

Peptide cyclization *via* ring-closing metathesis: the *N*-alkenoxy peptide approach†

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The preparation of cyclic hexapeptides from *N*-hydroxy tripeptides building blocks is described. Introduction of an unsaturated chain on the hydroxamate oxygen followed by fragment coupling leads to *N,N'*-dialkenoxy hexapeptides that are efficiently cyclized through a ring-closing metathesis reaction. The length of the alkene chains allows the modulation of the ring size: the synthesis of 17- and 18-membered cycles is reported.

Introduction

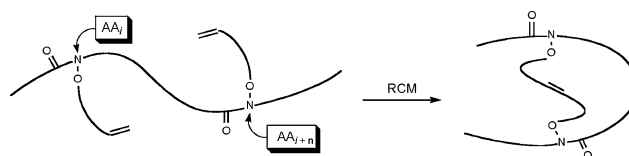
The preparation and study of cyclic analogues is a standard procedure in the development of therapeutical agents starting from one identified linear peptide binding to a target receptor. Constraining peptides through cyclization was first introduced as a tool to investigate bioactive conformations of hormones.¹ These researches led the way to considerable developments in drug design: cyclization often results in higher receptor binding affinities than the linear counterpart thanks to a smaller loss of entropy in the binding; moreover, the constrained structure can also enhance the selectivity of the ligand and its stability towards enzymatic degradation.²

With the discovery of air-stable and functional-group tolerant catalysts, ring-closing olefin metathesis³ (RCM) has emerged as a powerful tool to prepare cyclic peptides. RCM has for instance been used in the synthesis of molecules binding to the Grb2 SH2 domain⁴ or in the development of inhibitors of the hepatitis C virus NS3 protease.⁵ Analogues of cystine-⁶ or lanthionine⁷-containing biologically active peptides have been prepared by RCM-mediated substitution of a carbon-carbon double bond for the disulfide or monosulfide bridge. Introduction, through RCM, of an all-hydrocarbon link between two amino acid residues has also been studied as a way to stabilize β -turn⁸ or α - and 3_{10} -helices.^{9,10}

The cyclization staples the linear peptide between two amino acid residues referred to as *i* and *i* + *n* positions. The unsaturated chains involved in the ring closure can be introduced *via* derivatization of the terminal amine and/or carboxylic acid,¹¹ as part of an amino acid side-chain, or by modification of the peptide backbone. The most common solution is the incorporation of an amino acid presenting an unsaturated side-chain such as allylglycine, homo- and bishomo-allylglycine or more complex molecules.¹² This strategy lacks flexibility since several peptides have to be prepared if the ring size is to be studied. On the other hand, to cope with the preparation (or cost) of these unnatural

amino acids, *O*-allyl-serine, homoserine or tyrosine can be used.¹³ Nevertheless, in all cases, side-chains involved in the ring closure are not available anymore to interact with other molecules, such as molecular receptors. When biological properties are at stake, this loss of interaction can modify the response of the peptide more than the studied cyclization does. Thus, another possibility is the introduction of an alkene functionality at the nitrogen of peptide bonds¹⁴ but here also the unsaturated amino acid has to be prepared before its incorporation in the peptide chain.

We recently described the preparation of *N*-hydroxy peptides and their modification to *N*-acyloxy peptides through acylation of the hydroxamate oxygen.¹⁵ It is thus possible to introduce unsaturated acyl groups in the peptide. However, these acyl groups are rather reactive and the corresponding cyclized peptide would probably not be stable *in vivo*. We therefore considered that *N*-alkenoxy peptides (Scheme 1) could present an interesting alternative for the cyclization of peptides *via* RCM.



Scheme 1 RCM-mediated cyclization of *N,N'*-dialkenoxy peptide.

In this article, we demonstrate the feasibility of this approach *via* the convergent synthesis of *N,N'*-dialkenoxy hexapeptides **1** and **2** (Fig. 1) and their RCM-mediated cyclization to form 17- and 18-membered rings.

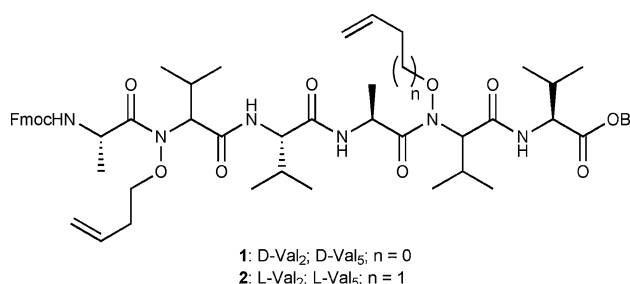


Fig. 1 Target *N,N'*-dialkenoxy hexapeptides.

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† Electronic supplementary information (ESI) available: Copies of ¹H and ¹³C NMR spectra for all new compounds, representative structure of **11** and **12** from conformational analysis data. See DOI: 10.1039/b812611a

Results and discussion

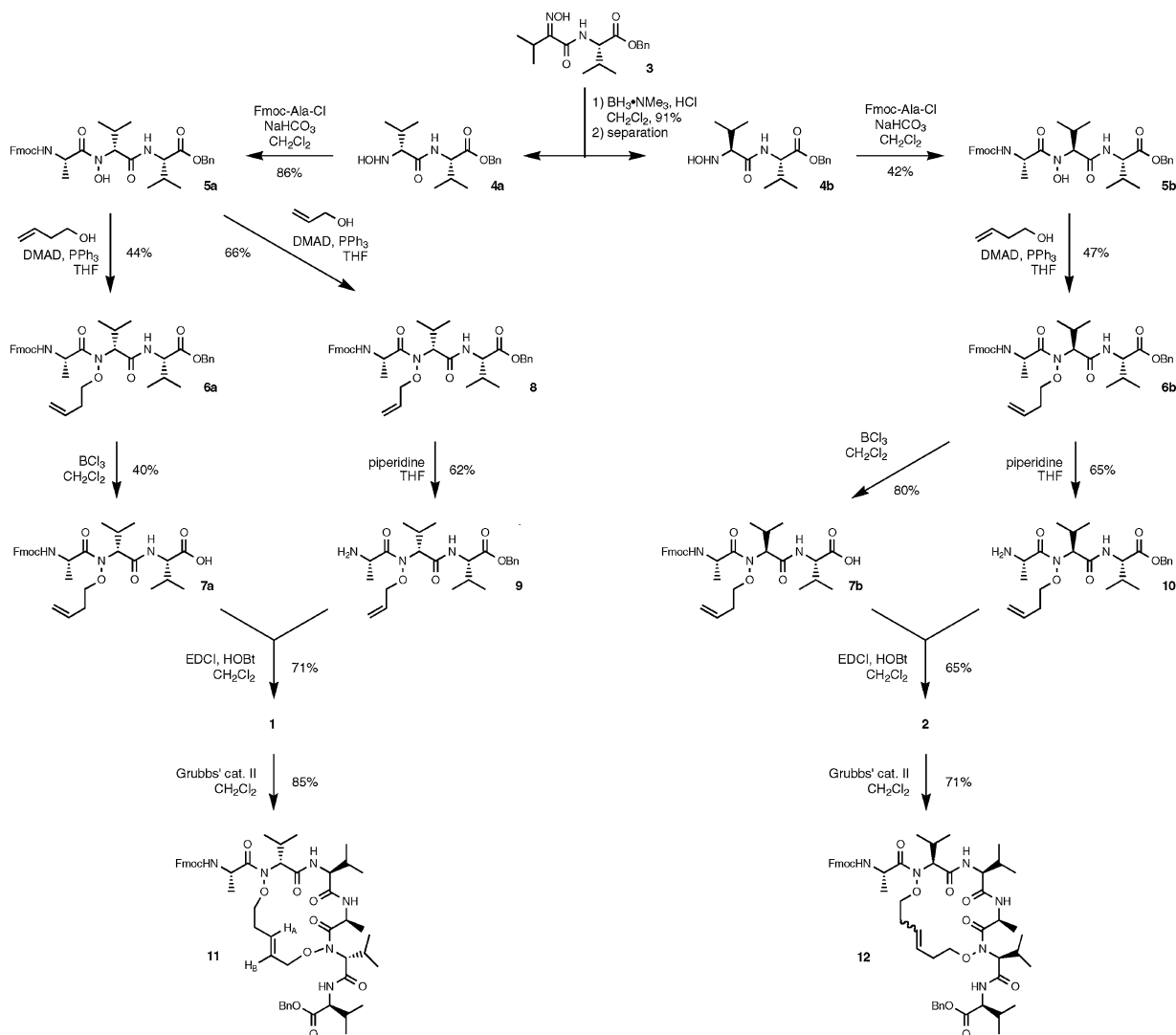
A fragment coupling strategy was chosen as it allows the obtention of a *N,N'*-dialkenoxy hexapeptide presenting two alkene chains of different lengths. The synthesis (Scheme 2) started from the previously reported reduction of oxime **3**.¹⁵

