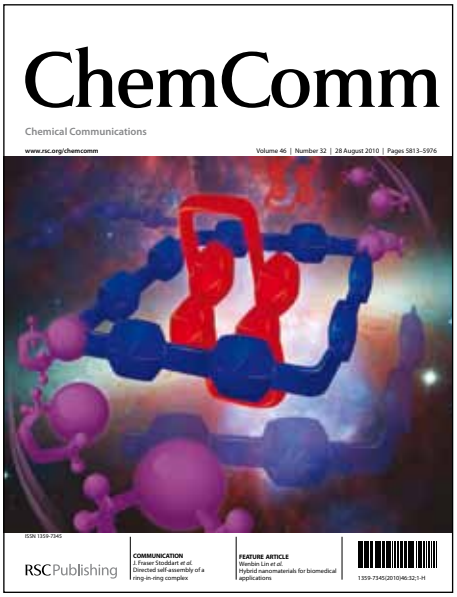
ChemComm

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: H. Ueno, T. Iwata, N. Koshiba, D. Takahashi and K. Toshima, *Chem. Commun.*, 2013, DOI: 10.1039/C3CC45275A.



This is an *Accepted Manuscript*, which has been through the RSC Publishing peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, which is prior to technical editing, formatting and proof reading. This free service from RSC Publishing allows authors to make their results available to the community, in citable form, before publication of the edited article. This *Accepted Manuscript* will be replaced by the edited and formatted *Advance Article* as soon as this is available.

To cite this manuscript please use its permanent Digital Object Identifier (DOI®), which is identical for all formats of publication.

More information about *Accepted Manuscripts* can be found in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics contained in the manuscript submitted by the author(s) which may alter content, and that the standard **Terms & Conditions** and the **ethical guidelines** that apply to the journal are still applicable. In no event shall the RSC be held responsible for any errors or omissions in these *Accepted Manuscript* manuscripts or any consequences arising from the use of any information contained in them.

Dynamic Article Links ▶
View Article Online
DOI: 10.1039/C3CC45275A

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Design, synthesis and evaluation of a boronic acid based artificial receptor for L-DOPA in aqueous media

Haruna Ueno, Takayuki Iwata, Nozomi Koshiba, Daisuke Takahashi* and Kazunobu Toshima*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A designed and synthesised boronic acid based artificial receptor selectively and effectively bound to a neurotransmitter, L-DOPA (1), in aqueous media. In addition, the synthetic receptor was found to effectively inhibit the DDC (L-DOPA decarboxylase) enzymatic reaction under physiological conditions.

L-DOPA (3,4-dihydroxyphenylalanine) (**1**) is a precursor of one of the important neurotransmitters, dopamine (**2**). L-DOPA is converted to **2** by L-DOPA decarboxylase (DDC, E.C. 4.1.1.28) in the presence of the co-enzyme, pyridoxal-5'-phosphate (PLP). In addition, **1** is generally accepted as the gold standard for drugs for alleviating the symptoms of Parkinson's disease (PD).[1] PD is a neurodegenerative disease mainly caused by the progressive loss of **2** in the central nervous system (CNS).[2] Although administration of exogenous **1** to PD patients compensates for inadequate dopamine synthesis and often dramatically alleviates the symptoms, only 1% of an orally administered dose of **1** reaches the dopaminergic neurons of the brain. The remainder is metabolized by the peripheral DDC to **2**.[3] Therefore, co-administration of **1** with peripheral DDC inhibitors (such as carbidopa[4] and benserazide[5]), which cannot cross the blood-brain barrier (BBB), is the most effective symptomatic treatment for PD. However, early clinical studies comparing carbidopa/**1** with **1** monotherapy showed that more than 75% of patients treated with carbidopa/**1** experienced marked dyskinesia as a severe side effect.[6] Therefore, the development of novel therapeutic agents which can prolong the peripheral half-life of **1** without causing serious side effects is urgently needed. Herein we describe the molecular design, chemical synthesis, and evaluation of artificial receptors which can selectively bind to **1** and reduce the rate of first-pass metabolism by DDC under physiological conditions. To the best of our knowledge, this is the first report of an artificial receptor that shows high affinity and selectivity for **1** over **2** under neutral aqueous conditions.

