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Synthesis and evaluation of new ω-borono-α-amino acids as rat liver arginase inhibitors

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Abstract—Recent studies have demonstrated that arginase plays important roles in pathologies such as asthma or erectile dysfunctions. We have synthesized new ω-borono-α-amino acids that are analogues of the previously known arginase inhibitors *S*-(2-boronoethyl)-L-cysteine (BEC) and 2-amino-6-boronohexanoic acid (ABH) and evaluated them as inhibitors of purified rat liver arginase (RLA). In addition to the distance between the $B(OH)_2$ and the α-amino acid functions, the position of the sulfur atom in the side chain also appears as a key determinant for the interaction with the active site of RLA. Furthermore, substitution of the alkyl side chain of BEC by methyl groups and conformational restriction of ABH by incorporation of its side chain in a phenyl ring led to inactive compounds. These results suggest that subtle interactions govern the affinity of inhibitors for the active site of RLA.

1. Introduction

In mammalian cells, L-arginine is metabolized by two major pathways. Arginases catalyze its hydrolysis to L-ornithine and urea, whereas NO synthases (NOSs) catalyze its oxidation to L-citrulline and nitric oxide, NO.[1] The general importance of arginases lies in their 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] NO is an important biological molecule involved in vasodilation, neurotransmission, and cytotoxicity.[3–5] Since NOSs and arginases can be found in similar tissues and cells, and because their expression may be regulated in response to the same stimuli (cytokines, endotoxines), both enzymes are believed to participate in the regulation of NO biosynthesis by competing for the common substrate, L-arginine.[6] Conversely, N^{ω}-hydroxy-L-arginine (NOHA), an intermediate in the reaction catalyzed by NOSs, is a potent competitive inhibitor of arginases with K_i value of 10–40 μM.[7,8] This resulted in the hypothesis that under various physiological situations, arginases may be essential in the regulation of NOSs activities by modulating local L-arginine concentration. Recent studies support this hypothesis and in cells producing NO, localized NOHA and L-arginine concentrations were found to modulate arginase activity and inhibition of arginases enhanced the formation of NO.[9–11] The inhibition of arginases by selective and potent inhibitors thus became the focus of potential therapies for treating several NO-dependent smooth muscle disorders, including asthma and erectile dysfunctions.[12–15]

Arginine hydrolysis by arginases is achieved by a metal-activated water molecule that bridges a $(Mn^{II})_2$ cluster at their active site.[16] 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.[17] This proposal resulted in the synthesis of the first boronic analogues of L-arginine, 2-amino-6-boronohexanoic acid (ABH),[18], and *S*-(2-boronoethyl)-L-cysteine (BEC) that act as high-affinity and slow-binding inhibitors of arginase (Scheme 1).[14,19]

Keywords: Rat liver arginase; Inhibitors; α-Amino acids; Boronic acids.
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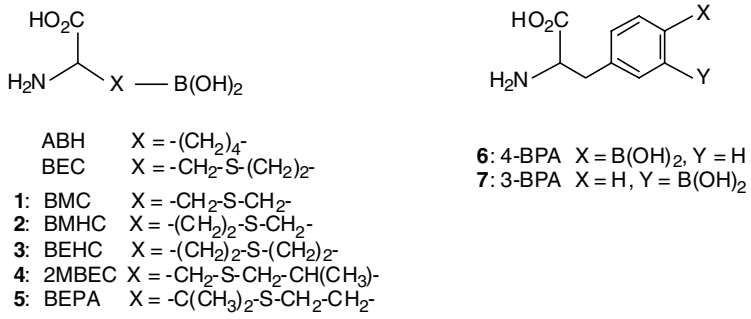


Scheme 1. Structure of ABH, BEC, and ω-borono-α-amino acids **1**–**7**.

In order to define better relationships between the chemical structure of ω-borono-α-amino acids and their recognition by RLA, we have synthesized five new α-amino acids, compounds **1**–**5**, that are BEC analogues. The distance between the two functions has been modified by introducing the boronic acid at the end of an alkyl side chain containing 2, 3, or 4 C-atoms. The distance between the B- and S-atoms of BEC has been reduced, and one or two methyl groups have been introduced in the alkyl side chain of BEC. We also synthesized two conformationally restricted ABH analogues, racemic 4- and 3-boronophenylalanine, **6** and **7**, that contain a phenyl ring in the alkyl side chain (Scheme 1). These BEC and ABH analogues have been evaluated as inhibitors of purified rat liver arginase (RLA) and we used the recent crystallographic data to discuss our results.

