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Versatile synthesis of oligosaccharide-containing fullerenes

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Abstract—Oxylamine-functionalized fullerene derivatives have been synthesized in order to attach oligosaccharides to the fullerene surface by simple chemical ligation. This method greatly simplifies the synthetic process for a variety of oligosaccharide-containing fullerenes without any complicated chemical modification.
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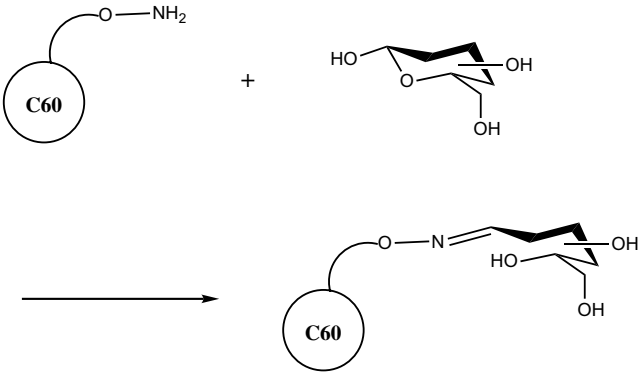
1. Introduction

Fullerene is well known for its ability to generate singlet oxygen under visible light irradiation and for scavenging superoxide radicals. Fullerenes have applications such as cytotoxin or radical scavengers in biological systems because of this ability. Since combining fullerenes with carbohydrates results in water solubility and biological recognition, they are often modified in this manner. Some sugar-containing fullerenes[1] have been synthesized using cycloaddition reactions of glycosyl azides[2] or glycosyl diazirine,[3] coupling reactions of malonic acid fullerenes and oligosaccharide derivatives containing alkylamines,[4] and sulfide-connection following a multivalent Grignard addition.[5] These synthetic strategies need the chemical modification of the carbohydrate part in order for the coupling reaction with fullerene to occur. However, the chemical synthesis of carbohydrates is normally a complicated, time-consuming process. Thus, it is not practical to attach oligosaccharides with a relatively large molecular weight to the fullerene molecule using these coupling reactions.

Herein, we chose a synthetic strategy using chemical ligation between free sugars and oxylamine-functionalized fullerenes (Scheme 1). A specific feature of free carbohydrates is the hemiacetal terminus, which can readily react with the oxylamino group to form a stable oxime linkage.[6,7] Although this method has generated a mixture of *cis*- and *trans*-isomers of the oxime bond, it has the advantage that unprotected sugars can be used, which greatly simplifies the process. Recently, we applied this chemoselective reaction in order to isolate *N*-glycans from glycoproteins using oxyamine-functionalized polymers for rapid glycoform analysis.[8] The oxylamine-functionalized fullerenes **1a**–**c** (Fig. 1) were synthesized for effective conjugation with various oligosaccharides without chemical modification in any way.



Scheme 1. Concept of saccharide-displayed fullerene.

2. Results and discussion

Scheme 2 describes the synthetic strategy leading to the fullerene derivatives **1a** and **1b**, which have linkers that

