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Detection of hydrogen-bonded adenine-thymine base-pair complexes by electrospray mass spectrometry

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Bibracchial lariat ether compounds having three-carbon sidearms terminated in either adenine or thymine have the potential to form a molecular box in which the "ends" are crown ethers and the "sides" are hydrogen-bonded base pairs. Previous solution studies relying upon NMR and vapor pressure osmometry confirmed the formation of complexes. In the present study, four diaza-18-crown-6 derivatives having either one or two sidearms each terminated in either adenine or thymine have been studied in a mixture of $CHCl_3$ and CH_3OH. Either acetic acid or sodium chloride was added to the solutions. For the two-armed systems, the preferred complex was the dimer involving adenines on one monomer and thymines on the other. Homodimers and other complexes were detected as well. The preferred single-armed complex was that occurring between the monomer whose sidearm was terminated in thymine and either H^+ or Na^+.

INTRODUCTION

A critical feature in the reactions of biological compounds is the remarkable selectivity that results from their ability to recognize each other. The interactions of biological chemicals normally involve relatively weak forces such as hydrogen bonding and hydrophobic effects. What makes the recognition process even more remarkable is that the complexes involved often cannot be the most stable ones possible as these would reduce the reversibility of the biochemical reactions which is an essential element for living systems.

In earlier studies we attempted to achieve cation binding selectivity[1] using the flexible ligand systems that we have called lariat ethers.[2] These compounds were designed to achieve three-dimensional cation complexation as do the cryptands[3] but retain the binding dynamics of natural ionophores such as valinomycin.[4] We have similarly used crown ethers having short sidearms terminated in nucleotide bases as complexation partners for crown ethers designed to be their complements.[5] Nuclear magnetic resonance and vapor pressure osmometry studies have shown that such compounds form hydrogen-bonded complexes in solution. In the latter case, our ability to assess selectivity was limited by the complexity of the species in solution.

Mass spectrometric techniques have increasingly been applied to complexation problems in recent years. Cation-ligand interactions[6] and crown-cation interactions have been assessed recently by mass spectrometry, the latter in the absence of bulk solvent. The results of these studies have often, but not always, paralleled solution studies of the same systems.[7] Various mass spectrometric techniques have permitted the study of cation binding interactions[8] in host molecules such as tris(crown ethers) that could not be assessed in more traditional ways.[9] The advent of electrospray ionization mass spectrometry (ESI-MS)[10] has proved to be particularly valuable in assessing the complexation of cationic guests by hosts. Recently, mass spectrometric techniques have been used to study non-covalent complexes,[11] aqueous solutions of metal salts,[12] charged clusters of amino acids,[13] peptide-metal ion interactions in solution,[14] bipyridyl amino acid-metal complexes,[15] alkali metal binding by valinomycin,[16] hydration of doubly protonated diamines,[17] human erythrocyte plasma membrane phospholipids,[18] and hydrogen-bonded complexes.[19]

We now report the application of electrospray ionization mass spectrometry as an approach to assess complexation between the two complementary molecular

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partners and between each of these monomers and sodium cation.

