REGIOSELECTIVE ENZYME-CATALYZED SYNTHESIS OF PYRAZOLE-CONTAINING PODANDS

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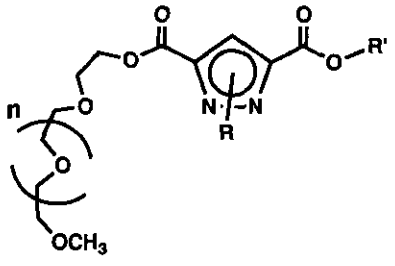
Abstract — Initial results are described about a new synthesis of a family of the acyclic cation-complexing compounds named podands containing a pyrazole moiety as a part of the molecule. This synthesis was carried out by lipase-catalyzed transesterification of the corresponding pyrazole-3,5-dicarbethoxy derivative with di- and triethyleneglycol monomethylether; it afforded good yields and a high degree of regioselectivity in *N*-substituted pyrazoles where 3 and 5 positions are not equivalents. As an initial approach, a simpler model of transesterification with n-octanol was studied.

INTRODUCTION

The group of acyclic polyethers named podands displays the property of complexing cations,[1] they are something like "acyclic crown-ethers." Several publications describe the enhancement of this feature by the presence of a sp^2 nitrogen as a part of a heteroaromatic ring.[2] As an extension of our line on the synthesis and properties of polyether pyrazolic macrolactones,[3] we studied the chemistry of 3,5-dioxycarbonylpyrazole system in order to achieve podands with a general structure (**1**). Very often, the obtention of these compounds using classical synthetic methods is a difficult task because a lot of secondary products are formed and troublesome purifications are required. At present, lipase-catalyzed transesterification in organic solvents is a common synthetic tool[4] but, although it has become a useful alternative to the chemical methods, examples of aromatic or heteroaromatic esters as acyl donors[5] are hardly known and, certainly, there is none involving a pyrazolyl group; these few reactions take place rather slowly and in a short extension.[6] Moreover, to our knowledge, there is not any published application of enzymatic catalysis to the synthesis of these acyclic or cyclic polyether cation-complexing compounds either. In this paper, we describe our initial results on the lipase-catalyzed transesterification of the diethyl pyrazole-3,5-

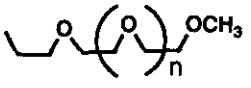
dicarboxylate system (**2**) and its first promising application to the synthesis of the podands derived from di- and triethyleneglycol monomethyl ether (DEG-Me and TEG-Me respectively). Under this lack of precedents, our initial approach was to study a previous easier reaction that could be carried out and analyzed more readily than the final podands.



n=0,1

R=H, CH_3, $CH_2C_6H_5$

R'=C_2H_5,



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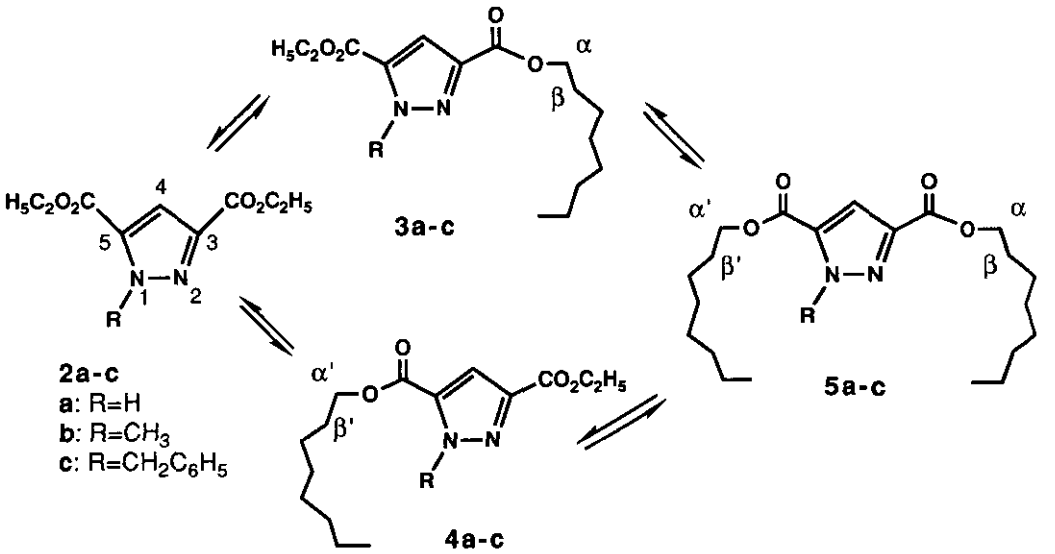
Three pyrazole derivatives were selected as acyl donors: *N*-hydrogen (**2a**), *N*-methyl (**2b**), and *N*-benzyl (**2c**), ranking in volume and lipophylicity (both **2a<2b<2c**); reactant nucleophile was n-octanol in all cases (Scheme 1). Prototropic tautomerism of the *N*-hydrogen atom makes **2a** a symmetrical compound, so both **3a** and **4a** are the same product with equivalent 3- and 5-carbethoxy groups.[7] Moreover, the ionization capability of this atom confers desirable cation-complexing properties to the podands or macrolactones,[8] but it may form hydrogen bonds in the active center of the enzyme, with unknown effects on the reactivity. On the other hand, *N*-substitution eliminates the possibility of these linkages, increases molecular volume and fixes positions in the cycle making unequivalents 3- and 5- groups: rate and regioselectivity of the reaction and how it is affected by a small (methyl, **2b**) or large (benzyl, **2c**) group can be studied.

RESULTS AND DISCUSSION

Initial studies. Transesterifications with n-octanol

Several commercially available lipases[9] and experimental conditions were tested in the reaction of **2a**: the preparation of immobilized *Mucor miehei* lipase MML (Lipozyme IM20 from Novo Nordisk) displayed the highest conversion (Table 1). The MML-catalyzed reaction in anhydrous diisopropyl ether was stabilized in 7 days with conversion of 50% to **3a=4a** and 10% to **5a**. The recovered

enzyme displayed 85% of its original activity; attempts were made to displace the equilibrium by increasing the concentration of n-octanol to 0.5M, 1M and neat, but it produced a progressive fall in the conversion. No conversion was detected in a blank reaction (Reaction 0) without enzyme. Direct enzymatic esterification of free dicarboxylic acid was also investigated but all attempts were unsuccessful.



Scheme 1

As it was expected, the reaction is much slower than those on aliphatic substrates[10] and, although the overall conversion is satisfactory, conversion into disubstituted compound (**5a**) was low. No more attempts were made to optimize conditions.

