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Total Synthesis of Glycinocins A–C

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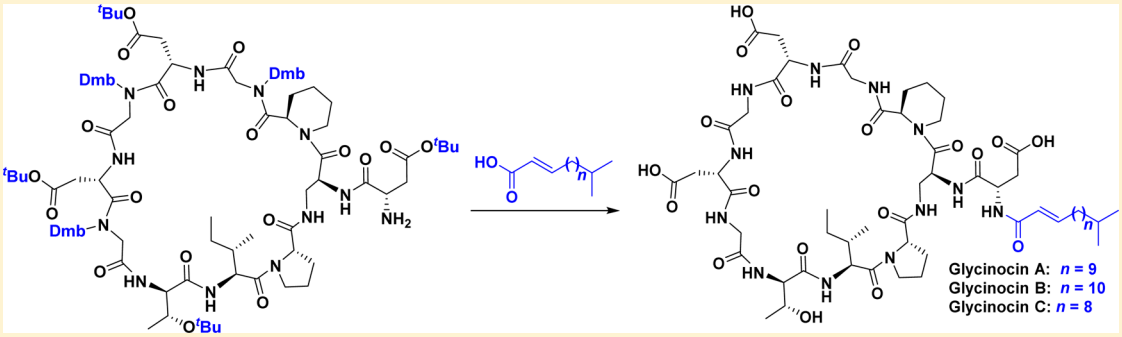
Supporting Information



ABSTRACT: The glycinocins are a class of calcium-dependent, acidic cyclolipopeptide antibiotics structurally related to the clinically approved daptomycin. Herein, we describe a divergent total synthesis of glycinocins A–C, which differ in the structure of a branched α,β-unsaturated fatty acyl moiety. The three natural products exhibited calcium-dependent antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* with MICs ranging from 5.5 to 17 μM.

The glycinocins belong to a family of acidic lipopeptide antibiotics (including daptomycin and friulimicin) that possess Ca^{2+}-dependent antimicrobial activity.[1,2] These secondary metabolites are produced by Actinobacteria through the action of nonribosomal peptide synthases, and many possess significant antimicrobial activity against a range of Gram-positive bacteria, including drug-sensitive and drug-resistant *Staphylococcus aureus* strains.[1,2] The recent FDA approval of daptomycin[3] for skin and skin structure infections, *S. aureus* bacteraemia and *S. aureus* endocarditis, has prompted a renewed interest in this class of compounds. Indeed, with recent resistance to daptomycin observed in strains of *S. aureus* and *Enterococcus* spp.,[4−6] an examination of other members of this natural product family, especially those that exhibit a novel mechanism of action, has become of significant interest.[2,7]

Glycinocin A (**1**), originally named laspartomycin C, was initially reported in 1967 by Naganawa et al.[8,9] following isolation from *Streptomyces viridochromogenes*. The natural product was subsequently shown to have antimicrobial activity against vancomycin-resistant *S. aureus* (VRSA) and methicillin-resistant *S. aureus* (MRSA) strains.[8−11] Kleijn et al. recently proposed a putative mechanism of action of glycinocin A (**1**) together with a synthesis of the natural product.[12] Specifically, the authors demonstrated the formation of a high affinity complex with undecaprenyl phosphate, an essential cell wall precursor in bacteria. Glycinocin A was shown to block lipid II synthesis in a dose-dependent manner, thereby preventing peptidoglycan synthesis which induces bacterial cell death. This mechanism mirrors that of fellow acidic lipopeptide antibiotic family members, amphomycin and friulimicin B, but differs from that of daptomycin.[13,14] In a separate report, glycinocin A (**1**) has also been reported to reduce viral cytopathogenicity in herpes simplex type 1 (HSV-1) infected HeLa cells.[15] Importantly, this suggests a possible broad-spectrum polymicrobial activity for the family of natural products.

Decades after its initial isolation, glycinocin A (**1**) was reisolated along with its congeners, glycinocin B (**2**), C (**3**), and D (**4**), from the fermentation broth of an unidentified terrestrial *Actinomycete* species. In this 2003 report, Kong and Carter[16] also provided the first complete structural characterization of glycinocins A–D (**1**–**4**). A follow-up study in 2007 by Borders et al.[17] confirmed the structure of the *Actinomycete*-derived glycinocin A to be identical to *S. viridochromogenes*-derived laspartomycin C. Whereas the biological activities of glycinocins B–D (**2**–**4**) have not yet been determined, their potential as antibiotic leads is also worth investigating. The innate production of these congeners reveals a natural process of metabolite optimization by the producing *Actinomycete* species. It would thus be interesting to investigate the effect of modifying chain length, specifically in glycinocins A–C (**1**–**3**), on the biological activity and physiochemical properties of these natural products. The aim of the present study therefore was to conduct a total synthesis of glycinocins A–C (**1**–**3**) and

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to investigate the effect of the fatty acyl moiety upon biological activity.

The structure of glycinocins A–D (**1**–**4**) (Figure 1) consists of a cyclodecapeptide that is bound, through an exocyclic L-Asp

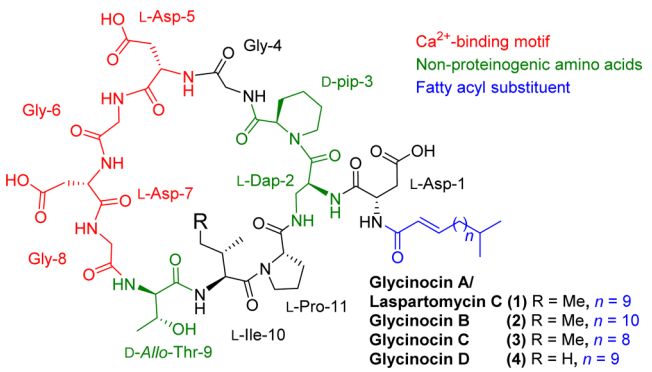


Figure 1. Glycinocins A–D with key structural features highlighted in red (Ca^{2+}-binding motif), green (nonproteinogenic amino acids), and blue (fatty acyl moiety).

amino acid, to a range of 2,3-unsaturated iso-fatty acids of various lengths. Several features are conserved with other members of the acidic lipopeptide family,[2] including the position of nonproteinogenic amino acids and the L-Asp-Gly-L-Asp-Gly motif, which is thought to be essential for Ca^{2+} complexation.[1] In glycinocins A–D (**1**–**4**), the nonproteinogenic amino acid loci are occupied with L-2,3-diaminopropionic acid (L-Dap), D-pipecolic acid (D-Pip), and D-allo-Thr, which appear at positions 2, 3, and 9, respectively. Glycinocins A–C (**1**–**3**) share a common peptidic core, which incorporates an L-Ile amino acid at position 10. In contrast, glycinocin D (**4**) possesses a L-Val amino acid at this position. The attachment of these peptidic macrocycles to lipid chains confers an amphipathic nature which is likely to play an important role in the physiochemical properties and biological activity of glycinocins A–D (**1**–**4**). However, as is common in this class of natural products, and depending on the availability of fatty acid precursors in the producing bacteria, various fatty acid linkages are observed. Importantly, despite the difference in the carbon-chain length, all attached lipids present common α,β-unsaturation as well as a terminating isopropyl moiety.

To date, only the total synthesis of glycinocin A (**1**) has been reported. This was achieved through the solid-phase synthesis of a branched peptide precursor followed by a final step macrolactamization.[12] Herein, we sought a synthetic strategy which would facilitate late-stage installation of the fatty acyl moiety and therefore provide highly divergent access to glycinocins A–C (**1**–**3**), as well as an avenue to fatty acid modified analogues in the future. Retrosynthetically, we envisioned that glycinocins A–C (**1**–**3**) could all be accessed through solution-phase acylation of the key common protected cyclic peptide precursor **5**, which in turn could be accessed from macrolactamization of the orthogonally protected linear peptide precursor **6**, prepared through SPPS (Figure 2).

