HETEROCYCLES, Vol. 96, No. 1, 2018, pp. 144 - 151. © 2018 The Japan Institute of Heterocyclic Chemistry
Received, 30th October, 2017, Accepted, 4th December, 2017, Published online, 13th December, 2017
DOI: 10.3987/COM-17-13832

NOVEL TRIVALENT C_3-SYMMETRICAL PHENYLBORONIC ACID PINACOL ESTERS AND THEIR BIOLOGICAL EVALUATION

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Abstract – We report the preparation of newly designed trivalent C_3-symmetrical cyclic phenylboronic acid derivatives constructed on a symmetrical benzene or a cyclohexane ring. The synthesis of these C_3-symmetrical molecules **4** was accomplished by an amide bond formation reaction using amino-substituted phenylboronic acid pinacol esters **2** and C_3-symmetrical benzene-1,3,5-tricarboxylic acid trichloride **3a** or cyclohexane-1,3,5-tricarboxylic acid trichloride **3b** in the presence of Et_3N. We confirmed that this procedure is conventionally applicable to the preparation of targeted C_3-symmetrical cyclic boronic acid derivatives **4** in good to excellent yields. We also report the results of biological evaluation of the prepared compounds.

Many host receptors that consist of homo-oligometric units (homo-multiligands) often construct symmetric macromolecule architectures such as C_2- or C_3-symmetrical geometry receptor systems. Molecular recognition of two-fold (C_2) or three-fold (C_3) geometric symmetrical macromolecules is one of the common features in several important biological processes.[1,2] With reference to the molecular symmetry, small oligovalent molecules having C_2- or C_3-symmetrical geometry have frequently appeared in various biologically active substances.[2-4] An oligovalent molecule is generally expected to show enhanced biological potential compared to that of the corresponding monovalent molecule.[4] On the other hand, regarding lectin-like carbohydrate recognition molecules, much attention has recently been paid to the design of synthetic receptors for carbohydrates.[5] We have been interested in small molecules that

interfere with such carbohydrate recognition stages in order to find new bioactive leads.[6-8] Regarding carbohydrate (sugar chain) recognition molecules, we have been particularly interested in boronic acid derivatives because many boronic acid functionalities (**A**) have a property to react with various 1,2-diol functionalities included in carbohydrates and generate cyclic derivatives (**B**) formed with reversible covalent bonds (see Figure 1).[9,10] From this point of view, we have recently synthesized a few C_2-symmetrical cyclic phenylboronic acid esters as new targeted bivalent molecules.[11,12] Among previously targeted C_2-symmetrical bivalent phenylboronic acid ester derivatives in this series, we have found an interesting bivalent seed molecule **1** (Figure 1) possessing significant antibacterial and anti-herpes simplex virus type 1 (anti-HSV-1) activities. The identified bivalent C_2-symmetrical molecule **1** has a C7-methylene linker and two *meta*-oriented boronic acid pinacol ester functionalities in the molecule. Compound **1** showed a high level of antibacterial activity (MIC=27.1 μM) against a Gram-positive strain [*Staphylococcus aureus* (*S. aureus*)] and anti-HSV-1 activity [50% effective concentration (EC_{50})=8.0 μM].[11]