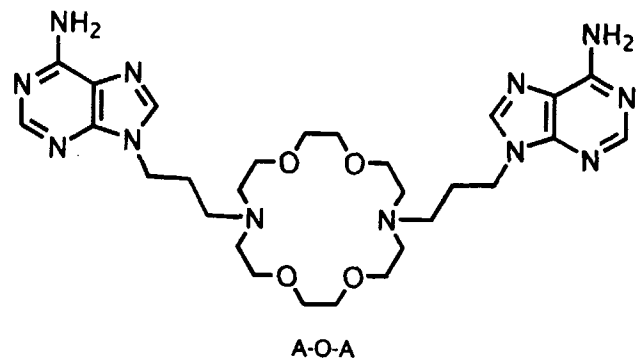
RESULTS AND DISCUSSION

The compounds utilized in this study have been described previously.[5] We use a shorthand notation to simplify our description of these substances. Each of them is based upon the 4,13-diaza-18-crown-6 framework and each has either one or two trimethylene sidearms. The sidearms are terminated in either adenine or thymine. Our shorthand represents the macrocycle as "**O**", the short alkyl chains as "-", and the bases as either "**A**" or "**T**". Thus, the adenine-crown-adenine system Ade-$(CH_2)_3$-diaza-18-crown-6-$(CH_2)_3$-Ade is referred to here as "**A-O-A**." Likewise, Thy-$(CH_2)_3$-diaza-18-crown-6-H is called "**T-O**." The structures of the four compounds are shown.

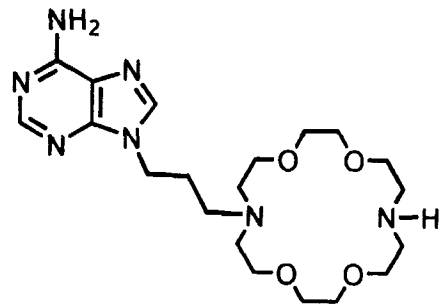
Multiple hydrogen bond interactions are possible with these systems and it has been shown in previous work that complexation is possible for **A-O-A** and **T-O-T** with each other and with themselves. We were interested to assess complexation selectivity by a technique that would permit us to directly observe the various permutations. Electrospray ionization mass spectrometry constitutes such a technique. The electrospray interface is often referred to as a "soft" ionization method and non-covalent complexes are known to survive passage from solution into the gas phase. The electrospray ionization (ESI) spectra were acquired on a triple quadrupole tandem mass spectrometer equipped with an electrospray interface. Information concerning the ion source may be found in published literature[20] and in the experimental section of this paper. The essential details are as follows. The temperature of the drying gas (N_2) was set at 100°C and held at a constant pressure of 25 psi as it entered the ionization chamber. Signal was averaged for 4 min for each spectrum. Sample solutions were infused directly into the ionization chamber at ≈ 25°C by using a syringe pump (rate = 2 μL/min). Experiments were normally replicated three times.

A-O-A and T-O-T complexes. Three solutions of **A-O-A** and **T-O-T** monomers were prepared in 2:1 (v/v) $CHCl_3$:CH_3OH at a concentration of 3.33 mM each. Acetic acid was added in concentrations of 1.5% and 5% (v/v) to two of the solutions and NaCl (0.33 mM) was added to the third solution. The mass spectra were acquired as described above and the results are recorded in Table 1.[21]

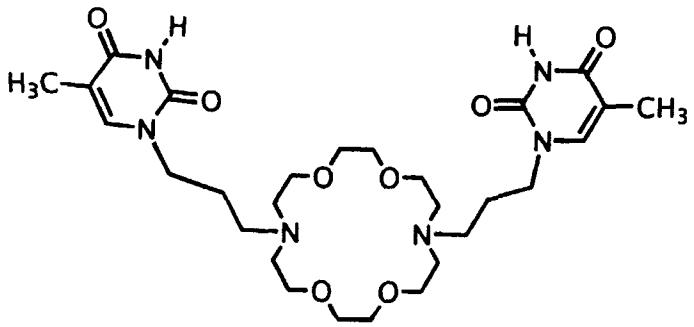
It should be noted at the outset that only ions having relative abundances of 2% or greater are recorded in Table 1. Further, the spectra acquired when 1.5% of acetic acid was added were recorded over a range of only 400–1600 amu so the $[(\mathbf{TOT})_2 \cdot \mathbf{AOA} \cdot H]^+$ peak was not detected in that case. A dozen ions were observed which had significant intensities. It is interesting that in cases in



