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Substituent effect on anthracene-based bisboronic acid glucose sensors

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Abstract—Earlier we communicated an anthracene-based bisboronic acid sensor for glucose. Aimed at understanding the substituent effect, we have introduced various functional groups, such as the cyano, nitro, and fluoro group on the boronic acid moiety of this glucose sensor. Fluorescent binding studies indicated that the cyano-substituted sensor (**4a**) has the highest affinity (K 2540 M^{-1}) for glucose, but the lowest selectivity (three-fold over fructose); the fluoro-substituted compound (**4c**) shows the lowest affinity (630 M^{-1}) and a modest selectivity (15-fold over fructose); and the unsubstituted one (**1a**) shows the highest selectivity over fructose (43-fold) and a modest affinity (1472 M^{-1}).

1. Introduction

Carbohydrates are considered critical to various biological processes.[1–5] The most prominent among them is glucose, which is a critical energy supplier to cells, and its elevated concentration is the primary symptom of diabetes.[6] Just as important are oligosaccharides (glycans) that are part of glycoproteins or glycolipids.[1,2] Sensors for these biologically important carbohydrates have the potential to be used as diagnostics, new imaging agents, as well as therapeutics.[7–9] In this area, there is especially strong interest in developing boronic acid-based sensors[10–16] because of the ability of the boronic acid group to form reversible and tight complexes with diol-containing compounds.[10,13,14,17–25] Boronic acids have been used to develop fluorescent sensors,[11,13,14,21,22,26–28] color sensors,[8,11–13,15,16,26,29–36] sensors for cell recognition based on surface carbohydrate biomarkers,[22] carbohydrate transporters,[37–42] and chromatographic stationary materials.[43–48] Because monoboronic acids have certain intrinsic preference for various carbohydrates, the design of selective sensors for a particular sugar often relies on the introduction of additional functional group interactions, such as a second boronic acid unit, with a proper scaffold to afford selectivity.[22] Such an approach has been successfully used in various examples.[10,11,15,32,49] Modulation of the affinity of a monoboronic acid for diols can be achieved through the introduction of various substituents on an arylboronic acid. Generally speaking, arylboronic acids with lower pK_a values tend to have higher affinities for diols, although one also needs to consider the pH of the solution and other factors.[19] For example, 2-fluoro-5-nitrophenylboronic acid has a pK_a of about 6.0, which is 2.8 pK_a units lower than that of phenylboronic acid. Consequently, the binding constant between glucose and 2-fluoro-5-nitrophenylboronic acid at physiological pH is about ten-fold higher than that of phenylboronic acid.[19] However, to the best of our knowledge there have not been studies that directly probe the effect of substituents on the affinity and selectivity of bisboronic acid sensors. Recently, our group reported a highly selective anthracene-based boronic acid sensor for glucose (**1a**, Fig. 1).[22] The sensor design used the Shinkai fluorescent reporter,[10] which showed an increase in

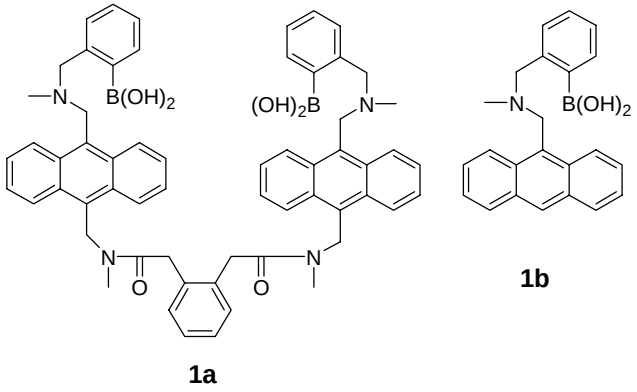

Figure 1. Dianthraceneboronic acid (**1a**) and anthracenemonoboronic acid (**1b**).

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fluorescence intensity upon binding with a diol due to protonation of the amine nitrogen upon binding.[50] Compound **1a** showed about 43-fold selectivity for glucose over fructose and 49-fold selectivity over galactose.

In this study, we have introduced various electron-withdrawing substituents on the arylboronic acid portion of the bisboronic acid sensor aimed at achieving a better understanding of the substituent effect. Specifically, there are two questions we wish to answer: (1) will the affinity-enhancing effect of electron-withdrawing groups such as fluoro, nitro, and cyano substituents be translated into enhanced affinity of the bisboronic acid sensors for saccharide and (2) how will such electron-withdrawing groups affect the selectivity of the parent boronic acid sensors **1a** and **1b**. With that in my mind we synthesized three analogs of **1a**[22] and **1b**[10] with the cyano, nitro, and fluoro functional groups placed at a position para to the boronic acid. Their binding constants with various sugars have also been determined.

