

Optical detection of complexation of labeled saccharides to macrocyclic synthetic receptors

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Abstract

The interaction of optically labeled saccharides with resorcin[4]arenes as synthetic saccharide receptors has been investigated by determination of the partition of the saccharides over an aqueous and 1-octanol phase in the absence and presence of the synthetic receptor. It is shown that the complexation of merocyanine labeled glucose, galactose and ribose with the resorcin[4]arenes can be optically detected and that the chosen receptors have a modest selectivity towards the labeled glucose.

Keywords: Optical detection of complexation; Saccharides; Macrocyclic synthetic receptors

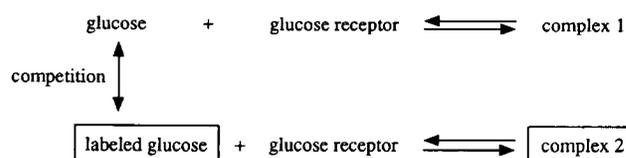
1. Introduction

One of the objectives in supramolecular chemistry is to apply selective complexation of analytes in solution for sensors. For example, derivatized calix[4]arenes have been incorporated as selective ion receptors in chemically modified field effect transistors (CHEM-FETs) to determine the concentration of sodium [1], potassium [2], silver, copper, cadmium, and lead ions [3]. Although it is possible to measure the complexation of charged species like cations by their charge, it is more difficult to determine the complexation of neutral species, like glucose. The detection of this compound is of large interest for the treatment of diabetes [4] and the control of fermentation processes [5]. As glucose contains no charge, nor does it show absorbance or fluorescence, the direct detection of glucose is simply not possible. The usual detection methods are based on the glucose oxidase catalyzed oxidation of glucose in which the consumption of oxygen or the production of hydrogen peroxide is measured amperometrically [4,5]. Detection of glucose without the need of using enzymes that show denaturation and chemical conversions, would be of advantage for the development of a durable sensor. Therefore we are currently investigating the possibilities of using competitive binding between glucose and la-

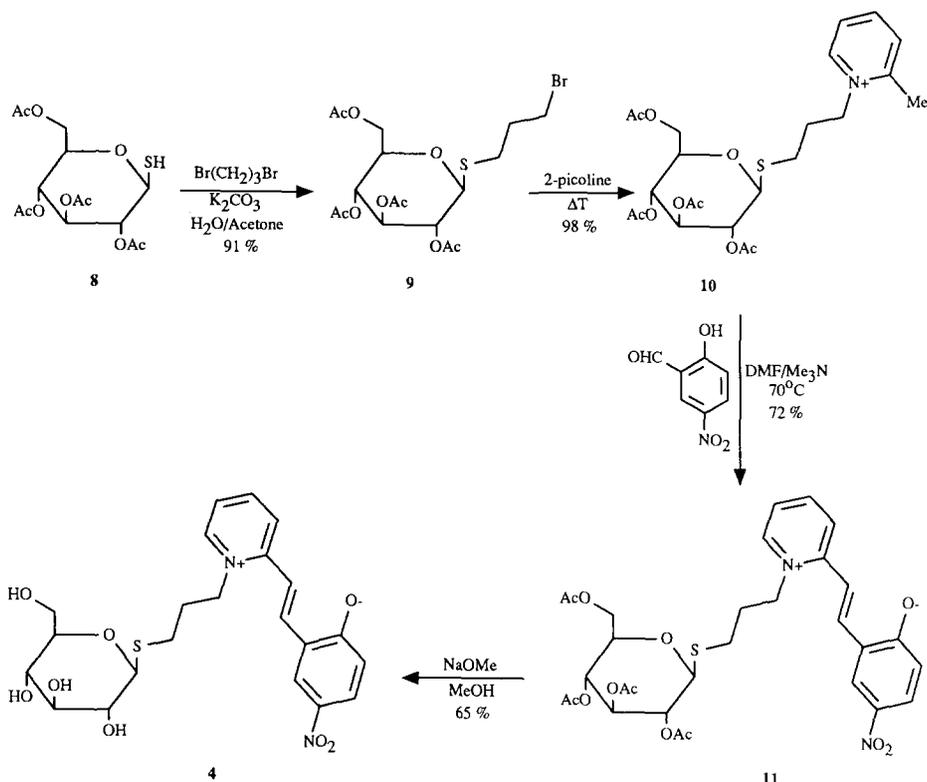
beled glucose derivatives to a common synthetic receptor molecule for the optical detection of glucose. The principle of the competitive binding is depicted in Scheme 1. The concentration of glucose in the analyte solution influences both the concentration of labeled glucose and that of complex 2. In principle, both species can be optically detected.

