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Synthesis, resolution and assignment of absolute configuration of *trans* 3-amino-1-oxyl-2,2,5,5-tetramethylpyrrolidine-4-carboxylic acid (POAC), a cyclic, spin-labelled β-amino acid

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Abstract

Racemic *trans* 3-(9-fluorenylmethyloxycarbonylamino)-1-oxyl-2,2,5,5-tetramethylpyrrolidine-4-carboxylic acid (Fmoc-POAC-OH), prepared by conventional methods, was resolved upon esterification with (a*R*)-2,2′-dihydroxy-1,1′-binaphthyl. Separation of the obtained diastereomeric monoesters Fmoc-(±)-*trans*-POAC-*O*-(a*R*)-binaphthol by crystallization/chromatography, and removal of the chiral auxiliary by saponification of the aryl ester function furnished both enantiomers (+)-(3*R*,4*R*)-Fmoc-POAC-OH and (−)-(3*S*,4*S*)-Fmoc-POAC-OH. The absolute configuration of the asymmetric C^3, C^4 carbons of POAC were assigned from the induced circular dichroism of a flexible biphenyl probe present in the terminally protected dipeptide derivatives Boc-Bip-(+)-POAC-OMe and Boc-Bip-(−)-POAC-OMe (Bip, 2′,1′:1,2;1″,2″:3,4-dibenzcyclohepta-1,3-diene-6-amino-6-carboxylic acid). This assignment was confirmed by X-ray diffraction analysis of the diastereomeric monoester Fmoc-(+)-*trans*-POAC-*O*-(a*R*)-binaphthol, shown to be (a*R*,3*R*,4*R*). Solution synthesis of peptides to the hexamer level, based on the (3*R*,4*R*)-POAC enantiomer combined with (1*S*,2*S*)-2-aminocyclopentane-1-carboxylic acid, was carried out to examine coupling conditions at both C- and N-termini of the POAC residue, in view of further syntheses and 3D-structural investigations.
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Keywords: β-Amino acids; Binaphthol esters; Circular dichroism; Chiral nitroxides; POAC; Resolution; Spin-labelled amino acids

1. Introduction

Nitroxide free radicals derived from TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) have found applications in chemistry and medicine as spin labels,[1] spin traps,[2] oxidizing agents,[3] anti-oxidants[4] and MRI contrast agents.[5] To date, one of their most common uses is in the study of conformation and structural mobility of peptides and proteins by EPR spectroscopy. Spin labels have been introduced into peptides by a variety of nitroxide-bearing amino acids, where the nitroxide function has been placed either in the side chain of α-amino acids,[6] or incorporated into cyclic structures of C^{α}-tetrasubstituted α-amino acids (TOAC,[7] PTOAC[8]), β-amino acids (POAC,[7a,9] β-TOAC[10]) or γ-amino acids.[11] By far the most popular, the achiral C^{α}-tetrasubstituted α-amino acid TOAC (4-amino-1-oxyl-2,2,6,6-tetramethylpiperidine-4-carboxylic acid)[7] (Fig. 1), has been widely used to label peptides at N-terminal and internal positions for biological studies and

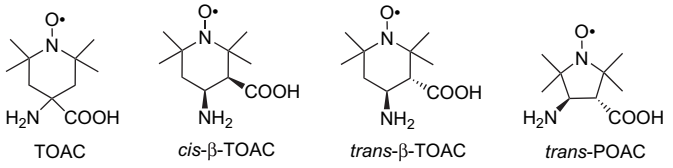

Figure 1. Chemical structures of the spin-labelled amino acids TOAC, β-TOAC and POAC.

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conformational analysis by ESR methods,[12] as well as 3D-structural investigations involving intramolecular spin–spin interactions (ESR), energy transfer (fluorescence quenching), or spin polarization (CIDEP) effects in designed rigid systems.[13] However, while the tetrasubstituted α-carbon of TOAC is interesting for its ability to induce β-turn or 3_{10}/α-helical structures in peptides,[12,13] it is also responsible for the reduced reactivity of the α-amino group. We wished to synthesize nitroxide spin-labelled, cyclic, chiral β-amino acids, which retain the conformationally rigid character of TOAC while allowing milder peptide coupling conditions. The β-amino acids have been extensively studied in the last 10 years since it was demonstrated that their oligomers may fold into helical conformations (stable in organic and aqueous solvents) and are resistant to enzymatic hydrolysis.[14] β-TOAC (4-amino-1-oxyl-2,2,6,6,-tetramethylpiperidine-3-carboxylic acid), a β-amino acid form of TOAC, was initially designed, both cis and trans isomers of which (Fig. 1) could be obtained in enantiopure form.[10]

The synthesis of enantiopure *trans*-POAC (4-amino-1-oxyl-2,2,5,5-tetramethylpyrrolidine-3-carboxylic acid) (Fig. 1), first reported as a racemic mixture by Rassat and Rey[7a] and later prepared and used as such,[15] also attracted our attention[9] as its easy insertion in a peptide synthesized by solid-phase methods had been demonstrated,[15] whereas similar syntheses with the TOAC residue proved more problematic.[12,16] Furthermore, the *trans*-POAC residue has a similar structure to that of *trans*-ACPC (2-aminocyclopentane-1-carboxylic acid), whose oligomers have been shown by Gellman and co-workers[14c] to adopt a helix stabilized by a hydrogen-bonded 12-membered ring (the '12-helix') in the crystal state and organic solvents. Consequently *trans*-POAC could be used as a spin-probe to study the 12-helical β-peptide secondary structure in the same way as TOAC and *trans*-β-TOAC have been exploited in the case of α/3_{10}-helical α-peptides[12] and 3_{14}-helical β-peptides,[10c] respectively. In the present paper we report: (i) the full experimental details of the synthesis and resolution of *trans*-POAC,[9] (ii) the assignment of its absolute configuration by the 'Bip method',[17,18] involving CD spectroscopy of the dipeptide derivatives Boc-Bip-(+)-*trans*-POAC-OMe and Boc-Bip-(−)-*trans*-POAC-OMe (Boc, *tert*-butyloxycarbonyl, Bip, 2′,1′:1,2;1″,2″:3,4-dibenzcyclohepta-1,3-diene-6-amino-6-carboxylic acid, OMe, methoxy) of both enantiomers (+)-*trans*-POAC and (−)-*trans*-POAC,[18] and (iii) its use as a building block, in combination with *trans*-ACPC, in the solution synthesis of the hexapeptide Boc-{(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-(1*S*,2*S*)-ACPC}$_2$-OMe, as a first step towards the preparation of a set of related peptides for 3D-structural investigations of their postulated 12-helical conformation.

2. Results and discussion

2.1. Synthesis

Racemic POAC was prepared by reported procedures,[7a] in which 2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide was first oxidized to 1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide **1**[19] (Fig. 2) using an acetonitrile, methanol, hydrogen peroxide, sodium tungstate and sodium hydrogen carbonate oxidative system.[20] This compound was then treated by *p*-toluenesulfonyl chloride in pyridine to afford 3-cyano-1-oxyl-2,2,5,5-tetramethyl-3-pyrroline **2**[19] in 79% overall yield. Aza-Michael addition of aqueous ammonia to the α,β-unsaturated nitrile **2** afforded 3-cyano-4-amino-1-oxyl-2,2,5,5-

