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Dodecaborate-Functionalized Anchor Dyes for Cyclodextrin-Based Indicator Displacement Applications

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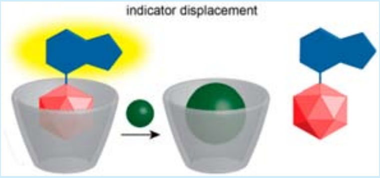
Supporting Information

ABSTRACT: A new type of water-soluble anchor dyes, that is, dyes which carry an auxiliary unit for strong binding to macrocyclic host molecules, has been synthesized. It consists of 7-nitrobenzofurazan (NBD) as a dye and the dodecaborate cluster ($B_{12}H_{11}R$) as a dianionic, globular, and purely inorganic anchoring group for cyclodextrins ($K_a > 10^5$ M^{-1}). The synthesized dodecaborate-substituted dyes show marked changes in their photophysical properties (UV−vis and fluorescence) upon complexation with cyclodextrins (β-CD and γ-CD), such that the resulting host·dye complexes (1:1 stoichiometry) present sensitive reporter pairs for indicator displacement applications.



The use of macrocycles for sensing applications has become very attractive due to the ability of the host molecule to encapsulate a wide range of guest molecules, including dye molecules.[1−5] The encapsulation of chromophores inside a macrocycle usually causes large photophysical alterations. In aqueous solutions, these are mainly due to relocation into the more hydrophobic environment offered by the host cavity.[4,6−10] Dyes which display both a high affinity to a host molecule and a significant optical response upon encapsulation are in high demand for the development of indicator displacement applications, where the presence of an analyte leads to dye displacement, which in turn can be sensitively monitored through changes in fluorescence and/or absorption.[1,2,4,11] Indicator displacement assays (IDAs) with macromolecular receptors have been used in numerous (bio)analytical applications.[5,12] The principle has been extended to the real-time monitoring of changes in analyte concentration as they occur, for example, during enzymatic reactions[3] or in biomembrane transport processes.[13,14] One particular challenge is to find suitable dyes for macrocycles with large cavity sizes, such as γ-cyclodextrin (γ-CD) or cucurbit[8]uril;[4,15] their cavities are generally sufficiently large to encapsulate simultaneously two dye molecules by formation of ternary complexes, which complicates the analysis of indicators displacement titration data.[16,17] In contrast, for the smaller homologues, such as β-CD or cucurbit[7]uril, well-suited reporter molecules are well-known.[4]

One synthetic approach is the use of chromophore-tethered macrocycles.[1,11,18,19] Another approach consists of the modification of indicator dyes with anchoring groups, which are being encapsulated by the parent macrocycles instead of the chromophores themselves. In this manner, the anchoring groups can be selected (i) to be specific for a particular host, (ii) to bind to the host with the required, generally high, affinity, and (iii) to be selected with the correct size and shape to form exclusively stoichiometric (1:1) complexes.[20−25] Our present study was driven by the absence of a suitable indicator dye for γ-CD,[4] for which we have recently identified boron clusters as a potential anchoring motif.[26]

Boron clusters have found numerous applications.[27−29] The discovery of boron clusters in the 1960s opened access to a new form of boron carrier for neutron capture therapy (NCT) of cancer.[28] Dodecaborate anions, with their chaotropic nature,[26] interact with proteins, carbohydrates, and membranes.[30−32] Recently, we have reported the supramolecular host−guest complexation of dodecaborate clusters of the type $B_{12}X_{12}{}^{2-}$ and $B_{12}X_{11}Y^{2-}$ (X = H, Cl, Br, I and Y = OH, SH, $NR_3{}^+$) with CDs.[26] Strong inclusion complexes were formed, particularly with γ-CD, reaching record micromolar affinities in aqueous solution.[26]

