2009

Synthesis of novel N-protected hydrophobic phenylalanines and their application in potential antibacterials

Timothy P. Boyle
University of Wollongong

J. B. Bremner
University of Wollongong, jbremner@uow.edu.au

Jonathan A. Coates
Avexa Ltd

John Deadman
Avexa Ltd

Paul A. Keller
University of Wollongong, keller@uow.edu.au

See next page for additional authors

Publication Details

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au
Synthesis of novel N-protected hydrophobic phenylalanines and their application in potential antibacterials

Abstract
An efficient synthesis of two new N-acetyl-4'-arylphenylalanines is described together with their incorporation into a number of cationic peptoid antibacterial agents, one of which had an MIC of 7.8 μg/mL against Staphylococcus aureus.

Keywords
CMMB

Disciplines
Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

Authors

This journal article is available at Research Online: http://ro.uow.edu.au/scipapers/4575
Synthesis of novel N-protected hydrophobic phenylalanines and their application in potential antibacterials

Timothy P. Boyle,a John B. Bremner,a* Jonathan A. Coates,b John Deadman,b Paul A. Keller,*a Stephen G. Pyne* a and Kittiya Somphola

aSchool of Chemistry, University of Wollongong, Wollongong, NSW 2522 Australia. Fax: +61 2 4221 3509; fax: +61 2 4221 4287
bAvexa Ltd, 576 Swan St, Richmond, Vic 3121 Australia.

*Corresponding authors: Tel: Tel: +61 2 4221 3509; fax: +61 2 4221 4287

E-mail address: keller@uow.edu.au or spyne@uow.edu.au

Abstract: An efficient synthesis of two new N-acetyl-4’-arylphenylalanines is described together with their incorporation in to a number of cationic peptoid antibacterial agents, one of which had an MIC of 7.8 μg/mL against Staphylococcus aureus.

Keywords: Anthracenyl phenylalanines; Phenanthrenyl phenylalanines; Cationic peptoids; Antibacterials
1. Introduction
With the increasing spread of antibacterial resistance,\textsuperscript{1-3} including resistance by pathogenic bacteria to vancomycin,\textsuperscript{4,5} there is a compelling imperative for new antibacterials.\textsuperscript{6,7} In this context, we have undertaken a program investigating the design and synthesis of cyclic cationic peptoids linked by a hydrophobic scaffold as potential antibacterial agents, and thus far, have shown the binaphthyl\textsuperscript{8} and carbazole scaffolds\textsuperscript{9,10} within these cyclic peptoids produce antibacterial agents, whilst the smaller indole based cyclic peptoids\textsuperscript{11} failed to inhibit bacterial growth. Therefore, as part of this program targeting new peptoid derivatives as antibacterial agents and attempting to address the resistance mechanism against vancomycin, we investigated the synthesis of novel hydrophobic amino acids and their subsequent incorporation into acyclic cationic peptides. These peptides were designed to further explore the effect of hydrophobicity and the role of cationic residues within the peptide. The synthesis and methodology of the novel hydrophobic amino acids, their incorporation into cationic peptides and aspects of their in vitro antibacterial activity are reported in this paper.\textsuperscript{12}

2. Chemistry
The strategy employed to prepare the two hydrophobic amino acids preceded via a common trimethylstannyl amido acid 5, which was prepared from phenylalanine in four steps (Scheme 1). This common intermediate was then coupled to either 9-bromoanthracene or 9-bromophenanthrene via a Stille coupling\textsuperscript{13} protocol followed by subsequent saponification to yield the hydrophobic amido acids 7a and 7b (Scheme 1).
Therefore, iodination of phenylalanine was performed as previously described\textsuperscript{14} to produce \( p \)-iodophenylalanine \( 2 \), which was isolated in quantitative yield. Esterification of \( 2 \) with MeOH/SOCl\(_2\) afforded the methyl ester \( 3 \) as the hydrochloride salt in excellent yield, which was carried forward to the \( N \)-acetyl derivative \( 4 \) without further purification (Scheme 1). The key trimethylstannyl intermediate \( 5 \) was prepared following the procedure of Morera et al.,\textsuperscript{15} in 76\% yield. This methodology was favoured over previously reported methods,\textsuperscript{16} due to the faster reaction time in preparing the aryltrimethylstannane over the aryltributylstannane, significantly decreasing the possibility of racemization at the \( \alpha \) position of the amido ester.

The hydrophobic amido esters \( 6a \) and \( 6b \), were prepared via a Stille coupling methodology\textsuperscript{14,15} in 67\% and 59\% yields, respectively (Scheme 1). The ligand of choice for this reaction was tri-\( o \)-tolylphosphine, as phenyl transfer to the amino acid was observed when triphenylphosphine was present as the ligand. It was also found that increasing the temperature above 85 \( ^\circ \)C resulted in faster reaction times and also resulted in partial racemization of the \( \alpha \)-stereocentre of the amido ester. However, at 70 \( ^\circ \)C, no racemization was observed. Partial racemization of these products formed at the higher temperature was detected from \(^1\)H NMR analysis of their products \( 12a,b \) (Scheme 2) that showed NMR signals for a minor diastereomer. The desired free acid form was obtained by saponification to afford \( 7a \) and \( 7b \) in 90\% and 55\% yield, respectively.
Scheme 1

Reagents and conditions: (a) NaIO₃, AcOH, H₂SO₄, 70 °C, 16 h, 100% (b) SOCl₂, MeOH, 0 °C – RT, 16 h, 99% (c) Ac₂O, AcONa(aq), 0 °C, 56% (d) (SnMe₃)₂, Pd(OAc)₂, PPh₃, PhMe, 100 °C, 30 min, 76% (e) a, 9-bromoanthracene, Pd(OAc)₂, P(o-tol)₃, DMF, 70 °C, 16 h, 6a: 67%; b. 9-bromophenanthrene, Pd(OAc)₂, P(o-tol)₃, DMF, 70 °C, 16 h, 6b: 59% (f) LiOH, THF/H₂O, RT, 16 h, 7a: 90%, 7b: 55%.
The peptide fragment 10 was prepared employing a well established EDCI peptide coupling methodology and a Fmoc protection/deprotection protocol.\(^8\text{--}^\text{11}\) This fragment was coupled to 7a and 7b to give the protected tripeptoids 11a and 11b, respectively (Scheme 2). \(N\)-Boc deprotection of 11a and 11b by exposure to TFA, followed by anion exchange with HCl provided the hydrochloride salts 12a and 12b, respectively (Scheme 2).

Scheme 2

Reagents and conditions: (a) Fmoc-\text{L-lysine}(Boc)OH, EDCI, HO\textbf{B}t, CH\textsubscript{2}Cl\textsubscript{2}, DMAP, RT, 16 h, 87%. (b) 1% Piperidine, MeCN, RT, 3 h, 100% (c) 7a, EDCI, HO\textbf{B}t, DMF, RT, 16 h, 11a: 59%, 11b: 50%. (d) TFA/CH\textsubscript{2}Cl\textsubscript{2} (1 : 1), RT, 3 h, then HCl/ether, 12a: 61%, 12b: 69%.
This chemistry was further expanded to include the dicationic tetrapeptoids \textbf{20a} and \textbf{20b}, that also incorporated the hydrophobic amido acids \textbf{7a,b} and the less hydrophobic \textit{O}-allyltyrosine peptoid analogue \textbf{20c} (Scheme 3). Allyl glycine \textbf{13} was converted to its benzyl ester \textbf{14} which was coupled to Fmoc-D-arginine(Pmc)OH to give the dipeptide \textbf{15}. Selective base catalysed removal of the N-Fmoc protecting group of \textbf{15} gave the free amine \textbf{16} that was coupled to Fmoc-D-lysine(Boc)OH to give the protected tripeptoid \textbf{17}. N-Fmoc removal from \textbf{17} and coupling of the resulting amine \textbf{18} with \textbf{7a} or \textbf{7b} gave the protected tetrapeptoids \textbf{19a} and \textbf{19b}, respectively. Coupling of \textbf{18} with \textit{N}-acetyl-\textit{O}-allyl-L-tyrosine\textsuperscript{17} gave the tetrapeptoid \textbf{19c}. Acid catalysed deprotection of \textbf{19a}, \textbf{19b} and \textbf{19c}, followed by anion exchange with HCl, gave the bis-hydrochloride salts of tetrapeptoids \textbf{20a}, \textbf{20b} and \textbf{20c}, respectively (Scheme 3).
Scheme 3

Reagents and conditions: (a) BnOH, SOCl₂, 16 h, RT, 68%  (b) Fmoc-D-arginine(Pmc)OH, EDCI, HOBT, DMF, 16 h, RT, 51%. (c) 1% Piperidine, MeCN, RT, 3 h, 70%  (d) Fmoc-D-lysine(Boc)OH, EDCI, HOBT, DMF, 16 h, RT, 51%. (e) 1%
Piperidine, MeCN, RT, 3 h, 93% (f) 7a or 7b or N-Ac-O-allyl-L-tyrosine, EDCI. HOBt, DMF, 16 h, RT, 19a: 36%, 19b: 80%, 19c: 85% (g) TFA/CH₂Cl₂ (1:1), RT, 3 h, then HCl/ether, 20a: 88%, 20b: 79%, 20c: 85%.

2.2. In vitro antibacterial activity

The synthesized hydrophobic and cationic peptoids 12a, 12b, 20a, 20b and 20c were tested against the Gram-positive bacterium *S. aureus* (ATCC6538) and showed MIC values of 31.3, 15.6, 15.6, 7.8 and >125 µg/mL, respectively. The positive control, vancomycin, showed a MIC value of 1.95 µg/mL. In stark contrast to 12a,b and 20a,b the less hydrophobic tetrapeptoid 20c was not active (MIC > 125 µg/mL). The 9-phenanthrenyl peptoids, 12b and 20b, were more active than their respective 9-anthracenyl counterparts, 12a and 20a. The dicationic peptoids, 20a,b were more active than their respective monocationic analogues 12a,b. However it should be noted that peptides 12a,b have a L-lysine residue whereas the peptides 20a,b have a D-lysine residue. These differences limit further structure-activity comparisons to be made between the tripeptides 12a,b and tetrapeptides 20a,b. These biological results are consistent with the pharmacophore model proposed by Svendsen\textsuperscript{12} for peptide compounds which indicates that two hydrophobic and two cationic sites are important for antibacterial activity. In our case the benzyl ester moiety in 20a,b would represent the second, albeit considerably smaller, hydrophobic group.