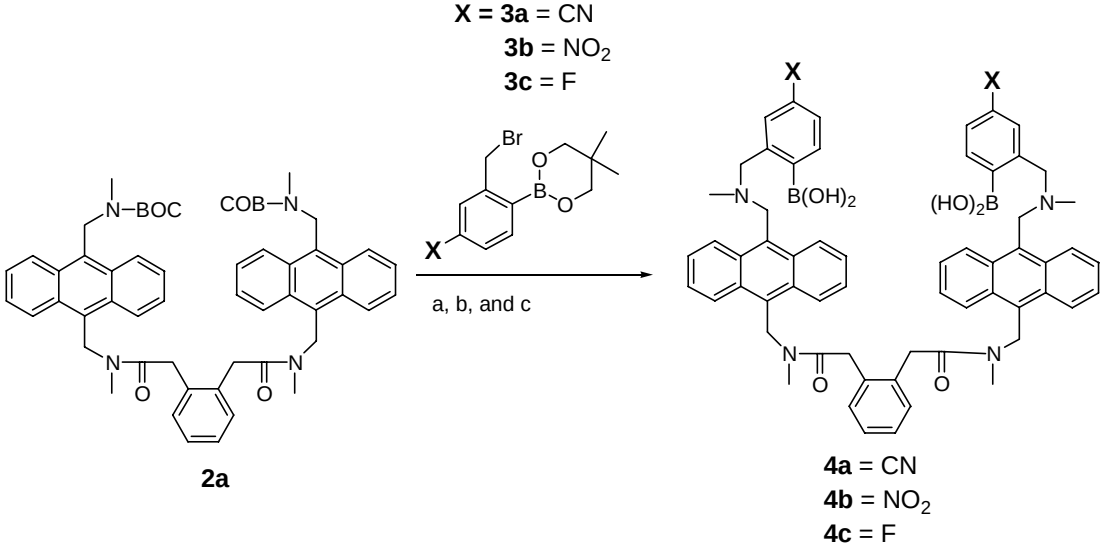
2. Results and discussions

2.1. Synthesis

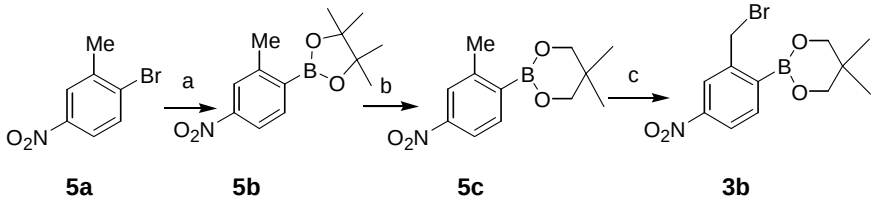
Three dianthraceneboronic acid compounds, **4a–c**, and three anthracenemonoboronic acid compounds, **6a–6c** with cyano, nitro, and fluoro groups, respectively, were prepared by following the procedure for the synthesis of the parent compounds (Schemes 1 and 3).[22,36] The key to the synthesis of these analogs was the preparation of protected arylboronic acids (**3a–c**) with various substitutions, which were used in the preparation of the bisboronic (**4a–c**) and monoboronic (**6a–6c**) acid products through alkylation (Schemes 1 and 3). The nitro-substituted pinacolato boronate **5b** was obtained by borylation of commercially available bromo-2-methyl-4-nitro-benzene using pinacolatodiboron in the presence of a palladium catalyst at 80 °C. The pinacolato protecting group of **5b** was then converted to the neopentyl glycol (**5c**)[51,52] protecting group for the ease of deprotection at the end of the synthesis. This was accomplished by reaction with neopentyl glycol at 250 °C. Bromination of **5c** in presence of NBS and AIBN yielded **3b**[51] (Scheme 2) in 82% yield. The cyano and fluoro-substituted boronates **3a** and **3c** were synthesized according to literature procedures.[51]

For the synthesis of the bisboronic acid sensors, compound **2a** was prepared following procedure reported earlier and deprotected using trifluoroacetic acid (Scheme 1). The deprotected amino group was then reacted with **3a–c** in the presence of K_2CO_3 in acetonitrile at rt to give the corresponding boronate esters of **4a–c**. Hydrolysis of the protected boronic acids under basic conditions in the presence of $NaHCO_3$ gave the free boronic acids **4a** and **4b**; whereas free boronic acid **4c** was obtained under acidic condition in the presence of HCl.[22]

