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Synthesis and Chiral Recognition Properties of L-Ala-Crown(3)-L-Ala Capped β-Cyclodextrin

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Abstract

The synthesis and full spectroscopic characterization (IR, 1D and 2D TOCSY and ROESY NMR, MS) of a novel capped β-cyclodextrin bearing a $6^A,6^D$-dideoxy-N,N'-3,6,9-trioxa-undecanoyl-(L,L)-bis-alanyl bridge are described. The chiral recognition properties of the product towards Dns-amino acids have been examined by capillary electrophoresis. © 1999 Elsevier Science Ltd. All rights reserved.

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Owing to their ability to distinguish between size and shape and to their intrinsic chirality, cyclodextrins (CDs) have been used as efficient molecular and chiral selectors in analytical applications,[1] especially in GC,[2] HPLC[3] and CE[4] analyses. Selective functionalization of CDs with different groups generally improved the recognition properties by increasing the asymmetry and allowing for more specific interactions between the host and the guest such as hydrogen bonding, electrostatic interactions and metal coordination.[5]

Recently, cyclodextrins capped with chiral moieties have been proposed as enzyme models (three-dimensional CDs). In particular, a β-CD capped with the cyclic dipeptide L-hystidyl-L-hystidyl was synthesized and its complexing ability towards the copper(II) ion examined.[6] Furthermore, a trehalose capped β-CD has been synthesized as the first example of an extended cavity with a sugar moiety.[7] Whether or not the capping of CDs with chiral or achiral bridges will enhance the enantioselectivity has not been studied to our knowledge.

Here, we report the synthesis and the enantiomeric recognition ability of the new capped $6^A,6^D$-dideoxy-N,N'-3,6,9-trioxa-undecanoyl-(L,L)-bis-alanyl-β-CD: L-Ala-Crown(3)-L-Ala-β-CD (**1**) (Fig. 1). Our aim was that of obtaining a water soluble selector to be used as an additive to the buffer in capillary electrophoresis. The bridge had already been used by us as a chiral selector in GC for the enantiomeric discrimination of alkyl esters of TFA-amino acids.[8].

Recently, we have performed very good enantiomeric separation of Dns-amino acids by CE with two positively charged histamine-functionalized β-CDs.[9] A model was devised to account for the chiral separation in which the inclusion mode of the Dns-moiety was opposed to that generally assumed for an unmodified β-CD. In order to verify the inclusion mode of the dansyl-amino acids in the case in which only one side of the CD cavity is accessible to the

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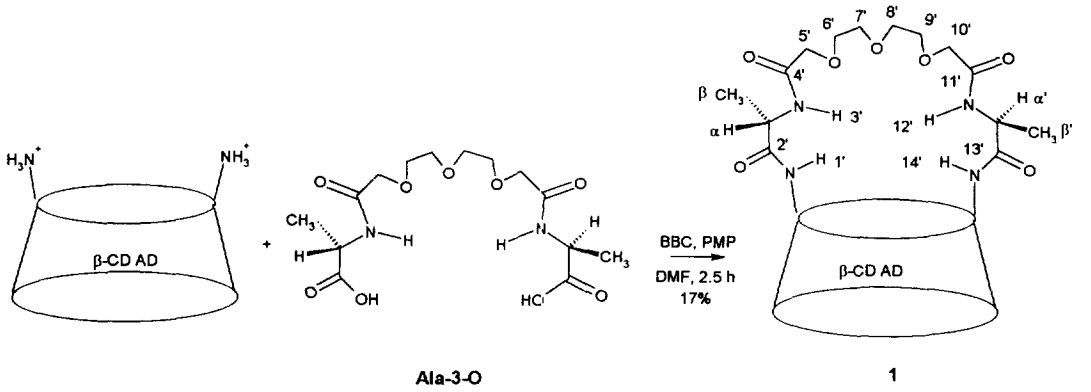
present capped neutral CD with the data previously observed with unmodified or modified CDs.The synthesis of **1** was carried out in a convergent way by reacting *N,N'*-3,6,9-trioxaundecanoyl-(L,L)-bis-alanine (Ala-3-O) [8] with the $6^A,6^D$-dideoxy-$6^A,6^D$-diamino-β-CD[10] in the presence of a coupling reagent (benzotriazolyloxy-bis(pyrrolidino)-carbonium hexafluorophosphate, BBC)[11] and a sterically hindered base (pentamethylpiperidine, PMP) (Fig.1).



Ala-3-O 1

Fig. 1. Synthesis, general formula, and numbering of **1**.

