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Selective Dopamine Receptors: Synthesis, Complexing Properties, and Molecular Modelling Studies of New Podands Derived from 4-Hydroxy-1*H*-Pyrazole

María Isabel Rodríguez-Franco,* Patricia San Lorenzo, Ana Martínez, and Pilar Navarro

Instituto de Química Médica (C.S.I.C.), Juan de la Cierva 3, 28006-Madrid, Spain.

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Abstract: New podands derived from 4-hydroxy-1*H*-pyrazole have been prepared. Their complexing properties towards cations (Na^+, K^+, NH_4^+) and some neurotransmitters (dopamine and norepinephrine) have been studied, using biphasic extraction experiments, molecular modelling, and a NMR titration. Podand **10**, 1-benzyl-4-hydroxy-3,5-bis(2, 5, 8, 11-tetraoxadodecan-1-yl)-1*H*-pyrazole, showed an interesting selective complexation of dopamine.

Keywords: Host compounds; Ammonium salts; Complexation; Molecular modelling.

INTRODUCTION

The molecular recognition of neurotransmitters (acetylcholine, dopamine, norepinephrine, etc.) is currently an active area of biological and chemical research,[1] because of their importance in neurodegenerative and some mental illnesses (Alzheimer's disease, Parkinsonism, depressive disorders, etc.), where the levels of such molecules are reduced.[2] In this field, our group have reported the synthesis of crowns and podands containing pyrazolic[3] or propylendipyrazolic[4] units, with different size, flexibility, steric hindrance, number and type of donor atoms. We have also studied their complexing properties towards alkali and ammonium cations of physiological interest, including neurotransmitter catecholamines.[5] In this way, we have found that the polyether crowns (**1**, X=H_2) bound alkylammonium cations better than their counterparts, the ester crowns (**1**, X=O) (Figure 1).

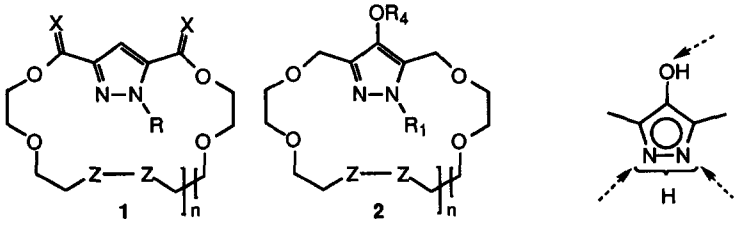

FIGURE 1

* E-mail: iqmrf32@pinar1.csic.es

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Taking into account that the binding affinity and selectivity of crown ethers may be modified by introducing appended chains into the crown ring,[6] now we are interested in the development of new non-ester pyrazolic receptors with different side arms (**2**), to improve and/or modify their complexing properties. Our target was to find a synthetic method that permits us to link selectively a chain in position 1 or in position 4 of the pyrazolic nucleus, and the 4-hydroxy-1*H*-pyrazole subunit (Figure 1) seemed to be useful, because its two different heteroatoms (in positions 1 and 4) could be differently functionalized using common organic reactions.

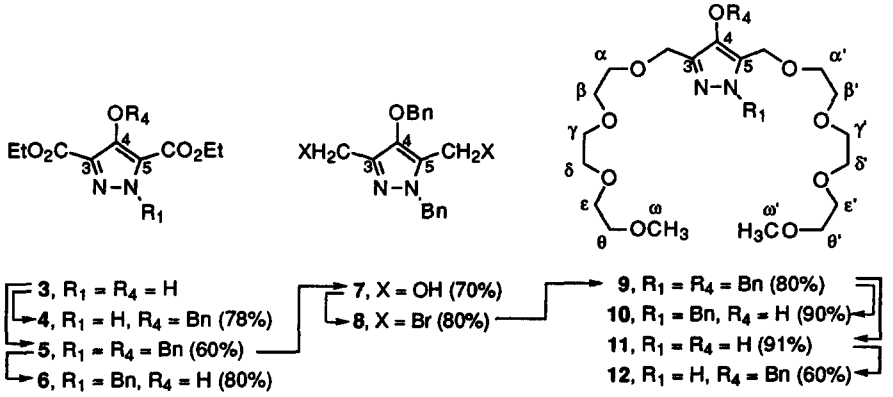
In this work we describe a synthetic route to achieve selective benzylation-debenzylation in podands derived from 4-hydroxy-1*H*-pyrazole, that opens the way to obtain receptors with the appropriate side arms, specially designed for molecular recognition. The complexing properties of the new podands towards cations (Na^+, K^+, NH_4^+) and some neurotransmitters (dopamine and norepinephrine) have been studied, using biphasic extraction experiments, molecular modelling, and a NMR titration.

RESULTS AND DISCUSSION

SYNTHESIS

The experimental conditions to obtain the selective functionalization of the 1,4-pyrazolic positions were checked out using as starting substrate 3,5-bis(ethoxycarbonyl)-4-hydroxy-1*H*-pyrazole (**3**), that was synthesised by cycloaddition of ethyl diazoacetate and ethyl malonate in the presence of sodium ethoxide, following a described method.[7] Selective *O*-benzylation of **3** was achieved in water using sodium hydroxide and benzyl bromide, both in equimolar proportions. This reaction yielded, almost exclusively, the *O*-benzylated pyrazole **4** in 78% yield (Scheme 1).