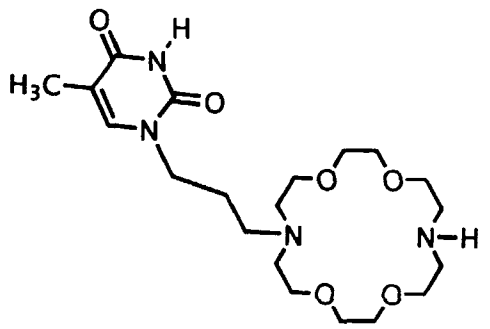
A-O-A



A-O



T-O-T



T-O

Table 1 ESI-MS data for AOA and TOT complexes.[a]

Ion observed	m/z	Relative intensity ($CHCl_3$:MeOH) 5% HOAc[b]	1.5% HOAc[c]	NaCl added[d]
$[TOT{\cdot}H]^+$	595.4	100	100	3
$[AOA{\cdot}H]^+$	613.3	82	81	<2
$[TOT{\cdot}Na]^+$	617.3	16	20	100
$[AOA{\cdot}Na]^+$	635.4	9	8	12
$[(TOT)_2{\cdot}H]^+$	1189.4	9	10	<2
$[TOT{\cdot}AOA{\cdot}H]^+$	1207.9	30	30	2
$[(TOT)_2{\cdot}Na]^+$	1211.2	<2	<2	2
$[(AOA)_2{\cdot}H]^+$	1225.8	3	3	<2
$[TOT{\cdot}AOA{\cdot}Na]^+$	1229.9	3	2	8
$[(AOA)_2{\cdot}Na]^+$	1247.8	<2	<2	<2
$[(TOT)_3{\cdot}(AOA)_2{\cdot}H]^{2+}$	1514.4	3	3	<2
$[(TOT)_2{\cdot}AOA{\cdot}H]^+$	1802.3	4	–[e]	<2

[a]See experimental section for conditions. Peaks having a relative intensity of less than 2 are not listed. [b][**AOA**] = [**TOT**] = 3.33 mM; 5% by volume HOAc in $CHCl_3$:MeOH (2:1 v/v). [c][**AOA**] = [**TOT**] = 3.33 mM; 1.5% by volume HOAc in $CHCl_3$:MeOH (2:1 v/v). [d][**AOA**] = [**TOT**] = 3.33 mM; [NaCl] = 0.33 mM in $CHCl_3$:MeOH (2:1 v/v). [e]The spectrum was recorded only between m/z = 400 and m/z = 1600.

which no sodium cation was deliberately added, the sodium complexes (adducts) exhibit intensities similar to those observed when the cation was intended to be present.

There is little difference in the spectra obtained when acetic acid was present to the extent of 1.5% or 5%. Only six major ions were observed in either case. In both experiments, protonated **T-O-T** (*i.e.* **T-O-T**·H^+) was the base peak. Protonated **A-O-A** was nearly as intense having a relative abundance of ≈80% in either case. Likewise, sodiated **T-O-T** exhibits a relative intensity of 16–20% in acid although it is the base peak when sodium, rather than acid, was added. The only other significant peak observed in the acidic solutions was the protonated dimer [**A-O-A·T-O-T**·H]$^+$. When sodium was added, the sodiated dimer [**A-O-A·T-O-T**·Na]$^+$ was also observed (relative intensity 8%) as was [**A-O-A**·Na]$^+$ (12%). In acidic solutions, the largest peak observed except for the protonated monomers was apparent at m/z 1207.9 and corresponded to [**A-O-A·T-O-T**·H]$^+$.

The most important result obtained in this series of experiments is that outside of protonated monomers, [**A-O-A·T-O-T**·H]$^+$ is the major peak. Its relative intensity (30%) is ten–fold greater than for [(**A-O-A**)$_2$·H]$^+$ (3%) and three-fold greater than for [(**T-O-T**)$_2$·H]$^+$ (9–10%). A strength of ESI mass spectrometry is that for certain systems, the gas phase results reflect solution phenomena. This has been demonstrated by Wilson and Wu for alkali metal complexation by valinomycin[16] and has also been noted more generally by Cheng *et al.*[12] although the latter results have been questioned.[22] Our own recent studies of dibenzyl ether complexation of sodium and potassium cations tend to support the relationship between gas and solution phase measurements.[23] The structure of the **A-O-A·T-O-T** complex presumed from the design, the solution data,[5] and supported by data obtained here is shown in Figure 1.