As synthetic receptor for glucose, derivatives of resorcin[4]arenes have been investigated. Resorcin[4]arenes are cyclic tetramers of the condensation reaction between resorcin and aliphatic or aromatic aldehydes. As was shown in the excellent work of Aoyama et al. with NMR spectroscopy, these compounds show (selective) interaction with various saccharides [6–8].

In order to investigate the use of the resorcin[4]arenes for the optical detection of glucose, we have determined the association and selectivity of resor-



Scheme 1. The competition principle for the detection of glucose.



Scheme 2. Synthesis of merocyanine labeled glucose 4.

cin[4]arenes **1** with the newly synthesized merocyanine labeled glucose, galactose and ribose derivatives **4**, **5** and **6** by UV-Vis spectroscopy. This has been performed by measuring the partition of the labeled saccharides over an aqueous and 1-octanol phase in the absence and presence of **1**. The contribution of the saccharide moiety could be evaluated by comparison of the association constants of the labeled saccharides with that of **7**, lacking the sugar moiety. Resorcin[4]arene **1** contains eight hydroxyl groups on one face for complexation and four decenyl hydrocarbon tails which makes this com-

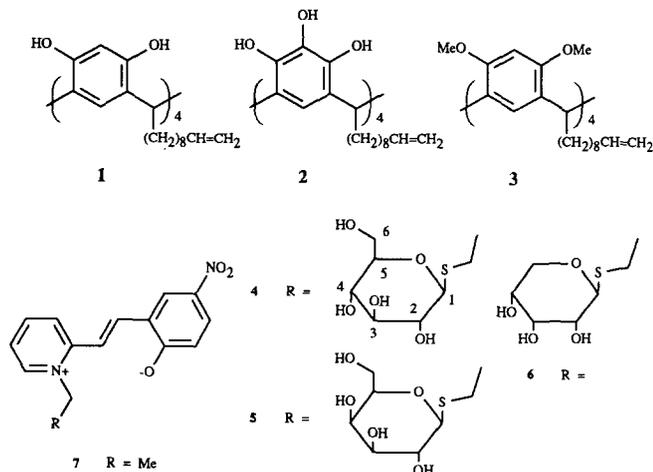
pound sufficiently lipophilic to dissolve completely in the 1-octanol phase. The terminal alkene group offers the possibility for eventual immobilization of the receptor [9]. By comparison of the association behavior of **1** with that of **2** and **3** the specific role of the hydroxyl groups could be further evaluated.

2. Experimental

2.1. Chemicals

The synthesis of resorcin[4]arenes **1**, **2** and **3** has been reported elsewhere [9]. 4-Hexylresorcinol which was used as a reference compound was purchased from Janssen Chimica. The merocyanine labeled saccharides were synthesized according to the route depicted for the glucose derivative **4** in Scheme 2.

Glucose derivative **9** was synthesized according to literature procedures [10] using a large excess of the bifunctional spacer in the coupling reaction to glucose derivative **8**. The deacetylation of **11** was performed according to Deferrari et al. [11] without using the Dowex-50 W resin because the reaction product precipitated after the deacetylation. The merocyanine labeled galactose **5** and labeled ribose derivatives **6** were synthesized similarly. Merocyanine **7** was used as a reference compound. All synthesized compounds showed elemen-



tal analysis data, ^1H NMR, ^{13}C NMR, IR and mass spectra consistent with their structures.

