HETEROCYCLES, Vol. 96, No. 6, 2018, pp. 1088 - 1100. © 2018 The Japan Institute of Heterocyclic Chemistry
Received, 6th April, 2018, Accepted, 8th May, 2018, Published online, 15th May, 2018
DOI: 10.3987/COM-18-13899

PREPARATION OF NOVEL BIVALENT LINKER MODE PHENYLBORONIC ACID DERIVATIVES AND THEIR BIOLOGICAL EVALUATION

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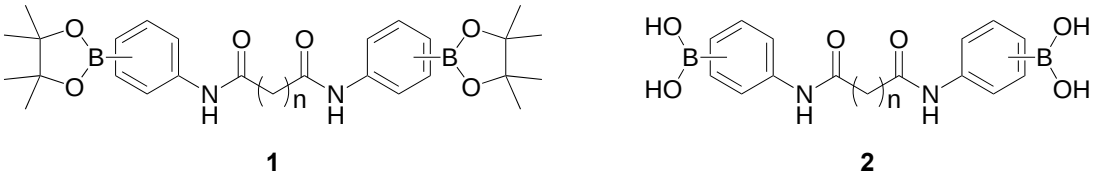
Abstract – We report a new route to the preparation of C_2-symmetrical bivalent phenylboronic acids having alkyl linker groups in the molecule and results of biological evaluation of their biological activity and cytotoxic activity against Vero cells. Among the tested compounds, C_2-symmetrical bivalent *meta*-oriented phenylboronic acid **2f** (n=7) showed high cytotoxic activity (CC_{50}=5.43 μM) against Vero cells. The results of an SAR study suggested that the presence of a C7-methylene linker group in the molecule is an important structural factor for expression of potential cytotoxic activities. A sugar recognition property of this C_2-symmetrical geometric molecule was suggested by NMR analysis of compound **2f** with methyl α-D-glucopyranoside **6**.

For various cell-to-cell communications, including the processes of tumor metastasis and bacterial or viral infection,[1-3] interactions between carbohydrate-containing glycoproteins, proteoglycans and glycolipids on the cell surface are important biological stages. On the other hand, molecular recognition by macromolecules with two-fold (C_2) or three-fold (C_3) symmetrical geometry is one of the common features in many important biological responses.[4] These supramolecular interactions of bio-macromolecules have encouraged us to develop new multivalent symmetrical synthetic molecules to find new bioactive compounds or leads. Regarding such geometrical molecules, we have recently designed and synthesized a few new symmetrical molecules and evaluated their bioactivities in order to find new types of bioactive compounds.[5-10] We have been particularly interested in phenylboronic acid

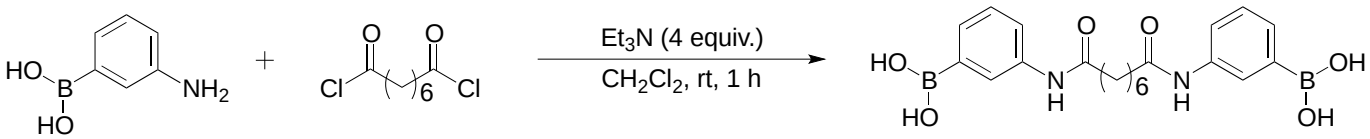
derivatives because many boronic acid functionalities have a property to react with various 1,2-diol functionalities included in sugars (sugar-chains) and generate cyclic boronic acid ester derivatives formed with reversible covalent bonds.[11] In connection with the above projects, we have recently reported the preparation of a few C_2-symmetrical bivalent phenylboronic acid pinacol esters and the results of biological evaluation of the synthesized symmetrical phenylboronic acid derivatives.[7,8] Among previously targeted C_2-symmetrical phenylboronic acid derivatives, we found that the C_2-symmetrical *meta*-oriented phenylboronic acid pinacol ester (**1f**; n=7) with a linear methylene linker showed a high level of cytotoxic activity (CC_{50}=25.2 μM) against Vero cells,[7] and it is considered to be a potential new lead in the search for cytotoxic compounds such as anticancer active molecules (Figure 1). In this paper, we describe the preparation of some free C_2-symmetrical phenylboronic acids (**2**) and evaluation of their biological activities. We also describe the sugar recognition property of bivalent symmetrical phenylboronic acids and the structure-activity relationships (SARs) of these symmetrical phenylboronic acid-related derivatives.



1 **2**

Figure 1

One example of symmetrical free *meta*-oriented phenylboronic acid derivatives (**2e**; n=6) was previously synthesized from amino-substituted phenylboronic acid (**3**) as a starting material using a direct *N*-acylation reaction by dicarboxylic acid dihalide (**4**) (Scheme 1). The details for the preparation of this compound (**2e**) were reported in our previous paper.[8] As an alternative new conventional route for the

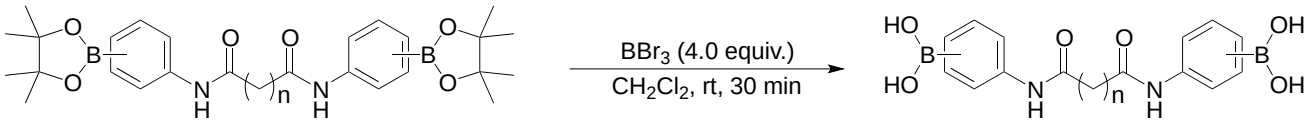


3 2 equiv. **4** 1 equiv. **2e** 35% yield

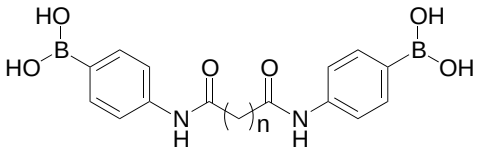
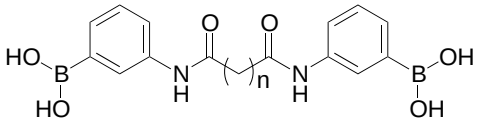
Scheme 1

preparation of free C_2-symmetrical phenylboronic acid derivatives (**2**), we conducted direct hydrolysis of C_2-symmetrical phenylboronic acid pinacol esters (**1**) with Lewis acid BBr_3, because pinacol ester derivatives (**1**) were easily obtained by *N*-acylation reactions of phenylboronic acid pinacol esters as a stable material. As can be seen in Table 1, the yields in the procedure (entries 1~7) with BBr_3 from bivalent pinacol esters to target free C_2-symmetrical phenylboronic acid (**2**) were good, and this method

