Modular Solid-Phase Synthetic Approach To Optimize Structural and Electronic Properties of Oligoboronic Acid Receptors and Sensors for the Aqueous Recognition of Oligosaccharides

Duane Stones, Sukhdev Manku, Xiaosong Lu, and Dennis G. Hall*[a]

Abstract: This article describes the design and optimization of the first entirely modular, parallel solid-phase synthetic approach for the generation of well-defined polyamine oligoboronic acid receptors and fluorescence sensors for complex oligosaccharides. The synthetic approach allows an effective building of the receptor polyamine backbone, followed by the controlled diversification of the amine benzylic side chains. This approach enabled the testing, in a modular fashion, of the effect of different arylboronic acid units substituted with unencumbering *para* electron-withdrawing or electron-donating groups. The feasibility of this approach toward automated synthesis was also investigated with the assembly of a sublibrary of receptors by means of the Irori MiniKan technology. Several sublibraries of anthracene-capped sensors containing two or three arylboronic acids were synthesized, and their binding to a series of model disaccharides was examined in neutral aqueous media. The calculation of association constants by fluorescence titrations confirmed that subtle changes in the structures of the interamine spacers in the polyamine backbone can have a significant effect on the stability of the resulting complexes. Most importantly, this study led to the determination of the preferred electronic characteristics for the arylboronate units, and suggests that a new generation of receptors containing very electron-poor arylboronic acids could lead to a significant improvement of binding affinities.

Keywords: boronic acids · combinatorial chemistry · fluorescence sensors · oligosaccharides · solid-phase synthesis

Introduction

The availability of small molecules capable of binding tightly and selectively to complex biomolecules is crucial for advancements in chemical biology and medicinal chemistry. Considerable success has been achieved with two of the three types of natural biopolymers: polypeptides (proteins and enzymes),[1] and nucleic acids (DNA and RNA).[2] In contrast, there is yet no general approach for the generation of selective ligands (or receptors) for complex oligosaccharides.[3] Carbohydrate recognition under physiological conditions still poses a formidable challenge to organic chemists. Small oligosaccharide-binding molecules may enable effective control of biological recognition events involving soluble and surface-associated oligosaccharides, which often act as specific cell markers.[4] Their potential applications are numerous, and include therapeutic uses, such as inhibition of viral and bacterial invasion, diagnosis and selective drug delivery, use as biological probes or analytical biosensors, and supports for affinity purification. The use of boronic acids in the reversible formation of cyclic boronate esters with sugar diols,[5] as demonstrated by Wulff[6], James, and Shinkai[7] with monosaccharides,[8] shows promise in the aqueous recognition of complex oligosaccharides. Because of their structural and conformational complexity, oligosaccharides may be best targeted with a combinatorial strategy based on the screening of large libraries of oligoboronic acid receptors.[9] The successful implementation of a combinatorial strategy, however, relies on the availability of an efficient solid-phase approach to the controlled synthesis of structurally diverse sets of receptors. Herein, we describe the first entirely modular, parallel solid-phase approach to generate well-defined polyamine oligoboronic acid receptors and fluorescence sensors. Their binding to model disaccharides was examined in a systematic fashion that led to the determination of the preferred electronic characteristics for the arylboronate units.

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Results and Discussion

Design of oligoboronic acid receptors: Although several simple diboronic acid receptors for monosaccharides have been reported,[7] there are no reports addressing the possibility of increasing a receptor's potency by modulating the electronic characteristics of the arylboronate units. Also left unexplored is the potential role of a third boronic acid, especially in view of binding disaccharides and more complex oligosaccharides.[10] Our design approach, shown in Figure 1, addressed these two issues. Receptors would include up to three *ortho*-alkylaminoboronic acid units with two interamine spacers (CH_2R^1 and CH_2R^2). The choice of boronic acid units with an *ortho*-methylamino group is based on the well-precedented ability of this B–N coordination motif to facilitate diol binding under neutral aqueous conditions.[7] The synthetic approach should also allow the controlled diversification of the amines' benzylic side chains to test, in a modular fashion, the inclusion of arylboronic acid units substituted with unencumbering *para* electron-withdrawing or electron-donating groups (X^1, X^2, X^3).

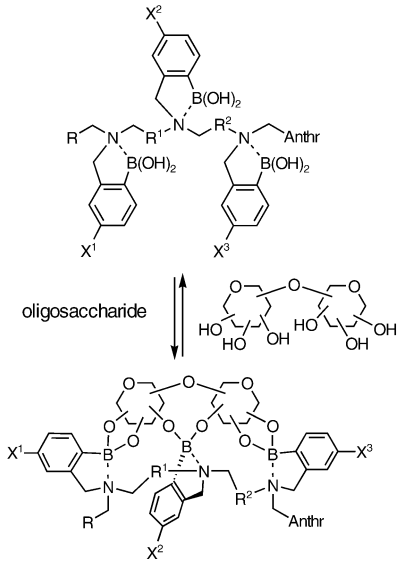


Figure 1. Modular approach to oligoboronic acid receptors for complex oligosaccharides capped with an anthracene sensing unit. R = solid support or free linker. R^1, R^2 = interamine spacers. X^1, X^2, X^3 = H, EWG or EDG substituents.

With the objective of assessing the efficiency of our synthetic scheme and examining the role of a third boronate unit in disaccharide binding, we first planned a small prototypical set of triboronic acid receptors devoid of substituents (X^1, X^2, X^3 = H) (Figure 2). Seven pseudosymmetric triboronic acid receptors capped with an anthracene sensor were made in a parallel fashion by varying one of the two interamine positions R^1 and R^2 with four reduced amino acid residues (Amb^R, Amc^R, Acc^R, Ahx^R; see the Experimental Section for the abbreviations), while one position was kept constant with the reduced aminobenzoic acid unit (Amb^R). The anthracene unit was incorporated to allow the determination of oligosaccharide binding affinities by monitoring photoelectron transfer (PET)-induced fluorescence enhancement.[11] Thus, in the first sublibrary, R^1 was kept constant as an Amb^R residue and R^2 was varied with the four residues (Figure 2). The second sublibrary had R^2 conserved as the Amb^R residue. It was anticipated that these two sublibraries featuring pairs of isomeric sequences, which only differed in the nature of the interamine spacer adjacent to the anthracene sensor (R^2), would address the role of a third arylboronate unit in oligosaccharide binding. Indeed, when compared to the corresponding diboronic acid receptors, a larger association constant of similar value for two pseudosymmetric sequences would indicate that all three boronic acids are involved in the complex. Conversely, a significantly higher K_a for one of the two sequences would suggest a complex spanning only two boronic acids, with a preference for the interamine spacer adjacent to the anthracene unit.

