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Regioselective Lipase Catalyzed Synthesis of Diester Crowns. New Asymmetric Macrocycles Containing a 1,3-Bis(1*H*-Pyrazol-1-yl)Propane Unit

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Abstract: Regioselective lipase catalyzed intramolecular transesterification of dipyrazolic tetraester **1** with di-, tri- and tetraethyleneglycol afforded symmetric and, in smaller amounts, asymmetric diester crowns including a 1,3-bis(1*H*-pyrazol-1-yl)propane unit. Their structures have been unequivocally elucidated after their ^{1}H and ^{13}C NMR spectra and INEPT experiments.

INTRODUCTION

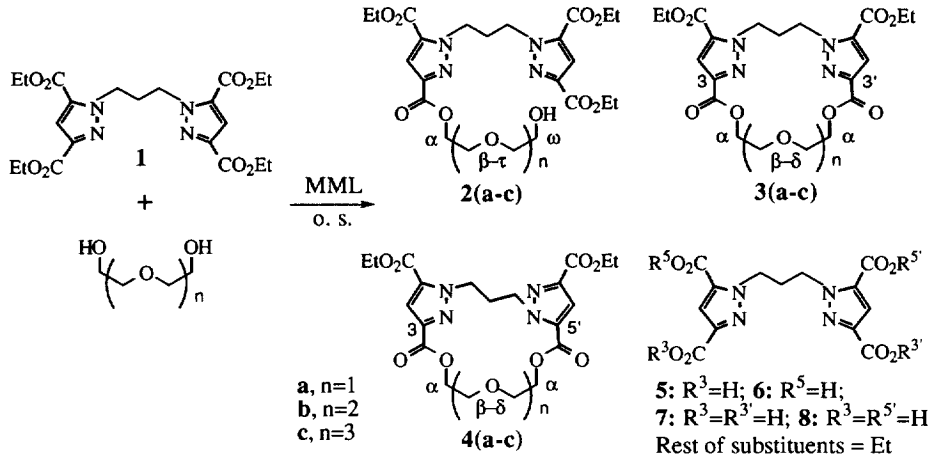
The binding affinity of crown ethers can be modulated by introducing nitrogen atoms as part of the macrocyclic ring, with the subsequent effect of an increased selective affinity for ammonium *vs.* alkali cations.[1] Taking into account the important role played by organic ammonium cations in the biological systems, many crowns and podands (pseudocyclic crown ethers) including nitrogen-containing heterocyclic systems have been synthesized and their complexing properties studied.[2] In this field, our group have reported the synthesis of crowns and podands containing pyrazolic[3] or propylendipyrazolic units,[4] and their ionophoric properties as carriers of alkali and neurotransmitter ammonium cations of physiological interest.

We have reported before[5] the enzymatic synthesis of new diesters crowns containing a 1,3-bis(1*H*-pyrazol-1-yl)propane moiety, the acyclic intermediates and some by-products. Recently, an alternative more versatile synthesis of these esters crowns *via* the regioselective enzymatic hydrolysis of the dipyrazolic tetraester **1** was studied[6] and we found that some dicarboxylic acid derivatives of **1** remained attached to the commercial enzyme preparation[7] used after a standard extractive procedure. In the present work we complete the initial published results[5] about the *Mucor miehei* lipase (MML from now on) catalyzed transesterification of **1** with polyethyleneglycols when the products were exhaustively extracted from the solid residue (the enzyme preparation plus the products adsorbed on it) with refluxing methanol in a Soxhlet apparatus.

RESULTS AND DISCUSSION

SYNTHESIS

First of all, the reactions were carried out at a preparative scale, following standard experimental conditions (see *Experimental Section*) in order to obtain samples of the resulting products that were unequivocally identified by its ^{1}H- and ^{13}C-NMR spectra (see below, *Structural Elucidation* part) or by comparison with previously obtained pure samples: besides the expected acyclic compounds **2a-c** and 3,3'-cycles **3a-c**, new products were also found: asymmetric 3,5'-cycles **4a-c** and small amounts of mono **5-6** and diacids **7-8** (Scheme 1).



