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Practical resolution of an atropoisomeric α,α-disubstituted glycine with L-phenylalanine cyclohexylamide as chiral auxiliary

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Abstract

L-Phenylalanine cyclohexylamide has been used as a chiral auxiliary for the medium-scale resolution of 2′,1′:1,2;1″,2″:3,4-dinaphthcyclohepta-1,3-diene-6-amino-6-carboxylic acid (Bin), an α,α-disubstituted glycine with only axial dissymmetry. Coupling of X–Bin–OH (X=Ac; Bz) with H–(L)-Phe–NH–C_6H_{11} by the EDC/HOBt method gave the dipeptide diastereoisomers X–(*R*)-Bin–(L)-Phe–NH–C_6H_{11} and X–(*S*)-Bin–(L)-Phe–NH–C_6H_{11}, which were separated by crystallization (X=Bz) and/or chromatography. Extensive acidic hydrolysis, followed by esterification of the resulting free amino acid enantiomers, led to enantiomerically pure (−)-(*R*)-H–Bin–OMe and (+)-(*S*)-H–Bin–OMe with high yields.

1. Introduction

In recent years, the non-proteinogenic open-chain and cyclic α,α-disubstituted-α-amino acids have acquired considerable importance in the design of peptide analogs, because of their tendency to induce and stabilize rather well defined secondary structures such as turns, β-sheet-like arrangements and 3_{10}- or α-helical conformations, when incorporated into peptides.[1] We have recently reported the synthesis of 2′,1′:1,2;1″,2″:3,4-dinaphthcyclohepta-1,3-diene-6-amino-6-carboxylic acid (Bin),[2] a new α,α-disubstituted glycine possessing only an axial chirality due to the hindered rotation along the 1–1′ bond of the 1,1′-binaphthyl moiety (Fig. 1). The Bin residue was later shown to behave as a helix inducer in short-chain peptides.[3]

Although the (*S*)-Bin enantiomer could be obtained in our previous work,[2] we were interested in the investigation of a practical procedure for the resolution of (*RS*)-Bin for several reasons. Indeed, (*R*)-Bin and (*S*)-Bin represent useful precursors or building blocks for the synthesis of new chiral catalysts, applicable to a variety of reactions. Furthermore, access to relatively large quantities of both enantiomerically pure (*R*)-Bin and (*S*)-Bin could allow the use of chirospectroscopic techniques in

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Fig. 1. Structure of the axially dissymmetric (*R*)- and (*S*)-Bin

structural analysis, especially for the study of the as-yet unexplored relationship between *axial chirality* and *helix handedness* in peptides. The impact of the α-carbon chirality of α,α-disubstituted glycines on the 3_{10}-helix screw sense is already known to be side-chain dependent: (*S*)-amino acids with a β-branched side chain such as L-(αMe)Val or L-Iva (isovaline) give right-handed helical structures, the same as shown by protein (*S*)-amino acids, while (*S*)-α,α-disubstituted amino acids with a γ-branched side chain such as L-(αMe)Leu, L-(αMe)Trp, L-(αMe)Phe or L-(αEt)Phe, give rise to opposite left-handed helicities.[4] Finally, the interesting results of Obrecht et al.[5] in which L-phenylalanine cyclohexylamide has been recognized as a simple and powerful chiral auxiliary for the resolution of open-chain α,α-disubstituted glycines H_2N–C(R^1)(R^2)–COOH (R^1=Me; R^2=*i*-Pr, cyclo-Pr, *t*-Bu, Ph, $PhCH_2$, 4-MeO–C_6H_4–CH_2) as well as of cyclic ones, all possessing α-carbon chirality, prompted us to check the ability of this reagent to effect the resolution of an atropoisomeric amino acid.

2. Results and discussion

The racemic Bin amino acid *tert*-butyl ester (*RS*)-**2** was first prepared on a moderate scale in 68% yield, by phase transfer bis-alkylation of the 4-chlorobenzylidene derivative of glycine *tert*-butyl ester, using racemic 2,2′-bis(bromomethyl)-1,1′-binaphthyl (*RS*)-**1**[6] as the alkylating agent (Fig. 2), as previously described,[2] except that the cleavage of the resulting Schiff base was accomplished by acidic hydrolysis on silica gel.

In order to follow as closely as possible Obrecht's resolution conditions,[5] we chose to protect the amino function of Bin either by an acetyl or a benzoyl group. Thus the amino ester (*RS*)-**2** was treated with either acetic anhydride or benzoic anhydride in acetonitrile at room temperature, to give the fully protected derivatives (*RS*)-**3** in 70% yield after crystallization and (*RS*)-**4** in 83% yield after chromatography. Acidic cleavage of the *tert*-butyl ester group of (*RS*)-**3** and (*RS*)-**4** in TFA/CH_2Cl_2 at room temperature gave the N-protected amino acids Ac–Bin–OH (*RS*)-**5** in 99% yield (crude) and Bz–Bin–OH (*RS*)-**6** in 86% yield after crystallization. The aminoamide resolving agent L-phenylalanine cyclohexylamide was readily prepared as previously described,[5] by coupling Boc-(L)-phenylalanine with cyclohexylamine by the mixed anhydride method using ethyl chloroformate and N-methyl morpholine at −15°C, followed by N-deprotection of the Boc protecting group in TFA/CH_2Cl_2 at room temperature.

The coupling of Ac–Bin–OH (*RS*)-**5** and Bz–Bin–OH (*RS*)-**6** with L-phenylalanine cyclohexylamide was first realized on a small scale in order to check the experimental conditions of both reaction and work-up. This study allowed us to draw the following conclusions:

(i) The desired dipeptides **7** and **8** could be obtained quantitatively as 1:1 mixtures of diastereoisomers, by using the EDC/HOBt method in CH_2Cl_2/THF at room temperature.

(ii) In both cases, the two diastereoisomers could be separated by column chromatography on silica gel, furnishing the pure compounds Ac–(*R*)-Bin–(L)-Phe–NH–C_6H_{11} **7a** (87%);[7] $[\alpha]_{546}^{25}$=+92.8 (c 0.13; MeOH) and Ac–(*S*)-Bin–(L)-Phe–NH–C_6H_{11} **7b** (97%);[7] $[\alpha]_{546}^{25}$=−5.3 (c 0.16; MeOH) or

