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Synthesis of derivatives of fullerenes C_{60} and C_{70} containing pharmacophore groups

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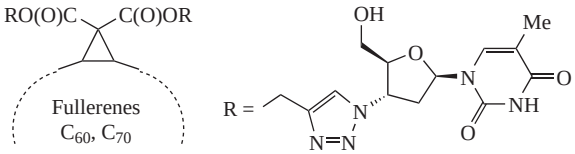
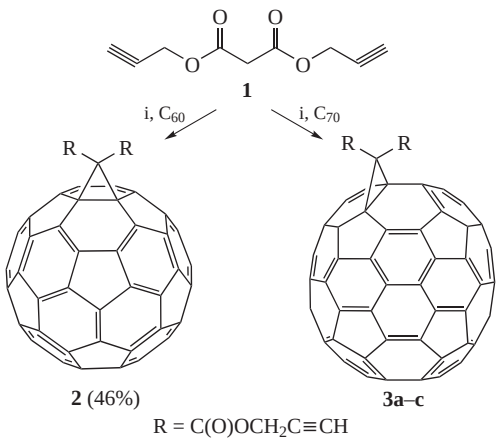
New derivatives of fullerenes C_{60} and C_{70} containing triazole and deoxythymidine fragments are obtained by the Bingel–Hirsch and 'click-chemistry' reactions from acetylene methanofullerene and azido thymidine derivatives.



Currently fullerene (C_{60} and C_{70}) chemistry is intensively investigated for obtaining biologically active compounds. The size of the fullerene, its structure and properties are attractive to medicine since C_{60} (C_{70}) molecule can penetrate into cell and coordinate in the hydrophobic parts of cells and enzymes, trap free radicals and generate singlet oxygen. In recent years, a large quantity of clinical trials on new fullerene-based drugs that can effectively combat cancer and inhibit HIV have been implemented.[1–19]

Natural biologically active substances on the basis of monosaccharides, amino acids and pyrimidine bases, containing various pharmacophore groups, are also promising for the treatment of tumor diseases.[8] Created in 1964 for cancer treatment 3-azido-2,3-dideoxythymidine proved to be a drug effective against retroviral infections.[9–11] It is also known that many triazole-containing compounds possess anti-HIV, antihepatitis activity and pronounced cytotoxic effect.[20–22]

A number of synthetic approaches towards various fullerene C_{60} derivatives are documented.[23–25] Earlier, we converted dipropargyl malonate **1** into a new alkyne derivative of fullerene C_{60} **2** (Scheme 1) and carried out its further modification by the method of 'click-chemistry' thus obtaining new triazole methanofullerenes.[26] It was also shown that for preparing triazole-containing methanofullerenes the 'click-chemistry' cycloaddition within azides and alkyne-containing methanofullerenes is preferable as compared to the Bingel–Hirsch reaction, since it leads only to the target compound in high yield. The fullerene under these conditions does not react with the azide.



Scheme 1 *Reagents and conditions*: i, fullerene (1.0 equiv.), **1** (1.5 equiv.), CBr_4 (1.5 equiv.), DBU (1.5 equiv.), *o*-DCB/toluene, 20 °C.

The aim of this study was to obtain new promising compounds for application in medicine, which combine useful properties of the above-listed individual substances, by different approaches for functionalization of fullerenes C_{60} and C_{70}. For this purpose, we used a simple synthetic method of 'click-chemistry', which allows one to design complex organic molecules and perform their functionalization. [2 + 1]-Cycloaddition of dipropargyl malonate **1** to the fullerenes C_{60} and C_{70} under the Bingel–Hirsch reaction conditions in the presence of DBU and CBr_4 afforded dipropargyl methanofullerenes **2** and **3**† (see Scheme 1).The reaction progress was monitored by HPLC.

† *71,71-Bis(propargyloxycarbonyl)methano[70]fullerene* **3**. A solution of dipropargyl malonate (0.0811 g, 0.45 mmol) in toluene (15 ml), CBr_4 (0.1495 g, 0.45 mmol) in toluene (15 ml) and DBU (0.0684 g, 0.45 mmol) were sequentially added to a solution of fullerene C_{70} (0.252 g, 0.3 mmol) in *o*-dichlorobenzene (15 ml) with subsequent stirring under the flow of dry argon at room temperature for 2 h. The unreacted fullerene C_{70} (0.070 g) was isolated by flash chromatography on silica eluting with a mixture hexane–toluene (8 : 1). Three isomeric monoadducts **3a** (0.072 g, 32%), **3b** (0.010 g, 4.5%) and **3c** (0.003 g, 1.4%) were isolated by elution with a mixture of hexane–toluene–acetonitrile in various ratios (in particular, 9 : 30 : 1; 9 : 30 : 7). The yield was calculated based on the consumed fullerene C_{70}.