SCHEME 1

Non-symmetric heterocycles **4a-c** can be explained because the cyclic compounds are formed after two well differentiated reactions. A first transesterification took place in a highly regioselective manner: the acyclic intermediates **2a-c** were identified as 3-substituted derivatives and 5-analogs were not detected by chromatographic or spectroscopic techniques: the two pyrazolic rings of the acyl donor **1** are equivalent and there is a kinetic preference for the 3- *vs.* 5-ester group, probably due to steric hindrance.[8] A dramatic difference marks the second step: the reaction is now intramolecular, acyl donor and nucleophile are two moieties of a single molecule. Even though 3-ester is the preferred group to form the acyl–Ser bond, the nucleophilic attack may have its own preference depending on the lower-energy conformation of the second ring and its hydroxyl-polyether chain inside the active site of the enzyme (Figure 1). In fact, the regioselectivity of this second step is not so clear and the radio **3:4** diminished to approximately 3:1. Small amounts of mono- and diacids **5-8** were also found as an expected consequence of the 10 % w/w of water present in the enzyme preparation and the long reaction times required.

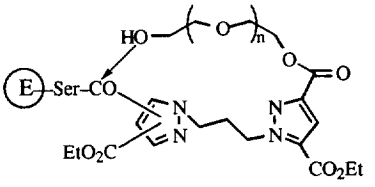


FIGURE 1

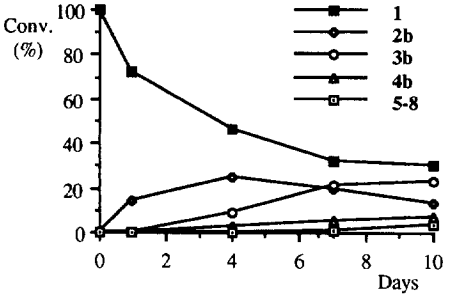
The enzymatic study was carried out using analytical scale reactions and finally validated in preparative conditions. Three parallel reactions were carried out at a 1.5 mL scale with **1** and DEG, TEG and TTEG as nucleophiles. Unfortunately, the composition of the mixture could not be known by direct HPLC analysis of aliquots but every vial had to be completely processed and then studied. So, four vials of each of the three reactions were incubated, processed and HPLC analyzed after 1, 4, 7 and 10 days.

All the three showed a similar behaviour. Reaction **b** (TEG) may be taken as a characteristic example (Table 1, Figure 2): starting tetraester **1** decreased to about 50 % in 3-4 days while the acyclic intermediate **2b** reached its peak and gradually changed into the cyclic compound **3b** and to a lesser extent **4b**. In an

attempt to increase the conversion of **1** into cyclic compounds, this water was reduced by desiccating the enzyme[9] to 2.5, 1.2 and nearly 0 % w/w but the activity of MML also decreased and 59, 70 and 100 % respectively of unconverted **1** was present after a 4 days reaction. When anhydrous diisopropylether was used instead of toluene (Table 1) the activity was also reduced (50 % of unconverted **1**) and hydrolysis products **5-8** become predominant.

TABLE 1, FIGURE 2.— **1** (25 mM), TEG (25 mM) and MML (25 mg/mL) in Anhydrous Toluene (a) and Diisopropylether (b) at 60°C. Analytical Scale, HPLC Data.

Days	**1**	**2b**	**3b**	**4b**	**5-6**	**7-8**
1[a]	72	14	0	0	0	0
4[a]	46	25	9	3	0	0
7[a]	32	20	21	6	1.3	0
10[a]	30	15	23	7	2.5	0.7
10[b]	50	9	0	0	14.0	3.0

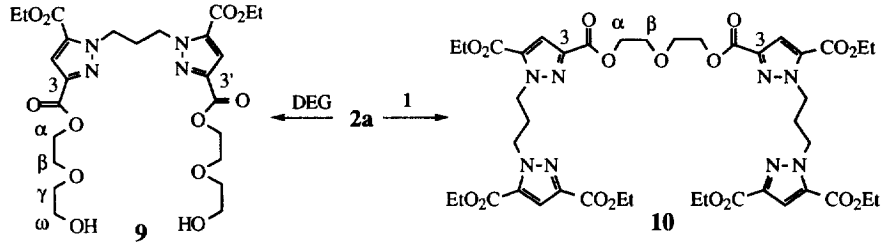


Finally preparative reactions in anhydrous toluene afforded pure compounds in percentages shown in Table 2. Acids **5-8** were not isolated.

