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### Abstract

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### Keywords

cyclic peptoid, antibacterial, ring-closing metathesis, indole, CMMB

### Disciplines

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## Synthesis of some cyclic indolic peptoids as potential antibacterials

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*Keywords:* cyclic peptoid; antibacterial; ring-closing metathesis; indole

**Abstract**— The synthesis of cyclic peptoids containing an indole hydrophobic scaffold has been realised through the ring-closing metathesis of diallylated precursors. The precursors and their cyclic counterparts possessed poor antibacterial activity in contrast to previously reported cyclic peptoids containing hydrophobic scaffolds.

### 1. Introduction

The development and clinical application of antibiotics in the late 1930's enabled the control of the majority of bacterial infections. However, by the late 1950's up to 85% of clinical staphylococci isolates were found to be penicillin resistant.<sup>1</sup> Bacteria now have the ability to pass genetic information onto different strains leading to the widespread resistance of bacteria to many of the currently used antibiotics. The recent report of resistance to vancomycin is of the greatest concern as it represents the last line of defence against infections of multidrug resistant staphylococci and enterococci.<sup>2</sup> Resistance to vancomycin was first documented in 1988,<sup>3</sup> from hospitals in both Europe,<sup>4</sup> and the USA.<sup>5</sup> This emergence of vancomycin resistant enterococci (VRE) is significant and points to the prospect of widespread vancomycin resistance to multi-drug resistant pathogenic bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA). 'Super-resistant' bacterial strains such as these have been

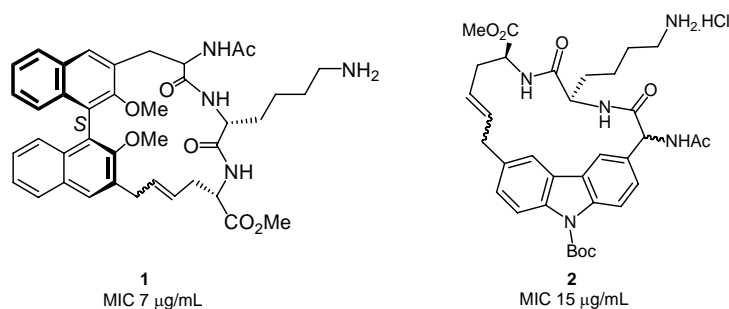
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demonstrated in a controlled environment, highlighting the ease in which resistance may spread to pathogenic bacteria,<sup>3</sup> and there are now cases of fully resistant isolates of *Staphylococcus aureus* being reported.<sup>6</sup>

The advent of untreatable multidrug resistant bacteria has created an unmet medical need to create new antibacterial agents. The development of new therapeutic agents is even more critical considering only one new class of antibacterial agents, the oxazolidinone linezolid,<sup>7</sup> has been launched in the last 35 years, and already resistance to this therapeutic has started to emerge.<sup>8-10</sup>

With the increasing spread of antibacterial resistance, including resistance by pathogenic bacteria to vancomycin, there is a compelling imperative for new antibacterials.<sup>10</sup> We have undertaken a program investigating the design and synthesis of cyclic peptoids linked by a hydrophobic scaffold as potential antibacterial agents, and thus far, have shown the binaphthyl<sup>11</sup> and carbazole scaffolds<sup>12,13</sup> within these cyclic peptoids produce antibacterial agents, for example **1** and **2**, of reasonable potency (Figure 1). Therefore, as part of this programme targeting the design and synthesis of new peptoid derivatives as antibacterial agents and attempting to address the resistance mechanism against vancomycin, we investigated the synthesis of some acyclic and cyclic indole-based derivatives. These derivatives were designed to explore the effect on activity of a smaller rigid scaffold in which the indolic nitrogen was to serve as one of the anchor points. The results are reported in this paper.



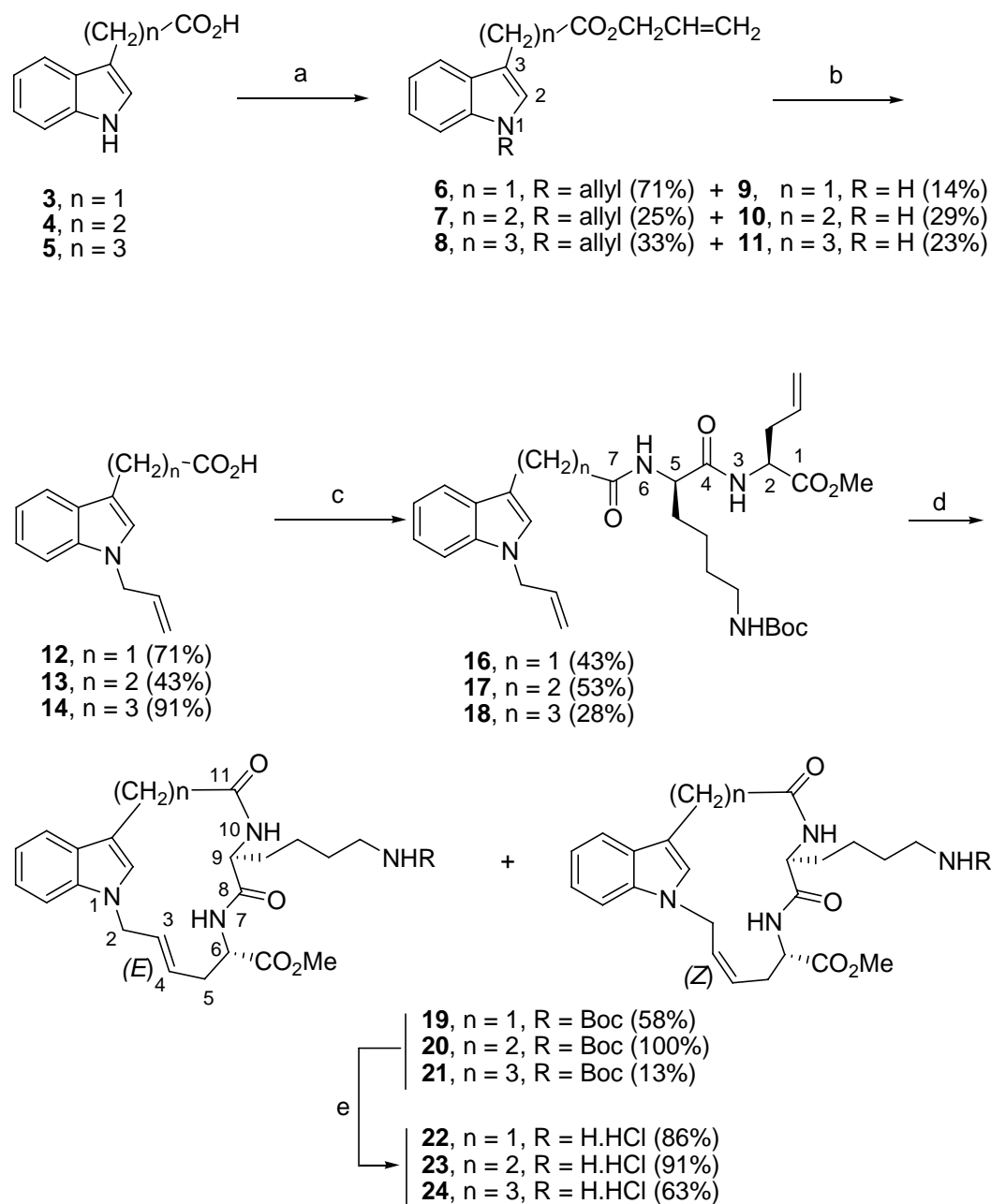
**Figure 1.** Cyclic peptoids containing a hydrophobic scaffold that show anti-bacterial activity.<sup>11-13</sup> MIC = minimum inhibitory concentration and activities are against a wild type *S. aureus* strain.

## 2. Results and Discussion

### 2.1. Synthesis of Indole-Based Peptoids

The synthesis of cyclic peptoids incorporating an indolic scaffold is outlined in Scheme 1. The strategy involved the addition of a dipeptide to an indole unit that contained a linking group at C3 as well as utilising the N1 position as a linking group via the addition of an allyl substituent. Cyclisation was to be achieved using reliable ring-closing metathesis (RCM) reactions.<sup>11-14</sup> While the choice of dipeptide to use is potentially extensive, a limitation is that one of the amino acids must also contain an additional allyl group such that the ring-closing metathesis reaction is possible. Cyclic peptoids of different sizes were synthesised by extending the number of methylene linkers between the indole C3 position and the first carboxylic acid unit.

Therefore, treatment of commercially available indole acids **3-5** with base, followed by an excess of allyl bromide gave a mixture of both the allyl esters **9-11** and the diallylated products **6-8** (Scheme 1). Subsequent saponification with LiOH yielded the desired carboxylic acids **12-14**. This 2-step process was the most efficient method of *N*-allylation in the presence of the carboxylic acid, although with the lengthening of the C3 chain, the yield of the monoallyl ester byproducts, **10** and **11**, became more prominent. The coupling of the dipeptide, L-allylGlyOMe-D-Lys **15**, to **12-14** was achieved through well established EDCI coupling. The key RCM of dienes **16-18** proceeded efficiently in the case of the products **19-21** producing a discernible mixture of both *E* and *Z* isomers, which could not be separated by silica gel chromatography. In contrast, the yield of **21** was lower, presumably due to the difficulties in forming the larger ring. However, further optimisation of this reaction may improve this outcome. The structures of the cyclised products were elucidated on the basis of high resolution mass spectrometric and NMR spectroscopic evidence. In the <sup>1</sup>H-NMR spectrum of **19**, for example, the signal ascribed to

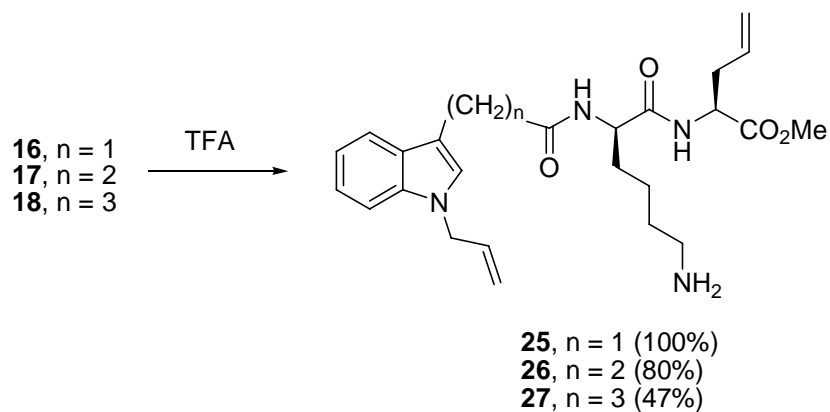


**Scheme 1.** a) NaH (2.2 equiv.), allyl bromide (2.5 equiv.), DMF, RT, 12h. b) 0.15 M LiOH, THF, water, 0 °C. c) L-allylGlyOMe-D-Lys **15**, EDCI, DMAP, MeCN, CH<sub>2</sub>Cl<sub>2</sub>, RT, 18 h. d) 10 mol% Grubbs I catalyst, CH<sub>2</sub>Cl<sub>2</sub>, reflux 18 h. e) TFA, CH<sub>2</sub>Cl<sub>2</sub>, RT, 2 h.

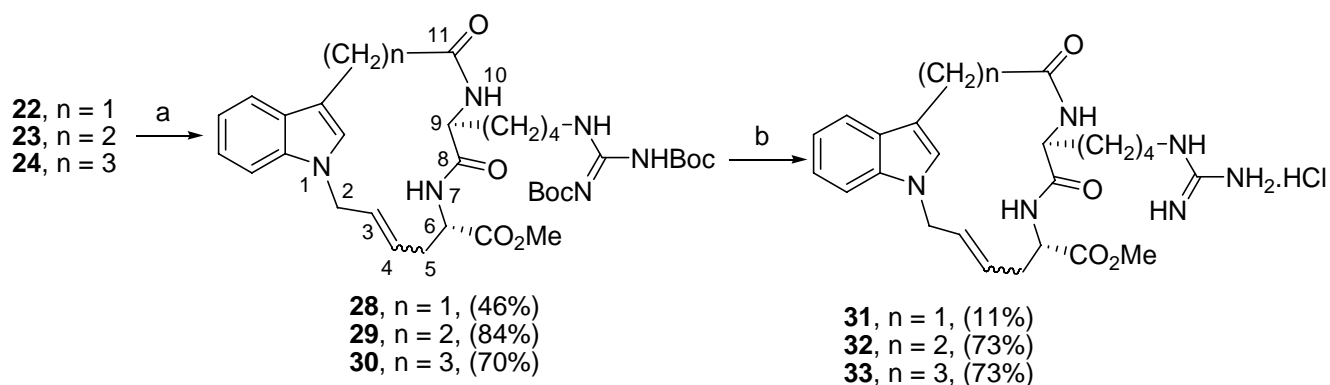
H3 appeared as a doublet of triplets at  $\delta$ 5.52 ( $J = 15, 6$  Hz) with an associated multiplet at  $\delta$  5.90 assigned as either the corresponding *Z* isomer or rotamers. The larger coupling constants of 15-16 Hz for H3 in **19** and **20** were consistent with the *E*-geometry of the major isomers of these compounds. Unfortunately,  $J_{3,4}$  for **22** could not be determined due to peak overlap. The *E/Z* ratio of **19**, **20**, and **21**

was thus estimated as 4:1, 7:3 and 3:2 respectively, from  $^1\text{H-NMR}$  analysis. Final unmasking of the lysine side chain and crystallisation from diethyl ether.HCl solution gave the cyclic peptoids as their hydrochloride salts **22-24**.

