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Design and Synthesis of Boron-Containing Noncanonical Amino Acids With Enhanced Stability and Solubility

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ABSTRACT

The development of noncanonical amino acids (ncAAs) provides a powerful strategy to expand the chemical space of proteins beyond the natural repertoire, thereby overcoming intrinsic pharmacodynamic limitations of peptides and proteins. Among these, boron-containing ncAAs are of particular interest due to the versatile reactivity of boron and its proven therapeutic relevance in clinically approved drugs. However, poor aqueous solubility, instability under physiological conditions, and oxidative degradation have hindered their broader biological application. Here, we report the design and synthesis of a new class of boron-containing ncAAs with enhanced solubility and stability. Structural modifications around the boron center and optimized substituents were employed to improve compatibility with biological systems while retaining functional reactivity. Moreover, fluorescence analysis revealed distinct photophysical properties, indicating potential applications in protein engineering and biosensing. These results highlight the utility of cyclic boron architectures as a versatile platform for the development of boron-based amino acid analogs with broad implications in chemical biology, drug discovery, and biomolecular design.

1 | Introduction

The design and synthesis of noncanonical amino acids (ncAAs) have played crucial roles in drug discovery by addressing the inherent pharmacodynamic limitations of proteins [1] and peptides composed of the 20 canonical amino acids [2]. Natural peptides often exhibit poor absorption due to low permeability and limited metabolic stability, which restricts their utility in therapeutic applications [3–5]. To overcome these drawbacks, the incorporation of ncAAs has emerged as an effective strategy [2, 6]. Beyond improving drug-like properties, ncAAs offer unique opportunities to enhance structural complexity, modulate physicochemical parameters, and introduce novel functionalities inaccessible to the natural amino acid repertoire.

Among these, boron-containing amino acids represent a particularly intriguing class of ncAAs because of the versatile chemistry of boron and its relevance in medicinal chemistry [7–12] and enzymology [13, 14]. Boron atoms display distinctive electronic and structural features, including the ability to form reversible covalent bonds and interact with diols [15], nucleophiles [16], and other electron-rich species [17]. These properties enable boron-containing molecules to act as enzyme inhibitors [18, 19],

Eunsol Jo, Hyojung Ahn, Guldana Issabayeva, and On-Yu Kang equally contributed to this work.

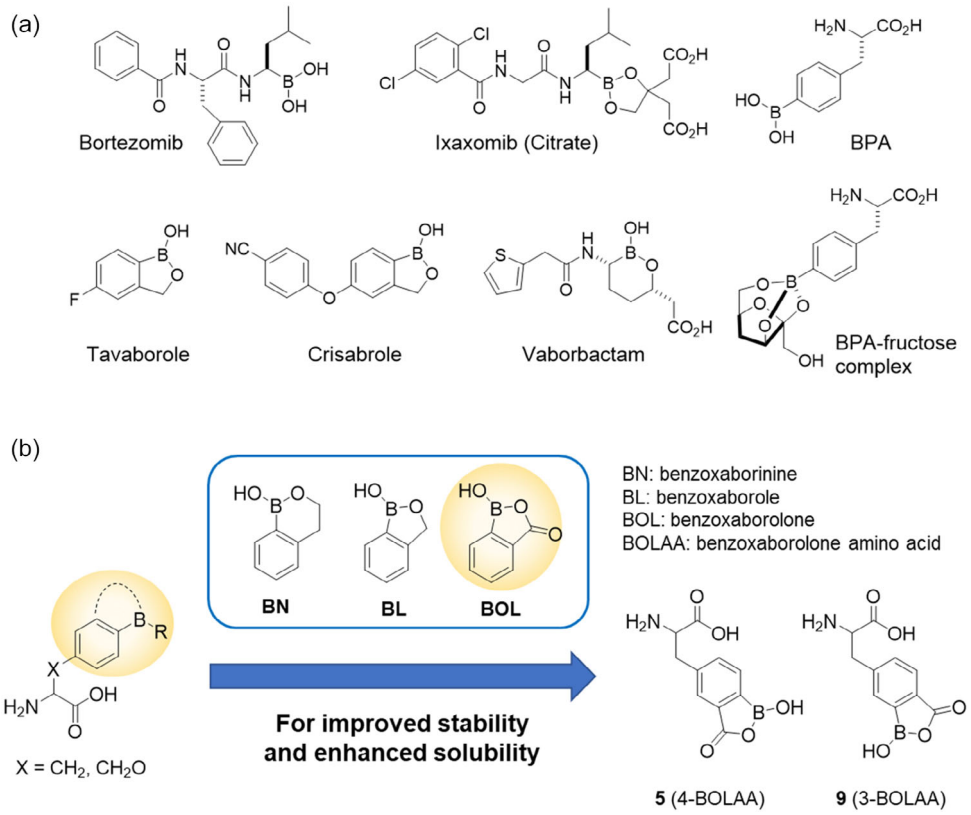
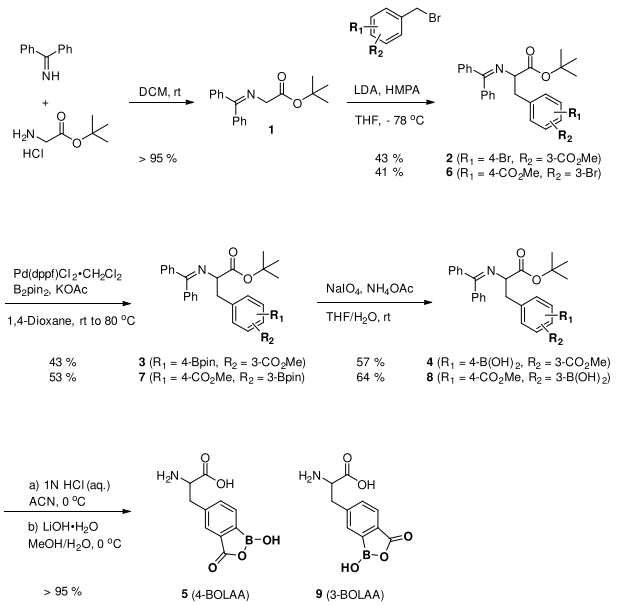


