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Stereoselective, nonracemic synthesis of ω-borono-α-amino acids

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Abstract

ω-Unsaturated α-amino acids are synthesized through condensation of allyl and propargyl bromides or of 9-bromoundecene with a Ni(II) complex of the Schiff base derived from glycine and BPB. Hydroboration with Ipc_2BH followed by oxidation with acetaldehyde affords enantiomerically pure ω-borono-α-aminocarboxylic acids. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

The increasing interest in the biological applications of boron containing biomolecules stems from the medicinal applications of bononeutrotherapy (BNCT).[1] Boron analogues of amino acids and peptides constitute a second topic of major importance. α-Aminoboronic acids and related peptides have been extensively investigated.[1,2] Among them are found the most potent inhibitors of serine proteases achieving subnanomolar affinity.[3] They function by forming stable tetrahedral complexes with serine in the active site of the enzymes.

The present work deals with the synthesis of nonracemic ω-borono-α-aminocarboxylic acids. Amino acids with a carboxylic group in the side chain, such as aspartic, glutamic or 2-aminoadipic acids, play a central role in a number of biological processes and it could be of interest to synthesize some boronic analogues. Potential applications are in the field of neuromediators[4] or enzyme inhibition. ω-Boronic analogues of aspartate[5] or of carbamylaspartate[6] have been investigated as antineoplastic agents, inhibitors of dihydroorotase in the pyrimidine biosynthetic pathway. 2-Amino-6-boronohexanoic acid has also been demonstrated to be an efficient inhibitor of arginase ($IC_{50} \approx 0.5$ μM)[7]. In the present work, a long chain ω-borono-α-aminocarboxylic acid was also investigated as an amphiphile.

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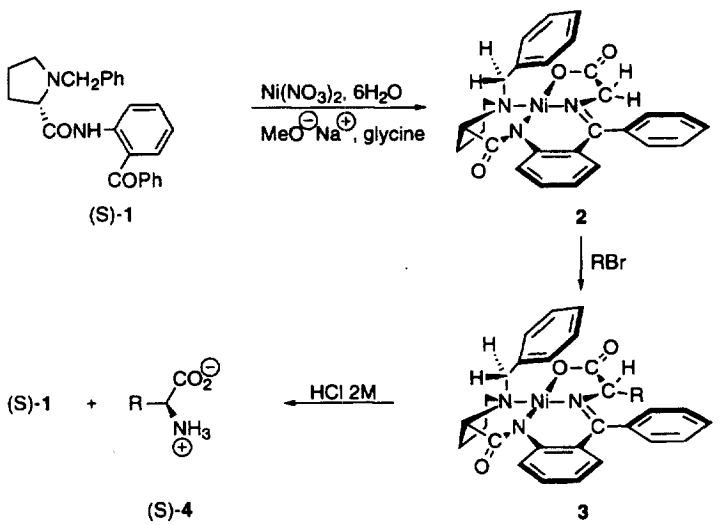
A common retrosynthetic pathway cannot be considered for all the ω-borono-α-amino acids and we report herein the approach based on hydroboration of ω-unsaturated, nonracemic, α-amino acids. Two examples of racemic derivatives were previously reported in a preliminary work.[8]

2. Synthesis of ω-unsaturated, nonracemic, α-amino acids

The methodology developed by Y. N. Belokon[9] was used. The Ni(II) complex **2** of the Schiff base derived from glycine and (S)-2-[N′-(N-benzylprolyl)amino]benzophenone (BPB, **1**) was reacted with alkenyl or alkynyl bromides under thermodynamic equilibrating conditions (Scheme 1).



Scheme 1.

Diastereoisomeric ratios were close to 95/5 (^{1}H NMR determination on benzylic hydrogens) and the major diastereoisomer was purified by column chromatography or recrystallization prior to decomplexation in 2 M HCl. One of the major advantages of this approach is that the chiral auxiliary, BPB **1**, is recovered in ≈90% yield and can be re-used. Results are summarized in Table 1. (S)-Configurations were attributed according to specific rotations reported in the literature for the known amino acids **4a** and **4c**. We have postulated the same configuration for the long chain derivative **4b**. As a general rule, (S)-configurations result in these alkylations, the *Si*-face of the glycine enolate being largely favored.[9]

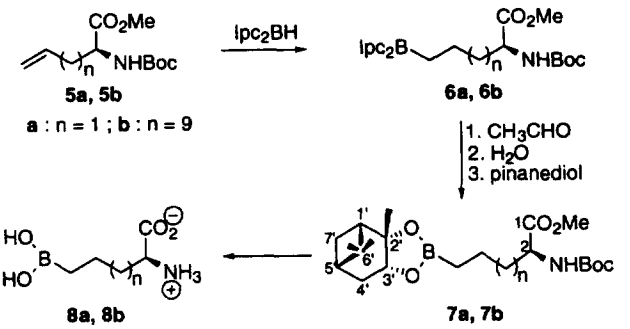
Table 1
Enantioselective synthesis of ω-unsaturated-α-amino acids

Electrophile	3		4	
	de[a]	%yield[b]	$[\alpha]_D^{20}$ [c]	%yield
a : $BrCH_2CH{=}CH_2$	98/2	80	-6.4[d]	90
b : $Br(CH_2)_9CH{=}CH_2$	92/8	76	-	70
c : $BrCH_2C{\equiv}CH$	97/3	76	-32.9[e]	90

[a] ^{1}H NMR determination. [b] Yield of the pure (S,S)-diastereoisomer.
[c] **a**: c = 2.1 g/100mL (HCl 6M); **b**: not measured (insoluble); **c**: c = 1 g/100mL (H_2O).
[d] : lit[10]: -6.4; [e] : lit[11]: -35.

3. Hydroboration of ω-unsaturated α-amino acids 4a, 4b and 4c

Protection of amino and carboxylic acid functions were first performed, under standard procedures, as the NHBoc and methyl carboxylic ester derivatives **5** (Scheme 2). To avoid these protection steps, one attempt to hydroborate directly complex **3a** was done, but without success. However, the following reactions were performed in a one-pot procedure.