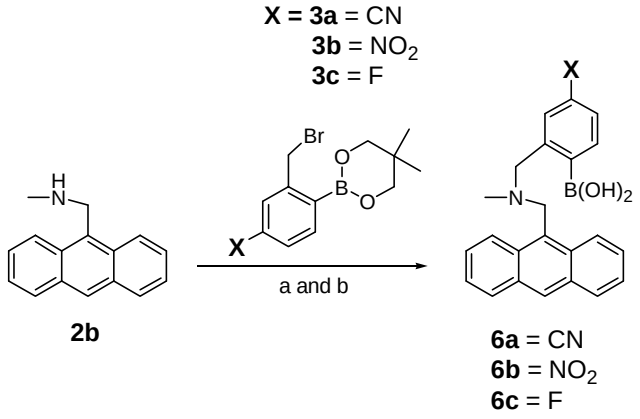
For the synthesis of the monoboronic acids, commercially available anthracen-9-ylmethyl-methylamine (**2b**) was reacted with **3a–c** in the presence of K_2CO_3 in acetonitrile at rt to give the corresponding boronate esters (Scheme 3).



Scheme 1. (a) TFA, DCM; (b) **3**(**a–c**), K_2CO_3, KI, CH_3CN; (c) **4a** and **4b**: DCM, 10% $NaHCO_3$, H_2O; **4c**: acetone–H_2O (4/1), 1 N HCl.



Scheme 2. (a) $PdCl_2$(dppf), KOAc, DMSO, (pinacolato)diboron, 80 °C; (b) neopentyl glycol, 250 °C; (c) NBS, AIBN, benzene, reflux.



Scheme 3. (a) **3**(**a**–**c**), K_2CO_3, KI, CH_3CN; (b) **6a** and **6b**: DCM, 10% $NaHCO_3$, H_2O; **6c**: acetone–H_2O (4/1), 1 N HCl.

Hydrolysis of the protected boronic acids was done under the same conditions as mentioned above to obtain free boronic acids **6a**–**c**.[22]

2.2. Fluorescent studies

As discussed earlier, these compounds were expected to increase fluorescence upon sugar binding. Furthermore, we were interested in seeing whether substitutions would change the binding affinity of the bisboronic acid sensors for diols parallel to that of the monoboronic acid unit. Therefore, fluorescence experiments were conducted to determine the appropriate binding constants of **4a**–**c** and **6a**–**c** for various sugars. Since the anthracene-based compounds are fairly lipophilic, a 1:1 mixture of methanol and phosphate buffer (pH 7.4) was used as the solvent with a sensor concentration of about 1×10^{-6} M. The sugars studied include glucose, fructose, and galactose. As expected, upon sugar addition, all three substituted glucose sensor analogs (**4a**–**c**) showed dramatically increased fluorescence intensity. Figure 2 shows two typical sets of fluorescent spectral changes upon sugar addition using compound **4a**–**b** as examples. In the past, it was proposed that the increase in fluorescence intensity for such anthracene-based boronic acids upon addition of a saccharide was due to increased B–N bond strength upon sugar binding, which in turn resulted in reduced fluorescence quenching by photoinduced electron transfer (PET).[10] Recently, our group has proposed a different mechanism, the hydrolysis mechanism, for the increase in the fluorescence intensity upon addition of a sugar.[50] As shown in Scheme 4, without sugar addition, there is a weak B–N bond. Under such a circumstance PET can happen from the amine lone pair electrons to the anthracence ring in the excited state. Upon sugar addition, the increased acidity of the boron[18,19] results in a pK_a switch so that the first pK_a is that of the boron. In such a case, the addition of a sugar allows for the breaking of the weak B–N bond and the formation of the anionic boron species **8b**, which helps to stabilize the protonated form of the amine nitrogen. Such protonation abolishes the PET process, turns off the fluorescence quenching, and results in increased fluorescence intensities. One would expect these new analogs to have a similar mechanism in inducing the fluorescent changes upon sugar binding, although this was not specifically studied.

