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Total Synthesis, Stereochemical Assignment, and Antimalarial Activity of Gallinamide A

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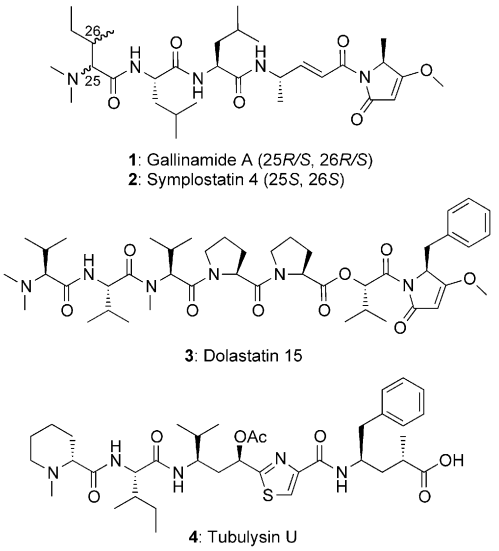
Abstract: The total synthesis and stereochemical assignment of gallinamide A, an antimalarial depsipeptide of cyanobacterial origin, is described. Synthesis of the four possible N-terminal diastereoisomers of gallinamide A (including the natural product symplostatin 4) was achieved using a divergent strategy from a common imide fragment. The natural product and corresponding diastereoisomers were synthesized in 30–33% overall yield in a longest linear sequence of 8 steps. Comparative NMR spectroscopic studies of the four synthetic diastereoisomers with the isolated natural product demonstrated that gallinamide A possesses a dimethylated L-isoleucyl residue at the N-terminus. As such, we have shown that gallinamide A is structurally and stereochemically identical to symplostatin 4. Gallinamide A and its N-terminal diastereoisomers were also shown to possess significant antimalarial activity with IC_{50} values in the nanomolar range against the 3D7 strain of *Plasmodium falciparum*.

Keywords: biological activity · malaria · natural products · parasiticides · peptides · total synthesis

Introduction

Malaria is a mosquito-borne infectious disease caused by protozoan parasites of the genus *Plasmodium*, with the most severe human form of malaria being caused by *Plasmodium falciparum* (*P. falciparum*).[1] In 2008, there were 247 million reported cases and nearly one million deaths as a result of malaria, with approximately 40% of the world's population living in endemic areas.[2] Poor compliance with existing antimalarial drug regimens has resulted in the emergence of strains of *P. falciparum* resistant to the majority of the frontline antimalarials. As such, there is currently an urgent need for the elucidation of new therapies for the treatment of malaria that operate through novel modes of action.[2c]

Gallinamide A (**1**, Scheme 1) is a linear depsipeptide natural product, first isolated in 2008 by Linington and coworkers from a *Schizothrix* species of cyanobacteria near Piedras Gallinas (Caribbean coast of Panama).[3] Gallinamide A possesses the identical linear structure to symplostatin 4 (compound **2**, Scheme 1), a depsipeptide isolated by Luesch and co-workers in 2010 from cyanobacteria of the genus *Symploca* collected from Key Largo (Florida Keys).[4] Interesting structural features of these cyanobacterial secondary metabolites include an aliphatic peptidic backbone, a dimethylated N-terminal amino acid, an unusual 4(*S*)-amino-2(*E*)-pentenoic acid moiety, and a C-terminal *N*-acylpyrrolinone unit. Unlike symplostatin 4, the absolute configuration of the *N,N*-dimethylisoleucyl (C25 and C26 in Scheme 1) stereocenters of gallinamide A were not elucidat-



Scheme 1. Gallinamide A (**1**) and structurally related marine natural products.

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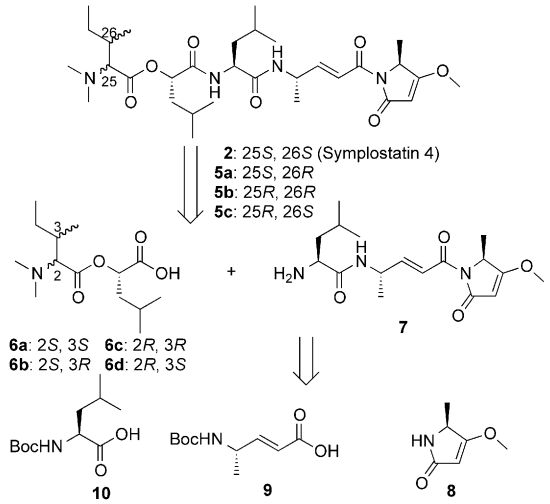
ed, owing to a lack of isolated material.[3] However, the spectral data provided for isolated symplostatin 4 suggested that these two natural products were diastereomeric.[4]

Gallinamide A (**1**) and symplostatin 4 (**2**) share several structural features with a number of remarkably potent, tubulin-modifying cytotoxic agents including symplostatin 1,[5] the dolastatins[6], and tubulysins[7] (e.g., compounds **3** and **4**, Scheme 1), derivatives of which have entered phase II clinical trials as anticancer agents.[8] Although dolastatins 10 and 15 have been shown to possess extremely potent antimalarial activity,[9] use of these compounds in a clinical setting is restricted by their significant cytotoxicity. Encouragingly, symplostatin 4 has been shown to possess only moderate cytotoxic activity (IC_{50} values of 12 and 53 μM against HeLa cervical carcinoma cells and HT-29 colon adenocarcinoma cells, respectively)[4] and potent inhibition of growth of the 3D7 strain of *P. falciparum*.[10] Similarly, gallinamide A has been reported to exhibit moderate antimalarial activity (IC_{50} of 8.4 μM) against the chloroquine resistant W2 strain of *P. falciparum*, only moderate activity against mammalian Vero cells, and surprisingly, no detectable cytotoxicity to NCI-H460 lung tumor or neuro-2a mouse neuroblastoma cell lines.[3]

The encouraging pharmacological profiles of gallinamide A and symplostatin 4 suggests that these natural products, and derivatives thereof, may serve as promising lead structures for the elucidation of antimalarial agents with low cytotoxicity. Accordingly, we were inspired to develop an efficient and general synthetic route towards these natural products that would facilitate the complete stereochemical assignment of gallinamide A and prove amenable to the preparation of structurally related analogues for evaluation as antimalarial agents in the future.

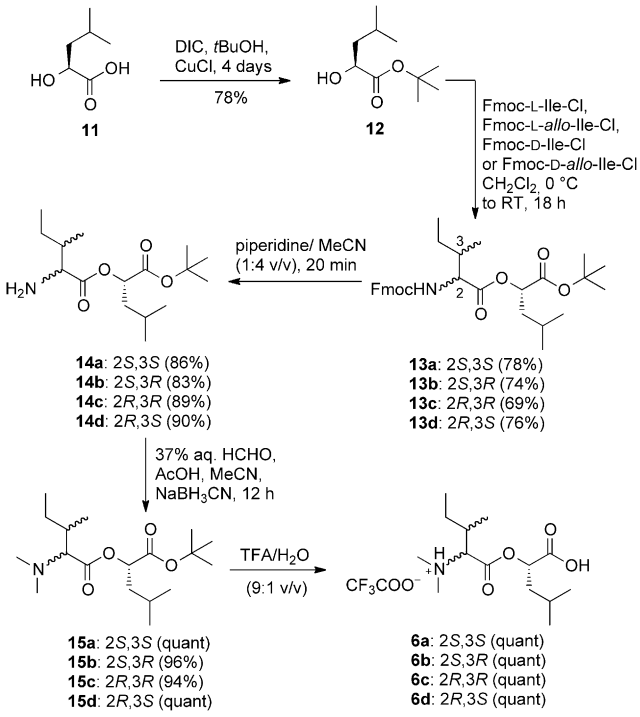
Results and Discussion

To determine the absolute stereochemistry of the N-terminal *N,N*-dimethylisoleucyl moiety in gallinamide A, and to establish the effect of N-terminal stereochemistry on antiparasitic activity, we embarked on the synthesis of each of the possible N-terminal stereoisomers of the natural product (**2** and **5a–c**, Scheme 2). We envisaged that each of the N-terminal diastereoisomers could be synthesized through a final fragment condensation between the corresponding N-terminal isomeric fragments **6a–d** and a key C-terminal imide fragment **7**. Synthesis of isomeric depsipeptide fragments **6a–d** could be achieved by esterification of a suitably protected (*S*)-2-hydroxy isocaproic acid (Hic) unit and the corresponding isomeric isoleucine (Ile) building blocks, namely 9-fluorenylmethoxycarbonyl (Fmoc)-protected L-Ile, L-*allo*-Ile, D-Ile, and D-*allo*-Ile. Finally, we proposed to access the C-terminal fragment **7** from the convergent assembly of three suitably protected building blocks: pyrrolinone unit **8**, *tert*-butoxycarbonyl (Boc)-protected 4(*S*)-amino-2(*E*)-pentenoic acid (Apa) **9**, and Boc-L-leucine-OH **10**.



Scheme 2. Retrosynthesis of four possible N-terminal diastereoisomers of gallinamide A.

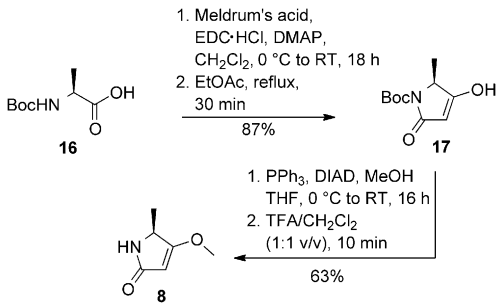
Synthesis of fragments **6a–d** began with carboxyl protection of (*S*)-2-hydroxyisocaproic acid (**11**) as the corresponding *tert*-butyl ester.[10] Treatment of **11** with in situ generated *tert*-butyl isourea[11] provided **12** in 78% yield (Scheme 3). Installation of the requisite N-terminal stereochemistry was achieved through subsequent esterification of **12** with the corresponding acid chlorides, derived from each of the four commercially available diastereoisomers of Fmoc-Ile-OH. Analysis of the ^1H and ^{13}C NMR spectra indicated that the stereochemical integrity of the isoleucyl moiety had been re-



Scheme 3. Synthesis of N-terminal amino esters **6a–d**. DIC = *N,N'*-diisopropylcarbodiimide; AcOH = acetic acid; TFA = trifluoroacetic acid.

tained in the product amino esters **13a–d** (see the Supporting Information). In preparation for N-terminal derivatization, removal of the Fmoc protecting group was achieved by treatment with 20% piperidine in acetonitrile to afford **14a–d** in 83–90% yield. Reductive alkylation of the free amino functionality with aqueous formaldehyde and sodium cyanoborohydride subsequently gave the desired *N,N*-dimethylated amino esters **15a–d**, in excellent yields. Acidolysis of the C-terminal ester with trifluoroacetic acid (TFA) completed the preparation of the desired isomeric amino esters **6a–d** in quantitative yields in all cases.

