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

James Lawrence, Muriel Jourdan, Yannick Vallée and Véronique Blandin*

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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.

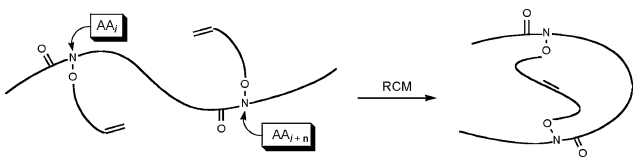
Introduction

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 metathesis[3] (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 domain[4] or in the development of inhibitors of the hepatitis C virus NS3 protease.[5] Analogues of cystine-[6] or lanthionine[7]-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-turn[8] or α- and 3_{10}-helices.[9,10]

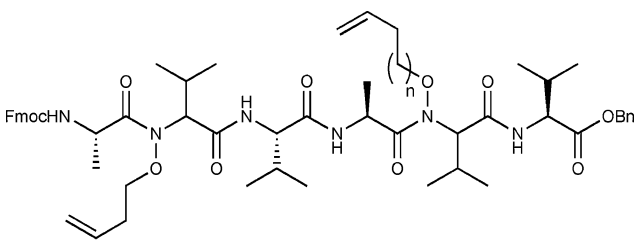
The cyclization staples the linear peptide between two amino acid residues referred to as *i* and *i* + n positions. The unsaturated chains involved in the ring closure can be introduced *via* derivatization of the terminal amine and/or carboxylic acid,[11] as part of an amino acid side-chain, or by 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 bonds[14] 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.



1: D-Val$_2$; D-Val$_5$; n = 0
2: L-Val$_2$; L-Val$_5$; n = 1

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

Département de Chimie Moléculaire, UMR-5250, ICMG FR-2607, CNRS, Université Joseph Fourier, BP-53, 38041, Grenoble Cedex 9, France. E-mail: Veronique.Blandin@ujf-grenoble.fr; Fax: +33 476 635 983; Tel: +33 476 514 803

