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Synthesis of [60]fulleropyrrolidine glycoconjugates using 1,3-dipolar cycloaddition with *C*-glycosyl azomethine ylides

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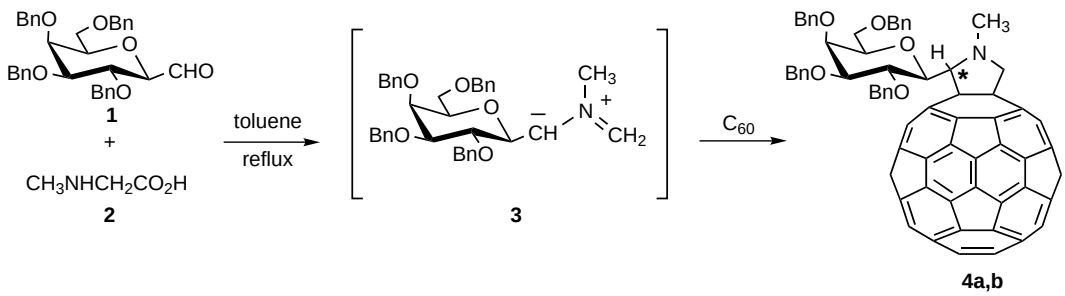
Abstract—Heating a mixture of [60]fullerene, *N*-methylglycine (sarcosine), and a sugar aldehyde in refluxing toluene resulted in the formation of a complex mixture of products from which the fulleropyrrolidine monocycloadduct was isolated in 14, 10, and 12% yield for formyl *C*-galactopyranoside, formyl *C*-glucopyranoside, and formyl *C*-mannofuranoside, respectively. © 2002 Elsevier Science Ltd. All rights reserved.

Given the broad range of biological activities[1] shown by fullerene derivatives[2] and their potential application in medicinal chemistry,[3] the functionalization of fullerenes with organic residues which are historically rich in biological activity is a topic of current interest. In addition to the extensive work on fullerenic acids and peptides,[3b,4] noteworthy are the recent reports on the synthesis of cyclodextrin–fullerenes,[5] steroid–fullerene adducts,[6] and a hybrid fullerene–trimethoxyindole–oligonucleotide conjugate.[7] Two approaches have been earlier carried out to introduce saccharide residues into fullerenes, one by Vasella, Diederich and their co-workers via cyclopropanation by cycloaddition with nucleophilic glycosylidene carbenes,[8] the other by Kobayashi and co-workers via [5,6]-azafulleroid formation by cycloaddition of glycosyl azides.[9] Glycosylation of fullerenes may not only give rise to ambiphilic products with improved pharmacokinetic and pharmacodynamic properties but also provide fulleroglycoconjugates with new biological activities arising from specific molecular recognition events involving the carbohydrate moieties. In fact complex oligosaccharides as part of glycoconjugates play an important role in biological communication processes including cell growth and differentiation, cell-to-cell communication, modulation of protein function, cancer metastasis, chronic inflammation, and viral and microbial infection.[10] Hence we would like to report here on a new approach to sugar fullerenes which relies on the Prato method of fullerene functionalization via fulleropyrrolidine formation based on the 1,3-dipolar cycloaddition reaction of azomethine ylide to C_{60}.[11] This opportunity of *C*-glycosyl [60]fulleropyrrolidine synthesis was provided by the availability in our laboratory of various configurationally stable sugar aldehydes (formyl *C*-glycosides) via the thiazole based formylation of sugars through their lactones.[12]



Scheme 1.

Keywords: [60]fullerene; 1,3-dipolar cycloaddition; azomethine ylide; fulleropyrrolidine.