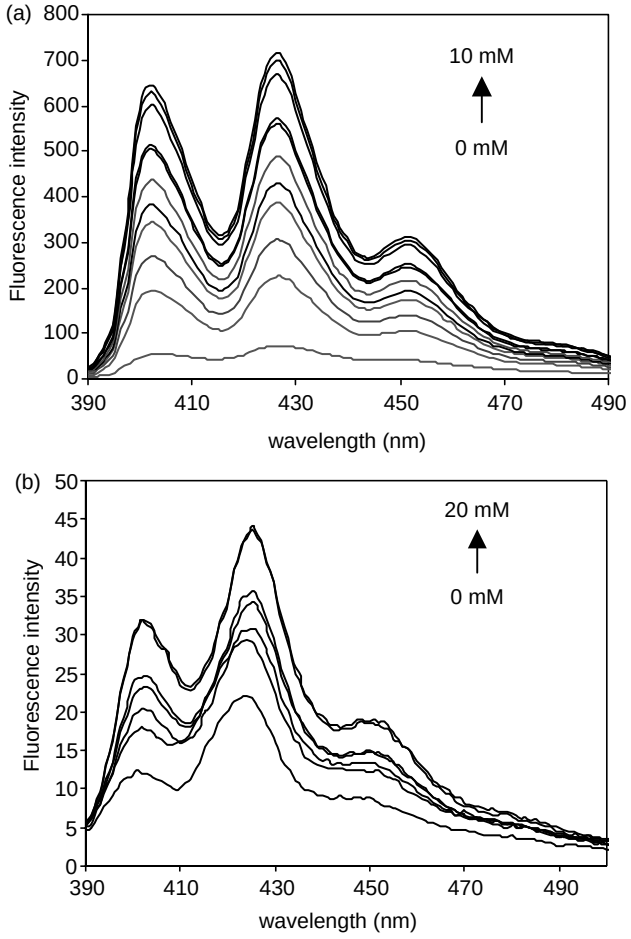
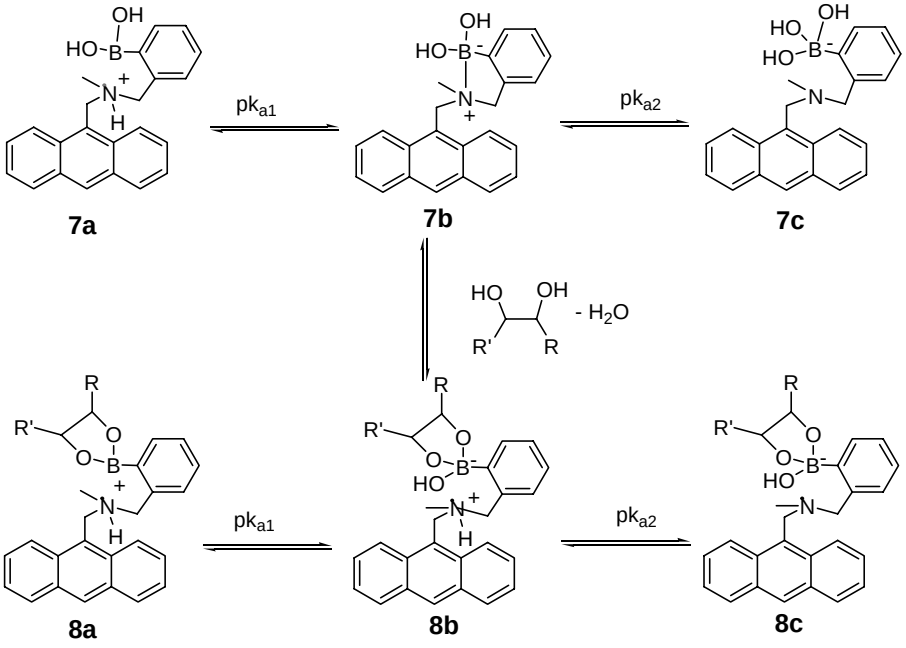


Figure 2. (a) Fluorescence spectra of **4a** (1.0×10^{-6} M) with D-glucose (0–10 mM); (b) fluorescence spectra of **4b** (1.0×10^{-6} M) with D-glucose (0–20 mM) at 25 °C in 50% MeOH/0.1 M aqueous phosphate buffer at pH 7.4: $\lambda_{ex}=370$ nm.

With the four analogs of dianthraceneboronic acid in hand, the cyano-substituted compound (**4a**) showed the highest change in fluorescence intensity with a ten-fold increase upon glucose addition. It also showed the highest binding affinity with an apparent binding constant (K_a) of 2540 M^{-1} for glucose (Table 1). It is interesting to see that the nitro-substituted compound (**4b**) showed the lowest fluorescent intensity changes (a maximum of two folds), while having the second highest affinity for glucose (1808 M^{-1}). The fluoro-substituted analog (**4c**) showed only four-fold fluorescent intensity changes with a binding constant (630 M^{-1}) that is even smaller than that of the parent compound (**1a**, 1472 M^{-1}). The binding of these bisboronic acids was compared with that of the corresponding monoboronic acid analogs (**6a**–**6c**, Table 2). It is interesting to note that the cyanophenylboronic acid compound (**6a**) also showed much higher affinity than either the nitro (**6b**) or fluoro (**6c**) substituted ones. Therefore, the results of the bisboronic acids parallel that of the monoboronic acids in this case.