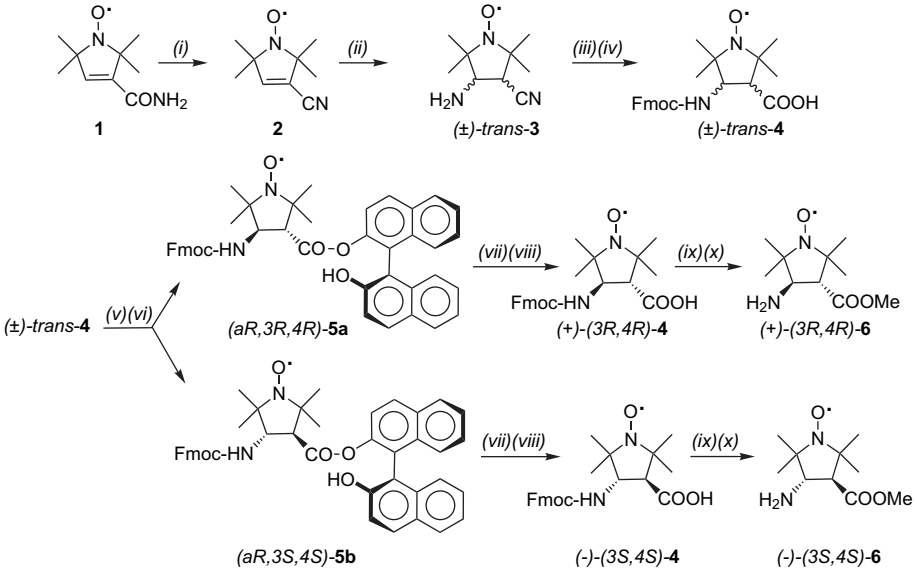

Figure 2. Synthetic path for the preparation of racemic Fmoc-POAC-OH, (±)-*trans*-**4**,[15] and resolution through its (a*R*)-2,2′-dihydroxy-1,1′-binaphthyl monoesters (the trans spatial relationship between the C^4–N bond and the C^3–CN or C^3–COOH bond of (±)-*trans*-**3** and (±)-*trans*-**4** is not represented to avoid confusion with enantiomerically pure compounds). (i) TsCl, pyridine, rt, 36 h. (ii) NH_3 (aq), rt, 72 h. (iii) NaOH, H_2O, MeOH, reflux, 48 h. (iv) Fmoc-OSu, $NaHCO_3$, acetone/H_2O 2:1, rt, 18 h. (v) (a*R*)-2,2′-dihydroxy-1,1′-binaphthyl, EDC, DMAP, CH_2Cl_2/CH_3CN 1:1, 0 °C, 2 h. (vi) Crystallization, then chromatography. (vii) NaOH, H_2O, MeOH, THF (tetrahydrofuran), 50 °C, 3 h. (viii) Fmoc-OSu, $NaHCO_3$, acetone/H_2O 2:1, rt, 18 h. (ix) MeOH, NMM, EDC, HOAt. (x) CH_2Cl_2/Et_2NH 5:1.

tetramethylpyrrolidine (±)-*trans*-**3**[7a] in only 40% yield in the best of several runs, accompanied by 23% of the starting nitrile **2** and 32% of the amide **1**, which both could be recycled in further runs [*note*: this reaction has been reported to be more efficient when performed under pressure in an autoclave, using liquid ammonia in the presence of a small amount of water[7a]]. In the ^{1}H NMR spectrum of the amino nitrile **3**, performed after sodium dithionite reduction of the nitroxide group,[21] the observed high coupling constant for the C^3H-C^4H protons ($J^3 \sim 11.3$ Hz) confirmed that only the trans isomer was formed under these conditions, as previously demonstrated by X-ray diffraction analysis.[15] Basic hydrolysis of (±)-*trans*-**3** with NaOH in methanol/water at 100 °C for 48 h provided the free amino acid (±)-*trans*-H-POAC-OH,[7a] which was not characterized but directly N^{α}-protected by treatment with Fmoc-OSu (Fmoc, 9-fluorenylmethyloxycarbonyl; OSu, succinimidyloxy), to afford 4-(Fmoc-amino)-1-oxyl-2,2,5,5-tetramethylpyrrolidine-3-carboxylic acid (±)-*trans*-Fmoc-POAC-OH **4**[15] in 70% overall yield.

For resolution of (±)-*trans*-**4** we discarded methods involving amide bond formation, with either amines or amino acid derivatives as chiral auxiliaries, because of the harsh acidic conditions required for removal of the chiral auxiliary from the separated diastereomers, incompatible with the presence of the nitroxide group.[12] Resolution through formation of diastereomeric pairs of esters by reaction with chiral alcohols was considered instead.[22] To this end, we selected 2,2′-dihydroxy-1,1′-binaphthyl as a potentially efficient chiral auxiliary, taking into account the known 'opposite' resolution of related 1,1′-binaphthyl diols by means of their esterification with chiral amino acids.[23] Treatment of (±)-*trans*-**4** by (a*R*)-2,2′-dihydroxy-1,1′-binaphthyl (binaphthol) in the presence of EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] and DMAP (4-dimethylaminopyridine) to form the diastereomeric monoesters Fmoc-(±)-*trans*-POAC-*O*-(a*R*)-binaphthol (a*R*,3*R*,4*R*)-**5** and (a*R*,3*S*,4*S*)-**5** (Fig. 2) allowed the separation of two diastereomers **5a** and **5b**, isolated in 40% and 41% yield (80% and 82% of theoretical yield), respectively, by standard column chromatography on silica gel. Advantageously, for resolution up to a gram-scale, it was also possible, prior to chromatography, to partially purify the mixture by crystallization, as the diastereomer with a lower R_f (**5b**) has an appreciably poorer solubility in non-polar solvents than that with a higher R_f (**5a**).

Alkaline hydrolysis of the esters **5a** and **5b** was accompanied by a partial cleavage of the Fmoc protecting group, as expected.[24] Subsequent reprotection of the amine function gave the desired Fmoc-POAC-OH amino acid enantiomers (+)-*trans*-**4** in 57% yield and (−)-*trans*-**4** in 43% yield, respectively (Fig. 2), the ee of each was demonstrated to be >99.5% by chiral HPLC.[9b] The corresponding amino esters H-POAC-OMe (+)-*trans*-**6** and (−)-*trans*-**6** (Fig. 2) were also obtained in two steps from (+)-*trans*-**4** and (−)-*trans*-**4**, respectively, by initial esterification with methanol in the presence of EDC/HOAt (7-aza-1-hydroxy-1,2,3-benzotriazole)/NMM (*N*-methyl-morpholine) (avoiding acidic conditions incompatible with the presence of the nitroxide group), followed by N-deprotection of the resulting amino esters Fmoc-POAC-OMe by treatment with CH_3CN/Et_2NH 9:1.

2.2. Assignment of the absolute configuration of the POAC enantiomers

To determine the absolute configuration of the enantiomeric pair of POAC derivatives (+)-/(−)-*trans*-**6**, we first applied a spectroscopic method recently introduced by our groups for configurational assignment of chiral α- and β-amino acids.[17] The so-called 'Bip method' is based on transfer of the central chirality of an α- or β-amino acid residue Xaa* to an axial chirality in the biphenyl core of 2′,1′:1,2;1″,2″:3,4-dibenzcyclohepta-1,3-diene-6-amino-6-carboxylic acid (Bip), a conformationally labile, atropoisomeric, C^{α}-tetrasubstituted α-amino acid.[26] This effect is observed in the terminally protected dipeptides Boc-Bip-Xaa*-OMe and results in a preferred diastereomeric conformer (Fig. 3) and the onset of an induced circular dichroism (ICD).

Specifically, C-terminal L-α-Xaa*,[17a,b] L-β^3-Xaa* as well as cyclic $\beta^{2,3}$-Xaa* residues (1*S*,2*S*)-ACHC (2-aminocyclohexane-1-carboxylic acid) and (1*S*,2*S*)-ACPC,[17c,d,18] preferentially induce a negative Cotton effect at about 240–250 nm, the A band of Suzuki,[27] related to a *P* torsion of the $C_{Ar}-C_{Ar}$ bond[28] of the Bip biphenyl chromophore. The Bip

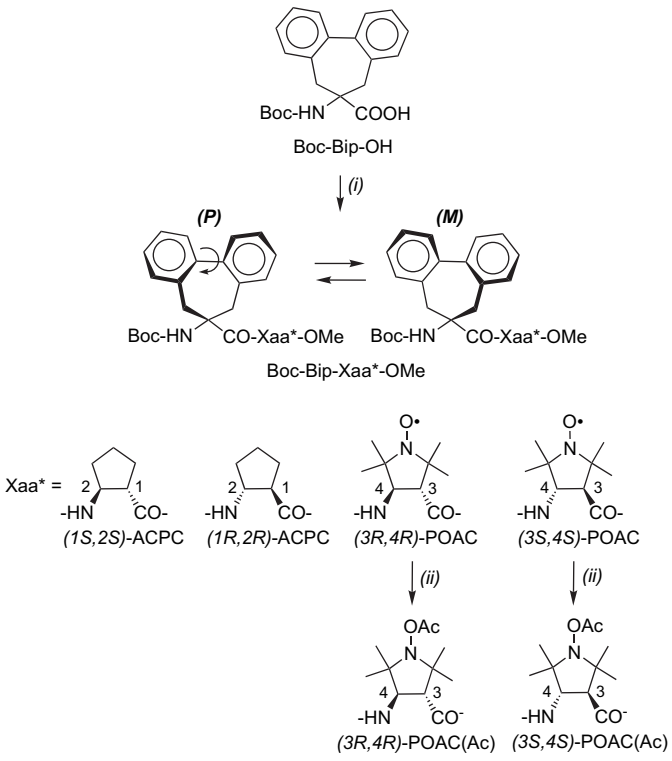

Figure 3. Conformational equilibrium of the Boc-Bip-Xaa*-OMe dipeptides [Xaa*=(1*S*,2*S*)-ACPC,[18] (1*R*,2*R*)-ACPC,[18] (3*R*,4*R*)-POAC, (3*S*,4*S*)-POAC, (3*R*,4*R*)-POAC(Ac) (Ac, acetyl), (3*S*,4*S*)-POAC(Ac)], and synthetic path for the preparation of Boc-Bip-(−)-*trans*-POAC-OMe and Boc-Bip-(+)-*trans*-POAC-OMe and the *N*-*O*-acetyl protected derivatives Boc-Bip-(−)-*trans*-POAC(Ac)-OMe and Boc-Bip-(+)-*trans*-POAC(Ac)-OMe. (i) (−)-*trans*-**6** or (+)-*trans*-**6**, NMM, EDC, HOAt. (ii) (1) H_2, 10% Pd/C, THF, rt, 30 min. (2) AcCl, Et_3N, rt, 10 min.

method was valid for Boc-Bip-Xaa*-OMe derivatives of the spin-labelled $\beta^{2,3}$-amino acids *cis*/*trans*-β-TOAC of known absolute configuration,[18] which was interesting in view of its application to POAC.

