Dimeric Building Blocks for Solid-Phase Synthesis of α-Peptide–β-Peptoid Chimeras

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Abstract: Recently, a novel type of antimicrobial and proteolytically stable peptidomimetic oligomers having an α-peptide–β-peptoid chimeric backbone was reported. The present paper describes efficient protocols for the preparation of a wide range of dimeric building blocks, displaying different types of side-chains, for use in solid-phase synthesis (SPS) of libraries of this type of oligomers. The β-peptoid monomers were obtained by microwave-assisted aza-Michael additions to acrylic esters. Subsequent solution-phase peptide coupling with suitably protected α-amino acids afforded dimeric intermediates. Even sluggish peptide couplings, involving sterically hindered *N*-alkyl-β-alanines or amino acids with bulky side-chains, gave high yields on multigram-scale when using microwave (MW) irradiation. Protecting group and side-chain manipulations were performed as one-pot solution-phase procedures to afford ten different building blocks in good to excellent yields. Finally, the efficiency of SPS oligomerization of a representative dimer was demonstrated by preparing 10- to 16-residue homomers and by the assembly of four different building blocks to give a diversely functionalized octamer.

Key words: peptides, peptoids, Michael addition, solid-phase synthesis, microwave irradiation

Peptidomimetic backbone constructs are of broad interest due to their new structural features and possible biological activities.[1–3] Distinct folding properties of oligomeric mimics of peptides (**1**) have been observed for several unnatural backbones constructed from, for example, β-amino acids (**2**),[4] *N*-alkylglycine moieties (peptoids; **3**),[5] γ-amino acids,[6] or heterocycles.[7] Combining the structural features of β-peptides and peptoids to give β-peptoids (**4**)[8,9] results in a potentially valuable extension to the existing ensemble of peptidomimetic structures (see Figure 1). Inspired by earlier heterogeneous backbones,[10] a chimeric design with alternating chiral β-peptoid and α-amino acid residues was probed.[11] Biological evaluation of the initial array of oligomers confirmed that this backbone design has potential in the development of non-hemolytic antibacterial peptidomimetics that resist proteolysis.[11] An advantage of such novel heteromers over β-peptoid homomers is the possibility of convenient diversification of the side-chain functionalities via the inclusion of a wide range of commercially available α-amino acids. In the present paper, optimized synthetic protocols for the preparation of different types of such dimeric α-peptide–β-peptoid building blocks are reported. In addition, conditions for efficient solid-phase synthesis (SPS) oligomerization to afford α-peptide–β-peptoid chimeras are described.

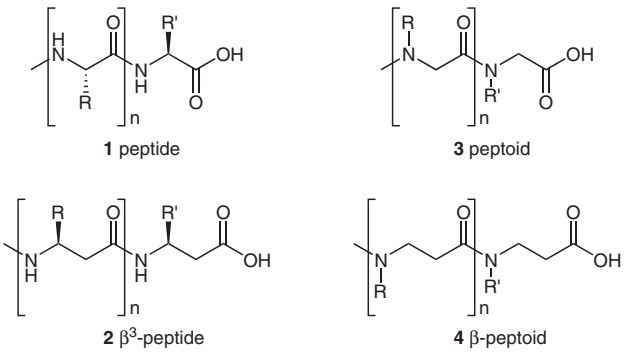

Figure 1 Structures of homomeric foldamer backbones

A general synthetic strategy involving coupling of commercial N^{α}-Fmoc-protected amino acids with an N-alkylated β-alanine ester was considered the most versatile and straightforward. Thus, a microwave (MW)-assisted aza-Michael addition protocol was developed for the preparation of N-alkylated β-alanine esters. The optimal conditions for the nucleophilic addition of (*S*)-1-phenethylamine (**5**) to *tert*-butyl acrylate (**6**) to give **7** (93%) were found to be two hours at 150 °C using a two-fold excess of the acrylate to ensure full conversion of the more expensive chiral amine component. This procedure also gave satisfactory results for the more sterically hindered (*S*)-1-naphthylethylamine and (*S*)-1-cyclohexylethylamine, as well as for the less nucleophilic benzylamine (Scheme 1). By contrast, only about 50% yield of **7** was obtained after conventional heating to 70 °C for several days. Furthermore, the purity of the aza-Michael adducts **7** and **10**–**11** were satisfactory (as judged by ^{1}H and ^{13}C NMR, and analytical RP-HPLC) after a simple work-up, followed by removal of excess alkyl acrylate under reduced pressure. Due to incomplete conversion of the amine component, adducts **8** and **9** required chromatographic purification.

A recently reported alternative to these conditions is an aza-Michael addition performed in a heterogeneous mixture containing water,[12] which parallels previous SPS of

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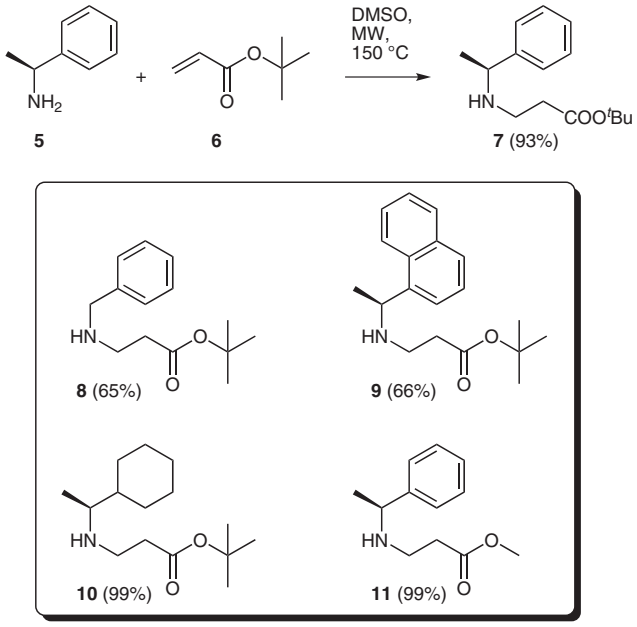


Scheme 1 Microwave-assisted aza-Michael addition of amines to alkyl acrylates

homomeric β-peptoids.[9a] Even though the 'on-water' reaction concept allows considerable rate accelerations of various transformations,[13] only insignificant formation of compound **9** was observed under such conditions even after 16 hours.

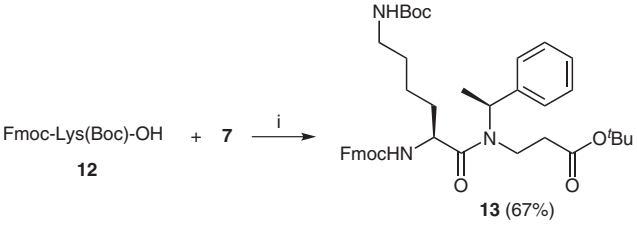
The required dimeric intermediates (**13**–**20**) comprise combinations of non-chiral (Bn) as well as chiral N-alkyl side-chains in β-peptoid units with neutral, basic, and acidic amino acid moieties. The gram-scale preparation of dimers **13** and **17**–**19** in 64–88% yield using standard coupling conditions with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) was considered satisfactory, since SPPS assembly of α-chiral peptoids proved difficult (Scheme 2).[14] By contrast, preparation of sterically congested building blocks led to lowered yields [e.g., 28% of **14** using TBTU and 46% of **15** using tetramethylfluoroformamidinium hexafluorophosphate (TFFH)[15]]. The peptide coupling leading to compound **16** appeared to be an extreme case, as standard TBTU conditions resulted in less than 10% conversion. An attempted improvement by changing the coupling reagent to TFFH was equally unsuccessful. Again, use of MW irradiation was an obvious choice to enhance the coupling efficiency, in analogy with a recently reported MW-assisted SPS assembly of peptoid monomers.[14]

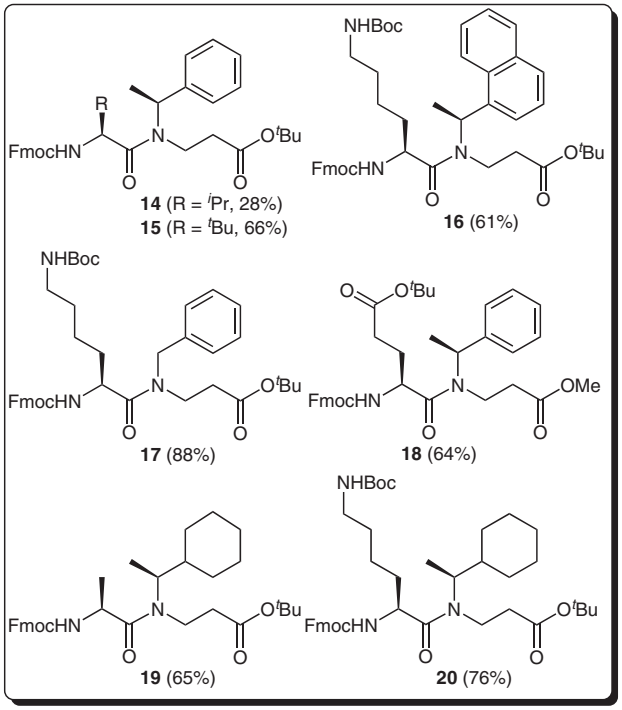
However, application of MW irradiation at 60, 80 or 100 °C did not improve the conversion to any acceptable degree when performing the reactions in DMF. On the other hand, the use of less polar solvents [MeCN or dichloroethane (DCE)] gave rise to significantly higher conversions, and further optimization was performed as shown in Table 1. In addition to TFFH, (tris)pyrrolidinophosphonium hexafluorophosphate (PyBOP) was investigated as a coupling reagent in DCE, as high yields of MW-assisted peptide formation were previously reported with this reagent,[16] but only an inferior conversion into compound **16** was observed (entry 3, Table 1).

