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An efficient, regioselective and fast enzymatic glycosylation for cyclodextrins

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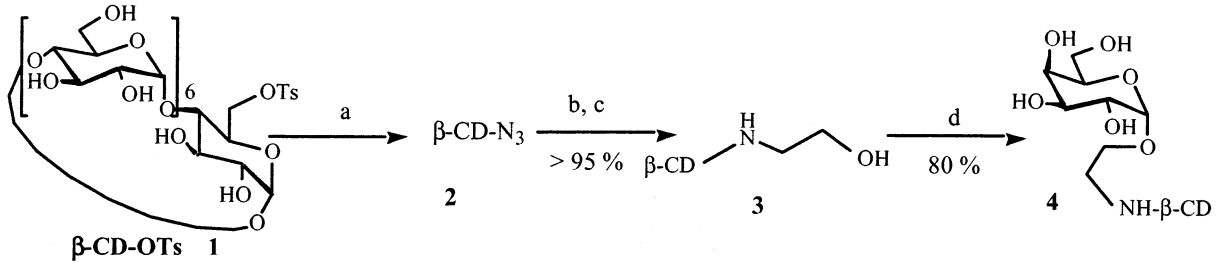
Abstract—The introduction of a short spacer arm was necessary to obtain complete regioselectivity in the glycosylation of β-cyclodextrin (β-CD) mediated by glycosidases. Thus 6-*N*-(2-aminoethyl-α-D-galactopyranosyl)-6-deoxycyclomaltoheptaose was prepared in four steps from β-cyclodextrin with 30% overall yield using, in the key step, the transfer activity of green coffee bean α-galactosidase.

Applications of cyclodextrins (CDs) as drug carriers have been severely limited due to the moderate water solubility of these compounds. Thus, in recent years, extensive work has been undertaken to graft hydrophilic substituents onto the hydroxyl groups.[1] One can divide the strategies used into two groups: complete or partial functionalisations and single modifications. The former methods present some severe drawbacks such as incomplete hydroxyl transformations, thus leading to complex mixtures of statistical regioisomers and to undesired modifications of the complexation properties of native CDs. Monofunctionalisations look preferable for these reasons. Furthermore, a small number of efficient methods have already been described for this purpose. This is the case, for instance, in the preparation of 6-monotosyl-CDs[2,3] which allowed an easy synthesis of reactive intermediates such as 6-deoxy-6-azido-CDs,[4] 6-deoxy-6-amino-CDs,[4,5] 6-deoxy-6-thio-CDs[6] or 6-oxo-CD.[7] Similarly, methods have also been developed to graft sulfonyl groups at the O-2 position of CDs.[8]

Glycosylation of cyclodextrins is one of the most popular methods for enhancing their solubility. This transformation can be achieved via *N*-or *S*-glycosylation reactions starting from the latter derivatives.[6,9–16] Mono-6-*O*-glucosyl-CDs were also synthesised as co-products during the formation of CDs from starch in the presence of CGTase.[17,18] Using the transfer activity of glycoside hydrolases in the presence of a convenient glycoside donor and with CDs as acceptors, Japanese researchers have described the enzymatic synthesis of a



Scheme 1. *Reagents and conditions*: (a) NaN_3; (b) PPh_3, DMF; (c) $NH_2CH_2CH_2OH$; (d) *p*-NP α-gal, α-galactosidase.

Keywords: cyclodextrins; cyclomalto-oligosaccharides; monoglycosylation; glycosidases.
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large variety of branched CDs.[19–22] Unfortunately, the selectivity and the yield of these reactions were low since di- and triglycosyl-CDs were also synthesised. Furthermore, the higher reactivity of the glycoside branched hydroxyl groups, induced the further grafting of additional glycosyl units. With the aim of obtaining monoglycosylated CDs and taking into account this experimental result, we have decided to introduce a short hydroxylated spacer arm on β-CD. It was hoped that the additional hydroxyl group would act as a selective acceptor in a transglycosylation catalyzed by α-glycosidase.

The strategy used to illustrate and test this concept is shown in Scheme 1. The selective synthesis of mono-6-ethanolamino-6-deoxy-β-CD **3** was achieved in three steps from β-CD. The monotosyl-β-CD **1** was prepared according to the procedure of Defaye et al.[3] (43% yield). The following step gave 6-azido-β-CD **2** in quantitative yield. Thus, the Staudinger reaction was performed with **2** in the presence of triphenylphosphine followed by the condensation of ethanolamine. The branched CD **3** was obtained as the sole product in almost quantitative yield. The synthesis of **3** has been previously described by means of the direct condensation of ethanolamine with monotosyl-β-CD **1** (yield 60%).[23]

