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SUPPORTING INFORMATION

A Multinuclear NMR Perspective on the Complexation between Bisboronic acids and Bisbenzoxaboroles with *cis*-Diols

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Table of Contents

UV-Visible studies of the complexation of boronic acids and benzoxaboroles with diols

Experimental details on the UV-vis measurements:

Absorbance measurements were performed on an Analytik Jena (Specord 210Plus) apparatus. Cuvettes made of Quartz Suprazil, with a light path of 10 mm, were used. Absorption scans were recorded at room temperature, from 300 nm to 750 nm, with a step size of 0.1 nm and a scanning speed of 0.10 $nm.s^{-1}$.

Operating procedure:

A solution of 10^{-4} M Alizarin Red S (ARS) and 10^{-3} M organoboron molecule (in phosphate buffer or DMSO/buffer depending on the case) was prepared and immediately analyzed by UV-Visible spectroscopy by successive scans over a period of 1 hour. The second diol (D-fructose or catechol) was then added in excess (1000 equiv. compared to ARS) in the cuvette and, after homogenization, immediately analyzed by UV-Visible spectroscopy by successive scans over a period of 1 hour.

For each organoboron molecule/*cis*-diol couple, the time required for the absorbance to become stable (generally < 10 minutes) was taken into account for the subsequent fluorescence studies. Moreover, from the study of the complexation with ARS, the wavelength at the maximum of absorbance (λ_m) was derived (Table S1), which corresponds to the excitation wavelength $\lambda_{exct°}$ used in the subsequent fluorimetric studies.

Table S1 : λ_m wavelengths provided by the UV-Visible studies.

	Phosphate Buffer[1]	DMSO / Phosphate buffer[2]
	λ_m (nm) [3]	λ_m (nm) [3]
BBzx	459	465
PBA	468	483
bisPBBzx	-	466
PBB	-	479

[1] Aqueous potassium phosphate buffer: pH = 7.4, 40 mM.
[2] DMSO/phosphate buffer mixture (60/40 v/v), prepared using an aqueous potassium phosphate buffer (pH = 7.4 ; 40 mM).
[3] Wavelength corresponding to the $\lambda_{exct°}$ used in the fluorimetric studies.

Fluorescence studies of the complexation of boronic acids or benzoxaboroles with diols

Experimental details on the fluorescence measurements:

Fluorescence spectra were recorded on an Edinburgh Instruments apparatus (Xe 900, NIR 300/2). Cuvettes made of Quartz Suprazil, with a light path of 10x10 mm, were used.

The emission scans were recorded at room temperature from {$\lambda_{exct°}$+15 nm} to 750 nm (with $\lambda_{exct°}$ depending on the organoboron molecule/*cis*-diol couple, as indicated in Table S1). A step size of 1 nm was used, and a dwell time of 1 s. Spectra were each accumulated three times.

Methodology:

Four solutions (***A***, ***B***, ***C*** and ***D***) were prepared using the afore-mentioned phosphate buffer or the DMSO/phosphate buffer mixture, depending on the case.

Table S2 : Composition of the solutions used in the fluorescence studies.

	[ARS]	[Organoboron molecule]	[Diol]
Solution ***A***	9.0×10^{-6} M	-	-
Solution ***B***	9.0×10^{-6} M	3.0×10^{-3} M	-
Solution ***C***	9.0×10^{-6} M	2.0×10^{-3} M	-
Solution ***D***	9.0×10^{-6} M	2.0×10^{-3} M	5.0×10^{-1} M

The first constant, $K_{app.1}$, between the organoboron molecule and the ARS, was determined with solutions ***A*** and ***B***. First, the fluorescence of the initial cuvette (Solution ***A***) was recorded. Then, progressively solution ***B*** was added to the cuvette. After each addition, the cuvette was manually agitated and left to set for a few minutes, depending on the complexation kinetics of the studied couple (previously evaluated by UV-vis spectroscopy). The

organoboron molecule was then added until reaching 200 equivalents with respect to the ARS. The data was then analyzed using the methodology described by Springsteen and Wang (which is recalled below) to derive $K_{app.1}$.[1] All the measurements were performed at least three times for each couple, and the $K_{app,\ 1}$ values were averaged. An illustration of this methodology for the ARS/PBA couple in the DMSO-phosphate buffer mixture is shown in Figure S1 (supporting information).

The second constant, $K_{app.2}$, between the organoboron molecule and the second diol (D-fructose or catechol) was determined from measurements performed using solutions ***C*** and ***D***. First, the fluorescence of the initial cuvette (solution ***C***) was recorded. Then, progressively solution ***D*** was added to the cuvette. After each addition, the cuvette was manually agitated and left to set for a few minutes, depending on the complexation kinetics of the studied couple (previously evaluated by UV-vis spectroscopy). Solution ***D*** was added progressively until the fluorescence of the initial solution had decreased by at least 60%. The data was then analyzed using the methodology described by Wang *et al* (which is recalled below) to derive $K_{app.2}$.[1] All the measurements were performed at least three times times for each couple, and the $K_{app,\ 2}$ values were averaged. An illustration of this methodology for the BBzx/D-fructose couple is provided in Figure 2 (main text).

