Novel ring expansion methods for the synthesis of functionalized macrocycles

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Abstract

Macrocycles, are a class of interesting compounds with extensive applications; most notable is the increasing interest in their applications in drug discovery in recent years. Despite constant efforts made to develop new chemistry to study macrocycles, making them efficiently is still considered to be a major challenge for synthetic chemistry.

In this work, different synthetic methods for macrocycle synthesis are introduced, with special emphasis on novel successive ring expansion reactions (SuRE) which has been recently developed in York. Distinct from other macrocyclization methods, SuRE was developed as two step ring expansion reactions based on intermolecular acylation, followed by intramolecular cyclization/rearrangement reactions. Instead of going through the a challenging macrocyclization steps, SuRE has achieved macrocyclization by annulation of intramolecularly cyclized 5 or 6 membered rings onto the existing cyclic compound, and utilizes thermodynamic driving forces to collapse the fused bicyclic bridge, release ring strain and producing enlarged monocyclic compound. SuRE enables macrocycles to be 'grown' from smaller rings, without ever performing a macrocyclization step.

In this thesis, three sub-projects connected to the Unsworth's SuRE research programme are described. Following a short review of ring expansion reactions and introduction, first the ring expansion and elaboration of benzosuberone derivatives as part of a successful collaboration with the European Lead factory is described (Chapter 2). Then, focus shifts to describing our efforts to prepare and expand novel medium sized ring scaffolds to expand the scope of SuRE (chapter 3). Finally, we describe progress towards an ambitious new ring expansion concept based on the pre-organization of long PEG side chains and our attempts to promote ring expansion via their insertion into lactams.

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And the last, I would like to thank Rachel from graduate office, for always being responsive and help me dealing with all administration related issues. Thank you so much for all your efforts, making York such an enjoyable place to live and learn.

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.

Chapter 1. Macrocyclization methods and Successive Ring Expansion

1.1 Macrocycles and macrocyclic compounds

Macrocycles occupy a unique planet in the chemistry universe – quiet and lonely planet as it used to be, relatively unknown to people and yet, thanks to recent developments in biological chemistry and synthetic methodology in past decades, we are now getting closer to sharpen the understanding of this under-explored area and starting to reveal the enigma of these macrocycles and attempting to fully release their potential in various fields of application. By definition, any compounds with a ring size over twelve atoms are considered to be macrocyclic. Macrocycles have been found to have a wide range of utilizations, such as in supramolecular chemistry,[1] catalysis,[2] material science,[3] nanotechnology,[4] and with a lot of emphasis on medicinal chemistry,[5] where majority of recent macrocycle containing publications are leading to medicinal chemistry and drug discovery.[6] Consequently, a wide range of macrocycles were studied extensively against most biological target classes have demonstrated enormous potential of macrocycles in medicinal chemistry.[5, 7]

With some important macrocycles as illustrated in Figure 1 as follows, Rifabutin **1**, is a spiro-piperidyl-rifamycin derived from ansamycins family, has been recently recognized as a WHO (World Health Organization) Essential Medicine and widely used as antibiotics for infection treatments.[8] Tacrolimus **2**, as a ascomycin

macrolide subclass approved drug, was proven to be an very effective immunosuppression agent after transplantation or refractory organ injection.[9] Approved by FDA,[10] Temsirolimus (Torisel) **3** is a dihydroxypivalate ester prodrug derived from rapamycin. As a mammalian target of rapamycin (mTOR) inhibitor, its applications on kidney (renal) cancer treatments and mantle cell lymphoma was also well studied.[10-11] Another novel ansamycin antibiotic, semisynthetic Rifalazil **4** with long half-life was also developed for chlamydia and tuberculosis treatments.[12] The cyclopeptide Voclosporin **5** and Pasireotide **6**, where voclosporin is a novel immunomodulatory calcineurin enzyme inhibitor can be administrated orally, it was reported to reduce inflammation for autoimmune diseases treatments.[13] Such as association with treatment of lupus nephritis,[14] plaque psoriasis[15] and noninfectious uveitis in recent publications.[13] On the other side, analogues to endogenous cyclic peptide hormone somatostatin,[16] Pasireotide is an oncology drug acting as growth hormone release inhibitor. Having applications on Cushing's disease, acromegaly and neuroendocrine tumor treatments.[16] Additionally, it's worth noting that cyclic peptides have many favorable properties such as good binding affinity, target selectivity and low toxicity that make them an attractive modality for the development of therapeutics, which all leads to the research on synthesis macrocyclic compounds.[16] Due to the nature of SuRE reactions, macrocyclic compound synthesized via this route will endorse cyclic peptide structure feature and can be programmed in such way to expand the cyclic peptide further and afford desired

sequence of drug **5** or **6** with incorporation of designated building blocks.

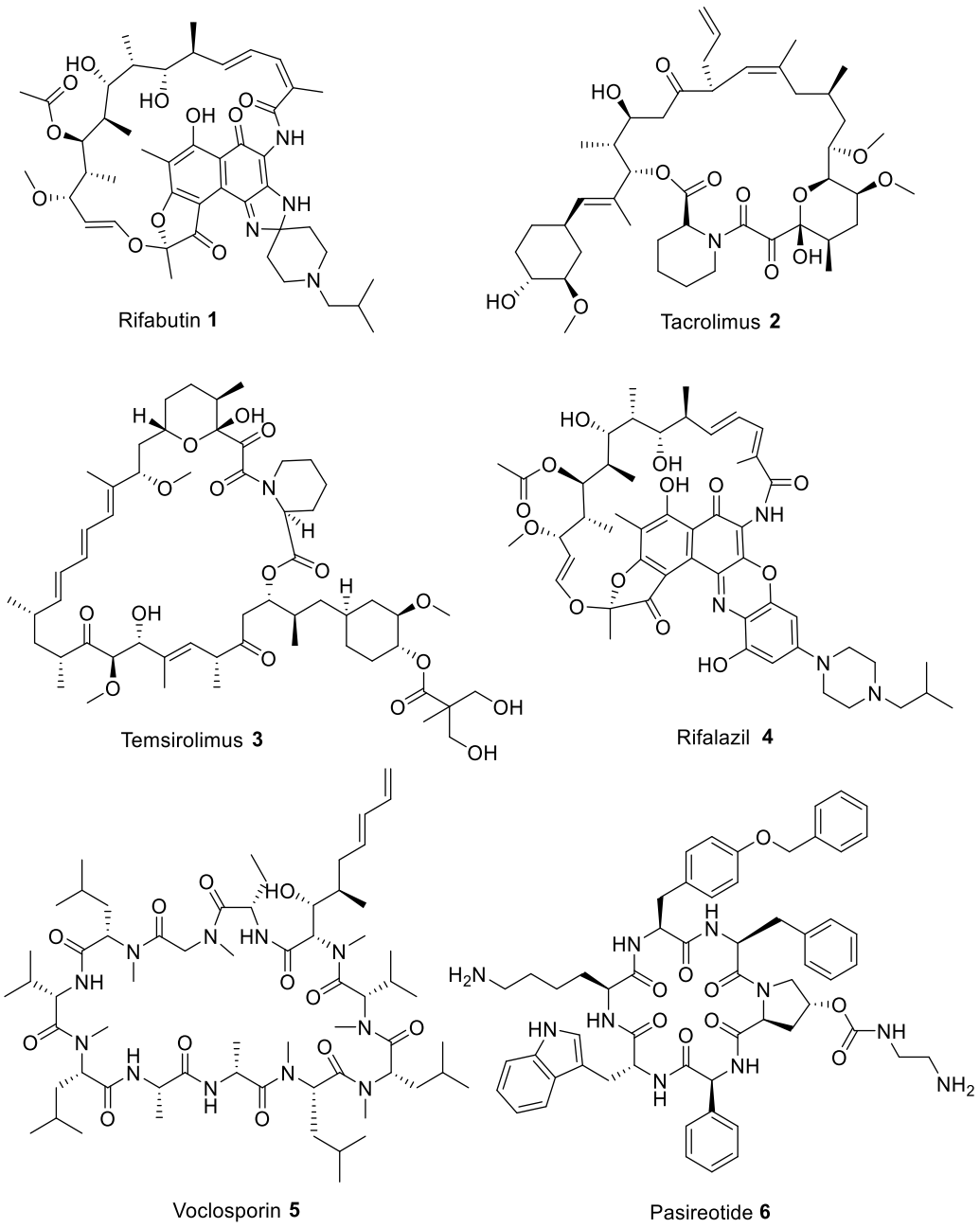


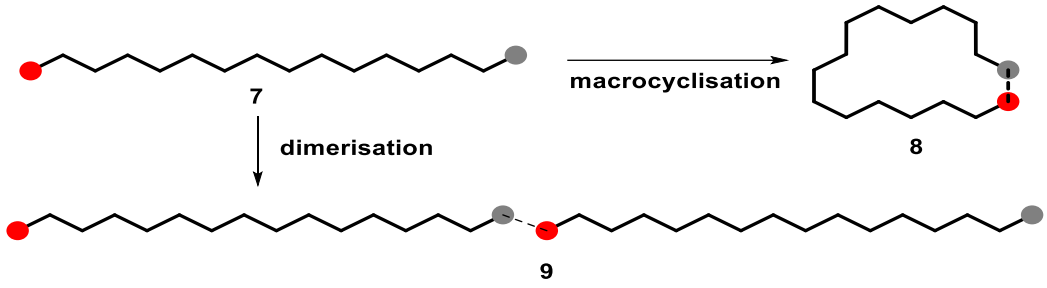
Figure 1 Some important marcocyclic drugs

1.2 Ring expansion methods for macrocycle synthesis

Since the discovery of natural occurring macrocyclic compounds, continuous efforts have been made to duplicate Nature and achieve their synthesis under laboratory conditions, wherein growing numbers of natural macrocycles have

been successfully made in the laboratory, driven by the advancing of synthetic methodology. During the past decade, the exploration of macrocyclic compounds in drug discovery has been advancing rapidly, making macrocycles an attractive class of compound to start drug developments. In recent years, arising interest on macrocyclic compound was dominated by search of new drugs for increasingly challenging drug targets, such as sophisticated protein-protein interactions.[17] As well as the growing demand particularly for compound with medicinal aiming macrocyclic scaffolds.[17b, 18] Therefore, new synthetic methodologies have been developed to meet these needs.

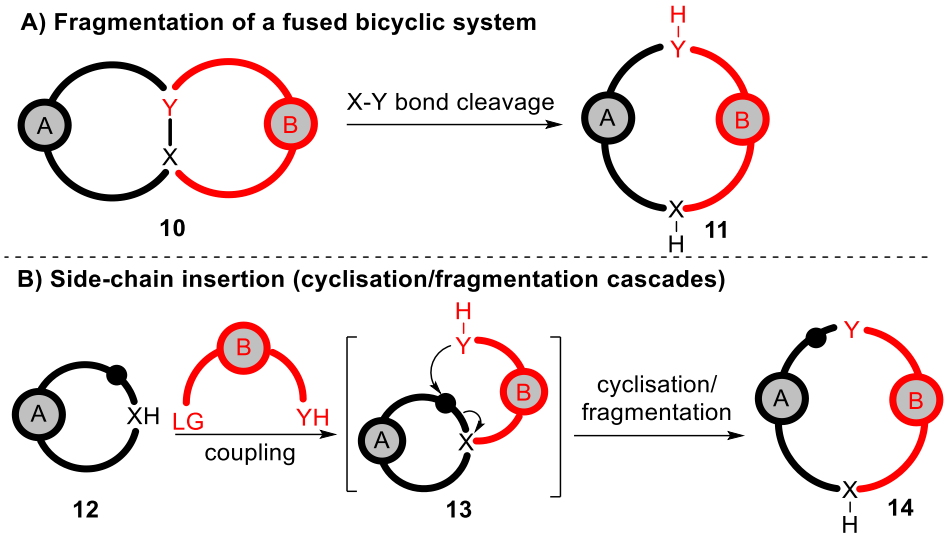
Classically, macrocyclization reactions are usually achieved by the cyclization of linear monomers (e.g. via amide bond formation); however, this method can be very inefficient, where dimerization competes with macrocyclization in most scenarios, leading to thermodynamically favorable long chain polymeric linear products instead of cyclized product desired as illustrated in Scheme 1. Attempted improvements were made to refine the reaction outcome, such as the incorporation of high dilution conditions, however this leads to another issue that such method is highly substrate dependent and impractical for scale up synthesis in industry.[19] Over the years new synthetic methods were developed as a better alternative and have offered access to new range of macrocycles, gradually progressing closer to naturally produced macrocyclic compounds.



Scheme 1 Comparison of macrocyclization and dimerization[19]

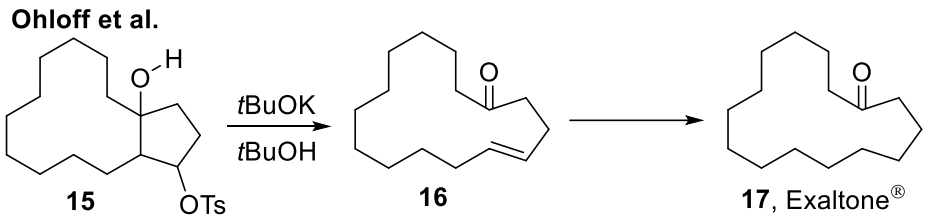
1.2.1 Fragmentation methods for ring expansion reaction

Ring expansion strategies, incorporating the concept of fragmentation, span a range of successfully expanded macrocycles through integration of additional fragments. In general, two major types of fragmentation methods are commonly used for macrocycle synthesis, fragmentation via fused bicyclic systems,[20] and fragmentation following side chain insertions.[21] For the former fused bicyclic method, such bicyclic structures can be synthesized by different means, and the ring expansion is initiated by collapse of a fused bridge (X-Y), leading to the amalgamation of two cyclic structures into one larger cycle (Scheme 2A). Whereas side-chain insertion utilizing simple linear fragment followed by attachment of one end onto existing smaller ring system, and go through intramolecular cyclization breaking a C-C bond from existing cyclic structure with formation of new C-C bond, as shown in Scheme 2B.



Scheme 2 Fragmentation type of ring expansions reactions. For a review, see reference 10.[19]

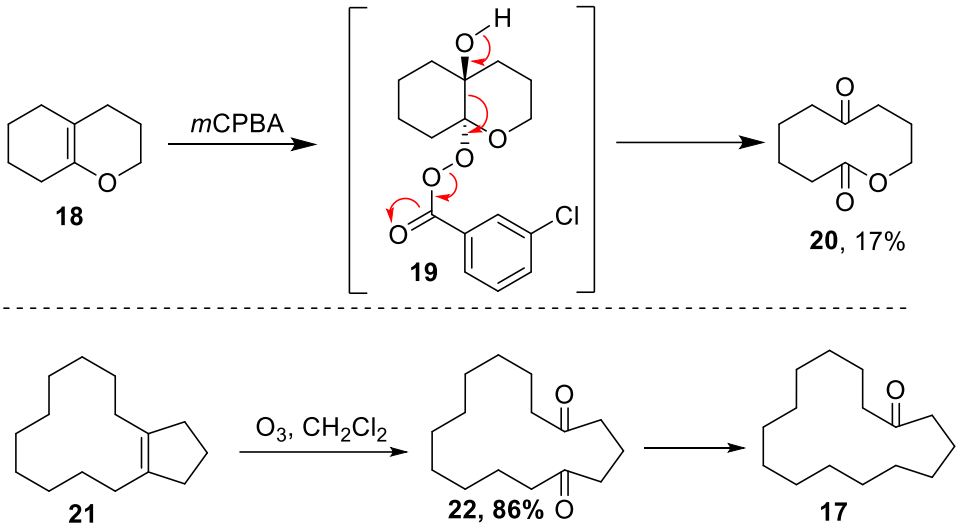
Fused bicyclic ring expansions can be further classified into elimination-type fragmentation reactions and redox driven fragmentations. One of the typical examples for elimination type fragmentation reaction is used in the synthesis of macrocyclic musks in fragrance chemistry; owning to the limited musk supply from natural sources, as well as reduced synthetic supply of nitro- and polycyclic musk due to adverse effects over health and environmental concerns, macrocyclic musk became attractive alternatives to meet the demanding markets. The synthesis of macrocyclic lactones and macrocyclic ketones are most extensively studied, and an early approach to synthesis macrocyclic ketone type musk incorporating elimination type fragmentation reaction ring expansion is shown in Scheme 3.[20] Note that the isolated yield for this reaction was not quoted in the publication.



Scheme 3 Elimination type approach ring expansion to Exaltone, yield not reported.[20]

Starting with tosylate compound **15**, elimination of the toslyate group leads to collapse of fused C-C bridge and merges the 5-membered ring onto parental 12-membered cycloalkane, forming a 15-membered cyclic product **16**, and the macrocyclic musk **17** was afforded followed by extra hydrogenation step.

Redox driven fragmentation type reactions are commonly achieved via oxidation of fused bridging double bond into diketone and consequently leads to ring expansion, with an example illustrated in Scheme 4 as follows; bicyclic enol ether **18** was oxidized by reacting with mCBPA, forming an intermediate **19** which undergo ring expansion into lactone **20** in one pot via the elimination of meta-chlorobenzoic acid.[22] Comparably, oxidative ozonolysis can be employed in place of mCPBA to cleave the alkene bridge in macrocycles showing below, leading to the alternative synthesis of musk compound **17** from macrocyclic diketone **22**.[23]



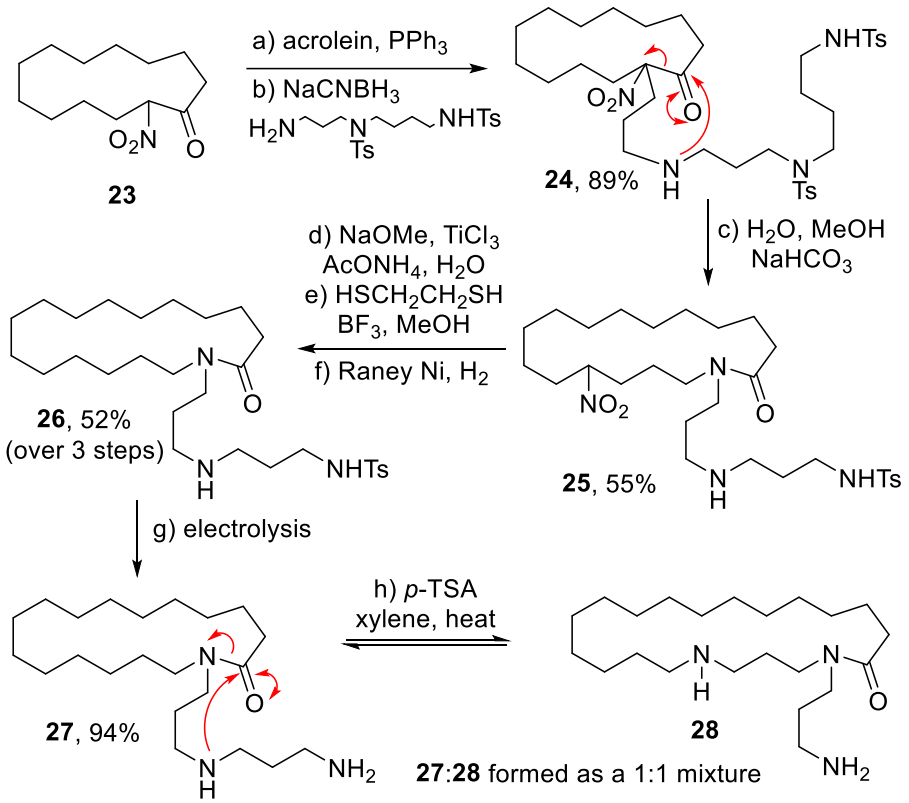
Scheme 4 Redox driven fragmentation reactions, *m*CPBA = *meta*-chloro perbenzoic acid.[20, 22]

1.2.2 Side chain insertion methods for ring expansion

Parallel to fused bicyclic ring expansions, side chain insertion ring expansion can be sub-divided into C-N bond formation, C-O bond formation and C-C bond formation side chain insertion reactions

The foremost work carried out for C-N bond formation type ring expansion was done by Hesse and coworkers, based upon the incorporation of nitrogen containing side chains, where series of ring expansion reactions was developed. [24] The extensive study of such pioneering work has led to a solid milestone laid in the synthesis of macrocyclic compounds via ring expansion methods. As revealed synthetic strategy in Scheme 5, starting material **23** was subject to Michael addition and reductive amination treatments, afforded compound **24** which was treated with aqueous sodium bicarbonate in methanol, leading to the side chain insertion via nucleophilic attack of secondary amine into cyclic ketone, consequent rearrangements producing carbanion and stabilized by adjoining nitro group, and

the first ring expansion was achieved as a result.[25]

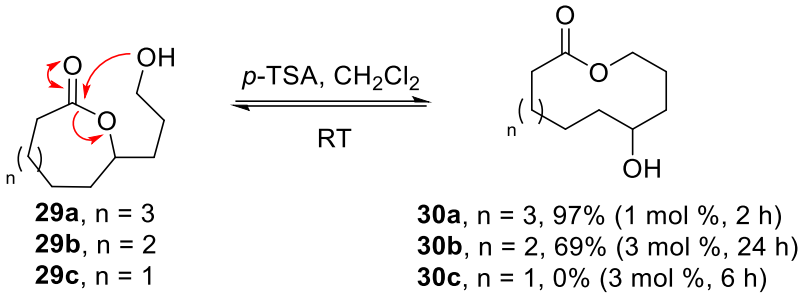


Scheme 5 Synthesis of macrocyclic alkaloid derivative **28**. *p*-TSA = *para*-toluene sulfonic acid.

The nitro group from first step is crucial to the overall reaction; other than stabilizing the carbanion, also it activates the adjacent carbonyl group for nucleophilic attack, driving the overall reaction process. The amine group introduced after first ring expansion can replace the function of nitro group and, therefore, the nitro group was effectively removed in next step. Followed by three-step reductive cleavage and electrolysis, the nitro and tosyl protecting groups were removed, generating precursor **27** for second fragment insertion and follow up ring expansion reactions. Cyclization of compound **27** was achieved by refluxing in *p*-xylene with *p*-TSA, affording ring expanded product **28** as 1:1 equilibrating mixture to its isomeric compound **27**.[25] The most significant impact of this work is contribution towards synthesis nitrogen bearing macrocycles, which

remain as frontier research until today for synthesizing macrocyclic peptides and peptide mimetics. [26]

As for C-O bond formation ring expansion reactions, among other similar transesterification reactions being one of the most typical side chain insertion examples. Comparable to transamination described in Scheme 5 above, the composition of functional groups was remaining untouched after overall transformation. This is demonstrated in Scheme 6 as follows: 9-membered cyclic lactone **29a** was successfully expanded with treatment of 1 mol% of para-toluenesulfonic acid, where free hydroxyl group initiates nucleophilic attack onto adjacent carbonyl group, followed by formation of new C-O bond in replace of old C-O bond after rearrangement, resulting overall ring expansion and generates 11-membered cyclic lactone **30b**.[27] Similar reaction was also performed in smaller ring sizes, however the yields are lowered with decrease in ring size and no product observed from 7-membered ring expansion attempts. This indicates that ring size dictated reversible reactions can reflect the relative stabilities of each individual ring system in yields, for smaller sized rings, the thermodynamic driving force for spontaneous ring expansion was diminished, therefore, it was difficult to afford ring expanded product. Such side chain insertion methods will be more applicable in larger sized macrocyclic compounds where macrocyclization is generally more thermodynamically favorable.

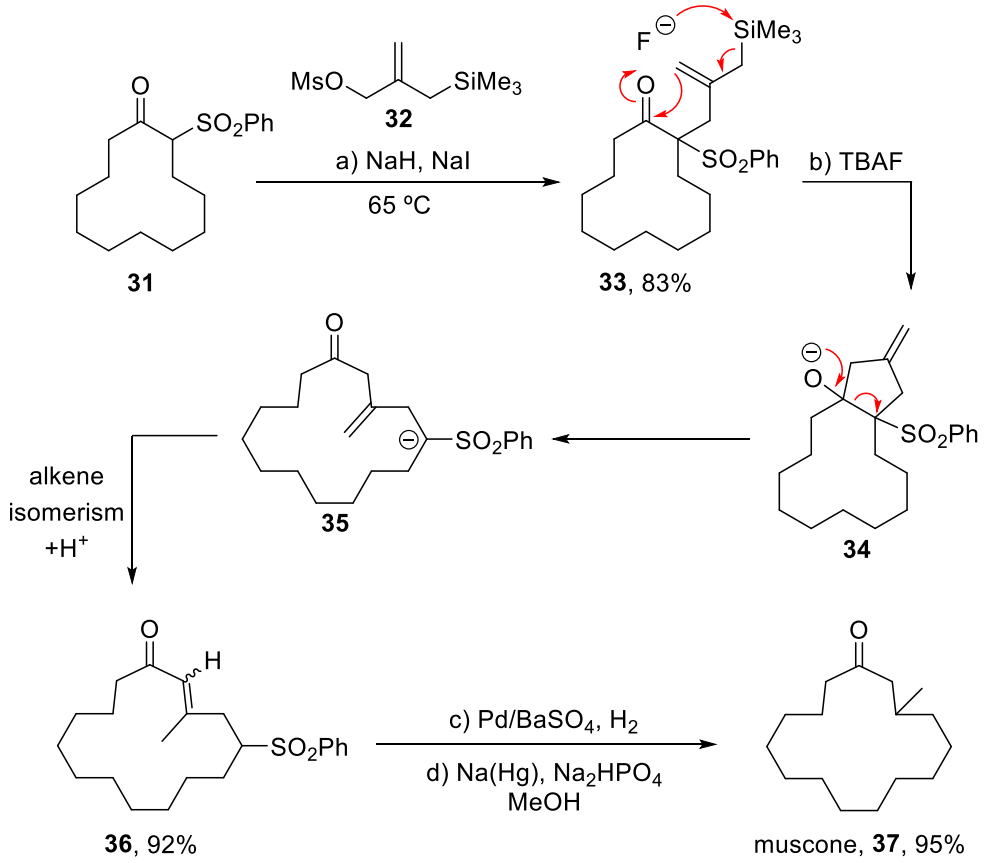


29a, n = 3
29b, n = 2
29c, n = 1

30a, n = 3, 97% (1 mol %, 2 h)
30b, n = 2, 69% (3 mol %, 24 h)
30c, n = 1, 0% (3 mol %, 6 h)

Scheme 6 Side chain insertion via C-O bond formation reactions[27]

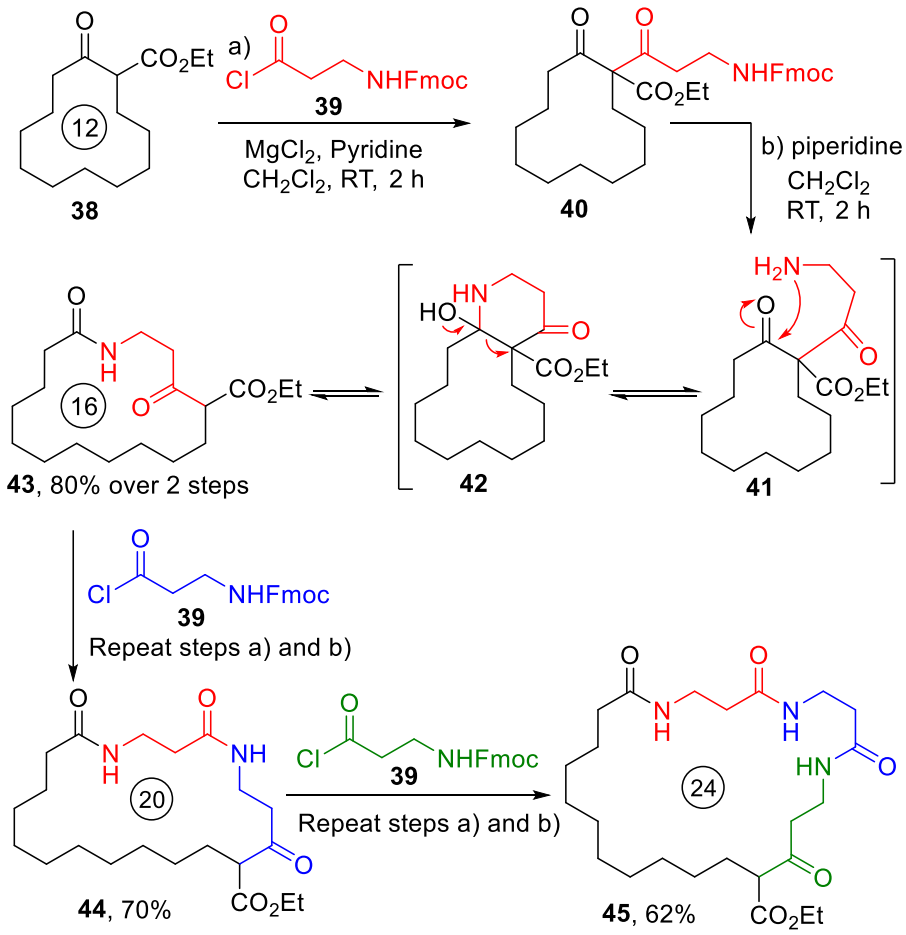
Other than two type of side chain insertion introduced above, C-C bond formation is also an important part in side chain insertion ring expansion reactions. With example work carried on synthesis of muscone **37** illustrated in Scheme 7. In this work, a novel three-carbon ring expansion was incorporated to achieve such synthesis.[28] Starting with β-ketosulfone **31**, ayllylation was carried out with mesylate **32** using Finkelstein conditions and precursor **33** for ring expansion reaction was afforded. Followed by allyl C-C insertion of allyl group into adjacent carbonyl group with catalytic amount of TBAF presence, a fused methylene cyclopentane **34** intermediate via intramolecular carbonyl addition was therefore generated. Fragmentation of such fused intermediate leads to formation of bridge collapse and integrate two rings into a larger cyclic β,γ-unsaturated ketone **35**. The isomerization was then undertaken with enone **36** formed, followed by hydrogenation and sulfone removal, generating compound **37** as product.



Scheme 7 Side chain inserting via C-C bond formation. TBAF = tetrabutylammonium fluoride

1.3 Successive ring expansion methods for macrocycle synthesis

Evolved from the ring expansion reactions introduced above, successive ring expansion strategies has been developed within our group in recent years. Based upon previous work on ring expansions reactions, successive ring expansion (SuRE) has merged the prerequisite β-ketoester group as driving force for spontaneous ring expansions and the capabilities to expand further with telescoped two-step procedure as illustrated in Scheme 8.[29]



Scheme 8 Telescoped 2-step successive ring expansion via C-N bond formation[19, 30]

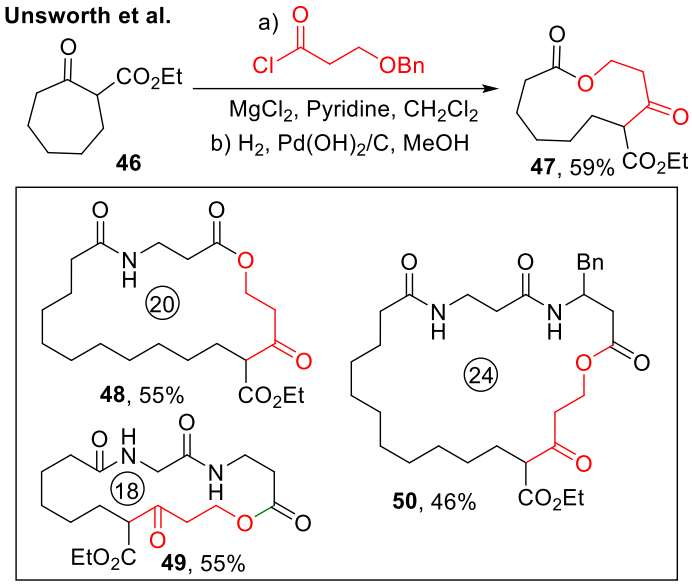
Starting from parent ring 12-membered cyclo-β-ketoester **38**, linear building block **39** is attached to the parental cyclic-β-ketoester via C-acylation, generating tricarbonyl species **40**, followed by base treatment, leading to Fmoc clevage and release the free amine group as key intermediate for ring expansion. The spontaneous rearrangement of intermediate **41** leads to the ring expansion after bringing bicyclic compound **42** falls apart, with a good yield afforded for ring expanded product **43**. The reactions work well because a reactive amine and unconjugated ketone intermediate in the starting material are converted into a more stable overall situation, i.e. an amide and conjugated β-ketoester in

compound **43** after rearrangement. This means that there should be a good thermodynamic driving force for ring expansion and also indicates that similar reaction can be also well adapted into wide range of ring systems, with variable ring sizes possible for ring expansion reactions. Additionally, the β-ketoester needed in starting material is regenerated after ring expansion, therefore, successive ring expansion is possible by performing same acylation and deprotection cycle, consequently leading to successive ring expansions. As a result, repeating same procedure will leads growing in ring size of macrocyclic compounds.

The reaction has shown to be well tolerant to different functionalized linear fragments, where both α- and β-amino acids have been used in an attempt at synthesizing precisely sequenced macrocyclic polypeptide with satisfactory yields.[29, 31] Additionally, the reaction could be carried out in practical concentrations of 0.1M and scale up to 5 g with ease. In addition to current work on side C-N bond formation on cyclo-β-ketoesters, investigation was carried out on similar C-N bond formation reaction modes with cyclic lactam ring systems,[31] with preliminary results published showing promising prospective over current cyclo-β-ketoester systems which will be introduced in part of this work in chapter 2.[31]

It has also been shown that the successive ring expansion method introduced above tolerates neutral hydrogenation conditions and the capability of the successive ring expansion methods on C-O bond formation insertion reactions to

make lactones is illustrated in Scheme 9.[31]



Scheme 9 Successive ring expansion via C-O bond formation

A two-step telescoped conversion of 7-membered β-ketoester into 11-membered ring product **47** was achieved, using method in analogues to C-N bond formation ring expansion reactions as introduced above. Further ring expanded product **48-50** were also afforded by repeating ring expansions based on compound **47**, in order to demonstrate successive ring expansion potential for similar reaction modes, since the presence of stabilized β-ketoester group in fragmentation step provides driving force and promotes cyclization reaction.[30] Notably, the C-O bond formation ring expansion was achieved via hydrogenation of benzyl ether protected hydroxyl groups, to free up hydroxyl group undertake nucleophilic attack, similar to the corresponding amine ring expansion reactions described above.[29-31]

1.4 Summary

Overall, with ever growing interest on macrocyclic compounds over their huge potential and broad-spectrum applications, attentions focused on developing range of macrocyclization methods in past decades were introduced. With emphasis on successive ring expansion methods developed within our research group, where different reaction mode was demonstrated, such as exploration on amine linear side chain insertion via C-N bond formation, and hydroxyl linear side chain insertion via C-O bond formation employing both cyclic-β-ketoester and cyclic lactam ring systems were addressed to be viable with promising reaction outcomes. Successive ring expansion method offers an alternative solution to achieve the synthesis of commonly inaccessible macrocyclic scaffolds by avoiding inpractical high dilution cyclistion conditions. This should help enable the development of macrocyclization reactions to contribute towards the synthesis of clinically important macrocycles and challenging macrocycle scaffolds. This thesis is focused on expanding the scope and application of SuRE reactions, with three distinct projects described, split by chapter. Chapter 2 is focused on making medicinally relevant scaffolds using SuRE as part of a collaboration with the European Lead Factory. Chapter 3 describes how to prepare medium-sized ring starting materials, and exploring their ring expansion, in order to more fully elaborate the scope of existing SuRE-type processes. Chapter 4 concerns early efforts towards the development of a new variant of SuRE that could enable long PEG chains to be incorporated into ring expanded products, based on metal

templated pre-organizations.

Chapter 2. Ring expansion reactions of benzosuberone derivative: preparation of medium-sized ring scaffolds for European Lead Factory submission

2.1 Medicinal potential and biological evaluation of benzosuberone and related derivates

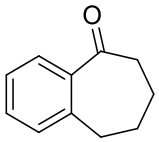


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Figure 2 Molecular structure of benzosuberone

Benzosuberone has the potential to be used as a precursor for the synthesis of new lead compounds in the field of drug discovery and development. As Figure 2 shows above, its basic structure features a six-membered aromatic ring and fused seven membered cycloketone, forming a bicyclic scaffold which can be found in various natural products with significant medicinal and pharmaceutical activities.[32]A wide range of inhibitory effects in derived from benzosuberone derivatives, from numerous reports, have been described, for example anti-inflammatory,[33] antiestrogenic,[34] anti-microbial,[35] anti-tumor,[36] anti-obesity/diabetes,[37] anti-malarial,[38] beta-amyloid production,[32] CNS-depressant/stimulant[39] in addition and anti-Alzheimer[40] and anti-tuberculosis activities.[41] Several derivatives are receptor antagonists, abnormalities such as sleep, eating and reproductive disorders can be potentially treated with related

derivatives according to literature,[39] additional benzosuberone based compounds were discovered as vascular disrupting agents, specifically targeting micro vessel feeding tumors and exhibit good anti-tumor potency.[32]

Many benzosuberone derivatives were found to be highly biologically active, demonstrating good potency on anti-inflammatory, immunosuppressive, antitumor activities and inhibitory of many enzymes as described above, which could be further developed into promising therapeutic drugs or as lead compound.[32] Some examples of benzosuberone based natural products are presented in Figure 3 with structural similarities addressed.

Colchicine, as a typical natural product bearing a benzosuberone scaffold, originated from plants of genus colchicum, was found to be the first and the oldest medication used in treatment of gout for centuries.[42] Colchicine can inhibit neutrophil motility activities, consequently regulates the immune system which leads to anti-inflammatory effect, therefore used as a common treatment on acute flares from inflammatory arthritis.[42-43] Further research also described the role of colchicine as a mitosis inhibitor, exhibits cytotoxicity through interaction with tubulin, which is a major component that polymerize into microtubules.[42] The microtubule polymerization can be inhibited owning to reduced availability of tubulin protein, leading to interrupted cell mitosis in metaphase; as such the compound can be further developed into valuable antiproliferative anticancer agents.[44] On the other hand, while alleviating flares, colchicine also exhibits many adverse effects as a secondary metabolite, such as fever, gastrointestinal upset,

muscle pain, low blood cell counts, and organ failure in rare scenarios.[45]

Despite the side-effects and toxicity of colchicine, market demands have grown rapidly in past decades for effective treatment on gout and other related symptoms. As an alternative for patients with nonsteroidal anti-inflammatory drug intolerances, even without FDA approval, still the colchicine was used in an unapproved drugs initiative scheme. However, this has cause problems where no FDA approved dosage guideline, drug interactions and prescribing information were given, which could be potentially dangerous whereas colchicine can be toxic when ingested, subsequent overdose often leads to unwanted side effects. Due to the above concerns, colchicine was only approved in 2009 by FDA,[45a] one hundred years after the first discovery of its medicinal value, and the approval was made in combination with other ingredient on treatment of gout. The market price was consequently rose over 2000 percent and generates $ 1.2 billion in revenue for licensed pharmacy company Tekeda Pharmaceuticals. Due to problematic clinical trials, the marketing of unapproved single ingredient oral colchicine was halted by the FDA in 2010, and a replacement is therefore in need to meet the market demand.

