

Tautomeric equilibria and binding selectivity of pyridineboronic acids

Luis Ramón Ortega-Valdovinos, José Guadalupe Chino-Cruz and Anatoly K. Yatsimirsky*

Facultad de Química, Universidad Nacional Autónoma de México, 04510 México D. F, México.

E-mail: iatsimirski46@comunidad.unam.mx

Supporting Information

S1. Synthesis of (N-Methyl)-3-pyridiniumboronic acid mesylate (3-(NMe)Py⁺BA).

Synthesis of 3-(NMe)Py⁺BA was performed by procedure described in the literature ¹ with minor modifications. 3-Pyridineboronic acid (50 mg, 0.407 mmole) and methyl mesylate (130 mg, 1.15 mmole) were dissolved in dry nitromethane (15 mL). Solution was heated at 73°C for 3 days. The cooled solution was extracted with water (2 x 15 mL). Combined aqueous extracts were washed with diisopropyl ether (3 x 10 mL) and water was evaporated to yield a pale yellow solid (0.81 g, 79.2%)

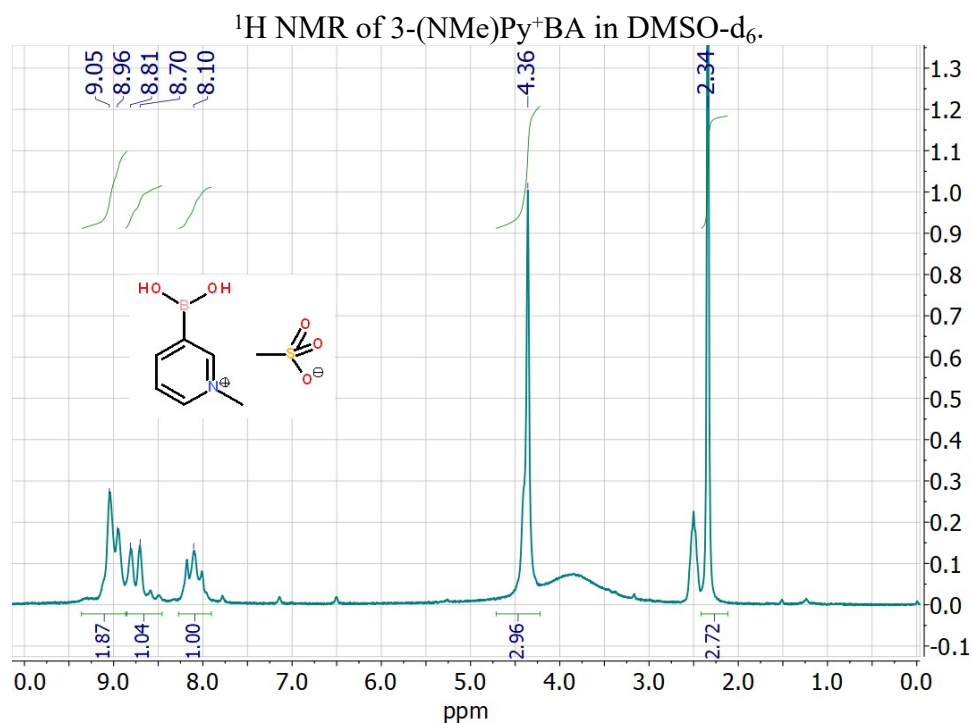


Table S1. Results of potentiometric titrations of pyridineboronic acids alone and in the presence of added diols in water (0.1 M NaCl, 25°C). Numbers in parenthesis are standard deviations in the last significant digit.

