Synthesis and evaluation of ω-borono-α-amino acids† as active-site probes of arginase and nitric oxide synthases

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Enantiomerically pure ω-borono-α-amino acids of various chain lengths have been synthesized according to a general methodology involving condensation of alkenyl and alkynyl bromides with Ni^{II} complex of the Schiff base derived from glycine and (*S*)-2-[*N*′-(*N*-benzylprolyl)amino]benzophenone, hydroboration of the intermediate ω-unsaturated α-amino acids with diisopinocampheylborane, and oxidation with acetaldehyde. Some of these compounds act as potent inhibitors of rat liver and murine macrophage arginases, demonstrating that distance between the $B(OH)_2$ and α-amino acid groups is a key determinant for their interaction with arginase. In contrast, they are without effect on neuronal and inducible NO synthases.

In mammalian cells, L-arginine is metabolized by two major pathways: arginase catalyses its hydrolysis to L-ornithine and urea in the first step of the urea cycle, whereas NO synthases (NOS) catalyse its oxidation to L-citrulline and nitric oxide.[1] The general biological importance of arginase lies in its roles in controlling nitrogen excretion and cellular levels of L-arginine and L-ornithine involved in protein synthesis, as well as production of creatine, proline and polyamines.[2,3] NO synthases are flavohaemoproteins that require NADPH, O_2, tetrahydrobiopterin and calmodulin for the stepwise oxidation of L-arginine to initially produce N^{ω}-hydroxy-L-arginine (NOHA, Chart 1) and, in a second step, to form L-citrulline and NO.[4,5]

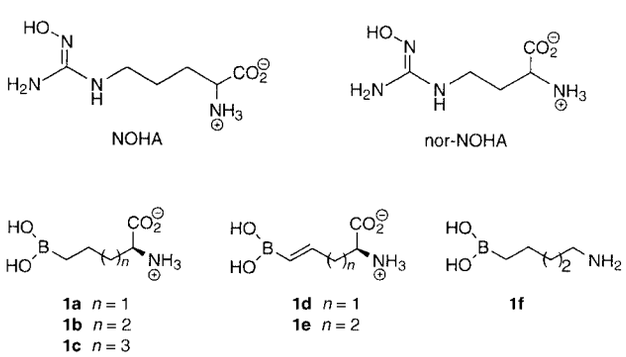


Chart 1 Structure of N^{ω}-hydroxy-L-arginine (NOHA), N^{ω}-hydroxy-nor-L-arginine (nor-NOHA), ω-borono-α-amino acids **1a**–**e**, and decarboxylated analogue **1f**.

NO thus produced is a key biological molecule involved in vasodilation, neurotransmission and cytotoxicity.[6]

Arginine hydrolysis by mammalian arginases is achieved by a metal-activated water molecule that bridges a Mn^{II}_2 cluster at the active site.[2,7] The hydrolysis is postulated to proceed through a tetrahedral intermediate resulting from the nucleophilic attack of metal-bridging hydroxide ion at the guanidinium carbon of L-arginine.[7] Although arginases and NOSs share the same substrate, they differ in their affinity for L-arginine with K_m-values of 1–10 mM and 1–15 μM respectively.[3,4] The two enzymes also have different patterns of inhibition. Most of the enzymology of NOSs and the pharmacological roles of NO have been determined by experiments using N^{ω}-substituted analogues of L-arginine like N^{ω}-methyl-L-arginine and N^{ω}-nitro-L-arginine.[4,8] In contrast, few compounds are known as potent arginase inhibitors.[3]

Since the two enzymes can be found in similar tissues and cells, and because their expression may be regulated in response to the same stimuli (cytokines, endotoxines),[9–12] the regulation of arginases and NOSs by selective and potent inhibitors may have considerable importance in therapy and could allow clear estimation of their roles in tumour growth or rejection.[13] Many studies now support the hypothesis that arginase may be essential in the regulation of NOS activity by modulating local L-arginine concentration.[14–16] It recently appeared that the NOS's intermediate NOHA is a potent inhibitor of rat liver and murine macrophage arginases.[15,17,18] Several studies confirmed the importance of NOHA as an endogenous arginase inhibitor increasing L-arginine availability for NO-biosynthesis.[14,15] Some analogues of NOHA have been synthesized and evaluated as inhibitors of NOSs and arginases. This has led to the discovery of the new α-amino acid N^{ω}-hydroxy-nor-L-arginine (nor-NOHA) as one of the most potent arginase inhibitors (K_i 0.5 ± 0.1 μM).[19,20] A possible explanation for the specific effects of this molecule is that it could act by replacement of the H_2O (or OH) bridging ligand of the Mn^{II}_2-cluster by its *N*-hydroxy function.[2,19,20] Almost simultaneously, the proposal of a tetrahedral transition state in L-arginine hydrolysis resulted in the evaluation of borate anion as arginase inhibitor and in the synthesis of the first boronic analogue of L-arginine, (*S*)-2-amino-6-(dihydroxyboryl)hexanoic acid.[21,22] The high affinity of this compound for arginase (IC_{50} 0.8 μM) corroborates the postulated mechanism for inhibition.

Boronic acid analogues have been previously found to be very potent inhibitors of serine proteases, dipeptidyl peptidases and dihydroorotase.[23–25] The rationale for the effects of such compounds was the well characterized hydration of the electron-deficient boron atom to a stable tetrahedral borate

† In this paper, 'borono' is used as a term for 'dihydroxyboryl'.