Table 1. Preparation and Biological Activity of C_2-Symmetrical Phenylboronic Acids from Their Corresponding Pinacol Esters

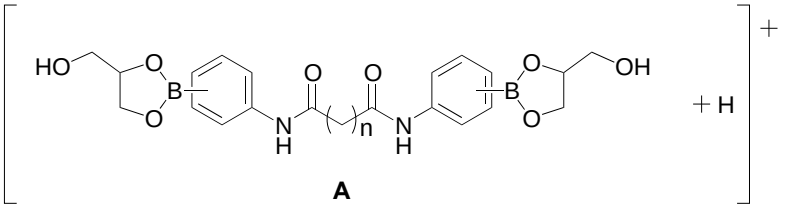


1 **2**

Entry	Product **2**	n		Yield (%)[a]	EC_{50} (*m*M) Anti-HSV-1 activity	CC_{50} (*m*M) Cytotoxic activity
1		4	**2a**	59	>100	>200
2		6	**2b**	50	>100	>200
3		8	**2c**	66	>100	>200
4		4	**2d**	34	>100	76.2
5		6	**2e**	67	>100	>200
6		7	**2f**	69	>100	5.43
7		8	**2g**	62	>100	>200

[a]Isolated yield.

for hydrolysis of *para*- and *meta*-substituted bivalent phenylboronic acid pinacol esters was reproducible and useful for a procedure for bivalent phenylboronic acids from corresponding pinacol esters. However, unfortunately, the *ortho*-substituted pinacol ester derivatives were unsuccessfully applied to give complex unknown mixtures as reaction products. The presence of a reactive *ortho*-amido functionality in the molecules (**2**) to the generated free phenylboronic acid group[12] is thought to be the reason for the formation of a complex mixture. The structures of the obtained bivalent free phenylboronic acids (**2**) were established by spectroscopic methods and elemental analysis. In high-resolution positive FAB-MS spectra of C_2-symmetrical free phenylboronic acids (**2a**~**2g**), all observed molecular ion peaks corresponded to the ion **A** (Figure 2) directly derived from the reaction of compounds **2a**~**2g** with two molar amounts of the matrix used (1,2,3-propanetriol). The appearance of these positive characteristic ions in FAB-MS may be due to the formation of 5-membered dioxabororane **A** by reaction of two boronic acid functionalities in molecules **2a**~**2g** with 1,2-diol functionality of glycerin. The values obtained by elemental analysis of these positive ions were identical with the composition **A** derived from



A

Figure 2

representative phenylboronic acid (**2**) (see EXPERIMENTAL). The cytotoxicities of compounds (**2**) (CC_{50}) against Vero cells are summarized in Table 1. Among the tested free C_2-symmetrical phenylboronic acid derivatives in this study, C_2-symmetrical bivalent phenylboronic acid (**2f**; n=7) showed the highest cytotoxic activity (CC_{50}=5.43 μM) against Vero cells. Other compounds listed in Table 1 showed almost no cytotoxic activity, indicating that the presence of a C7-methylene linker and two *meta*-substituted phenylboronic acid groups is a preferable structure for the expression of a high level of cytotoxic activity against Vero cells.

Throughout the NMR operation of the pinacol ester **1f**,[7] we observed that the two pinacol ester functionalities in the C_2-symmetrical molecule were hydrolyzed to free phenylboronic acid **2f** in DMSO-d_6-containing deuterated water (DMSO-d_6/D_2O=9/1) (Figure 3).[12,13] After standing for 30 days at 25 °C, ca 60% of the pinacol ester **1f** was estimated to be hydrolyzed to give the corresponding free phenylboronic acid **2f** by a comparison of the integrations of both aromatic protons.[14,15] We considered that the observed cytotoxic activity (CC_{50}=25.2 μM) of the pinacol ester **1f** may be due to the corresponding free boronic acid **2f** generated in the bioassay system.[16] We then further examined by NMR analysis whether these compounds form complexes with methyl α-D-glucopyranoside **6**. In the NMR experiments using phenylboronic acid pinacol ester **1f** and methyl α-D-glucopyranoside **6**, no spectroscopic change was observed by blending except for a sharp or slightly complicated appearance at the same δ value positions for proton signals ascribable to methyl α-D-glucopyranoside **6** (Figure 4). This

