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


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 of the initial array of oligomers confirmed that this backbone design has potential in the development of non-hemolytic antibacterial peptidomimetics that resist peptidase. 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.

Peptidomimetic backbone constructs are of broad interest due to their new structural features and possible biological activities. Distinct folding properties of oligomeric mimics of peptides have been observed for several unnatural backbones constructed from, for example, α-amino acids, N-alkylglycine moieties (peptoids), γ-amino acids, or heterocycles. Combining the structural features of β-peptides and peptoids to give β-peptoids results in a potentially valuable extension to the existing ensemble of peptidomimetic structures (see Figure 1). Inspired by earlier heterogeneous backbones, a chimeric design with alternating chiral β-peptoid and α-amino acid residues was probed. 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 peptidase. 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.

A general synthetic strategy involving coupling of commercial Nα-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-phenethyamine to tert-butyl acrylate 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 1H and 13C 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, which parallels previous SPS of...
homomeric β-peptoids. Even though the ‘on-water’ reaction concept allows considerable rate accelerations of various transformations, 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-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) was considered satisfactory, since SPPS assembly of α-chiral peptoids proved difficult (Scheme 2). 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)]. 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.

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)pyrrolidino-phosphonium hexafluorophosphate (PyBOP) was investigated as a coupling reagent in DCE, as high yields of MW-assisted peptide formation were previously reported with this reagent, 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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagent</th>
<th>9 (equiv)</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>Temp (°C)</th>
<th>16,9*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TFFH</td>
<td>1</td>
<td>MeCN</td>
<td>0.5</td>
<td>60</td>
<td>0.19</td>
</tr>
<tr>
<td>2</td>
<td>TFFH</td>
<td>1</td>
<td>DCE</td>
<td>0.5</td>
<td>60</td>
<td>0.38</td>
</tr>
<tr>
<td>3</td>
<td>PyBOP</td>
<td>1</td>
<td>DCE</td>
<td>0.5</td>
<td>60</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>TFFH</td>
<td>1</td>
<td>DCE</td>
<td>1</td>
<td>60</td>
<td>0.38</td>
</tr>
<tr>
<td>5</td>
<td>TFFH</td>
<td>2</td>
<td>DCE</td>
<td>0.5</td>
<td>60</td>
<td>0.47</td>
</tr>
<tr>
<td>6</td>
<td>TFFH</td>
<td>2</td>
<td>DCE</td>
<td>2</td>
<td>60</td>
<td>1.46</td>
</tr>
<tr>
<td>7</td>
<td>TFFH</td>
<td>2</td>
<td>DCE</td>
<td>2</td>
<td>80</td>
<td>2.12</td>
</tr>
</tbody>
</table>

*a Estimated by HPLC (220 nm).

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

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, CHCl3, 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 17, showing that MW conditions allowed sterically congested α-peptide–β-peptoid building blocks (i.e., 15, 16, and 17) to be obtained 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–CH₂Cl₂ allowed for 2-(trimethylsilyl)ethoxy carbonyl (Teoc)¹⁷ protection of the side-chain amino group to give 21, while guanidinylation with N,N'-bis-Boc-1H-pyrazole-1-carboxamidiné¹⁸ 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–CH₂Cl₂ 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–CH₂Cl₂ 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 di-amino-functionalized compounds 16, 17 and 20 were converted into the corresponding guanidinylated building blocks as described above for compound 22 (Scheme 4).

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–CH₂Cl₂, r.t. 0.5–1 h. For the synthesis of 25 and 26: 10% TFA–CH₂Cl₂, 0 °C, 7–10 h; (ii) N,N'-bis-Boc-1H-pyrazole-1-carboxamidine, DIPEA, CH₂Cl₂ (solvent used for 29: CH₂Cl₂–DMF, 1:1), r.t., 16 h; (iii) (a) 1 M NaOH, EtOH, r.t., 3 h; (b) Fmoc-Cl in 10% aq Na₂CO₃–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. However, the allyl ester would require cleavage with Pd(PPh₃)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) Ac₂O–DIPEA–DMF (1:2:3), r.t., 0.5 h; (v) 95% TFA–CH₂Cl₂, r.t., 1 h.