In contrast to the activity shown against *S. aureus*, the cationic peptoids 12a, 12b, 20a, and 20b, were not active against *Enterococcus faecalis* strains (both vancomycin sensitive and resistant strains); MIC values >125 µg/mL were obtained against these strains and the same results were seen with the peptoid 20c.
3. Conclusions

We have developed a useful method for preparing the novel biaryl hydrophobic amido acids 7a,b via Stille coupling reactions. This method could potentially be employed to prepare other novel biaryl phenylalanine derivatives. We have shown that incorporation of these hydrophobic amido acid residues into cationic peptides resulted in peptoids having significant antibacterial activity against *S. aureus* when compared to a less hydrophobic, *O*-allyltyrosine analogue 20c. These results highlight the importance of hydrophobicity within the peptoid for antibacterial activity and provide a platform for further development of antimicrobial agents with improved activity against *S. aureus*.

4. Experimental

4.1. Chemistry

Chemical Ionisation (CI) mass spectra were obtained on a Shimadzu QP-5000 mass spectrometer by a direct insertion technique (electron beam density 70 eV). Electrospray ionization (ESI) mass spectra were obtained on a VG Quattro spectrometer. High-resolution mass spectra (HRMS) were determined on a VG Autospec spectrometer or on a micromass QTof2 spectrometer using polyethylene glycol as the internal standard. The *m/z* values are stated with their peak intensity percentages in parentheses. Optical rotations were measured using a Jasco DIP-370 digital polarimeter with a 10 mm path length. Proton and carbon nuclear magnetic resonance (NMR) spectra were determined in
CDCl₃ solution at 300 MHz (¹H NMR) or 75 MHz (¹³C NMR) unless otherwise stated, using a Varian Mercury 300 MHz or Varian Inova 500 MHz spectrometer. TMS was used as the internal standard and all chemical shifts (δ) were measured relative to the internal standard. Analytical thin layer chromatography (TLC) was carried out on Merck Silica gel 60 F₂₅₄ pre-coated aluminium plates with a 0.2 mm adsorbant thickness. All column chromatography was performed under ‘flash’ conditions on Merck Silica gel 60 (230-400 mesh). ¹H NMR assignments were achieved with the aid of gCOSY, and in some cases NOESY and TOCSY experiments. ¹³C NMR assignments were based upon DEPT, gHSQC and sometimes gHMBC experiments. All compounds were homogeneous by TLC analysis and judged to be of >95% purity based upon ¹H NMR analysis. Compound numbering is based on that of compound 20 as shown in Scheme 3. All compounds were judged to be greater than 95% purity based upon ¹H NMR and TLC analysis. Solvent ratios are vol/vol.

4.2 General Procedures

4.2.1. General synthetic procedure for N-Boc and Pmc Deprotection (Procedure A)

The N-Boc or Pmc protected amine was stirred for 3 h in 1:1 CH₂Cl₂/TFA (5 mL / 0.1 mmol of substrate) solution at RT. The solvent was removed under reduced pressure, and the residue was resuspended in a minimal volume of methanol. The solution was then treated with an excess of 1M HCl/ether solution and the solvent evaporated. The crude product was purified by precipitation from CH₂Cl₂ and/or MeOH by addition of diethyl ether.

4.2.2. General synthetic procedure for peptide coupling (Procedure B)
To a solution of the acid (1 equiv.) in DMF (10 mL per 1 mmol of substrate) at room temperature was added HOBt (1.1 equiv.), EDCI (1 equiv.) and the amine (1.2 equiv.). If the amine was a hydrochloride salt, DIPEA (1 equiv.) was also added. The mixture was allowed to stir for 16 h before dilution with EtOAc (30 mL) and washing with water (30 mL) and brine (30 mL). The organic fraction was dried (MgSO₄) and further purified by column chromatography if required.

4.2.3. General synthetic procedure for N-Fmoc Deprotection (Procedure C)

The Fmoc protected amine was stirred in 1% piperidine/acetonitrile (5 mL per 1 mmol of substrate) for 3 h at RT. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (15:1, CH₂Cl₂/MeOH) to yield the free amine.

4.2.4. (S)-2-Amino-3-(4-iodophenyl)propanoic acid 2

To a solution (S)-2-amino-3-phenylpropanoic acid (4.01 g, 24.3 mmol) in acetic acid (22 mL) was added sulfuric acid (2.9 mL, 5.14 mmol), iodine (2.47 g, 4.7 mmol) and sodium iodate (1.02 g, 5.14 mmol). The mixture was heated to 70 °C and allowed to stir at this temperature for 16 h before an additional portion of sodium iodate (1.02 g, 5.14 mmol) was added. The reaction was left for a further 2 h before being concentrated, dissolved in MeOH (20 mL) and treated with NaOH (60 mL). The mixture was left to precipitate out of the basic solution overnight and the resulting solid was filtered by vacuum filtration to yield the title compound 2 (7.07 g, 24.3 mmol, 100%) as a pink solid, which had spectral
data in agreement with that reported.\textsuperscript{14} [\alpha]_D\textsuperscript{21} -10.6 (c. 0.3, HCl). Mp 258-260 °C (lit. 261-262 °C).\textsuperscript{14}

4.2.5. Methyl (2S)-2-amino-3-(4-iodophenyl)propanoate hydrochloride 3

To a solution of 2 (2.00 g, 6.87 mmol) in MeOH (10 mL) at 0 °C was added thionyl chloride (2 mL) and the resulting solution was allowed to stir for 16 h whilst equilibrating to RT. The reaction was evaporated to dryness \textit{in vacuo} to yield the title compound 3 (2.25 g, 6.80 mmol, 99%) as a white solid, which had spectral data in agreement with that reported.\textsuperscript{14} [\alpha]_D\textsuperscript{21} -9.3 (c. 0.15, HCl). Mp 195-198 °C (lit. 199.5-200.5 °C).\textsuperscript{14}

4.2.6. Methyl (2S)-2-acetamido-3-(4-iodophenyl)propanoate 4

To a solution of 3 (2.25 g, 6.80 mmol) in 10% HCl (10 mL) at 0 °C was added 4M sodium acetate (115 mL) and the resulting reaction was allowed to stir whilst equilibrating to 0 °C. Acetic anhydride (50 mL) was added and the reaction allowed to proceed with vigorous stirring. After 1 h the product was collected by vacuum filtration, dissolved in ethyl acetate (30 mL) and washed with 2M sodium bicarbonate (2 x 30 mL). The organic layer was dried and evaporated to yield the title compound 4 (1.31 g, 3.79 mmol, 56%) as a white solid. Mp 118-120 °C. [\alpha]_D\textsuperscript{27} +93.8 (c. 0.1, CHCl\textsubscript{3}). \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz): \delta 7.61 (d, J = 8.4 Hz, 2H, ArH2’ and ArH6’); 6.84 (d, J = 8.1 Hz, 2H, ArH3’ and ArH5’); 5.92 (d, J = 7.2 Hz, 1H, NH); 4.87 (m, 1H, H2); 3.73 (s, 3H, OCH\textsubscript{3}); 3.11 (dd, J = 6.0, 13.8, Hz, 1H, H3\textsubscript{a}); 3.03 (dd, J = 5.4, 13.8 Hz, 1H, H3\textsubscript{b}); 1.99 (s, 3H, NCOCH\textsubscript{3}). \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 75 MHz): \delta 171.8, C1; 169.5, NCO; 137.6, ArCH2’ and ArCH6’; 135.5, ArC4’; 131.2, ArCH3’ and ArCH5’; 94.1, ArC1’; 52.9, C2; 52.4, OCH\textsubscript{3};
37.4, C3; 23.1, NCOCH3. Mass Spectrum (CI+) m/z 348 (100%) [MH+]. HRMS calcd for C12H15NO3I 348.0097, found 348.0104.

4.2.7. Methyl (2S)-2-acetamido-3-(4-trimethylstannylphenyl)propanoate 5

A solution of 4 (590 mg, 1.7 mmol), hexamethyldistannane (781 mg, 2.38 mmol), palladium acetate (20 mg, 0.085 mmol), and triphenylphosphine (45 mg, 0.17 mmol) in toluene (7 mL) was flushed with nitrogen for 15 min and then heated at 100 °C for 30 min under N2. The brown mixture was filtered through a short pad of silica, diluted with diethyl ether (40 mL) and washed twice with water. The organic layer was dried and evaporated to yield the title compound 5 (497 mg, 1.29 mmol, 76%) as a clear oil. [α]D27 +13.7 (c. 0.3, CHCl3). 1H NMR (CDCl3, 300 MHz): δ 7.41 (d, J = 7.5 Hz, 2H, ArH2’ and ArH6’); 7.07 (d, J = 7.8 Hz, 2H, ArH3’ and ArH5’); 6.25 (d, J = 7.8 Hz, 1H, NH); 4.87 (m, 1H H2); 3.72 (s, 3H, OCH3); 3.12 (dd, J = 5.7, 14.1 Hz, 1H, H3a); 3.04 (dd, J = 6.0, 13.9 Hz, 1H, H3b); 1.98 (s, 3H, NCOCH3); 0.27 (t, J = 27.6 Hz, 9H, Sn(CH3)3). 13C NMR (CDCl3, 75 MHz): δ 172.1, C1; 169.7, NCO; 140.6, ArC4’; 135.9, ArCH2’ and ArCH6’; 135.9, ArC1’; 128.7, ArCH3’ and ArCH5’; 53.0, C2; 52.1, OCH3; 37.5, C3; 23.9, NCOCH3; -9.7, Sn(CH3)3. Mass Spectrum (CI+) m/z 386 (50%) [MH+], 382 (10%) [MH+] (112Sn), 85 (100%). HRMS calcd for C15H24NO3Sn (112Sn) 382.0754 found 382.0756.

