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Regioselective *Mucor miehei* Lipase Catalyzed Synthesis of Podands Containing a 1,3-Bis(1*H*-Pyrazol-1-yl)Propane Unit

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Abstract: The synthesis of podands (acyclic crown ethers) including a 1,3-bis(1*H*-pyrazol-1-yl)propane unit in their molecule is described. The key step of this synthesis was a regioselective lipase-catalyzed transesterification of the dipyrazolic tetraethylester **1** with monomethylether polyethyleneglycols. The ionophoric properties of the podands **4a-d** as carriers of cations were evaluated. Molecular modelling studies and quantum-chemical calculations were used in order to elucidate the superestructures of the complexes with ammonium cations.

INTRODUCTION

Since organic ammonium cations play an important role in biological systems, the development of receptor molecules capable of recognizing them is of special interest.[1,2] In previous papers, we have described the synthesis and ionophoric properties of crowns and podands containing 1-methyl and 1*H*-substituted pyrazole units[3] and the first synthesis of pyrazolic podands *via* a regioselective enzymatic transesterification.[4] Very recently, we also described the initial results on the first enzymatic synthesis of macrocycles, including a different new structure, 1,3-bis(1*H*-pyrazol-1-yl)propane.[5]

In the same way, we report here the synthesis of podands with the above mentioned heterocyclic structure, by regioselective lipase-catalyzed transesterification of 1,3-bis[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]propane (**1**) with polyethyleneglycol monomethylethers as nucleophiles, and their properties as carriers of cations. The two sp^2 nitrogen atoms are included in two distinct pyrazolic rings linked by a 1,3-propylene fragment that allows free rotation and a suitable length to reach conformations capable of trapping cations by electrostatic interactions with the sp^2 electron pairs of the nitrogen atoms of the pyrazoles and the ethereal oxygens of the two arms. Our target was to obtain acyclic derivatives of **1** that were capable of conforming a pseudo-cavity bearing the sp^2 electron pairs of both the pyrazolic nitrogen atoms and the polyether chains. Looking at this aim, we tried to transesterify only the ester groups situated at the 3 position of the pyrazolic rings, contiguous with the sp^2 nitrogen atoms, while those on the 5 position remained unchanged for future transformations.

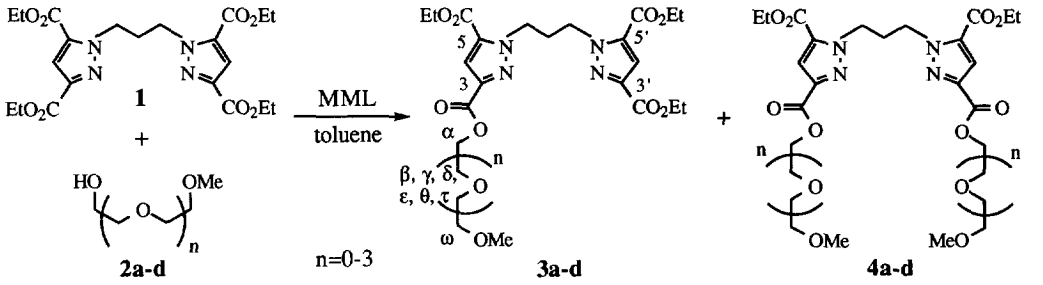
RESULTS AND DISCUSSION

SYNTHESIS

The starting substrate 1,3-bis[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]propane **1** was obtained[5] by condensation of two equivalents of 3,5-bis(ethoxycarbonyl)-1*H*-pyrazol[6] with 1,3-dibromopropane, in the presence of K_2CO_3.

Initially, the enzymatic reactions were performed on an analytical scale (in sealed 2 mL vials). They were carried out in anhydrous toluene at 60°C using mono- (n = 0, **2a**), di- (n = 1, **2b**), tri- (n = 2, **2c**) and tetra-

ethyleneglycol monomethylether (n = 3, **2d**) as nucleophiles (Scheme 1), in the presence of molecular sieves 3Å powder. Based on our own experience,[4] the only catalyst we used was *Mucor miehei* lipase[7] (MML).



Scheme 1

Aliquots were periodically taken and analyzed by hplc. As equilibrium was reached in all cases after a period of 10 - 14 days, conversions after 12 days were taken as the standard measure for comparison (see Table 1). No conversion was detected in parallel blank reactions without enzyme.

The best results were obtained with diethyleneglycol monomethylether (**2b**), both in mono- (**3b**) and disubstituted (**4b**) compounds. Overall conversions using tri- (**2c**) and tetra- ethyleneglycol monomethylether (**2d**) were acceptable, but the formation of disubstituted **4c** and **4d** appeared more difficult than that of **4b**, probably due to the steric and electronic hindrance of progressively longer polyether chains. Reaction with ethyleneglycol monomethylether (**2a**) displayed low conversion into **4a**. No attempts were made to improve these results.