Encouraged by the high affinity of these dianionic, purely inorganic clusters, we aimed to use them as novel anchoring groups for organic dyes. The synthesis (Figure 1) builds upon a monosubstituted cluster ($B_{12}H_{11}SH^{2-}$) and 4-chloro-7-nitro-

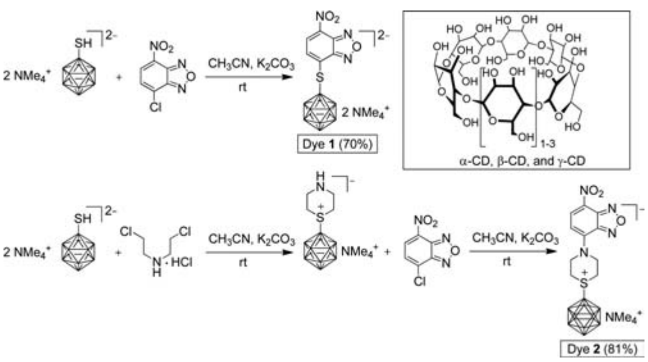


Figure 1. Synthesis of dodecaborate-anchored NBD dyes and molecular structure of cyclodextrins (inset).

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benzofurazan, a reactive precursor for NBD,[33,34] a widely used dye to label peptides, proteins, drugs, and biomolecules.[35−38] The fluorescence of NBD is highly sensitive to its environment and generally weak in water and strong in organic solvents.[39] The anchor dye **1** was obtained by direct nucleophilic aromatic substitution with the thiol, while dye **2** incorporates a spacer unit, which allowed the introduction of an amino group.

The interaction of dyes **1** and **2** with CDs was studied by ^1H NMR spectroscopy, which was facilitated by the high water solubility of the dyes (>2 mM). The formation of host−guest complexes could be directly followed through the signals of the CD hosts (see the Supporting Information). In particular, a marked broadening of several β-CD protons was observed upon complexation with **1** and **2**, while with γ-CD, the inner H5 protons showed a pronounced downfield shift, indicative for the formation of inclusion complexes.[26] No significant effects were observed upon addition of the smallest homologue, α-CD, suggesting that its cavity is too small to accommodate the cluster.

The guest protons were inspected in order to unambiguously identify which site (cluster versus NBD) of the ditopic guest molecules protruded into the CD cavities. Although the very broad signals of the B−H protons (from −0.5 to +2.0 ppm) were less diagnostic in this context, the small absolute changes of the NBD protons and the fact that the complexation-induced shifts were smallest for the most distal NBD protons (see the Supporting Information) demonstrated a preferential binding of the dodecaborate cluster, consistent with their recently established high affinity for the larger CDs. Indeed, while the parent cluster shows an exceptionally strong binding with the larger CDs, simple NBD derivatives such as 4-chloro-7-nitrobenzofurazan or 4-nitro-7-piperazinobenzofurazan show no indication for binding (see the Supporting Information). The cluster serves therefore as the essential molecular recognition element.

The experimentally observed preferential inclusion of the cluster was also reproduced by PM3 calculations. The optimized structures (Figure 2) showed that two different orientations of the encapsulated dye are principally possible, one with the dodecaborate cluster deeply immersed (orientation A) and the other one with the NBD moiety inside the cavity (orientation B). However, the interaction with NBD led to a more loosely bound, shallower complex with an energy higher (by 40 kcal mol^{-1}) than that for the deeply encapsulated cluster. The combined structural assignments establish therefore that the encapsulation of the inorganic dianionic cluster is much preferred, justifying its classification as an *anchor*.

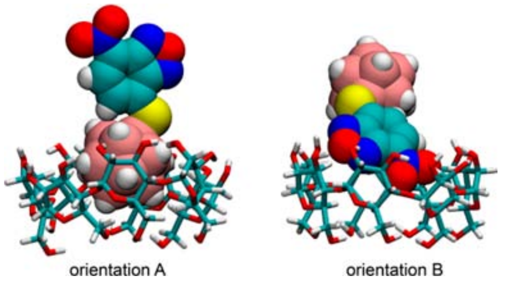

Figure 2. PM3-optimized structures of two different orientations of dye **1** in β-CD.

