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ORIGINAL ARTICLE

Oligosaccharide tagged β-cyclodextrins: synthesis and biological affinity towards Concanavalin A

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Abstract An original synthetic route based on multi-glycosylation and selective protection–deprotection steps has been developed which allows a fast access to complex oligomannosides with both α-(1,3),α-(1,6) and α-(1,3),α-(1,4) cores. The later have been linked to modified β-cyclodextrins bearing spacing arms of varying chemical structure and length through peptidic-like coupling, leading to the formation of a range of oligomannosyl cyclodextrin conjugates. Complexation studies with sodium anthraquinone-2-sulfonate (ASANa) and sodium adamantane 1-carboxylate (ACNa) as guest molecules demonstrated that the β-cyclodextrin inclusion properties are preserved. Binding affinity studies using the mannose specific lectin Concanavalin A (Con A) demonstrated the key role of the density and tridimensional structure of the sugar ligand in recognition events.

Keywords β-cyclodextrin · Manno-oligosaccharide · Biological interaction · Concanavalin A

Introduction

Many drugs present physical and chemical properties (hydrophobicity, high molecular weight...) which do not allow them to pass over the biological barriers separating the administering sites and the action site. Often the only way to obtain effective drug concentration close to the desired action site is to increase the injected quantity of the therapeutic agent. The compound activity on healthy cells, however, may be increased and so causes side effects. Thus, the specific targeting of therapeutic agents to a given organ or an infected cell represents one of the main challenges in drug development [1]. In the particular case of the HIV treatment, we have approached this problem using oligosaccharide tagged cyclodextrins as drug carrier system. Cyclodextrins (CDs) are cyclic oligosaccharides made up of six to eight D-glucose units (α, β, γCD, respectively) which form natural cages with an internal hydrophobic cavity of defined dimensions. This structural feature allows CDs to form inclusion complexes with hydrophobic guest molecules and, thus, to protect them from the environment [2]. In order to render cyclodextrins specific for HIV infected macrophage cells, we have modified the primary crown with a specific recognition pattern.

Some studies have reported the composition of various oligosaccharide motifs of the gp 120 viral glycoprotein, which is known to intervene in the first step of the recognition between the virus and macrophage cells. Among the described carbohydrate derivatives involved in the recognition phenomena are high-mannose oligosaccharide structures [3]. We attempted to synthesize truncated parts of such high-mannose moiety to use them as mimics of the natural structure. A fast and versatile strategy involving multi-glycosylation and selective protection/deprotection steps has been disclosed for this purpose. In a convergent methodology, this synthetic part was then grafted onto

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mono-6-substituted βCD scaffolds varying by the length and the nature of the spacing arm.

The aim of this study is, firstly, to present the synthetic methodology to access gp 120-related mannooligosaccharide-βCD conjugates and, secondly, to evaluate the influence of structural modifications at the primary rim of βCD in the inclusion properties. In the last part of the study the lectin affinity of the synthesized derivatives has been investigated using Con A as a model.

Experimental part

βCD was purchased from WACKER. Compounds 6–10 were prepared as described previously [4]. All reactions were carried out under an argon atmosphere in oven-dried glassware. Dichloromethane was distilled over calcium hydride. Fully deprotected compounds were purified by HPLC (prevail C18 and ALLtima amino ES 5u). ^{1}H-NMR spectra were recorded in D_2O on a Brucker DMX 300 and AMX 500 spectrometers operating at 300.13 MHz and 500.13 MHz using standard pulse programs from Bruker library. In all experiments, the probe temperature was maintained at 298 K. Chemical shifts are given in ppm downfield from external TMS and the length of the 90° pulse was ca 7.0 μs.

Complexation experiments were carried out with commercial anthraquinone-2-sulphonic acid and 1-adamantanecarboxylic acid converted in the respective sodium salts (ASANa and ACNa). Both species, host and guest, were freeze-dried twice in D_2O before the experiments and finally dissolved in D_2O. Series containing variable ratios of host molecules (1–5) and guest molecules (ASANa or ACNa) were prepared, no precipitation being observed in the whole range of relative proportions.

