# Molecular Recognition of Mono- and Di-saccharides by Phenylboronic Acids in Solvent Extraction and as a Monolayer

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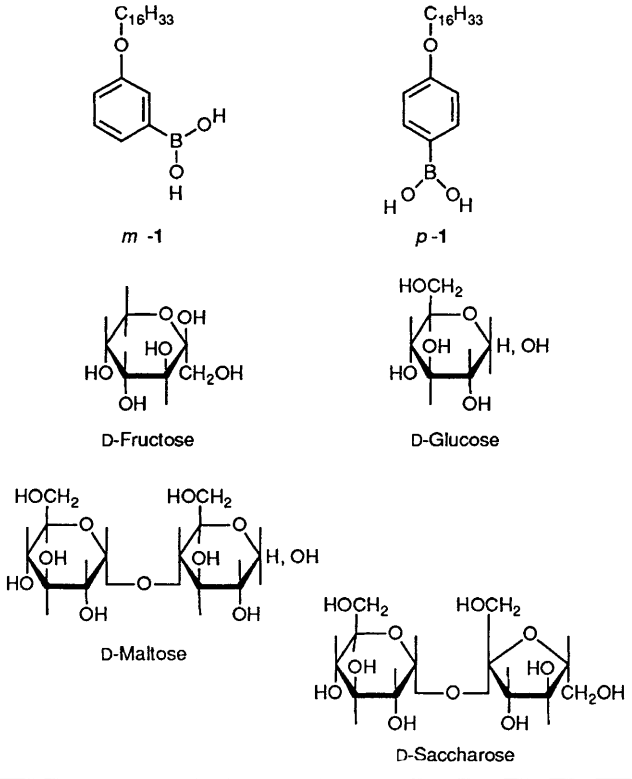
*m*- and *p*-Hexadecyloxyphenylboronic acids (*m*-**1** and *p*-**1**, respectively) selectively extract saccharides; the monolayer of *m*-**1** at the air–water interface selectively responds to these saccharides, the order of the change in $\pi$–*A* isotherms being similar to that of the extractability.



The development of receptor molecules that can precisely recognize and specifically bind guest molecules has been the focus of much recent attention.[1,2] In the design of such artificial receptors hydrogen-bonding interactions play a central role.[3–7] However, more precise molecular recognition may be achieved through the formation of covalent bonds rather than through non-covalent interactions. In fact, Wulff *et al.*[8,9] demonstrated that certain saccharide molecules are precisely recognized by phenylboronic acids immobilized in polymer matrices. In this paper, we report molecular recognition of mono- and di-saccharides by *m*- and *p*-hexadecyloxyphenylboronic acids (*m*-**1** and *p*-**1**, respectively) in solvent extraction and monolayer (at the air–water interface) systems.[10]

The treatment of *m*- and *p*-hexadecyloxybromobenzenes with trimethyl borate in the presence of butyllithium yielded dimethyl esters of *m*- and *p*-hexadecyloxyphenylboronate, respectively. The acid hydrolysis of these products resulted in *m*-**1** and *p*-**1** in 35 and 23% yield, respectively.† The products were identified on the basis of IR and NMR spectral evidence and elemental analysis.† Solvent extraction of saccharides was carried out at 25 °C under three different conditions: solid–liquid ($CDCl_3$) extraction (method *A*), extraction from neutral aqueous solution to the organic phase ($CDCl_3$) (method *B*) and extraction from alkaline aqueous solution to the organic phase ($CDCl_3$) (method *C*). The equilibria were attained after about 6 h. The extractability was determined by measuring the concentration of extracted saccharides in the organic phase with $^1H$ NMR spectroscopy. The results are summarized in Table 1.

Examination of Table 1 reveals that the order of the extractability for four saccharides tested herein is, regardless of the extraction method, D-fructose > D-glucose > D-maltose > D-saccharose. It is known that phenylboronic acid forms a five-membered ring with a *cis*-1,2-diol group.[12] In addition, it can form a six-membered ring with a *trans*-CH(OH)–$CH(CH_2OH)$-diol group although the stability is inferior to that of the five-membered ring.[8,9,11] The order of the




† Compound *m*-**1**: m.p. 81–82 °C; $\nu_{max}$ (KBr) 1240 (ArOC) $cm^{-1}$; $\delta_H$ ($CDCl_3$, 25 °C) 0.95–2.01 (31H, m, $C_{15}H_{31}$), 4.01 (2H, t, $OCH_2$), 7.05–7.90 (4H, m, ArH). The IR spectrum and elemental analysis indicated that the product we recovered by preparative TLC [silica gel, chloroform–hexane (4:1 v/v), $R_f$ = 0.20] is boronic acid anhydride ($C_{16}H_{33}O$-*p*-$C_6H_4$-BO). When treated with aqueous solution, this compound was rapidly hydrolysed to $C_{16}H_{33}O$-*p*-$C_6H_4$-$B(OH)_2$. Compound *p*-**1**: m.p. 78–81 °C; $\nu_{max}$ (KBr) 3100–3600 (OH) and 1240 (ArOC) $cm^{-1}$; $\delta_H$ ($CDCl_3$, 25 °C) 1.10–1.70 (31H, m, $C_{15}H_{31}$), 3.93 (2H, t, $OCH_2$), 6.99 and 7.67 [4H, d each (*J* 9.0 Hz), ArH].