A second interesting observation concerns the difference in relative intensities for the protonated monomers. The ratio of [**T-O-T**·H]$^+$:[**A-O-A**·H]$^+$ is (100:80). If the gas phase results do reflect solution, this suggests that the former is somewhat more stable than the latter. We noted in the solution phase that **T-O** is slow to alkylate.[5] We attributed this to sidearm participation by the thymine carbonyl group in stabilizing a ring bound cation in the presence of alkali metal carbonates. Protonation could occur at either, equivalent nitrogen. In addition, either sidearm could interact with the proton. We surmise from an examination of CPK molecular models that if stabilization occurs between the protonated nitrogen and the trans-annular side-arm, the potential for steric hindrance is much greater than when an 8-membered ring structure such as shown in Figure 2 forms.

No donor group corresponding to the thymine urea carbonyl is available in adenine. A stabilized structure of the type shown is therefore likely with **T-O-T** but not with **A-O-A**. The trans-annular possibility for **A-O-A**

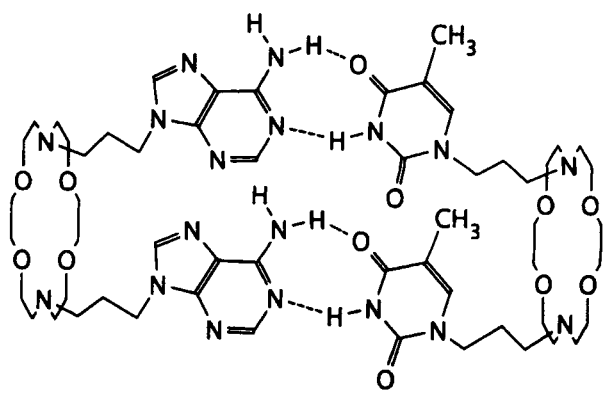


Figure 1 Presumed structure of the dimer complex formed between **A-O-A** and **T-O-T**

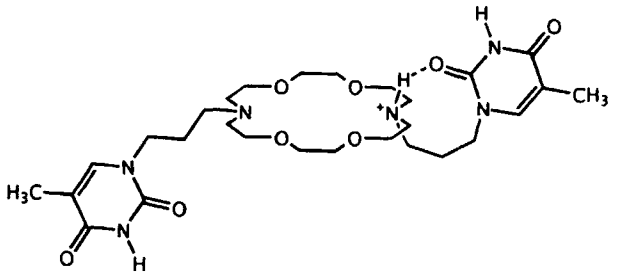


Figure 2 Monoprotonated **T-O-T** complex in which the ammonium salt is stabilized by the thymine urea carbonyl

appears stabilizing to the protonated nitrogen. The steric issue raised above makes such an interaction less likely.

A lariat ether complex of this type would be expected to differentiate the two monomers even more when sodium rather than hydrogen cation is present in the solution. Indeed, the base peak is $[\mathbf{T\text{-}O\text{-}T}\cdot Na]^+$ and the corresponding $[[\mathbf{A\text{-}O\text{-}A}\cdot Na]^+$ ion has a relative intensity of only 12%. No other ion exceeds a relative intensity of 10% in this system.

A-O and T-O complexes. The crown ether derivatives having single sidearms terminated in either adenine or thymine were studied in the presence of either protons or sodium cations. It was anticipated that sodium binding by **T-O** would be superior to that observed for **A-O**. This was anticipated from previous results[5] as well as from the data presented above. Indeed, the base peak observed in the presence of sodium cation was $[\mathbf{T\text{-}O}\cdot Na]^+$. This peak was threefold more intense than for $[\mathbf{A\text{-}O}\cdot Na]^+$ (relative intensity 31%).