The corresponding hydrochloride salts of the deprotected acyclic precursors (**25-27**) were also synthesised using typical acidic conditions starting from **15-17** (Scheme 2). Further, the corresponding guanidine derivatives of the cyclic peptoids **31-33** were also produced via **28-30**, again using standard reaction conditions starting from **22-24**, and using *N,N'*-diBoc-*N*-triflylguanidine in the key guanidation step (Scheme 3).



**Scheme 2.** Deprotection of acyclic indole peptides.



**Scheme 3.** Reagents: a) *N,N'*-diBoc-*N*-triflylguanidine,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ . b) TFA,  $\text{CH}_2\text{Cl}_2$ .

The chemical yields for all transformations were not optimised, leading to some inconsistencies in outcome for individual steps. However, the ease of formation of all derivatives ensured facile access to the desired cyclic derivatives for testing.

## 2.2. Antibacterial Results

The synthesised cyclic dipeptoids **22-24** and **31-33** were tested against the Gram-positive bacterium *S. aureus* ATCC6538 and showed MIC values greater than  $\geq 125$   $\mu\text{g/mL}$ , indicating that these indole-based scaffolds are not good hydrophobic units for incorporation into the design program for antibacterial development. In view of the success of some of our previous cyclic peptoid structures that showed antibacterial activities of MIC 7  $\mu\text{g/mL}$  and 15  $\mu\text{g/mL}$  for **1** and **2**, respectively (Figure 1), the biological results for the new indole-based molecules were disappointing. The binaphthyl scaffold would contain a degree of flexibility by comparison, and might be able to orientate more easily into an active conformation. However, the carbazole scaffold in **2** is quite rigid, a conformational characteristic which is anticipated to be closer to that shown by the indole-based molecules. Although the mode of action of this class of compounds has not yet been established, we anticipate that the lack of activity for the new derivatives must arise from the wrong 3-dimensional array of the relevant substituents. The smaller macrocyclic ring sizes in **22-24** compared with those in **1** or **2** may also be relevant in this context. Given that the same dipeptide is used in all three cases, we therefore presume that the presence of a particular hydrophobic moiety becomes an additional important element in establishing antibacterial activity, and that the indole scaffold itself is simply not appropriate. We are currently investigating other hydrophobic scaffolds in order to improve antibacterial activity.

## Acknowledgments

We would like to thank Amrad Corporation Ltd and the Institute for Biomolecular Science, University of Wollongong, for financial support and Daniel Coghlan and Helen Witchard for initial work associated with dipeptide synthesis.



### 3. Experimental

#### 3.1 General

All NMR spectra were determined in CDCl<sub>3</sub> solution at 300 MHz (<sup>1</sup>H NMR) or 75 MHz (<sup>13</sup>C NMR) unless otherwise indicated. On occasions, the <sup>13</sup>C and <sup>1</sup>H NMR spectra indicated the presence of a minor component which could be either rotamers or geometric isomers through the doubling of peaks. These have been reported and are assigned with an asterix (\*). Petroleum spirit has a bp range of 40-60 °C. TLC was performed on Merck Al-backed plates. Other general experimental procedures have been reported previously.<sup>13</sup>

#### 3.2 Allylation of Indolyl-3-carboxylates – General Procedure A

##### Allyl 1-allyl-1*H*-indole-3-acetate (**6**) and Allyl 1*H*-indole-3-acetate (**9**)

To a suspension of sodium hydride (1.0 g, 25.1 mmol, 60% in paraffin), that had been washed twice with petroleum spirit under a N<sub>2</sub> atmosphere, in dry DMF (8 mL) was added a solution of 1*H*-indole acetic acid **3** (2.00 g, 11.4 mmol) in DMF (5 mL) under N<sub>2</sub> at rt. The mixture was stirred for 30 min before allyl bromide (2.5 mL, 28.6 mmol) was added dropwise. The reaction mixture was stirred for 18 h, concentrated and the residue partitioned between water (20 mL) and diethyl ether (2 x 20 mL). The combined extracts were washed (water), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated. The crude product was subjected to flash silica gel column chromatography (20% CH<sub>2</sub>Cl<sub>2</sub> in petroleum spirit) to produce **6** (2.08 g, 71%) as an oil. TLC (petroleum spirit/DCM 5:1) R<sub>f</sub> = 0.89; <sup>1</sup>H NMR δ = 7.68 (d, *J* = 8 Hz, 1 H, ArH4), 7.34 (d, *J* = 8 Hz, 1 H, ArH7), 7.26 (apparent t, *J* = 7 Hz, 1 H, ArH6), 7.18 (apparent t, *J* = 7 Hz, 1 H, ArH5), 7.14 (s, 1 H, ArH2), 5.99 (m, 2 H, 2×CH=CH<sub>2</sub>), 5.37-5.10 (m, 4 H, 2×CH=CH<sub>2</sub>), 4.70 (d, *J* = 5 Hz, 2 H, CH<sub>2</sub>CH=CH<sub>2</sub>), 4.66 (d, *J* = 5 Hz, 2 H, CH<sub>2</sub>CH=CH<sub>2</sub>), 3.86 (s, 2 H, CH<sub>2</sub>CO); <sup>13</sup>C NMR δ = 171.1 (CO), 136.0 (ArC), 133.2 and 131.9 (CH=CH<sub>2</sub>), 127.6 (ArC), 126.6, 121.5 and 119.1 (ArCH), 118.9 (CH=CH<sub>2</sub>), 117.9 (ArCH), 117.1 (CH=CH<sub>2</sub>), 109.4 (ArCH), 106.9 (ArC), 65.2 (OCH<sub>2</sub>), 48.6

(NCH<sub>2</sub>), 31.1 (CH<sub>2</sub>); MS (CI) *m/z* 256 (100% MH<sup>+</sup>). HRMS (CI) calcd for C<sub>16</sub>H<sub>18</sub>NO<sub>2</sub>: 256.1338; found: 256.1338.

Further elution with 50% CH<sub>2</sub>Cl<sub>2</sub> in petroleum spirit gave **9** (0.35 g, 14%). TLC (petroleum spirit/CH<sub>2</sub>Cl<sub>2</sub> 1:1) R<sub>f</sub> = 0.54; <sup>1</sup>H NMR δ = 8.2 (1H, bs, NH), 7.68 (d, *J* = 7 Hz, 1 H, ArH<sub>4</sub>), 7.31-7.17 (m, 3 H, ArH<sub>7,6,5</sub>), 7.01 (d, *J* = 3 Hz, 1 H, ArH<sub>2</sub>), 5.98 (ddt, *J* = 17, 10, 5 Hz, 1 H, CH=CH<sub>2</sub>), 5.35 (dd, *J* = 16, 1 Hz, 2 H, CH=CH<sub>2</sub>), 5.27\* (dd, *J* = 10, 1 Hz, 2 H, CH=CH<sub>2</sub>), 4.68 (dd, *J* = 5, 1 Hz, 2 H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 3.86 (s, 2 H, CH<sub>2</sub>CO); <sup>13</sup>C NMR δ = 171.2 (CO), 135.9 (ArC), 132.0 (CH=CH<sub>2</sub>), 127.0 (ArC), 123.3, 121.9 and 119.4 (ArCH), 118.6 (CH=CH<sub>2</sub>), 118.2 (ArCH), 111.2 (ArCH), 107.7 (ArC), 65.4 (OCH<sub>2</sub>), 31.2 (CH<sub>2</sub>); MS (CI) *m/z* 216 (100% MH<sup>+</sup>). HRMS (CI) calcd for C<sub>13</sub>H<sub>14</sub>NO<sub>2</sub>: 216.1024; found: 216.1021.

#### **Allyl 1-allyl-1*H*-indole-3-propanoate (7) and Allyl 1*H*-indole-3-propanoate (10)**

These were prepared by *general procedure A* using 1*H*-indole-3-propanoic acid **4** (2.36 g, 12.5 mmol), NaH (660 mg, 16.5 mmol) allyl bromide (2.7 mL, 31.2 mmol) and DMF (9 mL). The crude residue was subjected to flash silica gel column chromatography (20% CH<sub>2</sub>Cl<sub>2</sub> in petroleum spirit) to give **7** (0.84 g, 25%) as an oil. TLC (petroleum spirit/DCM 5:1) R<sub>f</sub> = 0.92; <sup>1</sup>H NMR δ = 7.67 (d, *J* = 8 Hz, 1 H, ArH<sub>4</sub>), 7.34 (d, *J* = 8 Hz, 1 H, ArH<sub>7</sub>), 7.27 (t, *J* = 8 Hz, 1 H, ArH<sub>6</sub>), 7.18 (t, *J* = 8 Hz, 1 H, ArH<sub>5</sub>), 6.96, (s, 1 H, ArH<sub>2</sub>), 6.07-5.90 (m, 2 H, 2×CH=CH<sub>2</sub>), 5.38-5.08 (m, 4 H, CH=CH<sub>2</sub>), 4.69 (d, *J* = 5 Hz, 2 H, CH<sub>2</sub>CH=CH<sub>2</sub>), 4.65 (d, *J* = 5 Hz, 2 H, CH<sub>2</sub>CH=CH<sub>2</sub>), 3.19 (t, *J* = 8 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CO), 2.81 (t, *J* = 8 Hz, 2 H, CH<sub>2</sub>CO); <sup>13</sup>C NMR δ = 172.8 (CO), 136.2 (ArC), 133.5 and 132.1 (CH=CH<sub>2</sub>), 127.6 (ArC), 125.1 and 121.5 (ArCH), 118.8 (ArCH), 118.7 (CH=CH<sub>2</sub>), 118.0 (ArCH) 116.9 (CH=CH<sub>2</sub>), 113.6 (ArC), 109.5 (ArCH), 64.9 (OCH<sub>2</sub>), 48.4 (NCH<sub>2</sub>), 34.9 and 20.5 (CH<sub>2</sub>); MS (ES) *m/z* 270 (100% MH<sup>+</sup>), 212 (25%), 170 (88%). HRMS (ES) calcd for C<sub>17</sub>H<sub>19</sub>NO<sub>2</sub>: 269.1416; found: 269.1412.

Further elution with 50% petroleum spirit in DCM gave **10** (0.84 g, 29%): TLC (petroleum spirit/DCM 1:2) R<sub>f</sub> = 0.75; <sup>1</sup>H NMR δ = 8.16 (br s, 1 H, NH), 7.70 (dd, *J* = 8, 2 Hz, 1 H, ArH<sub>4</sub>), 7.36 (dd, *J* = 8, 2

Hz, 1 H, ArH7), 7.28 (dd,  $J = 8$  Hz, 1 H, ArH6), 7.22 (dt,  $J = 8$ , 2 Hz, 1 H, ArH5), 6.98 (d,  $J = 3$  Hz, 1 H, ArH2), 5.98 (ddt,  $J = 17$ , 10, 6 Hz, 1 H,  $\underline{\text{CH}}=\text{CH}_2$ ), 5.37 (ddd,  $J = 17$ , 2, 1 Hz, 2 H,  $\text{CH}=\underline{\text{CH}}_2$ ), 5.30\* (ddd,  $J = 10$ , 2, 1 Hz, 2 H,  $\text{CH}=\underline{\text{CH}}_2$ ), 4.68 (2xt,  $J = 6$  Hz, 2 H,  $\underline{\text{CH}}_2\text{CH}=\text{CH}_2$ ), 3.22 (t,  $J = 8$  Hz, 2 H,  $\text{CH}_2\text{CO}$ ), 2.85 (t,  $J = 8$  Hz, 2 H,  $\underline{\text{CH}}_2\text{CH}_2\text{CO}$ );  $^{13}\text{C}$  NMR  $\delta = 173.1$  (CO), 136.1 (ArC), 132.0 ( $\underline{\text{CH}}=\text{CH}_2$ ), 126.9 (ArC), 121.7, 121.4 and 119.0 (ArCH), 118.4 ( $\text{CH}=\underline{\text{CH}}_2$ ), 118.0 (ArCH), 114.3 (ArC), 111.1 (ArCH), 65.0 (OCH<sub>2</sub>), 34.7 and 20.4 (CH<sub>2</sub>); MS (ES)  $m/z$  230 (100% MH<sup>+</sup>), 172 (55%), 130 (75%). HRMS (ES) calcd for C<sub>14</sub>H<sub>15</sub>NO<sub>2</sub>: 229.1103; found: 229.1100.

