

Characterization of newly synthesized dendron-type sugars with self-assembling properties

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Abstract: Dendron-type sugars with self-assembling properties were synthesized from conjugation of 2-azidoethoxylactose as hydrophilic block, dendron as divergent block, and 1-azidodocosane as hydrophobic block employing click chemistry for the each block. The dendron and 1-azidodocosane was coupled employing in the presence of copper sulfate and sodium ascorbate, followed by deprotection of *tert*-butyldimethylsilyl groups. Then, the deprotected alkyne and 2-azidoethoxylactose were condensed in the same catalyst to provide 2 and 4-divergent dendron-type sugars in good yield (up to 97%). The completion of the conjugation process was established by both ¹H-NMR and ESI-MS spectroscopy. The interfacial properties of dendron-type sugars were investigated by surface tension and dynamic light scattering measurements.

Key words: carbohydrate-protein interaction, glycodendrimer, click chemistry, self-assembly

1. Introduction

Many recognition events important in biology are mediated via multivalent interactions between relevant oligosaccharides and multiple saccharide receptors present on lectins, viruses, toxins, and cell surfaces. Because of the important role on protein-carbohydrate interactions in these recognition events including human diseases, considerable effort has been devoted toward the development of multivalent polymeric ligands for carbohydrate-binding proteins. Recently, a dendrimer covered with sugar ligands, glycodendrimer, was employed as an artificial multivalent glycans (1), so it has the potential to realize multivalent carbohydrate-protein interactions, i.e., glycocluster effect (2). Particularly, the size of dendrimer and sugar numbers can be controlled by changing the generation. Moreover, the maximum size is to the extent of 10nm [1] and is a moderate order with nanoparticles having the ability to agglutinate acceptor proteins (3, 4]. Nanoparticles usually absorb cells through endocytosis because of their size. Thus, a large size particle could be demanded to induce the cell-cell interaction via glyconanoparticles like emulsion particles (5). This study demonstrates the glyconanoparticles with the ability to control size and ligand structure, to achieve optimal cell-cell interaction; the dendron-type sugars with self-assembling character were synthesized with click chemistry (6). Since the relation between the branch structure and the interfacial property significantly determines the nanoparticle formation, the relation was intensively investigated.

2. Experimental

2.1. Materials and Synthesis

Reagents were purchased at the highest commercial quality and were used without further purification. ¹H-NMR spectra were recorded with a JEOL JNM-LA500 spectrometer with chloroform (in chloroform-d) or dimethylsulfoxide (in dimethylsulfoxide d-6) as an internal standard. Chemical shifts are reported in ppm (δ units) downfield from internal tetramethylsilane (CDCl₃ and DMSO). Mass spectral analysis was carried out by a Bruker ESI-TOF MS.

2.2. Characterization of dendron-type sugars in aqueous solution at 298K

For the surface tension, aqueous solutions of dendron-type sugars were measured by KRÜSS Processor Tensiometer K 100 with the Wilhelmy plate technique.

The mean diameter and the size distribution of the micells in aqueous solution were measured by dynamic light scattering equipment (DLS-7000, Otsuka Electronics, Japan).

3. Results and Discussion

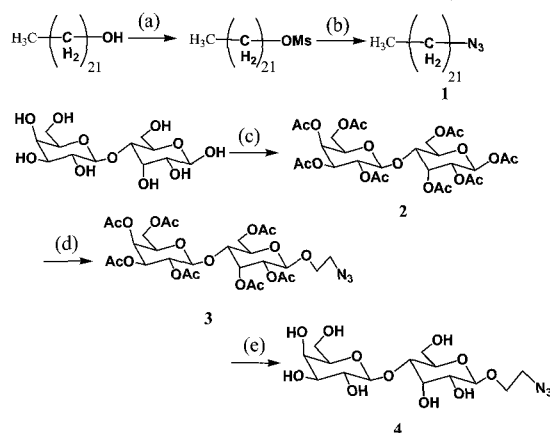
3-1. Synthesis of the dendron-type sugars

1-azidodocosane **1** and 2-azidoethoxylactose **4** were synthesized by the following procedure (Scheme 1).