boronic acid, M	diol, M	pK_{a1}	pK_{a2}	$\log\beta_{110}$	$\log\beta_{11-1}$
3-PyBA, 0.0054	none	4.34(0.02)	8.19(0.02)		
3-PyBA, 0.0055	fructose, 0.052			2.78(0.03)	-4.59(0.03)
3-PyBA, 0.0050	sialic acid, 0.0065			5.60(0.03)	-0.9(0.1)
3-PyBA, 0.0053	lactic acid, 0.0053			5.10(0.04)	-1.47(0.08)
3-PyBA, 0.0055	glucose, 0.20			1.72(0.02)	-5.95(0.03)
3-PyBA, 0.0055	catechol, 0.0107			3.35(0.03)	-3.72(0.04)
4-PyBA, 0.0075	none	3.85(0.02)	7.83(0.02)		
4-PyBA, 0.0085	fructose, 0.050			2.92(0.04)	-4.16(0.05)
4-PyBA, 0.0063	sialic acid, 0.0075			5.76(0.02)	-0.47(0.04)
3-(NMe)Py ⁺ BA, 0.0039	none	4.36(0.02)			
3-(NMe)Py ⁺ BA, 0.0039	sialic acid, 0.0054				1.17(0.02)

Table S2. Results of potentiometric titrations of pyridineboronic acids alone and in the presence of added diol in 50% vol. aqueous organic solvents (0.1 M NaCl, 25°C). Numbers in parenthesis are standard deviations in the last significant digit.

co-solvent	boronic acid, M	diol, M	pK_{a1}	pK_{a2}	$\log\beta_{110}$	$\log\beta_{11-1}$
dioxane	3-PyBA, 0.0050	none	4.40(0.03)	9.20(0.03)		
	none	sialic acid, 0.0074	3.53(0.05)			
	3-PyBA, 0.0056	fructose, 0.049			2.32(0.03)	-4.97(0.03)
	3-PyBA, 0.0052	sialic acid, 0.0074			5.45(0.06)	-2.2(0.2)
MeOH	3-PyBA, 0.0042	none	4.44(0.02)	8.08(0.02)		
	3-PyBA, 0.0042	fructose			2.54	-4.82
EtOH	3-PyBA, 0.0065	none	4.48(0.02)	8.97(0.01)		
	3-PyBA, 0.0062	fructose, 0.050			2.52(0.02)	-4.81(0.02)
MeCN	3-PyBA, 0.0058	none	4.32(0.07)	7.72(0.07)		
	3-PyBA, 0.0051	fructose, 0.050			2.37(0.04)	-4.91(0.04)
DMSO	3-PyBA, 0.0051	none	4.73(0.04)	9.42(0.05)		
	3-PyBA, 0.0049	fructose, 0.051			2.16(0.03)	-5.26(0.04)
dioxane	4-PyBA, 0.0065	none	4.20(0.02)	8.64(0.02)		
	4-PyBA, 0.0068	fructose, 0.050			2.61(0.03)	-4.51(0.03)
	4-PyBA, 0.0046	sialic acid, 0.0074			5.57(0.05)	-1.08(0.06)

Table S3. Results of potentiometric titrations of substituted phenylboronic acids (**X**-C₆H₄B(OH)₂) alone and in the presence of added fructose in 50% vol. aqueous dioxane (0.1 M NaCl, 25°C). Numbers in parenthesis are standard deviations in the last significant digit.

X, M	[fructose], M	pK_a	logβ₁₁₋₁	logK_{tet}
H, 0.013	none	11.18(0.01)		
H, 0.013	0.055		-6.37(0.01)	4.81
3-Cl, 0.0064	none	10.20(0.01)		
3-Cl, 0.0064	0.050		-5.69(0.01)	4.51
3-CF₃, 0.0057	none	10.20(0.02)		
3-CF₃, 0.0057	0.050		-5.68(0.01)	4.52
3-NO₂, 0.0068	none	9.13(0.02)		
3-NO₂, 0.0068	0.055		-4.83(0.01)	4.30

S2. Spectrophotometric titrations of substituted phenylboronic acids by sialic acid

Titration were performed at pH 5.5 when all boronic acids used in titration experiments are present in solution as neutral species. The observed binding constants determined under these conditions correspond to K'_{tet} values.

