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NOVEL C_2 -SYMMETRICAL PHENYLBORONIC ACID PINACOL ESTERS WITH A FEW TYPES OF LINKERS AND THEIR BIOLOGICAL ACTIVITIES

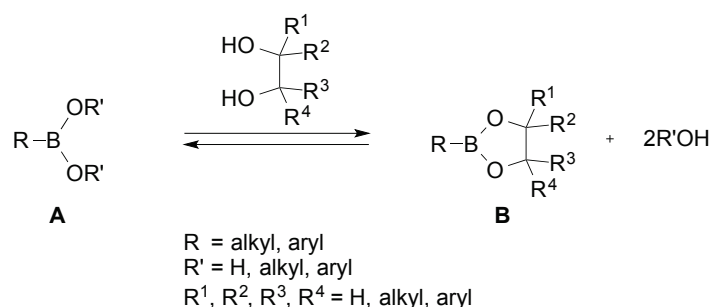
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Abstract – We report the preparation of new C_2 -symmetrical cyclic phenylboronic acid derivatives and their biological activities. New targeted C_2 -symmetrical molecules (**1**) were obtained in good to excellent yields by a primitive amide bond formation reaction using the reported method starting with amino-substituted phenylboronic acid pinacol esters **2**. A few C_2 -symmetrical phenylboronic acid ester analogues such as **5** and **7** that have two urea groups in the linker junction were also prepared in order to evaluate antibacterial and antiviral activities. The results of a structure-activity relationship study are also described.

Many bioactive C_2 -symmetrical bivalent molecules have been studied for the development of new agents to treat various infectious diseases or for new valuable ligands to treat diseases caused by dysfunctions of various receptors.¹⁻³ A multivalent molecule is generally expected to show enhanced biological potential compared to that of the corresponding monovalent molecule.⁴ On the other hand, much attention has recently been paid to the design of synthetic receptors and detectors for carbohydrates in order to find new lectin-like molecules for recognition of saccharides (sugar chains).⁵ We have reported a few molecules that have carbohydrate recognition properties for new bioactive leads.⁶⁻⁸ From this point of view, we have been interested in boronic acids and related derivatives because many boronic acids (**A**) have a property to react with various 1,2-diol functionalities such as sugars and form cyclic derivatives

(B) with reversible covalent bonds (see Scheme 1).^{9,10}



Scheme 1

We have already synthesized a few C_2 -symmetrical cyclic boronic acid esters as new targeted bivalent molecules.^{11,12} The bivalent C_2 -symmetrical phenylboronic acid derivatives (**1**) reported previously have considerably large molecular weights and belong to a new class of non-peptide mid-size molecules (Figure 1). Among the compounds synthesized previously, we found that a few derivatives ($n=6$ and 8) showed significant bioactivities such as antibacterial or antiviral activity [anti-herpes simplex virus type 1 activity (anti-HSV-1 activity)].¹¹ We considered that a similar flexible methylene linker and two cyclic phenylboronic acid pinacol esters with a *meta*-oriented amide group in C_2 -symmetrical molecules are important structural features of the molecules for expressing bioactivities. In order to investigate the structure-activity relationships (SARs) of this series in detail, we prepared two additional compounds **1bb** and **1bd** and evaluated their bioactivities to determine the most effective linker length for these C_2 -symmetrical phenylboronic acid pinacol esters.

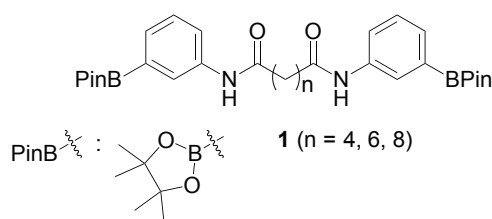
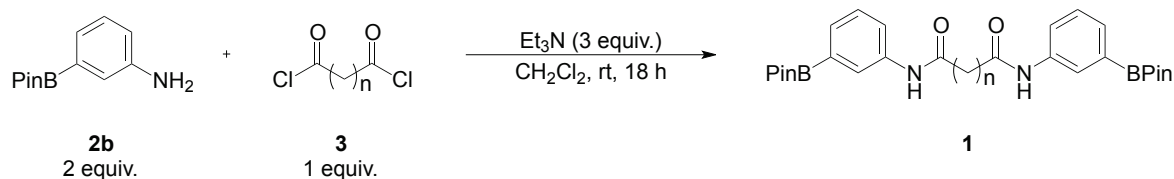


Figure 1

In this article, we describe the biological activities of synthesized bivalent C_2 -symmetrical phenylboronic acid derivatives and the results obtained for SARs of these C_2 -symmetrical molecules.