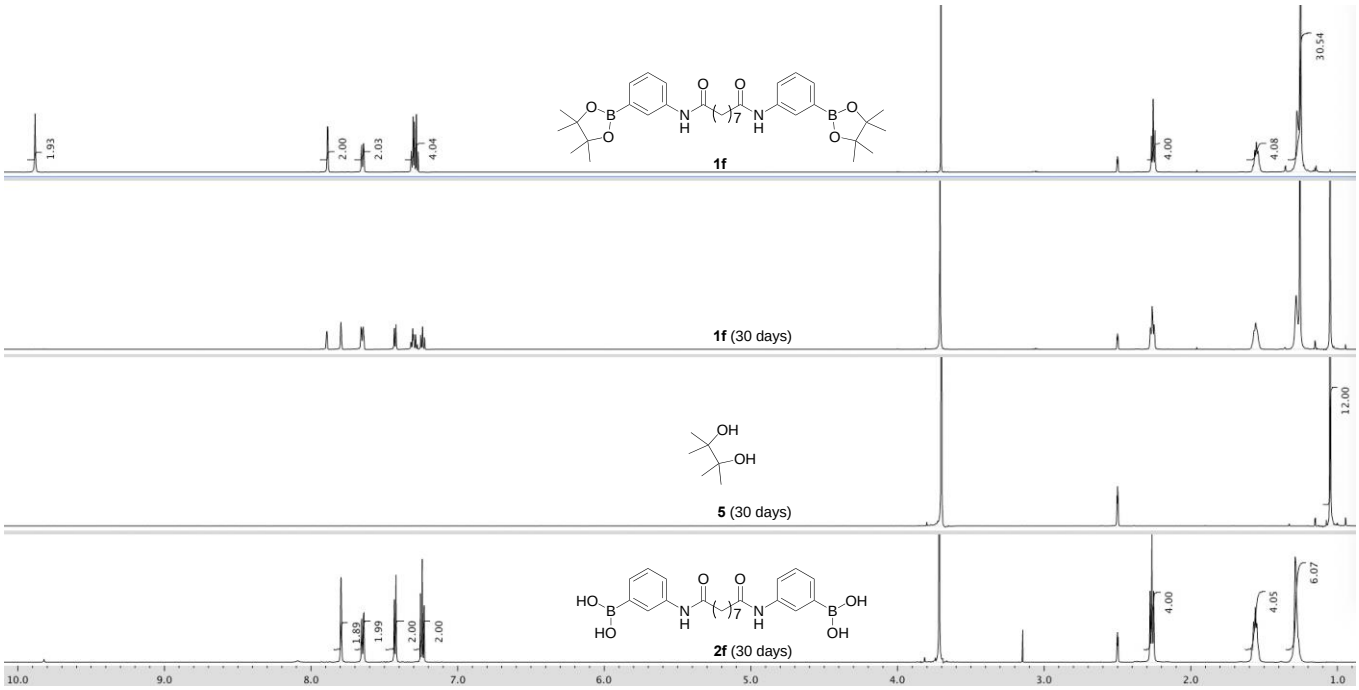


Figure 3. NMR study in DMSO-d_6/D_2O=9/1 at 25 °C

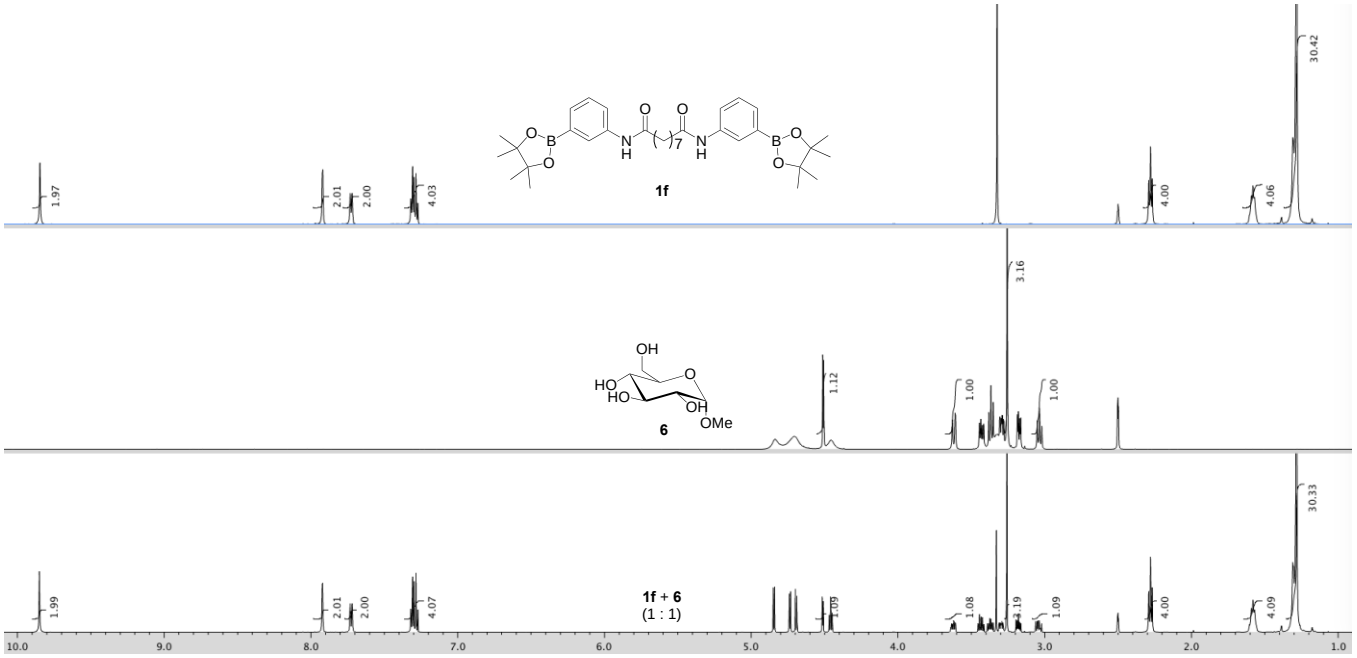


Figure 4. NMR study in DMSO-d_6 at 25 °C

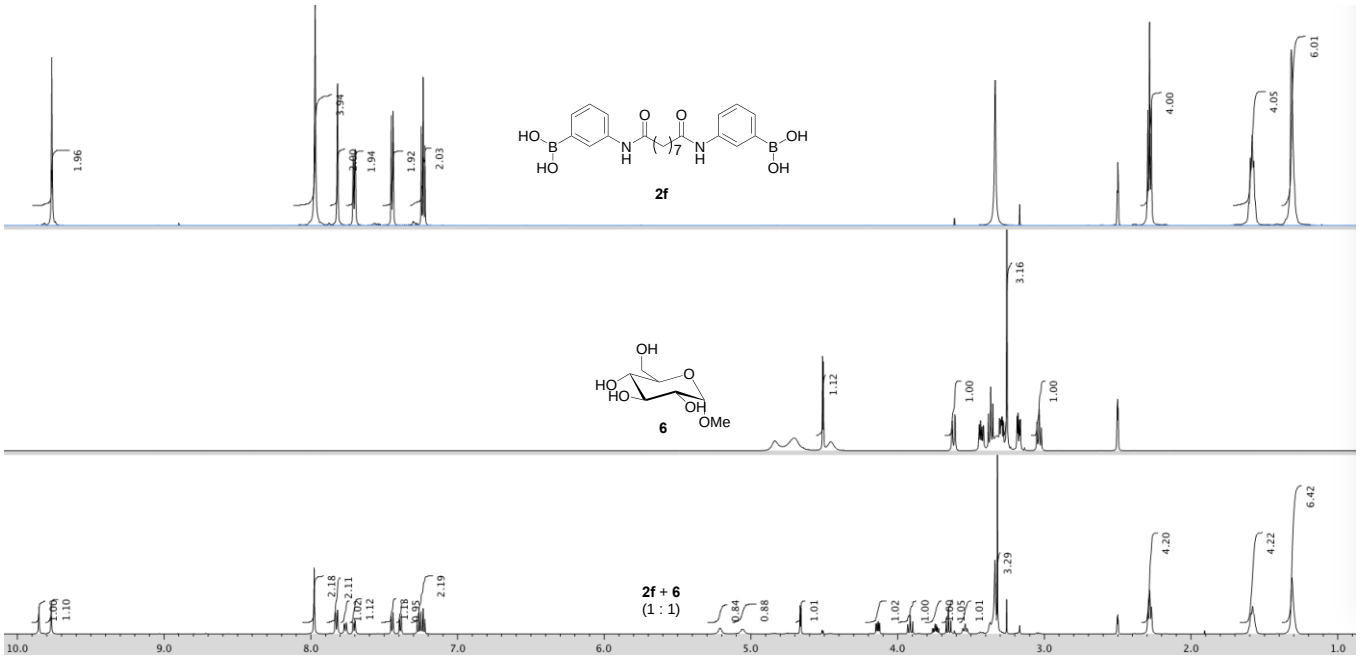
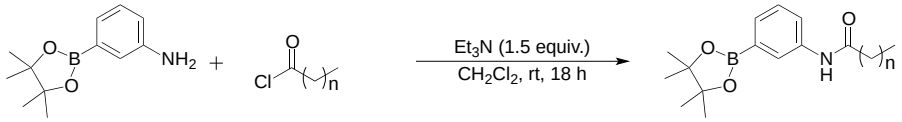
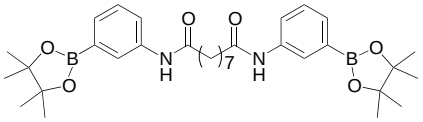
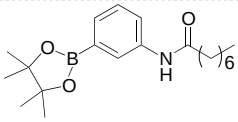
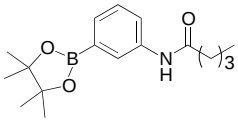
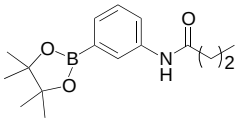