Satisfactory elemental analyses were obtained for *m*-**1** and *p*-**1**.

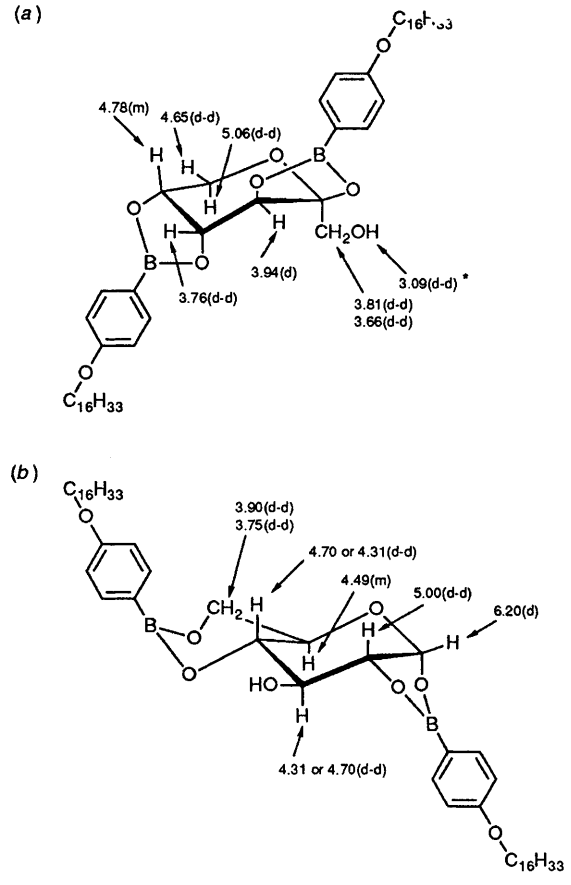



**Fig. 1** Assignment of (*a*) $(p\text{-}1)_2\cdot$ β-D-fructose and (*b*) $(p\text{-}1)_2\cdot$ α-D-glucose complexes. The numbers indicate the chemical shifts (δ in $CDCl_3$ at 25 °C). The splitting patterns are shown in parentheses. The splitting pattern for the OH peak in the $(p\text{-}1)_2\cdot$ β-D-fructose complex was determined in $CDCl_3$–$CD_3CN$ (1:1) solution (δ 3.09: marked with *) because it could not be observed clearly in $CDCl_3$ solution (δ 1.75–1.85) because of overlapping with the $C_{16}H_{33}$ protons.

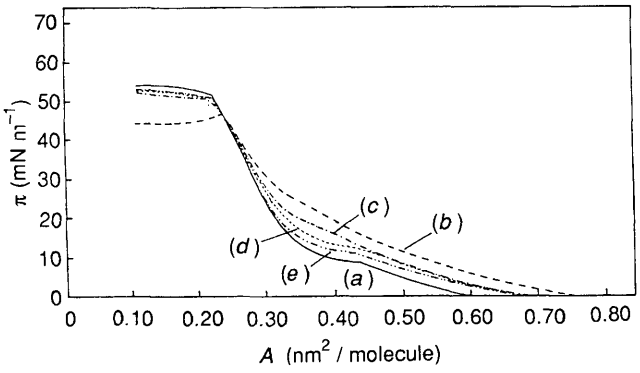



**Fig. 2** Surface pressure–area ($\pi$–$A$) isotherms of monolayer *m*-**1**. The saccharide concentration in the subphase (pH 10.0 with 0.20 mol dm$^{-3}$ carbonate buffer) is 10 mmol dm$^{-3}$. The $\pi$–$A$ curves were obtained at 20 ± 0.1 °C and a compression rate 0.4 mm s$^{-1}$ on a 478 × 150 mm trough with a computer-controlled film balance (San-esu Keisoku Co., model FSD-20). (*a*) None, (*b*) D-fructose, (*c*) D-glucose, (*d*) D-maltose, (*e*) D-saccharose.

