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Lipophilic Complexes with Cholic Acid-Based Cleft Compounds Including an Allosteric System

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Abstract

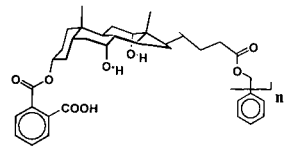
The complexation properties of the three cleft compounds **2**, **3** and **4** with two, three and four cholic acid arms coupled to a benzene core were studied in water with four fluorescence dyes **F1** to **F4** as guest structures, and compared with the parent mono-cholic acid derivative **1**. The cholic acid derivatives showed no aggregation or micellation behavior at up to mM concentrations. The coupling of three cholic acid arms to a trene unit yields an allosteric host **5**, which shows complexation of some aromatic fluorescence dyes as guest molecules only after addition of Zn(II) salts.

Introduction

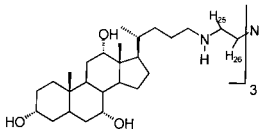
Steroids exert non-covalent lipophilic interactions, which are of interest for many biological systems, but also for artificial supramolecular complexes [1]. Cholic acid derivatives have been implemented in a variety of efficient synthetic receptors for e.g., sugar derivatives or for anions and other solutes [2–5]. Most of these, however, are restricted to the use of non-aqueous media, whereas in nature bile acids function in water. We wanted to explore the use of cholic acids in synthetic hosts which are hydrophilic enough to evaluate intermolecular lipophilic interactions in aqueous media. Until now there are only a few investigations with such hydrophilic steroidal host compounds [6]. Of particular interest was to see if such systems could be made allosteric.

A relatively inexpensive way to artificial receptors used also in the present work consists in the synthesis of clefts or tweezers, which can embrace guest molecules quite efficiently. The need to restrict the conformational mobility of hosts in the sense of optimal preorganization has been overestimated, as shown recently in a systematic investigation of host-guest complexes with a variable number of rotatable bonds [7]. That effective complexation is indeed possible with an open chain host has been shown earlier with the use of podands instead of crown ethers [8], and, e.g., with dimeric porphyrins which can complex nucleotides or nucleosides with affinities approaching μM ranges [9], as well as with inexpensive anion receptors [10].

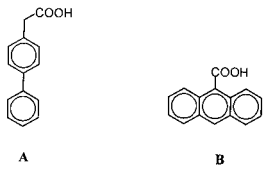
Self-association and micelle formation of the hosts were investigated using pinacyanolchloride as probe [11]. Measurements with the clefts **2**, **3** and **4** showed between 5×10^{-6} M and 1×10^{-3} M not the typical changes for micelle formation; however, the spectrum of pinacyanolchloride in the presence of e.g., the host **2** was similar to that reported by West and Pearce [12] for the dimeric dye. The results indicate that the new cholic acid derivatives do not aggregate below mM concentrations, but can induce dimer formation of the dye, for which a dimerization constant of 7×10^3 M was reported [12].



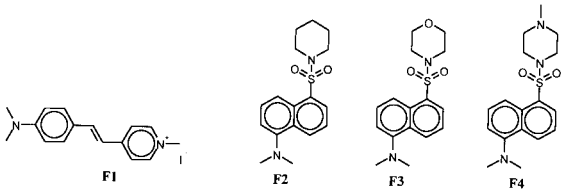
1, n = 1
2, n = 2 (benzene position 1,4)
3, n = 3 (benzene position 1,3,5)
4, n 4 (benzene position 1,2,4,5)

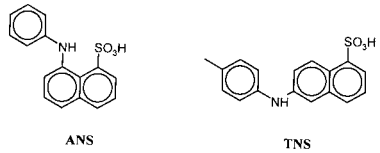


5



A B C





ANS TNS

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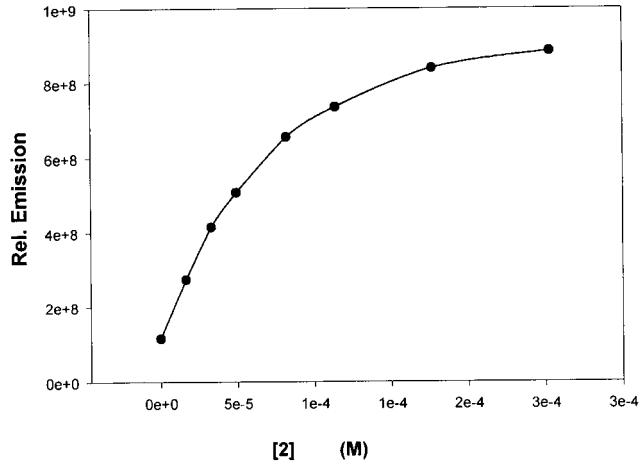

Figure 1. Titration curve with non-linear least-squares fitting for a 1 : 1 model of the complex between **2** and **F2**.