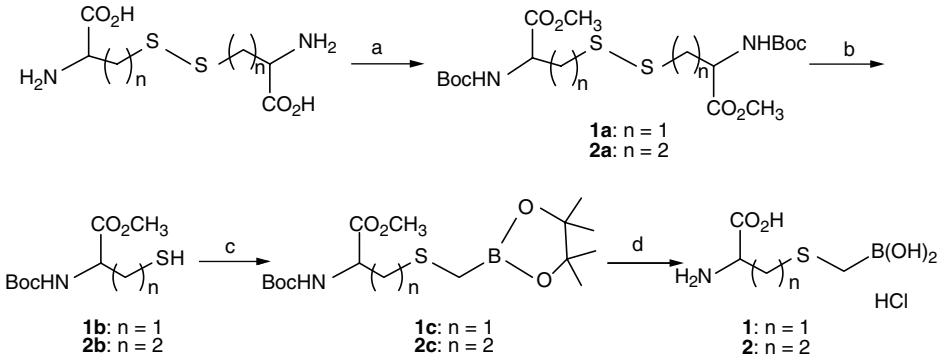
2. Results

2.1. Synthesis of *S*-(boronomethyl)-L-cysteine, BMC 1 and *S*-(boronomethyl)-L-homocysteine, BMHC 2

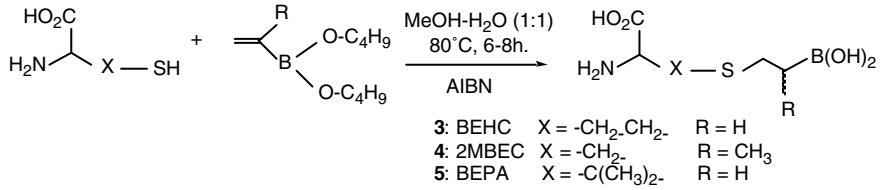
Our strategy to prepare BEC analogues **1** and **2** is based on the nucleophilic attack of orthogonally protected L-cysteine and L-homocysteine on bromomethylboronic ester (Scheme 2). Methyl esters of Boc-L-cysteine and Boc-L-homocysteine were synthesized from L-cystine and L-homocystine, respectively, following a described procedure.[20] The S-alkylation of methyl Boc-L-cysteinate with 2-(bromomethyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane[21] in THF led to the expected product in a 87% yield. In the case of L-homocysteine derivative, the thiolate anion was generated in the presence of the electrophile to avoid the formation of *tert*-butyl(2-oxotetrahydro-3-thienyl)carbamate resulting from an intramolecular cyclization. Under those conditions, the desired *S*-alkylated adduct was obtained in 90% yield. Removal of the protective groups of the α-amino acid function was carried out using treatments with aqueous solutions of NaOH and HCl. Boronic acids of *S*-(boronomethyl)-L-cysteine, BMC **1** and *S*-(boronomethyl)-L-homocysteine, BMHC **2**, were obtained from boronic esters by reacting phenylboronic acid in a two-phase ether/water system (Scheme 2).[22]

2.2. Synthesis of *S*-(boronoethyl)-D/L-homocysteine, BEHC 3, *S*-(2-methyl-2-boronoethyl)-L-cysteine, 2MBEC 4, and *S*-(boronoethyl)-D-penicillamine, BEPA 5

The strategy previously described to prepare BEC allowed us to get a simple access to the higher homologue BEHC **3**, and to compound **5**, BEPA, that contains a *gem*-dimethyl group adjacent to the α-CH of L-cysteine.[14,23] Addition of D/L-homocysteine and D-penicillamine to di(*n*-butyl)-vinylboronate[24] in the presence of AIBN led to the formation of BEHC and BEPA in 55% and 35% yields, respectively (Scheme 3). Compound **4** that contains a methyl group adjacent to the B-atom was similarly obtained in 42% yield by reacting L-cysteine and di(*n*-butyl)-2-propeneboronate.[24]



Scheme 2. Synthesis of BMC, **1** (*n* = 1) and BMHC, **2** (*n* = 2). Reagents and conditions: (a) (i) $SOCl_2$, MeOH, reflux, 12 h; (ii) NaOH, dioxane/H_2O (2:1) $(Boc)_2O$, rt, 12 h; (b) Ph_3P, NaOAc, MeOH/H_2O/AcOH, reflux; (c) 2-(bromomethyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, NaH, THF, rt, 2 h; (d) (i) 1 M aq NaOH, 50 °C, 2 h, (ii) 2 M aq HCl, Et_3O, $PhB(OH)_2$ rt, 9 h.



Scheme 3. Synthesis of BEHC, **3**, 2MBEC, **4**, and BEPA, **5**.

2.3. Synthesis of 4-(dihydroxyboryl)-D/L-phenylalanine, 4-BPA 6 and 3-(dihydroxyboryl)-D/L-phenylalanine, 3-BPA 7

The synthesis of 3-BPA, **7**, was based on the Horner–Emmons–Wittig reaction of carbonyl compounds with *N*-acylphosphonoglycine trimethyl ester following the methodology previously described for the synthesis of 4-BPA, **6** (Scheme 4).[25] Condensation of protected 3-formylphenylboronic acid with *N*-(benzyloxycarbonyl)-phosphonoglycine trimethyl ester[26] using DBU in CH_2Cl_2 was successfully achieved at ambient temperature to provide the intermediate α-amino acrylate in a 55% yield and predominantly in the form of the *Z*-isomer. Amino acrylate was hydrogenated using 10% Pd–C to afford the protected boronophenylalanine with concomitant debenzylation. Finally, successive treatments with NaOH and HCl in the presence of phenylboronic acid provided 3-BPA **7** in an overall yield of 35% from 3-formylphenylboronic acid.[22]

