A new tool for photoaffinity labeling studies: a partially constrained, benzophenone based, α-amino acid†

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The novel α-amino acid BpAib, a partially conformationally restricted analogue of the currently extensively used 3-(4-benzoylphenyl)alanine (Bpa) photoaffinity label, was synthesized, optically resolved, fully characterized, and appropriately derivatized. An intermolecular photocrosslinking experiment highlighted its regioselective reactivity towards the S-methyl side-chain group of a Met-based dipeptide, which is closely comparable to that of Bpa.

Introduction

Incorporation of photoreactive α-amino acids chemically or biosynthetically into peptides or proteins has been extensively exploited in biochemistry, molecular biology, and chemical proteomics to map the interfaces in ligand–receptor and protein–protein interactions.[1,2] Among the α-amino acids successfully proposed, the benzophenone-containing residue Bpa, 3-(4-benzoylphenyl)alanine (Fig. 1) is the most popular probe for photoaffinity labeling.[3–13] After photoexcitation at 350 nm, the benzophenone chromophore of Bpa is believed to remove by far most frequently a hydrogen atom from the side chain of a Met residue, followed by covalent C–C bond formation of the resulting radical pair.[1–14] In addition to these literature studies of *intermolecular* interactions, we recently investigated the chemical and 3D-structures of the products arising from the *intramolecular* Paternò-Yang photocyclization in Bpa-spacer-Met helical peptides.[15,16]

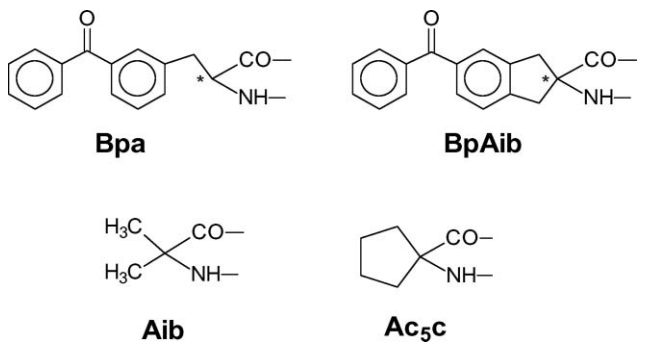

Fig. 1 Chemical structures of Bpa, BpAib, Aib and Ac_5c.

Bpa is a flexible amino acid.[17] Although it is known that flexibility is needed for efficient crosslinking of proteins by benzophenone photoligands,[18] for specific interactions a reduced conformational mobility might be beneficial. In this work, we designed, synthesized, resolved, configurationally and conformationally characterized, and *intermolecularly* photoreacted with a Met-based peptide partner, the novel, main-chain and side-chain partially restricted, chiral α-amino acid 2-amino-5-benzoyl-2,3-dihydro-1*H*-indene-2-carboxylic acid (BpAib, Fig. 1), belonging to the class of C^{α}-tetrasubstituted α-amino acids (strong turn and helix promoters in peptides), the prototypes of which are α-aminoisobutyric acid (Aib)[19–21] and 1-aminocyclopentane-1-carboxylic acid (Ac_5c)[21–24] (Fig. 1). A limited part of this work has already been reported in two communications to Peptide Symposia.[25,26]

Results and discussion

Synthesis

The 3,4-*bis*(bromomethyl)benzophenone **3** (Fig. 2) was synthesized in three steps from 3,4-dimethylbenzoic acid **1** by formation of the acid chloride, using thionyl chloride, followed by Friedel–Crafts acylation of benzene affording **2**, and finally by free radical side-chain bromination in the presence of *N*-bromosuccinimide

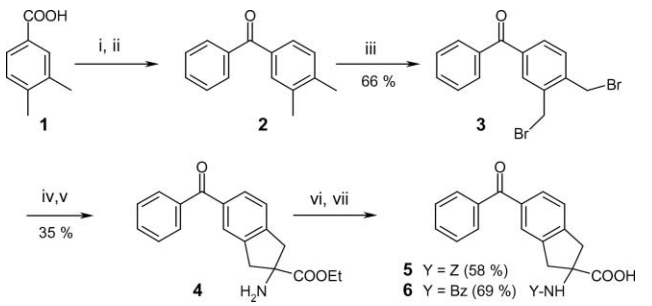

Fig. 2 Synthesis of N^{α}-protected (blocked) BpAib. (i) $SOCl_2$; DMF; rt. (ii) $AlCl_3$, benzene; DCE; rt. (iii) NBS, benzoyl peroxide; CCl_4; 90 °C. (iv) NC–CH_2–COOEt; K_2CO_3; $nBu_4N^+ \cdot HSO_4^-$ (cat.); CH_3CN; 80 °C. (v) 10 N aq. HCl; abs. EtOH; 0 °C to rt. (vi) Z-OSu or $(Bz)_2O$; CH_3CN; rt. (vii) NaOH; MeOH–THF–H_2O; 60 °C.