2.2. Measurements

The partition of the merocyanine labeled saccharides 4–6 and the reference merocyanine 7 over the water–1-octanol system was determined by preparing 10 solutions of 4 ml of the appropriate compound in the concentration range $(0.10\text{--}1.00) \times 10^{-3}$ M, from a 10^{-3} M stock solution, and stirring these solutions with 4 ml of 1-octanol for 24 h at 25 °C. After the stirring of this two-phase system, 2 ml of the 1-octanol layer were separated and the UV–Vis spectrum was recorded with a Hewlett-Packard 8452A diode array spectrophotometer. From the absorbance at 402 nm the concentrations of the labeled saccharides or the merocyanine in the 1-octanol solution were calculated using the extinction coefficient of each compound in water-saturated 1-octanol solutions determined separately from 24 absorbance measurements in the range 7.30×10^{-6} to 1.10×10^{-4} M.

Similarly, the partition of the labeled saccharides 4–6 and reference merocyanine 7 in the presence of 10^{-4} M resorcin[4]arenes 1–3 in the 1-octanol solution was measured. From the difference in partition of the labeled saccharides in the absence and presence of saccharide receptor in the 1-octanol phase the association constants were determined according to Eq. (3). Slopes and intercepts were determined with the least-squares method.

3. Results and discussion

The selectivities of the resorcin[4]arene receptors 1, 2, 3 were studied by determining the association constant (K_{ass}) of complex formation between the merocyanine labeled saccharides and the receptor molecules in 1-octanol solution. The association constants were determined from extraction experiments, in which the labeled saccharide was extracted from an aqueous source phase into an organic receiving phase containing the resorcin[4]arene. The amount of labeled saccharide extracted into the 1-octanol phase was monitored by measuring the UV–Vis spectrum of the organic layer and the amount is determined by contributions from complexation of the resorcin[4]arene and partition of the merocyanine labeled saccharide over the aqueous phase and the organic phase. The partitions over the aqueous phase and the 1-octanol phase in the absence of receptor were determined in separate extraction experiments. These partition coefficients P together with the extinction coefficients ϵ of labeled saccharides 4–6 and merocyanine 7 in water-saturated 1-octanol are given in Table 1.

As can be seen from Table 1, the partition of labeled ribose 6 to the 1-octanol phase is about 2.5 times larger

Table 1
1-Octanol/water partition coefficients (P) and extinction coefficients (ϵ) in water-saturated 1-octanol of labeled saccharides 4–6 and merocyanine 7 at 25 °C

	4	5	6	7
P	0.0540	0.0494	0.136	0.102
ϵ^a	14.6	9.4	12.5	5.6

^a ϵ determined at 402 nm; ϵ in $10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

than that of labeled glucose 4 and galactose 5. This may be attributed to the extra hydroxyl group at the C_6 position of labeled saccharides 4 and 5 giving these compounds a higher hydrophilicity. Remarkably, the extinction coefficients of the merocyanine derivatives in the 1-octanol phase are distinctly different, although the chromophoric unit is the same in all compounds. The extinction coefficient of merocyanine 7 is considerably lower than that of the labeled saccharides. Apparently, microenvironmental effects due to interaction of the merocyanine with the sugar moiety play a dominant role in the transition dipole moment, as can also be seen from the difference in extinction coefficient of labeled glucose 4 with the hydroxyl group at C_4 in the equatorial position and galactose 5 with the hydroxyl group in the axial position.

