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Synthesis of Cyclodextrin Derivatives Carrying Bio-Recognisable Saccharide Antennae

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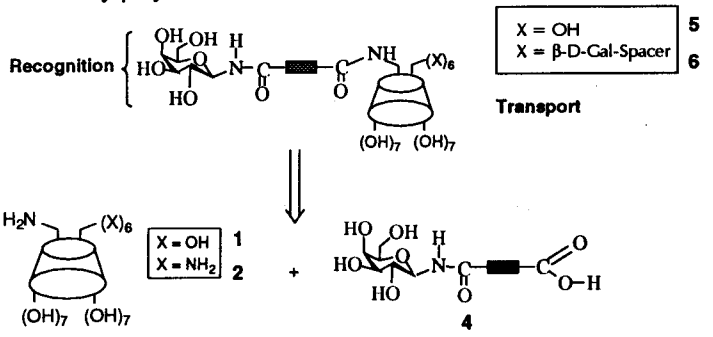
Abstract: The use of coupling saccharide antenna onto cyclodextrins allows mobility for the biologically active galactose head group, and allows the recognition process by the lectin (KbCWL). This paper reports a chemical synthesis of β-CD derivatives using spacer arms 3, 4, 5, 6 and 9 carbon atoms. Preliminary results suggested that recognition is strongly dependent on the length of the spacer chain between the cyclodextrin and the sugar head group. © 1997 Elsevier Science Ltd.

Cyclodextrins (CDs) are interesting candidates for the transport of biologically active molecules.[1] They are a class of cyclic oligosaccharides having 6,7 or 8 glucopyranosyl units linked α-1,4 (α-, β-, or γ-CD). The internal cavity of CD is slightly apolar. In this way, CDs are capable of including a wide range of organic molecules by interaction between host and guest molecules.[2] This type of molecular encapsulation may protect a great number of sensitive products. In particular, β-CD and its derivatives have been widely used for the solubilisation and non-specific transport of biologically active molecules.[3] In this work, the strategy consists of increasing the capacity and specificity of the transport and recognition properties by formation of a neo-glycoconjugate *via* an attached saccharide antenna.

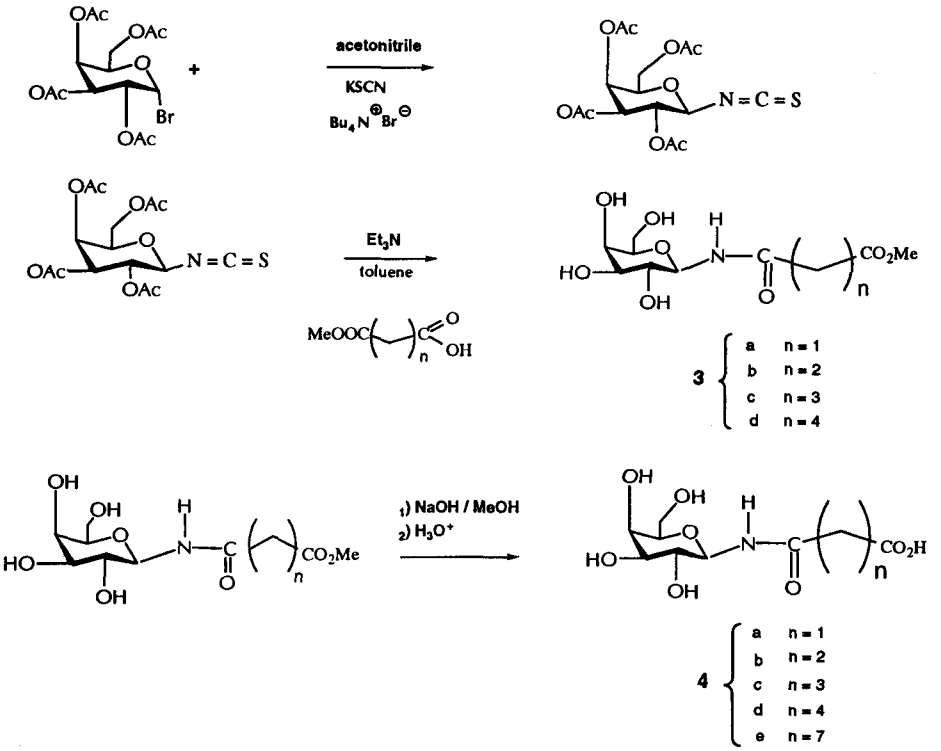
In preceding work, we demonstrated the capacity of galactosyl-β-cyclodextrin derivatives to be recognized by a galactose specific cell wall lectin (KbCWL).[4] This preliminary work suggested that recognition is strongly dependent on the length of spacer chain between the cyclodextrin and the sugar head group and also on the number of sugars presented to the lectin.