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(NBS) and benzoyl peroxide. *bis*-Alkylation of the Gly equivalent ethyl (Et) isocyanoacetate by **3** under phase-transfer conditions,[27] followed by acid hydrolysis, afforded the pentatomic cyclic, C^{α}-tetrasubstituted, BpAib α-amino ester **4**. Protection of the α-amino group of **4** by Z (benzyloxycarbonyl) or Bz (benzoyl) using the succinimidyl ester (OSu) or symmetrical anhydride, and subsequent saponification of the ethyl esters, gave the *N*-acylated α-amino acids **5** and **6**.

For the separation of the BpAib enantiomers, the chiral auxiliary H-(*S*)-Phe-NHChx (Chx, cyclohexyl), known to be an effective resolving agent for C^{α}-tetrasubstituted α-amino acid derivatives,[28,29] was reacted with the N^{α}-acylated (*R*,*S*)-BpAib compounds **5** and **6** using HATU [2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] and diisopropylethylamine (DIEA) in tetrahydrofuran (THF) to afford the corresponding N^{α}-acylated dipeptide alkylamides (Fig. 3).

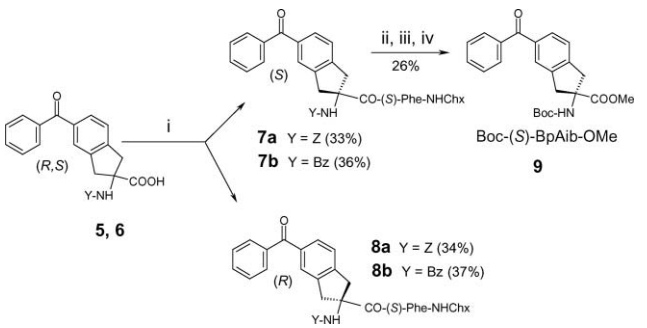

Fig. 3 Optical resolution of (*R*,*S*)-BpAib. (i) H-(*S*)-Phe-NHChx, HATU, DIEA; THF; rt. (ii) 10 M HCl, 1,4-dioxane; 100 °C. (iii) $SOCl_2$; MeOH; rt. (iv) $(Boc)_2O$; CH_3CN–CH_2Cl_2; rt.

Chromatographic separations of the dipeptide diastereomers afforded the Z-protected **7a** and **8a**, and the Bz-blocked **7b** and **8b** dipeptides. The crystalline (*R*,*S*) diastereomer with Y = Z (**8a**) proved to be suitable for an X-ray diffraction study (see below). Strong acid hydrolysis of **7b**, followed by esterification with methanol (MeOH)/thionyl chloride and treatment with $(Boc)_2O$ (di-*tert*butyl-dicarbonate) gave the Boc-protected BpAib α-amino methyl ester (*S*)-enantiomer **9**.

3D-Structural analysis

The crystal-state 3D-structure of the Z-BpAib-(*S*)-Phe-NHChx diastereomeric dipeptide analyzed (Fig. 4) unambiguously showed that the configuration at the α-carbon of its BpAib residue is (*R*), based on the known configuration of (*S*)-Phe. In addition, this Z-protected dipeptide cyclohexylamide (**8a**) was found to be folded in an intramolecularly C=O···H–N H-bonded β-turn conformation,[30–32] clearly induced by the BpAib residue. Indeed, this is a typical property of C^{α}-tetrasubstituted α-amino acids, originally authenticated for Aib,[19–21] the prototype of this class of conformationally-restricted compounds, and the related Ac_5c residue.[21–24]

The conformationally informative backbone torsion angles are helical [$\varphi_1 = -64.9(6)°$, $\psi_1 = -23.8(7)°$] for (*R*)-BpAib and distorted helical [$\varphi_2 = -88.6(7)°$, $\psi_2 = -5.0(7)°$] for (*S*)-Phe. These values indicate that the β-turn formed is type-I. Moreover, the signs of the helical (*R*)-BpAib φ,ψ torsion angles are both negative, characteristic of a right-handed screw sense,[33] but reminiscent

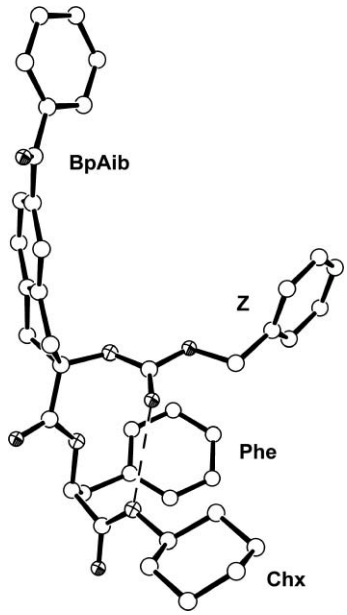

Fig. 4 X-Ray diffraction structure of the N^{α}-protected dipeptide alkylamide Z-(*R*)-BpAib-(*S*)-Phe-NHChx **8a**. The intramolecular C=O···H–N H-bond is represented by a dashed line.

of those of an oppositely configured C^{α}-trisubstituted (protein) α-amino acid. The intramolecular H-bond is weak, as the O···N distance is 3.136(5) Å.[34]

Spectroscopic characterization

Using the amino acid derivative Boc-(*S*)-BpAib-OMe **9**, we characterized the spectroscopic properties of this novel benzophenone-based residue. The near-UV absorption spectrum in MeOH solution (Fig. 5) typically shows a very weak shoulder at about 340 nm (n → π* transition of the benzophenone chromophore), an excellent "window" region for photoexcitation of peptides,