Figure 5. NMR study in DMSO-d_6 at 25 °C

Table 2. Preparation and Biological Activity of Monovalent Phenylboronic Acid Pinacol Esters



7 1 equiv. **8** 1 equiv. **9**

Entry	Product **9**		Yield (%)[a]	MIC [*m*M (*m*g/mL)] *S. aureus*	MIC [*m*M (*m*g/mL)] *E. coli*	EC_{50} (*m*M) Anti-HSV-1 activity	CC_{50} (*m*M) Cytotoxic activity
1		**1f**	-	27.1[b] (16[b])	216.8[b] (128[b])	8.0[b]	25.2[b]
2		**9a**	68	>370.7 (>128)	370.7 (128)	>100	196.9
3		**9b**	54	>422.2 (>128)	>422.2 (128)	>100	98.3
4		**9c**	75	>442.6 (>128)	442.6 (128)	>100	>200

[a]Isolated yield. [b]Data were taken from ref 7.

phenomenon may arise from a low degree of freedom of the exchange mobility of protons in the sugar molecule **6** by a weak hydrogen-bonding interaction between compound **6** and amide (or boronate) groups of **1f** in solution. However, the NMR spectrum of a mixture of the corresponding free boronic acid **2f** and methyl α-D-glucopyranoside **6** showed a significant influence on the NMR signal pattern (*i.e.*, the appearance of new signals by the formation of a complex of the compound **2f** with this sugar), suggesting its sugar recognition property (see Figure 5). A new signal at 9.85 (1H, s, N<u>H</u>) and four new signals for aromatic regions at δ 7.21-7.29 (1H, m, Ar H-5), 7.39 (1H, d, J = 6.6 Hz, Ar H-4), 7.77 (1H, d, J = 7.8 Hz, Ar H-6), 7.83 (1H, s, Ar H-2), and 3.32 (3H, s, C<u>H</u>$_3$O) apparently indicated the formation of a new C_2-symmetrical phenylboronic acid derivative (a 1:2 complex of the compound **2f** with this sugar **6**) (see EXPERIMENTAL). Regarding the sugar recognition property of this highly bioactive twin-drug type compound **2f**, we are planning to carry out calorimetric experiments with sugar derivatives.

For the purpose of comparison of the biological activity of twin-drug type phenylboronic acid pinacol ester (**1f**) with the activities of single-drug type molecules, we prepared simple *N*-acylaminosubstituted phenylboronic acid derivatives (**9a**~**9c**) by *N*-acylation of *meta*-aminophenylboronic acid pinacol ester as the single-drug type molecule. The results of biological evaluations are shown in Table 2. Only compound **9b** showed moderate cytotoxic activity (CC_{50}=98.3 μM), but the value was only about a quarter of the value for the twin-drug type bivalent molecule (**1f**). This result clearly indicates that a bivalent symmetrical structure of phenylboronic acid (**2f**) is responsible for expression of the high

specification of such biological activity.

On the basis of the information obtained by the above-described biological activities of the phenylboronic acid series together with recent information on the C_3-type phenylboronic acid series,[17,18] further molecular modifications of the highest cytotoxic active phenylboronic acid **2f** are now under investigation in order to find new promising anticancer active compounds.

EXPERIMENTAL

IR spectra were measured on a Shimadzu FT/IR-8100 spectrometer. ^{1}H- 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 ^{1}H-NMR [(DMSO-d_6) (2.50 ppm)] and ^{13}C-NMR [DMSO-d_6 (39.52 ppm)]. The signal assignments were confirmed by ^{1}H-^{1}H two-dimensional (2D) correlation spectroscopy (COSY), ^{1}H-^{13}C heteronuclear multiple-quantum coherence (HMQC), and ^{1}H-^{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 Starting *C*$_2$-Symmetrical Phenylboronic Acid Pinacol Esters (1).

Compounds **1a**~**1g** were prepared by an amide bond formation reaction using 4- or 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline and dicarboxylic acid dichloride in the presence of a base such as Et_3N by the same procedure as that reported previously.[5] Physical and spectroscopic data of compounds **1a**~**1g** were presented in our previous papers.[5,7,8]

Preparation of *C*$_2$-Symmetrical Phenylboronic Acid Derivatives from the Corresponding Phenylboronic Acid Pinacol Esters (2).

General Procedure: To a solution of C_2-symmetrical phenylboronic acid pinacol ester (**1**) (1.00 mmol) in CH_2Cl_2 (8.05 mL) was added a 1.0 M solution of BBr_3 in CH_2Cl_2 (4.000 mL, 4.00 mmol). The resulting mixture was stirred for 30 min at room temperature and diluted with CH_2Cl_2. The precipitate was filtered and washed sequentially with CH_2Cl_2, sat $NaHCO_3$ aq, and water. The obtained crude material was washed with CH_2Cl_2/MeOH/*n*-hexane and water (ca. 500 mL) to give the desired C_2-symmetrical phenylboronic acid derivatives (**2**). The results obtained are shown in Table 1.

