Electronic Supplementary Information

Reversible covalent interactions of β-aminoboronic acids with carbohydrate derivatives

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Contents

1.0 General considerations

Commercial reagents were purchased and used as received with the following exceptions: Methylene chloride (DCM/CH$_2$Cl$_2$), methanol (MeOH) and triethylamine were distilled from CaH$_2$ under nitrogen. Acetonitrile (MeCN) was distilled from activated 4 Å MS under nitrogen. Toluene was purified by being passed through columns of activated alumina under argon (Innovative Technology, Inc). Water used in spectroscopy was obtained by passing deionized water through a Millipore Synergy Water Purification System. Deuterated chloroform-d$_1$ was purchased from Cambridge Isotope. Deuterated methanol-d$_4$ was purchased from Sigma Aldrich. All other solvents were of reagent grade quality and dried over 4 Å MS prior to use.

2.0 Instrumentation

2.1 Chromatography

Flash column chromatography was carried out using Silicycle 230-400 mesh silica gel, or ISCO Teledyne Combiflash R$_f$ 200 Flash system. Thin-layer chromatography (TLC) was performed on Macherey Nagel pre-coated glass backed TLC plates (SIL G/UV254, 0.25 mm) and visualized using a UV lamp (254 nm), KMnO$_4$ or curcumin stain. Reverse-phase chromatography was carried out using Redi*Sep* Rf Gold C18 Columns.

2.2 Nuclear Magnetic Resonance (NMR) spectroscopy

^{1}H NMR, ^{13}C, and 2D NMR spectra were recorded on Varian Mercury 300 MHz, 400 MHz, 500 MHz, 600 MHz or 700 MHz spectrometers. ^{11}B NMR were recorded using Bruker 400/500 MHz spectrometer at 128/160 MHz and referenced to an external standard of BF$_3$·Et$_2$O (δ = 0 ppm). ^{1}H NMR spectra chemical shifts (δ) are reported in parts per million (ppm) referenced to residual protonated solvent peak (CD$_3$CN δ = 1.94, DMSO-d_6, δ = 2.49, CD$_3$OD δ = 3.31 center line). Spectral data is reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, ddt = doublet of doublet of triplets, dtd = doublet of triplet of doublets, m = multiplet, br = broad), coupling constant (*J*) in Hertz (Hz), and integration. ^{13}C NMR spectra chemical shifts (δ) are reported in parts per million (ppm) were referenced to carbon resonances in the NMR solvent (CD$_3$CN δ = 118.3, DMSO-d_6, δ = 39.5, CD$_3$OD δ = 49.0; center line). Carbons exhibiting significant line broadening brought about by boron substituents were not reported (quadrupolar relaxation). Carbons that were observed are noted (**C**-B).

2.3 Mass spectroscopy

2.3.1 High resolution

High resolution mass spectra were obtained on a VG 70- 250S (double focusing) mass spectrometer at 70 eV or on an ABI/Sciex Qstar mass spectrometer with ESI source, MS/MS and accurate mass capabilities.

2.3.2 RP-HPLC/MS

Low-resolution mass spectra (ESI) were collected on an Agilent Technologies 1200 series HPLC paired to a 6130 Mass Spectrometer. Compounds were resolved on Phenomenex's Kinetex 2.6μ C18 50x4.6mm column at room temperature with a flow of 1 mL/min. The gradient consisted of eluents A (0.1% formic acid in double distilled water) and B (0.1% formic acid in HPLC-grade acetonitrile). *Method*: A linear gradient starting from 5% of B to 95% over 4 min at a flow rate of 1.0 mL/min. Stays constant at 95% for 1 min and then returns to 5% over 0.5 min

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2.5 UV/Vis spectroscopy

Absorption spectra were recorded on a Varian Cary 5000 UV-Vis-NIR spectrophotometer at 25 °C.

2.6 Fluorescence spectroscopy

Fluorescence spectra were collected using a Photon Technology International (PTI) QuantaMaster 40-F NA spectrofluorometer with a photomultiplier detector and a xenon arc lamp source.

2.7 Infrared spectroscopy

Infrared FTIR spectra from *in situ* reaction monitoring were obtained on a Mettler-Toledo ReactIR™ 15 instrument equipped with 6.3 mm AgX DiComp probe.

2.8 pH Measurements

pH measurments were obtained on either a Thermo Scientific™ Orion™ 8103BNUWP ROSS Ultra™ pH Electrode coupled to VWR Symphony SB20 meter or a Oakton Acorn pH 6 pH meter. pH meters were calibrated with Orion Buffer solutions of pH = 4.01, 7.00, and 10.01 prior to measurement.

3.0 Synthetic methods
3.1 Preparation of β-amino MIDA-boronates
3.1.1 2-(2-((anthracen-9-ylmethyl)(methyl)amino)ethyl)-6-methyl-1,3,6,2-dioxazaborocane-4,8-dione
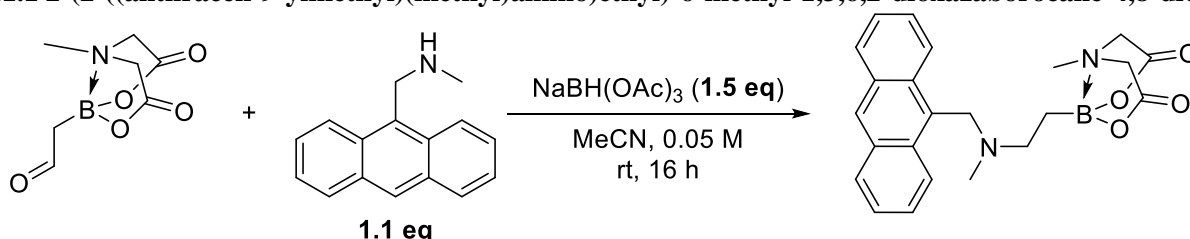
To a flame-dried 25 mL round bottom flask equipped with a magnetic stir bar and rubber septum was added α-MIDA boryl aldehyde **1** (1.0 eq, 53.6 mg, 0.269 mmol),[1] acetonitrile (0.05 M) and 1-(anthracen-9-yl)-N-methylmethanamine (1.1 eq, 61.4 mg, 0.296 mmol). The resultant mixture was left to stir at room temperature for 20 minutes under an inert atmosphere of nitrogen. Sodium tris(acetoxy)borohydride (1.5 eq, 85.6 mg, 0.404 mmol) was then added and left to stir for 16 h. Amberlite IRA743 resin was added to the solution and stirred for 15 minutes. The solution was passed through a plug of Celite, and the filtrate was concentrated under reduced pressure and purified by flash column chromatography.

Crude product was loaded directly onto a silica gel column deactivated with 1% triethylamine. A solvent mixture of EtOAc:MeCN 8:2→5:5→2:8 with a constant 1% triethylamine was used to obtain the product as a yellow solid (265 mg, 0.76 mmol, 86% yield).

^{1}H NMR (400 MHz, CD3CN) δ 0.86 - 1.01 (m, 2H), 2.23 (s, 3H), 2.59 - 2.68 (m, 2H), 2.80 (s, 3H), 3.64 (d, J = 17.0 Hz, 2H), 3.82 (d, J = 17.0 Hz, 2H), 4.45 (s, 2H), 7.43 - 7.61 (m, 5H), 8.05 (d, J = 8.2 Hz, 2H), 8.47 - 8.59 (m, 3H).

^{13}C NMR NMR (126 MHz, CD$_3$CN) δ 169.0, 132.4, 132.2, 129.9, 128.4, 126.7, 126.2, 126.0, 62.6, 54.5, 53.9, 46.7, 41.6. (signal for ipso-carbon on the anthracene ring was not observed. Attempts to resolve this signal in CDCl$_3$ were unsuccessful).

^{11}B NMR (128 MHz, CD3CN) δ 12.8.

HRMS (DART-TOF) [M+H$^+$] calculated for $C_{23}H_{26}B_1N_2O_4$ = 405.1986, found = 405.1985.

3.2 Preparation of β-amino boronic acid
3.2.1 N-(anthracen-9-ylmethyl)-2-borono-N-methylethan-1-aminium formate (5)
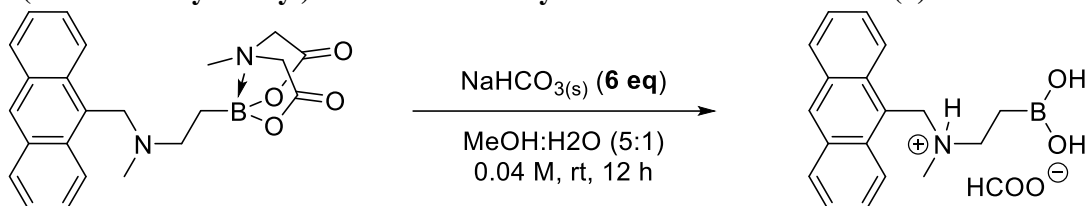
To a 2-dram vial equipped with a magnetic stir bar and screw-cap lid, 2-(2-((anthracen-9-ylmethyl)(methyl)amino)ethyl)-6-methyl-1,3,6,2-dioxazaborocane-4,8-dione (1.0 eq, 150 mg, 0.37 mmol) and MeOH:H2O (5:1, 0.04 M) was added. $NaHCO_{3(s)}$ (6 eq, 187 mg, 2.23 mmol) was then added and the reaction mixture was stirred vigorously at room temperature for 12 h while monitoring by LC-MS. The reaction was diluted with a MeCN (10 mL) and Celite was added. All volatiles were removed in vacuo and the crude product adsorbed onto the Celite was purified by reverse-phase chromatography (H2O:MeCN 5:95 → 95:5, with 0.1% formic acid). The fractions containing product (as indicated by LC-MS) were pooled and lyophilized to obtain the product as a yellow solid (85 mg, 0.29 mmol, 78% yield)

LC-MS retention time = 2.95 min;

^{1}H NMR (500 MHz, CD3OD) δ 1.11 (t, J = 7.8 Hz, 2H), 2.73 (s, 3H), 3.43 (t, J = 7.7 Hz, 2H), 5.35 (s, 2H), 7.52 - 7.65 (m, 2H), 7.73 (ddd, J = 9.0, 6.6, 1.3 Hz, 2H), 8.12 - 8.22 (m, 2H), 8.41 (dd, J = 8.9, 1.1 Hz, 2H), 8.47 (s, 1H), 8.73 (s, 1H) (formic acid, C-H).

^{13}C NMR (126 MHz, CD3OD) δ 169.5 (formic acid, C=O) 132.9, 132.9, 132.0, 130.8, 129.1, 126.6, 124.3, 122.3, 57.4, 52.7, 49.3, 49.0, 40.1.

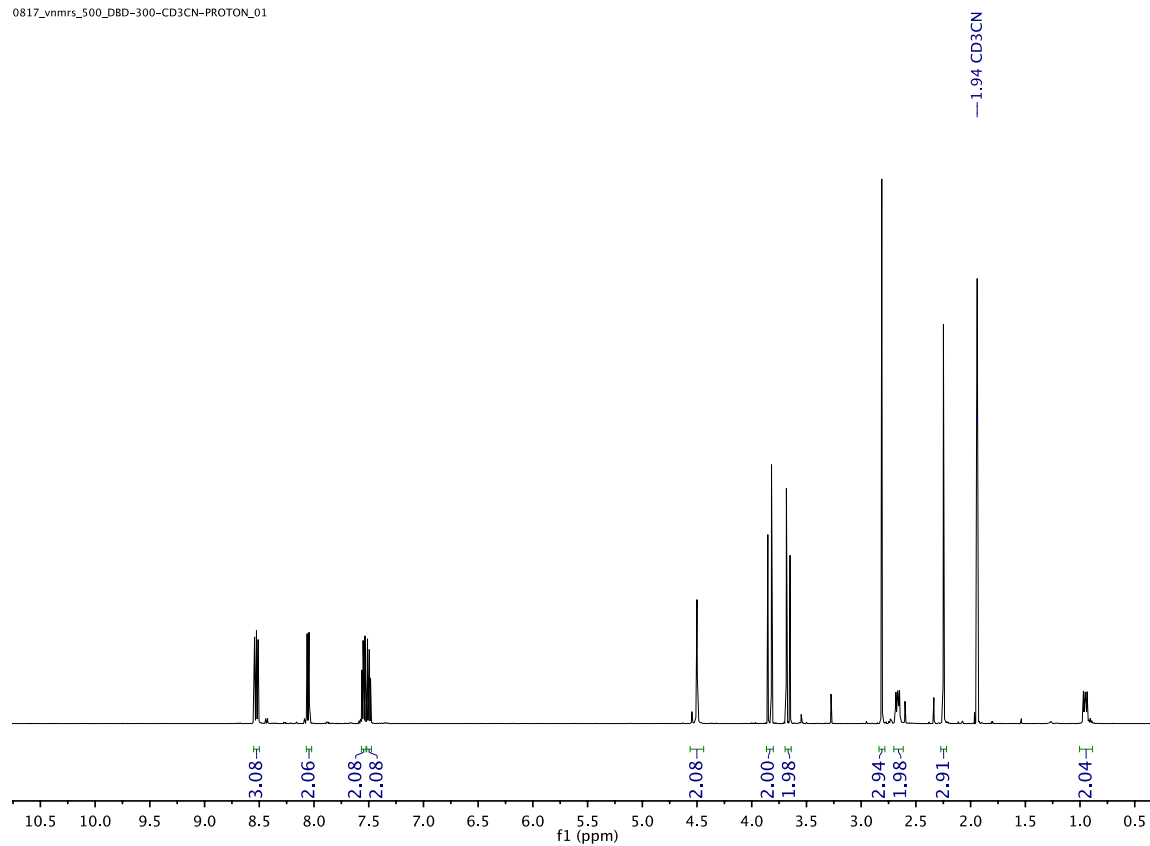**^{11}B NMR** (128 MHz, MeOD) δ 20.4.