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

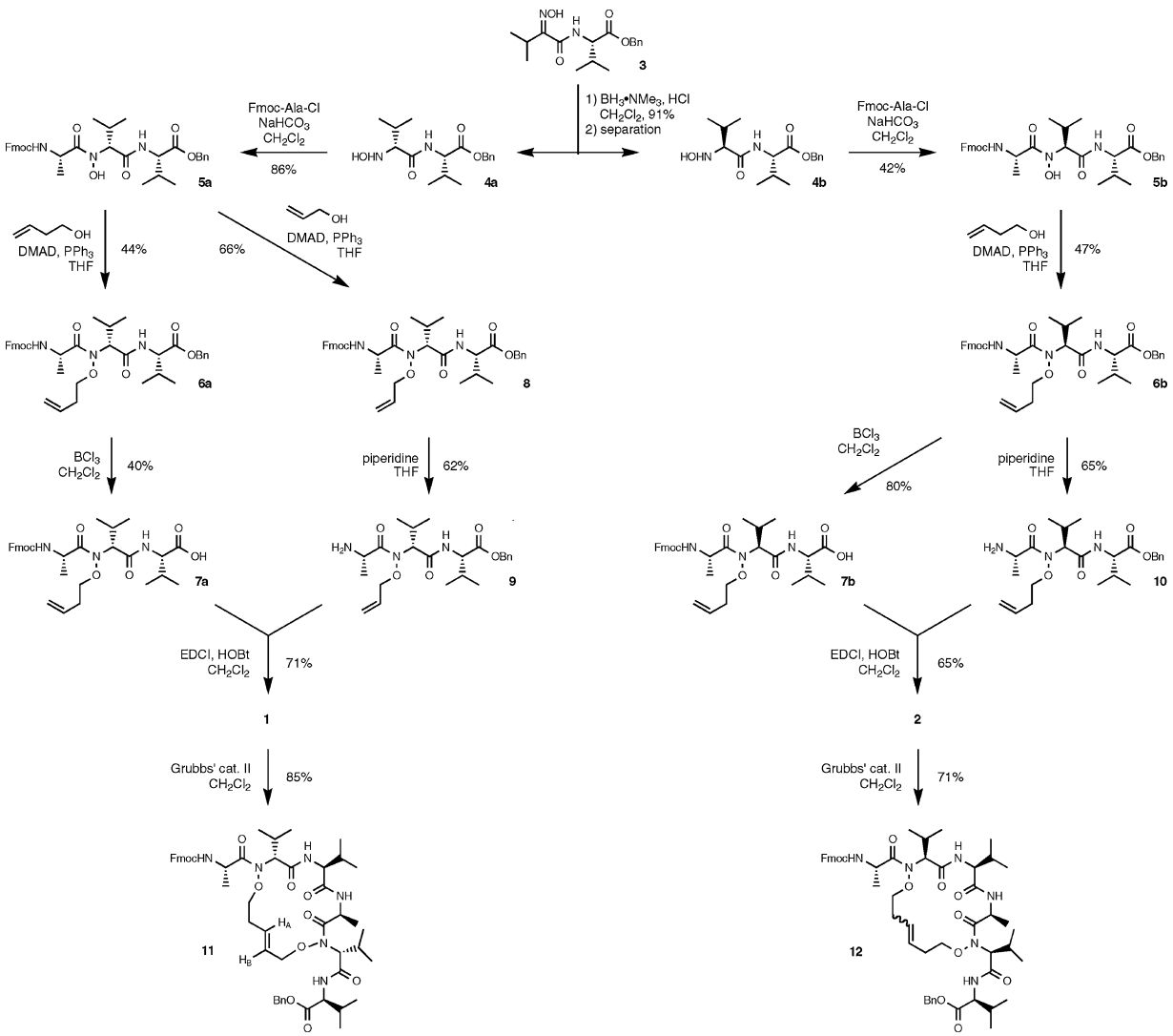
Results and discussion

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 chloride[16] 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 BCl_3 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 ^{13}C 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 bishomoallylglycine[22] 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 ^{1}H NMR and COSY experiments (in $CDCl_3$) revealed that several proton resonances were splitted into two: NH Ala_4 (3:1 ratio), CH_α Val_5, CH_α Val_3. 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 Ala_4 and Val_5 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. CH_2Cl_2 and THF were freshly distilled from CaH_2 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 F_{254}). TLC spots were viewed under ultraviolet light and by heating the plate after treatment with an appropriate staining solution ($KMnO_4$, 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}. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were run on a Bruker Advance300 spectrometer, and obtained from $CDCl_3$ (δ_C 77.2 ppm; standard for ^{1}H 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 $\delta_A - \delta_B$ (for AB spin system) are reported in Hz. ^{1}H and ^{13}C 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-Ala_1-ψ[CO-N(OH)]-Val_2-Val_3-OBn (5b)[25]. To a solution of **4b** (185.8 mg, 576 µmol) in CH_2Cl_2 (15 mL) were added $NaHCO_3$ (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, CH_2Cl_2-MeOH 100:0 to 98:2) gave **5b** (148.5 mg, 241 µmol, 42% yield) as a white solid which can be recrystallized from CH_2Cl_2/cyclohexane. Mp: 112–113 °C. R_f 0.44 (CH_2Cl_2-MeOH 95:5). $[\alpha]_D^{25}$ +19.8 (*c* 1.50 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.73 (1H, s, NOH), 8.47 (1H, d, *J* 9.1, NH Val_3), 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 Ala_1), 5.30-5.20 (1H, m, CH_α Val_2), 5.16 (2H, ABq, *J* 12.0, δ_A-δ_B 56.2, CH_2 -OBn), 5.13-5.02 (1H, m, CH_α Ala_1), 4.74 (1H, dd, *J* 4.5 and 9.2, CH_α Val_3), 4.42-4.15 (3H, m, CH Fmoc, CH_2 Fmoc), 2.50-2.33 (1H, m, CH_{iPr} Val_2), 2.25-2.12 (1H, m, CH_{iPr} Val_3), 1.29 (3H, d, *J* 6.9, CH_3 Ala_1), 0.99 (6H, d, *J* 6.7, CH_3 Val_2), 0.84 (3H, d, *J* 6.9, CH_3 Val_3), 0.73 (3H, d, *J* 6.9, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 173.2 (C=O Val_2), 172.6 (C=O Ala_1), 171.7 (C=O Val_3), 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 (CH_2 -OBn, CH_2 Fmoc), 63.3 (CH_α Val_2), 56.6 (CH_α Val_3), 47.4 (CH Fmoc), 47.1 (CH_α Ala_1), 32.0 (CH_{iPr} Val_3), 27.6 (CH_{iPr} Val_2), 19.9, 19.1, 18.9, 18.1, 17.5 (CH_3). IR: 3378, 3299, 3067, 2964, 2929, 2875, 1724, 1634. HRMS (ESI +) *m/z*: found, 638.2836; $C_{35}H_{41}N_3O_7Na$ requires 638.2842.