Liquid chromatography allowed the separation of the diastereomeric *N*-hydroxy dipeptides **4a** and **4b**. They were then coupled with Fmoc-protected alanine acid chloride¹⁶ in the presence of sodium hydrogencarbonate in dichloromethane. In these conditions selective *N*-acylation is possible.^{15,17} *O*-Alkylation of the hydroxamate group in the *N*-hydroxy tripeptides **5a** and **5b** was performed by reaction with 3-buten-1-ol under Mitsunobu conditions,¹⁸ leading to the *N*-homoallyloxy peptides **6a** and **6b**. Cleavage of the benzylic ester using BCl₃ gave the corresponding carboxylic acid fragments **7a** and **7b**. On the other hand, reaction of the *N*-hydroxy tripeptide **5a** with allyl alcohol under Mitsunobu conditions allowed the preparation of *N*-allyloxy peptide **8**. Piperidine-mediated deprotection of the terminal amine of **8** gave the amine fragment **9**. Similarly, deprotection of **6b** led to the second amine fragment **10**. Hexapeptides **1** and **2** were

obtained in good yields by reaction of tripeptides **7a** and **9**, and **7b** and **10** respectively, using the coupling agent EDCI (1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide hydrochloride) in conjunction with HOBt (1-hydroxybenzotriazole). Ring-closing was then performed through a metathesis reaction in the presence of Grubbs' catalyst II (2nd generation),¹⁹ under high dilution conditions.²⁰

The 17-membered cyclic peptide **11** was isolated in 85% yield. According to ¹H and ¹³C NMR spectra, the cyclization led to a single stereoisomer. A vicinal coupling constant of 15.4 Hz could be determined for the ethylenic proton H_A, indicating an *E* geometry for the alkene bond. Other examples of RCM-mediated formation of 17-membered rings in peptides involve connections between allylglycine residues at *i* and *i* + 4 positions.²¹ Using Grubbs' catalyst II, mixture of *E* and *Z* isomers in various proportions were obtained. The cyclic peptide **11** is, to our knowledge, the first example of an (*i*, *i* + 3)-linked 17-membered cyclic peptide prepared through RCM.

On the other hand, several RCM-mediated connections between residues at *i* and *i* + 3 positions leading to 18-membered cycles are described. They involve bishomoallylglycine²² or *O*-allylserine^{10,11b}



Scheme 2 Preparation and cyclization of *N,N'*-dialkenoxy hexapeptides **1** and **2**.

residues. In the latter case, cyclization of a tetrapeptide gave a 1:1 mixture of *E* and *Z* isomers.²³ Longer peptide sequences and/or C^α-tetrasubstituted α-amino acid residues – such as 2-aminoisobutyric acid residues (Aib) – induce conformational restrictions and thus higher selectivities are obtained: up to 20:1 in favor of the *E* isomer when an Aib-rich octapeptide is at stake.¹⁰ In the case of the hexapeptide **2**, the 18-membered ring **12** was obtained in 71% yield as a mixture of stereoisomers. Analysis of the spectra from ¹H NMR and COSY experiments (in CDCl₃) revealed that several proton resonances were splitted into two: NH Ala₄ (3:1 ratio), CH_α Val₅, CH_α Val₃. A vicinal coupling constant of 15.4 Hz could be determined for one ethylenic proton. From this estimation, we may conclude that the geometry of the major isomer is *E*, in accordance with literature results.

In order to determine some structural features of **11** and **12**, further NMR studies and restrained molecular dynamics using an explicit chloroform solvent model were performed. The structures obtained from molecular modeling are representative of the experimental NOE.²⁴ The cyclic parts of molecules **11** and **12** have similar backbone features but do not present any particular secondary structure. Nevertheless, the presence, in the NOESY spectra, of significant correlations between the ethylenic protons and the H_α proton of Ala₄ and Val₅ indicates the cyclization constrains the peptide.

Conclusions

Alkylation of *N*-hydroxy peptides building blocks allows the introduction of alkene chains in the peptide at a late stage of the synthesis. Modulation of the ring size is thus easy to achieve. These *N*-alkenoxy tripeptides can be elongated using a classical peptide synthesis sequence. In the resulting cyclized peptide, all the native amino acid residues are still available for molecular recognition. The *N*-alkenoxy peptides approach we propose for the RCM-mediated cyclization of peptides could be a versatile option for the incorporation of a constraining moiety into biologically relevant peptides.

Experimental (see also ESI†)

General experimental methods

All non-aqueous reactions were performed under an atmosphere of argon. Standard inert atmosphere techniques were used in handling all air and moisture sensitive reagents. CH₂Cl₂ and THF were freshly distilled from CaH₂ and sodium benzophenone ketyl respectively. Purchased reagents were used without prior purification. For chromatographic purification, reagent grade solvents were used as received. Reactions were monitored by thin layer chromatography (TLC) using commercial aluminum-backed silica gel plates (Merck, Kieselgel 60 F₂₅₄). TLC spots were viewed under ultraviolet light and by heating the plate after treatment with an appropriate staining solution (KMnO₄, ninhydrin, basic TTC (2,3,5-triphenyltetrazolium chloride)). Product purification by flash chromatography was performed using Macherey Nagel Silica Gel 60M (230–400 mesh).

Melting points were determined with a Büchi B-540 apparatus and are given uncorrected. Optical rotations were measured on a

Perkin Elmer 341 polarimeter; the corresponding concentration is reported in g per 100cm⁻³. ¹H NMR (300 MHz) and ¹³C NMR (75 MHz) spectra were run on a Bruker Advance300 spectrometer, and obtained from CDCl₃ (δ_C 77.2 ppm; standard for ¹H spectra: tetramethylsilane δ_H 0.0 ppm). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet; coupling constants *J* and δ_A – δ_B (for AB spin system) are reported in Hz. ¹H and ¹³C resonance assignments were performed using conventional 1D and 2D techniques (DEPT, COSY, HMQC and HMBC experiments). Infrared (IR) spectra were obtained either from neat films, from a thin film of a dichloromethane solution of the compound on sodium chloride discs, or from a dispersion of the compound in a KBr plate. IR spectra were recorded on a Nicolet Impact-400 FT-IR spectrometer and the data are reported as absorption maxima in cm⁻¹. Mass spectra (LRMS) were recorded on a ThermoFinnigan PolarisQ EI/CI ion-trap spectrometer (DCI; ammonia-isobutane 62:38). High resolution mass spectra (HRMS) were recorded at the LCOSB, UMR 7613, Université Pierre et Marie Curie, Paris. Experimental errors for HRMS data are estimated between 1 and 2 ppm.