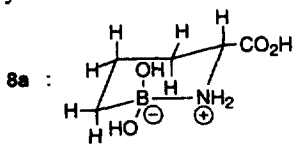


Scheme 2.

readily available diisopinocampheylborane (Ipc_2BH) proved to be the most regioselective hydroborating reagent, affording exclusively boranes **6**. It should be noted that a slight excess (2.0 equivalents for **5a** and **5b** and 1.2 equivalents for **5c**) of Ipc_2BH was necessary to ensure a complete reaction. This fact is probably related to the relative acidity of the NHBoc hydrogen.

Boranes **6** were converted, in situ, into the boronic esters **7** according to the procedure reported by H. C. Brown.[12] Oxidation by an excess of acetaldehyde afforded α-pinene and diethylboronates that were then converted to isolable and thoroughly characterized pinanediol boronates **7**. These boronic esters can be chromatographed without decomposition.

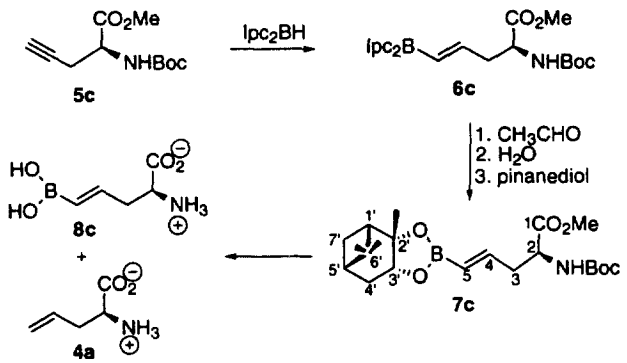
Total deprotection of the 2-aminoadipic analogue **7a** was performed in an almost quantitative manner by refluxing with 6 M HCl, followed by Dowex (H^+) column chromatography, affording 5-borono-2-aminopentanoic acid **8a**. It was isolated as an amorphous, hygroscopic powder and characterized by NMR and mass spectroscopy. It should be noted that ^1H and ^{11}B NMR spectra are strongly dependent on pH. In an acidic medium (**8a**, HCl), one open-chain structure is likely with $\delta^{11}B$ near 33 ppm, indicative of a three coordinated boron atom, and methylenic protons in the 4 or 5 position appearing equivalent. At biological pH (7.4), $\delta^{11}B$ shifts to 14 ppm and all the methylenic protons are nonequivalent, giving rise to a complex pattern. As previously reported,[3b,5] intramolecular complexation must be considered. We were unable to isolate any crystal of this boronic α-amino acid for X-ray structural determination. Tentatively, a cyclic borate complex may be considered.



The long chain boronic α-amino acid **8b** was isolated and characterized as the hydrochloride. It was slightly soluble only in trifluoroacetic acid.

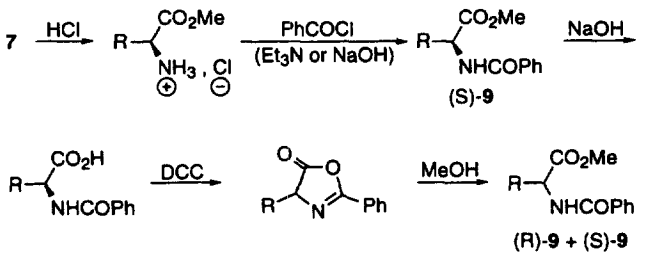
The vinyl boronic derivative **8c** (Scheme 3) was obtained according to the same scheme but, during the deprotection step, a partial protonolysis of the carbon–boron bond occurred (10%, NMR determination). The 5-borono-2-aminopentenoic acid **8c** was isolated after column chromatography on silica gel (ethanol:14 M NH_3, 2:1). NMR data are strongly indicative that intramolecular complexation does not

occur. At pH 7.4, $\delta^{11}B$=27 ppm and, in ^1H NMR, the coupling constants are in good agreement with a trans configuration of the double bond.



Scheme 3.

Enantiomeric purities were measured by HPLC, using a chiral column, on the protected boronates **7**, after derivatization as the benzamides **9** for UV detection (Scheme 4). Samples of racemic derivatives were obtained through a subsequent deprotection of the carboxylic group with sodium hydroxide, oxazolone formation with DCC[13] and opening of the heterocycle with methanol. Enantiomeric excesses were found superior to 98% for the three derivatives.



Scheme 4.

To ensure that no racemisation occurred during the hydrolysis step with 6 M HCl, a sample of the free 5-boronic-2-aminopentanoic acid **8a** was converted to **9a** and submitted to a new chromatographic determination without appreciable change.

4. Conclusion

Hydroboration of nonracemic ω-unsaturated α-amino acids opens a convenient access to enantiomerically pure ω-borono-α-amino acids. The Ni(II) complex of the Schiff base derived from BPB and glycine allows the synthesis of the enantiomerically pure unsaturated α-amino acids on a preparative scale and the protected ω-borono-α-amino acids are then obtained in a simple one-pot procedure.

The synthesis of the boronic analogues of aspartic and glutamic acids, through a different approach, will be described later.

5. Experimental

Melting points were determined with a Köfler apparatus and are uncorrected. NMR spectra were recorded on Brucker AM WB 300 (300 MHz, 75 MHz and 96 MHz for ^{1}H, ^{13}C and ^{11}B, respectively) and ARX 200 (200 MHz and 50 MHz for ^{1}H and ^{13}C, respectively) spectrometers, using $CDCl_3$ as a solvent, unless otherwise stated. Chemical shifts are reported in ppm [relative to internal TMS (^{1}H, ^{13}C) or external BF_3/OEt_2 (^{11}B)] and coupling constants in hertz. When necessary, assignments were determined after selective decoupling and two-dimensional experiments. Multiplicities of the ^{13}C NMR were assigned using DEPT sequence. Rotations were measured on a Perkin–Elmer 341 polarimeter. The enantiomeric excesses were determined by HPLC on a Perkin–Elmer chromatograph 250 equipped with a PIRKLE covalent (S,S) Whelk-01 column (UV detection, λ=225 nm, flow rate=1 ml/mn). Mass spectra were recorded on Varian MAT 311 (electron impact, EI) or Micromass ZABSpec TOF [LSIMS or electrospray (CH_3CN/H_2O), as stated] spectrometers by the 'Centre Régional de Mesures Physiques de l'Ouest' (Rennes). Elemental analyses were performed by the 'Laboratoire Central de microanalyse du CNRS' (Lyon). Column chromatography was carried out using Merck silica gel 60 (40–63 μm).

