

OPTICAL DETECTION OF GLUCOSE BINDING TO CONCAVALIN A AND MACROCYCLIC RECEPTORS

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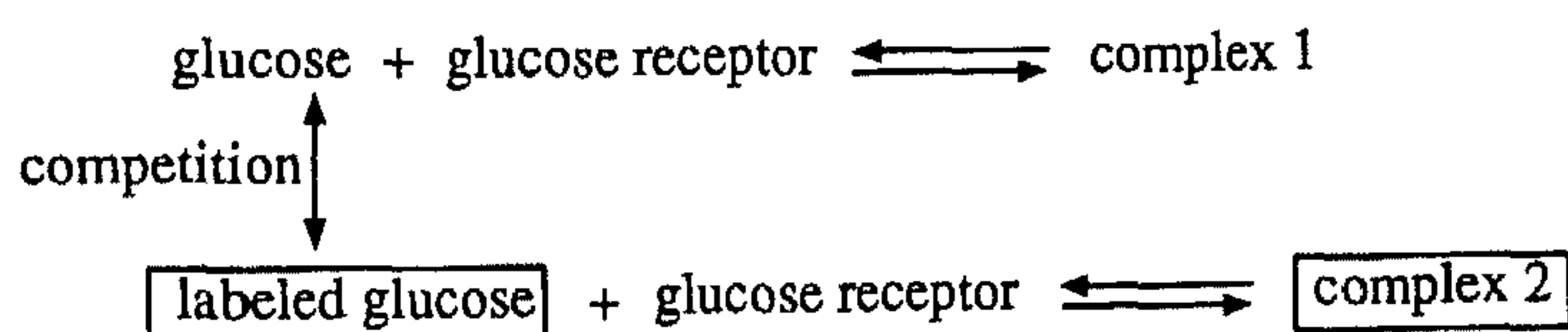
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Introduction

One of the objectives in supramolecular chemistry is to apply complexation for detection of analytes in solution. For example, in ISFET (Ion Sensitive Field Effect Transistor) sensor technology it is possible to determine the concentration of charged species in solution. One example is the detection of sodium ions using derivatized calix[4]arenes as selective ion receptors [1]. A second example is the detection of silver, copper, cadmium and lead ions by using derivatized calix[4]arenes as ion receptors [2]. 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. A good example in this case is glucose. As glucose contains no charge, nor does it show absorption or fluorescence, it is not possible to detect glucose complexation directly. In order to detect glucose, competitive binding of glucose and a labeled glucose derivative to receptor molecules was used to measure glucose concentrations. This approach is explained in more detail in the following section.

Detection method

The principle of competitive binding of glucose and labeled glucose to the glucose receptor is given in scheme 1.



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

From this scheme it follows that glucose forms with a glucose receptor a complex 1 and that the labeled glucose forms a complex 2 with the same glucose receptor. Complex 2 is the complex which can be detected since the glucose in this complex is labeled. If both complexes are formed at the same time, glucose and labeled glucose will compete for the available binding sites of the glucose receptor and the concentration of complex 2 will be dependent upon the glucose concentration. In the work presented two different glucose derivatives with optical labels have been investigated for their utility in optical glucose detection.

Complexation between merocyanine labeled glucose and resorcin[4]arenes

To study the interactions between labeled glucose derivatives and synthetic glucose receptors a merocyanine labeled glucose (1) was designed and synthesized. The structure of this molecule and that of the reference merocyanine (2) is shown in figure 1:

