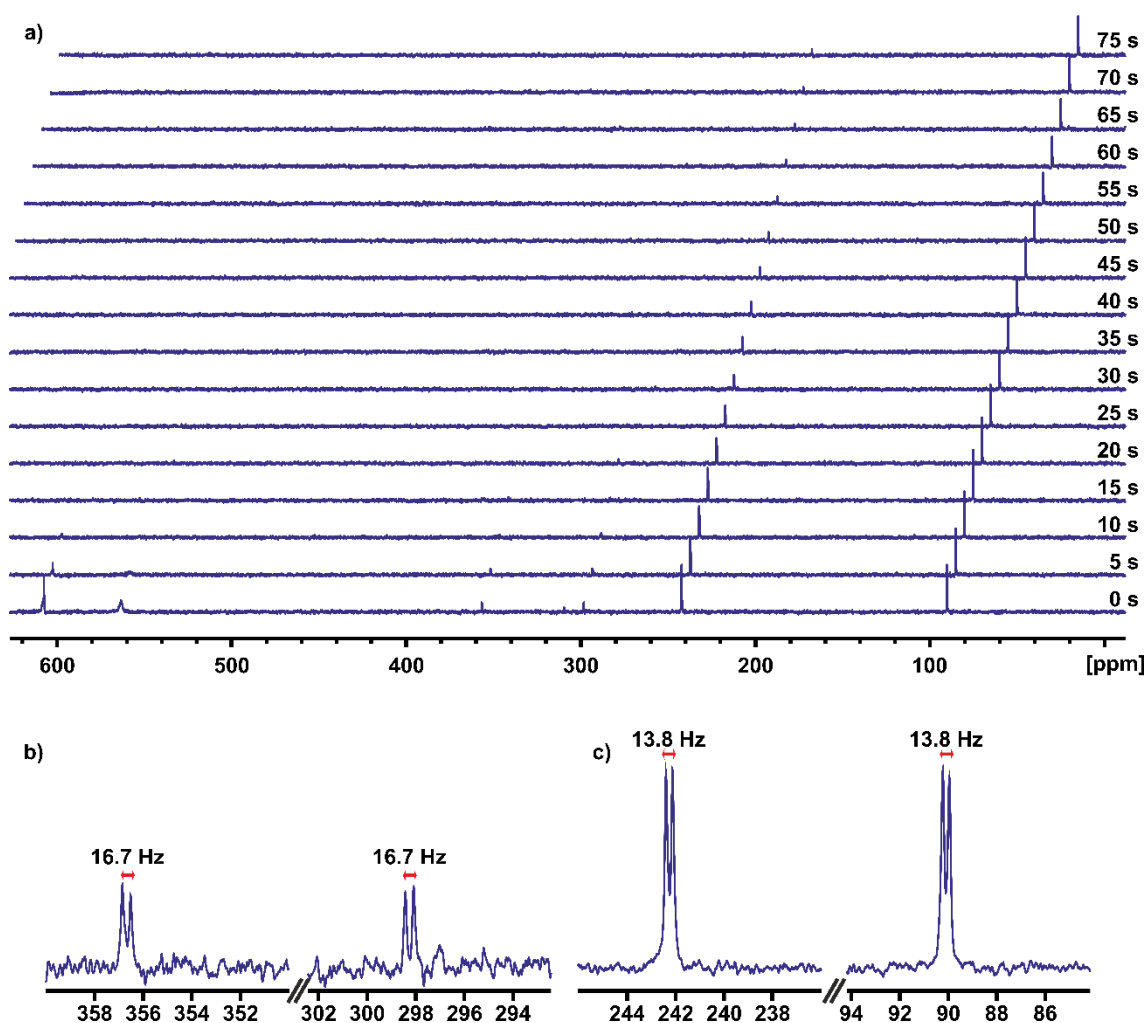


Figure 3.14 a) Series of ^{15}N NMR spectra obtained at different time points after a hyperpolarised sample of **Y** was mixed with excess ^{15}N -aniline, HCl and sodium azide. These NMR spectra were acquired consecutively at 11.7 T using a 9° degree pulse with a delay of 5 seconds between them. For ease of viewing an offset of 10 ppm is applied to each spectrum so only the bottom spectrum displays the true ^{15}N chemical shift. b) Expansion of the ^{15}N NMR signals at δ 356.8 and 298.2 to show their doublet splitting. c) Expansion of the ^{15}N NMR signals at δ 242.2 and 90.1 to show their doublet splitting.

Collectively these data revealed several new ^{15}N NMR resonances, indicating a number of products could be detected. This study starts with using the ^{15}N labelled diazo precursor. Transiently detectable doublets at δ 356.8 and 298.2 that share a common J_{NN} coupling of 16.7 Hz were seen. These signals were assigned to a ^{15}N centre attached to the phenyl ring in a cyclic intermediate and its neighbour. This assignment was made on the basis that when the ^{15}N labelled diazo precursor is the only ^{15}N labelled reactant, these are the only ^{15}N signals that show for this product. Strong ^{15}N NMR responses were also observed at δ 242.2 and 90.1, which now shared a common J_{NN} coupling of 13.8 Hz. These signals were attributed to the final phenyl azide product. Again, these signals were attributed to the ^{15}N attached to the phenyl ring and its neighbour as the only labelled ^{15}N nuclei present in structure. It was noted, that this reaction must proceed extremely quickly

as even after the 5 seconds of transfer time to the 11.7 T magnet for acquisition, the phenyl azide product was clearly the dominating species in solution.

When this experiment was repeated using ^{15}N labelled aniline and *mono*- ^{15}N labelled sodium azide, the situation was further complicated by the formation of two isotopologues of the cyclic intermediate and phenyl azide product. It was the introduction of the extra ^{15}N label that confirm the cyclic nature of the intermediate involved in this reaction. A typical ^{15}N NMR spectrum from this reaction, which also highlights the change in appearance of the originally detected ^{15}N NMR signals is shown in Figure 3.15.

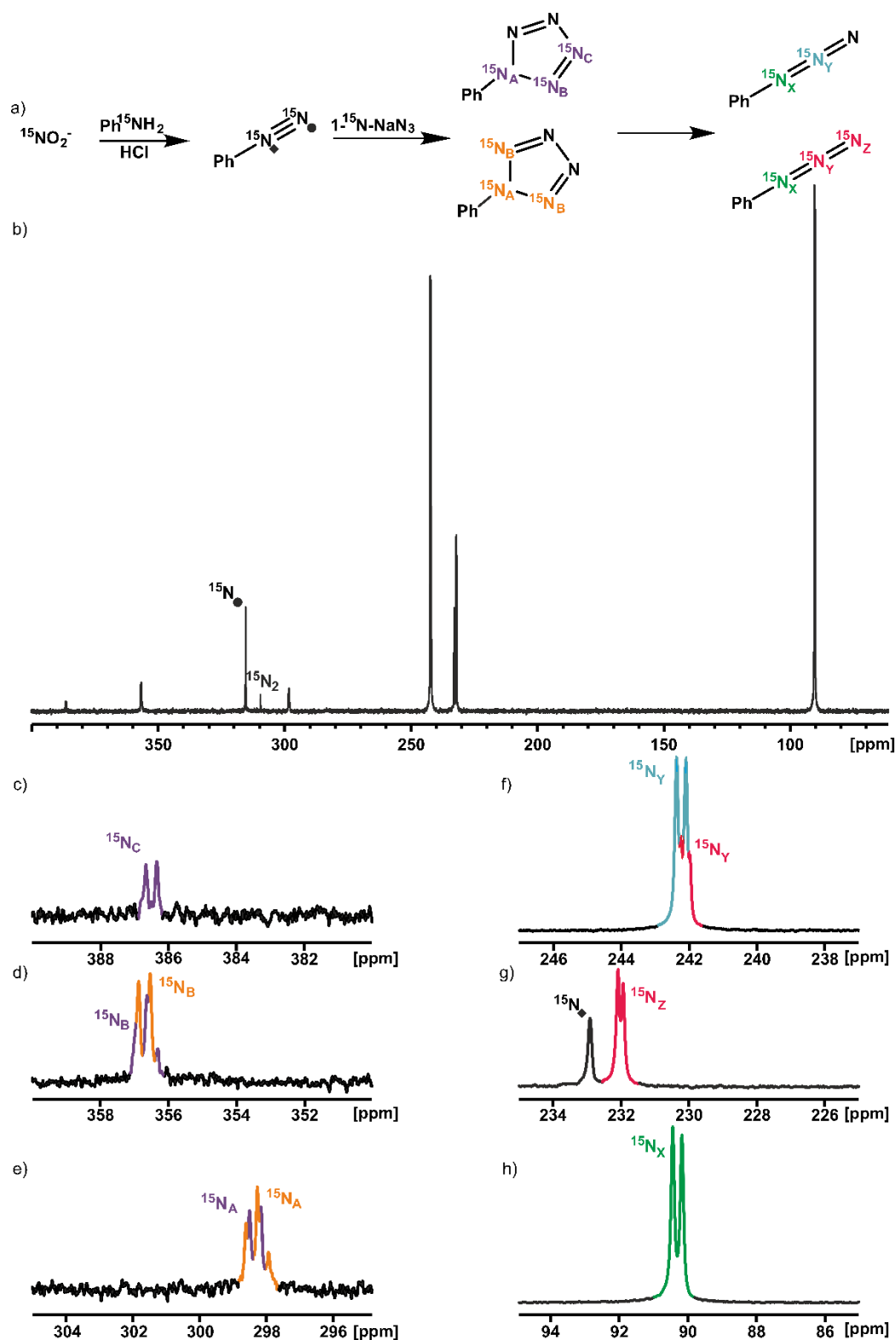


Figure 3.15 a) Reaction scheme showing where the ^{15}N labels are located during the formation of phenyl azide from Y, ^{15}N -phenyl aniline and 1- ^{15}N sodium azide. b) A typical ^{15}N NMR spectrum showing the product signals seen during this reaction. This NMR spectrum was collected using a 9° pulse at an 11.7 T field. c) An expansion showing the fine structure associated with the ^{15}N NMR signal at δ 386.5 d) An expansion showing the fine structure associated with the ^{15}N NMR signal at δ 356.8 e) An expansion showing the fine structure associated with the ^{15}N NMR signal at δ 298.2 f) An expansion showing the fine structure associated with the ^{15}N NMR signal at δ 242.2 g) An expansion showing the fine structure associated with the ^{15}N NMR signals at δ 233.0 and δ 232.0 h) An expansion showing the fine structure associated with the ^{15}N NMR signal at δ 90.1

The ^{15}N NMR resonance at δ 298.2 was confirmed to be that of the site labelled $^{15}\text{N}_\text{A}$ in Figure 3.15 due to its triplet character, rather than the previously witnessed 16.7 Hz doublet. This indicates the introduction of a ^{15}N label into the site for its neighbour, and is only possible if the *penta*-cyclic arrangement of Figure 3.15 is adopted. If a 5 membered nitrogen chain were formed instead, the ^{15}N NMR signal at δ 386.5 for N_C would appear as a singlet due to its terminal N nature. This reaction also causes added complexity to the peak at δ 356.8, which shows the previous doublet of 16.7 Hz, overlaid with a triplet (really a doublet of doublets with similar couplings). These three ^{15}N NMR signals satisfy the unique resonances needed for a 5 membered ring, no evidence was seen for the 5 membered chain implying that if this did form, it was in very small quantities or very short lived. As the ^{15}N NMR signals of the resulting phenyl azide are still easily visible in one scan it was hypothesised that the reactivity of the azide itself could now be probed to monitor further transformations.

3.3.3 Copper free click chemistry – formation of a 1,2,3-triazole

The next section utilises hyperpolarised phenyl azide, which either contained two ^{15}N labels or had all three positions ^{15}N labelled. These samples of hyperpolarised phenyl azide were rapidly mixed with what should be a stoichiometric amount of the reactive alkyne (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**Z**). This resulted in the formation of a triazole product. This reaction, as well as typical ^{15}N NMR spectra, are shown in Figure 3.16.

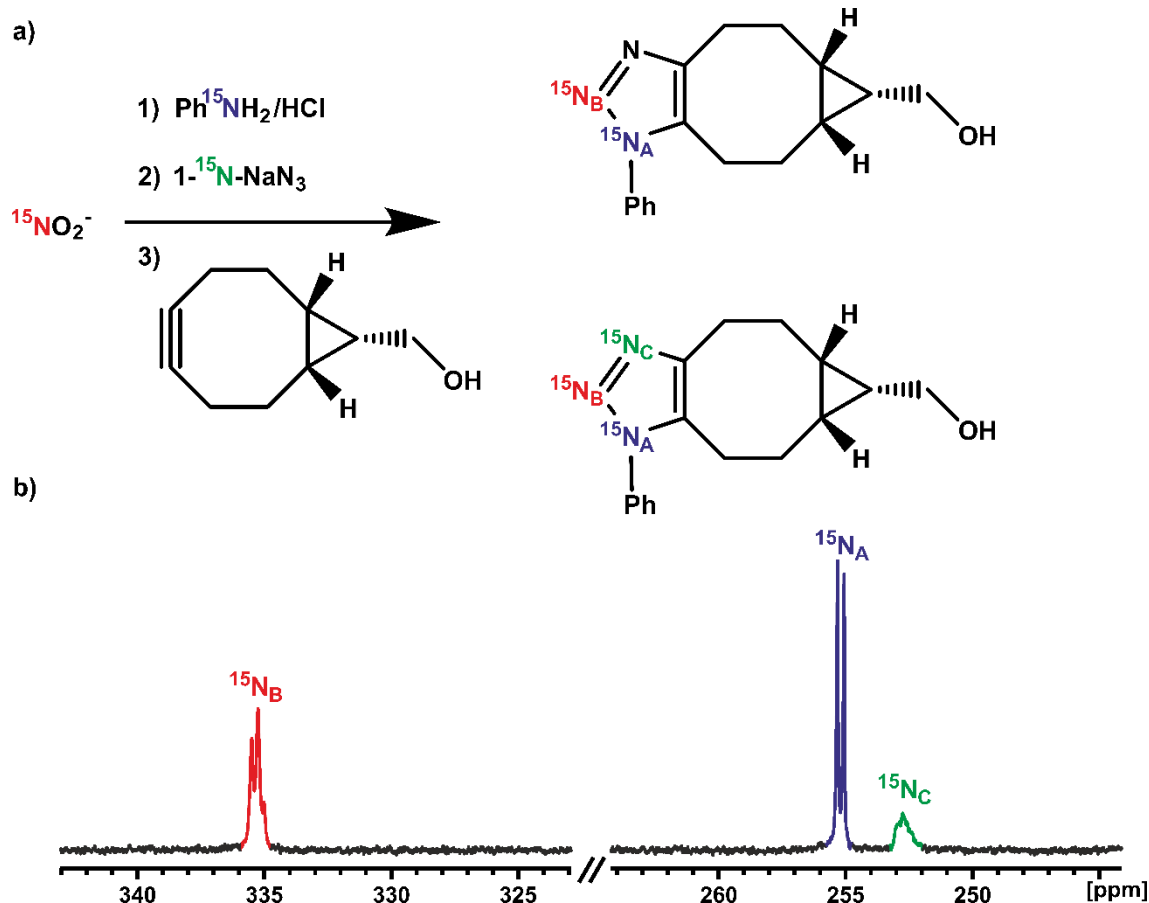


Figure 3.16 a) Reaction scheme for ¹⁵N-Y (150 mM) reacting in 0.6 mL methanol-d₄ with an excess of ¹⁵N-aniline and HCl followed by the addition of an excess of mono ¹⁵N labelled sodium azide. The addition of a stoichiometric amount of the reactive alkyne the followed this step, and formed the isotopologues the 1,2,3-triazole shown. b) A typical ¹⁵N NMR spectrum recorded directly after the addition of these reactants at 11.7 T is shown. This NMR spectrum was collected circa 12 s after the hyperpolarisation of Y took place and involved a 38° r.f. pulse.

In these data, three ¹⁵N NMR signals are observed, at δ 335.4 (N_B), 255.3 (N_A) and 252.7 (N_C) that were not seen if the reaction was carried out in absence of the octyne. These signals are therefore assigned to the triazole product. This assignment was confirmed by repeating this study when only the Y was labelled. Now, a single hyperpolarised resonance is seen at δ 335.4, which confirms the origin of this ¹⁵N NMR signal as resulting from the labelled Y. This experiment was also repeated with labelled Y and labelled aniline. A new peak at δ 255.3 results, confirming again the chemical shift and position of the nitrogen centre previously located in aniline in the product.

A control experiment was then carried out to see if the azide itself could be polarised. This involved performing a shake and drop experiment on a fresh sample that initially contained 2 mg **1**, 25 equivalents of mono ¹⁵N labelled sodium azide and 6 equivalents of **G**. This sample was first exposed to a pressure of 3 bar of H₂ for 20 min before shaking it under a pressure of 3 bar *p*-H₂ in a -0.35 μ T field for 10 s. It was then

transferred into a 9.4 T magnet for ^{15}N NMR signal acquisition. The resulting data revealed a strong ^{15}N signal for the azide at δ 95.7 reflective of 3.2% ^{15}N polarisation thereby indicating SABRE had been successful.

Further analysis revealed that this sample contained complex **14**, alongside new ^1H NMR signals for hydride ligands at δ -25.0 and -23.1 that proved to exhibit a mutual coupling of 8 Hz. Low temperature ^{15}N - ^1H HMQC analysis revealed the hydride ligand resonating at δ -25.0 coupled to a terminal ^{15}N of bound azide with a corresponding ^{15}N NMR signal at δ 216.3 ($J_{\text{HN}} = 21.6$ Hz). This reflects a large shift from its free ^{15}N NMR signal at δ 95.7. Although the large change in chemical shift was unexpected, the assignment is robust as only the azide was labelled and therefore readily detectable. The significant transfer of electron density from azide to iridium was therefore indicated. Unfortunately, full characterisation on this complex could not be achieved due to low stability. The sample degraded rapidly after just 30 minutes at 243 K. For context, conducting a shake on drop experiment on this sample after degradation resulted in no ^{15}N NMR signals under SABRE in agreement with the fact that no hydride ligand ^1H NMR signals remain.

The earlier rapid HMQC measurements carried out on this sample revealed no other azide ligand ^{15}N NMR signals, implying only one azide ligand was bound. Hence a complex of the form $[\text{Ir}(\text{H})_2(\text{G})_2(\text{IMes})([^{15}\text{N}=\text{N}^+=\text{N}^-])]$ was suggested as the active species. As the polarisation level achieved here directly with azide was quite impressive, it was hypothesised similar reactivity to that in Figure 3.16 could be monitored using the azide as a lone source of ^{15}N labelling. The resulting ^{15}N NMR spectrum from this reaction can be found in Figure 3.17.

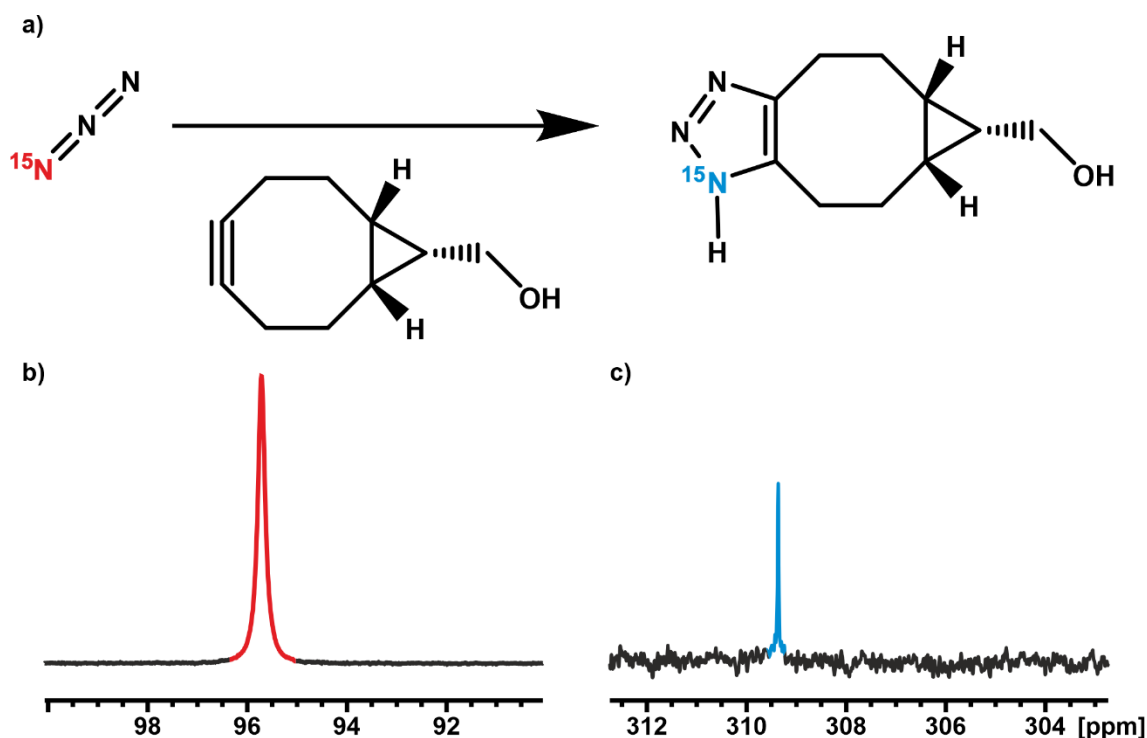


Figure 3.17 a) Reaction scheme between a hyperpolarised $1\text{-}^{15}\text{N}$ -sodium azide (150 mM) in 0.6 mL methanol- d_4 with a stoichiometric amount of the reactive alkyne to form the 1,2,3-triazole product shown. b) Hyperpolarised ^{15}N NMR signal from sodium azide collected from a 90° pulse acquisition sequence at 9.4 T. The sample was shaken for 10 s at $-0.35\ \mu\text{T}$ under 3 bar of $p\text{-H}_2$. c) Hyperpolarised ^{15}N NMR signal of the 1,2,3-triazole product, note this is an average signal across all tautomers of the product.

In these data, a new ^{15}N NMR signal is observed arising from the 1,2,3-triazole product. This signal was easily visible in just one scan with a 38° pulse angle which allows progress of the reaction to be monitored across several time points. Full characterisation data for the triazole product can be found in Section 7.4.30. Note that the sample utilised for characterisation experiments was prepared separately. This result therefore illustrates the existence of a further route to reaction monitoring, this time using a hyperpolarised ^{15}N synthon that is different from **Y**.

3.3.4 Formation of gaseous $^{15}\text{N}_2$

As shown previously, a diazonium salt can decompose to release nitrogen gas. This is a process known to occur photochemically, or with the aid of a catalyst such as CuI, known as the Sandmeyer reaction.¹⁵⁶ This reaction is responsible for converting arylamines into arylhalides, an important step in cross-coupling reactions.^{131, 164} Alternatively, nitrogen gas can also be produced spontaneously through the reaction of **Y** with ammonia in acidic conditions. The Sandmeyer reaction was examined with SABRE,

and the production of nitrogen from the reaction of **Y** and ammonia is shown in Figure 3.18 along with a typical hyperpolarised ^{15}N NMR signal for N_2 .

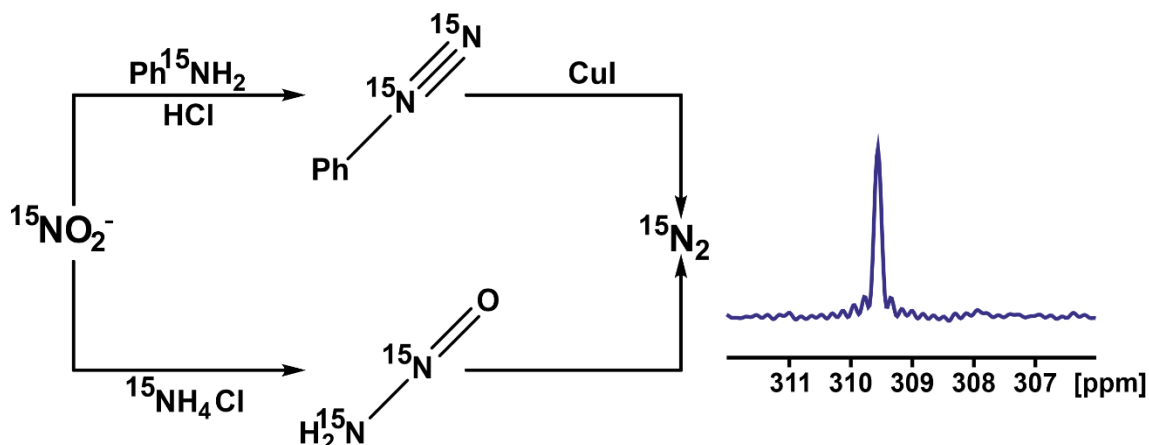


Figure 3.18 Two alternate routes for the production of $^{15}\text{N}_2$ from hyperpolarised **Y**; either via a Sandmeyer reaction with ^{15}N -aniline and HCl followed by CuI or a spontaneous creation reacting with ^{15}N ammonium chloride.

These data show both methods produce a larger ^{15}N NMR signal at δ 309.5 for $^{15}\text{N}_2$ gas, dissolved in the solvent. Unfortunately, no polarisation level could be estimated, although a 36-fold increase in signal to noise for the $^{15}\text{N}_2$ signal was deduced under SABRE. This was due to the fact the $^{15}\text{N}_2$ signal remained visible for a few seconds, and rapid outgassing of the solvent was seen. Hence, a very short T_1 and poor solubility was indicated.

3.4 Implementation of an automated flow system for hyperpolarisation of $[\text{}^{15}\text{NO}_2]^-$ and hyperpolarised ^{15}N diffusion spectroscopy.

This section of the project utilised a Bruker ‘Polariser’ unit (flow system) which allows automatic $p\text{-H}_2$ bubbling in a set PTF before pneumatic shuttling to a 9.4 T spectrometer for ^{15}N NMR signal acquisition. The full method for using the Polariser unit is outlined in the experimental Section 7.1.5. The advantage of using this flow system is that it allows for high data reproducibility and is easier to utilise if numerous ‘shake and drop’ style measurements need to be undertaken. The flow system also allows reproducibility to be achieved between labs.

3.4.1 Hyperpolarisation of **Y** using a flow system

Although the flow system has been widely utilised for SABRE studies, it was necessary to conduct a comparison study to ensure the optimised ratio of catalyst and co-ligand developed for **Y** still gave the necessary polarisation levels to conduct more

complex NMR experiments. A sample was therefore made containing 10 mg of **1** with 25 equivalents of **Y** and 6 equivalents of **G** in 3 mL of methanol-*d*₄ and inserted into the flow cell. The sample was then simultaneously degassed and activated by bubbling through the flow cell with 3 bar of *p*-H₂ for 30 s before leaving it to stand for 20 min. After the activation process, an equivalent of the shake and drop experiment was conducted in which the sample was bubbled for 10 s with 3 bar of *p*-H₂ for 10 s at a PTF of -0.30 μ T before transfer to the spectrometer. The solution transfer time from the flow system is now only 0.5 s, and hence much quicker than the 5-7 s taken for a manual shake and drop measurement. This means that less polarisation was wasted due to T_1 relaxation during the transfer process. The resulting SABRE study yielded a 9650 ± 300 fold increase in ¹⁵N NMR signal intensity, which translated to a $3.18 \pm 0.09\%$ ¹⁵N polarisation. The lower polarisation yield than that observed with the analogous shake and drop method is expected as the pressure of *p*-H₂ used is now 3 bar and it is of 92% purity, opposed to 4 bar and >99% purity used in the shake and drop experiments. Nonetheless, the polarisation level achieved here is still high enough to try more complex studies.

3.4.2 Polarisation transfer field determination

Due to the increased reproducibility gained by using the flow system it was appropriate to repeat the field sweep study to determine the optimum PTF, as in Figure 3.1. A sample was therefore prepared containing 10 mg of **1** with 25 equivalents of **Y** and 4 equivalents of **D** in 3 mL of methanol-*d*₄. The sample was loaded into a flow cell and activated. The sample was then exposed to 3 bar of *p*-H₂ for 10 s in a defined PTF before shuttling into a 9.4 T spectrometer for ¹⁵N NMR data acquisition. This process was repeated three times per PTF, which ranged from 0.90 to -1.10 μ T. The measurements were made 30 at a time before the sample was reconstituted as some solvent loss was seen. These samples were screened with their PTF fields randomised and normalisation data was collected. The resulting combined field sweep is shown in Figure 3.19.

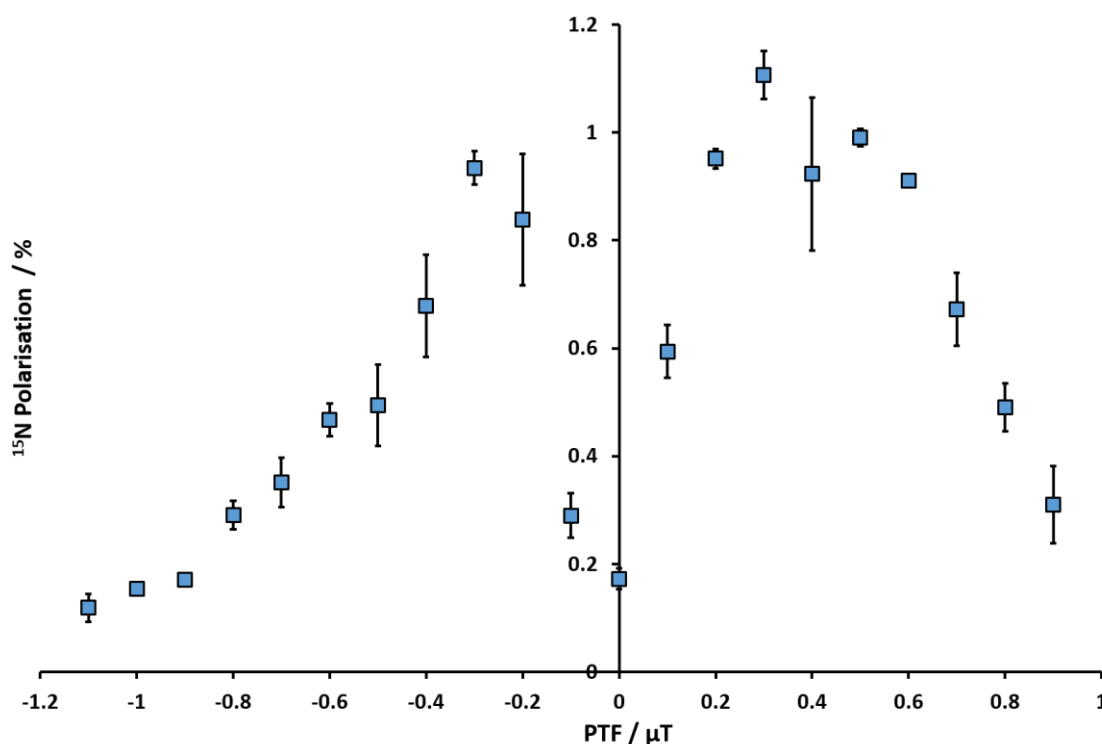


Figure 3.19 ^{15}N NMR signal acquired at 9.4 T as a function of polarisation transfer field. The sample consisted of 10 mg of **1** with 25 equivalents of **Y** and 4 equivalents of **D** in 3 mL of methanol- d_4 . At each PTF the sample was bubbled with 3 bar of fresh $p\text{-H}_2$ for 10 s.

Interestingly, the PTF at which maximum polarisation was achieved for **Y** was 0.30 μT , as opposed to the -0.35 μT of Figure 3.1. The maximum ^{15}N polarisation achieved with this method was also higher than the shake and drop method at $1.10 \pm 0.04\%$ which is likely to be due to the shorter sample transfer time. Thirty consecutive experiments were selected as testing demonstrated no solvent loss effects were apparent within the associated data. This screen involved a PTF of -0.30 μT , and the results are shown in Figure 3.20. The ^{15}N NMR signal intensity is not effected by solvent loss although there is a random variation in performance that is taken into account by averaging.

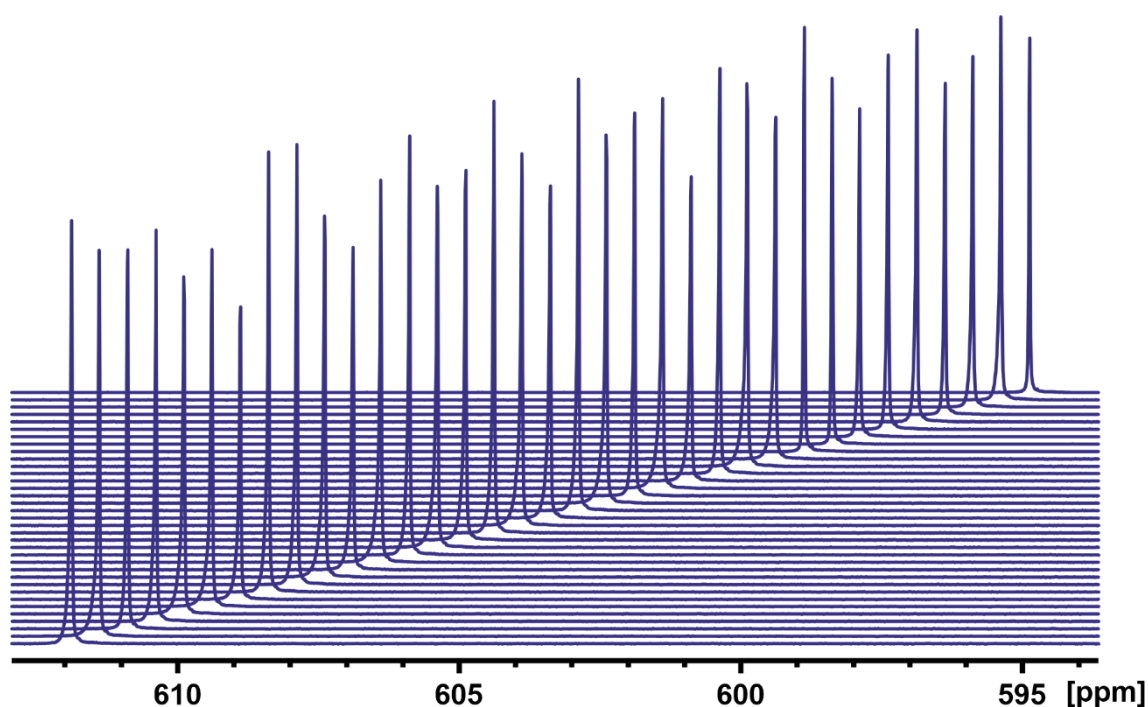


Figure 3.20 Series of ^{15}N NMR spectra acquired on a sample containing **1** (10 mg) with 25 equivalents of **Y** and 4 equivalents of **D** in 3 mL of methanol. For each spectrum the sample was bubbled for 10 s with 3 bar of $p\text{-H}_2$ before immediate pneumatic shuttling to a 9.4 T spectrometer for ^{15}N NMR signal acquisition. Note that the ^{15}N NMR spectra are stacked with an offset on the ppm axis. Note that time increases from bottom to top in these spectra. For ease of viewing an artificial shift of 10 ppm is applied to each subsequent spectrum and so only the bottom spectrum shows the true ^{15}N chemical shift value.

3.4.3 Diffusion spectroscopy - control study using pyridine

As the previous study showed many measurements could be undertaken on the flow system with high repeatability, it was natural to test a more complex pulse sequence. DOSY was selected to extract a diffusion coefficient for **Y**. To ensure the diffusion coefficients calculated by this method were accurate control studies were needed. The three major areas for concern when conducting these experiments were; the effect of the catalyst on the diffusion coefficient, the effect of sample shuttling on the diffusion coefficient and lastly whether probing ^{15}N instead of ^1H affected the diffusion coefficient. These three points will be probed, and discussed separately.

The first issue tested was the effect of the catalyst on the diffusion coefficient. As already introduced in the introduction, and shown in Figure 2.11 of Chapter 2, DOSY diffusion coefficients can be greatly affected by chemical exchange. Consequently, the concentration of catalyst and substrate relative to one another was hypothesised as potentially affecting the diffusion coefficient. If the substrate spends more time bound to the catalyst over the course of an experiment, it will appear to travel slower than a purely

free substrate. A series of samples were therefore made containing **1** (2 mg) with varying concentrations of pyridine in methanol- d_4 . The substrate **D** was used here instead of **Y** due to it containing ^1H nuclei that are much easier to observe at these concentrations and there is a known literature diffusion coefficient for comparison. Each sample was degassed and activated with 3 bar of H_2 before being probed on a 9.4 T instrument at 298 K, using a standard DOSY pulse sequence with convection compensation. A separate sample containing only pyridine in methanol- d_4 at a concentration of 25 mM was also probed for comparison. These data are presented in the Figure 3.21; the diffusion coefficients were calculated using the DOSY toolbox software of the University of Manchester NMR methodology Group as outlined in the experimental.

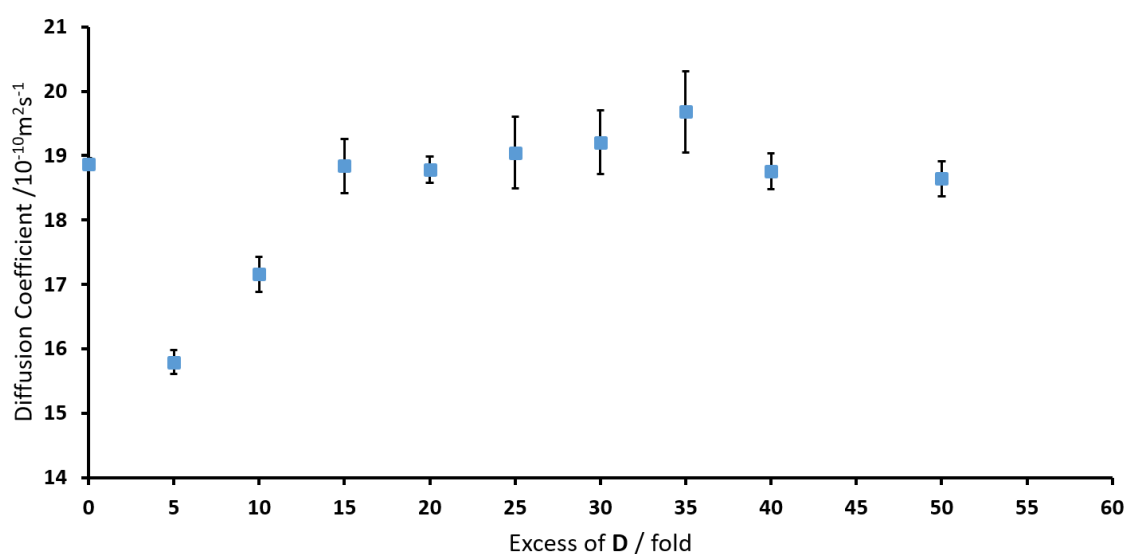


Figure 3.21 Diffusion coefficient *D* as a function of the relative excess of *D* (iridium complex at 5 mM) determined by ^1H measurement. The data point at 0 had no iridium present. All measurements presented here were conducted at 298 K on a 9.4 T NMR spectrometer.

As expected, there is a clear affect arising from the catalyst **1** being present. For a low excess of **D**, when each molecule has a greater chance of being bound, there is a stronger perturbation away from the diffusion coefficient of ‘free’ **D**. Furthermore, as the concentration of **D** increases, this effect becomes less pronounced and the diffusion coefficient returns to that of ‘free’ **D**. It is expected though that the ratio at which this plateau occurs will be heavily dependent on the rate of substrate exchange, as this ultimately determines how much time the substrate will remain bound to the catalyst. Therefore the point of plateauing may not be the same for each substrate. It is predicted though, that with a 50-fold excess, no effect will result.

The second issue to be addressed is that of the flow system itself. Using a pneumatic flow to shuttle the sample into the spectrometer involves a force that causes

advection and potentially artificially increases the diffusion coefficient. A sample was therefore made containing 10 mg of **1** with 50 equivalents of **D** in 3 mL of methanol- d_4 and loaded into the flow system. The sample was simultaneously degassed and activated with 3 bar of $p\text{-H}_2$ for 1 min before leaving for 30 mins. The bubbling gas was then changed to N_2 to remove any SABRE effects from the study. A series of DOSY measurements were then collected on the sample in which each bubble, shuttle and acquire sequence became a slice of the 2D DOSY spectrum, increasing in gradient strength for each spectrum from 20% to 80% over 10 points according to a quadratic ramping function. The DOSY pulse sequence was therefore modified to allow the bubble and shuttle process to take place between acquisitions at each gradient strength. This was essential for SABRE to be successfully implemented with DOSY as shown later. This process was repeated until data for each gradient strength had been collected, and the data was then processed as before. Figure 3.22 shows a typical DOSY plot from this study.

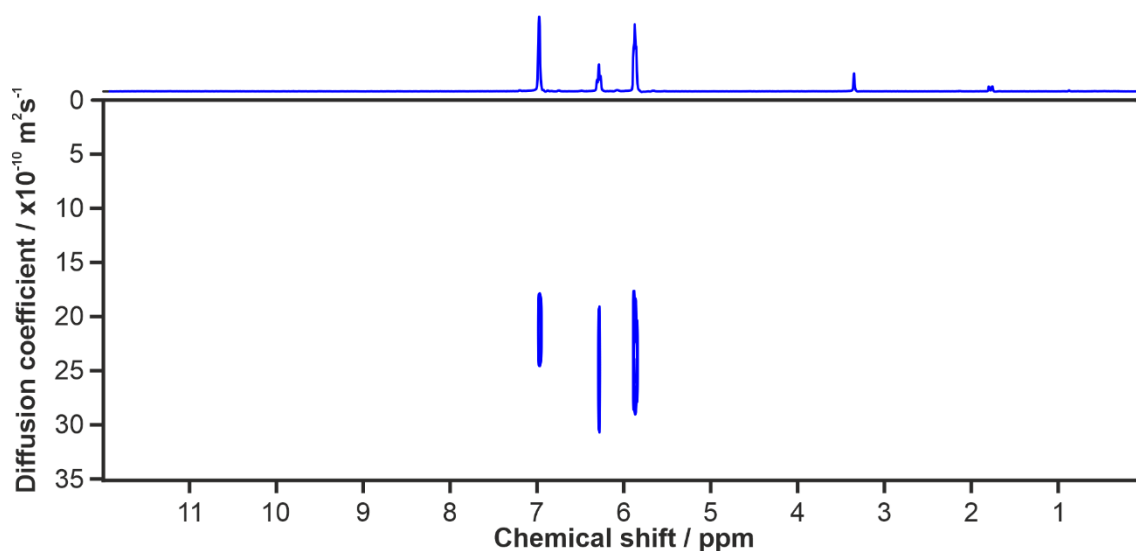


Figure 3.22 A typical ^1H DOSY plot of a sample containing 2 mg of **1** with 50 equivalents of **D**. The spectrum was acquired in the flow probe after bubbling with 3 bar of N_2 for 10 s and pneumatic shuttling into a 9.4 T spectrometer prior to initiating the DOSY pulse sequence.

As expected, the determined diffusion coefficient is much larger than that expected. Hence, **D** is moving quicker than it should. It was therefore hypothesised that a short settling delay should be added before data acquisition to quench advection. Consequently, this experiment was repeated with an increasing settling time. The diffusion coefficients gained in this way are shown in Figure 3.23. As expected, the resulting diffusion constants plateau as the mixing time increases.

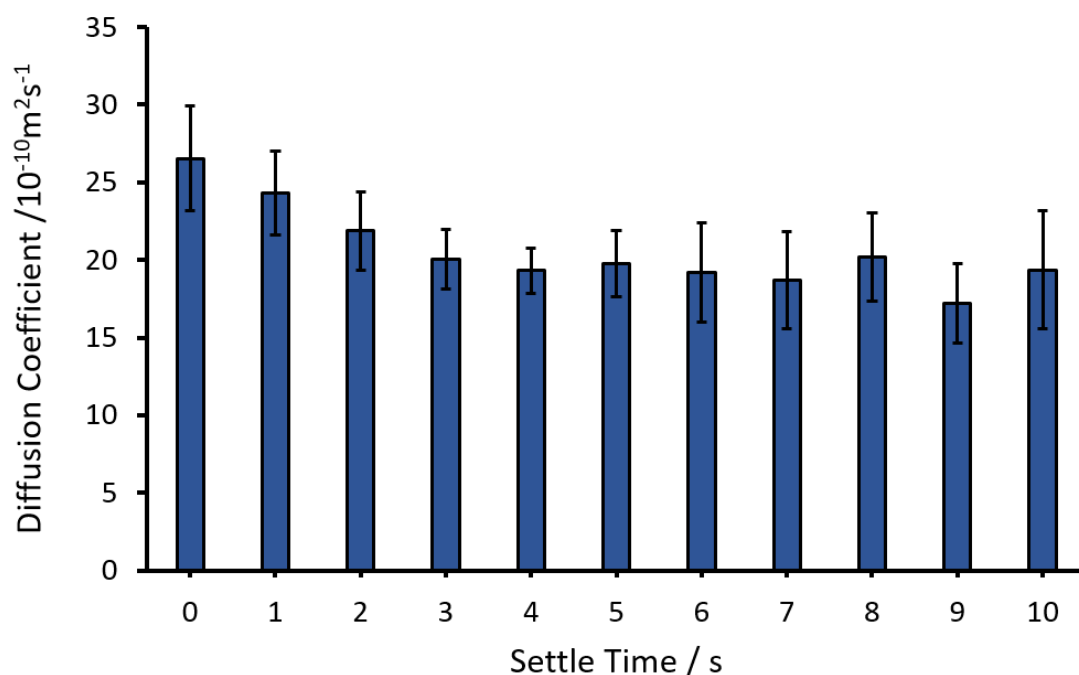


Figure 3.23 A plot showing how the diffusion coefficient of **D**, via ^1H data acquired using a flow system changes with settling time allowed in the spectrometer before application of the DOSY pulse sequence.

These data show the effects of advection are prominent at short settling times. After 3 s of settling the value of **D** returns to $20.03 \pm 1.93 \times 10^{-10} \text{m}^2 \text{s}^{-1}$, which matches that expected. This also matches the diffusion coefficient presented by Chan and Chan at $20.70 \pm 0.20 \times 10^{-10} \text{m}^2 \text{s}^{-1}$.¹⁶⁵ The flow DOSY experiment was then repeated with *p*-H₂ to gain a hyperpolarised response and see if SABRE affects the diffusion coefficient. This experiment yielded a very similar diffusion coefficient of $21.61 \pm 3.15 \times 10^{-10} \text{m}^2 \text{s}^{-1}$; there is a slight increase in the error in the measurement but this is due to the resulting signal now being dependent on the SABRE success. A previous study by Huber *et al.* has carried out ^1H SABRE DOSY via a one shot sequence known as Spatially ENcoded DOSY (SPEN-DOSY). They reported the diffusion coefficient of pyridine to be $14.46 \pm 0.47 \times 10^{-10} \text{m}^2 \text{s}^{-1}$,¹⁶⁶ the ratio of polarisation transfer catalyst : pyridine here was 1 : 5 respectively matching with the observation in Figure 3.21 that the catalyst is playing a significant role in affecting the diffusion coefficient of pyridine. Hence the work presented in this thesis demonstrates a method to more accurate diffusion coefficients by SABRE.

The last issue to be dealt with is whether the ^{15}N and ^1H DOSY data align. In order to test this, a measurement was performed on a sample containing the same 10 mg of **1** and 50 equivalents of **D**, but this time a ^{15}N NMR signal was used. **D** was therefore fully ^{15}N labelled. It was quickly found that the signal did not attenuate enough to extract an

accurate diffusion coefficient for the same gradient strengths as proton. As the gyromagnetic ratio of ^{15}N is much lower than that of ^1H , a much more powerful gradient is needed to encode its diffusion. An alternative to this is to allow the ^{15}N more time to travel in the sample, and so move to a region where the frequency difference is sufficient to dephase the ^{15}N and lower its signal intensity. Increasing the gradient pulse length from 1.0 ms to 1.5 ms and increasing the diffusion encoding time from 0.05 s to 5.00 s solved this problem. The extracted diffusion coefficient was now $22.19 \pm 1.39 \times 10^{-10} \text{ m}^2\text{s}^{-1}$. Hence as the hyperpolarised ^{15}N DOSY yielded an accurate diffusion coefficient, the diffusion coefficient of **Y** could now be probed in a similar manner.

3.4.4 Application for ^{15}N diffusion spectroscopy

The diffusion coefficient of **Y** in water has already been determined *via* ^{15}N DOSY measurements by Graham *et al.* using ^{15}N labelled samples with concentrations ranging from 0.1 to 12 mol dm^{-3} .¹⁴⁵ The study performed here utilises **Y** at a concentration of 0.25 mol dm^{-3} and demonstrates the ability to run SABRE in tandem with ^{15}N DOSY which paves the way for much lower concentration samples or even natural ^{15}N abundance samples to be probed in the same way. This in turn reduces the monetary cost as ^{15}N labelling is not required and also reduces the time cost as these experiments can be run in a single scan per gradient strength. A sample was therefore made containing **1** (10 mg) with 50 equivalents of **Y** and 6 equivalents of **G** in 3 mL of methanol- d_4 , loaded into the flow cell and fully activated with H_2 . The DOSY experiment used for **D** was then repeated for this sample of **Y** using a settling time of 5 s and a diffusion time of 5 s. The DOSY spectra of hyperpolarised **Y** were then processed using the DOSY toolbox which yielded the spectrum shown in Figure 3.24.

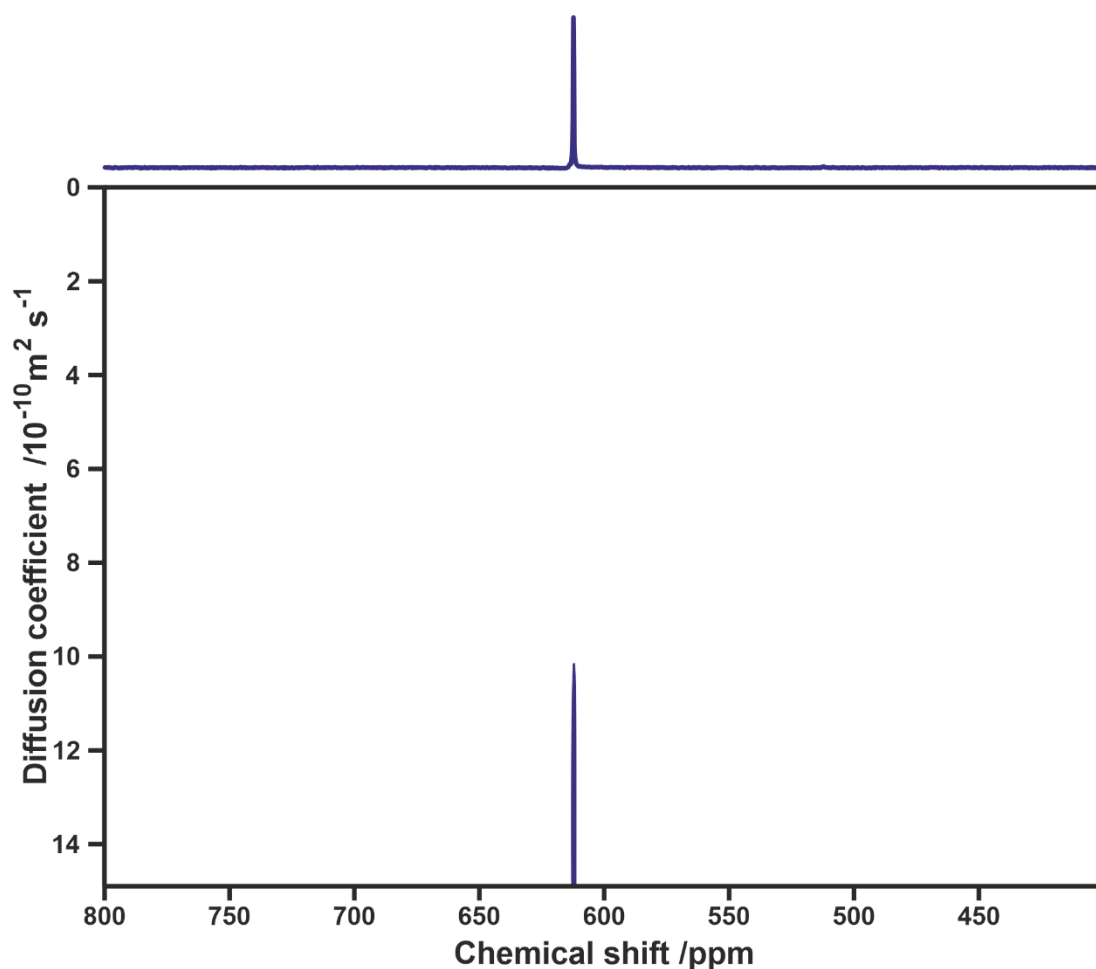


Figure 3.24 Typical ^{15}N DOSY spectrum gained from a sample containing 5 mM of **1** with 50 equivalents of **Y** and 6 equivalents of **G** in methanol- d_4 . The spectrum was acquired using a flow system bubbling the sample with 3 bar of $p\text{-H}_2$ for 10 s before pneumatic shuttling of the sample to a 9.4 T spectrometer where it was allowed to settle for a period of 5 s before application of the DOSY pulse sequence to acquire one gradient slice.

The diffusion coefficient gained from this DOSY spectrum was $13.54 \pm 1.48 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ which is similar to the literature value reported in D_2O of $14 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$.¹⁴⁵ The natural progression from this work is now to attempt these experiments on complex mixtures, use non-deuterated solvents and use natural abundance ^{15}N samples.

3.5 Conclusions

In this chapter, the ^{15}N hyperpolarisation of **Y** by SABRE was assessed, optimised and put to use in a variety of applications associated with reaction monitoring and diffusion spectroscopy. The chapter started monitoring the reactivity of **Y** towards the iridium pre-catalyst $[\text{IrCl}(\text{COD})(\text{IMes})]$ (**1**). It was found that **Y** was capable of displacing the Cl^- ligand forming the species $[\text{Ir}(\text{COD})(\text{IMes})(\text{Y})]$ (**8**). Upon further reaction with H_2 it was found that the octahedral dihydride intermediate $[\text{Ir}(\text{H})_2(\text{COD})(\text{IMes})(\text{Y})]$ (**9**) formed. Unfortunately, warming to 298 K revealed no stable SABRE active catalyst with many hydride ligand signals being observed although it is noted a 0.04% ^{15}N polarisation

of **Y** was achieved implying SABRE activity is possible. This mixture was therefore abandoned as a SABRE candidate.

The method of utilising the co-ligand pyridine (**D**) was successful in producing a stable SABRE active complex for the hyperpolarisation of **Y**. When monitoring this catalyst formation it was found that **Y** outcompetes **D** for binding, to again form **9**, in keeping with the relative pK_a of the corresponding acid for the two compounds being 3.16 and 5.14 respectively.^{167, 168} Interestingly, addition of H_2 causes labilisation of the **Y** ligand and known $[Ir(H)_2(COD)(IMes)(D)]Cl$ (**10**) is also formed. Allowing the sample to warm to room temperature saw the formation of the species $Na[Ir(H)_2(IMes)(D)(Y)_2]$ (**11**) and $[Ir(H)_2(IMes)(D)_2(Y)]$ (**12**) in the ratio 15 : 85. **11** and **12** proved to be stable and yielded an improved 0.29% ^{15}N polarisation for **Y**. Understanding the formation of these species through characterisation by multi-nuclear NMR was essential in allowing further optimisation as it allowed easy identification of analogous compounds when changing the catalyst further. A range of co-ligands from Figure 3.2 were tested for their ability to form **11** and **12** analogues and hyperpolarise **Y**. The study found the ratio of **11** to **12** made insignificant contributions to the performance of ^{15}N polarisation of **Y** and therefore the effects observed were likely down to the exchange rates of the catalytic species involved instead.^{54, 123} The best performing co-ligand species was that of dimethylaminopyridine (**G**) which formed **11** and **12** in a ratio of 5 : 95 respectively. Further studies found the concentration of **Y** and **G** played a role on the ratio of these species formed and proved that **12** was optimum for ^{15}N polarisation of **Y**. The optimum ratio of **1**, **Y**, and **G** was therefore determined to be 1 : 25 : 6 respectively. This yielded an improved ^{15}N polarisation of $3.0 \pm 0.1\%$. Selective deuteration of **G** proved very effective in giving further improvement to $4.6 \pm 0.2\%$ ^{15}N polarisation.

It was revealed that the exchange rate plays a huge impact on the efficiency of the polarisation transfer.⁵⁴ By probing the exchange of **G** in various complexes of the form $[Ir(H)_2(NHC)(D)_2(Y)]$, where NHC is a *N*-heterocyclic carbene from Figure 3.4, it was possible to observe that polarisation transfer efficiency for **Y** increased alongside exchange rate of **G** as shown in Figure 3.5 and Table 3.4. The optimum system here was determined to be the electron rich and bulky **IPr^{NMe2}** now yielding $7.18 \pm 0.21\%$ ^{15}N polarisation for **Y**. This finding is in agreement with a faster exchange being needed for the larger 27.7 Hz J_{HN} coupling determined for this complex as described by Barksy et al.¹²³

Further studies showed the ^{15}N polarisation could be improved by reducing spin dilution, a finding in agreement with that observed for ^1H by Rayner *et al.* through deuteration of species in the system.¹⁴⁹ By lowering the amount of ^{15}N nuclei present it was possible to achieve a maximum ^{15}N polarisation of $38.1 \pm 0.42\%$ for unlabelled **Y**, representing one of the highest ^{15}N polarisation levels achieved by SABRE for any substrate. The ^{15}N hyperpolarisation of **Y** utilising DNP (another hyperpolarisation technique), has also been investigated by Katz-Brull *et al.* who achieved *circa* 1% ^{15}N polarisation and so the results displayed in this thesis represent a remarkable improvement on this.¹⁶⁹ It is also observed that increasing concentration of H_2 yields higher ^{15}N polarisation transfer and that it is possible, by lowering the amount of catalyst and substrate to observe a plateau in achievable polarisation, the point where *p*- H_2 is no longer limiting. This observation is made for ^1H by Adams *et al.* but requires much higher levels of *p*- H_2 .¹⁵²

Harnessing the reactive nature of **Y** allowed several novel reaction monitoring studies to be undertaken for the first time through the eye of ^{15}N NMR. The incredible levels of ^{15}N polarisation achieved by this optimised system allowed for the detection of a number of short-lived, low concentration reaction intermediates. Notably, in the reaction sequence shown in Figure 3.15a all expected reaction products are detected, starting from only one hyperpolarised substrate (**Y**). This achievement opens up new opportunities for reaction monitoring by SABRE allowing for relay of polarisation through reaction. In the reaction observed, determination of a pentacyclic intermediate was possible and so provided mechanistic insights for the debated mechanism of Scheme 3.5.¹⁶¹⁻¹⁶³ This reactivity also shows the production of N_2 , thereby providing a facile route to hyperpolarised $^{15}\text{N}_2$ gas. Alternatively, if the reaction is done at a high magnetic field,⁴⁶ production of *paranitrogen* (*p*- N_2), a potentially very desirable lung imaging agent, is possible.^{47, 62}

The chapter finishes by investigating another application for the high polarisation levels achieved for **Y** in the form of SABRE hyperpolarised ^{15}N DOSY. It was discovered that the iridium catalyst plays a major role on the diffusion coefficient determined of the substrate. Therefore increasing the substrate excess past a certain point can yield much more accurate diffusion coefficients for that of the free material. These findings were in agreement with observations by Huber *et al.* where the diffusion coefficient of pyridine was reported as $14.46 \pm 0.47 \times 10^{-10} \text{ m}^2\text{s}^{-1}$,¹⁶⁶ lower than that of free pyridine which was found to have a diffusion coefficient of $20.03 \pm 1.93 \times 10^{-10} \text{ m}^2\text{s}^{-1}$, matching that reported

Chapter 3

by Chan and Chan of $20.70 \pm 0.20 \times 10^{-10} \text{ m}^2\text{s}^{-1}$.¹⁶⁵ The diffusion coefficient determined from the ^{15}N SABRE measurements on pyridine were also found to be in agreement with this coming out at $22.19 \pm 1.39 \times 10^{-10} \text{ m}^2\text{s}^{-1}$. The confidence in these measurements gives backing to the method and therefore to the ^{15}N diffusion coefficient determined for **Y** which was $13.54 \pm 1.48 \times 10^{-10} \text{ m}^2\text{s}^{-1}$ which is similar to the literature value reported in D_2O by Graham *et al.* of $14 \times 10^{-10} \text{ m}^2\text{s}^{-1}$.¹⁴⁵

Chapter 4. Evaluation of sodium nitrite as an MRI contrast agent

4.1 Introduction

As discussed previously, sodium nitrite plays an important role in the biology of plants but it has also been found to have medical use in humans and other animals. The action of $[\text{NO}_2]^-$ (**Y**), in the form NaNO_2 , has been shown to be beneficial in the treatment of cyanide poisoning and remains as one of the primary antidotes for acute intoxication.¹⁷⁰ Interestingly, sodium nitrite has also proved to be a candidate in erectile pharmacotherapy through its production of nitric oxide (NO), a reactive gas that plays a role in many cellular functions,¹⁷¹ by the enzyme nitric oxide synthase (NOS).¹⁷² The production of NO has also been explored for its antioxidant abilities through reacting with free radical species preventing cell damage and apoptosis.¹⁷³ It has been linked to regulatory functions related to diseases such as arthritis, diabetes and cancers so it is clear that NO is an extremely important gasotransmitter in the human body.^{174, 175} This behaviour makes **Y** an ideal candidate for use as an MRI contrast agent as it has already been shown that the hyperpolarised state on **Y** can be transferred to the products of the reactions it undergoes, potentially allowing us to observe the functions of NO in the body through MRI.

Traditional MRI contrast agents serve to artificially create contrast in tissues by injecting *paramagnetic* gadolinium-based materials known as T_1 contrast agents.¹⁷⁶ These T_1 contrast agents artificially decrease the T_1 of surrounding water molecules in the tissues.^{177, 178} This works through a dipolar coupling between the unpaired electrons on Gd and the coordinated water molecules.¹⁷⁸ The type of contrast agent being proposed in the form of hyperpolarised **Y** works in a different way. The hyperpolarised imaging agents utilise their larger than Boltzmann magnetic resonance signal to simply be more visible than the ‘noise’ of the rest of the body. The use of heteronuclei is especially beneficial in this method due to the incredibly low background signal in the body. Recent studies have been shown by Schmidt *et al.*^{65, 67} and Theis *et al.*⁶⁶ on metabolic imaging of pyruvate, an important metabolite involved in cellular energy pathways and a popular candidate in hyperpolarised magnetic resonance imaging. Pyruvate is an important part of the Krebs cycle. Biomolecules such as this can be important markers in spotting metabolic differences or irregularities which may be present in cancer cells.^{67, 179-181} To function as an effective agent in this way, **Y** must have a long T_1 so the biological processes can be monitored effectively. Many long lived lifetimes on ^{15}N have been reported with reports of over 9 min for $^{15}\text{N}_2\text{O}$,^{182, 183} which can be produced from **Y** as shown in Figure 3.10.¹³¹

The new type of imaging agents offer an advantage over the alternative Positron Emission Tomography (PET) in the fact they are completely non-ionising. While ^{18}F -fluorodeoxyglucose has been shown to act as a molecular sensor of these abnormal metabolic behaviours exhibited by cancer cells,^{184, 185} the use of PET has been associated with carcinogenic risk.⁷⁶ MRI on the other hand is completely non-ionising, with approved hyperpolarised contrast agents for lung imaging approved in the form of SEOP hyperpolarised noble gases such as ^{129}Xe .¹⁸⁶⁻¹⁸⁸ These agents look very promising as the next generation of metabolic tracers.