Ni(II) complex **2** of the Schiff base derived from glycine and (S)-2-[N′-(N-benzylprolyl)amino]benzophenone (BPB) was prepared according to the literature method.[14]

5.1. Alkylation of complex 2 with allylbromide: Ni(II) complex of the Schiff base derived from BPB and allylglycine, 3a

To a stirred mixture of **2** (5 g, 10 mmol) in dry CH_3CN (45 ml) were added, under N_2, finely powdered NaOH (1 g, 25 mmol) and allylbromide (1.3 ml, 1.5 equiv.). After 3 hours, the reaction mixture was treated with 150 ml of 0.1 M HCl. The red product was then extracted with CH_2Cl_2 (4×100 ml), dried over $MgSO_4$ and the solvent removed in vacuo. The two diastereoisomers (98:2) were separated by chromatography on silica gel (CH_2Cl_2:Me_2CO, 2:1) and **3a** was obtained as a red amorphous solid that was recrystallized in AcOEt. Yield: 4.3 g (80%); m.p. 217°C (lit.[15] 210–212°C). ^{1}H NMR δ=2.00–2.20 (m, 2H); 2.50–2.60 (m, 1H); 2.73–2.83 (m, 1H) (β-Hpro and γ-Hpro); 3.45 (dd, J=10.7 and 6.1, 1H, α-Hpro); 3.45–3.59 (m, 2H, δ-Hpro); 3.60 and 4.45 (AB system, J_{AB}=12.7, 2H, CH_2Ph); $=C^2H–C^3H_2–C^4H=C^5H_2$ part: 2.39 (m, $J_{H^{3a}H^{3b}}$=14.0, $J_{H^{3a}H^{2}}$=6.5, $J_{H^{3a}H^{4}}$=7.6, 1H, H^{3a}), 2.45 (m, $J_{H^{3b}H^{2}}$=4.2, $J_{H^{3b}H^{4}}$=7.3, 1H, H^{3b}), 4.03 (dd, 1H, H^{2}), 5.19 (dd, $J_{H^{5a}H^{5b}}$=1.7, $J_{H^{5a}H^{4}}$=17.0, 1H, H^{5a}), 5.40 (dd, $J_{H}5b_{H}4$=10.0, 1H, H^{5b}), 6.44 (ddt, 1H, H^{4}); 6.55–8.20 (m, 14H, Ar–H). ^{13}C NMR δ=22.3 (γ-Cpro); 29.8 (β-Cpro); 37.5 (C^3); 55.9 (δ-Cpro); 62.2 (CH_2Ph); 69.3, 69.4 (α-Cpro and C^2); 118.8 (C^5); 119.7–141.5 (C^4 and 18 Ar–C); 169.8 (C=N); 177.8 (NC=O); 179.4 (C^1). HRMS (LSIMS): m/z=538 $[M+H]^+$, calcd for $C_{30}H_{30}N_3O_3Ni$: 538.1641. Found: 538.164.

5.2. Alkylation of complex 2 with 11-undecenylbromide: Ni(II) complex of the Schiff base derived from BPB and 11-undecenylglycine, 3b

The reaction was carried out as above but in dry DMF (12 ml) with 3 equivalents of NaOH and 1.1 equivalents of the bromide derivative. After 10 minutes, the mixture was neutralized with AcOH (2 ml) and poured into water (200 ml). Compound **3b** was extracted with CH_2Cl_2 and purified by chromatography on silica gel (CH_2Cl_2:Me_2CO, 95:5). Yield 4.9 g (76%); red amorphous solid. ^{1}H NMR δ=1.00–1.45 (m, 12H), 1.50–1.75 (m, 2H), 1.85–2.25 (m, 6H), 2.40–2.60 (m, 1H), 2.65–2.85 (m, 1H) ($9CH_2$, β-Hpro and γ-Hpro); 3.47 (dd, J=10.8 and 5.9, 1H, α-Hpro); 3.46–3.60 (m, 2H, δ-Hpro); 3.58 and 4.44 (AB system, J_{AB}=12.6, 2H, CH_2Ph); 3.92 (dd, J=3.1 and 7.8, 1H, H^{2}); 4.93 (dd, J=10.3 and 1.5,

1H) and 4.99 (dd, J=16.8 and 1.5, 1H) (H_2C=); 5.81 (ddt, J=16.8, 10.3 and 6.6, 1H, HC=); 6.63–8.15 (m, 14H, Ar–H). ^{13}C NMR δ=23.6 (γ-Cpro); 25.4, 28.9, 29.1, 29.3, 29.4, 29.5, 29.7, 30.7, 33.8, 35.3 ($9CH_2$ and β-Cpro); 57.0 (δ-Cpro); 63.1 (CH_2Ph); 70.3, 70.4 (α-Cpro and C^2); 114.1 (=CH_2); 120.7–142.2 (=CH and 18 Ar–C); 170.2 (C=N); 179.5 (NC=O); 180.3 (C^1). HRMS (LSIMS): m/z=650 $[M+H]^+$, calcd for $C_{38}H_{46}N_3O_3Ni$: 650.2892. Found 650.289. Anal. calcd for $C_{38}H_{45}N_3O_3Ni$ (650.51): C, 70.16; H, 6.97; N, 6.46. Found: C, 69.94; H, 6.98; N, 6.33.