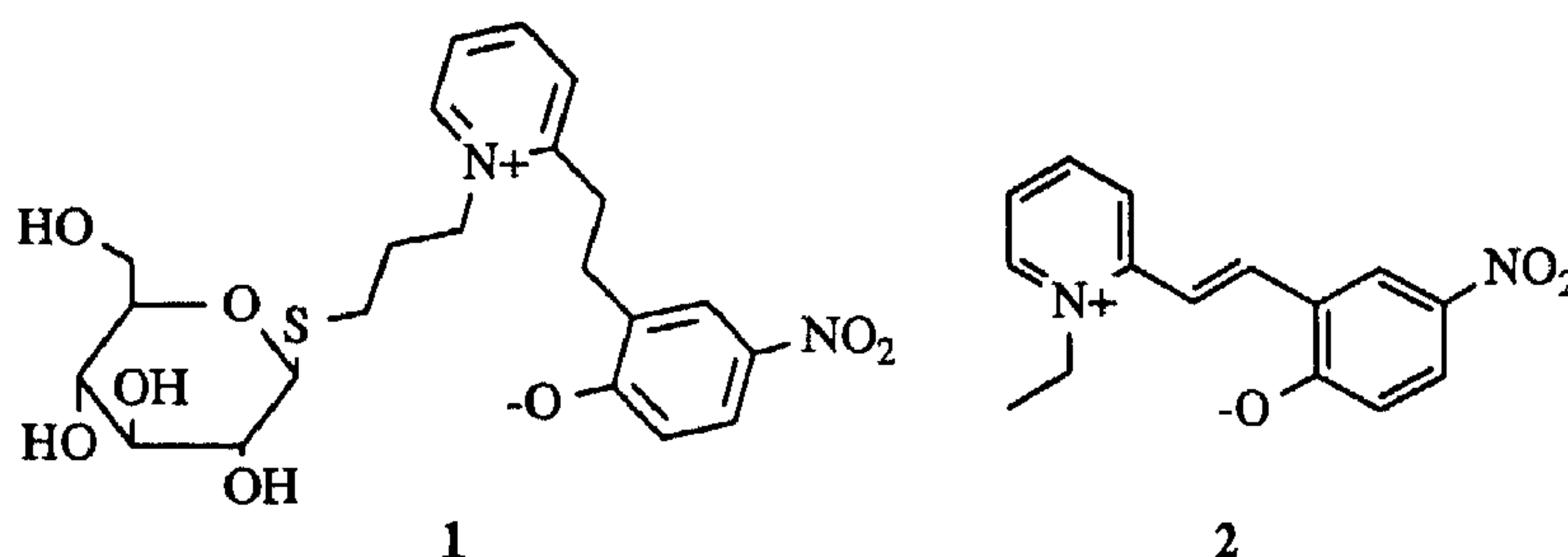


Figure 1: Merocyanine labeled glucose (1) and merocyanine (2).

In the study of the complexation of 1 with a synthetic glucose receptor, resorcin[4]arenes 3 and 4 were used as synthetic glucose receptors [3]. Resorcin[4]arenes are the cyclic products of a condensation reaction between resorcinol and aliphatic or aromatic aldehydes. These compounds show (selective) interaction with various saccharides as was shown by Aoyama et al. with NMR-spectroscopy [4a-c]. To study the complexation of the labeled glucose with the resorcin[4]arene, the resorcin[4]arene was dissolved in an organic phase, 1-octanol, and the extraction of 1 and reference compound 2 from the water phase was studied by UV-VIS spectroscopy.