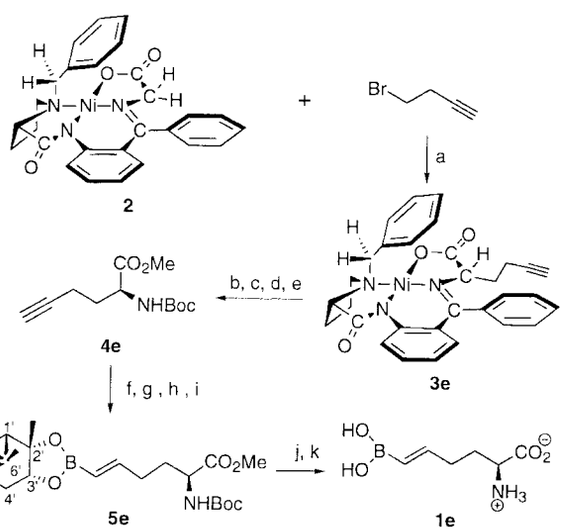
complex which is very close to the intermediate involved in the hydrolysis. Unlike the unstable sp^3 carbon species, the analogous tetrahedral boronic structures are stable and thus inhibit the target enzyme.[26]

We recently described a new stereoselective synthesis of ω-borono-α-amino acids based on hydroboration of ω-unsaturated, non-racemic, α-amino acids obtained through a general methodology.[27] The present work describes the synthesis of ω-borono-α-amino acids of various chain lengths **1a–c** (Chart 1). As amino boronic acids and related oligopeptides are very prone to give internal complexes,[28,29] we also synthesized conformationaly restricted unsaturated analogues **1d,e**. The importance of an intact α-amino acid function for arginase inhibition was evaluated with the decarboxylated analogue **1f** synthesized according to a previously described procedure.[30] This paper reports a study of the effects of compounds **1a–e** and of nor-NOHA as arginase inhibitors of purified rate liver arginase (RLA) and of arginase contained in murine macrophages. In addition, compounds **1a–f** were also studied as possible inhibitors of purified recombinant neuronal and inducible NOSs.

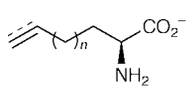
Results and discussion

Synthesis of ω-borono-α-amino acids

The synthesis of the ω-borono-α-amino acids was performed as outlined in Scheme 1 for the unsaturated analogue **1e**. Enantiomerically pure ω-unsaturated α-amino acids were synthesized through alkylation of the Ni^{II} complex **2** of the Schiff base derived from glycine and (*S*)-2-[*N*′-(*N*-benzylprolyl)amino]-benzophenone (BPB) with an ω-bromoalkene or ω-bromoalkyne.[27] This general methodology, developed by Belokon,[31] affords very good diastereoisomeric excess (de) under thermodynamic equilibrating conditions and the major diastereoisomers are readily isolated, after crystallization or chromatography, with yields in the 60–80% range. Decomplexation of the free amino acids **6a–e** occurs without racemization under mild conditions with recovery of the chiral auxiliary.



Scheme 1 *Reagents and conditions*: a, NaOH, DMF, r.t., 10 min; b, 2 M HCl, 60 °C, 1 h; c, Dowex (H^+); d, $SOCl_2$, MeOH, reflux, 3 h; e, Boc_2O, NEt_3, CH_3CN, r.t., 1.5 h; f, Ipc_2BH, THF, −20 °C, 1 h and r.t., 5 h; g, CH_3CHO, 0 °C, 1 h and r.t. 24 h; h, H_2O, r.t., 1 h; i, (+)-pinanediol, Et_2O, r.t., 15 h; j, 6 M HCl, 70 °C, 5 h; k, SiO_2 (EtOH–14 M NH_3).



6a–e $n = 0–2$

Protection of amino and carboxylic acid functions was performed under standard procedures as the NHBoc and methyl carboxylic ester derivatives **4a–e** in a one-pot procedure.[27] Protected amino acids **4a–e** were hydroborated with diisopinocampheylborane (Ipc_2BH) which allows a complete chemo- and regioselectivity. The resulting boranes were oxidized *in situ* by an excess of acetaldehyde[32] and the intermediate diethylboronates were converted to isolable and thoroughly characterized pinanediol boronates **5a–e**.[27] Total deprotection of **5a–e** was performed in an almost quantitative manner by refluxing with 6 M or 12 M HCl followed by chromatography, and afforded free ω-borono-α-amino acids **1a–e** as hygroscopic white powders. Enantiomeric excesses were measured by HPLC after derivatization and synthesis of samples of racemic acids **1a–e**. Compounds **1a** and **1d** have been previously prepared following our general methodology[27] whereas **1f** has already been described.[30] Previously obtained in 1.3% overall yield protected L-glutamic acid,[22] compound **1b** was obtained in 24% overall yield from 4-bromobut-1-ene following our general methodology.

Biological results and discussion

Three ω-borono-α-amino acids **1b**, **1c** and **1e** led to a clear concentration-dependent inhibition of purified RLA activity. Compound **1b** was as potent as nor-NOHA to inhibit RLA activity (IC_{50}-values of 1.6 ± 0.5 and 1.3 ± 0.4 μM, respectively, at physiological pH) (Table 1). This result is in good accord with the previously published IC_{50}-value for **1b** (0.8 μM)[22] and shows that differences in enzyme preparation or conditions of incubation weakly affect its potent inhibitory effect. The shorter analogues of **1b**, **1a** and **1d**, were almost without effect at the highest concentration used (5 mM) whereas the longer analogue, **1c**, displayed an IC_{50}-value 150-fold higher than **1b**. Interestingly, the unsaturated analogue of **1b**, compound **1e**, displayed an IC_{50}-value 10-fold higher than **1b**, and the decarboxylated analogue, **1f**, was almost without effect (IC_{50}-value close to 3 mM). These results indicate that changing chain length by the addition or loss of only one methylene group strongly affects the affinity for the active site of RLA. Similar effects have been observed when we compared α-amino acids bearing either an *N*-hydroxyguanidino group, such as nor-NOHA, NOHA and homo-NOHA,[20] or an *N*-hydroxylamino function, N^{δ}-hydroxy-L-ornithine and N^{ϵ}-hydroxy-L-lysine.[19] Furthermore, introduction of a double bond in compound **1b** restricts chain flexibility and modifies the distance between the α-amino acid and the $B(OH)_2$ groups. Removal of the carboxylic acid group (comparing **1b** and **1f** in Table 1), or modifications of the α-amino acid function,[18,19] give almost inactive products. These results demonstrate that the presence of a free α-amino acid is crucial for RLA recognition and reinforce the proposal that there must be an optimal distance between the α-amino acid function and the OH group of the studied compounds to achieve good inhibition.[19,20]

