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Synthesis of Fullerene Glycoconjugates via a Copper-Catalyzed Huisgen Cycloaddition Reaction

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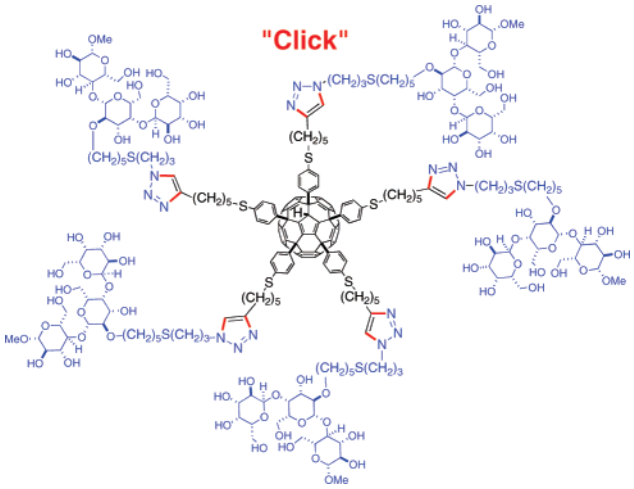
ABSTRACT



The synthesis of fullerene–carbohydrate conjugates using a copper-catalyzed [3 + 2] cycloaddition reaction to facilitate the union of an azido-functionalized sugar and a pentaalkynyl[60]fullerene is straightforward. Thus, fullerenes bearing five oligosaccharides such as Gb3-trisaccharide can be readily accessed. Nanometer-scale molecular architectures presenting as many as 15 sugar moieties in C_5-symmetry are readily produced. The cycloaddition reaction proceeds quantitatively under mild conditions without the need to protect the sugar hydroxyl groups.

The multivalent display of saccharides is an important principle of molecular recognition in biological systems, and a number of tailor-made ligands for natural protein receptors have been created.[1] The interaction of the globotriaosylceramide (Gb-3), a trisaccharide, in multivalent form to Shiga-like toxins, pentameric protein, is representative.[2] A

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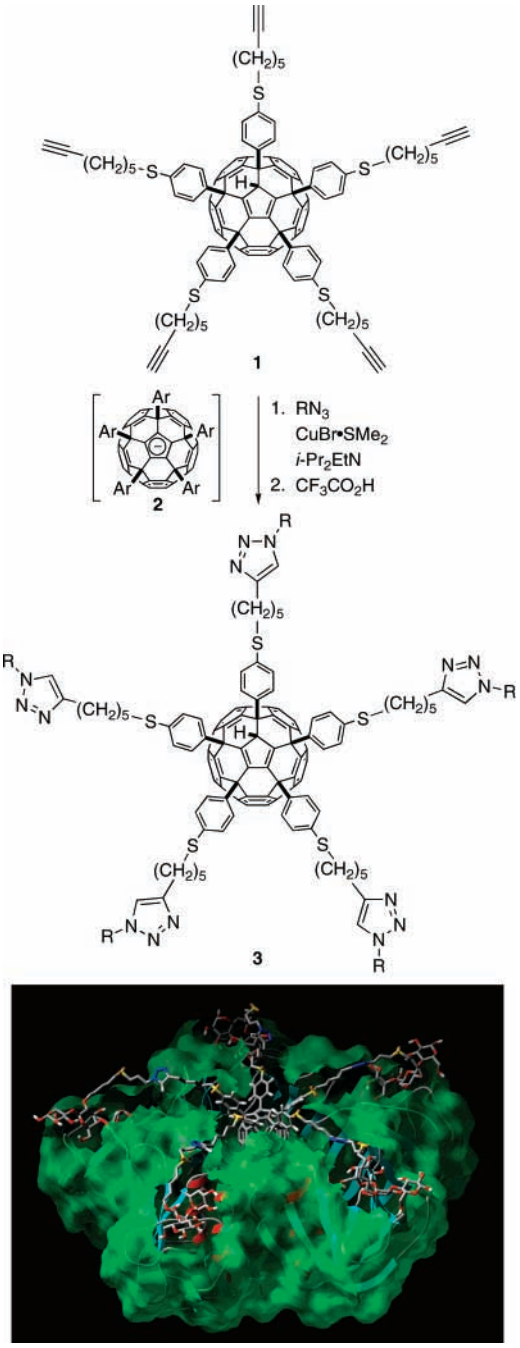
synthetic, C_5-symmetric molecule bearing five P^k trisaccharides of Gb-3 with suitable spatial orientation (see the Abstract and Scheme 1) should result in much tighter binding