This project considers the synthesis of carriers of biologically active molecules. In a first series, Gal head groups were connected to mono-6-amino-6-deoxy-β-cyclodextrin using varying spacer chain lengths (3,4,5,6 and 9 carbon atoms). In the second part, a carrier having seven Gal head groups was constructed from the per-6-amino-6-deoxy-β–cyclodextrin.

The synthesis was carried out *via* standard peptide coupling of the β-D-Gal spacer molecule onto mono[5] **1** and heptakis-6-amino-6-deoxy-β-cyclodextrin[6] **2**.



The β-cyclodextrin derivatives **5** and **6** were synthesised respectively by condensation of 1.2 equiv of β -D-Gal spacer **4** with mono-6-amino-6-deoxy-β-cyclodextrin **1** and of 8.4 equiv of **4** with heptakis-6-amino-6-deoxy-β-cyclodextrin **2** in dry DMF at 15°C using dicyclohexylcarbodiimide/1-hydroxybenzotriazole hydrate (DCC/HOBT, 1 equiv /1.4 equiv) as coupling reagents. The glycoconjugates **4a-e** were synthesised from the corresponding tetra-O-acetyl-α–D-bromo galactose in three steps. The synthetic route depends on the easy conversion of the saccharide isothiocyanate compounds into amides[7]. The starting compounds are prepared in a phase transfer reaction of the corresponding halides with KSCN and tetrabutyl ammonium bromide (Bu_4N^+Br) in acetonitrile in the presence of molecular sieves (4·Å). The tetra-O-acetyl-β-D-galactosyl isothiocyanate (3.85 mmol) is condensed with the mono-methyl-esters corresponding to malonic acid (C_3), succinic acid (C_4), glutaric acid (C_5), adipic acid (C_6) and nonanoic acid (C_9) (1.4 equiv) in anhydrous toluene (20ml) in the presence of triethylamine (0.1 equiv). This new products **3a-d** were purified by flash chromatography (toluene/acetone: 8/2).[8] Complete ester hydrolysis (acetate and methylester) could be achieved with methanolic sodium hydroxide (1M) during 4 h at room temperature.[9]



The final products **5a-d** and **6** are obtained in 35-52% yields after purification on silica gel chromatography (n-butanol/EtOH/H_2O: 5/4/3) and characterised by ^1H NMR (300MHz and 400MHz)[10] and electrospray mass spectrometry. The H-1 signals of β–cyclodextrin were observed at 4.84 ppm (d, J=1.5Hz for the mono CD derivatives **5** and non resolved signal for the per-CD-derivative **6**) and the H-1 signals of the β–D-Gal residues

at 4.68 ppm (J=7.5Hz). The broadening of the β-CD H-1 signals in the case of the per-substituted system, may arise from slow conformational rearrangement of the amido substituents as observed for polysubstituted amino-acid derivatives of β-CD by Stoddart.[6]

In order to confirm complete substitution electrospray mass spectroscopy (negative mode) was carried out, this showed mass peaks corresponding to m/z= M-3H$^+$/3 at 1147. NOE difference experiments show interactions between the Gal H-1 proton and the adjacent amide proton. In the β-D-Gal-spacer-β–CD-derivatives no NOESY and ROESY effects are seen between the Gal-amide proton and the H-1 β–cyclodextrin protons. Thus saccharide antennae are directed out of the cavity of the β-CD into the aqueous environment. Hence this system is available for the transport.

