Supporting Information

Borononucleotides: synthesis and formation of a new reversible boronate internucleosidic linkage

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Experimental procedures

Unless otherwise stated, materials were purchased from commercial suppliers and used without further purification. Alizarine red S and phenyl boronic acid were purchased from Aldrich, and were used as received. All reactions were carried out under argon atmosphere. TLC was performed on Merck silica coated plates 60F254 (art. 5554). Compounds were revealed on UV light (254 nm) and after spraying with 5% sulphuric acid ethanol solution and heating.

The water used for the $K_a$ studies was distilled and further purified with a filtration system. Phosphate buffer was prepared using potassium phosphate monobasic purchased from Merck.

All the synthetized compounds were characterized by NMR and Mass Spectroscopy. $^1$H and $^{13}$C NMR spectra were acquired at 298°K on Bruker AM 300 and DRX 400 spectrometers, locked on deuterium frequency. The samples were dissolved in CDCl$_3$, CD$_3$OD, DMSO-d$_6$, using the solvent residual peak as reference (7.26 ppm, 3.31 and 2.49 ppm respectively). Experiments in D$_2$O were also realized using 1% dioxane-d$_6$ as internal reference for $^1$H and $^{13}$C NMR spectra (3.75 ppm and 67.2 ppm respectively), and BF$_3$.Et$_2$O in CDCl$_3$ (0.0 ppm) for $^{11}$B spectra as external reference. Chemical shifts are given in ppm referenced to the solvent residual peak and coupling constants are given in Hertz (Hz).

The structure of the final compound 5 was checked by a complete assignment of the $^1$H and $^{13}$C nuclei, based on the analysis of 2D COSY and $^1$H-$^{13}$C HSQC.

FAB-MS spectra were recorded on a JEOL SX102 mass spectrometer using nitrobenzylalcohol (NBA) as a matrix. ESI spectra were recorded on a Q-Tof Micromass spectrometer. All compounds were further characterized by High Resolution Mass Spectrometry.

UV spectra were recorded on a Varian Cary 300 Bio UV/Vis spectrometer. Quartz cuvettes were used in all studies. All data was plotted on Microsoft Excel.
Synthesis of 5’-deoxy-5’-ethynyl-3’-O-tertbutyldimethylsilyl thymidine 2:

The Bestmann-Ohira\textsuperscript{1} reagent was prepared in one step by diazo transfer of tosyl azide to commercially available dimethyl-2-oxopropylphosphonate.

5’-aldehyde-3’-O-tertbutyldimethylsilylthymidine 1\textsuperscript{2} (9.00 g, 25 mmol) was first dissolved in anhydrous MeOH (50 mL) and stirred under argon at room temperature. Anhydrous K\textsubscript{2}CO\textsubscript{3} (6.83 g, 40 mmol) and Bestmann-Ohira reagent (6.50 g, 50 mmol) were added to the reaction mixture and stirring continued 12 h at room temperature. Solvent was evaporated, and the remaining residue was dissolved in ethyl acetate (200 mL) and washed with saturated NH\textsubscript{4}Cl (3×50 mL). The organic layer was dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (100% CH\textsubscript{2}Cl\textsubscript{2} (80-20)–0% ethyl acetate) to give 6.76 g (76%) of 2 as a white solid.

