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SYNTHESIS OF NEW OLIGOSACCHARIDYL-THIO-β-CYCLODEXTRINS (CDS): A NOVEL FAMILY OF POTENT DRUG-TARGETTING VECTORS.

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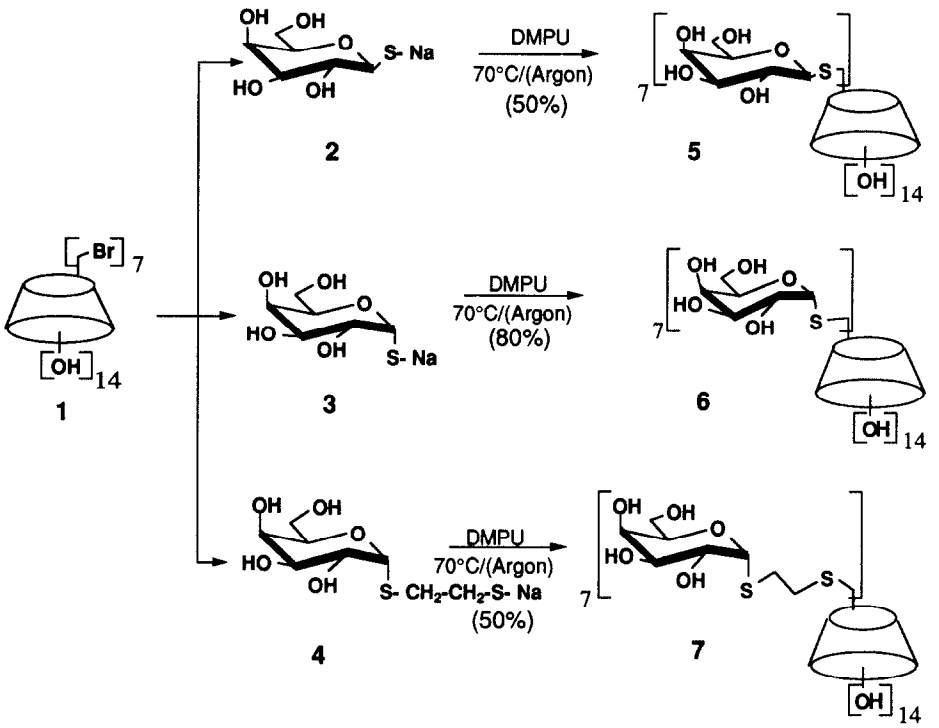
Abstract: Six potential carriers incorporating one or more thio-D-galactosyl residue(s) linked to β-cyclodextrin as complexing tool, were synthesized and evaluated *in vitro* for their specific recognition towards a cell wall galactose specific lectin (KbCWL). The inhibition of yeast flocculation by the compounds **5** -**10** was compared to reference compounds as, *p*-nitrophenyl-galactose or disaccharides containing an α or β-galactose non reducing terminal unit. Among the tested molecules, the heptakis galactosyl-CD derivatives were found the most efficient compounds.

In the precedent paper [1] we demonstrated the capacity of two new oligosaccharidyl-18-6 crown ethers to recognize a cell wall galactose specific lectin (KbWCL). To extend their limited complexation properties we decided to explore the utilization of modified CDs as hosts for a larger class of neutral guests.