For **3a**: ^{1}H NMR ($CDCl_3$) δ: 2.64 (t, 1H, CH, J_{HH} 2.4 Hz), 5.05 (d, 2H, OCH_2, J_{HH} 2.4 Hz). ^{13}C NMR ($CDCl_3$) δ: 35.74 (1C, C^{71}), 54.58 (2C, OCH_2), 66.14 (1C, sp^3), 66.60 (1C, sp^3), 76.26 (2C, ≡C), 76.64 (2C, CH), 130.81 (2C), 130.96 (4C), 132.80 (2C), 133.52 (2C), 136.49 (2C), 141.07 (2C), 141.59 (2C), 142.34 (2C), 142.48 (2C), 142.89 (2C), 143.58 (2C), 143.83 (2C), 143.95 (2C), 144.88 (2C), 145.87 (2C), 145.98 (2C), 147.00 (2C), 147.29 (2C), 147.49 (2C), 147.55 (2C), 148.47 (4C), 148.61 (2C), 148.78 (2C), 149.11 (2C), 149.27 (2C), 149.33 (2C), 150.56 (2C), 150.69 (2C), 151.15 (2C), 151.26 (2C), 151.33 (2C), 154.97 (2C), 162.44 (2C, C=O). UV (CH_2Cl_2, λ_{max}/nm): 326, 354, 371, 406, 458. IR (KBr, ν/cm^{-1}): 458 (C_{70}), 533 (C_{70}), 558, 579 (C_{70}), 631, 673 (C_{70}), 726, 795 (C_{70}), 991, 1085, 1136, 1160, 1211, 1268, 1366, 1429 (C_{70}), 1750 (C=O), 2130 (≡CH), 2921, 3294. MS (MALDI-TOF), *m*/*z*: 1018.97 $[M]^+$, 1019.93 $[M+H]^+$ (calc. for $C_{79}H_6O_4$, *m*/*z*: 1018.02).

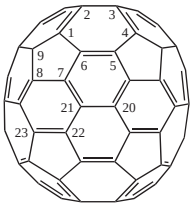


Figure 1 Fullerene C_{70} bond types.

Whereas fullerene C_{60} contains only a single type of [6,6] bond, fullerene C_{70} has four types of nonequivalent [6,6] bonds (Figure 1), which could lead to at least three monoadducts involving C^1–C^2, C^5–C^6 and C^7–C^{21} bonds. Previous quantum chemical calculations demonstrated that addition to the bonds of C^1–C^2 and C^5–C^6 types is greatly preferable for the formation of fullerene C_{70} products.[27]

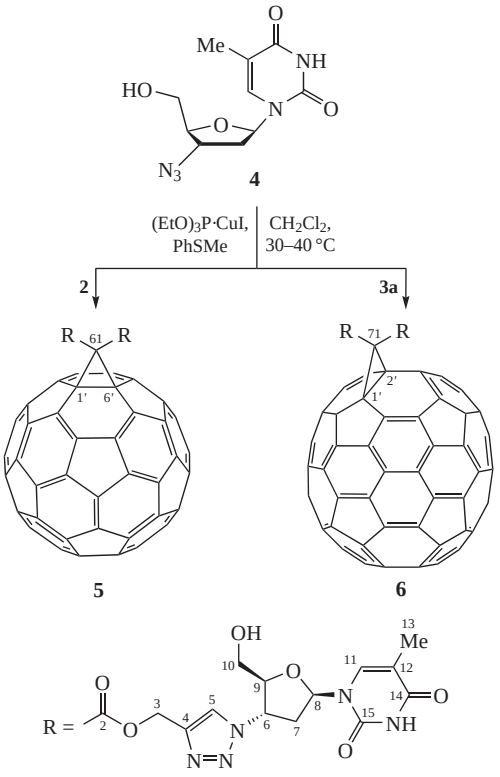
Indeed, the HPLC analysis of the reaction mixture contained peaks of three isomeric monoadducts **3a**, **3b** and **3c** with different retention time (Figure S1, see Online Supplementary Materials). However, column chromatography on SiO_2 allowed us to separate only one pure isomer **3a**, in which the cycloaddition occurred to more reactive C^1–C^2 double bond. Two other isomers **3b** and **3c** were isolated in scanty quantity with admixture of bis-adducts.

The chemical structure and composition of compounds **2** and **3** were confirmed by ^{13}C NMR data and results of MALDI-TOF mass spectrometry and HPLC. The mass spectra of compounds **2** and **3** (see Figure S1) exhibited peaks of the molecular ions with *m*/*z* 898.02 and 1018.97 $[M]^+$, respectively.

