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Cyclooctyne [60]Fullerene Hexakis Adducts: a Globular Scaffold for Copper-Free Click Chemistry

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The synthesis of a new highly symmetric hexakis adduct of C_{60} appended with 12 cyclooctyne moieties has been carried out. This compound has been used for the copper-free strain-promoted cycloaddition reaction to a series of azides with excellent yields. This strategy for the obtention of clicked adducts of [60]fullerene is of special interest for biological applications.

[60]Fullerene hexakis adducts with T_h symmetry constitute an attractive class of compounds that allow the globular disposition of substituents around the C_{60} core.[1] These compounds have attracted much attention in the last recent years, both in the areas of materials science and biomedicine. Thus, some of them present liquid crystal behavior,[2] have been employed for the study of electronic and energy transfer processes,[3] as organic connectivity centers for the synthesis of Metal Organic Frameworks (MOFs),[4] or as catalysts.[5] On the other hand, in the area of biological applications, their activity has been tested in different fields showing interesting properties and good biocompatibility. Hexakis adducts of [60]fullerene have been tested as gene transfection vectors,[6] multiplying units for photodynamic therapy,[7] glycosidase and glycosyltransferase inhibitors,[8] or efficient antibacterial[9] or antiviral systems.[10]

The synthesis of these adducts was first studied by Hirsch by the one-pot addition of malonates templated by 9,10-dimethylanthracene[11] and later modified by Sun.[12] However, these procedures are often limited by the size of the malonates, as the steric aspects limit the yield of the reaction. To overcome this drawback, Nierengarten proposed the employment of an azide appended malonate to obtain a clickable hexakis adduct of C_{60} to effectively allow the covalent functionalization of [60]fullerene by Cu(I) alkyne-azide cycloaddition (CuAAC) reaction.[13] Lately, Nierengarten and we described the 12-alkyne modified hexakis adduct of [60]fullerene and its use in the click chemistry addition of azides and alkynes.[14] This effective reaction requires, however, the use of copper (I) as catalyst, which involves a subsequent purification step for the removal of copper, especially with those materials for bio-medical applications, owing to its high cytotoxicity. This step can be hampered by the presence in the final products of functional groups, and even the triazole rings themselves, capable of binding copper, thus limiting their biological applications.[15]

A strategy to avoid this purification step is to employ of strain promoted alkyne-azide cycloaddition (SPAAC) click reaction. This reaction, developed by Bertozzi and coworkers,[16] consists of the reaction of strained cyclooctynes with azides and has received considerable attention owing to its simplicity and faster reaction rates, avoiding the use of a metal as catalyst. As it does not require the use of copper, it has especial relevance for biological applications.

In the present communication we report the synthesis of a new hexakis adduct of [60]fullerene substituted with twelve cyclooctyne moieties to further carry out SPAAC reactions. The versatility of this new derivative has been tested with the addition of different azides appended with polar and non-polar chains, natural products such as biotin, amino acids such as phenylalanine and peptide nucleic acid (PNAs) monomers such as thymine. All compounds were obtained with high yields and short reaction times under mild conditions, and the complete characterization of all new derivatives is also reported.