In addition to gout, colchicine was also used to treat familial Mediterranean fever and pericarditis,[46] benzosuberone as a pharmacophore merged into many synthetic ring systems was also found to be a versatile candidate against many diseases as Figure 3 showing below.

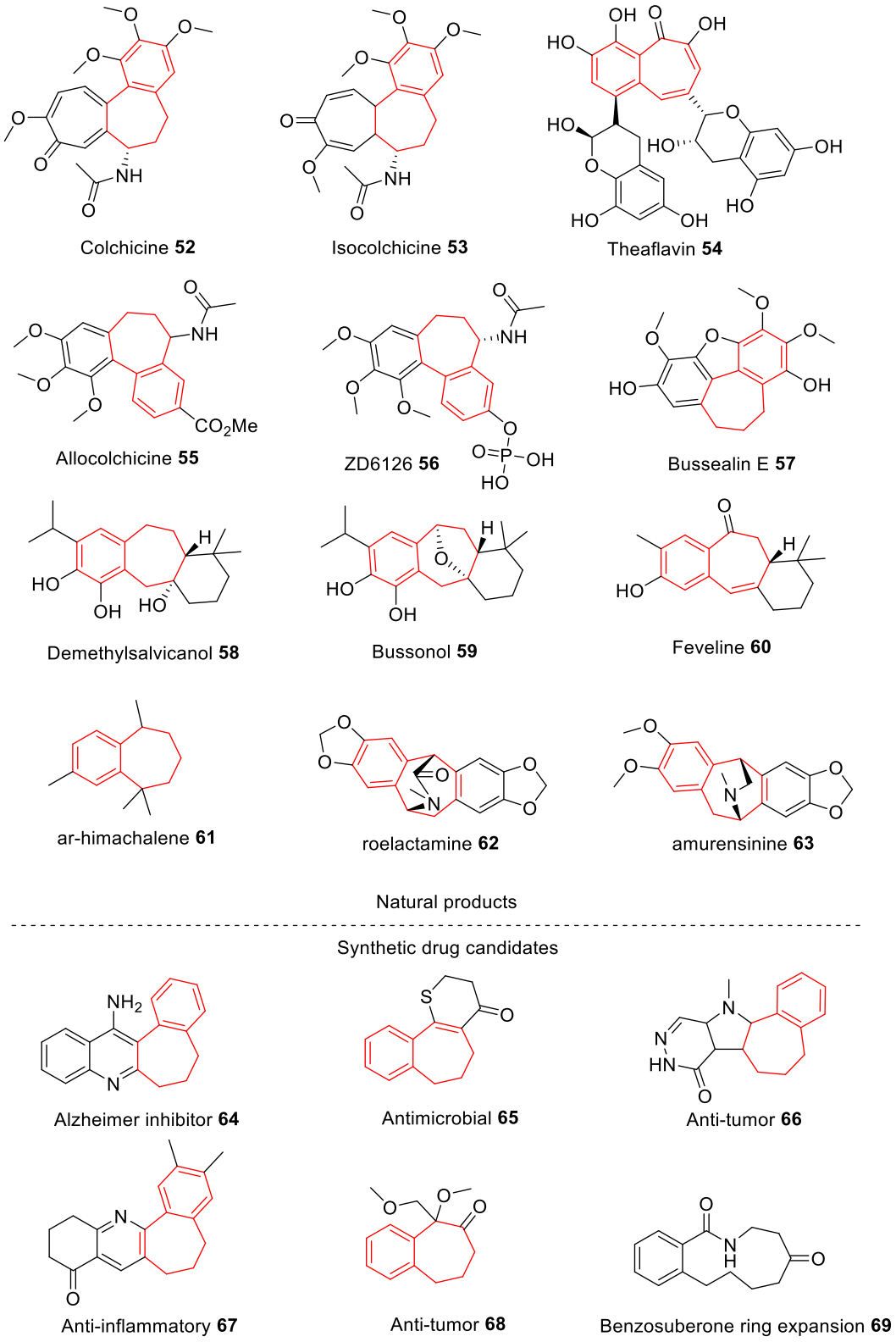


Figure 3 Reported natural products and synthetic benzosuberone derivatives with biological activities compared to benzosuberone ring expansion for European Lead Factory submission.[32, 36a]

In addition to natural products, many synthetic benzosuberone containing

compounds have been tested in the past few years. Broad-spectrum activities were reported from these compounds as anticipated,[32] despite the efforts made on benzosuberone bearing scaffold synthesis and the evaluation of its biological activities, the exploration of macrocyclic or medium sized ring derivatives of benzusuberone are unusual. Where benzosuberone derivatives discussed above tends to be structurally rigid, with little flexibility and more entropy cost when binding to substrates, the structural feature of ring expanded benzosuberones could offer advantageous solution with greater flexibility and less entropy penalty when binding to substrate.

The huge market demand stands behind which indicates considerable commercial interest could be potentially generated from benzosuberone related compounds. With clinical importance addressed, rather novel macrocyclic drug based on benzosuberone could offer breakthroughs from different prospect compared to conventional benzosuberone based drug synthesis, and the successive ring expansion will be a powerful tool to achieve ring enlargements.

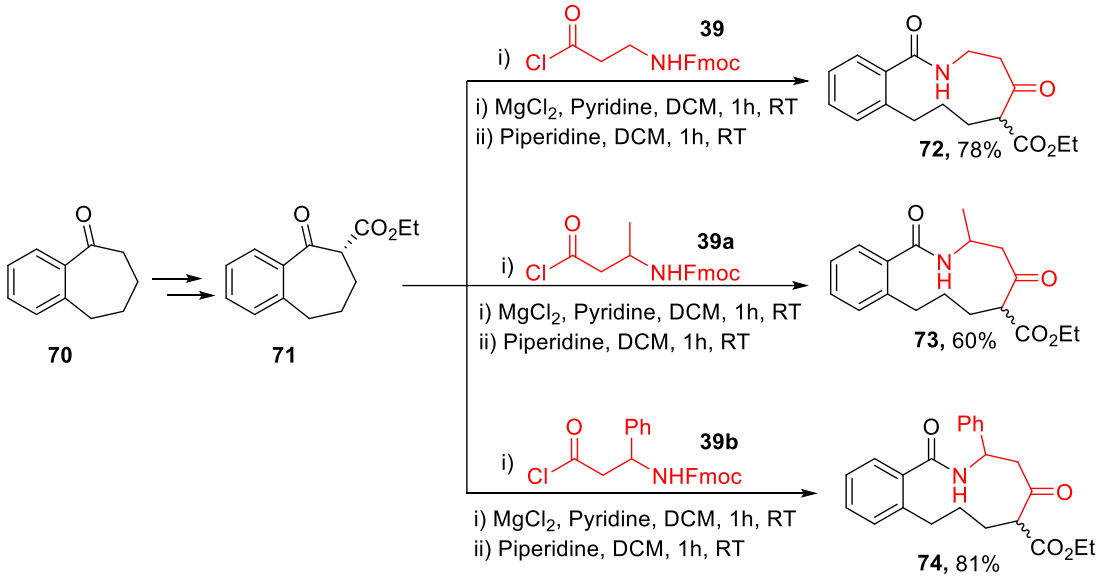
As described above, despite previous success on active benzosuberone and its derivatives, one of the major advantage of macrocyclic compound in medicinal chemistry is structural flexibility, where better binding can be enabled against drug targets, which means further exploration on the binding pockets and extended binding possibilities compared with structurally rigid benzosuberone alone, this

will contribute towards fetching information of binding sites for structural biology study on drug target. Pharmacokinetically, despite promising result from preliminary studies, many drug candidates were failed in later stage of drug discovery process due to poor pharmacokinetic properties or side effects. Successive ring expansion method offers highly customizable and modulated fragments for ring growth with corresponding character. As such, a customized macrocyclic compound blending both macrocyclic and benzosuberone character can help to enhance the potential of benzosuberone as pharmacophore. Lastly, referring to some famous accidental discoveries made from history, Sildenafil, originally designed as heart condition treatments, or Saccharin, a world changing sweetener derived from coal tar, it would be fascinating to see the potentials of these class of new compounds and letting chemistry do the rest magic. The European lead factory considered to enlist this compound would be based on its own merits other than medicinal properties such as enhance its compound library diversity. The overall aim of this section of work was set to use benzosuberone as precursor to generate a medium sized ring building block, and could be elaborated further. Collaborating with European Lead Factory partners, the aim was set to generate compounds that would become part of their shared high throughput screening compound library collection.

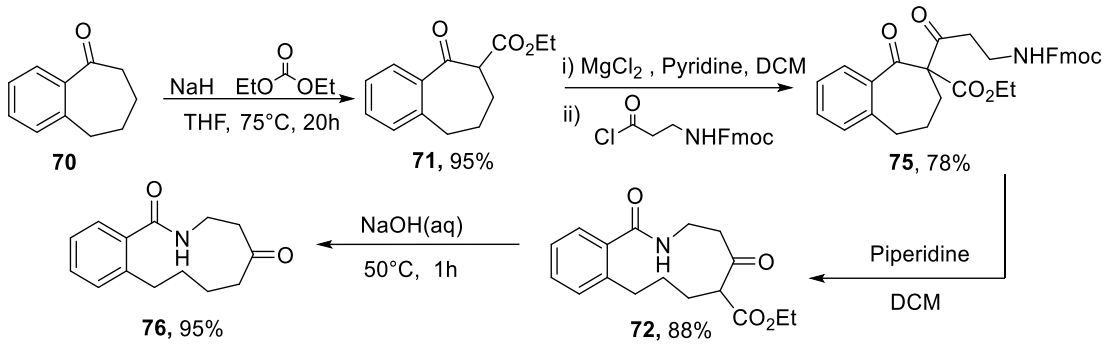
2.2 Benzosuberone and successive ring expansion

Starting from commercially available starting material benzosuberone **70**, a set of synthetic transformations were planned to convert the cyclic ketone into a cyclic

β -ketoester carboxylate **71**, followed by the application well-developed successive ring expansion methods from our group to expand the ring size by 4 atoms in one step, into 11-membered ring **72.** Further steps to elaborate this molecule into building block **76** were also planned (Scheme 11). To further address the capabilities to incorporate different linear fragments, the method was also demonstrated with integration of methyl and phenyl groups in benzosuberone ring expansions apart from simple β-alanine used showing in Scheme 10 (to form **73** and **74**). This indicates a range of different functionalities could be potentially introduced, thus providing rapid access to a diverse collection of functionalized macrocycles for compound library synthesis.

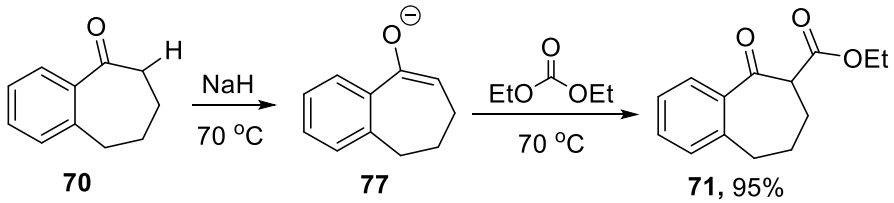


Scheme 10 Literature reported incorporation of various linear fragments into benzosuberone ring expansion reactions.[30]



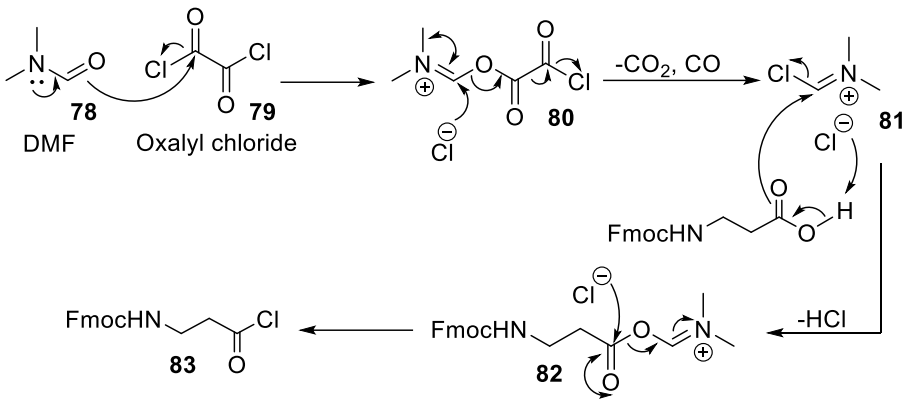
Scheme 11 Synthesis plan for benzosuberone ring expansions

The chemistry starts from benzosuberone **70**, using some standard enolate chemistry reactions; thus sodium hydride was used as a base to deprotonate α-carbonyl group, generating enolate **77** at 70 °C, with tetrahydrofuran as solvent, and then diethyl carbonate was added as the electrophile to produce β-keto ester scaffold **71** in 95% yield as Scheme 12 illustrates.



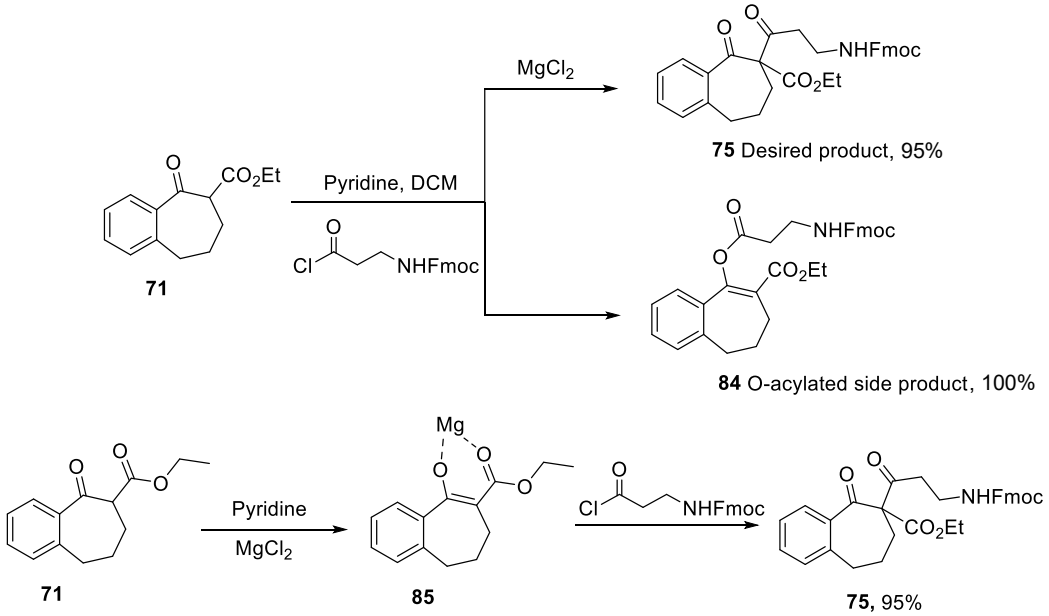
Scheme 12 Synthesis of benzosuberone carboxylate for ring expansion reactions

Upon the successful synthesis of benzosuberone β-ketoester **71** above, a fluorenylmethyloxycarbonyl (Fmoc) protected β-alanine was treated with excess oxalyl chloride in dichloromethane and produce Fmoc-β-alanine chloride, with DMF as a catalyst, as general procedure with mechanism shown in Scheme 13 as follows.



Scheme 13 General procedure for acid chloride formation

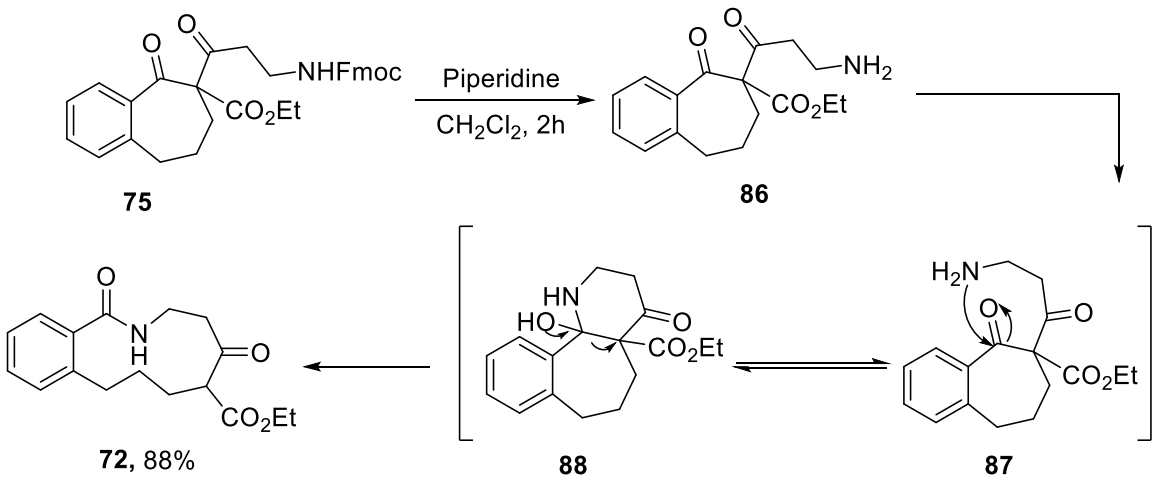
For the acylation step, pyridine was used as a base which deprotonates the carbon between two carbonyls to promote acylation. However, the acylation reaction can be a problematic step where the more electronegative oxygen atom often competes with carbon during acylation, which leads to oxygen acylation instead of desired acylation on the carbon site in many scenarios. Magnesium chloride was, therefore, added into the reaction as an additive that can bind strongly with both oxygen atoms, and the coordination between carbonyl groups subsequently helps to direct acylation onto the desired carbon site.



Scheme 14 Directed C-acylation using Fmoc-β-Alanine and $MgCl_2$ compared to competing O-acylations without $MgCl_2$

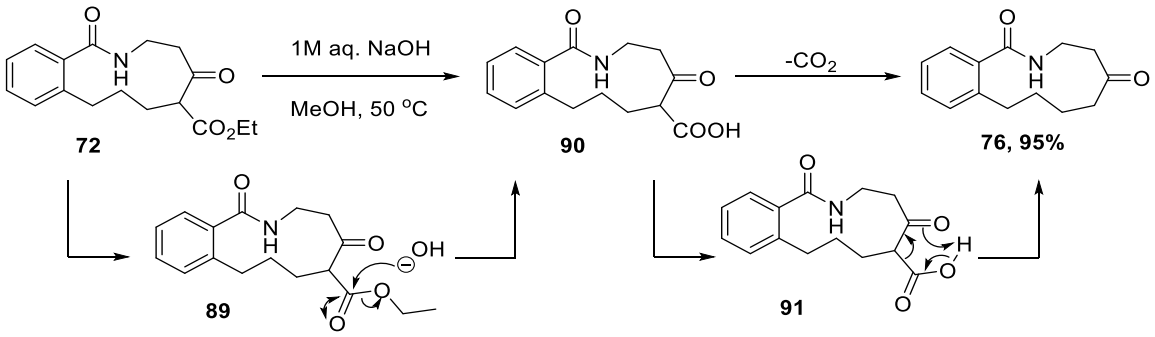
The acylated tricarbonyl compound **75** was purified by column chromatography with good yield of 95%, and was characterized by IR, NMR and high-resolution mass spectrometry. The correct mass acquired indicate successful acylation, where the characteristic CH proton peak from the β-ketoesters, (the hydrogen next to β-ketoester group around 3.5 ppm) was not observed, which confirmed the formation of desired C-acylated product with IR data reassuring some of the characteristic functional groups such as ketone absorption peaks instead of allyl group. ^{13}C NMR confirmed that the product has 4 carbonyl groups, with two being ketones (ca. 200 ppm), thus confirming that **75** rather than **84** had formed.

Piperidine was then used as base to treat the acylated compound **75** in dichloromethane and initiate protecting group cleavage and ring expansion reaction. In this step the Fmoc protecting group was effectively removed after piperidine treatment at rt in DCM, leaving a free amine group, which is a good nucleophile and thus attacks the δ carbonyl site (via a 6-membered ting transition state), resulting in spontaneous ring expansion after rearrangement via the mechanism shown in Scheme 15:



Scheme 15 Ring expansion of benzosuberone ester with Fmoc-β-alanine as the linear fragment.

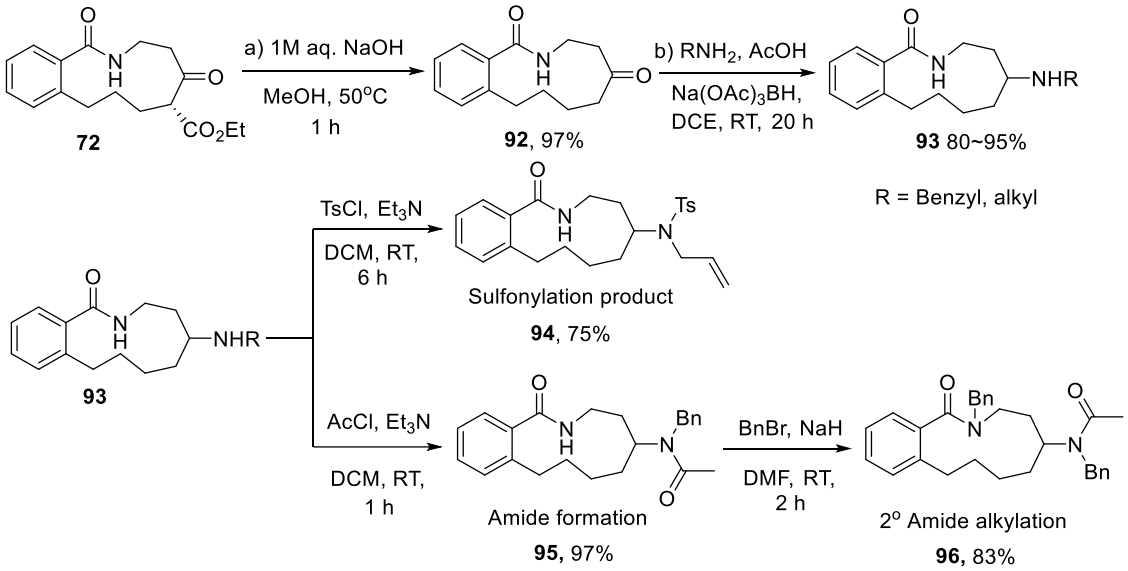
In this 11-membered ring product, there is a metabolically labile β-ketoester motif, meaning that realistically the compound itself would probably not be a favorable drug candidate, and therefore this required additional modifications before submission for screening of potential lead compounds. Thus, to cleave the ester group, a simple one pot reaction was carried out using a 1M NaOH aqueous solution, with methanol as the solvent, yielding ketone **76** after hydrolysis and decarboxylation. The reaction worked very well in general, with a 95% high yield as scheme show as follows:



Scheme 16 One-step hydrolysis and decarboxylation of β-ketoester for European Lead Factory submission

For European Lead Factory submission, it is important to show that a submitted

building block can be elaborated further to make multiple derivatives, and compound **76** was demonstrated to be a versatile building block amenable to additional chemistry from previous group work as demonstrated in Scheme 17. Ketone was converted into a secondary amine **93** after reductive amination with both benzyl and alkyl group afforded in good yields. The secondary amine can be further sulfonylated to compound **94** in 75% yield. Alternatively, the reductive amination product can also be converted to amide **95** again with excellent yield, furthermore, additional secondary amide alkylation was proven to be successful with benzyl group introduced.



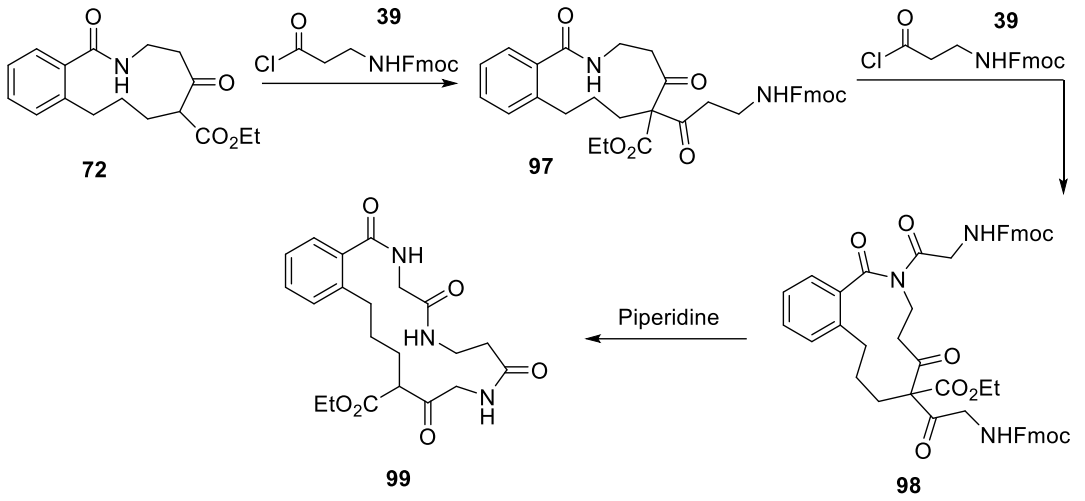
Scheme 17 Decarboxylation and reductive amination and follow up transformations showing extensive reaction potential for ring expanded benzosuberone as a potential drug candidate

Overall, the compound was demonstrated to have the potential and the ability to serve as a valuable precursor for further decorations or optimizations into lead compound candidate for screening. Indeed, using variants of these methods,

European Lead Factory collaborators have successfully prepared over 50 derivatives based on the 2 grams of compound **92** that we synthesized and sent to project partners during this project. These compounds are now a part of the European Lead Factory high throughput screening compound collections, and for this work the group was awarded a European Lead Factory Chemical Library Creativity Award (with €5000 prize) in recognition of its importance to the project.

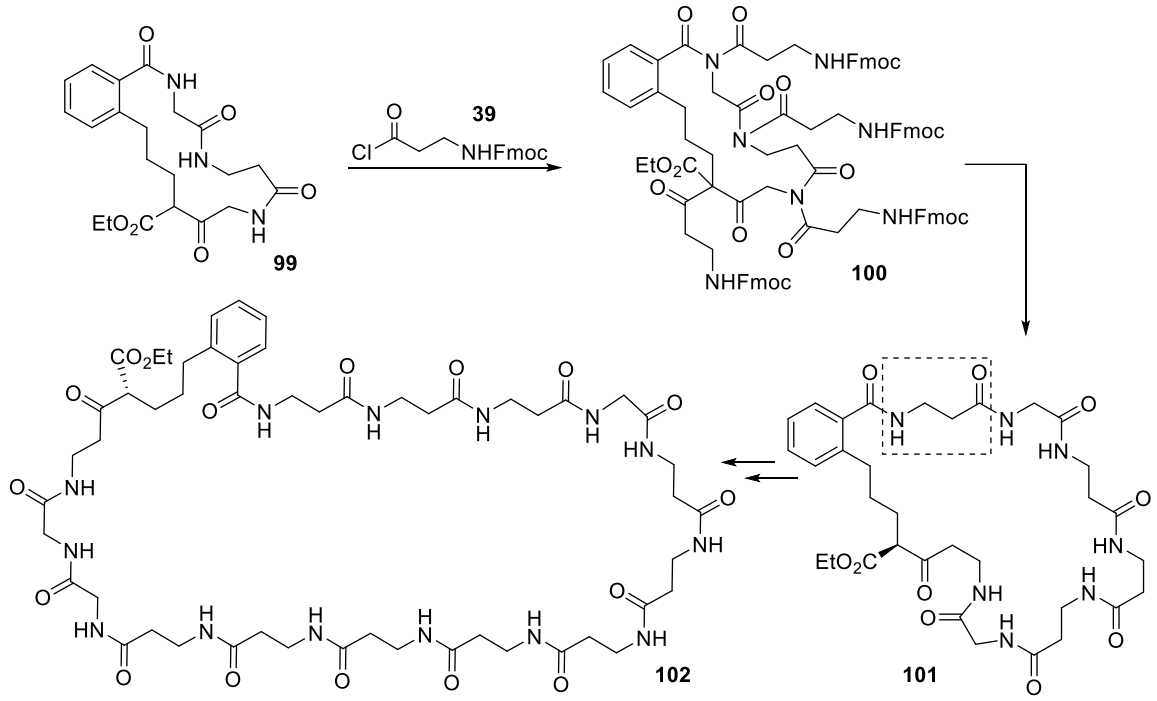
2.3 Double acylation and successive ring expansion on ring expanded Benzosuberone β-ketoester carbocycle.

We also decided to examine more complex ring expansion processes from 11-membered ring **72**. Within the Unsworth group, a new successive ring expansion method was discovered whereby *N*-acylation of a lactam can promote ring expansion in a method that analogues to beta-ketoesters as described. We therefore reasoned that *C*- and *N*-acylation of compound **72**, to make doubly acylated compound **98**, which might enable a double ring expansion reaction which leads to the formation of much larger macrocyclic compound **99** in one step, as showing below in Scheme 18.



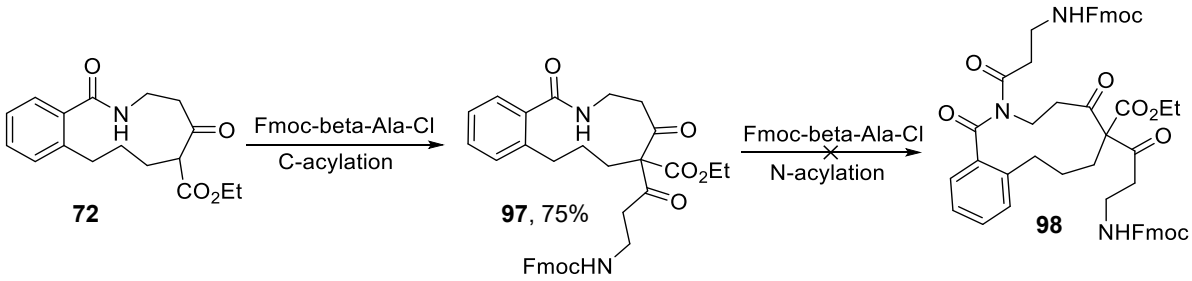
Scheme 18 Proposed reaction route for carbon and nitrogen acylation and one step ring expansion on expanded benzosuberone β-ketoester

Additionally, even more ambitious potential ring expansion that involves multiple acylation fragments with additional functional groups could be imagined, to integrate several functionalities in few steps as illustrated in Scheme 19.



Scheme 19 Proposed multiple acylation could expand the ring lot more efficient compare to single step successive ring expansion

We started by exploring a two-step double acylation of **72**, where we aimed to acylate the carbon and nitrogen site separately with a basic linear fragment Fmoc-β-alanine, and deprotect with piperidine at the same time with followed by spontaneous ring expansion as scheme illustrated as follows:

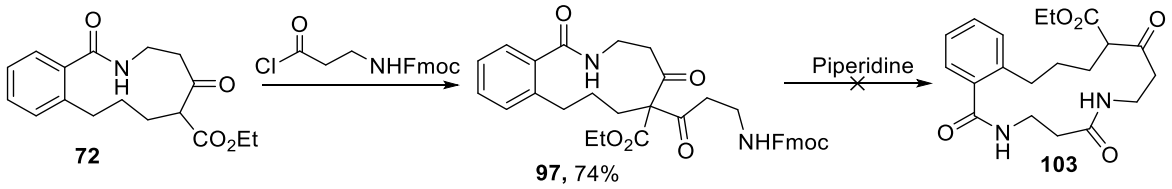


Scheme 20 Proposed reaction for double acylation of expanded benzosuberone

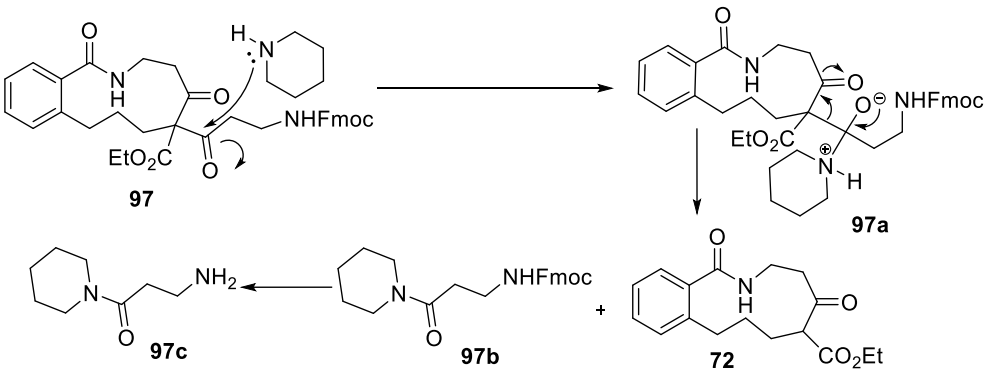
As Scheme 20 shows above, ring expanded β-ketoester **72** was acylated with simple Fmoc-β-alanine chloride as linear fragment, the acylation went smoothly as expected with 75% yield afforded, the acylated compound **97** was isolated and refluxed overnight again with Fmoc-β-alanine chloride. Attempted acylation on nitrogen site using 4-dimethylaminopyridine (DMAP) as catalyst (instead of the $MgCl_2$ used in standard procedure for C-acylation, for lactam acylation DMAP is required) to explore the plausibility of additional acylation on the lactam nitrogen. Since the successive ring expansion method works very well in a good range of ring systems, the acylation and expansion can often be done for most of the reaction in one pot without further purification and thin layer chromatography can generally be used to monitor the progress and confirm the completion of acylation reactions in this case. Unfortunately, no reaction had taken place after overnight reflux and the acylation was determined to be unsuccessful according to TLC and mass spectrometry analytics. The proton α- to the β-ketoester in the

starting material was clearly observable, in the NMR spectrum of this reaction, and we speculate means that the conformation of this particular starting material is not well suited to acylation for steric reasons.

Despite the unsuccessful attempts of performing a second nitrogen acylation reaction on compound **97**, there is still the positive result that the first carbon acylation reaction was successful. Therefore, it is worthy to attempt the expansion of mono-acylated compound **97**. Thus, repeating standard procedure, ring expanded benzosuberone β-ketoester **72** was treated with Fmoc-β-alanine chloride as linear fragment to make **97**, and this was followed by the addition of piperidine to promote ring expansion as shown in Scheme 21. Regrettably, despite completion of carbon acylation being confirmed by TLC and mass spectrometry, the follow up ring expansion with piperidine was unsuccessful again based on TLC and mass spectrometry, whereby starting material **72** was isolated. This suggests that instead of coupling and rearrangement of free amine group, the acylated compound had undergone degradation, cleaving the liner fragment into β-alanine and restoring the β-ketoester starting material, as proposed in Scheme 22.



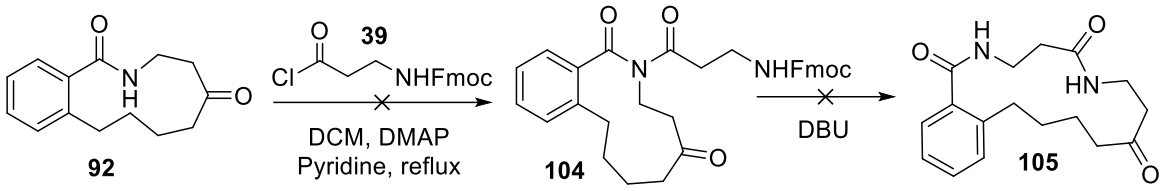
Scheme 21 Acylation of β-ketoester with Fmoc-β-alanine as liner fragment for ring expansion



Scheme 22 Proposed mechanism for acylated β-alanine cleavage with piperidine

Interestingly, no acylated free amine **105** was isolated after deprotection of compound **104**, where all the deprotected free amine acylation compounds looked at have endured decomposition, falling apart and back to starting material **72** and amide **97b** (which has been isolated previously in the Unsworth group); this may suggest that the piperidine in this reaction could be too nucleophilic. In this regard, alternative to secondary amine piperidine, the less nucleophilic tertiary amine could be used in replace of piperidine as base, such as the more bulky 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) which could be tested for further reactions. Additionally, investigation of the properties of different bases during ring expansion step is also essential to optimize the reaction conditions.

Another parallel reaction was carried out on hydrolyzed, decarboxylated and expanded benzosuberone ring **92**, to eliminate potential effect of β-ketoester group from the previous unsuccessful nitrogen acylations. The reaction was set up in accordance to previous conditions without additional modification, but unfortunately, no acylated product **104** was detected according to TLC analysis after overnight reflux with only starting material **92** recovered from column chromatography as in Scheme 23.



Scheme 23 Proposed nitrogen acylation and follow up successive ring expansion

In addition to DBU and piperidine, sodium hydride was also used in similar reactions as an alternative but unfortunately no positive result was found.

2.4 Conclusion and future works

To summarize, a ring expanded benzosuberone derivative **92** was synthesized successfully and prepared and submitted to the European Lead Factory library. This compound could be further functionalized and its derivatives are now part of the ELF screening collection. As such, biological screening against range of drug targets such as anti-inflammatory or anti-tumor activities as described previously can now be performed to evaluate the potential as lead compound followed by additional optimization or decoration for further pharmacological studies. Additional ring expansion reactions were also examined starting from **72** and **92**, however, more work will be necessary to help to understand the function of different bases and solvent effects during ring expansion step, where attempts at successive ring expansion on expanded benzosuberone β-ketoester were unsuccessful. Potentially this could be due to the steric hinderance from adjoining planar aromatic ring which restricts the carbonyl site for deprotected free amine. Therefore, making it less accessible which agrees with lowest energy conformation calculated for compound **104**. Furthermore, the acylation could be a problem sometimes where attempted second acylation on C-acylated cyclo-β-ketoester

97 was also unsuccessful. Since benzosuberone selected in this project could be more complicated to analyze, due to the impact from adjoining aromatic group, starting with simplified cyclic substrates such as 11-membered cyclo-β-ketoester or lactam could be a solution to examine the impact of the aromatic group for steric hinderance. As stated, more work is in need to be carry on, and overcome the failed acylation, and this will help us to understand multiple acylation reactions in more complicated systems. As such, altering the ring size could also be considered, instead of medium sized cyclic ring expansions, slightly larger macrocycles can be tested, where the advantage over medium sized ring is, they are tending to be less structurally rigid with greater extend of flexibility to accommodate intramolecular cyclization to take place.