TABLE 1. Transesterifications of tetraester **1**. Conversion after 12 days.[a]

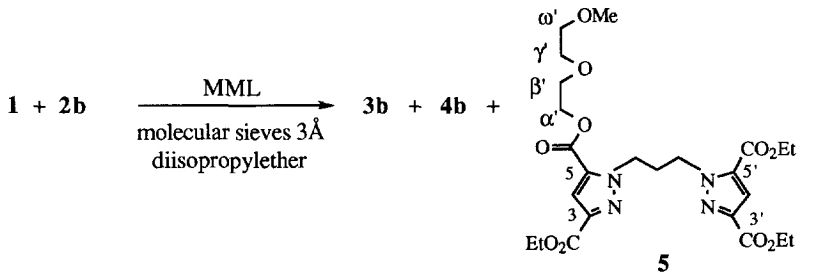
Nucleophile	Recovered **1**	Monosubstituted **3a-d**	Disubstituted **4a-d**
2a	71	18	4
2b	31	37	27
2c	48	29	10
2d	51	32	9

[a] No conversion was detected in absence of enzyme.

Preparative scale reactions were carried out in 100 mL volumes in order to confirm the structure of the compounds and check their cation complexing properties. Molecular sieves were omitted but ethanol released in the medium was continuously distilled off by formation of an azeotrope with toluene:[4,8] the reactions were stirred in a rotary evaporator without vacuum while they were heated at 60°C and periodically refilled with dry toluene to keep the volume of the solvent roughly constant.

Following the above mentioned procedure, the reactions took place in a regioselective manner and 3-mono- and 3,3'-disubstituted products were obtained (see below, *Structural Elucidation* part). We also obtained 5-monosubstituted regioisomer **5** when the reaction of tetraester **1** with **2b** was carried out in

diisopropylether (Scheme 2). We described in a previous work[9] that kinetic differences between two ester groups bore by non-equivalent positions on a heteroaromatic ring were significantly reduced when the solvent used was diisopropylether instead of toluene. As a result, reaction rate increases while regioselectivity diminishes. Following the general experimental procedure, a 7 days reaction of **1** with **2b** in diisopropylether (see Experimental part) yielded 3-mono- **3b**, 5-mono- **5** (32 and 4.5% respectively, regioselective ratio 88:12) and 3,3'-disubstituted **4b** (11%). Starting product **1** was also recovered (39%).



Scheme 2

STRUCTURAL ELUCIDATION

The structural assignment of the new compounds was made by ^{1}H NMR, since in N-alkylated pyrazoles the 3-alkoxy groups are always more deshielded than their counterparts of the 5 position.[10] In the ^{1}H NMR spectra of the disubstituted compounds **4a-d**, the two 3-ethyl groups from the starting substrate **1** (δ = 4.40 and 1.39 ppm) had disappeared, while those from the 5 pyrazolic positions ($\delta \approx$ 4.30 and 1.34 ppm, in **1** and **4a-d**) remained unchanged. Besides, in the spectra of the monosubstituted compounds **3a-d** it was possible to observe two ethyl groups attached to the 5 pyrazolic positions ($\delta \approx$ 4.29 and 1.33 ppm) and one ethyl group on the 3 position ($\delta \approx$ 4.37 and 1.37 ppm). In addition, the 5-monosubstituted regioisomer (**5**) showed two ethyl groups on the 3-pyrazolic position (δ = 4.31 and 1.30 ppm), and only one corresponding to the 5 position (δ = 4.24 and 1.28 ppm). The rest of their spectroscopic and microanalytical data are also consistent with the proposed structures.

TRANSPORT PROPERTIES

Podands **4a-d** had been evaluated as carriers of Li^+, Na^+, K^+, NH_4^+, and norepinephrine picrates. We used a chloroform liquid membrane that contains one of the products and which separates two aqueous phases. The guest cation salts are complexed with the host molecule from the first aqueous phase and transported to the second one by using a classical U-tube (see Experimental Section). The transport process occurs by carrier-mediated facilitated diffusion along the concentration gradient of the guest salts, and the results are gathered in Table 2.

TABLE 2. Transport rates (μmol h^{-1}) of alkali, ammonium and norepinephrine picrate across a $CHCl_3$ phase containing 7.10^{-4} M of carrier

Carrier	Li^+	Na^+	K^+	NH_4^+	norepinephrine
4a	1.89	2.41	1.49	3.15	10.46
4b	1.30	1.59	0.80	2.55	1.53
4c	11.85	4.51	4.15	6.18	1.60
4d	0.87	2.01	6.37	6.76	4.57

In general, ammonium was better transported than alkali cations by the podands **4a-d**. The selectivities observed in favour of NH_4^+ were 7.8 - 1.7 for Li^+, 3.4 - 1.3 for Na^+, and 3.2 - 1.1 for K^+. One exception was found, the podand derived from triethyleneglycol monomethyl ether (**4c**) exhibited the highest transport rate for Li^+ ion (11.85 μmol h^{-1}): it is about threefold the rates observed for Na^+ (4.51 μmol h^{-1}) and K^+ (4.15 μmol h^{-1}) with the same podand.

Since a decrease in the norepinephrine transmission had been found in the etiology of depressive disorders,[11] we tested our podands as carriers of this neurotransmitter. For the smaller acyclic compound **4a**, we obtained a high transport level of norepinephrine picrate (10.46 μmol h^{-1}) and an interesting selectivitiy in relation to alkali cations (norepinephrine / Li^+ = 5.5; norepinephrine / Na^+ = 4.3; and norepinephrine / K^+ = 7.0).