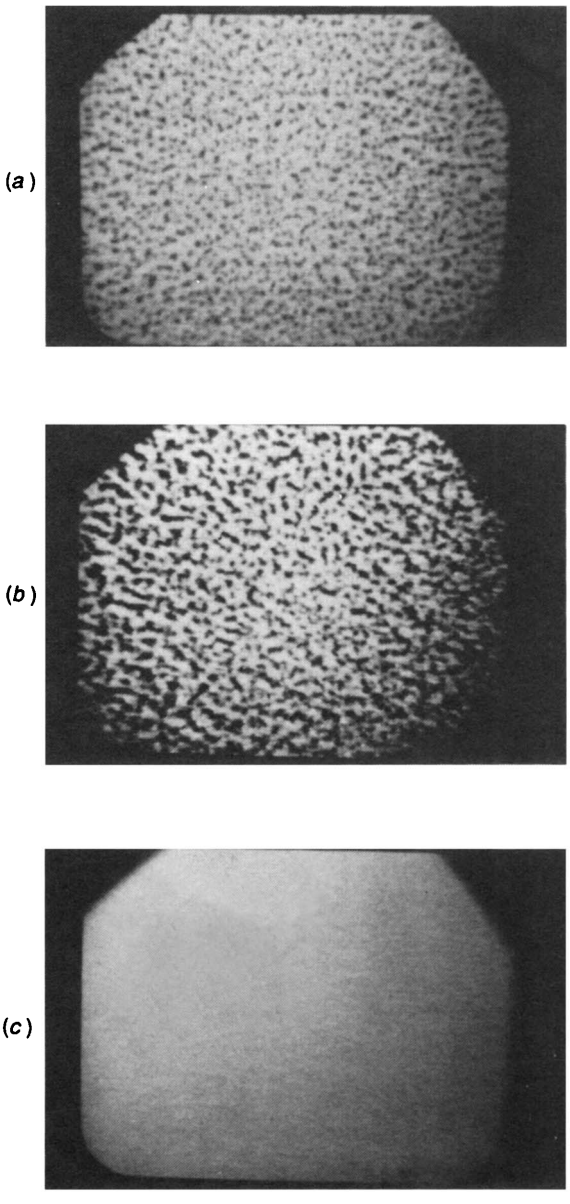



**Fig. 3** Optical microscopic morphologies of *m*-**1** (containing 0.25 mol% of DPPE Rhodamine B) at $A$ = 0.40 nm$^2$: the subphase is (*a*) buffer solution, (*b*) 10 mmol dm$^{-3}$ D-saccharose and (*c*) 10 mmol dm$^{-3}$ D-fructose. The size of the picture is 540 μm (from the left edge to the right edge).

**Table 1** Solvent extraction of saccharides

| Boronic acid | Saccharide | Extractability (%) Method $A^a$ | Method $B^b$ | Method $C^c$ |
|---|---|---|---|---|
| *m*-**1** | D-Fructose | 98[d] | 29[d] | 71[d] |
| | D-Glucose | 1[d] | 0 | 14 |
| | D-Maltose | 0 | 0 | 12 |
| | D-Saccharose | 0 | 0 | 0 |
| *p*-**1** | D-Fructose | 98[d] | 43[d] | 74[d] |
| | D-Glucose | 57[d] | 0 | 20 |
| | D-Maltose | 40[d] | 0 | 2 |
| | D-Saccharose | 0 | 0 | 0 |

[a] Solid–liquid extraction: 0.10 g saccharide in 3 ml $CDCl_3$ containing 1.00 × 10$^{-3}$ mol dm$^{-3}$ **1**. [b] Aqueous phase (5 ml), 0.10 mol dm$^{-3}$ saccharide; organic phase ($CDCl_3$ 2 ml), 1.00 × 10$^{-3}$ mol dm$^{-3}$ **1**. [c] Aqueous phase (5 ml, pH 10.0 with 0.20 mol dm$^{-3}$ carbonate buffer), 0.10 mol dm$^{-3}$ saccharide; organic phase ($CDCl_3$ 2 ml), 1.00 × 10$^{-3}$ mol dm$^{-3}$ **1**. [d] The stoichiometry of extracted species was confirmed to be **1** : saccharide = 2 : 1.

extractability is rationalized on the basis of these ring-formation modes.‡

In $^1$H NMR measurements of the $CDCl_3$ solution, the chemical shifts of the aromatic protons in complexed *m*-**1** and *p*-**1** are different from those of 'free' *m*-**1** and *p*-**1** (for the aromatic protons in *p*-**1**, for example, δ 6.99 and 7.67 for free *p*-**1** and δ 6.90, 6.93, 7.74 and 7.79 for the *p*-**1**·D-fructose complex). One can thus estimate the stoichiometry for the complexes: they always showed **1** : saccharide = 2 : 1. To obtain further insights into the complexation mode, we examined the $^1$H NMR spectrum of *p*-**1**·D-fructose and *p*-**1**·D-glucose complexes in detail. By using the decoupling method, Karplus rule and two-dimensional (COSY) method,[12] we could assign all peaks as shown in Fig. 1. Clearly, *p*-**1** forms 2 : 1 complexes with β-D-fructose and α-D-glucose. The $J_{HH}$ between 1-H and 2-H in the *p*-**1**·D-glucose complex was 4.0 Hz. This value is in line with the *gauche* conformation required for α-D-glucose.