The results were both interesting and somewhat different from expectations for these compounds in the presence of 1.5% acetic acid. As anticipated, protonated **T-O** has a relative intensity about twice that of protonated **A-O**. The surprising observation was that the next largest peak (25%) corresponded to the protonated 1:1 complex of **A-O** and **T-O**.

The cation complexing ability of **T-O** with Na^+ is anticipated to be better than that of **A-O**. The thymine urea oxygen atom of **T-O** is a stronger donor than N-1 of adenine and it can readily be directed to the center of the macroring when Na^+ complexation occurs. The structural arrangement shown in figure 3 for **T-O** is also possible for **A-O** although an identical sidearm conformation places H-2 rather than N-1 in the corresponding axial position. It should be borne in mind that although the mass spectrometric data accord nicely with binding data and the conformational possibilities apparent from CPK molecular model studies, this explanation remains somewhat speculative. We do not have a solid state structure to confirm it although many supporting structures are now available for relatives of this compound.

The drawing, which attempts to accurately reflect the molecular models, suggests that sodium binding by **T-O** should be more favorable than binding by **A-O**. The actual difference observed in the mass spectra suggest a 2:1 stability ratio. This difference seems more reasonable in the context of the known binding constants for 4,10-diaza-15-crown-5 and 4,13-diaza-18-crown-6. Two factors should be noted. First, azacrowns are generally weaker alkali metal cations binders than all-oxygen crowns. Second, sodium and potassium sleectivity is reversed for some azacrown compounds compared to the all-oxygen counterparts. For the 15-membered ring compound, the cation binding constants ($\log_{10} K_S$) are (in anhydrous methanol at 25°C) $Na^+ < 1.5$, $K^+ < 1.5$.[24] Binding is slightly more selective for the 18-membered ring analogs but is still poor: $Na^+ < 1.5$, $K^+ < 1.8$. These values compare with 18-crown-6 as follows: Na^+ 4.35, K^+ 6.08.

The selective formation of a **T-O·A-O** complex poses an interesting question. Presumably, its formation results from an interaction of adenine with thymine by H-bonding although whether the Hoogsteen or Watson-

Table 2 ESI-MS data for AOA and TOT complexes.[a]

Ion observed	m/z	Relative intensity ($CHCl_3$:MeOH) 1.5% HOAc[c]	NaCl added[d]
$[TOT\cdot H]^+$	429.1	100	11
$[AOA\cdot H]^+$	438.1	54	9
$[TOT\cdot Na]^+$	451.2	8	100
$[AO\cdot Na]^+$	460.1	4	31
$[(TO)_2\cdot H]^+$	857.7	11	4
$[TO\cdot AO\cdot H]^+$	866.8	25	7
$[(AO)_2\cdot H]^+$	875.7	6	<2
$[(TO0_2\cdot Na]^+$	879.7	2	12
$[TO\cdot AO\cdot Na]^+$	888.9	3	14

a. See experimental section for conditions. Peaks having a relative intesity of less than 2 are not lsited. b. [**AO**] = [**TO**] = 3.33 mM: 1.5% by volume HOAc in $CHC1_3$: meOH (2:1 v/v). c. [**AO**] = [**TO**] = 3.33 mM; [NaC1] = 0.33 mM in $CHC1_3$:MeOH (2:1 v/v).