Compound 2, yield: 76%; $\delta_H$ (300 MHz, CDCl\textsubscript{3}) 0.0 (6 H, s, Si-(CH\textsubscript{3})\textsubscript{2}), 0.78 (9 H, s, $^1$Bu-Si), 1.82 (3 H, s, CH\textsubscript{3}), 2.05 (1 H, m, H$_2$'), 2.29 (1 H, m, H$_2$'), 2.64 (1 H, s, H$_6$'-ethynyl), 4.38-4.46 (2 H, m, H$_3$; H$_4$'), 6.29 (1 H, t, $J$ 6.9, H$_1$'), 7.40 (1 H, s, H$_6$), 9.08 (1 H, br, NH); $\delta_C$ (75 MHz, CDCl\textsubscript{3}) 4.9 (Si-(CH\textsubscript{3})\textsubscript{2}), 12.7 (CH\textsubscript{3} thymine), 18.0 (Si-CIV), 25.7 (Si-$^1$Bu), 40.6 (C2'), 76.6 (C3'), 77 (C6'), 77.7 (C4'), 80.6 (C5'), 86.9 (C1'), 111.2 (C5), 135.6 (C6), 150.4 (C2), 163.8 (C4); MS (ESI$^+$) m/z 351.1 ([M+H]$^+$, 100%); HMRS-ESI$^+$ m/z calcd for C$_{17}$H$_{27}$O$_4$N$_2$Si [M+H]$^+$ 351.1740 Found 351.1732.


Synthesis of 5′-deoxy-5′-vinyl-3’-O-tertbutyldimethylsilyl thymidine 3.

A 57 mM solution of terminal alkyne 2 (1g, 2.86 mmol) in anhydrous methanol (50 mL) was reduced under hydrogen using Lindlar catalyst (130 mg, 15%) to yield the desired alkene. The reaction was monitored by $^1$H NMR and the theoretical amount of hydrogen was adsorbed in 12 hrs. The mixture was filtered through a bed of celite, and concentrated to dryness.

Compound 3, yield: 99%; $\delta_H$ (300 MHz, CDCl$_3$) -0.02 (6 H, s, Si-(CH$_3$)$_2$), 0.82 (9 H, s, tBu-Si), 1.64 (3 H, s, CH$_3$ thymine), 1.86-1.88 (1 H, m, H$_2''$), 2.00-2.05 (1 H, m, H$_2''$), 4.09-4.19 (2 H, m, H$_3$, H$_4$), 5.21-5.36 (2 H, m, 2H$_6$), 5.83-5.91 (1 H, m, H$_5$), 6.16 (1 H, t, $J$ 6.4, H$_1''$), 7.12 (1 H, s, H$_6$), 8.92 (1 H, br, NH); $\delta_C$ (75 MHz, CDCl$_3$) $\delta$ -4.9 (Si-(CH$_3$)$_2$), 12.7 (CH$_3$ thymine), 17.9 (Si-C$_{IV}$), 25.7 (Si-tBu), 40.5 (C$2'$), 75.2 (C$3'$), 85.2 (C$4'$), 87.5 (C$1'$), 110.9 (C$5$), 118.1 (C$6'$), 135.1 (C$5'$), 135.3 (C$6$), 150.1 (C$2$), 163.7 (C$4$); MS (ESI$^+$) m/z 353.0 ([M+H]$^+$, 100%); HMRS-FAB$^+$ (NBA) m/z calcd for C$_{17}$H$_{29}$O$_4$N$_2$Si [M+H]$^+$ 353.1897 Found 353.1899.

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Synthesis of 1-(5’-deoxy-3’-O-tertbutyldimethylsilyl thymidine)methylboronic acid 4:

A 20 mL round bottom flask was charged with borane dimethylsulfide (0.41 mL, 4.26 mmol) and dry Et₂O (10 mL) under an argon atmosphere. The mixture was warmed to reflux and α-pinene (1.56 mL, 9.94 mmol) was added dropwise. This mixture was stirred for 4 hrs under reflux. The resulting suspension was next allowed to reach room temperature and a solution of the alkene 3 (500 mg, 1.42 mmol) in THF (2 mL) was added dropwise and stirred at room temperature for an additional 12 hrs. Freshly distilled acetaldehyde (0.45 mL) was added to the mixture which was stirred overnight, HCl 0.1 M (2mL) was added and the solution was evaporated. The residue was dissolved in ethyl acetate (200 mL) and washed with brine (3x20 mL).

The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (80% CH₂Cl₂ (50/50)-20% ethyl acetate then 48/50/2 CH₂Cl₂/ethyl acetate/MeOH) to give 405 mg (72%) of 4 as a white foam.

