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Dynamic Multivalent Lactosides Displayed on Cyclodextrin Beads Dangling from Polymer Strings

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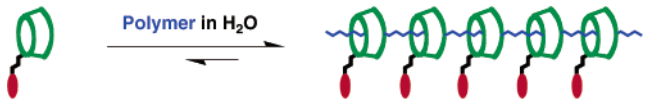
ABSTRACT



Lactose-appended cyclodextrin derivatives have been synthesized and threaded onto hydrophobic polymers in aqueous solution to form dynamic multivalent lactosides for binding to lectins. The threading process, which proceeds quickly, can be observed by one- and two-dimensional NMR spectroscopies.

Protein−carbohydrate interactions mediate both physiological and pathological phenomena,[1] typically using multivalent binding interactions[2] to enhance the relatively weak affinity of a single saccharide ligand for its dispersed and orientation-specific receptor. Multivalent constructs such as glycoclusters,[3] glycopolymers,[4] glycodendrimers,[5] and glycoproteins,[6] which display a large number of saccharide ligands, have been developed as probes to gain a better understanding of protein−carbohydrate interactions in the regulation of biological processes. Recently, interest[7] has been focused upon investigating how the architectural features, particularly those that control the presentation of the ligands, of the glycoconjugates determine their avidity for protein receptors. While these covalent architectures present their ligands in a somewhat constrained manner, we have become interested in developing dynamic systems in which the saccharide ligands can orient themselves with many fewer constraints in a way that maximizes their overall binding interaction with a dispersed and orientation-specific receptor.

Cyclodextrins (CDs), which are cyclic oligosaccharides typically comprised of six, seven, or eight (α-, *β*-, or *γ*-CD,

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respectively) α-1,4-linked D-glucopyranosyl residues have a long recorded history[8] of forming inclusion complexes with hydrophobic guests within their largely hydrophobic cavities. In addition to many 1:1 and 1:2 host−guest interactions, the CDs also form pseudopolyrotaxanes[9] with linear polymers such as poly(ethylene)glycol (PEG),[10] poly(propylene) glycol (PPG),[11] and poly(tetrahydropyran) (PTHP),[12] i.e., numerous CD "beads" thread spontaneously onto polymer "strings." Polyrotaxanes, either formed by reacting[13] bulky stopper groups with the two terminal ends of a pseudopolyrotaxane or by slipping[14] the CD beads over an appropriately sized stopper, have been synthesized using both α- and β-CD. This concept has been utilized in the formation of multivalent structures for the display of a valine−lysine dipeptide[15] and of biotin.[16] Herein, we present the synthesis of pseudopolyrotaxanes comprised of lactose-appended CD beads (Figure 1), which have been threaded onto polymer strings with the

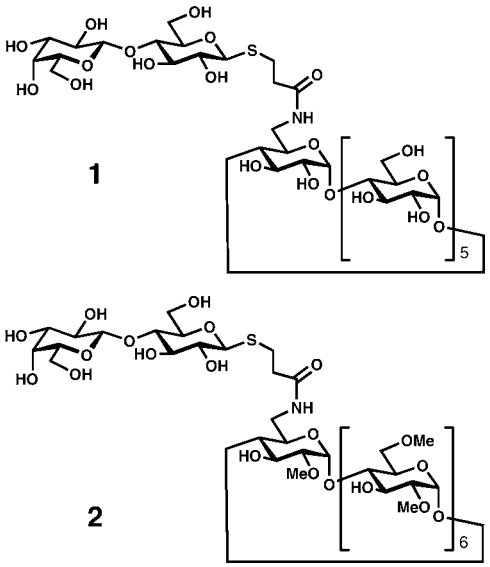
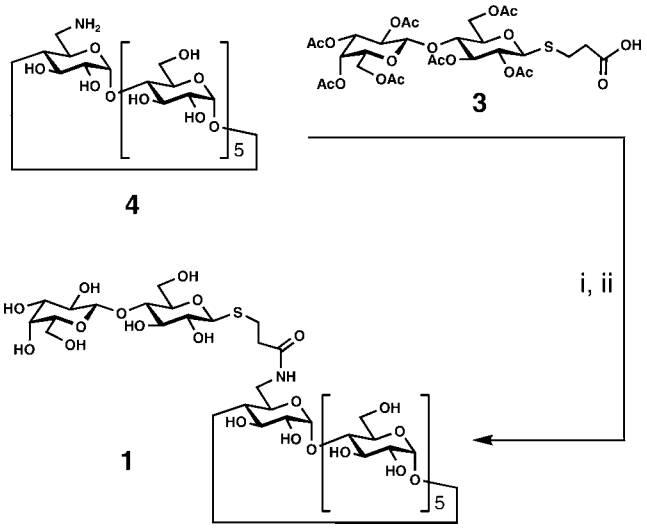