Table 1 Optimization of the Formation of **16** from **9** and **12**

Entry	Reagent	**9** (equiv)	Solvent	Time (h)	Temp (°C)	**16**:**9**[a]
1	TFFH	1	MeCN	0.5	60	0.19
2	TFFH	1	DCE	0.5	60	0.38
3	PyBOP	1	DCE	0.5	60	0.05
4	TFFH	1	DCE	1	60	0.38
5	TFFH	2	DCE	0.5	60	0.47
6	TFFH	2	DCE	2	60	1.46
7	TFFH	2	DCE	2	80	2.12

[a] Estimated by HPLC (220 nm).





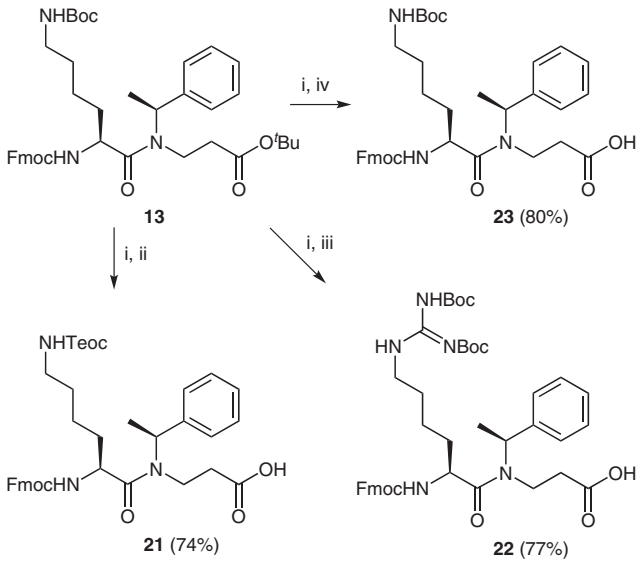
Scheme 2 Peptide couplings to afford dimeric intermediates **13**–**20**. *Reagents and conditions*: (i) For the synthesis of **13**, **14** and **17**–**19**: corresponding Fmoc-protected amino acid, TBTU, DIPEA, CH_2Cl_2, r.t., 16 h. For the synthesis of **15** and **20**: TFFH, DIPEA, DCE, MW 60 °C, 0.5 h. For the synthesis of **16**: TFFH, DIPEA, DCE, MW 80 °C, 2 h.

In order to detect possible instability of the reaction components under MW conditions, the individual components were subjected to irradiation and the resulting mixtures were analyzed by RP-HPLC. Only the acid was prone to degradation, thus it was decided to use a two-fold excess of the acid and TFFH. Comparison of entries 4 and 5 in Table 1 shows that this resulted in a slight increase in conversion within a shorter time. Finally, the coupling was optimized with respect to reaction time and temperature (entries 5–7). Thus, the use of TFFH in DCE with MW heating at 80 °C for two hours provided a satisfactory 61% yield of compound **16**.

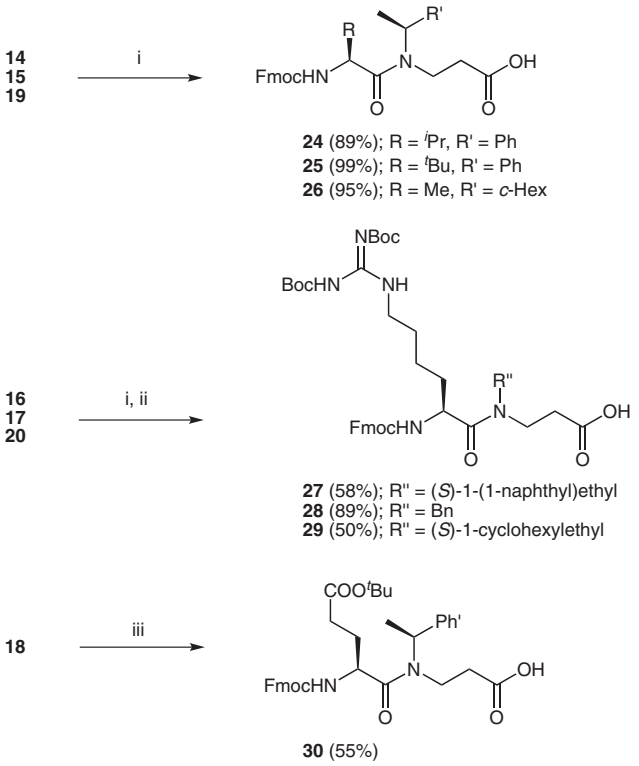
Synthesis of *tert*-butylglycine intermediate **15** in DCE using TFFH as the coupling reagent at room temperature afforded 46% yield, but MW irradiation at 60 °C for 30 minutes raised the yield to 66%. Similarly, MW-assisted coupling gave 76% yield of **20**, showing that MW conditions allowed sterically congested α-peptide–β-peptoid building blocks (i.e., **15**, **16**, and **20**) to be obtainable in good yields on a gram-scale (Scheme 2).

Cleavage of the *tert*-butyl ester group in **13** with concomitant cleavage of the Boc group using TFA–CH_2Cl_2, allowed for 2-(trimethylsilyl)ethoxycarbonyl (Teoc)[17] protection of the side-chain amino group to give **21**, while guanidinylation with *N*,*N*′-bis-Boc-1*H*-pyrazole-1-carboxamidine[18] gave **22**. Furthermore, the Boc group could be re-installed (one-pot from **13**) to give **23** (Scheme 3), which was considered more straightforward than applying an orthogonal three-dimensional protecting group strategy, which would require the synthesis of an additional dipeptide intermediate.

Cleavage of the *tert*-butyl ester group in **14**, **15** and **19** was similarly accomplished with 40% TFA–CH_2Cl_2 at room temperature, to give the corresponding Fmoc-protected building blocks in moderate to high yields after purification by vacuum liquid chromatography (VLC; Scheme 4). The use of milder conditions (10% TFA–CH_2Cl_2 at 0 °C) and a prolonged reaction time, followed by a simple washing procedure, allowed chromatography-free preparation of **25** and **26** in 99% and 95% yield, respectively. The diamino-functionalized compounds **16**, **17** and **20** were converted into the corresponding guanidinylated building blocks as described above for compound **22** (Scheme 4).



Scheme 3 Conversion of intermediate **13** into building blocks **21**–**23**. *Reagents and conditions*: (i) 40% TFA–CH_2Cl_2, r.t., 0.5 h; (ii) 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate, DIPEA, CH_2Cl_2, r.t., 16 h; (iii) *N*,*N*′-bis-Boc-1*H*-pyrazole-1-carboxamidine, DIPEA, CH_2Cl_2, r.t., 16 h; (iv) Boc_2O, DIPEA, THF, r.t., 16 h.

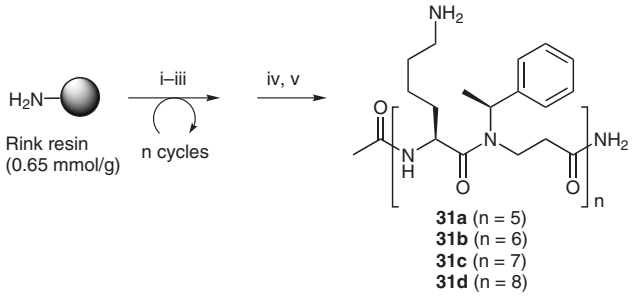


Scheme 4 Conversion of intermediates **14**–**17** and **20** into building blocks **24**–**29**. *Reagents and conditions*: (i) For the synthesis of **24** and **27**–**29**: 40% TFA–CH_2Cl_2, r.t., 0.5–1 h. For the synthesis of **25** and **26**: 10% TFA–CH_2Cl_2, 0 °C, 7–10 h; (ii) *N*,*N*′-bis-Boc-1*H*-pyrazole-1-carboxamidine, DIPEA, CH_2Cl_2 (solvent used for **29**: CH_2Cl_2–DMF, 1:1), r.t., 16 h; (iii) (a) 1 M NaOH, EtOH, r.t., 3 h; (b) Fmoc-Cl in 10% aq Na_2CO_3–dioxane, r.t., 2 h.

After several unsuccessful attempts to achieve selective hydrolysis of the methyl ester group in **18** without affecting the Fmoc group, as reported in other cases,[19] it was found more convenient to remove both groups under alkaline conditions and then re-install the Fmoc group. This furnished the dipeptide building block **30** in 55% overall yield in a one-pot procedure (Scheme 4). A truly orthogonal three-dimensional protecting group strategy could also be envisioned for this building block using an allyl ester instead of the methyl ester.[20] However, the allyl ester would require cleavage with $Pd(PPh_3)_4$, raising the cost of a large-scale synthesis of this building block considerably.

To demonstrate the utility of the prepared building blocks, compound **23** was submitted to SPS in order to generate a series of chimeric oligomers up to the hexadecamer length (Scheme 5 and Table 2). By using only two equivalents of

23 in each coupling step, good yields of the oligomers were obtained. In addition to the standard Fmoc deprotection, subsequent treatment with DBU–piperidine–*N*-methylpyrrolidinone (2:2:96) proved beneficial, as also described for the SPS of β-peptides.[21]



Scheme 5 SPS oligomerization of building block **23**. *Reagents and conditions*: (i) **23**, PyBOP, DIPEA, DMF, r.t., 1.5 h; (ii) 20% piperidine–DMF (2 × 10 min); (iii) 2% DBU and 2% piperidine in NMP (10 min, and then repeated for 5 min); (iv) Ac_2O–DIPEA–DMF (1:2:3), r.t., 0.5 h; (v) 95% TFA–CH_2Cl_2, r.t., 1 h.