Fmoc-Ala_1-ψ[CO-N($O(CH_2)_2CH=CH_2$)]-D-Val_2-Val_3-OBn (6a). A mixture of **5a** (100.0 mg, 162 µmol) and PPh_3 (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 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 **6a** (47.4 mg, 71 μmol, 44% yield) as a colourless oil. R_f 0.65 (CH_2Cl_2-MeOH 95:5). $[\alpha]_D^{25}$ +25.3° (*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 Val_3), 5.80-5.62 (1H, m, CH=), 5.55 (1H, d, *J* 8.2, NH Ala_1), 5.12-4.92 (4H, m, CH_2 –OBn, CH_2=), 4.70-4.60 (1H, m, CH_α Ala_1), 4.41 (1H, dd, *J* 4.6 and 8.4, CH_α Val_3), 4.33-4.25 (2H, m, C*H*H Fmoc, CH Fmoc), 4.22-4.05 (3H, m, CH*H* Fmoc, N–O–CH*H*, CH_α Val_2), 3.85-3.75 (1H, m, N–O–C*H*H), 2.55-2.40 (1H, m, CH_{iPr} Val_2), 2.31-2.20 (2H, m, N–O–CH_2–CH_2), 2.18-2.05 (1H, m, CH_{iPr} Val_3), 1.31 (3H, d, *J* 6.7, CH_3 Ala_1), 0.93 (3H, d, *J* 6.5, CH_3 Val_2), 0.89 (3H, d, *J* 6.4, CH_3 Val_2), 0.83 (3H, d, *J* 6.9, CH_3 Val_3), 0.77 (3H, d, *J* 6.8, CH_3 Val_3). 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 Val_2), 67.1, 67.0 (CH_2 Fmoc, CH_2 -OBn), 57.5 (CH_α Val_3), 48.0 (CH_α Ala_1), 47.4 (CH Fmoc), 32.6 (N–O–CH_2–*C*H_2), 30.8 (CH_{iPr} Val_3), 26.4 (CH_{iPr} Val_2), 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; $C_{39}H_{47}N_3O_7K$ requires 708.30456.

Fmoc-Ala_1-ψ[CO-N($O(CH_2)_2CH=CH_2$)]-Val_2-Val_3-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). $[\alpha]_D^{25}$ +4.1° (*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 Val_3), 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 Ala_1), 5.20-5.00 (4H, m, CH_2 –OBn, CH_2=), 4.80-4.70 (1H, m, CH_α Ala_1), 4.49 (1H, dd, *J* 4.3 and 8.4, CH_α Val_3), 4.45-4.32 (2H, m, C*H*H Fmoc, CH Fmoc), 4.28-4.20 (1H, m, CH*H* Fmoc), 4.10-3.85 (3H, m, N–O–CH_2, CH_α Val_2), 2.75-2.60 (1H, m, CH_{iPr} Val_2), 2.40-2.30 (2H, m, N–O–CH_2–CH_2), 2.28-2.15 (1H, m, CH_{iPr} Val_3), 1.40 (3H, d, *J* 6.8, CH_3 Ala_1), 1.00 (3H, d, *J* 6.8, CH_3 Val_2), 0.97 (3H, d, *J* 6.8, CH_3 Val_2), 0.92 (3H, d, *J* 6.9, CH_3 Val_3), 0.85 (3H, d, *J* 6.9, CH_3 Val_3). 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 Val_2), 67.2, 67.0 (CH_2 Fmoc, CH_2 -OBn), 57.5 (CH_α Val_3), 48.1 (CH_α Ala_1), 47.4 (CH Fmoc), 32.5 (N–O–CH_2–*C*H_2), 30.8 (CH_{iPr} Val_3), 27.3 (CH_{iPr} Val_2), 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; $C_{39}H_{47}N_3O_7K$ requires 708.30456.