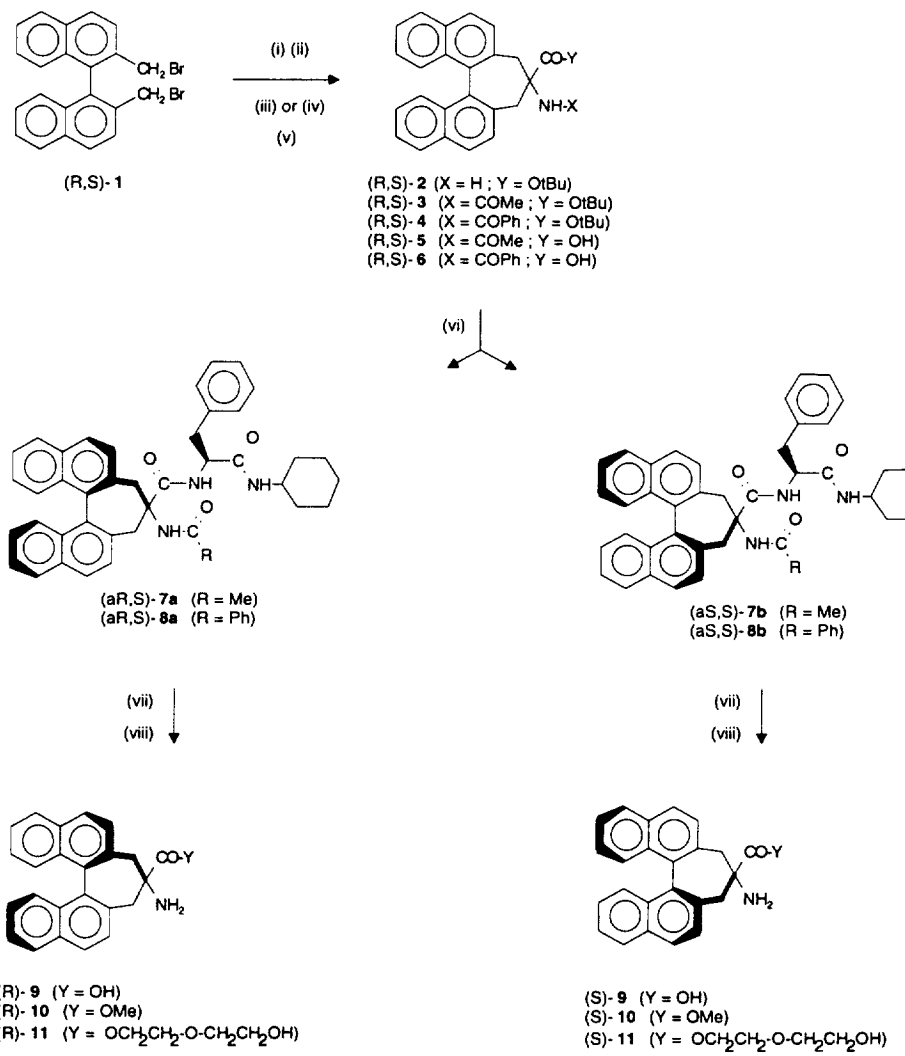

Fig. 2. Synthesis and resolution of (*RS*)-Bin. (i) *p*-Cl–Ph–CH=N–CH_2–COO*t*Bu; KOH powder; K_2CO_3; $nBu_4N^+Br^-$; CH_2Cl_2; r.t. (ii) SiO_2; CH_2Cl_2/MeOH. (iii) $(MeCO)_2O$; CH_3CN/r.t. (iv) $(PhCO)_2O$; CH_3CN/r.t. (v) TFA/CH_2Cl_2; r.t. (vi) H–(L)-Phe–NH–C_6H_{11}; EDC; HOBt; CH_2Cl_2/THF; r.t. (vii) 35% aq. HCl (10 M)/dioxane (1:1 vol:vol); 110°C; 6–8 days. (viii) 1. MeOH/98% H_2SO_4 (cat); reflux; 3 days. 2. Extraction aq. $NaHCO_3$/Et_2O

Bz–(*R*)-Bin–(L)-Phe–NH–C_6H_{11} **8a** (95%);[7] $[\alpha]_{246}{}^{25}$=+68.9 (c 0.1; MeOH) and Bz–(*S*)-Bin–(L)-Phe–NH–C_6H_{11} **8b** (90%);[7] $[\alpha]_{546}{}^{25}$=−22.2 (c 0.13; MeOH), respectively, with excellent yields.

(iii) Attempted crystallizations of the pure diastereoisomers **7a** and **7b** with an N-acetyl group were unsuccessful in our hands, both compounds being amorphous solids. However, in the N-benzoyl series, while the isomer **8b** was also an amorphous solid which could not be crystallized, the isomer **8a** showed a very high crystallinity from ethyl acetate. Pure crystals suitable for X-ray analysis, in which EtOAc was included, were obtained with 78% yield after a single crystallization from a dilute solution.

(iv) The absolute configuration of the binaphthyl unit in the two pairs of diastereoisomers, which was suspected from the specific rotations of the samples as (+)-(*aR,S*) for **7a** and **8a** and (−)-(*aS,S*) for **7b** and **8b**, by comparison with previous series of similar compounds Boc–(*S*)-Bin–(L)-Ala–OMe,[3a]

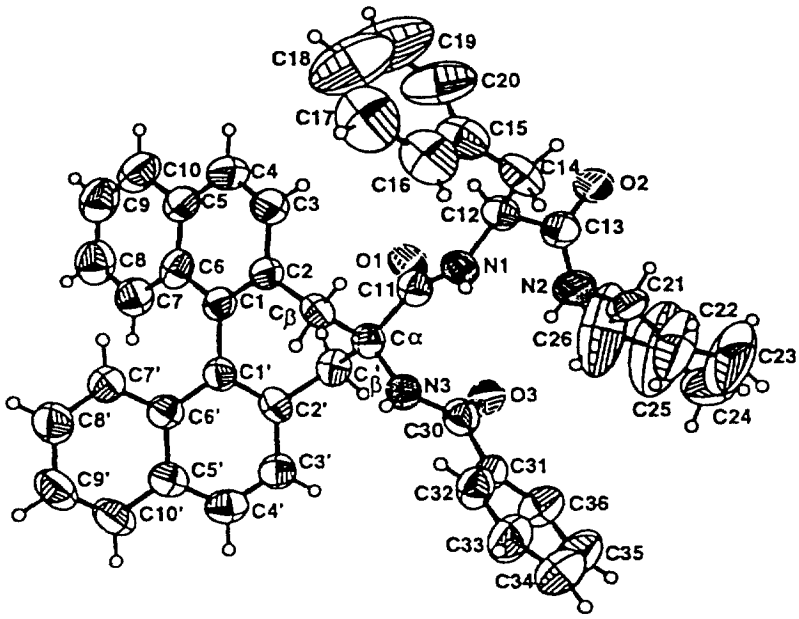

Fig. 3. The X-ray structure of Bz–(*R*)-Bin–(L)-Phe–NH–C_6H_{11} **8a**[8]

was confirmed by the X-ray structural determination of (+)-(*aR,S*) **8a** (Fig. 3),[8] as well as by the further recovery of H–(*R*)-Bin–OH from **8a** and H–(*S*)-Bin–OH from **8b** after acidic hydrolysis (*vide infra*).

In view of these results, the N-benzoyl protection was chosen for the resolution procedure on a moderate scale. A combination of crystallization and chromatography of the crude mixture of isomers obtained after EDC/HOBt coupling of (*RS*)-**6** with H–(L)-Phe–NH–C_6H_{11} led to **8a** (92%);[7] $[\alpha]_{546}^{25}$=+67.6 (c 0.1; MeOH) as white crystals containing ca. 1 equivalent mol/mol of EtOAc by ^{1}H NMR and **8b** (97%);[7] $[\alpha]_{546}^{25}$=−20.7 (c 0.13; MeOH) as an amorphous white powder. A second crystallization of **8a** from EtOAc did not raise its melting point or specific rotation to a significant extent.

Acid hydrolysis of **8a** and **8b** was performed in a 1:1 mixture of aqueous 10 N HCl and dioxane at 110°C for several days, which allowed the cleavage of all the amide functions in a single step. The resulting free amino acids H–Bin–OH, HCl (*R*)-**9**; $[\alpha]_{546}^{25}$=−418.9 (c 0.19; MeOH) and (*S*)-**9**;[2] $[\alpha]_{546}^{25}$=+415.8 (c 0.21; MeOH), respectively, were directly esterified to the corresponding amino esters H–Bin–OMe in refluxing methanol/H_2SO_4 in order to allow an easier purification by chromatography. The pure enantiomers (*R*)-**10** (78.3%); $[\alpha]_{546}^{25}$=−396.6 (c 0.21; MeOH) and (*S*)-**10** (73.3%); $[\alpha]_{546}^{25}$=+393.5 (c 0.21; MeOH), were obtained with excellent yields, although contaminated by a side product H–Bin–O$(CH_2)_2$O$(CH_2)_2$–OH (*R*)-**11** or (*S*)-**11** (ca. 3% content by NMR), presumably resulting from the acidic cleavage of dioxane during the vigorous conditions of hydrolysis of **8a** and **8b**, which was quite difficult to remove by chromatography.[9]

As already pointed out, the absolute configurations of all series of compounds **8–10** were deduced from both the X-ray analysis of **8a** and the sign of the specific rotations of the recovered amino acids (*R*)-**9** and (*S*)-**9**, by reference to earlier work.[2b] The enantiomeric purities of the obtained enantiomers (*R*)-**10** and (*S*)-**10** were shown to be very high: treatment of an aliquot of both samples with (−)-(*S*)-α-methoxy-α-trifluoromethyl-α-phenyl-acetic acid anhydride (resulting from the reaction of the Mosher's acid with 0.5 equiv. of EDC),[2b] led to the diastereoisomeric amido ester (*S*)-Ph$(OCH_3)(CF_3)$C–CO–(*R*)-Bin–OMe with >96% *de* from (*R*)-**10** and (*S*)-Ph$(OCH_3)(CF_3)$C–CO–(*S*)-Bin–OMe with >98% *de* from (*S*)-**10**, by