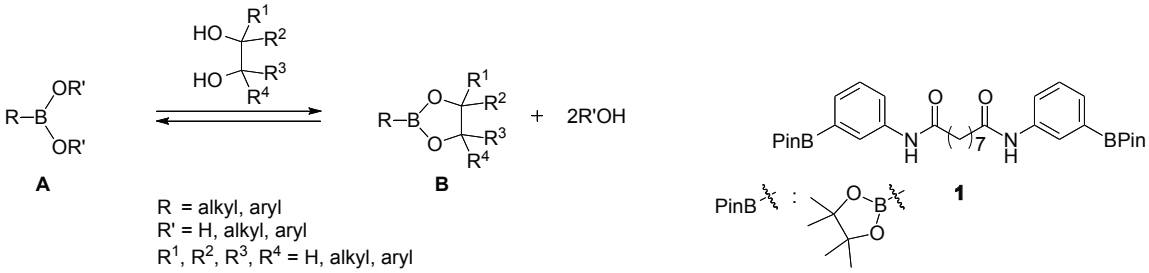
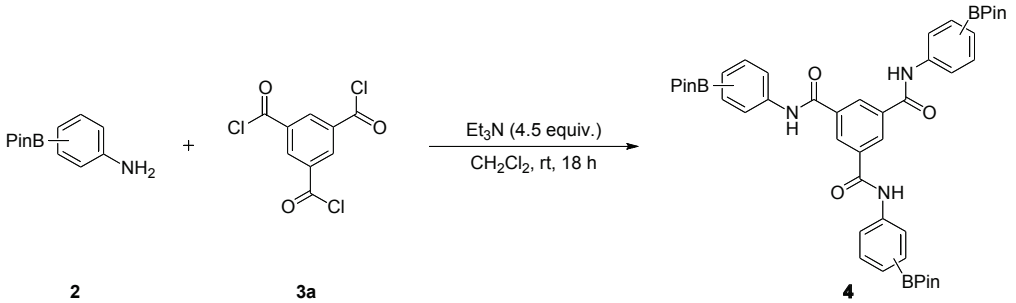


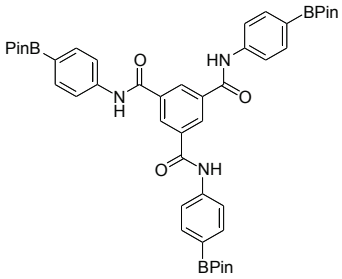
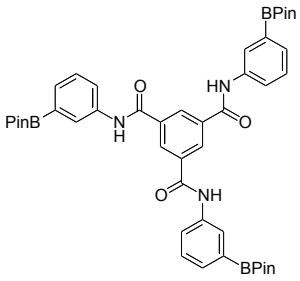
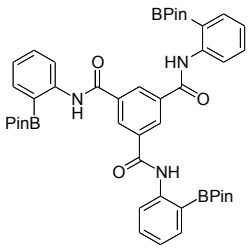
Figure 1

As an extension of this study, we carried out further investigations of these symmetrical classes of compounds. In this paper, we report the synthesis of new trivalent C_3-symmetrical phenylboronic acid derivatives incorporating a benzene or cyclohexane ring as a symmetrical template in the molecules and we report results of biological evaluations of the obtained C_3-symmetrical phenylboronic acid derivatives. The results for synthesis of new C_3-symmetrical cyclic boronic acid derivatives constructed on a benzene ring or a cyclohexane ring having three amide bonds are summarized in Tables 1 and 2. The synthesis of these target trivalent C_3-symmetrical molecules **4aa**~**4ca** constructed on a benzene ring was accomplished by an amide bond formation reaction using amino-substituted phenylboronic acid pinacol esters **2** and benzene-1,3,5-tricarboxylic acid trichloride **3a** in the presence of a base such as Et_3N. The preparation and physical and spectroscopic data of compounds **4aa**~**4ca** have been reported in detail as a separated paper.[13] New C_3-symmetrical compounds **4ab** and **4bb** constructed on a cyclohexane ring were also prepared by a procedure similar to that for compounds **4aa**~**4ca** with cyclohexane-1,3,5-tricarboxylic acid trichloride in good yields (see EXPERIMENTAL). The structures of targeted trivalent C_3-symmetrical products **4ab** and **4bb** were confirmed by both spectroscopic data and elemental analysis.[14,15] The

Table 1. Preparation of Trivalent C_3-Symmetrical Phenylboronic Acid Pinacol Esters