Another priority when assessing a new contrast agent is ensuring safe administration. This means removing any toxic agents such as solvents and the Ir based catalyst. Various methods have been proposed to do this; Manoharan *et al.* proposed a biphasic separation method using an immiscible solvent mixture,¹⁸⁹ Adams *et al.* have proposed rapid solvent evaporation followed by dissolution in a biocompatible solvent such as water,¹⁵² a method adopted by both Schmidt and Theis for their imaging studies.^{65,}

66

This chapter starts by assessing the possibility of hyperpolarising **Y** in dichloromethane- d_2 for the biphasic separation of **Y** using a mixture of dichloromethane- d_2 and D_2O . The biphasic sample is then probed by MRI to assess the distribution of **Y** in the mixture. The next part of the chapter then focuses on assessing the T_1 of **Y** and other related hyperpolarised ^{15}N compounds from the previous chapter.

4.2 Hyperpolarisation of $^{15}\text{NO}_2^-$ in different solvents

As already introduced, the use of ^{15}N detection for *in vivo* imaging is preferable over the currently used ^1H target as the abundance of ^{15}N within the body is very low and so there is virtually no background signal. This hypothetically means the optimised system for hyperpolarising **Y** by SABRE can be utilised to yield sufficient signal to monitor processes in the body. Before this hypothesis can be tested it must be ensured that the sample of **Y** is of high purity and contains no Ir catalyst or toxic solvent. In order to ensure this the hyperpolarisation of **Y** must take place in a solvent that can be easily separated from the mixture. Unfortunately, this makes the use of methanol redundant for this purpose as it is completely miscible with water. Therefore a solvent mixture must be selected that is not miscible with water.

4.2.1 ^{15}N Polarisation in dichloromethane- d_2

As already discussed, it has been shown in the literature by Manoharan *et al.* that extraction of a hyperpolarised substrate is possible by biphasic extraction from a dichloromethane/water mixture.¹⁸⁹ This process involves the substrate being able to travel between the aqueous and organic layers, while the catalyst stays in the organic layer. This ensures the substrate can interact with the catalyst to hyperpolarise whilst also being able to enter the other layer for ease of separation for the newly hyperpolarised sample. To ensure the success of SABRE in dichloromethane a sample containing 2 mg of **1** with 25 equivalents of **Y** and 6 equivalents of **G** was dissolved in dichloromethane- d_2 (0.6 mL). Unfortunately, there was a large amount of **Y** that did not dissolve and so when the sample was shaken in an appropriate PTF under a pressure of 3 bar $p\text{-H}_2$ no signal for **Y** resulted. Further investigation by NMR spectroscopy revealed that only $[\text{Ir}(\text{H})_2(\text{IMes})(\text{G})_3]\text{Cl}$ (**14**), which showed a ^1H NMR signal at δ -22.73, formed as a product in this solvent. Full characterisation data for this complex in methanol- d_4 can be found in Section 7.4.27.

In order to combat the low solubility of **Y** in dichloromethane, the macrocyclic ligand 15-crown-5 was added, which has a high affinity for chelation to Na^+ . A sample was therefore prepared with **1** (2 mg), 25 equivalents of **Y**, 25 equivalents of 15-crown-5 and 6 equivalents of **G**. The impact of the 15-crown-5 on the solubility of **Y** was immediately evident as the amount of solid dramatically decreased upon addition of the solvent. After sonicating the sample for around 30 min at a temperature of 303 K all of the solid **Y** had now dissolved. The sample was then placed under a pressure of 3 bar $p\text{-H}_2$ for around 2 hours to allow full activation of the catalyst. Probing the mixture at 298 K by ^1H NMR spectroscopy at this point revealed two hydride ligand resonances at δ -22.66 and -23.00 both sharing a common 7 Hz coupling. Cooling to 253 K revealed a slight shift to δ -21.60 and -22.86, respectively. These signals were attributed to **12G**. Further characterisation by a ^1H - ^{15}N HMQC measurement using a coupling constant of 20 Hz revealed a ^{15}N NMR signal for a bound **Y** ligand at δ 508.7 ($J_{\text{HN}} = 27$ Hz) and a further signal for bound **G** at δ 218.2. These two ^{15}N resonances therefore proved to be *trans* to the hydride ligands, with ^1H NMR signals at δ -21.60 and -22.85, respectively. A ^{13}C NMR signal was then located for the carbene carbon of the NHC ligand at δ 153.5 via coupling to the hydride ligand signals by ^1H - ^{13}C HMQC measurement. These data therefore fully satisfy the coordination sphere of the iridium centre in this solvent and confirm the species **12G** is now the sole product (>99%).

This sample was then shaken in a $-0.35\ \mu\text{T}$ field for 10 s with a pressure of 3 bar $p\text{-H}_2$ before being transferred to a 9.4 T magnet for ^{15}N NMR signal acquisition. This procedure revealed a large ^{15}N response for **Y** showing the use of the 15-crown-5 had indeed been successful. The ^{15}N response acquired showed a 3580 ± 330 -fold enhancement at 9.4 T which equates to $1.18 \pm 0.10\%$ ^{15}N polarisation. Although this is much lower than the maximum polarisation achieved when using methanol as the solvent, it is a significant improvement over the failed SABRE of **Y** in dichloromethane alone. The next stage of this study was to explore if the optimisation carried out in methanol translated to use in the dichloromethane solvent also. To test this, a further sample was made which contained 2 mg of the **IPr**^{NMe₂} pre-catalyst along with the same 25 equivalents of **Y**, 25 equivalents of 15-crown-5 and 6 equivalents of **G**. The sample was then shaken under the same conditions as the previous study so that a comparison could be drawn between the two pre-catalysts. After shaking the ^{15}N NMR signal that resulted proved to be 7250 ± 670 -fold in enhancement at 9.4 T equating to $2.4 \pm 0.2\%$ polarisation.

4.2.2 Use of a combination of dichloromethane- d_2 and D_2O

For biological use it is obviously highly desirable to produce contrast agents in water as this is a clear choice as a biologically-safe solvent compared to the organic solvents used so far. To evaluate the possibility of using water as a solvent for the SABRE here a sample was made containing 2 mg of the pre-catalyst **IPr**^{NMe₂} with 25 equivalents of **Y** and 6 equivalents of **G** in 0.6 mL of D_2O . It was immediately clear that the pre-catalyst was not very soluble in water as evidenced by the solution still appearing colourless, and the orange solid of the pre-catalyst still readily visible even after 30 min of sonication at 40 °C. Regardless of this, the sample was placed in a Schlenk tube and degassed carefully as outlined in the experimental. The flask was then transferred into a glove box where the mixture could be transferred to a 5 mm J. Youngs NMR tube for SABRE analysis. The sample was then exposed to a pressure of 4 bar H_2 and analysed by ^1H NMR spectroscopy. After leaving the sample to activate for 30 mins a spectrum was recorded which showed no visible hydride ligand ^1H NMR signals after 16 signal averages. At this point a SABRE study was conducted in which the sample was pressurised with 4 bar of $p\text{-H}_2$ and shaken in the fringe field of a 9.4 T spectrometer before transferring into the field for ^1H NMR signal acquisition. This study still showed no visible hydride signals and no hyperpolarisation of **G** was observed. Hence it was

concluded that a suitable catalyst species does not form in D₂O and so the solvent alone is not suitable for SABRE of **Y**.

A further sample was therefore made to trial the possibility of a biphasic separation approach. This sample consisted of 2 mg of **1** with 25 equivalents of **Y**, 6 equivalents of **G** and 25 equivalents of 15-crown-5 dissolved in 0.3 mL of dichloromethane-*d*₂. The sample was then exposed to 4 bar of H₂ until the formation of **12G** was complete (around 30 min). Now the addition of 0.3 mL of D₂O under a nitrogen atmosphere was undertaken. A SABRE study was then completed at -0.35 μ T under a pressure of 4 bar *p*-H₂ for 10 s, before transfer to a 9.4 T magnetic field for ¹⁵N NMR signal acquisition. The new ¹⁵N NMR spectrum contained two distinct ¹⁵N NMR peaks, both attributed to the free **Y**. These peaks appeared at δ 618 and 609 and are due to it being dissolved in dichloromethane-*d*₂ and D₂O respectively; based on comparison with spectra recorded in separate samples in each of the solvents. Interestingly these peaks showed a clear difference in intensity implying the distribution of polarised **Y** was not even between the two solvents. **Y** was partitioned 1 : 70 between dichloromethane-*d*₂ and D₂O, respectively. When calculating the signal enhancement from this experiment it was assumed that all of **Y** was dissolved in the D₂O layer and only this hyperpolarised response was used in calculating the enhancement at 9.4 T and the % polarisation also. This method will give an underestimate of the ¹⁵N polarisation achieved but will still give an idea of the SABRE success.

The calculated enhancement for the sample proved to be 4670 ± 440 -fold which yields a $1.54 \pm 0.14\%$ ¹⁵N polarisation. It is known in the literature that the 15-crown-5 added can act as a phase transfer catalyst and so help **Y** transport between the two solvent layers in order to build a higher level of polarisation. Therefore to the sample tested, a further 25 equivalents of 15-crown-5 were added and the SABRE study repeated. This resulted in a dramatic improvement in ¹⁵N signal gain to 13800 ± 1000 -fold at 9.4 T equal to a $4.6 \pm 0.3\%$ ¹⁵N polarisation. Interestingly, the addition of further amount of 15-crown-5 did not improve the ¹⁵N polarisation yield on **Y** any more with all further additions resulting in the same polarisation within error implying the phase transfer of **Y** is no longer limiting.

It has already been shown that the species **12G** exchanges **Y** at a much slower rate than needed for optimum polarisation transfer. The exchange rate has previously been shown to increase by changing the carbene ligand of the catalyst. It can, however, also be

increased by altering the temperature of the sample before shaking. This was done by warming the sample in a water bath at 308 K for 5 min before conducting the shake and drop experiment and heating the spectrometer to 308 K to ensure the temperature difference between the sample and the spectrometer was minimised to avoid fluctuations in temperature. While this method is a very coarse approach as the sample is still shaken at room temperature it does provide insight into the usefulness of a temperature controlled shaker. Using the temperature to tune the exchange rate would be much simpler than using the steric and electronic effects of the ligands. The resulting ^{15}N NMR signal enhancement gained when using this method on the same sample used previously was 26330 ± 8270 fold at 9.4 T corresponding to a ^{15}N polarisation of $8.7 \pm 2.7\%$. The large error on these measurements reflects the fact that the temperature cannot be precisely controlled using this method, and so although a clear improvement is seen, further developments need to be made to develop a temperature controlled shaker to carry out a more rigorous study.

In order to improve the ligand exchange rate, the pre-catalyst $\text{IPr}^{\text{NMe}_2}$ (2 mg) was examined with 25 equivalents of **Y**, 6 equivalents of **G** and 50 equivalents of 15-crown-5 in 0.3 mL of dichloromethane- d_2 . The sample was once again pre-activated by exposing it to a pressure of 4 bar of H_2 for 30 min before adding the 0.3 mL of D_2O under a nitrogen atmosphere. The sample was then shaken in a $-0.35 \mu\text{T}$ PTF, with a 10 s shake time under 4 bar of $p\text{-H}_2$. This study revealed a further improvement on the polarisation yielding a ^{15}N NMR signal enhancement of 29600 ± 2610 -fold at 9.4 T which gives a ^{15}N polarisation of $9.8 \pm 0.86\%$. To set this achievement in context, the best polarisation achieved by DNP for **Y** in D_2O at the time of writing was only *circa* 1% and so it is clear this reflects a remarkable improvement.¹⁶⁹

4.2.3 Magnetic resonance imaging of $[\text{}^{15}\text{NO}_2]^-$ across a biphasic solvent system.

In order to confirm the distribution of **Y** across the two phases, a separate study was conducted in which a sample containing the $\text{IPr}^{\text{NMe}_2}$ pre-catalyst (10 mg) with the same 25 equivalents of **Y**, 6 equivalents of **G** and 50 equivalents of 15-crown-5 was prepared. The sample was dissolved in 3 mL of dichloromethane- d_2 in a 10 mm diameter J. Young's topped NMR tube before the same pre-activation process and addition of 3 mL of D_2O took place. A 'shake and drop' style experiment was then conducted shaking the sample for 15 s at a pressure of 4 bar $p\text{-H}_2$ and a field of $-0.35 \mu\text{T}$. This time, the

sample was transferred to a 7 T pre-clinical MRI scanner for collection of a series of ^{15}N MRI images rather than single scan ^{15}N NMR spectra, as shown in Figure 4.1. It is noted though that the ^{15}N polarisation yield suffers in this study due to a number of factors. The first is the $p\text{-H}_2$ purity is now 92%. The second is due to the longer transport distance for the sample which adds 20 seconds before signal acquisition, and therefore the degree of relaxation that takes place before measurement is increased. The last reason is the 10 mm tube itself. Although a greater volume of $p\text{-H}_2$ is now in the headspace of the tube, the actual shaking of the tube is more difficult due to the increased weight leading to less efficient sample mixing. These factors therefore combined to result in a ^{15}N polarisation of $\sim 5\%$ being achieved.

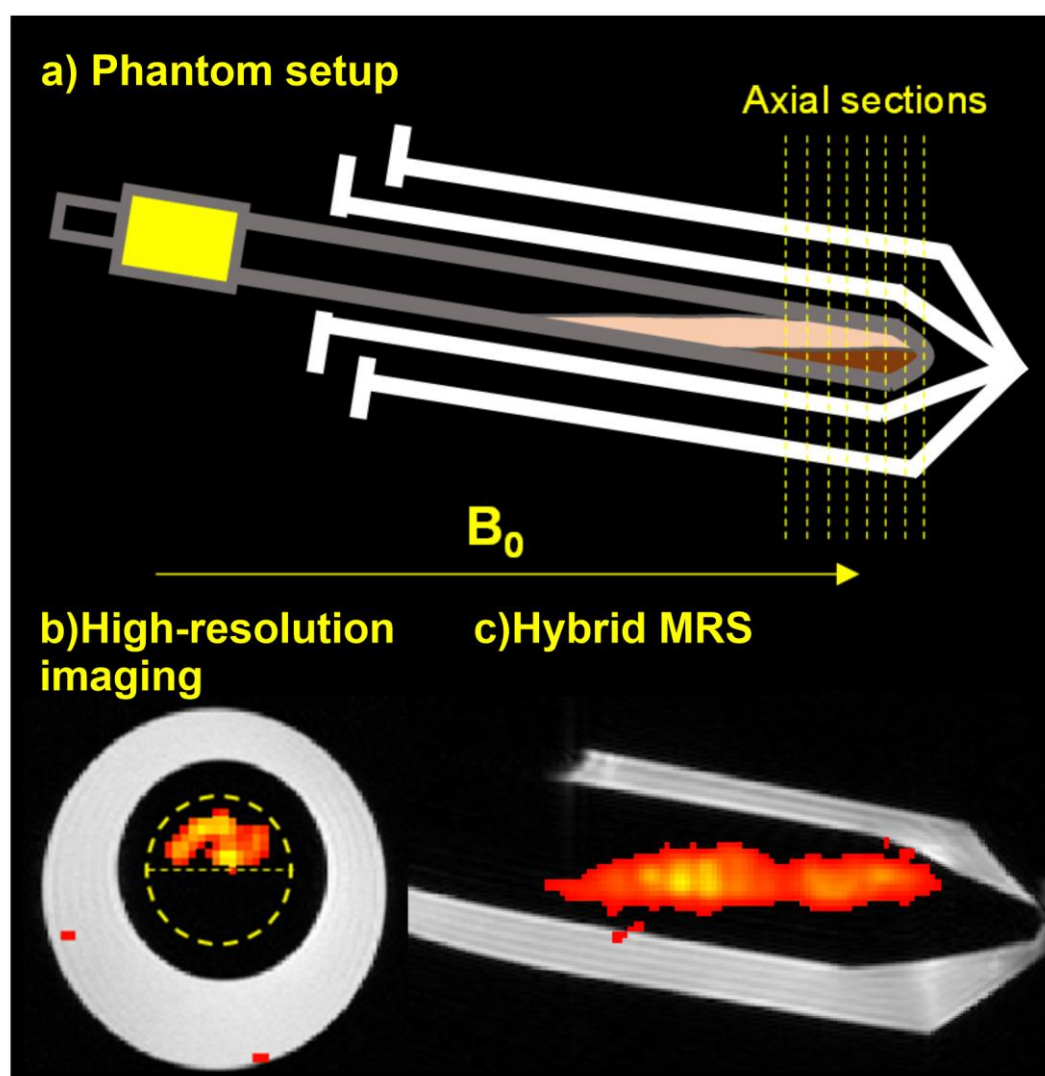


Figure 4.1 a) Setup for hyperpolarised imaging at 7 T of a reaction cell used for the biphasic preparation of $[\text{}^{15}\text{NO}_2]^-$ top solvent layer here is D_2O and bottom layer dichloromethane- d_2 ; b) high-resolution three-dimensional axial acquisition using spiral (out) encoding of 6 cm^3 over a $64 \times 64 \times 8$ matrix with a 6 s acquisition time; c) hybrid MRS(I) data collected using an EPSI pulse sequence and a 64×64 matrix (512 spectral points).

It is very clear from these data that **Y** is entirely located in the aqueous layer (top) as evidenced by the strong ^{15}N NMR signal coming from this area of the tube. These data therefore prove that the hyperpolarised **Y** can be produced in a biocompatible medium and that the signal strengths achieved are sufficient for high sensitivity ^{15}N imaging to take place.

4.3 Evaluation of T_1 lifetime for $[\text{}^{15}\text{NO}_2]^-$

The second important property of a potential hyperpolarised MRI contrast agent is the signal lifetime. It was therefore appropriate to determine the T_1 of **Y**.

4.3.1 Assessment of hyperpolarised ^{15}N NMR signal lifetime of $[\text{}^{15}\text{NO}_2]^-$

The first assessment of T_1 lifetime for **Y** was undertaken on a sample of **1** (2 mg) with 50 equivalents of **Y** and 6 equivalents of **G** in methanol- d_4 . This sample was fully activated under a pressure of 4 bar H_2 before being shaken for 10 s under 4 bar of $p\text{-H}_2$ and a PTF of $-0.35\ \mu\text{T}$. All further samples in this chapter were shaken under these conditions whenever a shake and drop experiment was undertaken. Upon transfer to an 9.4 T NMR spectrometer, a series of low tip angle pulses then acquire the ^{15}N NMR spectra that were collected. Each of these spectra used a pulse length equivalent to 10° , and loss is assessed in the analysis, see experimental Section 7.2.5. The resulting loss in signal gain is thus quantified as a function of T_1 relaxation. Figure 4.2 demonstrates a stacked plot of these 10° pulses, showing the ^{15}N signal of **Y** attenuating in an exponential manner.

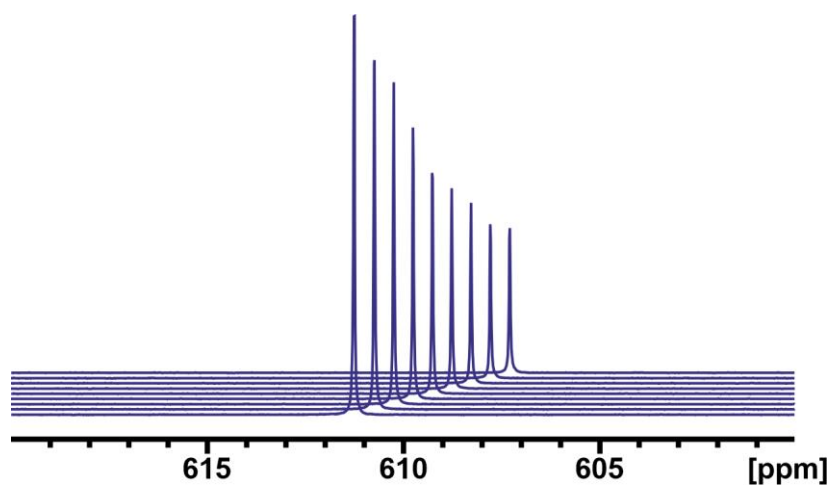


Figure 4.2 A series of ^{15}N NMR spectra, obtained from a hyperpolarised sample of **Y** to encode T_1 . These NMR spectra were acquired consecutively at 9.4 T using a 10° degree pulse with a delay of 5 seconds between each acquisition.

From these data, it is possible to fit an exponential function and extract a value for T_1 . This analysis was undertaken and is shown in Figure 4.3.

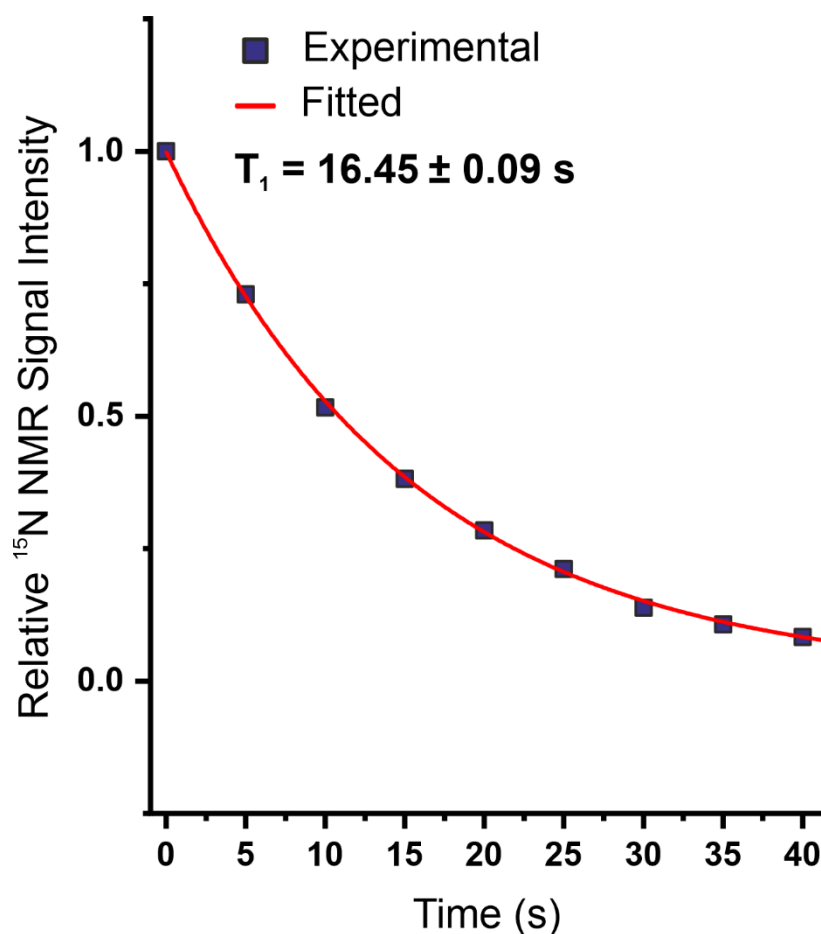
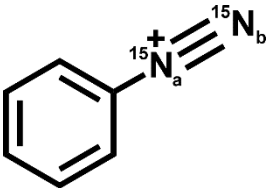
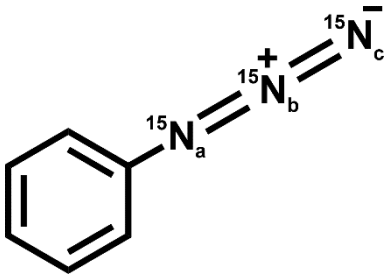
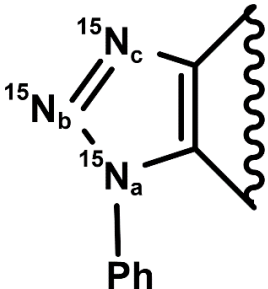


Figure 4.3 Exponential decay curve, fitted to the experimental ^{15}N NMR data to estimate T_1 relaxation. The sample was made up in methanol- d_4 stored at a field of 9.4 T and a temperature of 298 K. It contained 50 equivalents of **Y** relative to the $\text{IPr}^{\text{NMe}_2}$ pre-catalyst used.

The calculated T_1 value of 16.45 ± 0.09 s lies close to that previously reported for **Y** of 14.8 s¹⁶⁹ However, as this value was reported in D_2O as a solvent and at a storage field of 5.8 T the two values are not comparable. It has been shown by Theis *et al.* that the T_1 of hyperpolarised ^{15}N material can extend with lower storage fields,⁶³ a trend known for thermally polarised material for many years.⁶⁹ The effects caused by solvent and magnetic storage field will be explored shortly for **Y**. This signal lifetime gives ample time for the reactions carried out in the previous chapter to be observed as there is roughly 48 s ($3 \times T_1$) of available signal to probe. Using this small tip angle approach it was possible to measure the T_1 of reaction products formed in the previous chapter. These data are shown in Table 4.1. It is important to note that only data points from the complete reaction could be used. This is due to the complexity of the system. There is initial growth in signal from the conversion and then decay due to T_1 relaxation and also further reaction.

The modelling of these systems was attempted but a convincing fit could not be determined.

Table 4.1 Calculated T_1 values at 11.7 T for various reaction products from Chapter 3.

Chemical Structure	^{15}N Chemical Shift / ppm	T_1 / s
Diazonium 	a = 315.0	39 ± 3
	b = 232.6	29 ± 2
Azide 	a = 90.3	56 ± 8
	b = 242.5	192 ± 43
	c = 232.0	101 ± 19
Triazole 	a = 255.3	7 ± 2
	b = 335.4	5 ± 2
	c = 252.7	—*

* Signal from the hyperpolarised N_c site was too weak to determine a T_1 value.

The T_1 values calculated for the phenyl diazonium and azide products are much longer than **Y** and this explains why they are readily detected by the small tip angle approach used in Chapter 3; not much signal is lost before they are detected. It is noteworthy that the T_1 of N_b for the phenyl azide revealed a much larger fitting error than the other resonances. This is likely due to contributions from *bi*- or *tri*- ^{15}N labelled products. When repeating the study with the ^{15}N label of the azide at natural abundance,

its T_1 value was 211 ± 15 s. It is also noted there is also a large increase in signal to noise since only a single isotopologue forms. Surprisingly, even though the two triazole resonances exhibit low T_1 values, it is still readily detectable as a reaction product. The high errors for these again arise from low signal strength.

4.3.2 Impact of solvent on the T_1 value of $[^{15}\text{NO}_2]^-$.

It has already been shown that the hyperpolarised ^{15}N NMR signal of **Y** remains detectable in the biphasic mixture for long enough to complete an imaging study. However, it is important to know precisely how long the signal will last to ensure there is enough lifetime to prepare a sample for injection by separating the aqueous and organic layers. To probe this process, a series of samples were made; the first contained 2 mg of **1** with 50 equivalents of **Y**, 6 equivalents of **G** and 50 equivalents of 15-crown-5 in 0.6 mL of a 50/50 dichloromethane- d_2 /D $_2$ O mixture. The next contained the same amounts of **1**, **Y**, **G** and 15-crown-5, but instead was dissolved in 0.6 mL of dichloromethane. The last sample contained no catalyst, but the same amount of **Y** (10.9 mg) as the other samples in 0.6 mL of D $_2$ O. T_1 measurements were then carried out as discussed previously. These T_1 data are presented in Table 4.2.

Table 4.2 T_1 values for **Y** at 9.4 T in the indicated solvent. Each sample contained the same amount of **Y**, with all apart from D $_2$ O initially containing the pre-catalyst **1** (2 mg), 6 equivalents of **G** and 50 equivalents of 15-crown-5.

Solvent	^{15}N chemical shift / ppm	T_1 / s
methanol- d_4	612	16.45 ± 0.09
dichloromethane- d_2	618	14.33 ± 0.08
D $_2$ O*	609	18.32 ± 0.13
dichloromethane- d_2 /D $_2$ O	609	17.24 ± 0.15

*calculated using an inversion recovery pulse sequence.

It can be seen from these data that the solvent had an effect on the T_1 value. The longest T_1 value was obtained in D $_2$ O when no catalyst was present. The shortest T_1 for **Y** was obtained in dichloromethane- d_2 .

Subsequently, samples containing the same amount of **Y** were prepared in methanol- d_4 and dichloromethane- d_2 (note the dichloromethane- d_2 sample still contained

a stoichiometric amount of 15-crown-5 to aid solvation) and T_1 values measured by inversion recovery. These were 20.05 ± 0.36 and 25.36 ± 2.09 s for methanol- d_4 and dichloromethane- d_2 respectively. This confirms that while the solvent had an effect on T_1 (likely due to polarity and viscosity changes) it is clear the catalyst also had an effect.

4.4 Extension of T_1 lifetime

A number of factors relating to SABRE have been reported in the literature as effecting the T_1 relaxation such as the external magnetic field around the sample, the amount of substrate relative to the polarisation transfer catalyst, and the temperature at which the sample is stored. If these effects can be minimised it might be possible to further extend the visible lifetime of a signal from **Y**.

4.4.1 T_1 lifetime as a function of $[^{15}\text{NO}_2]^-$ concentration

As shown previously the Ir catalyst can have a significant impact on the T_1 of nuclei of interest, which is due to the substrate, in this case **Y**, experiencing a greater number of spin interactions while attached to the larger catalyst, which increases its relaxation rate. To probe this effect a series of samples were prepared each containing 2 mg of **1** with 6 equivalents of **G** and varying amounts of **Y** (from 5 to 65 equivalents compared to Ir) in a 0.6 mL solution of methanol- d_4 . These samples were then analysed by ^{15}N NMR spectroscopy to determine the T_1 of **Y** as shown in Figure 4.4. All these studies used the hyperpolarised T_1 method introduced previously.

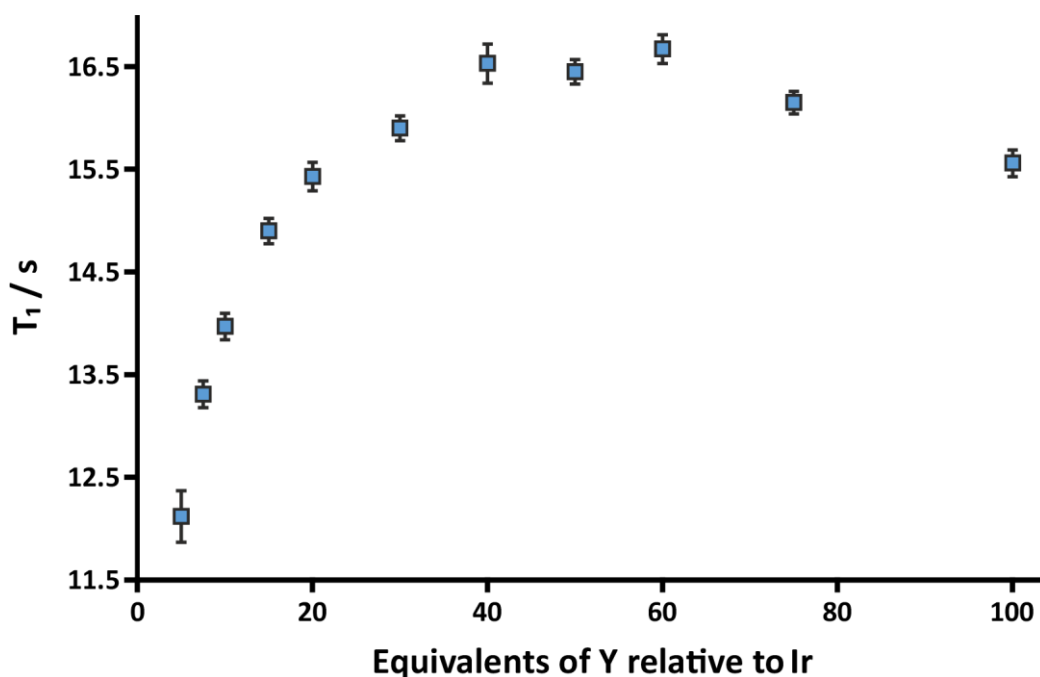


Figure 4.4 T_1 of **Y** as a function of ratio of **Y** to iridium. For all samples, the concentration of **1** was 5 mM and the concentration of co-ligand **G** 30 mM. T_1 data was collected at a field of 9.4 T using the hyperpolarised signal decay method already outlined.

These data show a clear effect from having the catalyst present. At low equivalents of **Y**, the T_1 is lowered significantly. However, at higher equivalents of **Y** the effect of the catalyst becomes less pronounced and the T_1 reaches a plateau around 16.5 s. It is noted that this is still lower than the ~20 s T_1 achieved for **Y** in the methanol without any catalyst present. Hence, the catalyst (or $[\text{NO}_2]^-$) must still have an intrinsic intramolecular dipole-dipole effect that aids T_1 . These data demonstrate, however, that the enhanced exchange contribution can be minimised. This is consistent with higher concentrations of **Y** resulting in lower T_1 values due to increased spin-spin interactions from other ^{15}N nuclei. To maximise T_1 , the optimum ratio of **Y** lies between 40 and 60 equivalents compared to the catalyst.

It was also hypothesised therefore that lowering the amount of catalyst present would limit the effect it had on T_1 . To test this hypothesis, another series of samples were made, this time fixing the concentration of **Y** at 250 mM and altering the amount of **1**. The concentration of **G** was also kept at 30 mM. These samples were then studied in the same way as before. The resulting data are presented in Figure 4.5.

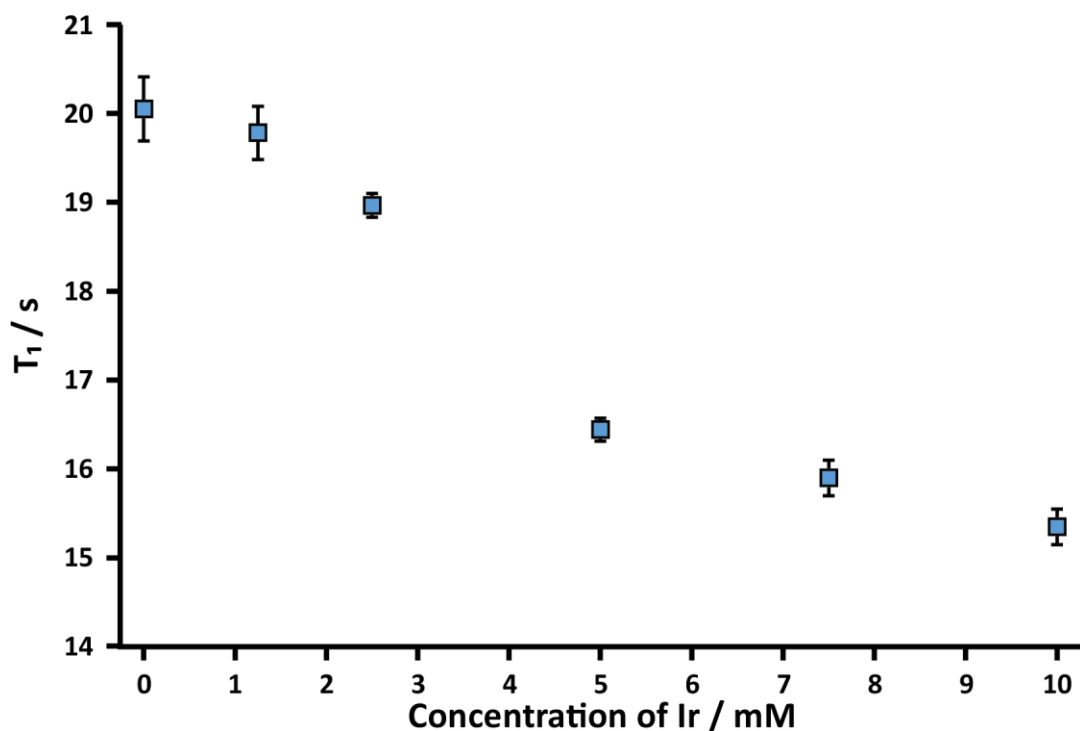


Figure 4.5 T_1 of **Y** given as a function of concentration of **1** present in the sample. All samples contained 250 mM of **Y** and 30 mM of **G**. All T_1 values were collected at 9.4 T and calculated using the hyperpolarised signal decay method outlined previously apart from that with 0 mM Ir, which was calculated by inversion recovery.

As predicted, these data shows that low concentrations of **1** allow the T_1 of **Y** to return to the same level as when no **1** was present in the sample, thus negating any effect from **1**. Therefore, when using a 250 mM concentration of **Y** it is optimum to only have ~1.25 mM of catalyst present in the solution.

4.4.2 T_1 lifetime of $[\text{}^{15}\text{NO}_2]^-$ as a function of magnetic storage field

The ‘storage field’, here, refers to the field the sample is placed into after the polarisation transfer step (shaking the sample under a pressure of $p\text{-H}_2$ in a μ -metal shield) is complete. This doesn’t necessarily have to be the same field the ^{15}N NMR signal will ultimately be acquired in, but it can simply be where the sample is located while it is cleaned-up for use in imaging. Three different methods were tested to probe the T_1 lifetime of **Y** across these different storage fields. These methods and their results will be introduced separately for clarity before comparing them.

The first study was implemented on a sample containing a 250 mM concentration of **Y** in methanol- d_4 (0.6 mL) with no catalyst or co-ligand present. The sample was degassed using the freeze-pump-thaw method and filled to a pressure of 4 bar H_2 . The T_1 was determined by inversion recovery, on various NMR spectrometers capable of ^{15}N

signal acquisition ranging from 7.05 T to 16.4 T. For proton precession frequency, this ranges from 300 to 700 MHz. Figure 4.6 shows a typical T_1 inversion recovery plot gained from this method at a 16.4 T field and the rest of these data are presented in Table 4.3.

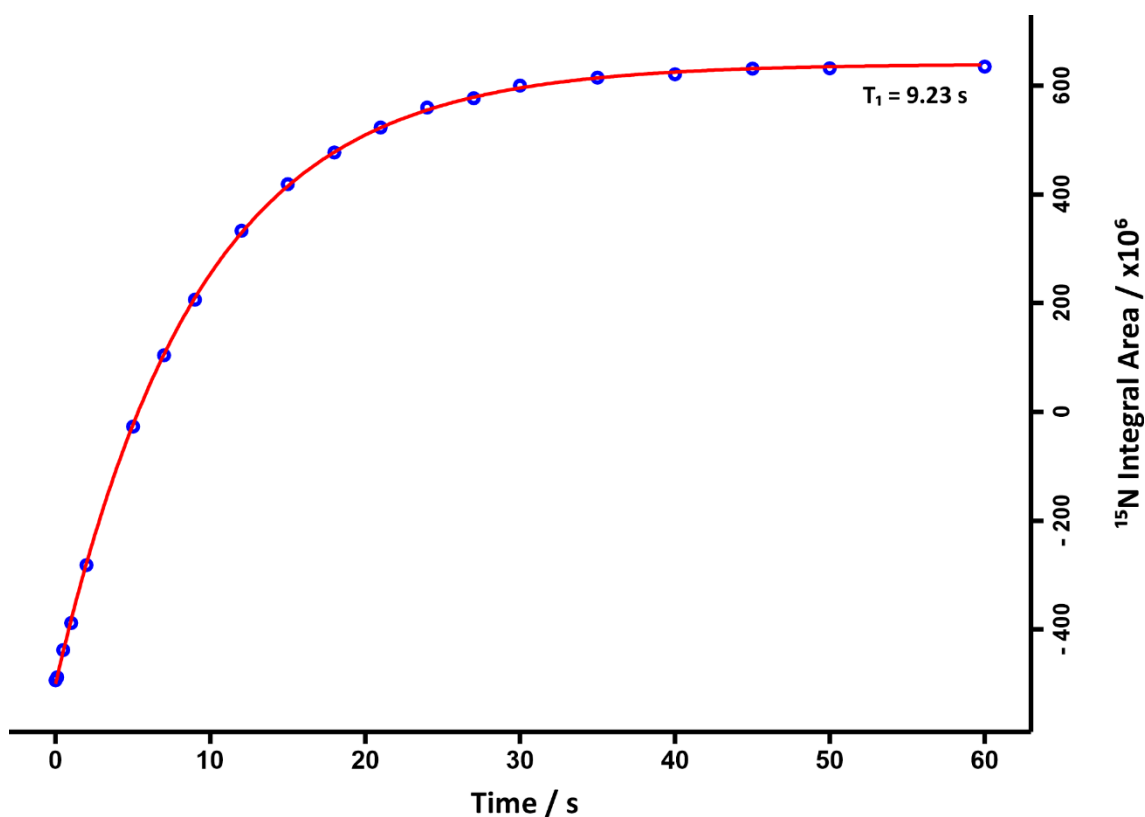


Figure 4.6 Typical T_1 inversion recovery curve gained from a 250 mM sample of **Y** in methanol- d_4 at a field of 16.4 T.

Table 4.3 T_1 values for **Y** gathered at various magnetic fields using a T_1 inversion recovery pulse sequence. All data was gathered on a sample of **Y** (250 mM) in methanol- d_4 under a hydrogen atmosphere of 4 bar.

Magnetic Field / T	Spectrometer ¹ H Frequency / MHz	¹⁵ N T_1 of Y / s
7.05	300	23.07 ± 0.50
9.4	400	20.05 ± 0.36
11.7	500	14.13 ± 0.15
14.1	600	11.42 ± 0.07
16.4	700	9.23 ± 0.05

The data gathered from this study show a clear trend where T_1 increases as the storage field strength decreases. Unfortunately, access to lower field spectrometers with capability to detect at the frequency required for ^{15}N was not possible. Therefore to measure the T_1 at lower fields alternate methods needed to be implemented.

The second method for measuring the T_1 of **Y** at these lower fields involved conducting a series of ‘shake and drop’ style experiments. In these experiments, a sample containing **1** (1.25 mM), **Y** (250 mM) and **G** (30 mM) was degassed and filled with 4 bar of $p\text{-H}_2$ and shaken in a field of $-0.35\ \mu\text{T}$ for 10 s storing the sample inside a 1 T field for varying lengths of time before transporting the sample to a 9.4 T instrument for immediate ^{15}N signal acquisition. For ease of recall, this will be referred to as the ‘ T_1 shake and store’ approach and is outlined in the experimental. The 1 T field used here was provided a 43 MHz permanent magnet located in a ‘spinsolve’ benchtop NMR spectrometer manufactured by Magritek. The sample was refilled with fresh $p\text{-H}_2$ before each shake was conducted. This method yields data that could then be fit to a hyperpolarised T_1 decay curve as shown in Figure 4.3.

It is important to note that there will be a considerable error in these measurements due to differences in the field the sample experiences during transport and acquisition. However, the timings of each step were recorded throughout the study and found to only differ by ± 1 s. Previous shake and drop studies with **Y** have produced polarisation levels with a 10% variation in signal intensity, so the maximum source of error is likely to come from the magnetic field variations in the sample transfer pathway which cannot be controlled with the current setup. Figure 4.7 shows the hyperpolarised T_1 decay curve for these data. If all other aspects of the experiment have successfully been kept the same, the decay observed here should reflect the effect of storing the sample at 1 T for different amounts of time.

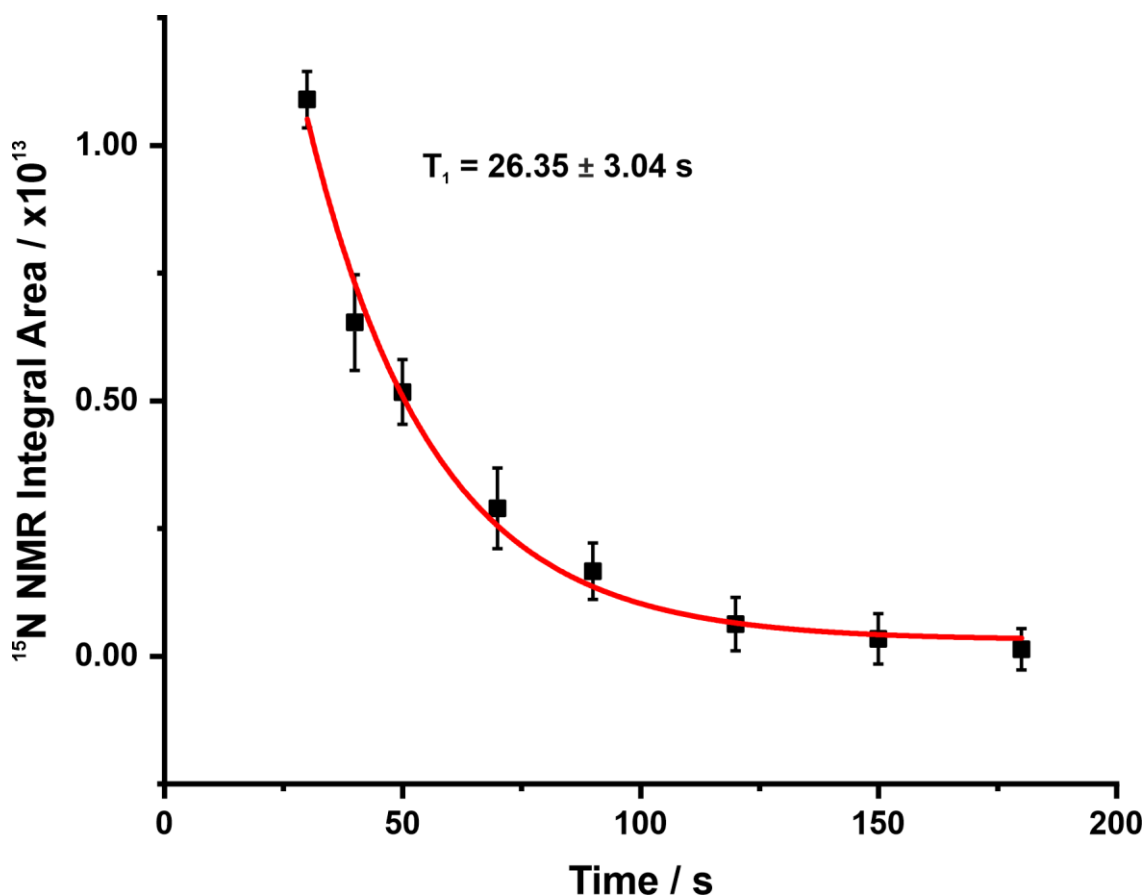


Figure 4.7 Average ^{15}N NMR signal integral area for *Y* plotted against time at a field of 1 T (40 MHz ^1H precession frequency). The sample consisted of 0.5 mg of *1* with 200 equivalents of *Y* and 24 equivalents of *G*. Each data point consisted of three repeat shakes of 10 s at a field of -0.35 μT with a pressure of 4 bar *p*- H_2 .

While there is an improvement in T_1 , it is less pronounced than hoped for. Nonetheless, it still fits the trend shown in Table 4.3. Notably, it was not possible in this study to probe the T_1 in storage fields between 1 and 7.05 T due to lack of magnets in this field range. It was however possible to evaluate the T_1 at lower fields using the ‘ T_1 shake and store method’ and the study was therefore repeated using the earth’s magnetic field as a storage field. While this field varies naturally between 25 and 65 μT it averages around 50 μT and so this is the value that will be reported here. The study was repeated, storing the sample at a distance greater than 3 m away from any spectrometers or permanent magnets for different lengths of time before transfer to a 9.4 T spectrometer for signal acquisition, the data for which is shown in Figure 4.8.

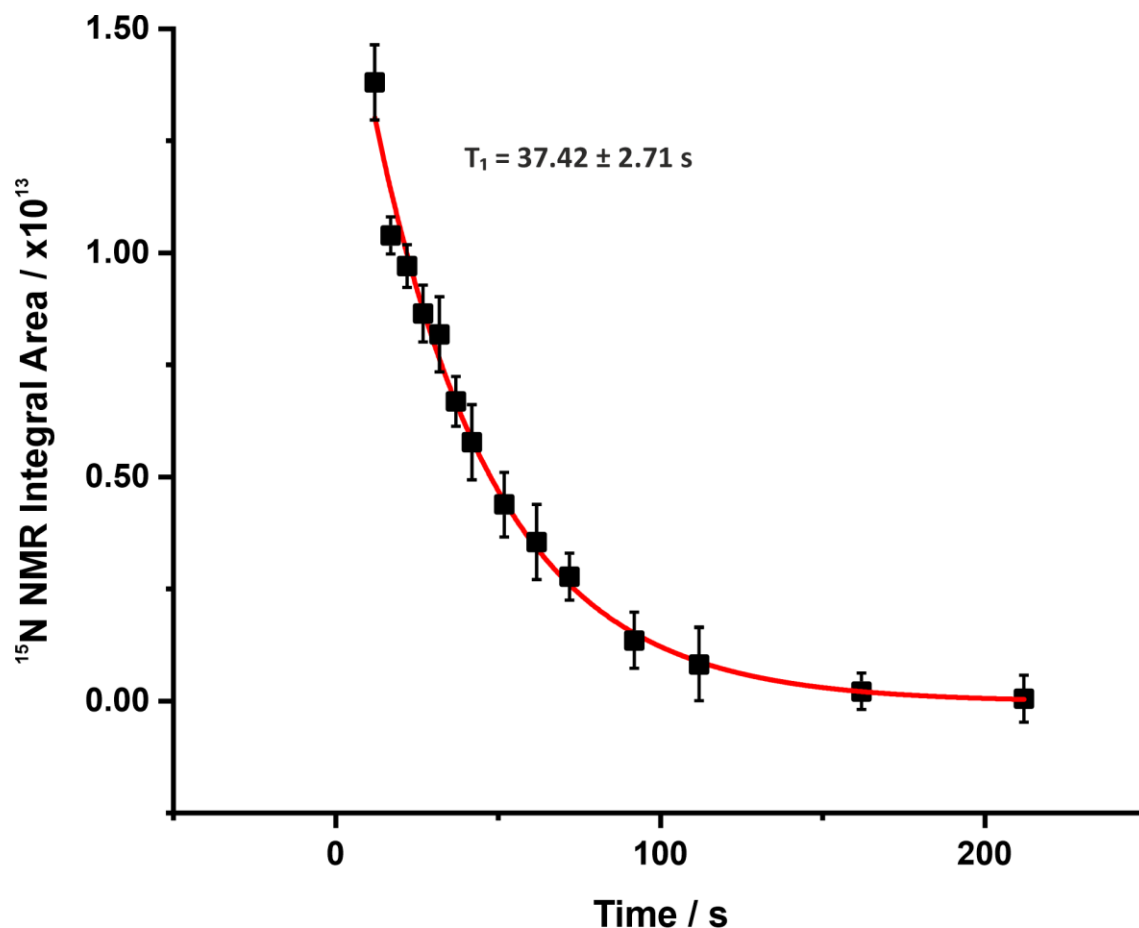


Figure 4.8 Average ^{15}N NMR signal integral area for **Y** plotted against time at a field of $50\ \mu\text{T}$ (Earth's Field). The sample consisted of $0.5\ \text{mg}$ of **1** with 200 equivalents of **Y** and 24 equivalents of **G**. Each data point consisted of three repeat shakes of $10\ \text{s}$ at a field of $-0.35\ \mu\text{T}$ with a pressure of $4\ \text{bar } p\text{-H}_2$.

As shown by the Figure 4.8, the trend of increasing T_1 with lower field remains. This is ideal for sample manipulation, as no extra equipment is needed to store the sample at the Earth's magnetic field meaning the field can be easily accessed anywhere. The ^{15}N T_1 of **Y** gained from this data was $37.2 \pm 1.7\ \text{s}$ meaning that there would be around $110\ \text{s}$ (around $3 \times T_1$) to manipulate the sample for injection. To probe even lower fields a new and final method of ^{15}N hyperpolarisation was employed.

This final method utilised the flow system discussed previously in Chapter 3. The sample containing **1** ($1.25\ \text{mM}$), **Y** ($250\ \text{mM}$) and **G** ($30\ \text{mM}$) was remade and probed by recording a series of ^{15}N NMR spectra. These spectra were recorded as separate experiments adding a variable delay after transfer to the spectrometer before signal acquisition. Before each experiment the sample was bubbled with fresh $p\text{-H}_2$ for $10\ \text{s}$ followed by immediate transfer to the spectrometer for ^{15}N NMR signal acquisition after the aforementioned delay had passed. This method generated an exponential decay curve for **Y** stored at $9.4\ \text{T}$ which revealed a T_1 of $17.14 \pm 0.07\ \text{s}$ which is comparable to that

achieved by hyperpolarised T_1 decay and inversion recovery methods. As this experiment showed that similar results could be achieved with the flow system, further experiments were undertaken whilst storing the sample at a field of $-0.30 \mu\text{T}$ and 0 T . This was achieved by placing a variable delay in the pulse sequence of the flow system method in which the sample would be stored inside the solenoid of a μ -metal shield for varying lengths of time before transfer to the 9.4 T spectrometer followed by immediate ^{15}N NMR signal acquisition. The T_1 decay curves generated at $0.00 \mu\text{T}$ and $-0.35 \mu\text{T}$ field are shown in Figure 4.9.

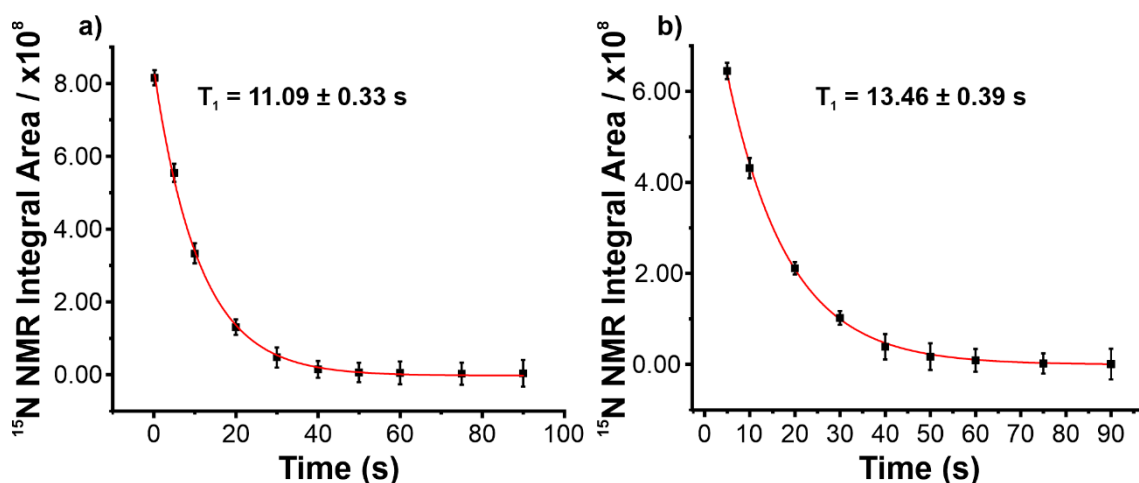


Figure 4.9 Average ^{15}N NMR signal integral area for **Y** plotted against time at a field of a) $0.10 \mu\text{T}$ and b) $0.35 \mu\text{T}$. The sample consisted of 0.5 mg of **1** with 200 equivalents of **Y** and 24 equivalents of **G**. Each data point consisted of three repeats of bubbling 10 s of $3 \text{ bar } p\text{-H}_2$ at a field of $-0.35 \mu\text{T}$ followed by storage in specified field for the set time.

A dramatic drop in T_1 is observed when the storage field is decreased here. The T_1 observed when **Y** was stored in a field of -0.35 and $0.00 \mu\text{T}$ was 13.46 ± 0.39 and $11.09 \pm 0.33 \text{ s}$ respectively. It is perhaps not surprising that the T_1 is slightly extended in the $-0.35 \mu\text{T}$ as SABRE operates in this regime and so will slightly replenish observable magnetisation until $p\text{-H}_2$ has been depleted in solution. Figure 4.10 displays the T_1 of **Y** at all the fields collected for easy comparison.

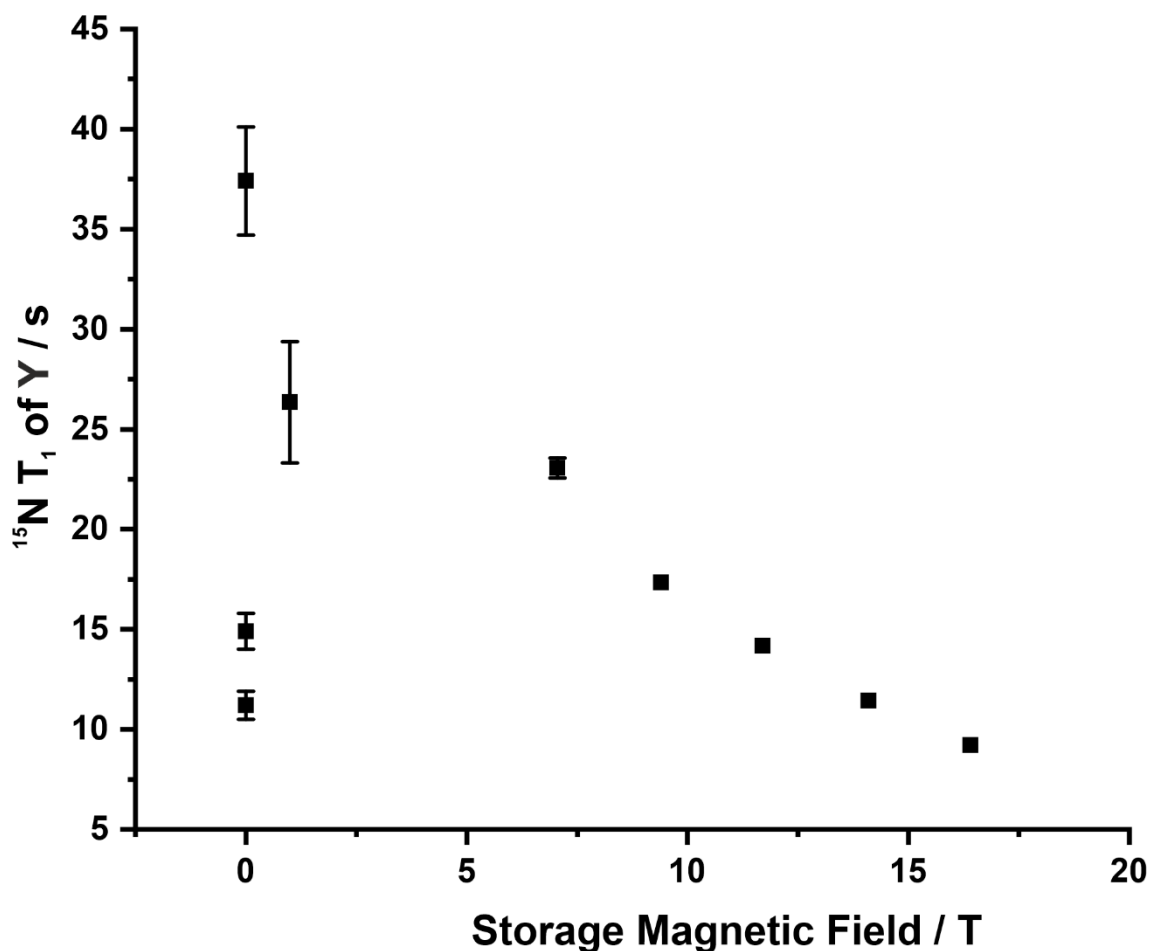


Figure 4.10 $^{15}\text{N } T_1$ values for **Y** shown as a function of the absolute magnetic field used to store the sample. The methods used to collect each T_1 value did differ and so directly comparing the exact values of data points at the different fields should be treated with caution.

From these data it is clear to see the ^{15}N NMR signal lifetime of **Y** increases until 50 μT is reached. While the actual optimum storage field is still not known, the Earth's magnetic field may reflect a good compromise. Here, the T_1 of ~37 s results in two minutes of observable signal ($3 \times T_1$) and so allows for the sample to be treated with ease without the need for extra equipment such as a permanent magnet for the storage field. While this is a very positive result, the effect of temperature still needed to be considered.

4.4.3 T_1 lifetime of $[\text{}^{15}\text{NO}_2]^-$ as a function of temperature

It is known from the literature that the T_1 of nuclei vary with temperature as this affects the motion of molecules in solution.^{69, 190} Ideally, the hyperpolarised sample of **Y** should be stored at a temperature that moves the speed of this motion away from the precession frequency of ^{15}N . To determine this temperature, a sample containing 250 mM of **Y** in methanol- d_4 was probed by ^{15}N NMR spectroscopy. A series of T_1 inversion

recovery experiments were performed at temperatures ranging from 233 to 318 K to see the effect of temperature on the ^{15}N T_1 lifetime, a plot for which is shown in Figure 4.11.

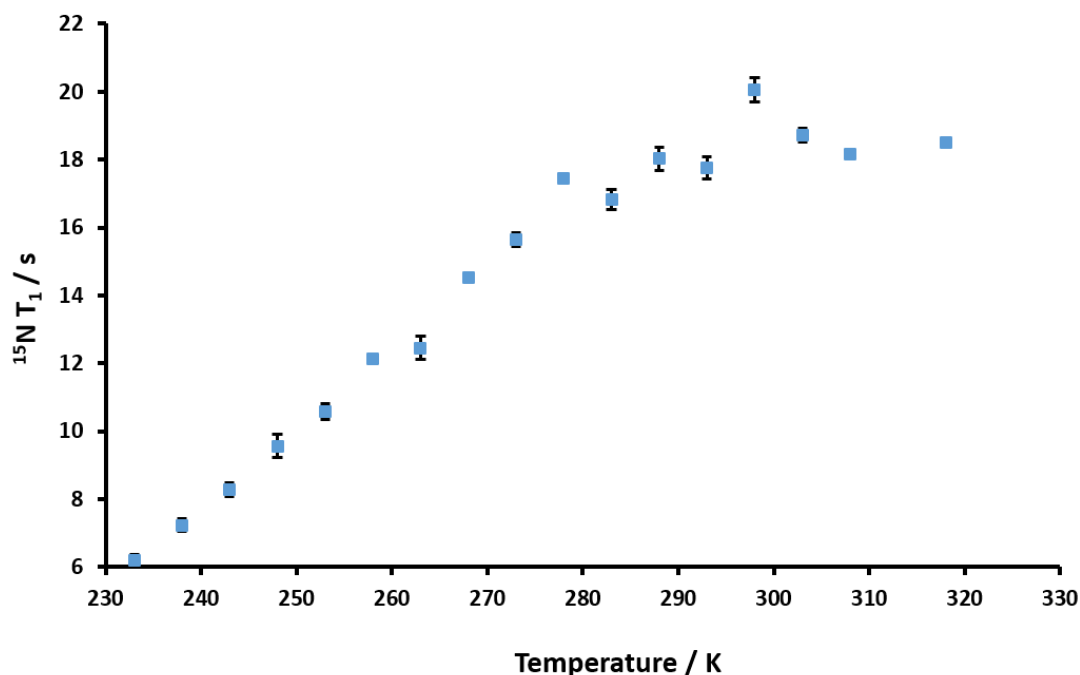


Figure 4.11 ^{15}N T_1 lifetime of **Y** presented as a function of temperature. These data were collected on a 250 mM sample of **Y** in methanol- d_4 at a field of 9.4 T.

There is a very clear effect of temperature on the ^{15}N T_1 of **Y**. Thankfully, the T_1 reaches a plateau at around 280 K and maintains a maximum somewhere between 17 and 20 s. Knowing the optimum storage temperature of hyperpolarised **Y** is around room temperature is ideal as it once again means no specialist equipment is needed and the sample can be treated in the Earth's field at around 20°C without any considerable increased loss in signal due to T_1 . This, therefore, concludes the studies of optimising storage conditions for **Y** in solution.

