Organic & Biomolecular Chemistry

ARTICLE

Synthesis and Evaluation of Analogues of the Glycinocin Family of Calcium-Dependent Antibiotics

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Leo Corcilius,[a] Dennis Y. Liu,[b] Jessica L. Ochoa,[c] Roger G. Linington,[b,c] and Richard J. Payne[a]*

The glycinocins are a class of calcium-dependent, acidic cyclolipopeptide antibiotics that are structurally related to the clinically approved antibiotic daptomycin. In this article, we describe the synthesis of a small library of glycinocin analogues that differ by variation in the exocyclic fatty acyl substituent. The glycinocin analogues were screened against a panel of Gram-positive bacteria (as well as Gram-negative *P. aeruginosa*). These analogues exhibited similar calcium-dependent activity to the parent natural products against Gram positive bacteria but showed no activity against *P. aeruginosa*. The length of the fatty acid was shown to be important for optimal biological activity, while the hybridisation at the α,β position and branching within the fatty acyl chain had only subtle effects on activity.

Introduction

The glycinocins (also called the laspartomycins) are members of a family of calcium-dependent antibiotics (CDAs), which includes daptomycin, the amphomycins and the fruilimicins.[1-3] CDAs are assembled by non-ribosomal peptide synthases within various Actinobacteria and are characterised by number of common structural features, namely, (1) a 10-amino acid cyclic (depsi)peptide core encompassing multiple non-proteinogenic amino acids, (2) an acidic Ca^{2+}-binding domain (Asp-X-Asp-Gly), and (3) an exocyclic portion bearing an *N*-terminal fatty acyl side chain.[1-3] Many of these CDAs exhibit antimicrobial activity against Gram-positive bacteria including drug-sensitive and drug-resistant *Staphylococcus aureus* strains, and despite having been known for several decades, have only gained traction as suitable clinical candidates over the past 20 years, culminating in the clinical approval of daptomycin (under the trade name Cubicin®). Daptomycin was approved in 2003 for the treatment of skin and skin structure infections, *S. aureus* endocarditis, and bacteraemia caused by vancomycin-resistant enterococci (VRE) and methicillin- and vancomycin-resistant *S. aureus* strains (MRSA and VRSA).[4] The recent emergence of clinical resistance to Daptomycin[5] has prompted the investigation of other members of this natural products family, especially those that may operate *via* a distinct mechanism of action.

Glycinocin A (**1**), originally named laspartomycin C, was reported in 1967, having been isolated from *S. viridochromogenes*.[6, 7] Decades later, it was re-isolated from an unidentified terrestrial Actinomycete species and the structure fully determined, along with three congeners, glycinocins B-D.[8] A later report established the common identity of glycinocin A (**1**) and the initially isolated laspartomycin C.[9] The structure of glycinocins A-D (**1**-**4**) (Figure 1) consists of a cyclodecapeptide core containing the Ca^{2+} binding motif Asp-Gly-Asp-Gly and the non-proteinogenic amino acids, D-*Allo*-Thr, D-pipecolic acid (D-Pip) and L-diaminopropionic acid (L-Dap). The L-Dap residue is bound to the C-terminal L-Pro through the side chain amino functionality *via* an amide bond, and is additionally bound to an exocyclic L-Asp residue with an α,β unsaturated and terminal branched fatty acyl group. Glycinocins A-C differ only in the length of the fatty acyl portion, whereas glycinocin D differs by substitution of the endocyclic L-Ile residue for L-Val.

[a] *School of Chemistry, The University of Sydney, Sydney, NSW 2006, Australia*
[b] *Department of Chemistry, Simon Fraser University, Burnaby, British Columbia BC V5A 1S6, Canada*
[c] *Department of Chemistry and Biochemistry, University of California, Santa Cruz, 1156 High Street, Santa Cruz, California 95064, United States*

† Electronic Supplementary Information (ESI) available: NMR Spectra of fatty acids and glycinocin analogues, tabulated ^{1}H and ^{13}C NMR shifts of analogues derived from HSQC and HMBC, analogue screening data. See DOI: 10.1039/x0xx00000x

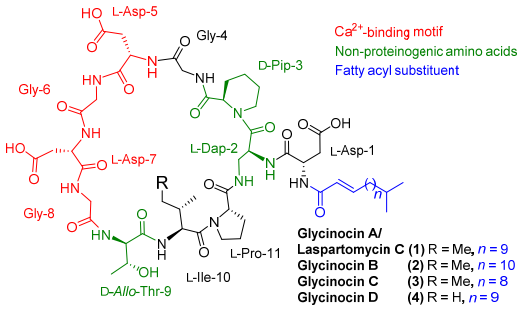


Figure 1. Glycinocins A-D with key structural features highlighted in red (Ca^{2+}-binding motif), green (non-proteinogenic amino acids) and blue (fatty acyl moiety).

Despite their structural similarity to daptomycin, the glycinocins (as well as the amphomycins and the fruilimicins) have been shown to operate *via* a distinct mechanism of action. Rather than directly associating with the cell membrane, they appear to exert their antimicrobial activity *via* sequestration of the cell membrane precursor undecaprenyl phosphate ($C_{55}P$),[10, 11] and may therefore be able to circumvent conventional resistance mechanisms. Indeed, it has been reported that it has not been possible to elicit resistance in a lab setting against recently-discovered antibiotics which target the downstream Lipid II cell membrane precursor.[12, 13]

The total synthesis of glycinocin A/laspartomycin C has been reported by Kleijn et. al., who also showed it to be a tight binder of undecaprenyl phosphate in the presence of Ca^{2+}, with a K_d of 7.3 ± 3.8 nM.[11] In a follow-up publication, the same authors a reported a co-crystal structure of glycinocin A with Ca^{2+} and model substrate geranyl phosphate ($C_{10}P$, stoichiometry = 1:2:1), which provided a rationale for this tight binding.[14] In this dimeric structure, the cyclic L-Pro and D-Pip residues force the ring to adopt a saddle-like conformation around the $C_{10}P$ phosphate head group. The phosphate is co-ordinatively saturated by H-bonding to the peptide backbone and by co-ordination to the two Ca^{2+} ions. The two Ca^{2+} ions are also co-ordinatively saturated by several carbonyls of the peptide backbone, two molecules of H_2O, the two carboxylates of the aforementioned Ca^{2+}-binding domain and the exocyclic carboxylate. Importantly, this interaction was also stabilised by hydrophobic interactions between the fatty acyl group and the polyprenyl chain of the $C_{10}P$. The crystal structure provides a rationale for the inactivity of previous semisynthetic glycinocin analogues which lack either the exocyclic L-Asp residue or contain exocyclic peptidic insertions.[15]

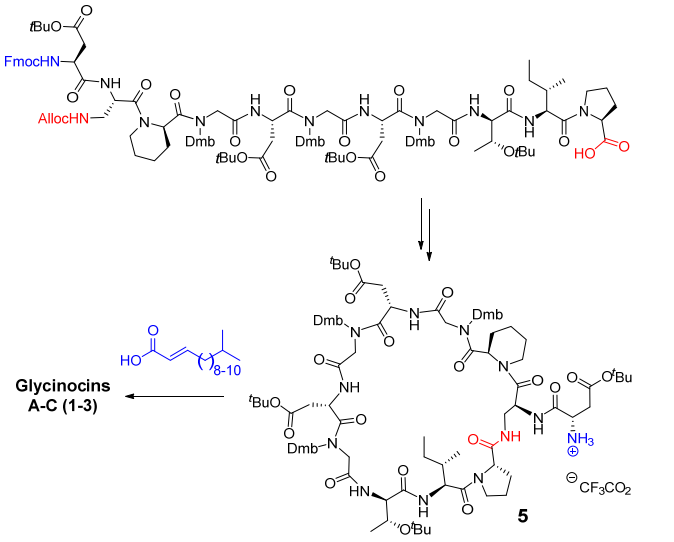


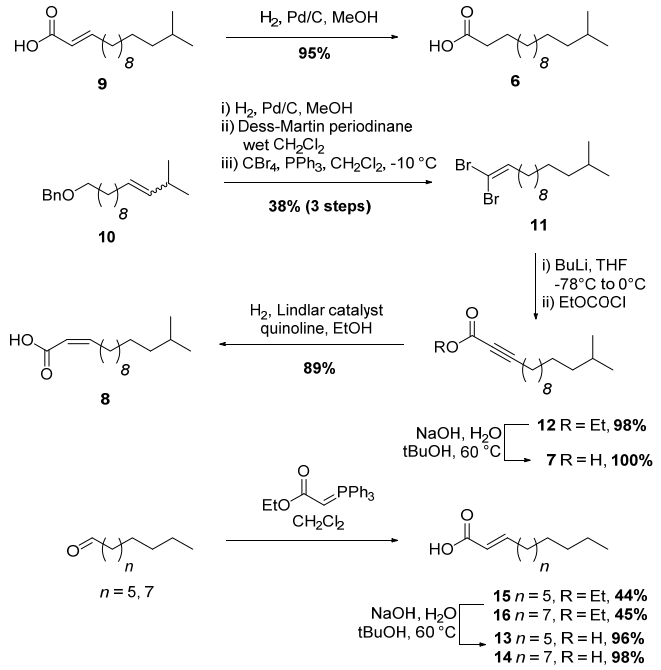
Figure 2. Previous total synthesis of glycinocins A-C (**1-3**).

We also recently reported a total synthesis of glycinocin A along with glycinocins B and C *via* a divergent strategy allowing for late stage installation of the fatty acyl side chain (Figure 2).[16] In this strategy, the side chain-protected cyclic peptide precursor **5**, bearing a free amine, was acylated with the corresponding glycinocin A-C fatty acids, followed by acidolytic deprotection of the side chain protecting groups to afford the natural products. Herein, we report the utilisation of our efficient, late stage synthetic strategy to the natural products for the generation of a library of glycinocin analogues bearing the glycinocin A-C core and exocyclic L-Asp residue, but with variation of the fatty acyl substituent. Specifically, we sought to evaluate the extent to which both the length, hybridisation, α,β-stereochemistry and substitution affected the antimicrobial activity of the natural product.

Results and Discussion

In order to determine the importance of hybridisation and stereochemistry at the fatty acyl α,β positions, we prepared fatty acids **6**-**8**, corresponding to the dihydro (**6**), didehydro (**7**) and (*Z*)-configured (**8**) variants of the glycinocin B fatty acyl side chain (Scheme 1). Saturated fatty acid **6** was prepared through hydrogenation of the previously prepared glycinocin B precursor fatty acid **9**.[16] Likewise, the alkyne and (*Z*)-olefin variants **7** and **8** were prepared from benzyl ether intermediate **10**.[16] First, hydrogenation of the alkene and concomitant hydrogenolysis of the benzyl ether afforded the alcohol, which was oxidised to the aldehyde using Dess-Martin periodinane. The aldehyde was then homologated under Corey-Fuchs conditions to give the *gem*-dibromoalkene **11**. Subsequent lithiation and elimination was followed by trapping of the intermediate alkynyllithium with ethyl chloroformate to form ethyl alkynoate derivative **12** in excellent yield. Hydrolysis of the ethyl ester, then afforded

alkynoic acid **7**, which was subsequently partially hydrogenated to the (*Z*)-alkenoic acid **8** with Lindlar catalyst.