Figure 1. Lactoside-bearing CD derivatives **1** and **2**.

objective of using them as dynamic multivalent glycoconjugates for binding lectins. In principle, the individual CD beads should be able to slide along and to rotate around the polymer axes, allowing the lactose ligands to orient themselves to achieve the most favorable presentation to their protein receptors and thus to maximize their binding interactions.

Scheme 1. Synthesis[a] of Lactose-Appended α-CD **1**



[a] Reaction conditions: (i) HBTU−BF_4/DMF; (ii) NaOMe/MeOH.

The lactose-bearing α-CD **1** was obtained[17] (Scheme 1) by coupling the lactosyl propionic acid derivative **3**[18] with the monoamino cyclodextrin **4**[19] using HBTU−BF_4 to activate the carboxylic acid. The crude product was deacetylated under Zémplen conditions to afford[20] **1**, which was purified by gel filtration chromatography. A pseudopolyrotaxane was formed by first dissolving **1**[21] in D_2O, followed

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(17) **Representative Experimental Procedure.** To a stirred solution of the lactosyl propionic acid **3** (0.88 mmol), HBTU−BF_4 (1.96 mmol), and *i*Pr_2NEt (150 μL) was added the monoamino CD derivative (0.515 mmol) under an argon atmosphere. The reaction mixture was stirred for 24 h and concentrated in vacuo, and the residue was redissolved in EtOAc. The organic layer was then washed with 1 M HCl and brine, dried (Na_2SO_4), and filtered. The organic solution was evaporated to dryness to afford an oil that was dissolved in 1:1 H_2O/MeOH (50 mL) and 0.5 M methanolic NaOMe solution (0.8 mL) and stirred at room temperature for 5 h. The reaction was neutralized using Amberlite IR-120 (H^+ form) and the solvent evaporated to afford a crude oil. The residue was purified by gel filtration chromatography (G-25, 5% BuOH/H_2O) to afford the product.

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by the addition of bis-3-aminopropyl-terminated PTHP (M_n = 1100), which was insoluble in the aqueous solution in the beginning. Following sonication for 40 min, the insoluble material was no longer evident in the aqueous solution, an observation which suggested that solubilization of the hydrophobic polymer had occurred as a result of pseudopolyrotaxane formation. The formation of pseudopolyrotaxanes between the lactose-appended α-CD **1** and a range of hydrophobic polymers was investigated (see, e.g., Figure 2)