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

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>Yield (%)</th>
<th>Purity (%)</th>
<th>[M + nH] n</th>
<th>Found (m/z)</th>
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<tbody>
<tr>
<td>31a</td>
<td>56</td>
<td>99</td>
<td>526.01078</td>
<td>526.01066</td>
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<td>31b</td>
<td>47</td>
<td>99</td>
<td>627.07567</td>
<td>627.07536</td>
</tr>
<tr>
<td>31c</td>
<td>32</td>
<td>96</td>
<td>546.35724</td>
<td>546.35734</td>
</tr>
<tr>
<td>31d</td>
<td>30</td>
<td>99</td>
<td>622.15591</td>
<td>622.15589</td>
</tr>
</tbody>
</table>

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 laboratory.

In conclusion, practical synthetic protocols for the preparation of a variety of dimeric α-peptide–β-peptoid building blocks for SPS 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. The described MW-assisted aza-Michael additions, as well as 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–H₂O–TFA, 5:95:0.1) and B (MeCN–H₂O–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–H₂O–HCO₂H, 5:95:0.1) and D (MeCN–H₂O–HCO₂H, 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 30 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 13C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, using CDCl₃ or CD₃OD 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% HCO₂H, 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-phenyldiamine (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 H₂O (4 × 100 mL) and brine (100 mL). The organic layer was dried (Na₂SO₄), filtered, concentrated and lyophilized. The crude adduct 7 was used without further purification (as was the case for the other Michael adducts unless otherwise stated below).
Yield: 4.25 g (65%); RP-HPLC: 97.6% at 267 nm (t_R = 10.6 min).

1H NMR (300 MHz, CD3OD): δ = 0.90–1.50 (m, 6 H, c-Hex), 1.00 (d, J = 6.5 Hz, 3 H, CH3), 1.45 (s, 9 H, C(CH3)3), 1.64–1.83 (m, 5 H, c-Hex), 2.38–2.50 (m, 3 H, COCH2 and NCH3), 2.69–2.87 (m, 2 H, NCH3).

13C NMR (75 MHz, CD3OD): 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 + H]+ calcld for C35H43N2O5: 571.31665; found: 571.31658.

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

1H NMR (300 MHz, CD3OD): δ = 1.35 (d, J = 6.8 Hz, 3 H, CH3), 2.48 (br t, J = 6.5 Hz, 2 H, COCH2), 2.58–2.72 (m, 2 H, NCH2), 3.64 (s, 3 H, OCH3), 3.75 (q, J = 0.8 Hz, 1 H, NCH), 7.20–7.33 (m, 5 H, Ph).

13C NMR (75 MHz, CD3OD): δ = 24.0, 34.7, 43.7, 52.1, 59.2, 127.6, 128.0, 129.4, 145.6, 174.2.


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 CH3Cl2 (~10 mL/mL). The mixture was stirred for 10 min then the Michael adduct was added in a minimum amount of CH2Cl2. The mixture was stirred at r.t. under N2 for 16 h, after which the solvent was removed in vacuo. The residue was dissolved in EtOAc (20–40 mL/mmol) and washed with H2O (10–20 mL), 1 M HCl (2 × 10–20 mL), sat. NaHCO3 (10–20 mL), and brine (20–20 mL). Drying (Na2SO4), filtration and evaporation afforded the crude product, which was dissolved in CH2Cl2 and purified by VLC.

131 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).

1H NMR (300 MHz, CD3OD): δ = 1.00–1.50 (m, 6 H, c-Hex), 1.00 (d, J = 6.9 Hz, 3 H, CH3), 1.35 (s, 9 H, C(CH3)3), 1.40–1.83 (m, 5 H, c-Hex), 2.38–2.50 (m, 3 H, COCH2 and NCH3), 2.69–2.87 (m, 2 H, NCH3).

13C NMR (75 MHz, CD3OD): 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.


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 = 21.2 min).

1H NMR (300 MHz, CD3OD): δ = 0.90–1.50 (m, 6 H, c-Hex), 1.00 (d, J = 6.5 Hz, 3 H, CH3), 1.45 (s, 9 H, C(CH3)3), 1.64–1.83 (m, 5 H, c-Hex), 2.38–2.50 (m, 3 H, COCH2 and NCH3), 2.69–2.87 (m, 2 H, NCH3).

13C NMR (75 MHz, CD3OD): 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.

17 Prepared using general procedure A (1.1 equiv TBTU) and purified by VLC (6 × 5 cm; hexane–EtOAc, 10:1 → 2:1).
Yield: 6.30 g (88%); RP-HPLC: 96.7% at 267 nm (t<sub>g</sub> = 20.3 min).

19 Prepared using general procedure B (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<sub>g</sub> = 18.8 min).

Prepared using general procedure B (1.1 equiv TBTU) and purified by VLC (5 × 5 cm; hexane–EtOAc, 10:1 → 5:1).
Yield: 1.30 g (66%); RP-HPLC: 99.7% at 267 nm (t<sub>g</sub> = 23.9 min).

