Chemical–Enzymatic Synthesis and Bioactivity of Mono-6-[Gal-β-1,4-GlcNAc-β-(1,6′)-hexyl]amido-6-deoxy-cycloheptaamylose

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Transfer of a β-1,4-galactosyl moiety by galactosyltransferase to chemically synthetised mono-6-(2-acetamido-2-deoxy-β-D-glucopyranosylhexyl)amido-6-deoxy-cycloheptaamylose yields mono-6-[Gal-β-1,4-GlcNAc-β-(1,6′)-hexyl]amido-6-deoxycycloheptaamylose which shows recognition by the galactose specific *Kluveromyces bulgaricus* cell wall lectin (Kb CWL)

The cyclodextrins [cycloheptaamylose (β-CD) **1**, Fig. 1], have found wide application for the solubilisation and transport of biologically active molecules.[1] However, they cannot be used to target specific sites or organisms in the controlled delivery of drugs as they lack biological recognition sites. In view of the importance of carbohydrate structures for bio-recognition processes at cell surfaces,[2] the grafting of these molecules onto the cyclodextrins is a way of importing site and organism specificity into the cyclodextrin-mediated transport of drugs. Simple sugars such as galactose or mannose have been bound to β-CD either directly,[3] or *via* alkylamido spacer arms,[4] and have shown promise with regard to recognition by cell wall lectins.[5]

Complex multistage syntheses are required for the chemical preparation of each complex oligosaccharide involved in cellular recognition. This effectively rules out such a purely chemical strategy for the large scale application of such 'intelligent' transport systems. Thus, a mixed chemical–enzymatic strategy in which a monosaccharide acceptor is coupled *via* a spacer to the CD and further saccharide motifs are added *via* enzymatic coupling is highly appealing. Such a strategy has the ease of production of diverse saccharide systems and also from the point of view of ease of synthesis; but it is not evident that the steric bulk of the cyclodextrin macrocycle will not prevent the saccharide receptor from reaching the catalytically active site. Recent work on the use of solid supported sugars in enzyme catalysis was, however, very encouraging.[6]

In this communication we report the first such synthesis, in which galactose is coupled to a mono-6-(2-acetamido-2-deoxy-β-D-glucopyranosylhexyl)amido-6-deoxy-β-cyclodextrin molecule **4** using galactosyl-transferase to generate the first part of the typical *O*-glycoprotein sequence. We also show that such a system shows good molecular recognition by the *Kluveromyces bulgaricus* yeast cell wall lectin (KbCWL).

The synthetic route used is shown in Fig. 2, DCC–1-hydroxybenzotriazole hydrate (1.16 mmol) peptide coupling of mono-6-amino-6-deoxy-β-CD[4] **2** (0.88 mmol) of the 2-acetamido-2-deoxy-β-D-glucopyranosyl-hexanoic acid **3** (0.88 mmol) in DMF (20 ml) at room temp. gives **4** in 28% yield after purification by chromatography on a column of silica gel.†

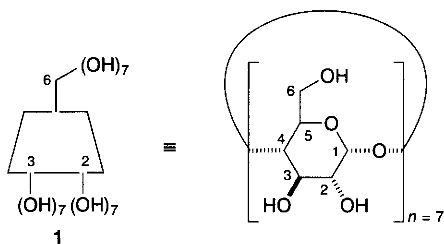

Fig. 1 Structure and schematic representation of β-CD

This molecule is the receptor for the enzymatic coupling reaction (Fig. 3). In view of the high cost of UDP-Gal, the relatively inexpensive precursor UDP-Glc was converted *in situ* to UDP-Gal using UDP-glucose 4-epimerase (EC 5.1.3.2).[7] The reaction was carried in the presence of $MnCl_2$ in aqueous solution at 37 °C during 36 h, with a Cacodylic acid buffer. An NAD–bovine serum albumin system was used to remove the UDP formed.[7]

The enzymatic coupling is highly efficient yielding the desired product mono-6-[Gal-β-1,4-GlcNAc-β-(1,6′)-hexyl]-amido-6-deoxy-β-cyclodextrin **5** in 68% yield.‡ Whilst the ^1H NMR spectra in D_2O of **4** and **5** are highly complex the anomeric protons of the conjugated saccharides are observed as a doublet at δ 4.47 (*J* 7.5 Hz) for **4** and two doublets at δ 4.47 (*J* 7.5 Hz) and δ 4.42 (*J* 7.5 Hz) corresponding to the β-GlcNAc and β-Gal for **5**. The β-CD anomeric protons are observed in both cases as an unresolved signal at δ 5.05.[8] FAB-MS of **4** and **5** are consistent with the proposed structures with $[M + Na]^+$ peaks at 1473 for **4** and 1635 for **5**.

The capability of **4** and **5** to be recognised by biological systems was tested by use of the galactose specific lectin *K. bulgaricus* cell wall lectin, KbCWL, as a function of the ability to inhibit flocculation of *K. bulgaricus* cells by the isolated lectin, KbCWL. The results are presented in Table 1, and show the minimum concentration of the molecule necessary to inhibit the flocculation activity of the isolated KbCWL in a solution corresponding to 3.4 Activity units/μg^{-1} of protein.[5]§

As expected from the galactose specificity of the lectin, β-CD itself and **4** show no activity towards inhibiting flocculation. Galactose itself inhibits flocculation at 3.75 mmol dm^{-3}, the mono-6-[Gal-β-1,4-GlcNAc-β-(1,6′)-hexyl]amido-6-deoxy-β-