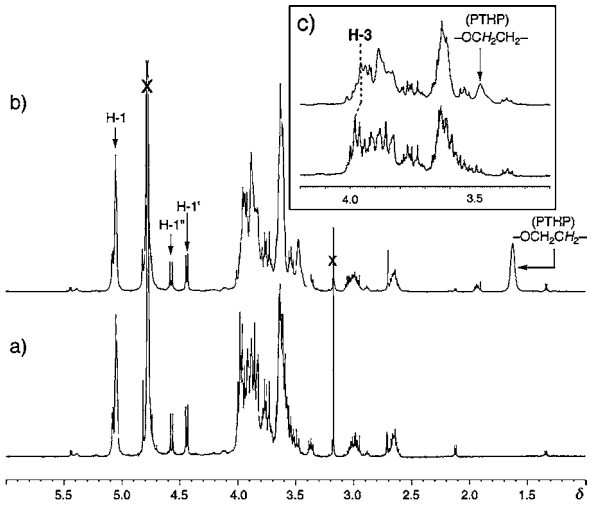

Figure 2. (a) Complete ^{1}H NMR spectrum of the α-CD **1** in D_2O recorded on a 500 MHz spectrometer at 298 K. Following the addition of PTHP and sonication for 40 min, (b) the ^{1}H NMR spectrum of the sample becomes broadened and (c) an expansion of the ^{1}H NMR spectrum shows the upfield shift of the H-3 signal.

by ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum[22] of **1** in D_2O broadened following the addition of PTHP to the solution. The H-3 and H-5-protons (Figure 3) of the D-glucopyranosyl residues comprising the CD point into the center of its cavity, thus allowing them to serve as probes for the complexation of a guest molecule. Upfield shifts were observed for the H-3 protons, an observation which indicates that the PTHP has threaded itself through the torus of the CD. Moreover, a two-dimensional TROESY NMR experiment (Figure 4), performed on the sample containing the

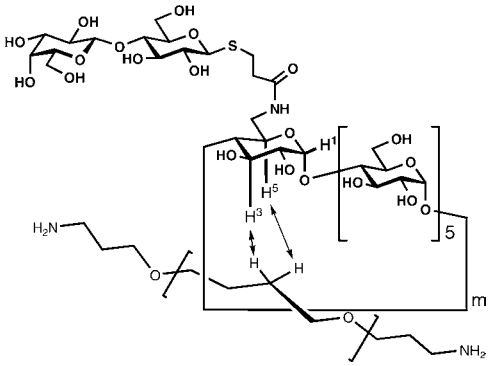

Figure 3. H-3 and H-5 hydrogen atoms of the CD torus point into the cavity and interact with the PTHP polymer chain.

pseudopolyrotaxane formed between **1** and PTHP, revealed cross-peaks between the H-3 and H-5 signals of the CD torus and the signals for the methylene protons in the polymer resonating at δ = 1.4 ppm. The presence of these cross-peaks, which is attributed to the close proximity of the H-3 and H-5 protons of the CD to the methylene protons in the polymer, supports strongly the formation of pseudopolyrotaxane. While water-soluble pseudopolyrotaxanes were formed initially, precipitation started after the aqueous solution had been standing for about 1 month. Precipitates also formed instantaneously when more concentrated solutions (42.7 mM) of the lactose-appended α-CD **1** were investigated.

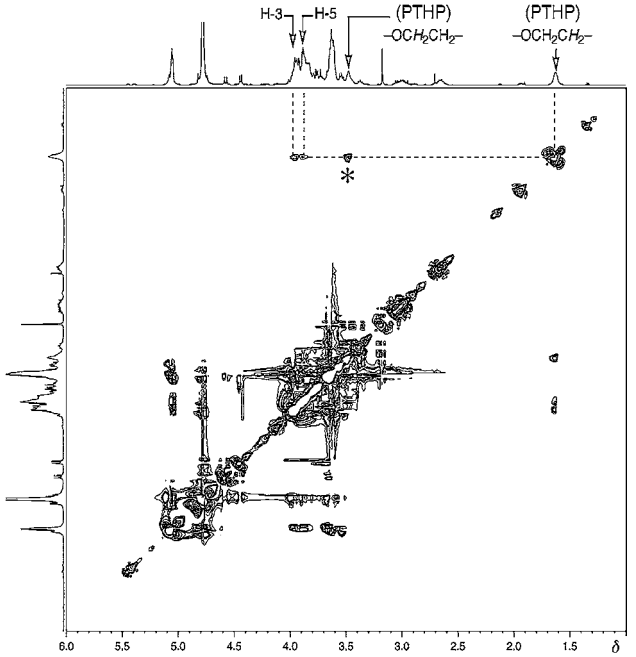

Figure 4. Two-dimensional TROESY NMR spectrum (500 MHz, 298 K) of the pseudopolyrotaxane formed by adding PTHP to a solution of the α-CD **1** in D_2O. The ROE cross-peak between adjacent methylenes of the polymer[26] present in the spectrum is denoted by an asterisk (*).