High dilution conditions were required to avoid polymer formation. Diamino-β-CD (361 mg, 0.3 mmol) and Ala-3-O (101 mg, 0.3 mmol) were dissolved in DMF (160 mL) in the presence of PMP (0.109 mL, 0.6 mmol) and added dropwise over 2h to a stirred solution of the condensing reagent (BBC) (250 mg, 0.6 mmol). After 30 min, the solvent was distilled under vacuum at 40°C. The residue was dissolved in DMF (2 mL) and precipitated with acetone (600 mL). The fluffy white solid obtained (440 mg) was shown to be a mixture of three products by TLC (reversed phase C_{18} silica gel plates, eluent MeOH/H_2O 1:1). The solid was dissolved in H_2O/MeOH 9:1 and separated on a reversed phase column (Lichroprep RP 8, height 25 cm, diameter 4.5 cm, applied pressure 1.75 atm). Step gradient elution was applied from H_2O/MeOH 9:1 to pure MeOH. The desired product was collected during the elution of the fraction H_2O/MeOH 75:25, the solvent was distilled under vacuum, the solid was dissolved in DMF (0.5 mL) and reprecipitated by addition of acetone (200 mL). Filtration on sintered glass (G5) and lyophilization afforded 75 mg of the pure product (17% yield).

The structure was confirmed by ESI-MS (m/z at 1461.9 (MH^+)) and by ^{1}H-NMR spectroscopy. The right integration ratio was found between the signals relative to the amidic protons (4H, 7.95 and 7.77 ppm), the secondary hydroxyls (14H, 5.82 and 5.65 ppm), the anomeric protons (7H, 4.81 and 4.87 ppm), and the methyl protons (6H, 1.23 and 1.25 ppm); the signals of the primary hydroxyl protons (5H) and of the CH_α of the alanyl moiety (2H) were present, although overlapping in the 4.48-4.28 ppm region (Fig. 2).

The molecular structure was definitely confirmed by bidimensional COSY and ROESY NMR spectra. The more significant correlations in the COSY and ROESY spectra are reported in Table 1.

By the COSY spectrum it was not possible to assign all the signals, owing to the high number of cross peaks near the diagonal.The correlations between the signals at 3.02 and 7.95 ppm and between those at 3.02 and 4.43 and 4.39, respectively, are due to long range couplings.

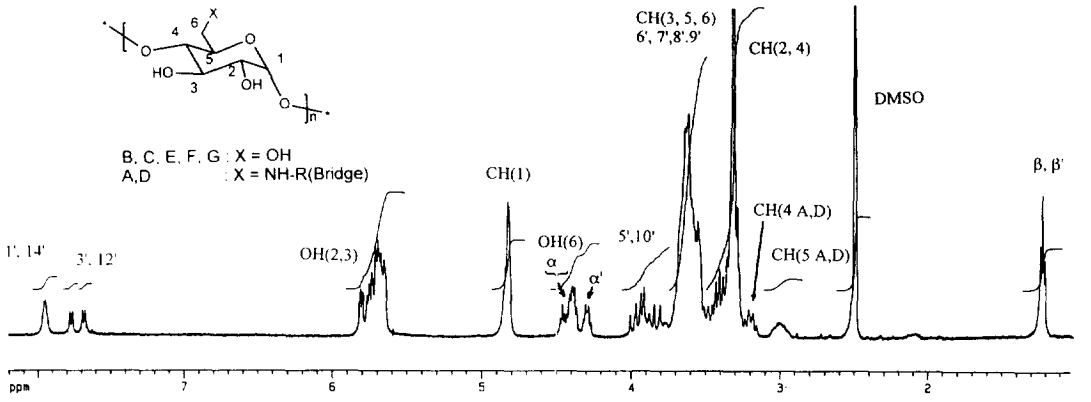


Fig. 2. ^{1}H-NMR of **1** in d6-dmso, 400 MHz.

Table 1.
COSY and ROESY cross-peaks for L-Ala-Crown(3)-L-Ala-β-CD (400 MHz, d6-dmso, 300 K).

