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Bip: a C^{α}-Tetrasubstituted, Axially Chiral α-Amino Acid. Synthesis and Conformational Preference of Model Peptides

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Abstract—By using the recently proposed biphenyl-based, C^{α}-tetrasubstituted, cyclic, axially chiral α-amino acid Bip we synthesised by solution methods a large set of model peptides, including the homo-oligomer series, to the pentamer level. All of the peptides were fully characterised and their preferred conformation was assessed in solution by means of a FT-IR absorption and ^{1}H NMR study. Results of X-ray diffraction analyses of two Bip derivatives and a terminally protected tripeptide with the sequence –Gly–Bip–Gly– are also presented. Our findings indicate that Bip tends to support β-turn and 3_{10}-helical structures, although in short peptides the fully-extended (C_5) conformation would also be populated to some extent.

Introduction

The last twenty years have witnessed a steady stream of new synthetic approaches to and conformational investigations of peptidomimetics, compounds that replace physiologically vulnerable peptide functionalities with chemical modules of increased stability and cellular penetration.[1] A generally applicable method for development of peptidomimetics involves preparation of conformationally restricted analogues. At the local level this target can be achieved by a modification of individual residues via incorporation of rigid structural elements, e.g. C^{α}-tetrasubstituted α-amino acids.[1g] In particular, in our ongoing study of the conformational preferences of peptides rich in this type of sterically demanding building block[2] we have already shown that peptides based on $C^{\alpha,\alpha}$-dibenzylglycine (Db_zg)[3] (Fig. 1) strongly prefer the fully-extended (C_5) conformation,[4] whereas those rich in 1-aminocycloheptane-1-carboxylic acid (Ac_7c)[5] (Fig. 1) exhibit a very high tendency to fold into β-turns[4a,6] and 3_{10}-helices.[7]

In this work we describe the synthesis and preferred conformation of peptides characterised by Bip (2′,1′: 1,2; 1″,2″: 3,4-dibenzcyclohepta-1,3-diene-6-amino-6-carboxylic acid) (Fig. 1), a C^{α}-tetrasubstituted α-amino acid which combines structural features of both Db_zg and Ac_7c.[8] More specifically, model peptides based on Bip/(*S*)-Ala and Bip/Gly sequences, along with Bip homo-oligomers, to the pentapeptide level have been synthesised by solution methods and fully characterised, and their conformation investigated in solution by FT-IR absorption and ^{1}H NMR techniques. X-Ray diffraction structures of two Bip derivatives and a terminally protected tripeptide with the sequence -Gly-Bip-Gly- have also been solved. In Bip, a mimic of the coded Phe residue, the chirality imposed by the biaryl axis (atropoisomerism) represents an additional, versatile tool

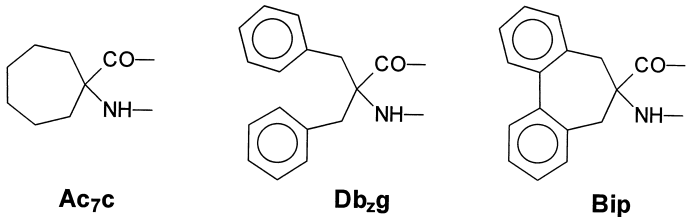

Figure 1. Chemical structure of Bip compared to those of Ac_7c and Db_zg.

Keywords: amino acids and derivatives; NMR; peptides; X-ray crystal structures.
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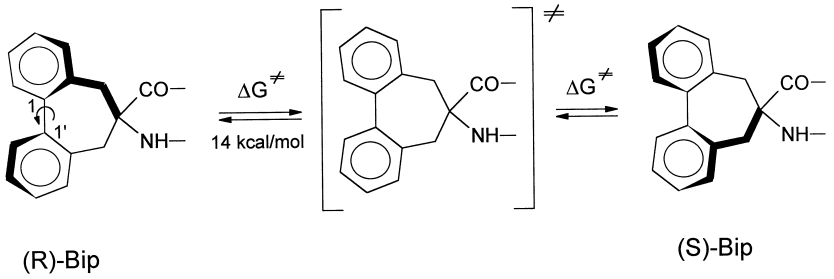

Figure 2. Interconversion between (*R*) and (*S*)-Bip.

for conformational restriction. However, the enantiomers of Bip and its derivatives have already been shown to interconvert in solution at room temperature.[8b] Preliminary accounts of a limited part of this work have been reported.[8a,9] The synthesis of a ring-dimethylated analogue of Bip has been published by Zavada and coworkers.[10]

Results and Discussion

Amino acid and peptide synthesis

The synthesis and characterization of Bip and its derivatives H-Bip-O*t*Bu (O*t*Bu, *tert*-butoxy), Boc-Bip-OH (Boc, *tert*-butyloxycarbonyl) and Z-Bip-OH (Z, benzyloxycarbonyl) used in this work were already reported.[8] Briefly, C^{α}-*bis*-alkylation of a Schiff base from H-Gly-O*t*Bu by 2,2′-*bis*(bromomethyl)-1,1′-biphenyl under phase-transfer conditions led to good yields (75%) of the α-amino ester H-Bip-O*t*Bu. Acidolysis of the amino ester in a 1:1 TFA/CH_2Cl_2 (TFA, trifluoroacetic acid) solution gave the free amino acid. The N^{α}-protected derivatives were prepared by standard procedures.

For the conformationally labile Bip and its derivatives broadened ^{1}H NMR signals were generally observed at room temperature, indicating a slow interconversion on the NMR time scale between the two conformers [(*R*)- and (*S*)-enantiomers] resulting from rotation about the C^1–$C^{1\prime}$ bond of the biphenyl moiety. The calculated rotational energy barrier is 14 kcal mol^{-1} (Fig. 2).[8b]

In this work the Bip/Ala and Bip/Gly peptides have been prepared via the step-by-step strategy in solution starting

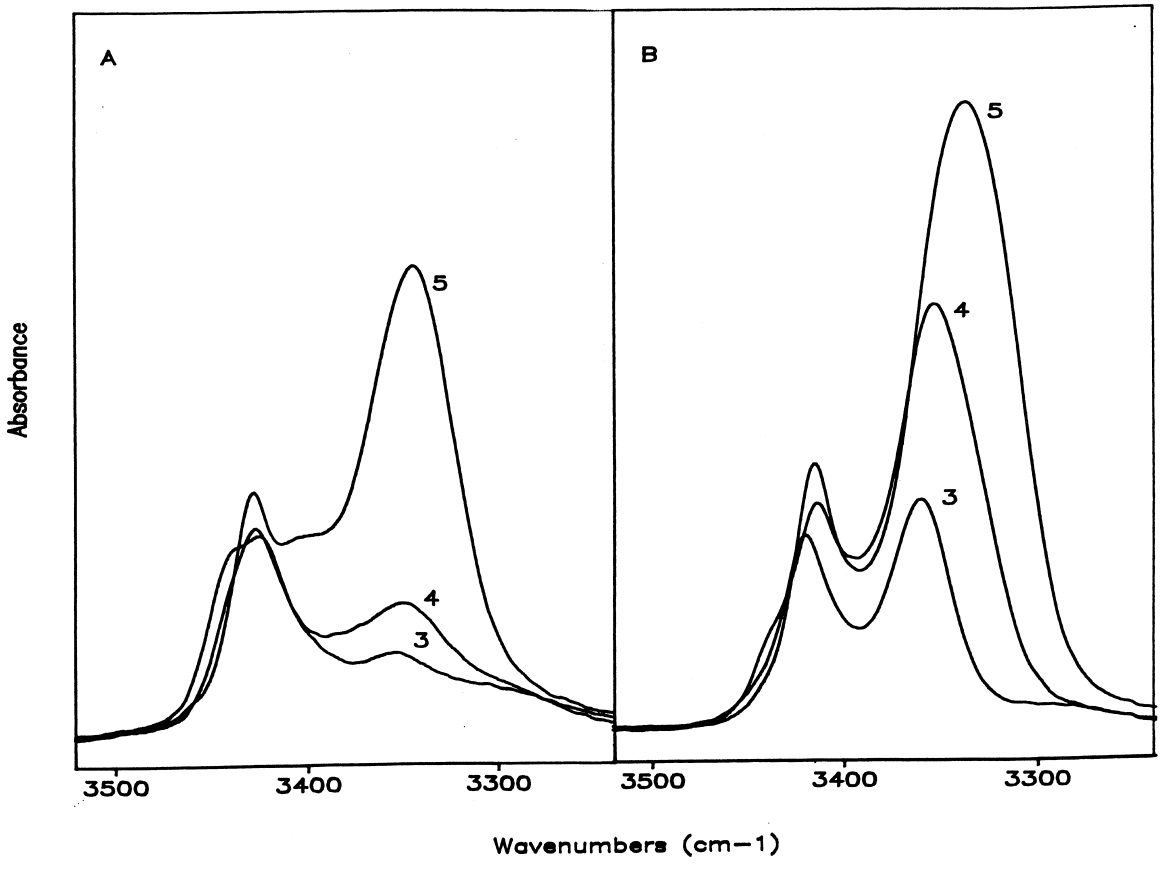

Figure 3. FT-IR absorption spectra (3500–3200 cm^{-1} region) in $CDCl_3$ solution of (**A**): Boc-(*S*)-Ala-Bip-(*S*)-Ala-OMe (**3**), Boc-[(*S*)-Ala]$_2$-Bip-(*S*)-Ala-OMe (**4**), and Z-Bip-[(*S*)-Ala]$_2$-Bip-(*S*)-Ala-OMe (**5**); (**B**): Z-(Bip)$_3$-O*t*Bu (**3**), Z-(Bip)$_4$-O*t*Bu (**4**), and Z-(Bip)$_5$-O*t*Bu (**5**). Peptide concentration: 1.0 mM.

from the C-terminus. Bip–Ala, Bip–Gly, Ala–Ala and Gly–Gly peptide bond formation has been achieved by the DCC/HOBt[11a] (DCC, *N*,*N*′-dicyclohexylcarbodiimide; HOBt, 1-hydroxy-1,2,3-benzotriazole) or the EDC/HOBt [EDC, *N*-ethyl,*N*′-(3-dimethylaminopropyl)-carbodiimide] method, while Ala–Bip and Gly–Bip couplings have been performed by the symmetrical anhydride procedure. Removal of the N^{α}-protecting Z-group was obtained by treatment with a 33% HBr/AcOH (acetic acid) solution, while removal of the Boc group was achieved using either a HCl/EtOAc (ethyl acetate) or a 1:1 TFA/CH_2Cl_2 solution. The fully-protected Bip homo-dipeptide has been prepared by means of the pivaloyl mixed anhydride method.[11b,c] The *tert*-butyl ester moiety has been removed by acidolysis (TFA/CH_2Cl_2 1:1). Then, a coupling strategy from the *N*-terminus, involving carboxyl group activation through the 5(4*H*)-oxazolone intermediate,[11b,c] has been used. Acylation of H-Bip-O*t*Bu by the oxazolones from Z-$(Bip)_{2\text{-}4}$-OH has required several days, but has given acceptable yields (60%) of the fully protected homo-oligomers Z-$(Bip)_n$-O*t*Bu (n=3–5), with the unreacted starting oxazolones generally also recovered.

Solution conformational analysis

A solution conformational analysis of the terminally protected Bip/Ala, Bip/Gly and $(Bip)_n$ peptides was carried out by using FT-IR absorption and ^{1}H NMR techniques in a structure-supporting solvent ($CDCl_3$). The longest peptides (pentamers) were also examined as a function of concentration and heating.

