A synthetic receptor for phosphocholine esters†

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A bifunctional Zn-salen modified cavitand, reminiscent of the enzyme phospholipase C, shows high efficiency and synergic effect in the binding of the phospholipid DOPC.

Non-covalent interactions are often involved in natural processes such as catalysis and recognition.[1] In particular Lewis acid–base and cation-π interactions play a role in the mechanisms of many enzymes[2] and biological receptors.[1b] Phosphocholine derivatives are natural targets of several enzymes and receptors that recognize and bind these substrates through weak non-covalent forces.[1a] An example is phospholipase C, a metallo-enzyme that catalyzes the hydrolysis of phospholipids.[3] This protein isolated from *Bacillus cereus* contains three zinc ions in its active site,[4a] and crystal structure analysis of several protein-ligand complexes revealed the importance of simultaneous zinc–phosphate coordination and cation-π interactions between the trimethylammonium group of the guest and Tyr/Phe amino acid residues of the protein.[4b] Some examples of synthetic receptors for the recognition of phosphate esters have been reported in literature.[5]

Inspired by a complex between phosphorylcholine and the McPC603 antibody,[6] de Mendoza *et al.* developed a guanidinium modified calix[6]arene[7] and established that binding of a phosphocholine derivative involved both hydrogen bonding and cation-π interactions.[7a] This receptor is efficient in the recognition even though the calix[6]arenes unit does not provide a preorganized cavity for the binding. Accordingly, the affinity is significantly lower than that expected for an additive contribution of the two interactions.

Recently we introduced the metal complex **1**-Zn[8] fused to a cavitand scaffold,[9] and we report its binding properties to the phospholipid 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC, Fig. 1). This complex features a deep and preorganized cavity for selective[8b,10b] cation-π interactions with the trimethylammonium head[10] of the guest and a Zn^{II} ion at the top of the cavity that is positioned to coordinate the phosphate group of the guest[11] (see Fig. 2).

The ^{1}H-NMR spectrum of **1**-Zn (2 mM) in $CDCl_3$ shows signals of the methine protons between 5.5 and 6 ppm, indicating that the cavitand is in the vase conformation.[9c] When DOPC is added to this solution, the signal for the methyl groups of the trimethylammonium appears at $\delta = -0.42$ ppm, with an upfield shift of $\Delta\delta = 3.8$ ppm compared to the free guest (Fig. 3). Free guest can only be observed upon the addition of more than one equivalent of guest, indicating a binding constant higher than 10^4 M^{-1}, with slow exchange on the NMR timescale. The ^{31}P-NMR spectrum of a **1**-Zn solution and more than 1 equivalent of DOPC also shows different signals for the bound and free guest. The signal of the bound guest is upfield shifted ($\Delta\delta = 3.3$ ppm) indicating a coordination of the phosphate diester to the zinc ion.[12] The formation of the **1**-Zn·DOPC complex was also observed in the gas phase by ESI mass spectrometry (see electronic

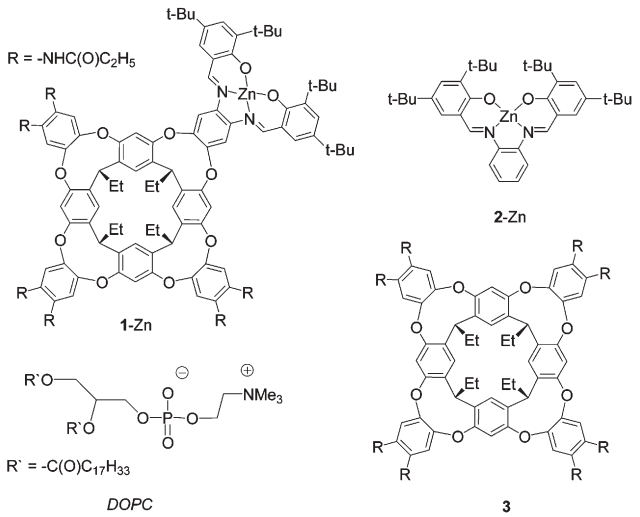

Fig. 1 Receptors and guest DOPC used in the binding studies.

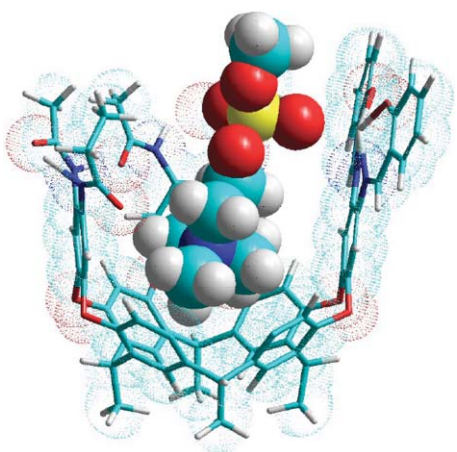

Fig. 2 Energy minimized structure (HyperChem, *PM3* semiempirical calculation) of a simplified model of the complex **1**-Zn·DOPC (one wall of the cavitand has been removed for viewing clarity).