Scheme 1. Synthesis of fatty acids.

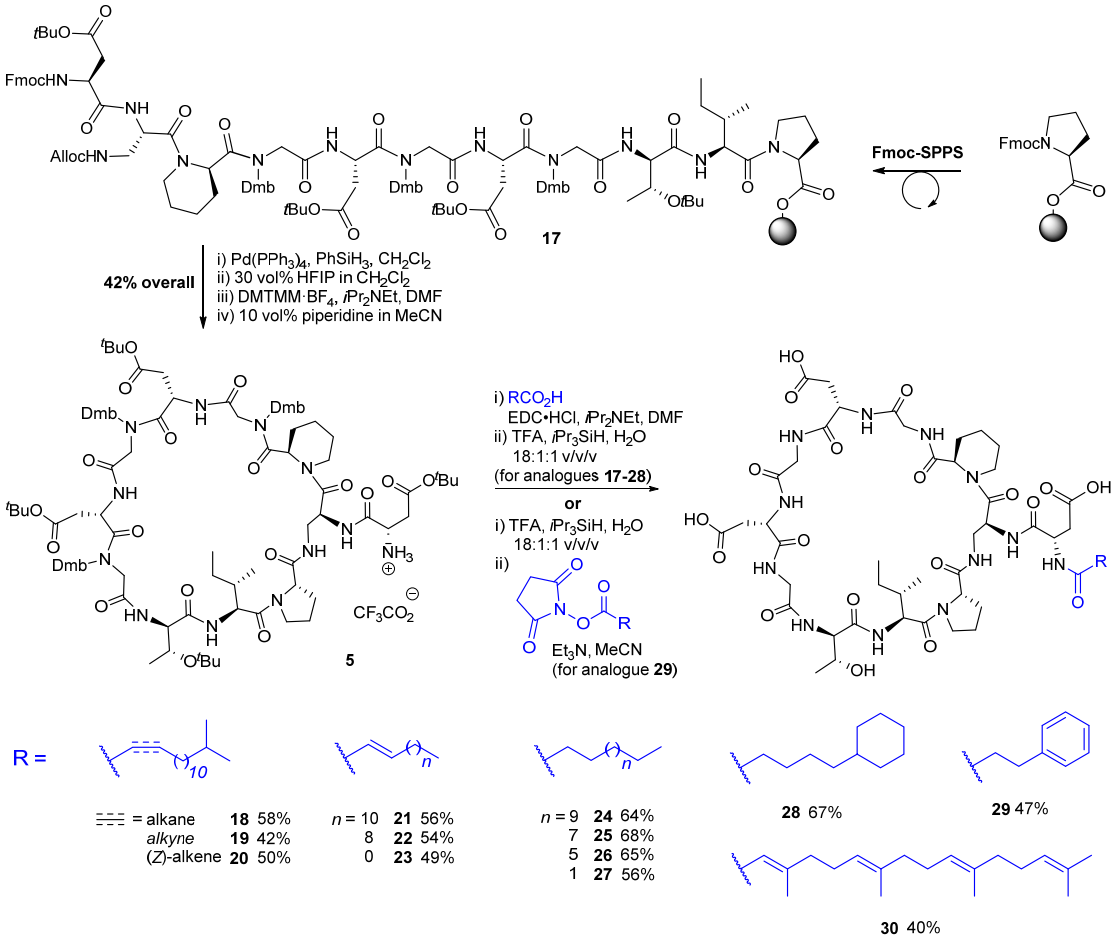
Finally, two (*E*)-α,β-unsaturated fatty acids **13** and **14** were prepared with a view to installing these into the natural product scaffold to assess the influence of the terminal methyl groups on antimicrobial activity. The fatty acid **14** corresponds to the 13-demethyl variant of the glycinocin A fatty acyl side chain and **13** corresponds to the 12,12-didemethyl variant of the glycinocin C fatty acyl side chain. Both fatty acids **13** and **14** were prepared through Wittig olefination of the corresponding aldehydes and subsequent hydrolysis of the resulting ethyl esters **15** and **16**.

Next, we turned our attention to the assembly of the target glycinocin analogues, beginning with the synthesis of the common side chain- and backbone-protected cyclic peptide **5** as previously described (Scheme 2).[16] Briefly, the protected linear peptide precursor **17** was prepared through iterative solid-phase peptide synthesis (SPPS) on 2-chlorotrityl chloride-functionalised polystyrene resin. This was then Alloc deprotected, cleaved from the resin using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), and cyclised under the promotion of 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM.BF_4). After Fmoc-deprotection and purification by reverse phase HPLC, the desired protected cyclic peptide **5** was obtained in 42% overall yield.

From here, the synthesis of glycinocin analogues **18**-**22** was carried out by acylation of cyclic peptide **5** with fatty acids **6**-**8**, **13** and **14** in DMF using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) as a coupling reagent in the presence of *i*Pr_2NEt, followed by global acidolytic deprotection with TFA/iPr_3SiH/H_2O 18:1:1 v/v/v. After purification by reversed-phase HPLC, the analogues **18**-**22** were obtained in 42-68% yield over 2 steps. Crotonic analogue **23**, and saturated analogues **24**-**29**, were prepared under identical conditions (using commercially available crotic acid and fatty acids, respectively) in 47-68% yield. The geranylgeranoyl analogue **30** was also prepared with the hope that the polyprenyl architecture would enhance hydrophobic interactions with the similarly structured undecaprenyl phosphate substrate. Mindful of the acid sensitivity of the polyprenyl motif, the geranylgeranoyl derivative was prepared through an alternative strategy involving acidolytic deprotection, followed by selective acylation of the amine with geranylgeranic *N*-hydroxysuccinimide ester in MeCN (see ESI for synthetic details). After purification by reversed-phase HPLC, geranylgeranoyl derivative **30** was isolated in 40% yield over 2 steps.

Organic & Biomolecular Chemistry

ARTICLE



Scheme 2. Synthesis of target glycinocin analogues **18**-**30**.

The antimicrobial activity of analogues **18**-**30** was next assessed against Gram-positive *Bacillus subtilis* (ATCC 23857), methicillin susceptible *Staphylococcus aureus* (MSSA) (ATCC 29213), as well as Gram-negative *Pseudomonas aeruginosa* (ATCC 27853) using daptomycin, rifampicin, vancomycin, gentamicin and synthetic glycinocins A-C as internal controls. Selected analogues were also screened against *Enterococcus faecium* (ATCC 6569). Antimicrobial activities were evaluated using a high throughput screening assay with cation adjusted Mueller-Hinton broth (MHB), containing either 0, 50, 100 and 200 mg/L Ca^{2+} (see Supplementary Information for details). Unsurprisingly, none of the compounds exhibited antimicrobial activity against Gram-negative *P. aeruginosa* or Gram-positive bacteria in the absence of Ca^{2+}. However, several compounds possessed significant activity in the presence of 50 mg/L Ca^{2+} (physiological calcium ion concentration) and potency further increased with increasing Ca^{2+} concentration (see Supplementary Information). The most notable structure-activity trend was a decrease in activity with decreasing chain length (Figure 3), with a very sharp decrease in potency when the acyl chain in a given analogue possessed 12 or less carbon atoms. This observation is in line with the previous finding that the decanoyl derivative (**26**) is inactive against *S. aureus*.[15] The dihydro analogue (**18**) of glycinocin B (**2**) was equipotent to the natural product, however a moderate drop in activity was noted for the didehydro (alkyne) and (*Z*)-configured analogues (**19** and **20**). The presence of terminal branching was also largely inconsequential (analogues **21** and **24**), except in cases where the chain length dropped below 13 carbon atoms. The geranylgeranoyl derivative (**30**), designed to bolster the hydrophobic interactions with the $C_{55}P$ substrate, led to a drop in antimicrobial activity, suggesting that increased chain branching does not significantly influence binding to the substrate.

Organic & Biomolecular Chemistry

ARTICLE

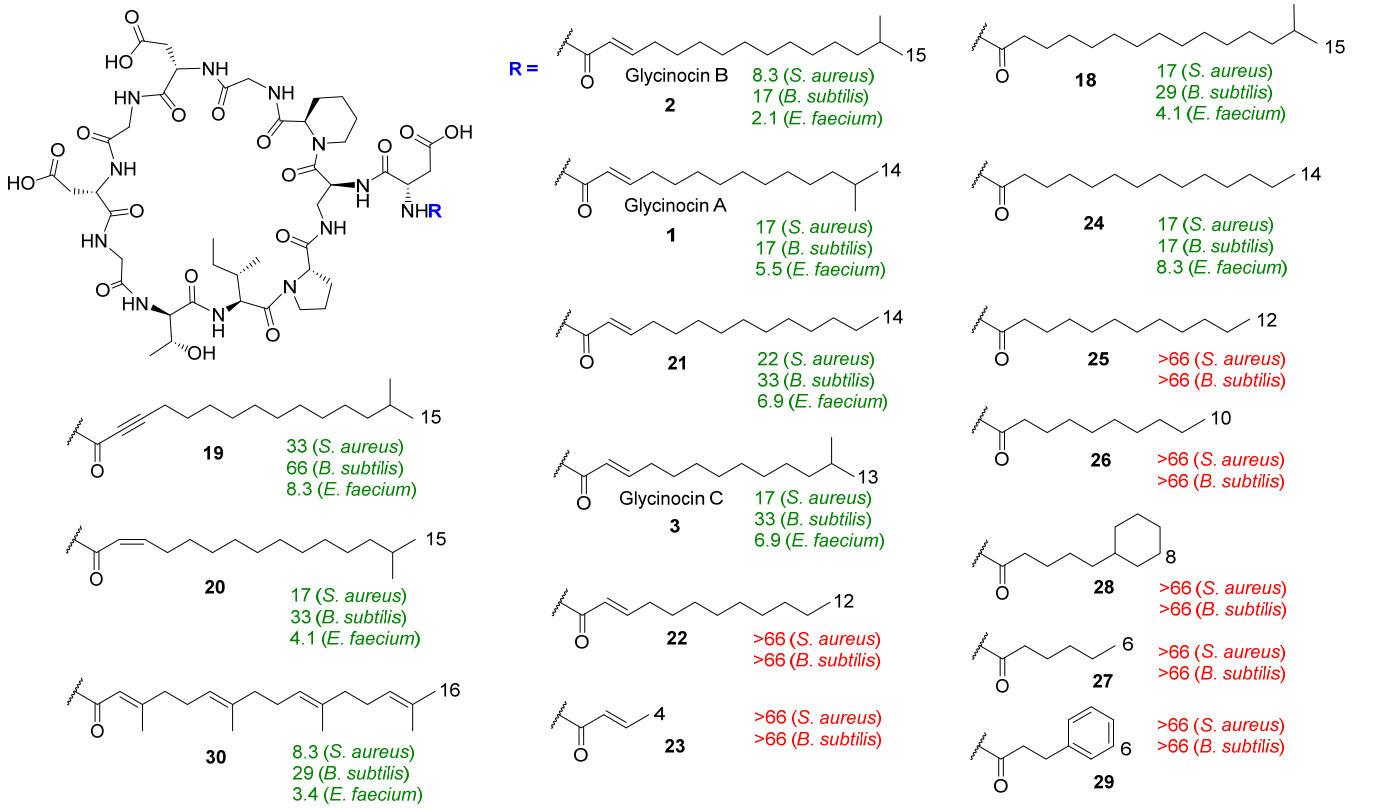


Figure 3. Antimicrobial activity of glycinocin analogues at 50 mg/L Ca^{2+} (MIC in μM).