^{1}H NMR and ^{19}F NMR. This demonstrates the absence of racemization of the binaphthyl unit under the rather drastic experimental conditions of hydrolysis of the dipeptides **8a** and **8b**, in agreement with the known very high configurational stability of 2,2′-bis(methylene)-substituted-1,1′-binaphthyls.[10]

In conclusion, the present study has illustrated the extension of the efficient method of Obrecht et al.[5] for the resolution of α,α-disubstituted glycines, using L-phenylalanine cyclohexylamide as a resolving agent, to an amino acid having an axial chirality instead of an α-carbon chirality. Starting from the readily available racemic 2′,1′:1,2;1″,2″:3,4-dinaphthcyclohepta-1,3-diene-6-amino-6-carboxylic acid *tert*-butyl ester (*RS*)-**2**, over 1 g samples of the corresponding amino acid methyl ester (*R*)-**10** and (*S*)-**10** of high enantiomeric purity, potentially useful for the synthesis of new catalysts and new conformationally constrained peptides with atropoisomerism, could be easily obtained in 50% overall yield.[7]

3. Experimental section

3.1. General

Racemic 2,2′-bis(bromomethyl)-1,1′-binaphthyl (*RS*)-**1** was prepared as previously described.[6] 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 at 300 MHz and 77 MHz, respectively, the solvent $CDCl_3$ or CD_3OD being used as an internal standard (δ=7.27 or 3.31 ppm for ^{1}H and 77.00 or 49.00 ppm for ^{13}C). Splitting patterns are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. The optical rotations were measured with an accuracy of 0.3%, in a 1 dm thermostated cell. Analytical thin layer chromatography (TLC) and preparative column chromatography were performed on Kieselgel F 254 and on Kieselgel 60 (0.040–0.063 mm) (Merck) respectively, with the following eluent systems: (I) CH_2Cl_2; (II) 2.5% MeOH–97.5% CH_2Cl_2; (III) 5% MeOH–95% CH_2Cl_2; (IV) 10% MeOH–90% CH_2Cl_2; (V) 5% Et_2O–95% CH_2Cl_2; (VI) 10% Et_2O–90% CH_2Cl_2; (VII) 20% Et_2O–80% CH_2Cl_2. UV light (254 nm) allowed visualization of the spots after TLC runs for all compounds, even at low concentration.

3.2. 2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-amino-6-carboxylic acid tert-butyl ester (RS)-2[2b]

A mixture of 2,2′-bis(bromomethyl)-1,1′-binaphthyl (*RS*)-**1** (8.80 g; 20 mmol), 4-chloro-benzylidene-glycine-*tert*-butyl ester (5.97 g; 22 mmol), tetrabutylammonium bromide (1.29 g; 4 mmol), anhydrous potassium carbonate (27.6 g; 200 mmol) and finely ground KOH pellets (11.2 g; 200 mmol), in CH_2Cl_2 (300 mL), was magnetically stirred at room temperature for 24 h. The reaction mixture was then evaporated *in vacuo* and the residue solubilized in water (250 mL) and diethyl ether (250 mL). The ethereal phase was washed with water and brine (2×250 mL), dried ($MgSO_4$), filtered and evaporated. To the resulting orange solid foam was added silica gel (100 g) and a solution of dichloromethane/methanol [eluent (II)]. The mixture was stirred at 60°C for 2 hours, then evaporated to dryness, left at room temperature for 4 days and directly chromatographed on a 3×80 cm column of silica gel with eluents (I) then (II) to give 6.59 g (68.3%) of H–Bin–O*t*Bu (*RS*)-**2** as a pale orange solid foam, which could be used in the next steps without further purification. ^{1}H NMR ($CDCl_3$): 7.98–7.22 [m, 12H, ArH], 3.13 and 2.39 [2×d, *J*=13.3, 2H, CH_2 β], 3.07 and 2.65 [2×d, *J*=13.1, 2H, CH_2 β′], 1.67 [s (broad), 2H, NH_2], 1.48 [s, 9H, O*t*Bu]. ^{13}C NMR ($CDCl_3$): 174.6 (C=O), 136.2, 134.5, 134.2, 133.7, 132.9, 132.7, 131.8, 131.7,

128.7, 128.4, 128.2, 128.1, 128.0, 127.4, 127.2, 127.16, 127.13, 125.7, 125.5, 125.1, 124.9 (CAr), 81.4 (O*t*Bu), 69.0 (Cα), 43.5 (Cβ), 43.2 (Cβ′), 27.9 (O*t*Bu). [Note: (*i*) yields of 76–78% in other runs have been previously reported; (*ii*) the chemical shifts in both ^{1}H NMR and ^{13}C NMR ($CDCl_3$) differ from the previously reported ones, probably because of a variation of concentration].[2b]

3.3. 2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-acetylamino-6-carboxylic acid tert-*butyl ester* (RS)-*3*

To a solution of (*RS*)-**2** (0.818 g; 2 mmol) in acetonitrile (30 mL) was added acetic anhydride (0.408 g; 4 mmol). The solution was stirred at room temperature for 24 h and evaporated *in vacuo*. The residue was dissolved in EtOAc (100 mL). The solution was extracted with 2 N NaOH (2×50 mL), washed with water (2×100 mL), dried ($MgSO_4$), filtered and evaporated. The crude product was dissolved in CH_2Cl_2 (70 mL), hexane (30 mL) was added and the clear solution was concentrated to ca. 25 mL. Crystallization occurred at room temperature. The solid was filtered, washed with a solution of CH_2Cl_2 (10 mL)/hexane (60 mL) and air dried, to give 0.628 g (69.6%) of Ac–Bin–O*t*Bu (*RS*)-**3** as a white crystalline powder. Mp=216°C. R_f=0.12 (I). ^{1}H NMR ($CDCl_3$): 7.96–7.22 [m, 12H, ArH], 5.55 [s, 1H, NHAc], 3.31 and 2.39 [2×d, *J*=12.9, 2H, CH_2 β], 3.19 and 3.05 [2×d, *J*=13.8, 2H, CH_2 β′], 1.97 [s, 3H, Ac], 1.46 [s, 9H, O*t*Bu]. ^{13}C NMR ($CDCl_3$): 170.9 (C=O), 169.3 (C=O), 134.9, 134.4, 134.3, 133.8, 133.1, 132.9, 131.8, 131.6, 128.3, 127.9, 127.2, 125.9, 125.7, 125.3, 125.2 (CAr), 81.6 (O*t*Bu), 69.9 (Cα), 42.0 (Cβ), 37.9 (Cβ′), 27.9 (O*t*Bu), 23.1 (Ac). Anal. $C_{30}H_{29}NO_3 \cdot 1.2H_2O$: calcd C 76.15, H 6.69, N 2.96; found C 75.82, H 6.26, N 2.77.