2.4. Effects of compounds 1–7 on rat liver arginase activity

The ability for compounds **1**–**7** to inhibit [^{14}C]urea formation from [Guanido-^{14}C]L-arginine in the presence of purified RLA was investigated following usual protocols[27,28] and compared to the inhibitory effects of BEC. Experiments were performed at physiological pH 7.4 and at pH 9.0 where RLA displays maximal activity.[2] As previously described,[14] BEC led to a potent concentration-dependent inhibition of purified RLA activity with half-maximal inhibition (IC_{50}) obtained in the presence of 5 ± 1 μM BEC (Table 1). The shorter and the longer analogues BMC **1** and BEHC **3**, were almost inactive at both pH (IC_{50} > 3 mM). Introduction of a methyl group in the α-position from the B-atom, in compound **4**, or of a *gem*-dimethyl group in close proximity to the α-amino acid function, in compound **5**, led to inactive compounds. Interestingly, BMHC **2**, which differs from BEC by the position of the S-atom in the side chain and the distance between the S-atom and the boronic acid function, was 70-fold less potent than BEC (IC_{50} = 350 ± 50 μM, Table 1). Finally, the two conformationally restricted ABH analogues, **6** and **7**, were almost inactive at both pH values (IC_{50} > 5 mM, Table 1).

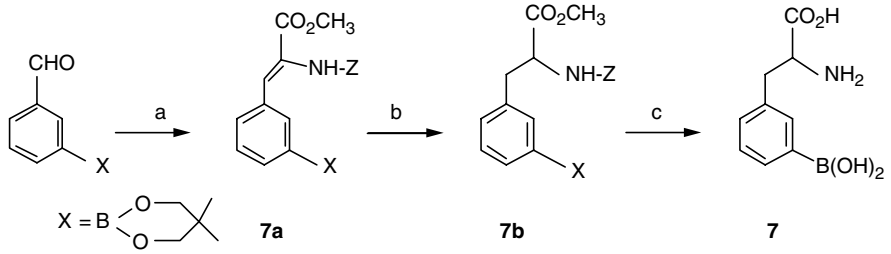
Table 1. Effects of BEC and ω-borono-α-amino acids **1**–**7** on the activity of purified RLA. Half-inhibitory concentrations (IC_{50}) values were measured at pH 7.4 and pH 9.0 following the hydrolysis of [^{14}C]L-arginine to [^{14}C] urea as indicated in Experimental

Compounds	IC_{50}	
	pH 7.4[a]	pH 9.0[a]
BEC	5 ± 1 μM	20 ± 4 μM
1 BMC	3.8 ± 0.5 mM	7.5 ± 1.0 mM
2 BMHC	350 ± 100 μM	>1 mM (40%)[b]
3 BEHC	>10 mM (35%)[b]	>10 mM (15%)[b]
4 2MBEC	9 ± 1 mM	>10 mM (10%)[b]
5 BEPA	>3 mM (15%)[b]	>3 mM (10%)[b]
6 4-BPA	6.0 ± 1.0 mM	>10 mM (20%)[b]
7 3-BPA	>10 mM (30%)[b]	>10 mM (10%)[b]

[a] Mean values ± SD from three independent experiments.
[b] % Inhibition at the concentration indicated.

2.5. Discussion

New amino acids **1**–**7** were easily obtained following previously described protocols and were tested as rat liver arginase inhibitors. The comparison of the inhibitory effects of BEC and its analogues **1** and **3** fully identified the distance between the α-amino acid function and the terminal OH group of the side chain as a key determinant in their binding at the active site of RLA. In the case of ABH analogues, such a key role for the distance between the two functions was similarly observed and one CH_2-group shorter or longer analogues of ABH were much less active.[29]



Scheme 4. Synthesis of 3-BPA **7**. Reagents and conditions: (a) *N*-(benzyloxycarbonyl)phosphonoglycine trimethyl ester, DBU/CH_2CI_2, rt, 3 h (*E*/*Z* = 30:70); (b) 10% Pd–CH_2/MeOH–H_2Cl_2 (2:1), rt, 5 atm, 21 h; (c) (i) 1 M aq NaOH, 50 °C, 2 h, (ii) 2 M aq HCI, Et_2O, $PhB(OH)_2$, rt, 9 h.

Dehydro-ABH, which only differs from ABH by introduction of a double-bond in the alkyl side chain, displayed lower inhibitory effects than ABH.[29] This could explain that introduction of the alkyl-chain of ABH in a phenyl ring, in compounds **6** and **7**, resulted in a complete loss of interaction with RLA. The determinants in the binding of these new ω-borono-α-amino acids to RLA thus seem very similar to those required for the binding of another group of potent RLA inhibitors, N^{ω}-hydroxy-guanidino-α-amino acids, NOHA, and nor-NOHA.[30,31]