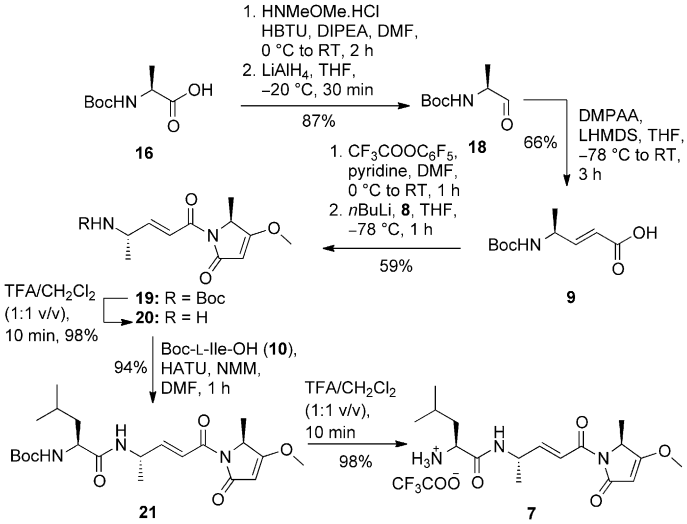
Having successfully synthesized the N-terminal fragments **6a–d**, we turned our attention to the synthesis of C-terminal fragment **7**. Preparation of the C-terminal pyrrolinone moiety **8** began from Boc-L-alanine **16**, which was condensed with Meldrum's acid using *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) and 4-(dimethylamino)pyridine (DMAP; Scheme 4).[12] The resulting



Scheme 4. Synthesis of pyrrolinone fragment **8**. EDC·HCl = *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride; DMAP = 4-(dimethylamino)pyridine; DIAD = diisopropyl azodicarboxylate.

acyl-Meldrum's adduct was not isolated, but instead, subsequent reflux in ethyl acetate effected the desired thermal cyclization, giving the desired pyrrolinone **17** as a 1:3 mixture of keto–enol tautomers (87% over 2 steps). Subsequent attempts at selective O-methylation using tosyl methoxide and potassium hexamethyldisilazide proved low yielding, and provided a mixture of regioisomeric products. However, the desired transformation was smoothly achieved through Mitsunobu alkylation with diisopropyl azodicarboxylate (DIAD), triphenylphosphine and methanol.[13] Acidolysis of the Boc carbamate completed the preparation of pyrrolinone **8** in good yield (63% over two steps). Interestingly, performing the Mitsunobu reaction following acidolysis of the Boc carbamate precluded alkylation of the enol functionality. This reflects the increased electron density in the pyrrolinone and the resulting diminished acidity, as evidenced by a shift in tautomeric forms (no enol tautomer observed by ^{1}H NMR spectroscopy).

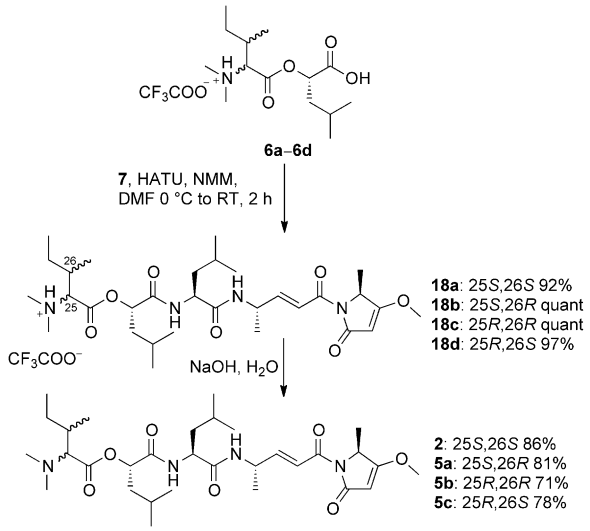
Having prepared pyrrolinone **8**, our attention turned to the preparation of Boc-protected Apa unit **9**. Previous experiments conducted in our laboratory have highlighted the susceptibility of the imide linkage in *N*-acylpyrrolinones to aminolysis. As such, we chose to protect the amino functionality of Apa as the corresponding Boc carbamate rather than the piperidine labile Fmoc carbamate. Synthesis of **9** began from Boc-L-alanine **16**, which was first converted to the corresponding Weinreb amide using *N,O*-dimethylhydroxylamine hydrochloride, HBTU, and DIPEA as a hindered base (Scheme 5). Subsequent reduction of the Weinreb amide with lithium aluminium hydride afforded the desired aldehyde **18** in 87% yield over two steps. A Horner–Wadsworth–Emmons reaction between **18** and dimethylphosphonoacetic acid (DMPAA) using lithium hexamethyldisilazide (LHMDS) as a base gave only the (*E*)-diastereoisomer of the requisite Boc-protected Apa fragment **9** in 66% yield.[10,14] In preparation for the key imide coupling between **9** and pyrrolinone **8**, the carboxylic acid of **9** was first activated as the corresponding pentafluorophenyl ester with pentafluorophenyl trifluoroacetate and pyridine.[15] Deprotonation of the pyrrolinone amide with *n*BuLi followed by the addition of the freshly prepared pentafluorophenyl ester provided *N*-acylpyrrolinone **19** in moderate yield. Analysis of ^{1}H and ^{13}C NMR spectra indicated that the reaction had proceeded without epimerization (see the Supporting Information). From here, acidolytic cleavage of the Boc carbamate provided **20**, which was coupled to Boc-L-Ile-OH using 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) as the coupling reagent and *N*-methylmorpholine (NMM) as the base to afford **21** in 94% yield. Treatment with TFA then provided the C-terminal fragment **7** in 98% yield as the corresponding trifluoroacetate salt.



Scheme 5. Synthesis of C-terminal fragment **7**. HBTU = *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; DIPEA = *N,N*-diisopropylethylamine; DMF = dimethylformamide; THF = tetrahydrofuran, DMPAA = dimethylphosphonoacetic acid; LHMDS = lithium hexamethyldisilazide; PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; NMM = *N*-methylmorpholine.

With C-terminal fragment **7** and the isomeric N-terminal fragments **6a–d** in hand, all that remained to complete the synthesis of the four gallinamide A diastereoisomers was the

fragment condensation. This was performed using HATU in the presence of NMM, and gave the desired isomeric forms of gallinamide A (as the trifluoroacetate salts) in excellent yields following purification by HPLC (Scheme 6). Notably, as the depsipeptide fragments are not capable of forming oxazoline intermediates in the C-terminal activation step, these reactions afforded the desired depsipeptides without epimerization (as determined by analytical HPLC and NMR spectroscopy). The trifluoroacetate salts **18a–d** were subsequently converted, using aqueous NaOH, to the corresponding free bases **2** and **5a–c**, which were obtained in good yields following purification by HPLC.



Scheme 6. Synthesis of the N-terminal diastereoisomers of gallinamide A (**2** and **5a–5c**).

Having prepared each of the four possible N-terminal diastereoisomers of gallinamide A (including symplostatin 4: compound **2**) we next sought to determine the absolute configuration of the C25 and C26 stereocentres of gallinamide A by comparison of the ^{1}H and ^{13}C NMR spectral data to that published for the isolated material. Not surprisingly, analysis of the NMR spectra of **2** and **5a–c** showed large degrees of similarity in chemical shift and coupling constants (see the Supporting Information). However, a number of subtle differences were observed for signals corresponding to the N-terminal region of the molecules in the ^{1}H NMR spectra. Specifically, the chemical shift for a multiplet corresponding to the N-terminal isoleucyl β-proton (i.e., at C26) for both D-*allo*-Ile and L-*allo*-Ile containing compounds **5a** and **5c**, respectively, were a poor match with the same signal (multiplet at $\delta = 1.16$ ppm) in the spectra of the isolated natural product (see black box in Figure 1). In contrast, the corresponding signals for the N-terminal L-Ile and D-Ile-containing compounds **2** and **5b** were both in excellent agreement to the resonances observed in the spectrum of the natural product. Analysis of the ^{1}H NMR signals corresponding to the α proton (C25) of the isoleucyl moiety (doublet at

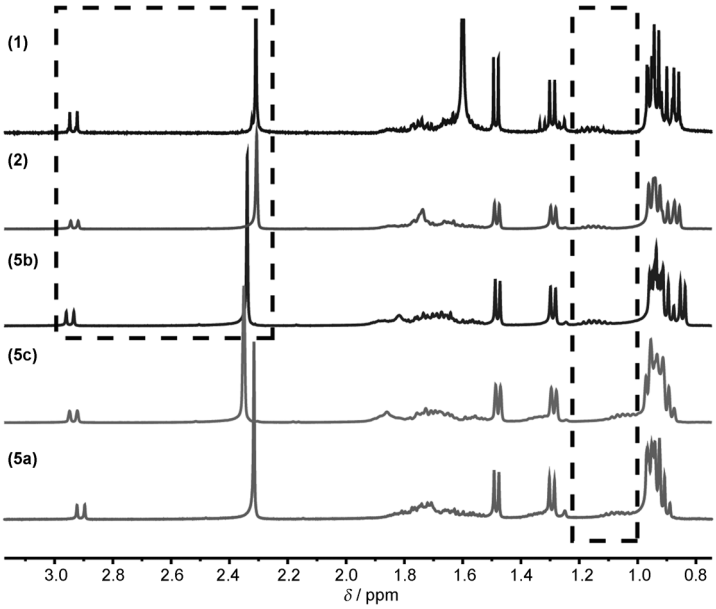


Figure 1. ^{1}H NMR chemical shift comparison (between $\delta = 0.8$ and 3.0 ppm) for isolated gallinamide A (**1**) and synthetic diastereoisomers **2** and **5a–c** (NMR solvent = $CDCl_3$).