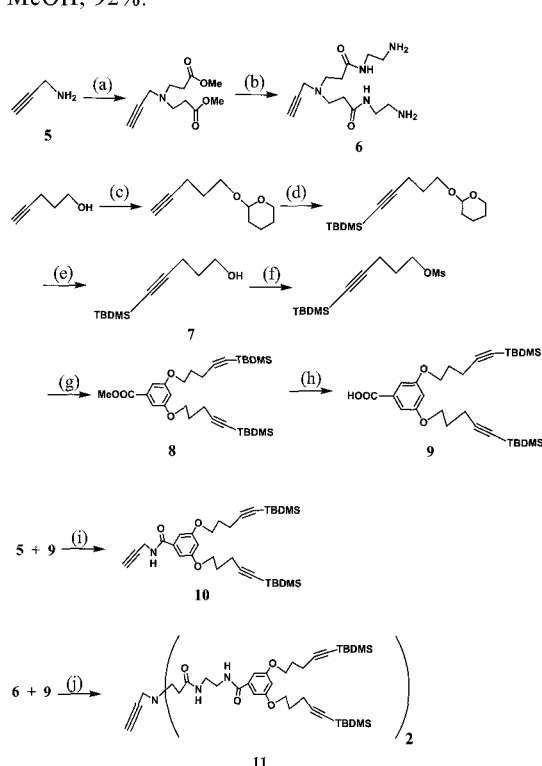
For the preparation of 1-azidodocosane **1**, 1-docosanol was treated with methanesulfonyl chloride (MsCl) to afford mesylated product. Reaction with sodium azide gave alkyl block, 1-azidodocosane **1** in 88% yield (2steps).

For preparation of 2-azidoethoxylactose **4**, D-lactose was protected with acetyl groups by treatment of acetic anhydride and pyridine. The acetylated lactose was reprecipitated with diethylether; β -D-lactose octaacetate **2** was obtained in 65% yield. **4** was synthesized by

previously reported procedure (7). **2** was treated with 2-azidoethanol in the presence of boron trifluoride diethylether complex to afford 2-azidoethylacetate lactose **3** in 65% yield; this was deprotected by sodium methoxide to get sugar ligand block, 2-azidoethylacetate lactose **4** in 92% yield.



Scheme 1. Synthesis of alkyl block **1** and sugar ligand block **4**. Reagents and Conditions: (a) MsCl, triethylamine, THF, rt; (b) NaN₃, ⁿBu₄NHSO₄, THF/H₂O, reflux, 88% (2steps); (c) Ac₂O, pyridine, rt, 65%; (d) 2-azidoethanol, BF₃·Et₂O, CH₂Cl₂, 0 °C to rt 65%; (e) NaOMe, MeOH, 92%.



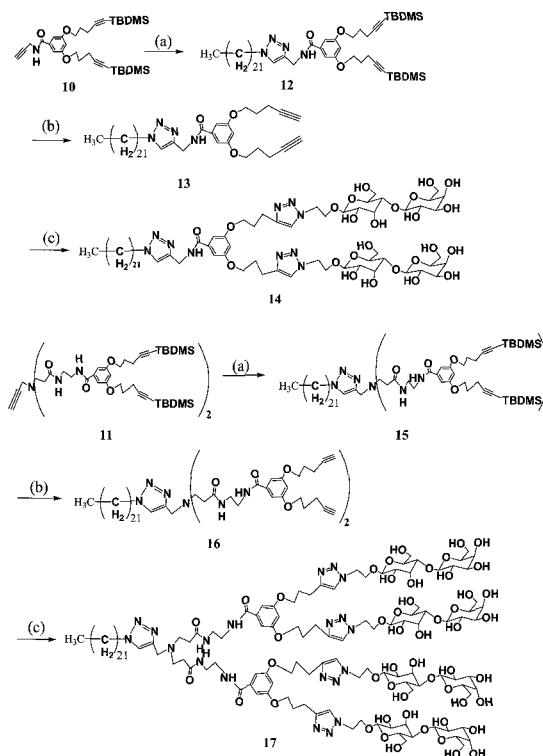
Scheme 2. Synthesis of dendron block **10** and **11**. Reagents and Conditions: (a) methylacrylate, MeOH, rt; (b) ethylenediamine, MeOH, rt; (c) dihydropyran, TsOH, CH₂Cl₂, 0 °C to rt; (d) ⁿBuLi, TBDMSCl, THF, -78 °C to rt; (e) TsOH, MeOH, rt, 57% (3steps); (f) MsCl, triethylamine, THF, rt; (g) Methyl 3,5-Dihydroxy benzoate, K₂CO₃, DMF, 85 °C, 99% (2steps); (h) KOH, EtOH/H₂O, reflux, DOWEX H⁺, 99%; (i) DCC, HOBT, CH₂Cl₂, rt, 90%; (j) DCC, HOBT, CH₂Cl₂,

rt, 85%.