The structure of compounds **2** and **3** was established by a number of 1D and 2D NMR correlation methods (^1H–^1H, ^1H–^{13}C).[29] Spectral data for **2** are described in our previous work.[23] The structure of methanofullerene C_{70} **3**† was proved similarly to that of compound **2**. In contrast to compound **2**, the ^{13}C NMR spectrum of methanofullerene **3a** has two signals of sp^3-hybridized nonequivalent carbon atoms C^1, C^2 (66.1 and 66.6 ppm) with the integral intensity of 1C. sp^2-Carbons of fullerene C_{70} resonate in the region δ_C 130.8–154.9 ppm (32 signals), thirty signals having integral intensity of 2C and two signals of 4C.

Coupling of alkyne methanofullerene **2** and azidodeoxythymidine **4** in the presence of $(EtO)_3P$·CuI catalyst gave compound **5**‡ in rather low yield (16% based on reacted methanofullerene) with the reaction proceeding very slowly (for several months).

Earlier,[28] a quick and highly efficient method has been developed for copper-catalyzed cycloaddition of water-insoluble azides and alkynes 'on water' at room temperature using 10 mol% CuBr as a catalyst and 50 mol% PhSMe as a ligand. Taking into account these data, we carried out **2** + **4** coupling with the use of the catalyst $(EtO)_3P$·CuI and ligand PhSMe. Indeed, the reaction accelerated significantly: after 20 h the brown precipitate of **5** formed in 5-fold increased yield (75%). We have also shown that solar light promotes more complete and rapid reaction proceeding, wherein the reaction mixture was heated to 30–40 °C. Similarly, cycloaddition of azide **4** to methanofullerene C_{70} alkyne **3a** afforded triazole methanofullerene C_{70} **6**§ in 74% yield. The



Scheme 2

‡ *61,61-Bis({1-[2-hydroxymethyl-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3-yl]-1,2,3-triazol-4-yl}methoxycarbonyl)methano[60]fullerene* **5**. A mixture of compound **2** (0.025 g, 0.028 mmol), azidodeoxythymidine **4** (0.0149 g, 0.056 mmol), catalyst $(EtO)_3P$·CuI (0.002 g, 0.006 mmol) and thioanisole (0.0035 g, 0.028 mmol) in anhydrous dichloromethane (20 ml) was stirred at 40 °C for 20 h. The precipitated brown product was filtered and multiply washed with toluene and dichloromethane to yield 0.03 g (75%) of product **5**. ^1H NMR (DMSO-d_6) δ: 1.79 (s, 6H, Me), 2.70 (d, 4H, CH_2, J_{HH} 6.14 Hz), 3.65 (d, 4H, CH_2OH, J_{HH} 34.64 Hz), 4.21 (s, 2H, CH), 5.27 (s, 2H, OH), 5.40 (s, 2H, CH), 5.62 (s, 4H, OCH_2), 6.40 (s, 2H, CH), 7.79 (s, 2H, CH), 8.50 (s, 2H, CH of triazole ring), 11.31 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) δ: 12.15 (2C, C^{13}), 37.02 (2C, C^7), 51.48 (1C, C^{61}), 59.34 (2C, C^6), 59.95 (2C, C^3), 60.65 (2C, C^{10}), 70.94 (2C, sp^3), 83.89 (2C, C^8), 84.39 (2C, C^9), 109.52 (2C, C^{12}), 125.25 (2C, C^5), 136.08 (2C, C^{11}), 138.30 (4C), 140.29 (4C), 140.83 (2C, C^4), 141.09 (4C), 141.55 (4C), 142.09 (2C), 142.22 (4C), 142.35 (4C), 143.05 (2C), 143.21 (4C), 143.91 (4C), 144.02 (4C), 144.20 (2C), 144.35 (4C), 144.42 (4C), 144.51 (4C), 144.61 (4C), 150.31 (2C, C^{15}), 162.20 (2C, C=O), 163.59 (2C, C^{14}). UV (DMSO, λ_{max}/nm): 324, 425, 462. IR (KBr, ν/cm^{-1}): 527 (C_{60}), 553, 582 (C_{60}), 704, 768, 890, 956, 1061, 1099, 1204, 1229, 1262, 1367, 1439 (C_{60}), 1467, 1688 (C=O), 2924, 3140, 3435. MS (MALDI-TOF), *m*/*z*: 1432.77 $[M]^+$, 1455.91 $[M+Na]^+$, 1494.83 $[M+Na+K]^+$ (calc. for $C_{89}H_{32}N_{10}O_{12}$, *m*/*z*: 1432.22).