13C NMR (75 MHz, CD<sub>3</sub>OD): δ = 17.1, 17.8, 18.3, 18.8, 27.0, 27.1, 27.2 (2 × C), 27.3, 27.4, 28.4, 30.1, 30.4 (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, 120.4, 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]<sup>+</sup> calcd for C<sub>34</sub>H<sub>36</sub>N<sub>2</sub>O<sub>7</sub>: 615.30643; found: 615.30649.

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, 15:1 → 9:1).
Yield: 0.77 g (66%); RP-HPLC: 99.3% at 267 nm (t<sub>g</sub> = 23.9 min).

13C NMR (75 MHz, CD<sub>3</sub>OD): 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, 128.5, 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]<sup>+</sup> calcd for C<sub>34</sub>H<sub>36</sub>N<sub>2</sub>O<sub>7</sub>: 615.30643; found: 615.30649.

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<sub>g</sub> = 23.7 min).

1 H NMR (300 MHz, CD<sub>3</sub>OD): δ = 1.20–1.68 (br m, 6 H, β-CH<sub>2</sub>-γ-CH, γ-CH<sub>2</sub>-δ-CH<sub>3</sub>), 1.30 [s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>], 1.39 [s, 9 H, C(CH<sub>3</sub>)<sub>3</sub>], 1.63 (d, J = 7.1 Hz, 3 H, CH<sub>3</sub>), 1.76 (m, 1 H, COCH<sub>2</sub>CH<sub>3</sub>), 2.06 (m, 1 H, COCH<sub>2</sub>CH<sub>3</sub>), 2.91–3.02 (br m, 2 H, Fmoc-CH<sub>2</sub>), 3.27 (m, 1 H, NCH<sub>2</sub>CH<sub>3</sub>), 3.48 (m, 1 H, NCH<sub>2</sub>CH<sub>3</sub>), 4.23 (br t, J = 6.8 Hz, 1 H, Fmoc-CHO), 4.32–4.45 (br m, 3 H, H-4, Fmoc-CHO), 6.48 (q, J = 7.1 Hz, 1 H, NCH<sub>2</sub>), 7.33 (br t, J = 7.3 Hz, 2 H, Fmoc ArH), 7.35 (br t, J = 7.3 Hz, 2 H, Fmoc ArH).
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).

13C NMR (75 MHz, CD3OD): δ = −1.3, 17.1*, 18.1, 18.7, 24.0, 24.1*, 30.5*, 36.0, 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*, 69.9*, 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 C34H33N3O7Si: 688.34125; found: 688.34121.

Analysis. Calcd for C34H33N3O7Si: C, 66.35; H, 7.18; N, 6.11. Found: C, 66.29; H, 7.31; N, 6.03.

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The crude product obtained from deprotection of 13 was dissolved in CH2Cl2 (5 mL/mmol) and DIPEA (5 equiv) was added followed by N,N′-bis-Boc-1H-pyrrozole-1-carboxamide (1.2 equiv) in CH2Cl2 (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 (tR = 18.9 min).

1H NMR as reported.

13C NMR (75 MHz, CD3OD): δ = 17.1*, 18.1, 21.4, 24.2*, 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 C34H33N3O7Si: 786.40725; found: 786.40687.

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The crude product obtained from deprotection of 13 was dissolved in THF (4 mL/mmol) and then Boc2O (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), H2O (8 × 100 mL until pH 7) and brine (1 × 100 mL), dried (Na2SO4), and evaporated in vacuo. The residue was purified on a VLC column (7.5 × 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 (tR = 15.5 min).

1H NMR (300 MHz, CD3OD): δ = 1.32–1.53 (br m, 4 H, γCH2, βCH–), 1.41 [s, 9 H, C(CH3)3], 1.55* (d, J = 7.0 Hz, 3 H, CH2), 1.61–1.78 (br m, 2 H, β–CH2), 1.67 (d, J = 7.0 Hz, 3 H, CH2), 2.14 (d, J = 15.8, 10.1, 5.3 Hz, 1 H, COCH2H2), 2.14 (d, J = 15.4, 10.1, 5.3 Hz, 1 H, COCH2H2), 2.48–2.70* (br m, 2 H, COCH2), 2.97–3.09 (br m, 2 H, γ–CH2), 3.19 (m, 1 H, NCH2H2), 3.38 (m, 1 H, NCH2H2), 3.48* (br t, J = 8.1 Hz, 2 H NCH2), 4.16–4.25 (br m, 1 H, Fmoc-CH), 4.33–4.38 (br m, 2 H, Fmoc-CH), 4.46* (dd, J = 8.4, 5.3 Hz, 1 H, H–q), 4.81 (dd, J = 8.4, 5.3 Hz, 1 H, H–a), 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, FmocArH), 7.80 (d, J = 7.9 Hz, 2 H, FmocArH). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