5.3. Alkylation of complex 2 with propargylbromide: Ni(II) complex of the Schiff base derived from BPB and propargylglycine, 3c

The reaction was carried out as with allylbromide (propargylbromide, 1.5 equiv.=1.1 ml). The two diastereoisomers (97:3) were separated by chromatography on silica gel (CH_2Cl_2:Me_2CO, 2:1) and **3c** was obtained as a red amorphous solid (lit.[16] 207–209°C). Yield 4.1 g (76%). ^1H NMR δ=2.05–2.15 (m, 2H, γ-Hpro); 2.49–2.62 (m, 1H), 2.80–2.90 (m, 1H) (β-Hpro); 3.50 (dd, J=10.6 and 6.5, 1H, α-Hpro); 3.60–3.72 (m, 2H, δ-Hpro); 3.71 and 4.50 (AB system, J_{AB}=12.7, 2H, CH_2Ph); $=C^2H–C^3H_2–C^4\equiv C^5H$ part: 2.31 (ddd, $J_{H^{3a}H^{3b}}$=17.2, $J_{H^{3a}H^2}$=6.9, $J_{H^{3a}H^5}$=2.8, 1H, H^{3a}), 2.59 (t, 1H, H^5), 2.69 (dt, 1H, $J_{H^{3b}H^2}=J_{H^{3b}H^5}$=2.8, H^{3b}), 4.07 (dd, 1H, H^2); 6.65–8.30 (m, 14H, Ar–H). ^{13}C NMR δ=23.2, 23.3 (γ-Cpro and C^3); 30.7 (β-Cpro); 56.9 (δ-Cpro); 63.3 (CH_2Ph); 67.6 (C^2); 70.4 (α-Cpro); 73.8 (C≡); 79.0 (HC≡); 120.6–142.8 (18 Ar–C); 171.9 (C=N); 178.5 (NC=O); 180.4 (C^1). HRMS (LSIMS): m/z=536 $[M+H]^+$, calcd for $C_{30}H_{28}N_3O_3Ni$: 536.1484. Found: 536.148.

5.4. Hydrolysis of 3a, 3b and 3c and recovery of 1

A solution of **3** (6 mmol) in MeOH (100 ml) was added to a warm 2 M HCl solution (70 ml). The mixture was refluxed for 1 hour. After cooling to room temperature, conc. NH_3 was added until the pH reached between 9 and 10. For **3a** and **3c**, ligand **1** was quantitatively recovered by extraction with CH_2Cl_2. The aqueous layer was concentrated to dryness and the residue was chromatographed with a cation exchange resin (Dowex 50X8 H^+) to obtain **4a** or **4c** in 90% yield. For **4b**, insoluble at pH 9–10, the solution was concentrated to dryness, then ligand **1** dissolved in acetone, and the insoluble **4b** was washed with water, and purified by crystallization from 1 M HCl.

5.4.1. (S)-2-Amino-4-pentenoic acid 4a

White powder; yield: 90%; m.p. 208°C (dec). $[\alpha]_D^{20}$ (c=2.1 g/100 ml, HCl 6 M)=−6.4 (lit.[10] −6.4). ^1H NMR (D_2O; δ_{H_2O}=4.80) δ=2.45–2.71 (m, 2H, $2H^3$); 3.75 (dd, J=6.8 and 5.0, 1H, H^2); 5.21 and 5.23 (2dm, 2H, $2H^5$); 5.74 (ddt, J=17.1, 10.1, 7.3, 1H, H^4). ^{13}C NMR (D_2O) δ=35.4 (C^3); 54.4 (C^2); 120.7 (C^5); 131.8 (C^4); 174.8 (C^1).

5.4.2. (S)-2-Amino-12-tridecenoic acid hydrochloride 4b, nHCl

White powder; yield: 70%; m.p. 260°C (dec). ^1H NMR (D_2O/CF_3CO_2H; δ_{H_2O}=4.80) δ=0.30–0.60 (m, 14H), 0.90–1.20 (m, 4H) ($9CH_2$); 3.12 (t, J=6.3, 1H, H^2); 4.00 (b.dd, J=10.0 and 1.5, 1H) and 4.07 (b.dd J=16.9 and 1.5, 1H) (H_2C=); 4.86 (ddt, J=16.9, 10.0 and 6.6, 1H, HC=). ^{13}C NMR (D_2O/CF_3CO_2H) δ=24.6, 28.5, 28.7, 28.8, 28.9, 29.0, 29.2, 29.9, 33.4 ($9CH_2$); 52.9 (C^2); 113.6 (=CH_2); 138.4 (=CH); 171.7 (C^1). HRMS (LSIMS): m/z=228 $[M+H]^+$, calcd for $C_{13}H_{26}NO_2$: 228.1964. Found: 228.196. Anal. calcd for $C_{13}H_{25}NO_2$, 0.37HCl (240.84): C, 64.83; H, 10.62; N, 5.82. Found: C, 64.75; H, 10.61; N, 5.82.

5.4.3. (S)-2-Amino-4-pentynoic acid ***4c***

White powder; yield: 90%; m.p. 228°C (dec). $[\alpha]_D^{20}$ (c=1 g/100 ml, H_2O)=−32.9 (lit.[11] −35). ^1H NMR (D_2O; δ_{H_2O}=4.80) δ=2.47 (t, J=2.6, 1H, H^5); 2.80 (dd; J=2.5 and 5.5, 2H, $2H^3$); 3.84 (t, J=5.5, 1H, H^2). ^{13}C NMR (D_2O) δ=23.4 (C^3); 55.6 (C^2); 76.0 (C^5); 80.0 (C^4); 175.9 (C^1).

5.5. Protected amino acids ***5***

To a suspension of amino acid **4** (5 mmol) in methanol (10 ml) was added, dropwise at 0°C, freshly distilled thionyl chloride (0.41 ml, 5.5 mmol) via syringe. The resulting solution was kept at reflux for 3 h. After removal of the solvent and excess of thionyl chloride in vacuo, the residue was dissolved in dry acetonitrile (5 ml), then triethylamine (0.77 ml, 5.5 mmol) and di-tert-butyl dicarbonate (Boc_2O) (1.20 g, 5.5 mmol) were added. The mixture was stirred for 1.5 h at room temperature. The solvent was removed in vacuo, 1 M $KHSO_4$ (10 ml) was added, and compound **5** was extracted with dichloromethane (3×10 ml). The organic layer was washed with 1 M $NaHCO_3$ (10 ml) and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (heptane:ethyl acetate, 9:1) to give pure protected amino acids **5**.