To date, several boronic acid based catecholamine receptors and chemosensors,[7,8] which can reversibly bind to a 1,2-diol in catecholamines in aqueous media at neutral pH, have been designed and synthesized as promising candidates. However, none of these receptors display selectivity for L-DOPA over dopamine and other catecholamines under aqueous conditions. In this context, to develop L-DOPA-specific receptors, we chose a phenylboronic acid for boronic ester formation with the 1,2-diol in **1**, and a guanidino group for ionic interaction with the carboxylic acid in **1**. Because no other catecholamines have a carboxylic acid in their structure, this ionic interaction may contribute to high affinity and selectivity for **1** (Figure 1B). To investigate our hypothesis, we designed nine compounds (**4a-c**, **5a-c**, and **6a-c**) by changing the position of an amide group to a boronyl group in the benzene ring (*ortho*, *para*, and *meta* isomers) and the length of the spacer between the benzene ring and the guanidino group (n = 1-3) (Figure 1A).

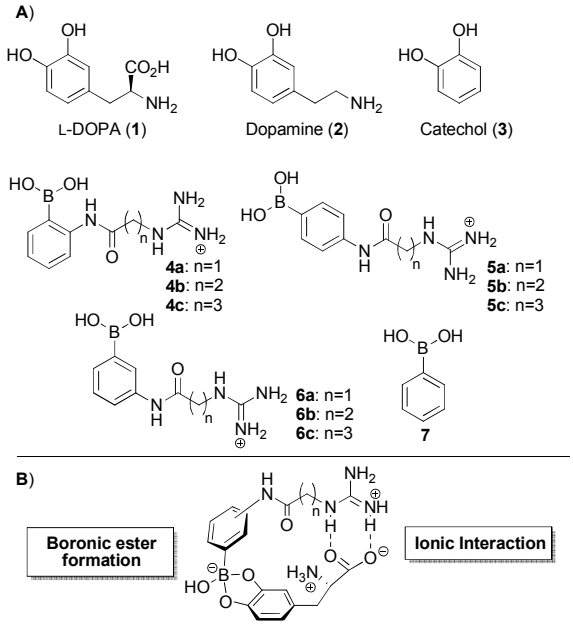


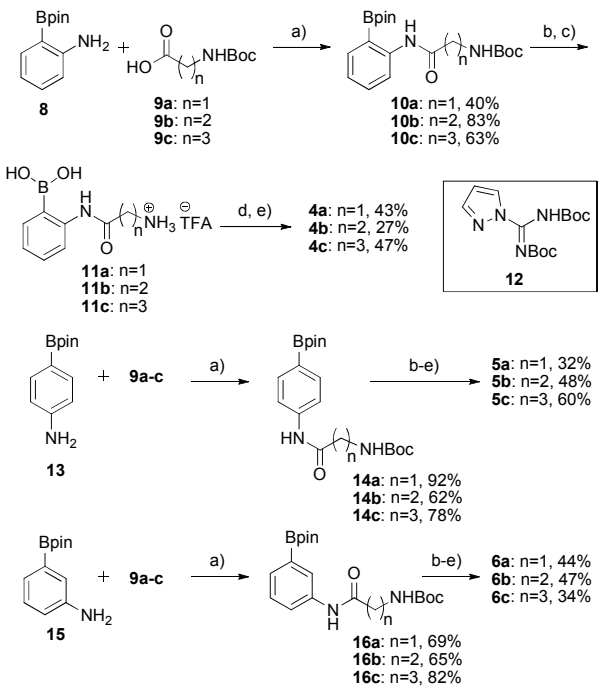
Figure 1. A) Chemical structures of L-DOPA (**1**), dopamine (**2**), catechol (**3**), designed artificial receptors **4-6**, and phenylboronic acid (**7**). B) Expected binding mode between designed artificial receptors and L-DOPA (**1**).

The synthesis of **4-6** is outlined in Scheme 1. Amidation reactions of *ortho*-anilineboronic acid (**8**) with Boc-glycine (**9a**), Boc-β-alanine (**9b**), and **9c** were performed using DMT-MM in MeOH to afford **10a-c**, respectively. Deprotection of the Boc group of **9a-c** and subsequent removal of pinacol ester provided

Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522 (Japan). E-mail: dtak@applc.keio.ac.jp, toshima@applc.keio.ac.jp; Fax: (+81) 45-566-1576.

† Electronic supplementary information (ESI) available: See DOI: 10.1039/b000000x/

View Article Online
DOI: 10.1039/C3CC45275A



Scheme 1. Synthetic scheme for **4-6**. *Reagents and conditions*: a) DMT-MM, MeOH, 40 °C; b) TFA, CH_2Cl_2; c) Acetone/H_2O; d) **12**, DIEA, MeCN/H_2O; e) 4.0 M HCl in 1,4-dioxane. pin = pinacol, Boc = *t*-butoxycarbonyl, TFA = trifluoroacetic acid, DMT-MM = 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride, DIEA = *N*,*N*-diisopropylethylamine.

ortho-substituted phenylboronic acid derivatives **11a-c**. Treatment of **11a-c** with **12**, followed by deprotection of the Boc groups, provided *ortho*-substituted artificial receptors **4a-c**, respectively. Similarly, *para*- and *meta*-substituted artificial receptors **5a-c** and **6a-c** were also synthesized in moderate yields.