$\delta = 2.94$ ppm) and the *N,N*-dimethyl protons (singlet at $\delta = 2.31$ ppm) for **5b** and **2** showed that the D-Ile-containing compound **5b** was a poor match to the isolated natural product spectrum. However, compound **2**, bearing the N-terminal *N,N*-dimethylated-L-isoleucyl moiety, provided signals that were almost identical in this region to those observed for isolated gallinamide A (see black box in Figure 1). Furthermore, upon inspection of all signals in the ^{1}H and ^{13}C NMR spectra for **2** and **5a–c**, compared to the authentic gallinamide A, compound **2** provided an extremely close correlation to isolated gallinamide A (Figure 2a). In contrast, chemical shifts for N-terminal diastereoisomers **5a–c** were not in complete agreement with all signals of the natural product (Figure 2b–2d). Although all four diastereoisomers provided similar measured specific rotations, **2** was in closest agreement in terms of magnitude. Taken together, these data provide strong evidence for a 25*S*, 26*S* diastereomeric configuration at the *N,N*-dimethylated isoleucyl residue of gallinamide A. This configuration is identical to that found in symplostatin 4 and, as such, these compounds do not have a diastereomeric relationship (as first predicted) but are identical natural products.

Having successfully prepared the four N-terminal diastereoisomers of gallinamide A we were next interested in investigating their antimalarial activity. The compounds were screened against the 3D7 strain of *P. falciparum* using a [^{3}H]-hypoxanthine incorporation assay. Gratifyingly, all four stereoisomers were found to potently inhibit *P. falciparum* growth, with IC_{50} values ranging from 37–104 nM (Table 1, see the Supporting Information for raw data). Importantly, these compounds displayed similar potencies to the widely employed front line antimalarial chloroquine ($IC_{50} = 17.8$ nM), which was included in these studies as a positive

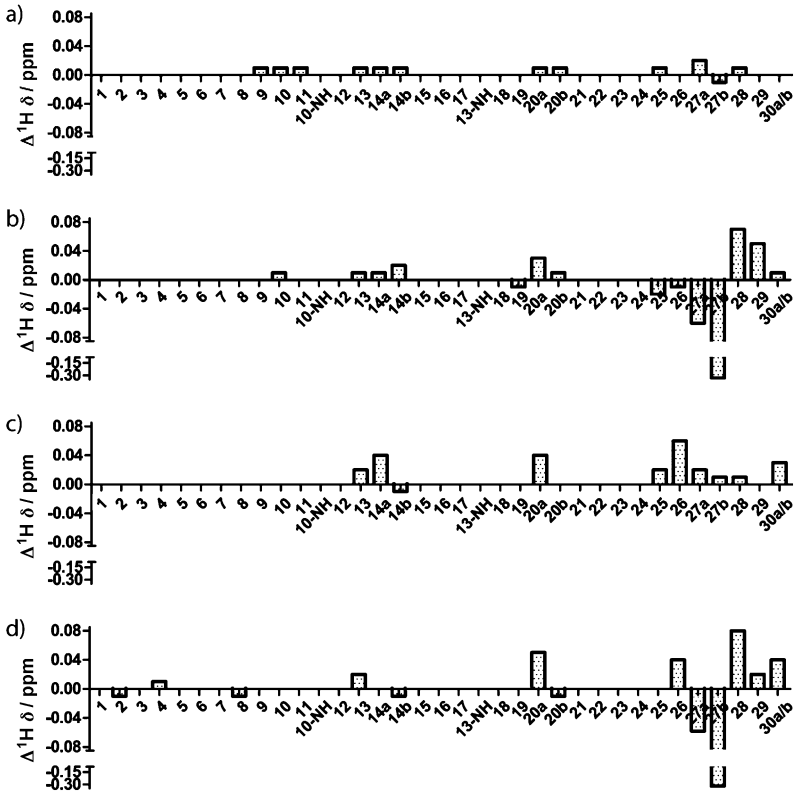


Figure 2. ^{1}H NMR chemical shift differences between isolated gallinamide A (**1**) and synthetic diastereoisomers: a) **2**, b) **5a**, c) **5b** and d) **5c**. NB: due to concentration effects, comparison of the majority of NH proton resonances are not included.

Table 1. Anti-parasitic activity of **2** (gallinamide A) and diastereoisomers **5a–c** against *P. falciparum* (3D7 strain).

Compound	IC_{50} [nM][a]
Chloroquine	17.8±2.3
2 (gallinamide A)	50.1±7.6
5a	104±33
5b	36.8±9.4
5c	54.8±8.9

[a] IC_{50} values were determined by the use of a [^{3}H]-hypoxanthine incorporation assay (see the Supporting Information for details and raw data).

control. These compounds therefore serve as promising leads for the development of natural product analogues with increased antiparasitic activity and biological stability.

Conclusion

In summary, we have reported an efficient synthesis of the four possible N-terminal diastereoisomers of the cyanobacterially derived natural products gallinamide A and symplostatin 4. The natural products (and derivatives) **2** and **5a–b** were synthesized in 30–33% overall yield in 8 steps in the longest linear sequence through a convergent assembly of two key building blocks, namely, depsipeptides **6a–d** and imide **7**. Comparative NMR studies of **2** and **5a** and **5b** with the spectra of the isolated natural product has enabled the absolute stereochemical configuration of gallinamide A to be assigned as 25*S*, 26*S* at the N-terminal *N,N*-dimethylisoleucyl moiety. As such, we have demonstrated that gallinamide A and symplostatin 4, isolated from two different species of cyanobacteria in Panama and Florida, respectively, are identical natural products. Each diastereoisomer was also shown to exhibit potent growth inhibitory activity (in the same range as chloroquine) against the 3D7 strain of *P. falciparum*. These results suggest that analogues of gallinamide A may serve as novel antimalarials; work towards which is currently underway in our laboratories.

Experimental Section

General procedure for esterification of isocaproate 12: Fmoc-Ile-OH (0.24 g, 0.66 mmol) was dissolved in neat $SOCl_2$ (1.9 mL, 27 mmol) and the reaction was stirred at room temperature for 24 h. The reaction was subsequently concentrated in vacuo to afford the acid chloride as a pale yellow solid which was used immediately in the next reaction. The acid chloride was dissolved in dry CH_2CH_2 (1 mL) and the reaction cooled to 0°C. A solution of *t*Bu-2-hydroxyisocaproate (Hic) **12** (0.10 g, 0.53 mmol) in dry CH_2CH_2 (0.3 mL) was added dropwise and the reaction was subsequently allowed to warm to room temperature and stirred for 24 h. The reaction was diluted with CH_2CH_2 (20 mL) and washed with saturated aqueous Na_2CO_3 solution (5 mL), 0.1 M HCl (5 mL) and brine (5 mL) before drying over $MgSO_4$. The solvent was concentrated in vacuo and the resulting residue purified by column chromatography.

Fmoc-L-*Ile-Hic-OtBu* (***13a***): Isocaproate **12** (0.10 g, 0.53 mmol) was esterified with Fmoc-L-Ile-OH according to the general procedure. The product was purified by column chromatography (eluent: 10% Et_2O/hexane) to afford **13a** as a colorless oil (0.22 g, 78%). R_f=0.4 (4:1 v/v hexane/Et_2O); $[\alpha]_D^{25}=-21.4$ (c=2.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.77 (d, 2H, J=7.6 Hz, 2×ArH), 7.60 (d, 2H, J=7.2 Hz, 2×ArH), 7.40 (app. t, 2H, J=7.4 Hz, 2×ArH), 7.32 (app. t, 2H, J=7.4 Hz, 2×ArH), 5.37 (d, 1H, J=9.2 Hz, NH), 4.92 (dd, 1H, J=9.0, 4.4 Hz, Hic α-H), 4.45–4.37 (m, 3H, Ile α-H, Fmoc CH_2), 4.24 (t, 1H, J=7.0 Hz, Fmoc CH), 2.03–1.96 (m, 1H, CH), 1.83–1.72 (m, 2H, CH_2), 1.68–1.56 (m, 2H, CH_2), 1.47 (s, 9H, 3×CH_3), 1.32–1.20 (m, 1H, CH), 1.07–0.85 ppm (m, 12H, 4×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=171.8, 169.4, 156.1, 144.0, 141.4, 127.8, 127.1, 125.2, 120.1, 82.3, 72.7, 67.1, 58.2, 47.3, 39.9, 38.2, 28.1, 24.9, 24.8, 23.0, 21.8, 15.4, 11.8 ppm; IR (thin film) $\tilde{\nu}$=3359, 2961, 2934, 2875, 1725, 1511, 1450, 1390, 1368, 1338 cm^{-1}; MS (ESI) m/z (%): 546 (100) $[M+Na]^+$; HRMS (ESI): m/z calcd for $C_{31}H_{41}NO_6Na$ $[M+Na]^+$: 546.2826; found: 546.2824.