5.5.1. (S)-2-tert-Butoxycarbonylamino-4-pentenoic acid methyl ester ***5a***

Oil; yield: 0.91 g (80%). $[\alpha]_D^{18}$ (c=1.5 g/100 ml, $CHCl_3$)=19.27 (lit.[17] $[\alpha]_D$=19.3). ^1H NMR δ=1.44 (s, 9H, $(CH_3)_3$); 2.48 (m, $J_{H^{3a}H^{3b}}$=14.0, $J_{H^{3a}H^2} \approx J_{H^{3a}H^4}$=6.7, 1H, H^{3a}); 2.55 (m, $J_{H^{3b}H^2}$=5.2, $J_{H^{3b}H^4}$=7.6, 1H, H^{3b}); 3.74 (s, 3H, OCH_3); 4.38 (m, $J_{H^2,NH}$=8.2, 1H, H^2); 5.07 (d, 1H, NH); 5.13 (m, $J_{H^{5a}H^{5b}}$=1.8, $J_{H^{5a}H^4}$=16.7, 1H, H^{5a}); 5.14 (m, $J_{H^{5b}H^4}$=10.1, 1H, H^{5b}); 5.70 (ddt, 1H, H^4). ^{13}C NMR δ=28.3 $[(CH_3)_3]$; 36.8 (C^3); 52.2 (OCH_3); 52.9 (C^2); 79.8 $[C(CH_3)_3]$; 119.1 (C^5); 132.4 (C^4); 155.2 (NC=O); 172.6 (C^1). HRMS (EI): m/z=170 $[M-\cdot CO_2CH_3]^+$, calcd for $C_9H_{16}NO_2$: 170.1181. Found: 170.118. Anal. calcd for $C_{11}H_{19}NO_4$ (229.28): C, 57.63; H, 8.35; N, 6.11. Found: C, 57.92; H, 8.39; N, 6.21.

5.5.2. (S)-2-tert-Butoxycarbonylamino-12-tridecenoic acid methyl ester ***5b***

Oil; yield: 1.59 g (93%). $[\alpha]_D^{20}$ (c=3 g/100 ml, $CHCl_3$)=9.63. ^1H NMR δ=1.23–1.40 (m, 14H, 7 CH_2); 1.44 (s, 9H, $(CH_3)_3$); 1.54–1.67 (m, 1H) and 1.70–1.83 (m, 1H) ($2H^3$); 2.04 (qt, ^3J=6.7, $^4J \approx$1.3, 2H, CH_2C=); 3.74 (s, 3H, OCH_3); 4.29 (m, 1H, H^2); 4.93 (ddt, ^2J=2.1, ^3J=10.2, ^4J=1.2, 1H) and 4.99 (ddt, ^3J=17.1, ^4J=1.5, 1H) (H_2C=); 4.99 (d, $J_{H^2,NH}$=8.3, 1H, NH); 5.81 (ddt, 1H, HC=). ^{13}C NMR δ=28.3 $[(CH_3)_3]$; 25.3, 28.9, 29.1, 29.2, 29.4, 29.45, 29.5, 32.8, 33.8 ($9CH_2$); 52.2 (OCH_3); 53.5 (C^2); 79.8 $[C(CH_3)_3]$; 114.2 (=CH_2); 139.2 (=CH); 155.4 (NC=O); 173.6 (C^1). HRMS (EI): m/z=282 $[M-\cdot CO_2CH_3]^+$, calcd for $C_{17}H_{32}NO_2$: 282.2433. Found: 282.244. Anal. calcd for $C_{19}H_{35}NO_4$ (341.49): C, 66.83; H, 10.33; N, 4.10. Found: C, 66.99; H, 10.25; N, 4.23.

5.5.3. (S)-2-tert-Butoxycarbonylamino-4-pentynoic acid methyl ester ***5c***

Oil; yield: 0.93 g (82%). $[\alpha]_D^{18}$ (c=3 g/100 ml, MeOH)=−4.7 (lit.[18] $[\alpha]_D^{20}$=−5.2). ^1H NMR δ=1.46 (s, 9H, $(CH_3)_3$); 2.05 (t, $^4J_{H^5H^3}$=2.5, 1H, H^5); 2.74 (dd, $J_{H^3H^2}$=4.8, 2H, $2H^3$); 3.79 (s, 3H, OCH_3); 4.48 (dt, $J_{H^2,NH}$=8.1, 1H, H^2); 5.39 (d, 1H, NH). ^{13}C NMR δ=22.8 (C^3); 28.3 $[(CH_3)_3]$; 52.0 (C^2); 52.6 (OCH_3); 71.6 (C^5); 78.5 (C^4); 80.2 $[C(CH_3)_3]$; 155.1 (NC=O); 171.1 (C^1). HRMS (EI): m/z=168 $[M-\cdot CO_2CH_3]^+$, calcd for $C_9H_{14}NO_2$: 168.1024. Found: 168.102. Anal. calcd for $C_{11}H_{17}NO_4$ (227.26): C, 58.14; H, 7.54; N, 6.16. Found: C, 57.89; H, 7.65; N, 6.13.