The capability of new compounds to be recognised by biological systems was tested by use of the galactose specific lectin K_bCWL (K_b:*Kluyveromyces bulgaricus*). These results show the minimum concentration necessary of the new molecules to inhibit the flocculation activity of the K_bCWL in a solution corresponding to 3.4 activity units $\mu g^{-1}$of protein.

Preliminary studies showed that β–CD derivatives **5** have a biological activity. The concentration inhibiting K_bCWL floculation is included between 3.5 mmol dm^{-3} for a short spacer and 1.75 mmol dm^{-3} for a long spacer. In contrast to our expectations of obtaining a "clustering effect" per- substitution of β-CD by the β–D-Gal-C9 head group **6** leads to only a 1.5 fold increase in recognition. This may on effect arise from cross linking of cells by the large (40 Å diameter) molecule leading to cell flocculation.

We have synthesised different types of cyclodextrin derivatives directed towards recognition by β–D-Gal specific cell wall lectins (five new carriers). Our results show that the chemical modification of β-CD by substitution of primary hydroxyl on the ring with galactose end arms induces their recognition by carbohydrate proteins. This is an "exo-recognition" process not involving the cyclodextrin cavity.

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8 **3-[N-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosylamino)] ethyl malonate 3a:** Yield (78%), Rf: 0.43,^{1}H RMN ($CDCl_3$), δ_H ppm, 1.21 (t, 3H, CH_3); 1.95,1.97,1.99,2.07 (s, 12H, CH_3-C=O); 3.33 (s, 2H, CH_2-C=O); 3.98 (m, 2H, H-6a et H-6b); 4.07 (q, 2H, CH_2); 4.11-4.18 (m, 1H, H-5); 5.09 (dd, 1H, $J_{2,3}$=9Hz, $J_{2,1}$=9Hz, H-2); 5.20 (dd, 1H, $J_{3,2}$=9Hz, $J_{3,4}$=4Hz, H-3); 5.28 (dd,1H, $J_{H1,NH}$=9Hz, $J_{1,2}$=9Hz, H-1); 5.37 (dd, 1H, $J_{4,3}$=4Hz, $J_{4,5}$=0,8Hz, H-4); 7.18 (d, 1H, $J_{NH,H1}$=9Hz, NH); 7.1 (q, 2H, CH_2).

4-[N-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosylamino)] methyl succinate 3b: Yield (80%), Rf: 0.40, ^{1}H RMN ($CDCl_3$), δ_H ppm, 1.27,1.31,1.37,1.41 (s, 12H, CH_3-C=O); 1.73 (t, 4H, $2CH_2$-C=O); 2.18 (s, 3H, OCH_3); 2.26-2.40 (m, 2H, H-6a et H-6b); 3.35-3.40 (m, 1H, H-5); 3.55 (dd, 1H, $J_{2,3}$=10Hz, $J_{2,1}$=7Hz, H-2); 3.8 (dd, 1H, $J_{3,2}$=10Hz, $J_{3,4}$=5Hz, H-3); 3.9 (dd, 1H, $J_{H1,NH}$=8Hz, $J_{1,2}$=7Hz, H-1); 4.62 (dd, 1H, $J_{4,3}$=5Hz, $J_{4,5}$=1Hz, H-4); 8.39 (d, 1H, $J_{NH,H1}$=8Hz, NH).

5-[N-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosylamino)] methyl glutarate 3c: Yield (72%), Rf: 0.42, ^{1}H RMN ($CDCl_3$), δ_H ppm, 1.91,1.97,1.99,2.11 (s, 3H, CH_3-C=O); 2.3- 2.4 (m, 2H, CH_2); 2.51 (t, 2H, $2CH_2$-C=O); 3.56 (s, 3H, OCH_3); 3.97-4.02 (m, 2H, H-6a et H-6b); 4.27-4.33 (m, 1H, H-5); 4.96-5.01(dd, 1H, $J_{2,3}$=10Hz,$J_{2,1}$=10Hz, H-2); 5.19 (dd, 1H, $J_{3,2}$=10Hz,$J_{3,4}$=3Hz, H-3); 5.28 (dd, 1H, $J_{H1,NH}$=10Hz, $J_{1,2}$=10Hz, H-1); 5.44 (dd, 1H, $J_{4,3}$=3Hz,$J_{4,5}$=1.2Hz,H-4); 8.8 (d, 1H, $J_{NH,H1}$=10Hz, NH).