HRMS (ESI) [M+H$^+$] calculated for $C_{18}H_{21}B_1NO_2$ = 293.1696, found = 293.1692.
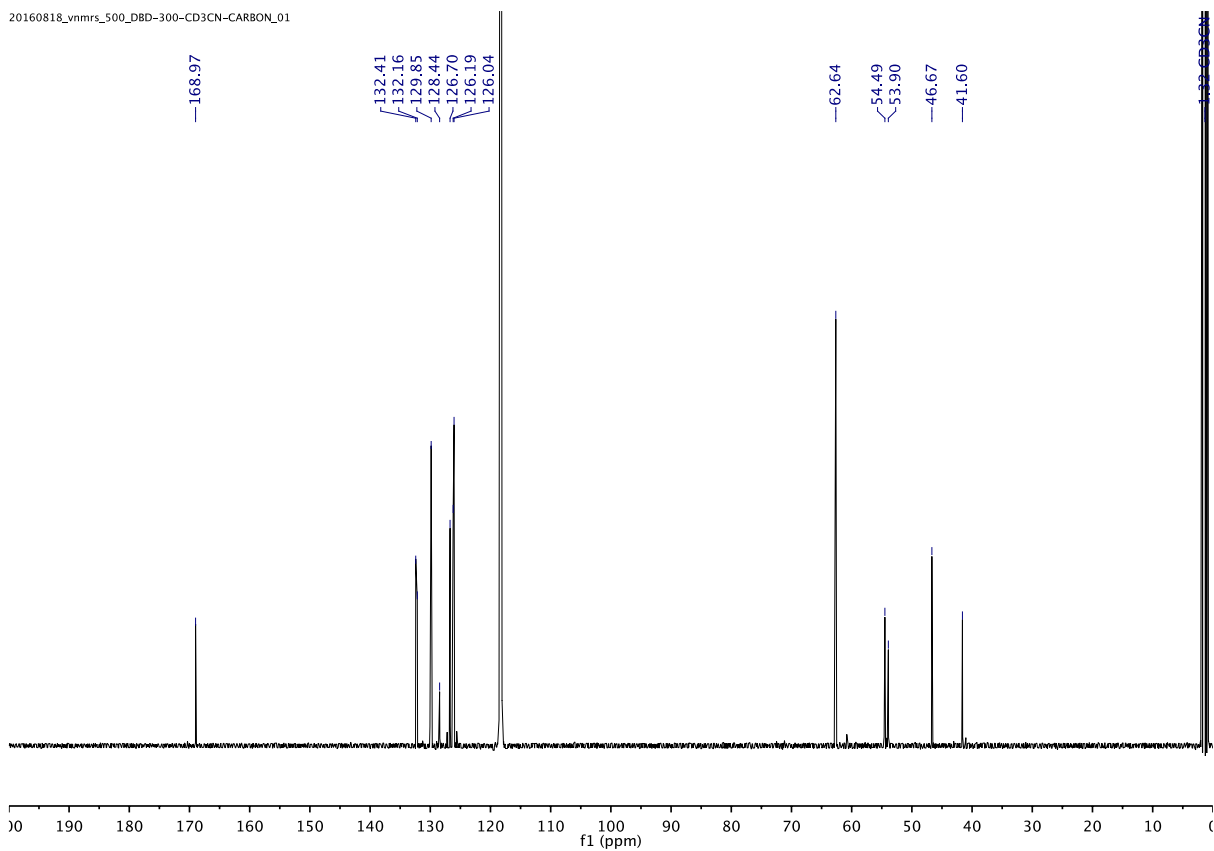
4.0 Spectral data
4.1 NMR
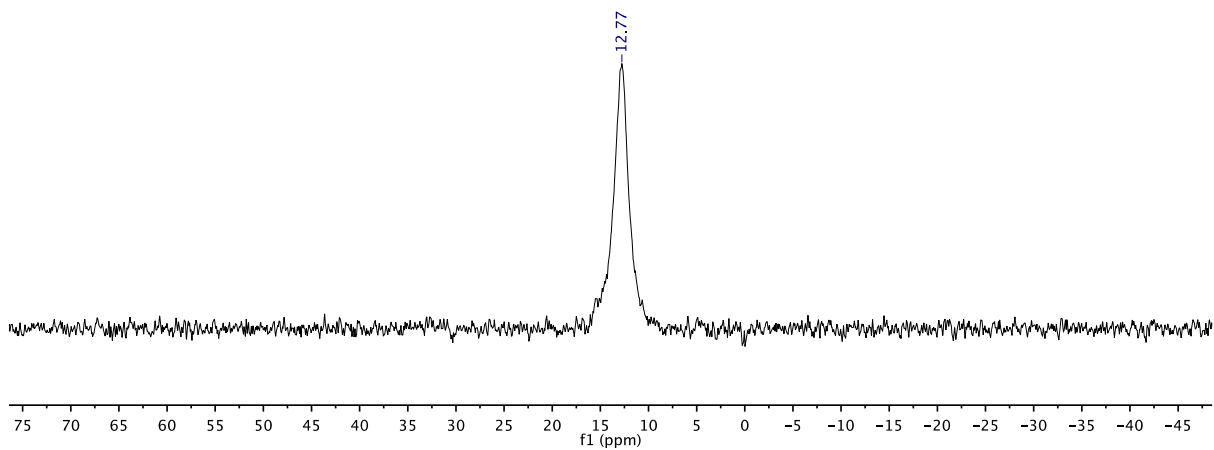**4.1.1 2-(2-((anthracen-9-ylmethyl)(methyl)amino)ethyl)-6-methyl-1,3,6,2-dioxazaborocane-4,8-dione**

0817_vnmrs_500_DBD-300-CD3CN-PROTON_01
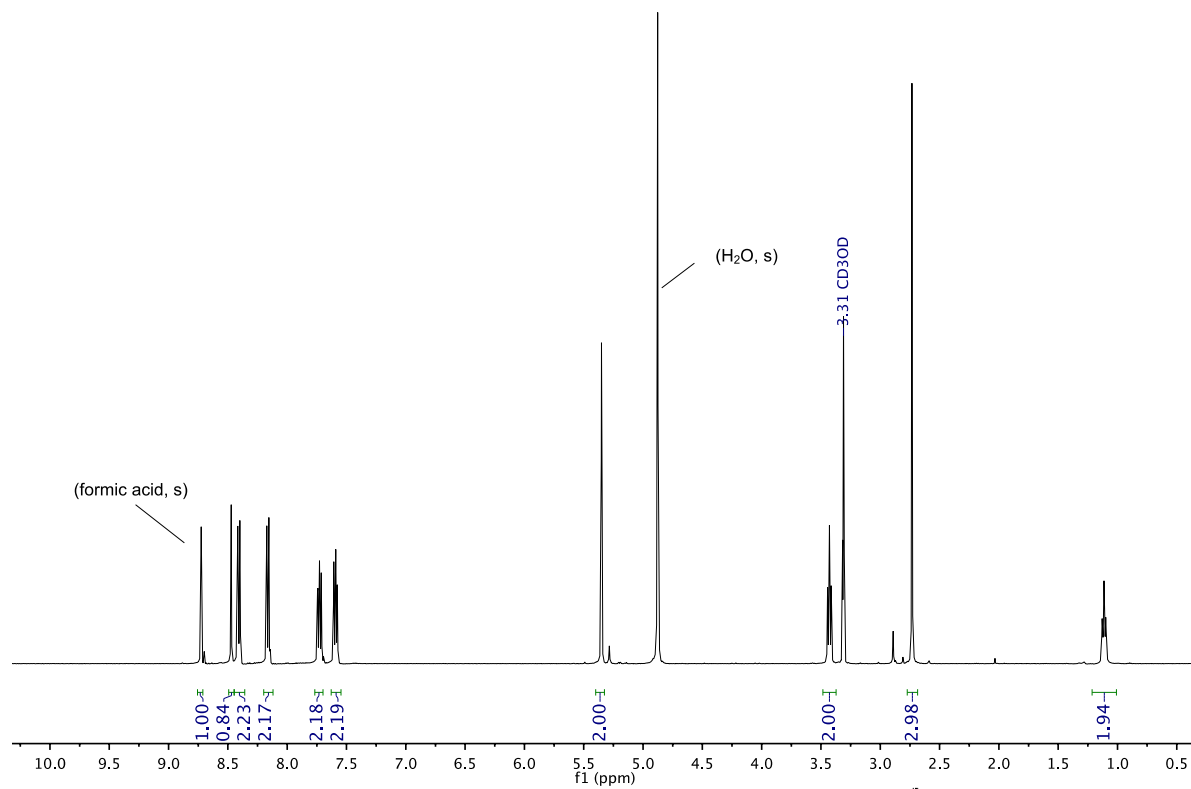
20160818_vnmrs_500_DBD-300-CD3CN-CARBON_01

168.97

132.41
132.16
129.85
128.44
126.70
126.19
126.04

62.64
54.49
53.90
46.67
41.60

1.32 CD3CN

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

DBD-300.4.fid
chem_Boron CD3CN /opt/data ddiaz 30

12.77

75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 -5 -10 -15 -20 -25 -30 -35 -40 -45
f1 (ppm)

6

4.1.2 N-(anthracen-9-ylmethyl)-2-borono-N-methylethan-1-aminium formate (5)

20151222_vnmrs_500_DBD-303-CD3OD-PROTON_01

~3.5mg

(formic acid, s)

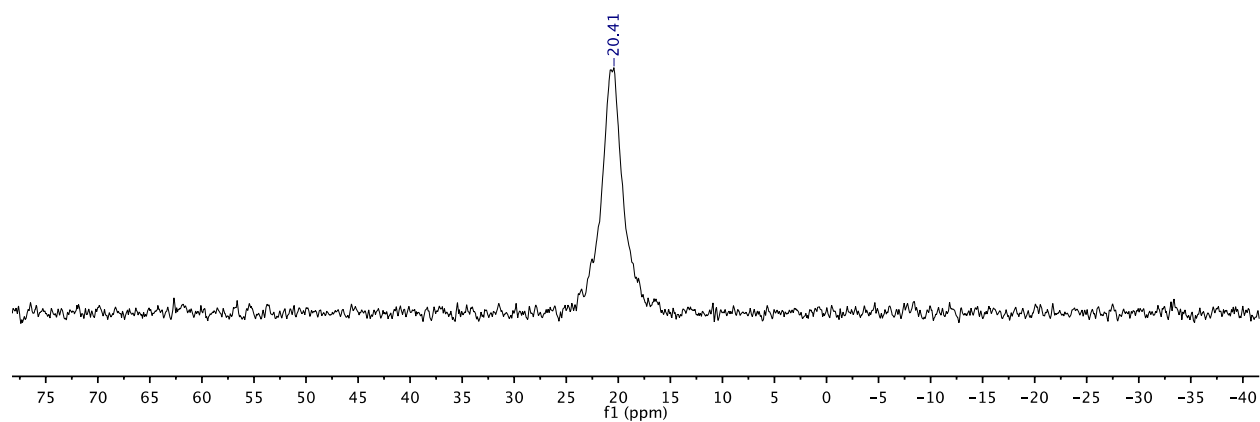(H₂O, s)

3.31 CD3OD

1.00 0.84 2.23 2.17 2.18 2.19 2.00 2.00 2.98 1.94

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

20151222_vnmrs_500_DBD-303-CD3OD-CARBON_01

~3.5mg

169.53

132.87 132.86 132.03 130.81 129.08 126.57 124.26 122.29

57.38 52.65 49.00 CD3OD 40.06

(Formic acid, **C**=O)

00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

7

—20.41

75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 −5 −10 −15 −20 −25 −30 −35 −40
f1 (ppm)