3.4. 2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-benzoylamino-6-carboxylic acid tert-*butyl ester* (RS)-*4*

A solution of (*RS*)-**2** (6.296 g; 15.4 mmol) and benzoic anhydride (6.96 g; 30.8 mmcl) in acetonitrile (125 mL) was stirred at room temperature for 24 h and evaporated *in vacuo*. The residue was dissolved in EtOAc (200 mL). The solution was extracted with 2 N NaOH (2×100 mL), washed with water (2×100 mL), dried ($MgSO_4$), filtered and evaporated. The obtained brown oil was chromatographed on a 3×60 cm column of silica gel with eluents (I) then (II), to give 6.580 g (83.3%) of pure Bz–Bin–O*t*Bu (*RS*)-**4** as a pale orange solid foam. Another run starting from 1.02 g (2.49 mmol) of (*RS*)-**2** gave 1.096 g (85.7 %) of (*RS*)-**4** after chromatography. Mp=158°C. R_f=0.71 (II). ^{1}H NMR ($CDCl_3$): 7.97–7.24 [m, 17H, ArH], 6.19 [s, 1H, NHBz], 3.46 and 2.55 [2×d, *J*=12.9, 2H, CH_2 β], 3.35 and 3.19 [2×d, *J*=13.9, 2H, CH_2 β′], 1.50 [s, 9H, O*t*Bu]. ^{13}C NMR ($CDCl_3$): 170.7 (C=O), 168.8 (C=O), 134.9, 134.5, 134.4, 134.3, 133.8, 133.1, 132.9, 131.8, 131.7, 131.6, 128.6, 128.4, 128.3, 128.2, 128.0, 127.9, 127.2, 127.1, 126.9, 125.9, 125.8, 125.3 (CAr), 81.6 (O*t*Bu), 70.1 (Cα), 42.1 (Cβ), 38.1 (Cβ′), 27.9 (O*t*Bu). Anal. $C_{35}H_{31}NO_3 \cdot 0.5H_2O$: calcd C 79.96, H 6.14, N 2.66; found C 80.29, H 6.09, N 2.61.

3.5. 2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-acetylamino-6-carboxylic acid (RS)-*5*

To a solution of (*RS*)-**3** (0.591 g; 1.31 mmol) in CH_2Cl_2 (5 mL) was added TFA (5 mL). The solution was stirred at room temperature for 3 h and evaporated *in vacuo*. The residue was dissolved in EtOAc (100 mL). The solution was washed with 0.5 N HCl (50 mL) then water (2×100 mL), dried ($MgSO_4$), filtered and evaporated, to give 0.523 g (100%) of crude Ac–Bin–OH (*RS*)-**5** which was used in the next step without further purification. An analytical sample was obtained by crystallisation of an aliquot from CH_2Cl_2/hexane. Mp=210°C. R_f=0.43 (III). ^{1}H NMR (CD_3OD): 7.93–7.14 [m, 12H, ArH], 3.11

and 2.60 [2×d, J=13.1, 2H, CH_2 β], 3.26 and 3.05 [2×d, J=13.7, 2H, CH_2 β′], 1.96 [s, 3H, Ac]. ^{13}C NMR (CD_3OD): 175.6 (C=O), 172.8 (C=O), 136.7, 135.5, 135.3, 134.7, 134.5, 134.3, 132.8, 129.3, 129.24, 129.21, 129.1, 128.7, 127.9, 127.8, 126.73, 126.70, 126.3, 126.1 (CAr), 70.5 (Cα), 42.0 (Cβ), 37.8 (Cβ′), 22.4 (Ac). Anal. $C_{26}H_{21}NO_3 \cdot H_2O$: calcd C 75.53, H 5.61, N 3.39; found C 75.85, H 5.75, N 3.04.

3.6. *2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-benzoylamino-6-carboxylic acid* (RS)-**6**

To a solution of (*RS*)-**4** (6.540 g; 12.75 mmol) in CH_2Cl_2 (25 mL) was added TFA (25 mL). The solution was stirred at room temperature for 2 h and evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 and the solution evaporated *in vacuo* at 50°C. This was repeated several times in order to remove most of the remaining TFA. EtOAc (100 mL) was then added to the residue, which remained insoluble. The mixture was stirred at 50°C for 0.5 h and left in a refrigerator overnight. The solid was filtered, washed with cold EtOAc and air dried to give 3.08 g of a white crystalline powder. The filtrate was evaporated to dryness and diethyl ether (50 mL) was added to the residue. The solution was concentrated and allowed to crystallize, to give 1.665 g of crystals. More crystals (0.231 g) were obtained after concentration of the filtrate and crystallization, to give a total of 4.976 g (85.4%) of pure Bz–Bin–OH (*RS*)-**6**. Mp=263.9°C. R_f=0.33 (IV). ^1H NMR ($CDCl_3$): 7.97–7.26 [m, 17H, ArH], 6.35 [s, 1H, NHBz], 3.63 and 2.55 [2×d, J=13.1, 2H, CH_2 β], 3.42 and 3.19 [2×d, J=14.0, 2H, CH_2 β′]. ^{13}C NMR ($CDCl_3$): 173.7 (C=O), 168.6 (C=O), 134.8, 134.2, 133.9, 133.4, 133.2, 133.1, 132.4, 131.9, 131.6, 128.8, 128.5, 128.4, 128.3, 128.2, 127.6, 127.3, 127.2, 127.1, 126.1, 125.8, 125.6, 125.5 (CAr), 70.3 (Cα), 41.7 (Cβ), 37.9 (Cβ′). Anal. $C_{31}H_{23}NO_3 \cdot 0.5H_2O$: calcd C 79.81, H 5.18, N 3.00; found C 79.83, H 5.17, N 2.95.

3.7. *N*-tert-*Butyloxycarbonyl-*(L)*-phenylalanine cyclohexylamide*

According to the procedure of Obrecht et al.[5], a solution of Boc-(L)-phenylalanine (10.60 g; 40 mmol) in EtOAc (150 mL) was strirred at −15°C (ice–salt bath) for 15 min and N-methylmorpholine (4.04 g; 40 mmol) was added, followed by ethyl chloroformate (4.34 g; 40 mmol). The resulting white suspension was stirred at −15°C for 20 min and cyclohexylamine (3.96 g; 40 mmol) was added. The mixture was stirred from −15°C to +10°C for 2 h and at room temperature for 1 h, then poured onto crushed ice (300 g). After addition of more EtOAc, the organic phase (300 mL) was extracted successively with 0.5 N HCl (3×100 mL), 5% $NaHCO_3$ (2×100 mL) then water (2×100 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo*. The crude product (13.35 g; 96.5%) was recrystallized from *tert*-butyl methyl ether (300 mL). The white crystals were filtered, washed with cold *t*BuOMe and air dried to give 10.30 g (74.4%) of pure Boc–(L)-Phe–NHC_6H_{11}. ^1H NMR ($CDCl_3$): 7.32–7.19 [m, 5H, ArH], 5.57 [d (broad), 1H, NH Phe], 5.19 [d (broad), 1H, NHC_6H_{11}], 4.23 [m, 1H, Hα Phe], 3.68 [m, 1H, CHC_5H_{10}], 3.09 [dd, J=6.3 and 13.6, 1H, $H\beta_A$ Phe] and 2.97 [dd, J=7.9 and 13.6, 1H, $H\beta_B$ Phe], 1.9–0.8 [m, 10H, C_5H_{10}], 1.42 [s, 9H, Boc]. ^{13}C NMR ($CDCl_3$): 169.8 (C=O), 155.2 (C=O Boc), 136.89, 129.3, 128.5, 126.8 (CAr), 79.9 (O*t*Bu Boc), 56.0 (Cα Phe), 38.9 (Cβ Phe), 48.0, 32.7, 32.6, 25.3, 24.6 (C_6H_{11}), 28.2 (*t*Bu Boc). Mp=143.1°C (lit.[5] mp=143.0–143.5°C). $[\alpha]_{589}^{25}$=+9.8; $[\alpha]_{578}^{25}$=+9.4; $[\alpha]_{546}^{25}$=+11.8; $[\alpha]_{436}^{25}$=+9.4; $[\alpha]_{365}^{25}$=+15.3 (c 0.2; $CHCl_3$), lit.[5] $[\alpha]_{589}$=+5.0 (c 0.2; $CHCl_3$).