In contrast, the preparation of *N*-benzylated pyrazole by direct alkylation of **3** was not possible. Using an equimolar amount of the alkylating agent in different experimental conditions (direct heating with the alkylating reactive,[8] or phase transfer catalysis) a mixture of all possible benzylated products was obtained. Therefore, we firstly prepared the *N,O*-dibenzylated pyrazole **5** (see Experimental Part), that was later subjected to several hydrogenolytic conditions to get the selective deprotection of the 4-pyrazolic position, without any change in the benzyl group attached in the N-1. This was achieved using soft hydrogenolytic conditions (Pd/C 10% at 5 psi for 10 minutes), that produced the 1-benzyl-4-hydroxy derivative **6** in good yield (80%).



SCHEME 1

Podands derived from 4-hydroxy-1*H*-pyrazole were obtained by the same methodology. Thus, using the *N,O*-dibenzylated diester pyrazole **5** as starting material, and following a three step reaction pathway (reduction, halogenation and Williamson ether formation) the corresponding *N,O*-dibenzylated podand **9** was obtained in good yield (80%) (Scheme 1). Its hydrogenolysis with soft conditions (Pd/C 10% at 10 psi for 30 minutes at room temperature) yielded the 4-hydroxy-1-benzylated podand **10** in excellent yield (90%), whereas when more vigorous conditions were used (Pd/C 10% at 50 psi for 48 hours at 50°C), the completely deprotected podand **11** was isolated, also in good yield (91%). Finally, the 4-benzyloxylated podand (**12**) was selectively synthesised from **11**, using experimental conditions closed to those of the diester series: sodium hydroxide and benzyl bromide (equimolar proportions) in water at 45-50°C.

The new compounds were characterised from their spectroscopic (^{1}H NMR, ^{13}C NMR, and MS) and analytical data. The chemical shifts of the quaternary pyrazolic carbons were unequivocally assigned using HMBC (Heteronuclear Multiple Bond Correlation) experiments.

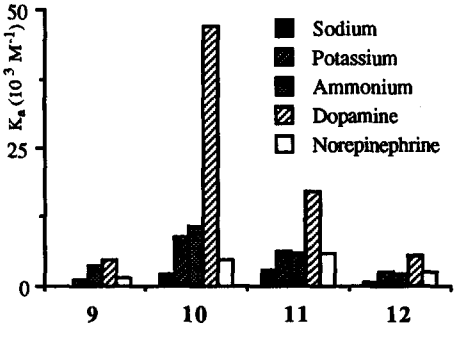
BIPHASIC EXTRACTION EXPERIMENTS

The affinities of podands **9-12** toward some cations of biological importance (Na^+, K^+, NH_4^+, dopamine, and norepinephrine) were examined by solvent extraction experiments, using a 1,2-dichloroethane—water biphasic system, following Cram's method.[9] The results are summarised in Table 1 and graphically compared in Figure 2.

TABLE 1 and FIGURE 2. Association constants (M^{-1})[a] of the new podands toward alkali, ammonium, dopamine (Dop), and norepinephrine (Nor) cations, using biphasic Cram's method at 25°C.

Host	Na^+	K^+	NH_4^+	Dop	Nor
9	140	980	3600	4800	1400
10	2100	8800	11000	47000	4800
11	2900	6400	5900	17000	6000
12	700	2700	2300	5600	2600

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



The new acyclic crowns showed almost the same cation selectivity pattern, with a clear preference for dopamine, specially the podand derived from 1-benzyl-4-hydroxy-1*H*-pyrazole (**10**) with an association constant of 4.7 10^4 M^{-1}, that is higher than the association constant described for others polyethyleneglycol podands in their interaction with alkylammonium cations ($K_a \approx 10^2$ M^{-1}).[10] Our pyrazolic acyclic receptor **10**, in addition to be a good complexing agent for dopamine, showed good selectivities dopamine / sodium and dopamine / potassium. Since the changes in the normal dopamine levels have been correlated with several illnesses (Alzheimer, Parkinsonism, etc.), podand **10** could be useful in the determination of dopamine in physiological fluids, where the sodium and potassium concentrations are higher than the neurotransmitter levels.[2b]

MOLECULAR MODELLING STUDIES

With the aim of obtaining information on the three-dimensional structures of the new receptors that could also explain their complexing properties, molecular modelling studies were carried out, using the

SYBYL program[11] implemented on a Silicon Graphics working station. Initial geometries for podands **9-12** were built from the standard molecular distances and angles within SYBYL program, assuming the planarity of the structures, that were firstly optimised with MAXIMIN. Semiempirical calculations were performed using the AM1 method[12] in MOPAC V5.0 program package[13]. In all cases, full geometry optimisations with Fletcher-Powell algorithm were carried out.

As it can be observed in the Figure 3, where the optimised structures of the four podands have been depicted, the interactions between the oxygens of the polyether chains and the hydrogens of the pyrazolic OH and NH, form macrocyclic pseudocavities that could act in the complexation process. In the best binder (podand **10**) the two polyether chains are involved in a pseudocavity around the hydroxyl group attached to the pyrazolic 4-position. The second best binder (**11**), derived from 4-hydroxy-1*H*-pyrazole, is able to form two pseudocavities, one around the OH and the other around the pyrazolic NH, yielding two independent complexation sites, as it can be observed in its Van der Waals surface. In the third in efficacy (podand **12**) only one polyether chain forms a pseudocavity around the pyrazolic NH, and in the worst binder (podand **9**) the two benzylic groups prevent this kind of interactions, and the pseudocavities do not take place.