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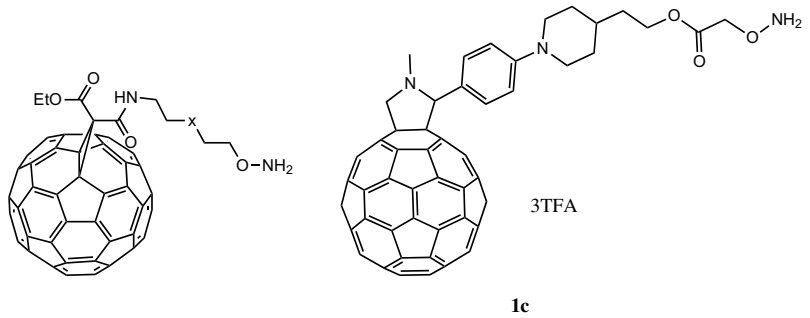
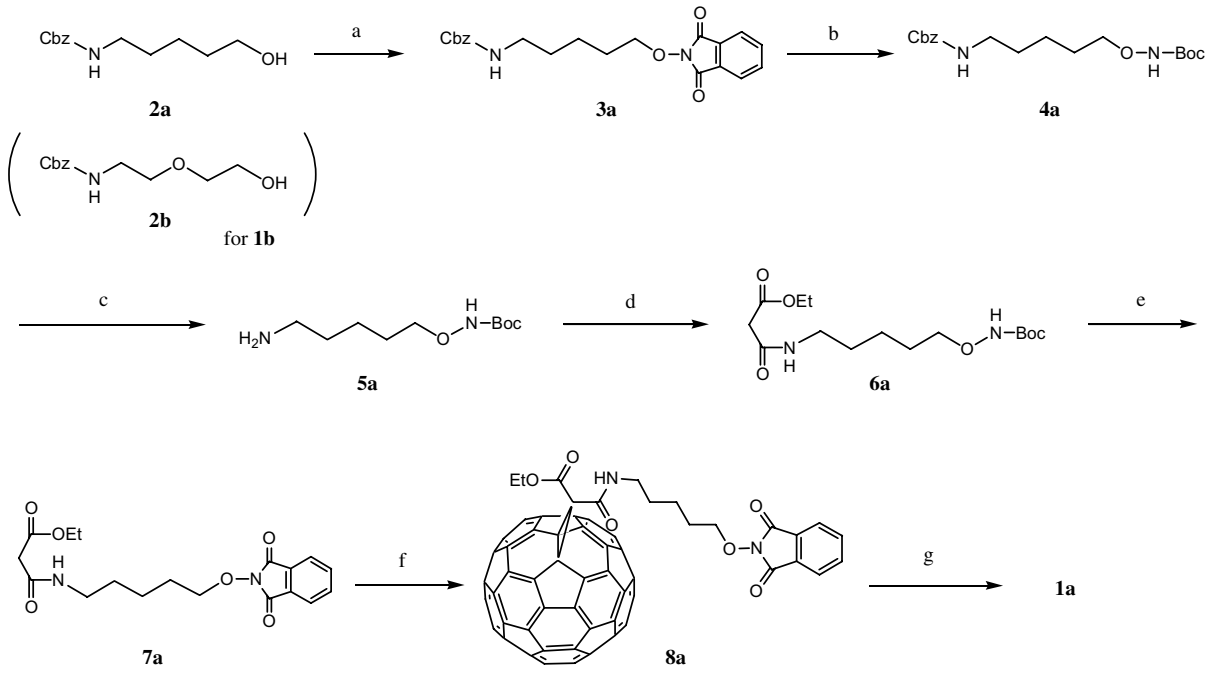


1a : X = CH_2
1b : X = O

1c

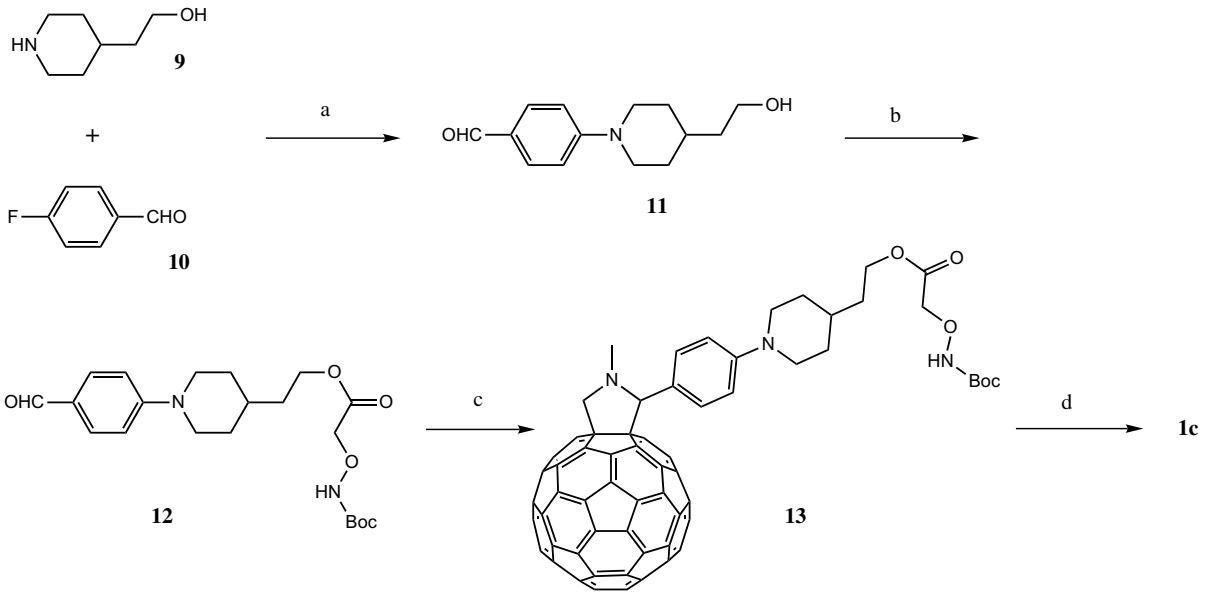
Figure 1. Structures of oxylamino group-containing fullerenes **1a**–**c**.