Very unexpected results resided in the poor inhibitory effects of BMHC compared to those of BEC. BMHC only differs from BEC by the position of its S-atom in the alkyl side chain. Shortening the distance between the S- and B-atoms by one CH_2-group resulted in a 70-fold decrease of the inhibitory effects. Introduction of one methyl group in the α-position to the B-atom, in 2MBEC **4**, or of a *gem*-dimethyl group, in BEPA **5**, completely abolished the inhibitory effects of BEC. Possible explanations could be found from the binding of BEC as observed in the X-ray crystal structure of the RLA–BEC complex.[14] This structure reveals that the S-atom of BEC is very close to His141 and His126 residues in the active site (pdb access 1HQ5).[14] Shortening the distance between the S-atom and the $B(OH)_2$ function could introduce steric and/or electronic interactions between the S-atom of BMHC and one (or both) of these His-residues. Similarly, introduction of a methyl group close to the B-atom could introduce steric constraints with one of these His-residues and reduce the affinity. The α-amino acid function of BEC establishes direct or water-mediated H-bonds with residues Asn130, Ser137, Asn139, Asp181, Asp183, and Glu186 of the protein.[14] Alteration of this network of H-bonds and restriction of the possible conformations by introduction of an hydrophobic bulky *gem*-dimethyl group could explain the low affinity of BEPA. Although the binding mode of BEC is close to that of ABH, subtle differences appear between the two inhibitors and can explain their 20-fold difference in affinity. Our results demonstrate for the first time that further modifications of the structure of BEC, by introduction of a methyl group close to the B-atom or by shortening the distance between the B- and S-atoms, strongly compromise the affinity for the active site of RLA. X-Ray studies are underway to more precisely explain these subtle differences.

3. Experimental

3.1. General procedures

The reagents were purchased from Acros and Aldrich and were used without further purification unless otherwise specified. Tetrahydrofuran was distilled from deep blue solutions of sodium/benzophenone ketyl prior to use. Methylene chloride was shaken with concentrated H_2SO_4, dried over K_2CO_3, and distilled. Merck silica gel was used for column chromatography. All melting points were determined on a Kofler apparatus and are uncorrected. ^{1}H NMR spectra were recorded on 200, 250 or 300 MHz Bruker spectrometers operating in the Fourier transform mode. ^{13}C NMR spectra were obtained with broadband proton decoupling. Chemical shifts are expressed as δ values in parts per million relative to tetramethylsilane, except for ^{11}B NMR where chemical shifts are relative to $BF_3{\cdot}OEt_2$. Coupling constants (J) are expressed in hertz. Optical rotations were measured using 10 cm path-length cell at 20 °C, and the concentration is expressed in grams per deciliter. HRMS were obtained with a Varian MAT 311 mass spectrometer at Centre Régional de Mesures Physiques de l'Ouest. Microanalysis were performed at the Central Laboratory for Analysis, CNRS (Solaize, France). 4-Dihydroxyboryl-D/L-phenylalanine hydrochloride, 4-BPA **6**, was prepared as previously described.[25]

3.2. *N*,*N*′-Bis[(*tert*-butyloxy)carbonyl]-L-cystine dimethyl ester 1a

Thionyl chloride (3.8 mL, 52 mmol) was added dropwise at 0 °C to 80 mL of methanol. L-Cystine (5 g, 20.8 mmol) was then added and the reaction mixture was refluxed overnight. The volatile reagents and solvent were evaporated in vacuo and a white solid (6.9 g, 97%) was obtained. L-Cystine dimethyl ester dihydrochloride was used directly in the next step without further purification. ^{1}H NMR (200 MHz, D_2O) δ 3.29–3.33 (m, 4H), 3.78 (s, 6H), 4.49 (m, 2H).

To a stirred solution of L-cystine dimethyl ester dihydrochloride (6.9 g, 20.2 mmol) in 1 M NaOH (40.4 mL) and dioxane/water (2:1, 60 mL), di-*tert*-butyl pyrocarbonate (10.1 g, 46.3 mmol) was added in one portion at 0 °C. The ice was removed, and the solution was stirred for 24 h at room temperature. After concentration of the reaction mixture, ethyl acetate (60 mL) was added. The pH of the mixture was adjusted to 3.0 with aqueous 2 M $KHSO_4$. The aqueous layer was extracted with ethyl acetate (2 × 30 mL) and the combined organic layers were washed with brine (30 mL) and water (30 mL), and dried over $MgSO_4$. Evaporation of ethyl acetate in vacuo and recrystallization from ethyl acetate/heptane gave *N*,*N*′-bis[(*tert*-butyloxy)carbonyl]-L-cystine dimethyl ester **1a** (7.3 g, 77%). Mp 96–98 °C; lit.[20] mp 96–97 °C; $[\alpha]_{589}^{20}$ +73.5 (*c* 1.08, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.49 (s, 18H), 3.20 (d, J = 5.3 Hz, 4H), 3.81 (s, 6H), 4.64 (m, 2H), 5.40 (br d, 2H). Anal. Calcd for $C_{18}H_{32}N_2O_8S_2$: C, 46.14; H, 6.88; N, 5.98. Found: C, 46.27; H, 6.93; N, 5.95.