Synthetic efforts began with the preparation of the three requisite fatty acids **7**–**9** (Scheme 1). Synthesis commenced with monobenzylation of 1,8-octanediol and 1,10-decanediol to provide alcohols **10** and **11**, respectively. Subsequent Swern oxidation and olefination with either isobutyl(triphenyl)-phosphonium bromide or isoamyl(triphenyl)phosphonium bromide yielded olefins **12**–**14** in 60–66% yield over two

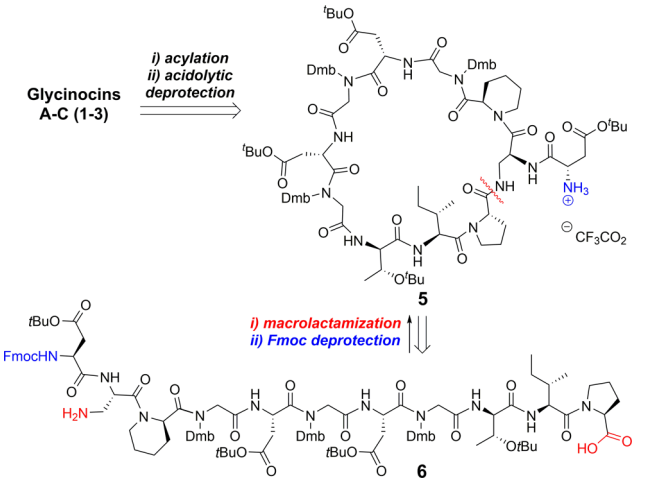
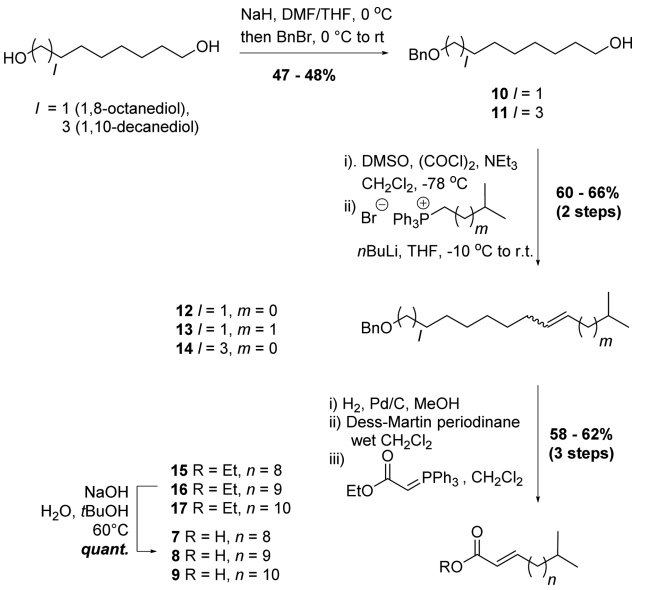


Figure 2. Proposed retrosynthesis of glycinocins A–C (**1**–**3**).

Scheme 1. Synthesis of Fatty Acids 7–9



steps. Simultaneous hydrogenation of the olefin and hydrogenolysis of the benzyl ether, followed by Dess-Martin oxidation, afforded the corresponding aldehydes. These were next subjected to a second Wittig olefination with ethyl-(triphenyl)phosphoranylidene acetate, to furnish (E)-α,β-unsaturated esters **15**–**17** in 58–62% overall yield over the three steps. Finally, hydrolysis of the ethyl ester provided the requisite fatty acids **7**–**9** in quantitative yield.

Next, we turned our attention to synthesis of the common side-chain- and backbone-protected cyclic peptide **5** (Scheme 2). Beginning with 2-chlorotrityl chloride-functionalized polystyrene resin, loading of the C-terminal proline residue, followed by iterative SPPS using commercially available *N*-Fmoc and side-chain-protected amino acids, afforded the requisite resin-bound undecapeptide **18**. Glycine residues were incorporated as their corresponding 2,4-dimethoxybenzyl (Dmb)-protected variants to prevent aspartimide formation during Fmoc deprotection steps and to function as turn-inducing elements for the subsequent cyclization. Furthermore, the L-Dap residue was incorporated as the side chain Alloc-protected variant to facilitate orthogonal deprotection on the

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Scheme 2. Synthesis of Glycinocins A–C

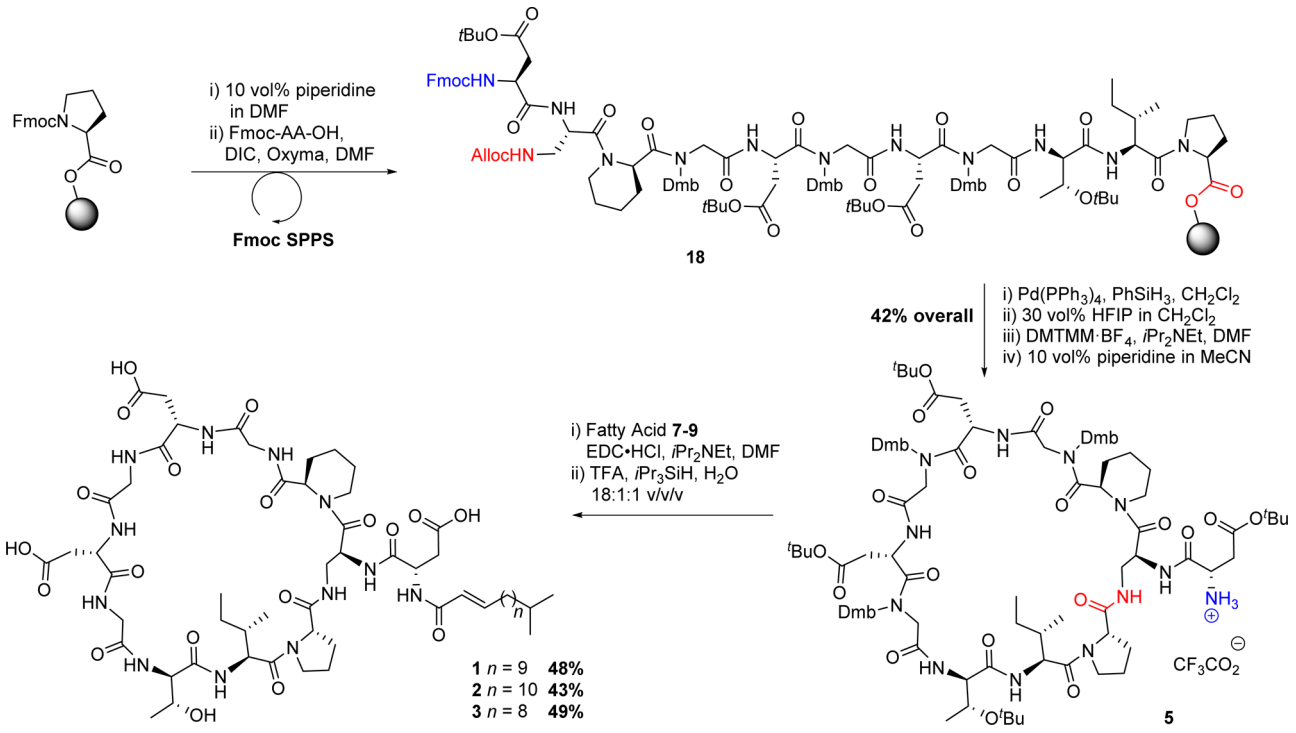

Table 1. MIC Values in μM (and μg/mL) of Synthetic Glycinocins A–C (1–3) and Control Antibiotics with and without the Presence of Ca^{2+} against *S. aureus* and *B. subtilis*

	S. aureus		*B. subtilis*	
species	0 mM Ca^{2+}	1.25 mM Ca^{2+}	0 mM Ca^{2+}	1.25 mM Ca^{2+}
glycinocin A (**1**)	>66 (>82)	11 (14)	>66 (>82)	17 (21)
glycinocin B (**2**)	>66 (>83)	5.5 (6.9)	>66 (>83)	8.3 (10)
glycinocin C (**3**)	>66 (>81)	17 (21.0)	>66 (>81)	11 (14)
rifampicin	0.006 (0.005)	0.008 (0.007)	0.15 (0.12)	0.11 (0.091)
daptomycin	22 (36)	0.34 (0.55)	22 (36)	0.94 (1.5)
vancomycin	0.94 (1.4)	0.51 (0.74)	0.097 (0.14)	0.11 (0.16)
gentamicin	1.3 (0.62)	4.4 (2.1)	0.39 (0.19)	0.064 (0.031)

solid phase. Thus, following assembly of the peptide chain, the Alloc group was removed via treatment of the resin-bound peptide with a solution of $Pd(PPh_3)_4$ and $PhSiH_3$ in CH_2Cl_2. Next, the peptide was treated with a mildly acidic solution of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in CH_2Cl_2 to facilitate selective cleavage from the resin without affecting the side chain protecting groups on the peptide. Gratifyingly, macrolactamization could be smoothly effected by treatment with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM·BF_4) with iPr_2NEt at high dilution (0.01 M). After Fmoc deprotection and purification by reverse-phase HPLC, the desired protected cyclic peptide **5** was obtained in an excellent yield of 42% over 36 linear steps (based on the original resin loading).