The terminally protected dipeptides Boc-Bip-(−)-*trans*-POAC-OMe and Boc-Bip-(+)-*trans*-POAC-OMe (Fig. 3) were synthesized by coupling Boc-Bip-OH[18b,26] with (−)-*trans*-**6** and (+)-*trans*-**6**, respectively, using the EDC/HOAt method, efficient in the C-activation of sterically demanding C^{α}-tetrasubstituted α-amino acids.[29] The corresponding *N*-*O*-acetyl (NO-Ac) protected derivatives Boc-Bip-(−)-*trans*-POAC(Ac)-OMe and Boc-Bip-(+)-*trans*-POAC(Ac)-OMe were also prepared, as it was possible that the nitroxide chromophore could interfere with the observed ICD in the UV region.[30]

The ICD resulting from the induced axial chirality in the biphenyl core of the Bip residue of the Bip/POAC dipeptides gives clear information on the absolute configuration of POAC. The CD spectra in MeOH solution of the enantiomeric pairs Boc-Bip-(−)/(+)-*trans*-POAC(Ac)-OMe (Fig. 4A) and Boc-Bip-(−)/(+)-*trans*-POAC-OMe (Fig. 4B) are mirror images, as expected, and all present an intense Cotton effect at 250 nm. For the dipeptides Boc-Bip-(−)-*trans*-POAC-OMe and Boc-Bip-(−)-*trans*-POAC(Ac)-OMe the Cotton effect is positive as for Boc-Bip-(1*R*,2*R*)-ACPC-OMe. The dipeptides Boc-Bip-(+)-*trans*-POAC-OMe and Boc-Bip-(+)-*trans*-POAC(Ac)-OMe show a negative Cotton effect, as does Boc-Bip-(1*S*,2*S*)-ACPC-OMe. Therefore, taking into account the different priority order of the substituents at C^3 and C^4 of POAC compared to ACPC, the absolute configurations (−)-(3*S*,4*S*) and (+)-(3*R*,4*R*) were assigned to the enantiomers of *trans*-POAC. The absolute configurations (a*R*,3*R*,4*R*)-**5**/(a*R*,3*S*,4*S*)-**5** (Fig. 2) may be assigned by direct filiation to the diastereomeric monoesters Fmoc-(±)-*trans*-POAC-*O*-(a*R*)-binaphthol **5a/5b**, respectively.

The configurational assignment achieved using the 'Bip method' was confirmed subsequently by X-ray diffraction analysis[31] (Fig. 5) of a suitable crystal of the diastereomer **5a** (Fig. 2), for which an (a*R*,3*R*,4*R*) absolute configuration was indeed found. There are two crystallographic independent molecules (**A** and **B**), with slightly different conformational properties, in the asymmetric unit of **5a**. The secondary Fmoc urethane group is in the usual trans disposition.[32] The −NH and −CO functions, pointing outwards from the POAC pyrrolidine ring system, are trans oriented, and the naphthyl groups of the binaphthyl moiety are nearly perpendicular to each other.

2.3. Synthesis of peptides based on (3R,4R)-POAC combined with (1S,2S)-ACPC

The solution synthesis of peptides to the hexamer level, based on the (3*R*,4*R*)-POAC enantiomer combined with

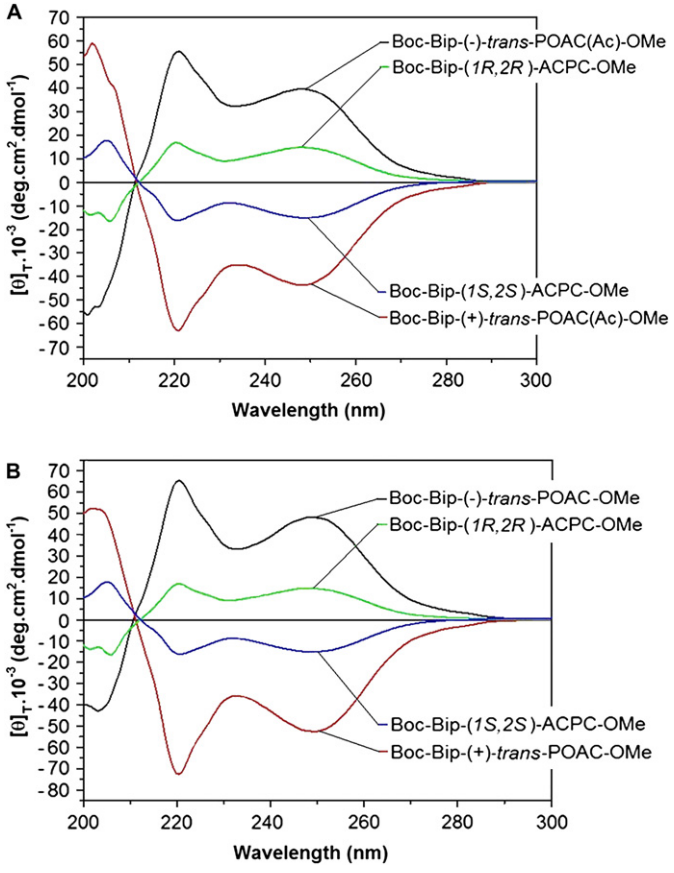

Figure 4. CD spectra (200−300 nm region) of the dipeptides Boc-Bip-(+) and (−)-*trans*-POAC(Ac)-OMe (**A**), and the corresponding dipeptides with a free nitroxide function Boc-Bip-(+) and (−)-*trans*-POAC-OMe (**B**), compared to Boc-Bip-(1*R*,2*R*)-ACPC-OMe and Boc-Bip-(1*S*,2*S*)-ACPC-OMe in MeOH solution (peptide concentration: 1×10^{-3} M).

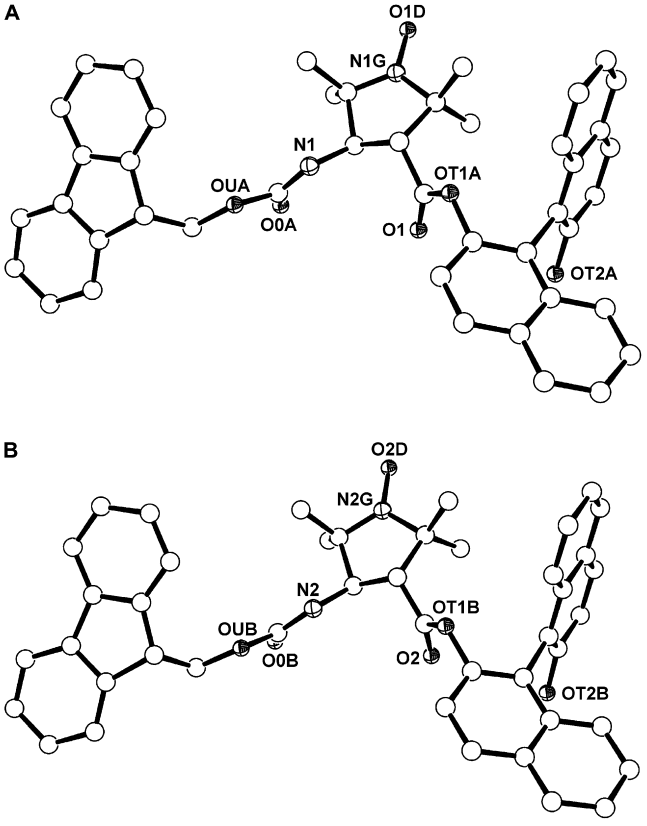

Figure 5. X-ray diffraction structure of (a*R*,3*R*,4*R*) Fmoc-(+)-*trans*-POAC-*O*-binaphthol **5a**. The two crystallographic independent molecules (**A** and **B**) in the asymmetric unit are shown.