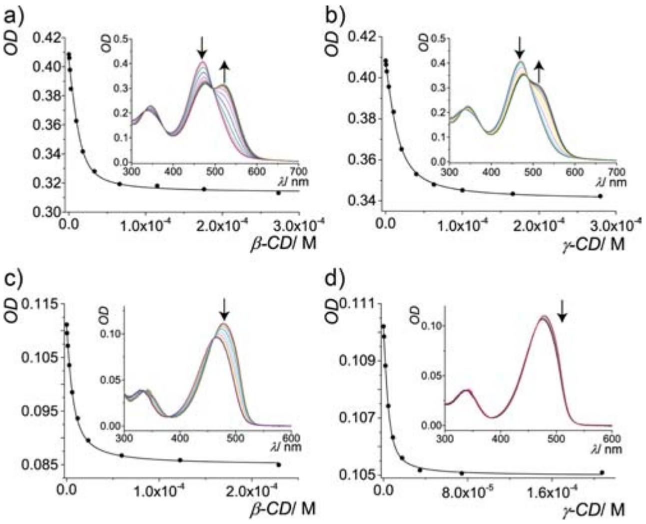

Figure 3. UV−vis titrations of dye **1** (15 μM, top) and **2** (5 μM, bottom) with β-CD (left) and γ-CD (right); the fittings (black lines) were done by assuming a 1:1 binding model from which the association constants were derived. Insets: Actual changes in the UV−vis spectra with increasing concentration of host.

The interaction of dyes **1** and **2** with β-CD and γ-CD was studied in more detail by optical spectroscopy. Marked changes in the course of the UV−vis titrations (Figure 3) were observed; the encapsulation of **1** afforded a bathochromic shift (40−50 nm), and that of **2** a smaller hypsochromic shift (3−13 nm). Dye **1** showed no detectable fluorescence in both its bound and unbound form, while dye **2** showed fluorescence in both forms. This trend is consistent with the nature of the aryl substituent in the 4-position, that is, NBD dyes with a 4-thiophene-yl substituent (similar to dye **1**) show no fluorescence, but those with a 4-dimethylamino group (akin to dye **2**) do fluoresce.[40] The fluorescence of **2**, which has the cluster separated through a spacer unit, was low in water (ϕ_f = 0.9%, at pH 7) but increased significantly upon complexation of the anchoring cluster with β-CD (ϕ_f = 3.3%) and γ-CD (ϕ_f = 1.3%), see Figure 4. In addition, a slight hypsochromic shift of the emission band was observed due to the relocation of the dye into a less polar environment.[41]

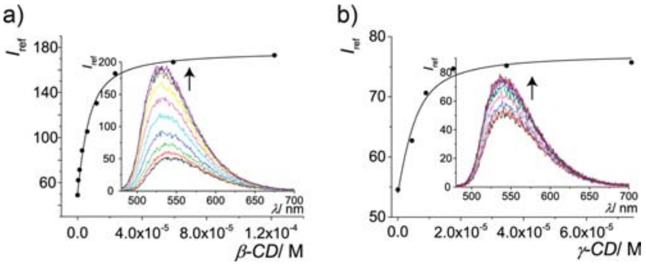

Figure 4. Fluorescence titrations of dye **2** (5 μM) with β-CD (left) and γ-CD (right); the fittings (black lines) were done by assuming a 1:1 binding model from which the association constants were derived. Insets: Actual changes in the fluorescence spectra (λ_{ex} = 460 nm) with increasing concentration of host.