Compound 4, yield: 72%; δH (300 MHz, CD₃OD) 0.00 (6 H, s, Si-(CH₃)₂), 0.80 (9 H, s, 3Bu-Si), 1.07-1.12 (2 H, m, H₆', H₆''), 1.56-1.64 (2 H, m, H₅', H₅''), 1.78 ( 1 H, s, CH₃ thymine), 2.06 (2 H, m, H₂', H₂''), 3.65-4.11 (2 H, m, H₃', H₄'), 6.06 (1 H, t, J 6.7; H₁'), 7.29 (1 H, s, H₆); δC (75 MHz, CD₃OD)-4.4-4.66 (Si-(CH₃)₂), 12.5 (CH₃ thymine), 18.8 (Si-Cını), 26.2 (Si-3Bu), 29.1 (C₅'), 40.9 (C₂'), 76.4 (C₃'), 86.0 (C₁'), 90.2 (C₄'), 111.8 (C₅), 137.6 (C₆), 152.3 (C₂), 166.3 (C₄); δB (128 MHz, CD₃OD, BF₃-Et₂O) 32.5; MS (ESI) m/z 511.1 ([M+CF₃COO]⁺, 100%); HMRS-ESI m/z calcd for C₁₉H₃₁BF₉O₈N₂Si [M+H]⁺ 511.1895 Found 511.1889.
Synthesis of 1-(5’-deoxythymidine)methylboronic acid 5:

![Diagram of molecules](attachment:image)

A 0.2 M solution of 3’-O-tertbutyldimethylsilyl thymidine boronic acid derivative 4 (400mg, 1 mmol) in aqueous HCl 3M (5mL) was stirred for 1 hr before removal of volatiles in vacuo. The remaining solution was dissolved in butanol (50 mL), washed with saturated NaHCO₃ solution (3x5 mL), brine and dried over sodium sulphate and concentrated in vacuo to furnish the crude product, which was not further purified.

**Compound 5**, yield: 98%; δ_H (300 MHz, D₂O, dioxane-d6) 0.87 (2 H, m, CH₂-B), 1.75 (2 H, m, H₅-H₆'), 1.89 (3 H, s, CH₃), 2.33 (2 H, m, H₂-H₂'), 3.87 (1 H, m, H₄'), 4.30 (1 H, m, H₃'), 6.25 (1 H, t, J 6.9, H₁), 7.43 (1 H, s, H₆); δ_C (75 MHz, D₂O; dioxane-d₆) 12.2 (CH₃), 28.2 (C₅'), 38.5 (C₂'), 73.9 (C₃'), 85.3 (C₁'), 88.8 (C₄'), 112.2 (C₅), 137.9 (C₆), 152.4 (C₂), 167.1 (C₄); δ_B (128 MHz; D₂O) 33.5 (br); MS (ESI⁺) m/z 285.1 ([M+H]⁺, 100%); HMRS-ESI⁺ m/z calcld for C₁₁H₁₈BO₆N₂ [M+H]⁺ 285.1258 Found 285.1265.
Figure S 1: $^1$H of compound 2

Figure S 2: $^{13}$C of compound 2
**Figure S 3**: DEPT of compound 2

**Figure S 4**: $^1$H NMR of compound 3
Figure S 5 : $^{13}$C of compound 3

Figure S 6 : DEPT of compound 3
Figure S 7: $^1$H NMR of compound 4

Figure S 8: $^{13}$C NMR of compound 4
Figure S9: DEPT NMR of compound 4

Figure S10: $^{11}$B NMR of compound 4
Figure S 11: $^1$H NMR of compound 5

Figure S 12: $^{13}$C NMR of compound 5
Figure S 13: DEPT NMR of compound 5

Figure S 14: $^{11}$B NMR of compound 5
Figure S 15 : COSY of compound 5

Figure S 16 : HSQC of compound 5
\(^1\)H NMR studies showing the dynamic binding of 5 to uridine in DMSO-\(d_6\) and D\(_2\)O

The complexed and uncomplexed forms of 5 with uridine were distinguishable by \(^1\)H NMR in DMSO-\(d_6\) and D\(_2\)O. Peak broadening of uridine protons was observed. These NMR experiments qualitatively confirm that the electronic environment of uridine undergoes significant changes as a result of boronic acid complexation with uridine.