6-[N-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosylamino)] methyl adipate 3d: Yield (85.4%), Rf: 0.45, ^{1}H RMN ($CDCl_3$), δppm 1.58 (m, 4H, 2 CH_2,); 1.95,1.99,2.01,2.04; (s, 12H, CH_3-C=O); 2.20 (t, 4H, 2 CH_2-C=O); 3.67 (s, 3H, OCH_3); 3.8 (m, 2H, H-6a et H-6b); 4.09 (m, 1H, H-5); 5.08 (dd, 1H, $J_{2,3}$=9Hz, $J_{2,1}$=9Hz, H-2); 5.17 (dd, 1H,

$J_{3,2}$=9Hz,$J_{3,4}$=3Hz, H-3); 5.20 (dd, 1H, $J_{H1,NH}$=9Hz,$J_{1,2}$=9Hz, H-1); 5.40 (dd, 1H, $J_{4,3}$=3Hz,$J_{4,5}$=1.5Hz, H-4); 6.44 (d, 1H, $J_{NH,H1}$=9Hz, NH).

9 The new products, very hygroscopic, were identified from their ^{1}H NMR spectra (DMSO-d_6):

3-(N-β-D-galactopyranosylamino)-malonic acid 4a Yield (46 %), δH ppm, 3.11 (s, 2H, CH_2-C=O); 3.28 (m, 1H, H-2); 3.4-3.71 (m, 4H gluc); 4.05 (m, 1H, H-3); 4.67 (m, 1H, H-1); 8.64 (d, 1H, $J_{NH,1}$=10Hz, NH).

4-[N-β-D-galactopyranosylamino)-succinic acid 4b: Yield (66%), δH ppm, 1.82 (t, 4H, 2CH_2-C=O);2.14-2.28 (m, H-2); 2.22-2.38 (m, 4H Gluc); 3.35-3.44 (m, H-3); 4.08-4.30 (m, 1H, H-1); 8.39 (d, 1H, $J_{NH,1}$=9.3Hz, NH).

5-(N-β-D-galactopyranosylamino)-glutaric acid 4c: Yield (72%) δH ppm, 1.36-1.40 (m, 2H, CH_2); 2.43 (t, 4H, 2CH_2-C=O); 3.06-3.15 (m, 1H, H-2); 3.16-3.20 (m, 4H Gluc); 3.35-3.39 (m, 1H, H-3); 4.58-4.66 (m, 1H, H-1); 8.49 (d, 1H, $J_{NH,1}$=9Hz, NH).

6-(N-β-D-galactopyranosylamino) adipic acid 4d: Yield (54%), δ ppm, 2.11 (t, 4H, 2CH_2-C=O); 3.25 3.33 (m, 1H, H-2); 3.48-3.59 (m, 4H Gluc); 4.28-4.52 (m, 1H, H-3); 4.60-4.69 (m, 1H, H-1); 8.35 (d, 1H, $J_{NH,1}$=9Hz, NH).

10 The new products were identified from their ^{1}H NMR spectrum (DMSO-d_6) and ES.MS negative mode: **Mono-6-[3-(β-D-galactopyranosylamino)-1,3-dioxopropyl]amino-6-deoxy-β-cyclodextrin, 5a:**Yield 50%, δppm 1.87 (s, 2H, CH_2-C=O); 3.06-3.35 (m, H Gluc β-CD and H-2 Gal); 3.64 (m, 7H, H-3 β-CD); 3.8 (m, 1H, H-3 Gal); 4.48 (m, 6H, OH-6 β-CD); 4.68 (d, 1H, H-1 Gal); 4.77 (d, 7H, H-1 β-CD); 5.71 (m, 16H, OH-2, OH-3 β-CD and OH-2, OH-3 Gal); 7.31 (m, 1H, NH β-CD); 7.58 (d, 1H, J=9Hz, NH Gal). ES.MS: m/z 1379.6 $[M\text{-}H^+]^-$, 689.3 $[M\text{-}2H^+]^{-2}$ /2.