Associations of the clefts with fluorescence dyes

As anionic dyes were unsuitable for complexation with the anionic hosts we have chosen the cationic styryl **F1** and the electroneutral analogs **F2**, **F3** and **F4** of dansylamide, which in contrast to dansylamide itself cannot deprotonate at the sulfonamide group; they are easily accessible from dansylchloride and the corresponding amines. Figure 1 illustrates that the titration curves gave satisfactory non-linear curve fitting for a 1 : 1 model by inclusion of one dye inside the clefts. The binding constants K (Table 1) are with the monosubstituted model derivative **1** too low for determination (<10 M^{-1}), but reach values up to 5.5×10^4 with the cleft compounds, higher than those observed with e.g., cyclodextrins. The K value with all clefts are about the same for each dye and is the highest with the most hydrophobic guest **F2**, which is in accord with nonspecific lipophilic binding forces. The larger constants observed with styryl (**F1**) are due to the complementary positive charge of this guest, although the effect may be diminished by the resulting higher hydrophilicity. The complexation is accompanied by emission wavelength shifts of around 55 nm and emission enhancements (Table 1), due to less quenching in the lipophilic clefts. Figure 2 illustrates the molecular model of such a complex between **2** and styryl **F1**, generated with the force field CHARMm.

Addition of dioxane to the complex **2** + **F4** leads to a strong decrease of emission and therefore complexation (Figure 3), in line with the hydrophobic binding mechanism; similar observations have been made by Regen *et al.* [13]. Added salts produce salting-in, only, however, at concentrations above 0.4 M. (Figure 4).

Complexes with an 'umbrella' host with allosteric behavior

The cholanyl compound **5**, which was obtained by reaction of trene with cholic acid methylester and subsequent reduction, contains a transition metal binding unit and three

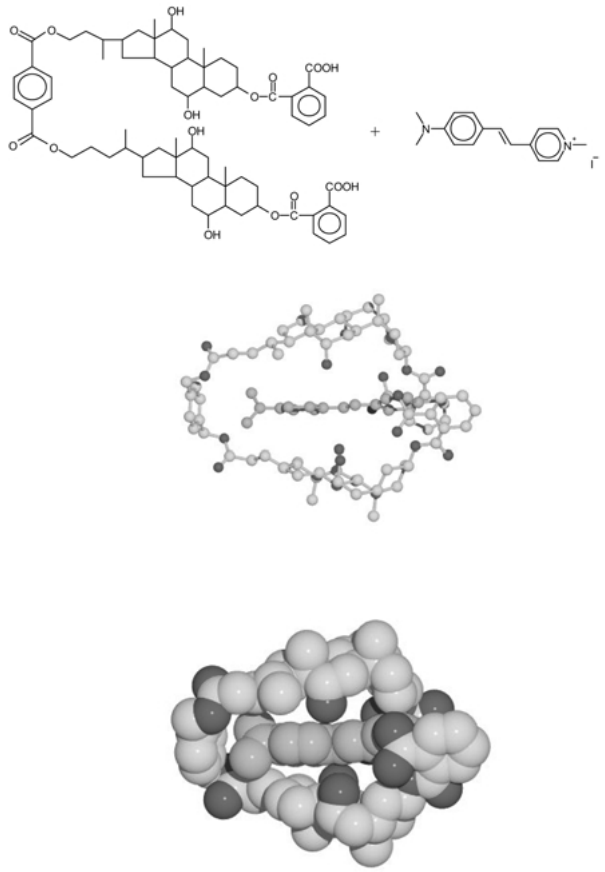

Figure 2. Simulation of the complex structure **2** + **F1** (CHARMm 6.02 gas phase calculations, DC = 3.0; Gasteiger charges.)