4.2 UV/Vis

$\Delta Abs = 0.00693[Host] - 0.0010$
$R^2 = 0.99999$
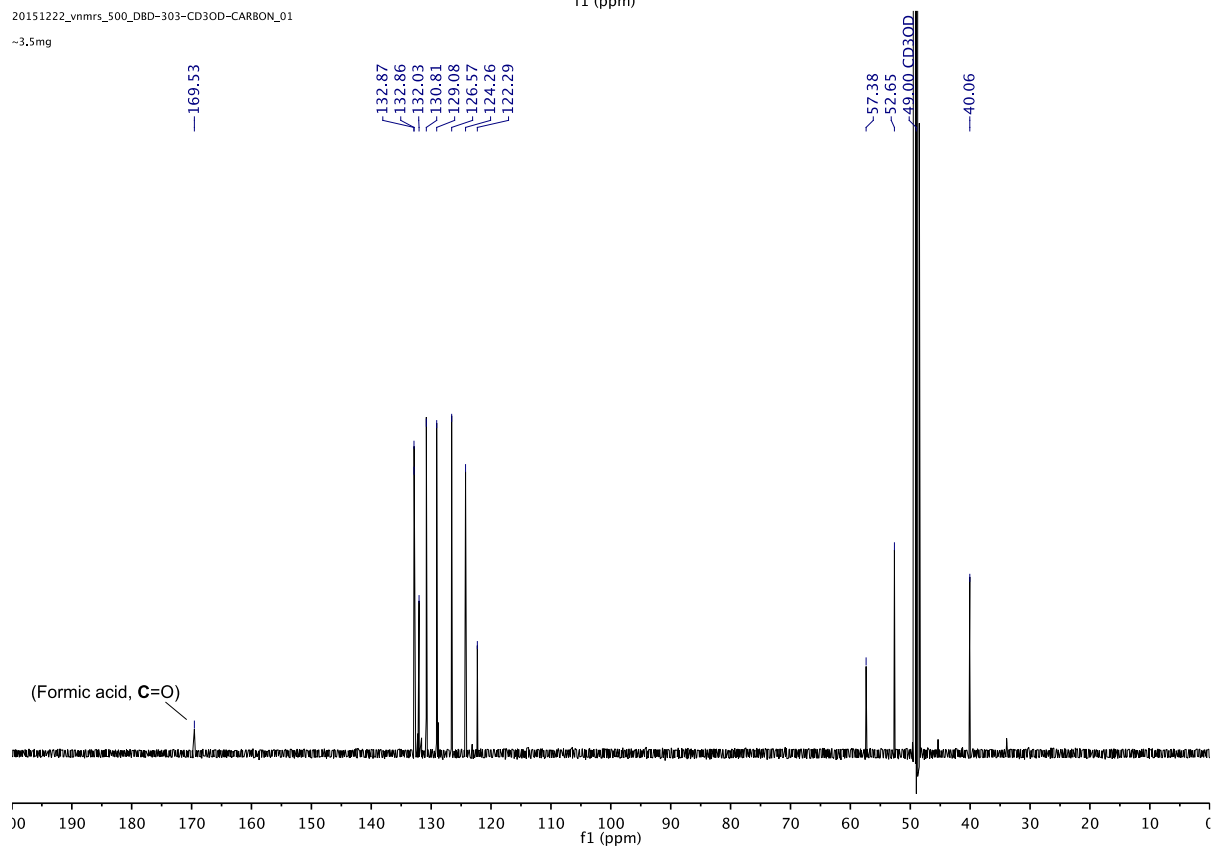
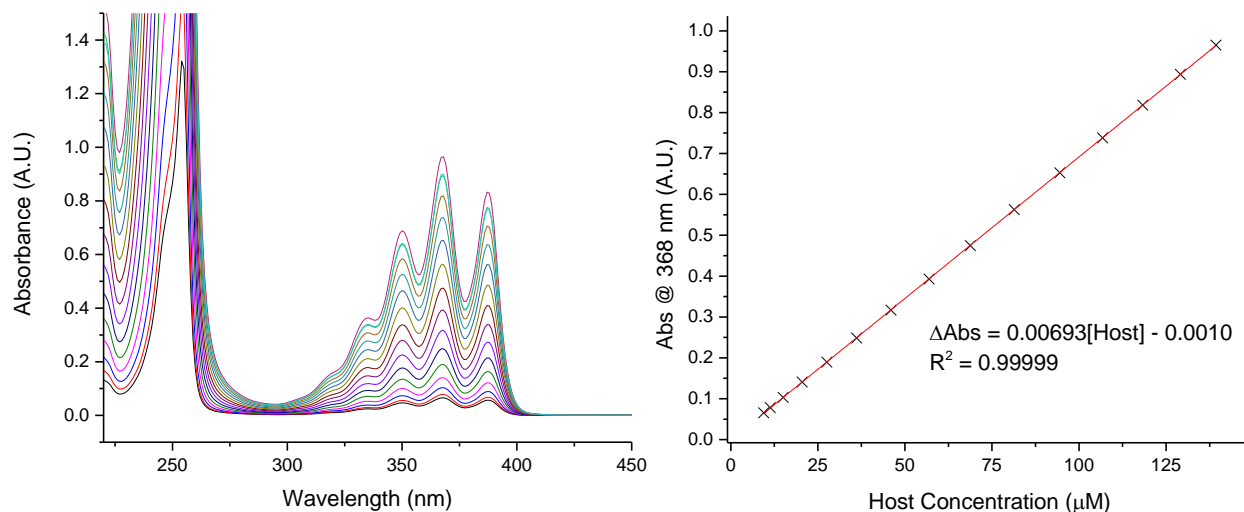**Figure S 1:** Concentration dependence of the UV/Vis spectrum of **5** obtained in 3:1 methanol water, buffered to pH 7.4 with 10 mM HEPES. (**left**) UV/Vis spectra at different concentrations of **5**. (**right**) Absorbance as a function of the concentration of **5**.
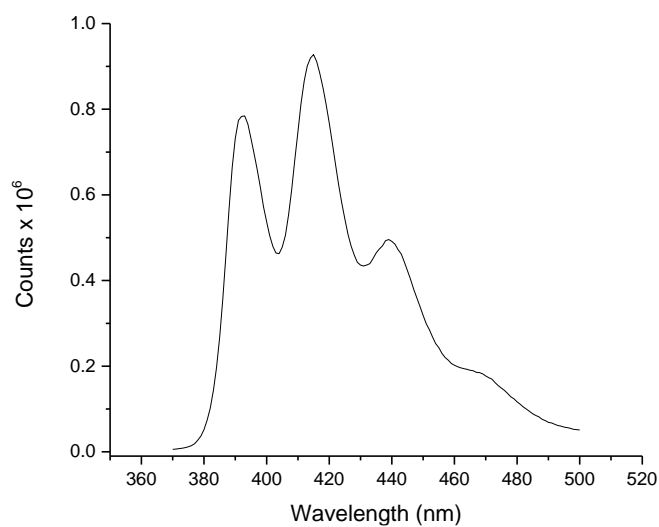
4.3 Fluorescence

Figure S 2: Fluorescence emission spectra of **5** obtained in a 3:1 methanol:water at pH 7.4 buffered with 10 mM HEPES. λ_{ex} = 350 nm.
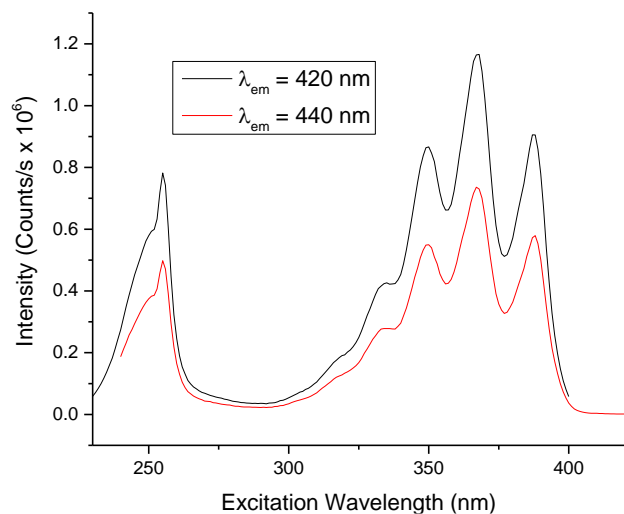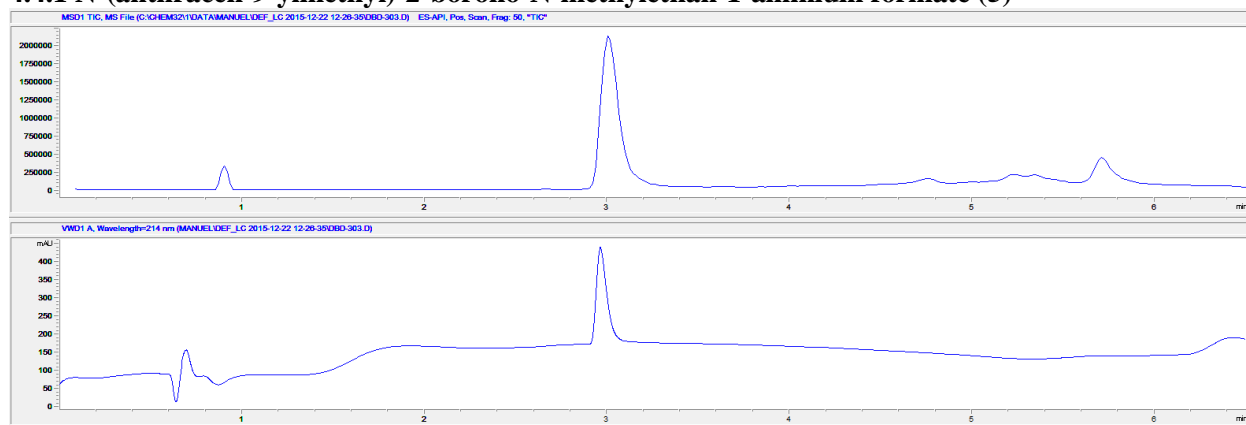

Figure S 3: Emission scan of **5** obtained in a 3:1 methanol:water at pH 7.4 buffered with 10 mM HEPES.

4.4 RP-HPLC/MS
4.4.1 N-(anthracen-9-ylmethyl)-2-borono-N-methylethan-1-aminium formate (5)

5.0 pH Titrations
5.1 pK$_a$s Determined by ^{1}H and ^{11}B spectroscopy
5.1.1 Methodology

To a solution of (2-((2-bromobenzyl)(methyl)amino)ethyl)boronic acid (**6**) (31.8 mg, 0.1 mmol) in 4.1 mL of CD$_3$OD was added 1.2 mL of HEPES buffer (0.10 M in D$_2$O) and was left to stir for 5 min. The pH was adjusted to ~3 by dropwise addition of 2.0 M HClO$_4$(aq). A 200 µL aliquot was taken for ^{1}H- and ^{11}B-NMR. The pH was increased by ~1 pH unit by addition of 2.0 M NaOH(aq) in D2O until a pH of ~13.5 was reached. At each pH, a 200 µL aliquot was taken for NMR. This experiment was repeated in duplicate. A scatter plot of ^{11}B-NMR (ppm) vs. pH was made for trials 1 and 2 and fit to a bi-dose-response curve in Origin 9.0 SR1.
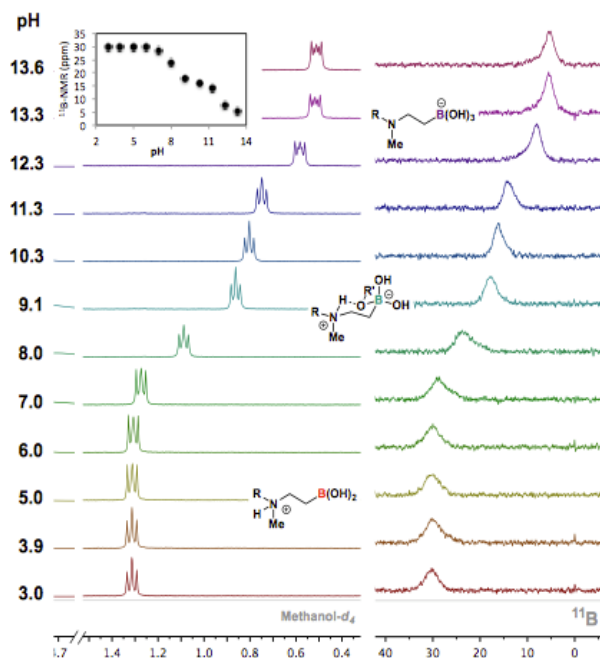
5.1.2 Results
5.1.2.1 ^{11}B and ^{1}H NMR spectra

Figure S 4: ^{1}H and ^{11}B NMR spectra of the pH titration of **6** (0.02 M) in 3:1 CD$_3$OD:D$_2$O with 0.02 M HEPES buffer.

5.1.2.2 Curve fitting

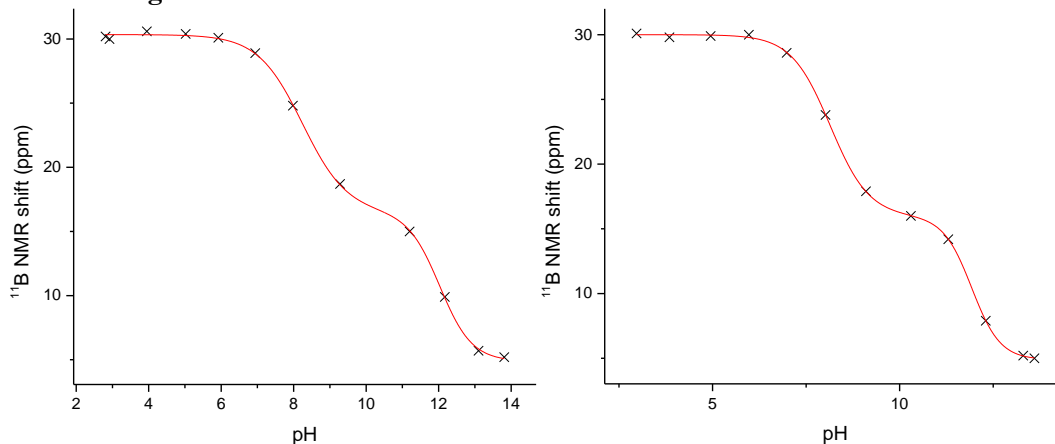**Figure S 5:** ^{11}B NMR chemical shifts of the pH titration of **6** (0.02 M) in 3:1 CD$_3$OD:D$_2$O with 0.02 M HEPES buffer. (**left**) Trial 1: pK$_{a1}$ = 8.2, pK$_{a1}$ = 12.0 (**right**) Trial 2: pK$_{a1}$ = 8.1, pK$_{a1}$ = 11.9.