Fmoc-Ala_1-ψ[CO-N($O(CH_2)_2CH=CH_2$)]-D-Val_2-Val_3-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). $[\alpha]_D^{25}$ +13.0° (*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 Val_3), 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 Ala_1), 5.20-5.05 (2H, m, CH_2=), 4.80-4.65 (1H, m, CH_α Ala_1), 4.42 (1H, dd, *J* 4.6 and 8.2, CH_α Val_3), 4.38-4.31 (2H, m, CH*H* Fmoc, CH Fmoc), 4.25-4.16 (1H, m, C*H*H Fmoc), 4.15-4.02 (2H, m, N–O–CH_2), 3.95 (1H, d, *J* 11.1, CH_α Val_2), 2.72-2.57 (1H, m, CH_{iPr} Val_2), 2.45-2.33 (2H, m, N–O–CH_2–CH_2), 2.30-2.15 (1H, m, CH_{iPr} Val_3), 1.39 (3H, d, *J* 6.8, CH_3 Ala_1), 1.05-0.80 (12H, m, CH_3 Val_2, CH_3 Val_3). 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–*C*H_2), 74.2 (CH_α Val_2), 67.3 (CH_2 Fmoc), 57.7 (CH_α Val_3), 48.1 (CH_α Ala_1), 47.3 (CH Fmoc), 32.6 (N–O–CH_2–*C*H_2), 30.4 (CH_{iPr} Val_3), 27.4 (CH_{iPr} Val_2), 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; $C_{32}H_{41}N_3O_7K$ requires 618.25761.

Fmoc-Ala_1-ψ[CO-N($O(CH_2)_2CH=CH_2$)]-Val_2-Val_3-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). $[\alpha]_D^{25}$ +10.9° (*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 Val_3), 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 Ala_1), 5.17-5.05 (2H, m, CH_2=), 4.81-4.69 (1H, m, CH_α Ala_1), 4.44 (1H, dd, *J* 4.5 and 8.1, CH_α Val_3), 4.40-4.30 (2H, m, CH*H* Fmoc, CH Fmoc), 4.25-4.15 (1H, m, C*H*H Fmoc), 4.15-3.95 (2H, m, N–O–CH_2), 4.01 (1H, d, *J* 11.3, CH_α Val_2), 2.70-2.55 (1H, m, CH_{iPr} Val_2), 2.43-2.30 (2H, m, N–O–CH_2–CH_2), 2.29-2.14 (1H, m, CH_{iPr} Val_3), 1.38 (3H, d, *J* 6.8, CH_3 Ala_1), 1.02-0.85 (12H, m, CH_3 Val_2, CH_3 Val_3). 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 Val_2), 67.3 (CH_2 Fmoc), 57.6 (CH_α Val_3), 48.1 (CH_α Ala_1), 47.3 (CH Fmoc), 32.6 (N–O–CH_2–*C*H_2), 30.5 (CH_{iPr} Val_3), 27.5 (CH_{iPr} Val_2), 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; $C_{32}H_{41}N_3O_7K$ requires 618.25761.

Fmoc-Ala_1-ψ[CO-N($OCH_2CH=CH_2$)]-D-Val_2-Val_3-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). $[\alpha]_D^{25}$ +17.7° (*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 Val_3), 6.00-5.80 (1H, m, CH=), 5.60 (1H, d, *J* 7.9, NH Ala_1), 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 Ala_1), 4.65-4.55 (1H, m,