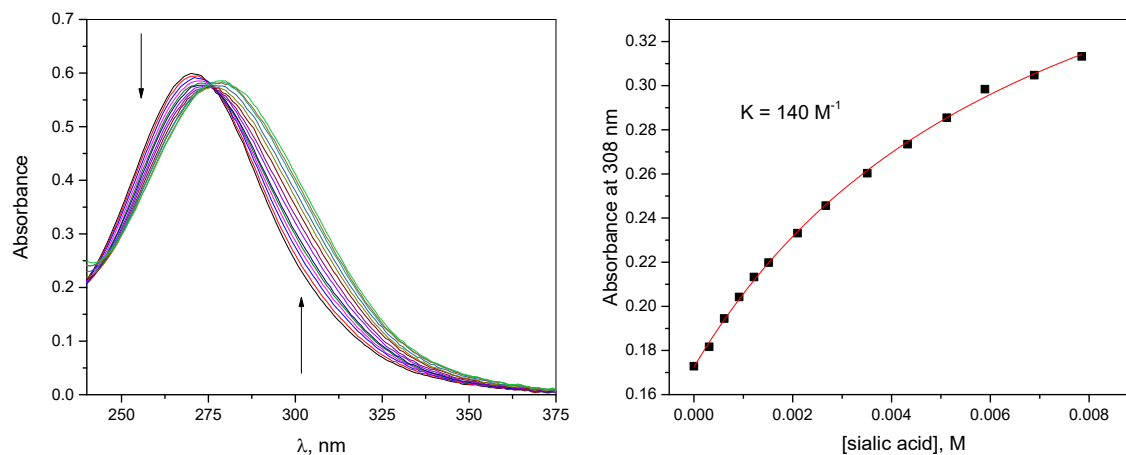


Fig. S1. Spectrophotometric titration of 0.066 mM 4-nitrophenylboronic acid by sialic acid at pH 5.5. Arrows show directions of spectral changes at increased concentration of sialic acid.

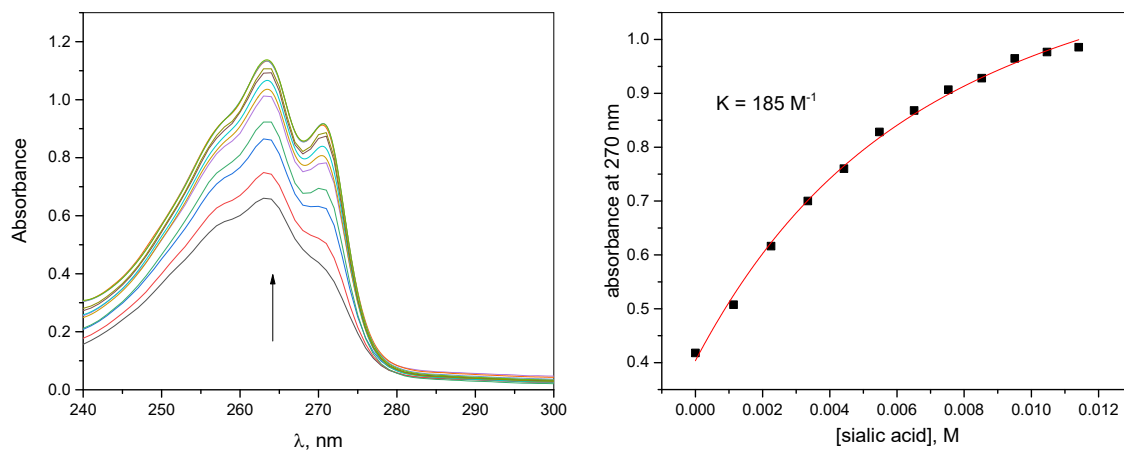


Fig. S2. Spectrophotometric titration of 2.4 mM 3,5-bis(trifluoromethyl)phenylboronic acid by sialic acid at pH 5.5. Arrow shows the direction of spectral changes at increased concentration of sialic acid.