((Adipoylbis(azanediyl))bis(4,1-phenylene))diboronic acid (2a). This compound was obtained from N^1,N^6-bis(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)adipamide (**1a**) (548.3 mg, 1.00 mmol) as a pale brown solid (227.0 mg, 59% yield). Mp 240-242 °C. IR (neat) 3312 (NH), 1662 cm^{-1} (C=O); FAB-MS (positive) *m*/*z* 497 (**A**+H)$^+$. HRMS (FAB) Calcd for $C_{24}H_{31}B_2N_2O_8^+$: *m*/*z* 497.2261 (**A**+H)$^+$. Found: 497.2281; ^{1}H-NMR (DMSO-d_6) δ 1.52-1.68 [4H, m, C(=O)-CH$_2$-C<u>H</u>$_2$-C<u>H</u>$_2$-CH$_2$-C(=O)], 2.28-2.40 [4H, m, C(=O)-C<u>H</u>$_2$-CH$_2$-CH$_2$-C<u>H</u>$_2$-C(=O)], 7.54 (4H, d, *J* = 7.8 Hz, Ar H-2, 6), 7.70 (4H, d, *J* = 7.8 Hz, Ar H-3, 5), 7.87 [4H, s, B(O<u>H</u>)$_2$], 9.90 (2H, s, N<u>H</u>). ^{13}C-NMR (DMSO-d_6) δ 24.9

[C(=O)-CH_2-$\underline{C}H_2$], 36.3 [C(=O)-$\underline{C}H_2$-CH_2], 117.8 (Ar C-2, 6), 134.8 (Ar C-3, 5), 140.9 (Ar C-1), 171.2 ($\underline{C}$=O). Anal. Calcd for $C_{18}H_{22}B_2N_2O_6$•0.55H_2O: C, 54.88; H, 5.91; N, 7.11. Found: C, 54.91; H, 5.77; N, 7.03.

((Octanedioylbis(azanediyl))bis(4,1-phenylene))diboronic acid (2b). This compound was obtained from N^1,N^8-bis(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)octanediamide (**1b**) (576.3 mg, 1.00 mmol) as a white solid (207.3 mg, 50% yield). Mp 243-247 °C. IR (neat) 3305 (NH), 1660 cm^{-1} (C=O); FAB-MS (positive) *m*/*z* 525 (**A**+H)$^+$. HRMS (FAB) Calcd for $C_{26}H_{35}B_2N_2O_8^+$: *m*/*z* 525.2574 (**A**+H)$^+$. Found: 525.2589; ^{1}H-NMR (DMSO-d_6) δ 1.26-1.38 [4H, m, C(=O)-CH_2-CH_2-C$\underline{H}_2$-C$\underline{H}_2$-CH_2-CH_2-C(=O)], 1.54-1.64 [4H, m, C(=O)-CH_2-C$\underline{H}_2$-CH_2-CH_2-C$\underline{H}_2$-CH_2-C(=O)], 2.30 [4H, t, *J* = 7.5 Hz, C(=O)-C$\underline{H}_2$-CH_2-CH_2-CH_2-CH_2-C$\underline{H}_2$-C(=O)], 7.54 (4H, d, *J* = 8.4 Hz, Ar H-2, 6), 7.70 (4H, d, *J* = 8.4 Hz, Ar H-3, 5), 7.87 [4H, s, B(O$\underline{H}$)$_2$], 9.87 (2H, s, N$\underline{H}$). ^{13}C-NMR (DMSO-d_6) δ 25.0 [C(=O)-CH_2-$\underline{C}H_2$-CH_2], 28.5 [C(=O)-CH_2-CH_2-$\underline{C}H_2$], 36.4 [C(=O)-$\underline{C}H_2$-CH_2-CH_2], 117.8 (Ar C-2, 6), 134.8 (Ar C-3, 5), 141.0 (Ar C-1), 171.3 ($\underline{C}$=O). Anal. Calcd for $C_{20}H_{26}B_2N_2O_6$•0.4H_2O: C, 57.29; H, 6.44; N, 6.68. Found: C, 57.29; H, 6.32; N, 6.65.

((Decanedioylbis(azanediyl))bis(4,1-phenylene))diboronic acid (2c). This compound was obtained from N^1,N^{10}-bis(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)decanediamide (**1c**) (604.4 mg, 1.00 mmol) as a white solid (292.5 mg, 66% yield). Mp 239-246 °C. IR (neat) 3312 (NH), 1661 cm^{-1} (C=O); FAB-MS (positive) *m*/*z* 553 (**A**+H)$^+$. HRMS (FAB) Calcd for $C_{28}H_{39}B_2N_2O_8^+$: *m*/*z* 553.2887 (**A**+H)$^+$. Found: 553.2902; ^{1}H-NMR (DMSO-d_6) δ 1.24-1.34 [8H, m, C(=O)-CH_2-CH_2-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-CH_2CH_2-C(=O)], 1.54-1.62 [4H, m, C(=O)-CH_2-C$\underline{H}_2$-CH_2-CH_2-CH_2-CH_2-C$\underline{H}_2$-CH_2-C(=O)], 2.29 [4H, t, *J* = 7.5 Hz, C(=O)-C$\underline{H}_2$-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-C$\underline{H}_2$-C(=O)], 7.54 (4H, d, *J* = 8.7 Hz, Ar H-2, 6), 7.70 (4H, d, *J* = 8.7 Hz, Ar H-3, 5), 7.87 [4H, s, B(O$\underline{H}$)$_2$], 9.86 (2H, s, N$\underline{H}$). ^{13}C-NMR (DMSO-d_6) δ 25.1 [C(=O)-CH_2-$\underline{C}H_2$-CH_2-CH_2], 28.7, 28.7 [C(=O)-CH_2-CH_2-$\underline{C}H_2$-$\underline{C}H_2$], 36.4 [C(=O)-$\underline{C}H_2$-CH_2-CH_2-CH_2], 117.8 (Ar C-2, 6), 134.8 (Ar C-3, 5), 141.0 (Ar C-1), 171.3 ($\underline{C}$=O). Anal. Calcd for $C_{22}H_{30}B_2N_2O_6$•0.2H_2O: C, 59.55; H, 6.91; N, 6.31. Found: C, 59.54; H, 6.91; N, 6.27.