In order to ascertain some information on the nature of the best complex of dopamine, a mechanical molecular modelling study was carried out, using the previously minimised geometries by AM1 of both podand **10** and dopamine, as initial geometries. Several possible complexes were built and optimised, using the MAXIMIN force field within the SYBYL program. In the more stable supramolecular structure (Figure 4) it can be seen that dopamine interacts with the receptor through two different sites: the ammonium group and the aromatic framework. The ammonium cationic head binds the polyether chain attached to the pyrazolic 3-position, through electrostatic forces of ion-dipole type and through hydrogen bonds. Two hydrogens of the ammonium cation are oriented to the two extreme oxygens of the polyethereal chain (O2 and O11), with typical hydrogen bond distances (1.73 and 1.71 Å).[14] The third ammonium hydrogen is placed between the two central oxygens (O5 and O8) with similar distances (1.73 and 1.85 Å). There is another hydrogen bond (1.97 Å) between a phenolic hydrogen of dopamine and the first oxygen (O2') of the polyether chain attached to the pyrazolic 5-position. Moreover, this oxygen keeps also its bond with the pyrazolic hydroxyl group, as it could see in the free ligand. In the CPK model of the complex one can observe the parallel spatial disposition between the aromatic framework of dopamine and the benzylic group belonging to the receptor. In this disposition π-aromatic interactions, that probably increase the stability of the supramolecular structure, can take place.

COMPLEXATION STUDY BY ^1H NMR SPECTROSCOPY

The stability constant (K_a) of the interaction in acetone-d_6 between the podand **10** (host) and dopamine picrate (guest) has been calculated by ^1H NMR spectroscopy at 26°C. During the experiment, the host concentration was held constant, while the concentration of the guest was increased (see Experimental Part).

Upon the addition of the guest, the polyether chain attached to the 3-pyrazolic position and the aromatic benzyl group protons of the host shifted upfield (about 0.3 and 0.2 ppm, respectively), while the polyether chain linked to pyrazolic-C5 moved downfield (0.1 ppm). This is in accord with the complex calculated by molecular modelling, where the strongest interaction occurred between the ammonium cationic head and the polyethereal chain at C3 in the pyrazole nucleus. The fact that the aromatic benzylic protons of the receptor moved also upfield, pointed up that π-interactions between this benzyl group and the catechol moiety of the neurotransmitter took place, as it could be observed in the modelling study.

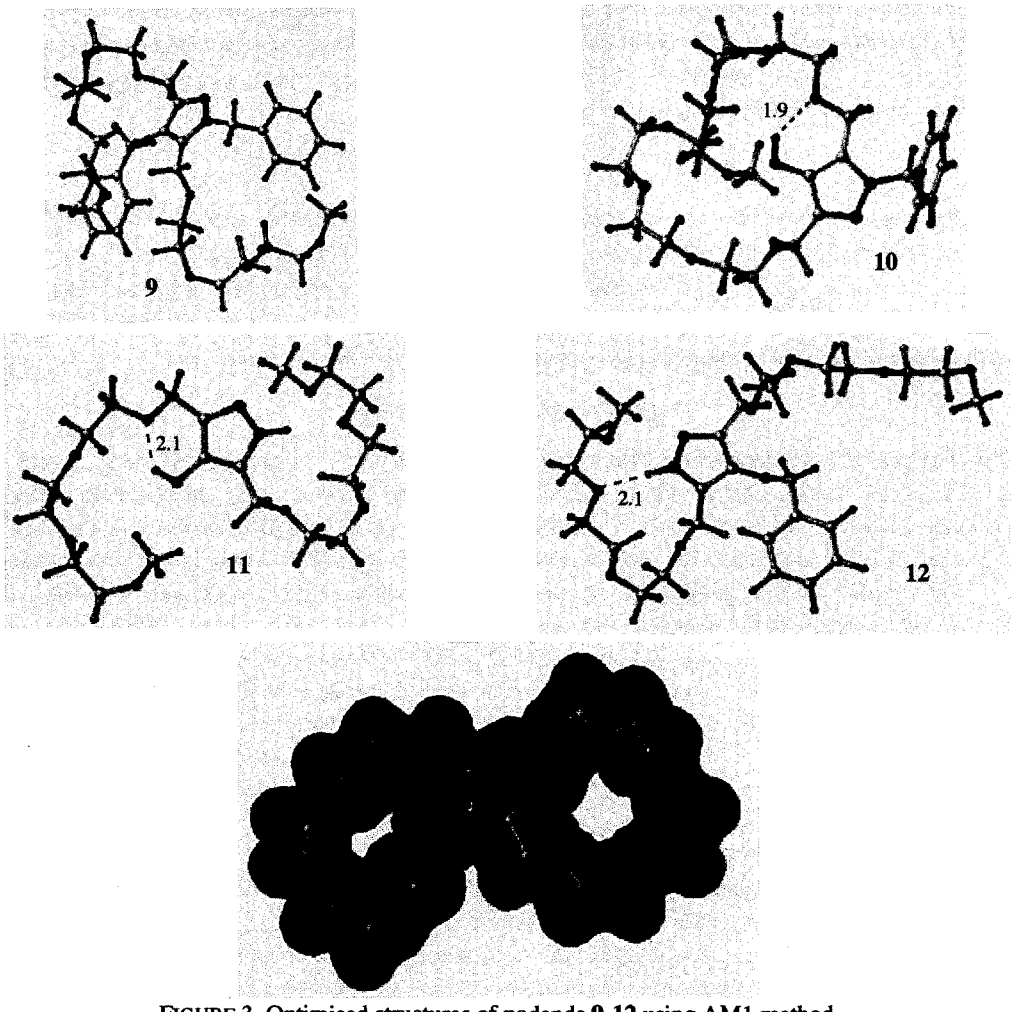


FIGURE 3. Optimised structures of podands **9-12** using AM1 method, and VdW surface of podand **11**. Distances in Å.

