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SYNTHESIS AND IMMOBILIZATION OF NEW CROWN ETHERS DERIVED FROM D-MANNITOL FOR THE RESOLUTION OF FREE AMINO ACIDS

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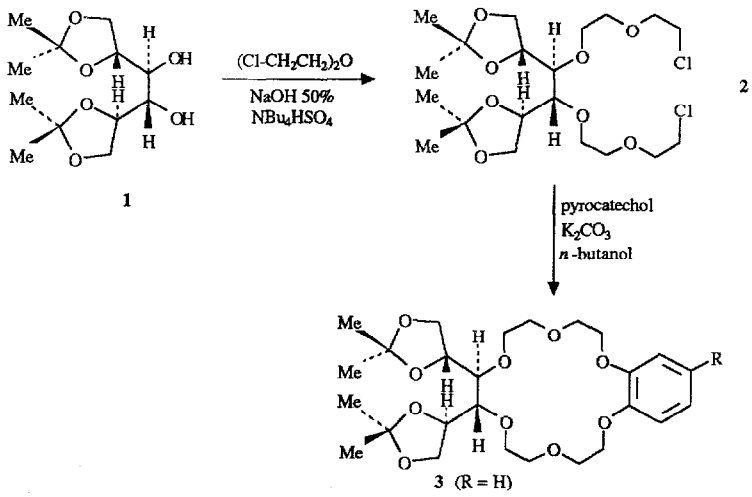
Summary: *A new crown ether of the 18-6 type, 1,2:5,6-di-O-isopropylidene-3,4-O-{1,2-bis[ethoxyethoxy]-benzenediyl}-D-mannitol, immobilized by dynamic coating on a C_{18}-silica, provides efficient chromatographic baseline separation of racemic phenylglycine into its enantiomers and partial separation for free norvaline, methionine, ethionine, leucine, phenylalanine. The 4-methyl and 4-nitro aromatic substituted derivatives of this host allow complete resolution of p-nitro-phenylalanine and tryptophan respectively.*

The separation of racemic amino acids into their enantiomers is usually achieved by ligand-exchange chromatography based upon the formation of diastereomeric species with cupric ion.[1] Cram and his co-workers first introduced in 1973 the use of optically active crown ethers bearing binaphthyl units.[2] By anchoring some of these compounds covalently to various supports, they were able to resolve many amino acids as their methylester salts.[3] Around the same time, Stoddart and his co-workers reported their work on new crown ethers synthesized from natural compounds such as tartaric acid or mannitol, which provide relatively inexpensive chiral crown ether frameworks.[4]

More recently, Shono and his co-workers have extended the *in situ* or dynamic coating method developed by Cassidy and Elchuk[5] to the immobilization of highly lipophilic crown ethers on an insoluble support with hydrophobic surface.[6] They succeeded in the separation of alkali metal iodides (Li^+, Na^+, Cs^+, Rb^+ and K^+) with a mixture of methanol and water as eluent. In 1987, Shinbo and his co-workers applied this procedure to the preparation of chiral crown ether packings for efficient chromatographic separation of many racemic underivatized amino acids when a dilute aqueous solution of perchloric acid was used as eluent at 2°C.[7] However, the synthesis of such chiral crown ethers as pure enantiomers always requires tedious optical resolution by recrystallization of cinchonine salts with low overall yields[8] or by liquid chromatography on chiral synthetic supports[9].

We now report the synthesis of three new chiral macrocyclic ethers derived from D-mannitol, a straightforward source of chirality, and their ability to resolve some free racemic amino acids under the conditions of HPLC without any acid in the eluent.

In a typical experiment, 2.62g (10 mM) of 1,2:5,6-Di-*O*-isopropylidene-D-mannitol **1** reacted in a two-phase system with 50 mL of bis-(2-chloroethyl)-ether (as solvent and reagent), 50 mL of 50% aq. NaOH and 6.80 g of NBu_4HSO_4 (2 eq.) during 8 h at room temperature according to literature[10] to give 3.57 g (75%) of **2** which was then cyclized with 1.05 eq. of pyrocatechol in presence of 2 eq. of dry potassium carbonate (boiling *n*-butanol, 6 h) to afford 2.69 g (70%) of **3** as an oil, $[\alpha]_D = +11°$ (c = 1.25, $CHCl_3$).



In similar conditions, 0.95 g of **2** was cyclized with 1.10 eq. of 4-methyl-catechol to give 0.42 g (40%) of **4** (R = Me) as an oil, $[\alpha]_D = +10°$ (c = 1.28, $CHCl_3$).

Compound **5** (R = NO_2) was obtained by controlled nitration of **3** (0.513 g in 40 mL of acetic anhydride) by 1 eq. of HNO_3 at 0°C for 4 h (0.097 g of 65% nitric acid in 15 mL acetic anhydride) as a brown gum in 69% yield, IR (neat) NO_2, ν_{AS}: 1520 cm^{-1}, ν_S: 1340 cm^{-1}.

A spectroscopic method was first used to estimate the chiral recognition of **3** towards the enantiomers of phenylglycine as methyl ester salts and to identify the more stable complex. For these determinations, 0.05 mM of **3** (25.6 mg) was dissolved in 1.0 mL of $CDCl_3$ and shaken for 1 mn at room temperature with 2.0 mL of D_2O containing 0.15 mM of racemic phenylglycine as methyl ester hydrochloride salt (or 0.075 mM of the R-isomer) and 8.0 mM of $LiPF_6$. The ^1H n.m.r. spectrum of the organic layer (400 MHz) gave the molar ratio of R and S directly by integration of the corresponding singlets of the protons on the stereogenic center on expanded spectra (10 Hz/cm) at 4.78 ppm (R) and at 4.84 ppm (S). The enantiomeric distribution of 55/45 was in favour of the D-isomer and the molar ratio of guest to host was 1.3/1.