N–O–C*H*H), 4.49 (1H, dd, *J* 4.5 and 8.3, CH_α Val_3), 4.42-4.32 (3H, m, CH Fmoc, C*H* Fmoc and N–O–C*H*H), 4.30-4.20 (2H, m, C*H* Fmoc and CH_α Val_2), 2.63-2.48 (1H, m, CH_{iPr} Val_2), 2.27-2.14 (1H, m, CH_{iPr} Val_3), 1.40 (3H, d, *J* 6.8, CH_3 Ala_1), 1.00 (3H, d, *J* 6.9, CH_3 Val), 0.97 (3H, d, *J* 6.9, CH_3 Val), 0.91 (3H, d, *J* 6.9, CH_3 Val), 0.85 (3H, d, *J* 6.9, CH_3 Val). δ_C (75 MHz; $CDCl_3$) 174.5 (C=O Ala_1), 171.2 (C=O Val_3), 170.1 (C=O Val_2), 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 (CH_2=), 120.2 (CH_{ar}), 77.8 (N–O–CH_2), 68.1, 67.2, 67.0 (CH_2 –OBn, CH_2 Fmoc, CH_α Val_2), 57.6 (CH_α Val_3), 48.0 (CH_α Ala_1), 47.4 (CH Fmoc), 30.9 (CH_{iPr} Val_3), 26.4 (CH_{iPr} Val_2), 19.8, 19.7, 19.3, 18.9, 17.7 (CH_3 Ala_1, Val_2, Val_3). IR: 3325, 3065, 2963, 2928, 2874, 1722, 1682, 1655. LMRS (DCI) *m*/*z*: 672.8 (M + NH_4)$^+$. HRMS (ESI +) *m*/*z*: found, 694.28843; $C_{38}H_{45}N_3O_7K$ requires 694.28891.

H-Ala_1-ψ[CO-N(OCH_2CH=CH_2)]-D-Val_2-Val_3-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, CH_2Cl_2-MeOH 100:0 to 95:5) gave **9** (22.3 mg, 51.4 μmol, 62% yield) as a yellow oil. R_f 0.11 (CH_2Cl_2-MeOH 95:5). $[\alpha]_D^{25}$ +48.3° (*c* 0.64 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.45-7.30 (5H, m, CH_{ar}), 7.17 (1H, d, *J* 8.5, NH Val_3), 5.95-5.78 (1H, m, CH=), 5.39-5.23 (2H, m, CH_2=), 5.14 (2H, ABq, *J* 12.3, δ_A-δ_B 14.1, CH_2 -OBn), 4.61-4.48 (2H, m, CH_α Val_3, N–O–C*H*H), 4.33-4.17 (2H, m, CH_α Val_2, N–O–C*H*H), 3.85 (1H, q, *J* 6.8, CH_α Ala_1), 2.62-2.47 (1H, m, CH_{iPr} Val_2), 2.30-2.16 (1H, m, CH_{iPr} Val_3), 1.28 (3H, d, *J* 6.8, CH_3 Ala_1), 0.99 (3H, d, *J* 6.7, CH_3 Val_2), 0.95 (3H, d, *J* 6.7, CH_3 Val_3), 0.93 (3H, d, *J* 6.9, CH_3 Val_2), 0.87 (3H, d, *J* 6.9, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 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 (CH_2=), 77.6 (N–O–CH_2), 68.1 (CH_α Val_2), 67.1 (CH_2 -OBn), 57.5 (CH_α Val_3), 47.8 (CH_α Ala_1), 30.7 (CH_{iPr} Val_3), 26.1 (CH_{iPr} Val_2), 20.9, 19.8, 19.7, 19.3, 17.7 (CH_3). IR: 3310, 2965, 2929, 2872, 1739, 1685, 1653. LRMS (DCI +) *m*/*z*: 434.1 (M + H)$^+$. HRMS (ESI +) *m*/*z*: found, 434.26451; $C_{23}H_{36}N_3O_5$ requires 434.26495.

H-Ala_1-ψ[CO-N($O(CH_2)_2CH$=CH_2)]-Val_2-Val_3-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, CH_2Cl_2-MeOH 100:0 to 90:10) gave **10** (20.3 mg, 45.4 μmol, 65% yield) as a yellow oil. R_f 0.16 (CH_2Cl_2-MeOH 90:10). $[\alpha]_D^{25}$ +0.8° (*c* 0.92 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.81 (1H, d, *J* 8.6, NH Val_3), 7.40-7.25 (5H, m, CH_{ar}), 5.85-5.65 (1H, m, CH=), 5.22-5.05 (4H, m, CH_2 -OBn, CH_2=), 4.51 (1H, dd, *J* 4.5 and 8.8, CH_α Val_3), 4.02-3.70 (4H, m, N–O–CH_2, CH_α Val_2, CH_α Ala_1), 2.75-2.60 (1H, m, CH_{iPr} Val_2), 2.40-2.18 (3H, m, N–O–CH_2–CH_2, CH_{iPr} Val_3), 1.29 (3H, d, *J* 6.8, CH_3 Ala_1), 0.99 (3H, d, *J* 6.8, CH_3 Val_2), 0.95 (3H, d, *J* 6.8, CH_3 Val_3), 0.94 (3H, d, *J* 6.8, CH_3 Val_2), 0.88 (3H, d, *J* 6.8, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 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 (CH_2=), 75.4 (N–O–CH_2), 74.5 (CH_α Val_2), 67.0 (CH_2 -OBn), 57.5 (CH_α Val_3), 47.8 (CH_α Ala_1), 32.5 (N–O–CH_2–CH_2), 30.8 (CH_{iPr} Val_3), 27.2 (CH_{iPr} Val_2), 20.6, 19.9, 19.7, 19.4, 17.6 (CH_3). IR: 3308, 3066, 3034, 2964, 2929, 2874, 1739, 1682. HRMS (ESI +) *m*/*z*: found, 448.28003; $C_{24}H_{38}N_3O_5$ requires 448.28060.

