Synthesis of Novel Cyclodextrin Derivatives Having Oligosaccharide Cluster

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A simple and facile method for the synthesis of novel 6-persubstituted ß-cyclodextrin derivatives having a sugar cluster through a spacer arm is described. The coupling reaction of per-6-iodo-ß-CD with each derivative of D-galactose (Gal) or *N*-acetyl-D-glucosamine (GlcNAc) smoothly proceeded and gave the corresponding cyclodextrin derivatives having seven sugar residues in excellent yields.

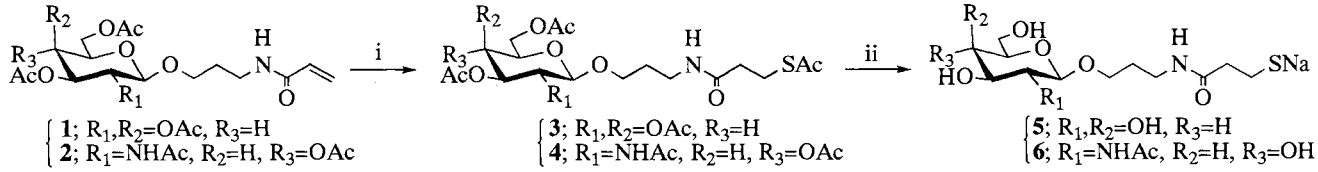
The significance of the sugar density on glycoproteins during the specific interaction between oligosaccharide chains and receptors was first reported by Lee et al.[1,2] on the basis of chemically designed cluster glycosides. Recently, the phenomenon referred to as the "sugar cluster effect" has been widely demonstrated using a variety of synthetic multivalent sugar-ligands.[3] Especially, low molecular ligands with a well-organized scaffold such as calix[4]arenes[4] and dendric wedges[5] have an advantage in which they can design monodisperse molecules with chemically well-defined carbohydrate densities. Therefore, we have noted cyclodextrin (CD) as a core moiety with a well-defined structure and biomedical function. CDs are well-known as cyclic oligosaccharides which possess a significant ability to form inclusion complexes by insertion of organic molecules into their hydrophobic intramolecular cavity and are also expected as carriers for a drug delivery system.[6] As an attempt to synthesize CD derivatives based on the sugar cluster effect, a synthetic method for the ß-CD derivatives binding sugar residues at all the C-6 positions has been reported by Lainé et al..[7] Although the models might be an efficient structure for the elucidation of physico-chemical properties, it is thought that no biochemical functions of branching carbohydrates have been fully demonstrated because sugar residues directly form glycoside linkages on the ß-CD. Thus, the suitable flexibility and distance between carbohydrates and the CD core seem to be necessary for the elucidation of biochemical properties by carbohydrates. In fact, the recognition capacity in regard to spacer length of the Gal-bearing CD derivatives toward a cell wall galactose specific lectin has been reported by Robertis et al.[8] and Kassab et al..[9] There was, however, a need for a more efficient method for the preparation of the CD derivatives in terms of their yield. In this communication, we report a simple and versatile method for the synthesis of ß-CD derivatives linked through a spacer arm of the sugar residues.

First, *N*-acryloylaminopropyl derivatives (**1**, **2**),[10] readily obtainable from the peracetate of Gal or GlcNAc, were converted to the *S*-acetyl-thiopropionylaminopropyl derivatives (**3**, **4**) by Michael addition onto the double bond of thioacetic acid[11] (Scheme 1). The monosaccharides (**3**, **4**)[12] were deacetylated in the presence of sodium methoxide (1.2 eq.) to afford the desired *S*-sodium salts (**5**, **6**). Next, coupling reactions of the glycomonomers (**5**, **6**) (8.4eq.; 1.2eq. per iodo-group) with per-6-iodo-ß-CD **7** in DMF were carried out at 70°C for 24 h under a nitrogen atmosphere (Scheme 2). After concentration, the obtained residues were subjected to size exclusion chromatography over Sephadex G-25 with H_2O as the eluent to afford the corresponding CD derivatives (**8**, **9**) in 70 and 88% yield (from H_2O-acetone), respectively.