Table 1. Screening of lipases in the transesterification of **2a** with n-octanol[a]

Enzyme[b]	React 0	PPL	CCL	MML	PSL	ANL
Conversion(%)[c]	0	31	28	48	29	24

a General conditions: Lipase (50 mg/ml) was added to a solution of **2a** (10 mM) and n-octanol (100 mM) in anhydrous diisopropyl ether (1.5 ml in vials of 2 ml) and incubated 3 days at 27°C.
b No reaction was detected when *Geotrichum candidum, Penicillium roqueforti* or *Rhizopus arrhizus* lipases were used.
c Determined by gas chromatography. Based in **3a=4a** and **5a** peaks.

Better results were obtained when the same experimental conditions were applied to the more lipophylic diethyl 1-methylpyrazole-3,5-dicarboxylate (**2b**). The five active lipases on **2a** (PPL, CCL, MML, PSL and ANL) were tested and MML showed the highest rate of conversion: 70% of monosubstituted ethyloctyl diesters (**3b**) and (**4b**), and 23% of disubstituted dioctyl ester (**5b**), when the equilibrium was reached at 12-14 days (Figure 1). MML was also the only enzyme that afforded disubstituted product.

Figure 1

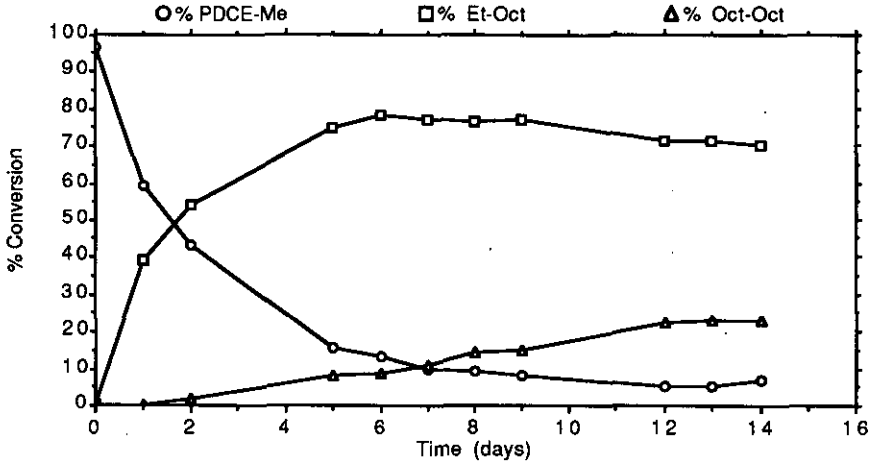


Figure 1. Experimental conditions: MML (10 mg/ml) was added to a solution of **2b** (20 mM) and n-octanol (100 mM) in anhydrous diisopropyl ether (1.5 ml in vials of 2 ml). Aliquots were taken and analyzed by gc: only one peak corresponding to monosubstituted products was detected.

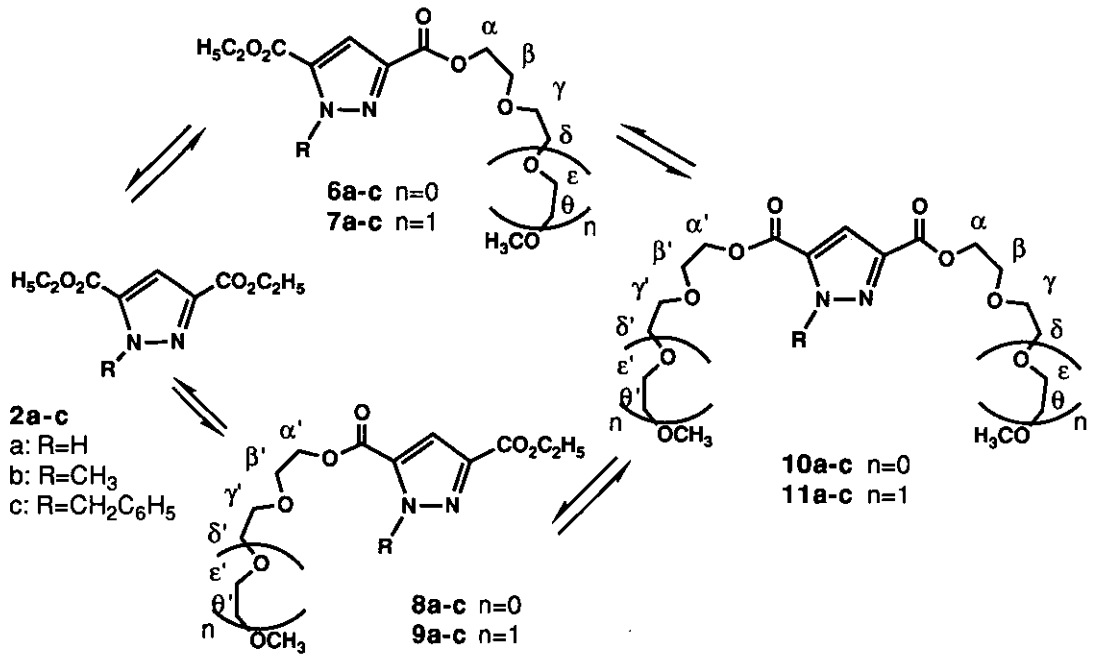
The reaction takes place in a regioselective manner and 3-octyl-5-ethyl 1-methylpyrazole-3,5-dicarboxylate (**3b**) was the major regioisomer obtained, as it was established by lanthanide induced shifts (LIS) in ^{1}H-nmr.[11] A bidentate interaction **2b**.Eu(fod)$_3$ was previously described by us:[3b] the lanthanide reagent was linked to both the nitrogen sp^2 (2-position) and the carbonyl oxygen at the contiguous 3-position of the pyrazole ring, but not to that at the distant 5-position and larger deshielding on the protons bound to carbethoxy group at 3-position than those at 5 were detected. The same effect was here observed on the triplet at δ=4.32 ppm (α-methylene from 3-octyloxy group) which underwent larger deshielding than the quartet at δ=4.34 ppm (methylene from 5-ethoxy group) and, on this base, structure (**3b**) was assigned to the major regioisomer.

Although only one peak appeared by gas chromatography from the couple of regioisomers (**3b**) and (**4b**), LIS experiments revealed the presence of about 7% of **4b** in the mixture. All attempts to isolate it from a preparative scale reaction failed. Concentration of (**4b**) lower than that of the final disubstituted product points out that, although formed in a little extension, it is a more effective substrate than its unfavoured regioisomer (**3b**) and probably even than the less lipophylic starting product (**2b**). Most of the small amount of **4b** that is being formed reacts efficiently to afford the majority of disubstituted **5b,** while **3b** contributes in a little extension as can be deduced from the slight decreasing of its concentration in Figure 1. Regioselectivity was not investigated in the other four lipases.

