Binding of Acetylcholine and other Molecular Cations by a Macrocyclic Receptor Molecule of Speleand Type[1]

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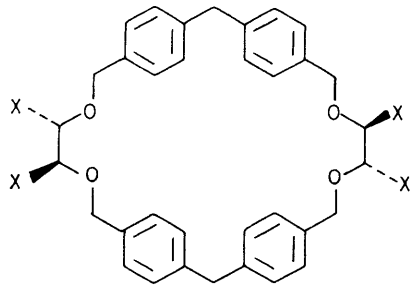
The chiral tetracarboxylic macrocycle (**1**) is a receptor molecule of the speleand type which binds strongly quaternary as well as primary ammonium cations, in particular acetylcholine and methylviologen.

Speleands have been defined as cryptand type receptor molecules connecting polar binding subunits with concave, more or less rigid apolar shaping components.[2,3]† Such a combination should be especially valuable‡ in designing molecular receptors capable of forming stable, selective supermolecules (speleates) by complexation of specific substrate species.[4]

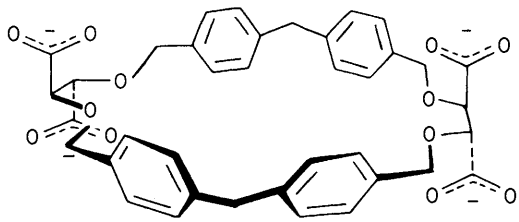
We now report the synthesis and some remarkable binding properties of a chiral, polyfunctional macrocyclic molecule [(**1**)-H], which combines two tartaric acid groups with two diphenylmethane fragments. The introduction of tartaric acid units into macrocycles[5] greatly increases the binding of metal and organic cations by polyether systems.[6] Also, the geometrical features of the diphenylmethane group make it a suitable structural element for the construction of molecules containing internal cavities capable of including substrate species.[7]

the bis-thallium salt of (*R,R*)-(+)-tartaric acid bis-dimethylamide (*N,N*-dimethylformamide, 60—65 °C), following a procedure described earlier,[5] gave a mixture from which the (2 + 2) macrocyclic tetramide (**2**) was isolated by chromatography on silica and crystallization from chloroform–diethyl ether (12% yield; m.p. > 350 °C; $[\alpha]_D = -84.3°$, *c* 2.06, $CHCl_3$).§ Nitrosation of (**2**) (N_2O_3, 0 °C, acetonitrile) gave a solution of the tetranitroso-amide (**3**) (not isolated; identified by n.m.r.) which, by treatment with aqueous NaOH, evaporation, and crystallisation from 50% aqueous methanol, gave the sodium salt [(**1**)-Na] as white hydrated crystals (55% yield; $[\alpha]_D = -6.9°$, *c* 0.78, H_2O). The tetracarboxylic acid [(**1**)-H] was obtained from [(**1**)-Na] by acid treatment (aqueous HCl) followed by crystallization from ethanol on addition of a small amount (*ca.* 5%) of water (m.p. > 350 °C; $[\alpha]_D$ + 10.4°, *c* 0.84, ethanol).¶

In its fully ionized form (**1**) is a large, water-soluble macrocycle of D_2 symmetry containing alternating polar and apolar groups. Although its molecular cavity may vary in size by rotation around single bonds, it should not collapse in view of the structural features of both the diphenylmethane and tartrate subunits. The average diameter and depth of the cavity in its extended form are both *ca.* 6—7 Å. In the schematic representation (**1S**) the plane of the phenyl rings[7a] and the carboxylate groups[6] are oriented more or less perpendicularly to the average plane of the ring.



(**1**) X = CO_2^-
[(**1**)-H] X = CO_2H
[(**1**)-Na] X = CO_2Na
(**2**) X = CONHMe
(**3**) X = CON(NO)Me



(**1S**)

When various ammonium cations were added to [(**1**)-Na] in aqueous solution (or conversely), the ^1H n.m.r. spectra of the cations displayed large upfield shifts, which reached a limiting value at high proportion of macrocycle. These shifts may be attributed to complex formation between the ammonium ions and the macrocycle (**1**). Plots of the shifts observed *vs.* the macrocycle:ammonium ion ratio agreed with a 1:1 stoicheiometry for the complexation and stability constants for the complexes were obtained. Some of the results obtained are listed in Table 1.