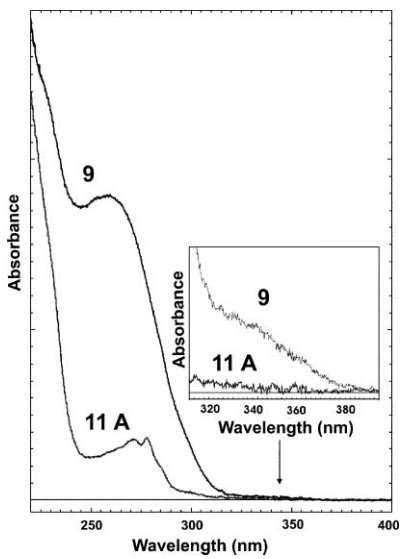

Fig. 5 Near-UV absorption spectra in MeOH solution of Boc-(*S*)-BpAib-OMe **9** and of the faster (**11A**) eluting of the two major HPLC peaks (see Fig. 7) arising from the photoreaction.

followed by a much more intense band near 260 nm ($\pi \rightarrow \pi^*$ transition).[35,36]

In the corresponding circular dichroism (CD) spectrum (Fig. 6), the 260 nm Cotton effect is clearly visible, negative for the (*S*)-enantiomer of the BpAib derivative **9**. Conversely, the band near 340 nm does not seem to be optically active, at least using the highest analyte concentration (1 mM) and the widest cell (1 cm) available to us. Below 240 nm (far-UV region), a very intense, negative Cotton effect dominates the CD spectrum of (*S*)-**9**.

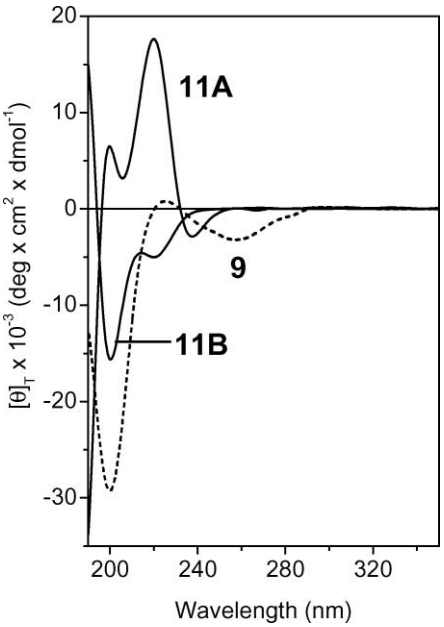

Fig. 6 Far- and near-UV CD spectra in MeOH solution of Boc-(*S*)-BpAib-OMe **9** and of the faster (**11A**) and slower (**11B**) eluting major HPLC peaks (see Fig. 7) arising from the photoreaction.

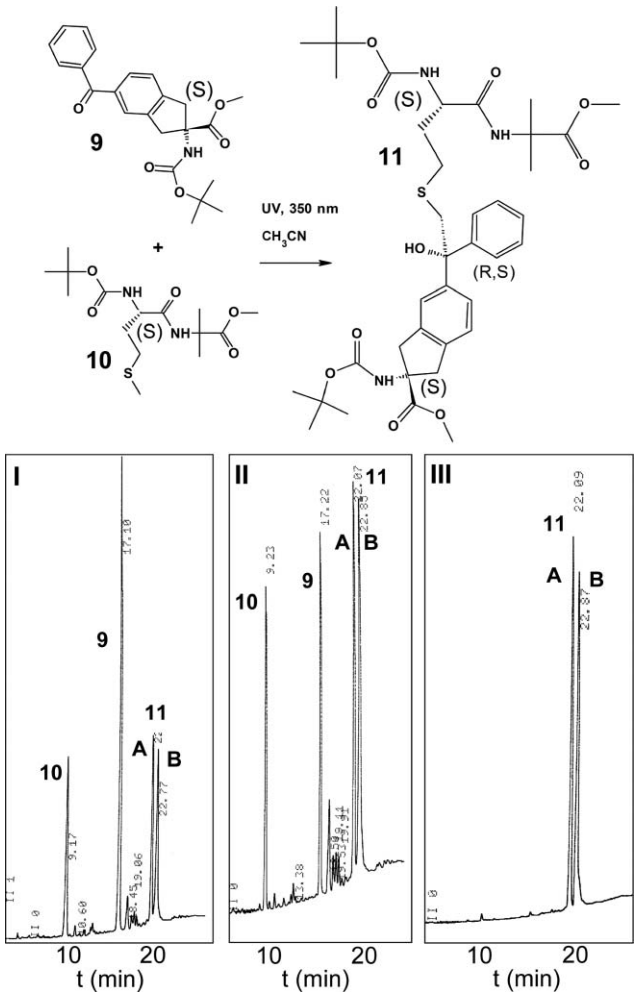

Fig. 7 Top: chemical structures of the Boc-(*S*)-BpAib-OMe **9** and Boc-(*S*)-Met-Aib-OMe **10** reagents and the products (**11**) of the photoreaction; bottom: reverse-phase HPLC profiles of the photoreaction mixture after 25 min (**I**) and 60 min (**II**). Part **III** shows the HPLC profile of the two photoreaction products after a flash chromatography purification of the crude mixture.