3.8. L-*Phenylalanine cyclohexylamide*[5]

A solution of Boc-(L)-phenylalanine cyclohexylamide (10.04 g; 29 mmol) in CH_2Cl_2 (60 mL) was stirred at 0°C and TFA (30 mL) was added. The solution was stirred at 0°C for 1 h, then at room temperature for 2 h and evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 (100 mL) and a solution of saturated $NaHCO_3$ (150 mL) was added. After shaking and decantation, the aqueous layer was extracted with CH_2Cl_2 (2×100 mL). The combined organic layer was dried over $MgSO_4$, filtered and evaporated *in vacuo*. The obtained white solid was recrystallized from *tert*-butyl methyl ether, to give 6.47 g (90.6%) of pure H-(L)-Phe–NHC_6H_{11} as white crystals. ^{1}H NMR ($CDCl_3$): 7.35–7.21 [m, 5H, ArH], 7.07 [d (broad), 1H, NHC_6H_{11}], 3.76 [m, 1H, Hα Phe], 3.59 [m (broad), 1H, CHC_5H_{10}], 3.25 [dd, J=4.0 and 13.6, 1H, $H\beta_A$ Phe] and 2.71 [dd, J=9.0 and 13.6, 1H, $H\beta_B$ Phe], 1.9–1.0 [m, 10H, C_5H_{10}]. ^{13}C NMR ($CDCl_3$): 173.0 (C=O), 137.9, 129.2, 128.5, 126.6 (CAr), 56.3 (Cα Phe), 41.0 (Cβ Phe), 47.5, 32.9, 32.8, 25.4, 24.7 (C_6H_{11}). Mp=100.3°C (lit.[5] mp=99–101°C). $[\alpha]_{589}^{25}$=−55.8; $[\alpha]_{578}^{25}$=−65.0; $[\alpha]_{546}^{25}$=−73.8; $[\alpha]_{436}^{25}$=−148.3; $[\alpha]_{365}^{25}$=−261.2 (c 0.2; $CHCl_3$), lit.[5] $[\alpha]_{589}$=−70.0 (c 0.2; $CHCl_3$). Another run in the same conditions gave, after just one extraction, a crude white solid with mp=99.8°C and $[\alpha]_{589}^{25}$=−58.2; $[\alpha]_{578}^{25}$=−64.0; $[\alpha]_{546}^{25}$=−71.3; $[\alpha]_{436}^{25}$=−147.4; $[\alpha]_{365}^{25}$=−268.7 (c 0.2; $CHCl_3$).

3.9. *Coupling of* (RS)-**5** *and* (RS)-**6** *with* L-*phenylalanine cyclohexylamide*

To a solution of (*RS*)-**5** (0.198 g; 0.5 mmol), H-(L)-Phe–NHC_6H_{11} (0.246 g; 1 mmol) and HOBt (N-hydroxybenzotriazole) (0.135 g; 1 mmol) in CH_2Cl_2 (5 mL) and THF (5 mL), was added EDC (N-ethyl-N′-dimethylaminopropyl-carbodiimide hydrochloride) (0.115 g; 0.6 mmol). The solution was stirred at room temperature for 3 days and evaporated *in vacuo*. The residue was dissolved in EtOAc (150 mL) and the solution extracted successively with 0.5 N HCl (2×100 mL), H_2O (100 mL), 5% $NaHCO_3$ (2×100 mL), H_2O (2×100 mL), then dried ($MgSO_4$), filtered and evaporated *in vacuo*. The residue was chromatographed on a 2.5×70 cm column of silica gel with eluents (VI) then (VII), to give 0.136 g (87%)[7] of pure diastereoisomer Ac–(*R*)-Bin–(L)-Phe–NHC_6H_{11} **7a** and 0.152 g (97%)[7] of pure diastereoisomer Ac–(*S*)-Bin–(L)-Phe–NHC_6H_{11} **7b**. Attempts for crystallization of **7a** and **7b** in EtOAc, MeOH or CH_2Cl_2/hexane were unsuccessful. No crystals deposited even from highly concentrated solutions and evaporation to dryness led to an amorphous white solid in both cases.

3.10. *Ac–*(R)*-Bin–*(L)*-Phe–*NHC_6H_{11} **7a**

Amorphous white powder. Mp=197.2°C. R_f=0.19 (VII). ^{1}H NMR ($CDCl_3$): 7.90 [m (t-like), 3H, ArH], 7.77 [d, J=8.3, 1H, ArH], 7.38 [m, 2H, ArH], 7.27 [m, 10H, ArH], 6.75 [d, J=8.3, 1H, ArH], 6.53 [d, J=8.1, 1H, NHC_6H_{11}], 6.47 [d, J=8.3, 1H, NH Phe], 5.65 [s, 1H, NHAc], 4.66 [ddd, J=8.3, 5.3 and 8.6, 1H, Hα Phe], 3.68 [m, 1H, CHC_5H_{10}], 3.36 [dd, J=5.3 and 14.1, 1H, $H\beta_A$ Phe] and 3.02 [dd, J=8.6 and 14.1, 1H, $H\beta_B$ Phe], 2.78 and 2.31 [2×d, J=12.9, 2H, CH_2 β Bin], 3.20 and 3.09 [2×d, J=13.8, 2H, CH_2 β′ Bin], 1.97 [s, 3H, Ac], 1.8–1.0 [m, 10H, C_5H_{10}]. ^{13}C NMR ($CDCl_3$): 171.0, 170.7, 169.5 (C=O Phe, C=O Bin, C=O Ac), 137.6, 134.7, 134.5, 133.9, 133.2, 132.9, 132.8, 131.7, 131.5, 129.3, 128.7, 128.33, 128.26, 128.20, 128.1, 127.7, 127.6, 127.1, 127.0, 126.0, 125.8, 125.4 (CAr), 70.8 (Cα Bin), 54.3 (Cα Phe), 48.5 ($NHCHC_5H_{10}$), 42.9 (Cβ Phe), 37.6 (Cβ Bin), 36.2 (Cβ′ Bin), 32.7, 32.5, 25.4, 25.0, 24.9 (C_5H_{10}), 23.7 (Ac). $[\alpha]_{589}^{25}$=+72.3; $[\alpha]_{578}^{25}$=+76.2; $[\alpha]_{546}^{25}$=+92.8; $[\alpha]_{436}^{25}$=+261.1; $[\alpha]_{365}^{25}$=+1082.4 (c 0.13; MeOH). Anal. $C_{41}H_{41}N_3O_3 \cdot 0.2H_2O$: calcd C 78.49, H 6.65, N 6.70; found C 78.46, H 6.66, N 6.59.

3.11. Ac–(S)-Bin–(L)-Phe–NHC_6H_{11} 7b

Amorphous white powder. Mp=177.8°C. R_f=0.11 (VII). ^1H NMR ($CDCl_3$): 7.92 [m, 4H, ArH], 7.53–7.25 [m, 8H, ArH], 6.90 [m (t-like), 1H, ArH], 6.74 [m (t-like), 2H, ArH], 6.64 [m (d-like), 2H, ArH], 6.73 [d, *J*=8.8, 1H, NHC_6H_{11}], 5.98 [d, *J*=8.6, 1H, NH Phe], 5.83 [s, 1H, NHAc], 4.68 [ddd, *J*=8.6, 5.2 and 6.6, 1H, Hα Phe], 3.70 [m, 1H, CHC_5H_{10}], 3.34 [dd, *J*=5.2 and 13.6, 1H, $H\beta_A$ Phe] and 2.72 [dd, *J*=6.6 and 13.8, 1H, $H\beta_B$ Phe], 3.45 and 2.29 [2×d, *J*=12.7, 2H, CH_2 β Bin], 3.11 and 2.54 [2×d, *J*=12.8, 2H, CH_2 β′ Bin], 1.94 [s, 3H, Ac], 1.8–1.1 [m, 10H, C_5H_{10}]. ^{13}C NMR ($CDCl_3$): 170.6, 169.7, 169.2 (C=O Phe, C=O Bin, C=O Ac), 136.7, 136.1, 134.7, 134.1, 133.7, 133.5, 133.2, 133.0, 131.7, 131.6, 129.3, 128.6, 128.5, 128.4, 128.3, 128.2, 127.8, 127.3, 127.1, 127.0, 126.8, 126.2, 126.0, 125.6 (CAr), 70.0 (Cα Bin), 53.5 (Cα Phe), 48.7 ($NHCHC_5H_{10}$), 41.7 (Cβ Phe), 36.8 (Cβ Bin), 32.8 (Cβ′ Bin), 32.7, 32.4, 25.4, 25.0, 24.5 (C_5H_{10}), 23.5 (Ac). $[\alpha]_{589}^{25}$=0.0; $[\alpha]_{578}^{25}$=−0.6; $[\alpha]_{546}^{25}$=−5.3; $[\alpha]_{436}^{25}$=−66.2; $[\alpha]_{365}^{25}$=−545.2 (c 0.16; MeOH). Anal. $C_{41}H_{41}N_3O_3 \cdot 0.7H_2O$: calcd C 77.38, H 6.72, N 6.60; found C 77.31, H 6.76, N 6.62.

