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An extension of the 'Bip method': induced axial chirality in a series of dipeptides based on Bip/β^{2,2}-HBip combined with Ala/β^3-HAla

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Abstract—In the search for an extension of the 'Bip method' for determining the absolute configuration of β-amino acids and β-peptides, dipeptides based on β^{2,2}-HBip/L(D)-Ala, Bip/L-β^3-HAla, and β^{2,2}-HBip/L-β^3-HAla were synthesized in solution and the induced circular dichroism (ICD) in their biphenyl core evaluated in comparison with the previously investigated Bip/L(D)-Ala series. Weak, poorly informative ICDs were observed in MeOH solution for the linear *N*-Boc protected dipeptide methyl esters based on β^{2,2}-HBip, as well as for those with Ala/β^3-HAla at the N-terminus of Bip/β^{2,2}-HBip. However, a significant ICD was recorded for Boc-Bip-L-β^3-HAla-OMe. These results were confirmed by low-temperature ^{1}H NMR spectroscopy studies of the dipeptides in $CDCl_3$ and CD_3OD solutions, showing two diastereoisomeric conformers in significantly different populations for Boc-Bip-L-β^3-HAla-OMe in CD_3OD. In general, ICDs were found to be weaker for dipeptides containing β-amino acids as compared to those of their α-amino acid counterparts.

1. Introduction

We have previously reported that in peptides as short as dimers, an axial chirality can be induced in the biphenyl moiety of 2′,1′:1,2;1″,2″:3,4-dibenzcyclohepta-1,3-diene-6-amino-6-carboxylic acid (Bip), a conformationally labile, atropoisomeric, turn/3_{10}-helix inducer, C$^\alpha$-tetrasubstituted α-amino acid previously investigated in our groups,[1–5] in the presence of a chiral α-amino acid Xaa* [Ala, Val, Leu, (αMe)Val and (αMe)Leu], resulting in the onset of an equilibrium between two diastereoisomeric conformers with unequal populations, which can be detected by CD and ^{1}H NMR techniques.[6,7] The magnitude of this effect is particularly remarkable when the chiral α-amino acid residue Xaa* is positioned at the C-terminus of Bip, and signs of the CD bands correlate with the absolute configuration of Xaa*. More specifically, the C-terminal L-Xaa* and D-Xaa* α-amino acid residues preferentially induce negative and positive Cotton effects at 250 nm and *P* and *M* torsions in the biphenyl chromophore, respectively. This phenomenon of induced circular dichroism (ICD) represents the basis for the 'Bip method', an easy and fast configurational assignment of chiral α-amino acids, organic acids, amines, and alcohols currently being developed in our laboratories. In the search for an extension of the Bip method to the field of β-amino acids and β-peptides, *N*-Boc protected dipeptide methyl esters based on Bip **1** and 2′,1′:1,2;1″,2″:3,4-dibenzcyclohepta-1,3-diene-6-aminomethyl-6-carboxylic acid β^{2,2}-HBip **2**, a conformationally labile, atropoisomeric C$^\alpha$-tetrasubstituted β-amino acid analogue of Bip,[8–10] combined with either L-Ala **a**, or D-Ala **a**′, or L-β^3-HAla **b** at their C-terminus **1**,**2**/**a**,**a**′,**b** and their N-terminus **a**,**a**′,**b**/**1**,**2** (Fig. 1) were synthesized by solution methods. The induced axial chirality in their biphenyl core was evaluated by ^{1}H NMR and CD techniques.

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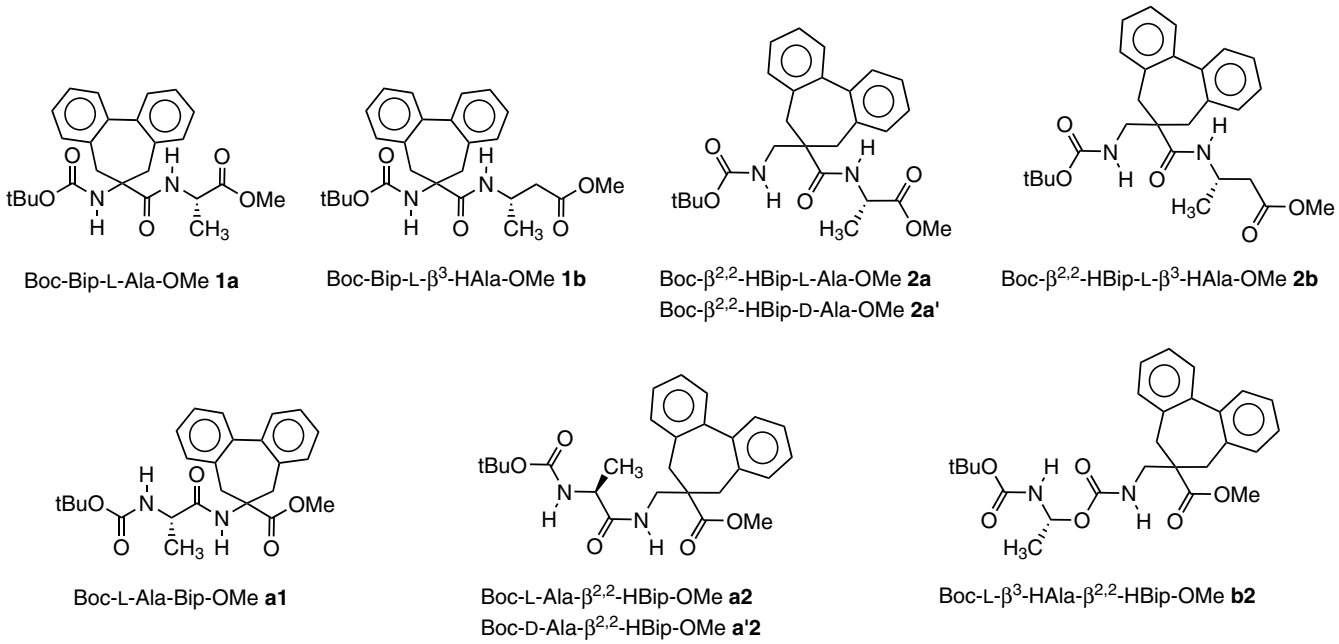

Figure 1. Chemical structures of the *N*-Boc protected dipeptide methyl esters discussed in this work.

2. Results and discussion

2.1. Synthesis

As for the previously synthesized Boc-Bip-L-Ala-OMe **1a**,[7] the terminally protected dipeptides Boc-Bip-L-β^3-HAla-OMe **1b** (86%), Boc-β^{2,2}-HBip-L-Ala-OMe **2a** (45%), Boc-β^{2,2}-HBip-D-Ala-OMe **2a′** (79%), and Boc-β^{2,2}-HBip-L-β^3-HAla-OMe **2b** (85%), with the Ala/β^3-HAla residue at the C-terminus of Bip/β^{2,2}-HBip, as well as Boc-L-Ala-Bip-OMe **a1**,[7] Boc-L-Ala-β^{2,2}-HBip-OMe **a2** (89%), Boc-D-Ala-β^{2,2}-HBip-OMe **a′2** (98%), and Boc-L-β^3-HAla-β^{2,2}-HBip-OMe **b2** (79%) (Fig. 1), were prepared in solution in high yields with EDC [*N*-ethyl,*N*′-(3-dimethylaminopropyl)-carbodiimide]/HOBt (1-hydroxy-1,2,3-benzotriazole)[11] or EDC/HOAt (7-aza-1-hydroxy-1,2,3-benzotriazole)[12] activation for coupling at the C-terminus of Bip and at both N,C-termini of β^{2,2}-HBip. These methods are known to be efficient in difficult cases involving sterically demanding C$^\alpha$-tetrasubstituted α-amino acids.[13]