5.6. *Hydroboration of compounds 5: boronic esters 7*

A suspension of diisopinocampheylborane (3 mmol) in freshly distilled THF (1 ml) was prepared according to H. C. Brown[19] and cooled to −20°C under dry nitrogen. A solution of the ω-unsaturated amino ester **5** (1.5 mmol for **5a**, **5b** and 2.5 mmol for **5c**) in THF (2.5 ml) was then transferred via cannula. After warming to room temperature the mixture was stirred for 24 h (**5a**, **5b**) or 5 h (**5c**). Then an excess of acetaldehyde (30 mmol for **5a**, **5b** and 25 mmol for **5c**) was added at 0°C and the resulting mixture was kept at room temperature for 24 h. After hydrolysis with water (2 ml), the solvent and excess acetaldehyde were removed under reduced pressure. Boronic acids were extracted with dichloromethane (3×20 ml). The organic layer was dried over $MgSO_4$, and the solvent removed in vacuo. The residue was dissolved in Et_2O (10 ml), and (+)-pinanediol (1 equiv./**5**) and a small amount of $MgSO_4$ were added. After stirring overnight at room temperature, the solvent was removed under reduced pressure and the resulting residue was chromatographed on silica gel (heptane:ethyl acetate, 8:2) to afford boronic esters **7**.

5.6.1. *(S)-2-(tert-Butoxycarbonylamino)-5-[(1S,2S,3R,5S)-(+)-pinanedioxaboranyl]-pentanoic acid methyl ester 7a*

Oil; yield: 0.40 g (65%). $[\alpha]_D^{22}$ (c=3 g/100 ml, $CHCl_3$)=18.3. ^{1}H NMR δ=0.83 (t, J=7.6, 2H, $2H^5$); 0.84 (s, 3H), 1.29 (s, 3H) and 1.38 (s, 3H) ($3CH_3$); 1.08 (d, J=10.8, 1H, $1H^{7'}$); 1.44 (s, 9H, $(CH_3)_3$); 1.41–1.55 (m, 2H, $2H^4$); 1.59–1.72 (m, 1H) and 1.75–1.87 (m, 1H) ($2H^3$); 1.83 (ddd, 1H) ($1H^{4'}$); 1.88–1.91 (m, 1H, $H^{5'}$); 2.04 (t, J=5.5, 1H, $H^{1'}$); 2.17–2.26 (m, 1H, $1H^{7'}$); 2.33 (ddt, 1H) ($1H^{4'}$); 3.73 (s, 3H, OCH_3); 4.21–4.31 (m, 1H, H^2); 4.25 (dd, J=8.7 and J=1.9, 1H, $H^{3'}$); 5.07 (d, J=8.1, 1H, NH). ^{13}C NMR δ=10.1 (C^5); 19.9 (C^4); 24.0, 27.1 and 28.7 ($3CH_3$); 26.5 ($C^{7'}$); 28.3 [$(CH_3)_3$]; 34.9 (C^3); 35.5 ($C^{4'}$); 38.1 ($C^{6'}$); 39.5 ($C^{5'}$); 51.2 ($C^{1'}$); 52.1 (OCH_3); 53.5 (C^2); 77.6 ($C^{3'}$); 79.7 [$C(CH_3)_3$]; 85.5 ($C^{2'}$); 155.4 (NC=O); 173.5 (C^1). ^{11}B NMR δ=33.1. HRMS (EI): m/z=350 $[M-\cdot CO_2CH_3]^+$, calcd for $C_{19}H_{33}NO_4B$: 350.2502. Found: 350.249. Anal. calcd for $C_{21}H_{36}NO_6B$ (409.34): C, 61.62; H, 8.86; N, 3.42. Found: C, 61.56; H, 8.45; N, 3.32.

5.6.2. *(S)-2-(tert-Butoxycarbonylamino)-13-[(1S,2S,3R,5S)-(+)-pinanedioxaboranyl]-tridecanoic acid methyl ester 7b*

Oil; yield: 0.55 g (69%). $[\alpha]_D^{20}$ (c=3 g/100 ml, $CHCl_3$)=14.6. ^{1}H NMR δ=0.81 (t, J=7.6, 2H, CH_2B); 0.84 (s, 3H), 1.29 (s, 3H) and 1.38 (s, 3H) ($3CH_3$); 1.12 (d, J=10.7, 1H, $1H^{7'}$); 1.21–1.34 (m, 18H, $9CH_2$); 1.44 (s, 9H, $(CH_3)_3$); 1.51–1.69 (m, 1H) and 1.73–1.88 (m, 1H) ($2H^3$); 1.86 (ddd, 1H) ($1H^{4'}$); 1.86–1.95 (m, 1H, $H^{5'}$); 2.05 (t, J=5.5, 1H, $H^{1'}$); 2.15–2.29 (m, 1H, $1H^{7'}$); 2.34 (ddt, 1H) ($1H^{4'}$); 3.73 (s, 3H, OCH_3); 4.20–4.35 (m, 1H, H^2); 4.25 (dd, J=8.6 and J=1.8, 1H, $H^{3'}$); 5.02 (d, J=8.3, 1H, NH). ^{13}C NMR δ=10.9 (BCH_2); 24.0, 27.1 and 28.7 ($3CH_3$); 26.5 ($C^{7'}$); 28.3 [$(CH_3)_3$]; 24.1, 25.3, 29.2, 29.4, 29.45, 29.5, 29.55, 29.6, 32.5, 32.8 ($10CH_2$); 35.6 ($C^{4'}$); 38.1 ($C^{6'}$); 39.6 ($C^{5'}$); 51.3 ($C^{1'}$); 52.1 (OCH_3); 53.5 (C^2); 77.5 ($C^{3'}$); 79.8 [$C(CH_3)_3$]; 85.3 ($C^{2'}$); 155.4 (NC=O); 173.5 (C^1). ^{11}B NMR δ=33.5. HRMS (EI): m/z=462 $[M-\cdot CO_2CH_3]^+$, calcd for $C_{27}H_{49}NO_4B$: 462.3754. Found: 462.377. Anal. calcd for $C_{29}H_{52}NO_6B$ (521.55): C, 66.79; H, 10.05; N, 2.69. Found: C, 66.37; H, 9.92; N, 2.68.