N-Benzyl **2c** reacts in a similar way displaying comparable conversion results with those obtained with *N*-methyl: 78% monosubstituted products with a **3c**:**4c** regioselectivity ratio of 95:5 and only 6% of disubstituted **5c**. In this case all the three compounds were detected and evaluated by gc. Structures were assigned by LIS in ^{1}H-nmr as described above.

Synthesis of podands. Transesterification with DEG-Me and TEG-Me

The reaction was then applied to the synthesis of podands with **2a-c** as acyl donors and di- and triethyleneglycol monomethyl ether (DEG-Me and TEG-Me, respectively) as nucleophiles (Scheme 2). A quick survey of lipases was performed with **2a** and DEG-Me; *Mucor miehei* lipase MML (Lipozyme from Novo) displayed again the highest results although modest anyway: Lipozyme (10 mg/ml) was added to a solution of **2a** (10 mM) and DEG-Me (50 mM) in anhydrous diisopropyl ether (300 ml), the suspension was placed in a sealed flask, stirred at 55-60ºC and analyzed by hplc. The equilibrium was reached in 7 days and, after following a standard process (see Experimental part), the resulting syrup was eluted on a silica gel column and the only three detected compounds were isolated: unchanged starting substrate (**2a**) (19%), 3-(3',6'-dioxaheptyl)-5-ethyl 1*H*-pyrazole-3,5-dicarboxylates (**6a=8a**) (13%) and finally 3,5-bis(3',6'-dioxaheptyl) 1*H*-pyrazole-3,5-dicarboxylate (**10a**) (3%). A similar reaction was carried out with TEG-Me affording unchanged substrate (**2a**) (49%), 3-(3',6',9'-trioxadecyl)-5-ethyl 1*H*-pyrazole-3,5-dicarboxylates (**7a=9a**) (26%) and 3,5-bis(3',6',9'-trioxadecyl) 1*H*-pyrazole-3,5-dicarboxylate (**11a**) (2%).



Scheme 2

It was supposed that ethanol released in the reaction and possible water present in the medium could play a significant role as competitive nucleophiles, what means unfavorable equilibrium and low yields. A first approach to minimize this effect was studied: the two pure intermediate diesters (**6a**=**8a**) and (**7a**=**9a**) reacted at the above described conditions with DEG-Me and TEG-Me but only 22% and 11% of the corresponding **10a** and **11a** were obtained.

Experimental system was then changed to get ethanol (and water if present) continuously distilled off by formation of an azeotrope with toluene. The flask containing a mixture of **2a** (10 mM), DEG-Me (50 mM) and suspended MML (10 mg/ml) in dry toluene (250 ml) was stirred in a rotary evaporator without vacuum while heated at 60°C in a silicone bath to allow a soft elimination of ethanol by azeotropic evaporation. It was periodically refilled with dry toluene to keep the solvent volume roughly constant. Aliquots were taken and analyzed by hplc. After 7 days, conversion reached 61.4% of monosubstituted **6a** and **8a** and 18.3% of disubstituted **10a** (Table 2). Similar conversions were also detected with TEG-Me, while no conversion was observed in a blank reaction without enzyme under the same experimental conditions. Slightly smaller conversions were observed with *N*-methyl derivative (**2b**) than with the *N*-unsubstituted (**2a**), while bulky *N*-benzyl **2c** reacts slower than both **2a** and **2b**.

Table 2. Conversion (%) at the 7th day.[a]

	N-H		N-Me		N-Bn	
	Monosubs.	Disubs.	Monosubs.	Disubs.	Monosubs.	Disubs.
DEG-Me	61.4	18.3	58.0	13.3	46.5	6.8
TEG-Me	64.8	16.3	51.1	7.6	39.5	6.8

a. General conditions: Lipozyme IM20 (50 mg/ml), pyrazoles **2a-c** (50 mM), DEG-Me or TEG-Me (125 mM), anhydrous toluene (250 ml), stirred in a rotary evaporator without vacuum at t=60°C and refilled periodically. Little vials are difficult to handle in these conditions.

Transesterifications on *N*-alkylated pyrazoles with DEG-Me and TEG-Me take also place regioselectively with a ratio 3-:5-monosubstituted diesters (**6b,c**:**8b,c** and **7b,c**:**9b,c**) of about 97:3 in all the four cases (hplc and gc data of isolated compounds). Complete structural assignments were made by ^{1}H-nmr. Four signals were studied in each ^{1}H-nmr spectrum of the mixtures of the intermediate products: two triplets from α, α'-protons of polyoxyethylene chains, and two quartets from the methylene of ethyl groups. The major triplet (δ=4.46-4.52 ppm) appears slightly deshielded in relation to the minor one (δ=4.40-4.41 ppm) while opposite situation is given with quartets: the minor quartet (δ=4.38-4.43 ppm) displays higher values of δ than the major one (δ=4.31-4.33 ppm). It is known that α-protons of 3-alkoxy groups in *N*-alkylated pyrazoles are more deshielded than their counterparts α' of 5 positions[7] and consequently, their signals appear at slightly higher values of δ. By comparison with initial diethyl (**2b,c**) and final dipolyoxyethylene esters (**10b,c**) spectra, it can be concluded that substitution takes place regioselectively on the 3 position and the major monopolyoxyethylene regioisomers afforded are **6b,c** and **7b,c**. Integrals of each couple of monosubstituted products are agree with the ratio calculated from hplc or gc data.

CONCLUSIONS

It is shown that lipase-catalysed transesterifications on aromatic esters can be used as a preparative synthetic method although they behave as worse substrates than aliphatic acyl donors and its limits are not yet studied. We have applied successfully this reaction to obtain podands containing a pyrazole unit. Moreover, the regioselectivity exhibited by the *Mucor miehei* lipase on *N*-substituted substrates (**2b,c**) opens a new way to asymmetric podands.

EXPERIMENTAL

Diethyl 1*H*-, 1-methyl and 1-benzylpyrazole-3,5-dicarboxylate (**2a-c**) were prepared by previously described methods.[12] Gc Hewlett-Packard apparatus was equipped with a 25 m capillary column of phenylmethyl silicone. Hplc analyses were performed in a Beckmann 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 silica gel, using the following techniques: flash column chromatography (on Kieselgel 60 Merck of 230-400 mesh), preparative thin layer chromatography (tlc, on 20x20 cm glass plates coated with a 2 mm layer of silica gel PF_{254}, Merck), and preparative centrifugal circular thin layer chromatography (cctlc, on a circular plate coated with a 1 mm layer of Kieselgel 60 PF_{254} gipshaltig, purchased from Merck, using a Chromatotron®). Compounds were detected with uv light (254 nm). Nmr spectra were recorded using a Varian XL-300 or a Gemini-200 spectrometers. Diisopropyl ether and toluene were both refluxed on sodium wire, distilled and stored on molecular sieves 4Å before using.