No attempt was made to enhance the lipophilicity of the crown ether **3** by introducing an alkyl chain at C-1 or C-6, since it was feared that the cleavage of the isopropylidene groups could lead to a less potent host.[11] Immobilization of the crown ether on the packing was achieved by flowing 60 mL of a MeOH/H_2O solution (67/33) containing 140 mg of **3** through a commercial octadecylsilanized silica (LiChrospher 100 RP-18, $d_p = 5\ \mu m$) prepacked column (250 x 4 mm, Merck 50838). The quantity of **3** immobilized on the

packing (*ca.* 100 mg) was estimated by difference between the initial amount and the evaporated eluted methanolic solution. To achieve an effective coating, the ratio of methanol was decreased stepwise from 67 to 10%.

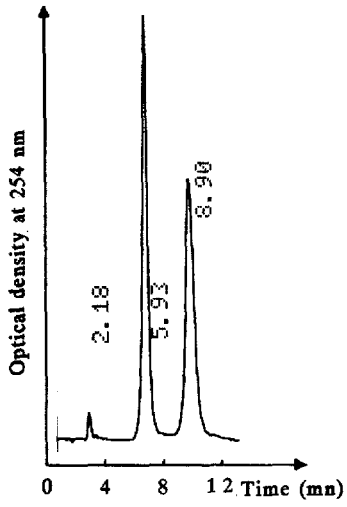
First chromatographic runs with a dilute aqueous solution of perchloric acid [10^{-3} M] as the mobile phase at room temperature showed no separation for racemic phenylglycine methyl ester hydrochloride (capacity factor, k = 17). A thorough study of the chromatogram revealed two tiny partially separed peaks at 8.8 mn which were assigned to the minor non-esterified part of DL-phenylglycine by comparison of their retention times with those of an authentic sample of free amino acid. The separation coefficient, α ($\simeq k_d/k_l$) was estimated to be about 1.1, which is too small a value for a baseline separation. Duplication of the run with pure water as eluent at room temperature increased the α value to 1.45 and allowed the complete separation of underivatized phenylglycine as its hydrochloride salt. Further investigations with various counterions (ClO_4^-, $MeCO_2^-$, PF_6^-) and without any acid in the sample, led to unchanged retention times and separation coefficients suggesting that the amino acid exists always mainly as a free amino acid in the host. S-phenylglycine was first eluted. This result accords with that of the one-plate extraction run with the corresponding methyl ester hexafluorophosphate and could indicate that the enantiomer forming the more stable complexe with this chiral crown ether was eluted after the one forming the less stable complexe. A close examination of the C.P.K. models of the two diastereoisomeric species, 3 + R-phenylglycine and 3 + S-phenylglycine, provides some evidence for a higher three-point interaction probability between the R-enantiomer and the crown ether: considering the $-NH_3^+$ group anchored in the center and on the top of the ring, the phenyl group of the amino acid oriented over the catechol, the formation of a hydrogen bond between the hydrogen atom of the carboxylic acid function and one of the oxygen atoms of the acetal seems to be easier with the R-enantiomer.

Lowering the column temperature led to an increase of the α value from 1.45 (at 20°C) to a maximum of 1.60 (at 2°C) and this temperature was kept constant for all the others runs (see Fig. 1).

Lowering the flow-rate from 1.0 to 0.5 mL/mn had no significative incidence either on the α value or on the resolution factor. A very slight separation could be detected when DL-phenylalanine was substitued for phenylglycine with an α close to 1.03. This poor result compared to the previous one can be attributed to steric restrictions on the existence of a similar three-point model in which the phenyl group is separated from the chiral center by a single carbon. An almost baseline separation was obtained for racemic ethionine with an α of 1.15 and a beginning separation for racemic methionine with an α of 1.11. The lowest values were calculated for DL-norvaline (α = 1.09) and for DL-leucine (α = 1.05) for which only partial separations were observed. No separation could be detected with racemic aspartic acid, asparagine, valine, cystine, lysine, threonine, tyrosine, proline and penicillamine eluted under the same conditions.

In a same manner, but with lower loadings on a 150 mm column, host **4** was able to resolve *p*-nitro-phenylalanine (α = 1.60) and host **5** tryptophane (α = 1.55) with a mixture of acetonitrile/water (10/90) as the eluent. This results confirm the placement of the phenyl group (or indole ring) of the guest in a plane roughly parallel to that of the host, allowing π-stacking interactions to stabilize one of the diastereomeric adsorbates.

We are now checking the possibilities of replacement of the isopropylidene group by the more bulky cyclohexylidene one in order to enhance the helicity and the lipophilicity of these hosts and to improve their chiral recognition towards the largest number of amino acids.



PEAK#	AREA%	RT
1	1.414	2.18
2	49.488	5.93
3	49.098	8.9
TOTAL	100.	

Figure 1: Chromatographic separation of racemic phenylglycine as free amino acid into its enantiomers on a reversed-phase packing coated with 3. Mobile phase: water at 2°C, 1 mL/mn. Elution sequence: 2nd peak: S-phenylglycine, 3rd peak: R-phenylglycine, RT (retention times) in mn.

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