182. Molecular Receptors. Structural Effects and Substrate Recognition in Binding of Organic and Biogenic Ammonium Ions by Chiral Polyfunctional Macrocyclic Polyethers Bearing Amino-Acid and Other Side-Chains[1])

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(14. VI. 82)

Summary

The stability constants of the complexes formed by the polyfunctional macrocyclic receptor molecules of type **1** with cationic substrates have been determined and analyzed in terms of structural factors. The binding strength is dominated by electrostatic interactions; the tetracarboxylate **1** (O^-) forms by far the most stable complexes of all known macrocyclic polyethers. Lipophilic groups also significantly enhance stabilities, stressing the role of such residues in biological receptor sites. The complexation selectivity of organic ammonium cations is determined by two main factors: *a) central discrimination,* resulting from the macrocyclic cavity, strongly favors binding of primary ammonium cations with respect to more highly substituted ones and also distinguishes among ($R\text{-}NH_3^+$)-substrates differently substituted on the C(α)-atom; *b) lateral discrimination* arises from interactions between the substrate and the side chains borne by the macrocycle; again electrostatic and lipophilic group effects play the major role; diammonium cations are especially strongly bound. Complexation of biogenic amines follows the same trends; for instance noradrenaline and norephedrine are selectively bound with respect to adrenaline and ephedrine, pointing towards potential applications in selective binding and transport of biologically active substances.

Introduction. - The design of synthetic molecular receptors requires the selection of structural units capable of interacting with the functional groups present in the substrates to be bound. Of special interest in this respect are macrocyclic polyethers and aza-ethers, which have attracted much attention in recent years by their ability to complex various inorganic and organic cations, in particular primary ammonium ions (see [1–13] and references therein). These properties are also attractive in view of their relation to those of natural ionophore antibiotics and their extension to the

[1]) Preliminary communication: [1].

[2]) E.R.A. N° 265 of the C.N.R.S.

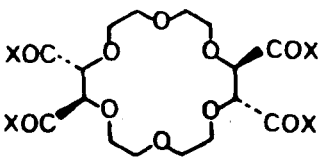
0018-019X/82/6/1853-15$01.00/0

complexation of biologically important substrates, like biogenic amines [6] [14]. Numerous studies have been devoted to the design and synthesis of a great variety of macrocycles with the aims of improving the stability and selectivity of the complexes [1–13], of achieving enantiomeric resolution with chiral macrocycles [5] [9] [11] [15], of performing molecular catalysis on the bound substrates with functionalized macrocycles [8] [16].

In our own approach to the design of receptor molecules for primary ammonium cations [1] [7] [8] [17], we developed a basic macrocyclic unit **1** which meets several important requirements: *1)* it is a direct derivative of 18-crown-6 (**2**), which, on the basis of the extensive work on numerous macrocyclic polyethers [2–13] is known to form the most stable complexes; *2)* it bears several functional groups, for attachment of a variety of side chains or incorporation into macropolycyclic structures; *3)* it is chiral of known absolute configuration; *4)* it is obtained in a one-step cyclization reaction from easily accessible starting materials, including the optically active components which are amides of L-(+)-tartaric acid.

A number of derivatives **1**(X) of the basic macrocyclic unit have been synthesized. Their remarkable binding properties have been briefly reported earlier [1] [7] [8] [18]. We now describe in detail the effect of structural factors on the stability and selectivity of the complexes formed by the macrocyclic receptor molecules of type **1** with K^+, NH_4^+ and alkylammonium cations. The results should *a)* allow to analyze the interactions responsible for the binding features; *b)* provide guidelines for further designed modification of the system, directed towards a specific set of binding properties; *c)* give insight into structural factors which may also play a role in substrate binding by biological receptor sites.

Results. – Ligands **1** (NMe_2) and **1** (NHMe) were obtained from the corresponding *(R,R)*-tartramides in one step *via* ring closure [17]. Acid-catalyzed hydrolysis of **1** (NMe_2) led to the tetracarboxylic acid **1** (OH). Conversion of **1** (OH) into the acid



1

X=	Designation	X=	Designation
NMe_2	**1** (NMe_2)	L-NHCH(COO^-)CH_2–(3-indolyl)	**1** ($TrpO^-$)
NHMe	**1** (NHMe)	L-(NHCH(COO^-)$CH_2CH_2COO^-$)	**1** ($GluO^{2-}$)
OH	**1** (OH)	NH–(6-sulfonato-2-naphthyl)	**1** (2,6-$Napht^-$)
$NHCH_2COOMe$	**1** (GlyOMe)	NH–(3-carboxylato-2-naphthyl)	**1** (2,3-$Napht^-$)
O^-	**1** (O^-)	L-NHCH(COO^-)$CH_2CH_2CH_2$-$NHC(NH_2)_2^+$)	**1** ($ArgO^{\pm}$)
$NHCH_2COO^-$	**1** ($GlyO^-$)	$NHCH_2CH_2NH_3^+$	**1** (ED^+)
	**1** ($ProO^-$)	$NHCH_2CH_2$–(3-carbamoyl-1-pyridinium)	**1** (Nic^+)
L-(NHCH(COO^-)$CH_2C_6H_5$	**1** ($PheO^-$)	H replacing COX	**2**

Table. *Stability constants* K_s[a]) *(1 mol^{-1}) in water for the complexation of cationic substrates by the tetrafunctional macrocyclic ligands* **1** *(X)*