To begin with, we optimized the most preferable methylene length for the original structure **1** (see compounds **1ba**~**1be** in Table 1). Compounds **1bb** and **1bd** were prepared from the reaction of **2b** with pimeloyl chloride **3b** or azelaoyl chloride **3d** by a method similar to that reported previously (see EXPERIMENTAL). Compounds **1ba**~**1be** showed similar antibacterial activities against a Gram-positive strain [*Staphylococcus aureus* (*S. aureus*)]. However, none of the prepared compounds **1ba**~**1be** that had

Table 1. C_2 -Symmetrical Phenylboronic Acid Pinacol Esters and Their Biological Evaluation

| Entry | n | Yield (%) ^a | MIC [μM ($\mu\text{g/mL}$)] | | EC ₅₀ (μM) Anti-HSV-1 activity | CC ₅₀ (μM) Cytotoxic activity |
|-------|---|------------------------|---|---------------------------------|---|--|
| | | | <i>S. aureus</i> | <i>E. coli</i> | | |
| 1 | 4 | 82 ^b | $\geq 233.4^b$ ($\geq 128^b$) | $\geq 233.4^b$ ($\geq 128^b$) | >100 ^b | >200 ^b |
| 2 | 5 | 62 | 227.6 (128) | 227.6 (128) | 62.7 | >200 |
| 3 | 6 | 98 ^b | 111.1 ^b (64 ^b) | $\geq 222.1^b$ ($\geq 128^b$) | 38.6 ^b | >200 ^b |
| 4 | 7 | 67 | 27.1 (16) | 216.8 (128) | 8.0 | 25.2 |
| 5 | 8 | 85 ^b | $\geq 211.8^b$ ($\geq 128^b$) | $\geq 211.8^b$ ($\geq 128^b$) | 41.1 ^b | >200 ^b |

^a Isolated yield. ^b Data were taken from ref 11.

C4~8 methylene length linkers showed antibacterial activity against a Gram-negative strain [*Escherichia coli* (*E. coli*)]. The highest levels of both antibacterial activity [minimum inhibitory concentration (MIC)=27.1 μM] against a Gram-positive strain (*S. aureus*) and anti-HSV-1 activity [50% effective concentration (EC₅₀)=8.0 μM] were observed for compound **1bd**, which had a C7-methylene length as the linker. Compound **1bd** also showed the highest level of cytotoxic activity [50% cytotoxic concentration (CC₅₀)=25.2 μM], indicating that the most preferable linker is a C7-methylene length linker in the general structure **1** for these bioactivities. The selectivity index (SI) value (CC₅₀/EC₅₀) for compound **1bd**, unfortunately, was small (SI=ca. 3.15) for an antiviral compound. The contrasting results regarding antibacterial activities against two strains (Gram-positive and Gram-negative strains) may be caused by the different natures of specific carbohydrates containing glycoproteins, proteoglycans and glucolipids at the cell surface that are involved in the processes of bacterial growth of these strains, as well as various cell-to-cell communications. We consider that the distance between two boronic acid ester functionalities in the C_2 -symmetrical target molecules is important for the molecular recognition ability of the derivatives **1**. Selectivity regarding the sugar recognition property has already been shown in a previous investigation of bivalent C_2 -symmetrical boronic acid derivatives as glucose-selective sensors.¹⁰

Based on the above information, we then prepared two C_2 -type geometric phenylboronic acid pinacol esters **5ba** and **5bb** that have two urea groups in the linker junction of the molecules from the reactions of compound **2b** with diisocyanates **4** (see Table 2). The structures of these bivalent urea-type C_2 -symmetrical derivatives **5ba** and **5bb** were confirmed by both spectroscopic data and elemental analysis. IR spectra of these phenylboronic acid esters showed typical absorption bands at 3406~3370 cm^{-1} and 1651~1469 cm^{-1} ascribable to urea N-H and C=O groups, respectively. In both ¹H-NMR spectra,

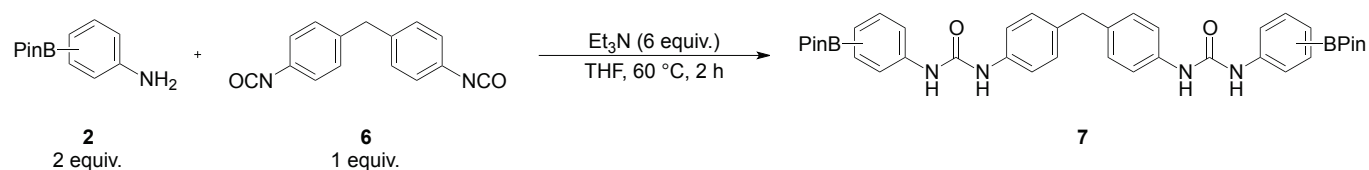
Table 2. Synthesis and Biological Activity of New C_2 -Symmetrical Phenylboronic Acid Pinacol Esters Having Two Urea Functionalities