4.2.8. Methyl (2S)-2-acetamido-3-(4-[9-anthracenyl]phenyl)-propanoate 6a

A solution of 5 (192 mg, 0.50 mmol), 9-bromoanthracene (141 mg, 0.55 mmol), palladium acetate (6 mg, 0.025 mmol), and tri-o-tolylphosphine (15 mg, 0.05 mmol) in
DMF (2 mL) was flushed with N₂ for 15 min then heated to 70 °C and allowed to stir for 16 h. The reaction was diluted with diethyl ether (20 mL) and washed with water (5 x 20 mL), dried and evaporated. The crude product was purified by flash column chromatography (15% EtOAc/hexane then 5% MeOH/CH₂Cl₂) to yield the title compound 6a (133 mg, 0.33 mmol, 67%) as an orange oil. [α]_D^{27} +66.9 (c. 0.1, CHCl₃).

^1^H NMR (CDCl₃, 300 MHz): δ 8.48 (s, 1H, ArH10”); 8.03 (dd, J = 0.9, 8.7 Hz, 2H, ArH3” and ArH6”); 7.63 (dd, J = 0.6, 9.0 Hz, 2H, ArH8” and ArH1”); 7.45 (m, 2H, ArH4” and ArH5”); 7.36 (m, 6H, ArH2” and ArH7”, 4 x ArH’); 5.40 (d, J = 7.8 Hz, 1H, NH); 5.04 (m, 1H, H2); 3.79 (s, 3H, OCH₃); 3.32 (dd, J = 6.3, 13.8 Hz, 1H, H3 a); 3.25 (dd, J = 6.3, 13.8 Hz, 1H, H3 b); 2.08 (s, 3H, COCH₃). ¹³C NMR (CDCl₃, 75 MHz): δ 172.2, C1; 169.8, COCH₃; 137.4, ArC9”; 136.4, ArC4”; 135.2, ArC1”; 132.0, ArC8a” and ArC9a”; 131.9, ArCH2” and ArCH7”; 131.3, ArCH2’ and ArCH6”; 129.2, ArCH3’ and ArCH5”; 128.3, ArCH4” and ArCH5”; 126.5, ArC4a” and ArC10a”; 125.3, ArCH8” and ArCH1”; 125.0, ArCH3” and ArCH6”, ArCH10”; 53.3, C2; 52.3, OCH₃; 37.8, C3; 23.1, COCH₃. Mass Spectrum (CI+) m/z 398 (100%) [MH⁺]. HRMS calcd for C_{26}H_{23}NO_{3} 397.1678, found 397.1675.

4.2.9. Methyl (2S)-2-acetamido-3-(4-[9-phenanthrenyl]phenyl)propanoate 6b

A solution of 5 (259 mg, 0.67 mmol), 9-bromophenanthrene (190 mg, 0.74 mmol), palladium acetate (8 mg, 0.034 mmol), and tri-o-tolylphosphine (20 mg, 0.067 mmol) in DMF (2 mL) was flushed with N₂ for 15 min then heated to 70 °C and allowed to stir for 16 h. The reaction was diluted with diethyl ether (20 mL) and washed with water (5 x 20 mL), dried and evaporated. The crude product was purified by flash column
chromatography (15% EtOAc/hexane then 5% MeOH/CH$_2$Cl$_2$) to yield the title compound 6b (157 mg, 0.40 mmol, 59%) as a clear oil. $[\alpha]_D^{27} +94.6$ (c. 0.1, CHCl$_3$). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.77 (d, $J = 9.0$ Hz, 1H, ArH4’’); 8.71 (d, $J = 8.1$ Hz, 1H, ArH3’’); 7.89 (m, 2H, ArH1’’ and ArH10’’); 7.61 (m, 5H, ArH7’, ArH6’, ArH5’, ArH2’’ and ArH1’’); 7.48 (d, $J = 8.4$ Hz, 2H, ArH2’ and ArH6’); 7.26 (d, $J = 8.1$ Hz, 2H, ArH3’ and ArH5’); 6.25 (d, $J = 7.5$ Hz, 1H, NH); 5.00 (m, 1H, H2); 3.79 (s, 3H, OCH$_3$); 3.30 (dd, $J = 5.7$, 13.8 Hz, 1H, H3a); 3.20 (dd, $J = 6.0$, 13.8 Hz, 1H, H3b); 2.05 (s, 3H, COCH$_3$). $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 172.1, C1; 169.7, COCH$_3$; 139.5, ArC4’; 138.2, ArC1’; 135.0, ArC9’; 131.4, ArC4b’; 130.9, ArC9a’; 130.6, ArC4a’; 130.1, ArCH2’ and ArCH6’; 129.9, ArC10a’; 129.1, ArCH3’ and ArCH5’; 128.5, ArCH1’; 127.4, ArCH7’; 126.8, ArCH6’; 126.7, ArCH1’; 126.5, ArCH5’; 126.4, ArCH10’; 126.3, ArCH2’; 122.9, ArCH4’; 122.4, ArCH3’; 53.2, C2; 52.3, OCH$_3$; 37.6, C3; 23.0, COCH$_3$. Mass Spectrum (Cl+) m/z 398 (100%) [MH$^+$]. HRMS (EI) calcd for C$_{26}$H$_{23}$NO$_3$ 397.1678, found 397.1680.

4.2.10. (2S)-2-Acetamido-3-(4-[(9-anthracenyl)phenyl]propanoic acid 7a

To a solution of 6a (80 mg, 0.20 mmol) in THF/water, 2:1 (3 mL) was added lithium hydroxide monohydrate (17 mg, 0.40 mmol) and the resulting suspension was allowed to stir for 16 h. The reaction mixture was diluted with water (30 mL) and the THF was removed by evaporation. The aqueous layer was washed with CH$_2$Cl$_2$ (40 mL) to remove unreacted starting material. The aqueous phase was acidified with 10% HCl and the resulting precipitate was extracted with CH$_2$Cl$_2$ (3 x 40 mL). The combined organics were dried and evaporated to yield the title compound 7a (69 mg, 0.18 mmol, 90%) as a
white solid. Mp 76 °C. [α]D\(^{20}\) +29.7 (c. 0.1, EtOH). \(^1\)H NMR (CDCl\(_3\), 300 MHz): δ 8.47 (s, 1H, ArH10’); 8.02 (d, \(J = 8.4\) Hz, 2H, ArH3” and ArH6”); 7.59 (d, \(J = 8.7\) Hz, 2H, ArH8” and ArH1”); 7.45 (m, 2H, ArH4” and ArH5”); 7.35 (m, 6H, ArH2” and ArH7”, 4 x ArH’); 6.27 (d, \(J = 6.6\) Hz, 1H, NH); 5.00 (m, 1H, H2); 3.39 (dd, \(J = 4.8, 12.9\) Hz, 1H, H3a); 3.26 (dd, \(J = 6.3, 14.4\) Hz, 1H, H3b); 2.07 (s, 3H, COCH\(_3\)). \(^{13}\)C NMR (CDCl\(_3\), 75 MHz): δ 174.2, C1; 171.2, COCH\(_3\); 137.5, ArC9”; 136.4, ArC4”; 135.0, ArC1”; 131.4, ArC8a”; 131.2, ArC9a”; 130.1, ArCH2” and ArCH7”; 129.3, ArCH2’ and ArCH6’; 128.8, ArCH3’ and ArCH5’; 128.3, ArCH4” and ArCH5”; 126.6, ArC4a” and ArC10a”; 125.3, ArCH8” and ArCH1”; 125.0, ArCH3” and ArCH6”, ArC10”; 53.5, C2; 37.3, C3; 22.9, COCH\(_3\)). Mass Spectrum (ESI+) \(m/z\) 383 (70%) [MH\(^+\)]. HRMS calcd for C\(_{25}\)H\(_{22}\)NO\(_3\) 384.1600, found 384.1610.

4.2.11. (2S)-2-Acetamido-3-(4-[9-phenanthrenyl]phenyl)propanoic acid \(7b\)

To a solution of \(6b\) (124 mg, 0.31 mmol) in THF/water, 2:1 (9 mL) was added lithium hydroxide monohydrate (26 mg, 0.62 mmol) and the resulting suspension was allowed to stir for 16 h. The reaction mixture was diluted with water (30 mL) and the THF was removed by evaporation. The aqueous layer was washed with CH\(_2\)Cl\(_2\) (40 mL) to remove unreacted starting material. The aqueous phase was acidified with 10% HCl and the resulting precipitate was extracted with CH\(_2\)Cl\(_2\) (3 x 40 mL). The combined organics were dried and evaporated to yield the title compound \(7b\) (65 mg, 0.17 mmol, 55%) as a white solid. Mp 128-132 °C. [α]D\(^{20}\) +36.8 (c. 0.1, EtOH). \(^1\)H NMR (CD\(_3\)OD, 300 MHz): δ 8.71 (d, \(J = 8.1\) Hz, 1H, ArH4”); 8.66 (d, \(J = 8.4\) Hz, 1H, ArH3”); 7.79 (s, 1H, ArH1”); 7.76 (s, 1H, ArH10”); 7.51 (m, 5H, ArH7”, ArH6”, ArH5”, ArH2” and ArH1”); 7.32 (m,
2H, Ar’H); 4.76 (dd, J = 5.1, 9.0 Hz, 1H, H2); 3.29 (dd, J = 4.8, 13.5 Hz, 1H, H3a); 3.03 (dd, J = 8.7, 13.5 Hz, 1H, H3b); 1.95 (s, 3H, COCH3). 13C NMR (CD3OD, 75 MHz): δ 174.8, C1; 173.2, COCH3; 140.5, ArC4'; 139.7, ArC1'; 137.7, ArC9"; 132.9, ArC4b"; 132.2, ArC8a"; 131.9, ArC4a"; 131.2, ArC10a"; 131.1, Ar CH2’ and ArCH6’; 130.2, ArCH3’ and ArCH5’; 129.6, ArCH1’; 128.3, ArCH3”; 127.9, ArCH6”; 127.7, ArCH1”; 127.7, ArCH5”; 127.6, ArCH10”; 127.5, ArCH2”; 124.0, ArCH4”; 123.5, ArCH3”; 55.2, C2; 38.2, C3; 22.4, COCH3. Mass Spectrum (ESI+) m/z 384 (50%) [MH+]. HRMS calcd for C25H22NO3 384.1600, found 384.1628.