Photocrosslinking experiments

To check the reactivity of the benzophenone moiety of BpAib under the classical conditions of intermolecular photoinduced covalent bond formation with a Met-containing peptide, equimolar (0.1 M) amounts of Boc-(*S*)-BpAib-OMe **9** and Boc-(*S*)-Met-Aib-OMe[17] (**10**) were dissolved in acetonitrile, placed in a quartz tube, and purged of oxygen [to avoid oxidation of the (*S*)-Met thioether side chain to a mixture of diastereomeric sulfoxides] by bubbling nitrogen for 20 min. After a 25 minute irradiation at 350 nm, the reverse-phase HPLC profile of the photoreaction mixture (Fig. 7**I**, bottom) showed two newly formed, roughly equivalent peaks (**11A** and **11B**). After a 60 minute irradiation, the amount of the two peaks **11** increases remarkably at the expense of the photoreactants **9** and **10** (Fig. 7**II**).

The mixture of the two peaks (Fig. 7**III**), obtained by flash-chromatography partial purification of the crude, was submitted to ESI-ToF mass spectrometry and NMR analyses. The combined chromatographic and spectrometric results (Fig. 7 and 8, and ESI, S12†) indicated that the two compounds are the two diastereomeric adducts originating from the regioselective photocrosslinking of the BpAib derivative **9** to the Met ε-CH_3 group of the dipeptide substrate **10**.

In particular, Fig. 8 clearly shows that the Met ε-CH_3 proton signal occurring at about $\delta = 2.1$ ppm in the spectrum of **10** is missing in that of the mixture of the two adducts, **11** (**A** + **B**). Moreover, this reaction involves the formation of an additional chiral center at the side-chain carbonyl carbon of the former BpAib residue[16,17] and consequently results in the production of two diastereomers (Fig. 7, top). The participation of the BpAib side-chain carbonyl group in the photoreaction was corroborated by the observation that the band near 260 nm is missing in the near-UV absorption spectrum of a representative diastereomeric product (**11A** in Fig. 5) and in the CD spectra of both **11A** and **11B** as well [the relatively weak UV absorption of **11A** with fine structure centered near 275 nm is associated with its substituted benzyl chromophores[37]]. Interestingly, most of the general features (apart from the relative intensities) of the CD spectra of the two diastereomers **11A** and **11B** reflect well their stereochemical relationship. It is worth pointing out that photocrosslinking of the BpAib derivative **9** to the Met γ-CH_2 group[14] of dipeptide **10**

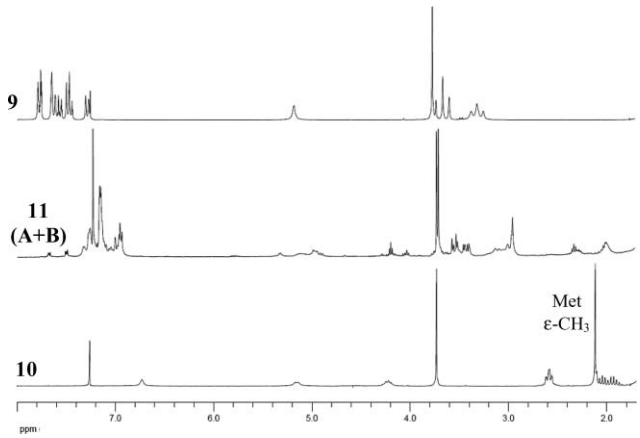

Fig. 8 400 MHz ^{1}H NMR spectra in $CDCl_3$ solution of Boc-(*S*)-BpAib-OMe **9**, Boc-(*S*)-Met-Aib-OMe **10**, and the mixture of the two diastereomeric products (**11A** and **11B**) isolated after the photoreaction.

would have created two additional chiral centers in the products with the resulting formation of four diastereomers.

Conclusions

In summary, we synthesized and resolved the two enantiomers of BpAib, a novel main-chain and side-chain partially restricted, α-amino acid. The configuration of one enantiomer was assigned *via* X-ray diffraction analysis of a diastereomeric dipeptide. The spectroscopic properties of this chromophoric residue were recorded. The usefulness of this new probe as a tool for the study of peptide–protein interactions was demonstrated by a photocrosslinking experiment, which showed that the regioselectivity of the BpAib intermolecular photoreaction towards a Met-containing substrate is closely related to that of its Bpa intramolecular counterpart.[16,17] We are currently extending the application of this photolabeled amino acid to actual peptide···protein interactions under the experimental conditions (solvent, pH, ionic strength) typically used in biochemical studies. However, it is worth recalling that the insertion of a turn/helix stabilizer, C^{α}-quaternary, α-amino acid,[19–24] such as BpAib, in a peptide sequence should be planned carefully, to avoid a potential, undesired conformational change of the ligand.

