Pergamon

SCIENCE DIRECT®

Tetrahedron: *Asymmetry* 14 (2003) 1879–1893

TETRAHEDRON: *ASYMMETRY*

Synthesis and conformational study of homo-peptides based on (*S*)-Bin, a C_2-symmetric binaphthyl-derived $C^{\alpha,\alpha}$-disubstituted glycine with only axial chirality

Jean-Paul Mazaleyrat,[a] Karen Wright,[a] Anne Gaucher,[a] Michel Wakselman,[a] Simona Oancea,[b] Fernando Formaggio,[b] Claudio Toniolo,[b,*] Vladimir Setnička,[c] Josef Kapitán[c] and Timothy A. Keiderling[c]

[a]*SIRCOB, UMR CNRS 8086, Bât. Lavoisier, University of Versailles, F-78035 Versailles, France*
[b]*Institute of Biomolecular Chemistry, CNR, Department of Organic Chemistry, University of Padua, I-35131 Padua, Italy*
[c]*Department of Chemistry, University of Illinois at Chicago, Chicago, IL 60607-7061, USA*

Received 21 March 2003; accepted 1 April 2003

Abstract—We synthesised the $[(S)\text{-Bin}]_n$ ($n=2$–5) homo-peptides by solution methods. Only the acid fluoride derivative Fmoc-(*S*)-Bin-F could be efficiently coupled with H-$[(S)\text{-Bin}]_n$-OMe ($n=2$–4). Spectroscopic experimental analyses of the $[(S)\text{-Bin}]_n$ homo-peptides in solution performed by CD, FT-IR absorption, and VCD, supported by VCD calculations, confirmed the (*S*) configuration of the Bin residues and identified the most largely populated secondary structures as right-handed β-turns which eventually evolve to 3_{10}-helices in the highest oligomers.

1. Introduction

The conformationally constrained $C^{\alpha,\alpha}$-symmetrically disubstituted glycines are among the simplest and most widely used structural units in the construction of peptides with a predetermined secondary structure.[1] In particular, it has been shown that peptides based on $C^{\alpha,\alpha}$-dibenzylglycine (Db_zg)[2] (Fig. 1) strongly prefer to adopt a fully-extended (C_5) conformation,[1k,1l] whereas those rich in 1-aminocycloheptane-1-carboxylic acid (Ac_7c) [3] exhibit a very high tendency to fold into β-turns and 3_{10}-helices.[1d] A conformational preference for β-turns and 3_{10}-helical structures, in the crystal state as well as in solution, was also demonstrated for a large set of model peptides based on 2′,1′:1,2;1″,2″:3,4-dibenzcyclohepta-1,3-diene-6-amino-6-carboxylic acid (Bip)[4,5] and 2′,1′:1,2;1″,2″:3,4-dinaphthcyclohepta-1,3-diene-6-amino-6-carboxylic acid (Bin)[4,6] (Fig. 1), in combination with Ala, Gly or Aib (α-aminoisobutyric acid) residues. The $[Bip]_n$ homo-oligomers are also folded into 3_{10}-helices.[5] Herein we report the synthesis of the corresponding $[(S)\text{-Bin}]_n$ homo-peptides to the pentamer level and their conformational study by electronic CD, FT-IR absorption, and VCD (vibrational CD) spectroscopy. The Bip and Bin residues combine structural features of Db_zg and Ac_7c. Both are C_2-symmetric and possess a chiral axis (atropoisomerism) along their 1-1′ bond.

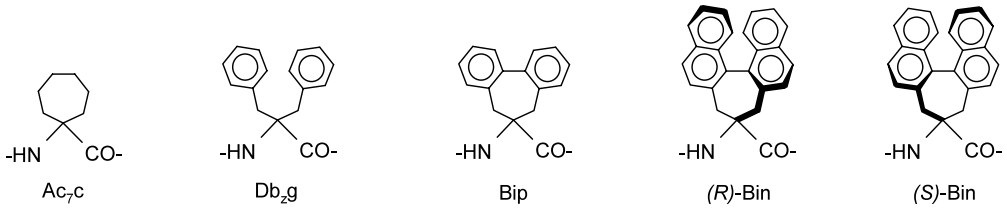

Figure 1. Chemical structures of the axially chiral (*R*)-Bin and (*S*)-Bin residues, compared to those of Ac_7c, Db_zg and Bip.

* Corresponding author. Fax: (+39)-049-827-5239; e-mail: claudio.toniolo@unipd.it

0957-4166/03/$ - see front matter
doi:10.1016/S0957-4166(03)00285-4

However, in contrast with the Bip residue, which is conformationally labile with non-isolable (*R*) and (*S*) interconverting enantiomers resulting from rotation along the 1-1′ bond of the biphenyl moiety (the rotational energy barrier is about 14 kcal mol^{-1}),[4b,5b] the Bin residue can be isolated in an enantiomerically pure state and has a very high enantiomeric stability,[4b,6] as expected.[7g] A variety of similar 2,2′-disubstituted 1,1′-binaphthyl systems with *highly stable*, *chiral*, *C_2-symmetric configurations* have been extensively exploited as chiral auxiliaries in enantioselective organic synthesis, in the preparation of chiral hosts for molecular recognition studies, and in the construction of new materials.[7] In this connection our targeted $[(S)\text{-Bin}]_n$ homo-peptides present several interesting properties: (i) access to a new class of 1,1′-binaphthyl oligomers as *potential chiral catalysts*[7g] *and photoactive fluorophores*,[8] (ii) *synthetic challenge* concerning the coupling of highly hindered Bin residues, (iii) *conformational study of unique peptide homo-oligomers with only axial chirality*, more specifically determination of their preferred secondary structure and its comparison with that of the conformationally labile $[\text{Bip}]_n$ series previously investigated,[5] and (iv) analysis of the relationship between *binaphthyl axial chirality* and *peptide helix handedness*.

2. Results

2.1. Synthesis

Our initial strategy was to apply the same synthetic methodology previously employed with success for the synthesis of the Z-$[\text{Bip}]_n$-O*t*Bu (Z, benzyloxycarbonyl; O*t*Bu, *tert*-butoxy) series,[5b] i.e. the use of the pivaloyl mixed anhydride coupling method for the homo-dipeptide $[(S)\text{-Bin}]_2$ and then successive C-deprotections and couplings from the N-terminus involving carboxyl group activation through the 5(4*H*)-oxazolone intermediates.[9] Therefore, our starting material, the amino ester H-(*S*)-Bin-OMe (OMe, methoxy) **1a** (Fig. 2), previously obtained in an enantiomerically pure state by resolution,[6a] was first treated with *N*-(benzyloxycarbonyloxy) succinimide (Z-OSu) in acetonitrile at room temperature to give Z-(*S*)-Bin-OMe **1b** (87%). Saponification of the ester function of **1b** with a large excess of aqueous 1N NaOH in MeOH, conducted for 24 h at ca 75°C, a relatively high temperature usually required for basic hydrolysis of $C^{\alpha,\alpha}$-disubstituted amino esters,[4b,10] afforded the N-protected amino acid Z-(*S*)-Bin-OH **1c**[4b] in high yield (91%).

Activation of **1c** as its pivaloyl (Piv) mixed anhydride Z-(*S*)-Bin-OPiv (not isolated), followed by reaction with H-(*S*)-Bin-OMe **1a** in toluene at 60°C for 18 h,[5b,9] led to the dipeptide Z-$[(S)\text{-Bin}]_2$-OMe **2b** in 59% yield (Fig. 3). Interestingly, the yield could be raised to 84% under even simpler experimental conditions, by applying the CIP (2-chloro-1,3-dimethylimidazolidinium hexafluorophosphate)/HOAt (7-aza-1-hydroxy-1,2,3-benzotriazole) coupling procedure.[11]

Saponification of the methyl ester function of **2b**, which was expected to present some difficulties,[9b] failed in our hands to afford the desired *N*-protected dipeptide Z-$[(S)\text{-Bin}]_2$-OH **2c**. No reaction occurred using aqueous 1N NaOH in MeOH at 60°C for 8 h, while either under similar experimental conditions with dioxane and toluene added for complete solubilisation, or when using tetrabutylammonium hydroxide as a base,[12] only unidentified decomposition products were obtained. Therefore, we turned to an alternative strategy of couplings from the C-terminus, starting with *N*-deprotection of **2b** by hydrogenolysis, which gave H-$[(S)\text{-Bin}]_2$-OMe **2a** in 96% yield (Fig. 3).

However, when coupling of **1c** with **2a** by the CIP/HOAt method, successfully employed for the coupling of **1c** with **1a** (vide supra), was attempted, only recov-

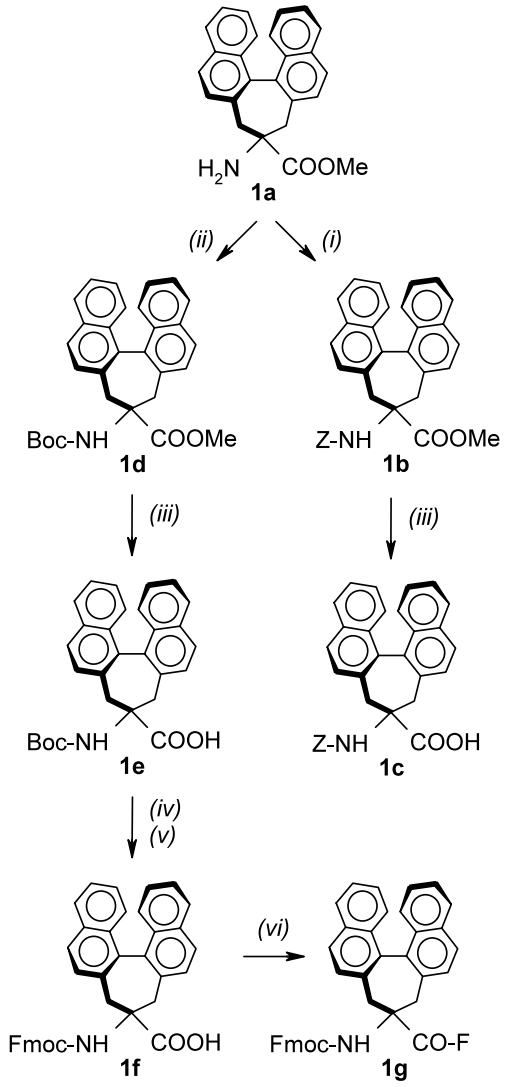

Figure 2. Synthesis of the activated and/or protected (*S*)-Bin residues used as building blocks, from H-(*S*)-Bin-OMe **1a**. (i) Z-OSu; CH_3CN; rt; 48 h; (ii) Boc_2O; CH_3CN; rt; 3 days; (iii) aq. 1N NaOH; MeOH; 75°C; 24 h; (iv) TFA/CH_2Cl_2 1:1; rt; 4 h; (v) Fmoc-OSu; DIEA; CH_3CN/CH_2Cl_2; 60°C; 10 h; (vi) cyanuric fluoride; pyridine; CH_2Cl_2; −15°C (1 h) to rt; 3 h.

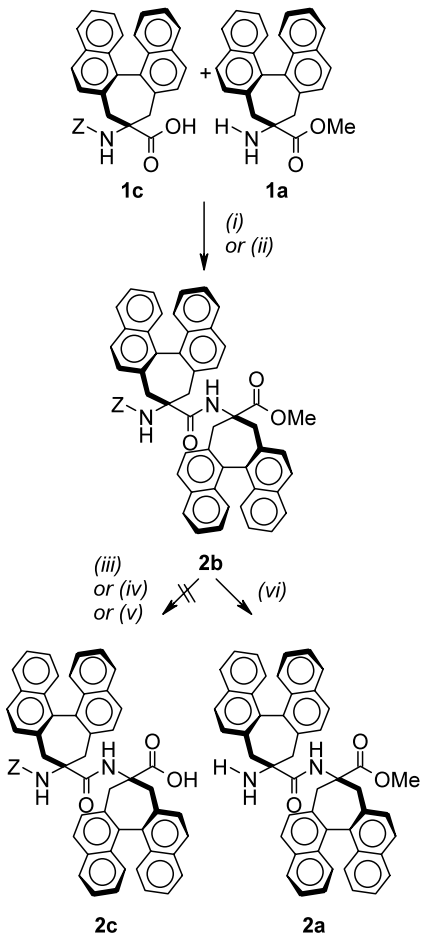

Figure 3. Synthesis of the protected [(*S*)-Bin]$_2$ dipeptides. (i) (1) TEA; Piv-Cl; toluene; −10°C to rt; 3h, (2) **1a**; toluene; 60°C; 18 h, (ii) CIP; HOAt; DIEA; CH_2Cl_2; rt; 3 days, (iii) aq. 1N NaOH; MeOH; 60°C; 8 h, (iv) aq. 2N NaOH; MeOH; toluene; dioxane; 70°C; 8 h, (v) nBu$_4$N$^+$OH$^-$; THF; rt; 4 days, (vi) H_2/Pd-C; MeOH; rt; 5 h.

ered **2a** (40%) and several unidentified compounds could be isolated, but no desired tripeptide Z-[(*S*)-Bin]$_3$-OMe. Finally, according to the well demonstrated efficiency of the amino acid fluoride activation for the coupling of hindered α-amino acids,[13] particularly those involving consecutive C$^{\alpha,\alpha}$-disubstituted glycines,[14,15] we considered the use of Fmoc-(*S*)-Bin-F (Fmoc, fluoren-9-ylmethyloxycarbonyl) **1g** (Fig. 2).[16] This compound was readily synthesised from resolved H-(*S*)-Bin-OMe **1a**, which was first *N*-protected by means of Boc_2O (Boc, *tert*-butyloxycarbonyl) in CH_3CN at room temperature, to give Boc-(*S*)-Bin-OMe **1d** (86%).[17] The ester function of **1d** was saponified as above for **1b** (Fig. 2), to afford Boc-(*S*)-Bin-OH **1e**[4b] in 94% yield. The Boc group of **1e** was then cleaved by trifluoroacetic acid (TFA) in CH_2Cl_2 and the resulting crude H-(*S*)-Bin-OH·TFA was *N*-acylated with Fmoc-OSu in the presence of a large excess of DIEA (*N*,*N*-di*iso*propylethylamine), in CH_3CN and CH_2Cl_2 at 60°C for several hours, to afford Fmoc-(*S*)-Bin-OH **1f** in 74% yield. Treatment of a solution of **1f** and pyridine in CH_2Cl_2 by cyanuric fluoride[13–15] at −15°C to room temperature, gave Fmoc-(*S*)-Bin-F **1g** in 58% yield.