Fmoc-L-*allo-Ile-Hic-OtBu* (***13b***): Isocaproate **12** (0.10 g, 0.53 mmol) was esterified with Fmoc-L-*allo*-Ile-OH according to the general procedure. The product was purified by column chromatography (eluent: 10%

Et_2O/hexane) to afford **13b** as a colorless oil (0.21 g, 74%). R_f=0.25 (5:1 v/v hexane/Et_2O); $[\alpha]_D^{25}$=−20.0 (c=1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ=7.76 (d, 2H, J=7.5 Hz, 2×Ar H), 7.62 (d, 2H, J=6.9 Hz, 2×Ar H), 7.40 (app. t, 2H, J=7.2 Hz, 2×Ar H), 7.32 (app. t, 2H, J=7.2 Hz, 2×Ar H), 5.40 (d, 1H, J=9.3 Hz, NH), 4.97 (dd, 1H, J=8.6, 4.2 Hz, Hic α-H), 4.58 (dd, 1H, J=9.5, 3.0 Hz, Ile α-H), 4.41 (d, 2H, J=7.2 Hz, Fmoc CH_2), 4.24 (t, 1H, J=7.2 Hz, Fmoc CH), 2.19–2.08 (m, 1H, CH), 1.87–1.58 (m, 4H, 2×CH_2), 1.49 (s, 9H, 3×CH_3), 1.36–1.25 (m, 1H, CH), 1.07–0.88 ppm (m, 12H, 4×CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ=172.2, 169.4, 156.4, 144.0, 141.3, 127.7, 127.1, 125.1, 120.0, 82.1, 72.4, 67.1, 56.9, 47.2, 39.8, 37.7, 28.0, 26.4, 24.7, 23.0, 21.8, 14.2, 11.8 ppm; IR (thin film) $\tilde{\nu}$=3347, 2962, 2937, 2876, 1729, 1514, 1450, 1388, 1368, 1330 cm^{-1}; MS (ESI) m/z (%): 546 (100) $[M+Na]^+$; HRMS (ESI): m/z calcd for $C_{31}H_{41}NO_6Na$ $[M+Na]^+$: 546.2826; found: 546.2831.

*Fmoc-D-Ile-Hic-OtBu (**13c**)*: Isocaproate **12** (0.10 g, 0.53 mmol) was esterified with Fmoc-D-Ile-OH according to the general procedure. The product was purified by column chromatography (eluent: 10% Et_2O/hexane) to afford **13c** as a colorless oil (0.19 g, 69%). R_f=0.25 (5:1 v/v hexane/Et_2O); $[\alpha]_D^{25}$=−22.5 (c=0.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ=7.77 (d, 2H, J=7.5 Hz, 2×Ar H), 7.61 (d, 2H, J=7.5 Hz, 2×Ar H), 7.40 (app. t, 2H, J=7.2 Hz, 2×Ar H), 7.32 (app. t, 2H, J=6.9 Hz, 2×Ar H), 5.33 (d, 1H, J=9.0 Hz, NH), 4.93 (dd, 1H, J=9.5, 3.3 Hz, Hic α-H), 4.47 (dd, 1H, J=9.0, 4.5 Hz, Ile α-H), 4.40 (d, 2H, J=7.2 Hz, Fmoc CH_2), 4.24 (t, 1H, J=6.9 Hz, Fmoc CH), 2.06–1.94 (m, 1H, CH), 1.84–1.55 (m, 4H, 2×CH_2), 1.46 (s, 9H, 3×CH_3), 1.32–1.18 (m, 1H, CH), 1.02–0.84 ppm (m, 12H, 4×CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ=171.6, 169.3, 156.1, 144.1, 141.4, 127.8, 127.2, 125.2, 120.1, 82.3, 72.5, 67.2, 58.6, 47.3, 39.8, 38.1, 28.0, 25.0, 24.7, 23.2, 21.4, 15.6, 11.7 ppm; IR (thin film) $\tilde{\nu}$=3350, 2962, 2935, 2876, 1728, 1608, 1513, 1450, 1391, 1368, 1328 cm^{-1}; MS (ESI) m/z (%): 546 (100) $[M+Na]^+$; HRMS (ESI): m/z calcd for $C_{31}H_{41}NO_6Na$ $[M+Na]^+$: 546.2826; found: 546.2831.

*Fmoc-D-allo-Ile-Hic-OtBu (**13d**)*: Isocaproate **12** (0.10 g, 0.53 mmol) was esterified with Fmoc-D-*allo*-Ile-OH according to the general procedure. The product was purified by column chromatography (eluent: 10% Et_2O/hexane) to afford **13d** as a colorless oil (0.21 g, 76%). R_f=0.25 (5:1 v/v hexane/Et_2O); $[\alpha]_D^{25}$=−16.0 (c=0.8, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ=7.77 (d, 2H, J=7.5 Hz, 2×Ar H), 7.61 (d, 2H, J=7.2 Hz, 2×Ar H), 7.40 (app. t, 2H, J=7.5 Hz, 2×Ar H), 7.32 (app. t, 2H, J=7.5 Hz, 2×Ar H), 5.30 (d, 1H, J=9.3 Hz, NH), 4.94 (dd, 1H, J=9.3, 3.6 Hz, Hic α-H), 4.59 (dd, 1H, J=9.3, 3.6 Hz, Ile α-H), 4.40 (d, 2H, J=7.2 Hz, Fmoc CH_2), 4.24 (t, 1H, J=6.9 Hz, Fmoc CH), 2.08–1.99 (m, 1H, CH), 1.85–1.59 (m, 4H, 2×CH_2), 1.46 (s, 9H, 3×CH_3), 1.39–1.30 (m, 1H, CH), 1.03–0.84 ppm (m, 12H, 4×CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ=172.0, 169.3, 156.3, 144.1, 141.4, 127.8, 127.2, 125.2, 120.1, 82.3, 72.5, 67.2, 57.3, 47.3, 39.8, 37.9, 28.0, 26.4, 24.8, 23.1, 21.5, 14.5, 11.8 ppm; IR (thin film) $\tilde{\nu}$=3370, 2961, 2935, 2874, 1729, 1512, 1450, 1390, 1369, 1325 cm^{-1}; MS (ESI) m/z (%): 546 (100) $[M+Na]^+$; HRMS (ESI): m/z calcd for $C_{31}H_{41}NO_6Na$ $[M+Na]^+$: 546.2826; found: 546.2831.

General procedure for Fmoc deprotection: Amino ester **13** (0.20 g, 0.38 mmol) was dissolved in a 4:1 v/v MeCN/piperidine solution (3 mL) and the reaction was stirred for 20 min. The reaction was subsequently concentrated in vacuo and the residue was purified by column chromatography.

*H_2N-L-Ile-Hic-OtBu (**14a**)*: Amino ester **13a** (0.20 g, 0.38 mmol) was Fmoc deprotected according to the general procedure. The product was purified by column chromatography (eluent: 2:1 v/v hexane/EtOAc + 1% Et_3N) to afford **14a** as a colorless oil (0.10 g, 86%). R_f=0.2 (2:1 v/v hexane/EtOAc + 1% Et_3N); $[\alpha]_D^{25}$=−11.9 (c=1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ=4.91 (dd, 1H, J=9.2, 4.4 Hz, Hic α-H), 3.40 (d, 1H, J=4.8 Hz, Ile α-H), 1.85–1.69 (m, 3H, CH, CH_2), 1.64–1.50 (m, 2H, CH_2), 1.46 (s, 9H, 3×CH_3), 1.31–1.20 (m, 1H, CH), 1.01–0.89 ppm (m, 12H, 4×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=175.2, 169.7, 81.9, 71.9, 59.1, 39.9, 39.0, 28.0, 24.7, 24.5, 23.0, 21.7, 15.6, 11.7 ppm; IR (thin film) $\tilde{\nu}$=2961, 2934, 2875, 1735, 1602, 1463, 1391, 1368 cm^{-1}; MS (ESI) m/z (%): 302 (100) $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{16}H_{32}NO_4$ $[M+H]^+$: 302.2326; found: 302.2326.

*H_2N-L-allo-Ile-Hic-OtBu (**14b**)*: Amino ester **13b** (0.20 g, 0.38 mmol) was Fmoc deprotected according to the general procedure. The product was purified by column chromatography (eluent: 2:1 v/v hexane/EtOAc + 1% Et_3N) to afford **14b** as a colorless oil (0.09 g, 83%). R_f=0.4 (1:1 v/v hexane/EtOAc + 1% Et_3N); $[\alpha]_D^{25}$=−16.7 (c=0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ=4.90 (dd, 1H, J=9.4, 4.0 Hz, Hic α-H), 3.51 (d, 1H, J=3.6 Hz, Ile α-H), 1.89–1.91 (m, 1H, CH), 1.81–1.73 (m, 2H, CH_2), 1.63–1.57 (m, 1H, C*H*H), 1.46 (s, 9H, 3×CH_3), 1.42–1.29 (m, 2H, CH*H*, CH), 0.99–0.88 ppm (m, 12H, 4×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=175.7, 169.8, 82.0, 71.8, 57.5, 39.9, 38.0, 28.0, 26.6, 24.7, 23.1, 21.7, 13.6, 12.0 ppm; IR (thin film) $\tilde{\nu}$=2961, 2935, 2875, 1737, 1601, 1464, 1368 cm^{-1}; MS (ESI) m/z (%): 302 (100) $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{16}H_{32}NO_4$ $[M+H]^+$: 302.2326; found: 302.2325.

*H_2N-D-Ile-Hic-OtBu (**14c**)*: Amino ester **13c** (0.17 g, 0.32 mmol) was Fmoc deprotected according to the general procedure. The product was purified by column chromatography (eluent: 2:1 v/v hexane/EtOAc + 1% Et_3N) to afford **14c** as a colorless oil (0.08 g, 89%). R_f=0.4 (1:1 v/v hexane/EtOAc + 1% Et_3N); $[\alpha]_D^{25}$=−59.5 (c=0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ=4.88 (dd, 1H, J=9.8, 3.6 Hz, Hic α-H), 3.42 (d, 1H, J=4.8 Hz, Ile α-H), 1.83–1.69 (m, 3H, CH, CH_2), 1.61–1.47 (m, 11H, CH_2, 3×CH_3), 1.26–1.13 (m, 1H, CH), 0.98–0.86 ppm (m, 12H, 4×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=175.6, 169.9, 82.1, 71.7, 59.1, 39.8, 38.7, 28.0, 24.7, 24.5, 23.2, 21.3, 15.9, 11.7 ppm; IR (thin film) $\tilde{\nu}$=2961, 2935, 2875, 1736, 1605, 1463, 1391, 1368 cm^{-1}; MS (ESI) m/z (%): 302 (100) $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{16}H_{32}NO_4$ $[M+H]^+$: 302.2326; found: 302.2325.