TABLE 2.- Transesterification of **1** with Anhydrous DEG, TEG, and TTEG, in Dry Toluene. Percentage of Pure Isolated Products.

Polyethylene glycol	Recovered **1**	Acyclic **2a-c**	3,3'-Cyclic **3a-c**	3,5'-Cyclic **4a-c**
a: DEG	30	9	11	1
b: TEG	31	11	17	5
c: TTEG	26	5	10	4

Two additional products **9** and **10** were isolated in small amounts (0.5 and 1%, respectively) when DEG was used as nucleophile (**a**). These acyclic compounds were formed from the intermediate **2a**: **9** when the 3'-ethoxycarbonyl group of **2a** was attacked by a second molecule of DEG and **10** when the hydroxy group of **2a** acted as nucleophile in an intermolecular substitution on a second molecule of **1** (Scheme 2). These compounds exhibited also the regioselectivity of 3- *vs.* 5-positions of the pyrazolic rings.



SCHEME 2

STRUCTURAL ELUCIDATION

The new compounds were characterized from their spectroscopic (^{1}H NMR, ^{13}C NMR and MS) and analytical data. The transesterification locations were identified by ^{1}H NMR, taking into account that in N-alkylated pyrazoles the 3-alkoxy groups are more deshielded than their counterparts of the 5-position.[10]

The structures of the symmetric crowns **3a-c** and the acyclic compounds **2a-c**, **9**, and **10** were established from the number and place of the glycol chains and the ethyl groups. The structural determinations of the asymmetric crowns **4a-c** were more complicated. In their proton spectra there were two different ethyl groups: one located on a 3-pyrazolic position and the other on a 5-position, and the glycol chains showed duplicated signals. These spectroscopic data were in accordance with two possible structures: (i) 3,5'-crown with the chain in different pyrazoles **4**, or (ii) 3,5-crown with the glycol chain attached to the same pyrazolic ring **4'** (Figure 3).

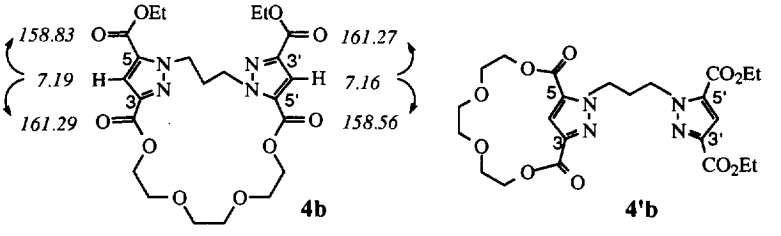


FIGURE 3— Selective INEPT experiments: unequivocal assignment of 3,5'-structure **4b** for the asymmetric crown.

With the aim of unequivocally fixing their structure we carried out a complete ^{1}H and ^{13}C NMR study of the asymmetric crown **4b** in $CDCl_3$. The corresponding HMQC and HMBC experiments allowed unambiguous assignment of the strategic carbonyl and pyrazol atoms. So, the resonances at 158.83 and 161.27 ppm were assigned to the carbonyl atoms in the CO_2Et groups and the resonances at 161.29 and 158.56 ppm to the carbonyl atoms in the $CO_2CH_2CH_2O$ groups. Unfortunally, correlations between the pyrazolic protons and the carbonyl carbons were not observed. To discriminate between the two possible structures, a series of selective INEPT experiments was carried out. The fact that each pyrazolic proton correlated with two carbonyl atoms, one belonging to a CO_2Et group and the other to a $CO_2CH_2CH_2O$ group, pointed out that **4b** was the 3,5'-macrocycle, in which the triethylene glycol chain was attached to the 3-position of one pyrazole and the 5'-position of the other (Figure 3).