Because of its low stability, the acid fluoride **1g** was purified only on one occasion by chromatography and then characterised by ^{1}H NMR. In the next coupling steps it was always freshly prepared in large excess relative to the amino component, identified by TLC and the crude solution used directly. Thus, coupling of crude **1g** (ca 2 equiv.) with the dipeptide **2a** gave Fmoc-[(*S*)-Bin]$_3$-OMe **3h** in 51% yield (Fig. 4). *N*-Deprotection of the Fmoc group with a 10% solution (vol/vol) of diethylamine (Et_2NH) in CH_3CN at room temperature for a few hours led to H-[(*S*)-Bin]$_3$-OMe **3a** in 73% yield. In the same manner, acylation of **3a** by crude **1g** (ca 3 equiv.) gave Fmoc-[(*S*)-Bin]$_4$-OMe **4h** in 66% yield. However, for unclear reasons, when H-[(*S*)-Bin]$_4$-OMe **4a**, obtained in 87% yield after cleavage of

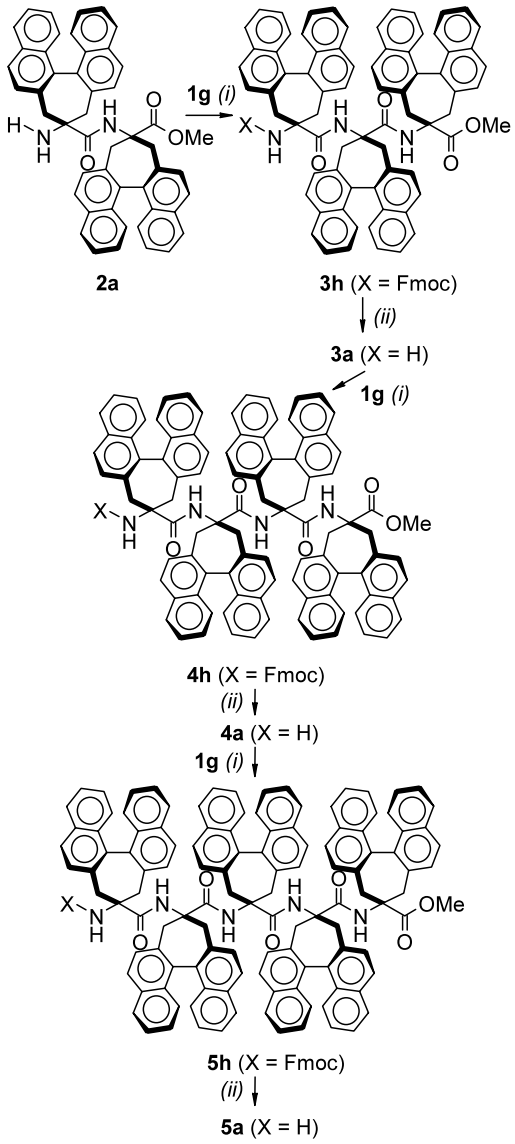

Figure 4. Synthesis of the protected homo-peptides Fmoc-[(*S*)-Bin]$_n$-OMe and H-[(*S*)-Bin]$_n$-OMe (n = 3–5). (i) NMM; CH_2Cl_2; rt; 10–14 days, (ii) 10% Et_2NH in CH_3CN; rt.

the Fmoc protecting group of **4h**, was reacted with crude **1g** (ca 3 equiv.), the desired pentapeptide Fmoc-[(*S*)-Bin]$_5$-OMe **5h** was isolated in only 18% yield. The deprotected amino ester H-[(*S*)-Bin]$_5$-OMe **5a** was obtained from **5h** in 38% yield, but chain elongation could not be pursued because of the lack of a sufficient amount of pentamer.

For the longest, N$^{\alpha}$-protected (Fmoc) or unprotected peptides Fmoc(or H)-[(*S*)-Bin]$_n$-OMe ($n=3$–5), all homogeneous by TLC, ^{1}H NMR was not informative for structural identification. However, the expected chemical structures were clearly confirmed by ES$^+$ MS of the Fmoc derivatives, with a single peak of correct m/z $(M+H)^+$ seen in each spectrum (Fig. 5).

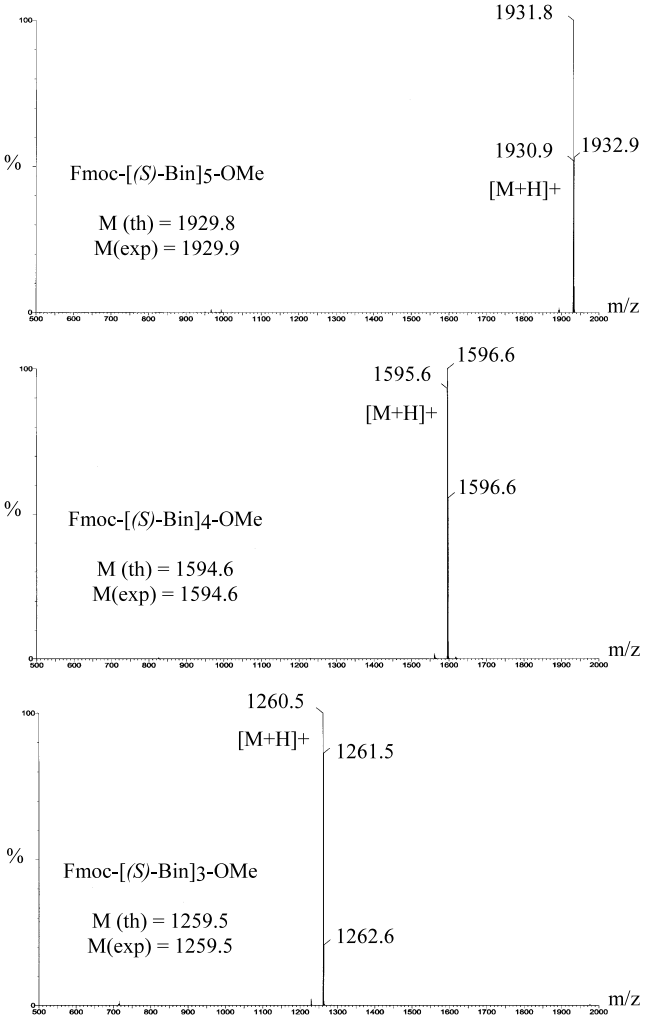

Figure 5. Mass spectra (ES$^+$) of the homo-peptides Fmoc-[(*S*)-Bin]$_n$-OMe ($n=3$–5).

2.2. Solution conformational analysis

The conformational preferences of the (*S*)-Bin homo-peptides were evaluated in structure supporting solvents (MeOH, $CHCl_3$ and $CHCl_3$/TFE mixtures) (TFE is 2,2,2-trifluoroethanol) by electronic CD, FT-IR absorption, and VCD techniques.

In Figure 6 the electronic CD spectra of the terminally protected (*S*)-Bin lowest homo-oligomers are reported. As already shown with simple Bin model peptides,[6b] the Cotton effects are extremely intense and dominated by the contribution of the split ^1B_b band ($\pi\rightarrow\pi^*$ transition) of the binaphthyl chromophore.[18] For this transition a positive band at longer wavelength ($\cong$232 nm) and a negative band at shorter wavelength ($\cong$218 nm) are typical of a binaphthyl moiety of (*S*) absolute configuration. No information can be extracted on the peptide secondary structure preference nor on the most populated screw sense of the putative turn/helix conformation. The spectra of the longest oligomers ($n=4$ and 5) are not shown in Figure 6 in view of the limited solubility of these two peptides in MeOH. In any case, qualitatively, their CD patterns closely resemble those of the lowest oligomers. Removal of the Z or Fmoc N$^{\alpha}$-protection does not change the overall shape of the CD curves [in the 1:9 (v/v) $CHCl_3$/TFE mixture, for solubility reasons; spectra not shown].

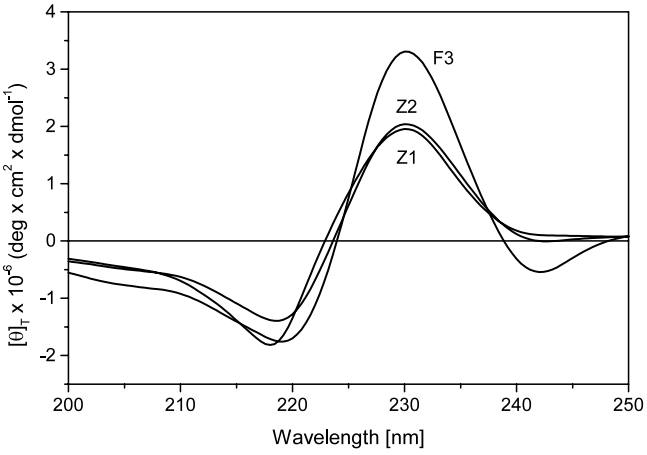

Figure 6. CD spectra in the 200–250 nm region of Z-(*S*)-Bin-OMe (Z1), Z-[(*S*)-Bin]$_2$-OMe (Z2), and Fmoc-[(*S*)-Bin]$_3$-OMe (F3) in MeOH solution. Peptide concentration: 1.0 mM.

The FT-IR absorption spectra of the N$^{\alpha}$-deprotected di- ($n=2$), tri- ($n=3$), and tetrapeptide ($n=4$) esters in the 98:2 (v/v) $CDCl_3$/TFE-OD solvent mixture over the spectral region 1780–1840 cm^{-1} are shown in Figure 7. The band at 1737–1739 cm^{-1} is assigned to the ν(C=O) vibration of the C-terminal protecting ester group. The amide I (C=O stretch) bands lie between 1700 and 1640 cm^{-1} with maxima at 1675, 1678, and 1669 cm^{-1} for $n=2$, 3, and 4, respectively. Contrary to what one expects for a uniform helical (or any repeating) peptide structure, the amide I absorption band for this series broadens with increasing peptide chain length and, most importantly, three distinct components are observed for the tetramer. Such a pattern indicates a dominant contribution from non-uniform secondary structures.[19]

To get another point of view from the IR absorption results, the spectra in the N–H stretching (amide A) region are shown in Figure 8 for the N- and C-protected peptide series in $CDCl_3$ solution. The spectra of

the highest oligomers are characterised by main bands at 3440–3428 cm^{-1} (free, solvated N–H groups) and at 3360–3348 cm^{-1} (H-bonded N–H groups).[20] The intensity of the low-frequency band relative to the high-frequency band increases as the peptide main chain is elongated. This effect is quite remarkable on going from the dimer to the trimer. We were also able to demonstrate that, even at 10 mM concentration, only quite limited changes take place in the spectra of the various oligomers. Therefore, the band at 3360–3348 cm^{-1} should be interpreted as arising almost exclusively from intramolecular C=O···H–N interactions.

This study provided convincing evidence that main-chain length dependent intramolecular H-bonding is a factor of paramount importance influencing the conformation of (*S*)-Bin peptides in $CDCl_3$ solution. The dramatic enhancement in the intensity of the band of H-bonded N–H groups from dimer to trimer is a strong indication of the onset of a significant population of β-turns in the conformational equilibrium mixture of the latter peptide. It is reasonable to assume that this propensity will propagate into a 3_{10}-helix[1d] for the longer oligomers, characterised by a steady increase in the intensity of the same band. However, the occurrence of other bands in the tetramer and pentamer points to the co-existence of other conformations in these peptides. More specifically, we observe concentration-independent shoulders at 3387–3388 cm^{-1}, present in both compounds but more evident in the tetramer and usually assigned to the fully-extended (C_5) conformation,[1k,11,19] and at about 3320 cm^{-1} in the pentamer, probably related to a different type of folded conformation.

