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Improved and High Yield Synthesis of the Potent Arginase Inhibitor: 2(*S*)-Amino-6-boronohexanoic Acid

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Abstract:

A simple three-step synthesis of the potent arginase inhibitor 2(*S*)-amino-6-boronohexanoic acid (ABH) has been developed. The key step was alkylation of the Ni^{II} complex of the Schiff base derived from glycine and (*S*)-2-[*N*′-(*N*-benzylprolyl)amino]-benzophenone (BPB) with pinacol 4-bromobutylboronate. Acidic hydrolysis afforded ABH in 50% overall yield, high enantiomeric excess, and quantitative recovery of the chiral auxiliary.

Introduction

In mammalian cells, L-arginine is metabolized by two major pathways: arginases catalyze its hydrolysis to L-ornithine and urea,[1] whereas NO synthases (NOSs) catalyze its oxidation to L-citrulline and nitric oxide, NO.[2,3] NO is an important biological molecule involved in vasodilation, neurotransmission, and immune responses.[4] Recent studies support the hypothesis that arginases may be essential in the regulation of NOSs activities by modulating local L-arginine concentration, and it was observed that inhibition of arginases led to an enhancement of NO production.[5−8] The search for selective and potent inhibitors of arginases thus became the focus of active studies.[9−12]

L-Arginine hydrolysis by arginases is achieved by a metal-activated water molecule that bridges a $(Mn^{II})_2$ cluster at their active site.[13] The hydrolysis is postulated to proceed through a tetrahedral intermediate resulting from nucleophilic attack of a $(Mn^{II})_2$-bound hydroxide ion at the guanidinium carbon of L-arginine. This proposal resulted in the synthesis of the first boronic analogue of L-arginine, 2(*S*)-amino-6-boronohexanoic acid (ABH), that acts as a very potent (inhibitory constant = 0.7 μM) and slow-binding inhibitor of arginases.[14] To date, only two syntheses of ABH have been described in the literature. The first one used 2(S)-*N*-(*tert*-butyloxycarbonyl)glutamic acid *tert*-butyl ester as starting material and furnished ABH in 1.3% overall yield after eight steps.[14] The second protocol incorporated the α-amino acid function by alkylation of the Ni^{II} complex of the Schiff base derived from glycine and (*S*)-2-[*N*′-(*N*-benzylprolyl)amino]benzophenone (BPB).[15] This general methodology, developed by Belokon, afforded good diastereoisomeric excess (de) under thermodynamic equilibrating conditions.[16,17] The following steps involved hydroboration, oxidation of the borane intermediate, and hydrolysis, resulting in an overall yield of 24% for ABH from 4-bromo-but-1-ene.[15] However, all these steps require tricky experimental conditions and high-cost reagents. We thus investigated alternative methodologies to gain a more straightforward access to this new important pharmacological tool. We herein report an improved and simpler synthesis of ABH from the recently commercially available catechol 4-bromo-butaneboronate, bromide **1**.

Results and Discussion

We previously used with success the general methodology of Belokon to prepare ABH.[15] Therefore, we tried to improve this methodology and discovered that we could gain a much easier access to ABH by direct alkylation of the BPB−Ni^{II} complex of glycine, **3**, with bromide **1**. Ligand BPB and BPB−Ni^{II}−glycine complex **3** are also commercially available and are easily synthesized in multigram amounts from cheap compounds following described procedures.[16] Preliminary experiments showed that simple alkylation of BPB−Ni^{II}−glycine complex **3** by bromide **1** led to untractable mixtures. Then, we prepared pinacol 4-bromo-butylboronate **2** in 85% yield after trans-esterification of **1** in the presence of excess pinacol in THF, as already described (Scheme 1).[18] To find the optimum conditions for the alkylation of **3** by

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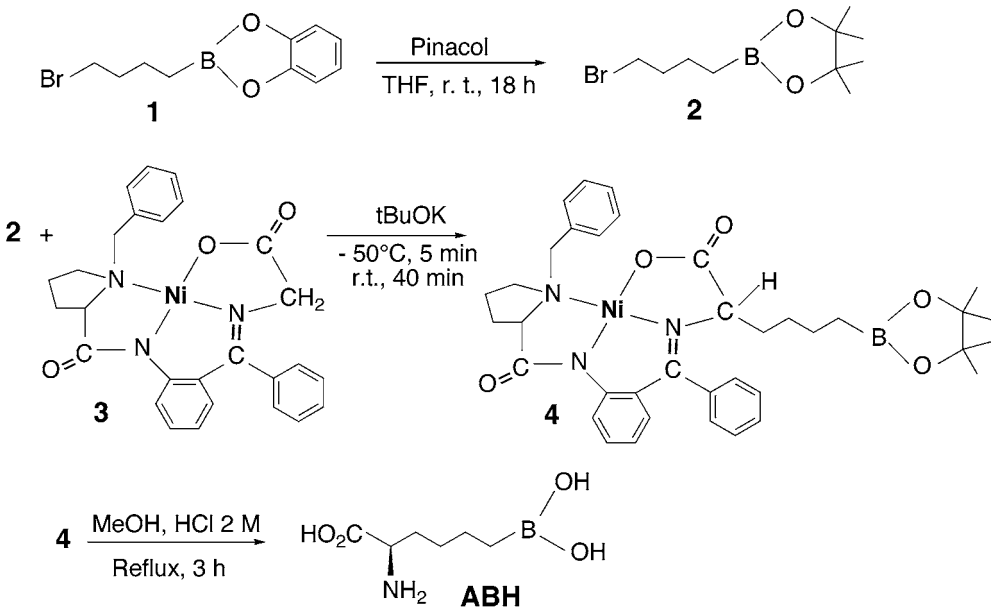
Scheme 1