Table 2 Synthesis, Yield, Purity and MS Data of Oligomers **31a–d**

Oligomer	Yield (%)[a]	Purity (%)[b]	$[M + nH]^{n+}$ calcd[c]	Found (*m/e*)[c]
31a	56	99	526.01078	526.01066
31b	47	99	627.07567	627.07536
31c	32	96	546.35724	546.35734
31d	30	99	622.15591	622.15589

[a] Upon purification by preparative HPLC (79, 79, 62 and 67 mg, respectively, were obtained).
[b] As judged by analytical HPLC.
[c] n = 3 for compounds **31a–b**; n = 4 for compounds **31c–d**.

Finally, we demonstrated that diversely functionalized chimeras may also be obtained, as four different building blocks (**24–27**) were efficiently coupled to give the α-peptide–β-peptoid chimera **32** in excellent yield (69% i.e., >96% per SPS step; Figure 2). Thus, the building blocks described here enable SPS of a wide variety of α-peptide–β-peptoid chimeras for future structural as well as biological investigations, which are in progress in our laboratories.

In conclusion, practical synthetic protocols for the preparation of a variety of dimeric α-peptide–β-peptoid building blocks for SPPS oligomerization have been developed. Importantly, only readily available starting materials were employed and the procedures allowed multigram-scale preparations. The utility of these building blocks for the preparation of α-peptide–β-peptoid chimeras of various lengths and diverse compositions using standard Fmoc SPPS conditions was also demonstrated. The biological evaluation of compounds **31a–d** have furnished the first non-hemolytic, antiplasmodial peptidomimetics with an unnatural backbone construct.[22] The described MW-assisted aza-Michael additions, as well as

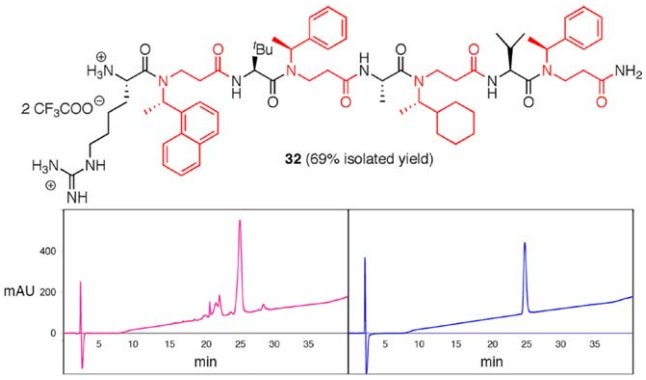


Figure 2 Structure and RP-HPLC traces (215 nm) of compound **32**; crude (left) and purified (right)

peptide couplings involving sterically hindered amines, may also prove generally useful for other applications in organic synthesis.

Starting materials were obtained from commercial suppliers and used without further purification. Water for HPLC was filtered through a 0.22 μm membrane filter. Vacuum liquid chromatography (VLC) was performed using silica gel 60H, 5–40 μm (average size 15 μm). The preparative HPLC system consisted of two preparative pump units, a UV detector, and a Phenomenex Luna C18(2) (5 μm) column (25 × 2.12 cm). Linear elution gradients were composed by mixing solvent A (MeCN–H_2O–TFA, 5:95:0.1) and B (MeCN–H_2O–TFA, 95:5:0.1) at a flow rate of 20 mL/min. Analytical HPLC separations were performed using a Phenomenex Luna C18(2) (3 μm) column (150 × 4.6 mm). Linear elution gradients were composed by mixing solvent C (MeCN–H_2O–HCO_2H, 5:95:0.1) and D (MeCN–H_2O–HCO_2H, 95:5:0.1) at a flow rate of 0.8 mL/min. A gradient with eluent D rising linearly from 0% to 80% during 5 min, followed by a linear rise to 100% D during 25 min was applied for the dimeric intermediates and final building blocks, whereas a gradient with eluent D rising linearly from 10% to 60% during 30 min followed by a linear rise to 100% D during 10 min was applied for the oligomers. ^1H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, using $CDCl_3$ or CD_3OD as solvents. Coupling constants (*J* values) are given in hertz (Hz). Multiplicities of the ^1H NMR signals are reported as follows: singlet (s), doublet (d), triplet (t), quartet (q), pentet (pent), multiplet (m) and broad (br). HRMS were recorded using a Fourier-transform mass spectrometer equipped with a 9.4 tesla superconducting cryomagnet and an external electrospray ion source. The spectra were externally calibrated with arginine clusters and measured in positive ion mode. The samples were dissolved in MeOH, further diluted with 50% MeOH containing 0.2% HCO_2H, and introduced using a syringe pump with a flow of 2 μL/min. A Biotage Initiator microwave reactor system was operated in the single mode using Emrys™ Process Vials (0.5–2.0 mL or 10–20 mL). The experiments were carried out using a fixed hold time with variable power to reach and maintain the set temperature in the vessel for the programmed period of time.

Aza Michael Addition; Typical Procedure

tert-Butyl acrylate (9.0 mL, 62 mmol) and (*S*)-1-phenethylamine (4.0 mL, 31 mmol) were dissolved in DMSO (7 mL) and stirred in a sealed vessel under MW irradiation at 150 °C for 2 h. The mixture was cooled to r.t., diluted with EtOAc (300 mL) and washed with H_2O (4 × 100 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and lyophilized. The crude adduct **7**[11] was used without further purification (as was the case for the other Michael adducts unless otherwise stated below).

7

Yield: 14.5 g (93%); RP-HPLC: 95.5% at 267 nm (t_R = 10.4 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 1.34 (d, *J* = 6.6 Hz, 3 H, CH_3), 1.42 [s, 9 H, $C(CH_3)_3$], 2.31–2.46 (m, 2 H, $COCH_2$), 2.56–2.67 (m, 2 H, NCH_2), 3.73 (q, *J* = 6.6 Hz, 1 H, CH), 7.19–7.35 (m, 5 H, Ph).

^{13}C NMR (75 MHz, CD_3OD): δ = 24.4, 28.4, 36.1, 43.9, 59.2, 81.6, 127.6, 128.0, 129.4, 145.7, 173.2.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{24}NO_2$: 250.18016; found: 250.18016.

8

Purified by VLC (6 × 6 cm; CH_2Cl_2–MeOH, 60:1 → 40:1).

Yield: 4.25 g (65%); RP-HPLC: 97.6% at 267 nm (t_R = 10.2 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 1.44 [s, 9 H, $C(CH_3)_3$], 2.46 (t, *J* = 6.8 Hz, 2 H, $COCH_2$), 2.79 (t, *J* = 6.8 Hz, 2 H, NCH_2), 3.74 (s, 2 H, $PhCH_2$), 7.21–7.36 (m, 5 H, Ph).

^{13}C NMR (75 MHz, CD_3OD): δ = 28.4, 35.8, 45.2, 54.2, 81.8, 128.2, 129.4, 139.9, 173.1.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{14}H_{22}NO_2$: 236.16451; found: 236.16450.

9

Purified by VLC (6 × 6 cm; CH_2Cl_2–MeOH, 50:1 to CH_2Cl_2–MeOH–NH_3 (concd), 300:10:1).

Yield: 6.89 g (66%); RP-HPLC: 97.1% at 267 nm (t_R = 10.6 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 1.40 [s, 9 H, $C(CH_3)_3$], 1.47 (d, *J* = 6.5 Hz, 3 H, CH_3), 2.42 (br t, *J* = 6.5 Hz, 2 H, $COCH_2$), 2.67–2.80 (m, 2 H, NCH_2), 4.67 (q, *J* = 6.5 Hz, 1 H, NCH), 7.43–7.55 (m, 3 H, 3 × ArH), 7.62 (br d, *J* = 7.1 Hz, 1 H, ArH), 7.76 (br d, *J* = 8.2 Hz, 1 H, ArH), 7.87 (br d, *J* = 8.0 Hz, 1 H, ArH), 8.16 (br d, *J* = 8.5 Hz, 1 H, ArH).

^{13}C NMR (75 MHz, CD_3OD): δ = 23.5, 28.4, 36.3, 44.0, 53.9, 81.8, 123.4, 123.6, 126.4, 127.0, 128.3, 129.9, 132.5, 135.3, 141.3, 173.4.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{19}H_{26}NO_2$: 300.19581; found: 300.19596.

10

Yield: 5.96 g (99%).

^{1}H NMR (300 MHz, CD_3OD): δ = 0.90–1.50 (m, 6 H, *c*-Hex), 1.00 (d, *J* = 6.5 Hz, 3 H, CH_3), 1.45 [s, 9 H, $C(CH_3)_3$], 1.64–1.83 (m, 5 H, *c*-Hex), 2.38–2.50 (m, 3 H, $COCH_2$ and NCH), 2.69–2.87 (m, 2 H, NCH_2).

^{13}C NMR (75 MHz, CD_3OD): δ = 16.4, 27.6, 27.7, 27.8, 28.4, 29.0, 31.1, 35.9, 43.5, 43.8, 58.7, 81.7, 173.5.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{30}NO_2$: 256.22711; found: 256.22718.

11

Yield: 6.35 g (99%); RP-HPLC: 99.5% at 267 nm (t_R = 9.5 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 1.35 (d, *J* = 6.8 Hz, 3 H, CH_3), 2.48 (br t, *J* = 6.5 Hz, 2 H, $COCH_2$), 2.58–2.72 (m, 2 H, NCH_2), 3.64 (s, 3 H, OCH_3), 3.75 (q, *J* = 6.8 Hz, 1 H, NCH), 7.20–7.33 (m, 5 H, Ph).

^{13}C NMR (75 MHz, CD_3OD): δ = 24.0, 34.7, 43.7, 52.1, 59.2, 127.6, 128.0, 129.4, 145.6, 174.2.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{12}H_{18}NO_2$: 208.13321; found: 208.13324.