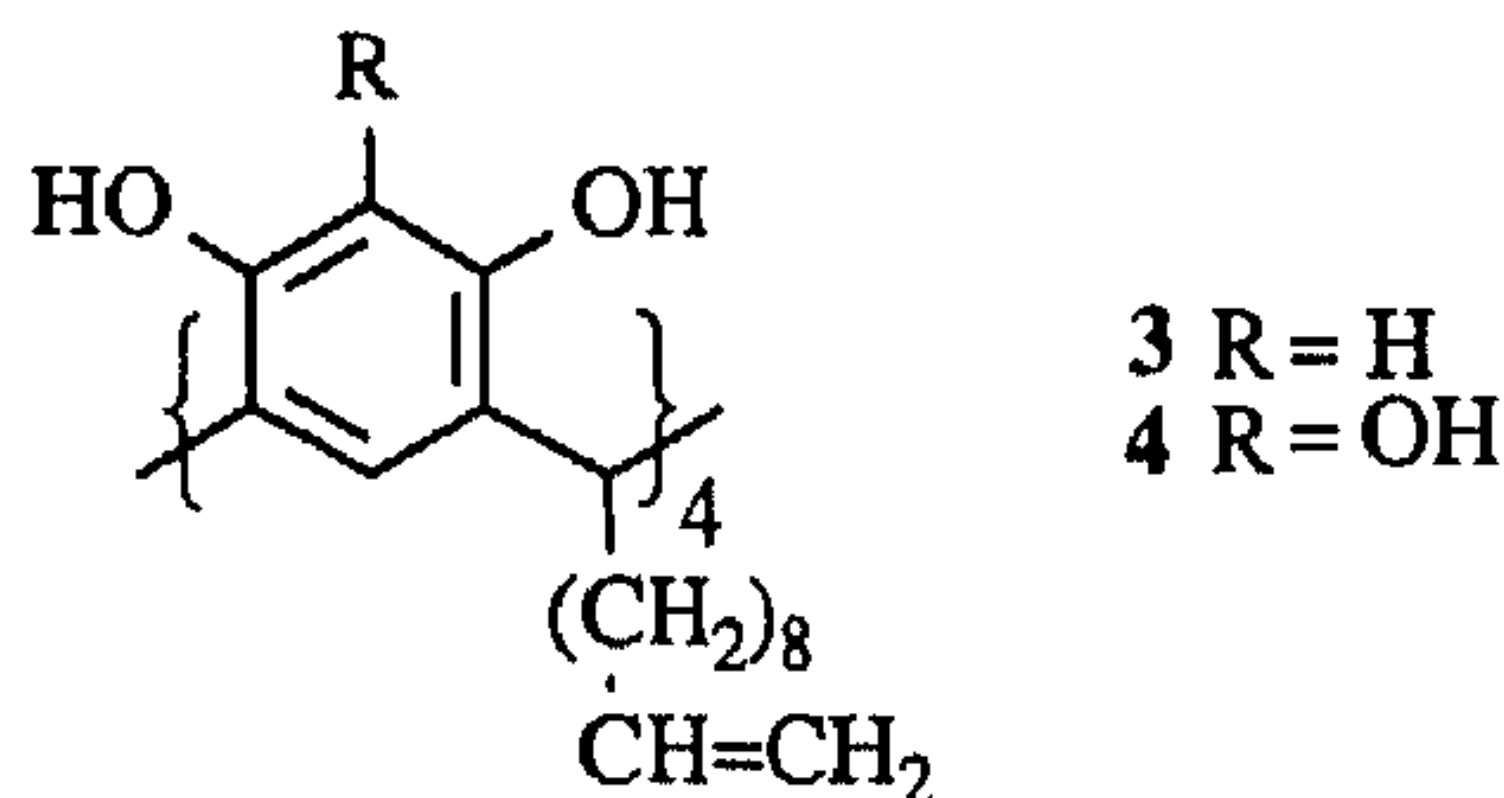


Figure 2: Resorcin[4]arenes as glucose receptors.

It is possible to calculate the association constant (K_{ass}) between the receptor and the labeled glucose derivative by equation 1, derived from the linearization of the relation between absorbance and concentration of labeled saccharide. In the equation, A is the measured absorption in the organic phase, $[L]_{w,0}$ is the initial concentration of labeled saccharide in the water phase, and $[\text{receptor}]_0$ is the initial concentration of the resorcin[4]arene in the organic phase. The parameter r_{par} is a

$$\frac{A_{\text{par}}}{A - A_{\text{par}}} = \frac{r_{\text{par}}}{\epsilon_{\text{com}} \cdot [\text{receptor}]_0} \cdot [L]_{w,0} + \frac{\epsilon_L}{\epsilon_{\text{com}} \cdot K_{\text{ass}} \cdot [\text{receptor}]_0} \quad \text{eq. 1}$$

correction parameter for the partition of the labeled saccharide between aqueous phase and organic phase in the absence of receptor molecules in the organic phase. A_{par} is the contribution to the absorbance A of the organic phase originating from

the partition of $[L]_{w,0}$ to the organic phase and ϵ_{com} and ϵ_L are the extinction coefficients of the complex and the labeled saccharide, respectively. A plot of $A_{par}/(A-A_{par})$ versus $[L]_{w,0}$ gives a linear relation as is shown in figure 3.