(*i*) The tetracarboxylate macrocycle (**1**) functions as a receptor molecule forming remarkably stable 1 : 1 complexes with a variety of molecular cationic substrates in aqueous solution.

† Cylindrical macropolycycles containing rigid bridges also present analogies with speleand structures; see references in ref. 2.

‡ This is strengthened by recent reports that a number of solid state adducts of macrocycles with neutral molecules are clathrate-type lattice inclusion compounds and not molecular inclusion complexes; *e.g.*: R. Hilgenfeld and W. Saenger, *Angew. Chem.*, 1982, **94**, 788; F. Vögtle, W. H. Müller, H. Puff, and E. Friedrichs, *Chem. Ber.*, 1983, **116**, 2344.

§ The (3 + 3) macrocyclic hexamide was also isolated in 5% yield. The compounds had spectroscopic (^1H, ^{13}C n.m.r., and mass spectra) and microanalytical properties in agreement with the proposed structures.

¶ The nitrosation followed a procedure developed in the course of other work (C. Burrows and J. M. Lehn, unpublished results); it requires carefully controlled reaction conditions; a detailed description of the procedure will be published later and is available on request.

Table 1. Stability constants (log K_s) and limiting ^{1}H n.m.r. upfield shifts ($\Delta\nu_c$) calculated for the complexes of receptor molecule (**1**) with molecular cationic substrates.[a]

Entry	Substrate	$\Delta\nu_{obs}$[b]	$\Delta\nu_c$[b]	log K_s[c]
1	NMe_4^+	53	265	2.4
2	NEt_4^+	[d]	[d]	*ca.* 2.0
3	$MeCO_2CH_2CH_2NMe_3^+$	110	315	2.7
4	$PhCO_2CH_2CH_2NMe_3^+$	150	315	2.9
5	$PhCH_2NMe_3^+$	97	300	2.7
6	*N*,*N*-Dimethylpiperidinium	46	230	2.4
7	$^+Me_3NCH_2CH_2NMe_3^+$	195	204	4.4
8	$^+Me_3N[CH_2]_3NMe_3^+$	158	166	4.3
9	$^+Me_3N[CH_2]_4NMe_3^+$	128	136	4.2
10	$MeCH_2CH_2NH_3^+$	71	290	2.5
11	$^+H_3N[CH_2]_3NH_3^+$	345	347	≥5
12	Methylviologen	67.7	68.5	≥5

[a] log K_s and $\Delta\nu_c$ are calculated from the plots of substrate chemical shift as a function of macrocycle : substrate ratio, obtained by addition of an aqueous solution of the ammonium salt to an 1.0—1.1 mM aqueous solution of [(**1**)-Na], adjusted to pH *ca.* 7.0, at *ca.* 23 °C. Competition experiments between $^+Me_3NCH_2CH_2NMe_3^+$ taken as reference substrate, and other cations (NEt_4^+, acetylcholine, and methylviologen) gave results consistent with the direct method. This competition method was also used for determining the sequence of alkali metal cation binding and for Et_3N^+–$[CH_2]_3$–NEt_3^+ (log K_s *ca.* 2.8). [b] Shifts of Me or (α-CH_2) signal given in Hz at 200 MHz; $\Delta\nu_{obs}$ is the largest upfield shift observed at the highest macrocycle: ammonium ion ratio. [c] log K_s ± 0.2; K_s in l mol^{-1}; determination of the largest stability constants is difficult owing to limitations of the method at high values; all values incorporate competition with Na^+ counterions of [(**1**)-Na]. [d] K_s determined by competition with $Me_3N^+CH_2CH_2NMe_3^+$, see footnote a.

Macrocycle (**1**) also binds alkali metal cations in the sequence $Li^+ < Na^+ < K^+ < Cs^+$ (log K_s *ca.* 1.7—2.8) (Table 1, footnote a).**

(*ii*) The strong binding observed incorporates a large macrocyclic effect, since no complexation was detected under the same conditions between the substrates of Table 1 and the dibenzyl ether of the tartaric acid dianion.

(*iii*) The structures of the complexes are not known in detail, but the results favour formation of macrocyclic speleates by more or less complete inclusion of the ammonium substrates into the molecular cavity of (**1S**). Thus the protons of the cations are subjected to the shielding effect of the aromatic groups and large upfield shifts are observed, as for other molecular inclusion complexes.[7,9] The 1:1 stoicheiometry and the large macrocyclic effect also indicate inclusion complexation. Binding of ^+R_3N–$[CH_2]_n$–NR_3^+ (n = 2,3; R=H, Me) to the chiral receptor (**1**) forms fast exchanging complexes in which the α-CH_2 protons are non-equivalent, showing a large difference in chemical shifts.