*H_2N-D-allo-Ile-Hic-OtBu (**14d**)*: Amino ester **13d** (0.09 g, 0.17 mmol) was Fmoc deprotected according to the general procedure. The product was purified by column chromatography (eluent: 2:1 v/v hexane/EtOAc + 1% Et_3N) to afford **14d** as a colorless oil (0.05 g, 90%). R_f=0.4 (1:1 v/v hexane/EtOAc + 1% Et_3N); $[\alpha]_D^{25}$=−55.5 (c=0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ=4.91 (dd, 1H, J=10.0, 4.0 Hz, Hic α-H), 3.56 (d, 1H, J=4.0 Hz, Ile α-H), 1.92–1.70 (m, 3H, CH, CH_2), 1.63–1.48 (m, 11H, CH_2, 3×CH_3), 1.42–1.22 (m, 1H, CH), 1.01–0.91 (m, 9H, 3×CH_3), 0.85 ppm (d, 3H, J=6.8 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=175.9, 169.9, 82.1, 71.7, 57.4, 39.8, 38.0, 28.0, 26.5, 24.7, 23.2, 21.3, 13.8, 11.9 ppm; IR (thin film) $\tilde{\nu}$=2960, 2935, 2875, 1737, 1605, 1462, 1369 cm^{-1}; MS (ESI) m/z (%): 302 (100) $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{16}H_{32}NO_4$ $[M+H]^+$: 302.2326; found: 302.2326.

General procedure for N,N-dimethylation: To amino ester **14** (0.08 g, 0.26 mmol) in MeCN (1 mL) was added 37% aqueous formaldehyde (210 μL, 2.59 mmol) followed by AcOH (10 μL). The reaction was stirred for 1 h before $NaBH_3CN$ (42 mg, 0.67 mmol) was added. The reaction was stirred for 18 h at room temperature and AcOH was added periodically to maintain the pH at 5–7. The reaction was subsequently concentrated in vacuo and the residue redissolved in EtOAc (30 mL). The organic fraction was then washed with saturated aqueous Na_2CO_3 solution (15 mL), brine (15 mL), dried ($MgSO_4$) and concentrated in vacuo to an oil which was purified by column chromatography.

*Me_2N-L-Ile-Hic-OtBu (**15a**)*: Amino ester **14a** (0.09 g, 0.29 mmol) was *N,N*-dimethylated according to the general procedure. The product was purified by column chromatography (eluent: 6:1 v/v hexane/EtOAc + 1% Et_3N) to afford **15a** as a colorless oil (0.10 g, quant). R_f=0.40 (5:1 v/v hexane/EtOAc + 1% Et_3N); $[\alpha]_D^{25}$=−51.1 (c=1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ=4.86 (dd, 1H, J=9.8, 3.6 Hz, Hic α-H), 2.90 (d, 1H, J=10.4 Hz, Ile α-H), 2.29 (s, 6H, 2×CH_3), 1.88–1.49 (m, 5H, CH, 2×CH_2), 1.44 (s, 9H, 3×CH_3), 1.19–1.08 (m, 1H, CH), 0.98–0.83 ppm (m, 12H, 4×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=171.2, 170.0, 81.9, 72.2, 71.4, 41.4, 40.0, 33.3, 28.0, 25.0, 24.6, 23.2, 21.3, 15.8, 10.4 ppm; IR (thin film) $\tilde{\nu}$=2961, 2935, 2874, 2833, 2789, 1751, 1732, 1456, 1368 cm^{-1}; MS (ESI) m/z (%): 330 (100) $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{18}H_{35}NO_4Na$ $[M+Na]^+$: 352.2458; found: 352.2460.

*Me_2N-L-allo-Ile-Hic-OtBu (**15b**)*: Amino ester **14b** (0.09 g, 0.29 mmol) was N,N-dimethylated according to the general procedure. The product was purified by column chromatography (eluent: 6:1 v/v hexane/EtOAc + 1% Et_3N) to afford **15b** as a colorless oil (0.09 g, 96%). R_f=0.40 (5:1 v/v hexane/EtOAc + 1% Et_3N); $[\alpha]_D^{25}$=−46.5 (c=0.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ=4.86 (dd, 1H, J=9.6, 3.9 Hz, Hic α-H), 2.89 (d, 1H, J=10.8 Hz, Ile α-H), 2.32 (s, 6H, 2×CH_3), 1.87–1.61 (m, 3H, C*H*H, CH_2), 1.59–1.46 (m, 11H, CH, CH*H*, 3×CH_3), 1.13–0.83 ppm (m, 13H,

CH, 4×CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ=171.2, 170.0, 81.9, 72.9, 71.6, 41.4, 40.1, 34.1, 28.1, 26.3, 24.7, 23.3, 21.4, 15.3, 11.3 ppm; IR (thin film) $\tilde{\nu}$=2961, 2934, 2873, 2833, 2789, 1750, 1732, 1455, 1391, 1368 cm^{-1}; MS (ESI) *m/z* (%): 330 (100) $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{18}H_{36}NO_4$ $[M+H]^+$: 330.2639; found: 330.2638.

Me_2N-D-Ile-Hic-OtBu (***15c***): Amino ester **14c** (0.08 g, 0.26 mmol) was N,N-dimethylated according to the general procedure. The product was purified by column chromatography (eluent: 6:1 v/v hexane/EtOAc + 1% Et_3N) to afford **15c** as a colorless oil (0.08 g, 94%). R_f=0.40 (5:1 v/v hexane/EtOAc + 1% Et_3N); $[\alpha]_D^{25}=-26.4$ (c=1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ=4.86 (dd, 1H, J=9.3, 4.2 Hz, Hic α-H), 2.90 (d, 1H, J=9.9 Hz, Ile α-H), 2.34 (s, 6H, 2×CH_3), 1.89–1.45 (m, 15H, 2×CH, 2×CH_2, 3×CH_3), 1.18–1.06 (m, 1H, CH), 0.96–0.83 ppm (m, 12H, 4×CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ=171.6, 170.1, 81.9, 72.4, 71.6, 41.7, 40.1, 33.4, 28.1, 25.3, 24.7, 23.2, 21.5, 15.6, 10.6 ppm; IR (thin film) $\tilde{\nu}$=2961, 2936, 2874, 2795, 1750, 1732, 1456, 1368 cm^{-1}; MS (ESI) *m/z* (%): 330 (100) $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{18}H_{36}NO_4$ $[M+H]^+$: 330.2639; found: 330.2639.

Me_2N-D-allo-Ile-Hic-OtBu (***15d***): Amino ester **14d** (0.04 g, 0.13 mmol) was N,N-dimethylated according to the general procedure. The product was purified by column chromatography (eluent: 6:1 v/v hexane/EtOAc + 1% Et_3N) to afford **15d** as a colorless oil (0.04 g, quant). R_f=0.40 (5:1 v/v hexane/EtOAc + 1% Et_3N); $[\alpha]_D^{25}=-30.7$ (c=0.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ=4.85 (dd, 1H, J=9.3, 4.2 Hz, Hic α-H), 2.88 (d, 1H, J=10.5 Hz, Ile α-H), 2.35 (s, 6H, 2×CH_3), 1.87–1.71 (m, 3H, C*H*H, CH_2), 1.61–1.48 (m, 11H, CH, CH*H*, 3×CH_3), 1.12–0.84 ppm (m, 13H, CH, 4×CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ=171.5, 170.1, 81.9, 72.7, 71.7, 41.5, 40.1, 33.9, 28.1, 26.2, 24.7, 23.2, 21.6, 15.4, 11.2 ppm; IR (thin film) $\tilde{\nu}$=2961, 2934, 2875, 2796, 1750, 1732, 1456, 1392, 1369 cm^{-1}; MS (ESI) *m/z* (%): 330 (100) $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{18}H_{36}NO_4$ $[M+H]^+$: 330.2639; found: 330.2639.

General procedure for *t*Bu ester acidolysis: Amino ester **15** (0.08 g, 0.23 mmol) was dissolved in 9:1 v/v TFA/H_2O (3 mL) and the reaction was stirred at room temperature for 1 h. The reaction was then concentrated in vacuo and the residue evaporated with toluene to afford **6a–d** which required no further purification.

TFA·Me_2N-L-Ile-Hic-OH (***6a***): Amino ester **15a** (0.55 g, 1.7 mmol) was deprotected at the C-terminus according to the general procedure, to afford **6a** as a pale yellow oil which solidified on standing (0.64 g, quant). $[\alpha]_D^{25}=-11.2$ (c=1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.97 (br s, 1H, COOH), 5.10 (dd, 1H, J=9.4, 3.6 Hz, Hic-αH), 3.95 (d, 1H, J=4.8 Hz, Ile-αH), 2.93 (s, 6H, 2×CH_3), 2.24–2.13 (m, 1H, CH), 1.90–1.76 (m, 2H, Hic-CH, Ile-C*H*H), 1.75–1.66 (m, 1H, Ile-CH*H*), 1.66–1.56 (m, 1H, Hic-C*H*H), 1.52–1.41 (m, 1H, Hic-CH*H*), 1.03–0.90 ppm (m, 12H, 4×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=171.3, 166.0, 73.1, 71.6, 39.4, 33.9, 25.8, 24.6, 22.7, 20.9, 14.6, 11.0 ppm; IR (thin film) $\tilde{\nu}$=2967, 2879, 1743, 1667, 1467, 1415, 1390, 1372 cm^{-1}; MS (ESI) *m/z* (%): 274 (100) $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{14}H_{28}NO_4$ $[M+H]^+$: 274.2013; found: 274.2013.