Results and discussion

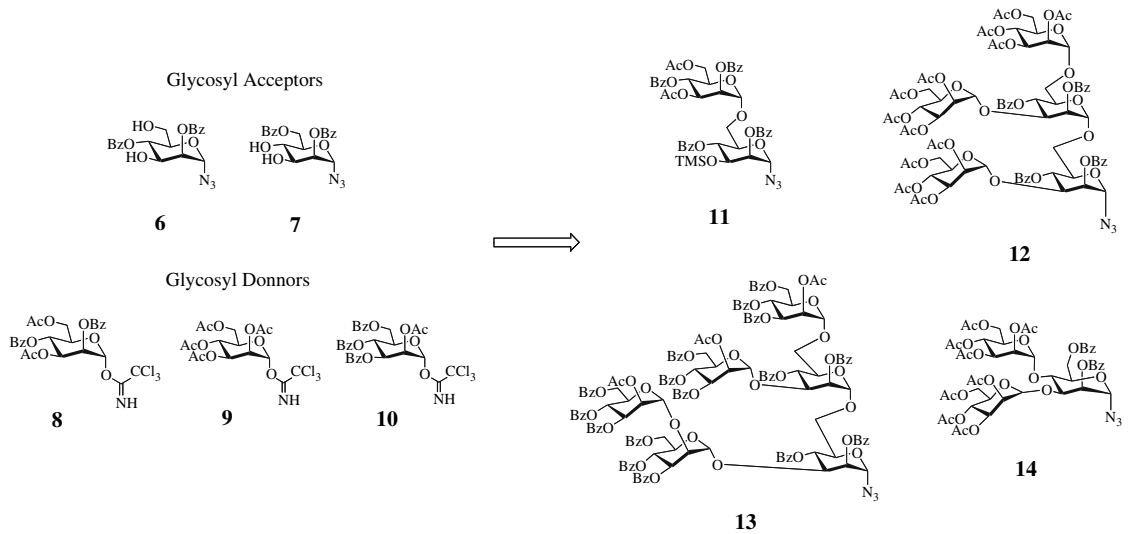
Preparation of the oligosaccharide moiety

An efficient route to the gp 120 oligosaccharide mimics which minimizes the number of steps has been developed using properly modified monosaccharides as starting materials. The strategy is based on multiglycosylation and selective deprotection steps and uses just five monosaccharide units, all of them obtained in few steps from commercial D-(+)-mannose, namely the glycosyl acceptors 6 and 7 and the glycosyl donors 8–10.

Regioselective 6-*O*-glycosylation of the acceptor 6 with the donor 8 afforded the key disaccharide 11 as already described [4]. An unified strategy has then been developed from this intermediate that allows an easy access to the following complex oligosaccharides containing a mannotrisaccharide core with α-(1,3), α-(1,6) linkages (Scheme 1) :

- the pentasaccharide 12,
- the hexasaccharide 13.

Moreover, the second acceptor 7 is used as starting material for the synthesis of the non-natural α-(1,3), α-(1,4) trisaccharide 14, which was included in the biological evaluation studies to determine the importance of the three-dimensional structure of the oligosaccharide in the interaction with specific receptors.



Scheme 1 Complex manno-oligosaccharides synthesised from the 5 monosaccharidic building blocks

Synthesis of the oligosaccharide/βCD glycoconjugates

The presence of an azido group in all these oligosaccharide structures makes possible their attachment to various scaffolds bearing carboxylic acid functional groups, by standard peptide coupling reactions, after a reduction step. Commercial βCD was modified by standard chemical methods (Scheme 2) [5] to afford 6^I-amino-6^I-deoxy-β-cyclodextrin, which allows the incorporation of two different spacing arms with terminal carboxylic functions in few steps, affording compounds 15 [6] and 16, respectively.

The newly synthesised oligosaccharides 12 and 14 have been attached to β-cyclodextrin via spacers of varying chemical structure and length. The peptidic-like coupling reaction between the reduced azido group on the oligosaccharide and modified cyclodextrin derivatives carrying acidic terminal groups in the presence of DIC and HOBt as activator, allowed the synthesis of a broad range of oligomannosyl linked cyclodextrins. Two types of derivatives, 1 to 5, bearing ''short'' and ''long'' spacing arms linked to an α-(1,3), α-(1,4) trisaccharide and to a pentasaccharide have been isolated after deprotection and an HPLC purification step (Scheme 3).