4.4.4 T_1 lifetime of $^{15}\text{NO}_2^-$ in the solid state.

The next section of the thesis looks at alternate storage methods for **Y**, this work is preliminary but is proving to be very promising in the extension of the hyperpolarised signal lifetime. It has previously been shown in the literature by Levitt *et al.* that nuclear spin order can be preserved by precipitation of the substrate after being hyperpolarised by DNP.¹⁹¹ This method of solid state storage of hyperpolarised materials has very recently been applied by Blank *et al.* utilising DNP also.¹⁹² Similar work has been undertaken by Ellis *et al.* preserving the ^{13}C NMR signal lifetime of biologically relevant fumarate hyperpolarised by PHIP through the reaction of acetylene dicarboxylate with *p*-

H_2 .¹⁹³ The work outlined here instead utilises SABRE as the hyperpolarisation method and **Y** as the substrate of interest. In this scenario, much like the work of Ellis *et al.*,¹⁹³ storage in the solid state would also allow easy separation from the SABRE catalyst and toxic methanol solvent. Before trialling the method with hyperpolarised material, it was important to first determine whether the ^{15}N of **Y** had a reasonable T_1 lifetime in the solid state. To do this a 3 mm rotor was packed with a sample of ^{15}N labelled **Y** and analysed by ^{15}N NMR spectroscopy. The sample was probed under 20 kHz magic angle spinning (MAS). A saturation recovery experiment was then undertaken on the sample, the data for which is shown in Figure 4.12. As the T_1 was hoped to be longer than that in solution (it would not be feasible to wait $5 \times T_1$ between data points) so saturation recovery approach was a more appropriate method choice.

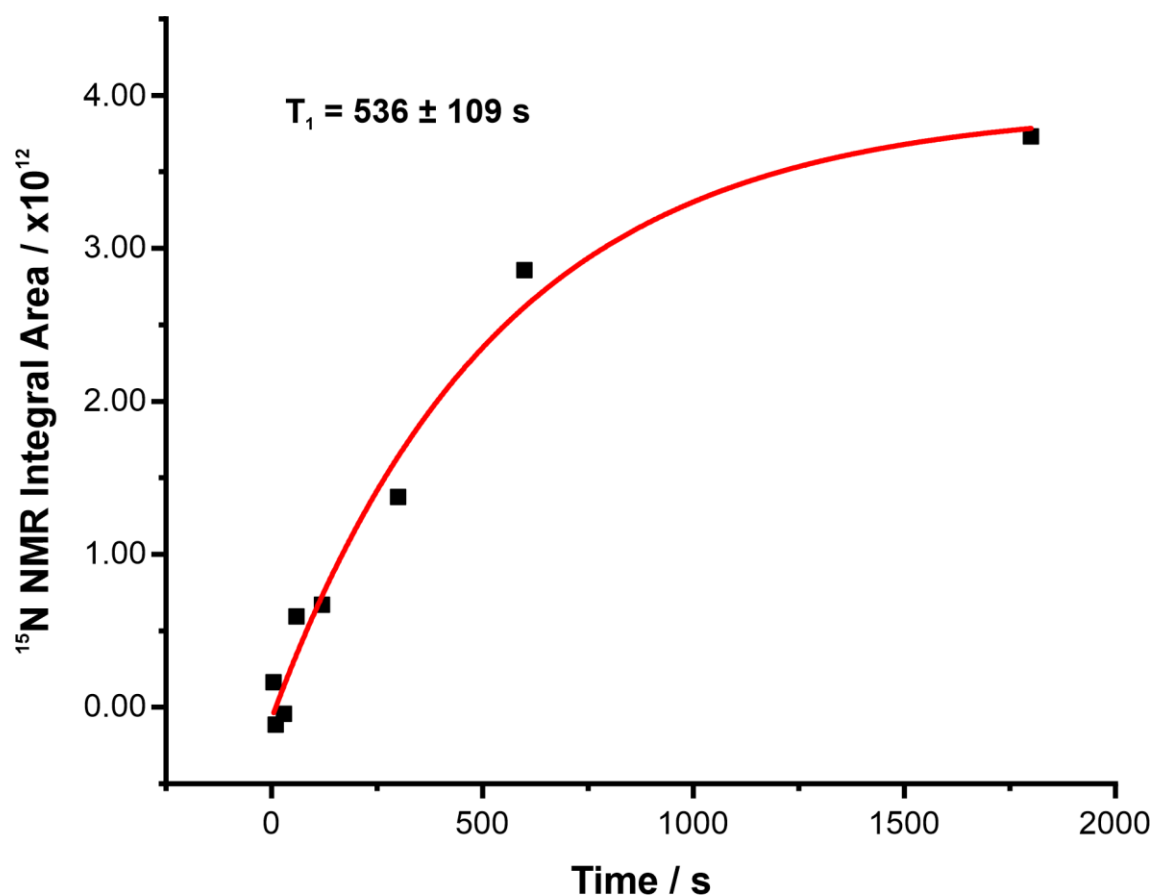


Figure 4.12 Typical ^{15}N T_1 saturation recovery plot from a sample containing **Y**. The experiment utilised a 3 mm rotor with 20 kHz MAS and was conducted at a field of 9.4 T

The ^{15}N T_1 of **Y** proved to extend to 536 ± 109 s, although there is a significant fitting error due to low signal to noise. Even if the lower bound is taken, this reflects a significant improvement in signal lifetime of over 11-fold. This T_1 value would provide 20 minutes of observable signal at 9.4 T, which is likely to extend even further at lower storage fields.⁶³ However, it still needed to be proven that the hyperpolarised signal of **Y**

survived the transformation from a solution in methanol- d_4 to the solid state, and then dissolution to return it to water as a biocompatible solvent. To test this a new solvent system had to be drawn up so that **Y** could be easily precipitated when required.

The solution to this was to use a solvent that was miscible with methanol, where **Y** was only sparingly soluble. The solvent identified for this was methylperfluorobutylether (**J**). A test was therefore undertaken to ensure that the detection of hyperpolarised **Y** was still possible in the presence of **J**- the introduction of the ^{19}F spins could promote ^{15}N relaxation. A sample was therefore prepared containing 2 mg of **1** with 25 equivalents of **Y** and 6 equivalents of **G** in a mixture of 0.3 mL methanol- d_4 and 0.3 mL of **J**. The sample was degassed before placing under an atmosphere of 3 bar H_2 for 30 minutes to activate it. Upon activation, the sample was analysed by ^1H NMR spectroscopy. This revealed hydride ligand ^1H NMR resonances at δ -21.74 and -22.64, matching those of complex **12G**. As already discussed, this complex is the major SABRE active species when a pure methanol- d_4 solvent is used and so confirms that the desired SABRE catalyst still forms in this solution.

A sample containing **12G** (5 mM) in 0.6 mL of a 1 : 1 mixture of methanol- d_4 and **J** respectively was then probed for ^{15}N NMR SABRE activity using the shake and drop method. The sample was shaken in a field of -0.30 μT for 10 s under a pressure of 3 bar $p\text{-H}_2$. It yielded a hyperpolarised ^{15}N NMR response for **Y** immediately after transporting the sample into a 9.4 T field following the polarisation transfer step. The ^{15}N signal enhancement achieved was 18090 ± 570 fold at a field of 9.4 T, which equates to $5.97 \pm 0.19\%$ ^{15}N polarisation. This represents a dramatic improvement over the polarisation gained from the same shaking conditions in methanol- d_4 alone that was presented in Table 3.3 ($2.95 \pm 0.13\%$). To determine the maximum polarisation achievable in this solvent, a further study was then undertaken to secure the optimum PTF. It was expected to be around -0.30 μT . The results of this study are shown in Figure 4.13.

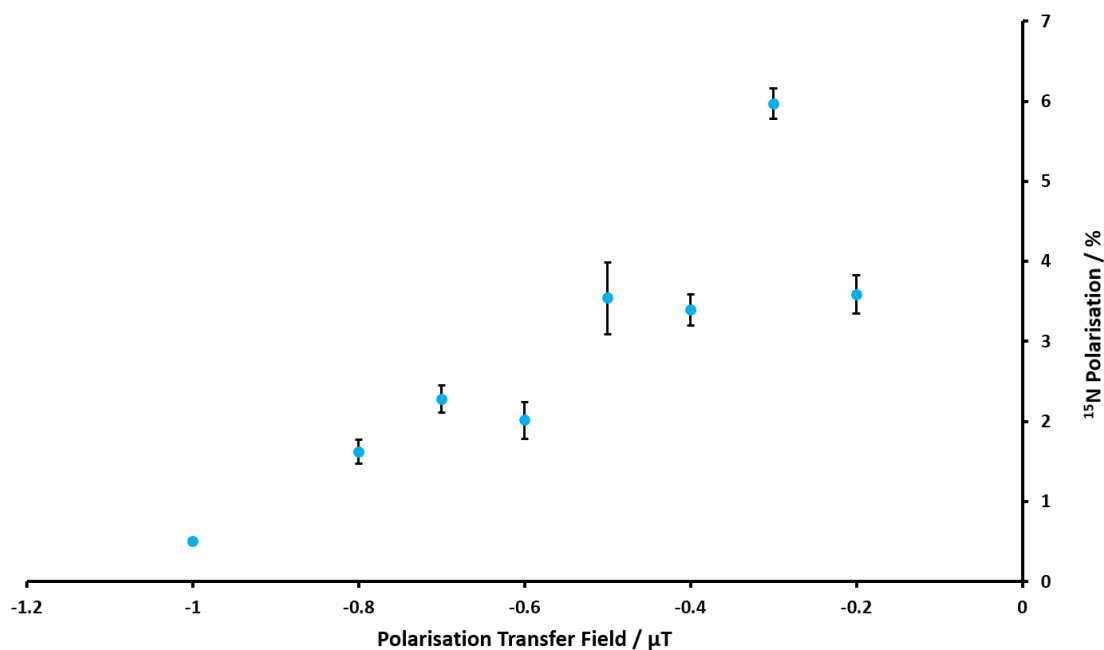


Figure 4.13 ^{15}N NMR signal acquired at 9.4 T as a function of polarisation transfer field used. The sample probed consisted of 2 mg of **1** with 25 equivalents of **Y** and 6 equivalents of **G** used as a co-ligand. At each PTF the sample was shaken with 3 bar of fresh $p\text{-H}_2$ for 10 s.

This study revealed that $-0.30\ \mu\text{T}$ was by far the most optimum transfer field tested. The improvement observed in the polarisation transfer to **Y** is of interest as it has already been shown the same complex forms, and so different catalytic species cannot be the reasoning behind the observation. Instead one of three reasons could potentially explain the difference; a different ligand dissociation rate in the solvent mixture, a longer T_1 for **Y** than in the pure methanol, or an increased amount of $p\text{-H}_2$ in the solution due to the better gas solubility in the solvent **J**.¹⁹⁴

The first of these hypotheses to be tested was the ligand dissociation rate in the new solvent **J**. A series of EXSY measurements were undertaken which revealed a dissociation rate of $0.48 \pm 0.01\ \text{s}^{-1}$ for **G** (c.f. $0.13 \pm 0.01\ \text{s}^{-1}$ for **G-d₂** in methanol- d_4 at 298 K) showing that there are clear solvent effects on the SABRE process. It has already been shown from the data in Table 3.4 and Figure 3.5 of Chapter 3 that an increased ligand dissociation rate is beneficial for the SABRE of **Y**. This improvement here should put the ^{15}N NMR signal enhancement at a similar level to that achieved with **SIMes** according to the Figure 3.5. However, the ^{15}N NMR signal enhancement achieved here is still higher than this so there must be other factors contributing to the improvement.

The next experiment was to evaluate the T_1 of **Y** in the methanol/**J** mixture. To undertake this study a fresh sample was made containing 2 mg of **1** with 25 equivalents of **Y** and 6 equivalents of **G** in 0.6 mL of a 1 : 1 mixture of methanol- d_4 and **J**. The sample

was degassed and activated with hydrogen before carrying out the T_1 experiments. The sample was shaken in a field of $-0.30\ \mu\text{T}$ for 10 s under a pressure of 4 bar $p\text{-H}_2$ before transfer to a 9.4 T magnetic field where a series of small tip angle pulses were applied as described in the experimental Section 6.2.5, hyperpolarised T_1 decay method. Fitting an exponential to these data revealed a ^{15}N T_1 for **Y** of 17.26 ± 0.07 s, which is significantly higher than the same conditions in pure methanol. This increase in T_1 allows an increased level of ^{15}N polarisation to be achieved before reaching a plateau as shown in the Figure 3.6. It was expected that the T_1 would increase even further in the mixture, although this has not yet been explored.

It is well known in the literature that perfluorocarbons (PFCs) such as **J** are very efficient at dissolving gases.^{194, 195} It has been shown earlier in this thesis in Figures 3.7 and 3.8 that an increased amount of $p\text{-H}_2$ led to higher levels of polarisation,¹⁵² and so it is likely that any increased H_2 solubility would be beneficial, especially when combined with the increased ligand dissociation rate and increased T_1 . The quantification of H_2 gas solubility in **J** will be explored in the next chapter when discussing SABRE in PFC solvents.

Interestingly, if an extra 0.3 mL of **J** is added to a sample containing 2 mg of **1** with 25 equivalents of **Y** and 6 equivalents of **G** in 0.6 mL of 1 : 1 mixture of methanol- d_4 and **J**, it causes the ionic **Y** to precipitate out of the solution as a white powder that sediments to the bottom of the tube. This opens the opportunity to hyperpolarise **Y** and then add **J** to precipitate it and thereby store the ^{15}N polarisation in the solid state. An example for ^{13}C was shown by Levitt *et al.* where the T_1 extended the lifetime of the biomolecule fumarate 6-fold. Figure 4.14 shows pictorially the method being used to hyperpolarise **Y** then precipitate into the solid, separate from the solution and re-dissolve for detection.

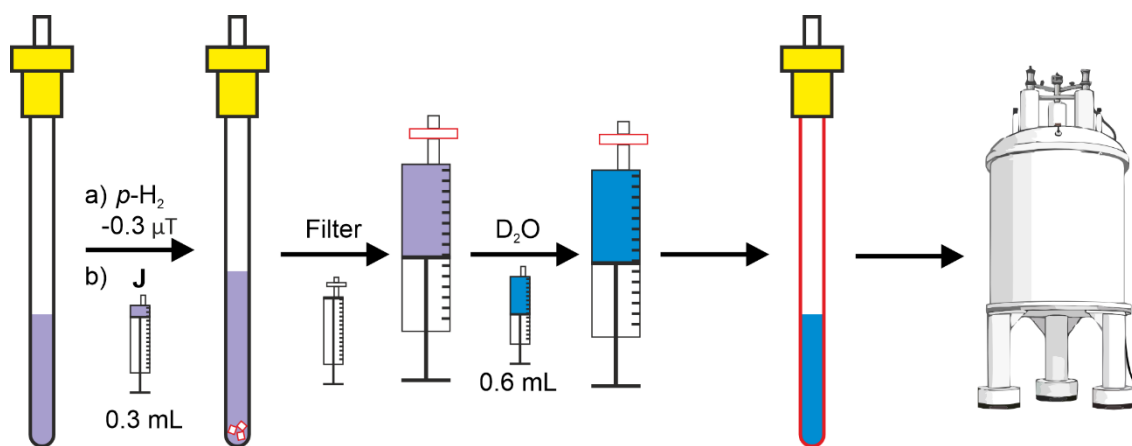


Figure 4.14 Method outline for the hyperpolarisation of **Y** followed by precipitation through addition of more **J**. The solution is filtered through a syringe filter to separate the precipitate before redissolving in water and transferring to the NMR spectrometer for signal acquisition. The red outline represents the location of hyperpolarised **Y**.

As shown in Figure 4.14, a syringe filter (0.45 μm) was utilised to extract the solid from the solvent mixture before re-dissolving the precipitate of **Y** in D_2O for ^{15}N NMR signal acquisition. It should be noted that this method comes with a high degree of uncertainty in the resulting ^{15}N NMR signal as there are many factors that contribute to signal losses such as the introduction of *paramagnetic* oxygen gas, some **Y** remaining dissolved in the **J**/methanol- d_4 mixture, some **Y** remaining dissolved in the D_2O on the filter and not re-entering the tube and the difference in crystallinity of the precipitate.

Despite these factors, the experiment was tested on the sample containing 2 mg of **1** with 50 equivalents of **Y** and 6 equivalents of **G** in 0.3 mL 1 : 1 mixture of methanol- d_4 and **J** was shaken for 10 s in a field of $-0.30 \mu\text{T}$ before carrying out the precipitation and dissolution procedure. The sample was then inserted into the 9.4 T spectrometer for immediate ^{15}N NMR signal acquisition. A control experiment was also carried out on the sample to measure the level of polarisation achieved before precipitation. To do this the sample was shaken under the same conditions, and yielded a ^{15}N NMR response 4770 ± 290 fold larger than the thermally equilibrated response, corresponding to around $1.57 \pm 0.10\%$ ^{15}N polarisation. The entire precipitation and dissolution procedure was timed as taking 28 s, and when tested it yielded a hyperpolarised ^{15}N NMR response for **Y** with a signal enhancement of 1241 ± 187 fold corresponding to a ^{15}N polarisation level of $0.41 \pm 0.06\%$

Equation 7.1 presented in the experimental Section 7.2.3 was used to fit the data of Figure 4.8 and hence determine roughly how much the polarisation level would have fallen by during this time in the earth's magnetic field. A value of 0.74% was predicted.

This shows that under these conditions precipitating **Y** and re-dissolving had some effect on the polarisation obtained. It is important to remember however that the sample travelled through many different magnetic fields while being processed all which will have effects on relaxation. It is also possible not all the **Y** precipitated and so the signal that survives is artificially lower than expected because of this. The important conclusion from the experiment is that the ^{15}N polarisation survives the processing and therefore transitioning to the solid phase and back into solution does not destroy the polarisation.

As the first experiment was successful in preserving some ^{15}N polarisation a second experiment was attempted, but increasing the storage time in solid state to 360 s. For this storage time all the hyperpolarised ^{15}N nuclei of **Y** should have relaxed fully to thermal equilibrium if the T_1 remains at 37 s. When this experiment was conducted, a weak signal was observed after the dissolution in D_2O corresponding to around 0.02% ^{15}N polarisation as shown in Figure 4.15.

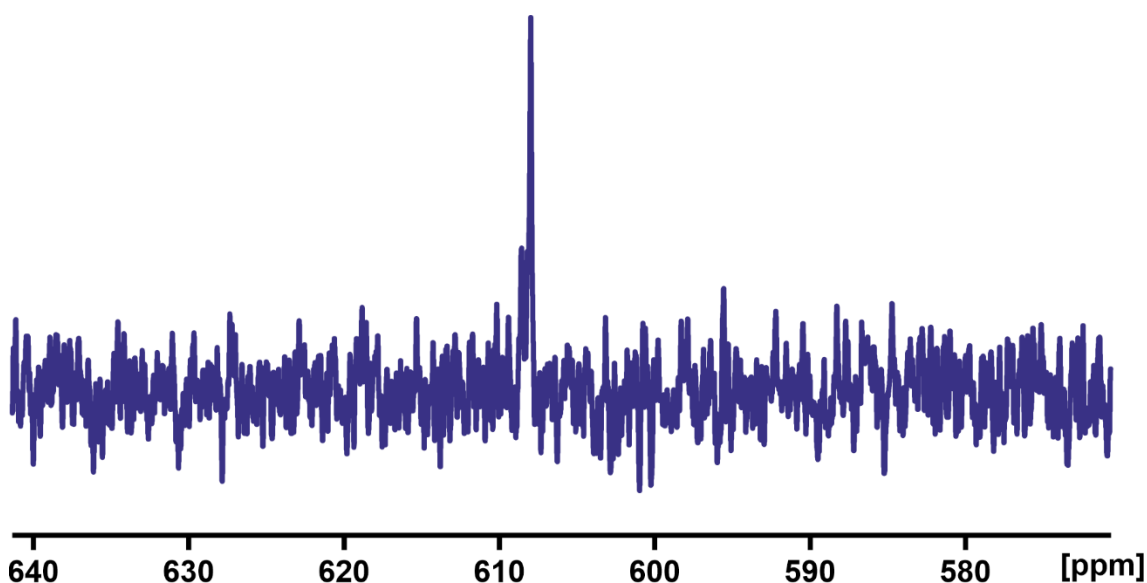


Figure 4.15 A typical ^{15}N NMR spectrum collected at a field of 9.4 T in a single scan acquisition on a sample initially containing 2 mg of **1** with 50 equivalents of **Y** and 6 equivalents of **G** dissolved in 0.6 mL of a 1 : 1 mixture of methanol- d_4 and **J**. The sample was treated as described in the text before acquiring the spectrum.

Hence, although the ^{15}N signal observed here is relatively weak compared to that achieved so far, this result represents a great milestone in preserving ^{15}N hyperpolarised signals by showing that it is possible. If a larger initial polarisation was produced using the optimum conditions laid out in Chapter 3, it can be assumed a higher residual polarisation would remain after the processing carried out here. Hence, there is now precedent to try this method for other highly polarisable substrates outlined in the

literature, such as metronidazole,^{43, 64} to establish if the ^{15}N polarisation can be sustained in the same way.

As mentioned the precipitation method carried out here could also double to ‘clean’ the sample ready for injection. To test the efficiency of this method the sample was probed after the precipitation method by ^1H NMR spectroscopy as shown compared to a typical spectrum before the precipitation in Figure 4.16.

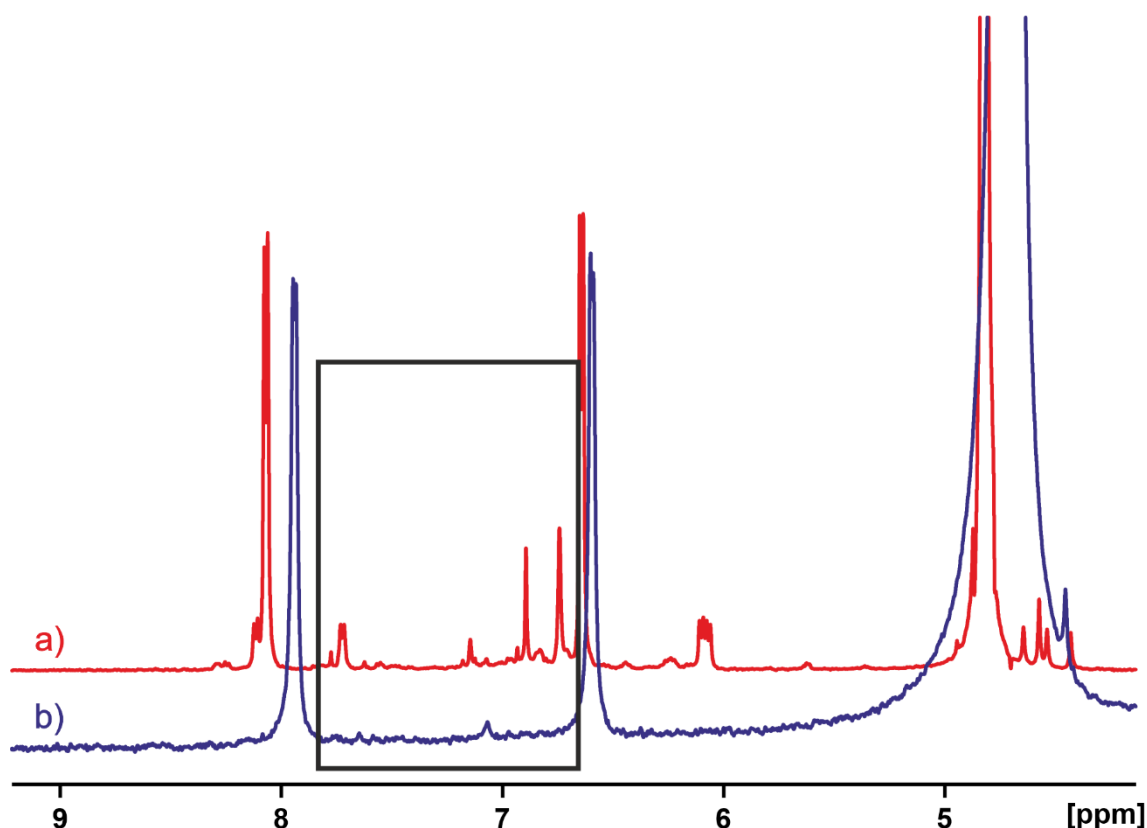


Figure 4.16 a) A typical ^1H NMR spectrum of a sample containing 2 mg of **1** with 50 equivalents of **Y** and 6 equivalents of **G** in 0.3 mL 1 : 1 mixture of methanol- d_4 and **J**. b) The ^1H NMR spectrum resulting from the same sample now in D_2O after the process in Figure 4.14 had been carried out. The spectrum now shows a lack of ^1H resonances typical of those for the Ir catalyst. Both spectra were recorded with 16 signal averages at 9.4 T.

Excitingly the spectrum acquired in Figure 4.16 shows no trace signals of the Ir catalyst present after 16 signal averages. This is a very promising result pushing towards bio-compatible SABRE for applications in *in vivo* imaging. It is noted, however, that a substantial amount of co-ligand **G** still remains after treatment and so a different co-ligand would have to be used, or a further removal procedure would have to be carried out as **G** is acutely toxic.

4.4.5 Assessment of T_1 lifetime of $^{15}\text{N}_2$ triplet state in solution

The previous attempts to measure the ^{15}N signal lifetime of $^{15}\text{N}_2$ produced were unsuccessful. This was put down to the low gas solubility of N_2 in methanol leading to the gas escaping too quickly to measure a T_1 . As previously mentioned the solvent **J** and other PFCs have a very high affinity for dissolving gas and so it is more likely for the determination of the ^{15}N T_1 of $^{15}\text{N}_2$ to be possible.

As shown previously, **Y** can be mixed with $^{15}\text{NH}_4\text{Cl}$ to spontaneously produce $^{15}\text{N}_2$. It was decided to implement this method again here, whilst ensuring no gas was released from the tube. The method of Figure 4.17 was utilised and the J. Young's cap of the tube replaced before mixing the two solutions. This method relies on the surface tension of the water holding the solution in place in the tube, preventing it from falling. This method could be further improved by providing a removable physical barrier such as a screw tap to allow the method to be carried out on a hyperpolarised sample.

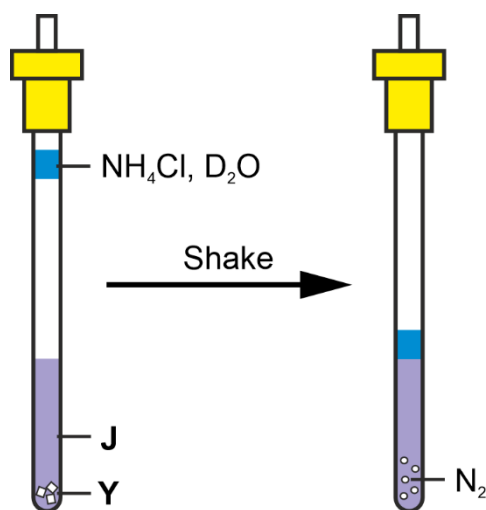


Figure 4.17 Formation of N_2 gas from the reaction of $[\text{NO}_2]^-$ and $[\text{NH}_4]^+$ as shown in Figure 3.18.

The method was therefore tested, measuring 42 mg of **Y** into the bottom of the NMR tube with a 0.6 mL of a **J**, and the top of the tube with a 0.1 mL of a solution of D_2O containing a stoichiometric amount of $^{15}\text{NH}_4\text{Cl}$. The lid was then replaced on the tube and the tube shaken to mix the contents and initiate the reaction. The amounts of both reactants were calculated to produce N_2 at a 3 bar pressure so as not to overpressure the NMR tube. This method was successful, and a thermal ^{15}N NMR peak at δ 308 could be easily seen for $^{15}\text{N}_2$ as shown in Figure 4.18. All further measurements in this chapter were then conducted on this same sample. For each experiment the sample was locked

and shimmed to a sample of pure methanol- d_4 of the same sample depth as the mixture presented here, experiments were then run with the lock turned off.

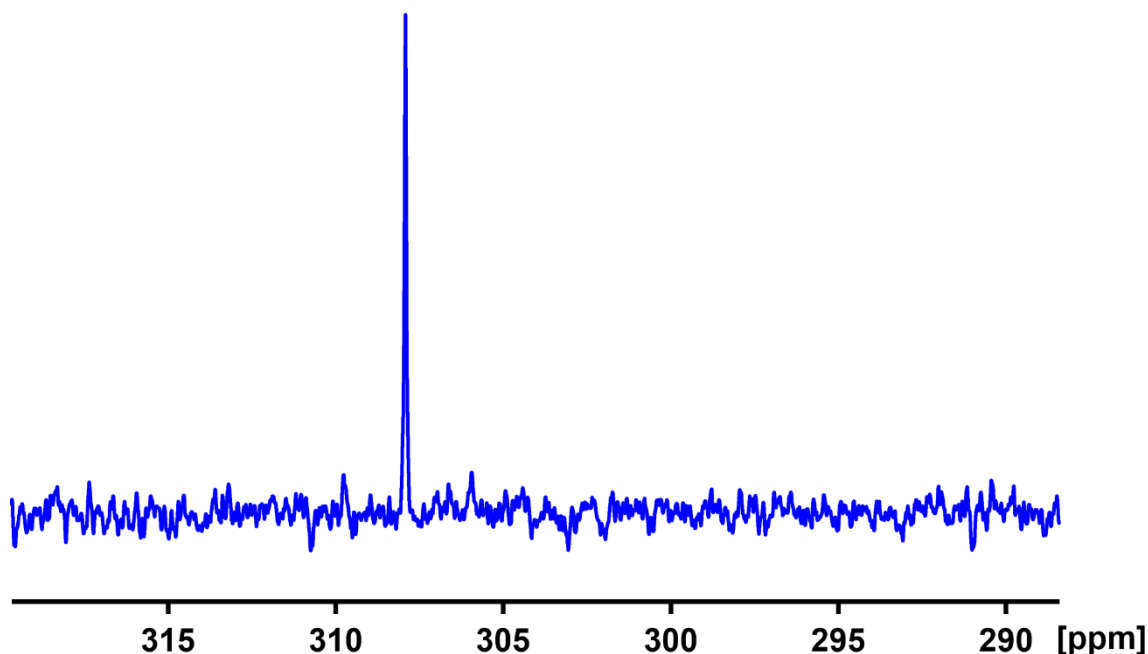


Figure 4.18 A typical ^{15}N NMR spectrum of a sample containing 0.6 mL of *J* and 0.1 mL of D_2O under a pressure of around 3 bar $^{15}\text{N}_2$. The signal at δ 308 is from $^{15}\text{N}_2$ dissolved in the mixture. The spectrum was acquired using 16 signal averages at 9.4 T.

The presence of dissolved $^{15}\text{N}_2$ gas now allowed for the determination of its T_1 by inversion recovery. To ensure a large enough relaxation delay, 100 s was used between each scan. This experiment revealed a T_1 growth curve yielding a T_1 value of just 2.30 ± 0.08 s as shown in Figure 4.19.

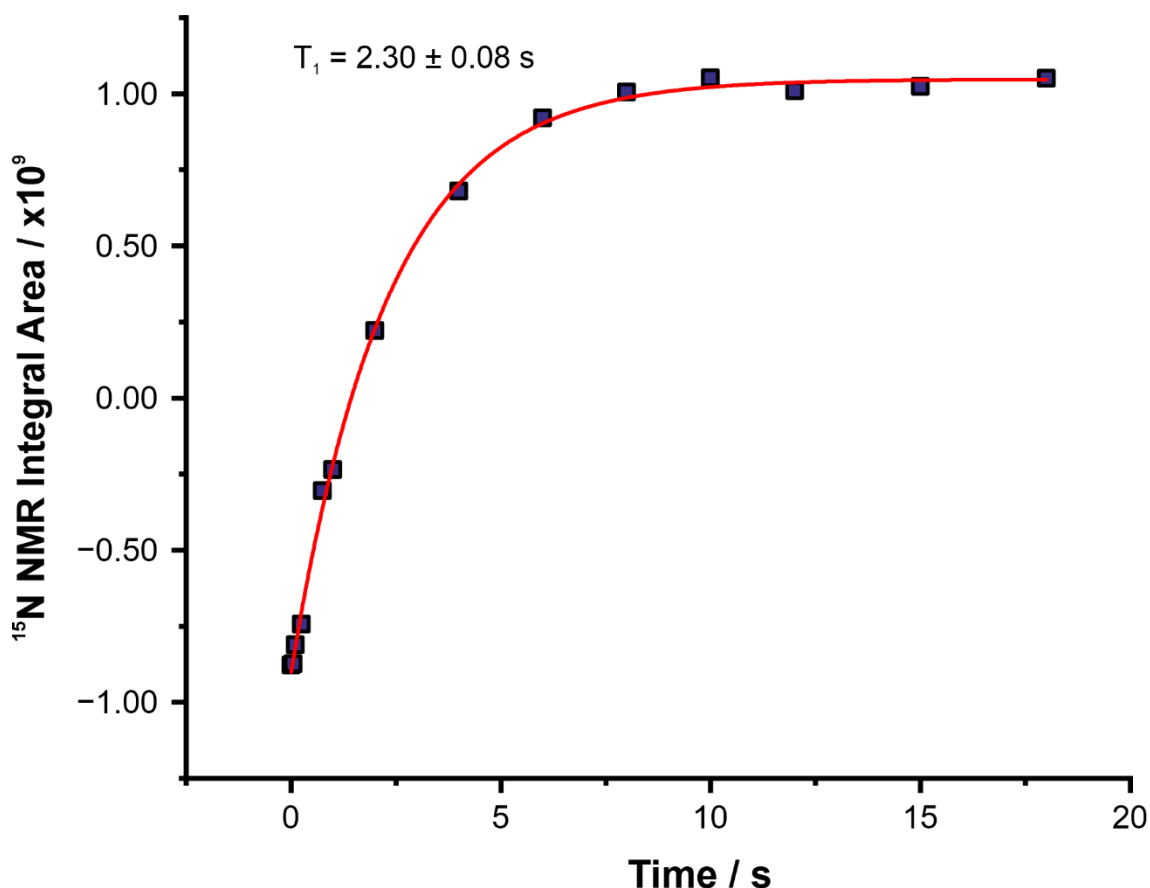


Figure 4.19 T_1 inversion recovery curve for the ^{15}N NMR signal of $^{15}\text{N}_2$ gained from a 0.6 mL of J and 0.1 mL of D_2O under a pressure of around 3 bar $^{15}\text{N}_2$. The study was carried out at a field of 9.4 T.

The T_1 of the $^{15}\text{N}_2$ is quite short in comparison to other ^{15}N T_1 values that have been observed throughout this thesis. This is likely partly due to the presence of *paramagnetic* oxygen present so ideally the experiment should be repeated in a glovebox. Unfortunately, this ^{15}N NMR signal lifetime is too short for MRI imaging of the lung space in a typical breath hold style experiment. If the T_1 could be extended slightly this would mean a hyperpolarised imaging agent could become a possibility. An alternative would be to generate *paranitrogen* ($p\text{-N}_2$) which is NMR silent like $p\text{-H}_2$.^{46, 47, 62} It is predicted that this would maintain the high spin ordered needed and exhibit slow conversion back to *orthonitrogen* ($o\text{-N}_2$) as the process is forbidden. However, once the $p\text{-N}_2$ enters the lung space the alveoli should inhibit the rotational motion of the $p\text{-N}_2$ and thereby allow signal detection. At this stage this is all hypothetical.

It has been shown already in Figure 4.10 that the ^{15}N T_1 can be extended significantly by storing the sample at lower magnetic fields. To test this method further the T_1 was determined for this sample of $^{15}\text{N}_2$ at various magnetic fields by the same inversion recovery pulse sequence, the data for which is shown in Figure 4.20. The 100 s relaxation time proved to be much longer than required for full relaxation ($5 \times T_1$) and so

for T_1 inversion recovery experiments for this sample at different fields a relaxation delay of 25 s was utilised instead. The range of magnetic fields tested in this study was only between 7.05 T and 16.40 T as the T_1 could now only be determined on a thermally equilibrated sample making the shake and store method used previously now obsolete. This means only the magnetic fields of spectrometers capable of ^{15}N NMR signal acquisition could be utilised.

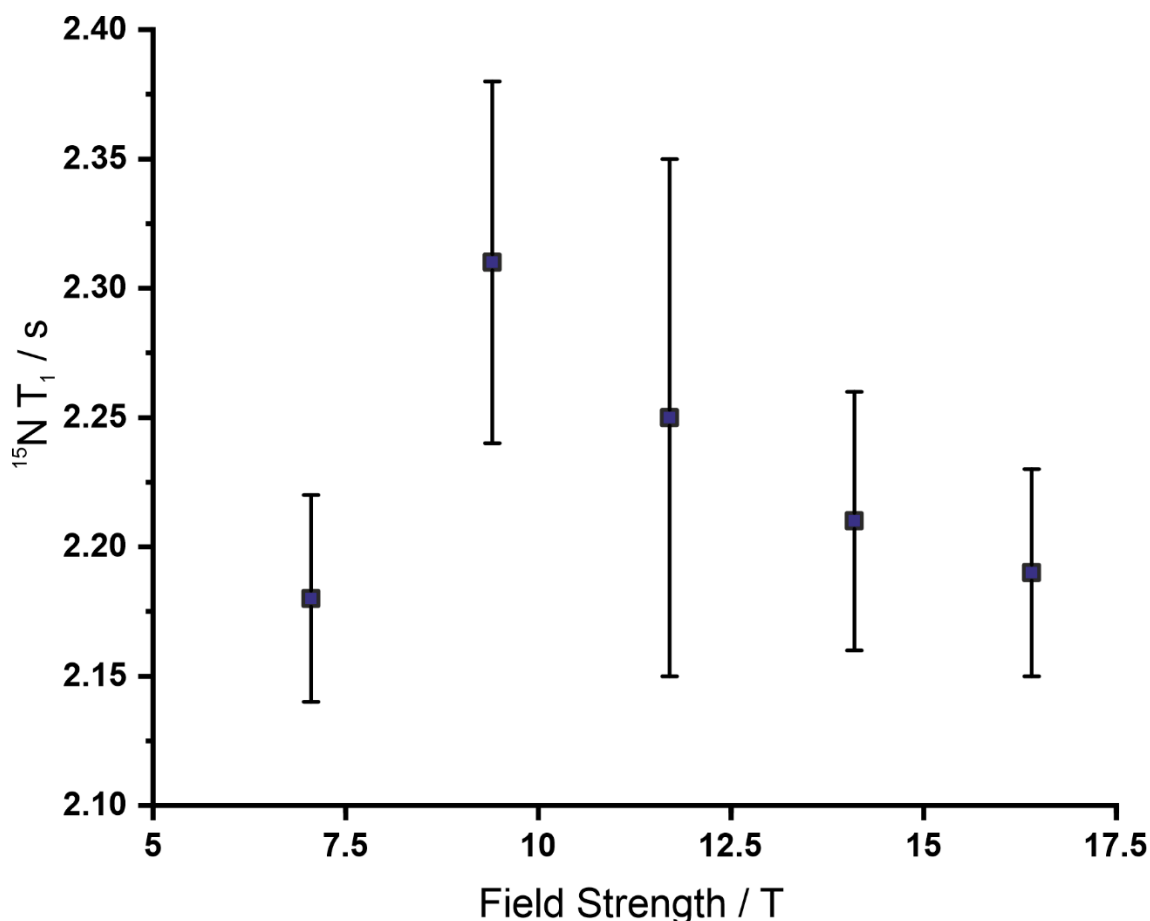


Figure 4.20 $^{15}\text{N } T_1$ values for $^{15}\text{N}_2$ dissolved in 0.6 mL of J and 0.1 mL of D_2O shown as a function of the magnetic field used to store the sample. All T_1 values were acquired using a T_1 inversion recovery pulse sequence.

Storing the sample at lower magnetic fields unfortunately did not have a significant impact on the T_1 value of $^{15}\text{N}_2$ with all tested fields yielding roughly the same T_1 value within error. This suggests the optimum magnetic storage field for this sample is much lower than tested here.^{63, 69} Unfortunately, there was not the opportunity to measure the T_1 of $^{15}\text{N}_2$ at lower fields during this research, but it is a logical next step and there are currently commercial instruments that would facilitate this. At the time of writing there is no known value for the T_1 of $^{15}\text{N}_2$ in the literature and so this represents another novel result from this research.

4.5 Conclusion

This chapter has assessed the ability of $[\text{NO}_2]^-$ (**Y**) to act as an MRI contrast agent. It was found that the hyperpolarisation of **Y** in the solvent dichloromethane- d_2 was successful, forming the optimum catalyst species from Chapter 3, $[\text{Ir}(\text{H})_2(\text{IPr}^{\text{NMe}_2})(\text{G})_2(\text{Y})]$, as the only product and yielded $2.4 \pm 0.2\%$ ^{15}N polarisation of **Y**. Importantly, it was found that the use of the macrocyclic ligand 15-crown-5 was essential in dissolving **Y** in the dichloromethane- d_2 and hence is essential to the polarisation transfer.

Utilising a mixture of D_2O and dichloromethane- d_2 eliminated the need for 15-crown-5 due to the high solubility of **Y** in the D_2O . The resulting ^{15}N polarisation here was 1.54 ± 0.14 with the system $[\text{Ir}(\text{H})_2(\text{IMes})(\text{G})_2(\text{Y})]$. However, when 15-crown-5 is added as a phase transfer catalyst the polarisation is dramatically improved to $4.6 \pm 0.3\%$ suggesting the partial solubility of **Y** in the dichloromethane- d_2 is a limiting factor to ^{15}N polarisation for this system. Further improvement was made to $8.7 \pm 2.7\%$ ^{15}N polarisation by raising the temperature of the solution before shaking to *circa* 308 K. This thus demonstrates the slow exchanging $[\text{Ir}(\text{H})_2(\text{IMes})(\text{G})_2(\text{Y})]$ benefiting from faster ligand dissociation. This was exemplified again utilising the more optimum $[\text{Ir}(\text{H})_2(\text{IPr}^{\text{NMe}_2})(\text{G})_2(\text{Y})]$; this system yielded $9.8 \pm 0.86\%$ ^{15}N polarisation of **Y**. For comparison Katz-Brull *et al.* achieved *circa* 1% ^{15}N polarisation in D_2O and so the results displayed in this thesis represent the largest ^{15}N polarisation for **Y** in a biocompatible solvent.¹⁶⁹ MRI measurements confirmed that the distribution of **Y** was indeed favoured towards D_2O making extraction by the method outlined by Manoharan *et al.* a definite possibility.¹⁸⁹

Another finding from this study was the ability to measure ^{15}N T_1 values for reaction products displayed in Chapter 3. It was however important to ensure the reaction had fully completed as not to add a growth term to the function due to more products forming. It is believed that now these T_1 values are known, rate data could be extracted from these hyperpolarised ^{15}N spectra for the processes outlined in Chapter 2. Reaction monitoring by SABRE has been shown by Semenova *et al.* for ^1H .¹⁹⁶ However, use of this data to extract rates would represent a first for the field.

It was revealed through hyperpolarised T_1 measurements of **Y** that the signal lifetime is *circa* 50 s ($3 \times$ a T_1 of 16.45 ± 0.09 s) at a 9.4 T storage field. Previous measurements by Katz-Brull *et al.* found the T_1 of **Y** to be 14.8 s when stored at a field of

5.8 T in D₂O.¹⁶⁹ Interestingly, it was found here that the T_1 of **Y** can actually be extended by storing the hyperpolarised samples at lower magnetic fields reaching a maximum T_1 value of *circa* 37 s at a storage field of 50 μ T. This is a trend also observed by Theis *et al.* for the anti-cancer drug anastrozole and so adds backing to this claim.⁶³ It is likely the lower lifetime observed by Katz-Brull *et al.* is due to the presence of highly relaxing *paramagnetic* oxygen present in the solvent. The lifetimes they observed could therefore be extended through degassing. Measurements were undertaken in this thesis which found no significant effect on the T_1 of **Y** from the solvent used. Therefore it is unlikely the difference in measurements undertaken by Katz-Brull and co-workers is due to the use of D₂O as a solvent.

Several studies were also undertaken to understand the effect of the Ir catalyst and the temperature on the T_1 of **Y**. It was found a significant effect is present from the catalyst, fitting with observations made by Rayner *et al.* on the effects of removing high γ ¹H nuclei. An amount of **Y** equal to 40 equivalents relative to the Ir were required to return the T_1 of **Y** to that of the free material (no Ir present). This substrate excess needed is likely related to the ligand dissociation rate as this determines the amount of substrate that will experience extra dipolar interactions from spins on the catalyst during the polarisation transfer process. The results of the temperature study revealed that the T_1 of **Y** suffered as temperature was decreased from 278 to 233 K with the optimum sample storage temperature being between 278 and 328 K, a promising result for potential use in metabolic imaging.

Perhaps the most staggering result of this chapter is the ability to extend the T_1 lifetime of **Y** through storage in the solid state. The T_1 of **Y** in the solid was measured to be *circa* 500 s on a crystalline sample of **Y**. Using the method outlined in Figure 4.14 it was shown that a 0.02% (60-fold ¹⁵N NMR signal enhancement) survives storage in the solid phase for 360 s, a time by which any residual ¹⁵N NMR signal would have decayed back to Boltzmann populations at this field. This builds on work shown by Levvit *et al.* who demonstrated a similar effect for the selectively deuterated biomolecule sodium fumarate-1-¹³C-2,3-*d*₂ shown in Figure 4.21.¹⁹¹ As shown in the Figure 4.14 this process also serves to remove the toxic Ir catalyst and so represents another catalyst removal method effectively applied to **Y** increasing confidence it could be utilised as a MRI contrast agent.

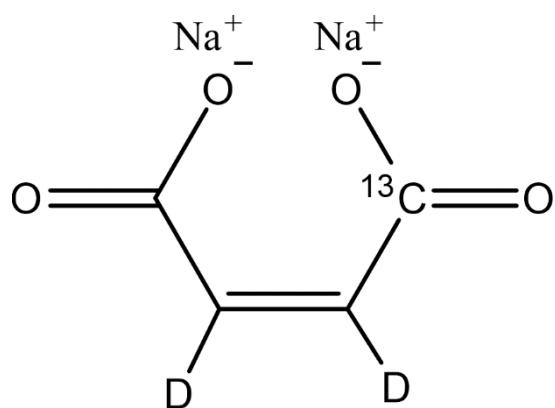


Figure 4.21 Structure of selectively deuterated biomolecule sodium fumarate-1- ^{13}C -2,3- d_2 used by Levvit et al. for precipitation studies.

The final section of this chapter explored the formation of N_2 in the highly solvating **J**. This method allowed the determination T_1 values for dissolved N_2 at various magnetic storage fields ranging from 7.0 to 16.4 T and found no significant change in the T_1 of the N_2 . This determination represents the first report of the ^{15}N T_1 of N_2 gas dissolved in a solvent. Use of the method outlined in Figure 4.17 could be utilised to aid in the generation of $p\text{-N}_2$ by performing the reaction with hyperpolarised **Y** in a suitable magnetic field.^{47, 197}

Chapter 5. Exploring the efficiency of SABRE in perfluorinated solvents

5.1 Introduction

This chapter focuses on investigating whether the high gas solubility in perfluorinated solvents is beneficial to SABRE. It has been shown in the previous chapters of this thesis that the molar amount of $p\text{-H}_2$ relative to the number of spins that are set to be polarised can limit the maximum polarisation level achieved for a substrate. It has also been shown that SABRE can operate in perfluorocarbon **J** with higher polarisation efficiency than methanol- d_4 .

Perfluorocarbons themselves have been utilised for their high O_2 retention where they facilitate ‘liquid breathing’ when the lungs of neonatal babies are underdeveloped and compromised.^{198, 199} They have also been highlighted as having potential applications in diving and aerospace.²⁰⁰ Interestingly, Clark and Gollan showed that small mammals can survive for weeks when completely submerged in a perfluorocarbon.²⁰¹ In the context of this work, they have also been utilised in MRI studies where lung-imaging studies of the ^{19}F response have been undertaken.^{202, 203} While there is consequently substantial literature data on O_2 and noble gas solubility,^{194, 204-206} little data is known about H_2 solubility in these solvents. Hence, this chapter starts with quantifying the H_2 solubility of **J** and the fifteen other solvents of Figure 5.1. Studies then on SABRE efficiency in these solvents are described that employ the substrate pyridine. The chapter finishes by exploring SABRE of biochemically relevant S-nicotine in the best of these solvents and comparing the resulting data to that obtained in methanol- d_4 .

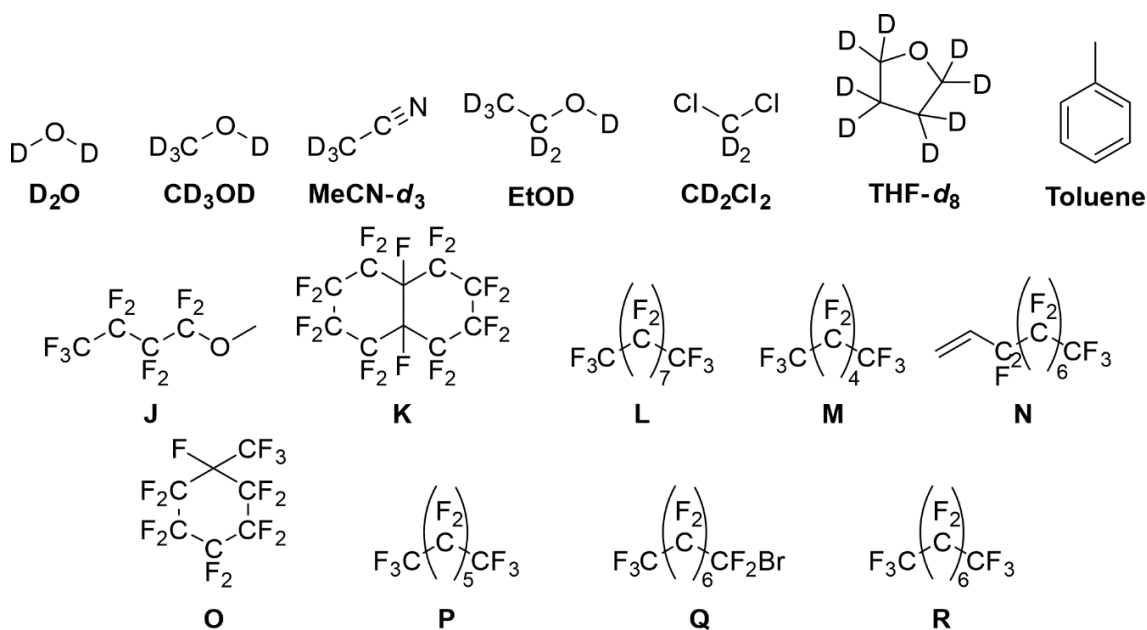


Figure 5.1 Chemical structures of solvents utilised for H_2 dissolution studies.

5.2 Gas solubility measurements

5.2.1 Method development

In order to determine the concentration of H₂ gas dissolved in these solvents a robust method utilising ¹H NMR spectroscopy was developed. This initially involved taking 0.7 mL of methanol-*d*₄, containing a 5 µL internal standard of dimethylsulfoxide (**A**), in a 7-inch (17.78 cm) J. Youngs NMR tube of 5mm diameter. The sample was degassed and then filled to a pressure of 0.5 bar of H₂. A dry ice / acetone bath at 195 K was used during this process to prevent solvent evaporation. The sample was thawed to room temperature and shaken thoroughly to ensure the gas was properly mixed with the solvent. Some of the H₂ gas will have dissolved in the solvent and so the pressure in the tube was re-equilibrated to 0.5 bar at this point to ensure the pressure above both solvents was indeed the same. The sample was then transferred into an 11.7 T NMR spectrometer for ¹H signal acquisition. This step consisted of recording four scans at 298 K. In order to ensure full relaxation of the H₂ and **A** ¹H nuclei, *T*₁ inversion recovery measurements were undertaken and found to be ~2 s and ~7 s respectively. Hence, a delay of 60 s between scans was utilised to ensure accurate signal integration. This process was repeated several times, increasing the pressure by 0.5 bar each time, until a total pressure of 4 bar was reached. These data were then plotted to give a graph from which the Henry constant, the proportionality factor for H₂ dissolution, in methanol could be determined as shown in Figure 5.2.

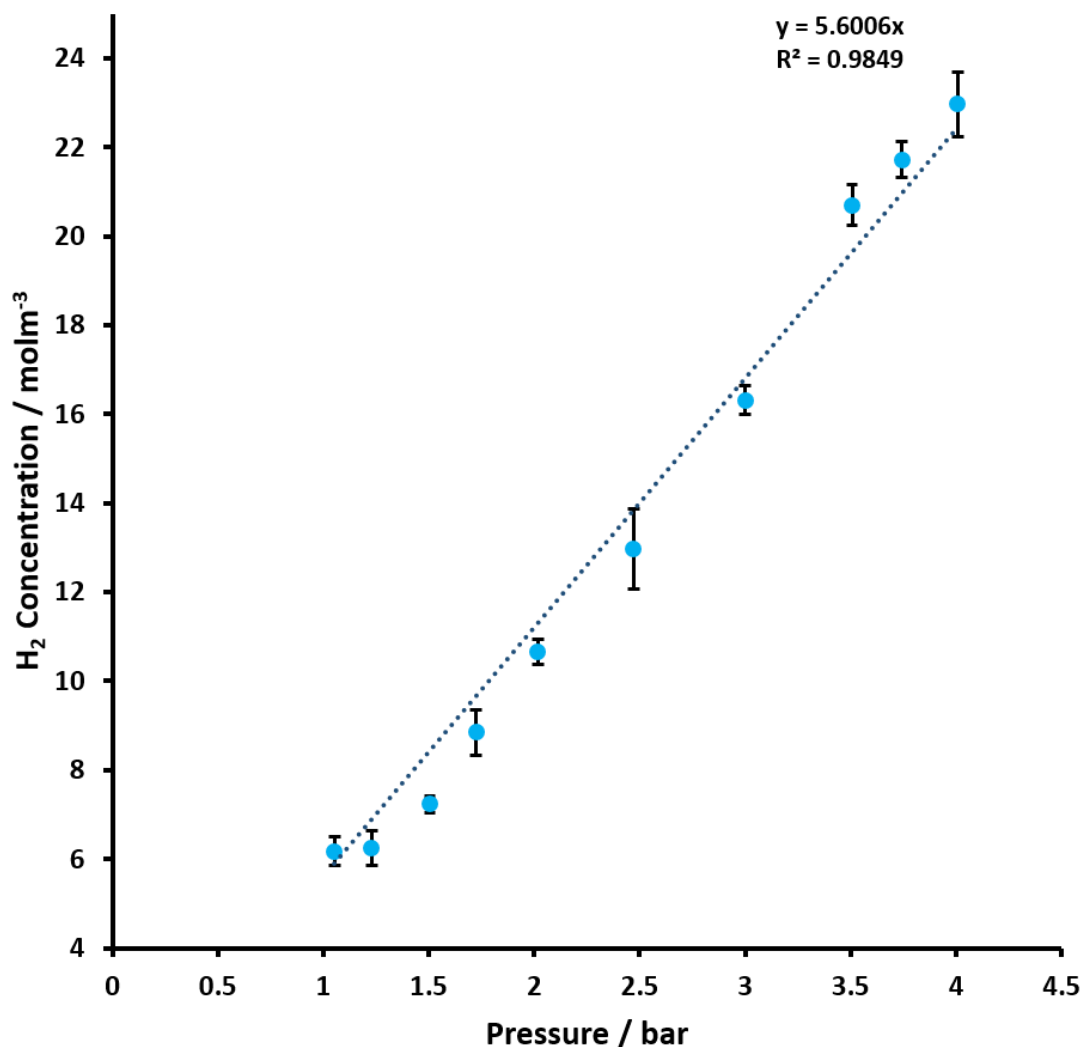


Figure 5.2 Plot of dissolved H_2 concentration versus external H_2 pressure for methanol- d_4 as measured by ^1H NMR spectroscopy. The sample contained $5\ \mu L$ of DMSO as an internal standard. All the ^1H NMR spectra were all recorded on an 11.7 T spectrometer at 298 K.

The resulting Henry constant is given by the gradient of this graph. The value of which is $5.60 \pm 0.21\ \text{molm}^{-3}\text{bar}^{-1}$. A more common literature unit for these results is from a mole fraction analysis and is actually presented as the inverse of the Henry constant, now k_H^{-1} , in MPa. This is $449.92 \pm 17.26\ \text{MPa}$ and closely matches the literature value of $440.20\ \text{MPa}$.²⁰⁷

5.2.2 Comparison between methanol and methylperfluorobutyl ether

This procedure was then applied to the solvent methylperfluorobutyl ether (**J**) of the last chapter and displayed in Figure 5.1. The result was the straight-line plot of Figure 5.3, which yields a Henry constant for H_2 in **J** of $9.96 \pm 0.26\ \text{molm}^{-3}\text{bar}^{-1}$ or a k_H^{-1} of $62.00 \pm 1.64\ \text{MPa}$.

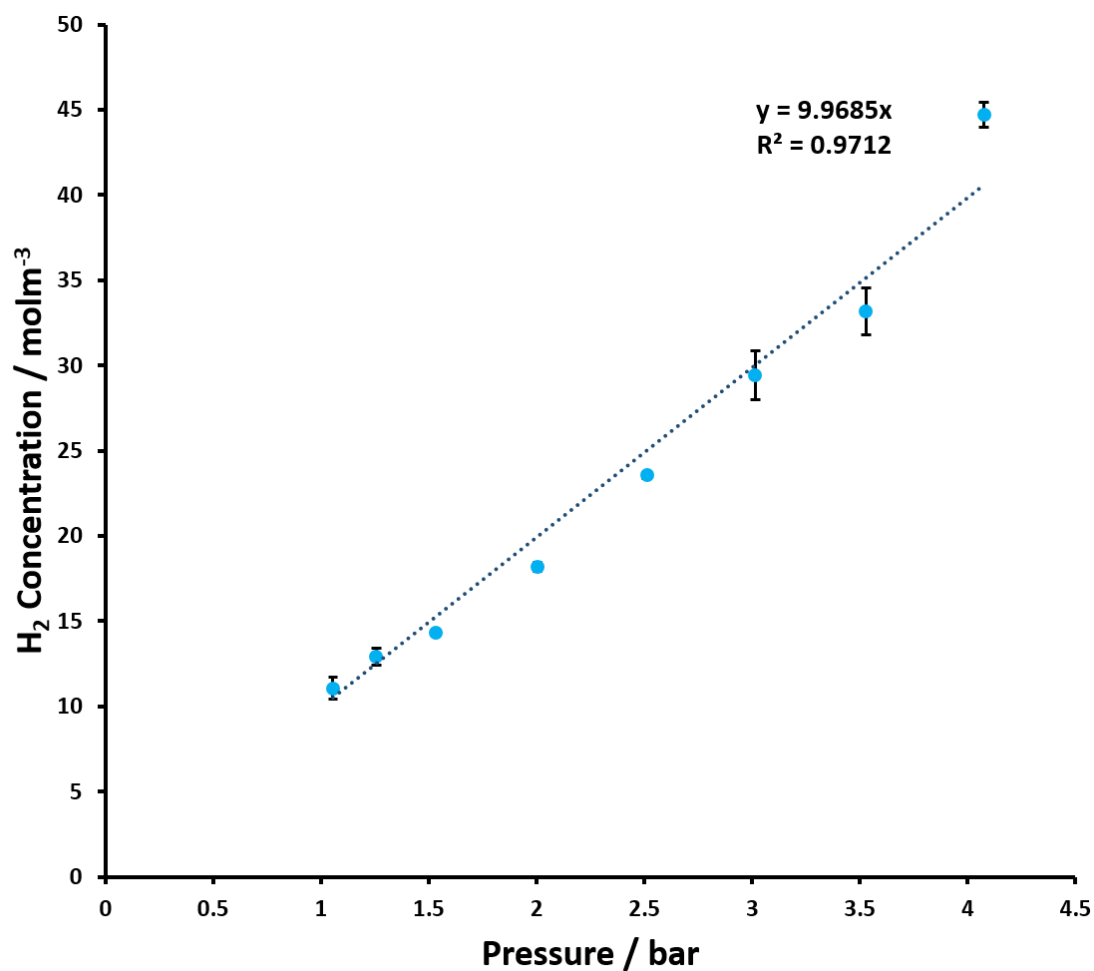


Figure 5.3 Plot of dissolved H_2 concentration versus external H_2 pressure for **J** as measured by ^1H NMR spectroscopy. The sample contained 5 μL of DMSO as an internal standard. ^1H NMR spectra were all gained on an 11.7 T spectrometer at 298 K.

These values mean that **J** can hold 1.8 times more H_2 than methanol- d_4 when analysing the Henry's constant values per m^3 of solvent in units of $molm^{-3}bar^{-1}$. This equates to around seven times more per mole of solvent when analysed by the mole fraction analysis giving values $2.2 \times 10^{-3} MPa^{-1}$ vs $16.0 \times 10^{-3} MPa^{-1}$ for methanol- d_4 and **J**, respectively.

Looking back at the previous chapter, **Y** achieved a 2 fold improvement in delivered ^{15}N polarisation level when using a 1 : 1 mixture of methanol- d_4 and **J** rather than pure methanol- d_4 as the solvent. This is clearly greater than the increase in H_2 concentration achieved by varying the solvent, and so the overall increase may reflect a combination of T_1 , exchange rate and H_2 solubility effects.

5.2.3 Extension to other solvents

The methodology described in Sections 5.2.1 and 7.2.9 was then applied to determine the Henry constant values for the remaining solvents of Figure 5.1. These are presented in Table 5.1.

Table 5.1 Henry constants (k_H) determined at 298 K for the solvents presented in Figure 5.1 alongside the literature values where they are known.

Solvent	k_H for H_2 / $\text{mol m}^{-3} \text{bar}^{-1}$	k_H^{-1} for H_2 / MPa	Literature value / MPa
D₂O	1.06 ± 0.03	5094.7 ± 152.2	7005 ²⁰⁸
CD₃OD	5.60 ± 0.21	449.92 ± 17.26	440 ²⁰⁷
MeCN – d₃	4.84 ± 0.19	394.54 ± 8.97	545 ²⁰⁹
EtOD	4.90 ± 0.10	346.48 ± 7.04	318 ²¹⁰ (513 ²¹¹)
CD₂Cl₂	4.24 ± 0.10	367.91 ± 9.00	537 ²¹²
THF-d₈	5.60 ± 0.15	226.51 ± 6.03	370 ²⁰⁹
Toluene	4.91 ± 0.19	190.18 ± 7.17	330 ²¹³
Methylperfluorobutylether (J)	9.96 ± 0.26	62.00 ± 1.64	-
Perfluorodecalin (K)*	7.55 ± 0.25	53.96 ± 1.78	-
Perfluorononane (L)*	8.80 ± 0.29	45.42 ± 1.49	-
Perfluorohexane (M)	15.79 ± 0.70	22.15 ± 0.98	-
Perfluoro-1-decene (N)	12.74 ± 0.51	20.14 ± 0.81	-
Methylperfluorocyclohexane (O)	24.72 ± 1.00	19.36 ± 0.78	-
Perfluoroheptane (P)	24.10 ± 1.22	18.96 ± 0.96	-
1-Bromoheptadecafluorooctane (Q)	28.92 ± 1.02	13.90 ± 0.49	-
Perfluorooctane (R)	59.34 ± 5.02	8.28 ± 0.70	-

**These solvents were probed using dioxane as the internal standard due to the low solubility of dimethylsulfoxide*

The Henry constants determined for these solvents show that in all cases the perfluorinated solvents are better at H_2 dissolution than widely used methanol. Hence, they could all be superior for SABRE. The following studies set out to test this hypothesis.

5.3 Exploring SABRE utilising pyridine as a substrate

5.3.1 Catalyst solubility in perfluorinated solvents

Firstly, although these solvents proved to be very efficient at dissolving H_2 they are very poor at dissolving the SABRE pre-catalyst **1**. To measure the catalyst solubility an internal standard of dioxane was utilised for all solvents. A series of samples was therefore prepared containing an initial 2 mg of **1** and 5 μL of dioxane in 0.6 mL of one of the solvents **J** to **R**. These samples were then transferred into an 11.7 T spectrometer for ^1H NMR signal acquisition. Table 5.2 shows the resulting solubility of **1** in each case in units of mg mL^{-1} . For context, the standard SABRE samples in this thesis routinely used 2 mg in 0.6 mL of solvent.