In a similar manner, a solution of (*RS*)-**6** (0.229 g; 0.5 mmol), H–(L)-Phe–NHC_6H_{11} (0.246 g; 1 mmol), HOBt (0.135 g; 1 mmol) and EDC (0.115 g; 0.6 mmol) in CH_2Cl_2 (5 mL)/THF (5 mL) was stirred at room temperature for 3 days and evaporated *in vacuo*. Extraction as above followed by chromatography on a 2×60 cm column of silica gel with eluents (V) then (VI), gave 0.162 g (95%)[7] of pure diastereoisomer Bz–(*R*)-Bin–(L)-Phe–NHC_6H_{11} **8a**, 0.130 g (76%)[7] of pure diastereoisomer Bz–(*S*)-Bin–(L)-Phe–NHC_6H_{11} **8b** with $[\alpha]_{546}^{25}$=−22.2 (c 0.13; MeOH) and 0.050 g (14%)[7] of **8b** contaminated with traces of **8a**. Attempts to purify the contaminated sample of **8b** by crystallization were again unsuccessful since no crystals could be obtained from concentrated solutions in various solvents. Crystallization from CH_2Cl_2/hexane gave only an amorphous solid. On the other hand, ca. 40 mL of boiling EtOAc was necessary to dissolve the pure isomer **8a** (0.162 g) and when the dilute clear colorless solution was left at room temperature overnight, a large amount of high quality shiny white crystals were deposited. The crystals were filtered, washed with cold EtOAc and air dried to give 0.142 g (78% yield for the crystallization process) of **8a** as EtOAc solvate (by ^1H NMR and X-ray), with mp=275.2°C; $[\alpha]_{546}^{25}$=+68.9 (c 0.1; MeOH). More crystals (ca. 0.025 g) were obtained after concentration of the mother liquor.

In a second run on a medium scale, a solution of (*RS*)-**6** (4.972 g; 10.9 mmol), H–(L)-Phe–NHC_6H_{11} (3.743 g; 15.2 mmol), HOBt (2.080 g; 15.4 mmol) and EDC (2.523 g; 13.2 mmol) in CH_2Cl_2 (300 mL)/THF (200 mL) was stirred at room temperature for 30 hours. More CH_2Cl_2 (200 mL) was added and the organic solution was extracted successively with 0.5 N HCl (2×200 mL), H_2O (200 mL), 5% $NaHCO_3$ (2×200 mL), H_2O (2×200 mL), then dried ($MgSO_4$), filtered and evaporated *in vacuo*. The obtained crude product (7.91 g) could not be totally solubilized in 200 mL of boiling EtOAc. After addition of CH_2Cl_2 (200 mL) and more EtOAc (100 mL), the resulting clear solution was concentrated at 90°C with complete elimination of dichloromethane. When crystallization started to occur from the boiling solution (ca. 200 mL), the flask was left at room temperature overnight. The white shiny crystals were filtered, washed with 150 mL of cold EtOAc and air dried to give 3.745 g (89.1%)[7] of pure diastereoisomer Bz–(*R*)-Bin–(L)-Phe–NHC_6H_{11} **8a** as EtOAc solvate by ^1H NMR, with mp=273.9°C; $[\alpha]_{546}^{25}$=+67.4 (c 0.1; MeOH). Recrystallization of these crystals (3.714 g) from EtOAc/(CH_2Cl_2) as above led to 3.643 g of crystals (**8a**:EtOAc ca. 1:1 mol:mol by ^1H NMR), with the same melting point, mp=273.9°C, and similar optical rotation $[\alpha]_{546}^{25}$=+72.1 (c 0.1; MeOH). The mother liquor from the first crop of crystals was evaporated *in vacuo* and the residue, dissolved in CH_2Cl_2 (10 mL), was chromatographed on a 3×56 cm column of silica gel with eluents (VI) then (VII), to give three fractions: (A) 0.323 g, consisting of a ca. 1:1 mixture of **8a** and **8b** (by TLC), (B) 0.844 g, consisting of **8b** with

traces of **8a** and (C) 2.772 g, consisting of pure **8b** with $[\alpha]_{546}^{25}=-20.7$ (c 0.13; MeOH). Fraction (A) was dissolved in CH_2Cl_2 (20 mL) and EtOAc (40 mL) was added. Upon concentration at 90°C to ca. 2 mL, with complete elimination of dichloromethane as above, crystallization started to occur and was completed at room temperature. The white shiny crystals were filtered, washed with abundant cold EtOAc and air dried to give 0.123 g of pure **8a** with $[\alpha]_{546}^{25}=+67.6$ (c 0.1; MeOH), for a total amount of 3.868 g (92.0%).[7] The mother liquor was evaporated *in vacuo* and the residue, combined with fraction (B), was chromatographed on a 3×56 cm column of silica gel with eluents (V) then (VI) then (VII), to give 0.830 g of pure **8b** for a total amount of 3.602 g (96.7%).[7]

3.12. *Bz-(R)-Bin-(L)-Phe-NHC$_6$H$_{11}$* **8a**

White crystals (EtOAc solvate). Mp=273.9°C. R_f=0.21 (VI). ^{1}H NMR ($CDCl_3$): 7.94–7.79 [m, 4H, ArH], 7.66–7.58 [m, 3H, ArH], 7.51–7.20 [m, 14H, ArH], 6.73 [d, *J*=8.3, 1H, ArH], 6.78 [d, *J*=8.4, 1H, NHC_6H_{11}], 6.41 [d, *J*=8.1, 1H, NH Phe], 6.13 [s, 1H, NHBz], 4.72 [ddd, *J*=8.1, 5.1 and 9.2, 1H, Hα Phe], 4.12 [q, *J*=7.1, 2H, EtOAc], 3.72 [m, 1H, C*H*C$_5$H$_{10}$], 3.46 [dd, *J*=5.1 and 14.1, 1H, $H\beta_A$ Phe] and 2.98 [dd, *J*=9.2 and 14.1, 1H, $H\beta_B$ Phe], 2.81 and 2.45 [2×d, *J*=12.8, 2H, CH_2 β Bin], 3.29 and 3.23 [2×d, *J*=14.1, 2H, CH_2 β′ Bin], 2.05 [s, 3H, EtOAc], 1.9–1.1 [m, 10H, C_5H_{10}], 1.27 [t, *J*=7.1, 3H, EtOAc]. ^{13}C NMR ($CDCl_3$): 171.1, 170.5, 169.4, 168.0 (C=O EtOAc, C=O Phe, C=O Bin, C=O Bz), 137.7, 134.7, 134.6, 133.9, 133.2, 133.1, 132.9, 132.7, 132.5, 131.7, 131.6, 129.3, 128.9, 128.7, 128.4, 128.3, 128.2, 128.1, 127.8, 127.2, 127.1, 127.0, 126.0, 125.8, 125.4 (CAr), 71.0 (Cα Bin), 60.4 (EtOAc), 54.6 (Cα Phe), 48.5 (NH*C*HC$_5$H$_{10}$), 43.3 (Cβ Phe), 37.5 (Cβ Bin), 36.4 (Cβ′ Bin), 32.8, 32.5, 25.5, 25.1, 24.9 (C_5H_{10}), 21.0 (EtOAc), 14.2 (EtOAc). $[\alpha]_{589}^{25}=+55.8$; $[\alpha]_{578}^{25}=+57.8$; $[\alpha]_{546}^{25}=+72.1$; $[\alpha]_{436}^{25}=+213.4$; $[\alpha]_{365}^{25}=+909.1$ (c 0.1; MeOH) (Δ necessary for dissolution). Anal. $C_{46}H_{43}N_3O_3\cdot 0.4$EtOAc (after drying at 50°C/0.1 mm): calcd C 79.28, H 6.46, N 5.83; found C 79.54, H 6.46, N 5.97.