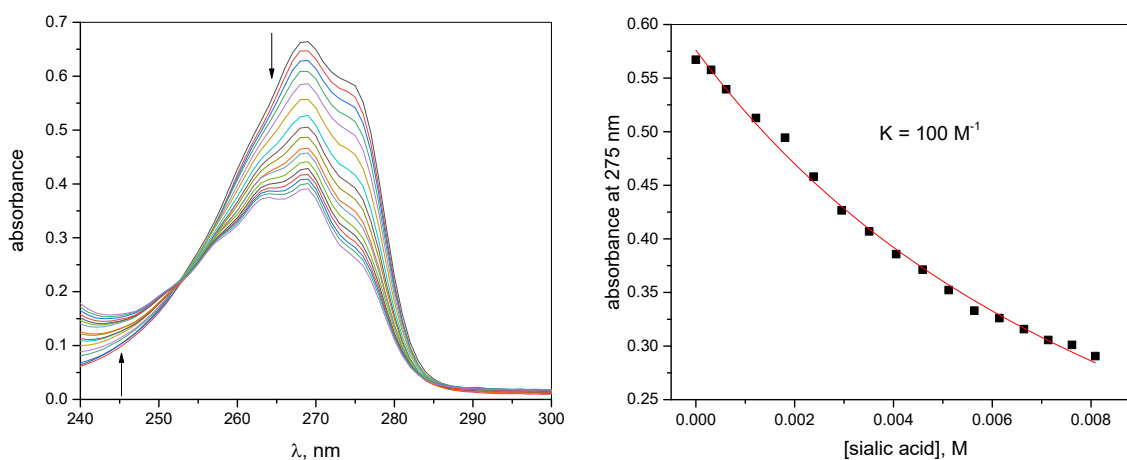


Fig. S3. Spectrophotometric titration of 0.7 mM 4-(trifluoromethyl)phenylboronic acid by sialic acid at pH 5.5. Arrows show directions of spectral changes at increased concentration of sialic acid.

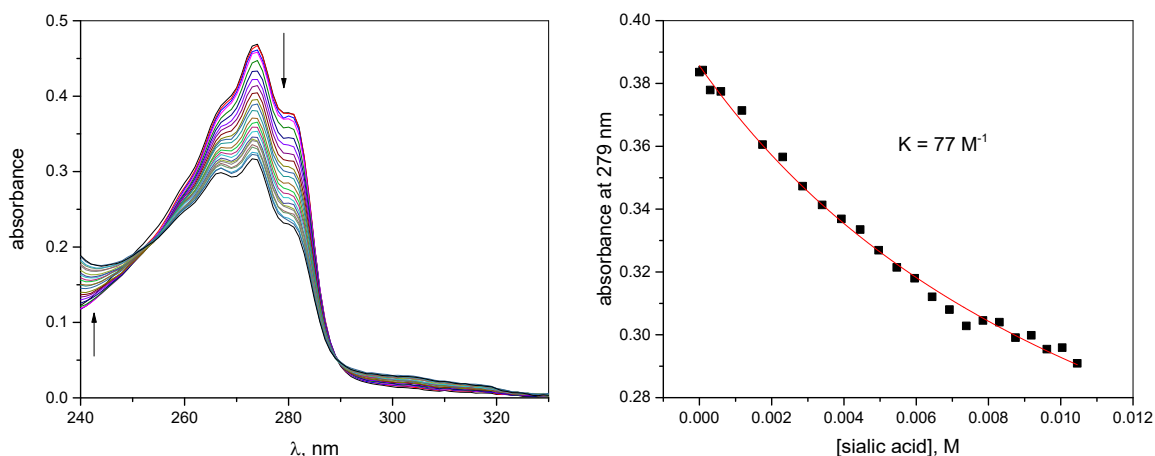


Fig. S4. Spectrophotometric titration of 0.78 mM 4-chlorophenylboronic acid by sialic acid at pH 5.5. Arrows show directions of spectral changes at increased concentration of sialic acid.