Fig. 3 shows the FT-IR absorption spectra in the conformationally informative 3500–3250 cm^{-1} (N–H stretching) region of the Bip/Ala and $(Bip)_n$ peptide series, while in Fig. 4 the spectra of four Bip/Ala and Bip/Gly tripeptides are illustrated. We assign: (i) The high-frequency band(s) found at ≥3420 cm^{-1} to free, solvated N–H groups. (ii) The medium-frequency band (shoulder) near 3405 cm^{-1} to weakly intramolecularly H-bonded N–H groups of fully-extended (C_5) conformers. (iii) The low-frequency band at 3360–3335 cm^{-1} to more strongly, intramolecularly H-bonded N–H groups of folded conformers.[12]

No marked differences are observed in the spectra of the conformationally relevant pentapeptides by heating the $CDCl_3$ solution to 50°C (data not shown). This result may suggest that Bip isomer inter-conversion and the related formation of diastereomeric compounds, that are expected to be facilitated at higher temperature, do not significantly alter the overall peptide conformational properties. In both peptide series the intensity of the low-frequency band, relative to the high-frequency band, tends to increase with peptide main-chain lengthening (Fig. 3). While this effect is gradual in the homo-peptide series, an abrupt enhancement is seen in the Bip/Ala series between the tetra- and pentapeptides. As in this series the intensity enhancement is rather modest between the tri- and tetrapeptides, these results, taken together, strongly support the view that the extent of folding is more enhanced as a consequence of the incorporation of an *N*-terminal Bip than an Ala residue. Concomitantly, the absorption maximum shifts to lower wavenumbers. In addition, a visual inspection of the spectra

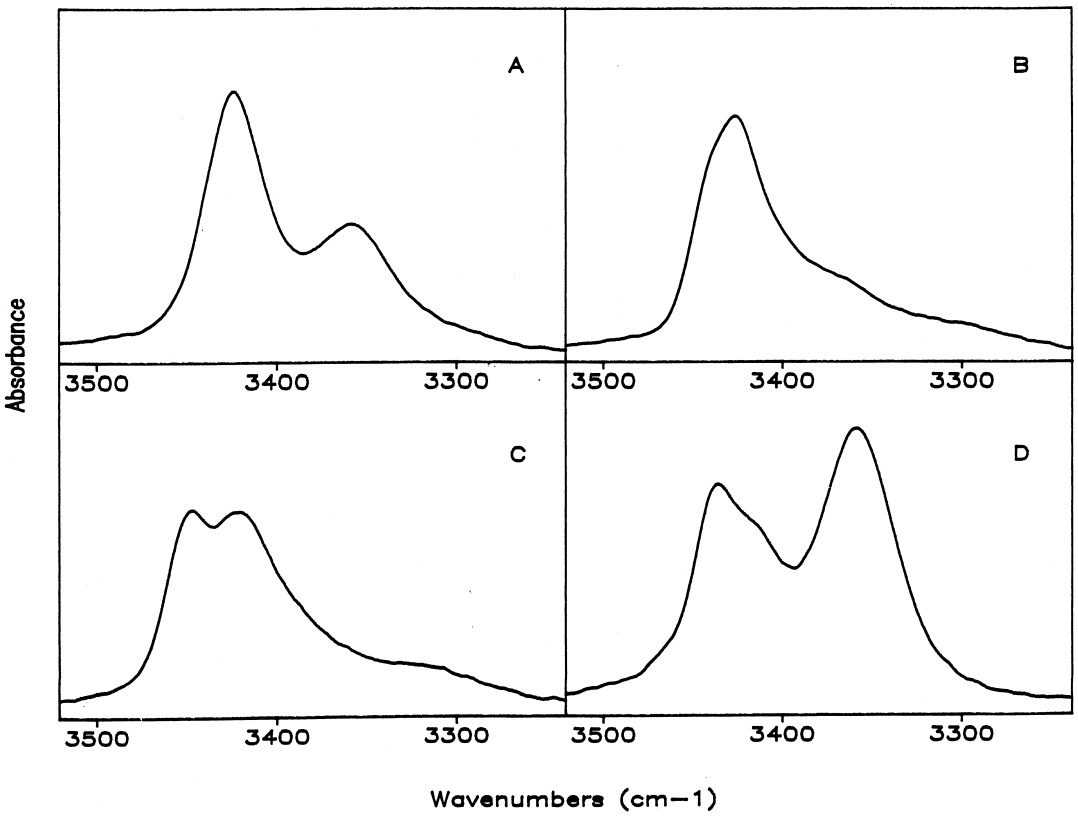

Figure 4. FT-IR absorption spectra (3500–3200 cm^{-1} region) in $CDCl_3$ solution of Z-Bip-[(*S*)-Ala]$_2$-OMe (**A**), Z-(*S*)-Ala-Bip-(*S*)-Ala-OMe (**B**), Z-Bip-$(Gly)_2$-OEt (**C**), and Z-Gly-Bip-Gly-OEt (**D**), Peptide concentration: 1.0 mM.

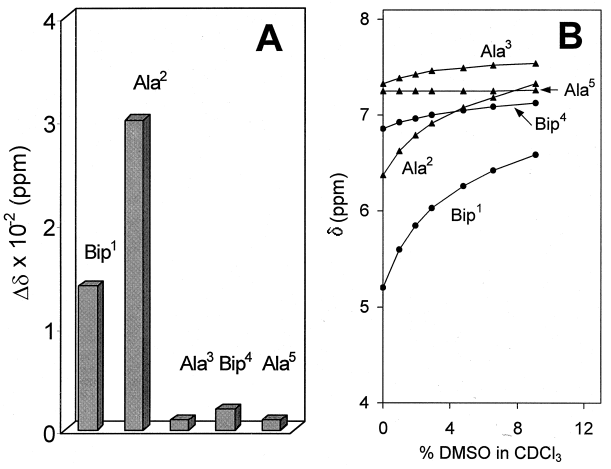

Figure 5. Plots of NH chemical shifts in the ^{1}H NMR spectra of Z-Bip-[(*S*)-Ala]$_2$-Bip-(*S*)-Ala-OMe at 323 K as a function of: (**A**) peptide concentration (between 10 mM and 1 mM), and (**B**) increasing percentages of DMSO added to the $CDCl_3$ solution (*v*/*v*) (peptide concentration: 1.0 mM).

of the four tripeptides (Fig. 4) clearly indicates that the amount of folding largely depends on a combination of type of protein amino acid and position of the Bip residue in the sequence. Interestingly, a remarkably high population of fully-extended conformers is seen in the –Bip–Gly–Gly– tripeptide.

To better understand the conformational tendency of the Bip residue in $CDCl_3$ solution we performed a ^{1}H NMR investigation of the pentapeptide Z-Bip-[(*S*)-Ala]$_2$-Bip-(*S*)-Ala-OMe (OMe, methoxy) (Fig. 5). To markedly reduce the signal broadening effect associated with Bip isomer interconversion this study was performed at 50°C. All NH proton resonances were unambiguously assigned by means of 2D ROESY experiments, starting from the urethane N(1)H proton known to resonate at higher fields. The pentapeptide weakly self-associates above 1.0 mM concentration and in this process only the N(1)H and N(2)H protons are involved. A detailed conformational analysis was carried out at 1.0 mM peptide concentration where self-association is absent. The participation of specific NH groups in intramolecular H-bonding was assessed by examining the behaviour of the NH resonances upon addition of the perturbing agent DMSO (dimethyl sulphoxide), a strong H-bonding acceptor solvent, to the $CDCl_3$ solution.[13] It is noteworthy that only the N(1)H and N(2)H proton chemical shifts are significantly sensitive to the addition of DMSO. All other NH protons display a behaviour characteristic of shielded protons, as their chemical shifts appear relatively insensitive to solvent composition. From our ^{1}H NMR analysis it is reasonable to conclude that the most populated conformation adopted by the terminally protected Bip/Ala pentapeptide is the 3_{10}-helix (originated by three consecutive β-turn conformations), as in this ordered secondary structure only the two N-terminal NH protons do not participate in the intramolecular H-bonding scheme. Two views of a molecular model of the pentapeptide in the 3_{10}-helical conformation are shown in Fig. 6. They clearly illustrate the overlapping of the two Bip side chains one on top of the other after one complete turn of the ternary helix.

Crystal-state conformational analysis

The molecular and crystal-structure of two Bip derivatives, H-Bip-O*t*Bu and Boc-Bip-O⊖, and one terminally protected Bip tripeptide, Z-Gly-Bip-Gly-OEt (OEt, ethoxy), were determined by X-ray diffraction. The molecular structures with the atomic numbering schemes are shown in Figs. 7 and 8. Selected N^{α}-protecting group, backbone and side-chain torsion angles[14] are given in Table 1. In Table 2 the intra- and intermolecular H-bond parameters are listed. Because of the relatively low rotation energy barrier about the C^1–$C^{1\prime}$ bond the enantiomers of the three compounds could not be separated, but rather they coexist in the crystals. For consistency, the torsion angles reported for all three compounds refer to the (*S*) isomer.

In H-Bip-O*t*Bu the neutral amino group has a pyramidal structure, the sum of bond angles at nitrogen being ≅327°. The Bip ψ_1 torsion angle is typical of a folded conformation. The ester moiety is *trans* planar (ω_1 torsion angle).[15a]

In the N^{α}-Boc derivative the Bip ϕ_1 torsion angle indicates a folded conformation. The *trans*, *trans* arrangement of the Boc–NH– moiety ($\omega_0$and θ^1 torsion angles) is that commonly reported for Boc-protected peptides (type *b* conformation).[15b]

The 1–2 sequence of the tripeptide is S-shaped. Indeed, Gly^1 is distorted helical, while Bip^2 is regular helical, but the handedness of the turn conformation is opposite in the two residues. The conformation of the C-terminal Gly residue is *semi*-extended. As described above for the Boc-derivative, the conformation of the related Z-NH- moiety in the tripeptide is the usual *trans*, *trans* (type *b*).[15c] The

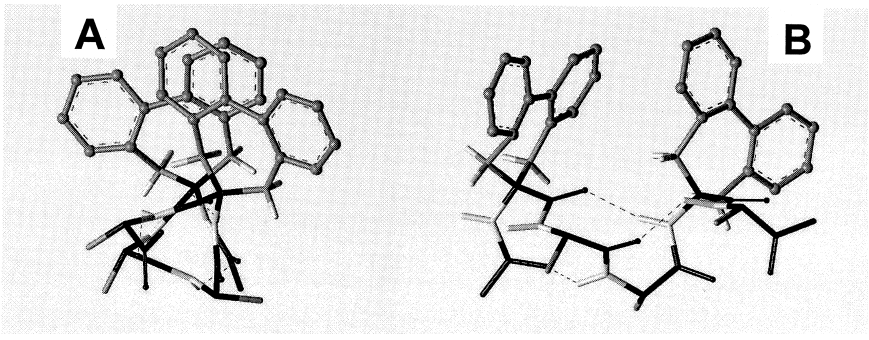

Figure 6. Molecular model of the 3_{10}-helical structure formed by the pentapeptide sequence -(*S*)-Bip-[(*S*)-Ala]$_2$-(*S*)-Bip-(*S*)-Ala-. (**A**) Top view; (**B**) side view.