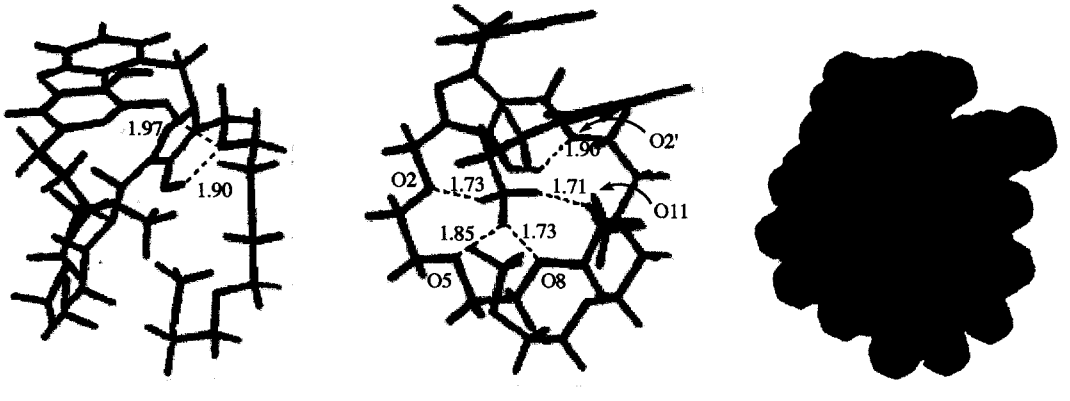


FIGURE 4. Optimised structure of the complex podand **10** : dopamine and its CPK model. Distances in Å.

The Job plot[15] using the chemical shift change of the methylene protons directly linked to pyrazolic C3 of the host had a maximum at a mole fraction of 0.5, indicative of formation of a 1:1 complex. To calculate the association constant (K_a) a nonlinear-least-square analysis of the same methylene was used,[16] providing 6 10^3 M^{-1} for the interaction **10**:dopamine picrate.

CONCLUSIONS

In this work, we have developed a synthetic route to obtain selective benzylation-debenzylation in podands derived from 4-hydroxy-1*H*-pyrazole. We have shown the potential usefulness of one of these podands as selective complexing agent of dopamine. The molecular modelling has made comprehensible the structures of the free podands, as well of the best complex of dopamine. The stabilising interactions showed by this study will be considered in future design of neurotransmitter selective receptors.

EXPERIMENTAL SECTION

Melting points were determined with a Reichert-Jung hot-stage microscope and are uncorrected. IR spectra were obtained with a Perkin-Elmer 681 infrared spectrophotometer. NMR spectra were recorded in $CDCl_3$ solutions, using Varian Unity-500, Varian XL-300, and Gemini-200 spectrometers. Mass spectra (MS) were obtained by electronic impact at 70 eV in a VG 12-250 spectrometer (VG Masslab). Elemental analyses were carried out in a Perkin-Elmer 240C equipment in the Centro de Química Orgánica 'Manuel Lora-Tamayo' (CSIC). Chromatographic separations were performed on silica gel, using the following techniques: flash column chromatography (Kieselgel 60 Merck of 230-400 mesh) and preparative centrifugal circular thin layer chromatography (CCTLC, on a circular plate coated with a 1 mm layer of Kieselgel 60 PF_{254} gishalting, Merck, using a Chromatotron®). Compounds were detected with UV light (254 nm) or iodine chamber. Most starting materials were commercially available products and were used without further purification. THF was freshly distilled from $LiAlH_4$ and commercial (Aldrich) triethylene glycol monomethyl ether was distilled *in vacuo*. 3,5-Bis(ethoxycarbonyl)-4-hydroxy-1*H*-pyrazole (**3**) was synthesised according to the literature[7] (mp = 128-129°C, lit. mp = 128-130°C).

4-Benzyloxy-3,5-bis(ethoxycarbonyl)-1*H*-pyrazole (4). To a solution of 100 mg (0.4 mmol) of **3** in 2 mL of 0.2 M aq. sodium hydroxide (0.4 mmol), heated at 90-100°C, 52 µL (75 mg, 0.4 mmol) of benzyl bromide was added. The solution was stirred at the same temperature until neutral pH was obtained (about 4 hours). After cooling to room temperature, the reaction mixture was extracted with CH_2Cl_2 (3 x 25 mL) and the organic solution was dried (Na_2SO_4) and evaporated to dryness *in vacuo*. The residual syrup was purified by flash chromatography on a silica gel column. The fractions of R_f = 0.4 (hexane : ethyl acetate, 1:1) were evaporated to dryness *in vacuo* and the residual solid was crystallized from hexane : ethyl acetate to afford 109 mg (78 % yield) of the *O*-benzylated product **4** as a white solid (mp = 117-118°C). IR (KBr): 3230 (broad, NH), 1725 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): 7.48-7.24 (m, 5H, $OCH_2C_6H_5$), 5.18 (s, 2H, $OCH_2C_6H_5$), 4.33 (q, 4H, J = 7.5 Hz, $CO_2CH_2CH_3$), 1.35 (t, 6H, J = 7.5 Hz, $CO_2CH_2CH_3$). ^{13}C NMR ($CDCl_3$): 159.6 (C=O), 147.6 (C3,5), 136.5, 128.4, 128.4, and 128.3 ($OCH_2C_6H_5$), 124.3 (C4), 77.5 (OCH_2Ph), 61.7 ($CO_2CH_2CH_3$), 14.2 ($CO_2CH_2CH_3$). MS m/z (rel intensity): 91 (100), 318 (M^+, 10), 319 (M^++1, 1). Anal. Calcd for $C_{16}H_{18}N_2O_5$ (318.3): C, 60.37; H, 5.70; N, 8.80. Found: C, 60.03; H, 5.53; N, 8.58.

