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Covalent Binding of C_{60} Fullerene to Quadricyclans: A Synthetic Avenue to Hexamethanofullerenes with Promising Antitumor Activity †

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Abstract: The Bingel-Hirsch reaction was used to synthesize two new hexamethanofullerenes containing six quadricyclane fragments. Preliminary experiments have established that the synthesized compounds have a high antitumor effect in combination with cisplatin on human T-lymphoblastic leukemia cells (Jurkat cells).

Keywords: [60]fullerene; quadricyclane; Bingel-Hirsh reaction; hexamethanofullerene; cytotoxic; antitumor activity

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1. Introduction

The ability of the quadricyclane molecule to cleave (break) strained C-C bonds in the presence of catalytic amounts of Pd or Pt ions with the release of about 100 kJ/mol [1,2] heat allowed us to put forward an original idea, consisting in the synthesis of derivatives of quadricyclanes, promising as effective antitumor drugs. We hypothesized [3] that, as a result of active metabolism, tumor cells, in contrast to healthy ones, will more intensively accumulate quadricyclan molecules and upon further introduction of Pd or Pt ions into the human body, for example, in the form of the well-known preparation cisplatin in a much lower concentration, scaffold the molecule will be cleaved with the release of heat, thereby exerting an additional thermal effect on tumor cells.

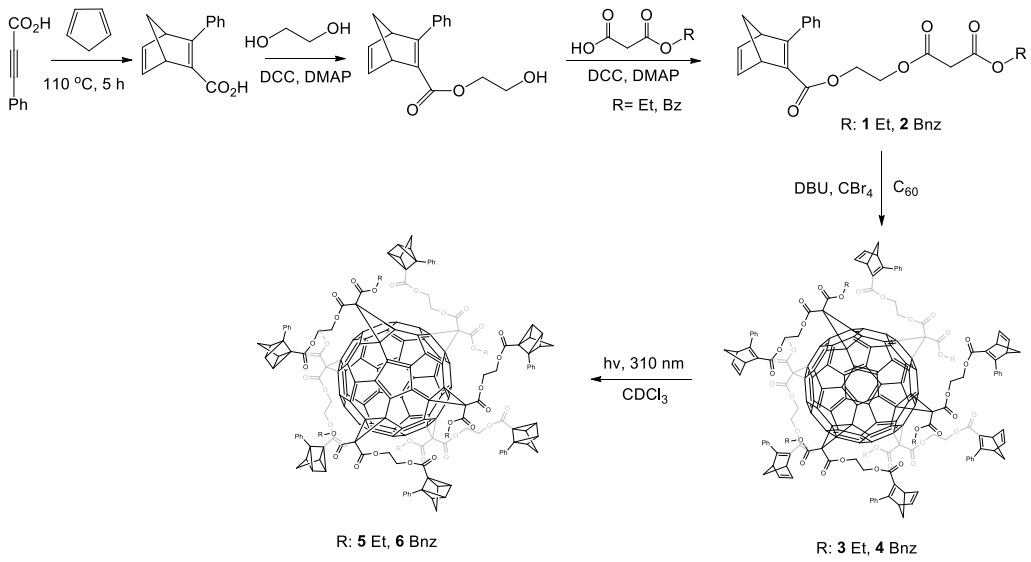
In the development of these studies, and also taking into account the prospects of using fullerenes for targeted delivery of various substances [4], which leads to an increase in the antitumor activity of already well-studied drugs [5], we carried out the covalent binding of C_{60} to quadricyclans by the Bingel-Hirsch reaction [6–8]. As a result of studying the cytotoxic effect of hybrid molecules in combination with cisplatin [7] or in its absence [8] on human T-lymphoblastic leukemia cells (Jurkat cells), we have shown that water-soluble polyvinylpyrrolidone complexes of methanofullerenes containing quadricyclane addends lead to a significant dose-dependent increase in the number of dead cells in each group, in comparison with the control. In this case, the activity of hybrid molecules is more than 100 times higher than the initial quadricyclanes that do not contain fullerene.

Given the promising potential of fullerene polyadducts for medicine for the treatment of bacterial [9], viral [10,11], tumor [12] and HIV [13] diseases, we assumed that by selectively synthesizing C_{60} adducts containing 6 quadricyclane addends, we would be able to increase the solubility of new hybrid molecules, as well as their antitumor activity due to the greater number of covalently attached quadricyclanes to the C_{60} carbon backbone.