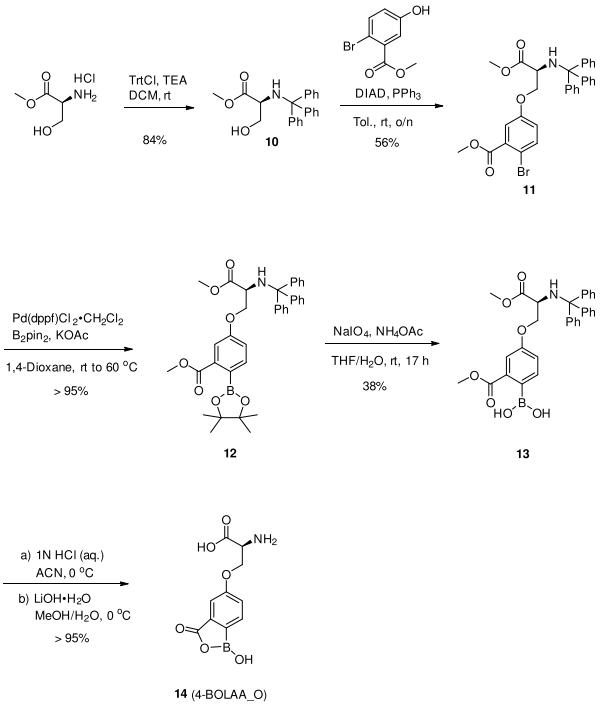
FIGURE 1 | FDA-approved boron-containing drugs and the concept of ncAA design.

chemical sensors [20], and a tool to modulate the function of proteins [21]. Notably, boronic acids and boronate esters are already employed in clinically approved drugs, such as Bortezomib [22] and Ixazomib [23], which function as proteasome inhibitors in cancer therapy [24, 25]. (Figure 1a).

Despite their promise, boron-containing ncAAs also present critical challenges that limit their broader use. Many boronic acid derivatives suffer from poor aqueous solubility, instability under physiological conditions, and susceptibility to oxidative degradation, collectively reducing their biological utility [26]. For example, boronophenylalanine (BPA) exhibits inferior solubility and stability compared to natural amino acids such as phenylalanine or tyrosine [27, 28]. Overcoming



SCHEME 1 | Synthetic scheme of **5** (4-BOLAA) and **9** (3-BOLAA).



SCHEME 2 | Synthetic scheme of **14** (4-BOLAA_O).

these limitations requires the development of new synthetic approaches that yield boron-containing ncAAs with improved stability, solubility, and compatibility with biological systems [29].

Recent advances in synthetic chemistry have explored strategies to stabilize boron moieties while preserving their functional reactivity. Approaches include steric protection of the boron center [30], design of cyclic boronates [31], and systematic tuning of substituents to enhance stability and aqueous compatibility [32]. Concurrently, progress in peptide synthesis and protein engineering provides opportunities to incorporate such improved boron-containing ncAAs into biomolecular frameworks, thereby enabling systematic evaluation of their structural and functional properties [33].

In this study, we report the synthesis of a new class of boron-containing ncAAs specifically designed to overcome the key limitations of solubility and stability (Figure 1b).