The partition of labeled glucose 4 into the organic phase in the absence (A) and presence (B) of resorcin[4]arene 2 is shown in Fig. 1. From line B in Fig. 1 the complexation effect of the resorcin[4]arene in the organic phase is clearly demonstrated. The strong increase of the absorbance in the lower concentration range of 4 levels off at higher concentrations. This is caused by the saturated complexation of resorcin[4]arene 2. Assuming 1:1 complexation, the association constant K_{ass} is given by Eq. (1).

$$K_{\text{ass}} = [\text{C}]/[\text{L}] \times [\text{R}] \quad (1)$$

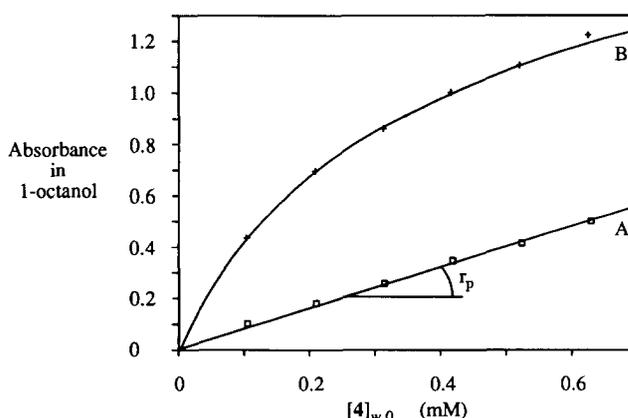


Fig. 1. Partition (line A) and extraction (line B) of labeled glucose 4 by resorcin[4]arene 2 at 25 °C. Absorbance recorded at 402 nm, $[2] = 9.72 \times 10^{-5}$ M.

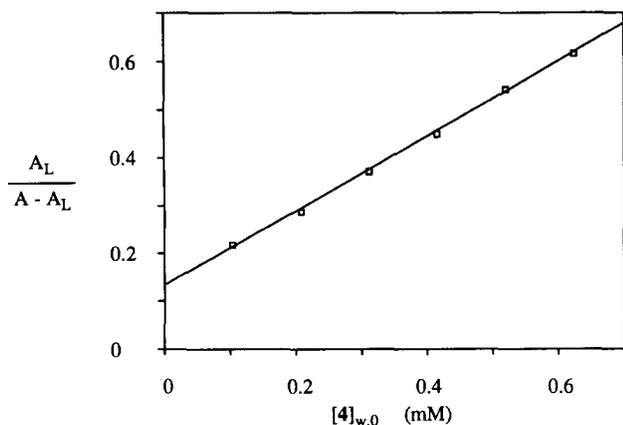


Fig. 2. Plot of $A_L/A - A_L$ vs. $[L]_{w,0}$. Change in absorbance due to the extraction of labeled saccharide **4** by resorcin[4]arene **2**. $[2] = 9.72 \times 10^{-5}$ M. $T = 25^\circ\text{C}$.

In this equation, $[C]$ is the concentration of the complex, $[L]$ is the concentration of the labeled saccharide, and $[R]$ the concentration of free resorcin[4]arene. The absorbance A of the organic phase is built up by a contribution from the partition of the free labeled compound in the organic phase (A_L) and a contribution from the labeled compound within the resorcin[4]arene complex (A_C).

$$A = A_L + A_C \quad (2)$$

Starting from these equations, a relation between the measured absorbance and the association constant can be derived.

$$\frac{A_L}{A - A_L} = \frac{r_p}{\epsilon_C [R]_{w,0}} [L]_{w,0} + \frac{\epsilon_L}{\epsilon_C K_{\text{ass}} [R]_{o,0}} \quad (3)$$

In this equation, r_p is the relation between the absorbance and the partition of the labeled saccharide given as the slope of line A in Fig. 1, ϵ_L is the extinction coefficient of the merocyanine labeled saccharide in the water saturated 1-octanol phase and $[L]_{w,0}$ and $[R]_{o,0}$ are the initial concentrations of labeled saccharide and receptor in the water and 1-octanol phase, respectively. A plot of $A_L/A - A_L$ versus $[L]_{w,0}$ gives a linear relation from which the extinction coefficient of the complex ϵ_C can be derived from the slope of Eq. (3) and the intercept with the Y-axis gives the association constant K_{ass} . In Fig. 2 the change in absorbance due to extraction of labeled saccharide **4** by resorcin[4]arene **2** is given according to Eq. (3).