The side-chain contributions to the spectra are evident in the strong IR absorption band at 1510 cm^{-1} (Fig. 7) consisting mainly of ν(C=C) stretching and δ(C–H) bending modes of the aromatic rings, which are overlapped by the broad amide II band to higher frequency. Other binaphthyl ν(C=C) vibrations are seen at 1620 and 1595 cm^{-1}. These assignments are confirmed by a comparison of the H-[(*S*)-Bin]$_4$-OMe experimental IR absorption and VCD spectra [again in the 98:2 (v/v) $CDCl_3$/TFE-OD solvent mixture] to the calculated spectra for just the binaphthyl side chains (Fig. 9). The VCD sign patterns and frequencies agree very well for these binaphthyl centred modes [especially taking into account the typical deviation to higher values for density functional theory (DFT) force fields (FFs)]. The VCD spectra of the $n=2$ and 3 peptides (data not shown) reveal almost the same shape, positions, and intensities for VCD bands in the region of 1620–1400 cm^{-1} as are shown for $n=4$ in Figure 9. The negative signs of the 1501 and 1465 cm^{-1} VCD bands are common to all these cases and agree well with those of the calculated spectra. Finally, we note that the amide I VCD is a *positive* couplet (centred at about 1670 cm^{-1}), a sign pattern normally associated with a *right* handed helical conformation (see Fig. 9).[21]

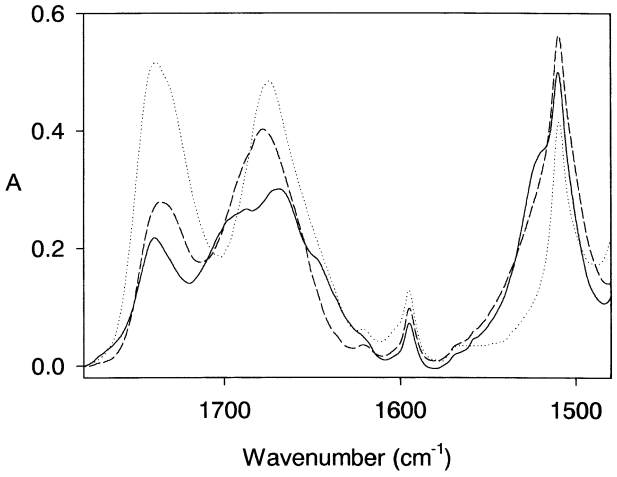

Figure 7. FT-IR absorption spectra (1780–1480 cm^{-1}) in the 98:2 (v/v) $CDCl_3$/TFE-OD solvent mixture of the H-[(*S*)-Bin]$_n$-OMe homo-peptides: $n=2$ (dotted line), $n=3$ (dashed line), $n=4$ (full line). Peptide concentration: 18 mg mL^{-1}.

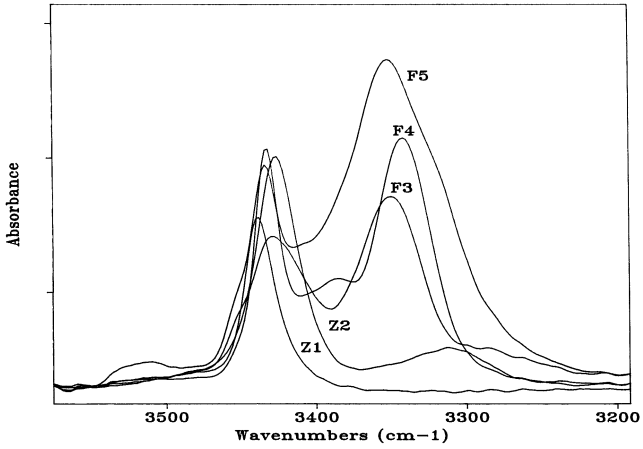

Figure 8. FT-IR absorption spectra (3550–3200 cm^{-1} region) in $CDCl_3$ solution of Z-(*S*)-Bin-OMe (Z1), Z-[(*S*)-Bin]$_2$-OMe (Z2), Fmoc-[(*S*)-Bin]$_3$-OMe (F3), Fmoc-[(*S*)-Bin]$_4$-OMe (F4), and Fmoc-[(*S*)-Bin]$_5$-OMe (F5). Peptide concentration: 1.0 mM.

2.3. Computational modelling

The fully optimised structure of the binaphthyl side chain was obtained as a single geometry minimum due to the bridge between the 1,1′-positions. IR absorption and VCD spectra computed for this side chain with or without methyl groups on the bridge 6-carbon atom were nearly identical (aside from specific -CH_3 modes) and agree well with observed spectra which do not change much among the oligopeptides studied in the 1620–1400 cm^{-1} region, suggesting that there is not strong intramolecular stacking interaction between binaphthyl aromatic side chains (Fig. 9). Lack of interactions justifies our calculating molecular property tensors for peptide backbone and aromatic side chains separately and suggests that transfer of the Cartesian FF, APT (atomic polar tensor) and AAT (atomic axial tensor) values from these segments onto the final tetrapeptide geometry will be satisfactorily accurate.

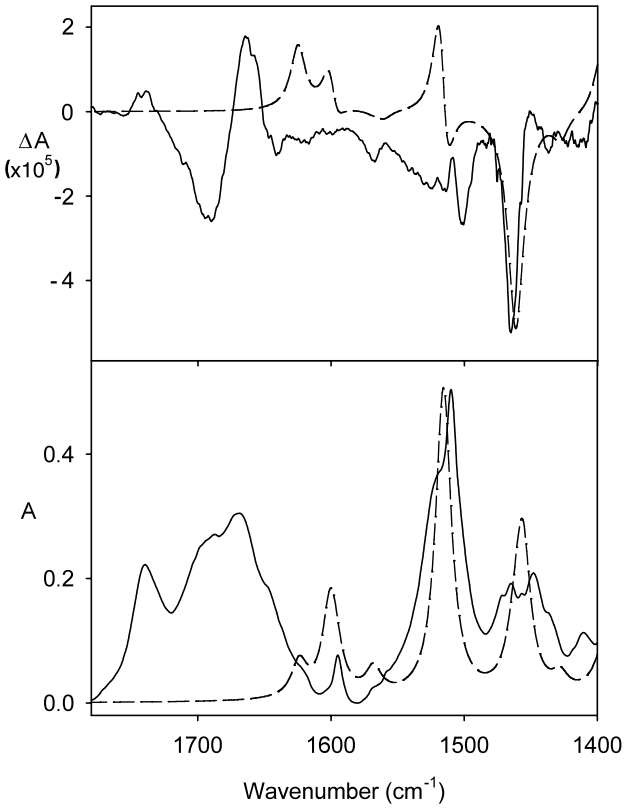

Figure 9. Experimental (full lines) VCD (upper frame) and FT-IR absorption (lower frame) spectra (1780–1400 cm^{-1}) in the 98:2 (v/v) $CDCl_3$/TFE-OD solvent mixture of H-[(*S*)-Bin]$_4$-OMe (peptide concentration: 18 mg mL^{-1}) and calculated spectra (dashed lines) of the simplified model compound 2′,1′:1,2:1″,2″:3,4-dinaphthcyclohepta-1,3-diene.

To find possible low-energy conformations of the H-[(*S*)-Bin]$_4$-OMe molecule, a series of molecular mechanics (MM) energy minimisation calculations were started from various regular geometries, including α-, 3_1 [poly (Pro)$_n$ II] and π-helices for both left- and right-handed forms. The final conformational features are mainly determined by the relatively large side chains, which, due to steric interference, prevent formation of some regular structures, such as *regular* 3_{10}-helix and β-sheet structures. To address this problem, some starting structures were based on *distorted* conformations, including β-sheet structures and 3_{10}-helices with ends distorted so that the side chains do not collide. Finally, simulations for H-[(*S*)-Bin]$_4$-OMe, started as β-turns of type I, II, I′ and II′, were allowed to minimise the energy by geometry optimisation. MM simulations were performed in both CFF91 and CVFF force fields (they were carried out using both the Cerius2 and InsightII programs). Due to small differences in the convergence algorithms and the implementation of the parametre sets, most of these conformations converged to slightly different minima with each method. All the minimised conformations differed from the starting geometries, the final states tending to have irregular structures and often developing distorted ends, with relative energies that lie within a range of 10 kcal mol^{-1} for CFF91 and 18 kcal mol^{-1} for CVFF (Table 1). These findings are close to the calculation error (as judged by the variation between methods), so it is not possible to reliably predict a single conformation that is most favourable from the MM results alone. However, some conformations were consistently low in all the MM calculations, and those were the focus of our follow-up spectral simulations.

Some binaphthyl stacking interactions were also observed, this being most apparent for distorted strand structures started from the β-sheet. This unique structure had also the lowest energy among all conformations, but was so 'encased' in interacting side chains, that only single-stranded species would be possible, thus effectively preventing H-bond formation between strands, but allowing two H-bonded 7-membered rings to form within the strand. Other promising low-energy geometries were the right-handed 3_1-helix, and left- and right-handed, distorted 3_{10}-helices. These conforma-

Table 1. MM minimised energies for H-[(*S*)-Bin]$_4$-OMe using the Cerius2 and InsightII programs

Starting conformation[a]	Force field[b]			
	Cerius2		InsightII	
	CFF91	CVFF300	CFF91	CVFF
α-Helix [right handed (RH)]	5	19	15	26
α-Helix [left handed (LH)]	7	1	9	16
Poly (Pro)$_n$ II (RH)	−1	4	6	12
Poly (Pro)$_n$ II (LH)	2	4	7	17
π-Helix (RH)	5	14	15	26
π-Helix (LH)	2	11	10	18
β-Turn I[22]	9	11	14	23
β-Turn I′	8	9	12	19
β-Turn II	3	1	12	21
β-Turn II′	7	10	12	23
Modified structures				
β-Sheet: $\psi_1=-29$, $\phi_4=-157$	6	6	7	17
β-Sheet: $\psi_1=151$, $\phi_4=-157$	2	12	11	23
β-Sheet: $\psi_1=-29$, $\phi_4=23$	3	5	0	0
β-Sheet: $\psi_1=151$, $\phi_4=23$	1	15	4	22
3_{10}-Helix 1 RH: $\psi_1=-116$	1	16	10	21
3_{10}-Helix 1 LH: $\psi_1=60$	8	4	9	16
3_{10}-Helix 2 RH: $\psi_1=30$	0	0	8	14
3_{10}-Helix 2 LH: $\psi_1=-90$	2	9	5	12

[a] Although all MM calculations in a given row were started with the same geometries, the final structures with different FF were not always the same (thus, comparison can be difficult). Therefore, the list primarily serves to identify low-energy conformations.

[b] Relative molecular energies in [kcal mol^{-1}].

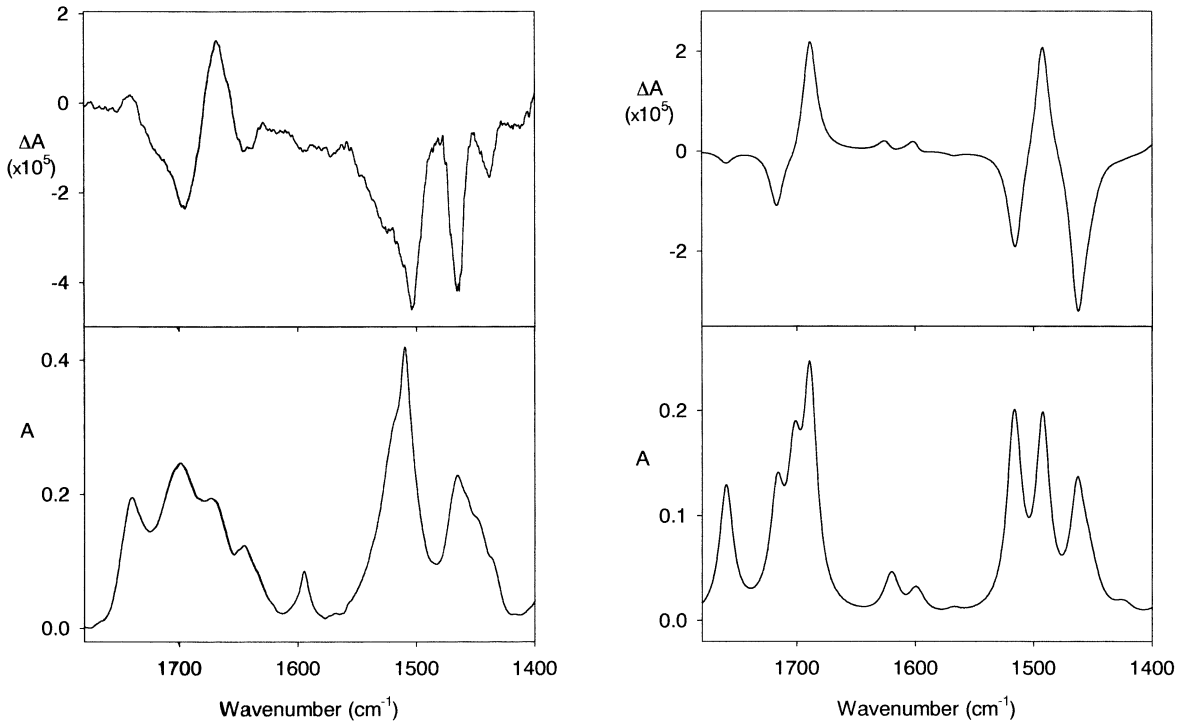

Figure 10. Comparison of experimental (left) and calculated (right) VCD (top) and IR absorption (bottom) spectra (1780–1400 cm^{-1}) of H-[(*S*)-Bin]$_4$-OMe. Experimental spectra were measured in $CDCl_3$ solution at a peptide concentration of 18 mg mL^{-1}.

tions were sources for the ϕ, ψ torsion angles used to constrain the DFT calculation of the backbone of the model tetrapeptide H-(Aib)$_4$-OMe, while all other internal coordinates (including the ω torsion angles) were relaxed. Then, the geometry of H-[(*S*)-Bin]$_4$-OMe for simulations was obtained by overlapping the H-(Aib)$_4$-OMe backbone and the side-chain binaphthyls, both optimised on the same DFT level, and eliminating the redundant atoms. Cartesian force field and intensity tensors of backbone and side chains were then transferred to the final geometry.

The best agreement with experiment is found for one of the consistently low-energy structures, the right-handed 3_{10}-helix, whose choice is justified below. Figure 10 shows a comparison of the experimental IR absorption and VCD spectra of H-[(*S*)-Bin]$_4$-OMe in $CDCl_3$ with the results of the simulated spectra in a distorted, right-handed 3_{10}-helical structure. The shape and the band assignment of the IR absorption spectrum are similar to those presented in Figure 9. There is a clear positive couplet for the amide I VCD and a large negative VCD correlated to the amide II. This latter is discriminated from the binaphthyl modes by both the calculated results and the lower VCD intensity seen experimentally for the amide II mode when the molecule is partially deuterated (as also seen in Figure 9). Table 2 lists the MM energy-optimised ϕ, ψ, and ω torsion angles for this most promising 3_{10}-helical conformation.