5.1.3 Boronic acid stability

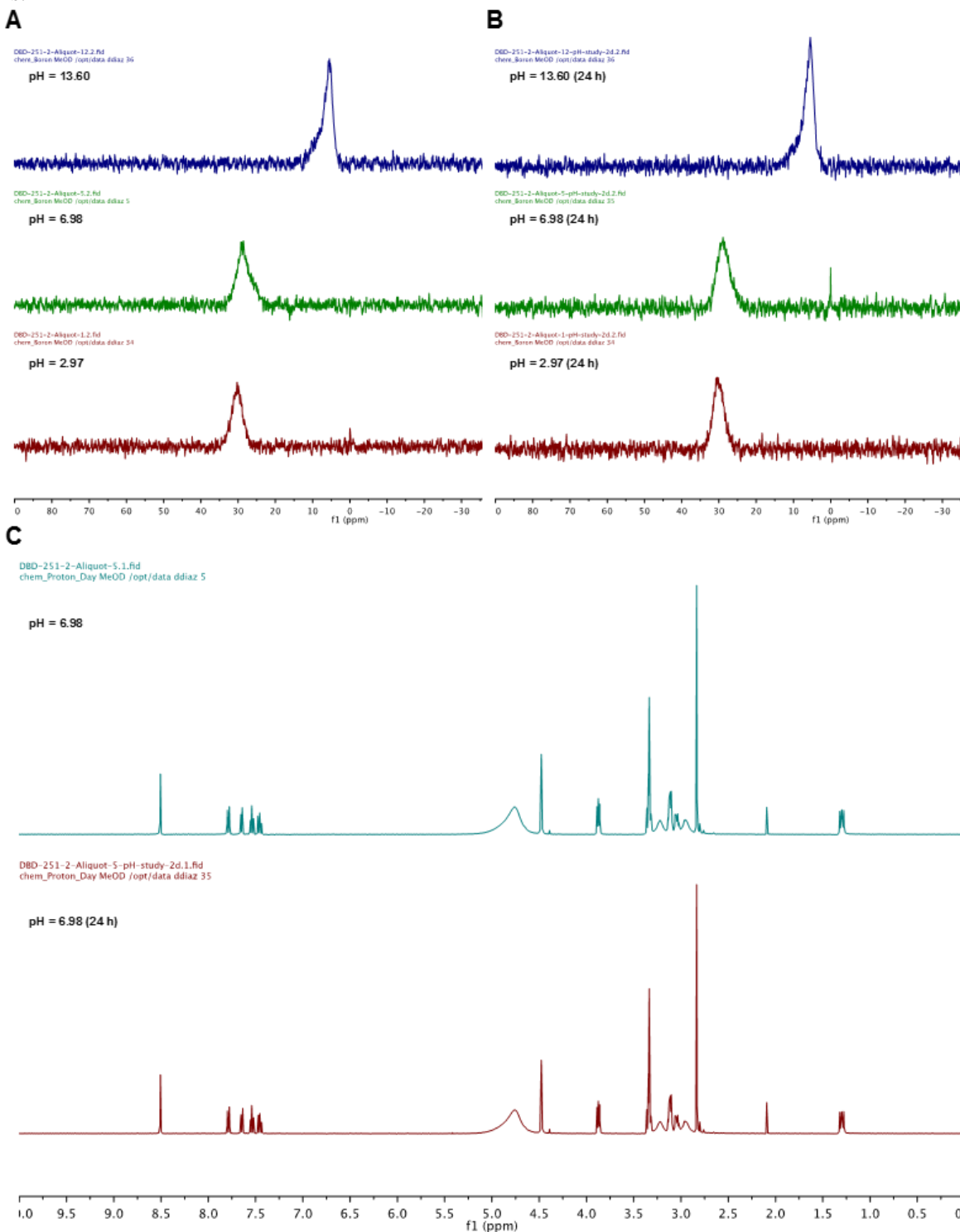^{1}H and ^{11}B NMR spectra obtained at pHs between 3.0 and 13.6 showed no degradation after 24 hours.

Figure S 6: pH stability of a β-aminoboronic acid in 3:1 CD$_3$OD:D$_2$O with 0.02 M HEPES buffer. (**A**) ^{11}B NMR spectra of compound 6 at pH 2.97 (red), 6.98 (green), 13.6 (blue). (**B**) ^{11}B NMR spectra of compound 6 after 24 at room temperature. (**C**) ^{1}H NMR spectra of compound 6 at a pH of 6.98 before (top) and after (bottom) 24 h.

5.2 pK$_a$s Determined by fluorescence spectroscopy

A pH titration was conducted in a 2:1 methanol:water containing 50 mM NaCl and 5.9 µM **5**. This was then used to create two solutions: one containing 48.4 mM HCl, obtained by serial dilution of concentration HCl$_{(aq)}$; and another containing 54.0 mM NaOH, obtained by serial dilution of NaOH pellets. The titration was conducted by slow addition of the basic host solution to the acidic host solution. The pH was allowed to equilibrate then an aliquot removed for fluorescence acquisition. The aliquot was then reintroduced to the original mixture and the process repeated. Fluorescence spectra obtained of both the acidic and basic host solutions showed no change at the end of the titration. To complete the curve at higher pH values, the process was repeated starting from the basic solution of host, with addition of the acidic solution.
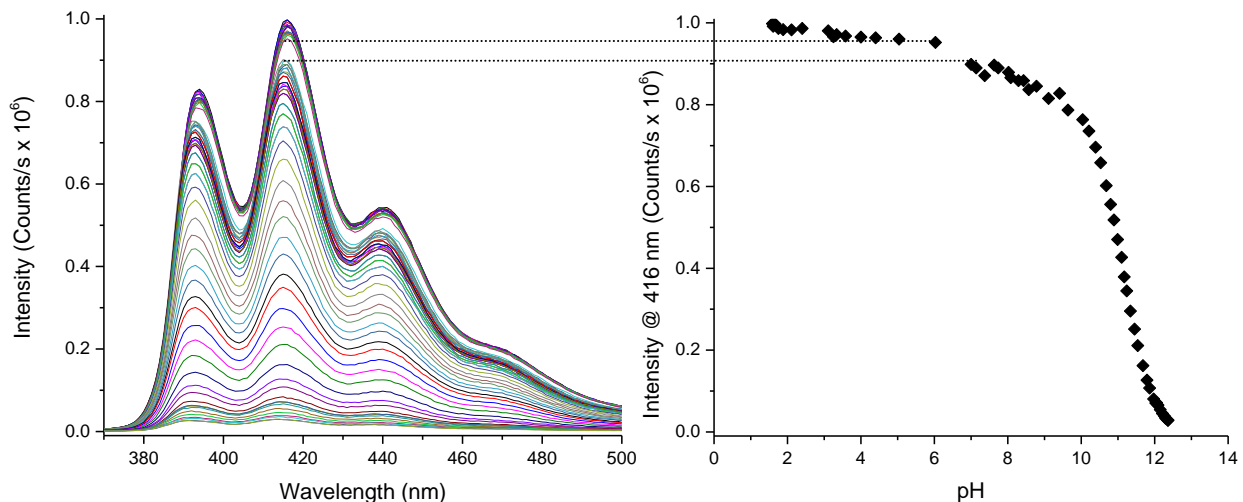

Figure S 7: pH titration of **5** (5.9 µM) in 2:1 methanol:water containing 50 mM NaCl. (**left**) Fluorescence spectral changes. (**right**) Fluorescence intensity at 416 nm, λ$_{em,max}$.
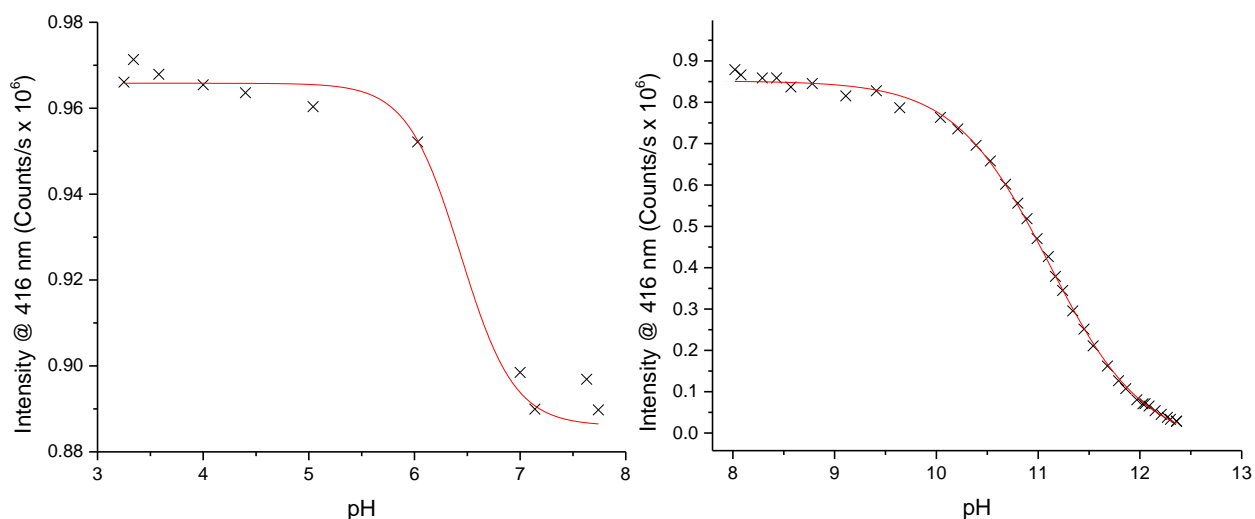

Figure S 8: Curve fitting of fluorescence data obtained from the pH titration of **5** in 3:1 methanol:water containing 50 mM NaCl. (**left**) pK$_{a1}$ = 6.4. (**right**) pK$_{a2}$ = 11.1.

6.0 Association constants determined by fluorescence spectroscopy
6.1 Methodology

A solution of boronic acid host **5** was prepared. In order to keep the concentration of the host constant throughout the titration, the host solution was used to prepare the guest solution. Fluorescnce titrations were carried out *via* serial dilution of the guest by adding aliquots of the guest solution to a known volume of host solution. The concentrations of the solutions used in the experiments were chosen such that the complexation ratio (β= [Complex]/[Host]$_T$) would be between 0.2 and 0.8.[2] Taken at the wavelength or peak of greatest change, graphs of ΔIntensity versus guest concentration were curve-fitted to a 1:1 binding isotherm[3] in Origin 9.0 SR1. Association constants were determined as averages from repeat experiments. The errors are reported as standard deviations. All association constants were determined at 25 °C. Association constants were determined in 3:1 Methanol:Water buffered to pH 7.4 with 10 mM HEPES to compare with previously reported boronic and borinic association constants in the literature.[4] The concentration of HEPES buffer has previously been shown to have a negligible effect of the association constants of boronic acid-carbohydrate equilibria.[5]

6.2 Association constant summary
Table S 1: Association constants (M^{-1}) with various guest in different solvents.

Guest	3:1 MeOH:Water[a]			Water[b]		
D-Fructose	4000	±	500			
1,2-O-Isopropylidine-α-D-glucofuranose	1040	±	70			
N-Acetyleneuraminic acid	115	±	6			
a-D-Glucose	102	±	6			
Catechol	11500	±	400	4100	±	200

a) Buffered to pH 7.4 using 10 mM HEPES. b) Buffered to pH 7.0 using 100 mM phosphate.

