Pergamon

Tetrahedron Letters 39 (1998) 9031–9034

TETRAHEDRON
LETTERS

Fullerene Glycoconjugates: A General Synthetic Approach via Cycloaddition of Per-*O*-Acetyl Glycosyl Azides to [60]Fullerene

Arihiro Yashiro, Yoshihiro Nishida, Masatomi Ohno,
Shoji Eguchi, and Kazukiyo Kobayashi*

Department of Molecular Design and Engineering, Graduate School of Engineering,
Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan

Received 8 July 1998; accepted 18 September 1998

Abstract: A general synthetic way to incorporate oligosaccharides into [60]fullerene via cycloadditon and deacetylation is presented. The cycloaddition reaction in refluxing chlorobenzene gave a mixture of two unseparable stereoisomers of *N*-β-glycopyranosyl [5,6]-azafulleroids in 13~28% yields for per-*O*-acetyl glycosyl azide of D-glucopyranose, D-galactopyranose, lactose, maltose, and maltotriose.

Keywords: fullerenes, glycosides, cycloadditions, singlet oxygen, azides

Oligosaccharides on cell surfaces are responsible for many cell-cell recognition events in biological systems, which include infection by certain viruses, pathogenic bacteria, and their producing toxins [1-3]. In our research projects to utilize biologically active oligosaccharides as new biomaterials [4], we have reported convenient synthetic ways to incorporate natural or synthetic oligosaccharides into artificial polymers [5]. In the course of our study, our interest was directed to another biologically active substance, [60]fullerene, which has been increasingly reported to exhibit many types of biological activites. By generating singlet oxygen under visible light, [60]fullerene shows, for example, DNA cleavage, enzyme inhibition, and cytotoxicity [6]. It is, therefore, expectable that these biological activities, coupled with oligosaccharides, may lead to a new class of biomaterials showing "catch and kill" functionality against pathogenic bacteria and toxins and also against tumor cells. In this paper, we wish to report a general synthetic approach to fullerene glycoconjugates utilizing a cycloaddition reaction between glycosyl azides and [60]fullerene.

Several approaches have already been reported to introduce monosaccharide or dendrimer saccharides into fullerenes. Diederich and co-workers reported the syntheses of fullerenes carrying *O*-benzylated or *O*-pivaloyl D-glucopyranose utilizing a cycloaddition of D-glycosidene carbenes to [60]fullerene [7]. Taylor and co-workers

PII: S0040-4039(98)02047-4

reported a synthesis of 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose linked fullerene through acylation at the O-6 position with aziridino[2',3':1,2][60]fullerene [8]. More recently, Cardullo *et al.* reported fullerene-glycodendron conjugates [9]. These methods, however, could not afford fullerene glycoconjugates carrying oligosaccharides with biological activities. Since most reducing oligosaccharides can be converted into the corresponding glycosyl azides, we examined a cycloaddion reaction between glycosyl azides and [60]fullerene to establish a general way to introduce di- and oligosaccharides into fullerenes utilizing the reaction of alkyl azides with fullerenes [10].

In order to conduct the cycloaddition reaction, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (56 mg, 1.5 equiv. to C_{60}) was allowed to react with C_{60} (72 mg) in refluxing chlorobenzene (16 ml) under a nitrogen atmosphere (Scheme 1, Table 1). The silica gel TLC indicated that a new reddish-purple spot (R_f = 0.6, toluene : ethyl acetate = 2:1) appeared in a few hours between fullerene (purple color, R_f = 0.95) and glycosyl azide (R_f = 0.5) in addition to minor spots between R_f = 0.1 and 0.5. After continuing the reflux for 10 h, the main product at R_f = 0.6 was isolated by silica gel column chromatography (toluene : ethyl acetate = 10 : 0 to 5 : 1) as a dark brown solid [30 mg, 28 % yield (57 % yield based on consumed C_{60})].

Scheme 1

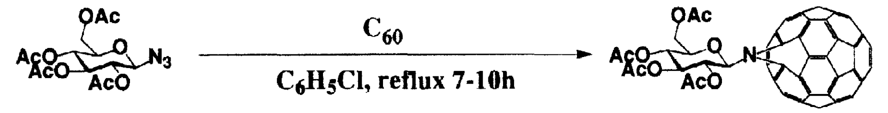
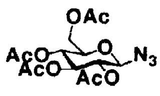
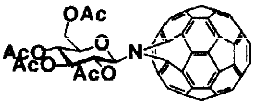
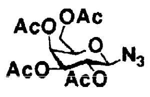
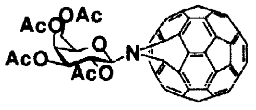
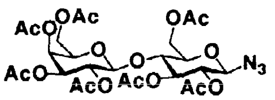
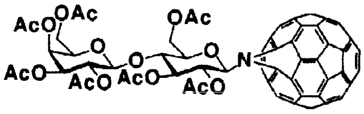
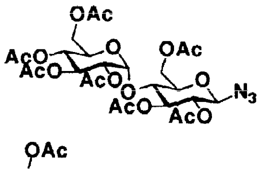
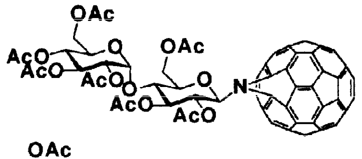
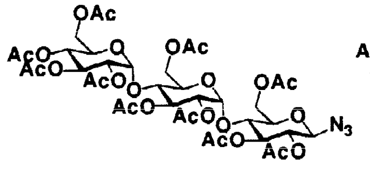
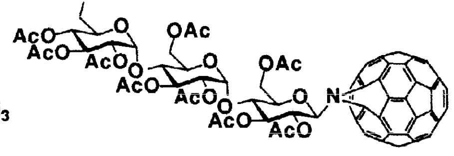