4.2.12. Methyl (2S,5S)-2-allyl-3-aza-9-(tert-butoxycarboxamido)-5-(9H-9-fluorenylmethyloxycarboxamido)-4-oxononanoate 9

To a solution of 818 (430 mg, 2.61 mmol) and Fmoc-L-lysine(Boc)OH (1.22 g, 2.61 mmol) in CH2Cl2 (10 mL) was added EDCI (500 mg, 2.61 mmol) and a catalytic quantity of DMAP. The resulting mixture was allowed to stir at RT for 16 h. The reaction was diluted with CH2Cl2 (25 mL), then the organic layer was washed with brine (2 x 25 mL) and water (2 x 25 mL) and dried, before being concentrated. The crude product was purified by flash column chromatography (25:1 CH2Cl2/ MeOH) to afford the title compound (1.31 g, 2.27 mmol, 87%) as a cream coloured solid. Mp 123-126 °C. 1H NMR (CDCl3, 300 MHz): δ 7.76 (d, J = 7.6 Hz, 2H, ArH1’ and ArH8’); 7.59 (d, J = 7.6 Hz, 2H, ArH4’ and ArH5’); 7.40 (t, J = 7.6 Hz, 2H, ArH3’ and ArH6’); 7.31 (ddd, J = 9.0, 7.2, 1.2 Hz, 2H, ArH2’ and ArH7’); 6.46 (bs, 1H, NH); 5.64 (m, 1H, H2’); 5.44 (s, 1H, NH); 5.10 (m, 2H, H3’); 4.65 (m, 1H, H2); 4.39 (d, J = 7.2 Hz, 2H, OCH2-H9’); 4.22 (m, 1H, H5); 4.17 (bs, 1H, H9’); 3.74 (s, 3H, OCH3); 3.11 (m, 2H, H9); 2.55 (m, 2H, H1’);
1.85 (m, 2H, H7); 1.65 (m, 2H, H6); 1.50 (m 2H, H8); 1.44 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 171.9, C4; 171.6, C1; 156.2, NCO₂; 143.7, ArC₈a” and ArC₉a”; 142.7, ArC₄a” and ArC₄b”; 131.9, C2’; 127.7, ArCH₃” and ArCH₆”; 127.0, ArCH₂” and ArCH₇”; 125.0, ArCH₁” and ArCH₈”; 119.9, C3’; 119.3, ArCH₄” and ArCH₅”; 79.1, C(CH₃)₃; 67.0, CH₂-C₉”; 54.5, C5; 52.4, OCH₃; 50.6, C2; 47.0, C₉”; 39.8, C9; 36.1, C1’; 32.0, C6; 29.9, C8; 28.3, C(CH₃)₃; 22.2, C7. Mass Spectrum (ESI+) m/z 580.5 (10%) [MH⁺], 130.5 (100%) [MH⁺ (less allylgly)]. HRMS calcd for C₃₂H₄₂N₃O₇ 580.3023, found 580.3025.

4.2.13. Methyl (2S,5S)-2-allyl-5-amino-3-aza-9-(tert-butoxycarboxamido)-4-oxononanoate 10

The title compound was synthesized using the general N-Fmoc deprotection procedure (Procedure C), from 9 (1.27 g, 2.19 mmol) to yield 10 (778 mg, 2.18 mmol, 100%) as a cream oil. ¹H NMR (CDCl₃, 300 MHz): δ 7.81 (d, J = 8.0 Hz, 1H, NH); 5.69 (m, 1H, H2’); 5.11 (m, 2H, H3’); 4.76 (bs, 1H, NH); 4.67 (m, 1H, H2); 3.75 (s, 3H, OCH₃); 3.39 (dd, J = 4.6, 7.6 Hz, 1H, H5); 3.12 (d, J = 6.3 Hz, 2H, H9); 2.54 (m, 2H, H1’); 1.52 (m, 8H, H6, H7, H8 and NH₂); 1.44 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 174.8, C4; 172.1, C1; 156.0, NCO₂; 132.2, C2’; 118.9, C3’; 78.9, C(CH₃)₃; 54.8, C5; 52.2, C2; 51.1, OCH₃; 40.0, C9; 36.4, C1’; 34.4, C6; 29.7, C8; 28.3, C(CH₃)₃; 22.6, C7. Mass Spectrum (ESI+) m/z 358.5 (85%) [MH⁺], 258.4 (100%) [MH⁺ (less Boc)]. HRMS calcd for C₁₇H₂₃N₃O₅ 358.2342, found 358.2339.

4.2.14. Benzyl (2S)-2-amino-4-pentenoate hydrochloride 14
To a solution of 13 (225 mg, 1.96 mmol) in benzyl alcohol (5 mL) was added thionyl chloride (2 mL) and the resulting mixture was allowed to stir for 16 h before addition of diethyl ether (30 mL) and extraction with water (3 x 30 mL). The aqueous layer was concentrated, diluted with 2M sodium bicarbonate (20 mL), and extracted with CH$_2$Cl$_2$ (3 x 30 mL). The combined organic fractions were dried and acidified with 1M HCl/diethyl ether (2 mL) and evaporated. The crude product dissolved in a minimal volume of MeOH and precipitated with diethyl ether to yield the title compound (322 mg, 1.34 mmol, 68%) as a white solid. [α]$_D^{20}$ – 40.6 (c. 0.1, H$_2$O). Mp 186-191 °C. $^1$H NMR (D$_2$O, 300 MHz): δ 7.28 (m, 5H, ArH); 5.51 (m, 1H, H4); 5.11 (m, 4H, H5 and ArCH$_2$); 4.08 (t, $J$ = 5.4 Hz, 1H, H2); 2.55 (m, 2H, H3). $^{13}$C NMR (D$_2$O, 75 MHz): δ 172.1, C1; 137.3, C4; 132.5, ArC1’; 131.7, ArC4’; 131.6, ArCH’; 131.4, ArCH’; 124.4, C5; 71.3, ArCH$_2$; 54.9, C2; 36.8, C3. Mass Spectrum (Cl+) m/z 205 (25%) [MH$^+$]. HRMS calcd for C$_{12}$H$_{16}$NO$_2$ 206.1181, found 206.1169.

4.2.14. Benzyl (2S,5R)-2-allyl-3-aza-5-(9H-9-fluorenylmethyloxycarboxamido)-4-oxo-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2H-6-chromenylsulfonyl)guanidino]octanoate 15

The title compound was synthesised using the general peptide coupling procedure (Procedure B), from 14 (155 mg, 0.65 mmol) and Fmoc-D-arginine(Pmc)OH (431 mg, 0.65 mmol) to afford 15 (280 mg, 0.33 mmol, 51%) as a white solid. Mp 78-74 °C. $^1$H NMR (CDCl$_3$, 300 MHz): δ 7.69 (d, $J$ = 7.5 Hz, 2H, ArH1” and ArH8”); 7.51 (d, $J$ = 7.5 Hz, 2H, ArH4” and ArH5”); 7.28 (m, 9H, ArH); 6.33 (m, 3H, NH); 5.68 (m, 1H, H2’); 5.61 (m, 1H, NH); 4.99 (m, 4H, ArCH$_2$ and H3’); 4.58 (m, 1H, H2); 4.24 (m, 3H, OCH$_3$-H9”and H5); 4.05 (dd, $J$ = 7.2, 7.2 Hz, 1H, H9”); 3.20 (m, 2H, H8); 2.57 (s, 3H, 7’’-
CH\textsubscript{3}); 2.54 (s, 3H, 5”-CH\textsubscript{3}); 2.52 (m, 4H, H3”’ and H1’); 2.05 (s, 3H, 8”’-CH\textsubscript{3}); 1.85 (m, 2H, H6); 1.69 (dd, J = 6.3, 6.3 Hz, H4”’); 1.58 (m, 2H, H7); 1.22 (s, 6H, 2 x 2”’-CH\textsubscript{3}).

\textsuperscript{13}C NMR (CDCl\textsubscript{3}, 75 MHz): \(\delta\) 172.2, C1; 171.4, C4; 156.4, ArC6”’; 156.3, NCO\textsubscript{2}; 153.5, ArC8a””; 143.7, CN\textsubscript{3}; 143.6, ArC8a” and ArC9a”; 141.0, ArC4a” and ArC4b”; 135.3, ArC7””; 135.1, ArC5””; 134.8, C2’; 128.5, ArC; 128.4, ArC; 128.3, ArC; 128.2, ArC; 127.6, ArCH2” and ArCH7””; 127.0, ArCH3” and ArCH6”; 125.1, ArCH4” and ArCH5””; 124.0, ArC8””; 119.8, ArCH1” and ArCH8”; 119.0, C3’; 117.9, ArC4a’”; 73.5, C2””; 67.0, ArCH\textsubscript{2}; 66.7, C\textsubscript{H2}-C9”; 54.7, C5; 53.8; 53.4, C2; 46.8, C9”; 39.0, C8; 35.7, C1’; 32.6, C4””; 29.8, C6; 26.6, 2”’-CH\textsubscript{3}; 22.4, C7; 21.3, C3””; 18.5, C7”-CH\textsubscript{3}; 17.5, C5”’-CH\textsubscript{3}; 12.0, C8”’-CH\textsubscript{3}. Mass Spectrum (ESI+) \(m/z\) 850 (100%) [MH\textsuperscript{+}]. HRMS calcd for C\textsubscript{47}H\textsubscript{56}N\textsubscript{5}O\textsubscript{8}S 850.3850, found 850.3855.