2 | Results and Discussion

To enhance the stability of boron-containing phenylalanine, it is essential to improve the robustness of the C—B bond through structural modification. Additionally, the designed structures could exhibit superior water solubility compared with BPA. We hypothesized that introducing cyclic boron-containing architectures would enable the development of next-generation boron-containing ncAAs.

Specifically, cyclic boron compounds exhibited a reduced rate of C—B bond dissociation relative to linear counterparts. This effect was especially pronounced in the boron oxalate-like (BOL) structure, which maintained its structural integrity even under strongly basic conditions, unlike alkyl-borates such as benzoxaborinine (BN) and benzoxaborole (BL) [32]. Such enhanced resistance to hydrolytic cleavage highlights the superior robustness of the BOL framework compared with other boron-based motifs, underscoring its potential

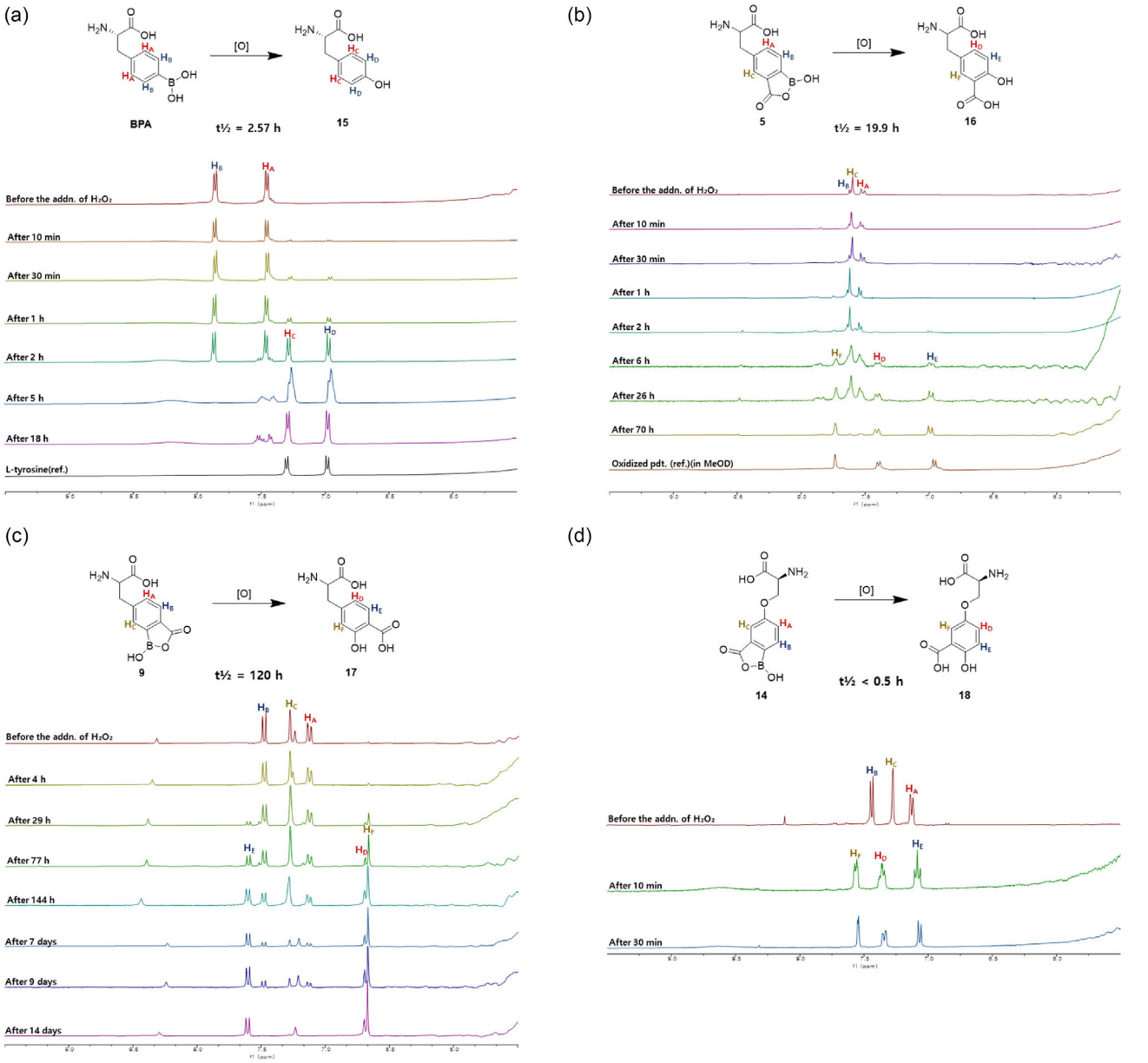


FIGURE 2 | Measurements of oxidative stability of BPA and BOLAAs by time-dependent ^{1}H NMR studies.

utility in applications demanding high stability across diverse environments.