13C NMR (75 MHz, CD3OD): δ = 17.1*, 18.1, 24.0, 24.2*, 28.3, 30.5*, 36.0, 33.1, 33.7, 36.2*, 40.3, 40.4*, 41.2, 48.4, 52.8, 53.2*, 53.4*, 56.0, 63.7*, 69.9*, 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 C34H33N3O7Si: 644.33303; found: 644.33300.

Analysis. Calcd for C34H33N3O7Si: 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 1

The ester was dissolved in 40% TFA–CH2Cl2 (5 mL/mmol) and stirred at r.t. for 0.5–1 h. The solvents were evaporated and the residue was co-concentrated with EtOAc or toluene.
Hydrolysis Procedure II

The ester was dissolved in 10% TFA–CH₂Cl₂ (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 CH₂Cl₂ (30 mL/mmol), washed with H₂O (4 × 30 mL/mmol), dried (Na₂SO₄), 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 (tₚ = 15.2 min).

1H NMR (300 MHz, CD₃OD): δ = 0.93* (s, 9 H, γ-CH₃), 1.05 (s, 9 H, γ-CH₃), 1.05* (d, J = 7.3 Hz, 3 H, CH₃), 1.64 (d, J = 7.0 Hz, 3 H, CH₃), 2.26 (dd, J = 15.9, 10.3, 5.6 Hz, 1 H, COCH₂H₂), 2.44 (ddd, J = 15.9, 10.2, 5.3 Hz, 1 H, COCH₂H₂), 2.51* (s, 9 H, γ-CH₃), 3.32–3.49 (brm, J = 15.9, 11.0, 5.5 Hz, 1 H, NCH₂H₂), 3.49 (m, 1 H, Fmoc-CH₃), 3.63 (dd, J = 15.9, 11.0, 5.5 Hz, 1 H, NCH₂H₂), 4.19 (br t, J = 6.8 Hz, 1 H, Fmoc-CH₃), 4.28–4.41 (brm, J = 15.9, 11.0, 5.5 Hz, 1 H, NCH₂H₂), 5.35 (q, J = 7.0 Hz, 1 H, CH₃), 5.85* (q, J = 7.3 Hz, 1 H, NCH₃), 7.15–7.40 (brm, 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.

13C NMR (75 MHz, CD₃OD): 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.


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–CH₂Cl₂ under stirring for 0.5–1 h. The mixture was washed with H₂O (3 × 50 mL), brine (1 × 50 mL), and then the organic phase was dried (Na₂SO₄) 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 (tₚ = 20.9 min).

1H NMR (300 MHz, CD₃OD): δ = 1.20–1.68 (br m, 4 H, γ-CH₂-Δ₂CH₃), 1.42 [s, 9 H (CH₂)], 1.45 [s, 9 H (CH₂)], 1.62 (d, J = 6.9 Hz, 3 H, CH₃), 1.68–1.85 (brm, 3 H, COCH₂-Δ₂CH₃, CH₃), 2.07–2.24 (brm, 1 H, COCH₂H₂), 3.24 (m, 2 H, CH₃), 3.28–3.40 (brm, 1 H, NCH₂H₂), 3.49 (m, 1 H, NCH₂H₂), 4.18 (br t, J = 6.8 Hz, 1 H, Fmoc-CH₃), 4.32 (brm, J = 6.8 Hz, 2 H, Fmoc-CH₂), 4.41 (dd, J = 8.2, 4.7 Hz, 1 H, H₆), 6.48 (q, J = 6.9 Hz, 1 H, NCH₂H₂), 7.28 (br t, J = 7.4 Hz, 2 H, Fmoc ArH), 7.36 (brm, J = 7.1 Hz, 2 H, Fmoc ArH), 7.44–7.56 (brm, 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).

13C NMR (75 MHz, CD₃OD): 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.


