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FIRST REGIOSELECTIVE *MUCOR MIEHEI* LIPASE CATALYZED SYNTHESIS OF DIESTER CROWNS. NEW MACROCYCLES CONTAINING A 1,3-BIS(1*H*-PYRAZOL-1-YL)PROPANE UNIT

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Abstract. Diester crowns **4a-c** have been prepared for the first time by regioselective lipase-catalyzed transesterification. The synthesized new ester crowns include a 1,3-bis(1*H*-pyrazol-1-yl)propane unit as a part of the macrocycle. Acyclic intermediates **3a-c** were also obtained.

The important role played by organic ammonium cations in biological systems has increased the interest for the development of receptors capable of recognizing them.[1,2] We have previously reported the synthesis and complexing properties of crowns containing 1-methyl and 1*H*-substituted pyrazole units[3-6] and we have found an interesting selectivity toward ammonium cation versus alkali ones. Since $^{+}$NH...N hydrogen bonding is stronger than $^{+}$NH...O,[7] selective binding of ammonium ions could be improved by adding another pyrazolic sp^2 nitrogen in the former crown structures. Thus the present paper is focused on macrocycles containing a 1,3-bis(1*H*-pyrazol-1-yl)propane unit, in order to study the effect of two pyrazolic nitrogens on the complexing properties.

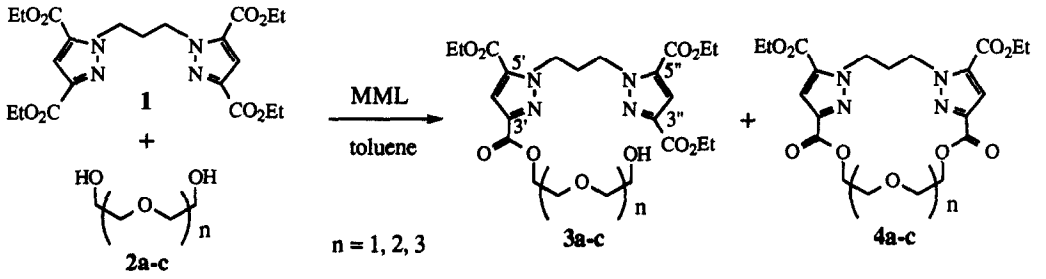
On the other hand, a recent and increasingly effective development in the field of the organic chemistry is the application of enzymes to the synthetic reactions. Nowadays it is well established that many families of enzymes are highly stable in organic solvents and can be used for transformations that would be difficult or impossible in water, but retaining the advantages of enzymatic reaction: selectivity (enantio, regio,...) and mild conditions.[8] Macrolactones have been synthesized by intramolecular enzyme-catalyzed transesterification in nearly anhydrous organic solvents[9] and, in the crowns field, we have recently published[10] the first enzymatic synthesis of podands, acyclic crown-like cation-complexing compounds.[11] However, to our knowledge, there are no reports about any enzyme-catalyzed synthesis of polyether macrolactones, ester crowns, yet.

We report here for the first time a direct synthesis of ester crowns through regioselective enzyme-catalyzed transesterification using polyethylene glycols as nucleophiles. The selected starting substrate was 1,3-bis(3,5-diethoxycarbonyl-1*H*-pyrazol-1-yl)propane **1**, which was prepared following a standard method: K_2CO_3 was suspended in a solution of 1,3-dibromopropane and 1*H*-3,5-diethoxycarbonylpyrazol[12] in acetone and refluxed for 8 hours. The suspended solid was filtered off and washed with acetone. The joined organic phases were evaporated and the residue recrystallised (m.p: 70°C, hexane, 64% yield).

Our target was to modify the ester groups situated in the 3 positions of the ring, contiguous to the sp^2 nitrogen atoms, while those on the 5 positions remained unchanged for future transformations. For this reaction

we have checked the enzymatic transformations as a regioselective synthetic method. A preparation of immobilized *Mucor miehei* lipase MML (Lipozyme from Novo Nordisk) was the enzyme selected because we recently demonstrated that it catalyzed the transesterification of 3,5-diethoxycarbonylpyrazole derivatives[10] and other aromatic and heteroaromatic esters[13] in a regioselective manner. Di- (**2a**, n=1), tri- (**2b**, n=2) and tetraethylene glycol (**2c**, n=3) were the nucleophiles employed; the three commercial glycols were distilled and stored on molecular sieves before using them.