Table 2. Backbone torsion angles for H-[(*S*)-Bin]$_4$-OMe in a distorted right-handed 3_{10}-helix[a]

Residue	ϕ (°)[b]	ψ (°)[b]	ω (°)[c]
(*S*)-Bin[1]		14.9	−160.8 (−167.1)
(*S*)-Bin[2]	−57.7	−35.0	−171.8 (−180.0)
(*S*)-Bin[3]	−64.5	−13.6	−173.4 (175.5)
(*S*)-Bin[4]	−1.3	−82.6	177.9 (178.3)

[a] Structure obtained from MM with the CFF force field.
[b] ϕ and ψ torsion angles were fixed.
[c] ω values in parentheses are ab initio optimised for the H-(Aib)$_4$-OMe peptide backbone.

3. Discussion

We have successfully synthesised by solution methods a novel series of [(*S*)-Bin]$_n$ homo-peptides to the pentamer level by use of acid fluoride C-activation, the only efficient methodology in our hands for the 1+2, 1+3 and 1+4 couplings of these extremely sterically hindered peptide bonds. Indeed, the CIP/HOAt procedure, which gave the highest yield in the 1+1 coupling, failed in peptide bond formation with amino components longer than the amino acid derivative.

Peptide structure is most often studied with NMR and X-ray diffraction techniques for atomic resolution and with electronic CD in the UV region or IR absorption for average conformational determination. For the binaphthyl substituted peptides investigated in this

work, these classical techniques become impossible or very difficult as series of large Bin side chains inhibit crystallisation and limit solubility. Due to their *homo*-oligopeptide nature, NMR resolution would also be compromised. The π–π* transitions of the binaphthyls and their intrinsic chirality have prevented any use of the electronic CD technique to determine the peptide secondary structure. Finally, normal IR absorption frequencies will not be very useful with both $C^{\alpha,\alpha}$-disubstituted peptides and aromatic side chains, in addition to the high probability of their being in distorted structures.[21a,23] However, MM calculations can address any of these compounds and with DFT techniques we can simulate their vibrational spectra. IR absorption and VCD spectra of the amide I and II bands do have the ability to focus on the peptide backbone and to sense regularity in the structure and the handedness of any helical form that is developed.

Several observations come to bear on our attempt to use optical spectra (IR absorption and VCD) to determine the solution conformation of the tetrapeptide H-[(*S*)-Bin]$_4$-OMe and the consequent effort to discriminate among the large number of possible computed structures. The amide A absorption spectra of the N^{α}-protected oligomers, showing the band at $\cong$3350 cm^{-1} to increase in relative intensity with peptide main-chain length, indicates occurrence of intramolecularly H-bonded amide N–H groups. The multiple component amide I absorption band for the $n=4$ oligomer suggests development of a distorted structure. Finally, the sign pattern of its amide I VCD favours a right-handed helical structure. Among our calculated most stable structures, the first observation discourages consideration of the β-sheet and 3_1-helix structures, which at best (in the β-sheet) would have weak H-bonds (interaction between N–H and C=O groups of the same residue in the sequence). The second observation eliminates regular α- or π-helices and favours either distorted helices or turn structures. Finally, the last observation favours a right-handed turn or helix. This logic and the shape of the VCD spectrum led us to explore the spectra for the distorted 3_{10}-helix, which was one of the low-energy structures in all our MM calculations. Considering all these approximations, its spectra prove to be an excellent match to what we observe experimentally (Fig. 10) as discussed below.

The most conformationally sensitive part of the IR absorption or VCD spectra of peptides is the amide I region, where side-chain vibrations do not appear (Fig. 9) and inter-residue coupling dominates the spectral character (IR absorption frequency and VCD sign pattern). The positive couplet (positive to low frequency) amide I VCD spectrum of H-[(*S*)-Bin]$_4$-OMe in $CDCl_3$ (Fig. 10) reflects published amide I results for right-handed 3_{10}- or α-helical structures.[19,21,23b,c,24] The negative amide II VCD signal at 1524 cm^{-1} is overlapped by two narrow, oppositely signed (couplet) VCD negative bands to lower frequency (seen best in Fig. 9, due to partial N–D deuteration weakening of the amide II band). The relative amide I and amide II intensities, which are useful for discrimination between 3_{10}-helix and α-helix, are complicated by this overlap, but the amide II is nonetheless still quite intense (Fig. 10), which would be consistent with the peptide forming a more tightly wound (increased number of H-bonds) structure like a 3_{10}-helix.[19,21a,b,24a] In this respect we further note that the α-helix energies were consistently calculated to be quite high in our MM optimisations (Table 1).

As stated above, the experimental VCD spectra of H-[(*S*)-Bin]$_4$-OMe are consistent: there is a positive couplet dominating the amide I and a negative broad amide II (at 1524 cm^{-1}), when measured in $CDCl_3$ or $CDCl_3$/TFE (data not shown), the latter being comparable in intensity to the negative component of the amide I. In $CDCl_3$/TFE the amide I positive couplet has the same shape and it is shifted for about 4 cm^{-1} to higher wavenumbers in comparison to the couplet of H-[(*S*)-Bin]$_4$-OMe in $CDCl_3$/TFE-OD with a minimum at 1691 cm^{-1} and a maximum at 1664 cm^{-1}. The intensity of the amide II band is significantly higher in $CDCl_3$ and $CDCl_3$/TFE than in $CDCl_3$/TFE-OD, reflecting the effects of N-deuteration in the latter sample (Fig. 9). In addition, the spectra of the tetrapeptide at a five times lower concentration (i.e. 3.6 mg mL^{-1}) are perfectly consistent in terms of shape, band positions, and even intensity to those plotted in Figure 10, indicating that no aggregation effects are operative. When the experimental spectra are compared (Fig. 10) to our simulated results, the agreement is particularly gratifying. While detailed intensities vary, the overall sign pattern and relative intensities are reproduced well for both the amides I and II as well as the major binaphthyl-based bands. Some level of disagreement between experiment and theory is expected for a distorted structure, since one is unlikely to predict its spectra correctly and the tetrapeptide is likely to sample many local conformations around the distorted segment. The results in Figure 10 fall well within the variation of the method itself. Since they were also consistently predicted to have relatively low MM energies, we also calculated the IR absorption and VCD spectra for a distorted left-handed 3_{10}-helix, a β-sheet structure with stacked side chains and a right-handed poly(Pro)$_n$-type helix backbone. None of these secondary structures gave simulated spectra in qualitative agreement with the experiments, leaving the distorted, right-handed 3_{10}-helix (Fig. 11) as yielding the optimal simulated spectra.

It is worth pointing out that our present conclusion on the conformation preferentially adopted by the Bin-rich peptides (distorted β-turn/3_{10}-helix) is not surprising in view of the C^{α}-tetrasubstituted nature of this α-amino acid.[1] Indeed, it was already proposed on the basis of an investigation of simpler model peptides containing one or two such residues.[6b,8]

4. Experimental

4.1. General experimental

Melting points were determined by means of a capillary tube immersed in an oil bath (Tottoli apparatus, Büchi) with a final temperature raise of 3°C min^{-1}, and are

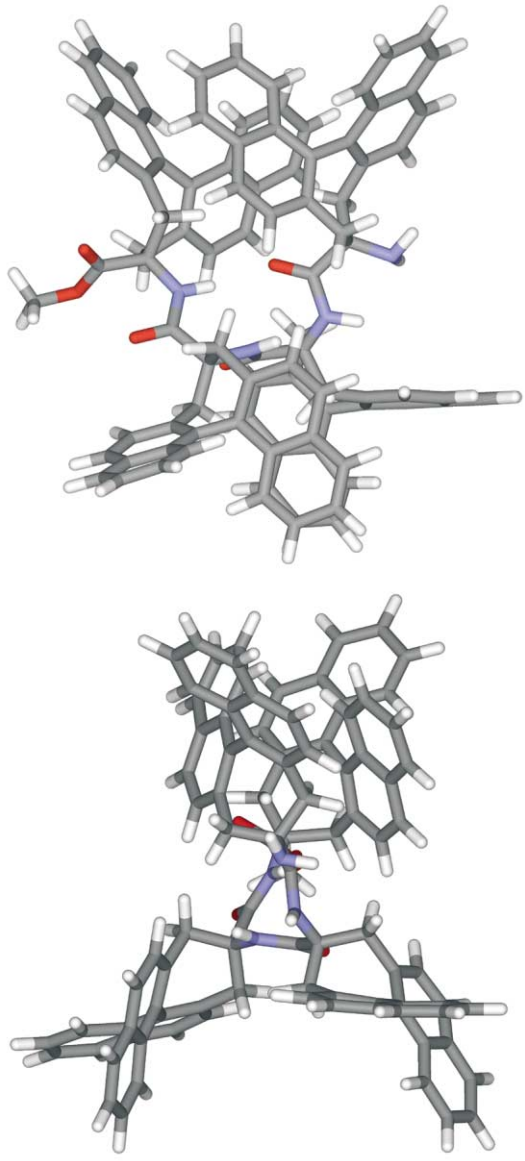

Figure 11. (Top) side view of the MM energy optimised, distorted, right-handed 3_{10}-helical conformation of H-[(*S*)-Bin]$_4$-OMe that shows the peptide main chain through the middle of the binaphthyl side chains. (Bottom) end view emphasising the triangular shape of the ternary 3_{10}-helix continuing to the distorted C-terminus (ester group) and the steric interference of the binaphthyl groups.

uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 MHz and 77 MHz, respectively, the solvent $CDCl_3$ being used as internal standard ($\delta = 7.27$ ppm for ^{1}H and 77.00 ppm for ^{13}C). Splitting patterns are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. The optical rotations were measured with an accuracy of 0.3%, in a 1 dm thermostated cell. Analytical TLC and preparative column chromatography were performed on Kieselgel F 254 and Kieselgel 60 (0.040–0.063 mm) (Merck) respectively, with the following eluent systems: CH_2Cl_2 (I); 1% MeOH–99% CH_2Cl_2 (II); 2.5% MeOH–97.5% CH_2Cl_2 (III); 5% MeOH–95% CH_2Cl_2 (IV); 10% MeOH–90% CH_2Cl_2 (V); 2.5% EtOAc (ethyl acetate)–97.5% CH_2Cl_2 (VI); 5% EtOAc–95% CH_2Cl_2 (VII); 20% EtOAc–80% CH_2Cl_2 (VIII). UV light ($\lambda = 254$ nm) allowed visualisation of the spots after TLC runs for all compounds, even at low concentration.

4.2. (*S*)-Methyl 2′,1′:1,2;1″,2″:3,4-dinaphthcyclohepta-1,3-diene-6-benzyloxycarbonylamino-6-carboxylate, Z-(*S*)-Bin-OMe 1b

To a mixture of resolved H-[(*S*)-Bin]-OMe **1a** (0.280 g; 0.76 mmol), containing traces of H-[(*S*)-Bin]-O-$(CH_2)_2$-O-$(CH_2)_2$-OH,[6a] and Z-OSu (0.209 g; 0.84 mmol), was added CH_3CN (10 mL). The resulting clear colourless solution was stirred at room temperature for 48 h and evaporated to dryness in vacuo. The residue was chromatographed on a 2.3×60 cm column of silica gel with eluents (I), then (II), to give 0.335 g (87%) of analytically pure **1b** as a white, solid foam. Rf = 0.50 (II). ^{1}H NMR ($CDCl_3$): δ 7.96 [m, 4H, ArH], 7.54–7.36 [m, 11H, ArH], 7.28 [m, 2H, ArH], 5.26 [s, 1H, NH], 5.24–5.16 [d-d (broad), $J \sim 12.3$–12.1, 1H-1H, CH_2 Z], 3.77 [s (broad), 3H, OMe], 3.35–2.46 [d-d, $J = 13.2$–13.2, 1H-1H, $ArCH_2\beta$], 3.25–3.15 [d (broad)-d, $J \sim 14.0$–14.1, 1H-1H, $ArCH_2\beta'$]. ^{13}C NMR ($CDCl_3$): δ 172.8 (C=O Bin), 155.0 (C=O Z), 136.1, 134.2, 134.1, 134.0, 133.6, 133.0, 132.8, 131.6, 131.5, 128.4, 128.2, 128.1, 128.05, 127.98, 127.92, 127.70, 126.96, 125.8, 125.7, 125.2 (CAr), 69.6 (Cα), 66.7 (CH_2 Z), 52.4 (OCH_3), 41.7 (Cβ), 38.2 (Cβ'). $[\alpha]^{25}_{589}$ +8; $[\alpha]^{25}_{578}$ +8; $[\alpha]^{25}_{546}$ +4; $[\alpha]^{25}_{436}$ −66; $[\alpha]^{25}_{365}$ −715 (*c* 0.2; MeOH). Anal. calcd for $C_{33}H_{27}NO_4 \cdot 0.5H_2O$ (510.562): C, 77.63; H, 5.53; N, 2.74. Found: C, 77.85; H, 5.61; N, 2.54%.

4.3. (*S*)-2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-benzyloxycarbonylamino-6-carboxylic acid, Z-(*S*)-Bin-OH 1c

To a clear solution of **1b** (0.242 g; 0.48 mmol) in MeOH (100 mL) was added aqueous 1N NaOH (50 mL). The resulting white suspension was stirred at 75°C, which gradually led to solubilisation of the precipitate. The obtained clear colourless solution was stirred for 24 h. Methanol was evaporated under reduced pressure with portions of water added. The resulting solution was ice-cooled and acidified by addition of a large excess of aq. 1N HCl. The resulting fine precipitate was filtered on a Büchner funnel, washed with water and air dried. The collected white solid was dried in vacuo at 80°C for 2 h to afford 0.214 g (91%) of pure **1c**, identical to an authentic sample.[4b] Mp = 150–180°C (indefinite). $[\alpha]^{25}_{589}$ +22, $[\alpha]^{25}_{578}$ +22, $[\alpha]^{25}_{546}$ +22, $[\alpha]^{25}_{436}$ −40, $[\alpha]^{25}_{365}$ −696 (*c* 0.5; MeOH). {Lit.[4b] Mp 167°C. $[\alpha]^{25}_{589}$ +15, $[\alpha]^{25}_{578}$ +15, $[\alpha]^{25}_{546}$ +13, $[\alpha]^{25}_{436}$ −56, $[\alpha]^{25}_{365}$ −712 (*c* 0.5; MeOH)}.