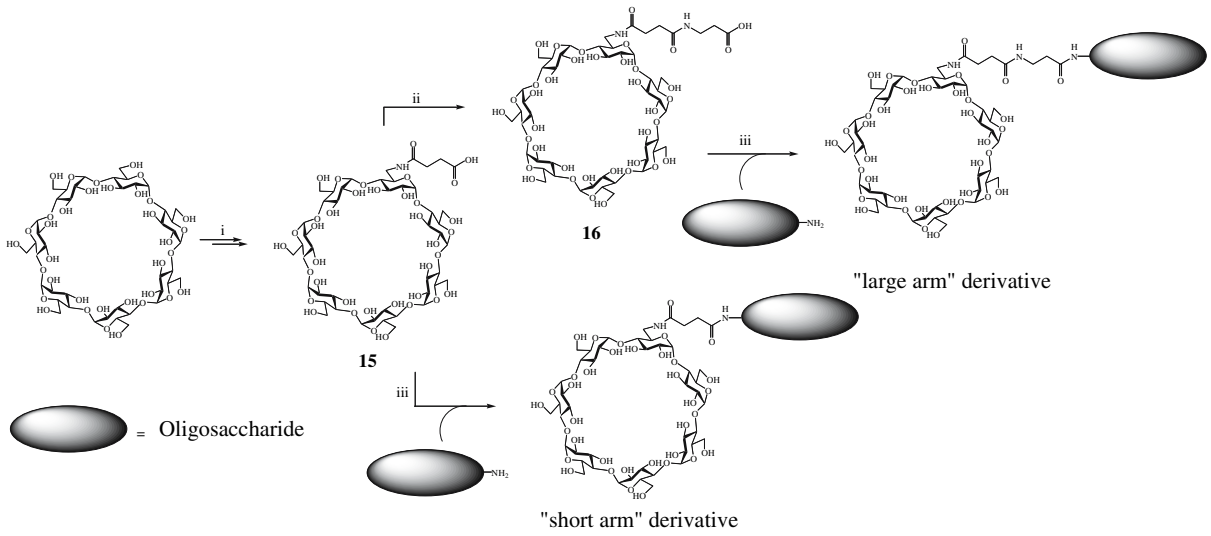
In order to evaluate both the influence of the cyclodextrin moiety and of the sugar linkage type on the oligosaccharide–protein interaction, we have also synthesized the fully unprotected oligosaccharide 17 and the βCD derivative 18 [7], bearing an α-(1,3), α-(1,6) mannotrisaccharide, as reference compounds (Scheme 4).

Inclusion properties

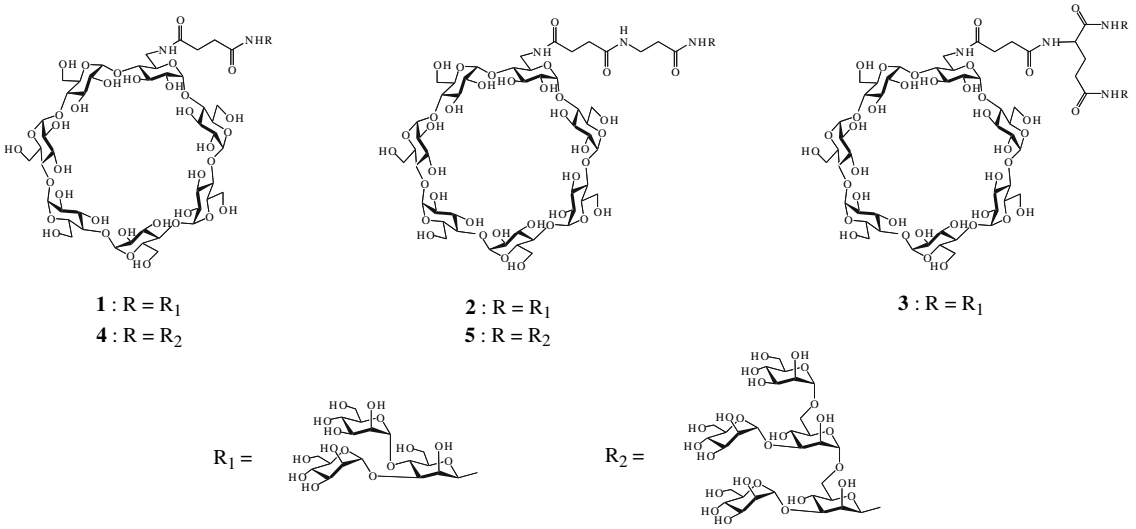
The complexing properties of the CD-oligosaccharide glycoconjugates 1–5 were investigated by ^{1}H-NMR in D_2O with ASANa and ACNa as guest molecules. These molecules have been shown to form inclusion complexes with natural β-cyclodextrin [8, 9]. The association constants at 298 K were experimentally determined by measuring the proton chemical shift changes of 2.5–5 mM solutions of the cyclodextrin derivatives 1–5 upon increased amounts of the guest (ASANa and ACNa). The ^{1}H NMR spectrum of each solution was recorded and the chemical shift of the diagnostic sugar protons obtained at the different host–guest concentration ratios were used in an iterative least-squares fitting procedure [6, 8].