(20) **Selected Spectroscopic Data for 1.** ^{1}H NMR (500 MHz, D_2O): δ = 2.58−2.70 (m, 2H, C(O)CH_2), 2.93−3.06 (m, 2H, SCH_2), 3.33−3.40 (m, 1H), 3.44−4.05 (m, 72H), 4.44 (d, $J_{1,2}$ = 7.8 Hz, H-1′), 4.57 (d, $J_{1,2}$ = 9.9 Hz, H-1″) 5.02−5.10 (overlapping d, 6H, H-1). Selected ^{13}C NMR (150 MHz, D_2O): δ = 26.5 (C(O)CH_2), 36.4 (SCH_2), 85.8 (C-1′), 101.2 (C-1), 101.3 (4 × C-1), 101.4 (C-1), 102. 9 (C-1″). MALDI-TOF: m/z = 1429 $[M + Na]^+$.

(21) Introduction of a substituent(s) onto one of the D-glucopyranosyl residues in a CD ring desymmetrizes the CD, leaving every proton and every carbon atom in the CD derivative heterotopic. Hence, signals in both the ^{1}H and ^{13}C NMR spectra for the anomeric protons and carbon atoms in the CD ring, for example, proliferate. For a further discussion of this phenomenon, see: Ashton, P. R.; Hartwell, E. Y.; Philp, D.; Spencer, N.; Stoddart, J. F. *J. Chem. Soc., Perkin Trans. 2* **1995**, 1263−1277.

(22) All of the ^{1}H NMR spectra have been referenced to tetramethylammonium chloride (TMACl) as described in: Funasaki, N.; Nomura, M.; Yamaguchi, H.; Ishikawa, S.; Neya, S. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 2727−2728.

In an effort to increase the water solubility of the pseudopolyrotaxane, we synthesized the lactose-appended β-CD **2**[23] starting from the mono(6-amino-6-deoxy-2-*O*-methyl)-hexakis-2,6-di-*O*-methyl-β-cyclodextrin[24] using a synthetic scheme[25] similar to the one outlined in Scheme 1 for the synthesis of **1**. The methoxyl groups serve to break both the inter- and intramolecular hydrogen bonding networks that form between the hydroxy groups in CDs.[12b]

The formation of the pseudopolyrotaxane in aqueous solution was confirmed by both one- and two-dimensional NMR experiments. Upfield shifts of the H-3 and H-5 proton signals were observed upon the addition of bis-2-aminopropyl-terminated PPG (M_n = 2000) to a solution of **2** in D_2O. Small changes were also observed in the chemical shifts of the MeO-2 and MeO-6 resonances.[19] In the two-dimensional TROESY NMR experiment (Figure 5) performed on this