MOLECULAR MODELLING STUDIES

In order to throw some light on the nature of the complexes with ammonium ions and due to the absence of X-ray crystal structures, a computer molecular modelling study using quantum-chemical calculations of carrier **4a** and its ammonium complex has been done using the Chem-X[12] software and the AM1[13] semiempirical method. First of all, the two possible dispositions of the 5-ethoxycarbonyl group (torsional angle a=0° or 180°) were considered. The ΔH_f^o values shown that the C=O group forms an stabilizing hydrogen bond with the hydrogen of the methylene moiety attached to the pyrazole ring (Figure1). In order to check the preferred conformations of the propylene chain, a systematic conformational analyses was performed according to the strategy described in experimental section. As a result, we found that the minimun is located in the staggered disposition of all the methylene groups (Figure1).

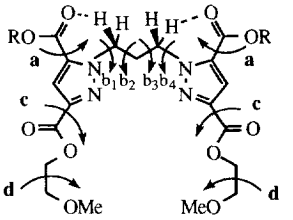


Figure 1

Taking into account these two results, AM1 calculations were performed on the four possible disposition of the podand **4a** (torsional angles c and d with values 0 and 180°). Optimized geometry of the minimun is represented in Figure 2 in which we can observed that the final OMe groups are situated one above and the other below the plane defined by the atoms of the molecule.

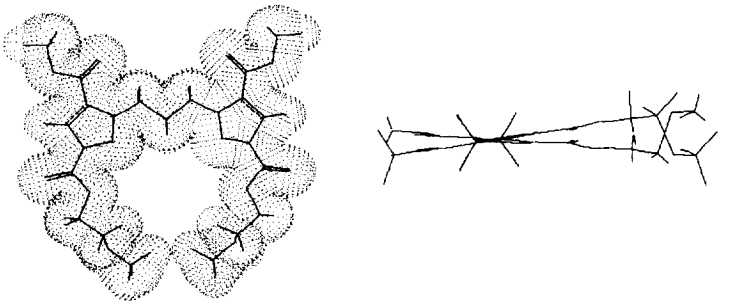


Figure 2.-Low-energy conformation and VDW surface of podand **4a**

Ammonium cation complex is calculated from this minimum conformation using AM1 method and full geometry optimization. The complex is depicted in Figure 3, in which we can observe that stabilization is due to the formation of hydrogen bond between the O-donor sites of the podand and the NH^+ centers. An important contribution to the complex formation is the electrostatic interactions between the NH^+ centers and the electron pairs of the nitrogen atoms of the pyrazoles.

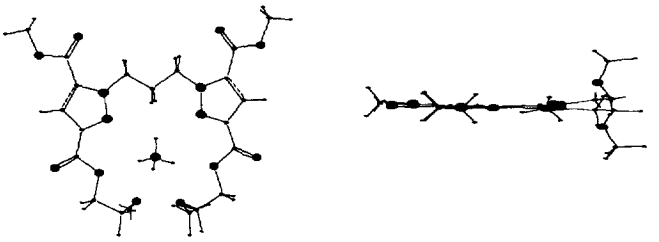


Figure 3.- Ammonium cation complex of podand **4a** optimized by AM1 method.

It is worthmentioning that torsional angles of ethyleneglycol chain has been modified in order to better acomodate the ammoniun cation closing the pseudocavity (Figure 4).

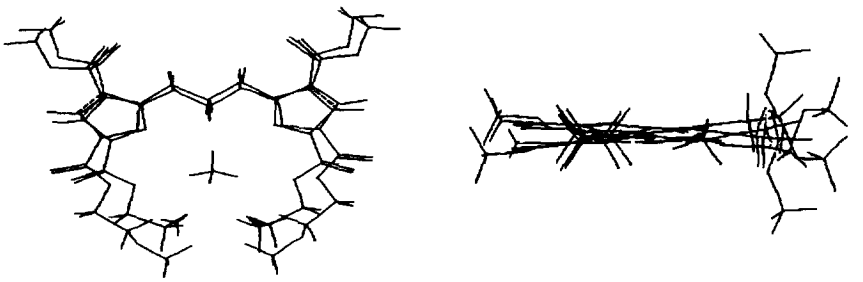


Figure 4.- Superimposition of podand **4a** and its ammonium cation complex

CONCLUSIONS

In this paper we have shown the potential usefulness of enzymatic transesterifications in the synthesis of pyrazolic podands. The regioselectivity and almost complete lack of secondary products of the reaction avoid a multistep time-consuming synthesis. The new podands **4a-d**, with two pyrazolic rings, showed selectivity toward ammonium and norepinephrine cations in relation to alkali ones. A preliminary molecular modelling and quantum-chemical study of one ammonium complex revealed its nature and the possible stabilizing interactions that will be considered in future design of ammonium selective carriers.