Conclusions

In summary, a small library of glycinocin analogues was designed that differed in the composition of the fatty acyl side chain in the natural products. The natural product analogues were rapidly synthesised by late stage acylation of a common cyclic peptide fragment, generated by solid-phase synthesis, with a range of fatty acids. The chain length of the analogues was found to be the most important contributor to activity, with a sharp decrease in antimicrobial potency when acyl chains less than 12 carbon atoms in length were installed. Fully saturated derivatives were almost identical in activity to the parent natural products, including in the Ca^{2+} dependency against Gram positive organisms, however isomerisation of the alkene, or further unsaturation to the alkyne caused moderate decreases in activity. This work lays the foundation for the rapid generation of further analogues with the view to developing more potent antimicrobials based on this family of natural products.

Experimental

General Procedures

Commercial materials, including solvents were used as received unless otherwise noted. Anhydrous solvents were obtained from a PURE SOLV™ solvent dispensing unit. Dry reactions were carried out under an atmosphere of dry nitrogen or argon.

Flash column chromatography was performed using 230–400 mesh Kieselgel 60 silica eluting with gradients as specified. Analytical thin layer chromatography (TLC) was performed on commercially prepared silica plates (Merck Kieselgel 60 0.25 mm F254).

^{1}H NMR, ^{13}C NMR and 2D NMR spectra were recorded at 300 K using a Bruker AVANCE600, DRX500, DRX400 or AVANCE300 spectrometer. Chemical shifts are reported in parts per million (ppm) and are referenced to solvent residual signals: chloroform-*d* δ 7.26 [^{1}H], and δ 77.16 [^{13}C], DMSO-d_6 δ 2.50 [^{1}H] and δ 39.52 [^{13}C] and methanol-d_4 δ 3.31 [^{1}H] and 49.0 [^{13}C] ppm. ^{1}H NMR data is reported as chemical shift,

multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublet of doublets, m = multiplet, br = broad), coupling constant (*J* Hz) and assignment where possible.

High resolution mass spectra were measured on a Thermo Velos Pro Orbitrap in Electrospray ionisation mode with syringe infusion using the inbuilt syringe pump. Low resolution ESI mass spectra were obtained on a Shimadzu 2020 ESI mass spectrometer operating in positive ion mode. Infrared (IR) absorption spectra were recorded on a Bruker ALPHA Spectrometer with Attenuated Total Reflection (ATR) capability. Compounds were deposited as films on the ATR plate *via* a CH_2Cl_2 solution.

Preparative reverse-phase HPLC was performed using a Waters 600 Multisolvent Delivery System and pump with Waters 486 Tuneable absorbance detector operating at 214 nm. Analytical reverse-phase UPLC was performed on a Waters Acquity UPLC system equipped with a PDA eλ detector (λ = 214 nm), using a Waters Acquity UPLC BEH C18 1.7 µm 2.1 × 50 mm column at a flow rate of 0.6 mL/min.

14-methylpentadecanoic acid (6): A solution of (*E*)-14-methylpentadec-2-enoic acid (**6**)[16] (11.5 mg, 0.0452 mmol) in MeOH (0.28 mL) with 10 wt% Pd/C (11 mg) was stirred under an atmosphere of H_2 (1 atm) for 3 h. The vessel was evacuated and flushed with N_2 before filtration through celite®. The filtrate was evaporated to dryness, yielding 14-methylpentadecanoic acid (**6**) (11.0 mg, 95%) as a white solid.

IR (thin film) ν_{max} = 2950, 2917, 2850, 1698, 1472, 1429, 1409, 1314, 1296, 1276, 1253, 1229, 1207, 1187, 917 cm^{-1}; **^{1}H NMR** (500 MHz, Methanol-d_4) δ 2.26 (t, *J* = 7.4 Hz, 2H, CH_2), 1.60 (m, 2H, CH_2), 1.53 (m, 1H, CH), 1.35-1.28 (m, 16H, 8 x CH_2), 1.18 (m, 2H, CH_2), 0.88 (d, *J* = 6.6 Hz, 6H, 2 x CH_3) ppm; **^{13}C NMR** (126 MHz, Methanol-d_4) δ 178.5 (C), 40.3 (CH_2), 35.6 (CH_2), 31.0 (CH_2), 30.8 (2 x CH_2), 30.8 (CH_2), 30.7 (CH_2), 30.6 (CH_2), 30.5 (CH_2), 30.3 (CH_2), 29.2 (CH), 28.5 (CH_2), 26.3 (CH_2), 23.0 (2 x CH_3) ppm. **HRMS:** (ESI-) Calcd for $C_{16}H_{31}O_2$ $[M-H]^-$, 255.2330, Found, 255.2331.

1,1-dibromo-13-methyltetradec-1-ene (11): A mixture of alkene **10**[16] (420 mg, 1.39 mmol) and 10 wt% Pd/C (420 mg) in methanol was stirred under a H_2 atmosphere at r.t. for 3 h. The reaction mixture was then filtered over celite, and the resulting filtrate was concentrated. A solution of the residue and Dess-Martin periodinane (883 mg, 2.08 mmol) in wet CH_2Cl_2 was stirred at r.t. for 1 h. Upon completion, saturated aqueous sodium hydrogen carbonate and sodium thiosulfate (1:1 v/v) were added and the mixture stirred for 20-30 min, until the lower organic layer was observed to transform from a white suspension to a colourless solution. The immiscible mixture was then separated and the aqueous layer was further extracted into CH_2Cl_2 (x3). The combined CH_2Cl_2 extracts were finally dried over anhydrous magnesium sulfate, and concentrated to yield the crude aldehyde (266 mg, 90%), which was used in the next step without further purification. To a solution of CBr_4 in CH_2Cl_2 (3 mL) at -10 °C was added PPh_3 (419 mg, 1.60 mmol) in portions. The temperature of the reaction was maintained at below -5°C during the addition. After 30 min at -10 to -5 °C, a solution of the above aldehyde (130 mg, 0.612 mmol) in CH_2Cl_2 (3 mL) was then added dropwise. The reaction was stirred for 3 h at -10 to -5 °C. After removal of the solvent, the residue was suspended in hexane and loaded onto a silica gel flash column. Elution with hexane (neat) afforded dibromoalkene **11** (94 mg, 42% [38% over 3 steps]) as a colourless oil.

IR (thin film) ν_{max} = 2951, 2922, 2852, 1625, 1465, 1383, 1366, 826, 801, 782, 721 cm^{-1}; **^{1}H NMR** ^{1}H NMR (300 MHz, Chloroform-*d*) δ 6.39 (t, *J* = 7.2 Hz, 1H, $CHCBr_2$), 2.09 (dt, *J* = 7.2, 7.2 Hz, 2H, CH_2), 1.53 (m, 1H, CH), 1.42 (m, 2H, CH_2), 1.33-1.22 (m, 16H, 8 x CH_2), 1.15 (m, 2H, CH_2), 0.87 (d, *J* = 6.6 Hz, 6H, 2 x CH_2) ppm; **^{13}C NMR** (75 MHz, Chloroform-*d*) δ 139.1 (*C*$HCBr_2$), 88.6 (CBr_2), 39.2 (CH_2), 33.2 (CH_2), 30.1 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.2 (CH_2), 28.1 (CH), 28.0 (CH_2), 27.6 (CH_2), 22.8 (2 x CH_3) ppm.

Ethyl 14-methylpentadec-2-ynoate (12): To a solution of dibromoalkene **11** (94 mg, 0.255 mmol) in THF (1.3 mL) at -78 °C was added *n*-BuLi (2.5 M, 204 µL, 0.511 mmol). The mixture was stirred at -78 °C for 1 h and then for 1 h at 0 °C. The reaction mixture was again cooled to -78 °C and ethyl chloroformate (61 µL, 0.638 mmol) was added dropwise. The reaction mixture was stirred for 1 h at -78 °C and then warmed gradually to rt and stirred for a further 30 min. The reaction was quenched with saturated aqueous $NaHCO_3$ at 0 °C and extracted with Et_2O (3 x 5 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to dryness. The residue was purified by silica gel flash chromatography (0 to 10 vol% EtOAc in hexane), affording alkynoate ester **12** (70 mg, 98%) as a colourless oil.

IR (thin film) ν_{max} = 2924, 2854, 2234, 1711, 1465, 1366, 1244, 1072, 752 cm^{-1}; **^{1}H NMR** (300 MHz, Chloroform-*d*) δ 4.21 (q, *J* = 7.1 Hz, 2H, CH_3CH_2), 2.32 (t, *J* = 7.1 Hz, 2H, $CCCH_2$), 1.62 – 1.47 (m, 3H, CH_2, CH), 1.38 (m, 2H, CH_2), 1.33 – 1.24 (m, 15H, 6 x CH_2, CH_3), 1.15 (m, *J* = 6.2 Hz, 2H, CH_2), 0.86 (d, *J* = 6.6 Hz, 6H, 2 x CH_3) ppm; **^{13}C NMR** (75 MHz, Chloroform-*d*) δ 154.0 (C=O), 89.6 ($CCCH_2$), 73.3 ($CCCH_2$), 61.9 (CH_2), 39.2 (CH_2), 30.1 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 28.1 (CH), 27.7 (CH_2), 27.5 (CH_2), 22.8 (2 x CH_3), 18.8 (CH_2), 14.2 (CH_3) ppm; **HRMS:** (ESI+) Calcd for $C_{18}H_{33}O_2$: $[M+H]^+$, 281.2475, Found, 281.2471.