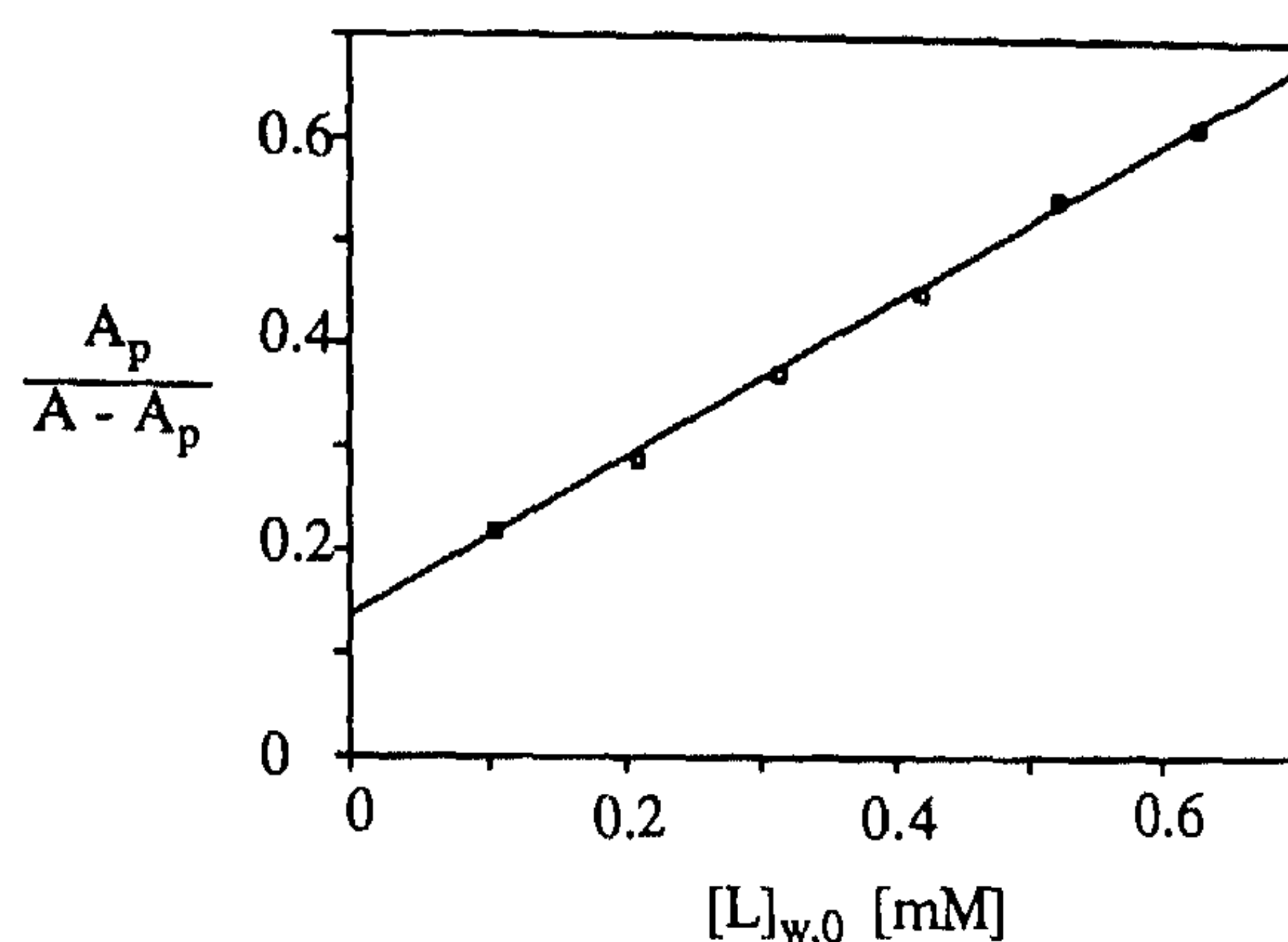


Figure 3: Plot of $A_{par}/(A-A_{par})$ versus $[L]_{w,0}$ of labeled glucose 1 with receptor 4.