2.2. ^{1}H NMR analysis

The ^{1}H NMR spectra of the dipeptides in CD_3OD, $CDCl_3$, or CD_3CN solution at 233 K generally exhibit two sets of signals corresponding to the presence of two diastereoisomeric conformers (Fig. 2), exchanging slowly on the NMR time scale, as previously observed for the Bip/Ala series.[7] Fast-interconverting conditions, resulting in the presence of only a single set of signals, are reached at about 333 K, as expected from the rotational energy barrier of 14 kcal mol^{-1} along the 1–1′ bond of the biphenyl moiety.[2,5]

Evolution of the ^{1}H NMR signals as a function of temperature for the dipeptides Boc-Bip-L-β^3-HAla-OMe **1b**, Boc-β^{2,2}-HBip-L-Ala-OMe **2a**, and Boc-β^{2,2}-HBip-L-β^3-HAla-OMe **2b** in CD_3OD are shown as representative examples (Fig. 3). In most cases (except for **2b**), the benzylic protons from Bip/β^{2,2}-HBip are resolved at 333 K into two different pairs of doublets reflecting the inequivalency of both carbons and protons $ArCH_AH_B$–$ArC'H_AH_B$, in both $CDCl_3$ and CD_3OD solutions (only in $CDCl_3$ for **b2**). In analogy with the Bip/Ala series,[7] the two doublets at a lower field are always better resolved (sharper peaks) than the two doublets at higher field, which remain broad, because of a higher coalescence temperature related to a higher difference in the corresponding chemical shifts of the two diastereomeric conformers. In the same manner, the patterns for the NCH_2 protons from β^{2,2}-HBip in **2a**, **a2**, **2b**, and **b2** are quite well resolved at 333 K into either a singlet (**b2** in CD_3OD), or an AB quartet (**a2** in CD_3OD), or a doublet-like peak (in the other cases). Similarly, those for the CH_2CO protons from β^3-HAla in **1b**, **2b**, and **b2** are well resolved at 333 K into either an AB quartet (in CD_3OD) or a doublet-like peak (in $CDCl_3$). At 233 K, slow exchange between the two conformers results in a complex superposition of pairs of doublets or AB quar-

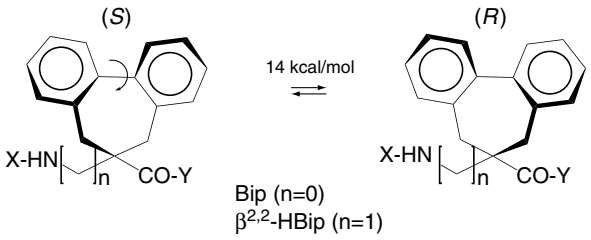

Figure 2. Conformational equilibrium between the two diastereoisomeric conformers present in the dipeptides in which Bip or β^{2,2}-HBip is combined with L-/D-Ala or L-β^3-HAla at its C-terminus **1a**, **1b**, **2a**, **2a′**, **2b** or at its N-terminus **a1**, **a2**, **a′2**, **b2**.

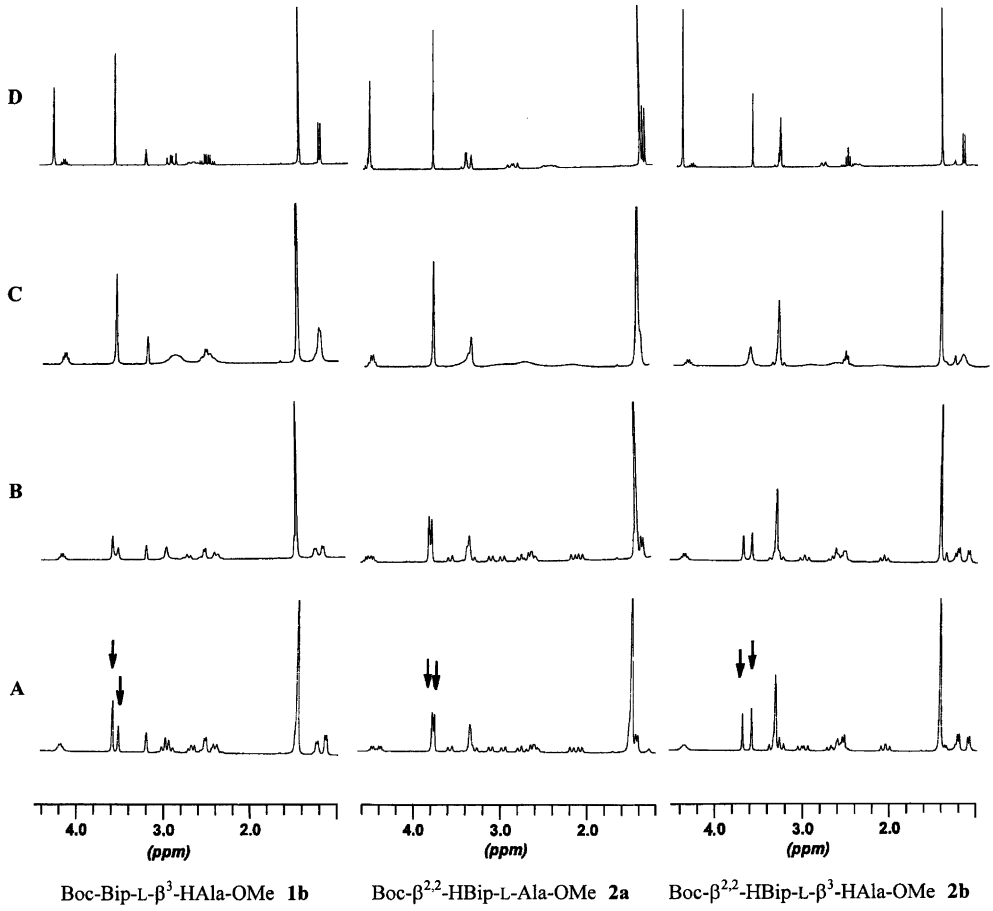

Figure 3. ^{1}H NMR signals (1.0–4.5 ppm) of the dipeptides Boc-Bip-L-β^3-HAla-OMe **1b**, Boc-β^{2,2}-HBip-L-Ala-OMe **2a**, and Boc-β^{2,2}-HBip-L-β^3-HAla-OMe **2b** in CD_3OD solution, as a function of temperature: 233 K (A) (the arrows indicate the separation of the –COOMe singlets in both diastereoisomeric conformers), 263 K (B), 293 K (C), and 333 K (D).

tets for all of the above-mentioned protons. On the other hand, the proton signals for the Boc, –COOMe, amide and/or carbamate NH groups, as well as the CH and CH_3 groups from Ala or β^3-HAla, can easily be identified at 333 K and 233 K (Table 1).

The diastereoisomeric ratio (dr) was determined at 233 K by integration of the two singlets relative to the COOMe group, which are generally (except for **a2** in $CDCl_3$ solution) well separated in both $CDCl_3$ and CD_3OD and therefore especially suitable for a high-accuracy calculation (Table 1). In most cases, the two singlets relative to the Boc group are separated in $CDCl_3$ solution (except for **2b**), but not in CD_3OD (except for **a2**). The amide and/or carbamate NH proton (in $CDCl_3$ and CD_3CN only), and the CH and CH_3 protons from Ala or β^3-HAla as well, are also more or less separated and could only be utilized occasionally.

The diastereoisomeric ratios (Table 2) are dependent on the nature of the solvent, with CD_3OD, also used in our CD experiments (vide infra), being the solvent of choice over $CDCl_3$ and CD_3CN to observe higher dr values, as found in our previous studies.[6,7] In CD_3OD, the dr values were found to be dependent (i) on the nature of the residue containing the diphenyl core (either Bip or β^{2,2}-HBip), with significant values in the case of Bip only, and (ii) as expected,[6,7] on the C-terminal (higher dr) or N-terminal (lower dr) position of the Ala or the β^3-HAla residue. Altogether, the diastereoisomeric ratios of dipeptides **2a**, **2b**, **a′1**, **a2**, and **b2** remained in the same, poorly significant, low range of 50:50 to ca. 55:45 in both $CDCl_3$ and CD_3OD solutions. Apart from our previously reported 'all-α' dipeptide Boc-Bip-D-Ala-OMe **1a′**,[7] the only compound of the present series showing a significant dr value (68:32) is Boc-Bip-L-β^3-HAla-OMe **1b** (in CD_3OD).

