Anthryl(alkylamino)cyclodextrin Complexes as Chemically Switched DNA Intercalators†

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The strong conformational coupling between heterotopic binding sites which can be achieved in synthetic supramolecular complexes[1] makes these promising candidates for the possible development of new drug delivery systems. With the idea of combining the known drug binding ability of cyclodextrins[2] with the potent DNA intercalating properties of anthrylamines,[3] we have synthesized by standard procedures (Scheme 1) a corresponding derivative **1**. The protonated amino groups in the spacer between cyclodextrin and the aryl units of **1** should provide for a superior water solubility in comparison to that of known analogous compounds[4] as well as for an affinity increase to the negatively charged grooves of double-strand DNA (ds-DNA). Spectroscopic examinations revealed that complexes of the anthrylcyclodextrin **1** display a new principle of an allosterically switched DNA intercalator.

The aromatic ^{1}H-NMR signals of **1** are substantially shifted to lower field after adding a known[5] strong intracavity complexer of β-cyclodextrin (β-CD) such as 1-adamantanol (AN) (Figure 1). In line with the known shielding effect of β-CD on aromatic guests,[6] the shifts of uncomplexed anthrylmethylamines are similar to the ones observed in **1** after addition of the guest AN. This signifies that the guest AN removes the anthryl unit out of the β-CD cavity where it was previously locked (first step in Scheme 2). This process is also visible in the circular dichroism spectra, which show a positive peak for **1** alone which is converted to a slightly negative peak upon addition of the guest AN . These observations are compatible with an orientation of the long anthracene axis parallel to the β-CD axis, which changes to a more perpendicular orientation outside the cavity upon addition of the competing guest AN.

The occupation of the CD cavity by AN frees the anthryl unit I, which then leads to strong intercalation with ds-DNA (Scheme 2). Whereas no significant change is observed in the

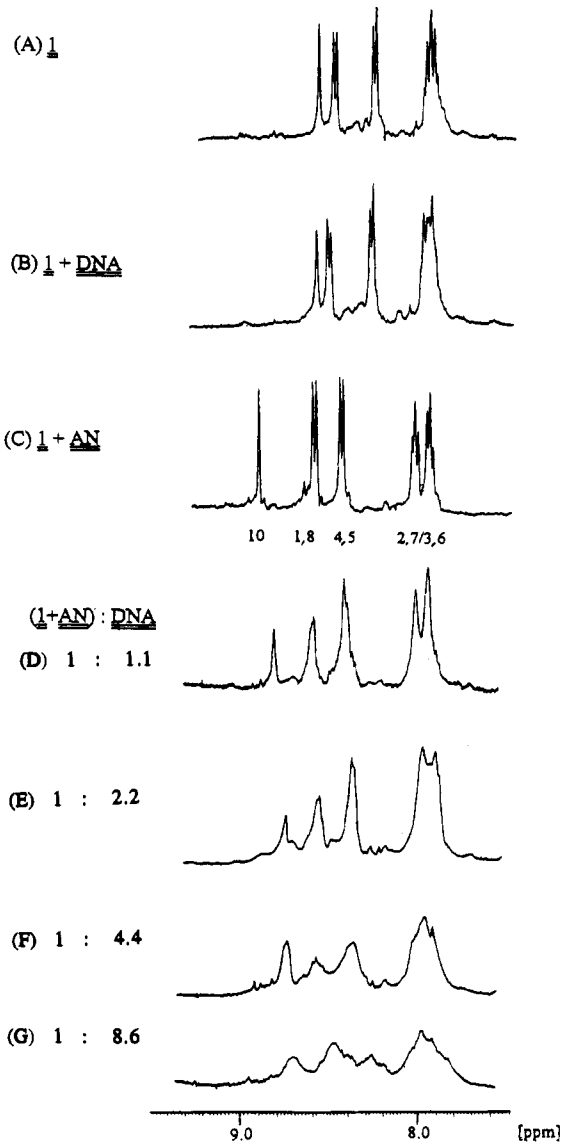
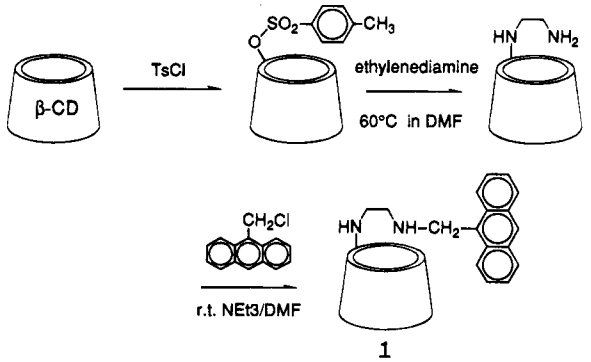


Figure 1. . ^{1}H-NMR spectra of the anthryl part I of 1 (D_2O, ambient temperature) without (A) or with added ds-DNA (B) or adamantanol-1 (AN) (C) and of AN + 1 (1:1) with ds-DNA added in increasing concentrations, with molar ratios of (AN + 1) to DNA base pair as indicated (D−G). Signal assigments (from COSY experiments and multiplicities) are indicated in spectrum C.

Scheme 1



† This is part 50 of the Saarbrücken series on Supramolecular Chemistry. For part 49, see: Baldes, R.; Schneider, H.-J. *Angew. Chem.*, in press.

