Novel Water-soluble Cyclodextrin–Calix[4]arene Host Molecules with Strongly Enhanced Binding Properties

Erik van Dienst, Bianca H. M. Snellink, Irma von Piekartz, Johan F. J. Engbersen and David N. Reinhoudt*

Laboratory of Organic Chemistry, University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands

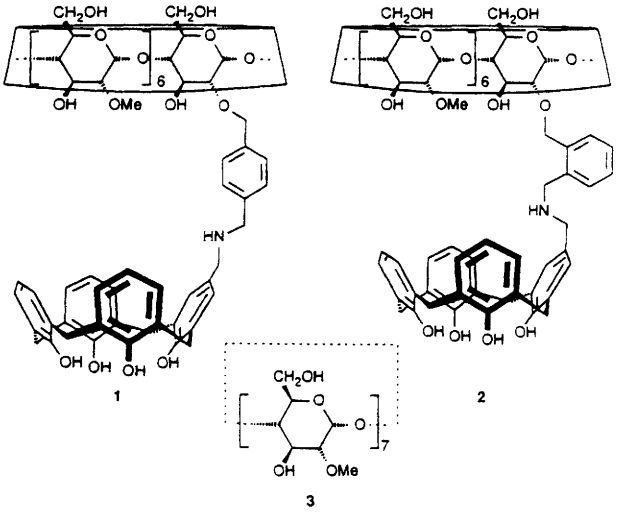
β-Cyclodextrins appended with a calix[4]arene moiety at the secondary face are very efficient host molecules for the fluorescent dyes 1-anilino-8-naphthalenesulfonate and 2-*p*-toluidino-6-naphthalenesulfonate with unprecedented high complexation constants.

Cyclodextrins are a unique group of naturally occurring cyclic D-glucose oligomers, capable of the complexation of hydrophobic guest molecules in aqueous solvents by predominantly hydrophobic interactions.[1] During complexation, the area of the hydrophobic surface, *i.e.* the interior of the cyclodextrin cavity and the hydrophobic part of the guest molecule, exposed to water is decreased. Binding constants of up to 10000 $dm^3\ mol^{-1}$ are known, but stronger binding of guests is restricted because of the open ends of the molecular cylinder. The first cyclodextrin derivatives with an enhanced hydrophobic cavity were developed by the groups of Breslow[2] and Tabushi[1,3] by functionalization of the primary hydroxy face. Introduction of a (flexible) 2-naphthalenesulfonyl cap at the primary face, combined with modification of the secondary face with a 4-toluenesulfonyl group was achieved by Ueno *et al.*[4] These cyclodextrin-based receptors show significantly increased binding affinities for several specific guest molecules because of additional shielding of the hydrophobic guest from the aqueous environment. Recently, D'Alessandro *et al.*[5] reported a cyclodextrin derivative with two different binding sites by linking the primary side of β-cyclodextrin (cycloheptaamylose) to one of the carboxyl groups of tetrakis(hydroxycarbonylmethoxy)calixarene *via* an ethylene diamine spacer. However, no binding properties of this compound were given.

In this paper we report the strongly enhanced binding properties of cyclodextrins modified with a calix[4]arene moiety at the secondary face. In these host molecules the upper rim of calix[4]arene[6] is facing *via* a xylyl spacer group to the wider opening of the cyclodextrin cavity. This arrangement can provide additional hydrophobic interaction and solvent shielding of a guest molecule accommodated in the cyclodextrin cavity. Binding interactions of the C–H hydrogens of organic guests with the π-arene system of calixarenes have been reported.[7] The host molecules **1** and **2** were prepared by reaction of the secondary hydroxy face of heptakis(6-*O*-*tert*-butyldimethylsilyl) β-cyclodextrin with α-bromotolunitrile, methylation of the remaining C(2)-hydroxys, conversion of the cyano group to the aminomethyl group, and reductive coupling with formylcalix[4]arene followed by desilylation.[8]

