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Note

Preparation and complexation ability of zwitterionic derivatives of cyclodextrins

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Abstract

Zwitterionic derivatives of β-cyclodextrin were obtained by acylation of 6^1-amino-6^1-deoxycyclomaltoheptaose with commercially available partially protected derivatives of aspartic and glutamic acids followed by removal of the protective groups. These products exhibited high solubility in water and were used to form soluble inclusion complexes of 5-fluorouracil. © 1997 Elsevier Science Ltd.

Keywords: Amino acid; Cyclodextrins; 5-Fluorouracil; Inclusion complexes

Grafting of amino acid derivatives on cyclodextrins has been achieved frequently [1–3]. Peptides have also been introduced on cyclodextrins [4]. In most cases these compounds are obtained by acylation of 6^1-amino-6^1-deoxycyclomaltoheptaose **1**. A thioethylamide linkage has also been used for the introduction of a polyglutamyl peptide [5]. Very few cyclodextrin derivatives bearing a carboxylic acid and an amine function have been described. Such compounds have, in most cases, been prepared as enzyme mimics [6]. Very recently, heptakis(6-cysteino-6-deoxy)cyclomaltoheptaose was prepared by the nucleophilic substitution of heptakis(6-deoxy-6-iodo)cyclomaltoheptaose by the thiolate anion of cysteine [7]. In this paper, we present a simple access to cyclodextrin derivatives bearing a free α-amino acid group and their use for the complexation of the antineoplastic agent, 5-fluorouracil (2,4-dihydroxy-5-fluoropyrimidine) (Scheme 1).

1. Experimental

Materials and general methods.—Amino acids: *N*-benzyloxycarbonyl-aspartic acid benzyl ester (Z-Asp-OBzl), *N*-benzyloxycarbonyl-glutamic acid benzyl ester (Z-Glu-OBzl), and *N-tert*-butoxycarbonyl-glutamic acid benzyl ester (Boc-Glu-OBzl) were obtained from Bachem. Dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, and 5-fluorouracil were purchased from Sigma. The commercial β-cyclodextrin (Roquette Frères, Lestrem, France) was dried under reduced pressure (1 mm Hg) at 100 °C for 1 h before

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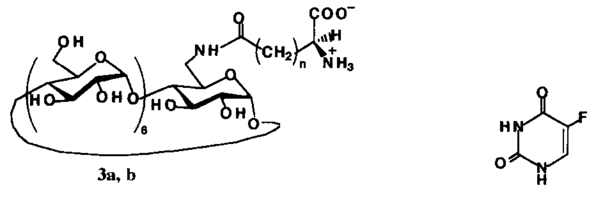


3a, b

5-fluorouracil

Scheme 1. **3a** ($n = 1$) and **3b** ($n = 2$).

use. Anhydrous solvents of analytical grade were stored over molecular sieves 4 Å. Catalytic hydrogenation was performed at atmospheric pressure. Reactions were controlled with thin-layer chromatography (TLC aluminium sheet Silica Gel 60F$_{254}$ from E. Merck, eluent A: 7:7:5:4 EtOAc–2-propanol–ammonia 25%–water; eluent B: 7:7:5 EtOAc–2-propanol–water), detected by UV 254 nm and 10% H_2SO_4 followed by heating. ^{1}H NMR spectra were recorded on Bruker 270, 400, or 500 MHz spectrometers. Melting points were measured with a Büchi instrument. Elemental analysis were performed by the Service de Microanalyse of the Centre Pharmaceutique de Chatenay-Malabry (Paris XI). 6^1-Amino-6^1-deoxycyclomaltoheptaose **1** was prepared in three steps from β-cyclodextrin according to [3] and was recrystallized from 4:1 water–MeOH.

Coupling of amino acids on 6^1-amino-6^1-deoxycyclomaltoheptaose.—6^1- Amino 6^1 deoxycyclomaltoheptaose (**1**) (1.5 g, 1.322 mmol) and the amino acid (1.6 mmol) were treated with dicyclohexylcarbodiimide (0.409 g, 1.983 mmol) and 1-hydroxybenzotriazole (0.267 g, 1.983 mmol) in Me_2NCHO (20 mL) at room temperature. The reaction was monitored by TLC. After 5 h at room temperature, the precipitated dicyclohexylurea (DCU) was filtered off. The soln was concd and the crude material recovered by precipitation in acetone. The resulting solid was solubilized in water and filtered over membrane filters (0.45 μm). The soln was concd and precipitated in acetone. All compounds were recrystallized from 4:1 water–MeOH and dried under reduced pressure.