1-Benzyl-4-benzyloxy-3,5-bis(ethoxycarbonyl)-1*H*-pyrazole (5). A mixture of **3** (1.75 g, 7.7 mmol), benzyl chloride (2.30 g, 18 mmol) and tetrabutylammonium bromide (0.87 g, 2.7 mmol) in dichloromethane (50 mL) was treated with 10 M aq. sodium hydroxide (50 mL) at 35° C for 10 hours. After cooling to room temperature, the organic phase was separated and the aqueous layer extracted with CH_2Cl_2 (3 x 50 mL). The resulting organic solution was dried (Na_2SO_4) and evaporated to dryness *in vacuo* to give a solid, which was purified by flash chromatography on a silica gel column. The fractions of R_f = 0.7 (hexane : ethyl acetate, 3:1)

were evaporated to dryness *in vacuo* and the residual solid was crystallized from ethanol : water to afford 1.88 g (60 % yield) of the dibenzylated pyrazole **5** as a white solid (mp = 54-55°C). IR (KBr): 1730, 1720 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): 7.0-7.4 (m, 10H, C_6H_5), 5.67 (s, 2H, NCH_2Ph), 5.05 (s, 2H, OCH_2Ph), 4.36 [q, 2H, J = 7.6 Hz, C(3)$CO_2CH_2CH_3$], 4.18 [q, 2H, J = 7.6 Hz, C(5)$CO_2CH_2CH_3$], 1.32 [t, 3H, J = 7.6 Hz, C(3)$CO_2CH_2CH_3$], 1.16 [t, 3H, J = 7.6 Hz, C(5)$CO_2CH_2CH_3$]. ^{13}C NMR ($CDCl_3$): 160.9 [C=O(3)], 158.7 [C=O(5)], 147.7 (C3), 134.4 (C5), 125.3 (C4), 136.6, 128.5, 128.3, and 128.2 ($OCH_2C_6H_5$), 136.1, 128.9, 127.9, and 127.3 ($NCH_2C_6H_5$), 77.5 (OCH_2Ph), 61.3 and 61.1 ($CO_2CH_2CH_3$), 56.9 (NCH_2Ph), 14.3 and 14.0 ($CO_2CH_2CH_3$). MS m/z (rel intensity): 91 (100), 408 (M^+, 8), 409 (M^++1, 2). Anal. Calcd for $C_{23}H_{24}N_2O_5$ (408.4): C, 67.63; H, 5.92; N, 6.86. Found: C, 67.79; H, 5.94; N, 6.88.

1-Benzyl-3,5-bis(ethoxycarbonyl)-4-hydroxy-1*H*-pyrazole (6). A solution of 20 mg (0.05 mmol) of the dibenzylated product **5** in 50 mL of methanol was hydrogenated over Pd/C 10 % at 5 psi for 10 minutes, at room temperature. After filtration, the solvent was removed under *vacuum* to give the *N*-benzylated diester, that was later crystallized from hexane to yield 12 mg (80 %) of **6** as a white solid (mp = 62-63°C). IR (KBr): 3520 (broad, OH), 1730, 1720 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): 7.23-7.10 (m, 5H, N-$CH_2C_6H_5$), 5.65 (s, 2H, NCH_2Ph), 4.40 [q, 2H, J = 6.2 Hz, C(3)$CO_2CH_2CH_3$], 4.28 [q, 2H, J = 6.2 Hz, C(5)$CO_2CH_2CH_3$], 1.36 [t, 3H, J = 6.2 Hz, C(3)$CO_2CH_2CH_3$], 1.23 [t, 2H, J = 6.2 Hz, C(5)$CO_2CH2CH3$]. ^{13}C NMR ($CDCl_3$): 163.3 [C=O(3)], 159.3 [C=O(5)], 150.0 (C3), 142.0 (C5), 135.9, 128.5, 127.9, and 127.3 ($NCH_2C_6H_5$), 118.0 (C4), 61.4 and 61.3 ($CO_2CH_2CH_3$), 56.8 (NCH_2Ph), 14.3 and 14.1 ($CO_2CH_2CH_3$). MS m/z (rel intensity): 91 (100), 318 (M^+, 12), 319 (M^++1, 2). Anal. Calcd for $C_{16}H_{18}N_2O_5$ (318.3): C, 60.37; H, 5.70; N, 8.80. Found: C, 60.07; H, 5.79; N, 8.67.