Table 1. Cleft compounds **1–4**: complexation constants K [10^3 M^{-1}], complexation free energies ΔG [kJ/mol], and fluorescence changes $\Delta\lambda/\Phi_{rel}$.[a]

Guest		Host			
		1	**2**	**3**	**4**
F1	K	<0.01	30	20	55
	ΔG	>−5.7	−25.5	−24.5	−27.0
	$\Delta\lambda/\Phi_{rel}$	<0 nm/1	36 nm/78	34 nm/63	38 nm/65
F2	K	<0.01	2.5	2.0	5.0
	ΔG	>−5.7	−19.4	−18.8	−21.1
	$\Delta\lambda/\Phi_{rel}$	<0 nm/1	54 nm/2.7	53 nm/2.0	56 nm/4.3
F3	K	<0.01	5.5	5.0	5.5
	ΔG	>−5.7	−21.3	−21.1	−21.3
	$\Delta\lambda/\Phi_{rel}$	<0 nm/1	55 nm/3.7	54 nm/3.6	53 nm/3.9
F4	K	<0.01	14	12	13.7
	ΔG	>−5.7 kJ/mol	−23.7	−23.3 kJ/mol	−23.6
	$\Delta\lambda/\Phi_{rel}$	<0 nm/1	56 nm/7.1	55 nm/6.0	59 nm/6.1

[a]Carbonate buffer pH = 10.0; 5 Vol% dioxane + 95% water; T = 298 K; [guest] = 2.10^{-6} M; error in K 15%; in $\Delta\lambda$: 2 nm; $\Delta\lambda$: emission wavelength change; Φ_{rel}: emission intensity, relative enhancement at 100% complexation (from least-squares fit).

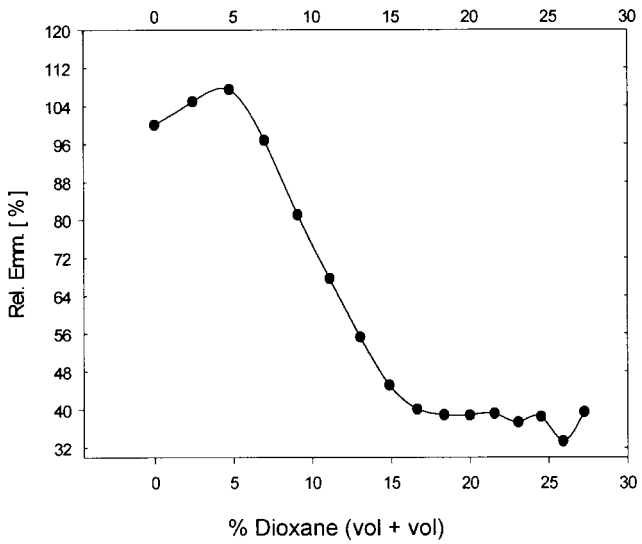

Figure 3. Solvent effect of added 1,4-dioxane on the fluorescence emission with the complex **2** + **F4** (2×10^{-6} M); carbonate buffer pH 10.0, ambient temperature.

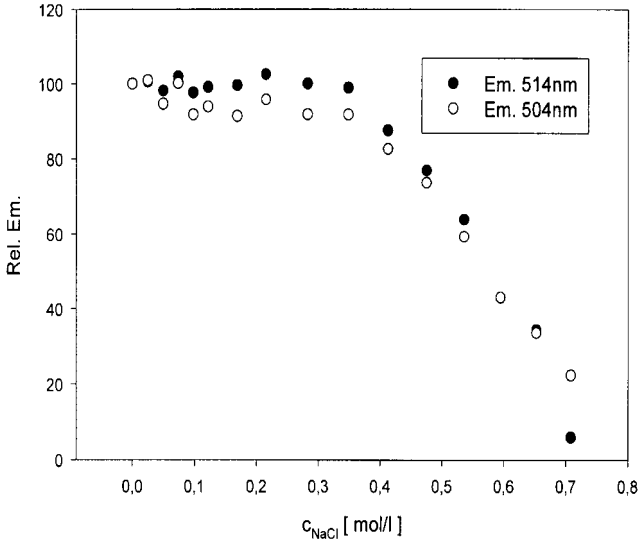

Figure 4. Salt effect of added sodium chloride on the fluorescence emission with the complex **2** + **F4** (2×10^{-6} M); carbonate buffer pH 10.0, ambient temperature.