The results of the extraction of merocyanine labeled saccharides **4–6** and reference merocyanine **7** are given in Table 2. Also the free energy of complex formation has been calculated in order to illustrate the contribution of saccharide and merocyanine residues towards the complexation.

From Table 2 it can be concluded that the labeled saccharides **4–6** and merocyanine **7** are bound by the

resorcin[4]arenes **1** and **2**. Extraction experiments with labeled glucose **4** and resorcin[4]arene **3**, where the hydroxyl groups of the resorcin[4]arene are replaced by methoxy groups, showed no complexation. This implies that the hydroxyl groups of the resorcin[4]arene are necessary for binding of the labeled saccharides. Extraction of **4** in 1-octanol solution containing four equivalents of 4-hexylresorcinol (with respect to the resorcin[4]arene) also showed no complexation, indicating that the cyclic geometry of the resorcin[4]arene is necessary for complexation of labeled saccharides.

The difference in ΔG_{ass} for labeled glucose **4** and merocyanine **7** is 6.2 kJ mol^{-1} for complexation with resorcin[4]arene **1**. This clearly indicates that there is a contribution of the saccharide residue in the binding of labeled glucose **4**. The ΔG_{ass} of labeled galactose **5** and ribose **6** are of the same order as that of merocyanine **7**. From these results it can be concluded that resorcin[4]arene **1** has selectivity for glucose among the labeled saccharides used and that the orientation of hydroxyl groups at the C_3 , C_4 and C_6 positions of the saccharides is important for complexation with **1**.

If resorcin[4]arene **2** is used as saccharide receptor some important differences could be observed. This receptor has four additional hydroxyl groups at the binding site (one from each aromatic unit) and therefore the number of possible hydrogen bonds to the saccharides is increased. This is reflected in higher association constants for all species. However, due to the decrease of orientational restrictions of the saccharides in the complex, the overall effect is a decrease of the selectivity of this receptor.

In conclusion, it has been shown that resorcin[4]arenes are useful candidates as synthetic receptors in the optical detection of glucose derivatives. The macrocyclic geometry of the resorcin[4]arenes in which the hydroxyl groups are oriented to one face is responsible for the complexation of merocyanine labeled saccharides. The complexation of the merocyanine labeled saccharides is caused by the formation of hydrogen bonds between the resorcin[4]arene and the labeled saccharide. The difference in ΔG_{ass} between merocya-

Table 2
Extinction coefficients, K_{ass} and ΔG_{ass} of complex formation between labeled saccharides **4–6**, merocyanine **7** and resorcin[4]arenes **1** and **2** ($T = 25^\circ\text{C}$)

		4	5	6	7
1	ϵ_c	5.3	12.3	23.7	7.5
	K_{ass}	89.6	11.0	11.3	7.6
	ΔG_{ass}	-28.3	-23.1	-23.1	-22.1
2	ϵ_c	9.1	11.2	18.7	8.3
	K_{ass}	118.6	88.0	44.0	13.3
	ΔG_{ass}	-28.9	-28.2	-26.5	-23.5

ϵ_c in $10^3 \text{ M}^{-1} \text{ cm}^{-1}$; K_{ass} in 10^3 M ; ΔG_{ass} in kJ mol^{-1} .

nine labeled glucose **4** and reference merocyanine **7** with resorcin[4]arenes **1** and **2** shows that the saccharide contributes towards the binding of the labeled saccharide. Resorcin[4]arene **1** shows selectivity towards the merocyanine labeled glucose. By introducing additional hydroxyl groups at the binding site of the resorcin[4]arene (**2**), the selectivity for labeled saccharides is decreased. Synthetic saccharide receptor **1** will be further investigated for its possibility to be applied in optical glucose sensing.