4.4. (*S*)-Methyl 2′,1′:1,2;1″,2″:3,4-dinaphthcyclohepta-1,3-diene-6-*tert*-butyloxycarbonylamino-6-carboxylate, Boc-(*S*)-Bin-OMe 1d

To a mixture of Boc_2O (0.654 g; 3.00 mmol) and resolved H-(*S*)-Bin-OMe **1a** (0.736 g; 2.00 mmol) containing traces of H-(*S*)-Bin-O-$(CH_2)_2$-O-$(CH_2)_2$-OH,[6a] was added CH_3CN (30 mL). The resulting clear colour-

less solution was stirred at room temperature for 3 days and evaporated to dryness in vacuo. The residue was chromatographed on a 2.3×53 cm column of silica gel with eluents (I), then (II), to give 0.807 g (86%) of analytically pure **1d** as a white, crystalline powder. Mp = 100–130°C (indefinite). Rf = 0.40 (I). ^{1}H NMR ($CDCl_3$): δ 7.93 [m, 4H, ArH], 7.49 [m, 4H, ArH], 7.27 [m, 2H, ArH], 4.81 [s, 1H, NH], 3.76 [s, 3H, OMe], 3.35–2.41 [d-d, J = 13.0–13.0, 1H-1H, $ArCH_2\beta$], 3.16–3.08 [d (broad)-d, $J \sim 13.8$–13.6, 1H-1H, $ArCH_2\beta'$], 1.48 [s, 9H, Boc]. ^{13}C NMR ($CDCl_3$): δ 173.2 (C=O Bin), 154.5 (C=O Boc), 134.5, 134.2, 134.0, 133.1, 133.0, 131.73, 131.68, 128.4, 128.23, 128.20, 128.1, 128.01, 127.97, 127.1, 125.9, 125.7, 125.4, 125.3 (CAr), 80.3 (C-O Boc), 69.6 (Cα), 52.4 (OCH_3), 42.0 (Cβ), 38.7 (Cβ'), 28.2 (CH_3 Boc). $[\alpha]^{25}_{589}$ +39; $[\alpha]^{25}_{578}$ +40; $[\alpha]^{25}_{546}$ +41; $[\alpha]^{25}_{436}$ +4; $[\alpha]^{25}_{365}$ −562 (c 0.5; MeOH). Anal. calcd for $C_{30}H_{29}NO_4 \cdot 1.2H_2O$ (489.159): C, 73.66; H, 6.47; N, 2.86. Found: C, 73.39; H, 5.99; N, 2.74%.

4.5. (*S*)-2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-*tert*-butyloxycarbonylamino-6-carboxylic acid, Boc-(*S*)-Bin-OH 1e

To a clear solution of **1d** (0.673 g; 1.44 mmol) in MeOH (100 mL) was added aqueous 1N NaOH (50 mL). The resulting milky solution clarified upon stirring at 75°C. After 24 h, the clear colourless solution was evaporated under reduced pressure with portions of water added in order to remove methanol. The obtained solution was ice-cooled, acidified by addition of a large excess of aq. 1N HCl, and extracted with CH_2Cl_2 (250 mL in three portions). The organic phase was washed with water (2×100 mL), dried ($MgSO_4$), filtered and evaporated. The collected white solid was dried in vacuo at 80°C for 2 h to afford 0.614 g (94%) of pure **1e**, identical to an authentic sample.[4b] Mp = 259–263°C. $[\alpha]^{25}_{589}$ +45, $[\alpha]^{25}_{578}$ +46, $[\alpha]^{25}_{546}$ +48, $[\alpha]^{25}_{436}$ +9, $[\alpha]^{25}_{365}$ −639 (c 0.5; MeOH). {Lit.[4b] Mp 264°C. $[\alpha]^{25}_{589}$ +46, $[\alpha]^{25}_{578}$ +47, $[\alpha]^{25}_{546}$ +50°, $[\alpha]^{25}_{436}$ +9, $[\alpha]^{25}_{365}$ −644 (c 0.5; MeOH)}.

4.6. (*S*)-2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-(9-fluorenylmethyloxycarbonyl-amino)-6-carboxylic acid, Fmoc-(*S*)-Bin-OH 1f

To a solution of **1e** (0.468 g; 1.10 mmol) in CH_2Cl_2 (10 mL) was added TFA (10 mL). The solution was stirred at room temperature for 4 h and evaporated to dryness in vacuo at 40°C. The residue was repeatedly dissolved in CH_2Cl_2 and the solution evaporated in vacuo to yield crude TFA·H-(*S*)-Bin-OH (not characterised) which was directly used in the next step. This compound was dissolved in CH_3CN (25 mL) and DIEA (0.550 mL; 3.16 mmol) was added. A heavy white precipitate formed. Addition of CH_2Cl_2 (ca. 40 mL) gave a milky solution to which Fmoc-OSu (0.100 g) was added. The mixture was magnetically stirred at 60°C for 4 h, with more Fmoc-OSu (0.330 g) added in portions every hour, then at room temperature for 3 days, then at 60°C again for 6 h, after addition of 0.024 g more Fmoc-OSu (for a total of 0.454 g; 1.34 mmol). The reaction mixture was evaporated in vacuo, and the residue taken up in CH_2Cl_2 (ca. 350 mL by portions) and 0.5N HCl (ca. 100 mL by portions) with stirring. The decanted CH_2Cl_2 solution was washed with more 0.5N HCl (100 mL), then water (2×100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was chromatographed on a 2.3×32 cm column of silica gel with eluents (IV), then (V), to afford 0.437 g (74%) of pure **1f** as a pale brown, amorphous solid. Mp = 160–210°C (indefinite). Rf = 0.43 (V). ^{1}H NMR ($CDCl_3$): δ 7.96 [m, 1H, ArH], 7.85 [m, 1H, ArH], 7.72 [m, 4H, ArH], 7.46 [m, 4H, ArH], 7.36–7.12 [m, 10H, ArH], 5.85 [s (broad), 1H, COOH], 4.94 [s (broad), 1H, NH], 4.53–4.21 [m (broad)-m (broad), 1H-1H, CH_2 Fmoc], 4.12 [m (broad), 1H, CH Fmoc], 3.21–2.19 [m (broad)-m (broad), 1H-1H, $ArCH_2\beta$], 3.07 [m (broad), 2H, $ArCH_2\beta'$]. ^{13}C NMR ($CDCl_3$): δ 176.9 (C=O Bin), 155.4 (C=O Fmoc), 143.7, 143.6, 141.3, 141.2, 134.5, 134.4, 134.1, 133.8, 133.0, 132.9, 131.7, 131.5, 128.2, 128.0, 127.6, 127.2, 127.0, 125.8, 125.6, 125.3, 125.2, 125.1, 124.9, 119.9 (CAr), 69.8 (Cα), 66.4 (CH_2 Fmoc), 47.2 (CH Fmoc), 41.6 (Cβ), 38.1 (Cβ'). $[\alpha]^{25}_{589}$ +101; $[\alpha]^{25}_{578}$ +101; $[\alpha]^{25}_{546}$ +113; $[\alpha]^{25}_{436}$ +132 (c 0.2; CH_2Cl_2). Anal. calcd for $C_{39}H_{29}NO_4 \cdot 1.5H_2O$ (602.654): C, 77.72; H, 5.35; N 2.32. Found: C, 77.96; H, 5.28; N, 2.05%.

4.7. (*S*)-2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-(9-fluorenylmethyloxycarbonyl-amino)-6-carboxylic acid fluoride, Fmoc-(*S*)-Bin-F 1g

To a solution of **1f** (0.044 g; 0.076 mmol) in CH_2Cl_2 (2 mL) was added pyridine (0.013 mL; 0.15 mmol). The solution was stirred under argon at ca. −15°C (ice-salt bath) and cyanuric fluoride (0.065 mL; 0.77 mmol) was added by syringe through a septum. The resulting suspension was stirred from −15°C to −5°C during 1 h and then at room temperature for 3 h. The mixture was diluted with CH_2Cl_2 and the solution was extracted twice with iced water, dried ($MgSO_4$), filtered and evaporated in vacuo at 40°C. The resulting crude product presented a main spot corresponding to **1g** on TLC, with traces of several other spots of unidentified compounds. Flash chromatography on a 1.5×23 cm column of silica gel with eluent (IV) led to 0.026 g (58%) of **1g** which was obtained as a white, amorphous solid after precipitation from CH_2Cl_2/hexane, but still presented several minor extra spots on TLC because of some decomposition occurring on silica gel. Rf = 0.75 (IV). ^{1}H NMR ($CDCl_3$): δ 7.94 [m, 4H, ArH], 7.80 [m, 2H, ArH], 7.62 [m, 2H, ArH], 7.51–7.26 [m, 12H, ArH], 4.93 [s (broad), 1H, NH], 4.76–4.48 [dd-m (broad), J = 5.9; 10.9-not seen, 1H-1H, CH_2 Fmoc], 4.26 [dd (t-like), $J \sim 5.9$, 1H, CH Fmoc], 3.26–2.47 [d (broad)-d (broad), $J \sim 12.8$–12.8, 1H-1H, $ArCH_2\beta$], 3.12 [m (broad), 2H, $ArCH_2\beta'$]. ^{19}F NMR ($CDCl_3$): δ 18.53.

4.8. Z-[(*S*)-Bin]$_2$-OMe 2b

4.8.1. By the pivaloyl mixed anhydride method. To a solution of Z-(*S*)-Bin-OH **1c** (0.083 g; 0.17 mmol) in toluene (2 mL) was added TEA (0.030 mL; 0.21 mmol). The solution was stirred under argon at ca. −10°C (ice-salt bath) and pivaloyl chloride (0.021 mL; 0.17

mmol) was added by syringe through a septum. Theresulting suspension was stirred from ca. −10°C to ca. +10°C for 1 h, and then at room temperature for 2 h. A solution of H-(*S*)-Bin-OMe **1a** (0.069 g; 0.19 mmol) in toluene (1 mL) was then added, the mixture was stirred in an oil bath at 60°C for 18 h, and evaporated in vacuo. The residue was solubilised in EtOAc (100 mL), and the solution was successively extracted with 0.5N HCl (2×50 mL), water (100 mL), 5% aq. $NaHCO_3$ (2×50 mL), water (2×100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was chromatographed on a 2.3×30 cm column of silica gel with eluent (VII) to afford 0.084 g (59%) of pure **2b**.

4.8.2. By the CIP/HOAt method. To a solution of **1c** (0.071 g; 0.14 mmol), **1a** (0.054 g; 0.14 mmol), HOAt (0.022 g; 0.16 mmol) and CIP (0.055 g; 0.20 mmol) in CH_2Cl_2 (2.5 mL), was added DIEA (0.100 mL; 0.57 mmol). The mixture was magnetically stirred at room temperature for 3 days. Addition of EtOAc (100 mL), followed by extraction of the organic phase as above, and then by preparative TLC of the crude product on silica gel with eluent (VII) gave 0.103 g (84%) of pure **2b**, obtained as a white, amorphous solid. Mp = 186–188°C. Rf = 0.54 (VII). ^{1}H NMR ($CDCl_3$): δ 7.96–7.83 [m, 8H, ArH], 7.71 [d (broad), $J\sim7.7$, 1H, ArH], 7.61 [d (broad), $J\sim7.4$, 1H, ArH], 7.57 [d, $J=8.2$, 1H, ArH], 7.48–7.32 [m, 13H, ArH], 7.29–7.20 [m, 5H, ArH], 6.99 [s (broad), 1H, NH Bin^2], 5.12–4.85 [d (broad)-d, $J\sim11.6$–12.3, 1H-1H, CH_2 Z], 3.76 [s, 3H, OMe], 5.24–5.16 [d-d (broad), $J\sim12.3$–12.1, 1H-1H, CH_2 Z], 4.94 [s, 1H, NH Bin^1 (Z)], 3.73 [s, 3H, OMe], 3.52–2.37 [d-d, $J=12.8$–13.0, 1H-1H, $ArCH_2\beta$ Bin], 3.52–2.44 [d-d (broad), $J\sim12.8$–13.0, 1H-1H, $ArCH_2\beta$ Bin], 3.36–2.91 [d (broad)-d (broad), $J\sim13.4$–13.2, 1H-1H, $ArCH_2\beta'$ Bin], 3.13 [s, 2H, $ArCH_2\beta'$ Bin]. ^{13}C NMR ($CDCl_3$): δ 172.9, 171.3 (C=O Bin), 155.2 (C=O Z), 136.0, 134.7, 134.4, 134.1, 134.0, 133.8, 133.1, 133.0, 132.9, 131.8, 131.7, 131.6, 131.5, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.7, 127.3, 127.2, 127.1, 127.0, 125.9, 125.6, 125.34, 125.26, 125.21 (CAr), 70.4, 69.3 (Cα Bin), 66.9 (CH_2 Z), 52.4 (OCH_3), 41.9, 41.0 (Cβ Bin), 39.1, 37.2 (Cβ′ Bin). $[\alpha]_{589}^{25}$ +27; $[\alpha]_{578}^{25}$ +26; $[\alpha]_{546}^{25}$ +23; $[\alpha]_{436}^{25}$ −76; $[\alpha]_{365}^{25}$ −986 (*c* 0.1; MeOH). Anal. calcd for $C_{57}H_{44}N_2O_5\cdot0.5\ H_2O$ (845.946): C, 80.92; H, 5.36; N, 3.31. Found: C, 81.14; H, 5.57; N 3.17%.