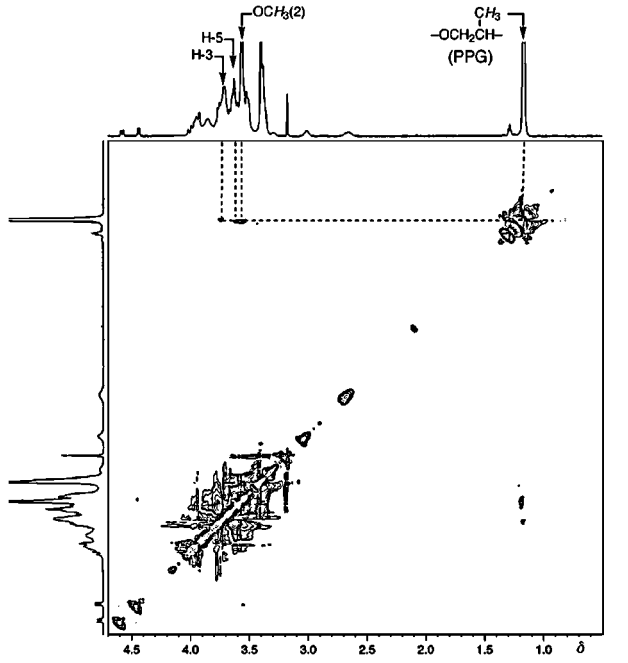

Figure 5. Two-dimensional TROESY NMR spectrum (500 MHz, 298 K) of the pseudopolyrotaxane formed by adding PPG to a solution of the β-CD **2** in D_2O.

pseudopolyrotaxane, ROE cross-peaks were observed between H-3 and H-5 of **2** and the methyl groups in the PPG derivative, indicating that **2** had indeed threaded onto the hydrophobic polymer. Similar observations were noted in the ^{1}H NMR spectra when the same experiment was repeated using PTHP as the polymer thread. As expected,[27] **2** does not form a complex with poly(ethylene glycol) (PEG), since the cavity of the β-CD derivative is simply too large to be able to facilitate complexation of the more hydrophilic polymer. The ^{1}H NMR spectrum of **2** in D_2O showed no changes at all upon addition of PEG (M_n = 2000), even after[25] a period of 4 days.

When either PPG or PTHP was added to a concentrated aqueous solution of **2**, neither of the two pseudopolyrotaxanes precipitated from solution over a period of several months. The lack of a precipitate reinforces the importance of the intermolecular hydrogen bonding network to the formation of a tightly packed pseudopolyrotaxane, a phenomenon that has been observed previously.[12b,28]

In conclusion, we have described the formation of pseudopolyrotaxanes from both lactose-bearing α- and β-CDs threaded onto hydrophobic polymers such as PTHP and PPG. The formation of the complexes was monitored using one- and two-dimensional NMR spectroscopies using the H-3 and H-5 hydrogens of the cyclodextrin as probes. Presently, we are pursuing the conversion of the pseudopolyrotaxanes into polyrotaxanes by both stoppering[13] and slippage[14] protocols with the goal of using them as dynamic water-soluble multivalent (supra)molecular polymers for binding to lectins. Specifically, we are targeting initially a family of proteins known[1d] as galectins, which recognize lactosyl residues in a paucivalent manner.

Acknowledgment. This research was supported by the National Science Foundation under equipment Grants CHE-9974928 and CHE-0092036.

Supporting Information Available: Full experimental protocols and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) **Selected Spectroscopic Data for 2.** ^{1}H NMR (500 MHz, D_2O): δ = 2.56−2.70 (m, C(O)CH_2), 2.92−3.06 (m, 2H, SCH_2), 3.19−3.92 (m, 57H), 4.40 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1′), 4.55 (d, $J_{1,2}$ = 9.8 Hz, 1H, H-1″), 5.08−5.28 (m, 7H, H-1). MALDI-TOF: m/z = 1750 $[M + Na]^+$.

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(25) Syntheses and ^{1}H NMR spectroscopic data are provided in Supporting Information.

(26) TROESY NMR spectra of the polymers are provided in Supporting Information.

(27) Okada, M.; Kamachi, M.; Harada, A. *J. Phys. Chem. B* **1999**, *103*, 2607−2613.

(28) Although the CDs are often assumed to adopt a head-to-head/tail-to-tail co-conformation along the polymer chain, a recent study has shown that the ratio of the formation of head-to-head versus head-to-tail co-conformations is 2:1. See: Miyake, K.; Yasuda, S.; Harada, A.; Sumaoka, J.; Komiyama, M.; Shigekawa, H. *J. Am. Chem. Soc.* **2003**, *125*, 5080−5085. The graphical representation shown in the abstract of this communication is an idealized one since we have no experimental data to allow us to be specific about co-conformational arrangements.