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New Oligosaccharidic Crown Ethers as Potential Drug-Targetting Vectors: Synthesis & Biological Evaluation

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Abstract: Two potential carriers (compounds **4** and **6**), incorporating one galactose residue linked to a 18-6 crown ether as complexing tool, were synthesized for the first time using the trichloracetimidate method and evaluated for their *in vitro* specific recognition of a cell wall galactose-specific yeast lectin (KbCWL).

Lectins are cellular non-immune membrane-glycoproteins receptors for free monosaccharides, conjugated oligosaccharides, or oligosaccharidyl-antenna of circulating macromolecules[1]. Moreover, it is well known that lectins precipitate glycolipids or glycoproteins and can also agglutinate cells and micro-organisms[2]. This affinity for simple and complex oligosaccharidic structures determines an important role in specific interactions between putative biotic or abiotic vectors and targets cells[3]. Thus, lectins (and selectins) are gaining a growing importance as cellular adhesion models[4] and some drugs-bounded neoglycoproteins have been reported in this sense for drug-targetting[5].

On the other hand, crown ethers form an important branch of the synthetic receptors family. Among them, chiral crown ethers have been extensively employed for enantiomeric recognition of racemic primary alkyl ammonium cations[6] including those from amino acids[7]. They have also been proposed as enzyme mimics[8] and enzyme analogs[9].

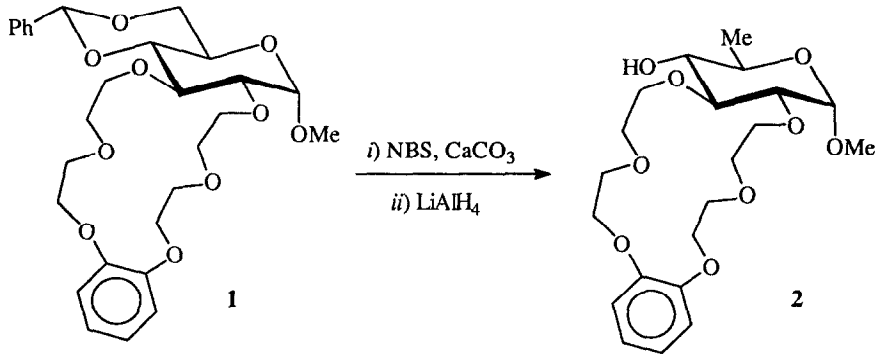
As the foremost part of a prospective chemistry research programme, we decided to design new oligosaccharidic carriers incorporating one non-reducing galactose residue linked to 18-6 crown ethers as complexing tools for cationic species (K^+, essential aminoacids, catecholamines, etc...) and to evaluate their *in vitro* recognition of a galactose-specific lectin isolated from the yeast cell wall.

We chose to use first a crown ether constructed on a D-glucose residue, available through our previously described methodology[10]. Both hydroxyl groups at C-6 and C-4 of the glucose residue of crown ether **1** can be used to link the galactose residue by a *O*-glycosidic bond.

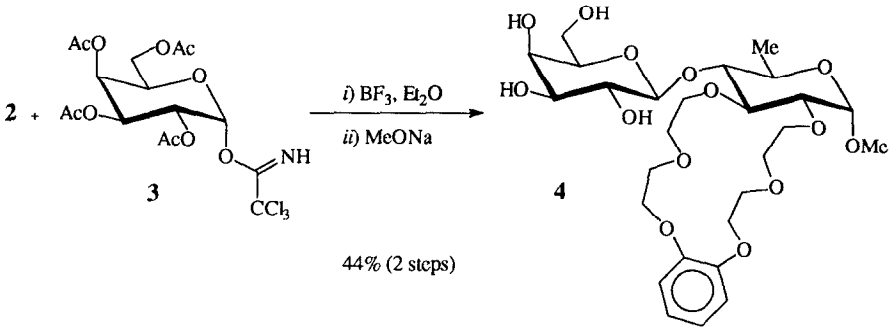
We decided to use the 6-deoxy-4-*O*-unprotected crown ether **2** as the simplest available *acceptor* to avoid any undesirable supplementary interaction of the lectin with the sugar framework incorporated into the crown ether.