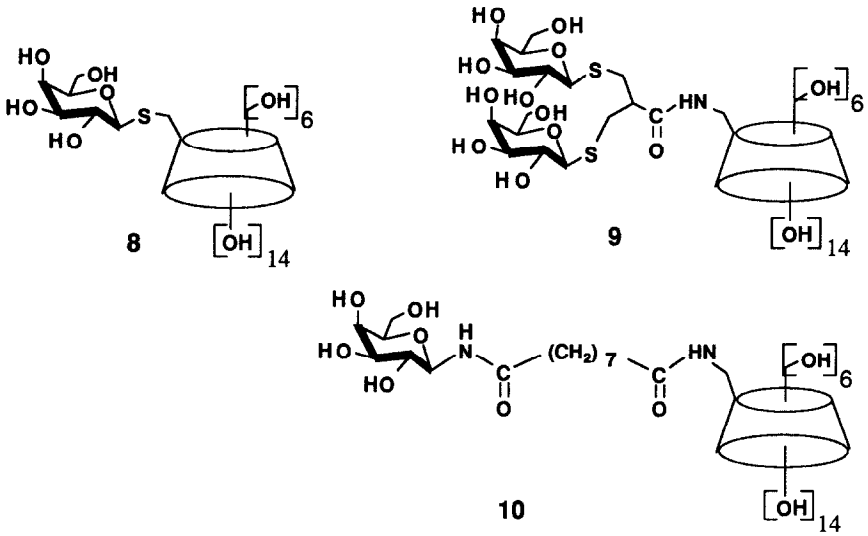
The present work deals about the synthesis of six carriers **5-10** incorporating one or more galactose residue(s) linked to a β-cyclodextrin as complexing tool for hydrophobic drugs and the *in vitro* evaluation of their specific recognition against a cell wall galactose specific lectin. Lectins have been selected as targets for potential vectors of drugs according to their biological status [2] (*i.e.* cell interaction molecules and specific sugar receptors). The later was previously isolated from a *Kluyveromyces bulgaricus* strain and works as aggregating agent in the yeast flocculation process [3].

The new β-CD derivatives **5**, **6** and **7** [7] were prepared in three steps from (6-bromo-6-deoxy)-cyclomaltoheptaose **1** [4] and either, the sodium salts of 1-thio-β-D-galactopyranose **2** [5], 1-thio-α-D-galactopyranose **3** [6] and 1,2-ethanedithio-α-D-galactopyranose **4** (Scheme 1). The final compounds **5** to **7** were obtained in moderate to good yields (50%, 80%) by crystallization from their aqueous solutions .

Syntheses of the 6-β-D-galactopyranosyl-6-thio-cyclomaltoheptaose **8** and of *N*-(6-deoxy-cyclomaltoheptaosyl)-2-(β-D-galactopyranosylthiomethyl)-3-(β-D-galactopyranosylthio)-propanamide **9** were described in earlier works of two coauthors of this paper [8,9] and (mono-6-(1-octanoyl-β-D-galactopyranosylamido)amino-6-deoxy-β-cyclodextrin) **10** (Scheme 2) was a friendly generous gift ¶ [10]. The *in vitro* flocculation-test were achieved following the method early described by Al-Mahmood *et al.*[11] on a lectin solution with decreasing concentrations of references and compounds **5** to **10** in the range of 2.10^{-2} mol/L to 1.10^{-5} mol/L, further incubated for 30 min. at 20°C in the presence of an E.D.T.A. deflocculated yeast cell-suspension. All results are given in the following tables 1 and 2.



Scheme 1



Scheme 2

Among the reference compounds the best activities were found with modified *p*-nitrophenylsaccharides (PNP) and melibiose. No effects were observed with unsubstituted α and β-CDs. Note also that the α-anomer was twice more efficient than the β-anomer for the α *vs.* β-PNP-galactoside. Concerning the inhibition level of respectively α and β anomers, similar difference were obtained with the modified CD **5** and **6**.This result suggests that configuration at the anomeric carbon in terminal galactosyl residue plays an important role in recognition. Likewise, the presence of a medium-length hydrophobic spacer between the terminal ose unit and the carrier in **7** and **10** gave better results than in the case of earlier aromatic 18-6 crown ethers (M.I.C. = 2.66 and 3.00 mM)[1].