Dendron block was synthesized from peptide like coupling of amine **5**, **6** and carboxylic acid **9** (Scheme 2).

Divergent amine was prepared from propargyl amine **5**; reaction of Michael addition with methyl acrylate and amidation with ethylenediamine [7], gave two divergent amine **6** in 86% yield (2steps). Carboxylic acid **9** was prepared from 4-pentyn-1-ol. The hydroxyl group was protected by tetrahydropyranyl (THP) group with treatment of dihydropyran in the presence of *p*-toluenesulfonic acid (TsOH) and the alkyne was protected by TBDMS group, with treatment of *n*-butyllithium and TBDMS chloride. Then, deprotection by treatment of MeOH in the presence of TsOH, gave 5-(tert-butyl-dimethyl-silyl)-pent-4-yn-1-ol **7** in 57% yield (3steps). **7** was mesylated by MsCl and reacted with methyl 3,5-dihydroxybenzoate in presence of potassium carbonate; methyl ester **8** was obtained in 99% yield. The methyl ester was hydrolyzed by potassium hydroxide and neutralized with DOWEX cation exchange resin; 3,5-Bis-[5-(tert-butyl-dimethyl-silyl)-pent-4-yn-1-yl]-benzoic acid **9** was obtained in 99% yield.

For the preparation of two or four divergent dendron, the carboxylic acid **9** and amine **5** or **6** were coupled by dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT); **10** and **11** were got in 90% and 88%, respectively.



Scheme 3. Synthesis of dendron-type sugars **14** and **17**. Reagents and Conditions: (a) **1**, CuSO₄·5H₂O, Sodium ascorbate, THF/H₂O (4/1), rt, 95% (**12**), 88% (**13**); (b) ⁿBu₄NF, THF, rt, 97% (**13**), 99% (**16**); (c) **2**, CuSO₄·5H₂O, Sodium ascorbate, ^tBuOH/H₂O (1/1), 40 °C, 99% (**14**), 60% (**17**).

For the preparation of dendron-type sugars **14** and **17**, the dendron was coupled with 1-azidodocosane **1** with

the Cu(I)-catalyzed azide-alkyne [3+2] cycloaddition reaction in presence of sodium ascorbate (20mol% per N_3) and copper sulfate (10mol% per N_3) and in THF-H₂O (4:1), then treated with tetrabutylene ammonium fluoride (TBAF) for deprotection of TBDMS-groups, led to **13** and **16** in 92% and 87% yield (2steps) respectively. Finally, when **13** or **16** was treated with 2-azidoethoxylactose **4** in presence of sodium ascorbate (40mol% per N_3) and copper sulfate (20mol% per N_3) in ^tBuOH-H₂O (1:1), pure dendron-type sugars **14** and **17** were obtained in 99% and 60% yield respectively, after purification by dialysis.

13 74 μ mol and **4** 178 μ mol were dissolved in 10ml ^tBuOH and 8ml H₂O at 40°C. Copper sulfate (20mol% per N_3) in 1ml H₂O and sodium ascorbate (40mol% per N_3) in 1ml H₂O added to the solution and stirred for 24 hours. The resulting solution was purified by dialysis (Mw: 3500 cut off) and freeze dried; dendron-type sugars **14** was obtained in 92% yield.

The complete incorporation of these dendron-type sugars was confirmed by ¹H-NMR and ESI-MS spectroscopy (Figure 1.).