However, the comparison between the nitro and fluoro-substituted compounds did not yield the same conclusions. In the monoboronic acid series, the nitro and fluoro-substituted boronic acids (**6b** and **6c**) had similar



Scheme 4. A hydrolysis mechanism for the fluorescence intensity changes of Shinkai's anthraceneboronic acid.

affinities for both fructose and glucose. On the other hand, in the bisboronic acid series, the nitro-substituted compound (**4b**) showed a much higher affinity than the fluoro-substituted one (**4c**). It is also worth noting that the fluoro-substituted bisboronic acid has an even lower affinity than that of the unsubstituted control (**1a**). Such results were somewhat unexpected because fluoro-substitution is electron-withdrawing. Furthermore, the size of the fluorine atom is small and one would not expect much perturbation of the conformation of the bisboronic acid compound by the fluorine atom. Consequently, one would expect **4c** to have a higher affinity for glucose than does **1a**.

The results of the selectivity studies were also somewhat surprising. Sensor **4a**, although having the highest affinity for glucose, showed the lowest selectivity with a three-fold preference for glucose over fructose in terms of the binding constants. This is in direct contrast to the 43-fold selectivity of **1a** for the same pair of sugars.[22] Compounds **4b** and **4c** were somewhere in between. In comparison with the monoboronic acids, it seems that the cyano-substituted one has a low selectivity problem too, only in this case it was the selectivity for fructose. Such results could mean that the cyano-substituted boronic acids have a lower propensity to discriminate among different sugars. However, the underlying reason for this is not clear.

Overall, the results indicate that the substituent effect on monoboronic acids can only be partially translated into the same kind effect when used for the preparation of bisboronic acid compounds. Other factors such as conformational changes may also need to be considered in designing analogs aimed at optimizing the affinity and selectivity of the interested sensors.

There is one additional point that is worth discussing, that is, why the substituent electron-withdrawing ability did not correlate with the apparent binding constants of the monoboronic acids (**1b**, and **6–c**) (Tables 1 and 2).

Table 1. Binding constant (K_a) for compounds **4a–4c** and **1a** with different saccharides

Compound	K_a (M^{-1}) D-glucose	K_a (M^{-1}) D-fructose	K_a (M^{-1}) D-galactose	Selectivity $K_{a\ glucose}/K_{a\ fructose}$	Fluorescence intensity changes for glucose
4a	2540±90.1	968±126.6	271±37.5	3	Ten-fold increase
4b	1808±130.6	198±32.8	132±60.5	9	Two-fold increase
4c	630±48.6	42±7.2	46±6.6	15	Four-fold increase
1a	1472	34	30	43	Seven-fold increase

Table 2. Binding constant (K_a) for monoboronic acids **6a–6c** and **1b** with different saccharides

Compound	K_a (M^{-1}) fructose	K_a (M^{-1}) glucose	Selectivity $K_{a\ fructose}/K_{a\ glucose}$	Fluorescence intensity changes for fructose
6a	1350±68.4	101±5.2	13	2.3-Fold increase
6b	714±51.3	40±4.0	18	Two-fold increase
6c	650±29.2	26±8.3	25	2.3-Fold increase
1b	940	50	18	Two-fold increase

As has been reported previously, the apparent binding constant of a particularly boronic acid can be affected by several factors including (1) the pK_a values of the boronic acid and the diol; (2) the optimal pH for a particular complexation reaction; (3) steric factors; (4) the concentration and nature of the buffer; (5) whether trivalent interaction is involved and (6) other idiosyncratic factors that have not been identified.[19] Among these factors, a shift of the optimal pH away from 7.4 to a lower pH is most likely the reason for the diminished intrinsic affinity of **6b** and **6c** for diols at pH 7.4 compared with the unsubstituted one (**1b**). Similar examples have been reported before, especially with boronic acids that have a very low pK_a value.[19]

3. Conclusions

We have synthesized three new anthracene-based bisboronic fluorescent sensors (**4a**–**4c**) and three monoboronic fluorescent sensors (**6a**–**c**). Both cyano- (**4a**) and nitro-substituted (**4b**) sensors had higher apparent binding constant for glucose (K 2540 and 1808 M^{-1}, respectively) than the parent sensor (**1a**) (1472 M^{-1}). Whereas fluoro-substituted bisfluoroboronic acid (**4c**) had a lower apparent binding constant (K 630 M^{-1}) but it has the most appropriate affinity and selectivity for glucose sensing under physiological conditions. The selectivity between glucose and fructose did diminish for all the new sensors (**4a**–**4c**) compared to **1a**. The monoboronic acid sensors (**6a**–**c**) also showed similar trend in the affinity for saccharides compared with their bisboronic acid analogs. Again, monofluoroboronic acid sensor (**6c**) had the lowest binding constant but showed a greater selectivity (25-fold) than **6a** for fructose over glucose. Overall, the introduction of an electron-withdrawing group does not always directly translate into enhanced affinity, and the affinity of the bisboronic sensors only partially tracks that of the monoboronic building blocks. The effect of the electron-withdrawing group in the selectivity of the bisboronic acid sensors is hard to predict.