Initial studies. Transesterifications with n-octanol. General procedure.

Lipozyme IM20 (6 g) was added to a solution of acyl donor (**2a-c**) (6 mmol) and n-octanol (4.72 ml, 30 mmol) in anhydrous diisopropyl ether (300 ml) in a tightly closed flask and stirred at 35-40ºC. The reaction was daily analyzed by gas chromatography. When it was stabilized, the enzyme was filtered off and the excess of n-octanol (bp 196ºC) was azeotropically removed by distillation with water in vacuum (azeotrope bp 99ºC). The residual syrup was dried and purified as indicated in each case.

Reaction of diethyl 1*H*-pyrazole-3,5-dicarboxylate (2a): The suspension was stirred during 7 days. The final residue was chromatographed on preparative silica gel tlc (hexane : ethyl acetate, 4:1). In agreement with the gas chromatograms, only three bands appeared, which were extracted with ethyl acetate:

The first band afforded pure dioctyl 1*H*-pyrazole-3,5-dicarboxylate (**5a**) (137 mg, 6% yield) as a syrup. ^{1}H-Nmr ($CDCl_3$): 7.32 (s, 1H, H4), 4.35 (t, 4H, J=6.8 Hz, α), 1.77 (quint, 4H, J=6.8 Hz, β), 1.60 (br, 1H, NH, disappears with D_2O), 1.33 (m, 20 H, rest of CH_2), 0.88 (t, 6H, J=6.8 Hz, CH_3). ^{13}C-Nmr: 160.41 (C=0), 140.19 (C3,5), 111.17 (C4), 65.71 (α), 31.72, 29.10, 28.59, 25.85, 22.55, 13.93 (CH_3). Anal. Calcd for $C_{21}H_{36}N_2O_4$: C, 66.28; H, 9.54; N, 7.36. Found: C, 65.98; H, 9.60; N, 7.10.

The second band yielded 3(5)-ethyl-5(3)-octyl 1*H*-pyrazole-3,5-dicarboxylate (**3a**=**4a**) (426 mg, 24%) again as a pure syrup. ^{1}H-Nmr ($CDCl_3$): 7.36 (s, 1H, H4), 4.45 (q, 2H, J=7.1 Hz, $CO_2C\underline{H}_2CH_3$), 4.38 (t, 2H, J=6.8 Hz, α), 1.80 (quint, 2H, J=6.8 Hz, β), 1.80 (br, 1H, NH, disappears with D_2O), 1.44 (t, 3H, J=7.1 Hz, $CO_2CH_2C\underline{H}_3$), 1.33 (m, 10 H, rest of CH_2), 0.91 (t, 3H, J=6.8 Hz, $(CH_2)_7C\underline{H}_3$). ^{13}C-Nmr : 160.34 (C=0), 140.22 (C3), 139.75 (C5), 111.11 (C4), 65.58 (α), 61.38 ($CO_2\underline{C}H_2CH_3$), 31.61, 29.00, 28.46, 25.74, 22.44, 14.00, 13.83. Anal. Calcd for $C_{15}H_{24}N_2O_4$: C, 60.79; H, 8.16; N, 9.45. Found: C, 60.59; H, 8.22; N, 9.26.

The third band corresponded to recovered original substrate (**2a**) (178 mg, 14% yield).

Reaction of diethyl 1-methylpyrazole-3,5-dicarboxylate (2b): According to the general procedure, the reaction was stirred 14 days, affording a mixture of three products that were separated by preparative silica gel tlc (hexane : ethyl acetate 5:1).

First band yielded pure dioctyl 1-methylpyrazole-3,5-dicarboxylate (**5b**) (220 mg, 9%) as a syrup. ^{1}H-Nmr ($CDCl_3$): 7.31 (s, 1H, H4), 4.38 (t, 2H, J=6.9 Hz, α), 4.34 (t, 2H, J=6.7 Hz, α'), 4.30 (s, 3H, NCH_3), 1.80 (quint, 4H, J=6.9 Hz, β β'), 1.39 (m, 20 H, rest of CH_2), 0.93 (t, 3H, J=6.6 Hz, CH_3), 0.92 (t, 3H, J=6.9 Hz, CH_3). ^{13}C-Nmr: 161.59 ($O=\underline{C}$-C3), 159.26 ($O=\underline{C}$-C5), 142.02 (C3), 133.93 (C5), 113.65 (C4), 65.48 (α'), 65.24 (α), 40.30 (NCH_3), 31.68, 29.06, 28.65, 25.85, 22.52, 13.92 (CH_3). Anal. Calcd for $C_{22}H_{38}N_2O_4$: C, 66.97; H, 9.71; N, 7.10. Found: C, 66.83; H, 9.54; N, 7.02.

Intermediate band afforded a syrup (660 mg, 35% yield), which was a mixture 93 : 7 of 3-octyl-5-ethyl 1-methylpyrazole-3,5-dicarboxylate (**3b**) and 3-ethyl-5-octyl 1-methylpyrazole-3,5-dicarboxylate (**4b**), respectively. Proportion was determined from LIS experiments (See Results and Discussion Section), but products were not possible to separate. ^{1}H-Nmr ($CDCl_3$): 7.31 (s, 1H, H4), 4.34 (q, 2H, J=7.1 Hz, $CO_2C\underline{H}_2CH_3$), 4.32 (t, 2H, J=6.9 Hz, α), 4.23 (s, 3H, NCH_3), 1.75 (quint, 2H, J=6.9 Hz, β), 1.37 (t, 3H, J=7.1 Hz, $CO_2CH_2C\underline{H}_3$), 1.28 (m, 10 H, rest of CH_2), 0.86 (t, 3H, J=6.8 Hz, $(CH_2)_7C\underline{H}_3$). ^{13}C-Nmr: 161.54 ($O=\underline{C}$-C3), 159.15 ($O=\underline{C}$-C5), 141.99 (C3), 133.90 (C5), 113.64 (C4), 65.28 (α), 61.30 ($CO_2\underline{C}H_2CH_3$), 40.26 (NCH_3), 31.66, 29.05, 28.61, 25.79, 22.49, 14.06, 13.90. Anal. Calcd for $C_{16}H_{26}N_2O_4$: C, 61.91; H, 8.44; N, 9.03. Found: C, 61.75; H, 8.21; N, 8.97.