Fmoc-Ala₁-ψ[CO-N(OH)]-Val₂-Val₃-OBn (5b)²⁵. To a solution of **4b** (185.8 mg, 576 μmol) in CH₂Cl₂ (15 mL) were added NaHCO₃ (60.1 mg, 715 μmol) and Fmoc-Ala-Cl (220.1 mg, 667 μmol). The mixture was stirred overnight. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (Silica gel, CH₂Cl₂-MeOH 100:0 to 98:2) gave **5b** (148.5 mg, 241 μmol, 42% yield) as a white solid which can be recrystallized from CH₂Cl₂/cyclohexane. Mp: 112–113 °C. *R*_f 0.44 (CH₂Cl₂-MeOH 95:5). [α]_D²⁵ +19.8 (*c* 1.50 in CHCl₃). δ_H (300 MHz; CDCl₃) 8.73 (1H, s, NOH), 8.47 (1H, d, *J* 9.1, NH Val₃), 7.73 (2H, d, *J* 7.6, CH_{ar} Fmoc), 7.67–7.60 (2H, m, CH_{ar} Fmoc), 7.45–7.15 (9H, m, CH_{ar}), 6.54 (1H, d, *J* 9.1, NH Ala₁), 5.30–5.20 (1H, m, CH_α Val₂), 5.16 (2H, ABq, *J* 12.0, δ_A-δ_B 56.2, CH₂ -OBn), 5.13–5.02 (1H, m, CH_α Ala₁), 4.74 (1H, dd, *J* 4.5 and 9.2, CH_α Val₃), 4.42–4.15 (3H, m, CH Fmoc, CH₂ Fmoc), 2.50–2.33 (1H, m, CH_{ipr} Val₂), 2.25–2.12 (1H, m, CH_{ipr} Val₃), 1.29 (3H, d, *J* 6.9, CH₃ Ala₁), 0.99 (6H, d, *J* 6.7, CH₃ Val₂), 0.84 (3H, d, *J* 6.9, CH₃ Val₃), 0.73 (3H, d, *J* 6.9, CH₃ Val₃). δ_C (75 MHz; CDCl₃) 173.2 (C=O Val₂), 172.6 (C=O Ala₁), 171.7 (C=O Val₃), 156.2 (C=O Fmoc), 144.2, 144.1, 141.40, 141.37 (C_{ar} Fmoc), 135.1 (C_{ar} -OBn), 128.8, 128.7, 127.8, 127.1, 125.5, 125.4, 120.0 (CH_{ar}), 67.6, 67.1 (CH₂ -OBn, CH₂ Fmoc), 63.3 (CH_α Val₂), 56.6 (CH_α Val₃), 47.4 (CH Fmoc), 47.1 (CH_α Ala₁), 32.0 (CH_{ipr} Val₃), 27.6 (CH_{ipr} Val₂), 19.9, 19.1, 18.9, 18.1, 17.5 (CH₃). IR: 3378, 3299, 3067, 2964, 2929, 2875, 1724, 1634. HRMS (ESI +) *m/z*: found, 638.2836; C₃₅H₄₁N₃O₇Na requires 638.2842.

Fmoc-Ala₁-ψ[CO-N(O(CH₂)₂CH=CH₂)]-D-Val₂-Val₃-OBn (6a). A mixture of **5a** (100.0 mg, 162 μmol) and PPh₃ (106.4 mg, 406 μmol) was dissolved in THF (10 mL). 3-buten-1-ol (34.7 μL, 406 μmol) and DMAD (methyl azodicarboxylate, 61.4 μL, 408 μmol) were added to the solution. After 4 hours of reaction, the solvent was evaporated and the crude material was taken up with CH₂Cl₂. The mixture was washed with brine and the aqueous phase was extracted with CH₂Cl₂. The organic layers were gathered and dried over Na₂SO₄, filtrated and evaporated. Purification by flash chromatography (Silica gel, pentane-EtOAc

9:1 to 1:1) gave **6a** (47.4 mg, 71 μmol , 44% yield) as a colourless oil. R_f 0.65 (CH_2Cl_2 -MeOH 95:5). $[\alpha]_{\text{D}}^{25} +25.3^\circ$ (c 0.75 in CHCl_3). δ_{H} (300 MHz; CDCl_3) 7.68 (2H, d, J 7.4, CH_{ar} Fmoc), 7.58-7.48 (2H, m, CH_{ar} Fmoc), 7.35-7.15 (9H, m, CH_{ar}), 6.95 (1H, d, J 8.2, NH Val₃), 5.80-5.62 (1H, m, CH=), 5.55 (1H, d, J 8.2, NH Ala₁), 5.12-4.92 (4H, m, CH_2 -OBn, CH_2 =), 4.70-4.60 (1H, m, CH_α Ala₁), 4.41 (1H, dd, J 4.6 and 8.4, CH_α Val₃), 4.33-4.25 (2H, m, CHH Fmoc, CH Fmoc), 4.22-4.05 (3H, m, CHH Fmoc, N-O- CHH , CH_α Val₂), 3.85-3.75 (1H, m, N-O- CHH), 2.55-2.40 (1H, m, CH_{ipr} Val₂), 2.31-2.20 (2H, m, N-O- CH_2 - CH_2), 2.18-2.05 (1H, m, CH_{ipr} Val₃), 1.31 (3H, d, J 6.7, CH_3 Ala₁), 0.93 (3H, d, J 6.5, CH_3 Val₂), 0.89 (3H, d, J 6.4, CH_3 Val₂), 0.83 (3H, d, J 6.9, CH_3 Val₃), 0.77 (3H, d, J 6.8, CH_3 Val₃). δ_{C} (75 MHz; CDCl_3) 174.4, 171.2, 170.3 (C=O), 155.8 (C=O Fmoc), 144.2, 144.0, 141.5, 141.4 (C_{ar} Fmoc), 135.6 (C_{ar} -OBn), 133.7 (CH=), 128.7, 128.5, 128.4, 127.8, 127.2, 125.4, 125.3, 120.1 (CH_{ar}), 117.9 (CH_2 =), 75.9 (N-O- CH_2), 68.0 (CH_α Val₂), 67.1, 67.0 (CH_2 Fmoc, CH_2 -OBn), 57.5 (CH_α Val₃), 48.0 (CH_α Ala₁), 47.4 (CH Fmoc), 32.6 (N-O- CH_2 - CH_2), 30.8 (CH_{ipr} Val₃), 26.4 (CH_{ipr} Val₂), 19.9, 19.7, 19.3, 18.8, 17.7 (CH_3). IR: 3325, 3067, 3028, 2964, 2929, 2868, 1723, 1681, 1649. LRMS (DCI +) m/z : 687.0 (M + NH_4)⁺. HRMS (ESI +) m/z : found, 708.30366; $\text{C}_{39}\text{H}_{47}\text{N}_3\text{O}_7\text{K}$ requires 708.30456.

Fmoc-Ala₁- ψ [CO-N(O(CH₂)₂CH=CH₂)]-Val₂-Val₃-OBn (**6b**).

The title compound was prepared as described for **6a** using **5b** (144.1 mg, 234 μmol). Purification by flash chromatography (Silica gel, pentane-EtOAc 9:1 to 1:1) gave **6b** (74.3 mg, 111 μmol , 47% yield) as a colourless oil. R_f 0.56 (CH_2Cl_2 -MeOH 95:5). $[\alpha]_{\text{D}}^{25} +4.1^\circ$ (c 1.09 in CHCl_3). δ_{H} (300 MHz; CDCl_3) 7.75 (2H, d, J 7.6, CH_{ar} Fmoc), 7.72-7.65 (1H, m, NH Val₃), 7.64-7.58 (2H, m, CH_{ar} Fmoc), 7.43-7.23 (9H, m, CH_{ar}), 5.85-5.70 (1H, m, CH=), 5.58 (1H, d, J 7.7, NH Ala₁), 5.20-5.00 (4H, m, CH_2 -OBn, CH_2 =), 4.80-4.70 (1H, m, CH_α Ala₁), 4.49 (1H, dd, J 4.3 and 8.4, CH_α Val₃), 4.45-4.32 (2H, m, CHH Fmoc, CH Fmoc), 4.28-4.20 (1H, m, CHH Fmoc), 4.10-3.85 (3H, m, N-O- CH_2 , CH_α Val₂), 2.75-2.60 (1H, m, CH_{ipr} Val₂), 2.40-2.30 (2H, m, N-O- CH_2 - CH_2), 2.28-2.15 (1H, m, CH_{ipr} Val₃), 1.40 (3H, d, J 6.8, CH_3 Ala₁), 1.00 (3H, d, J 6.8, CH_3 Val₂), 0.97 (3H, d, J 6.8, CH_3 Val₂), 0.92 (3H, d, J 6.9, CH_3 Val₃), 0.85 (3H, d, J 6.9, CH_3 Val₃). δ_{C} (75 MHz; CDCl_3) 174.6, 171.4, 170.3 (C=O), 155.8 (C=O Fmoc), 144.2, 144.0, 141.5, 141.4 (C_{ar} Fmoc), 135.7 (C_{ar} -OBn), 133.5 (CH=), 128.7, 128.5, 128.4, 127.9, 125.4, 125.3, 120.1 (CH_{ar}), 118.1 (CH_2 =), 75.8 (N-O- CH_2), 74.6 (CH_α Val₂), 67.2, 67.0 (CH_2 Fmoc, CH_2 -OBn), 57.5 (CH_α Val₃), 48.1 (CH_α Ala₁), 47.4 (CH Fmoc), 32.5 (N-O- CH_2 - CH_2), 30.8 (CH_{ipr} Val₃), 27.3 (CH_{ipr} Val₂), 19.9, 19.7, 19.3, 18.7, 17.6 (CH_3). IR: 3323, 3067, 3033, 2964, 2930, 2868, 2851, 1725, 1681. HRMS (ESI +) m/z : found, 708.30371; $\text{C}_{39}\text{H}_{47}\text{N}_3\text{O}_7\text{K}$ requires 708.30456.