(1*S*,2*S*)-ACPC for configurational homogeneity of the amino acid components (Fig. 3), was carried out to examine coupling conditions at the N- and C-termini of the POAC residue, in view of further syntheses and 3D-structural investigations of related Boc/OMe hexapeptides. The synthetic strategy used relied on the need to avoid acidic N-deprotection of the Boc group (i.e., TFA/CH_2Cl_2 or HCl/dioxane) in all peptide segments containing the POAC residue, as these conditions were reported to cause protonation followed by disproportionation and decomposition of the nitroxide group.[12] For this reason, we selected N— to —C chain elongation of *N*-Boc protected peptide segments (Fig. 6).

For convenience, we started from the terminally protected derivative Fmoc-(3*R*,4*R*)-POAC-*O*-(a*R*)-binaphthol **5a**, which was N-deprotected (in CH_3CN/Et_2NH) then coupled with Boc-(1*S*,2*S*)-ACPC-OH by the HATU [2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] activation method[25,33] in the presence of DIEA (*N*,*N*,*N*-diisopropylethylamine), to afford Boc-(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-*O*-(a*R*)-binaphthol **7** in 92% yield. This dipeptide ester was saponified with sodium hydroxide in MeOH/H_2O/THF at 50 °C. The resulting crude Boc-(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-OH **8** was coupled with HCl·H-[(1*S*,2*S*)-ACPC]$_2$-OMe (obtained by N-deprotection of Boc-[(1*S*,2*S*)-ACPC]$_2$-OMe **9**), by the HATU method to afford the N-terminal protected tetrapeptide Boc-(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-[(1*S*,2*S*)-ACPC]$_2$-OMe **10** in 80% yield. The C-terminal dipeptide fragment was obtained by the coupling of (3*R*,4*R*)-**4** with HCl·H-(1*S*,2*S*)-ACPC-OMe[18b] by the HATU method to afford Fmoc-(3*R*,4*R*)-POAC-(1*S*,2*S*)-ACPC-OMe **11** in 62% yield. Finally, segment coupling between the tetrapeptide Boc-(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-[(1*S*,2*S*)-ACPC]$_2$-OH, resulting from saponification of **10**, and the dipeptide H-(3*R*,4*R*)-POAC-(1*S*,2*S*)-ACPC-OMe, resulting from N-deprotection of **11**, gave the hexapeptide Boc-{(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-(1*S*,2*S*)-ACPC}$_2$-OMe **12** in 51% yield.

3. Conclusions

Both enantiomers of POAC could be obtained by resolution of their binaphthol esters, an advantage for future incorporation of this labelled building block into peptides for 3D-structural investigations, previously performed only with racemic material.[15] The absolute configuration of POAC was assigned by CD analysis of dipeptide derivatives containing a biphenyl probe, which constitutes a further application of our previously reported 'Bip method'[17] to a spin-labelled β-amino acid residue.[18] Confirmation of this spectroscopic assignment of absolute configuration was obtained by X-ray diffraction analysis. Finally, the solution synthesis of the hexapeptide Boc-{(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-(1*S*,2*S*)-ACPC}$_2$-OMe is a first step towards synthesis, 3D-structural analysis and ESR studies of a series of related bis spin-labelled hexapeptides, which will be performed by our groups.

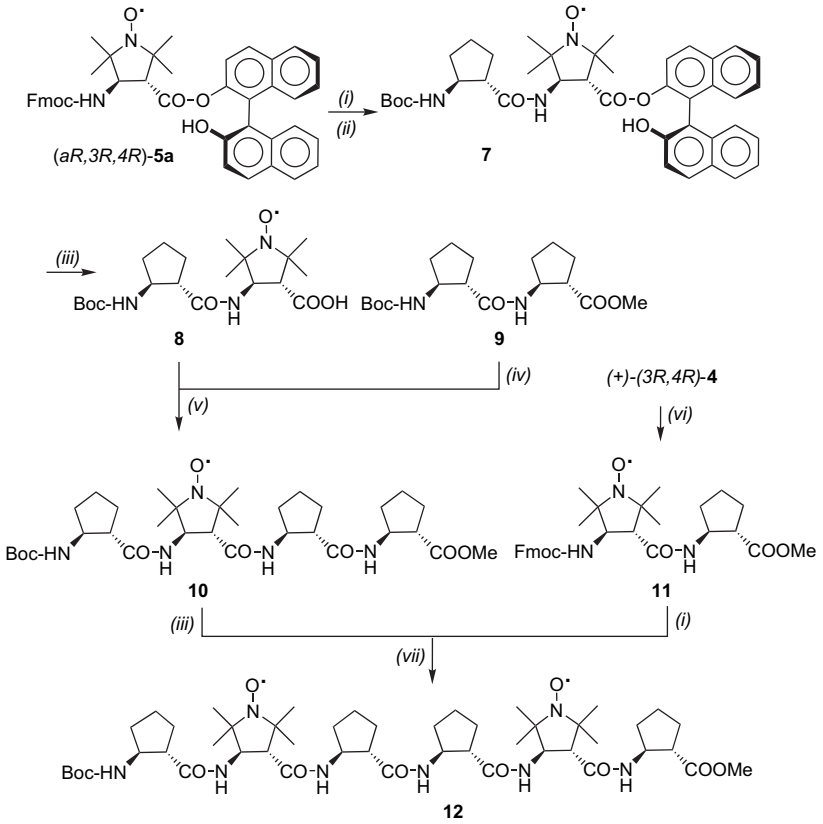

Figure 6. Synthetic path for the preparation of the hexapeptide Boc-{(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-(1*S*,2*S*)-ACPC}$_2$-OMe **12**. (i) CH_3CN/Et_2NH 4:1, rt, 3 h. (ii) Boc-(1*S*,2*S*)-ACPC-OH, HATU, DIEA, THF, rt, 18 h. (iii) NaOH, MeOH/H_2O/THF, 50 °C. (iv) HCl/dioxane, 0 °C (30 min) to rt (1 h). (v) HATU, DIEA, THF, rt, 48 h. (vi) HCl·H-(1*S*,2*S*)-ACPC-OMe, HATU, DIEA, THF, rt, 48 h. (vii) HATU, DIEA, THF, rt, 6 days.

4. Experimental

4.1. General experimental information

Melting points were determined with a temperature raise of 3 °C/min and are uncorrected. NMR spectra were recorded at 300 MHz for ^{1}H and at 77 MHz for ^{13}C, on a Bruker AC300 spectrometer, the solvent $CDCl_3$ (δ=7.27 ppm for ^{1}H and 77.0 ppm for ^{13}C) or D_2O/acetone-d_6 (δ=2.22 ppm for ^{1}H and 30.9 ppm for ^{13}C) being used as internal standard. Those involving spin-labelled compounds were performed after sodium dithionite reduction of the nitroxide group.[21] Splitting patterns are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. The CD spectra were obtained on a Jasco J-715 spectropolarimeter. Cylindral fused quartz cells (Hellma) of 0.1 mm path length were used. The values are expressed in terms of $[\theta]_T$, total molar ellipticity (deg cm^2 dmol^{-1}). Spectrograde methanol (Fluka) was used as solvent. MS analyses were performed by Mr. Vincent Steinmetz (ILV), on a Hewlett–Packard HP5989MS spectrometer. High resolution mass spectra (TOF ES mode) were performed by the C.N.R.S. Analytical Service, Vernaison (France) or the University of Amiens (France). Elemental analyses were performed by the C.N.R.S. Service of Microanalyses at Gif-sur-Yvette (France). The optical rotations were measured with an accuracy of 0.3%, in a 1-dm thermostatted cell. Analytical thin layer chromatography (TLC), preparative TLC and column chromatography were performed on silica gel F 254 (Merck), silica gel G-25 (1 mm) (Macherey-Nagel) and kieselgel gel 60 (0.040–0.063 mm) (Merck), respectively. The starting materials 2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide and Boc-(1*S*,2*S*)-ACPC-OH were purchased from Alpha Aesar and NeoMPS, respectively, while Boc-Bip-OH and HCl·H-(1*S*,2*S*)-ACPC-OMe were prepared as previously described.[18b]

4.2. 3-Cyano-1-oxyl-2,2,5,5-tetramethyl-3-pyrroline **2**[19]