14-methylpentadec-2-ynoic acid (7): A solution of alkynoate ester **12** (65 mg, 0.177 mmol) in *t*BuOH (1 mL) and 1 M aqueous NaOH (1 mL) was heated at 60 °C for 6 h. The mixture was cooled to rt and acidified with 0.5 M aqueous HCl before being extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to dryness affording alkynoic acid **7** (45 mg, 100%) as a white solid.

IR (thin film) ν_{max} = 2923, 2853, 2238, 1684, 1466, 1410, 1277 cm^{-1}; **^{1}H NMR** (400 MHz, Methanol-d_4) δ 2.35 (t, *J* = 7.1 Hz, 2H, $CCCH_2$), 1.61 – 1.48 (m, 3H, CH, CH_2), 1.43 (m, 2H, CH_2), 1.35 – 1.27 (m, 12H, 6 x CH_2), 1.18 (m, 2H, CH_2), 0.88 (d, *J* = 6.6 Hz, 6H, 2 x CH_3) ppm; **^{13}C NMR** (101 MHz, Methanol-d_4) δ 156.7 (C=O), 90.1 ($CCCH_2$), 74.5 ($CCCH_2$), 40.2 (CH_2), 31.0 (CH_2), 30.8

(CH_2), 30.7 (CH_2), 30.6 (CH_2), 30.1 (CH_2), 29.9 (CH_2), 29.1 (CH), 28.8 (CH_2), 28.5 (CH_2), 23.0 (2 x CH_3), 19.1 (CH_2) ppm. **HRMS:** (ESI-) Calcd for $C_{16}H_{27}O_2$: $[M-H]^-$, 251.2017, Found, 251.2015.

(*E*)-14-methylpentadec-2-enoic acid (8): To a solution of alkynoic acid **7** (23.0 mg, 0.0895 mmol) in ethanol was added Lindlar catalyst (9 mg), followed by quinoline (1.1 µL, 9.0 µmol). The solution was placed under an atmosphere of H_2 (1 atm) for 24 h. After this time, the vessel was evacuated and flushed with N_2. The mixture was filtered through celite® and evaporated to dryness. The residue was partitioned between EtOAc (10 mL) and 0.5 M aqueous HCl (10 mL). The resulting organic layer was washed once more with 0.5 M aqueous HCl and then dried over $MgSO_4$, filtered and evaporated to dryness yielding alkynoic acid **8** as a white solid.
IR (thin film) ν_{max} = 2953, 2923, 2853, 1695, 1639, 1465, 1435, 1293, 1238, 930, 825, 721 cm^{-1}; **^{1}H NMR** (300 MHz, Chloroform-*d*) δ 6.35 (dt, *J* = 11.5, 7.5 Hz, 1H, HO_2CCHCH), 5.78 (dt, *J* = 11.5, 1.8 Hz, 1H, HO_2CCHCH), 2.69 – 2.61 (m, 2H, CH_2), 1.58 – 1.40 (m, 3H, CH, CH_2), 1.35 – 1.24 (m, 14H, 7 x CH_2), 1.18 – 1.11 (m, 2H, CH_2), 0.86 (d, *J* = 6.6 Hz, 6H, 2 x CH_3) ppm. **^{13}C NMR** (75 MHz, Chloroform-*d*) δ 171.8 (C), 153.7 (CH), 119.1 (CH), 39.2 (CH_2), 30.1 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.4 (CH_2), 29.1 (CH_2), 28.1 (CH), 27.6 (CH_2), 22.8 (CH_3) ppm. **HRMS:** (ESI-) Calcd for $C_{16}H_{29}O_2$: $[M-H]^-$, 253.2173, Found, 253.2171.

Ethyl (*E*)-alkenoates 15 and 16: A solution of decanal or dodecanal (1 equiv.) and ethyl 2-(triphenylphosphoranylidene)acetate (1 equiv.) was stirred overnight at rt. The solution was evaporated and the residue was purified by silica gel flash chromatography (4% Et_2O in pentane), affording alkenoic esters **15** and **16** as colourless oils.
Data for **15**: Prepared from decanal (100 mg, 0.655 mmol), yield = 65 mg, 44%. **IR** (thin film) ν_{max} = 2955, 2925, 2855, 1723, 1655, 1655, 1465, 1367, 1309, 1264, 1180, 1127, 1045, 981 cm^{-1}; **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 6.96 (dt, *J* = 15.6, 7.0 Hz, 1H, HO_2CCHCH), 5.81 (dt, *J* = 15.7, 1.7 Hz, 1H, HO_2CCHCH), 4.18 (q, *J* = 7.1 Hz, 2H, CH_2O), 2.21 – 2.16 (m, 2H, CH_2), 1.48 – 1.42 (m, 2H, CH_2), 1.32 – 1.26 (m, 15H, 6 x CH_2, CH_3), 0.88 (t, *J* = 6.8 Hz, 3H, CH_3) ppm; **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 167.0 (C), 149.7 (CH), 121.4 (CH), 60.3 (CH_2), 32.4 (CH_2), 32.0 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 28.2 (CH_2), 22.8 (CH_2), 14.4 (CH_3), 14.2 (CH_3) ppm. **HRMS:** (ESI+) Calcd for $C_{14}H_{26}O_2Na$ $[M+Na]^+$ 249.1825, Found 249.1825.
Data for **16**: Prepared from dodecanal (100 mg, 0.543 mmol), yield = 62 mg, 45%. **IR** (thin film) ν_{max} = 2955, 2923, 2854, 1722, 1655, 1465, 1367, 1308, 1265, 1178, 1127, 1044, 978 cm^{-1}; **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 6.96 (dt, *J* = 15.5, 6.9 Hz, 1H, HO_2CCHCH), 5.81 (dt, *J* = 15.7, 1.6 Hz, 1H, HO_2CCHCH), 4.18 (q, *J* = 7.1 Hz, 2H, CH_2O), 2.21 – 2.16 (m, 2H, CH_2), 1.48 – 1.42 (m, 2H, CH_2), 1.33 – 1.22 (m, 19H, 8 x CH_2, CH_3), 0.88 (t, *J* = 6.8 Hz, 3H, CH_3) ppm; **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 167.0 (C), 149.7 (CH), 121.4 (CH), 60.3 (CH_2), 32.4 (CH_2), 32.1 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 28.2 (CH_2), 22.8 (CH_2), 14.4 (CH_3), 14.3 (CH_3) ppm. **HRMS:** (ESI+) Calcd for $C_{16}H_{31}O_2$ $[M+H]^+$ 255.2319, Found, 255.2316.

(*E*)-alkenoic acids 13 and 14: A solution of (*E*)-alkenoate ester **15** or **16** in *t*BuOH (1 mL) and 1 M aqueous NaOH (1 mL) was heated at 60 °C for 6 h. The mixture was cooled to rt and acidified with 0.5 M aqueous HCl before being extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to dryness affording alkynoic acid **13** or **14** as a white solid.

Data for **13**: Prepared from ethyl (*E*)-dodec-2-enoate (90 mg, 0.397 mmol), yield = 76 mg, 96%. **IR** (thin film) ν_{max} = 2955, 2923, 2854, 1694, 1650, 1465, 1419, 1284, 1229, 982, 936 cm^{-1}; **^{1}H NMR** (500 MHz, Methanol-d_4) δ 6.95 (dt, *J* = 15.5, 7.0 Hz, 1H, HO_2CCHCH), 5.79 (dt, *J* = 15.6, 1.3 Hz, 1H, HO_2CCHCH), 2.24 – 2.20 (m, 2H, CH_2), 1.50 – 1.43 (m, 2H, CH_2), 1.37 – 1.27 (m, 12H, 6 x CH_2), 0.90 (t, *J* = 6.7 Hz, 3H, CH_3) ppm; **^{13}C NMR** (126 MHz, Methanol-d_4) δ 170.1 (C), 151.2 (CH), 122.5 (CH), 33.1 (CH_2), 33.0 (CH_2), 30.6 (CH_2), 30.5 (CH_2), 30.4 (CH_2), 30.3 (CH_2), 29.2 (CH_2), 23.7 (CH_2), 14.4 (CH_3) ppm; **HRMS:** (ESI-) Calcd for $C_{12}H_{21}O_2$ $[M-H]^-$ 197.1547, Found, 197.1549.

Data for **14**: Prepared from ethyl (*E*)-tetradec-2-enoate (27 mg, 0.106 mmol), yield = 24 mg, 98%. **IR** (thin film) ν_{max} = 2955, 2922, 2853, 1696, 1651, 1465, 1420, 1284, 1230, 979, 936 cm^{-1}; **^{1}H NMR** (500 MHz, Methanol-d_4) δ 6.94 (dt, *J* = 15.6, 7.0 Hz, 1H, HO_2CCHCH), 5.79 (dt, *J* = 15.6, 1.4 Hz, 1H, HO_2CCHCH), 2.24 – 2.19 (m, 2H, CH_2), 1.50 – 1.43 (m, 2H, CH_2), 1.35 – 1.28 (m, 16H, 8 x CH_2), 0.90 (t, *J* = 6.8 Hz, 3H, CH_3) ppm; **^{13}C NMR** (126 MHz, Methanol-d_4) δ 170.3 (C), 151.1 (CH), 122.6 (CH), 33.1 (CH_2), 33.1 (CH_2), 30.7 (CH_2), 30.7 (CH_2), 30.7 (CH_2), 30.5 (CH_2), 30.5 (CH_2), 30.3 (CH_2), 29.2 (CH_2), 23.7 (CH_2), 14.4 (CH_3) ppm; **HRMS:** (ESI-) Calcd for $C_{14}H_{25}O_2$: $[M-H]^-$, 255.1860, Found, 255.1859.

Glycinocin analogues 18 – 29: To an Eppendorf tube containing cyclic peptide trifluoroacetate salt **5** (1 equiv.) was added a freshly prepared solution of fatty acid (5 equiv.), *i*Pr_2NEt (10 equiv.) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC.HCl, 5 equiv.) in DMF (to a total concentration of 50 mM with respect to the cyclic peptide **5**). The solution was vortexed and allowed to stand at rt for 18 h. The solution was concentrated by centrifugal evaporation, and the residue was resuspended in a freshly prepared mixture of TFA/*i*Pr_3SiH/H_2O (18:1:1 v/v/v) and allowed to stand for 2.5 h at r.t. The resulting solution was evaporated under a stream of nitrogen gas, and the residue dissolved in H_2O/MeCN (1:1 v/v), filtered, and purified by preparative reverse phase HPLC (Sunfire C18 5 µm, 18 x 150 mm, A = H_2O with 0.1% formic acid, B = MeCN with 0.1% formic acid with UV detection at 214 or 230 nm). In cases where the cyclic lipopeptide co-eluted with a non-peptidic impurity, a second pass purification was performed using buffers of 0.1% TFA in MeCN and H_2O under otherwise identical conditions. The cyclic lipopeptides **18-29** were obtained as white fluffy solids after lyophilisation.