After chemical synthesis of the designed compounds, the binding abilities of **4-7** with **1-3** were examined using ^{1}H NMR titration binding assays[9] in phosphate buffer (D_2O, 100 mM, pH 7.4). The association constants (K_a) were calculated based on the integration of the selected peak area corresponding to the free receptor (boronic acid) and the guest complex (see Figures S1a and S1b in ESI). The results are summarized in Figure 2. It was found that **7**, which does not possess a guanidino group, moderately bound to the 1,2-diol in **1-3**; the K_a values obtained for **7** with respect to **1-3** were 770, 800 and 560 M^{-1}, respectively. In contrast, the *ortho*-substituted compounds **4a**-c did not bind to **1-3** (K_a <10). These results suggested that the *ortho*-substituents in **4a-c** are close to the boron atom, and **4a-c** exist mainly in equilibrium between their stable cyclic form **4'** and **4''**, owing to the partial aromatic character of the boron in heterocycles, as shown in Figure 3A.[10] The ^{11}B NMR chemical shift of **4a** also supports the existence of the cyclic form (see Table S1 in ESI).

The K_a values for the *para*-substituted compounds **5a-c** bound to **1-3** showed a similar tendency to the values obtained for **7** with respect to **1-3**. Differences in the length of the spacer in **5a-c** did not affect binding. These results clearly indicated that the positively charged guanidino side chain of **5a-c** could not reach the negatively charged carboxyl group in **1**, and thus the guanidino and carboxyl groups could not ionically interact. In contrast, when the *meta*-substituted compounds **6a-c** were used

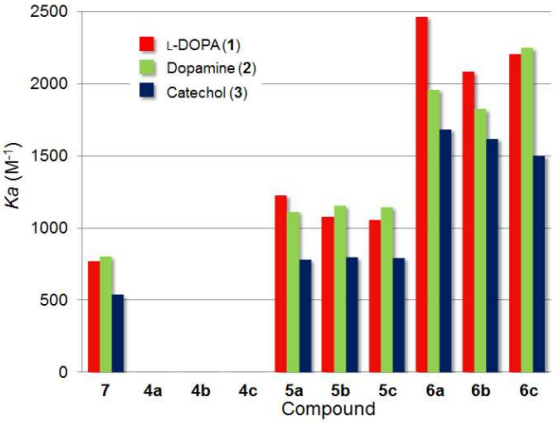


Figure 2. Association constants (K_a) obtained for **4-7** with **1-3**. The K_a values were determined by ^{1}H NMR titrations in phosphate buffer (D_2O, 100 mM, pH 7.4) and are the average of at least two reproducible measurements; see Electronic Supplementary Information (ESI).

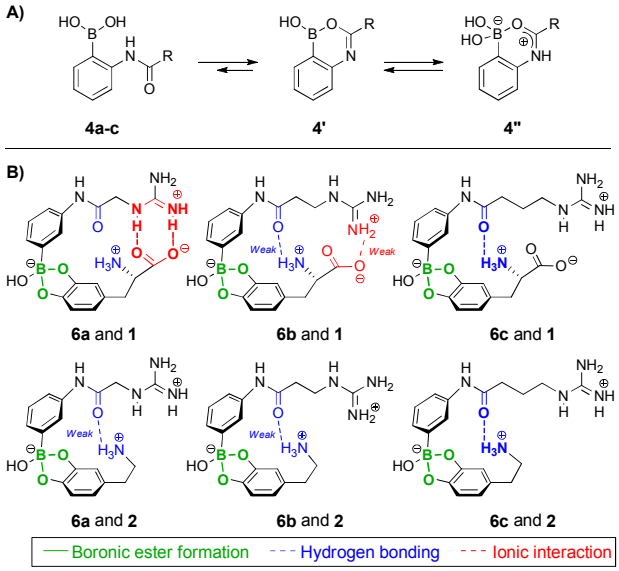


Figure 3. A) Boron containing heterocycles **4'** and **4''** and the formation of **4a-c**. B) Proposed binding mode between **6a-c** and **1** or **2**.