CONCLUSIONS

In this paper we report the synthesis of dipyrazolic diester crowns *via* enzymatic transesterification. After the results obtained, the reaction is highly regioselective for the 3- *vs.* 5-ester group, but the intramolecular transesterification of the acyclic intermediates **2** is affected by the conformation of the second ring and its 3-hydroxypolyether chain in the active site of the enzyme and, besides the regioselective 3,3'-cycles **3**, little amounts of 3,5'-asymmetric cycles **4** are also obtained.

EXPERIMENTAL SECTION

Elemental analyses of new products were carried out in a Perkin-Elmer 240C equipment in the Centro de Química Orgánica 'Manuel Lora-Tamayo' (CSIC). Mass spectra (MS) were obtained by electronic impact at 70 eV in a VG-12-250 spectrometer. NMR spectra were recorded in $CDCl_3$ solutions, using a Varian Unity-500, a Varian XL-300, and a Gemini-200 spectrometers. For compound **4b** several drops of C_6D_6 were added as shift reagent to avoid overlapping in the resonances corresponding to carbonyl carbons. HMQC (Heteronuclear Multiple Quantum Correlation) and HMBC (Heteronuclear Multiple Bond Correlation) were obtained in standard conditions. The INEPTLR (Insensitive Nuclei Enhanced by Polarization Transfer) experiment was carried out using low-power DANTE-type selective decoupler pulses. The experiment was optimized for 6 Hz couplings with selective excitation of the H4 pyrazolic protons of compound **4b**. Water content was measured in a Karl-Fischer apparatus. 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) and compounds were detected with UV light (254 nm). 1,3-bis[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]propane **1** was synthesized by us.[4a] Commercial (Aldrich) di-, tri-, and tetraethyleneglycol were distilled in vacuo before using them.

Enzymatic Transesterifications. General Procedure. Lipozyme (25 mg/mL) was added to a solution of 1,3-bis[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]propane **1** (25 mM) and the corresponding nucleophile (DEG, TEG, or TTEG: 25 mM) in dry toluene or diisopropylether.

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 (250 rpm) at 60°C. Tipically, four vials containing the same reaction mixture were incubated and then evaporated to dryness, extracted with boiling methanol (10 mL), and analyzed by HPLC after 1, 4, 7, and 10 days.

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 for 12 days. The solution was daily refilled with fresh dry toluene to the original volume. Afterwards, the suspended solid was filtered off and extracted with boiling methanol (100 mL) during 3-4 hours in a Soxhlet apparatus. The toluene and methanol solutions were jointly evaporated to dryness and the residue was purified on a silica gel column, using mixtures of hexane:chloroform:acetone of increasing polarity. Eluents and initial amounts are specified in each case.

Reaction with DEG (a). Reaction of **1** (1.16 g, 2.5 mmol), diethyleneglycol (0.24 mL, 2.5 mmol) and Lipozyme (2.50 g) in toluene (100 mL) during 12 days afforded a syrup that was chromatographed on a silica gel column, using first hexane:chloroform:acetone (10:8:1), changing gradually to chloroform:acetone (10:1).

The first product was initial substrate **1** (348 mg, 30%).

*1,9-Bis[1-{3-[3,5-bis(ethoxycarbonyl)-1*H*-pyrazol-1-yl]propyl}-5-ethoxycarbonyl-1*H*-pyrazol-3-yl]-1,9-dioxo-2,5,8-trioxanonane* **10**. The second band afforded the acyclic product **10** as a pure syrup (24 mg, 1%). MS m/z (rel intensity) 897 (M^+ - C_2H_5O, 1), 252(100). Anal. Calcd for $C_{42}H_{54}N_8O_{17}$: C, 53.50; H, 5.73; N, 11.89. Found: C, 53.30; H, 6.00; N, 11.80.

13,20-Bis(ethoxycarbonyl)-3,6,9-trioxa-14,18,19,22-tetraazatricyclo[16.3.0.1^{11,14}]docosa-1(21),11(22),12,19-tetraen-2,10-dione **4a**. The third product was the asymmetric crown **4a** (12 mg, 1%) as a white solid (mp: 221-2°C). MS m/z (rel intensity) 479 (MH^+, 5), 478 (M^+, 13), 252(100). Anal. Calcd for $C_{21}H_{26}N_4O_9$: C, 52.72; H, 5.44; N, 11.72. Found: C, 52.45; H, 5.15; N, 11.59.

