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The Sodium Salt of Diethyl 1*H*-pyrazole-3,5-dicarboxylate as an Efficient Amphiphilic Receptor for Dopamine and Amphetamines. Crystal Structure and Solution Studies

Felipe Reviriego,[†] Maria Isabel Rodríguez-Franco,[†] Pilar Navarro,*[,†] Enrique García-España,*[,‡] Malva Liu-González,[§] Begoña Verdejo,[‡] and Antonio Domènech[§]

Instituto de Química Médica, Centro de Química Orgánica Manuel Lora Tamayo, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain, Departamento de Química Inorgánica, Instituto de Ciencia Molecular, Universidad de Valencia, Edificio de Institutos de Paterna, Apartado de correos 22085, 46071 Valencia, Spain, and Servicio Central de Soporte a La Investigación Experimental and Departamento de Química Analítica, Universidad de Valencia, Dr. Moliner 50, 46100 Burjasot (Valencia), Spain

Received June 30, 2006; E-mail: enrique.garcia-es@uv.es

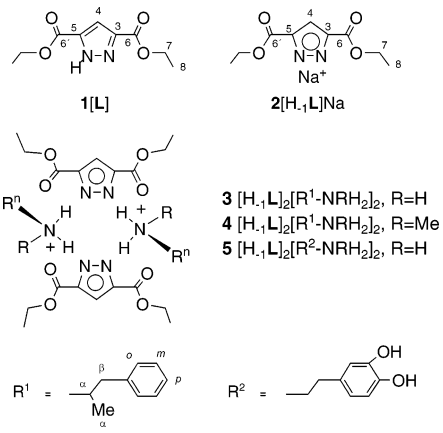
Amphetamine (AMPH) and methamphetamine (METH) are synthetic drugs which exert profound effects on mental function and behavior.[1,2] It is thought that the high lipophilic character of these abuse drugs facilitates its rapid entry into the brain, and that its most prominent biological effect is to raise extracellular levels of dopamine (DA) via a nonexocytic mechanism.[3] A large body of evidence exists justifying that such anomalous high levels generate reactive oxygen species (ROS) involved in DA-mediated neurodegeneration.[4] This accounts for the interest in developing synthetic receptors potentially able to cross the blood−brain barrier and complex amphetamine and/or dopamine.

Over the last 10 years, a wide range of efficient and selective artificial hosts for DA and/or norepinephrine (NA) which afford stable complexes in organic[5] or aqueous[6] media have been prepared. However, artificial hosts for the binding of AMPH have received much less attention.[7] In the search of abuse drug receptors, we previously reported on a series of 26-membered tetraester crowns containing two proton-ionizable 3,5-disubstituted 1*H*-pyrazole rings able to afford solid sodium dipyrazolate salts, which in DMSO solution behave as efficient and selective monotopic hosts for AMPH and METH in relation to DA and NA.[8] However, such macrocyclic salts showed scarce or null solubility in water. Now we report that the diethyl 1*H*-pyrazole-3,5-dicarboxylate **1** [**L**] (mp 53−54 °C) by treatment with NaOH in anhydrous ethanol forms a stable sodium pyrazolate salt **2**[H_{-1}**L**] (mp 212−214 °C) (Chart 1) which shows interesting complexation properties.

Reaction of sodium salt **2**[H_{-1}**L**] with (+)-amphetamine sulfate or (+)-methamphetamine hydrochloride in chloroform solution afforded, respectively, solid (2:2) complexes **3** (mp 113−116 °C) and **4** (mp 83−85 °C) (Chart 1), whose structures were established on the basis of their analytical and spectroscopic (IR, FAB-MS, ^{1}H and ^{13}C NMR) data. Furthermore, crystallization of complex **3** from ethanol afforded crystals suitable for X-ray diffraction studies.