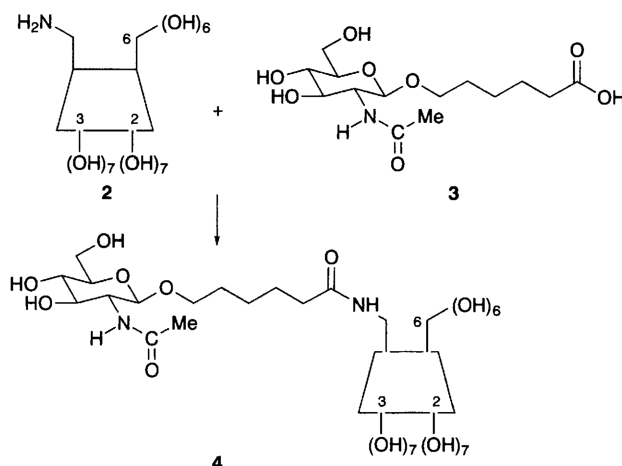

Fig. 2 Chemical synthesis of the mono-6-[GlcNAc-β-(1,6′)hexyl]amido-6-deoxy cycloheptaamylose **4**

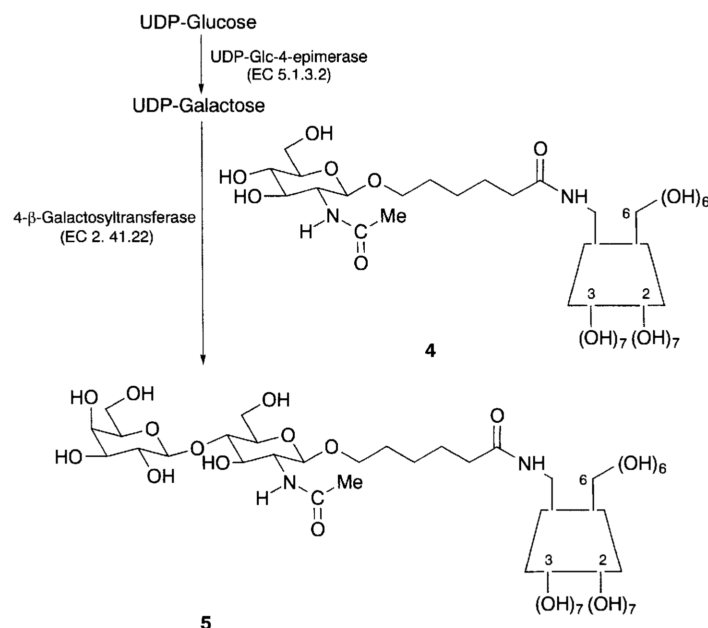

Fig. 3 Enzymatic synthesis of the mono-6-[Gal-β-1,4-GlcNAc-β-(1,6′)-hexyl]amido-6-deoxy cycloheptaamylose **5**

Table 1 Biological recognition properties as measured by inhibition of KbCWL flocculation activity

Products	Concentration inhibiting KbCWL flocculation activity/mmol dm^{-3}
β-CD	Negative[a]
Galactose	3.75
4	Negative[a]
5	1.75

[a] No inhibition of the lectin is observed at saturation concentrations.

CD derivative **5** inhibits the floculation at 1.75 mmol dm^{-3} showing higher biological activity. This activity is similar to that observed for galactose coupled to β-CD *via* a nine-carbon atom spacer.[5] Preliminary studies show that these molecules have a much lower haemolytic activity than the parent β-cyclodextrin **1**.[9]

The 2-acetamido-2-deoxy-β-D-glucopyranosyl-hexanoic acid **3** was kindly donated by D. Charon (CNRS, URA 1118, Centre Pharmaceutique, Châtenay-Malabry, 92296, France).

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Footnotes

† The product was identified from its ^{1}H NMR spectrum (D_2O, 400 MHz) δ_H 1.3 (m, 2H, CH_2), 1.55 (m, 4H, 2 CH_2), 2.0 (s, 3H, Me–CO), 2.25 (m, 2H, CH_2 α to CO), 3.2–4.0 (m, H β-CD), 4.47 (d, *J* 7.5 Hz, 1H, H-1 GlcNAc), 5.05 (d, 7H, H-1 β-CD).

‡ To a solution of **4** (80 mg, 54 μmol) in a cacodylic acid buffer (0.1 mol dm^{-3}, pH 7 qsp 4 ml) in the presence of Mn Cl_2 (5 μmol dm^{-3}), NAD (1 μmol dm^{-3}), bovine serum albumin (0.1%), sodium azide (0.01%) were added UDP glucose (37 mg, 65 μmol), UDP-glucose 4-epimerase (EC 5.1.3.2, 0.4 U) and 4-β galactosyl transferase (EC 2.4.1.22, 0.1 U). The resultant solution was incubated 36 h at 37 °C. Flash chromatography of the residue on a column of silica gel with 5 : 4 : 3 BuOH–EtOH–H_2O gave **5**, R_f 0.20 (68%). ^{1}H NMR (D_2O, 400 MHz) δ_H 1.3 (m, 2H, CH_2), 1.50 (m, 4H, 2 CH_2), 2.0 (s, 3H, Me–CO), 2.25 (m, 2H, CH_2 α to CO), 3.2–4.0 (m, H β-CD), 4.42 (d, *J* 7.5 Hz, 1H, H-1 Gal), 4.47 (d, *J* 7.5 Hz, 1H, H-1 GlcNAc), 5.05 (d, 7H, H-1 β-CD).

§ A minimum of six flocculation tests were carried out for each system, the results show a standard deviation (SD : ±10%).

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