3.13. *Bz-(S)-Bin-(L)-Phe-NHC$_6$H$_{11}$* **8b**

Amorphous white powder. Mp=222.6°C. R_f=0.14 (VI). ^{1}H NMR ($CDCl_3$): 7.96 [m, 4H, ArH], 7.64 [m (d-like), 2H, ArH], 7.56–7.26 [m, 11H, ArH], 6.89 [m (t-like), 1H, ArH], 6.70 [m, 4H, ArH], 6.97 [d, *J*=8.1, 1H, NHC_6H_{11}], 6.38 [s, 1H, NHBz], 6.01 [d, *J*=8.6, 1H, NH Phe], 4.76 [ddd, *J*=8.6, 5.1 and 6.3, 1H, Hα Phe], 3.75 [m, 1H, C*H*C$_5$H$_{10}$], 3.37 [dd, *J*=5.1 and 13.6, 1H, $H\beta_A$ Phe] and 2.77 [dd, *J*=6.3 and 13.6, lH, $H\beta_B$ Phe], 3.60 and 2.42 [2×d, *J*=12.7, 2H, CH_2 β Bin], 3.15 and 2.67 [2×d, *J*=12.9, 2H, CH_2 β′ Bin], 1.9–1.1 [m, 10H, C_5H_{10}]. ^{13}C NMR ($CDCl_3$): 171.1, 169.3, 167.6 (C=O Phe, C=O Bin, C=O Bz), 136.1, 134.8, 134.1, 133.8, 133.5, 133.3, 133.2, 133.1, 132.3, 131.8, 131.6, 129.3, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.2, 127.1, 127.0, 126.8, 126.3, 126.1, 125.7, 125.6 (CAr), 70.2 (Cα Bin), 53.4 (Cα Phe), 48.7 (NH*C*HC$_5$H$_{10}$), 41.9 (Cβ Phe), 37.4 (Cβ Bin), 36.7 (Cβ′ Bin), 32.9, 32.5, 25.6, 25.1 (C_5H_{10}). $[\alpha]_{589}^{25}=-14.8$; $[\alpha]_{578}^{25}=-16.3$; $[\alpha]_{546}^{25}=-22.2$; $[\alpha]_{436}^{25}=-108.0$; $[\alpha]_{365}^{25}=-647.0$ (c 0.13; MeOH). Anal. $C_{46}H_{43}N_3O_3\cdot 0.5H_2O$: calcd C 79.51, H 6.38, N 6.05; found C 79.18, H 6.37, N 5.88.

3.14. *Acidic hydrolysis of* **8a** *and* **8b**

To a solution of Bz-(*S*)-Bin-(L)-Phe-NHC_6H_{11} **8b** (3.408 g; 4.97 mmol) in dioxane (40 mL) was added 35% (10 N) HCl (40 mL), resulting in the formation of a white precipitate. The mixture was magnetically stirred at 110°C and the precipitate gradually disappeared to give a clear pale yellow

solution after 20 h. The solution was stirred at 110°C for 6 days, then evaporated to dryness *in vacuo* at 80°C. Water (ca. 40 mL) was added to the residue and the mixture was evaporated again *in vacuo* at 80°C, this operation being repeated several times in order to remove most of the excess of HCl. The residue was then triturated in water (40 mL) to give a solid precipitate and a turbid supernatant which clarified upon standing at room temperature overnight. The solid was filtered on a Büchner funnel, washed with water, air dried for ca. 2 h, then washed with abundant ether and triturated, then air dried, to give 2.250 g of a pale yellow–brown crystalline powder. The crude solid was dissolved in boiling MeOH (200 mL), activated charcoal was added and the mixture was filtered on paper, the filter was washed with abundant hot methanol. The solution was then evaporated to dryness *in vacuo* for elimination of possible residual water, the residue was dissolved in methanol (50 mL) and the solution was concentrated to near dryness (ca. 2 mL) at 60°C. Crystallization rapidly occurred at room temperature. The mixture was left in a refrigerator overnight, then diluted with a cold solution of MeOH:Et_2O 1:1 (10 mL). The crystals were filtered, rapidly washed with a cold solution of MeOH:Et_2O (1:1) (10 mL) and air dried, to give 0.482 g (24.8%) of pure H–Bin–OH, HCl (*S*)-**9** as a white crystalline powder. Another sample of (*S*)-**9** (1.537 g) contaminated by several impurities was also recovered after evaporation of the mother liquor. Both samples of pure (0.471 g) and impure (1.537 g) amino acid (*S*)-**9** were separately esterified in refluxing MeOH (100 mL)/98% H_2SO_4 (2 mL) for 3 days. In both cases, the solution was concentrated *in vacuo* at 30°C to ca. 10 mL and crushed ice (100 mL) then water (50 mL) were added to the stirred mixture, followed by small portions of solid $NaHCO_3$ up to a large excess. The basic aqueous phase was extracted with ether (250 mL), the organic phase was washed with water (3×200 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo*. The obtained crude product of the run involving impure (*S*)-**9** had to be repeatedly chromatographed on 2.3×60 cm columns of silica gel with eluent (II), then on preparative TLC plates, in order to allow the separation of the side product (*S*)-**11** [R_f close to the one of the desired compound (*S*)-**10**]. Chromatography of both crude products gave the following combined samples:

(1) Bz–(*S*)-Bin–OMe resulting from incomplete hydrolysis (0.015 g; 0.6%), R_f=0.70 (II), identified by ^{1}H NMR ($CDCl_3$): 7.93 [m, 4H, ArH], 7.72 [m, 2H, ArH], 7.46 [m, 9H, ArH], 7.27 [m, 2H, ArH], 6.31 [s, 1H, NH], 3.78 [s, 3H, OCH_3], 3.50 and 2.57 [2×d, *J*=13.0, 2H, CH_2 β Bin], 3.33 and 3.21 [2×d, *J*=13.8, 2H, CH_2 β′ Bin] as the first side product;

(2) H–Bin–$OCH_2CH_2OCH_2CH_2$–OH (*S*)-**11** (0.009 g; 0.4%), R_f=0.23 (II), identified by ^{1}H NMR ($CDCl_3$): 7.92 [m, 4H, ArH], 7.56 [m, 2H, ArH], 7.44 [m, 2H, ArH], 7.36 [m, 2H, ArH], 7.24 [m, 2H, ArH], 4.31 [m (t-like), 2H, OCH_2], 3.71 [m, 2H, OCH_2], 3.64 [m, 2H, OCH_2], 3.50 [m, 2H, OCH_2], 3.19 and 2.41 [2×d, *J*=13.2, 2H, CH_2 β Bin], 3.06 and 2.70 [2×d, *J*=13.2, 2H, CH2 β′ Bin], 1.79 [s, 3H, NH_2 and OH] as the second side product;

(3) H–Bin–OMe (*S*)-**10** (0.772 g; 42.3%), R_f=0.28 (11);

(4) H–Bin–OMe (*S*)-**10** (0.567 g; 3 1.0%) contaminated by ca. 5% (by ^{1}H NMR) of (S)-**11**.

In the same manner, to a solution of Bz–(*R*)-Bin–(L)-Phe–NHC_6H_{11} **8a**:EtOAc ca. 1:1 (3.622 g; 4.68 mmol) in dioxane (50 mL) was added 35% (10 N) HCl (50 mL), resulting in the formation of a white precipitate. The mixture was magnetically stirred at 110°C and the precipitate gradually disappeared to give a clear pale yellow solution after 20 h. The solution was stirred at 110°C for 8 days, then evaporated to dryness *in vacuo* at 80°C. The solid residue was treated exactly as above, to give 0.770 g (42.1%) of pure H–Bin–OH, HCl (*R*)-**9** as a white crystalline powder and 1.003 g (54.9%) of (*R*)-**9** contaminated by several impurities. Both samples of pure and impure amino acid (*R*)-**9** were separately esterified in refluxing MeOH (100 mL)/98% H_2SO_4 (2 mL) for 3 days. Work-up as above followed by repeated chromatography with eluent (II), led for the combined two runs, to small amounts of Bz–(*R*)-Bin–OMe and H–Bin–$OCH_2CH_2OCH_2CH_2$–OH (*R*)-**11**, both identified only by TLC, to 0.704 g (41.0%) of pure H–Bin–OMe (*R*)-**10** and to 0.642 g (37.3%) of (*R*)-**10** contaminated by ca. 5% (by ^{1}H NMR) of (*R*)-**11**.