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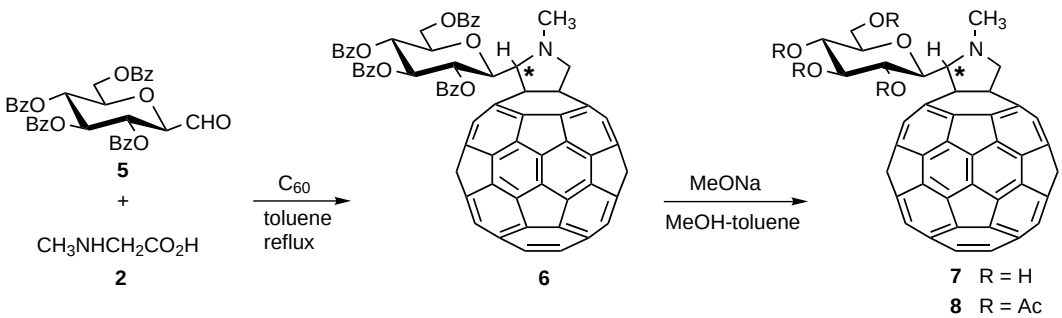
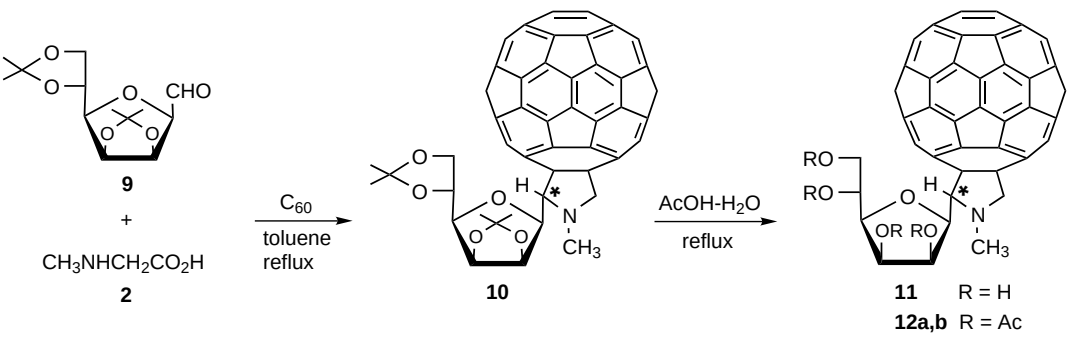
A model reaction was generated by refluxing in toluene for 1 h an equimolar mixture (5 mM) of [60]fullerene, perbenzylated formyl *C*-galactopyranoside[12] **1**, and *N*-methylglycine (sarcosine) **2** (Scheme 1). The reaction should proceed through the formation of the 1,3-dipolar sugar azomethine ylide intermediate **3** as, after workup[13] of the complex reaction mixture, the diastereomeric monocycloadducts **4a**,**b** were isolated by column chromatography (10:1 to 5:1 toluene–AcOEt) in a 2.5:1 ratio and 14% overall yield (56% based on C_{60} conversion). Each individual diastereomer was characterized[14] as a [60]fulleropyrrolidine glycoconjugate arising from the attack of the *C*-glycosyl azomethine ylide **3** on a 6,6-ring junction of C_{60}. Both compounds showed the signals for the diagnostic[11] quaternary sp^3-hybridized carbon atoms of attachment to the pyrrolidine ring at δ 71–76 ppm, while the signals for the other two carbons of the heterocycle were found at $\delta \sim 70$ and ~ 76 ppm. Unfortunately, attempts at deprotection of the sugar fullerenes **4a**,**b** by selective removal of the *O*-benzyl protective groups of the glycoside residue via hydrogenolysis over palladium produced a complex mixture of products due to the decomposition of C_{60}.

Having realized the need for protecting groups which could be removed under reaction conditions that do not affect the C_{60} moiety, we considered the use of the perbenzoylated formyl *C*-glucopyranoside[15] **5** since it has been demonstrated[9] that the removal of ester groups is tolerated by C_{60}. The sarcosine **2** promoted cycloaddition to C_{60} under the standard conditions[13] afforded a single isolable fulleropyrrolidine monocycloadduct[16] **6** in 10% yield, 52% based on C_{60} conversion (Scheme 2). In this case the crude reaction mixture appeared even more complex than in the reaction of the sugar aldehyde **1** due to the partial transformation of **5** into the corresponding α,β-unsaturated aldehyde. Quite rewardingly the removal of the benzoyl groups from **6** was successfully carried out by transesterification using sodium methoxide in a mixture of methanol–toluene (rt, 1 h). The hydroxy unprotected sugar fulleropyrrolidine **7** was characterized as the peracetylated derivative[16] **8** (67% from **6**).

Also, the acetonide protecting groups appeared promising for our aim because their cleavage proceeds under mild acidic conditions which are known to be compatible with C_{60}. Thus, on heating a mixture of C_{60}, sarcosine **2**, and formyl *C*-mannofuranoside diacetonide[12] **9** in refluxing toluene for 1 h and workup of the reaction mixture,[13] afforded the corresponding *C*-glycosyl [60]fulleropyrrolidine **10** as a pair of diastereomers (2:1 ratio) in 12% overall yield (62% based on C_{60} conversion).[17] For both compounds the quaternary sp^3-hybridized carbon atoms of the fullerene residue appeared at δ 71–75 ppm, thus confirming the 6,6-ring junction on the C_{60} cage. Treatment of this mixture with 6:1 AcOH–H_2O at reflux for 18 h resulted in the complete removal of the acetonide protecting groups to give the hydroxy free glycoconjugate **11**. After acetylation of the crude mixture (Ac_2O, pyridine, rt, 6 h) the two diastereomers **12a** and **12b** were separated by column chromatography on silica gel (3:1 cyclohexane–AcOEt) in 72% overall yield and each individual product was characterized by spectroscopic means (Scheme 3).[18]



Scheme 2.