Fmoc-Ala₁- ψ [CO-N(O(CH₂)₂CH=CH₂)]-D-Val₂-Val₃-OH (**7a**).

To a solution of **6a** (35.4 mg, 52.9 μmol) in CH_2Cl_2 (4 mL) was added BCl_3 (1M in hexane) (0.5 mL, 0.5 mmol). The mixture was stirred overnight. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (Silica gel, CH_2Cl_2 -MeOH 100:0 to 95:5) gave **7a** (12.3 mg, 21.2 μmol , 40% yield) as a yellow oil. R_f 0.11 (CH_2Cl_2 -MeOH 95:5). $[\alpha]_{\text{D}}^{25} +13.0^\circ$ (c 0.71 in CHCl_3). δ_{H} (300 MHz; CDCl_3) 7.75 (2H, d, J 7.6, CH_{ar} Fmoc), 7.73-7.65 (1H, m, NH Val₃), 7.62-7.53 (2H, m, CH_{ar} Fmoc), 7.42-7.25 (4H, m, CH_{ar} Fmoc), 5.85-5.70 (1H, m, CH=), 5.57 (1H, d, J 8.2, NH Ala₁), 5.20-5.05 (2H, m, CH_2 =),

4.80-4.65 (1H, m, CH_α Ala₁), 4.42 (1H, dd, J 4.6 and 8.2, CH_α Val₃), 4.38-4.31 (2H, m, CHH Fmoc, CH Fmoc), 4.25-4.16 (1H, m, CHH Fmoc), 4.15-4.02 (2H, m, N-O- CH_2), 3.95 (1H, d, J 11.1, CH_α Val₂), 2.72-2.57 (1H, m, CH_{ipr} Val₂), 2.45-2.33 (2H, m, N-O- CH_2 - CH_2), 2.30-2.15 (1H, m, CH_{ipr} Val₃), 1.39 (3H, d, J 6.8, CH_3 Ala₁), 1.05-0.80 (12H, m, CH_3 Val₂, CH_3 Val₃). δ_{C} (75 MHz; CDCl_3) 174.9, 173.9, 170.6 (C=O), 156.0 (C=O Fmoc), 144.1, 144.0, 141.5 (C_{ar} Fmoc), 133.5 (CH=), 127.9, 127.3, 125.4, 125.3, 120.2 (CH_{ar}), 118.2 (CH_2 =), 75.9 (N-O- CH_2), 74.2 (CH_α Val₂), 67.3 (CH_2 Fmoc), 57.7 (CH_α Val₃), 48.1 (CH_α Ala₁), 47.3 (CH Fmoc), 32.6 (N-O- CH_2 - CH_2), 30.4 (CH_{ipr} Val₃), 27.4 (CH_{ipr} Val₂), 19.8, 19.7, 19.4, 18.4, 17.7 (CH_3). IR: 3314, 3068, 2965, 2931, 1716, 1651. LRMS (DCI +) m/z : 580.1 (M + H)⁺, 597.6 (M + NH_4)⁺. HRMS (ESI +) m/z : found, 618.25684; $\text{C}_{32}\text{H}_{41}\text{N}_3\text{O}_7\text{K}$ requires 618.25761.

Fmoc-Ala₁- ψ [CO-N(O(CH₂)₂CH=CH₂)]-Val₂-Val₃-OH (**7b**).

To a solution of **6b** (42.1 mg, 62.9 μmol) in CH_2Cl_2 (4 mL) was added BCl_3 (1M in hexane) (315 μL , 315 μmol). The mixture was stirred for 2 hours before a second addition of BCl_3 (157 μL , 157 μmol). The mixture was stirred further for 2 hours. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (Silica gel, CH_2Cl_2 -MeOH 98:2 to 90:10) gave **7b** (29.2 mg, 50.4 μmol , 80% yield) as a yellow oil. R_f 0.13 (CH_2Cl_2 -MeOH 95:5). $[\alpha]_{\text{D}}^{25} +10.9^\circ$ (c 1.27 in CHCl_3). δ_{H} (300 MHz; CDCl_3) 7.74 (2H, d, J 7.4, CH_{ar} Fmoc), 7.64 (1H, d, J 7.8, NH Val₃), 7.60-7.55 (2H, m, CH_{ar} Fmoc), 7.42-7.25 (4H, m, CH_{ar} Fmoc), 5.85-5.72 (1H, m, CH=), 5.63 (1H, d, J 8.1, NH Ala₁), 5.17-5.05 (2H, m, CH_2 =), 4.81-4.69 (1H, m, CH_α Ala₁), 4.44 (1H, dd, J 4.5 and 8.1, CH_α Val₃), 4.40-4.30 (2H, m, CHH Fmoc, CH Fmoc), 4.25-4.15 (1H, m, CHH Fmoc), 4.15-3.95 (2H, m, N-O- CH_2), 4.01 (1H, d, J 11.3, CH_α Val₂), 2.70-2.55 (1H, m, CH_{ipr} Val₂), 2.43-2.30 (2H, m, N-O- CH_2 - CH_2), 2.29-2.14 (1H, m, CH_{ipr} Val₃), 1.38 (3H, d, J 6.8, CH_3 Ala₁), 1.02-0.85 (12H, m, CH_3 Val₂, CH_3 Val₃). δ_{C} (75 MHz; CDCl_3) 174.9, 174.3, 170.4 (C=O), 156.1 (C=O Fmoc), 144.1, 144.0, 141.5 (C_{ar} Fmoc), 133.5 (CH=), 127.9, 127.3, 125.4, 125.3, 120.1 (CH_{ar}), 118.2 (CH_2 =), 76.0 (N-O- CH_2), 73.7 (CH_α Val₂), 67.3 (CH_2 Fmoc), 57.6 (CH_α Val₃), 48.1 (CH_α Ala₁), 47.3 (CH Fmoc), 32.6 (N-O- CH_2 - CH_2), 30.5 (CH_{ipr} Val₃), 27.5 (CH_{ipr} Val₂), 19.8, 19.7, 19.3, 18.4, 17.7 (CH_3). IR: 3319, 3067, 2965, 2932, 2868, 1723, 1652. HRMS (ESI +) m/z : found, 618.25679; $\text{C}_{32}\text{H}_{41}\text{N}_3\text{O}_7\text{K}$ requires 618.25761.

Fmoc-Ala₁- ψ [CO-N(OCH₂CH=CH₂)]-D-Val₂-Val₃-OBn (**8**).

A mixture of **5a** (100.0 mg, 162 μmol) and PPh_3 (106.4 mg, 406 μmol) was dissolved in THF (10 mL). Allyl alcohol (28 μL , 412 μmol) and DMAD (methyl azodicarboxylate, 61 μL , 405 μmol) were added to the solution. After 4 hours, the solvent was evaporated and the crude material was taken up with CH_2Cl_2 . The mixture was washed with brine and the aqueous phase was extracted with CH_2Cl_2 . The organic layers were gathered and dried over Na_2SO_4 , filtrated and evaporated. Purification by flash chromatography (Silica gel, pentane-EtOAc 9:1 to 1:1) gave **8** (70.1 mg, 107 μmol , 66% yield) as a colourless oil. R_f 0.56 (CH_2Cl_2 -MeOH 95:5). $[\alpha]_{\text{D}}^{25} +17.7^\circ$ (c 0.83 in CHCl_3). δ_{H} (300 MHz; CDCl_3) 7.75 (2H, d, J 7.4, CH_{ar} Fmoc), 7.63-7.55 (2H, m, CH_{ar} Fmoc), 7.43-7.20 (9H, m, CH_{ar}), 7.00 (1H, d, J 8.1, NH Val₃), 6.00-5.80 (1H, m, CH=), 5.60 (1H, d, J 7.9, NH Ala₁), 5.40-5.25 (2H, m, CH_2 =), 5.08 (2H, ABq, J 12.3, δ_{A} - δ_{B} 12.5, CH_2 -OBn), 4.85-4.70 (1H, m, CH_α Ala₁), 4.65-4.55 (1H, m,