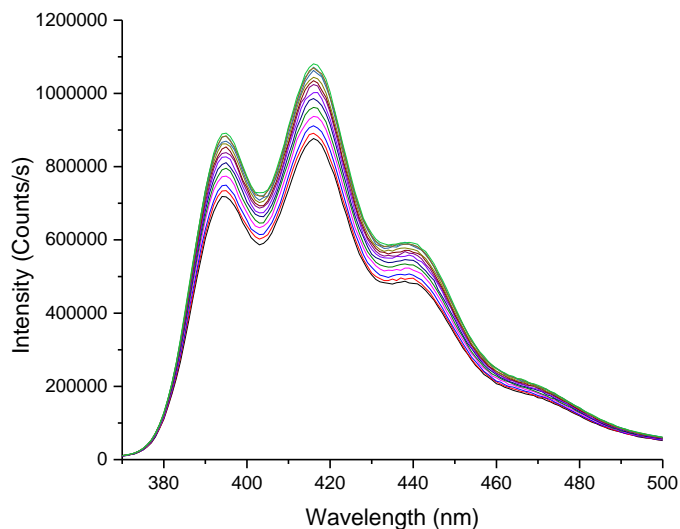## 6.3 Spectral changes and curve fitting
6.3.1 3:1 Methanol:Water
Association constants were determined in the presence of 10 mM HEPES, buffered to pH 7.4
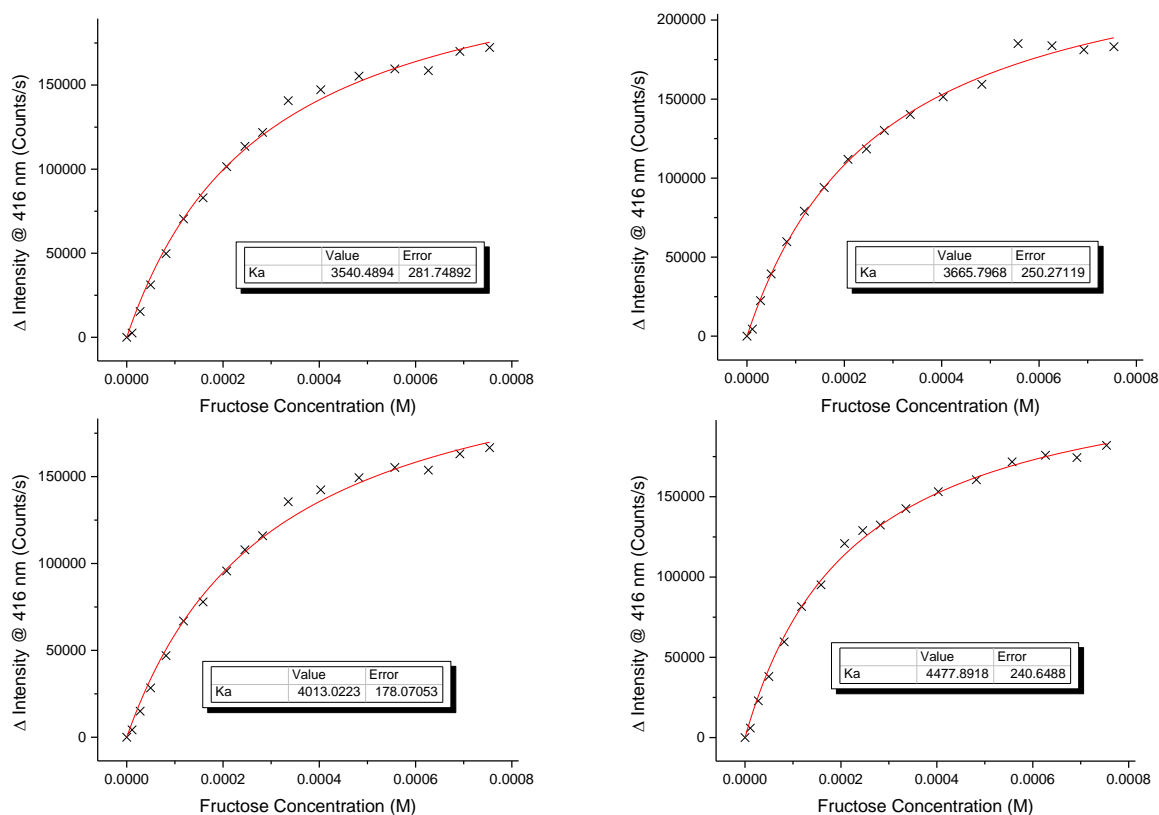### 6.3.1.1 D-Fructose
6.3.1.1.1 Spectrum
Figure S 9: Spectral change of **5** upon the addition of D-fructose in 3:1 methanol water, buffered to pH 7.4 with 10 mM HEPES. Titrations carried out at 6.5 μM **5**.
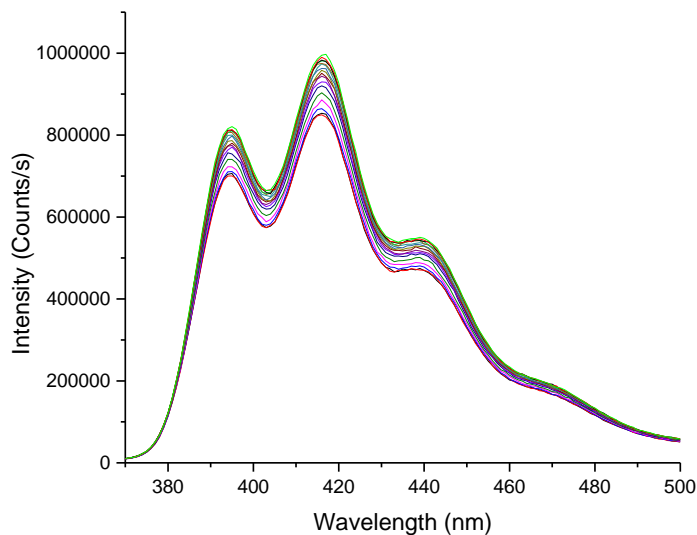

6.3.1.1.2 Titrations
Figure S 10: Plots of ΔIntensity versus fructose concentration for the fluorescence titration of **5** in 3:1 methanol:water, buffered to pH 7.4 with 10 mM HEPES. Titrations carried out at 4.3 μM **5**. From left to right, K_a = 3540 M^{-1}, 3666 M^{-1}, 4013 M^{-1}, 4478 M^{-1}.

## 6.3.1.2 1,2-O-Isopropylidine-α-D-glucofuranose (A)
6.3.1.2.1 Spectrum
Figure S 11: Spectral change of **5** upon the addition of 1,2-O-isopropylidine-a-D-glucofuranose (**A**) in 3:1 methanol water, buffered to pH 7.4 with 10 mM HEPES. Titrations carried out at 4.3 µM **5**.
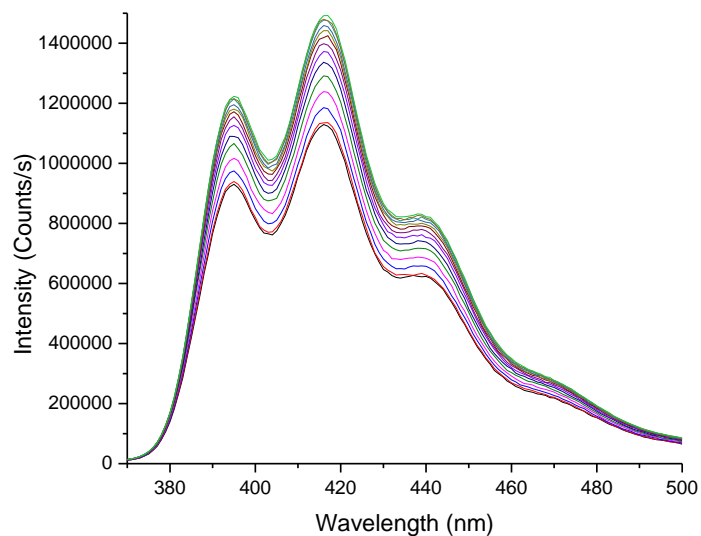

6.3.1.2.2 Titrations
Figure S 12: Plots of ΔIntensity versus 1,2-O-isopropylidine-a-D-glucofuranose (**A**) concentration for the fluorescence titration of **5** in 3:1 methanol:water, buffered to pH 7.4 with 10 mM HEPES. Titrations carried out at 4.3 µM **5**. From left to right, K_a = 1059 M^{-1}, 1112 M^{-1}, 949 M^{-1}, 1035 M^{-1}.
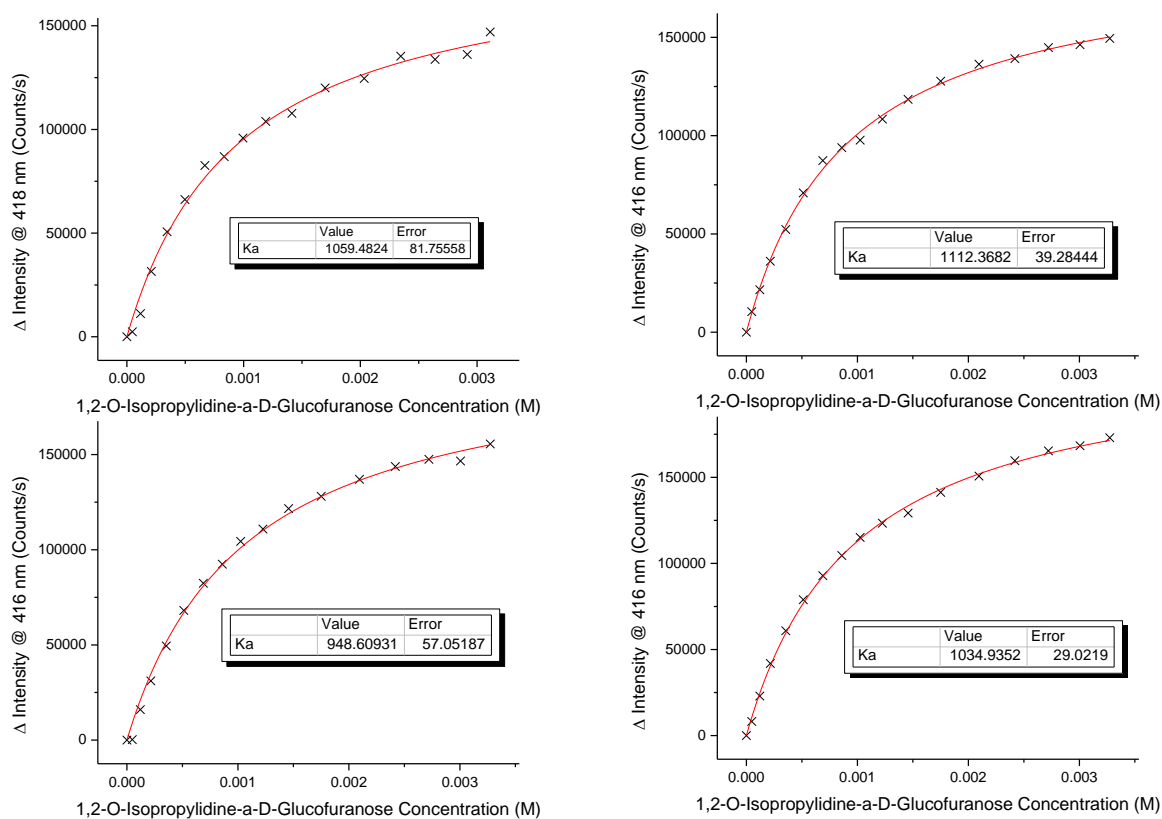
6.3.1.3 N-Acetylneuraminic acid (B)
### 6.3.1.3.1 Spectrum
Figure S 13: Spectral change of **5** upon the addition of N-Acetylneuraminic acid (**B**) in 3:1 methanol water, buffered to pH 7.4 with 10 mM HEPES. Titrations carried out at 4.3 μM **5**.