Table 1. Synthesis and Characterization of Fullerene Glycoconjugate *O*-Acetates

1-Azidosaccharides	Adducts	Yields[a] [ ][b] (%)	FAB-MS (m/z)	IR (KBr, cm^{-1})
		28 [57]	1065, 720	1755, 1427, 1365, 1216, 1033, 526
		18 [30]	1065, 720	1749, 1427, 1365, 1217, 1043, 526
		13 [27]	1353, 1097, 720	1751, 1429, 1365, 1218, 1036, 526
		18 [50]	1353, 1066, 720	1751, 1429, 1369, 1228, 1038, 526
		16 [35]	1641, 720	1751, 1427, 1367, 1224, 1036, 526

[a] isolated yield, [b] based on consumed C_{60}

Based on analyses using FAB-MS [m/z = 1066, 1065 (M^+), 720 (C_{60}), matrix = *m*-nitrobenzyl alcohol], IR [KBr film (cm^{-1}) 1755s ($OCOCH_3$), 1427m (C_{60}), 1033m (C_{60}), 526s (C_{60})], ^{13}C- and ^1H-NMR spectroscopies, the product was determined to be a 1:1 glucose-fullerene adduct [11]. However, the ^1H-NMR analysis (Figure 1) revealed that this product was a mixture of two isomers in a molar ratio of *ca.* 2:1 (adducts A and B in Figure 2). Compared with the ^{1}H-NMR data of per-*O*-acetyl-β-D-glucopyranosyl azide, both adducts showed large downfield shifts for all ring protons (H-1 to H-5), in which significant chemical shift differences were observed between A and B [Figure 1, downfield shift value (Δppm) of H-1 : +0.64 (A), +0.44 (B) ; H-2 : +0.48 (A), +0.83 (B) ; H-3 : +0.46 (A), +0.56 (B) ; H-4 : +0.01 (A), +0.15 (B)]. Judging from the unchanged vicinal couplings of the ring protons, it was concluded that the adducts A and B are structural or steric isomers which retain a common per-*O*-acetyl-β-D-glucopyranosyl residue.

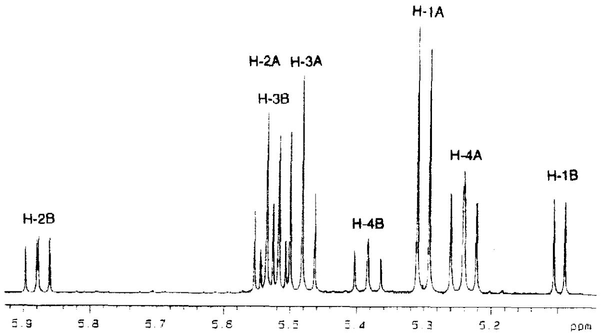
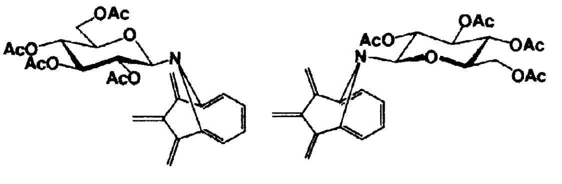


Figure 1. Partial ^{1}H-NMR spectrum of 1 : 1 adducts A and B (500 MHz, $CDCl_3$, 25 ℃). Assignments of A and B may be changed.



1:1 adduct A

1:1 adduct B

Figure 2. Possible structures of adducts A and B.

There is a possibility that two structural isomers can be yielded in the cycloaddition of alkyl or acyl azides with [60]fullerene ascribed to the bridge nitrogen spanning over either a 5,6-ring junction or a 6,6-ring junction. Alkyl azides are known to be preferred to afford 5,6-addition type azafulleroids [8,10,12,13], while acyl azides preferentially give aziridinofullerenes *via* the 6,6-addition. In order to identify the two possible types for adducts A and B, ^{13}C-NMR and UV characterizations were performed according to the reported methods [8,10,12]. In the ^{13}C-NMR spectrum, in addition to twelve sp^3 carbons of the glucose moiety (Adducts A and B) between 60 and 90 ppm, multiple overlapping 73 lines due to the sp^2 carbons of the C_{60} moiety were separated in the region of 135-150 ppm. The sp^3 carbon of C_{60} characteristic of aziridinofullerene could not be detected between 80-90 ppm. In the UV-vis spectrum, the absorption bands were observed at 260, 266, and 327 nm which are characteristic to fullerene derivatives, while the absorption bands between 420-440 nm characteristic of aziridinofullerenes were absent. These NMR and UV data enabled us to eliminate the possibility of a 6,6-addition type for both A and B.