2.3. CD analysis

The biphenyl chromophore present in the Bip/β^{2,2}-HBip residues is characterized by an intense electronic transition at about 240–250 nm, assigned to the A band,[14] followed by a very intense transition at ca. 210–215 nm (C band). The wavelength of the absorption maximum of the A band is strongly dependent on θ, the biphenyl axial torsion angle. Several studies have established that in the CD spectra of biphenyl-based chiral molecules a negative maximum corresponding to the A band is related to a *P* torsion of the C_{Ar}–C'_{Ar} bond [(*S*)-configuration of Bip or β^{2,2}-HBip], and a positive maximum to an *M* torsion.[15–17]

Table 1. Chemical shifts (ppm) of selected ^{1}H NMR signals of the Bip dipeptides in $CDCl_3$ and CD_3OD solutions at 233 and 333 K

NMR signals	$CDCl_3$		CD_3OD	
	233 K	333 K	233 K	333 K
Boc-Bip-L-β^3-HAla-OMe **1b**				
NH β^3-HAla	Masked	6.95	—	—
NH Bip	5.06 and 4.93	4.82	—	—
CH β^3-HAla	4.43	4.38	4.36	4.30
COOMe	3.70 and 3.66	3.68	3.72 and 3.65	3.69
CH_3 Boc	1.44 and 1.43	1.49	1.49	1.49
CH_3 β^3-HAla	1.27 and 1.21	1.27	1.26 and 1.16	1.24
Boc-β^{2,2}-HBip-L-Ala-OMe **2a**				
NH Ala	6.18	6.13	—	—
NH β^{2,2}-HBip	5.61 and 5.49	5.30	—	—
CH Ala	4.48	4.55	4.43 and 4.34	4.43
COOMe	3.80 and 3.77	3.76	3.74 and 3.72	3.73
CH_3 Boc	1.45 and 1.42	1.46	1.47	1.45
CH_3 Ala	1.41 and 1.35	1.38	1.42	1.41
Boc-β^{2,2}-HBip-L-β^3-HAla-OMe **2b**				
NH β^3-HAla	6.38 and 6.19	6.12	—	—
NH β^{2,2}-HBip	5.47 and 5.41	5.22	—	—
CH β^3-HAla	4.37	4.37	4.33	4.32
COOMe	3.64 and 3.58	3.64	3.68 and 3.58	3.63
CH_3 Boc	1.43	1.46	1.46	1.44
CH_3 β^3-HAla	1.17	1.20	1.25 and 1.13	1.19
Boc-L-Ala-β^{2,2}-HBip-OMe **a2**				
NH Ala	5.08 and 5.05	4.87	—	—
NH β^{2,2}-HBip	6.95 and 6.78	6.51	—	—
CH Ala	4.17 and 4.12	4.15	4.02	4.08
COOMe	3.73	3.75	3.70 and 3.69	3.72
CH_3 Boc	1.44 and 1.42	1.48	1.45 and 1.43	1.45
CH_3 Ala	1.37	1.38	1.30 and 1.28	1.30
Boc-L-β^3-HAla-β^{2,2}-HBip-OMe **b2**				
NH β^3-HAla	5.55 and 5.44	5.07	—	—
NH β^{2,2}-HBip	6.63 and 6.55	6.08	—	—
CH β^3-HAla	4.01	3.97	3.95	3.93
COOMe	3.75 and 3.72	3.75	3.72 and 3.70	3.72
CH_3 Boc	1.40 and 1.39	1.45	1.41	1.41
CH_3 β^3-HAla	1.23	1.26	1.15	1.18

Table 2. Dr values for the two diastereoisomeric conformers of title *N*-Boc protected dipeptide methyl esters in CD_3OD, $CDCl_3$, and CD_3CN solutions at 233 K, calculated by integration of the COOMe singlets (see Section 4)

Dipeptides	CD_3OD	$CDCl_3$	CD_3CN
Boc-Bip-D-Ala-OMe **1a′**[7]	81:19[7]	56:44[7]	76:24[7]
Boc-β^{2,2}-HBip-L-Ala-OMe **2a**	51:49	57:43	—
Boc-Bip-L-β^3-HAla-OMe **1b**	68:32	52:48	—
Boc-β^{2,2}-HBip-L-β^3-HAla-OMe **2b**	54:46	56:44	56:44
Boc-D-Ala-Bip-OMe **a′1**[7]	51:49[7]	54:46[7]	—
Boc-L-Ala-β^{2,2}-HBip-OMe **a2**	52:48	53:47	—
Boc-L-β^3-HAla-β^{2,2}-HBip-OMe **b2**	52:48	52:48	54:46

Our CD analysis in MeOH solution of the terminally protected Bip linear dipeptides allowed us to draw the following conclusions: (i) The previously reported ICD in the biphenyl core of the Boc-Bip-L/D-Ala-OMe dipeptides, giving a clear information on the Ala configuration,[7] is also shown by the Boc-Bip-L-β^3-HAla-OMe dipeptide **1b**, in which the L-Ala residue at the C-terminal position of Boc-Bip-L-Ala-OMe **1a** is replaced by a L-β^3-HAla residue, although with a weaker, but still informative, CD signal (Fig. 4); (ii) the replacement of

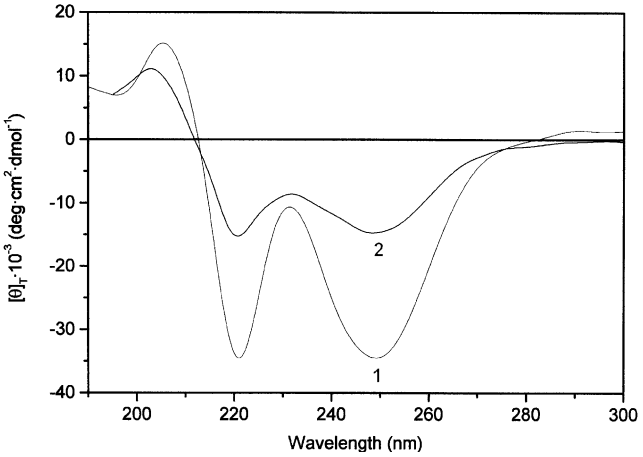

Figure 4. CD spectra of Boc-Bip-L-Ala-OMe **1a**[7] (1) and Boc-Bip-L-β^3-HAla-OMe **1b** (2) in MeOH solution (concentration 1×10^{-3} M).

Bip by β^{2,2}-HBip is always accompanied by a significant decrease of the CD signal, resulting in a less informative signature of the A band. That is the case for the dipep-

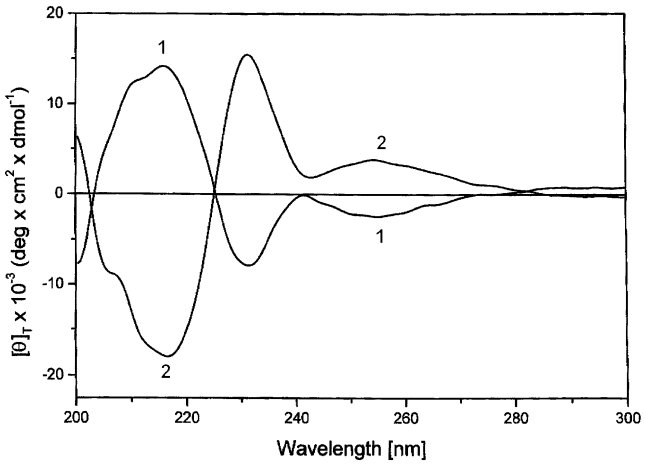

Figure 5. CD spectra of Boc-β^{2,2}-HBip-L-Ala-OMe **2a** (1) and Boc-β^{2,2}-HBip-D-Ala-OMe **2a′** (2) in MeOH solution (concentration 4×10^{-3} M).

tide Boc-β^{2,2}-HBip-L-Ala-OMe **2a** (Fig. 5) compared to **1a** (Fig. 4). Still, it can be clearly observed that the spectra of the enantiomeric Boc-β^{2,2}-HBip-L-Ala-OMe **2a** and Boc-β^{2,2}-HBip-D-Ala-OMe **2a′** dipeptides are mirror images (Fig. 5), as expected; (iii) The CD curves of the dipeptides **2b**, **b2**, and **a2/a′2**, all involving β^{2,2}-HBip, present very weak, not informative, Cotton effects in the A band region of the biphenyl absorption (not shown); (iv) in dipeptides **1b**, **2a**, and **2a′** a *P* torsion of the biphenyl axial bond [(*S*)-configuration of both Bip and β^{2,2}-Hbip] is preferentially induced by both L-Ala and L-β^3-HAla C-terminal residues, and a *M* torsion by a C-terminal D-Ala residue.

