Synthesis and comparative lectin-binding affinity of mannosyl-coated β-cyclodextrin-dendrimer constructs†

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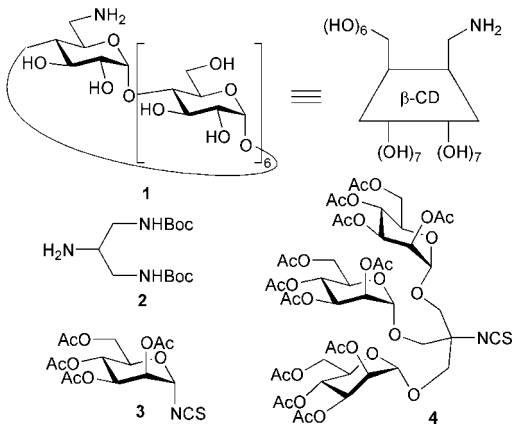
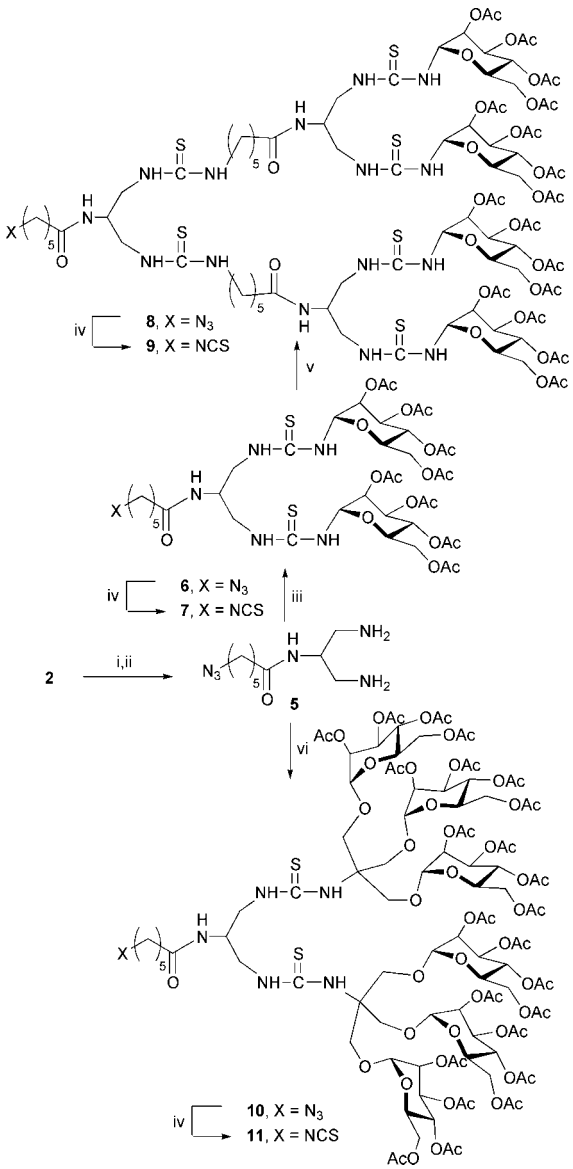
Targeted drug delivery systems have been built from β-cyclodextrin by monoconjugation with mannosyl-coated dendritic branches following an iterative thiourea-forming convergent strategy; the multivalent adducts showed high Concanavalin A lectin binding ability and intact inclusion capabilities.

Grafting biorecognisable saccharide epitopes onto suitable molecular carriers may provide new vectors for site-specific delivery of therapeutics. Several examples of oligosaccharide-branched cyclodextrins (CDs) have been reported in recent years for this purpose, taking advantage of the known ability of CDs to include a variety of hydrophobic compounds *via* host-guest complexation.[1] The majority of such conjugates are monosubstituted derivatives at a single primary hydroxy group position of the CDs.[2] A few per-(C-6)-substituted branched-CDs have also been synthesised to comply with the need for a multivalent presentation pattern of the saccharide markers.[2a,d,3] However, although lectin-binding capacity is substantially increased when compared with the monovalent counterparts, polysubstitution at the primary face of CDs may seriously impair inclusion and complex stabilisation of potential guests. Our own results indicated approximately a 10-fold decrease in the water solubilisation power of branched β-CDs, measured for the anticancer drug Taxotère®, upon heptafunctionalisation.[2c,d]