1-Benzyl-4-benzyloxy-3,5-bis(hydroxymethyl)-1*H*-pyrazole (7). To a suspension of 0.33 g (8.8 mmol) of $LiAlH_4$ in anhydrous THF (25 mL) a solution of **5** (1.79 g, 4.4 mmol) in THF (25 mL) was added at 0°C. After the mixture reached room temperature, it was refluxed until a TLC control (silica gel, hexane : ethyl acetate, 2:1) indicated that the reaction was completed (1 hour). The excess of hydride was destroyed with ice (50 mL) and the aluminium salts were precipitated with 3 N aq. sodium hydroxide (50 mL). The precipitate was filtered off and washed with several portions of dichloromethane. The solvent was removed under *vacuum* and the residue was crystallized from ethyl acetate : hexane, to yield 1.0 g (70 %) of **7** as a white solid (mp = 71-72°C). IR (KBr): 3560, 3320 (broad) cm^{-1}. ^1H NMR (Acetone-d_6): 7.1-7.4 (m, 10H, C_6H_5), 5.30 (s, 2H, NCH_2Ph), 5.01 (s, 2H, OCH_2Ph), 4.50 [d, 2H, J = 5.9 Hz, CH_2-(3), turns into a singlet with D_2O], 4.30 [d, 2H, J = 5.9 Hz, CH_2-(5), turns into a singlet with D_2O], 4.09 [t, 1H, J = 5.9 Hz, HO-(3), disappears with D_2O], 3.95 [t, 1H, J = 5.9 Hz, HO-(5), disappears with D_2O]. ^{13}C NMR (Acetone-d_6): 142.4 (C3), 138.5 (C4), 136.8, 128.4, 127.7, and 126.8 ($NCH_2C_6H_5$), 136.6, 129.1, 128.6, and 128.5 ($OCH_2C_6H_5$), 133.1 (C5), 77.5 (OCH_2Ph), 56.2 [CH_2-(3)], 53.8 (NCH_2Ph), 52.3 [CH_2-(5)]. MS m/z: 91 (100), 324 (M^+, 1), 325 (M^++1, 0.4). Anal. Calcd for $C_{19}H_{20}N_2O_3$ (324.4): C, 70.35; H, 6.21; N, 8.64. Found: C, 70.26; H, 6.03; N, 8.42.

1-Benzyl-4-benzyloxy-3,5-bis(bromomethyl)-1*H*-pyrazole (8). Phosphorous tribromide (0.7 mL, 7.4 mmol) was slowly added to a stirred solution of **7** (1.0 g, 3.1 mmol) in anhydrous THF (50 mL). The mixture was stirred at room temperature for 10 hours and then was poured into 50 g of crushed ice, vigorously stirred and neutralized with sodium carbonate. The organic layer was separated and the aqueous one was extracted with dichloromethane (3 x 50 mL). All the organic extracts were dried (Na_2SO_4) and evaporated to dryness *in vacuo* to give a white solid, which was crystallized from ethyl acetate : hexane, yielding 1.11 g (80 %) of **8** (mp = 85-86°C), as a white solid. IR (KBr): 770, 730, 715 cm^{-1}. ^1H NMR ($CDCl_3$): 7.07-7.41 (m, 10H, C_6H_5), 5.32 (s, 2H, NCH_2Ph), 5.05 (s, 2H, OCH_2Ph), 4.39 [s, 2H, CH_2-(3)], 3.99 [s, 2H, CH_2-(5)]. ^{13}C NMR ($CDCl_3$): 140.1 (C4), 139.59 (C3), 136.4, 128.7, 128.7, and 126.8 ($NCH_2C_6H_5$), 135.8, 128.9, 128.6, and 128.1 ($OCH_2C_6H_5$), 129.9 (C5), 76.7 (OCH_2Ph), 54.6 (N CH_2Ph), 22.2 [CH_2-(3)], 18.0 [CH_2-(5)]. MS m/z (rel intensity): 91 (100), 448 (M^+, 0.1), 450 (M^++2, 0.2), 452 (M^++4, 0.1). Anal. Calcd for $C_{19}H_{18}Br_2N_2O$ (450.2): C, 50.69; H, 4.03; N, 6.22; Br, 35.50. Found: C, 50.98; H, 4.29; N, 5.98; Br, 35.75.

1-Benzyl-4-benzyloxy-3,5-bis(2, 5, 8, 11-tetraoxadodecan-1-yl)-1*H*-pyrazole (9). To a stirred suspension of NaH (6 mmol, oil free) in dry THF (50 mL) under nitrogen, was added a solution of triethylene glycol monomethyl ether (0.8 mL, 5 mmol), and the mixture was warmed to 60°C for 10 minutes. Then, a solution of **8** (1.1 g, 2.4 mmol) in dry THF (50 mL) was slowly added at the same temperature. After the addition was completed, the reaction was kept for 2 h at 60°C and then cooled to room temperature over night. The resulting mixture was treated with a 15 % aqueous solution of ammonium chloride until pH = 7. The aqueous layer was decanted and extracted with CH_2Cl_2 (3 x 50 mL), the organic extracts were dried (Na_2SO_4) and evaporated to dryness to give a syrup, which was purified by flash chromatography on a silica gel column. From the fractions of $R_f = 0.8$ (dichloromethane : methanol, 20:1) the dibenzylated podand **9** was obtained (1.2 g, 80 % yield) as a pure syrup. IR (neat): 1170-1080 (C-O-C) cm^{-1}. ^1H NMR ($CDCl_3$): 7.05-7.35 (m, 10H, C_6H_5), 5.30 (s, 2H, NCH_2Ph), 4.99 (s, 2H, OCH_2Ph), 4.56 [s, 2H, CH_2-(3)], 4.09 [s, 2H, CH_2-(5)], 3.69-3.36 (m, 24 H, α-θ, α'-θ'), 3.35 [s, 3H, ω], 3.34 [s, 3H, ω']. ^{13}C NMR ($CDCl_3$): 141.1 (C4), 139.3 (C3), 130.0 (C5), 137.1, 128.7, 128.3, and 128.1 ($OCH_2C_6H_5$), 137.0, 128.5, 127.4, and 126.9 ($NCH_2C_6H_5$), 77.5 (OCH_2Ph), 71.8 and 71.7 (θ, θ'), 70.5, 70.4, and 70.2 (β-ε, β'-ε'), 69.2 (α), 68.9 (α'), 64.3 [CH_2-(3)], 60.0 [CH_2-(5)], 58.9 and 58.8 (ω, ω'), 54.1 (NCH_2Ph). MS m/z (rel intensity): 91 (100), 616 (M^+, 2), 617 (M^++1, 0.3). Anal. Calcd for $C_{33}H_{48}N_2O_9$ (616.7): C, 64.27; H, 7.84; N, 4.54. Found: C, 64.14; H, 7.98; N, 4.97.