3. Conclusion

Taken together, the results of the present extension of the 'Bip method' have revealed that a substantial central-to-axial induction of chirality from a C-terminal L-β^3-HAla to the pro-atropoisomeric, C$^\alpha$-tetrasubstituted α-amino acid Bip residue occurs in simple linear dipeptides, and results in a marked ICD effect, the negative maximum of the A band of which is associated to an *S* configuration of Bip. Conversely, a poor ICD is observed for the corresponding β^{2,2}-HBip dipeptides. Therefore, the Bip (rather than β^{2,2}-HBip) residue is proposed to be used as a convenient CD probe for the determination of the absolute configuration of not only α-amino acids, as previously reported,[7] but of β-amino acids as well. A further extension of this study to a series of dipeptides, in which Bip will be combined to other β-amino acids, for comparison with β^3-HAla, is currently in progress in our laboratories.

4. Experimental

4.1. Synthesis of peptides

Melting points were measured on a Mettler apparatus with a final temperature raise of 3 °C/min or by means of a capillary tube immersed in an oil bath (Tottoli apparatus, Büchi) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker WM300 spectrometer operating at 300 and 77 MHz, respectively, the solvent $CDCl_3$ (^{1}H: $\delta = 7.27$ ppm; ^{13}C: $\delta = 77.00$ ppm) or CD_3OD (^{1}H: $\delta = 3.31$ ppm) or CD_3CN (^{1}H: $\delta = 1.94$ ppm) being used as the internal standard. Splitting patterns are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (q) quartet, and (m) multiplet. The optical rotations were measured in a 1-dm thermostated cell on a Perkin–Elmer 241 polarimeter, with an accuracy of 0.3%. Elemental analyses were performed by the CNRS Service of Microanalyses in Gif-sur-Yvette (France). Analytical and/or preparative TLC and column chromatography were performed on Kieselgel 60 F254 and Kieselgel 60 (0.040–0.063 mm) (Merck), respectively, with the following eluant systems: 5% EtOAc (ethyl acetate)–95% CH_2Cl_2 (I); 10% EtOAc–90% CH_2Cl_2 (II), 20% EtOAc–80% CH_2Cl_2 (III); 5% MeOH (methanol)–95% CH_2Cl_2 (IV). UV light ($\lambda = 254$ nm) allowed visualization of the spots after TLC runs for all compounds. Except when stated, all starting materials and solvents were obtained from commercial suppliers and were used as received. The syntheses and characterizations of the Bip derivatives Boc-Bip-OH,[2] Boc-β^{2,2}-HBip-OH,[10] and H-β^{2,2}-HBip-OMe[10] have been previously reported. Boc-L-β^3-HAla-OH was purchased from Fluka.

4.2. Peptide coupling: general procedure

A solution (or a suspension) of the *N*-Boc-protected aminoacid, the amino ester (or its hydrochloride) and HOAt (or HOBt) in CH_2Cl_2/THF was cooled to 0 °C. In case of an amino ester hydrochloride, NMM [*N*-methyl morpholine] (or TEA [triethylamine]) was added. This was followed by the addition of EDC. The reaction mixture was allowed to warm up to rt, magnetically stirred for ca. 3 days and concentrated in vacuo. The residue was dissolved in EtOAc (150 mL), the solution was successively extracted with 0.5 M HCl (2×75 mL), H_2O (100 mL), 5% $NaHCO_3$ (2×75 mL), and H_2O (2×100 mL), then dried over $MgSO_4$, filtered, and evaporated in vacuo at 40 °C, to yield a crude product which was purified by chromatography.

4.3. HCl·H-L-β^3-HAla-OMe

The N-protected amino acid Boc-L-β^3-HAla-OH (0.150 g, 0.74 mmol) was dissolved in CH_2Cl_2 (7.5 mL), the solution cooled to 0 °C and TFA (trifluoroacetic acid) (7.5 mL) added. The solution was magnetically stirred at 0 °C for 15 min and then at rt for 2.5 h. The solution was evaporated in vacuo at 25 °C and the residue repeatedly co-evaporated in vacuo with CH_2Cl_2 at 40 °C. The crude TFA·H-L-β^3-HAla-OH obtained was dissolved in MeOH (6 mL), the solution cooled to 0 °C and $SOCl_2$ (1.20 mL) added dropwise. The resulting solution was stirred at rt for 1 week, and evaporated to dryness in vacuo. The obtained crude residue was repeatedly co-evaporated in vacuo with MeOH at 40 °C. The crude HCl·H-L-β^3-HAla-OMe obtained (0.127 g) was pure by ^{1}H NMR (CD_3OD): δ 3.74 [s, 3H, OCH_3], 3.69 [m (partly masked), 1H, CH βAla], 2.80 [dd, $J = 6.8$ and 17.1 Hz, 1H] and 2.73 [dd,

$J = 6.3$ and 17.1 Hz, 1H] [CH_2 βAla], and was used in the next coupling steps without further treatment.