6^1-(N^4-L-O^1-Benzyl-N^2-benzyloxycarbonyl-asparagino)-6^1-deoxy-cyclomaltoheptaose (**2a**).—Isolated as a white powder (1.623 g, 83%; 60% after recrystallization): mp 262–264 °C; R_f 0.49 (eluent B); ^{1}H NMR (Me_2SO-d_6, 500 MHz): δ 2.55 (m, 2 H, CO–CH_2), 3.05–3.75 (m, 42 H, H-2, H-6), 3.97 (m, 1 H, CH–CH_2), 4.25–4.42 (m, 6 H, O_6–H), 4.85 (d, 6 H, H-1), 4.88 (d, 1 H, H-1'), 5.06 (s, 2 H, NHCOOCH_2–C_6H_5), 5.12 (s, 2 H, CH_2–C_6H_5), 5.61–5.85 (m, 14 H, O_2–H, O_3–H), 7.34–7.38 (m, 10 H, Ar–H), 7.83 (d, 1 H, CH_2–NH), 7.90 (m, 1 H, CH–NH); $J_{1,2} = J_{1',2'} = 3.5$ Hz. Anal. Calcd for $C_{61}H_{88}O_{39}N_2$: C, 49.68; H, 5.97; N, 1.9. Found: C, 49.44; H, 6.23; N, 1.71.

6^1-(N^5-L-O^1-Benzyl-N^2-benzyloxycarbonyl-glutamino)-6^1-deoxy-cyclomaltoheptaose (**2b**).—Isolated as a white powder (1.711 g, 87%, 62% after recrystallization): mp 260–262 °C; R_f 0.60 (eluent B); ^{1}H NMR (Me_2SO-d_6, 270 MHz): δ 1.82–1.91 (m, 2 H, CH_2–CH_2–CO), 2.22 (brt, 2 H, CH_2–CH_2–CO), 3.25–3.63 (m, 42 H, H-2, H-6), 4.08 (m, 1 H, CH–NH), 4.42–4.50 (m, 6 H, O_6–H), 4.80 (d, 6 H, H-1), 4.85 (d, 1 H, H-1'), 5.02 (s, 2 H, NHCOOCH_2–C_6H_5), 5.10 (s, 2 H, CH_2–C_6H_5), 5.62–5.83 (m, 14 H, O_2–H, O_3–H), 7.34 (m, 10 H, Ar–H), 7.74 (m, 1 H, N–H); $J_{1,2} = J_{1',2'} = 3.5$, $J_{CH_2-CH_2}$ 6.3 Hz. Anal. Calcd for $C_{62}H_{90}O_{39}N_2$: C, 50.02; H, 6.05; N, 1.88, Found: C, 49.86; H, 6.28; N, 2.11.

*6^1-(N^5-L-O^1-Benzyl-N^2-*tert*-butyloxycarbonyl-glutamino)-6^1-deoxy-cyclomaltoheptaose* (**2c**).—Isolated as a white powder (1.633 g, 85%, 60% after recrystallization: mp 215–217 °C; R_f 0.53 (eluent B); ^{1}H NMR (Me_2SO-d_6, 400 MHz): δ 1.4 (s, 9 H, Boc), 1.78–1.86 (m, 2 H, CH_2–CH_2–CO), 2.2 (brt, 2 H, CH_2–CH_2–CO), 3.35–3.75 (m, 42 H, H-2, H-6), 4.15 (m, 1 H, CH–NH), 4.45–4.50 (m, 6 H, O_6–H), 4.81 (d, 6 H, H-1), 4.83 (d, 1 H, H-1'), 5.22 (s, 2 H, CH_2–C_6H_5), 5.62–5.81 (m, 14 H, O_2–H, O_3–H), 7.35 (m, 5 H, Ar–H); $J_{1,2} = J_{1',2'} = 3.5$ Hz. Anal. Calcd for $C_{59}H_{92}O_{39}N_2$: 48.71; H, 6.33; N, 1.92). Found: C, 48.53; H, 6.57; N, 2.15.

6^1-(N^5-L-O^1-Benzyl-glutamino)-6^1-deoxy-cyclomaltoheptaose (**2d**).—To the suspension of **2c** (1.453 g, 1 mmol) in 1:1 dioxane–water (150 mL) was added 1N HCl (10 mL). The reaction was monitored by TLC. After 30 min, the solvent was evaporated in vacuo. The solid collected was recrystallized from 1:1 MeOH–water and dried overnight over P_2O_5 to give **2d** (1.095 g, 81%, 55% after recrystallization) as white powder: mp 220–224 °C; R_f 0.30 (eluent A); ^{1}H NMR (Me_2SO-d_6, 400 MHz): δ 1.80–1.91 (m, 2 H, CH_2–CH_2–CO), 2.22 (brt, 2 H, CH_2–CH_2–CO), 3.35–3.75 (m, 42 H, H-2, H-6), 4.20 (m, 1 H, CH–NH), 4.45–4.51 (m, 6 H, O_6–H), 4.82 (d, 6 H, H-1), 4.84 (d, 1 H, H-1'), 5.24 (s, 2 H, CH_2–C_6H_5), 5.67–5.91 (m, 14 H, O_2–H, O_3–H), 7.34 (brs, 5 H, Ar–H), $J_{1,2} = J_{1',2'} = 3.5$, $J_{CH_2-CH_2}$ 6.3 Hz. Anal. Calcd for $C_{54}H_{84}O_{37}N_2$: C, 47.88; H, 6.20; N, 2.07. Found: C, 47.98; H, 6.34; N, 2.22.

