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Selective Carriers of Norepinephrine and Ammonium Ions: Ionophoric Properties and Molecular Modelling Studies of Diester Crown Compounds Containing a 1,3-Bis(1*H*-pyrazol-1-yl)propane Unit

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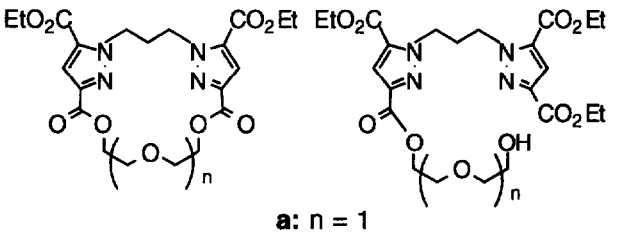
Abstract—Ionophoric properties of dipyrazolic crowns and podands containing a 1,3-bis(1*H*-pyrazol-1-yl)propane unit in their structure are described. They show selectivity of ammonium vs alkali cations and interesting norepinephrine transport rates. A molecular modelling study has been used to elucidate the superstructures of the crown and ammonium cation complexes. © 1997, Elsevier Science Ltd. All rights reserved.

Introduction

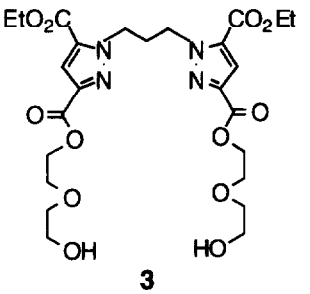
Dopamine and norepinephrine are catecholamine neurotransmitters, the levels of which are reduced in neurodegenerative and in mental illnesses, like parkinsonism, schizophrenia, depressive disorders, and Alzheimer's disease.[1] Currently, there are some treatments with drugs that affect the dopaminergic or noradrenergic systems, but these therapies are far from being satisfactory.[2] The interest for the development of selective carriers of dopamine/norepinephrine has grown in the last years[3] because they can increase the level of these neurotransmitters and, as a consequence, display potential clinical use in replenishment strategies[4] against these diseases. Most of the studies on catecholamine transport have been carried out with the classical crown ethers (cyclic oligoethers exclusively containing oxygen as donor atom)[5] that, in general, show selectivity for alkaline cations versus alkylammonium derivatives.[6] Taking into account that $R—NH_3^+$ centres form stronger hydrogen bonds with nitrogen than with oxygen atoms,[7] selective binding of ammonium ions could be achieved using oxa-aza macrocycles, with sp^3 or sp^2 nitrogen atoms.[8]

In previous works we studied the transport properties of some heteroaromatic crowns in which 3,5-disubstituted 1-methyl and 1*H*-pyrazole units are linked to chains of tetraethylene glycol.[9] We found that, in general, potassium was carried better than ammonium cations when the pyrazolic sp^2 nitrogens could not be incorporated into the macrocyclic cavity. In that case, the complexing behaviour of these coronands was similar to the classical crown ethers. On the other hand, the coronands exhibited the desired selectivity of ammonium over potassium cations when electron donor nitrogen atoms could be included into the cavity.

The above-mentioned findings prompted us to look for novel selective receptors of ammonium and neurotransmitter cations. We designed new macrocyclic (coronands) and pseudomacrocyclic (podands) molecules (**1a–c**, **2a–c**, and **3**) containing a 1,3-bis(1*H*-pyrazol-1-yl)propane unit, which exhibit the following features: (a) All the heteroatoms, including the pyrazolic nitrogens, have their unshared electron pairs set in the macrocyclic cavity or pseudocavity with the possibility of being active in the cation complexation. (b) The 1,3-propylene fragment allows conformational mobility that makes easier the accommodation of the cations inside the electrostatic cavity or pseudocavity.



1a-c **a:** n = 1 **b:** n = 2 **c:** n = 3 **2a-c**



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In a previous work we described the synthesis of these crowns and acyclic structures by regioselective *Mucor*

miehei lipase catalysis.[10] Now we wish to report here their ionophoric properties as carriers of alkali cations and neurotransmitters of physiological interest, and also a molecular modelling study to get a better understanding of the nature of the complexes with ammonium cations.

Results and Discussion

Crowns **1a–c** and acyclic compounds **2a–c** and **3** were evaluated as carriers of Li^+, Na^+, K^+, NH_4^+, dopamine and norepinephrine picrates using a bulk liquid membrane (see Experimental). The results are shown in Table 1.