4.4. Boc-Bip-L-β³-HAla-OMe 1b

The N-protected amino acid Boc-Bip-OH[2] (0.261 g, 0.74 mmol) and the amino ester hydrochloride HCl·H-L-β³-HAla-OMe (0.114 g, 0.74 mmol) were reacted with HOAt (0.201 g, 1.48 mmol), NMM (0.164 mL, 1.48 mmol) and EDC (0.212 g, 1.11 mmol) in THF (6 mL) and CH_2Cl_2 (13 mL), according to the general procedure of peptide coupling. The crude product obtained after extraction was purified by column chromatography on silica gel, and eluted successively with eluants (I) and (II), to afford 0.288 g (86%) of pure dipeptide **1b** as a solid. Mp = 197 °C. $R_f = 0.32$ (II). ^{1}H NMR (293 K, $CDCl_3$): δ 7.5–7.2 [m, 8H, ArH Bip], 7.04 [br m, 1H, NH βAla], 4.87 [s, 1H, NH Bip], 4.39 [m, 1H, CH βAla], 3.68 [s, 3H, OCH_3], 3.3–2.1 [br m, 4H, $ArCH_2$ Bip and $ArC'H_2$ Bip], 2.57 [m (d-like), $J = 5.3$ Hz, 2H, CH_2 βAla], 1.47 [s, 9H, CH_3 Boc], 1.26 [d, $J = 6.8$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (333 K, $CDCl_3$): δ 7.45–7.25 [m, 8H, ArH Bip], 6.95 [br d, 1H, NH βAla], 4.82 [s, 1H, NH Bip], 4.38 [m, 1H, CH βAla], 3.68 [s, 3H, OCH_3], 3.23 [d, $J = 12.8$ Hz, 1H] and 2.66 [br d, $J \sim 13.0$ Hz, 1H] [$ArCH_2$ Bip], 3.18 [d, $J = 13.0$ Hz, 1H] and 2.66 [br d, $J \sim 13.0$ Hz, 1H] [$ArC'H_2$ Bip], 2.57 [m (d-like), $J = 5.3$ Hz, 2H, CH_2 βAla], 1.49 [s, 9H, CH_3 Boc], 1.27 [d, $J = 6.7$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (233 K, $CDCl_3$): δ 7.6–7.1 [m, 9H, ArH Bip and NH βAla], 5.06 (ca. 52%) and 4.93 (ca. 48%) [s, 1H, NH Bip], 4.43 [br m, 1H, CH βAla], 3.70 (ca. 52%) and 3.66 (ca. 48%) [s, 3H, OCH_3], 3.4–2.2 [m, 6H, $ArCH_2$ Bip, $ArC'H_2$ Bip and CH_2 βAla], 1.44 (ca. 50%) and 1.43 (ca. 50%) [s, 9H, CH_3 Boc], 1.27 (ca. 50%) and 1.21 (ca. 50%) [d, $J \sim 6.7$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (293 K, CD_3OD): δ 7.5–7.1 [m, 8H, ArH Bip], 4.32 [m, 1H, CH βAla], 3.69 [s, 3H, OCH_3], 3.15–2.30 [br m, 6H, $ArCH_2$ Bip, $ArC'H_2$ Bip and CH_2 βAla], 1.49 [s, 9H, CH_3 Boc], 1.24 [br m, 3H, CH_3 βAla]. ^{1}H NMR (333 K, CD_3OD): δ 7.45–7.10 [m, ArH, 8H], 4.30 [m, 1H, CH βAla], 3.69 [s, 3H, OCH_3], 3.03 [d, $J = 13.6$ Hz, 1H] and 2.71 [br d, $J \sim 13.9$ Hz, 1H] [$ArCH_2$ Bip], 2.97 [d, $J = 13.6$ Hz, 1H] and 2.77 [br d, $J \sim 13.9$ Hz, 1H] [$ArC'H_2$ Bip], 2.62 [dd, $J = 6.1$ and 15.4 Hz, 1H] and 2.52 [dd, $J = 6.4$ and 15.4 Hz, 1H] [CH_2 βAla], 1.49 [s, 9H, CH_3 Boc], 1.24 [d, $J = 6.7$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (233 K, CD_3OD): δ 7.5–7.1 [m, 8H, ArH Bip], 4.36 [br m, 1H, CH βAla], 3.72 (ca. 68%) and 3.65 (ca. 32%) [s, 3H, OCH_3], 3.2–2.4 [m, 6H, $ArCH_2$ Bip, $ArC'H_2$ Bip and CH_2 βAla], 1.49 [s, 9H, CH_3 Boc], 1.26 (ca. 32%) and 1.16 (ca. 68%) [br d, $J \sim 6.2$ Hz, 3H, CH_3 βAla]. ^{13}C NMR (293 K, $CDCl_3$): δ 172.3, 172.0 [C=O βAla and Bip], 154.8 [C=O Boc], 140.7, 135.6, 130.2, 128.3, 127.8, 127.7 [C^{Ar}], 77.9 [C–O Boc], 69.8 [C^{α} Bip], 51.7 [OCH_3], 42.2 [CH βAla], 40.2–39.8 (br) [$ArCH_2$ and $ArC'H_2$ Bip], 40.0 [CH_2 βAla], 28.4 [CH_3 Boc], 20.1 (CH_3 βAla). $[\alpha]^{25}_{589} = -18.2$; $[\alpha]^{25}_{578} = -22.1$; $[\alpha]^{25}_{546} = -26.3$; $[\alpha]^{25}_{436} = -54.7$; $[\alpha]^{25}_{365} = -95.8$ (*c* 0.3, CH_2Cl_2). Anal. Calcd for $C_{26}H_{32}N_2O_5$ (452.532): C, 69.00; H, 7.13; N, 6.19. Found: C, 68.50; H, 7.12; N, 5.83.

4.5. Boc-β^{2,2}-HBip-L-Ala-OMe 2a

The N-protected amino acid Boc-β^{2,2}-HBip-OH[10] (0.092 g, 0.25 mmol) and the amino ester hydrochloride HCl·H-L-Ala-OMe (0.070 g, 0.50 mmol) were reacted with HOBt (0.067 g, 0.50 mmol), TEA (0.070 mL; 0.50 mmol), and EDC (0.071 g, 0.37 mmol) in THF (2.5 mL) and CH_2Cl_2 (5 mL), according to the general procedure of peptide coupling. The crude product obtained after extraction was purified by column chromatography on silica gel eluted with eluant (II), to afford 0.051 g (45%) of pure dipeptide **2a** as a solid. Mp = 75 °C. $R_f = 0.33$ (II). ^{1}H NMR (293 K, $CDCl_3$): δ 7.5–7.2 [m, 8H, ArH βBip], 6.14 [br m, 1H, NH Ala], 5.41 [br m, 1H, NH βBip], 4.52 [dq, $J \sim 7.2$ Hz and 7.2 Hz, 1H, CH Ala], 3.76 [s, 3H, OCH_3], 3.40 [m (br, d-like), 2H, NCH_2 βBip], 3.2–2.1 [br m, 4H, $ArCH_2$ βBip and $ArC'H_2$ βBip], 1.45 [s, 9H, CH_3 Boc], 1.38 [d, $J = 7.2$ Hz, 3H, CH_3 Ala]. ^{1}H NMR (333 K, $CDCl_3$): δ 7.45–7.25 [m, 8H, ArH βBip], 6.13 [br d, $J \sim 6.2$ Hz, 1H, NH Ala], 5.30 [br m, 1H, NH βBip], 4.55 [dq, $J \sim 7.1$ and 7.1 Hz, 1H, CH Ala], 3.76 [s, 3H, OCH_3], 3.49 [m (d-like), $J \sim 6.5$ Hz, 2H, NCH_2 βBip], 2.83 [d, $J \sim 12.5$ Hz, 1H] and 2.51 [br d, $J \sim 13.3$ Hz, 1H] [$ArCH_2$ βBip], 2.79 [d, $J \sim 13.0$ Hz, 1H] and 2.57 [br d, $J \sim 13.2$ Hz, 1H] [$ArC'H_2$ βBip], 1.46 [s, 9H, CH_3 Boc], 1.38 [d, $J = 7.1$ Hz, 3H, CH_3 Ala]. ^{1}H NMR (233 K, $CDCl_3$): δ 7.5–7.2 [m, 8H, ArH βBip], 6.18 [br m, 1H, NH Ala], 5.61 (ca. 57%) and 5.49 (ca. 43%) [br m, 1H, NH βBip], 4.48 [m, 1H, CH Ala], 3.80 (ca. 43%) and 3.77 (ca. 57%) [s, 3H, OCH_3], 3.42 and 3.33 [m, 2H, NCH_2 βBip], 3.0–2.2 [m, 4H, $ArCH_2$ and $ArC'H_2$ βBip], 1.45 (ca. 57%) and 1.42 (ca. 43%) [s, 9H, CH_3 Boc], 1.41 and 1.35 [d (partly masked), $J \sim 7.1$ Hz, 3H, CH_3 Ala]. ^{1}H NMR (293 K, CD_3OD): δ 7.5–7.1 [m, 8H, ArH βBip], 4.42 [m (q-like), 1H, CH Ala], 3.73 [s, 3H, OCH_3], 3.34 [br m, 2H, NCH_2 βBip], 3.2–1.9 [br m, 4H, $ArCH_2$ βBip and $ArC'H_2$ βBip], 1.46 [s, 9H, CH_3 Boc], 1.45 [d (masked), 3H, CH_3 Ala]. ^{1}H NMR (333 K, CD_3OD): δ 7.40–7.20 [m, 8H, ArH βBip], 4.43 [q, $J = 7.2$ Hz, 1H, CH Ala], 3.73 [s, 3H, OCH_3], 3.37 [m (d-like), $J \sim 4.1$ Hz, 2H, NCH_2 βBip], 2.88 [d, $J \sim 13.8$ Hz, 1H] and 2.40 [br d, 1H] [$ArCH_2$ βBip], 2.82 [d, $J \sim 13.6$ Hz, 1H] and 2.48 [br d, $J \sim 13.4$ Hz, 1H] [$ArC'H_2$ βBip], 1.45 [s, 9H, CH_3 Boc], 1.41 [d, $J = 7.3$ Hz, 3H, CH_3 Ala]. ^{1}H NMR (233 K, CD_3OD): δ 7.6–7.1 [m, 8H, ArH βBip], 4.43 (ca. 50%) and 4.34 (ca. 50%) [br q, 1H, CH Ala], 3.74 (ca. 51%) and 3.72 (ca. 49%) [s, 3H, OCH_3], 3.6–2.0 [m, 6H, NCH_2 βBip, $ArCH_2$ βBip and $ArC'H_2$ βBip], 1.47 [s, 9H, CH_3 Boc], 1.42 [m (partly masked), 3H, CH_3 Ala]. ^{13}C NMR (293 K, $CDCl_3$): δ 175.4, 173.6 [C=O Ala and βBip], 156.4 [C=O Boc], 140.8, 130.5, 128.6, 127.9, 127.8 [C^{Ar}], 79.4 [C–O Boc], 58.3 [C^{α} βBip], 52.7 [OCH_3], 48.6 [CH Ala], 45.8 [NCH_2 βBip], 38.1 (br) [$ArCH_2$ and $ArC'H_2$ βBip], 28.6 [CH_3 Boc], 18.0 (CH_3 Ala). $[\alpha]^{25}_{589} = -9.1$; $[\alpha]^{25}_{578} = -12.8$; $[\alpha]^{25}_{546} = -14.6$; $[\alpha]^{25}_{436} = -41.1$; $[\alpha]^{25}_{365} = -69.4$ (*c* 0.1, CH_2Cl_2). Anal. Calcd for $C_{26}H_{32}N_2O_5$ (452.532): C, 69.00; H, 7.13; N, 6.19. Found: C, 68.84; H, 7.16; N, 6.31.