Entry	Ligand	Substrate	K_s[b])	Entry	Ligand	Substrate	K_s[b])
1	**1**(NMe_2)	K^+	75	42		NH_4^+	30
2		NH_4^+	<5	43[c])		$^+H_3NCH_2CH_2NH_3^+$	160
3	**1**(NHMe)	K^+	20	44	**1**($ProO^-$)	NH_4^+	50
4	**1**(GlyOMe)	K^+	20	45	**1**($PheO^-$)	NH_4^+	150
5	**1**(O^-)	K^+	3.0×10^5	46	**1**($TrpO^-$)	K^+	5.5×10^4
6		NH_4^+	3.4×10^3	47		NH_4^+	440
7		$CH_3NH_3^+$	750	48		$CH_3NH_3^+$	100
8		$CH_3CH_2NH_3^+$	270	49		$CH_3CH_2NH_3^+$	70
9		$CH_3(CH_2)_3NH_3^+$	210	50		$C_6H_5CH_2NH_3^+$	150
10		$(CH_3)_2CHNH_3^+$	40	51		$C_6H_5CH_2CH_2NH_3^+$	100
11		$(CH_3)_3CNH_3^+$	40	52		$C_6H_5CH(OH)CH_2NH_3^+$	150
12		$(CH_3)_2NH_2^+$	<5	53		$HOCH_2CH_2NH_3^+$	150
13		$C_6H_5CH_2NH_3^+$	650	54[c]) [d])		$^+H_3N(CH_2)_2NH_3^+$	1.0×10^4
14		$C_6H_5CH_2CH_2NH_3^+$	310	55[d]		$^+H_3N(CH_2)_3NH_3^+$	1.7×10^3
15		$C_6H_5CH_2CH_2NH_2^+CH_3$	25	56		$^+H_3N(CH_2)_4NH_3^+$	600
16		$C_6H_5CH_2CH_2N^+(CH_3)_3$	<5	57[c]) [f])		$Nic^+(CH_2)_2NH_3^+$	2.3×10^3
17		Phenylalanine±	30	58[f])		$Nic^+(CH_2)_4NH_3^+$	400
18		Glycinemethylester$^+$	700	59	**1**($GluO^{2-}$)	K^+	2.7×10^4
19		$HOCH_2CH_2NH_3^+$	500	60		NH_4^+	250
20		$HOCH_2CH_2NH_2^+CH_3$	10	61[d])		$^+H_3N(CH_2)_2NH_3^+$	800
21		$HOCH_2CH_2NH_2^+(CH_3)_2$	5	62		$^+H_3N(CH_2)_3NH_3^+$	430
22		$HOCH_2CH_2CH_2NH^+$	300	63		$^+H_3N(CH_2)_4NH_3^+$	180
23		$HOCH_2CH(CH_3)NH_3^+$	40	64	**1**(2,6 $Napht^-$)	K^+	1.0×10^3
24		$HOCH(C_6H_5)CH_2NH_3^+$	550	65		NH_4^+	50
25		Noradrenaline$^+$	530	66		$^+H_3N(CH_2)_4NH_3^+$	150
26		Adrenaline$^+$	60	67		$^+H_3N(CH_2)_6NH_3^+$	250
27		Norephedrine$^+$	100	68[g])		$^+H_3N(CH_2)_8NH_3^+$	450
28		Ephedrine$^+$	20	69	**1**(2,3-$Napht^-$)	K^+	1.7×10^3
29		Tryptamine$^+$	320	70[h])		$HOCH_2CH_2NH_3^+$	65
30		Serotonine$^+$	320	71	**1**($ArgO^{\pm}$)	K^+	380
31[c])		Histamine$^+$	6×10^3	72		$^-O_3S(CH_2)_2NH_3^+$	<5
32[c])		Imidazole$^+$	10	73		$^-O_2C(CH_2)_3NH_3^+$	<5
33		Guanidine$^+$	45	74		$^-O_2C(CH_2)_5NH_3^+$	<5
34[c]) [d])		$^+H_3N(CH_2)_2NH_3^+$	4×10^4	75	**1**(ED^+)	K^+	20
35[d])		$^+H_3N(CH_2)_3NH_3^+$	6.0×10^3	76	**1**(Nic^+)	K^+	~5
36		$^+H_3N(CH_2)_4NH_3^+$	1.7×10^3	77	**2**	K^+	110 [2]
37		$^+H_3N(CH_2)_6NH_3^+$	1.2×10^3	78		NH_4^+	17 [33] [34]
38[e])		$^+H_3N(CH_2)_8NH_3^+$	<900	79		$CH_3NH_3^+$	13 [34]
39[c]		$(CH_3)_2NH^+(CH_2)_2NH_3^+$	2.5×10^4	80		$CH_3CH_2NH_3^+$	10 [34]
40		$(CH_3)_2NH^+(CH_2)_3NH_3^+$	4.1×10^3	81		$(CH_3CH_2)_2NH_2^+$	7 [34]
41	**1**($GlyO^-$)	K^+	650	82		$(CH_3CH_2)_3NH^+$	5 [34]

a) Determined with K^+ or NH_4^+ ion-selective electrodes (by competition with NH_4^+ for the substituted salts), in 0.1M aqueous tetramethylammonium phosphate or triethanolamine HCl buffers pH 7.0, 25°.
b) Accuracy ±10% for K^+, NH_4^+ and ±20% for the others; due to the experimental procedure used, K_s differences of *ca.* 10% are significant within a series of substrates.
c) Corrected for incomplete protonation at pH 7; accuracy ±30%.
d) A lower value was measured in the phosphate buffer, possibly because of the formation of a diammonium hydrogenphosphate complex.
e) Assuming a 1:1 stoichiometry.
f) Nic^+ = 3-carbamoyl-1-pyridinium.
g) The complex with dodecamethylenediammonium precipitated.
h) All other complexes formed, including ethylenediammonium, precipitated.

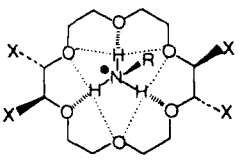
chloride **1** (Cl) and condensation with the required amines gave the corresponding tetraamide derivatives, **1** (NHR) listed above.

The association constants K_S for the complexation of these ligands with a variety of cationic substrates have been determined potentiometrically in aqueous buffer

using ion-selective (K^+, NH_4^+) membrane electrodes. The measurements were performed either directly for K^+ and NH_4^+, or indirectly for the other substrates, by competitive displacement of K^+ or NH_4^+ in presence of the substituted ammonium cation studied. The results are given in the *Table;* they may be discussed in the light of the features which synthetic molecular receptors should achieve: high stability and selectivity of complexation.

Discussion. – 1) *Structure of the complexes of* **1** *with primary ammonium cations.* In the complexes formed by macrocyclic polyethers of 18-crown-6 type, the cationic substrates interact with the ether O-atoms and are held in the centre of the circular cavity, as shown by the crystal structures of the complexes of 18-crown-6 (**2**) itself with K^+ [19] and with ammonium cations (NH_4^+ [20], $C_6H_5CH_2NH_3^+$ [21]). Similar features have been found for the crystal structures of complexes formed by substituted macrocycles of type **1**: the K^+-complex of **1** (NMe_2) [22]; the ethylenediammonium complex of a dicarboxylate of **1** (OH) [23]; the Ca^{2+}-, Sr^{2+}- and Na^+-complexes of diamide derivatives of **1** (OH) [24].

In the complexes with R–NH_3^+, the substrate is anchored in the centre and on top of the ring, and the R-group extends above the macrocycle. Since theoretical [25] [26] and experimental results [27] indicate that in NH_4^+- and in R–NH_3^+-groups the positive charge is mainly distributed over the H-atoms (and not located on the N-atoms, as implied by the valence bond picture) the *mode of binding* rests on their electrostatic interactions N–$H^{\delta+}\cdots$O with the O-sites. In view of the linear N–H$\cdots$O arrangement found in the crystal structures of complexes [20] [21] [28], a simplified picture **3** of the binding schem may be considered to involve [8] an array of three linear N–$H^{\delta+}\cdots$O H-bonds, completed by a set of six electrostatic interactions between the partial charges on the H-atoms with the neighbouring O-atoms, which may also be taken as six weaker, bent H-bonds. Less regular binding arrays may of course occur in the complexes of substituted, less symmetrical macrocycles.



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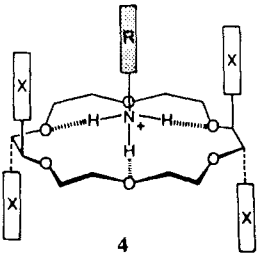

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In the substituted macrocycles of type **1**, the lateral appendages X are found in axial orientation with respect to the mean plane of the ring [22–24] and the $^+H_3NCH_2CH_2$-residue of the ethylenediammonium complex is located between two carboxy groups of the dicarboxylate of **1** (OH). Furthermore, the appearance of a charge-transfer band in the complex between **1** ($TrpO^-$) and a pyridinium substrate (*Table,* entry 57), has been taken as indication that in solution too, the substrate is sandwiched between the more or less axially oriented side arms X [29]. On the basis

of these results one may expect that in the complexes formed by R–NH_3^+-substrates with the receptor molecules of type **1**, the R-residues are located between the lateral groups X, so that the structure of these complexes may be represented schematically by **4**. Such an arrangement should allow lateral interactions between the X-groups and the R-residues, a feature of much interest for complexation selectivity.

2) *Stability of the complexes.* The intrinsic binding abilities of the ligands are best revealed by comparing the stability constants of their potassium or ammonium complexes, which are measurable in all cases, and where in principle no lateral-group effects interfere (see next *Section*). These K_s-values are plotted in *Figure 1* and listed in the *Table*; they may be understood on the basis of structural and of electrostatic considerations.