As an attempt to combine both high biological receptor binding ability and efficient inclusion capabilities, a series of monosubstituted multivalent β-CD carriers have now been synthesised. For this purpose, we have developed an efficient preparation of dendritic wedges suitable for sequential external carbohydrate coating and covalent attachment to the β-CD core through a convergent iterative methodology that exploits the reactivity of isothiocyanate and amine functionalised mono-





Scheme 1 *Reagents and conditions*: i, 6-azidohexanoyl chloride, 1:1 DMF–collidine, rt, 96%; ii, 1:1 TFA–water, rt, 2 h, 98%; iii, pyridine, 24 h, 87%; iv, PPh_3, CS_2, toluene, rt, 16 h, 90%; v, **7** + **5**, 1:1 water–acetone, pH 8 ($NaHCO_3$), 55%; vi, **4**, 1:1 water–acetone, pH 8 ($NaHCO_3$), 70%.

† Dedicated to Dr R. U. Lemieux, Emeritus Professor, University of Alberta (Edmonton, Canada), on the occasion of his 80th birthday.

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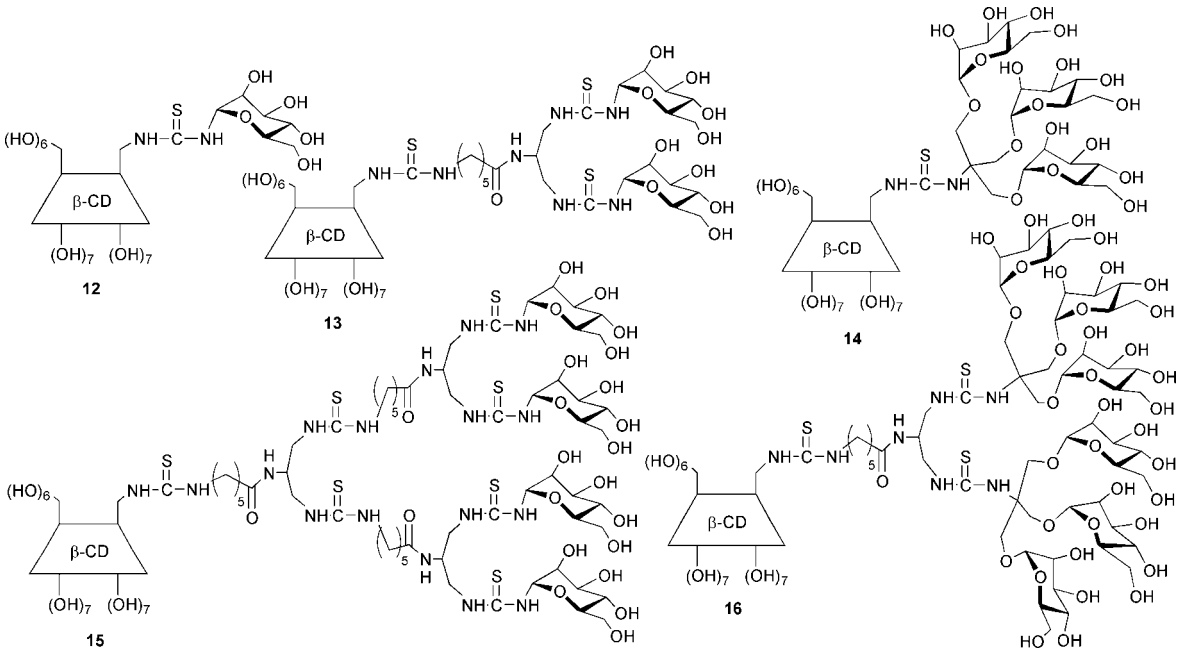

mers.[4] The key templates are 6^{I}-amino-6^{I}-deoxy-β-CD (**1**), for which an improved synthetic route has been recently elaborated,‡[5] the selectively protected 1,2,3-triaminopropane branching element **2**,[6] and the isothiocyanate functionalised α-D-mannopyranosyl derivatives **3**[4] and **4**. The latter was prepared by reaction of the known tris-α-mannopyranoside amine[7] with thiophosgene. Intercalation of a six-carbon spacer has also been considered to ensure the accessibility of the grafted bioactive components to molecular recognition events.