Anal. Calcd for C₃₂H₃₇N₅O₉: C, 76.53; H, 8.67; N, 8.38. Found: C, 76.61; H, 7.10; N, 8.27.
Building Blocks for SPS of α-Peptide–β-Peptidomimetic Chimeras

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The residue (from 4.08 mmol of 17) was dissolved in CH₂Cl₂ (25 mL) and DIPEA (8 equiv) was added, followed by N,N'-bis-Boc-1H-pyrazole-1-carboxamide (1.2 equiv) in CH₂Cl₂ (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 (tₚ = 18.8 min).

1H NMR as reported.¹¹

¹¹C NMR (75 MHz, CD₂OD): 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, 52.5, 67.8, 80.3, 84.4, 120.8, 125.6*, 126.1, 125.9, 142.4, 144.9, 145.1*, 153.9, 157.3, 181.1, 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]+ calc'd for C₁₇H₂₃N₇O₆: 372.3916; found: 372.39152.


29

The residue (from 4.56 mmol of 20) was dissolved in CH₂Cl₂–DMF (1:1; 80 mL), and DIPEA (7.5 equiv) and N,N'-bis-Boc-1H-pyrazole-1-carboxamide (1.2 equiv) in CH₂Cl₂ (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), H₂O (3 × 75 mL), brine (1 × 75 mL), dried (Na₂SO₄), and concentrated. The residue was purified on a VLC column (4 × 4 cm; hexane–EtOAc–AcOH, 10:1 to hexane–EtOAc–AcOH, 1:1:0.1).

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

¹¹H NMR (300 MHz, CD₂OD): δ = 0.81–1.30 (br m, 5 H, c-Hex), 1.18* (d, J = 6.9 Hz, 3 H, CH₃), 1.26 (d, J = 6.6 Hz, 3 H, CH₃), 1.30–1.84 (br m, 12 H, β-CH₂, γ-CH₃, δ-CH₂–c-Hex), 1.46 [s, 9 H, C(CH₃)₃], 1.48 [s, 9 H, C(CH₂)₃], 2.47 (dd, J = 15.7, 9.9, 5.5 Hz, 1 H, COCH₂H₂), 2.60–2.74 (br m, 1 H, COCH₂H₂), 2.95* (m, 2 H, 2-Hmoc–CH₂), 3.20–3.38 (m, 2 H, c-Hex), 3.54–3.67 (m, 3 H, N-CH₃), 3.60* (ddd, J = 16.8*, 17.8, 10.7 Hz, 1 H, NCH₂), 3.78* (ddd, J = 16.8*, 17.8, 10.7 Hz, 1 H, NCH₂), 4.21 (br t, J = 7.0 Hz, 1 H, Fmoc-CH), 4.23–4.38 (br m, 2 H, Fmoc-CH₂), 4.48* (br t, J = 7.1 Hz, 1 H, Ac-O), 4.66 (dd, J = 6.9, 5.2 Hz, 1 H, H-α), 7.30 (br t, J = 7.4 Hz, 2 H, Fmoc Ar-H), 7.38 (br t, J = 7.3 Hz, 2 H, Fmoc Ar-H), 7.66 (m, 2 H, Fmoc Ar-H), 7.79 (d, J = 7.4 Hz, 2 H, Fmoc Ar-H). Signals designated with * correspond to additional peaks due to the presence of a minor rotamer.

HRMS: m/z [M + H]+ calc'd for C₁₃H₂₀N₇O₄: 238.1729; found: 238.17312.

Analytical Calc'd for C₁₃H₂₀N₇O₄: C, 69.97; H, 6.71; N, 9.46. 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 Scanysys 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 CH₂Cl₂ (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 aminogroups were capped with Ac₂O–DIPEA–DMF (1:2:3, 3 mL, 0.5 h) and the resins were washed with DMF, MeOH, and CH₂Cl₂ (3 × 5 mL, 5 min each). The crude products were cleaved from the support with 95% TFA–CH₂Cl₂ (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 preincubated (10 min) mixture of building blocks 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 N₂ for 2 h, and washed with MeOH, DMF and CH₂Cl₂ (3 × 5 mL, 5 min each). Fmoc-deprotection with 20% piperidine–DMF (4 mL, 2 × 10 min) followed by 2% DNB and 2% piperidine in NMP¹¹ (3 mL, 10 + 5 min) followed by the above washing procedure. This two-step coupling/deprotection sequence was repeated with building blocks 26, 27, and 28 to give the resin-bound oligomer. The crude product was cleaved from the support with 95% TFA–CH₂Cl₂ (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 (tₚ = 24.8 min).

HRMS: m/z [M + 2H]+ calc'd for C₈H₁₀O₂N₁₂O₄: 614.40436; found: 614.40439.

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References