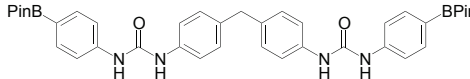
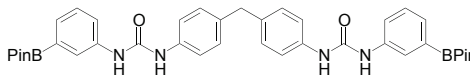
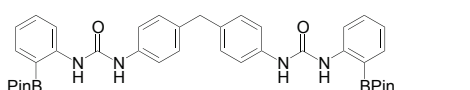
| Entry | Product 5 | Yield (%) ^a | MIC [μ M (μ g/mL)] | | EC ₅₀ (μ M) | CC ₅₀ (μ M) |
|-------|------------------|------------------------|------------------------------|----------------|-----------------------------|-----------------------------|
| | | | <i>S. aureus</i> | <i>E. coli</i> | Anti-HSV-1 activity | Cytotoxic activity |
| 6 | 5ba | 95 | 221.3 (128) | 221.3 (128) | >100 | >200 |
| 7 | 5bb | 95 | 105.5 (64) | 105.5 (64) | >100 | 141.2 |

^a Isolated yield.

four methyl groups on the phenylboronic acid pinacol ester were observed at δ 1.28 ppm as a sharp singlet, and NH protons of the two urea groups at δ 6.06-6.09 and 8.42-8.44 ppm were also observed as a triplet and a broad singlet, respectively. Other signals for aromatic protons and methylene protons in the linker moiety were in good agreement with represented structures **5** shown in Table 2 (see EXPERIMENTAL for detail). The C_2 -symmetrical structures of compounds **5ba** and **5bb** were confirmed by ^{13}C -NMR spectroscopic analysis. Both cyclic phenylboronic acid pinacol esters **5** displayed magnetically equivalent carbon signals assignable to half of the molecules that showed C_2 -symmetrical molecular structures in solution, except for the carbon in a phenyl ring connected to a substituent (boronic acid esters).¹³ The evidence obtained from FAB-MS spectroscopic (molecular ion) measurement and elemental analysis is consistent with the characteristic ^{13}C -NMR behavior (see EXPERIMENTAL for detail). As can be seen in Table 2, compound **5ba** showed no antibacterial activity (MIC \Rightarrow 200 μM) against two strains (Gram-positive and Gram-negative strains) and no anti-HSV-1 activity (EC \Rightarrow 100 μM). Compound **5ba** also showed no significant cytotoxic activity (CC₅₀ \Rightarrow 200 μM), indicating that the linker feature having a C4-methylene length linker in the urea-type C_2 -symmetrical structure **5** is not appropriate for these bioactivities. However, compound **5bb** showed moderate antibacterial activities (MIC=105.5 μM) against both strains (Gram-positive and Gram-negative strains) and no anti-HSV-1 activity (EC₅₀ \Rightarrow 100 μM). Compound **5bb** also showed a low level of cytotoxic activity (CC₅₀=141.2 μM) (see Table 2).

Since we have recently reported that the biphenyl methylene linker moiety is effective for expression of antibacterial activities in twin-drug type hydantoin derivatives,^{7,15} we also tried to prepare a few additional examples with a biphenyl methylene linker that has two urea groups connected with

Table 3. Synthesis and Biological Activity of a C_2 -Symmetrical Phenylboronic Acid Pinacol Ester Having a Biphenyl Methylene Linker with Two Urea Functionalities

| Entry | Product 7 | Yield (%) ^a | MIC [μM ($\mu\text{g/mL}$)] | | EC ₅₀ (μM) Anti-HSV-1 activity | CC ₅₀ (μM) Cytotoxic activity |
|-------|---|------------------------|---|----------------|---|--|
| | | | <i>S. aureus</i> | <i>E. coli</i> | | |
| 8 |  7a | 89 | >185.9 (>128) | >185.9 (>128) | >100 | 28.8 |
| 9 |  7b | 92 | 93.0 (64) | >185.9 (>128) | >100 | 178.1 |
| 10 |  7c | 93 | 93.0 (64) | 93.0 (64) | >100 | >200 |

^a Isolated yield.