3.3. *N*-[(*tert*-Butyloxy)carbonyl]-L-cysteine methyl ester 1b

N,*N*′-Bis[(*tert*-butyloxy)carbonyl]-L-cystine dimethyl ester **1a** (2.5 g, 5.34 mmol), triphenylphosphine (1.48 g, 5.49 mmol), and sodium acetate (0.17 g, 2.11 mmol) were suspended in a mixture of methanol (20 mL), water (10 mL), and glacial acetic acid (0.17 mL) and heated under reflux for 24 h. The mixture was diluted with 260 mL CH_2Cl_2, washed with 100 mL of water, 50 mL of saturated sodium chloride solution, and dried over $MgSO_4$. The solvent was removed in vacuo and the residue purified by column chromatography on silica gel

(ethyl acetate/heptane 2:8) to give **1b** (2.3 g, 91%) as a colorless oil. $[\alpha]_{589}^{20}$ +97.7 (*c* 2.12, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.50 (s, 9H), 2.90 (dd, $J = 4.3$, 8.9 Hz, 2H), 3.80 (s, 3H), 4.65 (m, 1H), 5.46 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.7, 28.7, 53.1, 55.2, 80.1, 155.5, 171.2. Anal. Calcd for $C_9H_{17}NO_4S$: C, 45.94; H, 7.28; N, 5.95. Found: C, 46.10; H, 7.30; N, 6.02.

3.4. *N*-[(*tert*-Butyloxy)carbonyl)]-*S*-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methyl]-L-cysteine methyl ester 1c

To a suspension of NaH (0.15 g, 3.8 mmol) in THF (10 mL) at 0 °C, *N*-[(*tert*-butyloxy)carbonyl]-L-cysteine methyl ester **1b** (0.8 g, 3.40 mmol) in THF (5 mL) was added dropwise under N_2. The mixture was warmed to room temperature, stirred 30 min and 2-(bromomethyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane[21] (0.75 g, 3.4 mmol) in THF (5 mL) was added dropwise. After stirring for 12 h at room temperature, the mixture was quenched by the addition of H_2O (20 mL), THF was evaporated and the residue extracted with ethyl acetate (3×20 mL). The organic phase was washed with brine (20 mL), dried over $MgSO_4$, and evaporated. Flash column chromatography on silica gel (ethyl acetate/heptane 2:8) gave pure **1c** (1.1 g, 87%) as a colorless oil. $[\alpha]_{589}^{20}$ +14.7 (*c* 1.15, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.31 (s, 12H), 1.48 (s, 9H), 2.03 (m, 2H), 3.02 (m, 2H), 3.80 (s, 3H), 4.56 (m, 1H), 5.51 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.8, 27.3, 35.7, 51.6, 51.9, 79.0, 81.1, 157.3, 170.8. Anal. Calcd for $C_{16}H_{30}BNO_6S$: C, 51.21; H, 8.06; N, 3.73. Found: C, 51.25; H, 8.09; N, 3.74.

3.5. *S*-[(Dihydroxyboryl)methyl]-L-cysteine 1

A mixture of **1c** (0.7 g, 1.87 mmol) and 1 M aq NaOH (16 mL) was stirred at 45–50 °C for 2 h and the pH of the mixture was adjusted to 1 with aqueous 3 M HCl. After being stirred at ambient temperature for 12 h, phenylboric acid (1.87 mmol) and ether (16 mL) were added. After 3 h at room temperature, the ether layer was removed and replaced by fresh ether (16 mL). This treatment was repeated two-fold, and the aqueous solution was concentrated and dried under high vacuum. After silica gel chromatography ($CHCl_3$/MeOH/NH_3/H_2O 6:6:0.5:1.5) compound **1** was obtained (181 mg, 49%) as an amorphous solid. $[\alpha]_D^{20}$ −7.2 (*c* 0.8, H_2O); $[\alpha]_{365}^{20}$ −13.7 (*c* 0.8, H_2O); ^1H NMR (200 MHz, D_2O + DCl) δ 1.94 (d, $J = 14.1$ Hz, 1H), 2.02 (d, $J = 14.1$ Hz, 1H), 2.97 (dd, $J = 14.6$, 7.6 Hz, 1H), 3.10 (dd, $J = 14.6$, 4.5 Hz, 1H), 4.22 (dd, $J = 7.6$, 4.5 Hz, 1H); ^{13}C NMR (50 MHz, D_2O) δ 39.9, 51.9, 170.7; ^{11}B NMR (96 MHz, D_2O) δ 30.1. HRMS: *m/z*: calcd for $C_{18}H_{21}N_3O_8SB$ with matrix 3-nitrobenzyl alcohol: 450.1142. Found 450.1144 $[M+H]^+$.