Experimental

Synthesis and analytical data

General methods. Melting points were measured by means of a capillary tube immersed in an oil bath (Tottoli apparatus, Büchi) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker WM300 spectrometer operating at 300 MHz and 77 MHz, respectively, the solvent being used as the internal standard: $CDCl_3$ (^{1}H: δ = 7.26 ppm; ^{13}C: δ = 77.00 ppm). Splitting patterns are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. Elemental analyses were performed by the CNRS Service of Microanalyses in Gif-sur-Yvette (France). Mass spectra (electrospray mode) were recorded on a Hewlett-Packard HP5989MS spectrometer by Mr Vincent Steinmetz (ILV, Versailles). Analytical TLC and preparative column chromatography were performed on Kieselgel F 254 and Kieselgel 60 (0.040–0.063 mm) (Merck), respectively.

The TLC profile of the Z-(*RS*)-BpAib-(*S*)-Phe-NHChx separation, ^{1}H and ^{13}C NMR spectra for all newly prepared compounds and X-ray diffraction data of Z-(*R*)-BpAib-(*S*)-Phe-NHChx may be found in the ESI.†

3,4-*bis*-(Bromomethyl)benzophenone 3. 3,4-Dimethylbenzophenone **2** (18.0 g, 85.7 mmol) was dissolved in carbon tetrachloride (250 mL), and NBS (29.0 g, 162.8 mmol) and benzoyl peroxide (1.0 g) were added. The mixture was stirred at reflux under argon for 2 h. The mixture was allowed to cool to rt, then filtered through Celite. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–cyclohexane (5 : 95) as eluent to give the *bis*-bromomethyl derivative as an oil (20.8 g, 66%). R_f 0.27 (pentane–ethyl acetate 95 : 5); δ_H ($CDCl_3$) 4.68, 4.69 (2 s, 4H), 7.50 (t, J = 7.5 Hz, 3H), 7.62 (m, 1H), 7.71 (m, 1H), 7.78 (m, 3H); δ_C ($CDCl_3$) 28.8, 29.2, 128.4, 129.9, 130.8, 131.0, 132.4, 132.8, 136.8, 136.9, 138.3, 140.7, 195.3. Found: C, 49.45; H, 3.42. Calc. for $C_{15}H_{12}Br_2O$: C, 48.94; H 3.28. CI-MS m/z 369 $[M + H]^+$.

Ethyl-2-amino-5-benzoyl-2,3-dihydro-1*H*-indene-2-carboxylate [H-(*RS*)-BpAib-OEt] 4. To a solution of 3,4-*bis*-(bromomethyl)-benzophenone **3** (10.15 g, 27.6 mmol) in acetonitrile (540 mL) was added tetrabutylammonium hydrogen sulfate (1.83 g, 5.4 mmol), potassium carbonate (22.4 g, 162.0 mmol) and ethyl isocyanoacetate (3 mL, 25.6 mmol). The mixture was refluxed under argon for 24 h, then filtered. The collected solids were washed with dichloromethane and the filtrate was concentrated under reduced pressure. The crude isonitrile obtained was dissolved in a mixture of dichloromethane (15 mL) and ethanol (150 mL). Concentrated hydrochloric acid (3.5 mL) was added. The reaction mixture was stirred for 3 h at rt. The mixture was diluted with water (100 ml) and volatiles were evaporated under reduced pressure. The resulting mixture was further diluted with 0.5 M aqueous hydrochloric acid and washed twice with diethyl ether. The aqueous phase was made alkaline by the slow addition of sodium hydrogen carbonate, and extracted with three portions of dichloromethane. The combined dichloromethane extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue obtained was purified by column chromatography using ethyl acetate–cyclohexane (7 : 3) as eluent to afford the α-amino ester as a solid (2.76 g, 35% yield). R_f 0.32 (dichloromethane–MeOH 95 : 5); m.p. 58–63 °C; δ_H ($CDCl_3$) 1.29 (t, J = 7.1 Hz, 3H), 2.94 (dd, 2H, J = 4.8, 16.6 Hz), 3.60 (dd, 2H, J = 9.4, 16.7 Hz), 4.23 (q, J = 7.1, 14.2 Hz, 2H), 7.31 (d, J = 7.7 Hz, 1H), 7.46 (t, J = 7.7 Hz, 2H), 7.54–7.68 (m, 3H), 7.78 (d, 2H, J = 5.0 Hz). δ_C ($CDCl_3$) 14.1, 45.8, 46.0, 61.5, 65.1, 124.5, 126.5, 128.2, 129.5, 129.9, 132.1, 136.6, 137.9, 140.9, 145.8, 176.2, 196.6. Found: C, 73.24; H, 6.21; N, 4.66. Calc. for $C_{19}H_{19}NO_3$: C, 73.76; H, 6.19; N, 4.53%. ESI-MS m/z 310.2 $[M + H]^+$.