N–O–CHH), 4.49 (1H, dd, *J* 4.5 and 8.3, CH_α Val₃), 4.42–4.32 (3H, m, CH Fmoc, CHH Fmoc and N–O–CHH), 4.30–4.20 (2H, m, CHH Fmoc and CH_α Val₂), 2.63–2.48 (1H, m, CH_{IPr} Val₂), 2.27–2.14 (1H, m, CH_{IPr} Val₃), 1.40 (3H, d, *J* 6.8, CH₃ Ala₁), 1.00 (3H, d, *J* 6.9, CH₃ Val), 0.97 (3H, d, *J* 6.9, CH₃ Val), 0.91 (3H, d, *J* 6.9, CH₃ Val), 0.85 (3H, d, *J* 6.9, CH₃ Val). δ_c (75 MHz; CDCl₃) 174.5 (C=O Ala₁), 171.2 (C=O Val₃), 170.1 (C=O Val₂), 155.8 (C=O, Fmoc), 144.2, 144.0, 141.51, 141.46 (CH_{ar} Fmoc), 135.6 (C_{ar} –OBn), 130.8 (CH=), 128.7, 128.53, 128.49, 127.9, 127.2, 125.4, 125.3 (CH_{ar}), 121.0 (CH₂=), 120.2 (CH_{ar}), 77.8 (N–O–CH₂), 68.1, 67.2, 67.0 (CH₂ –OBn, CH₂ Fmoc, CH_α Val₂), 57.6 (CH_α Val₃), 48.0 (CH_α Ala₁), 47.4 (CH Fmoc), 30.9 (CH_{IPr} Val₃), 26.4 (CH_{IPr} Val₂), 19.8, 19.7, 19.3, 18.9, 17.7 (CH₃ Ala₁, Val₂, Val₃). IR: 3325, 3065, 2963, 2928, 2874, 1722, 1682, 1655. LMRS (DCI) *m/z*: 672.8 (M + NH₄)⁺. HRMS (ESI +) *m/z*: found, 694.28843; C₃₈H₄₅N₃O₇K requires 694.28891.

H-Ala₁-ψ[CO-N(OCH₂CH=CH₂)]-D-Val₂-Val₃-OBn (9). To a solution of **8** (54.0 mg, 82.4 μmol) in THF (4 mL) was added piperidine (1 mL). The mixture was stirred for 1h. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (Silica gel, CH₂Cl₂-MeOH 100:0 to 95:5) gave **9** (22.3 mg, 51.4 μmol, 62% yield) as a yellow oil. *R_f* 0.11 (CH₂Cl₂-MeOH 95:5). [α]_D²⁵ +48.3° (*c* 0.64 in CHCl₃). δ_H (300 MHz; CDCl₃) 7.45–7.30 (5H, m, CH_{ar}), 7.17 (1H, d, *J* 8.5, NH Val₃), 5.95–5.78 (1H, m, CH=), 5.39–5.23 (2H, m, CH₂=), 5.14 (2H, ABq, *J* 12.3, δ_A-δ_B 14.1, CH₂ –OBn), 4.61–4.48 (2H, m, CH_α Val₃, N–O–CHH), 4.33–4.17 (2H, m, CH_α Val₂, N–O–CHH), 3.85 (1H, q, *J* 6.8, CH_α Ala₁), 2.62–2.47 (1H, m, CH_{IPr} Val₂), 2.30–2.16 (1H, m, CH_{IPr} Val₃), 1.28 (3H, d, *J* 6.8, CH₃ Ala₁), 0.99 (3H, d, *J* 6.7, CH₃ Val₂), 0.95 (3H, d, *J* 6.7, CH₃ Val₃), 0.93 (3H, d, *J* 6.9, CH₃ Val₂), 0.87 (3H, d, *J* 6.9, CH₃ Val₃). δ_c (75 MHz; CDCl₃) 178.5, 171.6, 170.7 (C=O), 135.6 (C_{ar}), 131.0 (CH=), 128.73, 128.69, 128.6, 128.4 (CH_{ar}), 120.6 (CH₂=), 77.6 (N–O–CH₂), 68.1 (CH_α Val₂), 67.1 (CH₂ –OBn), 57.5 (CH_α Val₃), 47.8 (CH_α Ala₁), 30.7 (CH_{IPr} Val₃), 26.1 (CH_{IPr} Val₂), 20.9, 19.8, 19.7, 19.3, 17.7 (CH₃). IR: 3310, 2965, 2929, 2872, 1739, 1685, 1653. LRMS (DCI +) *m/z*: 434.1 (M + H)⁺. HRMS (ESI +) *m/z*: found, 434.26451; C₂₃H₃₆N₃O₅ requires 434.26495.

H-Ala₁-ψ[CO-N(O(CH₂)₂CH=CH₂)]-Val₂-Val₃-OBn (10). To a solution of **6b** (47.1 mg, 70.4 μmol) in THF (5 mL) was added piperidine (1 mL). The mixture was stirred for 1h. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (Silica gel, CH₂Cl₂-MeOH 100:0 to 90:10) gave **10** (20.3 mg, 45.4 μmol, 65% yield) as a yellow oil. *R_f* 0.16 (CH₂Cl₂-MeOH 90:10). [α]_D²⁵ +0.8° (*c* 0.92 in CHCl₃). δ_H (300 MHz; CDCl₃) 7.81 (1H, d, *J* 8.6, NH Val₃), 7.40–7.25 (5H, m, CH_{ar}), 5.85–5.65 (1H, m, CH=), 5.22–5.05 (4H, m, CH₂ –OBn, CH₂=), 4.51 (1H, dd, *J* 4.5 and 8.8, CH_α Val₃), 4.02–3.70 (4H, m, N–O–CH₂, CH_α Val₂, CH_α Ala₁), 2.75–2.60 (1H, m, CH_{IPr} Val₂), 2.40–2.18 (3H, m, N–O–CH₂-CH₂, CH_{IPr} Val₃), 1.29 (3H, d, *J* 6.8, CH₃ Ala₁), 0.99 (3H, d, *J* 6.8, CH₃ Val₂), 0.95 (3H, d, *J* 6.8, CH₃ Val₃), 0.94 (3H, d, *J* 6.8, CH₃ Val₂), 0.88 (3H, d, *J* 6.8, CH₃ Val₃). δ_c (75 MHz; CDCl₃) 178.5, 171.6, 171.0 (C=O), 135.7 (C_{ar}), 133.5 (CH=), 128.7, 128.6, 128.4 (CH_{ar}), 118.0 (CH₂=), 75.4 (N–O–CH₂), 74.5 (CH_α Val₂), 67.0 (CH₂ –OBn), 57.5 (CH_α Val₃), 47.8 (CH_α Ala₁), 32.5 (N–O–CH₂-CH₂), 30.8 (CH_{IPr} Val₃), 27.2 (CH_{IPr} Val₂), 20.6, 19.9, 19.7, 19.4, 17.6 (CH₃). IR: 3308, 3066, 3034, 2964,

2929, 2874, 1739, 1682. HRMS (ESI +) *m/z*: found, 448.28003; C₂₄H₃₈N₃O₅ requires 448.28060.