The final steps in the synthesis involved coupling cyclic peptide **5** to fatty acids **7**–**9** followed by global deprotection. Toward this end, the protected cyclic peptide **5** was treated with a solution of each of the fatty acids **7**–**9** (2 equiv) in DMF using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC·HCl) as a coupling reagent in the presence of iPr_2NEt. The couplings were monitored by LC-MS, which indicated that the reactions had reached completion after 16 h. At this point, the reaction mixtures were concentrated to dryness and then treated with a mixture of TFA, iPr_3SiH, and H_2O (18:1:1 v/v/v) to effect acidolytic deprotection of the backbone Dmb and *t*Bu side chain protecting groups. Subsequent purification by reverse-phase HPLC afforded glycinocins A–C (**1**–**3**) in 43–49% yield. Pleasingly, the NMR spectra and optical rotations of the synthetic natural products were in close agreement with those reported for isolated glycinocin A–C (see Supporting Information).[16]

Having successfully synthesized the target natural products, we next investigated the antimicrobial activity of **1**–**3** against both Gram-positive and Gram-negative bacterial strains, with and without the presence of Ca^{2+} in the media (Table 1). Specifically, growth inhibition of Gram-positive *Bacillus subtilis* 168, methicillin susceptible *Staphylococcus aureus* (MSSA) (ATCC 29213), *Enterococcus faecium* (ATCC 6569), as well as Gram-negative *Pseudomonas aeruginosa* (ATCC 27853) were assessed using a high-throughput screening assay reported previously[18] using cation adjusted Mueller-Hinton broth (MHB), containing 0 or 1.25 mM Ca^{2+} (see Supporting Information for details). In addition to synthetic glycinocins A–C (**1**–**3**), rifampicin, daptomycin, vancomycin, and gentamicin were included as controls.

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All synthetic glycinocins **1**–**3** exhibited antimicrobial activity against Gram-positive *S. aureus* and *B. subtilis* in the presence of 1.25 mM Ca^{2+} (physiological concentration) with MICs ranging from 5.5 to 17 μM; however, the natural products lost this activity in the absence of Ca^{2+} in the media. All compounds were inactive against Gram-positive *E. faecium* and Gram-negative *P. aeruginosa* both in the presence and in the absence of Ca^{2+} (see Supporting Information for data). To date, only isolated glycinocin A (**1**) has been assessed for antimicrobial activity.[8,12] Importantly, the Ca^{2+} dependency and activities observed for synthetic glycinocins A–C (**1**–**3**) in this study are consistent with the prior data reported for **1**. It is interesting to note that the order of observed antimicrobial activity is **3** < **1** < **2**, which reflects the increasing chain length of the fatty acyl substituent. It is therefore tempting to speculate that the increasing lipophilicity of the fatty acyl substituent aids in binding to the proposed undecaprenyl phosphate target of the natural products. This work therefore provides a potential direction for analogue design, which will be the subject of future work in our laboratories.

In summary, a high yielding total synthesis of glycinocins A–C (**1**–**3**) has been accomplished by utilizing a highly divergent late-stage acylation of a common cyclic peptide precursor. The natural products exhibited calcium-dependent antimicrobial activity against the Gram-positive pathogens *S. aureus* and *B. subtilis*. Current work in our laboratory is focused on the preparation of glycinocin analogues to improve activity and to better understand structure–activity relationships.

EXPERIMENTAL SECTION

General Procedures. Commercial materials, including solvents, were used as received unless otherwise noted. Anhydrous MeOH, DMF, and CH_2Cl_2 were obtained from a PURE SOLV solvent dispensing unit. Solution-phase reactions were carried out under an atmosphere of dry nitrogen or argon, unless otherwise specified.

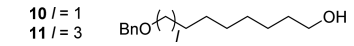
Flash column chromatography was performed using 230–400 mesh Kieselgel 60 silica eluting with gradients as specified. Analytical thin layer chromatography (TLC) was performed on commercially prepared silica plates (Merck Kieselgel 60 0.25 mm F254).

^{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: $CDCl_3$ δ 7.26 [^{1}H] and δ 77.16 [^{13}C] and DMSO-d_6 δ 2.50 [^{1}H] and δ 39.52 [^{13}C]. ^{1}H NMR data are reported as chemical shifts, 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. 1D peak assignments for cyclic peptide **5** were made using COSY, TOCSY, HSQC, and HMBC where appropriate. Peak assignments for synthetic glycinocins A–C (**1**–**3**) were made using HSQC and HMBC through comparison with literature assignments.[16]

High-resolution ESI+ mass spectra were measured on a Bruker–Daltonics Apex Ultra 7.0T Fourier transform mass spectrometer (FTICR). 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. Optical rotations were recorded at ambient temperature (293 K) on a Perkin-Elmer 341 polarimeter at 589 nm (sodium D line) with a cell path length of 1 dm, and the concentrations are reported in g/100 mL.

Preparative reverse-phase HPLC was performed using a Waters 600 multisolvent delivery system and pump with Waters 486 tunable absorbance detector operating at 214 nm. Analytical reverse-phase HPLC was performed on a Waters 2695 separation module equipped with a 2996 DAD detector operating at 214 nm.

Monobenzylated Alcohols 10 and 11.

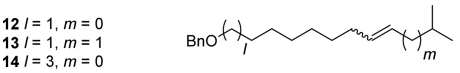


Sodium hydride (60% oil dispersion, 1.05 equiv) was suspended in DMF (20 mL) and subsequently cooled to 0 °C. A solution of alkanediol (1 equiv) in THF/DMF (1:2 v/v, 20 mL) was then slowly added, and the reaction mixture was stirred at 0 °C for 2 h. Benzyl bromide (1.05 equiv) was then added dropwise to the cooled reaction mixture. Following addition, the reaction mixture was warmed to rt and stirred for 16 h. Upon completion, the reaction mixture was cooled to 0 °C and quenched with H_2O (20 mL). The mixture was then partitioned between EtOAc (150 mL) and H_2O (150 mL). The organic extract was washed with H_2O (5 × 100 mL), followed by brine, and dried ($MgSO_4$). The solvent was then removed by rotary evaporation, and the crude mixture was purified by column chromatography to yield both the dibenzylated and monobenzylated products (an eluent gradient of 10–20 vol % EtOAc in hexane provided the dibenzylated product, whereas a gradient of 20–30 vol % EtOAc in hexane provided the desired monobenzylated alcohol **10** or **11**).