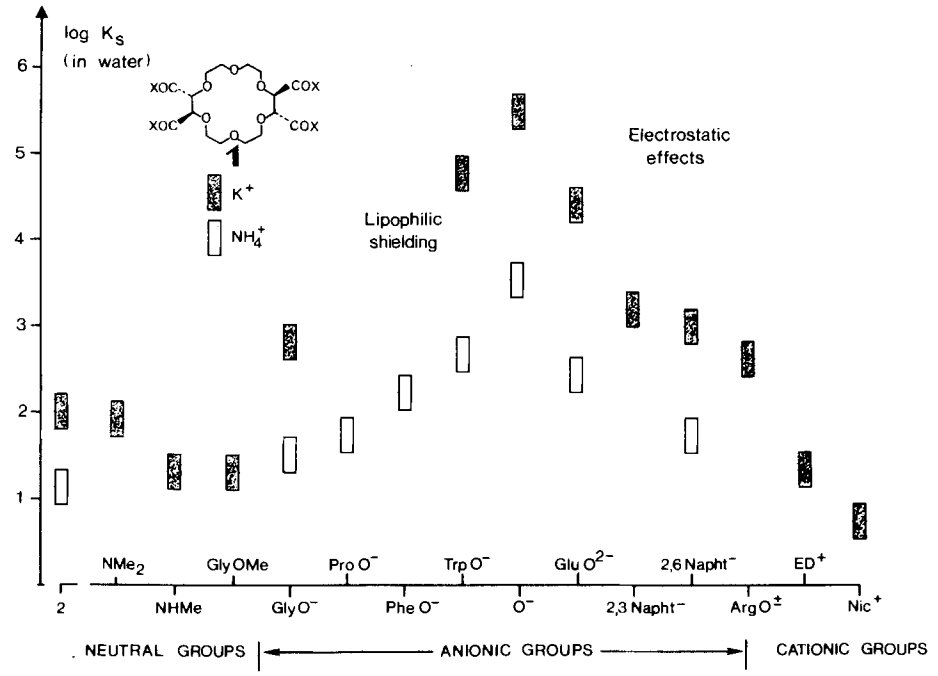

Fig. 1. *Variation of the stability of the K^+- and NH_4^+-complexes of receptor molecules* **1** *and of the parent macrocycle* **2** *with the nature of the side chain X borne by the macrocycle*

a) With respect to the *basic structure of receptor molecules* for primary ammonium ions as well as for K^+ and NH_4^+, one may note at the outset, that most complexes reported, except those of the **1** (X) ligands bearing neutral or cationic X-chains, are stronger than those of the parent 18-crown-6 (**2**) and are *very stable even in aqueous solution.* This justifies our initial aim to search for macrocyclic ligands based on the 18-crown-6 unit itself. Further confirmation is found in extensive investigations of the effect of structural parameters on association constants, which indicate that most modifications of the 18-crown-6 ring cause a marked decrease in complexation ability [2–13] [30] even when polar side chains are present [31].

b) Many *conformational similarities* exist among the ligands based on **1**. The crystal structures of various complexes [22–24] show that they maintain a remar-

kably constant overall shape, imposed by the two tartaric-acid residues. The approximately C_2-symmetry observed, is lower and in principle less favorable to complex formation than the D_{3d}-symmetry found in 18-crown-6 complexes of K^+ [19] and NH_4^+ [20], a fact which may contribute to the decrease of K_s-values observed between **2** and simple secondary amide derivatives like **1** (NHMe) and **1** (GlyOMe).

Interestingly, the nature of the amide groups which connect the lateral branches to the macrocycle has an orientational effect on the side chains. On the basis of crystal structure data [24], secondary amide groups in **1** (X = CONHR) tend to form a cyclic H-bond with the nearest ether O-atom in the ring, so that the carbonyl groups of O = C–NHR are directed away from the internal space of the ligand. On the contrary, with tertiary amide groups (X = CONRR'), as in the K^+-complex of **1** (NMe_2) [22], there is no such intramolecular bonding and furthermore inside positioning of NRR'-group would lead to marked steric interactions with the ring atoms; as a result, the carbonyl groups are turned inside and may take part in cation binding [22], thus increasing the stability of the K^+-complex of **1** (NMe_2) with respect to **1** (NHMe).

c) *Electrostatic interactions,* when present, *dominate the binding strength,* which leads to the following sequence with respect to the nature of the side chain X: cationic < neutral << anionic *(Fig. 1, Table).* The most pronounced effect is found for the parent tetracarboxylate **1** (O^-) where the anionic groups are directly bound to the macrocycle and line closely the periphery of the circular cavity. The complexes of **1** (O^-) are *by far the most stable* among all known polyether macrocyclic ligands. The stability of the K^+-complex of **1** (O^-) is higher than that of the K^+-complex of 18-crown-6 (**2**) by a factor of about 4000 and falls into the range of the most stable alkali cation cryptates [32]. The complexes with primary ammonium ions are also much more stable than those of **2** [33] [34] (see *Table*).

When the negative charges are farther from the ring, their effect on stability is less pronounced but still marked. The four carboxylate groups in **1** ($GlyO^-$) cause a 30 fold increase of K_s of the K^+-complex with respect to the tetraester **1** (GlyOMe). However, the stability remains sensitive to a further increase in the number of charges, as illustrated by its 40 fold increase in going from **1** ($GlyO^-$) to the octaanionic receptor **1** ($GluO^{2-}$) *(Table).*

d) *Lipophilic shielding* and *hindrance to solvation* of the polar groups involved in substrate binding, may explain the remarkable increase in complex stability as the glycinate groups of **1** ($GlyO^-$) are replaced by amino-acid residues with more and more lipophilic side chains: prolinate in **1** (PrO^-), phenylalaninate in **1** ($PheO^-$), tryptophanate in **1** ($TrpO^-$). Thus, the stability of the K^+-complex increases by a factor of about 100 from **1** ($GlyO^-$) to **1** ($TrpO^-$). The contribution of a lipophilic group may also play a role in the binding ability of the ligands bearing naphthalene residues **1** (2,6-$Napht^-$) and **1** (2,3-$Napht^-$).

The introduction of lipophilic groups in **1** (X) markedly reinforces the electrostatic interactions with the substrate, resulting in more efficient ligands and much tighter binding. The effect observed here is also a model of similar effects which have been considered to occur in substrate binding to biological receptors, and active sites containing hydrophobic residues in proteins [35].

3) *Selectivity of complexation of organic ammonium cations.* In view of their role both in organic and biological chemistry, a study of the various factors which influence the binding of molecular ammonium cations by the tetrafunctional receptors **1** is of special interest. It is convenient to separate the effects introduced by the presence of a macrocyclic structure from those imputable to the lateral groups X.

a) *Central discrimination* proceeds from the presence of a circular macrocyclic cavity of given diameter which may discriminate substrates of different size, as well as from the perturbation of the N–H$^+\cdots$O H-bonding scheme established between ligand and substrate. The NH_3^+-group of primary ammonium cations has a size suitable for anchoring the substrate into a macrocyclic cavity of 18-crown-6-type by an array of H-bonds (see above; **3** and **4**). Replacement of protons by larger groups at the N-site diminishes both the number of H-bonds, which the substrate may form with the macrocycle, and their strength. As the size of the ammonium group increases, steric interactions with the macrocycle make it more and more difficult for the substrate to approach towards the O-sites, thus lengthening the intermolecular bonds.