Fmoc-Ala₁-ψ[CO-N(O(CH₂)₂CH=CH₂)]-D-Val₂-Val₃-Ala₄-ψ[CO-N(OCH₂CH=CH₂)]-D-Val₅-Val₆-OBn (1). To a mixture of **7a** (9.8 mg, 16.9 μmol), **9** (8.3 mg, 19.2 μmol) and HOBt (1-hydroxybenzotriazole, 3.5 mg, 25 μmol) in CH₂Cl₂ (1.5 mL), was added EDCI (1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide hydrochloride, 5.0 mg, 26.1 μmol). The mixture was stirred overnight. The solution was then washed with brine and the organic layer dried over Na₂SO₄. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (Silica gel, pentane-EtOAc 9:1 to 1:1) gave **1** (12.0 mg, 12.1 μmol, 71% yield) as a yellow oil. *R_f* 0.40 (CH₂Cl₂-MeOH 95:5). [α]_D²⁵ +22.8° (*c* 0.69 in CHCl₃). δ_H (300 MHz; CDCl₃) 7.76 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.65–7.55 (2H, m, CH_{ar} Fmoc), 7.45–7.25 (10H, m, CH_{ar}, NH Val 6), 7.05–6.90 (1H, m, NH Val₃), 6.70–6.60 (1H, m, NH Ala₄), 5.95–5.70 (3H, m, 2x CH=, NH Ala₁), 5.40–4.95 (6H, m, CH₂ –OBn, 2x CH₂=), 4.95–4.86 (1H, m, CH_α Ala₄), 4.82–4.75 (1H, m, CH_α Ala₁), 4.67–4.55 (1H, m, NO–CHH–CH=), 4.52–4.35 (3H, m, CH_α Val₆, CH_α Val₅, NO–CHH–CH=), 4.34–4.15 (6H, m, CH_α Val₂, CH_α Val₅, CH Fmoc, CH₂ Fmoc, N–O–CHH), 4.00–3.90 (1H, m, N–O–CHH), 2.63–2.41 (2H, m, CH_{IPr} Val₂, CH_{IPr} Val₅), 2.40–2.29 (2H, m, N–O–CH₂-CH₂), 2.28–2.17 (2H, m, CH_{IPr} Val₃, CH_{IPr} Val₆), 1.45–1.20 (6H, m, CH₃ Ala₁, CH₃ Ala₄), 1.05–0.80 (24H, m, CH₃ Val₂, CH₃ Val₅, CH₃ Val₃, CH₃ Val₆). δ_c (75 MHz; CDCl₃) 174.2, 171.4, 171.2, 170.1, 169.8 (C=O), 155.8 (C=O Fmoc), 144.1, 144.0, 141.5 (CH Fmoc), 135.7 (C_{ar} –OBn), 133.7 (CH=), 130.9 (NO–CH₂-CH=), 128.8, 128.7, 128.54, 128.49, 127.9, 127.3, 125.3 (CH_{ar}), 121.0 (N–O–CH₂-CH=CH₂), 120.2 (CH_{ar}), 117.9 (N–O–(CH₂)₂CH=CH₂), 77.9, 76.0 (N–O–CH₂), 68.4, 67.9 (CH_α Val₂, CH_α Val₅), 67.2, 67.0 (CH₂ –OBn, CH₂ Fmoc), 58.9, 57.6 (CH_α Val₃, CH_α Val₆), 48.4 (CH_α Ala_{1 or 4}), 47.4 (CH Fmoc), 46.6 (CH_α Ala_{4 or 1}), 32.7 (N–O–CH₂-CH₂), 30.8, 30.5 (CH_{IPr} Val₃, CH_{IPr} Val₆), 26.59, 26.49 (CH_{IPr} Val₂, CH_{IPr} Val₅), 19.9, 19.8, 19.7, 19.5, 19.2, 18.7, 18.6, 17.9, 17.8, 17.7 (CH₃). IR: 3324, 3067, 2965, 2932, 2874, 1724, 1650. HRMS (ESI +) *m/z*: found, 1033.50339; C₅₅H₇₄N₆O₁₁K requires 1033.50472.

Fmoc-Ala₁-ψ[CO-N(O(CH₂)₂CH=CH₂)]-Val₂-Val₃-Ala₄-ψ[CO-N(O(CH₂)₂CH=CH₂)]-Val₅-Val₆-OBn (2). The title compound was prepared as described for **1** using **7b** (25.4 mg, 43.8 μmol) and **10** (18.4 mg, 41.1 μmol). Purification by flash chromatography (Silica gel, pentane-EtOAc 9:1 to 1:1) gave **2** (26.9 mg, 26.7 μmol, 65% yield) as a yellow oil. *R_f* 0.40 (CH₂Cl₂-MeOH 95:5). [α]_D²⁵ –12.7° (*c* 0.94 in CHCl₃). δ_H (300 MHz; CDCl₃) 7.76 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.63–7.55 (2H, m, CH_{ar} Fmoc), 7.47 (1H, d, *J* 8.7, NH Val₆), 7.44–7.26 (10H, m, CH_{ar} Fmoc, NH Val₃), 6.69 (1H, d, *J* 7.9, NH Ala₄), 5.90–5.60 (3H, m, 2x CH=, NH Ala₁), 5.24–5.02 (6H, m, CH₂ –OBn, 2x CH₂=), 5.01–4.90 (1H, m, CH_α Ala₄), 4.83–4.70 (1H, m, CH_α Ala₁), 4.48 (1H, dd, *J* 4.3 and 8.4, CH_α Val₆), 4.40–3.90 (10H, m, CH_α Val₃, CH_α Val₂, CH_α Val₅, CH Fmoc, CH₂ Fmoc, (2x) N–O–CH₂), 2.70–2.50 (2H, m, CH_{IPr} Val₂, CH_{IPr} Val₅), 2.50–2.27 (4H, m, (2x) N–O–CH₂-CH₂), 2.27–2.12 (2H, m, CH_{IPr} Val₃, CH_{IPr} Val₆), 1.41 (3H, d, *J* 6.7, CH₃ Ala₁), 1.32 (3H, d, *J* 6.9, CH₃ Ala₄), 1.07–0.80 (24H, m, CH₃ Val₂, CH₃ Val₅, CH₃ Val₃, CH₃ Val₆). δ_c (75 MHz; CDCl₃) 174.8, 174.2, 171.4, 170.1, 170.0, 169.8 (C=O), 155.8 (C=O Fmoc), 144.1, 144.0, 141.5 (C_{ar} Fmoc), 135.7 (C_{ar} –OBn), 133.6, 133.5 (CH=), 128.7, 128.5, 128.4, 127.9, 127.2, 125.3, 120.1 (CH_{ar}), 118.1, 118.0

(CH₂=), 76.0, 75.9 (N–O–CH₂), 73.5, 72.8 (CH_α Val₂, CH_α Val₅), 67.2, 67.0 (CH₂ Fmoc, CH₂ –OBn), 58.8 (CH_α Val₆), 57.4 (CH_α Val₃), 48.0 (CH_α Ala₁), 47.3 (CH Fmoc), 46.1 (CH_α Ala₄), 32.6, 32.5 (N–O–CH₂–CH₂), 30.7 (CH_{ipr} Val₃, CH_{ipr} Val₆), 27.32, 27.28 (CH_{ipr} Val₂, CH_{ipr} Val₅), 19.9, 19.8, 19.7, 19.5, 19.3, 18.5, 17.9, 17.7 (CH₃). IR: 3312, 3067, 2963, 2931, 2874, 1744, 1724, 1682, 1641. HRMS (ESI +) *m/z*: found, 1047.51887; C₅₆H₇₆N₆O₁₁K requires 1047.52037.

Fmoc-Ala₁-ψ[CO-N(O(CH₂)₂CH^{*})]-D-Val₂-Val₃-Ala₄-ψ[CO-N(OCH₂CH^{*})]-D-Val₅-Val₆-OBn (11). To a solution of **1** (7.4 mg, 7.4 μmol) in CH₂Cl₂ (15 mL), Grubbs' catalyst II (0.6 mg, 0.7 μmol) was added and the reaction was stirred for 12 hours. The mixture was washed with brine and extracted with CH₂Cl₂. The organic phases were gathered, dried over Na₂SO₄, filtered and evaporated. Flash chromatography (Silica gel, diethyl ether) gave **11** (6.1 mg, 6.3 μmol, 85% yield) as a colourless oil. *R_f* 0.23 (CH₂Cl₂-MeOH 95:5). [α]_D²⁵ +26.7° (*c* 0.56 in CHCl₃). δ_H (300 MHz; CDCl₃) 7.76 (2H, d, *J* 7.5, CH_{ar} Fmoc), 7.71 (1H, d, *J* 7.6, NH Val₆), 7.63-7.54 (2H, m, CH_{ar} Fmoc), 7.44-7.36 (2H, m, CH_{ar} Fmoc), 7.35-7.25 (7H, m, CH_{ar}), 6.62 (1H, d, *J* 6.9, NH Val₃), 6.42-6.37 (1H, m, NH Ala₄), 5.87-5.72 (2H, m, N–O–CH₂–CH_B=, NH Ala₁), 5.57 (1H, dt, *J* 6.5 and 15.4, CH_A=), 5.14 (2H, ABq, *J* 12.5, δ_A-δ_B 8.3, CH₂ –OBn), 4.92-4.81 (1H, m, CH_α Ala₁), 4.78-4.70 (1H, m, CH_α Ala₄), 4.70-4.60 (1H, m, N–O–CHH–CH=), 4.57 (1H, d, *J* 11.0, CH_α Val₂), 4.50 (1H, dd, *J* 5.7 and 7.9, CH_α Val₆), 4.42-4.22 (4H, m, CH₂ Fmoc, CH Fmoc, CH_α Val₅), 4.18-4.08 (1H, m, N–O–CHH–CH=), 4.05-3.84 (3H, m, N–O–CH₂–CH₂, CH_α Val₃), 2.59-2.44 (2H, m, CH_{ipr} Val₂, CH_{ipr} Val₅), 2.42-2.28 (1H, m, N–O–CH₂–CHH), 2.25-2.12 (1H, m, N–O–CH₂–CHH), 2.08-1.96 (2H, m, CH_{ipr} Val₃, CH_{ipr} Val₆), 1.45-1.40 (3H, m, CH₃ Ala₁), 1.27-1.20 (3H, m, CH₃ Ala₄), 1.09-0.80 (24H, m, CH₃ Val₂, CH₃ Val₃, CH₃ Val₅, CH₃ Val₆). δ_C (75 MHz; CDCl₃) 175.3, 175.1, 171.9, 170.6, 170.2, 169.3 (C=O), 156.0 (C=O Fmoc), 144.2, 143.9, 141.53, 141.46 (C_{ar} Fmoc), 135.8 (C_{ar} –OBn), 135.7 (CH=), 128.7, 128.5, 128.3, 127.94, 127.91, 127.2 (CH_{ar}), 125.6 (CH=), 125.4, 125.2, 120.2 (CH_{ar}), 76.5, 75.3 (N–O–CH₂), 67.4, 66.9 (CH₂ –OBn, CH₂ Fmoc, CH_α Val_{2 or 5}), 64.5 (CH_α Val_{2 or 5}), 61.2, 58.0 (CH_α Val₃, CH_α Val₆), 48.2, 48.1 (CH_α Ala_{1 and 4}), 47.3 (CH Fmoc), 34.4 (N–O–CH₂–CH₂), 30.8, 30.5 (CH_{ipr} Val₃, CH_{ipr} Val₆), 27.6, 26.8 (CH_{ipr} Val₂, CH_{ipr} Val₅), 20.3, 19.95, 19.85, 19.5, 19.4, 19.3, 18.8, 18.1, 17.5 (CH₃). IR: 3320, 2961, 2925, 2872, 2853, 1736, 1660. HRMS (ESI +) *m/z*: found, 1005.47242; C₅₅H₇₀N₆O₁₁K requires 1005.47342.