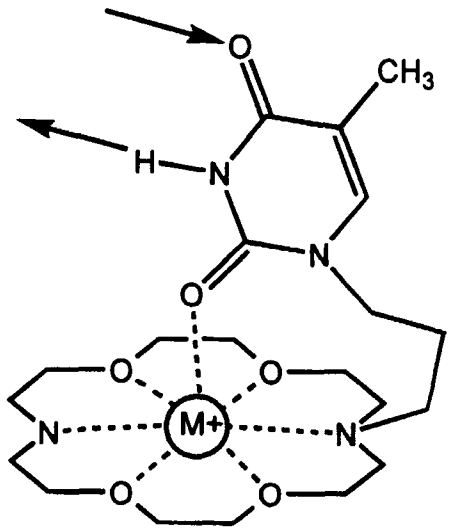


Figure 3 Lariat conformations for **T-O** binding a metal ion (M^+). Arrows indicate hydrogen bond donor (←) and acceptor (→) sites.

Crick modes predominate cannot be discerned from the mass spectrometric results. Addition of Na^+ to the **T-O** + **A-O** mixture should lead to significant **T-O** complexation. Formation of the **T-O** complex with Na^+ would, if the discussion above is correct, involve the critical carbonyl group in thymine and diminish association of **T-O** with **A-O**. The relative intensities for the peaks corresponding to $[(\mathbf{TO})_2 \cdot Na]^+$ (12%) and $[\mathbf{TO \cdot AO \cdot} Na]^+$ (14%) are essentially identical suggesting that complex formation as in $[(\mathbf{TO})_2 \cdot Na]^+$ competes with selective interactions between **T-O** and **A-O**. The selectivity of the corresponding protonated complexes $[(\mathbf{TO})_2 \cdot H]^+$ (11%) and $[\mathbf{TO \cdot AO \cdot} H]^+$ (25%) is approximately 2:1 in favor of the latter indicating that protonation of either monomer does not preclude more favorable A:T pairing.

CONCLUSION

The data presented here serve two purposes. The mass spectrometric method permits a more detailed assessment of the molecular complexes that form from the nucleotide-containing monomers. Even the extensive and detailed NMR studies previously reported do not give information about minor complexes as do the mass spectrometric results. Clear selectivities are observed for complexes in which adenine and thymine are paired. This is true even when a single sidearm is attached to the crown ether. Second, the general correlation of previously obtained solution data with the observations encourage the use of the ESI-MS method as a probe of supramolecular complexation phenomena.

ACKNOWLEDGMENT

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Experimental Section

ESI mass spectra were acquired on a triple quadrupole tandem mass spectrometer (Finnigan MAT TSQ 700, San Jose, CA) equipped with an electrospray interface (Analytica of Brandford, Branford, CT). The detector of the instrument is an off-axis continuous dynode electron multiplier operable from −400 to −3000V, with a variable post-acceleration/conversion dynode voltage from −3 kV to −20 kV for detection of positive ions. The electrospray ion source and its functional parts have been described in detail.[25] For all experiments, both the electrospray needle and the skimmer were operated at ground potential, whereas the electrospray chamber (*i.e.*, cylindrical electrode) and metallized entrance of the glass capillary were operated at −3.5 kV. A +90 V potential was applied to the metallized exit of the glass capillary. A separate +175 V potential was placed on the tube lens for acquisition of the positive ions. The temperature of nitrogen drying gas as it entered the electrospray chamber was set at 100°C and the drying gas was held at a constant pressure of 25 psi. A 4-min period of signal averaging was employed for each spectrum. 20% Chloroform in methanol (v/v) was used as solvent in all experiments. Sample solution was infused directly into the ESI chamber with a syringe pump at a flow rate of 2 μL/min. All the sample solution was prepared and then kept at room temperature (≈25°C).

A-O, **T-O**, **A-O-A**, and **T-O-T** were prepared as previously described.[5]

Solutions suitable for study were prepared by dissolving an amount of the monomer in 10% MeOH:chloroform to afford a final concentration of 10 mM. Solutions of the specified concentrations and solvent compositions were made by appropriate mixing of the above, glacial acetic acid, or 1 mM NaCl in anhydrous methanol. The resulting solutions were vortexed and allowed to stand for ≥30 min prior to use.

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