Solution-Phase Peptide Coupling; General Procedure (A)

The amino acid derivative (1.1 equiv), TBTU (1.1 or 1.5 equiv), and DIPEA (2.5 equiv) were dissolved in CH_2Cl_2 (~10 mL/mmol). The mixture was stirred for 10 min then the Michael adduct was added in a minimum amount of CH_2Cl_2. The mixture was stirred at r.t. under N_2 for 16 h, after which the solvent was removed in vacuo. The residue was dissolved in EtOAc (20–40 mL/mmol) and washed with H_2O (10–20 mL), 1 M HCl (2 × 10–20 mL), sat. $NaHCO_3$ (10–20 mL), and brine (10–20 mL). Drying (Na_2SO_4), filtration and evaporation afforded the crude product, which was dissolved in CH_2Cl_2 and purified by VLC.

13[11]

Prepared using general procedure A (1.1 equiv TBTU) and purified by VLC (6 × 6 cm; hexane–EtOAc, 10:1 → 3:1).

Yield: 2.45 g (67%); RP-HPLC: 99.8% at 267 nm (t_R = 21.2 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 1.30–1.57 (br m, 4 H, γ-CH_2, δ-CH_2), 1.37 [s, 9 H, $C(CH_3)_3$], 1.40* [s, 9 H, $C(CH_3)_3$], 1.41 [s, 9 H, $C(CH_3)_3$], 1.53* (d, *J* = 7.0 Hz, 3 H, CH_3), 1.60–1.78 (br m, 2 H, β-CH_2), 1.67 (d, *J* = 6.8 Hz, 3 H, CH_3), 2.05 (ddd, *J* = 15.5, 10.0, 5.3 Hz, 1 H, $COCH_AH_B$), 2.37 (ddd, *J* = 15.5, 10.6, 5.8 Hz, 1 H, $COCH_AH_B$), 2.50* (m, 2 H, $COCH_2$), 2.98–3.09 (br m, 2 H, ε-CH_2), 3.10–3.40 (br m, 2 H, NCH_2), 3.45* (br t, *J* = 7.6 Hz, 2 H, NCH_2), 4.15–4.27 (br m, 1 H, Fmoc-CH), 4.32–4.40 (br m, 2 H, Fmoc-CH_2), 4.45* (dd, *J* = 7.9, 5.3 Hz, 1 H, H-α), 4.79 (dd, *J* = 7.9, 5.3 Hz, 1 H, H-α), 5.42 (q, *J* = 6.8 Hz, 1 H, NCH), 5.81* (q, *J* = 7.0 Hz, 1 H, NCH), 7.20–7.45 (m, 9 H, Ph and Fmoc ArH), 7.68 (m, 2 H, Fmoc ArH), 7.80 (d, *J* = 7.6 Hz, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, CD_3OD): δ = 17.1*, 18.2, 24.1, 24.2*, 28.3, 28.8, 30.5*, 30.6, 32.9, 35.2, 37.6*, 40.2, 40.3*, 40.9, 41.0*, 48.4, 52.8, 53.1*, 53.4*, 56.0, 67.9, 79.7, 81.6, 82.1*, 120.8, 126.1, 128.0, 128.2, 128.3*, 128.5, 128.6, 128.7*, 129.6, 140.7, 141.6*, 142.4, 145.0, 145.1*, 158.2 (2 × C), 171.6*, 172.3, 173.9, 174.8*. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{41}H_{54}N_3O_7$: 700.39563; found: 700.39541.

14

Prepared using general procedure A (1.5 equiv TBTU) and purified by VLC (6 × 6 cm; hexane–EtOAc, 15:1 → 6:1).

Yield: 0.51 g (28%); RP-HPLC: 95.6% at 267 nm (t_R = 21.9 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 0.93* (d, *J* = 6.9 Hz, 6 H, γ-CH_3), 0.98 (d, *J* = 6.6 Hz, 6 H, γ-CH_3), 1.37 [s, 9 H, $C(CH_3)_3$], 1.38* [s, 9 H, $C(CH_3)_3$], 1.51* (d, *J* = 7.1 Hz, 3 H, CH_3), 1.64 (d, *J* = 6.9 Hz, 3 H, CH_3), 1.98–2.22 (br m, 3 H, $COCH_AH_B$, β-CH_2), 2.30–2.46 (br m, 1 H, $COCH_AH_B$), 3.14 (ddd, *J* = 15.9, 10.2, 5.6 Hz, 1 H, NCH_2), 3.34 (m, 1 H, NCH_2), 3.52* (m, 2 H, NCH_2), 4.16–4.24 (br m, 1 H, Fmoc-CH), 4.27* (d, *J* = 8.2 Hz, 1 H, H-α), 4.32–4.40 (br m, 2 H, Fmoc-CH_2), 4.62 (d, *J* = 8.2 Hz, 1 H, H-α), 5.52 (q, *J* = 6.9 Hz, 1 H, NCH), 5.80* (q, *J* = 7.1 Hz, 1 H, NCH), 7.20–7.50 (m, 9 H, Ph and Fmoc ArH), 7.66 (m, 2 H, Fmoc ArH), 7.80 (d, *J* = 7.4 Hz, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, CD_3OD): δ = 17.1*, 18.2, 18.4, 18.7*, 20.0*, 20.3, 28.3, 32.2, 32.5*, 35.1, 37.7*, 40.0, 40.4*, 48.4, 53.7*, 56.2, 58.0, 58.4*, 67.8*, 68.0, 81.7, 82.0*, 120.8, 126.1, 128.0, 128.1, 128.3, 128.5*, 128.6 (2 × C), 128.7*, 129.5, 140.7, 141.5*, 142.4, 145.0, 145.1*, 158.3,* 158.4, 171.5*, 172.3, 173.6, 174.1*. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{35}H_{43}N_2O_5$: 571.31665; found: 571.31659.

17

Prepared using general procedure A (1.5 equiv TBTU) and purified by VLC (6 × 6 cm; hexane–EtOAc, 10:1 → 2:1).

Yield: 6.30 g (88%); RP-HPLC: 96.7% at 267 nm (t_R = 20.3 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 1.28–1.77 (br m, 6 H, β-CH_2, γ-CH_2, δ-CH_2), 1.37 [s, 9 H, $C(CH_3)_3$], 1.40 [s, 9 H, $C(CH_3)_3$], 1.42 [s, 9 H, $C(CH_3)_3$], 2.30–2.71 (br m, 2 H, $COCH_2$), 2.96 (m, 1 H, H_A-ε), 3.04 (m, 1 H, H_B-ε), 3.45–3.72 (br m, 2 H, NCH_2), 4.15–4.25 (br m, 1 H, Fmoc-CH), 4.30–4.44 (br m, 2 H, Fmoc-CH_2), 4.50–4.82 (br m, 3 H, H-α and $PhCH_2$), 7.21–7.41 (br m, 9 H, Ph and Fmoc ArH), 7.66 (m, 2 H, Fmoc ArH), 7.79 (d, *J* = 7.4 Hz, 2 H, Fmoc ArH).

^{13}C NMR (75 MHz, CD_3OD): δ = 24.0, 24.1*, 26.9, 28.4, 30.6, 32.9, 35.6, 40.9, 41.0*, 44.2, 44.3*, 48.4, 52.6, 52.8, 67.9, 79.7, 81.8, 120.8, 126.1, 128.0, 128.3*, 128.6, 129.5, 129.8 (2 × C), 138.1, 138.4*, 142.4, 145.0, 145.1*, 158.3 (2 × C), 172.4, 174.6, 174.7*. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{40}H_{52}N_3O_7$: 686.37998; found: 686.37981.

18

Prepared using general procedure A (1.1 equiv TBTU) and purified by VLC (5 × 5 cm; hexane–EtOAc, 10:1 → 5:1).

Yield: 1.31 g (64%); RP-HPLC: 99.8% at 267 nm (t_R = 18.8 min).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.46* [s, 9 H, $C(CH_3)_3$], 1.47 [s, 9 H, $C(CH_3)_3$], 1.58* (d, *J* = 7.0 Hz, 3 H, CH_3), 1.73 (d, *J* = 6.8 Hz, 3 H, CH_3), 1.88 (br m, 1 H, H_A-β), 1.96–2.20 (br m, 1 H, H_B-β), 2.28–2.46 (br m, 2 H, γ-CH_2), 2.47–2.79 (br m, 2 H, $COCH_2$), 3.28 (ddd, *J* = 15.9, 10.6, 5.3 Hz, 1 H, NCH_AH_B), 3.39* (ddd, *J* = 15.9, 10.6, 5.3 Hz, 2 H, NCH_2), 3.49–3.70 (br m, 1 H, NCH_AH_B), 3.63 (s, 3 H, OCH_3), 3.66* (s, 3 H, OCH_3), 4.34 (br t, *J* = 7.2 Hz, 1 H, Fmoc-CH), 4.35–4.46 (br m, 2 H, Fmoc-CH_2), 4.66* (m, 1 H, H-α), 5.04 (m, 1 H, H-α), 5.41 (q, *J* = 6.8 Hz, 1 H, NCH), 6.00* (q, *J* = 7.0 Hz, 1 H, NCH), 5.76* (d, *J* = 8.8 Hz, 1 H, NH), 5.88 (d, *J* = 8.2 Hz, 1 H, NH), 7.28–7.38 (br m, 7 H, Ph, Fmoc ArH), 7.43 (br t, *J* = 7.3 Hz, 2 H, Fmoc Ar-H), 7.64 (m, 2 H, Fmoc ArH), 7.79 (d, *J* = 7.3 Hz, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.9*, 17.9, 28.2, 28.6*, 28.8, 30.8, 31.1*, 33.1, 35.6*, 39.1, 47.2, 50.6, 51.1*, 51.7, 51.9*, 54.7, 67.1, 80.8, 120.0, 125.2, 126.9, 127.1, 127.2*, 127.7*, 127.9*, 128.7, 128.8, 139.2, 140.2*, 141.2, 143.7, 143.9*, 156.1, 156.2*, 171.1*, 171.5, 172.0 (2 × C), 172.5*. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{36}H_{43}N_2O_7$: 615.30643; found: 615.30649.