3.6. *N*,*N*′-Bis[(*tert*-butyloxy)carbonyl]-L-homocystine dimethyl ester 2a

Thionyl chloride (0.7 mL, 9.31 mmol) was added dropwise with stirring to 15 mL of cold methanol. L-Homocystine (1.0 g, 3.7 mmol) was added and the reaction mixture was refluxed overnight. After evaporation of the reaction mixture in vacuo, a white solid (1.3 g, 95%) was obtained. L-Homocystine dimethyl ester dihydrochloride was used directly in the next step without further purification. ^1H NMR (200 MHz, D_2O) δ 2.30 (m, 2H), 2.79 (t, $J = 6.9$ Hz, 2H), 3.76 (s, 3H), 4.22 (t, $J = 6.5$ Hz, 1H). L-Homocystine dimethyl ester dihydrochloride (1.3 g, 3.52 mmol) was dissolved in dioxane/water (2:1, 12 mL). Sodium carbonate (0.8 g, 7.54 mmol) in water (4 mL) was added to the stirred solution and the mixture was cooled to 0–5 °C. Di-*tert*-butyl pyrocarbonate (1.9 g, 8.7 mmol) was added in one portion and the mixture was stirred at room temperature for 18 h. The mixture was concentrated to about 3 mL in vacuo. The precipitate was filtered off and resuspended in hot ethanol (12 mL). Insoluble particles were filtered off and the filtrate was kept at 0–5 °C for 18 h. The resulting colorless crystals were collected by filtration and dried in vacuo (1.43 g, 82%). Mp 100–102 °C; $[\alpha]_{589}^{20}$ +26.7 (*c* 0.52, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.48 (s, 18H), 1.88–2.15 (m, 4H), 2.75 (t, $J = 7.6$ Hz, 4H), 3.80 (s, 6H), 4.43 (m, 2H), 5.15 (br s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.7, 33.0, 34.9, 52.8, 52.9, 80.1, 156.5, 173.0. Anal. Calcd for $C_{20}H_{36}N_2O_8S_2$: C, 48.37; H, 7.31; N, 5.64. Found: C, 48.52; H, 7.35; N, 5.74.

3.7. *N*-[(*tert*-Butyloxy)carbonyl]-L-homocysteine methyl ester 2b

N,*N*′-bis[(*tert*-butyloxy)carbonyl]-L-homocystine dimethyl ester **2a** (1.26 g, 2.54 mmol), triphenylphosphine (0.7 g, 2.67 mmol), and sodium acetate (82 mg, 1 mmol) were suspended in a mixture of 9.8 mL of methanol, 4.7 mL of water, and 81 μL of glacial acetic acid and heated under reflux for 30 min. The mixture was diluted with 120 mL CH_2Cl_2, washed with 50 mL of water, 25 mL of brine, and dried over $MgSO_4$. The solvent was removed in vacuo yielding 2.0 g of a clear oil, which was purified by column chromatography on silica gel (ethyl acetate/heptane 3:7) to give **2b** (1.2 g, 95%) as a colorless oil. $[\alpha]_D^{20}$ +19.4 (*c* 1.5, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.65 (s, 9H), 2.00 (m, 1H), 2.20 (m, 1H), 2.78 (t, $J = 7.9$ Hz, 2H), 3.80 (s, 3H), 4.67 (m, 1H), 5.40 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.0, 28.6, 37.5, 52.6, 52.8, 80.4, 155.8, 173.2. Anal. Calcd for $C_{10}H_{19}NO_4S$: C, 48.17; H, 7.68; N, 5.62. Found: C, 48.30; H, 7.75; N, 5.71.

3.8. *N*-[(*tert*-Butyloxy)carbonyl)]-*S*-[(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methyl]-L-homocysteine methyl ester 2c

To a suspension of NaH (96 mg, 2.40 mmol) in THF (4 mL), 2-(bromomethyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.48 g, 2.16 mmol) in THF (3.5 mL) was added under N_2. *N*-[(*tert*-butyloxy)carbonyl]-L-homocysteine methyl ester **2b** (0.54 g, 2.16 mmol) in THF (4 mL) was added dropwise at 0 °C. The mixture was warmed to room temperature, stirred for 2 h, and quenched by the addition of H_2O (15 mL). THF was evaporated and the residue extracted with ethyl acetate

(3 × 15 mL). The organic phase was washed with brine (15 mL), dried, and evaporated. Flash column chromatography on silica gel (ethyl acetate/heptane 2:8) gave pure **2c** (0.76 g, 90%) as a colorless oil. $[\alpha]_D^{20}$ +7.6 (*c* 7.2, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.29 (s, 12H), 1.45 (s, 9H), 1.97 (s, 2H), 2.15 (m, 2H), 2.60 (t, J = 7.6 Hz, 2H), 3.75 (s, 3H), 4.35 (m, 1H), 5.20 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.6, 27.3, 29.0, 31.0, 51.4, 51.9, 81.9, 83.0, 154.3, 171.9; ^{11}B NMR (96 MHz, D_2O) δ 31.8. Anal. Calcd for $C_{17}H_{32}BNO_6S$: C, 52.45; H, 8.28; N, 3.60. Found: C, 52.48; H, 8.29; N, 3.63.

3.9. *S*-[(Dihydroxyboryl)methyl]-L-homocysteine 2

A mixture of **2c** (0.60 g, 1.56 mmol) and 1 M aq NaOH (15 mL) was stirred at 45–50 °C for 2 h and the pH of the mixture was adjusted to 1 with aqueous 3 M HCl. After being stirred at ambient temperature for 12 h, phenylboric acid (1.56 mmol) and ether (15 mL) were added. After 3 h at room temperature, the ether layer was removed and replaced by fresh ether (15 mL). This treatment was repeated two-fold and the aqueous phase was concentrated and dried under high vacuum. After silica gel chromatography ($CHCl_3$/MeOH/NH_3/H_2O 6:6:0.5:1.5), compound **2** was obtained (100 mg, 30%) as an hygroscopic colorless solid. $[\alpha]_D^{20}$ −11.7 (*c* 0.48, H_2O); ^1H NMR (200 MHz, D_2O + DCl) δ 1.85 (s, 2H), 2.02 (m, 2H), 2.51 (t, J = 7.6 Hz, 2H), 3.72 (dd, J = 7.1, 5.5 Hz, 1H); ^{13}C NMR (50 MHz, D_2O) δ 29.1, 54.2, 54.6, 175.2; ^{11}B NMR (96 MHz, D_2O) δ 29.5; HRMS: *m*/*z*: calcd for $C_{19}H_{23}N_3O_8SB$ with matrix 3-nitrobenzyl alcohol: 464.1299. Found 464.1295 $[M+H]^+$.