Purified analogues **18**-**29** were analyzed by UPLC (Acquity UPLC BEH C18, 1.7 µm, 2.1 x 50 mm, 0 to 100% MeCN [0.1% TFA] in H_2O [0.1% TFA] over 5 min, 214 nm, samples dissolved in MeCN/H_2O 1:1 v/v), ^{1}H NMR and both high and low-resolution ESI+ mass spectrometry (See ESI for traces and spectra). ^{1}H NMR signal assignments were made by HSQC and HMBC, COSY and/or 2D TOCSY. 2D NMR spectra and tabulated ^{1}H/ ^{13}C resonances (as derived from HSQC and HMBC) for each compound can be found in the ESI.

14-methylpentadecanoyl derivative (18): Prepared on a 12.0 mg (6.6 µmol) scale and purified with 40-100% B over 45 min. Yield = 4.8 mg, 58% (2 steps). **Analytical HPLC** Rt = 5.2 min. **LRMS (**ESI+) 1264 $[M+H]^+$, 1286 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{58}H_{94}N_{12}O_{19}Na$ $[M+Na]^+$ 1285.6650, found 1285.6649. **^{1}H NMR** (600 MHz, DMSO-d_6) *major rotamer* δ 8.35 (d, *J* = 4.8 Hz, 7Asp2NH), 8.24 (d, *J* = 7.8 Hz, 5Asp2NH), 8.17 – 8.13 (m, 2Dap2NH, 6Gly2NH), 8.08 (m, 4Gly2NH), 8.03 (d, *J* = 7.9 Hz, 1Asp2NH), 7.90 – 7.89 (m, 8Gly2NH, 9Thr2NH), 7.76 (d, *J* = 5.4 Hz, 10Ile2NH), 7.52 (m, 2Dap3NH), 4.78 (m, 3Pip2), 4.66 (m, 2Dap2), 4.58 (m, 5Asp2), 4.53 (m, 1Asp2), 4.48 (m, 7Asp2), 4.35 (m, 3Pip6a), 4.32 – 4.25 (m, 9Thr2, 10Ile2), 4.19 (m, 11Pro2), 3.98 (dd, *J* = 16.8, 6.7 Hz, 4Gly2a), 3.85 – 3.64 (m, 4Gly2b, 6Gly2, 8Gly2, 9Thr3, 11Pro5a), 3.55 – 3.50 (m, 2Dap3a, 11Pro5b), 3.14 (m, 2Dap3b), 2.86 (m, 3Pip6b), 2.72 – 2.52 (m, 1Asp3a, 5Asp3, 7Asp3), 2.44 (dd, *J* = 16.5, 7.9 Hz, 1Asp3b), 2.19 (m, 3Pip3a), 2.09 (m, FA2), 2.01 (m, 11Pro3a), 1.93 (m, 11Pro4a), 1.83 – 1.72 (m, 10Ile3, 11Pro3b, 11Pro4b), 1.59 – 1.33 (m, FA3, FA14, 3Pip3b, 3Pip4, 3Pip5a, 10Ile4a), 1.23 (m, 3Pip5b, FA3-12), 1.12 (m, FA13), 1.08 – 1.02 (m, 9Thr4, 10Ile4b), 0.87 (d, *J* = 6.7 Hz, 10Ile3Me), 0.84 (d, *J* = 6.6 Hz, FA15, FA15'), 0.78 (t, *J* = 7.2 Hz, 10Ile5) ppm.

14-methylpentadec-2-ynoyl derivative (19): Prepared on a 12.0 mg (6.6 µmol) scale and purified with 40-100% B over 45 min. Yield = 4.2 mg, 50% (2 steps). **Analytical HPLC** Rt = 5.2 min. **LRMS (**ESI+) 1260 $[M+H]^+$, 1282 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{58}H_{90}N_{12}O_{19}Na$ $[M+H]^+$ 1281.6337, found 1281.6337. **^{1}H NMR** (500 MHz, DMSO-d_6) *major rotamer* δ 8.69 (d, *J* = 7.6 Hz, 1Asp2NH), 8.35 (d, *J* = 5.9 Hz, 7Asp2NH), 8.28 (d, *J* = 7.6 Hz, 2Dap2NH), 8.26 (d, *J* = 7.6 Hz, 5Asp2NH), 8.14 (m, 6Gly2NH), 8.09 (m, 4Gly2NH), 7.92 (m, 8Gly2NH), 7.88 (d, *J* = 8.4 Hz, 9Thr2NH), 7.71 (d, *J* = 8.4 Hz, 10Ile2NH), 7.50 (m, 2Dap3NH), 4.79 (m, 3Pip2), 4.64 (m, 2Dap2), 4.59 – 4.46 (m, 1Asp2, 5Asp2, 7Asp2), 4.36 – 4.24 (m, 3Pip6a, 9Thr2, 10Ile2), 4.19 (m, 11Pro2), 3.97 (dd, , *J* = 16.8, 6.9 Hz, 4Gly2a), 3.82 (m, 9Thr3), 3.78 – 3.62 (m, 4Gly2b, 6Gly2, 8Gly2, 11Pro5a), 3.58 – 3.50 (m, 2Dap3a, 11Pro5b), 3.12 (m, 2Dap3b), 2.86 (m, 3Pip6b), 2.75 – 2.47 (m, 1Asp3, 5Asp3, 7Asp3), 2.32 (t, *J* = 7.1 Hz, FA4), 2.18 (m, 3Pip3a), 2.04 – 1.92 (m, 11Pro3a, 11Pro4a), 1.84 – 1.71 (m, 10Ile3, 11Pro3b, 11Pro4b), 1.59 – 1.45 (m, FA5, FA14, 3Pip3b, 3Pip4a, 3Pip5a, 10Ile4a), 1.42 – 1.32 (m, FA6, 3Pip4b), 1.24-1.22 (m, FA7-12, 3Pip5b), 1.13 (m, FA13), 1.07 – 1.02 (m, 9Thr4, 10Ile4b), 0.87 (d, *J* = 6.7 Hz, 10Ile3Me), 0.84 (d, *J* = 6.6 Hz, FA15, FA15'), 0.78 (t, *J* = 7.5 Hz, 10Ile5) ppm.

(*Z*)-14-methylpentadec-2-enoyl derivative (20): Prepared on a 12.0 mg (6.6 µmol) scale and purified with 40-100% B over 45 min. Yield = 3.5 mg, 42% (2 steps). **Analytical HPLC** Rt = 5.2 min. **LRMS (**ESI+) 1262 $[M+H]^+$, 1284 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{58}H_{93}N_{12}O_{19}$ $[M+H]^+$ 1261.6674, found 1261.6681. **^{1}H NMR** (500 MHz, DMSO-d_6) *major rotamer* δ 8.34 (m, 7Asp2NH), 8.29 – 8.24 (m, 2Dap2NH, 5Asp2NH), 8.18 – 8.13 (m, 1Asp2NH, 6Gly2NH), 8.10 (m, 4Gly2NH), 7.95 (m, 8Gly2NH), 7.88 (d, *J* = 8.2 Hz, 9Thr2NH), 7.74 (d, *J* = 7.0 Hz, 10Ile2NH), 7.53 (m, 2Dap3NH), 5.95 (dt, *J* = 11.5, 7.0 Hz, FA3), 5.79 (d, *J* = 11.2 Hz, FA2), 4.79 (m, 3Pip2), 4.65 (m, 2Dap2), 4.58 – 4.54 (m, 1Asp2, 5Asp2), 4.48 (m, 7Asp2), 4.35 – 4.24 (m, 3Pip6a, 9Thr2, 10Ile2), 4.19 (m, 11Pro2), 3.96 (dd, *J* = 16.1, 5.1 Hz, 4Gly2a), 3.86 – 3.45 (m, 4Gly2b, 6Gly2, 8Gly2, 9Thr3, 11Pro5a), 3.16 (m, 2Dap3b), 2.84 (m, 3Pip6b), 2.74 – 2.45 (m, FA4, 1Asp3, 5Asp3, 7Asp3), 2.18 (m, 3Pip3a), 2.01 (m, 11Pro3a), 1.91 (m, 11Pro4a), 1.82 – 1.68 (m, 10Ile3, 11Pro3b, 11Pro4b), 1.58 – 1.44 (m, FA14, 3Pip3b, 3Pip4a, 3Pip5a, 10Ile4a), 1.38 – 1.31 (m, 3Pip4b), 1.23 – 1.21 (m, FA5-12, 3Pip5b), 1.12 (m, FA13), 1.06 – 1.00 (m, 9Thr4, 10Ile4b), 0.86 (d, *J* = 6.5 Hz, 10Ile3Me), 0.83 (d, *J* = 6.6 Hz, FA15', FA15), 0.77 (t, *J* = 7.2 Hz, 10Ile5) ppm.

(*E*)-Tetradec-2-enoyl derivative (21): Prepared on a 12.0 mg (6.6 µmol) scale and purified with 40-100% B over 45 min. Yield = 4.0 mg, 49% (2 steps). **Analytical HPLC** Rt = 4.9 min. **LRMS (**ESI+) 1234 $[M+H]^+$, 1256 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{56}H_{89}N_{12}O_{19}$ $[M+H]^+$ 1233.6361, found 1233.6367. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.34 (d, *J* = 6.0 Hz, 7Asp2NH), 8.27 (d, *J* = 7.5 Hz, 5Asp2NH), 8.24 (d, *J* = 7.8 Hz, 2Dap2NH), 8.15 – 8.14 (m, 1Asp2NH, 6Gly2NH), 8.09 (m, 4Gly2NH), 7.93 (m, 8Gly2NH), 7.88 (d, *J* = 8.3 Hz, 9Thr2NH), 7.73 (d, *J* = 7.7 Hz, 10Ile2NH), 7.52 (m, 2Dap3NH), 6.63 (dt, *J* = 15.0, 6.9 Hz, FA3), 5.92 (d, *J* = 15.4 Hz, FA2), 4.80 (m, 3Pip2), 4.66 (m, 2Dap2), 4.61 – 4.55 (m, 1Asp2, 5Asp2), 4.48 (m, 7Asp2), 4.35 – 4.26 (m, 3Pip6a, 9Thr2, 10Ile2), 4.19 (dd, *J* = 6.9, 5.8 Hz, 11Pro2), 3.97 (dd, *J* = 16.6, 6.9 Hz, 4Gly2a), 3.85 – 3.63 (m, 4Gly2b, 6Gly2, 8Gly2, 9Thr3, 11Pro5a), 3.58 – 3.44 (m, 2Dap3a, 11Pro5b), 3.14 (m, 2Dap3b), 2.85 (m, 3Pip6b), 2.75 – 2.46 (m, 1Asp3, 5Asp3, 7Asp3), 2.18 (m, 3Pip3a), 2.12 (m, FA4), 2.00 (m, 11Pro3a), 1.91 (m, 11Pro4a), 1.84 – 1.70 (m, 10Ile3, 11Pro3b, 11Pro4b), 1.58 – 1.40 (m, 3Pip3b, 3Pip4a, 3Pip5a, 10Ile4a), 1.39 – 1.36 (m, FA5, 3Pip4b), 1.28 – 1.23 (m, FA6-13, 3Pip5b), 1.06 – 1.00 (m, 9Thr4, 10Ile4b), 0.87 – 0.83 (m, FA14, 10Ile3Me), 0.78 (t, *J* = 7.3 Hz, 10Ile5) ppm.