phenylboronic acid ester groups (see compounds **7a**~**7c** in Table 3). The biphenyl methylene linker moiety in the molecules **7** is considered to be a more rigid structural framework than that of the flexible methylene linkers in the bivalent structure **1**. The targeted C_2 -symmetrical phenylboronic acid derivatives **7a**~**7c** with a biphenyl methylene linker were prepared from the reactions of methylenediphenyl 4,4'-diisocyanate **6** with amino-substituted phenylboronic acid ester derivatives **2** in excellent yields (see Table 3 and EXPERIMENTAL). As can be seen in Table 3, all of these additional C_2 -symmetrical phenylboronic acid ester derivatives **7a**~**7c** showed some kind of bioactivity: *para*-oriented derivative **7a** showed a high level of cytotoxic activity (CC₅₀=28.8 μM), *meta*-oriented derivative **7b** showed a significant antibacterial activity (MIC=64 μM) against only a Gram-positive strain and *ortho*-oriented derivative **7c** showed significant antibacterial activities against both strains (Gram-positive and Gram-negative strains) (MIC=64 μM and 64 μM , respectively). We consider that such delicate selective activity is attributable to the biphenyl methylene linker moiety as a rigid structural framework. Notably, compound **7a** showed a high level of cytotoxic activity (CC₅₀=28.8 μM) but no antibacterial or antiviral activity. Although additional experiments are needed, we now consider that this molecule may provide a new anticancer lead.

For further structural modifications of new types of symmetrical molecules of these multivalent phenylboronic esters, we consider that the results obtained provide valuable information regarding an efficient linker structure for bioactivities of phenylboronic acid ester-related series to find new leads or candidates.

EXPERIMENTAL

IR spectra were measured on a Shimadzu FT/IR-8100 spectrometer. ^1H - and ^{13}C -NMR spectra were obtained on a JEOL JNM ECZ600R at 25 °C. Chemical shifts are expressed in δ ppm relative to the solvent peaks for ^1H -NMR [dimethyl sulfoxide- d_6 (DMSO- d_6) (2.50 ppm)] and ^{13}C -NMR [DMSO- d_6 (39.50 ppm)]. The signal assignments were confirmed by ^1H - ^1H two-dimensional (2D) correlation spectroscopy (COSY), ^1H - ^{13}C heteronuclear multiple-quantum coherence (HMQC), and ^1H - ^{13}C heteronuclear multiple-bond connectivity (HMBC) spectra. High-resolution FAB-MS spectra [HRMS (FAB)] were obtained by a JEOL JMS-700T mass spectrometer.

Preparation of C_2 -Symmetrical Cyclic Phenylboronic Acid Derivatives.

N^1, N^7 -Bis(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)heptanediamide (1bb). A solution of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**2b**) (438.2 mg, 2.00 mmol) and CH_2Cl_2 (4.348 mL) was cooled to 4.0 °C, and then Et_3N (415.9 μL , 3.00 mmol) and pimeloyl chloride (**3b**) (157.7 μL , 1.00 mmol) were added to the resulting solution. The resulting mixture was stirred for 18 h at room temperature and then water (ca. 100 mL) was added. The obtained solution was extracted with AcOEt (x3) and the combined organic extract was dried over Na_2SO_4 . After filtration, the solvents that were used were evaporated under reduced pressure. The obtained crude material was washed with $\text{CH}_2\text{Cl}_2/n$ -hexane to give the desired product (**1bb**) (350.4 mg, 62% yield) as a white solid. Mp 198-203 °C. IR (KBr) 3465 (NH), 1661 cm^{-1} (C=O); FAB-MS (positive) m/z 563 (M+H) $^+$. HRMS (FAB) Calcd for $\text{C}_{31}\text{H}_{45}\text{B}_2\text{N}_2\text{O}_6^+$: m/z 563.3458 (M+H) $^+$. Found: 563.3466; ^1H -NMR (DMSO- d_6) δ 1.29 (24H, s, CH_3), 1.20-1.42 [2H, m, C(=O)-CH $_2$ -CH $_2$ -CH $_2$ -CH $_2$ -CH $_2$ -C(=O)], 1.52-1.70 [4H, m, C(=O)-CH $_2$ -CH $_2$ -CH $_2$ -CH $_2$ -C(=O)], 2.30 [4H, t, J = 6.3 Hz, C(=O)-CH $_2$ -CH $_2$ -CH $_2$ -CH $_2$ -CH $_2$ -C(=O)], 7.29 (2H, dd, J = 6.9, 7.8 Hz, Ar H-5), 7.32 (2H, d, J = 7.8 Hz, Ar H-4), 7.73 (2H, d, J = 6.9 Hz, Ar H-6), 7.92 (2H, s, Ar H-2), 9.87 (2H, s, NH). ^{13}C -NMR (DMSO- d_6) δ 24.7 (CH_3), 24.9 [C(=O)-CH $_2$ -CH $_2$ -CH], 28.2 [C(=O)-CH $_2$ -CH $_2$ -CH], 36.2 [C(=O)-CH $_2$ -CH $_2$ -CH], 83.6 (B-O-C-O-B), 121.9 (Ar C-6), 125.0 (Ar C-2), 128.2 (Ar C-5), 128.9 (Ar C-4), 138.9 (Ar C-1), 171.2 (C=O). Anal. Calcd for $\text{C}_{31}\text{H}_{44}\text{B}_2\text{N}_2\text{O}_6 \cdot 0.5\text{H}_2\text{O}$: C, 65.17; H, 7.94; N, 4.90. Found: C, 65.17; H, 7.99; N, 4.96.