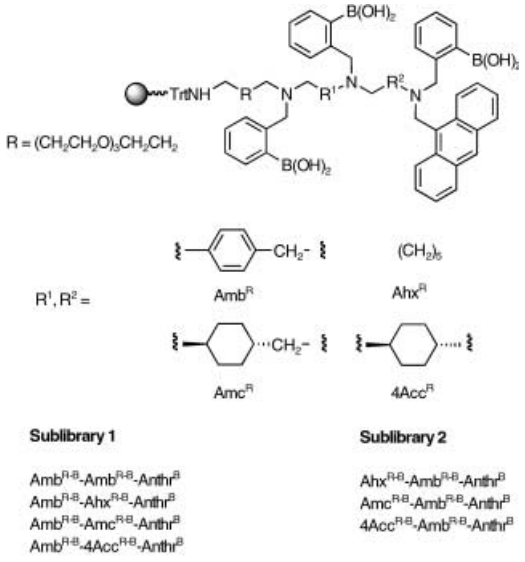
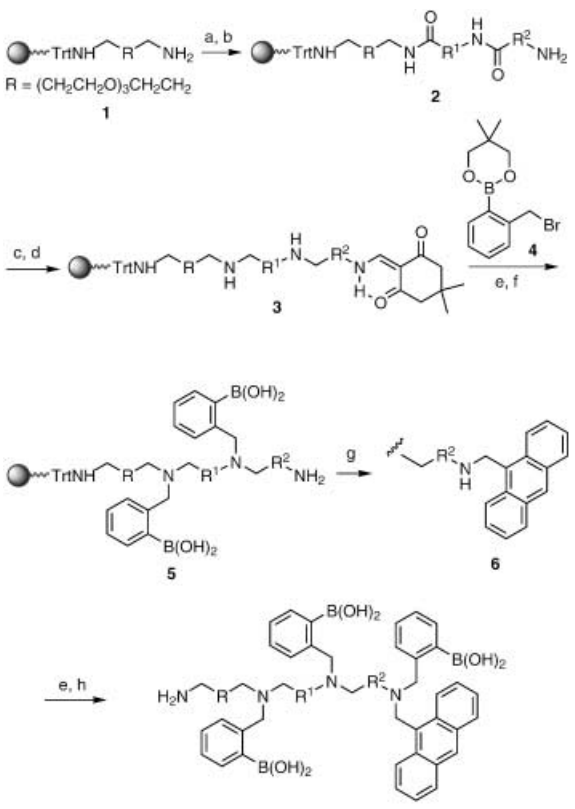


Figure 2. Sublibraries 1 and 2: triboronic acid receptors with different interamine spacers. Diboronic acid sublibrary 3 is a truncated version with only one interamine spacer (R^1). Superscripted R = reduced amino acid, superscripted B = *o*-boronobenzyl-substituted.

Solid-phase synthesis and evaluation of triboronic acid receptors against model disaccharides: For each sequence (Scheme 1), the Fmoc-terminal dipeptide was assembled from the diamine-derivatized trityl resin **1**. A polar diamine linker was chosen in order to optimize the solubility in aqueous solutions while its length should help avoid interference from the anchoring amine, which could be used eventually for conjugation purposes. After cleavage of the Fmoc group, the resulting deprotected dipeptide **2** was reduced with diborane,[12] and the terminal primary amine was selectively protected by reaction with 2-acetyldimedone.[13] The resulting intermediate **3** was subjected to a double alkylation of the secondary amines with boronate-containing benzyl bromide **4**. Hydrolysis of the cyclic dioxaborolanes and cleavage of the Dde group[14] provided intermediate **5**. The latter was functionalized with the requisite anthracene unit through a carefully optimized reductive amination protocol with 9-anthracenyl aldehyde to afford **6**. Final alkyla-

tion of the secondary amine of **6** followed by cleavage of the supported product with dilute trifluoroacetic acid yielded the crude triboronic acid receptors. Despite being assembled through more than ten consecutive steps on a solid support, the crude materials were obtained in >80% purity according to an HPLC analysis with ES-MS and UV detection at several wavelengths.[15] Quite importantly, the HPLC/UV trace at the optimal absorption for the anthracene unit revealed the presence of a single peak. Receptors that were not of satisfying homogeneity were purified by semipreparative HPLC. All receptors showed the presence of the molecular ion $[M+H]^+$ and/or the diagnostic dehydration peaks $[M+H-nH_2O]$ from gaseous boronic anhydride formation.

The receptors were evaluated for binding to a set of four model disaccharides: D-lactulose, D-melibiose, D-turanose and D-trehalose (Figure 3). The first three share the ability to isomerize between pyranose and furanose forms while the glycoside trehalose is locked in the isomer shown as a result of the absence of a reducing hydroxyl end. All these disaccharides possess at least three 1,2- or 1,3-diol units that may form cyclic boronate esters. Stability constants were measured for each complex by monitoring the increase in fluorescence intensity (λ = 423 nm) of the receptors upon addition of each disaccharide. All titrations were performed in 0.010 M phosphate buffer (pH 7.8) in a 1:1 water/methanol mixture. The association constants listed in Table 1 (sublibraries 1 and 2) were determined from the titration curves with a modified Benesi–Hildebrand equation,[16] which assumed a 1:1 binding stoichiometry.[15] Although other oligosaccharides may behave differently, inspection of the data reveals that the formation of complexes involving three tight boronate esters between the model disaccharides and the triboronic acid receptors is unlikely. For example, lactulose binds to the $Amb^{R\text{-}B}$-$Amc^{R\text{-}B}$-$Anthr^{B}$ receptor with twice the affinity (K_a = 390 M^{-1}) as the inverse sequence $Amc^{R\text{-}B}$-$Amb^{R\text{-}B}$-$Anthr^{B}$ (200 M^{-1}) and the diboronic acid receptor $Amb^{R\text{-}B}$-$Anthr^{B}$ (220 M^{-1}). These results suggest a preference for a putative diboronate complex spanning the Amc^{R} interamine spacer, which is supported by the higher K_a (520 M^{-1}) found for the diboronic acid receptor $Amc^{R\text{-}B}$-$Anthr^{B}$ (Table 1, sublibrary 3).[17] Thus, this study also demonstrates that small differences in the structure of interamine spacers can have a significant effect on the stability of the resulting complexes. For all receptors, none or negligible binding was observed with trehalose. These observations support the recent conclusions of Norrild and co-workers regarding the requirement for at least one rigid furanose *cis*-1,2-diol unit,[18] which can only be attained with disaccharides possessing an isomerizable reducing end. The higher affinity of the receptors for lactulose may be explained by its converging furanose triol unit, which could provide an alkoxyboronate ion.[18] In principle, the second boronic acid unit could bind the 4,6-diol unit on the hexose ring.



Scheme 1. Modular solid-phase synthesis of anthracenyl-capped triboronic acid receptors (sublibraries 1 and 2). a) i) HO_2C-R^1-NHFmoc, HBTU, HOBT (2 equiv each), $(iPr)_2EtN$ (4 equiv), DMF, 2 h; ii) piperidine/DMF 1:4, 0.5 h. b) i) HO_2C-R^2-NHFmoc, HBTU, HOBT (2 equiv each), $(iPr)_2EtN$ (4 equiv), DMF, 2 h; ii) 1:4 Piperidine/DMF, 0.5 h. c) i) 1.0 M BH_3·THF, 65 °C, 24–48 h; ii) piperidine, 65 °C, 16 h. d) 2-Acetyldimedone (Dde-OH, 1.5 equiv), DMF, 30 min. e) i) **4**, pempidine, THF, 65 °C, 24 h; ii) 1:4 H_2O/THF. f) 10% Ethanolamine in 1:2 EtOH/THF, 16 h. g) i) 9-Anthracenyl aldehyde, TMOF/DMF; ii) $NaBH_4$, AcOH, DMF. h) 5% TFA/CH_2Cl_2.

Solid-phase synthesis and evaluation of diboronic acid receptors with *para*-substituted arylboronic acid units: It was hoped that by incorporating various electron-donating and electron-withdrawing groups onto the arylboronic acid units, thus altering the Lewis acidity of the boron atom and the strength of B–N coordination, stronger binding affinities to the disaccharides could be observed. This was initially tested through the incorporation of different substituents *para* to the boronic acid functionality. Therefore, the same approach was used as that described for the triboronic acid receptors: we synthesized and evaluated a series of $Amc^{R\text{-}B}$-$Anthr^{B}$ diboronic acid receptors with the electron-withdrawing substituents cyano, fluoro, as well as the electron-donating methoxy group (Figure 4, sublibrary 4).[15,19] The binding measurements performed with lactulose as the model disaccharide clearly evidenced a qualitative trend showing that electron-poor arylboronic acid units are preferable in this system (Table 1, sublibrary 4). These findings are consistent with the hypothesis that a strongly coordinated tetrahedral B–N adduct should help in alleviating ring strain in the resulting boronic ester.[6,7] Further support for this important conclusion comes from the best lactulose receptor, $Ahx^{R\text{-}B(CN)}$-$Anthr^{B(CN)}$, which showed a six-fold improvement over the unsubstituted analogue $Ahx^{R\text{-}B}$-$Anthr^{B}$ (K_a = 1870 vs 315 M^{-1}).