RLA shows highest activity at pH 9.0,[2,3] and the inhibitory effects of **1a–f** were then studied at this pH. By comparison to experiments performed at pH 7.4, IC_{50}-values for **1a–f** significantly increased (5- to 10-fold) at pH 9.0 (Table 1). Similar results have been observed with compounds bearing an N–OH function.[19] It was postulated that RLA strongly recognizes positively charged compounds (L-arginine, NOHA or nor-NOHA) but weakly interacts with non-protonated hydroxylamines or amidoximes.[19] In the case of boronic analogues **1a–e**, the strongest interaction is observed at pH 7.4, a pH-value where the tricoordinated boron atom predominates. At pH 9.0, the boron atom should be mainly tetracoordinated and could less strongly interact with the OH group bridging the two Mn^{II} atoms at the RLA active site.

The ability of boronic compounds **1a–f** and nor-NOHA to

Table 1 Inhibition of rat liver and murine macrophage arginases by ω-borono-α-amino acids **1a**–**f**

	IC_{50} (μM)		
	RLA[a]		
Compound	pH 7.4	pH 9.0	mMA[b]
1a	5000	>5000 (10%)[c]	1000
1b	1.6 ± 0.8	18 ± 8	5 ± 1
1c	300 ± 40	350 ± 50	5000
1d	>5000 (30%)[c]	>5000 (10%)[c]	>10000 (20%)[d]
1e	15 ± 5	65 ± 15	100 ± 20
1f	3000	>5000 (40%)[c]	5000

[a] Assays quantitated the [^{14}C]urea produced from L-[G-^{14}C]Arg according to a previously described method.[19] A typical assay was performed in 0.1 cm^3 of 0.2 M Tris-HCl (pH 7.4) containing 10 mM L-Arg, 0.05 μCi of L-[G-^{14}C]Arg and variable concentrations of inhibitors. The reactions were initiated by the addition of purified RLA.[19] Protein amounts were adjusted to yield less than 15% substrate conversion. After 10 min at 37 °C, 0.15 cm^3 of cold stop buffer (0.25 M acetic acid and 7 M urea) was added and [^{14}C]urea was separated from unchanged L-[G-^{14}C]Arg by mixing with 0.25 cm^3 of a 1:1 slurry of Dowex AG-50W-X8 (H^+-form) in stop buffer, and centrifugation. Aliquots of the supernatant were counted after addition of Pico-Fluor 40. IC_{50} mean values ± SD from 3 to 5 independent experiments. [b] Murine macrophages (mMA) were obtained from C3H/HeN mice injected intraperitoneally with thioglycolate broth (Institut Pasteur, France) 3 days before the cells were harvested.[18] Macrophages were washed and resuspended (2×10^6 cm^{-3}) in RPMI medium containing 600 μM L-Arg, 0.05 μCi L-[G-^{14}C]Arg and appropriate concentration of inhibitors. Cells were incubated 2 h at 37 °C under a 5% CO_2 atmosphere. Arginase activity was measured as above after addition of 0.15 cm^3 of cold stop buffer, 0.25 cm^3 of a slurry of H^+ Dowex, centrifugation, and counting of [^{14}C]urea contained in aliquots of the supernatant. IC_{50} mean values ± SD from 3 independent experiments. [c] Highest concentration 5 mM. [d] Highest concentration 10 mM.

inhibit arginase activity contained in murine macrophages was also investigated (Table 1). Compounds **1b** and **1e** demonstrated the highest potency to inhibit [^{14}C]urea formation with IC_{50} of 5 ± 1 and 100 ± 20 μM, respectively, whereas nor-NOHA displayed an IC_{50}-value of 3.0 ± 1.0 μM. Compounds **1a**, **1c**, **1d** and **1f** were far less active, with IC_{50}-values in the mM range. Although the experimental conditions were different [substrate concentration 10 mM in RLA assays but only 0.6 mM in RPMI (cell culture medium) containing macrophages], almost identical IC_{50}-values were measured for the two arginases. These results suggest that compounds **1b** and **1e** which display similar potency against the macrophage and the rat liver enzymes penetrate into the intracellular medium.

When tested on recombinant purified neuronal and inducible NOSs, compounds **1a**–**f** exhibited very weak effects and failed to inhibit (less than 10% inhibition at 5 mM) the oxidation of L-[^{14}C]arginine to L-[^{14}C]citrulline and NO when followed by two classical methods used to measure NOS activity.[33,34] In addition, compounds **1a**–**f** did not significantly modify the haem absorption spectra of the oxygenase domain of inducible NOS.[4,5]

Further studies are necessary to understand the mechanisms of inhibition of RLA by compounds **1b** and **1e**. However, our results show a very different behaviour for **1b** and **1e** towards RLA and NOSs. Compound **1b** is a strong inhibitor of RLA and of murine macrophage arginase, as potent as nor-NOHA, but without effects towards NOSs. It does not bear an *N*-hydroxyguanidino function and should be less sensitive to oxidation mediated by superoxide ions or haem proteins than are nor-NOHA and NOHA. Compound **1b** is a useful tool for future comparisons of the active sites of these two classes of enzymes that use L-arginine as substrate as well as for pharmacological studies that require selective and potent inhibition of arginase.