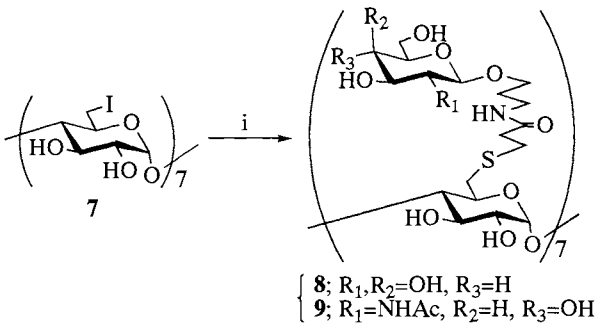
The fully assigned ^{1}H-NMR spectrum of the ß-CD derivative **8** is shown in Figure 1 along with the notes.[12] The H-1 signals of the Glc residues for the ß-CD and Gal residues are observed at 5.03 and 4.31 ppm, respectively, and the relative integration showed approximate values. It is clearly suggested that the iodo groups of each Glc residue of the ß-CD are perfectly substituted by the Gal derivative **5**. The ^{13}C-NMR spectrum of the ß-CD derivative **9** is also shown in Figure 2. All signals for the carbon atoms could be clearly observed, and the fully assigned chemical shifts are indicated in the notes.[13] The integrity of the clustered CD derivatives was further confirmed by the Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectra[14] and elemental analyses.[15]

The inhibitory assay of the hemagglutination of human erythrocytes by wheat germ agglutinin (WGA), which is known as *N*-acetylglucosamine-specific lectin, was also performed using a ß-CD derivative **9** prepared in this study. As a preliminary result, the minimum inhibitory concentration based on a GlcNAc unit of the ß-CD derivative **9** was approximately 20-fold lower than that of the GlcNAc monomer. This result shows the specific interaction between the GlcNAc-bearing ß-CD derivative **9** and WGA.

In conclusion, an efficient synthetic procedure for ß-CD derivatives having a sugar cluster was established. It was obviously suggested that the model shows a good clustering effect. An application of this synthetic method to ß-CD derivatives bearing much longer oligosaccharides is now under investigation and the results will be reported in the near future.



Scheme 1. Reagents and Conditions: i) AcSH, Et_3N / EtOAc, rt., 96% for **5**, 84% for **6**; ii) NaOMe / MeOH.



Scheme 2. Reagents and Conditions : i) **5** or **6** / DMF, 70 °C, 24h, 70% for **8**, 88% for **9**.

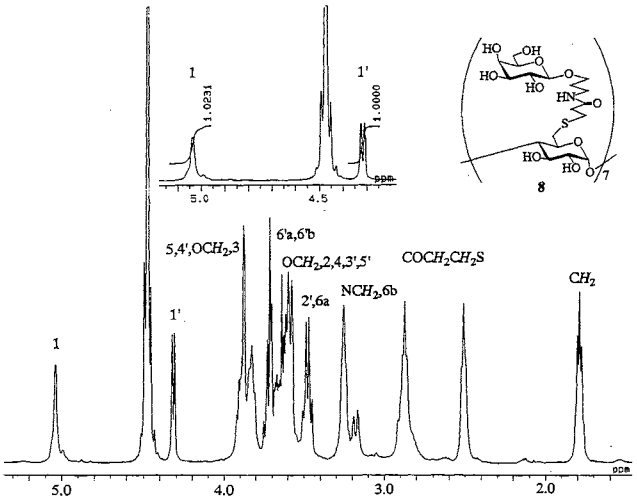


Figure 1. ^{1}H-NMR spectrum of clustered ß-CD derivative **8** measured in D_2O.

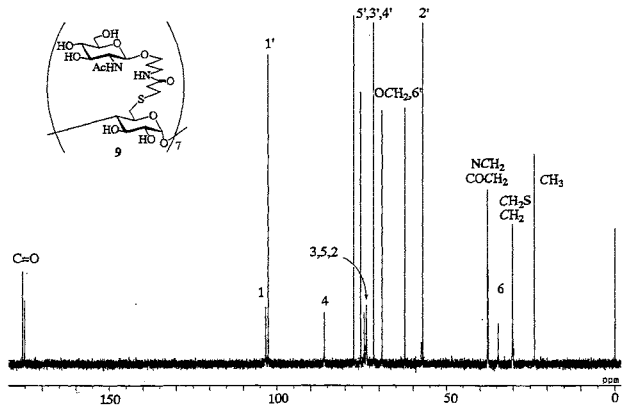


Figure 2. ^{13}C-NMR spectrum of clustered ß-CD derivative **9** measured in D_2O.