The alternative possibility allowed us to identify the 5,6-addition mechanism for both products. At present, it could be suggested that the pyramidal inversion at the nitrogen atom might be "frozen" by some steric or electric restrictions [10] to afford two N-invertomers of azafulleroids A and B (Figure 2). The significant ^{1}H-NMR chemical shift change in H-1 and H-2 between A and B, as described above, may be rationalized by the large conformational difference in the two isomers around the anomeric position. Increase of the temperature for ^{1}H-NMR measurements up to 100℃ in DMSO-d_6 as well as for reactions up to 180℃ in *o*-dichlorobenzene, however, did not substantially change the ratio of A and B .

Deacylation of per-*O*-acetyl β-D-glucopyranosyl fullerenes (A and B) was performed using a catalytic amount of sodium methoxide in a methanol-dichloromethane mixture (room temperature for 3 h). The mixture gave directly a brown precipitate in 69 % yield. The IR spectrum [KBr film (cm^{-1}): 3363s (OH), 2923s (CH), 1435m (C_{60}), 1076s, 1035w(C_{60}), 525s (C_{60})] showed the complete disappearance of the *O*-acetyl group signal (1755 cm^{-1}) and simultaneous appearance of a strong broad OH band. The spectrum indicated the complete deacetylation to afford azafulleroids carrying *N*-β-D-glucopyranoside. Measurements of the other spectral data are in progress, which are, however, hampered by the poor solvent solubility probably due to the high aggregation property of amphiphilic fullerenes [9].

In order to confirm the generality of the reaction between glycosyl azides and [60]fullerene, a series of mono-, di-, and trisaccharides were converted into the corresponding glycosyl azides (Table 1). Through S_N2 replacement of the 1-bromo group of per-*O*-acetyl glycosyl bromides with an azide group, each reducing saccharide gave the corresponding glycosyl azide in higher than 65% yield. The cycloaddition using the di- and trisaccharide azides afforded the corresponding fullerene glycoconjugates as per-*O*-acetates in 13~18% yields (21~50%, based on consumed C_{60}). They all gave two stereoisomers in ratios similar to the case of D-glucose. Thus, the present method was found to be valid for the synthesis of fullerene glycoconjugates carrying a variety of mono-, di-, and trisaccharides. Biological evaluations of the derived fullerene glycoconjugates are in progress and will be reported in due course elsewhere.

References

[1] Varki A. Glycobiology **1993** ; 3 : 97-130.

[2] Varki A. Proc. Natl. Acad. Sci. U.S.A. **1994** ; 91 : 7390-7397.

[3] Sharon N, Lis H. Sci. Am. **1993** ; 1 : 74-81.

[4] Kobayashi K, Kobayashi A, Tobe S, Akaike T. in Neoglycoconjugates: Preparation and Applications (Lee YC, Lee RT, eds.) Academic Press, San Diego **1994** ; 261-282.

[5] Kobayashi K, Tsuchida A, Usui A, Akaike T. Macromolecules **1997** ; 30 : 2016-2020

[6] For a review of biological application of fullerene, Jensen AW, Wilson SR, Schuster DI. Bioorg. Med. Chem. **1994** ; 4 : 767-779.

[7] Vasella A, Uhlmann P, Waldraff CAA, Diederich F, Thilgen C. Angew. Chem. Int. Ed. Engl. **1992** ; 31 : 1388-1390.

[8] Banks MR, Cadogan JIG, Gosney I, Hodgson PKG, Langridge-Smith PRR, Millar JRA, Taylor A. J. Chem. Soc., Chem. Commun. **1995** : 885-886.

[9] Cardullo F, Diederich F, Echegoyen L, Habicher T, Jayaraman N, Leblanc RM, Stoddart JF, Wang S. Langmuir **1998** ; 14 : 1955-1959.

[10] Prato M, Li QC, Wudl F. J. Am. Chem. Soc. **1993** ; 115 : 1148-1150.

[11] The possibility of a 2:1 adduct of glucose and fullerene (*i.e*, bisazafulleroid [12]) could be ruled out by the absence of the ^{13}C signal at *ca.* 160 ppm as well as by FAB-MS measurement.

[12] Grosser T, Prato M, Lucchini V, Hirsch A, Wudl F. Angew. Chem. Int. Ed. Engl. **1995** ; 34 : 1343-1345.

[13] Hummelen JC, Prato M, Wudl F. J. Am. Chem. Soc. **1995** ; 117 ; 7003-7004.

[14] Schick G, Grosser T, Hirsch A. J. Chem. Soc., Chem. Commun. **1995** : 2289-2290.