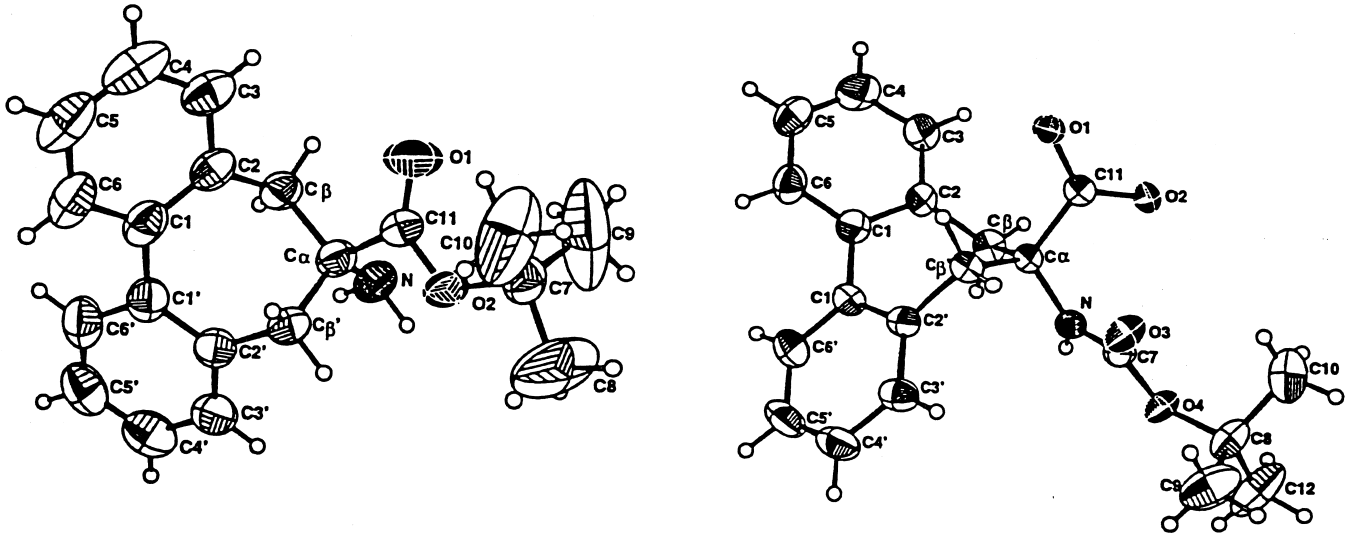

Figure 7. ORTEP views of the H-Bip-O*t*Bu molecule (left) and the Boc-Bip-O⊖ molecule (right) with numbering of the atoms. In both cases only the structure of the (*S*)-Bip isomer is shown. Anisotropic displacement ellipsoids are drawn at the 50% probability level.

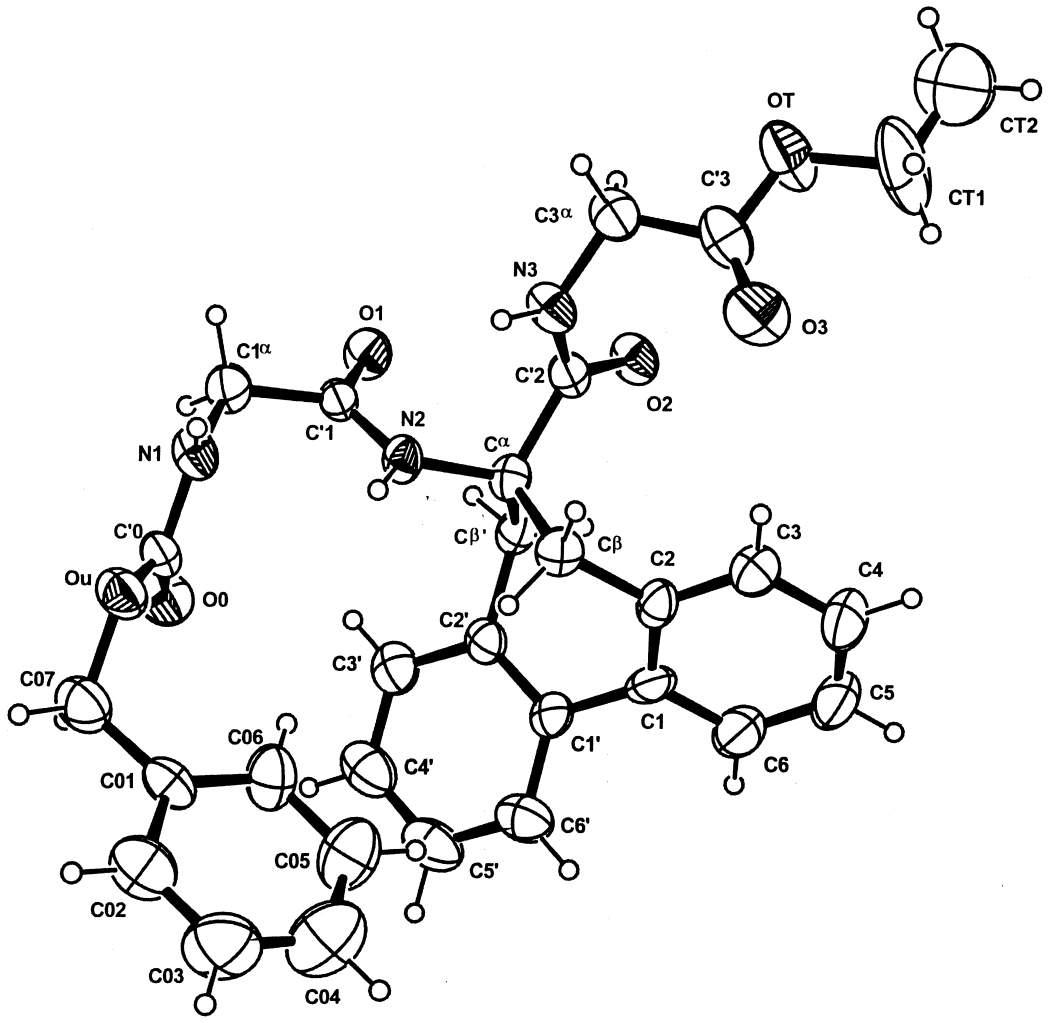

Figure 8. ORTEP view of the Z-Gly-Bip-Gly-OEt molecule with numbering of the atoms. Only the structure of the (*S*)-Bip isomer is shown. Anisotropic displacement ellipsoids are drawn at the 30% probability level.

peptide,[15d,e] and ester[15a] groups (ω_1, ω_2, and ω_3 torsion angles) are *trans* planar.

It is worth mentioning that in the N^{α}-Boc derivative and the tripeptide the sign of the Bip ϕ torsion angle is positive, thus implying a left-handed screw sense preference for the (*S*) isomer.

An analysis of the geometry of the Bip residues in the three structures shows that tricyclic skeleton deformations, compared to the saturated ring of the parent Ac_7c, may be produced by unfavourable steric interactions. More specifically, in each Bip residue the seven-membered cycle is pseudo-symmetrical with a non crystallographic C_2 axis passing through C^{α} and the middle of the opposite bond, and the two phenyl rings being not coplanar [the dihedral angle between the planes of the two rings is about 45° in the two derivatives and 48.0(1)° in the tripeptide]. The torsion angle about the C^1–$C^{1\prime}$ bond is 46.6(5)° in H-Bip-O*t*Bu, 46.7(4)° in Boc-Bip-O⊖, and 46.6(3)° in the tripeptide. The conformation of the seven-membered ring in the three compounds is close to a twist-boat (TB),[16] with a bisectional arrangement of the C^{α}–N and C^{α}–C' bonds relative to the average ring plane. The puckering parameters[17] are Q_T=1.028(4) Å, ϕ_2=−87.5(2)°, ϕ_3=−76.7(16)°, and θ_2=82.6(2)° for H-Bip-O*t*Bu; Q_T=1.049(3) Å, ϕ_2=−86.8(1)°, ϕ_3=−81.8(11)°, and θ_2=82.8(1) for Boc-Bip-O⊖; and Q_T=1.058(4) Å, ϕ_2=−89.1(2)°, ϕ_3=−88.5(20)°, and θ_2=82.7(2)° for the tripeptide. According to literature data, in the family of conjugated cycloheptadienes the seven-membered ring adopts a boat or a flat boat conformation.[18] The vicinal C=C double bond moiety is intermediate between the *cis* and *gauche* conformations. If two aromatic rings are vicinally fused to cycloheptane, they are rotated about the joining C^1–$C^{1\prime}$ bond forming a *gauche* conformation with a torsion angle in the range ±45–50°. All seven-membered rings of this type are classified as distorted boats, most of them adopting a conformation with the two torsion angles about the fusion bonds (C^{β}–C^2–C^1–$C^{1\prime}$ and $C^{\beta\prime}$–$C^{2\prime}$–$C^{1\prime}$–C^1) close to 0°. Indeed, the absolute values observed for these two torsion angles in our three compounds are in the range 0.4–4.9°.

The H-bonding scheme in H-Bip-O*t*Bu is determined by a single weak interaction of the pyramidal α-NH_2 group as a donor with the ester carbonyl O1=C′1 as an acceptor.[19] The

Table 1. Selected N^{α}-protecting group backbone and side-chain torsion angles (deg) for the Bip derivatives and peptide (the torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ, ω) are described in Ref. 14. For the torsion angles for rotation about bonds of the Boc- and Z-protecting groups (θ^1 and θ^2) see Ref. 15b and 15c, respectively)

Torsion angle	H-Bip-O*t*Bu [a]	Boc-Bip-O⊖ [a] water/ methanol solvate	Z-Gly-Bip-Gly-Oet [a]
N^{α}-protecting group			
θ^2			96.3(5)
θ^1		169.7(2)	−177.5(4)
ω_0		−168.9(2)	−173.3(4)
Backbone			
ϕ_1		58.9(3)	−87.3(6)
ψ_1	−72.5(3)[b]		−6.2(7)
ω_1	176.1(3)[c]		−179.8(4)
ϕ_2			59.9(6)
ψ_2			43.9(6)
ω_2			171.5(4)
ϕ_3			−71.6(6)
ψ_3			159.3(5)
ω_3			178.5(5)
Bip side chain			
C^{α}–C^{β}–C^{2}–C^{1}	−71.1(4)	−73.3(3)	−76.4(4)
C^{α}–$C^{\beta\prime}$–$C^{2\prime}$–$C^{1\prime}$	−74.1(4)	−75.0(3)	−75.6(4)
C^{β}–C^{2}–C^{1}–$C^{1\prime}$	3.3(5)	4.9(4)	4.0(4)
$C^{\beta\prime}$–$C^{2\prime}$–$C^{1\prime}$–C^{1}	1.1(5)	0.4(4)	1.9(4)
C^{2}–C^{1}–$C^{1\prime}$–$C^{2\prime}$	46.6(5)	46.7(4)	46.6(3)
C^{2}–C^{β}–C^{α}–$C^{\beta\prime}$	37.7(4)	37.8(3)	42.3(5)
$C^{2\prime}$–$C^{\beta\prime}$–C^{α}–C^{β}	47.9(4)	48.3(3)	44.6(5)

[a] Torsion angles for the (*S*)-isomer are reported.
[b] N–C^{α}–C′–OT torsion angle.
[c] C^{α}–C′–OT–CT torsion angle.

H-bond forms infinite chains along the *b* axis. Between the chains a hydrophobic zone, packed with the biphenyl and *tert*-butyl moieties, is observed.

The structure of Boc-Bip-O⊖ is a centrosymmetrical Ca-complex with a six-coordinated cation. Four coordination sites are provided by co-crystallised solvent molecules: one water molecule [Ca···O_W 2.340(2) Å] and one methanol molecule [Ca···O_M 2.364(2) Å], while two coordination sites are given by the carboxylate ligand uniquely via the oxygen O2 atom [Ca···O2 2.356(2) Å]. The orientation of the Boc-NH– fragment (see above) is not favourable for other cation-ligand interactions. In the crystal structure both water H-atoms participate in strong H-bonds with the carbonyl groups O1 and O0. The H-bond with O1 generates a planar six-membered pseudo-ring [the deviations of atoms from the plane O1···H_2–O_W···Ca···O2–C′1 are less than 0.08 Å], while the second H-bond involves the O0 atom of the urethane carbonyl function of a symmetry-related molecule forming a two-dimensional infinite chain along the *a* axis. The methanol molecule, coordinated with Ca^{++}, is H-bonded to the carboxylic O1 atom, which acts as a double acceptor (three-centre H-bonding).[20] Between the layer-like H-bond network a spacious hydrophobic zone, formed by the bulky biphenyl moieties, is observed.