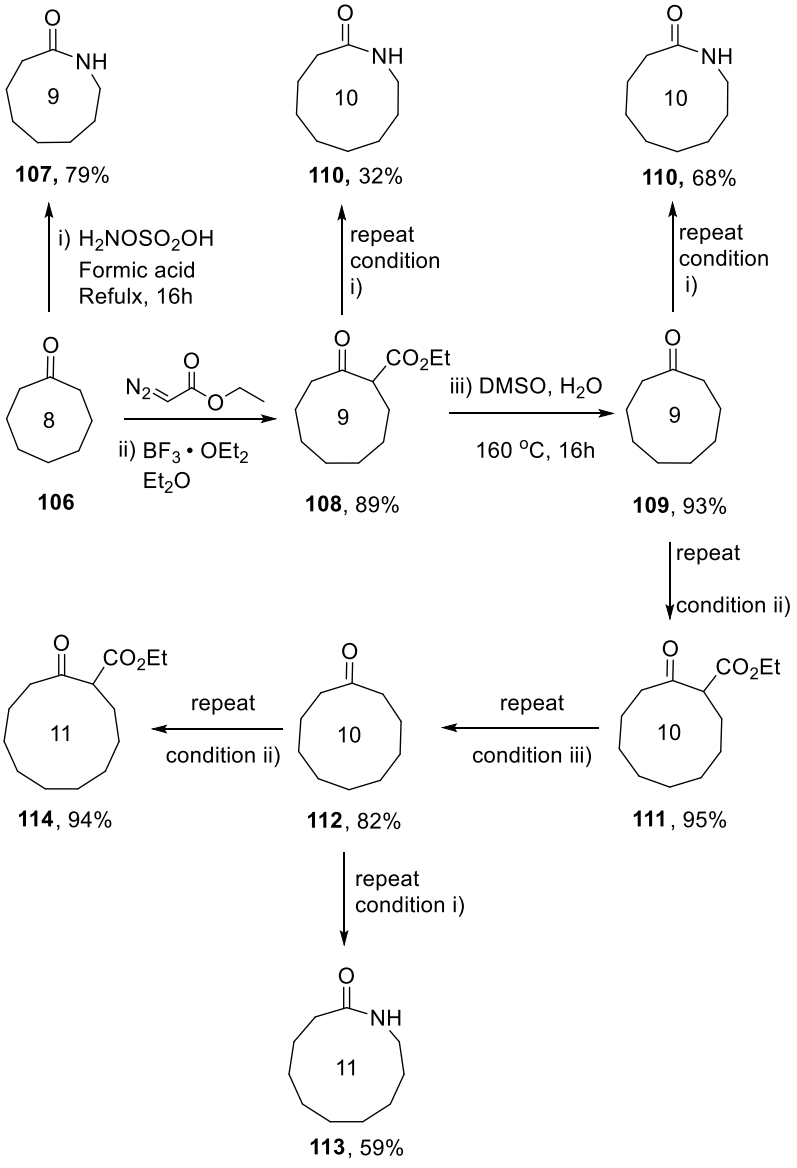
Chapter 3. Ring expansion reactions on medium-sized rings to macrocycles

3.1 Medical potential of macrocyclic compounds.

Macrocycles derived from natural product origin have been known for over one hundred years with some examples illustrated in Figure 1. Many macrocycle containing natural products were found to be biologically active with impressive record of efficacy owning to the diverse functionality, stereochemical complexity, and pre-organized ring structure, leading to the capabilities to address biochemical space in many drug targets that most conventional small molecule drugs cannot. Consequently, macrocycles can exhibit high affinity towards protein targets and remain highly potent and target selective, whereas retaining extensive bioavailability to reach intracellular locations. [6b] It is reported that the relatively large surface area of macrocyclic drugs means they bind over extended areas and hence are well suited to modulate protein-protein interactions, protein-nucleic acid interactions, transcription factors, protease and phosphatases as drug targets,.[6a] However, due to the challenging synthesis of macrocyclic drugs and poor compliance with well recognized drug likeness rules as for small molecules, this structural class has been under explored in drug discovery. To address this, our ring expansion methods may offer a solution towards synthesis of diverse functionalized macrocyclic lead compound libraries. For a summary of existing macrocyclisation methods, see chapter 1.

3.2 Synthesis of medium-sized rings for successive ring expansion

For the ring expansion reactions developed in the Unsworth group, suitable starting materials are required, but not all are easily available from commercial sources. Thus, the main aim of the work in this chapter was to develop a reliable route to prepare medium sized cyclic β-ketoester and lactam starting materials to be tested in the group's ring expansion chemistry (SuRE). An overall synthetic summary is given in Scheme 24, with more detail to follow.



Scheme 24 Ring expansion methods for synthesis commercially unavailable starting materials.

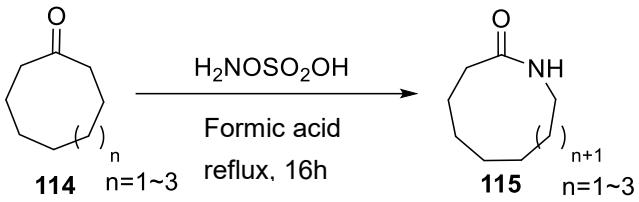
One of our aims was to make 9 to 11 membered cyclo-β-ketoesters **108**, **111** and **114**, thus filling the gaps in examples previously completed within the group and then attempt to complete the ring expansion series (ring size 5–8 and 12-membered starting materials had been completed prior to this project, but none 9–11-membered rings tested). The first problem needs to be resolved is that the 9, 10 and 11 membered cyclo-β-ketoester starting material are not available from commercial sources (or are at prohibitively high price for a very small quantity). Therefore, the first work that needs to be done is to provide readily available stock of cyclic-β-ketoesters for subsequent ring expansion reactions.

3.3 Ring expansion methods for cyclic starting material synthesis

Starting from available stock cyclooctanone, a series of reactions were designed to progressively generate desired 9, 10 and 11 membered cyclo-β-ketoesters after repetition of the Büchner–Curtius–Schlotterbeck reaction followed by decarboxylation.[31] Originated from linear compounds, such reaction can be facilitated to synthesis cyclo-β-ketoesters by one carbon ring expansion when the substrate ketone is cyclic, as illustrated in Scheme 30 below. Furthermore, the ketone precursors **106**, **109** and **112** were considered to be useful precursors to 9-, 10-, and 11-membered lactams, as they could also be prepared alongside the esters via the Beckmann rearrangement,[31] which is useful as these lactams are also commercially unavailable. Therefore, we had planned a route that could potentially allow us to generate difficult to access starting materials for two

separate reactions series from common intermediates. The successful realization of both strategies is described together below.

3.3.1 Beckmann rearrangement on cycloketones for ring expansion

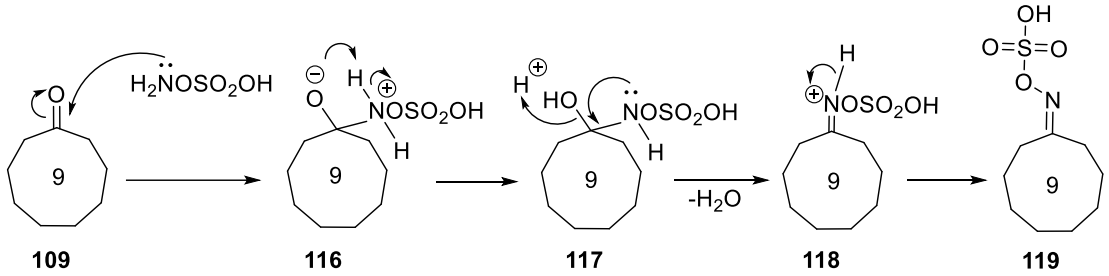


Scheme 25 General strategy of ring enlargement from cyclo-ketone to lactam via Beckmann rearrangement reaction

First reported in 1886, the conversion of ketoximes into substituted amides, the Beckmann rearrangement has been extensively studied.[47] One of the most important application of such reaction is to convert cyclo-ketoximes into lactams, as shown in Scheme 25 above. A famous example is the industry production of caprolactam, a 7-membered lactam from cyclohexanone, which then used in manufacture of a high strength material polycaprolactam widely known as Nylon 6 after ring opening polymerization under high temperature. [48]

For this project, we based the reaction conditions on literature conditions for the Beckmann rearrangement of related systems. Thus, the expansion of cyclooctenone **106** into lactam **107** as in scheme 24 above, achieved at good yield of 79% and was performed in a two-step sequence. The first step is the formation

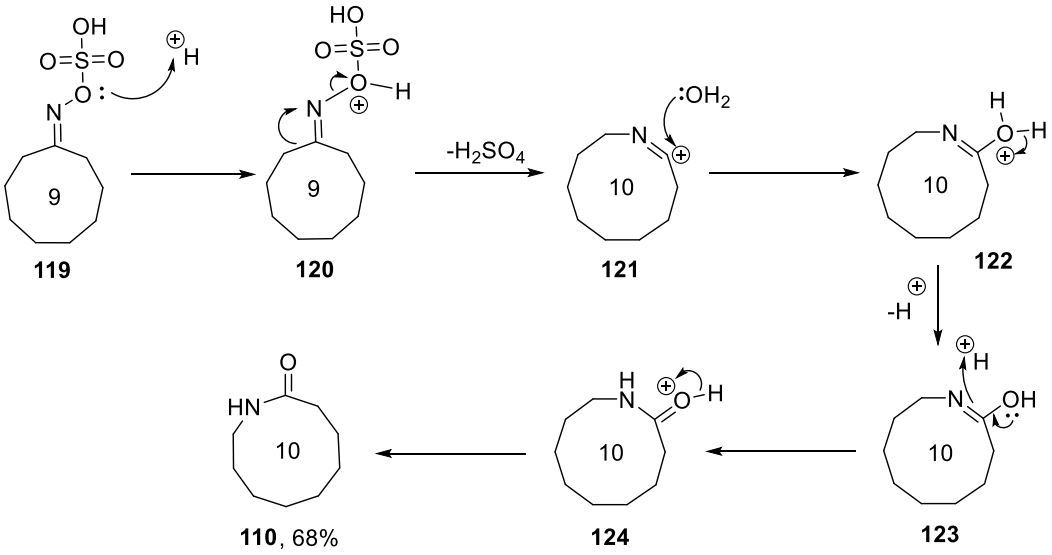
of an oxime via the addition of hydroxylamine sulfonic acid to cycloketone **109** via mechanism as in Scheme 26



Scheme 26 Formation of oxime for Beckmann rearrangement

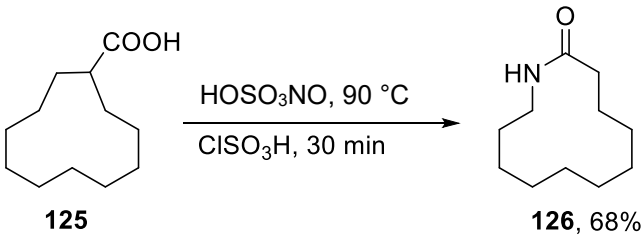
The second step is the Beckmann rearrangement, while the protonation of hydroxylamine sulfonic acid oxime results in the formation of sulfuric acid cation $H_2SO_4^+$, which is a much better leaving group compared to hydrogen sulfate HSO_4^- anion. The electron rich nitrogen as thermodynamic driving force then leads to rearrangement taking place, as the rate determining step for the overall reaction, the carbon-carbon bond breaks and migrates to nitrogen, with a new carbon-nitrogen bond formed and loss of sulfuric acid as leaving group. A free carbocation is formed as a result of rearrangement, and the addition of water is followed by tautomerisation, to produce the final expanded lactam **110**, as shown in Scheme 27. The reaction overall is similar to all other 1,2-rearrangement reactions such as hydride, or alkyl shifts,[49] as well as a rearrangement for synthesizing cyclo-β-ketoesters, which will be introduced later. Microwave heating was used for precise control in this case instead of conventional heating methods, where temperature was constantly monitored throughout the reaction,

microwave output wattage would change accordingly to minimal temperature fluctuation and maintain accurate control over reaction temperature compared to conventional oil bath.



Scheme 27 Formation of lactam after Beckmann rearrangement of oxime

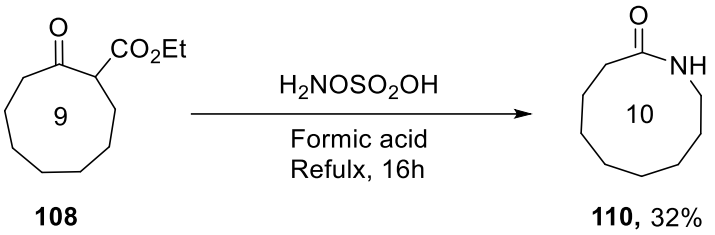
Additionally, a slightly unusual Beckmann reaction was performed on cyclo-carboxylic acid, instead of aliphatic carbonyl, the reaction started on carboxylic carbonyl site, leading to the rearrangement and generates lactam **126** as Scheme 28 showing as follows, where cyclocarboxylic acid was converted into lactam with moderate yields.[50]



Scheme 28 Beckmann rearrangement of cyclic carboxylic acid

This raised our interest on the plausibility of similar reactions on cyclic β-ketoester systems and potentially skipping the decarboxylation step. As Scheme 29 suggests,

attempts to perform a Beckmann rearrangement were made on cyclic-β-ketoester **108** directly, but the yield was found to be only 32%, which is lower than 68% yield compared to two step syntheses from cyclo-β-ketoester to lactam with additional step of decarboxylateion performed.



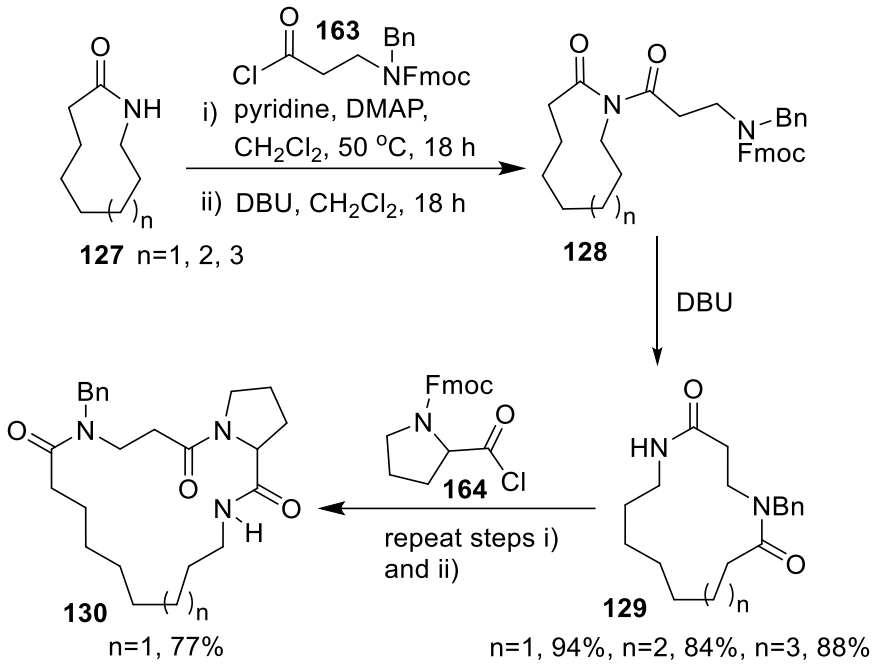
108 **110,** 32%

Scheme 29 Beckmann rearrangement on cyclic-β-ketoesters

The 10-membered lactam was, therefore, afforded on larger scale via the two-step synthesis, yielding around 68% of target compound and was identified by comparing its spectroscopic data to those previously obtained in the literature.[51] As described above, 9- and 11- membered cyclic lactams were also synthesized with 79% and 59% yield afforded respectively, the products were confirmed by NMR and mass spec, with data in accordance to those reported in literature. [51]

All cyclic lactams synthesized via Beckmann reaction were tested within the group using SuRE ring expansion method as presented in our recent publications for synthesis cyclic peptide mimetics,[31] with some example listed below in Scheme 19. All three products **107**, **110** and **113** were reacted with acid chloride to form imides followed by Fmoc cleavage with DBU, expanded successfully to produce 13- 14- and 15 membered novel lactams in good yields. In one case, where lactam was expanded for a second time using proline derived acid chloride as the

linear fragment in the SuRE reaction to form 17- membered macrocyclic lactam.[31]

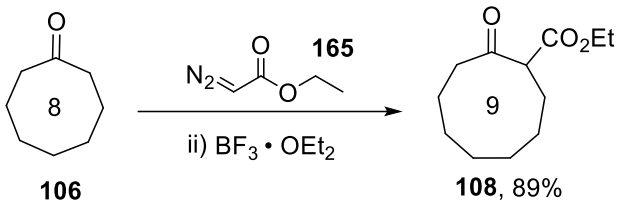


Scheme 30 Further ring expansions based on 9-11 membered cyclic lactams

3.3.2 Büchner–Curtius–Schlotterbeck reaction for ring expansion on cyclo-β-ketoesters

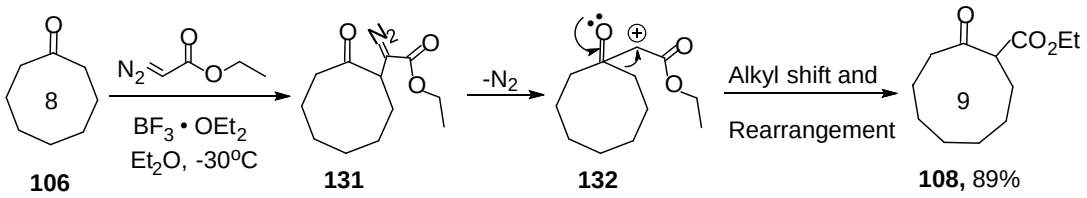
Enlarged 9-11 membered cyclo-β-ketoesters **108**, **111** and **114** were also targeted using a reaction conceptually similar to the Beckmann rearrangement via with one carbon expansion achieved by carbanion migration, namely the Büchner–Curtius–Schlotterbeck reaction, initially described as a reaction of aldehyde or ketone with aliphatic diazoalkanes to synthesize homologated ketones, which was latterly extended to cyclo-β-ketoesters.[52] For this instance, ketones or aldehydes are reacted with aliphatic diazoalkanes to produce homologated cyclic ketones. However, in this reaction the condition was changed slightly, whereby ethyl diazoacetate was used for to generate β-ketoesters. Some

literature also reported the use of Meerwein reagent in dilute CH_2Cl_2 solution in addition to ethyl diazoacetate,[53] however, such reactions involving Meerwein salts need to be closely monitored to due to the stability issues of the reagent itself.[53] As such, a similar reaction was taken into consideration, in which the sensitive Meerwein reagents replaced with more stable boron trifluoride diethyl etherate. Pleasingly, the method worked very well in general under controlled low temperature at 0 °C as shows below, enabling ketone **106** to be converted into expanded β-ketoester **108** in 89% yield.



Scheme 31 Ring enlargement via Büchner reaction

A general mechanism is shown in Scheme 32. The initial step of this reaction is nucleophilic addition of a resonance form of the diazo-ester nitrogen into the cyclic ketone, after which the compound undergoes decomposition in the presence of boron trifluoride, release nitrogen gas and forming a carbocation.[54] Owning to instability of the carbocation, the 1,2-rearrangement happens at this stage where the carbonyl group is reformed and the adjacent alkyl group migrates to the carbocation as shown.



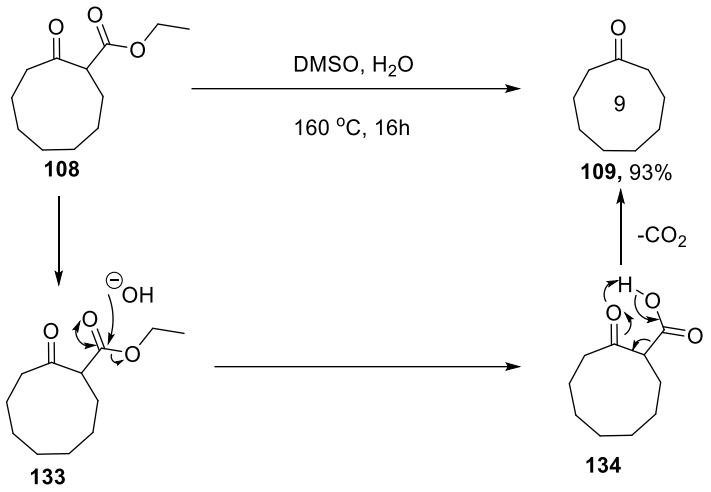
Scheme 32 Büchner reaction on cyclooctaone for one carbon ring enlargement

Notably, due to the scale of such reaction (c.a. 15 grams), conventional column chromatographic methods for purification became impractical in this case. Alternatively, fractional distillation under high vacuum was utilized to access the ring expanded cyclo-β-ketoesters with good purity. Once again, 10- and 11 membered cyclo-β-ketoesers **111** and **114** were afforded with similar manner adopted, with matching mass obtained from mass spec and integration ratio of α-H to aliphatic region from NMR (where 16H and 18H was observed for compound **111** and **114** respectively), carbon NMR shows distinctive carbonyl peaks for ketone at around 210ppm and ester around 170ppm; additional low temperature diagnostic carbon NMR reveals the enol form of the compound which also reassures the formation of desired product.

2.2.3 Decarboxylation of cyclo-β-ketoesters for further ring expansion

After synthesis of the cyclo-β-ketoester above, efforts were made to remove the ester group and provide access to the expanded cycloketone **109**. after decarboxylation methods were tested, in accordance with a literature report.[53] With rather simple reaction conditions, the decarboxylation was carried out by the mixture of cyclic β-ketoester **108** in water and dimethyl sulfoxide as solvent at

160 °C with overnight stirring.



Scheme 33 One-step hydrolysis and decarboxylation of cyclo-β-ketoester into cycloketone

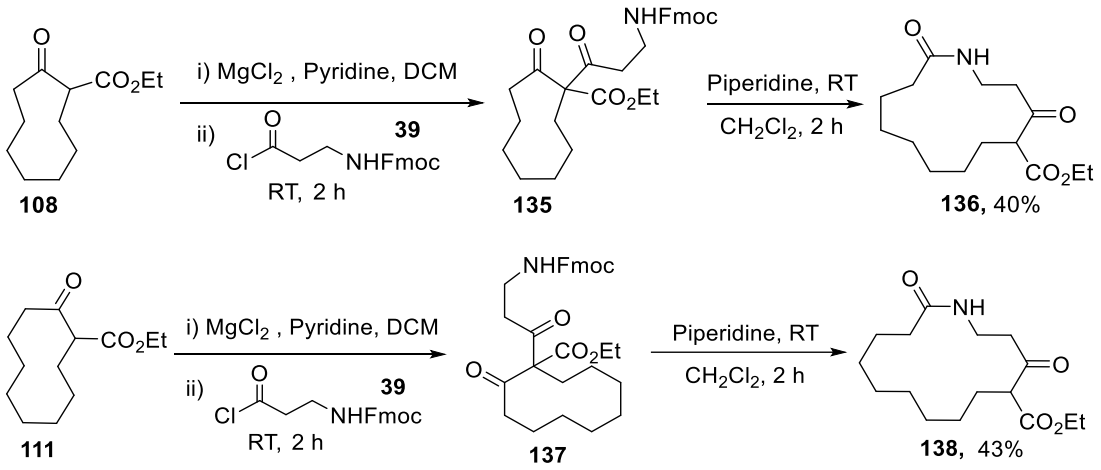
As Scheme 33 shows above, the reaction is entropically driven and is based on rather straightforward chemistry. The decarboxylation reaction generally consists of two parts; the first stage is nucleophilic attack of hydroxide on the ester **133**, this eliminates ethanol and produce a carboxylic anion which then decompose into carbon dioxide as gas evolved leaving an enolate. Resulting carbanion was stabilized via enol formation, the enolate formed will be then interconverted into cyclo-ketone via tautomerization to afford the desired substrate. Thanks to the nature of the reaction, both ethanol and carbon dioxide generated from the reaction as side produce could be removed easily, which leads to efficient reaction outcome with cycloketone product acquired at good purity. 10-membered cyclic ketone **112** was also afforded in similar manner with all data collected in line with those previously reported.

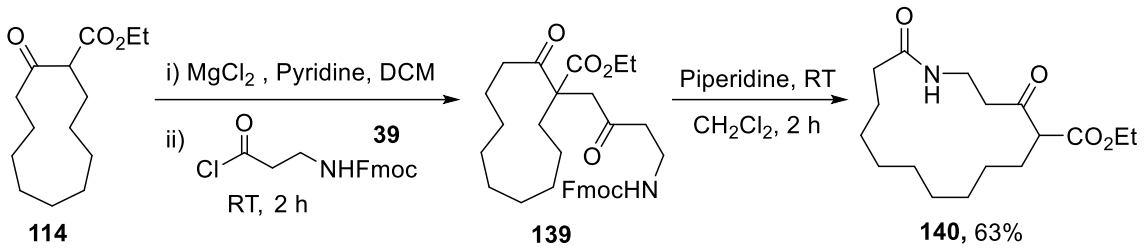
3.4 Successive ring expansion method on cyclo-β-ketoester ring expansions

After successful preparation of required substrates for the next step in the

synthesis, attempts were made to expand cyclo-β-ketoesters **108**, **111** and **114** with Fmoc-β-alanine as the linear fragment using the group's published successive ring expansion methods. To reiterate, 5- to 8- and 12- membered cyclo-β-ketoesters had been tested before[29-30], but 9- to 11- membered cyclo-β-ketoesters **108**, **111** and **114** were not, so these results are new variants of the method, performed on these ring sizes for the first time.

As illustrated in Scheme 34 , cyclo-β-ketoesters of 8 to 11 membered rings were subject to C-acylation of linear fragment Fmoc-β-alanine with $MgCl_2$ and pyridine in dichloromethane as solvent at ambient temperature. The intermediate tricarbonyl species was produced upon treatment with piperidine in dichloromethane, which led to cleavage of fluorenylmethoxycarbonyl (Fmoc) protecting group and release the free amine. Amine, attack onto the adjacent cyclic ketone, is followed by spontaneous ring expansion via fused bicyclic mechanism introduced in section 1.3 from the previous chapter.





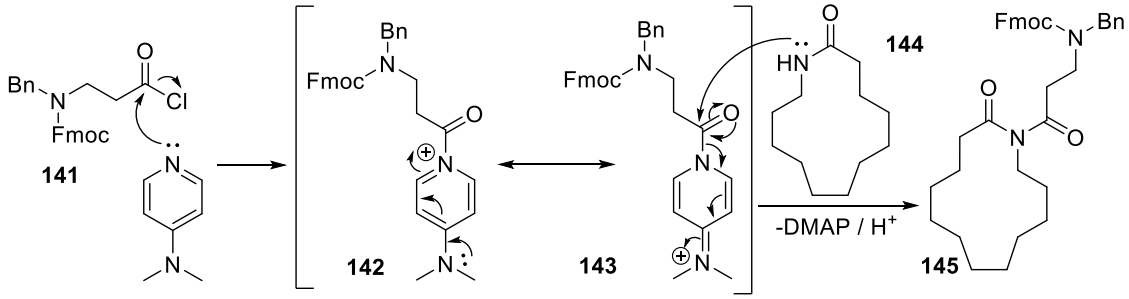
Scheme 34 Successive ring expansion method on 9 to 11 membered cyclo-β-ketoesters.

As described above, the reaction worked generally well with the desired ring expansion product afforded after a telescoped two-step sequence to our expectation. Since a well-established foundation was laid on cyclo-β-ketoesters from our previous work, the new ring expanded compound was identified by spectroscopic methods and mass spectrometry with ease in line with previous published results, where proton NMR gave the characteristic peak for broad amide around 6.3 ppm and triplet at 3.30 from its α-proton with J value of 6.8Hz; ^{13}C NMR gave a ketone peak and two ester/amide peak with same pattern, where 207ppm for ketone and 174, 170ppm for ester/amide in addition to matching number from mass spectrometry.[29] In summary, the ring expansion concept has now been verified to be successful on a complete series of 5 to 12 membered cyclo-β-ketoesters into 9 to 16 membered rings. Showing that the reaction works on all ring sizes is important is demonstrating its versatility.

3.5 Synthesis of cyclic peptide mimetics by the successive ring expansion of lactams

With the synthesis of 9 to 11 membered cyclo-β-ketoesters, cyclic lactams of ring size from 9 to 11 were also produced. Despite the emphasized potentials of successive ring expansion method, the heavy dependence on its relatively labile β-ketoester motif has limited the application of ring expanded product. Especially in medicinal and pharmaceutical utilities,[29] meaning additional steps such as decarboxylation are required to eliminate metabolically labile β-ketoester group. Due to the limitations of β-ketoester ring expansions described above, alternative cyclic lactam as a different ring system was taken into account, as a result, acylation reactions on cyclic amides was developed, with ring expansion attempts followed by base treatments.

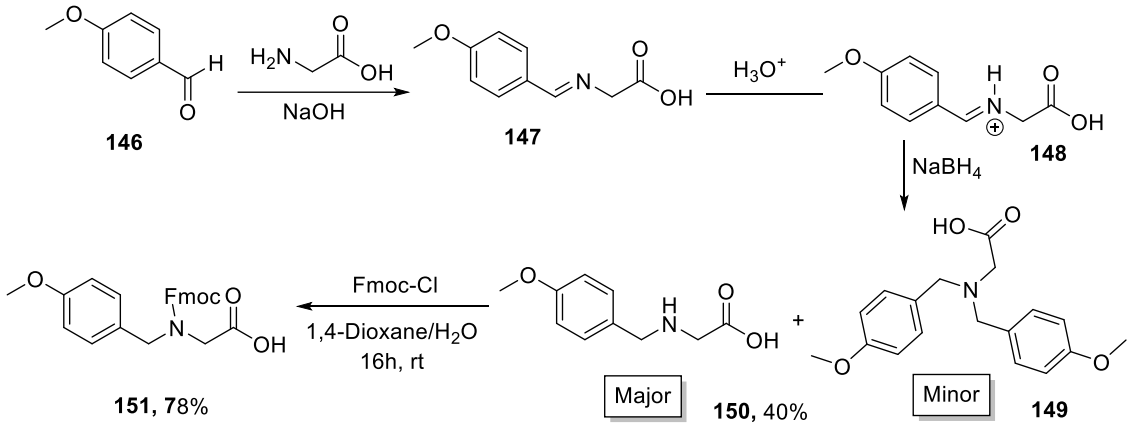
Owning to the relatively unreactive cyclic structure of lactam and higher pKa value of amide hydrogen compare to previous α-hydrogen from cyclic-β-ketoesters, acylation reactions on cyclic amide would be very slow and requires further kinetic control such as additional catalyst or higher temperature. The reaction conditions were, therefore, optimised with catalytic 0.1 mol equivalent of DMAP added and amide acylation was carried out under overnight reflux at 50 °C in DCM, and it is still a much slower acylation even with catalysts involved in contrast to 2 hours acylation of cyclo-β-ketoesters at ambient temperature as described above. Showing in Scheme 35 below, with pyridine present as a base, DMAP act as a catalyst during acylation on cyclic lactams.



Scheme 35 DMAP catalysed cyclic lactam acylation

3.5.1 Linear fragment synthesis and ring expansion on cyclic lactams

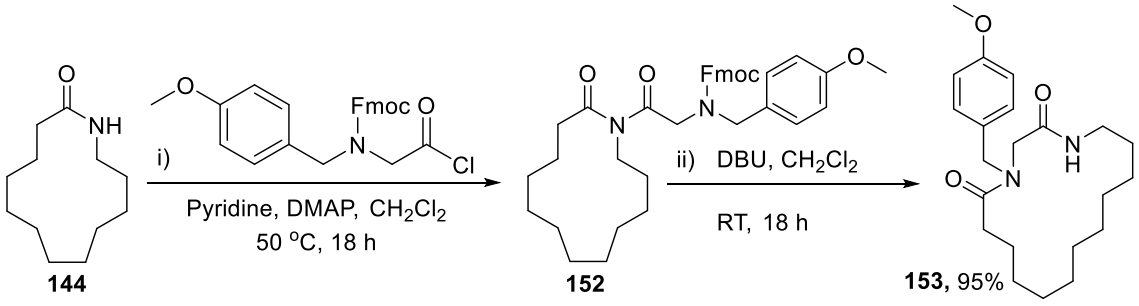
Having discovered an efficient ring system for ring expansion reactions with laurolactam **144** been successively expanded, the exploration of reaction scope has become a new interest to us, including the integration of successive ring expansion methods into different ring sizes accompanied by incorporation of various linear fragments. In this regard many of α-amino acid fragments were constructed, one of them being Fmoc protected (4-methoxybenzyl) glycine.



Scheme 36 Synthesis of Fmoc protected (4-methoxybenzyl) glycine fragment for ring expansion

According to standard procedure of lactam acylation, in order to acylate 1 equivalent of lactam, additional 3 equivalents of protected amine fragment are required for the reaction. As such, targeted Fmoc-protected amino acid was in need to be synthesized in gram scale to satisfy reaction consumption showing

above in Scheme 36. Where inexpensive 4-anisaldehyde and glycine were used as starting material in this case, forms imine under base catalysis. Sodium borohydride was then used to perform reductive animation, producing (4-methoxybenzyl) glycine and di-substituted amide species as minor side product, which could be removed easily by gravitational filtration. The secondary amine produced was pure enough to carry on next step according to NMR, where accurate proton integration with splitting pattern was found, chemical shift of two doublet aromatic peaks at 7.06 ppm and 6.77 ppm accordingly with J value of 8.5 Hz, a distinctive singlet at 3.69 with integration number of 3 from remote methoxyl group, and distinctive singlet from CH_2 group at 4.52ppm, all peaks was found to be exact match of literature publication without any impurity peaks, with exact mass fount at 397.2462 for $C_{22}H_{34}NNaO_3$. Fmoc protected amino acid linear fragment was produced in this way after overnight reaction with Fmoc chloride in 1,4-dioxiane and water as solvent at ambient temperature.

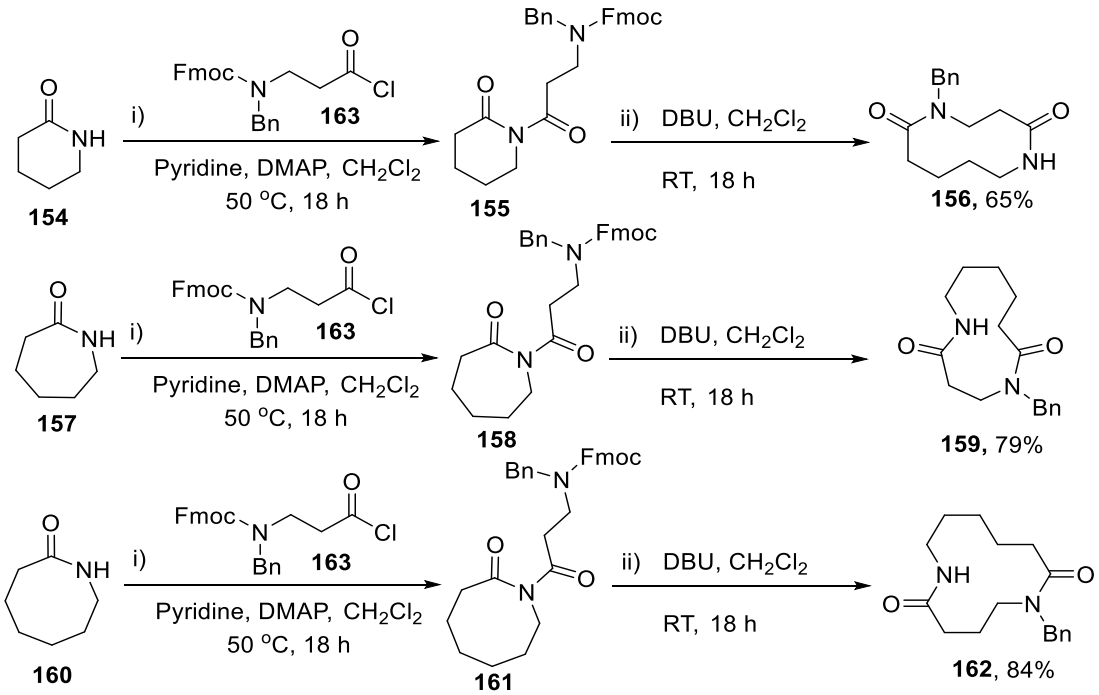


Scheme 37 Ring expansion on laurolactam with (4-methoxybenzyl) glycine as linear fragment.

The Fmoc protected fragment was then utilized in ring expansion of laurolactam **144**, after consecutive two step synthesis as described, showing above in Scheme 37. However, the yield was relatively low and isolation by column chromatography was difficult due to very close in R_f values of starting material and ring expanded

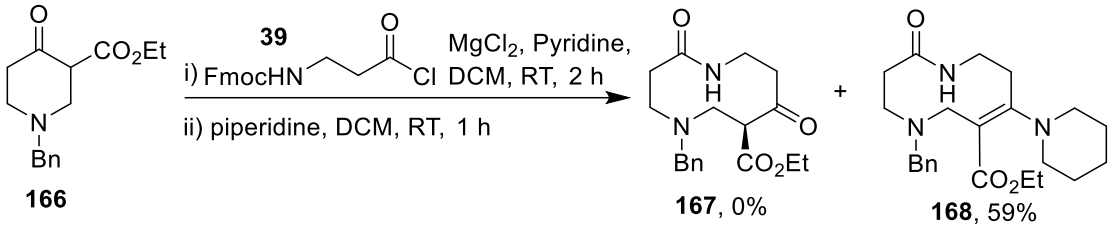
compound, further optimization is necessary to improve the acylation outcome, presumably longer reaction time and slightly higher temperature. Otherwise the purification of acylated intermediate would be necessary to ensure the purity of final ring expanded product, since acylated lactam with Fmoc group will have significant difference than starting material in polarity for easier isolation.

Above example has demonstrated the potential for different amino acid derivatives to be used for ring expansion reactions, also we wish to reveal the impact of ring expansion methods upon variation of ring sizes. Thus, 6 to 8 membered cyclic lactams were acylated with same linear fragment as used previously in laurolactam **144** ring expansion, and standard procedure was applied. Showing the consistency of ring expansion methods, all three lactams were successfully expanded after overnight treatment of DBU in dichloromethane, all product with good yields were observed showing below in Scheme 38.