According to our initial design, the new dipyrazolic receptors transport selectively ammonium better than potassium cations. The selectivities range from 3.3 (crown derived from diethyleneglycol **1c**) to 1.3 (acyclic compound **2c**, with a tetraethyleneglycol chain). Only one exception was found, the crown derived from triethyleneglycol **1b**, which showed the opposite selectivity: $K^+ > NH_4^+$. In general, dopamine was worse transported than ammonium cation, but the change from dopamine to norepinephrine, with an extra hydroxyl group in the molecule, increases the transport rate in all the receptors.

In general, crowns **1a–c** transport alkali and NH_4^+ cations better than the neurotransmitters. In fact, **1b** showed the highest potassium and ammonium transport rates (73.83 and 48.25 μmol h^{-1}, respectively) among the receptors evaluated here. It was also possible to find a correlation between the macrocyclic hole and the radius of the alkali cation best transported. Thus, the smallest crown **1a** (18-membered) transports preferably Na^+, whereas K^+ is preferred by the 21-membered crown **1b**.

The four acyclic structures (**2a–c** and **3**) showed almost the same cation selectivity pattern (graphically compared in Fig. 1), with a clear preference for the neurotransmitters (dopamine and norepinephrine), as well as for the ammonium cation, whereas the transport rates of the alkali cations were lower. In these acyclic compounds, the neurotransmitter transport increased as long as the glycol length decreased. In fact, the acyclic compound **2a**, with the shortest chain, was the best carrier for dopamine (6.27 μmol h^{-1}) and norepinephrine (24.51 μmol h^{-1}). Surprisingly, the podand **3** with two diethyleneglycol chains showed lower transport rates than its counterpart **2a** with only one chain.

In order to ascertain some information on the nature of the complexes between crowns **1a–c** and ammonium ion, which could also explain the experimental ammonium transport rates, a computer molecular modelling study (Chem-X[11] software and AM1[12] semiempirical method) using quantum-chemical calculations of crowns and their ammonium complexes was done.

In a previous work,[9d] we studied the interactions between a podand (acyclic crown compound) and the ammonium ion, using the same molecular modelling method. We found that the most stable complex (Fig. 2) showed: (a) a stabilizing hydrogen bond between the C=O group in the 5-pyrazolic position and the hydrogen of the methylene moiety attached to the heterocycle, (b) all the methylene groups of the propylene chain arranged in zigzag order, and (c) the 3,3′-carbonyl groups oriented outside the pseudocavity.

Now, using these previous results as initial geometries we have constructed crowns **1a–c** that were optimized using AM1 calculations. Then, ammonium cation complexes were calculated from these minimum conformations using AM1 method and full geometry optimization. The final optimized complexes are depicted in Figure 3. We can observe that, in general, stabilization is due to interactions between the NH^+ ammonium centres and both the O-donor sites of the polyethyleneglycol chain and the sp^2 pyrazolic nitrogen atoms.

Crown **1a**, which shows the smallest ammonium transport rate (4.47 μmol h^{-1}), also has the smallest cavity (18-crown-5). Because of its little size the ammonium ion is located outside the macrocyclic hole (Fig. 3a), with the consequent loss of the complex stability. The cation directs three hydrogen atoms to the three oxygen atoms of the cavity, with distances very close (≈2.2 Å) to typical hydrogen bond distances[13] (1.7–2.1 Å). The fourth ammonium hydrogen is equally situated

Table 1. Transport rates (μmol h^{-1})[a] of alkali, ammonium, dopamine, and norepinephrine ions across a $CHCl_3$ phase containing 7×10^{-4} M of carrier

Carrier	Li^+	Na^+	K^+	NH_4+	Dopamine	Norepinephrine	Selectivity NH_4^+/K^+
1a	2.10	5.21	1.36	4.47	1.40	3.84	3.3
1b	1.77	1.15	73.83	48.25	0.13	2.51	0.7
1c	0.40	2.16	4.00	7.47	0.64	1.19	1.9
2a	0.56	0.92	1.68	2.51	6.27	24.51	1.5
2b	0.77	1.55	2.71	3.85	5.08	7.87	1.4
2c	1.09	1.19	2.82	3.60	0.26	5.15	1.3
3	0.61	0.47	1.48	3.35	0.10	4.66	2.3

[a]Each transport rate value (±15%) is the average of three independent determinations.