Subsequently, we tested if *m*-**1** and *p*-**1** form stable monolayers at the air–water interface and if they selectively respond to saccharides dissolved in the aqueous phase. Compound *p*-**1** did not form a stable monolayer on water (pH 6.1–11.3). This was confirmed by (*i*) the lack of the reproducibility, (*ii*) the production of layered white crystals at the interface and (*iii*) the formation of the huge crystal phase [detected through the observation of a *p*-**1**-DPPE Rhodamine B§ (0.25 mol%) mixed system with an optical microscope]. In contrast, *m*-**1** gave a stable monolayer (Fig. 2). The pressure–area ($\pi$–$A$) isotherm was reproducible and the monolayer (containing 0.25 mol% of DPPE Rhodamine B: the $\pi$–$A$ isotherm was not affected by the addition of DPPE Rhodamine B) was observed as a well-dispersed island structure [Fig. 3(*a*)]. The $\pi$–$A$ isotherm was affected by the addition of

‡ The $^1$H NMR measurements established that D-fructose and D-glucose are extracted by *p*-**1** as β-anomer and α-anomer, respectively (see Fig. 1).

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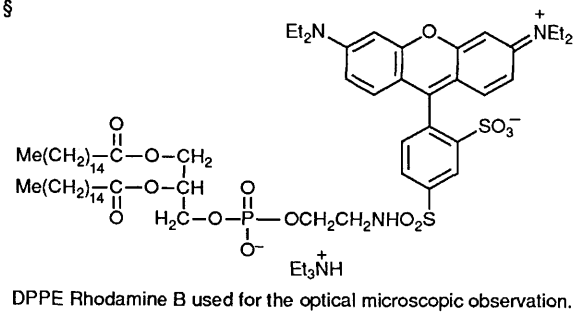


DPPE Rhodamine B used for the optical microscopic observation.

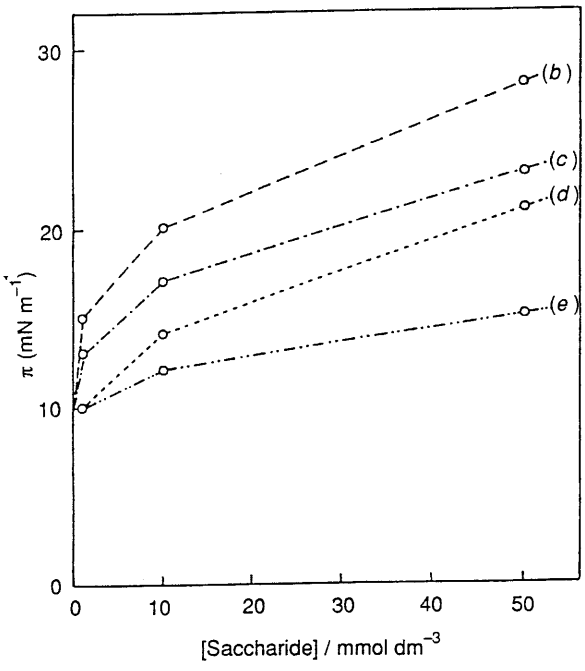


**Fig. 4** Dependence of π at $A$ = 0.40 nm² on the saccharide concentration (*b–e* as in Fig. 2)

saccharides in the subphase. As shown in Fig. 2, a plateau at around $A$ 0.40 nm² becomes less apparent. Thus, we plotted π at $A$ 0.40 nm² against the saccharide concentration (Fig. 4). It is seen from Fig. 4 that the sensitivity of π to saccharide concentration is exactly equal to that of the extractability. The optical microscopic observation indicated that the structure of the monolayer is scarcely affected in the presence of D-saccharose [Fig. 3(*b*)], whereas in the presence of D-fructose the domain structure of *m*-**1** disappears and a homogeneous, fluorescent monolayer is formed [Fig. 3(*c*)]. These observations establish that D-saccharose is hardly bound to *m*-**1** while D-fructose is covalently bound to *m*-**1** and retards crystallization of the monolayer.

Although mechanistic differences may exist between the solvent extraction and the monolayer behaviour, both of these results suggest that the present system acts as a new sensory system for sugar molecules.[13]

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