Scheme 3.

In conclusion, we have demonstrated the feasibility of fullerene glycoconjugate synthesis via a three-component approach involving [60]fullerene, a sugar aldehyde, and sarcosine. The key step of the glycosylation process is constituted by a 1,3-dipolar cycloaddition reaction of a *C*-glycosyl *N*-methyl azomethine ylide on the 6,6-ring junction of C_{60}. Subtle reaction conditions are required to achieving enough reactivity and nevertheless producing the monocycloadduct in acceptable yet modest yield. A continuation of this work dealing with the isolation of bis-cycloadducts and their characterization is in progress.

Acknowledgements

We gratefully thank the University of Ferrara for financial support.

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13. After evaporation of the toluene, the residue was triturated sequentially with 1:1 CH_2Cl_2–AcOEt, MeOH, again with 1:1 CH_2Cl_2–AcOEt. The remaining insoluble material was essentially pure C_{60}. The combined extracts were concentrated and the residue was eluted from a column of silica gel. First eluted was the monoadduct followed by unreacted aldehyde and polyadducts.
14. Faster moving diastereomer **4a**. ^{1}H NMR ($CDCl_3$): δ 7.47–7.21 (m, 20H, 4Ph), 5.20 and 5.01 (2d, 2H, $J=11.3$ Hz, PhCH_2), 5.07 and 4.66 (2d, 2H, $J=11.6$ Hz, PhCH_2), 4.97 and 4.24 (2d, 2H, $J=10.3$ Hz, CH_2N), 4.84 and 4.72 (2d, 2H, $J=11.6$ Hz, PhCH_2), 4.65 (d, 1H, $J_{1,CHN}=1.0$ Hz, CHN), 4.58 (dd, 1H, $J_{1,2}=9.8$, $J_{2,3}=8.8$ Hz, H-2), 4.41 (s, 2H, PhCH_2), 4.37 (dd, 1H, H-1), 4.08 (dd, 1H, $J_{3,4}=2.5$, $J_{4,5}=0.8$ Hz, H-4), 3.78 (dd, 1H, $J_{5,6a}=8.2$, $J_{6a,6b}=8.8$ Hz, H-6a), 3.77 (dd, 1H, H-3), 3.59 (dd, 1H, $J_{5,6b}=5.0$ Hz, H-6b), 3.50 (ddd, 1H, H-5), 3.13 (s, 3H, CH_3N). ^{13}C NMR ($CDCl_3$) selected data: δ 85.6 (C-3), 79.1 (C-1), 77.2 (C-5), 76.2 (C-2), 75.9 (sp^3-C of C_{60}), 75.6 (CHN), 75.1, 74.6, 73.4, and 72.1 (4PhCH_2), 73.8 (C-4), 70.8 (CH_2N), 68.3 (C-6), 42.5 (CH_3N). MALDI-TOF MS: 1302.5 (M+2H). Slower moving diastereomer **4b**. ^{1}H NMR ($CDCl_3$): δ 7.42–7.12 (m, 20H, 4Ph), 5.02 and 4.70 (2d, 2H, $J=10.9$ Hz, PhCH_2), 5.00 and 4.24 (2d, 2H, $J=9.9$ Hz, CH_2N), 4.78 (dd, 1H, $J_{1,2}=10.0$, $J_{2,3}=8.7$ Hz, H-2), 4.78 and 4.67 (2d, 2H, $J=11.6$ Hz, PhCH_2), 4.71 and 4.44 (2d, 2H, $J=10.8$ Hz, PhCH_2), 4.66 (d, 1H, $J_{1,CHN}=1.0$ Hz, CHN), 4.51 (s, 2H, PhCH_2), 