### Allyl 1-allyl-1H-indole-3-butanoate (8) and allyl 1H-indole-3-butanoate (11)

These were prepared by *general procedure A* using 1H-indole-3-butanoic acid **5** (1.00 g, 4.9 mmol), NaH (217 mg, 5.4 mg), allyl bromide (1.1 mL, 12.7 mmol) and DMF (6 mL). The crude product was subjected to flash silica gel column chromatography (50% DCM in petroleum spirit) to give **8** (0.46 g, 33%) as an oil. TLC (petroleum spirit/DCM 2:1)  $R_f = 0.91$ ;  $^1\text{H}$  NMR  $\delta = 7.58$  (dt,  $J = 8$ , 1 Hz, 1 H, ArH4), 7.24 (dd,  $J = 8$ , 1 Hz, 1 H, ArH7), 7.17 (dt,  $J = 8$ , 1 Hz, 1 H, ArH6), 7.08 (dt,  $J = 8$ , 1 Hz, 1 H, ArH5), 6.84 (s, 1 H, ArH2), 5.90 (m, 2 H,  $2 \times \underline{\text{CH}}=\text{CH}_2$ ), 5.28 (ddd,  $J = 17$ , 2, 1 Hz, 2 H,  $\text{CH}=\underline{\text{CH}}_2$ ), 5.19\* (ddd,  $J = 11$ , 2, 1 Hz, 2 H,  $\text{CH}=\underline{\text{CH}}_2$ ), 5.13\* (ddd,  $J = 10$ , 2, 1 Hz, 2 H,  $\text{CH}=\underline{\text{CH}}_2$ ), 5.03 (ddd,  $J = 18$ , 2, 1 Hz, 2 H,  $\text{CH}=\underline{\text{CH}}_2$ ), 4.57 (dd,  $J = 5$ , 1 Hz, 4 H,  $\underline{\text{CH}}_2\text{CH}=\text{CH}_2$ ), 2.78 (t,  $J = 7$  Hz, 2 H,  $\underline{\text{CH}}_2\text{CH}_2\text{CH}_2\text{CO}$ ), 2.38 (t,  $J = 7$  Hz, 2 H,  $\text{CH}_2\text{CO}$ ), 2.03 (pent,  $J = 7$  Hz, 2 H,  $\underline{\text{CH}}_2\text{CH}_2\text{CO}$ );  $^{13}\text{C}$  NMR  $\delta = 173.2$  (CO), 136.3 (ArC), 133.5 and 132.2 ( $\underline{\text{CH}}=\text{CH}_2$ ), 127.9 (ArC), 125.1, 121.4 and 118.9 (ArCH), 118.6 ( $\text{CH}=\underline{\text{CH}}_2$ ), 118.0 (ArCH) 116.9 ( $\text{CH}=\underline{\text{CH}}_2$ ), 114.3 (ArC), 109.4 (ArCH), 64.8 (OCH<sub>2</sub>), 48.4 (NCH<sub>2</sub>), 33.7, 25.3 and 24.3 (CH<sub>2</sub>); MS (CI)  $m/z$  284 (100% MH<sup>+</sup>). HRMS (CI) calcd for C<sub>18</sub>H<sub>22</sub>NO<sub>2</sub>: 284.1650; found: 284.1639.

Further elution with 50% petroleum spirit in DCM gave **11** (0.27 g, 23%). TLC (petroleum spirit/DCM 1:2)  $R_f = 0.66$ ;  $^1\text{H}$  NMR  $\delta = 8.0$  (br s, 1 H, NH), 7.59 (d,  $J = 8$  Hz, 1 H, ArH4), 7.34 (d,  $J = 8$  Hz, 1 H, ArH7), 7.17 (t,  $J = 8$  Hz, 1 H, ArH6), 7.09 (t,  $J = 8$  Hz, 1 H, ArH5), 6.97 (d,  $J = 2$  Hz, 1 H, ArH2), 5.89

(ddt,  $J = 10, 5, 1$  Hz, 1 H,  $\underline{\text{CH}}=\text{CH}_2$ ), 5.29 (dd,  $J = 16, 1$  Hz, 2 H,  $\text{CH}=\underline{\text{CH}}_2$ ), 5.21\* (dd,  $J = 10, 1$  Hz, 2 H,  $\text{CH}=\underline{\text{CH}}_2$ ), 4.55 (d,  $J = 6$  Hz, 2 H,  $\underline{\text{CH}}_2\text{CH}=\text{CH}_2$ ), 2.80 (t,  $J = 7$  Hz, 2 H,  $\underline{\text{CH}}_2\text{CH}_2\text{CH}_2\text{CO}$ ), 2.40 (t,  $J = 7$  Hz, 2 H,  $\text{CH}_2\text{CO}$ ), 2.05 (t,  $J = 7$  Hz, 2 H,  $\underline{\text{CH}}_2\text{CH}_2\text{CO}$ );  $^{13}\text{C}$  NMR  $\delta = 173.2$  (CO), 136.2 (ArC), 132.1 ( $\underline{\text{C}}\text{H}=\text{CH}_2$ ), 127.3 (ArC), 121.8, 121.3 and 119.1 (ArCH), 118.8 ( $\text{CH}=\underline{\text{C}}\text{H}_2$ ), 118.1 (ArCH), 115.5 (ArC), 111.0 (ArCH), 65.0 (OCH<sub>2</sub>), 33.9, 29.8, 25.4 and 24.5 (CH<sub>2</sub>); MS (CI)  $m/z$  244 (100% MH<sup>+</sup>), 186 (45% M<sup>+</sup>-OCH<sub>2</sub>CH=CH<sub>2</sub>). HRMS (CI) calcd for C<sub>15</sub>H<sub>18</sub>NO<sub>2</sub>: 244.1338; found: 244.1332.

### 3.2 Hydrolysis of Allyl Esters – General Procedure B

#### 1-Allyl-1H-indole-3-acetic acid (12)

To a solution of **6** (1.84 g, 7.2 mmol) in THF/water (5:2, 35 mL) was added lithium hydroxide (0.3 g, 12.5 mmol) and the reaction mixture was placed in an ice bath and stirred at 0 °C for 3 h. It was then concentrated and the residue was partitioned between diethyl ether and water (2 x 30 mL). The combined aqueous layers were acidified with HCl (10%) to pH < 2. The aqueous layer was then saturated with sodium chloride and extracted with DCM (20 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to give **12** (1.1 g, 71%) as an oil.  $^1\text{H}$  NMR  $\delta = 7.60$  (d,  $J = 8$  Hz, 1 H, ArH4), 7.30 (d,  $J = 8$  Hz, 1 H, ArH7), 7.22 (dt,  $J = 8, 1$  Hz, 1 H, ArH6), 7.13 (dt,  $J = 8, 1$  Hz, 1 H, ArH5), 7.08 (s, 1 H, ArH2), 5.98 (ddt,  $J = 17, 11, 5$  Hz, 1 H,  $\underline{\text{C}}\text{H}=\text{CH}_2$ ), 5.20\* (dd,  $J = 9, 1$  Hz, 2 H,  $\text{CH}=\underline{\text{C}}\text{H}_2$ ), 5.11 (dd,  $J = 16, 1$  Hz, 2 H,  $\text{CH}=\underline{\text{C}}\text{H}_2$ ), 4.68 (dd,  $J = 5, 1$  Hz, 2 H,  $\text{NCH}_2\text{CH}=\text{CH}_2$ ), 3.80 (s, 2 H, CH<sub>2</sub>CO);  $^{13}\text{C}$  NMR  $\delta = 177.5$  (CO), 136.1 (ArC), 133.2 ( $\underline{\text{C}}\text{H}=\text{CH}_2$ ), 127.6 (ArC), 126.8, 121.8 and 119.3 (ArCH), 118.9 ( $\text{CH}=\underline{\text{C}}\text{H}_2$ ), 117.4 and 109.6 (ArCH), 106.4 (ArC), 48.8 (NCH<sub>2</sub>), 31.0 (CH<sub>2</sub>); MS (CI)  $m/z$  216 (100% MH<sup>+</sup>). HRMS (CI) calcd for C<sub>13</sub>H<sub>14</sub>NO<sub>2</sub>: 216.1024; found: 216.1039.

#### 1-Allyl-1H-indole-3-propanoic acid (13)

This was prepared by *general procedure B* from **7** (0.650 g, 2.4 mmol), LiOH (0.220 g, 9.2 mmol) and 35 mL of solvent to give **13** (0.24 g, 43%) as an oil.  $^1\text{H}$  NMR  $\delta$  7.59 (d,  $J = 8$  Hz, 1 H, ArH4), 7.34 (d,  $J = 8$  Hz, 1 H, ArH7), 7.16-7.01 (m, 3 H, ArH2,5,6), 6.07-5.94 (m, 1 H,  $\underline{\text{C}}\text{H}=\text{CH}_2$ ), 5.12\* (dd,  $J = 10, 1$

Hz, 2 H, CH=CH<sub>2</sub>), 5.04 (dd,  $J = 17, 1$  Hz, 2 H, CH=CH<sub>2</sub>), 4.77 (d,  $J = 3$  Hz, 2 H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 3.04 (t,  $J = 10$  Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CO), 2.68 (t,  $J = 8$  Hz, 2 H, CH<sub>2</sub>CO); <sup>13</sup>C NMR  $\delta = 179.3$  (CO), 136.4 (ArC), 133.5 (CH=CH<sub>2</sub>), 127.7 (ArC), 125.2, 121.7 and 118.9 (ArCH), 118.8 (CH=CH<sub>2</sub>), 117.1 (ArCH), 113.5 (ArC), 109.6 (ArCH), 48.6 (NCH<sub>2</sub>), 31.5 and 20.3 (CH<sub>2</sub>); MS (CI)  $m/z$  230 (100% MH<sup>+</sup>); (ES) 170 (M<sup>+</sup>-CH<sub>2</sub>COOH). HRMS (CI) calcd for C<sub>14</sub>H<sub>16</sub>NO<sub>2</sub>: 230.1181; found: 230.1181.

### 1-Allyl-1*H*-indole-3-butanoic acid (**14**)

This was prepared by *general procedure B* from **8** (0.460 g, 1.6 mmol), LiOH (0.220 g, 9.2 mmol) and solvent (35 mL) to give **14** (0.36 g, 91%) as an oil. <sup>1</sup>H NMR  $\delta = 7.61$  (d,  $J = 8$  Hz, 1 H, ArH<sub>4</sub>), 7.30 (d,  $J = 8$  Hz, 1 H, ArH<sub>7</sub>), 7.21 (t,  $J = 8$  Hz, 1 H, ArH<sub>6</sub>), 7.12 (t,  $J = 8$  Hz, 1 H, ArH<sub>5</sub>), 6.91 (s, 1 H, ArH<sub>2</sub>), 5.99 (ddt,  $J = 17, 11, 5$  Hz, 1 H, CH=CH<sub>2</sub>), 5.20\* (dd,  $J = 10, 2$  Hz, 2 H, CH=CH<sub>2</sub>), 5.10 (dd,  $J = 17, 2$  Hz, 2 H, CH=CH<sub>2</sub>), 4.69 (d,  $J = 5$  Hz, 2 H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 2.84 (t,  $J = 7$  Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO), 2.45 (t,  $J = 7$  Hz, 2 H, CH<sub>2</sub>CO), 2.07 (t,  $J = 7$  Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>CO); <sup>13</sup>C NMR  $\delta = 179.7$  (CO), 136.4 (ArC), 133.6 (CH=CH<sub>2</sub>), 127.9 (ArC), 125.3, 121.5 and 119.0 (ArCH), 118.8 (CH=CH<sub>2</sub>), 117.0 (ArCH), 114.3 (ArC), 109.5 (ArCH), 48.6 (NCH<sub>2</sub>), 33.5, 15.1 and 24.3 (CH<sub>2</sub>); MS (ES)  $m/z$  244 (100% MH<sup>+</sup>), 226 (55% MH<sup>+</sup>-H<sub>2</sub>O). HRMS (CI) calcd for C<sub>15</sub>H<sub>18</sub>NO<sub>2</sub>: 244.1337; found: 244.1336.

### 3.3 Preparation of

#### Methyl (2*S*,5*R*)-2-Allyl-5-amino-3-aza-9-(*tert*-butoxycarbonyl)amino-4-oxononanoate -

#### L-allylGlyOMe-D-Lys (**15**)

To a mixture of *N*-Fmoc-D-Lys(Boc)OH (1.22 g, 2.61 mmol), methyl (*S*)-2-amino-4-pentenoate hydrochloride (430 mg, 2.61 mmol) and 4-dimethylaminopyridine (DMAP) (1 crystal) was added dry DCM (10 mL) followed by *N,N*-diisopropylethylamine (0.45 mL, 2.61 mmol) under a nitrogen atmosphere. The mixture was stirred at rt for 5 min before EDCI (500 mg, 2.61 mmol) was added, and the reaction mixture was then stirred for 23.5 h. After this period, the reaction mixture was diluted with DCM, washed with brine and then water, and the DCM layer was dried and evaporated. The crude