In the crystal structure of the tripeptide Z-Gly-Bip-Gly-OEt the molecules are connected through (urethane) N1–H···O1=C′1 (peptide) and (peptide) N3–H···O2=C′2 (peptide) intermolecular H-bonds which give rise to infinite rows along the *a* axis. This structure is further stabilised by van der Waals interactions between the apolar moieties.

In summary, as far as the backbone conformation of Bip is concerned, this crystal-state analysis clearly indicates a tendency of this C^{α}-tetrasubstituted α-amino acid to fold, as already observed for Aib (α-aminoisobutyric acid), the prototype of this family, and 1-aminocycloalkane-1-carboxylic acids, including Ac_7c.[2,5] However, in the tripeptide this propensity is apparently not strong enough to overcome that of the Gly residue, known to dislike incorporation into position 1 of a β-turn conformation.

Conclusions

In this paper we have reported on the synthesis and preferred conformation of peptides built from Bip, a C^{α}-tetrasubstituted α-amino acid which combines the characteristics of both Ac_7c, a $C_i^{\alpha}\leftrightarrow C_i^{\alpha}$ cyclized residue,[1g] and Db_zg, a $C^{\alpha,\alpha}$-symmetrically di-substituted glycine without cyclization between the two side chains. The former class of amino acids is known to induce β-turns and 3_{10}-helices,[5] while the latter class tend to force the peptide main chain into a fully-extended disposition.[3] Therefore, it is not surprising that the results described here would indicate that: (i) Bip is a turn/helix former, although less effective than Ac_7c. (ii) A detectable amount of the fully-extended conformation populates the equilibrium mixtures of some of the short Bip peptides.

In protein amino acids the chirality depends exclusively on the presence of at least one stereogenic centre (the asymmetric α-carbon atom). By contrast, the chirality of Bip, the biphenyl-based α-amino acid investigated in this work, results from a different kind of molecular dissymmetry, the biaryl axis. In this work we have been able to show that in the *crystal* state, in contrast to protein amino acids, a (*S*)-Bip residue tends to adopt a *left*-handed turn/helical structure. However, a slow interconversion between the two enantiomers of Bip does take place in *solution* at room

Table 2. Intra- and intermolecular H-bond parameters for the Bip derivatives and peptide

Compound	Donor D-H	Acceptor A	Symmetry operation	Distance (Å) D···A	Distance (Å) H···A	Angle (deg) D-H···A
H-Bip-O*t*Bu	N1–H	O1	$x-1/2, -y+3/2, z$	3.144(4)	2.34(3)	139(2)
Boc-Bip-O⊖ water/methanol solvate	O_W–H1	O0	$1-x,-y,-z$	2.788(3)	2.04(2)	169(2)
	O_W–H2	O1	x, y, z	2.697(3)	1.85(2)	166(1)
	O_M–H	O1	$1+x, y, z$	2.621(3)	1.73(2)	175(1)
Z-Gly-Bip-Gly-OEt	N1–H	O1	$x-1/2, -y+3/2, z$	2.756(5)	2.14	128
	N3–H	O2	$x-1/2, -y+3/2, z$	2.920(5)	2.08	165

temperature, a clear indication of a residual mobility in the biphenyl system. This property, in turn, tends to dramatically complicate the structural assignment of Bip-rich peptides, as numerous diastereomeric species may concomitantly be present in these compounds. For this reason we are currently synthesizing and investigating the preferred conformation of peptides based on rigidified Bip congeners.

Experimental

General

Melting points were determined with a temperature raise of 3°C/min and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 MHz and 77 MHz, respectively, on a Bruker model AC-300. Splitting patterns are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. The optical rotations were measured with a Perkin–Elmer model 241 polarimeter equipped with a 1 dm thermostated cell. Analytical thin-layer chromatography (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: 2.5% MeOH-97.5% CH_2Cl_2 (A); 5% MeOH-95% CH_2Cl_2 (B); 10% MeOH-90% CH_2Cl_2 (C); CH_2Cl_2 (D). UV light (254 nm) allowed visualisation of the spots after TLC runs for all compounds, even at low concentrations.

Synthesis of Bip/(*S*)-Ala peptides

Boc-Bip-(*S*)-Ala-OMe. To a suspension of Boc-Bip-OH[8b] (0.088 g, 0.25 mmol), HCl·H-(*S*)-Ala-OMe (0.070 g, 0.5 mmol) and HOBt (0.068 g, 0.5 mmol) in a mixture of THF (tetrahydrofuran) (2 ml) and CH_2Cl_2 (2 ml), a solution of TEA (triethylamine) (0.052 g, 0.5 mmol) in CH_2Cl_2 (0.5 ml) was added, followed by a solution of DCC (0.062 g, 0.3 mmol) in CH_2Cl_2 (0.5 ml). The reaction mixture was magnetically stirred at room temperature overnight, and then evaporated in vacuo. The residue was stirred for a few minutes in the presence of EtOAc (50 ml) and the insoluble solid (*N*,*N*′-dicyclohexylurea, DCU) was filtered off. The solution was extracted with 5% $NaHCO_3$ (2×50 ml), H_2O (100 ml), 0.5 N HCl (2×50 ml), H_2O (2×100 ml), dried over $MgSO_4$, filtered and evaporated in vacuo. The crude product was chromatographed on a 1.5×39 cm column of silica gel with eluent (B) to give 0.103 g (94%) of pure title dipeptide. Crystallization of a sample from abs EtOH gave very fine needles. Mp 207°C. Found: C, 68.09; H, 6.91; N, 6.38%; $C_{25}H_{30}N_2O_5$ (438.506) requires C, 68.47; H, 6.90; N, 6.39%. $[\alpha]_{589}^{25}=-112$; $[\alpha]_{578}^{25}=-117$; $[\alpha]_{546}^{25}=-137$; $[\alpha]_{436}^{25}=-263$; $[\alpha]_{365}^{25}=-498$ (*c* 1; MeOH). R_f=0.75 (B). ^{1}H NMR: 7.45–7.30 (m, 8H, ArH); 7.12 (broad m, 1H, NH Ala); 4.88 (s, 1H, NH Bip); 4.64 (dq, 1H, Hα Ala, *J*=7.3; 7.2 Hz); 3.76 (s, 3H, OMe); 3.22 (broad m, 2H, Hβ Bip); 2.58 (broad m, 2H, Hβ Bip); 1.48 (s, 9H, Boc); 1.44 (d, 3H, Hβ Ala, *J*=7.2 Hz).

H-Bip-(*S*)-Ala-OMe. To Boc-Bip-(*S*)-Ala-OMe (0.092 g, 0.21 mmol), dissolved in EtOAc (4 ml), a 4.8 N solution of HCl in EtOAc was added (4 ml). The resulting solution was stirred at room temperature for 2 h and evaporated in vacuo. The residue was dissolved in EtOAc and the solution was extracted with 5% $NaHCO_3$ (50 ml), H_2O (100 ml), dried over $MgSO_4$, filtered and evaporated in vacuo. The crude product (0.068 g, 96%) was chromatographed on a 1.5×37 cm column of silica gel with eluent (B) to give 0.055 g (78%) of pure title dipeptide as a glass. Found: C, 69.73; H, 6.57; N, 7.99%; $C_{20}H_{22}N_2O_3$·0.3 H_2O (347.796) requires C, 69.87; H, 6.62; N, 8.15%. $[\alpha]_{589}^{25}=-53$; $[\alpha]_{578}^{25}=-55$; $[\alpha]_{546}^{25}=-64$; $[\alpha]_{436}^{25}=-123$; $[\alpha]_{365}^{25}=-227$ (*c* 0.5; MeOH). R_f=0.40 (B). ^{1}H NMR: 7.80 (d, 1H, NH Ala, *J*=7.9 Hz); 7.45–7.25 (m, 8H, ArH); 4.58 (dq, 1H, Hα Ala, *J*=7.9; 7.2 Hz); 3.77 (s, 3H, OMe); 3.14 (broad d, 2H, Hβ Bip); 2.33 (d, 2H, Hβ Bip); 1.84 (broad s, 2H, NH Bip); 1.46 (d, 3H, Hβ Ala, *J*=7.2 Hz). ^{13}C NMR: 175.2, 173.5 (C=O Ala and Bip), 140.5–127.3 (CAr), 67.8 (Cα Bip), 52.3 (OMe), 47.7 (Cα Ala), 44.1 (Cββ′ Bip), 18.1 (Cβ Ala).

Z-(*S*)-Ala-Bip-(*S*)-Ala-OMe. To an ice-cold solution of Z-(*S*)-Ala-OH (0.135 g, 0.6 mmol) in CH_3CN (2 ml) DCC (0.061 g, 0.3 mmol) was added. The mixture was stirred at 0°C for 1 h, filtered through glass wool for elimination of the DCU precipitate, and added to an ice-cold solution of H-Bip-(*S*)-Ala-OMe (0.050 g, 0.15 mmol) in CH_3CN (2 ml). The resulting solution was stirred from 0°C to room temperature overnight, and then evaporated in vacuo. The residue was dissolved in EtOAc and the solution was extracted with 0.5 M HCl (2×100 ml), H_2O (100 ml), 5% $NaHCO_3$ (2×100 ml), H_2O (2×100 ml), dried over $MgSO_4$, filtered and evaporated in vacuo. The crude product was chromatographed on a 1.5×39 cm column of silica gel with eluent (C) to give 0.078 g of pure title tripeptide as a glass. Crystallization from EtOAc (ca 2 ml) led to crystals which were filtered, washed with hexane and air dried. Yield 0.076 g (94%). Mp 210°C. Found: C, 68.09; H, 6.33; N, 7.56%; $C_{31}H_{33}N_3O_6$·0.3 H_2O (549.003) requires C, 67.81; H, 6.17; N, 7.65%. $[\alpha]_{589}^{25}=-153$; $[\alpha]_{578}^{25}=-159$; $[\alpha]_{546}^{25}=-186$; $[\alpha]_{436}^{25}=-356$; $[\alpha]_{365}^{25}=-670$ (*c* 0.5; MeOH). R_f=0.75 (C). ^{1}H NMR (Z-Ala^1-Bip-Ala^2-OMe): 7.45–7.22 (m, 13H, ArH); 7.25 (masked d, 1H, NH Ala^2); 6.53 (s, 1H, NH Bip); 5.43 (d, 1H, NH Ala^1, *J*=6.4 Hz); 5.06 (m, 2H, CH_2 Z); 4.54 (dq, 1H, Hα Ala^2, *J*=7.3; 7.2 Hz); 4.09 (dq, 1H, Hα Ala^1, *J*=6.4; 7.2 Hz); 3.72 (s, 3H, OMe); 3.25 (broad d, 1H, Hβ Bip); 3.17 (d, 1H, Hβ Bip); 2.98–2.64 (broad m, 2H, Hβ Bip); 1.39 (d, 6H, Hβ Ala^1 and Ala^2, *J*=7.2 Hz). ^{1}H NMR (213 K; two diastereoisomers D^1 ~75% and D^2 ~25%, are observed): 7.13 (d, ~0.3H, NH Ala^2 D^2, *J*=6.7 Hz); 7.04 (d, ~0.7H, NH Ala^2 D^1, *J*=7.1 Hz); 6.85 (s, ~0.25H, NH Bip D^2); 6.32 (s, ~0.75H, NH Bip D^1); 5.68 (broad s, ~0.7H, NH Ala^1 D^1); 5.63 (broad s, ~0.3H, NH Ala^1 D^2); 5.10 and 4.88 (two d, ~1.4H, CH_2 Z D^1, *J*=12.0 Hz); 5.03 (m, ~0.6H, CH_2 Z D^2); 4.50 (m, 1H, Hα Ala^2 D^1 D^2); 4.07 (m, ~0.3H, Hα Ala^1 D^2); 3.91 (m, ~0.7H, Hα Ala^1 D^1); 3.78 (s, ~2.25H, OMe D^1); 3.68 (s, ~0.75H, OMe D^2). ^{1}H NMR (333 K): 7.22 (d, 1H, NH Ala^2, *J*=7.1 Hz); 6.34 (s, 1H, NH Bip); 5.17 (d, 1H, NH Ala^1, *J*=6.6 Hz); 5.10 (m, 2H, CH_2 Z); 4.58 (dq, 1H, Hα Ala^2, *J*=7.1; 7.2 Hz); 4.12 (dq, 1H, Hα Ala^1, *J*=6.6; 7.2 Hz); 3.74 (s, 3H, OMe);); 3.29 (d, 1H, Hβ Bip); 3.23 (d, 1H, Hβ Bip); 2.84 (broad d, 1H, Hβ Bip); 2.68 (d, 1H, Hβ Bip); 1.42 (d, 3H, Hβ Ala, *J*=7.2 Hz); 1.41 (d, 3H, Hβ Ala, *J*=7.2 Hz). ^{13}C NMR: 173.5, 172.3, 171.4 (C=O Ala^1, Ala^2 and Bip), 156.3 (C=O Z), 140.6–127.5

(CAr), 70.0 (Cα Bip), 67.1 (CH_2 Z), 52.2 (OMe), 51.2, 48.2 (Cα Ala[1], Ala[2]), ~40 (broad, Cββ′ Bip), 17.7,17.5 (Cβ Ala[1], Ala[2]).