Table 5.2 Solubility of [IrCl(COD)(IMes)] (1) in 0.6 mL in each of the perfluorinated solvents J-Q of Figure 5.1.

Solvent	[IrCl(COD)(IMes)] (1) solubility / mgmL ⁻¹
Methylperfluorobutylether (J)	0.89
Perfluoro-1-decene (N)	0.22
1-Bromoheptadecafluorooctane (Q)	0.38
K, L, M, O, P and R	<0.1

Obviously, for a noticeable SABRE effect to be observed, sufficient active catalyst must be present to carry out polarisation transfer. Due to these results, the solvents **K, L, M, O, P and R** were not taken forward for SABRE studies. It is notable though that the remainder span the necessary H₂ solubility levels needed to draw robust conclusions from a SABRE study.

5.3.2 SABRE of pyridine in solvents J, N and Q

In order to test the efficiency of SABRE in the solvents **J, N, and Q**, a series of samples was prepared containing 2 mg of **1**, 1.6 µL of pyridine (**D**) and 0.6 mL of the solvent. While not all the pre-catalyst **1** dissolved in each case, this method was deemed the simplest route to produce real sample data. These samples were then degassed as outlined in the Experimental Section 7.2.9 before filling to a pressure of 4 bar H₂ to activate the complex. In each case the known complex [IrCl(H)₂(**D**)₂(IMes)],¹⁵⁴ formed as the only detectible product. Consequently, two inequivalent hydride ligand signals were observed in the corresponding ¹H NMR spectra at ~δ -23.73 and ~-24.00. These data suggest the nonpolar perfluorocarbons favour formation of neutral SABRE catalysts, thereby departing again from cationic catalyst [Ir(H)₂(**D**)₃(IMes)]Cl that forms in polar organic solvents.

These samples were then probed using the shake and drop approach, where they were shaken for 10 s in fields of 6 mT, 0.1 µT or 0.4 µT to target polarisation transfer to ¹H, ¹³C and ¹⁵N, respectively. The samples were shaken under a pressure of 4 bar *p*-H₂ before transfer into a 9.4 T NMR spectrometer for immediate signal acquisition of the

respective nuclei. Table 5.3 shows the resulting NMR signal enhancements for these samples and the corresponding polarisation values for ^{15}N , where the polarisation level is greater than 1% in all cases.

Table 5.3 NMR signal enhancement data presented for pyridine (D) from 1.6 μL samples in solvents J, N, Q and methanol- d_4 with 2 mg of 1. NMR spectra were recorded at 9.4 T after shaking with 4 bar $p\text{-H}_2$ as described in the text.

Solvent	Signal enhancement at 9.4 T / fold			^{15}N Polarisation / %
	^1H	^{13}C	^{15}N	
J	93 ± 6 (ortho)	77 ± 7 (meta)	$25,400 \pm 300$	8.3 ± 0.1
	75 ± 6 (meta)			
	44 ± 3 (para)			
N	3 ± 1 (ortho)	No signal observed	$6,200 \pm 300$	2.0 ± 0.1
	2 ± 1 (meta)			
	1 ± 1 (para)			
Q	52 ± 1 (ortho)	No signal observed	$14,750 \pm 300$	4.8 ± 0.1
	30 ± 2 (meta)			
	45 ± 2 (para)			
methanol- d_4	600 ± 20 (ortho)	No signal observed	$10,300 \pm 1200$	3.4 ± 0.4
	190 ± 20 (meta)			
	670 ± 40 (para)			

All these solvents proved to yield high ^{15}N polarisation values thereby showing their benefit to SABRE. The solvents **J** and **Q** actually show higher polarisation levels than those achieved in methanol- d_4 despite the issues with catalyst solubility. Interestingly, this polarisation level trend for the perfluorocarbons follow the same trend as catalyst solubility thereby suggesting that the concentration of SABRE catalyst is itself limiting the scale of the polarisation transfer. A further sample was therefore prepared for

J, the best performing solvent tested here, using less **1** to ensure its full dissolution. This sample used 0.5 mg of **1** with 20 equivalents of **D** in 0.6 mL of **J**. The sample was left to activate fully under 4 bar of H₂ before shake and drop experiments were repeated as before. Now, shaking with *p*-H₂ at 6 mT yields ¹H NMR signal enhancements for pyridine of 170 ± 10-fold, 130 ± 8-fold and 180 ± 10-fold for the *ortho*, *meta*, and *para* sites, respectively at 9.4 T. This is approximately a 2.5-fold improvement over the previous conditions tested. A greater improvement in ¹⁵N NMR signal enhancement was achieved though, which increased from 8.3 ± 0.1% to 13.0 ± 1.1%. For comparison, a sample with the same catalyst and pyridine concentrations was prepared in methanol-*d*₄ and probed in the same manner. This yielded ¹H NMR signal enhancements of 810 ± 110-fold, 270 ± 35-fold and 1410 ± 60-fold for the *ortho*, *meta*, and *para* sites respectively which are significantly higher for that achieved in **J**. The sample was then shaken for 10 s at a field of 0.4 μT under a pressure of 4 bar *p*-H₂ before immediate transfer to the 9.4 T spectrometer for immediate acquisition of a ¹⁵N NMR spectrum. In contrast to the ¹H NMR data, the ¹⁵N polarisation achieved in **J** is significantly higher than that achieved under analogous conditions in methanol-*d*₄ which was 13.0 ± 1.1% compared to 8.5 ± 0.6%. These data are presented in Figure 5.4 for ease of comparison. It should be noted that this difference in SABRE performance is partly due to the different catalyst speciation, as [Ir(H)₂(**D**)₃(IMes)]Cl forms in methanol-*d*₄.

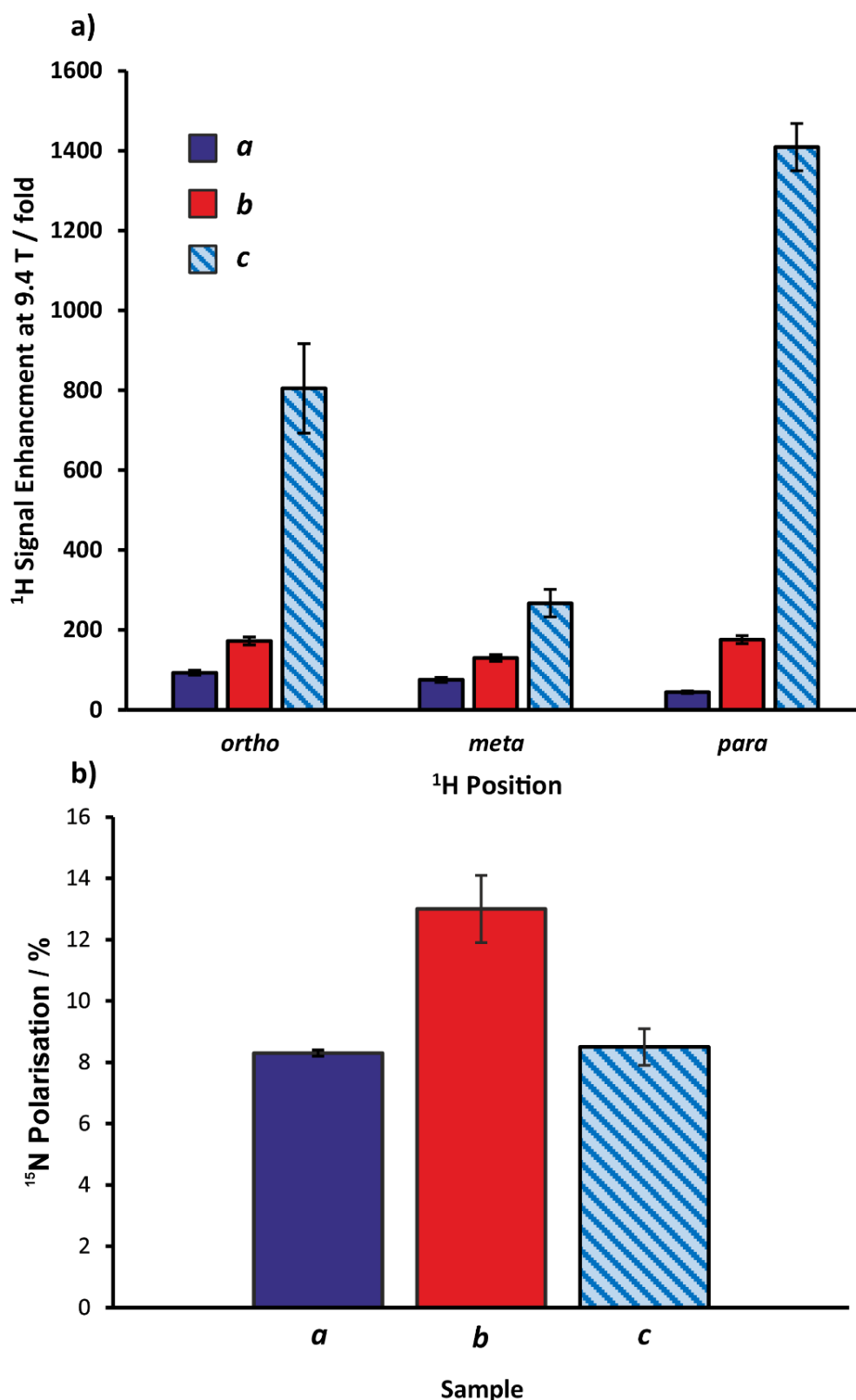


Figure 5.4 a) ¹H NMR signal enhancements at 9.4 T for ortho meta and para positions of pyridine hyperpolarised in three different samples *a* *b* and *c*, when they were shaken in a 6 mT field. *a* contained 2 mg of **1**, 1.6 μ L of **D** (would be 6 equivalents if all of **1** dissolved in **J**) and 0.6 mL of **J**. *b* contained 0.5 mg of **1**, 20 equivalents of **D** and 0.6 mL of **J**. *c* contained 0.5 mg of **1**, 20 equivalents of **D** and 0.6 mL of methanol- d_4 . b) ¹⁵N NMR polarisation levels achieved for these samples when shaken in a 4 μ T field. For both a) and b) the samples were shaken in the indicated fields for 10 s under 4 bar of *p*-H₂.

In order to rule out the effect of catalyst speciation, a new sample was prepared containing 2 mg of **1** with the same 20 equivalents of **D** but now in 0.6 mL of dichloromethane-*d*₂. This change will favour the formation of neutral, [IrCl(H)₂(**D**)₂(IMes)], the species present in the perfluorocarbons. This sample was degassed and then left to activate under a pressure of 4 bar H₂. Once activated, the sample was analysed by ¹H NMR spectroscopy at a field of 9.4 T. This revealed two hydride ligand ¹H NMR resonances now at δ -23.86 and -24.47 for [IrCl(H)₂(**D**)₂(IMes)] which was the only active species present in solution. Shake and drop style experiments were then conducted on this sample to assess both ¹H and ¹⁵N polarisation transfer efficiency. This was achieved by shaking in fields of 6 mT and -0.3 μ T respectively. The resulting ¹H NMR signal enhancements were 920 ± 8 -fold, 400 ± 120 -fold and 750 ± 9 -fold at 9.4 T. This confirms that the level of ¹H polarisation is superior in dichloromethane-*d*₂ despite the lower H₂ solubility. The associated ¹⁵N NMR signal enhancement level was found to be $25,000 \pm 300$ -fold ($8.3 \pm 0.1\%$). This is directly comparable with that in **J** and suggests that the higher level of H₂ solubility is a direct benefit. This is evident as the amount of catalyst in **J** is far less, so the same ¹⁵N polarisation is being achieved by fewer molecules of catalyst hence showing higher polarisation transfer efficiency.

It was hypothesised that the large discrepancy in polarisation transfer performance in **J** for ¹H and ¹⁵N stems from a large difference in *T*₁ values for these two nuclei in the solvent. Therefore, it was deemed appropriate to conduct *T*₁ measurements on the pyridine in both methanol-*d*₄ and **J** as presented in the next section.

5.3.3 *T*₁ measurements of pyridine

In order to ensure that the effect of relaxation in these solvents was quantified, three samples were prepared that contained 50 μ L of ¹⁵N-**D** in 0.6 mL of solvent. The samples were degassed and exposed to a pressure of 4 bar H₂ to match the conditions experienced during the earlier SABRE studies. The *T*₁ evaluations were then carried out using a standard *T*₁ inversion recovery pulse sequence. These data are presented in Table 5.4.

Table 5.4 Calculated T_1 values for ^{15}N -D in different solvent systems. Each sample contained the same amount of ^{15}N -D (50 μL) and was under a pressure of 4 bar H_2 .

Solvent	T_1 / s	
	^1H	^{15}N
methanol- d_4	24.8 ± 1.6 (ortho)	52.5 ± 1.4
	18.6 ± 1.1 (meta)	
	33.2 ± 0.7 (para)	
dichloromethane- d_2	72.8 ± 0.7 (ortho)	50.6 ± 1.3
	58.20 ± 1.0 (meta)	
	59.8 ± 0.6 (para)	
J	16.2 ± 0.2 (ortho)	53.6 ± 2.5
	14.9 ± 0.2 (meta)	
	17.8 ± 0.2 (para)	

It is clear from these data that the T_1 of the ^1H nuclei in **J** is reduced relative to methanol- d_4 and dichloromethane- d_2 , while the ^{15}N T_1 value remains essentially constant. Both of these values may reduce further at the low fields where polarisation transfer takes place. Hence, these data explain the success of ^{15}N -SABRE in **J** over methanol- d_4 . The key point to this deduction is that the ^{19}F precession frequency is much closer to that of ^1H than ^{15}N , and hence any dipole-dipole contributions to relaxation will be much greater for ^1H .⁶⁹

5.4 Extension to S-nicotine

As the use of **J** has resulted in higher ^{15}N polarisation levels it was deemed sensible to extend this study to include the examination of nicotine (**T**), a material that is present in many cigarette products. Electronic cigarette use is becoming increasingly popular, with an estimated 35 million users worldwide as of 2019.²¹⁴ The idea of the electronic cigarette has been around since 1930 when J. Robinson first patented a

design.²¹⁵ However, their wide-spread use is quite recent when compared to the traditional cigarette. Electronic cigarettes typically heat the cigarette fluid, to vaporise it, thereby allowing the user to breathe in the chemical mixture. With many cardiovascular and oral or dental diseases being related to electronic cigarette use,^{216, 217} it is essential we understand these mixtures better. Improving the NMR signal that can be gained from components in these mixtures, such as **T**, would allow the detection of low concentration components of such a mixture, shown by Tickner *et. al.*²¹⁸ This thereby allows for a better understanding of these vapour mixtures to be formed. This section focusses on achieving the maximum polarisation possible for **T**.

5.4.1 Comparison of SABRE of S-nicotine in methanol-*d*₄ and **J**

In order to compare the SABRE efficiency in methanol-*d*₄ and **J**, two samples were made, both containing 2 mg of **1** with 5 μ L of **T** dissolved in 0.6 mL of each solvent. As before, the solvent **J** did not fully dissolve **1** but the resulting solutions were transferred to a NMR tube fitted with a J. Young's cap and degassed. The samples were then filled to a pressure of 4 bar H₂ and allowed to fully activate before probing by NMR spectroscopy. Shake and drop experiments were then conducted to probe both ¹H and ¹⁵N polarisation using a PTF of 6 mT and -0.3 μ T, respectively. These samples were shaken for 10 s at the respective field before being transferred to a 9.4 T spectrometer for immediate signal acquisition. Table 5.5 summarises the ¹H NMR signal enhancements gained from these samples for S-nicotine. Figure 5.5 displays the structure of nicotine with the corresponding peak positions labelled for clarity.

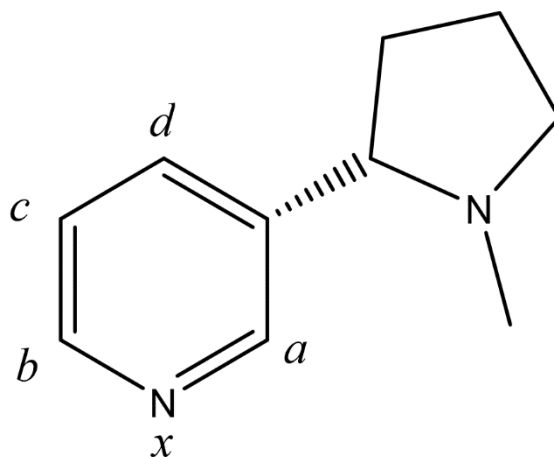


Figure 5.5 Structure of nicotine (**T**) with relevant positions of the nuclei labelled to link to the corresponding data presented in Table 5.5, and in the text.

Table 5.5 ^1H NMR signal enhancements at 9.4 T resulting for the indicated ^1H nuclei as detailed in Figure 5.5 after SABRE.

	Position on T as shown in Figure 5.5	Solvent	
		Methanol- d_4	J
^1H Signal Enhancement at 9.4 T / Fold	<i>a</i>	220.4 ± 57.3	60.8 ± 26.1
	<i>b</i>	374.4 ± 87.2	61.6 ± 24.2
	<i>c</i>	22.4 ± 14.5	33.6 ± 13.4
	<i>d</i>	105.4 ± 36.9	49.4 ± 20.3

It is clear from these data that similar trends are observed to those found with **D**. Namely, **J** is detrimental to the observed ^1H NMR signal enhancements of **T**. Once again it is important to explore possible reasoning for the difference in SABRE performance between the two solvents. The first of these is catalyst speciation. In the methanol, a cationic complex of the form $[\text{Ir}(\text{H})_2(\text{T})_3(\text{IMes})]\text{Cl}$ results that shows two inequivalent hydride ligand signals at δ -22.63 and -22.47 due to the chiral nature of the nicotine. In solvent **J**, the neutral complex $[\text{IrCl}(\text{H})_2(\text{T})_2(\text{IMes})]$ forms instead, with its hydride ligand ^1H NMR signals appearing at δ -24.10 and -24.43. This complex will clearly have different ligand exchange rates and therefore different inherent SABRE activity.

It was hypothesised that **J** was likely to have a negative impact on the T_1 of the ^1H NMR resonances of **T**, and so the samples were probed again by ^1H NMR to determine them in both solvents. These data are gathered together in Table 5.6.

Table 5.6 T_1 values for the ^1H NMR signals in the labelled positions on Figure 5.5 all measured at 9.4 T.

	Position on T as shown in Figure 5.5	Solvent	
		Methanol- d_4	J
T_1 at 9.4 T and 298 K / s	<i>a</i>	4.73 ± 0.02	4.32 ± 0.03
	<i>b</i>	5.17 ± 0.01	5.13 ± 0.02
	<i>c</i>	5.29 ± 0.03	4.57 ± 0.03
	<i>d</i>	5.34 ± 0.02	4.50 ± 0.06

Interestingly, the ^1H T_1 values obtained for **T** were not as significantly impacted as those of pyridine, shown previously in Table 5.4. This finding suggests that the T_1 , at least in the case of **T**, had a very small impact on the observed level of ^1H signal enhancement. It is therefore concluded that the difference in ^1H SABRE performance is due to the change in catalyst.

Comparing the two solvents, this time for ^{15}N , revealed a contrast with the ^1H data with **J** dramatically outperforming methanol- d_4 . The ^{15}N NMR signal enhancements for the pyridyl like ^{15}N labelled **x** in Figure 5.5 were 11740 ± 870 and 49090 ± 800 -fold for methanol- d_4 and **J**, respectively. This corresponds to polarisation values of $3.8 \pm 0.3\%$ and $16.0 \pm 0.5\%$. Typical ^{15}N NMR responses gained under these conditions are displayed in Figure 5.6. No hyperpolarised ^{15}N NMR signal was observed for the second nitrogen at δ 59.2 although a second ^{15}N NMR signal was observed in **J** assigned to be a bound **T** ligand. This was a similar value to that obtained in the characterisation step carried out in methanol- d_4 , and comparing to literature values assist in confirming its identity as a bound nicotine.

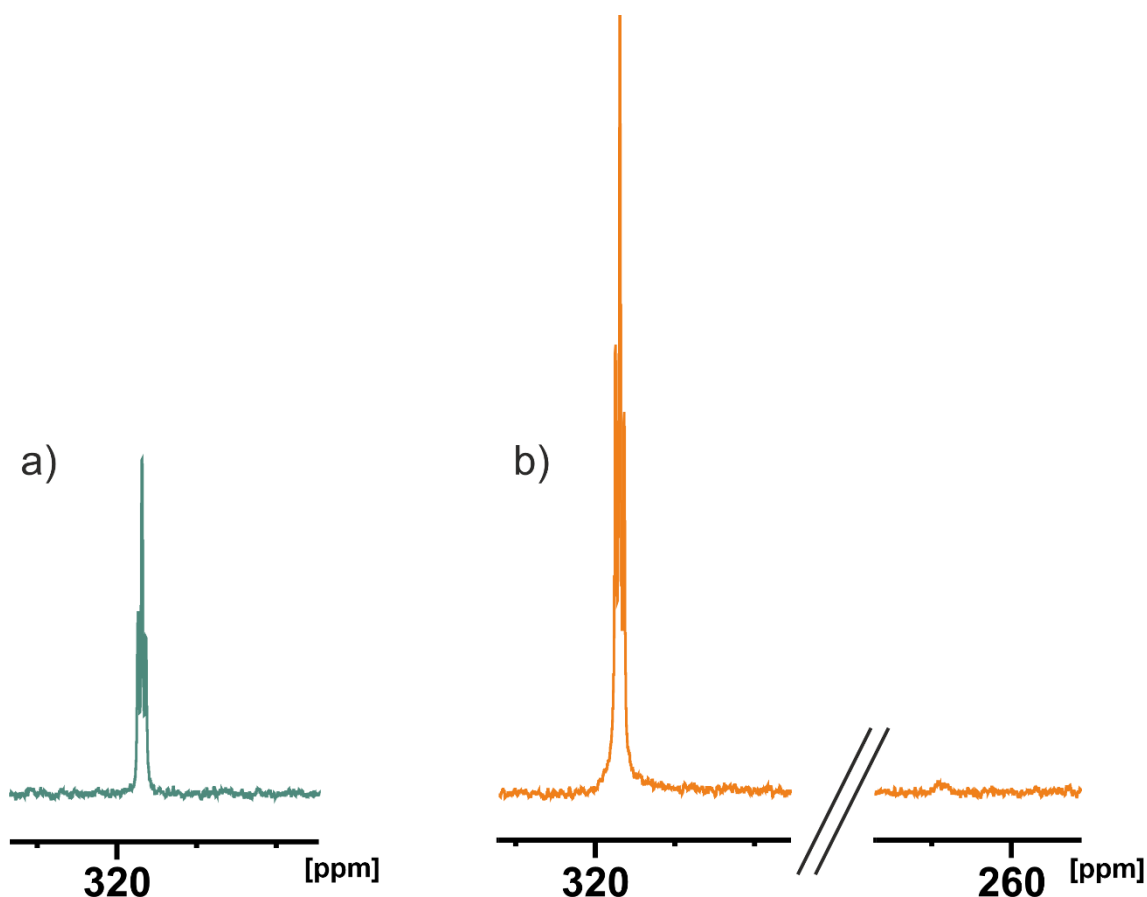


Figure 5.6 Typical hyperpolarised ^{15}N NMR spectra for nicotine in a) A sample containing 2 mg of **1** with 10 equivalents of *S*-nicotine dissolved in 0.6 mL of methanol- d_4 b) A sample containing 2 mg of **1** with 10 equivalents of nicotine in 0.6 mL of the solvent **J** (note that not all of **1** dissolved in this case so the concentration is lower than expected). Both samples were shaken for 10 s at a field of 0.3 μT under a pressure of 4 bar $p\text{-H}_2$.

It was at this point T_1 measurements were repeated on the two samples, probing ^{15}N . A similar trend to that observed for the ^1H was again observed with T_1 values of 30.6 ± 3.9 s and 28.6 ± 1.4 s for methanol- d_4 and **J**, respectively. Again, there is a slight decrease in T_1 observed in **J**, but this difference does not explain the dramatic improvement in ^{15}N NMR signal gain that is observed. This therefore supports the conclusions raised for pyridine that catalyst speciation plays a major role in SABRE performance in these cases. As this dramatic improvement arises from a different catalytic species, it was likely the polarisation transfer field for the two would be different and therefore this ^{15}N polarisation value could be improved further.

5.4.2 Optimising the polarisation transfer field for SABRE *S*-nicotine in **J**

Optimisation of the ^{15}N response of **T** further required a polarisation transfer sweep to be conducted. It was decided to carry out this experiment in both solvents to ensure that **J** outperformed the methanol at all PTFs. This involved preparing fresh

samples of 2 mg of **1** with 10 equivalents of **T** and 0.6 mL of either methanol- d_4 or **J**. These samples were then probed by ^{15}N NMR spectroscopy using shake and drop experiments. The samples were shaken for 10 s under a pressure of 4 bar $p\text{-H}_2$ at PTFs ranging from -0.2 to -1 μT . The data for these studies are presented in Figure 5.7.

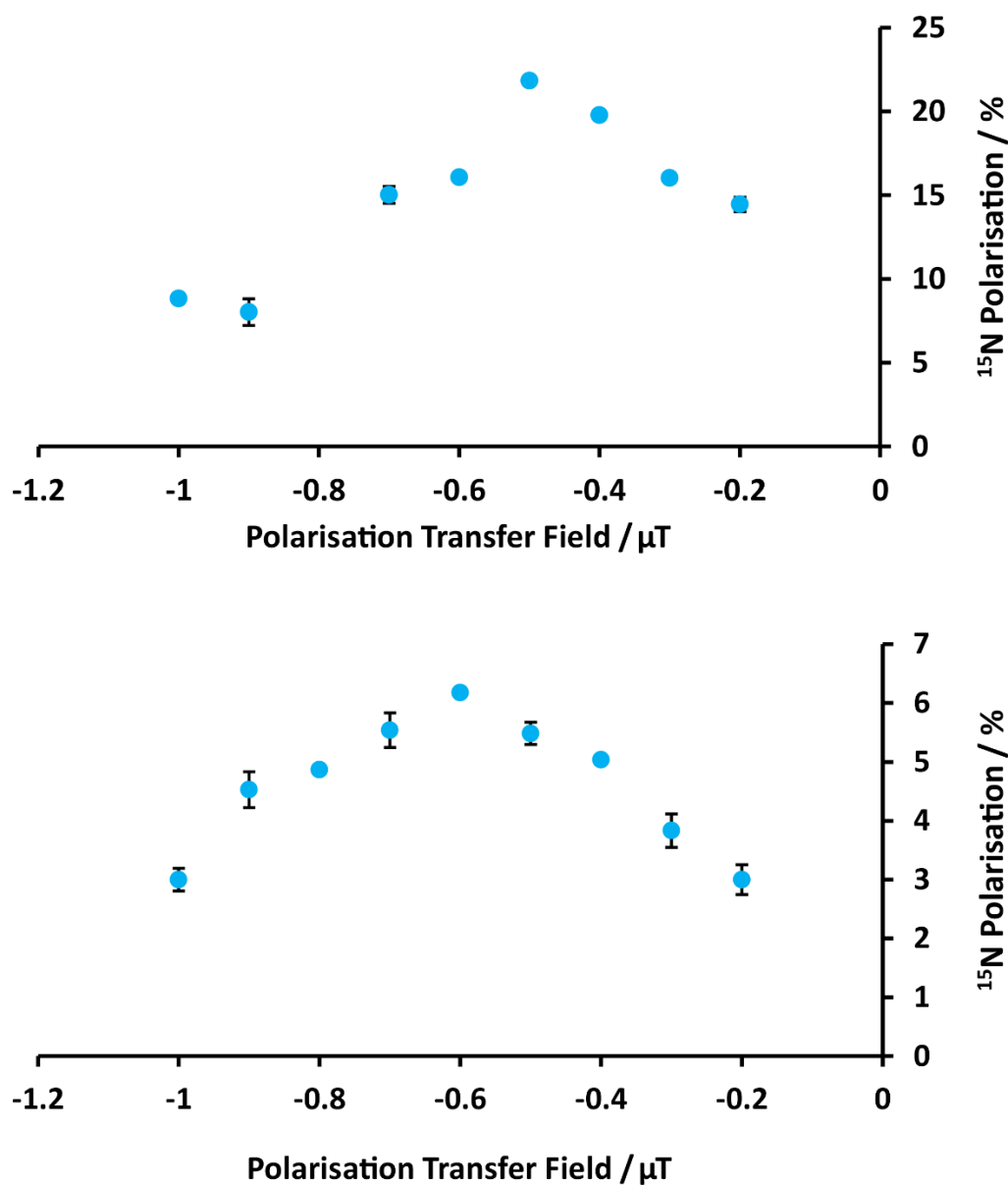


Figure 5.7 Both of these plots show ^{15}N NMR signal acquired at 9.4 T as a function of polarisation transfer field for S-nicotine. Top shows the resulting data for a sample containing 2 mg of **1**, 5 μL of **T** in solvent **J**. Bottom shows the resulting data for a sample containing 2 mg of **1**, 5 μL of **T** in methanol- d_4 . Both samples were shaken in the various fields each for 10 s with 4 bar $p\text{-H}_2$.

Notably, both polarisation transfer field sweeps revealed that -0.3 μT was not the optimum for SABRE efficiency, and the polarisation transfer benefitted greatly from conducting the sweep leading to a much larger observed ^{15}N NMR signal in both cases.

The maximum ^{15}N polarisation achieved was now $6.0 \pm 0.1\%$ (18900 ± 300 -fold signal enhancement at 9.4 T) and $21.8 \pm 0.2\%$ (66840 ± 610 -fold signal enhancement at 9.4 T) for methanol- d_4 at $-0.6 \mu\text{T}$ and **J** at $-0.5 \mu\text{T}$, respectively. This incredibly large level of ^{15}N polarisation is very promising in suggesting that it will be possible to undertake reaction monitoring with nicotine of metabolic processes in the future.

5.5 Conclusions

In this chapter, hydrogen gas solubility has been determined in the solvents of Figure 5.1. It was shown in Chapter 3, and by Adams *et al.*, that the concentration of hydrogen gas in solution has a direct effect on the level of polarisation transfer achieved.¹⁵² Therefore by using solvents with a higher solubility of H_2 it should be possible to improve SABRE of any substrate without the need for working with dangerous high pressure samples. By measuring the integral area of the H_2 peak in solution and comparing to an internal standard it was possible to determine the H_2 concentration and therefore the Henry constant for all these solvents. The Henry constant values were on the same order of magnitude as those reported in literature and generally showed the same trend of gas solubility, increasing confidence in the results. This method developed for the hydrogen solubility determination is arguably much simpler than the methodology outlined in the comparative literature as it can be performed with minimal training on a benchtop NMR and does not need any other specialist equipment. It was found that the perfluorinated solvents, as expected, all dissolved hydrogen well and the data for this are presented in Table 5.1.

When testing for SABRE activity in the perfluorinated solvents, it was found that the resulting polarisation levels followed the catalyst solubility trend directly and so implies in these systems it is the catalyst that becomes limiting. The perfluorocarbons with the highest solubility of **1** and therefore the best SABRE performance were found to be methylperfluorobutylether (**J**), perfluoro-1-decene (**N**), and 1-bromoheptadecafluorooctane (**Q**) with solubilities for **1** of 0.89 mgmL^{-1} , 0.22 mgmL^{-1} and 0.38 mgmL^{-1} respectively. The three solvents showed poor ^1H polarisation for pyridine in comparison to methanol- d_4 . However, solvents **J** and **Q** outperformed methanol- d_4 for ^{15}N polarisation transfer efficiency with solvent **J** yielding $13.0 \pm 1.1\%$ compared to $8.5 \pm 0.6\%$ in methanol- d_4 when comparable samples were tested.

Notably, different catalyst species were found to form in the two solvents with the cationic $[\text{Ir}(\text{H})_2(\mathbf{D})_3(\text{IMes})]\text{Cl}$ forming in methanol- d_4 while **J** favoured the formation of the neutral $[\text{IrCl}(\text{H})_2(\mathbf{D})_2(\text{IMes})]$. Control measurements were carried out using dichloromethane- d_2 which also favours the neutral *bis-D* complex. This found comparable levels of ^{15}N polarisation at $8.3 \pm 0.1\%$. This suggests that the higher level of H_2 solubility in **J** indeed improved the polarisation transfer efficiency for ^{15}N as the same level of ^{15}N polarisation can be achieved with less SABRE catalyst in solution.

Exploring the reasoning behind this behaviour found that the T_1 played a major impact on polarisation transfer efficiency in the perfluorinated solvents with ^1H T_1 values for **D** suffering in the perfluorinated **J** (*ortho* ^1H $T_1 = 16.2 \pm 0.2$ s) compared with methanol- d_4 (*ortho* ^1H $T_1 = 24.8 \pm 1.6$ s) and dichloromethane- d_2 (*ortho* ^1H $T_1 = 72.8 \pm 0.7$ s) hence explaining the poor performance. Comparatively, the ^{15}N T_1 values in the three solvents for **D** were all the same, within error, and so helps explain why the ^{15}N polarisation in **J** is more efficient. The difference in the T_1 behaviour was rationalised to be down to the presence of ^{19}F in **J** causing greater dipole-dipole contributions for ^1H than ^{15}N .

Extending the study to nicotine (**T**) saw the same trend, with **J** outperforming methanol- d_4 for ^{15}N polarisation yielding; $6.0 \pm 0.1\%$ (18900 ± 300 -fold signal enhancement at 9.4 T) and $21.8 \pm 0.2\%$ (66840 ± 610 -fold signal enhancement at 9.4 T) in methanol- d_4 and **J** respectively. The same poor performance of ^1H polarisation in **T** was also observed in the solvent **J**. T_1 Studies again revealed that the ^1H lifetimes suffered in **J** whereas ^{15}N T_1 values remained the same in both solvents at *circa* 30 s. This incredible improvement in ^{15}N polarisation efficiency over typical SABRE solvents like methanol- d_4 and dichloromethane- d_2 represents a significant result in the field as a facile way to improve ^{15}N signal gains whilst using biocompatible solvent mixture. It should be noted that a very recent study by R. Swenson *et al.* has shown the use of perfluorinated solvents as a route to biocompatible SABRE.²¹⁹ However, no improvement in SABRE performance was noted or rationalisation of the higher hydrogen solubility made and therefore this work is still unique in its achievements.

Chapter 6. Analysis and quantification of Nitrile functionalised compounds using SABRE.

6.1 Introduction

The next family of compounds studied in this thesis are nitrile based. These compounds find a wide variety of uses, including as building blocks in organic chemistry,²²⁰ are used in many rubber products^{221, 222} and the functional group appears in a wide variety of drug molecules. Here, their therapeutic use ranges from treating Parkinson's disease,²²³ through to fungal infections^{224, 225} and the treatment of breast cancer.²²⁶ This wide variety of uses reflects a huge market and so they are widely produced on a large scale in industry. This is especially true in the pharmaceutical industry, where quality control testing of these products must be thorough and rigorous. This is usually achieved by employing techniques such as HPLC and GCMS to separate and quantify materials and their impurities.²²⁷ Whilst these techniques have very good limits of detection, they are costly and require a lot of maintenance to perform optimally in a high pressure and result critical environment.

Benchtop NMR provides a low cost, low maintenance alternative to compound identification and the quantification of impurities. However, it suffers itself from the lower chemical shift separation, and a low polarisation level that comes with the lower magnetic field. It has already been demonstrated in this thesis how SABRE hyperpolarised ^{15}N NMR can be utilised to identify low concentration reaction intermediates in a mixture and so the precedent is set for testing if mixtures can be probed using this route. As ^{15}N benefits from a wide chemical shift window, even chemically similar species should still yield well-resolved ^{15}N NMR signals in the resulting spectra.

This chapter starts by looking at the optimisation of the ^{15}N hyperpolarisation of acetonitrile and therefore follows on from work that was laid out in Chapter 2. This work then extends to look at a wider variety of nitrile containing products, including the isomers 2-, 3- and 4-cyanopyridine, the aim being to test the ability of this technique to resolve similar materials in a complex mixture. This chapter then goes on to assess the ability of these compounds to act as MRI contrast agents by optimising the ^{15}N signal lifetimes by varying the storage field of the hyperpolarised samples as outlined in Chapter 4. The chapter then finishes by applying all the derived optimisation methods to the pharmaceutically relevant anastrozole,²²⁸ which is a treatment for breast cancer and one of the most widely prescribed pharmaceuticals containing this moiety.²²⁹

6.2 Effect of co-ligand on nitrile ^{15}N polarisation

It has already been shown in this thesis that complexes of the form $[\text{Ir}(\text{NHC})(\text{H})_2(\text{substrate})_2(\text{co-ligand})]\text{Cl}$ or $[\text{Ir}(\text{NHC})(\text{H})_2(\text{substrate})(\text{co-ligand})_2]\text{Cl}$ can dramatically outperform the more traditional SABRE catalysts which have the form $[\text{Ir}(\text{NHC})(\text{H})_2(\text{substrate})_3]\text{Cl}$. This is associated with improving the efficiency of polarisation transfer by enabling the rates of H_2 loss and substrate ligand exchange to match with the propagating couplings. The fact that under these conditions, the two protons of *parahydrogen* optimally share their polarisation with a single ligand is also beneficial, as this acts to reduce polarisation wastage. Given that it is the asymmetry in coupling between the hydrides and the target nucleus that enables polarisation flow, it is also possible to transfer polarisation into the axial ligands, albeit very weakly, as the *cis* couplings are now so small. Given the potential for structural isomers within these ligand arrangements, further complications arise in terms of catalyst specificity. The selection of co-ligands used to test whether improved ^{15}N polarisation of acetonitrile, **C**, could be achieved is shown in Figure 6.1.

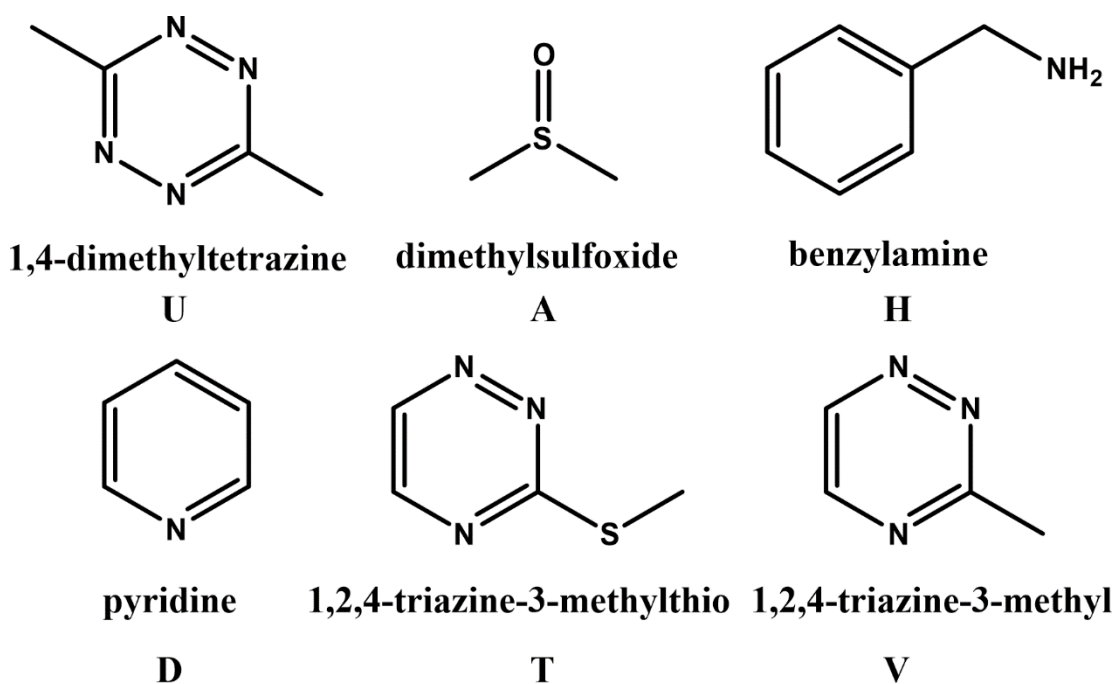


Figure 6.1 Chemical structures of co-ligands used in this study.

6.2.1 Utilising $[\text{Ir}(\text{H})_2(\text{C})_3(\text{IMes})]\text{Cl}$ to hyperpolarise acetonitrile.

For context, when no co-ligand is present, $[\text{Ir}(\text{H})_2(\text{C})_3(\text{IMes})]\text{Cl}$ (**17**) forms with **C** and **1** under H_2 .⁴⁴ This active catalyst relies on magnetic inequivalence for polarisation transfer. When examined here, no visible ^{15}N signals result when a 5 mM sample of **1**

with a 5-fold excess of **C** is examined in methanol- d_4 without ^{15}N labelling after polarisation transfer shaking at a $0.3\ \mu\text{T}$ field under 4 bar of $p\text{-H}_2$ in a single measurement at 9.4 T. This finding agrees with that reported early, when much less was understood about SABRE.⁴⁴ It should be noted that under these conditions, but with an appropriate transfer field of 6 mT, the ^1H CH_3 NMR signal of acetonitrile shows a 3.4-fold signal gain at a 9.4 T acquisition field as shown in Figure 6.2.

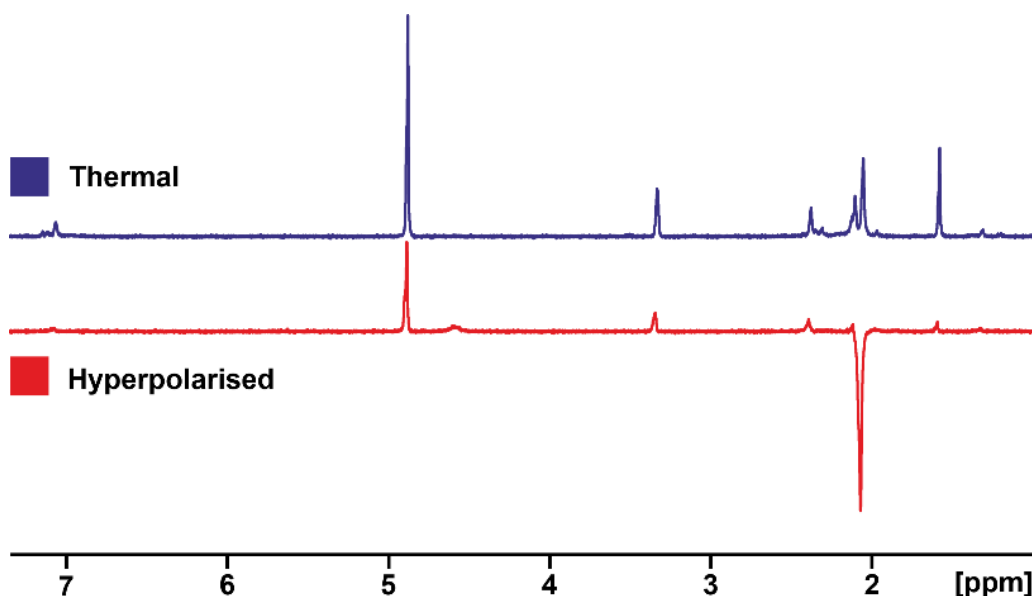


Figure 6.2 ^1H NMR spectra acquired at 9.4 T after shaking for 10 s at a field of 6 mT with a pressure of 3 bar $p\text{-H}_2$

It was clear from the ^1H NMR spectra in this figure that the acetonitrile CH_3 peak at $\delta\ 2.06\ \text{ppm}$ was hyperpolarised due to its negative appearance. As mentioned previously, this is a feature very commonly seen in SABRE hyperpolarisation.

As discussed in Chapter 2, the use of a combination of acetonitrile and 3-methylthio-1,2,4-triazine (**X**) with **1** led to the detection of a large ^{15}N response for free **C- d_3** . This method was further developed here for a sample containing **1** with 5 equivalents of ^1H containing **C** and 5 equivalents of **X**. Initially, no ^{15}N response was seen in a single scan measured at 9.4 T when the sample was shaken for 10 s with $p\text{-H}_2$ in a PTF of $-0.3\ \mu\text{T}$. This poor return from the *protio*-acetonitrile could be due to polarisation wastage to ^1H , or increased relaxation. The proton response was therefore examined, repeating the 10 s shake at $-0.3\ \mu\text{T}$ with 4 bar $p\text{-H}_2$, but acquiring a ^1H NMR spectrum. A 27-fold signal enhancement was then observed. This reflects a significant improvement on the sample without a co-ligand, but is still lower than that reported previously in the literature.⁴⁴ As the aim of this chapter is to explore the optimisation of the ^{15}N signal, the

successful **C-d₃** was used going forward in the studies and so little ¹H NMR data was gathered for **C** in the subsequent optimisation measurements.

6.2.2 Screening the ¹⁵N hyperpolarisation of acetonitrile-*d*₃ as a function of co-ligand.

A series of six samples containing 2 mg of **1** and 5 equivalents of both the specified co-ligand from Figure 6.1, and **C-d₃** in methanol-*d*₄ were exposed to a pressure of 4 bar of H₂ for 2 hours. The resulting samples were then shaken for 10 s in a field of -0.3 μT for polarisation transfer, before being moved to the magnet for ¹⁵N NMR signal acquisition. Fortuitously, all of these samples yielded a hyperpolarised ¹⁵N response, as quantified in Table 6.1. All of these co-ligand containing matrices performed better than **17**, with 1,2,4-triazine-3-methyl (**V**) yielding the best results of >6%. All the signal enhancements were calculated as detailed in the experimental section using an external standard measured under the same conditions.

Table 6.1 ¹⁵N NMR signal with enhancement and % polarisation for the free ¹⁵N signal of **C** at δ 240.8 in methanol-*d*₄; a typical sample contained **1** (5 mM), a 5-fold excess of **C** and a 5-fold excess of the co-ligand stated. These data utilised 4 bar of *p*-H₂ and a shake time of 10 s

Co-ligand	¹⁵ N (raw signal)	¹⁵ N (enhancement, fold at 400 MHz)	¹⁵ N (% polarisation)
No Co-ligand	Signal not observed	Signal not observed	Signal not observed
1,4-dimethyl tetrazine (U)	100100 ± 5670	2470 ± 140	0.81 ± 0.05
DMSO (A)	217840 ± 23670	5370 ± 580	1.77 ± 0.19
Benzylamine (H)	266850 ± 14760	6570 ± 360	2.17 ± 0.12
Pyridine (D)	339160 ± 50540	8350 ± 1240	2.76 ± 0.41
1,2,4-triazine-3-methylthio (X)	653400 ± 32430	16090 ± 790	5.31 ± 0.26
1,2,4-triazine-3-methyl (V)	861060 ± 17440	21210 ± 430	6.99 ± 0.14

Figure 6.3 shows a typical hyperpolarised ¹⁵N NMR spectrum for the pyridine (**D**) containing sample, and reveals both substrates receive significant polarisation from *p*-H₂. Despite the fact that **X**, **V**, **U** and **H** also bind through an N-donor, there were no detected signals beyond those for **C** in the corresponding measurements. These effects have been commented on previously.

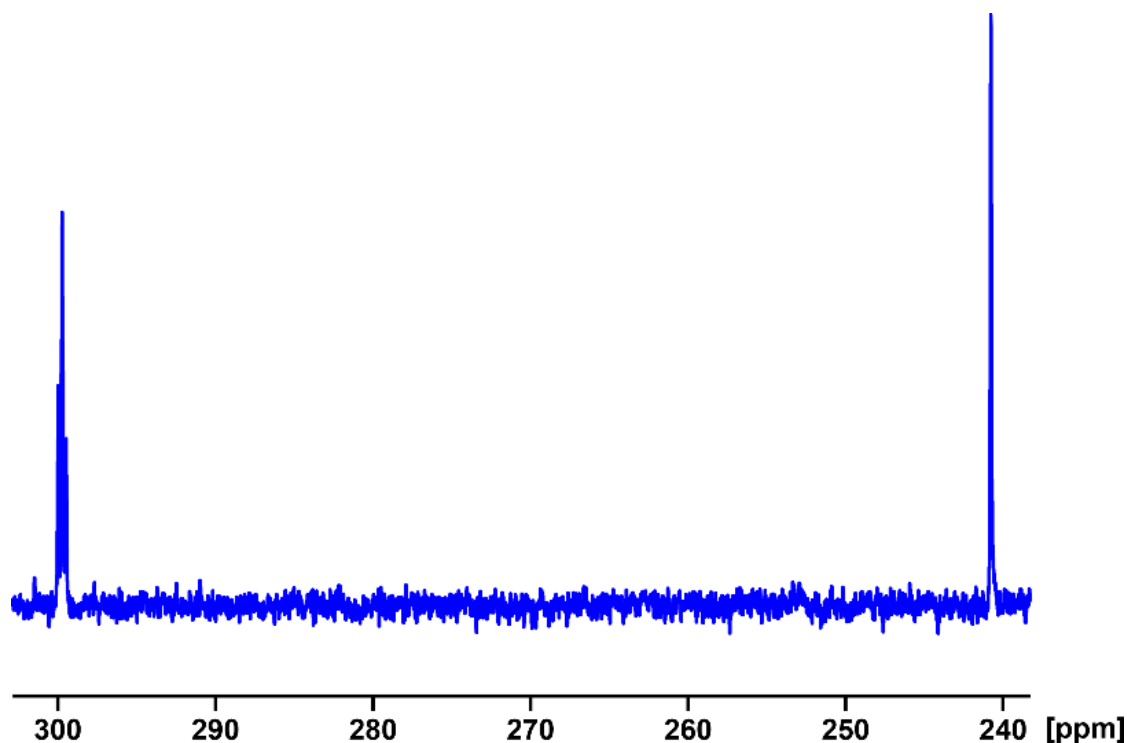


Figure 6.3 ^{15}N NMR spectrum, at 9.4 T, acquired from a sample containing 2 mg of **1** with 5 equivalents of **C-d₃** and 5 equivalents of **D**. The sample was shaken in a field of $-0.3\ \mu\text{T}$ for 10 s with a pressure of 4 bar $p\text{-H}_2$. The ^{15}N NMR peak for **D** was observed at $\delta\ 299.7\ \text{ppm}$ while **C** is at $\delta\ 240.8\ \text{ppm}$.

The ^{15}N NMR spectrum shown in Figure 6.3 represents a signal enhancement of $13,460 \pm 1650$ -fold at 9.4 T for the pyridine ^{15}N resonance at 299 ppm. This is equivalent to $4.40\% \pm 0.53\%$ ^{15}N polarisation. This study was then repeated using **D-d₅** in order to demonstrate the effect that the presence of the ^1H nuclei had on this measurement. This study found that the observed ^{15}N polarisation level of **D** dropped to $1.91 \pm 0.06\%$ as only a signal enhancement of 5860 ± 170 -fold at 9.4 T was recorded. The polarisation of the ^{15}N resonance of **C-d₃** increased to $4.77 \pm 0.21\%$ though representing a 14610 ± 640 -fold enhancement. Interestingly, the ^{15}N resonance of the carbene ligand was now also evident in the single scan acquisition with a chemical shift of $\delta\ 191.7$. The ^{15}N polarisation achieved on the carbene was $2.39 \pm 0.17\%$, representing an enhancement of 7260 ± 510 -fold at 9.4 T.

At these low polarisation transfer fields in the μT region, polarisation transfer to ^{13}C has also been widely reported.^{49, 51} When this possibility was examined by repeating the shaking process with 4 bar of fresh $p\text{-H}_2$ and field of $-0.3\ \mu\text{T}$, no observable ^{13}C signals resulted for **A**, **U** or **H**. However in the case of **D**, **X**, and **V**, ^{13}C polarisation transfer was observed. A typical ^{13}C NMR spectrum gained for the sample containing **X** is shown in Figure 6.4 to illustrate this effect. Notably, it is the ^{13}C NMR response of the carbene ligand at $\delta\ 174.8$ that is strongest in these spectra. The ^{13}C NMR resonance for free **C-d₃**

nitrile carbon is also observed at δ 116.9. The observed ^{13}C responses around 150 ppm matched those for the free **X** ligand. Interestingly, they all appear in antiphase which means a two spin-order state is involved, similar to that observed for the ^{15}N response in Chapter 2.

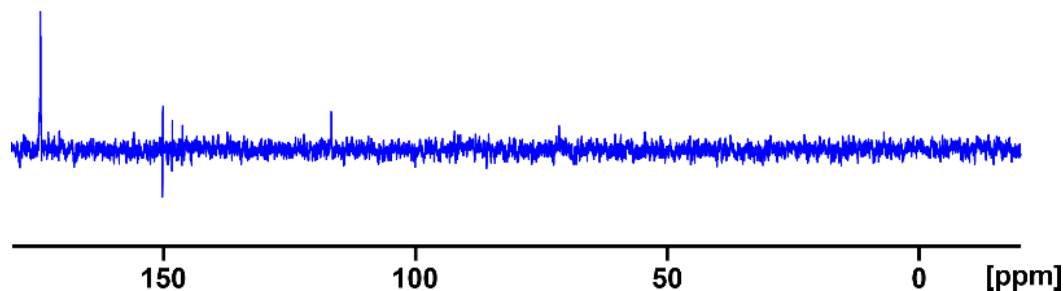
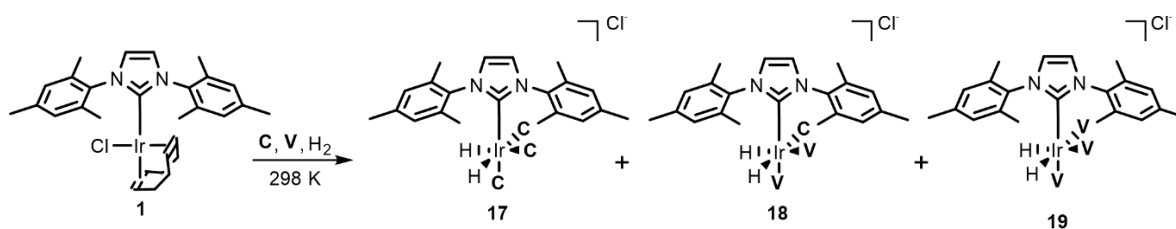


Figure 6.4 A typical hyperpolarised ^{13}C NMR spectrum at 9.4 T acquired from a sample containing 2 mg of **1** with 5 equivalents of C-d_3 and 5 equivalents of **X**. The sample was shaken in a field of $-0.3\ \mu\text{T}$ for 10 s with a pressure of 4 bar $p\text{-H}_2$.

As the system containing 1,2,4-triazine-3-methyl (**V**) was found to yield the highest ^{15}N response, it is important to know the catalytic species present in the mixture. The sample was therefore probed by ^1H NMR spectroscopy. This revealed a complicated hydride region, and when shaken with $p\text{-H}_2$, the hyperpolarised response revealed even further complexity. Only the major species were characterised, as these are the species that will be responsible for the SABRE effects observed. They were revealed to be $[\text{Ir}(\text{H})_2(\text{C})(\text{IMes})(\text{V})_2]\text{Cl}$ (**18**) and $[\text{Ir}(\text{H})_2(\text{IMes})(\text{V})_3]\text{Cl}$ (**19**) of Scheme 6.1, present in a ratio of 1 : 6 thereby showing that the ligand **V** outcompetes **C-d**₃ in catalyst binding.



Scheme 6.1 Reaction of $\text{IrCl}(\text{COD}(\text{IMes}))$ (**1**) with **C**, **V**, H_2 in methanol- d_4 leads to several dihydride derived reaction products.

Concentrated samples containing **1**, **V** and *protio-C* were prepared in the same ratio as previously described in order to characterise the complexes in the sample through multinuclear NMR analysis at a temperature of 243 K. Complex **18** proved to yield two hydride ligand resonances at δ -20.23 and -21.20, with a common J_{HH} coupling of 7.4 Hz. ^1H - ^{15}N HMQC NMR measurements were then undertaken, which revealed binding of **V** *trans* to the hydride ligand at δ -20.23 typical of N_1 binding and consistent with that seen

in Chapter 2 for **X**. The associated ^{15}N resonance appears at δ 340.23, shifted from the free resonance at δ 413.20. These measurements revealed a further ^{15}N resonance at δ 171.15 that is due to a group *trans* to the other hydride ligand at δ -21.20. This resonance was attributed to a bound **C** ligand shifted from the free ^{15}N resonance at δ 240.70. ^1H - ^{13}C HMQC measurements were carried out which revealed a cross peak to a ^{13}C NMR signal at δ 153.14 assigned to that of the bound carbene ligand. ^1H NMR NOESY measurements were undertaken which revealed nOe connections from the hydride ligands to that of a second bound **V** ligand with a ^1H NMR resonances at δ 9.22 which revealed a cross peak to a further bound ^{15}N resonance at δ 370.21 thus completing the coordination sphere of **18**.

The major product **19** proved to yield a singlet hydride ligand ^1H NMR resonance at δ -20.39. Analysis by ^1H NMR NOESY measurements revealed two bound **V** ligands through nOe connections to ^1H NMR signals at δ 9.08 and δ 9.54. These resonances integrated in a 2 : 1 ratio confirming their positions as the equatorial and axial **V** ligands respectively. Low temperature ^1H - ^{15}N HMQC measurements revealed cross peaks for the ^1H NMR signals at δ 9.08 and δ 9.54 in the ^{15}N domain at δ 343.82 and δ 322.00, once again confirming the preferential binding through N_1 . Further integration and analysis by ^1H - ^{13}C HMQC measurements revealed a ^{13}C NMR resonance at δ 150.44 for the carbene ligand. This fully satisfies all the ligands surrounding the metal centre. Full characterisation data for both complexes and **17** can be found in Section 7.4.33. As the complex **19** will clearly not hyperpolarise **C-d₃**, it was concluded that **18** is the main catalytic species involved in the polarisation transfer process, therefore improving the concentration of this would be beneficial to the SABRE performance. This mixture containing **1**, **C-d₃** and **V**, as the best performing system tested, was therefore taken forward for further optimisation. Note that concentrated samples using *protio*-**C** were used for characterisation studies. These consisted of 10 mg of pre-catalyst and the same ratio of **C** and **V**.

6.3 Exploring acetonitrile-*d*₃ ^{15}N polarisation as a function of the iridium pre-catalyst utilised.

Modifying the catalyst further to optimise the exchange rate is possible, as shown in the previous chapters, by changing the steric and electronic properties of the carbene ligand. A broad selection of these NHC derived catalysts are shown in Figure 6.5, and

they were examined here to explore their effect on polarisation transfer efficiency to the ^{15}N resonance of **C**. Consequently, a series of samples were made containing 2 mg of the corresponding pre-catalyst $[\text{IrCl}(\text{COD})(\text{NHC})]$ (where the NHC is one of the carbene ligands shown in Figure 6.5) alongside 5 equivalents of **C-d₃** and 5 equivalents of **V** relative to iridium, all dissolved in 0.6 mL of methanol- d_4 . These samples were degassed and exposed to a pressure of 4 bar H_2 to activate the SABRE complex. Once activated, these samples were shaken in a field of $-0.3\ \mu\text{T}$ for 10 s with a pressure of 4 bar $p\text{-H}_2$. Given the very broad range of available catalysts, the study was split into two subsections, the first providing a coarse refinement of steric control to identify an optimum region within the catalyst profile, and the second a more rigorous evaluation using NHC's that operate close to this point according to previous work to hone in on the optimum solution.

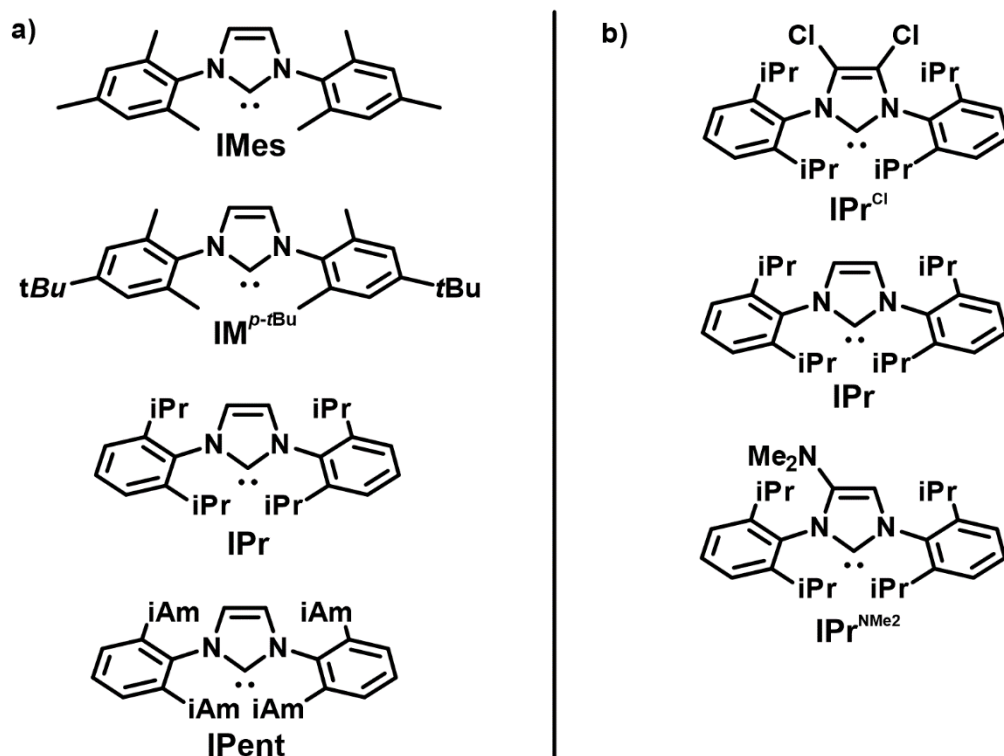


Figure 6.5 NHC ligands used in the pre-catalysts $[\text{IrCl}(\text{COD})(\text{NHC})]$ for the optimisation of ^{15}N hyperpolarisation for **C**. a) Broad changes in steric bulk utilised to identify optimum ligand exchange window. Increasing steric bulk shown from top to bottom. b) Fine tuning associated with change in electron donation to more precisely match SABRE conditions to outcome. Electron donating ability increases top to bottom.

The first refinement showed that the steric effects associated with **IPent** delivered the optimum SABRE outcome (Figure 6.6). However, utilising the ^1Pr manifold and additional substituents to alter the electronic properties of the NHC enabled further refinement. This showed the **IPr^{Cl}** based ligand of Figure 6.5 proved to operate optimally.

The polarisation gain on the ^{15}N resonance using this system is $13.4 \pm 0.7\%$, which corresponds to a ^{15}N NMR signal enhancement of 41100 ± 2100 -fold at a field of 9.4 T. These data, for the full array of catalysts utilised are presented in Figure 6.6.