Peak broadening was observed in DMSO-\(d_6\) for the H\(_1\) proton of thymidine boronic acid 5 due to boroxine formation.

Figure S 17 : Boronic ester formation between 5 and uridine in DMSO-\(d_6\)

Figure S 18 : Boroxine formation in DMSO-\(d_6\) (a non hydroxylic solvent) due to the release of three molecules of water.
Figure S19: Selected areas of the $^1$H NMR spectra of (a) 23 mM of uridine, (b) 92 mM of uridine and 23 mM of 5, (c) 23 mM of uridine and 23 mM of 5, (d) 23 mM of uridine and 69 mM of 5; (e) 23 mM of 5. The samples were dissolved in DMSO-\(d_6\) and the spectra were acquired at 300.13 MHz on a Bruker AM300 spectrometer locked on the deuterium frequency. The solvent residual peak was used as reference (2.49 ppm).
Peak broadening of NH-uridine proton was observed. One noticed the apparition of peak at 11.40 assigned to the NH of bounded.

Figure S 20: Selected areas of the $^1$H NMR spectra of (a) 23 mM of uridine, (b) 92 mM of uridine and 23 mM of 5, (c) 23 mM of uridine and 23 mM of 5, (d) 23 mM of uridine and 69 mM of 5; (e) 23 mM of 5. The samples were dissolved in DMSO-d$_6$ and the spectra were acquired at 300.13 MHz on a Bruker AM300 spectrometer locked on the deuterium frequency. The solvent residual peak was used as reference (2.49 ppm).
The 2 hydroxyl groups borne by the boron atom disappeared with an increasing quantity of uridine.

Figure S 21: Selected areas of the $^1$H NMR spectra of (a) 23 mM of uridine, (b) 92 mM of uridine and 23 mM of 5, (c) 23 mM of uridine and 23 mM of 5, (d) 23 mM of uridine and 69 mM of 5; (e) 23 mM of 5. The samples were dissolved in DMSO-d$_6$ and the spectra were acquired at 300.13 MHz on a Bruker AM300 spectrometer locked on the deuterium frequency. The solvent residual peak was used as reference (2.49 ppm).
The addition of a molecular sieve (4Å) to the stoechiometric mixture of uridine with 5 yields to the observation of the only bound species resonances.

Figure S 22: Selected areas of the $^1$H NMR spectra of (a) 23 mM of uridine and 23 mM of 5 in DMSO-\textit{d}_6 without molecular sieve, (b) 23 mM of uridine and 23 mM of 5 in DMSO-\textit{d}_6 with molecular sieve 4Å after 30 minutes.
Similar study realized in D$_2$O showing that the boronate ester also exists in aqueous conditions.

Figure S 23: Selected areas of the $^1$H NMR spectra of (a) 23 mM of uridine, (b) 92 mM of uridine and 23 mM of 5, (c) 23 mM of uridine and 23 mM of 5, (d) 23 mM of 5. The samples were dissolved in D$_2$O and the spectra were acquired at 300.13 MHz on a Bruker AM300 spectrometer locked on the deuterium frequency. The solvent residual peak was used as reference (4.79 ppm).
Methodology and examples for $K_a$ measurement by ARS method of $5$ to different nucleosides

It is well-known that spectroscopic methods are generally more sensitive than methods using NMR. However, for this to happen, the binding event needs to trigger a change in the spectroscopic properties of the boronic acid component. To address this issue, the Wang’s group developed a generally applicable, highly sensitive method for the determination of the binding constants between boronic acid and diols. This method uses Alizarine Red S. (ARS) as a reporter compound in a three-component competition assay.