The complexation behaviour of these water-soluble cyclodextrin–calix[4]arene host molecules was studied with the fluorescent guests 1-anilino-8-naphthalenesulfonate (ANS) and 2-*p*-toluidino-6-naphthalenesulfonate (TNS).[1,9] The fluorescence emission maximum of ANS in pH 7.0 buffered aqueous solution ($\lambda_{em,max}$ = 528 nm) shows a distinct blue shift and an increase in fluorescence intensity upon addition of β-cyclodextrin to the solution ($\lambda_{em,max}$ = 508 nm). Both effects are indicative of a shielding of ANS from the aqueous environment by (partial) inclusion in the cyclodextrin cavity.[1,9] Methylation of the C(2)-hydroxys at the secondary hydroxy face[10] to give **3** increases the shielding properties of the cavity, causing a further blue shift of the emission maximum ($\lambda_{em,max}$ = 489 nm) and increase of the fluorescence intensity. In compounds **1** and **2**, the secondary face is both methylated at the C(2)-hydroxy and functionalised with a calix[4]arene moiety *via* a *p*-xylyl and *o*-xylyl spacer, respectively. This modification leads to a distinct further blue shift of the emission maximum of ANS (**1**, $\lambda_{em,max}$ = 469 nm; **2**, $\lambda_{em,max}$ = 466 nm) and a strong enhancement of the fluorescence quantum yield, suggesting considerable additional shielding of the complexed fluorescent probe by the calix[4]arene cap. The increase in fluorescence intensity (ΔF) of ANS for the various hosts is of the order: β-cyclodextrin < **3** < **1** < **2** (Fig. 1).

The changes in fluorescence intensities of solutions in which the guest concentration was kept constant and the host concentrations of **1** and **2** were steadily increased (Fig. 2) show that the host molecules have high binding affinities for the fluorescent guests. In very diluted solutions of TNS (1.30×10^{-5} mol dm^{-3}) addition of only a fourfold excess of the *para*-linked host **1** is sufficient to reach the maximum fluorescence emission, whereas for the *ortho*-linked host **2** a 12-fold excess is necessary. The binding of ANS is weaker than that of TNS, as is illustrated by the more gradual increase of the fluorescence intensity upon increment of the host concentration. Complete complexation of ANS could not be achieved because the solution starts to become turbid at higher host concentrations. Analysis of the data using a linear least squares regression



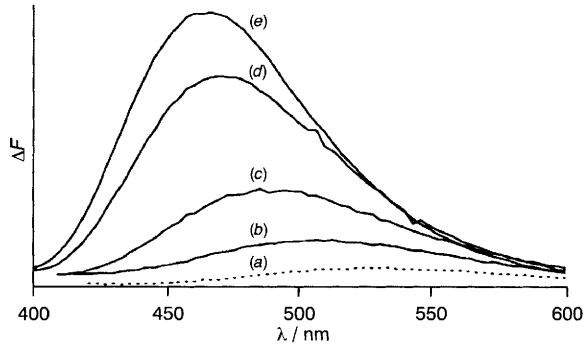


Fig. 1 Fluorescence emission spectra of (*a*) ANS (10 × amplified), ANS with (*b*) β-cyclodextrin, (*c*) **3**, (*d*) **1**, and (*e*) **2**. ([ANS] = 10^{-5} mol dm^{-3}, λ_{ex} = 354 nm, λ_{em} = 467 nm, pH 7.0, 25 °C).

method gave good fits for 1 : 1 complexes, with the association constants listed in Table 1.

The high association constants of hosts **1** and **2** for ANS and TNS show that the presence of the calixarene cap at the secondary side of the cyclodextrin results in a large increase in binding ability compared to the parent cyclodextrin. The association constant of 153 000 dm^3 mol^{-1} of **1** with TNS is the highest observed for derivatised cyclodextrins with this guest.[1,9,11] For comparison, cyclodextrin dimers[11] bind TNS with binding constants up to 74 000 dm^3 mol^{-1},[11b] but in these cases the high binding capacity originates mainly from the possibility that the two aromatic groups of the fluorescent probe can accommodate in a different cyclodextrin cavity. Although the binding of ANS is weaker than that of TNS the presence of the calixarene group induces a larger enhancement of the binding affinity for this guest. In β-cyclodextrin, the phenyl ring of ANS is included in the cavity *via* the secondary hydroxy face and the naphthalene moiety is residing outside the hydrophobic cavity.[12] In **1** and **2** the C(2)-methylated and calix[4]arene capped secondary hydroxy face provides additional shielding of the relatively large part of the probe outside the cyclodextrin cavity.