EXPERIMENTAL

HPLC analyses were performed in a Beckman equipment with an Ultrasphere 25 cm C-18 column, eluted with different proportions of acetonitrile/phosphoric acid:triethylamine pH 3.5 buffer at a flow rate of 1 mL/min and UV detector at λ:233 nm. Chromatographic separations were performed on columns, using the flash

chromatography technique on silica gel (Merck, 230-400 mesh). Compounds were detected with UV light (254 nm). NMR spectra were recorded using a Varian XL-300 or a Gemini-200 spectrometer, and elemental analyses were carried out in the Centro Nacional de Química Orgánica (CSIC). Mono-, di-, and triethyleneglycol monomethylether were commercially available products (Aldrich) and were purified by destillation in vacuo before use them. Tetraethyleneglycol monomethylether **2d**[14] was synthesized following a standard procedure,[15] and the microanalytical and spectroscopic data of the product obtained were in accordance with its structure. Toluene was refluxed on sodium wire, distilled and stored on molecular sieves 4Å before use.

1,3-bis[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]propane (1). A mixture of 3,5-bis(ethoxycarbonyl)-1*H*-pyrazole (5.30 g, 25 mmol), 1,3-dibromopropane (2.50 g, 12.5 mmol), and K_2CO_3 (3.46 g, 25 mmol) in acetone (150 mL) was refluxed for 8 hours.[6] After cooling to room temperature, the residual solid was filtered off and the resulting solution evaporated to dryness. The residue was dissolved in chloroform (100 mL) and washed repeatedly with water (3 x 100 mL). The organic layer was dried ($MgSO_4$), evaporated to dryness under reduced pressure, and the residue recrystallised yielding 4.86 g (84%) of **1** (m.p: 70°C, hexane). ^{1}H NMR ($CDCl_3$): 7.33 (s, 2H, H_4), 4.71 (t, 4H, J=7.1 Hz, NCH_2), 4.40 (q, 4H, J=7.0 Hz, $CH_3CH_2CO_2$-C_3), 4.37 (q, 4H, J=7.0 Hz, $CH_3CH_2CO_2$-C_5), 2.48 (quint, 2H, J=7.1 Hz, $NCH_2CH_2CH_2N$), 1.39 (t, 6H, J=7.0 Hz, $CH_3CH_2CO_2$-C_3), 1.37 (t, 6H, J=7.0 Hz, $CH_3CH_2CO_2$-C_5). ^{13}C NMR ($CDCl_3$): 161.52 (O=*C*-C_3), 158.97 (O=*C*-C_5), 142.41 (C_3), 133.63 (C_5), 114.15 (C_4), 61.44 ($CH_3CH_2CO_2$-C_5), 61.18 ($CH_3CH_2CO_2$-C_3), 50.14 (NCH_2), 30.79 ($NCH_2CH_2CH_2N$), 14.33 ($CH_3CH_2CO_2$-C_3), 14.13 ($CH_3CH_2CO_2$-C_5). Anal. Calcd for $C_{21}H_{28}N_4O_8$: C, 54.31; H, 6.03; N, 12.07. Found: C, 54.66; H, 6.19; N, 12.30.

GENERAL PROCEDURE OF ENZYMATIC TRANSESTERIFICATIONS IN TOLUENE

Lipozyme (20 mg/mL) was added to a solution of 1,3-bis[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]propane **1** (20 mM) and the corresponding nucleophile **2a-d** (100 mM) in dry toluene.

Analytical scale.- The reactions were carried out in sealed screw-cap 2 mL vials containing 1.5 mL of the reaction mixture and molecular sieves 3Å powder (20 mg/mL), stirred in an orbital shaker at 60°C. Aliquots were periodically withdrawn and analyzed by hplc.

Preparative scale.- The reactions were carried out in 500 mL round-bottom flasks containing 100 mL of the reaction mixture in a rotary evaporator without vacuum while heated at 60°C in a silicone bath, and controlled by hplc. The solution was daily refilled with fresh dry toluene to the original volume. When the reaction was stabilized, the enzyme was filtered off and washed with chloroform, and the clear solution was evaporated to dryness. The excess of starting alcohol was separated by dissolving the mixture in chloroform (100 mL) and washing the solution repeatedly with water (5 x 100 mL). Then it was dried (Na_2SO_4), evaporated to dryness and the residue was purified on a silica gel column. Eluents and initial amounts are specified in each case.

Reaction of 1 and ethyleneglycol monomethylether 2a. Reaction of **1** (928 mg, 2 mmol), **2a** (0.78 mL, 10 mmol) and Lipozyme (2.00 g) in toluene (100 mL) during 14 days afforded a syrup that was eluted on a silica gel column (chloroform : acetone, 10:1).

The first product was initial substrate **1** (620 mg, 67%).