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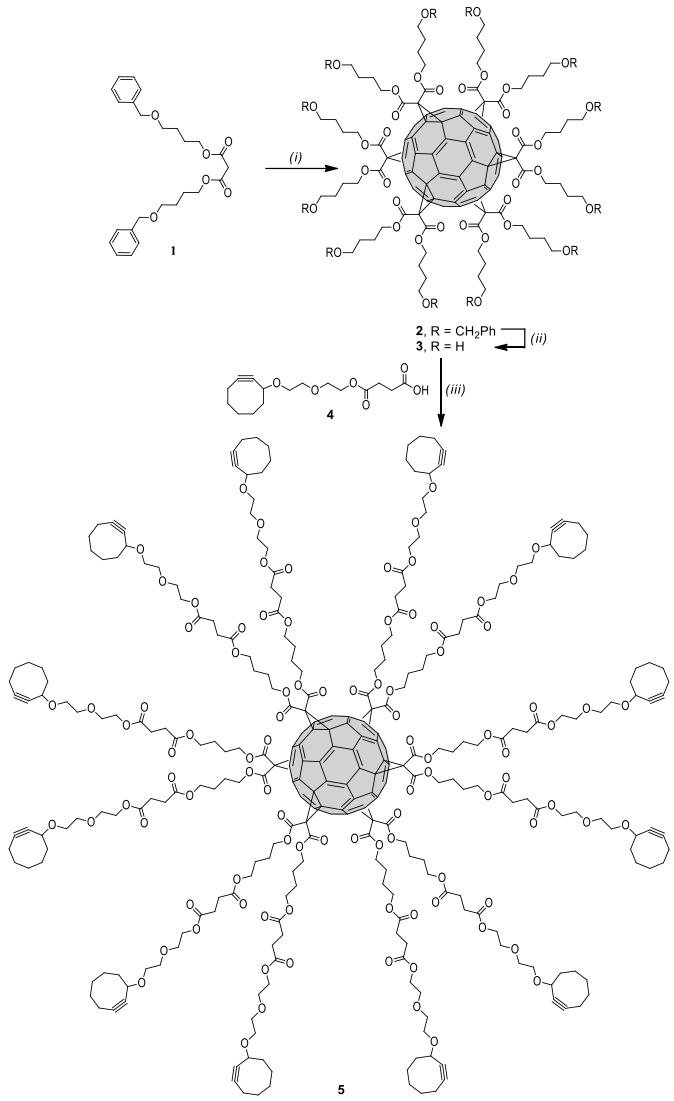
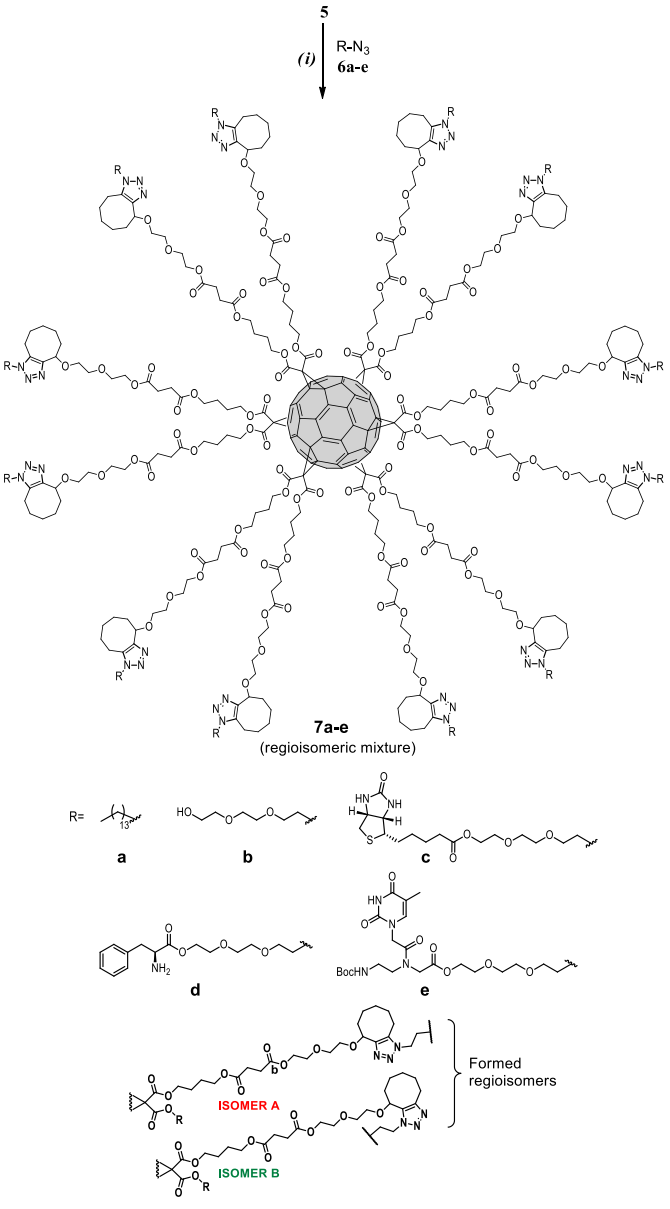


Scheme 1 Synthesis of compound **5**. *Reagents and conditions*: (*i*) C_{60}, DBU, CBr_4, ODCB, rt, 72h (50%); (*ii*) H_2, Pd-C, DCM/MeOH, rt, overnight (100%); (*iii*) DPTS, DCC, DCM/DMF, rt, overnight (99%).

The synthesis of compound **5** by direct Bingel addition of the corresponding cyclooctyne substituted malonate under the conditions reported by Sun[12] for the preparation of hexakis adducts of C_{60} yielded a complex mixture of compounds where the bromination of the alkyne moiety was observed. Therefore, we decided to follow a synthetic strategy in three steps, as depicted in Scheme 1. Malonate **1** (12 equiv.) was added to C_{60} (1 equiv.) in the presence of DBU (20 equiv.) and CBr_4 (100 equiv.) in ODCB at room temperature. After 72 h, hexakis adduct **2** was obtained in 50% yield after column chromatography. Deprotection of the hydroxyl groups was carried out by hydrogenation at atmospheric pressure, yielding compound **3** in quantitative yield. Finally, the esterification of cyclooctyne carboxylic acid **4** (see SI for synthetic details) with hexakis adduct **3** using DCC/DPTS led to compound **5** in a quantitative yield, which was purified by size-exclusion chromatography employing Sephadex.