19

Prepared using general procedure A (1.1 equiv TBTU) and purified by VLC (5 × 5 cm; hexane–EtOAc, 10:1 → 4:1).

Yield: 1.39 g (65%); RP-HPLC: 99.7% at 267 nm (t_R = 24.0 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 0.80–1.50 (br m, 5 H, *c*-Hex), 1.15* (d, *J* = 7.0 Hz, 3 H, CH_3), 1.22 (d, *J* = 6.5 Hz, 3 H, CH_3), 1.25 (d, *J* = 7.0 Hz, 3 H, β-CH_3), 1.28* (d, *J* = 6.9 Hz, 3 H, β-CH_3), 1.44 [s, 9 H, $C(CH_3)_3$], 1.50–1.83 (br m, 6 H, *c*-Hex), 2.42 (ddd, *J* = 15.9, 10.0, 5.9 Hz, 1 H, $COCH_AH_B$), 2.52–2.67 (br m, 1 H, $COCH_AH_B$), 2.91* (m, 2 H, $COCH_2$), 3.24 (m, 1 H, NCH_AH_B), 3.45–3.62 (br m, 2 H, NCH, NCH_AH_B), 4.18 (br t, *J* = 6.9 Hz, 1 H, Fmoc-CH), 4.23–4.37 (br m, 2 H, Fmoc-CH_2), 4.50* (q, *J* = 6.9 Hz, 1 H, H-α), 4.69 (q, *J* = 7.0 Hz, 1 H, H-α), 7.28 (br t, *J* = 7.3 Hz, 2 H, Fmoc ArH), 7.36 (br t, *J* = 7.3 Hz, 2 H, Fmoc ArH), 7.65 (m, 2 H, Fmoc ArH), 7.77 (d, *J* = 7.3 Hz, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, CD_3OD): δ = 17.1*, 17.8, 18.3*, 18.8, 27.0*, 27.1, 27.2 (2 × C), 27.3, 27.4*, 28.4, 31.0*, 31.3 (2 × C), 31.6*, 35.3, 37.5*, 38.9, 41.9*, 42.8, 48.3 (2 × C), 48.6* (2 × C), 59.4, 67.9, 81.8, 82.1*, 120.8, 126.1, 126.2*, 128.0, 128.6*, 142.4, 145.0, 145.1*, 157.6, 157.9*, 171.9*, 172.5, 174.6, 175.4*. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{33}H_{45}N_2O_5$: 549.33230; found: 549.33240.

Solution-Phase Peptide Coupling with Microwave Irradiation; General Procedure (B)

The amino acid derivative (2–4 mmol; 1.1 or 2.0 equiv), TFFH (1.1 or 2.0 equiv), and DIPEA (1.5 or 2.5 equiv) were dissolved in DCE (5–10 mL) and stirred for 10 min in an Emrys Process Vial (20 mL) for use in the Biotage Initiator MW reactor. The Michael adduct in DCE (3–4 mL) was added, the vessel was sealed, and the mixture was heated (MW) to 60 °C or 80 °C for 0.5–2 h applying the power necessary to reach and maintain the set temperature. Upon cooling to r.t., the reaction mixture was diluted with EtOAc (150 mL) and washed successively with 1 M HCl (3 × 75 mL), H_2O (1 × 75 mL), 0.1 M NaOH (3 × 75 mL) and brine (1 × 75 mL), dried (Na_2SO_4), and evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (10–15 mL) and purified by VLC.

15

Prepared using general procedure B (1.1 equiv TFFH, and MW to 60 °C for 0.5 h) and purified by VLC (5 × 5 cm; hexane–EtOAc, 15:1 → 9:1).

Yield: 0.77 g (66%); RP-HPLC: 99.3% at 267 nm (t_R = 23.9 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 0.98* (s, 9 H, γ-CH_3), 1.05 (s, 9 H, γ-CH_3), 1.39 [s, 9 H, $C(CH_3)_3$], 1.49* (d, *J* = 7.1 Hz, 3 H, CH_3), 1.63 (d, *J* = 6.8 Hz, 3 H, CH_3), 2.15 (ddd, *J* = 16.1, 10.0, 5.3 Hz, 1 H, $COCH_AH_B$), 2.32–2.60* (br m, 2 H, $COCH_2$), 2.38 (ddd, *J* = 16.1, 10.3, 5.6 Hz, 1 H, $COCH_AH_B$), 3.10 (ddd, *J* = 15.3, 10.0, 5.6 Hz, 1 H, NCH_AH_B), 3.19–3.47 (br m, 1 H, NCH_AH_B), 3.60* (ddd, *J* = 15.6, 10.3, 5.0 Hz, 1 H, NCH_AH_B), 4.15–4.24 (br m, 1 H, Fmoc-CH), 4.26–4.49 (br m, 3 H, H-α, Fmoc-CH_2), 5.52 (q, *J* = 6.8 Hz, 1 H, NCH), 5.81* (q, *J* = 7.1 Hz, 1 H, NCH), 7.13–7.41 (m, 9 H, Ph, Fmoc ArH), 7.64 (d, *J* = 7.4 Hz, 2 H, Fmoc ArH), 7.78 (m, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, CD_3OD): δ = 17.1*, 17.9, 27.1*, 27.2, 28.3, 35.2, 36.7, 36.9*, 37.8*, 39.8, 40.8*, 48.4, 48.5*, 53.5*, 56.8, 58.3, 59.1*, 67.6*, 68.1, 81.7, 82.0*, 120.8, 126.0*, 126.1, 128.0, 128.1, 128.5*, 128.6, 128.7, 129.5, 141.0, 141.6*, 142.4, 145.0, 145.1*, 158.1*, 158.2, 171.6*, 172.3, 173.0, 173.2*. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{36}H_{45}N_2O_5$: 585.33230; found: 585.33246.

16

Prepared using general procedure B (2.0 equiv TFFH, and MW at 80 °C for 2 h) and purified by VLC (7 × 8 cm; heptane–EtOAc, 5:1 → 4:1 then heptane–acetone, 10:1 → 4:1).

Yield: 3.04 g (61%); RP-HPLC: 99.1% at 267 nm (t_R = 23.7 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 1.20–1.68 (br m, 6 H, β-CH_2, γ-CH_2, δ-CH_2), 1.30 [s, 9 H, $C(CH_3)_3$], 1.39 [s, 9 H, $C(CH_3)_3$], 1.63 (d, *J* = 7.1 Hz, 3 H, CH_3), 1.76 (m, 1 H, $COCH_AH_B$), 2.06 (m, 1 H, $COCH_AH_B$), 2.91–3.02 (br m, 2 H, ε-CH_2), 3.27 (m, 1 H, NCH_AH_B), 3.48 (m, 1 H, NCH_AH_B), 4.23 (br t, *J* = 6.8 Hz, 1 H, Fmoc-CH), 4.32–4.45 (br m, 3 H, H-α, Fmoc-CH_2), 6.48 (q, *J* = 7.1 Hz, 1 H, NCH), 7.33 (br t, *J* = 7.3 Hz, 2 H, Fmoc ArH), 7.35 (br t, *J* = 7.3 Hz,

2 H, Fmoc ArH), 7.47 (m, 3 H, ArH), 7.70 (m, 3 H, ArH), 7.79–7.83 (m, 3 H, ArH, Fmoc ArH), 7.90 (m, 2 H, ArH).

^{13}C NMR (75 MHz, CD_3OD): δ = 17.0, 24.3, 28.3, 28.8, 30.6, 33.3, 37.3, 39.6, 41.1, 48.4, 50.0, 53.2, 67.8, 79.7, 81.9, 120.8, 124.1, 126.1, 126.2, 127.0, 127.6, 128.0, 128.6, 129.9, 133.0, 135.0, 136.1, 142.4, 145.1, 158.3 (2 × C), 171.2, 174.4.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{45}H_{56}N_3O_7$: 750.41128; found: 750.41131.

20

Prepared using general procedure B (2.0 equiv TFFH, and MW to 60 °C for 0.5 h) and purified by VLC (5 × 5 cm; hexane–EtOAc, 10:1 → 5:1).

Yield: 1.05 g (76%); RP-HPLC: 98.7% at 267 nm (t_R = 25.7 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 0.80–1.50 (br m, 5 H, *c*-Hex), 1.16* (d, *J* = 6.8 Hz, 3 H, CH_3), 1.25 (d, *J* = 6.5 Hz, 3 H, CH_3), 1.30–1.86 (br m, 6 H, β-CH_2, γ-CH_2, δ-CH_2), 1.41 [s, 9 H, $C(CH_3)_3$], 1.45 [s, 9 H, $C(CH_3)_3$], 1.50–1.86 (br m, 6 H, *c*-Hex), 2.41 (ddd, *J* = 16.1, 10.3, 5.6 Hz, 1 H, $COCH_AH_B$), 2.53–2.67 (br m, 1 H, $COCH_AH_B$), 2.88* (m, 2 H, $COCH_2$), 3.03 (m, 2 H, ε-CH_2), 3.23 (m, 1 H, NCH_AH_B), 3.48–3.66 (br m, 2 H, NCH, NCH_AH_B), 4.20 (br t, *J* = 6.8 Hz, 1 H, Fmoc-CH), 4.27–4.38 (br m, 2 H, Fmoc-CH_2), 4.45* (br t, *J* = 6.5 Hz, 1 H, H-α), 4.65 (dd, *J* = 8.2, 4.6 Hz, 1 H, H-α), 7.29 (br t, *J* = 7.6 Hz, 2 H, Fmoc ArH), 7.38 (br t, *J* = 7.3 Hz, 2 H, Fmoc ArH), 7.66 (m, 2 H, Fmoc ArH), 7.78 (d, *J* = 7.6 Hz, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, CD_3OD): δ = 17.2*, 17.9, 23.8, 24.2*, 27.0*, 27.1, 27.2, 27.3, 27.4*, 28.4, 28.9, 30.6, 31.1*, 31.2, 31.4, 31.7*, 33.0*, 33.4, 35.3, 37.6*, 39.0, 40.9*, 41.1, 41.8*, 42.8, 48.4, 52.6, 53.3*, 59.5, 67.8, 67.9*, 79.7, 81.8, 82.2*, 120.8, 126.1, 126.2*, 128.0, 128.6*, 142.4, 144.9*, 145.1, 158.0, 158.3, 171.9*, 172.5, 174.0, 174.7*. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{41}H_{60}N_3O_7$: 706.44258; found: 706.44223.