2,2,5,5-Tetramethyl-3-pyrroline-3-carboxamide (5.0 g; 29.76 mmol) was dissolved in a mixture of methanol (50 mL) and acetonitrile (5 mL). The solution was cooled on an ice bath. Sodium tungstate dihydrate (320 mg; 0.97 mmol) and sodium hydrogen carbonate (2.2 g) were added. Hydrogen peroxide 35% (15 mL) was added slowly in portions over 1 h. The reaction was stirred at rt for 24 h. The mixture was concentrated under reduced pressure and the residue was taken up in CH_2Cl_2 and washed with brine. The aqueous phase was extracted repeatedly with CH_2Cl_2 until the extracts were colourless. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give crude 1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide **1**[19] as a yellow solid residue. ^{1}H NMR (D_2O/acetone-d_6): δ 6.46 [s, 1H, CH], 1.40 [s, 6H, CH_3] and 1.31 [s, 6H, CH_3]. This solid was dissolved in pyridine (60 mL) and the solution was cooled on an ice bath. Tosyl chloride (10.9 g; 57.18 mmol) was added. The mixture was stirred at rt for 36 h. A solution of potassium hydroxide (3.8 g) in water (100 mL) was added and the mixture was heated at 80 °C for 75 min. The mixture was allowed to cool and concentrated to ≈30 mL under reduced pressure. The mixture was taken up in CH_2Cl_2 and washed twice with a 0.5 N HCl solution, then with a saturated $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford 3.88 g (79%) of 1-oxyl-2,2,5,5-tetramethyl-3-cyano-3-pyrroline **2**[19] as an orange oil that crystallized on standing. R_f 0.58 (cyclohexane/EtOAc 6:4). ^{1}H NMR (D_2O/acetone-d_6): δ 6.78 [s, 1H, C_3H], 1.34 [s, 6H, CH_3] and 1.30 [s, 6H, CH_3]. ^{13}C NMR (D_2O/acetone-d_6): δ 152.8 [C_4], 119.2 [C_3], 116.4 [CN], 71.4, 71.0 [C_2, C_5], 25.6, 25.4 [CH_3]. CIMS *m/z* (%): 166 $[M+H]^+$ (45).

4.3. 3-Cyano-4-amino-1-oxyl-2,2,5,5-tetramethyl-pyrrolidine (±)-*trans*-**3**[7a]

A solution of concentrated (32%) aqueous ammonia (75 mL) was added to the nitrile **2** (1.460 g; 8.8 mmol). The flask was firmly stoppered and the mixture was stirred at rt for 72 h. Sodium chloride was added to the mixture to saturation point. The mixture was stirred for a further 3 h, during which time a precipitate formed. The mixture was extracted three times with CH_2Cl_2, and the combined extracts were dried over $MgSO_4$, filtered and evaporated. Analytical TLC of this residue (MeOH/CH_2Cl_2 5:95) revealed three compounds: the starting nitrile **2** (R_f 0.68), the amide **1** (R_f 0.14) and the desired amino nitrile **3** (R_f 0.25; ninhydrin positive spot). Diethyl ether was added to the residue and the mixture was cooled on an ice bath. A precipitate formed and the mixture was filtered. The solid collected was the amide **1** (0.527 g; 32%). The filtrate was evaporated and the resulting residue was purified by column chromatography on silica gel with eluant MeOH/CH_2Cl_2 5:95 to give successively the starting nitrile **2** (0.344 g; 23%) and 3-cyano-4-amino-1-oxyl-2,2,5,5-tetramethyl-pyrrolidine (±)-*trans*-**3**[7a] (0.652 g; 40%). ^{1}H NMR (D_2O/acetone-d_6): δ 3.21 [d, J=11.4 Hz, 1H, C_3H], 2.91 [d, J=11.2 Hz, 1H, C_4H], 1.30 [s, 6H, CH_3], 1.22 [s, 3H, CH_3] and 1.05 [s, 3H, CH_3]. ^{13}C NMR (D_2O/acetone-d_6): δ 121.8 [CN], 69.1 [C_3], 67.8, 66.7 [C_2, C_5], 63.7 [C_4], 26.2, 25.6 [CH_3]. CIMS *m/z* (%): 183 $[M+H]^+$ (100).

4.4. 4-(Fmoc-amino)-1-oxyl-2,2,5,5-tetramethylpyrrolidine-3-carboxylic acid (±)-*trans*-Fmoc-POAC-OH **4**[15]

The amino nitrile (±)-*trans*-**3** (2.64 g; 14.5 mmol) was dissolved in MeOH (100 mL) and a solution of NaOH (12.5 g) in water (50 mL) was added. The mixture was stirred and refluxed for 48 h, then allowed to cool to rt and concentrated under reduced pressure to remove the remaining MeOH. The solution was then cooled to 0 °C and the pH was adjusted to ≈6 by the addition of 2 N HCl. To the resulting cold solution of crude 4-amino-1-oxyl-2,2,5,5-tetramethylpyrrolidine-3-carboxylic acid (±)-*trans*-H-POAC-OH[7a] was added sodium bicarbonate (12 g), and then a solution of Fmoc-OSu (6.10 g; 18.10 mmol) in acetone (190 mL). The mixture was stirred from 0 °C to rt overnight. A further portion of Fmoc-OSu (530 mg; 1.57 mmol) was added and the mixture was stirred

for a further 6 h at rt. The mixture was concentrated under reduced pressure to remove acetone. The resulting aqueous mixture was washed twice with diethyl ether. The aqueous phase was then acidified with 2 N HCl and extracted three times with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over $MgSO_4$, filtered and evaporated. The residue was recrystallized from CH_2Cl_2/cyclohexane to afford 4.32 g (70%) of (±)-*trans*-**4**.[15] Mp 174–176 °C. ^{1}H NMR (D_2O/acetone-d_6): δ 7.87 [m (d-like), 2H, ArH Fmoc], 7.72 [m (t-like), 2H, ArH Fmoc], 7.50–7.37 [m, 4H, ArH Fmoc], 4.49–4.25 [m, 4H, CH_2 Fmoc, CH Fmoc and C_3H], 2.73 [d, J=11.4 Hz, 1H, C_4H], 1.42 [s, 3H, CH_3], 1.31 [s, 3H, CH_3], 1.21 [s, 3H, CH_3] and 1.08 [s, 3H, CH_3]. ESI$^+$ MS *m/z* (%): 446 $[M+Na]^+$ (100).

4.5. Fmoc-(±)-trans-POAC-O-(aR)-binaphthol diastereomers 5a and 5b

To an ice-cold solution of (a*R*)-2,2′-dihydroxy-1,1′-binaphthyl (1.220 g; 4.25 mmol) and DMAP (43 mg; 0.35 mmol) in CH_2Cl_2 (40 mL) was added EDC (1.010 g; 5.31 mmol). A solution of (±)-*trans*-**4** (1.500 g; 3.54 mmol) in CH_2Cl_2 (50 mL) and CH_3CN (50 mL) was then added dropwise to the mixture over 30 min. The mixture was stirred for a further 90 min at 0 °C, then concentrated under reduced pressure. The resulting residue was taken up in CH_2Cl_2 and washed with 0.5 M HCl solution, then with brine. The organic phase was dried over $MgSO_4$, filtered and concentrated. The residue was dissolved in CH_2Cl_2 (≈30 mL) and the same volume of diethyl ether was added. The mixture was left to stand at rt overnight and the resulting precipitate was filtered off. The solid collected was recrystallized from warm CH_2Cl_2 (≈15 mL) and diethyl ether (≈15 mL) to give 0.600 g of pure diastereomer **5b**. The combined filtrates were purified by repeated column chromatography on silica gel with cyclohexane/EtOAc 7:3 as eluant to give further 0.395 g of diastereomer **5b** (total 0.995 g; 41%), later assigned (see text) (a*R*,3*S*,4*S*)-**5** {mp 234–236 °C. R_f 0.27 (cyclohexane/EtOAc 7:3). $[\alpha]_{589}^{25}$ −36; $[\alpha]_{578}^{25}$ −43; $[\alpha]_{546}^{25}$ −61; $[\alpha]_{436}^{25}$ −7; $[\alpha]_{365}^{25}$ +232 (*c* 0.26, CH_2Cl_2). ESI$^+$ MS *m/z* (%): 714.6 $[M+Na]^+$. Anal. Calcd for $C_{44}H_{39}N_2O_6\cdot H_2O$ (709.784): C, 74.45; H, 5.82; N, 3.95. Found: C, 74.48; H, 5.51; N, 3.95. HRMS (TOF ES MS) calcd for $C_{44}H_{39}N_2O_6Na$: 714.2706; found: 714.2693}, and 0.987 g (40%) of pure diastereomer **5a**, later assigned (see text) (a*R*,3*R*,4*R*)-**5** {mp 141–143 °C. R_f 0.35 (cyclohexane/EtOAc 7:3). $[\alpha]_{589}^{25}$ +131; $[\alpha]_{578}^{25}$ +134; $[\alpha]_{546}^{25}$ +167; $[\alpha]_{436}^{25}$ +223; $[\alpha]_{365}^{25}$ +402 (*c* 0.26, CH_2Cl_2). ESI$^+$ MS *m/z* (%): 714.6 $[M+Na]^+$. Anal. Calcd for $C_{44}H_{39}N_2O_6$ (691.768): C, 76.39; H, 5.68; N, 4.05. Found: C, 76.48; H, 6.15; N, 3.61. HRMS (TOF ES MS) calcd for $C_{44}H_{39}N_2O_6Na$: 714.2706; found: 714.2700}.