Z-Bip-(*S*)-Ala-(*S*)-Ala-OMe. To a suspension of Z-Bip-OH[8b] (0.097 g, 0.25 mmol), HCl·H-(*S*)-Ala-(*S*)-Ala-OMe (0.105 g, 0.5 mmol) and HOBt (0.068 g, 0.5 mmol) in a mixture of THF (2 ml) and CH_2Cl_2 (2 ml), a solution of TEA (0.052 g, 0.5 mmol) in CH_2Cl_2 (1 ml) was added, followed by a solution of DCC (0.065 g, 0.3 mmol) in CH_2Cl_2 (1 ml). The reaction mixture was magnetically stirred at room temperature overnight and then evaporated in vacuo. The residue was treated as described above for Boc-Bip-(*S*)-Ala-OMe to give, after repeated chromatography on silica gel with eluent (C), 0.100 g (74%) of pure title tripeptide as a solid. Mp 113°C. Found: C, 68.26; H, 6.09; N, 7.66%; $C_{31}H_{33}N_3O_6$ (543.598) requires C, 68.49; H, 6.12; N, 7.73%. $[\alpha]^{25}_{589}=-31$; $[\alpha]^{25}_{578}=-32$; $[\alpha]^{25}_{546}=-37$; $[\alpha]^{25}_{436}=-66$; $[\alpha]^{25}_{365}=-110$ (*c* 0.5; MeOH). R_f=0.75 (C). ^{1}H NMR: 7.42–7.17 (m, 13H, ArH); 7.03 (broad d, 1H, NH Ala); 6.77 (d, 1H, NH Ala, *J*=7.2 Hz); 5.23 (s, 1H, NH Bip); 5.13 (s, 2H, CH_2 Z); 4.53 (m, 2H, Hα Ala[1] and Ala[2]); 3.73 (s, 3H, OMe); 3.27 (broad d, 1H, Hβ Bip); 3.15 (broad d, 1H, Hβ Bip); 2.66 (broad m, 2H, Hβ Bip); 1.41 (d, 6H, Hβ Ala[1] and Ala[2], *J*=7.0 Hz). ^{1}H NMR (213 K; two diastereoisomers D[1] ~55% and D[2] ~45%, are observed): 7.66 and 7.54 (two broad d, NH Ala); 7.03 and 6.88 (two broad d, NH Ala); 5.50 and 5.46 (two s, NH Bip); 5.16–4.99 and 5.13–4.99 (two m, CH_2 Z); 4.62 (m, Hα Ala); 4.46 and 3.67 (two m, Hα Ala); 3.75 and 3.67 (two s, ~55/45, OMe); ^{1}H NMR (333 K): 6.90 (broad d, 1H, NH Ala); 6.80 (d, 1H, NH Ala, *J*=7.3 Hz); 5.21 (s, 1H, NH Bip); 5.14 (s, 2H, CH_2 Z); 4.53 (m, 2H, Hα Ala[1] and Ala[2]); 3.73 (s, 3H, OMe); 3.25 (d, 1H, Hβ Bip); 3.18 (d, 1H, Hβ Bip); 2.71 (broad d, 1H, Hβ Bip); 2.66 (broad d, 1H, Hβ Bip); 1.41 (d, 3H, Hβ Ala, *J*=7.0 Hz); 1.39 (d, 3H, Hβ Ala, *J*=7.0 Hz). ^{13}C NMR: 173.0, 171.8 (C═O Ala[1], Ala[2] and Bip), 155.4 (C═O Z), 140.6–127.7 (CAr), 69.8 (Cα Bip), 67.2 (CH_2 Z), 52.3 (OMe), 49.1, 48.2 (Cα Ala[1], Ala[2]), ~40 (broad, Cββ′ Bip), 17.8 (Cβ Ala[1], Ala[2]).

Z-Bip-(*S*)-Ala-OMe. To a suspension of Z-Bip-OH (0.387 g, 1 mmol), HCl·H-(*S*)-Ala-OMe (0.279 g, 2 mmol) and HOBt (0.270 g, 2 mmol) in a mixture of THF (10 ml) and CH_2Cl_2 (5 ml), a solution of TEA (0.202 g, 2 mmol) in CH_2Cl_2 (2.5 ml) was added, followed by a solution of EDC (0.230 g, 1.2 mmol) in CH_2Cl_2 (2.5 ml). The reaction mixture was magnetically stirred at room temperature overnight and then evaporated in vacuo. The residue was dissolved in a mixture of EtOAc (100 ml) and 0.5 M HCl (100 ml). The organic phase was extracted with 0.5 M HCl (100 ml), followed by H_2O (100 ml), 5% $NaHCO_3$ (2×100 ml), H_2O (2×100 ml), dried over $MgSO_4$, filtered and evaporated in vacuo. The crude product was chromatographed on a 2.3×60 cm column of silica gel with eluent (A) to give 0.396 g of pure title dipeptide as a solid. Mp 155°C. Found: C, 71.06; H, 6.12; N, 6.08%; $C_{28}H_{28}N_2O_5$ (472.520) requires C, 71.17; H, 5.97; N, 5.93%. $[\alpha]^{25}_{589}=-112$; $[\alpha]^{25}_{578}=-115$; $[\alpha]^{25}_{546}=-133$; $[\alpha]^{25}_{436}=-256$; $[\alpha]^{25}_{365}=-482$ (*c* 0.5; MeOH). R_f=0.60 (A). ^{1}H NMR (333 K): 7.45–7.24 (m, 13H, ArH); 6.97 (broad d, 1H, NH Ala, *J*=7.3 Hz); 5.16 (s, 2H, CH_2 Z); 5.06 (s, 1H, NH Bip); 4.60 (dq, 1H, Hα Ala, *J*=7.3; 7.2 Hz); 3.75 (s, 3H, OMe); 3.28 (d, 1H, Hβ Bip); 3.23 (d, 1H, Hβ Bip); 2.75 (broad d, 1H, Hβ Bip); 2.68 (broad d, 1H, Hβ Bip); 1.39 (d, 3H, Hβ Ala, *J*=7.2 Hz). ^{13}C NMR: 173.5, 171.8 (C═O Ala, Bip), 155.2 (C═O Z), 140.5–127.6 (CAr), 69.8 (Cα Bip), 67.0 (CH_2 Z), 52.3 (OMe), 48.2 (Cα Ala), ~40 (broad, Cββ′ Bip), 18.1 (Cβ Ala).

Boc-(*S*)-Ala-Bip-(*S*)-Ala-OMe. A solution of Z-Bip-(*S*)-Ala-OMe (0.397 g, 0.8 mmol) in 33% HBr/AcOH (7 ml) and Et_2O (diethyl ether) (7 ml) was stirred at room temperature for 2 h, then diluted with EtOAc (100 ml) and made basic by addition of an excess of 5% $NaHCO_3$. The extracted organic phase was washed with water, dried over $MgSO_4$, filtered and evaporated in vacuo. The obtained crude H-Bip-(*S*)-Ala-OMe (0.269 g, 97%) was dissolved in CH_3CN (4 ml) and CH_2Cl_2 (10 ml). The solution was cooled to −5°C and added to a cold solution of [Boc-(*S*)-Ala]$_2$O, just previously prepared by stirring a solution of Boc-(*S*)-Ala-OH (0.825 g, 4.4 mmol) and EDC (0.417 g, 2.2 mmol) in CH_3CN (6 ml) at −5°C for 1 h. The reaction mixture was magnetically stirred from −5°C to room temperature overnight and then evaporated in vacuo. The residue was treated as described above for Z-(*S*)-Ala-Bip-(*S*)-Ala-OMe to give, after repeated chromatography on silica gel with eluent (B), 0.176 g (43%) of pure tripeptide as a solid. Crystallization from MeOH gave needles (0.150 g). Mp 235°C. Found: C, 65.65; H, 6.96; N, 8.14%; $C_{28}H_{35}N_3O_6$ (509.584) requires C, 65.99; H, 6.92; N, 8.25%. $[\alpha]^{25}_{589}=-323$; $[\alpha]^{25}_{578}=-332$; $[\alpha]^{25}_{546}=-358$; $[\alpha]^{25}_{436}=-527$; $[\alpha]^{25}_{365}=-837$ (*c* 0.2; MeOH). R_f=0.35 (B). ^{1}H NMR (Boc-Ala[1]-Bip-Ala[2]-OMe) (333 K): 7.45–7.21 (m, 9H, ArH and masked NH Ala[2]); 6.47 (s, 1H, NH Bip); 4.86 (d, 1H, NH Ala[1], *J*=6.9 Hz); 4.59 (dq, 1H, Hα Ala[2], *J*=7.3; 7.2 Hz); 4.07 (dq, 1H, Hα Ala[1], *J*=6.9; 7.0 Hz); 3.75 (s, 3H, OMe); 3.30 (d, 1H, Hβ Bip); 3.24 (d, 1H, Hβ Bip); 2.83 (broad d, 1H, Hβ Bip); 2.73 (broad d, 1H, Hβ Bip); 1.44 (d, 3H, Hβ Ala[2], *J*=7.2 Hz); 1.38 (d, 3H, Hβ Ala[1], *J*=7.0 Hz); 1.41 (s, 9H, Boc).