Scheme 38 Ring expansion of lactams in variation of ring sizes

Instead of using structurally simple Fmoc-β-alanine, this linear fragment was further modified with additional benzyl group attached to the secondary amine, converting to a tertiary amine as a building block for ring expansion reactions, and ring expanded product with integration of linear fragment **163** shown above in Scheme 38. Moreover, DBU was used in place of piperidine as our research finding showed DBU as a base would produce a reliable result and better consistency, whereas less bulky and more nucleophilic piperidine could leads to erratic product formation shown in Scheme 39 as follows.[29]



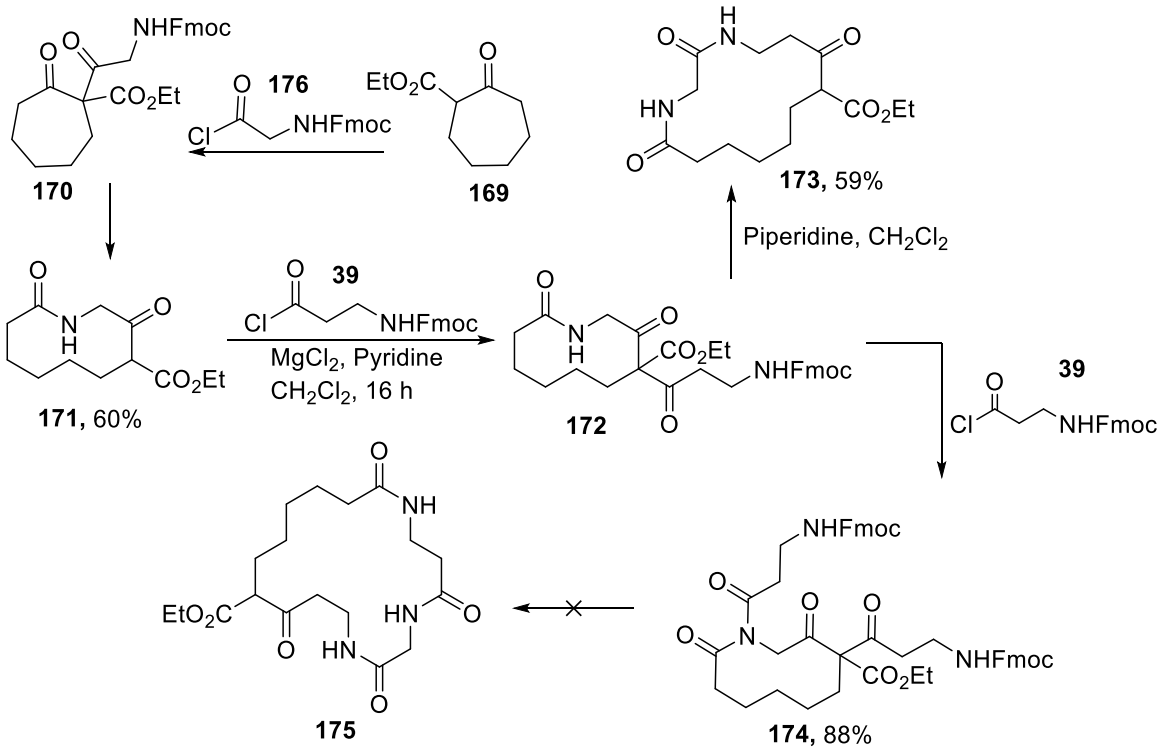
Scheme 39 Piperidine as base forming adduct to the product and leads to erratic results

3.6 Attempted successive acylation reactions on cyclic lactam

Referring back to the previous double acylation work on benzosuberone ring expansions in Chapter 2, due to unsuccessful attempts on double acylated ring expansion reactions, a ring expanded compound **171** with both amide and β-ketoester functionality were selected to further simplify the ring structure, and eliminate potential steric impact of aromatic group from previous ring expansion reactions. Starting from 7-membered compound **170**, the reaction was brought forward with a simple linear fragment acylated, expanded using general reaction procedure as described before. The ring expanded substrate **171** was further

acylated with linear fragment followed by the treatment of piperidine which was expected to lead to the successive ring expanded product **175**.[30]

Knowing from previous work in the group that the compound **171** could undergo successive ring expansion after C-acylation next to β-ketoester, precursor **172** was isolated and further decorated with same linear fragment at nitrogen site, using the general procedure as for nitrogen acylation reactions. The double acylated compound **174** was isolated as a pale-yellow solid in good yield (88%), the compound was generally stable at ambient temperature and characterized by proton NMR where distinct amide peak at 8.26 ppm was missing compared to starting material, and a triplet at 1.25 ppm with J value ot 7.1 Hz indicates the survival of ester group after a set of reactions, carbon NMR gave 2 ketone peaks at 202.2 ppm and 199.9 ppm, with 5 amine/ester peaks at 175.2, 171.3, 167.6, 156.4 and 144.5 ppm respectively, overlapped aromatic peaks also gives a good indication of fomc protecting group presence. High-resolution mass spectrometry gave the correct mass found at 850.3310 in accordance to calculated mass. After the confirmation of successive acylation reaction, the di-acylated compound was then subject to ring expansion investigations, with DBU used as the base.[29]



Scheme 40 Attempted bi-acylated ring expansion on cyclic lactam

However, despite efforts made on best reduce possible side reactions, attempted ring expansion on double acylated compound was found to be unsuccessful. Only deacylated compound **171** was recovered after an overnight reaction with DBU at room temperature. This suggests that the acylated fragments were cleaved and returned starting material, the multiple acylation reactions are more complicated than expected.

3.7 Conclusion and future prospects

This project was tailored to fit into the overall research trajectory within the group, and, therefore, different ring expansion methods were applied to synthesize commercially unavailable 9 to 11 membered cyclic β-ketoesters and lactams. The

ring expansion series was completed with all 5 to 12 membered cyclic β-ketoesters successfully expanded using Fmoc-β-alanine as linear building block. successfully

Access of 9 to 11 membered lactams from Beckmann rearrangement have also filled in the gap of 4 to 13 membered cyclic lactam ring expansions. The reaction scope was explored extensively from variation of ring sizes, with successful integration of series of building blocks of medicinal interest, such as α and β-amino acids, peptiod sub-monomers, hydroxy acids and even with the application on elongation of linear compounds.[31]

Moreover, the impact on different reagent and condition to ring expansion reactions were developed in regards to previous ring expansion reactions. As for the acylation reactions, solvent and pyridine were used with consistency, where acylation of lactam would usually require overnight reflux and addition of DMAP in contrast to $MgCl_2$ with completed reaction in 2 hours. The ratio of linear fragment to substrate was found to be optimal using 1.5 equivalents instead of 3 equivalents for cyclo-β-ketoester reactions, the significant reduction of material consumption would also help the synthesis since some of the slightly complicated linear fragments for ring expansion reactions are very time consuming to make.

The choice of amine protecting group is another important factor and was also

examined to test the impact on efficiency of ring expansion reactions. Other than the typically used Fmoc group, the Cbz group was also taken into consideration; the advantage of using the Cbz group is that the deprotection of this group is considered to be generally clean, where hydrogenation leads to the evolution of carbon dioxide and toluene, both which can be removed without effort. Thus, reducing the potential effect of base used in common deprotection reactions and simplifies the result for further analysis, similar reaction could also be tested on benzyl and Boc groups. Later in this thesis (compound **202** from chapter 4) examples of related reactions which Cbz is used are included.

Furthermore, all cyclo-β-ketoesters and cyclic lactams synthesized from first step ring expansion could be subject to further ring expansions. Leading to successive ring expansion reactions of more peptide bearing macrocycles, such compound could be constructed with wide range of functionalities introduced. On top of this, the diacylated ring expansion could be optimized based on preliminary unsuccessful ring expansions from benzosuberone and cyclo-β-ketoesters ring expansions. According to typical conformational effects, medium sized rings usually have relatively large transannular ring strains, which could have an effect on the efficiency of the ring expansions, making them less likely to proceed well. However larger ring sized macrocycles could be tested such as previously expanded 24-membered cyclo-β-ketoester, presumably with much lower ring strain to overcome for ring expansion reactions.[55]

Compared to the arithmetic growth of successive ring expansions, multiple acylation could expand the macrocycle geometrically, leading to the synthesis of macrocycles with enormous molecular weight, potentially offering a polymerization approach to acquire macrocyclic peptide or even protein as described in Chapter 1.

It is worth noting that all products generated from ring expansion reactions can be utilized for further ring expansions, standing on current construction blocks tested, a wide range of compound library with diverse functionalization can be synthesized as such, further screening can be performed to evaluate the biological activities and potential as lead candidates.

Chapter 4. Template effects and the synthesis of polyethylene glycol linkers for ring expansion reactions

4.1 Polyethylene glycol monomer as linear fragments for ring expansion

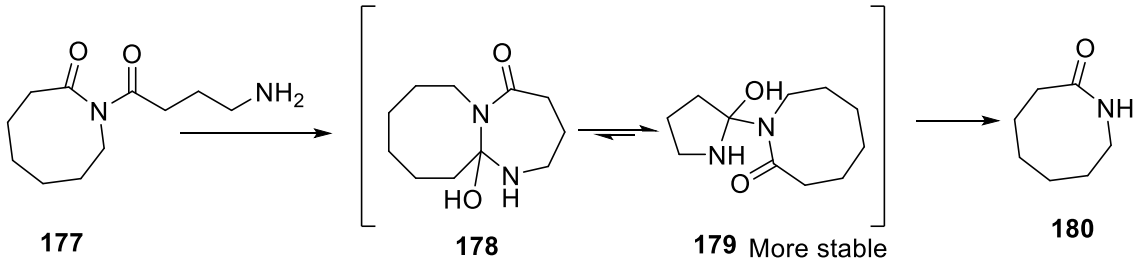
Nearly all ring expansion reactions carried out to date in the Unsworth group have used linear fragments of 3- or 4-members in length, and therefore proceed via 5- or 6-membered intermediates which are kinetically favorable. Proceed via 5- or 6-exo-trig ring closure, and lead to spontaneous ring expansion with breaking of a C-C bond after intermediate cyclol formation according to Baldwin's rules for ring closure summarized in Table 1 below.[55]

Ring size	3		4		5		6		7	
type	exo	endo	exo	endo	exo	endo	exo	endo	exo	endo
tet	Y		Y		Y	N	Y	N	Y	N
trig	Y	N	Y	N	Y	N	Y	Y	Y	Y
dig	N	Y	N	Y	Y	Y	Y	Y	Y	Y

Table 1 Baldwin's rules for ring closure prediction

However, in practice, the formation of cyclized cyclol intermediates from longer linear fragments (> 5-members) have all failed; the ring expansion step was found to be unsuccessful with decomposition of acylated intermediate and gave back to

starting material. This is due to the nature of cyclization intermediates, where 5- and 6-membered cyclization are usually always kinetically and thermodynamically favorable compared with larger ring sizes, such as 7-membered cyclization reactions. This often leads to the formation of much more stable 5- and 6-membered intermediate via attack into the exocyclic imide carbonyl, followed by self-cleavage of acylated compound **177**, as example shown in Scheme 41 below.



Scheme 41 Kinetic and thermodynamic competition on formation of intermediate cyclols

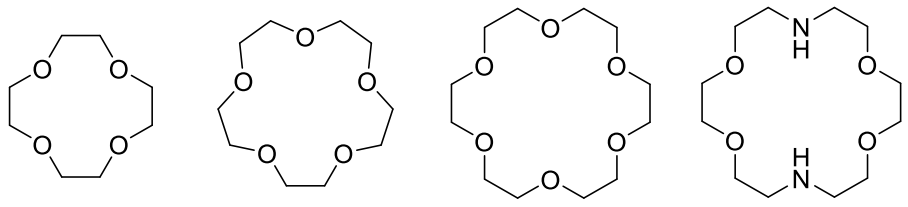
Nonetheless, our ambition was not restricted by such limitations, since one step expansion of macrocycles with much longer linear fragments involved remain as a challenging topic in macrocycle synthesis and to achieve such one step synthesis of longer linear fragments would significantly improve the efficiency of macrocycle synthesis.

4.1.1 Crown ether and PEG related compounds

Our focus has therefore reached crown ether type synthesis; the plan was developed to incorporate ideas from the crown ether synthesis strategy into ring expansion reactions, using metal cation as template to synthesis a crown ether

cyclol intermediate followed by ring expansion, producing macrocyclic polyether structure in addition to parent cyclic substrate. Thus, lengthy linear fragments can be integrated to ring expansion reactions.

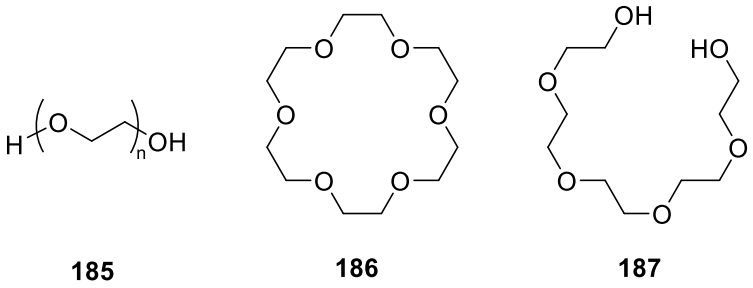
Crown ethers, or macrocyclic polyethers are a class of cyclic compound consisting of repeating ether groups (-CH_2CH_2O-) and a size variable cavity in the center depending on number of repeating ether units. Due to the presence of the lone pairs, oxygen atoms from ether group can act as Lewis base. Such compound was considered to be the first subclass of macrocycles ever synthesized with explicit function to structure relationships, as shown in Scheme 42 below. The discovery of crown ethers has led to the award of the Nobel prize in 1987. [56]



Scheme 42 Structures of common crown ethers, **181)** 12-crown-4, **182)** 15-crown-5, **183)** 18-crown-6, **184)** diaza-18-crown-6

The unique structure feature of crown ethers offers some of the most interesting properties among other macrocyclic compounds, such as strong affinity on binding to certain cations, such as Li^+, Na^+, K^+ and Mg^{2+} ions, this enables the versatile utilities of crown ether ranging from phase transfer catalysis,[57] isotope enrichment,[58] metal extraction,[59] and molecular probing.[60] Diaza-crown ethers,

with additional nitrogen in place of existing oxygen atom, derived from crown ethers with enhanced selectivity which binds to Mg^{2+} and Ca^{2+} ions, such property leads to the application on ion selection electrodes,[61] recent literature also reported the fluorophore modified aza-crown ether has demonstrated the selective binding towards Cu^{2+} ion, which could be used as molecular sensors.[62] Metal ion such as Zn^{2+} complexed with aza-crown ethers was also reported to exhibit catalytic activities in nucleic acid, phosphate ester and protein hydrolysis.[63] This class of compound is of particular interest to us is that proposed ring expansion product will have cyclic amine group in parallel to polyether structures, of which may share more similar characteristics in common.



185 **186** **187**

Scheme 43 General structure of polyethylene glycol and structure similarity of pentaethylene and 18-crown-6

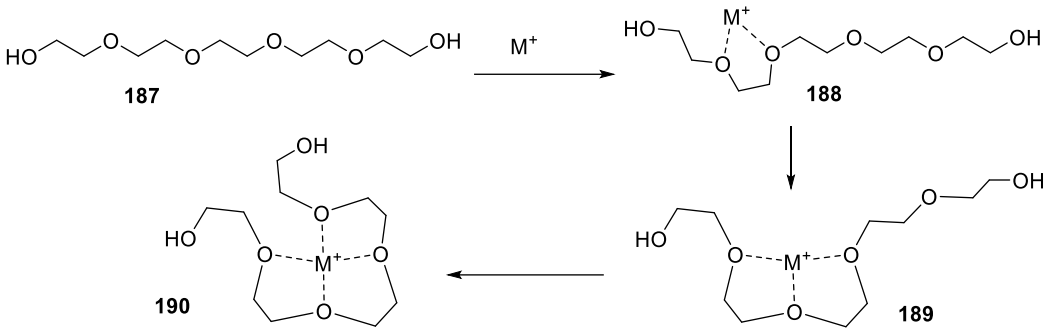
Polyethylene glycols (PEGs), as a precursor for crown ether synthesis, can be regarded as linear form of cyclized crown ether, containing repeat subunit of ether groups to the corresponding crown ether, and hydroxyl group at both ends. Such structural features have many attractive properties and this has led to numerous industrial and commercial applications since they were first discovered, such as

use in cosmetics, detergents, paper coating and polyurethane productions.[64] Since PEG compounds are generally biological inert and considered to be non-toxic and non-immunogenic, additional interesting features found on this class of compounds to have wide impacts on biological activities have also attracted growing attention in pharmaceutical researches during past decades.[65] Functionalized PEG derivatives have also demonstrated to be a valuable reagents that participate in a wide range of reactions based on active functional groups attached, with reported applications on fluorescent probing for imaging and therapy,[66] tissue engineering,[67] and anti-tumor prodrug synthesis.[68] PEG modified peptides were demonstrated to have improved solubility, prolong stability and reduced immunogenicity, which could be used to assist protein drug formulation and deliveries,[69]

4.1.2 Template effects and ring expansion

For all general ring expansion reactions, particularly for cyclization of macrocycles, the reaction rate for cyclization is considerably slow where most cyclization reaction of crown ethers would require few days under high dilution conditions.[19] In order to accelerate the rate of cyclization reactions, preorganization is required for correct alignments of linear fragments into a cyclic conformation where cyclization can take place in a relatively faster reaction rate. Owning to the polyether structure, metal ions can be employed to coordinate on oxygen atoms, acting as template for polyether side chain to wrap around, preorganization can

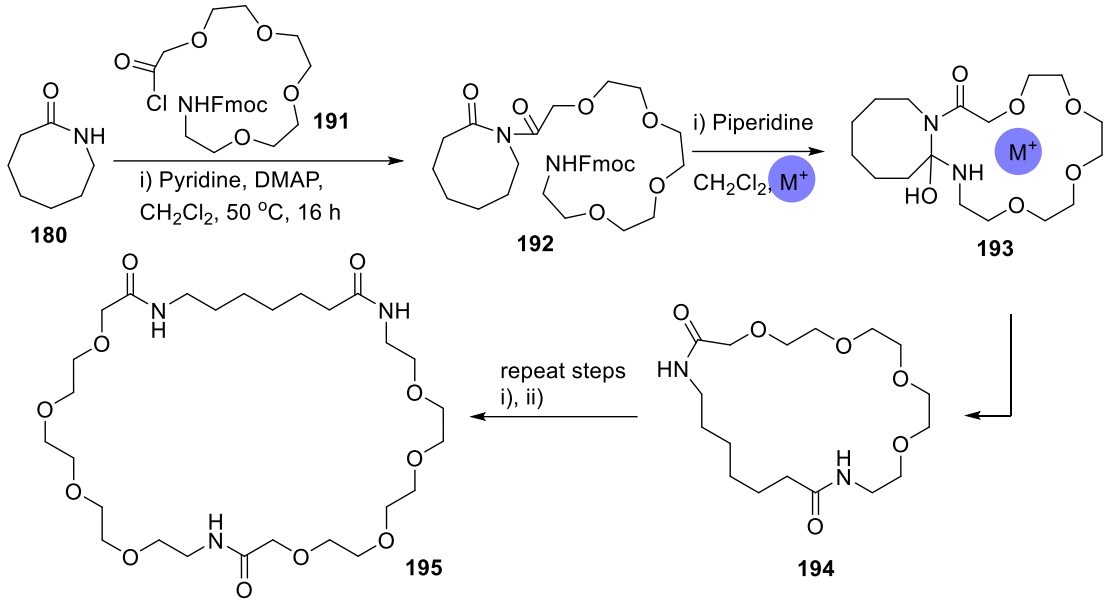
be reached efficiently in this approach without high dilution conditions.



Scheme 44 Preorganization of PEGs with metal cation as template for cyclization

Overall, it could be interesting to synthesize a macrocyclic compound combining impressive features from both crown ethers and polyethylene glycols described above, using successive ring expansion methods. A new project was therefore planned to incorporate polyethylene glycol compounds as linear fragments, which can be cyclized into fused cyclol crown ether intermediate as described above. Since the most important compound of crown ether class is tetramer **4.3**, pentamer **4.4**, and hexamer **4.5**, to address this, linear tetraethylene glycol (4EG), pentaethylele glycol (5EG) and hexaethylene glycol (6EG) as precursors corresponding to crown ethers were selected as building blocks to synthesis ring expanded compounds bearing similar structural features from both crown ether and polyethylene glycol. Macrocycles such as 12-membered β-ketoester, 13-membered laurolactam as well as 8-mambered cyclic lactam were used as substrates for PEGylation and consequent attempts of ring expansion reactions. As illustrated in Scheme 45 below, the idea is to expand the parent lactam by implementing template effects which analogues to crown ether synthesis, with modified polyethylene glycol as linear fragment acylate onto cyclic substrates, the

PEG chain can then cyclize with aid from metal cations M^+, leads to transannulation of PEGs, forming a crown ether like cyclol intermediate **193** followed by spontaneous ring enlargements. The successive ring expansion could also perform on such ring expanded products **194**, leads to geometric growth of macrocycles **195**, showing as follows



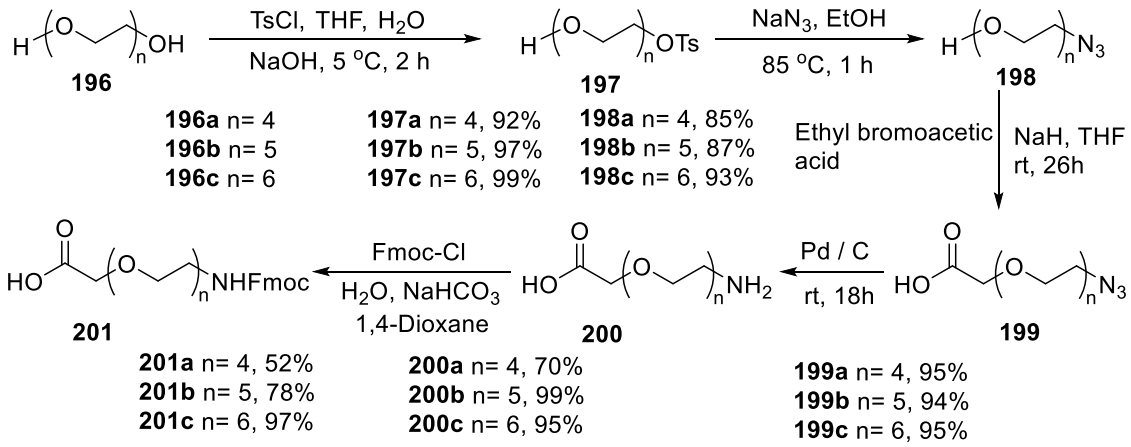
Scheme 45 Proposed successive ring expansion with tetrathylene glycol as building fragment to achieve massive ring expansion in one step.

Macrocycles synthesized via this strategy will have more lipophilic character compared with crown ethers while retaining certain degree of flexibility for coordination, which offers a good starting point for development of phase transfer catalysts and drug transporters.

4.2 Synthesis of 'PEGylated' amino acids for ring expansion reactions

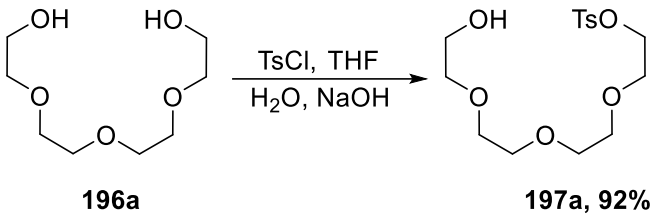
For employment of successive ring expansion methods on PEG compounds, the linear building block needs to be modified with well-established two step

synthesis sequences, by converting two hydroxyl ends into carboxylic acid and protected amine, respectively. Since most of our previous work on ring expansion reactions was carried out with Fmoc as protecting group for amine protections, the initial attempt of side chain synthesis was also brought forward in line with previous work, where Fmoc was used to protect amine group. Thus, the synthesis of Fmoc protected PEG linkers with different length ranging from 4 to 6 were proposed as workflow showing in Scheme 46 below.



Scheme 46 Workflow of synthesis Fmoc protected PEG linkers for PEGylation

According to previous literature synthesis, the most commonly used tetraethylene glycol (4EG) **196a** was used in large excess of 10 to 1 equivalent of 4-toluenesulfonyl chloride in mixture of water and THF as solvent, with presence of sodium hydroxide as base.[70] Such an overwhelmingly excess helps to ensure the formation of desired mono-tosylated instead of ditosylated side product after nucleophilic substitution, showing below in Scheme 47.



196a **197a, 92%**

Scheme 47 Mono tosylation of tetraethylene glycol.

In consideration of such excessive usage of PEGs for tosylation, it is worth noting that starting pentamer and hexamer PEG compounds **196b** and **196c** are relatively expensive, and given the fact that the same reaction has been repeated numerous times over the past decades, it is surprising that no publication has ever questioned the redundancy of using such excess 10 equivalents of reagent for tosylation reactions, to the best of our knowledge. Promoting green chemistry is also important for chemists to reduce waste and protect environments. As such, additional work was carried out to push the limit with minimal reagent usage while achieving good mono-tosylation on PEG compounds as from Scheme 47, results summarized in Table 2 below.

PEG (eq)	TsCl (eq)	Mono-tosylation	Di-tosylation	Yield (%)
10	1	100 %	0 %	89
8	1	100 %	0 %	90
6	1	100 %	0 %	94
4	1	> 99.9 %	< 0.1 %	98
2	1	85.7 %	14.3 %	Mixture

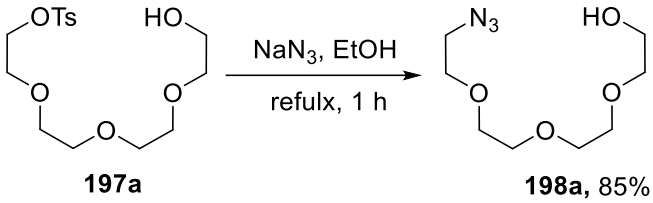
Table 2 Variation of 4EG equivalencies in tosylation reactions

Setting up a rather straightforward reaction, different equivalencies of tetramer PEG was tested at small scale with TsCl, where 8 eq, 6 eq, 4 eq, and 2 eq of PEG

was tested on tosylation of 1 eq of TsCl compound, the result is rather satisfactory where 8 and 6 eq of PEG showing excellent mono-tosylation and 4 eq of PEG has also produces good mono-tosylated PEG, based on their ^{1}H NMR spectra, where ratio of mono-tosylation to di-tosylation can be determined by comparing proton integrations from aromatic region. With trace amounts of di-tosylated PEG only detectable using sensitive high-resolution mass spectrometry, where 2 eq of PEG leads to formation of di-toyslated PEG with 1:6 ratio to mono-toyslated PEG according to its proton NMR spectra integrations. Using different equivalent of PEG was found to have no significant impact on the yields of reaction with consistent 90 to 98% mono-toyslated PEGs afforded, however, the less PEG used means more mono-toyslated PEG could be generated from same amount of PEG input, therefore this leads to higher efficiency and greater overall yield. In summary, we found that using 4 equivalents of PEG is sufficient to produce mono-toyslated PEG compounds with good purity. Using less PEG precursor will reduce waste and lower costs, which also is more favorable to the environment especially when the compound was synthesized on scaling up industrial productions.

The mono-tosylated compound **197a** was afforded after an aqueous wash with 92% yield identified with good purity according to ^{1}H NMR and mass spec without needing further purification. For the next step, sodium azide was used as nucleophile to substitute the tosylate group, which was done in ethanol at reflux (85 $^{\circ}$C), thus, introducing azide group into the molecule as in Scheme 48. The yield was good in general with around 85% of being typical, again with excellent purity

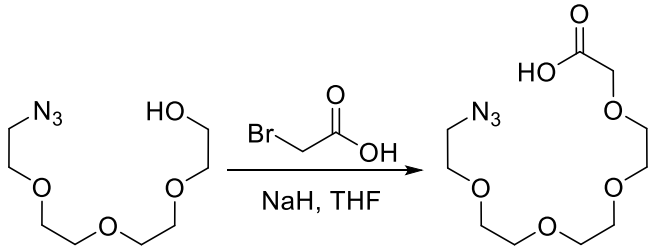
and further purification not required based on TLC analysis.



197a **198a,** 85%

Scheme 48 Formation of monosubstituted azide ethylene glycol

The solid bromoacetic acid is a relatively strong alkylating agent widely used in industry, and was used to treat compound **198a** acquired from previous step to convert the free alcohol into carboxylic end. Different combination of base and solvent were tested for optimal reaction conditions; a combination of 5 equivalent of potassium hydroxide in DMF, in comparison to 2 equivalent of sodium hydride in THF were tested. The later was shown to be far superior with alkylated compound **199a** acquired at 95% yield after reaction overnight under inert atmosphere as in Scheme 49.

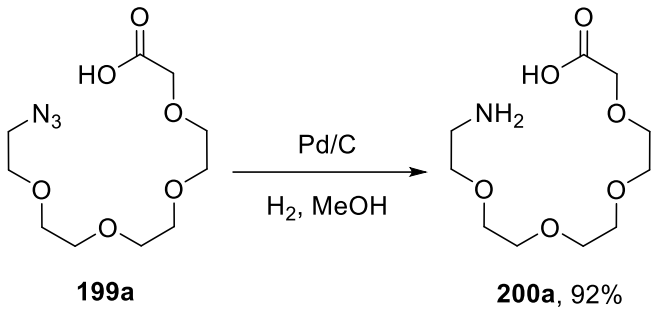


198a **199a,** 95%

Scheme 49 Alkylation of PEG azide with bromoacetic acid

The resulting azide-4EG-acid **199a** was subject to hydrogenation, for the reduction of azide to amine group, of which 10 molar equivalents of 10% palladium on carbon as catalyst in methanol was used to dissolve compound **200a**, followed

by overnight reaction under hydrogen atmosphere.



199a **200a**, 92%

Scheme 50 Hydrogenation of Azide-4EG-acid forming amino-4EG-acid

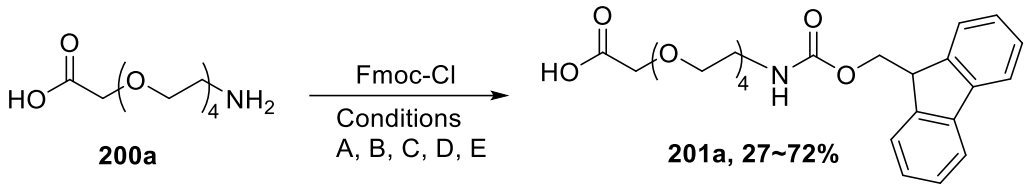
Thus, we showed that the proposed reaction route has worked smoothly to make linear tetramer PEG amino acid **200a**, with excellent multistep yields, and the product synthesized with this work flow was determined to have excellent purity according to proton NMR where exact integration for compound **200a** was acquired, particularly clean in polyether region with reference to singlet of $COCH_2$ and reoccurring NH_2 signals, the integration reassures the starting material **199a** has gone depletion. Hence, the same procedure was applied to pentamer and hexamer PEGs to synthesis modified amino-PEG-acid compounds as demonstrated in Scheme 46, all with excellent multistep yields ranging between 85 to 95% and good purity, this eases the difficulty of column chromatographic purification of such polar amino acid polyether compounds.

4.3 Protecting strategies on polyethylene glycol linkers

Before implementing the template effects for crown ether synthesis, tetramer, pentamer and hexamer PEG chains needed their free amine group needs to be protected before attempting acylation and ring expansions.

4.3.1 Fluorenylmethyloxycarbonyl protection on amino-PEG-acids

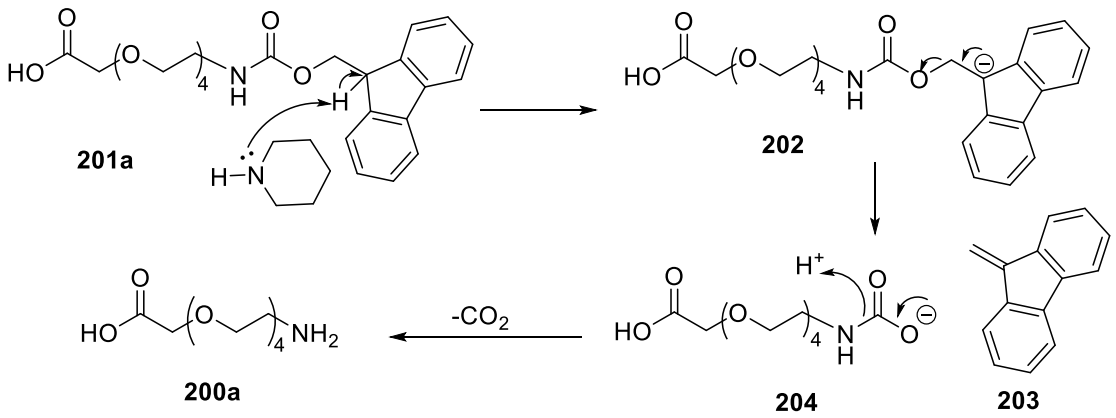
The 9-fluorenylmethoxycarbonyl (Fmoc) group is a bulky aromatic tricyclic compound with two phenyl groups fused onto a five membered ring, which binds to amine via forming a β-nitroethyl acetate, commonly used protecting group for amines showing below in Scheme 51.



Scheme 51 Fmoc protection of amino-4EG-acid as linear fragment for ring expansion reactions

With exceptional resistance toward acids, carboxylic acids can be converted to acid chlorides with oxalyl chloride where protected amine group remain untouched as commonly used in our general procedure acid chloride preparations. The delocalized aromatic ring releases intense florescence under UV light after protection, which leads to easy identification of the desired product via TLC analysis. Moreover, the amino-PEG-acid compounds are particularly polar compounds and very difficult to purify with polar silica columns, Fmoc as protecting group will have significant impact on polarity of molecule, dramatically reducing the polarity of protected PEG chain and easing the purification with the use of medium polarity solvent systems. The introduction of Fmoc groups can be relatively easy, with Fmoc chloride as most commonly used reagent, reacting with amino-PEG-acid as nucleophile under base catalysis and evolves HCl. Removal of Fmoc groups is also usually straightforward, where addition of piperidine as base deprotonates the relative acidic fluorenyl proton which leads lineal descendent of

β-nitroethyl acetate, where negative charge from intermediate **202** can be stabilized by adjacent aromatic system, carbamate **204** was then cleaved and release carbon dioxide after decomposition. The reaction overall undergo elimination via E1cb-type mechanism, thus freeing up the amine group for further reactions showing in Scheme 52. As a result, the Fmoc group was considered as first option for amine-PEG-acid protections.



Scheme 52 Piperidine catalyzed deprotection of Fmoc protecting group

The Fmoc group was successfully introduced to our amino-PEG-acid compounds, with sodium carbonate as base catalyst in a solvent mixture of water and 1,4-dioxane. Although it is more common to use water in 1,4-dioxane procedure for Fmoc protection on amines, with over 90% reported yield on related systems, replicate reactions conducted on our PEG chains only gave Fmoc-amino-4EG-acid **201a** with only 52% of isolated yield. With regards to trivial steps and time cost on synthesis of amino acid **200a**, efforts were spent on improving the Fmoc protection yields, and several reaction conditions were tested on this compound with different combination of base and solvents. As indicated in Scheme 51, variation in reaction conditions were tested to improve overall yield with results

summarized in Table 3 below.

Condition (rt, 16 h)	Amino acid (eq)	FmocCl (eq)	Base (eq)	Solvent	Yield (%)
A	1	1.2	1.5 $NaHCO_3$	H_2O Dioxane	52
B	1	1.2	1.8 Na_2CO_3	H_2O Dioxane	43
C	1	1.2	3 Na_2CO_3 60°C	H_2O	70
D	1	1.5	1.2 Na_2CO_3	H_2O Dioxane	22
E	1	1.2	3 Na_2CO_3	H_2O EtOH	65

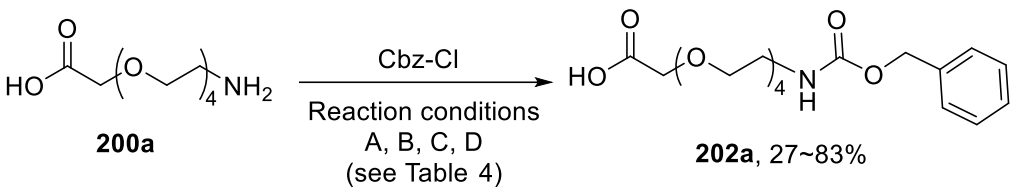
Table 3 Optimization of reaction conditions for Fmoc protection on amino-4EG-acid

The optimal conditions we found for Fmoc protection used rather unusual condition compare with conventional Fmoc protecting procedures; instead of a mixture of organic and inorganic solvents, solely water was used with sodium carbonate as base, with the temperature set to 60 °C overnight. [71] The yield for Fmoc protected amino acid from this reaction was found to be much higher, boasting 72% isolated yield in contrast to other conditions. Notably, all data summarized from table above were solely produced from protection of amino-4EG-acids for determining optimal reaction conditions, yields on variation in chain length are therefore not comparable, further experiments are necessary employing identical reaction conditions for pentamer and hexamer PEGs to produce consistent results.

4.3.2 Carboxybenzyl protection on amino-PEG-acids

To better study the roles of protecting group in ring expansion reactions, another commonly used protecting group in peptide synthesis was also considered. Carboxybenzyl (Cbz), is a carbamate which was also widely adopted as amine protecting group in peptide synthesis.[72] Analogous to Fmoc protections, Cbz protection can be achieved by reacting benzyl chloroformate with amine in the presence of base in common solvents. One of the major advantages of using Cbz over Fmoc is in the deprotection step, where cleavage of Cbz via catalytic Pd/C hydrogenation under atmospheric pressure results the formation toluene and carbon dioxide as side products, of which can be removed without effort and the overall deprotection can be considered as clean and efficient as described above.

Fmoc deprotection, however, produces white crystalline side product **203** which requires additional purification step, also piperidine adduct can be formed in some cases and complicates the reaction for analysis. Previous studies have also employed similar methodology to redeem amino function as prelude for macrocyclization to the naturally occurring angiotensin converting enzyme inhibitor.[73] Thus, Cbz was used as alternative to Fmoc for amine protection, all 4 to 6-membered amino-PEG-acids synthesized above were subject to Cbz protections.