*8-(Benzyloxy)-1-octanol (**10**):* Prepared from 2.00 g (13.7 mmol) of 1,8-octanediol; yield = 1.53 g, 47%, colorless oil; R_f [30 vol % EtOAc/hexane] = 0.4; IR (thin film) ν_{max} = 3350, 2928, 2855, 1718, 1703, 1453, 1362, 1314, 1275, 1097, 1071, 1056, 1027, 737, 713, 697, 611 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.55–7.23 (5H, m, aromatic CH), 4.48 (2H, s, benzylic CH_2), 3.61 (2H, t, *J* 6.6 Hz, CH_2OH), 3.44 (2H, t, *J* 6.6 Hz, CH_2OBn), 2.61 (1H, s, OH), 1.59–1.52 (4H, m), 1.40–1.30 (8H, m) ppm. Data are in agreement with that reported by Madda et al.[19] and Subba Reddy et al.[20]

*10-(Benzyloxy)-1-decanol (**11**):* Prepared from 1.20 g (6.89 mmol) of 1,10-decanediol; yield = 879 mg, 48%, colorless oil; R_f [30 vol % EtOAc/hexane] = 0.4; IR (thin film) ν_{max} = 3366, 2926, 2853, 1454, 1362, 1205, 1100, 1074, 1058, 1028, 735, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.25 (5H, m, aromatic CH), 4.49 (2H, s, benzylic CH_2), 3.62 (2H, t, *J* 6.5 Hz, CH_2OH), 3.45 (2H, t, *J* 6.5 Hz, CH_2OBn), 1.62–1.53 (4H, m, 2 × CH_2), 1.39–1.28 (12H, m, 6 × CH_2) ppm; LRMS (ESI+) *m*/*z* 287 [(M + Na)$^+$, 100%]. Data are in agreement with that reported by Mash et al.[21] and Penov Gasi et al.[22]

Alkenes 12–14.



To a solution of oxalyl chloride (3 equiv) in CH_2Cl_2 (15 mL) at −78 °C was added dimethylsulfoxide (3.3 equiv). The mixture was stirred at −78 °C for 30 min before the slow addition of a solution of benzyl alcohol **10** or **11** (1 equiv) in CH_2Cl_2 (10 mL). The mixture was stirred at −78 °C for a further 30 min. Triethylamine (5.1 equiv) was finally added to the cooled reaction mixture, which was then warmed to rt and stirred for 1 h. The reaction was quenched with H_2O (50 mL) and the resulting mixture extracted into chloroform (3 × 50 mL). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate, and the solvent removed by rotary evaporation. The resulting aldehyde was used in the next step without further purification.

n-Butyllithium (2.5 M, 2 equiv) was added slowly to a cooled (−10 °C) suspension of isobutyl- or isoamyl(triphenyl)phosphonium bromide (1.2 equiv) in THF (2.8 mL). While being stirred at −10 °C for 1 h, the reaction mixture slowly transformed from a white suspension to an orange solution. A solution of the aldehyde from the previous step (1 equiv) in THF (1 mL) was then added slowly to the yellow solution at −10 °C. The reaction mixture was stirred at −10 °C for 10 min and at rt for a further 2 h. After this time, cold H_2O (0 °C) was added slowly to quench the reaction, which was then extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate, and the solvent removed by rotary evaporation. Finally, purification by column chromatography (an eluent gradient of 0–3 vol % EtOAc in hexane was used to remove any byproducts, followed by a gradient of 4–10%

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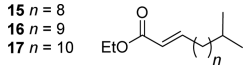
EtOAc in hexane to yield the purified alkenes) provided the desired alkene products **12**–**14**.

*(E/Z)-11-(Benzyloxy)-2-methylundec-3-ene (**12**):* Prepared from 1.98 mmol of 8-(benzyloxy)-1-octanol **10** and isobutyl(triphenyl)-phosphonium bromide; yield = 0.36 g, 66%, ∼5:1 inseparable diasteromeric mixture, colorless oil; R_f [10 vol % EtOAc/hexane] = 0.4; IR (thin film) ν_{max} = 3370, 2952, 2925, 2855, 1721, 1466,1453, 1274, 1113, 1070, 1027, 712 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *diastereomeric mixture* δ 7.34–7.25 (5H, m, aromatic CH), 5.36–5.14 (2H, m, C*H*=C*H*), 4.50 (2H, s, benzylic CH_2), 3.46 (2H, t, *J* 6.7 Hz, CH_2OBn), 2.58, 2.22 (1H, 2m, C*H*$(CH_3)_2$), 2.02, 1.95 (2H, 2m, CH=CHCH_2), 1.65–1.59 (2H, m, CH_2), 1.40–1.26 (8H, m, 4 × CH_2), 0.96, 0.94 (6H, 2d, *J* 6.6 Hz, CH(CH_3)$_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) *major isomer* δ 138.7, 137.5, 128.3, 127.6, 127.5, 72.9, 70.5, 29.9, 29.8, 29.4, 29.2, 27.3, 26.4, 26.2, 23.2 ppm; LRMS (ESI+) *m*/*z* 313 [(M + K)$^+$, 100%]; HRMS (ESI+) calcd for $C_{19}H_{30}O_1Na$ [M + Na]$^+$, 297.2189; found [M + Na]$^+$, 297.2193.

*(E/Z)-12-(Benzyloxy)-2-methyldodec-4-ene (**13**):* Prepared from 3.59 mmol of 8-(benzyloxy)-1-octanol and isoamyl(triphenyl)-phosphonium bromide; yield = 0.64 g, 62%, ∼5:1 inseparable diasteromeric mixture, colorless oil; R_f [10 vol % EtOAc/hexane] = 0.4; IR (thin film) ν_{max} = 2951, 2926, 2854, 1721, 1463, 1365, 1273, 1099, 1028, 734, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *diastereomeric mixture* δ 7.36–7.27 (5H, m, aromatic CH), 5.45–5.16 (2H, m, C*H*=C*H*), 4.52 (2H, s, benzylic CH_2), 3.54, 3.49 (2H, 2t, *J* 6.6 Hz, CH_2OBn), 2.05–1.87 (4H, m, 2 × CH=CHCH_2), 1.67–1.59 (3H, m, C*H*$(CH_3)_2$, CH_2), 1.42–1.29 (10H, m, 5 x CH_2), 0.92, 0.89 (6H, 2d, *J* 6.6 Hz, CH(CH_3)) ppm; LRMS (ESI+) *m*/*z* 311 [(M + Na)$^+$, 100%]; HRMS (ESI+) calcd for $C_{20}H_{32}ONa$ [M + Na]$^+$, 311.2345; found [M + Na]$^+$, 311.2348. Data are in agreement with that reported by Shiori and Irako.[23]

*(E/Z)-13-(Benzyloxy)-2-methyltridec-3-ene (**14**):* Prepared from 3.20 mmol of 10-(benzyloxy)decanol and isobutyl(triphenyl)-phosphoium bromide; yield = 0.58 g, 60%, ∼5:1 inseparable diasteromeric mixture, colorless oil; R_f [10 vol % EtOAc/hexane] = 0.4; IR (thin film) ν_{max} = 2925, 2854, 1463, 1438, 1361, 1202, 1117, 1102, 733, 696, 542 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *diastereomeric mixture* δ 7.34–7.33 (4H, m, aromatic *ortho/meta*-CH), 7.28 (1H, m, aromatic *para*-CH), 5.36–5.15 (2H, m, C*H*=C*H*), 4.50 (2H, s, benzylic CH_2), 3.46 (2H, t, *J* 6.7 Hz, CH_2OBn), 2.59, 2.22 (1H, 2m, C*H*$(CH_3)_2$), 2.02, 1.96 (1H, 2m, CH=CHCH_2), 1.64–1.58 (2H, m, CH_2), 1.38–1.28 (12H, m, 6 × CH_2), 0.96, 0.94 (6H, 2d, *J* = 6.7 Hz, CH(CH_3)$_2$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) *major isomer* δ 138.9, 137.6, 128.5, 127.8, 127.7, 127.6, 73.0, 70.7, 30.1, 29.9, 29.7, 29.6, 29.4, 27.5, 26.6, 26.3, 23.4 ppm; LRMS (ESI+) *m*/*z* 325 [(M+Na)$^+$, 100%]; HRMS (ESI+) calcd for $C_{21}H_{34}ONa$ [M + Na]$^+$, 325.2502; found [M + Na]$^+$, 325.2505.

Alkenoates 15–17.