(*iv*) The strong binding of substituted ammonium ions in aqueous solution yields by far the most stable complexes known to date for such cations.†† Aromatic quaternary ammonium cations associate with a paracyclophane dicarboxylate[10] and the diquat cation is bound by large polyether macrocycles in acetone.[11] Whereas [18]-O_6 type macrocycles do not bind substituted ammonium ions, the present complexes formed by (**1**) are much more stable than even the strongest RNH_3^+ or alkali metal cation complexes of 18-crown-6.

(*v*) Of special importance from the biological point of view, is the appreciable binding by receptor (**1**) of the neurotransmitter acetylcholine and of related quaternary ammonium ions.[12] This provides one specific answer to the general question of how acetylcholine can be bound. It may shed light on the still poorly understood problem of the nature of the interactions which play a role in neurotransmitter–receptor binding,[13] in particular for biological acetylcholine receptors, which are the subject of current research.

Also of interest is the very strong binding of methylviologen, MV^{2+}, a substance which has been much studied recently in relation to electron transfer and photochemical energy storage processes. It should modify the redox potential and electron transfer rates of the MV^{2+}/MV^+ couple.

(*vi*) Receptor (**1**) also represents a new type of binding unit for primary ammonium cations. The stabilities of the complexes with the propylammonium and propylenediammonium ions are much greater than those observed with the [18]-O_6 type of ligands; only the bis-tartro-[18]-O_6 displays similar binding strength.[6] Thus, the highly polar tartrate fragments of (**1**) provide the electrostatic interactions for strong RNH_3^+ binding, indicating that a macrocyclic polyether structure is not required. However, a more detailed description of the anchoring mode of RNH_3^+ must await a crystal structure determination of a complex.

(*vii*) A very high binding selectivity in favour of quaternary ammonium ions exists at high pH where less substituted ammonium ions are deprotonated.

(*viii*) Both electrostatic and hydrophobic effects contribute to the stability of the complexes of receptor (**1**), underlining the basic principle of speleand design.[2,3] Methylation of the RNH_3^+ groups lowers the stability, owing to diminished electrostatic (and hydrogen bonding) interactions (Table 1, entries 8 and 11), but the decrease is modest in view of the drastic change undergone by the cationic site. The increase in stability from monoammonium to diammonium ions (Table 1, entries 1 and 7—9, 10, and 11) and for cations containing organic groups of larger size (Table 1, entries 1 and 5, 3 and 4) also indicates the operation of electrostatic and lipophilic effects respectively. However, substitution of Et for all three Me groups on N decreases binding (Table 1, entries 1 and 2, 8 and footnote a), perhaps because of too large an increase in size.

(*ix*) Ligand (**1**) is also a ditopic coreceptor[3] in which the distance of the two tartrate binding subunits should enable linear recognition of a dicationic substrate of complementary length, as demonstrated earlier for coreceptors of linear diammonium[3,7d,9] and dicarboxylate[14] substrates.

These results support the idea that especially attractive features may be conferred upon speleands and speleates by the simultaneous operation of electrostatic and hydrophobic effects.[2,3] Further enhancement of binding strength and selectivity should result from constructing deeper cavities of other shapes and sizes, adding bridges to enter the polycyclic manifold, and locating strategically suitable binding subunits as well as reactive functional groups for developing molecular catalysts. Strong and selective complexation of biologically important molecules, like neurotransmitters, amino-acids, nucleic bases and nucleotides, cofactors, allosteric effectors *etc.*, would provide models of biological receptor–substrate interactions, while, at the same time, expanding the ability to manipulate molecular interactions for the design of supermolecular systems.

** The presence of weaker 2:1 complexes cannot be excluded (in particular for the monocations) when the substrate is in excess.

†† In the complex of a highly lipophilic $RNMe_3^+$ ion with γ-cyclodextrin, the R group is in the cavity and the cationic group is located outside: N. J. Turro, T. Okubo, and C.-J. Chung, *J. Am. Chem. Soc.*, 1982, **104**, 1789. Similar behaviour is found with a 18-crown-6 polymer: B. Roland and J. Smid, *J. Am. Chem. Soc.*, 1983, **105**, 5269.

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