4.2.15. Benzyl (2S,5R)-2-allyl-5-amino-3-aza-8-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2H-6-chromenylsulfonylamido)guanidino]-4-oxooctanoate 16

The title compound was synthesized using the general N-Fmoc deprotection procedure (Procedure C), from 15 (278 mg, 0.33 mmol) to yield 16 (144 mg, 0.23 mmol, 70%) as a cream semi-solid. Mp 66-68 °C. \(^1\)H NMR (CDCl\textsubscript{3}, 300 MHz): \(\delta\) 7.85 (d, J = 7.8 Hz, 1H, NH); 7.60 (d, J = 7.8 Hz, 1H, NH); 7.32 (m, 5H, ArH); 6.33 (m, 2H, NH\textsubscript{2}); 5.63 (s, 1H, H2’); 5.14 (m, 4H, ArCH\textsubscript{2} and H3’’); 4.56 (m, 1H, H2); 3.40 (m, 1H, H5); 3.16 (m, 2H, H8); 3.09 (m, 2H, H1’); 2.61 (t, J = 6.9 Hz, 2H, H4”’); 2.56 (s, 3H, 7”-CH\textsubscript{3}); 2.55 (s, 3H, 5”-CH\textsubscript{3}); 2.09 (s, 3H, 8”’-CH\textsubscript{3}); 1.78 (t, J = 7.2 Hz, 2H, H3”’); 1.68 (m, 4H, H6 and NH\textsubscript{2}); 1.54 (m, 2H, H7); 1.29 (s, 6H, 2 x 2”’-CH\textsubscript{3}). \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 75 MHz): \(\delta\) 171.4, C1; 171.2, C4; 156.2, ArC6””; 153.4, ArC8a’”; 146.0, CN\textsubscript{3}; 135.2, ArC7’’; 135.1, ArC5’’;
4.2.16. Benzyl (2S,5R,8R)-2-allyl-3,6-diaza-12-(tert-butoxycarboxamido)-8-(9H-9-fluorenylmethoxy-carboxamido)-5-((2,2,5,7,8-pentamethyl-3,4-dihydro-2H-6-chromenylsulfonamido)guanidino)propyl)-4,7-dioxododecanoate 17

The title compound was synthesised using the general peptide coupling procedure (Procedure B), from 16 (200 mg, 0.32 mmol) and Fmoc-D-lysine(Boc)OH (151 mg, 0.32 mmol) to afford 17 (202 mg, 0.19 mmol, 59%) as a white solid. Mp 116 °C. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.72 (d, $J = 7.8$ Hz, 2H, ArH$_1$”” and ArH$_8$””); 7.55 (d, $J = 7.8$ Hz, 2H, ArH$_4$”” and ArH$_5$””); 7.45 (m, 1H, NH); 7.29 (m, 11H, ArH); 6.25 (m, 3H, NH); 5.64 (m, 1H, H$_2'$); 5.03 (m, 4H, ArCH$_2$, H$_3'$); 4.59 (m, 1H, H$_2$); 4.51 (m, 1H, H$_5$); 4.29 (m, 1H, H$_8$); 4.20 (m, 2H, OCH$_2$-$H_9$””); 3.98 (m, 1H, H$_9$””); 3.18 (m, 2H, H$_3$”’); 2.50 (m, 4H, H$_4$”’ and H$_1$’); 2.03 (s, 3H, 8’’’-CH$_3$); 1.95 (m, 4H, H$_1$”” and H$_9$); 1.74 (m, 2H, H$_3$””); 1.67 (m, 4H, H$_2$”” and H$_10$); 1.59 (m, 2H, H$_11$); 1.41 (s, 6H, 2 x 2’’’-CH$_3$). $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 173.0, C$_1$; 171.7, C$_4$; 170.7, C$_7$; 156.8, ArC$_6$””; 156.2, NCO$_2$; 153.5, NCO$_2$; 144.0, CN$_3$; 143.5, ArC$_8$a”” and ArC$_9$a””; 141.1, ArC$_4$a”” and ArC$_4$b””; 135.3, ArC$_7$””; 135.2, ArC$_5$””; 134.8, C$_2$’; 128.4, ArC; 128.2, ArC; 128.1, ArC; 127.5, ArC; 126.9, ArCH$_2$”” and ArCH$_7$””; 125.2, ArCH$_3$”” and ArCH$_6$””; 125.0, ArCH$_4$”” and ArCH$_5$””; 124.0, 134.7, C$_2$’; 128.5, ArC; 128.3, ArC; 128.3, ArC; 128.2, ArC; 123.9, ArC$_8$””; 119.2, C$_3$’; 117.8, ArC$_4$a”; 73.5, C$_2$””; 67.1, ArCH$_2$; 54.2, C$_5$; 53.4, C$_2$; 40.8, C$_8$; 35.9, C$_1$’; 32.7, C$_4$””; 30.8, C$_6$; 29.3, C$_7$; 26.6, 2’’-CH$_3$; 21.3, C$_3$’”; 18.4, C$_7$’’-CH$_3$; 17.4, C$_5$’’-CH$_3$; 12.0, C$_8$’’-CH$_3$. Mass Spectrum (ESI+) m/z 628 (100%) [MH$^+$]. HRMS caleed for C$_{32}$H$_{46}$N$_5$O$_6$S 628.3169, found 628.3157.
4.2.17. Benzyl (2S,5R,8R)-2-allyl-8-amino-3,6-diaza-12-(tert-butoxycarboxamido)-5-((2,2,5,7,8-pentamethyl-3,4-dihydro-2H-6-chromenylsulfonamido)guanidino)propyl)-4,7dioxododecanoate 18

The title compound was synthesized using the general N-Fmoc deprotection procedure (Procedure C), from 17 (202 mg, 0.19 mmol) to yield 18 (157 mg, 0.18 mmol, 93%) as a cream oil. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.00 (d, $J = 7.2$ Hz, 1H, NH); 7.58 (d, $J = 7.2$ Hz, 1H, NH); 7.32 (m, 5H, ArH); 6.44 (m, 3H, NH); 5.63 (m, 1H, H2$'$); 5.09 (m, 4H, ArCH$_2$ and H3$'$); 4.61 (m, 2H, H2 and H5); 3.36 (m, 1H, H8); 3.22 (m, 2H, H3$''$); 3.05 (m, 2H, H1$'$); 2.62 (m, 2H, H4$''$); 2.58 (s, 3H, 7$''$-CH$_3$); 2.56 (s, 3H, 5$''$-CH$_3$); 2.47 (m, 2H, H1$''$); 2.15 (m, 2H, H1$''$); 2.10 (m, 3H, 8$''$-CH$_3$); 1.89 (m, 2H, H9); 1.80 (t, $J = 6.3$ Hz, H3$'''$); 1.72 (m, 4H, H2$''$ and H10); 1.58 (m, 4H, H11 and NH$_2$); 1.42 (s, 9H, C(CH$_3$)$_3$); 1.31 (s, 6H, 2 x 2$''$-CH$_3$). $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 175.7, C1; 171.6, C4; 171.3, C7; 156.2, ArC6$'''$ and NCO$_2$; 153.4, ArC8$''''$; 135.2, ArC7$''''$; 135.1, ArC5$''''$; 133.3, ArC; 132.2, C2$'$; 128.4, ArC; 128.2, ArC; 128.0, ArC; 123.8, ArC8$''''$; 118.9, C3$'$; 117.8, ArC4a$''''$; 78.9, C(CH$_3$)$_3$; 73.5, C2$''$; 66.9, ArCH$_2$; 54.8, C8; 53.3, C2; 51.8, C5;
40.3, C3”; 40.0, C12; 35.9, C1’; 34.5, C2”; 32.6, C4””; 29.6, C9; 28.3, C(CH3)3; 26.6, 2”’-CH3; 25.4, C10; 22.6, C11; 21.3, C4”; 18.4, 7”’-CH3; 17.4, 5”’-CH3; 15.3, C1”; 12.0, 8”’-CH3. Mass Spectrum (ESI+) m/z 856 (100%) [MH+]. HRMS calcd for C43H66N7O9S 856.4643, found 856.4655.

4.2.18. Methyl (2S,5S,8S)-2-allyl-8-(4-[9-anthrecenyl]benzyl)-3,6,9-triaza-5-(4-[tert-butoxycarboxamido]butyl)-4,7,10-trioxoundecanoate 11a

The title compound was synthesised using the general peptide coupling procedure (Procedure B), from 10 (35 mg, 0.098 mmol) and 7a (20 mg, 0.052 mmol) to afford the title compound (22 mg, 0.030 mmol, 59%) as a cream solid. Mp 128 °C. 1H NMR (CDCl3, 300 MHz): δ 8.49 (s, 1H, ArH10”’); 8.04 (d, J = 8.7 Hz, 2H, ArH2”’ and ArH6”’); 7.64 (d, J = 8.4 Hz, 2H, ArH3”’ and ArH5”’); 7.38 (m, 8H, ArH”’); 6.72 (d, J = 7.2 Hz, 1H, NH); 6.48 (d, J = 7.2 Hz, 1H, NH); 6.37 (bs, 1H, NH); 5.59 (m, 1H, H2’); 5.06 (m, 2H, H3’); 4.82 (m, 1H, H8); 4.60 (dd, J = 6.9, 14.1 Hz, 1H, H2); 4.45 (m, 1H, H5); 3.73 (s, 3H, OCH3); 3.24 (m, 2H, ArCH2); 3.08 (m, 2H, H4”); 2.47 (m, 2H, H1”); 2.07 (s, 3H, H11); 1.93 (m, 2H, H1”’); 1.68 (m, 2H, H3”’); 1.50 (m, 2H, H2”’); 1.44 (s, 9H, C(CH3)3). 13C NMR (CDCl3, 75 MHz): δ 171.9, OCH3; 171.3, C4; 171.1, C10; 170.4, C7; 156.2, NCOOC; 137.3, ArC9”; 136.5, ArC4”; 135.7, ArC1”; 131.9, ArC8a”; 131.4, ArC9a”; 131.3, C2’; 130.1, ArCH2”’ and ArCH7”; 129.2, ArCH4”’ and ArCH6”; 129.1, ArCH2”’ and ArCH6”; 128.3, ArCH3”’ and ArCH5”; 126.8, ArCH10”; 126.5, ArCH4a” and ArC10a”; 125.3, ArCH8”’ and ArCH1”’; 123.4, ArCH3”’ and ArCH6”; 119.2, C3’; 79.0, C(CH3)3; 54.4, C8; 52.9, OCH3; 52.4, C2; 51.8, C5; 40.0, C4”; 38.2, ArCH2; 36.1, C1”; 32.2, C1”; 29.7, C3”; 29.3, C2”; 28.4, C(CH3)3; 23.1, C11.
Mass Spectrum (ESI+) m/z 745 (50%) [MNa⁺], 723 (20%) [MH⁺], 623 (100%) [M-Boc].

