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Synthesis of novel benzoxaborole-containing phenylalanine analogues

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ABSTRACT

The synthesis of novel benzoxaborole-containing phenylalanine analogues **2** and **3** has been developed. The key steps involve the preparation of appropriate precursors from the readily available amino acids and the formation of benzoxaborole ring directly in the corresponding amino acid fragment. The resulting compounds **2–3** show improved water solubility at physiological pH, suggesting their potential use as boron delivery agents for boron neutron capture therapy.

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p-Boronophenylalanine (BPA, **1** in Fig. 1) is a boronated amino acid which exhibits a specific affinity for tumor cells. Its ¹⁰B-enriched form has been employed in Boron Neutron Capture Therapy (BNCT) for treatments of patients affected by different types of cancer.¹ Upon irradiation with thermal neutrons, BPA absorbs neutrons and self-destructs to release lithium ion (⁷Li) and α -particles (⁴He) of high energy in tumor cells, leading to radiation-induced cell death. However, the clinical use of BPA is limited by its poor water solubility at physiological pH. There has been lack of new boron delivery agents to achieve effective neutron capture therapy of cancer.² To facilitate the delivery of BPA to tissue sites, the complex formation of BPA with fructose or mannitol has been proposed to produce a water soluble form of BPA for BNCT.³ Also, the continued development of new boron delivery agents, including boron-containing amino acids⁴ or BPA analogues with water-solubilizing groups,⁵ is the subject of extensive research. In addition, BPA-containing molecules may be used as potential biosensors⁶ or as chemical warheads⁷ for various biological applications. Furthermore, BPA and its derivatives have been widely employed as building blocks to construct biologically active molecules in drug discovery.⁸

Benzoxaboroles are boron-containing heterocycles that have emerged as a new class of potential therapeutics.^{9,10} Recently, several benzoxaborole-based compounds have entered human clinical trials for antifungal, anti-inflammatory, and antibacterial indications, exemplified by AN2690,^{9a,b} AN2728,^{9c} and AN3365

(GSK'052).^{9d} These benzoxaborole-containing molecules are metabolically stable and exhibit excellent drug-like properties. The benzoxaborole core has been shown to have a lower pK_a value (pK_a 7.2)¹¹ compared to that of phenylboronic acid (pK_a 8.9),¹² suggesting that it exists about 50% in anionic tetrahedral form at physiological pH and thus has an enhanced water solubility. Based on these considerations, we postulated that the incorporation of benzoxaborole in phenylalanine would result in a new class of potential boron delivery agents with improved physicochemical properties including water solubility. This Letter describes the synthesis of benzoxaborole-containing phenylalanine **2** and its close derivative with an additional oxygen linker **3** (Fig. 1).

Results and discussion

As shown in Figure 2, two different synthetic routes can be envisioned to prepare the target benzoxaboroles. The first route (route a) is based on carbon–carbon bond formation between the

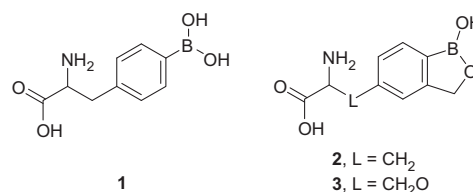


Figure 1. *p*-Boronophenylalanine **1** and benzoxaborole-containing phenylalanine analogues **2–3**.