1-Benzyl-4-hydroxy-3,5-bis(2, 5, 8, 11-tetraoxadodecan-1-yl)-1*H*-pyrazole (10). A solution of 200 mg (0.32 mmol) of the dibenzylated podand **9** in 100 mL of methanol was hydrogenated over Pd/C 10% at 10 psi for 30 minutes, at room temperature. After filtration, the solvent was removed under *vacuum* to give a syrup, which was purified by flash chromatography on a silica gel column. The fractions of $R_f = 0.4$ (dichloromethane : methanol, 20:1) gave 154 mg (90%) of the *N*-benzylated podand **10** as a pure syrup. IR (neat): 3490 (OH), 1170-1080 (C-O-C) cm^{-1}. ^1H NMR ($CDCl_3$): 7.29-7.09 (m, 5H, C_6H_5), 5.25 (s, 2H, NCH_2Ph), 4.63 [s, 2H, CH_2-(3)], 4.41 [s, 2H, CH_2-(5)], 3.70-3.35. (m, 24 H, α-θ, α'-θ'), 3.34 (s, 6H, ω, ω'). ^{13}C NMR ($CDCl_3$): 139.8 (C4), 135.7 (C3), 125.0 (C5), 137.3, 128.4, 127.4, and 126.9 ($NCH_2C_6H_5$), 71.8 and 71.7 (θ, θ'), 70.4, 70.3, and 70.2 (β-ε, β'-ε'), 69.0 (α), 68.4 (α'), 65.7 [CH_2-(3)], 60.0 [CH_2-(5)], 58.9 and 58.8 (ω, ω'), 53.8 (NCH_2Ph). MS m/z (rel intensity): 91 (100), 526 (M^+, 1), 527 (M^++1, 0.2). Anal. Calcd for $C_{26}H_{42}N_2O_9$ (526.6): C, 59.30; H, 8.04; N, 5.32. Found: C, 58.98; H, 8.25; N, 5.42.

4-Hydroxy-3,5-bis(2, 5, 8, 11-tetraoxadodecan-1-yl)-1*H*-pyrazole (11). A solution of 150 mg (0.24 mmol) of the dibenzylated podand **9** in 100 mL of methanol was hydrogenated over Pd/C 10 % at 50 psi and 50°C, for 48 hours. After filtration, the solvent was removed under *vacuum* to give a syrup, which was purified by flash chromatography on a silica gel column. Using dichloromethane : methanol (20:1) as eluent, and collecting the fractions of $R_f = 0.3$, the podand **11** was obtained (97 mg, 91 % yield) as a pure syrup. IR (neat): 3460 (broad, OH-NH), 1170-1070 (C-O-C) cm^{-1}. ^1H NMR ($CDCl_3$): 4.60 [CH_2-(3)], 4.60 [CH_2-(5)], 3.64-3.34 (m, 24 H, α-θ, α'-θ'), 3.35 (s, 6H, ω, ω'). ^{13}C NMR ($CDCl_3$): 138.0 (C4), 131.5 (C3,5), 71.8 (θ, θ'), 70.6, 70.4, and 70.3 (β-ε, β'-ε'), 68.9 (α, α'), 63.4 [CH_2-(3,5)], 58.8 (ω, ω'). MS m/z (rel intensity): 45 (100), 436 (M^+, 0.5), 437 (M^++1, 0.1). Anal. Calcd for $C_{19}H_{36}N_2O_9$ (436.5): C, 52.28; H, 8.31; N, 6.42. Found: C, 51.95; H, 8.17; N, 6.14.

4-Benzyloxy-3,5-bis(2, 5, 8, 11-tetraoxadodecan-1-yl)-1*H*-pyrazole (12). A solution of 3.6 mg (0.09 mmol) of sodium hydroxide in 2 mL of water was added to 40 mg (0.09 mmol) of podand **11**. After some minutes, 10.9 μL (15.7 mg, 0.091 mmol) of benzyl bromide was added and the reaction was kept at 40-50 ºC over night. The resulting mixture, that showed neutral pH, was extracted with ethyl acetate and the organic layer dried (Na_2SO_4) and evaporated to dryness *in vacuo* to give a syrup, which was purified by centrifugal circular thin layer chromatography. From the fractions of $R_f = 0.5$ (dichloromethane : methanol, 20:1) the *O*-benzylated podand **12** was obtained (29 mg, 60 % yield) as a pure syrup. IR (neat): 3250 (NH), 1180-1060 (C-O-C) cm^{-1}. ^1H NMR ($CDCl_3$): 7.32-7.25 (m, 5H, $OCH_2C_6H_5$), 4.94 (s, 2H, OCH_2Ph). 4.41 [s, 4H, CH_2-(3,5)], 3.64-3.35 (m, 24H, α-θ, α'-θ'), 3.34 (s, 6H, ω, ω'). ^{13}C NMR ($CDCl_3$): 139.2 (C4), 135.7

