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

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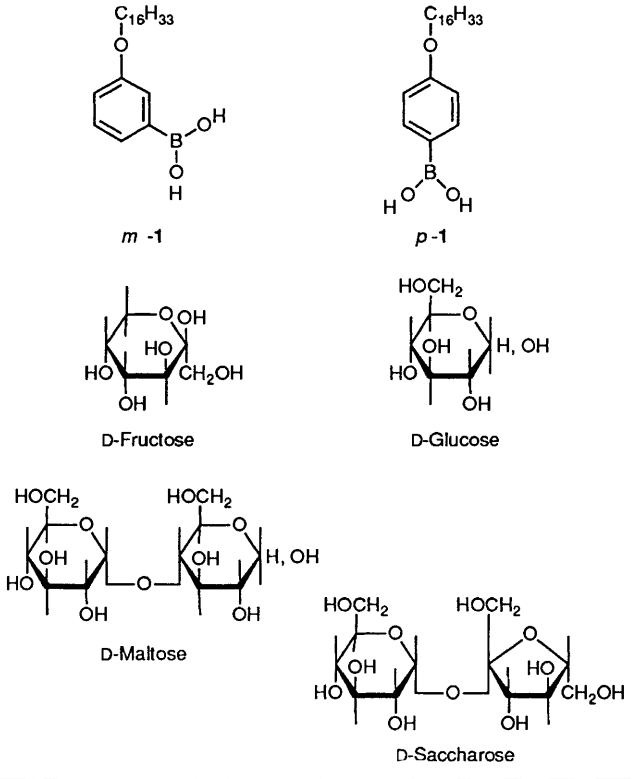
m- and *p*-Hexadecyloxyphenylboronic acids (*m*-**1** and *p*-**1**, respectively) selectively extract saccharides; the monolayer of *m*-**1** at the air–water interface selectively responds to these saccharides, the order of the change in π–*A* isotherms being similar to that of the extractability.

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



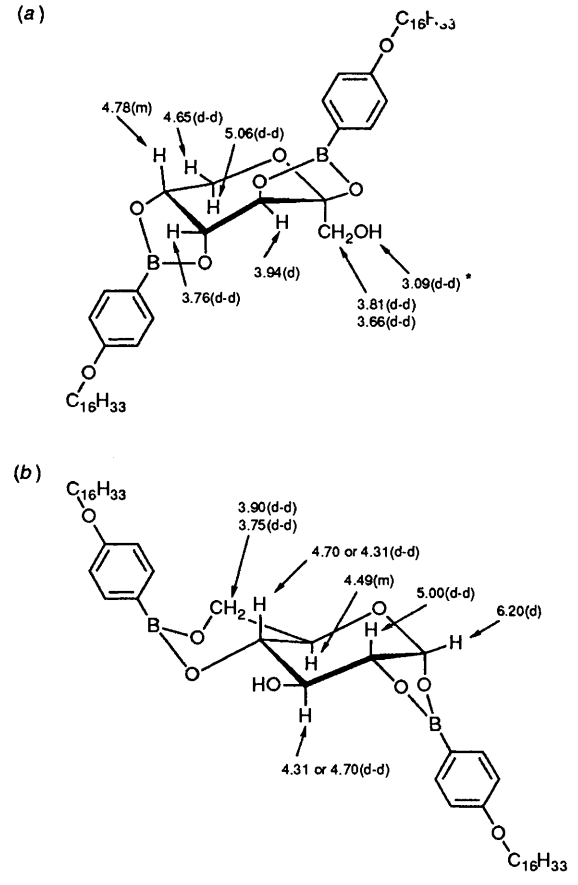


Fig. 1 Assignment of (*a*) (*p*-**1**)$_2$·β-D-fructose and (*b*) (*p*-**1**)$_2$· α-D-glucose complexes. The numbers indicate the chemical shifts (δ in $CDCl_3$ at 25 °C). The splitting patterns are shown in parentheses. The splitting pattern for the OH peak in the (*p*-**1**)$_2$·β-D-fructose complex 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.

† Compound *m*-**1**: m.p. 81–82 °C; ν_{max} (KBr) 1240 (ArOC) cm^{-1}; δ_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; ν_{max} (KBr) 3100–3600 (OH) and 1240 (ArOC) cm^{-1}; δ_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**.

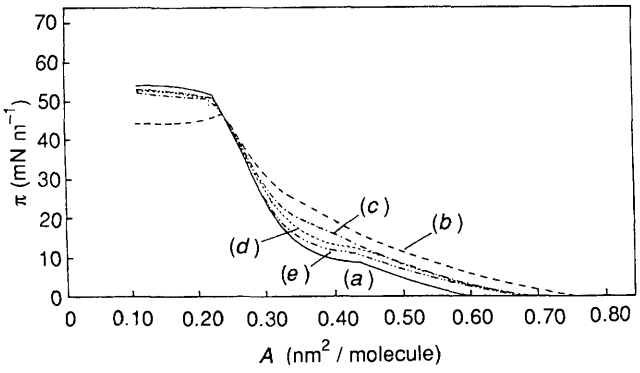


Fig. 2 Surface pressure–area (π–A) isotherms of monolayer *m*-**1**. The saccharide concentration in the subphase (pH 10.0 with 0.20 mol dm^{-3} carbonate buffer) is 10 mmol dm^{-3}. The π–A curves were obtained at 20 ± 0.1 °C and a compression rate 0.4 mm s^{-1} on a 478 × 150 mm trough with a computer-controlled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