(*E*)-Dodec-2-enoyl derivative (22): Prepared on a 20.0 mg (11.0 µmol) scale and purified with 20-100% B over 45 min. Yield = 7.2 mg, 54% (2 steps). **Analytical HPLC** Rt = 3.4 min. **LRMS (**ESI+) 1205 $[M+H]^+$, 1227 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{54}H_{84}N_{12}O_{19}Na$ $[M+Na]^+$ 1227.5870, found 1227.5880. **^{1}H NMR** (500 MHz, DMSO-d_6) *major rotamer* δ 8.34 (d, *J* = 7.4 Hz, 7Asp2NH), 8.27 (d, *J* = 7.5 Hz, 5Asp2NH), 8.23 (d, *J* = 7.7 Hz, 2Dap2NH), 8.15 – 8.07 (m, 1Asp2NH, 4Gly2NH, 6Gly2NH), 7.92 (m, 8Gly2NH), 7.85 (d, *J* = 7.9 Hz, 9Thr2NH), 7.68 (m, 10Ile2NH), 7.50 (m, 2Dap3NH), 6.63 (dt, *J* = 15.2, 6.8 Hz, FA3), 5.91 (d, *J* = 15.3 Hz, FA2), 4.79 (m, 3Pip2), 4.68 – 4.55 (m, 1Asp2, 2Dap2, 5Asp2), 4.48 (m, 7Asp2), 4.35 – 4.25 (m, 3Pip6a, 9Thr2, 10Ile2), 4.18 (dd, *J* = 6.6, 5.2 Hz, 11Pro2), 3.96 (m, 4Gly2a), 3.84 – 3.50 (m, 2Dap3a, 4Gly2b, 6Gly2, 8Gly2, 9Thr3, 11Pro5), 3.13 (m, 2Dap3b), 2.86 (m, 3Pip6b), 2.74 (dd, *J* = 16.4, 5.4 Hz, 5Asp3a), 2.70 (dd, *J* = 16.6, 4.7 Hz, 7Asp3a), 2.63 (dd, *J* = 16.4, 5.5 Hz, 1Asp3a), 2.55 – 2.46 (m, 1Asp3b, 5Asp3b,

7Asp3b), 2.17 (m, 3Pip3a), 2.12 (m, FA4), 2.00 (m, 11Pro3a), 1.91 (m, 11Pro4a), 1.83 – 1.69 (m, 10Ile3, 11Pro3b, 11Pro4b), 1.59 – 1.47 (m, 3Pip3b, 3Pip4a, 3Pip5a, 10Ile4a), 1.39 – 1.36 (m, FA5, 3Pip4b), 1.27 – 1.23 (m, FA6, FA7, FA8, FA9, FA10, FA11, 3Pip5b), 1.06 – 1.00 (m, 9Thr4, 10Ile4b), 0.89 – 0.83 (m, FA12, 10Ile3Me), 0.77 (t, *J* = 7.4 Hz) ppm.

(*E*)-But-2-enoyl derivative (23): Prepared on a 20.0 mg (11.0 µmol) scale and purified with 0-100% B over 45 min. Yield = 6.8 mg, 56% (2 steps). **Analytical HPLC** Rt = 2.1 min. **LRMS (**ESI+) 1093 $[M+H]^+$, 1115 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{46}H_{68}N_{12}O_{19}Na$ $[M+Na]^+$ 1115.4616, found 1115.4602. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.34 (d, *J* = 7.3 Hz, 7Asp2NH), 8.26 (d, *J* = 7.7 Hz, 5Asp2NH), 8.22 (d, *J* = 7.6 Hz, 2Dap2NH), 8.14 – 8.07 (m, 1Asp2NH, 4Gly2NH, 6Gly2NH), 7.91 (m, 8Gly2NH), 7.86 (d, *J* = 8.5 Hz, 9Thr2NH), 7.70 (d, *J* = 8.4 Hz, 10Ile2NH), 7.49 (m, 2Dap3NH), 6.63 (m, FA3), 5.94 (m, FA2), 4.80 (m, 3Pip2), 4.68 – 4.56 (m, 1Asp2, 2Dap2, 5Asp2), 4.49 (m, 7Asp2), 4.36 – 4.26 (m, 3Pip6a, 9Thr2, 10Ile2), 4.18 (dd, *J* = 7.4, 6.1 Hz, 11Pro2), 3.97 (dd, *J* = 16.5, 6.6 Hz, 4Gly2a), 3.84 – 3.49 (m, 2Dap3a, 4Gly2b, 6Gly2, 8Gly2, 9Thr3, 11Pro5), 3.12 (m, 2Dap3b), 2.86 (m, 3Pip6b), 2.77 – 2.46 (m, 1Asp3, 5Asp3, 7Asp3), 2.17 (m, 3Pip3a), 2.01 (m, 11Pro3a), 1.92 (m, 11Pro4a), 1.83 – 1.68 (m, 10Ile3, 11Pro3b, 11Pro4b, FA4), 1.59 – 1.48 (m, 3Pip3b, 3Pip4a, 3Pip5a, 10Ile4a), 1.39 (m, 3Pip4b), 1.23 (m, 3Pip5b), 1.07 – 1.00 (m, 9Thr4, 10Ile4b), 0.87 (d, *J* = 6.7 Hz, 10Ile3Me), 0.78 (t, *J* = 7.4 Hz, 10Ile5) ppm.

Tetradecanoyl derivative (24): Prepared on a 20.0 mg (11.0 µmol) scale and purified with 20-100% B over 45 min. Yield = 7.6 mg, 56% (2 steps). **Analytical HPLC** Rt = 3.9 min. **LRMS (**ESI+) 1235 $[M+H]^+$, 1257 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{56}H_{90}N_{12}O_{19}Na$ $[M+Na]^+$ 1257.6337, found 1257.6338. **^{1}H NMR** (600 MHz, DMSO-d_6) *major rotamer* δ 8.37 (m, 7Asp2NH), 8.22 – 8.18 (m, 5Asp2NH, 6Gly2NH), 8.14 (d, *J* = 7.5 Hz, 2Dap2NH), 8.07 (m, 4Gly2NH), 8.03 (d, *J* = 7.9 Hz, 1Asp2NH), 7.96 – 7.91 (m, 8Gly2NH, 9Thr2NH), 7.77 (m, 10Ile2NH), 7.52 (m, 2Dap3NH), 4.78 (m, 3Pip2), 4.65 (m, 2Dap2), 4.59 – 4.45 (m, 1Asp2, 5Asp2, 7Asp2), 4.35 (m, 3Pip6a), 4.31 – 4.24 (m, 9Thr2, 10Ile2), 4.19 (m, 11Pro2), 3.98 (dd, *J* = 16.9, 6.0 Hz, 4Gly2a), 3.85 – 3.64 (m, 4Gly2b, 6Gly2, 8Gly2, 9Thr3, 11Pro5a), 3.57 – 3.50 (m, 2Dap3a, 11Pro5b), 3.15 (m, 2Dap3b), 2.85 (m, 3Pip6b), 2.70 – 2.51 (m, 1Asp3a, 5Asp3, 7Asp3), 2.44 (dd, *J* = 16.4, 7.8 Hz, 1Asp3b), 2.19 (m, 3Pip3a), 2.09 (m, FA2), 2.01 (m, 11Pro3a), 1.93 (m, 11Pro4a), 1.83 – 1.72 (m, 10Ile3, 11Pro3b, 11Pro4b), 1.62 – 1.33 (m, FA3, 3Pip3b, 3Pip4, 3Pip5a, 10Ile4a), 1.29 – 1.23 (m, FA4-13, 3Pip5b), 1.08 – 1.01 (m, 9Thr4, 10Ile4b), 0.87 – 0.84 (m, FA14, 10Ile3Me), 0.78 (m, *J* = 7.4 Hz) ppm.

Dodecanoyl derivative (25): Prepared on a 20.0 mg (11.0 µmol) scale and purified with 20-100% B over 45 min. Yield = 8.7 mg, 65% (2 steps). **Analytical HPLC** Rt = 3.5 min. **LRMS (**ESI+) 1207 $[M+H]^+$, 1229 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{54}H_{86}N_{12}O_{19}Na$ $[M+Na]^+$ 1229.6024, found 1229.6014. **^{1}H NMR** (600 MHz, DMSO-d_6) *major rotamer* δ 8.33 (d, *J* = 7.1 Hz, 7Asp2NH), 8.25 (d, *J* = 7.9 Hz, 5Asp2NH), 8.14 – 8.12 (m, 2Dap2NH, 6Gly2NH), 8.07 (m, 4Gly2NH), 8.03 (d, *J* = 7.8 Hz, 1Asp2NH), 7.88 – 7.87 (m, 8Gly2NH, 9Thr2NH), 7.74 (d, *J* = 8.4 Hz, 10Ile2NH), 7.51 (m, 2Dap3NH), 4.79 (m, 3Pip2), 4.66 (m, 2Dap2), 4.60 – 4.49 (m, 5Asp2, 1Asp2, 7Asp2), 4.36 (m, 3Pip6a), 4.29 (m, 9Thr2, 10Ile2), 4.19 (m, 11Pro2), 3.99 (dd, *J* = 16.6, 6.5 Hz, 4Gly2a), 3.81 – 3.64 (m, 4Gly2b, 6Gly2, 8Gly2, 9Thr3, 11Pro5a), 3.56 – 3.51 (m, 2Dap3a, 11Pro5b), 3.10 (m, 2Dap3b), 2.86 (m, 3Pip6b), 2.75 – 2.53 (m, 1Asp3a, 5Asp3, 7Asp3), 2.44 (dd, *J* = 16.3, 8.0 Hz, 1Asp3b), 2.19 (m, 3Pip3a), 2.09 (m, FA2), 2.02 (m, 11Pro3a), 1.94 (m, 11Pro4a), 1.84 – 1.70 (m, 11Pro4b, 10Ile3, 11Pro3b), 1.58 – 1.32 (m, 3Pip3b, 3Pip4, 3Pip5a, 10Ile4a, FA3), 1.28 – 1.23 (m, 3Pip5b, FA4-FA11), 1.06 – 1.00 (m, 9Thr4, 10Ile4b), 0.90 – 0.84 (m, FA12, 10Ile3Me), 0.78 (m, 10Ile5) ppm.