Mathematical treatment of the fluorescence data:

The following notations are used below:

A = ARS, B = organoboron molecule, D = *cis*-diol,

AB = ARS/organoboron molecule complex, DB = *cis*-diol/organoboron molecule complex

$$K_{app,1} = \frac{[AB]}{[A].[B]} \qquad Q = \frac{[A]}{[AB]} \qquad K_{app,2} = \frac{[DB]}{[D].[B]}$$

$$[A]_0 = [A] + [AB] \qquad [B]_0 = [B] + [AB] + [DB] \qquad [D]_0 = [D] + [DB]$$

The first complexation constant, $K_{app.1}$, was determined as follows. The variation of the fluorescence ΔI_f of the solution after addition of B (ΔI_f being determined at the wavelength of the maximum fluorescence of the ARS/organoboron complex AB, *i.e.* λ_{max}) is proportional to the concentration of AB complex formed, meaning that

$$[AB] = \beta.\Delta I_f \ (\beta \text{ being the proportionality constant})$$

Hence,

$$[A]_0 = [A] + [AB] = [A] + \beta.\Delta I_f = \frac{[AB]}{K_{app,1}.[B]} + \beta.\Delta I_f = \beta.\Delta I_f.\frac{1 + K_{app,1}.[B]}{K_{app,1}.[B]}$$

This leads to $\Delta I_f = [A_0] \Big/ \left(\beta.\frac{1+K_{app,1}.[B]}{K_{app,1}.[B]}\right)$ and hence $\frac{1}{\Delta I_f} = \frac{\beta}{K_{app,1}.[A]_0}.\frac{1}{[B]} + \frac{\beta}{[A]_0}$ (Eq 1)

Consequently, by plotting $\frac{1}{\Delta I_f}$ as a function of $\frac{1}{[B]}$, $K_{app,1}$ can be determined.

The $K_{app.2}$ complexation constant was determined as follows. The [A], [B] and [D] concentrations are re-expressed as functions of the initial concentrations (noted $[A]_0$, $[B]_0$ and $[D]_0$), $K_{app,1}$ and Q.

$$[D]_0 = [D] + [DB] = [D].\left(1 + \frac{[DB]}{[D]}\right) = [D].\left(1 + \frac{K_{app,2}}{Q.K_{app,1}}\right) \quad => \quad [D] = \frac{[D]_0}{1 + \frac{K_{app,2}}{Q.K_{app,1}}}$$

$$\text{Moreover } [B] = \frac{1}{Q.K_{app,1}}$$

$$\text{and } [A]_0 = [A] + [AB] = [A]\left(1 + \frac{1}{Q}\right) \quad => \quad [A] = \frac{[A]_0.Q}{Q+1}$$

The different complex concentrations are then expressed by the equations below :

$$[AB] = K_{app,1}.[A].[B] = K_{app,1}.\frac{[A]_0.Q}{Q+1}.\frac{1}{Q.K_{app,1}} \quad => \quad [AB] = \frac{[A]_0}{Q+1}$$

$$[DB] = K_{app,2}.[D].[B] = K_{app,2}.\frac{[D]_0}{1+\frac{K_{app,2}}{Q.K_{app,1}}}.\frac{1}{Q.K_{app,1}} \quad => \quad [DB] = \frac{K_{app,2}.[D]_0}{K_{app,2}+Q.K_{app,1}}$$

$$\text{Hence, } [B]_0 = [B] + [AB] + [DB] = \frac{1}{Q.K_{app,1}} + \frac{[A]_0}{Q+1} + \frac{K_{app,2}.[D]_0}{K_{app,2}+Q.K_{app,1}}$$

$$[B]_0 - \frac{1}{Q.K_{app,1}} - \frac{[A]_0}{Q+1} = \frac{K_{app,2}.[D]_0}{K_{app,2}+Q.K_{app,1}}$$

=> by setting the first half of the equation to P,

$$P = \frac{K_{app,2}.[D]_0}{K_{app,2}+Q.K_{app,1}}$$

$$\text{Hence, } \frac{[D]_0}{P} = \frac{Q.K_{app,1}}{K_{app,2}} + 1 \qquad \text{(Eq 2)}$$

Consequently, by plotting $\frac{[D]_0}{P}$ as a function of Q, $K_{app,2}$ can be determined.