HRMS calcd for C₄₄H₄₉N₄O₇ 745.3601, found 745.3590.

4.2.19. Methyl (2S,5S,8S)-2-allyl-3,6,9-triaza-5-(4-\{tert-butoxycarboxamido\}butyl)-4,7,10-trioxo-8-(4-[9-phenanthrenyl]benzyl)undecanoate 11b

The title compound was synthesised using the general peptide coupling procedure (Procedure B), from 10 (28 mg, 0.078 mmol) and 7b (15 mg, 0.039 mmol) to afford 11b (14 mg, 0.019 mmol, 50%) as a cream solid. Mp 132-134 °C. ¹H NMR (CDCl₃, 300 MHz): δ 8.76 (d, J = 8.1 Hz, 1H, ArH₄’’’); 8.71 (d, J = 8.4 Hz, 1H, ArH₃’’’); 7.88 (m, 2H, ArH1’’’’ and ArH10’’’’); 7.60 (m, 5H, ArH7’’’, ArH6’’’, ArH5’’’, ArH₂’’’ and ArH1’’’’); 7.45 (d, J = 7.8 Hz, 2H, ArH2’’’’ and ArH6’’’’); 7.33 (d, J = 7.8 Hz, 2H, ArH3’’’’ and ArH5’’’’); 7.10 (d, J = 8.4 Hz, 1H, NH); 6.94 (d, J = 8.7 Hz, 1H, NH); 6.74 (d, J = 8.1 Hz, 1H, NH); 5.61 (m, 1H, H₂’’); 5.06 (m, 2H, H3’’); 4.90 (m, 1H, H8); 4.57 (m, 2H, H2 and H5); 3.72 (s, 3H, OCH₃); 3.20 (m, 2H, ArCH₂); 3.08 (m, 2H, H4’’); 2.47 (m, 2H, H1’’); 2.04 (s, 3H, OCH₃); 1.92 (m, 2H, H1’’); 1.68 (m, 2H, H3’’); 1.48 (m, 2H, H2’’); 1.42 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃, 75 MHz): δ 171.9, OCH₃; 171.4, C4; 171.0, C10; 170.4, C7; 156.1, NOOC; 139.4, ArC4’’’; 138.3, ArC1’’’; 135.6, ArC9’’’; 132.0, ArC4b’’’’; 131.5, ArC8a’’’’; 131.0, ArC4a’’’’; 130.6, ArC10a’’’’; 130.2, ArCH2’’’’ and ArCH6’’’’; 129.9, ArCH3’’’’ and ArCH5’’’’; 129.2, ArCH1’’’’; 128.6, ArCH3’’’’; 127.5, ArCH6’’’’; 126.8, ArCH1’’’’; 126.6, ArCH5’’’’; 126.5, ArCH10’’’’; 122.8, ArCH2’’’’; 122.5, ArCH4’’’’; 119.3, ArCH3’’’’; 79.1, C(CH₃)₃; 54.4, C8; 52.9, OCH₃; 52.4, C2; 51.8, C5; 40.0, C4’’; 38.0, ArCH₂; 36.1, C1’’; 32.1, C1’’; 29.7, C3’’; 29.3, C2’’; 28.4, C(CH₃)₃; 23.1,
C11. Mass Spectrum (ESI+) \( m/z \) 745 (60\%) [MNa\(^{+}\)], 723 (20\%) [MH\(^{+}\)], 623 (100\%) [M-Boc]. HRMS calcd for C\(_{42}\)H\(_{51}\)N\(_{4}\)O\(_{7}\) 723.3758, found 723.3767.

4.2.20. Methyl (2S,5S,8S)-2-allyl-5-(4-aminobutyl)-8-(4-[9-anthrecenyl]benzyl)-3,6,9-triiaza-5-butylamino-4,7,10-trioxoundecanoate hydrochloride 12a

The title compound was synthesized using the general N-Boc deprotection procedure (Procedure A), from 11a (20 mg, 0.028 mmol) to yield 12a (13 mg, 0.017 mmol, 61\%) as a light yellow solid. Mp 194-202 °C. \(^1\)H NMR (CD\(_3\)OD, 300 MHz): δ 8.53 (s, 1H, ArH10\(^{"}\)); 8.26 (m, 3H, exchanging NH’s); 8.06 (d, \( J = 8.1 \) Hz, 2H, ArH2\(^{"}\) and ArH6\(^{"}\)); 7.64 (d, \( J = 9.0 \) Hz, 2H, ArH3\(^{"}\) and ArH5\(^{"}\)); 7.38 (m, 8H, ArH\(^{"}\)); 5.68 (m, 1H, H2\(^{\prime}\)); 5.02 (m, 2H, H3\(^{\prime}\)); 4.67 (m, 1H, H8); 4.45 (m, 2H, H2 and H5); 3.69 (m, 3H, OCH\(_3\)); 2.93 (m, 4H, H4\(^{"}\) and ArCH\(_2\)); 2.44 (m, 2H, H1\(^{\prime}\)); 2.00 (s, 3H, H11); 1.69 (m, 4H, H1\(^{"}\) and H3\(^{"}\)); 1.50 (m, 2H, H2\(^{\prime}\)). \(^13\)C NMR (CD\(_3\)OD, 75 MHz): δ 174.4, C7; 173.7, C1; 173.6, C4; 173.5, C10; 138.7, ArC4\(^{"}\); 137.8, ArC1\(^{"}\); 137.7, ArC9\(^{"}\); 134.1, C2\(^{\prime}\); 132.9, ArCH2\(^{"}\) and ArCH6\(^{"}\); 132.4, ArC4a\(^{"}\) and ArC10a\(^{"}\); 131.5, ArC8a\(^{"}\) and ArC9a\(^{"}\); 130.4, ArCH4\(^{"}\) and ArCH5\(^{"}\); 130.1, ArCH3\(^{"}\) and ArCH5\(^{"}\); 129.5, ArCH10\(^{"}\); 127.7, ArCH8\(^{"}\) and ArCH1\(^{"}\); 126.5, ArCH2\(^{"}\) and ArCH7\(^{"}\); 126.2, ArCH3\(^{"}\) and ArCH6\(^{"}\); 118.8, C3\(^{\prime}\); 56.7, C5; 53.8, OCH\(_3\); 53.6, C8; 52.7, C2; 40.5, C4\(^{\prime}\); 38.6, ArCH\(_2\); 36.6, C1\(^{\prime}\); 32.8, C1\(^{\prime}\); 28.1, C3\(^{\prime}\); 23.4, C11; 22.4, C2\(^{\prime}\). Mass Spectrum (ESI+) \( m/z \) 623 (100\%) [M\(^{+}\)]. HRMS calcd for C\(_{37}\)H\(_{43}\)N\(_{4}\)O\(_{5}\) 623.3233, found 623.3215.

4.2.21. Methyl (2S,5S,8S)-2-allyl-5-(4-aminobutyl)-3,6,9-triiaza-5-butylamino-4,7,10-trioxo-8-(4-[9-[phenanthrenyl]benzyl]undecanoate hydrochloride 12b
The title compound was synthesized using the general N-Boc deprotection procedure (Procedure A), from \textit{11b} (24 mg, 0.033 mmol) to yield \textit{12b} (15 mg, 0.023 mmol, 69%) as a light yellow solid. Mp 198 °C. \textit{1}H NMR (CD$_3$OD, 300 MHz): \(\delta\) 8.84 (d, \(J = 7.8\) Hz, 1H, ArH$^4''''$); 8.78 (d, \(J = 8.1\) Hz, 1H, ArH$^5''''$); 8.30 (d, \(J = 7.2\) Hz, 1H, exchanging NH); 8.15 (d, \(J = 8.1\) Hz, 1H, exchanging NH); 7.90 (m, 2H, ArH$^1''''$ and ArH$^{10''''}$); 7.60 (m, 5H, ArH$^{7''''}$, ArH$^{6''''}$, ArH$^5''''$, ArH$^2''''$ and ArH$^1''''$); 7.45 (d, \(J = 8.4\) Hz, 2H, ArH$^2''''$ and ArH$^6''''$); 7.40 (d, \(J = 8.7\) Hz, 2H, ArH$^3''''$ and ArH$^5''''$); 5.68 (m, 1H, H$^2'$); 4.98 (m, 2H, H$^3'$); 4.61 (m, 1H, H$^8'$); 4.40 (m, 2H, H$^2$ and H$^5$); 3.67 (s, 3H, OCH$_3$); 2.93 (t, \(J = 7.5\) Hz, 2H, H$^4'$); 2.40 (m, 2H, H$^1'$); 1.99 (s, 3H, H$^{11}$); 1.83 (m, 4H, H$^1''$ and ArCH$_2$); 1.69 (m, 2H, H$^3''$); 1.49 (m, 2H, H$^2''$). \textit{13}C NMR (CD$_3$OD, 75 MHz): \(\delta\) 173.7, C7; 173.6, C1; 173.5, C4; 173.4, C10; 140.7, ArC4'''; 139.8, ArC1'''; 137.5, ArC9'''''; 134.0, C2'; 133.0, ArC8a'''''; 132.3, ArC4b'''''; 132.0, ArC4a''''''; 131.3, ArCH2''''' and ArCH6''''''; 131.2, ArC10a''''''; 130.3, ArCH3''''' and ArCH5''''''; 129.7, ArCH1''''''; 128.5, ArCH7''''''; 128.0, ArCH6''''''; 127.9, ArCH1''''''; 127.8, ArCH5''''''; 127.7, ArCH10''''''; 127.6, ArCH2''''''; 124.2, ArC4'''''; 124.1, ArCH3''''''; 118.8, C3'; 56.7, C5; 53.7, OCH$_3$; 53.6, C8; 52.7, C2; 40.5, C4'''; 38.5, ArCH$_2$; 36.5, C1'; 32.8, C1'''; 28.0, C3''''; 23.3, C11; 22.4, C2'''. Mass Spectrum (ESI+) \(m/z\) 623 (100%) [MH$^+$]. HRMS calcd for C$_{37}$H$_{43}$N$_4$O$_5$ 623.3233, found 623.3262.