4.6. 4-(Fmoc-amino)-1-oxyl-2,2,5,5-tetramethylpyrrolidine-3-carboxylic acid (+)-trans-4 and (−)-trans-4

The Fmoc-(±)-*trans*-POAC-*O*-(a*R*)-binaphthol diastereomer **5a** (0.150 g; 0.22 mmol) was dissolved in THF (6 mL), MeOH (2 mL) and H_2O (1 mL). Sodium hydroxide (75 mg) was added and the mixture was heated on a 50 °C bath for 3 h. The mixture was allowed to cool, concentrated under reduced pressure and H_2O (3 mL) was added. The mixture was neutralized by careful addition of 1 M HCl solution, then cooled on an ice bath. Acetone (3 mL), $NaHCO_3$ (0.182 g; 2.17 mmol) and Fmoc-OSu (0.095 g; 0.28 mmol) were added and the mixture was stirred at rt overnight. The mixture was concentrated under reduced pressure and H_2O was added. The resulting solution was washed twice with diethyl ether. The aqueous phase was acidified by careful addition of 1 M HCl solution. This solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel with CH_2Cl_2/MeOH 9:1 as eluant to afford pure Fmoc-POAC-OH (+)-*trans*-**4** (0.084 g; 91%) as a yellow solid, later assigned (see text) (3*R*,4*R*)-**4** {R_f 0.54 (CH_2Cl_2/MeOH 85:15). Mp 138–139 °C. ^{1}H NMR (D_2O/acetone-d_6): see (±)-*trans*-**4** (Section 4.4). $[\alpha]_{589}^{25}$ +73; $[\alpha]_{578}^{25}$ +77; $[\alpha]_{546}^{25}$ +103; $[\alpha]_{436}^{25}$ +122; $[\alpha]_{365}^{25}$ −239 (*c* 0.1, MeOH). ESI$^+$ MS *m/z* (%): 446 $[M+Na]^+$(100). Anal. Calcd for $C_{24}H_{27}N_2O_5.H_2O$ (441.488): C, 65.29; H, 6.62; N, 6.34. Found: C, 65.76; H, 6.71; N, 6.01. HRMS (TOF ES MS) calcd for $C_{24}H_{27}N_2O_5Na$: 446.1818; found: 446.1819}.

Treatment of the Fmoc-(±)-*trans*-POAC-*O*-(a*R*)-binaphthol diastereomer **5b** (0.286 g; 0.41 mmol) under the same experimental conditions and work-up as above, gave pure Fmoc-POAC-OH (−)-*trans*-**4** (0.147 g; 84%) as a yellow solid, later assigned (see text) (3*S*,4*S*)-**4** {R_f 0.54 (CH_2Cl_2/MeOH 85:15). ^{1}H NMR (D_2O/acetone-d_6): see (±)-*trans*-**4** (Section 4.4). Mp 136–138 °C. $[\alpha]_{589}^{25}$ −63; $[\alpha]_{578}^{25}$ −64; $[\alpha]_{546}^{25}$ −107; $[\alpha]_{436}^{25}$ −122; $[\alpha]_{365}^{25}$ +254 (*c* 0.1, MeOH). ESI$^+$ MS *m/z* (%): 446 $[M+Na]^+$(100). Anal. Calcd for $C_{24}H_{27}N_2O_5$ (423.472): C, 68.06; H, 6.43; N, 6.61. Found: C, 68.06; H, 6.81; N, 6.07. HRMS (TOF ES MS) calcd for $C_{24}H_{27}N_2O_5Na$: 446.1818; found: 446.1828}.

4.7. Methyl 4-(amino)-1-oxyl-2,2,5,5-tetramethylpyrrolidine-3-carboxylate (+)-trans-6 and (−)-trans-6

To a solution of Fmoc-POAC-OH (+)-*trans*-**4** (44 mg; 0.104 mmol) and HOAt (29 mg; 0.213 mmol) in MeOH (3 mL) were added NMM (0.02 mL) and then EDC (30 mg; 0.156 mmol). The mixture was stirred at rt for 3 h and then concentrated under reduced pressure. The resulting residue was taken up in CH_2Cl_2. The solution was washed successively with a 0.5 N HCl solution, H_2O and a saturated $NaHCO_3$ solution. The organic phase was dried ($MgSO_4$), filtered and evaporated to give crude Fmoc-POAC-OMe (41 mg). This crude product was dissolved in CH_2Cl_2 (3 mL) and diethylamine (0.6 mL) was added. The solution was stirred at rt for 3 h and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with CH_2Cl_2/MeOH 95:5 as eluant to give 17 mg (76%) of H-POAC-OMe (+)-*trans*-**6** as a yellow solid, later assigned (see text) (3*R*,4*R*)-**6** {mp 42–44 °C. R_f 0.29 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR (D_2O/acetone-d_6):

δ 3.84–3.78 [m, 4H, OCH_3, C_3H], 3.05 [d, J=10.3 Hz, 1H, C_4H], 1.41 [s, 3H, CH_3], 1.36 [s, 3H, CH_3], 1.25 [s, 3H, CH_3] and 1.09 [s, 3H, CH_3]. ^{13}C NMR (D_2O/acetone-d_6): δ 173.4 [C=O], 65.4, 64.7 [C_2, C_5], 57.6 [C_3], 55.3 [C_4], 54.1 [OCH_3], 27.8, 25.7, 21.6, 21.3 [CH_3]. $[\alpha]_{589}^{25}$ +148; $[\alpha]_{578}^{25}$ +164; $[\alpha]_{546}^{25}$ +224 (c 0.22, CH_2Cl_2). ESI$^+$ MS m/z (%): 238 [M, Na]$^+$. HRMS (TOF ES MS) calcd for $C_{10}H_{19}N_2O_3Na$: 238.1293; found: 238.1288}.

In the same manner, to a solution of Fmoc-POAC-OH (−)-*trans*-**4** (120 mg; 0.280 mmol) and HOAt (77 mg; 0.560 mmol) in MeOH (9 mL) were added NMM (0.06 mL) and then EDC (81 mg; 0.425 mmol). The mixture was stirred at rt for 3 h and then concentrated under reduced pressure. The resulting residue was taken up in CH_2Cl_2. The solution was washed successively with a 0.5 N HCl solution, H_2O and a saturated $NaHCO_3$ solution. The organic phase was dried ($MgSO_4$), filtered and evaporated to give crude Fmoc-POAC-OMe (115 mg). This crude product was dissolved in CH_2Cl_2 (8 mL) and diethylamine (2 mL) was added. The solution was stirred at rt for 3 h and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with CH_2Cl_2/MeOH 95:5 as eluant to give 37 mg (62%) of H-POAC-OMe (−)-*trans*-**6** as a yellow solid, later assigned (see text) (3*S*,4*S*)-**6** {mp 43–44 °C. R_f 0.29 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR: see above. $[\alpha]_{589}^{25}$ −149; $[\alpha]_{578}^{25}$ −163; $[\alpha]_{546}^{25}$ −222 (c 0.22, CH_2Cl_2). ESI$^+$ MS m/z (%): 238 [M, Na]$^+$. HRMS (TOF ES MS) calcd for $C_{10}H_{19}N_2O_3Na$: 238.1293; found: 238.1301}.

4.8. Boc-Bip-(+)-(3R,4R)-POAC-OMe

To a suspension of Boc-Bip-OH[18b] (0.036 g; 0.10 mmol), the enantiomer H-POAC-OMe (+)-*trans*-**6** (0.020 g; 0.093 mmol) and HOAt (0.028 g; 0.20 mmol) in CH_2Cl_2 (4.0 mL) were added NMM (0.02 mL; 0.18 mmol) and then EDC (0.029 g; 0.15 mmol). The reaction mixture was stirred at rt for 48 h and evaporated in vacuo. The residue was solubilized in CH_2Cl_2 and the solution (ca. 100 mL) was washed successively with 0.5 N HCl (2×100 mL), H_2O (100 mL), 5% $NaHCO_3$ (100 mL) and H_2O (2×100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by preparative TLC on silica gel with CH_2Cl_2/MeOH 99:1 as eluant to afford 0.071 g (97%) of pure Boc-Bip-(+)-*trans*-POAC-OMe, later assigned Boc-Bip-(+)-(3*R*,4*R*)-POAC-OMe, as a yellow glass. R_f 0.39 (cyclohexane/EtOAc 2:1). $[\alpha]_{589}^{25}$ +25 (c 0.12, $CHCl_3$). ESI$^+$ MS (m/z): 573 [M, Na]$^+$. HRMS (TOF ES MS) calcd for $C_{31}H_{40}N_3O_6Na$: 573.2815; found: 573.2812.