Preliminary studies performed on PBA-ARS in DMSO/Phosphate buffer

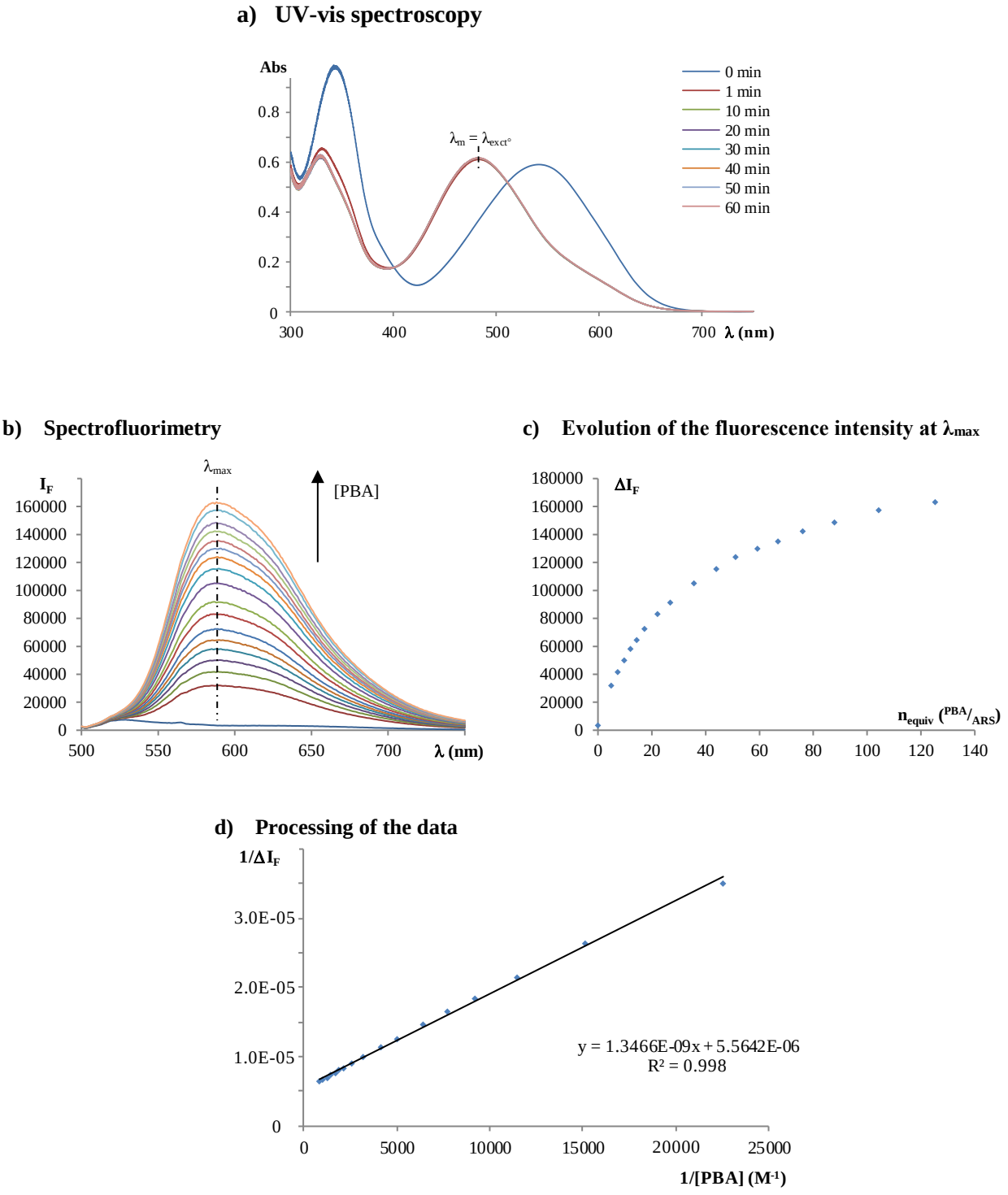


Figure S1. a/ UV-Visible spectroscopy study of the complexation between ARS and PBA, in DMSO/Phosphate Buffer (60/40, v/v %). The complex was obtained in less than 10 min, and the $\lambda_{exct°}$ to be subsequently used for fluorescence measurements was equal to 483 nm; b/ Fluorescence spectroscopy study of the complexation between ARS and PBA, in the DMSO/Phosphate Buffer solvent mixture (60/40, v/v %): illustration of the increase in fluorescence (I_F) upon addition of PBA in the cuvette; c/ Representation of the change in fluorescence of the spectra at λ_{max} = 588 nm; d/ Fitting of the data shown in c) using Eq. 1 (Page S3), in order to extract the $K_{app,1}$ constant ($K_{app.1,ARS/PBA,DMSO/PBS\ (n\,=\,5)}$ = 3977 ± 315 M^{-1}).

Fluorimetric studies of the complexation between 1,4-phenylenediboronic acid (PBB) and diols