Mono- and trivalent mannosylated thioureido β-CDs **12** and **14** were obtained in high yield by direct coupling of **1** with **3** and **4**, respectively, followed by removal of the *O*-acetyl groups.§ Reaction of **2** with 6-azidohexanoyl chloride and further TFA-catalysed hydrolysis of the Boc N-protecting groups afforded dendron **5**, which was subsequently reacted with **3** and **4** to give the di- and hexavalent ligands **6** and **10** (Scheme 1). Aza-Wittig type isothiocyanation reaction of the terminal azido group using the triphenylphosphine–CS_2 system led to the corresponding bridging armed glycosyl clusters **7** and **11**, which were conjugated with monoamine **1** and deacetylated to yield the mannosyl labelled β-CD carriers **13** and **16**. The potential of the approach was further examined by constructing the second-generation tetravalent homologue **15** *via* nucleophilic addition of diamine **5** to the divalent isothiocyanate **7** (→**8**), isothiocyanation of the resulting adduct (→**9**), conjugation with the β-CD reagent **1** and final deacetylation.¶

Comparative protein-affinity evaluation of the mannosyl-coated thioureido β-CDs **12**–**16** towards horseradish peroxidase-labelled concanavalin A (Con A) was effected by performing the enzyme-linked lectin assay (ELLA) test.[8] The corresponding IC_{50} values for inhibition of Con A-yeast mannan binding[9] reflected the expected amplification of lectin-binding strength for the higher-valent representatives. Nonetheless, preliminary Taxotère® solubilisation experiments in water showed solubility values (*e.g.* 4.5 g L^{-1} in a 50 mM solution of **14**) that were similar to those obtained for monobranched CDs.

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Notes and references

‡ A much better yield for the key precursor of **1**, namely the corresponding C-6 monotosyl derivative, was achieved by using the following procedure: To a solution of β-CD (11.35 g, 10 mmol) in water (500 mL), $CuSO_4$ (7.5 g, 30 mmol) in water (750 mL) and NaOH (10 g, 250 mmol) in water (250 mL) were successively added. After 10 min, tosyl chloride (15 g, 79 mmol) in acetonitrile (100 mL) was added dropwise within 1 h. The mixture was stirred for 4.5 h, then neutralised (1 M HCl, 50 mL), the salts were filtered off and the solution was concentrated by freeze-drying to $\frac{2}{3}$ of its original volume. The crystallised solid was washed with acetone (2 × 40 mL), ether (2 × 30 mL) and dried. After two recrystallisations from water, pure monotosyl β-CD (6.33 g, 48%) was obtained.

§ Coupling yields 45–75%. Removal of the acetyl groups was effected by a mixed transterification–saponification process (see ref. 2*d*). In the cases of compounds **12**, **13** and **15** this step was performed at 0 °C to avoid anomerization of the external mannosylthioureido subunits (see ref. 10).

¶ All new compounds gave microanalytical, mass spectral (FAB or MALDI-TOF) and ^{13}C NMR data (125.7 MHz, D_2O) in agreement with the proposed structures.

Selected data for **12**: $[\alpha]_D$ +107.1 (*c* 0.7, H_2O); ^{13}C NMR δ 182.8 (CS), 102.6–101.9 (C-1$^{I–VII}$), 82.8 (C-1′), 46.3 (C-6^{I}). For **13**: $[\alpha]_D$ +105.2 (*c* 1, H_2O); ^{13}C NMR δ 183.3 (CS), 177.3 (CO), 102.7–101.8 (C-1$^{I–VII}$), 83.0 (C-1′). For **14**: $[\alpha]_D$ +74.0 (*c* 1.1, H_2O); ^{13}C NMR δ 182.2 (CS), 102.7–102.0 (C-1$^{I–VII}$), 100.8 (C-1′) 46.8 (C-6^{I}). For **15**: $[\alpha]_D$ +54.6 (*c* 1.4, H_2O); ^{13}C NMR δ 182.5 (CS), 177.0 (CO), 102.1–101.7 (C-1$^{I–VII}$), 82.5 (C-1′). For **16**: $[\alpha]_D$ +32.0 (*c* 1.1, H_2O); ^{13}C NMR δ 182.9 (CS), 177.3 (CO), 102.1–101.9 (C-1$^{I–VII}$), 100.9 (C-1′).

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