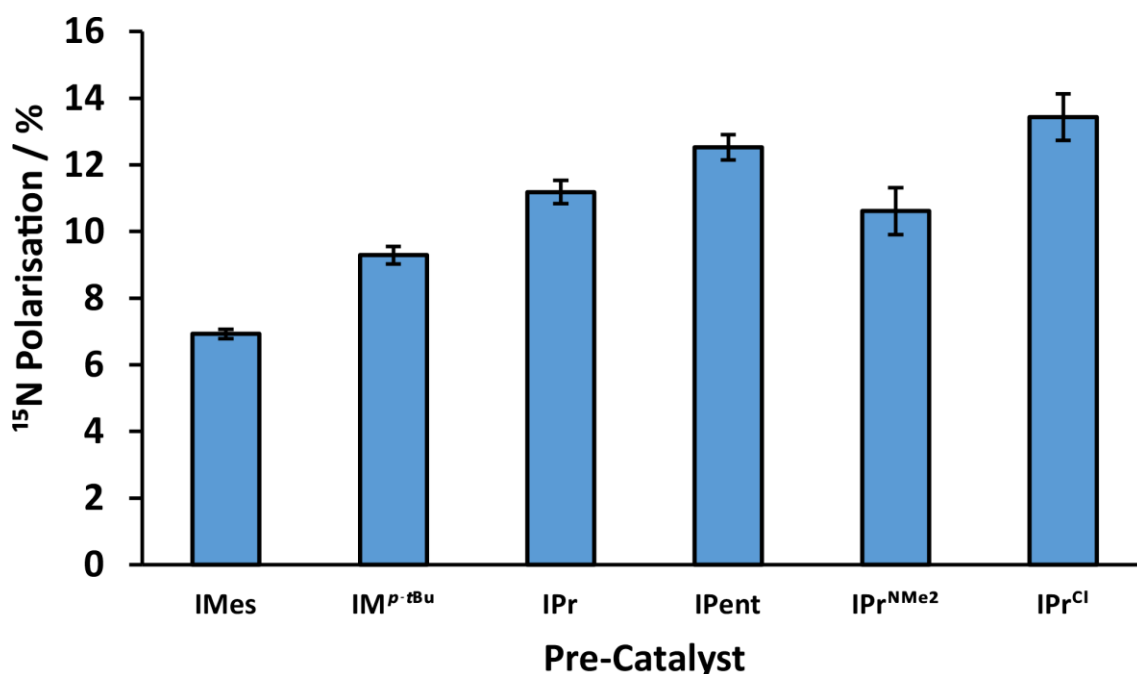


Figure 6.6 Effect of changing steric and electronic properties of the SABRE catalyst on the ^{15}N polarisation of acetonitrile. Samples all contained 2 mg of the respective pre-catalyst with 5 equivalents of C-d_3 and V shaken at a field of $-0.3 \mu\text{T}$ for 10 s with 4 bar pressure of $p\text{-H}_2$.

It is notable, that upon switching to sterically bulkier carbene ligands compared to IMes, the hydride region of the corresponding ^1H NMR spectra simplifies. Now analogous products to **18** and **19** form as $>95\%$ of the material present. Interestingly, the species containing $\text{IM}^{p\text{-tBu}}$, **IPr** and IPr^{Cl} all formed **18** and **19** in a 1 : 1 ratio whereas with $\text{IPr}^{\text{NMe}_2}$ this ratio changed to 1 : 1.7 for the **18** and **19** type species. Consequently, there is less catalyst present for the hyperpolarisation of C-d_3 . For the **IPent** type system, the analogous complex to **18** formed as the only product and so it is surprising this did not outperform the IPr^{Cl} . The hydride ligand signals in the ^1H NMR spectrum for **IPent** appear very broad in nature implying fast exchange. Hence it is believed the **IPent** catalyst pushes the exchange rate of C-d_3 past the optimum. Unfortunately, due to using C-d_3 no EXSY measurements were carried out on the sample. Therefore only the IPr^{Cl} was taken forward, and the next section considers how to maximise the presence of $[\text{Ir}(\text{H})_2(\text{C})(\text{IPr}^{\text{Cl}})(\text{V})_2]\text{Cl}$ (**20**), (analogous to **18** of Scheme 6.1) in more detail.

6.4 Catalyst speciation as a function of acetonitrile- d_3 and 1,2,4-triazine-3-methyl concentration.

To understand the formation of **20** in more detail, and confirm all the species involved in the sample mixture, the reaction of the $[\text{IrCl}(\text{COD})(\text{IPr}^{\text{Cl}})]$ pre-catalyst with a 5 fold excess of **C** in methanol- d_4 was considered alone. The result is the formation of $[\text{Ir}(\text{H})_2(\text{C})_3(\text{IPr}^{\text{Cl}})]\text{Cl}$ (**21**), analogous to the complex **17** of Scheme 6.1, which provides a well-defined hydride ligand response at δ -21.07 and 298 K. When this catalyst is considered for SABRE with **C**, a ^1H NMR signal enhancement of around 12-fold at 9.4 T results when the sample was shaken for 10 s at a field of 6 mT under a pressure of 4 bar $p\text{-H}_2$. This shows the presence on the IPr^{Cl} is beneficial to ^1H polarisation transfer, although yielding enhancement values much lower than in the literature.⁴⁴ At this point the ^{13}C and ^{15}N responses were tested again shaking the sample with 4 bar of $p\text{-H}_2$ at a -0.3 μT polarisation transfer field. Unfortunately, no hyperpolarised signals were observed in either case. However, when **C- d_3** is examined, a ^{15}N level of 0.89% can be obtained. A similar process was carried out to confirm the hydride ligand ^1H NMR chemical shift for the complex $[\text{Ir}(\text{H})_2(\text{IPr}^{\text{Cl}})(\text{V})_3]\text{Cl}$ (**22**), analogous to complex **19** of Scheme 6.1. A sample was prepared containing 2 mg of the IPr^{Cl} pre-catalyst with 5 equivalents of **V** in methanol- d_4 . The sample was degassed and allowed to activate under a 4 bar pressure of H_2 before analysis by ^1H NMR spectroscopy. This revealed a hydride ligand resonance at δ -20.28 for **22**. A fresh sample containing 2 mg of the IPr^{Cl} pre-catalyst with 5 equivalents of both **C- d_3** and **V** was then prepared. This sample showed **20** and **22** present in the same 1 : 1 ratio as before and no **21** was detected under these conditions. At this point the sample was taken and shaken at a field of 6 mT with a pressure of 4 bar fresh $p\text{-H}_2$ before transfer to a 9.4 T spectrometer for immediate ^1H signal acquisition as shown in Figure 6.7.

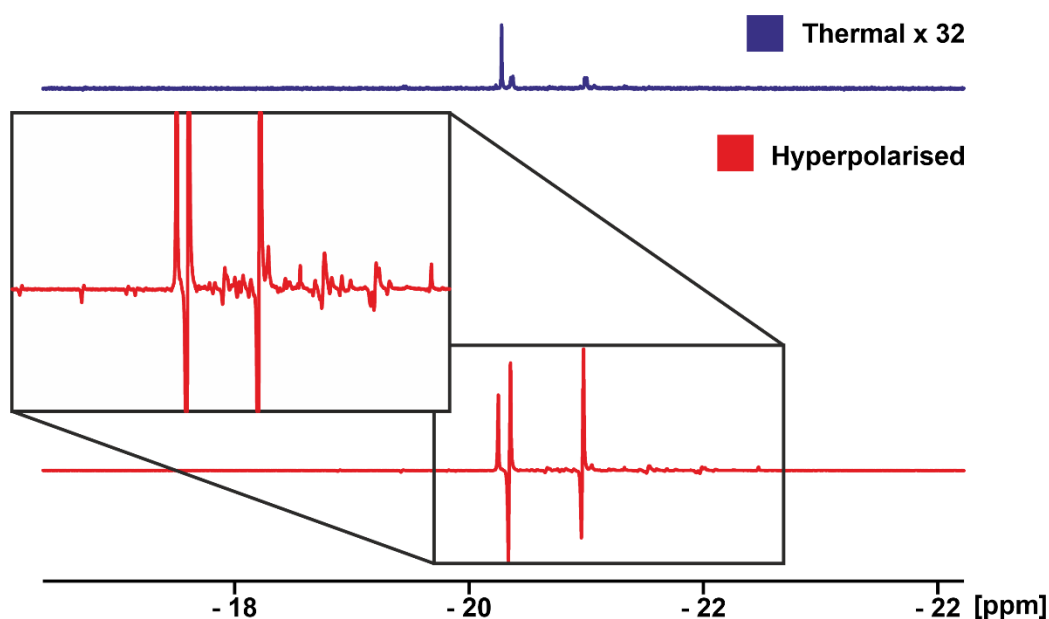


Figure 6.7 Hydride section of two ^1H NMR spectra obtained from a sample containing 2 mg of IPr^{Cl} with 5 equivalents of **V** and 5 equivalents of **C-d₃**. a) Thermally equilibrated acquisition collected for 32 signal averages. b) Hyperpolarised single scan NMR spectrum showing PHIP signal complexity; the sample was shaken for 10 s at a 6 mT field with a pressure of 4 bar $p\text{-H}_2$ before acquisition.

The ^1H NMR spectrum revealed hyperpolarised responses for the hydride ligands in **20** and **22** as expected. The spectrum also revealed several other hyperpolarised hydride ligand resonances suggesting that the catalyst speciation in the mixture is actually a lot more complex than it first appears. However, these species are only detected when $p\text{-H}_2$ is used and so must be in very low concentrations and will have minimal effect on the polarisation transfer to **C-d₃** when compared to **20**.

In order to maximise the formation of **20**, a series of samples were prepared to examine how sensitive the ratio of these species was to the excess of **V** used. These consisted of 2 mg of IPr^{Cl} pre-catalyst, 5 equivalents of **C-d₃** and varying concentrations of the co-ligand **V**. The results of this study, alongside the resulting ^{15}N polarisation level and raw signal gain are shown in Figure 6.8. A very substantial variation in signal enhancement was observed, with the optimum ratio of IPr^{Cl} pre-catalyst : **C-d₃** : **V** loading proving to be 1 : 5 : 5. Under these conditions, the ratio of **20** : **22** was 1 : 1 as discussed previously. A sharp decline in enhancement level is seen on moving from 5 to 10-fold co-ligand loading which is reflected in the corresponding increase in proportion of the inactive **22**.

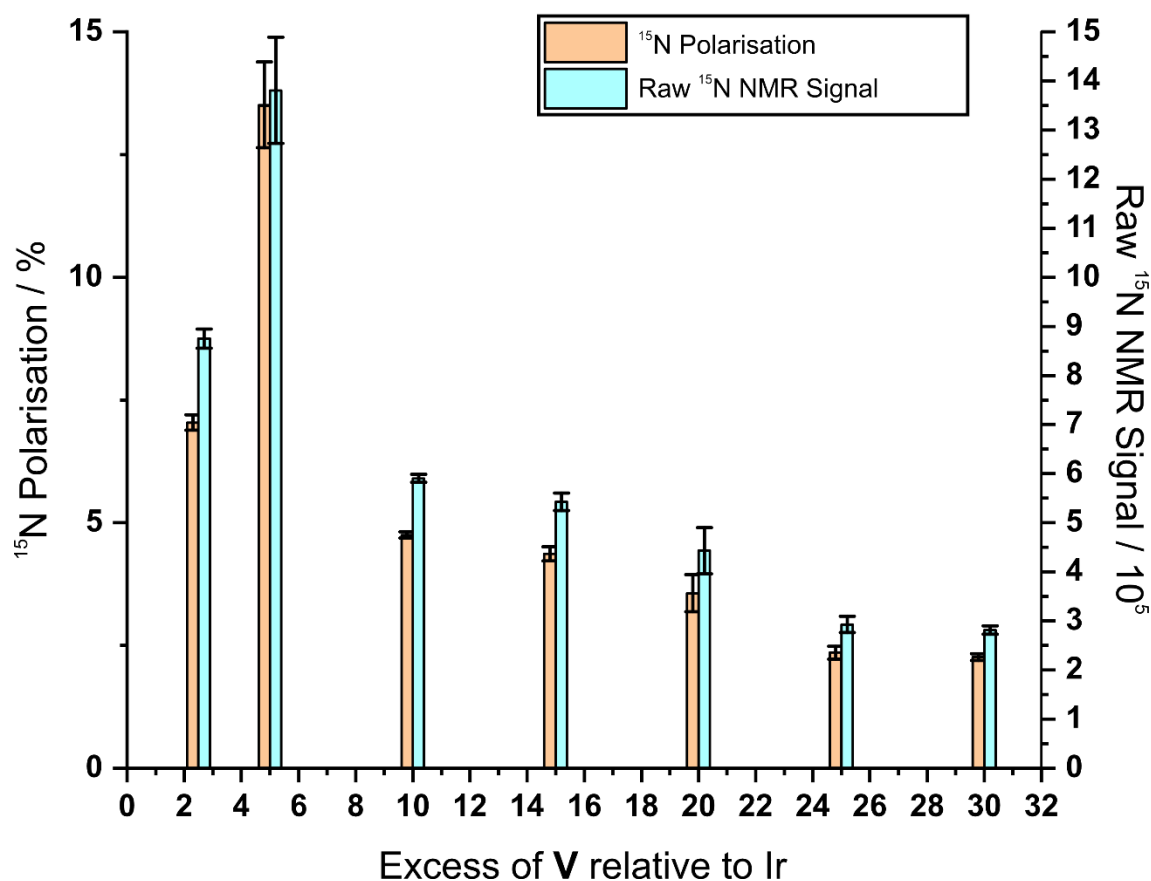


Figure 6.8 ¹⁵N polarisation displayed as a function of the excess of **V** on a series of samples containing 2 mg of the *IPr*^{Cl} pre-catalyst and 5 equivalents of *C*-d₃. All samples were shaken for 10 s at a field of -0.3 μT with a pressure of 4 bar *p*-H₂.

As expected, the hydride region of each of these NMR spectra conveys the information needed to explain this trend. This is revealed in Figure 6.9 which shows hydride regions of the corresponding ¹H NMR spectra for the samples with 2.5, 5, 15 and 25-fold excesses of the co-ligand **V**. The concentration of **20** is maximised with the 1 : 5 : 5 reagent loading. At higher loading it is evidenced that this acetonitrile polarising complex drops to an undetectable concentration when measured with 16 signal averages at a 9.4 T field.

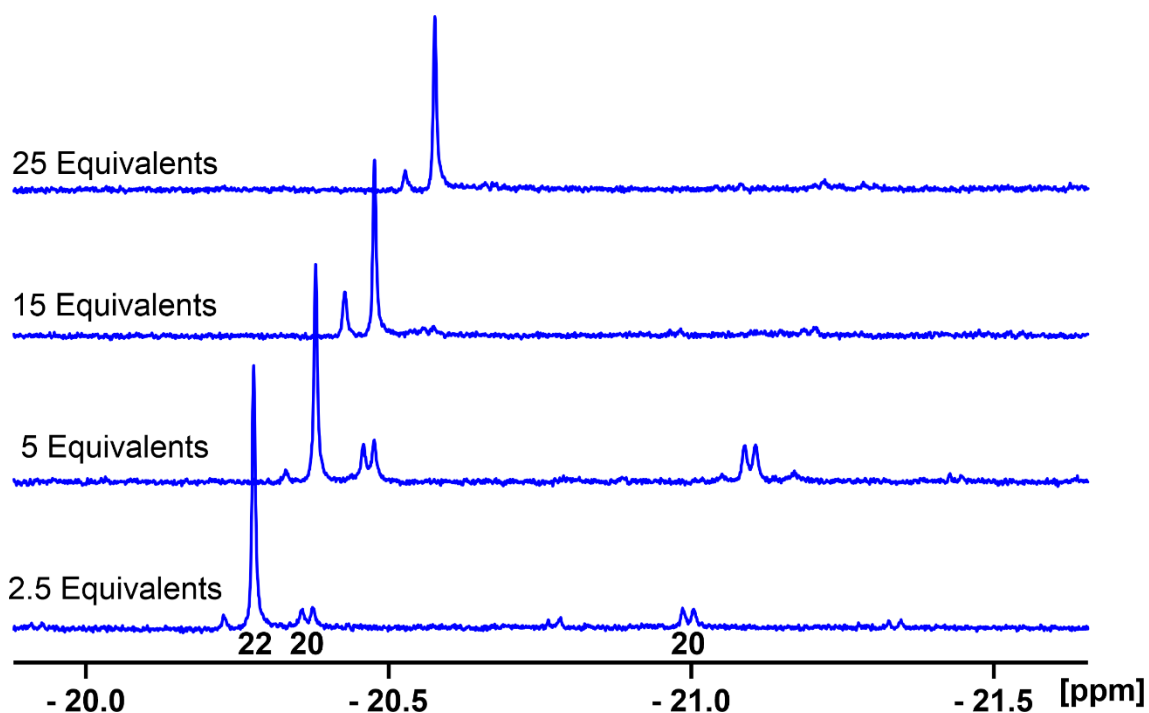


Figure 6.9 shows the effect on catalyst speciation caused by the increasing co-ligand concentration compared to catalyst.

A similar study was then completed to determine the effect of **C- d_3** concentration on the ^{15}N response for samples containing 2 mg of **IPr^{Cl}** (5mM) and 5 equivalents of **V** and various excesses of **C- d_3** in methanol- d_4 . The results of this study are shown in Figure 6.10; samples were shaken for 10 s at $-0.3\ \mu\text{T}$ with a pressure of 4 bar $p\text{-H}_2$. The maximum polarisation level for the ^{15}N NMR signal results when the ratio of the pre-catalyst **IPr^{Cl}** : **C- d_3** : **V** is 1 : 5 : 5. However, for the maximum signal, a trend is shown where raw ^{15}N NMR signal obtained from the hyperpolarised spectra increases with substrate concentration. This is despite the overall polarisation level falling.

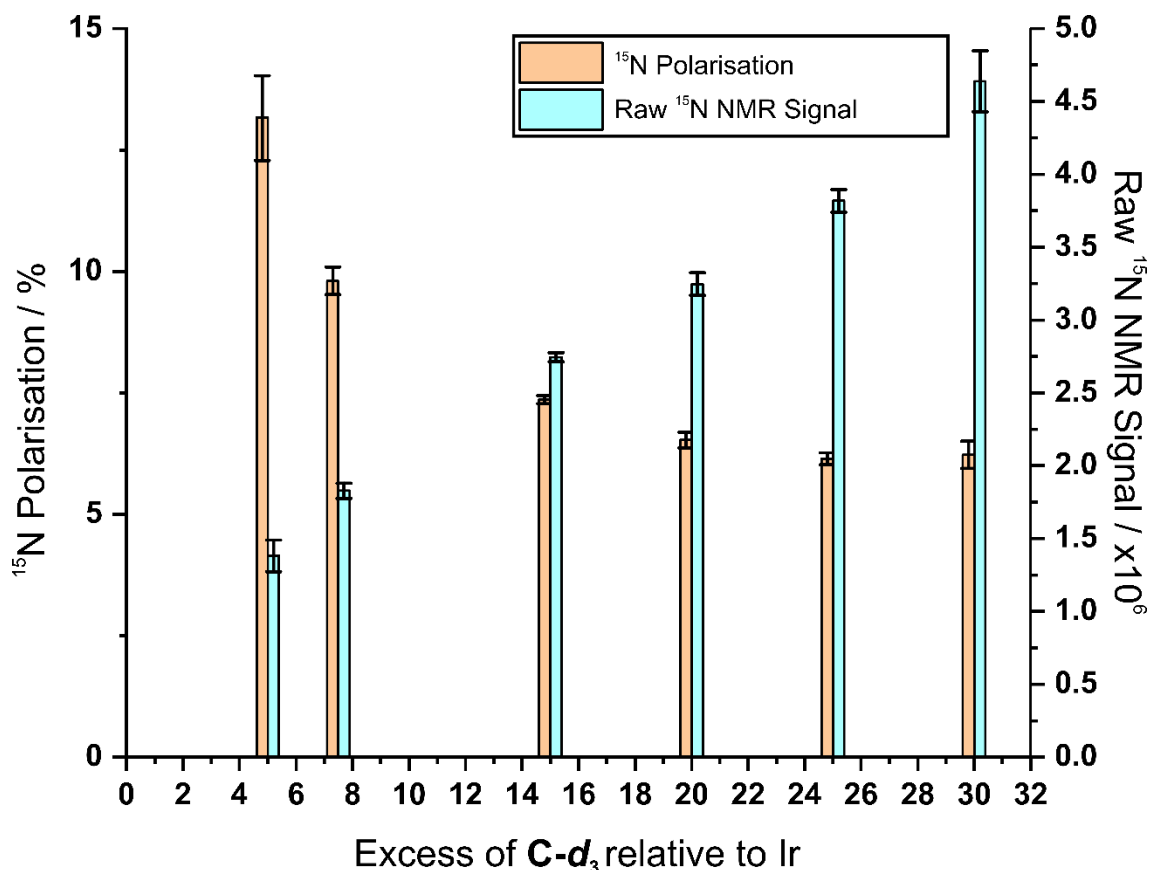


Figure 6.10 ^{15}N polarisation and raw ^{15}N NMR integral area of C-d_3 displayed as a function of the excess of C-d_3 itself. The series of samples each contained 2 mg of the IPr^{Cl} pre-catalyst and 5 equivalents of **V**. All samples were shaken for 10 s at a field of $-0.3 \mu\text{T}$ with a pressure of 4 bar $p\text{-H}_2$.

The largest polarisation value obtained is therefore still $13.4 \pm 0.7\%$, representative of 41100 ± 2100 -fold enhancement in ^{15}N NMR signal at 9.4 T. Moving to higher concentrations of acetonitrile resulted in a progressive drop in ^{15}N polarisation level which appears to plateau at around 6% within error of the measurement. Figure 6.11 shows the corresponding hydride ligand region of these samples ^1H NMR spectra. It is clear from these NMR spectra that the concentration of the unwanted and inactive **22**, and its isomeric form at $\delta -20.23$ decrease with increased C-d_3 concentration. However, the amount of poorly polarising **21** also increases. Table 6.2 shows quantitatively the amounts of each catalyst species as a % of overall catalyst species present for ease of comparison.

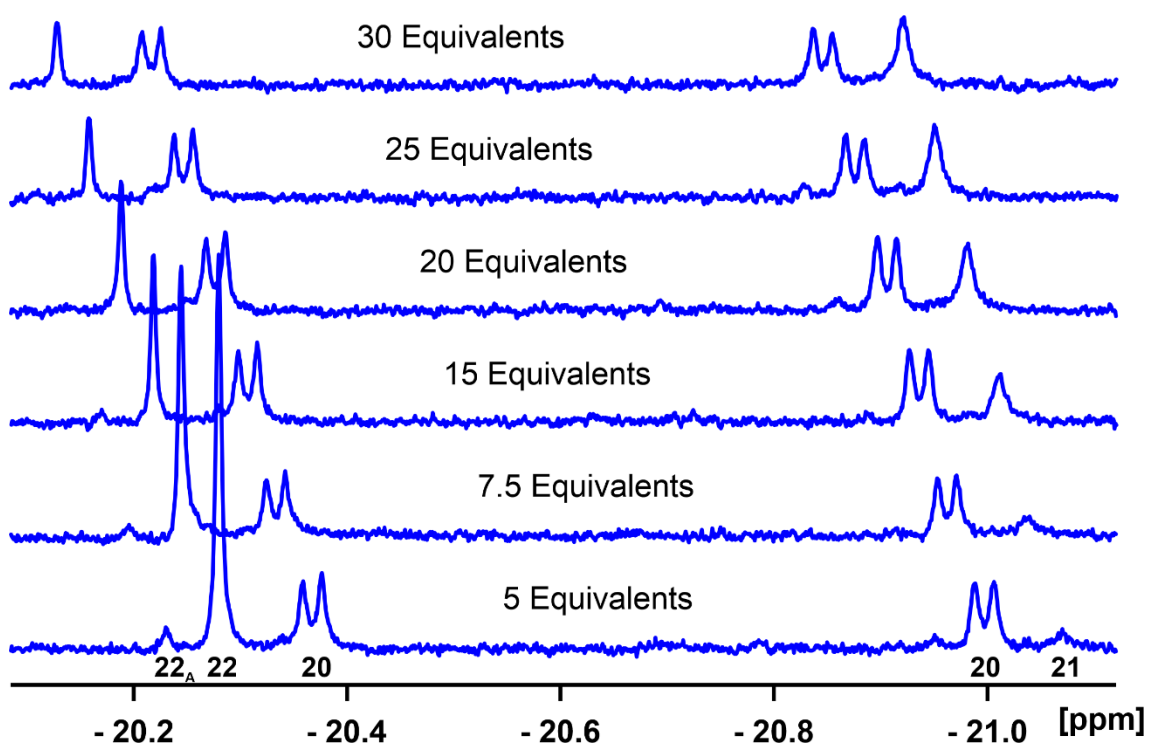


Figure 6.11 A series of ^1H NMR spectra showing the effect on catalyst speciation caused by the increasing substrate concentration compared to the Ir catalyst.

Table 6.2 Relative amount of **20**, **21** and **22** in solution as a % of the overall signal from all hydride ligands. Note that **22_A** is an isomeric form of **22** with similar behaviour to **4a** and **4b** described in Chapter 2.

Substrate Excess	Species Relative Abundance / %			
	22 _A	22	20	21
5	2.89	45.89	44.39	6.83
7.5	0.34	40.05	50.74	8.91
15	-	24.31	57.85	17.83
20	-	18.03	57.47	24.49
25	-	14.00	58.35	27.64
30	-	13.87	55.44	30.69

As the amount of **20** stays approximately the same throughout this study, a similar polarisation level might be expected to result. This is clearly not the case and so it is likely that $p\text{-H}_2$ destruction via **21** occurs.

6.5 Effect of ^{15}N polarisation of acetonitrile- d_3 as a function of polarisation transfer field.

As discussed in previous chapters, a condition of polarisation transfer is to match the frequency of the *parahydrogen* derived hydride ligand and the target nucleus. This resonance condition can be met at a LAC, as outlined in the introduction. This LAC may be met at multiple transfer fields and so a field sweep is necessary to explore the optimum polarisation that can be achieved.

In order to probe this a sample containing the optimum ratio of containing 5 mM IPr^{Cl} with 5 equivalents of both **V** and **C- d_3** was taken and shaken for 10 s under a pressure of 4 bar *p*- H_2 at PTFs ranging from 0.0 to $-1.0\ \mu\text{T}$. The solenoid used to generate the PTF is outlined in the experimental. The field sweep for the sample is presented in Figure 6.12.

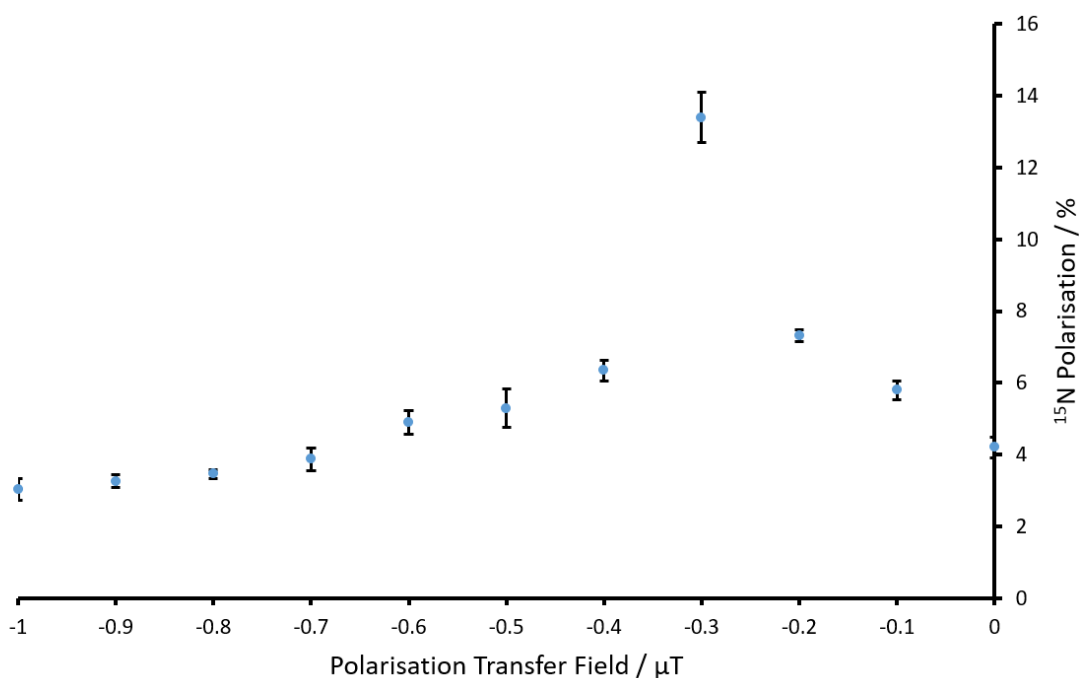


Figure 6.12 ^{15}N Polarisation (%) of free **C- d_3** as a function of polarisation transfer field (μT); the sample consisted of 2 mg of IPr^{Cl} with 5 equivalents of **C- d_3** and 5 equivalents of **V** in methanol- d_4 shaken for 10 s with a pressure of 4 bar *p*- H_2 at the corresponding PTF.

The PTF sweep showed clearly that the optimum PTF field was $-0.3\ \mu\text{T}$ achieving a maximum polarisation of $13.4 \pm 0.7\%$. This shows that the PTF used in previous studies was at the maximum for the transfer to the ^{15}N of **C- d_3** . In order to illustrate the PTF is dependent on the catalyst species a separate sample was taken with 2 mg of **1**, 5 equivalents of **C- d_3** and 5 equivalents of **V** and a field sweep conducted. The ^{15}N polarisation as a function of PTF is shown in Figure 6.13.

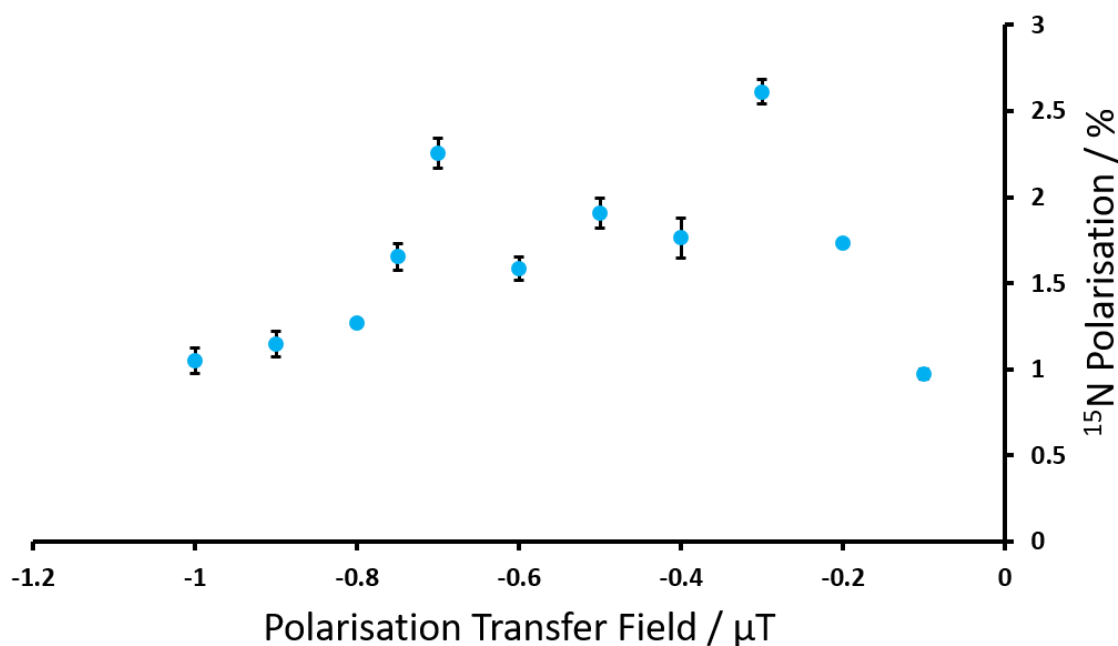


Figure 6.13 ^{15}N Polarisation (%) of free C-d_3 as a function of polarisation transfer field (μT); the sample consisted of 2 mg of **1** with 5 equivalents of C-d_3 and 5 equivalents of **V** in methanol- d_4 shaken for 10 s with a pressure of 4 bar $p\text{-H}_2$ at the corresponding PTF.

These data show, as predicted, that the PTF sweep looks very different for the same C-d_3 substrate with **1** as the pre-catalyst used. Now the PTF sweep shows fine structure at around -0.3 and -0.7 μT showing two different LAC occur. The polarisation here is notably much lower than with the catalyst IPr^{Cl} , with a maximum polarisation of only $2.12 \pm 0.08\%$ at -0.3 μT . The -0.7 μT field used showed a polarisation of $1.97 \pm 0.18\%$ which within error is the same as the -0.3 μT field.

The reason behind the difference in the appearance of the field sweeps shown between Figure 6.12 and Figure 6.13 is likely due to the difference in catalytic species. The LAC is reliant on the chemical shifts of the two bound species and as the electronics of both catalysts are different, the *parahydrogen* derived hydrides and the ^{15}N resonance will both show a different chemical shift between species. With the IPr^{Cl} derived catalyst the hydride signals for the species $[\text{Ir}(\text{H})_2(\text{IPr}^{\text{Cl}})(\text{C-d}_3)(\text{V})_2]\text{Cl}$ appear at δ -20.37, -21.00 on the ^1H spectrum, and bound acetonitrile shows a peak at δ 174.56 on the ^{15}N spectrum, as mentioned previously. In the IMes species the bound ^{15}N acetonitrile signal is now shifted to δ 171.15 with ^1H resonances of the hydrides now at δ -20.23 and -21.20.

The system containing 5 mM IPr^{Cl} with 5 equivalents of both **V** and C-d_3 now represents a fully optimised system for polarisation from C-d_3 in terms of steric bulk, electronics and polarisation transfer field. As this optimised system works so well for C-d_3

d_3 ^{15}N polarisation, it was deemed appropriate to extend the study to other nitrile containing species to see if the same effect was observed.

6.6 Expansion of optimisation to other nitrile containing compounds

As mentioned in the introduction to this chapter, nitriles are very common in molecules with a wide variety of uses. Due to this it is important that the optimisation works for all nitrile containing species if it is to be implemented to detect them. To test this a variety of nitrile compounds were selected to see if the optimisation was universal to all nitriles or selective to acetonitrile only. The nitriles selected for the study are shown in Figure 6.14.

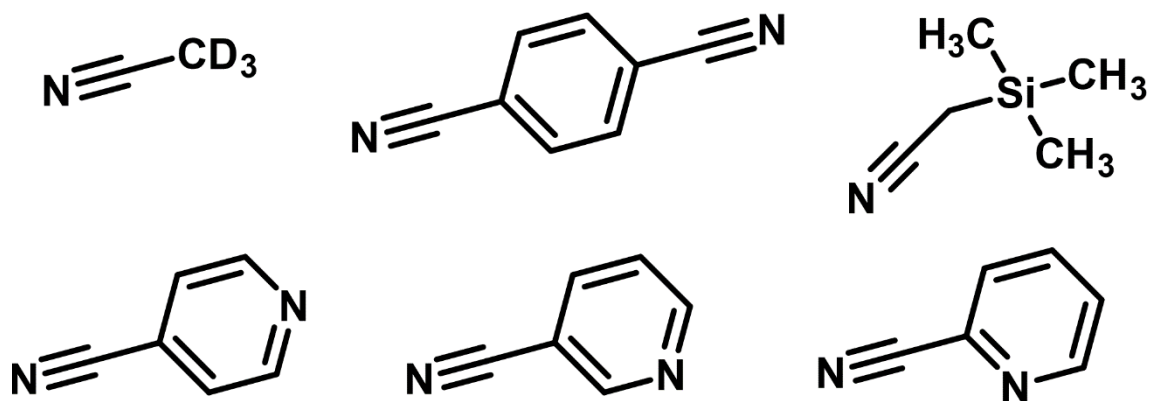


Figure 6.14 shows the structures of the nitrile containing compounds selected for this study.

In order to test this, an unoptimised sample of **1** (2 mg) with 5 equivalents of each nitrile compound was taken and shaken in a field of $-0.3\ \mu\text{T}$ and a pressure of 4 bar $p\text{-H}_2$. All samples were found to give no corresponding nitrile signal observable in a one scan acquisition. In order to gain a ^{15}N response, fresh samples were made containing the same ratio of **1** : nitrile substrate but now with 5 equivalents of **V** also added. It was found that some ^{15}N resonances were now observable in a one scan acquisition at 9.4 T after shaking under the same conditions. Modest polarisation levels were observed for all nitrile compounds shown in Table 6.3. To further this study and show the capability of the fully optimised catalytic species a series, of samples were made with 2 mg of IPr^{Cl} and the same ratio of 5 equivalents of substrate and **V** and shaken under the same conditions as previously described. It was expected that polarisation of all nitrile species would improve considerably and that it may even be possible to observe the ^{15}N NMR resonances of the pyridine type N-donors present on some compounds also. These data are presented in Table 6.3.

Table 6.3 % polarisation values for the ^{15}N responses of the nitriles shown in Figure 6.14; all samples were shaken at a field of $-3\ \mu\text{T}$ with a pressure of 4 bar $p\text{-H}_2$ for 10 s with either ‘un-optimised’ or ‘optimised’ conditions as explained in the text above.

Substrate	δ / ppm	% polarisation	
		un-optimised	optimised
Acetonitrile- d_3	240.8	2.10 ± 0.10	13.7 ± 0.70
2-cyanopyridine	253.4	Signal not observed	4.57 ± 0.16
	314.0	Signal not observed	Signal not observed
3-cyanopyridine	262.1	0.80 ± 0.07	4.29 ± 0.06
	310.5	Signal not observed	1.20 ± 0.04
4-cyanopyridine	263.4	1.00 ± 0.14	9.66 ± 0.11
	319.2	Signal not observed	0.80 ± 0.09
1,4-dicyanobenzene	260.4	0.90 ± 0.04	4.46 ± 0.05
Trimethylsilyl acetonitrile	240.7	0.30 ± 0.03	10.38 ± 0.13

As expected, impressive improvements were seen for all nitrile compounds with the largest improvement of 34-fold being observed for trimethylsilylacetonitrile now achieving a $10.38 \pm 0.13\%$ ^{15}N polarisation, representing a 31700 ± 200 -fold enhancement in signal at a 9.4 T acquisition field. Due to the incredibly high polarisation levels achieved on the ^{15}N , the hyperpolarisation of ^{29}Si was attempted here by shaking the sample with 4 bar of fresh $p\text{-H}_2$ at fields of $-0.3\ \mu\text{T}$ and 6 mT for 10 s before transferring to a 9.4 T magnet for ^{29}Si NMR signal acquisition. However, no ^{29}Si was observed in a single scan acquisition for trimethylsilylacetonitrile under these conditions. The highest polarisation level still remained with **C- d_3** , although it is noted that **C- d_3** benefits uniquely in this range of substrates from its deuterated form. It is predicted that the other substrates may benefit from the same polarisation increase that comes with deuteration of the substrate, although this has not been attempted in this work.

In order to illustrate just how selective the optimum PTF for compounds could be, samples of the isomers 2-, 3- and 4-cyanopyridine were prepared containing 2 mg of the **IPr^{Cl}** pre-catalyst with 5 equivalents of the co-ligand **V** and 5 equivalents of the corresponding nitrile being studied. The samples were degassed before filling to a pressure of 4 bar H_2 and allowed to activate for 30 min. The samples were then shaken at

various fields ranging from 0.0 to 1.0 μT for 10 s under a pressure of 4 bar $p\text{-H}_2$ before being transferred to a 9.4 T magnet for immediate ^{15}N NMR signal acquisition. Figure 6.15 displays the polarisation of the ^{15}N nitrile resonance for each of the 2-, 3- and 4-cyanopyridine displayed alongside **C-d₃**, for ease of comparison to the above Figure 6.12.

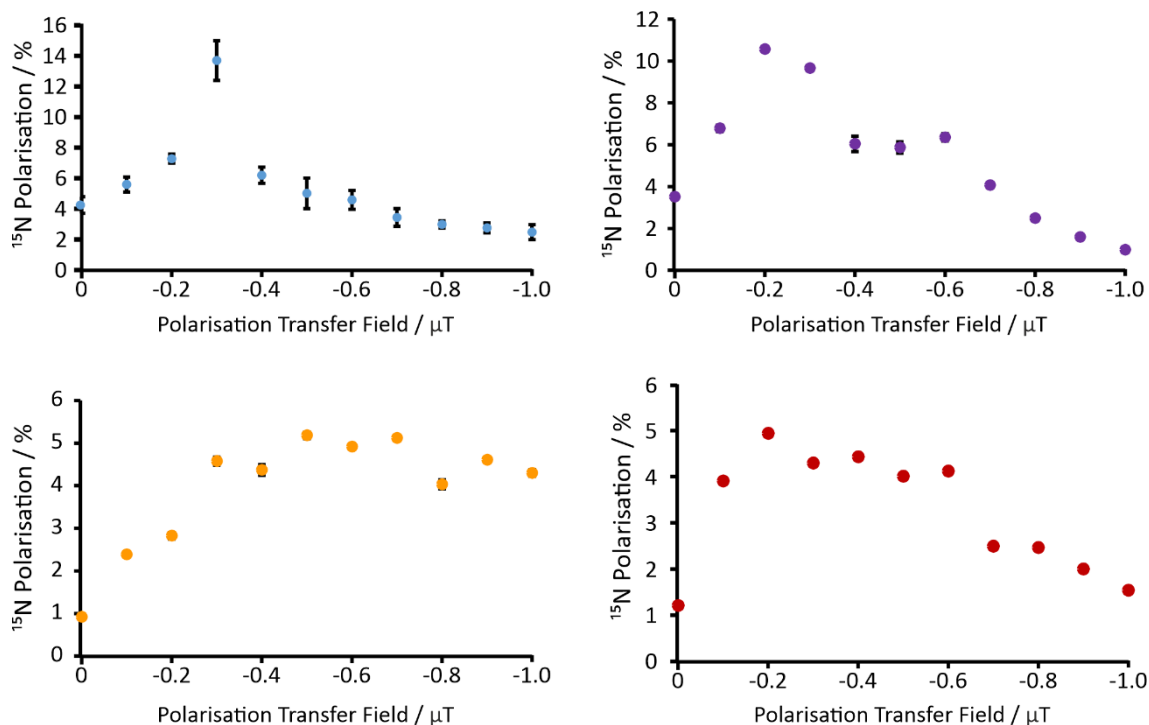


Figure 6.15 Polarisation as a function of polarisation transfer field. All samples consisted of 2 mg of IPr^{Cl} with 5 equivalents of **V** and 5 equivalents of the corresponding nitrile compound. Top left – acetonitrile- d_3 . Top Right – 4-cyanopyridine. Bottom left – 3-cyanopyridine. Bottom right – 2-cyanopyridine.

All the nitriles tested showed a unique polarisation transfer field. Notably, all seem to show a maximum polarisation (or close to) at *circa* $-0.3 \mu\text{T}$ which, as mentioned, was found to be superior for $[\text{}^{15}\text{NO}_2]^-$ (**Y**) in Chapter 3. It is also interesting to note that 2-cyanopyridine showed a relatively close to maximum polarisation yield all the way out to $-1.0 \mu\text{T}$. This was not observed for any other nitriles which all decreased to a much lower proportion of their maximum polarisation yield. It was hypothesised that this unique characteristic could be exploited to selectively polarise 2-cyanopyridine in a mixture of the nitriles. In order to test this hypothesis, it first had to be determined whether competition for the catalyst caused by more nitriles being present would affect polarisation transfer.

6.7 Analysis of Mixtures of Nitriles

During pharmaceutical manufacture it is essential to be able to identify impurities and unwanted isomeric products at each stage of a synthetic procedure. These isomeric

products can be hard to identify by ^1H NMR or even mass spectrometry due to the similarity between the products involved. ^{15}N NMR offers a great advantage of an increased chemical shift range over ^1H and low background signal. A sample was prepared containing 2 mg IPr^{Cl} pre-catalyst, 5 equivalents of **V** and 5 equivalents of both 3- and 4-cyanopyridine. This sample was then shaken at a field of $-0.3\ \mu\text{T}$ and transferred to a 9.4 T magnet for ^{15}N NMR signal acquisition. Figure 6.16 shows a typical ^{15}N NMR spectrum achieved by this process and shows two clear signals for the ^{15}N nitrile signal for 3- and 4-cyanopyridine at δ 262.1 and 263.4 respectively.

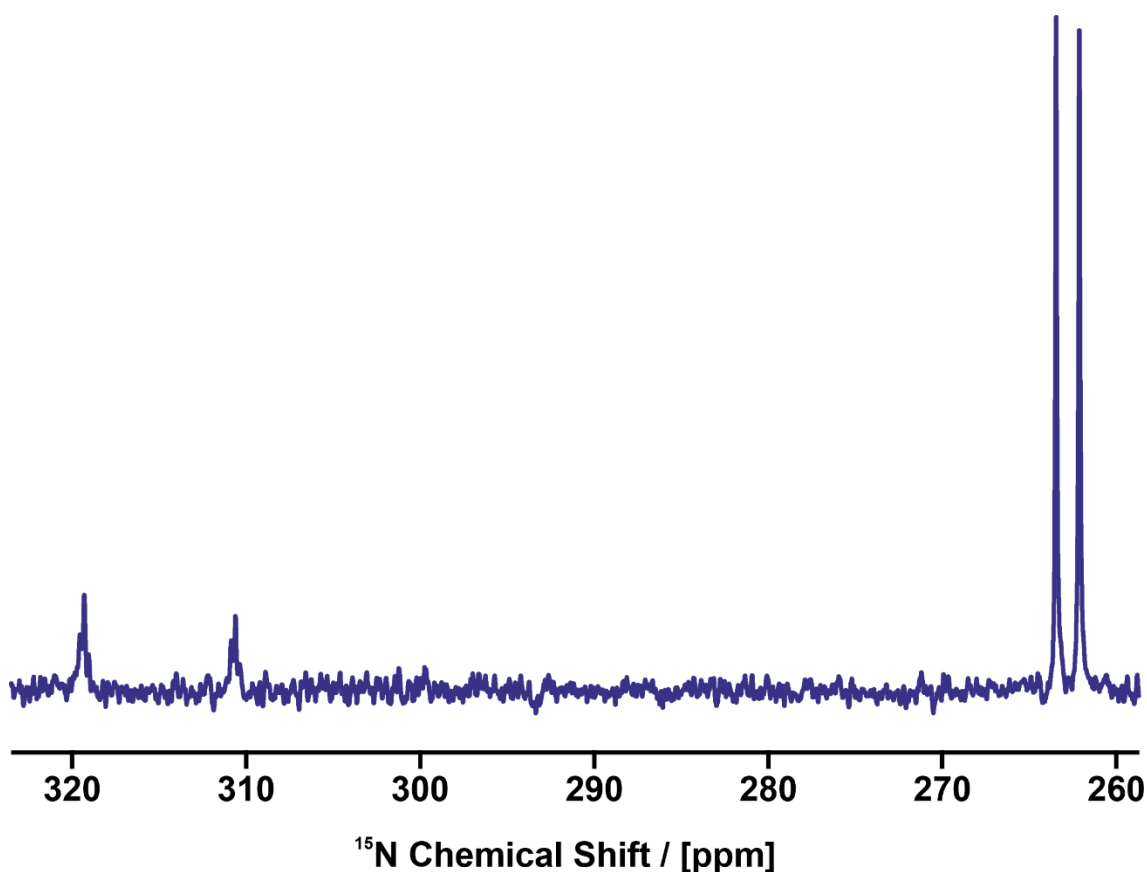


Figure 6.16 Typical SABRE hyperpolarised ^{15}N spectrum of a mixture containing 2 mg IPr^{Cl} with 5 equivalents of **V** and of both 3- and 4-cyanopyridine. Sample was shaken at a field of $-0.3\ \mu\text{T}$ for 10 s with 4 bar of $p\text{-H}_2$ and acquired at a field of 9.4 T.

The ^{15}N NMR signals for the pyridyl nitrogen on each compound are also visible here at δ 310.5 and 319.2 for 3- and 4-cyanopyridine respectively. Table 6.4 shows the relative polarisation and enhancement values for these four ^{15}N resonances of the two species made visible in one scan by SABRE hyperpolarisation.

Table 6.4 ^{15}N polarisation and enhancement values at 9.4 T for the ^{15}N resonances present in 3- and 4-cyanopyridine.

Sample	^{15}N Chemical Shift / ppm	Enhancement / Fold	Polarisation / %
3-cyanopyridine	262.1	17100 ± 700	5.6 ± 0.2
	310.5	3100 ± 750	1.0 ± 0.2
4-cyanopyridine	263.4	17000 ± 650	5.6 ± 0.2
	319.2	5500 ± 450	1.8 ± 0.2

Under these conditions, the pyridyl ^{15}N -donor on both compounds is still polarised to a much lower level than the nitrile showing the binding of nitriles is still selective even though catalyst speciation will be even more complex here. Interestingly the 4-cyanopyridine suffers under these conditions whereas the 3-cyanopyridine is polarised to the same extent as when it was by itself.

To extend this principle of polarising mixtures further, an extremely complicated sample of all the nitrile containing compounds in Figure 6.14, apart from trimethylsilylacetonitrile, was probed. This mixture contained 2 mg of the pre-catalyst **IPr**^{Cl}, 5 equivalents of all nitrile compounds and 5 equivalents of **V** also. The sample was shaken at a PTF of -0.3 μT with a shake time of 10 s and a pressure of 4 bar $p\text{-H}_2$. This sample yielded a clear ^{15}N response for all nitriles in the mixture as outlined in Figure 6.17. Even the isomeric cyanopyridines that suffered from signal overlap in the ^1H spectra now yield distinct response in the ^{15}N NMR spectrum obtained. All resonances showed lower levels of polarisation under these conditions. Table 6.5 shows the relative polarisation and enhancement at 9.4 T under these conditions.

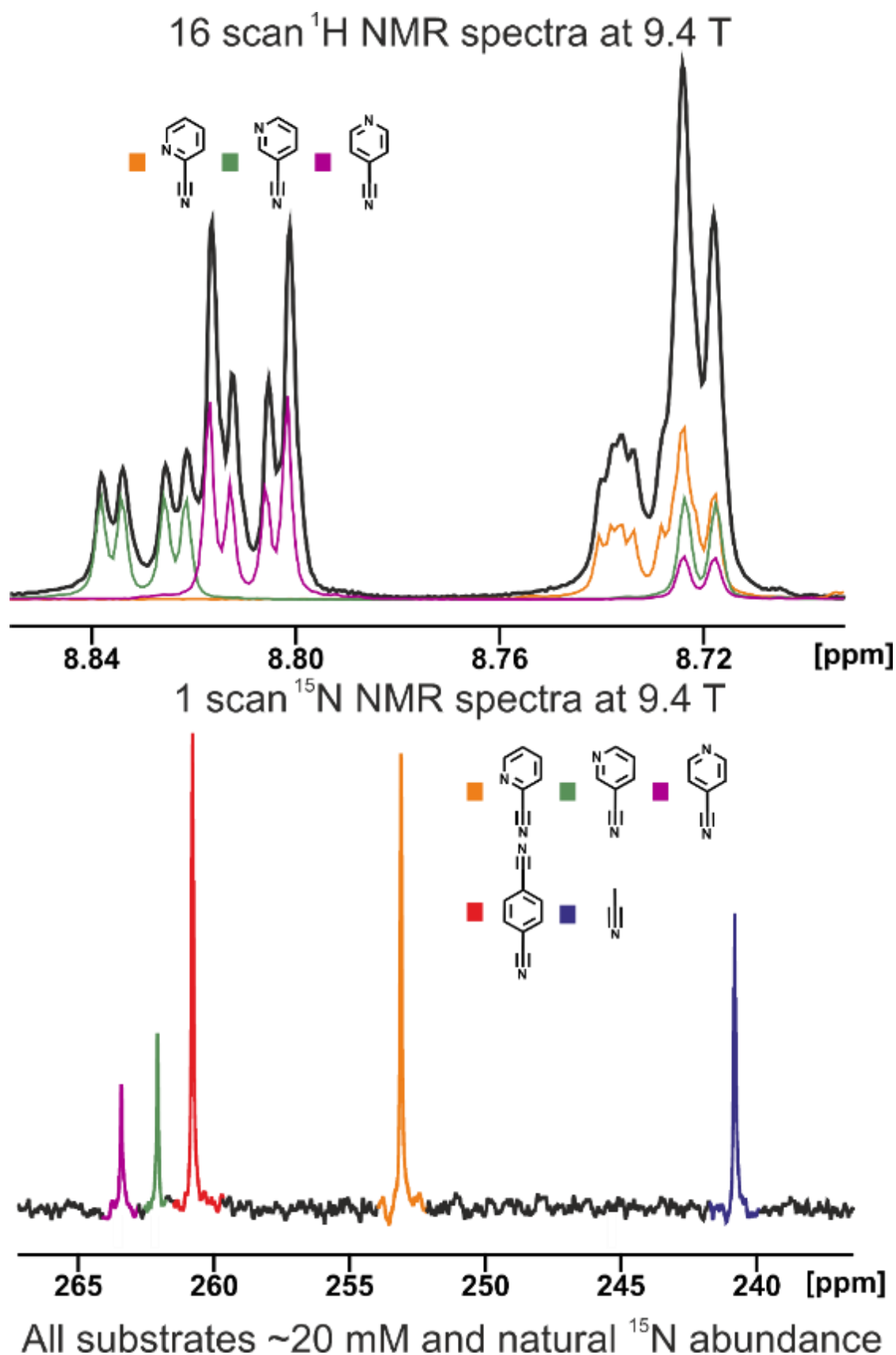


Figure 6.17 (Top) ^1H NMR spectrum of 2-, 3- and 4-cyanopyridine shown to overlap at 9.4 T. (Bottom) ^{15}N NMR spectrum, gained after shaking for 10 s at $-0.3\ \mu\text{T}$ with a pressure of 4 bar $p\text{-H}_2$. The spectrum was also collected at a field of 9.4 T.

This level of polarisation on such a complicated mixture opens up the opportunity for benchtop analysis of complex mixtures in a one scan experiment. This kind of analysis is relatively low cost and requires very little maintenance when compared with mass spectrometry or high field NMR for an example. Benchtop NMR spectroscopy is also very portable and could easily be placed inside a fume hood. The resonance frequency of ^{15}N on a standard benchtop at 1.4 T (60 MHz ^1H frequency) would be around 6 MHz which is extremely low and requires specialist probes. Due to this, these mixtures were not able to be tested on a benchtop spectrometer during this research, but this is an obvious extension of the work and commercial systems are available with this capability.

Table 6.5 ^{15}N polarisation and enhancement values at 9.4 T for the ^{15}N resonances present in the complicated mixture.

Substrate	δ / ppm	Enhancement / Fold	Polarisation / %
Acetonitrile- d_3	240.8	7800 ± 700	2.50 ± 0.20
2-cyanopyridine	253.4	11800 ± 930	3.90 ± 0.30
	314.0	Signal not observed	Signal not observed
3-cyanopyridine	262.1	4800 ± 370	1.60 ± 0.10
	310.5	Signal not observed	Signal not observed
4-cyanopyridine	263.4	3400 ± 500	1.10 ± 0.20
	319.2	Signal not observed	Signal not observed
1,4-dicyanobenzene	260.4	6700 ± 600	2.30 ± 0.20

The above experiment was then repeated in *protio*-methanol, as it was observed there is no longer the requirement for expensive deuteration of the solvent when probing ^{15}N resonances. To carry out this process, the sample was locked and shimmed to a sample of methanol- d_4 of the same sample depth. The test sample was then filled to a pressure of 4 bar with $p\text{-H}_2$ before being shaken at a field of $-0.3 \mu\text{T}$ for 10 s and transferred into the 9.4 T magnet for ^{15}N NMR signal acquisition, now with the lock turned off. The observed ^{15}N NMR spectrum acquired showed the same five nitrile peaks, however it is noted slightly lower polarisation levels were achieved in all cases although remained roughly the same within error. The slight fall in achieved ^{15}N polarisation is likely due to the presence of many more ^1H nuclei to interact with causing increased T_1 relaxation. On a

high field instrument, like the 9.4 T instrument used in this study, the lock signal is required to keep the sample referenced correctly. Running repeat studies of this sample with the lock off could lead to slight deviations in the chemical shift of the ^{15}N response. However, many benchtop NMR spectrometers do not have this issue as they utilise an external lock feature and so this *protio*-solvent approach would be beneficial to reducing cost yet again.

The above experiments have now shown that these complex mixtures of nitriles yield high levels of polarisation. The next step was to see if one of these chemically similar species could be selected out of the mixture and selectively polarised utilising the differences in the optimum PTF observed in Figure 6.15. This study utilised the same sample as that in Figure 6.17, only now the sample was shaken at a field of $-1\ \mu\text{T}$ instead of $-0.3\ \mu\text{T}$. The results of the study shaking at $-0.3\ \mu\text{T}$ and $-1.0\ \mu\text{T}$ are presented in Figure 6.18.

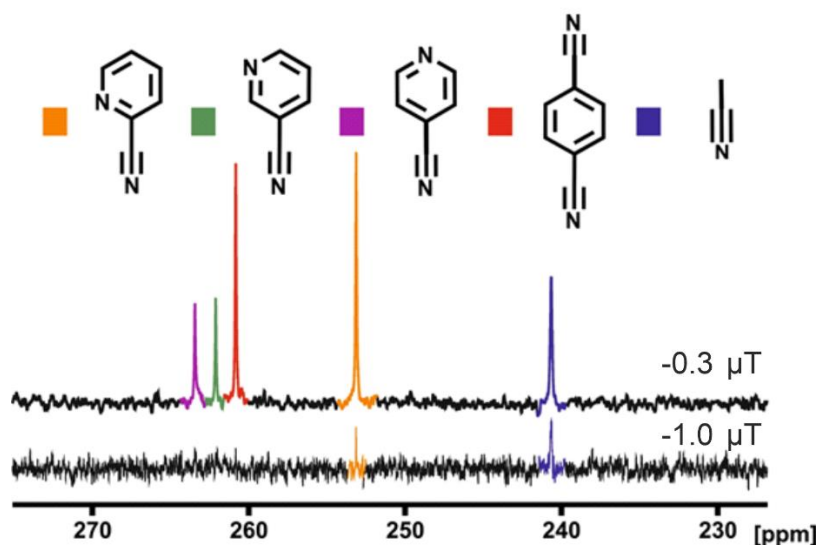


Figure 6.18 Typical SABRE hyperpolarised ^{15}N NMR spectra gained from a sample containing 2 mg of the pre-catalyst IPr^{Cl} , 5 equivalents of all nitrile compounds in Table 6.5 and 5 equivalents of V also. The sample was shaken at a PTF of either -0.3 or $-1.0\ \mu\text{T}$ with a shake time of 10 s and a pressure of 4 bar $p\text{-H}_2$.

The ^{15}N NMR spectra show that the selective detection of only some of the nitriles in the mixture is possible by varying the PTF used. When the field of $-1.0\ \mu\text{T}$ is utilised the polarisation level of 3- and 4-cyanopyridine, and 1,4-dicyanobenzene drop to undetectable levels in the one scan acquisition whereas 2-cyanopyridine and acetonitrile are still readily detected at 0.02% (70-fold signal enhancement) and 0.32% (980-fold signal enhancement) ^{15}N polarisation, respectively.

Identifying impurities within industrial processes is important but quantifying these impurities is even more important. As detailed in the previous section, the competition for the catalyst caused by having more than one substrate present does indeed have an effect on the polarisation level achieved and so quantification of a complicated sample is not possible due to this. Quantification of a simpler sample though where only one nitrile concentration changes at a time could be possible as the concentration of this nitrile is increased, catalysts containing this species should be favoured and so polarisation should increase. To exemplify this, the data in Figure 6.10 was taken and plotted as a scatter graph to fit a linear function shown in Figure 6.19.

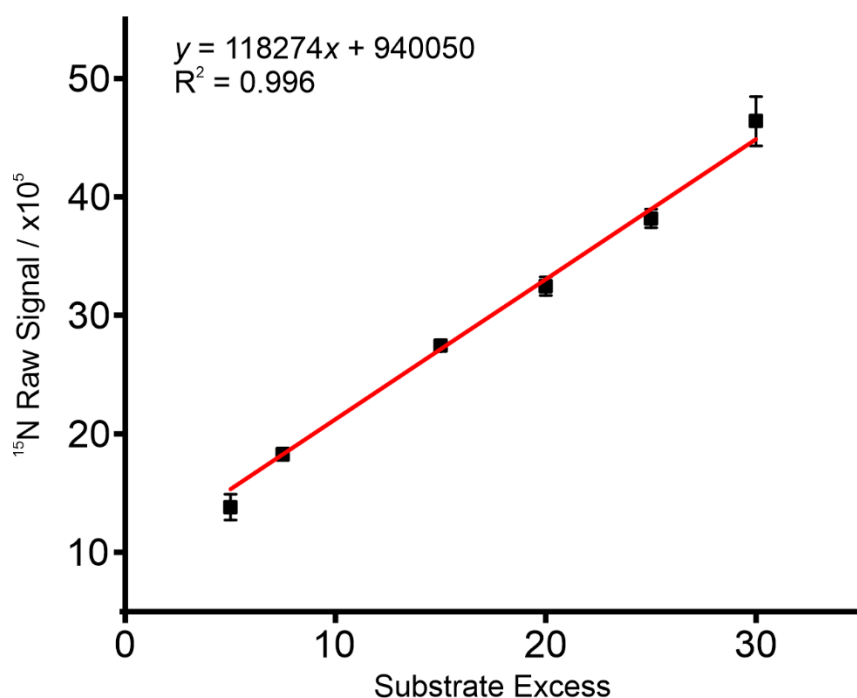


Figure 6.19 Raw signal of the detected ^{15}N acetonitrile response as a function of acetonitrile excess. All samples were shaken for 10 s at a field of $-0.3 \mu\text{T}$ with a pressure of 4 bar $p\text{-H}_2$.

These data show a good linear response evidenced by the high R^2 of 0.996. This linear trend has previously been shown by Fekete *et al.* with samples containing pyrazine, pyridine and metronidazole as examples.⁴³ Here, the linear trend is extended, being observed for nitriles also. Interestingly the linear response is maintained despite the change in catalyst speciation observed in Figure 6.11. It was observed in the previous section how competition for the catalyst between these nitrile species, and thus catalyst speciation, can affect the ^{15}N signal obtained from a sample. In the case of a single substrate it does not seem to have an effect.

6.8 Work towards nitriles as potential MRI contrast agents

In order to be useful as an MRI imaging agent, a substrate must have sufficient observable signal to be detectable in a single scan in the bloodstream. For SABRE to operate successfully, it must also survive the time period taken to prepare the biocompatible sample for injection. The first of these issues has already been maximised by optimising the polarisation transfer system to deliver 10% polarisation.

The next study involved taking the optimum system of 2 mg **IPr**^{Cl} 5 equivalents of **C-d₃** and 5 equivalents of **V** and measuring the lifetime of the polarised signal to see whether it would be suitable as an imaging agent. This was carried out as detailed in the experimental using a hyperpolarised T_1 pulse sequence. It was hypothesised that due to the ^{15}N site of the acetonitrile being so isolated, the lifetime of the ^{15}N resonance would be very long. Also, due to the fact that the sample is deuterated, the ^{15}N should experience less dipolar relaxation due to the low γ of deuterium compared with ^1H nuclei. A series of typical polarised ^{15}N NMR spectra are presented in Figure 6.20 that show the resulting decay in signal intensity with time. Next to this in the figure is a plot showing how the attenuating signal can be fitted to an exponential decay to obtain T_1 .

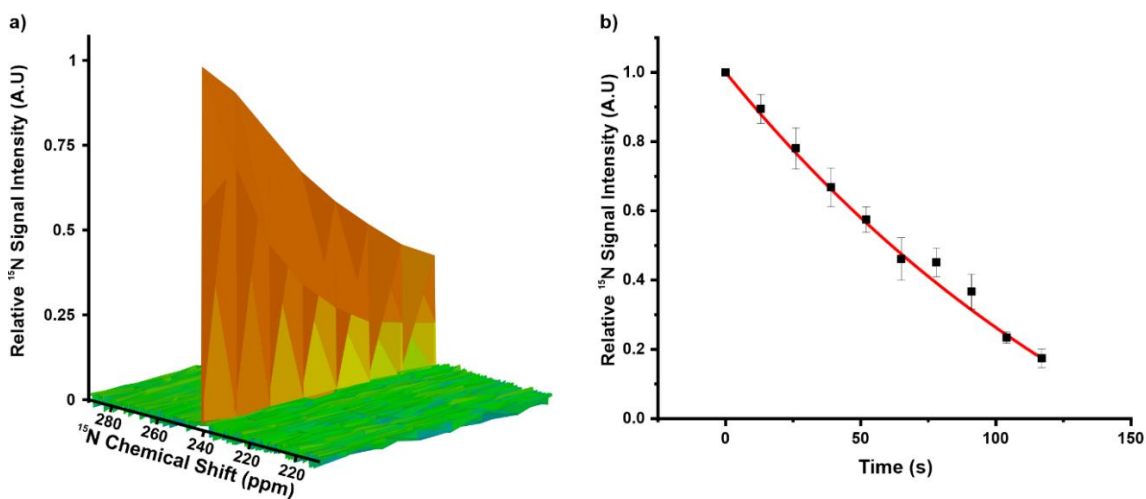


Figure 6.20 a) A series of hyperpolarised ^{15}N NMR intensity data acquired to show the impact of ^{15}N signal relaxation. b) Typical plot showing the resulting ^{15}N signal intensity corrected for magnetisation loss against time for to an exponential decay linked to T_1 . All data was collected at 9.4 T. Each slice was collected using a pulse length equating to an angle of 14 degrees. The sample was shaken for 10 s in a field of $-0.3\ \mu\text{T}$ with a pressure of 4 bar $p\text{-H}_2$ before transfer to the magnet for data acquisition.