2. Results and Discussion

To date, one of the most selective methods for the synthesis of poly adducts of fullerenes in preparative quantities can be considered the Bingel-Hirsch reaction, leading to hexamethanofullerenes [14–16].

Malonic acid esters 1 and 2 were synthesized as precursors of α-halogencarbanions (Scheme 1). The choice of these monomers is due to several reasons. Thus, disubstituted norbornadiene lends itself more readily to monosubstituted isomerized to the corresponding quadricyclanes [17,18] The presence of the phenyl substituent of said most efficiently promotes isomerization. The use of a diethylene glycol spacer in ethers **1** and **2**, as shown in our previous works [6,7], promotes the stabilization of the quadricyclane addend in the hybrid molecule and prevents its spontaneous destruction. The synthesis of hexamethanofullerenes using norbornadiene esters of malonic acid, as well as the isomerization of norbornadiene fragments in methanofullerenes **3** and **4**, were carried out according to the method described by Hirsch et al. [19].



Scheme 1. Synthesis of hexamethanofullerenes containing quadricyclane moieties.

Preliminary studies of the antitumor activity of the obtained hexamethanofullerenes 5 and 6 in combination with cisplatin on human T-lymphoblastic leukemia cells (Jurkat cells) showed a significant (10-fold) increase in the number of dead cells in comparison with the previously synthesized monomethanofullerenes 6 containing one quadricyclane fragment.

Currently, the Laboratory of Molecular Design and Biological Screening of Candidate Substances for the Pharmaceutical Industry at the Institute of Petrochemistry and Catalysis of RAS is conducting more detailed studies of the antitumor activity of synthesized hexamethanofullerenes using a wide range of cancer cells as an example.

3. Materials and Methods

All reactions were performed under an argon atmosphere and in anhydrous solvent. The solvents and reagents were dried or refined according to the literature procedures. Commercially available [60]fullerene (99.5 % pure, Sigma-Aldrich) and cisplatin (ABCR) were used. The reaction products were analyzed on a HPLC chromatograph Shimadzu SPD-20A (Japan) equipped with the UV detector at 313 or 340 nm. The mixtures were separated on a preparative column Cosmosil Buckyprep Waters (250×10 mm) at ~20 °C. Toluene was used as eluent, the flow rate was 3.0 mL·min^{-1}. The ^{1}H and ^{13}C NMR spectra were run on a Bruker Avance-500 spectrometer. A mixture of $CDCl_3$ and CS_2 (1:5) was used as a solvent. The chemical shifts are reported as δ values in parts per million relative

to internal standard Me_4Si. The coupling constants (J) are reported in Hertz. The mass spectra were obtained on an UltraFlex III TOF/TOF (Bruker Daltonik GmbH, Germany) operating in linear (TOF) and reflection (TOF/TOF) positive and negative ion modes. S_8 and DCTB (trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenyliden]malononitrile) were used as the matrix. For the application on a metal target, toluene solutions of the samples were used. Hamamatsu Lightning cure LC-8 150 W was used for UV irradiation of norbornadienes.

Procedure for the synthesis of hexamethanofullerenes **3 и 4**.

Under inert atmosphere and the exclusion of light, C_{60} (1.0 eq., $8.33 \cdot 10^{-5}$ mol, 60 mg) was dissolved in 20 mL of 1,2-dichlorobenzene. The solution was degassed with argon for 10 min. Afterwards the norbornadiene malonates 1 (10 eq., $8.33 \cdot 10^{-4}$ mol, 307.5 mg) or 2 (10 eq., $8.33 \cdot 10^{-4}$ mol, 358 mg), CBr_4 (100 eq., 8.33 mmol, 2.76 g) and DBU (10 eq., $8.33 \cdot 10^{-4}$ mol, 125μL) were added. The reaction mixture was stirred overnight and another 25 μL of DBU were added. After another day, the reaction mixture was concentrated and separated by the HPLC, eluent was toluene. The product was obtained after removal of the solvent *in vacuo*. The method corresponds to the literature data [19].

Hexamethanofullerene **3**

Brown powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 7.50–7.49 (m, 6H, CH (Ph)), 7.33–7.28 (m, 24H, CH (Ph)), 6.98–6.91 (m, 12H, CH), 4.45–4.23 (m, 24H, CH_2), 4.07–4.04 (m, 12H, CH_2), 3.84 (m, 12H, CH), 2.25–2.09 (m, 12H, CH_2), 1.35–1.23 (m, 18H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) =167.76, 164.27, 163.11, 162.94, 162.80, 162.60, 145.87, 143.92, 141.03, 140.95, 140.54, 138.37, 135.45, 128.90, 127.98, 127.78, 70.63, 69.02, 65.75, 64.20, 62.89, 61.52, 61.11, 58.91, 53.21, 45.09, 14.19. HRMS (MALDI TOF) $[M]^-$ calcd. for $C_{186}H_{120}O_{36}$ 2928.7559; Found 2928.7557. Yield 42 mg, 70%.