Conversion of Compound 13 into Building Blocks 21–23

The ester **13** (3.5–9.5 mmol) was dissolved in 40% TFA–CH_2Cl_2 (5–15 mL/mmol) and stirred at r.t. for 0.5 h. The mixture was concentrated and the residue was co-concentrated with Et_2O or toluene. The crude intermediate was treated with the respective reagents at r.t. in CH_2Cl_2 or THF.

21[11]

The above crude intermediate was dissolved in CH_2Cl_2 (10 mL/mmol), and DIPEA (5 equiv) was added followed by (2-trimethylsilyl)ethyl *p*-nitrophenyl carbonate (1.2 equiv) in CH_2Cl_2 (5 mL/mmol). The mixture was stirred for 16 h then evaporated and the residue was purified on a VLC column (5.5 × 6 cm; hexane–EtOAc, 10:1 to hexane–EtOAc–AcOH, 50:50:0.1).

Yield: 1.77 g (74%); RP-HPLC: 98.8% at 267 nm (t_R = 17.3 min).

^{1}H NMR as reported.[11]

^{13}C NMR (75 MHz, CD_3OD): δ = –1.3, 17.1*, 18.1, 18.7, 24.0, 24.1*, 30.5*, 30.6, 33.0*, 33.1, 33.7, 36.2*, 40.3, 40.4*, 41.2, 48.4, 52.8, 53.2*, 53.4*, 56.0, 63.7, 67.9*, 68.0, 120.8, 126.1, 128.0, 128.2, 128.3*, 128.5, 128.6*, 128.7*, 129.6, 140.7, 141.6*, 142.4, 145.0*, 145.1, 158.2, 158.3*, 159.0, 174.0, 174.1*, 174.9. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{38}H_{50}N_3O_7Si$: 688.34125; found: 688.34121.

Anal. Calcd for $C_{38}H_{49}N_3O_7Si$: C, 66.35; H, 7.18; N, 6.11. Found: C, 66.29; H, 7.31; N, 6.03.

22[11]

The crude product obtained from deprotection of **13** was dissolved in CH_2Cl_2 (5 mL/mmol), and DIPEA (5 equiv) was added followed by *N*,*N*′-bis-Boc-1*H*-pyrazole-1-carboxamidine (1.2 equiv) in CH_2Cl_2 (5 mL/mmol). The mixture was stirred at r.t. for 16 h then concentrated and purified by VLC (6 × 8 cm; hexane–EtOAc, 5:1 to hexane–EtOAc–AcOH, 66:33:0.1).

Yield: 5.70 g (77%); RP-HPLC: 96.0% at 267 nm (t_R = 18.9 min).

^{1}H NMR as reported.[11]

^{13}C NMR (75 MHz, CD_3OD): δ = 17.1*, 18.1, 24.1, 24.3*, 28.3, 28.6, 29.8, 33.0*, 33.2, 33.8, 36.3*, 40.4, 40.5*, 41.5, 41.6*, 48.4, 52.7, 53.1*, 53.5*, 56.0, 67.9, 80.3, 84.3, 120.8, 126.1, 128.0, 128.2, 128.3*, 128.5*, 128.6, 128.7, 129.6, 140.7, 141.6*, 142.4, 144.9*, 145.1, 154.0, 157.3, 158.2, 158.3*, 164.3, 173.9, 174.1*, 174.8*, 174.9. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{43}H_{56}N_5O_9$: 786.40725; found: 786.40687.

23

The crude product obtained from deprotection of **13** was dissolved in THF (4 mL/mmol) and then Boc_2O (1.2 equiv) in THF (1 mL/mmol) and DIPEA (6 equiv) were added. The mixture was stirred for 16 h then diluted with EtOAc (200 mL) and washed successively with 1 M HCl (3 × 100 mL), H_2O (8 × 100 mL until pH 7) and brine (1 × 100 mL), dried (Na_2SO_4), and evaporated in vacuo. The residue was purified on a VLC column (7 × 5.5 cm; hexane–EtOAc, 10:1 to hexane–EtOAc–AcOH, 50:50:0.1).

Yield: 2.92 g (80%); RP-HPLC: 96.0% at 267 nm (t_R = 15.5 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 1.32–1.53 (br m, 4 H, γ-CH_2, δ-CH_2), 1.41 [s, 9 H, $C(CH_3)_3$], 1.55* (d, *J* = 7.0 Hz, 3 H, CH_3), 1.61–1.78 (br m, 2 H, β-CH_2), 1.67 (d, *J* = 7.0 Hz, 3 H, CH_3), 2.14 (ddd, *J* = 15.8, 10.1, 5.3 Hz, 1 H, $COCH_AH_B$), 2.44 (ddd, *J* = 15.8, 10.1, 5.3 Hz, 1 H, $COCH_AH_B$), 2.48–2.70* (br m, 2 H, $COCH_2$), 2.97–3.09 (br m, 2 H, ε-CH_2), 3.19 (m, 1 H, NCH_AH_B), 3.38 (m, 1 H, NCH_AH_B), 3.48* (br t, *J* = 8.1 Hz, 2 H NCH_2), 4.16–4.25 (br m, 1 H, Fmoc-CH), 4.33–4.38 (m, 2 H, Fmoc-CH_2), 4.46* (dd, *J* = 8.4, 5.3 Hz, 1 H, H-α), 4.81 (dd, *J* = 8.4, 5.3 Hz, 1 H, H-α), 5.43 (q, *J* = 7.0 Hz, 1 H, NCH), 5.82* (q, *J* = 7.0 Hz, 1 H, NCH), 7.23–7.44 (br m, 9 H, Ph, Fmoc ArH), 7.68 (m, 2 H, Fmoc ArH), 7.80 (d, *J* = 7.9 Hz, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, CD_3OD): δ = 17.1*, 18.1, 24.0, 24.2*, 28.8, 30.5*, 30.6, 33.1, 33.7, 36.2*, 40.3, 40.4*, 40.9, 48.4, 52.8, 53.2*, 53.4*, 56.0, 67.9, 79.7, 120.8, 126.1, 128.0, 128.2, 128.3*, 128.5, 128.6, 128.7*, 129.6, 140.7, 141.6*, 142.4, 144.9*, 145.1, 158.2, 158.8, 174.0, 174.9. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{37}H_{46}N_3O_7$: 644.33303; found: 644.33300.

Anal. Calcd for $C_{37}H_{45}N_3O_7$: C, 69.03; H, 7.05; N, 6.53. Found: C, 68.71; H, 7.13; N, 6.48.

Conversion of Compounds 14, 15 and 19 into Building Blocks 24–26

Hydrolysis Procedure I

The ester was dissolved in 40% TFA–CH_2Cl_2 (5 mL/mmol) and the mixture was stirred at r.t. for 0.5–1 h. The solvents were evaporated and the residue was co-concentrated with Et_2O or toluene.

Hydrolysis Procedure II

The ester was dissolved in 10% TFA–CH_2Cl_2 (10 mL/mmol) and the mixture was stirred at 0 °C for 7–10 h, whilst following the reaction by analytical HPLC. Upon completion of the reaction, the mixture was diluted with CH_2Cl_2 (30 mL/mmol), washed with H_2O (4 × 30 mL/mmol), dried (Na_2SO_4), and evaporated in vacuo to give the acid.

24

Compound **14** was hydrolyzed using procedure I and purified by VLC (4 × 4 cm; hexane–EtOAc, 10:1 to hexane–EtOAc–AcOH, 80:20:0.1).

Yield: 0.37 g (89%); RP-HPLC: 97.7% at 267 nm (t_R = 15.2 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 0.93* (d, *J* = 6.8 Hz, 3 H, γ-CH_3), 0.97* (d, *J* = 6.8 Hz, 3 H, γ-CH_3), 0.98 (d, *J* = 6.5 Hz, 6 H, γ-CH_3), 1.53* (d, *J* = 7.3 Hz, 3 H, CH_3), 1.64 (d, *J* = 7.0 Hz, 3 H, CH_3), 1.99–2.22 (br m, 2 H, COCH_AH$_B$, β-CH), 2.37–2.51 (br m, 1 H, COCH$_A$$H_B$), 3.17 (ddd, *J* = 15.6, 10.3, 5.0 Hz, 1 H, NCH_AH$_B$), 3.38 (m, 1 H, NCH$_A$$H_B$), 3.54* (m, 2 H, NCH_2), 4.17–4.24 (br m, 1 H, Fmoc-CH), 4.28* (d, *J* = 8.2 Hz, 1 H, H-α), 4.32 (d, *J* = 7.1 Hz, 2 H, Fmoc-CH_2), 4.38* (d, *J* = 6.8 Hz, 2 H, Fmoc-CH_2), 4.62 (d, *J* = 8.2 Hz, 1 H, H-α), 5.53 (q, *J* = 7.0 Hz, 1 H, NCH), 5.82* (q, *J* = 7.3 Hz, 1 H, NCH), 7.20–7.49 (m, 9 H, Ph, Fmoc ArH), 7.68 (m, 2 H, Fmoc ArH), 7.80 (m, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, CD_3OD): δ = 17.0*, 18.1, 18.3, 18.7*, 19.9*, 20.2, 32.2, 32.6*, 33.7, 36.2*, 40.1, 40.4*, 48.4, 53.7*, 56.2, 58.0, 58.4*, 67.8*, 68.0, 120.8, 126.1, 128.0, 128.1*, 128.3, 128.4*, 128.6, 128.7, 129.5, 140.7, 141.5*, 142.4, 145.0, 145.1*, 158.4, 173.6, 174.0*, 174.2*, 174.9. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{31}H_{35}N_2O_5$: 515.25406; found: 515.25422.