Boc-(*S*)-Ala-(*S*)-Ala-Bip-(*S*)-Ala-OMe. To a solution of Boc-(*S*)-Ala-Bip-(*S*)-Ala-OMe (0.150 g, 0.29 mmol) in CH_2Cl_2 (5 ml) TFA (5 ml) was added. The solution was stirred at room temperature for 3 h and evaporated in vacuo. The residue was dissolved in EtOAc (50 ml). The solution was extracted with 5% $NaHCO_3$ (50 ml), dried over $MgSO_4$, filtered and evaporated in vacuo. The residue was chromatographed on a 2.5×47 cm column of silica gel with eluent (C) to give 0.063 g (52%) of H-(*S*)-Ala-Bip-(*S*)-Ala-OMe which was immediately used in the next coupling step. A solution of a mixture of this compound (0.063 g, 0.15 mmol), Boc-(*S*)-Ala-OH (0.044 g, 0.23 mmol) and HOBt (0.042 g, 0.31 mmol) in THF (5 ml) and CH_2Cl_2 (2.5 ml) was stirred at −5°C. Then, a solution of EDC (0.044 g, 0.23 mmol) in CH_2Cl_2 (2.5 ml) was added. The reaction mixture was magnetically stirred from −5°C to room temperature overnight and evaporated in vacuo. The residue was treated as described above for Z-(*S*)-Ala-Bip-(*S*)-Ala-OMe to give, after chromatography on a 1.6×38 cm column of silica gel with eluent (C), 0.077 g (78%) of pure title tetrapeptide as a solid. Mp 230°C. Found: C, 63.12; H, 7.01; N, 9.43%; $C_{31}H_{40}N_4O_7$0.5 H_2O (589.670) requires C, 63.14; H, 7.01; N, 9.50%. ESI$^+$ MS *m/z* (relative intensity): 581 $(M,H)^+$ (33); 603 $(M,Na)^+$ (100); 619 $(M,K)^+$ (8). $[\alpha]^{25}_{589}=-146$; $[\alpha]^{25}_{578}=-157$; $[\alpha]^{25}_{546}=-182$;

$[\alpha]_{436}^{25}=-341$; $[\alpha]_{365}^{25}=-628$ (*c* 0.2; MeOH). R_f=0.45 (C). ^{1}H NMR (Boc-Ala^1-Ala^2-Bip-Ala^3-OMe) (333 K): 7.45–7.22 (m, 9H, ArH and masked NH Ala^3); 6.62 (s, 1H, NH Bip); 6.56 (d, 1H, NH Ala^2, *J*=6.0 Hz); 4.85 (d, 1H, NH Ala^1, *J*=6.7 Hz); 4.57 (dq, 1H, Hα Ala^3, *J*=7.2; 7.1 Hz); 4.30 (dq, 1H, Hα Ala^2, *J*=6.0; 6.7 Hz); 4.08 (dq, 1H, Hα Ala^1, *J*=6.7; 7.1 Hz); 3.75 (s, 3H, OMe); 3.26 (d, 1H, Hβ Bip); 3.24 (d, 1H, Hβ Bip); 2.85 (broad d, 1H, Hβ Bip); 2.79 (broad d, 1H, Hβ Bip); 1.43 (d, 3H, Hβ Ala^3, *J*=7.1 Hz); 1.41 (s, 9H, Boc); 1.40 (masked d, 3H, Hβ Ala^2); 1.33 (d, 3H, Hβ Ala^1, *J*=7.1 Hz). ^{13}C NMR: 173.5, 173.2, 171.8, 171.5 (C=O Ala^1, Ala^2, Ala^3 and Bip), 155.7 (C=O Boc), 140.6–127.5 (CAr), 80.7 (Boc), 70.2 (Cα Bip), 52.2 (OMe), 50.5, 50.0, 48.3 (Cα Ala^1, Ala^2, Ala^3), ~40 (broad, Cββ′ Bip), 17.9, 17.7, 17.2 (Cβ Ala^1, Ala^2, Ala^3).

Z-Bip-(*S*)-Ala-(*S*)-Ala-Bip-(*S*)-Ala-OMe. To a solution of Boc-(*S*)-Ala-(*S*)-Ala-Bip-(*S*)-Ala-OMe (0.056 g, 0.1 mmol) in CH_2Cl_2 (5 ml) TFA (5 ml) was added. The solution was stirred at room temperature for 3 h and evaporated in vacuo. The residue was dissolved in EtOAc (50 ml). The solution was extracted with 5% $NaHCO_3$ (50 ml), dried over $MgSO_4$, filtered and evaporated in vacuo, to give 0.042 g (91%) of crude H-(*S*)-Ala-(*S*)-Ala-Bip-Ala-OMe which was immediately used in the next coupling step. A solution of a mixture of this compound (0.042 g, 0.09 mmol), Z-Bip-OH (0.035 g, 0.09 mmol), HOBt (0.018 g, 0.13 mmol) and EDC (0.020 g, 0.11 mmol) in CH_2Cl_2 (10 ml) and THF (10 ml) was stirred at room temperature overnight and evaporated in vacuo. The residue was treated as described above for Z-(*S*)-Ala-Bip-(*S*)-Ala-OMe and purified by preparative TLC on silica gel with eluent (C) to give 0.039 g (52%) of pure title pentapeptide as a solid. Mp 118°C. Found: C, 69.75; H, 6.21; N, 8.01%; $C_{50}H_{51}N_5O_8$·0.5 H_2O (858.956) requires C, 69.91; H, 6.10; N, 8.15%. $[\alpha]_{589}^{25}=+18$; $[\alpha]_{578}^{25}=+19$; $[\alpha]_{546}^{25}=+22$; $[\alpha]_{436}^{25}=+46$; $[\alpha]_{365}^{25}=+94$ (*c* 0.2; MeOH). R_f=0.40 (C). ^{1}H NMR (Z-Bip^1-Ala^2-Ala^3-Bip^4-Ala^5-OMe) (333 K): 7.45–6.99 (m, 23H, ArH and masked NH Ala^5, NH Ala^3); 6.88 (s, 1H, NH Bip^4); 6.48 (d, 1H, NH Ala^2, *J*=5.4 Hz); 5.27 (s, 1H, NH Bip^1); 5.13 (m, 2H, CH_2 Z); 4.56 (dq, 1H, Hα Ala^5, *J*=7.0; 7.2 Hz); 4.29 (dq, 1H, Hα Ala^3, *J*=6.8; 7.2 Hz); 4.25 (dq, 1H, Hα Ala^2, *J*=5.4; 7.2 Hz); 3.70 (s, 3H, OMe); 3.23 (d, 1H, Hβ Bip); 3.19 (d, 1H, Hβ Bip); 3.09 (broad d, 2H, Hβ Bip); 2.95 (d, 1H, Hβ Bip); 2.87 (broad d, 1H, Hβ Bip); 2.55 (d, 1H, Hβ Bip); 2.43 (broad d, 1H, Hβ Bip); 1.50 (d, 3H, Hβ Ala^3, *J*=7.2 Hz); 1.46 (d, 3H, Hβ Ala^5, *J*=7.2 Hz); 1.42 (d, 3H, Hβ Ala^2, *J*=7.2 Hz). ^{13}C NMR: 173.3 (broad), 172.7 (broad), 172.2, 172.1 (C=O Bip^1, Bip^4, Ala^2, Ala^3 and Ala^5), 156.2 (C=O Z), 140.5–127.1 (CAr), 70.0, 69.4 (Cα Bip^1 and Bip^4), 67.2 (CH_2 Z), 52.1 (OMe), 51.1, 50.4, 48.3 (Cα Ala^2, Ala^3 and Ala^5), ~41.5, ~35.5 (broad, Cββ′ Bip^1 and Bip^4), 17.8, 16.9, 16.6 (Cβ Ala^2, Ala^3 and Ala^5).

Synthesis of Bip/Gly peptides

Z-Bip-Gly-Gly-OEt. To a suspension of Z-Bip-OH (0.193 g, 0.5 mmol), HCl·H-Gly-Gly-OEt (0.196 g, 1 mmol) and HOBt (0.135 g, 1 mmol) in a mixture of THF (5 ml) and CH_2Cl_2 (2 ml), a solution of NMM (*N*-methylmorpholine) (0.101 g, 1 mmol) in CH_2Cl_2 (1 ml) was added, followed by a solution of EDC (0.106 g, 0.55 mmol) in CH_2Cl_2 (2 ml). The reaction mixture was magnetically stirred at room temperature overnight and then evaporated in vacuo. The residue was treated as described above for Z-(*S*)-Ala-Bip-(*S*)-Ala-OMe to give, after repeated chromatography on silica gel with eluent (B) followed by crystallization from a hexane/CH_2Cl_2 solution, 0.185 g (70%) of pure title tripeptide as crystals. Mp 195°C. Found: C, 67.33; H, 5.82; N, 7.71%; $C_{30}H_{31}N_3O_6$·0.3 H_2O (534.977) requires C, 67.35; H, 5.95; N, 7.85%. R_f=0.20 (B). ^{1}H NMR (293 K): 7.48 (m t-like, 1H, NH Gly); 7.42–7.21 (m, 13H, ArH); 6.96 (t, 1H, NH Gly, *J*=6.0 Hz); 5.31 (s, 1H, NH Bip); 5.12 (s, 2H, CH_2 Z); 4.19 (q, 2H, OEt, *J*=7.1 Hz); 4.07 (d, 2H, Hα Gly, *J*=6.0 Hz); 4.05 (d, 2H, Hα Gly, *J*=5.8 Hz); 3.26 (broad d, 2H, Hβ Bip); 2.63 (broad m, 2H, Hβ Bip); 1.27 (t, 3H, OEt, *J*=7.1 Hz). ^{1}H NMR (333 K): 7.45–7.08 (m, 14H, ArH and masked NH Gly); 6.87 (broad t, 1H, NH Gly); 5.18 (s, 1H, NH Bip); 5.15 (s, 2H, CH_2 Z); 4.21 (q, 2H, OEt, *J*=7.1 Hz); 4.04 (d, 2H, Hα Gly, *J*=6.0 Hz); 4.03 (d, 2H, Hα Gly, *J*=5.6 Hz); 3.26 (d, 2H, Hβ Bip); 2.67 (d, 2H, Hβ Bip); 1.27 (t, 3H, OEt, *J*=7.1 Hz). ^{1}H NMR (223 K): 7.99 (t, 1H, NH Gly, J~5.9 Hz); 7.50–7.07 (m, 13H, ArH); 6.87 (partly masked t, 1H, NH Gly); 5.61 (s, 1H, NH Bip); 5.06 (m, 2H, CH_2 Z); 4.25–3.92 (m, 4H, four Hα Gly); 4.14 (q, 2H, OEt, *J*=7.1 Hz); 3.53 (d, 1H, Hβ Bip); 3.13 (d, 1H, Hβ Bip); 2.74 (d, 1H, Hβ Bip); 2.34 (d, 1H, Hβ Bip); 1.25 (t, 3H, OEt, *J*=7.1 Hz). ^{13}C NMR: 172.7, 170.0, 169.7 (C=O Gly^1, Gly^2 and Bip), 156.0 (C=O Z), 140.5–127.8 (CAr), 69.9 (Cα Bip), 67.4 (CH_2 Z), 61.3 (OEt), 43.1, 41.0 (Cα Gly^1, Gly^2), ~40 (broad, Cββ′ Bip), 14.1 (OEt).

Z-Gly-Bip-O*t*Bu. To a solution of Z-Gly-OH (0.418 g, 2 mmol) in CH_3CN (10 ml) DCC (0.206 g, 1 mmol) was added. The mixture was stirred at room temperature for 1 h, filtered through glass wool and added to a solution of H-Bip-O*t*Bu[8b] (0.160 g, 0.52 mmol) in CH_3CN (5 ml). The resulting solution was stirred at room temperature for 20 h and evaporated in vacuo. The residue was treated as described above for Z-(*S*)-Ala-Bip-(*S*)-Ala-OMe and purified by column chromatography on silica gel with eluent (C) to give 0.250 g (96%) of pure title dipeptide as a solid. Crystallization from a hexane/CH_2Cl_2 solution gave crystals (0.170 g). Mp 181°C. Found: C, 71.91; H, 6.35; N, 5.46%; $C_{30}H_{32}N_2O_5$ (500.572) requires C, 71.98; H, 6.44; N, 5.60%. R_f=0.75 (C). ^{1}H NMR: 7.47–7.20 (m, 13H, ArH); 6.64 (s, 1H, NH Bip); 5.70 (m t-like, 1H, NH Gly); 5.29 (s, 2H, CH_2 Z); 3.84 (d, 2H, Hα Gly, *J*=5.5 Hz); 3.12 (broad d, 2H, Hβ Bip); 2.7 (broad m, 2H, Hβ Bip); 1.46 (s, 9H, OtBu). ^{13}C NMR: 170.8, 168.4 (C=O Gly and Bip), 156.6 (C=O Z), 140.9–126.9 (CAr), 81.7 (O*t*Bu), 69.3 (Cα Bip), 67.0 (CH_2 Z), 44.5 (Cα Gly), 40.9 (broad, Cβ Bip), 38.2 (broad, Cβ′ Bip), 14.1 (O*t*Bu).