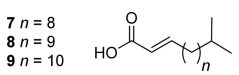
A mixture of alkenes **12**–**14** and 5 wt % palladium on carbon (0.05 equiv) in methanol was stirred under a H_2 atmosphere at rt for 3 h. The reaction mixture was then filtered over Celite, and the resulting filtrate was concentrated to yield the desired alcohol, which was used in the next step without further purification. A solution of the above alcohol (1 equiv) and Dess-Martin periodinane (1.5 equiv) in wet CH_2Cl_2 was stirred at rt for 1 h. Upon completion, saturated aqueous sodium hydrogen carbonate and sodium thiosulfate (1:1 v/v) were added, and the mixture was stirred for 20–30 min, until the lower organic layer was observed to transform from a white suspension to a colorless solution. The immiscible mixture was then separated, and the aqueous layer was further extracted into CH_2Cl_2 (3×). The combined CH_2Cl_2 extracts were finally dried over anhydrous magnesium sulfate and concentrated to yield the desired aldehyde, which was used in the next step without further purification. A mixture of the above aldehyde (1 equiv) and ethyl 2-(triphenylphosphoranylidene)acetate (1.5 equiv) in CH_2Cl_2 was stirred at rt for 16 h. The solvent was removed by rotary evaporation, and the crude residue was purified by column chromatography (0–5 vol % EtOAc in hexane) to yield pure α,β-unsaturated ethyl ester **15**–**17**.

*Ethyl (E)-12-Methyltridec-2-enoate (**15**):* Prepared from 0.46 mmol of (*E*/*Z*)-11-(benzyloxy)-2-methylundec-3-ene (**12**); yield = 66 mg, 56% over three steps, colorless oil; R_f [5 vol % EtOAc/hexane] = 0.2; IR (thin film) ν_{max} = 3000, 2954, 2627, 2826, 2044, 1724, 1694, 1651, 1445 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.96 (1H, dt, *J* = 7.0 Hz and 15.6 Hz, EtO_2CCH=C*H*), 5.81 (1H, dt, *J* = 1.6 Hz and 15.6 Hz, EtO_2CC*H*=CH), 4.18 (2H, q, *J* = 7.1 Hz, CH_3CH_2O), 2.17 (2H, m, CH=CHCH_2), 1.51 (1H, m, C*H*$(CH_3)_2$), 1.48–1.41 (2H, m, CH_2), 1.33–1.24 (13H, m, 5 × CH_2 and CH_3CH_2O), 1.14 (2H, m, CH_2), 0.86 (6H, d, *J* = 6.6 Hz, CH(CH_3)$_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0 (C_q=O); 149.7 (EtO_2CCH=CH), 121.4 (EtO_2CCH=CH), 60.3 (OCH_2CH_3), 39.2, 32.4, 30.0, 29.7, 29.6, 29.3, 28.2 (7 × CH_2), 28.1 ($CH(CH_3)_2$), 27.5 (CH_2), 22.8 ($CH(CH_3)_2$), 14.4 (OCH_2CH_3) ppm; LRMS (ESI+) *m*/*z* 277 [(M + Na)$^+$, 100%]; HRMS (ESI+) calcd for $C_{16}H_{30}O_2Na$ [M + Na]$^+$, 277.2138; found [M + Na]$^+$, 277.2141.

*Ethyl (E)-13-Methyltetradec-2-enoate (**16**):* Prepared from 0.23 mmol of (*E*/*Z*)-12-(benzyloxy)-2-methyldodec-4-ene (**13**); yield = 39 mg, 61% over 3 steps, colorless oil; R_f [5 vol % EtOAc/hexane] = 0.2; IR (thin film) ν_{max} = 2953, 2925, 2854, 1723, 1655, 1466, 1367, 1309, 1265, 1180, 1045, 979 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.96 (1H, dt, *J* = 7.0 Hz and 15.6 Hz, EtO_2CCH=C*H*), 5.81 (1H, dt, *J* = 1.6 Hz and 15.6 Hz, EtO_2CC*H*=CH), 4.18 (2H, q, *J* = 7.1 Hz, CH_3CH_2O), 2.19 (2H, m, CH=CHCH_2), 1.56–1.41 (3H, m, C*H*$(CH_3)_2$ and CH_2), 1.33–1.24 (15H, m, 6 × CH_2 and CH_3CH_2O), 1.15 (2H, m, CH_2), 0.86 (6H, d, *J* = 6.6 Hz, CH(CH_3)$_2$) ppm; LRMS (ESI+) *m*/*z* 291 [(M+Na)$^+$, 100%]; HRMS (ESI+) calcd for $C_{17}H_{32}O_2Na$ [M + Na]$^+$, 291.2295; found [M + Na]$^+$, 291.2297. Data are in agreement with that reported by Shiori et al.[23] and Suami et al.[24]

*Ethyl (E)-14-Methylpentadec-2-enoate (**17**):* Prepared from 0.13 mmol of (*E*/*Z*)-13-(benzyloxy)-2-methyltridec-3-ene (**14**); yield = 23 mg, 62% over three steps, colorless oil; R_f [5 vol % EtOAc/hexane] = 0.2; IR (thin film) ν_{max} = 2956, 2920, 2859, 1731, 1649 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.96 (1H, dt, *J* = 7.0 Hz and 15.6 Hz, EtO_2CCH=C*H*), 5.80 (1H, dt, *J* = 1.6 Hz and 15.6 Hz, EtO_2CC*H*=CH), 4.18 (2H, q, *J* = 7.1 Hz, CH_3CH_2O), 2.19 (2H, m, CH=CHCH_2), 1.55–1.41 (3H, m, C*H*$(CH_3)_2$ and CH_2), 1.33–1.24 (17H, m, 7 × CH_2 and CH_3CH_2O), 1.15 (2H, m, CH_2), 0.86 (6H, d, *J* = 6.6 Hz, CH(CH_3)$_2$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.0 (C=O), 149.7 (EtO_2CCH=CH), 121.4 (EtO_2CCH=CH), 60.3 (OCH_2CH_3), 39.2 ($CH_2CH(CH_3)_2$), 32.4 (CH=CHCH_2), 30.1, 29.8, 29.8, 29.7, 29.5, 29.3, 28.2 (7 × CH_2), 28.1 ($CH(CH_3)_2$), 27.6 (CH_2), 22.8 ($CH(CH_3)_2$), 14.4 (OCH_2CH_3) ppm; LRMS (ESI+) *m*/*z* 305 [(M + Na)$^+$, 100%]; HRMS (ESI+) calcd for $C_{18}H_{34}O_2Na$ [M + Na]$^+$, 305.2451; found [M + Na]$^+$, 305.2453.

Alkenoic Acids 7–9.



A solution of α,β-unsaturated ethyl esters **15**–**17** in NaOH (1 M) and *tert*-butyl alcohol (1:1 v/v, 2 mL) was stirred at 60 °C for 6 h. After being cooled to rt, the reaction was acidified with $HCl_{(aq)}$ (0.5 M, 2 mL) and extracted into CH_2Cl_2 (3 × 3 mL). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate, and concentrated to dryness to yield the α,β-unsaturated fatty acids **7**–**9**, which were used without further purification.