steroidal arms. These could form an umbrella-type conformation [13] with inclusion of guest molecules [14], possibly enhanced upon metalation [15], and reminiscent of a system we had investigated earlier [16]. In view of their possible shielding effects on the steroid protons the aromatic guest compounds **A**, **B** and **C** were used in NMR experiments with **5** in methanol as a suitable solvent. The titration results from non-linear least squares fitting (Table 2) showed relatively small affinities, and CIS values (shifts at 100% complexation from the non-linear fit) which were always deshielding, reaching up to 0.118 ppm. Measurements in the absence of added Zn(II) salts showed **no shifts above 0.002 ppm**, this proves the allosteric effect of the metalation on the inclusion inside the formed 'umbrella' cleft (Figure 5). Although structure **5** is flexible, inclusion in the absence of the metal cation obviously costs too much conformation change; the concomitant strain must also be compensated (or 'paid') during complexation with the lipophilic guest molecules, which may contribute to their relatively small affinities. Methanol as solvent also decreases the association, for which reason attempts were made to complex the fluorescence dyes **ANS** and **TNS** in water. Unfortunately, the limited solubility of the hosts prevented full titrations, but with both dyes a distinct fluorescence increase with concomitant wavelength changes were observed **only** in presence of Zn(II), not with the host **5** alone. Approximate titrations yielded for **TNS** a K value around 4×10^4 M^{-1}, not far from the affinities observed with the other non allosteric hosts. (In contrast to the tren derivative **5** no cooperativity with added Zn(II) is observed with clefts based on coupling of desoxycholic acid to α,α-bipyridyl; obviously the conformational change induced by metalation on this system is too small to bring about a sizeable allosteric effect on binding of various fluorescence dyes tried in this context.)

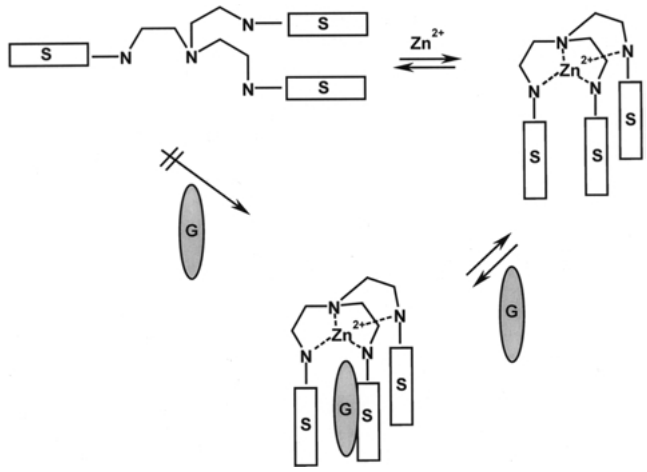

Figure 5. Allosteric effect of the metalation on the inclusion inside the formed 'umbrella' cleft.

Conclusions

Cholic acid-based host compounds hold particular promise in view of their biocompatibility and possible use for transmembrane transport of entrapped materials [14]. Open-chain derivatives in the form of clefts are attractive in view of their relatively easy synthetic availability and the wide tolerance for various guest structures, due to their built-in flexibility. This ability is also of interest for their influence on the kinetics of fatty acid ester hydrolysis [17]. The combination of steroidal clefts and polyamine scaffolds offers new ways to control the function of such supramolecular entities by allosteric interactions with metal ions.

Table 2. Association of the allosteric cleft compound **5** + Zn(II) with selected aromatic acids **A**, **B**, **C**; complexation constants K [M^{-1}]/induced H-NMR shifts at 100% complexation (CIS).*)

Guest			Proton						
			C_3H	$C_{12}H$	C_7H	$C_{19}H$	$C_{18}H$	$C_{21}H$	$C_{25}H$
A	K		–	26	26	[a]	24	[a]	28
	CIS	(0.0457)[c]	0.0523	0.0524			0.0725		0.1583
B	K		[a]	[a]	[a]	187	211	[a]	[a]
	CIS					0.012	0.118		
C	K		65/0.0649	200/0.331	170/0.279	123/0.186	122	146	[a]
	CIS						0.667	0.262	

*At 298 K in CD_3OD; CIS in [ppm], always shielding; error in K <20%.
[a]Too small shift changes for evaluation.
[b]Overlapping signals.
[c]CIS calculated with average K from other signals.

Acknowledgements

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