Decanoyl derivative (26): Prepared on a 20.0 mg (11.0 µmol) scale and purified with 20-100% B over 45 min. Yield = 8.9 mg, 68% (2 steps). **Analytical HPLC** Rt = 3.1 min. **LRMS** (ESI+) 1179 $[M+H]^+$, 1201 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{52}H_{82}N_{12}O_{19}Na$ $[M+Na]^+$ 1201.5711, found 1201.5691. **^{1}H NMR** (500 MHz, DMSO-d_6) *major rotamer* δ 8.33 (d, *J* = 7.1 Hz, 7Asp2NH), 8.25 (d, *J* = 7.9 Hz, 5Asp2NH), 8.12 – 8.11 (m, 2Dap2NH, 6Gly2NH), 8.06 (m, 4Gly2NH), 8.02 (d, *J* = 7.8 Hz, 1Asp2NH), 7.90 (m, 8Gly2NH), 7.86 (d, *J* = 8.5 Hz, 9Thr2NH), 7.70 (d, *J* = 8.4 Hz, 10Ile2NH), 7.51 (m, 2Dap3NH), 4.78 (m, 3Pip2), 4.66 (m, 2Dap2), 4.61 – 4.47 (m, 1Asp2, 5Asp2, 7Asp2), 4.36 – 4.26 (m, 3Pip6a, 9Thr2, 10Ile2), 4.19 (dd, *J* = 8.0, 5.8 Hz, 11Pro2), 3.98 (dd, *J* = 16.6, 6.7 Hz, 4Gly2a), 3.84 – 3.74 (m, 6Gly2, 8Gly2, 9Thr3, 11Pro5a), 3.66 (dd, *J* = 15.6, 4.5 Hz, 4Gly2b), 3.53 (m, 2Dap3a, 11Pro5b), 3.11 (m, 2Dap3b), 2.86 (m, 3Pip6b), 2.76 – 2.52 (m, 1Asp3a, 5Asp3, 7Asp3), 2.44 (dd, *J* = 16.5, 7.9 Hz, 1Asp3b), 2.18 (m, 3Pip3a), 2.09 (m, FA2), 2.02 (m, 11Pro3a), 1.94 (m, 11Pro4a), 1.83 – 1.70 (m, 10Ile3, 11Pro3b, 11Pro4b), 1.59 – 1.32 (m, FA3, 3Pip3b, 3Pip4, 3Pip5a, 10Ile4a), 1.28 – 1.23 (m, FA4-9, 3Pip5b), 1.07 – 1.00 (m, 9Thr4, 10Ile4b), 0.87 – 0.83 (m, FA10, 10Ile3Me), 0.78 (t, *J* = 7.5 Hz, 10Ile5) ppm.

Hexanoyl derivative (27): Prepared on a 20.0 mg (11.0 µmol) scale and purified with 0-100% B over 45 min. Yield = 8.0 mg, 64% (2 steps). **Analytical HPLC** Rt = 2.4 min. **LRMS (**ESI+) 1123 $[M+H]^+$, 1145 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{48}H_{74}N_{12}O_{19}Na$ $[M+Na]^+$ 1145.5085, found 1145.5084. **^{1}H NMR** (500 MHz, DMSO-d_6) *Major rotamer* δ 8.34 (d, *J* = 7.1 Hz, 7Asp2NH), 8.27 (d, *J* = 7.9 Hz, 5Asp2NH), 8.13 – 8.07 (m, 2Dap2NH, 4Gly2NH, 6Gly2NH), 8.04 (d, *J* = 7.8 Hz, 1Asp2NH), 7.93 (m, 8Gly2NH), 7.85 (d, *J* = 8.3 Hz, 9Thr2NH), 7.69 (d, *J* = 8.4 Hz, 10Ile2NH), 7.51 (m, 2Dap3NH), 4.78 (m, 3Pip2), 4.66 (m, 2Dap2), 4.60 – 4.46 (m, 1Asp2, 5Asp2, 7Asp2), 4.36 – 4.25 (m, 3Pip6a, 9Thr2, 10Ile2), 4.19 (dd, *J* = 7.3, 6.0 Hz, 11Pro2), 3.96 (dd, *J* = 16.7, 6.7 Hz, 4Gly2a), 3.84 – 3.64 (m, 4Gly2b, 6Gly2, 8Gly2, 9Thr3, 11Pro5a), 3.57 – 3.49 (m, 2Dap3a, 11Pro5b), 3.12 (m, 2Dap3b), 2.86 (m, 3Pip6b), 2.76 – 2.51 (m, 1Asp3a, 5Asp3, 7Asp3), 2.45 (dd, *J* = 16.5, 8.0 Hz, 1Asp3b), 2.18 (m, 3Pip3a), 2.09 (m, FA2), 2.02 (m, 11Pro3a), 1.93 (m, 11Pro4a), 1.84 – 1.70 (m, 10Ile3, 11Pro3b, 11Pro4b), 1.59 – 1.34 (m, FA3, 3Pip3b, 3Pip4, 3Pip5a, 10Ile4a), 1.28 – 1.19 (m, FA4, FA5, 3Pip5b), 1.06 – 1.00 (m, 9Thr4, 10Ile4b), 0.87 – 0.82 (m, FA6, 10Ile3Me), 0.77 (t, *J* = 7.5 Hz, 10Ile5) ppm.

5-Cyclohexanepentanoyl derivative (28): Prepared on a 20.0 mg (11.0 µmol) scale and purified with 20-100% B over 45 min. Yield = 8.8 mg, 67% (2 steps). **Analytical HPLC** Rt = 3.1 min. **LRMS (**ESI+) 1191 $[M+H]^+$, 1213 $[M+Na]^+$. **HRMS** (ESI+) calcd

for $C_{53}H_{82}N_{12}O_{19}Na$ $[M+Na]^+$ 1213.5711, found 1213.5695. **^{1}H NMR** (500 MHz, DMSO-d_6) *major rotamer* δ 8.34 (d, *J* = 7.2 Hz, 7Asp2NH), 8.24 (d, *J* = 7.8 Hz, 5Asp2NH), 8.14 – 8.11 (m, 2Dap2NH, 6Gly2NH), 8.07 (m, 4Gly2NH), 8.02 (d, *J* = 7.8 Hz, 1Asp2NH), 7.92 (m, 8Gly2NH), 7.87 (d, *J* = 8.4 Hz, 9Thr2NH), 7.71 (d, *J* = 8.5 Hz, 10Ile2NH), 7.52 (m, 2Dap3NH), 4.78 (m, 3Pip2), 4.66 (m, 2Dap2), 4.60 – 4.46 (m, 1Asp2, 5Asp2, 7Asp2), 4.36 – 4.26 (m, 3Pip6a, 9Thr2, 10Ile2), 4.19 (dd, *J* = 7.4, 6.2 Hz, 11Pro2), 3.98 (dd, *J* = 16.9, 6.6 Hz, 4Gly2a), 3.85 – 3.64 (m, 4Gly2b, 6Gly2, 8Gly2, 9Thr3, 11Pro5a), 3.57 – 3.50 (m, 2Dap3a, 11Pro5b), 3.13 (m, 2Dap3b), 2.86 (m, 3Pip6b), 2.74 – 2.52 (m, 1Asp3a, 5Asp3, 7Asp3), 2.44 (dd, *J* = 16.5, 7.8 Hz, 1Asp3b), 2.18 (m, 3Pip3a), 2.10 (m, FA2), 2.01 (m, 11Pro3a), 1.93 (m, 11Pro4a), 1.84 – 1.71 (m, 10Ile3, 11Pro3b, 11Pro4b), 1.66 – 1.36 (m, FA3, 5 x CyH, 3Pip3b, 3Pip4, 3Pip5a, 10Ile4a), 1.26 – 1.01 (m, FA4, FA5, 4 x CyH, 3Pip5b, 9Thr4, 10Ile4b), 0.87 – 0.76 (m, 2 x CyH, 10Ile3Me, 10Ile5) ppm.

3-Phenylpropanoyl derivative (29): Prepared on a 20.0 mg (11.0 µmol) scale and purified with 0-100% B over 45 min. Yield = 6.0 mg, 47% (2 steps). **Analytical HPLC** Rt = 2.4 min. **LRMS (**ESI+) 1157 $[M+H]^+$, 1179 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{51}H_{72}N_{12}O_{19}Na$ $[M+Na]^+$ 1179.4929, found 1179.4921. **^{1}H NMR** (500 MHz, DMSO-d_6) *major rotamer* δ 8.35 (d, *J* = 7.1 Hz, 7Asp2NH), 8.27 (d, *J* = 7.8 Hz, 5Asp2NH), 8.19 (d, *J* = 7.4 Hz, 2Dap2NH), 8.15 (d, *J* = 7.9 Hz, 1Asp2NH), 8.11 (m, 6Gly2NH), 8.08 (m, 4Gly2NH), 7.94 (m, 8Gly2NH), 7.84 (d, *J* = 8.7 Hz, 9Thr2NH), 7.68 (d, *J* = 8.6 Hz, 10Ile2NH), 7.52 (m, 2Dap3NH), 7.26 (t, *J* = 7.5 Hz, 2 x ArH), 7.20 – 7.15 (m, 3 x ArH), 4.80 (m, 3Pip2), 4.67 (m, 2Dap2), 4.61 – 4.46 (m, 1Asp2, 5Asp2, 7Asp2), 4.35 (m, 3Pip6a), 4.31 – 4.25 (m, 9Thr2, 10Ile2), 4.20 (dd, *J* = 7.1, 6.0 Hz, 11Pro2), 3.97 (dd, *J* = 16.7, 6.5 Hz, 4Gly2a), 3.84 – 3.65 (m, 4Gly2b, 6Gly2, 8Gly2, 9Thr3, 11Pro5a), 3.62 – 3.47 (m, 2Dap3a, 11Pro5b), 3.10 (m, 2Dap3b), 2.88 – 2.52 (m, FA2, 1Asp3a, 3Pip6b, 5Asp3, 7Asp3), 2.46 – 2.41 (m, FA3, 1Asp3b), 2.18 (m, 3Pip3a), 2.04 – 1.89 (m, 11Pro3a, 11Pro4a), 1.84 – 1.68 (m, 10Ile3, 11Pro3b, 11Pro4b), 1.60 – 1.35 (m, 3Pip3b, 3Pip4, 3Pip5a, 10Ile4a), 1.24 (m, 3Pip5b), 1.07 – 1.00 (m, 9Thr4, 10Ile4b), 0.83 – 0.74 (m, 10Ile3Me, 10Ile5) ppm.