The synthetic procedures for the designed structures were developed based on general schemes for the synthesis of unnatural amino acids. Using protected glycine **1** as a starting material, alkylation with substituted benzyl bromide afforded 4-bromo phenylalanine derivatives (**2** and **6**) in good yields (Scheme 1). The bromo group was subsequently converted into pinacolborane (**4** and **8**) by a Pd-catalyzed coupling reaction [17]. Hydrolysis, followed by cyclization under basic condition, gave the desired cyclic boron structures (4-BOLAA **5** and 3-BOLAA **9**) in good yields. The structures of the boron-containing molecules were assigned by proton, carbon, and boron NMR spectroscopy, which clearly identified the cyclic nature of the structures. For the oxygen-linked structure, protected L-serine **10** was used as a starting material (Scheme 2). *O*-arylation, followed by subsequent borylation, afforded the desired product **12** in good yields. Hydrolysis of the borane **12** and the resulting boric acid **13** was cyclized under basic condition to obtain **14** (4-BOLAA_O).

Using the designed structures, the oxidative stability of the three ncAAs and BPA was evaluated through time-dependent NMR studies. Under conditions similar to physiological environments, with hydrogen peroxide as the oxidant, the stability of ncAAs in water was assessed. Compared to BPA ($t^{1/2} = 2.57$ h), **5** (4-BOLAA) had more than seven times greater, as shown in Figure 2a–b. For **9** (3-BOLAA), the increase in stability was even more pronounced, as illustrated in Figure 2c. In contrast, the oxygen-linked **14** (4-BOLAA-O) showed poor stability under the same condition (Figure 2d). This suggests that the position and electronic properties of the boron atom play a key role in enhancing stability.

The water solubility of the two compounds was measured using ICP-OES, revealing that the newly synthesized BOL derivatives exhibited significantly higher solubility (approximately 12 g/L) compared to BPA (0.55 g/L). Both **9** (3-BOLAA) and **5** (4-BOLAA) showed similar solubility profiles (Table 1). The increased solubility of cyclic BOLAA derivatives compared to BPA can be attributed to the reduction of the inherent hydrophobicity of the boronic acid group in BPA, enhancing interactions with water. The cyclic structure likely decreases the extent of intramolecular interactions that are present in BPA, thereby facilitating better solvation and solubility in aqueous environments.

TABLE 1 | Aqueous solubilities of the boron-containing ncAAs.

Compound	Water solubility, g/L			Mean value, g/L
BPA	1.1	1.1	0.9	1.03 ± 0.12
5(4-BOLAA)	35.9	35.3	35.5	35.57 ± 0.31
9(3-BOLAA)	29.4	31.0	36.4	32.27 ± 3.65

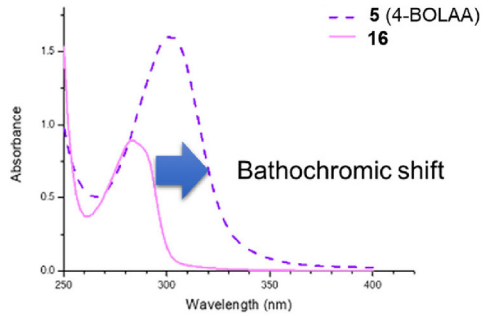


FIGURE 3 | UV spectra of 4-BOLAA and the deboronated compound **16**.

Furthermore, fluorescence analysis revealed bathochromic shifts in spectra of the boron-containing ncAA **5** (4-BOLAA) compared to their deboronated counterpart **16** (Figure 3), indicating potential applications in fluorescence-based assays and biosensing [32].

3 | Conclusion

In this study, we successfully developed a new class of boron-containing ncAAs that address the long-standing limitations of solubility and stability in conventional boron amino acid derivatives. By adopting a cyclic boron architecture, we demonstrated that C—B bond stability can be substantially improved, resulting in enhanced oxidative resistance and significantly higher water solubility than BPA. Our findings establish a robust design principle for the creation of boron-containing amino acids with improved biological compatibility, paving the way toward their broader use in therapeutic, diagnostic, and biomaterials applications. Applications of the developed boron-containing ncAAs for medicinal purposes are currently underway, and the results will be reported in due course.

