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Synthesis of a New Molecular Carrier: N-(Leu-enkephalin)yl 6-amido-6-deoxy-Cyclomaltoheptaose.

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Abstract: The synthesis of N-(Leu-enkephalin)yl 6-amido-6-deoxy-cyclomaltoheptaose has been preformed in high yield. The final derivative has been characterized by proton NMR in terms of chemical structure and inclusion properties and represents a new class of target-directed transporters.

The capacity of cyclomaltooligosaccharides (cyclodextrins) to include a variety of guest molecules into their hydrophobic cavity has allowed the solubilization and the transport of hydrophobic drugs[1]. This vectorization is, however, not specific and the drug is not targeted towards a specific site.

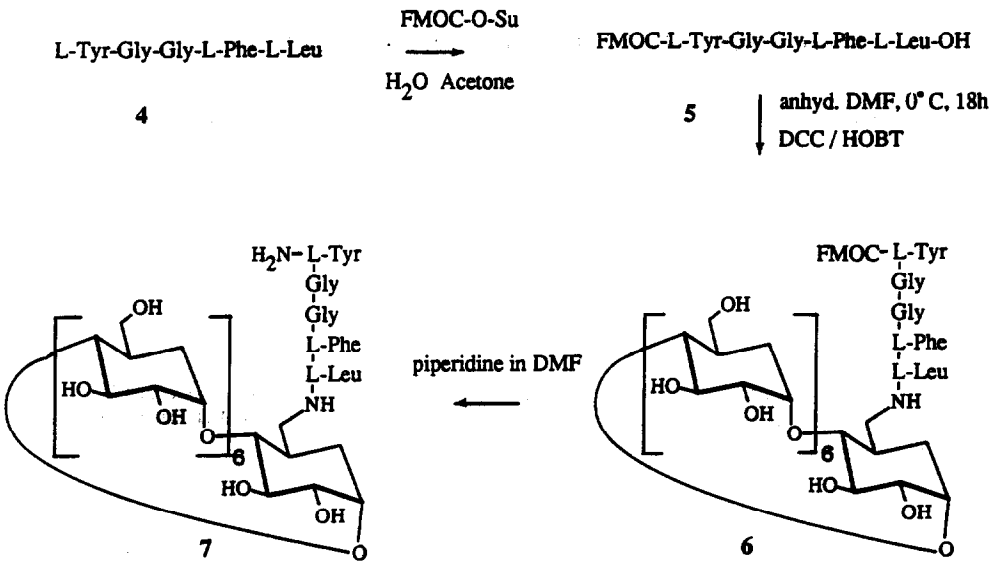
Grafting bio-active peptides onto cyclodextrins may provide new vectors carrying signal molecules for targetting purposes. The chemical substitution should, however, neither preclude the recognition of the signal by the receptor nor the inclusion of the drug. For this reason only unmodified β-cyclodextrin has to be used since the hydroxyl groups may play a key-role in the stabilization of the inclusion complexes [2].

We describe here the synthesis of N-(Leu-enkephalin)yl 6-amido-6-deoxy-cyclomaltoheptaose by grafting Leu-enkephalin, a neuropeptide having opiate properties[3], onto 6-amino-6-deoxy-cyclomaltoheptaose and its NMR characterization. Coupling Leu-enkephalin onto 6-amino-6-deoxy-per(2,3,6-tri-*O*-methyl)-cyclomaltoheptaose has been already described [4] but the poor overall yield and the permethylation of the hydroxyl groups of the cyclodextrin preclude the use of this compound as molecular carrier.

6-Amino–6-deoxy-cyclomaltoheptaose was obtained from 6-*O*-p-tolylsulfonyl cyclomaltoheptaose **1** [5]. This compound was converted into the 6-azido-6-deoxy derivative **2** by action of lithium azide in water in good yield (83%). Compound **2** was reduced smoothly by treatement with triphenylphosphine[6] in anhydrous dimethylformamide and then addition of concentrated ammonium hydroxide. Recrystallization from water afforded the 6-amino-6-deoxy-cyclomaltoheptaose **3** in 99% yield. However, the NMR characterization of this compound has shown the presence of free β-cyclodextrin (up to 15%) which could be removed by ion-exchange chromatography[7] (elution with 6% aqueous NH_4OH). After this purification step, **3** was obtained in 90% yield.