This system has two competing equilibria. The first equilibrium, between the boronic acid and the UV reporter compound (ARS) can be directly measured. The addition of a diol sets up a second equilibrium between the boronic acid and the diol to give a new complex (BA-diol). This perturbs the ARS/boronic acid equilibrium, resulting in a change in the absorbance of the solution. The solution shows a $\lambda_{\text{max}}$ change and a corresponding visible color change from deep red to yellow upon addition of boronic acid.

In a such experiment, the boronic acid species needs to be in excess (at least 10-fold) compared with the diol.

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Figure S 24: Cis-diol detection by using a competitive boronic acid receptor

Methodology for screening thymidine boronic acid: qualitative ARS assay

**Solution A:** 50 mL of $10^{-3}$ M stock solution of ARS solution in 0.10 M sodium phosphate monobasic buffer was diluted 10-fold with 0.10 M sodium phosphate monobasic buffer in a 500 mL volumetric flask. The pH of the solution was adjusted to 7.6 with 2M NaOH (a portable pH meter was used which gave pH values within 0.01 units). The resultant solution containing $10^{-4}$ M solution of ARS in 0.10 M phosphate monobasic buffer at pH 7.6 would be referred to as solution A.

**Solution B:** The controls were prepared by dissolving the boronic acid (0.10 mmol) in solution A in a 5 mL volumetric flask to give 0.015 M solution with respect to the boronic acid. The pH was adjusted to 7.6 with 2M NaOH before diluting to the 5 mL mark with ARS solution A.

**Solution C:** The sugar or nucleoside solutions (2M for D-ribose, D-fructose, D-glucose and uridine, 1.5 M for 2’-deoxyuridine, 0.67 M for cytidine, 0.5 M for 5’-monophosphate uridine, 5’-monophosphate cytidine, 5’-monophosphate adenosine, 5’-monophosphophate guanosine) were prepared by adding the appropriate quantity of sugar or nucleoside to the control solution B. The pH was adjusted to 7.6 with 2M NaOH.

Colorimetric assays were tried out with the solution with uridine.

**Selected assays (pH=7.6) with uridine**

![Figure S 25: Vials of ARS (10^{-4} M in 0.1 M phosphate buffer) and 5 (3mM) containing A, no uridine neither 5 ; B, no uridine; C, 0.1 M uridine, 0.6 M uridine.](image-url)
Methodology and examples for Ka measurements by ARS method

There are literature precedents for the use of absorbance changes in the three-component system for the determination of binding constant. Consequently, there are also well-established mathematical models for the determination of the respective equilibrium constants.

Values were obtained by ARS competitive experiments as follows:

In the complex mixture of boronic acid receptor (R), ARS Indicator (I) and sugar substrate (S), there exist the following equilibria:

\[
R + I \rightleftharpoons \text{RI} \quad K_{\text{ars}}
\]

\[
\text{RI} + S \rightleftharpoons \text{RS} \quad K_{\alpha}
\]

Where RI is the boronic acid-ARS complex and RS is the substrate complex.

Equations for association constant determinations:

\[
\frac{1}{\Delta A} = \frac{1}{\Delta K p_o I_o K_{\text{ars}}} \ast \frac{1}{[S]} + \frac{1}{\Delta K p_o I_o}
\]

Equation 1

Where \( \Delta K \), \( p_o \), \( I_o \) are all the parameters of the UV spectrophotometer.

\[
Q = \frac{[I]}{[\text{RI}]} = \frac{A_{\text{RI}} - A}{A - A_I}
\]

Equation 2

Where A is measured absorbance, \( A_{\text{RI}} \) is absorbance of the receptor-indicator complex, and \( A_I \) is absorbance of free indicator.

\[
P = [R] \cdot \frac{1}{Q K_{\text{ars}}} - \frac{[I_o]}{Q + 1}
\]

Equation 3

Where \([I_o]\) is total indicator concentration (ARS).