Hexamethanofullerene **4**

Brown powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 7.56–7.54 (m, 12H, CH (Ph)), 7.38–7.28 (m, 48H, CH (Ph)), 7.01–6.92 (m, 12H, CH), 5.27–5.21 (m, 12H, CH_2), 4.34–4.07 (m, 24H, CH_2), 3.86 (m, 6H, CH), 2.24–2.07 (m, 12H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) =168.30, 168.07, 167.96, 164.98, 164.72, 163.44, 163.35, 146.10, 145.56, 143.92, 143.77, 141.04, 140.78, 140.74, 140.65, 138.47, 135.49, 134.52, 129.03, 128.90, 128.65, 128.62, 128.41, 127.84, 127.77, 70.58, 69.82, 68.61, 65.80, 64.41, 61.65, 61.29, 58.72, 53.00, 44.89. HRMS (MALDI TOF) $[M]^-$ calcd. for $C_{216}H_{132}O_{36}$ 3300.8498; Found 3300.8501. Yield 54 mg, 90%.

Procedure for photoisomerization of norbornadiene moieties to quadriclane in hexamethanofullerenes **3** and **4**.

Quadricylane **5** and **6** was prepared by photoisomerization of corresponding hexamethanofullerenes **3** and **4**. For this purpose, 10 mg of compound **3** or **4** were dissolved in thoroughly degassed $CDCl_3$ (2.5 mL). The solution was transferred into a quartz cuvette which was sealed under argon atmosphere. Afterwards, the cuvette was irradiated with a Hamamatsu Lightning cure LC-8 150 W of a wavelength of 310 nm. The reaction was followed by ^{1}H NMR spectroscopy, which indicated full conversion after six hours for the 3 and five hours for the 4. The method corresponds to the literature data [19].

Hexamethanofullerene **5**

Brown powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 7.28–7.20 (m, 24H, CH (Ph)), 7.19 (m, 6H, CH (Ph)), 4.39–4.28 (m, 24H, CH_2), 4.20 (m, 12H, CH_2), 2.63–2.16 (m, 12H, CH), 1.73–1.60 (m, 12H, CH), 1.44–1.28 (m, 18H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) =171.46, 164.81, 163.45, 145.90, 143.85, 141.17, 141.01, 140.67, 136.71, 128.70, 127.71, 126.30, 70.61, 69.08, 65.84, 64.34, 63.07, 61.10, 58.70, 52.96, 45.10, 37.65, 32.77, 32.19, 31.82, 31.33, 30.01, 20.87, 14.06. HRMS (MALDI TOF) $[M]^-$ calcd. for $C_{186}H_{120}O_{36}$ 2928.7559; Found 2928.7556.

Hexamethanofullerene **6**

Brown powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 7.41–7.24 (m, 48H, CH (Ph)), 7.18 (m, 12H, CH (Ph)), 5.32–5.19 (m, 12H, CH_2), 4.24–4.07 (m, 24H, CH_2), 2.59–2.12 (m, 12H, CH), 1.70–1.60 (m, 12H, CH). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) =171.40, 163.37, 145.47, 143.89, 141.02, 140.69, 136.75, 134.54, 130.54, 128.86, 128.67, 127.71, 126.28, 70.57,

69.79, 68.98, 68.61, 65.72, 64.44, 60.88, 44.88, 37.69, 32.82, 32.16, 31.79, 31.30, 30.06, 20.91. HRMS (MALDI TOF) $[M]^-$ calcd. for $C_{216}H_{132}O_{36}$ 3300.8498; Found 3300.8497.

4. Conclusions

The Bingel-Hirsch reaction was used to synthesize two new hexamethanofullerenes containing six quadricyclane fragments. Preliminary studies of the antitumor activity of hexamethanofullerenes in combination with cis-platinum on human T-lymphoblastic leukemia cells (Jurkat cells) revealed a significant (10-fold) increase in the number of dead cells in comparison with previously synthesized monomethanofullerenes containing one quadricyclane fragment.

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