The ability of our efficient synthetic scheme to vary the benzylic side chain of the amines in a controlled fashion could be of paramount importance in the design of second-

Table 1. Association constants K_a [M^{-1}] between oligoboronic acid receptors and the four model disaccharides of Figure 3.[a]

Triboronic acid receptors[b]	Disaccharides			
	Lactulose	Melibiose	Turanose	Trehalose
sublibrary 1				
$Amb^{R\text{-}B}$-$Amb^{R\text{-}B}$-$Anthr^{B}$	125	7	13	–[c]
$Amb^{R\text{-}B}$-$Ahx^{R\text{-}B}$-$Anthr^{B}$	430	60	150	–
$Amb^{R\text{-}B}$-$Amc^{R\text{-}B}$-$Anthr^{B}$	390	6	4	–
$Amb^{R\text{-}B}$-$4Acc^{R\text{-}B}$-$Anthr^{B}$	215	5	8	–
sublibrary 2				
$Ahx^{R\text{-}B}$-$Amb^{R\text{-}B}$-$Anthr^{B}$	140	12	15	–
$Amc^{R\text{-}B}$-$Amb^{R\text{-}B}$-$Anthr^{B}$	200	11	14	–
$4Acc^{R\text{-}B}$-$Amb^{R\text{-}B}$-$Anthr^{B}$	185	23	18	–
diboronic acid receptors[b]				
sublibrary 3				
$Amb^{R\text{-}B}$-$Anthr^{B}$	220			
$Ahx^{R\text{-}B}$-$Anthr^{B}$	315			
$Amc^{R\text{-}B}$-$Anthr^{B}$	520			
sublibrary 4				
$Amc^{R\text{-}B(OMe)}$-$Anthr^{B(OMe)}$	150			
$Amc^{R\text{-}B(F)}$-$Anthr^{B(F)}$	585			
$Amc^{R\text{-}B(CN)}$-$Anthr^{B(CN)}$	1020			
$Ahx^{R\text{-}B(CN)}$-$Anthr^{B(CN)}$	1870			
sublibrary 5				
$Amc^{R\text{-}B(OMe)}$-$Anthr^{B(H)}$	590			
$Amc^{R\text{-}B(F)}$-$Anthr^{B(H)}$	320			
$Amc^{R\text{-}B(CN)}$-$Anthr^{B(H)}$	290			
$Amc^{R\text{-}B(CO_2Me)}$-$Anthr^{B(H)}$	280			
$Amc^{R\text{-}B(NO_2)}$-$Anthr^{B(H)}$	185			

[a] Conditions for K_a measurements:[14] a titration was carried out by adding aliquots of saccharide to a solution of receptor in MeOH/H_2O 1:1 at pH 7.8 (0.010 M phosphate buffer). Fluorescence intensity was observed at $\lambda = 423$ nm. Most entries over 100 M^{-1} were duplicated. R^2 range: 0.95–0.99. [b] See Figures 2 and 4 for structures. Superscripted R = reduced amino acid, superscripted B = *o*-boronobenzyl-substituted. [c] No significant binding was observed.

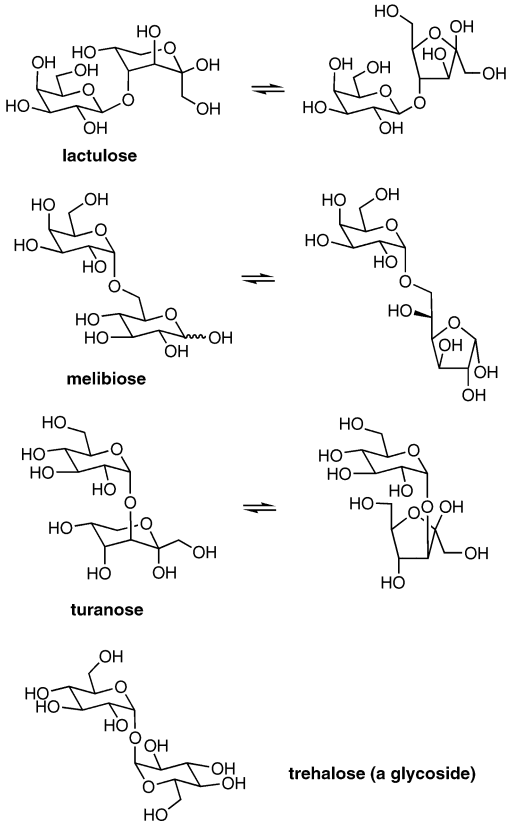


Figure 3. Set of four model disaccharides used in the binding measurement studies (Table 1).

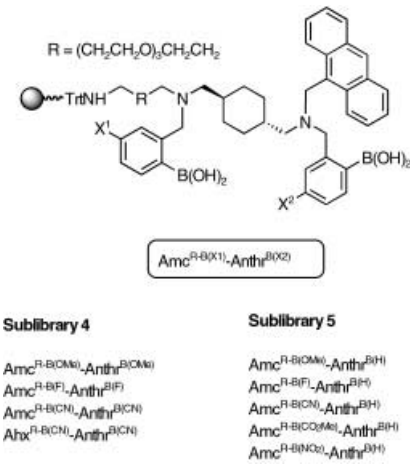


Figure 4. Sublibraries 4 and 5: Amc^{R} based diboronic acid receptors with different arylboronic acid units to probe the electronic requirements in oligosaccharide binding. Superscripted R = reduced amino acid, superscripted B = *o*-boronobenzyl-substituted.

generation libraries. To demonstrate the potential of our modular solid-phase approach, another small set of $Amc^{R\text{-}B}$-$Anthr^{B}$ diboronic acid receptors was synthesized by means of the IRORI MiniKan rf-encoding technology (Figure 4, sublibrary 5).[20] Instead of diversifying both nitrogens with various arylboronic acid units, it was decided that the first site only would be altered for this proof of concept. The entire sequence for synthesizing the diboronic acid sensors,

including the harsh exhaustive reduction step, was performed in the MiniKans, and employed an approach similar to that described in Scheme 1.[15] At the first alkylation stage, fifteen MiniKans were separated into five pools of three, then the pools were treated with one of *p*-cyano, *p*-nitro, *p*-fluoro, *p*-methoxycarbonyl, and *p*-methoxy-*o*-bromomethylphenylboronate. Following this operation, all the vessels were combined and underwent all subsequent modifications together. Final compounds cleaved from the solid support showed comparable purity to reactions performed on loose resin. Although the apparent K_a values obtained for lactulose binding are difficult to interpret on account of the unsymmetrical nature of these receptors, the inverse electronic trend compared to sublibrary 4 reflects the expected bias for the binding of the furanose diol to the most electron-deficient of the two arylboronic acid units. Indeed, in all of these receptors, except for $Ahx^{R\text{-}B(OMe)}$-$Anthr^{B(H)}$, the tightest boronate complex is located away from the sensing unit and thus cannot contribute to the fluorescence response. Most importantly, this sublibrary made with the MiniKan technology suggests that a much larger number of receptors could be rapidly synthesized by exhaustively diversifying the interamine spacer (R^1) and the second boronic acid unit (X^2) as well.

Conclusion

In summary, we have described the first entirely modular solid-phase synthetic approach to oligoboronic acid receptors and sensors for complex oligosaccharides. This approach allowed a systematic evaluation of the structural requirements for disaccharide binding with receptors displaying two and three boronic acid units. While new types of boronic acid units will be required for effective binding to glycosides, our study led to the identification of the preferred electronic characteristics for the arylboronate units in the binding of isomerizable sugars. By leading us to the design and synthesis of very electron-poor boronic acid units, this work constitutes an exciting prelude to the use of large libraries of receptors for the selective recognition of complex oligosaccharides.