Variations of the chemical shifts as a function of the host:guest ratio were observed. The absence of new peaks arising from the complex indicated that the inclusion process is in a fast exchange regime in the NMR time scale. Thus, the protons located inside the βCD cavity (H3 and H5) were shielded at high magnetic field in the presence of ASANa or ACNa, while protons, situated on the outer face (H2 and H4) were unaffected. Conversely, the ASANa aromatic protons showed a downfield shift characteristic of an inclusion phenomenon involving an aromatic guest.

NMR titration experiments with both guests were consistent with the 1:1 stoichiometry, with association constants (K_{as}) in the same range than those reported for the native βCD (K_{as} = 350 M^{-1} [8] and $K_{as} = 3.9 \times 10^4$ M^{-1} [9], respectively). In fact values of association constant are contained between 290 M^{-1}

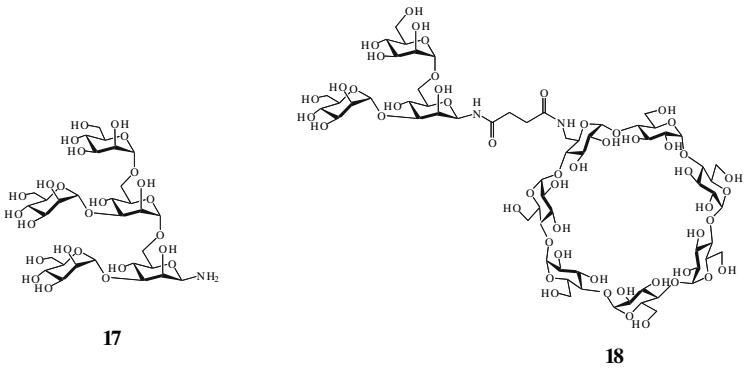


Scheme 2 Selective modification of βCD for the synthesis of the glycoconjugate derivatives



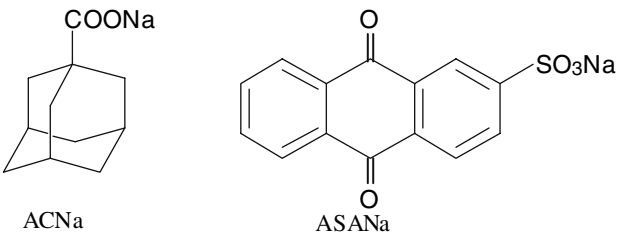
Scheme 3 Oligosaccharide tagged βCD conjugates

Scheme 4 Reference compounds



and 380 M^{-1} when ASANa is used as guest and 1.6×10^4 M^{-1} and 3.5×10^4 M^{-1} with ACNa, respectively. Taking account the experimental errors, it should be postulated that the presence of the saccharidic moiety does not inhibit the inclusion properties of the cyclodextrin part.

Moreover, inclusion complex formation was further demonstrated by T-ROESY experiments, which showed strong dipolar cross peaks between protons located in the cavity of the cyclodextrin derivatives (H3 and H5) and aromatic protons of ASANa (Scheme 5, Fig. 1).



Scheme 5 Structure of the guest molecules

Lectin binding evaluation

Evaluation of the biological receptor recognition properties of the new conjugates was carried out by ELLA (Enzyme Linked Lectin Assay) [10], using horseradish peroxidase-labelled Concanavalin A (HRP-Con A) as a model lectin. Con A is known to posses an extended binding site for the branched trisaccharide 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyaranose, resulting in a binding affinity (log *K* 5.4) much higher than that encountered for trivalent or even higher-valency mannopyranosides [11]. Although Con A is a plant lectin, recent results have shown a parallelism with the mannose/fucose specific receptor at the surface of macrophages in the recognition of oligomannoside ligands [12]. Figure 2 collects the IC_{50} values for the inhibition of the Con A–yeast mannan association, considered to be inversely proportional to the corresponding binding affinities, by compounds 1–5, 17 and 18.