The T_1 proved to be 183 ± 26 s, thereby providing around 900 s ($5 \times T_1$) of measurement time. Hence sample separation should be feasible. It was hypothesised

however that this time could be extended by storage of the sample in a predefined magnetic field as demonstrated previously in Chapter 4. Consequently, the T_1 value was evaluated at 11.7 T and 7 T where values were 149 ± 43 s and 241 ± 34 s, respectively. This lifetime is expected to extend even further at lower storage fields. As most clinical MRI machines operate at a field of 3 T or less, the hope this ^{15}N signal lifetime may extend is highly promising. A similar method was then used to evaluate the ^{15}N lifetimes of the remaining nitriles of Figure 6.14. As predicted, a similar beneficial trend is seen for all. Table 6.6 shows the corresponding T_1 lifetime data at the different storage fields. Given the major difference though between **C- d_3** and the *protio*-version, we might predict that a similar increase in T_1 may be achieved for other substrates possibly having huge gains of the lifetime of observable signal for other substrates. Deuterated samples of these substrates can be prepared but this was beyond the scope of this study, although it is noted that this would be a productive follow up study from this work.

Table 6.6 T_1 lifetime data collected at 11.7 T, 9.4 T and 7 T for a series of nitrile compounds for which the SABRE hyperpolarisation has been optimised.

Substrate	T_1 (s)		
	11.7 T	9.4 T	7 T
Acetonitrile- d_3	149 ± 43	183 ± 26	241 ± 34
^{15}N - Acetonitrile- h_3 *	91 ± 9	93 ± 2	121 ± 4
2-cyanopyridine	74 ± 8	68 ± 9	118 ± 13
3-cyanopyridine	51 ± 9	61 ± 7	76 ± 6
4-cyanopyridine	44 ± 6	52 ± 3	89 ± 8
1,4-dicyanobenzene	33 ± 3	55 ± 7	72 ± 15
Trimethylsilyl acetonitrile	38 ± 3	43 ± 3	65 ± 4

***From a ^{15}N labelled sample of *protio*-acetonitrile acquired thermally at each field using a T_1 inversion recovery pulse sequence outlined in the experimental.**

It is noted that the acquisition of the *protio*- ^{15}N -acetonitrile lifetime, although 99.8% ^{15}N labelled, took around 4 days of acquisition time due to the $5 \times T_1$ delay needed between each acquisition. This measurement could have been done in around 12 hrs using a T_1 saturation recovery instead but the aim was to illustrate the enormous length of time these measurements take, even with expensive labelling. Without labelling, at the same concentration of acetonitrile, this sample would have taken around 4 years of acquisition

time to achieve the same level of signal to noise as in this experiment. Due to the improved polarisation level developed by the methods implemented in this chapter, utilising SABRE hyperpolarised signals can achieve the same spectra in just 3 minutes, a much more agreeable time commitment representing a 700,000-fold time saving.

To ensure the ^{15}N labelling was not the reason for the sudden decrease in T_1 lifetime a control sample of pure acetonitrile was taken and a T_1 inversion recovery undertaken over a period of 5 days at a field of 11.7 T. This resulted in a T_1 of 91 ± 14 s which is in agreement, within measurement error, with that obtained by the ^{15}N labelled sample.

As the long lifetime and high polarisation levels were achieved for all the nitrile compounds tested, it was decided to try to extend the study to a biologically relevant nitrile compound as this would be much more relevant as an MRI contrast agent. The drug molecule commonly known as anastrozole was selected for study. The skeletal formula for anastrozole is displayed in Figure 6.21.

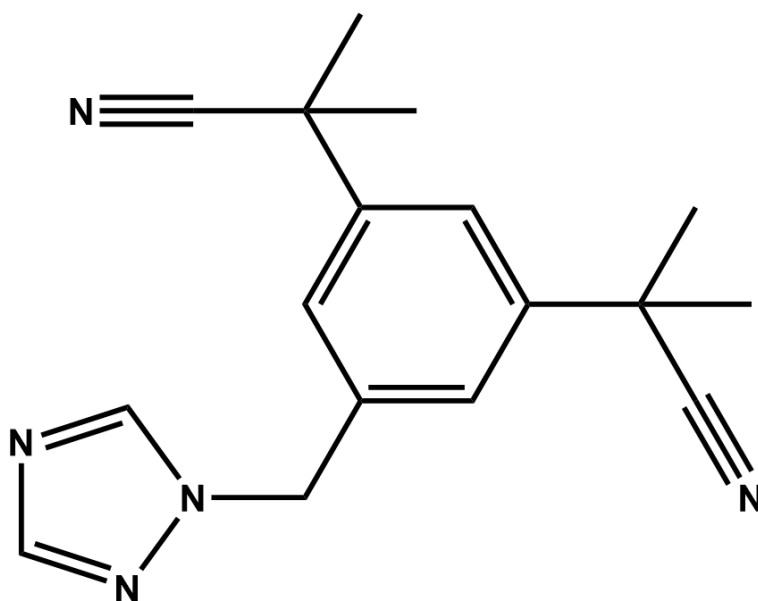


Figure 6.21 Skeletal structure of the drug molecule anastrozole; IUPAC name 2,2'-[5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]bis(2-methylpropanenitrile).

Anastrozole was chosen as it is currently used as a treatment both in a preventative and a curative manner for certain types of breast cancer caused by the overproduction of oestrogen.²³⁰ Anastrozole works by inhibiting aromatase enzymes responsible for producing oestrogens in the body. By stopping or slowing the production of these oestrogens it is possible to stop the cancer forming in the tissue or starve it leading to the carcinoma regressing.²³⁰ Interestingly, anastrozole has multiple potential N-donor sites

available. Due to this, the electronics of the SABRE system developed in this chapter are expected to be key in favouring binding through one of the nitrile groups and not through the N-substituted triazole ring functionality present.

The polarisation of anastrozole was optimised in the same way as previous substrates. The same optimised catalyst system of **IPr**^{Cl} (2 mg) with 5 equivalents of both anastrozole and **V** were taken in 0.6 mL of methanol-*d*₄. The sample was degassed and activated under 4 bar of H₂ for 30 min. At this point the sample was shaken in a field of -0.3 μT; this was found to give a polarisation on each ¹⁵N nitrile resonance of 10.70 ± 0.20% which represented a 32900 ± 700-fold improvement in signal detection for the two nitrile signals in the free material measured at a 9.4 T acquisition field. However, using a sample of the un-optimised catalyst **1** (2 mg), with 5 equivalents of both anastrozole and **V** under the same conditions only gave 1.7 ± 0.4% polarisation.

As the catalyst system was shown again to be an improvement over an unoptimized catalyst, a study was carried out to determine the optimum polarisation transfer field. For this, a fresh sample of 2 mg of **IPr**^{Cl} with 5 equivalents of anastrozole and **V** was shaken at various polarisation transfer fields with 4 bar of *p*-H₂. Figure 6.22 shows the polarisation of each of the ¹⁵N resonances of the nitrile groups present. The maximum polarisation was gained at -0.3 μT and so no improvement was made on the previous study.

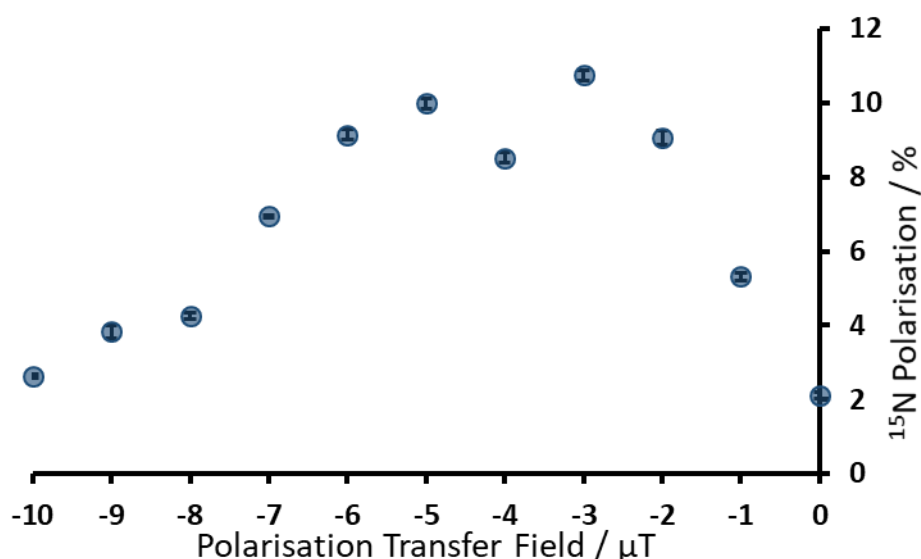


Figure 6.22 ¹⁵N polarisation of anastrozole measured as a function of polarisation transfer field. The sample consisted of 2 mg of **IPr**^{Cl} and 5 equivalents of anastrozole and **V** and was shaken for 10 s with 4 bar of *p*-H₂ at each field.

Further polarisation can be built up overtime in solution by shaking for longer with $p\text{-H}_2$ in the optimum transfer field, provided the $p\text{-H}_2$ is not fully spent during the shaking process. If this occurs, the sample will relax according to its T_1 in that field. In the next study as sample was prepared according to the optimum ratio described previously and shaken for different times at a field of $-0.3\text{ }\mu\text{T}$. Figure 6.23 shows that the polarisation reaches a plateau after 15 s of shaking time achieving $12.0 \pm 0.1\%$ polarisation now representative of 36800 ± 50 -fold enhancement at a 9.4 T acquisition field. This implies this is the maximum polarisation that can be achieved as it is presumably the polarisation and T_1 balance out.

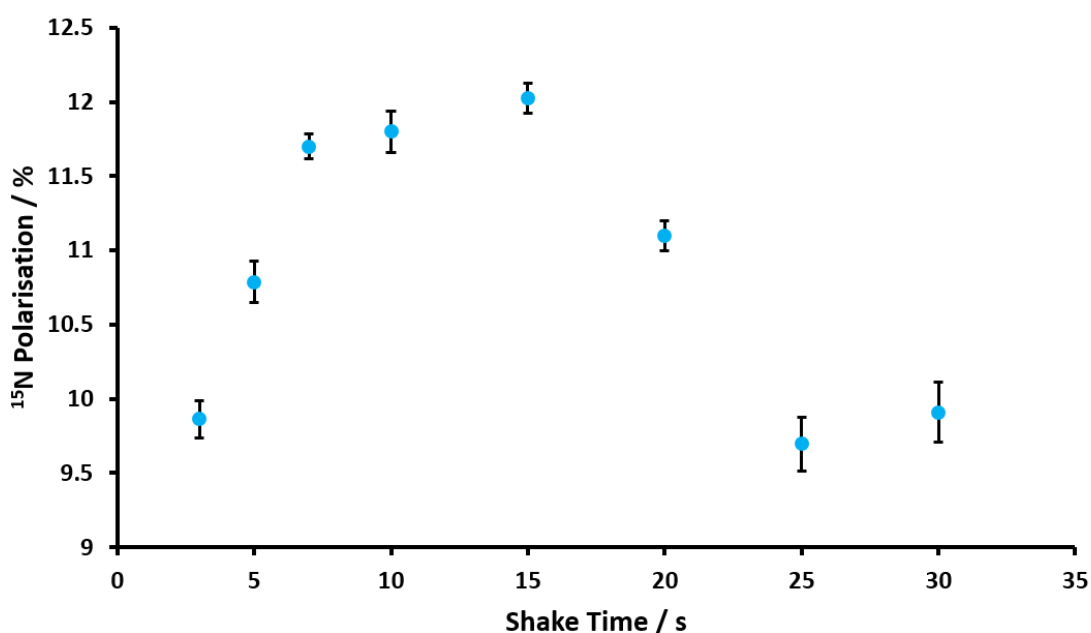


Figure 6.23 ^{15}N polarisation for anastrozole plotted as a function of the time the sample was shaken before signal acquisition. The sample consisted of 2 mg of IPr^{Cl} with 5 equivalents of V and 5 equivalents of anastrozole.

The same storage field study was then undertaken on the optimised system to see how long the polarised signal could be stored for. As expected, the same trend was observed showing T_1 of the ^{15}N signal to be $19.36 \pm 1.44\text{ s}$ at 11.7 T, extending to $41.29 \pm 8.67\text{ s}$ at 7 T. The T_1 was found to be the same, within error when moving between 11.7 and 9.4 T yielding $20.58 \pm 5.80\text{ s}$ at the 9.4 T field. A lifetime of $41.29 \pm 8.67\text{ s}$ provides ample time for sample separation and imaging. This finding is in agreement with Theis and co-workers who published the same trend with anastrozole during the writing of this thesis.⁶³

6.9 Conclusions

This chapter has seen the creation of another set of novel polarisation transfer catalysts optimised for the ^{15}N polarisation of nitriles. It was observed that the use of co-ligands in forming an optimal species was again key to efficient polarisation transfer with the co-ligand, 3-methyl-1,2,4-triazine (**V**) proving to yield the greatest ^{15}N polarisation of acetonitrile (**C**) in an un-optimised system. It was found that deuteration of **C** was also key in yielding sufficient levels of ^{15}N polarisation (maximum ^{15}N polarisation at this point = $6.99 \pm 0.14\%$). This was believed to be due to polarisation wastage to ^1H or increased relaxation in the *protio* form. Carrying **V** forward in the optimisation, the exchange rate of **C-d₃** was then optimised (evidenced by increasing ^{15}N polarisation levels) by altering once again the steric and electronic properties of the NHC ligand. This revealed the Ir pre-catalyst **IPr^{Cl}** of Figure 6.5 was optimal, now yielding $13.4 \pm 0.7\%$ ^{15}N polarisation for **C-d₃**. Utilising rate data from Chapter 3, it is possible to infer that the rate of ligand exchange for **IPr^{Cl}** sits somewhere between the **IPent** ($3.42 \pm 0.02 \text{ s}^{-1}$ for **G**, see Table 3.4) and the **IPr^{NMe₂}** ($4.24 \pm 0.01 \text{ s}^{-1}$ for **G**, see Table 3.4). This Goldilocks type behaviour is typical of the optimisation process.

Carrying forward this system for further optimisation it was found the relative concentrations of **V** and **C-d₃** played a significant role in the polarisation transfer efficiency as they affected catalyst speciation. Varying the amounts of the two relative to **IPr^{Cl}** saw the formation of three catalyst species; $[\text{Ir}(\text{H})_2(\text{C})_2(\text{IPr}^{\text{Cl}})(\text{V})]\text{Cl}$ (**20**), $[\text{Ir}(\text{H})_2(\text{C})_3(\text{IPr}^{\text{Cl}})]\text{Cl}$ (**21**) and $[\text{Ir}(\text{H})_2(\text{IPr}^{\text{Cl}})(\text{V})_3]\text{Cl}$ (**22**) all of which were fully characterised. The studies showed that the optimum ratio of **IPr^{Cl}**, **C-d₃** and **V** had already been achieved at a ratio of 1 : 5 : 5 respectively and so no further improvement on the $13.4 \pm 0.7\%$ ^{15}N polarisation was observed. Achieving this level of polarisation was only possible at a specific polarisation transfer field (PTF) which was observed to be very specific for the catalytic species being probed.

Extending the study to other nitrile containing compounds saw that this optimised system could be utilised to improve the ^{15}N polarisation transfer of all nitriles tested with significant ^{15}N NMR signal gains being made for all substrates. Substrates with more than one possible N-donor saw the near selective hyperpolarisation of only the nitrile ^{15}N and so this method proves to be an out of box optimal polarisation transfer tool for detecting nitrile containing compounds. Mixtures of nitriles akin to more ‘real-world’ samples showed facile ^{15}N detection and identification of all nitriles in the mixture as each showed

a distinct ^{15}N chemical shift value thanks to its large chemical shift window. All nitrile compounds were at natural ^{15}N abundance and 25 mM concentration. Detecting all compounds in a one scan acquisition shows the power of this technique; detecting 25 mM at natural ^{15}N abundance demonstrates the ability to detect μM amounts of labelled material. It was also shown that the unique PTFs of the nitrile compounds could be utilised in order to selectively polarise just one out of three nitrile isomers in the mixture, a technique that could be applied to many different systems and see selective screening for certain compounds.

Interestingly, it was shown that quantification of **C-d₃** was possible using a SABRE hyperpolarised sample in super-stoichiometric (more substrate than catalyst) regime. It has been shown by Tessari *et al.* that quantification of hyperpolarised mixtures is possible in a sub-stoichiometric (less substrate than catalyst) regime through ^1H NMR by utilising a strongly binding triazole ligand to maintain a species of the form $[\text{Ir}(\text{H})_2(\text{triazole})_2(\text{NHC})(\text{substrate})]\text{Cl}$ as the only active species in solution, thus eliminating catalyst competition by substrates and therefore catalyst speciation effects.⁶⁰ It has however been shown by Fekete *et al.* that quantification of ^{15}N by SABRE is possible instead with an excess of a single substrate compared with Ir, a regime like the one presented here.⁴³ Therefore it is unlikely that quantification of a mixture of nitriles would be possible under the current system but utilising Tessari's method with the strongly binding **V** needed for efficient polarisation transfer could reveal ^{15}N quantification in mixtures also. It is also noted that these mixtures could now be tested utilising the proven ^{15}N DOSY from Chapter 3 in order to further easy identification based on molecule size or diffusivity.

Nitrile compounds were highlighted as potential ^{15}N MRI contrast agents proving to all achieve high levels of ^{15}N polarisation selectively on the nitrile ^{15}N site. They also all were shown to have long T_1 values that extended at lower magnetic storage fields as shown previously in this thesis and by Theis *et al.* also.^{63, 69} The longest T_1 lifetime observed was for that of **C-d₃** which proved to be 241 ± 34 s when stored at 7 T. It is believed this incredibly long signal lifetime of around 12 minutes ($3 \times T_1$) would extend even further at lower fields providing ample time for sample modification prior to injection.

A more biologically relevant molecule, the aromatase inhibitor anastrozole (an approved treatment for certain breast cancers), proved to yield *circa* 12% ^{15}N polarisation

on each nitrile site in the structure. The T_1 lifetime of anastrozole was found to be 41.29 ± 8.67 s at a field of 7 T which has been shown to extend to 7 min at a field of 1 T.⁶³ This gives real promise to the use of anastrozole as a future hyperpolarised contrast agent.

Chapter 7. Experimental

7.1 Equipment and materials

7.1.1 NMR spectrometers

The NMR measurements undertaken were carried out on a range of spectrometers due to the variation in magnetic field undertaken in this thesis. The Table 7.1 summarises the type of spectrometer utilised for each field.

Table 7.1 A summary of the NMR spectrometers utilised showing the magnetic field strength, ^1H NMR resonance frequency and the model of spectrometer

Magnetic Field / T	^1H Resonance Frequency / MHz	Spectrometer Information
7	300	300 MHz Fourier Magnet, Bruker Avance III console, BBO probe with Z-gradient.
9.4	400	400 MHz Ultrashield™ Magnet, Bruker Avance III console, BBI probe with Z-gradient. Flow measurements conducted on TXO 200 μL flow probe ($^1\text{H}/^{13}\text{C}/^{15}\text{N}$).*
11.7	500	500 MHz Ascend™ Magnet, Bruker Avance III HD console, BBI probe with Z-gradient.
14.1	600	600 MHz Widebore, Bruker Avance III HD console, PA BBO 600W2/S4 BBF-H-D-05 Z SP.
16.7	700	700 MHz Ultrashield™ Magnet, Bruker Avance NEO console, Prodigy TCI Cryoprobe ($^1\text{H}/^{19}\text{F}/^{13}\text{C}/^{15}\text{N}$) with a Z gradient.

***Note that solid state measurements were undertaken on 400MHz Ultrashield™ Widebore Magnet, with an Avance III HD console and a PH MAS 400WB VL2. 5 N-P/F-H probe spinning at 20 kHz.**

7.1.2 *Para*-hydrogen generation

The *para*hydrogen (*p*-H₂) used in all ‘shake and drop’ studies was produced by passing hydrogen gas from a cylinder over a *paramagnetic* iron (III) oxide catalyst at ~27 K. This facilitates the spin-transfer process to produce a constant supply of *p*-H₂ with a purity of > 99%. Pressures for these experiments were read as absolute pressure. The pressure gauge used on this set-up was a MKS Baratron® which reads the pressure relative to the atmospheric pressure and hence 3 bar on this gauge corresponds to 4 bar absolute pressure.

All *p*-H₂ used for flow and imaging studies was produced using a Bruker *p*-H₂ generator which cooled to a temperature of 36 K corresponding to around 92% *p*-H₂ purity. The hydrogen used here was produced using a FDGS-Alliance hydrogen generator that creates constant H₂ through electrolysis of water. The pressure used for bubbling in these studies is quoted as an absolute pressure.

All studies using ‘normal’ hydrogen utilised hydrogen from a cylinder without further purification and have pressures quoted in absolute pressure rather than the gauge pressure.

7.1.3 Solvents and substrates

Substrates and solvents were purchased from Alfa-Aesar, Fluorochem or Sigma Aldrich and were used without any further processing or purification. All ¹⁵N labelled substrates were purchased from Cambridge Isotope Laboratories or Sigma-Aldrich and used without further processing or purification. All the iridium pre-catalysts utilised in this work were synthesised by Dr. Victoria Annis, Dr. Peter J. Rayner or Dr. Kieren J. Evans according to literature procedures.^{59, 231-233} The co-ligand **G-d₂** was synthesised by Dr Peter J. Rayner according the literature procedure.¹⁴⁷ The co-ligand 1,4-dimethyltetrazine (**U**) was synthesised by the group of Dr. Valery Kozhevnikov at Northumbria University.

7.1.4 X-ray Crystallography

All crystal X-ray experiemnts were carried out by Dr. Adrian Whitwood, Dr. Richard Gammons or Dr. Theo Tanner. A suitable crystal was selected and (50

micrometer micromount) on a XtaLAB Synergy, Single source at home/near, HyPix-Arc 100 diffractometer. The crystal was kept at 110.00 K during data collection. Using Olex2,²³⁴ the structure was solved with the SHELXT²³⁵ structure solution program using Intrinsic Phasing and refined with the SHELXL²³⁶ refinement package using Least Squares minimisation. Crystal structures are presented with solvent molecules omitted for clarity. Any relevant crystallographic data can be found in appendix Section A.2.

7.1.5 Flow measurements

Flow measurements were carried out using a Bruker automated ‘Polariser’ unit. The device employs a pneumatic shuttle system to transfer the sample between a mixing chamber where bubbling with *p*-H₂ can occur and the probe head for measurement. The device used N₂ gas from a cylinder for shuttling and for bubbling utilised hydrogen generated as described in Section 6.1.2. The bubbling and shuttling stages are all controlled by transistor-transistor logic (TTL) triggers with custom pulse sequences to automatically implement these into the experiment.

The mixing chamber itself was housed within a tuneable copper coil. This coil could operate between 50 μ T and 15 mT when powered directly from the polariser control board. Alternatively, the coil could be powered through an external power supply with the addition of an appropriate resistance to the circuit until operating field between 0 and 1 μ T was achieved. Figure 7.1 shows the plot of voltage vs. resulting magnetic field for this setup utilised.

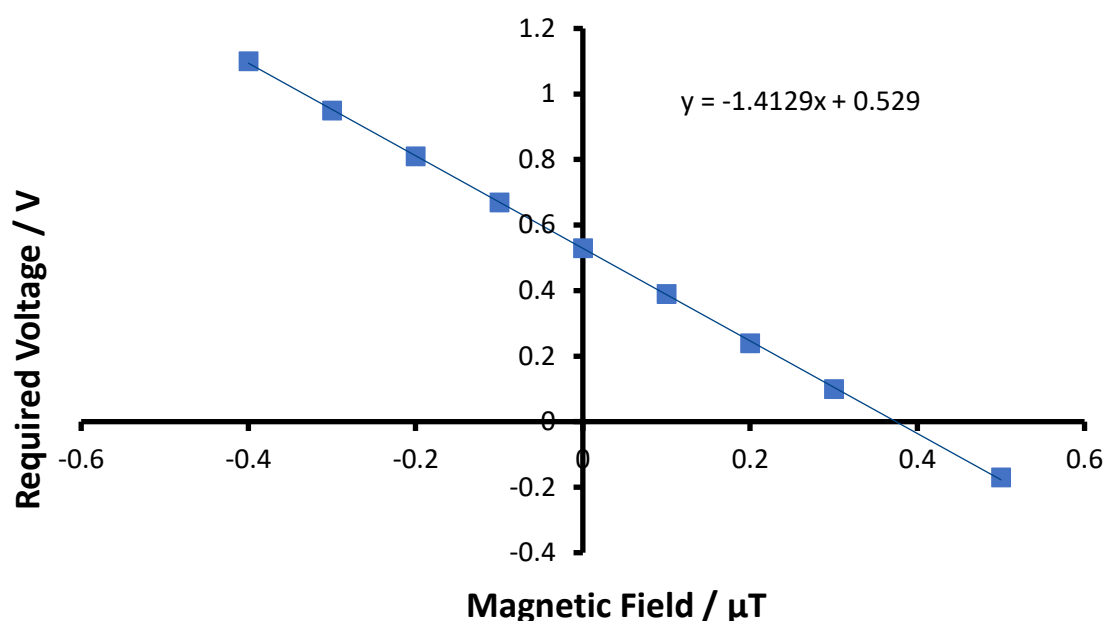


Figure 7.1 Graph showing the magnetic field outcome (μT) as a function of the voltage applied (V) to the coil.

The coil and mixing chamber are fully surrounded by a *mu*-metal casing providing shielding from the earth's magnetic field. The casing leaves behind a small residual field of 0.1 μT but all magnetic fields quoted for experiments in the mixing chamber are corrected for this.

7.1.6 MRI Measurements

All imaging studies were carried out on a 7 T (300 MHz) preclinical Bruker horizontal bore (BioSpec 70/30) imaging system with a preinstalled BGA-12S gradient coil (600 mT/m). All ^{15}N measurements were undertaken using a 72 mm ID dual tuned $^1\text{H} / ^{15}\text{N}$ linear volume resonator (Bruker Biospec T20075V3). Samples were loaded onto an AutoPac (motor driven positioning cradle). Tuning and matching for each coil channel was carried out during setup and parameterised on a phantom containing water was completed. Automatic frequency shimming and zero point were completed on the phantom and a ^1H localiser carried out to locate the phantom (TR = 110 ms, TE = 2.5 ms, FA = 30°, FOV = 90 mm², image size = 256 x 256, slice thickness = 2 mm, 3 orthogonal imaging planes).

Following set up a single ^{15}N spectroscopic sequence was carried out. Acquisition duration lasted 300 ms with 2048 points over 6 kHz, dwell time was 83.2 μs , 90° flip angle). Each time the sample was re-polarised using 92% $p\text{-H}_2$ from a Bruker

parahydrogen generator as described in section 7.1.2. Power levels were set from the resulting FIDs to maximise signal output over the multiple shakes.

For imaging, a hybrid chemical shift imaging sequence was used (FA = 7°, TR = 122 ms, TE = 1.1 ms, image size 16 x 16 voxels, FOV = 60 mm², slice thickness 10 mm, 100 ms acquisition duration with 320 points over 3.2 kHz bandwidth and dwell time 156 ms).

For high resolution imaging, a fast FID based 3D spiral approach was taken. (TR = 100 ms, TE = 1.23 ms, centric encoded (spiral out), FA = 10°, 8 spirals per image were acquired across a bandwidth of 50 kHz, final image size 50 x 50 x 8 across 60 x 60 x 60 mm.) Echo Planar Spectroscopic Imaging (EPSI) sequence was also tested. (TR = 187.5 ms, TE = 4.2 ms, image size 64 x 64 over 80 x 80 mm² FOV, 7mm slice thickness, 200 kHz bandwidth, spectral size 256).

7.2 General experimental procedures

7.2.1 Shake and drop method

Samples for shake and drop studies were prepared in a 5 mm NMR tube fitted with a J. Young's cap. Details of the contents in each sample are presented where relevant in the text but samples were usually prepared in 0.6 mL of solvent. The samples were then degassed by three freeze-pump-thaw cycles using dry / ice acetone for methanol-*d*₄ samples and liquid N₂ for all other solvents. The tube was then filled to a pressure of 4 bar H₂ to activate the SABRE catalyst.

To conduct the shake and drop procedure, the sample prepared above was pressurised with *p*-H₂ and shaken, before transferring to the NMR spectrometer for signal acquisition. The sample was shaken at a pressure of 4 bar *p*-H₂ for 10 s unless otherwise stated. Samples had to be shaken at a specific PTF in order for efficient polarisation transfer to occur to the nuclei of interest. For ¹H measurements this was usually a 6 mT field generated by an array of permanent magnets positioned in a shaker to achieve the desired field as measured by a gauss meter. For other experiments where the PTF ranged from 3 to 14 mT the same style shaker was used with the magnet array altered in each case to reflect the correct field. For ¹⁵N measurements the field range utilised was typically in the μT region, which was generated using a tuneable copper coil connected to a standard

power supply. The copper coil had an appropriate amount of resistance added to the circuit so that the magnetic field achieved operated between 0.0 and 2.0 μT . The coil was housed in a handheld sized *mu*-metal shaker providing shielding from the earth's magnetic field. The casing leaves behind a small residual field of 0.1 μT but all magnetic fields quoted for experiments in the mixing chamber are corrected for this.

7.2.2 SABRE measurements under flow conditions

Samples for investigation by flow measurements were instead prepared in 3 mL of the relevant solvent to accommodate the much larger volume of the sample mixing chamber. Details of the contents in each sample are presented where relevant in the text. The samples were simultaneously degassed of air and activated by bubbling through the sample with a constant flow of H_2 gas for between 1 and 5 min. The experiments consisted of a 'bubble, shuttle and acquire' process which was all controlled as outlined previously in Section 7.1.5.

7.2.3 T_1 inversion recovery

Inversion recovery T_1 measurements were conducted using the Bruker standard pulse sequence. The pulse sequence works by applying a 180° *r.f.* pulse which inverts the magnetisation of the probed nuclei so it is now $-z$ on an axis. A variable time delay (τ) is encoded into the sequence through a variable delay list. This time period allows the spins to partially relax back to $+z$ with the amount of relaxation proportional to the time delay encoded. Immediately after this delay a 90° *r.f.* pulse is applied which rotates any remaining magnetisation into the *xy* plane for NMR signal detection in the form of a free induction decay of signal. The NMR spectrum is then obtained through a Fourier transformation of this data. It is important that a time period of $5 \times T_1$ is applied between each acquisition to allow full recovery of the magnetisation to the $+z$ direction. The data is then fitted to the Equation 7.1.

$$M_\tau = M_0 \left(1 - Ae^{\left(\frac{-\tau}{T_1}\right)} \right) \quad (7.1)$$

Where M_τ is the magnetisation measured in the *xy* plane at time t , M_0 is the magnetisation at thermal equilibrium, A is a constant left as a fitting variable to account

for any imperfections in timings during the pulse sequence, τ is the variable time delay applied from a variable delay list and T_1 is the spin-lattice relaxation time.

7.2.4 T_1 saturation recovery

Saturation recovery T_1 measurements were conducted using a Bruker standard pulse sequence. It works in a similar way to the inversion recovery pulse sequence described previously. However, at the start of each acquisition any remaining magnetisation is saturated so that the net magnetisation of the sample is zero, the magnetisation is then allowed to recover in the z axis. The saturation is achieved through applying a chain of 90° pulses in rapid succession effectively scrambling the remaining magnetisation. The data is then fit to the same Equation 7.1 to extract the T_1 value. This method was utilised over the inversion recovery method in order to probe the ^{15}N T_1 lifetime of NaNO_2 in the solid phase as it benefits from not having to wait the time period of $5 \times T_1$ between acquisitions which greatly reduces the time of the experiment. The inversion recovery method was utilised mostly in this thesis as it is more robust method.

7.2.5 Hyperpolarised T_1 decay

Hyperpolarised T_1 decay experiments utilised a series of low tip angle *r.f* pulses of equal pulse length with an acquisition period after each pulse. The pulse length was decided upon on a case by case basis but usually was selected to maximise the NMR signal gained from the final pulse-acquire sequence in the series as found by Equation 7.2.

$$y = \cos(x)^n \sin(x) \quad (7.2)$$

Where y is the proportion of magnetisation xy plane on the final pulse-acquire in the series, x is the pulse angle in degrees applied to the sample and n is the number of pulses to be applied in the series. The equation above was plotted in a graphing calculator and the x value at the maximum value of y taken as the pulse angle to be used in the study. The pulse length then utilised was then calculated using Equation 7.3.

$$PL_x = PL_{\frac{\pi}{2}} \times \frac{PA_x}{90^\circ} \quad (7.3)$$

Where PL_x is the pulse length in μs required to tip the magnetisation by the desired angle, $PL_{\frac{\pi}{2}}$ is the pulse length in μs required to tip the magnetisation a full 90° into the xy

plane and PA_x is the desired pulse angle in degrees. With is consecutive pulse applied, less z magnetisation is remaining for the subsequent measurements and so results in less xy magnetisation also. When processing the results, the magnetisation measured through the NMR signal observed must be corrected for this loss. The loss in z magnetisation was calculated according to Equation 7.4.

$$M_z = M_z^* \times \cos\left(PA_x \times \frac{\pi}{180}\right) \quad (7.4)$$

Where M_z is the remaining z magnetisation. M_z^* is the remaining z magnetisation from the previous run of the series and PA_x is the pulse angle used in degrees. The amount of xy magnetisation generated on each run was calculated as shown in Equation 7.5.

$$M_{xy} = M_z \times \sin\left(PA_x \times \frac{\pi}{180}\right) \quad (7.5)$$

Where M_{xy} is the magnetisation flipped into the xy plane, M_z is the remaining z magnetisation, and PA_x is the pulse angle used in degrees. The integrals of each resulting NMR spectrum were then divided by the xy magnetisation adjust for the loss as shown in Equation 7.6.

$$SI_{corr} = \frac{S_x}{M_{xy}} \quad (7.6)$$

Where SI_{corr} is the corrected NMR integral area accounting for the loss of z magnetisation from previous runs in the series, S_x is the acquired NMR integral area from the current run in the series and M_{xy} is the xy magnetisation calculated for the current run in the series. Once the corrected NMR integral areas had been calculated they could be plotted as before according to Equation 7.1, in order to extract T_1 .

7.2.6 EXSY measurements

Observing the exchange processes within the complexes in this thesis were done by acquiring exchange spectroscopy (EXSY) data. These measurements utilised a Bruker standard selective NOESY experiment with the pulse program name ‘selnoggp’. It works by applying a single pulsed-field-gradient echo, which is a 180° pulse after an initial 90° pulse selective *r.f.* pulse to a the ^1H NMR resonance of interest before leaving a time period known as the ‘mixing time’ where the sample is allowed to evolve and chemical exchange can take place. This mixing time can be altered to reflect the exchange time of

the reaction, in the case of the complexes here, between 0.05 s and 2.0 s. A standard gradient echo is then applied to refocus the selected magnetisation. This and the exchanged resonances are then detected using a further 90° *r.f.* pulse. The resulting integral areas of the exchanged species can then be fit to a kinetic model to reflect the integral area at each time point from which a rate can be extracted. Standard ligand dissociation was fit to the model in Equation 7.7.

$$[Bound]_{t+\delta t} = k_{-1}[Free] - k_1[Bound]\delta t \quad (7.7)$$

Where the $[Bound]_{t+\delta t}$ represents the change in the concentration of the bound material per unit of time. k_{-1} and k_1 are the rate constants for the transition from free to bound and bound to free respectively, $[Free]$ and $[Bound]$ represent the relative amounts of the free and bound ligand respectively and δt is the incremental time difference.

7.2.7 Determination of rate constant values

The rate data for the experiments in Chapter 2 and presented in Figure 2.13 were fitted to Equations 7.8 to 7.10.

$$[1] = -k_1[1] \quad (7.8)$$

$$[6] = k_{obs}[1] - 2k_2[6] \quad (7.9)$$

$$[7] = k_2[6] \quad (7.10)$$

Where $[X]$ refers to the concentration of the species in the text, k_{obs} is the observed rate constant for the reaction of **1** to **6** and k_2 is the rate constant for the formation of **7** from **6**. All rates were then calculated from the fits using Microsoft excel solver package and used to calculate the observed activation parameters for the reaction steps by fitting them to the Eyring Equation 7.11.

$$\ln \frac{k}{T} = \frac{-\Delta H^\ddagger}{R} \times \frac{1}{T} + \ln \frac{k_B}{h} + \frac{\Delta S^\ddagger}{R} \quad (7.11)$$

Where k is the experimental rate constant determined at a specific temperature, T is the temperature, $\Delta H^\ddagger$ is the standard enthalpy of activation, R is the molar gas constant, k_B is the Boltzmann constant, h is Planck's constant, $\Delta S^\ddagger$ is the standard entropy of activation. In this linear form of the equation the gradient is equal to $\frac{-\Delta H^\ddagger}{R}$ and the y

intercept is equal to $\ln \frac{k_B}{h} + \frac{\Delta S^\ddagger}{R}$. Once these activation parameters have been determined the Gibbs free energy, $\Delta G^\ddagger$, can be determined as shown in Equation 7.12 where all components of the equation are the same as defined in the previous equation.

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \quad (7.12)$$

7.2.8 DOSY measurements

DOSY measurements undertaken on non-hyperpolarised samples were carried out using the pulse sequence ‘dstebpgp3s’, a convection compensated sequence utilising a double stimulated echo, developed by Mueller and Jerschow.²³⁷ Measurements were carried out from 5 to 95% gradient strength with a quadratic ramping function. Hyperpolarised DOSY spectra were carried out using a modified version of the pulse sequence DONESHOT (DOSY Oneshot) developed by Hammond *et al.*²³⁸ which was edited to include the bubbling and transfer steps of the flow system control. Measurements were carried out from 20 to 80% gradient strength with a quadratic ramping function. These data were processed as outlined in section 7.3.3.

7.2.9 Hydrogen solubility measurements

For comparison between solvents H₂ solvating ability Henry constants were calculated using Henry’s Law shown in Equation 7.13.

$$C = k_H P \quad (7.13)$$

Where C is the concentration of dissolved H₂, k_H is the Henry’s Law constant and P is the partial pressure of the gas above the solvent. The units for k_H vary greatly in the literature and so the units of C and P required, change alongside this. In this thesis Henry’s constant is presented in units of $\text{mol dm}^{-3} \text{bar}^{-1}$ meaning C is in units of mol dm^{-3} and P in bar. The most widely cited units for k_H in literature are MPa^{-1} where C is given as a mole fraction, so has no units, and P is given in MPa, values are then often presented as k_H^{-1} for ease of comparison. Where values need to be compared to the literature in the text, both versions of k_H are presented.

To calculate the amount of H₂ in solution ¹H NMR spectroscopy was utilised. To make the samples a 7 inch (17.78 cm) J. Youngs NMR tube with a diameter of 5 mm was

placed in a bespoke holder on a 5 decimal place balance and 0.7 mL of the solvent and 5 μ L of either dimethylsulfoxide or dioxane internal standard added. The exact amounts added in mL added were then calculated from the mass change on the balance and the density of the solvent / internal standard. This was done to ensure accuracy of the amounts of each material being added. The sample was then degassed using 5 x freeze-pump-thaw cycles using liquid N₂ for all solvents except D₂O which was degassed by a separate method. The D₂O was degassed by using a passive vacuum to evacuate the headspace of the sample. The sample was then sonicated for 1 min, to encourage gas evolution from the solvent, before evacuating the headspace again. This process was repeated until no change in the barometer reading was registered when venting the headspace. While this will not achieve the same $\times 10^{-4}$ vacuum as the other solvents it was deemed the safest route to avoid shattering the NMR tube.

The samples were then exposed to 0.5 bar of H₂ whilst cooling to 195 K using a dry ice / acetone slurry to avoid solvent loss. Again, the D₂O was an exception here and was cooled using an ice bath instead. The sample was thawed to room temperature and shaken to ensure proper mixing between the gas and solvent. The process was then repeated to ensure a pressure of 0.5 bar remained in the headspace after gas dissolution. The sample was then transferred to an 11.7 T spectrometer where ¹H signal acquisition took place. The integral of H₂ could then be compared to that of the internal standard to calculate a concentration, making sure to account for the number of protons in the H₂ and the standard. The integral of H₂ was divided by 0.75 to account for the 25% NMR silent *p*-H₂ present at 298 K.

The method was then repeated with increasing pressures of 0.5 bar up to 4 bar. These data were then plotted as a graph of pressure in MPa vs. the concentration, presented as mole fraction. From this graph a Henry constant could be extracted as the inverse of the gradient.

Where no deuterium was present to lock and shim, a 'lock' sample of methanol-*d*₄ was prepared to the same liquid depth to allow shimming to be performed. The 'lock' sample was then swapped for the actual sample and the spectrometer lock turned off for the duration for the experiment.

7.3 General data processing

7.3.1 Signal enhancement and polarisation calculations

To calculate signal enhancements for ^1H and ^{15}N labelled SABRE experiments the formula in Equation 7.14 was used.

$$\varepsilon = \frac{SI(hyp)}{SI(thermal)} \quad (7.14)$$

Where ε is the enhancement of the NMR signal in fold, $SI(hyp)$ is the integral of the hyperpolarised NMR signal, $SI(thermal)$ is the integral of the corresponding thermally polarised NMR signal. Both spectra were recorded using identical acquisition parameters.

For calculating enhancements when natural abundance ^{15}N labelled material was used, a thermal ^{15}N NMR signal could not be acquired in an appropriate time window and so an external ^{15}N labelled standard was utilised instead. The external standard consisted of a 5 M solution of $^{15}\text{NH}_4\text{Cl}$ in 0.6 mL of D_2O . The hyperpolarised signal was then compared to that of this external standard, making sure to account for the difference in concentration between the two samples as shown in Equation 7.15.

$$\frac{SI(sub)}{SI(standard)} \times \frac{\text{mmol of standard}}{\text{mmol of substrate}} \quad (7.15)$$

Where $SI(sub)$ is the integral area for the NMR signal of the substrate, $SI(standard)$ is the integral area for the NMR signal of the standard. Once again both spectra were recorded using identical acquisition parameters.

The polarisation of any nuclei is given by the Equation 7.16

$$P = \frac{\gamma B_0 \hbar}{2k_B T} \quad (7.16)$$

Where γ is the gyromagnetic ratio of the nuclei, B_0 is the applied magnetic field, $\hbar$ is Planck's constant divided by 2π , k_B is the Boltzmann constant and T is temperature at which the measurement takes place. The percentage polarisation, presented in this thesis as a measure for SABRE success, is then given by Equation 7.17.

$$\%P = P \times \varepsilon \times 100 \quad (7.17)$$

Where %*P* is the % polarisation of the nuclei achieved and ε is the enhancement observed for the resulting NMR signal of the nuclei as described in Equation 7.14.

7.3.2 Error calculations

Errors calculated for all enhancement and polarisation data were calculated as standard errors using Equation 7.18.

$$\text{Standard Error} = \frac{SD}{\sqrt{n}} \quad (7.18)$$

Where *SD* is the standard deviation of the data and *n* is the number of repeats undertaken for the experiment.

All errors quoted for *T*₁ values are fitting errors extracted from the plot using origin.

Fitting data resulting from EXSY measurements was done using Microsoft Excel's Solver package, however no fitting error is given from this. Instead the errors were calculated using the Jack-knife method.²³⁹ This is very time consuming method requiring repeat fits to be undertaken on the data removing one data point for each fit and changing the data point that has been removed each time a fit is calculated. This process is repeated until fits have been calculated for the data with all the possibilities of removing one data point. Rather than doing this manually a python script was employed to automate the process (See appendix Section A.5 for script), the necessary control measurements were undertaken to ensure the python script came out with the same value as repeat fits by solver.

7.3.3 Software

All NMR data was processed using Topspin version 3.6.1 or Topspin 4.1. Data was processed using various window functions, but processing parameters were kept the same when processing a series of data or when comparing between two separate experiments.

Origin was used to obtain exponential fits for data for all *T*₁ measurements utilising the pre-set non-linear curve fit 'expdec1'.

DOSY toolbox from the University of Manchester's General NMR Analysis Toolbox (GNAT) was utilised to process all DOSY measurements. All measurements were processed in the same way, spectra were individually phased and a 5th order baseline correction applied before feeding into the DOSY plotting tool. Diffusion coefficients were taken straight from the processed DOSY spectra in DOSY toolbox and fitting errors displayed in the text from this processing also.

All images were processed using bespoke code in MatLab.

7.4 Characterisation of complexes

7.4.1 General characterisation procedures

Characterisation data was carried out on a variety of NMR spectrometers listed in Table 7.1. Generally, a temperature of 243 or 263 K was used to carry out the characterisation measurements. Characterisation generally consisted of COSY, NOESY, TOCSY, HMQC and HMBC measurements.

7.4.2 Characterisation of 1,2,4-triazine-3-methylthio (X)

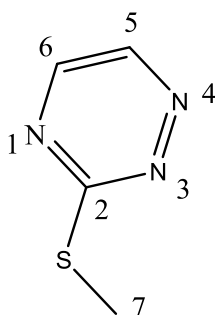


Figure 7.2 Structure of X determined from data given in Table 7.2.

Table 7.2 NMR data collected in methanol-*d*₄ at 298 K for X shown in Figure 7.2. Resonance labels are stated according to Figure 7.2

Resonance number	¹ H (ppm)	¹³ C (ppm)	¹⁵ N (ppm)
1	-	-	292.20 $J_{\text{HN}} = 12.0$ Hz (N ₄)
2	-	174.60	-
3	-	-	355.60 $J_{\text{HN}} = 4.9$ Hz (N ₂)
4	-	-	415.20 $J_{\text{HN}} = 15.4$ Hz (N ₁)
5	9.01 d, $J_{\text{HH}} = 2.5$ Hz	145.43	-
6	8.55	149.28	-
7	2.66	12.34	-

7.4.3 Characterisation for complex [IrCl(COD)(IMes)] (1)

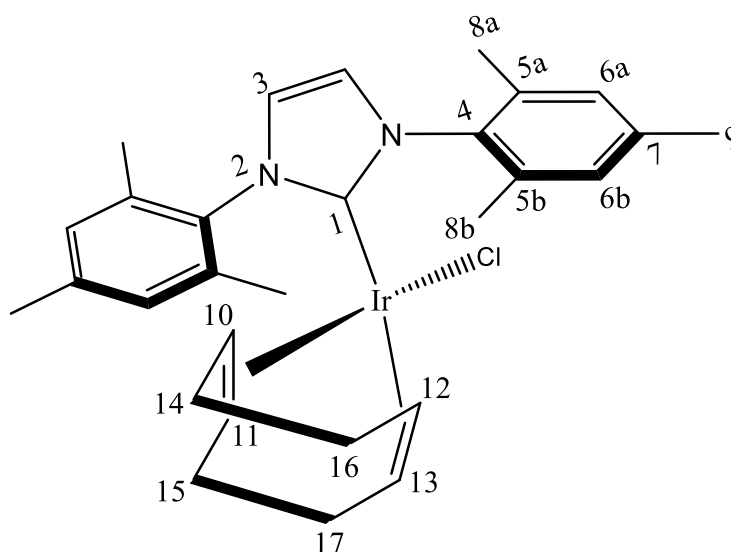


Figure 7.3 Structure of 1 determined from data given in Table 7.3.

Table 7.3 NMR data collected in methanol- d_4 at 263 K for 1 shown in Figure 7.3. Resonance labels are stated according to Figure 7.3

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	179.10	-
2	-	-	191.64
3	7.24	123.89	-
4	-	136.15	-
5a	-	134.47	-
5b	-	136.79	-
6a	7.05	127.95	-
6b	7.03	128.93	-
7	-	138.50	-
8a	2.20	17.16	-
8b	2.32	18.59	-
9	2.38	19.76	-
10, 11	3.11	51.71	-
12, 13	4.03	81.54	-
14	1.29	32.98	-
15	1.66	32.86	-
16	1.72	28.13	-
17	1.36	28.35	-

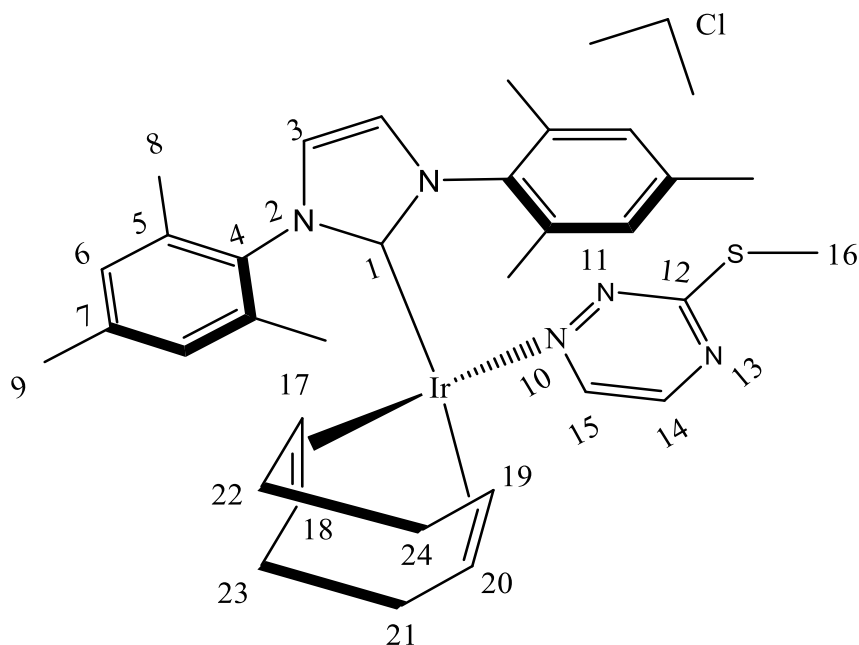
7.4.4 Characterisation for complex $[\text{Ir}(\text{COD})(\text{IMes})(\text{X})]\text{Cl}$ (2)

Figure 7.4 Structure of 2 determined from data given in Table 7.4.

Table 7.4 NMR data collected in methanol- d_4 at 263 K for 2 shown in Figure 7.4. Resonance labels are stated according to Figure 7.4.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	172.00	-
2	-	-	196.16 (273 K)
3	7.29	123.93	-
4	-	135.60	-
5	-	136.00	-
6	7.09	129.18	-
7	-	139.99	-
8	2.22	17.66	-
9	2.44	19.80	-
10	-	-	332.30
11	-	-	341.70
12	-	176.20	-
13	-	-	291.80
14	8.25	152.29	-
15	7.93	147.64	-
16	2.58	12.72	-
17, 18*	3.50	82.94	-
19, 20*	3.77	68.06	-
21, 24*	1.38, 1.87	32.24, 32.92	-
22, 23*	1.77, 2.05	28.58, 22.88	-

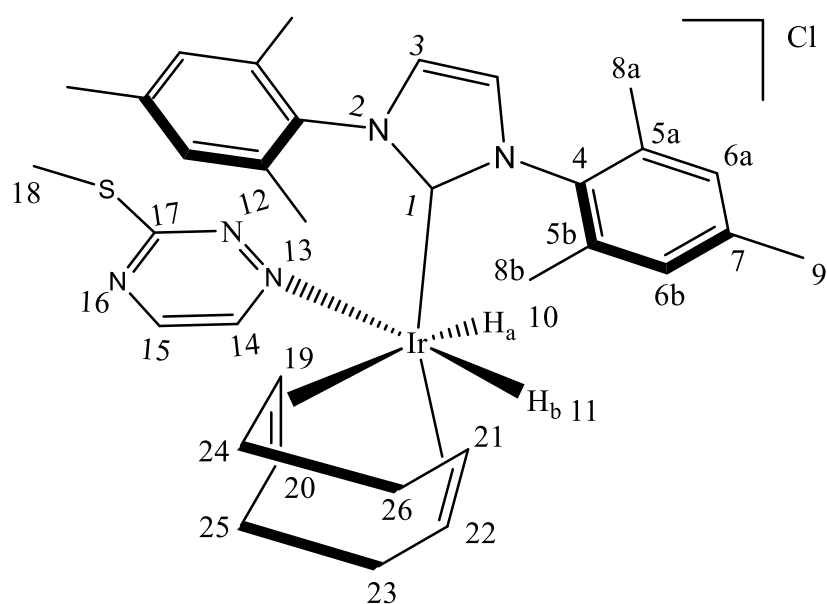
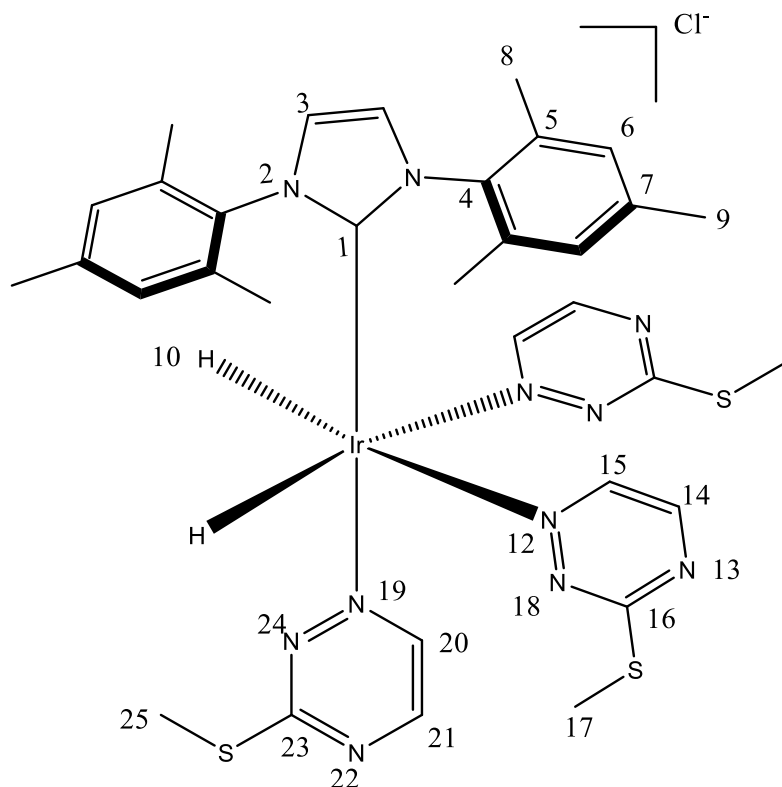
7.4.5 Characterisation for complex [Ir(H)₂(COD)(IMes)(X)]Cl (3)

Figure 7.5 Structure of 3 determined from data given in Table 7.5.

Table 7.5 NMR data collected in methanol-*d*₄ at 243 K for 3 shown in Figure 7.5. Resonance labels are stated according to Figure 7.5.

Resonance number	¹ H (ppm)	¹³ C (ppm)	¹⁵ N (ppm)
1	-	154.06	-
2	-	-	197.21
3	7.42	124.03	-
4	-	136.67	-
5a	-	135.40	-
5b	-	135.00	-
6a	7.05	129.04	-
6b	6.97	129.10	-
7	-	139.96	-
8a	2.07	17.18	-
8b	2.10	17.27	-
9	2.38	20.04	-
10	-13.47	-	-
11	-17.34 (<i>J</i> _{HN} = 24.5 Hz)	-	-
12	-	-	356.30
13	-	-	318.60
14	9.05	156.50	-
15	8.42	153.40	-
16	-	-	292.54
17	-	175.97	-
18	2.62	12.52	-
19	4.73	94.53	-
20	4.97	96.50	-
21	3.75	84.20	-
22	5.05	81.20	-
23	1.91, 2.06	30.60	-
24	1.60, 2.36	33.84	-
25	1.67, 1.96	25.66	-
26	1.25, 2.38	30.95	-

7.4.6 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IMes})(\text{X})_3]\text{Cl}$ (**4a**)Figure 7.6 Structure of **4a** determined from data given in Table 7.6.Table 7.6 NMR data collected in methanol- d_4 at 263 K for **4a** shown in Figure 7.6. Resonance labels are stated according to Figure 7.6.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	150.70	-
2	-	-	194.73
3	7.31	123.01	-
4	-	135.61	-
5	-	137.15	-
6	6.79	128.47	-
7	-	139.05	-
8	2.09	17.49	-
9	2.29	19.82	-
10	-20.19 ($J_{\text{HN}} = 22.6$ Hz)	-	-
12	-	-	344.50
13	8.69	151.23	-
14	8.33	151.89	-
15	-	-	291.43
16	-	174.50	-
17	2.65	12.47	-
18	-	-	354.07
19	-	-	321.70
20	9.35	155.33	-
21	8.32	152.36	-
22	-	-	293.16
23	-	174.53	-
24	-	-	354.71
25	2.46	12.61	-
22	5.05	81.20	-
23	1.91, 2.06	30.60	-
24	1.60, 2.36	33.84	-
25	1.67, 1.96	25.66	-

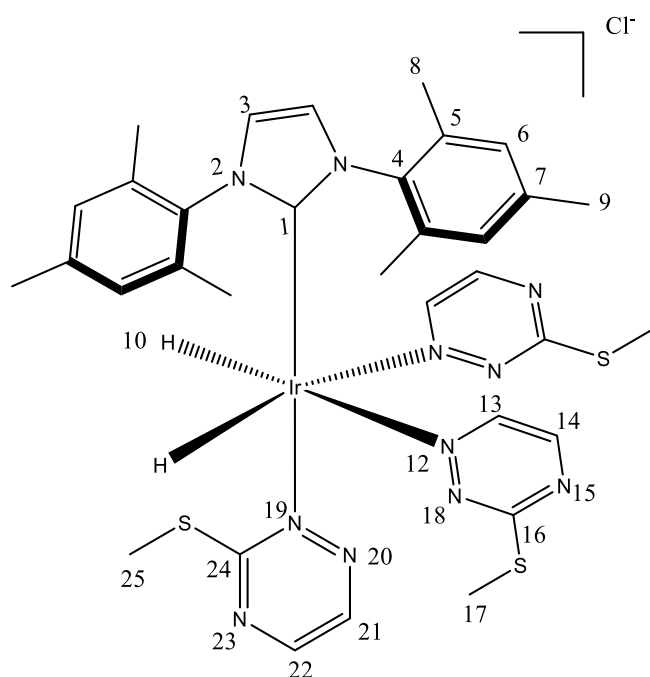
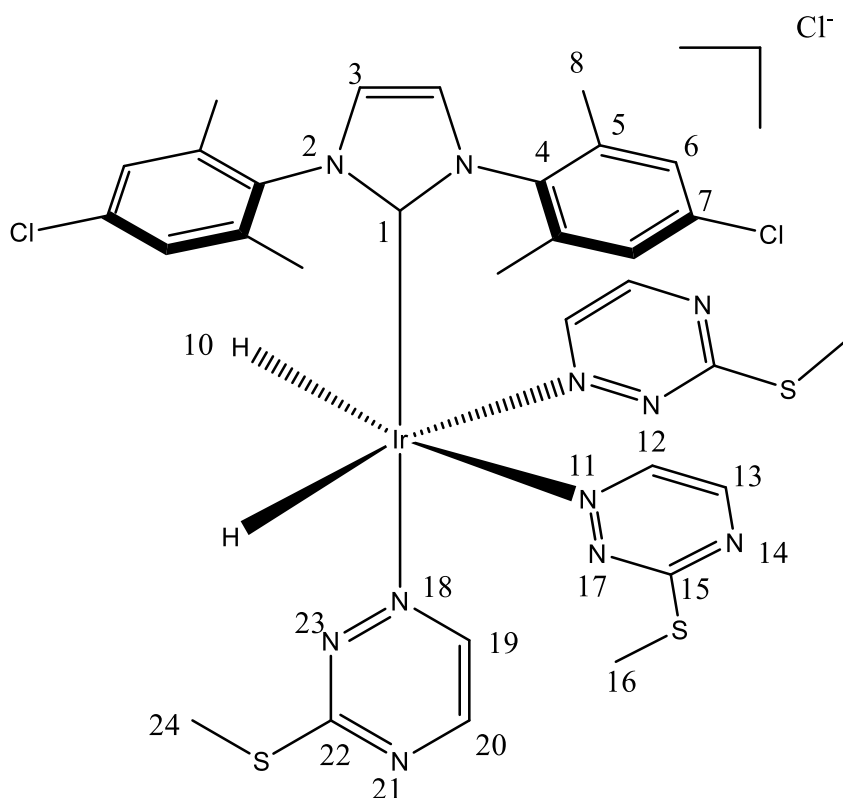
7.4.7 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IMes})(\text{X})_3]\text{Cl}$ (**4b**)

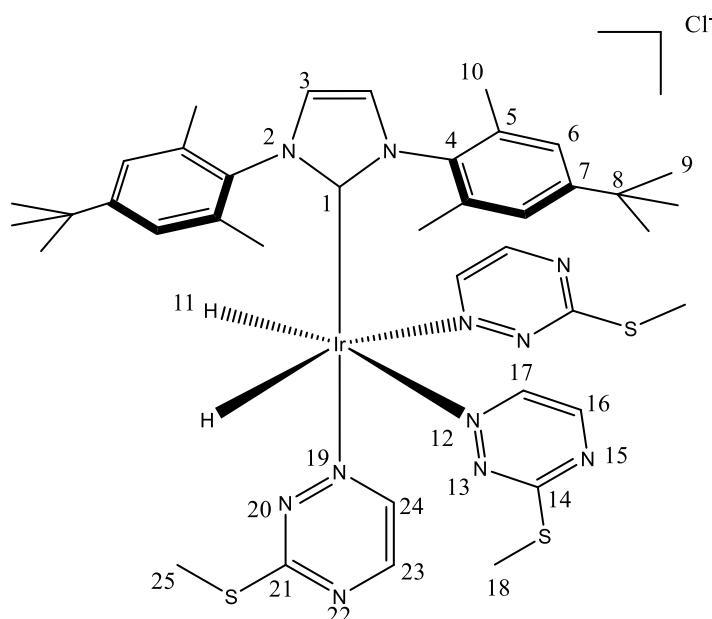
Figure 7.7 Structure of **4b** determined from data given in Table 7.7.