Experimental Section

Reagents and apparatus: Fmoc-protected amino acids were purchased from NovaBiochem (La Jolla, California) or Advanced Chemtech (Louisville, Kentucky). Fmoc amino acids not commercially available were prepared by means of the procedure of Lapatsanis et al.[21] Polystyrene trityl chloride resin (90–150 mm, and 150–300 mm) was purchased from Rapp-Polymere (Tübingen, Germany). The loading specified by the supplier was used in all cases. THF (used in reactions and for resin washing) was dried by distillation over sodium/benzophenone and used the same day; CH_2Cl_2 and triethylamine over calcium hydride. Anhydrous DMF and NMP were obtained from Aldrich. Commercially available boronic acids were purchased from CombiBlocks (San Diego, California). All other chemicals were purchased from Aldrich and used without further purification. Solid-phase reactions that did not require external heat were performed in polypropylene (PP) reaction vessels (10 mL, 20 mL or 70 mL, BioRad Laboratories, Hercules, California). Sublibrary 5 was prepared with a IRORI MiniKans, (Discovery Partners, La Jolla, California) and 25–30 mg of resin (dry weight). The MiniKans were then fitted with a radiofrequency (RF) tag, and the reactions sorted with the IRORI AccuTag 100 sorting system.

Compound characterization and binding measurements: NMR spectra were acquired on Varian INOVA 300 and 500 MHz spectrometers. The residual solvent protons (^1H) or solvent carbons (^{13}C) were used as internal standards. ^1H NMR data is presented as follows: chemical shift (δ) downfield from tetramethylsilane (multiplicity, coupling constant, integration). The following abbreviations are used in reporting NMR data: s: singlet; brs: broad singlet; d: doublet; t: triplet; q: quartet; qt: quartet of triplets; dd: doublet of doublets; dt: doublet of triplets. In the ^{13}C NMR of the *para*-substituted bromomethylphenyl boronate esters, some resonances may be missing because of the weakly intense peaks of quaternary aromatic carbons. *NOTE*: NMR analysis of the final, cleaved oligoboronic acid receptors was not carried out for two reasons: i) their limited solubility, and ii) the very complex spectra obtained, which was partly due to the formation of equilibrating boronic anhydrides that result in several indistinguishable peaks. HPLC/UV/ESMS was the main method employed for assessing identity and purity of the oligoboronic acids (see below). These analyses were carried out on a Hewlett-Packard/Agilent 1100 MSD system (see below for specific conditions). High-resolution electrospray mass spectra (HRMS) were recorded by the University of Alberta Mass Spectrometry Services Laboratory with either electron impact (EI) or electrospray (ES) ionization. As is customary with boronic acids, peaks corresponding to gaseous dehydrative anhydride formation are often observed ($[M+H-(H_2O)_n]$), and consequently the molecular ion peak may be weak or absent. In some cases, a peak corresponding to an oxidized phenol form of one boronic acid, $[M-27]$, $[M+H-BOH]$, was observed in the HRMS and LCMS of the final compounds. We tentatively attribute this to a gas-phase detection phenomenon as only a single peak is always obtained on the HPLC chromatogram. Fluorescence titrations were carried out on a Photon Technology International (PTI) fluorimeter in a Hellma quartz glass SUPRASIL cuvette with a path length of 1 cm.

Abbreviations: The following abbreviations were used throughout the paper and the Experimental Section: Amb = 4-aminomethyl benzoyl, Amc = 4-aminomethyl cyclohexanoyl, Acc = 4-aminocyclohexane carboxyl, Ahx = 6-aminohexanoyl; R = any of the above interamine residues; R^R: superscript R indicates that the amide moiety of the residue has been reduced to a methylene, $R^{R\text{-}B(H)}$: superscript R-B indicates that the reduced amide, a secondary amine, has been alkylated with the (bromomethyl)phenyl boronate ester. The use of different *para*-substituted boronate esters is indicated by the letters following B and also corresponds to the use of the corresponding alkylating agents: $R^{R\text{-}B(H)}$ = (2-bromomethyl)phenyl boronic acid (ester), $R^{R\text{-}B(CN)}$ = (2-bromomethyl-4-cyano)phenyl boronic acid (ester), $R^{R\text{-}B(F)}$ = (2-bromomethyl-4-fluoro)-phenyl boronic acid (ester), $R^{R\text{-}B(NO_2)}$ = (2-bromomethyl-4-nitro)phenyl boronic acid (ester), $R^{R\text{-}B(CO_2Me)}$ = (2-bromomethyl-4-methoxycarbonyl)-phenyl boronic acid (ester), $R^{R\text{-}B(OMe)}$ = (2-bromomethyl-4-methoxy)-phenyl boronic acid (ester).

General procedures

Exhaustive amide reductions with BH_3·THF: The resin was weighed into a 50 mL round-bottomed flask fitted with a condenser, under an inert atmosphere. The resin was allowed to swell, and was then suspended in dry THF (5 mL). To this was added BH_3·THF (1 M in THF, ≈10 equiv per amide bond). The reaction mixture was then heated and stirred at 65 °C for 24–48 h. The suspension was allowed to cool to RT. The resin was filtered, washed with methanol, and re-suspended in piperidine (10–15 mL). The suspension was reheated to 65 °C and gently stirred for 24 h at this temperature. The suspension was allowed to cool to RT, and the resin was filtered, washed with dry THF, methanol and dichloromethane (3× each), then dried under a high vacuum overnight (< 0.5 mm Hg).

Dde protection of the terminal primary amine: To the resin in a 20 mL PP vessel was added 2-acetyldimedone (1.5 equiv) and anhydrous DMF (8 mL). The resulting suspension was gently vortexed for 30 min, filtered, washed with DMF, methanol and dichloromethane (3× each), and dried under high vacuum.

N-Alkylation with *o*-bromomethylphenyl boronate esters: The resin was weighed into a 25 mL round-bottomed flask with a stirring bar and fitted

with a condenser, under an inert atmosphere. The resin was suspended in dry THF (10 mL) and to the suspension was added 1,2,2,6,6-pentamethylpiperidine (5 equiv per alkylation site), followed by the bromomethylboronate ester to be attached (5 equiv per alkylation site), as a solution in dry THF. The suspension was then stirred and heated to 65 °C for 24–48 h, after which time it was allowed to cool to RT. The resin was filtered, washed with dry THF (4×), and dried under high vacuum.

Removal of the Dde protecting group: The removal of the Dde protecting group from the primary amine was done by one of two methods:

Method A: The resin was suspended in a 2% *v*/*v* solution of hydrazine in DMF (5 mL), gently vortexed for 10 min, and then the solution was drained. This process was repeated twice. The resin was then washed with DMF, methanol, and dichloromethane (3× each), and dried under high vacuum.

Method B: The resin was placed in a 25 mL round-bottomed flask fitted with a condenser and suspended in a 10% *v*/*v* solution of ethanolamine in ethanol (5 mL). To this suspension was added dry THF (5 mL) to facilitate swelling of the resin. The resulting suspension was then stirred at 65 °C for 24 h. The suspension was allowed to cool to RT, and the resin was filtered, washed with THF, methanol and dichloromethane, and dried under a high vacuum. (The resulting resin gave a positive Ninhydrin test in each case.)