Z-Gly-Bip-Gly-OEt. To a solution of Z-Gly-Bip-O*t*Bu (0.240 g, 0.48 mmol) in CH_2Cl_2 (5 ml) TFA (5 ml) was added. The solution was stirred at room temperature for 3 h and evaporated in vacuo. Dissolution of the residue in CH_2Cl_2 and evaporation in vacuo were repeated several times in order to remove the excess of TFA. Then, the residue was precipitated from a concentrated CH_2Cl_2 solution by addition of hexane. The precipitate was collected and dried in vacuo to give 0.191 g (90%) of crude Z-Gly-Bip-OH as a powder (mp 175°C), which was used in the

next coupling step without further purification. To a suspension of a mixture of this compound (0.177 g, 0.40 mmol), HCl·H-Gly-OEt (0.111 g, 0.80 mmol) and HOBt (0.108 g, 0.80 mmol) in THF (5 ml) and CH_2Cl_2 (2 ml), a solution of NMM (0.084 g, 0.80 mmol) in CH_2Cl_2 (1 ml) was added, followed by a solution of EDC (0.092 g, 0.48 mmol) in CH_2Cl_2 (2 ml). The reaction mixture was magnetically stirred at room temperature overnight and then evaporated in vacuo. The residue was treated as described above for Z-(*S*)-Ala-Bip-(*S*)-Ala-OMe to give, after column chromatography on silica gel with eluent (B), 0.173 g (82%) of pure title tripeptide. Crystallization from a hexane/CH_2Cl_2 solution gave crystals (0.141 g). Mp 152°C. Found: C, 67.56; H, 5.77; N, 7.84%; $C_{30}H_{31}N_3O_6$ (529.572) requires C, 68.04; H, 5.90; N, 7.93%. R_f=0.40 (B). ^{1}H NMR (Z-Gly^1-Bip-Gly^2-OEt) (293 K): 7.45–7.27 (m, 13H, ArH); 7.25 (partly masked t, 1H, NH Gly^2); 6.45 (s, 1H, NH Bip); 5.45 (broad t, 1H, NH Gly^1); 5.11 (s, 2H, CH_2 Z); 4.21 (q, 2H, OEt, *J*=7.1 Hz); 4.02 (broad d, 2H, Hα Gly^2, J∼5.0 Hz); 3.83 (d, 2H, Hα Gly^1, *J*=5.7 Hz); 3.26 (broad m, 2H, Hβ Bip); 2.68 (broad m, 2H, Hβ Bip); 1.28 (t, 3H, OEt, *J*=7.1 Hz). ^{1}H NMR (333 K): 7.45–7.27 (m, 13H, ArH); 7.19 (broad t, 1H, NH Gly^2); 6.32 (s, 1H, NH Bip); 5.30 (broad t, 1H, NH Gly^1); 5.13 (s, 2H, CH_2 Z); 4.23 (q, 2H, OEt, *J*=7.1 Hz); 4.03 (d, 2H, Hα Gly^2, *J*=5.3 Hz); 3.83 (d, 2H, Hα Gly^1, *J*=5.8 Hz); 3.30 (d, 2H, Hβ Bip); 2.76 (broad d, 2H, Hβ Bip); 1.29 (t, 3H, OEt, *J*=7.1 Hz). ^{1}H NMR (223 K): 7.59 (broad t, 1H, NH Gly^2); 7.45–7.05 (m, 13H, ArH); 6.74 (s, 1H, NH Bip); 6.00 (broad t, 1H, NH Gly^1); 5.00 (m, 2H, CH_2 Z); 4.04 (broad q, 2H, OEt); 4.00 (m, 2H, Hα Gly^2); 3.75 (broad d, 2H, Hα Gly^1); 3.18 (d, 2H, Hβ Bip); 2.38 (d, 1H, Hβ Bip); 3.07 (d, 1H, Hβ Bip); 1.23 (t, 3H, OEt, *J*=7.1 Hz). ^{13}C NMR: 172.3, 170.0, 169.4 (C═O Gly^1, Gly^2 and Bip), 156.8 (C═O Z), 140.6–127.8 (CAr), 70.2 (Cα Bip), 67.5 (CH_2 Z), 61.4 (OEt), 45.4, 41.6 (Cα Gly^1, Gly^2), ∼40 (broad, Cββ′ Bip), 14.1 (OEt).

Synthesis of Bip homo-peptides

Z-Bip-Bip-O*t*Bu. To a solution of Z-Bip-OH (0.388 g, 1 mmol) and TEA (0.101 g, 1 mmol) in CH_2Cl_2 (0.5 ml) and toluene (1 ml) cooled to −5°C, a solution of Piv-Cl (Piv, pivaloyl) (0.121 g, 1 mmol) in toluene (1 ml) was added. The resulting suspension was stirred at −5°C for 2 h, then at room temperature for 1.5 h, and finally filtered through glass wool. The solution was evaporated in vacuo at 30°C and to the crude solid Z-Bip-OPiv a solution of H-Bip-O*t*Bu (0.341 g, 1.1 mmol) in toluene (5 ml) was added. The reaction mixture was stirred for 3 h at 60°C and then evaporated in vacuo. The residue was dissolved in EtOAc (150 ml) and the solution was extracted with 0.5 M HCl (2×100 ml), H_2O (100 ml), 5% $NaHCO_3$ (100 ml), H_2O (2×100 ml), dried over $MgSO_4$, filtered and evaporated in vacuo. The crude product was chromatographed on a 2.3×50 cm column of silica gel with eluent (A) to give 0.414 g (61%) of pure title dipeptide as a foam. Mp 116°C. Found: C, 77.44; H, 6.41; N, 4.06%; $C_{44}H_{42}N_2O_5$ (678.792) requires C, 77.85; H, 6.24; N, 4.13%. R_f=0.75 (A). ^{1}H NMR (Z-Bip^1-Bip^2-O*t*Bu): 7.49–7.27 (m, 21H, ArH); 7.12 (broad s, 1H, NH Bip^2); 5.09 (s, 3H, CH_2 Z and NH Bip^1); 3.5–2.2 (very broad m, 8H, Hββ′ Bip^1, Bip^2); 1.44 (s, 9H, O*t*Bu). ^{13}C NMR: 171.1 (C═O Bip^1, Bip^2), 156.3 (C═O Z), 140.7–127.4 (CAr), 81.3 (O*t*Bu), 69.7, 69.2 (Cα Bip^1, Bip^2), 69.9 (CH_2 Z), ∼40 (very broad, Cββ′ Bip^1, Bip^2), 27.8 (O*t*Bu).

Z-Bip-Bip-OH. To a solution of Z-Bip-Bip-O*t*Bu (1.091 g, 1.61 mmol) in CH_2Cl_2 (15 ml) TFA (15 ml) was added. The solution was stirred at room temperature for 3 h and evaporated in vacuo. The residue was dissolved in EtOAc (150 ml). The solution was extracted with H_2O (2×100 ml), dried over $MgSO_4$, filtered and evaporated in vacuo. The residue was dissolved in MeOH, and the solution was filtered and evaporated in vacuo to give 0.929 g (93%) of crude title dipeptide as a solid, which was used in the next step without further purification. Mp 260°C. Found: C, 74.64; H, 5.67; N, 4.24%; $C_{40}H_{34}N_2O_5H_2O$ (640.704) requires C, 74.98; H, 5.66; N, 4.37%.

5(4H)-Oxazolone from Z-Bip-Bip-OH. A suspension of Z-Bip-Bip-OH (0.903 g, 1.45 mmol) in Ac_2O (25 ml) was stirred at 115–120°C for 40 min. Then, the resulting solution was evaporated in vacuo. The residue was repeatedly dissolved in toluene and the solution evaporated in vacuo to give 0.877 g (quantitative yield) of crude title oxazolone, which was used in the next step without further purification. This next coupling reaction being uncomplete (see Z-Bip-Bip-Bip-O*t*Bu), separation by column chromatography gave a small sample of title oxazolone, obtained as a powder. Mp 168°C. Found: C, 79.31; H, 5.45; N, 4.65%; $C_{40}H_{32}N_2O_4$ (604.672) requires C, 79.45; H, 5.33; N, 4.63%. R_f=0.45 (D). ^{1}H NMR (oxazolone from Z-Bip^1-Bip^2-OH): 7.45–7.22 (m, 21H, ArH); 5.19 (s, 2H, CH_2 Z); 5.14 (s, 1H, NH Bip^1); 3.4–2.4 (very broad m, 8H, Hββ′ Bip^1, Bip^2). ^{13}C NMR: 179.0 (O–C═O Bip^2), 163.2 (O–C═N Bip^1), 154.6 (C═O Z), 140.7–127.7 (CAr), 78.0, 65.0 (Cα Bip^1, Bip^2), 67.1 (CH_2 Z), ∼40.5, ∼38.8 (very broad, Cββ′ Bip^1, Bip^2).

Z-Bip-Bip-Bip-O*t*Bu. A solution of the 5(4*H*)-oxazolone from Z-Bip-Bip-OH (0.518 g, 0.86 mmol) and H-Bip-O*t*Bu (0.300 g, 0.97 mmol) in CH_3CN (25 ml) was refluxed for 14 d and evaporated in vacuo. The residue was dissolved in EtOAc (150 ml). The solution was extracted with 5% citric acid (2×50 ml), H_2O (2×100 ml), dried over $MgSO_4$, filtered and evaporated in vacuo to give 0.652 g of crude neutral part. The acidic aqueous phase was made basic by addition of an excess of 1 M NaOH and then extracted with EtOAc. The organic phase was washed with H_2O (2×100 ml), dried over $MgSO_4$, filtered and evaporated in vacuo to give 0.080 g (26%) of crude recovered H-Bip-O*t*Bu. The crude neutral part was chromatographed on a 2.3×55 cm column of silica gel with eluent (D), then (A), to give 0.115 g (22%) of recovered pure oxazolone and 0.559 g (59%) of pure title tripeptide as a powder. Mp 204°C. Found: C, 77.35; H, 6.25; N, 4.34%; $C_{60}H_{55}N_3O_6H_2O$ (932.080) requires C, 77.31; H, 6.16; N, 4.51%. R_f=0.80 (A). ^{1}H NMR (Z-Bip^1-Bip^2-Bip^3-O*t*Bu): 7.66 (s, 1H, NH Bip); 7.49–7.14 (m, 29H, ArH); 6.62 (broad s, 1H, NH Bip); 5.09 (s, 1H, NH Bip^1); 4.90 (broad m, 2H, CH_2 Z); 3.6–2.3 (very broad m, 12H, Hββ′ Bip^1, Bip^2, Bip^3); 1.49 (s, 9H, O*t*Bu). ^{13}C NMR: 171.7, 171.1, 170.8 (C═O Bip^1, Bip^2, Bip^3), 155.6 (C═O Z), 140.8–127.2 (CAr), 81.2 (O*t*Bu), 70.0, 69.8, 69.7 (Cα Bip^1, Bip^2, Bip^3), 67.3 (CH_2 Z), ∼40 (very broad, Cββ′ Bip^1, Bip^2, Bip^3), 27.9 (O*t*Bu).

Z-Bip-Bip-Bip-OH. Tripeptide Z-Bip-Bip-Bip-O*t*Bu (0.657 g, 0.72 mmol) was C-deprotected in TFA (10 ml) and CH_2Cl_2 (10 ml), as reported above for Z-Bip-Bip-OH, to give 0.586 g (95%) of crude title tripeptide as a solid, which was used in the next step without further purification. Mp 219°C. Found: C, 75.05; H, 5.78; N, 4.58%; $C_{56}H_{47}N_3O_6 2\ H_2O$ (893.992) requires C, 75.23; H, 5.75; N, 4.70%.