As a result, the *site effect,* due to direct substitution at the N-atom, leads to the selectivity sequence $N^+ < NH^+ < NH_2^+ < NH_3^+ < NH_4^+$ (*Table,* entries $12 << 7 < 6$, $16 < 15 < 14$, $21 < 20 < 19$). Similar results have been found for the complexation of substituted ammonium ions by 18-crown-6 (**2**) itself [34], but the selectivities were less pronounced than with the present ligands. Thus, for **2** the ratio of stability constants $K_s\,(CH_3CH_2NH_3^+)/K_s\,(CH_3CH_2)_2NH_2^+) = 1.3$ (in aqueous solution) [34] is much smaller than $K_s(CH_3NH_3^+)/K_s(CH_3)_2NH_2^+) > 150$ obtained here with **1** (O^-). Macrocycle **2** reaches comparable selectivity ratio only in methanol solution [36]. This may be related to the fact that central discrimination between substituted ammonium ions should be higher for stronger complexes, where the ammonium group is expected to penetrate deeper into the macrocyclic cavity, as found in the crystal structure of the ethylenediammonium complex with the dianion of **1** (OH) [23]; the same factor may contribute to the selectivity sequence found for the following entries in the *Table:* $27/28 < 25/26 < 7/12$. The comparatively low stability of the imidazolium and guanidinium complexes (*Table;* entries 32, 33) results from a combination of a cavity-size effect, a decrease in number of H-bonds and a decrease in electrostatic interaction by delocalization of the positive charge. One may, however, note that a K_s-value of 45 for guanidinium in aqueous solution, is still quite remarkable, but is much lower than the value of 9000 $l \cdot mol^{-1}$ found for its complex with the carboxylate of a larger macrocycle (27-crown-9 hexacarboxylate [37]) which possesses a cavity of suitable size.

The *vicinal effect* resulting from steric hindrance introduced by substitution at the C-atom next to the cationic head, markedly decreases the stability of the complexes of R–NH_3^+ with **1** (O^-) along the sequence R = *t*-Bu < *i*-Pr < Et < Me (*Table,* entries 7, 8, 10, 11). A similar, but less pronounced effect is found for the complexes of **2** [34] [36]. Larger hydrocarbon substituents produce a relative increase in stability: it may be attributed to a compensation of the steric bulk effect by *lipophilic stabilization* (*Table,* entries $8 < 13$, $49 < 50$). The latter factor is apparently also present, although weaker, at longer range: there is a systematic increase in stability when an H-atom in β-position is replaced by a phenyl group (*Figure 2; Table,* entries $9 \sim 8$; $8 < 14, 29, 30$).

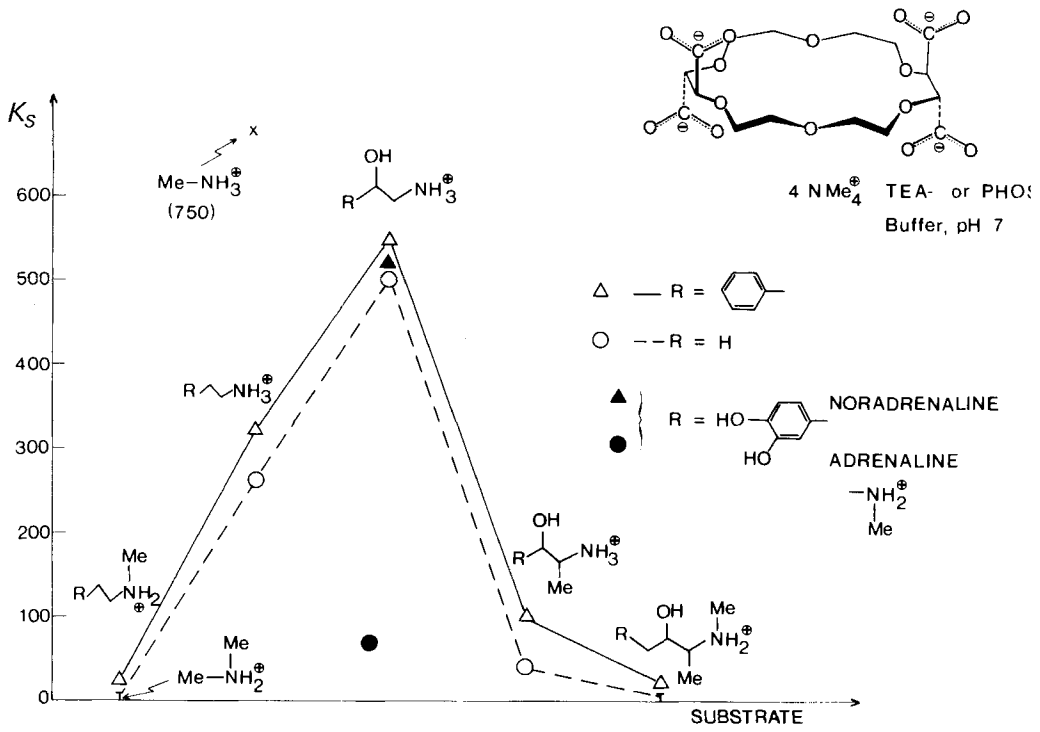

Fig. 2. *Variation of the stability of ammonium complexes of the tetracarboxylate receptor* **1** *(O^-) with the structure of the substrate*

Finally, one may note that the *K^+/R–NH_3^+-selectivities* are in all cases in favor of K^+. Thus, although ammonium ions are strongly bound by the present receptors, they are displaced by K^+. Binding selectivities in favor of R–NH_3^+-ions have been achieved by introducing N-binding sites, as found in azaoxa-macrocycles [38] and in a spherical macrotricyclic cryptand which is a highly selective receptor for NH_4^+ with respect to K^+ [7] [8] [39].

b) *Lateral discrimination* may result from interactions between a bound substrate and additional binding sites contained in side chains borne by the macrocyclic unit. As discussed above, solution and crystal structure data support the view that in complexes **4** the lateral appendages X are in axial arrangement, which allows optimal secondary interaction with the bound substrate.

Electrostatic interactions, as already mentioned in the previous section, are the prime stabilizing factor for complexes with anionic ligands. Lateral cation-anion interactions make also a marked contribution, yielding very stable complexes with diammonium substrates *(Figure 3),* for which the usual selectivity sequence (R–$NH_3^+ < NH_4^+$) may even be reversed (*Table,* entries 6 < 34, 35, 40; 42 < 43; 47 < 54–57; 60 < 61, 62; 65 < 66–68). The ($^+H_3NCH_2CH_2NH_3^+/CH_3CH_2NH_3^+$)-selectivity is very high (> 100, *Table*).