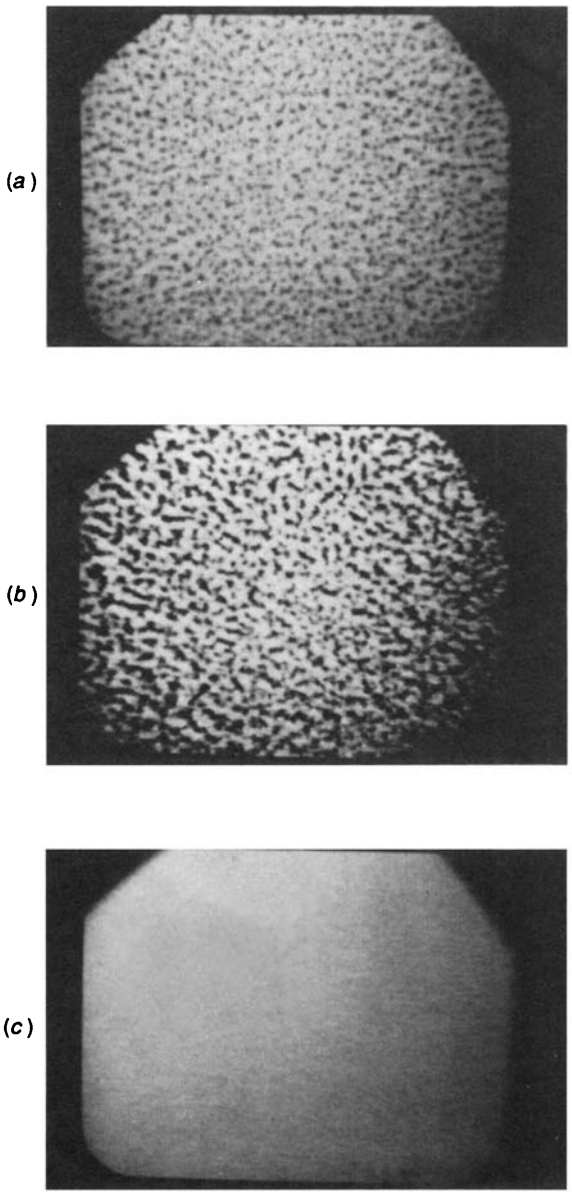


Fig. 3 Optical microscopic morphologies of *m*-**1** (containing 0.25 mol% of DPPE Rhodamine B) at A = 0.40 nm^2: the subphase is (*a*) buffer solution, (*b*) 10 mmol dm^{-3} D-saccharose and (*c*) 10 mmol dm^{-3} D-fructose. The size of the picture is 540 μm (from the left edge to the right edge).

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 ^1H 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 ^1H 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 (π–A) isotherm was reproducible and the monolayer (containing 0.25 mol% of DPPE Rhodamine B: the π–A isotherm was not affected by the addition of DPPE Rhodamine B) was observed as a well-dispersed island structure [Fig. 3(*a*)]. The π–A isotherm was affected by the addition of

‡ The ^1H 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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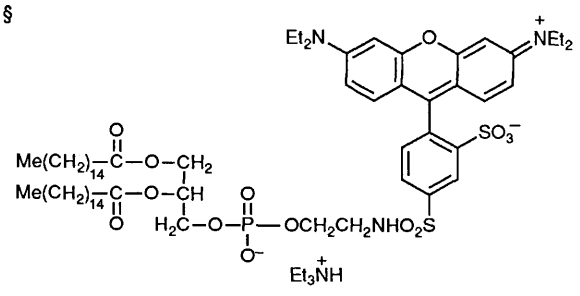

DPPE Rhodamine B used for the optical microscopic observation.

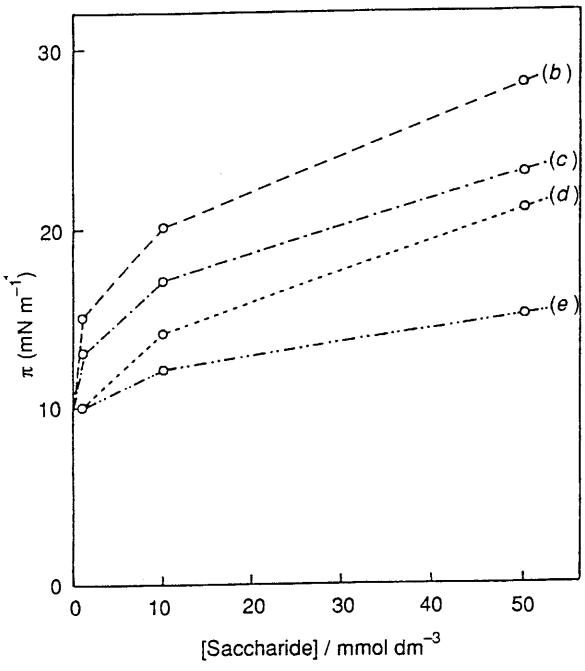

Fig. 4 Dependence of π at A = 0.40 nm² on the saccharide concentration (*b–e* as in Fig. 2)

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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