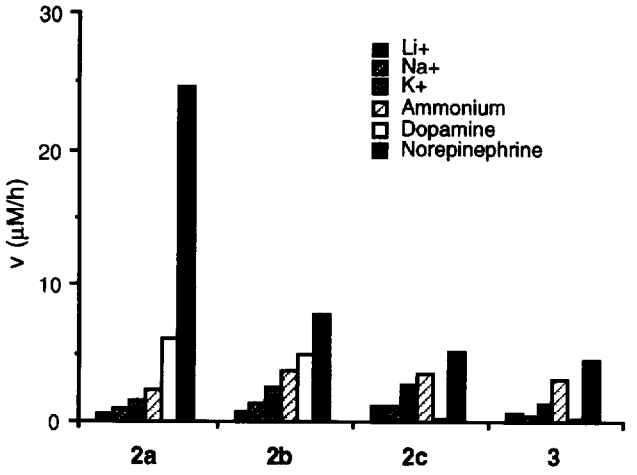

Figure 1. Graphical comparison of the Li^+, Na^+, K^+, NH_4+, dopamine, and norepinephrine transport rates of the acyclic compounds **2a–c** and **3**.

between the two sp^2 pyrazolic nitrogens, at slightly larger distances (≈2.6 Å), probably due to a steric repulsion with the central methylene group of the propylene chain. It is worth mentioning that complexation induces drastic conformation changes in the macrocycle, in order to better bind the ammonium ion: the pyrazoles adopt a 'butterfly form', directing their sp^2 nitrogens toward the cation, and the polyether chain modifies their torsional angles closing the cavity (Fig. 3b).

Crown **1b** has a bigger macrocyclic hole than **1a** and can place the ammonium cation inside the electrostatic cavity (Fig. 3c), affording the most stable inclusion complex and the highest ammonium transport rate (48.25 μmol h^{-1}), among the dipyrazolic crowns here studied. In this case, the ammonium cation directs two hydrogen atoms to the two ester oxygens, at the same distances (≈2.2 Å) than in the previous crown. Another hydrogen is situated between the two central oxygens of the cavity (2.0 and 2.5 Å), and the fourth ammonium hydrogen is also shared by the two sp^2 pyrazolic nitrogens (≈2.7 Å). In crown **1b** complexation origins smaller conformation changes than in **1a**.

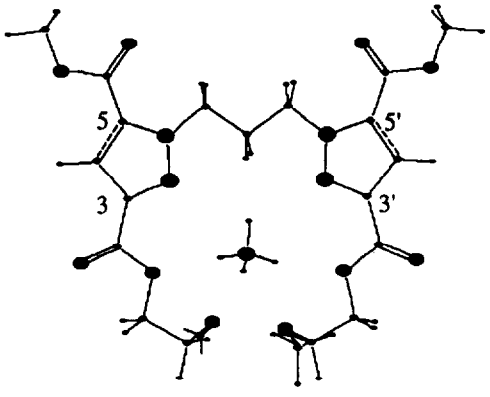

Figure 2. Ammonium cation complex of a podand optimized by AM1 method.[9d].

The two pyrazoles remain in the same plane and only the cavity has been slightly closed in order to approach donating heteroatoms to ammonium cation.

Crown **1c** is too big to complex ammonium ion efficiently, and for this reason exhibits a lower transport rate (7.47 μmol h^{-1}) than **1b**. Although the cation is also located inside the cavity (Fig. 3d), the complex is less stable because the ammonium–heteroatom distances are larger than in crown **1b**, and the whole superstructure has less symmetry. In addition, ammonium ion is better transported by **1c** than by **1a**, because the biggest crown **1c** may form a more stable inclusion complex, while **1a** cannot form it.

Conclusions

In this work, we have shown the potential usefulness of crowns and acyclic compounds containing a 1,3-bis(1*H*-pyrazol-1-yl)propane unit as selective carriers of ammonium and neurotransmmitter cations of physiological interest. AM1 semiempirical calculations made comprehensive the possible nature of the complexes and the experimental ammonium transport rates of crowns **1a–c**. The stabilizing interactions showed by this molecular modelling will be considered in future design of neurotransmitter selective carriers.

Experimental

Transport rate properties

The transport experiments were performed at 30 °C in a U-tube (9 mm, i.d.). The membrane phase (3 mL of chloroform Uvasol, Merck), in which carrier is dissolved (7×10^{-4} mol L^{-1}), lay below and bridged the two aqueous phases. The first aqueous phase (1 mL) contained 5×10^{-5} mol L^{-1} of LiOH, 10^{-1} mol L^{-1} of alkali nitrate or ammonium nitrate or neurotransmitter ammonium chloride, and 2×10^{-3} mol L^{-1} of the corresponding picrate. The second aqueous phase contained 1 mL of deionized water. The membrane phase was slowly and constantly stirred by a magnetic bar. A similar experiment was carried out in the absence of carrier. The picrate concentration in the second aqueous phase, monitored spectroscopically by UV ($\lambda = 355$ nm), was confirmed to increase linearly with running time (<12 h), and the initial transport rates were calculated. The values indicated in Table 1 were estimated from the differences in the transport rates of carrier-containing systems and blank systems (no carrier present).