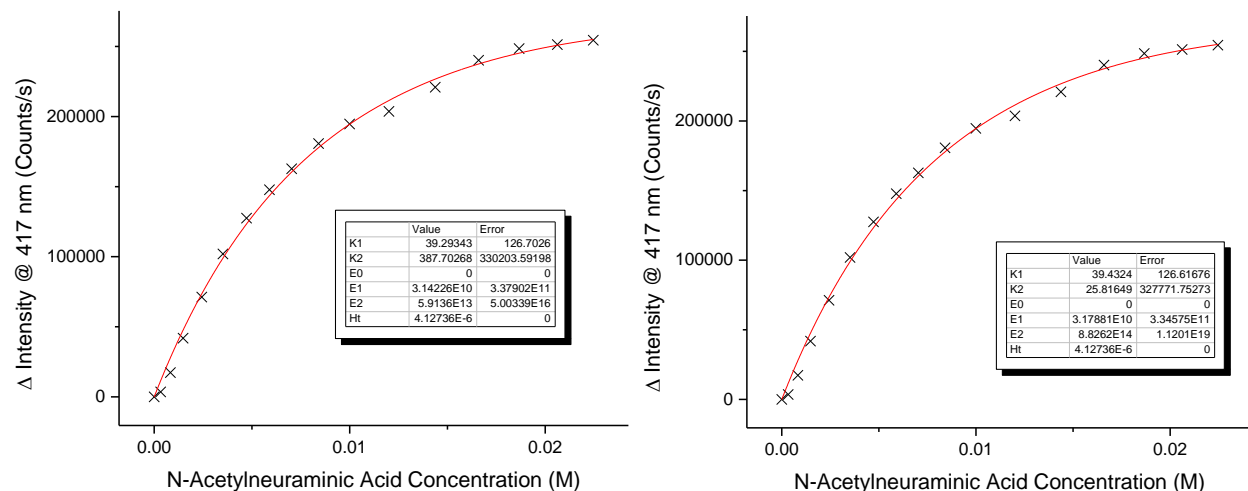### 6.3.1.3.2 Titrations
6.3.1.3.2.1 1:1 Binding Isotherm
Figure S 14: Plots of ΔIntensity versus N-Acetylneuraminic acid (**B**) concentration for the fluorescence titration of **5** in 3:1 methanol:water, buffered to pH 7.4 with 10 mM HEPES. Titrations carried out at 4.3 μM **5**. From left to right, K_a = 109 M^{-1}, 114 M^{-1}, 121 M^{-1}.
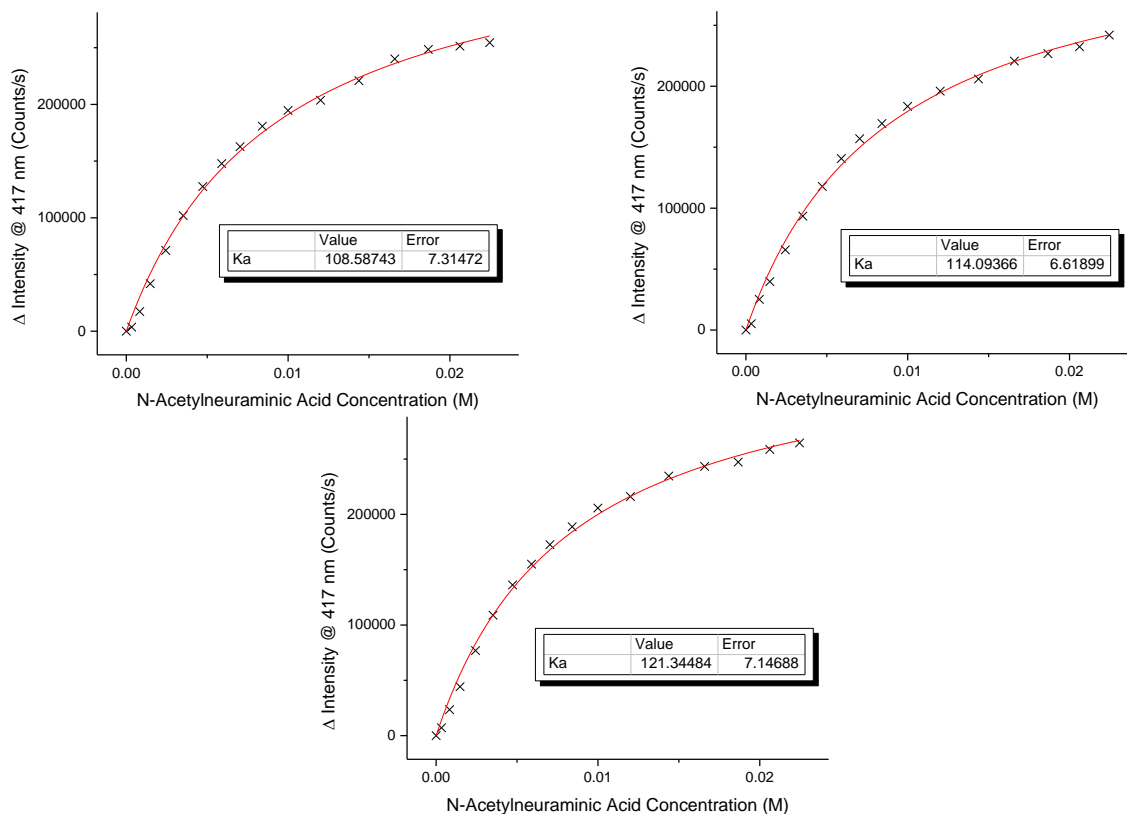
6.3.1.3.2.2 2:1 Binding Isotherm

Due to the presence of multiple locations on N-Acetylneuraminic acid (**B**) where the boronic acid can bind, the obtained titration data was also fit to a 2:1 (**5**:**B**) binding isotherm starting from different initial values of K_1, K_2, ε_1 and ε_2. The fitting procedures failed to converge, and produced different values of K_1, K_2, ε_1 and ε_2 when starting from different initial parameter, with significant errors on the fit. Below are representative fits of the same data, starting from different initial conditions.
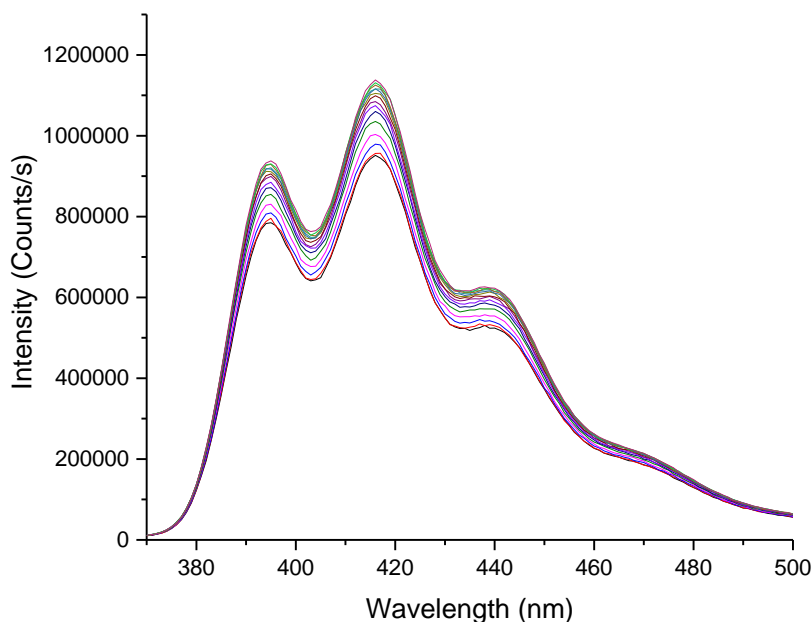
Figure S 15: Plots of ΔIntensity versus N-Acetylneuraminic acid (**A**) concentration for the fluorescence titration of **5** in 3:1 methanol:water, buffered to pH 7.4 with 10 mM HEPES. Titrations carried out at 4.3 μM **5**. Data fit to a 2:1 host:guest binding isotherm, errors presented are those of the fitting procedure. (**left**) $K_1 = 40 \pm 130$ M^{-1}, $K_2 = 400 \pm 300000$ M^{-1}. (**right**) $K_1 = 40 \pm 130$ M^{-1}, $K_2 = 30 \pm 300000$ M^{-1}.

	Value	Error
K1	39.29343	126.7026
K2	387.70268	330203.59198
E0	0	0
E1	3.14226E10	3.37902E11
E2	5.9136E13	5.00339E16
Ht	4.12736E-6	0

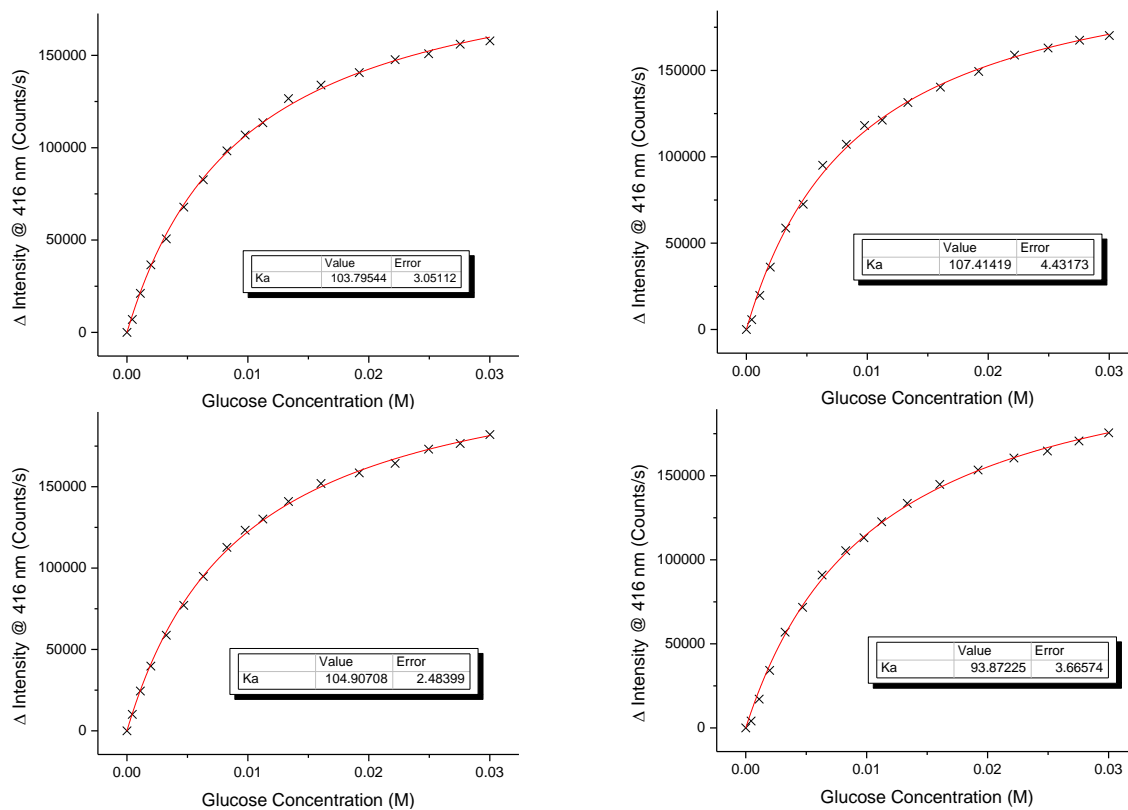|	Value	Error
K1	39.4324	126.61676
K2	25.81649	327771.75273
E0	0	0
E1	3.17881E10	3.34575E11
E2	8.8262E14	1.1201E19
Ht	4.12736E-6	0

17

6.3.1.4 α-D-Glucose
6.3.1.4.1 Spectrum

Figure S 16: Spectral change of **5** upon the addition of α-D-glucose in 3:1 methanol water, buffered to pH 7.4 with 10 mM HEPES. Titrations carried out at 4.3 μM **5**.
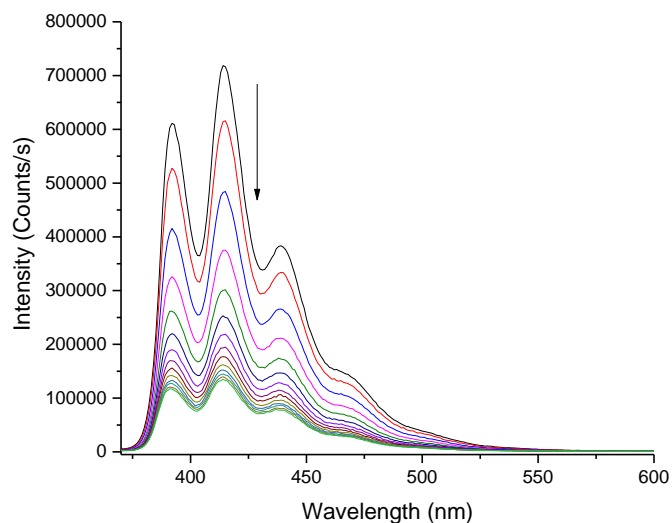

6.3.1.4.2 Titrations

Figure S 17: Plots of ΔIntensity versus glucose concentration for the fluorescence titration of **5** in 3:1 methanol water, buffered to pH 7.4 with 10 mM HEPES. Titrations carried out at 4.7 μM **5**. From left to right, $K_a = 104$ M^{-1}, 107 M^{-1}, 105 M^{-1}, 94 M^{-1}.

	Value	Error
Ka	103.79544	3.05112

	Value	Error
Ka	107.41419	4.43173

	Value	Error
Ka	104.90708	2.48399

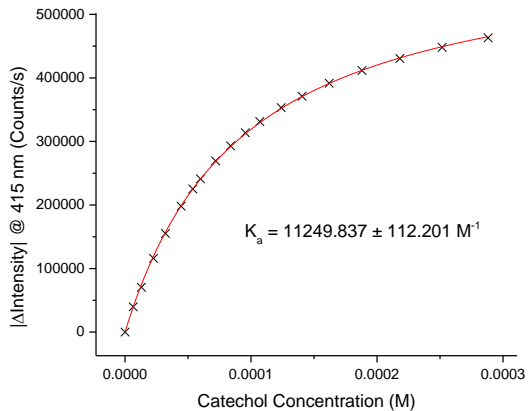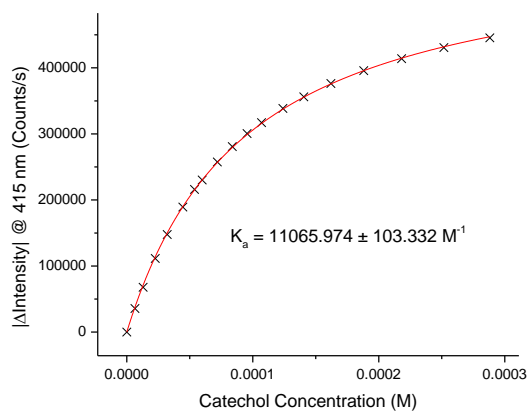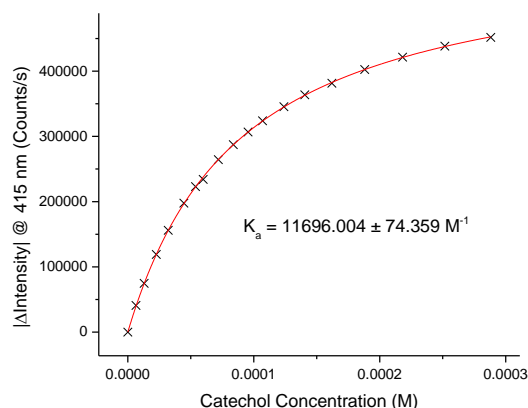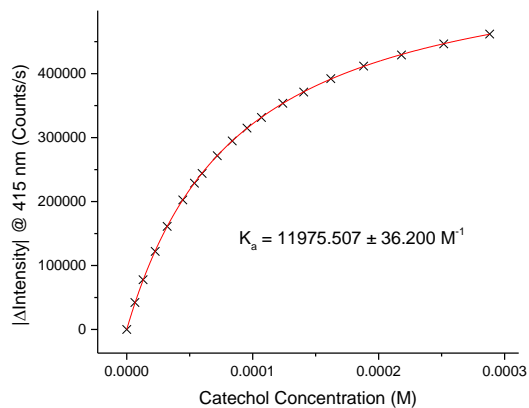|	Value	Error
Ka	93.87225	3.66574

6.3.1.5 Catechol
6.3.1.5.1 Spectrum
Figure S 18: Spectral change of **5** upon the addition of catechol in 3:1 methanol water, buffered to pH 7.4 with 10 mM HEPES. Titrations carried out at 6.5 µM **5**.