The second isolated product was 1-[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]-3-[3-(3-oxabutoxycarbonyl)-5-ethoxycarbonyl-1*H*-pyrazol-1-yl]propane (**3a**) as a pure sirup (76 mg, 7%). ^{1}H NMR ($CDCl_3$): 7.30 (s, 1H, H_4 or $H_{4'}$), 7.28 (s, 1H, $H_{4'}$ or H_4), 4.65 (t, 4H, J=7.1 Hz, NCH_2), 4.42 (t, 2H, J=4.8 Hz, α), 4.34 (q, 2H, J=7.0 Hz, $CH_3CH_2CO_2$-$C_{3'}$), 4.27 (q, 4H, J=7.0 Hz, $CH_3CH_2CO_2$-$C_{5,5'}$), 3.65 (t, 2H, J=4.8

Hz, ω), 3.35 (s, 3H, OCH_3), 2.41 (quint, 2H, J=7.1 Hz, $NCH_2CH_2CH_2N$), 1.33 (t, 3H, J=7.0 Hz, $CH_3CH_2CO_2$-$C_{3'}$), 1.31 (t, 6H, J=7.0 Hz, $CH_3CH_2CO_2$-$C_{5,5'}$). ^{13}C NMR ($CDCl_3$): 161.47 and 161.32 (O=*C*-$C_{3,3'}$), 158.82 (O=*C*-$C_{5,5'}$), 142.22 and 141.76 ($C_{3,3'}$), 133.47 ($C_{5,5'}$), 114.19 and 114.05 ($C_{4,4'}$), 70.22 (ω), 63.86 (α), 61.37 ($CH_3CH_2CO_2$-$C_{5,5'}$), 61.11 ($CH_3CH_2CO_2$-$C_{3'}$), 58.89 (OCH_3), 50.04 (NCH_2), 30.67 ($NCH_2CH_2CH_2N$), 14.24 ($CH_3CH_2CO_2$-$C_{3'}$), 14.05 ($CH_3CH_2CO_2$-$C_{5,5'}$). Anal. Calcd for $C_{22}H_{30}N_4O_9$: C, 53.44; H, 6.07; N, 11.34. Found: C, 53.11; H, 5.87; N, 11.67.

The third product was 1,3-bis[3-(3-oxabutoxycarbonyl)-5-ethoxycarbonyl-1*H*-pyrazol-1-yl]propane (**4a**) (22 mg, 2%) again as a syrup. ^1H NMR ($CDCl_3$): 7.34 (s, 2H, H_4), 4.68 (t, 4H, J=7.1 Hz, NCH_2), 4.46 (t, 4H, J=4.8 Hz, α), 4.31 (q, 4H, J=7.0 Hz, $CH_3CH_2CO_2$-C_5), 3.69 (t, 4H, J=4.8 Hz, ω), 3.39 (s, 6H, OCH_3), 2.44 (quint, 2H, J=7.1 Hz, $NCH_2CH_2CH_2N$), 1.34 (t, 6H, J=7.0 Hz, $CH_3CH_2CO_2$-C_5). ^{13}C NMR ($CDCl_3$): 161.45 (O=*C*-C_3), 158.94 (O=*C*-C_5), 141.94 (C_3), 133.61 (C_5), 114.30 (C_4), 70.34 (ω), 63.94 (α), 61.45 ($CH_3CH_2CO_2$-C_5), 58.98 (OCH_3), 50.15 (NCH_2), 30.74 ($NCH_2CH_2CH_2N$), 14.13 ($CH_3CH_2CO_2$-C_5). Anal. Calcd for $C_{23}H_{32}N_4O_{10}$: C, 52.67; H, 6.11; N, 10.69. Found: C, 53.01; H, 5.76; N, 10.90.

Reaction of 1 and diethyleneglycol monomethylether 2b. Reaction of **1** (928 mg, 2 mmol) and **2b** (1.18 mL, 10 mmol) catalyzed by Lipozyme (2.00 g) in anhydrous toluene (100 mL) following the general procedure in 12 days gave a mixture that was separated by chromatography on a silica gel column (hexane : chloroform : acetone, 10:8:1).

Initial substrate **1** (158 mg, 17%) was the first product isolated.

The second product: 1-[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]-3-[3-(3,6-dioxaheptyloxycarbonyl)-5-ethoxycarbonyl-1*H*-pyrazol-1-yl]propane (**3b**) was isolated as a pure sirup (347 mg, 32%). ^1H NMR ($CDCl_3$): 7.31 (s, 1H, H_4 or $H_{4'}$), 7.30 (s, 1H, $H_{4'}$ or H_4), 4.67 (t, 4H, J=7.1 Hz, NCH_2), 4.47 (t, 2H, J=5.0 Hz, α), 4.36 (q, 2H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{3'}$), 4.30 (q, 4H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{5,5'}$), 3.79 (t, 2H, J=5.0 Hz, β), 3.64 (m, 2H, γ), 3.52 (m, 2H, ω), 3.34 (s, 3H, OCH_3), 2.44 (quint, 2H, J=7.1 Hz, $NCH_2CH_2CH_2N$), 1.36 (t, 3H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{3'}$), 1.33 (t, 6H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{5,5'}$). ^{13}C NMR ($CDCl_3$): 161.49 and 161.37 (O=*C*-$C_{3,3'}$), 158.90 (O=*C*-$C_{5,5'}$), 142.30 and 141.87 ($C_{3,3'}$), 133.54 ($C_{5,5'}$), 114.25 and 114.13 ($C_{4,4'}$), 71.85 (ω), 70.49 (γ), 69.00 (β), 63.98 (α), 61.44 ($CH_3CH_2CO_2$-$C_{5,5'}$), 61.18 ($CH_3CH_2CO_2$-$C_{3'}$), 59.03 (OCH_3), 50.11 (NCH_2), 30.74 ($NCH_2CH_2CH_2N$), 14.31 ($CH_3CH_2CO_2$-$C_{3'}$), 14.11 ($CH_3CH_2CO_2$-$C_{5,5'}$). Anal. Calcd for $C_{24}H_{34}N_4O_{10}$: C, 53.53; H, 6.32; N, 10.41. Found: C, 53.80; H, 6.04; N, 10.56.