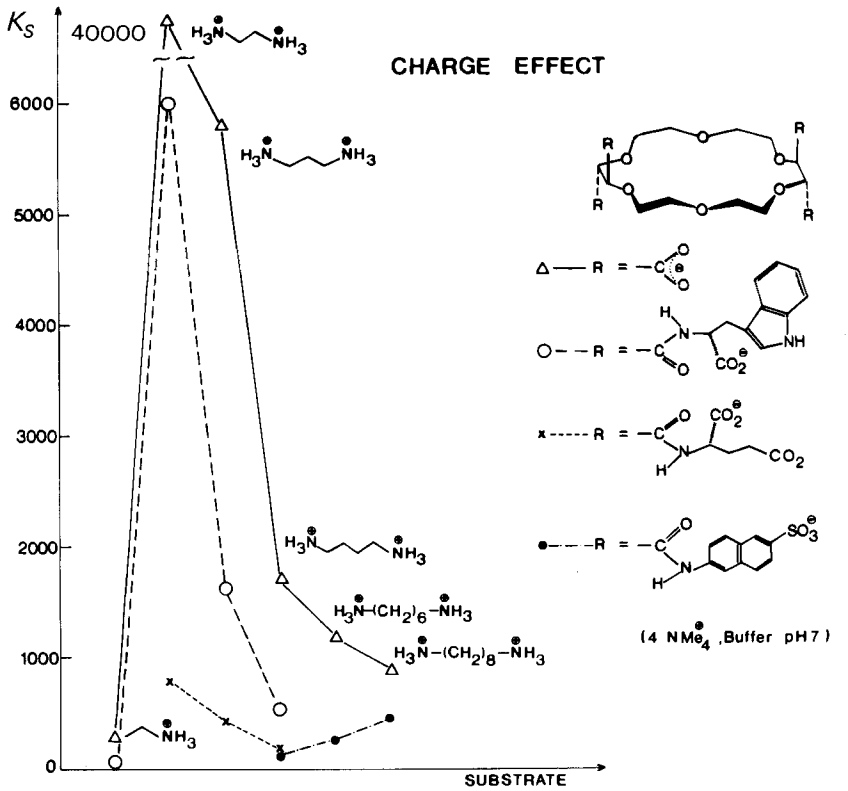

Fig. 3. *Variation of the stability of the complexes of different receptor molecules* **1** *with diammonium substrates* $^{+}H_3N$–$(CH_2)_n$–NH_3^{+} *as a function of charge separation in the dication*

A plot of the stability constants of the complexes of a given ligand *vs.* the chain length of ($^{+}H_3N$–$(CH_2)_n$–NH_3^{+})-substrates *(Fig. 3)*, displays a steep increase as the chain length decreases, *i. e.* as the charge density in the substrate increases; this is especially true for ligand **1** (O^{-}), which has itself the highest charge density.

The only exception is **1** (2,6-Napht^{-}), which forms the most stable complex with the octamethylenediammonium substrate, whose length corresponds to the distance between the macrocycle and the negative charges located at the opposite end of the naphthalene residues, thus allowing binding of one NH_3^{+}-group to the macrocycle and interaction of the other with the SO_3^{-}-groups. This suggests a chain-length discrimination process in favor of substrates of length complementary to the distance between the binding sites, as has been achieved for ($^{+}H_3N$–$(CH_2)_n$–NH_3^{+})-cations with cylindrical macrotricyclic ligands [40].

The presence of lipophilic groups like indole increases selective recognition of dicationic substrates by **1** (TrpO^{-}) with respect to **1** (GlyO^{-}) (*Table*, entries 43/42 < 54/47). The binding strength also depends on the charge density and H-bonding ability of the second cationic head, as may be judged from the decrease in stability observed for substitution on the second N-atom (*Table*, entries

31 < 39 < 34; 40 < 35; 57 < 54; 58 < 56). Finally, unfavorable anionic repulsion is revealed by the low association constant between **1** (O^-) and the phenylalanine zwitterion (*Table*, entries 17 < 14).

H-Bonding between the OH-group of hydroxyl-containing substrates with the carboxylate groups of the ligand may explain the consistently higher stabilities found for the complexes of **1** (O^-) and **1** ($TrpO^-$) with the cations of amino-alcohols; the stability sequence is *n*-alkylamine < γ-amino-alcohol < β-amino-alcohol (*Fig. 2, Table*, entries 8, 9 < 22 < 19; 14 < 24; 49 < 53).

c) *Biogenic amine complexation* deserves some special comments. Noradrenaline and adrenaline, which have very important and opposite physiological effets, as well as norephedrine and ephedrine, differ only by the degree of substitution at the ammonium site. It is seen that **1** (O^-) discriminates efficiently between these pairs of substrates, complexing preferentially primary ammonium nor-compounds (*Table*, entries 26 < 25, 28 < 27). Comparing (β-hydroxy-β-phenylethyl)ammonium with (β-phenylethyl)ammonium shows again a twofold stability increase due to the OH-group (*Table*, entries 14 < 24); the lower stability of the norephedrine complex of **1** (O^-) compared to the (β-hydroxy-β-phenylethyl)ammonium complex arises from the CH_3-substitution on the C-atom next to the N-site, as discussed above *(Fig. 2)*. The neurotransmitters serotonine and histamine are also distinguished (*Table*, entries 30 < 31); the latter, which possesses an imidazolium group able to interact with the carboxylate functions of **1** (O^-), is more strongly bound than the former.

Thus, the structural effects found for complexation of the simple ammonium cations allow to rationalize the stability sequences of the complexes of the biogenic amines, whose physiological activity depends on a CH_3-substituent at or next to the ammonium site, and on hydroxyl, phenyl or imidazolium groups on the C(β)-atom. The selective complexation processes observed point to potential applications in selective binding and transport of various drugs by receptor molecules of type **1** or of the diamid-diacid type derived from **1** (OH) [24]. It has already been shown that selective transport of various biogenic amines and related drugs by dicyclohexyl-18-crown-6 through artificial liquid membranes [41] or in erythrocytes [42] displays similar structural effects as found here for complexation. Similar effects have been observed with natural ionophores [14].

d) It is clear also that from the *analytical-chemistry* point of view, it should be possible to devise efficient separation procedures, for instance between biogenic amines or isomeric ammonium salts. Of special interest in this respect would be the attachment of the **1** (OH) binding unit on solid phase in order to produce new materials for chromatographic separation and analysis (for earlier applications see for instance [43]).

Experimental Part

General remarks. Abbreviations: *i. v.* = *in vacuo*, r. t. = room temperature. – ^1H-and ^{13}C-NMR. spectra were taken on *Varian A60* (60 MHz) and *XL100-15* (25 MHz) spectrometers respectively. Chemical shifts are given in ppm downfield from internal TMS (in $CDCl_3$) or sodium (trimethylsilyl)propanesulfonate (TMPS) (in D_2O). Since most compounds synthesized complex Na^+, the ^{13}C-shifts in H_2O were measured with respect to internal NMe_4^+ and converted to TMPS reference (+57.9 ppm). Coupling constants *(J)* are given in Hz. Alumina was purchased from *Merck* (activity II–III). Amines and amino acids used as substrates or starting materials for the synthesis of ligands were commercially available; only the nicotinamide bearing amines were prepared according to a literature procedure [44].

Preparation of the macrocycles bearing four amino-acid residues. – *General procedure.* A solution of **1** (C1) [17] (1mmol) in CH_2Cl_2 (10 ml) was added dropwise with stirring to a mixture of *(S)*-amino acid methyl ester hydrochloride (5 mmol) and triethylamine (10 mmol), or of the *(S)*-amino acid methyl ester (5 mmol) and triethylamine (5 mmol), in CH_2Cl_2 (20 ml). After 30 min the solvent was removed *i. v.* The purification steps depend on the nature of the amino acids and are given below. Hydrolysis of the carboxymethyl groups was performed for all amino esters by the same procedure in basic conditions. A 10% aqueous or methanolic NMe_4OH-solution (4.1 mmol) was added to the tetraester (1 mmol) in CH_3OH (20 ml). After stirring for 24 h at r. t., the solvent was removed *i. v.*, the residue dissolved in H_2O (20 ml) and the pH adjusted to 7 with HCl-solution. Removal of the water *i. v.* led to a glass which was further dried overnight *i. v.* The purity of the resulting tetracarboxylate salts was checked by ^{1}H-and ^{13}C-NMR. spectroscopy. The spectra were in agreement with the expected structures and showed the salts to be at least 97% pure. Stock solutions for the measurements were prepared by dissolving the salts in aqueous buffer solution.