3.10. *S*-[2-(Dihydroxyboryl)ethyl]-D/L-homocysteine 3

Di-(*n*-butyl)-vinylboronate[24] (0.37 g, 2.0 mmol) and D/L-homocysteine (0.345 g, 2.0 mmol) were mixed in MeOH (5 mL) and water (3 mL) under an inert atmosphere. AIBN (40 mg) was added and the mixture was heated at 80 °C for 1 h. Twenty milligram of AIBN were further added and the mixture was heated for 6 h at 80 °C. The solvents were evaporated and the ammonium salt of (dihydroxyboryl)ethyl-D/L-homocysteine **3** was obtained after silica gel chromatography ($CHCl_3$/MeOH/NH_3/H_2O 1:4:0.5:1) as an hygroscopic colorless solid (245 mg, 55%); ^1H NMR (200 MHz, D_2O + DCl) δ 1.12 (t, J = 7.9 Hz, 2H), 2.15 (m, 2H), 2.69 (m, 4H), 3.82 (t, J = 5.6 Hz, 1H); ^{13}C NMR (50 MHz, D_2O) δ 15.2 (br s), 26.4, 26.5, 29.6, 51.9, 171.7; ^{11}B NMR (96 MHz, D_2O) δ 30.2; HRMS: *m*/*z*: calcd for $C_{20}H_{25}O_8N_3SB$ with matrix 3-nitrobenzyl alcohol: 478.1343. Found 478.1462 $[M+H^+]$.

3.11. *S*-[2-Methyl-2-(dihydroxyboryl)ethyl]-L-cysteine 4

Reaction of di-(*n*-butyl)-2-propeneboronate[24] (0.435 g, 2.2 mmol) and L-cysteine (0.270 g, 2.2 mmol) in MeOH (4 mL) and water (3 mL) following the protocol previously described for compound **3** yielded S-[2-methyl-2-(dihydroxyboryl)ethyl]-L-cysteine **4** as a white powder (210 mg, 42%). ^1H NMR (250 MHz, D_2O) δ 1.10 (d, J = 7.2 Hz, 3H), 1.36–1.48 (m, 1H), 2.66–2.77 (m, 2H), 3.07–3.23 (m, 2H), 3.98 (m, 1H); ^{13}C NMR (50 MHz, D_2O + DCl) δ 14.6, 20.6 (br s), 31.5, 35.6, 52.8, 170.8; ^{11}B NMR (96 MHz, D_2O) δ 31.3; HRMS: *m*/*z*: calcd for $C_{20}H_{25}O_8N_3SB$ with matrix 3-nitrobenzyl alcohol: 478.1343. Found: 478.1462 $[M+H^+]$.

3.12. *S*-[2-(Dihydroxyboryl)ethyl]-D-penicillamine 5

Reaction of di-(*n*-butyl)-vinylboronate (0.37 g, 2.0 mmol) and D-penicillamine (0.340 g, 2.1 mmol) following the protocol described for compound **3** provided *S*-[2-(dihydroxyboryl)ethyl]-penicillamine **5** as an hygroscopic white solid (165 mg, 35%). ^1H NMR (300 MHz, D_2O + DCl) δ 0.95 (t, J = 7.8 Hz, 2H), 1.25 (s, 3H), 1.44 (s, 3H), 3.62 (m, 2 H), 3.98 (s, 1H); ^{13}C NMR (50 MHz, D_2O + DCl) δ 23.2, 23.9, 26.8, 46.7, 53.6, 169.5; ^{11}B NMR (96 MHz, D_2O) δ 31.4; HRMS: *m*/*z*: calcd for $C_{21}H_{27}O_8N_3SB$ with matrix 3-nitrobenzyl alcohol: 492.1612. Found 492.1610 $[M+H^+]$.