bromide **2**, different conditions were tested. The highest yield (83%, after column chromatography) and diastereoselectivity (90/10, determined by ^{1}H NMR) for alkylated complex **4** were obtained by the addition at −50 °C of 3.0 equiv of *t*BuOK to a mixture of bromide **2** (1.25 equiv) and Ni^{II} complex **3** dissolved in THF, followed by stirring for 40 min at room temperature (Scheme 1). When reactions were performed in another solvent (DMF or CH_3CN), in the presence of another base (NaOH or NaH), for longer reaction times (up to 5 h), at higher temperature (up to 50°C), or using different ratios for bases, **2** and **3**, all resulted in lower yields and de for alkylated complex **4**. It has been described that such reactions are highly susceptible to thermodynamic factors as well as intramolecular interactions[17] and the precise factors that govern the selectivity of the reaction remain to be established in this case.

In some experiments, we prepared pinanediol ester of 4-bromobutaneboronic acid by trans-esterification of bromide **1** in the presence of pinanediol.[19] However, alkylation of **3** by this compound under the experimental conditions leading to the highest yields and de for complex **4** from bromide **2** led to many more byproducts and lower yields. The pinacol ester **2** was thus preferred for an improved synthesis of ABH.

Decomplexation of the alkylated glycine and deprotection of the boronic acid occurred simultaneously by refluxing purified complex **4** at 70 °C for 3 h in MeOH/2 M HCl (3:2).[15,16] ABH was then obtained in 50% overall yield from bromide **1** after ion-exchange chromatography (Scheme 1). The chiral auxiliary BPB was recovered and quantitatively recycled. Separation of the two enantiomers of ABH was performed by chiral HPLC. The S- to R-isomer ratio measured after hydrolysis of a 90/10 mixture of complex **4** was found to be 90/10. This indicated that no epimerisation had occurred during acid hydrolysis. Further recrystallization of purified complex **4** led to a ratio between the two diastereoisomers close to 98/2. Hydrolysis of this enriched complex **4** enhanced the enantiomeric excess (ee) of ABH from 80% to 97%. Racemic ABH, used as reference, was synthesized by alkylation of ethyl *N*-diphenylmethyleneglycinate by bromide **2** in the presence of *t*BuOK, following a well-known protocol.[20]

Different batches of ABH obtained with this new protocol were tested as inhibitors of purified rat liver arginase following well-established methods.[9,10] Their half-inhibitory concentration values were found identical to those of ABH synthesized following our previous protocol.[15]

In summary, this new synthesis of ABH highlights the usefulness of the glycine−BPB−Ni^{II} complex alkylation reaction for the synthesis of various α-amino acids. Only simple operational conditions are required. The high overall yield of ABH, the low cost of the reagents, and the recycling of BPB make this process a very practical alternative to the existing methods. It can be easily adapted to multigram synthesis and will allow further investigations of the pharmacological properties of ABH.

Experimental Section

Catechol 4-bromobutaneboronate **1**, (*S*)-*N*-benzylproline, (*S*)-2-[*N*′-(*N*-benzylprolyl)amino] benzophenone, and BPB−Ni^{II}−glycine complex, **3**, were obtained from Aldrich. All other reagents were obtained from Aldrich or Acros and were used without further purification. NMR spectra were recorded on a Bruker ARX 250 spectrometer, and chemical shifts δ are expressed in ppm relative to TMS as internal reference (^{1}H and ^{13}C) or external BF_3-OEt_2 (^{11}B). High-resolution mass spectrometry was performed at Ecole Normale Superieure (Paris) on a Nermag mass spectrometer. Satisfactory spectroscopic data (^{1}H and ^{13}C NMR and MS) have been obtained for all compounds synthesized in this study and previously described in the literature.[16,18,19]