*(2*R,*3*R,*11*R,*12*R*)-2,3,11,12-Tetrakis[*N*-(methoxycarbonylmethyl)carbamoyl]-1,4,7,10,13,16-hexaoxacyclooctadecane* (**1** (GlyOMe)). The crude mixture was dissolved in MeOH and deionized on a weak acid cation exchange resin (50 g *Amberlite CG-50*). The acidic methanolic fraction was evaporated to dryness, dissolved in the minimum amount of $CHCl_3$ and put on an alumina column (10 g). Elution with $CHCl_3/CH_3OH$ 98:2 gave the tetramethylester which was recrystallized from $MeOH/Et_2O$ (66% yield); m. p. 188–189°. – ^{1}H-NMR ($CDCl_3$): 3.7 (*m*, 28 H, 8 OCH_2, 4 OCH_3); 4.09 (*d*, J=6.0, 8 H, 4 NCH_2); 4.33 (*s*, 4 H, 4 OCH); 7.6–7.9 (*t*, 4 H, 4 NH). – ^{13}C-NMR. ($CDCl_3$): 41.0 (NCH_2); 52.1 (OCH_3); 69.3, 72.3 (OCH_2); 82.0 (OCH); 170.4, 170.7 (CO).

$C_{25}H_{44}N_4O_{18}$ (724) Calc. C 46.40 H 6.12 N 7.73% Found C 46.25 H 6.06 N 7.90%

*(2*R,*3*R,*11*R,*12*R*)-2,3,11,12-Tetrakis[*N*-(carboxylatomethyl)carbamoyl]-1,4,7,10,13,16-hexaoxacyclooctadecane* (**1** ($GlyO^-$)). – ^{13}C-NMR.(D_2O): 45.9 (NCH_2); 71.8, 74.2 (OCH_2); 83.7 (OCH); 174.5, 178.5 (CO).

*(2*R,*3*R,*11*R,*12*R*)-2,3,11,12-Tetrakis{*N*-[(2*S*)-2-(methoxycarbonyl)-1-pyrrolidinyl]carbonyl}-1,4,7,10,13,16-hexaoxacyclooctadecane* (**1** (ProOMe)). The crude residue was dissolved in $CHCl_3$ (50 ml), washed with 0.1M HCl (3 × 50 ml) and then with H_2O until neutral. The organic phase was concentrated *i. v.* and passed over a short alumina column with $CHCl_3/CH_3OH$ 96:4. Solvent removal led to a glass (55% yield). – ^{1}H-NMR. ($CDCl_3$): 2.0 (*m*, 16 H, 2 H-C(3) and 2 H-C(4) of 4 pyrrolidinyl units; 3.66–3.73 (*m*, 36 H, 8 OCH_2, 4 OCH_3, 4 NCH_2); 4.39 (*m*, 4 H, 4 NCH); 4.72 (*s*, 4 H, 4 OCH). – ^{13}C-NMR. ($CDCl_3$): 24.9 (C(4) of pyrrolidinyl); 29.0 (C(3) of pyrrolidinyl); 47.1 (NCH_2); 52.1 (OCH_3); 58.8 (NCH); 67.3, 70.7 (OCH_2); 75.3 (OCH); 168.6, 172.5 (CO).

*(2*R,*3*R,*11*R,*12*R*)-2,3,11,12-Tetrakis{*N*-[(2*S*)-2-carboxylato-1-pyrrolidinyl]carbonyl}-1,4,7,10,13,16-hexaoxacyclooctadecane* (**1** ($ProO^-$)). – ^{13}C-NMR. (D_2O): 27.0 (C(4) of pyrrolidinyl); 31.3 (C(3) of pyrrolidinyl); 50,6 (NCH_2); 62.1 (NCH); 71.0, 72.3 (OCH_2); 80.2 (OCH); 171.6, 177.8 (CO).

*(2*R,*3*R,*11*R,*12*R*)-2,3,11,12-Tetrakis{*N*-[(1*S*)-1-(methoxycarbonyl)-2-phenylethyl]carbamoyl}-1,4,7,10,13,16-hexaoxacyclooctadecane* (**1** (PheOMe)). Isolation as for **1** (ProOMe). The compound crystallized from $MeOH/Et_2O$ (60% yield); m. p.: 188–189°. – ^{1}H-NMR. ($CDCl_3$): 3.20 (*m*, 24 H, 8 OCH_2, 4 CH_2-Ph); 3.60 (*s*, 12 H, 4 OCH_3); 4.36 (*s*, 4 H, 4 OCH); 4.63 (*m*, 4 H, 4 NCH); 7.04 (*s*, 20 H, arom. H); 7.6 (*d*, J=7.7, 4 H, 4 NH). – ^{13}C-NMR. ($CDCl_3$): 37.0 (arom. C); 52.1, 53.5 (NCH, OCH_3); 69.8, 70.6 (OCH_2); 80.9 (OCH); 126.8, 128.6, 129.4, 137.1 (C_6H_5); 170.9, 172.1 (CO).

$C_{56}H_{68}N_4O_{18}$ (1084) Calc. C 61.98 H 6.32 N 5.16% Found C 62.11 H 6.28 N 5.33%

*(2*R,*3*R,*11*R,*12*R*)-2,3,11,12-Tetrakis{*N*-[(1*S*)-1-carboxylato-2-phenylethyl]carbamoyl}-1,4,7,10,13,16-hexaoxacyclooctadecane* (**1** ($PheO^-$)). – ^{13}C-NMR. (D_2O): 39.7 (CH_2-Ph); 58.4 (NCH); 72.3 (OCH_2); 83.4 (OCH); 129.5, 131.3, 131.9, 140.2 (C_6H_5); 172.8, 179.9 (CO).

*(2*R,*3*R,*11*R,*12*R*)-2,3,11,12-Tetrakis{*N*-[(1*S*)-2-(3-indolyl)-1-(methoxycarbonyl)ethyl]carbamoyl}-1,4,7,10,13,16-hexaoxacyclooctadecane* (**1** (TrpOMe)). Isolation as for **1** (ProOMe). The compound was obtained as a white powder by precipitation from $CHCl_3$ with ether (82% yield), m. p.: 135–140°. – ^{1}H-NMR. ($CDCl_3$): 2.60–3.25 (*m*, 24 H, 8 OCH_2, 4 CH_2–indolyl); 3.52 (*s*, 12 H, 4 OCH_3); 4.10 (*s*, 4 H, 4 OCH); 4.69 (*m*, 4 H, 4 NCH); 7.07 (*m*, 28 H, Indole-H, NH). – ^{13}C-NMR. ($CDCl_3$): 26.8 (CH_2-indolyl); 52.2, 53.1 (NCH, OCH_3); 70.2 (OCH_2); 80.6 (OCH); 110.2, 111.4, 118.5, 119.5, 122.0, 123.5, 127.5, 136.3 (Indole-C); 170.9, 172.5 (CO).

$C_{64}H_{72}N_8O_{18}$ (1277) Calc. C 60.17 H 5.99 N 8.77% Found C 60.45 H 5.93 N 9.00%

(2R,3R,11R,12R)-2,3,11,12-Tetrakis{N-[(1S)-1-carboxylato-2-(3-indolyl)ethyl]carbamoyl}-1,4,7,10,13,16-hexaoxacyclooctadecane (**1** (TrpO$^-$)). – ^{13}C-NMR. (D_2O): 29.5 (CH_2-indolyl); 57.9 (NCH); 71.8, 72.0, (OCH_2); 83.2 (OCH); 112.7, 114.3, 121.2, 121.8, 124.4, 126.5, 130.0, 138.6 (Indole-C); 172.6, 180.5 (CO).