5.6.3. *(S)-2-(tert-Butoxycarbonylamino)-5-[(1S,2S,3R,5S)-(+)-pinanedioxaboranyl]-4-pentenoic acid methyl ester 7c*

Oil; yield: 0.66 g (65%). $[\alpha]_D^{20}$ (c=1.5 g/100 ml, $CHCl_3$)=34.3. ^{1}H NMR δ=0.85 (s, 3H), 1.29 (s, 3H), and 1.40 (s, 3H) ($3CH_3$); 1.12 (d, J=10.9, 1H, $1H^{7'}$); 1.44 (s, 9H, $(CH_3)_3$); 1.86 (ddd, 1H) ($1H^{4'}$); 1.87–1.95 (m, 1H, $H^{5'}$); 2.06 (t, J=5.5, 1H, $H^{1'}$); 2.16–2.26 (m, 1H, $1H^{7'}$); 2.34 (ddt, 1H) ($1H^{4'}$); 2.56

(m, $J_{H^{3a}H^{3b}}$=14.5, $J_{H^{3a}H^{2}}$=6.7, $J_{H^{3a}H^{4}}$=6.9, 1H, H^{3a}); 2.66 (m, $J_{H^{3b}H^{2}}$=4.6, $J_{H^{3b}H^{4}}$=6.9, 1H, H^{3b}); 3.74 (s, 3H, OCH_3); 4.30 (dd, J=8.7 and J=1.9, 1H, $H^{3'}$); 4.43 (m, $J_{H^{2},NH}$=8.2, 1H, H^{2}); 5.03 (d, 1H, NH); 5.56 (dt, $J_{H^{5}H^{4}}$=17.9, $J_{H^{5}H^{3}}$=1.3, 1H, H^{5}); 6.45 (dt, 1H, H^{4}). ^{13}C NMR δ=24.0, 27.1 and 28.6 (3 CH_3); 26.4 ($C^{7'}$); 28.3 [$(CH_3)_3$]; 35.4 ($C^{4'}$); 38.2 ($C^{6'}$); 38.7 (C^{3}); 39.5 ($C^{5'}$); 51.3 ($C^{1'}$); 52.3 (OCH_3); 52.7 (C^{2}); 77.8 ($C^{3'}$); 79.9 [$C(CH_3)_3$]; 85.8 ($C^{2'}$); 122.5 (C^{5}); 146.9 (C^{4}); 155.1 (NC=O); 172.4 (C^{1}). ^{11}B NMR δ=29.4. HRMS (EI): m/z=351 [M−C_4H_8], calcd for $C_{17}H_{26}NO_6B$: 351.1853. Found: 351.186. Anal. calcd for $C_{21}H_{34}NO_6B$, H_2O: C, 59.30; H, 8.53; N, 3.29. Found: C, 59.08; H, 8.08; N, 3.21.

5.7. ω-Borono-α-amino acids 8

A mixture of boronic esters **7a** or **7c** (0.5 mmol) in 6 M HCl (2 ml) was kept at 70°C for 2 h. The resulting brown solution was evaporated to dryness and the residue was dissolved in water. The aqueous phase was washed with ethyl acetate and water removed in vacuo to give **8a** and **8c** as the hydrochlorides. Compound **8a** was isolated after ion exchange chromatography (Dowex 50X8 H^+; 2 M ammonia as eluent). Compound **8c** was purified by chromatography on silica gel (EtOH:14 M NH_3; 2:1).

Hydrolysis of **7b** (0.5 mmol) was performed in 12 M HCl (10 ml) at 70°C over 5 h. After evaporation to dryness, the residue was chromatographed on silica gel (AcOEt/MeOH of increasing polarity) to afford **8b** as the hydrochloride.

5.7.1. (S)-2-Amino-5-boronopentanoic acid, hydrochloride 8a, HCl

^{1}H NMR (D_2O; δ_{H_2O}=4.80) δ=0.78 (t, J=7.8, 2H, $2H^{5}$); 1.35–1.55 (m, 2H, $2H^{4}$); 1.77–1.99 (m, 2H, $2H^{3}$); 4.02 (t, J=6.1, 1H, H^{2}). ^{13}C NMR (D_2O) δ=16.3 (C^{5}); 21.8 (C^{4}); 34.8 (C^{3}); 56.1 (C^{2}); 174.6 (C^{1}). ^{11}B NMR (D_2O) δ=32.6. HRMS (LSIMS): m/z=203 $[M+H+CH_3CN]^+$, calcd for $C_7H_{16}N_2O_4B$: 203.1203. Found: 203.120.

5.7.2. (S)-2-Amino-5-boronopentanoic acid 8a

White powder; yield 73%; m.p. 260°C (dec). ^{1}H NMR (H_2O/ext DSS) (for clarity, the numbering of atoms is the same as in open-chain derivatives) δ=0.39 (m, $J_{H^{5a}H^{5e}}$=14.4, $J_{H^{5a}H^{4a}}$=9.0, $J_{H^{5a}H^{4e}}$=6.1, 1H, H^{5a}); 0.59 (m, $J_{H^{5e}H^{4a}}$=5.2, $J_{H^{5e}H^{4e}}$=5.4, 1H, H^{5e}); 1.39 (m, $J_{H^{4a}H^{4e}}$=11.2, $J_{H^{4a}H^{3a}}$=8.5, $J_{H^{4a}H^{3e}}$=3.7, 1H, H^{4a}); 1.49 (m, $J_{H^{3a}H^{3e}}$=14.3, $J_{H^{3a}H^{4e}}\approx$0, $J_{H^{3a}H^{2a}}$=9.5; 1H, H^{3a}); 1.61 (m, $J_{H^{4e}H^{3e}}$=6.6, 1H, H^{4e}); 1.92 (m, $J_{H^{3e}H^{2a}}$=3.9, 1H, H^{3e}); 3.51 (dd, 1H, H^{2a}). ^{13}C NMR (H_2O/ext DSS) δ=18.6 (C^{5}); 23.9 (C^{4}); 34.5 (C^{3}); 59.1 (C^{2}); 180.0 (C^{1}). ^{11}B NMR (H_2O) δ=14.4.

