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Simple preparation of multi-valent cyclodextrin–carbohydrate conjugates

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Abstract

Several β-cyclodextrin (CD) derivatives conjugated with carbohydrates via aminohexyl linkages have been prepared. These CD-conjugates were demonstrated to be multi-valent ligands by lectin-binding assay.

Because of their unique structure and capability of accommodating hydrophobic 'guest' molecules, cyclodextrins (CDs) have found a variety of applications, including drug delivery.[1] In terms of chemical modification of CDs, however, tedious chemical manipulations (protection/deprotection) are usually required.[2]

Recently Chang and Robyt reported a very efficient way to oxidize the primary hydroxyl groups of CDs without protecting the remaining secondary hydroxyl groups.[3] This report stimulated us to design a new class of multi-valent ligand based on the CD framework[4] which is illustrated below (Fig. 1).

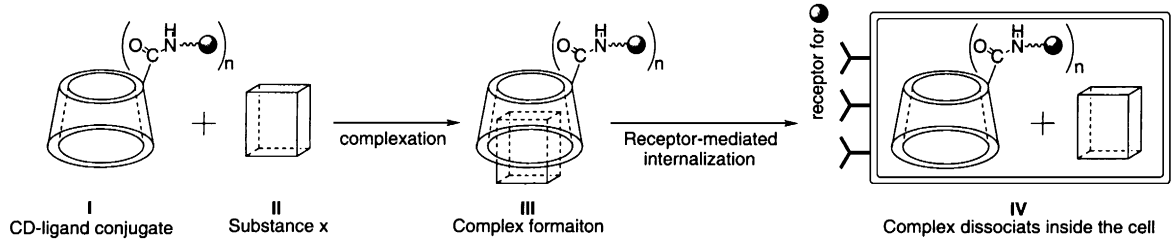

Fig. 1. CD–ligand conjugate: a new carrier molecule with multi-valency

The hydrophobic cavity of CD–ligand conjugate **I** accommodates a hydrophobic molecule (**II**, Substance X) to form a complex **III**. The multi-valent ligands on the complex **III** facilitate the receptor-

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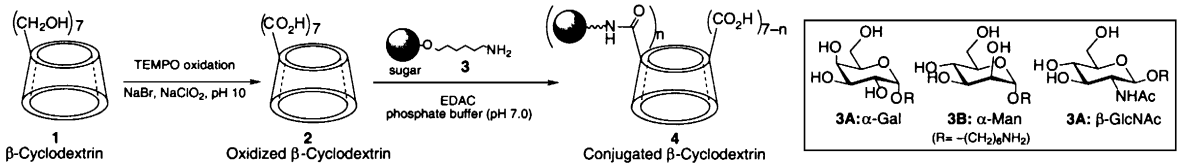
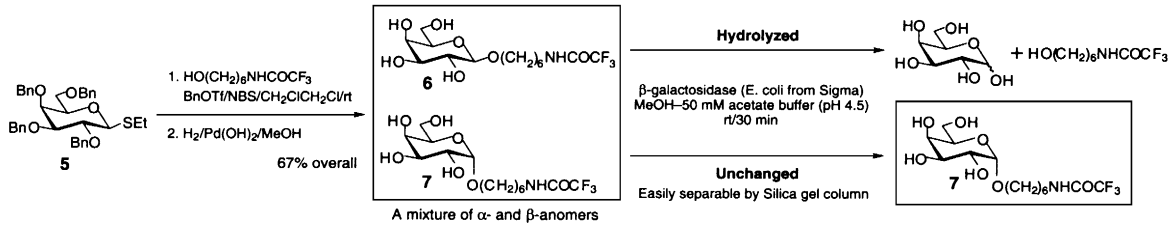
mediated internalization and dissociate inside the cell (**IV**) to release the substance X. In this communication, we describe our initial goals: synthesis and characterization of the CD–ligand conjugates using carbohydrates and their binding activity to lectins.

Synthesis of CD–carbohydrate conjugates: As outlined in Scheme 1, CD–carbohydrate conjugates linked via an aminohexyl group were easily prepared. β-CD **1** was oxidized by the Robyt method[3] (TEMPO–$NaClO_4$) to give a β-CD carboxylate derivative **2**. Coupling of a well-dried **2** and 7 equivalents (one ligand per one CO_2H of **2**) of 6-aminohexyl glycoside of carbohydrate **3** was carried out in the presence of 20 equivalents of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) in phosphate buffer (pH 7.0) overnight at room temperature. The coupling product was purified by Sephadex gel permeation chromatography (eluted with water).