Table S4. Observed stability constants of sialic acid complexes with substituted phenylboronic acids obtained by fitting results shown in Figures S1-S4.

substituent	σ	$\text{p}K_a^{\text{B}}$	$\log K_{\text{obs}}$
4-NO ₂	0.78	7.1	2.20±0.01
3,5-(CF ₃) ₂	0.86	7.2	2.27±0.06
4-CF ₃	0.54	7.39	2.00±0.06
3-Cl	0.37	7.85	1.88±0.02

S3. Observed stability constants of boronate esters at variable pH.

The observed equilibrium constant K_{obs} for ester formation between a boronic acid RB(OH)_2 and a diol D(OH)_2 is given by equation (S1).²

$$K_{\text{obs}} = \beta_{11-1}[\text{H}^+]/\{(K_a^{\text{D}} + [\text{H}^+])(K_a^{\text{B}} + [\text{H}^+])\} \quad (\text{S1})$$

Considering that $\beta_{11-1} = K_{\text{tet}}K_a^{\text{B}} = K'_{\text{tet}}K_a^{\text{D}}$ one obtains expressions (S2) and (S3).

$$K_{\text{obs}} = K_{\text{tet}}K_a^{\text{B}}[\text{H}^+]/\{(K_a^{\text{D}} + [\text{H}^+])(K_a^{\text{B}} + [\text{H}^+])\} \quad (\text{S2})$$

$$K_{\text{obs}} = K'_{\text{tet}}K_a^{\text{D}}[\text{H}^+]/\{(K_a^{\text{D}} + [\text{H}^+])(K_a^{\text{B}} + [\text{H}^+])\} \quad (\text{S3})$$

Here K_a^{B} and K_a^{D} are the acid dissociation constants of boronic acid and diol respectively and K_{tet} is the stability constant of the tetrahedral complex formed through reaction (S4).



Alternatively, the tetrahedral complex can be formed through reaction (S5) with the respective stability constant K'_{tet} .



Stability constants K_{tet} and K'_{tet} are mutually related through equation (S6).

$$K_{\text{tet}} = K'_{\text{tet}} K_{\text{a}}^{\text{D}} / K_{\text{a}}^{\text{B}} \quad (\text{S6})$$

Equations (S1) - (S3) are used in calculation of profiles shown in Fig. 12. They predict a bell-shaped profile of K_{obs} vs pH for any combination of boronic acid and diol with a maximum value of K_{obs} at the optimum pH given by equation (S7).

$$\text{pH}_{\text{opt}} = (\text{p}K_{\text{a}}^{\text{B}} + \text{p}K_{\text{a}}^{\text{D}}) / 2 \quad (\text{S7})$$

All three equations (S1) - (S3) are identical but use different expressions for the cumulative constant β_{11-1} . Introducing in any of them, for instance in (S3) the expression for $[\text{H}^+]_{\text{opt}} = (K_{\text{a}}^{\text{B}} K_{\text{a}}^{\text{D}})^{0.5}$ following from eqn (S7) one obtains for the maximum value of K_{obs} the equation (S8).

$$K_{\text{obs}}^{\text{MAX}} = K'_{\text{tet}} / (1 + (K_{\text{a}}^{\text{B}} / K_{\text{a}}^{\text{D}})^{0.5})^2 \quad (\text{S8})$$

If $K_{\text{a}}^{\text{D}} \gg K_{\text{a}}^{\text{B}}$ (the case of sialic acid) one obtains from (S8) $K_{\text{obs}}^{\text{MAX}} = K'_{\text{tet}}$, but if $K_{\text{a}}^{\text{B}} \gg K_{\text{a}}^{\text{D}}$ (the case of fructose) one obtains $K_{\text{obs}}^{\text{MAX}} = K'_{\text{tet}} K_{\text{a}}^{\text{D}} / K_{\text{a}}^{\text{B}} = K_{\text{tet}}$ (see Fig. 12).