4 | Experimental Section

4.1 | Chemistry

Commercially available reactants and solvents were used without additional purification. Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F_{254} glass plates precoated with a 0.2 mm thickness of silica gel. The TLC plates were visualized by shortwave (254 nm). Medium-pressure liquid chromatography (MPLC) was performed on CombiFlash NextGen 300+ apparatus using Buchi FlashPure EcoFlex silica cartridges with 50 μm particle size. Preparatory TLC was performed on Kieselgel 60 F_{254} glass plates precoated with a 1.0 mm thickness of silica gel. ^{1}H NMR spectra were obtained at 300 MHz, 400 MHz, or 500 MHz (Bruker). ^{13}C NMR spectra were acquired at 100 and 125 MHz (Bruker). ^{11}B NMR spectra were obtained at 160 MHz (Bruker). Liquid chromatography mass spectrometry (LCMS) with an electrospray ionization (ESI) method was used to obtain mass spectra. High-resolution mass spectra (HRMS) were recorded with a fast atom bombardment (FAB) using a sector field mass analyzer. Compound purity was measured using a Shimadzu Nexera lite HPLC system. Data acquisition and processing were performed using LabSolutions software.

The compounds 1, 2, 6, 10, and 11 were synthesized according to the reported procedure [34–36].

4.2 | General Procedure 1: Synthesis of Aryl Boronic Esters via Miyaura Borylation

To a round-bottom flask equipped with a stirring bar were added appropriate bromo benzoate (3.8 mmol, 1.0 eq.),

$Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ ([1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II), complex with dichloromethane) (0.76 mmol, 0.2 eq.), bis(pinacolato)diboron (5.7 mmol, 1.5 eq.), and potassium acetate (9.5 mmol, 2.5 eq.). The round-bottom flask was capped with a rubber septum, evacuated, and backfilled with Argon. The reactants were dissolved in anhydrous 1,4-dioxane at room temperature. The reaction mixture was heated up to 80°C and stirred overnight. After the reaction was completed, the resulting mixture was filtered through celite and washed with EtOAc. The filtrate was extracted with water. The organic layer was collected and dried over anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by MPLC (EtOAc /n-hexane, 1:4) to afford the target compound.

4.3 | General Procedure 2: Synthesis of Boronic Acid via Hydrolysis of Boronic Ester

To a solution of the requisite boronic ester, prepared according to General Procedure 1 (0.31 mmol, 1.0 eq.) in THF/H_2O (4:1) was added sodium (meta)periodate (1.40 mmol, 4.5 eq.) and ammonium acetate (0.93 mmol, 3.0 eq.) at 0°C. The reaction mixture was stirred at 30°C overnight. After the reaction was completed, the reaction mixture was quenched with water and was extracted with EtOAc. The organic layer was collected and dried using anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. Afterward, the crude product was purified by MPLC (MeOH/DCM, 1:9) to afford the target compound.

4.4 | General Procedure 3: Synthesis of BOLAA via Deprotection and Intramolecular Cyclization

To a solution of the requisite boronic acid, prepared according to General Procedure 2 (0.17 mmol, 1.0 eq.) in acetonitrile was added dropwise 1 N aqueous solution of HCl (0.85 mmol, 5.0 eq.) at 0°C. The reaction mixture was stirred at 0°C for 3 h. After the reaction was completed, the reaction solvent was evaporated under blower. The residue was dissolved in diethyl ether and extracted with water. The aqueous layer was collected, and water was removed under blower. Following that, the residue was dissolved in $MeOH/H_2O$ (2:1) and lithium hydroxide monohydrate ($LiOH{\cdot}H_2O$) (0.85 mmol, 5.0 eq.) was added to the above reaction mixture at 0°C. The reaction mixture was stirred at 0°C for 5 h. After the reaction was completed, the reaction solvent was evaporated under blower. The crude product obtained was dissolved in a minimum amount of MeOH and was purified by reverse prep TLC ($nBuOH/AcOH/H_2O$, 3:1:1) to afford the target compound.

4.5 | Synthesis of Methyl 5-(3-(tert-Butoxy)-2-((diphenylmethylene)amino)-3-Oxopropyl)-2-(4,4,5,5-Tetramethyl-1,3,2-Dioxaborolan-2-Yl) benzoate (3)

The desired product was synthesized according to General Procedure 1 using **2** (2.0 g, 3.8 mmol, 1.0 eq.), [1,1′-bis(diphenylphosphino) ferrocene] dichloropalladium(II) complex with dichloromethane (0.6 g, 0.76 mmol, 0.2 eq.), bis(pinacolato)diboron (1.4 g, 5.7 mmol, 1.5 eq.), and potassium acetate (0.90 g, 9.5 mmol, 2.5 eq.), affording the desired product in 43% yield (0.87 g, 1.6 mmol).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, J = 1.6 Hz, 1H), 7.60–7.53 (m, 2H), 7.40–7.27 (m, 7H), 7.25–7.21 (m, 1H), 6.67 (d, J = 6.8 Hz, 2H), 4.13 (dd, J = 8.5, 4.9 Hz, 1H), 3.84 (s, 3H), 3.28–3.16 (m, 2H), 1.45 (s, 9H), 1.39 (s, 12H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 173.51, 171.91, 169.59, 140.59, 140.28, 137.09, 134.68, 134.37, 133.14, 131.51, 131.16, 129.74, 129.71, 129.27, 128.95, 128.52, 85.30, 82.82, 79.32, 78.99, 78.67, 68.57, 52.77, 40.05, 28.30, 25.16, 25.13; ^{11}B NMR (160 MHz, MeOD-d_4) δ 31.91 (bs), 18.64 (s); HRMS (FAB) calcd. for C34H40BNO6 m/z: 569.2949, found m/z: 570.3028 [M+H]+.