Scheme 1. Synthesis of CD–carbohydrate conjugates

The 6-aminohexyl α-mannoside **3B**[5] and β-*N*-acetylglucosaminide **3C**[6] were synthesized from the corresponding carbohydrates; however, we had a problem with the synthesis of 6-aminohexyl α-galactoside **3A** in isolating the pure anomer. We solved this problem by using β-galactosidase to eliminate the unwanted β-anomer (Scheme 2).



Scheme 2. Chemo-enzymatic synthesis of an α-Gal aminohexylglycoside

Glycosylation reaction of a galactosyl donor **5** and 6-(*N*-trifluoroacetyl)-aminohexanol[7] was carried out with Kusumoto's condition[8] (Bu_4NOTf and NBS) in ether and gave an inseparable (on silica gel chromatography) mixture of α- and β-anomers. This mixture was hydrogenated to give a mixture which was tedious to separate on a silica gel column on a preparative scale: α-anomer **7** (R_f=0.59 in $CHCl_3$:EtOAc:MeOH 5:2:3; 0.64 in 2-PrOH:H_2O:NH_4OH 7:2:1) and β-anomer **6** (R_f=0.53 in $CHCl_3$:EtOAc:MeOH 5:2:3; 0.60 in 2-PrOH:H_2O:NH_4OH 7:2:1). After unsuccessful attempts of examining a number of solvent systems that might give a better separation on silica gel TLC, we decided to use an enzymatic reaction. When the mixture of **6** and **7** was treated with β-galactosidase (*E. coli* from Sigma) in 50 mM acetate buffer (pH 4.5) with MeOH as a co-solvent (7% of the total volume) for 30 min at room temperature, the β-anomer **6** completely disappeared on TLC (2-PrOH:H_2O:NH_4OH 7:2:1). The reaction mixture was then concentrated and the desired α-anomer **7**[9] was recovered by silica gel chromatography ($CHCl_3$:EtOAc:MeOH 5:2:0.5).

Characterization of the CD–sugar conjugates: The average number of ligands was estimated with a ^1H NMR spectrum by comparing the integral ratio of the alkyl protons of the aminohexyl group and protons of the carbohydrate frames. Based on these analyses, we obtained the average number of ligands was five for the CD–α-Gal conjugate and four for the CD–β-GlcNAc conjugate. These numbers were also confirmed with a MALDI mass spectrum[10] as a dominant peak. In the case of CD–α-Gal conjugate,

the *m/z* (mass/charge) of the molecular ion at 2541.7 corresponding to a molecular weight of CD–α-Gal conjugate with five ligands. It has the hightest relative intensity when compared to other peaks at *m/z* 2279.4 (with four ligands; 36% intensity compared to the one at 2541.7) and *m/z* 2804.6 (with six ligands; 30% intensity compared to the one at 2541.7) (Fig. 2).