PBB-D-fructose complexation in DMSO/phosphate buffer

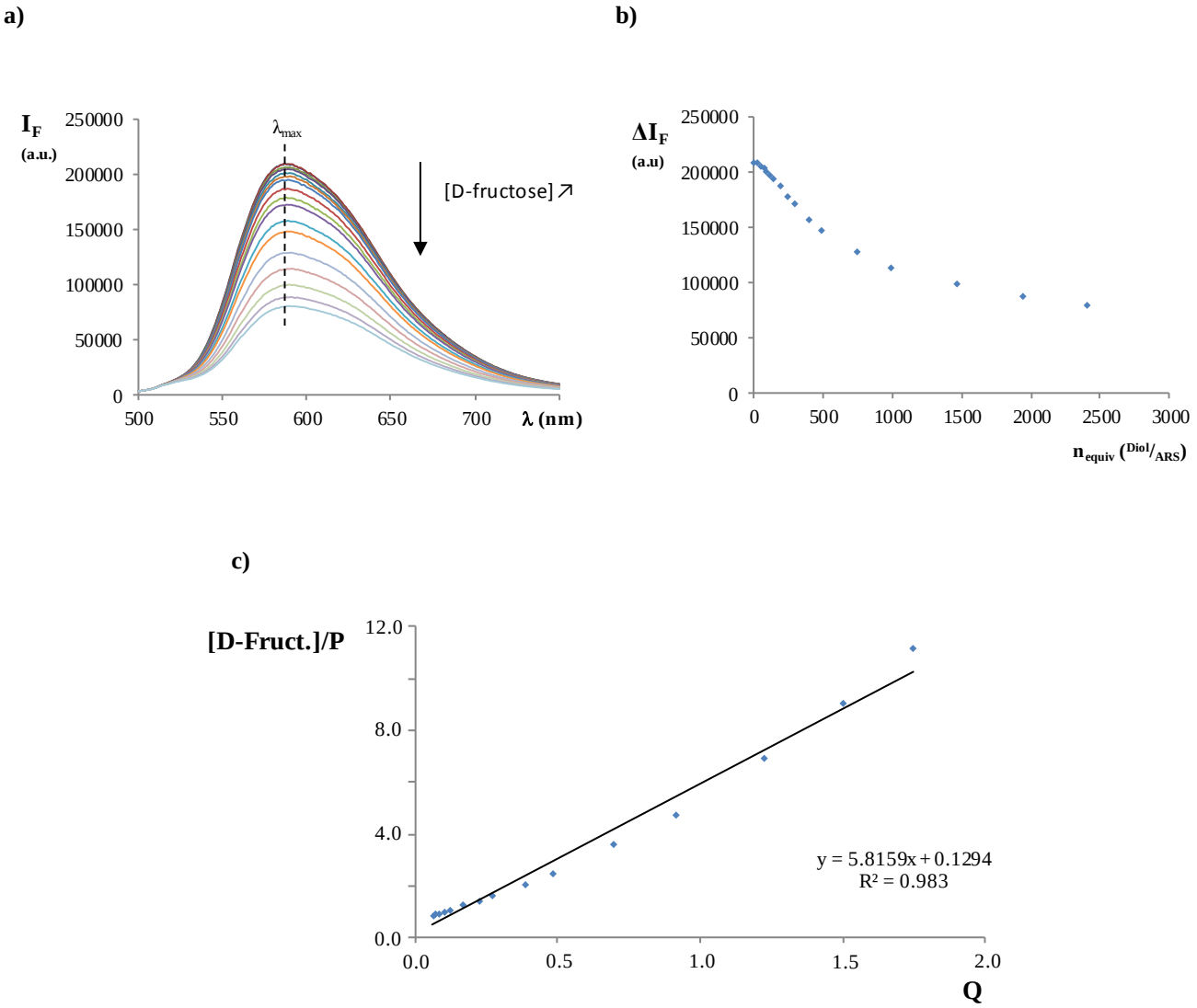
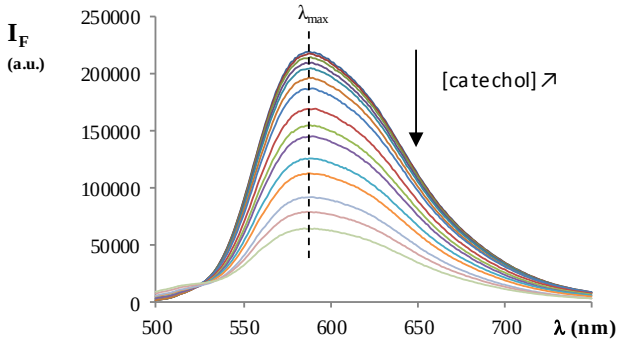


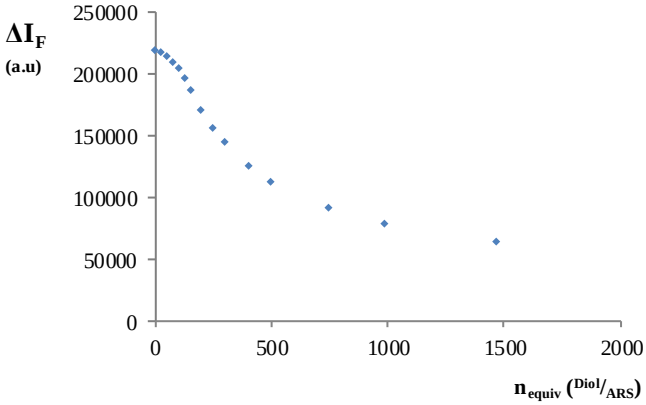
Figure S2. a/ Decrease in fluorescence (I_F) upon addition of D-fructose in the cuvette containing the ARS-PBB complex; b/ Representation of the change in fluorescence at λ_{max} = 586 nm; c/ Fitting of the data using Eq. 2 (page S4); the lack of linearity suggests that these equations are inappropriate for this system.

