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Diboronic Acids as Fluorescent Probes for Cells Expressing Sialyl Lewis X

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Abstract—A series of fluorescent diboronic acids was synthesized in nine steps as potential sensors for sialyl Lewis X (sLex). The fluorescent binding studies of these sensors with sLex were carried out in a mixed aqueous solution. Compound **7e** was found to show the strongest fluorescence enhancement upon binding with sLex. Using cell cultures, **7e** was shown to label sLex-expressing HEPG2 cells at 1 μM, while non-sLex-expressing cells were not labeled. © 2002 Elsevier Science Ltd. All rights reserved.

It is well known that cell surface carbohydrate structures, as part of glycosylated proteins and lipids, form characteristic signatures of different cell types.[1,2] Certain cell surface carbohydrates, such as sialyl Lewis X (sLex), sialyl Lewis a (sLea), Lewis X (Lex) and Lewis Y (Ley), have been associated with the development and progression of many types of cancers.[3–6] For instance, over-expression of sLex (Fig. 1) containing mucins has been associated with the development of gastrointestinal, pancreatic, and breast cancer.[7,8] Therefore, the development of compounds (sensors) that recognize sLex could help the diagnosis and early detection of cancer. Such compounds could also be used as vectors for targeted drug delivery. To the best of our knowledge, only one artificial synthetic receptor for Lewis oligosaccharides using CD spectroscopy method has been reported recently;[9] synthetic fluorescent sensors targeting sLex-expressing cells has not been reported yet.

To develop such sensors or molecular tags targeted on sLex, it is desirable to use recognition moieties that can recognize unique structural features on carbohydrates with high affinities. It has been known since the 1940's that boronic acids can bind compounds with a vicinal diol (dihydroxyl) structural motif with high affinity,[10] and such vicinal diol structures are commonly found in carbohydrates. Taking this advantage, many molecular recognition systems based on boronic acid moieties have been developed recently.[11–18] Our group has developed a new method of making fluorescent sensors for sugars through template-directed polymerization of boronic acid monomers.[19,20] Herein, we plan to extend the application of such fluorescent boronic acid compounds for the preparation of small molecule fluorescent sensors for sLex. By linking two boronic acid moieties with various spacers, the artificial receptors with a special spatial arrangement of the two boronic acid moieties, which are complementary to the spatial arrangement of the diol structures of sLex, could be used as a selective and sensitive sensors for the target carbohydrate, sLex.

With this design in mind, we synthesized a series of diboronic acids **7** with two different types of linkers as shown in Schemes 1 and 2. The first type of linkers consists of linear aliphatic chains with different length, such as compounds **7a**–**d**. The second type consists of different kinds of four-carbon liners (counted between the two amide carbonyl carbons) (**7e**–**j**). Starting from the readily available hydroxyaldehyde **1**,[21] upon reductive

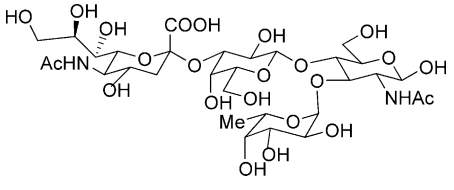

Figure 1. Sialyl Lewis X tetrasaccharide.

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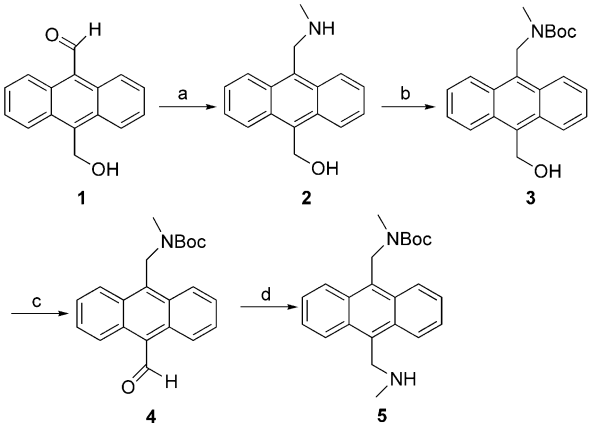
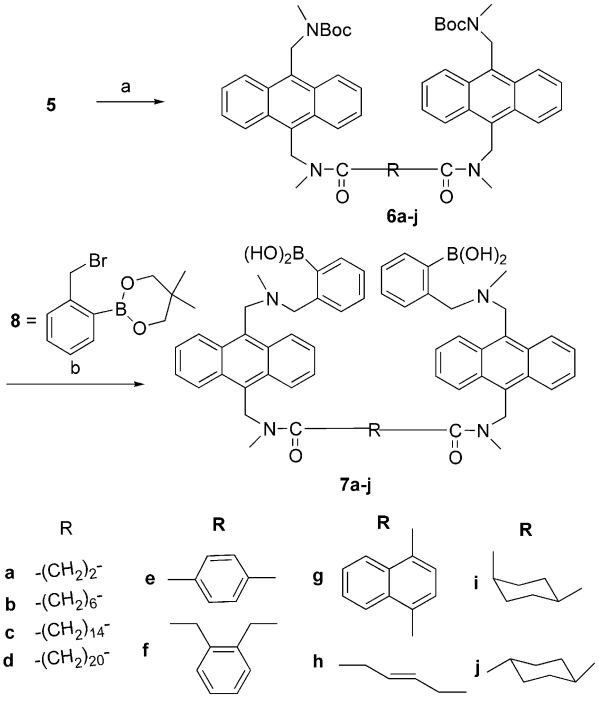