***Z*-(*RS*)-BpAib-OH 5.** A solution of ethyl-2-amino-5-benzoyl-2,3-dihydro-1*H*-indene-2-carboxylate **4** (187 mg, 0.6 mmol) in dichloromethane (10 mL) was cooled on an ice bath and Z-OSu (226 mg, 0.91 mmol) was added. The reaction mixture was allowed to warm to rt and stirred for 18 h, then concentrated under reduced pressure. The residue obtained was purified by column chromatography using cyclohexane–ethyl acetate (7 : 3) as eluent

to afford the intermediate protected α-amino ester. This residue was dissolved in a mixture of tetrahydrofuran (3 mL), MeOH (1.5 mL) and water (0.75 mL). Sodium hydroxide (60 mg) was added and the mixture was stirred at 55 °C for 2 h. The mixture was allowed to cool to rt and diluted with water. Volatiles were removed under reduced pressure. The mixture was cooled on an ice bath and acidified by addition of 2 M aqueous hydrochloric acid. The resulting solid was filtered and dried under vacuum (146 mg, 58%). R_f 0.39 (dichloromethane–methanol 90 : 10); m. p. 66–70 °C; δ_H ($CDCl_3$) 3.32 (t, J = 15.4 Hz, 2H), 3.66 (t, J = 15.8 Hz, 2H), 5.03 (s, 2H), 7.37 (m, 3H), 7.24 (m, 6H), 7.44 (m, 2H), 7.56 (m, 3H), 7.73 (d, 2H, J = 7.0 Hz). δ_C ($CDCl_3$) 29.7, 43.6, 67.1, 124.2, 126.1, 127.9, 128.2, 128.5, 129.6, 129.9, 132.3 132.8, 135.7, 136.7, 137.7, 140.3, 145.2, 196.6. Found: C, 72.36; H, 5.37; N, 3.27. Calc. for $C_{25}H_{21}NO_5$: C, 72.27; H, 5.09; N, 3.37. ESI-MS m/z 438.1 $[M + Na]^+$.

Bz-(*RS*)-BpAib-OH 6. A solution of ethyl-2-amino-5-benzoyl-2,3-dihydro-1*H*-indene-2-carboxylate **4** (5.51 g, 17.8 mmol) in acetonitrile (150 mL) was cooled on an ice bath and benzoic anhydride (8.1 g, 35.7 mmol) was added. The reaction mixture was allowed to warm to rt and stirred for 48 h, then concentrated under reduced pressure. The residue was taken up in dichloromethane and washed twice with 2 M aqueous sodium hydroxide and once with water. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue obtained was purified by column chromatography using dichloromethane–*iso*propanol (98 : 2) as eluent to afford the intermediate protected α-amino ester. This residue was dissolved in a mixture of tetrahydrofuran (50 mL), MeOH (20 mL) and water (5 mL). Sodium hydroxide (1.20 g) was added and the mixture was stirred at 55 °C for 90 min. The mixture was allowed to cool to room temperature and diluted with water. Volatiles were removed under reduced pressure. The mixture was cooled on an ice bath and acidified by addition of 2 M aqueous hydrochloric acid. The resulting solid was filtered and dried under vacuum (4.73 g, 69%). R_f 0.57 (dichloromethane–methanol 90 : 10); m.p. 176–179 °C; δ_H (CD_3OD) 3.51 (dd, 2H, J = 9.1, 17.7 Hz), 3.76 (dd, 2H, J = 17.5, 16.7 Hz), 7.37 (m, 3H), 7.47 (m, 3H), 7.59 (m, 3H), 7.73 (m, 4H). δ_C (CD_3OD) 44.1, 44.3, 67.6, 125.5, 127.1, 128.5, 129.4, 129.5, 130.4, 130.9, 132.8, 133.7, 135.2, 137.8, 139.1, 142.2, 147.6, 170.7, 176.5, 198.8. Found: C, 71.53; H, 5.04; N, 3.47. Calc. for $C_{24}H_{19}NO_4 \cdot H_2O$: C, 71.44; H, 5.25; N, 3.47. ESI-MS m/z 408.3 $[M + Na]^+$, 793.5 $[2M + Na]^+$.

Z-(*RS*)-BpAib-(*S*)-Phe-NHChx (7a and 8a). A suspension of Z-(*RS*)-BpAib-OH **5** (231 mg, 0.56 mmol) and HCl·H-(*S*)-Phe-NHChx (188 mg, 0.67 mmol) in tetrahydrofuran (7 mL) was cooled on an ice bath. HATU (254 mg, 0.67 mmol) and DIEA (0.25 mL) were added. The reaction mixture was allowed to warm to rt and stirred for 48 h. The mixture was concentrated under reduced pressure and the resulting residue was taken up in dichloromethane. The solution was washed twice with 0.5 M aqueous hydrochloric acid, once with water, and once with saturated aqueous $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue obtained was purified by column chromatography using cyclohexane–ethyl acetate (1 : 1) as eluent affording first Z-(*S*)-BpAib-(*S*)-Phe-NHChx **7a** (119 mg, 33%), then Z-(*R*)-BpAib-(*S*)-Phe-NHChx **8a** (122 mg, 34%).