Geranylgeranoyl derivative (30): To an eppendorf tube containing cyclic peptide trifluoroacetate salt **5** (12.0 mg, 6.6 µmol, 1 equiv.) was added a freshly-prepared mixture of TFA/iPr$_3$SiH/H_2O (18:1:1 v/v/v, 0.5 mL). The mixture was vortexed and then allowed to stand for 2 h at rt before concentrating under a stream of nitrogen gas. The residue was suspended in diethyl ether (1 mL) and the resulting suspension was centrifuged. The supernatant was removed and the pellet was air-dried to yield the fully deprotected cyclic peptide.

In a separate eppendorf, a solution of geranylgeranic acid (20.1 mg, 66.0 µmol, 10 equiv.), EDC (25.4 mg, 132 µmol, 20 equiv.) and *N*-hydroxysuccinimide (15.2 mg, 132 µmol, 20 equiv.) in CH_2Cl_2 (130 µL) was incubated at rt for 16 h. The mixture was washed with 1 M aqueous HCl (3 x 300 µL), followed by H_2O (1 x 300 µL), and then concentrated under a stream of nitrogen gas. The resulting geranylgeranic *N*-hydroxysuccinimide ester was dissolved in MeCN (140 µL) and treated with iPr$_2$NEt (9.2 µL, 53 µmol, 8 equiv.) and this solution was added to the above fully deprotected cyclic peptide (1 equiv.). The solution was incubated at rt for 24h, then diluted with MeCN/H_2O 1:1 and purified by HPLC (Sunfire C18 5 µm, 18 x 150 mm, 40 to 100% MeCN [0.1% formic acid] in H_2O [0.1% formic acid] over 45 min) with UV detection at 214 nm, yielding geranylgeranoyl derivative **30** (3.5 mg, 40% over 2 steps) as a fluffy white solid.

Analytical HPLC Rt = 5.1 min. **LRMS (**ESI+) 1312 $[M+H]^+$, 1334 $[M+Na]^+$. **HRMS** (ESI+) calcd for $C_{62}H_{94}N_{12}O_{19}Na$ $[M+Na]^+$ 1333.6650, found 1333.6654. **^{1}H NMR** (500 MHz, DMSO-d_6) *major rotamer* δ 8.34 (d, *J* = 4.7 Hz, 7Asp2NH), 8.27 (d, *J* = 7.7 Hz, 5Asp2NH), 8.19 – 8.14 (m, 2Dap2NH, 6Gly2NH), 8.09 (m, 4Gly2NH), 7.99 (d, *J* = 7.7 Hz, 1Asp2NH), 7.94 (m, 8Gly2NH), 7.87 (d, *J* = 8.0 Hz, 9Thr2NH), 7.72 (d, *J* = 8.3 Hz, 10Ile2NH), 7.52 (m, 2Dap3NH), 5.70 (s, FACH), 5.11 – 5.04 (m, 3 x FACH), 4.79 (m, 3Pip2), 4.66 (m, 2Dap2), 4.62 – 4.55 (m, 1Asp2, 5Asp2), 4.48 (m, 7Asp2), 4.35 – 4.25 (m, 3Pip6a, 9Thr2, 10Ile2), 4.19 (dd, *J* = 7.6, 5.8 Hz, 11Pro2), 3.97 (dd, *J* = 17.1, 6.4 Hz, 4Gly2a), 3.84 – 3.64 (m, 4Gly2b, 6Gly2, 11Pro5a, 8Gly2, 9Thr3), 3.58 – 3.49 (m, 2Dap3a, 11Pro5b), 3.14 (m, 2Dap3b), 2.85 (m, 3Pip6b), 2.75 – 2.44 (m, 1Asp3, 5Asp3, 7Asp3), 2.18 (m, 3Pip3a), 2.12 – 1.99 (m, 4 x $FACH_2$ $FACH_3$, 11Pro3a), 1.96 – 1.91 (m, 2 x $FACH_2$, 11Pro4a), 1.83 – 1.70 (m, 10Ile3, 11Pro3b, 11Pro4b), 1.63 (s, $FACH_3$), 1.57 – 1.47 (m, 3Pip3b, 3Pip4a, 3 x $FACH_3$, 3Pip5a), 1.45 – 1.36 (m, 3Pip4b), 1.23 (m, 3Pip5b), 1.06 – 1.00 (m, 9Thr4, 10Ile4b), 0.86 (d, *J* = 6.6 Hz, 10Ile3Me), 0.77 (t, *J* = 7.4 Hz, 10Ile5) ppm.

Antimicrobial Screening Methods

Antimicrobial susceptibility tests for select bacteria against glycinocin analogues were performed using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI). Bacterial test strains were grown on fresh LB/TSB agar plates as recommended by the American Type Culture Collection (ATCC) cultivation protocol. Individual colonies were used to inoculate 3 mL of sterile non-cation adjusted Mueller-Hinton Broth (MHB). Inoculated cultures were grown overnight with shaking (225 RPM; 35 °C). Saturated overnight cultures were diluted 1:1000 or 1:100 according to turbidity to achieve approximately 5 x 10^5 CFU of final inoculum density and dispensed into sterile clear polypropylene 384-well microplates with a final screening volume of 30 µL. All cultures were diluted and grown in both MHB and cation-adjusted Mueller-Hinton Broth (CAMHB) containing 10-15 mg/L of Mg^{2+} and 50-55 mg/L of Ca^{2+} (CAMHB50). *B. subtilis, S. aureus and E. faecium* were grown in two additional CAMHB media containing 100-105 mg/L of Ca^{2+} (CAMHB100) and 200-205 mg/L of Ca^{2+} (CAMHB200). DMSO solutions of test compounds were pinned into each well (200 nL) at T0 using a high-throughput pinning robot (Tecan Freedom EVO 100). In each 384-well plate lanes 1 and 2 were reserved for DMSO vehicle and culture medium, while lanes 23 and 24 contained vehicle, culture medium and the test organism. After compound addition, assay plates were read at OD600 using an automated plate reader (Molecular Devices SpectraMax i3x). Post-absorbance reading, assay plates were sealed and incubated at 35 °C for 18-20 hours before obtaining OD600 readings again. The resulting growth absorbance data

for each dilution series were used to determine MIC values for all test compounds following standard procedures. The MIC for *E. faecium* was observed visually as the lowest concentration of compound for which there is no visible growth.

Bacterial strains used in the screen: Gram-positive: Bacillus subtilis 168 (ATCC 23857)*, methicillin-susceptible Staphylococcus aureus* (MSSA) (ATCC 29213), *Enterococcus faecium* (ATCC 6569)*, Pseudomonas aeruginosa* (ATCC 27853).

Conflicts of interest

There are no conflicts to declare

Acknowledgements

The authors acknowledge Dr Ian Luck and Dr. Nick Proschogo (School of Chemistry, The University of Sydney) for technical support with NMR spectroscopy and high-resolution mass spectrometry, respectively. We would like to acknowledge an Australian Research Council Future Fellowship FT130100150 (R.J.P.) and a Natural Sciences and Engineering Research Council of Canada Grant RGPIN-2016-03962 (R.G.L.) for funding.

Notes and references

1 R. H. Baltz, V. Miao and S. K. Wrigley, *Nat. Prod. Rep.*, 2005, **22**, 717-741.
2 M. Strieker and M. A. Marahiel, *ChemBioChem*, 2009, **10**, 607-616.
3 L.H. Kleijn, N.I. Martin (2017) The Cyclic Lipopeptide Antibiotics. In: . Topics in Medicinal Chemistry. Springer, Berlin, Heidelberg..
4 B. I. Eisenstein, J. F. B. Oleson and R. H. Baltz, *Clin. Infect. Dis.*, 2010, **50**, S10-S15.
5 S. Stefani, F. Campanile, M. Santagati, M. L. Mezzatesta, V. Cafiso and G. Pacini, *Int. J. Antimicrob. Agents*, 2015, **46**, 278-289.
6 H. Naganawa, M. Hamada, K. Maeda, Y. Okami, T. Takeuchi and H. Umezawa, *J. Antibiot.*, 1968, **21**, 55-62.
7 H. Naganawa, T. Takita, K. Maeda and H. Umezawa, *J. Antibiot.*, 1970, **23**, 423-424.
8 F. Kong and G. T. Carter, *J. Antibiot.*, 2003, **56**, 557-564.
9 D. B. Borders, R. A. Leese, H. Jarolmen, N. D. Francis, A. A. Fantini, T. Falla, J. C. Fiddes and A. Aumelas, *J. Nat. Prod.*, 2007, **70**, 443-446.
10 T. Schneider, K. Gries, M. Josten, I. Wiedemann, S. Pelzer, H. Labischinski and H.-G. Sahl, *Antimicrob. Agents Chemother.*, 2009, **53**, 1610-1618.
11 L. H. Kleijn, S. F. Oppedijk, P. 't Hart, R. M. Van Harten, L. A. Martin-Visscher, J. Kemmink, E. Breukink and N. I. Martin, *J. Med. Chem.*, 2016, **59**, 3569-3574.
12 L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller, T. F. Schäberle, D. E. Hughes, S. Epstein, M. Jones, L. Lazarides, V. A. Steadman, D. R. Cohen, C. R. Felix, K. A. Fetterman, W. P. Millett, A. G. Nitti, A. M. Zullo, C. Chen and K. Lewis, *Nature*, 2015, **517**, 455.
13 B. M. Hover, S.-H. Kim, M. Katz, Z. Charlop-Powers, J. G. Owen, M. A. Ternei, J. Maniko, A. B. Estrela, H. Molina, S. Park, D. S. Perlin and S. F. Brady, *Nat. Microbiol.*, 2018, **3**, 415-422.
14 L. H. J. Kleijn, H. C. Vlieg, T. M. Wood, J. Sastre Toraño, B. J. C. Janssen, N. I. Martin, *Angew. Chem. Int. Ed.* 2017, **56**, 16546-16549.
15 W. V. Curran, R. A. Leese, H. Jarolmen, D. B. Borders, D. Dugourd, Y. Chen and D. R. Cameron, *J. Nat. Prod.*, 2007, **70**, 447-450.
16 L. Corcilius, N. T. Elias, J. L. Ochoa, R. G. Linington and R. J. Payne, *J. Org. Chem.*, 2017, **82**, 12778-12785.