4.6 | Synthesis of (4-(3-(tert-Butoxy)-2-((diphenyl Methylene)amino)-3-Oxopropyl)-2-(methoxy Carbonyl)phenyl)boronic Acid (4)

The desired product was synthesized according to General Procedure 2 using **3** (150 mg, 0.31 mmol, 1.0 eq.), sodium (meta)periodate (290 mg, 1.4 mmol, 4.5 eq.), and ammonium acetate (70 mg, 0.93 mmol, 3.0 eq.), affording the product in 57% yield (86 mg, 0.18 mmol).

^{1}H NMR (300 MHz, MeOD-d_4) δ 7.72 (d, J = 1.5 Hz, 1H), 7.52–7.44 (m, 2H), 7.44–7.21 (m, 8H), 6.64 (d, J = 7.3 Hz, 2H), 4.20–4.11 (m, 1H), 3.85 (s, 3H), 3.20 (dtd, J = 22.1, 13.3, 4.2 Hz, 2H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 173.39, 171.97, 169.67, 140.46, 139.92, 137.32, 135.31, 133.36, 131.76, 131.65, 131.34, 129.91, 129.83, 129.39, 129.09, 128.67, 85.38, 82.84, 68.82, 52.88, 40.12, 28.29, 25.19; ^{11}B NMR (160 MHz, MeOD-d_4) δ 30.31 (bs); LCMS (ESI): 488.29 [M+H]+.

4.7 | Synthesis of 2-Amino-3-(1-Hydroxy-3-Oxo-1,3-Dihydrobenzo[c [1, 2] Oxaborol-5-Yl)propanoic Acid (5)

The desired product was synthesized according to General Procedure 3 using **4** (83 mg, 0.17 mmol, 1.0 eq.), 1 N aqueous HCl (0.85 mL, 0.85 mmol, 5.0 eq.), and lithium hydroxide monohydrate ($LiOH{\cdot}H_2O$, 35 mg, 0.85 mmol, 5.0 eq.), affording the product in 95% yield (38 mg, 0.16 mmol).

^{1}H NMR (300 MHz, MeOD-d_4) δ 7.59 (s, 1H), 7.48 (d, J = 7.3 Hz, 1H), 7.41 (dd, J = 7.4, 1.5 Hz, 1H), 3.62 (dd, J = 9.1, 4.2 Hz, 1H), 3.27 (d, J = 4.3 Hz, 1H), 2.88 (dd, J = 14.0, 9.1 Hz, 1H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 179.05, 176.13, 160.06, 137.21, 136.69, 132.69, 128.87, 124.38, 57.10, 39.71; ^{11}B NMR (160 MHz, MeOD-d_4) δ 8.81 (bs); HRMS (FAB) calcd. for $C_{10}H_{10}BNO_5$ m/z: 235.0652, found m/z: 236.1215 $[M+H]^+$.

4.8 | Synthesis of Methyl 4-(3-(tert-Butoxy)-2-((diphenylmethylene)amino)-3-Oxopropyl)-2-(4,4,5,5-Tetramethyl-1,3,2-Dioxaborolan-2-Yl) benzoate (7)

The desired product was synthesized according to General Procedure 1 using **6** (1.5 g, 3.0 mmol, 1.0 equiv), [1,1′-bis (diphenylphosphino)ferrocene] dichloropalladium(II) complex with dichloromethane (0.49 g, 0.6 mmol, 0.2 equiv), bis(pinacolato) diboron (1.1 g, 4.5 mmol, 1.5 equiv), and potassium acetate (0.74 g, 7.5 mmol, 2.5 equiv), affording the product in 53% yield (0.91 g, 1.6 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 8.1 Hz, 1H), 7.59–7.53 (m, 2H), 7.37–7.27 (m, 6H), 7.19 (d, J = 1.7 Hz, 1H), 7.13 (dd, J = 8.0, 1.8 Hz, 1H), 6.69–6.60 (m, 2H), 4.11 (dd, J = 8.5, 5.1 Hz, 1H), 3.87 (s, 3H), 3.28–3.17 (m, 2H), 1.45 (s, 9H), 1.35 (d, J = 11.3 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.94, 170.66, 168.53, 142.83, 139.51, 136.39, 133.84, 131.46, 130.70, 130.25, 128.86, 128.83, 128.38, 128.33, 128.07, 127.73, 83.99, 81.50, 67.47, 52.29, 39.57, 28.19, 24.97, 24.93; ^{11}B NMR (160 MHz, MeOD-d_4) δ 30.15 (bs), 18.25 (s); LCMS (ESI): 570.10 $[M+H]^+$.