The crystal structure of **3** consists of (+)-amphetamine and diethyl 1*H*-pyrazole-3,5-dicarboxylate units in the form of ammonium cations and pyrazolate anions, respectively, interconnected between them through an extended hydrogen bond network forming a supramolecular double helix structure.[9] Figure 1 shows a view of the double helix along the *c*-axis. One of the strands would be defined by the diethyl ester pyrazolate units and the other one by the ammonium cations.

Chart 1



As seen in Figure 1, every ammonium subunit is sticking together three pyrazolate subunits forming T-shaped hydrogen bonds with the carbonyl oxygen atom of an ester group (N3−O6B 2.87 Å), the nitrogen atom N2 of a pyrazolate placed at the same side (N2−N3 2.913 Å), and with N1 of a pyrazolate located at the opposite side (N1−N3 2.863 Å) of the chain. The angles of the hydrogen bond arrangement are N1−N3−N2 118.9°, N1−N3−O6B 100.7°, and O6B−N3−N2 61.8°. This pattern is alternatively repeated along

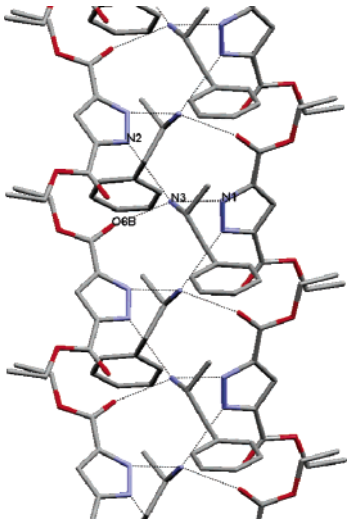


Figure 1. View of the crystal structure of **3** along the *c*-axis.

[†] CSIC.
[‡] Departamento de Química Inorgánica, Universidad de Valencia.
[§] Departamento de Química Analítica, Universidad de Valencia.

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the whole double helix. The angle formed by the planes of the benzene rings at each side of the chain is 59° and that between the rotated pyrazole rings is 54°. The distance between repeating benzene rings is 6.12 Å and that between repeating pyrazole units is 3.7 Å. Interestingly enough, there is no participation of a $\pi-\pi$ stacking interaction in the crystal. Also of note is that the double helices are isolated in the crystal structure. Therefore, the present structure constitutes a very nice example of a double-strand helical structure packed together by hydrogen bonds.

We have performed preliminary studies on the interaction of **2**[H_{-1}**L**] with (+)-AMPH, (+)-METH, and DA in DMSO and in water. First, formation constants for the interaction of **2**[H_{-1}**L**] with (+)-AMPH in DMSO (0.10 M Bu_4NPF_6) were determined by square wave voltammetry at platinum working electrodes using a previously reported procedure.[8a] Application of a generalization of the molar ratio method provided a 2:2 (+)-AMPH:**2** stoichiometry, and this was the stoichiometry found for all the systems (Chart 1).[10]

The constant obtained was $K_s = (1.8 \pm 0.4) \times 10^9$ M^{-3}, which represents a 36% complexation of (+)-AMPH for 10^{-3} M equimolar solutions of both reactants. Interaction with (+)-METH provided a very close value of $K_s = (4.2 \pm 0.7) \times 10^9$ M^{-3}, which represents a 43% complexation under the same conditions. Evaluation of the DA binding by **2**[H_{-1}**L**] provided a larger constant of $K_s = (1.2 \pm 0.1) \times 10^{10}$ M^{-3} (60% complexation), which indicates some selectivity for DA.