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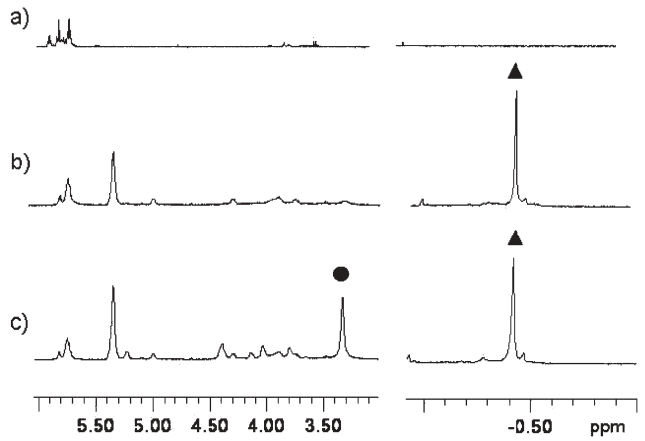

Fig. 3 ^{1}H-NMR spectra (600 MHz, $CDCl_3$, 300 K) of a 2 mM solution of **1**-Zn (a) without any guest (b) with 0.9 and (c) 1.5 equivalents of DOPC, respectively. $-N(CH_3)_3^+$ of the bound (▲) and unbound (•) DOPC.

supplementary information, ESI†). Isothermal titration calorimetry (ITC) confirmed a high binding constant between DOPC and **1**-Zn ($K = 6.0 \times 10^5$ M^{-1})† whereas the binding with the nonfunctionalized octaamide **3**[13] is about 3000 times lower (see Table 1, entry 3).

Furthermore, the signal of the trimethylammonium protons of the bound DOPC within **3** is broader (suggesting a faster exchange of the guest in and out of its cavity), and less upfield shifted ($\delta = -0.32$ ppm) indicating that the trimethylammonium group is, on average, not as deep in the cavity of **3** as it is in **1**-Zn. The Zn–phosphate interaction likely forces the guest to stay deeper in the cavity of **1**-Zn.

The binding of the DOPC toward the Zn-salen unit **2**-Zn was also investigated to evaluate the independent contribution of the metal cation. The association constant was 3.8×10^3 M^{-1} (Table 1, entry 2), about 150 times lower than the binding with **1**-Zn. These results clearly demonstrate the synergic effect between the metal center and the cavity.

UV-Vis titration experiments with DOPC and the receptors **1**-Zn and **2**-Zn were carried out at different wavelengths (380–450 nm, 300 K, $CHCl_3$). The spectra of the metal complexes slightly change adding increasing amounts of DOPC† providing further evidence for the interaction of the guest with the Zn-salen unit of the receptors.

Thermodynamic parameters for the binding of DOPC to the different receptors were measured by ITC and NMR titrations experiments at different temperatures (Table 1). Interestingly, all binding equilibria are endothermic and entropically driven.

Table 1 Thermodynamic data for the binding of DOPC to **1**-Zn, **2**-Zn, and **3** (chloroform, 300 K)[a]

Entry	Receptor	K/M^{-1}	ΔH°/kcal mol^{-1}	ΔS°/e.u.
1[b]	**1**-Zn	6.0×10^5	7.1	50
2[b]	**2**-Zn	3.8×10^3	2.0	23
3[c]	**3**	180	8.3	38

[a] Error limit ± 15%, see ESI. [b] Isothermal titration calorimetry experiments, $CHCl_3$. [c] Titration experiment monitored by NMR, $CDCl_3$.

Apparently, the complexation of the DOPC results in the liberation of solvent molecules bound in the cavity, to the Zn^{II} ion and to the free solute. Semiempirical quantum mechanical calculations (*PM3*, HyperChem 7.51) were carried out on a simplified model of the complex DOPC·**1**-Zn (Fig. 2). These calculations show the geometrical complementarity between host and guest and indicate simultaneous cation-π interaction and a metal–phosphate coordination.

In conclusion, the metallo-cavitand **1**-Zn is reminiscent of the protein Phospholipase C, and represents a nearly ideal recognition site for the naturally-occurring DOPC. The combination of a deep, selective binding pocket[8b,10b] and a well-positioned metal center results in additive contributions of the two binding subunits and shows significantly higher synergic effect than previously achieved.[7a]

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