Reductive amination: The resin and 9-anthraldehyde (10 equiv) were weighed into a 20 mL PP vessel. To this was added anhydrous trimethylorthoformate (1 mL) followed by anhydrous DMF (6 mL). The resulting suspension was vortexed for 5 min and shaken for 6 h. The suspension was filtered, washed with DMF (5×), and partially dried under vacuum. Sodium borohydride (10 equiv) was added to the dry resin which was suspended in anhydrous DMF (5 mL) and vortexed gently for 2 min. To this suspension was added glacial acetic acid (10 equiv), and the vessel was shaken for 2 h, filtered, washed with DMF, methanol, dichloromethane (3× each), then dried under a high vacuum overnight.

Final N-alkylation with bromomethylphenyl boronate esters: The N-alkylation of the final secondary amine was carried out as outlined above for the first alkylation. After stirring at 65 °C for 24–48 h, the resin was filtered and washed with dry THF and dried under a high vacuum. The dry resin was resuspended in 1:1 H_2O/THF and vortexed for 1 h. The resin was filtered, washed with 1:1 H_2O/THF, and THF. The resin was then dried under a high vacuum overnight.

Cleavage of oligoboronic acid receptors from the solid support: The resin was weighed into a 10 mL vial, with a stirbar, and suspended in a 5% *v*/*v* solution of TFA in dichloromethane. After stirring for 40 min, the resin beads had turned a deep red color. The resin was filtered through a glass wool plug and washed with dichloromethane, 5% TFA in dichloromethane and methanol (2× each). The combined filtrates were evaporated under reduced pressure, giving a green/brown oil which was dried overnight under a high vacuum.

Analysis of cleaved oligoboronic acid receptors by HPLC: LCMS/UV analyses were performed on a Hewlett-Packard/Agilent 1100 MSD by means of one of the following separation methods.

Method A: Column: SB-C8 ZORBAX, 3.5 μm, 4.6×50 mm; eluent: 5% MeCN (0.1% TFA) to 85% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 5 min, then hold for 7 min, at 0.7 mL min^{-1}. Post run of 10 min.

Method B: Column: SB-C8 ZORBAX, 3.5 μm, 4.6×50 mm; eluent: 5% MeCN (0.1% TFA) to 85% MeCN (0.1% TFA) in H_2O (0.1% TFA) over 5 min, then hold for 7 min, at 0.5 mL min^{-1}. Post run of 10 min.

In both cases, detection was carried out with a UV diode array detector (λ = 210 nm, 254 nm, and 370 nm (anthracene absorption)), coupled to a ESMS detector (fragmentor voltage: 80 eV).

Semipreparative purifications: These were carried out when required by means of the following general method: column: RX-C8 semipreparative, 5 μm, 9.4×250 mm; eluent: 5% MeCN (0.1% TFA) to 85% MeCN (0.1% TFA) over 5 min, then hold for 7 min at 3 mL min^{-1}. Post run of 10 min, with manual collection of required peaks, determined by absorption at 370 nm.

All purities quoted for the final oligoboronic acid receptors is prior to any purification by semipreperative HPLC. Post-HPLC purities >95% in all cases.

Synthesis of sublibraries 1 and 2

4,7,10-Trioxy-1,13-tridecanediamine trityl polystyrene resin (1): In a 500 mL round-bottomed flask, 4,7,10-trioxa-1,13-tridecanediamine (73.1 g, 72.8 mL, 331 mmol) was dissolved in dry dichloromethane (300 mL) and gently stirred. To this stirring solution was added chlorotrityl polystyrene resin (12 g, 1.38 mmol g^{-1} Cl, 16.6 mmol) in portions (2–3 g per addition, 20–30 min between additions). After complete addition of the resin, the suspension was stirred gently overnight. The resin was then transferred to a large PP vessel, washed with methanol, DMF/NEt_3 4:1, methanol and dichloromethane (3× each), then dried overnight under a high vacuum.

Synthesis of resin-bound Amb-R^{2}NH$_2$: Resin **1** (5 g, 1.1 mmol g^{-1}, 5.5 mmol) and Fmoc-Amb-OH (4.1 g, 11 mmol) were weighed into a 100 mL round-bottomed flask, suspended in anhydrous DMF (20 mL) and stirred gently under an inert atmosphere. HOBt (1.68 g, 11 mmol) and HBTU (4.17 g, 11 mmol) were each dissolved in anhydrous DMF and added to the stirring suspension. After stirring for a few min, DIPEA (2.84 g, 3.83 mL, 22 mmol) was added. The suspension was stirred for 6 h, and then the resin was filtered, washed with DMF, methanol and dichloromethane (3× each), and dried under a high vacuum. The Fmoc protecting group was removed by two treatments with 20% piperidine in DMF [i) 15 min, ii) 45 min], then the mixture was filtered and washed with DMF, methanol and dichloromethane (3× each), and dried under a high vacuum. The resin (750 mg, 0.72 mmol) and the second residue (2 equiv, 1.44 mmol) were weighed into a 20 mL PP vessel. HOBt (0.3 M in DMF, 4.8 mL, 1.44 mmol) and HBTU (0.3 M in DMF, 4.8 mL, 1.44 mmol) were added to the PP vessel, and the mixture was vortexed until all the second amino-acid residue had dissolved. DIPEA (0.37 g, 0.50 mL, 2.9 mmol) was then added and the mixture vortexed overnight. The resin was filtered, washed with DMF, methanol and dichloromethane (3× each) and dried under a high vacuum. Removal of the Fmoc group with two treatments of 20% piperidine in DMF, as outlined above, was performed again. The resin was washed with DMF, methanol and dichloromethane (3× each) and dried overnight under a high vacuum.

Synthesis of resin-bound R^1-AmbNH$_2$: Resin **1** (0.90 g, 1.1 mmol g^{-1}, 1.0 mmol) and the first residue (2 equiv, 2 mmol) were weighed into a 20 mL PP vessel. HOBt (0.3 M in DMF, 6.7 mL, 2 mmol) and HBTU (0.3 M in DMF, 6.7 mL, 2 mmol) were added to the PP vessel, and the mixture was vortexed until the first residue had dissolved. DIPEA (0.52 g, 0.70 mL, 4.0 mmol) was then added, and the suspension was vortexed for 4 h. After this time, the resin was filtered, washed with DMF, methanol and dichloromethane (3× each) and dried under a high vacuum. The Fmoc protecting group was removed by two treatments with 20% piperidine in DMF [i) 15 min, ii) 45 min], then filtered and washed with DMF, methanol and dichloromethane (3× each), and dried under a high vacuum. The resin was then treated with the second residue, Fmoc-Amb-OH (2 equiv, 2 mmol). HOBt (0.3 M in DMF, 6.7 mL, 2 mmol) and HBTU (0.3 M in DMF, 6.7 mL, 2 mmol) were added to the PP vessel, and the mixture was vortexed until the Fmoc-Amb-OH had dissolved. DIPEA (0.52 g, 0.70 mL, 4.0 mmol) was then added and the mixture was vortexed overnight. The resin was filtered, washed with DMF, methanol and dichloromethane (3× each), and dried under a vacuum. Removal of the Fmoc group with two treatments of 20% piperidine in DMF, as outlined above, was performed again. The resin was washed with DMF, methanol and dichloromethane (3× each), and dried overnight under a high vacuum.

Exhaustive amide reduction of resin-bound Amb-R^{2}NH$_2$ and R^1-AmbNH$_2$: This was carried out according to the general procedure.

Dde protection of resin-bound Amb^R-R$^{2.R}$NH$_2$ and R$^{1.R}$-Amb^{R}NH$_2$: This was carried out according to the general procedure.

Synthesis of resin-bound Amb$^{R\text{-}B(H)}$-R$^{2.R\text{-}B(H)}$NHDde and R$^{1.R\text{-}B(H)}$-Amb$^{R\text{-}B(H)}$NHDde: This was carried out according to the general procedure for N-alkylation.

Dde deprotection of resin-bound Amb$^{R\text{-}B(H)}$-R$^{2.R\text{-}B(H)}$NHDde and R$^{1.R\text{-}B(H)}$-Amb$^{R\text{-}B(H)}$NHDde: This was carried out according to the general procedure (Method A).