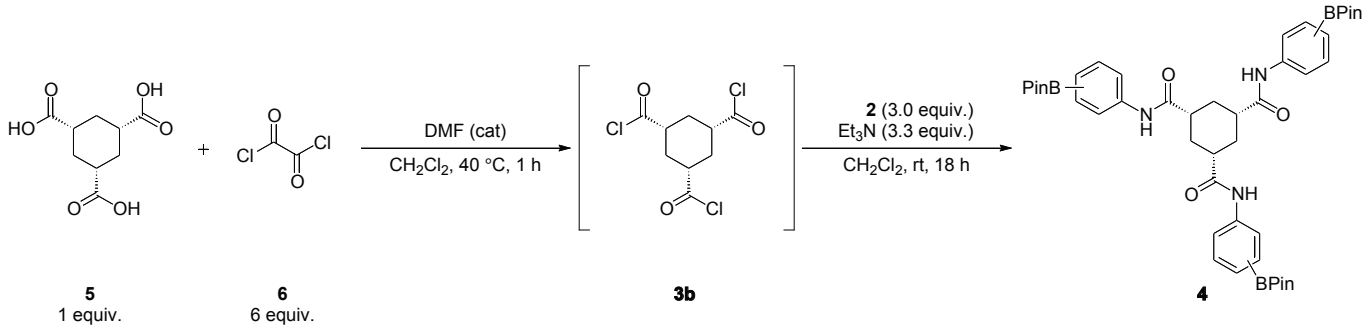
2 3 equiv. **3a** 1 equiv. **4**

Entry	Product **4**		Yield (%)[a]	MIC [μM (μg/mL)] *S. aureus*		*E. coli*		EC_{50} (μM) Anti-HSV-1 activity	CC_{50} (μM) Cytotoxic activity
1		**4aa**	91[b]	>157.4	(>128)	157.4	(128)	>100	101.0
2		**4ba**	81[b]	157.4	(128)	>157.4	(>128)	>100	>200
3		**4ca**	36[b] (76[b,c])	157.4	(128)	>157.4	(>128)	>100	>200

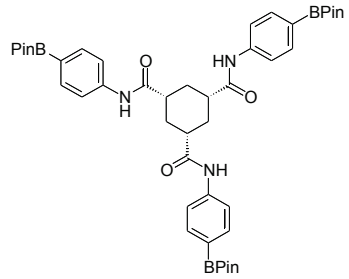
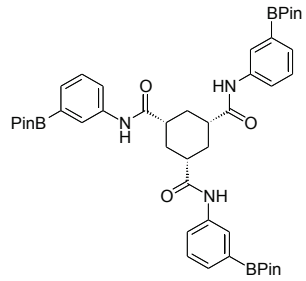
[a] Isolated yield. [b] Data were taken from ref.13. [c] Estimated crude yield.

C_3-symmetrical structures of synthesized boronic acid derivatives **4ab** and **4bb** were confirmed by ^{13}C-NMR spectroscopic analysis. All C_3-symmetrical cyclic phenylboronic acid pinacol esters **4** showed magnetically equivalent carbon signals assignable to a third of the molecules that indicated C_3-symmetrical molecular structures in solution, except for the carbon in a phenyl ring connected to a substituent (boronic acid esters).[14] The structures of the C_3-symmetrical phenylboronic acid derivatives **4** and the results of evaluation of biological activities (antibacterial and anti-HSV-1 activities) are summarized in Tables 1 and 2. All C_3-symmetrical molecules **4aa**~**4ca** constructed on a benzene ring were inactive by assays for antibacterial activities [against *S. aureus* and *Escherichia coli* (*E. coli*)] and anti-HSV-1 activities. Only *para*-substituted compound **4aa** showed a low level of cytotoxic activity [50%

Table 2. Preparation of Trivalent C_3-Symmetrical Phenylboronic Acid Pinacol Esters[15]



5 1 equiv. **6** 6 equiv. **3b** **4**

Entry	Product **4**		Yield (%)[a]	MIC [μM (μg/mL)] *S. aureus*	*E. coli*	EC_{50} (μM) Anti-HSV-1 activity	CC_{50} (μM) Cytotoxic activity
1		**4ab**	46	156.2 (128)	>156.2 (>128)	>100	>200
2		**4bb**	77	156.2 (128)	>156.2 (>128)	65.2	>200

[a] Isolated yield.

cytotoxic concentration (CC_{50})=101.0 μM].