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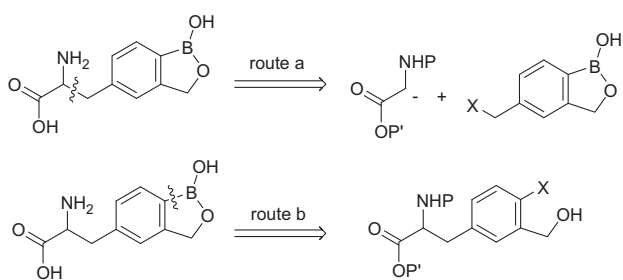
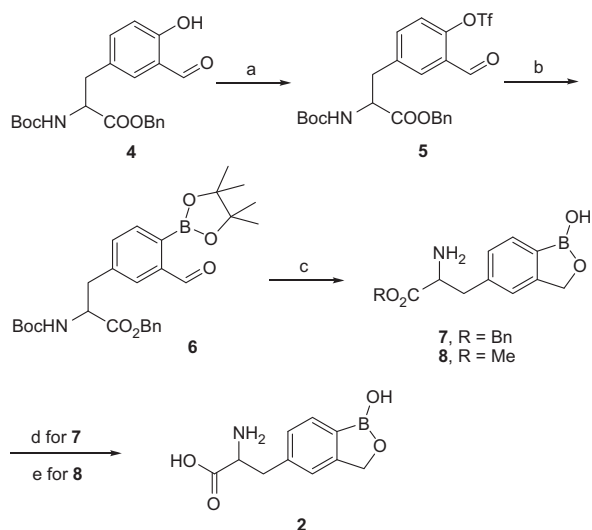


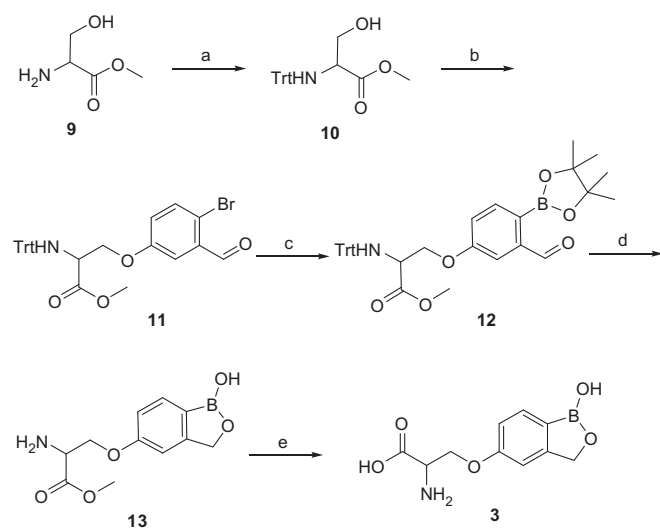
Figure 2. Two proposed approaches to prepare benzoxaborole-containing phenylalanine **2**.

synthetic equivalent of an amino acid and benzoxaborole-containing bromide or aldehyde fragment. The second route (route b) includes the introduction of benzoxaborole ring directly into the phenylalanine fragment. We chose the latter since this approach allows the use of readily available amino acids and the method developed with DL-amino acids can be directly extended to make corresponding chiral compounds. Also, this route introduces boron atom toward the end of synthesis and would involve minimized transformation of the ^{10}B -enriched product.

The synthesis of compound **2** is shown in Scheme 1. Hydroxybenzaldehyde **4**, prepared using Reimer-Tiemann reaction according to the reported procedure,¹³ was converted into its triflate derivative **5** in 80% yield by reaction with triflate chloride in dichloromethane. Without further purification, crude **5** was allowed to react with pinacol diboron in the presence of Pd(dppf)Cl₂¹⁴ to afford boronate **6** in 55% yield. Subsequently, formation of benzoxaborole ring was accomplished by using a one-pot procedure as previously described.¹⁵ This included the treatment of **6** with sodium borohydride in methanol to convert the aldehyde into hydroxymethyl group, which simultaneously cyclized to the adjacent boronate and subsequently hydrolyzed to form the corresponding benzoxaborole ring upon addition of aqueous hydrochloric acid. Boc protecting group was also removed in this step resulting in the desired benzyl ester **7** in 32% yield. Also methyl ester **8** was isolated in 39% yield due to transesterification in methanol under these reaction conditions. Combination of two



Scheme 1. Reagents and conditions: (a) Triflate chloride, DMAP, dichloromethane, 0 °C, 1 h, 80%; (b) pinacol diboron, Pd(dppf)Cl₂, KOAc, dioxane, N₂, 80 °C, 16 h, 55%; (c) NaBH₄, MeOH, rt, 2 h, then 6 N HCl, 12 h, 32% for **7**, 39% for **8**; (d) Pd/C, EtOH, H₂, 10 h, 89%; (e) LiOH, MeOH, H₂O, 30 min, rt, 64%.