Reductive amination of resin-bound Amb$^{R\text{-}B(H)}$-R$^{2.R\text{-}B(H)}$NH$_2$ and R$^{1.R\text{-}B(H)}$-Amb$^{R\text{-}B(H)}$NH$_2$: This was carried out according to the general procedure.

Synthesis of resin-bound Amb$^{R\text{-}B(H)}$-R$^{2.R\text{-}B(H)}$-Anthr$^{B(H)}$ and R$^{1.R\text{-}B(H)}$-Amb$^{R\text{-}B(H)}$-Anthr$^{B(H)}$: This was carried out according to the general procedure for final N-alkylation.

Cleavage of resin-bound $Amb^{R\text{-}B(H)}$-$R^{2,R\text{-}B(H)}$-$Anthr^{B(H)}$ and $R^{1,R\text{-}B(H)}$-$Amb^{R\text{-}B(H)}$-$Anthr^{B(H)}$: This was carried out according to the general procedure.

$Amb^{R\text{-}B(H)}Amb^{R\text{-}B(H)}Anthr^{B(H)}$: HPLC, Method B, t_R = 8.337 min; purity >85%; HRMS (ES): calcd for $C_{62}H_{68}B_3N_4O_6$: 997.541802; found: 997.542094 $[M+H-3H_2O]^+$.

$Amb^{R\text{-}B(H)}Amc^{R\text{-}B(H)}Anthr^{B(H)}$: HPLC, Method B, t_R = 8.645 min; purity >80%; HRMS (ES): calcd for $C_{62}H_{80}B_3N_4O_9$: 1057.620447; found: 1057.620346 $[M+H]^+$.

$Amb^{R\text{-}B(H)}Acc^{R\text{-}B(H)}Anthr^{B(H)}$: HPLC, Method B, t_R = 8.558 min; purity >80%; HRMS (ES): calcd for $C_{61}H_{72}B_3N_4O_9$: 1041.589147; found: 1041.590924 $[M+H-2H]^+$.

$Amb^{R\text{-}B(H)}Ahx^{R\text{-}B(H)}Anthr^{B(H)}$: HPLC, Method B, t_R = 8.042 min; purity >80%; HRMS (ES): calcd for $C_{60}H_{67}B_3N_4O_6$: 977.573103; found: 977.573344 $[M+H-3H_2O]^+$.

$Amc^{R\text{-}B(H)}Amb^{R\text{-}B(H)}Anthr^{B(H)}$: HPLC, Method A, t_R = 6.833 min; purity >76%; HRMS (ES): calcd for $C_{62}H_{74}B_3N_4O_6$: 1003.588753; found: 1003.588169 $[M+H-3H_2O]^+$.

$Acc^{R\text{-}B(H)}Amb^{R\text{-}B(H)}Anthr^{B(H)}$: HPLC, Method A, t_R = 6.778 min; purity >75%; HRMS (ES): calcd for $C_{61}H_{72}B_3N_4O_6$: 989.573103; found: 989.573733 $[M+H-3H_2O]^+$.

$Ahx^{R\text{-}B(H)}Amb^{R\text{-}B(H)}Anthr^{B(H)}$: HPLC, Method A, t_R = 7.175 min; purity >85%; HRMS (ES): calcd for $C_{60}H_{72}B_3N_4O_6$: 977.573101; found: 977.573073 $[M+H-3H_2O]^+$.

Synthesis of sublibrary 3

Synthesis of resin-bound R^1NH_2: Resin **1** was weighed into a 20 mL PP vessel. To this was added the first amine residue, R^1, either Fmoc-Amb-OH, Fmoc-Amc-OH or Fmoc-Ahx-OH (2 equiv). To this was added HOBt (2 equiv) and HBTU (2 equiv) both as solutions in DMF. The suspension was vortexed for 5 min until the first amine residue had dissolved, and then DIPEA (4 equiv) was added. The PP vessel was shaken for 6 h, then the suspension was filtered, washed with DMF, methanol and dichloromethane, and dried under a high vacuum. Removal of the Fmoc group with two treatments of 20% piperidine in DMF, as outlined above, was performed again. The resin was washed with DMF, methanol and dichloromethane (3× each) and dried overnight under a high vacuum.

Exhaustive amide reduction of resin-bound R^1NH_2: This was carried out according to the general procedure.

Dde protection of $R^{1.R}NH_2$: This was carried out according to the general procedure.

Synthesis of resin-bound $R^{1,R\text{-}B(H)}$NHDde: This was carried out according to the general procedure for N-alkylation.

Dde deprotection of resin-bound $R^{1,R\text{-}B(H)}$NHDde: This was carried out according to the general procedure (Method B).

Reductive amination of resin-bound $R^{1,R\text{-}B(H)}NH_2$: This was carried out according to the general procedure.

Synthesis of resin-bound $R^{1,R\text{-}B(H)}$-$Anthr^{B(H)}$: This was carried out according to the general procedure for final N-alkylation.

Cleavage of sublibrary 3: This was carried out according to the general procedure.

$Amc^{R\text{-}B(H)}Anthr^{B(H)}$: HPLC, Method B, t_R = 8.462 min; purity >80%; LCMS: for $C_{47}H_{63}B_2N_3O_7$: 768 $[M+H-2H_2O]$, 786 $[M+H-H_2O]$, 804 $[M+H]$, 826, $[M+Na]^+$.

$Amb^{R\text{-}B(H)}Anthr^{B(H)}$: HPLC, Method B, t_R = 8.652 min; purity >85%; LCMS: for $C_{47}H_{57}B_2N_3O_7$: 780 $[M+H-H_2O]$, 762 $[M+H-2H_2O]$.

$Ahx^{R\text{-}B(H)}Anthr^{B(H)}$: HPLC, Method B, t_R = 8.338 min; purity >80%.

Synthesis of sublibrary 4

Synthesis of resin-bound $AmcNH_2$: Resin **1** (0.44 g, 1.1 mmol g^{-1}, 0.48 mmol) and Fmoc-Amc-OH (0.36 g, 0.96 mmol) were weighed into a 20 mL PP vessel. Anhydrous DMF (3 mL) was added and the mixture vortexed until the amino acid had dissolved. HOBt (47 mg, 0.96 mmol) and HBTU (0.36 g, 0.96 mmol) were added as solutions in DMF. The mixture was vortexed for 2 min and DIPEA (0.25 g, 0.33 mL, 1.9 mmol) added. The mixture was vortexed again for 2 min, then gently shaken overnight. The resin was filtered, washed with DMF, methanol and dichloromethane (3× each), and dried at the pump. Treatment of the resin with two rounds of 20% piperidine in DMF [i) 15 min, ii) 45 min], afforded the supported free primary amine.

Exhaustive amide reduction of resin-bound $AmcNH_2$: This was carried out according to the general procedure.

Dde protection of resin-bound Amc^RNH_2: This was carried out according to the general procedure.

Synthesis of resin-bound $Amc^{R\text{-}B(X)}$NHDde: This was carried out according to the general procedure for N-alkylation.

Dde deprotection of resin-bound $Amc^{R\text{-}B(X)}$NHDde: This was carried out according to the general procedure (Method B).

Reductive amination of resin-bound $Amc^{R\text{-}B(X)}NH_2$: This was carried out according to the general procedure.

Synthesis of resin-bound $Amc^{R\text{-}B(X)}Anthr^{B(X)}$: This was carried out according to the general procedure for final N-alkylation.

Cleavage of resin-bound $Amc^{R\text{-}B(X)}Anthr^{B(X)}$: This was carried out according to the general procedure.

$Amc^{R\text{-}B(OMe)}Anthr^{B(OMe)}$: HPLC, Method B, t_R = 9.270 min; purity >75%; HRMS (ES): calcd for $C_{49}H_{67}B_2N_3O$: 864.514167; found: 864.513954 $[M+H]^+$.