product was purified by column chromatography (PS with gradient elution to DCM:MeOH 10:1) to afford the protected coupled dipeptide (1.25 g, 2.16 mmol, 83%) as a pale yellow solid, mp 118-119 °C.  $R_f$ : 0.52 in 10% MeOH in DCM.  $^1\text{H NMR}$   $\delta$  7.73 (d,  $J = 7.5$  Hz, 2H, ArH4 and ArH5), 7.56 (d,  $J = 7.2$  Hz, 2H, ArH1 and ArH8), 7.36 (dd,  $J = 7.3, 7.2$  Hz, 2H, ArH3 and ArH6), 7.27 (dd,  $J = 7.3, 7.2$  Hz, 2H, ArH2 and ArH7), 6.82 (d,  $J = 6.6$  Hz, 1H, NH-3), 5.76-5.52 (m, 2H,  $\text{CH}_2\text{CH}=\text{CH}_2$  and  $\text{NH}\text{Boc}$ ), 5.05 (d,  $J = 16.5$  Hz, 1H,  $\text{CH}_2\text{CH}=\text{CHH}$ ), 5.04 (d,  $J = 10.5$  Hz, 1H,  $\text{CH}_2\text{CH}=\text{CHH}$ ), 4.64 (m, 2H, NCH-2 and  $\text{NH}\text{Fmoc}$ ), 4.35 (d,  $J = 6.9$  Hz, 2H,  $\text{OCH}_2$ ), 4.18 (t,  $J = 6.9$  Hz, 2H, Fmoc CH and NCH-5 (obscured)), 3.67 (s, 3H,  $\text{OCH}_3$ ), 3.07 (m, 2H,  $\text{NCH}_2(\text{CH}_2)_3$ ), 2.50 (m, 2H,  $\text{CH}_2\text{CH}=\text{CH}_2$ ), 1.82 (m,  $\text{N}(\text{CH}_2)_3\text{CH}_2$ ), 1.63 (m,  $\text{N}(\text{CH}_2)_3\text{CH}_2$ ), 1.53-1.25 (m, 4H,  $\text{NCH}_2(\text{CH}_2)_2\text{CH}_2$ ), 1.40 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ).  $^{13}\text{C NMR}$   $\delta$  171.8 ( $\text{COOCH}_3$ ), 171.4 (CO-4), 156.2 (Fmoc CO), 156.1 (Boc CO), 143.7 and 143.6 (ArC8a and ArC9a), 141.2 (ArC4a and ArC4b), 132.0 ( $\text{CH}_2\text{CH}=\text{CH}_2$ ), 127.7 (ArCH3 and ArCH6), 127.0 (ArCH2 and ArCH7), 125.0 (ArCH1 and ArCH8), 119.9 (ArCH4 and ArCH5), 119.3 ( $\text{CH}_2\text{CH}=\text{CH}_2$ ), 79.1 ( $\text{C}(\text{CH}_3)_3$ ), 67.1 ( $\text{OCH}_2$ ), 54.6 (NCH5), 52.4 ( $\text{OCH}_3$ ), 51.5 (NCH2), 47.0 (Fmoc CH), 39.8 ( $\text{NCH}_2(\text{CH}_2)_3$ ), 36.3 ( $\text{CH}_2\text{CH}=\text{CH}_2$ ), 32.1 ( $\text{N}(\text{CH}_2)_3\text{CH}_2$ ), 29.6 ( $\text{NCH}_2\text{CH}_2(\text{CH}_2)_2$ ), 28.3 ( $\text{C}(\text{CH}_3)_3$ ), 22.3 ( $\text{N}(\text{CH}_2)_2\text{CH}_2\text{CH}_2$ ). MS (ES)  $m/z$  580 (100%  $\text{MH}^+$ ), 524 (88%), 480 (66%). HRMS (ES) calcd for  $\text{C}_{32}\text{H}_{42}\text{N}_3\text{O}_7$ : 580.3023; found: 580.3002.

To a solution of the protected dipeptide (500 mg, 0.86 mmol) in anhydrous  $\text{CH}_3\text{CN}$  (10 mL) was added a solution of piperidine (0.04 mL, 0.43 mmol) in anhydrous  $\text{CH}_3\text{CN}$  under a nitrogen atmosphere, and the reaction mixture was stirred at rt for 3.25 h. The reaction solvent was evaporated and the crude product was purified by column chromatography (PS with gradient elution to DCM:MeOH 10:1) to afford **15** (308 mg, 0.86 mmol, 100%) as a yellow oil.  $R_f$ : 0.24 in 10% MeOH in DCM.  $^1\text{H NMR}$   $\delta$  7.66 (d,  $J = 8.1$  Hz, 1H, NH-3), 5.60 (ddt,  $J = 17.1, 10.5, 6.9$  Hz, 1H,  $\text{CH}_2\text{CH}=\text{CH}_2$ ), 5.03 (dd,  $J = 16.5, 1.2$  Hz, 1H,  $\text{CH}_2\text{CH}=\text{CHH}$ ), 5.02 (dd,  $J = 10.8, 0.9$  Hz, 1H,  $\text{CH}_2\text{CH}=\text{CHH}$ ), 4.79 (br s, 1H,  $\text{NH}\text{Boc}$ ), 4.52 (dt,  $J = 8.1, 6.0$  Hz, 1H, NCH-2), 3.64\* (s,  $\text{OCH}_3$ ), 3.64 (s,  $\text{OCH}_3$ ), 3.27 (m, 1H, NCH-5), 3.01 (m, 2H,

NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>, 2.45 (m, 2H, CH<sub>2</sub>CH=CH<sub>2</sub>), 1.80-1.64 (m, NH<sub>2</sub> and N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.56-1.25 (m, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 1.33 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>). <sup>13</sup>C NMR δ 174.9 (COOCH<sub>3</sub>), 172.0 (CO-4), 156.0 (Boc CO), 132.2 (CH<sub>2</sub>CH=CH<sub>2</sub>), 118.8 (CH<sub>2</sub>CH=CH<sub>2</sub>), 78.8 (C(CH<sub>3</sub>)<sub>3</sub>), 54.7 (NCH-5), 52.1 (OCH<sub>3</sub>), 51.1 (NCH-2), 39.9 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 36.1 (CH<sub>2</sub>CH=CH<sub>2</sub>), 34.3 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 29.6 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 28.2 (C(CH<sub>3</sub>)<sub>3</sub>), 22.4 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>). MS (ES) *m/z* 358 (89% MH<sup>+</sup>), 302 (100%), 258 (93%). HRMS (ES) calcd for C<sub>17</sub>H<sub>32</sub>N<sub>3</sub>O<sub>5</sub>: 358.2342; found: 358.2329.

### 3.4 Coupling of Indole Carboxylates with Dipeptides – General Procedure C.

#### Methyl (2*S*,5*R*)-2-allyl-8-(*N*-allyl-1*H*-indol-3-yl)-3,6-diaza-5-(*tert*-butoxycarbonylamino)butyl-4,7-dioxooctanoate (16)

To a solution of **12** (0.048 g, 0.2 mmol) in DCM (3 mL) was added **15** (0.080 g, 0.22 mmol) in acetonitrile (3 mL) and a catalytic amount of DMAP. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (0.043 g, 0.2 mmol) was then added and the mixture was stirred for 18 h under a N<sub>2</sub> atmosphere at rt. The solvents were evaporated and the mixture was partitioned between DCM and water. The DCM layer was separated and washed several times with water, dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated. The crude product was chromatographed on a flash silica gel column (2% methanol in DCM) to produce **16** (0.090 g, 43%) as a solid. TLC (DCM/MeOH 50:1) R<sub>f</sub> = 0.22; <sup>1</sup>H NMR δ = 7.52 (d, *J* = 8 Hz, 1 H, ArH4), 7.29 (d, *J* = 8 Hz, 1 H, ArH7), 7.20 (t, *J* = 8 Hz, 1 H, ArH6), 7.10 (t, *J* = 8 Hz, 1 H, ArH5), 7.06 (s, 1 H, ArH2), 6.78 (d, *J* = 8 Hz, 1 H, NH-3), 6.21 (d, *J* = 8 Hz, 1 H, NH-6), 5.97 (ddt, *J* = 17, 11, 5 Hz, 1 H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 5.61 (m, 1 H, CHCH<sub>2</sub>CH=CH<sub>2</sub>), 5.18 (d, *J* = 10 Hz, 1 H, CH=CHH), 5.09-5.04 (m, 3 H, CH=CH<sub>2</sub> + CH=CHH), 4.69 (d, *J* = 6 Hz, 2 H, NCH<sub>2</sub>-CH=CH<sub>2</sub>), 4.53 (m, 1 H, H2), 4.43 (m, 1 H, H5), 3.72 (s, 2 H, H8), 3.68 (s, 3 H, OCH<sub>3</sub>), 2.93 (d, *J* = 5 Hz, 2 H, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.53-2.35 (m, 2 H, CHCH<sub>2</sub>CH=CH<sub>2</sub>), 1.77-1.66 (m, 1 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.41 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 1.45-1.27 (m, 4 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub> + NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 1.15-1.08 (m, 2 H, N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR δ = 171.7 (7-CO),

171.5 ( $\text{COOCH}_3$ ), 170.9 (5-CO), 155.8 ( $\text{NCO}_2\text{Bu}^t$ ), 136.4 (ArC7), 133.1 ( $\text{NCH}_2\text{CH}=\text{CH}_2$ ), 132.0 ( $\text{CH}_2\text{CH}=\text{CH}_2$ ), 127.4 (ArC3a), 127.2 (ArC2), 122.1 (ArC6), 119.6 (ArC4), 119.1 ( $\text{CH}_2\text{CH}=\text{CH}_2$ ), 118.7 (ArCH5), 117.4 ( $\text{NCH}_2\text{CH}=\text{CH}_2$ ), 109.8 (ArCH7), 107.4 (ArC3), 79.1 ( $\text{OCMe}_3$ ), 52.7 (C2), 52.4 ( $\text{OCH}_3$ ), 51.6 (C5), 48.8 ( $\text{NCH}_2\text{CH}=\text{CH}_2$ ), 40.1 ( $\text{NCH}_2(\text{CH}_2)_3$ ), 36.3 (C8), 33.3 ( $\text{CHCH}_2\text{CH}=\text{CH}_2$ ), 31.5 ( $\text{N}(\text{CH}_2)_3\text{CH}_2$ ), 29.5 ( $\text{NCH}_2\text{CH}_2(\text{CH}_2)_2$ ), 28.5 ( $\text{C}(\text{CH}_3)_3$ ), 22.5 ( $\text{N}(\text{CH}_2)_2\text{CH}_2\text{CH}_2$ ); MS (ES)  $m/z$  555 (95%  $\text{MH}^+$ ), 499 (89%), 455 (100%). HRMS (ES) calcd for  $\text{C}_{30}\text{H}_{43}\text{N}_4\text{O}_6$ : 555.3184; found: 555.3163.

**Methyl (2S,5R)-2-allyl-9-(N-allyl-1H-indol-3-yl)-3,6-diaza-5-(tert-butoxycarbonylamino)butyl-4,7-dioxononanoate (17)**

This was prepared by *general procedure C* using **13** (0.220 g, 0.96 mmol), **15** (0.210 g), DCM (3 mL), AcCN (6 mL) and EDCI (0.114 g), to give **17** (0.290 g, 53%). TLC (DCM/MeOH 50:1)  $R_f$  = 0.45;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500MHz)  $\delta$  = 7.57 (d,  $J$  = 8 Hz, 1 H, H4), 7.25 (d,  $J$  = 8 Hz, 1 H, H7), 7.17 (dd,  $J$  = 8, 4 Hz, 1 H, H6), 7.07 (dd,  $J$  = 8, 4 Hz, 1 H, H5), 7.01 (d,  $J$  = 4 Hz, 1 H, NH-3), 6.88 (s, 1 H, H2), 6.49 (d,  $J$  = 4 Hz, 1 H, NH-6), 5.97-5.88 (m, 1 H,  $\text{NCH}_2\text{CH}=\text{CH}_2$ ), 5.65 (ddt,  $J$  = 17, 10, 4 Hz, 1 H,  $\text{CH}_2\text{CH}=\text{CH}_2$ ), 5.15-5.03 (m, 4 H,  $2\times\text{CH}=\text{CH}_2$ ), 4.67-4.61 (m, 2 H,  $\text{NCH}_2\text{CH}=\text{CH}_2$  and  $\text{NHBoc}$ ), 4.62 (m, 1 H, H2), 4.47 (dd,  $J$  = 14, 7 Hz, 1 H, H5), 3.69 (s, 3 H,  $\text{OCH}_3$ ), 3.15-3.00 (m, 2 H, H9), 3.05-2.93 (m, 2 H,  $\text{NCH}_2(\text{CH}_2)_3$ ), 2.72 (m, 1 H, H8), 2.60 (m, 1 H, H8), 2.60-2.52 (m, 1 H,  $\text{CH}-\text{CH}_2-\text{CH}=\text{CH}_2$ ), 1.74-1.66 (m, 1 H,  $\text{N}(\text{CH}_2)_3\text{CH}_2$ ), 1.54-1.44 (m, 2 H,  $\text{N}(\text{CH}_2)_3\text{CH}_2$ ), 1.41 (s, 9 H,  $\text{C}(\text{CH}_3)_3$ ), 1.42-1.34 (m, 2 H,  $\text{NCH}_2(\text{CH}_2)_2$ ), 1.22-1.14 (m, 2 H,  $\text{N}(\text{CH}_2)_2\text{CH}_2\text{CH}_2$ );  $^{13}\text{C}$  NMR  $\delta$  = 176.8\* (7-CO), 172.9 (7-CO), 171.5 ( $\text{COOCH}_3$ ), 171.5 (4-CO), 155.9 ( $\text{NCO}_2\text{Bu}^t$ ), 136.3 (C7), 133.4 ( $\text{CH}=\text{CH}_2$ ), 132.0 ( $\text{CH}=\text{CH}_2$ ), 127.7 (ArC3a), 125.2 (ArC2), 125.0\* (ArC2), 121.5 (ArC6), 119.1 ( $\text{CH}_2\text{CH}=\text{CH}_2$ ), 118.9 (ArC4 and ArC5), 118.8\* (ArC4), 118.7\* (ArC5), 117.0 ( $\text{NCH}_2\text{CH}=\text{CH}_2$ ), 113.7\* (ArC3), 113.6 (ArC3), 109.4 (ArC7), 79.0 ( $\text{OCMe}_3$ ), 52.7 (C5), 52.4 ( $\text{OCH}_3$ ), 51.7 (C2), 48.6 ( $\text{NCH}_2-\text{CH}=\text{CH}_2$ ), 40.0 ( $\text{NCH}_2(\text{CH}_2)_3$ ), 37.2 (H8), 36.3 ( $\text{CHCH}_2-\text{CH}=\text{CH}_2$ ), 34.8\* (H8), 31.9 ( $\text{N}(\text{CH}_2)_3\text{CH}_2$ ), 29.5 ( $\text{NCH}_2-\text{CH}_2(\text{CH}_2)_2$ ), 28.4



(C(CH<sub>3</sub>)<sub>3</sub>), 22.4 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 21.3 (C<sub>9</sub>), 20.5\* (C<sub>9</sub>); MS (ES) *m/z* 569 (100% MH<sup>+</sup>), 513 (49% MH<sup>+</sup>-C(Me<sub>3</sub>)), 469 (64% MH<sup>+</sup>-Boc). HRMS (ES) calcd for C<sub>31</sub>H<sub>45</sub>N<sub>4</sub>O<sub>6</sub>: 569.3339; found: 569.3308.