Scheme 1. Synthetic Scheme of Pentameric Saccharide Display and a Molecular Model of Shiga-like Toxin (the Molecular Surface Shown in Green) Complexed with a P^k Trisaccharide Conjugate **3g** (Stick Model)



than any construct bearing a single Gb-3 molecule. One chemical problem associated with the design of such ligands is their size, which is comparable to that of the target protein. Given the scarcity of such gigantic molecules in the conventional repertoire of organic molecules, new avenues need to be explored. To this end, we sometime ago developed a synthetic route to C_5-symmetric fullerene derivatives connected to five monosaccharides via sulfide linkages.[3] Upon application of the methodology to large oligosaccharides, the reaction slowed down and produced, under forcing conditions, many side products that were difficult to remove.[3,4] Clearly, a more powerful coupling reaction that preferably takes place under mild conditions was required. A copper-catalyzed version of the Huisgen [3 + 2] cycloaddition introduced by Meldal[5] and Sharpless[6] has found widespread use.[7] Here, we report the successful application of this "click" reaction to the efficient and quick synthesis of glycoconjugates bearing as many as 15 sugar moieties displayed on a C_5 symmetric framework. We synthesized a fullerene displaying five P^k trisaccharide moieties spanning several nanometers in stretched form in nearly quantitative yield without protection of the sugar hydroxyl groups.

The synthetic strategy of the 5-fold copper-catalyzed [3 + 2] cycloaddition approach is shown in Scheme 1. The fullerene core was synthesized via the quantitative organocopper addition reaction we reported previously.[8] Connection of the fullerene to 5 mol of an alkynyl group was achieved quantitatively through sulfide formation (Supporting Information).[3] The development of a quantitative reaction that does not generate byproducts was mandatory since the separation of fullerene molecules with different numbers of sugar side chains is not possible. We were concerned whether the reaction would tolerate the use of excess azide and if the excess azide could be recovered because the application of Huisgen cycloaddition to fullerene has not been reported previously. Recovery of the starting materials is a particularly important issue when precious oligosaccharides are used.

The viability of the synthetic scheme was examined initially for simple compounds (Table 1, entries 1−3). The pentaalkynylfullerene **1** was synthesized from the corresponding penta(4-sulfanylphenyl)fullerene (see the Supporting Information) and was treated with a slight excess of 1-azidopentane in the presence of copper bromide (50 mol %) and diisopropylethylamine (DIPEA, 5.0 equiv) in DMSO at room temperature for 24 h. The reaction was so clean that the target **3a** was obtained in high purity after washing away excess azide with toluene and copper salts with aqueous ammonia. The reaction also proceeded smoothly in toluene, and **3a** was obtained in 94% yield. Likely, cyclopentadienide **2** is formed in situ by deprotonation of **1** by DIPEA, since a brown precipitate formed in toluene and redissolved after

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(4) Hamasaki, R.; Matsuo, Y.; Nakamura, E. *Chem. Lett.* **2004**, *33*, 328−329.

(5) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057−3064.

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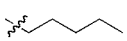
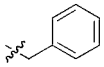
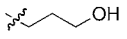
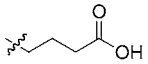
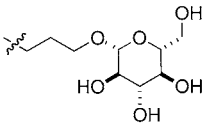
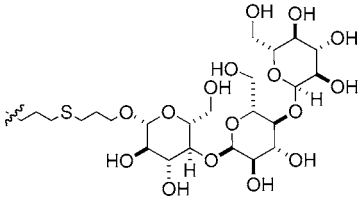
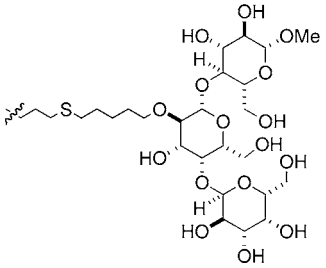
(7) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128−1137.