*(E)-12-Methyltridec-2-enoic Acid (**7**):* Prepared from 47 mg (0.19 mmol) of ethyl (*E*)-12-methyltridec-2-enoate **15**; yield = 41 mg, 98%, colorless oil; IR (thin film) ν_{max} = 2951, 2924, 2853, 1696, 1650, 1466, 1420, 1308, 1285, 980, 939 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.08 (1H, dt, *J* = 7.3 Hz and 14.6 Hz, HO_2CCH=C*H*), 5.81 (1H, d, *J* = 15.5 Hz, HO_2CC*H*=CH), 2.23 (2H, m, CH=CHCH_2), 1.56–1.43 (3H, m, CH_2, C*H*$(CH_3)_2$), 1.33–1.24 (10H, m, 5 × CH_2), 1.15 (2H, m, CH_2), 0.85 (6H, d, *J* = 6.5 Hz, CH(CH_3)$_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2 (C=O), 152.6 (HO_2CCH=CH), 120.5 (HO_2CCH=CH), 39.2 ($CH_2CH(CH_3)_2$), 32.5 (CH=CHCH_2), 30.0 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 28.1 (CH_2), 28.0

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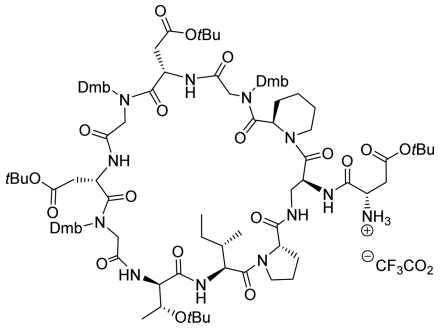
($CH(CH_3)_2$), 27.5 (CH_2), 22.8 ($CH(CH_3)_2$) ppm; LRMS (ESI+) m/z 225 [$(M - H)^+$, 100%]; HRMS (ESI+) calcd for $(C_{14}H_{26}O_2)_2Na$ [2M + Na]$^+$, 476.3792; found [2M + Na]$^+$, 476.3792.

*(E)-13-Methyltetradec-2-enoic Acid (**8**):* Prepared from 28 mg (0.10 mmol) of ethyl (*E*)-13-methyltetradec-2-enoate **16**; yield = 24 mg, 98%, colorless oil; IR (thin film) ν_{max} = 2955, 2923, 2853, 1722, 1699, 1464 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (1H, dt, J = 7.0 and 15.5 Hz, $HO_2CCH{=}CH$), 5.83 (1H, dt, J = 1.5 and 15.6 Hz, $HO_2CCH{=}CH$), 2.23 (2H, m, $CH{=}CHCH_2$), 1.56−1.43 (3H, m, $CH(CH_3)_2$ and CH_2), 1.34−1.24 (12H, m, 6 × CH_2), 1.15 (2H, m, CH_2), 0.86 (6H, d, J = 6.6 Hz, $CH(CH_3)_2$) ppm; LRMS (ESI+) m/z 239 [$(M + H)^+$, 100%]; HRMS (ESI+) calcd for $C_{15}H_{27}O_2$ [$M - H]^-$, 239.2017; found [$M - H]^-$, 239.2020. Data are in agreement with that reported by Suami et al.[24]

*(E)-14-Methylpentadec-2-enoic Acid (**9**):* Prepared from 15 mg (53 μmol) of (*E*)-14-methylpentadec-2-enoate **17**; yield = 13 mg, 96%, colorless oil; IR (thin film) ν_{max} = 2952, 2925, 2854, 1698, 1651, 1466, 1421, 1308, 1285 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (1H, dt, J = 15.6 and 7.0 Hz, $HO_2CCH{=}CH$), 5.83 (1H, dt, J = 15.6 and 1.5 Hz, $HO_2CCH{=}CH$), 2.23 (2H, m, $CH{=}CHCH_2$), 1.57−1.43 (3H, m, $CH(CH_3)_2$ and CH_2), 1.33−1.24 (14H, m, 7 × CH_2), 1.15 (2H, m, CH_2), 0.86 (6H, d, J = 6.6 Hz, $CH(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 ($C_q{=}O$), 152.5 ($HO_2CCH{=}CH$), 120.3 ($HO_2CCH{=}CH$), 39.2 ($CH_2CH(CH_3)_2$), 32.5 ($HO_2CCH{=}CHCH_2$), 30.1, 29.8, 29.8, 29.7, 29.5, 29.3, 28.1 (7 × CH_2), 28.0 ($CH(CH_3)_2$), 27.6 (CH_2), 22.8 ($CH(CH_3)_2$) ppm; LRMS (ESI+) m/z 277 [$(M + Na)^+$, 100%]; HRMS (ESI+) calcd for $C_{16}H_{30}O_2Na$ [M + Na]$^+$, 277.2138; found [M + Na]$^+$, 277.2141.

Cyclic Peptide 5.



Resin Loading. 2-Chlorotrityl chloride resin (100−200 mesh) with 1% divinylbenzene (474 mg, 1.14 mmol g^{-1}, 0.540 mmol, 1 equiv) was allowed to swell in dry CH_2Cl_2 (5 mL) for 30 min. After being drained, the resin was suspended in a solution of Fmoc-L-Pro-OH (364 mg, 1.08 mmol, 2 equiv) and iPr$_2$NEt (376 μL, 2.16 mmol, 4 equiv) in DMF/CH_2Cl_2 (1:1 v/v, 10 mL) and shaken for 16 h. The resin was subsequently washed with DMF (5 × 5 mL), CH_2Cl_2 (5 × 5 mL), and DMF (5 × 5 mL) before being capped with a mixture of CH_2Cl_2/MeOH/iPr$_2$NEt (17:2:1 v/v/v, 5 mL) for 1 h. The resin was again washed with DMF (5 × 5 mL), CH_2Cl_2 (5 × 5 mL), and DMF (5 × 5 mL).

Fmoc-SPPS. An iterative strategy of Fmoc deprotection and amino acid coupling was repeated sequentially for Fmoc-L-Ile-OH, Fmoc-D-allo-Thr(*t*Bu)-OH, Fmoc-(Dmb)Gly-OH, Fmoc-L-Asp(*t*Bu)-OH, Fmoc-(Dmb)Gly-OH, Fmoc-L-Asp(*t*Bu)-OH, Fmoc-(Dmb)Gly-OH, Fmoc-D-Pip-OH, Fmoc-L-Dap(Alloc)-OH, and Fmoc-L-Asp(*t*Bu)-OH.

Fmoc Deprotection: A solution of 10 vol % piperidine in DMF (5 mL) was added to the resin and shaken for 3 min (2×). The resin was subsequently washed with DMF (5 × 5 mL), CH_2Cl_2 (5 × 5 mL), and DMF (5 × 5 mL). Following the initial Fmoc deprotection, the efficiency of amino acid loading was determined by spectroscopic measurement of the resulting fulvene piperidine adduct at λ = 301 nm (ε = 7800 M^{-1} cm^{-1}). ***Caution: Resin cross-linking was found to occur upon extended standing after the initial Fmoc deprotection (as evidenced by resin clumping and the complete inability to acylate the resin-bound proline residue). Therefore, it was crucial to couple the second amino acid residue immediately after the initial Fmoc deprotection.***

Standard Amino Acid Coupling: For standard amino acids (Fmoc-L-Ile-OH and Fmoc-L-Asp(*t*Bu)-OH), a solution of the protected amino acid (2.70 mmol, 5 equiv), DIC (423 μL, 2.70 mmol, 5 equiv), and oxyma (384 mg, 2.70 μmol, 5 equiv) in DMF (5 mL) was added to the resin and shaken. After 1 h, the resin was washed with DMF (5 × 5 mL), CH_2Cl_2 (5 × 5 mL), and DMF (5 × 5 mL).

Nonstandard Amino Acid Coupling: For Fmoc-D-allo-Thr(*t*Bu)-OH, Fmoc-(Dmb)Gly-OH, Fmoc-D-Pip-OH, Fmoc-L-Dap(Alloc)-OH), a solution of the protected amino acid (0.810 mmol, 1.5 equiv), DIC (127 μL, 0.810 mmol, 1.5 equiv), and oxyma (115 mg, 0.810 mmol, 1.5 equiv) in DMF (4 mL) were added to the resin and shaken for 4 h. The resin was washed with DMF (5 × 5 mL), CH_2Cl_2 (5 × 5 mL), and DMF (5 × 5 mL).

This strategy was followed to yield the resin-bound linear peptide: Fmoc-L-Asp(^{t}Bu)-L-Dap(Alloc)-D-Pip-(Dmb)Gly-L-Asp(^{t}Bu)-(Dmb)-Gly-L-Asp(^{t}Bu)-(Dmb)Gly-D-allo-Thr(^{t}Bu)-L-Ile-L-Pro-resin.