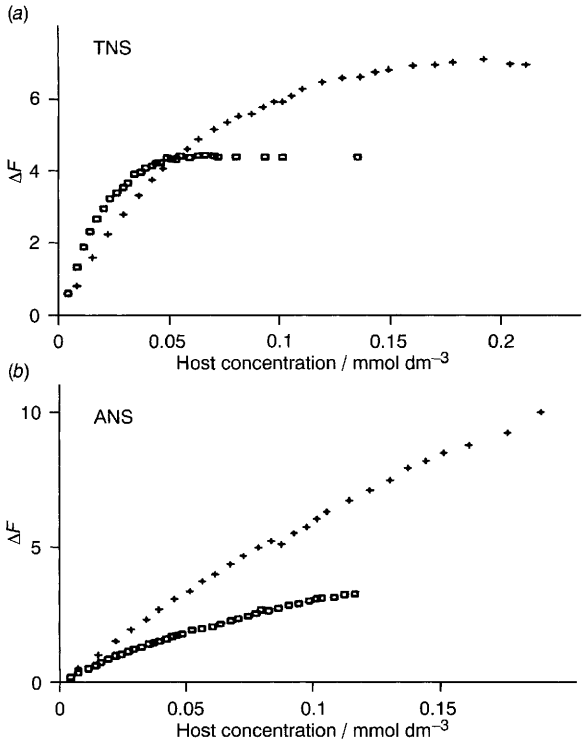


Fig. 2 Fluorescence titration with cyclodextrin–calix[4]arene hosts **1** (□) and **2** (+) of (*a*) TNS (1.30×10^{-5} mol dm^{-3}, λ_{ex} = 358 nm, λ_{em} = 440 nm) and (*b*) ANS (0.94×10^{-5} mol dm^{-3}, λ_{ex} = 354 nm, λ_{em} = 467 nm), pH 7.0, 25 °C

Table 1 Association constants K_{ass} (±2%) of β-cyclodextrin and the β-cyclodextrin–calixarenes **1** and **2** at 25 °C, pH 7.0

	K/mol dm^3	
	ANS	TNS
β-cyclodextrin	—	2 000
	(65)[a]	(1 980)[b]
1	24 800	153 000
2	2 300	30 500

[a] See ref. 12*a*. [b] See ref. 13.

The lower binding affinity observed for the *ortho*-xylyl isomer **2** compared to the *para*-xylyl isomer **1** is probably due to competitive intramolecular inclusion of part of the calix[4]arene moiety of **2** in the cyclodextrin cavity. The close vicinity of the calixarene moiety to the cyclodextrin cavity is also expressed in the higher fluorescence quantum yields found with **2** (Fig. 1) which show that this host gives a better shielding of the fluorescent probes from the aqueous environment. The cyclodextrin–calix[4]arenes **1** and **2** are also efficient hosts for other guest molecules, *e.g.* steroids and terpenes, as was shown in competition experiments. For example, a clear reduction of the relative fluorescence intensity (I/I_0) of a solution containing 5×10^{-5} mol dm^{-3} **1** and 1.2×10^{-5} mol dm^{-3} TNS is observed upon addition of 4×10^{-4} mol dm^{-3} of cholic acid (0.71), cholic acid methyl ester (0.62), prednisolone acetate (0.62), and (−)-borneol (0.51).

In summary, the novel cyclodextrin–calix[4]arene receptor molecules **1** and **2** are very effective hosts for the fluorescent dyes ANS and TNS with association constants much larger than reported thus far in literature for cyclodextrins capped at the primary or secondary hydroxy face. Also other compounds can be effectively complexed by these novel water-soluble host compounds. The strongly increased binding capacity is attributed to the additional environmental shielding of the guest by the upper rim of the calix[4]arene which can move over the secondary hydroxy face of the cyclodextrin cavity.

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