Scheme 53 Cbz protection of amino-PEG-acid as linear fragment for ring expansion reactions

As anticipated, like Fmoc protections, initial attempts on the commonly used protection procedure did not work very well on amino-PEG-acids, with around 30% isolated yield obtained. Regarding to the relative time cost on trivial synthetic steps of starting material **200a**, as well as mutil-equivalent consumption of protected amino-PEG-acid in follow up PEGylation reactions, the reaction of Cbz protection in needs to be optimized to achieve higher yield at this stage and provide reliable source of Cbz protected amino-PEG-acids for ring expansion attempts. Therefore, several conditions were tested for Cbz protection, as indicated in Scheme 53 above. Where conditions as summarized in Table 4 as follows.

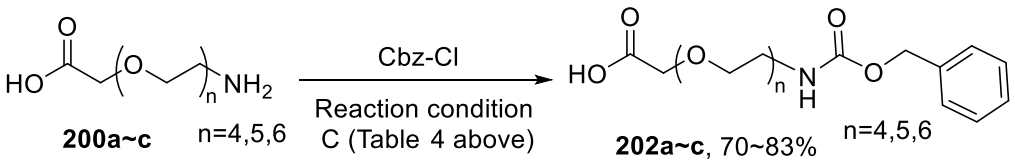
Condition (rt, 16 h)	Amino acid (eq)	CbzCl (eq)	Base (eq)	Solvent	Yield (%)
A	1	1.2	1.3 Et_3N	DCM	30
B	1	1.1	1 NaOH	DCM	27
C	1	1.1	2 Na_2CO_3 1 $NaHCO_3$	30 H_2O 4 Acetone	83
D	1	1.1	2 Na_2CO_3	1 H_2O 1 Dioxane	50

Table 4 Variation in reaction conditions for Cbz protection on amino-4EG-acids

Comparable with Fmoc protections, the optimal condition found for Cbz

protection was again derived from rather unusual conditions in contrast to other commonly used protection procedures listed above. Both sodium carbonate and sodium bicarbonate (together) were found improve the yield of Cbz protection. Water used as solvent will also affect the yield where 30 volumes was reported to be optimal.[72] This caused may due to mixing acetone with water has enhanced the solubility or chemical exposure of Fmoc chloride to PEG chains, therefore more accessible for free amines to react with. Unfortunately, we have been unable to satisfactorily explain the differences in yields under the various conditions, with the high polarity and associated purification problems potentially complicating this problem.

Overall, the reaction conditions of Cbz protection was investigated, with 4, 5 and 6 membered Cbz protected amino-PEG acids been successfully synthesized using optimized reaction condition. All at good yields showing in Table 5, with no considerable change in yield with alternating PEG chain lengths.



200a~c **202a~c**, 70~83%

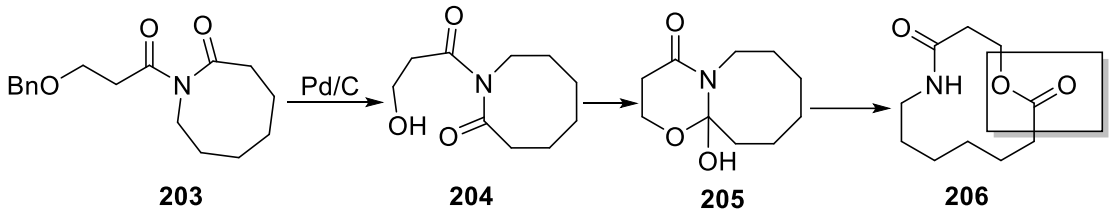
Chain length	Yield (%)
Cbz-amino-4EG-acid (202a)	78
Cbz-amino-5EG-acid (202b)	83
Cbz-amino-6EG-acid (202c)	70

Table 5 Variation in chain lengths for optimized Cbz protection condition

4.3.3 Benzyl ether protection of PEGs for templated hydroxyl ring expansion

Along with the progress of other group projects, ring expansion was also found to be possible for using acylated alcohols **204** as nucleophile forming macrocyclic lactones after ring expansion. Lactones, as class of cyclic ester compound found expansively in nature, spanning from medium sized daily antioxidant vitamin C, food addictive gluconolactone and enzyme lactonase to macrocyclic anthelmintic Ivermectin,[74] antibiotic macrolides,[75] and anticancer drug epothilones,[76] with highlighted interest in a range of biological activities exhibited to macrocyclic lactones. Despite many appealing features of macrocyclic lactones, its synthesis was mainly proposed through intramolecular lactonization or intermolecular dimerization,[77] such reaction was limited by target molecule ring sizes and synthesis much larger macrocyclic lactones can be challenging, hence, versatile ring expansion methods can therefore apply into synthesis of macrocyclic lactone without restrictions.

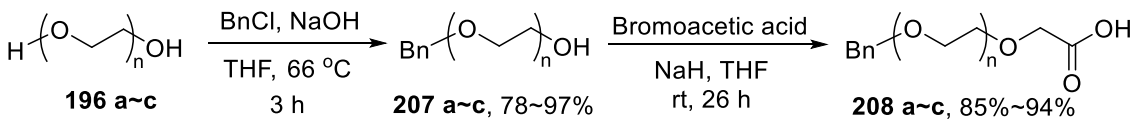
Shown below in Scheme 54, instead of deprotected free amines, alcohols can be used in the Unsworth group's SuRE chemistry, which results integration of lactone functionality into ring expanded macrocycles rather than formation of lactam from previous nitrogen-based ring expansions series.



Scheme 54 Introduction of lactone functionality with alcohol ring expansion

PEG compounds with two hydroxyl ends are more than ideal for development of hydroxyl linear fragment for such ring expansions, and benzyl ether was selected to be the protecting group for the free alcohol. Similar to methoxy groups, benzyl ethers are considered to be robust protecting groups and stable in wide range of solvents, well tolerant to acidic and basic conditions, it is also chemically inert to most of mild oxidizing and reducing agents, and most importantly, the use of benzyl ether as protecting group can be done using easy and clean deprotection steps i), where hydrogenolysis catalyzed by Pd on carbon is particularly useful.

Differing from Fmoc protected amines, the work up from benzyl ether hydrogenolysis simply involves removal of catalyst by filtration, without column purification and this simplifies the reaction, especially for polar compounds. Therefore, the reaction plan was designed to synthesis benzyl ether protected hydroxyl-PEG-carboxylic acids using tetramer, pentamer and hexamer PEGs as Scheme 55 revealed below.



Scheme 55 Synthesis of benzyl ether protected hydroxyl-PEG-carboxylic acids

A telescoped two step reaction was exercised on selected PEG compounds, where first step involves benzyl chloride protection of PEG hydroxyl group. Correspond to previously described tosylation reactions, the PEG compounds were again start with 10 to 1 equivalent to benzyl chloride and ensure the formation of mono-benzylated PEGs. Due to limited availability of pentamer 5EG and hexamer 6EG, readily available tetramer 4EG were used for 10 to 1 synthesis to test the viability of such reactions. To start with, 1 equivalent of benzyl chloride was used to react with 10 equivalents of compound 4EG at 100 °C in 50% NaOH solution for 12 hours, mono-benzylated 4EG was afforded at 97% yield after simple work up with excellent purity according to NMR spectra, where clear singlets gave 2H integration around 5.05 and 3.5 ppm suggests CH_2 groups next to aromatic and carboxylic acid group, mass spec also yields accurate value correspond to calculated mass. At this stage, another test similar to tosylation reactions was carried out to reduce redundancy usage of PEGs and examine the minimal ratio for producing mono-benzylated compounds, where 10, 4 and 2 equivalents of 4EG were used for test, with results showing in Table 6 below,

Equivalents	Mono-benzylated	Di-benzylated (%)	Yield (%)	Efficiency (%)
10：1	100	0	97	18.9
4：1	100	0	83	41.3
2：1	> 99	< 1	70	70

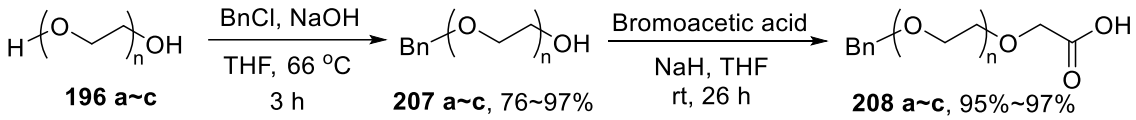
Table 6 Variation in equivalencies for optimized 4EG benzylation reaction ratio

As summarized in above, surprisingly 2 equivalents of 4EG was found to be more than sufficient to achieve mono-benzylation, with only trace amount of di-

benzylated 4EG found under sensitive high-resolution mass spectrometry. However, notably, with the decreasing usage of 4EG compounds, the yield of reaction also decreases, compare to previously tested tosylation reactions, where no significant change in yield were observed. This may cause by electrophilicity of compound used in reactions, where TsCl used from previous tosylation is much more reactive electrophile compare to benzyl chloride used in this reaction, the excess amount of benzyl chloride used will not only ensure the mono-benzylated product, the yield was also affected. However, in this case, the decrease in yield are negligible compare to reduced material waste, as indicated in efficiently column showing above, every 100% of starting material consumed will produce 70% yield of product from 2:1 reaction, 41.3% yield from 4:1 reaction and 18.9% from 10:1 reaction in even with 70% yield from benzylation, therefore the benzylation of 4EG **196a** was shown to be most efficient using 2 equivalents of benzyl chloride to minimize the waste of starting material.

The benzylated 4EG was then subject to further reaction and introduce carboxylic acid into the compound, where same procedure from previous amino-PEG-acid synthesis was employed without modification. Using 60% sodium hydride in mineral oil as base, bromoacetic acid were used to react with benzylated 4EG **207 a~c** in THF, producing benzylated 4EG-acid **208 a~c** with good yield and purity after overnight reaction at rt. Due to the NaH in mineral oil used during the reaction, first batch of product afforded was mixed with mineral oil and subject to

column purification, owing to the polarity of polyether and carboxylic group, the column requires solvent system with extreme polarity and more difficult to purify, to address the second bath were carried out with additional organic wash, which eliminates mineral oil and produce desired product as viscous oil with excellent purity confirmed by NMR, the compound was later brought forward without further purifications. Apart from BnO-4EG-acid, benzylated 5- and 6-membered PEG acids were also synthesized with good yield as data listed in Table 7 below.



Chain length	Benzylation yield (%)	Carboxylic acid yield (%)
BnO-4EG-acid 207a (10：1)	97	97
BnO-5EG-acid 207b (2：1)	78	95
BnO-6EG-acid 207c (2：1)	76	97

Table 7 Variation in chain lengths for two-step synthesis of benzyl ether protected PEG acids

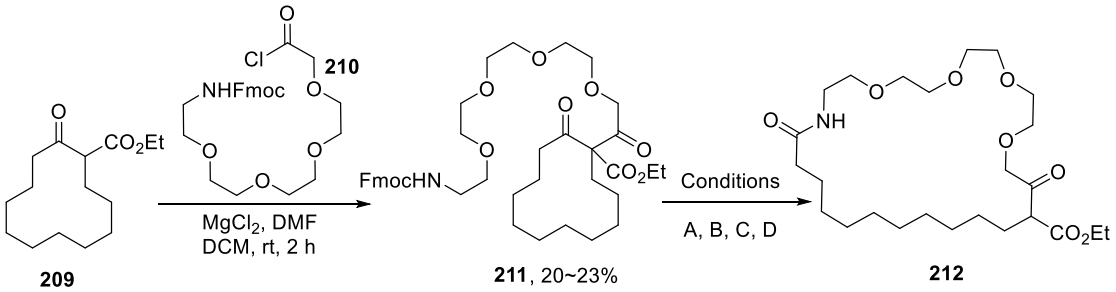
As reflected from data above, the trend is obvious that benzylation using 5EG and 6EG with 2 equivalents of benzyl chloride used will produce nomo-benzylated product at lower yields, result in line with reaction carried out in 4EG attempts, where using 2 equivalents of 4EG will leads to 70% yield, the as for the second step there are no notable affects in yields or purity. Thus, all three linear fragments were successfully synthesized with excellent yield and purity and can be used into next step without further purifications.

4.4 Ring expansion attempts with modified polyethylene glycol as linear building blocks with incorporation of metal templates

The initial project was planned to investigate the ring expansion with all linear fragments and three selected parenting cyclic substrates. However, unfortunately due to time constraint set on the project, it is has not been possible to test all combinations. Some initial results have been obtained however, and are described below

4.4.1 Attempted acylation and ring expansion with Fmoc protected PEG chains

Purified linear fragment Fmoc protected amino acid **201a** synthesized from previous steps were subject to the acid chloride forming general procedure, using oxalyl chloride in DCM catalyzed by DMF for acid chloride formation. Synthesized Fmoc-4EG-acid **201a** chloride was tested with established 12-membered cyclo-β-ketoester with existing reaction conditions without further modification, showing in Scheme 56.



Scheme 56 Attempted acylation of Fmoc-4EG-chloride onto 12-membered cyclo-β-ketoester and follow up ring expansions

As Scheme 56 illustrated above, attempted acylation of linear fragment chloride **210** were determined to be successfully acylated on to the parental ring **209**, however, the isolated yield was constantly low around 22% and no observable improvement after few attempts. The acylated compound was characterized and confirmed by spectroscopic methods, where characteristic proton peak for α-H next to ester group has disappeared after acylation with correct HRMS mass value match, then subject to deprotection of Fmoc and release free amine for initial test on ring expansion reactions. Different reaction conditions were tested, mainly the alternation in bases over concerns of nucleophilicity of piperidine used in the deprotection step, as scheme above stated, the deprotection condition A have used piperidine, and condition B used DBU as a weaker nucleophilic base for Fmoc deprotection.

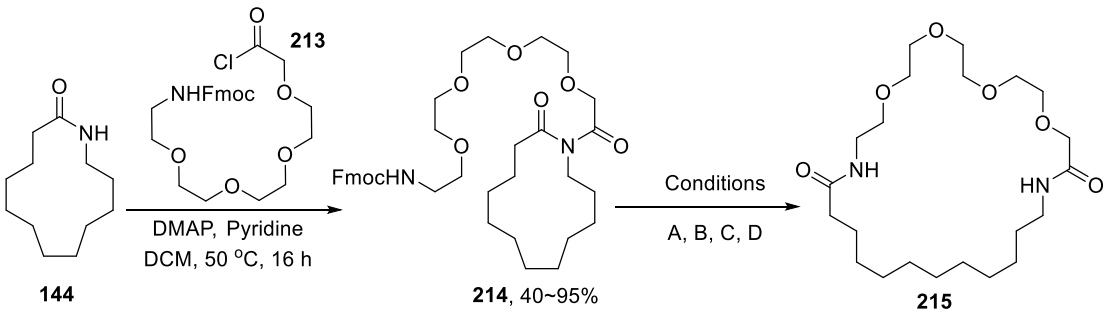
Conditions	Reagent base	Template
A	Pieridine	None
B	DBU	None
C	DBU	NaCl
D	MeOH	NaOMe

Table 8 Deprotection reaction conditions for attempted ring expansion reactions

As anticipated, no obvious change was observed for such reactions, where all Fmoc groups were cleaved according to TLC. The intramolecular cyclization for such long polyether chain has shown to be unsuccessful without presence of metal templates, regardless of altering base for deprotections, additionally, both conditions has led to fully decomposition of deprotected free amine with only starting material isolated, again regardless of base used. In conclusion, the reaction has demonstrated the successful acylation can be performed on such

long polyether chains but attention needs to be taken in detail and improve low yielding, and intramolecular cyclization of long polyether chain was demonstrated unlikely to happen without certain degrees of preorganization, and the last, base used for Fmoc deprotection have no observable impact on overall cyclization or free amine intermediate stabilizations, in contrast to significant yield improvements achieved by reducing degradation from common cyclic lactam ring expansion reactions.

Due to the unpredictable properties of cyclic β-ketoester acylation reactions, for concerns over $MgCl_2$ salt used could potentially complicate the reaction for such low yielding, our vision has reached to another similar macrocyclic system. Laurolactam, as a 13-membered cyclic lactam **144** described in Chapter 3 above, has been extensively tested with our successive ring expansion methods and proven to have promising reaction outcome, well tolerant to a wide range of functionalities introduced with good yields generated from ring expanded products.[31] The reaction condition has eliminated the usage of magnesium salt during acylation reactions, instead, catalytic amount of DMAP were added to promote acylation reactions. This makes such ring system an ideal starting point to test long chain polyether intramolecular cyclization tests, as we were hoping the use of DMAP catalyst will improve the yield and promising result from PEG acylation reactions, with planed reaction scheme showing as follows



Scheme 57 Acylation on 13-membered lactam with Fmoc-4EG-acid chloride as linear fragment and follow up ring expansion attempts

Started with laurolactam **144**, the 13-membered ring has been successfully acylated with significant improvement over yields, and we doubled the yield to 40%, compared to the initial test compared cyclo-β-ketoester **211**, and improved this to a pleasingly high 95% yield after few more attempts. This is a great step ahead as long chain acylation reaction from our experience are challenging and mostly tend to be low yielding. The acylated product was successfully isolated and identified with spectroscopy methods, where missing amide peak in parallel to accurate HRMS value confirms the formation of product **214**. After confirmation of acylated product **214**, next investigation step was carried out attempting ring expansion on such ring systems, duplicated from cyclo-β-ketoester reactions above, preliminary test were performed again with piperidine (Condition A) and DBU (condition B) as base, to examine the nucleophilicity of based used in relating to self-decomposition of deprotected free amine. Again as expected, the results are in accordance to cyclo-β-ketoester acylation reactions, where all deprotected long chain amine has fallen apart and gave back to starting material, observations of significant elevation on rate of free amine survival from our cyclic lactam

expansions cannot be reproduced in long chain deprotections with less nucleophilic base used. Additional condition C was tested, this time with a metal template introduced. Taken from literature reports of crown ether synthesis, the metal template introduced this time was taken the cyclized intermediate into account, to appropriate estimate the size of cyclized cavity and use the right metal template, the cyclol intermediate **216** was rationalized to 15-crown-5 **218** with slightly larger in ring size by 1 atom and additional proton donor from nitrogen as showing in Scheme 58.

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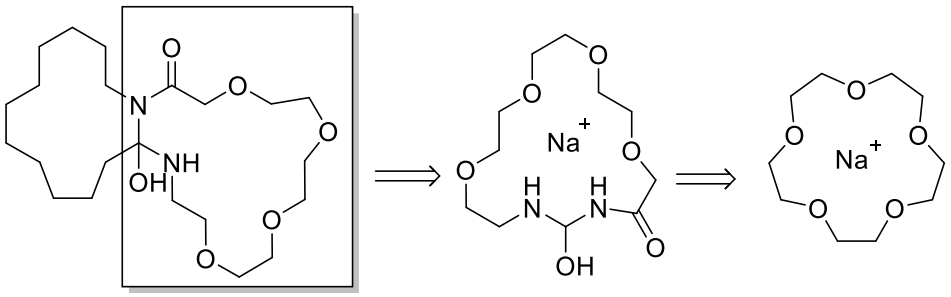


Scheme 58 Cyclol intermediate for polyether ring expansions analogues to 15-crown-5 for templated ring expansion

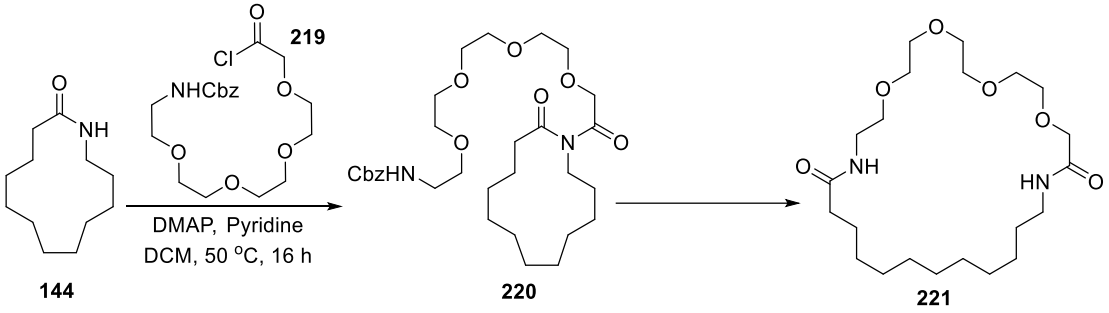
As a result, condition C and D was developed accordingly with sodium as template cation, where condition C used sodium chloride as template provider and DBU as base, after overnight reaction only decomposed starting material was found. Whereas in condition D, sodium methoxide used acting both as base and template in methanol as solvent. After overnight reaction, the reaction again was shown to be unsuccessful, with starting material and cleaved polyether long chain fragment returned according to their spectroscopic data. However, the result from TLC looks rather strange, where a very weak streaky spots with R_f value ranging

from 0.2 to 0.5 was found, which was never observed in previous attempts of ring expansions. In order to investigate the new spot, efforts were made on column chromatography and attempting isolate the spot, around 13 mg of white solid was afforded from ultra-polar column. Unfortunately, upon isolation, its NMR spectrum was rather messy without any useful information given. But our high-resolution mass spectrometry has given the correct mass for the exact sample submitted for NMR, with a sodium cation. Although, for the ionization method used, sodium ion coordination cannot be used as a proof for cyclization reactions, however such result from mass spec was still encouraging. As summarized from our previous attempts on such ring expansion reaction, all linear fragment has fallen apart and gave back to starting material, wherefore it is highly unlikely for the isolated product to exist as linear acylated from of free amine.

In conclusion, cyclic lactams **114** were demonstrated to be a better ring system for PEGylation reactions compared to β-ketoesters. The decomposition from the free amine may suggests that relative long polyether amine chain after depotentiation are unstable due to entropic factors, and underwent self-degradation without cyclization into desired cyclol intermediates. However, in the sodium templated cyclization, the correct mass acquired by HRMS gives hope that the desired product may have been formed, but more work is needed to verify this.

4.4.2 Attempted acylation and ring expansion reactions with Cbz protected PEG chains

As previous attempts on Fmoc-protected cyclization has shown to be problematic, Cbz was introduced as alternative eliminate formation of side products during deprotection step. Unlike Fmoc deprotections, the Cbz group can be deprotected under hydrogenolysis, without base, and toluene formed as side product step can be easily removed which leads to much easier purification for analysis after simple filtration. The acylation strategy was carried on from previous attempts, with proposed ring expansion route showing in Scheme 59, and only 13-membered laurolactam was used for acylation due to unpredictable β-ketoester acylation described above.



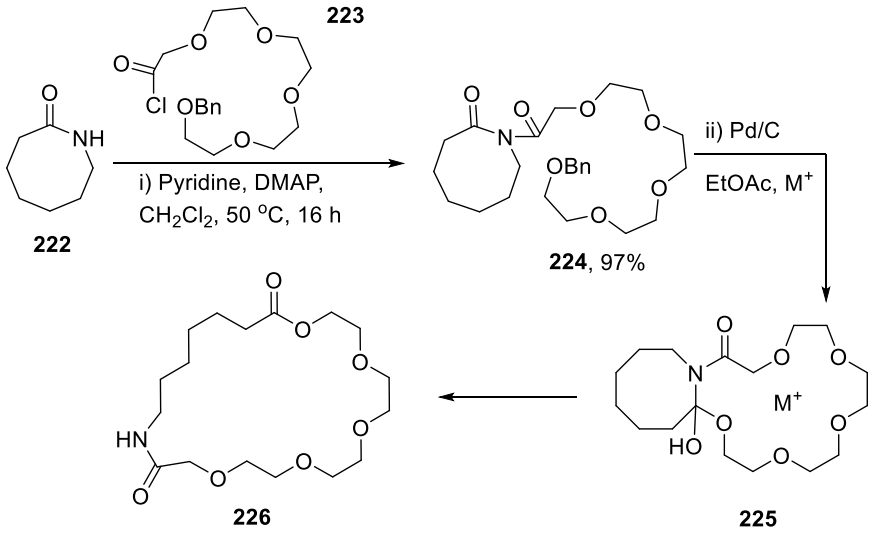
Scheme 59 Proposed ring expansion via Cbz deproction

Due to time constraint, only 1 attempt was carried out for Cbz protected linear fragment acylation, where reaction was found to be largely incomplete after overnight reflux, with over 75% of starting material **219** recovered, the acylated product **220** was formed but nearly inseparable with identical R_f value around 0.5 as starting material **144** in 100% EtOAc. Overall the yield of reaction was found to be very low, the inseparable starting material make the reaction even harder to carry on, for such reason our research has focused on benzylated ring expansions.

4.4.3 Attempted acylation and ring expansion reactions with OBn protected PEG chains

Once again, with the progressing of our mainline successive ring expansion methods, it was found that using linear fragments with free hydroxyl group can also undertake ring expansion reactions, instead of formation of lactam from linear amine used for ring expansion, employing acylated linear fragments with hydroxyl group end can form a form a lactam after ring expansion as introduced above. For this instance, the reaction was set up with 8-membered cyclic lactam, where such cyclic system was used for developing lactone ring expansion methods and well understood, therefore offers a good starting point in parallel to existing 13-membered laurolactam **144** acylation.

As Scheme 60 illustrates benzyl ether protected tetra ethylene carboxylic acid **208 a~c** was converted into acid chloride using general procedure, the acylation was then carried out with procedure employed from laurolactam acylation as described above, acylated product **224** was afforded after overnight reaction with excellent yield of 97% in analogues to laurolactam **144**, compound was characterized by NMR with missing amide H signal and accurate mass spec value match with calculated mass.

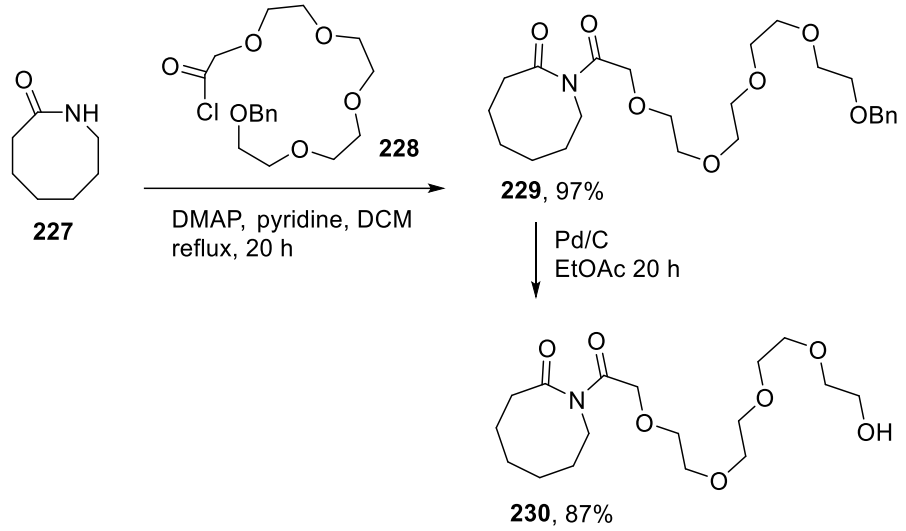


Scheme 60 Proposed ring expansion with Benzyl ether protected PEG for introducing lactone functionality

Similarly, pentamer and hexamer amino acids were successfully acylated onto parental cyclic lactam **222**, employing same procedure, successfully afforded acylated compound analogues to **224**with different chain length, all at ~97% yield and good purity confirmed by HMRS with accurate mass matching with calculated data and missing amide H signal from proton NMR spectra.

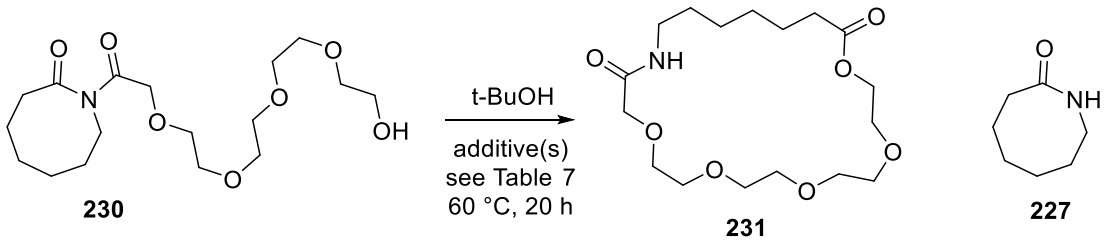
As follow up deprotection step been set up comparable to Cbz deprotections, palladium catalyst was used for overnight hydrogenation, although no ring expansion occurred as expected, however, more importantly, for the first time ever the deprotected acylation compound **230** was afforded without self-degradation at 87% yield. The deprotected hydroxyl group **230** was shown to be much more stable compared to deprotected free amine acylation; partial decomposition of deprotected hydroxyl compound was observed later but at very slow rate with over a month time scale. As such, the reaction strategy is different than previous amine expansions, where metal ions could be screened against imide **230** with template effect incorporated rather than deprotection with template, as the self-

cleavage of free amine always kinetically favorable and competing with very slow polyether cyclization reactions, as such, relatively stable imide **230** offers an opportunity to carry out screening against range of metal templates without competing with self-degradation.



Scheme 61 Deprotection of hydroxyl group without self-decomposition

As a result, imide **230** was then reacted at 60 °C in t-BuOH with various basic/group one metal additives in an attempt to promote cyclisation and ring expansion to form 23-membered product **231**. The results are summarized below in Table 9



Scheme 62 Attempted screening on templets for hydroxyl ring expansion

ENTRY	ADDITIVE(S)	% STARTING MATERIAL 230	% MACROCYCLE 231	% 8-MEMBERED LACTAM 227
1	none	>95	0	trace
2	LiOH (2=)	0	0	>95
3	NaOH (2=)	0	0	>95
4	KOH (2=)	0	0	>95
5	LiI (2=)	>95	0	trace
6	NaI	>95	0	trace
7	KI	>95	0	trace
8	LiI (2=) Et_3N (2=)	0	0	>95
9	NaI (2=) Et_3N (2=)	0	0	>95
10	KI (2=) Et_3N (2=)	20	0	80

Table 9 Screening test for hydroxyl ring expansion reactions

Disappointly, none of these early screens were successful. First, we found that the solvent and reactions conditions alone led to little or no background reaction (entry 1). Then, LiOH, NaOH, and KOH were all tested (entries 2–4), but all led to complete hydrolysis of imide **230** into 8-membered lactam **227**. We thought that the basicity and presence of hydroxide was likely the cause of this, so tried simple iodides salts (LiI, NaI and KI, entries 5–7), but all three resulted in little no reaction. As the group's lactone-forming ring expansion reactions are typically done in the presence of a tertiary amine base, we decided to run reactions with the same additives (LiI, NaI and KI) but also include triethylamine, but sadly, these led to either full or partial hydrolysis, and again, no evidence of macrocycle formation

was obtained (entries 8–10), All of the results above were obtained via integration of the ^{1}H NMR spectra of the reactions.

While we are disappointed not to be able to validate the key idea, there are many opportunities for future work that offer promise. For example, various other solvents, additives, temperature and reaction times should be screened, as well as the other substrates prepared (i.e. longer/shorter PEG chains and other lactams). The reliable methods for the preparation of the linear PEG containing precursors done during this project will certainly help the next Unsworth group member who works on this project to develop this idea further.

4.5 Conclusion and future work

Overall, a range of modified PEG compounds were synthesized with different chain length and protecting group, followed by acylation onto selected ring systems. The cyclization of long chain polyether fragment was attempted for expected spontaneous ring expansion. Different combination of parental cyclic compound (cyclic β-ketoesters and lactams) and linear fragments (amine or hydroxyl as nucleophile for side chain insertion). Although no much positive results been generated so far, still there are many improvements can be made to cyclize the polyether sidechain, such as using metal templating prior to deprotection, where time consuming preorganization is required to pre-cyclize the polyether chain, which may improve the chance for ring to expand, use slightly more diluted condition with extend reaction time can also be a solution, whereas

degradation was shown to occur even without base presence as from Cbz deprotections, and free amine itself is a good nucleophile which may undergo intermolecular reaction which leads to decomposition. Based on current results, more screening in needs to be carried out, with additional selection of templates such as Zn^{2+} or Cu^{2+}, where literature reports compare to tetramer PEG, pentamer and hexamer are more likely to cyclize in the emphasis on pentaethylene glycol, which has the preference over 15-crown-5 for potassium ion coordination, therefore higher binding affinity.[78] Moreover, based on current reaction mode, the product formed would be more tend to like aza-crown rather than crown ether, as such, the cyclization of aza-crown can be studied to assist current ring expansion work.[63] Additionally, highly biocompatible and multi-functional polyglycerol analogues to polyethylene glycol, can also use as linear fragment for ring expansion attempts, where hydroxyl side chain leads to intramolecular hydrogen bonding to parental ring, which may assist the cyclization process as reported in literature. [64]

Chapter 5. Conclusion and Future Work

Overall, the successive ring expansion reactions has introduced a novel and efficient way to access diverse range of macrocycles. Whereas ring expansion can be performed constitutively with repetitive addition of linear fragments, which leads to arithmetic growth of ring expanded macrocycles, a wide range of functionalities could also be introduced with the addition of building blocks.

With reliance on successful acylation of linear fragments and most importantly, cyclisation of intermediates, where thermodynamic driving force leads to spontaneous ring expansion from cyclized intermediates. To address this, further study on acylation reactions are necessary to figure out the optimal condition especially on resolving previous unsuccessful acylation attempts and conceivably offers a solution for successive acylation reactions. The theoretic ground of intermediate cyclisation in this method was also supported by the empirical Baldwin's rules, where most of the ring expansions we have performed are exo-trig reactions, which exhibits the preference of spontaneous cyclisation, ranging from 3 to 7 membered cyclization. with few exceptions such as PEG compounds of being much larger ring sizes.

As presented in this work, with detailed attention spend on understating these crucial steps, the huge potential behind successive ring expansion method could be released and navigate the synthesis of much larger macrocycle with versatile functionalities, with more valuable applications to be discovered in wide discipline.

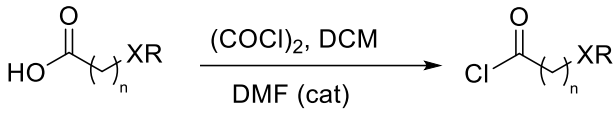
Starting with cyclo-β-ketoesters, where our successive ring expansion methods were based upon, range of macrocyclic system were utilized for promising ring expansion reactions. With reliance on successful incorporation of wide range of linear fragments and most importantly, cyclisation of intermediates followed by hassle-free spontaneous ring expansion, library of macrocycles with versatile functionalities can be therefore synthesized, awaiting to open the door reshape the world of chemistry.

Chapter 6. Experimental

Except where stated, all reagents were purchased from commercial sources and used without further purification. Except where stated, all experimental procedures were carried out under an atmosphere of argon. Anhydrous CH_2Cl_2, toluene and DMF were obtained from an Innovative Technology Inc. PureSolv® solvent purification system. Anhydrous THF was obtained by distillation over sodium benzophenone ketyl immediately before use. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL ECX400 or JEOL ECS400 spectrometer, operating at 400 MHz and 100 MHz, respectively, or a Bruker DRX500 spectrometer, operating at 500 MHz and 125 MHz, respectively. All spectral data was acquired at 295 K. Chemical shifts (δ) are quoted in parts per million (ppm). The residual solvent peak, δH 7.26 and δC 77.0 for $CDCl_3$ was used as a reference. Coupling constants (*J*) are reported in Hertz (Hz) to the nearest 0.1 Hz. The multiplicity abbreviations used are: s singlet, d doublet, t triplet, q quartet, m multiplet. Signal assignment was achieved by analysis of DEPT, COSY, NOESY, HMBC and HSQC experiments where required. Infrared (IR) spectra were recorded on a PerkinElmer UATR two spectrometer as a thin film. Mass-spectra (low and high resolution) were obtained by the University of York Mass Spectrometry Service, using electrospray ionisation (ESI) on a Bruker Daltonics, Micro-tof spectrometer. Melting points were determined using Gallenkamp apparatus and are uncorrected. Thin layer chromatography was carried out on Merck silica gel $60F_{254}$ pre-coated

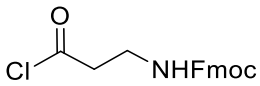
aluminium foil sheets and were visualised using UV light (254 nm) and stained with either basic aqueous potassium permanganate or ethanolic p-anisaldehyde as appropriate. Flash column chromatography was carried out using slurry packed Fluka silica gel (SiO_2), 35–70 μm, 60 Å, under a light positive pressure, eluting with the specified solvent system.

General procedure for acid chloride formation[30]



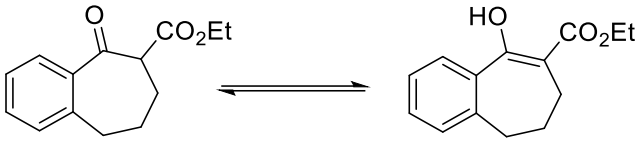
Oxalyl chloride (3 mmol) was added to a suspension of carboxylic acid (1 mmol) in DCM (5 mL), followed by a catalytic amount of DMF (1 drop/mmol of carboxylic acid). The resulting mixture was stirred at RT for 1 h and concentrated *in vacuo* to remove all the solvent and excess oxalyl chloride.

(9H-fluoren-9-yl)methyl (3-chloro-3-oxopropyl)carbamate (39)



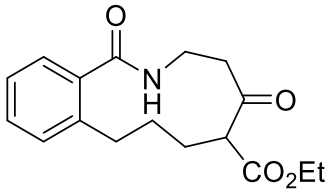
Oxalyl chloride (3 mmol) was added to a suspension of Fmoc-β-alanine (1.00 mmol) in dichloromethane (5 mL), followed by a catalytic amount of DMF (1 drop/mmol of carboxylic acid). The resulting mixture was stirred at RT for 1h (in general the initial suspension became homogeneous over this period) and concentrated *in vacuo* to remove all the solvent and excess oxalyl chloride.