Mono-6-[4-(β-D-galactopyranosylamino)-1,4-dioxybutyl]amino-6-deoxy-β-cyclodextrin, 5b: Yield 46%, δppm 2.09 (t, 4H, 2CH_2-C=O); 3.35-3.59 (m, H Gluc β-CD, H-2 Gal); 3.61 (m, 7H, H-3 β-CD); 3.65 (m, 1H, H-3 Gal); 4.52 (m, 6H, OH-6 β-CD); 4.67 (d, 1H, H-1 Gal); 4.84 (d, 7H, H-1 β-CD); 5.76 (m, 16H, OH-2, OH-3 β-CD and OH-2, OH-3 Gal); 7.58 (m, 1H, NH β-CD); 7.70 (d, 1H, J=9Hz, NH Gal). ES.MS: m/z, 1393.6 $[M\text{-}H^+]^-$, 696.3 $[M\text{-}2H^+]^{2-}$ /2.

Mono-6-[5-(β-D-galactopyranosylamine)-1,5-dioxopentyl]amino-6-deoxy-β-cyclodextrin, 5c: Yield 54.5%, δ ppm 2.09 (m, 2H, CH_2 β to C=O); 2.51 (t, 4H, 2CH_2-C=O); 3.4 (m, H Gluc β-CD and H-2 Gal); 3.61 (m, 7H, H-3 β-CD); 3.64 (m, 1H, H-3 Gal); 4.47 (m, 6H, OH-6 β-CD); 4.7 (d, 1H, H-1 Gal); 4.89 (d, 7H, H-1 β-CD); 5.76 (dd, 16H, OH-2, OH-3 β-CD and OH-2, OH-3 Gal); 7.2 (m, 1H, NH β-CD); 7.6 (d, 1H, J=9.1Hz NH). ES.MS: m/z, 1407.6 $[M\text{-}H^+]^-$, 703.3 $[M\text{-}2H^+]^{2-}$ /2.

Mono-6-[6-(β-D-galactopyranosylamino)-1,6-dioxohexyl]amino-6-deoxy-β-cyclodextrin, 5d: Yield 52%, δ ppm 2.41 (m, 4H, 2CH_2 β to C=O); 2.51 (t, 4H, 2CH_2-C=O); 2.74 (m, H Gluc β-CD, H-2 Gal); 2.90 (m, 7H, H-3 β-CD); 3.35 (m, 1H, H-3 Gal); 3.64 (m, 6H, OH-6 β-CD); 4.48 (d, 1H, H-1 Gal); 4.83 (d, 7H, H-1 β-CD); 5.7 (m, 16H, OH-2 and OH-3 β-CD and OH-2 et OH-3 Gal); 7.2 (m, 1H, NH β-CD); 7.96 (d, 1H, J=10Hz, NH Gal). ES-MS: m/z, 1421.6 $[M\text{-}H^+]^-$, 710.3 $[M\text{-}2H^+]^{2-}$ /2.

Per-6-[9-(β-D-galactopyranosylamino)-1,9-dioxononyl]amino-6-deoxy-β-cyclodextrin, 6: Yield 35%, (D_2O) δ 1.3 (m, 42H, CH_2 spacer); 1.55 (m, 28H, CH_2 β to C=O); 2.3 (m, 28H, CH_2 α to C=O); 3.4-3.9 (brd, H Gluc β-CD and H Gal); 3.95 (d, 7H, J=1Hz, H-4 Gal); 4.87 (d, 7H, J=7.5Hz, H-1 Gal); 5.05 (brd, 7H, H-1 β-CD). ES.MS: m/z, 1147 $[M\text{-}3H^+]^{3-}$ /3.

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