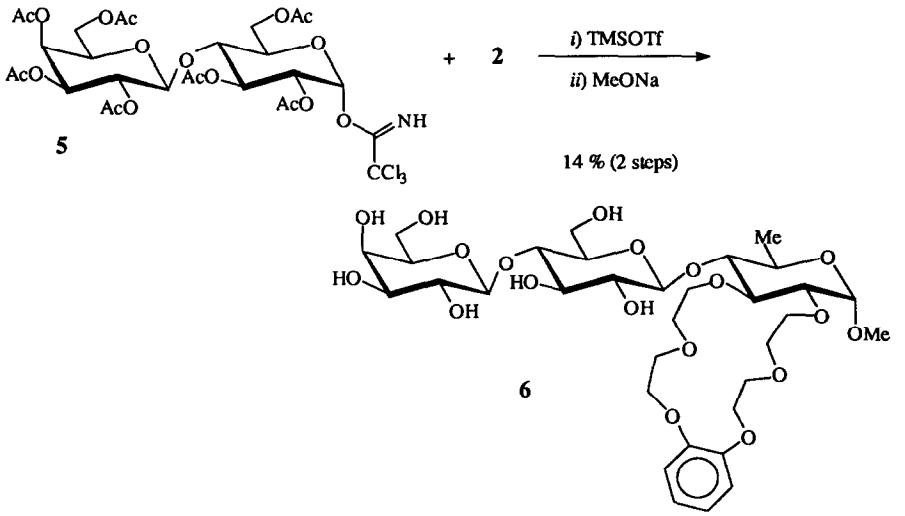
The *acceptor* **2** was readily obtained from crown ether **1**[11] in two steps:

i) ring-opening reaction with *N*-bromosuccinimide[12] in refluxing benzene in 58% yield,
ii) reduction of the 4-*O*-benzoyl-6-bromo-6-deoxy-α-D-glucopyranoside intermediate with $LiAlH_4$[13] (4 eq.) in 87% yield after recrystallization from *i*-PrOH/*n*-hexane as depicted below:



The key step was the *O*-glycosylation between the *acceptor* **2** and the trichloracetimidates of D-galactose **3** or of D-lactose **5** as highly reactive glycosyl *donors*, which were prepared by standard chemistry[14,15]. Different catalysts were tested and using BF_3/Et_2O or TMSOTf (CH_2Cl_2, 4Å molecular sieves, rt), the desired 1-4 β-glycosidic linkage was formed exclusively as seen from ^1H NMR data. A subsequent deprotection (Zemplén deacetylation) afforded the desired target carriers **4** and **6** quantitatively from their peracetylated precursors[16]:





Attempts are actually performed to improve the yield of **6** by using another catalyst such as $ZnBr_2$[15] or/and CH_3CN as the solvent[17]. The *in vitro* flocculation-tests were realized using the method early described by Al-Mahmood *et al.*[18] on a lectin solution, with decreasing concentrations of compounds to be tested 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. The results are summarized in the following table:

Table: *In vitro* flocculation inhibitory capacity of references and oligosaccharidic crown ethers towards KbCWL[a]

Compounds	M.I.C (mM)[b]
Galactose	3.50
Methyl-β-D-galactose	1.75
Lactose	1.25
4	2.65
6	3.00

[a] KbWCL solution activity was 3,4 A.U./µg of protein (1 A.U. = inhibition capacity of a 0,5 mg/ml lectin solution towards a given yeast cell suspension)[19].

[b] The M.I.C. is the Minimum Inhibitory Concentration in mM that totally inhibits flocculation (mean value of 3 measurements)

In summary, two potential drug-targetting vectors, compounds **4** and **6**, incorporating one non-reducing galactose residue linked to a 18-6 crown ether, were synthesized for the first time using the trichloroacetimidate method[14]. Their good *in vitro* specific recognition of a cell wall galactose-specific lectin indicate that the biological activity of the galactose moiety is fairly conserved after the glycosylation stage with the crown ether. The introduction of an hydrophilic glucose moiety as spacer in **6** seems to have no appreciable influence on its final bioactivity.

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16. All new compounds displayed satisfactory spectral data in full accord with their structures.
    For instance, **6**: yield 0.17g (99%), $[\alpha]_{365}$ +109.2° (c = 1, $CHCl_3$, peracetylated precursor); MS (CI): *m/z* 775 (M + Na^+); ^{13}C NMR (CD_3CN): δ (ppm) 149.0 (C-1/C-2 ar), 122.7 (C-4/C-5 ar), 115.1/114.9 (C-3/C-6 ar), 103.9 (C'-1), 103.1 (C-1), 97.7 (C-1 glu), 82.6 (C-4), 80.6 (C'-5), 79.8 (C-5), 79.6 (C-3), 76.2 (C-2 glu), 75.8 (C-3 glu), 75.3 (C-2), 74.4 (C-5 glu), 73.5 (C-4 glu), 72.7 (OCH_2), 71.8 (C'-3), 71.5, 71.0, 70.4, 70.0, 69.9, 69.7, 69.5 (7 x OCH_2), 69.4 (C'-2), 67.5 (C'-4), 61.9 (C'-6), 61.3 (C-6), 55.5 (OMe), 17.7 (C-6 glu).
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