From the slope of the line in figure 3, the ϵ_{com} can be calculated and the intercept of the Y-axis contains the parameter which should be determined (see eq. 1). From the determination of the intercept it is possible to calculate K_{ass} . The association constants of glucose derivative 1 (Gluc) with receptors 3 and 4 are given in table 1. In order to study the selectivity of the receptors also the complexation with the merocyanine labeled galactose (Gal) and ribose (Rib) and that of the reference compound 2 (Label) was studied.

Table 1: K_{ass} and ΔG_{ass} of the complexes between labeled saccharides and resorcin[4]arenes.

	Gluc	Gal	Rib	Label
3 K_{ass}	89.6	11.0	11.3	7.6
ΔG_{ass}	-28.3	-23.1	-23.1	-22.1
4 K_{ass}	118.6	88.0	44.0	13.3
ΔG_{ass}	-28.9	-28.2	-26.5	-23.5

K_{ass} in $10^3 \cdot l \cdot mol^{-1}$, ΔG_{ass} in $kJ \cdot Mol^{-1}$

From the data in the table it can be concluded that, when comparing K_{ass} and ΔG_{ass} of labeled glucose 1 and label 2, there is a clear contribution of the glucose residue towards the complexation. Moreover, resorcin[4]arene 3 shows the highest selectivity towards glucose, compared to the other saccharides. Experiments with labeled glucose and glucose in competitive binding of these compounds by resorcin[4]arenes are in progress.

Complexation between Malvin and Concanavalin A

Another labeled glucose derivative which was investigated is Malvin (5, figure 4). This molecule is an anthocyanin, a naturally occurring flower pigment, which shows the copigmentation effect [5]. The copigmentation effect can be found in, for example, plants and is the phenomenon that the intensity of the absorption of the pigment is strongly increased when a pigment is complexed by a copigment (6, figure 4). A review of this phenomenon was published by Goto and Kondo [6].