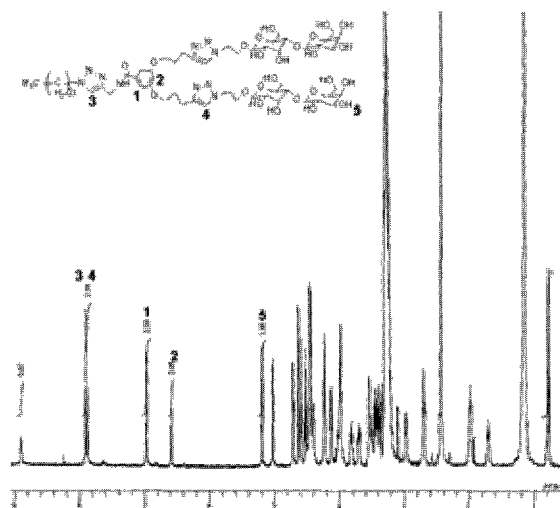


Figure 1. ¹H-NMR spectroscopy of **14**

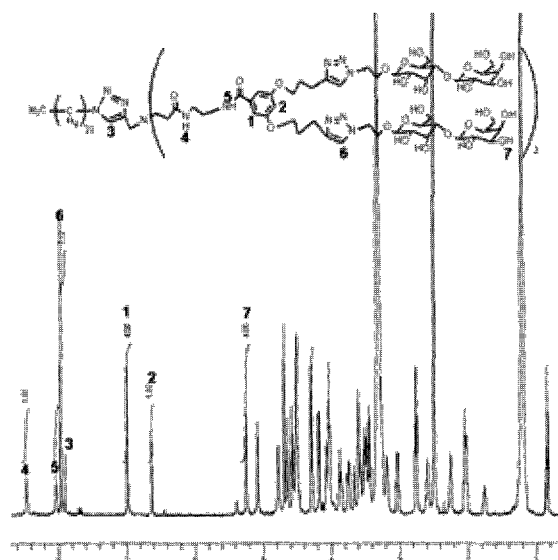


Figure 2. ¹H-NMR spectroscopy of **17**

The distinguishing ¹H peaks were belonged to triazol unit, aromatic and lactose peak ratio of their proton. For the sample **14**, the presence of two signals at 7.91 and 7.88 ppm (3H), corresponding to the triazol proton, of a doublet at 7.04 ppm and triplet at 6.66 ppm (1:2 ratio), due to the aromatic protons, and a doublet at 5.14 ppm (2H), due to hydroxyl group protons of lactose, indicates the presence of two lactose units. For the sample **17**, the resulting 4-divergent sugars showed NMR features similar to those of **14**.

14 was detected as $[M+H]^+$: 1497.8 by ESI-MS spectroscopy. But, the 4-divergent sugars **17** could not detected because the compound was difficult for ionization, so precursor of the compound, **16**, was measured and detected as $[M+H]^+$: 1171.8. The precursor structure and peak ratio of triazole and lactose was well corresponding to theoretical figure, so **17** could be synthesized.

3-2. Characterization of dendron-type sugars in aqueous solution at 298K

Table.1 Data calculated from surface tension measurement for dendron-type sugars in aqueous solution at 298 K. Critical micell concentration, critical surface tension and molecular area were denoted as cmc , Γ_{cmc} , and A , respectively.

	cmc /mmol dm ⁻³	Γ_{cmc} /mN m ⁻¹	A /nm ² molecule ⁻¹
2divergent	0.0654	42.38	0.723
4divergent	0.0276	43.31	1.178

Comparing 2-divergent sugars **14** with 4-divergent sugars **17**, molecular area for **17** was larger than **14**, so molecular number oriented at air-liquid interface decreased due to the bulky structure of hydrophilic blocks. Γ_{cmc} was the same trend as molecular area. Because **17** has the bulky hydrophilic sugar groups, it is not easy to densely orient at air-liquid interface. At the same time, cmc for **17** was smaller than **14**, this is presumably because the bulky structure is not so related with the intermolecular interactions. It is further suggested that **17** has high self-assembling ability due to the desired hydrophilic-hydrophobic balance. This assumption was ensured by dynamic light scattering measurement. **17** formed micells with the diameter 15nm, but **14** didn't, suggesting that **17** has higher self-assembling ability. To obtain larger glycoparticles, emulsion polymerization with these amphiphilic molecules is now investigation.

4. Conclusion

We have shown a novel amphiphilic dendron-type sugars that combine the conjugation of three separation blocks: alkyne-derived and protected dendron, 1-azidodocosane, and 2-azidoethoxylactose. The amphiphilic structure of 4-divergent sugars form micell with the diameter 15nm, but 2-divergent sugars didn't form. This was suggested from surface tension measurement.

The result of these self-assembling properties

will be applied to synthesis emulsions which have multivalent ligands at their surface, looking like large glycodendrimer. So they can be used as glyconano or micro particle for cell agglutination and separation.

5. References

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