N^1, N^9 -Bis(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)nonanediamide (1bd). A solution of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**2b**) (438.2 mg, 2.00 mmol) and CH_2Cl_2 (4.348 mL) was cooled to 4.0 °C, and then Et_3N (415.9 μL , 3.00 mmol) and azelaoyl chloride (**3d**) (195.7 μL , 1.00 mmol) were added to the resulting solution. The resulting mixture was stirred for 18 h at room temperature and then water (ca. 100 mL) was added. The obtained solution was extracted with AcOEt (x3) and the combined organic extract was dried over Na_2SO_4 . After filtration, the solvents that were used were evaporated under reduced pressure. The obtained crude material was washed with $\text{CH}_2\text{Cl}_2/n$ -hexane to give the desired product (**1bd**) (395.3 mg, 67% yield) as a white solid. Mp 93-115 °C. IR (KBr) 3444

(NH), 1662 cm^{-1} (C=O); FAB-MS (positive) m/z 591 (M+H)⁺. HRMS (FAB) Calcd for $\text{C}_{33}\text{H}_{49}\text{B}_2\text{N}_2\text{O}_6^+$: m/z 591.3771 (M+H)⁺. Found: 591.3779; ¹H-NMR (DMSO-*d*₆) δ 1.29 (24H, s, CH₃), 1.25-1.36 [6H, m, C(=O)-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-C(=O)], 1.50-1.64 [4H, m, C(=O)-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-C(=O)], 2.28 [4H, t, $J = 7.2$ Hz, C(=O)-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-C(=O)], 7.28 (2H, dd, $J = 7.2, 7.8$ Hz, Ar H-5), 7.31 (2H, d, $J = 7.2$ Hz, Ar H-4), 7.73 (2H, d, $J = 7.8$ Hz, Ar H-6), 7.90-7.95 (2H, m, Ar H-2), 9.85 (2H, s, NH). ¹³C-NMR (DMSO-*d*₆) δ 24.7 (CH₃), 25.1 [C(=O)-CH₂-CH₂-CH₂-CH], 28.5, 28.6 [C(=O)-CH₂-CH₂-CH₂-CH or C(=O)-CH₂-CH₂-CH₂-CH], 36.3 [C(=O)-CH₂-CH₂-CH₂-CH], 83.6 (B-O-C-C-O-B), 121.9 (Ar C-6), 125.0 (Ar C-2), 128.2 (Ar C-5), 128.8 (Ar C-4), 138.9 (Ar C-1), 171.2 (C=O). Anal. Calcd for $\text{C}_{33}\text{H}_{48}\text{B}_2\text{N}_2\text{O}_6$: C, 67.14; H, 8.20; N, 4.75. Found: C, 67.38; H, 8.37; N, 4.65.

The preparation and spectroscopic data of three compounds **1ba**, **1bc** and **1bd** in Table 1 have already been reported.^{11,12}

1,1'-(Butane-1,4-diyl)bis(3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea) (5ba). To a solution of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**2b**) (438.2 mg, 2.00 mmol) in THF (2.174 mL) were added Et₃N (831.0 μL , 6.00 mmol) and 1,4-diisocyanatobutane (**4a**) (126.2 μL , 1.00 mmol). The resulting mixture was stirred at 60 °C for 2 h. After cooling to room temperature, the solution was diluted with Et₂O (4.348 mL) and the precipitate was filtered and washed with Et₂O to afford the desired product (**5ba**) (551.1 mg, 95% yield) as a white solid. Mp 225-232 °C. IR (KBr) 3406 (NH), 1651 cm^{-1} (C=O); FAB-MS (positive) m/z 579 (M+H)⁺. HRMS (FAB) Calcd for $\text{C}_{30}\text{H}_{45}\text{B}_2\text{N}_4\text{O}_6^+$: m/z 579.3520 (M+H)⁺. Found: 579.3527; ¹H-NMR (DMSO-*d*₆) δ 1.28 (24H, s, CH₃), 1.41-1.49 (4H, m, N-CH₂-CH₂-CH₂-CH₂-N), 3.04-3.14 (4H, m, N-CH₂-CH₂-CH₂-CH₂-N), 6.09 (2H, t, $J = 6.0$ Hz, C₆H₄-NH-C(=O)-NH), 7.16-7.20 (2H, m, Ar H-4 in B-C₆H₄-N), 7.21 (2H, dd, $J = 7.2$ Hz, 7.8 Hz, Ar H-5 in B-C₆H₄-N), 7.42-7.49 (2H, m, Ar H-6 in B-C₆H₄-N), 7.76-7.79 (2H, m, Ar H-2 in B-C₆H₄-N), 8.44 (2H, s, C₆H₄-NH-C(=O)-NH). ¹³C-NMR (DMSO-*d*₆) δ 24.7 (CH₃), 27.3 (N-CH₂-CH₂-CH₂-CH₂-N), 38.8 (N-CH₂-CH₂-CH₂-CH₂-N), 83.5 (B-O-C-C-O-B), 120.6 (Ar C-6 in B-C₆H₄-N), 123.6 (Ar C-2 in B-C₆H₄-N), 127.0 (Ar C-4 in B-C₆H₄-N), 128.2 (Ar C-5 in B-C₆H₄-N), 140.1 (Ar C-1 in B-C₆H₄-N), 155.2 (C=O). Anal. Calcd for $\text{C}_{30}\text{H}_{44}\text{B}_2\text{N}_4\text{O}_6 \cdot 0.5\text{H}_2\text{O}$: C, 61.35; H, 7.72; N, 9.54. Found: C, 61.22; H, 7.45; N, 9.67.