On the other hand, among the C_3-symmetrical compounds constructed on a cyclohexane ring, *meta*-substituted compound **4bb** showed a moderate level of anti-HSV-1 activity (EC_{50}=65.2 μM) with a low level of cytotoxic activity (CC_{50}=>200 μM). The results of biological evaluation of previously prepared compounds **1** with a flexible methylene linker indicated that the length of a linker is important for the expression of biological activities.[11] Regarding the C_3-type molecules described in this paper, a saturated flexible cyclohexane core (compound **4bb**) seems to be a preferable structure rather than a rigid unsaturated benzene core for the expression of anti-HSV-1 activity. Trivalent C_3-symmetrical phenylboronic acid pinacol esters **4bb** in this study also had an *meta*-substituted cyclic boronic acid ester group on the phenyl rings. Regarding C_3-type molecules in this study, it is noteworthy that *meta*-substituted cyclic boronic acid ester (**4bb**) was again biologically active compound and that compound **4bb** with a flexible cyclohexane ring as the C_3-template showed remarkable anti-HSV-1 activity (EC_{50}=65.2 μM) compared to that of compound **4ba** with a rigid benzene ring as the C_3-template.

On the basis of the structural information obtained by the above-described modifications for biological activities in the C_2- and C_3-symmetrical phenylboronic acid ester series together with recent information on other receptor-type molecule series, further molecular modifications to other new types of molecules are now under investigation in order to develop new promising bioactive leads.

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 [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 ^{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 C_3-Symmetrical Cyclic Phenylboronic Acid Pinacol Esters.

The preparation of and spectroscopic data for the three compounds **4aa**~**4ca** in Table 1 have already been reported.[13] New C_3-symmetrical compounds **4ab** and **4bb** constructed on a cyclohexane ring were also prepared by a procedure similar to that for compounds **4aa**~**4ca** with (1s,3s,5s)-cyclohexane-1,3,5-tricarboxylic acid **5** in good yields (46-77%).

(1s,3s,5s)-N^1,N^3,N^5-Tris(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)cyclohexane-1,3,5-tricarboxamide (4ab). To a solution of (1s,3s,5s)-cyclohexane-1,3,5-tricarboxylic acid (**5**) (216.2 mg, 1.00 mmol) in CH_2Cl_2 (6.000 mL) were added oxalyl chloride (**6**) (514.1 μL, 6.00 mmol) and DMF (2 drops). The resulting mixture was stirred at reflux for 1 h. After evaporation, CH_2Cl_2 (16.00 mL), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**2a**) (657.3 mg, 3.00 mmol), and Et_3N (457.5 μL, 3.30 mmol) were successively added to the mixture. The resulting solution was stirred for 18 h at room temperature and then water (ca. 100 mL) was added. The mixture was extracted with CH_2Cl_2 (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 product (**4ab**) (377.1 mg, 46% yield) as a white solid. Mp >250 °C; IR (KBr) 3450 (NH), 1655 cm^{-1} (C=O); FAB-MS (positive) *m*/*z* 820 $(M+H)^+$. HRMS (FAB) Calcd for $C_{45}H_{61}B_3N_3O_9^+$: *m*/*z* 820.4681 $(M+H)^+$. Found: 820.4696; ^{1}H-NMR (DMSO-d_6) δ 1.27 (36H, s, C$\underline{H}_3$), 1.66 [3H, dd, J = 13.2, 25.8 Hz, C(=O)-CH-C$\underline{H}_2$], 1.97-2.05 [3H, m, C(=O)-CH-C$\underline{H}_2$], 2.51-2.60 [3H, m, C(=O)-C$\underline{H}$-CH$_2$], 7.60 (6H, d, J = 8.4 Hz, Ar H-3, H-5 in B-C$_6\underline{H}_4$-N), 7.63 (6H, d, J = 8.4 Hz, Ar H-2, H-6 in B-C$_6\underline{H}_4$-N), 10.01 (3H, s, N$\underline{H}$); ^{13}C-NMR (DMSO-d_6) δ 24.7 (C$\underline{H}_3$), 31.1 [C(=O)-CH-C$\underline{H}_2$], 43.5 [C(=O)-C$\underline{H}$-CH$_2$], 83.4 (B-O-C$\underline{-}$C-O-B), 118.3 (Ar C-2, C-6 in B-C$_6$H$_4$-N), 135.2 (Ar C-3, C-5 in B-C$_6$H$_4$-N), 142.0 (Ar C-1 in

B-C$_6$H$_4$-N), 173.2 (C=O). Anal. Calcd for $C_{45}H_{60}B_3N_3O_9$•1.7H_2O: C, 63.58; H, 7.52; N, 4.94. Found: C, 63.55; H, 7.25; N, 4.85.