Anal. Calcd for $C_{31}H_{34}N_2O_5$: C, 72.35; H, 6.66; N, 5.44. Found: C, 72.70; H, 6.82; N, 5.52.

25

Compound **15** was hydrolyzed using procedure II.

Yield: 0.34 g (99%); RP-HPLC: 95.0% at 267 nm (t_R = 16.0 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 0.98* (s, 9 H, γ-CH_3), 1.05 (s, 9 H, γ-CH_3), 1.50* (d, *J* = 7.3 Hz, 3 H, CH_3), 1.64 (d, *J* = 7.0 Hz, 3 H, CH_3), 2.26 (ddd, *J* = 15.9, 10.3, 5.6 Hz, 1 H, COCH_AH$_B$), 2.44 (ddd, *J* = 15.9, 10.2, 5.3 Hz, 1 H, COCH$_A$$H_B$), 2.51* (ddd, *J* = 15.9, 11.0, 5.6 Hz, 1 H, COCH_AH$_B$), 2.69* (ddd, *J* = 15.9, 10.8, 5.5 Hz, 1 H, COCH$_A$$H_B$), 3.14 (ddd, *J* = 15.6, 10.3, 5.3 Hz, 1 H, NCH_AH$_B$), 3.26–3.49 (br m, 1 H, NCH$_A$$H_B$), 3.63* (ddd, *J* = 15.9, 11.0, 5.5 Hz, 1 H, NCH$_A$$H_B$), 4.19 (br t, *J* = 6.8 Hz, 1 H, Fmoc-CH), 4.28–4.41 (br m, 3 H, H-α, Fmoc-CH_2), 5.53 (q, *J* = 7.0 Hz, 1 H, NCH), 5.85* (q, *J* = 7.3Hz, 1 H, NCH), 7.15–7.40 (br m, 9 H, Ph, Fmoc ArH), 7.65 (d, *J* = 7.3 Hz, 2 H, Fmoc ArH), 7.78 (m, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, CD_3OD): δ = 17.2*, 17.8, 27.1*, 27.2, 33.8, 36.2*, 36.7, 37.0*, 39.9, 40.8*, 48.4, 48.5*, 53.4*, 56.8, 58.3, 59.1*, 67.7*, 68.1, 120.8, 126.1, 127.9, 128.0, 128.1*, 128.5*, 128.6, 128.7, 129.5, 141.0, 141.6*, 142.4, 145.0, 158.2, 172.2*, 173.0, 174.1*, 174.9. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{32}H_{37}N_2O_5$: 529.26970; found: 529.26983.

Anal. Calcd for $C_{32}H_{36}N_2O_5$: C, 72.70; H, 6.86; N, 5.30. Found: C, 72.37; H, 6.99; N, 5.20.

26

Compound **19** was hydrolyzed using procedure I and purified by VLC (5 × 5 cm; hexane–EtOAc, 10:1 to hexane–EtOAc–AcOH, 75:25:0.1).

Yield: 0.85 g (86%).

Hydrolysis by procedure II afforded a yield of 95%. RP-HPLC: 98.8% at 267 nm (t_R = 15.5 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 0.80–1.50 (br m, 5 H, *c*-Hex), 1.16* (d, *J* = 6.9 Hz, 3 H, CH_3), 1.23 (d, *J* = 6.9 Hz, 3 H, CH_3), 1.25 (d, *J* = 6.9 Hz, 3 H, β-CH_3), 1.30* (d, *J* = 6.9 Hz, 3 H, β-CH_3), 1.51–1.84 (br m, 6 H, *c*-Hex), 2.49 (ddd, *J* = 15.8, 9.9, 5.5 Hz, 1 H, COCH_AH$_B$), 2.61–2.73 (br m, 1 H, COCH$_A$$H_B$), 3.00* (m, 2 H, $COCH_2$), 3.50–3.64 (m, 2 H, NCH, NCH_AH$_B$), 3.28 (m, 1 H, NCH$_A$$H_B$), 4.18 (m, 1 H, Fmoc-CH), 4.24–4.36 (br m, 2 H, Fmoc-CH_2), 4.51* (q, *J* = 6.9 Hz, 1 H, H-α), 4.69 (q, *J* = 6.9 Hz, 1 H, H-α), 7.28 (br t, *J* = 7.4 Hz, 2 H, Fmoc ArH), 7.36 (br t, *J* = 7.4 Hz, 2 H, Fmoc ArH), 7.65 (m, 2 H, Fmoc ArH), 7.77 (d, *J* = 7.4 Hz, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, CD_3OD): δ = 17.1*, 17.7, 18.3*, 18.8, 27.0*, 27.1, 27.2 (2 × C), 27.3, 27.4*, 31.0*, 31.3 (2 × C), 31.6*, 33.9, 36.1*, 38.9, 41.9*, 42.8, 48.3 (2 × C), 48.6* (2 × C), 59.4, 67.9, 120.8, 126.1, 128.0, 128.6, 142.4, 145.0, 145.1*, 157.7, 158.0*, 174.4*, 174.7, 175.1, 175.6*. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{29}H_{37}N_2O_5$: 493.26970; found: 493.26993.

Conversion of Compounds 16, 17 and 20 into Building Blocks 27–29

The *N*-Boc-protected *tert*-butyl ester derivative was treated with 40% TFA–CH_2Cl_2 under stirring for 0.5–1 h. The reaction mixture was concentrated, and the residue was co-evaporated with Et_2O.

27

The residue (from 0.53 mmol of **16**) was dissolved in CH_2Cl_2 (10 mL) and DIPEA (10 equiv) and *N*,*N*′-bis-Boc-1*H*-pyrazole-1-carboxamidine (1.2 equiv) in CH_2Cl_2 (2 mL) were added successively. The mixture was stirred at r.t. for 16 h, diluted with EtOAc (100 mL), washed with 0.1 M HCl (2 × 50 mL), H_2O (3 × 50 mL), brine (1 × 50 mL), and then the organic phase was dried (Na_2SO_4) and concentrated. The residue was purified on a VLC column (4 × 4 cm; hexane–EtOAc, 10:1 to hexane–EtOAc–AcOH, 66:33:0.1).

Yield: 0.26 g (58%); RP-HPLC: 96.7% at 267 nm (t_R = 20.9 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 1.20–1.68 (br m, 4 H, γ-CH_2, δ-CH_2), 1.42 [s, 9 H, C(CH_3)$_3$], 1.45 [s, 9 H, C(CH_3)$_3$], 1.62 (d, *J* = 6.9 Hz, 3 H, CH_3), 1.68–1.85 (br m, 3 H, COCH_AH$_B$, β-CH_2), 2.07–2.24 (br m, 1 H, COCH$_A$$H_B$), 3.24 (m, 2 H, ε-$CH_2$), 3.28–3.40 (br m, 1 H, NCH_AH$_B$), 3.49 (m, 1 H, NCH$_A$$H_B$), 4.18 (br t, *J* = 6.8 Hz, 1 H, Fmoc-CH), 4.32 (br d, *J* = 6.8 Hz, 2 H, Fmoc-CH_2), 4.41 (dd, *J* = 8.2, 4.7 Hz, 1 H, H-α), 6.48 (q, *J* = 6.9 Hz, 1 H, NCH), 7.28 (br t, *J* = 7.4 Hz, 2 H, Fmoc ArH), 7.36 (br t, *J* = 7.1 Hz, 2 H, Fmoc ArH), 7.44–7.56 (br m, 3 H, ArH), 7.65 (m, 3 H, ArH, Fmoc ArH), 7.76 (d, *J* = 7.4 Hz, 2 H, Fmoc ArH), 7.78–7.90 (m, 3 H, ArH).

^{13}C NMR (75 MHz, CD_3OD): δ = 17.1, 24.3, 28.2, 28.6, 29.8, 33.3, 35.8, 39.7, 41.5, 48.4, 50.0, 53.3, 67.9, 80.3, 84.3, 120.8, 124.2, 126.1, 126.2 (2 × C), 127.6, 127.9, 128.0, 128.6, 129.9, 130.0, 133.0, 135.0, 136.1, 142.4, 145.0, 153.9, 157.3, 158.3, 164.3, 173.7, 174.4.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{47}H_{58}N_5O_9$: 836.42290; found: 836.42255.

Anal. Calcd for $C_{47}H_{57}N_5O_9$: C, 67.53; H, 6.87; N, 8.38. Found: C, 67.61; H, 7.10; N, 8.27.

28[11]

The residue (from 4.08 mmol of **17**) was dissolved in CH_2Cl_2 (25 mL) and DIPEA (8 equiv) was added, followed by *N*,*N*′-bis-Boc-1*H*-pyrazole-1-carboxamidine (1.2 equiv) in CH_2Cl_2 (5 mL). The mixture was stirred at r.t. for 16 h, then concentrated and purified by VLC (6 × 7 cm; hexane–EtOAc, 10:1 to hexane–EtOAc–AcOH, 50:50:0.1).

Yield: 2.8 g (89%); RP-HPLC: 96.7% at 267 nm (t_R = 18.8 min).

