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# Synthesis of Fullerene Glycoconjugates through Sulfide Connection in Aqueous Media

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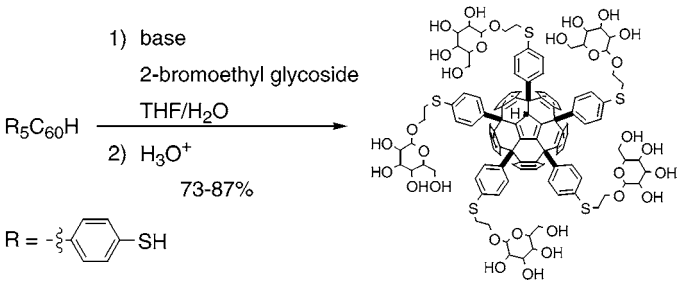
## ABSTRACT




**A thiolate/alkyl halide coupling reaction in aqueous media provides a one-step synthesis of fullerene glycoconjugates bearing five carbohydrate groups in good yield by using stoichiometric amounts of reactants, without recourse to hydroxy group protection. The sulfide-connection methodology is also useful for synthesis of simpler amphiphilic fullerene molecules, such as one bearing five carboxylic acid groups.**

Biological recognition through carbohydrate-protein interactions often relies on multivalent contacts and has hence aroused much interest in multivalent saccharide displays.[1] Among various low molecular weight displays that bear several carbohydrate groups, $C_5$-symmetric glycoconjugates possessing five carbohydrate units show promise for interaction with some important pentameric receptors.[2] On the basis of the recent development of extremely efficient synthesis of $C_5$ or quasi-$C_5$ symmetric fullerene derivatives $R_5C_{60}H$ (cf. Scheme 1),[3] we conjectured that such $R_5C_{60}H$ molecules serve as a nanometer-scale scaffold to which five carbohydrates can be attached. Our own experience in functional fullerene molecules indicated that the synthesis and the isolation of hydroxy-free carbohydrate/fullerene conjugates are rather difficult tasks owing to the vastly different solvophilicity of fullerene and unprotected sugar. Our recent experience with water-soluble fullerene anions[4] suggested to us that the use of a stable fullerene anion (e.g., **3**) instead of a neutral fullerene molecule may allow the synthesis to be carried out in an aqueous phase and hence allow the use of unprotected carbohydrates for synthesis. We report herein that the NaOH-mediated alkylation of a pentathiol compound

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**Scheme 1**

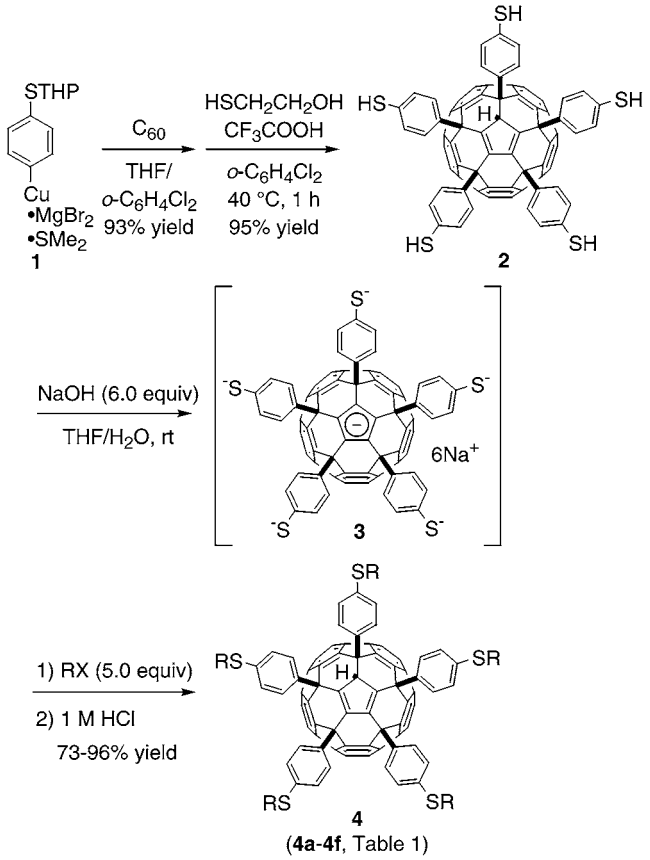
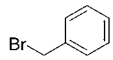
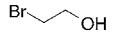
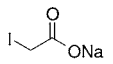
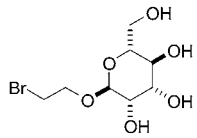
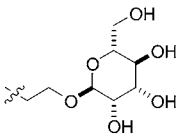
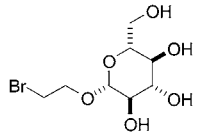
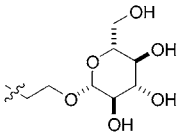
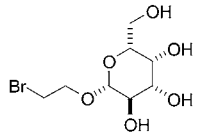
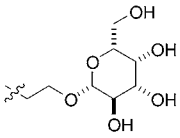