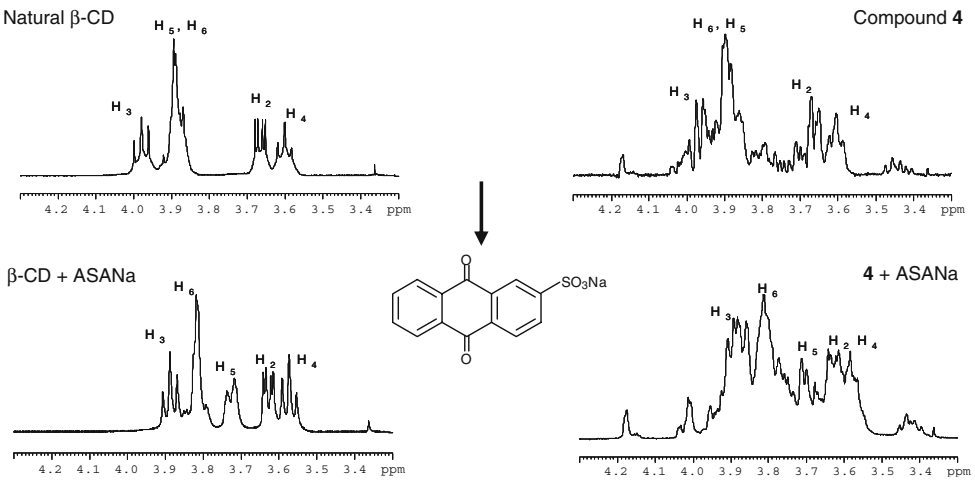


Fig. 1 Partial NMR spectra of (β-CD and compound 4 in presence and in absence of ASANa as guest molecule (500 MHz, 298 K, 10 mM in D_2O)

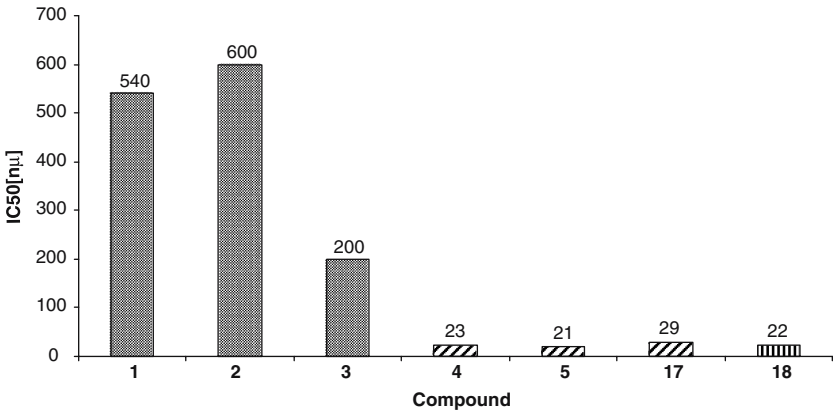


Fig. 2 ELLA results (IC_{50} for the inhibition of the ConA–yeast mannan binding) for compounds 1 to 5, 17 and 18

From the above data, several conclusions with regard to the lectin/oligosaccharide interactions can be drawn. Thus, the IC_{50} values determined for compounds 2 (600 μM) and 3 (200 μM), bearing an α-(1,3),α-(1,4) trisaccharide motif, confirm the major importance of the ligand density in the binding affinity, according to the cluster effect. The length of the spacing arm appears to have only a weak incidence on the accessibility to the saccharide ligand, as inferred from the results obtained for compounds 1 and 2. This observation is confirmed for the pentasaccharide substituted derivatives, for which the difference between the ''short armed'' (4) and the ''long armed'' (5) compounds (ΔIC_{50} = 2 μM) falls within the experimental error. The fact that the oligosaccharide 17 exhibited a IC_{50} value almost identical to that of the CD conjugates 4 or 5 suggests that the CD moiety does not interfere in the mannoligosaccharide-lectin recognition phenomena. In contrast, the oligosaccharide ramification pattern plays a critical role in lectin binding strength. Accordingly, for the same mannosyl ligand density, the ''natural'' α-(1,3),α-(1,6) trisaccharide derivative 18 exhibited a far higher affinity (IC_{50} = 22 μm) than the synthetic α-(1,3),α-(1,4) derivative 1 (IC_{50} = 540 μm).

Conclusion

A fast access to complex oligosaccharides has been developed using properly substituted monosaccharide building blocks. A range of mannosylated βCD conjugates varying by the length of the spacing arm and the nature of the carbohydrate moiety has been synthesised by peptidic-like coupling. These compounds have shown to retain the ability of the cyclodextrin moiety in terms of inclusion of hydrophobic guest molecules. Affinity studies proved the crucial importance of the saccharidic density and linkage type on the recognition phenomena.

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