1,1'-(Hexane-1,6-diyl)bis(3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea) (5bb). To a solution of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**2b**) (438.2 mg, 2.00 mmol) in THF (2.174 mL) were added Et₃N (831.0 μL , 6.00 mmol) and hexamethylene diisocyanate (**4b**) (160.2 μL , 1.00 mmol). The resulting mixture was stirred at 60 °C for 2 h. After cooling to room temperature, the solution was diluted with Et₂O (4.348 mL) and the precipitate was filtered and washed with Et₂O to afford the desired product (**5bb**) (578.0 mg, 95% yield) as a white solid. Mp 228-232 °C; IR (KBr) 3370

(NH), 1649 cm^{-1} (C=O); FAB-MS (positive) m/z 607 (M+H)⁺. HRMS (FAB) Calcd for C₃₂H₄₉B₂N₄O₆⁺: m/z 607.3833 (M+H)⁺. Found: 607.3842; ¹H-NMR (DMSO-*d*₆) δ 1.28 (24H, s, CH₃), 1.20-1.37 (4H, m, N-CH₂-CH₂-CH₂-CH₂-CH₂-N), 1.37-1.48 (4H, m, N-CH₂-CH₂-CH₂-CH₂-CH₂-N), 3.02-3.11 (4H, m, N-CH₂-CH₂-CH₂-CH₂-CH₂-N), 6.06 (2H, t, J = 5.7 Hz, C₆H₄-NH-C(=O)-NH), 7.16-7.20 (2H, m, Ar H-4 in B-C₆H₄-N), 7.21 (2H, dd, J = 7.8 Hz, 7.8 Hz, Ar H-5 in B-C₆H₄-N), 7.42-7.47 (2H, m, Ar H-6 in B-C₆H₄-N), 7.75-7.79 (2H, m, Ar H-2 in B-C₆H₄-N), 8.42 (2H, s, C₆H₄-NH-C(=O)-NH); ¹³C-NMR (DMSO-*d*₆) δ 24.7 (CH₃), 26.1 (N-CH₂-CH₂-CH₂-CH₂-CH₂-N), 29.8 (N-CH₂-CH₂-CH₂-CH₂-CH₂-N), 39.0 (N-CH₂-CH₂-CH₂-CH₂-CH₂-N), 83.5 (B-O-C-C-O-B), 120.6 (Ar C-6 in B-C₆H₄-N), 123.6 (Ar C-2 in B-C₆H₄-N), 126.9 (Ar C-4 in B-C₆H₄-N), 128.2 (Ar C-5 in B-C₆H₄-N), 140.1 (Ar C-1 in B-C₆H₄-N), 155.2 (C=O). Anal. Calcd for C₃₂H₄₈B₂N₄O₆•0.4H₂O: C, 62.64; H, 8.02; N, 9.13. Found: C, 62.55; H, 7.95; N, 9.41.