13,19-Bis(ethoxycarbonyl)-3,6,9-trioxa-14,18,21,22-tetraazatricyclo[16.2.1.1^{11,14}]docosa-1(21),11(22),12,19-tetraen-2,10-dione **3a**. The fourth band yielded the symmetric crown **3a** (132 mg, 11%) as a white solid (mp: 234-5°C).

MS m/z (rel intensity) 479 (MH^+, 13), 478 (M^+, 25), 252(100). Anal. Calcd for $C_{21}H_{26}N_4O_9$: C, 52.72; H, 5.44; N, 11.72. Found: C, 53.10; H, 5.20; N, 11.48.

1-[3, 5-Bis(ethoxycarbonyl)-1H-pyrazol-1-yl]-3-[5-ethoxycarbonyl -3- (5-hydroxy-3-oxapentyloxycarbonyl)-1H-pyrazol-1-yl]propane **2a.** The next product was the monosubstituted **2a** (118 mg, 9%), that was isolated as a pure syrup. MS m/z (rel intensity) 525 (MH^+, 44), 252(100). Anal. Calcd for $C_{23}H_{32}N_4O_{10}$: C, 52.67; H, 6.11; N, 10.69. Found: C, 52.91; H, 6.30; N, 10.51.

1,3-bis[5-ethoxycarbonyl-3-(5-hydroxy-3-oxapentyloxycarbonyl)-1H-pyrazol-1-yl]propane **9.** The last fractions of the column gave the disubstituted product **9**, again as a pure syrup (8 mg, 0.5%). MS m/z (rel intensity) 585 (MH^+, 1), 45(100). Anal. Calcd for $C_{25}H_{36}N_4O_{12}$: C, 51.37; H, 6.16; N, 9.59. Found: C, 51.60; H, 5.90; N, 9.31.

TABLE 3.—^{1}H-NMR data (δ, ppm) of new compounds.

	Acyclic intermediates			Symmetric crowns			Asymmetric crowns				
	2a	**2b**	**2c**	**3a**	**3b**	**3c**	**4a**	**4b**	**4c**	**9**	**10**
H(4,4')[a]	7.32	7.32	7.31	7.30	7.28	7.31	7.37	7.33	7.28	7.33	7.30
	7.30	7.29	7.29				7.29	7.30	7.19		7.29
N-CH_2[b]	4.68	4.66	4.66	4.78	4.75	4.73	4.69	4.71	4.65	4.68	4.64
								4.69	4.61		
N-CH_2-CH_2[c]	2.45	2.43	2.43	2.77	2.63	2.56	2.80	2.51	2.43	2.45	2.40
(3)$CO_2CH_2CH_3$[d]	4.37	4.35	4.36	—	—	—	4.37	4.38	4.34	—	4.33
(3)$CO_2CH_2CH_3$[e]	1.36	1.34	1.35	—	—	—	1.42	1.38	1.33	—	1.32
(5)$CO_2CH_2CH_3$[d]	4.30	4.29	4.32	4.38	4.36	4.35	4.36	4.35	4.29	4.34	4.26
(5)$CO_2CH_2CH_3$[e]	1.34	1.32	1.33	1.42	1.39	1.38	1.37	1.37	1.31	1.38	1.29
α[f]	4.48	4.45	4.47	4.53	4.47	4.47	4.46	4.48	4.42	4.46	4.43
							4.45	4.39	4.36		
β[f]	3.80	3.78	3.76	3.82	3.74	3.80	3.84	3.82	3.76	3.82	3.79
							3.84	3.74	3.71		
γ[g]	3.62	3.64	3.61	—	3.77	3.70	—	3.68	3.63	3.56	—
								3.68	3.59		
δ[g]	—	3.64	3.61	—	—	3.70	—	—	3.57	—	—
ε[g]	—	3.55	3.61	—	—	—	—	—	—	—	—
ϕ,τ[g]	—	—	3.61	—	—	—	—	—	—	—	—
ω[h]	3.70	3.65	3.61	—	—	—	—	—	—	3.65	—
OH[h]	2.40	2.36	2.30	—	—	—	—	—	—	2.63	—

[a] Singlet. [b] Triplet (J ≈ 6.9 Hz). [c] Quint (J ≈ 6.9 Hz). [d] Quartet (J ≈ 7.1 Hz). [e] Triplet (J ≈ 7.1 Hz). [f] Triplet (**2a-c**, **9**, **10**: J ≈ 4.8 Hz; **3a-c**, **4a-c**: J ≈ 4.4 Hz). [g] Multiplet. [h] Disappear with D_2O.