The third product was 1,3-bis[3-(3,6-dioxaheptyloxycarbonyl)-5-ethoxycarbonyl-1*H*-pyrazol-1-yl]-propane (**4b**) (220 mg, 18%) again as a syrup. ^1H NMR ($CDCl_3$): 7.32 (s, 2H, H_4), 4.68 (t, 4H, J=7.1 Hz, NCH_2), 4.48 (t, 4H, J=5.0 Hz, α), 4.31 (q, 4H, J=7.1 Hz, $CH_3CH_2CO_2$-C_5), 3.80 (t, 4H, J=5.0 Hz, β), 3.65 (m, 4H, γ), 3.53 (m, 4H, ω), 3.35 (s, 6H, OCH_3), 2.44 (quint, 2H, J=7.1 Hz, $NCH_2CH_2CH_2N$), 1.35 (t, 6H, J=7.1 Hz, $CH_3CH_2CO_2$-C_5). ^{13}C NMR ($CDCl_3$): 161.38 (O=*C*-C_3), 158.90 (O=*C*-C_5), 141.89 (C_3), 133.54 (C_5), 114.26 (C_4), 71.87 (ω), 70.50 (γ), 69.01 (β), 64.00 (α), 61.46 ($CH_3CH_2CO_2$-C_5), 59.06 (OCH_3), 50.14 (NCH_2), 30.86 ($NCH_2CH_2CH_2N$), 14.13 ($CH_3CH_2CO_2$-C_5). Anal. Calcd for $C_{27}H_{40}N_4O_{12}$: C, 52.94; H, 6.54; N, 9.15. Found: C, 53.15; H, 6.59; N, 9.39.

Reaction of 1 and triethyleneglycol monomethylether 2c. According to the general procedure, **1** (928 mg, 2 mmol), **2c** (1.57 mL, 10 mmol), and Lipozyme (2.00 g) in anhydrous toluene (100 mL) in 12 days gave a mixture that was eluted on a silicagel column (chloroform : acetone, 10:1).

The first band corresponded to recovered original substrate **1** (442 mg, 48%).

The second band afforded pure 1-[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]-3-[3-(3,6,9-trioxadecyloxycarbonyl)-5-ethoxycarbonyl-1*H*-pyrazol-1-yl]propane (**3c**) as a sirup (254 mg, 22%). ^{1}H NMR ($CDCl_3$): 7.32 (s, 2H, $H_{4,4'}$), 4.68 (t, 4H, J=7.1 Hz, NCH_2), 4.46 (t, 2H, J=5.0 Hz, α), 4.38 (q, 2H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{3'}$), 4.31 (q, 4H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{5,5'}$), 3.79 (t, 2H, J=5.0 Hz, β), 3.66 (m, 6H, γ,δ,ε), 3.51 (m, 2H, ω), 3.34 (s, 3H, OCH_3), 2.45 (quint, 2H, J=7.1 Hz, $NCH_2CH_2CH_2N$), 1.37 (t, 3H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{3'}$), 1.35 (t, 6H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{5,5'}$). ^{13}C NMR ($CDCl_3$): 161.08 and 160.98 (O=*C*-$C_{3,3'}$), 158.55 (O=*C*-$C_{5,5'}$), 142.03 and 141.64 ($C_{3,3'}$), 133.28 ($C_{5,5'}$), 113.84 and 113.74 ($C_{4,4'}$), 71.58 (ω), 70.28 (δ,ε), 70.21 (γ), 68.66 (β), 63.70 (α), 61.09 ($CH_3CH_2CO_2$-$C_{5,5'}$), 60.76 ($CH_3CH_2CO_2$-$C_{3'}$), 58.58 (OCH_3), 49.77 (NCH_2), 30.37 ($NCH_2CH_2CH_2N$), 13.98 ($CH_3CH_2CO_2$-$C_{3'}$), 13.79 ($CH_3CH_2CO_2$-$C_{5,5'}$). Anal. Calcd for $C_{26}H_{38}N_4O_{11}$: C, 53.61; H, 6.53; N, 9.62. Found: C, 53.85; H, 6.21; N, 9.81.