Scheme 1. (a) (i) MeOH, THF, $MeNH_2$ (40%, wt); (ii) $NaBH_4$, 90%; (b) MeOH, TEA, $(Boc)_2O$, 85%; (c) DMSO, TEA, $Py{\cdot}SO_3$, 100%; (d) (i) MeOH, THF, $MeNH_2$ (40%, wt); (ii) $NaBH_4$, 85%.



Scheme 2. (a) CH_2Cl_2, EDC, HOOCRCOOH, 30–80%; (b) (i) TFA, CH_2Cl_2; (ii) CH_3CN, **8**, K_2CO_3, 50–80%.

amination with methylamine in MeOH/THF and $NaBH_4$, amine **2** was obtained in 90% yield. The Boc-protected compound **3** was obtained in 85% yield by treatment of **2** with di-*tert*-butyldicarbonate in MeOH in the presence of triethylamine (TEA). This was followed by oxidation with pyridine sulfur trioxide in DMSO in the presence of TEA to give aldehyde **4** in quantitative yield. The resulting aldehyde **4** was then converted to amine compound **5** in 85% yield through reductive amination. This amine **5** was coupled with various diacids using EDC as the activating reagent to furnish compounds **6**[22] in 30–80% yields. After deprotection of compounds **6** with trifluoroacetic acid (TFA), the unprotected free amines were then reacted with boronate **8**[11] in the presence of potassium carbonate to give the diboronic acids **7**[23] in 50–80% yields.

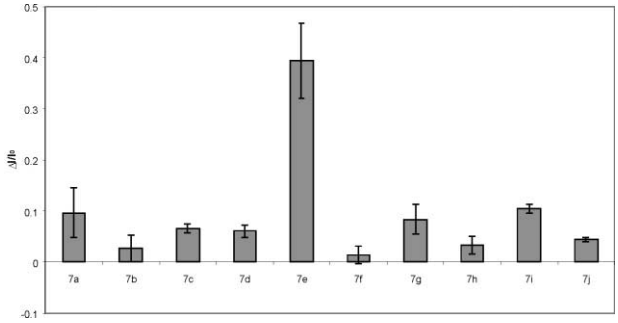


Figure 2. The fluorescence intensity change profile of the diboronic acids **7a–j** (1×10^{-5} or 1×10^{-6} M) upon binding with sLex (60 μM), $\lambda_{ex}=370$ nm, $\lambda_{em}=426$ nm.

The 'monomeric' unit of **7** is known to show increased fluorescence upon binding with a diol.[24,25] The fluorescence intensity increase upon binding was thought to be due to the diminished fluorescence quenching by the nitrogen lone pair electrons upon boronate ester formation, which increases the Lewis acidity of the boron and consequently increases the ability of the boron atom to 'mask' the nitrogen lone pair electrons through B–N bond formation.[11,25,26] Based on the same rationale, it was expected that binding of our diboronic acid compounds **7** with the target sugar should increase their fluorescence intensity. This was indeed the case. The fluorescent binding experiments of the diboronic acids **7** thus prepared with sLex were conducted in a mixture of MeOH and 0.1 M phosphate buffer (pH 7.4) (1:1, v/v). The sensor (**7**) concentration was fixed at 1×10^{-5} M,[27] and the concentration of sLex was set at 60 μM. The fluorescence intensity change profile for these diboronic acids is shown in Figure 2. Most of the diboronic acids showed a small enhancement (<10%) of the fluorescence intensity upon binding with sLex except for compound **7e**. This compound (**7e**) with a phenyl ring linker in a 1,4 relationship exhibited the highest fluorescence intensity enhancement (nearly 40%). However, the other diboronic acids **7f–j** with a four-carbon linker but different spatial or geometric arrangements, such as a *trans* double bond (**7h**), *trans* and *cis* cyclohexane rings (**7i**,**j**), and aromatic rings (**7f**,**g**), showed much less fluorescent enhancement compared to **7e** upon binding with sLex. These results indicate that the unique spatial relationship of the two boronic acid units in compound **7e** allows for its more favorable interactions with sLex than the other diboronic acid compounds prepared (Scheme 2).