The whole reaction consisted of two enzyme-catalyzed steps (Scheme 1): tetraethylester **1** was regioselectively transesterified to the acyclic intermediates **3a-c** that underwent an intramolecular second transesterification to the macrolactones **4a-c**. The experimental conditions, low concentration and 1:1 ratio nucleophile:acyl donor (that means two hydroxy for four ester groups), were projected to favour the formation of the monomer cycles **4a-c** over other possible products without caring about yields.



Scheme 1

The general procedure followed was: MML (2.5 g) was added to a solution of **1** (1.16 g, 2.5 mmol) and the corresponding glycol **2a-c** (2.5 mmol) in dry toluene (100 ml). The suspension was stirred in a rotary evaporator without vacuum while heated at 60°C in a silicone bath for 12 days. The solution was daily evaporated to about a half of the original volume and refilled with fresh dry toluene.[14] Afterwards, the enzyme was filtered off and washed with chloroform,[16] the clear solution of the combined filtrate and wash was evaporated and the residue was dissolved in chloroform (100 ml). This solution was washed with distilled water (3 x 100 ml), dried (Na_2SO_4) and evaporated to dryness. The resulting mixture was separated by chromatography on a silica gel column (hexane: chloroform: acetone 10:8:1 changing to chloroform: acetone 10:1), affording intermediates **3a-c** as syrups and cycles **4a-c** as solid products (see Table). The reaction took place regioselectively and no 5-substituted derivatives were detected by chromatographic and spectroscopic techniques. No conversion was detected in parallel blank reactions without enzyme.

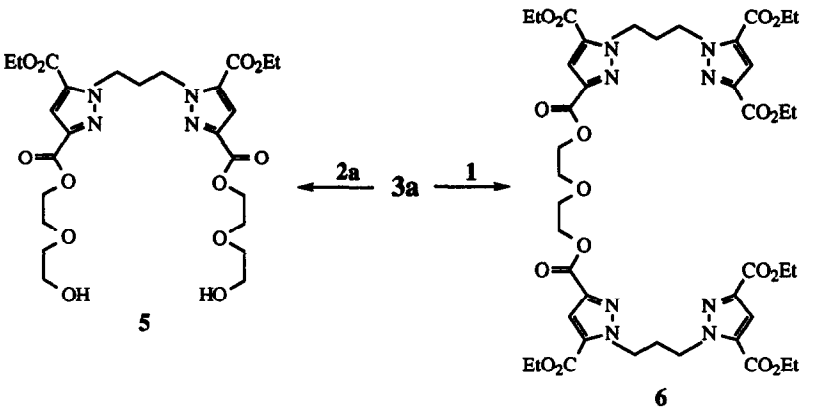
The structural assignment of the new compounds were made by ^1H NMR, since it is known that the 3-alkoxy groups in N-alkylated pyrazoles are always more deshielded than their counterparts of the 5 position.[17] The signals belonging to the 3-ethyl groups ($\delta \approx 4.40$ and 1.39 ppm, in **1**) had disappeared from the ^1H NMR spectra of the macrocycles **4a-c**, while those from the 5 pyrazolic positions ($\delta \approx 4.32$ and 1.35 ppm, in **1** and **4a-c**) remained unchanged. In addition, two ethyl groups attached to the 5 pyrazolic positions ($\delta \approx 4.30$ and 1.33 ppm) and one ethyl group on the 3 position ($\delta \approx 4.38$ and 1.38 ppm) can be observed in the spectra of the acyclic intermediates **3a-c**. The rest of their spectroscopic (^{13}C NMR and Mass) and microanalytical data are also in accordance with the proposed structures.