The third band yielded 1,3-bis[3-(3,6,9-trioxadecyloxycarbonyl)-5-ethoxycarbonyl-1*H*-pyrazol-1-yl] propane (**4c**) (114 mg, 8%) again as a pure syrup. ^{1}H NMR ($CDCl_3$): 7.27 (s, 2H, H_4), 4.65 (t, 4H, J=7.2 Hz, NCH_2), 4.42 (t, 4H, J=5.0 Hz, α), 4.29 (q, 4H, J=7.1 Hz, $CH_3CH_2CO_2$-C_5), 3.78 (t, 4H, J=5.0 Hz, β), 3.64 (m, 12H, γ,δ,ε), 3.58 (m, 4H, ω), 3.32 (s, 6H, OCH_3), 2.44 (quint, 2H, J=7.2 Hz, $NCH_2CH_2CH_2N$), 1.33 (t, 6H, J=7.1 Hz, $CH_3CH_2CO_2$-C_5). ^{13}C NMR ($CDCl_3$): 161.18 (O=*C*-C_3), 158.73 (O=*C*-C_5), 141.81 (C_3), 133.44 (C_5), 114.03 (C_4), 71.75 (ω), 70.44 (δ,ε), 70.38 (γ), 68.83 (β), 63.86 (α), 61.27 ($CH_3CH_2CO_2$-C_5), 58.78 (OCH_3), 49.95 (NCH_2), 30.54 ($NCH_2CH_2CH_2N$), 13.96 ($CH_3CH_2CO_2$-C_5). Anal. Calcd for $C_{31}H_{48}N_4O_{14}$: C, 53.14; H, 6.86; N, 8.00. Found: C, 53.47; H, 6.78; N, 8.33.

Reaction of 1 and tetraethyleneglycol monomethylether 2d. From **1** (742 mg, 1.6 mmol), **2d** (1.65 mL, 8 mmol), and Lipozyme (1.60 g) in anhydrous toluene (80 mL), and following the general procedure, after 14 days a sirup was obtained; it was purified by column chromatography (chloroform : acetone, 10:1), affording three bands.

Initial substrate **1** (217 mg, 29%) was recovered from the first band.

The second band yielded pure 1-[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]-3-[3-(3,6,9,12-tetraoxatridecyloxycarbonyl)-5-ethoxycarbonyl-1*H*-pyrazol-1-yl]propane (**3d**) as a sirup (391 mg, 39%). ^{1}H NMR ($CDCl_3$): 7.28 (s, 1H, H_4 or $H_{4'}$), 7.27 (s, 1H, $H_{4'}$ or H_4), 4.64 (t, 4H, J=7.1 Hz, NCH_2), 4.42 (t, 2H, J=5.0 Hz, α), 4.33 (q, 2H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{3'}$), 4.27 (q, 4H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{5,5'}$), 3.75 (t, 2H, J=5.0 Hz, β), 3.59 (m, 10H, γ,δ,ε,φ,τ), 3.48 (m, 2H, ω), 3.31 (s, 3H, OCH_3), 2.41 (quint, 2H, J=7.1 Hz, $NCH_2CH_2CH_2N$), 1.32 (t, 3H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{3'}$), 1.31 (t, 6H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{5,5'}$). ^{13}C NMR ($CDCl_3$): 161.12 and 161.08 (O=*C*-$C_{3,3'}$), 158.61 (O=*C*-$C_{5,5'}$), 142.02 and 141.66 ($C_{3,3'}$), 133.30 ($C_{5,5'}$), 113.86 ($C_{4,4'}$), 71.63 (ω), 70.30 (ε,φ,τ), 70.01 (δ), 69.92 (γ), 68.74 (β), 63.80 (α), 61.21 ($CH_3CH_2CO_2$-$C_{5,5'}$), 60.93 ($CH_3CH_2CO_2$-$C_{3'}$), 58.73 (OCH_3), 49.89 (NCH_2), 30.51 ($NCH_2CH_2CH_2N$), 14.08 ($CH_3CH_2CO_2$-$C_{3'}$), 13.91 ($CH_3CH_2CO_2$-$C_{5,5'}$). Anal. Calcd for $C_{28}H_{42}N_4O_{12}$: C, 53.67; H, 6.71; N, 8.95. Found: C, 53.88; H, 6.42; N, 8.67.

The third band corresponded to 1,3-bis[3-(3,6,9,12-tetraoxatridecyloxycarbonyl)-5-ethoxycarbonyl-1*H*-pyrazol-1-yl]propane (**4d**) (40 mg, 3%) again as a pure syrup. ^{1}H NMR ($CDCl_3$): 7.31 (s, 2H, H_4), 4.67 (t, 4H, J=7.2 Hz, NCH_2), 4.45 (t, 4H, J=5.0 Hz, α), 4.30 (q, 4H, J=7.1 Hz, $CH_3CH_2CO_2$-C_5), 3.78 (t, 4H, J=5.0 Hz, β), 3.62 (m, 20H, γ,δ,ε,φ,τ), 3.52 (m, 4H, ω), 3.33 (s, 6H, OCH_3), 2.45 (quint, 2H, J=7.2 Hz, $NCH_2CH_2CH_2N$), 1.34 (t, 6H, J=7.1 Hz, $CH_3CH_2CO_2$-C_5). ^{13}C NMR ($CDCl_3$): 161.30 (O=*C*-C_3), 158.84 (O=*C*-C_5), 141.89 (C_3), 133.54 (C_5), 114.15 (C_4), 71.75 (ω), 70.54 (δ,ε,φ,τ), 70.42 (γ), 68.93 (β), 63.96 (α), 61.38 ($CH_3CH_2CO_2$-C_5), 58.90 (OCH_3), 50.07 (NCH_2), 30.67 ($NCH_2CH_2CH_2N$), 14.06

($CH_3CH_2CO_2$-C_5). Anal. Calcd for $C_{35}H_{56}N_4O_{16}$: C, 53.30; H, 7.11; N, 7.11. Found: C, 53.01; H, 7.24; N, 7.47.