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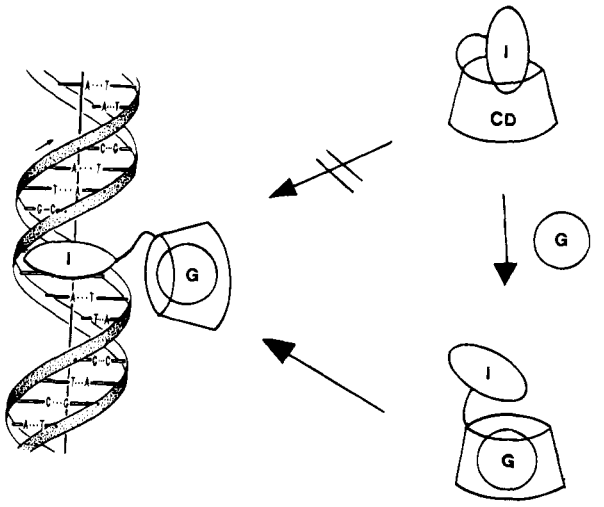
Scheme 2



^{1}H-NMR spectra of **1** after addition of calf thymus DNA alone, dramatic upfield shifts of up to 0.4 ppm as well as broadening by up to 55 Hz of the anthryl signals clearly indicate intercalation if both **1** *and* AN are present in increasingly concentrated DNA solutions (Figure 1). Such shifts as the result of nucleobase anisotropy effects are known to be typical for strong intercalators such as anthrylamines,[7] whereas groove binders exhibit usually shift differences below 0.1 ppm and broadening of only 5−10 Hz.[7,8]

The UV spectra of **1** show a small red shift upon addition of DNA *only* in the presence of the guest AN, also supporting intercalation of the anthracene unit I. Fluorescence excitation spectra, observed at 440 nm, exhibit a new band around 290−310 nm in the red edge region of DNA again only in the presence of **1** and AN, in accordance with intercalation induced by the spectroscopically inert guest AN (Figure 2) . With excitation at 392 nm, near the isosbestic point of the UV spectra, addition of DNA leads to a continous fluorescence decrease (Figure 3), which is the consequence of intercalation, exhibiting more quenching than inclusion of the anthryl part in the β-CD cavity does.

In summary, the combined use of UV, of fluorescence, and in particular of NMR spectroscopy unequivocally establishes intercalation of the anthryl part I of **1** after addition of a guest molecule such as AN. The absence of any spectroscopic changes in mixtures of **1** and DNA alone shows that the complex represents a system with high positive cooperativity between heterotopic binding sites. Future studies will quantify both the strength of cooperativity and the binding affinity to ds-DNA. The principle shown in the present Communication may find use in nucleic acid reactions of medicinal and/or biotechnological importance, particularly in view of the many established

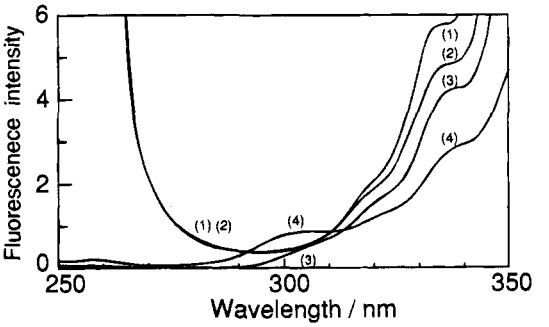


Figure 2. Fluorescence excitation spectra of **1** (10 μM) λ_{em} = 440 nm: (1) **1** alone, (2) with AN (5 mM), (3) with DNA (500 μM), and (4) with AN (5 mM) and DNA (500 μM).

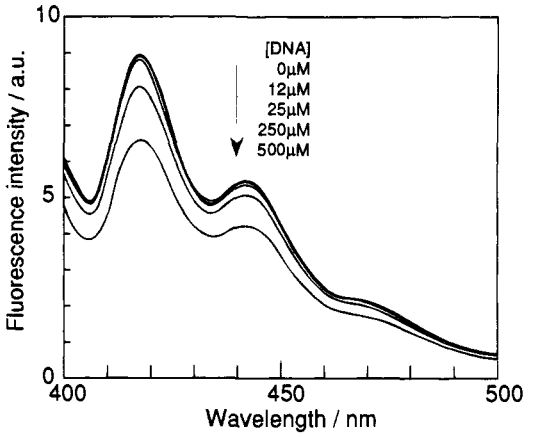


Figure 3. Fluorescene spectra of **1** (10 μM) with AN (5 mM) in the absence and in the presence of DNA, with excitation at 392 nm.

methods to modify cyclodextrins for specific interactions with a wide range of substrates.

Experimental Procedures. 6-(2-(9-Anthrylmethylamino)-ethylamino)-6-deoxy-β-CD (**1**) was prepared from 6-*O*-(*p*-tosyl)-β-CD,[9] converted into 6-(2-aminoethyl)amino-6-deoxy-β-CD by reaction with ethylendiamine,[10] then allowed to react with 9-(chloromethyl)anthracene in dry DMF in the presence of triethylamine at room temperature for 6 h. After precipitation with acetone and purification on CM-Sephadex C-25, **1** was obtained and characterized by TLC, elemental analysis, and ^{1}H-NMR. Calf thymus DNA was purchased from Pharmacia and purified by phenol extraction as described elswhere.[3] Purity and concentration of DNA were determined by absorption spectroscopy.

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