Synthesis of Singly and Doubly Spin-labeled Maltoses and Their EPR Spectra

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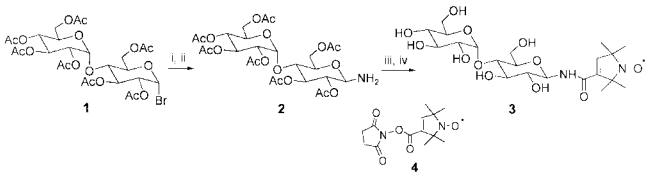
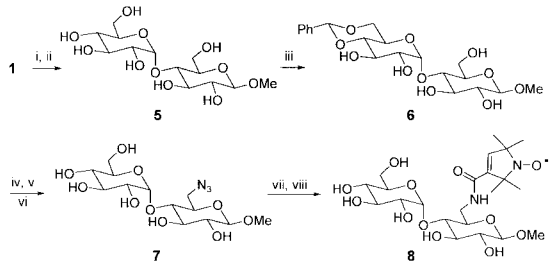
The cell-surface oligosaccharides are involved in many crucial biological processes including cell-cell communication, inflammation, cell growth, bacterial and viral infection.[1] Many of their biological functions result from the initial recognition of oligosaccharides through carbohydrate-protein interactions.[2] Until recently, understanding these important interactions has primarily relied upon NMR,[3] circular dichroism (CD)[4] and fluorescence resonance energy transfer (FRET)[4b,5] as well as affinity labeling experiments as a biochemical tool.[6]

Spin-labeled biomolecules such as nucleic acids, peptides/proteins and lipids have been widely employed to elucidate their biological processes by EPR spectroscopy. For example, the conformational changes and local dynamics of DNA double helices have been evaluated using nitroxide-labeled nucleic acids.[7] EPR studies of singly or doubly spin-labeled peptides/proteins have provided valuable information on their local and global mobility, and conformational changes by interaction with small ligands or receptors.[8] The lipid chain dynamics in hydrated binary mixtures have been studied with spin-labeled lipids.[9] Therefore, it is expected that EPR spectroscopy using synthetic spin-labeled carbohydrates could be an alternative method to probe the protein-carbohydrate interactions.

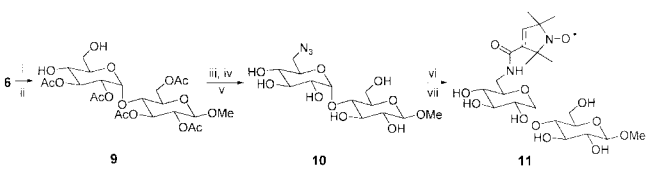
Spin-label(s) was incorporated into maltose at three different positions – anomeric, C-6 and C-6' – *via* an amide bond since a nitroxide spin-label is sensitive to its local environment. Initially, maltose (**3**) labeled at the anomeric position was prepared in two steps from acetylated maltosylamine (**2**), which was synthesized by treatment of **1** with sodium azide and a subsequent reduction (Scheme 1).[10] Coupling of **2** to spin-labeled *N*-hydroxysuccinimide ester **4**,[11,12] followed by removal of acetyl groups, produced the desired product **3** in good yield.[13] Maltose derivatives **8** and **11** containing a single radical at C-6 and C-6' were synthesized from 4',6'-benzylidene protected maltose (**6**) as a common precursor that was prepared from benzylidenation of **5** with benzaldehyde dimethyl acetal in the presence of a catalytic amount of *p*-TsOH (Scheme 2). A selective tosylation of a primary alcohol in **6** followed by a substitution reaction by azide and the removal of a benzylidene group under acidic conditions yielded azide **7**. Reduction of **7** and a subsequent coupling of the resultant amine to **4** provided a singly spin-labeled maltose **8** at C-6 in moderate yield.[13] Synthesis of **11** possessing a nitroxide at C-6' was initiated by peracetylation of **6** and a subsequent debenzylidenation to afford **9** (Scheme 3). A selective tosylation of a primary alcohol in **9** followed by a substitution reaction and deacetylation gave **10**. Subsequent steps are analogous to those shown in Scheme 2, except that the reactive site is C-6' position instead of C-6 position.[13] A maltose derivative **13** bearing two spins at C-6 and C-6' was synthesized by the reactions shown in Scheme 4. Two primary alcohols in **5** were selectively tosylated and the resultant tosyl groups were converted to azide groups to afford **12**. Reduction of azides in **12** and a coupling reaction



Scheme 1. *Reagents and conditions*: i, NaN_3, tetrabutylammonium hydrogen sulfate, sat. $NaHCO_3$, CH_2Cl_2, 2 h, 95%; ii, Pd/C, H_2, MeOH, 1 h, 99%; iii, **4**, MeOH, 5 h, 70%; iv, NaOMe, MeOH, 1 h, 91%.



Scheme 2. *Reagents and conditions*: i, HgO, $HgBr_2$, $CaSO_4$, MeOH, $CHCl_3$, 12 h, 86%; ii, NaOMe, MeOH, 1 h, 81%; iii, benzaldehyde dimethyl acetal, *p*-TsOH, CH_3CN, 5 h, 40%; iv, TsCl, Pyr, 4 h, 62%; v, NaN_3, DMSO, 80 °C, 7 h, 83%; vi, TFA, CH_2Cl_2, 5 h, 70%; vii, Pd/C, H_2, MeOH, 1 h, 99%; viii, **4**, MeOH, 5 h, 41%.