Scheme 2. Reagents and conditions: (a) TrtCl, Et₃N, dichloromethane, addition over 1.5 h, then rt, 16 h, 100%; (b) 2-bromo-5-hydroxybenzaldehyde, dry toluene, PPh₃, DIAD, N₂, 0 °C, 30 min, then 60 °C, 16 h, 24%; (c) pinacol diboron, Pd(dppf)Cl₂, KOAc, dioxane, N₂, 80 °C, 16 h, 56%; (d) NaBH₄, MeOH, rt, 2 h, then 6 N HCl, 2 h, 79%; (e) LiOH, MeOH, H₂O, rt 10 min, 27%.

compounds **7** and **8** was accounted for the 71% yield. Hydrogenation of benzyl ester **7** or basic hydrolysis of methyl ester **8** afforded the desired compound **2** as a white solid.¹⁶

The synthesis of compound **3** was carried out using DL-serine methyl ester **9** according to Scheme 2. A key step in the synthesis was to convert the hydroxyl group of serine methyl ester into phenyl ether by Mitsunobu reaction. During our initial attempts, we found that the reaction of Boc or Cbz-protected serine ester with 2-bromo-5-hydroxybenzaldehyde in the presence of DIAD and PPh₃ failed to give any desired ether product. The β -elimination product was isolated instead, which is attributed to the fact that the relative acidity of the α -proton of the carbamate-protected serine ester causes its facile elimination under Mitsunobu conditions.¹⁷ Subsequently, compound **9** was protected with trityl group, a non-carbamate type protecting group, by reacting with trityl chloride in the presence of triethylamine to convert into ester **10**. Mitsunobu reaction of **10** and 2-bromo-5-hydroxybenzaldehyde in the presence of DIAD and PPh₃ afforded phenyl ether compound **11**. Full conversion of **10** to **11** was observed, and low isolated yield (24%) was due to compound loss during column purification and was not optimized. Compound **11** was reacted with pinacol diboron in the presence of Pd(dppf)Cl₂ to afford boronate **12** in 56% yield. Reduction of **12** with sodium borohydride in methanol converted the aldehyde into its hydroxymethyl group which simultaneously cyclized to the adjacent boronate and hydrolyzed to give benzoxaborole, upon addition of 6 N HCl. Under these conditions, trityl protecting group was also removed resulting in benzoxaborole **13** in 79% yield. Hydrolysis of methyl ester **13** gave the desired compound **3** as off white solid.¹⁸

The water solubility of **1–3** was determined in 0.1 M, pH 7.4 phosphate buffer.¹⁹ BPA **1** has a solubility of 1.7 mg/mL, which is consistent with the reported value (1.6 mg/mL).^{3a,5a} Compared to **1**, compounds **2** and **3** exhibit improved water solubility of 5.2 and 3.6 mg/mL, respectively. Their better solubility could be attributable to the lower pK_a of benzoxaborole since the benzoxaborole would exist in anionic tetrahedral form to a more significant extent at physiological pH. Compound **2** is more soluble than **3**. One potential explanation is that the presence of the electron-donating oxygen atom *para* to the benzoxaborole in **3** would increase its pK_a value and thus decrease its water solubility.

In summary, we have developed the first synthesis of novel benzoxaborole-containing phenylalanine analogues **2** and **3**. The key steps include the preparation of appropriate precursors from the readily available amino acids and the formation of benzoxaborole ring directly in the corresponding amino acid fragment. The resulting compounds **2–3** show improved water solubility at physiological pH, suggesting their potential use as boron delivery agents for boron neutron capture therapy.