4.6. Boc-β^{2,2}-HBip-D-Ala-OMe 2a′

Prepared as **2a** from Boc-β^{2,2}-HBip-OH[10] (0.037 g, 0.10 mmol), HCl·H-D-Ala-OMe (0.028 g, 0.20 mmol), HOBt (0.027 g, 0.20 mmol), TEA (0.028 mL, 0.20 mmol), and EDC (0.029 g, 0.15 mmol) in THF (1 mL) and CH_2Cl_2 (2 mL), according to the general procedure of peptide coupling. The crude product obtained after extraction was purified by column chromatography on silica gel eluted with eluant (II), to afford 0.036 g (79%) of pure dipeptide **2a′** as a solid. Mp = 82 °C. ^{1}H NMR (293 K, $CDCl_3$) and ^{13}C NMR (293 K, $CDCl_3$): as **2a**. $[\alpha]^{25}_{589} = +28.4$; $[\alpha]^{25}_{578} = +28.4$; $[\alpha]^{25}_{546} = +24.2$; $[\alpha]^{25}_{436} = +27.4$; $[\alpha]^{25}_{365} = +54.7$ (*c* 0.1, CH_2Cl_2). Anal. Calcd for $C_{26}H_{32}N_2O_5{\cdot}0.5H_2O$ (461.612): C, 67.65; H, 7.21; N, 6.07. Found: C, 67.75; H, 7.41; N, 5.85.

4.7. Boc-L-Ala-β^{2,2}-HBip-OMe a2

The N-protected amino acid Boc-L-Ala-OH (0.151 g, 0.80 mmol) and the amino ester H-β^{2,2}-HBip-OMe[10] (0.112 g, 0.40 mmol) were reacted with HOBt (0.216 g, 1.60 mmol) and EDC (0.230 g, 1.20 mmol) in THF (8 mL) and CH_2Cl_2 (8 mL), according to the general procedure of the peptide coupling. The crude product obtained after extraction was purified by column chromatography on silica gel eluted with eluant (III) to afford 0.161 g (89%) of pure dipeptide **a2** as a solid. Mp = 86 °C. $R_f = 0.22$ (III). ^{1}H NMR (293 K, $CDCl_3$): δ 7.5–7.1 [m, 8H, ArH βBip], 6.78 [br m, 1H, NH βBip], 5.17 [d, $J = 7.3$ Hz, 1H, NH Ala], 4.16 [br m, 1H, CH Ala], 3.72 [s, 3H, OCH_3], 3.7–3.2 [br m, 2H, NCH_2 βBip], 3.2–2.1 [br m, 4H, $ArCH_2$ βBip and $ArC'H_2$ βBip], 1.45 [s, 9H, CH_3 Boc], 1.37 [d, $J = 7.0$ Hz, 3H, CH_3 Ala]. ^{1}H NMR (333 K, $CDCl_3$): δ 7.45–7.25 [m, 8H, ArH βBip], 6.51 [br m, 1H, NH βBip], 4.87 [br d, $J \sim 5.7$ Hz, 1H, NH Ala], 4.15 [dq, $J \sim 7.1$ and 7.1 Hz, 1H, CH Ala], 3.75 [s, 3H, OCH_3], 3.55 [m, 2H, NCH_2 βBip], 2.91 [d, $J \sim 13.8$ Hz, 1H] and 2.47 [br d, 1H] [$ArCH_2$ βBip], 2.89 [d, $J \sim 13.8$ Hz, 1H] and 2.51 [br d, 1H] [$ArC'H_2$ βBip], 1.48 [s, 9H, CH_3 Boc], 1.38 [d, $J = 7.1$ Hz, 3H, CH_3 Ala]. ^{1}H NMR (233 K, $CDCl_3$): δ 7.5–7.1 [m, 8H, ArH βBip], 6.95 (ca. 53%) and 6.78 (ca. 47%) [br m, 1H, NH βBip], 5.08 (ca. 47%) and 5.05 (ca. 53%) [br d, $J \sim 7.9$ Hz, 1H, NH Ala], 4.17 (ca. 50%) and 4.12 (ca. 50%) [m, 1H, CH Ala], 3.73 [s, 3H, OCH_3], 3.64 [m, 1H] and 3.35 [m, 1H] [NCH_2 βBip], 3.0–2.2 [m, 4H, $ArCH_2$ and $ArC'H_2$ βBip], 1.44 (ca. 47%) and 1.42 (ca. 53%) [s, 9H, CH_3 Boc], 1.37 [m (partly masked), 3H, CH_3 Ala]. ^{1}H NMR (293 K, CD_3OD): δ 7.5–7.1 [m, 8H, ArH βBip], 4.07 [m (q-like), 1H, CH Ala], 3.71 [s, 3H, OCH_3], 3.59 [m, 1H] and 3.35 [m, 1H] [NCH_2βBip], 3.2–1.9 [br m, 4H, $ArCH_2$ βBip and $ArC'H_2$ βBip], 1.44 [s, 9H, CH_3 Boc], 1.29 [d, $J = 7.2$ Hz, 3H, CH_3 Ala]. ^{1}H NMR (333 K, CD_3OD): δ 7.40–7.20 [m, 8H, ArH βBip], 4.08 [q, $J = 7.2$ Hz, 1H, CH Ala], 3.72 [s, 3H, OCH_3], 3.58 [d, $J = 13.6$ Hz, 1H] and 3.39 [d, $J = 13.6$ Hz, 1H] [NCH_2 βBip], 2.87 [d, $J \sim 13.5$ Hz, 1H] and 2.45 [br d, 1H] [$ArCH_2$ βBip], 2.85 [d, $J \sim 13.5$ Hz, 1H] and 2.45 [br d, 1H] [$ArC'H_2$ βBip], 1.45 [s, 9H, CH_3 Boc], 1.30 [d, $J = 7.2$ Hz, 3H, CH_3 Ala]. ^{1}H NMR (233 K, CD_3OD): δ 7.5–7.2 [m, 8H, ArH βBip], 4.02 [br q, 1H, $J \sim 6.9$ Hz, CH Ala], 3.70 (ca. 50%) and 3.69 (ca. 50%) [s, 3H, OCH_3], 3.72 [d (partly masked), 0.5H]-3.35 [d (partly masked), 0.5H] (ca. 50%) and 3.57 [d, $J = 13.4$ Hz, 0.5H]-3.20 [d, $J = 13.3$ Hz, 0.5H] (ca. 50%) [NCH_2 βBip], 3.1–2.1 [m, 4H, $ArCH_2$ βBip and $ArC'H_2$ βBip], 1.45 (ca. 48%) and 1.43 (ca. 52%) [s, 9H, CH_3 Boc], 1.30 (ca. 50%) and 1.28 (ca. 50%) [d (superimposed), $J \sim 6$ Hz, 3H, CH_3 Ala]. ^{13}C NMR (293 K, $CDCl_3$): δ 175.5, 173.0 [C=O Ala and βBip], 155.7 [C=O Boc], 140.8, 135.7, 130.2, 128.3, 127.7, 127.6 [C^{Ar}], 80.2 [C–O Boc], 58.4 [C^{α} βBip], 52.4 [OCH_3], 50.5 [CH Ala], 44.1 [NCH_2 βBip], 38.2 (br) [$ArCH_2$ βBip], 36.2 (br) [$ArC'H_2$ βBip], 28.5 [CH_3 Boc], 18.3 (CH_3 Ala). $[\alpha]^{25}_{589} = -6.7$; $[\alpha]^{25}_{578} = -14.3$; $[\alpha]^{25}_{546} = -14.3$; $[\alpha]^{25}_{436} = -47.6$; $[\alpha]^{25}_{365} = -82.9$ (*c* 0.1, CH_2Cl_2). Anal. Calcd for $C_{26}H_{32}N_2O_5$ (452.532): C, 69.00; H, 7.13; N, 6.19. Found: C, 68.90; H, 7.20; N, 6.25.