The title compound was synthesised using the general peptide coupling procedure (Procedure B), from 18 (40 mg, 0.045 mmol) and 7a (17 mg, 0.045 mmol) to afford 19a (20 mg, 0.016 mmol, 36%) as a white solid. Mp 108-110 °C. 1H NMR (CDCl₃, 300 MHz): δ 8.48 (s, 1H, ArH10””’); 8.03 (m, 2H, ArH); 7.58 (m, 2H, ArH); 7.44 (m, 2H, ArH); 7.30 (m, 11H, ArH); 6.82 (bs, 1H, NH); 6.36 (bs, 2H, NH₂); 5.77 (m, 1H, H2’); 5.12 (m, 4H, H3’ and PhCH₂O); 4.85 (m, 1H, H11); 4.59 (m, 1H, H2); 4.44 (m, 1H, H5); 4.31 (m, 1H, H8); 3.19 (m, 2H, 11-CH₂); 2.95 (m, 4H, H4”’ and H3”’); 2.56 (s, 3H, 7””’-CH₃); 2.54 (s, 3H, 5””’-CH₃); 2.52 (m, 4H, H4”” and H1’); 2.06 (s, 3H, 8””-CH₃); 1.97 (m, 2H, H3”’’); 1.94 (s, 3H, H14); 1.74 (m, 4H, H1”’ and H1”’’); 1.71 (m, 2H, H3”’); 1.62 (m, 2H, H2”); 1.38 (m, 2H, H2”’); 1.36 (s, 9H, C(CH₃)₃); 1.23 (s, 6H, 2 x 2””-CH₃). 13C NMR (CDCl₃, 75 MHz): δ 173.0, C13; 172.2, C1; 172.0, C4; 171.8, C7; 170.6, C10; 156.3, ArC6”’; 156.2, NCO₂; 153.6, ArC8a””’; 142.8, CN₃; 140.0, ArC; 139.9, ArC; 136.5, ArC7”’’; 135.4, ArC5”’’; 133.2 ArC; 132.5, C2’; 131.5, ArC; 131.3, 2 x ArCH; 130.1, ArCH; 129.2, ArCH; 128.1, ArCH; 127.9, ArCH; 127.6, ArCH; 127.5, ArCH; 126.6, ArC; 125.3, ArCH; 125.1, ArCH; 124.1, ArC; 123.5, ArC8”’’; 119.0, C3’; 118.0, ArC4a”’; 79.0, C(CH₃)₃; 73.7, C2”’; 67.0, CH₂-ester; 57.7, C11; 54.6, C2; 53.2, C5; 52.3, C8; 40.7, C3”’; 39.8, C4”’’; 37.5, C1’; 36.0, C2’’; 32.7, C4”’; 29.7, C1”’’; 29.3, 11-CH₂; 28.4, C(CH₃)₃; 27.1, C1”’; 26.7, 2””-CH₃; 25.3, C2”’’; 22.9, C14; 22.8, C3”’’; 21.4, C3”; 18.6, 7””-CH₃; 17.5, 5””-CH₃; 12.1, 8””-CH₃. Mass Spectrum (ESI+) m/z 1221 (10%) [MH⁺]; 282 (100%). HRMS calcd for C₆₈H₇₅N₈O₁₁S 1221.6059, found 1221.6089.

4.2.23. Benzyl (2S,5R,8R,11S)-2-allyl-3,6,9,12-tetraaza-8-(4-[tert-butoxycarboxamido]butyl)-5-[[2,2,5,7,8-pentamethyl-3,4-dihydro-2H-6-
The title compound was synthesised using the general peptide coupling procedure (Procedure B), from 18 (38 mg, 0.044 mmol) and 7b (16 mg, 0.042 mmol) to afford 19b (41 mg, 0.034 mmol, 80%) as a white solid. Mp 108 °C. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.72 (m, 2H, ArH); 7.58 (m, 16H, ArH); 6.40 (bs, 2H, NH); 5.71 (m, 1H, H2’); 5.13 (m, 2H, PhCH$_2$O); 5.03 (m, 2H, H3’); 4.83 (m, 1H, H11); 4.60 (m, 1H, H2); 4.59 (m, 1H, H5); 4.29 (m, 1H, H8); 3.12 (m, 2H, 11-CH$_2$); 2.94 (m, 4H, H4”’ and H3”); 2.56 (s, 3H, 7”’-CH$_3$); 2.54 (s, 3H, 5”’-CH$_3$); 2.53 (m, 4H, H4”’ and H1’); 2.07 (s, 3H, 8”’-CH$_3$); 1.91 (s, 3H, H14); 1.82 (m, 4H, H1” and H1’’’); 1.72 (t, $J = 6.6$ Hz, 2H, H3’’’); 1.62 (m, 4H, H2” and H3’’’); 1.39 (m, 2H, H2”’); 1.34 (s, 9H, C(CH$_3$)$_3$); 1.23 (s, 6H, 2 x 2”’-CH$_3$). $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 173.0, C13; 172.4, C1; 172.0, 171.9, C10; C4; 171.7, C7; 156.3, ArC6”’; 156.1, NCO$_2$; 153.6, CN$_3$; 139.3, ArC8a”’; 138.2, ArC; 135.3, ArC and ArC7”’; 134.7, ArC5”’; 133.2, C2’; 132.7, ArC; 132.5, ArC; 131.4, ArC; 130.8, ArC; 130.6, ArCH; 130.2, ArCH; 129.8, ArC; 129.2, ArC; 128.6, ArCH; 128.5, ArCH; 128.3, ArCH; 128.1, ArCH; 127.4, ArCH; 126.8, ArCH; 126.6, 2 x ArCH; 126.4, ArCH; 126.2, ArCH; 124.0, ArCH; 122.9, ArC8”’; 122.4, ArCH; 118.9, C3’; 118.0, ArC4a”’; 77.9, C(CH$_3$)$_3$; 73.6, C2’’’; 66.9, CH$_2$-ester; 55.4, C11; 54.5, C8; 53.2, C5; 52.2, C2; 40.6, C3”; 39.8, C4”’; 37.6, 11-CH$_2$; 36.0, C4”’; 32.6, H1’; 30.6, H1’’; 29.6, C1”’; 29.4, H14; 28.3, C(CH$_3$)$_3$; 26.7, 2”’-CH$_3$; 25.4, C2”’; 22.9, C3”’; 22.8, C2”’; 21.5, 7”’-CH$_3$; 18.6, 5”’-CH$_3$; 17.5, C3”; 12.1, 8”’-CH$_3$. Mass Spectrum (ESI+) $m/z$ 1221 (100%) [MH$^+$]. HRMS caled for C$_{68}$H$_{82}$N$_8$O$_{11}$S 1221.6059, found 1221.6045.
4.2.24. Benzyl (2S,5R,8R,11S)-2-allyl-11-(4-allyloxybenzyl)-3,6,9,12-tetraaza-8-(4-tert-butoxycarboxamido)butyl)-5-[[2,2,5,7,8-pentamethyl-3,4-dihydro-2H-6-chromenylsulfonamido]guanidino]propyl)-4,7,10,13-tetraoxotetradecanoate 19c

The title compound was synthesised using the general peptide coupling procedure (Procedure B), from N-acetyl-O-allyl-L-tyrosine17 (60 mg, 0.069 mmol) and 16 (18 mg, 0.068 mmol) to afford the 19c (65 mg, 0.058 mmol, 85%) as a white solid. Mp 94-102 °C. 1H NMR (CDCl3, 300 MHz): δ 7.76 (bs, 1H, NH); 7.54 (bs, 1H, NH); 7.41 (bs, 1H, NH); 7.31 (m, 5H, ArH); 7.09 (d, J = 8.7 Hz, 2H, ArH2' and ArH6''); 6.77 (d, J = 8.4 Hz, 2H, ArH3' and ArH5''); 6.39 (bs, 3H, 3 x NH); 6.02 (m, 1H, H2''); 5.70 (m, 1H, H2'); 5.39 (dd, J = 1.5, 17.1 Hz, 1H, H3a''); 5.26 (dd, J = 1.2, 10.5 Hz, 1H, H3b''); 5.06 (m, 2H, PhCH2O); 4.65 (dd, J = 6.9, 13.5 Hz, 1H, H11); 4.57 (dd, J = 8.1, 13.5 Hz, 1H, H2); 4.50 (m, 1H, H5); 4.45 (d, J = 5.4 Hz, 2H, H1''); 4.41 (m, 1H, H8); 4.14 (bs, 1H, NH); 3.15 (m, 2H, H3''); 2.92 (m, 4H, H4' and 11-CH2); 2.58 (m, 4H, H1' and H4''); 2.53 (s, 3H, 7''-CH3); 2.52 (s, 3H, 5''-CH3); 2.08 (s, 3H, H14); 1.94 (m, 4H, H1'' and H1'''); 1.84 (s, 3H, 8''-CH3); 1.78 (m, 2H, H3'''); 1.69 (m, 4H, H2'' and H2'''); 1.55 (m, 2H, H3'''); 1.40 (s, 9H, C(CH3)3); 1.30 (s, 6H, 2 x 2'''-CH3). 13C NMR (CDCl3, 75 MHz): δ 172.2, C1; 172.0, C4; 171.6, C7; 157.5, C10; 156.2, C13 and NCO2; 156.1, ArC6’’'; 153.5, ArC8a’’'; 135.3, ArC7’’'; 134.7, ArC5’’'; 133.1, C2’’’; 132.5, C2’; 130.5, ArC4’’’'; 130.2, ArCH2’’’ and ArCH6’’’'; 128.5, ArC1’’’; 128.4, ArCH; 128.3, ArCH; 128.2, ArCH; 128.1, ArC; 124.0, ArC8’’'; 118.8, C3’; 118.0, C3’’’'; 117.6, ArC4a’’'; 114.7, ArCH3’’’' and ArCH5’’’'; 78.9, C(CH3)3; 73.7, C2’; 68.7, C1’’’'; 66.9, ArCH2; 55.6, C11; 54.5, C5; 53.1, C8; 52.2, C2; 41.2, C3’; 40.0, C4’'’; 37.2, 11-CH2; 35.9, C1’; 34.0, C4’’; 32.7, C2’’'; 31.1, C2’; 29.4, C1’’’; 28.4,
C(CH$_3$)$_3$; 26.7, 2$''$-CH$_3$; 22.9, C3$'''$; 22.6, C14; 21.4, C3$''''$; 18.5, 7$''$-CH$_3$; 17.5, 5$''$-CH$_3$; 12.1, 8$''$-CH$_3$. Mass Spectrum (ESI+) $m/z$ 1101 (30%) [MH$^+$]; 288 (100%). HRMS calcd for C$_{57}$H$_{81}$N$_8$O$_{12}$S 1101.5695, found 1101.5731.