Ethyl 5-oxo-6,7,8,9-tetrahydro-5H-benzo[7]annulene-6-carboxylate (71)



To a suspension of sodium hydride (1.16 g, 48.2 mmol, 60% in mineral oil) and diethyl carbonate (5.8 mL, 48.2 mmol) in THF (30 mL) at RT was added a solution of 1-benzosuberone (3.86 g, 24.1 mmol) in THF (75 mL) was added dropwise. The mixture was heated to 75 °C and stirred for 20 h, then cooled to RT and acidified to pH 5 with 1M aq. HCl followed by diethyl ether exaction, the combined organic extracts were dried over $MgSO_4$ and concentrated. Purification by column chromatography (hexane:ethyl acetate 1:0 → 20:1 → 10:1) to obtain the title compound 2 as a 4:1 mixture of enol:keto tautomers (5.36 g, 95%) as a yellow oil. R_f (hexane:ethyl acetate 10:1) = 0.58; δ_H (400 MHz, $CDCl_3$): 12.68 (1H, s, OH, enol), 7.74–7.18 (8H, m, aromatics, both), 4.27 (2H, q, *J* = 6.8, OCH_2, enol), 4.23–4.16 (2H, m, OCH_2, ketone), 3.79 (1H, dd, CH, *J* = 10.4, 4.2, ketone), 2.97–2.91 (2H, m, CH_2, ketone), 2.62 (2H, t, *J* = 7.1, CH_2, enol), 2.19–2.02 (4H, m, CH_2, both), 1.34 (3H, t, CH_3, *J* = 6.8, enol), 1.24 (3H, t, CH_3, *J* = 7.1, ketone). The spectroscopic data are consistent with those reported in the literature.[29]

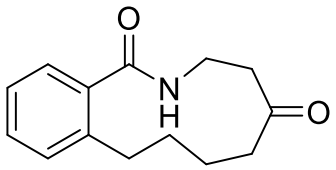
Ethyl 1,5-dioxo-2,3,4,5,6,7,8,9-octahydro-1H-benzo[c][1]azacycloundecine-6-carboxylate (72)



A mixture of ethyl 5-oxo-6,7,8,9-tetrahydro-5H-benzo[7]annulene-6-carboxylate **71** (3.99 g, 17.22 mmol), $MgCl_2$ (4.92 g, 51.66 mmol) and pyridine (8.32 mL, 103.3 mmol) in dichloromethane (125 mL) under an argon atmosphere was stirred at RT for 30 mins. Next, a solution of acid chloride **1** (34.44 mmol) in dichloromethane (65 mL) was added and the reaction mixture was stirred for 1 h at RT. The mixture was then diluted with dichloromethane (150 mL) and washed with 10% aq. HCl (200 mL). The aqueous layer was extracted with dichloromethane (3 × 150 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was then re-dissolved in dichloromethane (125 mL) and piperidine (17.01 mL, 172.2 mmol) was added. The resulting mixture was stirred for 1 h at RT, before the solvent was removed *in vacuo*. Purification by column chromatography (SiO_2, 10:1 → 1:1 hexane:ethyl acetate → pure ethyl acetate) afforded the title compound **72** (4.59 g, 88%) as a white solid; m.p. 162–164 °C (chloroform); R_f 0.20 (1:1 hexane:ethyl acetate); δ_H (400 MHz, $CDCl_3$) 7.27–7.06 (4H, m, ArH), 6.26 (1H, s, NH), 4.11 (2H, q, *J* = 7.1, OCH_2), 3.92 (1H, dd, *J* = 10.7, 4.6, CH), 3.89–3.78 (1H, m), 3.56–3.39 (2H, m), 2.79–2.59 (2H, m), 2.56–2.44 (1H, m), 2.12–2.00 (1H, m), 1.92–1.78 (1H, m), 1.69–1.54 (1H, m), 1.36–1.25 (1H, m), 1.21 (3H, t, *J* = 7.1, CH_3). The spectroscopic data are

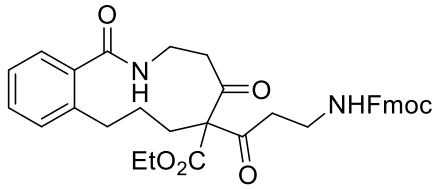
consistent with those reported in the literature. [29]

3,4,6,7,8,9-Hexahydro-1H-benzo[c][1]azacycloundecine-1,5(2H)-dione (76)



To a solution of β-ketoester **72** (598 mg, 14.5 mmol) in ethanol (5.92 mL) was added a 1M aq. NaOH solution (145 mL, 145 mmol). The resulting mixture was stirred at 50 °C for 1 h, before acidifying the mixture with 1 M aq. HCl and stirring at 50 °C for an additional 30 min. The reaction was then cooled, diluted with water (250 mL), extracted with ethyl acetate (4 x 100 mL) and dried over $MgSO_4$. Purification by column chromatography (hexane:ethyl acetate 10:1 → 1:1 → 1:2) to obtain the title compound **76** (3.19 g, 97%) as a white solid; m.p. 135–137 °C; R_f 0.24 (1:2 hexane:ethyl acetate); δ_H (400 MHz, $CDCl_3$) 7.29–7.10 (4H, m, Ar), 5.95 (1H, br s, NH), 3.72 (2H, app. q, *J* = 6.3, CH_2), 2.92 (2H, t, *J* = 6.1, CH_2), 2.66–2.57 (4H, m, 2 × CH_2), 1.78 (2H, app. quin, *J* = 6.3, CH_2), 1.55–1.45 (2H, m, CH_2). The spectroscopic data are consistent with those reported in the literature.[29]

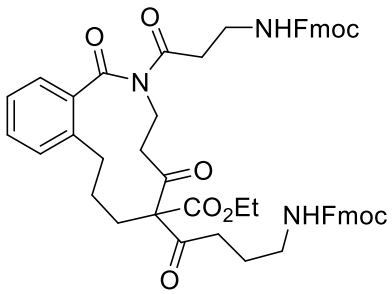
ethyl 6-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-1,5-dioxo-2,3,4,5,6,7,8,9-octahydro-1H-benzo[c][1]azacycloundecine-6-carboxylate (97)



A mixture of ethyl 5-oxo-6,7,8,9-tetrahydro-5H-benzo[7]annulene-6-carboxylate **72** (380 mg, 1.25 mmol), $MgCl_2$ (356 mg, 6.45 mmol) and pyridine (0.6 mL, 7.5 mmol) in dichloromethane (12.5 mL) under an argon atmosphere was stirred at RT for 30 mins. Next, a solution of acid chloride **39** (2.5 mmol) in dichloromethane (6.5 mL) was added and the reaction mixture was stirred for 1 h at RT. The mixture was then diluted with dichloromethane (15 mL) and washed with 10% aq. HCl (20 mL). The aqueous layer was extracted with dichloromethane (3 × 15 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (hexane: ethyl acetate 10:1 → 1:1 → 1:2) to obtain the title compound **97** (530 mg, 74%) as pale-yellow solid. δ_H (400 MHz, $CDCl_3$) 7.76 (2H, d, *J* = 7.5Hz, ArH), 7.58 (2H, d, *J* = 7.5Hz, ArH), 7.38 (2H, t, *J* = 7.5, ArH), 7.30 (2H, t, *J* = 7.5, ArH), 7.13–7.29 (4H, m, ArH), 4.39 (2H, d, *J*=7.1Hz, CH_2), 4.16 (2H, m, CH_2), 3.15-1.5 (14H, m, CH_2), 1.26 (3H, t, *J* = 7.1Hz, CH_3). δ_c (100 MHz, $CDCl_3$) 208.7 (CO), 176.6 (CO), 171.3 (CO), 169.1 (CO) 156.5 (CO) 144.0 (ArC), 141.5 (ArC), 139.2 (ArC), 137.0 (ArC), 129.9 (ArC), 129.8 (ArC), 128.0 (ArC), 127.0 (ArC), 126.4 (ArC) 126.1 (ArC), 125.2 (ArC), 120.1 (ArC), 66.9 (C), 61.7 (CH_2), 57.0 (CH_2), 47.3 (CH), 41.7 (CH_2), 36.7 (CH_2), 36.5 (CH_2) 34.2

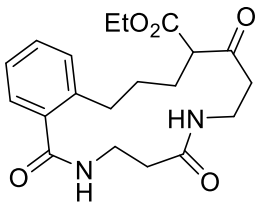
(CH_2), 31.5 (CH_2), 29.1 (CH_2), 27.7 (CH_2), 14.2 (CH_3); HRMS (ESI^+): Found: 619.2407, $C_{35}H_{36}N_2NaO_7$(MNa^+) Requires: 619.2420 (1.1 ppm error).

Ethyl 2,6-bis((((9H-fluoren-9-yl)methoxy)carbonyl)glycyl)-1,5-dioxo-2,3,4,5,6,7,8,9-octahydro-1H-benzo[c][1]azacycloundecine-6-carboxylate (98)



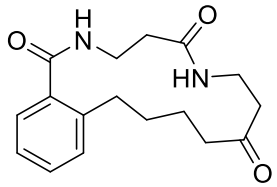
A mixture of compound **97** (510 mg, 0.86 mmol), DMAP (10 mg, 0.086 mmol) and pyridine (0.42 mL, 5.16 mmol) in DCM (12.5 mL) under an argon atmosphere was stirred at RT for 30 mins. Next, a solution of acid chloride **39** (1.34 mmol, 1.50 equiv., freshly prepared using the general procedure) in DCM (10 mL) was added and the resulting mixture was refluxed at 50 °C for 16 h. The mixture was then diluted with DCM (15 mL) and washed with 10% aq. HCl (15 mL). The aqueous layer was then extracted with DCM (3 × 15 mL) and the combined organic extracts dried over $MgSO_4$ and concentrated *in vacuo*. No reaction happened according to TLC and mass spec, NMR confirmed the existence of starting material from reaction mixture.

Ethyl 1,5-dioxo-1,2,3,4,5,6,7,8,9,10,11,12-dodecahydrobenzo[g][1,5] diazacyclotetradecine-9-carboxylate



Compound **72** (100 mg, 0.167 mmol) was dissolved in dichloromethane (12.5 mL) and piperidine (0.165 mL, 1.67 mmol) was added. The resulting mixture was stirred for 1 h at RT, before the solvent was removed *in vacuo*. Purification by column chromatography (hexane: ethyl acetate 1:2 → 0:1), afforded the compound **97**, no desired product obtained.

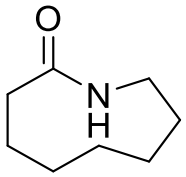
2,3,4,5,6,7,9,10,11,12-decahydrobenzo[f][1,4]diazacyclotetradecine-1,8-dione (105)



A mixture of compound **92** (92 mg, 0.4 mmol), DMAP (5 mg, 0.04 mmol) and pyridine (0.2 mL, 2.4 mmol) in DCM (12.5 mL) under an argon atmosphere was stirred at RT for 30 mins. Next, a solution of acid chloride **1** (0.6 mmol, 1.50 equiv., freshly prepared using the general procedure) in DCM (5 mL) was added and the resulting mixture was refluxed at 50 °C for 16 h. The mixture was then diluted with DCM (15 mL) and washed with 10% aq. HCl (15 mL). The aqueous layer was then extracted with DCM (3 × 15 mL) and the combined organic extracts dried over

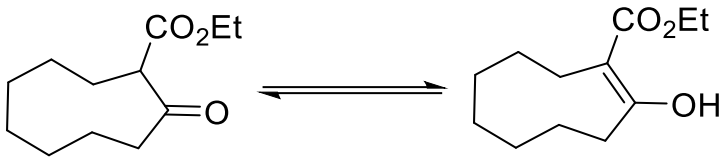
$MgSO_4$ and concentrated *in vacuo*. The crude material was then re-dissolved in DCM (8 mL) and DBU (0.61 mL, 4 mmol) was added, followed by stirring at RT overnight, before the solvent was removed in vacuo. Purification by flash column chromatography (SiO_2, hexane: ethyl acetate 1:2 → 0:1) afforded the compound **2.5,** no desired product obtained.

Azonan-2-one (107)



Cyclooctanone **106** (633 mg, 5.0 mmol) in formic acid (5 mL) was added a solution of hydroxylamine-osulphonic acid (850 mg, 7.5 mmol) in formic acid (2.5 mL) and heated to 110 C for 16 h. Purification by flash column chromatography (SiO_2, 2:1 hexane:ethyl acetate → ethyl acetate → 9:1 ethyl acetate:methanol) afforded the title compound as an orange solid (560 mg, 79%), δ_H (400 MHz, $CDCl_3$) 5.57 (1H, br s, NH), 3.23 (2H, q, *J* = 6.2, $NHCH_2$), 2.18 (2H, m, $COCH_2$), 1.51-1.43 (4H, m, CH_2) 1.35-1.25 (6H, m, CH_2). Data consistent with those reported in the literature.[51]

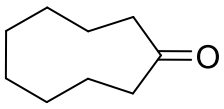
Ethyl 2-oxocyclononane-1-carboxylate (108)



To a solution of cyclooctenone **107** (5.04 g, 40.0 mmol) in dry diethyl ether at 0 °C, slowly added a solution of boron trifluoride diethyl etherate (8.52 g, 60 mmol) in dry diethyl ether (30 mL). A solution of ethyl diazoacetate (7.9 g, 60.0 mmol) in 30 mL dry Et_2O was then added over a period of 30 min. The resulting mixture was stirred at room temperature under an argon atmosphere. After 24 h, the mixture was cooled to 0 °C and neutralized with saturated aqueous solution of $NaHCO_3$. The resulting mixture is extracted with chloroform (5 x 40 mL) and the combined extracts were washed with a saturated solution of NaCl, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuum. Purification by fractional distillation (90 - 110 °C) and column chromatography (hexane:ethyl acetate 50:1 → 25:1 → 10:1) to obtain the carboxylate as title compound **108** (11.7 g, 89%) as light yellow oil. R_f 0.75 & 0.65 ketone/enol tautomers (DCM); ν_{max}/cm^{-1} (neat); 2927, 1745, 1706, 1640, 1609; δ_H (400 MHz, $CDCl_3$) 12.74 (1H, s, OH, enol), [4.19 (2H, q, J = 7.3 Hz, OCH_2CH_3), 4.12 (2H, q, J = 6.9 Hz, OCH_2CH_3), ketone and enol], 3.66–3.54 (1H, m, $CHCO_2Et$, ketone), [2.67–2.50 (2H, m, CH_2COCH), 2.38 (2H, t, J = 6.0 Hz, CH_2COH), 2.35–2.28 (2H, m, CH_2), 2.13–2.03 (2H, m, CH_2), 1.94–1.79 (2H, m, CH_2), 1.77–1.67 (2H, m, CH_2), 1.67–1.32 (16H, m, 8 × CH_2), 1.28 (3H, t, J = 7.3 Hz, CH_3), 1.21 (3H, t, J = 6.9 Hz, CH_3), ketone and enol]; δ_C (100 MHz, $CDCl_3$) 212.0 ($COCH_2$, ketone),

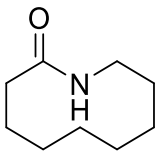
175.9 (CO_2Et, ketone), 173.5 (COH, enol), 170.7 (CO_2Et, enol), 100.3 (CCO2Et, enol), 61.3 (OCH_2, enol), 60.3 (OCH_2, ketone), 58.9 ($CHCO_2$Et, ketone), 42.5 (CH_2COCH, ketone), [31.5 (CH_2), 27.1 (CH_2), 27.0 (CH_2), 26.2 (CH_2), 25.9 (CH_2), 25.2 (CH_2), 25.0 (CH_2), 24.7 (CH_2), 24.64 (CH_2), 24.56 (CH_2), 24.4 (CH_2), 24.01 (CH_2), 23.96 (CH_2), ketone and enol], 14.4 (CH_3, enol), 14.1 (CH_3, ketone); HRMS (ESI^+): calculated for $C_{12}H_{20}NaO_3$, 235.1305. Found: $[MNa]^+$, 235.1304 (0.7 ppm error)].

Cyclononanone (109)



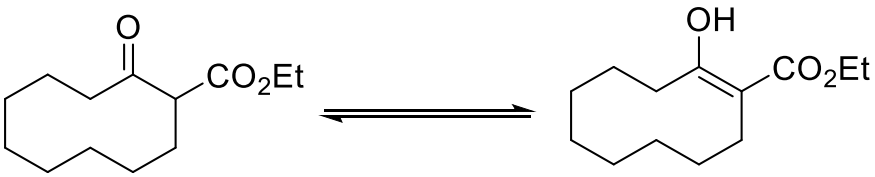
A degassed solution of ethyl 2-oxocyclononane-1-carboxylate **(108)** (8.49 g, 40.1 mmol) in DMSO (50 mL) and H_2O (8 mL) was stirred at 150 °C (bath temperature) for 16 h. After reaching ambient temperature, the mixture was partitioned between water (35 mL) and Et_2O (4 × 40 mL), the combined organic phases were dried by filtering through $MgSO_4$ and then evaporated. The crude material obtained was purified by distillation under reduced pressure (b.p. 85 – 97 °C) and column chromatography (hexane: ethyl acetate 100:1 → 50:1 → 30:1) to afford title compound as a light-yellow oil (4.6 g, 82 %). The analytical data are in accord with those reported in the literature. δ_H (400 MHz, $CDCl_3$): δ 2.47-2.28 (4H, m), 1.90-1.68 (4H, m), 1.62-1.41 (4H, m), 1.39-1.24 (4H, m); δ_C (100 MHz, $CDCl_3$): δ 218.4 (C=O), 43.5 ($O=CCH_2$), 26.9 (CH_2), 25.1 (CH_2), 24.3 (CH_2). Data consistent with those reported in the literature. [53]

Azecan-2-one (110)



To a stirring solution of Cyclononanone **(109)** (252 mg, 1.80 mmol) and formic acid (2 mL, 95% - 97%), slowly add solution of hydroxylamine-o-sulfonic acid (350 mg, 3.00 mmol) in formic acid (2 mL, 95% - 97%) at RT over 10 mins. The resulting solution was then microwave heated to 108 °C and reflux for 7 h. The dark brown mixture then cooled and quenched with ice water, neutralized to pH 7 with 5% aq. NaOH. Followed by chloroform extraction (3 x 10 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*, yielding the crude title compound 5 (200 mg, 73%) as a light brown solid. δ_H (400 MHz, $CDCl_3$) 5.59 (1H, br s, NH), 3.32 (2H, q, J = 6.2, $NHCH_2$), 2.22 (2H, m, $COCH_2$), 1.77-1.61 (4H, m, CH_2) 1.45-1.52 (4H, m, CH_2) 1.33-1.41 (4H, m, CH_2). The spectroscopic data are consistent with those reported in the literature.[79]

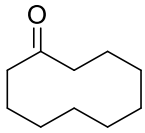
Ethyl 2-oxocyclodecane-1-carboxylate (111)



To a solution of cyclononanone **(109)** (1.12 g, 8.10 mmol) in dry diethyl ether at 0 °C, slowly added a solution of boron trifluoride diethyl etherate (1.75 g, 12.2 mmol) in dry diethyl ether (6 mL). A solution of ethyl diazoacetate (1.62 g, 12.2 mmol) in 5 mL dry Et_2O was then added over a period of 10 min. The resulting

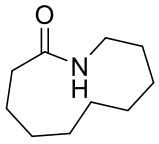
mixture was stirred at room temperature under an argon atmosphere. After 24 h, the mixture was cooled to 0 °C and neutralized with saturated aqueous solution of $NaHCO_3$. The resulting mixture is extracted with chloroform (3 x 30 mL) and the combined extracts were washed with a saturated solution of NaCl, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuum. Purification by column chromatography (hexane:ethyl acetate 50:1 → 25:1 → 10:1) to obtain the pale orange coloured oil as title compound **111** with 3:2 mixture of ketone:enol tautomers (1.75 g, 95%). R_f 0.72 & 0.64 ketone/enol tautomers (DCM); ν_{max}/cm^{-1} (neat) 2926, 1744, 1707, 1638, 1602; δ_H (400 MHz, $CDCl_3$) 12.95 (1H, s, OH, enol), 4.20 (2H, q, J = 7.3 Hz, OCH_2CH_3, enol), 4.12 (2H, q, J = 7.3 Hz, OCH_2CH_3, keto), 3.81 (1H, dd, J = 3.2, 11.0 Hz, $CHCO_2Et$, keto), 2.76–2.64 (2H, m, CH_2CO, keto), [2.45–2.34 (2H), 2.24–2.04 (2H), 2.00–1.87 (2H), 1.86–1.70 (4H), 1.69–1.31 (20H), 7 × CH2 (keto and enol) and CH_2CO (enol)], 1.29 (3H, t, J = 7.3 Hz, OCH_2CH_3, enol), 1.22 (3H, t, J = 7.3 Hz, OCH_2CH_3, keto); δ_C (100 MHz, $CDCl_3$) data for the ketone tautomer only: 209.0 (COCH), 169.9 (COOEt), 61.4 (OCH_2), 57.9 (CH), 42.2 (CH_2CO), 30.2 (CH_2), 27.3 (CH_2), 25.3 (CH_2), 25.2 (CH_2), 24.5 (CH_2), 23.7 (CH_2), 21.1 (CH_2), 14.1 (CH_3). Diagnostic ^{13}C NMR resonances for the enol tautomer: 175.1, 173.7, 99.9 (CCO_2Et), 60.3 (OCH_2), 38.5 (CH_2CO). HRMS (ESI^+): calculated for $C_{13}H_{22}NaO_3$, 249.1461. Found: $[MNa]^+$, 249.1463 (−0.7 ppm error)].

Cyclodecanone (112)



A degassed solution of Ethyl 2-oxocyclodecane-1-carboxylate **(111)** (1.55 g, 10.0 mmol) in DMSO (8 mL) and H_2O (4 mL) was stirred at 150 °C (bath temperature) for 16 h. After reaching ambient temperature, the mixture was partitioned between water (20 mL) and Et_2O (4 × 20 mL), the combined organic phases were dried by filtering through $MgSO_4$ and then evaporated. The crude material obtained was purified by distillation under reduced pressure (b.p. 85 – 97 °C) and column chromatography (hexane:ethyl acetate 100:1 → 50:1 → 30:1) to give title compound **112** as a pale orange liquid (1.08 g, 82 %). δ_H (400 MHz, $CDCl_3$) 2.38 (4H, m, CH_2), 1.80 (4H, m, CH_2), 1.52 (4H, m, CH_2), 1.32 ppm (4H, m, CH_2); δ_C (100 MHz, $CDCl_3$): δ=218.4 (CO), 43.5 (CH_2), 26.9 (CH_2), 25.1 (CH_2), 24.3 (CH_2) ppm.

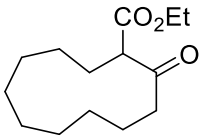
Azacycloundecan-2-one (113)



Cyclodecanone **112** (1.240 g, 8.04 mmol) in formic acid (9 mL) was added a solution of hydroxylamine-osulphonic acid (1.37 g, 12.1 mmol) in formic acid (4 mL) and heated to 110 C for 16 h. Purification by flash column chromatography (SiO2, 2:1 hexane:ethyl acetate → ethyl acetate → 9:1 ethyl acetate:methanol)

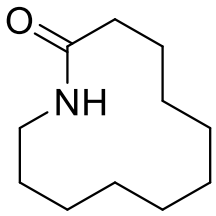
afforded the title compound as an orange solid (803 mg, 59%). Data consistent with those reported in the literature.[51]

Ethyl 2-oxocycloundecane-1-carboxylate (114)



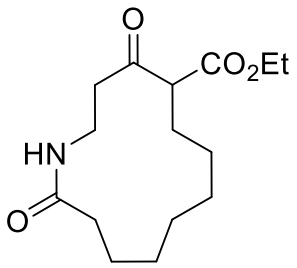
To a suspension of sodium hydride (114 mg, 4.25 mmol, 60% in mineral oil) and diethyl carbonate (0.55 mL, 4.25 mmol) in THF (2.5 mL) at RT was added a solution of cycloundecanone **112** (350 mg, 2.11 mmol) in THF (8 mL) was added dropwise until completion. The mixture was stirred at 75 °C for 20 h, then cooled to RT and acidified with 1M aq. HCl, followed by diethyl ether exaction, the combined organic extracts were dried over $MgSO_4$ and concentrated. Purification by column chromatography (hexane:ethyl acetate 20:1 → 10:1 → 5:1) to obtain the carboxylate title compound **114** as a yellow oil (470 mg, 94%). R_f (hexane:ethyl acetate 10:1) = 0.35; δ_H (400 MHz, $CDCl_3$) 4.12 (2H, q, *J* = 7.1 Hz, CH_2CH_3), 3.66 (1H, dd, *J* = 10.5, 3.0 Hz, O=CCH), 2.73 (1H, ddd, *J* = 16.1, 9.4, 2.9 Hz, O=CCH_2), 2.57 (1H, ddd, *J* = 16.1, 8.6, 3.1 Hz, O=CCH_2), 2.14-1.86 (2H, m, $CHCH_2$), 1.86-1.61 (2H, m, CH_2), 1.47–1.25 (12H, m, CH_2) 1.24-1.20 (3H, t, *J* = 7.06 Hz, CH_2CH_3). The spectroscopic data are consistent with those reported in the literature.[80]

Azacyclododecan-2-one (113)



To a stirring solution of cycloundecanone **112** (168 mg, 0.99 mmol) and formic acid (1 mL, 95% - 97%), with slowly add a solution of hydroxylamine-o-sulfonic acid (175 mg, 1.5 mmol) in formic acid (2 mL, 95% - 97%) at RT over 10 mins. The resulting solution was then microwave heated to 108 °C and reflux for 5.5 h. The dark brown mixture then cooled and quenched with ice water, neutralized with 5% aq. NaOH. Followed by chloroform extraction (3 x 10 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*, yielding the crude title compound **113** (90 mg, 47%) as a light brown to white solid. δ_H (400 MHz, $CDCl_3$) 5.62 (1H, br s, NH), 3.31 (2H, q, *J* = 6.0 $NHCH_2$), 2.17-2.24 (2H, m, $COCH_2$) 1.64-1.72 (2H, m, CH_2) 1.52-1.63 (4H, m, CH_2) 1.36-1.46 (4H, m, CH_2) 1.28-1.36 (6H, m, CH_2). The spectroscopic data are consistent with those reported in the literature.[79]

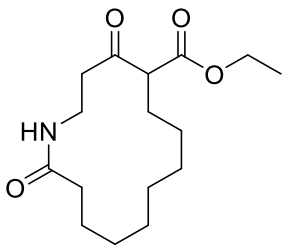
4,13-Dioxo-1-azacyclotridecane-5-carboxylate (136)



A mixture of ethyl 2-oxocyclononane-1-carboxylate **(108)** (425 mg, 2 mmol), $MgCl_2$ (571 mg, 6 mmol) and pyridine (0.97 mL, 12 mmol) in dichloromethane (15 mL) under an argon atmosphere was stirred at RT for 30 mins. To a solution of Fmoc-β-alanine (1245 mg, 4 mmol) in dichloromethane (7.5 mL), oxalyl chloride (0.86 mL, 10 mmol) was added followed by a catalytic amount of DMF (2 drops) under inert atmosphere protection. The resulting mixture was then stirred at RT for 1 h (the initial milky suspension became a homogeneous yellow transparent solution over the period) and concentrated *in vacuo* to remove all the solvent and excess oxalyl chloride. The resulting dried solid was then re-dissolved in dichloromethane (12 mL) and added to the ethyl 2-oxocyclononane-1-carboxylate mixture, stirred for 2 h at RT. The mixture solution was then diluted with dichloromethane (30 mL) and washed with 1M aq. HCl (45 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was re-dissolved in dichloromethane (10 mL) and piperidine (2.01 mL, 20 mmol) was added, followed by stirring at RT for 2 h, the solvent was then evaporated and the crude material dried *in vacuo*. Purification by column chromatography (SiO_2, 10:1 hexane: ethyl acetate → 2:1 hexane: ethyl acetate → ethyl acetate) afforded

title compound (230 mg, 40%) as a white solid; R_f 0.28 (3:1 hexane: ethyl acetate); mp 85-88℃; ν_{max}/cm^{-1} (thin film) 3299, 2933, 2860, 1741, 1708, 1641, 1537, 1444, 1367, 1266, 1183; δ_H (400 MHz, $CDCl_3$) δ 6.61 (1H, br s, NH), 4.01 (2H, q, *J* = 7.1, OCH_2CH_3), 3.44-3.55 (1H, m, $NHCH_2$), 3.30 (1H, t, *J* = 6.8 O=CCHC=O), 3.25-3.36 (1H, m, overlapped, $NHCH_2$), 2.69-2.86 (2H, m, $O=CCH_2$), 1.95-2.12 (2H, m, $CHCH_2$), 1.67-1.76 (2H, m, CH_2), 1.26–1.02 (13H, m CH_2); δ_C (100 MHz, $CDCl_3$) δ 206.9 (C=O, ketone), 173.7 (C=O, ester/amide), 169.4 (C=O, ester/amide), 61.2 (OCH_2), 58.3 (CH), 42.1 ($O=CCH_2$), 36.3 ($NHCH_2$), 33.9 (CH_2), 27.4 (CH_2), 26.1 (CH_2), 25.45 (overlapped $2CH_2$), 24.3 (CH_2), 24.1 (CH_2), 14.1 (CH_3).; HRMS (ESI^+): Found: 284.1849, $C_{15}H_{26}NO_4$ (MH^+) Requires: 284.1856 (2.2 ppm error), 306.1664, $C_{15}H_{25}NNaO_4$ (MNa^+) Requires: 306.1676 (3.9 ppm error).

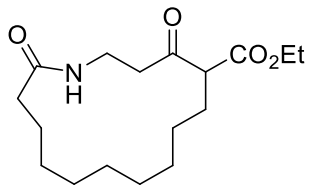
Ethyl 4,14-dioxoazacyclotetradecane-5-carboxylate (138)



A mixture of ethyl 2-oxocyclodecane-1-carboxylate **(109)** (340 mg, 1.50 mmol), $MgCl_2$ (285 mg, 3.01 mmol) and pyridine (0.72 mL, 9.00 mmol) in dichloromethane (15 mL) under an argon atmosphere was stirred at RT for 30 mins. To a solution of Fmoc-β-alanine (1.41 g, 4.50 mmol) in dichloromethane (8.5 mL), oxalyl chloride (1.12 mL, 13.5 mmol) was added followed by a catalytic amount of DMF (2 drops) under inert atmosphere protection. The resulting mixture was then stirred at RT for 1 h (the initial milky suspension became to homogeneous yellow transparent solution over the period) and concentrated *in vacuo* to remove all the solvent and excess oxalyl chloride. The resulting dried solid was then re-dissolved in dichloromethane (12 mL) and added to the ethyl 2-oxocyclodecane-1-carboxylate mixture and stirred for 2 h at RT. The mixture solution was then diluted with dichloromethane (30 mL) and washed with 1M aq. HCl (45 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was re-dissolved in dichloromethane (10 mL) and piperidine (1.48 mL, 15.0 mmol) was added, followed by stirring at RT for 2 h, the solvent was then evaporated yields crude material was dried *in vacuo*. Purification by column chromatography (SiO_2, 10:1 hexane: ethyl acetate → 2:1 hexane: ethyl acetate → ethyl acetate)

afforded title compound (195 mg, 43%) as a white solid; R_f 0.31 (1:1 hexane: ethyl acetate); mp 73-75°C; ν_{max}/cm^{-1} (thin film) 3300, 2928, 2858, 1739, 1710, 1642, 1535, 1441, 1366, 1261, 1180; δ_H (400 MHz, $CDCl_3$) 6.35 (1H, br s, NH), 4.06 (2H, q, *J* = 7.1, OCH_2CH_3), 3.43-3.52 (2H, m, $NHCH_2$), 3.30 (1H, dd, *J* = 10.8 O=CCHC=O), 2.72-2.79 (2H, m, $HCO=CCH_2$), 2.08 (t, 2H, *J* = 6.2, $HNO=CCH_2$), 1.65-1.97 (2H, m, $CHCH_2$), 1.26–1.02 (15H, m CH_2); δ_c (100 MHz, $CDCl_3$) δ 206.4 (C=O, ketone), 172.9 (C=O, ester/amide), 169.5 (C=O, ester/amide), 61.4 (OCH_2), 58.2 (CH), 41.5 ($O=CCH_2$), 34.5 ($NHCH_2$), 33.2 (CH_2), 28.6 (CH_2), 26.7 (CH_2), 26.1 (CH_2), 25.3 (CH_2), 24.9 (CH_2), 24.7 (CH_2), 24.5 (CH_2), 14.1 (CH_3); HRMS (ESI^+): Found: 298.2010, $C_{16}H_{28}NO_4$ (MH^+) Requires: 297.1900 (1.1 ppm error), 320.1817, $C_{16}H_{27}NNaO_4$ (MNa^+) Requires: 320.1800 (4.3 ppm error).

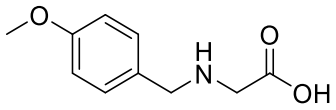
Ethyl 4,15-dioxoazacyclopentadecane-5-carboxylate (140)



A mixture of ethyl 2-oxocycloundecane-1-carboxylate **(110)** (250 mg, 1.05 mmol), $MgCl_2$ (190 mg, 2.00 mmol) and pyridine (0.51 mL, 6.25 mmol) in dichloromethane (10 mL) under an argon atmosphere was stirred at RT for 30 mins. To a solution of Fmoc-β-alanine (972 mg, 3.12 mmol) in dichloromethane (5.5 mL), oxalyl chloride (0.81 mL, 9.3 mmol) was added followed by a catalytic amount of DMF (2 drops) under inert atmosphere protection. The resulting mixture was then stirred at RT for 1 h (the initial milky suspension became to homogeneous yellow transparent solution over the period) and concentrated *in vacuo* to remove all the solvent and excess oxalyl chloride. The resulting dried solid was then re-dissolved in dichloromethane (12mL) and added to the ethyl 2-oxocycloundecane-1-carboxylate mixture and stirred for 2h at RT. The mixture solution was then diluted with dichloromethane (20 mL) and washed with 1M aq. HCl (30 mL). The aqueous layer was extracted with dichloromethane (4 × 15 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was re-dissolved in dichloromethane (10 mL) and piperidine (1.48 mL, 15.0 mmol) was added, followed by stirring at RT for 2 h, the solvent was then evaporated yields crude material was dried *in vacuo*. Purification by column chromatography (SiO_2, 10:1 hexane: ethyl acetate → 2:1 hexane: ethyl acetate → ethyl acetate) afforded the title compound **140** (176 mg, 63%) as a white crystalline solid; R_f 0.27

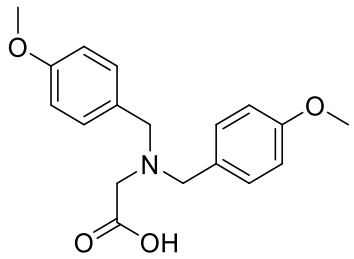
(1:1 hexane: ethyl acetate); mp 74-75℃; ν_{max}/cm^{-1} (thin film) 3325, 2926, 2855, 1740, 1711, 1640, 1535, 1430, 1365, 1281, 1255, 1180; δ_H (400 MHz, $CDCl_3$) 6.17 (1H, br s, NH), 4.12 (2H, qd, *J* = 7.1, 1 Hz, OCH_2CH_3), 3.71-3.61 (1H, m, $NHCH_2$), 3.44 (1H, dd, *J* = 10.3, 3.9 Hz, O=CCHC=O), 3.29 (1H, ddt, *J* = 13.9, 7.2, 5.2 Hz, $CHCH_2$), 2.33-2.24 (1H, m, $NHCH_2$), 2.89-2.82 (2H, m, $HCO=CCH_2$), 2.22 (1H, ddd, *J* = 14.4, 7.5, 3.6 Hz, $HNO=CCH_2$), 2.01 (2H, ddd, *J* = 14.2, 9.9, 3.5 Hz, $O=CCH_2$), 1.51 (1H, m, $HNO=CCH_2$), 1.36–1.05 (13H, overlapped m CH_2), 1.21 (3H, overlapped t, *J* = 7.2 Hz, CH_2CH_3); δ_C (100 MHz, $CDCl_3$) 206.6 (C=O, ketone), 173.4 (C=O, ester/amide), 169.5 (C=O, ester/amide), 61.6 (OCH_2), 57.8 (CH), 43.6 ($O=CCH_2$), 36.0 ($NHCH_2$), 33.9 (CH_2), 28.4 (CH_2), 27.8 (CH_2), 27.5 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 26.7 (CH_2), 26.5 (CH_2), 24.9 (CH_2), 14.2 (CH_3); HRMS (ESI$^+$): Found: 334.1982, $C_{17}H_{29}NNaO_4$ (MNa^+) Requires: 334.2000 (1.7 ppm error).