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\[
\frac{[S]}{P} = \frac{K_{\text{ars}}}{K_a} \times Q + 1
\]

Equation 4

Where \([S]\) is substrate concentration.

The association constant of the ARS-5 (\(K_{\text{ars}}\)) is the quotient of the intercept and the slope in a plot of \(1/\Delta A\) versus \(1/[S]\). (equation 1).

Following the procedure of Wang et al.,\(^4\) a 0.1 mM ARS solution was prepared in 0.1 M phosphate solution buffered at pH 7.6. A solution of 5 (15mM) was prepared so as to prepare mixtures of 5 (0-15mM) and UV absorbances were taken at 471 nm. Two experiments were carried out to determine an average value of \(K_a\). To validate our experimental techniques, we reproduced the binding constant between 5 and the ARS indicator and obtained an average value of 1400 M\(^{-1}\) compared to 1300 M\(^{-1}\) obtained by Dr. Wang’s laboratory and for the complexation between phenyl boronic acid and fructose, the binding constant was found to be 155 M\(^{-1}\) as compared to 160 M\(^{-1}\) from Dr. Wang’s laboratory.

The above thymidine boronic acid solution (15mM) in ARS was diluted with the ARS solution (0.1 mM) to give a 3 mM solution in the phosphate buffered solution at pH 7.6. 5 mL of this solution was used to make a 2.0 M nucleoside solutions at pH 7.6. By mixing the two solutions together in the UV cuvette, a range of nucleoside concentrations (0-2M) was obtained. The absorbances were determined at 471 nm and equations 3 and 4 were used to find \(K_a\).

According to equation 4, the y-intercept should be 1, but in our studies, it was not so. This could be attributed to the UV instrumental parameters, and when we determined the binding constants of phenyl boronic acid and fructose according to the description of Wang et al., they matched the reported ones (in their case, too, the intercept was not 1). Similar observations were made by D. G. Hall et al.\(^5\)
Results and discussions:

As the concentration of thymidine boronic acid is increased, a visible colour change from burgundy to yellow was observed. Fig. S 26 shows the absorption changes with added boronic acid. The absorbance of the free ARS at 516 nm decreases as 5 is added, and a new absorbance at 471 nm appears. The stability constant $K_{ars}$ of ARS with 5 was calculated by fitting the absorbance at 471 nm versus boronic acid concentration. The calculated $K_{ars}$ value is 430 M$^{-1}$.

Figure S 26: Absorption spectral changes of ARS (1.10$^{-4}$M) with increasing concentration of 5 (0-15mM).

Figure S 27: $1/\Delta\lambda$ versus $1/[5]$ plot for determination of $K_{ars}$ in the presence ARS (0.1 mM) and 5 (0-15mM) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.
The association constants of 5 to sugar and different nucleosides in water (pH= 7.6) were measured by the ARS method based on the competitive release of alizarine red S.

As the concentration of nucleoside (or sugar) is increased, a visible color change from yellow to burgundy was observed. Fig. S 28 (example of uridine) shows the absorption changes with added nucleoside. The absorbance of the free ARS at 471 nm decreases as nucleoside is added, and a new absorbance at 516 nm appears.

![Figure S 28: Absorption spectral changes of 5-ARS complex (5: 3mM, ARS: 1.10^{-4}M) with increasing concentration of uridine (0-2M).](image)

![Figure S 29: Absorption spectral changes at 471 nm of 5-ARS complex (5: 3mM, ARS: 1.10^{-4}M) with increasing concentration of uridine (0-2M).](image)
The stability constant $K_a$ of 5 with each nucleoside was calculated by fitting [nucleoside]/P versus Q factor. The calculated $K_a$ values are given in Table 1.

Figure S 30: [uridine]/P versus Q plot for determination of $K_a$ in the presence ARS (0.1 mM) and 5 (3 mM) with increasing concentration of uridine (0-2M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.