Cleavage of the benzyl groups by hydrogenolysis. —Compounds **2a**, **2b**, and **2d** (1 mmol) were suspended in 1:1 MeOH–water (100 mL). After addition of 60 mg of 10% Pd–C, these mixtures were hydrogenated at atmospheric pressure for 4 h at room temperature. The catalyst was filtered off and washed twice with water (5 mL). The mixture were concd and precipitated in acetone. The resulting solids were solubilized in water and filtered over membrane filters (0.45 μm). The solns were evaporated and the residues recrystallized from 2:1 MeOH–water to give compounds **3a** and **3b** as white powders. These products were purified with a copper(II) complex method: To a soln of cyclodextrin derivative **3a** or **3b** in water (1 g, 5 mL) was added with caution 1 equiv of copper(II) acetate. The resulting soln was stirred for 5 h at room temperature. The light blue copper complex precipitate formed was collected on a filter, washed with water (2 × 2 mL) and dried in vacuum. The finely powdered copper complex was suspended in water (5 mL), treated with 2 g of Duolite® GT-73 Resin (Supelco, Sigma). After stirring for 3 h at room temperature, the chelating resin was filtered off and the colourless soln evaporated to afford the desired products.

6^I-(N^4-L-Asparagino)-6^I-deoxy-cyclomaltoheptaose (**3a**).—Isolated as a white solid (1.174 g, 94%, 65% after purification by complexation with Cu^{2+}): mp 210–214 °C; R_f 0.12 (eluent B); ^1H NMR (Me_2SO-d_6, 500 MHz): δ 2.54 (m, 2 H, CO–CH_2), 3.05–3.72 (m, 42 H, H-2, H-6), 4.04 (m, 1 H, CH–CH_2), 4.24–4.41 (m, 6 H, O_6–H), 4.83 (d, 6 H, H-1), 4.87 (d, 1 H, H-1'), 5.61–5.87 (m, 14 H, O_2–H, O_3–H), 7.89 (m, 1 H, CH_2–NH); $J_{1,2} = J_{1',2'} = 3.5$ Hz. Anal. Calcd for $C_{46}H_{76}O_{37}N_2$: C, 44.19; H, 6.08; N, 2.24. Found: C, 44.03; H, 6.31; N, 2.05.

6^I-(N^5-L-Glutamino)-6^I-deoxy-cyclomaltoheptaose (**3b**).—From **2b**: (1.162 g, 93%, 52% after purification by complexation with Cu^{2+}); From **2d:** (1.237 g, 95%, 62% after purification by complexation with Cu^{2+}): mp 224–226 °C; R_f 0.08 (eluent B); ^1H NMR (Me_2SO-d_6, 500 MHz): δ 1.80–1.92 (m, 2 H, CH_2–CH_2–CO), 2.25 (t, 2 H, CH_2–CH_2–CO), 3.22–3.75 (m, 42 H, H-2, H-6), 4.13 (m, 1 H, CH–NH_2), 4.40–4.44 (m, 6 H, O_6–H), 4.81 (d, 6 H, H-1), 4.88 (d, 1 H, H-1'), 5.61–5.79 (m, 14 H, O_2–H, O_3–H); $J_{1,2} = J_{1',2'} = 3.5$, $J_{CH_2-CH_2}$ 6.3 Hz. Anal. Calcd for $C_{47}H_{78}O_{37}N_2$: C, 44.65; H, 6.18; N, 2.21. Found: C, 44.34; H, 6.51; N, 1.87.

Preparation of the inclusion complexes..—Inclusion compounds were prepared by the satd soln method. To a warm soln (70 °C) of **3a–b** (1 mmol) in 1 mL of water was added 5-FU (0.13 g, 1 mmol) in five portions. The mixtures were stirred at 70 °C for 1 h. After cooling to 5 °C, the resulting complexes were separated by filtration and dried *in vacuo* (complex **3a**-5FU: 0.98 g, 71% and complex **3b**-5FU: 1.03 g, 75%). The stability constant of the complex **3b**-5FU, determined by the competition method, using methyl orange as a dye [8], was 450 M^{-1} (7:3 EtOH–H_2O) and 950 M^{-1} (8:2 EtOH–H_2O).