1,1'-(Methylenebis(4,1-phenylene))bis(3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea) (7a). To a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**2a**) (438.2 mg, 2.00 mmol) in THF (2.174 mL) were added Et₃N (831.8 μ L, 6.00 mmol) and methylenediphenyl 4,4'-diisocyanate (**6**) (250.3 mg, 1.00 mmol). The resulting mixture was stirred at 60 °C for 2 h. After cooling to room temperature, the solution was diluted with Et₂O (4.348 mL) and the precipitate was filtered and washed with Et₂O to afford the desired product (**7a**) (614.2 mg, 89% yield) as a white solid. Mp 290-295 °C. IR (KBr) 3465 (NH), 1654 cm^{-1} (C=O); FAB-MS (positive) m/z 689 (M+H)⁺. HRMS (FAB) Calcd for C₃₉H₄₇B₂N₄O₆⁺: m/z 689.3676 (M+H)⁺. Found: 689.3701; ¹H-NMR (DMSO-*d*₆) δ 1.28 (24H, s, CH₃), 3.82 (2H, s, C₆H₄-CH₂-C₆H₄), 7.12 (4H, d, J = 8.1 Hz, Ar H-3, H-5 in C₆H₄-CH₂-C₆H₄), 7.36 (4H, d, J = 8.1 Hz, Ar H-2, H-6 in C₆H₄-CH₂-C₆H₄), 7.46 (4H, d, J = 8.4 Hz, Ar H-2, H-6 in B-C₆H₄-N), 7.58 (4H, d, J = 8.4 Hz, Ar H-3, H-5 in B-C₆H₄-N), 8.63 (2H, s, B-C₆H₄-NH-C(=O)-NH-C₆H₄-CH₂-), 8.77 (2H, s, B-C₆H₄-NH-C(=O)-NH-C₆H₄-CH₂-). ¹³C-NMR (DMSO-*d*₆) δ 24.7 (CH₃), 39.8 (C₆H₄-CH₂-C₆H₄), 83.3 (B-O-C-C-O-B), 117.0 (Ar C-2, C-6 in B-C₆H₄-N), 118.5 (Ar C-2, C-6 in C₆H₄-CH₂-C₆H₄), 128.9 (Ar C-3, C-5 in C₆H₄-CH₂-C₆H₄), 135.2 (Ar C-4 in C₆H₄-CH₂-C₆H₄), 135.4 (Ar C-3, C-5 in B-C₆H₄-N), 137.4 (Ar C-1 in C₆H₄-CH₂-C₆H₄), 142.7 (Ar C-1 in B-C₆H₄-N), 152.3 (C=O). Anal. Calcd for C₃₉H₄₆B₂N₄O₆: C, 68.04; H, 6.73; N, 8.14. Found: C, 68.20; H, 6.84; N, 8.21.

1,1'-(Methylenebis(4,1-phenylene))bis(3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea) (7b). To a solution of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**2b**) (438.2 mg, 2.00 mmol) in THF (2.174 mL) were added Et₃N (831.8 μ L, 6.00 mmol) and methylenediphenyl 4,4'-diisocyanate (**6**) (250.3 mg, 1.00 mmol). The resulting mixture was stirred at 60 °C for 2 h. After cooling to room temperature, the solution was diluted with Et₂O (4.348 mL) and the precipitate was filtered and washed with Et₂O to afford the desired product (**7b**) (634.7 mg, 92% yield) as a white solid.

Mp 312-314 °C. IR (KBr) 3457 (NH), 1649 cm^{-1} (C=O); FAB-MS (positive) m/z 689 (M+H)⁺. HRMS (FAB) Calcd for $\text{C}_{39}\text{H}_{47}\text{B}_2\text{N}_4\text{O}_6^+$: m/z 689.3676 (M+H)⁺. Found: 689.3687; ¹H-NMR (DMSO-*d*₆) δ 1.29 (24H, s, CH_3), 3.82 (2H, s, $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 7.12 (4H, d, $J = 8.7$ Hz, Ar H-3, H-5 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 7.24-7.31 (4H, m, Ar H-4, H-5 in B- $\text{C}_6\text{H}_4\text{-N}$), 7.36 (4H, d, $J = 8.7$ Hz, Ar H-2, H-6 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 7.44-7.50 (2H, m, Ar H-6 in B- $\text{C}_6\text{H}_4\text{-N}$), 7.84-7.90 (2H, m, Ar H-2 in B- $\text{C}_6\text{H}_4\text{-N}$), 8.53 (2H, s, B- $\text{C}_6\text{H}_4\text{-NH-C(=O)-NH-C}_6\text{H}_4\text{-CH}_2\text{-}$), 8.66 (2H, s, B- $\text{C}_6\text{H}_4\text{-NH-C(=O)-NH-C}_6\text{H}_4\text{-CH}_2\text{-}$). ¹³C-NMR (DMSO-*d*₆) δ 24.7 (CH_3), 39.8 ($\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 83.6 (B-O-C-C-O-B), 118.4 (Ar C-2, C-6 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 121.2 (Ar C-6 in B- $\text{C}_6\text{H}_4\text{-N}$), 124.0 (Ar C-2 in B- $\text{C}_6\text{H}_4\text{-N}$), 127.8 (Ar C-4 in B- $\text{C}_6\text{H}_4\text{-N}$), 128.3 (Ar C-5 in B- $\text{C}_6\text{H}_4\text{-N}$), 128.9 (Ar C-3, C-5 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 135.1 (Ar C-4 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 137.5 (Ar C-1 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 139.3 (Ar C-1 in B- $\text{C}_6\text{H}_4\text{-N}$), 152.5 (C=O). Anal. Calcd for $\text{C}_{39}\text{H}_{46}\text{B}_2\text{N}_4\text{O}_6 \cdot 0.3\text{H}_2\text{O}$: C, 67.51; H, 6.77; N, 8.07. Found: C, 67.45; H, 6.80; N, 8.30.