Fmoc-Ala₁-Ψ[CO-N(O(CH₂)₂CH^{*})]-Val₂-Val₃-Ala₄-Ψ[CO-N(O(CH₂)₂CH^{*})]-Val₅-Val₆-OBn (12). To a solution of **2** (18.8 mg, 18.6 μmol) in CH₂Cl₂ (15 mL), Grubbs' catalyst II was added in three times: 1.6 mg (1.9 μmol); 0.8 mg (0.9 μmol) after 6h; 0.8 mg (0.9 μmol) after 12h. The reaction was stirred further for 2 hours. The mixture was washed with brine and extracted with CH₂Cl₂. The organic phases were gathered, dried over Na₂SO₄, filtered and evaporated. Flash chromatography (Silica gel, pentane-EtOAc 9:1 to 1:1) gave **12** (12.9 mg, 13.2 μmol, 71% yield) as a 3:1 mixture of two stereoisomers. *R_f* 0.27 (CH₂Cl₂-MeOH 95:5). δ_H (300 MHz; CDCl₃) 7.75 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.63-7.50 (2H, m, CH_{ar} Fmoc), 7.46 (1H, d, *J* 7.0, NH Val₆), 7.42-7.25 (10H, m, CH_{ar}, NH Val₃), 6.37-6.28 and 6.00-5.95 (1H, ratio 3:1, m, NH Ala₄), 5.75-5.55 (2H, m, CH=, NH Ala₁), 5.55-5.43 (1H, dt, *J* 6.1 and 15.4, CH=), 5.12 (2H, ABq, *J*

12.3, δ_A-δ_B 57.1, CH₂ –OBn), 4.85-4.65 (2H, m, CH_α Ala₁, CH_α Ala₄), 4.45 (1H, dd, *J* 4.4 and 8.3, CH_α Val₆), 4.40-4.25 (1H, m, CHH Fmoc), 4.25-4.17 (2H, m, CHH Fmoc, CH Fmoc), 4.15-4.00 (minor diastereomer: m, CH_α Val₅, CH_α Val₃), 3.95-3.75 (m, 4H, NO–CH₂ + major diastereomer: m, CH_α Val₅, CH_α Val₃), 3.69 (1H, d, *J* 11.0, CH_α Val₂), 2.80-2.50 (2H, m, CH_{ipr} Val₂, CH_{ipr} Val₅), 2.40-2.00 (4H, m, N–O–CH₂–CH₂, CH_{ipr} Val₃, CH_{ipr} Val₆), 1.50-1.25 (6H, m, CH₃ Ala₁, CH₃ Ala₄), 1.10-0.78 (24H, m, CH₃ Val₂, CH₃ Val₃, CH₃ Val₅, CH₃ Val₆). δ_C (75 MHz; CDCl₃) for the major diastereomer: 174.45, 174.38, 172.7, 171.2, 170.4, 170.2 (C=O), 155.8 (C=O Fmoc), 144.3, 143.9, 141.5, 141.4 (C_{ar} Fmoc), 136.0 (C_{ar} –OBn), 130.7 (CH=), 128.6, 128.4, 127.8, 127.2 (CH_{ar}), 126.7 (CH=), 125.4, 125.3, 120.1 (CH_{ar}), (overlapped by CDCl₃: N–O–CH₂, CH_α Val_{2 or 5}), 73.8 (CH_α Val_{2 or 5}), 67.2, 66.7 (CH₂ –OBn, CH₂ Fmoc), 60.8 (CH_α Val₃), 57.6 (Val₆), 48.6 (CH_α Ala_{1 or 4}), 47.4 (CH Fmoc), 46.6 (CH_α Ala_{1 or 4}), 31.1, 30.6, 30.3 (2x N–O–CH₂–CH₂, CH_{ipr} Val₃, CH_{ipr} Val₆), 27.3 (CH_{ipr} Val_{2 and 5}), 20.5, 19.85, 19.82, 19.7, 19.4, 19.3, 19.2, 19.0, 17.6 (CH₃). IR: 3318, 3063, 3033, 2965, 2924, 2872, 1714, 1664. HRMS (ESI +) *m/z*: found, 981.53157; C₅₄H₇₃N₆O₁₁ requires 981.5318.

Analysis of conformational preferences of **11** and **12**

NMR experiments were carried out using a Varian Unity+ 500 MHz spectrometer. The NOESY experiments were recorded with 512 (t1) × 2048 (t2) complex data points and 64 scans were acquired over a spectra width of 5000 Hz in both dimensions. They were acquired with mixing times of 250 and 400 ms. Data processing and analysis were performed using the VNMR program (Varian) on a Sunray workstation.

Molecular modeling: calculations were performed on a workstation using InsightII 2005 and Discover 2004.1 (Biosym/Molecular Simulation). The molecules **11** and **12** were constructed in InsightII using the Biopolymer module. In the case of **12**, we only studied the major *E* isomer. Calculations were performed using an explicit solvent model in chloroform (cubic box of 40 Å side length and 417 CHCl₃ molecules) and the cvff force-field. Periodic bounded conditions were applied.

After minimization (100 steps of steepest descents then 2000 of conjugate gradients), a restrained molecular dynamics protocol was applied. The structure was heated to 900 K in 1.5 ps, then slowly cooled to 300 K by steps of 100 K. At each step the system was allowed to stay 10 ps. 100 structures were saved over the last 10 ps dynamics calculation and each was subjected to a further minimization (2000 steps of conjugate gradient). The 6 structures with the lowest energy were averaged and energy minimized to lead to the representative structure.

The restraints used in the calculations were derived from observed intermolecular NOE classified semiquantitatively into three categories, strong (1.8–3.0 Å), medium (2.5–4.0 Å) and weak (3.5–5.0 Å). Only one violation greater than 0.2 Å (0.25 Å) was detected in the averaged structures.