Table[a] — Transesterification of **1** with Polyethylene Glycols **2a-c**.
Percentage of isolated products

Polyethylene glycol	Recovered **1**	Acyclic **3a-c**	Cyclic **4a-c**
2a	57	12	9
2b	14	3	5
2c	42	7	7

[a] Mass, ^{1}H NMR and ^{13}C NMR spectroscopic data and microanalysis were consistent with the proposed structures of the new compounds **1**, **3a-c** and **4a-c**.

Two expected byproducts, **5** and **6**, were isolated in little amounts, less than 1%, in the reaction with diethylene glycol **2a**. They both were formed from the intermediate **3a** (Scheme 2): **5** when the 3"-ethoxycarbonyl group of **3a** was attacked by a second molecule of the **2a**, and **6** when the hydroxy group of **3a** acted as nucleophile in an inter- instead of intramolecular substitution of **1**. Corresponding derivatives were not detected in the reactions with **2b** and **2c**. The compounds **5** and **6** were also identified by their Mass, ^{1}H NMR and ^{13}C NMR spectroscopic and microanalytical data.



5 6

Scheme 2

In conclusion, here we have described the preliminary results of the first enzymatic synthesis of ester crowns and opened a new route to obtain these macrocyclic compounds. Work is now in progress in order to study the complexing and other biological properties of the new structures. More work will be devoted to improve the initially modest yields if the biological results are promising enough.

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References and Notes

1. Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89-112.
2. Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J.; Sen, S. *Chem. Rev.* **1985**, *85*, 271-339.
3. Elguero, J.; Navarro, P.; Rodríguez-Franco, M. I. *Chem. Lett.* **1984**, 425-428.
4. Navarro, P.; Rodríguez-Franco, M. I. *J.Chem. Soc., Chem. Commun.* **1988**, 1365-1367.
5. Navarro, P.; Rodríguez-Franco, M. I.; Foces-Foces, C.; Cano, F.; Samat, A. *J. Org. Chem.*, **1989**, *54*, 1391-1398.
6. Acerete, C.; Bueno, J. M.; Campayo, L.; Navarro, P.; Rodríguez-Franco, M. I.; Samat, A. *Tetrahedron*, **1994**, *50*, 4765-4774.
7. Lehn, J. M.; Vierling, P. *Tetrahedron Lett.* **1980**, *21*, 1323-1326.
8. Santaniello, E.; Ferraboschi, P.; Grisenti, P. *Enzyme Microb. Technol.*, **1993**, *15*, 367-382.
9. a) Makita, A.; Nihira, T.; Yamada, Y. *Tetrahedron Lett.*, **1987**, *28*, 805-808. b) Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.*, **1988**, *110*, 1999-2001. c) Yamada, H.; Sugai, T.; Ohta, H.; Yoshikawa, S. *Agric. Biol. Chem.*, **1990**, *54*, 1579-1580.
10. Fierros, M.; Rodríguez-Franco, M. I.; Navarro, P.; Conde, S. *Heterocycles*, **1993**, *36*, 2019-2034.
11. Vögtle, F. "Supramolecular Chemistry", ed. by F. Vögtle, Wiley, Chischester, 1991.
12. Iturrino, L.; Navarro, P.; Rodríguez-Franco, M. I.; Contreras, M.; Escario, J. A.; Martínez, A.; Pardo, R.M. *Eur. J. Med. Chem.*, **1987**, *22*, 445-451.
13. Martín-Muñoz, M. G.; Fierros, M.; Rodríguez-Franco, M. I.; Conde, S. *Tetrahedron.*, **1993**, *50*, 6999-7008.
14. The methodology of removing water or ethanol by azeotropic evaporation has recently been applied by us[10] and other group.[15]
15. Lin, G.; Liu, S. H. *Org. Prep. Proc. Int.* **1993**, *25*, 463-466.
16. The products are poorly soluble in toluene.
17. Elguero, J. in "Comprehensive Heterocyclic Chemistry", Vol.5, ed. by K. T. Potts; Pergamon Press, Oxford, 1984, pp. 167-303.

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