**Table 1.** Alkylation of Thiol **2** with an Alkyl Halide

| entry | RX | product | R | yield |
|---|---|---|---|---|
| 1 |  | **4a** |  | 96% |
| 2 |  | **4b** |  | 87% |
| 3 |  | **4c** |  | 84% |
| 4 |  | **4d** |  | 73% |
| 5 |  | **4e** |  | 79% |
| 6 |  | **4f** |  | 87% |

(**2**) in an aqueous solution permits high-yield connection of the fullerene molecule with five R groups that may bear free carbohydrate groups. The nucleophilicity of the thiolate anion is so high that the coupling could be achieved by using stoichiometric amounts of the fullerene, the base, and the alkyl halide. Thus, the fullerene glycoconjugates **4** containing mannose, glucose, and galactose have been synthesized in one step and in good yields without recourse to hydroxy protection.

The key pentathiol **2** was synthesized by the 5-fold addition reaction of the copper reagent **1** prepared from the corresponding Grignard reagent and CuBr·$SMe_2$ (93% isolated yield) followed by deprotection (95% isolated yield) under slightly unconventional conditions (Scheme 1).[5]

The nucleophilic substitution reaction of the thiolate in aqueous THF was optimized first for benzyl bromide. Pentathiol **2** does not dissolve in THF but dissolves freely in aqueous THF in the presence of 6 equiv of sodium hydroxide. Practically, **2** was mixed with sodium hydroxide (6.0 equiv) and benzyl bromide (5.0 equiv) under nitrogen in a degassed 2:1 THF/$H_2O$ mixture. After 2 h of stirring at room temperature, both reactants completely disappeared as analyzed by TLC, and a clear orange solution resulted. Acidification of this solution with aqueous 1 M HCl protonated the cyclopentatadienyl anion and gave insoluble orange precipitates, which were washed repeatedly with water to obtain the benzylated compound **4a** (R = $PhCH_2$) in 96% isolated yield (Table 1, entry 1).[6] Exclusive reaction at the thiolate sites rather than at the cyclopentadienyl anion in **3** is likely due to steric effects of the phenyl groups.

The same reaction conditions used for 2-bromoethanol and sodium iodoacetate gave the desired coupling products **4b** and **4c** in 87% and 84% yield, respectively, starting with stoichiometric amounts of the reactants (Table 1, entries 2 and 3). The presence of hydroxy and carboxylate groups did not affect the efficiency of the sulfide formation reaction at all.

With these results in hand, we next examined the reaction of **2** with 2-bromoethyl mannoside.[7] The reaction with 2-bromoethyl α-D-mannoside was carried out in a manner exactly the same as the one described for benzyl bromide and afforded the glycoconjugate **4d** in 73% isolated yield (Table 1, entry 4). Owing to the poor solubility in various solvents, this compound posed considerable problems of structural determination. In addition, the $^1H$ NMR spectrum of **4d** in DMSO-$d_6$ was complicated because of the $C_1$ symmetry of the compound. However, addition of a small amount of NaOH/$D_2O$ to the DMSO-$d_6$ solution dramatically simplified the spectrum by establishing fast equilibrium of the H form **4d** with the corresponding cyclopentadienyl anion

(5) Block, E.; Aslam, M. *J. Am. Chem. Soc.* **1985**, *107*, 6729. Watanabe, H.; Ogo, S.; Yamamura, T. *Chem. Lett.* **1996**, 999.

(6) Even in the case of incomplete reaction, it is likely that the intermediary products that contain thiol group(s) were oxidized, during workup, into oligomeric disulfides of extremely large molecular weights.