Compound **5** was completely characterized by the usual analytical and spectroscopic techniques. The ^{13}C NMR spectrum contains only three signals for the C_{60} carbons, two for the Csp^2 at δ ~ 145.8, 141.05 and one for the Csp^3 at δ ~ 69.1 (see ESI). Two different carbonylic signals for the succinic and the malonate appear respectively at δ ~ 172.2 and 163.6 and the Csp of the alkyne are observed at 110.1 and 92.8 for the carbons d and c of the cyclooctyne moiety, respectively (see Figure 1).

The ability of compound **5** to be clicked to different azides was tested with compounds **6a-e** (Scheme 2). The addition reactions were carried out in DMSO under microwave irradiation at 50ºC during 30 min. Compounds **7a-e** (obtained respectively from **6a-e**) were purified by size-exclusion chromatography using Sephadex (see ESI). The yields obtained were over 90% in all cases.



Scheme 2. Synthesis of clicked adducts **7a-e**. *Reagents and conditions*: *(i)* DMSO, 50°C under MW, 30 min (93-99%). The two possible regioisomers are represented at the bottom.

Cycloadducts **7a-e** were fully characterized by FTIR, ^{1}H and ^{13}C NMR and MS and the assignment of the signals in NMR was elucidated by COSY and HSQC NMR spectroscopies (see supporting information). The lack of the typical band of the azide group in the FTIR spectra (~2100 cm^{-1}) of the clicked adducts was indicative of the absence of unreacted azide after purification. ^{1}H NMR spectra show two different signals for the CH of the cyclooctyne adjacent to triazole moiety (i.e. δ~ 4.82 and 4.69 for **7a**). Several other signals appear duplicated, indicating the presence of the two expected regioisomers (see Scheme 2). Once assigned the signals of the ^{1}H and ^{13}C spectra using COSY and HSQC experiments (see ESI), the ratio of regioisomers was calculated by integration of the signals in the ^{1}H NMR spectrum. For all the compounds, almost equal proportion of the two isomers was obtained (**7a**: 57% (A), 43% (B); **7b**: 54% (A), 46% (B); **7c**: 53% (A), 47% (B); **7d**: 55% (A), 45% (B); **7e**: 57% (A), 43% (B)), with a slight excess of isomer A, with less steric hindrance than isomer B (see Figure 1). This result is comparable to previous studies on monomeric compounds, indicating that the globular presentation of cyclooctynes provided by the hexakis adduct of C_{60} **5** does not favour the formation of one regioisomer over the other.[17]

^{13}C NMR also shows the presence of the two possible regioisomers (see Figure 1). Thus, while two signals are observed for the Csp^2 of C_{60} for **7a-e** (*i.e.*: 145.8 and 141.1 for **7a**), four signals appear in the ^{13}C NMR spectrum of each compound corresponding to the two carbons of the triazole ring of the two regioisomers (*i.e.*: 144.9, 144.8, 133.5 and 132.4 for **7a**). The rest of the signals of the cyclooctyne moiety also appear duplicated (see ESI).

MS data obtained by MALDI-TOF spectrometry confirmed the presence of the molecular ion peak for **7a,b,e**. For **7c-d**, however, high level of occurring fragmentation avoided the observation of the expected molecular ion peaks.

In conclusion, we have carried out the synthesis of a new building block for the synthesis of hexakis adducts of [60]fullerene by using SPAAC. This compound is obtained in three steps by cyclopropanation (Bingel reaction), deprotection and esterification, with very good yields. The use of this building block was tested in reactions with a series of azides, leading to the clicked adducts with yields over 90%. Most importantly, this methodology avoids the use of copper as a catalyst in the click cycloaddition reaction, being of special interest for the preparation of globular fullerene derivatives for biological studies.

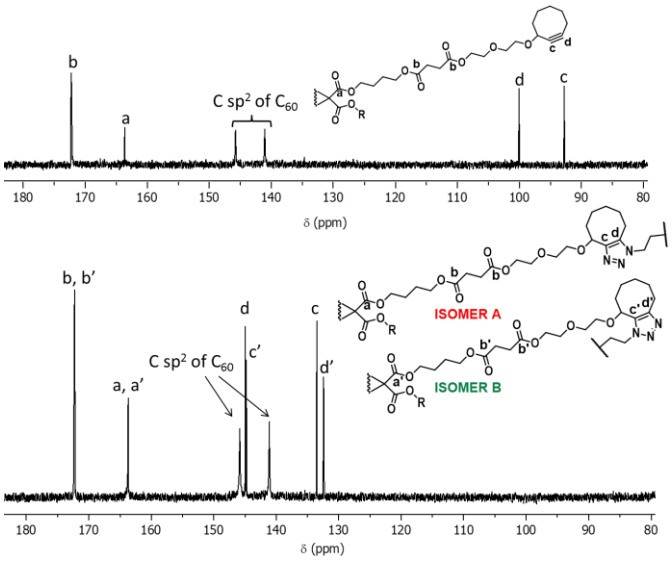


Figure 1. ^{13}C NMR spectra of compounds **5** (up) and **7a** (down) ($CDCl_3$, 125.8 MHz).

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