Second, by using pH-metric titrations, we have performed preliminary studies on the systems (+)-AMPH:**2**, (+)-METH:**2**, and DA:**2** in pure water, observing that even in this medium, where hydrogen bonding should be much weaker, the interaction persists at some extent.[11] At pH = 7.4, for the systems (+)-AMPH:**2** and (+)-METH:**2**, species of 2:2:4 substrate:receptor:proton stoichiometries are found, while in the system DA:**2**, at the same pH, a species of 2:2:6 stoichiometry is formed (Table 3S collects the stability constants obtained). These stoichiometries indicate that, at this pH, pyrazole is interacting as a neutral species with the cationic ammonium forms of the substrates. The maximum percentages of formation of these adducts change with the concentration of reactants at pH 7.4 for a 1:1 substrate:receptor mole ratio and 10^{-3} and 2×10^{-3} M concentrations in both reactants (ca. 4 and 15% for (+)-AMPH:**2**, ca. 22 and 45% for (+)-METH:**2**, and ca. 5 and 19% for DA:**2**). Interestingly, in water, the stability trend changes with respect to DMSO, with (+)-METH as the substrate interacting strongest with **2**.

The stoichiometries of the species formed in water were supported by electrospray ionization (ESI-MS). The ESI-MS spectra of aqueous solutions resulting from the reaction of **2** and (+)-amphetamine sulfate, (+)-methamphetamine hydrochloride, or dopamine hydrochloride analyzed at physiological pH (7.4) show peaks at *m*/*z* (%) 835 (73), 889.5 (12), and 916.1 (80), corresponding to species in which two neutral diester units with the pyrazole ring in the protonated form are complexing two substrate molecule (Table 4S and Figure 2S). The found stoichiometries agree with the 2:2:4 and 2:2:6 stoichiometries derived from the potentiometric measurements at neutral pH.

Finally, considering that the parallel artificial membrane permeability assay (PAMPA), first introduced by Kansy et al.[12a] and later modified by Di et al.,[12b] has been widely used in the pharmaceutical industry as a high-throughput permeability assay to predict passive blood−brain barrier (BBB) penetration of potential CNS drugs, we have evaluated the new pyrazolate salt **2**[H_{-1}**L**], in a PAMPA−BBB assay using a lipid extract of porcine brain.[13] Assay validation was made comparing the experimental permeabilities of 10 commercial drugs with their reported values,[12b] which gave a good linear correlation. From the straight-line equation, we established that compounds with permeability values over 2.2×10^{-6} cm s^{-1} should cross the blood−brain barrier. The amphiphilic salt **2**[H_{-1}**L**] showed a permeability value ($P_e = 4.2 \times 10^{-6}$ cm s^{-1}) that suggested that it could potentially reach the central nervous system.

In conclusion, this simple pyrazolate receptor shows very appealing characteristics for binding (+)-METH (speed), (+)-AMPH, and DA and provides very nice double helical crystal arrangements.

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Supporting Information Available: Synthesis and spectroscopic data of **2**−**4** (Tables 1S and 2S) and comments on their structures (Figures 1S and 2S). Description of the electrochemical and emf measurements. Stability constants derived by pH-metric titrations (Table 3S). ESI-MS (positive mode) data of 2:2 complexes formed from **2**[H_{-1}L] and (+)-AMPH, (+)-METH, and DA in aqueous solution at physiological pH (Table 4S). PAMPA−BBB permeability assays (Table 5S). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Crystals of **3** have been structurally characterized with the formula $C_{18}H_{26}N_3O_4$. Colorless crystals: $M_w = 929.56$, monoclinic, space group P_{21}, $a = 11.4180(3)$, $b = 6.8800(3)$, $c = 12.6820(3)$ Å, $\beta = 108.8690(2)°$, $V = 942.71(3)$ Å^3, $Z = 2$, $D_{calcd} = 1.227$ g cm^{-3}, $\mu = 0.175$ mm^{-1}, $T = 293(2)$ K; 1530 unique of 2497 reflections collected; 2497 observed reflections were used for the full matrix least-squares refinement on F^2; $R_1 = 0.0404$ ($I > 2\sigma(I)$), $R_1 = 0.0656$ (all data); $wR_2 = 0.1081$ ($I > 2\sigma(I)$), $wR_2 = 0.1300$ (all data).

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