Experimental

Mps were determined with a Köfler apparatus and are uncorrected. NMR spectra were recorded on a Bruker 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 (δ) are reported in ppm [relative to internal TMS (^{1}H, ^{13}C) or external $BF_3 \cdot OEt_2$ (^{11}B)] and coupling constants (J) in Hz. If necessary, assignments were determined after selective decoupling and two-dimensional experiments. J Values are only given for the first described protons in coupled systems. Multiplicities of the ^{13}C NMR spectra were assigned using DEPT sequence. Optical rotations were measured on the sodium D-line (589.3 nm) using a Perkin-Elmer 341 polarimeter and were recorded in units of 10^{-1} deg cm^2 g^{-1}. The enantiomeric excesses (ees) were determined by HPLC on a Perkin-Elmer chromatograph 250. 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, France). Elemental analyses were performed by the 'Service de microanalyse du CNRS' (Gif-sur-Yvette, France). 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 BPB was prepared according to the literature method.[35]

Alkylation of complex 2 with 4-bromobut-1-ene: Ni^{II} complex of the Schiff base derived from BPB and but-3-enylglycine, 3b

Finely powdered NaOH (1 g, 25 mmol) and 4-bromobut-1-ene (1.5 cm^3, 1.5 equiv.) were added, under N_2, to a stirred mixture of **2** (5 g, 10 mmol) in dry CH_3CN (45 cm^3). After 3 h, the reaction mixture was treated by 150 cm^3 of 0.1 M HCl. The red product was then extracted with CH_2Cl_2 (4×100 cm^3), dried ($MgSO_4$), and the solvent was removed *in vacuo*. The two diastereoisomers (98:2) were separated by chromatography on silica gel (CH_2Cl_2–Me_2CO, 3:1) and **3b** was obtained as a *red solid* (3.4 g, 62%), mp 210 °C (from AcOEt) (Found: C, 67.2; H, 5.8; N, 7.6. $C_{31}H_{31}N_3NiO_3$ requires C, 67.4; H, 5.65; N, 7.6%); δ_H 2.00–2.10 (1 H, m, 1δ-Hpro), 2.10–2.23 (1 H, m, 1γ-Hpro), 2.46–2.60 (1 H, m, 1β-Hpro), 2.66–2.80 (1 H, m, 1β-Hpro), 3.48 (1 H, dd, J 11.2 and 5.8, α-Hpro), 3.43–3.62 (2 H, m, 1γ-, 1δ-Hpro), 3.56 and 4.43 (2 H, AB system, J 12.6, CH_2Ph), ethylenic chain 1.64–1.75 (1 H, m, 3-H), 2.10–2.23 (1 H, m, 3-H′), 2.23–2.34 (1 H, m, 4-H), 2.66–2.80 (1 H, m, 4-H′), 3.91 (1 H, dd, J 8.5 and 3.5, 2-H), 4.87 (1 H, dm, $J_{6,5}$ 10.2, $J_{6,6'}$ 1.7, $J_{6,4}$ 1.3, 6-H), 4.96 (1 H, dq, $J_{6',5}$ 17.1, $J_{6',4}$ 1.5, 6-H′), 5.53 (1 H, ddt, $J_{5,4}$ 6.5, 5-H), 6.63–8.12 (14 H, m, ArH); δ_C 23.8 (γ-Cpro), 29.5 (C-4), 30.8 (β-Cpro), 35.1 (C-3), 57.1 (δ-Cpro), 63.1 (*C*H_2Ph), 69.8 (C-2), 70.3 (αCpro), 115.8 (C-6), 120.8–142.2 (18 × Ar-C), 136.6 (C-5), 170.4 (C=N), 179.2 (NC=O), 180.4 (C-1); *m*/*z* (LSIMS) 552.179 [(M^+ + H). $C_{31}H_{32}N_3NiO_3$ requires *m*/*z*, 552.1797].

Alkylation of complex 2 with 5-bromopent-1-ene: Ni^{II} complex of the Schiff base derived from BPB and pent-4-enylglycine, 3c

The reaction was carried out as above with 2.9 g (5.8 mmol) of **2**. The two diastereoisomers (96:4) were separated by chromatography on silica gel (CH_2Cl_2–Me_2CO, 2:1) and **3c** was obtained as a *red solid* (1.7 g, 52%), mp 192 °C (from AcOEt) (Found: C, 67.9; H, 5.9; N, 7.35. $C_{32}H_{33}N_3NiO_3$ requires C, 67.85; H, 5.85; N, 7.4%); δ_H 1.93–2.07 (1 H, m, 1δ-Hpro), 2.08–2.29 (1 H, m, 1γ-Hpro), 2.45–2.59 (1 H, m, 1β-Hpro), 2.72–2.81 (1 H, m, 1β-Hpro), 3.47 (1 H, dd, J 11.0 and 5.8, α-Hpro), 3.44–3.62 (2 H, m, 1γ-, 1δ-Hpro), 3.59 and 4.45 (2 H, AB system,

J 12.7, C*H*$_2$Ph), ethylenic chain 1.59–1.74 (2 H, m, 3-, 4-H), 1.86–2.07 (3 H, m, 3-H′, 5-H$_2$), 2.08–2.29 (1 H, m, 4-H′), 3.91 (1 H, dd, *J* 8.1 and 3.5, 2-H), 4.96 (1 H, dm, $J_{7,6}$ 10.3, 7-H), 4.98 (1 H, dm, $J_{7',6}$ 17.0, 7-H′), 5.73 (1 H, ddt, $J_{6,5}$ 6.6, 6-H), 6.63–8.15 (14 H, m, ArH); δ_C 23.6 (γ-Cpro), 24.6 (C-4), 30.7 (β-Cpro), 33.3 (C-5), 34.8 (C-3), 57.0 (δ-Cpro), 63.1 (*C*H$_2$Ph), 70.3 (α-Cpro), 70.4 (C-2), 115.2 (C-7), 120.7–142.2 (18 × Ar-C), 137.7 (C-6), 170.3 (C=N), 179.3 (NC=O), 180.4 (C-1); *m*/*z* (LSIMS) 566.195 [(M$^+$ + H). $C_{32}H_{34}N_3NiO_3$ requires *m*/*z*, 566.1954].