REACTION OF **1** AND DIETHYLENEGLYCOL MONOMETHYLETHER **2b** IN DIISOPROPYLETHER

Lipozyme (2.50 g) and molecular sieves 3Å powder (2.50 g) were added to a solution of 1,3-bis[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]propane **1** (1.16 g, 2.5 mmol) and diethyleneglycol monomethylether **2b** (1.18 mL, 10 mmol) in dry diisopropylether (100 mL). The reaction vessel was sealed and stirred at 60°C during 7 days. Then the enzyme and molecular sieves were filtered off and washed with chloroform, and the clear solution was evaporated to dryness. The excess of diethyleneglycol monomethylether was separated by dissolving the mixture in chloroform (100 mL) and washing the solution repeatedly with water (5 x 100 mL). Then it was dried over Na_2SO_4, evaporated to dryness and the residue was purified on a silica gel column, using hexane : chloroform : acetone (10:8:1) as eluent.

The first band corresponded to recovered original substrate **1** (455 mg, 39%).

The second one afforded pure 1-[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]-3-[5-(3,6-dioxaheptyloxy-carbonyl)-5-ethoxycarbonyl-1*H*-pyrazol-1-yl]propane (**5**) as a sirup (61 mg, 4.5%). ^{1}H NMR ($CDCl_3$): 7.29 (s, 1H, H_4 or $H_{4'}$), 7.25 (s, 1H, $H_{4'}$ or H_4), 4.62 (t, 4H, J=7.2 Hz, NCH_2), 4.62 (t, 2H, J=4.9 Hz, α'), 4.31 (q, 4H, J=7.2 Hz, $CH_3CH_2CO_2$-$C_{3,3'}$), 4.24 (q, 2H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{5'}$), 3.71 (t, 2H, J=4.9 Hz, β'), 3.59 (m, 2H, γ'), 3.47 (m, 2H, ω'), 3.31 (s, 3H, OCH_3), 2.39 (quint, 2H, J=7.2 Hz, $NCH_2CH_2CH_2N$), 1.30 (t, 6H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{3,3'}$), 1.28 (t, 3H, J=7.1 Hz, $CH_3CH_2CO_2$-$C_{5'}$). ^{13}C NMR ($CDCl_3$): 161.50 (O=*C*-$C_{3,3'}$), 158.97 and 158.95 (O=*C*-$C_{5,5'}$), 142.44 ($C_{3,3'}$), 133.63 and 133.33 ($C_{5,5'}$), 114.46 and 114.15 ($C_{4,4'}$), 71.95 (ω'), 70.64 (γ'), 68.88 (β'), 64.42 (α'), 61.45 ($CH_3CH_2CO_2$-$C_{5'}$), 61.18 ($CH_3CH_2CO_2$-$C_{3,3'}$), 59.04 (OCH_3), 50.14 (NCH_2), 30.80 ($NCH_2CH_2CH_2N$), 14.33 ($CH_3CH_2CO_2$-$C_{3,3'}$), 14.14 ($CH_3CH_2CO_2$-$C_{5'}$). Anal. Calcd for $C_{24}H_{34}N_4O_{10}$: C, 53.53; H, 6.32; N, 10.41. Found: C, 53.44; H, 6.56; N, 10.19.

The third and fourth bands yielded **3b** (361 mg, 32%) and **4b** (170 mg, 11%), respectively.

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 x 10^{-4} mol L^{-1}), lies below two aqueous phases and bridges them. The first aqueous phase (1 mL) contains 5 x 10^{-5} mol L^{-1} of LiOH, 10^{-1} mol L^{-1} of alkali nitrate or ammonium nitrate or neurotransmitter ammonium chloride, and 2 x 10^{-3} mol L^{-1} of the corresponding picrate. The second aqueous phase contains 1 mL of deionized water. The membrane phase is stirred slowly and constantly by magnetic stirrer. A similar experiment was carried out in the absence of carrier. The picrate concentration in the second aqueous phase, monitored spectroscopically by UV (λ=355 nm), was confirmed to increase linearly with running time (<12 h), and the initial transport rates were calculated. The values indicated in Table 2 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[12] assuming the planarity of the system.

Conformational analysis was performed by rotating the torsional angles of the propylene chain of **4a** in 60° increments, given rise to 1296 conformations. 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.[16]

Semiempirical calculations has been performed using the AM1 method[13] in MOPAC V5.0 program package[17]. In all cases, the Chem-QM interface[18] was used and full geometry optimizations with Fletcher-Powell algorithm[19] were carried out.

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