PBB-catechol complexation in DMSO/phosphate buffer:

a)



b)



c)

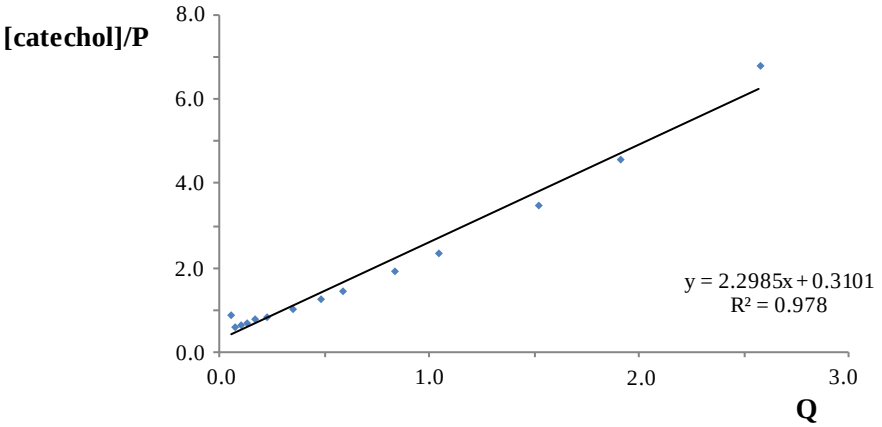


Figure S3. a/ Decrease in fluorescence (I_F) upon addition of catechol in the cuvette containing the ARS-PBB complex; b/ Representation of the change in fluorescence at λ_{max} = 587 nm; c/ Fitting of the data using Eq. 2 (page S4); the lack of linearity suggests that these equations are inappropriate for this system.

Comparison of fluorescence measurements with literature values

Table S3: Apparent complexation constants $K_{app,2}$ determined by fluorimetry between organoboron molecules (PBA, BBzx, PBB and bisPBBzx) and *cis*-diols (D-fructose and catechol). The values are the average of at least three independent measurements. The data reported for the bifunctional molecules (PBB and bisPBBzx) are in italic, because they were derived as a first approximation from fits based on the equations reported in supporting information (page S3), which is unsatisfactory (as shown in Figures S2 and S3 for PBB). For measurements on PBA and BBzx in phosphate buffer, a comparison to values reported in the literature is provided below the table.

	Organoboron molecule	Phosphate buffer[a]	DMSO/ phosphate buffer[b]
D-Fructose	PBA	180 ± 40[c]	1020 ± 100
	BBzx	560 ± 80[d]	4000 ± 200
	PBB	ND[e]	*1940 ± 410*
	bisPBBzx	ND[e]	*2410 ± 570*
Catechol	PBA	900 ± 200[f]	2200 ± 190
	BBzx	1470 ± 20	10660 ± 610
	PBB	ND[e]	*6450± 2500*
	bisPBBzx	ND[e]	*24100 ± 1240*

[a] Aqueous potassium phosphate buffer (pH = 7.4; 40 mM).
[b] DMSO/phosphate buffer mixture (60/40 v/v), prepared using an aqueous potassium phosphate buffer (pH = 7.4 ; 40 mM).
[c] Other values in the literature for PBA vs D-fructose:

- K = 160 M^{-1} (ARS assay, phosphate buffer at pH ~ 7.4 - see Springsteen *et al*, *Tetrahedron*, 2002, **58**, 5291)
- K = 160 M^{-1} (ARS assay, phosphate buffer at pH ~ 7.4 - see Mahalingam *et al*, *Mol. Pharmaceutics* 2011, **8**, 2465)
- K = 210 ± 2 M^{-1} (ITC in phosphate buffer at pH ~ 7.4 – see Schumacher *et al*, *J. Mol. Recogn.* 2011, **24**, 953)
- K = 79 M^{-1} (^{1}H NMR, phosphate buffer at pH ~ 7.4 - see Dowlut *et al*, *JACS* 2006, **128**, 4226)

[d] Other values in the literature for BBzx vs D-fructose:

- K = 664 M^{-1} (ARS assay, phosphate buffer at pH ~ 7.4 - see Mahalingam *et al*, *Mol. Pharmaceutics* 2011, **8**, 2465)
- K = 508 ± 7 M^{-1} (ITC in phosphate buffer at pH ~ 7.4 – see Schumacher *et al*, *J. Mol. Recogn.* 2011, **24**, 953)
- K = 606 M^{-1} (^{1}H NMR, phosphate buffer at pH ~ 7.4 - see Dowlut *et al*, *JACS* 2006, **128**, 4226)

[e] Not determined due to solubility issues.
[f] Other value in the literature for PBA vs catechol:

- K = 830 M^{-1} (ARS assay, phosphate buffer at pH ~ 7.4 - see Springsteen *et al*, *Tetrahedron*, 2002, 58, 5291)