Alloc Deprotection. A solution of $Pd(PPh_3)_4$ (624 mg, 0.540 mmol, 1 equiv) and $PhSiH_3$ (1.1 mL, 8.6 mmol, 16 equiv) in CH_2Cl_2 (20 mL) was added to the resin and shaken for 1 h. The resin was subsequently washed with CH_2Cl_2 (5 × 5 mL), DMF (5 × 5 mL), and CH_2Cl_2 (5 × 5 mL).

Cleavage. A solution of 30 vol % HFIP in CH_2Cl_2 (20 mL) was added to the resin and shaken for 30 min before the resin was filtered off and rinsed with CH_2Cl_2. This process was repeated a further three times for 10 min, and the combined filtrates were concentrated in vacuo. The residue was azeotroped three times with CH_2Cl_2 and dried in vacuo to yield the crude linear peptide as an off-white foam.

Macrolactamization. iPr$_2$NEt (189 μL, 1.08 mmol, 2 equiv) was added to a solution of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM·BF_4, 192 mg, 0.651 mmol, 1.2 equiv) and the crude linear peptide (0.540 mmol, 1 equiv) in DMF (50 mL, 0.01 M). The resulting reaction mixture was stirred at rt for 16 h before the solvent was removed in vacuo.

Final Fmoc Deprotection and Purification. The crude Fmoc-protected cyclic peptide was dissolved in a solution of 10 vol % piperidine in MeCN (10 mL), and the mixture was stirred for 30 min at rt. The mixture was then concentrated in vacuo and azeotroped with toluene (2×) and CH_2Cl_2 (2×). Finally, the residue was dissolved in a mixture of MeCN and H_2O, filtered, and purified by preparative reverse-phase HPLC (Waters Sunfire C18 5 μm, 30 × 150 mm, 40 mL·min^{-1}, 30−100% MeCN [0.1% TFA] in H_2O [0.1% TFA] over 30 min).

The above steps afforded pure cyclic peptide **5** (416 mg, 42% over 36 steps) as a fluffy white solid following lyophilization: analytical HPLC R_t = 4.6 min; 0 to 100% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 5 min; acquity UPLC BEH C18, 1.7 μm, 2.1 × 50 mm, 214 nm; sample dissolved in MeCN/H_2O 1:1; LRMS (ESI+) 1549 [M − Dmb]$^+$, 1700 [M + H]$^+$, 1721 [M + Na]$^+$; HRMS (ESI+) calcd for $C_{85}H_{127}N_{12}O_{24}Na$ [M + Na + H]$^{2+}$, 861.4487; found, 861.4497; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.45 (1H, d, J = 7.7 Hz, Dap NH-2), 8.31−8.29 (2H, m, Thr NH-2, Asp NH-2), 8.23 (1H, d, J = 7.0 Hz, Ile NH-2), 8.07 (1H, d, J = 3.9 Hz, Asp NH-2), 7.73 (3H, d, J = 3.5 Hz, Asp NH_3^+-2), 7.13−6.74 (4H, m, Dap NH-3, 3 × Dmb ArH), 6.58−6.35 (6H, m, 6 × Dmb ArH), 5.42 (1H, m, Pip H2), 5.27 (1H, m, Asp H2), 5.15−5.09 (2H, m, Dap H2, DmbCH$_2$a), 4.86 (1H, m, Asp H2), 4.76−4.71 (2H, m, Gly H2a, DmbCH$_2$a), 4.58 (1H, d, J = 14.6 Hz, DmbCH$_2$a), 4.43−4.38 (2H, m, Asp H2, Gly H2a), 4.29 (1H, t_{apt}, J = 8.9 Hz, Ile H2), 4.06 (1H, t_{apt}, J = 9.2 Hz, Thr H2), 3.97−3.60 (28H, m, 3 × DmbCH$_2$b, Pro CH$_2$-5, Gly CH$_2$-2, 6 × Dmb ArOCH$_3$, Thr H3, Pip H6a, Pro H2), 3.31−3.25 (2H, m, Dap H3a, Gly H2b), 3.11−3.06 (2H, m, Pip H6b, Dap H3b), 3.01−2.88 (3H, m, Gly H2b, 2 × Asp H3a), 2.82−2.56 (3H, m, Asp H3a, 2 × Asp H3b), 2.37 (1H, m, Asp H3b), 2.08 (1H, m, Pip H3a), 2.01−1.91 (2H, m, Pro H4a, Pip H4a), 1.85−1.71 (3H, m, Pro H4b, Pro H3a, Ile H3), 1.61 (1H, m, Ile H4a), 1.64−1.51 (11H, m, Pro H3b, Pip H4b, CO$_2$*t*Bu), 1.45−1.22 (22H, m, 2 × CO$_2$*t*Bu, Pip H3b, Ile H4b, Pip CH$_2$-5), 1.12−0.83 (18H, m, Thr CH$_3$-4, O*t*Bu, Ile CH$_3$-4, Ile CH$_3$-5) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.4−166.3 (14 × C═O), 160.8−157.9 (6 × ArO), 131.7, 128.8, 128.5 (3 × ArH), 116.0, 116.0, 115.9 (3 × ArC), 105.7−104.2 (3 × ArH), 98.6−97.6 (3 × ArH), 81.9, 80.5, 79.8, 73.7

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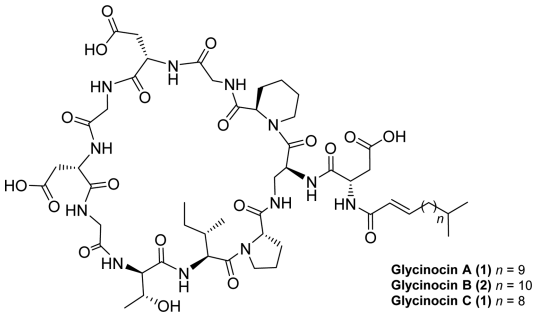
(4 × $C(CH_3)_3$), 65.9 (Thr C3), 60.2 (Pro C2), 59.2 (Thr C2), 55.6–55.2 (3 × OCH_3) 54.9 (Ile C2), 50.7 (Pip C2), 49.9 (Asp C2), 49.8 (Gly C2), 49.2 (Gly C2), 49.0 (Dap C2), 47.6 (Pro C5), 46.4 (Asp C2), 45.9 (Asp C2), 45.7 ($DmbCH_2$), 45.4 ($DmbCH_2$), 44.5 (Gly C2), 43.7 (Pip C6), 43.5 ($DmbCH_2$), 42.3 (Dap C3), 37.2 (Asp C3), 35.7 (Ile C3), 35.3 (Asp C3), 35.1 (Asp C3), 28.4–27.6 (4 × $C(CH_3)_3$), 27.9 (Pro C3), 25.9 (Pip C3), 24.9 (Ile C4), 24.8 (Pro C4), 21.0 (Thr C4), 19.8 (Pip C5), 19.7 (Pip C4), 14.3 (Ile C4′), 10.4 (Ile C5) ppm. See Supporting Information for tabulated HSQC cross-peaks.

Glycinocins A–C (1–3).



To an Eppendorf tube containing cyclic peptide trifluoroacetate salt **5** (12 mg, 6.6 μmol, 1 equiv) were added a freshly prepared solution of fatty acid **7–9** (2 equiv), iPr_2NEt (4.6 μL, 4 equiv), and *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl, 2.5 mg, 2 equiv) in DMF (130 μL, 50 mM with respect to cyclic peptide). 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/iPr_3SiH/H_2O (18:1:1 v/v/v) and allowed to stand for 2.5 h at rt. 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, 19 × 150 mm, 7 mL min^{-1}, 20 for 1 min to 100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 45 min, UV at 230 nm). In cases where the cyclic lipopeptide coeluted with a nonpeptidic impurity, a second pass purification was performed using buffers of 0.1% TFA in MeCN and H_2O under conditions otherwise identical to those stated above. The cyclic lipopeptides **1–3** were obtained as white fluffy solids after lyophilization.