Alkylation of complex 2 with 4-bromobut-1-yne: Ni^{II} complex of the Schiff base derived from BPB and but-3-ynylglycine, 3e

4-Bromobut-1-yne was prepared from but-3-ynyl toluene-*p*-sulfonate[36] according to the literature method.[37]

The reaction with **2** (1 mmol) was carried out as above but in dry DMF (1.2 cm^3) with 3 equiv. of NaOH and 1.1 equiv. (0.15 g) of 4-bromobut-1-yne. After 10 min, the mixture was neutralized with AcOH (0.25 cm^3) and poured into water (25 cm^3). Crude **3e** was extracted with CH_2Cl_2 and the two diastereoisomers (95 : 5) were separated by chromatography on silica gel (CH_2Cl_2–Me_2CO, 4 : 1) to give pure **3e** as *red crystals* (330 mg, 60%), mp 218 °C (from AcOEt) (Found: C, 66.4; H, 5.4; N, 7.5. $C_{31}H_{29}N_3NiO_3$·0.5 H_2O requires C, 66.55; H, 5.4; N, 7.5%); δ_H 2.03–2.12 (1 H, m, 1δ-Hpro), 2.15–2.24 (1 H, m, 1γ-Hpro), 2.45–2.59 (1 H, 1β-Hpro), 2.69–2.84 (1 H, m, 1β-Hpro), 3.48 (1 H, dd, *J* 10.8 and 5.6, α-Hpro), 3.46–3.70 (2 H, m, 1γ-, 1δ-Hpro), 3.58 and 4.43 (2 H, AB system, *J* 12.6, C*H*$_2$Ph), ethylenic chain 1.80 (1 H, t, $J_{6,4}$ 2.5, 6-H), 1.83–1.92 (1 H, m, 3-H), 2.27–2.36 (1 H, m, 3-H′), 2.35–2.44 (1 H, m, 4-H), 2.69–2.84 (1 H, m, 4-H′), 3.99 (1 H, dd, $J_{2,3}$ 8.8, $J_{2,3'}$ 3.5, 2-H), 6.61–8.13 (14 H, m, ArH); δ_C 15.1 (C-4), 23.9 (γ-Cpro), 30.7 (β-Cpro), 34.1 (C-3), 57.1 (δ-Cpro), 63.1 (*C*H$_2$Ph), 69.4 (C-2), 69.6 (C-6), 70.2 (α-Cpro), 82.7 (C-5), 120.8–142.3 (18 × Ar-C), 171.0 (C=N), 178.8 (NC=O), 180.4 (C-1); *m*/*z* (LSIMS) 550.164 [(M$^+$ + H). $C_{31}H_{30}N_3NiO_3$ requires *m*/*z*, 550.1641].

Hydrolysis of 3b, 3c, 3e and recovery of BPB

Hydrolysis of the complexes was performed as previously described.[27] A solution of a complex **3** (6 mmol) in MeOH (100 cm^3) was added to warm 2 M HCl (70 cm^3). The mixture was refluxed for 1 h, then cooled to room temperature, and conc. NH_3 was added up to pH 9–10. BPB 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 the amino acids **6**.

(*S*)-2-Aminohex-5-enoic acid 6b.—White powder (96%), mp 248 °C (decomp.); $[\alpha]_D^{20}$ 13.6 (*c* 0.9 in H_2O) (lit.,[38] 13.5); δ_H [D_2O, δ(H_2O) 4.80] 1.80–2.00 (2 H, m, 3-H$_2$), 2.09–2.16 (2 H, m, 4-H$_2$), 3.68 (1 H, dd, *J* 6.8 and 5.4, 2-H), 5.04 (1 H, dm, $J_{6,5}$ 10.2, $J_{6,6'}$ 1.9, 6-H), 5.09 (1 H, dm, $J_{6',5}$ 17.2, 6-H′), 5.84 (1 H, ddt, $J_{5,4}$ 6.5, 5-H); δ_C (D_2O) 31.3 (C-4), 32.5 (C-3), 57.0 (C-2), 118.5 (C-6), 139.7 (C-5), 177.6 (C-1).

(*S*)-2-Aminohept-6-enoic acid 6c.—White powder (70%), mp 224 °C (decomp.); $[\alpha]_D^{20}$ 8.4 (*c* 0.5 in H_2O); δ_H (D_2O) 1.26–1.40 (2 H, m, 4-H$_2$), 1.58–1.71 (2 H, m, 3-H$_2$), 1.98 (2 H, br q, $J_{5,4} = J_{5,6} \approx 7.0$, 5-H$_2$), 3.43 (1 H, t, *J* 6.2, 2-H), 4.89 (1 H, dm, $J_{7,6}$ 10.2, 7-H), 4.96 (1 H, dm, $J_{7',6}$ 17.3, 7-H′), 5.77 (1 H, ddt, $J_{6,5}$ 6.7, 6-H); δ_C (D_2O) 24.2 (C-4), 31.8 (C-5), 33.0 (C-3), 55.5 (C-2), 115.2 (C-7), 139.4 (C-6), 178.5 (C-1).

(*S*)-2-Aminohex-5-ynoic acid 6e.—White powder (84%), mp 244 °C (decomp.); $[\alpha]_D^{18}$ 3.7 (*c* 1 in H_2O) (lit.,[39] 3.8); δ_H (D_2O) 1.97 (1 H, m, $J_{3,3'}$ 14.3, 3-H), 2.07 (1 H, m, 3-H′), 2.34–2.37 (2 H, m, 4-H$_2$), 2.39 (1 H, s, 6-H), 3.82 (1 H, dd, $J_{2,3}$ 7.2, $J_{2,3'}$ 5.5, 2-H); δ_C (D_2O) 17.0 (C-4), 31.8 (C-3), 56.5 (C-2), 73.5 (C-6), 85.6 (C-5), 176.8 (C-1).