4.9. Boc-Bip-(−)-(3S,4S)-POAC-OMe

To a suspension of Boc-Bip-OH (0.025 g; 0.072 mmol), the enantiomer H-POAC-OMe (−)-*trans*-**6** (0.012 g; 0.056 mmol) and HOAt (0.020 g; 0.14 mmol) in CH_2Cl_2 (2.5 mL) were added NMM (0.01 mL; 0.11 mmol) and then EDC (0.021 g; 0.11 mmol). The reaction mixture was stirred at rt for 48 h and evaporated in vacuo. The residue was solubilized in CH_2Cl_2 and the solution (ca. 100 mL) was washed successively with 0.5 N HCl (2×100 mL), H_2O (100 mL), 5% $NaHCO_3$ (100 mL) and H_2O (2×100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by preparative TLC on silica gel with CH_2Cl_2/MeOH 99:1 as eluant to afford 0.021 g (68%) of pure Boc-Bip-(−)-*trans*-POAC-OMe, later assigned Boc-Bip-(−)-(3*S*,4*S*)-POAC-OMe, as a yellow glass. R_f 0.39 (cyclohexane/EtOAc 2:1). $[\alpha]_{589}^{25}$ −27 (c 0.12, $CHCl_3$). ESI$^+$ MS (m/z): 573 [M+Na]$^+$. HRMS (TOF ES MS) calcd for $C_{31}H_{40}N_3O_6Na$: 573.2815; found: 573.2809.

4.10. Boc-Bip-(+)-(3R,4R)-POAC(Ac)-OMe

The dipeptide Boc-Bip-(+)-(*trans*)-POAC-OMe (0.030 g; 0.054 mmol) was dissolved in THF (4.0 mL) and 10% Pd/C (0.005 g) was added. The mixture was stirred under H_2 for 30 min. The reaction vessel was then flushed with Ar, and triethylamine (0.1 mL) then acetyl chloride (0.05 mL) were added. The mixture was stirred for 10 min and cyclohexane (10 mL) was added. The insoluble material was filtered through a cotton plug and the filtrate was evaporated in vacuo. The crude product was purified by preparative TLC on silica gel with cyclohexane/EtOAc 2:1 as eluant to afford 0.022 g (69%) of pure Boc-Bip-(+)-*trans*-POAC(Ac)-OMe, later assigned Boc-Bip-(+)-(3*R*,4*R*)-POAC(Ac)-OMe, as a foam. R_f 0.36 (cyclohexane/EtOAc 2:1). ^{1}H NMR ($CDCl_3$): δ 7.45–7.31 [m, 8H, CH^{Ar} Bip], 6.86 [br m, 1H, NH POAC], 4.81 [br m, 2H, NH Bip, H^4 POAC], 3.72 [br s, 4H, H^3 POAC and $COOCH_3$], 3.3–2.9 [br m, 2H, $ArCH_A^{\beta}$ and $ArC'H_A^{\beta}$ Bip], 2.8–2.6 [br m, 2H, $ArCH_B^{\beta}$ and $ArC'H_B^{\beta}$ Bip], 2.13 [s, 3H, $COCH_3$], 1.47 [s, 9H, CH_3 Boc], 1.33 [s, 6H, 2 CH_3 POAC], 1.17 [s, 3H, CH_3 POAC], 1.11 [s, 3H, CH_3 POAC]. ^{13}C NMR ($CDCl_3$): δ 170.3 [C=O Bip, C=O Ac, C=O POAC], 154.8 [C=O Boc], 140.5, 135.6, 134.7 [C^{Ar} Bip], 130.1, 129.8, 128.0, 128.0, 127.6, 127.4 [CH^{Ar} Bip], 80.4 [C–O Boc], 77.5, 77.4 [$C^{3,4}$ POAC], 69.9 [C^{α} Bip], 51.9 [OCH_3], 30.1 [CH_3 POAC], 28.1 [CH_3 Boc], 26.8 [CH_3 POAC], 18.9 [CH_3 Ac]. $[\alpha]_{589}^{25}$ −24; $[\alpha]_{578}^{25}$ −24; $[\alpha]_{546}^{25}$ −25; $[\alpha]_{436}^{25}$ −91; $[\alpha]_{365}^{25}$ −235 (c 0.4, MeOH). ESI$^+$ MS (m/z): 594 [M+H]$^+$. Anal. Calcd for $C_{33}H_{43}N_3O_7$ (593.698): C, 66.76; H, 7.30; N, 7.08. Found: C, 67.05; H, 7.78; N, 6.75.

4.11. Boc-Bip-(−)-(3S,4S)-POAC(Ac)-OMe

The dipeptide Boc-Bip-(−)-(*trans*)-POAC-OMe (0.020 g; 0.036 mmol) was dissolved in THF (3.0 mL) and 10% Pd/C (0.005 g) was added. The mixture was stirred under H_2 for 30 min. The reaction vessel was then flushed with Ar, and triethylamine (0.1 mL) then acetyl chloride (0.05 mL) were added. The mixture was stirred for 10 min and cyclohexane (10 mL) was added. The insoluble material was filtered through a cotton plug and the filtrate was evaporated in vacuo. The crude product was purified by preparative TLC on silica gel with cyclohexane/EtOAc 2:1 as eluant to afford 0.014 g (65%) of pure Boc-Bip-(−)-*trans*-POAC(Ac)-OMe, later assigned Boc-Bip-(−)-(3*S*,4*S*)-POAC(Ac)-OMe, as a foam. R_f

0.36 (cyclohexane/EtOAc 2:1). ^{1}H NMR and ^{13}C NMR ($CDCl_3$): same as above for Boc-Bip-(+)-(3*R*,4*R*)-POAC(Ac)-OMe. $[\alpha]_{589}^{25}$ +20; $[\alpha]_{578}^{25}$ +20; $[\alpha]_{546}^{25}$ +21; $[\alpha]_{436}^{25}$ +79; $[\alpha]_{365}^{25}$ +205 (*c* 0.2, MeOH). ESI$^+$ MS (*m/z*): 594 $[M+H]^+$. Anal. Calcd for $C_{33}H_{43}N_3O_7$ (593.698): C, 66.76; H, 7.30; N, 7.08. Found: C, 66.62; H, 7.94; N, 6.77.

4.12. Boc-(1S,2S)-ACPC-(3R,4R)-POAC-O-(aR)-binaphthol **7**

The diastereomer **5a** (204 mg; 0.29 mmol) was dissolved in CH_3CN (8 mL) and diethylamine (2 mL) was added. The mixture was stirred at rt for 3 h, then evaporated in vacuo. To the resulting crude product containing the N-deprotected amino ester H-(3*R*,4*R*)-POAC-(a*R*)-binaphthol (not isolated) were added Boc-(1*S*,2*S*)-ACPC-OH (56 mg; 0.24 mmol) and then THF (5 mL). The solution was cooled on an ice bath, and HATU (93 mg; 0.24 mmol) and DIEA (0.05 mL) were added. The mixture was stirred at rt for 18 h, then concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 and the organic phase was washed successively with a 0.5 M HCl solution, H_2O, a saturated $NaHCO_3$ solution, then dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was dissolved in a minimum of warm CH_2Cl_2 and diethyl ether was added. The mixture was left to stand at rt overnight. The resulting precipitate of dipeptide **7** (96 mg) was filtered off. The filtrate was concentrated and purified by preparative TLC on silica gel with CH_2Cl_2/EtOAc 8:2 as eluant to give a further 22 mg of dipeptide **7** (total 118 mg; 92%) as a solid {mp 254–256 °C (dec). R_f 0.51 (CH_2Cl_2/MeOH 95:5). $[\alpha]_{589}^{25}$ +87; $[\alpha]_{578}^{25}$ +96; $[\alpha]_{546}^{25}$ +123; $[\alpha]_{436}^{25}$ +149; $[\alpha]_{365}^{25}$ +305 (*c* 0.15, CH_2Cl_2). ESI$^+$ MS *m/z* (%): 703.4 $[M+Na]^+$. HRMS (TOF ES MS) calcd for $C_{40}H_{46}N_3O_7Na$: 703.3233; found: 703.3220}.