3.13. Methyl (*E*/*Z*)-2-(benzyloxycarbonylamino)-3-[3-(5,5-dimethyl-1,3,2-dioxaboran-2-yl)phenyl] acrylate 7a

To a stirred solution of *N*-(benzyloxycarbonyl)phosphonoglycine ester (1.38 g, 4.16 mmol) in anhydrous CH_2Cl_2 (5 mL) was added DBU (0.57 mL, 3.78 mmol). After 10 min at room temperature, a solution of 2-(3-formylphenyl)-5,5-dimethyl-1,3,2-dioxaborane (0.8 g, 3.65 mmol) in anhydrous CH_2Cl_2 (6 mL) was added dropwise to the mixture and stirring was continued for 3 h. Then, the solution was diluted with CH_2Cl_2 (75 mL), washed with 0.5 M aq H_2SO_4 (20 mL) and then with H_2O (4 × 40 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by silica gel chromatography (ethyl acetate/heptane 3:7) to give a *E*/*Z* mixture of **7a** (0.97 g, 55%, *E*/*Z* = 30/70) as a colorless solid. ^1H NMR (200 MHz, $CDCl_3$) δ 1.06 (s, 6H), 3.65 (s, 0.9H), 3.71 (s, 1.1H), 3.79 (s, 2.9H), 3.85 (s, 2.1H), 5.17 (s, 1.4H), 5.22 (s, 0.6H), 6.43 (br s, 0.7H), 7.05 (br s, 0.3H), 7.33–8.00 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.3, 22.4, 32.3, 53.0, 53.8, 67.8, 68.1, 72.7, 124.4, 128.3, 128.6, 128.8, 128.9, 129.0, 131.9, 132.7, 133.2, 135.4, 136.3, 136.4, 154.3, 154.5, 166.2, 166.8. Anal. Calcd for $C_{23}H_{26}BNO_6$: C, 65.27; H, 6.19; N, 3.31. Found: C, 65.21; H, 6.37; N, 3.46.

3.14. Methyl 2-amino-3-[3-(5,5-dimethyl-1,3,2-dioxaboran-2-yl)phenyl]propionate 7b

To a stirred solution of (*E*/*Z*)-**7a** (0.67 g, 1.58 mmol) in anhydrous MeOH/CH_2Cl_2 (2:1, 18 mL) was added 10% Pd–C (35 mg). After 21 h under 5 atm of hydrogen, the mixture was passed through a silica plug and the filtrate was concentrated in vacuo to give **7b** (0.45 g, 98%) as a colorless oil. The product was used in the next step without further purification. ^1H NMR (200 MHz, $CDCl_3$) δ 1.05 (s, 6H), 2.96 (dd, J = 7.8, 13.6 Hz, 1H), 3.20 (dd, J = 5.0, 13.6 Hz, 1H), 3.76 (s, 3H), 3.79 (s, 4H), 3.94 (m, 1H), 7.28–7.35 (m, 2H), 7.62–7.78 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.3, 32.3, 40.6, 52.6, 56.0, 72.7, 128.3, 132.1, 133.0, 135.1, 136.0, 174.6; ^{11}B NMR (96 MHz, $CDCl_3$) δ 29.1.

3.15. 3-Dihydroxyboryl-D/L-phenylalanine 7

A mixture of **7b** (0.45 g, 1.55 mmol) and 1 M aq NaOH (6 mL) was stirred at 45–50 °C for 2 h and then, the pH was adjusted to 1–2 by addition of 3 M aq HCl to the cooled (0 °C) solution. After stirring at ambient temperature for 12 h, phenylboric acid (1.55 mmol) and ether (14 mL) were added. After 3 h at room temperature, the ether layer was removed and replaced by fresh ether (14 mL). This treatment was repeated two-fold and the resulting aqueous phase was washed with ether. The aqueous solution was concentrated and dried under high vacuum. After silica gel chromatography ($CHCl_3$/MeOH/NH_3/H_2O 6:6:0.5:1.5), compound **7** was obtained (257 mg, 73%) as a colorless solid. Mp 128–130 °C; ^{1}H NMR (200 MHz, D_2O + DCl) δ 3.01 (dd, J = 7.6, 14.6 Hz, 1H), 3.17 (dd, J = 5.5, 14.6 Hz, 1H), 3.87 (dd, J = 5.5, 7.6 Hz, 1H), 7.23–7.40 (m, 2H), 7.45–7.62 (m, 2H); ^{13}C NMR (50 MHz, D_2O + DCl) δ 36.7, 56.3, 128.9, 132.0, 133.0, 134.7, 174.3; ^{11}B NMR (96 MHz, D_2O + DCl) δ 29.2. HRMS: *m/z*: calcd for $C_{23}H_{23}N_3O_8B$ with matrix 3-nitrobenzyl alcohol: 480.1578. Found 480.1584 $[M+H]^+$.

3.16. Biological assays

Rat liver arginase was purified from male Sprague–Dawley rats using an Amicon-Green dye ligand media as previously described.[16] Assays quantitated the [^{14}C]urea produced from [Guanido-^{14}C]L-arginine following a usual protocol.[27,28] A typical assay was performed in a total volume of 100 μL. Increasing concentrations of the tested compounds and RLA were preincubated for 10 min at room temperature in 0.2 M Tris buffer pH 7.4 or 9.0 and the assays were initiated by the addition of L-arginine (final concentration 10 mM) and 10^6 cpm [Guanido-^{14}C]L-arginine. The reactions were stopped after 10 min at 37 °C by the addition of 150 μL of a stop-buffer containing 0.25 M acetic acid and 7 M urea. [^{14}C]Urea was separated from unreacted [Guanido-^{14}C]L-arginine by mixing with 250 μL of a 1:1 v/v slurry of Dowex (H^+ form) in stop-buffer and centrifugation of the mixture. [^{14}C]Urea was quantitated by mixing 200 μL aliquots of the supernatants to 2 mL of Pico-Fluor 40 scintillation cocktail and counting in a Packard 2100 scintillation counter.

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