**Methyl (2*S*,5*R*)-2-allyl-10-(*N*-allyl-1*H*-indol-3-yl)-3,6-diaza-5-(*tert*-butoxycarbonylamino)butyl-4,7-dioxodecanoate (18)**

This was prepared by *general procedure C* using **14** (0.200 g, 0.79 mmol), **15** (0.230 g), DCM (3 mL), AcCN (6 mL) and EDCI (0.124 g) to give **18** (130 mg, 28%) as a solid. TLC (DCM/MeOH 50:1) *R<sub>f</sub>* = 0.45; <sup>1</sup>H NMR δ = 7.56 (d, *J* = 8 Hz, 1 H, ArH<sub>4</sub>), 7.27 (d, *J* = 8 Hz, 1 H, ArH<sub>7</sub>), 7.17 (dt, *J* = 8, 1 Hz, 1 H, ArH<sub>6</sub>), 7.07 (t, *J* = 8 Hz, 1 H, ArH<sub>5</sub>), 6.99 (d, *J* = 8 Hz, 1 H, 3-NH), 6.88 (s, 1 H, ArH<sub>2</sub>), 6.34 (d, *J* = 8 Hz, 1 H, 6-NH), 5.96 (ddt, *J* = 18, 11, 6 Hz, 1 H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 5.66 (ddt, *J* = 17, 9, 7 Hz, 1 H, CHCH<sub>2</sub>CH=CH<sub>2</sub>), 5.18-5.03 (m, 4 H, 2×CH=CH<sub>2</sub>), 4.71-4.65 (m, 3 H, NCH<sub>2</sub>CH=CH<sub>2</sub> and NHBoc), 4.60 (dd, *J* = 6, 2 Hz, 1 H, H<sub>2</sub>), 4.50 (dt, *J* = 6, 8 Hz, 1 H, H<sub>5</sub>), 3.68 (s, 3 H, OCH<sub>3</sub>), 3.06 (d, *J* = 6 Hz, 2 H, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.77 (t, *J* = 7 Hz, 2 H, H<sub>10</sub>), 2.52 (ddd, *J* = 15, 9, 5 Hz, 2 H, CHCH<sub>2</sub>CH=CH<sub>2</sub>), 2.28 (t, *J* = 7 Hz, 2 H, CH<sub>2</sub>8), 2.03 (pent, *J* = 7 Hz, 2 H, H<sub>9</sub>), 1.81 (m, 1 H, N(CH<sub>2</sub>)<sub>3</sub>CHH), 1.63 (m, 1 H, N(CH<sub>2</sub>)<sub>3</sub>CHH), 1.41 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 1.48-1.29 (m, 4 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub> and N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR δ = 173.2 (7-CO), 171.7 (COOCH<sub>3</sub>), 171.4 (4-CO), 156.1 (NCO<sub>2</sub><sup>t</sup>Bu), 136.4 (ArC<sub>7a</sub>), 133.6 (NCH<sub>2</sub>CH=CH<sub>2</sub>), 132.1 (CH<sub>2</sub>CH=CH<sub>2</sub>), 127.9 (ArC<sub>3a</sub>), 125.2 (ArC<sub>2</sub>), 121.4 (ArC<sub>6</sub>), 119.2 (ArC<sub>4</sub>), 119.0 (CH<sub>2</sub>CH=CH<sub>2</sub>), 118.7 (ArC<sub>5</sub>), 117.0 (NCH<sub>2</sub>CH=CH<sub>2</sub>), 114.4 (ArC<sub>3</sub>), 109.4 (ArC<sub>7</sub>), 79.0 (OC(CH<sub>3</sub>)<sub>3</sub>), 52.6 (C<sub>2</sub>), 52.3 (OCH<sub>3</sub>), 51.6 (C<sub>5</sub>), 48.5 (NCH<sub>2</sub>CH=CH<sub>2</sub>), 39.9 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 36.2 (CHCH<sub>2</sub>CH=CH<sub>2</sub>), 35.9 (H<sub>8</sub>), 31.9 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 29.6 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 28.3 (C(CH<sub>3</sub>)<sub>3</sub>), 26.0 (H<sub>10</sub>), 24.4 (H<sub>9</sub>), 22.4 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (ES) *m/z* 583 (100% MH<sup>+</sup>), 527 (44%), 483 (46%). HRMS (ES) calcd for C<sub>32</sub>H<sub>47</sub>N<sub>4</sub>O<sub>6</sub>: 583.3496; found: 583.3506.

**3.5 Amino Acid Deprotection – General Procedure D.**

**Methyl (2*S*,5*R*)-2-allyl-8-(*N*-allyl-1*H*-indol-3-yl)-3,6-diaza-5-(4-aminobutyl)-4,7-dioxooctanoate hydrochloride (25)**

To a solution of **16** (0.07 g, 0.1 mmol) in DCM (2 mL) was added trifluoroacetic acid (2 mL). The reaction mixture was stirred under a N<sub>2</sub> atmosphere at rt for 2 h. The solvents were evaporated and the residue was dissolved in methanol. Hydrochloric acid (1M, 0.2 mL, 0.2 mmol) in diethyl ether was added and the solution was evaporated. Recrystallization from a minimum amount of petroleum spirit and DCM/diethyl ether produced **25** as a brown solid in quantitative yield, Mp 103-106 °C; TLC (MeOH) R<sub>f</sub> = 0.74; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ = 7.56 (d, *J* = 8 Hz, 1 H, ArH4), 7.28 (d, *J* = 8 Hz, 1 H, ArH7), 7.15 (s, 1 H, ArH2), 7.10 (t, *J* = 8 Hz, 1 H, ArH6), 7.00 (t, *J* = 8 Hz, 1 H, ArH5), 6.00-5.92 (m, 1 H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 5.67-5.54 (m, 1 H, CH<sub>2</sub>CH=CH<sub>2</sub>), 5.09 (d, *J* = 10 Hz, 1 H, CH=CHH), 5.11-4.86 (m, 4 H, 2×CH=CH<sub>2</sub>), 4.71 (d, *J* = 6 Hz, 2 H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 4.37 (m, 2 H, H2 and H5), 3.68 (s, 2 H, H8), 3.62 (s, 3 H, OCH<sub>3</sub>), 2.70 (br s, 2 H, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.49-2.41 (m, 1 H, CHCHHCH=CH<sub>2</sub>), 2.38-2.24 (m, 1 H, CHCHHCH=CH<sub>2</sub>), 1.84-1.68 (br s, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.68-1.46 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 1.34-1.22 (m, 2 H, N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR δ = 174.1 (7-CO), 173.2 (COOCH<sub>3</sub>), 172.8 (4-CO), 137.8 (ArC7a), 135.1 (NCH<sub>2</sub>CH=CH<sub>2</sub>), 134.0 (CH<sub>2</sub>CH=CH<sub>2</sub>), 128.9 (ArC3a), 128.4 (ArH2), 122.6 (ArH6), 120.0 (ArH4), 119.8 (ArH5), 118.8 (CH<sub>2</sub>CH=CH<sub>2</sub>), 116.9 (N-CH<sub>2</sub>CH=CH<sub>2</sub>), 110.8 (ArH7), 109.1 (ArC3), 54.1 (C2), 53.3 (OCH<sub>3</sub>), 52.7 (C5), 49.4 (NCH<sub>2</sub>CH=CH<sub>2</sub>), 40.4 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 36.7 (CHCH<sub>2</sub>CH=CH<sub>2</sub>), 33.8 (CH<sub>2</sub>8), 32.6 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 28.0 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 23.5 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (ES) *m/z* 455 (100% MH<sup>+</sup>), 326 (79%). HRMS (ES) calcd for C<sub>25</sub>H<sub>35</sub>N<sub>4</sub>O<sub>4</sub>: 455.2658; found: 455.2663.

**Methyl (2*S*,5*R*)-2-allyl-9-(*N*-allyl-1*H*-indol-3-yl)-3,6-diaza-5-(4-aminobutyl)-4,7-dioxononanoate hydrochloride (26)**

This was prepared by *general procedure D* using **17** (70 mg, 0.12 mmol) giving **26** as a solid (0.050 g, 80%). Mp 145-148 °C; TLC (MeOH) R<sub>f</sub> = 0.67; <sup>1</sup>H NMR δ 7.94 (br s, 1 H, 3-NH), 7.54 (d, *J* = 7 Hz, 1 H, ArH4), 7.24-7.04 (m, 4 H, ArH2,5,6,7), 6.86 (br s, 1 H, 6-NH), 5.87 (m, 1 H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 5.64 (m, 1 H, CH<sub>2</sub>CH=CH<sub>2</sub>), 5.12-4.97 (m, 4 H, 2×CH=CH<sub>2</sub>), 4.62-4.44 (m, 4 H, N-CH<sub>2</sub>-CH=CH<sub>2</sub> and H2

and H5), 3.64 (s, 3 H, OCH<sub>3</sub>), 3.09-2.81 (m, 4 H, H9 and NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.75-2.37 (m, 4 H, H8 + CHCH<sub>2</sub>CH=CH<sub>2</sub>), 1.81-1.52 (m, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.40-1.25 (m, 4 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub> and N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR δ = 175.1 (7-CO), 172.3 (COOCH<sub>3</sub>), 171.9 (4-CO), 136.3 (ArC7a), 133.5 (NCH<sub>2</sub>CH=CH<sub>2</sub>), 132.3 (CH<sub>2</sub>CH=CH<sub>2</sub>), 127.7 (ArC3a), 125.6 (ArC2), 121.6 (ArC6), 119.2 (ArC4), 119.0 (CH<sub>2</sub>CH=CH<sub>2</sub>), 118.9 (ArC5), 117.1 (NCH<sub>2</sub>CH=CH<sub>2</sub>), 113.1 (ArC3), 109.6 (ArC7), 52.9 (C2), 52.5 (OCH<sub>3</sub>), 52.2 (C5), 48.6 (NCH<sub>2</sub>CH=CH<sub>2</sub>), 39.2 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 36.7 (CHCH<sub>2</sub>CH=CH<sub>2</sub>), 35.8 (H8), 31.8 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 27.3 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 22.0 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 21.3 (H9); MS (ES) *m/z* 469 (100% MH<sup>+</sup>). HRMS (ES) calcd for C<sub>26</sub>H<sub>37</sub>N<sub>4</sub>O<sub>4</sub>: 469.2815; found: 469.2805.

**Methyl (2*S*,5*R*)-2-allyl-10-(*N*-allyl-1*H*-indol-3-yl)-3,6-diaza-5-(4-aminobutyl)-4,7-dioxodecanoate hydrochloride (27)**

This was prepared by *general procedure D* using **18** (0.070 g, 0.12 mmol) giving **27** as a solid (0.030 g, 47%). Mp 121-123 °C; TLC (MeOH) R<sub>f</sub> = 0.75; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ = 7.43 (d, *J* = 8 Hz, 1 H, ArH4), 7.17 (d, *J* = 8 Hz, 1 H, ArH7), 7.00 (t, *J* = 7 Hz, 1 H, ArH6), 6.92-6.88 (m, 2 H, ArH2,5), 5.88 (ddt, *J* = 17, 10, 5 Hz, 1 H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 5.61 (m, 1 H, CH<sub>2</sub>CH=CH<sub>2</sub>), 5.03-4.85 (m, 4 H, 2×CH=CH<sub>2</sub>), 4.61 (dd, *J* = 5 Hz, 2 H, NCH<sub>2</sub>CH=CH<sub>2</sub>), 4.37-4.26 (m, 2 H, H2 and H5), 3.56 (s, 3 H, OCH<sub>3</sub>), 3.20-3.19 (m, 2 H, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.78\* (t, *J* = 7 Hz, H10), 2.67 (t, *J* = 7 Hz, 2 H, H10), 2.51-2.29 (m, 2 H, CHCH<sub>2</sub>CH=CH<sub>2</sub>), 2.22 (t, *J* = 7 Hz, 2 H, H8), 1.89 (pent, *J* = 7 Hz, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.77-1.62 (m, 1 H, N(CH<sub>2</sub>)<sub>3</sub>CHH), 1.60-1.48 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 1.39-1.22 (m, 2 H, N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR δ = 175.2 (7-CO), 172.9 (COOCH<sub>3</sub>), 172.0 (4-CO), 136.9 (ArC7a), 134.4 (NCH<sub>2</sub>CH=CH<sub>2</sub>), 133.1 (CH<sub>2</sub>CH=CH<sub>2</sub>), 128.2 (ArC3a), 125.5 (ArC2), 121.2 (ArC6), 118.6 (ArC4), 118.4 (CH<sub>2</sub>CH=CH<sub>2</sub>), 117.8 (ArC5), 115.6 (NCH<sub>2</sub>CH=CH<sub>2</sub>), 114.4 (ArC3), 109.4 (ArC7), 53.0 (C2), 52.2 (OCH<sub>3</sub>), 51.6 (C5), 48.9 (NCH<sub>2</sub>CH=CH<sub>2</sub>), 39.3 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 35.6 (C8), 35.4 (CHCH<sub>2</sub>CH=CH<sub>2</sub>), 31.3 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 26.9 (C10), 24.4 (C9), 22.6 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>); MS (ES) *m/z* 483 (100% MH<sup>+</sup>). HRMS (ES) calcd for C<sub>27</sub>H<sub>39</sub>N<sub>4</sub>O<sub>4</sub>: 483.2971; found: 483.2991.