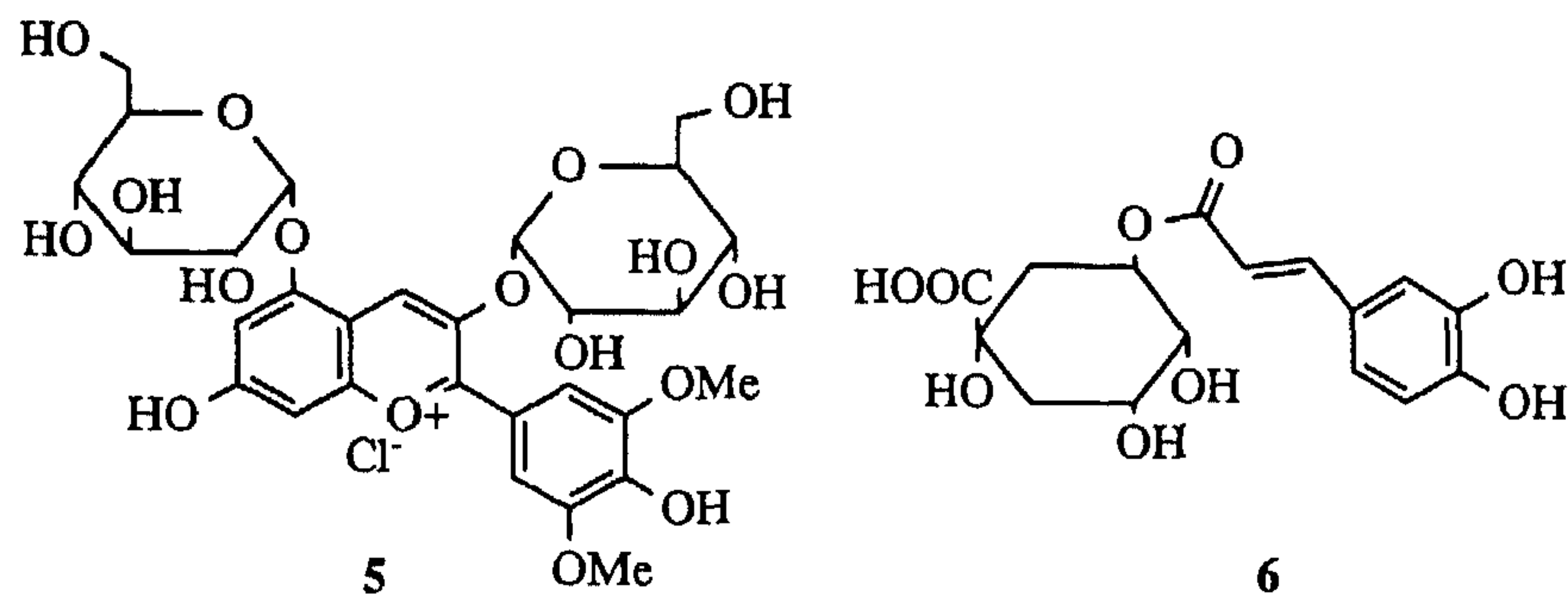
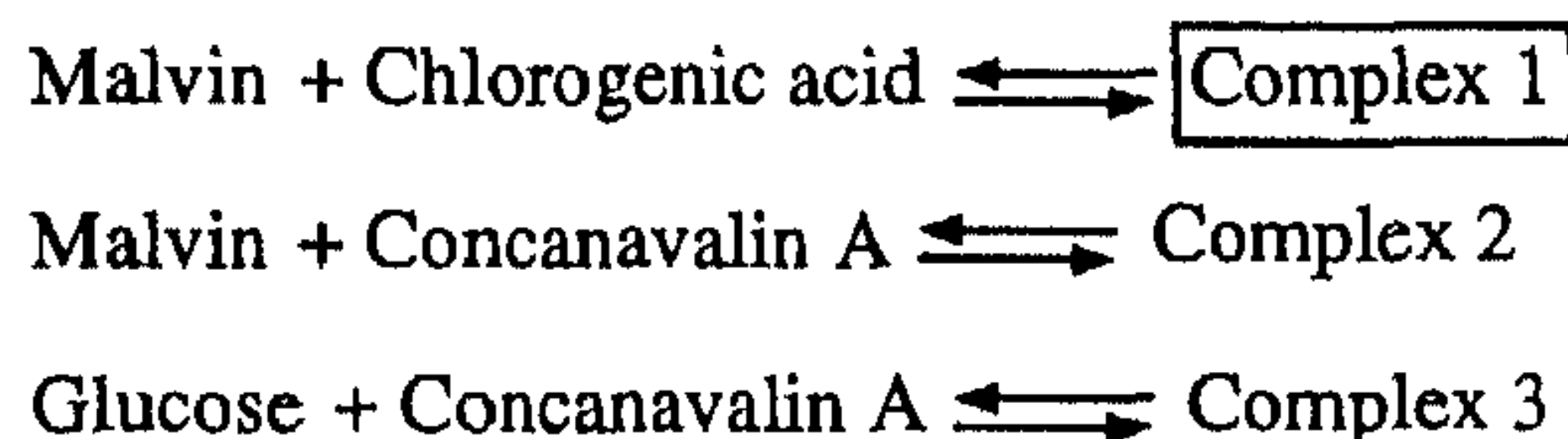


Figure 4: The naturally occurring pigment Malvin (5) and copigment Chlorogenic acid (6).

By influencing the concentration of the complex between Malvin and Chlorogenic acid (complex 1) via the complexation of the glucose residues of Malvin by a glucose receptor (complex 2), a direct response can be obtained for the glucose concentration (complex 3). As a glucose receptor Concanavalin A was chosen. Concanavalin A is a glucose/mannose selective protein which was studied earlier for sensor applications [7]. The interactions between the pigment Malvin, the copigment Chlorogenic acid and Concanavalin A are summarized in scheme 2:



Scheme 2: Complex formation between Malvin, Chlorogenic acid, Concanavalin A and glucose.

Complex 1 is a complex that can be detected at 500 nm. As Chlorogenic acid and Concanavalin A compete for complexation with the available Malvin, there is a relation between the concentration of complex 1 and the available binding sites of the glucose receptor Concanavalin A. Therefore, it should be possible to measure the glucose concentration in solution because the glucose concentration determines the concentration of free Concanavalin A, which in turn determines the concentration of complex 1. The result is a glucose response which is given in figure 5.