*Z-(*S*)-BpAib-(*S*)-Phe-NHChx **7a**.* R_f 0.45 (cyclohexane–ethyl acetate 1 : 1); m.p. 81–85 °C; δ_H ($CDCl_3$) 1.05–1.33 (m, 5H), 1.57–1.85 (m, 5H), 2.92–3.32 (m, 5H), 3.69–3.87 (m, 2H), 4.66 (m, 1H), 4.99 (m, 2H), 5.78 (s, 1H), 6.45 (d, 1H, J = 7.9 Hz), 6.61 (d, 1H, J = 8.1 Hz), 7.14 (d, 1H, J = 6.9 Hz), 7.21–7.34 (m, 10H), 7.47 (m, 3H), 7.57 (m, 3H), 7.75 (d, 2H, J = 7.0 Hz); δ_C ($CDCl_3$) 24.9, 25.4, 32.6, 32.8, 37.5, 42.5, 43.8, 48.5, 54.2, 67.4, 67.7, 72.6, 124.6, 126.1, 126.9, 128.0, 128.3, 128.5, 128.6, 128.7, 129.1, 129.9, 132.4, 135.4, 136.7, 136.8, 137.6, 139.2, 145.6, 155.6, 168.6, 169.3, 171.6, 196.6; $[\alpha]_{589}^{25}$ = +36 (*c* 0.27, MeOH); Found: C, 73.33; H, 6.85; N, 6.15. Calc. for $C_{40}H_{41}N_3O_5 \cdot 0.5H_2O$: C, 73.59; H, 6.48; N 6.43. ESI-MS m/z 666.4 $[M + Na]^+$.

*Z-(R)-BpAib-(S)-Phe-NHChx **8a**.* R_f 0.37 (cyclohexane–ethyl acetate 1 : 1); m.p. 127–130 °C; δ_H ($CDCl_3$) 1.03–1.21 (m, 5H), 1.49–1.71 (m, 5H), 2.92–3.35 (m, 5H), 3.68 (m, 2H), 4.60 (m, 1H), 4.94 (m, 2H), 5.56 (s, 1H), 6.24 (d, 1H, J = 8.0 Hz), 6.60 (d, 1H, J = 8.1 Hz), 7.07–7.34 (m, 12H), 7.39 (m, 2H), 7.52 (m, 3H), 7.67 (d, 2H, J = 7.0 Hz); δ_C ($CDCl_3$) 24.9, 25.4, 32.6, 32.8, 37.5, 42.5, 43.8, 48.4, 54.2, 67.3, 67.6, 72.6, 124.3, 126.4, 126.9, 128.0, 128.5, 128.6, 129.2, 129.6, 129.9, 132.4, 135.4, 136.7, 137.6, 140.6, 143.9, 155.5, 169.3, 171.6, 196.5 $[\alpha]_{589}^{25}$ = −2 (*c* 0.26, MeOH); Found: C, 74.58; H, 6.58; N, 6.51. Calc. for $C_{40}H_{41}N_3O_5$: C, 74.62; H, 6.42; N, 6.53. ESI-MS m/z 666.4 $[M + Na]^+$.

Bz-(*RS*)-BpAib-(*S*)-Phe-NHChx (7b and 8b). A suspension of Bz-(*RS*)-BpAib-OH **6** (2.02 g, 5.24 mmol) and HCl·H-(*S*)-Phe-NHChx (1.81 g, 6.40 mmol) in tetrahydrofuran (70 mL) was cooled on an ice bath. HATU (2.39 g, 6.28 mmol) and DIEA (2.5 mL) were added. The reaction mixture was allowed to warm to rt and stirred for 24 h. The mixture was concentrated under reduced pressure and the resulting residue was taken up in dichloromethane. The solution was washed twice with 0.5 M aqueous hydrochloric acid, once with water, and once with saturated aqueous $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue obtained was purified by column chromatography using cyclohexane–ethyl acetate (6 : 4) as eluent affording first Bz-(*S*)-BpAib-(*S*)-Phe-NHChx **7b** (1.176 g, 36%), then Bz-(*R*)-BpAib-(*S*)-Phe-NHChx **8b** (1.184 g, 37%).

*Bz-(*S*)-BpAib-(*S*)-Phe-NHChx **7b**.* R_f 0.32 (cyclohexane–ethyl acetate 1 : 1); m.p. 125–127 °C; δ_H ($CDCl_3$) 1.06–1.29 (m, 5H), 1.57–1.86 (m, 5H), 3.03 (m, 2H), 3.39 (m, 3H), 3.68 (m, 1H), 3.93 (d, 1H, J = 17.5 Hz), 4.72 (dd, 1H, J = 6.9, 8.1 Hz), 6.66 (d, 1H, J = 8.1 Hz), 6.79 (d, 1H, J = 8.4 Hz), 7.05–7.15 (m, 5H), 7.20–7.26 (m, 2H), 7.32–7.63 (m, 8H), 7.65–7.74 (m, 4H); δ_C ($CDCl_3$) 24.8, 24.9, 25.4, 32.6, 32.7, 37.2, 42.5, 43.6, 48.6, 53.9, 67.8, 124.6, 125.9, 126.9, 127.2, 128.3, 128.5, 129.1, 129.9, 132.2, 132.5, 132.8, 136.5, 136.7, 137.5, 139.5, 146.1, 168.1, 169.5, 171.8, 196.9; $[\alpha]_{589}^{25}$ = +98 (*c* 0.5, CH_2Cl_2); Found: C, 75.28; H, 6.47; N 6.73. Calc. for $C_{39}H_{39}N_3O_4 \cdot 0.5H_2O$: C, 75.21; H, 6.47; N, 6.75. ESI-MS m/z 636.5 $[M + Na]^+$.