4.13. Boc-(1S,2S)-ACPC-(3R,4R)-POAC-OH **8**

To a solution of Boc-(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-*O*-(a*R*)-binaphthol **7** (100 mg, 0.147 mmol) in THF (6 mL), MeOH (2 mL) and H_2O (1 mL) was added sodium hydroxide (75 mg). The mixture was heated on a 50 °C bath for 3 h, then concentrated under reduced pressure, diluted with H_2O (3 mL) and neutralized by careful addition of a 1 N HCl solution. The solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by column chromatography on silica gel with CH_2Cl_2/MeOH 9:1 as eluant to afford 60 mg (96%) of dipeptide **8** as a solid {mp 159–161 °C (dec). R_f 0.62 (CH_2Cl_2/MeOH 85:15). $[\alpha]_{589}^{25}$ +32; $[\alpha]_{578}^{25}$ +36; $[\alpha]_{546}^{25}$ +50 (*c* 0.4, CH_2Cl_2). ESI$^+$ MS *m/z* (%): 435.2 $[M+Na]^+$; HRMS (TOF ES MS) calcd for $C_{20}H_{34}N_3O_6Na$: 435.2345; found: 435.2361}.

4.14. Boc-[(1S,2S)-ACPC]$_2$-OMe **9**

To an ice-cold solution of Boc-(1*S*,2*S*)-ACPC-OH (120 mg; 0.52 mmol) and HCl·H-(1*S*,2*S*)-ACPC-OMe[18b] (80 mg; 0.44 mmol) in THF (10 mL) were added HATU (197 mg; 0.52 mmol) and DIEA (0.15 mL). The mixture was stirred at rt for 48 h and then evaporated in vacuo. The residue was taken up in CH_2Cl_2 and the organic phase was washed successively with a 0.5 M HCl solution, H_2O, a saturated $NaHCO_3$ solution, then dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by column chromatography on silica gel with CH_2Cl_2/MeOH 95:5 as eluant to give 132 mg (85%) of dipeptide **9** as a solid {mp 168–170 °C. R_f 0.36 (CH_2Cl_2/MeOH 95:5). $[\alpha]_{589}^{25}$ +41; $[\alpha]_{578}^{25}$ +44; $[\alpha]_{546}^{25}$ +50; $[\alpha]_{436}^{25}$ +87; $[\alpha]_{365}^{25}$ +146 (*c* 0.25, CH_2Cl_2). ESI$^+$ MS *m/z* (%): 377.4 [M, Na]$^+$. HRMS (TOF ES MS) calcd for $C_{18}H_{30}N_2O_5Na$: 377.2052; found: 377.2072}.

4.15. Boc-(1S,2S)-ACPC-(3R,4R)-POAC-[(1S,2S)-ACPC]$_2$-OMe **10**

The dipeptide **9** (42 mg; 0.12 mmol) was dissolved in CH_2Cl_2 (3 mL), the solution was cooled on an ice bath, and a ca. 4 N solution of HCl in dioxane (1 mL) was added. The solution was magnetically stirred at 0 °C for 30 min, then at rt for 1 h and evaporated in vacuo at 30 °C. The residue was repeatedly triturated in CH_2Cl_2 and the suspension evaporated in vacuo. To the resulting crude HCl·H-[(1*S*,2*S*)-ACPC]$_2$-OMe were added successively Boc-(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-OH **8** (45 mg; 0.11 mmol), HATU (44 mg; 0.115 mmol) and THF (3 mL). The resulting suspension was cooled to 0 °C and DIEA (0.03 mL; 0.18 mmol) was added. The reaction mixture was magnetically stirred from 0 °C to rt for 48 h and then diluted with CH_2Cl_2 (100 mL). The solution was washed successively with 0.5 N HCl (50 mL), H_2O (50 mL), 5% $NaHCO_3$ (50 mL) and H_2O (100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by chromatography on a preparative TLC plate of silica gel with CH_2Cl_2/MeOH 95:5 as eluant to afford 57 mg (80%) of pure tetrapeptide **10** as a solid {mp 74–76 °C. R_f 0.36 (CH_2Cl_2/MeOH 95:5). $[\alpha]_{589}^{25}$ +103; $[\alpha]_{578}^{25}$ +119; $[\alpha]_{546}^{25}$ +144; $[\alpha]_{436}^{25}$ +137 (*c* 0.23, CH_2Cl_2). ESI$^+$ MS *m/z* (%): 671.4 [M, Na]$^+$. HRMS (TOF ES MS) calcd for $C_{33}H_{54}N_5O_8Na$: 671.3870; found: 671.3864}.

4.16. Fmoc-(3R,4R)-POAC-(1S,2S)-ACPC-OMe **11**

To an ice-cold solution of (+)-(3*R*,4*R*)-**4** (40 mg; 0.094 mmol) and HCl·H-(1*S*,2*S*)-ACPC-OMe[18b] (20 mg; 0.11 mmol) in THF (2 mL) were added HATU (40 mg; 0.105 mmol) and DIEA (0.050 mL). The mixture was stirred at 0 °C to rt for 20 h and then evaporated in vacuo. The residue was taken up in CH_2Cl_2 and the organic phase was washed successively with a 0.5 M HCl solution, H_2O, a saturated $NaHCO_3$ solution, then dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by chromatography on a preparative TLC plate of silica gel with CH_2Cl_2/MeOH 97:3 as eluant to give 32 mg (62%) of pure dipeptide **11** as a solid {mp 152–154 °C. R_f 0.39 (CH_2Cl_2/MeOH 97:3). $[\alpha]_{589}^{25}$ +96; $[\alpha]_{578}^{25}$ +111; $[\alpha]_{546}^{25}$ +135; $[\alpha]_{436}^{25}$ +71; $[\alpha]_{365}^{25}$ −91 (*c* 0.20, CH_2Cl_2). ESI$^+$ MS *m/z* (%): 571.4 [M, Na]$^+$. HRMS

(TOF ES MS) calcd for $C_{31}H_{38}N_3O_6Na$: 571.2658; found: 571.2665}.

4.17. Boc-[(1S,2S)-ACPC-(3R,4R)-POAC-(1S,2S)-ACPC]$_2$-OMe **12**

To a solution of tetrapeptide **10** (35 mg, 0.054 mmol) in THF (2 mL), MeOH (1 mL) and H_2O (0.5 mL) was added sodium hydroxide (20 mg). The mixture was stirred at 50 °C overnight, then concentrated under reduced pressure, diluted with H_2O (3 mL) and neutralized by careful addition of a 0.5 N HCl solution. The solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried ($MgSO_4$), filtered and evaporated in vacuo to afford crude Boc-(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-[(1*S*,2*S*)-ACPC]$_2$-OH (34 mg; 0.054 mmol). In parallel, the dipeptide **11** (35 mg, 0.064 mmol) was dissolved in CH_3CN (2 mL) and diethylamine (0.2 mL) was added. The mixture was stirred at rt for 3 h, then evaporated in vacuo. The resulting crude H-(3*R*,4*R*)-POAC-(1*S*,2*S*)-ACPC-OMe was dissolved in THF (2 mL) and the solution added to the residue of crude Boc-(1*S*,2*S*)-ACPC-(3*R*,4*R*)-POAC-[(1*S*,2*S*)-ACPC]$_2$-OH. The resulting solution was cooled to 0 °C and HATU (24 mg; 0.064 mmol) was added, followed by DIEA (0.010 mL). The mixture was stirred at 0 °C for 2 h, at rt for six days, and then evaporated in vacuo. The residue was taken up in CH_2Cl_2 and the organic phase was washed successively with a 0.5 M HCl solution, H_2O, a saturated $NaHCO_3$ solution, then dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by chromatography on a preparative TLC plate of silica gel with CH_2Cl_2/MeOH 92.5:7.5 as eluant to afford 26 mg (51%) of pure hexapeptide **12** as a solid {mp 196–198 °C. R_f 0.42 (CH_2Cl_2/MeOH 97.5:7.5). $[\alpha]_{589}^{25}$ +128; $[\alpha]_{578}^{25}$ +140; $[\alpha]_{546}^{25}$ +181; $[\alpha]_{436}^{25}$ +146; (*c* 0.13, CH_2Cl_2). ESI$^+$ MS *m/z* (%): 966.0 (calcd 965.6) [M, Na]$^+$. Anal. Calcd for $C_{48}H_{78}N_8O_{11}$ (943.168): C, 61.12; H, 8.34. Found: C, 61.54; H, 8.71. HRMS (TOF ES MS) calcd for $C_{48}H_{78}N_8O_{11}Na$: 965.5688; found: 965.5748}.

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