The globular $B_{12}H_{11}$ anchor has an ideal shape for encapsulation to the truncated cone-shaped cavity of CDs. Moreover, the size of the cluster is too large (V = 152 Å^3)[26] to allow the simultaneous inclusion of more than one cluster into the cavity of both, β-CD (V = 262 Å^3) and γ-CD (V = 427 Å^3),[42] especially if one considers a preferred packing of 55%.[24,43,44] Indeed, the optical titration data could be well fitted according to a 1:1 complexation stoichiometry (Figures 3 and 4). The

Table 1. Association Constants of Dodecaborate-Anchored NBD Dyes and Selected Guest Molecules[a] with CDs in Aqueous Solution

	K_a (10^5 M^{-1})			K_a (10^3 M^{-1})
guest	β-CD	γ-CD	guest	β-CD
1	2.7	1.5	cyclopentanol	0.7[c]
2	2.6 (2.4)[b]	5.9 (3.8)[b]	cyclohexanol	1.6[c]
nandrolone	0.28[c]	0.23[c]	1-adamantol	61[c]
perfluoropentanoic acid	0.56[c]		*p*-chlorophenol	0.8[c]
$B_{12}Br_{12}^{2-}$[d]		10[e]	*p*-bromophenol	0.9[c]
			p-iodophenol	1.6[c]

[a] Determined by UV−vis titrations. [b] Values in parentheses obtained from fluorescence titrations. [c] Values were determined by fluorescence displacement titrations using CD·**2** as a reporter pair. [d] Measured as sodium salt; a value of 9.6 × 10^5 M^{-1} was previously reported by ITC.[26] [e] Value was determined by UV−vis displacement titrations using γ-CD·**1** as a reporter pair.

resulting affinity constants are found in Table 1. High-affinity binding (10^5 M^{-1}) was observed for both dyes, which makes them excellent choices for indicator displacement applications; in particular, it allows host and dye to be used in low micromolar concentrations. It should be mentioned here that binding constants on the order of 10^4 M^{-1} or higher have been fairly difficult to achieve for CDs, especially for the larger homologue, γ-CD.[4,45] Surprisingly, the binding constant of the two dyes to both CD homologues (β-CD and γ-CD) is one to 2 orders of magnitude higher than that of the parent cluster ($B_{12}H_{11}SH^{2-}$).[26] The higher affinity of the dyes is presumably due to the addition of the hydrophobic moiety to the cluster, such that the complexation of the anchoring group results in a concomitant partial desolvation of the NBD moiety.

The large shifts in the UV−vis spectra and the fluorescence enhancements of dyes **1** and **2** upon complexation by CDs allow their implementation in IDAs. IDAs have been used extensively in other and our laboratories to either determine association constants of optically transparent and/or sparingly water-soluble, highly hydrophobic guests, or to sense analytes with known affinity.[24,46] Typical displacement titrations are shown in Figure 5. For example, upon the addition of a competitive guest, in this case $B_{12}Br_{12}^{2-}$ (which was previously reported to bind strongly to γ-CD), dye **1** was displaced from the γ-CD cavity and its original UV−vis spectrum was fully restored. The binding constant of $B_{12}Br_{12}^{2-}$ was determined to be 1.0 × 10^6 M^{-1}, which is consistent with the reported value by direct NMR or isothermal calorimetric titrations.[26] Alternatively, when perfluoropentanoic acid was added to a solution with preformed β-CD·**2** complex, the fluorescence decreased by a factor of ca. 3, as expected, signaling the displacement of dye **2** by the known fluorocarbon binder.[47,48] The binding constant of perfluoropentanoic acid could therefore be readily determined by fluorescence, affording a high value of 5.6 × 10^4 M^{-1}. The method was also tested for additional alcohols and phenols (structures are shown in Figure 6a and K_a values are given in Table 1); in all cases, good agreement with literature values was found.[45]