Initial substrate (**2b**) (70 mg, 5%) was recovered from the third band.

Reaction of diethyl 1-benzylpyrazole-3,5-dicarboxylate (2c): The suspension was stirred 13 days and the residual syrup was eluted on a silica gel column (hexane : ethyl acetate, 5:1).

From the first band, dioctyl 1-benzylpyrazole-3,5-dicarboxylate (**5c**) was obtained (93 mg, 3.3%) as a pure syrup. ^{1}H-Nmr ($CDCl_3$): 7.30 (s, 1H, H4), 7.22 (s, 5H, C_6H_5), 5.79 (s, 2H, $NC\underline{H}_2C_6H_5$), 4.31

(t, 2H, J=6.9 Hz, α), 4.20 (t, 2H, J=6.7 Hz, α'), 1.73 (quint, 2H, J=6.9 Hz, β), 1.64 (quint, 2H, J=6.7 Hz, β'), 1.23 (m, 20 H, rest of CH_2), 0.84 (t, 6H, J=6.8 Hz, CH_3). ^{13}C-Nmr : 161.66 (O=C-C3), 159.09 (O=C-C5), 142.61 (C3), 136.21 (C2' benzyl group), 133.53 (C5), 128.53 (C4' benzyl group), 127.89 (C5' benzyl group), 127.64 (C3' benzyl group), 114.23 (C4), 65.56 (α'), 65.35 (α), 55.85 ($NCH_2C_6H_5$), 32.10, 31.73, 29.10, 28.70, 28.52, 25.86, 22.57, 13.97 (CH_3). Anal. Calcd for $C_{28}H_{42}N_2O_4$: C, 71.46; H, 8.99; N, 5.95. Found: C, 71.34; H, 8.78; N, 5.79.

The second band (syrup, 1.02 g, 44%) corresponded to a mixture 95:5 of 3-octyl-5-ethyl 1-benzylpyrazole-3,5-dicarboxylate (**3c**) and 3-ethyl-5-octyl 1-benzylpyrazole-3,5-dicarboxylate (**4c**), respectively. Proportion was determined both by gc and LIS experiments, but products could not be isolated. ^{1}H-Nmr ($CDCl_3$): 7.36 (s, 1H, H4), 7.28 (m, 5H, C_6H_5), 5.85 (s, 2H, $NCH_2C_6H_5$), 4.35 (t, 2H, J=6.8 Hz, α), 4.32 (q, 2H, J=7.1 Hz, $CO_2CH_2CH_3$), 1.78 (quint, 2H, J=6.8 Hz, β), 1.34 (t, 3H, J=7.1 Hz, $CO_2CH_2CH_3$), 1.29 (m, 10 H, rest of CH_2), 0.89 (t, 6H, J=6.8 Hz, $(CH_2)_7CH_3$). ^{13}C-Nmr: 161.64 (O=C-C3), 158.95 (O=C-C5), 142.59 (C3), 136.15 (C2' benzyl group), 133.55 (C5), 128.46 (C4' benzyl group), 127.83 (C5' benzyl group), 127.59 (C3' benzyl group), 114.25 (C4), 65.28 (α), 61.33 ($CO_2CH_2CH_3$), 55.82 ($NCH_2C_6H_5$), 31.70, 29.08, 28.65, 25.84, 22.53, 13.94, 13.71. Anal. Calcd for $C_{22}H_{30}N_2O_4$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.28; H, 7.74; N, 7.10.

The third band was the recovered original substrate (**2c**) (165 mg, 9%).

Synthesis of podands. Transesterifications with DEG-Me and TEG-Me. General procedure

Lipozyme IM20 was added into a flask containing a solution of acyl donor (**2a-c**) and DEG-Me or TEG-Me in dry toluene. The mixture was stirred in a rotary evaporator without vacuum, heated at 60°C in a silicone bath and controlled by hplc. Original volume was approximately kept constant by adding dry toluene once a day. When the reaction was stabilized, the enzyme was filtered off and the clear solution was evaporated to dryness. Excess of glycol-Me was separated by dissolving the mixture in chloroform (30 ml) and washing the solution repeatedly with water (10x30 ml). Then it was dried over 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 diethyl 1*H*-pyrazole-3,5-dicarboxylate (2a) and DEG-Me: Reaction of **2a** (212 mg, 1 mmol), DEG-Me (0.59 ml, 5 mmol) and Lipozyme (1 g) in toluene (50 ml) during 8 days afforded a syrup which was eluted on a silica gel column (chloroform : acetone, 6:1).

The first product was initial substrate **2a** (26 mg, 12%).

The second isolated product was 3-(3',6'-dioxaheptyl)-5-ethyl 1*H*-pyrazole-3,5-dicarboxylate (**6a=8a**) as a pure syrup (120 mg, 42%). ^{1}H-Nmr ($CDCl_3$): 7.29 (s, 1H, H4), 4.43 (t, 2H, J=4.8 Hz, α), 4.35 (q, 2H, J=7.1 Hz, $CO_2C\underline{H}_2CH_3$), 3.76 (t, 2H, J=4.8 Hz, β), 3.62 (t, 2H, J=4.5 Hz, γ), 3.51 (t, 2H, J=4.5 Hz, δ), 3.33 (s, 3H, OCH_3), 1.34 (t, 3H, J=7.1 Hz, $CO_2CH_2C\underline{H}_3$). ^{13}C-Nmr: 160.50 (O=$\underline{C}$-C3), 159.59 (O=$\underline{C}$-C5), 141.15 (C3,5), 111.59 (C4), 71.90 (δ), 70.56 (γ), 68.85 (β), 64.41 (α), 61.53 ($CO_2\underline{C}H_2CH_3$), 59.02 (OCH_3), 14.24 ($CO_2CH_2\underline{C}H_3$). Anal. Calcd for $C_{12}H_{18}N_2O_6$: C, 50.35; H, 6.34; N, 9.79. Found: C, 50.65; H, 6.58; N, 9.65.

The third product was 3,5-bis(3',6'-dioxaheptyl) 1*H*-pyrazole-3,5-dicarboxylate (**10a**) (37 mg, 10%) again as a syrup. ^{1}H-Nmr ($CDCl_3$): 7.36 (s, 1H, H4), 4.47 (t, 4H, J=4.8 Hz, α α'), 3.80 (t, 4H, J=4.8 Hz, β β'), 3.67 (t, 4H, J=4.5 Hz, γ γ'), 3.54 (t, 4H, J=4.5 Hz, δ δ'), 3.37 (s, 6H, OCH_3). ^{13}C-Nmr: 159.94 (*C*=O), 139.87 (*C*3,5), 111.62 (*C*4), 71.69 (δ), 70.55 (γ), 68.36 (β), 64.35 (α), 59.01 (OCH_3). Anal. Calcd for $C_{15}H_{24}N_2O_8$: C, 50.00; H, 6.71; N, 7.77. Found: C, 49.85; H, 6.46; N, 7.41.