Acknowledgments

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- 2-Amino-3-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yl)propanoic acid **2**: To a solution of compound **7** (550 mg, 1.77 mmol) in ethanol (20 ml) was added Pd/C (50 mg) portionwise. The solution was stirred at rt under H₂ atmosphere for 10 h. After the reaction was completed as indicated by HPLC, ethanol was evaporated in vacuo. The residue was purified by prep-HPLC (column: Luna 300 × 50.0 mm, 10 μm; mobile phase: [A-H₂O; B-MeCN + 0.1% TFA] B%: 0–10%, 20 min) to give compound **2** as white solid (350 mg, 89%). Alternatively, LiOH (252 mg, 6 mmol) in 5 mL water was added to a solution of compound **8** (500 mg, 2.12 mmol) in MeOH (20 mL) portionwise. The solution was stirred at rt for 30 min. After the reaction was completed as indicated by HPLC, the mixture was acidified to pH 5 with 2 N HCl, and MeOH was evaporated in vacuo. The residue was purified by prep-HPLC (column: Luna 300 × 50.0 mm, 10 μm; mobile phase: [A-H₂O; B-MeCN + 0.1% TFA] B%: 0–10%, 20 min) to give compound **2** as white solid (300 mg, 64%). ¹H NMR (400 MHz, MeOD) δ 7.65–7.67 (d, 1H, J = 7.6 Hz), 7.32 (s, 1H), 7.27–7.29 (d, 1H, J = 7.6 Hz), 5.07 (s, 2H), 4.20–4.23 (m, 1H), 3.35–3.40 (m, 1H), 3.16–3.21 (m, 1H); ¹³C NMR (400 MHz, MeOD) δ 170.27 (C=O), 154.91 (C), 137.56 (C), 130.51 (C), 128.03 (C), 121.84 (C), 70.75 (CH₂), 54.19 (CH₂), 36.35 (CH), carbon adjacent to boron was not observed²⁰; HRMS calcd for C₁₀H₁₃BNO₄ (M+H)⁺, 222.0938; found, 222.0938; HPLC purity: 99.4% (MaxPlot 190–370 nm), 99.4% (220 nm).
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- 2-Amino-3-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yloxy)propanoic acid **3**: To a solution of compound **13** (2 g, 8 mmol) in MeOH (60 mL) was added LiOH (1.3 g, 32 mmol) in 5 mL water portionwise. The solution was stirred at rt for 40 min. After the reaction was completed as indicated by HPLC, the mixture was acidified to pH 6–6.5 with 2 N HCl, to precipitate the product. The solid was collected by filtration and washed with methanol to give compound **3** as off white solid (515 mg, 27%). ¹H NMR: (400 MHz, MeOD): δ 7.59–7.61 (d, 1H, J = 8.0 Hz), 7.02 (s, 1H), 6.99–7.01 (d, 1H, J = 8.0 Hz), 5.03 (s, 2H), 4.43–4.53 (m, 2H), 4.36–4.38 (m, 1H); ¹³C NMR (400 MHz, MeOD) 169.50 (C=O), 161.91 (C), 157.98 (C), 132.79 (C), 116.08 (C), 107.69 (C), 72.10 (CH₂), 67.09 (CH₂), 54.07 (CH), carbon adjacent to boron was not observed²⁰; HRMS calcd for C₁₀H₁₃BNO₅ (M+H)⁺, 238.0887; found, 238.0889; HPLC purity: 98.3% (MaxPlot 190–370 nm), 98.5% (220 nm).
- Assay for solubility: Various amounts of compounds **1–3** were added to 0.1 M, pH 7.4 phosphate buffer. After vortex-mixing, the mixture was sonicated and incubated at room temperature for 24 h. If material is precipitated after incubation then the solubility is lower than the concentration assayed. If the solution becomes transparent, then solubility is equal to or higher than the concentration assayed. The solubility was calculated by dividing the amount of material by total volume of buffer in final transparent solution. Multiple replications of each experiment were carried out and the results were averaged.
- It has been reported that the carbon adjacent to boron is not observed in ¹³C NMR spectrum. For examples, see: (a) Adamczyk-Wozniak, A.; Madura, I.; Velders, A. H.; Sporzynski, A. *Tetrahedron Lett.* **2010**, *51*, 6181–6185; (b) Korner, C.; Starkov, P.; Sheppard, T. D. *J. Am. Chem. Soc.* **2010**, *132*, 5968–5969.