4.42 (dd, 1H, H-1), 4.09 (dd, 1H, $J_{3,4}=2.4$, $J_{4,5}=0.7$ Hz, H-4), 3.83 (ddd, 1H, $J_{5,6a}=5.5$, $J_{5,6b}=8.0$ Hz, H-5), 3.77 (dd, 1H, H-3), 3.74 (dd, 1H, $J_{6a,6b}=9.0$ Hz, H-6a), 3.58 (dd, 1H, H-6b), 3.01 (s, 3H, CH_3N). ^{13}C NMR ($CDCl_3$) selected data: δ 85.8 (C-3), 77.5 (C-1), 76.9 (C-5), 76.2 (CHN), 75.3 (C-2), 75.0, 74.9, 73.7, and 72.1 (4PhCH_2), 73.6 (C-4), 73.1 and 70.9 (sp^3-C of C_{60}), 69.5 (CH_2N), 68.2 (C-6), 40.3 (CH_3N). MALDI-TOF MS: 1302.9 (M+2H).
15. The hitherto unreported β-D-linked sugar aldehyde **5** was prepared by debenzylation (BCl_3, CH_2Cl_2, −60 to 0°C, 2 h, then MeOH, 0°C, 10 min) of the known[12a] *O*-perbenzylated thiazolyl *C*-glucopyranoside, followed by acetylation (Ac_2O, Py), hydrolysis (MeOH–Et_3N–H_2O), benzoylation (BzCl, Py), and finally by the improved thiazole-to-formyl transformation protocol (MeOTf, $NaBH_4$, $AgNO_3$–H_2O; for details see: Dondoni, A.; Marra, A.; Scherrmann, M.-C.; Bertolasi, V. *Chem. Eur. J.* **2001**, *7*, 1371 or write to the corresponding author). The purity of the crude aldehyde **5**, determined by NMR analysis, was ca. 90%. ^{1}H NMR ($CDCl_3$) selected data: δ 9.70 (d, 1H, $J_{1,2}=2.0$ Hz, H-1), 6.00 (dd, 1H, $J_{3,4}=9.6$, $J_{4,5}=9.4$ Hz, H-4), 5.76 (dd, 1H, $J_{5,6}=9.7$ Hz, H-5), 5.68 (dd, 1H, $J_{2,3}=9.9$ Hz, H-3), 4.72 (dd, 1H, $J_{6,7a}=2.8$, $J_{7a,7b}=12.5$ Hz, H-7a), 4.55 (dd, 1H, $J_{6,7b}=4.9$ Hz, H-7b), 4.26 (ddd, 1H, H-6), 4.19 (dd, 1H, H-2).
16. Cycloadduct **6**. ^{1}H NMR ($CDCl_3$): δ 8.08–7.88 and 7.60–7.31 (2m, 20H, 4Ph), 6.49 (dd, 1H, $J_{1,2}=J_{2,3}=9.5$ Hz, H-2), 6.11 (dd, 1H, $J_{3,4}=9.4$ Hz, H-3), 5.98 (dd, 1H, $J_{4,5}=10.5$ Hz, H-4), 5.36 and 4.64 (2d, 2H, $J=11.6$ Hz, CH_2N), 4.92 (dd, 1H, $J_{1,CHN}=1.5$ Hz, H-1), 4.84 (d, 1H, CHN), 4.77 (dd, 1H, $J_{5,6a}=3.0$, $J_{6a,6b}=12.2$ Hz, H-6a), 4.69 (dd, 1H, $J_{5,6b}=4.0$ Hz, H-6b), 4.25 (ddd, 1H, H-5), 3.49 (s, 3H, CH_3N). MALDI-TOF MS: 1357.7 (M+H). Cycloadduct **8**. ^{1}H NMR ($CDCl_3$): δ 5.98–5.92 (m, 1H, H-2), 5.41–5.36 (m, 2H, H-3, H-4), 5.26 and 4.63 (2d, 2H, $J=11.8$ Hz, CH_2N), 4.70 (d, 1H, $J_{1,CHN}=1.2$ Hz, CHN), 4.55 (dd, 1H, $J_{1,2}=9.8$ Hz, H-1), 4.38 (dd, 1H, $J_{5,6a}=4.0$, $J_{6a,6b}=12.6$ Hz, H-6a), 4.30 (dd, 1H, $J_{5,6b}=2.0$ Hz, H-6b), 3.76–3.70 (m, 1H, H-5), 3.43 (s, 3H, CH_3N), 2.24,

2.14, 2.11, and 2.07 (4s, 12H, 4Ac). MALDI-TOF MS: 1109.2 (M+H).