Scheme 2. Reagents and conditions: (a) hydroxyphthalimide, Ph_3P, DEAD, THF; (b) $MeNH_2$, MeOH; $(Boc)_2O$, diisopropylethylamine, 1,4-dioxane; 51% (from **2a**); (c) Pd–C, EtOAc; (d) mono-ethyl malonate, WSC, HOBt, DMF; 70% (from **4a**); (e) TFA, CH_2Cl_2; phthalic anhydride, toluene; 83%; (f) C_{60}, I_2, DBU, toluene; 24%; (g) $MeNH_2$, MeOH; quant.

include oxylamino groups. A solution of the diethylazodicarboxylate (DEAD) (1.2 equiv) in THF was added to a mixture of the compounds **2a** (1 equiv), hydroxyphthalimide (1.1 equiv) and triphenylphosphine (1.2 equiv) in THF at 0 °C. The mixture was then stirred for 2 h at room temperature. After the addition of a small amount of water, the solvent was evaporated. Compound **3a** was purified with silica gel chromatography. Compound **3a** was then subjected to the removal of the phthaloyl group with 40% methylamine/methanol, which was followed by the protection of the amino group with Boc_2O (1.1 equiv) and diisopropylethylamine (1.1 equiv) in order to afford compound **4a** in 51% yield from **2a**. Compound **4a** was subjected to the removal of the benzyl carbamate (Cbz) group, using 10% palladium–carbon under hydrogen atmosphere, which was followed by the condensation with the mono-ethyl malonate moiety in the presence of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (WSC) and 1-hydroxytriazole (HOBt) to give compound **6a** in 70% yield. Removal of the Boc group in compound **6a** was performed using trifluoroacetic acid (TFA). For the formation of the phthalimide group, phthalic anhydride (1 equiv) and triethylamine (1 equiv) were added to the solution of compound **6a** in toluene, and was then refluxed for 80 min.[9] The product was purified using chromatography in order to generate compound **7a** in 83% yield.[†] Using the cyclopropanation of C_{60} using stabilized α-halocarbanions, the so-called Bingel reaction,[10–12] compounds **1a** and **b** were synthesized under Nierengarten's

[†] NMR data for **7a**: ^1H NMR ($CDCl_3$): δ 1.29 (t, 3H, J = 7.1 Hz), 1.5–1.7 (m, 4H), 1.7–2.0 (m, 2H), 3.2–3.4 (m, 4H), 4.1–4.3 (m, 4H), 7.19 (s, 1H), 7.65–7.8 (m, 2H), 7.8–7.9 (m, 2H). TOF-Mass: 363 (M+H), 385 (M+Na), 401 (M+K).



Scheme 3. Reagents and conditions: (a) K_2CO_3, DMF; 42%; (b) $BocNHOCH_2COOH$, WSC, CH_2Cl_2; 89%; (c) C_{60}, $MeNHCH_2COOH$, toluene; 32%; (d) TFA, CH_2Cl_2; quant.

conditions, which were slightly modified to fit our experiments.[13]