4-methoxybenzyl)glycine (151)



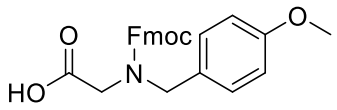
Glycine (1.5 g, 20 mmol) and NaOH (800 mg, 20 mmol) were dissolved in water (20 mL). A solution of *p*-methoxy-benzaldehyde (2.75 g, 20 mmol) in ethanol (7.5 mL) was added into above mixture. Resulting clear yellow solution was heated to 60 °C, and then stirred without heating for 2 h. After cooling to 10 °C in an ice bath, the solution was treated with $NaBH_4$ (250 mg, 6 mmol) with slow addition over 30 mins, The reaction was left in RT for 30 mins, followed by second addition of *p*-methoxy-benzaldehyde (1.36g, 10 mmol), resulting reaction mixture was then cooled to 10 °C again with slow addition of $NaBH_4$ (125 mg, 3 mmol). The reaction was then left at RT for 30 min followed by cooling back to 10 °C for third addition of *p*-methoxy-benzaldehyde (1.36g, 10 mmol) and $NaBH_4$ (150 mg, 4 mmol) over the period of 30 min. The reaction was then left overnight at 5 °C followed by solvent removal of EtOH, resulting aqueous solution was extracted with DCM (3 × 10 mL). The extracted aqueous layer was acidified to pH 6.5 with 1M HCl, followed by filtration of precipitated white solid **149**. The volume of filtrate was reduced to 10 mL, crystallized product **150** (160 mg, 40 %) was afforded after low temperature filtration and ice water wash. δ_H (400 MHz, d_6-DMSO) 7.35 (2H, d, J = 8.5, H_{Ar}), 6.89 (2H, d, J = 8.5, H_{Ar}), 3.96 (2H, s, CH_2), 3.69 (3H, s, CH_3), 3.21 (2H, s, CH_2). The spectroscopic data are consistent with those reported in the literature.[81]

bis(4-methoxybenzyl)glycine (149)



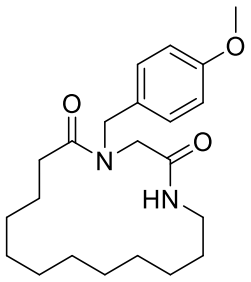
Title compound **149** afforded as white solid (100 mg) generated as side product from reaction of **150**. ν_{max}/cm^{-1} (thin film) 2837, 1683, 1607, 1509, 1449, 1380, 1300, 1247, 1175, 1031, 808, 549 δ_H (400 MHz, d_6-DMSO) 7.20 (4H, d, *J* = 8.5 Hz, H_{Ar}), 6.84 (4H, d, *J* = 8.5 Hz, H_{Ar}), 3.78 (2H, s, $COCH_2$), 3.68 (6H, s, OCH_3), 3.60 (4H, s, NCH_2) The spectroscopic data are consistent with those reported in the literature. [81]

N-(((9H-fluoren-9-yl)methoxy)carbonyl)-N-(4-methoxybenzyl)glycine (151)



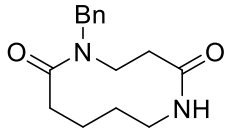
Compound **150** (160 mg, 0.8 mmol) was dissolved in a mixture of water (5 mL) and dioxane (5 mL) and the pH of the solution was brought to around pH 8 using solid sodium bicarbonate (1.5 equiv, 100 mg, 1.6 mmol). The mixture was then cooled to 0 °C and a solution of fluorenylmethyloxycarbonyl chloride (Fmoc-Cl, 1.2 equiv, 250 mg, 0.96 mmol) in 1,4-dioxane (1 mL) was added dropwise. The reaction was allowed to stir at RT overnight. The solvent was evaporated *in vacuo*, remained aqueous phase was washed with ethyl acetate and acidified with 10% aq. HCl. Extraction with ethyl acetate (5 × 15 mL) was followed by drying of the combined organic phases over $MgSO_4$ and evaporation of the solvent. The residue was subjected to chromatography on silica gel (CH_2Cl_2/MeOH 9:1) to yield Fmoc-protected amino acid **151** as white solid (257 mg, 74 %). R_f = 0.21 (silica, CH_2Cl_2/MeOH 9:1); δ_H (400 MHz, $CDCl_3$): 8.91 (1H, br s, OH), 7.77 (2H, d, J = 7.5 Hz, H_{Ar}), 7.75 (2H, d, J = 7.5 Hz, H_{Ar}), 7.56 (2H, d, J = 7.4 Hz, H_{Ar}), 7.32 (2H, d, J = 7.4 Hz, H_{Ar}), 7.16 (1H, m, H_{Ar}), 6.98 (1H, m, H_{Ar}), 6.85 (1H, m, H_{Ar}), 4.60 (2H, s, CH_2), 4.42 (2H, s, CH_2), 3.82 (3H, s, CH_3), δ_C (100 MHz, $CDCl_3$): 174.9 (COOH), 159.1 (C_{Ar}), 156.5 (CO), 143.7 (C_{Ar}), 141.3 (C_{Ar}), 129.6 (C_{Ar}), 129.0 (C_{Ar}), 127.7 (C_{Ar}), 127.1 (C_{Ar}), 124.9 (C_{Ar}), 120.0 (C_{Ar}), 114.1 (C_{Ar}), 67.9 (CH_2), 55.2 (CH_3), 50.5 (CH_2), 47.2 (CH_2), 46.5(CH). HRMS (ESI^+): Found: 440.1468, $C_{25}H_{23}NO_5$ (MH^+) Requires: 440.1472 (0.4 ppm error).

4-(4-methoxybenzyl)-1,4-diazacyclohexadecane-2,5-dione (153)



A mixture of laurolactam **144** (158.0 mg, 0.81 mmol), DMAP (9.6 mg, 0.081 mmol) and pyridine (0.384 mL, 4.79 mmol) in DCM (10 mL) under an argon atmosphere was stirred at RT for 30 mins. Next, a solution of acid chloride (1.18 mmol, 1.50 equiv., freshly prepared from **151** using the general procedure) in DCM (3 mL) was added and the resulting mixture was refluxed at 50 °C for 16 h. The mixture was then diluted with DCM (30 mL) and washed with 10% aq. HCl (30 mL). The aqueous layer was then extracted with DCM (3 × 30 mL) and the combined organic extracts dried over $MgSO_4$ and concentrated in vacuo. The crude material was then re-dissolved in DCM (8 mL) and DBU (1.2 mL, 7.96 mmol) was added, followed by stirring at RT overnight, before the solvent was removed in vacuo. Purification by flash column chromatography (SiO_2, 1:1 ethyl acetate: hexane → 9:1 ethyl acetate: methanol) afforded the title compound. δ_H (400 MHz, $CDCl_3$) 7.06 (2H, dd, *J* = 8.5 Hz, ArH), 6.77 (2H, d, *J* = 8.6 Hz, ArH), 4.52 (2H, s, CH_2), 3.86 (2H, s, CH_2), 3.69 (3H, s, CH_3), 3.17 (2H, m, CH_2), 2.40 (2H, m, CH_2), 1.66 – 1.52 (4H, m, $2CH_2$). HRMS (ESI^+): Found: 397.2462, $C_{22}H_{34}NNaO_3$ (MNa^+) Requires: 397.2463 (0.4 ppm error).

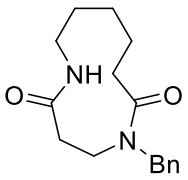
5-Benzyl-1,5-diazecane-2,6-dione (156)



A mixture of δ-valerolactam **154** (78.0 mg, 0.786 mmol), DMAP (10 mg, 0.0786 mmol) and pyridine (0.380 mL, 4.72 mmol) in DCM (5.5 mL) under an argon atmosphere was stirred at RT for 30 mins. Next, a solution of acid chloride **163** (1.18 mmol, 1.50 equiv., freshly prepared using the general procedure) in DCM (3 mL) was added and the resulting mixture was refluxed at 50 °C for 16 h. The mixture was then diluted with DCM (30 mL) and washed with 10% aq. HCl (30 mL). The aqueous layer was then extracted with DCM (3 × 30 mL) and the combined organic extracts dried over $MgSO_4$ and concentrated in vacuo. The crude material was then re-dissolved in DCM (8 mL) and DBU (1.2 mL, 7.86 mmol) was added, followed by stirring at RT overnight, before the solvent was removed in vacuo. Purification by flash column chromatography (SiO_2, 1:1 ethyl acetate: hexane → 9:1 ethyl acetate: methanol) afforded the title compound (as a 10:1 mixture of rotamers) as a colourless oil (129 mg, 65%); R_f 0.26 (9:1 ethyl acetate: methanol); ν_{max}/cm^{-1} (neat) 3326, 2929, 1645, 1607, 1535; δ_H (400 MHz, $CDCl_3$) 7.35–7.17 (10H, m, Ph, both rotamers), 6.00 (1H, d, *J* = 9.2 Hz, NH, minor), 5.85 (1H, br s, NH, major), 5.01 (1H, d, *J* = 14.5 Hz, NCH_2Ph, major), 4.84 (1H, d, *J* = 16.8 Hz, NCH_2Ph, minor), 4.28 (1H, d, *J* = 16.8 Hz, NCH_2Ph, minor), 4.20 (1H, d, *J* = 14.5 Hz, NCH_2Ph, major), 3.97– 3.82 (2H, m, $BnNCH_2$, both), 3.80–3.65 (2H, m, $BnNCH_2$, both), 3.30–3.20 (1H, m, CH_2NH, major), 3.19– 3.14 (1H, m, CH_2NH, minor), 2.97–2.90 (1H, m,

CH_2NH, minor), 2.89–2.78 (1H, m, CH_2NH, major), 2.75– 2.59 (2H, m, $COCH_2$, both), 2.50 (1H, m, $COCH_2$, major), 2.43–2.34 (1H, m, $COCH_2$, minor), 2.20–2.04 (8H, m, 2 × CH_2, both), 1.69–1.55 (4H, m, CH_2, both); δ_C (100 MHz, $CDCl_3$) data for the major rotamer only: 174.1 (CO), 171.2 (CO), 138.0 (CCH), 129.0 (CCH), 128.1 (CH), 127.8 (CH), 49.1 (CH_2Ph), 45.2 (CH_2), 39.3 (CH_2), 37.4 (CH_2), 28.3 (CH_2), 25.8 (CH_2), 24.0 (CH_2). Diagnostic 13C NMR resonances for the minor rotamer: 179.2 (CO), 176.4 (CO), 136.7 (Ph), 60.5 (CH_2). HRMS (ESI): calcd. for $C_{15}H_{20}N_2NaO_2$, 283.1417. Found: $[MNa]^+$, 283.1417 (−0.1 ppm error)].

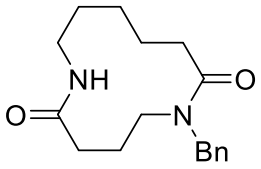
5-Benzyl-1,5-diazacycloundecane-2,6-dione (159)



A mixture of ε-caprolactam **157** (89 mg, 0.786 mmol), DMAP (10 mg, 0.0786 mmol) and pyridine (0.380 mL, 4.72 mmol) in DCM (5.5 mL) under an argon atmosphere was stirred at RT for 30 mins. Next, a solution of acid chloride **163** (1.18 mmol, 1.50 equiv, freshly prepared using the general procedure) in DCM (3 mL) was added and the resulting mixture was refluxed at 50 °C for 16 h. The mixture was then diluted with DCM (30 mL) and washed with 10% aq. HCl (30 mL). The aqueous layer was then extracted with DCM (3 × 30 mL) and the combined organic extracts dried over $MgSO_4$ and concentrated in vacuo. The crude material was then re-dissolved in DCM (8 mL) and DBU (1.20 mL, 7.86 mmol) was added,

followed by stirring at RT overnight, before the solvent was removed in vacuo. Purification by flash column chromatography (SiO_2, 5:1 hexane:ethyl acetate → 1:1 hexane:ethyl acetate → 9:1 ethyl acetate:methanol) afforded the title compound (as a 6:1 mixture of rotamers determined by ^1H NMR in $CDCl_3$ at RT) as a colourless oil (170 mg, 79%). Note, to aid characterisation the ^1H NMR data was recorded at 80 °C, which resolved the rotamer resonances. R_f = 0.37 (9:1 ethyl acetate:methanol); ν_{max}/cm^{-1} (thin film) 3289, 2931, 1620, 1554; δ_H (400 MHz, d_6-DMSO, 80 °C) 7.85 (1H, br s, NH), 7.39–7.15 (5H, m, Ph), 4.53 (2H, br s, $PhCH_2$), 3.50 (2H, br s, $BnNCH_2$), 2.96 (2H, br s, $NHCH_2$), 2.22 (4H, br s, 2 × CH_2), 1.68–1.41 (4H, m, 2 × CH_2), 1.27 (2H, br s, CH_2). Diagnostic ^1H NMR resonances recorded in $CDCl_3$ at RT, which confirm the presence of the two rotamer forms can be found at: 6.10 (1H, br s, NH, major), 5.97 (1H, br s, NH, minor); δ_C (100 MHz, $CDCl_3$) 173.5 (CO), 171.4 (CO), 138.5 (C), 129.0 (CH), 128.3 (CH), 127.9 (CH), 49.2 (CH_2Ph), 45.2 ($BnNCH_2$), 41.8 ($NHCH_2$), 37.07 (CH_2CONH), 28.6 (CH_2CO), 25.3 (CH_2), 24.4 (CH_2), 22.8 (CH_2); HRMS (ESI): calcd. for $C_{16}H_{22}N_2NaO_2$, 297.1573. Found: $[MNa]^+$, 297.1567 (2.2 ppm error).

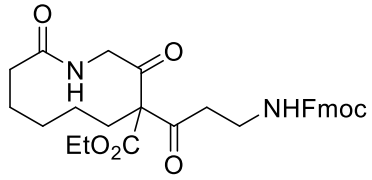
5-Benzyl-1,5-diazacyclododecane-2,6-dione (162)



A mixture of azocan-2-one **160** (100 mg, 0.786 mmol), DMAP (10 mg, 0.0786 mmol) and pyridine (0.380 mL, 4.72 mmol) in DCM (5.5 mL) under an argon atmosphere was stirred at RT for 30 mins. Next, a solution of acid chloride **163** (1.18 mmol, 1.50 equiv., freshly prepared using the general procedure) in DCM (3 mL) was added and the resulting mixture was refluxed at 50 °C for 16 h. The mixture was then diluted with DCM (30 mL) and washed with 10% aq. HCl (30 mL). The aqueous layer was then extracted with DCM (3 × 30 mL) and the combined organic extracts dried over $MgSO_4$ and concentrated in vacuo. The crude material was then re-dissolved in DCM (8 mL) and DBU (1.2 mL, 7.86 mmol) was added, followed by stirring at RT overnight, before the solvent was removed in vacuo. Purification by flash column chromatography (SiO_2, 1:1 ethyl acetate: hexane → 9:1 ethyl acetate: methanol) afforded the title compound (as a 5:1 mixture of rotamers determined by ^{1}H NMR in $CDCl_3$ at RT) as a colourless oil (190 mg, 84%). Note, to aid characterisation the ^{1}H NMR data was recorded at high temperature, which resolved the rotamer resonances. R_f = 0.42 (9:1 ethyl acetate: methanol); ν_{max}/cm^{-1} (neat) 3299, 2928, 1623, 1552, 1448; δ_H (400 MHz, d^6-DMSO, 120 °C) 7.60– 7.15 (6H, m, Ph, NH), 4.57 (2H, br s, $PhCH_2$), 3.15 (2H, s, $BnNCH_2$), 2.57–2.18 (4H, m, $NHCH_2$, CH_2CONH), 1.84–1.02 (10H, m, 5 × CH_2). Diagnostic ^{1}H NMR resonances recorded in $CDCl_3$ at RT, which confirm the presence of the two

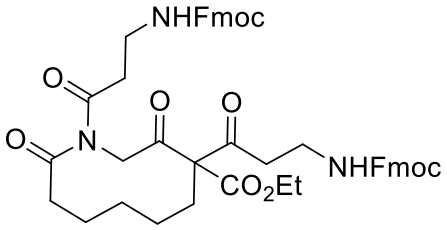
rotamer forms can be found at: 6.57 (1H, br s, NH, major), 5.71 (1H, br s, NH, minor); δ_C (100 MHz, $CDCl_3$) data for the major rotamer only: 175.9 (CO), 170.4 (CO), 136.6 (C), 129.0 (CH), 127.8 (CH), 126.5 (CH), 51.9 (CH_2Ph), 41.1 ($BnNCH_2$), 39.2 (CH_2NH), 35.1 (CH_2), 32.5 (CH_2), 27.3 (CH_2), 25.9 (CH_2), 24.0 (CH_2), 22.3 (CH_2). ^{13}C NMR resonances for the minor rotamer: 174.9 (CO), 171.0 (CO), 138.5 (C), 128.8 (CH), 128.6 (CH), 127.7 (CH), 60.5 (CH_2), 48.7 (CH_2), 44.9 (CH_2), 36.7 (CH_2), 30.9 (CH_2), 26.7 (CH_2), 23.6 (CH_2), 22.5 (CH_2), 14.3 (CH_2). HRMS (ESI): calcd. for $C_{17}H_{24}N_2NaO_2$, 311.1730. Found: $[MNa]^+$: 311.1718 (3.4 ppm error).

ethyl 4-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoyl)-3,10-dioxoazecane-4-carboxylate (172)



A mixture of macrocycle **169** (430 mg, 2.01 mmol), $MgCl_2$ (400 mg, 3.98 mmol) and pyridine (0.97 mL, 12.04 mmol) in DCM (12.5 mL) under an argon atmosphere was stirred at RT for 30 mins. Next, a solution of acid chloride **39** (4.47 mmol, prepared using the general procedure) in DCM (6.5 mL) was added and the reaction mixture was stirred for 2 h at rt. The mixture was then diluted with DCM (100 mL) and washed with 10% aq. HCl (100 mL). The aqueous layer was extracted with DCM (3 × 50 mL) and the combined organic extracts dried over $MgSO_4$ and concentrated *in vacuo.* Purification by flash column chromatography (SiO_2, ethyl acetate: hexane 1:9 → 1:1) afforded the title compound as pale-yellow solid (855 mg, 82%). δ_H (400 MHz, $CDCl_3$) 8.26 (1H, s, NH), 7.69 (2H, d, *J* = 7.5 Hz, H_{Ar}), 7.52 (2H, d, *J* = 7.4 Hz, H_{Ar}), 7.32 (2H, t, *J* = 7.4 Hz, H_{Ar}), 7.26 – 7.21 (2H, m, H_{Ar}), 4.25 (2H, m, CH_2), 4.19 – 4.02 (5H, m, overlapped CH,CH_2,CH_2O), 3.41 (2H, m, $NHCH_2$), 2.62 (2H, m, CH_2CO), 1.59 – 1.33 (4H, m, overlapped CH_2), 1.23 – 1.04 (5H, m, overlapped CH_2, CH_3). δ_C 205.24 (CO), 202.13 (CO), 175.70 (CONH), 170.19 (COO), 156.55 (NHCO), 143.7 (2 C_{Ar}), 141.1 (2 C_{Ar}), 127.6 (2 C_{Ar}), 126.9 (2 C_{Ar}), 124.9 (2 C_{Ar}), 119.9 (2 C_{Ar}), 77.4 (C), 66.7 (OCH_2), 61.2 (OCH_2), 49.8 (CH_2), 46.9 (CH), 37.8 (CH_2), 36.3 (CH_2), 36.1 (CH_2), 26.1 (CH_2), 25.1 (CH_2), 23.5 (CH_2), 22.7 (CH_2), 13.8 (CH_3).HRMS (ESI): calcd. for $C_{30}H_{34}N_2NaO_7$, 557.2240. Found: $[MNa]^+$, 557.2258 (3.5 ppm error).

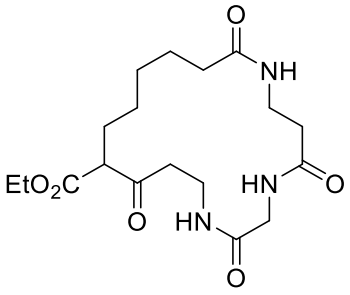
ethyl 1,4-bis(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoyl)-3,10-dioxoazecane-4-carboxylate (174)



A mixture of compound **172** (440 mg, 0.82 mmol), DMAP (5 mg, 0.046 mmol) and pyridine (0.15 mL, 1.46 mmol) in DCM (5.5 mL) under an argon atmosphere was stirred at RT for 30 mins. Next, a solution of acid chloride **39** (1.18 mmol, 1.50 equiv., freshly prepared using the general procedure) in DCM (3 mL) was added and the resulting mixture was refluxed at 50 °C for 16 h. The mixture was then diluted with DCM (30 mL) and washed with 10% aq. HCl (30 mL). The aqueous layer was then extracted with DCM (3 × 30 mL) and the combined organic extracts dried over $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography (SiO_2, ethyl acetate: hexane 1:9 → 1:1) afforded the title compound as pale-yellow solid (600 mg, 88%). ν_{max}/cm^{-1} (neat) 2947, 1707, 1523, 1250, 741. δ_H (400 MHz, $CDCl_3$) 7.75 (4H, d, *J* = 7.6 H_{Ar}), 7.56 (4H, d, *J* = 7.5, H_{Ar}), 7.37 (4H, t, *J* = 7.5 Hz, H_{Ar}), 7.32 – 7.28 (4H, m, H_{Ar}), 4.37 (3H, m, overlapped CH_2), 4.22 – 4.07 (3H, overlapped CH, CH_2), 3.58-3.20 (4H, m, CH_2NH), 2.75-2.55 (4H, m, CH_2), 1.25 (3H, t, *J* = 7.1 Hz, CH_3). δ_c (100MHz, $CDCl_3$) 202.1 (CO), 199.9 (CO), 175.2 (NCO), 171.3 (NCO), 167.6 (COO), 156.4 (NHCO), 156.3 (NHCO) 144.5 (4 C_{Ar}), 141.25 (4 C_{Ar}), 127.77 (4 C_{Ar}), 127.18 (4 C_{Ar}), 125.09 (4 C_{Ar}), 120.10 (4 C_{Ar}), 76.07

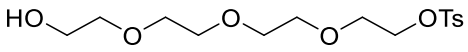
(C), 66.75 (CH_2), 62.80 (CH_2), 60.44 (CH_2), 55.01 (CH_2), 47.16 (CH), 40.30 (CH_2), 39.59 (CH_2), 36.77 (CH_2), 36.00 (CH_2), 34.13 (CH_2), 33.02 (CH_2), 26.78 (CH_2), 22.63 (CH_2), 21.06 (CH_2), 19.86 (CH_2), 13.92 (CH_3). HRMS (ESI): calcd. for $C_{48}H_{49}N_3NaO_{10}$, 850.3317. Found: $[MNa]^+$, 850.3310 (-1.2 ppm error).

ethyl 2,5,9,16-tetraoxo-1,4,8-triazacyclooctadecane-15-carboxylate (175)



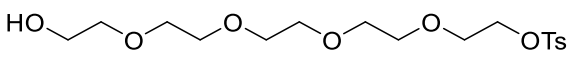
Compound **174** (200 mg, 0.25 mmol) was dissolved in dichloromethane (12.5 mL) and DBU (0.38 mL, 2.51 mmol) was added. The resulting mixture was stirred for overnight at RT, before the solvent was removed *in vacuo*. Purification by column chromatography (hexane: ethyl acetate 1:2 → 1:9 methanol: ethyl acetate), afforded the compound **171**, no desired product obtained.

2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (197a)



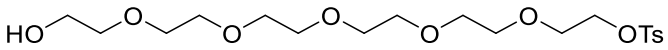
A solution of sodium hydroxide (237 mg, 6.85 mmol) in water (3 mL) was added to a solution of tetraethylene glycol **196a** (8.78 g, 45.2 mmol) in THF (2 mL). The resulting mixture was cooled to 0 °C and an ice cooled solution of p-toluenesulfonyl chloride (835 mg, 4.38 mmol) in THF (5 mL) was slowly added under stirring for 2 hours. After stirring at 0 °C for 3 hours, the reaction mixture was poured onto an ice water mixture (25 mL). The organic layer was separated, and the aqueous layer was extracted with dichloromethane (5 × 15 mL). The combined organic layers were washed twice with water (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*, afforded title compound **197a** (1.41 g, 92%) as a colorless clear oil. R_f 0.35 (5% MeOH in dichloromethane), δ_H (400MHz, $CDCl_3$) 7.78 (2H, d, *J* = 8.3 Hz, C_6H_4), 7.32 (2H, d, *J* = 8.3 Hz, C_6H_4), 4.13-4.18 (2H, m, $MeC_6H_4SO_3CH_2$), 3.77-3.51 (14H, m, OCH_2CH_2), 2.45 (1H, s, OH), 2.42 (3H, s, CH_3); HRMS (ESI^+): Found: 371.1135, $C_{15}H_{24}NaO_7S$ (MNa^+) Requires: 371.1100 (3.7 ppm error). These data are in accordance with those reported in the literature.[70]

14-hydroxy-3,6,9,12-tetraoxatetradecyl 4-methylbenzenesulfonate (197b)



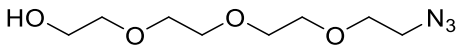
A solution of sodium hydroxide (320 mg, 8 mmol) in water (1.75 mL) was added to a solution of pentaethylene glycol **196b** (4.77 g 20.1 mmol) in THF (1.25 mL). The resulting mixture was cooled to 0 °C, an ice cooled solution of p-toluenesulfonyl chloride (954 mg, 5.01 mmol) in THF (5 mL) was slowly added under stirring for 1 hours. After stirring at 0 °C for another 2 hours, the reaction mixture was poured onto an ice water mixture (20 mL) and then partitioned between water and dichloromethane, followed by dichloromethane (5 × 10 mL) extraction of aqueous layer. The combined organic layers were washed twice with brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*, afforded title compound **197b** (1.92 g, 97%) as a colorless clear oil with good purity without further purification. R_f 0.34 (5% MeOH in dichloromethane), δ_H (400MHz, $CDCl_3$) 7.79 (2H, d, *J* = 8.3 Hz, C_6H_4), 7.34 (2H, d, *J* = 8.3 Hz, C_6H_4), 4.13-4.17 (2H, m, $MeC_6H_4SO_3CH_2$), 3.71-3.57 (18H, m, OCH_2CH_2), 2.56 (1H, s, OH), 2.44 (3H, s, CH_3); HRMS (ESI$^+$): Found: 393.1578, $C_{17}H_{38}O_8S$ (M^+), 415.1401, $C_{17}H_{38}NaO_8S$ (MNa^+) Requires: 392.1505 (0.7 ppm error). These data are in accordance with those reported in the literature.[82]

17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (197c)



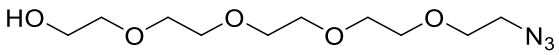
A solution of sodium hydroxide (223 mg, 5.62 mmol) in water (1.5 mL) was added to a solution of hexaethylene glycol **196c** (9.88 g 35.2 mmol) in THF (1.5 mL). The resulting mixture was cooled to 0 °C and an ice cooled solution of p-toluenesulfonyl chloride (668 mg, 3.50 mmol) in THF (5 mL) was slowly added under stirring for 2 hours. After stirring at 0 °C for 3 hours, the reaction mixture was poured onto an ice water mixture (20 mL). The organic layer was separated, and the aqueous layer was extracted with dichloromethane (5 × 10 mL). The combined organic layers were washed twice with water (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*, afforded title compound **197c** (1.50 g, 99%) as a colorless clear oil. R_f 0.35 (5% MeOH in dichloromethane), δ_H (400MHz, $CDCl_3$) 7.78 (2H, d, *J* = 8.4 Hz, C_6H_4), 7.32 (2H, d, *J* = 8.4 Hz, C_6H_4), 4.12-4.16 (2H, m, $MeC_6H_4SO_3CH_2$), 3.71-3.57 (22H, m, OCH_2CH_2), 2.78 (1H, s, OH), 2.43 (3H, s, CH_3); HRMS (ESI^+): Found: 459.1659, $C_{19}H_{32}NaO_9S$ (MNa^+) Requires: 459.1648 (2.7 ppm error). These data are in accordance with those reported in the literature.[83]

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-ol (198a)



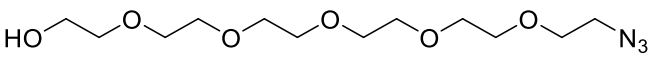
Sodium azide (522 mg, 8.35 mmol) was added to a solution of the monotosylated tetraethylene glycol derivative **197a** (1.4g, 4.05 mmol) in absolute ethanol (20 mL). The reaction mixture was then heated at 85 °C and reflux for 2 hours. The reaction was quenched with water (20 mL) and dichloromethane (25 mL) were added and the aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic phases were washed successively with water and brine, dried with $MgSO_4$ and concentrated *in vacuo*, afford the title compound **198a** (882 mg, 99%) as clear colorless oil. R_f 0.25 (5% MeOH in dichloromethane), δ_H (400MHz, $CDCl_3$) 3.72 (2H, m, CH_2OH), 3.69-3.64 (10H, m, OCH_2CH_2), 3.62-3.58 (2H, m, CH_2), 3.39 (2H, t, J = 5.0 Hz, CH_2O) 2.50 (2H, t, J = 6.3 Hz, CH_2N_3) 1.71 (1H, br s, OH); HRMS (ESI^+): Found: 242.1103, $C_8H_{17}N_3NaO_4$ (MNa^+) Requires: 242.1100 (3.4 ppm error). These data are in accordance with those reported in the literature.[70]

14-azido-3,6,9,12-tetraoxatetradecan-1-ol (198b)



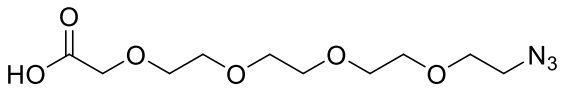
Sodium azide (651 mg, 10.00 mmol) was added to a solution of the monotosylated tetra ethylene glycol derivative **197b** (1.92g, 4.91 mmol) in absolute ethanol (25 mL). The reaction mixture was then heated and reflux at 85 °C for 2 hours. The mixture was quenched with water (20 mL) and dichloromethane (25 mL) were added. The aqueous layer was then extracted with dichloromethane (3 × 20 mL), combined organic phases were washed successively with water and brine, dried with $MgSO_4$ and concentrated *in vacuo*, afford the title compound **198b** (1.14 g, 87%) as clear colorless oil. R_f 0.27 (5% MeOH in dichloromethane), δ_H (400MHz, $CDCl_3$) 3.74 - 3.68 (2H, m, CH_2OH), 3.69 - 3.62 (16, m, OCH_2CH_2), 3.62-3.58 (2H, m, CH_2), 3.38 (2H, t, *J* = 5.1 Hz, CH_2O), 2.80 (1H, br s, OH), 1.74 (2H, t, *J* = 6.3 Hz, CH_2N_3); HRMS (ESI^+): Found: 286.1373 $C_{10}H_{21}N_3NaO_5$ (MNa^+) Requires: 286.1368 (1.9 ppm error). These data are in accordance with those reported in the literature.[83]

17-azido-3,6,9,12,15-pentaoxaheptadecan-1-ol (198c)



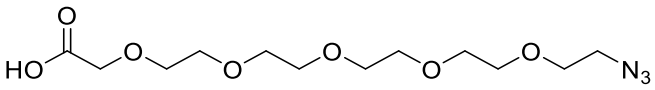
Sodium azide (458 mg, 7.35 mmol) was added to a solution of the monotosylated tetraethylene glycol derivative **197c** (1.6g, 3.60 mmol) in absolute ethanol (20 mL). The reaction mixture was then heated at 85 °C and reflux for 2 hours. The reaction was quenched with water (20 mL) and dichloromethane (25 mL) were added and the aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic phases were washed successively with water and brine, dried with $MgSO_4$ and concentrated *in vacuo*, afford the title compound **198c** (1041 mg, 93%) as clear colorless oil. R_f 0.25 (5% MeOH in dichloromethane), δ_H (400MHz, $CDCl_3$) 3.74 - 3.68 (2H, m, CH_2OH), 3.69 - 3.62 (16, m, OCH_2CH_2), 3.62-3.58 (2H, m, CH_2), 3.38 (2H, t, J = 5.1 Hz, CH_2O), 2.80 (1H, br s, OH), 1.74 (2H, t, J = 6.3 Hz, CH_2N_3); HRMS (ESI^+): Found: 330.1623 $C_{12}H_{25}N_3NaO_6$ (MNa^+) Requires: 330.1636 (3.8 ppm error). These data are in accordance with those reported in the literature.[83]

14-Azido-3,6,9,12-tetraoxatetradecanoic acid (199a)



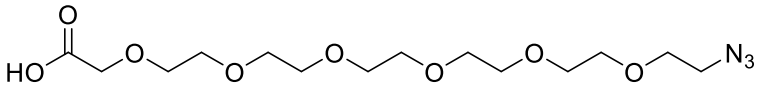
Compound alcohol azide **198a** 2.19 g, 10.0 mmol) and sodium hydride (803 mg, 20.0 mmol, 2.0 eq) were solubilised in dry THF (25 mL) and stirred at 0°C for 45 minutes. An ice cooled bromoacetic acid (1.38 g, 10.0 mmol, 1.0 eq) in dry THF (25 mL) solution was then added dropwise. The reaction was stirred under nitrogen at RT for 24 h. The solvent was removed *in vacuo* and the residue taken up in water (80 mL), acidified to pH 2 with 1M aq. HCl (20 mL) and then the aqueous phase was extracted with DCM (5 × 50 mL). The organic fractions were combined, dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to yield a crude, light orange oil (2.16 g, 95%). R_f 0.47 (65:25:4 $CHCl_3$/MeOH/H_2O, $KMnO_4$ stain). δ_H (400 MHz, $CDCl_3$), 4.14 (2H, s, $O{=}CCH_2$), 3.75 (2H, m, OCH_2) 3.72-3.61 (13H, m, CH_2CH_2O), 3.40 (2H, t, J = 4.96 Hz, CH_2N_3), HRMS (ESI^+): Found: 300.1169, $C_{10}H_{19}N_3NaO_6$ (MNa^+) Requires: 300.1200 (-9.9 ppm error). These data are in accordance with those reported in the literature.[83]

17-azido-3,6,9,12,15-pentaoxaheptadecanoic acid (199b)



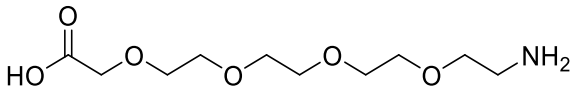
Compound alcohol azide **198b** 1.08 g, 3.50 mmol) and sodium hydride (330 mg, 7.01 mmol, 2.0 eq) were solubilised in dry THF (9 mL) and stirred at 0°C for 45 minutes. An ice cooled bromoacetic acid (483 mg, 3.51 mmol, 1.0 eq) in dry THF (9 mL) solution was then added dropwise. The reaction was stirred under nitrogen at RT for 24 h. The solvent was removed *in vacuo* and the residue taken up in water (50 mL), acidified to pH 2 with 1M aq. HCl (15 mL) and then the aqueous phase was extracted with DCM (5 × 20 mL). The organic fractions were combined, dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to yield a crude, light orange oil (2.16 g, 95%). R_f 0.47 (65:25:4 $CHCl_3$/MeOH/H_2O, $KMnO_4$ stain). δ_H (400MHz, $CDCl_3$) 4.14 (2H, s, $O{=}CCH_2$), 3.76-3.71 (2H, m, OCH_2) 3.72-3.61 (21H, m, CH_2CH_2O), 3.38 (2H, t, J = 4.9 Hz, CH_2N_3), HRMS (ESI^+): Found: 344.1428, $C_{12}H_{23}N_3NaO_7$ (MNa^+) Requires: 344.1423 (1.1 ppm error). These data are in accordance with those reported in the literature.[83]

20-azido-3,6,9,12,15,18-hexaoxaicosanoic acid (199c)



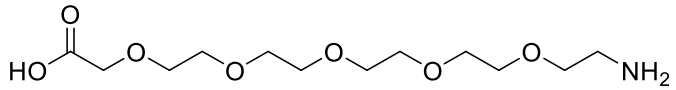
Compound alcohol azide **198c** 1.08 g, 3.50 mmol) and sodium hydride (330 mg, 7.01 mmol, 2.0 eq) were solubilised in dry THF (9 mL) and stirred at 0°C for 45 minutes. An ice cooled bromoacetic acid (483 mg, 3.51 mmol, 1.0 eq) in dry THF (9 mL) solution was then added dropwise. The reaction was stirred under nitrogen at RT for 24 h. The solvent was removed *in vacuo* and the residue taken up in water (50 mL), acidified to pH 2 with 1M aq. HCl (15 mL) and then the aqueous phase was extracted with DCM (5 × 20 mL). The organic fractions were combined, dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to yield a crude, light orange oil (2.16 g, 95%). R_f 0.47 (65:25:4 $CHCl_3$/MeOH/H_2O, $KMnO_4$ stain). δ_H (400MHz, $CDCl_3$) 4.14 (2H, s, $O=CCH_2$), 3.76-3.71 (2H, m, OCH_2) 3.72-3.61 (21H, m, CH_2CH_2O), 3.38 (2H, t, J = 4.9 Hz, CH_2N_3) These data are in accordance with those reported in the literature.[83]

14-Amino-3,6,9,12-tetraoxatetradecanoic acid (200a)



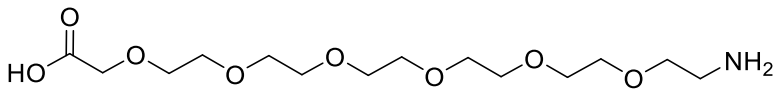
14-azido-3,6,9,12-tetraoxatetradecanoic acid **199a** (681 mg, 2.45 mmol) was dissolved in methanol (20 mL), flushed with nitrogen, and palladium on carbon (250 mg, 10% w/w) was added. Hydrogen filled balloons were used as hydrogen source until completion of the reaction as monitored by TLC (CH_2Cl_2/MeOH 1:1). The reaction mixture was then filtered over Celite and the solvent removed *in vacuo*, yield amino acid **200a** as colorless oil (428 mg, 70 %). R_f=0.13 (silica, 50 % MeOH in CH_2Cl_2); δ_H (400MHz, $CDCl_3$) 3.93 (s, 2H, CH_2C=O), 3.86 (2H, t, *J* = 4.9 Hz, CH_2O), 3.73 (2H, t, *J* = 4.6 Hz, CH_2O), 3.69-3.60 (12H, m, CH_2CH_2O), 3.11 (2H, t, *J* = 4.8 Hz, CH_2NH_2), 2.16 (1H, br s, NH_2). These data are in accordance with those reported in the literature.[84]

17-amino-3,6,9,12,15-pentaoxaheptadecanoic acid (200b)



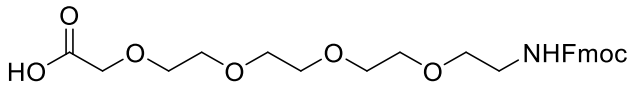
17-azido-3,6,9,12,15-pentaoxaheptadecanoic acid **199b** (1.49 g, 4.6 mmol) was dissolved in methanol (46 mL), flushed with nitrogen, and palladium on carbon (460 mg, 10% w/w) was added. Hydrogen filled balloons were used as hydrogen source until completion of the reaction as monitored by TLC (CH_2Cl_2/MeOH 1:1). The reaction mixture was then filtered over Celite and the solvent removed *in vacuo*, yield amino acid **200b** as light orange oil (1.14 mg, 99 %). R_f=0.11 (silica, 50 % MeOH in CH_2Cl_2); δ_H (400 MHz, $CDCl_3$), 3.92 (s, 2H, CH_2C=O), 3.86 (2H, t, J = 4.9 Hz, CH_2O), 3.74 (2H, t, J = 4.6 Hz, CH_2O), 3.69-3.60 (14H, m, CH_2CH_2O), 3.07 (2H, t, J = 4.8 Hz, CH_2NH_2), 1.98 (1H, br s, NH_2). HRMS (ESI^+): Found: 318.1523, $C_{12}H_{25}NNaO_7$ (MNa^+) Requires: 318.1515 (3.6 ppm error)

20-amino-3,6,9,12,15,18-hexaoxaicosanoic acid (200c)



20-azido-3,6,9,12,15,18-hexaoxaicosanoic acid **199c** (1.16 g, 3.17 mmol) was dissolved in methanol (30 mL), flushed with argon, and palladium on carbon (300 mg, 10% w/w) was added. Hydrogen filled balloons were used as hydrogen source until completion of the reaction as monitored by TLC (CH_2Cl_2/MeOH 1:1). The reaction mixture was then filtered through Celite followed by solvent removal *in vacuo*, yield amino acid **200c** as dark brown oil (1.02 g, 94.8 %). R_f=0.13 (silica, 50 % MeOH in CH_2Cl_2); δ_H (400 MHz, $CDCl_3$): 3.94 (s, 2H, CH_2C=O), 3.87 (2H, t, *J* = 4.9 Hz, CH_2O), 3.75 (2H, t, *J* = 4.6 Hz, CH_2O), 3.69-3.55 (16H, m, CH_2CH_2O), 3.04 (2H, t, *J* = 4.8 Hz, CH_2NH_2), 1.85 (1H, br s, NH_2).