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Notes and references

- 1 See in particular: (a) V. J. Hruby, *Life Sci.*, 1982, **31**, 189–199 and references therein; (b) H. Kessler, *Angew. Chem. Int. Ed. Engl.*, 1982, **21**, 512–523 and ref. therein.
- 2 For a review, see: (a) P. Li and P. P. Roller, *Curr. Top. Med. Chem.*, 2002, **2**, 325–341 and ref. therein; (b) For a recent study of the effect of backbone cyclization, see: S. Hess, Y. Linde, O. Ovadia, E. Safrai, D. E. Shalev, A. Swed, E. Halbfinger, T. Lapidot, I. Winkler, Y. Gabinet, A. Faier, D. Yarden, Z. Xiang, F. P. Portillo, C. Haskell-Luevano, C. Gilon and A. Hoffman, *J. Med. Chem.*, 2008, **51**, 1026–1034.
- 3 See for instance the review: (a) D. Astruc, *New J. Chem.*, 2005, **29**, 42–56, and the Nobel lectures: (b) Y. Chauvin, *Angew. Chem. Int. Ed.*, 2006, **45**, 3741–3747; (c) R. R. Schrock, *Angew. Chem. Int. Ed.*, 2006, **45**, 3748–3759; (d) R. H. Grubbs, *Angew. Chem. Int. Ed.*, 2006, **45**, 3760–3765.
- 4 (a) F. Liu, K. M. Worthy, L. K. Bindu, R. J. Fisher and T. R. Burke, Jr., *J. Org. Chem.*, 2007, **72**, 9635–9642 and ref. therein; (b) F. J. Dekker, N. J. de Mol, M. J. E. Fischer, J. Kemmink and R. M. J. Liskamp, *Org. Biomol. Chem.*, 2003, **1**, 3297–3303.
- 5 (a) F. Velázquez, S. Venkatraman, W. Wu, M. Blackman, A. Prongay, V. Girjavallabhan, N.-Y. Shih and F. G. Njoroge, *Org. Lett.*, 2007, **9**, 3061–3064; (b) Y. S. Tsanztrizos, J.-M. Ferland, A. McClory, M. Poirier, V. Farina, N. K. Yee, X. Wang, N. Haddad, X. Wei, J. Xu and L. Zhang, *J. Organomet. Chem.*, 2006, **691**, 5163–5171 and ref. therein.
- 6 See for instance: (a) D. D'Addona, A. Carotenuto, E. Novellino, V. Piccand, J. C. Reubi, A. Di Cianni, F. Gori, A. M. Papini and M. Ginanneschi, *J. Med. Chem.*, 2008, **51**, 512–520; (b) A. J. Robinson, J. Elaridi, B. J. Van Lierop, S. Mujcinovic and W. R. Jackson, *J. Pept. Sci.*, 2007, **13**, 280–285; (c) A. Mollica, G. Guardiani, P. Davis, S.-W. Ma, F. Porreca, J. Lai, L. Mannina, A. P. Sobolev and V. J. Hruby, *J. Med. Chem.*, 2007, **50**, 3138–3142; (d) I. Berezowska, N. N. Chung, C. Lemieux, B. C. Wilkes and P. W. Schiller, *J. Med. Chem.*, 2007, **50**, 1414–1417; (e) J. L. Stymiest, B. F. Mitchell, S. Wong and J. C. Vederas, *J. Org. Chem.*, 2005, **70**, 7799–7809; (f) V. Chaleix, V. Sol, M. Guilloton, R. Granet and P. Krausz, *Tetrahedron Lett.*, 2004, **45**, 5295–5299.
- 7 (a) V. R. Pattabiraman, J. L. Stymiest, D. J. Derksen, N. I. Martin and J. C. Vederas, *Org. Lett.*, 2007, **9**, 699–702 and ref. therein; (b) N. Ghalit, J. F. Reichwein, H. W. Hilbers, E. Breukink, D. T. S. Rijkers and R. M. J. Liskamp, *ChemBioChem*, 2007, **8**, 1540–1554 and ref. therein.
- 8 β -turn mimics via RCM-mediated cyclization between i and $i + 3$ amino acid residues: (a) J. Giovannoni, C. Didierjean, P. Durand, M. Marraud, A. Aubry, P. Renaut, J. Martinez and M. Amblard, *Org. Lett.*, 2004, **6**, 3449–3452; (b) S. Hanessian and M. Angiolini, *Chem. Eur. J.*, 2002, **8**, 111–117. Between i and $i + 1$: see for instance: (c) J. Einsiedel, H. Lanig, R. Waibel and P. Gmeiner, *J. Org. Chem.*, 2007, **72**, 9102–9113 and ref. therein.
- 9 (a) F. Bernal, A. F. Tyler, S. J. Korsmeyer, L. D. Walensky and G. L. Verdine, *J. Am. Chem. Soc.*, 2007, **129**, 2456–2457 and ref. therein; (b) D. Wang, M. Liu and P. S. Arora, *Angew. Chem. Int. Ed.*, 2008, **47**, 1879–1880 and the recent Account: (c) A. Patgiri, A. L. Jochim and P. S. Arora, *Acc. Chem. Res.*, 2008, DOI: 10.1021/ar700264k and ref. therein.
- 10 A. K. Boal, I. Guryanov, A. Moretto, M. Crisma, E. L. Lanni, C. Toniolo, R. H. Grubbs and D. J. O'Leary, *J. Am. Chem. Soc.*, 2007, **129**, 6986–6987.
- 11 (a) See in particular ref. 4b, 9b, c; (b) See also: U. Kazmaier, C. Hebach, A. Watzke, S. Maier, H. Mues and V. Huch, *Org. Biomol. Chem.*, 2005, **3**, 136–145.
- 12 (a) See ref. 4–8, 9a. See also: (b) P. W. R. Harris and M. A. Brimble, *Org. Biomol. Chem.*, 2006, **4**, 2696–2709 (c) N. Schmiedeberg and H. Kessler, *Org. Lett.*, 2002, **4**, 59–62; (d) A. S. Ripka, R. S. Bohacek and D. H. Rich, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 357–360; (e) J. Pernerstorfer, M. Schuster and S. Blechert, *Chem. Commun.*, 1997, 1949–1950.
- 13 (a) See ref. 5a, 6e, 10, 11b. See also: (b) H. E. Blackwell, J. D. Sadowsky, R. J. Howard, J. N. Sampson, J. A. Chao, W. E. Steinmetz, D. J. O'Leary and R. H. Grubbs, *J. Org. Chem.*, 2001, **66**, 5291–5302; (c) S. J. Miller, H. E. Blackwell and R. H. Grubbs, *J. Am. Chem. Soc.*, 1996, **118**, 9606–9614.
- 14 (a) See ref. 8c, 9b, 12b, 13c. See also: (b) J. Cluzeau, S. Oishi, H. Ohno, Z. Wang, B. Evans, S. C. Peiper and N. Fujii, *Org. Biomol. Chem.*, 2007, **5**, 1915–1923; (c) J. F. Reichwein, C. Versluis and R. M. J. Liskamp, *J. Org. Chem.*, 2000, **65**, 6187–6195 and ref. therein (d) J. F. Reichwein, B. Wels, J. A. W. Kruijtzter, C. Versluis and R. M. J. Liskamp, *Angew. Chem. Int. Ed.*, 1999, **38**, 3684–3687.
- 15 J. Lawrence, L. Cointeaux, P. Maire, Y. Vallée and V. Blandin, *Org. Biomol. Chem.*, 2006, **4**, 3125–3141.
- 16 Many amino acids, including amino acids bearing a functionalized side chain, can be introduced via the Fmoc-protected amino acid chloride. see: (a) L. A. Carpino, B. J. Cohen, K. E. Stephens, Jr., S. Y. Sadat-Aalae, J.-H. Tien and D. C. Langridge, *J. Org. Chem.*, 1986, **51**, 3732–3734, and for instance a recent example using Fmoc-Ser(*t*-Bu)-Cl: (b) A. C. Spivey, J. McKendrick, R. Srikanan and B. A. Helm, *J. Org. Chem.*, 2003, **68**, 1843–1851.
- 17 P. Maire, V. Blandin, M. Lopez and Y. Vallée, *Synlett*, 2003, 671–674.
- 18 The Mitsunobu reaction occurs under mild conditions and can be carried out in the presence of a wide range of functional groups. See the recent review: (a) T. Y. S. But and P. H. Toy, *Chem. Asian. J.*, 2007, **2**, 1340–1355 and ref. therein. The reaction is compatible with usual amino acid side chain protections. See for instance the study of *N*-methylation of *N*^α-arylsulfonyl peptides via Mitsunobu reaction: (b) E. Biron, J. Chatterjee and H. Kessler, *J. Peptide Sci.*, 2006, **12**, 213–219.
- 19 M. Scholl, S. Ding, C. W. Lee and R. H. Grubbs, *Org. Lett.*, 1999, **1**, 953–956.
- 20 Concentrations: 0.5–1.2 mM. Byproducts that could result from intermolecular RCM or cross metathesis reactions were not recovered during the purification process.
- 21 (a) See ref. 6f, 7a. See also: (b) N. Ghalit, D. T. S. Rijkers and R. M. J. Liskamp, *J. Mol. Catal. A*, 2006, **254**, 68–77.
- 22 See ref. 8b; the stereoselectivity of the ring-closing reaction is not given.
- 23 This result was obtained using the first generation of Grubbs' catalyst (ref. 11b).
- 24 See Experimental and Electronic Supplementary Information.
- 25 Compounds **3**, **4a**, **4b** and **5a** were previously described (ref. 15).