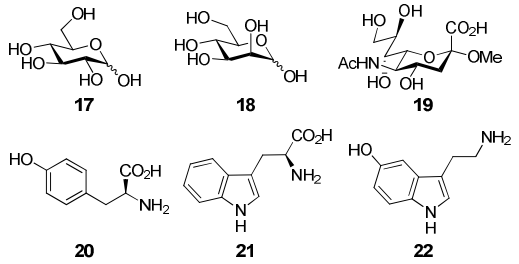
with **3**, K_a values in the range of 1500-1690 M^{-1} were obtained, which is almost three times greater than the value obtained for **7** with **3**. Although we have yet to obtain direct evidence, these results may suggest that NH/π interaction between the benzene ring of **3** and the hydrogen atom(s) in the guanidino group in **6a-c** contribute to the increase in K_a values. In addition, it was found that **6a** showed the highest affinity and selectivity for **1** over **2** and **3**. Furthermore, the order of the K_a values obtained for **6a-c** with **1** was **6a** > **6c** ≈ **6b**, whereas the order of the K_a values obtained for **6a-c** with **2** was **6c** > **6a** ≈ **6b**. A comparison of the K_a values for **6a-c** with **1** suggests that the expected ionic interaction between the guanidino group in **6a** and the carboxyl group in **1** is sufficient for selective recognition. In addition, from a comparison of the K_a values for **6a-c** with **2**, it appears that hydrogen bonding between the amide group in **6a-c** and the amino group in **1** or **2**, except in case of **6a** and **1**, is also an important factor in binding. However, we have not obtained direct evidence for the hydrogen bonding yet. Although the exact binding mode remains unclear, taking these results together indicates that **6a** can selectively and effectively bind to **1** by a

View Article Online
DOI: 10.1039/C3CC45275A

two-point interaction as follows: 1) boronic ester formation between **6a** and 1,2-diol in **1**[11]; and 2) ionic interaction between the guanidino group in **6a** and the carboxyl group in **1**[12] (Figure 3B).

Next, to confirm the selective binding ability of **6a**, binding assays were conducted under identical conditions using other biologically important potential guests in humans that may show affinity with **6a**. These compounds included sugars **17** and **18** possessing 1,2-diols, sialic acid **19** possessing both 1,2-diols and a carboxylic acid, amino acids **20** and **21** possessing both an aromatic ring and an α-amino acid structure, and neurotransmitter **22** which possesses a phenol, an amino group and an aromatic ring. These assays showed that **17**-**22** all have low affinity for **6a** (Table 1), and indicate that our designed molecule **6a** selectively binds to **1** with high affinity under neutral aqueous conditions.

Table 1 Association constants (K_a) obtained for **6a** with several guests.



Entry	Guest	K_a [M^{-1}][a]
1	D-Glucose (**17**)	<10
2	D-Mannose (**18**)	<10
3	Neu5Acα-OMe (**19**)	<10
4	L-Tyrosine (**20**)	<10
5	L-Tryptophan (**21**)	<10
6	Serotonin (**22**)	<10

[a]The K_a values were determined by ^{1}H NMR titrations in phosphate buffer (D_2O, 100 mM, pH 7.4), and are the average of at least two reproducible measurements; see Electronic Supplementary Information (ESI).

We next examined the inhibition activity of the DDC enzymatic reaction by **4a** and **6a**, which exhibit low and high affinity, respectively, towards **1**. The progress of the DDC enzymatic reaction was monitored by measuring the production of dopamine (**2**) from L-DOPA (**1**) with a spectrophotometric assay using 2,4,6-trinitrobenzene-1-sulfonic acid (TNBS).[13] The results are summarized in Figure 4. It was found that, due to its low affinity for **1**, **4a** did not inhibit the DDC enzymatic reaction. In sharp contrast, when equimolar amounts of **6a** to **1** were used, the DDC enzymatic reaction was significantly inhibited. These results clearly indicate that our designed and synthesized artificial receptor **6a** effectively binds to **1** and inhibits the DDC enzymatic reaction under physiological conditions.