6.3.1.5.2 Titrations
Figure S 19: Plots of ΔIntensity versus catechol concentration for the fluorescence titration of **5** in 3:1 methanol water, buffered to pH 7.4 with 10 mM HEPES. Titrations carried out at 6.5 µM **5**. From left to right, K_a = 11976 M^{-1}, 11696 M^{-1}, 11066 M^{-1}, 11250 M^{-1}.

$K_a = 11975.507 \pm 36.200$ M^{-1}

$K_a = 11696.004 \pm 74.359$ M^{-1}

$K_a = 11065.974 \pm 103.332$ M^{-1}

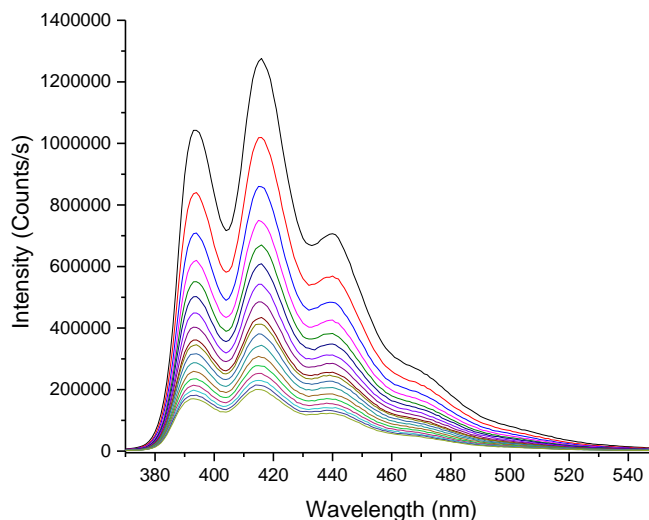$K_a = 11249.837 \pm 112.201$ M^{-1}

6.3.2 Water

Association constants were determined in the presence of 100 mM phosphate, buffered to pH 7.0

6.3.2.1 Catechol

6.3.2.1.1 Spectrum

Figure S 20: Spectral change of **5** upon the addition of catechol in water, buffered to pH 7.0 with 100 mM phosphate. Titrations carried out at 6.5 μM **5**.

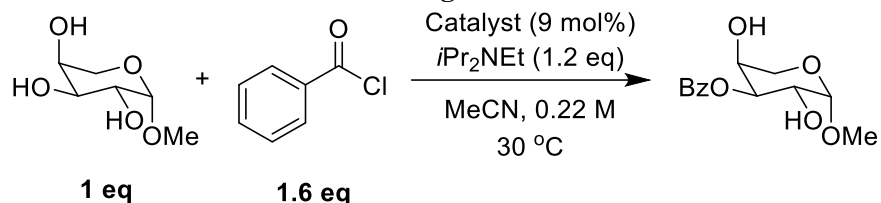### 6.3.2.1.2 Titrations

Figure S 21: Plots of ΔIntensity versus catechol concentration for the fluorescence titration of **5** in water, buffered to pH 7.0 with 100 mM phosphate. Titrations carried out at 6.5 μM **5**. From left to right, K_a = 3884 M^{-1}, 4282 M^{-1}, 3949 M^{-1}, 4175 M^{-1}.
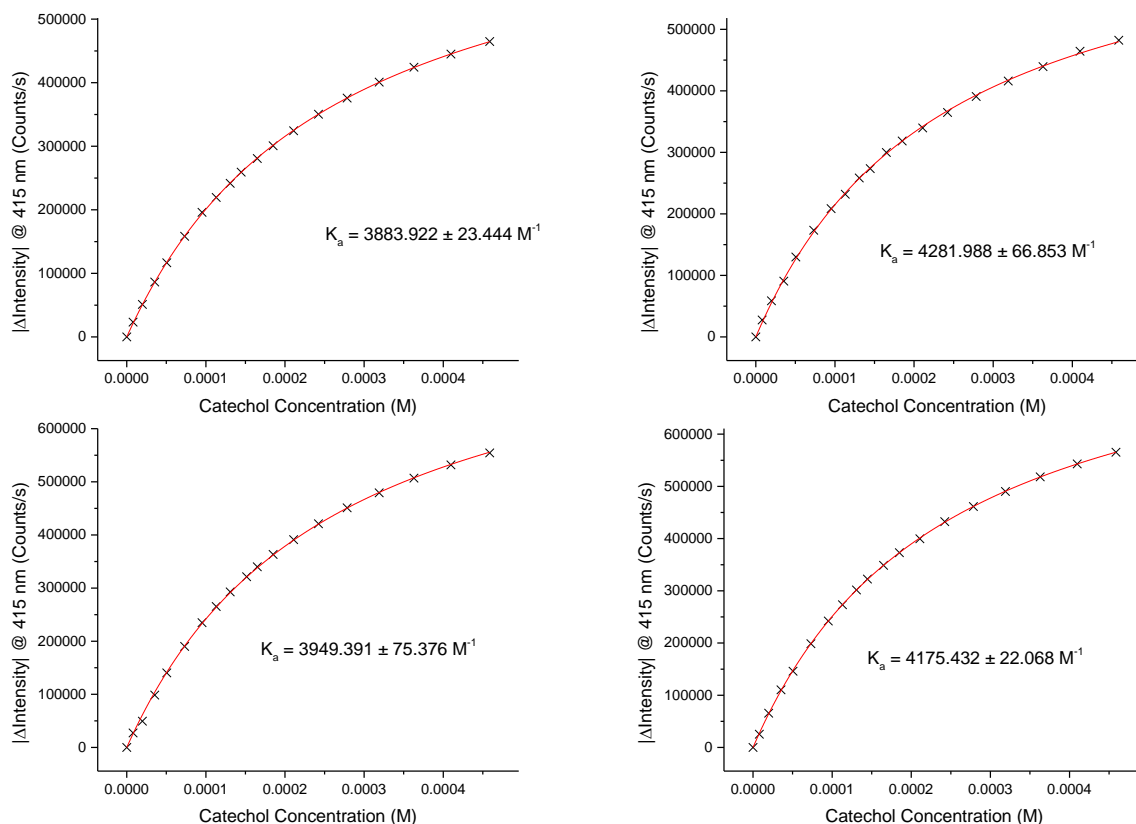
7.0 Reaction monitoring of carbohydrate acylations
7.1 General method for in situ reaction monitoring

1 eq   **1.6 eq**

Me-β-L-arabinopyranoside (26.5 mg, 0.16 mmol) was weighed into a two neck conical flask with a magnetic stir bar. The IR probe was inserted into the flask and the second neck sealed with a fresh septum. The flask was then purged with argon for 15 minutes. 235 µL of acetonitrile was then added to the flask. The vessel was submerged into an oil bath set to 30 °C and stirring set to 250 RPM. *N,N*-Diisopropylethylamine (DIPEA) (35 µL, 24.9 mg, 0.19 mmol) was added to the flask and allowed to stir. Weighed into a ½ dram vial in the glovebox, 1,1,3,3-tetraphenyldiboroxane, (Ph₂B)₂O, (3.0 mg, 0.01 mmol) or (2-((2-bromobenzyl)(methyl)amino)ethyl)boronic acid (**6**) (5.7 mg, 0.02 mmol) were dissolved in 500 µL of acetonitrile with sonication and light heating. Benzoyl chloride (**3a**) (30 µL, 36.1 mg, 0.26 mmol) was then injected into the flask followed immediately by 400 µL of the acetonitrile solution of either (Ph₂B)₂O or **6**. The final volume in the reaction was 700 µL, with total boron loading of 9 mol%.

Reaction progress was monitored by *in situ* FTIR. Formation of the acylation product was monitored using the height of the peak at 1278 cm^{-1} (C-O ester bond). The end of the reaction was characterized by the end of IR peak growth. Starting material concentration was determined assuming a that [SM] was inversely proportional to the concentration of product.
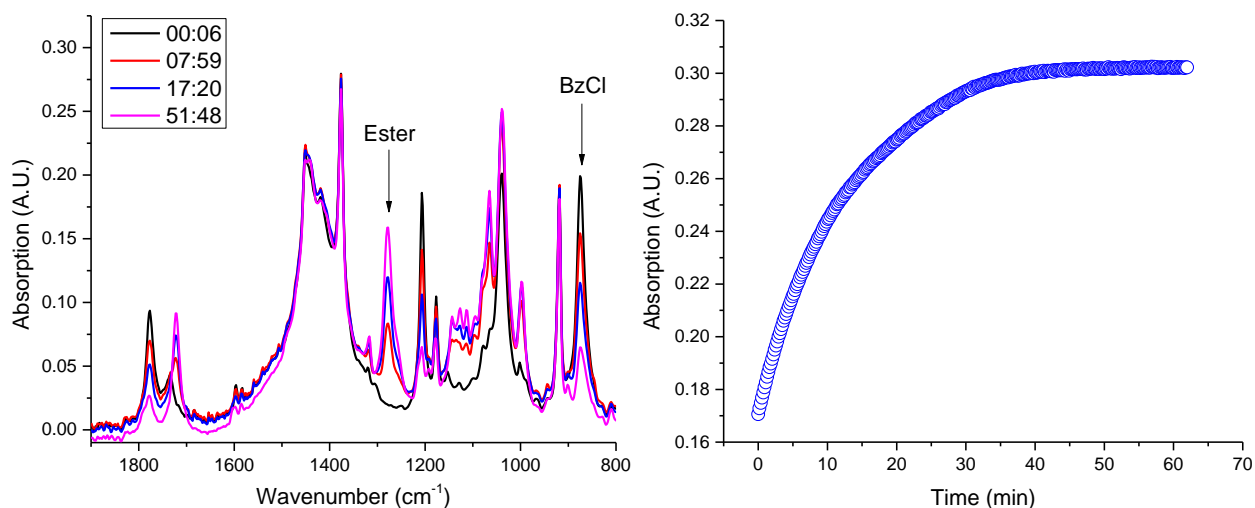
Figure S 22: *in situ* FTIR data obtained for the benzoylation of Me-β-L-arbinopyranoside catalyzed by (Ph₂B)₂O under the conditions of the general method. (right) FTIR spectral evolution over the course of the reaction. (right) Absorption versus time data obtained from the ester C-O stretch at 1278 cm^{-1}.

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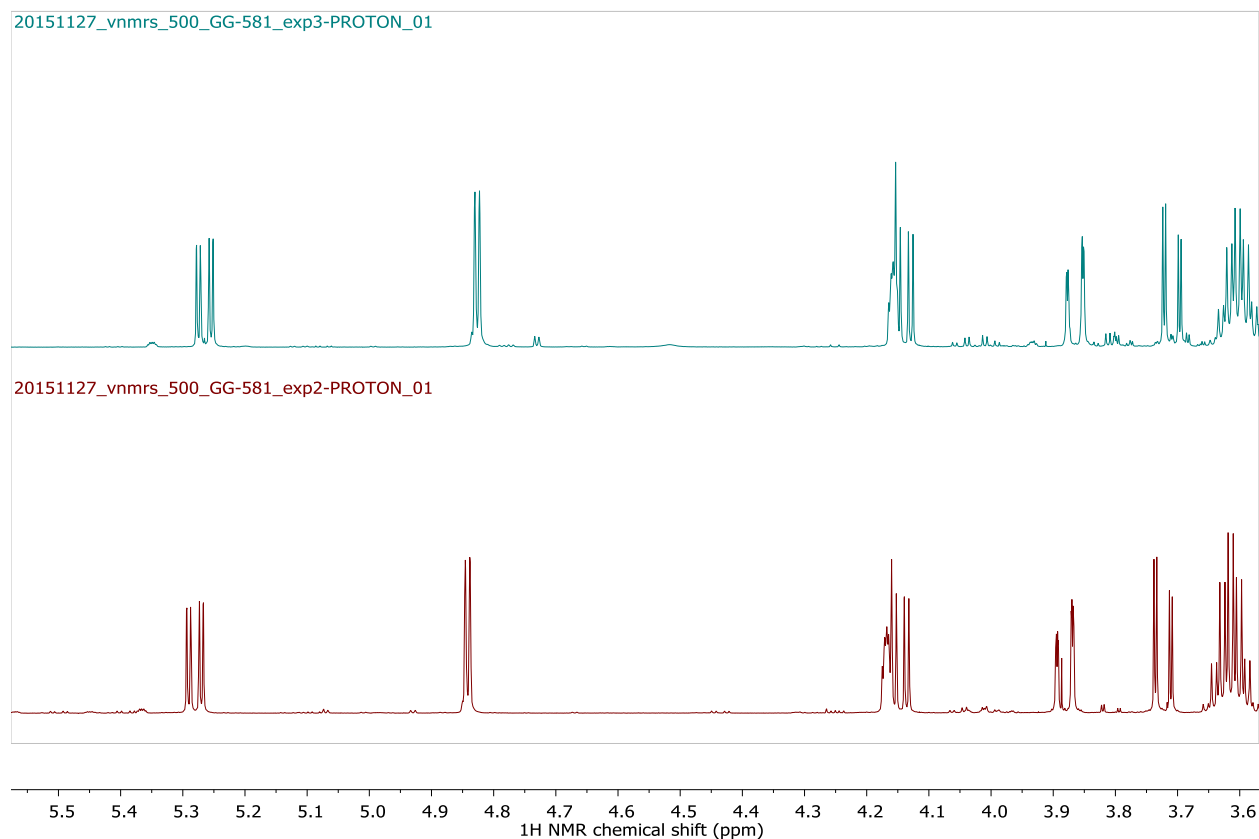Crude ^{1}H NMR spectroscopy of the final reaction mixtures showed clean conversion to product.[4]

Figure S 23: ^{1}H NMR spectra (CDCl₃) of crude reaction mixtures obtained after carrying out the benzoylation of Me-β-L-arabinopyranoside under the general method catalyzed by **6** (**top**) or (Ph₂B)₂O (**bottom**).