4.9. Attempts at saponification of 2b

To a clear warm solution of **2b** (0.226 g; 0.27 mmol) in MeOH (100 mL) was added aqueous 1N NaOH (25 mL). The resulting white suspension was stirred at 60°C for 8 h, but did not solubilise. The mixture was decanted at room temperature and the absence of starting material **2b** in the supernatant was checked by TLC. Methanol was partly evaporated in vacuo, the suspension was magnetically stirred, cooled by addition of crushed ice, acidified by addition of a large excess of aq. 1N HCl, and extracted with CH_2Cl_2 (ca. 200 mL in three portions). The organic phase was washed with water, dried ($MgSO_4$), filtered and evaporated in vacuo, to afford 0.214 g of a crude product consisting of starting material **2b** by TLC. As the absence of reaction could possibly be due to the insolubility of **2b** in MeOH/aq. 1N NaOH, the experiment was repeated with a solution of **2b** (0.021 g; 0.025 mmol) in MeOH (10 mL), aq. 2N NaOH (1 mL), toluene (1 mL) and dioxane (10 mL), which was stirred at 70°C for 8 h. Extraction as above gave a crude product in which several compounds were present, with no, or only traces of, starting material **2b**. Preparative TLC on silica gel with eluent (V) gave fractions of 0.0043 g (Rf = 0.70), 0.0012 g (Rf = 0.54), 0.0016 g (Rf = 0.37) and 0.0067 g (Rf = 0.03), of four unidentified compounds. In another experiment, a clear colourless solution of **2b** (0.013 g; 0.015 mmol) and aq. 40% tetrabutylammonium hydroxide (0.030 mL; 0.045 mmol) in THF (1 mL), was stirred at room temperature for 4 days, diluted with CH_2Cl_2 (75 mL), acidified with a large excess of 0.5N HCl (50 mL), extracted as above and chromatographed on a 1×23 cm column of silica gel with eluent (V) to give 0.0053 g of an unidentified compound. Rf = 0.37 (V).

4.10. H-[(*S*)-Bin]$_2$-OMe 2a

To a warm solution (heating was necessary for solubilisation) of **2b** (0.145 g; 0.17 mmol) in MeOH (75 mL) was added 10% palladium on charcoal (0.125 g) under an argon stream. The mixture was hydrogenated in a Parr apparatus at ca. 50 psi for 5 h at room temperature and filtered through paper. The solution was evaporated in vacuo at 40°C and the crude product was dissolved in CH_2Cl_2 (100 mL). The solution was washed with water (100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo after addition of hexane to afford 0.116 g (95%) of pure **2a**, obtained as a white, amorphous solid after precipitation from CH_2Cl_2/hexane. Mp = 185–230°C (indefinite). Rf = 0.18 (VII). ^{1}H NMR ($CDCl_3$): δ 7.96–7.88 [m, 8H, ArH], 7.72 [s, 1H, NH Bin^2], 7.68–7.18 [m, 16H, ArH], 3.75 [s, 3H, OMe], 3.51–2.34 [d-d, $J=13.4$–13.2, 1H-1H, $ArCH_2\beta$ Bin], 3.33–2.46 [d-d, $J=13.1$–13.1, 1H-1H, $ArCH_2\beta$ Bin], 3.22–3.14 [d-d, $J=13.8$–14.2, 1H-1H, $ArCH_2\beta'$ Bin], 3.07–2.38 [d-d, $J=13.2$–13.2, 1H-1H, $ArCH_2\beta'$ Bin]. ^{13}C NMR ($CDCl_3$): δ 172.9, 172.4 (C=O Bin), 134.5, 134.4, 134.0, 133.8, 133.1, 133.0, 132.8, 131.8, 131.7, 128.4, 127.3, 127.2, 125.9, 125.4 (CAr), 69.6, 69.2 (Cα Bin), 52.7 (OCH_3), 43.0, 41.6, 38.1 (Cβ Cβ′ Bin). $[\alpha]_{589}^{25}$ −19; $[\alpha]_{578}^{25}$ −22; $[\alpha]_{546}^{25}$ −33; $[\alpha]_{436}^{25}$ −203; $[\alpha]_{365}^{25}$ −1293 (*c* 0.1; MeOH). Anal. calcd for $C_{49}H_{38}N_2O_3\cdot0.5H_2O$ (711.818): C, 82.67; H, 5.52; N, 3.93. Found: C, 82.62; H, 6.01; N, 3.61%.

4.11. Attempt at coupling Boc-(*S*)-Bin-OH 1e with H-[(*S*)-Bin]$_2$-OMe 2a by the CIP/HOAt method

To a solution of **1e** (0.046 g; 0.10 mmol), **2a** (0.070 g; 0.10 mmol), HOAt (0.017 g; 0.12 mmol) and CIP (0.034 g; 0.12 mmol) in CH_2Cl_2 (3 mL), was added DIEA (0.070 mL; 0.40 mmol). The mixture was magnetically stirred at room temperature for 24 h, a TLC control showing a main spot corresponding to the starting material **2a**. Acetonitrile (3 mL) was added and the solution was stirred in an oil bath at 75°C (CH_2Cl_2 evaporated) for 8 h. Extraction as for the preparation of **2b** (vide supra), followed by preparative TLC of the

obtained crude product on silica gel with eluent (VII), gave 0.028 g (40%) of recovered **2a** and a mixture of several minor unidentified compounds.

4.12. Fmoc-[(*S*)-Bin]$_3$-OMe 3h

A solution of **1f** (0.230 g; 0.40 mmol) and pyridine (0.070 mL; 0.82 mmol) in CH_2Cl_2 (10 mL), was treated with cyanuric fluoride (0.180 mL; 2.13 mmol) under argon at ca. −15°C to −5°C (1 h) and then at room temperature (2 h), under the same experimental conditions and work-up as described earlier, to give Fmoc-(*S*)-Bin-F **1g** (vide supra). The extracted crude **1g** was not purified by flash chromatography, but directly treated with a solution of H-[(*S*)-Bin]$_2$-OMe **2a** (0.140 g; 0.20 mmol) and NMM (*N*-methylmorpholine) (0.050 mL; 0.45 mmol) in CH_2Cl_2 (4 mL). The resulting clear orange–brown solution was stirred at room temperature for 10 days, diluted with CH_2Cl_2 (150 mL), successively extracted with 0.5N HCl (2×50 mL), water (100 mL), 5% aq. $NaHCO_3$ (2×50 mL), water (2×100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was chromatographed on a 2.3×50 cm column of silica gel with eluent (VI) to afford 0.130 g (51%) of pure **3h**, as a white, amorphous solid. Mp = 226–230°C. Rf = 0.27 (VI); 0.56 (VII). ^{1}H NMR ($CDCl_3$): δ 7.97–7.88 [m, 8H, ArH], 7.83–7.56 [m, 8H, ArH], 7.61 [s, 1H, NH Bin], 7.32–7.09 [m, 27H, ArH], 6.76 [d, J = 8.3, 1H, ArH], 6.62 [s, 1H, NH Bin], 4.56 [s, 1H, NH Bin^1 (Fmoc)], 3.99–3.86 [dd-d, $J \sim$ 11.0; 5.2–13.0, 1H-1H, CH_2 Fmoc], 3.71 [dd (t-like), $J \sim$ 5.2, 1H, CH Fmoc], 3.49 [s, 3H, OMe], 3.39 [d, $J \sim$ 13.4, 1H], 3.37 [d, $J \sim$ 12.7, 1H], 3.36 [d, $J \sim$ 12.1, 1H], 3.28 [d, J = 14.0, 1H], 3.18 [d (partly masked), 1H], 3.15 [s, 2H], 2.96 [d-d (t-like), $J \sim$ 12.5, 2H], 2.74 [d, J = 13.1, 1H], 2.37 [d, J = 13.2, 1H], 2.01 [d, J = 12.9, 1H], [ArCH$_2$ββ′ Bin^{1,2,3}]. ^{13}C NMR ($CDCl_3$): δ 172.8, 171.7, 170.8 (C=O Bin), 155.4 (C=O Fmoc), 143.4, 142.9, 141.4, 141.2, 135.5, 135.4, 134.7, 134.44, 134.42, 134.3, 134.1, 133.9, 133.5, 133.45, 133.38, 133.1, 133.0, 132.9, 132.8, 132.7, 131.80, 131.76, 131.71, 131.6, 131.5, 131.3, 129.5, 129.2, 128.6, 128.4, 128.35, 128.27, 128.18, 128.11, 128.06, 127.9, 127.8, 127.7, 127.6, 127.5, 127.3, 127.2, 126.9, 126.8, 126.7, 126.0, 125.8, 125.7, 125.5, 125.4, 125.34, 125.29, 125.22, 125.0, 124.5, 119.8 (CAr), 70.6, 70.3, 69.8 (Cα Bin), 65.4 (CH_2 Fmoc), 52.3 (OCH_3), 47.1 (CH Fmoc), 42.0, 41.8, 41.0, 39.6, 38.4, 37.3 (Cβ Cβ′ Bin). $[\alpha]_{589}^{25}$ −123; $[\alpha]_{578}^{25}$ −132; $[\alpha]_{546}^{25}$ −159; $[\alpha]_{436}^{25}$ −422; $[\alpha]_{365}^{25}$ −1608 (*c* 0.1; MeOH; sparingly soluble). $[\alpha]_{589}^{25}$ −45; $[\alpha]_{578}^{25}$ −57; $[\alpha]_{546}^{25}$ −76; $[\alpha]_{436}^{25}$ −292; $[\alpha]_{365}^{25}$ −1516 (*c* 0.1; CH_2Cl_2). ESI$^+$ MS *m*/*z*: 1260.5 [*M*+H]$^+$ (see Fig. 5). Anal. calcd for $C_{88}H_{65}N_3O_6 \cdot 1.5\ H_2O$ (1287.448): C, 82.09; H, 5.32; N, 3.26. Found: C, 82.13; H, 5.41; N, 3.32%.

4.13. H-[(*S*)-Bin]$_3$-OMe 3a

A suspension of **3h** (0.126 g; 0.10 mmol) in CH_3CN (18 mL) was heated for a few minutes in order to obtain a clear solution which was allowed to cool to room temperature. Diethylamine (2 mL) was added, the resulting solution was stirred for 5 h and then evaporated to dryness in vacuo. The residue was dissolved in CH_3CN and the solution was again evaporated in vacuo at 40°C. The crude product was chromatographed on a 1.5×40 cm column of silica gel with eluent (VII) (elution of dibenzofulvene and traces of impurities of high Rf), then (VIII), to afford 0.103 g (73%) of pure **3a**, as a white, amorphous solid. Mp = 240–243°C. Rf = 0.20 (VII); 0.80 (VIII). ^{1}H NMR ($CDCl_3$): δ 8.06–7.18 [m, 38H, 36 ArH and 2 NH Bin^{2,3}], 3.70 [s, 3H, OMe], 3.72 [d (partly masked), 1H], 3.67 [d (partly masked), 1H], 3.57 [d, J = 13.3, 1H], 3.20 [d, $J \sim$ 12.8, 1H], 3.16 [d, $J \sim$ 12.8, 1H], 3.10–3.04 [d-d, $J \sim$ 13.8–13.8, 1H-1H], 2.86 [d, J = 13.6, 1H], 2.47 [d, J = 13.3, 1H], 2.38 [d, $J \sim$ 13.4, 1H], 2.34 [d, $J \sim$ 13.4, 1H], 2.17 [d, J = 13.3, 1H], [ArCH$_2$ββ′ Bin^{1,2,3}]. $[\alpha]_{589}^{25}$ −109; $[\alpha]_{578}^{25}$ −128; $[\alpha]_{546}^{25}$ −160; $[\alpha]_{436}^{25}$ −534; $[\alpha]_{365}^{25}$ −2430 (*c* 0.1; CH_2Cl_2). Anal. calcd for $C_{73}H_{55}N_3O_4 \cdot H_2O$ (1056.210): C, 83.01; H, 5.44; N, 3.98. Found: C, 83.11; H, 5.35; N, 3.84%.