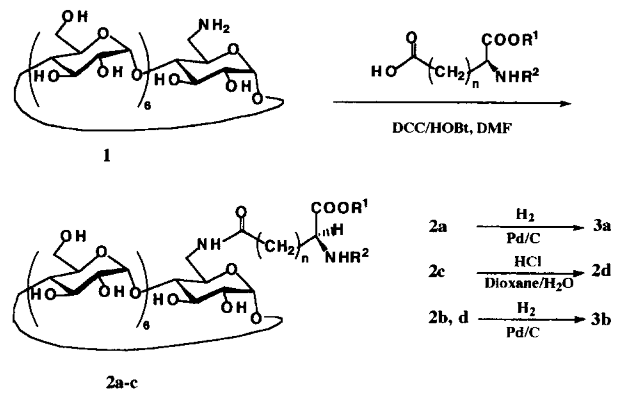
2. Results and discussion

The α-amino acid derivatives **3** of cyclomaltoheptaose(β-cyclodextrin) were obtained in two steps from 6^I-amino-6^I-deoxycyclomaltoheptaose (**1**). In the first step, acylation could be achieved by using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in dimethylformamide. Benzyl and benzyloxycarbonyl (Z) groups of the acylating agents were used for the protection of the acid and the amine functions, respectively. Apart from their easy and simultaneous removal, these groups conferred low solubility in water to the acylated products **2**, facilitating their purification by recrystallization. A glutamic acid protected on the amine function by a *tert*-butyloxycarbonyl group (Boc) was also prepared. This compound is suitable for incorporation of the amino acid bearing the cyclodextrin moiety into a peptide.

In a second step, removal of the protecting groups of **2a** and **2b** was achieved by hydrogenolysis to give **3a** and **3b**, respectively. In the case of **2c**, the *tert*-butyloxycarbonyl group was first removed by dilute hydrochloric acid. The obtained product (**2d**) could then be hydrogenated to **3b**.

Solubility studies.—The zwitterionic derivatives of cyclodextrin (**3a** and **3b**) were evaluated for their solubility in water at neutral pH at room temperature by visual observation. The compounds were added in small portions to 1 mL of water. The solubility of **3a** and **3b** was difficult to measure precisely but was greater than 150% (w/v) (Scheme 2).

Complexation studies.—5-Fluorouracil (5-FU) is a widely used anticancer agent that potentializes taxoids derivatives, and has a moderate solubility (1 g in 100 mL of water at room temperature) [9]. Inclusion complexes of 5-FU are of interest in order to decrease the volume of the solution required for its administration and to improve its bioavailability. The complexation of 5-FU is difficult since it lacks hydrophobic groups that can interact with the cavity of



Cyclodextrins	n	R^1	R^2
2a	1	$CH_2C_6H_5$	$COOCH_2C_6H_5$
2b	2	$CH_2C_6H_5$	$COOCH_2C_6H_5$
2c	2	$CH_2C_6H_5$	$COOC(CH_3)_3$
2d	2	$CH_2C_6H_5$	H
3a	1	H	H
3b	2	H	H

Scheme 2. Synthesis of **3a–c** from **1**.

the cyclodextrin. The preparation of inclusion complexes of 5-FU with cyclodextrins has already been attempted but no inclusion complex could be isolated and the association constant, determined by ^{19}F NMR, was low [10]. We have previously described the formation of a specific interaction between the guest, ellipticine and a modified cyclodextrin. The inclusion was stabilized by the formation of a charge transfer complex between the host and the guest [11]. This could not be applied to the case of 5-FU. Products **3a** and **3b** were used to form inclusion complexes with 5-FU. The precipitation of the complex was observed when 5-FU was gradually added to a concentrated solution of **3a** or **3b**. The stoichiometry was found by ^1H NMR to be 1:1. The solubility in water of complexed 5-FU was raised ten-fold. We failed to isolate inclusion complexes of 5-FU with α-cyclodextrin, β-cyclodextrin or 2,6-di-*O*-methyl-β-cyclodextrin.

The high solubility of **3a** and **3b** is difficult to explain. It can be noticed that a 30% rise in the solubility of β-cyclodextrin in water was observed by the addition of an equimolar amount of **3b** to a solution of β-cyclodextrin. This could be interpreted by the formation of dimers resulting from association of the secondary face of cyclodextrins. In a study of the interaction of amino acids with α-cyclodextrin, Barone et al. [12] have established that the amino acid group is not included in the cyclodextrin cavity. The amino acid group might close the primary end of the cavity. Capping the primary alcohol side [13] or both side [14] was shown in some cases to improve the inclusion property. This could then explain the inclusion of 5-FU.

In conclusion, the easily obtained new derivatives of cyclodextrins exhibited high solubility in water and valuable complexation ability. In the particular case under study, the stability constant of the complex was raised 50 times.

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