Table 7.7 NMR data collected in methanol- d_4 at 263 K for **4b** shown in Figure 7.7. Resonance labels are stated according to Figure 7.7.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	152.00	-
2	-	-	194.63
3	7.31	122.74	-
4	-	135.55	-
5	-	137.12	-
6	6.75	128.34	-
7	-	138.77	-
8	2.14	17.50	-
9	2.25	19.81	-
10	-20.53 ($J_{\text{HN}} = 22.1$ Hz)	-	-
12	-	-	346.82
13	8.60	150.71	-
14	8.24	151.64	-
15	-	-	290.58
16	-	174.43	-
17	2.45	12.62	-
18	-	-	321.76
19	-	-	291.84
20	-	-	411.50
21	8.33	144.55	-
22	8.56	147.20	-
23	-	-	298.48
24	-	180.96	-
25	2.48	16.95	-

7.4.8 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IM}^{p\text{-Cl}})(\text{X})_3]\text{Cl}$ (**4a** – $\text{IM}^{p\text{-Cl}}$)Figure 7.8 Structure of **4a** – $\text{IM}^{p\text{-Cl}}$ determined from data given in Table 7.8.Table 7.8 NMR data collected in methanol- d_4 at 263 K for **4a** – $\text{IM}^{p\text{-Cl}}$ shown in Figure 7.8. Resonance labels are stated according to Figure 7.8.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	142.82	-
2	-	-	193.50
3	7.34	123.3	-
4	-	147.1	-
5	-	138.1	-
6	7.03	127.64	-
7	-	134.50	-
8	2.12	17.35	-
9	-	-	-
10	-20.17 ($J_{\text{HN}} = 20.9$ Hz)	-	-
11	-	-	344.30
12	8.73	150.74	-
13	8.36	151.89	-
14	-	-	291.60
15	-	175.3	-
16	2.52	12.00	-
17	-	-	-
18	-	-	319.30
19	9.31	143.55	-
20	8.34	152.42	-
21	-	-	293.30
22	-	176.27	-
23	-	-	-
24	2.49	16.71	-

7.4.10 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IM}^{p\text{-tBu}})(\text{X})_3]\text{Cl}$ (**4a** – $\text{IM}^{p\text{-tBu}}$)Figure 7.10 Structure of **4a** – $\text{IM}^{p\text{-tBu}}$ determined from data given in Table 7.10.Table 7.10 NMR data collected in methanol- d_4 at 263 K for **4a** – $\text{IM}^{p\text{-tBu}}$ shown in Figure 7.10. Resonance labels are stated according to Figure 7.10.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	150.27	-
2	-	-	194.75
3	7.26	123.14	-
4	-	135.42	-
5	-	137.31	-
6	7.03	124.87	-
7	-	152.01	-
8	-	34.05	-
9	1.32	30.32	-
10	2.15	17.75	-
11	-20.09 ($J_{\text{NH}} = 22.4$ Hz)	-	-
12	-	-	346.14
13	-	-	-
14	-	174.66	-
15	-	-	291.40
16	8.27	151.67	-
17	8.63	151.14	-
18	2.47	12.55	-
19	-	-	321.80
20	-	-	-
21	-	174.66	-
22	-	-	291.40
23	8.32	152.33	-
24	9.36	155.18	-
25	1.81	12.03	-

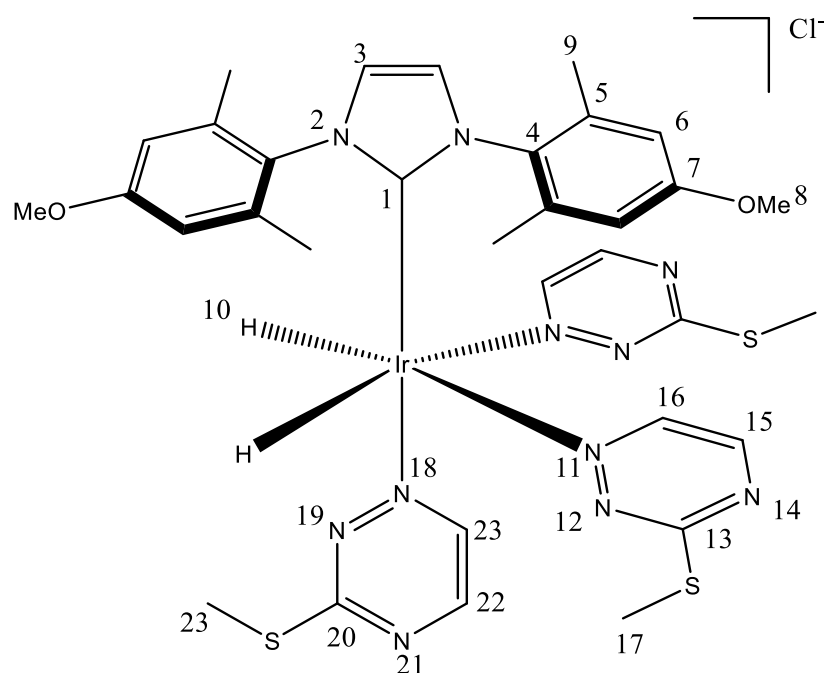
7.4.11 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IMP}^{\text{OMe}})(\text{X})_3]\text{Cl}$ (**4a** – IMP^{OMe})

Figure 7.11 Structure of **4a** – IMP^{OMe} determined from data given in Table 7.11.

Table 7.11 NMR data collected in methanol- d_4 at 263 K for **4a** – IMP^{OMe} shown in Figure 7.11. Resonance labels are stated according to Figure 7.11.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	151.90	-
2	-	-	194.28
3	7.28	123.16	-
4	-	137.27	-
5	-	132.60	-
6	6.51	112.54	-
7	-	159.60	-
8	3.78	54.32	-
9	2.09	17.73	-
10	-20.30 ($J_{\text{NH}} = 21.8$ Hz)	-	-
11	-	-	345.16
12	-	-	-
13	-	174.69	-
14	-	-	291.52
15	8.34	151.83	-
16	8.72	151.00	-
17	2.48	12.51	-
18	-	-	322.00
19	-	-	-
20	-	175.98	-
21	8.32	152.29	-
22	9.35	155.19	-
23	-	-	292.96
24	1.83	12.03	-

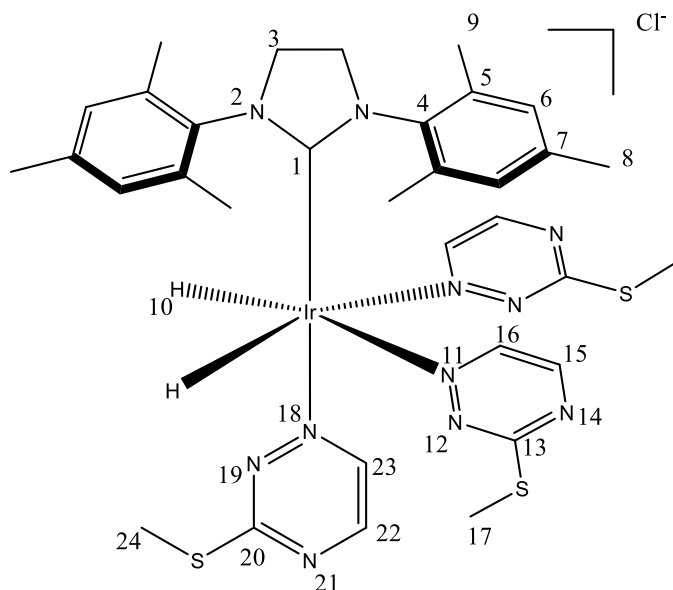
7.4.12 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{SIMes})(\text{X})_3]\text{Cl}$ (4a – SIMes)

Figure 7.12 Structure of 4a – SIMes determined from data given in Table 7.12.

Table 7.12 NMR data collected in methanol- d_4 at 263 K for 4a – SIMes shown in Figure 7.12. Resonance labels are stated according to Figure 7.12.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	178.40	-
2	-	-	-
3	3.99	50.23	-
4	-	136.39	-
5	-	137.79	-
6	6.71	128.80	-
7	-	138.03	-
8	2.23	19.72	-
9	2.29	17.41	-
10	-20.37 ($J_{\text{NH}} = 22.5$ Hz)	-	-
11	-	-	344.86
12	-	-	-
13	-	174.57	-
14	-	-	291.21
15	8.30	151.74	-
16	8.63	151.16	-
17	12.55	2.48	-
18	-	-	323.55
19	-	-	-
20	-	174.41	-
21	-	-	293.5
22	8.31	152.29	-
23	9.25	154.60	-
24	1.80	12.02	-

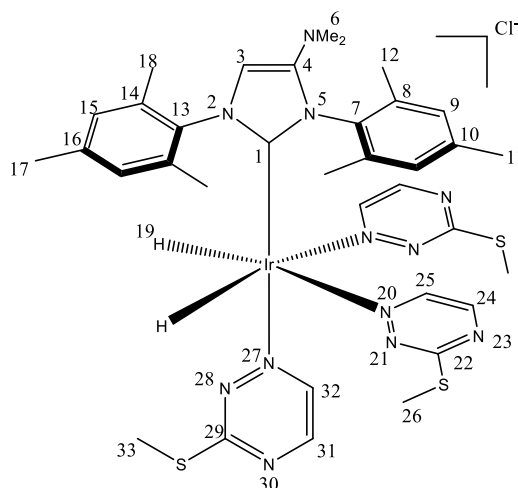
7.4.13 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IMes}^{\text{NMe}_2})(\text{X})_3]\text{Cl}$ (**4a** – $\text{IMes}^{\text{NMe}_2}$)

Figure 7.13 Structure of **4a** – $\text{IMes}^{\text{NMe}_2}$ determined from data given in Table 7.13.

Table 7.13 NMR data collected in methanol- d_4 at 263 K for **4a** – $\text{IMes}^{\text{NMe}_2}$ shown in Figure 7.13. Resonance labels are stated according to Figure 7.13.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	147.78	-
2	-	-	190.82
3	6.77	108.54	-
4	-	146.43	-
5	-	-	188.55
6	2.45	42.65	-
7	-	136.44	-
8	-	134.87	-
9	6.76	128.60	-
10	-	138.94	-
11	2.28	19.84	-
12	2.11	18.18	-
13	-	135.54	-
14	-	137.46	-
15	6.74	128.42	-
16	-	138.85	-
17	2.27	19.78	-
18	2.13	17.40	-
19	-20.40 ($J_{\text{NH}}=22.2$ Hz)	-	-
20	-	-	345.53
21	-	-	-
22	-	174.5	-
23	-	-	290.84
24	8.31	151.66	-
25	8.72	151.14	-
26	2.47	12.61	-
27	-	-	322.91
28	-	-	-
29	-	175.77	-
30	-	-	292.59
31	8.32	152.20	-
32	9.35	155.00	-
33	1.79	11.97	-

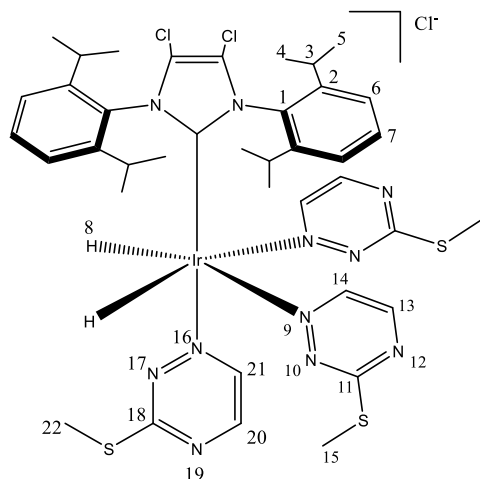
7.4.14 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IPr}^{\text{Cl}})(\text{X})_3]\text{Cl}$ (**4a** – IPr^{Cl})

Figure 7.14 Structure of **4a** – IPr^{Cl} determined from data given in Table 7.14.

Table 7.14 NMR data collected in methanol- d_4 at 263 K for **4a** – IPr^{Cl} shown in Figure 7.14. Resonance labels are stated according to Figure 7.14.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	134.84	-
2	-	146.33	-
3	2.90	28.65	-
4	1.14	21.91	-
5	1.23	24.01	-
6	7.25	124.62	-
7	7.41	130.66	-
8	-20.13 ($J_{\text{NH}}=21.3$ Hz)	-	-
9	-	-	345.49
10	-	-	-
11	-	175.46	-
12	-	-	291.19
13	8.44	152.38	-
14	8.51	149.60	-
15	2.48	12.22	-
16	-	-	317.52
17	-	-	-
18	-	176.77	-
19	-	-	293.62
20	8.27	152.70	-
21	8.91	155.67	-
22	2.11	12.18	-

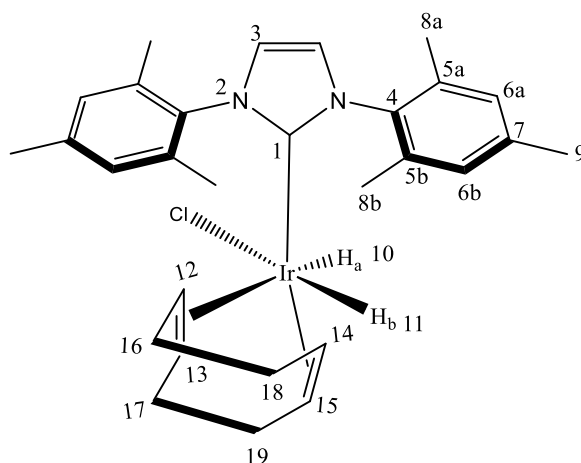
7.4.15 Characterisation for complex $[\text{IrCl}(\text{H})_2(\text{COD})(\text{IMes})]$ (5)

Figure 7.15 Structure of 5 determined from data given in Table 7.15.

Table 7.15 NMR data collected in dichloromethane- d_2 at 243 K for 5 shown in Figure 7.15. Resonance labels are stated according to Figure 7.15.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	157.00	-
2	-	-	197.59
3	6.91	122.91	-
4	-	128.65	-
5a	-	135.79	-
5b	-	136.05	-
6a	6.98	128.59	-
6b	6.98	128.59	-
7	-	138.86	-
8a	2.15	18.19	-
8b	2.27	18.75	-
9	2.35	21.16	-
10	-13.38	-	-
11	-18.08	-	-
19	4.48	86.10	-
20	3.67	92.30	-
21	4.62	74.00	-
22	3.87	88.78	-
23	1.66, 2.24	-	-
24	1.52, 1.83	-	-
25	2.01, 2.44	-	-
26	2.06, 2.64	-	-
23	-	-	290.84
24	8.31	151.66	-
25	8.72	151.14	-
26	2.47	12.61	-

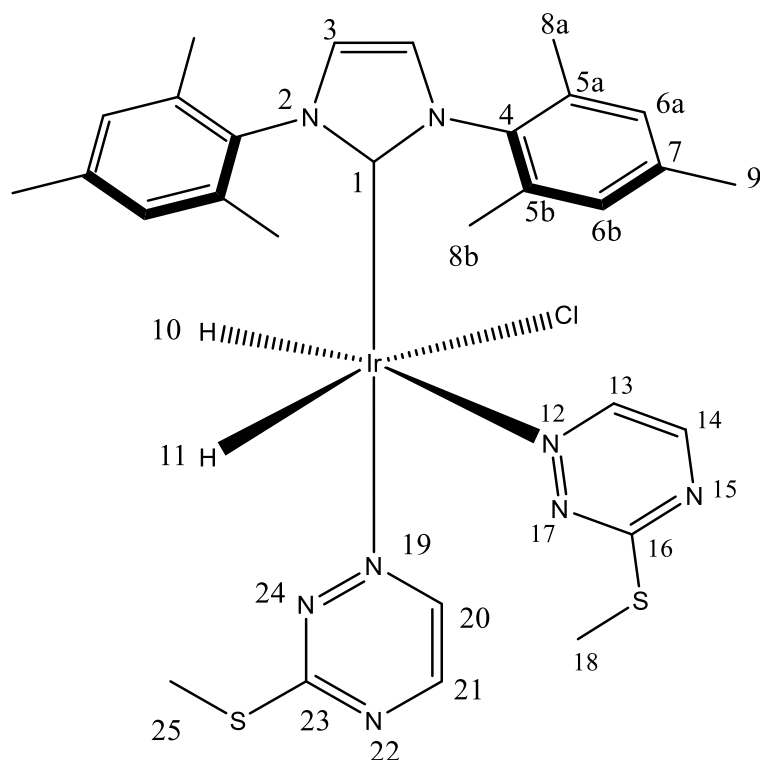
7.4.16 Characterisation for complex $[\text{IrCl}(\text{H})_2(\text{IMes})(\text{X})_2]$ (6)

Figure 7.16 Structure of 6 determined from data given in Table 7.16.

Table 7.16 NMR data collected in dichloromethane- d_2 at 243 K for 6 shown in Figure 7.16. Resonance labels are stated according to Figure 7.16.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1 (273 K)	-	153.71	-
2 (263 K)	-	-	193.7
3 (273 K)	6.90	122.01	-
4 (273 K)	-	138.77	-
5a (273 K)	-	136.85	-
5b (273 K)	-	135.70	-
6a (275 K)	6.73	128.08	-
6b (275 K)	6.83	128.49	-
7 (275 K)	-	138.37	-
8a (275 K)	2.18	18.11	-
8b (275 K)	2.21	18.44	-
9 (275 K)	2.25	20.85	-
10 (275 K)	-21.79	-	-
11 (275 K)	-23.10	-	-
12 (263 K)	-	-	353.00
13 (275 K)	9.61	145.62	-
14 (275 K)	7.95	149.90	-
15 (263 K)	-	-	282.97
16 (275 K)	-	173.55	-

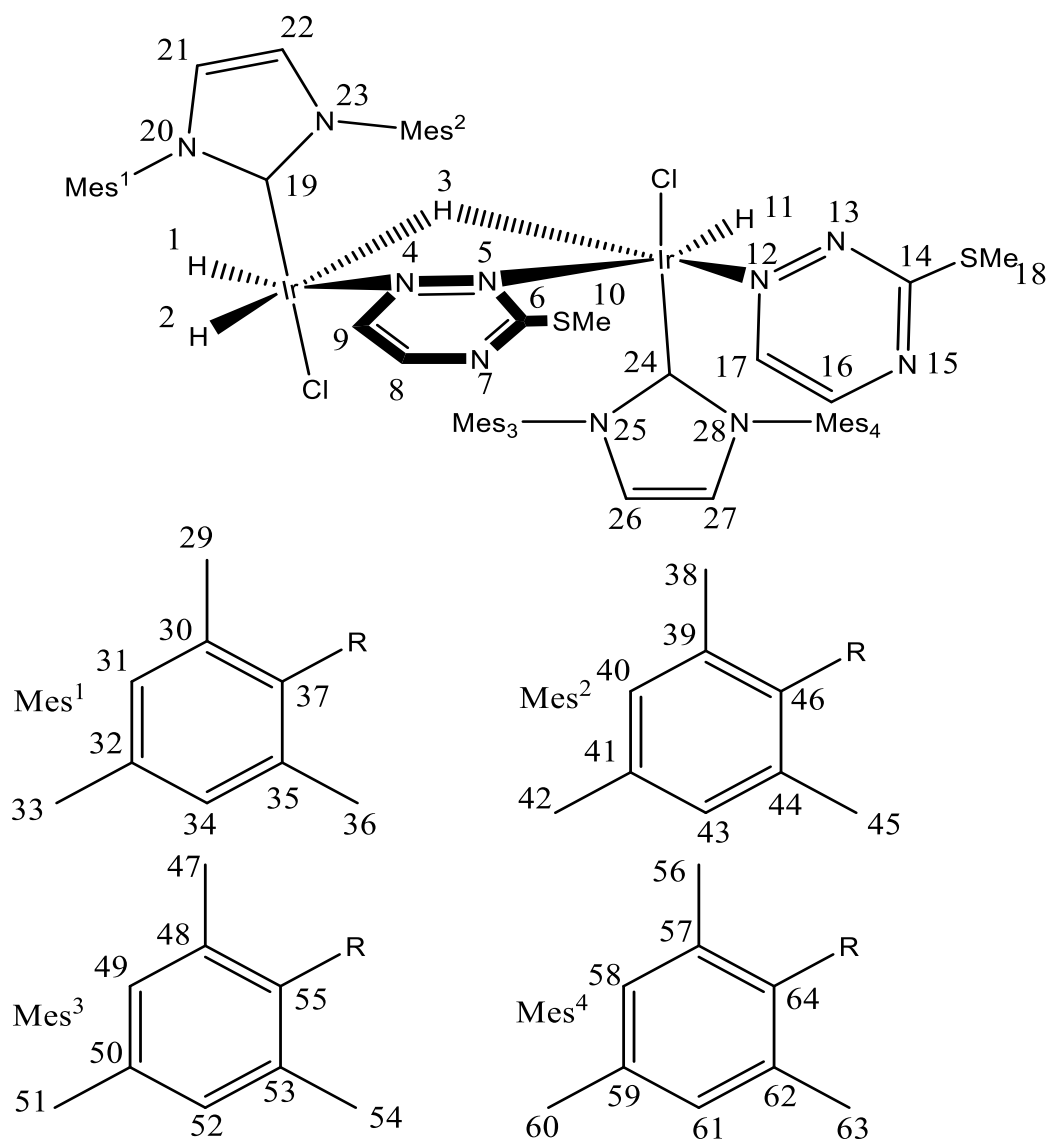
7.4.17 Characterisation for complex $[\text{Ir}_2\text{Cl}_2(\text{H})_4(\text{IMes})_2(\text{X})_2]$ (7)

Figure 7.17 Structure of 7 determined from data given in Table 7.17.

Table 7.17 NMR data collected in dichloromethane- d_2 at 243 K for 7 shown in Figure 7.17. Resonance labels are stated according to Figure 7.17.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-19.37	-	-
2	-20.54	-	-
3	-23.77	-	-
4	-	-	275.70
5	-	-	318.80
6	-	179.15	-
7	-	-	292.20
8	8.08	148.81	-
9	8.33	142.24	-
10	2.39	21.06	-
11	-20.03	-	-
12	-	-	295.80
13	-	-	455.50
14	-	176.85	-
15	-	-	295.40
16	8.14	147.83	-

Chapter 7

17	9.16	145.39	-
18	2.18	14.53	-
19	-	145.00	-
20	-	-	191.40
21	7.13	124.03	-
22	6.76	123.27	-
23	-	-	191.90
24	-	150.22	-
25	-	-	198.90
26	7.12	122.76	-
27	6.77	123.27	-
28	-	-	199.60
29	2.34	18.50	-
30	-	136.34	-
31	7.16	129.23	-
32	-	138.73	-
33	2.42	21.06	-
34	6.93	128.83	-
35	-	134.82	-
36	1.98	20.62	-
37	-	138.81	-
38	2.17	17.83	-
39	-	136.89	-
40	6.73	128.99	-
41	-	139.96	-
42	2.15	20.81	-
43	6.21	128.13	-
44	-	137.75	-
45	1.78	19.96	-
46	-	136.16	-
47	2.00	19.22	-
48	-	134.17	-
49	6.97	128.73	-
50	-	138.32	-
51	2.43	21.06	-
52	7.16	129.45	-
53	-	135.76	-
54	2.27	18.88	-
55	-	139.47	-
56	2.00	19.22	-
57	-	136.74	-
58	6.65	128.02	-
59	-	138.87	-
60	2.10	20.63	-
61	6.25	128.07	-
62	-	136.99	-
63	1.93	20.91	-
64	-	134.93	-

7.4.18 Characterisation for the product XZ ((6aS,7S,7aR)-3-(methylthio)-6,6a,7,7a,8,9-hexahydro-5H-cyclopropa[5,6]cycloocta[1,2-c]pyridin-7-yl)methanol.)

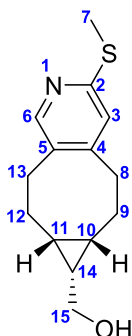


Figure 7.18 Structure of XZ determined from data given in Table 7.18.

Table 7.18 NMR data collected in methanol- d_4 at 243 K for XZ shown in Figure 7.18. Resonance labels are stated according to Figure 7.18.

Resonance number	^1H	^{13}C	^{15}N
1	-	-	289.80
2	-	157.90	-
3	6.88, d, $J_{\text{HH}} = 4.85$ Hz	121.80	-
4	-	150.70	-
5	-	134.70	-
6	8.14, d, $J_{\text{HH}} = 4.85$ Hz	145.90	-
7	2.66, s	12.50	-
8	2.85-2.77, m	33.20	-
9	2.27-2.19, m	23.40	-
10, 11	2.92-2.87, m	37.50	-
12	2.48-2.39, m	21.00	-
13	3.03-2.99, m	26.40	-
14	1.04, q, $J_{\text{HH}} = 8.40$ Hz	20.90	-
15	3.67, d, $J_{\text{HH}} = 7.34$ Hz	58.10	-

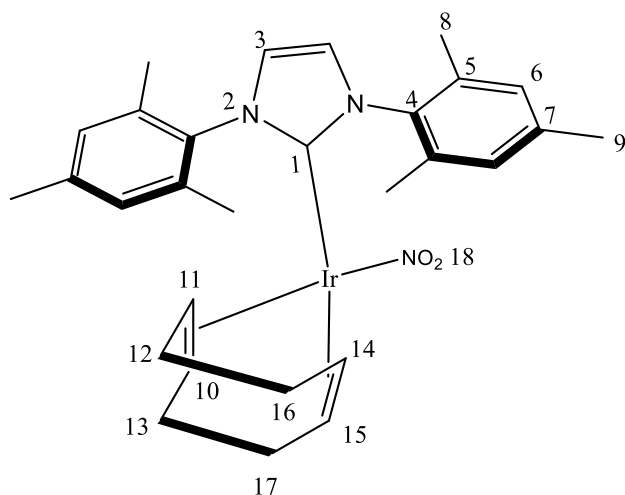
7.4.19 Characterisation for the complex $[\text{Ir}(\text{COD})(\text{IMes}) (\text{Y})] (\mathbf{8})$ 

Figure 7.19 Structure of **8** determined from data given in Table 7.19.

Table 7.19 NMR data collected in methanol- d_4 at 263 K for **8** shown in Figure 7.19. Resonance labels are stated according to Figure 7.19.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	174.20	-
2	-	-	143.90
3	7.34	124.30	-
4	-	155.00	-
5	-	135.70	-
6	7.09	128.60	-
7	-	139.00	-
8	2.22	17.40	-
9	2.42	19.90	-
10/11	3.79	119.60	-
12/13	1.52, 1.90	28.90	-
14/15	3.50	123.60	-
16/17	1.58, 1.80	32.10	-
18	-	-	490.70

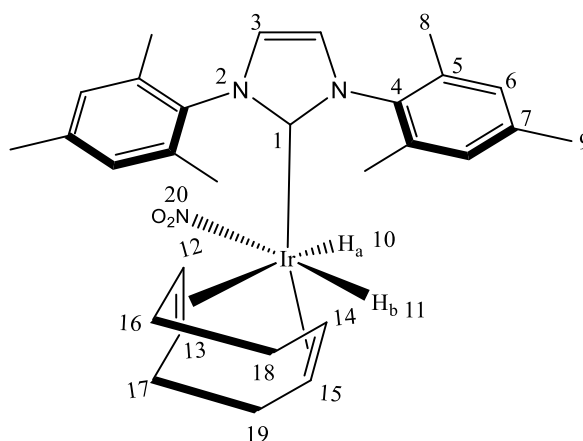
7.4.20 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{COD})(\text{IMes})(\text{Y})]$ (9)

Figure 7.20 Structure of 9 determined from data given in Table 7.20.

Table 7.20 NMR data collected in methanol- d_4 at 243 K for 9 shown in Figure 7.20. Resonance labels are stated according to Figure 7.20.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	154.78	-
2	-	-	198.10
3	7.25	124.05	-
4	-	119.13	-
5	-	115.75, 115.61	-
6	7.04, 7.02	128.80, 128.50	-
7	-	117.27	-
8	2.07, 2.30	16.74, 17.62	-
9	2.36	19.20	-
10	-14.17	-	-
11	-18.77 ($J_{\text{NH}} = 23.1$ Hz, $J_{\text{HH}} = 3.3$ Hz)	-	-
12	4.60	96.65	-
13	4.28	92.74	-
14	1.68, 1.39	27.53	-
15	2.02, 1.74	27.03	-
16	4.21	83.59	-
17	4.95	77.54	-
18	2.97, 2.13	37.92	-
19	2.32, 2.10	29.69	-
20	-	-	476.10

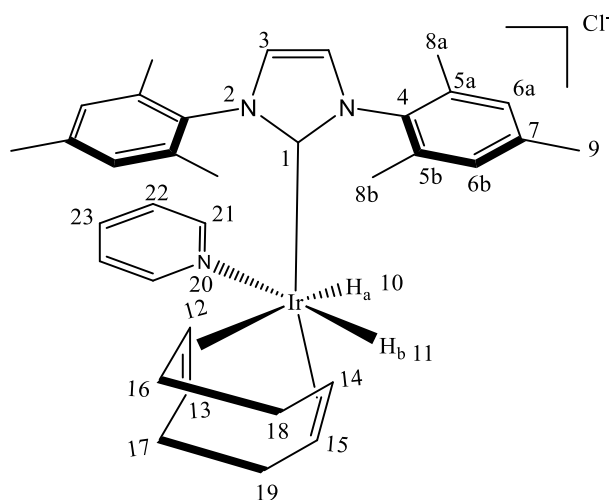
7.4.21 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{COD})(\text{D})(\text{IMes})]\text{Cl}$ (10)

Figure 7.21 Structure of 10 determined from data given in Table 7.21.

Table 7.21 NMR data collected in methanol- d_4 at 243 K for 10 shown in Figure 7.21. Resonance labels are stated according to Figure 7.21.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	153.80	-
2	-	-	194.22
3	7.37	124.46	-
4	-	136.87	-
5a	-	135.41	-
5b	-	135.16	-
6a	6.96	129.29	-
6b	7.10	129.03	-
7	-	139.63	-
8a	1.94	16.87	-
8b	1.99	16.97	-
9	2.41	16.71	-
10	-17.31	-	-
11	-12.07	-	-
12	4.98	80.80	-
13	3.68	84.60	-
14	4.25	96.50	-
15	4.69	91.20	-
16	2.00, 2.11	38.50	-
17	1.55, 1.90	27.20	-
18	1.80, 2.38	28.41	-
19	2.01, 2.40	30.07	-
20	-	-	220.63
21	8.41	148.55	-
22	7.24	124.45	-
23	7.96	137.55	-

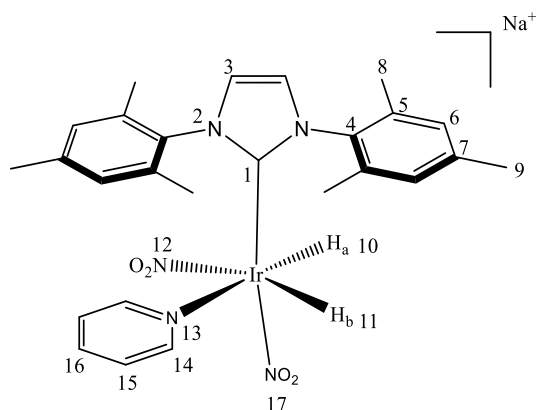
7.4.22 Characterisation for complex $\text{Na}[\text{Ir}(\text{H})_2(\text{D})(\text{IMes})(\text{Y})_2]$ (11)

Figure 7.22 Structure of 11 determined from data given in Table 7.22.

Table 7.22 NMR data collected in methanol- d_4 at 263 K for 11 shown in Figure 7.22. Resonance labels are stated according to Figure 7.22.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	154.16	-
2	-	-	194.94
3	7.01	122.03	-
4	-	137.72	-
5	-	135.66, 135.51	-
6	6.85, 6.66	128.28, 128.25	-
7	-	137.98	-
8	2.11, 2.08	17.05, 17.68	-
9	2.26	19.86	-
10	-22.05 ($J_{\text{NH}} = 20.0$ Hz, $J_{\text{HH}} = 7$ Hz)	-	-
11	-23.27 ($J_{\text{NH}} = 29.0$ Hz, $J_{\text{HH}} = 7$ Hz)	-	-
12	-	-	509.20
13	-	-	254.50
14	8.76	154.87	-
15	6.90	123.30	-
16	7.60	134.06	-
17	-	-	483.20

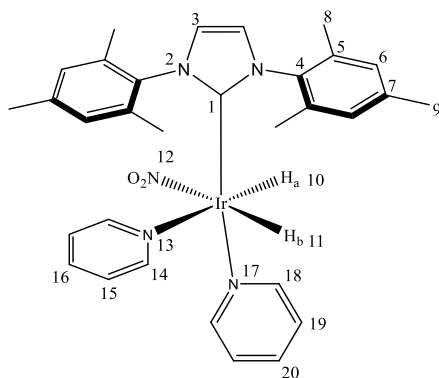
7.4.23 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{D})_2(\text{IMes})(\text{Y})]$ (12)

Figure 7.23 Structure of 12 determined from data given in Table 7.23.

Table 7.23 NMR data collected in methanol- d_4 at 263 K for 12 shown in Figure 7.23. Resonance labels are stated according to Figure 7.23.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	155.66	-
2	-	-	194.05
3	7.03	121.86	-
4	-	138.01	-
5	-	135.63, 135.58	-
6	6.75, 6.71	128.28	-
7	-	137.85	-
8	2.18, 2.14	17.70, 17.13	-
9	2.21	19.82	-
10	-22.53 ($J_{\text{NH}} = 20.0$ Hz, $J_{\text{HH}} = 8$ Hz)	-	-
11	-21.30 ($J_{\text{NH}} = 29.0$ Hz, $J_{\text{HH}} = 8$ Hz)	-	-
12	-	-	511.10
13	-	-	258.30
14	8.62	154.13	-
15	6.91	124.24	-
16	7.60	134.38	-
17	-	-	237.20
18	8.24	155.60	-
19	6.94	124.40	-
20	7.61	135.66	-

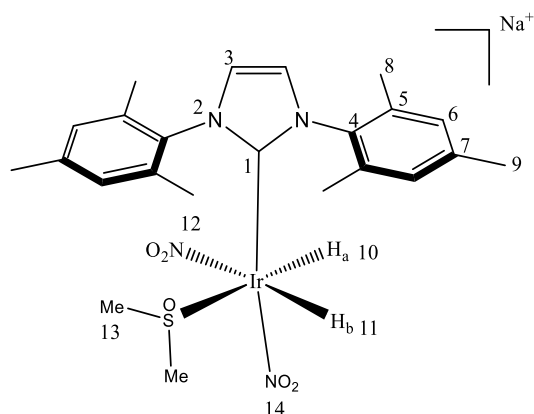
7.4.24 Characterisation for complex $\text{Na}[\text{Ir}(\text{H})_2(\text{A})(\text{IMes})(\text{Y})_2]$ (**11A**)

Figure 7.24 Structure of **11A** determined from data given in Table 7.24.

Table 7.24 NMR data collected in methanol- d_4 at 263 K for **11A** shown in Figure 7.24. Resonance labels are stated according to Figure 7.24.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	-	-
2	-	-	196.10
3	7.10	122.74	-
4	-	135.50	-
5	-	136.87, 137.22	-
6	6.97, 6.92	128.40, 128.60	-
7	-	138.45	-
8	2.17, 2.22	18.26, 17.57	-
9	2.36	19.96	-
10	$-22.17 \text{ J}_{\text{NH}} 29.6, \text{J}_{\text{HH}} 6.85$	-	-
11	$-15.57, \text{J}_{\text{HH}} 6.85$	-	-
12	-	-	500.65
13	2.92	48.56	-
14	-	-	468.72

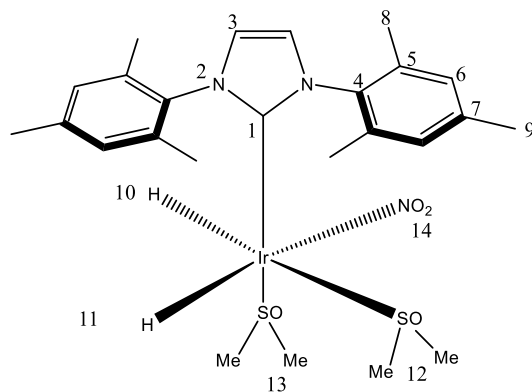
7.4.25 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{A})_2(\text{IMes})(\text{Y})]$ (12_{A})

Figure 7.25 Structure of 12_{A} determined from data given in Table 7.25.

Table 7.25 NMR data collected in methanol- d_4 at 263 K for 12_{A} shown in Figure 7.25. Resonance labels are stated according to Figure 7.25.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	-	-
2	-	-	-
3	7.30	123.67	-
4	-	-	-
5	-	-	-
6	6.99, 6.96	128.66	-
7	-	-	-
8	2.19, 2.17	18.11, 17.52	-
9	2.41	19.92	-
10	-16.08 ($J_{\text{HH}} = 6.0$ Hz)	-	-
11	-21.54 ($J_{\text{NH}} = 28.4$, $J_{\text{HH}} = 6.0$)	-	-
12	3.18, 2.80	42.21, 48.90	-
13	3.42, 3.06	57.56, 44.57	-
14	-	-	486.3

7.4.26 Characterisation for complex $\text{Na}[\text{Ir}(\text{H})_2(\text{IMes})(\text{pyridine})(\text{Y})_2]$ (13) – Both Y ligand equatorial

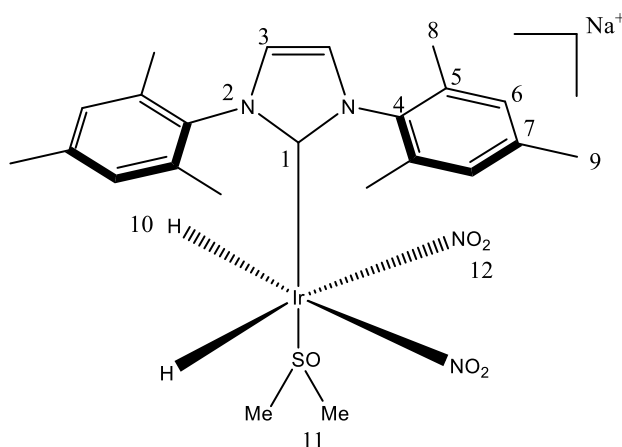


Figure 7.26 Structure of 13 determined from data given in Table 7.26.

Table 7.26 NMR data collected in methanol- d_4 at 263 K for 13 shown in Figure 7.26. Resonance labels are stated according to Figure 7.26.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	-	-
2	-	-	195.50
3	7.19	122.60	-
4	-	135.86	-
5	-	137.49	-
6	6.87	128.20	-
7	-	138.13	-
8	2.15	17.47	-
9	2.32	20.00	-
10	$-22.32 (J_{\text{NHcis}} + J_{\text{NHtrans}}) = 27.6 \text{ Hz}$	-	-
11	3.16	49.70	-
12	-	-	502.03

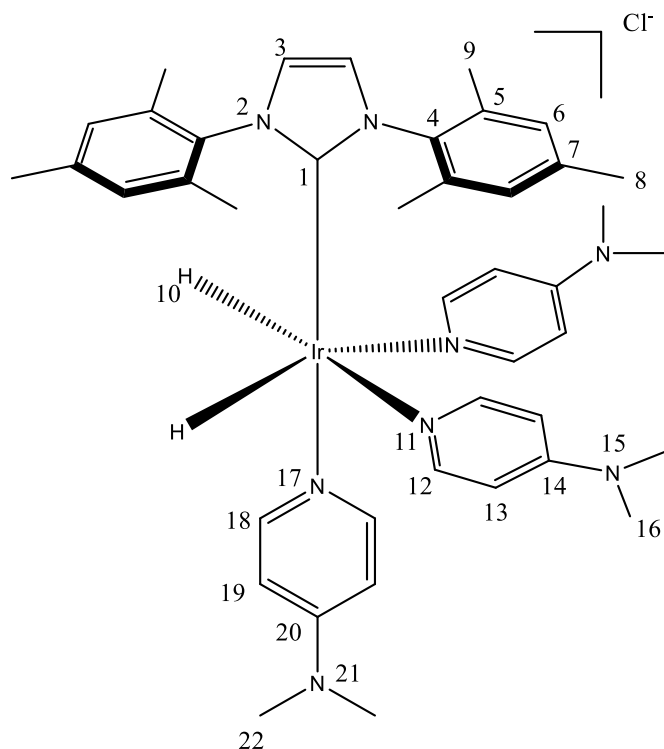
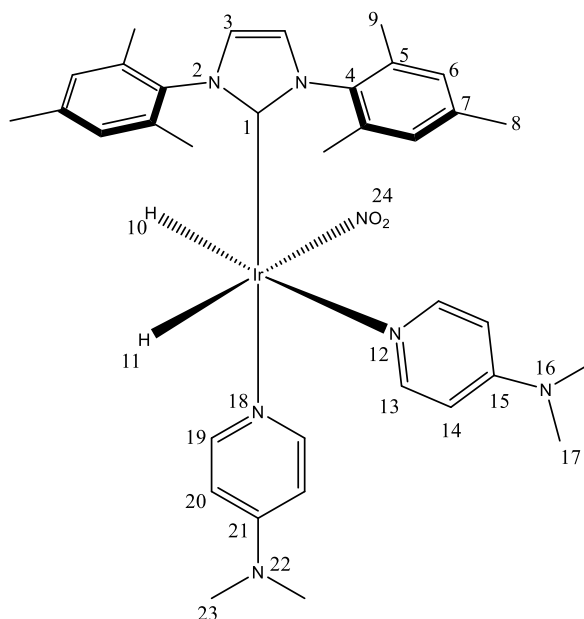
7.4.27 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{IMes})(\text{G})_3]\text{Cl}$ (14)

Figure 7.27 Structure of 14 determined from data given in Table 7.27.

Table 7.27 NMR data collected in methanol- d_4 at 263 K for 14 shown in Figure 7.27. Resonance labels are stated according to Figure 7.27.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	155.02	-
2	-	-	-
3	7.02	121.99	-
4	-	138.17	-
5	-	135.12	-
6	6.69	128.20	-
7	-	137.50	-
8	2.14	19.80	-
9	2.07	17.90	-
10	-22.76	-	-
11	-	-	212.80
12	7.81	153.22	-
13	6.26	107.02	-
14	-	-	-
15	-	-	59.50
16	3.01	37.87	-
17	-	-	196.10
18	7.48	154.10	-
19	6.15	106.68	-
20	-	152.70	-
21	-	-	60.55
22	2.86	37.78	-

7.4.28 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{G})_2(\text{IMes})(\text{Y})]$ (12_{G})Figure 7.28 Structure of 12_{G} determined from data given in Table 7.28.Table 7.28 NMR data collected in methanol- d_4 at 263 K for 12_{G} shown in Figure 7.28. Resonance labels are stated according to Figure 7.28.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	153.50	-
2	-	-	193.00
3	6.77	121.22	-
4	-	138.57	-
5	-	136.06	-
6	6.69	128.38	-
7	-	137.21	-
8	2.18	20.93	-
9	2.11	17.98	-
10	-21.60	-	-
11	-22.85	-	-
12	-	-	218.20
13	8.05	154.85	-
14	5.90	106.65	-
15	-	152.62	-
16	-	-	-
17	2.83	39.00	-
18	-	-	196.90
19	7.69	153.76	-
20	5.99	106.32	-
21	-	152.02	-
22	-	-	-
23	2.91	39.00	-
24	-	-	508.70

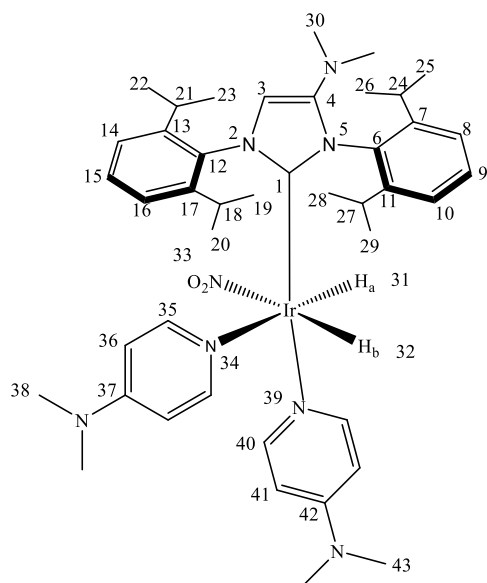
7.4.29 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{G})_2(\text{IPr}^{\text{NMe}_2})(\text{Y})]$ ($12_{\text{G}}\text{-IPr}^{\text{NMe}_2}$)

Figure 7.29 Structure of $12_{\text{G}}\text{-IPr}^{\text{NMe}_2}$ determined from data given in Table 7.29.

Table 7.29 NMR data collected in methanol- d_4 at 263 K for 12_G -IPr^{NMe₂} shown in Figure 7.29. Resonance labels are stated according to Figure 7.29.

Resonance number	¹ H (ppm)	¹³ C (ppm)	¹⁵ N (ppm)
1	-	153.25	-
2	-	-	183.66
3	6.47	110.95	-
4	-	144.14	-
5	-	-	190.81
6	-	137.04	-
7	-	146.71	-
8	-	123.81	-
9	-	128.36	-
10	-	123.88	-
11	-	146.45	-
12	-	139.18	-
13	-	145.98	-
14	-	122.71	-
15	-	128.57	-
16	-	123.67	-
17	-	145.99	-
18	3.53	27.91	27.91
19	1.48	22.07	-
20	1.23	25.61	-
21	3.43	28.05	-
22	1.24	25.61	-
23	1.02	24.75	-
24	3.25	28.54	-
25	1.45	23.89	-
26	1.19	23.52	-
27	3.22	28.85	-
28	1.46	23.44	-
29	1.19	23.47	-
30	2.92	17.73	-
31	-22.27	-	-
32	-21.16	-	-
33	-	-	-
34	-	-	217.2
35	7.81	153.62	-
36	5.96	106.49	-
37	-	-	-
38	2.92	37.76	-
39	-	-	196.1
40	7.69	154.88	-
41	6.10	106.03	-
42	-	-	-
43	2.86	37.72	-

7.4.30 Characterisation for ((5aR,6R,6aS)-1,4,5,5a,6,6a,7,8-Octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol6-yl)methanol

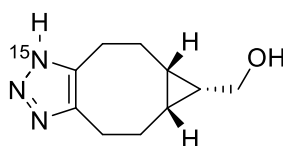


Figure 7.30 Structure of the compound ((5aR,6R,6aS)-1,4,5,5a,6,6a,7,8-Octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol6-yl)methanol

1-¹⁵N-Sodium azide (3.6 mg, 0.055 mmol, 1.1 eq.) was added to a solution of (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (7.5 mg, 0.05 mmol, 1 eq.) in methanol-d₄ inside an NMR tube fitted with a J. Young's Tap. The NMR tube was placed in warm water for 30 min and then analysed by ¹H NMR spectroscopy which showed completed conversion to the product. ¹H NMR (CD₃OH, 500 MHz): δ 3.67-3.64 (m, 4H), 3.09 (ddd, J = 14.5, 6.5, 2 Hz, 1H), 2.72 (ddd, J = 14.5, 10.5, 2 Hz, 1H), 2.20-2.17 (m, 1H), 1.50-1.42 (m, 1H), 1.33-1.26 (m, 1H), 1.17-1.12 (m, 1H), 1.11-1.05 (m, 1H), 0.94-0.85 (m, 2H); ¹³C NMR (CD₃OH, 125 MHz): δ 141.2, 58.5, 58.3, 28.6, 25.1, 23.3, 20.6, 20.5, 19.7; ¹⁵N NMR (CD₃OH, 54 MHz): δ 321.1 HRMS (ESI): m/z calculated for C₁₀H₁₅N₂¹⁵NONa 217.1078 (M + Na)⁺, found 217.1073 (error -2.1 ppm)

7.4.31 Characterisation for 1,2,4-triazine-3-methyl (V)

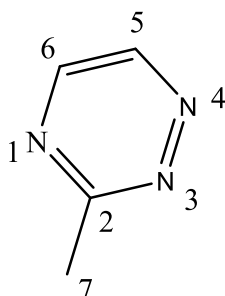


Figure 7.31 Structure of V determined from data given in Table 7.31.

Table 7.30 NMR data collected in methanol-d₄ at 263 K for V shown in Figure 7.31. Resonance labels are stated according to Figure 7.31.

Resonance Label	¹ H (ppm)	¹³ C (ppm)	¹⁵ N (ppm)
1	-	-	299.80 (J _{HN} =12.2 Hz)
2	-	167.66	-
3	-	-	362.40 (J _{HN} =6.2 Hz)
4	-	-	413.20 (J _{HN} =14.3 Hz)
5	9.19 (J _{HH} =2.36)	147.54	-
6	8.72	149.85	-
7	2.85	22.23	-

7.4.32 Characterisation for acetonitrile (C)

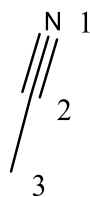


Figure 7.32 Structure of C determined from data given in Table 7.32.

Table 7.31 NMR data collected in methanol- d_4 at 263 K for C shown in Figure 7.32. Resonance labels are stated according to Figure 7.32.

Resonance Label	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1			240.70
2		116.84	
3	2.05	-0.58	

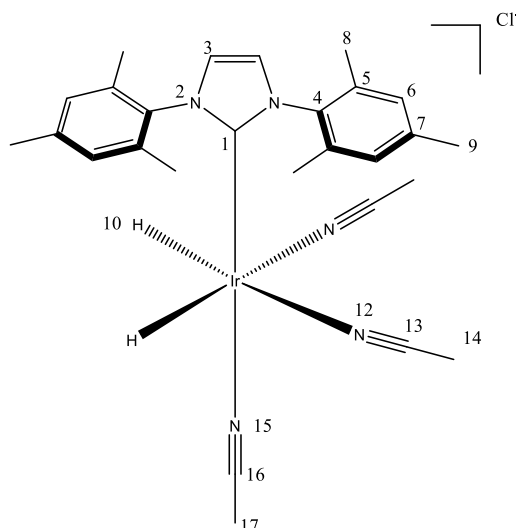
7.4.33 Characterisation for complex [Ir(H) $_2$ (C) $_3$ (IMes)]Cl (17)

Figure 7.33 Structure of 17 determined from data given in Table 7.33.

Table 7.32 NMR data collected in methanol- d_4 at 263 K for 17 shown in Figure 7.33. Resonance labels are stated according to Figure 7.33.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	148.20	-
2	-	-	194.09
3	7.17	122.19	-
4	-	137.43	-
5	-	135.81	-
6	7.08	128.36	-
7	-	138.64	-
8	2.11	16.99	-
9	2.39	19.85	-
10	-22.32	-	-
12	-	-	178.44 (243 K)
13	-	116.95	-
14	2.16	1.03	-
15	-	-	157.64 (243 K)
16	-	119.51	-
17	2.32	2.63	-

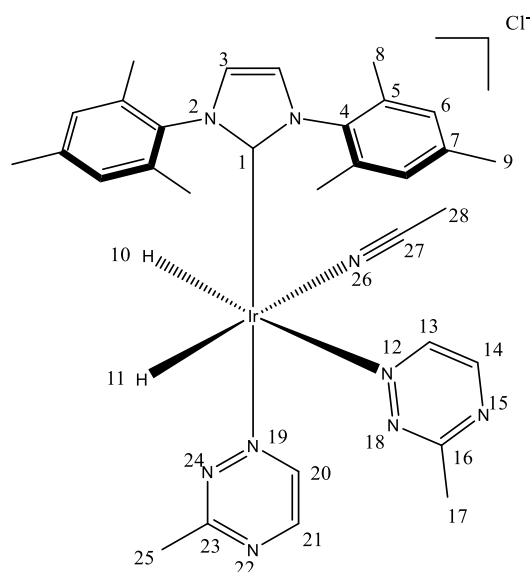
7.4.34 Characterisation for complex $[\text{Ir}(\text{H})_2(\text{C})(\text{IMes})(\text{V})_2]\text{Cl}$ (18)

Figure 7.34 Structure of 18 determined from data given in Table 7.34.

Table 7.33 NMR data collected in methanol- d_4 at 263 K for 18 shown in Figure 7.34. Resonance labels are stated according to Figure 7.34.

Resonance number	^1H (ppm)	^{13}C (ppm)	^{15}N (ppm)
1	-	153.14	-
2	-	-	190.43
3	7.20	123.31	-
4	-	-	-
5	-	-	-
6	6.84	128.90	-
7	-	-	-
8	2.11	17.59	-
9	2.45	19.91	-
10	-20.23	-	-
11	-21.20	-	-
12	-	-	340.23
13	9.07	153.14	-
14	8.33	153.40	-
15	-	-	299.90
16	-	167.30	-
17	2.81	22.59	-
18	-	-	365.10
19	-	-	370.21
20	9.22	157.21	-
21	8.24	155.30	-
22	-	-	299.40
23	-	170.22	-
24	-	-	360.21
25	2.26	20.01	-
26	-	-	171.15
27	-	117.87	-
28	2.19	0.90	-

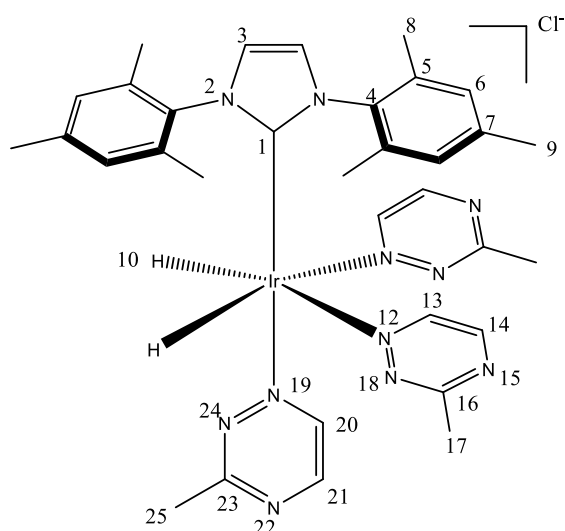
7.4.35 Characterisation for complex [Ir(H)₂(IMes)(V)₃]Cl (19)

Figure 7.35 Structure of 19 determined from data given in Table 7.35.

Table 7.34 NMR data collected in methanol-d₄ at 263 K for 19 shown in Figure 7.35. Resonance labels are stated according to Figure 7.35.

Resonance number	¹ H (ppm)	¹³ C (ppm)	¹⁵ N (ppm)
1	-	150.44	-
2	-	-	194.93
3	7.24	122.98	-
4	-	135.60	-
5	-	137.05	-
6	6.76	128.44	-
7	-	138.62	-
8	2.06	17.37	-
9	2.28	19.75	-
10	-20.39	-	-
12	-	-	343.82
13	9.08	152.88	-
14	8.52	152.17	-
15	-	-	299.5
16	-	168.03	-
17	2.70	22.14	-
18	-	-	364.30
19	-	-	322.00
20	9.54	156.81	-
21	8.48	152.72	-
22	-	-	299.20
23	-	169.51	-
24	-	-	358.91
25	2.19	17.27	-

Chapter 8. Conclusions

The central themes explored in this thesis are that of improving the detectability of ^{15}N nuclei through hyperpolarisation with SABRE. This was achieved through thorough optimisation of each system investigated, finding that the ligand dissociation rate of the substrate is an essential factor in efficient polarisation transfer for ^{15}N as has already described for ^1H .¹²³ This was done by altering the steric and electronic properties of the *N*-heterocyclic carbene (NHC) ligand, as well as introducing co-ligand species to help form more stable catalytic species and to further improve the exchange rate of the substrate.⁵⁹ This optimisation led to the novel systems $[\text{Ir}(\text{H})_2(\text{IPr}^{\text{NMe}_2})(\text{X})_3]\text{Cl}$, $[\text{Ir}(\text{H})_2(\text{IPr}^{\text{NMe}_2})(\text{G})_2(\text{Y})]$ and $[\text{Ir}(\text{H})_2(\text{C})_2(\text{IPrCl})(\text{V})]\text{Cl}$ being identified as the best polarisation transfer catalysts for 3-methylthio-1,2,4-triazine (**X**), $[\text{NO}_2]^-$ (**Y**) and acetonitrile (**C**) respectively. High levels of polarisation were achieved for all three substrates with ^{15}N polarisation levels of $4.7 \pm 0.2\%$, $38.1 \pm 0.4\%$ and $13.4 \pm 0.7\%$ achieved for **X**, **Y**, and **C** respectively. These results represent some of the highest polarisation levels achieved for ^{15}N by SABRE and are much higher than attempts made by DNP (1% ^{15}N polarisation for **Y**).¹⁶⁹

Many exciting applications of these high ^{15}N polarisation levels have been outlined throughout the thesis that can be extended into the future. It was exemplified, through the reactivity of **Y** and azide, that SABRE hyperpolarised ^{15}N NMR spectroscopy can be an incredibly useful tool in determining very short lived, low concentration reaction intermediates. This in turn can provide useful mechanistic insights into reactions inaccessible through current methods available. Notably, in the reaction of phenyl diazonium chloride with sodium azide (Figure 3.15a) all expected reaction products are detected, starting from only one hyperpolarised substrate (**Y**). This achievement opens up new opportunities for reaction monitoring by SABRE allowing for relay of polarisation through reaction. In the reaction observed, determination of a pentacyclic intermediate was possible and so provided mechanistic insights for the debated mechanism of Scheme 3.5.¹⁶¹⁻¹⁶³

Harnessing the reactivity of **Y** has allowed the transfer of polarisation, creating a facile route to hyperpolarised N_2 . This exciting result opens future opportunities to probe *paranitrogen* which has been proposed as an important contrast agent for lung imaging by MRI,⁴⁶ offering an alternative to expensive methods such as SEOP. This work could also be extended to produce other hyperpolarised gases such as N_2O (nitrous oxide) or NO (nitric oxide). It was shown in Chapter 4 that the T_1 of *ortho* N_2 gas is too short to be

useful as a hyperpolarised lung imaging agent quantifying it as *circa* 2.3 s at magnetic storage fields ranging from 7.0 to 16.4 T.

The extension of T_1 was thoroughly investigated and several interesting observations were drawn throughout the work. Firstly, the ^{15}N T_1 lifetime was found to extend at lower fields, as demonstrated for **Y**, which reached a maximum of *circa* 37 s at 50 μT . The effect was also observed for several nitrile containing substrates (Figure 6.14) with all compounds tested reaching an observed maximum at a field of 7 T. The ^{15}N T_1 of **C-d₃** was determined to be *circa* 240 s at a 7 T storage field and the aromatase inhibitor anastrozole reached a T_1 of *circa* 40 s at the same field. Combined with a >12% ^{15}N polarisation being achieved utilising the optimised catalytic system gives real promise to the use of anastrozole as a hyperpolarised imaging agent.

Other factors effecting T_1 were also observed such as temperature and effects from the catalyst. **Y** demonstrated lower T_1 values at temperatures lower than 278 K and was found to stay stable between 278 and 328 K, this again gives promise for use in imaging as ambient sample manipulation is preferential. Lowering the concentration of the polarisation transfer catalyst relative to **Y** was also beneficial in reducing the number of spin-spin interactions undergone by the substrate. The most interesting finding observed for **Y** was the ability to extend the T_1 through storage in the solid phase. Storing the sample in the solid phase at 50 μT saw the extension of the T_1 so that a 0.02% ^{15}N polarisation remained after 360 s. It is believed that storage at a suitable magnetic field will extend this lifetime even more so and prove to be a very useful tool in long term polarisation storage.

This method, shown in Figure 4.14, not only demonstrated T_1 lifetime extension but also successfully exemplified complete catalyst removal. No Ir species were detected by ^1H NMR after the processes had been undertaken. Thus, demonstrating an easy approach to long term ^{15}N polarisation storage and sample purification simultaneously. Another purification method *via* a biphasic approach was also successfully exemplified acquiring ^{15}N images of **Y** that show a high affinity for the biologically compatible water layer. These two demonstrations again increase the backing that hyperpolarised ^{15}N imaging agents will be widely up-taken in the near future. It is noted that the acutely toxic dimethylaminopyridine (**G**) did remain but utilising DMSO would eliminate this issue while still yielding high levels of polarisation.

The precipitation of **Y** was achieved by use of a perfluorous solvent methylperfluorobutyl ether (**J**). Interestingly, **Y** showed a higher level of polarisation in a 1 : 1 mixture of methanol-*d*₄ and **J** than in pure methanol-*d*₄. The SABRE efficiency in these perfluorous solvents was thoroughly investigated and found, due to their ability to dissolve higher concentrations of hydrogen, the SABRE of ¹⁵N benefited greatly. This trend was originally shown in Chapter 3 where the maximum SABRE efficiency (for the conditions used) was achieved by increasing the amount of hydrogen in solution. The improvement in ¹⁵N polarisation achieved by this simple method was astonishing yielding $21.8 \pm 0.2\%$ (66840 ± 610 -fold signal enhancement at 9.4 T) for the pyridyl ¹⁵N of nicotine (**T**); the comparative result in methanol-*d*₄ was only $6.0 \pm 0.1\%$ (18900 ± 300 -fold signal enhancement at 9.4 T). This discovery represents a potential turning point in the field for ¹⁵N hyperpolarisation *via* SABRE. As substrates can be easily extracted into water through the biphasic separation method outlined previously, it is easy to see this being applied for *in vivo* application in the near future.

This thesis also outlined many results to aid the progression of mixture analysis by ¹⁵N NMR spectroscopy. It was demonstrated that complex mixtures of nitrile compounds can be hyperpolarised to a high degree, allowing the easy distinction of all species in solution. Despite the sample being low concentration (25 mM) and natural ¹⁵N abundance, all nitriles in the mixture were readily detectible in just one scan. The quantification of these nitrile compounds was also shown to be possible *via* SABRE although further work is still required to avoid catalyst speciation effects when the quantification is attempted on mixtures of nitriles. The ¹⁵N DOSY of **D** and **Y** was exemplified in Chapter 2. The effects from ligand exchange and advection were explored and eliminated, yielding comparable results to the reported literature. Thanks again to the high chemical shift window of ¹⁵N, these results offer an accurate method of diffusion coefficient determination without signal overlap (a major struggle for traditional DOSY). However, this still needs to be applied to a mixture which could lead to complications due to catalyst speciation.

In conclusion, the results from this thesis have demonstrated a huge improvement in ¹⁵N hyperpolarisation *via* SABRE, not only in the high levels of ¹⁵N polarisation observed, but also in furthering understanding the wider effects at play leading to essential developments in applications towards MRI, mixture analysis, intermediate detection and reaction monitoring

References

1. P. J. Hore, *Nuclear Magnetic Resonance*, Oxford University Press, 1995.
2. R. Freeman, *Magnetic Resonance in Chemistry and Medicine*, Oxford University Press 2003.
3. A. M. Olaru, A. Burt, P. J. Rayner, S. J. Hart, A. C. Whitwood, G. G. R. Green and S. B. Duckett, *Chem. Commun.*, 2016, **52**, 14482-14485.
4. P. J. Rayner, M. Fekete, C. A. Gater, F. Ahwal, N. Turner, A. J. Kennerley and S. B. Duckett, *J. Am. Chem. Soc.*, 2022, **144**, 8756-8769.
5. M. H. Levitt, *Spin Dynamics*, John Wiley & Sons Ltd., 2008.
6. M. L. Hirsch, N. Kalechofsky, A. Belzer, M. Rosay and J. G. Kempf, *J. Am. Chem. Soc.*, 2015, **137**, 8428-8434.
7. D. T. Peat, M. L. Hirsch, D. G. Gadian, A. J. Horsewill, J. R. Owers-Bradley and J. G. Kempf, *Phys. Chem. Chem. Phys.*, 2016, **18**, 19173-19182.
8. G. A. Morris and R. Freeman, *J. Am. Chem. Soc.*, 1979, **101**, 760-762.
9. P. Moutzouri, Y. Chen, M. Foroozandeh, P. Kiraly, A. R. Phillips, S. R. Coombes, M. Nilsson and G. A. Morris, *Chem. Commun.*, 2017, **53**, 10188-10191.
10. J. A. Aguilar, S. Faulkner, M. Nilsson and G. A. Morris, *Angew. Chem. Int. Ed.*, 2010, **49**, 3901-3903.
11. A. I. Silva Terra, M. Rossetto, C. L. Dickson, G. Peat, D. Uhrin and M. E. Halse, *ACS Meas. Sci. Au*, 2023, **3**, 73-81.
12. M. Foroozandeh, R. W. Adams, N. J. Meharry, D. Jeannerat, M. Nilsson and G. A. Morris, *Angew. Chem. Int. Ed.*, 2014, **53**, 6990-6992.
13. R. W. Adams, J. A. Aguilar, K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, I. G. Khazal, J. N. López-Serrano and D. C. Williamson, *Science*, 2009, **323**, 1708-1711.
14. C. R. Bowers and D. P. Weitekamp, *Phys. Rev. Lett.*, 1986, **57**, 2645-2648.
15. C. R. Bowers and D. P. Weitekamp, *J. Am. Chem. Soc.*, 1987, **109**, 5541-5542.
16. R. Eisenberg, *Acc. Chem. Res.*, 1991, **24**, 110-116.
17. H. R. Ward and R. G. Lawler, *J. Am. Chem. Soc.*, 1967, **89**, 5518-5519.
18. P. F. Seidler, H. E. Bryndza, J. E. Frommer, L. S. Stuhl and R. G. Bergman, *Organometallics*, 1983, **2**, 1701-1705.
19. Y. Y. Milenko, R. M. Sibileva and M. A. Strzhemechny, *J. Low Temp. Phys.*, 1997, **107**, 77-92.
20. J. Natterer and J. Bargon, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1997, **31**, 293-315.
21. M. Born and R. Oppenheimer, *Ann. Phys.*, 1927, **389**, 457-484.
22. S. B. Duckett and C. J. Sleight, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1999, **34**, 71-92.
23. A. J. Parrott, P. Dallin, J. Andrews, P. M. Richardson, O. Semenova, M. E. Halse, S. B. Duckett and A. Nordon, *Appl. Spectrosc.*, 2019, **73**, 88-97.
24. R. A. Green, R. W. Adams, S. B. Duckett, R. E. Mewis, D. C. Williamson and G. G. R. Green, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2012, **67**, 1-48.
25. S. B. Duckett and D. Blazina, *Eur. J. Inorg. Chem.*, 2003, **2003**, 2901-2912.
26. S. B. Duckett and N. J. Wood, *Coord. Chem. Rev.*, 2008, **252**, 2278-2291.