Scheme 3. *Reagents and conditions*: i, Ac_2O, DMAP, Pyr, 5 h, 82%; ii, TFA, CH_2Cl_2, 5 h, 70%; iii, TsCl, Pyr, 4 h, 62%; iv, NaN_3, DMSO, 80 °C, 7 h, 83%; v, NaOMe, MeOH, 1 h, 81%; vi, Pd/C, H_2, MeOH, 1 h, 99%; vii, **4**, MeOH, 6 h, 41%.

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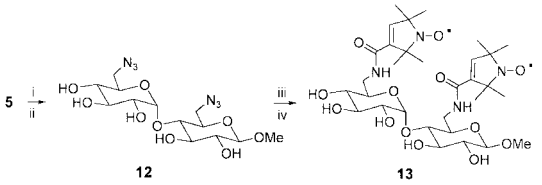

Scheme 4. *Reagents and conditions*: i, TsCl, Pyr, 5 h, 62%; ii, NaN_3, DMSO, 80 °C, 8 h, 83%; iii, Pd/C, H_2, MeOH, 1 h, 99%; iv, **4**, MeOH, 5 h, 41%.

of the amines to **4** furnished a doubly labeled maltose **13**.[13] To our knowledge, oligosaccharides labeled by two spins have never been synthesized for their structural and biological studies.

The EPR spectra of nitroxide-labeled maltose **3**, **8**, **11** and **13** in 0.15 M phosphate-buffered saline (pH 7.2) are shown in Figure 1. EPR spectra of singly spin-labeled maltose derivatives showed triplet (Figure 1a-1c) and EPR spectrum of doubly spin-labeled **13** showed an additional splitting as a result of the spin-spin interaction between two nitroxides (Figure 1d). However, the distance between two radicals seems to be long because the strength of the biradical interaction is weak.[14] A closer examination of EPR spectra of singly labeled maltose reveals that EPR spectra of **3** and **11** containing a spin-label at anomeric and C-6' position are superimposed but EPR spectrum of **8** labeled at C-6 is slightly different from those of **3** and **11**. This is probably due to the fact that the spin label in **8** is located in the central region, whereas it is labeled at terminal position in the other two cases. Numerical simulation of EPR spectra of singly labeled maltose using the NLSL/EPRLL program gives excellent agreement to the experimental data (Figure 1a-1c).[10] All (g-, hyperfine, rotational) tensors are assumed to have the same coordinate system and to be axially symmetric. Parameters used for simulation are $g_{iso} = 2.0061$, $a_{\|} = 34.97$ gauss, $R_{\perp} = 9.33 \times 10^8$ sec^{-1}, $R_{\|} = 10^{10}$ sec^{-1}, gib (Gaussian inhomogeneous broadening width) = 1.12 gauss. It was found that the perpendicular component of hyperfine tensor ($a_{\perp}$) is the major difference (6.55 gauss for **8** *vs*. 6.79 gauss for **3** and **11**). EPR spectra of singly and doubly labeled compounds are solvent dependent and their perpendicular rotational rate is slightly faster in water solvent than in MeOH and DMF, and inhomogeneous line broadening is the smallest in H_2O.[10]

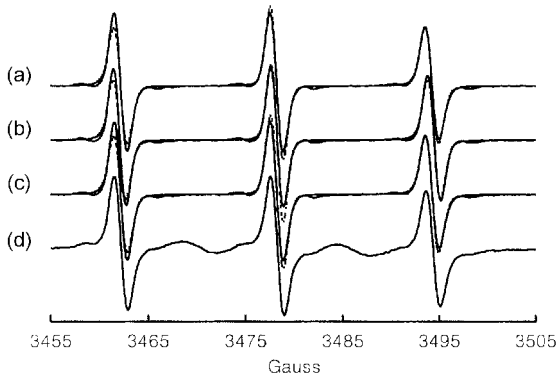

Figure 1: Experimental (solid lines) and simulated (dot lines) EPR spectra of spin-labeled maltose in 0.15 M phosphate-buffered saline (pH 7.2). EPR spectra were recorded on an X-band Bruker spectrometer at room temperature: microwave frequency, 9.77 GHz; microwave power, 1.00 mW; modulation frequency, 100.0 kHz; modulation amplitude, 0.52 G. (a) **3**. (b) **8**. (c) **11**. (d) **13**.

As an attempt to probe the mobility of carbohydrates and conformational change of glycosidic linkage between two monosaccharides in maltose upon binding to a protein, maltose bearing one or two spin-labels was incubated with *Vicia faba* agglutinin (VFA) as a model protein. The resulting EPR spectra show very slight change of line shape or splitting pattern (data not shown). It is known that maltose binds to VFA relatively weakly ($K_a = 200$ M^{-1}).[15] In addition, spin-labeling of the maltose may change its binding property considerably because the spin-label moiety is not so small compared to the disaccharide unit. Therefore, the labeled maltose is unlikely to bind to a protein. However, we note that EPR spectroscopy using optimized spin-labeled carbohydrates would expand the scope of the studies on the protein-carbohydrate interactions.

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13. Compound **3**: (FAB MS) calcd for $C_{21}H_{35}N_2O_{12}Na$ $[M+Na]^+$ 530, found 530. Compound **8**: (ESI-MS) (neg) calcd for $C_{22}H_{36}N_2O_{12}$ $[M-H]^+$ 520, found 520. Compound **11**: (ESI-MS) (neg) calcd for $C_{22}H_{36}N_2O_{12}$ $[M-H]^+$ 520, found 520. Compound **13**: (ESI-MS) (neg) calcd for $C_{31}H_{50}N_4O_{13}$ $[M-H]^+$ 685, found 685.
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