4.2.25. Benzyl (2S,5R,8R,11S)-2-allyl-8-(4-aminobutyl)-11-(4-[9-anthracenyl]benzyl)-3,6,9,12-tetraaza-5-(3-guanidinopropyl)-4,7,10,13-tetraoxotetradecanoate 20a

The title compound was synthesized using the general N-Boc deprotection procedure (Procedure A), from 19a (20 mg, 0.016 mmol) to yield 20a (13 mg, 0.014 mmol, 88%) as a white solid. Mp 218-220 °C. $^1$H NMR (CD$_3$OD, 300 MHz): δ 7.68 (m, 17H, ArH); 5.77 (m, 1H, H2$'$); 5.15 (m, 4H, H3$'$ and PhCH$_2$O); 4.82 (m, 1H, H11); 4.42 (m, 1H, H2); 4.25 (m, 1H, H5); 4.07 (m, 1H, H8); 3.18 (m, 2H, 11-CH$_2$); 2.88 (m, 4H, H4$''$ and H3$''$); 2.55 (m, 2H, H1$'$); 1.95 (s, 3H, H14); 1.85 (m, 2H, H1$''$); 1.65 (m, 2H, H1$''''$); 1.53 (m, 2H, H2$''$); 0.94 (m, 2H, H2$''''$). $^{13}$C NMR (CD$_3$OD, 75 MHz): δ 175.2, C13; 174.4, C1; 174.2, C4; 174.1, C10; 172.5, C7; 158.6, CN$_3$; 140.0, ArC; 139.9, ArC; 138.1, ArC; 137.4, ArC; 134.3, C2$'$; 133.2, ArC; 131.5, ArC; 131.3, ArCH; 130.1, ArCH; 129.2, ArC; 128.1, ArC; 127.9, ArCH; 127.6, ArCH; 127.5, ArCH; 126.6, ArCH; 125.9, ArCH; 125.8, ArCH; 125.6, ArCH; 124.2, ArCH; 119.1, C3$'$; 68.1, CH$_2$-ester; 57.9, C11; 55.3, C8; 54.7, C5; 54.2, C2; 42.1, C3$''$; 40.3, C4$''''$; 38.1, 11-CH$_2$; 36.7, C1$'$; 31.4, C1$': 29.4, C1$''$; 27.3, C14; 26.5, C2$''$; 23.6, C3$''''$; 22.5, C2$''''$. Mass Spectrum (ESI+) $m/z$ 855 (50%) [M$^{2+}$]; 428 (100%). HRMS calcd for C$_{49}$H$_{59}$N$_8$O$_6$ 855.4558, found 855.4539.

The title compound was synthesized using the general N-Boc deprotection procedure (Procedure A), from 19b (42 mg, 0.034 mmol) to yield 20b (25 mg, 0.027 mmol, 79%) as a white solid. Mp 215-220 °C. ¹H NMR (CD3OD, 300 MHz): δ 8.82 (m, 2H, ArH); 7.60 (m, 16H, ArH); 5.81 (m, 1H, H2’); 5.15 (m, 4H, PhCH₂O and H3’); 4.58 (m, 1H, H11); 4.43 (m, 1H, H2); 4.35 (dd, J = 4.8, 9.0 Hz, 1H, H5); 4.17 (dd, J = 4.8, 9.6 Hz, 1H, H8); 3.17 (m, 4H, H4” and H3”); 2.72 (m, 2H, 11-ArCH₂); 2.59 (m, 1H, H1’); 1.96 (s, 3H, H14); 1.80 (m, 4H, H1” and H1”’); 1.65 (m, 2H, H3”’); 1.51 (m, 2H, H2”’); 1.22 (m, 2H, H2’’’). ¹³C NMR (CD3OD, 75 MHz): δ 175.2, C13; 174.4, C1; 174.2, C4; 174.1, C10; 172.5, C7; 158.6, CN5; 140.7, ArC; 139.6, ArC; 137.4, ArC; 137.2, ArC; 134.3, C2’; 132.9, ArC; 132.1, ArC; 131.3, ArCH; 130.5, ArCH; 129.7, ArC; 129.6, ArC; 129.4, 2x ArCH; 128.5, ArCH; 128.1, ArCH; 127.9, ArCH; 127.8, ArCH; 127.6, ArCH; 124.2, ArCH; 123.7, ArCH; 12.4, ArCH; 122.1, ArCH; 121.8, ArCH; 119.0, C3’; 68.0, CH₂-ester; 57.7, C11; 55.2, C8; 54.7, C5; 54.0, C2; 42.0, C3”’; 40.1, C4”’; 38.1, 11-CH₂; 36.6, C1’; 31.3, C1”: 29.6, C1”’; 27.8, C14; 26.4, C2”’; 23.8, C3”’; 22.6, C2”’. Mass Spectrum (ESI+) m/z 855 (30%) [M²⁺], 428 (100%). HRMS calcd for C₄₉H₅₀N₆O₆ 855.4558, found 855.4528.

4.2.27. Benzyl (2S,5R,8R,11S)-2-allyl-11-(4-allyloxybenzyl)-8-(4-aminobutyl)-3,6,9,12-tetraaza-5-(3-[guanidino]propyl)-4,7,10,13-tetraoxotetradecanoate hydrochloride 20c

The title compound was synthesized using the general N-Boc deprotection procedure (Procedure A), from 19e (65 mg, 0.059 mmol) to yield 20e (39 mg, 0.048 mmol, 82%) as a cream solid. Mp 108°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.35 (m, 5H, ArH); 7.16 (d, J = 8.7 Hz, 2H, ArH2”’ and ArH6”’); 6.87 (d, J = 8.7 Hz, 2H, ArH3”’ and ArH5”’); 6.02 (m, 1H, H2”’); 5.78 (m, 1H, H2’); 5.39 (dd, J = 1.8, 17.1 Hz, 1H, H3a”’’); 5.24 (dd, J = 1.8,
10.5 Hz, 1H, H3′′); 5.10 (m, 4H, H3′ and PhCH2O); 4.52 (m, 2H, H1′′′′); 4.39 (m, 2H, H13 and H2); 4.24 (dd, J = 4.8, 9.0 Hz, 1H, H5); 3.98 (dd, J = 3.9, 9.9 Hz, 1H, H8); 3.16 (m, 2H, H3′′); 2.94 (m, 2H, 11-CH2); 2.84 (m, 2H, H4′′); 2.55 (m, 2H, H1′); 1.94 (s, 3H, H14); 1.87 (m, 2H, H1′′); 1.73 (m, 2H, H1′′′); 1.54 (m, 4H, H2′ and H2′′); 1.03 (m, 2H, H3′′′). 13C NMR (CDCl3, 75 MHz): δ 175.4, C1; 174.4, C4; 174.2, C7; 172.5, C10; 159.0, C13; 158.5, NCO; 137.2, ArC4′′′; 134.9, C2′′′′; 134.3, C2′; 131.5, ArC; 130.0, ArCH2′′′′ and ArCH6′′′′; 129.6, ArCH; 129.4, ArCH; 129.4, ArCH; 128.5, ArCl′′′; 119.0, C3′; 117.6, C3′′′′′′; 115.9, ArCH3′′′′ and ArCH5′′′′; 69.8, C1′′′′′′; 67.9, CH2-ester); 57.8, C11; 55.3, C5; 54.8, C8; 54.0, C2; 41.9, C3′; 40.3, C4′′′; 37.4, 11-CH2; 36.5, C1′; 31.2, C1′′′′; 29.5, C2′′′; 28.0, C2′′′′; 26.5, C14; 23.8, C3′′′′; 22.5, C1′′. Mass Spectrum (ESI+) m/z 735 [M+2] (70%), 368 (100%). HRMS calcd for C38H55N8O7 735.4194, found 735.4200.

4.3. In vitro antimicrobial activity

Antibacterial testing against *Staphylococcus aureus* ATCC6538P was performed at Avexa Corporation, 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 μL of liquid medium [Mueller-Hinton broth medium (MHB) and Mueller-Hinton agar medium (MHA)]. The peptoid compounds were dissolved in a 50% MeOH/H2O solution for the final concentration of 1 mg/mL. Test solution (50 μL) 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.
Acknowledgments: We thank Amrad and Avexa Limited, the University of Wollongong, the Australian Research Council (PhD scholarship to TPB), and the National Health and Medical Research Council (Development Grant 404528) for their support, and Drs Susan Cox and David Rhodes for their support in the initial development of this project.

References


Synthesis of novel N-protected hydrophobic phenylalanines and their application in potential antibacterials

Timothy P. Boyle, John B. Bremner, Jonathan A. Coates, John Deadman, Paul A. Keller,

Stephen G. Pyne and Kittiya Somphol