4.14. Fmoc-[(*S*)-Bin]$_4$-OMe 4h

A solution of **1f** (0.111 g; 0.19 mmol) and pyridine (0.035 mL; 0.41 mmol) in CH_2Cl_2 (5 mL), was treated with cyanuric fluoride (0.100 mL; 1.18 mmol), under the same experimental conditions and work-up as described earlier, to give crude Fmoc-(*S*)-Bin-F **1g** (vide supra), which was directly treated with a solution of H-[(*S*)-Bin]$_3$-OMe **3a** (0.067 g; 0.064 mmol) and NMM (0.025 mL; 0.23 mmol) in CH_2Cl_2 (2 mL). The resulting solution was stirred at room temperature for 12 days. Work-up as in the preparation of **3h** (vide supra), followed by chromatography of the obtained crude product on a 1.5×41 cm column of silica gel with eluent (VI) afforded 0.068 g (66%) of pure **4h**, as a white, amorphous solid. Mp = 260–262°C. Rf = 0.65 (VII). ^{1}H NMR ($CDCl_3$): δ 8.28 [d, J = 8.3, 1H], 8.09 [d, J = 8.3, 1H], 8.05 [d, J = 8.3, 1H], 7.96–7.76 [m, 15H], 7.68 [d, J = 7.4, 1H], 7.53 [m (t-like), J = 7.2, 2H], 7.49–7.28 [m, 19H], 7.23–7.06 [m, 15H], 7.00–6.89 [m, 2H], 6.59 [m (broad), 1H], [56 ArH and 2 NH Bin], 6.04 [s, 1H, NH Bin], 4.86–5.51 [m (dd-like)-m (dd-like), 1H-1H, CH_2 Fmoc], 4.39 [s, 1H, NH Bin^1 (Fmoc)], 4.19 [m (t-like), 1H, CH Fmoc], 3.47 [s, 3H, OMe], 4.15 [d (partly masked), 1H], 3.86 [d, J = 13.4, 1H], 3.46 [d (partly masked), 1H], 3.31–3.14 [m, 5H], 2.94 [d, J = 13.1, 1H], 2.63 [d, J = 13.2, 1H], 2.33 [d, J = 13.6, 1H], 2.20 [m, 2H], 1.85 [m (broad), 1H], 1.82 [d, J = 13.2, 1H], 1.45 [d, $J \sim$ 12.5, 1H], [ArCH$_2$ββ′ Bin^{1,2,3,4}]. ^{13}C NMR ($CDCl_3$): δ 173.2, 173.1, 171.8, 170.8 (C=O Bin), 155.3 (C=O Fmoc), 143.9, 143.3, 141.5, 141.4, 137.4, 135.9, 135.8, 135.1, 134.7, 134.6, 134.3, 134.2, 134.1, 134.0, 133.8, 133.2, 133.0, 132.95, 132.92, 132.83, 132.79, 132.6, 131.9, 131.84, 131.79, 131.75, 131.6, 131.5, 131.3, 130.8, 130.7, 130.5, 129.2, 128.7, 128.6, 128.4, 128.2, 128.1, 127.8, 127.76, 127.66, 127.60, 127.4, 127.3, 127.2, 127.1, 127.0, 126.9, 126.8, 126.7, 126.3, 126.0, 125.9, 125.7, 125.5, 125.1, 125.0, 124.7, 124.6, 120.0 (CAr), 70.5, 70.3, 69.8, 68.9 (Cα Bin), 65.3 (CH_2 Fmoc), 52.1 (OCH_3), 47.3 (CH Fmoc), 41.6, 41.4, 40.9, 40.6, 38.1, 36.6 (Cβ Cβ′ Bin). $[\alpha]_{589}^{25}$ −116; $[\alpha]_{578}^{25}$ −133; $[\alpha]_{546}^{25}$ −159; $[\alpha]_{436}^{25}$ −462; $[\alpha]_{365}^{25}$ −1806 (*c* 0.1; CH_2Cl_2). ESI$^+$ MS *m*/*z*: 1595.6 [*M*+H]$^+$ (see Fig. 5). Anal. calcd for $C_{112}H_{82}N_4O_7 \cdot 2\ H_2O$ (1631.840): C, 82.43; H, 5.31; N, 3.43. Found: C, 82.53; H, 5.31; N, 3.24%.

4.15. H-[(*S*)-Bin]$_4$-OMe 4a

A solution of **4h** (0.059 g; 0.037 mmol) in CH_3CN (9 mL) and diethylamine (1 mL) was stirred at room temperature for 5 h. Work-up as in the preparation of **3a** (vide supra), followed by chromatography of the obtained crude product on a 1.5×38 cm column of silica gel with eluent (VII), then (VIII), afforded 0.044 g (87%) of pure **4a**, as a white, amorphous solid. Mp = 240–260°C (indefinite). Rf = 0.10 (VII). ^{1}H NMR ($CDCl_3$): δ 8.06–6.90 [m, 51H, 48 ArH and 3 NH Bin^{2,3,4}], 3.46 [s, 3H, OMe], 3.87 [d, J = 13.4, 1H], 3.77 [d, $J \sim 13.4$, 1H], 3.72 [d, $J \sim 13.4$, 1H], 3.43 [d (partly masked), 1H], 3.19 [d, J = 13.2, 1H], 3.01–2.76 [m, 6H], 2.83 [m, 3H], 2.10 [d, J = 12.9, 1H], 1.68 [d, $J \sim 13.6$, 1H], [ArCH$_2$ββ′ Bin^{1,2,3,4}]. ^{13}C NMR ($CDCl_3$): δ 177.0, 173.0, 172.8, 172.1 (C=O Bin), 134.0, 133.9, 133.7, 133.6, 133.4, 133.1, 132.94, 132.90, 132.85, 132.81, 131.9, 131.8, 131.73, 131.68, 131.6, 131.5, 131.4, 129.5, 128.8, 128.7, 128.5, 128.4, 128.2, 128.1, 127.9, 127.8, 127.66, 127.7, 127.6, 127.5, 127.2, 127.0, 126.0, 125.8, 125.6, 125.5, 125.4, 125.3, 125.26, 125.20, 125.1, 125.0 (CAr), 71.4, 71.2, 69.6, 68.4 (Cα Bin), 52.1 (OCH_3), 45.1, 43.1, 41.4, 41.1, 40.9, 39.6, 39.0, 37.2 (Cβ Cβ′ Bin). $[\alpha]_{589}^{25}$ −122; $[\alpha]_{578}^{25}$ −142; $[\alpha]_{546}^{25}$ −176; $[\alpha]_{436}^{25}$ −526; $[\alpha]_{365}^{25}$ −2162 (*c* 0.1; CH_2Cl_2). Anal. calcd for $C_{97}H_{72}N_4O_5 \cdot 1.5\ H_2O$ (1400.602): C, 83.18; H, 5.40; N, 4.00. Found: C, 83.27; H, 5.88; N, 3.55%.

4.16. Fmoc-[(*S*)-Bin]$_5$-OMe 5h

A solution of **1f** (0.034 g; 0.059 mmol) and pyridine (0.011 mL; 0.13 mmol) in CH_2Cl_2 (2 mL), was treated with cyanuric fluoride (0.030 mL; 0.35 mmol), under the same experimental conditions and work-up as described earlier, to give crude Fmoc-(*S*)-Bin-F **1g** (vide supra), which was directly treated with a solution of H-[(*S*)-Bin]$_4$-OMe **4a** (0.0266 g; 0.019 mmol) and NMM (0.008 mL; 0.073 mmol) in CH_2Cl_2 (2 mL). The resulting solution was stirred at room temperature for 14 days. Work-up as in the preparation of **3h** (vide supra), followed by chromatography of the obtained crude product on a 1.5×37 cm column of silica gel with eluent (VI), then (VII), gave 0.0133 g (50%) of starting **4a** and 0.0094 g of the desired pentapeptide, which was further purified by preparative TLC on silica gel with eluent (VI) to afford 0.0069 g (18%) of pure **5h**, as a pale brown, amorphous solid. Mp = 250–270°C (indefinite). Rf = 0.46 (VII). $[\alpha]_{589}^{25}$ −185; $[\alpha]_{578}^{25}$ −208; $[\alpha]_{546}^{25}$ −246; $[\alpha]_{436}^{25}$ −621; $[\alpha]_{365}^{25}$ −2177 (*c* 0.1; CH_2Cl_2). ESI$^+$ MS *m*/*z*: 1930.9 $[M+H]^+$ (see Fig. 5). Anal. calcd for $C_{136}H_{99}N_5O_8 \cdot 4\ H_2O$ (2003.256): C, 81.54; H, 5.38; N, 3.49. Found: C, 81.81; H, 6.13; N, 3.02%.

4.17. H-[(*S*)-Bin]$_5$-OMe 5a

A solution of **5h** (0.0060 g; 0.003 mmol) in CH_3CN (9 mL) and diethylamine (1 mL) was stirred at room temperature for 5 h. Work-up as in the preparation of **3a** (vide supra), followed by preparative TLC of the obtained crude product on silica gel with eluent (VII), afforded 0.0020 g (38%) of pure **5a**, as a white, amorphous solid. Mp = 200–260°C (indefinite). Rf = 0.40 (VII). $[\alpha]_{589}^{25}$ −122; $[\alpha]_{578}^{25}$ −142; $[\alpha]_{546}^{25}$ −176; $[\alpha]_{436}^{25}$ −526; $[\alpha]_{365}^{25}$ −2162 (*c* 0.1; CH_2Cl_2). ESI$^+$ MS *m*/*z*: 1907.8 $[M+H]^+$, 854.9 $[M+2H]^{2+}$.

4.18. Electronic circular dichroism

Electronic CD spectra were recorded on a Jasco model J-715 spectropolarimeter. Cylindrical, fused quartz cells of 1.0 and 0.2 mm path lengths were employed. The data are expressed in terms of $[\theta]_T$, the total molar ellipticity (deg cm^{-2} dmol^{-1}). Spectrograde MeOH (Fluka), TFE (Acros), and $CHCl_3$ (Fluka) were used as solvents.

4.19. FT-IR absorption

The solution IR absorption spectra of the N$^\alpha$-protected (*S*)-Bin derivative and peptide esters in the 3550–3200 cm^{-1} region were recorded at UP using a Perkin–Elmer model 1720X FT-IR spectrophotometer, nitrogen flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Solvent (baseline) spectra were obtained under the same conditions. Cells with path lengths of 0.1, 1.0, and 10 mm (with CaF_2 windows) were used. Spectrograde deuterochloroform (99.8% d) was purchased from Fluka.

Samples of the N$^\alpha$-deprotected (*S*)-Bin peptide esters for IR absorption spectroscopy in the 1780–1480 cm^{-1} region were dissolved in deuterochloroform at both $\cong$3.6 and 18 mg mL^{-1} concentration and placed in a demountable cell (Specac) constructed of two CaF_2 windows separated by a 500 μm Teflon spacer. Additional experiments were performed in 98:2 (*v*/*v*) $CDCl_3$/TFE-OD (deuterated TFE). The spectra were recorded at UIC on a Digilab FTS-60 FT-IR spectrophotometer as an average of 256 scans at 4 cm^{-1} resolution with a zero filling factor of 4.

4.20. Vibrational circular dichroism

For the VCD spectroscopic study of the N$^\alpha$-deprotected (*S*)-Bin peptide esters, the same solutions were used as those described above for the FT-IR absorption investigation. VCD spectra over the amide I and II regions were obtained on the UIC dispersive VCD spectrophotometer, the design and the use of which have been previously described in detail.[25] Spectra were recorded at 10 cm^{-1} resolution and 10-s time constant, collected under the same experimental conditions, averaged for base line correction and subsequently subtracted from the averaged sample spectra.

4.21. Calculations

A series of MM energy minimisation calculations of the target molecule H-[(*S*)-Bin]$_4$-OMe were performed using the CFF and CVFF force fields as provided in the Cerius2 and InsightII program packages from MSI (San Diego, CA). These force fields are applicable to systems including peptide backbone and aromatic side chains. These MM calculations were used to determine possible equilibrium conformations of the tetrapeptide

ester molecule in vacuo. A large number of conformers, based on standard secondary structural types, were used as starting conformations for minimisations. The tetrapeptide ester structure consists of 178 atoms for which it was not possible for us to calculate the IR absorption and VCD spectra directly on an ab initio quantum mechanical level. However, it could be done in parts, peptide chain and side chains, and the results transferred to the complete molecule using our established techniques.[26] To simulate spectra for the backbone, ab initio calculations of the FF and intensity parameters (APT and AAT) were performed by means of DFT at the BPW91/6-31G** level using the Gaussian 98 program package.[27] Simulations were carried out for the H-$(Aib)_4$-OMe tetrapeptide ester as a model of the H-[(*S*)-Bin]$_4$-OMe backbone, but constrained to fixed ϕ, ψ angles from the most favourable conformation found in the MM calculations. All the remaining structural parameters were relaxed (including the ω angles). Separate DFT calculations were performed at the same level with full optimisation, for 6,6-dimethyl-2′,1′:1,2:1″,2″:3,4-dinaphthcyclohepta-1,3-diene, with two methyls on the central bridge carbon (equivalent to the Bin C^{α} atom), which was designed to mimic the electronic environment of the Bin side-chain group, to determine the spectral properties of the H-[(*S*)-Bin]$_4$-OMe peptide side-chain. Previously, it was shown that the BPW91 functional provides reliable simulations of IR absorption and VCD for larger spectral molecules[21a,28] as well as other systems.[29] The Cartesian FF, APT and AAT values from these binaphthyl side-chain and H-$(Aib)_4$-OMe backbone calculations were transferred onto appropriate position and with the proper orientation to match the complete H-[(*S*)-Bin]$_4$-OMe oligopeptide geometry using the previously described property tensor transfer technique.[26] Theoretical IR absorption and VCD spectra were simulated by placing Lorentzian bands with a half-bandwidth of 7 cm^{-1} on each transition scaled with the appropriate dipole (**D**) or rotational (*R*) strength, respectively.

Acknowledgements

One of the authors (T.A.K.) wishes to thank the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for a grant in partial support of this work. Thanks are due to Dr. P. Bour for discussion of the theoretical modelling.