((Adipoylbis(azanediyl))bis(3,1-phenylene))diboronic acid (2d). This compound was obtained from N^1,N^6-bis(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)adipamide (**1d**) (548.3 mg, 1.00 mmol) as a white solid (129.1 mg, 34% yield). Mp 252-258 °C. IR (neat) 3303 (NH), 1655 cm^{-1} (C=O); FAB-MS (positive) *m*/*z* 497 (**A**+H)$^+$. HRMS (FAB) Calcd for $C_{24}H_{31}B_2N_2O_8^+$: *m*/*z* 497.2261 (**A**+H)$^+$. Found: 497.2275; ^{1}H-NMR (DMSO-d_6) δ 1.56-1.70 [4H, m, C(=O)-CH_2-C$\underline{H}_2$-C$\underline{H}_2$-CH_2-C(=O)], 2.26-2.40 [4H, m, C(=O)-C$\underline{H}_2$-CH_2-CH_2-C$\underline{H}_2$-C(=O)], 7.24 (2H, dd, *J* = 6.9, 8.1 Hz, Ar H-5), 7.45 (2H, d, *J* = 6.9 Hz, Ar H-4), 7.71 (2H, d, *J* = 8.1 Hz, Ar H-6), 7.82 (2H, s, Ar H-2), 7.97 [4H, s, B(O$\underline{H}$)$_2$], 9.80 (2H, s, N$\underline{H}$). ^{13}C-NMR (DMSO-d_6) δ 25.0 [C(=O)-CH_2-$\underline{C}H_2$], 36.2 [C(=O)-$\underline{C}H_2$-CH_2], 121.1 (Ar C-6),

125.2 (Ar C-2), 127.6 (Ar C-5), 128.8 (Ar C-4), 138.4 (Ar C-1), 171.0 (C=O). Anal. Calcd for $C_{18}H_{22}B_2N_2O_6$•0.3H_2O: C, 55.52; H, 5.85; N, 7.19. Found: C, 55.51; H, 5.73; N, 7.18.

((Octanedioylbis(azanediyl))bis(3,1-phenylene))diboronic acid (2e).[8] This compound was obtained from N^1,N^8-bis(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)octanediamide (**1e**) (576.3 mg, 1.00 mmol) as a white solid (275.5 mg, 67% yield).

((Nonanedioylbis(azanediyl))bis(3,1-phenylene))diboronic acid (2f). This compound was obtained from N^1,N^9-bis(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)nonanediamide (**1f**) (590.4 mg, 1.00 mmol) as a white solid (293.4 mg, 69% yield). Mp 232-242 °C. IR (neat) 3319 (NH), 1662 cm^{-1} (C=O); FAB-MS (positive) *m*/*z* 539 (**A**+H)$^+$. HRMS (FAB) Calcd for $C_{27}H_{37}B_2N_2O_8^+$: *m*/*z* 539.2731 (**A**+H)$^+$. Found: 539.2744; ^{1}H-NMR (DMSO-d_6) δ 1.24-1.38 [6H, m, C(=O)-CH_2-CH_2-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-CH_2-CH_2-C(=O)], 1.52-1.64 [4H, m, C(=O)-CH_2-C$\underline{H}_2$-CH_2-CH_2-CH-C$\underline{H}_2$-CH_2-C(=O)], 2.28 [4H, t, *J* = 7.2 Hz, C(=O)-C$\underline{H}_2$-CH_2-CH_2-CH_2-CH_2-CH_2-C$\underline{H}_2$-C(=O)], 7.24 (2H, dd, *J* = 7.8, 7.8 Hz, Ar H-5), 7.45 (2H, d, *J* = 7.8 Hz, Ar H-4), 7.71 (2H, d, *J* = 7.8 Hz, Ar H-6), 7.82 (2H, s, Ar H-2), 7.98 [4H, s, B(O$\underline{H}$)$_2$], 9.77 (2H, s, N$\underline{H}$). ^{13}C-NMR (DMSO-d_6) δ 25.2 [C(=O)-CH_2-C$\underline{H}_2$-CH_2-CH], 28.6, 28.6 [C(=O)-CH_2-CH_2-C$\underline{H}_2$-CH], 36.4 [C(=O)-C$\underline{H}_2$-CH_2-CH_2-CH], 121.1 (Ar C-6), 125.2 (Ar C-2), 127.6 (Ar C-5), 128.8 (Ar C-4), 138.5 (Ar C-1), 171.1 (C=O). Anal. Calcd for $C_{21}H_{28}B_2N_2O_6$•0.3H_2O: C, 58.46; H, 6.68; N, 6.49. Found: C, 58.44; H, 6.66; N, 6.47.

((Decanedioylbis(azanediyl))bis(3,1-phenylene))diboronic acid (2g). This compound was obtained from N^1,N^{10}-bis(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)decanediamide (**1g**) (604.4 mg, 1.00 mmol) as a white solid (273.0 mg, 62% yield). Mp 227-240 °C. IR (neat) 3312 (NH), 1660 cm^{-1} (C=O); FAB-MS (positive) *m*/*z* 553 (**A**+H)$^+$. HRMS (FAB) Calcd for $C_{28}H_{39}B_2N_2O_8^+$: *m*/*z* 553.2887 (**A**+H)$^+$. Found: 553.2902; ^{1}H-NMR (DMSO-d_6) δ 1.18-1.32 [8H, m, C(=O)-CH_2-CH_2-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-C$\underline{H}_2$-CH_2CH_2-C(=O)], 1.48-1.60 [4H, m, C(=O)-CH_2-C$\underline{H}_2$-CH_2-CH_2-CH_2-CH_2-C$\underline{H}_2$-CH_2-C(=O)], 2.24 [4H, t, *J* = 7.5 Hz, C(=O)-C$\underline{H}_2$-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-C$\underline{H}_2$-C(=O)], 7.20 (2H, dd, *J* = 7.8, 7.8 Hz, Ar H-5), 7.41 (2H, d, *J* = 7.8 Hz, Ar H-4), 7.67 (2H, d, *J* = 7.8 Hz, Ar H-6), 7.78 (2H, s, Ar H-2), 7.93 [4H, s, B(O$\underline{H}$)$_2$], 9.72 (2H, s, N$\underline{H}$). ^{13}C-NMR (DMSO-d_6) δ 25.2 [C(=O)-CH_2-C$\underline{H}_2$-CH_2-CH_2], 28.7, 28.7 [C(=O)-CH_2-CH_2-C$\underline{H}_2$-C$\underline{H}_2$], 36.3 [C(=O)-C$\underline{H}_2$-CH_2-CH_2-CH_2], 121.1 (Ar C-6), 125.1 (Ar C-2), 127.6 (Ar C-5), 128.7 (Ar C-4), 138.5 (Ar C-1), 171.1 (C=O). Anal. Calcd for $C_{22}H_{30}B_2N_2O_6$•0.2H_2O: C, 59.55; H, 6.91; N, 6.31. Found: C, 59.54; H, 6.91; N, 6.29.