(1s,3s,5s)-*N*^{1},*N*^{3},*N*^{5}-Tris(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)cyclohexane-1,3,5-tricarboxamide (4bb). To a solution of (1s,3s,5s)-cyclohexane-1,3,5-tricarboxylic acid (**5**) (216.2 mg, 1.00 mmol) in CH_2Cl_2 (6.000 mL) were added oxalyl chloride (**6**) (514.1 μL, 6.00 mmol) and DMF (2 drops). The resulting mixture was stirred at reflux for 1 h. After evaporation, CH_2Cl_2 (16.00 mL), 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**2b**) (657.3 mg, 3.00 mmol), and Et_3N (457.5 μL, 3.30 mmol) were successively added to the mixture. The resulting solution was stirred for 18 h at room temperature and then water (ca. 100 mL) was added. The mixture was extracted with CH_2Cl_2 (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 product (**4bb**) (629.2 mg, 77% yield) as a white solid.

The structure of the product was easily established by spectroscopic data shown below.

Mp >250 °C; IR (KBr) 3444 (NH), 1658 cm^{-1} (C=O); FAB-MS (positive) *m*/*z* 820 $(M+H)^+$. HRMS (FAB) Calcd for $C_{45}H_{61}B_3N_3O_9^+$: *m*/*z* 820.4681 $(M+H)^+$. Found: 820.4682; ^{1}H-NMR (DMSO-d_6) δ 1.27 (36H, s, CH_3), 1.65 [3H, dd, *J* = 12.6, 25.8 Hz, C(=O)-CH-CH_2], 1.91-2.03 [3H, m, C(=O)-CH-CH_2], 2.43-2.56 [3H, m, C(=O)-CH-CH_2], 7.26-7.35 (6H, m, Ar H-4, H-5 in B-C$_6$H$_4$-N), 7.75 (3H, d, *J* = 6.6 Hz, Ar H-6 in B-C$_6$H$_4$-N), 7.91 (3H, s, Ar H-2 in B-C$_6$H$_4$-N), 9.87 (3H, s, NH); ^{13}C-NMR (DMSO-d_6) δ 24.7 (CH_3), 31.2 [C(=O)-CH-CH_2], 43.6 [C(=O)-CH-CH_2], 83.6 (B-O-C-C-O-B), 122.3 (Ar C-6 in B-C$_6$H$_4$-N), 125.4 (Ar C-2 in B-C$_6$H$_4$-N), 128.3 (Ar C-5 in B-C$_6$H$_4$-N), 129.1 (Ar C-4 in B-C$_6$H$_4$-N), 138.8 (Ar C-1 in B-C$_6$H$_4$-N), 173.0 (C=O).

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 μg/mL. The MIC of a standard strain was measured by the authentic microdilution method to monitor bacterial growth turbidity in Müller-Hinton broth according to the Japanese Society of Chemotherapy.[17,18] 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,[19] 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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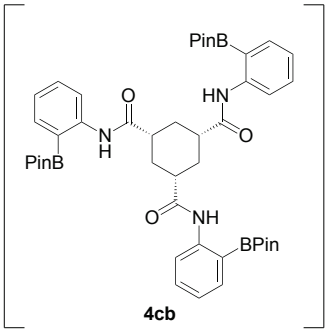
14. In ^{13}C-NMR spectra of compounds **4**, we consider that the difficulty in observing the corresponding signal of this quaternary aromatic carbon linked with a boronic acid ester functionality is attributable

to the quadrupolar relaxation of ^{11}B.[16]

15. In the reaction in which *ortho*-amino-substituted phenylboronic acid pinacol ester was used, the target *ortho*-substituted C_3-type compound **4cb** could not be isolated and the reaction provided a complex mixture including a few unknown compounds. Our attempts to isolate the target C_3-type *ortho*-substituted **4cb** were unsuccessful. However, the observation of a product showing an amide absorption [3444 (NH) and 1633 cm^{-1} (C=O)] and an ion peak [*m*/*z* 820 $(M+H)^+$] indicate formation of the C_3-type target compound **4cb**.



4cb

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