For sialic acid $K_{\text{a}}^{\text{D}} = 2.5 \times 10^{-3}$ M. Therefore, in the pH range from 5 to 7 the condition $K_{\text{a}}^{\text{D}} \gg [\text{H}^+]$ is fulfilled and the equation (S3) takes the form of (S9), which coincides with equation (22) in the main text.

$$K_{\text{obs}} = K'_{\text{tet}} [\text{H}^+] / (K_{\text{a}}^{\text{B}} + [\text{H}^+]) = K'_{\text{tet}} / (1 + K_{\text{a}}^{\text{B}} / [\text{H}^+]) \quad (\text{S9})$$

Table S5. Stability constants of sialate complexes with boronic acids at pH 6 (Fig. 13).

boronic acid	$\text{p}K_{\text{a}}^{\text{B}}$	$K_{\text{obs}}, \text{M}^{-1}$	Ref.
1	4.2	645	3
2	4.2	499	3
3	3.85	356	this work
4	4.34	252	this work
4	4.4	182	3
5	4.4	61	3
6	4.36	132	this work
7	6.2	490	3
8	6.3	419	3
9	6.65	358	3
10	7.2	175	this work
11	7.1	147	this work
12	7.39	96	this work
13	7.85	75	this work
14	7.99	34	3
15	8.3	40	3
16	8.9	20	4
17	7.3	51.2	5

Table S6. Stability constants of sialate and fructose complexes with boronic acids at pH 7.4 (Fig. 14) determined by affinity capillary electrophoresis. ⁶

tag	boronic acid	pK _a ^B	K _{obs} , M ⁻¹	
			sialic acid	fructose
<i>a</i>	phenylboronic acid	8.9	16.0	140
<i>b</i>	3-carboxyphenylboronic acid	8.14	35.7	261
<i>c</i>	4-(methylsulfonyl)benzene-boronic acid	7.4	104	1450
<i>d</i>	2,4-difluoro-3-formylphenyl-boronic acid	6.74	42.5	2790
<i>e</i>	2-furanboronic acid	7.88	23.7	372
<i>f</i>	3-furanboronic acid	8.65	9.2	139
<i>g</i>	2-thiopheneboronic acid	8.11	24.0	483
<i>h</i>	3-thiopheneboronic acid	8.77	8.8	169
<i>i</i>	3-pyridinylboronic acid	4.34	39.4	833
<i>j</i>	4-pyridinylboronic acid	3.85	35.7	794
<i>k</i>	pyrimidine-5-boronic acid	6.2	22.6	1020
<i>l</i>	N-Me-3-pyridinylboronic acid *	4.36	5.4	

* This work

¹ L. K. Mohler and A. W. Czarnik, *J. Am. Chem. Soc.* 1993, **115**, 2998-2999.

² M. A. Martínez-Aguirre, R. Villamil-Ramos, J. A. Guerrero-Alvarez and A. K. Yatsimirsky, *J. Org. Chem.* 2013, **78**, 4674-4684.

³ A. Matsumoto, A. J. Stephenson-Brown, T. Khan, T. Miyazawa, H. Cabral, K. Kataoka and Y. Miyahara, *Chem. Sci.*, 2017, **8**, 6165–6170.

⁴ G. Springsteen and B. Wang, *Tetrahedron* 2002, **58**, 5291-5300

⁵ A. Di Pasquale, S. Tommasone, L. Xu, J. Ma, and P. M. Mendes, *J. Org. Chem.* 2020, **85**, 8330–8338.

⁶ C. Lü, H. Li, H. Wang and Z. Liu, *Anal. Chem.* 2013, **85**, 2361–2369.