Molecular modelling studies

Input geometries were taken from the standard ones within Chem-X[11] assuming the planarity of the system. The calculation of the conformational energies was carried out using the van der Waals method implemented in Chem-X, and the initial charge distribution according to the Gasteiger and Marsili method.[14]

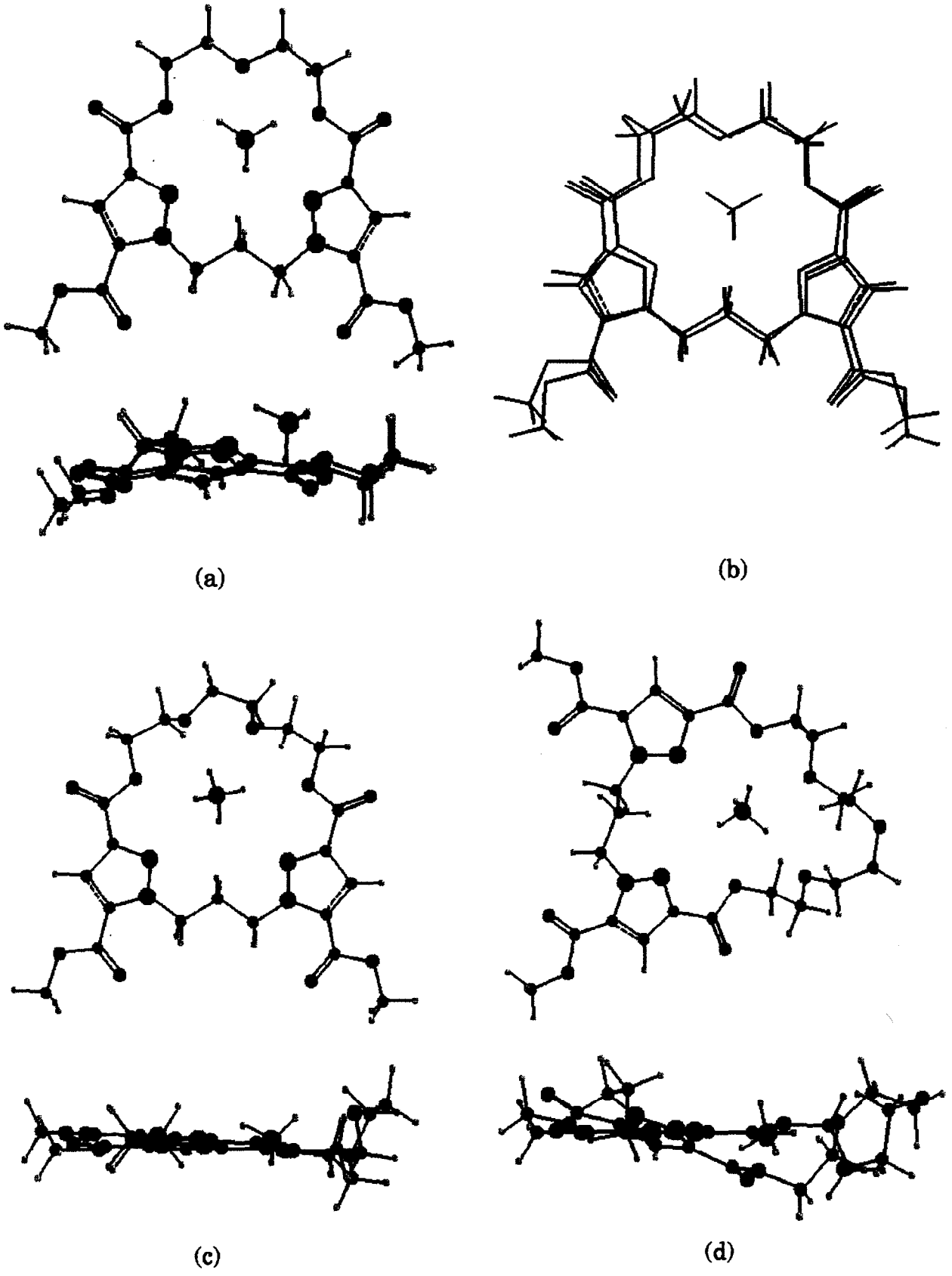

Figure 3. Ammonium cations complexes of crowns **1a–c**, optimized by AM1 method. (a) Crown **1a**: NH_4^+ complex. (b) Fitting of crown **1a** (blue) and its ammonium complex (green). (c) Crown **1b**: NH_4^+ complex. (d) Crown **1c**: NH_4^+ complex.

Semiempirical calculations has been performed using the AM1 method[12] in MOPAC V5.0 program package.[15] In all cases, the Chem-QM interface[16] was used and full geometry optimizations with Fletcher–Powell algorithm were carried out.

Acknowledgements

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