^{1}H NMR study of the complexation between benzoxaborole (BBzx) and catechol

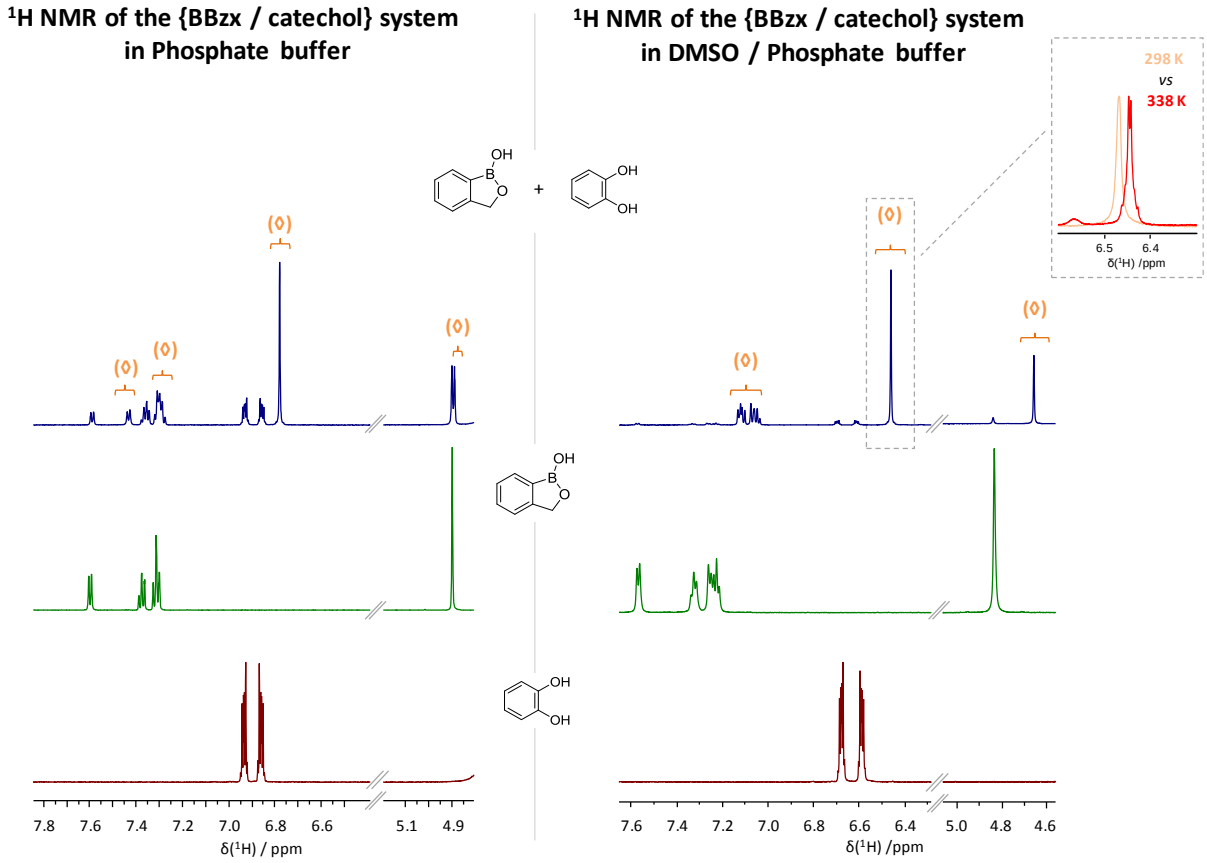


Figure S4. ^{1}H NMR study of the complexation of a 1:1 mixture between benzoxaborole (BBzx) and catechol in deuterated phosphate buffer (left) and the deuterated DMSO/phosphate buffer mixture (right). In the 1:1 complex, the expected inequivalence of the ^{1}H resonances of the catechol protons in the complex is observed when performing ^{1}H measurements at higher temperatures (see insert on the right).