Glycinocin A (1): Yield = 3.96 mg, 48% (two steps); $[\alpha]_D$ −9.9 (*c* = 0.27, MeOH), lit[16] −22; analytical HPLC R_t = 4.0 min; 0 to 100% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 5 min; acquity UPLC BEH C18, 1.7 μm, 2.1 × 50 mm, 214 nm; sample dissolved in MeCN/H_2O 1:1. LRMS (ESI+) 1247 $[M + H]^+$, 1269 $[M + Na]^+$; HRMS (ESI+) calcd for $C_{57}H_{90}N_{12}O_{19}Na$ $[M + Na]^+$ 1269.6337, found 1269.6349; ^1H NMR (600 MHz, DMSO-d_6) δ 8.41–7.67 (9H, m, 9 × NH), 7.50 (1H, t, *J* = 5.5 Hz, Dap NH-3), 6.62 (1H, dt, *J* = 7.0, 15.0 Hz, FA H3), 5.93 (1H, d, *J* = 15.0 Hz, FA H2), 4.79 (1H, m, Pip H2), 4.67–4.56 (3H, m, Dap H2, 2 × Asp H2), 4.50 (1H, m, Asp H2), 4.39–4.27 (3H, m, Pip H6a, Ile C2, Thr H2), 4.18 (1H, m, Pro H2), 3.99 (1H, dd, Gly H2a), 3.83–3.60, (7H, m, Gly H2b, 2 × Gly CH_2–2, Thr H3, Pro H5a), 3.59–3.45 (2H, m, Dap H3a, Pro H5b), 3.09 (1H, m, Dap H3b), 2.86 (1H, m, Pip H6b), 2.76–2.46 (6H, m, 3 × Asp CH_2–3), 2.18 (1H, m, Pip H3a), 2.11 (2H, m, FA CH_2–4), 2.01 (1H, m, Pro H3a), 1.92 (1H, m, Pro H4a), 1.81 (1H, m, Pro H4b), 1.76–1.70 (2H, m, Pro H3b, Ile H3), 1.59–1.46 (5H, m, Pip H5a, Pip H4a, Pip H3b, Ile H4a, FA H13), 1.41–1.36 (2H, m, FA CH_2–5, Pip H4b), 1.28–1.20 (13H, m, FA CH_2–6 to CH_2–11, Pip H5b), 1.12 (2H, m, FA CH_2–12), 1.07–0.99 (4H, m, Ile H4b, Thr CH_3-4), 0.90–0.77 (12H, m, Ile CH_3-4′, CH_3-14, CH_3-14′, Ile CH_3-5) ppm. See Supporting Information for tabulated HSQC cross-peaks and comparison with literature spectra.

Glycinocin B (2): Yield = 3.56 mg, 43% (two steps); $[\alpha]_D$ −6.2 (*c* = 0.23, MeOH), lit[16] −19; analytical HPLC R_t = 4.2 min; 0 to 100% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 5 min; acquity UPLC BEH C18, 1.7 μm, 2.1 × 50 mm, 214 nm; sample dissolved in MeCN/H_2O 1:1; LRMS (ESI+) 1261 $[M + H]^+$, 1283 $[M + Na]^+$; HRMS (ESI+) calcd for $C_{58}H_{92}N_{12}O_{19}Na$ $[M + Na]^+$ 1283.6494, found 1283.6506; ^1H NMR (600 MHz, DMSO-d_6) δ 8.38–7.68 (9H, m, 9 × NH), 7.50 (1H, m, Dap NH-3), 6.62 (1H, dt, *J* = 7.0, 15.0 Hz, FA H3), 5.93 (1H, d, *J* = 15.0 Hz, FA H2), 4.80 (m, 1H, Pip H2), 4.66–4.44 (4H, m, Dap H2, 3 × Asp H2), 4.36–4.17 (4H, m, Pip H6a, Ile C2, Thr H2, Pro H2), 3.99–3.60 (8H, m, 3 × Gly CH_2-2, Thr H3, Pro H5a), 3.56–3.47 (2H, m, Dap H3a, Pro H5b), 3.15 (1H, m, Dap H3b), 2.85 (1H, m, Pip H6b), 2.70–2.45 (6H, m, 3 × Asp CH_2-3), 2.19 (1H, m, Pip H3a), 2.12 (2H, m, FA CH_2-4), 2.01 (1H, m, Pro H3a), 1.92 (1H, m, Pro H4a), 1.82–1.70 (3H, m, Pro H4b, Pro H3b, Ile H3), 1.60–1.46 (5H, m, Pip H5a, Pip H4a, Pip H3b, Ile H4a, FA H14), 1.42–1.36 (2H, m, FA CH_2-5, Pip H4b), 1.28–1.20 (15H, m, FA CH_2-6 to CH_2-12, Pip H5b), 1.13 (2H, m, FA CH_2-13), 1.07–1.01 (4H, m, Ile H4b, Thr CH_3-4), 0.91–0.75 (12H, m, Ile CH_3-4′, CH_3-15, CH_3-15′, Ile CH_3-5) ppm. See Supporting Information for tabulated HSQC cross-peaks.

Glycinocin C (3): Yield 3.98 mg, 49% (two steps); $[\alpha]_D$ −13 (*c* = 0.28, MeOH), lit[13] −13; analytical HPLC R_t = 3.8 min; 0 to 100% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 5 min; acquity UPLC BEH C18, 1.7 μm, 2.1 × 50 mm, 214 nm; sample dissolved in MeCN/H_2O 1:1; LRMS (ESI+) 1233 $[M + H]^+$, 1255 $[M + Na]^+$; HRMS (ESI+) calcd for $C_{56}H_{88}N_{12}O_{19}Na$ $[M + Na]^+$ 1255.6181, found 1255.6191; ^1H NMR (600 MHz, DMSO-d_6) δ 8.39–7.69 (9H, m, 9 × NH), 7.50 (1H, m, Dap NH-3), 6.63 (1H, dt, *J* = 6.8, 15.4 Hz, FA H3), 5.93 (1H, d, *J* = 15.4 Hz, FA H2), 4.79 (1H, m, Pip H2), 4.67–4.56 (3H, m, Dap H2, 2 × Asp H2), 4.49 (1H, m, Asp H2), 4.37–4.25 (3H, m, Pip H6a, Ile C2, Thr H2), 4.18 (1H, m, Pro H2), 3.98 (1H, dd, Gly H2a), 3.85–3.46, (9H, m, Gly H2b, 2 × Gly CH_2-2, Thr H3, Pro H5a, Dap H3a, Pro H5b), 3.11 (1H, m, Dap H3b), 2.86 (1H, m, Pip H6b), 2.74–2.45 (6H, m, 3 × Asp CH_2-3), 2.19 (1H, m, Pip H3a), 2.12 (2H, m, FA CH_2-4), 2.02 (1H, m, Pro H3a), 1.93 (1H, m, Pro H4a), 1.84–1.69 (3H, m, Pro H4b, Pro H3b, Ile H3), 1.60–1.46 (5H, m, Pip H5a, Pip H4a, Pip H3b, Ile H4a, FA H12), 1.41–1.36 (2H, m, FA CH_2-5, Pip H4b), 1.28–1.22 (11H, m, FA CH_2-6 to CH_2-10, Pip H5b), 1.13 (2H, m, FA CH_2-11), 1.07–1.00 (4H, m, Ile H4b, Thr CH_3-4), 0.91–0.77 (12H, m, Ile CH_3-4′, CH_3-13, CH_3-13′, Ile CH_3-5) ppm. See Supporting Information for tabulated HSQC cross-peaks.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b01959.

Copies of the ^1H and ^{13}C NMR spectra for all novel fatty acids and intermediates; ^1H NMR spectra for previously reported fatty acids and intermediates; ^1H NMR, ^{13}C NMR, COSY, TOCSY, HSQC, and HMBC spectra for cyclic peptide **5**; key HSQC and HMBC data and spectra for **1–3**; analytical HPLC traces and low-resolution mass spectra for cyclic peptide **5** and **1–3**; and NMR spectral comparison of synthetic **1–3** with authentic material (PDF)

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Notes

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