Reaction with TEG (b). Following the general procedure, reaction of **1** (1.16 g, 2.5 mmol), triethyleneglycol (0.33 mL, 2.5 mmol) and Lipozyme (2.50 g) in toluene (100 mL) during 12 days afforded a syrup that was chromatographed on a silica gel column, using first hexane:chloroform:acetone (10:8:1), changing gradually to chloroform:acetone (10:1). The elution order was:

Initial substrate **1** (348 mg, 31%).

16, 23 - Bis (ethoxycarbonyl) - 3, 6, 9, 12- tetraoxa - 17, 21, 22, 25 - tetraazatricyclo [19. 3. 0. $1^{14,17}$]pentacosa-1(24),14(25),15,22-tetraen-2,13-dione **4b**, the asymmetric crown (66 mg, 5%), isolated as a white solid (mp: 130-1°C). MS m/z (rel intensity) 523 (MH^+, 5), 522 (M^+, 17), 121(100). Anal. Calcd for $C_{23}H_{30}N_4O_{10}$: C, 52.87; H, 5.75; N, 10.73. Found: C, 52.66; H, 6.04; N, 11.02.

16, 22 - Bis (ethoxycarbonyl) - 3, 6, 9, 12- tetraoxa - 17, 21, 24, 25 - tetraazatricyclo [19. 2. 1. $1^{14,17}$]pentacosa-1(24),14(25),15,22-tetraen-2,13-dione **3b** the symmetric crown (222 mg, 17%), isolated also as a white solid (mp: 142-3°C). MS m/z (rel intensity) 523 (MH^+, 1), 522 (M^+, 1), 121 (100). Anal. Calcd for $C_{23}H_{30}N_4O_{10}$: C, 52.87; H, 5.75; N, 10.73. Found: C, 52.57; H, 6.00; N, 10.91.

1-[3, 5-Bis (ethoxycarbonyl)-1H-pyrazol-1-yl]-3-[5-ethoxycarbonyl-3-(8-hydroxy-3, 6-dioxaoctyloxycarbonyl)-1H-pyrazol-1-yl]propane **2b,** the monosubstituted intermediate (156 mg, 11%), that was isolated as a pure syrup. MS m/z (rel intensity) 569 (MH^+, 31), 252(100). Anal. Calcd for $C_{25}H_{36}N_4O_{11}$: C, 52.82; H, 6.34; N, 9.86. Found: C, 53.10; H, 6.60; N, 9.78.

TABLE 4.—^{13}C-NMR data (δ, ppm) of new compounds.