TFA·Me_2N-L-allo-Ile-Hic-OH (***6b***): Amino ester **15b** (0.08 g, 0.23 mmol) was deprotected at the C-terminus according to the general procedure, to afford **6b** as a pale yellow oil which solidified on standing (0.09 g, quant) m.p.=142–144 °C; $[\alpha]_D^{25}=-26.0$ (c=1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ=5.18 (dd, 1H, J=9.5, 3.6 Hz, Hic α-H), 3.76 (d, 1H, J=10.2 Hz, Ile α-H), 3.04 (s, 6H, 2×CH_3), 2.08–1.96 (m, 1H, CH), 1.89–1.68 (m, 2H, Hic-CH, Hic-CH_2), 1.50–1.42 (m, 1H, Ile-C*H*H), 1.27–1.13 (m, 4H, Ile-CH*H*, CH_3), 1.07–0.91 ppm (m, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=172.0, 165.6, 73.0, 72.3, 39.4, 33.6, 25.6, 24.9, 23.1, 20.9, 14.5, 10.6 ppm; IR (thin film) $\tilde{\nu}$=2965, 2940, 2881, 1742, 1670, 1467, 1411, 1390, 1371 cm^{-1}; MS (ESI) *m/z* (%): 274 (100) $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{14}H_{28}NO_4$: $[M+H]^+$, 274.2013; found: 274.2013.

TFA·Me_2N-D-Ile-Hic-OH (***6c***): Amino ester **15c** (0.06 g, 0.18 mmol) was deprotected at the C-terminus according to the general procedure, to afford **6c** as a pale yellow oil which solidified on standing (0.07 g, quant). $[\alpha]_D^{25}=-26.8$ (c=1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ=5.12 (d, 1H, J=10.0 Hz, Hic α-H), 3.90 (d, 1H, J=6.8 Hz Ile α-H), 2.99 (s, 6H, 2×CH_3), 2.07 (m, 1H, CH), 1.89–1.61 (m, 4H, 2×CH_2), 1.37 (m, 1H, CH), 1.02–0.92 ppm (m, 12H, 4×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=172.0, 167.2, 73.2, 70.9, 39.1, 34.0, 26.0, 24.6, 23.1, 20.8, 14.3, 11.1 ppm; IR (thin film) $\tilde{\nu}$=2964, 2939, 2877, 1742, 1668, 1466, 1416, 1389, 1371 cm^{-1}; MS (ESI) *m/z* (%): 274 (100) $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{14}H_{28}NO_4$ $[M+H]^+$: 274.2013; found: 274.2016.

TFA·Me_2N-D-allo-Ile-Hic-OH (***6d***): Amino ester **15d** (0.04 g, 0.11 mmol) was deprotected at the *C*-terminus according to the general procedure, to afford **6c** as a pale yellow oil which solidified on standing (0.04 g, quant). $[\alpha]_D^{25}=-17.6$ (c=1.0, $CHCl_3$); ^{1}H NMR (100 MHz, $CDCl_3$) δ=5.10 (d, 1H, J=9.2 Hz, Hic α-H), 3.77 (d, 1H, J=8.4 Hz), 2.98 (s, 6H, 2×CH_3), 2.05 (m, 1H, CH), 1.89–1.72 (m, 3H, CH_2, C*H*H), 1.47 (m, 1H, CH*H*), 1.23–1.14 (m, 1H, CH), 1.66 (d, 3H, CH_3), 0.99–0.91 ppm (m, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=171.9, 167.5, 73.4, 70.7, 39.2, 34.0, 25.4, 24.7, 23.3, 21.0, 14.9, 10.6 ppm; IR (thin film) $\tilde{\nu}$=2961, 2938, 2875, 1740, 1672, 1469, 1414, 1390, 1371 cm^{-1}; MS (ESI) *m/z* (%): 274 (100) $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{14}H_{28}NO_4$ $[M+H]^+$: 274.2013; found: 274.2013.

Boc-Apa-pyAla-OMe (***19***): To a solution of amino acid **9**[10] (0.15 g, 0.60 mmol) in DMF (0.75 mL) at 0 °C was added pentafluorophenyl trifluoroacetate (0.13 mL, 0.78 mmol), followed by pyridine (48 μL, 0.60 mmol) and the reaction was allowed to warm to room temperature. The reaction was subsequently stirred for 1 h before diluting with EtOAc (10 mL) and washing with 0.1 M aqueous HCl (5 mL), saturated aqueous $NaHCO_3$ solution (5 mL) and brine (5 mL). The organic phase was then dried ($MgSO_4$), before concentrating in vacuo to afford the pentafluorophenyl ester as a pale yellow oil which was used immediately in the following reaction. To a solution of pyrrolinone 8 (120 mg, 0.95 mmol) in THF (10 mL) at −78 °C was added 2.41 M *n*BuLi in hexanes (166 μL, 0.40 μmol) and the reaction was stirred for 10 min. A solution of the previously prepared pentafluorophenyl ester (396 mg, 1.04 mmol) in THF (5 mL) was subsequently added dropwise over 15 min and the reaction was allowed to stir for a further 1 hour. The reaction was subsequently quenched with AcOH (200 μL) in THF (2 mL), before diluting with EtOAc (30 mL). The organic fraction was washed with water (5 mL), saturated aqueous $NaHCO_3$ solution (5 mL) and brine (5 mL) before concentrating in vacuo to a yellow oil which was purified by column chromatography (eluent: 3:2 v/v hexane/EtOAc) to afford imide 19 as an amorphous white solid (180 mg, 59%). R_f=0.25 (1:1 v/v hexane/EtOAc); $[\alpha]_D^{25}=+25.3$ (c=1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ=7.39 (dd, 1H, J=15.6, 1.5, CH), 6.98 (dd, 1H, J=15.6, 4.8 Hz, CH), 5.01 (s, 1H, CH), 4.70–4.55 (m, 2H, 2×CH), 4.44 (br s, 1H, NH), 3.84 (s, 3H, CH_3), 1.46 (d, 3H, J=6.6 Hz, CH_3), 1.43 (s, 9H, 3×CH_3), 1.27 ppm (d, 3H, J=6.9 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ=180.6, 169.7, 164.3, 154.9, 149.4, 121.8, 92.9, 79.6, 58.6, 55.6, 47.3, 28.3, 20.3, 17.0 ppm; IR (thin film) $\tilde{\nu}$=3345, 2978, 2934, 2851, 1718, 1674, 1626, 1516, 1454 cm^{-1}; MS (ESI) *m/z* (%): 347 (100) $[M+Na]^+$; HRMS (ESI): *m/z* calcd for $C_{16}H_{24}N_2O_5Na$ $[M+Na]^+$: 347.1577; found: 347.1579.

H_2N-Apa-pyAla-OMe·TFA (***20***): Boc-protected fragment **19** (24 mg, 74 μmol), was dissolved in 1:1 v/v TFA/DCM (2 mL) and the reaction was stirred for 10 min before the reaction was concentrated in vacuo. The residue was purified by preparative reverse phase HPLC (gradient: 0–40% MeCN over 40 min) to afford **20** as a hygroscopic white solid (24 mg, quant). R_t=10.0 min (gradient: 0–50% MeCN over 30 min); $[\alpha]_D^{25}=+16.4$ (c=0.5, $CHCl_3$); 1H NMR (400 MHz, CD_3OD) δ=7.57 (d, 1H, J=15.6 Hz, CH), 6.96 (dd, 1H, J=15.6, 6.4 Hz, CH), 5.26 (s, 1H, CH), 4.69 (q, 1H, J=6.4 Hz, CH), 4.14 (dq, 1H, J=6.4, 6.4 Hz, CH), 3.97 (s, 3H, CH_3), 1.50 (d, 3H, J=6.8 Hz, CH_3), 1.50 ppm (d, 3H, J=6.8 Hz, CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ=184.2, 172.8, 165.5, 144.2, 127.8, 94.5, 60.8, 58.0, 19.5, 18.1 ppm (one peak obscured by solvent); IR (thin film) $\tilde{\nu}$=3345, 2978, 2931, 2853, 2355, 1721, 1673, 1626, 1517, 1455 cm^{-1}; MS (ESI) *m/z* (%): 225 (100) $[M+H]^+$; HRMS (ESI): *m/z* calcd for $C_{11}H_{17}N_2O_3$ $[M+H]^+$: 225.1234; found: 225.1235.

Boc-L-Leu-Apa-pyAla-OMe (***21***): A solution of Boc-Leu-OH (233 mg, 0.93 mmol), HATU (355 mg, 0.93 mmol) and NMM (308 μL, 2.80 mmol in DMF (2.5 mL) was added to **20** (158 mg, 0.47 mmol) in DMF and the reaction stirred for 1 hour. The reaction was subsequently diluted with EtOAc (20 mL) and was washed with saturated aqueous $NaHCO_3$ solution (10 mL), 0.1 M HCl (10 mL) and brine (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated in vacuo to an oil which was

purified by column chromatography (eluent: 2:1 v/v EtOAc/Hexane) to afford **21** as a colorless oil (192 mg, 94%). R_f=0.2 (1:1 v/v EtOAc/Hexane); $[\alpha]_D^{25}$=+53 (c=0.3, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ=7.39 (d, 1H, J=15.5 Hz, CH), 6.96 (dd, 1H, J 15.5, 5.0, CH), 5.20 (s, 1H, CH), 4.68–4.61 (m, 2H, 2×CH), 4.13 (dd, 1H, J=9.8, 5.5 Hz, CH), 3.93 (s, 3H, OCH_3), 1.70 (m, 1H, CH), 1.62–1.45 (m, 14H, 4×CH_3, CH_2), 1.30 (d, 1H, J=3.5 Hz, CH_3), 0.95 ppm (m, 6H, 2×CH_3); ^{13}C NMR (100 MHz, 5:1 v/v $CDCl_3$/CD_3OD) δ=181.1, 172.8, 170.2, 164.6, 156.1, 148.7, 122.0, 92.7, 79.9, 58.8, 55.8, 52.8, 45.9, 41.6, 28.1, 24.6, 22.8, 21.6, 19.4, 16.9 ppm; IR (thin film) $\tilde{\nu}$=3302, 2975, 2934, 1670, 1625, 1528, 1454, 1356, 1328 cm^{-1}; MS (ESI) m/z (%): 460 (100) $[M+Na]^+$; HRMS (ESI): m/z calcd for $C_{22}H_{35}N_3O_6Na$ $[M+Na]^+$: 460.2418; found: 460.2423.