§ *71,71-Bis({1-[2-hydroxymethyl-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3-yl]-1,2,3-triazol-4-yl}methoxycarbonyl)methano[70]fullerene* **6**. A mixture of compound **3a** (0.050 g, 0.049 mmol) in anhydrous dichloromethane (15 ml) and toluene (10 ml), azidodeoxythymidine **4** (0.0263 g, 0.098 mmol), catalyst $(EtO)_3P$·CuI (0.0035 g, 0.0098 mmol) and thioanisole (0.0061 g, 0.049 mmol) in anhydrous dichloromethane (15 ml) was stirred at 40 °C for 20 h. The precipitated brown product was filtered and multiply washed with toluene and dichloromethane. The unreacted initial compound **3a** (0.01 g) was isolated by flash chromatography on silica. The yield of pure product **6** based on the consumed **3a** was 0.045 g (74%). ^1H NMR (DMSO-d_6) δ: 1.79 (s, 6H, Me), 2.67 (d, 4H, CH_2, J_{HH} 32.27 Hz), 3.63 (d, 4H, CH_2OH, J_{HH} 27.03 Hz), 4.21 (s, 2H, CH), 5.27 (s, 2H, OH), 5.40 (s, 2H, CH), 5.58 (s, 4H, OCH_2), 6.39 (s, 2H, CH), 7.78 (s, 2H, CH), 8.51 (s, 2H, CH of triazole ring), 11.30 (s, 2H, NH). ^{13}C NMR (DMSO-d_6) δ: 12.18 (2C, C^{13}), 36.48 (1C, C^{71}), 37.03 (2C, C^7), 59.42 (2C, C^6), 59.99 (2C, C^3), 60.64 (2C, C^{10}), 65.37 (1C, sp^3), 66.08 (1C, sp^3), 83.88 (2C, C^8), 84.38 (2C, C^9), 109.54 (2C, C^{12}), 125.41 (2C, C^5), 130.18 (4C), 130.29 (2C), 132.18 (2C), 132.89 (2C), 136.07 (2C, C^{11}), 136.17 (4C), 139.93 (2C), 140.81 (2C, C^4), 141.45 (2C), 142.08 (4C), 142.83 (2C), 143.15 (2C), 143.29 (2C), 144.09 (2C), 145.27 (4C), 146.31 (2C), 146.53 (2C), 146.83 (4C), 147.78 (8C), 148.00 (4C), 148.44 (2C), 148.54 (2C), 148.62 (4C), 150.02 (2C), 150.30 (2C, C^{15}), 150.70 (2C), 154.43 (2C), 162.04 (2C, C=O), 163.59 (2C, C^{14}). UV (DMSO, λ_{max}/nm): 351, 369, 408, 456, 484. IR (KBr, ν/cm^{-1}): 459, 535 (C_{70}), 579 (C_{70}), 673 (C_{70}), 694, 728, 768, 796 (C_{70}), 893, 971, 1059, 1095, 1161, 1214, 1271, 1369, 1429 (C_{70}), 1688 (C=O), 2924, 3067 (CH of triazole ring), 3390. MS (MALDI-TOF), *m*/*z*: 1552.57 $[M]^+$, 1575.90 $[M+Na]^+$ (calc. for $C_{99}H_{32}N_{10}O_{12}$, *m*/*z*: 1552.92).

obtained products **5** and **6** are poorly soluble in conventional for fullerene derivatives solvents, whereas well soluble in DMSO and pyridine. Mass spectra of **5** and **6** contain peaks of molecular ions with *m*/*z* 1432 and 1552, respectively.

The structures of compounds **5** and **6** were identified on the basis of ^{13}C and ^{1}H NMR 1D and 2D correlation experiments.[29] The principal heteronuclear correlations, which allowed us to establish the structures of the addends in compounds **5** and **6**, are shown in Online Supplementary Materials.

The 1D ^{13}C NMR spectra of compounds **5** and **6** exhibited 16 signals at δ_C 138–150 ppm and 24 signals at δ_C 130–154 ppm, respectively, with different integrated intensity in the region indicative for the sp^2-hybridized C atoms of the fullerene sphere (Figure S2). sp^3-Carbons C^1/C^6 are characterized by one signal at δ_C 70.9 ppm for compound **5** and two signals at δ_C 65.3 and 66.0 ppm for compound **6**.

In conclusion, we have obtained and characterized new alkyne and triazole derivatives of fullerenes C_{60} and C_{70}, including those containing thymidine fragment in their structure. These compounds are promising for further studies of their biological activity.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2017.03.033.

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