### 3.6 Ring Closing Metathesis Reaction – General Procedure E.

#### Methyl (6*S*,9*R*,3*E/Z*)-1,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1,13-metheno-8,11-dioxo-9-(4'-*tert*-butoxycarbonylaminobutyl)-2*H*-1,7,10-benzotriazacyclopentadecine-6-carboxylate (**19**)

To a solution of **16** (0.110 g, 0.20 mmol) in DCM (50 mL) was added benzylidene-bis-(tricyclohexylphosphine)-dichlororuthenium catalyst (0.016 g, 0.02 mmol, 10 mol%). The reaction mixture was heated at reflux under a N<sub>2</sub> atmosphere for 24 h. After the solvent was evaporated, the crude product was chromatographed on a flash silica gel column (1% MeOH in DCM) to produce **19** (0.060 g, 58%) as a brown amorphous solid. TLC (DCM/MeOH 10:1) R<sub>f</sub> = 0.85; <sup>1</sup>H NMR δ = 7.57 (d, *J* = 8 Hz, 1 H, ArH14), 7.53\* (d, *J* = 8 Hz, ArH14), 7.35\* (d, *J* = 7 Hz, ArH17), 7.29 (d, *J* = 8 Hz, 1 H, ArH17), 7.21 (t, *J* = 8 Hz, 1 H, ArH16), 7.11 (t, *J* = 8 Hz, 1 H, ArH15), 7.03 (s, 1 H, ArH18), 6.86 (d, *J* = 8 Hz, 1 H, 10-NH), 6.32 (d, *J* = 7 Hz, 1 H, 7-NH), 6.09\* (d, *J* = 8 Hz, 7-NH), 5.90\* (m, H3), 5.52 (dt, *J* = 15, 6 Hz, 1 H, H3), 4.93 (m, 1 H, H4), 4.79-4.71 (m, 2 H, CHH2 and NH-Boc), 4.63\* (d, *J* = 5 Hz, CHH2), 4.61-4.55 (m, 1 H, CHH2), 4.54-4.43 (m, 2 H, unresolved resonance from the (*Z*)-isomer and H9), 4.33 (ddd, *J* = 12, 7 Hz, 1 H, H6), 3.70 (d, *J* = 14 Hz, 1 H, CHH12), 3.65 (s, 3 H, OCH<sub>3</sub>), 3.64\* (s, OCH<sub>3</sub>), 3.53 (d, *J* = 14 Hz, 1 H, CHH12), 3.11-2.99 (m, 2 H, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.45-2.22 (m, 2 H, CH<sub>2</sub>5), 1.84-1.56 (m, 4 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub> and NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 1.38 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 1.34\* (s, C(CH<sub>3</sub>)<sub>3</sub>), 1.45-1.26 (m, 2 H, N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR δ = 174.1 (11-CO), 172.3 (8-CO), 172.1 (COOCH<sub>3</sub>), 156.2 (NCO<sub>2</sub><sup>t</sup>Bu), 137.2 (ArC17a), 130.5 (C3), 127.8 (ArC13a), 127.6 (C4), 126.5 (ArC17), 122.2 (ArC16), 119.7 (ArC15), 118.8 (ArC14), 113.1 (ArC13), 109.3 (ArC18), 79.1 (OCMe<sub>3</sub>), 53.7 (C6), 52.5 (OCH<sub>3</sub>), 52.3 (C9), 46.8 (C2), 39.8 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 33.6 (C12), 32.9 (C5), 31.3 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 29.7 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 28.5 (C(CH<sub>3</sub>)<sub>3</sub>), 22.6 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (ES) *m/z* 527 (45% MH<sup>+</sup>), 471 (56%), 453 (74%), 427 (100%). HRMS (ES) calcd for C<sub>23</sub>H<sub>31</sub>N<sub>4</sub>O<sub>4</sub>: 527.2870; found: 527.2870.

#### Methyl (6*S*,9*R*,3*E/Z*)-1,2,3,4,5,6,7,8,9,10,11,12,13,14-tetradecahydro-1,14-metheno-8,11-dioxo-9-(4'-*tert*-butoxycarbonylaminobutyl)-1,7,10-benzotriazacyclohexadecine-6-carboxylate (**20**)

This was prepared by *general procedure E* using **17** (0.040 g, 0.07 mol), Ru catalyst (5.86 mg), and DCM (17.5 mL) giving **20** as a brown solid (0.038 g, 100%). TLC (DCM/MeOH 50:1)  $R_f = 0.68$ ;  $^1\text{H}$  NMR  $\delta = 7.55$  (d,  $J = 8$  Hz, 1 H, ArH15), 7.56\* (d,  $J = 8$  Hz, ArH15), 7.32\* (d,  $J = 8$  Hz, ArH18), 7.23 (d,  $J = 8$  Hz, 1 H, ArH18), 7.17 (dt,  $J = 8$ , 1 Hz, 1 H, ArH17), 7.08 (dt,  $J = 8$ , 1 Hz, 1 H, ArH16), 6.91 (s, 1 H, ArH19), 6.88\* (s, ArH19), 6.53 (d,  $J = 7$  Hz, 1 H, 10-NH), 6.20 (d,  $J = 7$  Hz, 1 H, 7-NH), 6.12\* (d, 8 Hz, 7-NH), 5.96\* (m, H3), 5.67 (dt,  $J = 16$ , 5 Hz, 1 H, H3), 5.56\* (m, H4), 4.94 (dt,  $J = 16$ , 7 Hz, 1 H, H4), 4.62 (dd,  $J = 16$ , 5 Hz, 2 H, CHH2), 4.56-4.51 (m, 2 H, CHH2 and NHBoc), 4.50-4.42\* (m, H9), 4.40-4.26 (m, 2 H, H9 and H6), 3.68\* (s, OCH<sub>3</sub>), 3.62 (s, 3 H, OCH<sub>3</sub>), 3.37-3.21 (m, 1 H, H13), 3.11-2.90 (m, 4 H, NHCH<sub>2</sub> and H13 + CH<sub>2</sub>CH13 (Z)), 2.75-2.56 (m, 3 H, H12 and CHH5), 2.55-2.38 (m, 1 H, CHH5), 1.91-1.50 (m, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.46-1.22 (m, 4 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>) and N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.39 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 1.32\* (s, C(CH<sub>3</sub>)<sub>3</sub>), 1.23\* (s, C(CH<sub>3</sub>)<sub>3</sub>);  $^{13}\text{C}$  NMR  $\delta = 173.6^*$  (11-CO), 173.1 (11-CO), 171.6 (8-CO), 171.4 (COOCH<sub>3</sub>), 156.3 (NCO<sub>2</sub><sup>t</sup>Bu), 136.3 (ArC18a), 130.0 (C3), 127.5 (ArC14a), 126.2 (C4), 125.7 (ArC19), 121.6 (ArC17), 118.9 (ArC16), 118.6 (ArCH15), 114.1 (ArC14), 109.0 (ArC18), 79.2 (OCMe<sub>3</sub>), 54.2 (C6), 52.6 (OCH<sub>3</sub>), 52.4 (C9), 46.3 (C2), 39.6 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 36.4 (C12), 33.0 (C5), 30.8\* (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 29.8 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 28.5 (C(CH<sub>3</sub>)<sub>3</sub>), 22.4 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 19.6 (C13); MS (ES)  $m/z$  541 (100% MH<sup>+</sup>), 485 (56%). HRMS (ES) calcd for C<sub>29</sub>H<sub>41</sub>N<sub>4</sub>O<sub>6</sub>: 541.3026; found: 541.3014.

**Methyl (6*S*,9*R*,3*E/Z*)-1,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1,15-metheno-8,11-dioxo-9-(4'-*tert*-butoxycarbonylaminobutyl)-2*H*-1,7,10-benzotriazacycloheptadecine-6-carboxylate (21)**

This was prepared by *general procedure E* using **18** (0.80 g, 0.014 mmol), Ru cat (11.4 mg) and DCM (24 mL) giving **21** as a brown solid (0.013 g, 13%). TLC (DCM/MeOH 50:1)  $R_f = 0.59$ ;  $^1\text{H}$  NMR  $\delta = 7.54^*$  (d,  $J = 8$  Hz, 1 H, ArH16), 7.55 (d,  $J = 8$  Hz, 1 H, ArH16), 7.33-7.03 (m, 3 H, ArH17,18,19), 6.88 (s, 1 H, ArH20), 6.84 (d,  $J = 8$  Hz, 1 H, 10-NH), 6.74\* (s, ArH20), 6.44 (d,  $J = 6$  Hz, 1 H, 7-NH), 5.96-5.89\* (m, H3), 5.88-5.71 (m, 1 H, H3), 5.59-5.36 (m, 1 H, H4), 4.73-4.38 (m, 4 H, H2 and H9 and H6),

3.73\* (s, OCH<sub>3</sub>), 3.68\* (s, OCH<sub>3</sub>), 3.67\* (s, OCH<sub>3</sub>), 3.66 (3H, s, OCH<sub>3</sub>), 3.06-2.89 (m, 2 H, ArH14), 2.88-2.79\* (m, H14), 2.78-2.70 (m, 2 H, H2), 2.66-2.48 (m, 2 H, H12), 2.32-2.14 (m, 2 H, H5), 2.03-1.94 (m, 2 H, H13), 1.82-1.58 (m, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.44-1.23 (m, 4 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub> and N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.41-1.38 (m, 9 H, C(CH<sub>3</sub>)<sub>3</sub>); <sup>13</sup>C NMR δ = 174.4 (11-CO), 173.3 (8-CO), 171.9 (COOCH<sub>3</sub>), 156.0 (NCO<sub>2</sub><sup>t</sup>Bu), 136.3 (ArC19a), 130.1 (C3), 128.9 (ArC15a), 127.9\* (ArCH15a), 125.0 (ArC20), 121.6 (ArC18), 119.1 (ArC17), 118.8 (ArC16), 113.8 (C15), 108.9 (CH19), 79.1 (OCMe<sub>3</sub>), 53.4 (C6), 52.5 (OCH<sub>3</sub>), 52.0 (C9), 46.8 (C2), 40.7 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 33.8 (C12), 32.7 (C5), 31.3 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 29.7 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 28.5 (C(CH<sub>3</sub>)<sub>3</sub>), 23.2 (C14), 22.7 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 21.8 (C13); MS (ES) *m/z* 555 (100% MH<sup>+</sup>), 297 (53%). HRMS (ES) calcd for C<sub>30</sub>H<sub>43</sub>N<sub>4</sub>O<sub>6</sub>: 555.3183; found: 555.3187.

### 3.7 Amino Acid Deprotection – General Procedure F.

**Methyl (6*S*,9*R*,3*E/Z*)-1,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1,13-metheno-8,11-dioxo-9-(4-aminobutyl)-2*H*-1,7,10-benzotriazacyclopentadecine-6-carboxylate hydrochloride (22)**

To a solution of **19** (0.08 g, 0.011 mmol) in DCM (2 mL) was added trifluoroacetic acid (2 mL). The reaction mixture was stirred under a N<sub>2</sub> atmosphere at rt for 2 h. After the removal of solvent, DCM was added and the solution was re-evaporated. The residue was dissolved in methanol (5 mL), and then a soln of HCl (1M, 0.3 mL, 0.3 mmol, 2 molar equiv) in diethyl ether was added. The solvents were then evaporated. The product was precipitated from diethyl ether and a minimum amount of petroleum spirit followed by DCM to give **22** (0.06 g, 86%) as a brown solid. mp 168-170 °C; TLC (MeOH) R<sub>f</sub> = 0.81; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) δ = 7.39 (d, *J* = 8 Hz, 1 H, ArH14), 7.27\* (d, *J* = 8 Hz, ArH17), 7.21 (d, *J* = 8 Hz, 1 H, ArH17), 6.98 (t, *J* = 8 Hz, 1 H, ArH16), 6.93 (s, 1 H, ArH18), 6.88 (t, *J* = 8 Hz, 1 H, ArH15), 5.78\* (dd, *J* = 10, 5 Hz, H3), 5.52 (dt, *J* = 15, 5 Hz, 1 H, H3), 5.40\* (dd, *J* = 10, 5 Hz, H4), 4.88-4.83 (dt, *J* = 15, 8 Hz, 1 H, H4), 4.59 (dd, *J* = 9, 3 Hz, 1 H, CHH2), 4.36 (dd, *J* = 9, 6 Hz, 1 H, CHH2), 4.20 (t, *J* = 7 Hz, 1 H, H9), 4.10 (dd, *J* = 9, 5 Hz, 1 H, H6), 3.67 (d, *J* = 9 Hz, 1 H, CHH12),

3.51 (s, 3 H, OCH<sub>3</sub>), 3.31 (d,  $J = 9$  Hz, 1 H, CHH<sub>12</sub>), 2.74 (dd, 2 H, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.51-2.42\* (m, CHH<sub>5</sub>), 2.41-2.34 (m, 1 H, CHH<sub>5</sub>), 2.12 (ddd,  $J = 14, 6$  Hz, 1 H, CHH<sub>5</sub>), 1.69-1.49 (m, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.58-1.48 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 1.36-1.23 (m, 2 H, N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR (CD<sub>3</sub>OD)  $\delta = 174.9$  (11-CO), 174.8 (8-CO), 173.2 (COOCH<sub>3</sub>), 138.7 (ArC17a), 131.6 (C3), 129.2 (ArC13a), 129.2 (C4), 127.8 (ArC18), 122.5 (ArC16), 120.3 (ArC15), 119.6 (ArC14), 111.1 (ArC13), 110.3 (ArC17), 55.9 (C6), 54.4 (OCH<sub>3</sub>), 52.8 (C9), 47.5 (C2), 40.5 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 33.9 (C12), 33.5\* (C12), 32.2 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 27.9 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 23.7 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (ES)  $m/z$  427 (100% MH<sup>+</sup>). HRMS (ES) calcd for C<sub>23</sub>H<sub>31</sub>N<sub>4</sub>O<sub>4</sub>: 427.2345; found: 427.2343.