(2R,3R,11R,12R)-2,3,11,12-Tetrakis{N-[(1S)-1,3-bis(methoxycarbonyl)propyl]carbamoyl}-1,4,7,10,13,16-hexaoxacyclooctadecane (**1** (GluOMe)). The crude material was deionized in the same way as **1** (GlyOMe), and crystallized from hot CH_3OH (76% yield), m. p.: 159–160°. – ^{1}H-NMR. ($CDCl_3$): 2.41 (*m*, 16 H, 4 CH_2CH_2CO); 3.6–3.7 (*m*, 40 H, 8 OCH_2, 8 OCH_3); 4.6 (*m*, 8 H, 4 OCH, 4 NCH); 7.8 (*d*, J=7.7, 4 H, 4 NH). – ^{13}C-NMR. ($CDCl_3$): 26.8, 30.6 (CH_2CH_2CO); 51.9, 52.4 (NCH, OCH_3); 70.8 (OCH_2); 81.2 (OCH); 171.2, 172.3, 183.4 (CO).

$C_{44}H_{68}N_4O_{26}$ (1069) Calc. C 49.43 H 6.41 N 5.24% Found C 49.32 H 6.18 N 5.21%

(2R,3R,11R,12R)-2,3,11,12-Tetrakis{N-[(1S)-1.3-dicarboxylatopropyl]carbamoyl}-7,10,13,16-hexaoxacyclooctadecane (**1** (GluO^{2-}). – ^{13}C-NMR. (D_2O): 31.1, 36.5 (CH_2CH_2CO); 57.7 (NCH); 72.6, 72.9 (OCH_2); 83.3 (OCH); 172.9, 180.3, 183.9 (CO).

(2R,3R,11R,12R)-2,3,11,12-Tetrakis[N-(6-sulfonato-2-naphthyl)carbamoyl]-1,4,7,10,13,16-hexaoxacyclooctadecane (**1** (2,6-Napht$^-$)). Prepared from 2-amino-6-naphthalenesulfonic acid (5 mmol) and triethylamine (10 mmol) following the general procedure. The crude mixture was dissolved in $CHCl_3$ (50 ml) and extracted with H_2O (50 ml). The aqueous layer was reduced *i. v.* and deionized over a strong acid ion exchange column (*Dowex* 50 × 8). The acidic fraction was taken to dryness (red glass, 90% yield). The compound was found to be unstable in the tetrasulfonic acid form and was therefore converted immediately to its tetramethylammonium salt. – ^{1}H-NMR. (D_2O): 3.2 (*s*, 48 H, 4 $N(CH_3)_4^+$); 3.75 (*m*, 16 H, 8 OCH_2); 4.60 (*s*, 4 H, 4 OCH); 7.05–8.40 (*m*, 24 H, arom. H). – ^{13}C-NMR. (D_2O): 72.2, 72.9 (OCH_2); 83.6 (OCH); 119.7, 123.8, 125.5, 127.8, 131.2, 132.0, 132.2, 136.5, 138.2, 142.4 (arom. C); 172.1 (CO).

(2R,3R,11R,12R)-2,3,11,12-Tetrakis{N-[6-(methoxycarbonyl)-2-naphtyl]carbamoyl}-1,4,7,10,13,16-hexaoxacyclooctadecane (**1** (2,3-NaphtOMe)). The crude reaction mixture was chromatographed on alumina. Elution with $CHCl_3$ allowed the excess of starting amine to be recovered. The desired compound was eluted with $CHCl_3$/CH_3OH 98:2, and was recrystallized from CH_2Cl_2/MeOH (90% yield), m. p. 185–186°. – ^{1}H-NMR. ($CDCl_3$): 3.75 (*s*, 12 H, 4 OCH_3); 4.06 (*m*, 16 H, 8 OCH_2); 4.98 (*s*, 4 H, 4 OCH); 7.70 (*m*, 24 H, arom. H); 8.33 (*s*, 4 H, 4 NH). – ^{13}C-NMR. ($CDCl_3$): 59.3 (OCH_3); 77.4, 78.9 (OCH_2); 87.9 (OCH); 123.1, 124.9, 132.8, 135.0, 135.9, 136.2, 140.3, 142.9, 143.6 (arom. C); 174.9, 176.9 (CO).

$C_{64}H_{60}N_4O_{18}$ (1173) Calc. C 65.52 H 5.16 N 4.78% Found C 65.54 H 5.09 N 5.13%

(2R,3R,11R,12R)-2,3,11,12-Tetrakis[N-(3-carboxylato-2-naphthyl)carbamoyl]-1,4,7,10,13,16-hexaoxacyclooctadecane (**1** (2,3 Napht$^-$)). – ^{13}C-NMR. (D_2O): 73.3, 73.7 (OCH_2); 85.2 (OCH); 120.9, 128.6, 129.9, 130.8, 131.3, 132.2, 134.2, 136.5, 136.9 (arom. C); 172.4, 176.6 (CO).

(2R,3R,11R,12R)-2,3,11,12-Tetrakis{N[(1S)-(1-carboxylato-4-guanidinio)butyl]carbamoyl}-1,4,7,10,13,16-hexaoxacyclooctadecane (**1** (ArgO$^\pm$)). To a solution of *(S)*-arginine methylester dihydrochloride (5 mmol) and triethylamine (10 mmol) in dry DMF (20 ml) was added dropwise with stirring a solution of **1** (Cl) (1 mmol) in CH_2Cl_2 (10 ml). After stirring for 1 h, the precipitate formed was filtered off and the solution was evaporated *i. v.* The residue was dissolved in the minimum amount of hot 2-propanol and the oil which separated on cooling was washed with 2-propanol and dried (colorless glass, 50% yield). The ester functions were hydrolyzed following the general scheme, except that the NMe_4OH-solution was added portionwise over 24 h. – ^{13}C-NMR. (D_2O): 27.5, 31.6 ($CHCH_2CH_2$); 43.4 (NCH_2); 58.0 (NCH); 72.3, 73.0 (OCH_2); 82.7 (OCH); 159.5 (NCN); 173.0, 180.7 (CO).

(2R,3R,11R,12R)-2,3,11,13-Tetrakis-[N-(2-aminoetyhl)carbamoyl]}-1,4,7,10,13,16-hexaoxacyclooctadecane (**1** (ED)). A solution of **1** (Cl) (1 mmol) in CH_2Cl_2 (10 ml) was added dropwise with stirring to a solution of mono(*N*-benzyloxycarbonyl)ethylenediamine (4.5 mmol, see below) and triethylamine (4.5 mmol) in dry CH_2Cl_2 (10 ml). After stirring for 30 min, $CHCl_3$ (20 ml) and CH_3OH (10 ml) were added. The resulting clear solution was extracted successively with 0.1M HCl (50 ml) and water (50 ml). The organic layer was evaporated to dryness and the residue crystallized from $CHCl_3$/CH_3OH as a white powder (70% yield) m. p.: 214–215°. – ^{1}H-NMR. ($CDCl_3$): 3.0–3.7 (*m*, 32 H, 8 OCH_2, 8 NCH_2); 4.29 (*s*, 4 H, 4 OCH); 4.95 (*s*, 8 H, 4 CH_2–Ph); 7.13 (s, 20 H, arom. H). – ^{13}C-NMR. ($CDCl_3$): 39.2, 41.0 (NCH_2); 66.5 CH_2–Ph); 69.3, 71.3 (OCH_2CH_2); 81.4 (OCH); 128.0, 128.4, 136,4 (arom. C); 156.7 (COO); 171.1 (CON).