The terminal amino group of Leu-enkephalin was protected by the FMOC group (fluorenemethyloxycarbonyl) using the succinimide active ester (FMOC-O-Su) and affords **5** in 95% yield[8].

Reaction of **5** with 6-amino-6-deoxy-cyclomaltoheptaose **2** using the dicyclohexylcarbodiimide / hydroxybenzotriazole procedure[9] in anhydrous dimethylformamide at 0°C resulted in the formation of **6** in 93% yield after purification by ion-exchange chromatography as described for **3**. Quantitative removal of the FMOC protecting group was achieved by treatment with 50% piperidine in DMF. Purification by ion-exchange chromatography (eluant: H_2O:NH_4OH 6%) afforded **7** in 95% yield



Scheme 1

The chemical and optical integrity and purity of the final and intermediate compounds were checked by thin-layer chromatography, proton NMR, elemental analysis and FAB mass spectroscopy[10].

The NMR analysis performed in DMSO-d_6 demonstrated that the purified final compound **7** was free of any included by-products or reagents[14]. The monosubstitution of the cyclodextrin derivative has been shown by digital integration of NMR signals arising from peptide and cyclodextrin moieties.

The identification of all amino-acid spin systems and assignment of proton Hα of tyrosine which is not coupled to amide proton, were derived from a phase-sensitive double quantum filtered COSY experiment[11]. A phase sensitive NOESY experiment has then allowed sequential assignement of all protons of the peptide backbone[12].

The 500 MHz ^{1}H NMR spectrum of N-(L-Leu-enkephalin)yl 6-amido-6-deoxy-cyclomaltoheptaose **7** has been obtained in deuterium oxide and revealed several interesting features. Monosubstitution results in a C_1 symmetry in which every proton becomes inequivalent.

However we have shown that synthetic derivatives obtained by grafting specific amino-acids onto 6-amino-6-deoxy-cyclomaltoheptaose may give intramolecular self-inclusion complexes[13] in aqueous solution. One of

their behavior is to exhibit a very large spectral dispersion of the proton NMR signals in deuterium oxide. Preliminary comparaison of these spectra with that of the title compound in D_2O suggests that the cyclodextrin cavity of 7 is vacant and can be occupied by a guest. In view of the above, 7 should retain the inclusion properties of β-cyclodextrin. To support this assumption, we have investigated the interaction with neurotropic molecules. Preliminary NMR studies have shown that 7 formed inclusion complexes with these molecules and that these inclusion complexes seemed to be similar to those obtained with β-cyclodextrin in terms of stoichiometry and affinity constant.