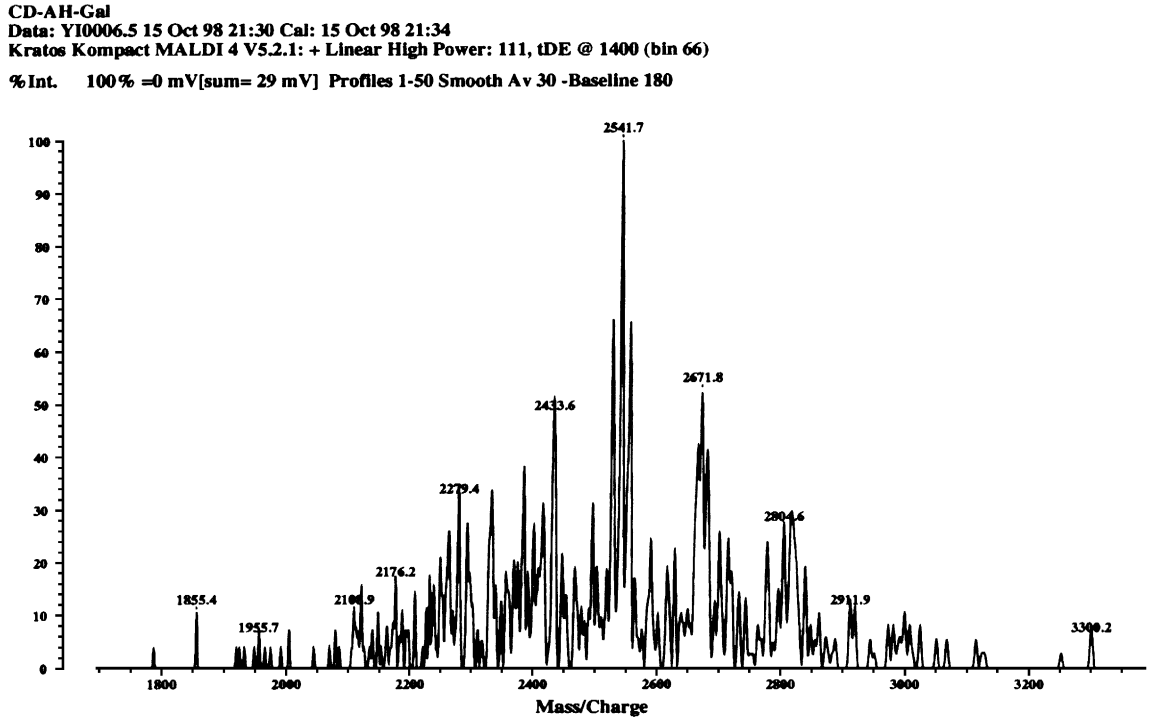


Fig. 2. MALDI mass spectrum of CD–α-Gal conjugate

Lectin-binding studies of CD–carbohydrate conjugates: Because carbohydrate-binding proteins (lectins) are known to precipitate when they are mixed with multi-valent carbohydrate ligands, they are often used to examine whether a substance contains specific carbohydrate residues with a multi-valency. We therefore used a galactose-binding protein[11] (*Griffonia simplicifolia* I, GSI) and an *N*-acetyl glucosamine-binding protein[12] (wheat germ agglutinin, WGA) to determine whether the CD–carbohydrate conjugates exhibit multi-valency (Fig. 3).

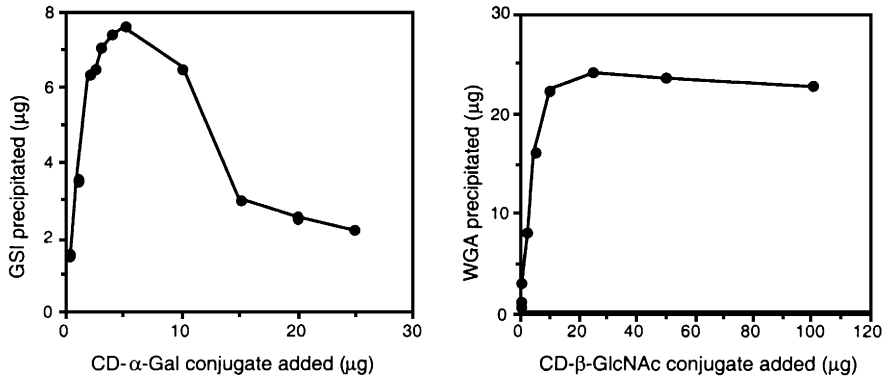


Fig. 3. Lectin-precipitation curves with the CD–carbohydrate conjugates

Typical precipitin-like curves were generated when increasing amounts of the CD–carbohydrate ligands were added to fixed quantities of the two lectins (10 μg of the GS I lectin; 40 μg of wheat germ agglutinin). These precipitin curves establish the multi-valency of the CD–carbohydrate ligands. For some unknown reason the CD–α-Man ligand did not precipitate the snowdrop lectin.[13]

Summary: We have designed a new class of multi-valent ligand using a simple coupling reaction of the carboxyl CD derivative and the amino-containing carbohydrate glycosides. We used a very convenient way to estimate the number of ligands introduced on the CD molecule with ^{1}H NMR spectrum which was also confirmed by MALDI mass spectral analysis. We have also shown that these CD-conjugates are multi-valent ligands which cause a precipitation with their corresponding carbohydrate-binding proteins (lectins). These CD-conjugates may have great potential as a new class of targeting vehicles to deliver a hydrophobic molecule inside the cell via receptor-mediated internalization. Work is in progress to explore this possibility.

Acknowledgements

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9. ^{1}H NMR for **7**: δ (D_2O) 1.35–1.48 (4H, m), 1.62–1.74 (4H, m), 2.99 (1H, d, J=7.62 Hz), 3.01 (1H, d, J=7.62 Hz), 3.50 (1H, dt, J=6.27, 9.94 Hz), 3.69–3.78 (3H, m), 3.82–3.84 (2H, m), 3.94 (1H, brt, J=6.19Hz), 3.97 (1H, brd, J=1.43 Hz), 4.94 (1H, d, J=3.03 Hz).
10. Spectra were acquired on a Kratos (Manchester, England) Kompact MALDI (Matrix Assisted Laser Desorption/Ionization) equipped with a nitrogen laser (337 nm) and a 20 kV extraction voltage, in the positive mode. The matrix was a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% ethanol.
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