Protected amino acids 4

Amino acids **6** were protected as methyl ester and *tert*-butyl carbamate, as previously described.[27]

(*S*)-2-(*tert*-Butoxycarbonylamino)hex-5-enoic acid methyl ester 4b.—Oil (83%); $[\alpha]_D^{18}$ −15.2 (*c* 1.2 in $CHCl_3$) (lit.,[40] $[\alpha]_D^{20}$ −17) (Found: C, 59.15; H, 8.55; N, 5.7. $C_{12}H_{21}NO_4$ requires C, 59.25; H, 8.7; N, 5.75%); δ_H 1.45 [9 H, s, (CH$_3$)$_3$], 1.63–1.76 (1 H, m, 3-H), 1.83–1.95 (1 H, m, 3-H′), 2.06–2.13 (2 H, m, 4-H$_2$), 3.72 (3 H, s, OCH$_3$), 4.27–4.34 (1 H, m, 2-H), 4.99 (1 H, dm, $J_{6,5}$ 10.1, $J_{6,6'}$ 1.8, 6-H), 5.01 (1 H, br d, NH), 5.03 (1 H, dm, $J_{6',5}$ 17.1, 6-H′), 5.76 (1 H, ddt, $J_{5,4}$ 6.6, 5-H); δ_C 28.3 [(CH$_3$)$_3$], 29.5 (C-4), 32.0 (C-3), 52.2 (OCH$_3$), 53.0 (C-2), 79.9 [*C*(CH$_3$)$_3$], 115.7 (C-6), 137.0 (C-5), 155.3 (NC=O), 173.3 (C-1); *m*/*z* (EI) 187.084 [(M$^+$ − C_4H_8). $C_8H_{13}NO_4$ requires *m*/*z*, 187.0844].

(*S*)-2-(*tert*-Butoxycarbonylamino)hept-6-enoic acid methyl ester 4c.—Oil (83%); $[\alpha]_D^{20}$ 14.1 (*c* 1.17 in $CHCl_3$) (lit.,[41] $[\alpha]_D^{24}$ 13.16) (Found: C, 60.8; H, 9.0; N, 5.35. $C_{13}H_{23}NO_4$ requires C, 60.7; H, 9.0; N, 5.45%); δ_H 1.37–1.52 (2 H, m, 4-H$_2$), 1.45 [9 H, s, (CH$_3$)$_3$], 1.57–1.69 (1 H, m, $J_{3,3'}$ 12.8, $J_{3,2}$ 6.8, 3-H), 1.75–1.87 (1 H, m, $J_{3',2}$ 5.0, 3-H′), 2.07 (2 H, qdd, $J_{5,4} = J_{5,6} = 6.7$, $J_{5,7}$ 1.3, $J_{5,7'}$ 2.7, 5-H$_2$), 3.74 (3 H, s, OCH$_3$), 4.27–4.34 (1 H, m, 2-H), 4.97 (1 H, dm, $J_{7,6}$ 10.3, $J_{7,7'}$ 2.0, 7-H), 5.03 (1 H, dm, $J_{7',6}$ 17.0, 7-H′), 5.05 (1 H, br d, NH), 5.77 (1 H, ddt, $J_{6,5}$ 6.6, 6-H); δ_C 24.6 (C-4), 28.3 [(CH$_3$)$_3$], 32.2 (C-5), 33.2 (C-3), 52.2 (OCH$_3$), 53.3 (C-2), 79.8 [*C*(CH$_3$)$_3$], 115.1 (C-7), 138.0 (C-6), 155.4 (NC=O), 173.4 (C-1); *m*/*z* (EI) 198.150 [(M$^+$ − $^{\bullet}CO_2CH_3$). $C_{11}H_{20}NO_2$ requires *m*/*z*, 198.1494].

(*S*)-2-(*tert*-Butoxycarbonylamino)hex-5-ynoic acid methyl ester 4e.—Oil (85%); $[\alpha]_D^{20}$ 17 (*c* 1.2 in $CHCl_3$) (Found: C, 59.5; H, 7.75; N, 5.5. $C_{12}H_{19}NO_4$ requires C, 59.75; H, 7.95; N, 5.8%); δ_H 1.45 [9 H, s, (CH$_3$)$_3$], 1.89 (1 H, m, $J_{3,3'}$ 13.7, $J_{3,2}$ 7.6, $J_{3,4} \approx J_{3,4'} \approx 7.0$, 3-H), 2.00 (1 H, t, $J_{6,4}$ 2.6, 6-H), 2.09 (1 H, m, $J_{3',4'} \approx J_{3',4} \approx 7.1$, $J_{3',2}$ 5.3, 3-H′), 2.26–2.32 (2 H, m, 4-H$_2$), 3.76 (3 H, s, OCH$_3$), 4.39 (1 H, m, $J_{2,NH}$ 6.7, 2-H), 5.15 (1 H, d, NH); δ_C 14.9 (C-4), 28.3 [(CH$_3$)$_3$], 31.5 (C-3), 52.4 (OCH$_3$), 52.7 (C-2), 69.3 (C-6), 80.0 [*C*(CH$_3$)$_3$], 82.8 (C-5), 155.3 (NC=O), 172.7 (C-1); *mz* (EI) 182.118 [(M$^+$ − $^{\bullet}CO_2CH_3$). $C_{10}H_{16}NO_2$ requires *m*/*z*, 182.1180].