**Methyl (6*S*,9*R*,3*E/Z*)-1,2,3,4,5,6,7,8,9,10,11,12,13,14-tetradecahydro-1,14-metheno-8,11-dioxo-9-(4-aminobutyl)-1,7,10-benzotriazacyclohexadecine-6-carboxylate hydrochloride (23)**

This was prepared by *general procedure F* using **20** (0.15 g, 0.28 mmol), TFA (3 mL) and DCM (3 mL) giving **23** (0.120 g, 91%). Mp 176-179 °C; TLC (MeOH)  $R_f = 0.76$ ; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz)  $\delta = 8.20^*$  (d,  $J = 8$  Hz, 10-NH), 8.00 (d,  $J = 7$  Hz, 1 H, 10-NH), 7.78 (d,  $J = 7$  Hz, 1 H, 7-NH), 7.59\* (d,  $J = 7$  Hz, 7-NH), 7.53 (d,  $J = 7$  Hz, 1 H, ArH15), 7.51 (d,  $J = 8$  Hz, 1 H, ArH15), 7.38\* (d,  $J = 8$  Hz, ArH18), 7.26 (d,  $J = 8$  Hz, 1 H, ArH18), 7.15\* (t,  $J = 7$  Hz, ArH17), 7.10 (t,  $J = 7$  Hz, 1 H, ArH17), 7.03\* (t,  $J = 7$  Hz, ArH16), 7.01 (t,  $J = 7$  Hz, 1 H, H16), 6.94\* (s, ArH19), 6.93 (s, 1 H, ArH19), 5.86-5.75\* (m, H3), 5.79-5.72 (dt,  $J = 16, 4$  Hz, 1 H, H3), 5.59-5.51\* (dt,  $J = 10, 5$  Hz, H4), 4.95-4.85 (m, 1 H, H4), 4.78-4.70 (dd,  $J = 16, 3$  Hz, 1 H, CHH<sub>2</sub>), 4.73-4.66\* (m, CHH<sub>2</sub>), 4.61-4.54\* (m, CHH<sub>2</sub>), 4.52 (dd,  $J = 16, 5$  Hz, 1 H, CHH<sub>2</sub>), 4.52-4.46\* (m, H6), 4.45-4.39\* (m, H9), 4.30-4.24 (m, 1 H, H9), 4.21-4.14 (m, 1 H, H6), 3.71\* (s, OCH<sub>3</sub>), 3.65 (s, 3 H, OCH<sub>3</sub>), 3.27-3.14 (m, 2 H, H13), 2.99-2.89\* (m, H13), 2.98-2.87\* (m, CHH<sub>5</sub>), 2.97-2.86 (m, 2 H, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.72-2.61\* (m, CHH<sub>5</sub>), 2.71-2.60 (m, 2 H, H12), 2.57-2.49 (m, 1 H, CHH<sub>5</sub>), 2.37-2.28 (m, 1 H, CHH<sub>5</sub>), 1.88-1.78\* (m, N(CH<sub>2</sub>)<sub>3</sub>CHH), 1.74-1.65\* (m, N(CH<sub>2</sub>)<sub>3</sub>CHH), 1.80-1.60 (m, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.73-1.62 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 1.55-1.36 (m, 2 H, N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR  $\delta = 174.3$  (11-CO), 174.2\* (11-CO), 174.0 (8-CO), 173.8\* (8-

CO), 172.1 (COOCH<sub>3</sub>), 171.8\* (COOCH<sub>3</sub>), 136.7 (ArC18a), 129.6 (C3), 127.9 (ArC14a), 127.3\* (C4), 126.4 (C4), 125.7 (ArC19), 121.5 (ArC17), 121.4 (ArC17), 118.6 (ArC16), 118.3 (ArC15), 113.8 (ArC14), 109.2 (ArC18), 109.0\* (ArC18), 54.6 (C6), 53.6\* (C6), 53.4 (C9), 52.5\* (C9), 52.1\* (OCH<sub>3</sub>), 51.9 (OCH<sub>3</sub>), 46.3 (C2), 41.2\* (C2), 39.4 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 35.7 (C12), 32.3 (C5), 32.0\* (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 31.5 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 28.5\* (C5), 26.9 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 22.6 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 20.4 (C13); MS (ES) *m/z* 441 (100% MH<sup>+</sup>). HRMS (ES<sup>+</sup>) calcd for C<sub>24</sub>H<sub>33</sub>N<sub>4</sub>O<sub>4</sub>: 541.3026; found: 541.3014.

**Methyl (6*S*,9*R*,3*E/Z*)-1,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1,15-metheno-8,11-dioxo-9-(4-aminobutyl)-2*H*-1,7,10-benzotriazacycloheptadecine-6-carboxylate hydrochloride (24)**

This was prepared by *general procedure F* using **21** (0.90 g, 0.16 mmol), TFA (2 mL) and DCM (2 mL) giving **24** (0.050 g, 63%). mp 156-159 °C; TLC (MeOH) R<sub>f</sub> = 0.69; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) δ = 7.43 (d, *J* = 8 Hz, 1 H, ArH16), 7.30\* (d, *J* = 8 Hz, ArH19), 7.25 (d, *J* = 8 Hz, 1 H, ArH19), 7.07\* (t, *J* = 8 Hz, ArH18), 7.03 (t, *J* = 8 Hz, 1 H, ArH18), 6.91 (t, *J* = 8 Hz, 1 H, ArH17), 6.76 (s, 1 H, ArH20), 6.70\* (s, ArH20), 5.82-5.75 (m, 1 H, H3), 5.61-5.53\* (m, H3), 5.52-5.44 (dt, *J* = 5, 8 Hz, 1 H, H4), 4.35-4.24\* (m, H4), 4.65-4.59 (dd, *J* = 5, 3 Hz, 1 H, CHH2), 4.49\* (d, CHH2), 4.44-4.41\* (d, CHH2), 4.38 (apparent t, *J* = 7 Hz, 1 H, CHH2), 4.35-4.26 (m, 1 H, CH9), 4.20 (dd, *J* = 9, 2 Hz, 1 H, H6), 3.66\* (s, OCH<sub>3</sub>), 3.62 (s, 3 H, OCH<sub>3</sub>), 2.84-2.77 (m, 4 H, H14 and NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.38-2.29 (m, 2 H, H12), 2.21 (dt, *J* = 16, 5 Hz, 1 H, CHH5), 2.13-2.05 (m, 1 H, CHH5), 1.97-1.80 (m, 2 H, H13), 1.75-1.50 (m, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.57 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 1.41-1.26 (m, 2 H, N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), <sup>13</sup>C NMR, δ = 175.0 (12-CO), 174.9 (8-CO), 173.3 (COOCH<sub>3</sub>), 172.9\* (COOCH<sub>3</sub>), 138.0 (ArC19a), 131.4 (C3), 129.4 (ArC15a), 128.9 (C4), 126.6 (ArC20), 122.3 (ArC18), 119.8 (ArC17), 119.6 (ArC16), 119.5 (ArC16), 114.2 (ArC15), 110.0 (ArCH17), 54.6 (CHN), 54.4 (OCH<sub>3</sub>), 53.0 (CHN), 47.4 (CH<sub>2</sub>), 40.4 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 34.3 (C12), 33.3 (C5), 32.6 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 27.8 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 23.7 (C14), 23.5 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 23.1 (CH<sub>2</sub>13); MS (ES) *m/z* 455 (100% MH<sup>+</sup>). HRMS (ES) calcd for C<sub>25</sub>H<sub>35</sub>N<sub>4</sub>O<sub>4</sub>: 455.2658; found: 455.2647.



### 3.8 Guanidation Reactions – General Procedure G.

#### Methyl (6*S*,9*R*,3*E/Z*)-1,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1,13-metheno-8,11-dioxo-9-[4-(*N,N'*-*di**tert*-butoxycarbonylguanidino)aminobutyl]-2*H*-1,7,10-benzotriazacyclopentadecine-6-carboxylate (**28**)

To a solution of *N,N'*-diBoc-*N*-triflylguanidine (0.024 g, 0.06 mmol) in DCM was added **22** (0.03 g, 0.06 mmol) and triethylamine (9.0  $\mu$ L, 0.06 mmol). Triethylamine (0.1 mL) in DCM (0.9 mL) was pre-prepared and a portion (0.1 mL) of this soln was taken for the reaction. The reaction mixture was stirred under a N<sub>2</sub> atmosphere at rt for 3 h. The solvent was evaporated and the reaction mixture was diluted with DCM and washed with sodium bisulfate (2M), saturated sodium bicarbonate and brine, dried and evaporated giving **28** (20 mg, 46%). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz)  $\delta$  = 7.66 (d, *J* = 8 Hz, 1 H, ArH14), 7.31 (d, *J* = 8 Hz, 1 H, ArH17), 7.23 (t, *J* = 8 Hz, 1 H, ArH16), 7.15 7.23 (t, *J* = 8 Hz, 1 H, ArH15), 7.08 (s, 1 H, ArH18), 6.36 (d, *J* = 5 Hz, 1 H, 10-NH), 6.04 (d, *J* = 5 Hz, 1 H, 7-NH), 5.61 (dt, *J* = 16, 5 Hz, 1 H, H3), 4.91 (dt, *J* = 16, 7 Hz, 1 H, H4), 4.60 (d, *J* = 6 Hz, 2 H, H2), 4.52 (dt, *J* = 12, 6 Hz, 1 H, H9), 4.43 (dd, *J* = 13, 6 Hz, 1 H, H6), 3.69\* (s, OCH<sub>3</sub>), 3.67 (s, 3 H, OCH<sub>3</sub>), 3.64-3.62 (m, 2 H, H12), 3.37 (dt, *J* = 8, 5 Hz, 2 H, NHCH<sub>2</sub>), 2.39 (dd, *J* = 4 Hz, 2 H, H5), 1.90-1.40 (m, 4 H, 2 $\times$ CH<sub>2</sub>), 1.51 (s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>), 1.47\* (s, C(CH<sub>3</sub>)<sub>3</sub>); <sup>13</sup>C NMR  $\delta$  = 171.9 (11-CO), 171.7 (8-CO), 163.4 (COOCH<sub>3</sub>), 162.1\* (COOCH<sub>3</sub>), 156.0\* (NCO<sub>2</sub><sup>t</sup>Bu), 151.2 (NCO<sub>2</sub><sup>t</sup>Bu), 137.4 (ArC17a), 130.7 (C2), 127.8 (ArC13a), 127.1 (C4), 126.5 (ArCH18), 122.5 (ArCH16), 121.3 (ArCH15), 117.0 (ArC13), 112.8 (ArC14), 109.5 (ArC17), 86.0 (CNHBoc), 83.1\* (NCMe<sub>3</sub>), 79.1 (OCMe<sub>3</sub>), 53.7 (C6), 52.8 (OCH<sub>3</sub>), 52.6 (C9), 46.9 (C2), 40.6 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 33.7 (C12), 32.9 (CH5), 31.4 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 29.7 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 28.4, 28.1 and 27.9 (C(CH<sub>3</sub>)<sub>3</sub>), 22.9 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (ES) *m/z* 669 (27% MH<sup>+</sup>). HRMS (ES) calcd for C<sub>34</sub>H<sub>49</sub>N<sub>6</sub>O<sub>8</sub>: 669.3612; found: 669.3624.