Preparation of Single Drug Type Mono-valent Phenylboronic Acid Pinacol Esters (9).

General Procedure: A solution of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**7**) (219.1 mg, 1.00 mmol) and CH_2Cl_2 (4.348 mL) was cooled to 4.0 °C, and then Et_3N (207.9 μL, 1.50 mmol) and alkanoyl chloride (**8**) (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 CH_2Cl_2/*n*-hexane to give the desired phenylboronic acid pinacol esters (**9**).

***N*-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)octanamide (9a).** This compound was obtained from the reaction of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**7**) and *n*-octanoyl chloride (**8a**) (171.4 μL, 1.00 mmol) as a white solid (235.7 mg, 68% yield). Mp 97-100 °C; IR (neat) 3299 (NH), 1661 cm^{-1} (C=O); FAB-MS (positive) *m*/*z* 346 $(M+H)^+$. HRMS (FAB) Calcd for $C_{20}H_{33}BNO_3^+$: *m*/*z* 346.2548 $(M+H)^+$. Found: 346.2548; ^{1}H-NMR (DMSO-d_6) δ 0.86 (3H, t, J = 7.2 Hz, C<u>H</u>$_3$-CH$_2$-), 1.14-1.42 (20H, m, CH$_3$-C<u>H</u>$_2$-C<u>H</u>$_2$-C<u>H</u>$_2$-C<u>H</u>$_2$-, C<u>H</u>$_3$ in boronate), 1.52-1.64 [2H, m, C(=O)-CH$_2$-C<u>H</u>$_2$-], 2.27 [2H, t, J = 7.8 Hz, C(=O)-C<u>H</u>$_2$-], 7.29 (1H, dd, J = 7.5, 8.1 Hz, Ar H-5), 7.32 (1H, d, J = 7.5 Hz, Ar H-4), 7.73 (1H, d, J = 8.1 Hz, Ar H-6), 7.92 (1H, s, Ar H-2), 9.85 (1H, s, N<u>H</u>); ^{13}C-NMR (DMSO-d_6) δ 13.9 (<u>C</u>H$_3$-CH$_2$-), 22.1 (CH$_3$-<u>C</u>H$_2$-CH$_2$- or CH$_3$-CH$_2$-<u>C</u>H$_2$-), 24.7 (<u>C</u>H$_3$ in boronate), 25.1 (C(=O)-CH$_2$-<u>C</u>H$_2$-), 28.5 (C(=O)-CH$_2$-CH$_2$-<u>C</u>H$_2$-), 28.6 (CH$_3$-CH$_2$-CH$_2$-<u>C</u>H$_2$-), 31.2 (CH$_3$-<u>C</u>H$_2$-CH$_2$- or CH$_3$-CH$_2$-<u>C</u>H$_2$-), 36.4 (C(=O)-<u>C</u>H$_2$-), 83.6 (B-O-<u>C</u>-<u>C</u>-O-B), 122.0 (Ar C-6), 125.0 (Ar C-2), 128.2 (Ar C-5), 128.9 (Ar C-4), 138.9 (Ar C-1), 171.3 (<u>C</u>=O). Anal. Calcd for $C_{20}H_{32}BNO_3$: C, 69.57; H, 9.34; N, 4.06. Found: C, 69.34; H, 9.50; N, 4.14.

***N*-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pentanamide (9b).** This compound was obtained from the reaction of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**7**) and valeryl chloride (**8b**) (121.1 μL, 1.00 mmol) as a white solid (164.3 mg, 54% yield). Mp 133-138 °C; IR (neat) 3252 (NH), 1656 cm^{-1} (C=O); FAB-MS (positive) *m*/*z* 304 $(M+H)^+$. HRMS (FAB) Calcd for $C_{17}H_{27}BNO_3^+$: *m*/*z* 304.2079 $(M+H)^+$. Found: 304.2088; ^{1}H-NMR (DMSO-d_6) δ 0.89 (3H, t, J = 7.2 Hz, C<u>H</u>$_3$-CH$_2$-), 1.29 [12H, s, C<u>H</u>$_3$ in boronate], 1.24-1.36 [2H, m, CH$_3$-C<u>H</u>$_2$-], 1.54-1.60 [2H, m, C(=O)-CH$_2$-C<u>H</u>$_2$], 2.28 (2H, t, J = 7.5 Hz, C(=O)-C<u>H</u>$_2$), 7.29 (1H, dd, J = 7.5, 8.1 Hz, Ar H-5), 7.32 (1H, d, J = 7.5 Hz, Ar H-4), 7.70-7.76 (1H, m, Ar H-6), 7.92 (1H, s, Ar H-2), 9.85 (1H, s, N<u>H</u>); ^{13}C-NMR (DMSO-d_6) δ 13.7 (<u>C</u>H$_3$-CH$_2$-), 21.8 (CH$_3$-<u>C</u>H$_2$-), 24.7 (<u>C</u>H$_3$ in boronate), 27.3 (C(=O)-CH$_2$-<u>C</u>H$_2$-), 36.1 (C(=O)-<u>C</u>H$_2$-), 83.6 (B-O-<u>C</u>-<u>C</u>-O-B), 122.0 (Ar C-6), 125.0 (Ar C-2), 128.2 (Ar C-5), 128.9 (Ar C-4), 138.9 (Ar C-1), 171.3 (<u>C</u>=O). Anal. Calcd for $C_{17}H_{26}BNO_3$: C, 67.34; H, 8.64; N, 4.62. Found: C, 67.22; H, 8.82; N, 4.67.