4.8. Boc-D-Ala-β^{2,2}-HBip-OMe a′2

Prepared as **a2** from Boc-D-Ala-OH (0.038 g, 0.20 mmol), H-β^{2,2}-HBip-OMe[10] (0.028 g, 0.10 mmol), HOBt (0.054 g, 0.40 mmol) and EDC (0.057 g, 0.30 mmol) in THF (2 mL) and CH_2Cl_2 (2 mL), according to the general procedure of peptide coupling. The crude product obtained after extraction was purified by column chromatography on silica gel eluted with eluant (III) to afford 0.045 g (98%) of pure dipeptide **a′2** as a solid. Mp = 83 °C. ^{1}H NMR (293 K, $CDCl_3$) and ^{13}C NMR (293 K, $CDCl_3$): as **a2**. $[\alpha]^{25}_{589} = +38.6$; $[\alpha]^{25}_{578} = +34.5$; $[\alpha]^{25}_{546} = +38.6$; $[\alpha]^{25}_{436} = +45.7$; $[\alpha]^{25}_{365} = +70.0$ (*c* 0.1, CH_2Cl_2). Anal. Calcd for $C_{26}H_{32}N_2O_5{\cdot}0.5H_2O$ (461.612): C, 67.65; H, 7.21; N, 6.07. Found: C, 67.89; H, 7.42; N, 5.62.

4.9. Boc-β^{2,2}-HBip-L-β^3-HAla-OMe 2b

The N-protected amino acid Boc-β^{2,2}-HBip-OH[10] (0.110 g, 0.30 mmol) and the amino ester hydrochloride HCl·H-L-β^3-HAla-OMe (0.092 g, 0.60 mmol) were reacted with HOBt (0.081 g, 0.60 mmol), TEA (0.084 mL; 0.60 mmol), and EDC (0.086 g, 0.45 mmol) in THF (3 mL) and CH_2Cl_2 (6 mL), according to the general procedure of peptide coupling. The crude product obtained after extraction was purified by column chromatography on silica gel eluted with eluant (II) to afford 0.119 g (85%) of pure dipeptide **2b** as a solid. Mp = 64 °C. $R_f = 0.30$ (II), 0.75 (IV). ^{1}H NMR (293 K, $CDCl_3$): δ 7.5–7.2 [m, 8H, ArH βBip], 6.18 [br m, 1H, NH βAla], 5.33 [br m, 1H, NH βBip], 4.36 [br m, 1H, CH βAla], 3.62 [s, 3H, OCH_3], 3.38 [br d, $J \sim 5.9$ Hz, 2H, NCH_2 βBip], 3.1–2.2 [br m, 4H, $ArCH_2$ βBip and $ArC'H_2$ βBip], 2.49 [br m (d-like), 2H, CH_2 βAla], 1.45 [s, 9H, CH_3 Boc], 1.19 [d, $J = 6.7$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (333 K, $CDCl_3$): δ 7.45–7.25 [m, 8H, ArH βBip], 6.12 [br d, $J \sim 7.1$ Hz, 1H, NH βAla], 5.22 [br m, 1H, NH βBip], 4.37 [m, 1H, CH βAla], 3.64 [s, 3H, OCH_3], 3.40 [d, $J = 6.4$ Hz, 2H, NCH_2 βBip], 2.75 [d, $J = 13.4$ Hz, 2H] and 2.50 [br d, $J \sim 12.8$ Hz, 2H] [$ArCH_2$ βBip and $ArC'H_2$ βBip], 2.49

[d, $J = 5.6$ Hz, 2H, CH_2 βAla], 1.46 [s, 9H, CH_3 Boc], 1.20 [d, $J = 6.7$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (233 K, $CDCl_3$): δ 7.5–7.1 [m, 8H, ArH βBip], 6.38 (ca. 44%) and 6.19 (ca. 56%) [br d, $J \sim 8.3$ Hz, 1H, NH βAla], 5.47 (ca. 56%) and 5.41 (ca. 44%) [br m, 1H, NH βBip], 4.37 [m br, 1H, CH βAla], 3.64 (ca. 44%) and 3.58 (ca. 56%) [s, 3H, OCH_3], 3.37 [br d, $J \sim 5.7$ Hz, 2H, NCH_2 βBip], 2.9–2.2 [m, 6H, $ArCH_2$ βBip, $ArC'H_2$ βBip and CH_2 βAla], 1.43 [s, 9H, CH_3 Boc], 1.17 [d br, $J \sim 5.3$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (293 K, CD_3OD): δ 7.5–7.1 [m, 8H, ArH βBip], 4.34 [br q, $J \sim 6.4$ Hz, 1H, CH βAla], 3.63 [br s, 3H, OCH_3], ~3.30 [m (masked), NCH_2 βBip], 3.2–2.0 [br m, 4H, $ArCH_2$ βBip and $ArC'H_2$ βBip], 2.53 [m, 2H, CH_2 βAla], 1.45 [s, 9H, CH_3 Boc], 1.20 [br m, 3H, CH_3 βAla]. ^{1}H NMR (333 K, CD_3OD): δ 7.45–7.25 [m, 8H, ArH βBip], 4.32 [q, $J = 6.6$ Hz, 1H, CH βAla], 3.63 [s, 3H, OCH_3], 3.36 [d, $J = 14.1$ Hz, 1H] and 3.29 [d (partly masked), 1H] [NCH_2 βBip], 2.81 [d, $J = 14.1$ Hz, 2H] and 2.42 [br d, $J \sim 13.6$ Hz, 2H] [$ArCH_2$ βBip and $ArC'H_2$ βBip], 2.57 [dd, $J = 6.9$ and 15.3 Hz, 1H] and 2.49 [dd, $J = 6.1$ and 15.3 Hz, 1H] [CH_2 βAla], 1.44 [s, 9H, CH_3 Boc], 1.19 [d, $J = 6.7$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (233 K, CD_3OD): δ 7.6–7.2 [m, 8H, ArH βBip], 4.33 [br m, 1H, CH βAla], 3.68 (ca. 46%) and 3.58 (ca. 54%) [s, 3H, OCH_3], 3.35 [d (partly masked), 1H] and 3.24 [d, $J = 14.1$ Hz, 1H] [NCH_2 βBip], 3.1–2.0 [m, 6H, $ArCH_2$ βBip, $ArC'H_2$ βBip and CH_2 βAla], 1.46 [s, 9H, CH_3 Boc], 1.25 (ca. 50%) and 1.13 (ca. 50%) [d, $J \sim 6.4$ Hz, 3H, CH_3 Ala]. ^{13}C NMR (293 K, $CDCl_3$): δ 174.4, 171.8 [C=O βAla and βBip], 156.2 [C=O Boc], 140.7, 135.5, 130.1, 128.4, 127.6, 127.5 [C^{Ar}], 79.3 [C–O Boc], 58.2 [C^{α} βBip], 51.9 [OCH_3], 45.5 [NCH_2 βBip], 42.3 [CH βAla], 39.9 [CH_2 βAla], 37.6 (br) [$ArCH_2$ βBip and $ArC'H_2$ βBip], 28.6 [CH_3 Boc], 20.2 (CH_3 βAla). $[\alpha]^{25}_{589} = +9.9$; $[\alpha]^{25}_{578} = +5.8$; $[\alpha]^{25}_{546} = +3.3$; $[\alpha]^{25}_{436} = -16.5$; $[\alpha]^{25}_{365} = -45.5$ (*c* 0.12, CH_2Cl_2). Anal. Calcd for $C_{27}H_{34}N_2O_5 \cdot 0.5H_2O$ (475.566): C, 68.19; H, 7.42; N, 5.89. Found: C, 67.90; H, 7.52; N, 5.83.