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Alkylation of Complex 3 with Pinacol 4-Bromobutylboronate: Ni Complex 4. BPB−Ni^{II}−glycine complex **3** (2.00 g, 4.0 mmol) was dissolved in 100 mL of anhydrous THF, and the solution was kept at −50 °C under dry nitrogen. Finely powdered *t*BuOK (1.35 g, 12.0 mmol) and bromide **2** (1.40 g, 5.0 mmol) were added at −50 °C. The mixture was warmed and stirred at room temperature for 40 min. The reaction was stopped by the addition at 0 °C of 5 mL of acetic acid in 100 mL of water. The red product was then extracted with CH_2Cl_2 (4 × 150 mL) and dried over $MgSO_4$, and the solvent was removed in vacuo. Purification of the crude complex **4** by chromatography on silica gel (CH_2Cl_2−acetone, 3:1) furnished pure complex **4** as a red solid (2.25 g, 83%). Mp: 73−74°C (dec); ^{1}H NMR ($CDCl_3$) (major isomer): δ 0.76 (t, 2H, J = 8.0 Hz), 1.16 (s, 12H), 1.26−2.80 (m, 10H), 3.40−3.60 (m, 4H), 3.89 (t, 1H, J = 4.2 Hz), 4.42 (d, 1H, J = 12.6 Hz), 6.62 (m, 2H), 6.92 (d, 1H, J = 7.1 Hz), 7.10−7.35 (m, 4H), 7.45 (m, 4H), 8.01 (d, 2H, J = 7.2 Hz), 8.15 (d, 2H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ 11.2, 22.9, 23.8, 24.2, 25.0, 28.2, 29.5, 31.0, 35.1, 57.1, 63.3, 70.5, 70.7, 83.1, 120.9, 123.9, 126.7, 127.3, 127.8, 129.1, 129.9, 131.8, 132.2, 133.4, 133.5, 134.1, 142.5 (total 18C Ar), 170.5, 179.7, 180.6; HRMS (FAB^+, matrix magic bullet) Calcd for $C_{37}H_{45}O_5N_3BNi$ 680.2806, Found 680.2827. The assignment of de of alkylated complex **4** was based on a ^{1}H NMR spectrum by integration of the characteristic aromatic protons: major isomer δ 8.15 ppm, minor isomer δ 8.49 ppm. Diastereomeric ratio was found to be 90/10 for purified **4** and was increased to 97/3 after recrystallization in a CH_2Cl_2−acetone (3:1) mixture.

Preparation of ABH: Complex **4** (2.35 g, 3.45 mmol) was dissolved in 60 mL of CH_3OH, and the solution was added to 40 mL of 2 M HCl at 60 °C. The mixture was refluxed (70 °C) for 3 h, cooled to room temperature, and evaporated in vacuo. Water (30 mL) and concentrated NH_4OH were added up to pH 9−10. Chiral auxiliary BPB was quantitatively recovered by extraction with CH_2Cl_2. The aqueous layer was concentrated to dryness, and the residue was chromatographed on a cation-exchange resin (Dowex 50 × 8, H^+ form, elution with 2 M NH_4OH) to yield ABH as a white powder (ammonium salt, 500 mg, 83%). Mp: >250 °C, decomp; TLC (SiO_2) ^{n}BuOH−CH_3CO_2H−H_2O, 80:1:10, one single spot at R_f: 0.15, lit.[14] R_f 0.15; ^{1}H NMR (D_2O) δ 0.85 (t, 2H, J = 6.9 Hz), 1.46 (m, 4H), 1.93 (m, 2H), 3.75 (t, 1H, J = 6.1 Hz); ^{13}C NMR (D_2O) δ 16.5, 25.9, 29.6, 32.8, 57.4, 177.7. Further treatment with 1 M HCl and lyophilization quantitatively yielded the hydrochloride salt of ABH. Mp 150−152 °C, dec, lit.[14] 148−150°C, dec >150 °C; $[\alpha]^{25}_D$ +18.2° (*c* 0.7, H_2O); ^{1}H NMR (D_2O) δ 0.85 (t, 2H, J = 6.9 Hz), 1.48 (m, 4H), 1.98 (m, 2H), 4.09 (t, 1H, J = 6.3 Hz); ^{11}B NMR (D_2O) δ 32.3; HRMS (FAB^+, matrix 3-nitrobenzyl alcohol) Calcd for $C_{20}H_{25}O_8N_3B$ 446.1739, Found 446.1733. The ee of ABH obtained from different batches of alkylated complex **4** were evaluated by chiral RP-HPLC using a Chrompack column and water containing $HClO_4$ (pH 2.0) as solvent. The flow rate was 0.4 mL/min, and detection was done at 210 nm. Retention times for R- and S-isomers were 5.7 and 9.3 min, respectively.

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