***N*-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)butyramide (9c).** This compound was obtained from the reaction of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**7**) and butyryl chloride (**8c**) (104.4 μL, 1.00 mmol) as a white solid (216.0 mg, 75% yield). Mp 126-129 °C; IR (neat) 3286 (NH), 1653 cm^{-1} (C=O); FAB-MS (positive) *m*/*z* 290 $(M+H)^+$. HRMS (FAB) Calcd for $C_{16}H_{25}BNO_3^+$: *m*/*z* 290.1922 $(M+H)^+$. Found: 290.1937; ^{1}H-NMR (DMSO-d_6) δ 0.91 (3H, t, J = 7.5 Hz,

C<u>H</u>$_3$-CH$_2$-), 1.29 (12H, s, C<u>H</u>$_3$ in boronate), 1.56-1.64 [2H, m, C(=O)-CH$_2$-C<u>H</u>$_2$], 2.26 (2H, t, *J* = 7.2 Hz, C(=O)-C<u>H</u>$_2$), 7.29 (1H, dd, *J* = 7.5, 8.1 Hz, Ar H-5), 7.32 (1H, d, *J* = 7.8 Hz, Ar H-4), 7.70-7.76 (1H, m, Ar H-6), 7.93 (1H, s, Ar H-2), 9.85 (1H, s, N<u>H</u>); ^{13}C-NMR (DMSO-*d*$_6$) δ 13.6 (<u>C</u>H$_3$-CH$_2$-), 18.5 (CH$_3$-<u>C</u>H$_2$-), 24.7 (<u>C</u>H$_3$ in boronate), 38.3 [C(=O)-<u>C</u>H$_2$-], 83.6 (B-O-<u>C</u>-<u>C</u>-O-B), 122.0 (Ar C-6), 125.0 (Ar C-2), 128.2 (Ar C-5), 128.9 (Ar C-4), 138.9 (Ar C-1), 171.1 (<u>C</u>=O). Anal. Calcd for $C_{16}H_{24}BNO_3$: C, 66.46; H, 8.37; N, 4.84. Found: C, 66.49; H, 8.62; N, 4.88.

NMR Study.

NMR Study Shown in Figure 3.

^{1}H-NMR of *C*$_2$-symmetrical phenylboronic acid pinacol ester (1f) in DMSO-*d*$_6$/D$_2$O=9/1: To a 20-mL vial were successively added *C*$_2$-symmetrical phenylboronic acid pinacol ester (**1f**) (17.71 mg, 30.00 μmol), DMSO-*d*$_6$ (945.0 μL), and D$_2$O (105.0 μL). A solution of 700.0 μL (20.00 μmol) of the resulting mixture was transferred into an NMR tube and ^{1}H-NMR was recorded. After 30 days, ^{1}H-NMR data were again obtained. ^{1}H-NMR spectra using pinacol (**5**) (3.546 mg, 30.00 μmol) and *C*$_2$-symmetrical phenylboronic acid (**2f**) (12.78 mg, 30.00 μmol) were also obtained in the same manner as that described above.

NMR Study Shown in Figure 4.

^{1}H-NMR of *C*$_2$-symmetrical phenylboronic acid pinacol ester (1f) in DMSO-*d*$_6$: To a 20-mL vial were added *C*$_2$-symmetrical phenylboronic acid pinacol ester (**1f**) (17.71 mg, 30.00 μmol) and DMSO-*d*$_6$ (1.050 mL). A solution of 700.0 μL (20.00 μmol) of the resulting mixture was transferred into an NMR tube and ^{1}H-NMR was recorded. ^{1}H-NMR spectra using methyl α-D-glucopyranoside (**6**) (5.826 mg, 30.00 μmol) were also obtained in the same manner as that described above.

^{1}H-NMR of a 1:1 mixture of *C*$_2$-symmetrical phenylboronic acid pinacol ester (1f) and α-D-glucopyranoside (6) in DMSO-*d*$_6$: To a 20-mL vial were successively added *C*$_2$-symmetrical phenylboronic acid pinacol ester (**1f**) (17.71 mg, 30.00 μmol), methyl α-D-glucopyranoside (**6**) (5.826 mg, 30.00 μmol), and DMSO-*d*$_6$ (1.050 mL). A solution of 700.0 μL (20.00 μmol) of the resulting mixture was transferred into an NMR tube and ^{1}H-NMR was recorded within 1 h.

NMR Study Shown in Figure 5.

^{1}H-NMR of *C*$_2$-symmetrical phenylboronic acid (2f) in DMSO-*d*$_6$: To a 20-mL vial were added *C*$_2$-symmetrical phenylboronic acid (**2f**) (12.78 mg, 30.00 μmol) and DMSO-*d*$_6$ (1.050 mL). A solution of 700.0 μL (20.00 μmol) of the resulting mixture was transferred into an NMR tube and ^{1}H-NMR was recorded.

^{1}H-NMR of a 1:1 mixture of *C*$_2$-symmetrical phenylboronic acid (2f) and α-D-glucopyranoside (6) in DMSO-*d*$_6$: To a 20-mL vial were successively added *C*$_2$-symmetrical phenylboronic acid (**2f**) (12.78

mg, 30.00 μmol), methyl α-D-glucopyranoside (**6**) (5.826 mg, 30.00 μmol), and DMSO-d_6 (1.050 mL). A solution of 700.0 μL (20.00 μmol) of the resulting mixture was transferred into an NMR tube and ^{1}H-NMR was recorded within 1 h. A new signal at δ 9.85 (1H, s, NH) and four new signals for aromatic regions at δ 7.21-7.29 (1H, m, Ar H-5), 7.39 (1H, d, J = 6.6 Hz, Ar H-4), 7.77 (1H, d, J = 7.8 Hz, Ar H-6), 7.83 (1H, s, Ar H-2), and 3.32 (3H, s, CH_3O) appeared by blending (in Figure 5), apparently indicating a new C_2-symmetrical phenylboronic acid derivative. The NMR spectrum of a mixture of free boronic acid **2f** and methyl α-D-glucopyranoside **6** (**2f** : **6** = 1:2) showed the same pattern of signals for aromatic and aliphatic regions (sugar and linker moieties). Successive full assignments for these complicated signals in aliphatic regions (sugar and linker moieties) were difficult.

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 phenylboronic acid derivatives were dissolved in DMSO or dimethylformamide (DMF) to a concentration of 1.280 μg/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.[19,20] 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 phenylboronic acid derivatives were measured by using a plaque reduction assay,[16] and their cytotoxicity against Vero cells (CC_{50}) was also evaluated.

ACKNOWLEDGEMENTS

This work was supported by a Grant-in-Aid for Young Scientists B (KAKEHI no. 17K15486) from the JSPS.

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13. In ^{13}C-NMR spectra of compounds **2** and **9**, we consider that the difficulty in observing the corresponding signal of this quaternary aromatic carbon linked with a boronic acid ester functionality is attributable to the quadrupolar relaxation of ^{11}B.[12]
14. During HPLC analysis of the pinacol ester **1f** in acetonitrile-containing water (MeCN/H_2O=9/1), we observed that the two pinacol ester functionalities in the C_2-symmetrical molecule were easily hydrolyzed to free phenylboronic acid **2f**. The formation of free aminoarylboronic acid by hydrolysis of the corresponding aminoarylboronic acid pinacol ester has been described in a recent paper (see reference 15).
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