In conclusion, our designed and synthesized boronic acid based artificial receptor **6a** was found to selectively and effectively bind to **1** through boronic ester formation and ionic interaction between the guanidino group in **6a** and the carboxyl group in **1**. In addition, we demonstrated that **6a** effectively binds to **1** and inhibits the DDC enzymatic reaction under physiological conditions. This approach should provide a new strategy for inhibiting the peripheral DDC catalysis of **1**. Although the binding selectivity of **6a** toward **1** is moderate, we hope the results presented here will contribute to the molecular design of

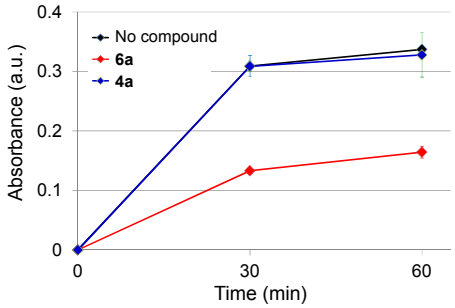


Figure 4. Time courses of DDC enzymatic reaction in the absence (black) or presence of **4a** (blue) and **6a** (red). DDC (3.5 μg/mL) was incubated with **1** (1.0 mM) and PLP (0.1 mM) in the absence or presence of each receptor molecule (1.0 mM) in 50 mM HEPES buffer (containing 100 mM NaCl, pH 7.2) at 37 °C for 30 or 60 min. To the reaction mixture was added TNBS (5% in H_2O), and the mixture was incubated at 37 °C for 20 min. Then, 2,4,6-trinitrophenyl (TNP)-dopamine, which was formed by reaction between **2** and TNBS, was extracted with benzene. The progress of the DDC enzymatic reaction was monitored by UV analysis (340 nm) of the extracted TNP-dopamine.

novel therapeutic agents that can regulate the concentration of **2** in the CNS without causing serious side effects.[14] The development of more specific and higher affinity binding receptors for **1** is now under investigation in our laboratory.

The authors wish to thank Prof. Dr. S. Shimizu, Faculty of Science and Technology, Keio University, for valuable discussion on cell viability assay.

Notes and references

1. A. H. V. Schapira, M. Emre, P. Jenner and W. Poewe, *Eur. J. Neurol.*, 2009, **16**, 982.
2. (a) H. Bernheimer, W. Birkmayer, O. Hornykiewicz, K. Jellinger and F. Seitelberger, *J. Neurol. Sci.*, 1973, **20**, 415; (b) J. A. Gingrich and M. G. Caron, *Annu. Rev. Neurosci.*, 1993, **16**, 299.
3. B. Zorc, M. Ljubić, S. Antolić, J. Filipović-Grčić, D. Maysinger, T. Alebić-Kolbah and I. Jalšenjak, *Int. J. Pharm.*, 1993, **99**, 135.
4. C. Diederich, L. Milakofsky, T. A. Hare, J. M. Hofford, M. Dadmarz and W. H. Vogel, *Pharmacology*, 1997, **55**, 109.
5. C. S. Myers, M. Witten, Y.-L. Yu and G. C. Wagner, *Mol. Chem. Neuropathol.*, 1998, **33**, 81.
6. A. Lieberman, A. Goodgold, S. Jonas and M. Leibowitz, *Neurology*, 1975, **25**, 911.
7. J. K. W. Chui and T. M. Fyles, *Supramol. Chem.*, 2008, **20**, 397 and references cited therein.
8. Although Akkaya's group have reported a fluorescent sensor for L-DOPA consisting of a phenyl boronic acid and a Lucifer yellow dye bound to **1** effectively (K_a = 1600 M^{-1}) in aqueous media [A. Coskun and E. U. Akkaya, *Org. Lett.*, 2004, **6**, 3107.], the K_a values obtained for the sensor with other catecholamines were not provided.
9. M. Maue and T. Schrader, *Angew. Chem. Int. Ed.*, 2005, **44**, 2265.
10. M. P. Groziak, A. D. Ganguly and P. D. Robinson, *J. Am. Chem. Soc.*, 1994, **116**, 7597.
11. (a) The ^{1}H and ^{11}B NMR studies showed prominent shifts of peaks for the protons corresponding to both the phenylboronic acid moiety in **6a** and catechol moiety in **1**, and a prominent shift of a peak for the boron atom after bindng (see Figures S1a and S2 in ESI). (b) H. Otsuka, E. Uchimura, H. Koshino, T. Okano and K. Kataoka, *J. Am. Chem. Soc.*, 2003, **125**, 3493.
12. (a) The ^{1}H NMR studies showed a relatively small shift of a peak for the protons corresponding to the methylene protons on the side chain in **6a** after binding (see Figure S1b in ESI); (b) S. Jin, M. Li, C. Zhu, V. Tran and B. Wang, *ChemBioChem*, 2008, **9**, 1431.
13. A. Charteris and R. John, *Anal. Biochem.*, 1975, **66**, 365.
14. It was confirmed that **6a** did not exhibit cytotoxicity against normal human lung fibroblast WI-38 cells (see Figure S3 in ESI).