Table 1. *In vitro* flocculation inhibitory ability of reference mono and oligosaccharides towards KbCWL[b]

Monosaccharides	M.I.C.(mM)[a)]	oligosaccharides	M.I.C.(mM)[a)]
D-Fucose	3.5	Melibiose	0.60
D-Arabinose	no inhibition	Raffinose	1.20
D-Glucose	no inhibition	β-cyclodextrin	no inhibition
PNP- α-galactoside	0.40	α-cyclodextrin	no inhibition.
PNP -β-galactoside	0.83		

[a] The M.I.C. is the Minimum Inhibitory Concentration in mM that totally inhibits flocculation (mean value of 3 measurements)
[b] KbCWL solution activity was 3.4 A.U./μg of protein. (1A.U. = Inhibition capacity of a 0.5mg/ml lectin solution towards a given yeast cell suspension.)[12]

Table 2. *In vitro* flocculation inhibitory capacity of compounds **5 -10** towards KbCWL

Compounds	M.I.C. (mM).
5	1.66
6	0.70
7	0.41
8	3.75
9	2.00
10	1.70

This indicates probably more the requirement of a minimum steric hindrance in close proximity of the receptor-binding unit, than the necessity of a lipophilic character of the spacer. Finally, the polysubstitution in **6, 7** by seven galactosyl residues on the upper ring of CDs leads to a better level of lectin recognition than the monosubstituted **8, 10** or the digalactosyl **9** CD derivatives.

In conclusion, the initial aim of this work was the synthesis of new vectors of xenobiotics bearing galactosyl residue(s) linked to 18-6 crown ethers or thio-β-cyclodextrins as complexing tools. We were able to demonstrate here their good recognition capacity towards a yeast cell wall lectin (KbWCL). The complexing abilities of these compounds and relative structures are actually under study in order to vectorize and enhance the potency and bioavailability of anticancer intercalating drugs.

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7. All new compounds gave satisfactory spectroscopic and analytical data in full accord with their structures. Selected mass spectra and ^{13}C NMR data for new compounds are included below.
S-α-D-galactopyranosyl-ethane-1,2-dithiol sodium salt 4: yield (0.92 g, 99%), MS (DCI) *m/z* 274 $[M+NH_4^+]^+$; ^{13}C NMR (D_2O): δ(ppm) 87.9 (C-1); 73.2, 72.1, 70.9, 69.7 (C-2, 3, 4, 5); 62.6 (C-6); 39.1, 30.7 (CH_2-S-).
Heptakis(6-deoxy-6-S-β-D-galactopyranosyl) 6-thio-cyclomaltoheptaose 5: yield (0.3g, 50%). $[\alpha]_D^{25}$ +62.7 (c 0.5, water). MS (FAB$^+$) *m/z* 2405 $[M+H^+ + Na^+]$; ^{13}C NMR (D_2O): δ(ppm) 103.4 (C-1); 88.6 (C'-1); 81.8, (C-4); 80.3, 75.5, 74.3, 73.5, 72.3, 71.8, 70.4 (C-2, 2', 3, 3', 4', 5, 5'); 62.5 (C-6'), 33.9 (C-6).
Heptakis(6-deoxy-6-S-α-D-galactopyranosyl) 6-thio-cyclomaltoheptaose 6: yield (0.44g, 80%). $[\alpha]_D^{25}$ +262.0 (c 0.5, water). MS (FAB$^+$) *m/z* 2405 $[M+H^+ + Na^+]$. ^{13}C NMR (D_2O) : δ(ppm) 103.3 (C-1); 87.7 (C'-1); 85.9 (C-4); 74.2, 73.5, 72.9, 72.8, 71.7, 70.6, 69.1 (C-2, 2', 3, 3', 4', 5, 5'); 62.5 (C-6'); 32.6 (C-6).
Heptakis(6-deoxy-6-S-β-D-galactopyranosyl-1',2'-dithioethyl) 6-thio-cyclomaltoheptaose 7: yield (0.3g, 50%). $[\alpha]_D^{25}$ +190.1 (c 0.5, water). MS (FAB$^+$) *m/z* 2824 $[M+H^+ + Na^+]$. ^{13}C NMR (D_2O) : δ(ppm) 103.2 (C-1); 87.7 (C'-1); 86 (C-4); 75.6, 74.5, 73.5, 72.8, 71.8, 70.6, 69.4 (C-2, 2', 3, 3', 4', 5, 5'); 62.1 (C-6'); 34.7 (CH_2-S); 31.8 (C-6).
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