1,8-Diazabicyclo[5,4,0]undec-7-ene (DBU, 5 equiv) had been added to a solution of C_{60} (100 mg), **7a** (1.1 equiv) and I_2 (3 equiv) in toluene at room temperature. After the mixture was stirred for 12 h, additional portions of **7a** (0.5 equiv) and I_2 (1 equiv) were added, and the mixture was stirred for another 12 h. The product was then purified by chromatography (compound **8a**, 36 mg, 24%).[‡] Finally, removal of the phthaloyl moiety in compound **8a** was performed using 40% methylamine/methanol, which resulted in the target compound **1a**. Another target compound, **1b**, was synthesized from compound **2b**, instead of **2a**. Compound **1b** was synthesized according to almost the same procedure as described above. The yield of the Bingel reaction for **1b** was 23%. In this synthetic route, there were two key reactions. When the Cbz group of compound **3a** was removed by a catalyst with 10% palladium–carbon, the N–O bond of compound **3a** was cleaved under the reaction conditions. Therefore, two more reactions (the removal of the phthaloyl group and the protection with the Boc group) were required to reach the intermediate **5a**. The second key step was the reaction for the modification of C_{60} with the maloimide derivative **7a**. When we used compound **6a** instead of compound **7a** for the Bingel reaction, the cycloaddition reaction did not proceed at all. From this result, it was found that protection of the oxylamino group with the phthaloyl moiety was suitable for this reaction. However, the reaction mechanism is not completely clear.

Another synthetic strategy was used for the synthesis of the fullerene-containing pyrrolidines via a process of 1,3-dipolar cycloaddition of azomethine (Scheme 3).[14–18] A mixture of compound **9** (1 equiv), compound **10** (1 equiv) and K_2CO_3 (1.2 equiv) in DMF was stirred for 14 h at room temperature, and then for 12 more hours at 50–60 °C. After the usual treatment, the product was then purified using chromatography, and compound **11** was produced in 42% yield. $BocNHOCH_2COOH$ (2 equiv), WSC (3 equiv) and 4-dimethylaminopyridine (0.1 equiv) were added to a solution of compound **11** (1 equiv) in CH_2Cl_2, and then stirred for 1.5 h at room temperature. The resulting mixture was purified by silica gel chromatography to afford compound **12a** in 89% yield.[§] Compound **12** (5 equiv) and *N*-methylglycine (1 equiv) were added to a solution of C_{60} (100 mg) in toluene. The solution was then refluxed for 2 h. The product was purified with chromatography to afford compound **13** (37 mg, 32%).[¶] Finally, removal of the Boc group in compound **13** was performed using TFA, to create target compound **1c** (20 mg, quant.).

Using the fullerenes **1a**–**c** with oxylamino moieties, the coupling reaction between oxylamino group and saccharide was performed. In a typical procedure, the oligosaccharide was dissolved with KH_2PO_4 buffer solution/methanol (1:1). The solution was then added to a solution of C_{60} derivative in chloroform/methanol (1:1).

[‡] NMR data for **8a**: ^1H NMR ($CDCl_3$): δ 1.45–1.5 (t, 3H, J = 7.1 Hz), 1.75–1.8 (m, 2H), 1.8–1.9 (m, 4H), 3.65 (q, 2H, J = 6.0 Hz), 4.25 (t, 2H, J = 5.8 Hz), 4.57 (q, 2H, J = 7.1 Hz), 7.7–7.8 (m, 4H). TOF-Mass: 1080 (M+H).

[§] NMR data for **12**: ^1H NMR ($CDCl_3$): δ 1.3–1.4 (m, 1H), 1.48 (s, 9H), 1.6–1.7 (m, 3H), 1.82 (d, 2H, J = 12.6 Hz), 2.91 (dt, 2H, J = 2.4, 12.6 Hz), 3.94 (d, 2H, J = 13.2 Hz), 4.27 (t, 2H, J = 6 Hz), 4.44 (s, 2H), 6.90 (d, 2H, J = 12 Hz), 7.74 (d, 2H, J = 6 Hz), 9.76 (s, 1H).

[¶] NMR data for **13**: ^1H NMR ($CDCl_3$): δ 1.3–1.45 (m, 2H), 1.47 (s, 9H), 1.47–1.6 (m, 1H), 1.66 (q, 2H, J = 6.6 Hz), 1.75–1.85 (m, 2H), 2.65–2.75 (m, 2H), 2.79 (s, 3H), 3.65–3.75 (m, 2H), 4.22 (d, 1H, J = 9 Hz), 4.26 (t, 2H, J = 7 Hz), 4.43 (s, 2H), 4.85 (s, 1H), 4.96 (d, 1H, J = 9 Hz), 6.97 (d, 2H, J = 8.4 Hz), 7.65 (s, 2H), 7.72 (s, 1H). TOF-Mass: 1154 (M+H).