$Amc^{R\text{-}B(F)}Anthr^{B(F)}$: HPLC, Method B, t_R = 9.432 min; purity >75%. LCMS (ES): 862 $[M+Na]^+$, 840 $[M+H]^+$, 822 $[M+H-H_2O]$.

$Amc^{R\text{-}B(CN)}Anthr^{B(CN)}$: HPLC, Method B, t_R = 9.221 min; purity >80%; LCMS (ES): 854 $[M+H]^+$, 836 $[M+H-H_2O]$. HRMS (ES): calcd for $C_{49}H_{61}B_2N_5O_7$: 854.483536; found: 854.483238 $[M+H]^+$.

$Ahx^{R\text{-}B(CN)}Anthr^{B(CN)}$: This compound was synthesized by means of the previously outlined procedure for the Amc compounds in sublibrary 4, substituting Fmoc-Ahx-OH for Fmoc-Amc-OH in the first step. HPLC, Method B, t_R = 8.743 min; purity >75%. LCMS (ES): 810 $[M+H-H_2O]$, 792 $[M+H-2H_2O]$.

IRORI Mini-Kan synthesis of sublibrary 5: All reactions in Kans were carried out according to the same general procedures as outlined for reactions with free resin except for the following points. All reaction times were extended by 1.5–2 times to allow for sufficient diffusion of solvents and reagents into the Kan. All reactions in THF were carried out at 60 °C as opposed to 65 °C on account of the instability of the polypropylene Kans at the higher temperature. Finally, all washing times and amounts were increased.

Synthesis of resin-bound $AmcNH_2$: Three IRORI Mini-kans (referred to as Kans from here on) were fitted with a radio frequency (RF) tag followed by 25 mg dry weight of resin **1**. The Kans were capped and suspended in DMF (30 mL) and stirred gently (*NOTE*: DMF was added to the Kans in order to suspend the resin and remove any air bubbles from the Kans). To the solution was added Fmoc-Amc-OH (0.15 g, 0.40 mmol, 4 equiv) followed by HOBt (61 mg, 0.40 mmol, 4 equiv) and HBTU (0.15 g, 0.40 mmol, 4 equiv). The mixture was stirred again for 5 minutes before DIPEA (0.10 g, 0.14 mL, 0.80 mmol, 8 equiv) was added. The mixture was then stirred at room temperature for 10 h. The Kans were filtered, washed with DMF, and then treated with two rounds of 20% piperidine in DMF to remove the Fmoc protecting group. After washing with DMF, methanol and dichloromethane, the Kans were dried under a high vacuum.

Exhaustive amide reduction of resin-bound $AmcNH_2$: This was performed according to the general procedure.

Dde protection of resin-bound $AmcRNH_2$: This was carried out according to the general procedure.

Synthesis of resin-bound $Amc^{R\text{-}B(X)}$NHDde: This was carried out according to the general procedure for N-alkylation. *NOTE*: After this first N-alkylation step, the Kans were all pooled together and underwent the rest of the reaction steps in the same pot.

Dde deprotection of resin-bound $Amc^{R\text{-}B(X)}$NHDde: This was carried out according to the general procedure (Method B).

Reductive amination of resin-bound $Amc^{R\text{-}B(X)}NH_2$: This was carried out according to the general procedure.

Synthesis of resin-bound $Amc^{R\text{-}B(X)}$-$Anthr^{B(H)}$: This was carried out according to the general procedure for final N-alkylation.

When dry, the Kans were deconvoluted into the five different compounds by scanning each Kan with the IRORI AccuTag-100 system.

Cleavage of resin-bound Amc$^{R\text{-}B(X)}$-Anthr$^{B(H)}$: This was carried out according to the general procedure.

Amc$^{R\text{-}B(OMe)}$Anthr$^{B(H)}$: HPLC, Method B, t_R = 8.989 min; purity >80%. LCMS (ES): 834 $[M+H]^+$, 816 $[M+H-H_2O]$.

Amc$^{R\text{-}B(F)}$Anthr$^{B(H)}$: HPLC, Method B, t_R = 8.796 min; purity >75%; HRMS (ES): calcd for $C_{47}H_{62}B_2FN_3O_7$: 822.483616; found: 822.483727 $[M+H]^+$.

Amc$^{R\text{-}B(CN)}$Anthr$^{B(H)}$: HPLC, Method B, t_R = 8.765 min; purity >75%; HRMS (ES): calcd for $C_{48}H_{62}B_2N_4O_7$: 822.483616; found: 822.483727 $[M+H]^+$.

Amc$^{R\text{-}B(CO_2Me)}$Anthr$^{B(H)}$: HPLC, Method B, t_R = 8.769 min; purity >85%; HRMS (ES): calcd for $C_{49}H_{65}B_2N_3O_9$: 862.498517; found: 862.498165 $[M+H]^+$.

Amc$^{R\text{-}B(NO_2)}$Anthr$^{B(H)}$: HPLC, Method B, t_R = 8.825 min; purity >80%; HRMS (ES): calcd for $C_{47}H_{62}B_2N_4O_9$: 849.478116; found: 849.478833 $[M+H]^+$.

Measurements of binding constants: Accurately measured solutions of the receptors were prepared by dissolution in a 0.010 M phosphate buffer (pH 7.8) in 1:1 water/methanol. A 3 mL amount of the solution was added to a quartz cell and titrated with a 1.0 M solution of the disaccharide dissolved in the buffer solution. The fluorescence emission spectrum (excitation wavelength: 370 nm) was taken after each addition of the saccharide solution. Titration curves were constructed by plotting the relative fluorescence intensity at 423 nm vs concentration of the saccharide in the cell. The stability constant, K_a, assumes 1:1 binding stoichiometry and was determined with the Benesi–Hildebrandt equation by plotting $1/(I-I_o)$ vs 1/[saccharide] and obtaining the ratio between the intercept and the slope.

Synthesis of *para*-substituted bromomethylphenyl boronate esters:

(2-Bromomethyl)phenyl boronate ester (4):[7] *o*-Tolylboronic acid (9.90 g, 72.8 mmol) and 2,2-dimethyl-1,3-propanediol (9.89 g, 94.7 mmol) were weighed into a round-bottomed flask and dissolved in toluene (250 mL). The mixture was then refluxed under Dean–Stark conditions for 20 h. After this time, the solvent was removed under reduced pressure, and the mixture dissolved in dichloromethane. The solution was then purified by flash chromatography through silica gel with dichloromethane as the eluent to give the desired compound (14.6 g, 99%) as a clear oil. To the oil (14.6 g, 71.7 mmol), dissolved in carbon tetrachloride (200 mL) was added *N*-bromosuccinimide (13.4 g, 75.2 mmol) and AIBN (0.16 g, 0.99 mmol). The mixture was stirred and heated at reflux for 16 h. The solution was allowed to cool to RT and was then filtered. The solvent was evaporated under reduced pressure to give the title product as a yellow oil (19.7 g, 97%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.78 (d, J = 7.4 Hz, 1H), 7.34 (m, 2H), 7.25 (m, 1H), 4.91 (s, 2H), 3.79 (s, 4H), 1.05 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 143.5, 135.6, 130.2, 127.6, 72.4, 34.5, 21.9.