TFA·H$_2$N-L-Leu-Apa-pyAla-OMe (**7**): Boc-protected fragment **21** (181 mg, 0.41 mmol), was dissolved in 1:1 v/v TFA/DCM (5 mL). The reaction was stirred for 5 min before the reaction was concentrated in vacuo. The residue was purified by preparative reverse phase HPLC (gradient: 0–60% MeCN over 40 min) to afford **7** as an amorphous white solid (183 mg, 98%). R_t=16.4 min (gradient: 0–100% MeCN over 30 min); m.p.=176–178 °C; $[\alpha]_D^{25}$=+31.2 (c=0.4, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ=7.41 (dd, 1H, J=15.4, 1.6 Hz, CH), 6.98 (dd, 1H, J=15.6, 4.8 Hz, CH), 5.20 (s, 1H, CH), 4.71 (m, 1H, CH), 4.64 (q, 1H, J=6.7 Hz, CH), 3.92 (s, 1H, OCH_3), 3.87 (m, 1H, CH), 1.90–1.70 (m, 3H, CH, CH_2), 1.46 (d, 3H, J=6.4 Hz, CH_3), 1.35 (d, 3H, J=7.2 Hz, CH_3), 1.07–0.99 ppm (m, 6H, 2×CH3); ^{13}C NMR (100 MHz, CD_3OD) δ=183.0, 171.7, 170.1, 165.6, 149.4, 123.1, 93.7, 59.8, 57.0, 53.1, 47.6, 41.9, 25.5, 23.2, 21.8, 19.9, 17.4 ppm; IR (thin film) $\tilde{\nu}$=3294, 3080, 2960, 1720, 1660, 1618, 1553, 1512, 1457, 1372, 1344, 1332 cm^{-1}; MS (ESI) m/z (%): 338 (100) $[M+H]^+$; HRMS (ESI): m/z calcd for $C_{17}H_{28}N_3O_4$: $[M+H]^+$, 338.2074; found: 338.2076.

General Procedure for fragment condensation with HATU: Imide fragment **7** (18 mg, 40 μmol), amino ester fragment **6** (20 mg, 52 μmol) and HATU (20 mg, 52 μmol) were dissolved in DMF (0.4 mL) before NMM (15 μL, 137 μmol) was added and the reaction was stirred for 2 h. The reaction was subsequently diluted with 0.1% aqueous TFA and purified by preparative reverse phase HPLC (gradient: 0–60% MeCN over 60 min).

General Procedure for neutralization of trifluoroacetate salts: The trifluoroacetate salts of depsipeptides **18a–d** (7.5 mg, 10 μmol) were dissolved in 9:1 v/v H_2O/MeCN (1 mL), 0.05 M aqueous NaOH (250 μL, 13 μmol) was added and the mixture was extracted into 3:1 v/v $CHCl_3$/*i*PrOH (3×10 mL). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo to an oil which was purified by preparative reverse phase HPLC (gradient: 0–80% MeCN over 40 min; eluent A: water, eluent B: MeCN).

Gallinamide A (**2**): Amino ester **6a** was condensed with fragment **7** (17.0 mg, 37.7 μmol) according to the general procedure. The product was purified by preparative reverse phase HPLC (gradient: 0–60% MeCN over 40 min) to afford the trifluoroacetate salt of gallinamide A **18a** as a white amorphous solid (24.4 mg, 92%). R_t=19.8 min (gradient: 0–100% MeCN over 30 min); $[\alpha]_D^{25}$−47 (c=0.06, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ=7.38 (dd, 1H, J=15.6, 1.2 Hz, CH), 7.21 (br d, 1H, J=6.6 Hz, NH), 6.94 (dd, 1H, J=15.6, 4.8 Hz, CH), 6.26 (br d, 1H, J=6.6 Hz, NH), 5.15 (dd, 1H, J=9.6, 4.8 Hz, CH), 5.04 (s, 1H, CH), 4.69 (m, 1H, CH), 4.60 (q, 1H, J=6.6, CH), 4.44 (m, 1H, CH), 3.87 (s, 3H, CH_3), 3.81 (d, 1H, J=6.0 Hz, NH), 2.95 (s, 6H, 2×CH_3), 2.03 (m, 1H, CH), 1.84 (m, 1H, C*H*H), 1.72–1.59 (m, 6H, CH_2, C*H*H, CH*H*, 2×CH), 1.48 (d, 3H, J=6.6 Hz, CH_3), 1.38 (m, 1H, CH), 1.30 (d, 3H, J=6.6 Hz, CH_3), 1.07 (d, 3H, J=7.2 Hz, CH_3), 1.00–0.91 ppm (m, 15H, 5×CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ=180.8, 171.1, 169.8, 169.3, 165.5, 164.1, 162.0 (q, J=30 Hz), 147.9, 122.5, 116.3 (q, J=285 Hz), 92.9, 75.2, 71.6, 58.8, 55.7, 52.0, 46.2, 41.7, 40.8, 40.5, 34.0, 26.4, 24.7, 24.5, 22.8, 22.8, 21.8, 21.4, 19.9, 17.1, 14.8, 11.3 ppm; IR (thin film) $\tilde{\nu}$=2962, 2928, 2874, 1725, 1671, 1624, 1546, 1458, 1326, 1292, 1181, 1133, 1057, 978 cm^{-1}; MS (ESI) m/z (%): 593 (100) $[M+H]^+$; Trifluoroacetate salt **18a** (7.5 mg, 10.6 μmol) was converted to the free base according to the general procedure to afford gallinamide A **2** as a white amorphous solid (5.4 mg, 86%). $[\alpha]_D^{25}$=−56 (c=0.06, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.41 (d, 1H, J=15.6 Hz, CH), 6.97 (dd, 1H, J=15.6, 5.2 Hz, CH), 6.44 (d, 1H, J=8.0 Hz, NH), 6.22 (d, 1H, J=8.0 Hz, NH), 5.16 (dd, 1H, J=9.2, 4.0 Hz, CH), 5.03 (s, 1H, CH), 4.72 (m, 1H, CH), 4.61 (q, 1H, J=6.4 Hz, CH), 4.43 (m, 1H, CH), 3.87 (s, 3H, CH_3), 2.94 (d, 1H, J=10.4 Hz, CH), 2.31 (s, 6H, 2×CH_3), 1.90–1.52 (m, 8H, 3×CH_2, 2×CH), 1.49 (d, 3H, J=6.4 Hz, CH_3), 1.30 (d, 3H, J=6.8 Hz, CH_3), 1.16 (m, 1H, CH), 1.00–0.85 ppm (m, 18H, 6×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=180.8, 171.2, 170.7, 170.6, 169.8, 164.3, 148.3, 122.5, 93.1, 72.6, 72.6, 58.9, 55.8, 51.6, 46.3, 41.8, 41.0, 41.0, 33.6, 25.3, 24.9, 24.6, 23.3, 23.1, 22.1, 21.6, 20.1, 17.2, 15.9, 10.6 ppm; IR (thin film) $\tilde{\nu}$=3287, 2958, 2927, 2917, 2876, 1728, 1652, 1628, 1550, 1460, 1378, 1351, 1328 cm^{-1}. HRMS (ESI): m/z calcd for $C_{31}H_{53}N_4O_7$ $[M+H]^+$: 593.3909; found: 593.3904.

Me$_2$N-L-allo-Ile-Hic-Leu-Apa-pyAla-OMe (**5a**): Amino ester **6b** was condensed with fragment **7** (18.0 mg, 39.9 μmol) according to the general procedure. The product was purified by preparative reverse phase HPLC (gradient: 0–60% MeCN over 40 min) to afford **18b** as the trifluoroacetate salt as a white amorphous solid (28.3 mg, quant). R_t=21.2 min (gradient: 0–100% MeCN over 30 min); $[\alpha]_D^{25}$=−101 (c=0.06, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.38 (dd, 1H, J=15.6, 1.2 Hz, CH), 6.97–6.93 (m, 2H, CH, NH), 6.45 (d, 1H, J=8.0 Hz, NH), 5.11 (dd, 1H, J=9.6, 4.0 Hz, CH), 5.03 (s, 1H, CH), 4.71 (m, 1H, CH), 4.60 (q, 1H, J=6.4, CH), 4.47 (m, 1H, CH), 3.86 (s, 3H, CH_3), 3.81 (d, 1H, J=8.8 Hz, NH), 2.95 (s, 6H, 2×CH_3), 2.03 (m, 1H, CH), 1.84 (m, 1H, C*H*H), 1.73–1.57 (m, 5H, 2×CH_2, CH*H*), 1.54–1.48 (m, 1H, CH), 1.48 (d, 3H, J=6.8 Hz, CH_3), 1.30 (d, 3H, J=6.8 Hz, CH_3), 1.26–1.17 (m, 1H, CH), 1.15 (d, 3H, J=6.8 Hz, CH_3), 0.98–0.89 ppm (m, 15H, 5×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=181.0, 171.3, 170.0, 169.3, 166.1, 164.3, 148.0, 122.6, 93.1, 74.9, 72.1, 58.9, 55.9, 52.0, 46.4, 41.5, 40.6, 40.6, 34.2, 25.4, 24.8, 24.8, 23.1, 21.9, 21.4, 20.0, 17.2, 15.2, 11.0 ppm; IR (thin film) $\tilde{\nu}$=3313, 2960, 2873, 1728, 1654, 1627, 1546, 1455, 1353, 1328 cm^{-1}; MS (ESI) m/z (%): 593 (100) $[M+H]^+$; Trifluoroacetate salt **18b** (7.5 mg, 10.6 μmol) was converted to the free base according to the general procedure to afford **5a** as a white amorphous solid (5.1 mg, 81%). $[\alpha]_D^{25}$=−108 (c=0.06, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.42 (dd, 1H, J=15.4, 1.2 Hz, CH), 6.97 (dd, 1H, J=15.4, 5.2 Hz, CH), 6.39 (d, 1H, J=8.0 Hz, NH), 6.18 (d, 1H, J=8.0 Hz, NH), 5.15 (dd, 1H, J=9.2, 4.0 Hz, CH), 5.03 (s, 1H, CH), 4.72 (m, 1H, CH), 4.61 (q, 1H, J=6.4 Hz, CH), 4.43 (m, 1H, CH), 3.87 (s, 3H, CH_3), 2.91 (d, 1H, J=10.4 Hz, CH), 2.32 (s, 6H, 2×CH_3), 1.88–1.52 (m, 8H, 3×CH_2, 2×CH), 1.49 (d, 3H, J=6.8 Hz, CH_3), 1.30 (d, 3H, J=6.8 Hz, CH_3), 1.07 (m, 1H, CH), 0.99–0.88 ppm (m, 18H, 6×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=180.8, 171.2, 170.7, 170.6, 169.8, 164.3, 148.3, 122.5, 93.2, 73.1, 72.6, 58.9, 55.8, 51.6, 46.3, 41.6, 41.0, 41.0, 34.2, 26.5, 24.9, 24.6, 23.3, 23.2, 22.1, 21.6, 20.1, 17.3, 15.4, 11.4 ppm; IR (thin film) $\tilde{\nu}$=3294, 2959, 2933, 2873, 1727, 1651, 1627, 1546, 1454, 1349, 1325 cm^{-1}. HRMS (ESI): m/z calcd for $C_{31}H_{53}N_4O_7$ $[M+H]^+$: 593.3909; found: 593.3913.