(7) Dahmén, J.; Frejd, T.; Magnusson, G.; Noori, G.; Carlström, A.-S. *Carbohydr. Res.* **1984**, *125*, 237. Davis, B. G.; Maughan, M. A. T.; Green, M. P.; Ullman, A.; Jones, J. B. *Tetrahedron: Asymmetry* **2000**, *11*, 245.

form. Under these conditions, the $^1$H NMR spectrum exhibited one set of doublets due to the five aryl moieties and one set of signals due to the carbohydrate moieties, clearly indicating the formation of five sulfide linkages. A broad singlet signal due to the anomeric position confirmed the retention of the C1-stereochemistry during the reaction. The ESI-TOF mass spectrum showed the molecular weight consistent with **4d** [$C_{130}H_{96}O_{30}S_5$ $(M - H)^-$ *m*/*z* 2296, calcd 2296].

The new amphiphile contains both fullerene and free carbohydrate moieties, which differ drastically from each other in their solvent compatibility. As a consequence, we could not find chromatographic conditions suitable for purification of **4d**. The purity of the product was, however, ascertained by TLC analysis that showed complete conversion of the starting materials, the $^1$H NMR spectrum in basic DMSO-$d_6$, and MALDI MS/MS analysis indicating the high purity of the product.

Connection to glucose and galactose was achieved in the same manner. Thus, the substitution reaction of **2** with 2-bromoethyl $\beta$-D-glucoside and 2-bromoethyl $\beta$-D-galactoside gave the glucosyl (**4e**) and galactosyl conjugates (**4f**) in 79% and 87% yield, respectively (Table 1, entries 5 and 6). The structures of **4e** and **4f** were also determined by $^1$H NMR and mass spectra.

In summary, we have developed an efficient method for the synthesis of fullerene glycoconjugates and expect that the method will be applicable to the attachment of larger sugar groups or other biologically relevant groups. The aqueous conditions that eliminate the use of protected sugar make the method unique among known methods for fullerene modification that are carried out in an aprotic organic solvent.[8] The use of the reported compounds as multivalent saccharide displays[1,9] and as building blocks for fullerene self-assembly[10,11] are among our interests in these compounds.

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**Supporting Information Available:** Experimental procedures and details of mass and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) See, for examples: Vasella, A.; Uhlmann, P.; Waldraff, C. A. A.; Diederich, F.; Thilgen, C. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1388. Yamago, S.; Tokuyama, H.; Nakamura, E.; Plato, M.; Wudl, F. *J. Org. Chem.* **1993**, *58*, 4796. Uhlmann, P.; Harth, E., Naughton, A. B.; Vasella, A. *Helv. Chim. Acta* **1994**, *77*, 2335. Cardullo, F.; Diederich, F.; Echegoyen, L.; Habicher, T.; Jayaraman, N.; Leblanc, R. M.; Stoddart, J. F.; Wang, S. *Langmuir* **1998**, *14*, 1955. Yashiro, A.; Nishida, Y.; Ohto, M.; Eguchi, S.; Kobayashi, K. *Tetrahedron Lett.* **1998**, *39*, 9031. Ishi-i, T.; Nakashima, K.; Shinkai, S.; Ikeda, A. *J. Org. Chem.* **1999**, *64*, 984. Kato, H.; Yashiro, A.; Mizuno, A.; Nishida, Y.; Kobayashi, K.; Shinohara, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2935. Dondoni, A.; Marra, A. *Tetrahedron Lett.* **2002**, *43*, 1649. Mikata, Y.; Takagi, S.; Tanahashi, M.; Ishii, S.; Obata, M.; Miyamoto, Y.; Wakita, K.; Nishisaka, T.; Hirano, T.; Ito, T.; Hoshino M.; Ohtsuki C.; Tanihara M.; Yano S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3289. Ito, H.; Tada, T.; Sudo, M.; Ishida, Y.; Hino, T.; Saigo, K. *Org. Lett.* **2003**, *5*, 2643.

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(11) Mirkin, C. A.; Caldwell, W. B. *Tetrahedron* **1996**, *52*, 5113. Georgakilas, V.; Pellarini, F.; Prato, M.; Guldi, D. M.; Melle-Franco, M.; Zerbetto, F. *Proc. Natl. Acad. Sci., U.S.A.* **2002**, *99*, 5075. Cassell, A. M.; Asplund, C. L.; Tour, J. M. *Angew. Chem., Int. Ed.* **1998**, *38*, 2403. Isobe, H.; Sugiyama, S.; Fukui, K.-i.; Iwasawa, Y.; Nakamura, E. *Angew. Chem., Int. Ed.* **2001**, *40*, 3364.