(2-Bromomethyl-4-cyano)phenyl boronate ester: 4-Bromo-3-methylbenzonitrile (1.0 g, 5.1 mmol) was dissolved in dry THF under N_2. The solution was cooled to −98 °C and *n*BuLi (1.58 M in hexanes, 4.84 mL, 7.65 mmol) was added dropwise to the solution. After completion of the addition, the solution was stirred for 15 min, then trimethyl borate (1.06 g, 1.16 mL, 10.2 mmol) was added slowly to the solution. Stirring was continued at −98 °C for 30 min and then the solution was allowed to warm to room temperature for 3 h. The solvent was removed under reduced pressure and the residue was dissolved in ether. After washing with 1 M HCl and water, the solution was evaporated to dryness. The crude compound was purified by column chromatography (silica gel, ethyl acetate/hexanes 3:1 to 100% ethyl acetate) to afford the 4-cyano-2-methylphenylboronic acid as a clear oil (0.63 g, 77%). The boronic acid was then protected and the methyl group brominated as outlined above for the unsubstituted analogue, to give the (2-bromomethyl-4-cyano)phenylboronate ester as a red/brown solid (1.22 g, 91% from (2-methyl-4-cyano)phenylboronic acid). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.87 (d, J = 7.0 Hz, 1H), 7.60 (d, J = 1.2 Hz, 1H), 7.51 (dd, J = 7.0 Hz, 1.2 Hz, 1H), 4.85 (s, 2H), 3.80 (s, 4H), 1.05 (S, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 144.7, 136.2, 133.1, 130.5, 118.4, 114.0, 72.6, 32.2, 21.8; HRMS (EI): calcd for $C_{13}H_{15}{}^{11}B^{79}BrNO_2$: 307.03793; found: 307.03762 $[M]^+$; for $C_{13}H_{15}{}^{11}B^{81}BrNO_2$: 309.03589; found: 309.03599 $[M]^+$.

(2-Bromomethyl-4-fluoro)phenyl boronate ester: (2-Methyl-4-fluoro)phenylboronic acid (0.40 g, 2.6 mmol) was protected with 2,2-dimethyl-1,3-propane diol (0.30 g, 2.9 mmol) as outlined above for the unsubstituted analogue. Bromination of the *ortho*-methyl group with *N*-bromosuccinimide (0.46 g, 2.8 mmol) and AIBN (14 mg, 0.08 mmol), under the conditions described above afforded the title compound as a clear yellow oil (0.73 g, 94% from (2-methyl-4-fluorophenyl)boronic acid). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.78 (t, J = 6.8 Hz, 1H), 7.05 (dd, J = 9.8 Hz, 2.4 Hz, 1H), 6.93 (td, J = 8.5 Hz, 2.4 Hz, 1H), 4.85 (s, 2H), 3.80 (s, 4H), 1.10 (S, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 165.4, 163.6, 146.3, 137.9, 117.0, 114.5, 72.3, 33.2, 21.8; HRMS (EI): calcd for $C_{12}H_{15}{}^{11}B^{79}BrFO_2$ $[M]^+$: 300.03326; found: 300.03358; for $C_{12}H_{15}{}^{11}B^{81}BrFO_2$ $[M]^+$: 302.03119; found: 302.03123.

(2-Bromomethyl-4-methoxy)phenyl boronate ester: (2-Methyl-4-methoxy)-phenylboronic acid (0.50 g, 3.0 mmol) was protected with 2,2-dimethyl-1,3-propane diol (345 mg, 3.31 mmol) as outlined above for the unsubstituted analogue. Bromination of the *ortho*-methyl group with *N*-bromosuccinimide (0.55 g, 3.34 mmol) and AIBN (16 mg, 0.09 mmol), under the conditions described above for the unsubstituted analogue afforded the title compound as a clear yellow oil (0.82 g, 85% from (2-methyl-4-methoxy)phenylboronic acid). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.74 (d, J = 8.4 Hz, 1H), 6.88 (s, 1H), 6.78 (d, J = 8.4 Hz, 1H), 4.88 (s, 2H), 3.80 (s, 3H), 3.76 (s, 4H), 1.02 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 161.2, 145.6, 137.5, 115.8, 113.0, 72.3, 55.1, 34.4, 21.9; HRMS (EI): calcd for $C_{13}H_{18}{}^{11}B^{79}BrO_3$ $[M]^+$: 312.05322; found: 312.05354; for $C_{13}H_{18}{}^{11}B^{81}BrO_3$ $[M]^+$: 314.05118; found: 314.05196.

(2-Bromomethyl-4-methoxycarbonyl)phenyl boronate ester: 4-Bromo-3-methylbenzoic acid (2.15 g, 10.0 mmol) was dissolved in dry diethyl ether (30 mL) and stirred under an argon atmosphere. The solution was cooled to −100 °C and *n*BuLi (1.6 M in hexanes, 18.8 mL, 30 mmol) was added dropwise to the solution, at a pace slow enough to maintain the temperature below −90 °C. After complete addition, the mixture was stirred for 30 min, then trimethylborate (5.7 mL, 50 mmol) was slowly added. The reaction was stirred at −100 °C for 1 hour then the bath was removed, and the reaction mixture was allowed to warm to room temperature under stirring for 5 h. After this time, the reaction mixture was poured onto 1 M aqueous HCl (30 mL) and extracted with ether. The boronic acid was then purified by column chromatography and isolated in 75% yield. The boronic acid was dissolved in methanol and refluxed for 16 h in the presence of catalytic sulfuric acid. The solvent was removed under reduced pressure and the boronic acid residue was protected with 2,2-dimethyl-1,3-propanediol (1.1 equiv), as described above for the unsubstituted analogue. The *ortho*-methyl group (320 mg, 1.22 mmol) was then brominated with NBS (239 mg, 1.34 mmol) and AIBN (61 mg, 0.37 mmol) as described above, to afford the title compound after flash column chromatography (silica gel) as an off-white/pink solid (340 mg, 70% yield from the boronic acid). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.98–7.83 (m, 3H), 4.90 (s, 2H), 3.89 (s, 3H), 3.79 (s, 4H), 1.04 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 166.5, 143.7, 135.6, 131.7, 130.8, 128.2, 72.5, 52.2, 33.6, 31.9, 22.0; HRMS (EI): calcd for $C_{14}H_{18}{}^{11}B^{79}BrO_4$ $[M]^+$: 340.04816; found: 314.04754; for $C_{14}H_{18}{}^{11}B^{81}BrO_4$ $[M]^+$: 342.04611; found: 342.04556.

(2-Bromomethyl-4-nitro)phenyl boronate ester: 2-Iodo-5-nitrotoluene (1.00 g, 3.80 mmol) was dissolved in dry diethyl ether (10 mL) and stirred under an argon atmosphere. The solution was cooled to −100 °C and *n*BuLi (1.6 M in hexanes, 3.60 mL, 5.76 mmol) was added dropwise to the solution. This addition was slow enough to maintain the reaction temperature below −90 °C. After complete addition, the mixture was stirred for 25 min, then trimethylborate (1.30 mL, 11.4 mmol) was slowly added to the solution. After addition, the solution was stirred at −100 °C for a further 40 minutes. The bath was removed, and the mixture allowed to warm to room temperature. After 5 h, the solution was poured into 1 M aqueous HCl (30 mL), and the product extracted with diethyl ether. The boronic acid was protected with 2,2-dimethyl-1,3-propanediol (1.1 equiv) as outlined above for the unsubstituted analogue. The *ortho*-methyl group was then brominated with *N*-bromosuccinimide (416 mg, 2.34 mmol) and AIBN (105 mg, 0.64 mmol) under the conditions described above to afford the title compound as a dark solid (570 mg, 82% from 2-iodo-5-nitrotoluene). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.17–7.94 (m, 3H), 4.91 (s, 2H), 3.82 (s, 4H), 1.06 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 149.1, 145.4, 136.7, 124.4, 121.8, 72.6, 32.2, 31.8, 21.9; HRMS (EI): calcd for $C_{12}H_{15}{}^{11}B^{79}BrNO_4$: 327.02774; found: 327.02748 $[M]^+$; calcd for $C_{12}H_{15}{}^{11}B^{81}BrNO_4$: 329.02570; found: 329.02595 $[M]^+$.

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