*Bz-(R)-BpAib-(S)-Phe-NHChx **8b**.* R_f 0.22 (cyclohexane–ethyl acetate 1 : 1); m.p. 108–111 °C; δ_H ($CDCl_3$) 1.01–1.33 (m, 5H), 1.55–1.81 (m, 5H), 3.04 (m, 2H), 3.41 (m, 3H), 3.64 (m, 1H), 3.79 (d, 1H, J = 16.9 Hz), 4.64 (dd, 1H, J = 6.6, 7.9 Hz), 6.51 (d, 1H, J = 8.1 Hz), 6.69 (d, 1H, J = 8.1 Hz), 7.05–7.16 (m, 5H), 7.21–7.26 (m, 2H), 7.31–7.61 (m, 8H), 7.63–7.78 (m, 4H); δ_C ($CDCl_3$) 24.8, 24.9, 25.5, 32.6, 32.8, 37.5, 42.7, 43.7, 48.6, 53.4, 54.3, 67.7, 124.4, 126.4, 126.9, 127.2, 128.3, 128.5, 128.6, 129.2, 129.5, 129.9,

132.3, 132.4, 133.1, 136.6, 137.1, 137.7, 140.9, 144.3, 168.0, 169.3, 171.9, 196.5; $[\alpha]_{589}^{25} = -11$ (*c* 0.5, CH_2Cl_2); Found: C, 74.06; H, 6.59; N, 6.56. Calc. for $C_{39}H_{39}N_3O_4 \cdot H_2O$: C, 74.14; H, 6.54; N, 6.65. ESI-MS *m/z* 636.5 $[M + Na]^+$.

Boc-(*S*)-BpAib-OMe 9. Bz-(*S*)-BpAib-(*S*)-Phe-NHChx **7b** (629 mg, 1.03 mmol) was dissolved in dioxane (15 mL) and 10 M hydrochloric acid (15 mL) was added. The mixture was heated at reflux for 72 h and concentrated under reduced pressure. MeOH was added to the residue and the mixture was concentrated under reduced pressure three times. The resulting brown oil was dissolved in MeOH (5 mL) and the solution cooled on an ice bath. Thionyl chloride (0.5 mL) was added. The reaction mixture was allowed to warm to rt and stirred for 18 h. The mixture was concentrated under reduced pressure. MeOH was added to the residue and the mixture was concentrated under reduced pressure repeatedly. The residue was dissolved in MeOH and the solution made basic by addition of aqueous ammonia (28%). The mixture was concentrated under reduced pressure and the residue was filtered through silica gel, eluting with dichloromethane–MeOH (95 : 5). The resulting residue was dissolved in acetonitrile (5 mL) and dichloromethane (2 mL), and $(Boc)_2O$ (327 mg, 1.5 mmol) was added. The mixture was stirred at rt for 24 h. The mixture was concentrated under reduced pressure and the residue obtained was purified by column chromatography using ethyl acetate–cyclohexane (3 : 7) as eluent to afford the product as a solid (106 mg, 26% yield). R_f 0.31 (ethyl acetate–cyclohexane 3 : 7); m.p. 168–170 °C; δ_H ($CDCl_3$) 1.40 (s, 9H), 3.29 (m, 2H), 3.65 (m, 2H), 3.74 (s, 3H), 5.38 (bs, 1H), 7.27 (d, 1H, $J = 7.7$ Hz), 7.44 (t, $J = 7.7$ Hz, 2H), 7.53–7.63 (m, 3H), 7.75 (d, 2H, $J = 6.9$ Hz). δ_C ($CDCl_3$) 28.1, 43.6, 52.6, 65.8, 80.2, 124.2, 126.0, 128.1, 129.5, 129.8, 132.2, 136.6, 137.7, 140.2, 145.2, 154.9, 173.8, 196.4. $[\alpha]_{589}^{25} = +21$ (c 0.5, CH_2Cl_2); Found: C, 68.57, H, 6.52; N 3.33. Calc. for $C_{23}H_{25}NO_5 \cdot 0.5H_2O$: C, 68.29; H, 6.48; N, 3.46. ESI-MS *m/z* 418.1 $[M + Na]^+$.

Photocrosslinking experiments

An equimolar 0.1 M solution of **9** and **10** in CH_3CN was placed in a 100 mL Suprasil quartz tube, purged with dry nitrogen for 20 min and sealed with a rubber cap. The reaction vessel was then placed in a Rayonet multilamp photochemical reactor (Southern New England Ultra Violet Company, Branford, CT), equipped with 12 × 350 nm lamps, and irradiated in parallel for the desired time (up to 60 min). The progress of the photocrosslinking reaction was monitored by means of an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA), equipped with a Phenomenex (Torrance, CA) C_{18} analytical column (UV detection at 220 nm). Binary gradients of H_2O–CH_3CN, containing 0.05% trifluoroacetic acid, were used for elution at a flow rate of 1 mL min^{-1}.

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