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12 ^{1}H-NMR spectroscopic data: **3**;($CDCl_3$) δ 1.79(m, 2H, CH_2), 1.99, 2.05, 2.07, 2.17(all s, 12H, 4OCOCH_3), 2.33(s, 3H, SCOCH_3), 2.52(m, 2H, COCH_2), 3.15(t, 2H, CH_2S), 3.30, 3.43, 3.58, 3.99(4m, 4H, CH_2), 3.91(ddd, 1H, H-5), 4.13 (dd, 1H, H-6b), 4.19(dd, 1H, H-6a), 4.44(d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 5.04(dd, 1H, H-3), 5.19(dd, 1H, H-2), 5.42(dd, 1H, H-4) and 6.02(brt, 1H, NH). **4** ; ($CDCl_3$) δ 1.66, 1.80 (2m, 2H, CH_2), 1.95, 2.02, 2.04, 2.08(all s, 12H, 4OCOCH_3), 2.34(s, 3H, SCOCH_3), 2.53(m, 2H, COCH_2), 3.09(m, 1H, CH_2), 3.15(t, 2H, CH_2S), 3.64, 3.69, 3.99 (3m, 3H, CH_2), 3.04(t, 1H, H-2), 4.14(dd, 1H, H-6b), 4.27 (dd, 1H, H-6a), 4.51(d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.10(t, 1H, H-4), 5.16(t, 1H, H-3), 6.23(brt, 1H, NH) and 6.49[d, 1H, NH(GlcNAc)]. **8** ; (D_2O) δ 1.78(brt, 2H, CH_2), 2.50(brs, 2H, COCH_2), 2.87 (brt, 2H, CH_2S), 4.31 (d, 1H, $J_{1',2'}$ 7.5 Hz, H-1') and 5.03 (s, 1H, H-1). **9** ; (D_2O) δ 1.69(brt, 2H, CH_2), 1.97 (s, 3H, NHCOCH_3), 2.48 (brs, 2H, COCH_2), 2.85(brt, 2H, CH_2S), 4.43(d, 1H, $J_{1',2'}$ 7.9 Hz, H-1') and 5.02 (d, 1H, $J_{1,2}$ 2.4 Hz, H-1).
13 ^{13}C-NMR spectroscopic data : **8** ; (D_2O) δ 30.5, 30.6, 34.9, 37.9, 38.2, 62.7, 69.4, 70.4, 72.6, 73.9, 74.0, 74.6, 74.7, 76.8, 86.2, 103.6, 104.7 and 175.5. **9** ; (D_2O) δ 23.8, 30.2, 30.3, 34.6, 37.6, 37.8, 57.2, 62.4, 69.2, 71.6, 73.7, 73.8, 74.3, 75.4, 77.4, 85.9, 102.6, 103.3, 175.1 and 175.8.
14 TOF-MASS (positive mode) spectral data : **8**; Calcd for $C_{126}H_{217}N_7O_{77}S_7$: 3286.59. Found: 3287 $(M+H)^+$. **9**; Calcd for $C_{140}H_{238}N_{14}O_{77}S_7$: 3573.96. Found: 3575 $(M+H)^+$.
15 elemental analyses : **8**; Found: C, 45.40; H, 6.77; N, 2.65; S, 6.85%. Calcd for $C_{126}H_{217}N_7O_{77}S_7 \cdot 2H_2O$: C, 45.55; H, 6.70; N, 2.95; S, 6.75%. **9** ; Found: C, 45.89; H, 6.81; N, 5.05; S, 6.10%. Calcd for $C_{140}H_{238}N_{14}O_{77}S_7 \cdot 4H_2O$: C, 46.12; H, 6.80; N, 5.38; S, 6.16%.