4.9 | Synthesis of (5-(3-(tert-Butoxy)-2-((diphenylmethylene)amino)-3-Oxopropyl)-2-(methoxycarbonyl)phenyl)boronic Acid (8)

The desired product was synthesized according to General Procedure 2 using 7 (0.57 g, 1.0 mmol, 1.0 eq.), sodium (meta) periodate (0.96 g, 4.5 mmol, 4.5 eq.), and ammonium acetate (0.23 g, 3.0 mmol, 3.0 eq.), affording the product in 64% yield (0.31 g, 0.64 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 8.2 Hz, 1H), 7.49 (ddt, J = 8.9, 3.1, 1.6 Hz, 2H), 7.34 (dd, J = 3.5, 1.8 Hz, 1H), 7.32–7.26 (m, 1H), 7.19 (s, 5H), 7.10 (tt, J = 7.3, 2.8 Hz, 1H), 6.69–6.53 (m, 2H), 4.17 (dt, J = 7.1, 3.8 Hz, 1H), 3.99 (t, J = 4.3 Hz, 3H), 3.31 (qd, J = 13.1, 8.2 Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.89, 170.80, 170.59, 143.50, 139.44, 137.67, 137.44, 136.27, 132.53, 131.53, 130.62, 130.36, 130.17, 128.88, 128.79, 128.54, 128.38, 128.31, 128.07, 127.99, 127.71, 127.59, 81.56, 67.32, 52.88, 39.62, 28.14; ^{11}B NMR (160 MHz, MeOD-d_4) δ 30.33 (bs); LCMS (ESI): 488.10 $[M+H]^+$.

4.10 | Synthesis of 2-Amino-3-(1-Hydroxy-3-Oxo-1,3-Dihydrobenzo[c [1, 2] Oxaborol-6-Yl)propanoic Acid (9)

The desired product was synthesized according to General Procedure 3 using 8 (97 mg, 0.20 mmol, 1.0 eq.), 1 N aqueous HCl (1.0 mL, 1.0 mmol, 5.0 eq.), and lithium hydroxide monohydrate ($LiOH{\cdot}H_2O$, 42 mg, 1.0 mmol, 5.0 eq.), affording the product in 95% yield (45 mg, 0.19 mmol).

^{1}H NMR (400 MHz, MeOD-d_4) δ 7.65 (d, J = 7.7 Hz, 1H), 7.48–7.39 (m, 1H), 7.27 (d, J = 7.8 Hz, 1H), 3.87 (dt, J = 9.3, 4.5 Hz, 1H), 3.49–3.39 (m, 1H), 3.09–2.98 (m, 1H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 177.35, 175.39, 140.91, 137.32, 130.86, 129.35, 125.56, 57.32, 38.50, 20.84; ^{11}B NMR (160 MHz, MeOD-d_4) δ 8.43 (bs); LCMS (ESI): 236.00 [M+H]+.

4.11 | Synthesis of Methyl (S)-5-(3-Methoxy-3-Oxo-2-(tritylamino)propoxy)-2-(4,4,5,5-Tetramethyl-1,3,2-Dioxaborolan-2-Yl)benzoate (12)

The desired product was synthesized according to General Procedure 1 using 11 (2.9 g, 5.1 mmol, 1.0 eq.), [1,1′-bis (diphenylphosphino)ferrocene] dichloropalladium(II) complex with dichloromethane (0.8 g, 1.0 mmol, 0.2 eq.), bis(pinacolato) diboron (1.6 g, 6.1 mmol, 1.5 eq.), and potassium acetate (1.3 g, 12.8 mmol, 2.5 eq.), affording the product in 95% yield (3.0 g, 4.8 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56–7.48 (m, 6H), 7.41 (dt, J = 4.9, 2.5 Hz, 2H), 7.26 (s, 6H), 7.18 (dt, J = 8.1, 4.0 Hz, 3H), 7.00 (dd, J = 8.2, 2.6 Hz, 1H), 4.28 (dt, J = 9.3, 3.2 Hz, 1H), 4.02 (dd, J = 9.2, 6.7 Hz, 1H), 3.90 (s, 3H), 3.71 (dt, J = 11.1, 5.5 Hz, 1H), 3.22 (s, 3H), 1.39 (s, 12H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 174.98, 169.74, 160.77, 147.12, 137.11, 135.14, 129.91, 128.97, 127.67, 119.40, 115.78, 85.30, 72.14, 71.37, 57.47, 52.97, 52.52, 25.18, 25.02; ^{11}B NMR (160 MHz, MeOD-d_4) δ 30.39 (bs), 18.56 (s); HRMS (FAB) calcd. for $C_{37}H_{40}BNO_7$ m/z: 621.2898, found m/z: 622.2989 $[M+H]^+$.