**Methyl (6*S*,9*R*,3*E/Z*)-1,2,3,4,5,6,7,8,9,10,11,12,13,14-tetradecahydro-1,14-metheno-8,11-dioxo-9-[4-(*N,N'*-ditert-butoxycarbonylguanidino)aminobutyl]-1,7,10-benzotriazacyclohexadecine-6-carboxylate (29)**

This was prepared by *general procedure G* using **23** (0.050 g, 0.11 mmol), *N,N'*-diBoc-*N*-triflylguanidine (0.040 g) and triethylamine (15  $\mu$ L) giving **29** (0.060 g, 84%). TLC (DCM/MeOH 50:1)  $R_f$  = 0.20;  $^1\text{H NMR}$   $\delta$  = 8.28 (br s, 1 H, NHBoc), 7.55 (d,  $J$  = 8 Hz, 1 H, ArH15), 7.31\* (d,  $J$  = 8 Hz, ArH15), 7.23 (d,  $J$  = 8 Hz, 1 H, ArH18), 7.17 (dt,  $J$  = 8, 1 Hz, 1 H, ArH17), 7.08 (dt,  $J$  = 8, 1 Hz, 1 H, ArH16), 6.91 (s, 1 H, ArH19), 6.88\* (s, ArH19), 6.68 (d,  $J$  = 8 Hz, 1 H, 10-NH), 6.18\* (d,  $J$  = 8 Hz, 7-NH), 6.02 (d,  $J$  = 8 Hz, 1 H, 7-NH), 5.95\* (m, H3), 5.65 (dt,  $J$  = 15, 5 Hz, 1 H, H3), 4.97 (dt,  $J$  = 15, 8 Hz, 1 H, H4), 4.68-4.44 (m, 3 H, H2 and H9), 4.41-4.33 (m, 1 H, H6), 3.67\* (s, OCH<sub>3</sub>), 3.63 (s, 3 H, OCH<sub>3</sub>), 3.36-3.24 (m, 2 H, H13), 3.03-2.92 (m, 1 H, CHH5), 2.75-2.72\* (m, CHH5), 2.71-2.67 (m, 1 H, CHH5), 2.59-2.54 (m, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 2.46 (dd,  $J$  = 13, 6 Hz, 2 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 1.95-1.83 (m, 2 H, N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.47 (s, 18 H, C(CH<sub>3</sub>)<sub>3</sub>), 1.45\* (s, C(CH<sub>3</sub>)<sub>3</sub>);  $^{13}\text{C NMR}$   $\delta$  = 172.9 (11-CO), 171.4 (8-CO), 171.3 (COOCH<sub>3</sub>), 163.4 (ArC), 156.0 and 153.1 (NCO<sub>2</sub>Bu<sup>†</sup>), 136.3 (ArC18a), 130.1 (C3), 127.5 (ArC14a), 126.3 (C4), 125.5 (ArCH19), 121.6 (ArCH17), 118.9 (ArCH16), 118.5 (ArCH15), 114.1 (ArC14), 109.0 (ArCH18), 83.1 (CNHBoc), 79.3 (OCMe<sub>3</sub>), 54.1 (C6), 52.5 (OCH<sub>3</sub>), 52.4 (C9), 46.3 (C2), 40.5 (NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 36.4 (C12), 33.0 (C5), 31.0 (N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 29.8 (NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 28.4 and 28.1 (C(CH<sub>3</sub>)<sub>3</sub>), 23.1 (N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 19.7 (ArC13); MS (ES)  $m/z$  683 (23% MH<sup>+</sup>). HRMS (ES) calcd for C<sub>35</sub>H<sub>51</sub>N<sub>6</sub>O<sub>8</sub>: 683.3768; found: 683.3792.

**Methyl (6*S*,9*R*,3*E/Z*)-1,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1,15-metheno-8,11-dioxo-9-[4-(*N,N'*-ditert-butoxycarbonylguanidino)aminobutyl]-2*H*-1,7,10-benzotriazacycloheptadecine-6-carboxylate (30)**

This was prepared by *general procedure G* using **24** (0.030 g, 0.06 mmol), *N,N'*-diBoc-*N*-triflylguanidine (0.023 g) and triethylamine (8.5  $\mu$ L) to give **30** (30 mg, 70%). TLC (DCM/MeOH 50:1)

$R_f = 0.20$ ;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 500 MHz)  $\delta = 8.26$  (s, 1 H, NHBoc), 7.56 (d,  $J = 8$  Hz, 1 H, ArH16), 7.32\* (d,  $J = 8$  Hz, ArH19), 7.24-7.16 (m, 2 H, ArH18,19), 7.08 (t,  $J = 7$  Hz, 1 H, ArH17), 6.87 (s, 1 H, ArH20), 6.74\* (s, ArH20), 6.43 (d,  $J = 7$  Hz, 1 H, 10-NH), 6.38\* (d,  $J = 7$  Hz, 7-NH), 5.94\* (m, H3), 5.80-5.71 (m, 1 H, H3), 5.65 (d,  $J = 9$  Hz, 1 H, 7-NH), 5.54\* (m, H4), 5.43 (dt,  $J = 15, 9$  Hz, 1 H, H4), 4.68-4.57 (m, 2 H,  $\text{CHH}_2$  and H9), 4.50-4.43 (m, 2 H,  $\text{CHH}_2$  and H6), 3.73\* (s,  $\text{OCH}_3$ ), 3.68 (s, 3 H,  $\text{OCH}_3$ ), 3.39-3.31 (m, 2 H, H14), 3.03-2.97 (m, 2 H,  $\text{NCH}_2(\text{CH}_2)_3$ ), 2.89-2.81 (m, 2 H, H12), 2.75\* (m, H12), 2.60-2.49 (m, 2 H, H5), 2.30-2.24 (m, 2 H, H13), 2.18-1.97 (m, 2 H,  $\text{N}(\text{CH}_2)_3\text{CH}_2$ ), 2.00-1.99 (m, 2 H,  $\text{NCH}_2\text{CH}_2(\text{CH}_2)_2$ ), 1.97-1.80 (m, 2 H,  $\text{N}(\text{CH}_2)_2\text{CH}_2\text{CH}_2$ ), 1.60 (s, 9 H,  $\text{C}(\text{CH}_3)_3$ ), 1.46 and 1.45\* (9H,  $\text{C}(\text{CH}_3)_3$ );  $^{13}\text{C NMR}$   $\delta = 173.2$  (11-CO), 171.7 (8-CO), 171.6 ( $\text{COOCH}_3$ ), 163.3 (ArC), 156.0 ( $\text{NCO}_2^t\text{Bu}$ ), 153.1\* ( $\text{NCO}_2^t\text{Bu}$ ), 136.3 (ArC19a), 130.1 (C3), 128.2 (ArC15a), 127.8 (C4), 126.4\* (C4), 124.9 (ArC20), 121.6 (ArC18), 119.1 (ArC17), 118.9 (ArC16), 113.8 (ArC15), 108.9 (ArC19), 83.1 ( $\text{CNHBoc}$ ), 79.3 ( $\text{OCMe}_3$ ), 53.7 (C6), 52.5 ( $\text{OCH}_3$ ), 51.9 (C9), 46.8 (C2), 40.4 ( $\text{NCH}_2(\text{CH}_2)_3$ ), 33.7 (C12), 32.7 (C5), 31.5 ( $\text{N}(\text{CH}_2)_3\text{CH}_2$ ), 29.8 ( $\text{NCH}_2\text{CH}_2(\text{CH}_2)_2$ ), 28.3 and 28.1 ( $\text{C}(\text{CH}_3)_3$ ), 23.2 (C14), 23.0 ( $\text{N}(\text{CH}_2)_2\text{CH}_2\text{CH}_2$ ), 21.9 (C13); MS (ES)  $m/z$  697 (100%  $\text{MH}^+$ ). HRMS (ES) calcd for  $\text{C}_{36}\text{H}_{53}\text{N}_6\text{O}_8$ : 697.3925; found: 697.3943.

### 3.9 Guanidation Deprotection – General Procedure H.

**Methyl (6S,9R,3E/Z)-1,3,4,5,6,7,8,9,10,11,12,13-dodecahydro-1,13-metheno-8,11-dioxo-9-(4-guanidylbutyl)-2H-1,7,10-benzotriazacyclopentadecine-6-carboxylate hydrochloride (31)**

To a solution of **28** (0.010 g, 0.11 mmol) in DCM (2 mL) was added trifluoroacetic acid (4 mL). The reaction mixture was stirred under a  $\text{N}_2$  atmosphere at rt for 2 h. The solution was evaporated and the crude product dissolved in methanol (5 mL). Hydrochloric acid (0.3 mL, 0.3 mmol, 2 molar equiv, 1M) in diethyl ether was added and the solvent was then evaporated. Recrystallization from a minimum amount of petroleum spirit and DCM/diethyl ether gave **31** (0.006 g, 11%) as a brown solid. mp 151-154 °C;  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta = 7.59$  (d,  $J = 8$  Hz, 1 H, ArH14), 7.47\* (d,  $J = 8$  Hz, ArH17), 7.41

(d,  $J = 8$  Hz, 1 H, ArH17), 7.18 (m, 1 H, ArH16), 7.10 (m, 2 H, ArH15,18), 5.99\* (m, H3), 5.70 (dt,  $J = 15, 6$  Hz, 1 H, H3), 5.62-5.58\* (m, H4), 5.06 (dt,  $J = 15, 9$  Hz, 1 H, H4), 4.89 (m, 1 H, CHH2), 4.59 (dd,  $J = 16, 7$  Hz, 1 H, CHH2), 4.42 (dd,  $J = 9, 7$  Hz, 1 H, H9), 4.33 (dd,  $J = 10, 4$  Hz, 1 H, H6), 3.81 (d,  $J = 15$  Hz, 1 H, CHH12), 3.70 (s, 3 H, OCH<sub>3</sub>), 3.64\* (s, OCH<sub>3</sub>), 3.53 (d,  $J = 9$  Hz, 1 H, CHH12), 3.19-3.16 (m, 2 H, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.57 (dt,  $J = 8, 3$  Hz, 1 H, CHH5), 2.35-2.26 (m, 1 H, CHH5), 1.86-1.80 (m, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.69-1.60 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 1.49-1.42 (m, 2 H, N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (ES)  $m/z$  469 (100% MH<sup>+</sup>). HRMS (ES) calcd for C<sub>24</sub>H<sub>33</sub>N<sub>6</sub>O<sub>4</sub>: 469.2563; found: 469.2576.

**Methyl (6*S*,9*R*,3*E/Z*)-1,2,3,4,5,6,7,8,9,10,11,12,13,14-tetradecahydro-1,14-metheno-8,11-dioxo-9-(4-guanidylbutyl)-1,7,10-benzotriazacyclohexadecine-6-carboxylate hydrochloride (32)**

This was prepared by *general procedure H* using **29** (0.020 g, 0.03 mmol), TFA (4 mL) and DCM (2 mL) to give **32** (0.010 g, 73%). mp 176-179 °C; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz)  $\delta = 7.47$  (d,  $J = 8$  Hz, 1 H, ArH15), 7.32\* (d,  $J = 8$  Hz, ArH18), 7.21 (d,  $J = 8$  Hz, 1 H, ArH18), 7.11-6.92 (m, 2 H, ArH16,17), 6.86 (s, 1 H, ArH19), 5.71 (dt,  $J = 15, 5$  Hz, 1 H, H3), 4.87-4.62 (m, 1 H, H4), 4.57-4.46 (m, 1 H, CHH2), 4.44-4.33 (m, 1 H, CHH2), 4.20 (t,  $J = 8$  Hz, 1 H, H9), 4.12 (dd,  $J = 11, 4$  Hz, 1 H, H6), 3.65\* (s, OCH<sub>3</sub>), 3.59 (s, 3 H, OCH<sub>3</sub>), 3.25-3.13 (m, 2 H, H13), 3.11-3.06 (m, 2 H, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.96-2.82 (m, 2 H, H12), 2.61-2.43 (m, 1 H, CHH5), 2.35-2.20 (m, 1 H, CHH5), 1.74-1.63 (m, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.58-1.47 (m, 4 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub> and N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (ES)  $m/z$  484 (100% MH<sup>+</sup>). HRMS (ES) calcd for C<sub>25</sub>H<sub>36</sub>N<sub>6</sub>O<sub>4</sub>: 484.2798; found: 484.2805.

**Methyl (6*S*,9*R*,3*E/Z*)-1,3,4,5,6,7,8,9,10,11,12,13,14,15-tetradecahydro-1,15-metheno-8,11-dioxo-9-(4-guanidylbutyl)-2*H*-1,7,10-benzotriazacycloheptadecine-6-carboxylate hydrochloride (33)**

This was prepared by *general procedure H* using **30** (0.020 g, 0.03 mmol), TFA (4 mL) and DCM (2 mL) to give **33** (0.010 g, 73%), as a light brown solid. Mp >228 °C. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz)  $\delta = 7.62^*$  (d,  $J = 8$  Hz, ArH16), 7.45 (d,  $J = 8$  Hz, 1 H, ArH16), 7.32\* (d,  $J = 8$  Hz, ArH19), 7.27 (1H, d,  $J = 8$  Hz, ArH19), 7.07-7.01 (m, 1 H, ArH18), 6.92 (t,  $J = 8$  Hz, 1 H, ArH17), 6.79 (s, 1 H, ArH20), 6.72\*

(s, ArH20), 5.83-5.76 (m, 1 H, H3), 5.68-5.56\* (m, H3), 5.62-5.48 (m, 1 H, H4), 5.34-5.30\* (m, H4), 4.84-4.63 (m, 2 H, H2), 4.41-4.31 (m, 1 H, H9), 4.25-4.21 (m, 1 H, H6), 3.68\* (s, OCH<sub>3</sub>), 3.64 (s, 3 H, OCH<sub>3</sub>), 3.07 (apparent t,  $J = 6$  Hz, 2 H, NCH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>), 2.82-2.81 (m, 1 H, CHH14), 2.65-2.62 (m, 2 H, H12), 2.34-2.32 (m, 2 H, H5), 2.22 (dt,  $J = 10, 3$  Hz, 1 H, CHH14), 2.12-2.08 (m, 2 H, H13), 1.98-1.88 (m, 2 H, N(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.72-1.66 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 1.56-1.51 (m, 2 H, N(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); MS (ES)  $m/z$  497 (100% MH<sup>+</sup>). HRMS (ES) calcd for C<sub>26</sub>H<sub>37</sub>N<sub>6</sub>O<sub>4</sub>: 497.2876; found: 497.2878.

### 2.2.1. Antibacterial Testing

Antibacterial testing against *Staphylococcus aureus* ATCC6538P was performed at Amrad Corporation Ltd, Melbourne, Australia.

Assay procedure: A standardised inoculate for assays was prepared in 1/10 dilution of seed culture. To a 96 well microtitre plate was added 50  $\mu$ L of liquid medium [Mueller-Hinton broth medium (MHB) and Mueller-Hinton agar medium (MHA)]. The peptoid compounds were dissolved in a 50% MeOH/H<sub>2</sub>O solution for the final concentration of 1 mg/mL. Test solution (50  $\mu$ L) was added into the top row of the plate. A dilution series was continued until it reached the last row of the plate the excess was discarded. The plates (2 peptoid samples were tested per plate) were incubated at 37 °C and shaken at 100 rpm for 18 h.

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