5.7.3. (S)-2-Amino-13-boronotridecanoic acid hydrochloride 8b, HCl

White powder; yield 71%; m.p. 266°C (dec). ^{1}H NMR (CF_3CO_2H/ext TMS) δ=1.04 (t, J=7.6, 2H, CH_2B); 1.36–1.68 (m, 18H, $9CH_2$); 2.10–2.32 (m, 2H, $2H^{3}$); 4.43 (b.sext., J≈6.0, 1H, H^{2}); 7.39 (s, 3H, NH_3^+). ^{13}C NMR (CF_3CO_2H/ext TMS) δ=15.6 (BCH_2); 25.2, 26.5, 30.5, 30.8, 30.9, 31.0, 31.1, 31.2, 32.1, 34.0 ($10CH_2$); 56.9 (C^{2}); 176.2 (C^{1}). ^{11}B NMR (CF_3CO_2H) δ=36.1. HRMS (LSIMS): m/z=296 $[M+Na]^+$, calcd for $C_{13}H_{28}NO_4BNa$: 296.2009. Found: 296.201; m/z=274 $[M+H]^+$, calcd for $C_{13}H_{29}NO_4B$: 274.2189. Found: 274.219.

5.7.4. (S)-2-Amino-5-borono-4-pentenoic acid 8c

White powder; yield 70%; m.p. 230°C (dec). ^{1}H NMR (H_2O/ext DSS) δ=2.66 (m, $J_{H^{3a}H^{3b}}$=14.8, $J_{H^{3a}H^{2}}$=$J_{H^{3a}H^{4}}$=7.0, $J_{H^{3a}H^{5}}$=1.3, 1H, H^{3a}); 2.72 (m, $J_{H^{3b}H^{2}}$=5.1, $J_{H^{3b}H^{4}}$=7.0, $J_{H^{3b}H^{5}}$=1.3, 1H, H^{3b}); 3.82 (dd, 1H, H^{2}); 5.63 (dt, $J_{H^{4}H^{5}}$=18.0, 1H, H^{5}); 6.34 (dt, 1H, H^{4}). ^{13}C NMR (H_2O/ext DSS) δ=39.4 (C^{3}); 56.7

(C^2); 131.4 (C^5); 146.1 (C^4); 176.8 (C^1). ^{11}B NMR (H_2O) δ=27.4. HRMS (LSIMS): m/z=182 $[M+Na]^+$, calcd for $C_5H_{10}NO_4BNa$: 182.0602. Found: 182.060.

5.8. *Measurement of enantiomeric excess*

5.8.1. *Preparation of enantiomerically pure boronates* **9**

A solution of boronate **7** (0.12 mmol) in dichloromethane (2.5 ml) was saturated with dry HCl. After 1 h at room temperature, the solvent was removed in vacuo and the residue dissolved in acetonitrile (1 ml). Triethylamine (0.13 mmol) and benzoyl chloride (0.13 mmol) were added and the mixture allowed to stand at room temperature for 4 h, then the solvent was removed in vacuo. The residue was dissolved in dichloromethane (2 ml), washed with 0.1 M HCl (2 ml) and saturated $NaHCO_3$ (2 ml) solutions, then dried over $MgSO_4$. Concentration of the mixture and purification by silica gel chromatography (heptane:ethyl acetate, 8:2) gave the N-benzoyl boronate **9** with ≈50% overall yield. ^1H NMR spectra are in good agreement with structures. **9a** was also synthesized from the free boronic α-amino acid **7a** through successive esterification, N-benzoylation and pinanediol protection.

5.8.1.1. *(S)-2-Benzoylamino-5-[(1S,2S,3R,5S)-(+)-pinanedioxaboranyl]-pentanoic acid methyl ester* ***9a***. HPLC (hexane:isopropanol, 90:10) t_R=32.6 min.

5.8.1.2. *(S)-2-Benzoylamino-13-[(1S,2S,3R,5S)-(+)-pinanedioxaboranyl]-tridecanoic acid methyl ester* ***9b***. HPLC (hexane:isopropanol, 95:5) t_R=33.4 min.

5.8.1.3. *(S)-2-Benzoylamino-5-[(1S,2S,3R,5S)-(+)-pinanedioxaboranyl]-4-pentenoic acid methyl ester* ***9c***. HPLC (hexane:isopropanol, 90:10) t_R=29.1 min.

5.8.2. *Racemic boronates* **9**

Pinanediol boronate **7** (0.12 mmol) was reacted with HCl as above. Water (35 μl) and 10 M NaOH (0.36 mmol) were added to the resulting hydrochloride and the reaction mixture was kept at room temperature for 1 h. Benzoyl chloride (0.12 mmol) was added and the mixture allowed to stand at room temperature for an additional 1 h, before treatment with HCl and extraction with dichloromethane. Oxazolone formation was then performed according to Höfle,[13] with DCC (0.12 mmol) in THF (150 μl) for 1 h at room temperature. After filtration of dicyclohexylurea, the solvent was removed in vacuo and the residue was refluxed in methanol for 2 h. Racemic derivatives were isolated as above, after removal of the solvent and silica gel chromatography.

5.8.2.1. *(R,S)-2-Benzoylamino-5-[(1S,2S,3R,5S)-(+)-pinanedioxaboranyl]-pentanoic acid methyl ester* ***9a***. HPLC (hexane:isopropanol, 90:10) t_{R1}=29.5 min; t_{R2}=32.2 min.

5.8.2.2. *(R,S)-2-Benzoylamino-13-[(1S,2S,3R,5S)-(+)-pinanedioxaboranyl]-tridecanoic acid methyl ester* ***9b***. HPLC (hexane:isopropanol, 95:5) t_{R1}=33.6 min; t_{R2}=35.0 min.

5.8.2.3. *(R,S)-2-Benzoylamino-5-[(1S,2S,3R,5S)-(+)-pinanedioxaboranyl]-4-pentenoic acid methyl ester* ***9c***. HPLC (hexane:isopropanol, 90:10) t_{R1}=26.2 min; t_{R2}=29.6 min.

In the three experiments, the peak related to (S)-**9** grew up after addition of pure (S)-**9**.

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