4. Experimental

4.1. General procedures

Commercially available reagents were used without additional purification unless otherwise indicated. Dichloromethane was distilled from CaH_2. THF was distilled from sodium and benzophenone. Mass spectrometry (MS) analyses were performed by the Mass Spectrometry Laboratories of Georgia State University. ^1H and ^{13}C NMR spectra were recorded at 75 and 100 MHz. Chemical shifts (δ) are given in ppm relative to TMS for ^1H spectra and relative to residual solvent for ^{13}C spectra.

4.2. Fluorescence binding study procedure

A Shimadzu RF-5301PC fluorometer was used for all the fluorescent studies. For a typical fluorescent measurement, a 2 ml of sensor stock solution in methanol (2.0×10^{-6} M) was mixed with 2 ml of saccharide solution in 0.1 M of phosphate buffer (pH 7.4) at various concentrations. The pH was checked and corrected if necessary. The mixture was allowed to mix for 20 min and fluorescence intensity was recorded. Triplicate measurements were taken for each sugar. The correlation coefficients for all determinations in fitting the 1:1 model were over 0.99.

4.2.1. 4,4,5-Trimethyl-2(2-methyl-4-nitro-phenyl)-[1,3,2]dioxaboroloane (5b). A mixture of bromo-2-methyl-4-nitro-benzene (**5a**, 11.6 mmol), $PdCl_2$(dppf) (0.1 mmol), KOAc (14.6 mmol), and bis(pinacolato)diboron (12.7 mmol) in DMSO was heated to 80 °C overnight. The reaction mixture was pour into 25 ml ice-water slush and extracted with ethyl acetate (3×10 ml) and dried over $MgSO_4$. After evaporation of solvent, crude product was chromatographed on silica column using hexane–ethyl acetate as the eluent to give **5b** in 53%. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.05 (6H, s), 2.59 (3H, s), 3.80 (4H, s), 7.86 (1H, d, J=8.0 Hz), 7.96 (2H, d, J=8.8 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.8, 22.3, 31.6, 72.4, 119.3, 124.1, 135.7, 145.7, 148.9 ppm. HRMS (EI): calcd for $C_{12}H_{18}BNO_4$ [M^+] 263.1329, found 263.1336.

4.2.2. 5-Methyl-2-(2-methyl-4-nitro-phenyl)-[1,3,2]-dioxaborinane (5c). Compound **5b** (4.7 mmol) and neopentyl glycol (62.5 mmol) were mixed and heated to 250 °C for 2 h and then reaction was cooled to rt. Crude product was chromatographed on silica column using hexane–ethyl acetate as eluent to give **5c** in 82%. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.36 (12H, s), 2.62 (2H, s), 7.90 (1H, d, J=8.0 Hz), 7.95 (1H, s), 7.98 (1H, s) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.4, 25.1, 84.5, 119.6, 124.2, 136.9, 146.8, 149.6 ppm. HRMS (ESI−): calcd for $C_{12}H_{16}BNO_4$ [M+CH_3OH] 280.1256, found 280.1252.