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References

- [1] J.A.J. Brunink, J.G. Bomer, J.F.J. Engbersen, W. Verboom and D.N. Reinhoudt, Effects of ionic sites on the selectivity of sodium-sensitive CHEMFETs, *Sensors and Actuators B*, 15/16 (1993) 195–198.
- [2] E.J.R. Sudhölter, P.D. van der Wal, M. Skrowronska-Ptasinska, A. van den Berg, P. Bergveld and D.N. Reinhoudt, Transduction of host-guest complexation into electronic signals: favoured complexation of potassium ions by synthetic macrocyclic polyethers using membrane-modified, ion-sensitive field-effect transistors (ISFETs), *Recl. Trav. Chim. Pays-Bas*, 109 (1990) 222–225.
- [3] P.L.H.M. Cobben, R.J.M. Egberink, J.G. Bomer, P. Bergveld, W. Verboom and D.N. Reinhoudt, Transduction of selective recognition of heavy metal ions by chemically modified field effect transistors (CHEMFETs), *J. Am. Chem. Soc.*, 114 (1992) 10573–10582.
- [4] F.J.B. Kremer, J.F.J. Engbersen, J. Kruijs, P. Bergveld, D.A.J. Starmans, J. Feijen and D.N. Reinhoudt, Immobilization and activity of Concanavalin A on tantalum pentoxide and silicon dioxide surfaces, *Sensors and Actuators B*, 13/14 (1993) 176–179.
- [5] J. Rishpon, Y. Shabtai, I. Rosen, Y. Zibenberg, R. Tor and A. Freeman, In situ glucose monitoring in fermentation broth by 'sandwiched' glucose-oxidase electrode (sge), *Biotech. Bioeng.*, 35 (1990) 103–107.
- [6] Y. Aoyama, Y. Tanaka and S. Sugahara, Molecular recognition. 5. Molecular recognition of sugars via hydrogen-bonding interaction with a synthetic polyhydroxy macrocycle, *J. Am. Chem. Soc.*, 111 (1989) 5397–5404.
- [7] K. Kurihara, K. Ohto, Y. Tanaka, Y. Aoyama and T. Kunitake, Molecular recognition of sugars by monolayers of resorcinol-dodecanal cyclotetramer, *J. Am. Chem. Soc.*, 113 (1991) 444–450.
- [8] Y. Kikuchi, Y. Tanaka, K. Sutarto, K. Kobayashi, H. Toi and Y. Aoyama, Highly cooperative binding of alkyl glucopyranosides to the resorcinol cyclic tetramer due to intracomplex guest-guest hydrogen-bonding: solvophobicity/solvophilicity control by an alkyl group of the geometry, stoichiometry, stereoselectivity, and cooperativity, *J. Am. Chem. Soc.*, 114 (1992) 10302–10306.
- [9] E.U. Thoden van Velzen, J.F.J. Engbersen and D.N. Reinhoudt, Self-assembled monolayers of receptor adsorbates on gold: preparation and characterization, *J. Am. Chem. Soc.*, 116 (1994) 3597–3598.
- [10] M. Černý, J. Stanek and J. Pacák, 2,3,4,6-Tetra-O-acetyl- β -D-galaktopyranosylmercaptan und dessen Anwendung zur Synthese von β -D-Thiogalaktosiden, *Monatsh. Chem.*, 94 (H1) (1963) 290–294.
- [11] J.O. Deferrari, E.G. Gros and I.O. Mastronardi, Methylation of carbohydrates bearing base-labile substituents, with diazomethane-boron trifluoride etherate II. A new synthesis of 2O-methyl-D-mannose, *Carbohydr. Res.*, 4 (1967) 432–434.

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