Fmoc-Ala_1-Ψ[CO-N($O(CH_2)_2CH$=CH_2)]-D-Val_2-Val_3-Ala_4-Ψ[CO-N(OCH_2CH=CH_2)]-D-Val_5-Val_6-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 CH_2Cl_2 (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 Na_2SO_4. 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 (CH_2Cl_2-MeOH 95:5). $[\alpha]_D^{25}$ +22.8° (*c* 0.69 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 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 Val_3), 6.70-6.60 (1H, m, NH Ala_4), 5.95-5.70 (3H, m, 2x CH=, NH Ala_1), 5.40-4.95 (6H, m, CH_2 -OBn, 2x CH_2=), 4.95-4.86 (1H, m, CH_α Ala_4), 4.82-4.75 (1H, m, CH_α Ala_1), 4.67-4.55 (1H, m, NO–CH*H*–CH=), 4.52-4.35 (3H, m, CH_α Val_6, CH_α Val_3, NO–C*H*H–CH=), 4.34-4.15 (6H, m, CH_α Val_2, CH_α Val_5, CH Fmoc, CH_2 Fmoc, N–O–C*H*H), 4.00-3.90 (1H, m, N–O–C*H*H), 2.63-2.41 (2H, m, CH_{iPr} Val_2, CH_{iPr} Val_5), 2.40-2.29 (2H, m, N–O–CH_2–CH_2), 2.28-2.17 (2H, m, CH_{iPr} Val_3, CH_{iPr} Val_6), 1.45-1.20 (6H, m, CH_3 Ala_1, CH_3 Ala_4), 1.05-0.80 (24H, m, CH_3 Val_2, CH_3 Val_5, CH_3 Val_3, CH_3 Val_6). δ_C (75 MHz; $CDCl_3$) 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–CH_2–C*H*=), 128.8, 128.7, 128.54, 128.49, 127.9, 127.3, 125.3 (CH_{ar}), 121.0 (N–O–CH_2–CH=CH_2), 120.2 (CH_{ar}), 117.9 (N–O–(CH_2)$_2$CH=CH_2), 77.9, 76.0 (N–O–CH_2), 68.4, 67.9 (CH_α Val_2, CH_α Val_5), 67.2, 67.0 (CH_2 –OBn, CH_2 Fmoc), 58.9, 57.6 (CH_α Val_3, CH_α Val_6), 48.4 (CH_α $Ala_{1\ or\ 4}$), 47.4 (CH Fmoc), 46.6 (CH_α $Ala_{4\ or\ 1}$), 32.7 (N–O–CH_2–CH_2), 30.8, 30.5 (CH_{iPr} Val_3, CH_{iPr} Val_6), 26.59, 26.49 (CH_{iPr} Val_2, CH_{iPr} Val_5), 19.9, 19.8, 19.7, 19.5, 19.2, 18.7, 18.6, 17.9, 17.8, 17.7 (CH_3). IR: 3324, 3067, 2965, 2932, 2874, 1724, 1650. HRMS (ESI +) *m*/*z*: found, 1033.50339; $C_{55}H_{74}N_6O_{11}K$ requires 1033.50472.