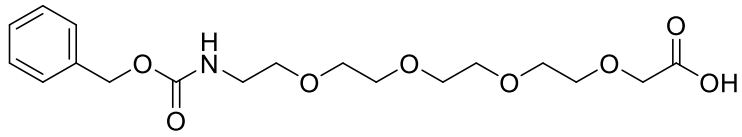
1-(9H-Fluoren-9-yl)-3-oxo-2,7,10,13,16-pentaoxa-4-azaoctadecan-18-oic acid (201a)



14-Amino-3,6,9,12-tetraoxatetradecanoic acid **200a** (428 mg, 1.71 mmol) was dissolved in a mixture of water (10 mL) and dioxane (10 mL) and the pH of the solution was brought to around pH 8 using solid sodium bicarbonate (1.5 equiv, 215 mg, 3.50 mmol). The mixture was then cooled to 0 °C and a solution of fluorenylmethyloxycarbonyl chloride (Fmoc-Cl, 1.2 equiv, 526 mg, 2.04 mmol) in 1,4-dioxane (1 mL) was added dropwise. The reaction was allowed to stir at RT overnight. The solvent was evaporated *in vacuo*, remained aqueous phase was washed with ethyl acetate and acidified with 10% aq. HCl. Extraction with ethyl acetate (5 × 30 mL) was followed by drying of the combined organic phases over $MgSO_4$ and evaporation of the solvent. The residue was subjected to chromatography on silica gel (CH_2Cl_2/MeOH 9:1) to yield Fmoc-protected amino acid **201a** as colorless oil (395 mg, 52 %). R_f = 0.21 (silica, CH_2Cl_2/MeOH 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75 (2H, d, J = 7.5 Hz, H_{Ar}), 7.61 (2H, d, J = 7.4 Hz, H_{Ar}), 7.38 (2H, t, J = 7.4 Hz, H_{Ar}), 7.30 (2H, t, J = 7.4 Hz, H_{Ar}.), 5.57 (1H, br s, N*H*), 4.38 (2H, d, J = 7.0Hz, CH_2Fmoc), 4.21 (1H, t, J = 7.2 Hz, C*H*Fmoc), 4.11 (2H, s, CH_2C=O), 3.79–3.53 (14H, m overlapped peaks, CH_2CH_2O), 3.50-3.36 ppm (2H, m, $NHCH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ=175.8 (C=O), 159.0 (C=O), 145.4 (C_{Ar}), 142.7 (C_{Ar}), 128.8 (C_{Ar}), 128.2 (C_{Ar}), 126.2 (C_{Ar}), 120.9 (C_{Ar}), 72.1 (CH_2), 71.9 (CH_2), 71.5 (CH_2), 71.4 (CH_2), 71.2 (CH_2), 71.1 (CH_2), 70.7 (CH_2), 67.6 (CH_2), 58.4 (CH), 41.7 (CH_2NH)

ppm; HRMS (ESI^+): Found: 474.2110, $C_{25}H_{32}NO_8$ (MH^+) Requires: 474.2100 (2.3 ppm error), 496.1900, $C_{25}H_{31}NNaO_8$ (MNa^+) Requires: 496.1929 (2.7 ppm error). These data are in accordance with those reported in the literature.[84]

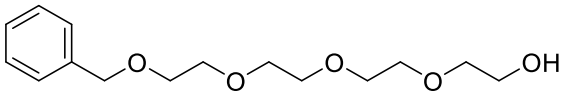
3-oxo-1-phenyl-2,7,10,13,16-pentaoxa-4-azaoctadecan-18-oic acid (202a)



14-Amino-3,6,9,12-tetraoxatetradecanoic acid **(200a)** (500 mg, 2.00 mmol) was dissolved in a mixture of water (10 mL) and dioxane (10 mL) and solid sodium carbonate (2 eq, 424 mg, 4.0 mmol) was used to adjust the solution to pH = 8. The mixture was then cooled to 0 °C and a solution of Benzyl chloroformate (Cbz-Cl, 1.1 equiv, 0.37 mL, 2.20 mmol) was added dropwise. The reaction was allowed to stir at RT overnight, followed by solvent was evaporation and concentrated *in vacuo*, remained aqueous phase was washed with ethyl acetate and acidified with 10% aq. HCl. Extraction with dichloromethane (5 × 30 mL) was followed by drying of the combined organic phases over $MgSO_4$ and solvent evaporation. The residue light yellow oil was subjected to chromatography on silica gel (CH_2Cl_2/MeOH 9:1) to yield Cbz-protected amino acid **202a** as colorless oil (600 mg, 78 %). R_f = 0.51 (silica, CH_2Cl_2/MeOH 9:1); ν_{max}/cm^{-1} (thin film) 3346, 2870, 1672, 1533, 1455, 1348, 1251, 1103, 740, 699, 563; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.30 (5H, m, H_{Ar}), 5.60 (1H, br s, NH), 5.05 (2H, s, CH_2Cbz), 4.63 (1H, m, CH_2O), 4.06 (1H,

m, CH_2O), 3.57 (12H, m, CH_2CH_2), 3.50 (2H, s, CH_2CH_2), 3.33 (2H, m, CH_2NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ=172.7 (C=O), 156.7 (C=O), 141.0 (C_{Ar}), 136.6 (C_{Ar}), 128.6 (C_{Ar}), 128.2 (C_{Ar}), 127.1 (2C, overlapped, C_{Ar}), 71.0 (CH_2), 70.6 (CH_2), 70.4 (CH_2), 70.3 (CH_2), 70.1 (CH_2), 68.7 (CH_2), 67.0 (CH_2), 66.7 (CH_2), 65.0 (CH), 40.8 (CH_2NH) ppm; HRMS (ESI^+): Found: 386.1816, $C_{18}H_{28}NO_8$ (MH^+) Requires: 386.1809 (1.8 ppm error), 408.1635, $C_{18}H_{27}NNaO_8$ (MNa^+) Requires: 408.1629 (0.9 ppm error).

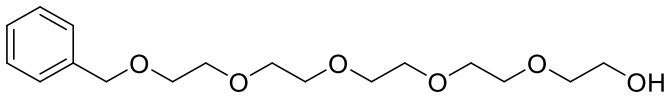
1-phenyl-2,5,8,11-tetraoxatridecan-13-ol (207a)



Tetraethylene glycol **196a** (10 g, 50mmol) was dissolved in 50% aqueous solution of NaOH (230 mg in 0.44 mL H_2O) with stirring, benzyl chloride (0.62 mL, 5.18 mmol) was added dropwise into reaction mixture at room temperature, upon completion of Benzyl chloride addition, the reaction was brought to 100 °C and left reflux for 12 h with stirring. Completed reaction was then cooled to RT and 50 mL of ice water were used to quench the reaction mixture. Followed by hexane wash (2 × 10 mL) and extracted with EtOAc. Resulting organic extraction was washed with ice water (2 × 10 mL) and dried over anhydrous magnesium sulfate Afforded title compound **207a** (1.41 g, 97 %) as colorless oil. δ_H (400MHz, $CDCl_3$): 7.26 – 7.37 (5H, m, H_{Ar}), 4.56 (2H, s, CH_2), 3.57 – 3.72 (m, 17H CH_2); HRMS (ESI^+): Found: 285.1697, $C_{15}H_{25}NO_5$ (MH^+) Requires: 285.1693 (1.8 ppm error), 307.1516,

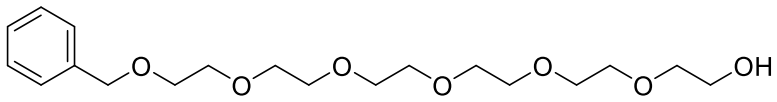
$C_{15}H_{24}NNaO_5$ (MNa^+) Requires: 307.1511 (1.3 ppm error). These data are in accordance with those reported in the literature.[85]

1-phenyl-2,5,8,11-tetraoxatridecan-13-ol (207b)



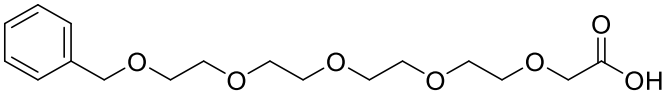
Pentaethylele glycol **196b** (2.38 g, 10mmol) was dissolved in 50% aqueous solution of NaOH (230 mg in 0.44 mL H_2O) with stirring, benzyl chloride (0.62 mL, 5.18 mmol) was added dropwise into reaction mixture at room temperature, upon completion of Benzyl chloride addition, the reaction was brought to 100 °C and left reflux for 12 h with stirring. Completed reaction was then cooled to RT and 50 mL of ice water were used to quench the reaction mixture. Followed by hexane wash (2 × 10 mL) and extracted with EtOAc. Resulting organic extraction was washed with ice water (2 × 10 mL) and dried over anhydrous magnesium sulfate, afforded title compound **207b** (1.41 g, 78 %) as colorless oil. δ_H (400MHz, $CDCl_3$): 7.38 – 7.27 (m, 5 H H_{Ar}). 4.55 (s, 2H CH_2); 3.51 – 3.74 (m, 19H CH_2); HRMS (ESI^+): Found: 329.1959, $C_{17}H_{29}NO_6$ (MH^+) Requires: 329.1960 (0.0 ppm error), 351.1780, $C_{17}H_{28}NaO_6$ (MNa^+) Requires: 351.1778 (-0.5 ppm error). These data are in accordance with those reported in the literature.[86]

1-phenyl-2,5,8,11,14,17-hexaoxanonadecan-19-ol (207c)



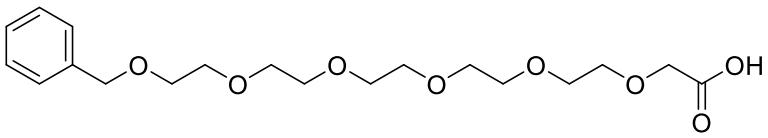
Hexaethylene glycol **196c** (2.82 g, 10mmol) was dissolved in 50% aqueous solution of NaOH (230 mg in 0.44 mL H_2O) with stirring, benzyl chloride (0.62 mL, 5.18 mmol) was added dropwise into reaction mixture at room temperature, upon completion of Benzyl chloride addition, the reaction was brought to 100 °C and left reflux for 12 h with stirring. Completed reaction was then cooled to RT and 50 mL of ice water were used to quench the reaction mixture. Followed by hexane wash (2 × 10 mL) and extracted with EtOAc. Resulting organic extraction was washed with ice water (2 × 10 mL) and dried over anhydrous magnesium sulfate, afforded title compound **207c** (1.45 g, 76 %) as colorless oil. δ_H (400MHz, $CDCl_3$): 7.38 – 7.27 (m, 5 H H_{Ar}). 4.55 (s, 2H CH_2); 3.51 – 3.74 (m, 19H CH_2); HRMS (ESI^+): Found: 390.2493, $C_{19}H_{36}NO_7$ (MH^+) Requires: 390.2486 (1.8 ppm error), 395.2406, $C_{19}H_{32}NaO_7$ (MNa^+) Requires: 395.2040 (-1.3 ppm error). These data are in accordance with those reported in the literature.[86]

1-phenyl-2,5,8,11,14-pentaoxahexadecan-16-oic acid (208a)



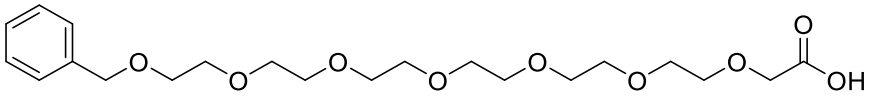
Compound **207a** 1.43 g, 5.01 mmol) and sodium hydride (402 mg, 10.01 mmol, 2.0 eq) were solubilised in dry THF (12.5 mL) and stirred at 0°C for 45 minutes. An ice cooled Bromoacetic acid (700 mg, 5.01 mmol, 1.0 eq) in dry THF (12.5 mL) solution was then added dropwise. The reaction was stirred under nitrogen at RT for 24 h. The solvent was removed *in vacuo* and the residue taken up in water (50 mL), washed with hexane (2 × 25 mL), and acidified to pH 2 with 1M aq. HCl (10 mL) and then the aqueous phase was extracted with DCM (5 × 25 mL). The organic fractions were combined, dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to yield a crude orange oil as title compound **208a** with excellent purity (1.62 g, 94%). δ_H(400MHz, $CDCl_3$) 7.26-7.34 (5H, m, H_{Ar}), 4.56 (2H, s, CH_2) 4.12 (2H, s, CH_2), 3.74-3.58 (17H, m, OCH_2CH_2), HRMS (ESI^+): Found: 365.1570, $C_{17}H_{26}NaO_7$ (MNa^+) Requires: 365.1571 (-1.9 ppm error). These data are in accordance with those reported in the literature.[83]

1-phenyl-2,5,8,11,14,17-hexaoxanonadecan-19-oic acid (208b)



Compound **207b** 1.33 g, 4.01 mmol) and sodium hydride (322 mg, 8.01 mmol, 2.0 eq) were solubilised in dry THF (12.5 mL) and stirred at 0°C for 45 minutes. An ice cooled Bromoacetic acid (560 mg, 4.01 mmol, 1.0 eq) in dry THF (12.5 mL) solution was then added dropwise. The reaction was stirred under nitrogen at RT for 24 h. The solvent was removed *in vacuo* and the residue taken up in water (50 mL), washed with hexane (2 × 25 mL), and acidified to pH 2 with 1M aq. HCl (10 mL) and then the aqueous phase was extracted with DCM (5 × 25 mL). The organic fractions were combined, dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to yield a crude drak orange oil as title compound **208b** with excellent purity (1.43 g, 94%). δ_H (400MHz, $CDCl_3$) 7.26-7.37 (5H, m, H_{Ar}), 4.56 (2H, s, CH_2) 4.13 (2H, s, CH_2), 3.74-3.58 (20H, m, OCH_2CH_2), HRMS (ESI^+): Found: 409.1831, $C_{19}H_{30}NaO_8$ (MNa^+) Requires: 409.1833 (1.0 ppm error). These data are in accordance with those reported in the literature.[83]

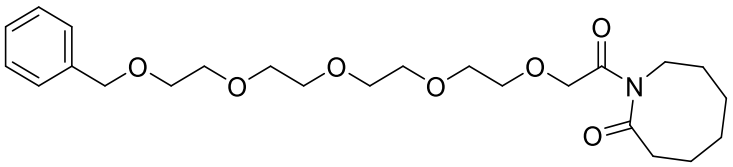
1-phenyl-2,5,8,11,14,17,20-heptaoxadocosan-22-oic acid (208c)



Compound **207c** 145 g, 3.91 mmol) and sodium hydride (322 mg, 8.01 mmol, 2.0 eq) were solubilised in dry THF (20 mL) and stirred at 0°C for 45 minutes. An ice cooled Bromoacetic acid (560 mg, 4.01 mmol, 1.0 eq) in dry THF (10 mL) solution

was then added dropwise. The reaction was stirred under nitrogen at RT for 24 h. The solvent was removed *in vacuo* and the residue taken up in water (50 mL), washed with hexane (2 × 25 mL), and acidified to pH 2 with 1M aq. HCl (10 mL) and then the aqueous phase was extracted with DCM (5 × 25 mL). The organic fractions were combined, dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to yield a crude green oil as title compound **208c** with excellent purity (1.41 g, 85%). δ_H (400MHz, $CDCl_3$) 7.26-7.34 (5H, m, H_{Ar}), 4.56 (2H, s, CH_2) 4.13 (2H, s, CH_2), 3.76-3.7 (2H, m, OCH_2) 3.69-3.57 (22, m, OCH_2CH_2), HRMS (ESI^+): Found: 453.2101, $C_{19}H_{30}NaO_8$ (MNa^+) Requires: 453.2095 (-0.9 ppm error). These data are in accordance with those reported in the literature.[83] [83]

1-(1-phenyl-2,5,8,11,14-pentaoxahexadecan-16-oyl)azocan-2-one (224)

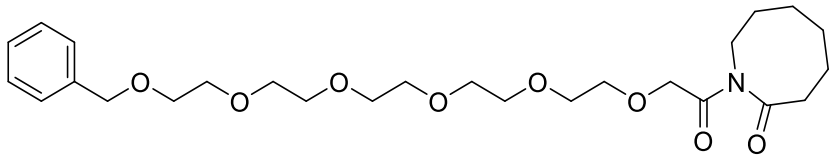


A mixture of azocan-2-one **222** (265 mg, 2.08 mmol), pyridine (1.01 mL, 12.5 mmol) and DMAP (25.4 mg, 0.208 mmol) in dichloromethane (20 mL) under an argon atmosphere was stirred at RT for 30 mins.

To a solution of 1-phenyl-2,5,8,11,14-pentaoxahexadecan-16-oic acid **208a** (940 mg, 3.12 mmol) in dichloromethane (15.5 mL), oxalyl chloride (0.817 mL, 19.36 mmol) was added followed by a catalytic amount of DMF (1 drop) under inert

atmosphere protection. The resulting mixture was then stirred at RT for 1 h (the initial milky suspension became to homogeneous yellow transparent solution over the period) and concentrated *in vacuo* to remove all of the solvent and excess oxalyl chloride. The resulting dried solid was then re-dissolved in dichloromethane (15 mL) and slowly, dropwise added to the 12-aminododecanolactam mixture and resulting solution was stirred and reflux at 50°C overnight. The mixture solution was then diluted with dichloromethane (20 mL) and washed with 1M aq. HCl (20 mL). The aqueous layer was extracted with dichloromethane (4 × 15 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to chromatography on silica gel (10:1 hexane: ethyl acetate → 2:1 hexane: ethyl acetate → ethyl acetate) afforded *title compound* (1.07 g, 97%) as a colorless oil; R_f 0.31 (5% MeOH in EtOAc); ν_{max}/cm^{-1} (thin film) 2921, 2861, 1691, 1453, 1381, 1175, 1122, 1110, 1090, 888, 740, 699. δ_H (400 MHz, $CDCl_3$) 7.37 – 7.25 (5H, m, H_{Ar}), 4.63 – 4.60 (2H, s, $PhCH_2$), 4.55 (2H, s, CH_2CO), 3.94 – 3.86 (2H, m, cyclic NCH_2), 3.73 – 3.58 (17H, m, OCH_2CH_2), 2.68 – 2.55 (2H, m, cyclic $COCH_2$), 1.90 – 1.81 (2H, m, cyclic CH_2), 1.72 (2H, m, cyclic CH_2H), 1.61 – 1.51 (2H, m, cyclic CH_2), 1.49 – 1.39 (2H, m, cyclic CH_2). δ_c (100 MHz, $CDCl_3$) 178.14 (cyclic NCO), 174.02 (NCO), 138.36 (C_{Ar}), 128.44 (2 C_{Ar}), 127.84 (2 C_{Ar}), 127.66 (C_{Ar}), 74.21 (PhC), 73.31 (OCH_2), 70.79 – 70.63 (multiple OCH_2CH_2), 43.41 (cyclic CH_2), 36.97 (cyclic CH_2), 29.25 (cyclic CH_2), 29.01 (cyclic CH_2), 26.26 (cyclic CH_2), 24.10 (cyclic CH_2). HRMS (ESI^+): Found: 474.2459, $C_{24}H_{37}NNaO_7$ (MNa^+) Requires: 474.2462 (0.7 ppm error).

1-(1-phenyl-2,5,8,11,14,17-hexaoxanonadecan-19-oyl)azocan-2-one (232)

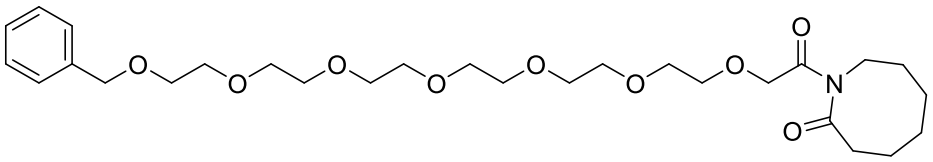


A mixture of azocan-2-one **227** (305 mg, 2.41 mmol), pyridine (1.51 mL, 19.5 mmol) and DMAP (37.4 mg, 0.24 mmol) in dichloromethane (20 mL) under an argon atmosphere was stirred at RT for 30 mins.

To a solution of 1-phenyl-2,5,8,11,14,17-hexaoxanonadecan-19-oic acid **208b** (1.43 g, 3.71 mmol) in dichloromethane (15.5 mL), oxalyl chloride (1.224 mL, 29.06 mmol) was added followed by a catalytic amount of DMF (1 drop) under inert atmosphere protection. The resulting mixture was then stirred at RT for 1 h (the initial milky suspension became to homogeneous yellow transparent solution over the period) and concentrated *in vacuo* to remove all of the solvent and excess oxalyl chloride. The resulting dried solid was then re-dissolved in dichloromethane (15 mL) and slowly, dropwise added to the 12-aminododecanolactam mixture and resulting solution was stirred and reflux at 50°C overnight. The mixture solution was then diluted with dichloromethane (20 mL) and washed with 1M aq. HCl (20 mL). The aqueous layer was extracted with dichloromethane (4 × 15 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to chromatography on silica gel (10:1 hexane: ethyl acetate → 2:1 hexane: ethyl acetate → ethyl acetate) afforded *title compound*

(1.16 g, 98%) as a colorless oil; R_f 0.28 (5% MeOH in EtOAc); ν_{max}/cm^{-1} (thin film) 2862, 1691, 1452, 1380, 1248, 1203, 1090, 740, 699. δ_H (400 MHz, $CDCl_3$) 7.36 – 7.28 (5H, m, H_{Ar}), 4.62 (2H, s, CH_2), 4.54 (2H, s, CH_2), 3.90 (2H, m, cyclic NCH_2), 3.80 – 3.54 (24H, m, OCH_2CH_2), 2.63 (2H, m, cyclic $COCH_2$), 1.85 (2H, m, cyclic CH_2), 1.67 (2H, m, cyclic CH_2), 1.63 – 1.52 (2H, m, cyclic CH_2), 1.49 – 1.38 (2H, m, cyclic CH_2), δ_c(100 MHz, $CDCl_3$) 178.1 (cyclic NCO), 174.0 (NCO), 138.3 (C_{Ar}), 128.4 (C_{Ar}), 127.8 (C_{Ar}), 127.6 (C_{Ar}), 74.2 (PhC), 73.3 (OCH_2), 71.2 – 70.2 (multiple OCH_2CH_2), 69.5 (OCH_2CO), 43.4 (cyclic CH_2), 36.9 (cyclic CH_2), 29.2 (cyclic CH_2), 29.0 (cyclic CH_2), 26.3 (cyclic CH_2), 24.1 (cyclic CH_2). HRMS (ESI^+): Found: 518.2708, $C_{26}H_{42}NNaO_8$ (MNa^+) Requires: 518.2724 (4.2 ppm error).

1-(1-phenyl-2,5,8,11,14,17,20-heptaoxadocosan-22-oyl)azocan-2-one (233)

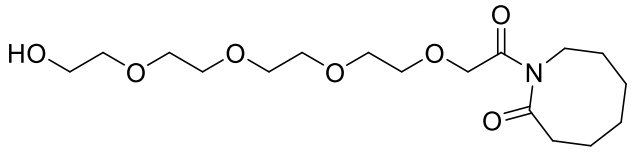


A mixture of azocan-2-one **227** (330 mg, 2.61 mmol), pyridine (1.31 mL, 15.6 mmol) and DMAP (40.4 mg, 0.26 mmol) in dichloromethane (20 mL) under an argon atmosphere was stirred at RT for 30 mins.

To a solution of 1-phenyl-2,5,8,11,14,17,20-heptaoxadocosan-22-oic acid **208c** (1.43 g, 3.91 mmol) in dichloromethane (15.5 mL), oxalyl chloride (1.04 mL, 23.4 mmol) was added followed by a catalytic amount of DMF (1 drop) under inert atmosphere protection. The resulting mixture was then stirred at RT for 1 h (the

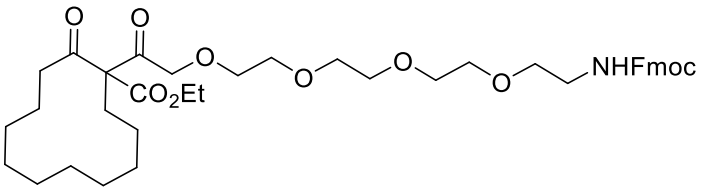
initial milky suspension became to homogeneous yellow transparent solution over the period) and concentrated *in vacuo* to remove all of the solvent and excess oxalyl chloride. The resulting dried solid was then re-dissolved in dichloromethane (15 mL) and slowly, dropwise added to the 12-aminododecanolactam mixture and resulting solution was stirred and reflux at 50°C overnight. The mixture solution was then diluted with dichloromethane (20 mL) and washed with 1M aq. HCl (20 mL). The aqueous layer was extracted with dichloromethane (4 × 15 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to chromatography on silica gel (10:1 hexane: ethyl acetate → 2:1 hexane: ethyl acetate → ethyl acetate) afforded *title compound* (1.32 g, 94.3%) as a colorless oil; R_f 0.23 (5% MeOH in EtOAc); ν_{max}/cm^{-1} (thin film) 2863, 1692, 1453, 1381, 1206, 1125, 1092, 741, 700, 596. δ_H (400 MHz, $CDCl_3$) 7.35 – 7.25 (5H, m, H_{Ar}), 4.62 (2H, s, CH_2), 4.55 (2H, s, CH_2), 3.91 (2H, m, cyclic NCH_2), 3.73 – 3.59 (27H, m, OCH_2CH_2), 2.63 (2H, m, cyclic $COCH_2$), 1.84 (2H, m, cyclic CH_2), 1.69 (2H, m, cyclic CH_2), 1.56 (2H, m, cyclic CH_2), 1.39 (2H, m, cyclic CH_2). δ_c (100 MHz, $CDCl_3$) 178.1 (cyclic NCO), 174.0 (NCO), 138.3 (C_{Ar}), 128.5 (C_{Ar}), 127.8 (C_{Ar}), 127.6 (C_{Ar}), 74.2 (PhC), 73.3 (OCH_2), 70.8 – 70.3 (multiple OCH_2CH_2), 69.5 (OCH_2CO), 43.4 (cyclic CH_2), 36.9 (cyclic CH_2), 29.2 (cyclic CH_2), 29.0 (cyclic CH_2), 26.3 (cyclic CH_2), 24.1 (cyclic CH_2). HRMS (ESI^+): Found: 562.2991, $C_{28}H_{45}NNaO_9$ (MNa^+) Requires: 562.2987 (-0.5 ppm error).

1-(14-hydroxy-3,6,9,12-tetraoxatetradecanoyl)azocan-2-one (230)



1-(1-phenyl-2,5,8,11,14-pentaoxahexadecan-16-oyl)azocan-2-one **229** (100 mg, 0.24 mmol) was dissolved in ethyl acetate (20 mL), flushed with nitrogen, and palladium on carbon (24 mg, 10% w/w) was added. Hydrogen filled balloons were used as hydrogen source until completion of the reaction as monitored by TLC (CH_2Cl_2/MeOH 1:1). The reaction mixture was then filtered over Celite and the solvent removed *in vacuo*, yield deprotected free alcohol title compound as colorless oil (76 mg, 87 %). R_f=0.13 (silica, 50 % MeOH in CH_2Cl_2); δ_H (400MHz, $CDCl_3$) 3.93 (s, 2H, CH_2C=O), 3.86 (2H, t, J = 4.9 Hz, CH_2O), 3.73 (2H, t, J = 4.6 Hz, CH_2O), 3.69-3.60 (12H, m, CH_2CH_2O), 3.11 (2H, t, J = 4.8 Hz, CH_2NH_2), 2.16 (1H, br s, NH_2). ν_{max}/cm^{-1} (thin film) 3463, 2923, 2864, 2175, 1950, 1692, 1447, 1381, 1206, 1127, 1091, 1007, 889, 707, 553, 506. δ_H (400 MHz, $CDCl_3$) 4.53 (2H, s, $COCH_2$), 3.82 (2H, m, cyclic NCH_2), 3.71 – 3.37 (16H, m, OCH_2CH_2), 2.53 (2H, m, cyclic $COCH_2$), 1.73 (2H, m, cyclic CH_2), 1.62 (2H, m, cyclic CH_2), 1.50 (2H, m, cyclic CH_2), 1.34 (2H, m, cyclic CH_2).δ_c(100 MHz, $CDCl_3$) 178.13 (cyclic NCO), 174.1 (NCO), 73.2 (OCH_2), 71.1 – 70.1 (multiple OCH_2CH_2), 69.5 (OCH_2CO), 43.4 (cyclic CH_2), 37.0 (cyclic CH_2), 29.2 (cyclic CH_2), 29.0 (cyclic CH_2), 26.2 (cyclic CH_2), 24.1 (cyclic CH_2). HRMS (ESI^+): Found: 384.1987, $C_{17}H_{31}NNaO_7$ (MNa^+) Requires: 384.1998 (0.3 ppm error).

Ethyl 1-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16-pentaoxa-4-azaoctadecan-18-oyl)-2-oxocyclododecane-1-carboxylate (211)

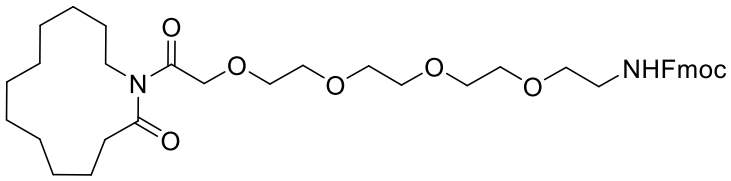


A mixture of ethyl 2-oxocyclododecane-1-carboxylate **209** (135 mg, 0.53 mmol), $MgCl_2$ (100 mg, 1.06 mmol) and pyridine (0.25 mL, 3.18 mmol) in dichloromethane (10 mL) under an argon atmosphere was stirred at RT for 30 mins.

To a solution of 1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16-pentaoxa-4-azaoctadecan-18-oic acid **(201a)** (378 mg, 0.79 mmol) in dichloromethane (5.5 mL), oxalyl chloride (0.21 mL, 2.30 mmol) was added followed by a catalytic amount of DMF (1 drop) under inert atmosphere protection. The resulting mixture was then stirred at RT for 1 h (the initial milky suspension became to homogeneous yellow transparent solution over the period) and concentrated *in vacuo* to remove all of the solvent and excess oxalyl chloride. The resulting dried solid was then re-dissolved in dichloromethane (12mL) and added to the ethyl 2-oxocyclododecane-1-carboxylate mixture and stirred for 2h at RT. The mixture solution was then diluted with dichloromethane (20 mL) and washed with 1M aq. HCl (20 mL). The aqueous layer was extracted with dichloromethane (4 × 15 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to chromatography on silica gel (10:1 hexane: ethyl acetate → 2:1 hexane: ethyl acetate → ethyl acetate) afforded *title compound* **211** (87 mg, 24%) as a clear oil; R_f 0.43 (1:1 hexane: ethyl acetate);

ν_{max}/cm^{-1} (thin film) 2928, 2865, 1727, 1693, 1450, 1335, 1216, 1141, 1109, 740; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (1H, s, NH), 7.76 (2H, d, *J* = 7.6 Hz, H_{Ar}), 7.54 (2H, d, *J* = 7.5 Hz, H_{Ar}), 7.40 (2H, t, *J* = 7.5 Hz, H_{Ar}), 7.31 (2H, t, *J* = 7.4 Hz, H_{Ar}), 4.66 (2H, d, *J* = 6.0 Hz, CHC**H**$_2$O), 4.27 (1H, t, *J* = 6.0 Hz, C**H**CH_2O), 4.15 (2H, q, *J* = 16.82 Hz, C**H**$_2CH_3$), 3.72 (2H, t, *J* = 6.0 Hz, CH_2O), 3.57 – 3.47 (12H, m, OCH_2CH_2O), 3.41 (2H, t, *J* = 6.0 Hz, C**H**$_2$NH), 3.15 (1H, ddd, *J* = 19.4, 11.5, 2.6 Hz, CH_2C=O), 2.61 (1H, dt, *J* = 19.3, 4.0 Hz), 2.14 (1H, ddd, *J* = 13.9, 12.2, 6.2 Hz, C**H**$_2$C=O), 2.00 (1H, td, *J* = 11.6, 9.8, 4.2 Hz), 1.92 (1H, td, *J* = 14.1, 13.5, 2.8 Hz), 1.71 (1H, s), 1.58 (1H, td, *J* = 13.0, 5.8 Hz), 1.21 (3H, overlapped t, *J* = 7.1 Hz, C**H**$_3CH_2$); 1.43-1.12 (15H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.8 (C=O, ketone), 202.1 (C=O, ketone), 168.2 (C=O, ester/amide), 162.62 (C=O, ester/amide), 143.1 (C_{Ar}), 141.4 (C_{Ar}), 128.2(C_{Ar}), 127.4(C_{Ar}), 124.7(C_{Ar}), 120.3(C_{Ar}), 75.2 (C), 73.3 (O=C**C**O), 70.6 (CH_2CH_2O), 70.6 (CH_2CH_2O), 70.6 (CH_2CH_2O), 70.6 (CH_2CH_2O), 70.4 (CH_2CH_2O), 70.1 (CH_2CH_2O), 68.7 (CH_2CH_2O), 67.8 (**C**HCH_2O), 61.8 (CH_3**C**H_2O), 46.9 (**C**HCH_2O), 39.9 (CH_2NH), 36.7 (**C**H_2C=O), 31.8 (CH_2), 27.1 (CH_2), 26.5 (CH_2), 23.7 (CH_2), 22.8 (CH_2), 22.7 (CH_2), 22.7 (CH_2), 21.2 (CH_2), 20.3 (CH_2), 14.1 (CH_3); HRMS (ESI$^+$): Found: 760.3654, $C_{40}H_{55}NNaO_{10}$(MNa^+) Requires: 710.3899.

(9H-Fluoren-9-yl)methyl (14-oxo-14-(2-oxoazacyclotridecan-1-yl)-3,6,9,12-tetraoxatetradecyl)carbamate (214)



A mixture of 12-aminododecanolactam **144** (130 mg, 0.66 mmol), pyridine (0.32 mL, 3.96 mmol) and DMAP (8 mg, cat.) in dichloromethane (10 mL) under an argon atmosphere was stirred at RT for 30 mins.

To a solution of 1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16-pentaoxa-4-azaoctadecan-18-oic acid **(201a)** (474 mg, 0.99 mmol) in dichloromethane (5.5 mL), oxalyl chloride (0.27 mL, 2.89 mmol) was added followed by a catalytic amount of DMF (1 drop) under inert atmosphere protection. The resulting mixture was then stirred at RT for 1 h (the initial milky suspension became to homogeneous yellow transparent solution over the period) and concentrated *in vacuo* to remove all of the solvent and excess oxalyl chloride. The resulting dried solid was then re-dissolved in dichloromethane (12mL) and slowly, dropwise added to the 12-aminododecanolactam mixture and resulting solution was stirred and reflux at 50°C overnight. The mixture solution was then diluted with dichloromethane (20 mL) and washed with 1M aq. HCl (20 mL). The aqueous layer was extracted with dichloromethane (4 × 15 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to chromatography on silica gel (10:1 hexane: ethyl acetate → 2:1 hexane: ethyl acetate → ethyl acetate) afforded *title compound* **214** (170 mg,

39.5%) as a white solid; R_f 0.41 (1:9 MeOH: DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (2H, d, *J* = 7.5 Hz, H_{Ar}), 7.59 (2H, d, *J* = 7.4 Hz, H_{Ar}), 7.37 (2H, t, *J* = 7.5 Hz, H_{Ar}), 7.29 (2H, t, *J* = 7.5 Hz, H_{Ar}), 5.55 (1H, br s, NH), 4.55 (2H, s, $OCH_2C{=}O$), 4.36 (2H, d, *J* = 7.1 Hz, C**H**$_2$CH), 4.19 (1H, t, *J* = 7.1 Hz, C**H**CH_2), 3.70 – 3.59 (10H, m, CH_2CH_2O), 3.55 (2H, t, *J* = 4.9 Hz, CH_2), 3.38 (2H, q, *J* = 5.6 Hz, CH_2), 3.27 (1H, dd, *J* = 10.9, 5.9 Hz), 2.49 – 2.41 (1H, m), 2.21 – 2.13 (1H, m), 1.76 – 1.59 (4H, m), 1.53 – 1.39 (2H, m), 1.30 (14H, overlapped br s, cyclic CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.5 (O=C, amide), 174.0 (O=C, amide), 173.6 (O=C, amide), 144.1 (2 C_{Ar}), 141.4 (2 C_{Ar}), 127.7 (2 C_{Ar}), 127.1 (2 C_{Ar}), 125.2 (C_{Ar}), 120.0 (C_{Ar}), 74.0 (O=C**C**H_2O), 70.8 (overlapped CH_2CH_2O), 70.7 (overlapped CH_2CH_2O), 70.6 (CH_2CH_2O), 70.5 (CH_2CH_2O), 47.3 (**C**HCH_2), 42.9 (CH_2N), 39.1 (C**H**$_2$NH), 37.1 (**C**$H_2C{=}O$), 28.42 (**C**H_2, cyclic), 26.8 (**C**H_2, cyclic), 26.2 (**C**H_2, cyclic), 25.9 (**C**H_2, cyclic), 25.8 (**C**H_2, cyclic), 25.7 (**C**H_2, cyclic), 25.0 (**C**H_2, cyclic), 24.8 (**C**H_2, cyclic), 24.7 (**C**H_2, cyclic), 24.0 (**C**H_2, cyclic), 23.9 (**C**H_2, cyclic), 23.7 (**C**H_2, cyclic), 23.5 (**C**H_2, cyclic). HRMS (ESI^+): Found: 675.3632, $C_{37}H_{52}N_2NaO_8$ (MNa^+) Requires: 675.3631 (-2.7 ppm error).

Abbreviations

4EG	Tetraethylele glycol
5EG	Pentaethylene glycol
6EG	Hexaethyelx glycol
Bn	Benzyl
CBz	Benzyloxy carbonyl
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
Et	Ethyl
Fmoc	9*H*-Fluorenylmethoxycarbonyl
HRMS	High Resolution Mass Spectroscopy
Me	Methyl
MeOH	Methanol
NMR	Nuclear magnetic resonance
PG	Protecting group
Ph	Phenyl
ppm	parts per million
SuRE	Successive ring expansion
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
Ts	Tosyl

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