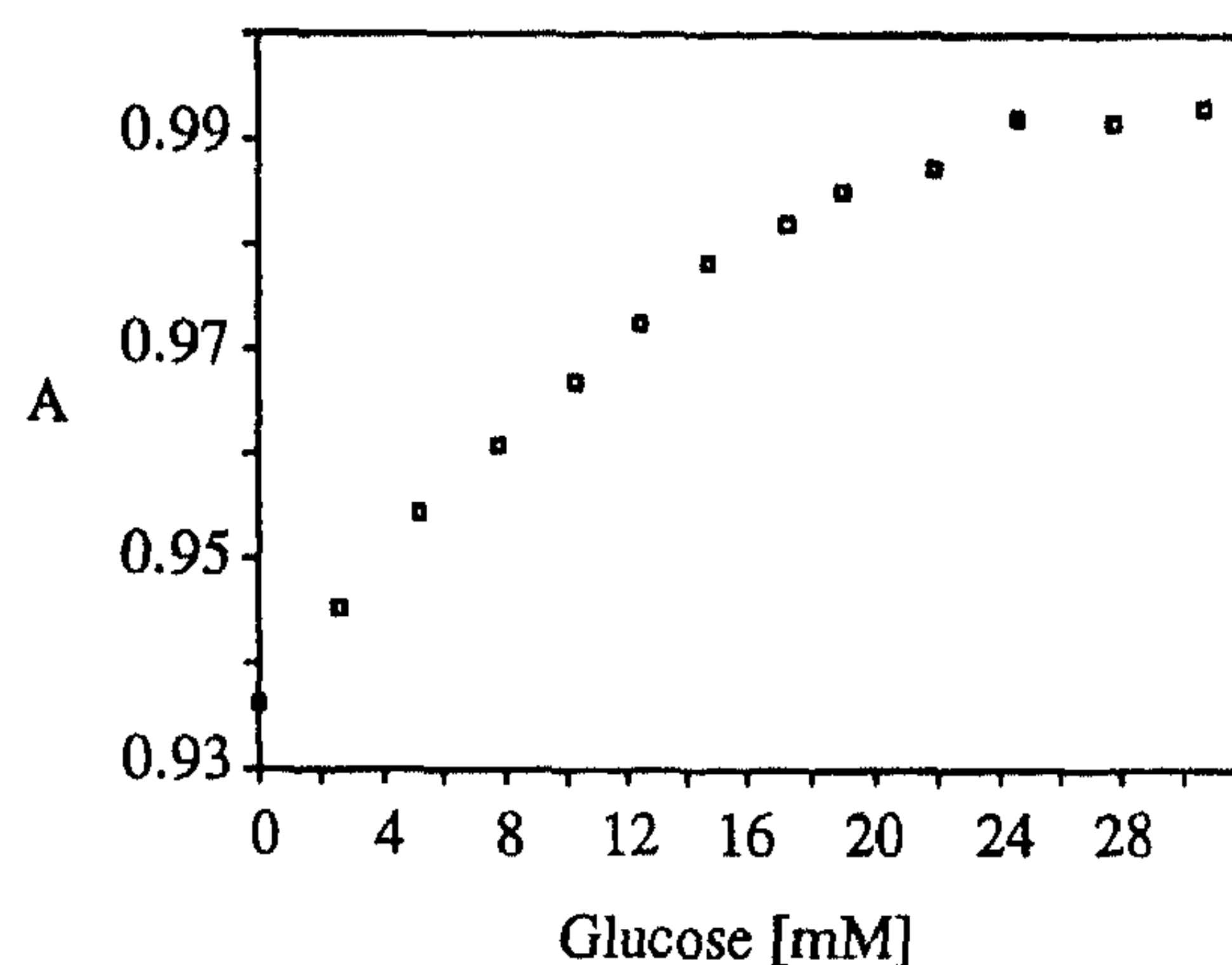


Figure 5: Glucose response with the copigmentation effect.

The response in figure 5 shows that for the first time the complexation of glucose can be followed optically.

Conclusions

It was shown that resorcin[4]arenes show selectivity towards glucose derivatives and that the contribution of glucose towards the association is significant. Also it was shown that the copigmentation effect between Malvin and Chlorogenic acid can be utilized to measure the reversible complexation of glucose with a glucose receptor.

Literature

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