Fmoc-Ala_1-ψ[CO-N($O(CH_2)_2CH$=CH_2)]-Val_2-Val_3-Ala_4-ψ[CO-N($O(CH_2)_2CH$=CH_2)]-Val_5-Val_6-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 (CH_2Cl_2-MeOH 95:5). $[\alpha]_D^{25}$ –12.7° (*c* 0.94 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 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 Val_6), 7.44-7.26 (10H, m, CH_{ar} Fmoc, NH Val_3), 6.69 (1H, d, *J* 7.9, NH Ala_4), 5.90-5.60 (3H, m, 2x CH=, NH Ala_1), 5.24-5.02 (6H, m, CH_2 -OBn, 2x CH_2=), 5.01-4.90 (1H, m, CH_α Ala_4), 4.83-4.70 (1H, m, CH_α Ala_1), 4.48 (1H, dd, *J* 4.3 and 8.4, CH_α Val_6), 4.40-3.90 (10H, m, CH_α Val_3, CH_α Val_2, CH_α Val_5, CH Fmoc, CH_2 Fmoc, (2x) N–O–CH_2), 2.70-2.50 (2H, m, CH_{iPr} Val_2, CH_{iPr} Val_5), 2.50-2.27 (4H, m, (2x) N–O–CH_2–CH_2), 2.27-2.12 (2H, m, CH_{iPr} Val_3, CH_{iPr} Val_6), 1.41 (3H, d, *J* 6.7, CH_3 Ala_1), 1.32 (3H, d, *J* 6.9, CH_3 Ala_4), 1.07-0.80 (24H, m, CH_3 Val_2, CH_3 Val_5, CH_3 Val_3, CH_3 Val_6). δ_C (75 MHz; $CDCl_3$) 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

(CH_2=), 76.0, 75.9 (N–O–CH_2), 73.5, 72.8 (CH_α Val_2, CH_α Val_5), 67.2, 67.0 (CH_2 Fmoc, CH_2 –OBn), 58.8 (CH_α Val_6), 57.4 (CH_α Val_3), 48.0 (CH_α Ala_1), 47.3 (CH Fmoc), 46.1 (CH_α Ala_4), 32.6, 32.5 (N–O–CH_2–CH_2), 30.7 (CH_{iPr} Val_3, CH_{iPr} Val_6), 27.32, 27.28 (CH_{iPr} Val_2, CH_{iPr} Val_5), 19.9, 19.8, 19.7, 19.5, 19.3, 18.5, 17.9, 17.7 (CH_3). IR: 3312, 3067, 2963, 2931, 2874, 1744, 1724, 1682, 1641. HRMS (ESI +) *m/z*: found, 1047.51887; $C_{56}H_{76}N_6O_{11}K$ requires 1047.52037.

Fmoc-Ala_1-ψ[CO-N($O(CH_2)_2CH$=*)]-D-Val_2-Val_3-Ala_4-ψ[CO-N(OCH_2CH*=)]-D-Val_5-Val_6-OBn (11). To a solution of **1** (7.4 mg, 7.4 μmol) in CH_2Cl_2 (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 CH_2Cl_2. The organic phases were gathered, dried over Na_2SO_4, filtrated 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 (CH_2Cl_2-MeOH 95:5). $[\alpha]_D^{25}$ +26.7° (*c* 0.56 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.76 (2H, d, *J* 7.5, CH_{ar} Fmoc), 7.71 (1H, d, *J* 7.6, NH Val_6), 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 Val_3), 6.42-6.37 (1H, m, NH Ala_4), 5.87-5.72 (2H, m, N–O–CH_2–CH_B=, NH Ala_1), 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, CH_2 -OBn), 4.92-4.81 (1H, m, CH_α Ala_1), 4.78-4.70 (1H, m, CH_α Ala_4), 4.70-4.60 (1H, m, N–O–CH–CH=), 4.57 (1H, d, *J* 11.0, CH_α Val_2), 4.50 (1H, dd, *J* 5.7 and 7.9, CH_α Val_6), 4.42-4.22 (4H, m, CH_2 Fmoc, CH Fmoc, CH_α Val_5), 4.18-4.08 (1H, m, N–O–C*H*–CH=), 4.05-3.84 (3H, m, N–O–CH_2–CH_2, CH_α Val_3), 2.59-2.44 (2H, m, CH_{iPr} Val_2, CH_{iPr} Val_5), 2.42-2.28 (1H, m, N–O–CH_2–C*H*), 2.25-2.12 (1H, m, N–O–CH_2–C*H*H), 2.08-1.96 (2H, m, CH_{iPr} Val_3, CH_{iPr} Val_6), 1.45-1.40 (3H, m, CH_3 Ala_1), 1.27-1.20 (3H, m, CH_3 Ala_4), 1.09-0.80 (24H, m, CH_3 Val_2, CH_3 Val_3, CH_3 Val_5, CH_3 Val_6). δ_C (75 MHz; $CDCl_3$) 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–CH_2), 67.4, 66.9 (CH_2 –OBn, CH_2 Fmoc, CH_α $Val_{2\ or\ 5}$), 64.5 (CH_α $Val_{2\ or\ 5}$), 61.2, 58.0 (CH_α Val_3, CH_α Val_6), 48.2, 48.1 (CH_α $Ala_{1\ and\ 4}$), 47.3 (CH Fmoc), 34.4 (N–O–CH_2–CH_2), 30.8, 30.5 (CH_{iPr} Val_3, CH_{iPr} Val_6), 27.6, 26.8 (CH_{iPr} Val_2, CH_{iPr} Val_5), 20.3, 19.95, 19.85, 19.5, 19.4, 19.3, 18.8, 18.1, 17.5 (CH_3). IR: 3320, 2961, 2925, 2872, 2853, 1736, 1660. HRMS (ESI +) *m/z*: found, 1005.47242; $C_{53}H_{70}N_6O_{11}K$ requires 1005.47342.