Figure S 31: [2'-deoxyuridine]/P versus Q plot for determination of $K_a$ in the presence ARS (0.1 mM) and 5 (3 mM) with increasing concentration of 2'-deoxyuridine (0-2M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.
Figure S 32: [α-uridine]/P versus Q plot for determination of $K_a$ in the presence ARS (0.1 mM) and 5 (3 mM) with increasing concentration of α-uridine (0-2M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.

$y = 9.9382x + 89.375$

$R^2 = 0.9628$

Figure S 33: [cytidine]/P versus Q plot for determination of $K_a$ in the presence ARS (0.1 mM) and 5 (3 mM) with increasing concentration of cytidine (0-0.5M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.

$y = 2.0075x + 79.666$

$R^2 = 0.9763$
Figure S 34: [UMP]/P versus Q plot for determination of $K_a$ in the presence ARS (0.1 mM) and 5 (3 mM) with increasing concentration of UMP (0-0.5M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.

\[ y = 2.8096x + 218.68 \]
\[ R^2 = 0.9893 \]

Figure S 35: [CMP]/P versus Q plot for determination of $K_a$ in the presence ARS (0.1 mM) and 5 (3 mM) with increasing concentration of CMP (0-0.5M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.

\[ y = 1.3307x + 105.56 \]
\[ R^2 = 0.993 \]
Figure S 36: \([\text{AMP}] / \mathcal{P} \) versus \( Q \) plot for determination of \( K_a \) in the presence \( \text{ARS} \) (0.1 mM) and \( 5 \) (1.5 mM) with increasing concentration of \( \text{AMP} \) (0-0.4M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.

\[
\begin{align*}
\text{y} &= 6.2696x + 184.4 \\
R^2 &= 0.9896
\end{align*}
\]

Figure S 37: \([\text{GMP}] / \mathcal{P} \) versus \( Q \) plot for determination of \( K_a \) in the presence \( \text{ARS} \) (0.1 mM) and \( 5 \) (1.5 mM) with increasing concentration of \( \text{GMP} \) (0-0.4M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.

\[
\begin{align*}
\text{y} &= 1.9923x + 121.2 \\
R^2 &= 0.9861
\end{align*}
\]
Figure S 38: \([\text{fructose}]_P\) versus Q plot for determination of \(K_a\) in the presence ARS (0.1 mM) and 5 (3 mM) with increasing concentration of fructose (0-0.6M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.

\[ y = 24.12x + 78.023 \]
\[ R^2 = 0.9831 \]

Figure S 39: \([\text{glucose}]_P\) versus Q plot for determination of \(K_a\) in the presence ARS (0.1 mM) and 5 (3 mM) with increasing concentration of glucose (0-1.5M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.

\[ y = 8.8911x + 300.04 \]
\[ R^2 = 0.9786 \]
The stability constant $K_{\text{ars}}$ of ARS with $n$-butyl boronic acid was calculated by fitting the absorbance at 471 nm versus boronic acid concentration. The calculated $K_{\text{ars}}$ value is 245 M$^{-1}$. 

Figure S 40: [ribose]/P versus Q plot for determination of $K_a$ in the presence ARS (0.1 mM) and 5 (3 mM) with increasing concentration of ribose (0-1.5M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.

Figure S 41: 1/∆A versus 1/[butyl BA] plot for determination of $K_{\text{ars}}$ in the presence ARS (0.1 mM) and $n$-butyl boronic acid (0-15mM) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm. $K_{\text{ars}}=245$ M$^{-1}$
The stability constant $K_a$ of $n$-butyl boronic acid with uridine was calculated by fitting [uridine]/P versus Q factor. The calculated $K_a$ value is 14 M$^{-1}$.

\[ y = 16.379x + 33.874 \]
\[ R^2 = 0.9538 \]

**Figure S 42:** [uridine]/P versus Q plot for determination of $K_a$ in the presence ARS (0.1 mM) and $n$-butyl boronic acid (3 mM) with increasing concentration of uridine (0-2M) at pH 7.6 in 0.1 M phosphate buffered solution with data being taken at 471 nm.