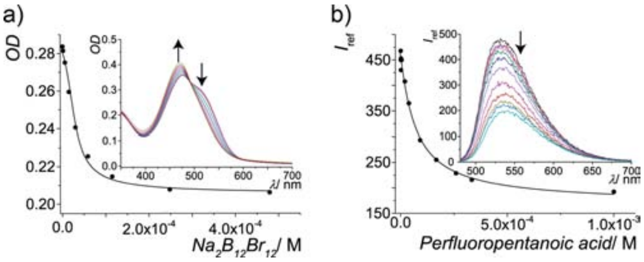

Figure 5. (a) UV−vis titration for the competitive displacement of dye **1** (15 μM) from γ-CD (30 μM) by $Na_2B_{12}Br_{12}$; the binding constant was found to be 1.0 × 10^6 M^{-1}. (b) Fluorescence titrations for the competitive displacement of dye **2** (3 μM) from β-CD (12 μM) by perfluoropentanoic acid; the binding constant was found to be 5.6 × 10^4 M^{-1}.

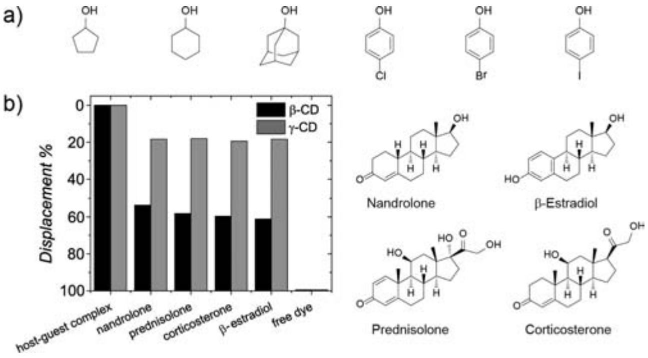

Figure 6. (a) Molecular structures of guest molecules for β-CD determined by competitive fluorescence titrations with dye **2** (2.5 μM **2** and 15 μM β-CD); binding constants are given in Table 1. (b) Displacement efficacy after the addition of 700 μM of different steroids to solutions containing 2.5 μM **2** and 15 μM of β-CD or 10 μM of γ-CD as reporter pairs; experiments were done by using a fluorescence microplate reader. All values were normalized to the intensity of the complexed dye (0% displacement) and the intensity of the free dye (100% displacement).

We also conducted the displacement experiments in a microplate reader, which allows the fast screening of analyte binding to β-CD and γ-CD. To illustrate this, we tested a set of steroids to obtain information on their relative binding affinities to CDs (Figure 6b). Addition of the same concentration of steroid to the preformed host/dye complexes led to different degrees of dye displacement, signaled by a fluorescence decrease. The results allowed quick conclusions on the binding propensities of the steroids to be drawn, in particular, that β-CD shows a higher affinity for steroid binding than γ-CD. The absolute binding constants of nandrolone to β-CD and γ-CD were independently determined through displacement titrations in cuvette format and found to be 2.8 and 2.3 × 10^4 M^{-1}, respectively. As can be seen, the dodecaborate-cluster tethered dyes are powerful indicators for guest binding to macrocycles, including large ones such as γ-CD for which no stoichiometric indicator dyes are at hand.[4,15] This opens the door to optimized indicator displacement assays and hitherto unexplored sensing applications.

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In summary, hybrid anchor dyes were designed by merging an organic dye with an inorganic residue which serves as an anchor to macrocycles. The dyes form strong 1:1 complexes with CDs through the encapsulation of the dodecaborate anchor, reaching micromolar affinities. Important to note, the new anchoring group is *orthogonal* to the known hydrophobic groups (e.g., adamantane or ferrocene), which have been abundantly used for both, β-CD and CBs as hosts.[49−55] In fact, beyond sensing, the use of dodecaborate as a tight anchor motif would further enable noncovalent chemistry in the direction of biolabeling,[49] self-assembled polymers and gels,[50−52] and materials surface modification.[53−55]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03611.

> Experimental details, synthetic procedures, and characterization of the new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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