$C_{56}H_{72}N_8O_{18}$ (1145) Calc. C 58.73 H 6.34 N 9.78% Found C 58.25 H 6.30 N 10.12%

Deprotection of the amine functions was performed in AcOH (5 ml) with 2.5M HBr/AcOH (4 ml). After a 1 h stirring, ethyl acetate (30 ml) was added and the precipitate was filtered, washed with ether and dried. **1** $(ED^+)\cdot 4\ Br^-$ crystallized from MeOH/EtOH (very hygroscopic). – ^{13}C-NMR. (CD_3OD): 38.4, 40.8 (NCH_2); 70.8, 72.8 (OCH_2); 82.6 (OCH); 173.4 (CO). The compound was dissolved in CH_3OH (2 ml) and deionized on a strong basic ion-exchange column (*Dowex* 1 × 8). The basic fraction was collected, taken to dryness *i. v.* and the residual oil crystallized upon standing (95% yield) m. p.: 175–177°. – ^{1}H-NMR. (CD_3OD): 2.95 (*t*, J=6.0, 8 H, 4 CH_2N); 3.53 (*t*, 8 H, 4 $CONCH_2$); 3.77 (*s*, 16 H, 8 OCH_2); 4.47 (*s*, 4 H, 4 OCH). – ^{13}C-NMR. (D_2O): 41.4, 43.4 (NCH_2); 70.9, 72.6 (OCH_2); 82.6 (OCH); 173.6 (CO). Mono(*N*-benzyloxycarbonyl)ethylenediamine was prepared by slow addition of one equivalent of benzyloxycarbonyl chloride to a buffered (AcOK/AcOH, pH 5) aqueous solution of ethylenediamine. The solution was filtered, then the pH was raised to 12 (25% NaOH-solution). Extraction with $CHCl_3$ allowed the desired compound to be isolated in about 30% yield.

(2R,3R,11R,12R)-2,3,11,12-Tetrakis{N-[(3-carbamoyl-1-pyridinio)ethyl]carbamoyl}-1,4,7,10,13,16-hexaoxacyclooctadecane (**1** (Nic^+)). A solution of N^1-(2,4-dinitrophenyl)nicotinamide chloride (1 mmol) in CH_3OH (5 ml) was added dropwise (1 h) to a stirred solution of **1** (ED) (0.25 mmol) in CH_3OH (5 ml) at 40–50°. The desired compound precipitated with ether (50 ml) as a slightly yellow powder (80% yield). – ^{13}C-NMR. (CD_3OD): 41.2, 62.9 (NCH_2); 71.0, 72.0 (OCH_2); 82.6 (OCH); 129.6, 135.6, 145.2, 147.0, 148.3 (C's of Py); 165.2, 173.0 (CO).

$C_{48}H_{72}N_{12}O_{18}Cl_4$ (1247) Calc. C 46.23 H 5.82 N 13.47% Found C 46.12 H 5.43 N 13.32%

Stability constant measurements. – The association constants given in the *Table* have been determined potentiometrically using K^+ or NH_4^+ ion-selective electrodes. Other techniques have been used for some complexes: polarimetry (**1** ($TrpO^-$)/K^+, NH_4^+, $MeNH_3^+$), optical rotatory dispersion (**1** (O^-)/K^+) and UV. spectroscopy (**1** ($TrpO^-$)/NH_3^+ $(CH_2)_n$Nic$^+$) [29]. The values found agree well with the potentiometric derived constants (±20%).

Apparatus. The apparatus consisted of an automatic titration unit *Tacussel TITRIMAX (TT 100, 200, 300)* connected to a digital burette *(ELECTROBUREX)*, a printing unit *(Tacussel 900)* and a recorder *(TARKAN NW 600)*. The measuring electrodes were liquid membrane K^+- and NH_4^+-selective electrodes *(Phillips IS 561)* together with a calomel reference electrode *(Tacussel)* in contact with the buffered solution through an ionic bridge filled with the same buffer as employed for the probe studied. The measuring cell (5 ml) was thermostated at 25 ± 1°.

Stoichiometries. The titration curves were consistent with a 1:1 stoichiometry. A direct determination was possible for the most stable complexes using concentrated solutions. Thus, a 10^{-2} molar solution of **1** (O^-) shows a potentiomeric wave for 1 ± 0.05 equivalent of K^+ or NH_4^+; the intensity of the charge-transfer band between **1** ($TrpO^-$) and pyridinium-bearing ammonium salts reaches a plateau for one equivalent of substrate [29]. Higher stoichiometries may intervene at high substrate concentration, but should not contribute significantly in the conditions used for the measurements.

K^+- and NH_4^+-stabilities. The electrode response was checked in 4 ml of buffer solution before and after each ligand titration. It was *Nernstian* between 5.10^{-6} and 10^{-2}M (slope 59 ± 1 mV), and almost driftless (± 2 mV/24 h). In a typical experiment, 4 ml of a 5 millimolar (the concentration range has to be $\approx K_s^{-1}$ for optimal precision) ligand solution buffered at pH 7.0 with tris(2-hydroxyethyl)amine/HCl or tetramethylammonium phosphate (0.1M) was introduced in the thermostated cell. Just before each incremental automatic addition (25μl) of a similarly buffered 10 millimolar NH_4^+-(or K^+-)solution, the *ddp* between the measuring and reference electrodes was printed out. The waiting time was set long compared with the response time of the electrode (< 10 s). For each increment, the stability constant was calculated from the relation: $K_s=(c-c_f)/[(c_L-c+c_f)\cdot c_f]$, where c and c_f are respectively the total and measured NH_4^+-(or K^+-)concentrations, and c_L the total ligand concentration. For increments corresponding to < 0.2 or > 0.8 equivalents of cation, the calculated K_s-values varied somewhat. The low-edge variation may be due to the presence of small amounts of cations, whereas for cation/ligand ratios close to 1, K_s becomes very sensitive to concentration errors. The K_s-value given in the *Table* is the average (the spread is < 5%). The accuracy may be estimated to ± 10%.

R–NH_3^+-stabilities. The stability constants have been determined by competitive inhibition of the NH_4^+ (or in some cases K^+) complexation using the corresponding cation-selective electrode. As none responded to R–NH_3^+, the electrode was calibrated in the same solution as that used for the titration. Competition experiments were performed in 4 ml of buffer solution containing the ligand and the am-

monium salt (ratio *ca.* 1:1) in the appropriate concentration range (see above). Incremental titration with a buffered NH_4^+ (or K^+) solution yielded the stability constants K_s^R from the following relation:

$$K_s^R = K_s^c \cdot \frac{1}{\alpha - 1} \cdot \frac{c_L - \alpha\beta}{\alpha\beta^2 + (c_R - c_L)\beta} \qquad \alpha = \frac{1}{K_s^c \cdot c_f} + 1; \quad \beta = c - c_f$$

where K_s^c is the known $NH_4^+(K^+)$ association constant, c_L, c, c_R are respectively the total ligand, NH_4^+ (K^+) and ammonium salt concentrations, and c_f is the measured free NH_4^+-(K^+-) concentration. In the *Table* are given the arithmetically averaged K_s values (spread *ca.* 5%). The estimated accuracy is ±20% but for a given ligand K_s differences of *ca.* 10% are significant.

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