1,1'-(Methylenebis(4,1-phenylene))bis(3-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-urea) (7c). To a solution of 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**2c**) (438.2 mg, 2.00 mmol) in THF (2.174 mL) were added Et₃N (831.8 μL , 6.00 mmol) and methylenediphenyl 4,4'-diisocyanate (**6**) (250.3 mg, 1.00 mmol). The resulting mixture was stirred at 60 °C for 2 h. After cooling to room temperature, the solution was diluted with Et₂O (4.348 mL) and the precipitate was filtered and washed with Et₂O to afford the desired product (**7c**) (641.9 mg, 93% yield) as a white solid. Mp 170-173 °C. IR (KBr) 3434 (NH), 1635 cm^{-1} (C=O); FAB-MS (positive) m/z 689 (M+H)⁺. HRMS (FAB) Calcd for $\text{C}_{39}\text{H}_{47}\text{B}_2\text{N}_4\text{O}_6^+$: m/z 689.3676 (M+H)⁺. Found: 689.3685; ¹H-NMR (DMSO-*d*₆) δ 1.18 (24H, s, CH_3), 3.89 (2H, s, $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 6.99 (2H, dd, $J = 7.2$ Hz, 7.5 Hz, Ar H-4 in B- $\text{C}_6\text{H}_4\text{-N}$), 7.14 (2H, d, $J = 7.8$ Hz, Ar H-6 in B- $\text{C}_6\text{H}_4\text{-N}$), 7.18 (4H, d, $J = 8.4$ Hz, Ar H-3, H-5 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 7.23 (2H, dd, $J = 7.2$ Hz, 7.8 Hz, Ar H-5 in B- $\text{C}_6\text{H}_4\text{-N}$), 7.34 (4H, d, $J = 8.4$ Hz, Ar H-2, H-6 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 7.42 (2H, d, $J = 7.5$ Hz, Ar H-3 in B- $\text{C}_6\text{H}_4\text{-N}$), 9.34 (2H, s, B- $\text{C}_6\text{H}_4\text{-NH-C(=O)-NH-C}_6\text{H}_4\text{-CH}_2\text{-}$), 9.45 (2H, s, B- $\text{C}_6\text{H}_4\text{-NH-C(=O)-NH-C}_6\text{H}_4\text{-CH}_2\text{-}$). ¹³C-NMR (DMSO-*d*₆) δ 25.6 (CH_3), 39.7 ($\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 80.8 (B-O-C-C-O-B), 116.6 (Ar C-6 in B- $\text{C}_6\text{H}_4\text{-N}$), 120.7 (Ar C-2, C-6 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 122.5 (Ar C-4 in B- $\text{C}_6\text{H}_4\text{-N}$), 128.7 (Ar C-5 in B- $\text{C}_6\text{H}_4\text{-N}$), 128.9 (Ar C-3, C-5 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 133.2 (Ar C-3 in B- $\text{C}_6\text{H}_4\text{-N}$), 135.7 (Ar C-1 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 136.6 (Ar C-4 in $\text{C}_6\text{H}_4\text{-CH}_2\text{-C}_6\text{H}_4$), 140.8 (Ar C-1 in B- $\text{C}_6\text{H}_4\text{-N}$), 154.3 (C=O). Anal. Calcd for $\text{C}_{39}\text{H}_{46}\text{B}_2\text{N}_4\text{O}_6 \cdot \text{H}_2\text{O}$: C, 66.31; H, 6.85; N, 7.93. Found: C, 66.34; H, 6.76; N, 8.09.

Assays for Antibacterial Activity

We used *S. aureus* ATCC6538P and *E. coli* NBRC14237 (NIHJ) (Gram-positive and Gram-negative bacteria, respectively) as target organisms. Synthesized compounds were dissolved in DMSO or dimethylformamide (DMF) to a concentration of 1.280 $\mu\text{g}/\text{mL}$. The MIC of a standard strain was measured by the authentic microdilution method to monitor bacterial growth turbidity in Muller-Hinton

broth according to the Japanese Society of Chemotherapy.^{16,17} The values of MIC are expressed as molar concentrations (μM) for discussion of structure-activity relations.

Antiviral Activity Assay and Cytotoxicity

The anti-HSV-1 activities (EC_{50}) of the synthesized symmetrical cyclic phenylboronic acid ester derivatives were measured by using a plaque reduction assay,¹⁸ and their cytotoxicity against Vero cells (CC_{50}) was also evaluated.

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