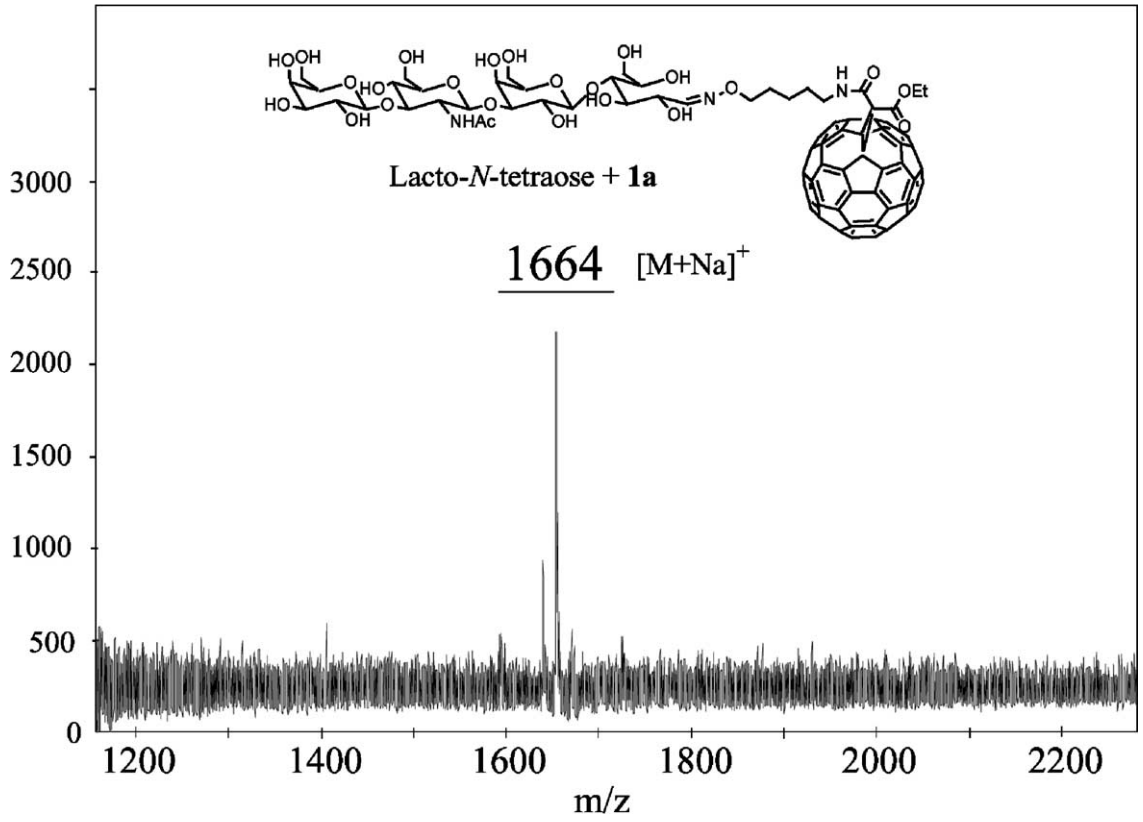


Figure 2. MALDI-TOF mass spectrum of lacto-*N*-tetraose displayed fullerene.

For the attachment of the saccharide, the solution was incubated for 1 h at 90 °C. The products were then determined by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker, UltraFlex). After the incubation of lacto-*N*-tetraose with **1a**, the signal corresponding to **1a** disappeared and a new signal corresponding to the oligosaccharide-containing fullerene appeared (Fig. 2). We succeeded in attaching various oligosaccharides, such as P1 antigene or Leiws A, on fullerene derivatives **1a**–**c** (data not shown). These results suggest that the fullerene derivatives with oxylamino group are capable of easily attaching oligosaccharides without using any special chemical modification.

3. Conclusion

In conclusion, we have synthesized oxyamine-attached fullerene derivatives **1a**–**c**, for the simple oligosaccharide-attachment on the surface of a fullerene. It was found that various oligosaccharides could be easily introduced to the fullerene without any chemical modification. We are now interested in the biological activity of these oligosaccharide-containing fullerenes, targeting microorganisms or cancer cells as antibacterial and anticancer drugs.

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