Due to the high fluorescent enhancement of compound **7e** upon binding with sLex, it was also tested in a cell culture system using sLex-expressing HEPG2 and non-expressing COS7 cells. The incubation of **7e** (and other compounds) with cells was carried out in six-well plates in darkness at 4°C. After 45 min, the cells were observed with a fluorescence microscope. At both 1 and 5 μM, compound **7e** selectively stained HEPG2 cells but not COS7 cells (Fig. 3). As a control, compound **7f** was also tested in cell labeling experiments and was found to be incapable of staining HEPG2 cells even at a higher concentration.

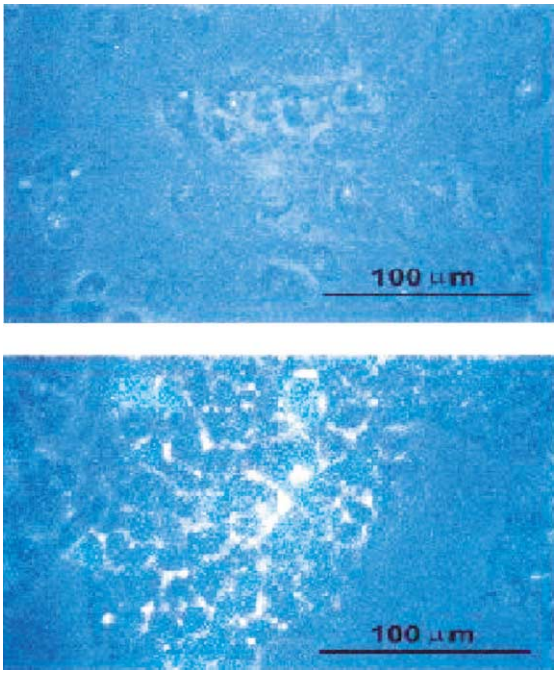


Figure 3. Fluorescent labeling studies of sLex-expressing HEPG2 cells (bottom) and non-expressing COS7 cells (top) with compound **7e** (5 μM), $\lambda_{ex} = 370$ nm, $\lambda_{em} = 426$ nm.

In conclusion, a series of fluorescent diboronic acids have been synthesized to target sLex. Compound **7e** was found to stain HEPG2 cells expressing sLex at single digit micromolar concentrations. To the best of our knowledge, this is the first time that a synthetic receptor has been used to fluorescently label sLex-expressing cells.

Acknowledgements

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22. For examples, compound **6e**: Yield (57%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.60–8.40 (m, 8H), 7.63–7.55 (m, 8H), 7.40 (s, 4H), 5.86 (s, 4H), 5.55 (s, 4H), 2.51 (s, 12H), 1.62 (s, 18H). IR (cm^{-1}): 1684, 1635. HRMS (FAB) calcd for $C_{54}H_{58}N_4O_6$ ($M^+ + H$) 859.4435; found 859.4451. Compound **6h**: Yield (31%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.46–8.43 (m, 4H), 8.36–8.32 (m, 4H), 7.59–7.56 (m, 8H), 5.79 (s, 2H), 5.68 (s, 4H), 5.52 (s, 4H), 3.24 (s, 4H), 2.56 (s, 6H), 2.48 (s, 6H), 1.56 (s, 18H). IR (cm^{-1}): 1689, 1642. HRMS (FAB) calcd for $C_{52}H_{60}N_4O_6$ ($M^+ + H$) 837.4591, found 837.4592. Anal. calcd for $C_{52}H_{60}N_4O_6 \cdot 2H_2O$: C, 71.55; H, 7.33; N, 6.42. Found: C, 71.70; H, 7.03; N, 6.31.
23. For examples, compound **7e**: Yield (70%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.60–8.40 (m, 4H), 8.32–8.20 (m, 4H), 7.72–7.52 (m, 12H), 7.50–7.20 (m, 8H), 5.81 (s, 4H), 5.06 (s, 4H), 4.34 (s, 4H), 2.47 (s, 6H), 2.39 (s, 6H). IR (cm^{-1}): 1626. MS-ESI: 969.5 ($M^+ + 3MeOH - 3H_2O + H$). Anal. calcd for $C_{58}H_{56}B_2N_4O_6 \cdot 2H_2O$: C, 72.28; H, 6.07; N, 5.82. Found: C, 72.27; H, 6.05; N, 5.87. Compound **7h**: Yield (71%). ^{1}H NMR ($CD_3OD + CDCl_3$, 300 MHz) δ 8.31–8.28 (m, 4H), 8.18–8.15 (m, 4H), 7.56–7.50 (m, 8H), 7.40–7.30 (m, 8H), 5.65 (s, 2H), 5.57 (s, 4H), 4.90 (s, 4H), 4.26 (s, 4H), 3.18 (s, 4H), 2.33 (s, 6H), 2.16 (s, 6H). IR (cm^{-1}): 1642. MS-ESI: 887.6 ($M^+ - H_2O + H$). Anal. calcd for $C_{56}H_{58}B_2N_4O_6$: C, 74.34; H, 6.46; N, 6.19. Found: C, 74.38; H, 6.73; N, 6.21.
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27. For compound **7d**, the concentration was set at 1×10^{-6} M due to its poor solubility in the aqueous solution.