Me$_2$N-D-Ile-Hic-Leu-Apa-pyAla-OMe (**5b**): Amino ester **6c** was condensed with fragment **7** (18.0 mg, 39.9 μmol) according to the general procedure. The product was purified by preparative reverse phase HPLC (gradient: 0–60% MeCN over 40 min) to afford **18c** as the trifluoroacetate salt as a white amorphous solid (28.2 mg, quant). R_t=21.5 min (gradient: 0–100% MeCN over 30 min); $[\alpha]_D^{25}$=−97 (c=0.06, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.36 (dd, 1H, J=15.6, 1.2 Hz, CH), 6.97 (m, 1H, NH), 6.94 (dd, 1H, J=15.4, 4.8 Hz, CH), 6.42 (d, 1H, J=7.6 Hz, NH), 5.07 (dd, 1H, J=9.6, 4.0 Hz, CH), 5.03 (s, 1H, CH), 4.68 (m, 1H, CH), 4.60 (q, 1H, J=6.8, CH), 4.48 (m, 1H, CH), 4.04 (d, 1H, J=6.4 Hz, NH), 3.86 (s, 3H, CH_3), 2.96 (s, 6H, 2×CH_3), 2.04 (m, 1H, CH), 1.86–1.58 (m, 7H, 3×CH_2, CH), 1.48 (d, 3H, J=6.8 Hz, CH_3), 1.39 (m, 1H, CH), 1.30 (d, 3H, J=6.8 Hz, CH_3), 1.01–0.91 ppm (m, 18H, 6×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ=181.0, 171.4, 170.0, 169.5, 167.4, 164.3, 148.1, 122.6, 93.1, 75.0, 69.9, 58.9, 55.9, 51.9, 46.4, 41.3, 40.6, 40.6, 34.2, 26.5, 24.9, 24.6, 23.1, 21.9, 21.4, 20.0, 17.2, 15.6, 11.2 ppm; IR (thin film) $\tilde{\nu}$=3289, 2960, 2935, 2871, 1726, 1651, 1627, 1549, 1456, 1353, 1329 cm^{-1}; MS (ESI) m/z (%): 593 (100) $[M+H]^+$; Trifluoroacetate salt **18c** (15.0 mg, 21.2 μmol) was converted to the free base according to the general procedure to afford **5b** as a white amorphous solid (9.0 mg, 71%). $[\alpha]_D^{25}$=−67 (c=0.06, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.41 (dd, 1H, J=15.6, 1.6 Hz, CH), 6.97 (dd, 1H, J=15.6, 4.8 Hz, CH), 6.53 (d, 1H, J=8.4 Hz, NH), 6.25 (d, 1H, J=8.0 Hz, NH), 5.17 (dd, 1H, J=9.2, 4.0 Hz, CH), 5.03 (s, 1H, CH), 4.72 (m, 1H, CH), 4.61 (q, 1H, J=6.8 Hz,

CH), 4.44 (m, 1H, CH), 3.86 (s, 3H, CH_3), 2.95 (d, 1H, $J=10.4$ Hz, CH), 2.34 (s, 6H, $2\times CH_3$), 1.92–1.53 (m, 8H, $3\times CH_2$, $2\times CH$), 1.49 (d, 3H, $J=6.4$ Hz, CH_3), 1.29 (d, 3H, $J=6.8$ Hz, CH_3), 1.16 (m, 1H, CH), 0.99–0.83 ppm (m, 18H, $6\times CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=180.8$, 171.3, 170.8, 170.7, 169.8, 164.3, 148.3, 122.5, 93.1, 72.5, 72.3, 58.8, 55.8, 51.5, 46.3, 41.7, 41.1, 40.9, 33.4, 25.3, 24.9, 24.6, 23.3, 23.1, 22.1, 21.6, 20.1, 17.2, 15.9, 10.5 ppm; IR (thin film) $\tilde{\nu}=3287$, 2959, 2937, 2873, 1728, 1651, 1629, 1548, 1455, 1350, 1327 cm^{-1}. HRMS (ESI): m/z calcd for $C_{31}H_{53}N_4O_7$ $[M+H]^+$: 593.3909; found: 593.3914.

*Me_2N-D-allo-Ile-Hic-Leu-Apa-pyAla-OMe (**5c**)*: Amino ester **6d** was condensed with fragment **7** (18.0 mg, 39.9 μmol) according to the general procedure. The product was purified by preparative reverse phase HPLC (gradient: 0–60% MeCN over 40 min) to afford **18d** as the trifluoroacetate salt as a white amorphous solid (27.5 mg, 97%). $R_t=21.5$ min (gradient: 0–100% MeCN over 30 min); $[\alpha]_D^{25}=-71$ ($c=0.06$, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) $\delta=7.36$ (dd, 1H, $J=15.6$, 1.6 Hz, CH), 6.94 (d, 1H, $J=15.6$, 4.8 Hz, CH), 6.85 (d, 1H, $J=8.4$ Hz, NH) 6.43 (d, 1H, $J=8.0$ Hz, NH), 5.06–5.02 (m, 2H, $2\times CH$), 4.68 (m, 1H, CH), 4.59 (q, 1H, $J=6.4$, CH), 4.48 (m, 1H, CH), 3.99 (d, 1H, $J=8.8$ Hz, NH), 3.86 (s, 3H, CH_3), 2.94 (s, 6H, $2\times CH_3$), 2.03 (m, 1H, CH), 1.87–1.56 (m, 6H, $3\times CH_2$), 1.48 (d, 3H, $J=6.4$ Hz, CH_3), 1.47–1.39 (m, 1H, CH), 1.29 (d, 3H, $J=6.8$ Hz, CH_3), 1.21–1.11 (m, 1H, CH), 1.16 (d, 3H, $J=6.8$ Hz, CH_3), 0.97–0.89 ppm (m, 15H, $5\times CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=181.0$, 171.3, 170.0, 169.5, 167.8, 164.3, 148.0, 122.6, 93.1, 74.8, 70.3, 58.9, 55.9, 51.8, 46.4, 41.5, 40.6, 40.6, 34.4, 25.7, 24.9, 24.6, 23.1, 21.9, 21.3, 20.0, 17.2, 15.4, 10.8 ppm; IR (thin film) $\tilde{\nu}=3286$, 2958, 2927, 2916, 2875, 2842, 1727, 1651, 1628, 1550, 1458, 1378, 1353, 1328 cm^{-1}; MS (ESI) m/z (%): 593 (100) $[M+H]^+$; Trifluoroacetate salt **18d** (15.0 mg, 10.3 μmol) was converted to the free base according to the general procedure to afford **5c** as a white amorphous solid (8.9 mg, 78%). $[\alpha]_D^{25}=-61$ ($c=0.06$, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) $\delta=7.41$ (d, 1H, $J=15.6$ Hz, CH), 6.97 (dd, 1H, $J=15.6$, 5.2 Hz, CH), 6.56 (d, 1H, $J=8.4$ Hz, NH), 6.28 (d, 1H, $J=8.0$ Hz, NH), 5.17 (dd, 1H, $J=8.8$, 3.6 Hz, CH), 5.03 (s, 1H, CH), 4.72 (m, 1H, CH), 4.61 (q, 1H, J 6.4 Hz, CH), 4.44 (m, 1H, CH), 3.87 (s, 3H, CH_3), 2.94 (d, 1H, $J=10.4$ Hz, CH), 2.36 (s, 6H, $2\times CH_3$), 1.93–1.52 (m, 8H, $3\times CH_2$, $2\times CH$), 1.49 (d, 3H, $J=6.8$ Hz, CH_3), 1.30 (d, 3H, $J=6.8$ Hz, CH_3), 1.06 (m, 1H, CH), 0.99–0.87 ppm (m, 18H, $6\times CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta=180.8$, 171.3, 170.7, 170.7, 169.8, 164.3, 148.3, 122.5, 93.4, 72.6, 72.4, 58.8, 55.8, 51.5, 46.3, 41.5, 41.0, 40.9, 33.8, 26.4, 24.9, 24.6, 23.3, 23.1, 22.1, 21.7, 20.1, 17.2, 15.5, 11.0 ppm; IR (thin film) $\tilde{\nu}=3299$, 2959, 2938, 2873, 1728, 1651, 1629, 1548, 1454, 1351, 1326 cm^{-1}. HRMS (ESI): m/z calcd for $C_{31}H_{53}N_4O_7$ $[M+H^+]$: 593.3909; found: 593.3915.

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