4.12 | Synthesis of (S)-(4-(3-Methoxy-3-Oxo-2-(tritylamino)propoxy)-2-(methoxycarbonyl)phenyl) boronic Acid (13)

The desired product was synthesized according to General Procedure 2 using 12 (2.9 g, 4.6 mmol, 1.0 eq.), sodium (meta) periodate (4.4 g, 20.7 mmol, 4.5 eq.), and ammonium acetate (1.0 g, 13.8 mmol, 3.0 eq.), affording the product in 38% yield (0.94 g, 1.8 mmol).

^{1}H NMR (300 MHz, MeOD-d_4) δ 7.54–7.47 (m, 6H), 7.33–7.12 (m, 12H), 4.28 (dd, J = 9.9, 5.0 Hz, 1H), 4.09 (dd, J = 9.9, 6.5 Hz, 1H), 3.92 (s, 3H), 3.69 (dd, J = 6.5, 5.0 Hz, 1H), 3.26 (s, 3H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 174.95, 169.43, 160.02, 147.09, 134.94, 133.15, 129.89, 128.96, 127.65, 120.68, 115.62, 72.12, 71.41, 57.48, 54.79, 53.05, 52.52; ^{11}B NMR (160 MHz, MeOD-d_4) δ 30.13 (bs), 18.42 (s); HRMS (FAB) calcd. for $C_{31}H_{30}BNO_7$ m/z: 539.2115, found m/z: 540.2153 [M+H]+.

4.13 | Synthesis of O-(1-Hydroxy-3-Oxo-1,3-Dihydrobenzo[c [1, 2] Oxaborol-5-Yl)-L-Serine (14)

The desired product was synthesized according to General Procedure 3 using 13 (0.8 g, 1.5 mmol, 1.0 eq.), 1 N aqueous HCl (15 mL, 15 mmol, 10 eq.), and lithium hydroxide monohydrate ($LiOH{\cdot}H_2O$, 0.3 g, 7.5 mmol, 5.0 eq.), affording the product in 95% yield (0.36 g, 1.43 mmol).

^{1}H NMR (400 MHz, MeOD-d_4) δ 7.44 (d, J = 7.9 Hz, 1H), 7.27 (d, J = 2.2 Hz, 1H), 7.12 (dd, J = 7.9, 2.3 Hz, 1H), 4.44 (d, J = 4.6 Hz, 2H), 4.18 (d, J = 4.5 Hz, 1H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 175.22, 159.28, 139.57, 131.06, 120.68, 110.00, 67.70, 54.99; ^{11}B NMR (160 MHz, MeOD-d_4) δ 18.55 (s), 8.85 (bs); LCMS (ESI): 252.10 $[M+H]^+$.

4.14 | Measurement of Oxidative Stability

A solution of the compound (0.1 M, 5 mL) in a DMSO-d_6/PBS mixture (1:4, v/v) was treated with hydrogen peroxide (H_2O_2, 30 wt%, 0.5 eq.) at 10–15°C in a water bath. At predetermined time intervals, aliquots (0.3 mL) were withdrawn and immediately quenched by addition to a preprepared solution of sodium bisulfite ($NaHSO_3$, 0.5 M in D_2O, 0.1 mL) in an NMR tube. The reaction was monitored by ^{1}H NMR spectroscopy.

4.15 | Measurement of Water Solubility

Precisely weighed samples of each compound (BPA, 3.0 mg; 5, 20.0 mg; 9, 3.0 mg) were suspended in water (45 μL) and stirred

at 25°C for 24 h. The suspensions were then centrifuged (10,000 rpm, 5 min) to remove any undissolved solids. The boron concentrations in the resulting supernatants were determined by ICP-OES (NFEC-2025-06−306 719) and ICP-MS (NFEC-2022-02−276 618).

4.16 | Measurement of Bathochromic Shift

A solution of the compound (4 mM, 5 mL) was prepared in a DMSO-d_6/PBS mixture (1:4, v/v). The UV–Vis absorption spectrum of the solution was recorded over the wavelength range of 250–400 nm at room temperature using a UV–Vis spectrophotometer. Subsequently, the solution was treated with hydrogen peroxide to induce a bathochromic shift, and the UV–Vis spectra were recorded again.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.