Hydroboration of compounds 4: boronic esters 5

The ω-unsaturated amino esters **4** were first hydroborated with Ipc$_2$BH (2 equiv. for **4b**, **4c** and 1.2 equiv. for **4e**). The resulting boranes were added to an excess of acetaldehyde (20 equiv. for **4b**, **4c** and 12 equiv. for **4e**), affording boronic acids after hydrolysis, which were converted to pinanediol boronates **5** by reaction with (+)-pinanediol (1 equiv.). Compounds **5** were purified by chromatography on silica gel (heptane–ethyl acetate, 8 : 2).[27]

(*S*)-2-(*tert*-Butoxycarbonylamino)-6-[(1*S*,2*S*,3*R*,5*S*)-(+)-pinanyl-2,3-dioxyboryl]hexanoic acid methyl ester 5b.—Oil (51%); $[\alpha]_D^{20}$ 23.6 (*c* 3 in $CHCl_3$) (Found: C, 61.6; H, 8.9; N, 3.1. $C_{22}H_{38}BNO_6$·0.33 H_2O requires C, 61.55; H, 9.1; N, 3.25%); δ_H 0.79 (2 H, *J* 7.6, 6-H$_2$), 0.82 (3 H, s), 1.26 (3 H, s) and 1.35 (3 H, s) (together 3 × CH$_3$), 1.06 (1 H, d, *J* 10.9, 7′-H), 1.41 [9 H, s, (CH$_3$)$_3$], 1.25–1.47 (4 H, m, 5-, 4-H$_2$), 1.53–1.65 (1 H, m) and 1.70–1.80 (1 H, m) (together 3-H$_2$), 1.80 (1 H, ddd, 4′-H), 1.85–1.91 (1 H, m, 5′-H), 2.01 (1 H, t, *J* ≈ 5.5, 1′-H), 2.15–2.23 (1 H, m, 7′-H), 2.30 (1 H, ddt, 4′-H), 3.72 (3 H, s, OCH$_3$), 4.20–4.28 (1 H, m, 2-H), 4.22 (1 H, dd, *J* 8.8 and 1.8, 3′-H), 4.98 (1 H, d, $J_{2,NH}$ 7.9, NH); δ_C 10.4 (C-6), 23.8 (C-5), 24.0, 27.1 and 28.7 (3 × CH$_3$), 26.5 (C-7′), 27.9 (C-4), 28.3 [(CH$_3$)$_3$], 32.4 (C-3), 35.5 (C-4′), 38.1 (C-6′), 39.5 (C-5′), 51.2 (C-1′), 52.1 (OCH$_3$), 53.4

(C-2), 77.6 (C-3′), 79.7 [*C*(CH_3)$_3$], 85.4 (C-2′), 155.4 (NC=O), 173.5 (C-1); δ_B 33.4; *m*/*z* (EI) 366.210 [(M^+ − $^{\bullet}C_4H_9$). $C_{18}H_{29}BNO_6$ requires *m*/*z*, 366.2088].

(*S*)-2-(*tert*-Butoxycarbonylamino)-7-[(1*S*,2*S*,3*R*,5*S*)-(+)-pinanyl-2,3-dioxyboryl]heptanoic acid methyl ester 5c.—Oil (60%); $[\alpha]_D^{20}$ 22.1 (*c* 1 in $CHCl_3$) (Found: C, 63.45; H, 9.3; N, 3.15. $C_{23}H_{40}BNO_6$ requires C, 63.15; H, 9.2; N, 3.2%); δ_H 0.80 (2 H, t, *J* 7.6, 7-H_2), 0.84 (3 H, s), 1.29 (3 H, s) and 1.38 (3 H, s) (together 3 × CH_3), 1.10 (1 H, d, *J* 10.8, 7′-H), 1.44 [9 H, s, (CH_3)$_3$], 1.27–1.47 (6 H, m, 6-, 5-, 4-H_2), 1.55–1.67 (1 H, m) and 1.73–1.83 (1 H, m) (together 3-H_2), 1.83 (1 H, ddd, 4′-H), 1.88–1.94 (1 H, m, 5′-H), 2.04 (1 H, t, *J* ≈ 5.5, 1′-H), 2.17–2.26 (1 H, m, 7′-H), 2.33 (1 H, ddt, 4′-H), 3.73 (3 H, s, OCH_3), 4.23–4.30 (1 H, m, 2-H), 4.24 (1 H, dd, *J* 8.7 and 1.9, 3′-H), 4.99 (1 H, d, $J_{2,NH}$ 8.2, NH); δ_C 10.6 (C-7), 23.9 (C-6), 24.0, 27.1 and 28.7 (3 × CH_3), 25.1 (C-4), 26.5 (C-7′), 28.3 [(CH_3)$_3$], 31.9 (C-5), 32.7 (C-3), 35.5 (C-4′), 38.1 (C-6′), 39.5 (C-5′), 51.3 (C-1′), 52.1 (OCH_3), 53.5 (C-2), 77.6 (C-3′), 79.8 [*C*(CH_3)$_3$], 85.3 (C-2′), 155.4 (NC=O), 173.5 (C-1); δ_B 33.6; *m*/*z* (EI) 378.283 [(M^+ − $^{\bullet}CO_2CH_3$). $C_{21}H_{37}BNO_4$ requires *m*/*z*, 378.2815].

(*S*)-2-(*tert*-Butoxycarbonylamino)-6-[(1*S*,2*S*,3*R*,5*S*)-(+)-pinanyl-2,3-dioxyboryl]hex-5-enoic acid methyl ester 5e.—Oil (50%); $[\alpha]_D^{20}$ 27.7 (*c* 1.5 in $CHCl_3$) (Found: C, 61.2; H, 8.45; N, 2.9. $C_{22}H_{36}BNO_6$·0.5 H_2O requires C, 61.4; H, 8.65; N, 3.25%); δ_H 0.85 (3 H, s), 1.29 (3 H, s) and 1.40 (3 H, s) (together 3 × CH_3), 1.13 (1 H, d, *J* 10.9, 7′-H), 1.44 [9 H, s, (CH_3)$_3$], 1.69–1.80 (1 H, m, 3-H), 1.84 (1 H, ddd, 4′-H), 1.87–1.99 (2 H, m, 3-, 5′-H), 2.04 (1 H, t, *J* 5.5, 1′-H), 2.12–2.25 (3 H, m, 4-H_2, 7′-H), 2.32 (1 H, ddt, 4′-H), 3.73 (3 H, s, OCH_3), 4.27 (1 H, dd, *J* 8.5 and 1.8, 3′-H), 4.23–4.34 (1 H, m, 2-H), 5.02 (1 H, d, $J_{2,NH}$ 8.1, NH), 5.47 (1 H, dt, $J_{6,5}$ 18.0, $J_{6,4}$ 1.5, 6-H), 6.56 (1 H, dt, $J_{5,4}$ 6.3, 5-H); δ_C 24.0, 27.1 and 28.6 (3 × CH_3), 26.4 (C-7′), 28.3 [(CH_3)$_3$], 31.25 and 31.35 (C-3 and -4), 35.5 (C-4′), 38.1 (C-6′), 39.5 (C-5′), 51.3 (C-1′), 52.3 (OCH_3), 53.1 (C-2), 77.7 (C-3′), 79.9 [*C*(CH_3)$_3$], 85.6 (C-2′), 119.5 (C-6), 152.0 (C-5), 155.3 (NC=O), 173.1 (C-1); δ_B 29.3; *m*/*z* (EI) 365.200 [(M^+ − C_4H_8). $C_{18}H_{28}BNO_6$ requires *m*/*z*, 365.2010].