(8) Sawamura, M.; Iikura, H.; Nakamura, E. *J. Am. Chem. Soc.* **1996**, *118*, 12850−12851. Nakamura, E. *J. Organomet. Chem.* **2004**, *689*, 4630−4635.

Table 1. Five-Fold Coupling Reaction of Pentaalkynylfullerene and Azide

entry	R	product	method[a]	yield[b]
1		**3a**	A	98%
2		**3a**	B	94%[c]
3		**3b**	A	95%
4		**3c**	A	97%
5		**3d**	A	98%
6		**3e**	A	98%
7		**3f**	C	91%
8		**3f**	D	93%
9		**3f**	E	88%
10		**3g**	E	86%

[a] Method A: The reaction was carried out in DMSO at ambient temperature for 24 h. Method B: The reaction was carried out in toluene at ambient temperature for 24 h. Method C: The reaction was carried out in DMSO at 50 °C for 3 d. Method D: The reaction was carried out in DMSO under irradiation of microwave (sealed vessel, 50 °C, 15 min). Method E: The small-scale reaction (ca. 400 μg of **1**) was carried out in DMSO at 40 °C for 3 d. [b] Isolated yield. [c] Aqueous NH_4Cl was used for protonation instead of CF_3CO_2H.

acidic workup. The reaction with benzyl azide gave **3b** in excellent yield (Table 1, entry 3).

Polar functional groups including unprotected alcohols and carboxylic acids did not affect the reaction. Thus, the desired coupling products **3c** and **3d** were obtained in 97% and 98% yield, respectively. The yields of the coupling products were consistently better than those achieved employing the sulfide coupling method.[3]

Next, the reaction was applied to an azide-bearing unprotected glucose. The reaction was as facile as the above reactions and afforded glycoconjugate **3e** in 98% isolated yield at room temperature after 24 h (Table 1, entry 6). The NMR spectrum of the C_1 symmetric cyclopentadiene **3e** was too complex to assign each signal but was indicative of the C_5 symmetry of the corresponding cyclopentadienide. Thus, deprotonation of **3e** with sodium hydroxide greatly simplified the spectra and allowed us to completely assign the ^{1}H and ^{13}C NMR signals (cf. conversion of **3** to **2**; see the Supporting Information).

The coupling of **1** with an oligosaccharide was found to be significantly more difficult. Thus, a maltotriose derivative bearing an azide group at the terminus of a long tether was prepared through sequential glycosidation, thiolation, and introduction of an azide group by alkylation (see the Supporting Information). The azide was unreactive at room temperature and was largely recovered. The reaction proceeded slowly upon warming the reaction mixture to 50 °C to afford the corresponding glycoconjugate **3f** in 91% yield after 3 days (Table 1, entry 7). Microwave irradiation dramatically accelerated the reaction[9] to furnish **3f** in 93% isolated yield after only 15 min (entry 8). No other side products were detected either by HPLC or mass spectral analysis of the crude product. Excess sugar azide was largely recovered when excess was used.

The neutral C_1 symmetric oligosaccharide **3f** was soluble in water but resulted in an ill-resolved ^{1}H NMR spectrum (in D_2O). The spectrum showed only carbohydrate signals but no signals indicative of the phenyl groups attached to the fullerene core. Dynamic light-scattering analysis of the aqueous solution (ca. 0.2 mM) showed an average hydrodynamic diameter of 8.3 ± 0.1 nm, suggesting that the molecules are aggregated in water. Upon conversion to the corresponding C_5 symmetric cyclopentadienide anion (cf. **2** in Scheme 1), a well-defined NMR spectrum in aqueous DMSO was obtained. The signals due to the sugar moiety were essentially the same as those in the azide reactant. The successful attachment of five trisaccharide units to the fullerene core was evident from these simplied spectra and was further supported by the mass spectrum of **3f** (Supporting Information).