(C3,5), 137.0, 128.5, 128.5, and 128.3 ($OCH_2C_6H_5$), 77.2 (OCH_2Ph), 71.8 (θ, θ'), 70.6 70.5, and 70.4 (β-ε, β'-ε'), 63.0 [CH_2-(3,5)], 58.9 (ω, ω'). MS m/z (rel intensity): 91 (100), 526 (M^+, 2), 527 (M^++1, 0.5). Anal. Calcd for $C_{26}H_{42}N_2O_9$ (526.6): C, 59.30; H, 8.04; N, 5.32. Found: C, 59.08; H, 7.85; N, 4.99.

BIPHASIC EXTRACTION EXPERIMENTS

These complexation experiments were made following the Cram's Method,[9] using a Perkin-Elmer 550 SE UV/VIS spectrometer and working at 380 nm at constant temperature (25°C). Mixtures of the picrate (2.10^{-3} M) and the chloride (10^{-2} M) of a given cation (sodium, potassium, ammonium, dopamine and norepinephrine) in deionizated water (milli-Q) in volumetric flasks (10 mL) were prepared. Solutions of each host **9-12**, 10^{-3} M in bidistilled 1,2-dichloroethane, were also prepared in 10 mL volumetric flasks. Into a 10-mL centrifuge tube a measured volume (0.5 mL) of cation aqueous solution and the same volume of organic host solution were introduced. To one tube 0.5 mL of deionized water was placed, instead the saline solution, to be used as a blank. The tubes were capped, vigorously shaken for 3 min., using a Vortex shaker, and then separated into two clear layers by centrifugation at 2000 rpm at 25°C for 10 min. Using a microsyringe, five aliquots of each organic phase were measured (25, 50, 75, 100 and 125 μL) and transferred into five 5-mL volumetric flask and diluted to the mark with acetonitrile (Scan-Lab, special for UV). The UV absortion of each new solution was measured at 380 nm and the resulting data processed by linear regression to obtain the concentration of the extracted cation in the organic phase. In order to calculate the association constants (K_a), summarized in table 1, it was necesary to know the salt distribution between the two phases, in ausence of the host. These distribution constants (K_d) were experimentally determined shaking 4 mL of the corresponding aqueous picrate solution with 4 mL of pure 1,2-dichloroethane, and following the same methology as before. All experiments were carried out in triplicate (with a reproducibility of ±15%) and the respective results were averaged. The experimental K_d (M^{-1} x 10^2) values were: 2.8 (Na^+), 4.2 (K^+), 6.3 (NH_4^+), 7.4 (dopamine), and 8.6 (norepinephrine).

Extintion coefficients (ε) for each salt in acetonitrile were measured at 380 nm, using solutions 10^{-6} to 10^{-4} M, prepared by dilutions from a solution 10^{-3} M, that was obtained by direct weight. The graphical representation of the salt concentration *vs.* the absorbance gave a straight line that was subjected to linear regression. At least, three independent determinations were made for each cation picrate and the averaged ε ($M^{-1}cm^{-1}$) values were: 13700 (Na^+), 14600 (K^+), 16000 (NH_4^+), 16000 (dopamine), and 16100 (norepinephrine).

MOLECULAR MODELLING STUDIES

SYBYL 6.3 program,[11] implemented on a Silicon Graphics working station, was used. Input geometries were taken from the standard ones within SYBYL program, assuming the planarity of the structures, that were firstly optimized with MAXIMIN. Semiempirical calculations were performed using the AM1 method[12] in MOPAC V5.0 program package.[13] In all cases, full geometry optimizations with Fletcher-Powell algorithm were carried out.

BINDING STUDY BY ^1H NMR SPECTROSCOPY

The stoichiometry of the complex **10** : dopamine was determinated following the Job's Method.[15] Separate solutions of host (podand **10**) and guest (dopamine picrate) in acetone-d_6, each of the same concentration ($6\ 10^{-4}$ M prepared in 5-mL volumetric flasks), were mixed in order to reach the same final volume (0.5 mL). The ^1H NMR spectrum of each solution was recorded at 26±1°C. The chemical shift diferences (Δδ, ppm) of the methylene linked to pyrazolic C-3 in the host **10** were ploted *vs.* the mole fraction, showing a maximun at mole fraction = 0.5.

The association constant (K_a) was calculated using a titration in ^1H NMR. A stock solution for the host ($5.7\ 10^{-4}$ M, solution A) was obtained by dissolving the receptor **10** in acetone-d_6, using a 5-mL volumetric

flask. Using a 2-mL volumetric flask, a stock solution for the guest (dopamine picrate, $5.9.10^{-3}$ M, solution B) was prepared from the stock solution A, in order to have a constant concentration of the host during the titration. To an initial volume of 0.5 mL of pure host (solution A), forty portions of solution B (ranging from 3 μL, in the begining of the titration, to 100 μL in the end) were added via microsyringe, and the ^{1}H NMR spectrum of each solution was recorded at 26±1°C. The chemical shifts of the CH_2-(3), that was a singlet at $\delta = 4.518$ ppm in the pure host, were used in an iterative least-squares fitting procedure,[16] in order to calculate the association constant (K_a). The infinite δ value calculated for the complex was 4.205 ppm. Two independent titrations were carried out and the association constant was averaged (with an estimated error of 10%).

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