Reaction of diethyl 1*H*-pyrazole-3,5-dicarboxylate (2a) and TEG-Me: Reaction of **2a** (212 mg, 1 mmol), TEG-Me (0.80 ml, 5 mmol) catalyzed by Lipozyme (1 g) in anhydrous toluene (50 ml) following the general procedure in 8 days gave a mixture that was separated by chromatography on a silica gel column (chloroform : acetone 15:1).

Substrate **2a** (16 mg, 8%) was the first product isolated.

The second compound: 3-(3',6',9'-trioxadecyl)-5-ethyl 1*H*-pyrazole-3,5-dicarboxylate (**7a=9a**) (133 mg, 40%) isolated as a syrup. ^{1}H-Nmr ($CDCl_3$): 7.39 (s, 1H, H4), 4.50 (t, 2H, J=4.6 Hz, α), 4.41 (q, 2H, J=7.1 Hz, $CO_2C\underline{H}_2CH_3$), 3.83 (t, 2H, J=4.6 Hz, β), 3.72 (m, 2H, γ), 3.66 (m, 4H, δ ε), 3.57 (m, 2H, θ), 3.41 (s, 3H, OCH_3), 1.40 (t, 3H, J=7.1 Hz, $CO_2CH_2C\underline{H}_3$). ^{13}C-Nmr: 160.94 (O=$\underline{C}$-C3), 159.23 (O=$\underline{C}$-C5), 141.80 (C3,5), 111.80 (C4), 71.66 (θ), 70.66 (ε), 70.41 (δ), 70.32 (γ), 68.67(β), 64.15 (α), 61.34 ($CO_2\underline{C}H_2CH_3$), 58.95 (OCH_3), 14.23 ($CO_2CH_2\underline{C}H_3$). Anal. Calcd for $C_{14}H_{22}N_2O_7$: C, 50.90; H, 6.71; N, 8.48. Found: C, 50.87; H, 6.35; N, 8.27.

The third product was 3,5-bis(3',6',9'-trioxadecyl) 1*H*-pyrazole-3,5-dicarboxylate (**11a**), as a syrup (62mg, 14%). ^{1}H-Nmr ($CDCl_3$): 7.37 (s, 1H, H4), 4.48 (t, 4H, J=4.6 Hz, α α'), 3.81 (t, 4H, J=4.6 Hz, β β'), 3.69 (m, 4H, γ γ'), 3.65 (m, 8H, δ δ' ε ε'), 3.55 (m, 4H, θ θ'), 3.38 (s, 6H, OCH_3). ^{13}C-Nmr: 160.57 (C=O), 140.24 (C3,5), 112.09 (C4), 71.87 (θ), 70.55 (ε), 70.44 (δ), 70.30 (γ), 68.92 (β), 64.05 (α), 58.95 (OCH_3). Anal. Calcd for $C_{19}H_{32}N_2O_{10}$: C, 50.89; H, 7.19; N, 6.25. Found: C, 50.71; H, 6.89; N, 6.01.

Reaction of diethyl 1-methylpyrazole-3,5-dicarboxylate (2b) and DEG-Me: According to the general procedure, reaction of **2b** (1.13 g, 5 mmol), DEG-Me (1.49 ml, 12.5 mmol) and Lipozyme (5 g) in anhydrous toluene (100 ml) during 6 days afforded a syrup that was chromatographed on a silica gel column (chloroform : acetone, 20:1) to isolate three compounds.

The first compound was recovered substrate **2b** (199 mg, 17%).

The second fraction (625 mg, 42%) was a mixture of monosubstituted products (**6b**:**8b**) in proportion 91:9 (hplc data). The main product, 3-(3',6'-dioxaheptyl)-5-ethyl 1-methylpyrazole-3,5-dicarboxylate (**6b**), was purified by preparative cctlc using an eluent dichloromethane : methanol (200:1) at a flow-rate 5ml/min. ^{1}H-Nmr ($CDCl_3$) : 7.33 (s, 1H, H4), 4.48 (t, 2H, J=5.0 Hz, α), 4.33 (q, 2H, J=7.1 Hz, $CO_2C\underline{H}_2CH_3$), 4.22 (s, 3H, NCH_3), 3.81 (t, 2H, J=5.0 Hz, β), 3.66 (m, 2H, γ), 3.52 (m, 2H, δ), 3.35 (s, 3H, OCH_3), 1.36 (t, 3H, J=7.1 Hz, $CO_2CH_2C\underline{H}_3$). ^{13}C-Nmr: 161.44 (O=C-C3), 159.21(O=$\underline{C}$-C5), 141.54 (C3), 133.93 (C5), 113.94 (C4), 71.89 (δ), 70.51 (γ), 69.02 (β), 63.99 (α), 61.42 ($CO_2\underline{C}H_2CH_3$), 59.04 (OCH_3), 40.44 (NCH_3), 14.15 ($CO_2CH_2\underline{C}H_3$). Anal. Calcd for $C_{13}H_{20}N_2O_6$: C, 51.99; H, 6.71; N, 9.33. Found: C, 52.10; H, 6.55; N, 9.42.

The third compound was pure 3,5-bis(3',6'-dioxaheptyl) 1-methylpyrazole-3,5-dicarboxylate (**10b**) (170 mg, 9%) as a syrup. ^{1}H-Nmr ($CDCl_3$): 7.36 (s, 1H, H4), 4.47 (t, 2H, J=5.0 Hz, α), 4.43 (t, 2H, J=4.7 Hz, α'), 4.21 (s, 3H, NCH_3), 3.80 (t, 2H, J=5.0 Hz, β), 3.77 (t, 2H, J=4.7 Hz, β'), 3.65 (t, 2H, J=3.7 Hz, γ), 3.63 (t, 2H, J=3.5 Hz, γ'), 3.52 (m, 4H, δ δ'), 3.35 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3). ^{13}C-Nmr: 161.38 (O=$\underline{C}$-C3), 159.04 (O=$\underline{C}$-C5), 141.54 (C3), 130.62 (C5), 114.20 (C4), 71.85 (δ, δ'), 70.56 (γ), 70.47 (γ'), 68.98 (β), 68.83 (β'), 64.14 (α'), 63.98 (α), 59.02 (OCH_3), 40.47 (NCH_3). Anal. Calcd for $C_{16}H_{26}N_2O_8$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.13; H, 7.05; N, 7.75.