^{1}H NMR study of the complexation between ARS and the bisphenylbenzoxaborole

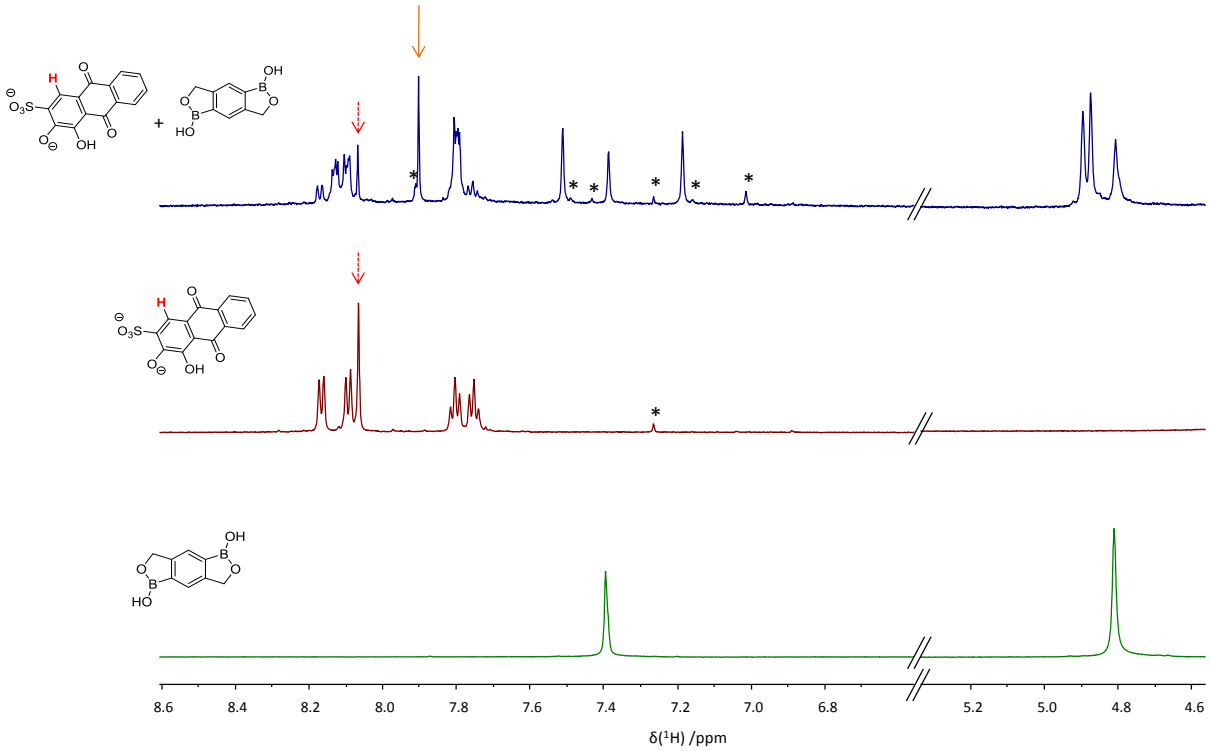


Figure S5. ^{1}H NMR analysis of the complexation between ARS and the bisbenzoxaborole in the DMSO/phosphate buffer mixture. The spectrum in blue corresponds to a ~ 50:50 molar ratio between both molecules. On the spectra of the ARS molecule alone (middle) and of the mixture (top), the ^{1}H resonance of the "red" aromatic proton of ARS is highlighted by an arrow, showing its shift to low frequencies upon formation of the complex. ("*" symbols correspond to yet unidentified minority species).

^{19}F NMR studies of the complexation between organoboron molecules and F-catechol

Complexation between p-fluorobenzeneboronic acid and F-catechol in phosphate buffer

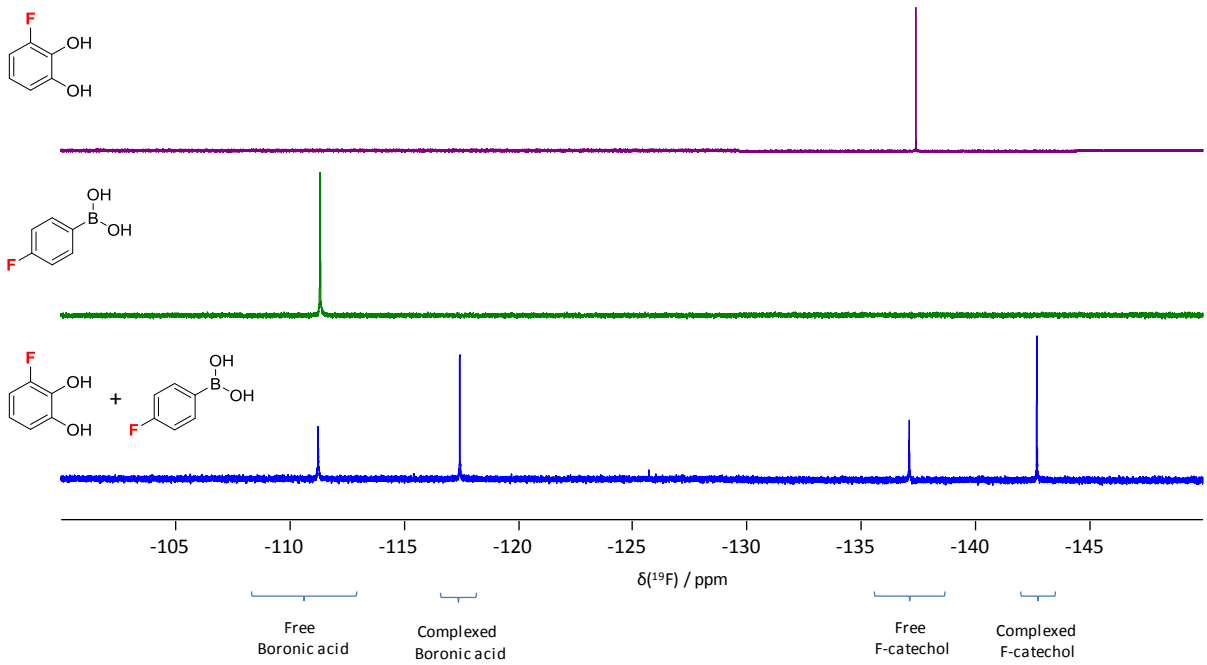


Figure S6. ^{19}F NMR analysis of the complexation between F-catechol and *p*-F-benzeneboronic acid in phosphate buffer (pH 7.4), confirming the shift towards low frequencies of the ^{19}F resonances of the complexed F-catechol.