After having successfully established a general and simple method to create fullerene derivatives densely functionalized with carbohydrates, we started the synthesis of P^k trisaccharide−fullerene glycoconjugate **3g**. Following the work on Shiga-like toxins by Bundle,[2] we installed the azide linker at the C2 position of central β-galactose (cf. Abstract and Scheme 1). The required trisaccharide was synthesized by the route shown in Scheme 2. Silyl triflate mediated glycosidation of **4** with glycosyl phosphate **5** and subsequent PMB deprotection afforded the disaccharide **6** in 54% yield. Introduction of the third galactose moiety by α-selective glycosidation with the phosphate **7** gave the protected Gb-3 trisaccharide **8** (R = Fmoc) in 37% yield. The Fmoc group in the central sugar was replaced by a five-carbon alkenyl chain to obtain **8** (R = $(CH_2)_3CH{=}CH_2$). Subsequent exhange of protective groups, thiolation, deprotection, and alkylation furnished the P^k trisaccharide **9** bearing an azide terminus ready to be used for the click coupling with the pentaalkynylfullerene **1**.

Due to the scarcity of P^k trisaccharide **9**, the coupling reaction was optimized using the maltotriose model and revealed that mild thermal conditions (40 °C in DMSO) with

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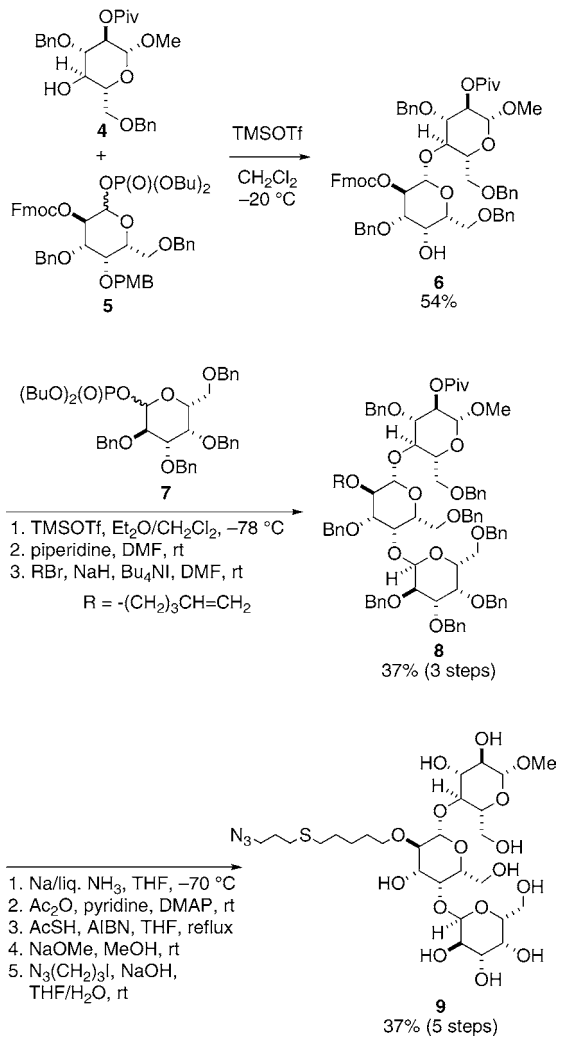
Scheme 2. Synthesis of P^k Trisaccharide Bearing a Long Tether Terminated with an Azide Group



excess azide are most suitable. Thus, **3f** was obtained in 88% yield in the reaction using ca. 400 μg of fullerene **1** (entry 9). Application of the same conditions afforded the desired glycoconjugate **3g** in 86% yield. Mass spectral analysis indicated that the reaction exclusively gave the pentacycloadduct **3g**. ^{1}H NMR analysis of **3g** (as for **3f**) was possible only for the corresponding anion.

In summary, we have developed an efficient method to construct molecular architecture of several nanometer diameter that bears as many as 15 sugar moieties radially arranged around a [60]fullerene core. Compounds **3f** and **3g** are among the most densely functionalized organic molecules prepared using click chemistry and suggest that the method will provide a powerful strategy for multivalent saccharide displays and related studies such as polynucleic acid arrays.[10,11] The aggregation behavior of the saccharide fullerene conjugates and the interaction of **3g** with Shiga-like toxin will be the subject of forthcoming studies.[12,13]

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Supporting Information Available: Experimental procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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