Compound **3** was used as an acceptor in the presence of a seven-fold amount of *p*-nitrophenyl α-galactopyranoside (*p*-NP α-gal) as a donor in the transglycosylation reaction catalysed by green coffee bean α-galactosidase. The analysis of the reaction medium showed the presence of galactose and disaccharides resulting from the self-condensation of *p*-NP α-gal and 6-*N*-(2-aminoethane-α-D-galactopyranosyl)-6-β-CD **4**. Under the conditions used for this reaction,[24] *p*-NP α-gal completely reacted while small amounts of unreacted **3** were still present. In addition, CDs branched with galactosyl units on the ring hydroxyls were not detected. Thus, this regioselective reaction indicates the preference of the galactosidase for outer hydroxyl groups. Finally, galactosyl-CD **4** was obtained in 30% yield starting from native β-CD, the limiting step being the synthesis of monotosyl-β-CD **1**. The structures of **3** and **4** were established by comparison with previous NMR data,[23] two-dimensional NMR sequences and by mass spectrometry.[24] The extension of this reaction to other glycosidases is now under investigation in our laboratory.

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24. *Mono-6-ethanolamino-6-deoxy-β-CD* ***3***: Compound **3** was prepared as follows: 50 mg (43 μmol, 1 equiv.) of 6-azido-β-CD **2** (see Refs. 3 and 5) was dissolved in 1.1 mL of DMF. To this solution, 27.8 mg (106 μmol, 2.5 equiv.) of triphenylphosphine and 682 μL (11.3 μmol, 263 equiv.) of ethanolamine were added. The mixture was stirred for 20 h at room temperature. The branched CD was then precipitated by the addition of 10 mL of acetone. The resulting solid was washed with 10 mL of acetone and 10 mL of ether. CD **3** was obtained as white

crystals in quantitative yield; mp 202°C (dec.); $[\alpha]_D^{20}=+96.2$ (c 0.5, H_2O). NMR δ_H (500 MHz, D_2O): 5.05 (7H, H-1 CD), 4.00–3.40 (42H, H CD), 3.50–3.35 (2H, m, CH_2N), 3.10 (NH_2), 2.85–2.75 (2H, m, CH_2N); δ_C (125 MHz, D_2O): 101.5 (C-1 CD), 80.8 (C-4 CD), 72.8 (C-3 CD), 71.8 (C-5 CD), 71.5 (C-2 CD), 62.2 (CH_2OH arm), 59.9 (C-6 CD), 42.9 and 41.6 (2 CH_2N). MS ES$^+$: 1134 ($M-^{\bullet}CH_2-CH_2OH+2H^+$).

Preparation of galactosyl branched β-CD **4**: 104 mg (88 μmol, 1 equiv.) of **3** was dissolved in 12 mL of water. The pH was adjusted to 6.6 with 0.1 M HCl and *p*-NP α-gal (184 mg, 611 μmol, 7 equiv.) was added with 18 units of green coffee bean α-galactosidase (Sigma). The mixture was incubated at 37°C for 40 min. The reaction was then quenched by the addition of 3 mL of methanol to inactivate the enzyme. Then, the solvent was partially evaporated under reduced pressure and *p*-nitrophenol was extracted with 3×5 mL of ethyl acetate. The aqueous phase was evaporated and **4** was purified on a silica gel column. Seymour eluent ($MeOH/CHCl_3/AcOH/H_2O$, 30:60:3:5) was used first to eliminate the mono- and disaccharides. This eluent was replaced by pure MeOH for the separation of **4** and unreacted **3**. The former was obtained as white crystals. Compound **4**: yield 80%; mp 185°C (dec.); $[\alpha]_D^{20}=+99.5$ (c 0.5, H_2O). NMR δ_H (500 MHz, D_2O): 5.05 (7H, H-1 CD), 4.93 (1H, d, $J=3.2$ Hz, H-1 Gal), 4.00–3.92 (2H, m, H-4 and H-5 Gal), 3.93–3.89 (7H, m, H-3 CD), 3.89–3.78 (21H, H-3 Gal, H-6 CD, H-5 CD, H-2 Gal), 3.73–3.68 (2H, m, H-6 and H-6′ Gal), 3.65–3.61 (2H, m, CH_2O), 3.61–3.58 (7H, m, H-2 CD), 3.58–3.54 (6H, m, H-4 CD), 3.54–3.49 (3H, m, CH_2N, H-4 CD), 3.45–3.40 (2H, m, CH_2N), 3.32 (NH_2); δ_C (125 MHz, D_2O): 101.3 and 100.5 (C-1 CD), 98.8 (C-1 Gal), 82.5, 80.6 and 80.0 (C-4 CD), 72.6 (C-3 CD), 71.6 (C-5 CD), 71.2 (C-2 CD), 70.5 (C-5 Gal), 68.9 (C-3 Gal), 68.8 (C-4 Gal), 67.9 (C-2 Gal), 62.2 (CH_2O), 60.5 (C-6 Gal), 59.9 (C-6 CD), 40.5 (2 CH_2N). MS ES$^+$: 1134 ($M-^{\bullet}CH_2-CH_2OGal+2H^+$).