4.2.3. Compound (4a). Boc protected compound **2** (127 mg, 14 mmol) was dissolved in 4 ml dry DCM and 2 ml of trifluoroacetic acid (TFA) was added to the flask. Reaction was stirred for 30 min at rt and solvent was removed and dried under vacuum. The deprotected product, **3a** (156 mg, 0.50 mmol), potassium carbonate (175 mg, 1.27 mmol) and KI (6 mg) was dissolved in dried acetonitrile and mixed for 12 h at rt. Solvent was removed and resulted yellow precipitate was dissolved in (10 ml) DCM and 5 ml 10% $NaHCO_3$ and stirred for 1 h at rt. The organic phase was washed with water (2×10 ml), and dried over $MgSO_4$ and solvent was removed in vacuo. The resulted residue was re-precipitated from DCM–ether to give **4a** in 39%. ^1H NMR (400 MHz, CD_3OD) δ: 2.37 (6H, s), 2.63 (6H, s), 3.81 (4H, s), 4.22 (4H, s), 5.06 (4H, s), 5.74 (4H, s), 7.24 (4H, s), 7.57 (9H, t, J=8.8 Hz), 7.83 (4H, d, J=7.2 Hz), 8.31 (4H, d, J=7.6 Hz), 8.45 (4H, d, J=8 Hz) ppm. HRMS (ESI+): calcd for $C_{62}H_{58}B_2N_6O_6$: 1005.4663; found: 1005.4663. We were unable to remove cleaved neopentyl glycol, in order to confirm the structure; the boronic acid was oxidized in the presence of acetic acid–water (1/1) and H_2O_2 to obtain pure NMR. ^1H NMR (400 MHz, CD_3Cl_3) δ: 2.41 (6H, s), 2.64 (6H, s), 3.81 (8H, s), 4.71 (4H, s), 5.72 (4H, s), 6.66 (2H, d, J=8.4 Hz), 7.23 (6H, s), 7.34–7.32 (3H, dd, J=2, 6.4 Hz), 7.54 (4H, t, J=8.8 Hz), 7.63 (4H, t, J=6.4 Hz), 8.44 (8H, q, J=9.2, 12 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.58, 29.62, 33.37, 39.35, 41.85, 53.59, 59.52, 102.32, 116.85, 119.12, 122.84, 124.37, 124.76, 125.28, 125.64, 126.26, 126.42, 127.32, 128.55, 129.98, 130.52, 130.89, 131.17, 132.22, 132.42, 132.98, 133.12,

134.42, 161.58, 170.77 ppm. HRMS (ESI+): calcd for $C_{62}H_{56}N_6O_4$: 949.4442; found: 949.4479.

4.2.4. Compound (4b). The procedure was same as the preparation of **4a** from **2**. Yield (20%). ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$) δ: 2.37 (6H, s), 2.63 (6H, s), 3.80 (4H, s), 4.21 (10H, s), 5.73 (4H, s), 7.23 (4H, s), 7.56 (6H, t, J= 8.0 Hz), 7.86 (2H, d, J=8.0 Hz), 8.07 (2H, s), 8.12 (2H, d, J=8 Hz), 8.30 (4H, d, J=8.4 Hz), 8.43 (4H, d, J= 8.4 Hz) ppm. HRMS (ESI+): calcd for $C_{60}H_{58}B_2N_6O_{10}$: 1045.4479; found: 1045.4523. We were unable to remove cleaved neopentyl glycol, in order to confirm the structure; the boronic acid was oxidized in the presence of acetic acid–water (1/1) and H_2O_2 to obtain pure NMR. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.44 (6H, s), 2.64 (6H, s), 3.80 (4H, s), 3.88 (4H, s), 4.73 (4H, s), 5.72 (4H, s), 6.64 (2H, d, J= 6 Hz), 7.26 (3H, s), 7.52 (4H, t, J=8 Hz), 7.62 (4H, t, J= 6.8 Hz), 7.87 (2H, s), 7.95–7.93 (4H, dd, J=2.4, 2.4 Hz), 8.41 (4H, t, J=10 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.71, 33.48, 39.36, 41.66, 42.01, 53.50, 59.39, 116.17, 121.81, 124.36, 124.65, 125.22, 125.32, 126.36, 126.57, 127.44, 128.38, 130.04, 130.82, 131.10, 134.35, 140.05, 163.70, 170.74 ppm. HRMS (ESI+): calcd for $C_{60}H_{56}N_6O_8$: 989.4238; found: 989.4252.