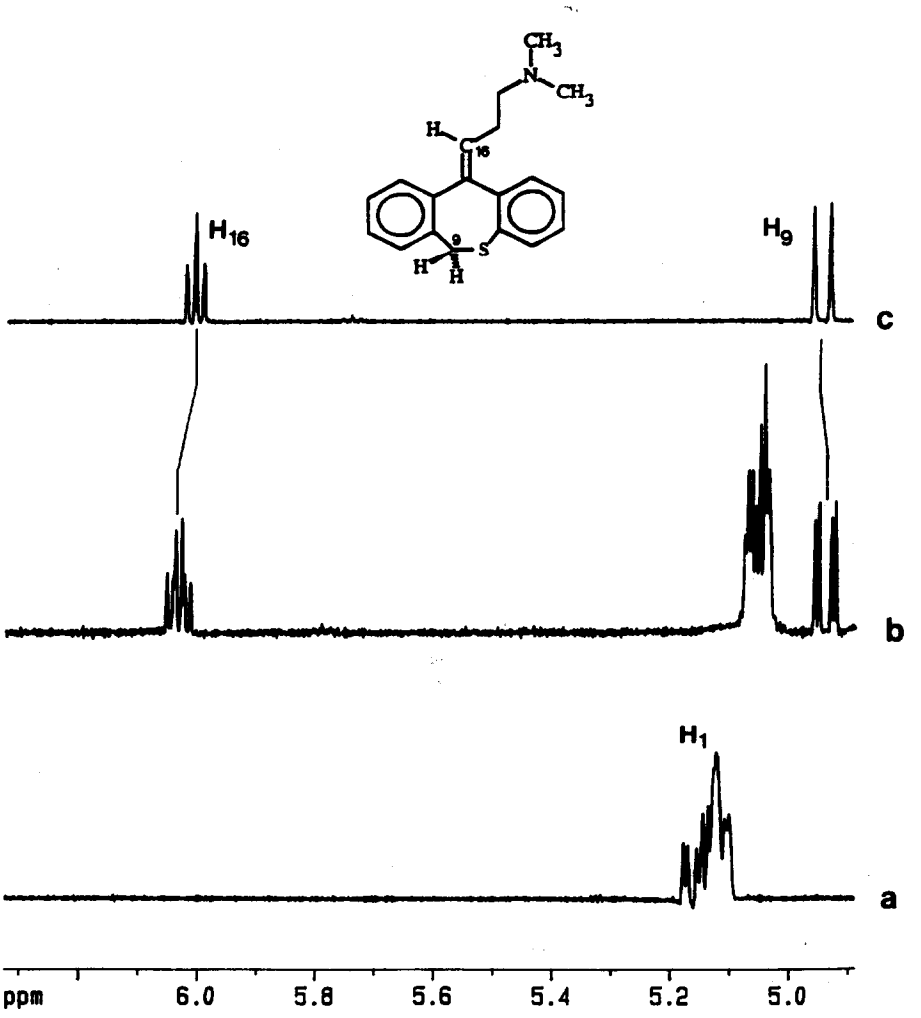


Figure 1

Figures 1a,b show a partial 500 MHz NMR spectrum of **7** in D_2O in the absence (a) and in the presence of the nootropic drug dothiepine (b). The corresponding spectrum of the free racemic drug is displayed in figure 1c. The modification of the pattern of the H1 anomeric protons of the host as well as the shifts of the signals from the intracyclic H9 and H16 protons of the guest are typical of the formation of an inclusion complex[15]. The duplication of the latter signals originates from the chiral separation associated with the inclusion process.

Determination of the 3-dimensional conformation in solution using dedicated NMR experiments is in progress to afford complete sequential assignment of protons from the cyclodextrin moiety. On the other hand, comparaison of the structure of the peptide part of **7** with the structure of free enkephalins in terms of affinity for opiate receptors has also been performed. The NMR studies performed in aqueous solution have shown that free and grafted Leu-enkephalins exhibit similar conformations in solution. These results suggest that the affinity of both neuro peptides should be similar. These complete NMR studies will be reported elsewhere.

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10. T.L.C.: Silica gel plates Merck F_{254}, detection by UV, ninhydrin spray or charring with 10% H_2SO_4. **2** : TLC: (n-butanol-DMF-H_2O 2:1:1) R_f = 0.8; I.R.: ν= 2100 cm^{-1} (azide function); **3** : TLC: (n-butanol-DMF-H_2O 2:1:1) R_f = 0.2; Anal. Cal. for $C_{42}H_{72}O_{39}NCl$: C 39.7, H 6.5, N 1.3, O 48.7; found : C 40.1, H 6.4, N 1.2, O 48.9. mp_{dec}: 242°C; **5** : mp : 152°C, **6** TLC: (n-butanol-acetic acid 60% 6:4) R_f = 0.45; Anal. Cal. for $C_{85}H_{124}O_{46}N_6$: C 51.8, H 6.3, O 33.4, N 4.0; found C 50.3, H 6.4, O 33.0, N 3.9. mp_{dec}: 248°C; **7** TLC: (n-butanol/DMF/H_2O 2:1:1) R_f = 0.7; FAB-MS: 1711 $[M+K]^+$, calc 1711, mp_{dec}: 248°C.
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