4.10. Boc-L-β^3-HAla-β^{2,2}-HBip-OMe b2

The N-protected amino acid Boc-L-β^3-HAla-OH (0.041 g, 0.20 mmol) and the amino ester H-β^{2,2}-HBip-OMe[10] (0.028 g, 0.10 mmol) were reacted with HOBt (0.027 g, 0.20 mmol) and EDC (0.029 g, 0.15 mmol) in THF (1 mL) and CH_2Cl_2 (2 mL), according to the general procedure of peptide coupling. The crude product obtained after extraction was purified by column chromatography on silica gel eluted with eluant (IV) to afford 0.036 g (76%) of pure dipeptide **b2** as a solid. Mp = 74 °C. $R_f = 0.50$ (IV). ^{1}H NMR (293 K, $CDCl_3$): δ 7.5–7.1 [m, 8H, ArH βBip], 6.25 [br m, 1H, NH βBip], 5.26 [br m, 1H, NH βAla], 3.99 [br m, 1H, CH βAla], 3.74 [s, 3H, OCH_3], 3.63 [br m, 1H] and 3.44 [br m, 1H] [NCH_2 βBip], 3.1–2.1 [br m, 4H, $ArCH_2$ βBip and $ArC'H_2$ βBip], 2.42 [br m, 2H, CH_2 βAla], 1.43 [s, 9H, CH_3 Boc], 1.25 [d, $J = 6.6$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (333 K, $CDCl_3$): δ 7.45–7.25 [m, 8H, ArH βBip], 6.08 [br m, 1H, NH βBip], 5.07 [br d, $J \sim 6.7$ Hz, 1H, NH βAla], 3.97 [m, 1H, CH βAla], 3.75 [s, 3H, OCH_3], 3.55 [br m (d-like), 2H, NCH_2 βBip], 2.92 [d, $J = 13.5$ Hz, 1H] and 2.48 [br d, 1H] [$ArCH_2$ βBip], 2.91 [d, $J = 13.6$ Hz, 1H] and 2.48 [br d, 1H] [$ArC'H_2$ βBip], 2.45 [dd, $J = 5.7$ and 14.4 Hz, 1H] and 2.39 [dd, $J = 5.0$ Hz and 14.4 Hz, 1H] [CH_2 βAla], 1.45 [s, 9H, CH_3 Boc], 1.26 [d, $J = 6.8$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (233 K, $CDCl_3$): δ 7.5–7.1 [m, 8H, ArH βBip], 6.63 (ca. 50%) and 6.55 (ca. 50%) [br m, 1H, NH βBip], 5.55 (ca. 50%) and 5.44 (ca. 50%) [d, $J = 8.7$ Hz, 1H, NH βAla], 4.01 [m br, 1H, CH βAla], 3.75 (ca. 48%) and 3.72 (ca. 52%) [s, 3H, OCH_3], 3.65 [m, 1H] and 3.39 [m, 1H] [NCH_2 βBip], 3.1–2.1 [m, 6H, $ArCH_2$ βBip, $ArC'H_2$ βBip and CH_2 βAla], 1.40 (ca. 52%) and 1.39 (ca. 48%) [s, 9H, CH_3 Boc], 1.23 [d br, $J \sim 7.1$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (293 K, CD_3OD): δ 7.5–7.2 [m, 8H, ArH βBip], 3.93 [m, 1H, CH βAla], 3.72 [s, 3H, OCH_3], 3.47 [br m, 2H, NCH_2 βBip], 3.2-2.1 [br m, 4H, $ArCH_2$ βBip and $ArC'H_2$ βBip], 2.41 [dd, $J = 7.1$ Hz and 13.6 Hz, 1H] and 2.27 [dd, $J = 7.1$ and 13.6 Hz, 1H] [CH_2 βAla], 1.41 [s, 9H, CH_3 Boc], 1.17 [d, $J = 6.6$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (333 K, CD_3OD): δ 7.45–7.25 [m, 8H, ArH βBip], 3.93 [q, $J = 6.7$ Hz, 1H, CH βAla], 3.72 [s, 3H, OCH_3], 3.48 [s, 2H, NCH_2 βBip], 2.87 [br d, $J \sim 13.3$ Hz, 2H] and 2.46 [br d (partly masked), 2H] [$ArCH_2$ βBip and $ArC'H_2$ βBip], 2.42 [dd, $J = 6.7$ Hz and 14.1 Hz, 1H] and 2.30 [dd, $J = 6.6$ and 14.1 Hz, 1H] [CH_2 βAla], 1.41 [s, 9H, CH_3 Boc], 1.18 [d, $J = 6.6$ Hz, 3H, CH_3 βAla]. ^{1}H NMR (233 K, CD_3OD): δ 7.5–7.2 [m, 8H, ArH βBip], 3.95 [br m, 1H, CH βAla], 3.72 (ca. 48%) and 3.70 (ca. 52%) [s, 3H, OCH_3], 3.6–3.3 [m, 2H, NCH_2 βBip], 3.1–2.1 [m, 6H, $ArCH_2$ βBip, and $ArC'H_2$ βBip and CH_2 βAla], 1.41 [s, 9H, CH_3 Boc], 1.15 [br m, 3H, CH_3 βAla]. ^{13}C NMR (293 K, $CDCl_3$): δ 175.7, 171.1 [C=O βAla and βBip], 156.7 [C=O Boc], 140.8, 135.6, 130.2, 128.4, 127.8, 127.7 [C^{Ar}], 79.6 [C–O Boc], 60.6 [C^{α} βBip], 52.5 [OCH_3], 44.4 [NCH_2 βBip], 43.2 [CH βAla and [CH_2 βAla], 38.1 (br) [$ArCH_2$ βBip], 36.5 (br) [$ArC'H_2$ βBip], 28.6 [CH_3 Boc], 20.8 (CH_3 βAla). $[\alpha]^{25}_{589} = 0$; $[\alpha]^{25}_{578} = 0$; $[\alpha]^{25}_{546} = 0$; $[\alpha]^{25}_{436} = -23.1$; $[\alpha]^{25}_{365} = -42.3$ (*c* 0.1, CH_2Cl_2). Anal. Calcd for $C_{27}H_{34}N_2O_5 \cdot 0.5H_2O$ (475.566): C, 68.19; H, 7.42; N, 5.89. Found: C, 68.50; H, 7.63; N, 5.58.

4.11. Circular dichroism

The CD spectra were obtained on a Jasco J-710 dichrograph. Cylindrical, fused quartz cells of 10-, 1-, 0.2- and 0.1-mm pathlengths (Hellma) were used. The values are expressed in terms of $[\Theta]_T$, the total molar ellipticity (deg cm^2 dmol^{-1}). Spectrograde MeOH (Acros Organics) was used as the solvent. Peptide concentration was from 1×10^{-3} to 4×10^{-3} M.

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