References

1. For leading review-articles on $C^{\alpha,\alpha}$-disubstituted glycines and their peptides, see: (a) Marshall, G. R. In *Intra-Science Chemistry Report*; Kharasch, N., Ed.; Gordon and Breach: New York, 1971; pp. 305–316; (b) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747–6756; (c) Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, *24*, 4004–4009; (d) Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350–353; (e) Balaram, P. *Curr. Opin. Struct. Biol.* **1992**, *2*, 845–851; (f) Toniolo, C. *Janssen Chim. Acta* **1993**, *11*, 10–16; (g) Benedetti, E. *Biopolymers* **1996**, *40*, 3–44; (h) Karle, I. L. *Biopolymers* **1996**, *40*, 157–180; (i) Karle, I. L. *Acc. Chem. Res.* **1999**, *32*, 693–701; (j) Andrews, M. J. I.; Tabor, A. B. *Tetrahedron* **1999**, *55*, 11711–11743; (k) Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. *Biopolymers (Peptide Sci.)* **2001**, *60*, 396–419; (l) Toniolo, C.; Benedetti, E. In *Molecular Conformation and Biological Interactions*; Balaram, P.; Ramaseshan, S., Eds.; Indian Academy of Sciences: Bangalore, 1991; pp. 511–521.
2. (a) Valle, G.; Crisma, M.; Bonora, G. M.; Toniolo, C.; Lelj, F.; Barone, V.; Fraternali, F.; Hardy, P. M.; Langran-Goldsmith, A.; Maia, H. L. S. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1481–1487; (b) Crisma, M.; Valle, G.; Bonora, G. M.; Toniolo, C.; Lelj, F.; Barone, V.; Fraternali, F.; Hardy, P. M.; Maia, H. L. S. *Biopolymers* **1991**, *31*, 637–641.
3. (a) Valle, G.; Crisma, M.; Toniolo, C.; Rao, R. B.; Sukumar, M.; Balaram, P. *Int. J. Peptide Protein Res.* **1991**, *38*, 511–518; (b) Toniolo, C.; Crisma, M.; Formaggio, F.; Benedetti, E.; Santini, A.; Iacovino, R.; Saviano, M.; Di Blasio, B.; Pedone, C.; Kamphuis, J. *Biopolymers* **1996**, *40*, 519–522; (c) Benedetti, E.; Di Blasio, B.; Iacovino, R.; Menchise, V.; Saviano, M.; Pedone, C.; Bonora, G. M.; Ettorre, A.; Graci, L.; Formaggio, F.; Crisma, M.; Valle, G.; Toniolo, C. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2023–2032.
4. (a) Mazaleyrat, J.-P.; Gaucher, A.; Wakselman, M.; Tchertanov, L.; Guilhem, J. *Tetrahedron Lett.* **1996**, *37*, 2971–2974; (b) Mazaleyrat, J.-P.; Gaucher, A.; Šavrda, J.; Wakselman, M. *Tetrahedron: Asymmetry* **1997**, *8*, 619–631; (c) Mazaleyrat, J.-P.; Gaucher, A.; Wakselman, M.; Toniolo, C.; Crisma, M.; Formaggio, F. In *Peptides* 1996; Ramage, R.; Epton, R., Eds.; Mayflower Scientific: Kingswinford, UK, 1998; pp. 623–624.
5. (a) Formaggio, F.; Crisma, M.; Toniolo, C.; Mazaleyrat, J.-P.; Wakselman, M. In *Peptides* 1998; Bajusz, S.; Hudecz, F., Eds.; Akadémiai Kiado: Budapest, 1999; pp. 352–353; (b) Formaggio, F.; Crisma, M.; Toniolo, C.; Tchertanov, L.; Guilhem, J.; Mazaleyrat, J. P.; Gaucher, A.; Wakselman, M. *Tetrahedron* **2000**, *56*, 8721–8734.
6. (a) Mazaleyrat, J.-P.; Boutboul, A.; Lebars, Y.; Gaucher, A.; Wakselman, M. *Tetrahedron: Asymmetry* **1998**, *9*, 2701–2713; (b) Formaggio, F.; Peggion, C.; Crisma, M.; Toniolo, C.; Tchertanov, L.; Guilhem, J.; Mazaleyrat, J. P.; Goubard, Y.; Gaucher, A.; Wakselman, M. *Helv. Chim. Acta* **2001**, *84*, 481–501.
7. For reviews on synthesis, molecular recognition, asymmetric catalysis and new materials involving 1,1′-binaphthyl monomers, dimers, oligomers and polymers, see: (a) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1009–1020; (b) Whitesell, J. K. *Chem. Rev.* **1989**, *89*, 1581–1590; (c) Noyori, R.; Takaya, H. *Acc. Chem. Res.* **1990**, *23*, 345–350; (d) Rosini, C.; Francini, L.; Raffaelli, A.; Salvadori, P. *Synthesis* **1992**, 503–517; (e) Blaser, H.-U. *Chem. Rev.* **1992**, *92*, 935–952; (f) Shibasaki, M.; Sasai, H.; Arai, T. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1236–1256; (g) Pu, L. *Chem. Rev.* **1998**, *98*, 2405–2494; (h) Putala, M. *Enantiomer* **1999**, *4*, 243–262.
8. The Bin residue has been used as fluorophore in photophysical studies involving intramolecular energy transfer (fluorescence quenching) and intramolecular spin polarisation (CIDEP) effects in designed, rigid peptide-based systems: (a) Toniolo, C.; Formaggio, F.; Crisma, M.; Mazaleyrat, J. P.; Wakselman, M.; George, C.; Deschamps, J. R.; Flippen-Anderson, J. L.; Pispisa, B.; Venanzi, M.; Palleschi, A. *Chem. Eur. J.* **1999**, *5*, 2254–

2264; (b) Corvaja, C.; Sartori, E.; Toffoletti, A.; Formaggio, F.; Crisma, M.; Toniolo, C.; Mazaleyrat, J. P.; Wakselman, M. *Chem. Eur. J.* **2000**, *6*, 2775–2782.
9. (a) Leplawy, M. T.; Jones, D. S.; Kenner, G. W.; Sheppard, R. C. *Tetrahedron* **1960**, *11*, 39–51; (b) Jones, D. S.; Kenner, G. W.; Preston, J.; Sheppard, R. C. *J. Chem. Soc.* **1965**, 6227–6239; (c) Slomczynska, U.; Beusen, D. D.; Zabrocki, J.; Kociolek, K.; Redlinski, A.; Reusser, F.; Hutton, W. C.; Leplawy, M. T.; Marshall, G. R. *J. Am. Chem. Soc.* **1992**, *114*, 4095–4106; (d) Brückner, H. In *Chemistry of Peptides and Proteins*; König, W. A.; Voelter, W., Eds.; Attempto: Tübingen, 1989; pp. 79–86.
10. Abele, S.; Guichard, G.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 2141–2156.
11. (a) Akaji, K.; Tamai, Y.; Kiso, Y. *Tetrahedron Lett.* **1995**, *36*, 9341–9344; (b) Akaji, K.; Kuriyama, N.; Kiso, Y. *J. Org. Chem.* **1996**, *61*, 3350–3357.
12. Abdel-Magid, A. F.; Cohen, J. H.; Maryanoff, C. A.; Shah, R. D.; Villani, F. J.; Zhang, F. *Tetrahedron Lett.* **1998**, *39*, 3391–3394.
13. (a) Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; DeSelms, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 9651–9652; (b) Carpino, L. A.; Beyermann, M.; Wenschuh, H.; Bienert, M. *Acc. Chem. Res.* **1996**, *29*, 268–274; (c) Fiammengo, R.; Licini, G.; Nicotra, A.; Modena, G.; Pasquato, L.; Scrimin, P.; Broxterman, Q. B.; Kaptein, B. *J. Org. Chem.* **2001**, *66*, 5905–5910.
14. (a) Polese, A.; Formaggio, F.; Crisma, M.; Valle, G.; Toniolo, C.; Bonora, G. M.; Broxterman, Q. B.; Kamphuis, J. *Chem. Eur. J.* **1996**, *2*, 1104–1111; (b) Gratias, R.; Konat, R.; Kessler, H.; Crisma, M.; Valle, G.; Polese, A.; Formaggio, F.; Toniolo, C.; Broxterman, Q. B.; Kamphuis, J. *J. Am. Chem. Soc.* **1998**, *120*, 4763–4770.
15. For a recent review-article on the coupling methods used in the case of $C^{\alpha,\alpha}$-disubstituted glycines, see: Formaggio, F.; Broxterman, Q. B.; Toniolo, In *Houben-Weyl: Methods of Organic Chemistry*, Vol. E 22c. *Synthesis of Peptides and Peptidomimetics*; Goodman, M.; Felix, A.; Moroder, L.; Toniolo, C., Eds.; Thieme: Stuttgart, 2003; pp. 292–310.
16. We chose to prepare Fmoc-(*S*)-Bin-F, rather than Z-(*S*)-Bin-F, for its potential use in solid-phase peptide synthesis.
17. *N*-Protection of H-[(*S*)-Bin]-OMe **1a** to Boc-[(*S*)-Bin]-OMe **1d** seems to be unnecessary since the Boc group is to be removed and replaced by a Fmoc group in the next steps. However, it allowed a further ultimate purification, as the sample of **1a** obtained after resolution was contaminated by traces of the side product H-[(*S*)-Bin]-O-$(CH_2)_2$-O-$(CH_2)_2$-OH.[6a]
18. Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy. Exciton Coupling in Organic Stereochemistry*; Oxford University Press: Oxford, 1983.
19. Keiderling, T. A.; Silva, R. A. G. D.; Yoder, G.; Dukor, R. K. *Bioorg. Med. Chem.* **1999**, *7*, 133–141.
20. (a) Mizushima, S.; Shimanouchi, T.; Tsuboi, M.; Souda, R. *J. Am. Chem. Soc.* **1952**, *74*, 270–271; (b) Bonora, G. M.; Mapelli, C.; Toniolo, C.; Wilkening, R. R.; Stevens, E. S. *Int. J. Biol. Macromol.* **1984**, *6*, 179–188.
21. (a) Kubelka, J.; Silva, R. A. G. D.; Keiderling, T. A. *J. Am. Chem. Soc.* **2002**, *124*, 5325–5332; (b) *Peptide and Protein Conformational Studies with Vibrational Circular Dichroism and Related Spectroscopies*, 2nd ed.; Keiderling, T. A.; Berova, N.; Nakanishi, K.; Woody, R. A., Eds.; Wiley-VCH: New York, 2000; pp. 621–666; (c) Yoder, G.; Keiderling, T. A.; Formaggio, F.; Crisma, M.; Toniolo, C.; Kamphuis, J. *Tetrahedron: Asymmetry* **1995**, *6*, 687–690.
22. (a) Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425–1436; (b) Toniolo, C. *C.R.C. Crit. Rev. Biochem.* **1980**, *9*, 1–44; (c) Rose, G. D.; Gierasch, L. M.; Smith, J. P. *Adv. Protein Chem.* **1985**, *37*, 1–109.
23. (a) Kennedy, D. F.; Crisma, M.; Toniolo, C.; Chapman, D. *Biochemistry* **1991**, *30*, 6541–6548; (b) Yasui, S. C.; Keiderling, T. A. *Biopolymers* **1986**, *25*, 5–15; (c) Yasui, S. C.; Keiderling, T. A.; Formaggio, F.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1986**, *108*, 4988–4993.
24. (a) Silva, R. A. G. D.; Yasui, S. C.; Kubelka, J.; Formaggio, F.; Crisma, M.; Toniolo, C.; Keiderling, T. A. *Biopolymers* **2002**, *65*, 229–243; (b) Yoder, G.; Polese, A.; Silva, R. A. G. D.; Formaggio, F.; Crisma, M.; Broxterman, Q. B.; Kamphuis, J.; Toniolo, C.; Keiderling, T. A. *J. Am. Chem. Soc.* **1997**, *119*, 10278–10285; (c) Yasui, S. C.; Keiderling, T. A.; Bonora, G. M.; Toniolo, C. *Biopolymers* **1986**, *25*, 79–89; (d) Yasui, S. C.; Keiderling, T. A. *J. Am. Chem. Soc.* **1986**, *108*, 5576–5581; (e) Baumruk, V.; Huo, D. F.; Dukor, R. K.; Keiderling, T. A.; Lelievre, D.; Brack, A. *Biopolymers* **1994**, *34*, 1115–1121; (f) Nafie, L. A. *Adv. Infrared Raman Spectr.* **1984**, *11*, 49–93; (g) Freedman, T. B.; Nafie, L. A.; Keiderling, T. A. *Biopolymers* **1995**, *37*, 265–279; (h) Keiderling, T. A. *Appl. Spectrosc. Rev.* **1981**, *17*, 189–226.
25. (a) *Vibrational Circular Dichroism of Peptides and Proteins*; Keiderling, T. A.; Gremlich, H. U.; Yan, B., Eds.; Dekker: New York, 2001; Vol. 24, pp. 55–100; (b) *Vibrational Circular Dichroism: Comparison of Techniques and Practical Considerations*; Keiderling, T. A.; Krishnan, K.; Ferraro, J. R., Eds.; Academic Press: New York, 1990; pp. 203–284.
26. Bour, P.; Sopkova, J.; Bednarova, L.; Malon, P.; Keiderling, T. A. *J. Comput. Chem.* **1997**, *18*, 646–659.
27. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 1998*, Gaussian Inc., Pittsburgh, PA, 1998. Revision A. 6th ed.
28. (a) Kubelka, J.; Keiderling, T. A. *J. Am. Chem. Soc.* **2001**, *123*, 6142–6150; (b) Kubelka, J.; Keiderling, T. A. *J. Am. Chem. Soc.* **2001**, *123*, 12048–12058; (c) Kubelka, J. In *IR and VCD Spectroscopy of Model Peptides. Theory and Experiment*; Kubelka, J., Ed.; University of Illinois at Chicago: Chicago, 2002.
29. (a) Bour, P.; Kapitán, J.; Baumruk, V. *J. Phys. Chem. A* **2001**, *105*, 6362–6368; (b) Bour, P.; Zaruba, K.; Urbanova, M.; Setnicka, V.; Matejka, P.; Fiedler, Z.; Kral, V.; Volka, K. *Chirality* **2000**, *12*, 191–198; (c) Bour, P.; McCann, J.; Wieser, H. J. *J. Phys. Chem. A* **1998**, *102*, 102–110; (d) Bour, P.; McCann, J.; Wieser, H. J. *J. Phys. Chem. A* **1997**, *101*, 9783–9790.