17. Faster moving diastereomer **10a**. ^{1}H NMR ($CDCl_3$): δ 5.33 (dd, 1H, $J_{1,2}=3.2$, $J_{2,3}=6.0$ Hz, H-2), 4.86 (d, 1H, $J_{1,CHN}=9.1$ Hz, CHN), 4.84 (dd, 1H, $J_{3,4}=3.7$ Hz, H-3), 4.67 and 4.46 (2d, 2H, $J=10.4$ Hz, CH_2N), 4.60 (dt, 1H, $J_{4,5}=7.6$, $J_{5,6}=5.2$ Hz, H-5), 4.51 (dd, 1H, H-1), 4.26 (d, 2H, 2H-6), 3.70 (dd, 1H, H-4), 3.24 (s, 3 H, CH_3N), 1.58, 1.51, 1.43, and 1.20 (4s, 12H, $4CH_3$). ^{13}C NMR ($CDCl_3$) selected data: δ 112.8 and 109.3 (2OCO), 83.4, 81.7, 80.8, 80.6, 75.9, 74.9 (sp^3-C of C_{60}), 73.1, 71.1, 67.2, 41.7 (CH_3N), 27.0, 25.9, 25.3, and 24.3 ($4CH_3$). MALDI-TOF MS: 1021.9 (M+2H). Slower moving diastereomer **10b**. ^{1}H NMR ($CDCl_3$): δ 5.27 (dd, 1H, $J_{1,2}=3.2$, $J_{2,3}=5.9$ Hz, H-2), 4.94 (dd, 1H, $J_{3,4}=3.6$ Hz, H-3), 4.86 and 4.52 (2d, 2H, $J=10.7$ Hz, CH_2N), 4.68 (d, 1H, $J_{1,CHN}=9.4$ Hz, CHN), 4.38 (ddd, 1H, $J_{4,5}=8.8$, $J_{5,6a}=6.2$, $J_{5,6b}=4.4$ Hz, H-5), 4.33 (dd, 1H, H-1), 3.86 (dd, 1H, $J_{6a,6b}=8.5$ Hz, H-6a), 3.50 (dd, 1H, H-6b), 3.43 (dd, 1H, H-4), 3.26 (s, 3H, CH_3N), 1.69, 1.53, 1.39, and 1.32 (4s, 12H, $4CH_3$). ^{13}C NMR ($CDCl_3$) selected data: δ 112.9 and 109.4 (2OCO), 83.3, 82.4, 82.3, 80.6, 75.4, 72.5, 71.9 (sp^3-C of C_{60}), 71.2, 67.1, 41.5 (CH_3N), 26.9, 26.1, 25.0, and 24.9 ($4CH_3$). MALDI-TOF MS: 1022.5 (M+2H).

18. Faster moving diastereomer **12a**. ^{1}H NMR ($CDCl_3$): δ 5.91 (dd, 1H, $J_{1,2}=4.0$, $J_{2,3}=5.7$ Hz, H-2), 5.82 (dd, 1H, $J_{3,4}=6.5$ Hz, H-3), 5.52 (ddd, 1H, $J_{4,5}=8.6$, $J_{5,6a}=2.5$, $J_{5,6b}=5.5$ Hz, H-5), 4.92 and 4.68 (2d, 2H, $J=10.5$ Hz, CH_2N), 4.86 (dd, 1H, $J_{1,CHN}=8.1$ Hz, H-1), 4.78 (dd, 1H, $J_{6a,6b}=12.0$ Hz, H-6a), 4.68 (d, 1H, CHN), 4.34 (dd, 1H, H-6b), 4.28 (dd, 1H, H-4), 3.30 (s, 3H, CH_3N), 2.15, 2.08, 2.05, and 2.01 (4s, 12H, 4Ac). MALDI-TOF MS: 1110.8 (M+2H). Slower moving diastereomer **12b**. ^{1}H NMR ($CDCl_3$): δ 6.02 (dd, 1H, $J_{1,2}=2.9$, $J_{2,3}=5.5$ Hz, H-2), 5.84 (dd, 1H, $J_{3,4}=7.5$ Hz, H-3), 5.36 (ddd, 1H, $J_{4,5}=8.5$, $J_{5,6a}=2.5$, $J_{5,6b}=5.2$ Hz, H-5), 4.85 and 4.51 (2d, 2H, $J=11.0$ Hz, CH_2N), 4.79 (dd, 1H, $J_{1,CHN}=9.3$ Hz, H-1), 4.74 (d, 1H, CHN), 4.57 (dd, 1H, $J_{6a,6b}=12.2$ Hz, H-6a), 4.18 (dd, 1H, H-4), 3.80 (dd, 1H, H-6b), 3.14 (s, 3H, CH_3N), 2.38, 2.06, 1.98, and 1.94 (4s, 12H, 4Ac). MALDI-TOF MS: 1110.2 (M+2H).