Fmoc-Ala_1-Ψ[CO-N($O(CH_2)_2CH$=*)]-Val_2-Val_3-Ala_4-Ψ[CO-N($O(CH_2)_2CH$*=)]-Val_5-Val_6-OBn (12). To a solution of **2** (18.8 mg, 18.6 μmol) in CH_2Cl_2 (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 CH_2Cl_2. The organic phases were gathered, dried over Na_2SO_4, filtrated 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 (CH_2Cl_2-MeOH 95:5). δ_H (300 MHz; $CDCl_3$) 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 Val_6), 7.42-7.25 (10H, m, CH_{ar}, NH Val_3), 6.37-6.28 and 6.00-5.95 (1H, ratio 3:1, m, NH Ala_4), 5.75-5.55 (2H, m, CH=, NH Ala_1), 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, CH_2 -OBn), 4.85-4.65 (2H, m, CH_α Ala_1, CH_α Ala_4), 4.45 (1H, dd, *J* 4.4 and 8.3, CH_α Val_6), 4.40-4.25 (1H, m, C*H*H Fmoc), 4.25-4.17 (2H, m, CHH Fmoc, CH Fmoc), 4.15-4.00 (minor diastereomer: m, CH_α Val_5, CH_α Val_3), 3.95-3.75 (m, 4H, NO–CH_2 + major diastereomer: m, CH_α Val_5, CH_α Val_3), 3.69 (1H, d, *J* 11.0, CH_α Val_2), 2.80-2.50 (2H, m, CH_{iPr} Val_2, CH_{iPr} Val_5), 2.40-2.00 (4H, m, N–O–CH_2–CH_2, CH_{iPr} Val_3, CH_{iPr} Val_6), 1.50-1.25 (6H, m, CH_3 Ala_1, CH_3 Ala_4), 1.10-0.78 (24H, m, CH_3 Val_2, CH_3 Val_3, CH_3 Val_5, CH_3 Val_6). δ_C (75 MHz; $CDCl_3$) 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 $CDCl_3$: N–O–CH_2, CH_α $Val_{2\ or\ 5}$), 73.8 (CH_α $Val_{2\ or\ 5}$), 67.2, 66.7 (CH_2 –OBn, CH_2 Fmoc), 60.8 (CH_α Val_3), 57.6 (Val_6), 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–CH_2–CH_2, CH_{iPr} Val_3, CH_{iPr} Val_6), 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 (CH_3). IR: 3318, 3063, 3033, 2965, 2924, 2872, 1714, 1664. HRMS (ESI +) *m/z*: found, 981.53157; $C_{54}H_{73}N_6O_{11}$ requires 981.53318.

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 $CHCl_3$ 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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