ω-Borono-α-amino acids 1

Hydrolysis of compounds **5** was performed in HCl solution at 70 °C (12 M HCl; 2 h for **5b** or **5c**; 6 M HCl; 5 h for **5e**).[27] Compounds **1** were purified by chromatography on silica gel (EtOH–14 M NH_3; 2:1).

(*S*)-2-Amino-6-(dihydroxyboryl)hexanoic acid 1b.—White powder (79%), mp 261 °C (decomp.); δ_H (D_2O) 0.80 (2 H, t, $J_{6,5}$ 7.6, 6-H_2), 1.26–1.47 (4 H, m, 4-, 5-H_2), 1.74–1.92 (2 H, m, 3-H_2), 3.70 (1 H, t, $J_{2,3}$ 6.1, 2-H); Hydrochloride salt[22] mp 148–150 °C, decomp. >150 °C, δ_H (CD_3OD) 0.82 (2 H, m), 1.45 (4 H, m), 1.90 (2 H, m), 3.95 (1 H, t); δ_C (D_2O) 16.5 (C-6), 25.9 (C-5), 29.6 (C-4), 32.8 (C-3), 57.4 (C-2), 177.7 (C-1); δ_B (D_2O) 32.6; *m*/*z* (Electrospray) 176.110 [(M^+ + H). $C_6H_{15}BNO_4$ requires *m*/*z*, 176.1094].

(*S*)-2-Amino-7-(dihydroxyboryl)heptanoic acid 1c.—White powder (91%) mp 246 °C (decomp.); δ_H (H_2O/ext DSS) 0.76 (2 H, t, $J_{7,6}$ 7.4, 7-H_2), 1.27–1.44 (6 H, m, 4-, 5-, 6-H_2), 1.77–1.88 (2 H, m, 3-H_2), 3.71 (1 H, dd, $J_{2,3}$ 6.4, $J_{2,3'}$ 5.8, 2-H); δ_C (D_2O) 16.6 (C-7), 25.8, 26.6, 33.0, 33.7 (C-6, -5, -4, -3), 57.7 (C-2), 177.7 (C-1); δ_B (D_2O) 33.2; *m*/*z* (Electrospray) 190.126 [(M^+ + H). $C_7H_{17}BNO_4$ requires *m*/*z*, 190.1251].

(*S*)-2-Amino-6-(dihydroxyboryl)hex-5-enoic acid 1e.—White powder (30%) mp 263 °C (decomp.); δ_H (H_2O/ext DSS) 1.86–2.07 (2 H, m, 3-H_2), 2.20–2.30 (2 H, m, 4-H_2), 3.73 (1 H, dd, $J_{2,3}$ 6.7, $J_{2,3'}$ 5.6, 2-H), 5.51 (1 H, dt, $J_{6,5}$ 18.1, $J_{6,4}$ 1.5, 6-H), 6.51 (1 H, dt, $J_{5,4}$ 6.2, 5-H); δ_C (D_2O) 32.1 and 33.0 (C-3 and -4), 57.2 (C-2), 126.0 (C-6), 152.7 (C-5), 177.4 (C-1); δ_B (H_2O) 27.9; *m*/*z* (Electrospray) 174.093 [(M^+ + H). $C_6H_{13}BNO_4$ requires *m*/*z*, 174.0938].

Measurement of optical purity

The ees of the protected boronates **5** derivatized as benzamides were measured by HPLC using a PIRKLE covalent (S,S) whelk-01 column.[27] Elution was performed with a mixture of hexane–propan-2-ol (9:1), flow rate was 1 cm^3 min^{-1}, and the UV detector was set at 225 nm. Samples of racemic derivatives were obtained through a subsequent deprotection of the carboxylic group with sodium hydroxide, oxazolone formation with DCC,[42] and opening of the heterocycle with methanol.[27] Ees were found to be superior to 98% for the three derivatives.

2-Benzoylamino-6-[(1*S*,2*S*,3*R*,5*S*)-(+)-pinanyl-2,3-dioxyboryl]hexanoic acid methyl ester from 5b.—(*S*): t_R 24.8 min; (*R*,*S*): t_{R1} 22.6 min; t_{R2} 25.0 min.

2-Benzoylamino-7-[(1*S*,2*S*,3*R*,5*S*)-(+)-pinanyl-2,3-dioxyboryl]heptanoic acid methyl ester from 5c.—(*S*): t_R 28.0 min; (*R*,*S*): t_{R1} 26.3 min; t_{R2} 29.0 min.

2-Benzoylamino-6-[(1*S*,2*S*,3*R*,5*S*)-(+)-pinanyl-2,3-dioxyboryl]hex-5-enoic acid methyl ester from 5e.—(*S*): t_R 27.8 min; (*R*,*S*): t_{R1} 25.9 min; t_{R2} 28.1 min.

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