3.15. 2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-amino-6-carboxylic acid (S)-9

Crystalline white powder. Mp=251.8°C (decomp.). $[\alpha]_{589}^{25}$=+349.4; $[\alpha]_{578}^{25}$=+363.8; $[\alpha]_{546}^{25}$=+415.8; $[\alpha]_{436}^{25}$=+687.5; $[\alpha]_{365}^{25}$=+481.2 (c 0.21; MeOH), lit.[2b] for H–(*S*)-Bin–OH, TFA salt: $[\alpha]_{546}^{25}$=+375.0 (c 0.62; MeOH). Anal. ($C_{24}H_{19}NO_2 \cdot 0.3HCl$): calcd C 79.11, H 5.62, N 3.84; found C 78.87, H 5.84, N 3.84.

3.16. 2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-amino-6-carboxylic acid (R)-9

Crystalline white powder. Mp=256.1°C (decomp.). $[\alpha]_{589}^{25}$=−352.1; $[\alpha]_{578}^{25}$=−367.8; $[\alpha]_{546}^{25}$=−418.9; $[\alpha]_{436}^{25}$=−691.6; $[\alpha]_{365}^{25}$=−482.1 (c 0.19; MeOH). Anal. ($C_{24}H_{19}NO_2 \cdot 0.5HCl$): calcd C 77.56, H 5.56, N 3.77; found C 77.58, H 5.71, N 3.79.

3.17. 2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-amino-6-carboxylic acid methyl ester (S)-10

White solid foam. Mp=106.6°C. ^{1}H NMR ($CDCl_3$): 7.93 [m, 4H, ArH], 7.55 [m, 2H, ArH], 7.45 [m, 2H, ArH], 7.37 [m, 2H, ArH], 7.24 [m, 2H, ArH], 3.73 [s, 3H, OCH_3], 3.17 and 2.41 [2×d, *J*=13.2, 2H, CH_2 β Bin], 3.07 and 2.69 [2×d, *J*=13.3, 2H, CH_2 β′ Bin], 1.73 [s, 2H, NH_2]. ^{13}C NMR ($CDCl_3$): 175.9 (C=O); 135.9, 134.3, 134.1, 133.8, 132.9, 132.8, 131.8, 128.4, 128.24, 128.21, 128.13, 128.10, 127.7, 127.2, 125.8, 125.6, 125.2, 125.1 (CAr); 68.8 (Cα); 52.2 (OCH_3); 43.5 (Cβ); 43.0 (Cβ′). $[\alpha]_{589}^{25}$=+331.3; $[\alpha]_{578}^{25}$=+345.1; $[\alpha]_{546}^{25}$=+393.5; $[\alpha]_{436}^{25}$=+630.0; $[\alpha]_{365}^{25}$=+311.2 (c 0.21; MeOH), lit.[2b] for H–(*S*)-Bin–OEt and H–(*S*)-Bin–O*t*Bu: $[\alpha]_{546}^{25}$=+376.8 and +358.4 (c 0.5; MeOH), respectively. Anal. $C_{25}H_{21}NO_2 \cdot 0.3H_2O$: calcd C 80.53, H 5.84, N 3.76; found C 80.57, H 5.87, N 3.65.

3.18. 2′,1′:1,2;1″,2″:3,4-Dinaphthcyclohepta-1,3-diene-6-amino-6-carboxylic acid methyl ester (R)-10

White solid foam. Mp=105.9°C. ^{1}H NMR and ^{13}C NMR ($CDCl_3$): see above. $[\alpha]_{589}^{25}$=−333.2; $[\alpha]_{578}^{25}$=−348.1; $[\alpha]_{546}^{25}$=−396.6; $[\alpha]_{436}^{25}$=−634.1; $[\alpha]_{365}^{25}$=−307.9 (c 0.21; MeOH). Anal. $C_{25}H_{21}NO_2$: calcd C 81.72, H 5.76, N 3.81; found C 81.52, H 5.61, N 3.75.

3.19. Coupling of (R)-10 and (S)-10 with (−)-(S)-α-methoxy-α-trifluoromethyl-α-phenyl-acetic acid

To a solution of (−)-(*S*)-α-methoxy-α-trifluoromethyl-α-phenyl-acetic acid (Aldrich, 99+%) (0.117 g; 0.5 mmol) in acetonitrile (4 mL) was added EDC (0.048 g; 0.25 mmol). After stirring at room temperature for 1 h the clear colorless solution of the resulting MTPA anhydride was divided into two portions of 2 mL each, which were added to samples of (*R*)-**10** (0.0183 g; 0.05 mmol) and (*S*)-**10** (0.0184 g; 0.05 mmol), respectively. The resulting clear colorless solutions were stirred at room temperature for 3 days. After addition of ether (100 mL) the organic phases were successively extracted with 5% $NaHCO_3$ (2×50 mL), then water (2×100 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo*. The residues were chromatographed on preparative TLC plates of silica gel with eluent (I), care being taken not to exercise a mechanical separation of one of the diastereoisomers over the other. From (*R*)-**10** was obtained the amido ester (*S*)-Ph(OCH_3)(CF_3)C–CO–(*R*)-Bin–OMe (0.025 g; 86.2%) with >96% *de*: ^{1}H NMR ($CDCl_3$): 7.95–7.10 [m, 17H, ArH], 6.90 [s, 1H, NH], 3.76 [s (>98%), 3H, OCH_3 Bin], 3.70 [s (<2%), OCH_3 Bin of the (*S,S*) isomer], 3.44 [m, 3H, OCH_3 MTPA], 3.43 (masked) and 2.53 [2×d, *J*=12.9, 2H, CH_2 β Bin], 3.17 and 3.11 [2×d, *J*=14.2, 2H, CH_2 β′ Bin]. ^{19}F NMR ($CDCl_3$): −68.87

[s (>98%), CF_3], −68.95 [s (<2%), CF_3 of the (*S,S*) isomer]. ^{13}C NMR ($CDCl_3$): 171.8, 165.8 (C=O Bin, C=O MTPA), 134.4–125.4 (CAr), 83.5 [q, *J*=26, CF_3], 69.2 (Cα Bin), 55.09 and 55.07 (OCH_3 MTPA), 52.6 (OCH_3 Bin), 41.6 (Cβ Bin), 37.9 (Cβ′ Bin). From (*S*)-**10** was obtained the amido ester (*S*)-Ph(OCH_3)(CF_3)C–CO–(*S*)-Bin–OMe (0.026 g; 89.6%) with >98% *de:* ^1H NMR ($CDCl_3$): 7.96–7.24 [m, 17H, ArH], 7.14 [s, 1H, NH], 3.70 [s (>99%), 3H, OCH_3 Bin], 3.76 [s (<1%), OCH_3 Bin of the (*S,R*) isomer], 3.30 [m, 3H, OCH_3 MTPA], 3.31 (masked) and 2.62 [2×d, *J*=12.9, 2H, CH_2 β Bin], 3.33 and 3.22 [2×d, *J*=14.1, 2H, CH_2 β′ Bin], ^{19}F NMR ($CDCl_3$): −68.96 [s (>99%), CF_3], −68.88 [s (<1%), CF_3 of the (*S,R*) isomer]. ^{13}C NMR ($CDCl_3$): 171.8, 165.8 (C=O Bin, C=O MTPA), 134.5–125.4 (CAr), 83.9 [q, *J*=26, CF_3], 69.4 (Cα Bin), 54.82 and 54.80 (OCH_3 MTPA), 52.5 (OCH_3 Bin), 41.9 (Cβ Bin), 37.6 (Cβ′ Bin).

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