5(4H)-Oxazolone from Z-Bip-Bip-Bip-OH. Tripeptide Z-Bip-Bip-Bip-OH (0.566 g, 0.66 mmol) was treated as described above for the oxazolone from Z-Bip-Bip-OH to give 0.554 g (quantitative yield) of crude title oxazolone, which was used in the next step without further purification. This next coupling reaction being uncomplete (see Z-Bip-Bip-Bip-Bip-O*t*Bu below), separation by column chromatography gave a small sample of title oxazolone, obtained as a powder. Mp 175°C. Found: C, 78.18; H, 5.62; N, 4.81%; $C_{56}H_{45}N_3O_5H_2O$ (857.960) requires C, 78.39; H, 5.52; N, 4.89%. R_f=0.30 (D); 0.80 (A). ^{1}H NMR (oxazolone from Z-Bip^1-Bip^2-Bip^3-OH): 7.51–7.30 (m, 29H, ArH); 7.02 (s, 1H, NH Bip^2); 5.15 (broad s, 2H, CH_2 Z); 5.12 (s, 1H, NH Bip^1); 3.5–2.3 (very broad m, 12H, Hββ′ Bip^1, Bip^2, Bip^3). ^{13}C NMR: 179.3 (O–C=O Bip^3), 171.5 (C=O Bip^1), 163.0 (O–C=N Bip^2), 155.2 (C=O Z), 140.8–127.5 (CAr), 78.0, 76.6, 64.7 (Cα Bip^1, Bip^2, Bip^3), 67.1 (CH_2 Z), ~40.2, ~39.1, ~37.5 (very broad, Cββ′ Bip^1, Bip^2, Bip^3).

Z-Bip-Bip-Bip-Bip-O*t*Bu. A solution of the 5(4*H*)-oxazolone from Z-Bip-Bip-Bip-OH (0.533 g, 0.63 mmol) and H-Bip-O*t*Bu (0.200 g, 0.65 mmol) in CH_3CN (25 ml) was refluxed for 17 d and treated as described above for Z-Bip-Bip-Bip-O*t*Bu. The crude product was chromatographed on a 2.3×52 cm column of silica gel with eluent (D), then (A), to give 0.078 g (15%) of recovered pure oxazolone and 0.459 g (63%) of pure title tetrapeptide as a powder. Mp 215°C. Found: C, 78.15; H, 6.01; N, 4.87%; $C_{76}H_{68}N_4O_7H_2O$ (1167.352) requires C, 78.19; H, 6.04; N, 4.80%. R_f=0.05 (D); 0.75 (A). ^{1}H NMR (Z-Bip^1-Bip^2-Bip^3-Bip^4-O*t*Bu): 7.84 (s, 1H, NH Bip); 7.57–7.02 (m, 38H, ArH and masked NH Bip); 6.66 (s, 1H, NH Bip); 5.17 (s, 1H, NH Bip^1); 5.11 (broad m, 2H, CH_2 Z); 3.7–2.2 (very broad m, 16H, Hββ′ Bip^1, Bip^2, Bip^3, Bip^4); 1.58 (s, 9H, O*t*Bu). ^{13}C NMR: 172.0 (broad), 171.6 (broad), 171.4, 170.7 (broad) (C=O Bip^1, Bip^2, Bip^3, Bip^4), 155.5 (C=O Z), 140.7–127.1 (CAr), 80.9 (O*t*Bu), 70.2, 69.8, 69.6, 69.4 (Cα Bip^1, Bip^2, Bip^3, Bip^4), 67.4 (CH_2 Z), ~40 (very broad, Cββ′ Bip^1, Bip^2, Bip^3, Bip^4), 27.9 (O*t*Bu).

Z-Bip-Bip-Bip-Bip-OH. Tetrapeptide Z-Bip-Bip-Bip-Bip-O*t*Bu (0.423 g, 0.37 mmol) was C-deprotected in TFA (10 ml) and CH_2Cl_2 (10 ml) as reported above for Z-Bip-Bip-OH to give 0.395 g (98%) of crude title tetrapeptide as a solid, which was used in the next step without further purification. Mp 238°C. Found: C, 76.59; H, 5.87; N, 4.95%; $C_{72}H_{60}N_4O_7 2\ H_2O$ (1129.264) requires C, 76.57; H, 5.71; N, 4.96%.

5(4H)-Oxazolone from Z-Bip-Bip-Bip-Bip-OH. Tetrapeptide Z-Bip-Bip-Bip-Bip-OH (0.382 g, 0.35 mmol) was treated as described above for the oxazolone from Z-Bip-Bip-OH to give 0.376 g (quantitative yield) of crude title oxazolone, which was used in the next step without further purification. This next coupling reaction being uncomplete (see Z-Bip-Bip-Bip-Bip-Bip-O*t*Bu below), separation by column chromatography gave a small sample of title oxazolone, obtained as a powder. Mp 175°C. Found: C, 77.86; H, 5.76; N, 4.91%; $C_{72}H_{58}N_4O_6 2\ H_2O$ (1111.248) requires C, 77.81; H, 5.62; N, 5.04%. R_f=0.10 (D); 0.80 (A). ^{1}H NMR (oxazolone from Z-Bip^1-Bip^2-Bip^3-Bip^4-OH): 7.75 (s, 1H, NH Bip); 7.45–7.25 (m, 37H, ArH); 6.33 (s, 1H, NH Bip); 5.10 (s, 1H, NH Bip^1); 4.90 (m, 2H, CH_2 Z); 3.6–2.3 (very broad m, 16H, Hββ′ Bip^1, Bip^2, Bip^3, Bip^4). ^{13}C NMR: 180.0 (O–C=O Bip^4), 171.0, 170.6 (C=O Bip^1, Bip^2), 163.2 (O–C=N Bip^3), 155.7 (C=O Z), 140.3–127.1 (CAr), 78.1, 70.0, 65.3 (Cα Bip^1, Bip^2, Bip^3, Bip^4), 67.4 (CH_2 Z), ~41.8, ~39.8, ~38.5, ~37.0 (very broad, Cββ′ Bip^1, Bip^2, Bip^3, Bip^4).

Z-Bip-Bip-Bip-Bip-Bip-O*t*Bu. A solution of the 5(4*H*)-oxazolone from Z-Bip-Bip-Bip-Bip-OH (0.363 g, 0.34 mmol) and H-Bip-O*t*Bu (0.209 g, 0.67 mmol) in CH_3CN (25 ml) was refluxed for 18 d and treated as described above for Z-Bip-Bip-Bip-O*t*Bu. Chromatography of the crude product on silica gel with gradient eluent from (D) to (A), gave 0.026 g (7%) of recovered pure oxazolone and 0.268 g (57%) of pure title pentapeptide as a solid. Mp 234°C. Found: C, 78.73; H, 6.04; N, 4.87%; $C_{92}H_{81}N_5O_8H_2O$ (1402.624) requires C, 78.77; H, 5.96; N, 4.99%. ESI$^+$ MS *m/z* (relative intensity): single peak 1385 $(M,H)^+$ (100). R_f=0.75 (A). ^{1}H NMR (Z-Bip^1-Bip^2-Bip^3-Bip^4-Bip^5-O*t*Bu): 7.90 (broad s, 1H, NH Bip); 7.56–7.02 (m, 46H, ArH and masked NH Bip); ~6.90 (broad s, 1H, NH Bip); 6.66 (broad s, 1H, NH Bip); 5.15 (broad s, 1H, NH Bip^1); 5.15–4.50 (broad m, 2H, CH_2 Z); 3.7–2.1 (very broad m, 20H, Hββ′ Bip^1, Bip^2, Bip^3, Bip^4, Bip^5); 1.51 (s, 9H, O*t*Bu). ^{1}H NMR (333 K): 7.72 (s, 1H, NH Bip); 7.47–7.05 (m, 45H, ArH); 6.90 (s, 1H, NH Bip); 6.88 (s, 1H, NH Bip); 6.63 (s, 1H, NH Bip); 5.01 (s, 1H, NH Bip^1); 4.85 (s, 2H, CH_2 Z); 3.30–2.35 (m, 20H, Hββ′ Bip^1, Bip^2, Bip^3, Bip^4, Bip^5); 1.46 (s, 9H, O*t*Bu). ^{13}C NMR: 172.6 (broad), 171.7 (broad), 170.3 (broad) (C=O Bip^1, Bip^2, Bip^3, Bip^4, Bip^5), 155.6 (C=O Z), 140.8–126.8 (CAr), 81.0 (O*t*Bu), 70.5, 70.4, 69.9, 69.5, 69.3 (Cα Bip^1, Bip^2, Bip^3, Bip^4, Bip^5), 67.5 (CH_2 Z), ~44–~34 (very broad, Cββ′ Bip^1, Bip^2, Bip^3, Bip^4, Bip^5), 28.0 (O*t*Bu).

FT-IR absorption

The solid-state infrared absorption spectra (KBr disk technique) were recorded with a Perkin–Elmer model 580 B spectrophotometer equipped with a Perkin–Elmer model 3600 IR data station. The solution IR absorption spectra were recorded using a Perkin–Elmer model 1720XFT–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. Cell 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.

Nuclear magnetic resonance

The ^{1}H NMR spectra were recorded with a Bruker model AM 400 spectrometer. Measurements were carried out in

deuterochloroform (99.96% d; Aldrich) and deuterated DMSO (99.96% d_6; Acros Organics) with tetramethylsilane as the internal standard.

X-Ray diffraction

The α-amino carboxylic ester H-Bip-O*t*Bu crystallises in the non-centrosymmetric space group An (No. 9) with unit cell parameters a=10.435(1) Å, b=17.256(2) Å, c=9.991(2) Å, β=103.44(1)°, while the Ca^{++} complex of the N^{α}-protected α-amino acid Boc-Bip-OH crystallises as a water/methanol solvate in the centrosymmetric space group P-1 with a=6.352(2) Å, b=11.465(2) Å, c=15.778(4) Å, α=76.47(2)°, β=79.98(2)°, γ=86.97(2)°. For these two structures data were collected on a Enraf–Nonius CAD-4 diffractometer with graphite monochromated CuKα (λ=1.54184 Å) radiation. The structures were solved by using the SHELXS 86 program[21a] and refined with the SHELXL 93 program.[21b] The amino H-atoms of H-Bip-O*t*Bu were located on the difference Fourier map and refined with fixed isotropic thermal parameters. In the two structures all H-atoms bound to carbons were placed in ideal positions and refined with a rigid model and fixed isotropic displacement parameters (Ux 1.2). The high temperature factors observed for the terminal *tert*-butyl groups in the two structures indicated a low accuracy in the determination of the position of their atoms. Final R-factors are R_1=0.0454 and wR_2=0.1234 for H-Bip-O*t*Bu, while R_1=0.0565 and wR_2=0.1596 for the Ca^{++} complex of Boc-Bip-OH.

The tripeptide Z-Gly-Bip-Gly-OEt crystallises in the centrosymmetric space group $P2_1/a$ with unit cell parameters a=9.924(2) Å, b=28.190(3) Å, c=10.515(2) Å, β=106.3(1)°. Data were collected on a Philips PW 1100 diffractometer with graphite monochromated MoKα (λ=0.71073 Å) radiation. The structure was solved by using the SHELXS 86 program[21a] and refined by full-matrix least-squares procedures on F^2, using all data, with the SHELXL 97 program.[21c] The C-terminal ethyl moiety is disordered. Its methyl group was isotropically refined on two sites (CT2 and CT2′) with occupancies 0.70 and 0.30, respectively. All other non-H atoms were anisotropically refined. H-atoms were calculated at idealised positions and during the refinement they were allowed to ride on their carrying atom, with U_{iso} set equal to 1.2 (or 1.5 for methyl groups) times the U_{eq} of the parent atom. The refinement converged to R_1=0.0466 and wR_2=0.1530.

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