Reaction of diethyl 1-methylpyrazole-3,5-dicarboxylate (2b) and TEG-Me: According to the general procedure, **2b** (565 mg, 2.5 mmol), TEG-Me (1.00 ml, 6.25 mmol) and Lipozyme (2.5 g) in anhydrous toluene (50 ml) in 6 days gave a mixture which was eluted on a silicagel column (hexane : chloroform : acetone, 4:8:1). Three compounds were isolated.

The first compound was initial substrate (**2b**) (90 mg, 16%).

The second fraction (336 mg, 39%) was a mixture of monosubstituted podands (**7b**:**9b**), in a proportion 93:7 (hplc data), which could not be separated at a preparative scale. ^{1}H-Nmr ($CDCl_3$): 7.34 (s, **9b**: H4), 7.31 (s, **7b**: H4), 4.46 (t, J=5.0 Hz, **7b**: α), 4.40 (t, J=4.8 Hz, **9b**: α'), 4.38 (q, J=7.1Hz, **9b**: $CO_2C\underline{H}_2CH_3$), 4.33 (q, J=7.2 Hz, **7b**: $CO_2C\underline{H}_2CH_3$), 4.22 (s, 3H, NCH_3), 3.79 (t, J=5.0 Hz, **7b**: β), 3.77 (t, J=4.8 Hz, **9b**: β'), 3.62 (m, 6H, **7b**: γ δ ε; **9b**: γ' δ' ε'), 3.50 (m, 2H, **7b**: θ; **9b**: θ'),

3.33 (s, 3H, OCH_3), 1.36 (t, J=7.1 Hz, **9b**: $CO_2CH_2C\underline{H}_3$), 1.35 (t, J=7.2 Hz, **7b**: $CO_2CH_2C\underline{H}_3$). ^{13}C-Nmr: 161.41 (O=$\underline{C}$-C3), 159.17 (O=$\underline{C}$-C5), 141.34 (C3), 133.69 (C5), 114.06 (**9b**: C4), 113.88 (**7b**: C4), 71.88 (θ), 70.57 (δ, ε), 70.53 (γ), 68.96 (**7b**: β), 68.80 (**9b**: β'), 64.36 (**9b**: α'), 64.00 (**7b**: α), 61.39 (**7b**: $CO_2\underline{C}H_2CH_3$), 61.19 (**9b**: $CO_2\underline{C}H_2CH_3$), 58.96 (OCH_3), 40.40 (NCH_3), 14.28 (**9b**: $CO_2CH_2\underline{C}H_3$), 14.12 (**7b**: $CO_2CH_2\underline{C}H_3$). Anal. Calcd for $C_{15}H_{24}N_2O_7$: C, 52.32; H, 7.02; N, 8.13. Found: C, 52.30; H, 7.21; N, 8.42.

Finally, the third compound was pure 3,5-bis(3',6',9'-trioxadecyl) 1-methylpyrazole-3,5-dicarboxylate (**11b**) (73 mg, 6%) as a syrup. ^{1}H-Nmr ($CDCl_3$): 7.36 (s, 1H, H4), 4.47 (t, 2H, J=5.0 Hz, α), 4.43 (t, 2H, J=4.8 Hz, α'), 4.22 (s, 3H, N-CH_3), 3.80 (t, 2H, J=5.0 Hz, β), 3.78 (t, 2H, J=4.8 Hz, β'), 3.64 (m, 12H, γ γ' δ δ' ε ε'), 3.51 (m, 4H, θ θ'), 3.34 (s, 6H, OCH_3). ^{13}C-Nmr: 161.10 (O=$\underline{C}$-C3), 159.05 (O=$\underline{C}$-C5), 141.58 (C3), 133.62 (C5), 114.18 (C4), 71.89 (θ θ'), 70.61 (γ γ' δ δ' ε ε'), 68.96 (β), 68.81 (β'), 64.37 (α'), 64.03 (α), 58.98 (OCH_3), 40.49 (NCH_3). Anal. Calcd for $C_{20}H_{34}N_2O_{10}$: C, 51.94; H, 7.41; N, 6.06. Found: C, 51.81; H, 7.60; N, 6.35.

Reaction of diethyl 1-benzylpyrazole-3,5-dicarboxylate (2c) and DEG-Me: From **2c** (1.51 g, 5 mmol), DEG-Me (1.47 ml, 12.5 mmol) and Lipozyme (5 g) in anhydrous toluene (100 ml), and following the general procedure, after 12 days a syrup was obtained; it was purified by column chromatography (dichloromethane:acetone 50:1), affording three bands.

Initial substrate was recovered (492 mg, 33%) from the first band as a white solid.

The second band (850 mg, 45%) yielded a mixture of products (**6c**) and (**8c**) in proportion 92:8 (gc data). ^{1}H-Nmr ($CDCl_3$): 7.39 (s, 1H, H4), 7.28 (s, 5H, C_6H_5), 5.84 (s, 2H, $NC\underline{H}_2C_6H_5$), 4.52 (t, J=5.0 Hz, **6c**: α), 4.43 (q, J=7.1 Hz, **8c**: $CO_2C\underline{H}_2CH_3$), 4.41 (t, J=5.0 Hz, **8c**: α'), 4.31 (q, J=7.1 Hz, **6c**: $CO_2C\underline{H}_2CH_3$), 3.84 (t, 2H, J=5.0 Hz, **6c**: β), 3.76 (t, J=5.0 Hz, **8c**: β'), 3.69 (m, **6c**: γ), 3.65 (m. **8c**: γ'), 3.56 (m, **6c**: δ), 3.53 (m, **8c**: δ'), 3.38 (s, **6c**: OCH_3), 3.37 (s, **8c**: OCH_3), 1.41 (t, J=7.1 Hz, **8c**: $CO_2CH_2C\underline{H}_3$), 1.33 (t, J=7.1 Hz, **6c**: $CO_2CH_2C\underline{H}_3$). ^{13}C-Nmr ($CDCl_3$): 161.47 (O=$\underline{C}$-C3), 158.91 (O=$\underline{C}$-C5), 142.09 (C3), 136.04 (C2' benzyl group), 133.50 (C5), 128.52 (C4' benzyl group), 127.92 (C5' benzyl group), 127.64 (C3' benzyl group), 114.50 (C4), 71.89 (δ), 70.52 (γ), 69.03 (β), 64.05 (α), 61.43 ($CO_2\underline{C}H_2CH_3$), 59.04 (OCH_3), 55.87($N\underline{C}H_2C_6H_5$), 14.08 ($CO_2CH_2\underline{C}H_3$). Anal. Calcd for $C_{19}H_{24}N_2O_6$: C, 60.63; H, 6.43; N, 7.44. Found: C, 60.74; H, 6.60; N, 7.40.