^{1}H NMR as reported.[11]

^{13}C NMR (75 MHz, CD_3OD): δ = 23.9, 24.2*, 28.3, 28.6, 29.6, 29.8*, 32.8*, 32.9, 34.3, 41.5, 41.6*, 44.1*, 44.5, 48.9, 52.4, 52.8, 67.8, 80.3, 84.4, 120.8, 125.6*, 126.1, 127.9, 128.6, 142.4, 144.9, 145.1*, 153.9, 157.3, 158.1, 164.3, 174.3, 174.6*, 174.7*, 174.9. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{42}H_{54}N_5O_9$: 772.39160; found: 772.39152.

Anal. Calcd for $C_{42}H_{53}N_5O_9$: C, 65.35; H, 6.92; N, 9.07. Found: C, 65.59; H, 7.04; N, 9.23.

29

The residue (from 4.56 mmol of **20**) was dissolved in CH_2Cl_2–DMF (1:1, 80 mL), and DIPEA (7.5 equiv) and *N*,*N*′-bis-Boc-1*H*-pyrazole-1-carboxamidine (1.2 equiv) in CH_2Cl_2 (5 mL) were added successively. The mixture was stirred at r.t. for 16 h, diluted with EtOAc (250 mL), then the organic phase was washed with 0.1 M HCl (2 × 75 mL), H_2O (3 × 75 mL), brine (1 × 75 mL), dried (Na_2SO_4), and concentrated. The residue was purified on a VLC column (4 × 4 cm; hexane–EtOAc, 10:1 to hexane–EtOAc–AcOH, 1:1:0.1%).

Yield: 1.80 g (50%); RP-HPLC: 95.2% at 267 nm (t_R = 21.9 min).

^{1}H NMR (300 MHz, CD_3OD): δ = 0.81–1.30 (br m, 5 H, *c*-Hex), 1.18* (d, *J* = 6.9 Hz, 3 H, CH_3), 1.26 (d, *J* = 6.6 Hz, 3 H, CH_3), 1.30–1.84 (br m, 12 H, β-CH_2, γ-CH_2, δ-CH_2, *c*-Hex), 1.46 [s, 9 H, $C(CH_3)_3$], 1.48 [s, 9 H, $C(CH_3)_3$], 2.47 (ddd, *J* = 15.7, 9.9, 5.5 Hz, 1 H, $COCH_AH_B$), 2.60–2.74 (br m, 1 H, $COCH_AH_B$), 2.95* (m, 2 H, $COCH_2$), 3.20–3.38 (m, 2 H, ε-CH_2), 3.54–3.67 (m, 3 H, NCH, NCH_2), 4.21 (br t, *J* = 7.0 Hz, 1 H, Fmoc-CH), 4.23–4.38 (br m, 2 H, Fmoc-CH_2), 4.48* (br t, *J* = 7.1 Hz, 1 H, H-α), 4.66 (dd, *J* = 6.9, 5.2 Hz, 1 H, H-α), 7.30 (br t, *J* = 7.4 Hz, 2 H, Fmoc ArH), 7.38 (br t, *J* = 7.3 Hz, 2 H, Fmoc ArH), 7.66 (m, 2 H, Fmoc ArH), 7.79 (d, *J* = 7.4 Hz, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, CD_3OD): δ = 17.2*, 17.9, 23.9, 24.3, 27.0, 27.2, 27.4, 28.3, 28.6, 29.7, 31.2, 31.4, 33.1*, 33.4, 34.0, 36.3*, 39.1*, 41.5, 41.6, 41.8*, 42.8, 48.4, 52.5, 53.3*, 59.6, 67.9, 80.3, 84.3, 120.8, 126.1, 128.0, 128.6, 142.4, 144.9*, 145.1, 154.0, 157.3, 157.9, 164.3, 173.9, 175.0. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{43}H_{62}N_5O_9$: 792.45421; found: 792.45434.

Conversion of Compound 18 into Dipeptide Building Block 30

Compound **18** (0.98 mmol) was dissolved in EtOH (10 mL) and then aq NaOH (1 M, 1.55 equiv) was added slowly (some precipitation was observed). The mixture was stirred for 3 h then aq Na_2CO_3 (10%, 10 mL) and Fmoc-Cl (1.5 equiv) in dioxane (10 mL) were added successively. After stirring at r.t. for 2 h, the mixture was acidified with 1 M HCl then extracted with EtOAc (4 × 50 mL). The combined organic layers were washed with brine (1 × 50 mL), dried (Na_2SO_4), and concentrated. The residue was dissolved in EtOAc and coated onto Celite (10–15 mL), which was loaded onto a VLC column (4 × 4 cm) and eluted with hexane–EtOAc, 10:1 to hexane–EtOAc–AcOH, 66:33:0.1.

Yield: 0.33 g (55%); RP-HPLC: 95.4% at 267 nm (t_R = 16.2 min).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.43* [s, 9 H, $C(CH_3)_3$], 1.44 [s, 9 H, $C(CH_3)_3$], 1.55* (d, *J* = 7.0 Hz, 3 H, CH_3), 1.70 (d, *J* = 6.8 Hz, 3 H, CH_3), 1.84 (m, 1 H, H_A-β), 1.92–2.14 (br m, 1 H, H_B-β), 2.23–2.76 (br m, 2 H, $COCH_2$), 2.36 (m, 2 H, γ-CH_2), 3.26 (ddd, *J* = 15.1, 10.2, 5.5 Hz, 1 H, NCH_AH_B), 3.32–3.54 (br m, 1 H, NCH_AH_B), 3.60* (ddd, *J* = 16.2, 10.7, 5.5 Hz, 1 H, NCH_AH_B), 4.22 (br t, *J* = 6.9 Hz, 1 H, Fmoc-CH), 4.32–4.44 (br m, 2 H, Fmoc-CH_2), 4.60* (dd, *J* = 9.8, 3.4 Hz, 1 H, H-α), 4.99 (dd, *J* = 8.9, 3.4 Hz, 1 H, H-α), 5.39 (q, *J* = 6.8 Hz, 1 H, NCH), 5.94* (q, *J* = 7.0 Hz, NCH), 7.24–7.36 (br m, 7 H, Ph, Fmoc ArH), 7.40 (br t, *J* = 7.4 Hz, 2 H, Fmoc ArH), 7.62 (d, *J* = 7.2 Hz, 1 H, Fmoc ArH), 7.63 (d, *J* = 7.2 Hz, 1 H, Fmoc ArH), 7.76 (d, *J* = 7.4 Hz, 2 H, Fmoc ArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.8*, 17.8, 28.2,* 28.3, 28.5*, 28.6, 30.9, 31.1*, 33.3, 35.5*, 39.0, 47.2, 50.6, 51.1*, 52.0*, 54.9, 67.2, 67.3, 80.9, 120.0, 125.2, 127.0, 127.1, 127.2*, 127.7*, 127.9*, 128.7, 128.9, 138.9, 139.9*, 141.2, 143.7, 143.9*, 156.3, 156.5*, 172.1, 172.2*, 172.9, 174.5*, 175.8. Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{35}H_{41}N_2O_7$: 601.29083; found: 601.29065.

Anal. Calcd for $C_{35}H_{40}N_2O_7$: C, 69.98; H, 6.71; N, 4.66. Found: C, 69.97; H, 6.79; N, 4.63.

Solid-phase Synthesis and Purification of 31a–d

SPS were performed in Teflon filter vessels on a Scansys PLS 4 × 6 Organic Synthesizer equipped with a heating block. Fmoc-protected Rink amide resin (100 mg, 0.065 mmol) was treated with 20% piperidine–DMF (4 mL, 2 × 10 min), and washed with DMF, MeOH, and CH_2Cl_2 (3 × 5 mL, 5 min each). Oligomerization was performed as previously described,[11] using building block **23** for the appropriate number of coupling/deprotection cycles. Terminal amino groups were capped with Ac_2O–DIPEA–DMF (1:2:3, 3 mL, 0.5 h) and the resins were washed with DMF, MeOH, and CH_2Cl_2 (3 × 5 mL, 5 min each). The crude products were cleaved from the support with 95% TFA–CH_2Cl_2 (3 mL, 1 h). The compounds were purified by preparative RP-HPLC. A gradient with eluent B rising linearly from 5% to 40% during 25 min followed by a linear rise to 100% during 10 min was applied. The isolated peptidomimetics were lyophilized from the HPLC solvents and stored at –20 °C. See Table 2 for yields and HRMS data.

Solid-phase Synthesis and Purification of 32

SPS was performed as above agitating the resin (100 mg) with a pre-incubated (10 min) mixture of compound **24** (67 mg, 0.13 mmol, 2 equiv), PyBOP (68 mg, 0.13 mmol, 2 equiv), and DIPEA (0.045 mL, 0.26 mmol, 4 equiv) in anhydrous DMF (1.5 mL) under N_2 for 2 h, and washed with MeOH, DMF and CH_2Cl_2 (3 × 5 mL, 5 min each). Fmoc-deprotection with 20% piperidine–DMF (4 mL, 2 × 10 min) followed by 2% DBU and 2% piperidine in NMP[21] (3 mL, 10 + 5 min) followed by the above washing procedure. This two-step coupling/deprotection sequence was repeated with building blocks **26**, **25** and **27**, to give the resin-bound oligomer. The crude product was cleaved from the support with 95% TFA–CH_2Cl_2 (3 mL, 1 h), and purified by preparative RP-HPLC. A gradient with eluent B rising linearly from 5% to 55% during 25 min followed by a linear rise to 100% B during 10 min was applied. The isolated oligomer was lyophilized from the HPLC solvent and stored at –20 °C.

Yield: 66 mg (69%); RP-HPLC: >99.5% at 215 nm (t_R = 24.8 min).

HRMS: *m*/*z* [M + 2H]$^{2+}$ calcd for $C_{69}H_{102}N_{12}O_8$: 614.40446; found: 614.40439.

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