8.0 Titrations of boronic acid 6 with Alizarin Red S

In order to obtain association constants with the non-florescent boronic acid receptor **6**, a dye displacement assay was attempted with the dye Alizarin Red S.[5-6] 1:1 Association constants with the dye to boronic acid **6** could not be determined, and the dye displacement assay could not be carried out.

8.1 Methodology

A solution of Alizarin Red S was prepared. In order to keep the concentration of Alizarin Red S constant throughout the titration, this solution was used to prepare the stock solution of **6**. Titrations were carried out using UV/Vis spectroscopy *via* serial dilution **6** by adding aliquots of the stock solution of **6** to a known volume of the Alizarin Red S solution. Taken at the wavelength or peak of greatest change, graphs of ΔAbsorbance versus concentration of **6** were curve-fitted to a 1:1 or 2:1 host:guest binding isotherm in Origin 9.0 SR1. All titrations were carried out at 25 °C.
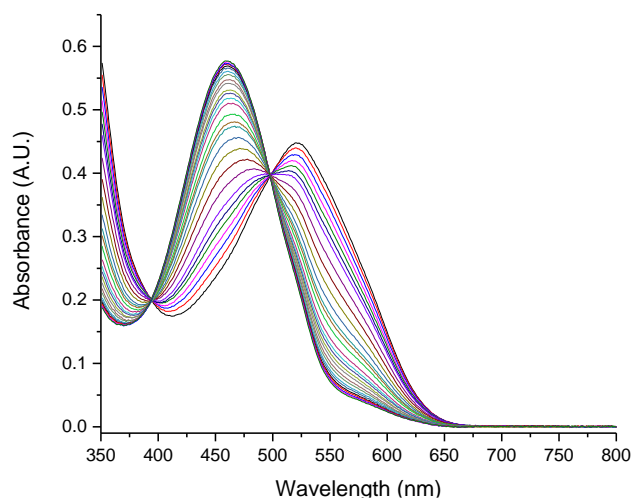
8.2 Titrations
8.2.1 2:1 Methanol:Water

Association constants were determined in the presence of 10 mM HEPES, buffered to pH 7.0
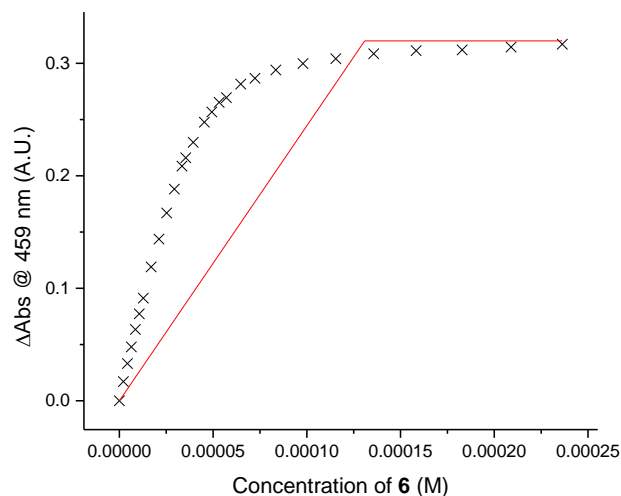
8.2.1.1 Spectrum

Figure S 24: Spectral change of **6** upon the addition of Alizarin Red S in 2:1 Methanol:Water, buffered to pH 7.0 with 10 mM HEPES. Titration carried out at 114.5 µM Alizarin Red S.

8.2.1.2 Titration

Figure S 25: Plot of ΔAbsorbance versus the concentration of **6** for the UV/Vis titration of Alizarin Red S in 2:1 Methanol:Water, buffered to pH 7.0 with 10 mM HEPES. Titration carried out at 114.5 µM Alizarin Red S. Data fit using a 1:1 binding isotherm. Binding too tight to obtain an accurate association constant. Break in the curve fitting occurs at a 1:1 stoichiometry of **6** to Alizarin Red S. Data could not be fit to a 2:1 binding isotherm.
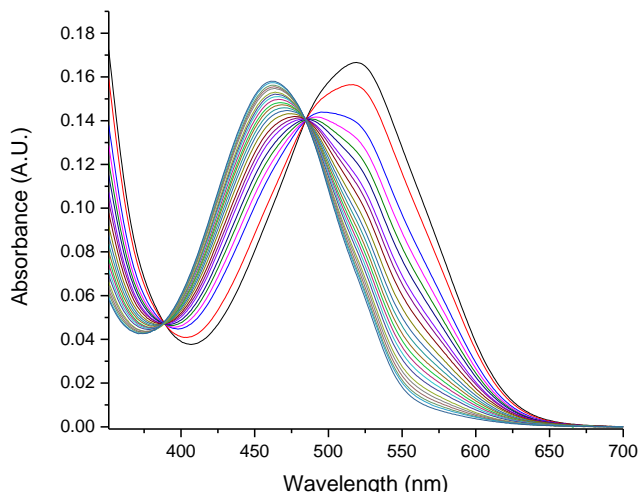
8.2.2 Water

Association constants were determined in the presence of 100 mM phosphate, buffered to pH 7.0

8.2.2.1 Spectrum

Figure S 26: Spectral change of **6** upon the addition of Alizarin Red S in Water, buffered to pH 7.0 with 100 mM phosphate. Titration carried out at 30.4 µM Alizarin Red S.


8.2.2.2 Titration

8.2.2.2.1 1:1 Binding isotherm

Figure S 27: Plots of ΔAbsorbance versus the concentration of **6** for the UV/Vis titration of Alizarin Red S in Water, buffered to pH 7.0 with 10 mM HEPES. Titration carried out at 30.4 µM Alizarin Red S. Data fit using a 1:1 binding isotherm. Data fit using a 2:1 (Alizarin Red S:**6**) binding isotherm.

$K_a = 144154.15351 \pm 18102.75619$ M^{-1}
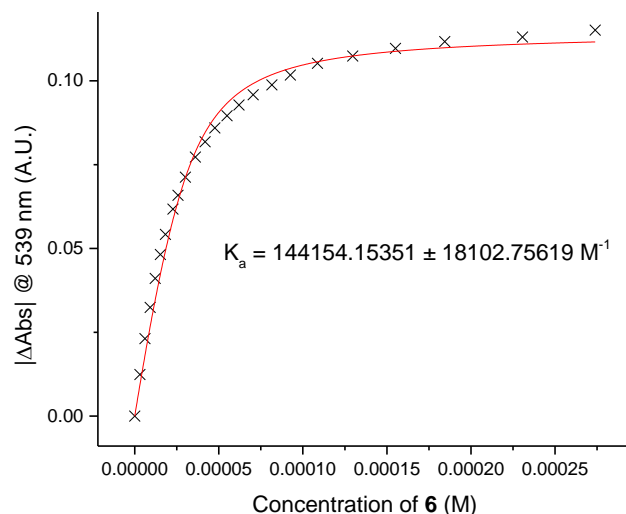
8.2.2.2.2 2:1 Binding isotherm

Figure S 28: Plots of ΔAbsorbance versus the concentration of **6** for the UV/Vis titration of Alizarin Red S in Water, buffered to pH 7.0 with 10 mM HEPES. Titration carried out at 30.4 µM Alizarin Red S. Data fit using a 2:1 (Alizarin Red S:**6**) binding isotherm.

$K_1 = 43038.09504 \pm 7624.9467$ M^{-1}

$K_2 = 23033.72912 \pm 1260.9572$ M^{-1}
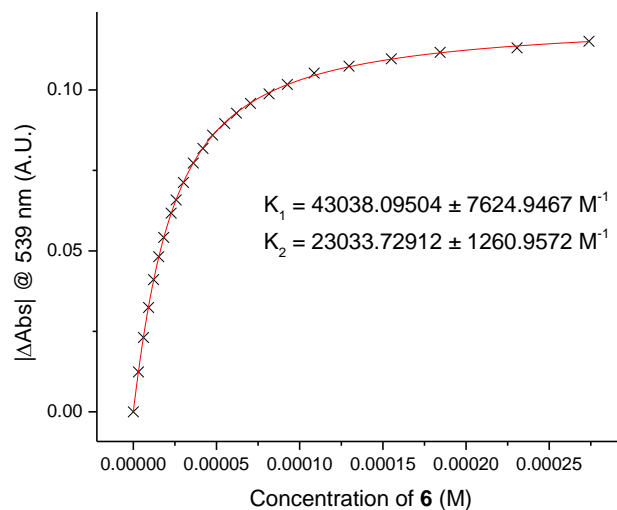
9.0 Origin code for curve fitting to a 2:1 and 1:2 host:guest binding isotherms
9.1 Methodology

The LabTalk scripts used for curve fitting to the 2:1 Alizarin Red S:**6** binding isotherms are shown below. For reference, an alternate LabTalk script for 1:2 binding isotherms is provided incase there are difficulties using methods previously reported in the literature.[6] Derivations for the binding isotherm have been previously reported.[7] The below scripts are written for UV/Vis titrations.

9.2 Origin LabTalk scripts

9.2.1 2:1 Host:Guest binding isotherm

```
a=K1*K2;
b=K1+2*K2*K1*x-K1*K2*Ht;
c=1+K1*x-K1*Ht;
d=-Ht;
a0=d/a;
a1=c/a;
a2=b/a;
p=(3*a1-a2^2)/3;
q=(9*a1*a2-27*a0-2*a2^3)/27;
C=q/2*(3/ABS(p))^(3/2);
root=0;

if (p > 0)
root=SINH(1/3*ASINH(C));
else
if (p < 0 && C >= 1)
root=COSH(1/3*ACOSH(C));
else
if (p < 0 && C <= -1)
root=-COSH(1/3*ACOSH(ABS(C)));
else
root=COS(1/3*ACOS(C));

H=2*SQRT(ABS(p)/3)*root-a2/3;

y=x*(E0+E1*K1*H+2*E2*K1*K2*H*H)/(1+K1*
H+K1*K2*H*H);
```

9.2.2 1:2 Host:Guest binding isotherm

```
a=K1*K2;
b=K1+2*K2*K1*Ht-K1*K2*x;
c=1+K1*Ht-K1*x;
d=-x;
a0=d/a;
a1=c/a;
a2=b/a;
p=(3*a1-a2^2)/3;
q=(9*a1*a2-27*a0-2*a2^3)/27;
C=q/2*(3/ABS(p))^(3/2);
root=0;

if (p > 0)
root=SINH(1/3*ASINH(C));
else
if (p < 0 && C >= 1)
root=COSH(1/3*ACOSH(C));
else
if (p < 0 && C <= -1)
root=-COSH(1/3*ACOSH(ABS(C)));
else
root=COS(1/3*ACOS(C));

G=2*SQRT(ABS(p)/3)*root-a2/3;

y=Ht*(E0+E1*K1*G+2*E2*K1*K2*G*G)/(1+K1*
G+K1*K2*G*G);
```

10.0 References

1.   St. Denis, J. D.; Zajdlik, A.; Tan, J.; Trinchera, P.; Lee, C. F.; He, Z.; Adachi, S.; Yudin, A. K., *Journal of the American Chemical Society* **2014,** *136* (50), 17669-17673.
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5.   Springsteen, G.; Wang, B., *Tetrahedron* **2002,** *58* (26), 5291-5300.
6.   Hargrove, A. E.; Zhong, Z.; Sessler, J. L.; Anslyn, E. V., *New Journal of Chemistry* **2010,** *34* (2), 348-354.
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