Complexation between PBB and bisPBBzx and F-catechol in DMSO/phosphate buffer

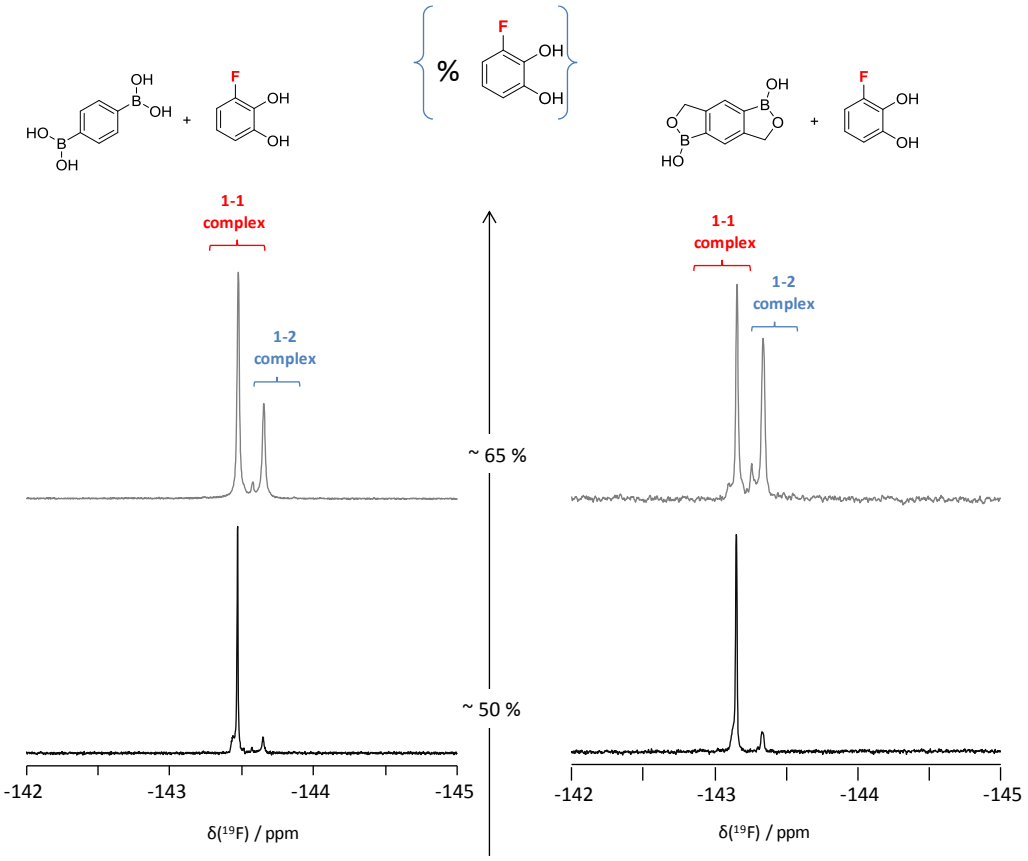


Figure S7. ^{19}F NMR analysis of the complexation between F-catechol and bi-functional organoboron molecules (PBB and bisPBBzx) in the DMSO/phosphate buffer mixture, for different proportions of F-catechol. Several yet-unidentified minority peaks are also present on the spectra.

^{1}H DOSY NMR study of the complexation between bisphenylbenzoxaborole and F-catechol

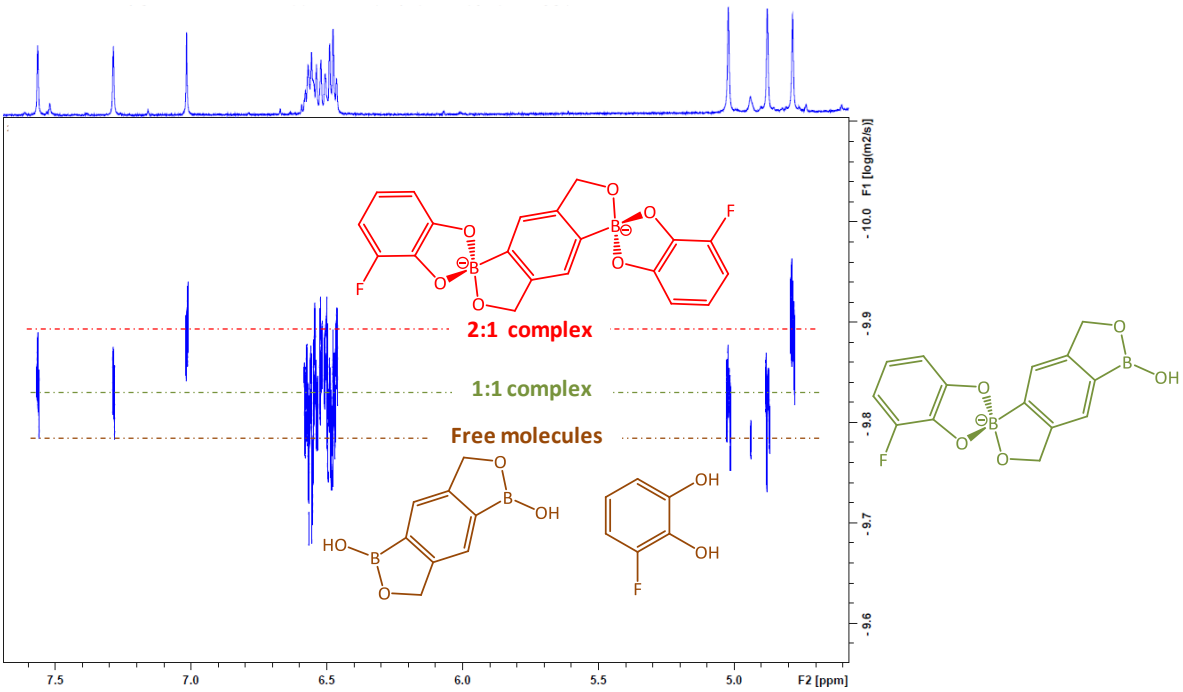


Figure S8. ^{1}H DOSY NMR analysis of the complexation between F-catechol and bisPBBzx in the DMSO/phosphate buffer mixture, highlighting the difference in diffusion properties of the free molecule, 1:1 complex, and 2:1 complex.

Reference

[1] G. Springsteen, B. H. Wang, *Tetrahedron*, 2002, **58**, 5291.