4.2.5. Compound (4c). The procedure was same as the preparation of **4a** from **2**, but the hydrolysis was done in solution of acetone–water (1/4) in total volume of 150 ml and 1 N HCl (10 ml). The reaction was stirred vigorously for 1 h at rt. The organic phase was washed with water (2× 10 ml), and dried over $MgSO_4$ and solvent was removed in vacuo. The resulted residue was re-precipitated from DCM–ether to give **4c** in 20%. ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$) δ: 2.21 (6H, s), 2.42 (6H, s), 3.72 (4H, s), 4.11 (4H, s), 4.60 (4H, s), 5.68 (4H, s), 7.09 (4H, q, J=8.4, 9.6 Hz), 7.24 (4H, s), 7.48 (8H, s), 7.68 (2H, s), 8.27 (4H, s), 8.42 (4H, d, J=6.4 Hz) ppm. HRMS (ESI+): calcd for $C_{60}H_{58}B_2F_2N_4O_6$–H_2O: 973.4483; found: 973.4464. We were unable to remove cleaved neopentyl glycol, in order to confirm the structure; the boronic acid was oxidized in the presence of acetic acid–water (1/1) and H_2O_2 to obtain pure NMR. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.34 (6H, s), 2.61 (6H, s), 3.79 (8H, s), 4.67 (4H, s), 5.29 (4H, s), 6.62 (2H, q, J=4.4, 4.8 Hz), 6.71 (2H, dd, J=2.8 Hz), 6.77 (2H, d, J= 2.8 Hz), 7.23 (4H, s), 7.52 (4H, t, J=8.4 Hz), 7.60 (4H, t, J=8.4 Hz), 8.40–8.42 (8H, dd, J=2.4, 2.8 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.71, 39.31, 41.66, 41.75, 53.59, 59.97, 114.78 (s (d_{C-F}), J=14 Hz), 115.01 (s (d_{C-F}), J=14 Hz), 116.49 (s (d_{C-F}), J=8 Hz), 122.69, 122.75, 124.63, 125.19, 126.29, 127.42, 129.14, 129.58, 130.08, 130.85, 131.07, 134.38, 153.11, 157.15 (s (d_{C-F}), J= 220 Hz), 170.72 ppm. HRMS (ESI+) calcd for $C_{60}H_{56}F_2N_4O_4$: 935.4348; found: 935.4351.

4.2.6. 2-[(Anthracen-9-ylmethyl-methyl-amino)-methyl]-4-cyano-boronic acid (6a). Anthracen-9-ylmethyl-methyl-amine (120 mg, 0.54 mmol), **3a** (183 mg, 0.60 mmol), potassium carbonate (299 mg, 2.17 mmol) and KI (7.2 mg) was dissolved in dried acetonitrile and mixed for 12 h at rt. Solvent was removed and resulted yellow precipitate was dissolved in (10 ml) DCM and 5 ml 10% $NaHCO_3$ and stirred for 1 h at rt. The organic phase was washed with water (2×10 ml), and dried over $MgSO_4$ and solvent was removed in vacuo. The resulted residue was re-precipitated from DCM–hexane to give **6a** in 12%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (3H, s), 4.23 (2H, s), 5.03 (2H, s), 7.55 (4H, m), 7.67 (1H, d, J=7.8 Hz), 7.89 (1H, d, J=7.5 Hz), 8.10–8.19 (4H, dd, J=8.4 Hz), 8.59 (1H, s) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 43.15, 51.55, 112.24, 119.33, 124.92, 125.88, 128.08, 128.34, 129.08, 129.32, 130.09, 131.00, 131.39, 131.72, 133.79, 144.71 ppm. HRMS (ESI+): calcd for $C_{24}H_{21}BN_2O_2$: 381.1774; found: 381.1774.

4.2.7. 2-[(Anthracen-9-ylmethyl-methyl-amino)-methyl]-4-nitro-boronic acid (6b). The procedure was same as the preparation of **6a**. Yield (29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.25 (3H, s), 4.54 (2H, s), 5.15 (2H, s), 7.58 (4H, m), 7.97 (1H, d, J=8 Hz), 8.14 (2H, d, J=8 Hz), 8.24–8.28 (4H, dd, J=5.6, 6 Hz), 8.64 (1H, s) ppm. ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ: 42.29, 121.80, 124.24, 124.90, 126.08, 126.77, 128.57, 129.17, 131.38, 131.95, 135.88, 141.96, 143.07, 148.50 ppm. HRMS (ESI+): calcd for $C_{23}H_{21}BN_2O_4$: 401.1672; found: 401.1670.

4.2.8. 2-[(Anthracen-9-ylmethyl-methyl-amino)-methyl]-4-fluoro-boronic acid (6c). The procedure was same as the preparation of **6a**. Yield (53%). ^{1}H NMR (300 MHz, CD_3OD) δ: 2.24 (3H, s), 3.98 (2H, s), 4.57 (2H, s), 7.06 (2H, m), 7.42–7.44 (4H, dd, J=2.7, 3.3 Hz), 7.79 (1H, s), 7.94–8.00 (4H, dd, J=3, 13.2 Hz), 8.39 (1H, s) ppm. ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ: 38.39, 61.24, 112.81 (s (d_{C-F}), J=18 Hz), 115.52 (s (d_{C-F}), J= 19 Hz), 122.32, 123.64, 125.38, 127.84, 128.28, 130.01, 130.16, 134.69, 141.96, 162.69 (s (d_{C-F}), J=243 Hz) ppm. HRMS (ESI+): calcd for $C_{23}H_{21}BFNO_2$: 374.1727; found: 374.1717.

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52. Compound **5c** synthesis has been reported in literature but not fully characterized. See Section 4 for full characterization of **5c**.