27. M. R. Gyton, C. G. Royle, S. K. Beaumont, S. B. Duckett and A. S. Weller, *J. Am. Chem. Soc.*, 2023, **145**, 2619-2629.
28. S. B. Duckett, C. L. Newell and R. Eisenberg, *J. Am. Chem. Soc.*, 1994, **116**, 10548-10556.
29. S. B. Duckett, C. L. Newell and R. Eisenberg, *J. Am. Chem. Soc.*, 1997, **119**, 2068-2068.
30. D. O. Zakharov, K. Chernichenko, K. Sorochkina, S. Yang, V. V. Telkki, T. Repo and V. V. Zhivonitko, *Eur. J. Chem*, 2022, **28**, e202103501.
31. K. Sorochkina, V. V. Zhivonitko, K. Chernichenko, V.-V. Telkki, T. Repo and I. V. Koptug, *J. Phys. Chem. Lett.*, 2018, **9**, 903-907.
32. K. V. Kovtunov, I. E. Beck, V. I. Bukhtiyarov and I. V. Koptug, *Angew. Chem. Int. Ed.*, 2008, **47**, 1492-1495.
33. L. Kaltschnee, A. N. Pravdivtsev, M. Gehl, G. Huang, G. L. Stoychev, C. Riplinger, M. Keitel, F. Neese, J.-B. Hövener, A. A. Auer, C. Griesinger, S. Shima and S. Glöggler, unpublished work.
34. M. G. Pravica and D. P. Weitekamp, *Chem. Phys. Lett.*, 1988, **145**, 255-258.
35. A. Svyatova, V. P. Kozinenko, N. V. Chukanov, D. B. Burueva, E. Y. Chekmenev, Y.-W. Chen, D. W. Hwang, K. V. Kovtunov and I. V. Koptug, *Sci. Rep.*, 2021, **11**, 5646.
36. O. G. Salnikov, I. A. Trofimov, Z. T. Bender, A. I. Trepakova, J. Xu, G. L. Wibbels, R. V. Shchepin, I. V. Koptug and D. A. Barskiy, *Angew. Chem. Int. Ed.*, 2024, **63**, e202402877.
37. J. McCormick, S. Korchak, S. Mamone, Y. N. Ertas, Z. Liu, L. Verlinsky, S. Wagner, S. Glöggler and L. S. Bouchard, *Angew. Chem. Int. Ed.*, 2018, **57**, 10692-10696.
38. T. C. Eisenschmid, J. McDonald, R. Eisenberg and R. G. Lawler, *J. Am. Chem. Soc.*, 1989, **111**, 7267-7269.
39. S. B. Duckett, C. L. Newell and R. Eisenberg, *J. Am. Chem. Soc.*, 1993, **115**, 1156-1157.
40. R. W. Adams, S. B. Duckett, R. A. Green, D. C. Williamson and G. G. R. Green, *J. Chem. Phys.*, 2009, **131**, 194505.
41. W. Iali, P. J. Rayner, A. Alshehri, A. J. Holmes, A. J. Ruddlesden and S. B. Duckett, *Chem. Sci.*, 2018, **9**, 3677-3684.
42. W. Iali, P. J. Rayner and S. B. Duckett, *Sci. Adv.*, 2018, **4**, eaao6250.
43. M. Fekete, F. Ahwal and S. B. Duckett, *J. Phys. Chem. B*, 2020, **124**, 4573-4580.
44. R. E. Mewis, R. A. Green, M. C. R. Cockett, M. J. Cowley, S. B. Duckett, G. G. R. Green, R. O. John, P. J. Rayner and D. C. Williamson, *J. Phys. Chem. B*, 2015, **119**, 1416-1424.
45. J. F. P. Colell, A. W. J. Logan, Z. Zhou, R. V. Shchepin, D. A. Barskiy, G. X. Ortiz, Jr., Q. Wang, S. J. Malcolmson, E. Y. Chekmenev, W. S. Warren and T. Theis, *J. Phys. Chem. C*, 2017, **121**, 6626-6634.
46. T. Theis, G. X. Ortiz, A. W. J. Logan, K. E. Claytor, Y. Feng, W. P. Huhn, V. Blum, S. J. Malcolmson, E. Y. Chekmenev, Q. Wang and W. S. Warren, *Sci. Adv.*, 2016, **2**, e1501438.
47. B. Procacci, S. S. Roy, P. Norcott, N. Turner and S. B. Duckett, *J. Am. Chem. Soc.*, 2018, **140**, 16855-16864.
48. A. W. J. Logan, T. Theis, J. F. P. Colell, W. S. Warren and S. J. Malcolmson, *Eur. J. Chem*, 2016, **22**, 10777-10781.
49. W. Iali, S. S. Roy, B. J. Tickner, F. Ahwal, A. J. Kennerley and S. B. Duckett, *Angew. Chem. Int. Ed.*, 2019, **58**, 10271-10275.

50. M. E. Gemeinhardt, M. N. Limbach, T. R. Gebhardt, C. W. Eriksson, S. L. Eriksson, J. R. Lindale, E. A. Goodson, W. S. Warren, E. Y. Chekmenev and B. M. Goodson, *Angew. Chem. Int. Ed.*, 2020, **59**, 418-423.
51. B. J. Tickner, P. J. Rayner and S. B. Duckett, *Anal. Chem.*, 2020, **92**, 9095-9103.
52. P. M. Richardson, A. J. Parrott, O. Semenova, A. Nordon, S. B. Duckett and M. E. Halse, *Analyst*, 2018, **143**, 3442-3450.
53. M. L. Truong, T. Theis, A. M. Coffey, R. V. Shchepin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren and E. Y. Chekmenev, *J. Phys. Chem. C*, 2015, **119**, 8786-8797.
54. B. J. Tickner, M. Dennington, B. G. Collins, C. A. Gater, T. F. N. Tanner, A. C. Whitwood, P. J. Rayner, D. P. Watts and S. B. Duckett, *ACS Catal.*, 2024, **14**, 994-1004.
55. A. M. Olaru, T. B. R. Robertson, J. S. Lewis, A. Antony, W. Iali, R. E. Mewis and S. B. Duckett, *ChemistryOpen*, 2018, **7**, 97-105.
56. S. S. Roy, K. M. Appleby, E. J. Fear and S. B. Duckett, *J. Phys. Chem. Lett.*, 2018, **9**, 1112-1117.
57. D. A. Barskiy, A. N. Pravdivtsev, K. L. Ivanov, K. V. Kovtunov and I. V. Koptug, *Phys. Chem. Chem. Phys.*, 2016, **18**, 89-93.
58. T.-L. Hwang, M. Kadkhodaei, A. Mohebbi and A. J. Shaka, *Magn. Reson. Chem.*, 1992, **30**, S24-S34.
59. P. J. Rayner, P. Norcott, K. M. Appleby, W. Iali, R. O. John, S. J. Hart, A. C. Whitwood and S. B. Duckett, *Nat. Commun.*, 2018, **9**, 4251.
60. N. Eshuis, B. J. A. Van Weerdenburg, M. C. Feiters, F. P. J. T. Rutjes, S. S. Wijmenga and M. Tessari, *Angew. Chem. Int. Ed.*, 2015, **54**, 1481-1484.
61. T. Theis, M. L. Truong, A. M. Coffey, R. V. Shchepin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren and E. Y. Chekmenev, *J. Am. Chem. Soc.*, 2015, **137**, 1404-1407.
62. J. Bae, Z. Zhou, T. Theis, W. S. Warren and Q. Wang, *Sci. Adv.*, 2018, **4**, eaar2978.
63. K. MacCulloch, A. Browning, P. TomHon, S. Lehmkuhl, E. Y. Chekmenev and T. Theis, *Anal. Chem.*, 2023, **95**, 7822-7829.
64. R. V. Shchepin, J. R. Birchall, N. V. Chukanov, K. V. Kovtunov, I. V. Koptug, T. Theis, W. S. Warren, J. G. Gelovani, B. M. Goodson, S. Shokouhi, M. S. Rosen, Y.-F. Yen, W. Pham and E. Y. Chekmenev, *Eur. J. Chem*, 2019, **25**, 8829-8836.
65. H. De Maissin, P. Groß, O. Mohiuddin, M. Weigt, L. Nagel, M. Herzog, Z. Wang, R. Willing, W. Reichardt, M. Pichotka, L. Heß, T. Reinheckel, H. Jessen, R. Zeiser, M. Bock, D. Von Elverfeldt, M. Zaitsev, S. Korchak, S. Glöggler, J.-B. Hövener, E. Chekmenev, F. Schilling, S. Knecht and A. B. Schmidt, unpublished work.
66. K. MacCulloch, A. Browning, D. O. Guarín Bedoya, S. J. McBride, M. B. Abdulmojeed, C. Dedesma, B. M. Goodson, M. S. Rosen, E. Y. Chekmenev, Y.-F. Yen, P. TomHon and T. Theis, *J. Magn. Reson. Open*, 2023, **16-17**, 100129.
67. S. Petersen, L. Nagel, P. Groß, R. Willing, L. Heß, J. Mitschke, N. Klemm, J. Treiber, C. Müller, S. Knecht, I. Schwartz, M. Weigt, M. Bock, D. Von Elverfeldt, M. Zaitsev, E. Chekmenev, J.-B. Hövener, A. Martins, F. Schilling, T. Reinheckel and A. Schmidt, unpublished work.
68. E. Terreno, D. D. Castelli and S. Aime, in *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, 2013, DOI: <https://doi.org/10.1002/9781118503652.ch9>, pp. 387-425.
69. V. I. Bakhmutov, *Practical NMR Relaxation for Chemists*, John Wiley & Sons Ltd., 2004.

70. I. M. Ismail, S. J. S. Kerrison and P. J. Sadler, *Polyhedron*, 1982, **1**, 57-59.
71. K. R. Keshari and D. M. Wilson, *Chem. Soc. Rev.*, 2014, **43**, 1627-1659.
72. B. Plainchont, P. Berruyer, J.-N. Dumez, S. Jannin and P. Giraudeau, *Anal. Chem.*, 2018, **90**, 3639-3650.
73. T. J. Reddy, T. Iwama, H. J. Halpern and V. H. Rawal, *J Org Chem*, 2002, **67**, 4635-4639.
74. S. Jannin, A. Comment, F. Kurdzesau, J. A. Konter, P. Hautle, B. van den Brandt and J. J. van der Klink, *J Chem Phys*, 2008, **128**, 241102.
75. M. S. Albert, G. D. Cates, B. Driehuys, W. Happer, B. Saam, C. S. Springer and A. Wishnia, *Nature*, 1994, **370**, 199-201.
76. R. A. Nievelstein, H. M. Quarles van Ufford, T. C. Kwee, M. B. Bierings, I. Ludwig, F. J. Beek, J. M. de Klerk, W. P. Mali, P. W. de Bruin and J. Geleijns, *Eur Radiol*, 2012, **22**, 1946-1954.
77. J. Jokisaari, in *Encyclopedia of Spectroscopy and Spectrometry (Third Edition)*, eds. J. C. Lindon, G. E. Tranter and D. W. Koppenaal, Academic Press, Oxford, 2017, DOI: <https://doi.org/10.1016/B978-0-12-409547-2.05191-X>, pp. 260-273.
78. I. Fan, S. Knappe-Grüneberg, J. Voigt, W. Kilian, M. Burghoff, D. Stollfuss, A. Schnabel, G. Wübbeler, O. Bodner, C. Elster, F. Seifert and L. Trahms, *Journal of Physics: Conference Series*, 2016, **723**, 012045.
79. B. D. Jordan and E. L. Wright, *AANA Journal*, 2010, **78**, 387-392.
80. K. Hecker, J. H. Baumert, N. Horn and R. Rossaint, *Minerva Anesthesiol*, 2004, **70**, 255-260.
81. R. H. Crabtree, *The Organometallic Chemistry of the Transition Metals*, John Wiley & Sons, 7th edn., 2019.
82. M. Álvarez, M. Besora, F. Molina, F. Maseras, T. R. Belderrain and P. J. Pérez, *J. Am. Chem. Soc.*, 2021, **143**, 4837-4843.
83. B. T. Boyle, N. W. Dow, C. B. Kelly, M. C. Bryan and D. W. C. MacMillan, *Nature*, 2024, **631**, 789-795.
84. E. O. Fischer and A. Maasböl, *Angew. Chem.*, 1964, **3**, 580-581.
85. A. Feliciano, J. L. Vázquez, L. J. Benítez-Puebla, I. Velazco-Cabral, D. Cruz Cruz, F. Delgado and M. A. Vázquez, *Eur. J. Chem*, 2021, **27**, 8233-8251.
86. W. D. Wulff, W. E. Bauta, R. W. Kaesler, P. J. Lankford, R. A. Miller, C. K. Murray and D. C. Yang, *J. Am. Chem. Soc.*, 1990, **112**, 3642-3659.
87. J. Barluenga and E. Aguilar, in *Adv. Organomet. Chem.*, ed. P. J. Pérez, Academic Press, 2017, vol. 67, pp. 1-150.
88. Y. Chauvin, *Angew. Chem. Int. Ed.*, 2006, **45**, 3740-3747.
89. R. R. Schrock, *J. Am. Chem. Soc.*, 1974, **96**, 6796-6797.
90. S. M. Rocklage, J. D. Fellmann, G. A. Rupprecht, L. W. Messerle and R. R. Schrock, *J. Am. Chem. Soc.*, 1981, **103**, 1440-1447.
91. C. D. Wood, S. J. McLain and R. R. Schrock, *J. Am. Chem. Soc.*, 1979, **101**, 3210-3222.
92. J. Heppekausen and A. Fürstner, *Angew. Chem. Int. Ed.*, 2011, **50**, 7829-7832.
93. E. S. Sattely, S. J. Meek, S. J. Malcolmson, R. R. Schrock and A. H. Hoveyda, *J. Am. Chem. Soc.*, 2009, **131**, 943-953.
94. X. Shen, T. T. Nguyen, M. J. Koh, D. Xu, A. W. Speed, R. R. Schrock and A. H. Hoveyda, *Nature*, 2017, **541**, 380-385.
95. H. W. Wanzlick and H. J. Schönherr, *Angew. Chem.*, 1968, **80**, 154-154.
96. K. Öfele, *J. Organomet. Chem.*, 1968, **12**, P42-P43.
97. A. J. Arduengo, III, R. L. Harlow and M. Kline, *J. Am. Chem. Soc.*, 1991, **113**, 361-363.
98. W. A. Herrmann, M. Elison, J. Fischer, C. Köcher and G. R. J. Artus, *Angew. Chem. Int. Ed.*, 1995, **34**, 2371-2374.

99. S. Diez-Gonzalez, N. Marion and S. P. Nolan, *Chem. Rev.*, 2009, **109**, 3612-3676.
100. K. Oisaki, Q. Li, H. Furukawa, A. U. Czaja and O. M. Yaghi, *J. Am. Chem. Soc.*, 2010, **132**, 9262-9264.
101. C. A. Tolman, *Chem. Rev.*, 1977, **77**, 313-348.
102. J. Huang, H.-J. Schanz, E. D. Stevens and S. P. Nolan, *Organometallics*, 1999, **18**, 5375-5380.
103. A. C. Hillier, W. J. Sommer, B. S. Yong, J. L. Petersen, L. Cavallo and S. P. Nolan, *Organometallics*, 2003, **22**, 4322-4326.
104. A. Poater, B. Cosenza, A. Correa, S. Giudice, F. Ragone, V. Scarano and L. Cavallo, *Eur. J. Inorg. Chem.*, 2009, **2009**, 1759-1766.
105. H. Clavier and S. P. Nolan, *Chem. Commun.*, 2010, **46**, 841.
106. C. A. Tolman, *J. Am. Chem. Soc.*, 1970, **92**, 2953-2956.
107. C. Köcher and W. A. Herrmann, *J. Organomet. Chem.*, 1997, **532**, 261-265.
108. X. Hu, I. Castro-Rodriguez, K. Olsen and K. Meyer, *Organometallics*, 2004, **23**, 755-764.
109. H. V. Huynh, Y. Han, R. Jothibasu and J. A. Yang, *Organometallics*, 2009, **28**, 5395-5404.
110. F. Krauth, H.-M. Dahse, H.-H. Rüttinger and P. Froberg, *Biorg. Med. Chem.*, 2010, **18**, 1816-1821.
111. Z. el-Gendy, J. M. Morsy, H. A. Allimony, W. R. Ali and R. M. Abdel-Rahman, *Pharmazie*, 2001, **56**, 376-383.
112. V. L. Rusinov, I. N. Egorov, O. N. Chupakhin, E. F. Belanov, N. I. Bormotov and O. A. Serova, *Pharm. Chem. J.*, 2012, **45**, 655-659.
113. H. Wang, L. Hu, X. Chang, Y. Hu, Y. Zhang, P. Zhou and X. Cui, *Food Chem.*, 2023, **399**, 134010.
114. A. L. Forgacs, M. L. D'Souza, I. T. Huhtaniemi, N. A. Rahman and T. R. Zacharewski, *Toxicol. Sci.*, 2013, **134**, 155-167.
115. K. M. Ellis, L. Lucantoni, M. Chavchich, M. Abraham, A. De Paoli, M. R. Luth, A. M. Zeeman, M. J. Delves, F. S. Terán, U. Straschil, J. Baum, C. H. M. Kocken, S. A. Ralph, E. A. Winzeler, V. M. Avery, M. D. Edstein, J. B. Baell and D. J. Creek, *Antimicrob. Agents Chemother.*, 2021, **65**, e0031121.
116. E. V. Shchegol'Kov, O. G. Khudina, A. E. Ivanova, Y. V. Burgart, E. V. Sadchikova, M. A. Kravchenko and V. I. Saloutin, *Pharm. Chem. J.*, 2014, **48**, 383-386.
117. E. D. Anderson and D. L. Boger, *J. Am. Chem. Soc.*, 2011, **133**, 12285-12292.
118. S. J. Siegl, R. Dzajak, A. Vázquez, R. Pohl and M. Vrabel, *Chem. Sci.*, 2017, **8**, 3593-3598.
119. L. A. Meirelles and D. K. Newman, *Mol. Microbiol.*, 2022, **117**, 1384-1404.
120. J. Lee, J. Park, S. Kim, I. Park and Y. S. Seo, *Mol. Plant Pathol.*, 2016, **17**, 65-76.
121. E. Sola, V. I. Bakmutov, F. Torres, A. Elduque, J. A. López, F. J. Lahoz, H. Werner and L. A. Oro, *Organometallics*, 1998, **17**, 683-696.
122. B. J. Tickner, J. S. Lewis, R. O. John, A. C. Whitwood and S. B. Duckett, *Dalton Trans.*, 2019, **48**, 15198-15206.
123. D. A. Barskiy, S. Knecht, A. V. Yurkovskaya and K. L. Ivanov, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2019, **114-115**, 33-70.
124. E. Belorizky and P. H. Fries, *J. Chem. Phys.*, 2021, **155**, 184108.
125. B. J. Tickner, Y. Borozdina, S. B. Duckett and G. Angelovski, *Dalton Trans.*, 2021, **50**, 2448-2461.
126. K. Takai, *Microbes Environ.*, 2019, **34**, 223-225.

127. R. Cammack, C. L. Joannou, X.-Y. Cui, C. Torres Martinez, S. R. Maraj and M. N. Hughes, *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1999, **1411**, 475-488.
128. E. Weitzberg, M. Hezel, J. O. Lundberg and D. S. Warner, *Anesthesiology*, 2010, **113**, 1460-1475.
129. J. S. W. Chui, W. T. Poon, K. C. Chan, A. Y. W. Chan and T. A. Buckley, *Anaesthesia*, 2005, **60**, 496-500.
130. H. H. Hodgson, *Chem. Rev.*, 1947, **40**, 251-277.
131. S. Mukhopadhyay and S. Batra, *Eur. J. Org. Chem.*, 2019, **2019**, 6424-6451.
132. A. Takeuchi, K. Sato, K. Sone, S. Yamada and K. Yamasaki, *Inorg. Chim. Acta*, 1967, **1**, 399-402.
133. D. M. L. Goodgame and M. A. Hitchman, *Inorg. Chem.*, 1964, **3**, 1389-1394.
134. I. Nakamura, Y. Funasako and T. Mochida, *Cryst. Growth Des.*, 2020, **20**, 8047-8052.
135. T. G. Appleton, K. J. Barnham, J. R. Hall and M. T. Mathieson, *Inorg. Chem.*, 1991, **30**, 2751-2756.
136. M. A. Hitchman and G. L. Rowbottom, *Coord. Chem. Rev.*, 1982, **42**, 55-132.
137. M. J. Cowley, R. W. Adams, K. D. Atkinson, M. C. R. Cockett, S. B. Duckett, G. G. R. Green, J. A. B. Lohman, R. Kerssebaum, D. Kilgour and R. E. Mewis, *J. Am. Chem. Soc.*, 2011, **133**, 6134-6137.
138. G. A. Morris, in *eMagRes*, 2009, DOI: <https://doi.org/10.1002/9780470034590.emrstm0119.pub2>.
139. V. Gilard, S. Trefi, S. Balayssac, M. A. Delsuc, T. Gostan, M. Malet-Martino, R. Martino, Y. Prigent and F. Taulelle, in *NMR Spectroscopy in Pharmaceutical Analysis*, eds. U. Holzgrabe, I. Wawer and B. Diehl, Elsevier, Amsterdam, 2008, DOI: <https://doi.org/10.1016/B978-0-444-53173-5.00011-1>, pp. 269-289.
140. S. Indris, R. Heinzmann, M. Schulz and A. Hofmann, *J. Electrochem. Soc.*, 2014, **161**, A2036.
141. J. Lee, S. H. Park, S. Cavagnero and J. H. Lee, *Anal. Chem.*, 2020, **92**, 5073-5081.
142. J. Hrabec, G. Kaur and D. N. Guilfoyle, *J. Med. Phys.*, 2007, **32**, 34-42.
143. E. O. Stejskal and J. E. Tanner, *J. Chem. Phys.*, 1965, **42**, 288-292.
144. J. E. Tanner and E. O. Stejskal, *J. Chem. Phys.*, 1968, **49**, 1768-1777.
145. T. R. Graham, Y. Wei, E. D. Walter, E. T. Nienhuis, J. Chun, G. K. Schenter, K. M. Rosso, C. I. Pearce and A. E. Clark, *Chem. Commun.*, 2023, **59**, 14407-14410.
146. L. S. Lloyd, A. Asghar, M. J. Burns, A. Charlton, S. Coombes, M. J. Cowley, G. J. Dear, S. B. Duckett, G. R. Genov, G. G. R. Green, L. A. R. Highton, A. J. J. Hooper, M. Khan, I. G. Khazal, R. J. Lewis, R. E. Mewis, A. D. Roberts and A. J. Ruddlesden, *Catal. Sci. Technol.*, 2014, **4**, 3544-3554.
147. M. C. Bagley, A. Alnomisy and H. I. Sharhan, *Synlett*, 2016, **27**, 2467-2472.
148. R. A. Kelly Iii, H. Clavier, S. Giudice, N. M. Scott, E. D. Stevens, J. Bordner, I. Samardjiev, C. D. Hoff, L. Cavallo and S. P. Nolan, *Organometallics*, 2008, **27**, 202-210.
149. P. J. Rayner, M. J. Burns, A. M. Olaru, P. Norcott, M. Fekete, G. G. R. Green, L. A. R. Highton, R. E. Mewis and S. B. Duckett, *PNAS*, 2017, **114**, E3188-E3194.
150. P. Norcott, M. J. Burns, P. J. Rayner, R. E. Mewis and S. B. Duckett, *Magn. Reson. Chem.*, 2018, **56**, 663-671.
151. P. Norcott, P. J. Rayner, G. G. R. Green and S. B. Duckett, *Eur. J. Chem*, 2017, **23**, 16990-16997.

152. A. Duchowny, J. Denninger, L. Lohmann, T. Theis, S. Lehmkuhl and A. Adams, *Int. J. Mol. Sci.*, 2023, **24**, 2465.
153. P. J. Rayner and S. B. Duckett, *Angew. Chem. Int. Ed.*, 2018, **57**, 6742-6753.
154. S. Knecht, S. Hadjiali, D. A. Barskiy, A. Pines, G. Sauer, A. S. Kiryutin, K. L. Ivanov, A. V. Yurkovskaya and G. Buntkowsky, *J. Phys. Chem. C*, 2019, **123**, 16288-16293.
155. W. Makulski, M. Wilczek and K. Jackowski, *Phys. Chem. Chem. Phys.*, 2018, **20**, 22468-22476.
156. T. Sandmeyer, *Ber. Dtsch. Chem. Ges.*, 1884, **17**, 1633-1635.
157. G. A. Olah, R. Herges, K. Laali and G. A. Segal, *J. Am. Chem. Soc.*, 1986, **108**, 2054-2057.
158. J. H. Enemark and R. D. Feltham, *Coord. Chem. Rev.*, 1974, **13**, 339-406.
159. S. Sun, J. Choe and J. Cho, *Chem. Sci.*, 2024, DOI: 10.1039/D4SC06820C.
160. M. A. Alvarez, M. E. García, D. García-Vivó, A. M. Guerra and M. A. Ruiz, *J. Organomet. Chem.*, 2024, **1022**, 123375.
161. C. D. Ritchie and D. J. Wright, *J. Am. Chem. Soc.*, 1971, **93**, 2429-2432.
162. R. N. Butler, A. Fox, S. Collier and L. A. Burke, *Journal of the Chemical Society, Perkin Transactions 2*, 1998, 2243-2248.
163. S. M. Joshi, A. de Cózar, V. Gómez-Vallejo, J. Koziorowski, J. Llop and F. P. Cossío, *Chem. Commun.*, 2015, **51**, 8954-8957.
164. R. Akhtar, A. F. Zahoor, N. Rasool, M. Ahmad and K. G. Ali, *Mol Divers*, 2022, **26**, 1837-1873.
165. M. L. Chan and T. C. Chan, *J. Phys. Chem.*, 1995, **99**, 5765-5768.
166. L. Guduff, P. Berthault, C. van Heijenoort, J.-N. Dumez and G. Huber, *ChemPhysChem*, 2019, **20**, 392-398.
167. G. da Silva, E. M. Kennedy and B. Z. Dlugogorski, *J. Phys. Chem. A*, 2006, **110**, 11371-11376.
168. E. A. Braude and F. C. Nachod, *Determination of organic structures by physical methods*, Academic Press, New York, 1955.
169. A. Gamliel, S. Uppala, G. Sapir, T. Harris, A. Nardi-Schreiber, D. Shaul, J. Sosna, J. M. Gomori and R. Katz-Brull, *J. Magn. Reson.*, 2019, **299**, 188-195.
170. V. S. Bebarta, M. Brittain, A. Chan, N. Garrett, D. Yoon, T. Burney, D. Mukai, M. Babin, R. B. Pilz, S. B. Mahon, M. Brenner and G. R. Boss, *Ann. Emerg. Med.*, 2017, **69**, 718-725.e714.
171. Y. Yang, Z. Huang and L.-L. Li, *Nanoscale*, 2021, **13**, 444-459.
172. G. F. Lasker, C. J. Matt, A. M. Badejo, Jr., D. B. Casey, J. S. Dhaliwal, S. N. Murthy and P. J. Kadowitz, *Can J Physiol Pharmacol*, 2010, **88**, 770-776.
173. Z. Huang, J. Fu and Y. Zhang, *J Med Chem*, 2017, **60**, 7617-7635.
174. L. J. Ignarro, C. Napoli and J. Loscalzo, *Circ Res*, 2002, **90**, 21-28.
175. D. A. Wink and J. B. Mitchell, *Free Radical Biol. Med.*, 1998, **25**, 434-456.
176. P. Nikolaou, B. M. Goodson and E. Y. Chekmenev, *Chemistry*, 2015, **21**, 3156-3166.
177. R. C. S. Mark A. Brown, *MRI: Basic Principles and Applications*, John Wiley & Sons, 2011.
178. H. S. Thomsen, in *Contrast Media: Safety Issues and ESUR Guidelines*, ed. H. S. Thomsen, Springer Berlin Heidelberg, Berlin, Heidelberg, 2006, DOI: 10.1007/3-540-27742-0_17, pp. 115-120.
179. E. Cavallari, C. Carrera, S. Aime and F. Reineri, *ChemPhysChem*, 2019, **20**, 318-325.
180. S. J. Nelson, J. Kurhanewicz, D. B. Vigneron, P. E. Z. Larson, A. L. Harzstark, M. Ferrone, M. Van Criekinge, J. W. Chang, R. Bok, I. Park, G. Reed, L. Carvajal, E. J. Small, P. Munster, V. K. Weinberg, J. H. Ardenkjaer-Larsen, A.

- P. Chen, R. E. Hurd, L.-I. Odegardstuen, F. J. Robb, J. Tropp and J. A. Murray, *Sci. Transl. Med.*, 2013, **5**, 198ra108-198ra191.
181. T. H. Witney, M. I. Kettunen, D. E. Hu, F. A. Gallagher, S. E. Bohndiek, R. Napolitano and K. M. Brindle, *Br. J. Cancer*, 2010, **103**, 1400-1406.
 182. G. Pileio, M. Carravetta, E. Hughes and M. H. Levitt, *J. Am. Chem. Soc.*, 2008, **130**, 12582-12583.
 183. M. H. Levitt, *J. Magn. Reson.*, 2019, **306**, 69-74.
 184. S. Basu, S. Hess, P.-E. Nielsen Braad, B. B. Olsen, S. Inglev and P. F. Høilund-Carlsen, *PET Clin.*, 2014, **9**, 355-370.
 185. J. W. Fletcher, B. Djulbegovic, H. P. Soares, B. A. Siegel, V. J. Lowe, G. H. Lyman, R. E. Coleman, R. Wahl, J. C. Paschold, N. Avril, L. H. Einhorn, W. W. Suh, D. Samson, D. Delbeke, M. Gorman and A. F. Shields, *J. Nucl. Med.*, 2008, **49**, 480.
 186. L. L. Walkup and J. C. Woods, *NMR Biomed.*, 2014, **27**, 1429-1438.
 187. J. P. Mugler and T. A. Altes, *J. Magn. Reson. Imaging*, 2013, **37**, 313-331.
 188. N. J. Stewart, L. J. Smith, H. F. Chan, J. A. Eaden, S. Rajaram, A. J. Swift, N. D. Weatherley, A. Biancardi, G. J. Collier, D. Hughes, G. Klafkowski, C. S. Johns, N. West, K. Ugonna, S. M. Bianchi, R. Lawson, I. Sabroe, H. Marshall and J. M. Wild, *Br J Radiol*, 2022, **95**, 20210207.
 189. A. Manoharan, P. J. Rayner, W. Iali, M. J. Burns, V. H. Perry and S. B. Duckett, *ChemMedChem*, 2018, **13**, 352-359.
 190. A. G. Redfield, *J Biomol NMR*, 2012, **52**, 159-177.
 191. J. Eills, J. Alonso-Valdesueiro, D. E. Salazar Marcano, J. Ferreira da Silva, S. Alom, G. J. Rees, J. V. Hanna, M. Carravetta and M. H. Levitt, *ChemPhysChem*, 2018, **19**, 40-44.
 192. I. Katz, A. Schmidt, I. Ben-Shir, M. Javitt, K. Kouřil, A. Capozzi, B. Meier, A. Lang, B. Pokroy and A. Blank, *Sci Adv*, 2024, **10**, eado2483.
 193. S. Knecht, J. W. Blanchard, D. Barskiy, E. Cavallari, L. Dagys, E. Van Dyke, M. Tsukanov, B. Bliemel, K. Münnemann, S. Aime, F. Reineri, M. H. Levitt, G. Buntkowsky, A. Pines, P. Blümmler, D. Budker and J. Eills, *PNAS*, 2021, **118**.
 194. A. M. A. Dias, C. M. B. Gonçalves, J. L. Legido, J. A. P. Coutinho and I. M. Marrucho, *Fluid Phase Equilib.*, 2005, **238**, 7-12.
 195. M. H. Abraham, W. E. Acree and E. Matteoli, *Fluid Phase Equilib.*, 2016, **421**, 59-66.
 196. O. Semenova, P. M. Richardson, A. J. Parrott, A. Nordon, M. E. Halse and S. B. Duckett, *Anal. Chem.*, 2019, **91**, 6695-6701.
 197. J. Bae, Z. Zhou, T. Theis, W. S. Warren and Q. Wang, *Sci. Adv.*, 2018, **4**, eaar2978.
 198. S. Tredici, R. Seetharamaiah, D. S. Foley and R. B. Hirschl, in *Manual of Neonatal Respiratory Care (Second Edition)*, eds. S. M. Donn and S. K. Sinha, Mosby, Philadelphia, 2006, DOI: <https://doi.org/10.1016/B978-032303176-9.50070-2>, pp. 437-441.
 199. M. L. Costantino, *Int J Artif Organs*, 1996, **19**, 284-290.
 200. Q. A. Tawfic and R. Kausalya, *Oman Med J*, 2011, **26**, 4-9.
 201. L. C. Clark and F. Gollan, *Science*, 1966, **152**, 1755-1756.
 202. L. Wu, F. Liu, S. Liu, X. Xu, Z. Liu and X. Sun, *Int J Nanomedicine*, 2020, **15**, 7377-7395.
 203. A. H. J. Staal, K. Becker, O. Tagit, N. Koen van Riessen, O. Koshkina, A. Veltien, P. Bouvain, K. R. G. Cortenbach, T. Scheenen, U. Flögel, S. Temme and M. Srinivas, *Biomaterials*, 2020, **261**, 120307.
 204. A. M. A. Dias, M. Freire, J. A. P. Coutinho and I. M. Marrucho, *Fluid Phase Equilib.*, 2004, **222-223**, 325-330.

205. J. G. Riess, *Artif. Cells, Blood Substitutes, Biotechnol.*, 2005, **33**, 47-63.
206. J. Jägers, A. Wrobeln and K. B. Ferenz, *Pflugers Arch*, 2021, **473**, 139-150.
207. Purwanto, R. M. Deshpande, R. V. Chaudhari and H. Delmas, *J. Chem. Eng. Data*, 1996, **41**, 1414-1417.
208. R. Sander, *Atmos. Chem. Phys.*, 2023, **23**, 10901-12440.
209. E. Brunner, *J. Chem. Eng. Data*, 1985, **30**, 269-273.
210. J. V. H. d'Angelo and A. Z. Francesconi, *J. Chem. Eng. Data*, 2001, **46**, 671-674.
211. T.-K.-H. Trinh, J.-C. de Hemptinne, R. Lugo, N. Ferrando and J.-P. Passarello, *J. Chem. Eng. Data*, 2016, **61**, 19-34.
212. K. Shirono, T. Morimatsu and F. Takemura, *J. Chem. Eng. Data*, 2008, **53**, 1867-1871.
213. J.-Z. Yin and C.-S. Tan, *Fluid Phase Equilib.*, 2006, **242**, 111-117.
214. A. Besaratinia and S. Tommasi, *EClinicalMedicine*, 2019, **17**, 100208.
215. USA Pat., US1775947A, 1927.
216. K. Nicolay, G. Strijkers and H. Grüll, in *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, 2013, DOI: <https://doi.org/10.1002/9781118503652.ch11>, pp. 449-487.
217. P. Marques, L. Piqueras and M.-J. Sanz, *Respir. Res.*, 2021, **22**, 151.
218. B. J. Tickner, S. Komulainen, S. Palosaari, J. Heikkinen, P. Lehenkari, V. V. Zhivonitko and V.-V. Telkki, *RSC Adv.*, 2022, **12**, 1479-1485.
219. J. Etteedgui, K. Yamamoto, B. Blackman, N. Koyasu, N. Raju, O. Vasalatiy, H. Merkle, E. Y. Chekmenev, B. M. Goodson, M. C. Krishna and R. E. Swenson, *Angew. Chem. Int. Ed.*, **63**, e202407349.
220. B. Hoff, *Synthesis*, 2018, **50**, 2824-2852.
221. K. Roy, S. C. Debnath, A. Pongwisuthiruchte and P. Potiyaraj, *ACS Omega*, 2021, **6**, 9975-9981.
222. J. K. Peter, R. Singh, A. K. Yadav, R. Kothari and P. K. Mehta, *J. Hazard. Mater. Adv.*, 2024, **13**, 100389.
223. L. C. Seeberger and R. A. Hauser, *Expert Rev Neurother*, 2009, **9**, 929-940.
224. A. C. Pasqualotto, K. O. Thiele and L. Z. Goldani, *Curr Opin Investig Drugs*, 2010, **11**, 165-174.
225. A. Schmitt-Hoffmann, B. Roos, J. Spickermann, M. Heep, E. Peterfaí, D. J. Edwards and K. Stoeckel, *Antimicrob. Agents Chemother.*, 2009, **53**, 4885-4890.
226. M. Milani, G. Jha and D. A. Potter, *Clin Med Ther*, 2009, **1**, 141-156.
227. A. Dispas, P. Y. Sacré, E. Ziemons and P. Hubert, *J Pharm Biomed Anal*, 2022, **221**, 115071.
228. P. E. Lønning, J. Geisler and M. Dowsett, *Breast Cancer Res Treat*, 1998, **49 Suppl 1**, S53-57; discussion S73-57.
229. F. F. Fleming, L. Yao, P. C. Ravikumar, L. Funk and B. C. Shook, *J. Med. Chem.*, 2010, **53**, 7902-7917.
230. M. Kalidas and P. Brown, *Clin. Breast Cancer*, 2005, **6**, 27-37.
231. R. Savka and H. Plenio, *Dalton Trans.*, 2015, **44**, 891-893.
232. O. Torres, M. Martín and E. Sola, *Organometallics*, 2009, **28**, 863-870.
233. P. J. Rayner, B. J. Tickner, W. Iali, M. Fekete, A. D. Robinson and S. B. Duckett, *Chem. Sci.*, 2019, **10**, 7709-7717.
234. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, *J. Appl. Crystallogr.*, 2009, **42**, 339-341.
235. G. M. Sheldrick, *Acta Crystallogr., Sect. A: Found. Adv.*, 2015, **71**, 3-8.
236. G. M. Sheldrick, *Acta Crystallogr., Sect. C: Struct. Chem.*, 2015, **71**, 3-8.
237. A. Jerschow and N. Müller, *J. Magn. Reson.*, 1997, **125**, 372-375.

238. M. D. Pelta, G. A. Morris, M. J. Stchedroff and S. J. Hammond, *Magn. Reson. Chem.*, 2002, **40**, S147-S152.
239. D. C. Harris, *J. Chem. Educ.*, 1998, **75**, 119.

List of Compounds

A-<i>h</i>₆	dimethylsulfoxide
A-<i>d</i>₆	dimethylsulfoxide- <i>d</i> ₆
B	diphenylsulfoxide
C-<i>h</i>₃	acetonitrile
C-<i>d</i>₃	acetonitrile- <i>d</i> ₃
D-<i>h</i>₅	pyridine
D-<i>d</i>₅	pyridine- <i>d</i> ₅
¹⁵N-D	¹⁵ N- pyridine
E	2,6-lutidine
F	methyl-4,6- <i>d</i> ₂ -nicotinate
G	dimethylaminopyridine
H-<i>d</i>₇	benzylamine- <i>d</i> ₇
I	aniline
J	methylperfluorobutyl ether
K	perfluorodecalin
L	perfluorononane
M	perfluorohexane
N	1H,1H,2H-perfluoro-1-decene
O	perfluoro(methylcyclohexane)
P	perfluoroheptane
Q	perfluorooctyl
R	perfluorooctane
T	S-nicotine
U	1,4-dimethyltetrazine
V	1,2,4-triazine-3-methyl
W	1,6-Dimethylpyrimido[5,4- <i>e</i>][1,2,4]triazine-5,7(1H,6H)-dione
X	1,2,4-triazine-3-methylthio
Y	NaNO ₂
Z	(1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethanol
XZ	((6aS,7S,7aR)-3-(methylthio)-6,6a,7,7a,8,9-hexahydro-5H-cyclopropa[5,6]cycloocta[1,2- <i>c</i>]pyridin-7-yl)methanol
1	[IrCl(COD)(IMes)]
2	[Ir(COD)(IMes)(X)]Cl
3	[Ir(H) ₂ (η ² -η ² -COD) ₂ (IMes)(X)]Cl
4a	[Ir(H) ₂ (IMes)(X ₁) ₃]Cl
4b	[Ir(H) ₂ (IMes)(X ₁) ₂ (X ₂)]Cl
5	[IrCl(H) ₂ (η ² -η ² -COD)]
6	[IrCl(H) ₂ (IMes)(X ₁) ₂]
7	[Ir ₂ Cl ₂ (H) ₂ (IMes) ₂ (X) ₂]
8	[Ir(COD)(IMes)(Y)]
9	[Ir(H) ₂ (COD)(IMes)(Y)]
10	[Ir(H) ₂ (COD)(D)(IMes)]
11	Na[Ir(H) ₂ (IMes)(D)(Y) ₂]

12	$[\text{Ir}(\text{H})_2(\mathbf{D})_2(\text{IMes})(\mathbf{Y})]$
11A-<i>d</i>₆	$\text{Na}[\text{Ir}(\text{H})_2(\mathbf{A-d}_6)(\text{IMes})(\mathbf{Y})_2]$
13	$\text{Na}[\text{Ir}(\text{H})_2(\text{IMes})(\mathbf{A-d}_6)(\mathbf{Y})_2]$
14	$[\text{Ir}(\text{H})_2(\mathbf{G})_3(\text{IMes})]\text{Cl}$
17	$[\text{Ir}(\text{H})_2(\mathbf{C})_3(\text{IMes})]\text{Cl}$
18	$[\text{Ir}(\text{H})_2(\mathbf{C})(\text{IMes})(\mathbf{V})_2]\text{Cl}$
19	$[\text{Ir}(\text{H})_2(\text{IMes})(\mathbf{V})_3]\text{Cl}$
20	$[\text{Ir}(\text{H})_2(\mathbf{C})_2(\mathbf{IPr}^{\text{Cl}})(\mathbf{V})]\text{Cl}$
21	$[\text{Ir}(\text{H})_2(\mathbf{C})_3(\mathbf{IPr}^{\text{Cl}})]\text{Cl}$
22	$[\text{Ir}(\text{H})_2(\mathbf{IPr}^{\text{Cl}})(\mathbf{V})_3]\text{Cl}$

List of Abbreviations

ALTADENA	Adiabatic Longitudinal Transport After Dissociation Engenders Nuclear Alignment
BBI	BroadBand Inverse
BBO	BroadBand Observe
CHyM	York Centre for Hyperpolarisation in Magnetic Resonance Imaging
CIDNP	Chemically Induced Dynamic Nuclear Polarisation
COA	CycloOctAne
COD	CycloOctaDiene
COSY	CORrelation SpectroscopyY
CSA	Chemical Shift Anisotropy
DNP	Dynamic nuclear polarisation
DONESHOT	DOSY ONESHOT
DOSY	Diffusion Spectroscopy
EPR	Electron <i>Paramagnetic</i> Resonance
EPSI	Echo Planar Spectroscopic Imaging
EXSY	EXchange SpectroscopyY
FA	Flip Angle
FID	Free Induction Decay
FOV	Field Of View
GCMS	Gas chromatography/Mass spectrometry
GNAT	General NMR Analysis Toolbox
HMBC	Heteronuclear multiple-bond coherence
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High-Performance Liquid Chromatography
HSQC	Heteronuclear Single-Quantum Correlation
INEPT	Insensitive Nuclei Enhanced by Polarisation Transfer
LAC	Level Anti-Crossing
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NHC	<i>N</i> -Heterocyclic Carbene
NMR	Nuclear Magnetic Resonance
nOe	nuclear Overhauser effect
NOESY	Nuclear Overhauser Effect SpectroscopyY
NOS	nitric oxide synthase
<i>o</i> -H ₂	<i>ortho</i> hydrogen
<i>o</i> -N ₂	<i>ortho</i> nitrogen
PASADENA	<i>Para</i> hydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment
PET	Positron Emission Tomography
<i>p</i> -H ₂	<i>para</i> hydrogen
PHIP	<i>Para</i> Hydrogen Induced Polarisation
<i>p</i> -N ₂	<i>para</i> nitrogen
PTF	Polarisation Transfer Field
r.f.	radiofrequency

SABRE	Signal Amplification By Reversible Exchange
SABRE-SHEATH	SABRE in SHield Enables Alignment Transfer to Heteronuclei
SAMVCA	SAlerno Molecular Buried Volume Calculation
SEOP	Spin Exchange Optical Pumping
SPEN-DOSY	Spatially ENcoded DOSY
TE	Echo Time
TEP	Tolman Electronic Parameter
TOCSY	TOtal Correlation SpectroscopY
TR	Relaxation Time
TTL	Transistor-Transistor Logic

Appendix

A.1 'T₁HyperP' Pulse sequence utilised for reaction monitoring studies and hyperpolarised *T*₁ measurements.

"d11=30m"

"acqt0=-p1*2/3.1416"

"DELTA=d20-aq-30m"

"ds=0"

"ns=1"

1 ze

autosuspend

d30

2 vp ph1

go=2 ph31

d11 wr #0 if #0 ivp

DELTA ; relaxation delay (to allow for more time between FIDs)

lo to 2 times td1

exit

ph1=0

ph31=0

;pl1 : f1 channel - power level for pulse (default)

;p1 : f1 channel - high power pulse (90 degree, not used)

;d11: delay for disk I/O [30 msec]

;d20: delay between FIDs (d20>aq) [1-2 sec]

;d30: delay for sample to settle: optional [1-2s or 0sec]

;ns: 1

;ds: 0

;td1: number of experiments = number of delays in vp-list

;define VPLIST

A.2 Crystallography data for Figure 3.13

Identification code sbd23007

Empirical formula C 42 H 48 Cl 7.33 Ir 2 N 6.67 O 2.67

Formula weight 1333.20

Temperature/K 110.00(10)

Crystal system orthorhombic

Space group Pbca

a/Å 16.22086(12)

b/Å 16.71712(16)

c/Å 35.3808(3)

$\alpha/^\circ$ 90

$\beta/^\circ$ 90

$\gamma/^\circ$ 90

Volume/Å³ 9594.08(14)

Z 8

ρ calc g/cm³ 1.846

μ /mm 1 14.701

F(000) 5173.2

Crystal size/mm 3 0.19 × 0.063 × 0.029

Radiation Cu K α (λ = 1.54184)

2 θ range for data collection/ $^\circ$ 7.394 to 134.156

Index ranges $-18 \leq h \leq 19$, $-18 \leq k \leq 19$, $-42 \leq l \leq 20$

Reflections collected 46564

Independent reflections 8563 [R int = 0.0361, R sigma = 0.0249]

Data/restraints/parameters 8563/12/567

Goodness-of-fit on F 2 1.166

Final R indexes [$I \geq 2\sigma(I)$] R 1 = 0.0645, wR 2 = 0.1343

Final R indexes [all data] R 1 = 0.0685, wR 2 = 0.1360

Largest diff. peak/hole / e Å⁻³ 3.67/-2.58

Table A.2.1 Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($\text{\AA}^2 \times 10^3$) for the structure presented in Figure 3.13. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij} tensor.

Atom	x	y	z	U(eq)
C1	3845(5)	2344(6)	2435(2)	27(2)
C2	2583(7)	2049(8)	2212(3)	42(3)
C3	2917(6)	2585(7)	1976(3)	38(2)
C4	3006(6)	1306(6)	2778(3)	32(2)
C5	2535(6)	1523(7)	3095(3)	33(2)
C6	2374(6)	939(7)	3361(3)	38(2)
C7	2651(7)	162(7)	3313(3)	37(2)
C8	3101(7)	-37(7)	2989(3)	41(3)
C9	3265(7)	536(7)	2713(3)	39(2)
C10	2217(7)	2369(8)	3147(3)	49(3)
C11	2458(8)	-480(8)	3604(3)	47(3)
C12	3709(8)	307(8)	2354(3)	54(3)
C13	4211(6)	3294(7)	1895(3)	36(2)
C14	4776(6)	2945(7)	1645(3)	33(2)
C15	5193(6)	3484(8)	1409(3)	44(3)
C16	5035(7)	4298(8)	1401(3)	48(3)
C17	4461(8)	4594(8)	1654(4)	58(4)
C18	4032(7)	4111(8)	1904(4)	48(3)
C19	4907(7)	2075(7)	1614(3)	41(3)
C20	5464(9)	4829(9)	1120(4)	69(4)
C21	3394(9)	4442(8)	2169(5)	77(5)
Cl1	5603(3)	3206(3)	2477.4(14)	50.6(14)
Cl2	5891.9(17)	2543(4)	3281.6(10)	100.3(18)
Cl3	4168(2)	3563(3)	3066.6(12)	85.0(14)
Cl4	5508.3(16)	1337.7(19)	2558.8(9)	51.0(7)
Ir1	4807.6(3)	2427.0(4)	2818.9(2)	44.60(16)
N1	3159(5)	1904(5)	2492(2)	30.9(18)
N2	3702(5)	2762(5)	2115(2)	31.1(18)
N3	4335(6)	1807(8)	3191(3)	65(4)
N4	5270(30)	3220(20)	2504(12)	50.6(14)
O1	4216(8)	1497(13)	3452(3)	167(9)
O2	5472(18)	3844(16)	2440(9)	50.6(14)
C22	3482(5)	2136(6)	5043(2)	23.7(19)
C23	4687(6)	2551(7)	5288(3)	35(2)
C24	4378(6)	1991(7)	5516(3)	37(2)
C25	4265(6)	3221(6)	4703(3)	31(2)

C26	4727(6)	2992(6)	4387(3)	31(2)
C27	4882(6)	3570(6)	4118(3)	34(2)
C28	4606(7)	4348(6)	4156(3)	40(3)
C29	4173(7)	4562(7)	4475(3)	41(3)
C30	4014(7)	4010(6)	4764(3)	36(2)
C31	5045(6)	2142(7)	4346(3)	37(2)
C32	4796(9)	4970(8)	3855(4)	54(3)
C33	3605(7)	4274(7)	5125(3)	44(3)
C34	3183(6)	1130(6)	5574(3)	28(2)
C35	2616(6)	1380(6)	5844(3)	33(2)
C36	2252(6)	810(7)	6072(3)	39(3)
C37	2456(7)	25(7)	6033(3)	37(2)
C38	3029(7)	-217(7)	5765(3)	37(2)
C39	3412(6)	329(6)	5532(3)	29(2)
C40	2424(7)	2262(7)	5891(3)	41(3)
C41	2075(9)	-624(8)	6286(4)	59(4)
C42	4067(7)	79(7)	5253(3)	40(3)
Cl5	1695(5)	1371(4)	5049(2)	37.0(14)
Cl6	1403.4(15)	1897(2)	4221.7(8)	58.6(9)
Cl7	1870.7(16)	3177.4(19)	4916.4(8)	47.9(7)
Cl8	3095.9(18)	861.2(18)	4428.8(8)	47.5(7)
Ir2	2510.5(3)	2035.8(3)	4669.2(2)	33.54(13)
N5	4130(5)	2648(5)	4996(2)	30.9(18)
N6	3646(5)	1734(5)	5367(2)	30.6(18)
N7	2968(5)	2666(6)	4294(2)	39(2)
N8	1810(30)	1510(30)	5012(13)	37.0(14)
O3	2971(10)	3093(9)	4066(4)	140(7)
O4	1257(12)	1357(11)	5182(5)	37.0(14)

Table A.2.2 Bond lengths for the structure presented in Figure 3.13.

Atom 1	Atom 2	Length/Å	Atom 1	Atom 2	Length/Å
C1	Ir1	2.074(9)	C22	Ir2	2.065(9)
C1	N1	1.348(12)	C22	N5	1.366(12)
C1	N2	1.352(12)	C22	N6	1.354(12)
C2	C3	1.339(15)	C23	C24	1.333(15)

C2	N1	1.382(13)	C23	N5	1.381(12)
C3	N2	1.396(12)	C24	N6	1.367(13)
C4	C5	1.404(14)	C25	C26	1.398(13)
C4	C9	1.374(15)	C25	C30	1.398(15)
C4	N1	1.444(12)	C25	N5	1.430(13)
C5	C6	1.382(15)	C26	C27	1.380(14)
C5	C10	1.516(16)	C26	C31	1.520(14)
C6	C7	1.386(16)	C27	C28	1.382(15)
C7	C8	1.401(15)	C28	C29	1.377(15)
C7	C11	1.519(14)	C28	C32	1.521(14)
C8	C9	1.391(15)	C29	C30	1.400(15)
C9	C12	1.511(15)	C30	C33	1.507(14)
C13	C14	1.399(15)	C34	C35	1.391(13)
C13	C18	1.396(17)	C34	C39	1.397(14)
C13	N2	1.441(12)	C34	N6	1.456(13)
C14	C15	1.402(15)	C35	C36	1.381(15)
C14	C19	1.475(15)	C35	C40	1.516(15)
C15	C16	1.385(18)	C36	C37	1.362(16)
C16	C17	1.385(19)	C37	C38	1.387(15)
C16	C20	1.502(16)	C37	C41	1.537(15)
C17	C18	1.385(17)	C38	C39	1.379(15)
C18	C21	1.503(18)	C39	C42	1.509(13)
Cl1	Ir1	2.196(5)	Cl5	Ir2	2.189(4)
Cl2	Ir1	2.411(3)	Cl6	Ir2	2.405(3)

Cl3	Ir1	2.334(4)	Cl7	Ir2	2.342(3)
Cl4	Ir1	2.336(3)	Cl8	Ir2	2.341(3)
Ir1	N3	1.841(10)	Ir2	N7	1.850(8)
Ir1	N4	1.889(17)	Ir2	N8	1.879(16)
N3	O1	1.079(12)	N7	O3	1.077(12)
N4	O2	1.115(18)	N8	O4	1.111(17)

Table A.2.3 Bond angles for the structure presented in Figure 3.13

Atom 1	Atom 2	Atom 3	Angle / °	Atom 1	Atom 2	Atom 3	Angle / °
N1	C1	Ir1	124.1(7)	N5	C22	Ir2	124.0(6)
N1	C1	N2	105.5(8)	N6	C22	Ir2	130.7(7)
N2	C1	Ir1	130.0(7)	N6	C22	N5	105.3(8)
C3	C2	N1	106.9(9)	C24	C23	N5	106.8(9)
C2	C3	N2	107.0(9)	C23	C24	N6	108.3(9)
C5	C4	N1	118.3(9)	C26	C25	N5	118.6(9)
C9	C4	C5	122.8(9)	C30	C25	C26	122.6(10)
C9	C4	N1	118.7(9)	C30	C25	N5	118.4(9)
C4	C5	C10	121.5(10)	C25	C26	C31	121.0(9)
C6	C5	C4	117.7(10)	C27	C26	C25	117.2(10)
C6	C5	C10	120.8(10)	C27	C26	C31	121.8(9)
C5	C6	C7	121.2(10)	C26	C27	C28	122.1(10)
C6	C7	C8	119.5(10)	C27	C28	C32	120.6(10)
C6	C7	C11	120.8(10)	C29	C28	C27	119.4(10)
C8	C7	C11	119.7(11)	C29	C28	C32	120.0(10)

C9	C8	C7	120.7(11)	C28	C29	C30	121.3(10)
C4	C9	C8	118.0(10)	C25	C30	C29	117.1(9)
C4	C9	C12	121.5(10)	C25	C30	C33	122.4(10)
C8	C9	C12	120.4(11)	C29	C30	C33	120.4(10)
C14	C13	N2	117.4(10)	C35	C34	C39	122.5(9)
C18	C13	C14	123.9(10)	C35	C34	N6	118.6(9)
C18	C13	N2	118.1(10)	C39	C34	N6	118.3(8)
C13	C14	C15	115.1(11)	C34	C35	C40	120.2(10)
C13	C14	C19	123.6(9)	C36	C35	C34	118.5(10)
C15	C14	C19	121.3(10)	C36	C35	C40	121.2(9)
C16	C15	C14	123.7(11)	C37	C36	C35	120.0(10)
C15	C16	C20	120.6(13)	C36	C37	C38	121.0(10)
C17	C16	C15	117.5(11)	C36	C37	C41	121.5(10)
C17	C16	C20	121.9(13)	C38	C37	C41	117.5(10)
C16	C17	C18	122.8(13)	C39	C38	C37	121.2(10)
C13	C18	C21	121.2(11)	C34	C39	C42	121.5(9)
C17	C18	C13	116.8(12)	C38	C39	C34	116.8(9)
C17	C18	C21	122.0(12)	C38	C39	C42	121.6(9)
C1	Ir1	Cl1	97.0(3)	C22	Ir2	Cl5	96.2(3)
C1	Ir1	Cl2	177.9(3)	C22	Ir2	Cl6	178.4(2)
C1	Ir1	Cl3	88.0(3)	C22	Ir2	Cl7	91.9(3)
C1	Ir1	Cl4	93.2(3)	C22	Ir2	Cl8	89.5(3)
Cl1	Ir1	Cl2	84.11(17)	Cl5	Ir2	Cl6	84.5(3)
Cl1	Ir1	Cl3	89.12(17)	Cl5	Ir2	Cl7	85.2(2)

Cl1	Ir1	Cl4	87.71(15)	Cl5	Ir2	Cl8	92.4(2)
Cl3	Ir1	Cl2	90.20(15)	Cl7	Ir2	Cl6	89.63(10)
Cl3	Ir1	Cl4	176.71(13)	Cl8	Ir2	Cl6	89.02(10)
Cl4	Ir1	Cl2	88.58(13)	Cl8	Ir2	Cl7	177.39(11)
N3	Ir1	C1	96.7(4)	N7	Ir2	C22	96.1(4)
N3	Ir1	Cl1	166.3(3)	N7	Ir2	Cl5	166.5(4)
N3	Ir1	Cl2	82.2(3)	N7	Ir2	Cl6	83.3(3)
N3	Ir1	Cl3	90.3(4)	N7	Ir2	Cl7	88.9(3)
N3	Ir1	Cl4	92.6(4)	N7	Ir2	Cl8	93.1(3)
N3	Ir1	N4	169.2(16)	N7	Ir2	N8	166.4(18)
N4	Ir1	C1	87.9(16)	N8	Ir2	C22	94.9(18)
N4	Ir1	Cl2	93.0(16)	N8	Ir2	Cl6	85.9(18)
N4	Ir1	Cl3	80.1(15)	N8	Ir2	Cl7	82.7(17)
N4	Ir1	Cl4	96.9(15)	N8	Ir2	Cl8	95.0(18)
C1	N1	C2	110.8(8)	C22	N5	C23	109.8(8)
C1	N1	C4	128.6(8)	C22	N5	C25	128.9(8)
C2	N1	C4	120.5(8)	C23	N5	C25	121.4(8)
C1	N2	C3	109.9(8)	C22	N6	C24	109.9(8)
C1	N2	C13	132.5(8)	C22	N6	C34	131.9(8)
C3	N2	C13	117.6(8)	C24	N6	C34	118.2(8)
O1	N3	Ir1	163.4(11)	O3	N7	Ir2	156.3(12)
O2	N4	Ir1	153(4)	O4	N8	Ir2	161(5)

A.3 Python Script for EXSY data processing.

```
import pandas as pd
import matplotlib.pyplot as plt
from scipy.optimize import curve_fit
import numpy as np
import math

def roundsf(num, n):
    if num == 0:
        return 0
    else:
        order_of_magnitude = int(math.floor(math.log10(abs(num))))
        scale_factor = 10**(n - 1 - order_of_magnitude)
        rounded_num = round(num * scale_factor) / scale_factor
        return rounded_num

def exp(t, k, c):
    return (100-c) * np.exp(-(k*t)) + c
#####
#####

#INSERT FILE PATH HERE
file_path = 'FILENAME.xlsx'

#####
#####

excel_file = pd.ExcelFile(file_path)
Names = excel_file.sheet_names

for name in Names:
    k_array = []
    c_array = []
    print(f'Sheet is: {name}')
    df = pd.read_excel(file_path, sheet_name=name)
    #print(df.head(), "\n")
    D8 = df['D8'].to_numpy()
    Bound = df['Bound'].to_numpy()
    Free = df['Free'].to_numpy()
    lower_bounds = [0,0]
    upper_bounds = [100,100]
    popt, pcov = curve_fit(exp, D8, Bound, p0=[5,5], bounds = (lower_bounds, upper_bounds),
maxfev=5000)
    k,c = popt[0],popt[1]
    t_fit = np.linspace(min(D8), 20*max(D8), 100)
    B_fit = exp(t_fit, k, c)
    F_fit = 100-B_fit

    plt.plot(D8,Bound,'o')
    plt.plot(D8,Free,'o')
    plt.plot(t_fit, B_fit, label = 'Bound')
    plt.plot(t_fit, F_fit, label = 'Free')
    plt.xlabel("Time (s)")
    plt.ylabel("% Bound / Free")
    plt.legend()
```

```

print(f"No points missing, k = {roundsf(k,6)}, c = {roundsf(c,4)}.")

for i in range(len(D8)):
    concD8 = np.concatenate((D8[:i],D8[i+1:]))
    concBound = np.concatenate((Bound[:i],Bound[i+1:]))
    concFree = np.concatenate((Free[:i],Free[i+1:]))
    lower_bounds = [0,0]
    upper_bounds = [100,100]
    popt, pcov = curve_fit(exp, concD8, concBound, p0=[5,5], bounds = (lower_bounds,
upper_bounds), maxfev=5000)
    k_array.append(popt[0])
    c_array.append(popt[1])

#     print(f"Point {i+1} missing, k = {knew}")

k_error = np.std(k_array, ddof=1)
c_error = np.std(c_array, ddof=1)
# print(f"Jackknife error is:{roundsf(k_error,3)}.")
# print(f"k = {roundsf(k,4)} +- {roundsf(k_error,3)}.")

plt.text(0.5*max(t_fit),0.5*max(B_fit),
         f"k = {roundsf(k,6)}+-{roundsf(k_error,3)} s^-1", fontsize = 14)
plt.text(0.5*max(t_fit),0.4*max(B_fit),
         f"c = {roundsf(c,3)} +- {roundsf(c_error,3)} %", fontsize = 14)
plt.savefig(name+".png")
plt.show()

print("\n\n\n")

```