	Acyclic intermediates			Symmetric crowns			Asymmetric crowns				
	2a	**2b**	**2c**	**3a**	**3b**	**3c**	**4a**	**4b**	**4c**	**9**	**10**
(3,3')C-*C*=O	161.34	161.45	161.46	161.28	161.28	161.27	161.38	161.40	161.44	161.41	161.49
	161.22	161.32	161.33								161.35
(5,5')C-*C*=O	158.79	158.90	158.87	159.19	159.05	158.88	158.89	159.03	158.57	158.92	158.94
	158.71	158.86	158.85				158.84	158.65	158.44		158.90
C(3,3')	142.21	142.33	142.33	141.64	141.95	141.82	142.31	142.39	142.45	141.90	142.39
	141.73	141.88	141.91				141.65	142.06	142.04		141.67
C(4,4')	114.06	114.23	114.21	114.11	113.94	114.10	114.57	114.52	114.67	114.25	114.24
	113.97	114.07	114.09				113.96	114.23	114.31		114.14
C(5,5')	133.59	133.62	133.59	133.49	133.82	133.66	133.86	133.86	133.79	133.76	133.62
	133.47	133.57	133.58				133.75	133.52	133.62		
N-CH_2	49.96	50.08	50.09	50.14	49.83	49.96	50.08	50.25	50.27	50.16	50.12
							49.82	49.70	50.11		
N-CH_2-*CH_2*	30.51	30.68	30.70	28.80	29.50	29.65	30.79	30.25	30.49	30.67	30.75
(3,3')CO_2*CH_2*CH_3	61.00	61.11	61.13	—	—	—	61.02	61.15	61.17	—	61.16
(3,3')CO_2CH_2*CH_3*	14.14	14.26	14.27	—	—	—	14.36	14.32	14.33	—	14.35
(5,5')CO_2*CH_2*CH_3	61.32	61.40	61.42	61.35	61.36	61.37	61.51	61.50	61.50	61.50	61.44
(5,5')CO_2CH_2*CH_3*	13.96	14.07	14.09	14.11	14.06	14.01	14.12	14.12	14.14	14.14	14.12
α	63.66	63.88	63.97	63.48	64.73	64.73	63.88	64.36	64.53	64.03	63.95
							63.08	64.22	64.24		
β	68.77	68.96	68.98	68.74	68.88	68.97	68.73	69.10	68.97	68.97	68.99
							68.32	68.94	68.90		
γ	72.37	70.32	70.29	—	70.96	70.65	—	71.03	70.79	72.48	—
								70.73	70.75		
δ	—	70.62	70.59	—	—	71.21	—	—	71.17	—	—
ε, ϕ	—	72.49	70.59	—	—	—	—	—	—	—	—
τ	—	—	72.48	—	—	—	—	—	—	—	—
ω	61.49	61.71	61.67	—	—	—	—	—	—	61.72	—

Reaction with TTEG (c). Reaction of **1** (1.16 g, 2.5 mmol), tetraethyleneglycol (0.43 mL, 2.5 mmol) and Lipozyme (2.50 g) in toluene (100 mL) during 12 days afforded a syrup that was chromatographed on a silica gel column, using chloroform:acetone (10:1).

The first product was initial substrate **1** (302 mg, 26%).

19, 26- Bis (ethoxycarbonyl)-3, 6, 9, 12, 15- pentaoxa-20, 24, 25, 28- tetraazatricyclo [22. 3. 0. $1^{17,20}$] octacosa-1(27),17(28),18,25-tetraen-2,16-dione **4c.** The second band yielded the asymmetric crown **4c** (60 mg, 4%) as a white solid (mp: 104-5°C). MS m/z (rel intensity) 567 (MH^+, 12), 566 (M^+, 7), 208 (100). Anal. Calcd for $C_{25}H_{34}N_4O_{11}$: C, 53.00; H, 6.01; N, 9.89. Found: C, 52.86; H, 5.95; N, 9.72.

19, 25- Bis (ethoxycarbonyl)-3, 6, 9, 12, 15- pentaoxa-20, 24, 27, 28- tetraazatricyclo [22. 2. 1. $1^{17,20}$] octacosa-1(27),17(28),18,25-tetraen-2,16-dione **3c.** The third product was the symmetric crown **3c** (142 mg, 10%) as a white solid (mp: 121-2°C). MS m/z (rel intensity) 567 (MH^+, 31), 566 (M^+, 5), 121 (100). Anal. Calcd for $C_{25}H_{34}N_4O_{11}$: C, 53.00; H, 6.01; N, 9.89. Found: C, 53.27; H, 5.87; N, 10.10.

1- [3, 5 - Bis (ethoxycarbonyl)-1H-pyrazol-1-yl]-3-[5-ethoxycarbonyl - 3 - (11 - hydroxy-3, 6, 9 - trioxaundecyl oxycarbonyl)-1H-pyrazol-1-yl]propane **2c.** The next product was the monosubstituted **2c** (77 mg, 5%), that was isolated as a pure syrup. MS m/z (rel intensity) 613 (MH^+, 3), 612 (M^+, 1), 252(100). Anal. Calcd for $C_{27}H_{40}N_4O_{12}$: C, 52.94; H, 6.53; N, 9.15. Found: C, 53.01; H, 6.81; N, 9.11.

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