The third band corresponded to pure disubstituted compound **10c** (60 mg, 3%). ^{1}H-Nmr ($CDCl_3$): 7.43 (s, 1H, H4), 7.28 (s, 5H, C_6H_5), 5.84 (s, 2H, $NC\underline{H}_2C_6H_5$), 4.52 (t, 2H, J=5.0 Hz, α), 4.41 (t, 2H, J=4.8 Hz, α'), 3.84 (t, 2H, J=5.0 Hz, β), 3.76 (t, 2H, J=4.8 Hz, β'), 3.69 (m, 2H, γ), 3.64 (m, 2H, γ'),

3.55 (m, 4H, δ δ'), 3.38 (s, 3H, OCH_3), 3.37 (s, 3H, OCH_3). ^{13}C-Nmr ($CDCl_3$): 161.69 (O=C-C3), 159.01 (O=C-C5), 142.12 (C3), 135.97 (C2' benzyl group), 133.44 (C5), 128.21 (C4' benzyl group), 127.93 (C5' benzyl group), 127.65 (C3' benzyl group), 114.80 (C4), 71.84 (δ δ'), 70.55 (γ), 70.51 (γ'), 69.00 (β), 68.80 (β'), 64.38 (α'), 64.05 (α), 59.01 (OCH_3), 55.90 ($NCH_2C_6H_5$). Anal. Calcd for $C_{22}H_{30}N_2O_8$: C, 58.66; H, 6.71; N, 6.22. Found: C, 58.69; H, 6.55; N, 6.10.

Reaction of diethyl 1-benzylpyrazole-3,5-dicarboxylate (2c) and TEG-Me: Reaction of **2c** (1.51 g, 5 mmol), and TEG-Me (1.96 ml, 12.5 mmol) catalysed by Lipozyme (5 g) in anhydrous toluene (100 ml) during 10 days gave a syrup that was chromatographed on silicagel column (hexane: chloroform: acetone 10:8:1).

From the first band original substrate (518 mg, 34%) was recuperated.

The second band (795 mg, 38%) gave a mixture of products (**7c**:**9c**) in proportion 95:5 (hplc data) that could not be separated. ^{1}H-Nmr ($CDCl_3$): 7.40 (s, 1H, **9c**: H4), 7.38 (s, 1H, **7c**: H4), 7.28 (s, 5H, C_6H_5), 5.84 (s, 2H, $NCH_2C_6H_5$), 4.51 (t, J=5.0 Hz, **7c**: α), 4.43 (q, J=7.1 Hz, **9c**: $CO_2CH_2CH_3$), 4.41 (t, J=4.9 Hz, **9c**: α'), 4.31 (q, J=7.1 Hz, **7c**: $CO_2CH_2CH_3$), 3.84 (t, J=5.0 Hz, **7c**: β), 3.76 (t, J=4.9 Hz, **9c**: β'), 3.68 (m, 6H, **7c**: γ δ ε; **9c**: γ' δ' ε'), 3.53 (m, 2H, **7c**: θ; **9c**: θ'), 3.36 (s, 6H, OCH_3), 1.41 (t, J=7.1 Hz, **9c**: $CO_2CH_2CH_3$), 1.33 (t, J=7.1 Hz, **7c**: $CO_2CH_2CH_3$). ^{13}C-Nmr ($CDCl_3$): 161.39 (O=C-C3), 158.80 (O=C-C5), 141.97 (C3), 135.93 (C2' benzyl group), 133.39 (C5), 128.44 (C4' benzyl group), 127.85 (C5' benzyl group), 127.55 (C3' benzyl group), 114.60 (**9c**: C4), 114.40 (**7c**: C4), 71.78 (θ), 70.51 (δ ε), 70.45 (γ), 68.90 (**7c**: β), 68.74 (**9c**: β'), 64.40 (**9c**: α'), 64.00 (**7c**: α), 61.37 (**7c**: $CO_2CH_2CH_3$), 61.17 (**9c**: $CO_2CH_2CH_3$), 58.91 (OCH_3), 55.78 ($NCH_2C_6H_5$), 14.00 ($CO_2CH_2CH_3$). Anal. Calcd for $C_{21}H_{28}N_2O_7$: C, 59.99; H, 6.71; N, 6.66. Found: C, 59.97; H, 9.78; N, 6.90.

Finally, a syrup of pure 3,5-bis(3',6',9'-trioxadecyl) 1-benzylpyrazole-3,5-dicarboxylate (**11c**) (200 mg, 7%) was obtained. ^{1}H-Nmr ($CDCl_3$): 7.43 (s, 1H, H4), 7.29 (s, 5H, C_6H_5), 5.84 (s, 2H, $NCH_2C_6H_5$), 4.52 (t, 2H, J=4.9 Hz, α), 4.41 (t, 2H, J=4.7 Hz, α'), 3.85 (t, 2H, J= 5.0 Hz, β), 3.76 (t, 2H, J=4.7 Hz, β'), 3.66 (m, 12H, γ γ' δ δ' ε ε'), 3.54 (m, 4H, θ θ'), 3.37(s, 6H, OCH_3). ^{13}C-Nmr ($CDCl_3$): 161.24 (O=C-C3), 158.56 (O=C-C5), 141.88 (C3), 135.76 (C2' benzyl group), 133.00 (C5), 128.34 (C4' benzyl group), 127.76 (C5' benzyl group), 127.44 (C3' benzyl group), 114.59 (C4), 71.64 (θ θ'), 70.38 (γ γ' δ δ' ε ε'), 68.76 (β), 68.56 (β'), 64.22 (α'), 63.90 (α), 58.81 (OCH_3), 55.72 ($NCH_2C_6H_5$). Anal. Calcd for $C_{26}H_{38}N_2O_{10}$: C, 57.98; H, 7.11; N, 5.20. Found: C, 58.17; H, 7.35; N, 5.33.

Lanthanide Induced Shifts (LIS) experiments.

Solutions of the appropriate product in $CDCl_3$ with increasing amounts of $Eu(fod)_3$ were prepared, and their ^1H-nmr spectra recorded at 30°C. Concentration ratios of [$Eu(fod)_3$]/[product] were about 0.05, 0.10, 0.15, and 0.20.

ACKNOWLEDGEMENTS

Generous gifts of enzymes from Amano Pharmaceutical Co., and Novo Nordisk[9] are gratefully acknowledged.

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Received, 15th February, 1993