COSY				ROESY		
ppm	ppm	assignment	J (Hz)	ppm	ppm	assignment
3.79	7.95	CH(6)--NH(1')	10.6	7.95	4.39	NH(1')--CH_α
3.91	7.94	CH(6)--NH(14')	10.6	7.94	4.43	NH(14')--$CH_{\alpha'}$
4.39	7.77	CH_α--NH(3')	7.1	7.77	3.99	NH(3')--H(5')
4.43	7.68	$CH_{\alpha'}$--NH(12')	7.6	7.77	3.83	NH(3')--H(5')
4.39	1.25	CH_α--$CH_{3\beta}$	7.4	7.68	3.97	NH(12')--H(10')
4.43	1.23	$CH_{\alpha'}$--$CH_{3\beta'}$	7.4	7.68	3.90	NH(12')--H(10')
3.02	7.95	CH(5)--NH(1',14')		7.77	4.39	NH(3')--CH_α
3.02	4.43	CH(5)--CH_α		7.68	4.43	NH(12')--$CH_{\alpha'}$
3.02	4.39	CH(5)--$CH_{\alpha'}$		7.95	1.25	NH(1')--$CH_{3\beta}$
				7.94	1.23	NH(14')--$CH_{3\beta'}$
				7.77	1.25	NH(3')--$CH_{3\beta}$
				7.68	1.23	NH(12')--$CH_{3\beta'}$

The ROESY spectrum allowed the correlation of the upper part of the bridge with the corresponding linking sites on the cyclodextrin. The amidic protons 1' and 14' gave an Overhauser effect with the CH_α of the alanine moieties: two signals are present in the spectrum, partially superimposed, assignable to the two parts of the bridge. Moreover, the amidic protons 3' and 12' appear to be placed near the CH_2 5' and 10' of the bridge, respectively. Both types of amidic protons gave correlation signals with CH_α, $CH_{\alpha'}$ $CH_{3\beta}$ and $CH_{3\beta'}$ of the alanyl moieties.

On the basis of these data it was not possible to assign the exact position to each part of the bridge (A or D). In any case, the bridge seems to be flattened upon the upper part of the CD, occluding the access to the cavity.

In order to verify the recognition properties of the new selector in capillary electrophoresis the enantiomeric separation of D- and L-dansyl-glutamic and D- and L-dansyl-aspartic acid was performed by adding **1** to the electrophoretic buffer in a constant voltage mode (5 kV) with a coated capillary (no electro-osmotic flow) and reverse polarity (anode to the detector). Under the conditions used (pH = 6), the analytes are negatively charged and migrate towards the anode. We selected Dns-Asp and Dns-Glu as analytes since the dansyl moiety fits well into the β-CD cavity and since, bearing a double negative charge, they should migrate to the

complexation equilibria in solution, and the complex should migrate more slowly. If the process is enantioselective, the enantiomer with the higher affinity for the selector is retained more, showing a higher migration time.

Indeed, D,L-Dns-Asp and D,L-Dns-Glu were very well separated with a migration order L<D: thus, the D-enantiomer is preferentially complexed by **1**, forming the more stable diastereomeric complex.

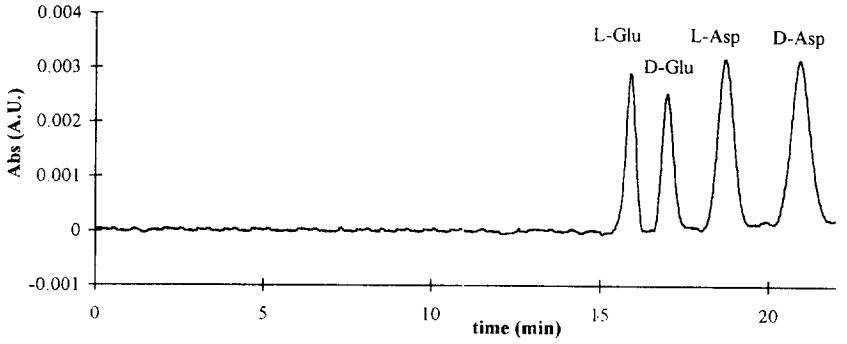


Fig. 3. Enantiomeric separation of Dns-Glu and Dns-Asp in CE. Conditions: buffer, 100 mM phosphate containing 1 mM **1**, pH = 6.0; coated capillary (27 cm x 75 μm I.D.); temperature 25°C. applied voltage 5 kV; detector UV (λ = 200 nm).

Under the same conditions, no separation was obtained with unmodified β-CD, which must be used in higher concentration (5 mM) in the presence of an organic modifier.[12] However, the same migration order was observed, thus confiming that complexation takes place via the same inclusion mode as with β-CD.

These data are consistent with a recognition model in which the dansyl moiety interacts with the cavity and the amino acid carboxylates protrude from the lower rim. The role of the bridge seems to be that of modifying the dimensions and the conformation of the CD, preventing access from the upper side of the cavity, thus increasing the enantioselectivity.

Spectroscopic studies are being carried out in order to determine the nature of the discriminating interactions.

Aknowledgements

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