

A water-soluble β -cyclodextrin derivative possessing a fullerene tether as an efficient photodriven DNA-cleavage reagent

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Abstract

A simple synthetic route for a water-soluble cyclodextrin–fullerene conjugate based on the Diels–Alder reaction between anthryl-cyclodextrin and fullerene has been presented. Aided by the fascinating biochemical functions of fullerene, the resultant cyclodextrin-C₆₀ conjugate displays a satisfactory DNA-cleavage ability under the visible-light irradiation.

Fullerene is generally considered as a powerful building block in material sciences and medicinal chemistry owing to the combination of its several unique properties, particularly with respect to its electron donor or acceptor capability as well as its photophysical and photochemical behavior.^{1–10} For example, C₆₀ and its derivatives are reported to be able to exhibit satisfactory enzyme-inhibiting,^{11–13} radical-quenching,¹⁴ and DNA-cleavage abilities.^{15–17} Notably among them, cyclodextrin (CD)-C₆₀ conjugates have attracted extensive interest of chemists and biologists.^{18–23} Compared with other C₆₀ derivatives, CD-C₆₀ conjugates possess an inherent advantage in the field of biochemistry due to the capability of the CD cavity to recognize various model substrates. Upon being associated with a biological guest molecule, the CD cavity can selectively bind with certain fragments of the guest, thus enabling the site-specific interaction between C₆₀ and the model substrate.^{18–20} To combine the drug binding ability of CD and photodriven DNA-cleavage properties of fullerene,²¹ we prepare a water-soluble β -CD derivative possessing a [60]fullerene tether. Herein we report a very simple method for synthesizing CD-C₆₀ conjugate by the Diels–Alder reaction of C₆₀

with anthryl- β -CD. Although [4+2] cycloaddition has been proved to be one of the most expeditious methods for selective functionalization of [60]fullerene at the 6,6-ring junctions,^{3,4,24-32} its application to the synthesis of CD-fullerene conjugates has never been reported, to the best of our knowledge. Our current work in this direction presents a new avenue for the design and preparation of water-soluble fullerene derivative. Moreover, benefiting from the good biological property of C₆₀, the obtained water-soluble CD-C₆₀ conjugate shows an efficient DNA-cleavage ability under the visible-light irradiation attributing to the sensitized singlet oxygen (¹O₂) by the photoexcitation of C₆₀ moiety.

CD-C₆₀ **2**³³ is prepared by the Diels–Alder reaction of C₆₀ with mono[6-(9-anthrylformamido)ethyleneamino-6-deoxy]- β -CD **1**³⁴ in DMF/toluene solution under nitrogen according to the procedure shown in Scheme 1. Owing to the introduction of β -CD as a solubilizer, CD-C₆₀ **2** exhibits a moderate water-solubility up to 2.5 g L⁻¹ at 25 °C and can remain stable for several weeks when stored in a freezer (-10 °C). Notably, since both **1** and **2** are readily soluble in water, the unconsumed C₆₀ can be easily reclaimed by filtration after the reaction.

The structure of **2** is confirmed by elemental analysis, UV–vis, FT-IR, and NMR spectra. The UV–vis absorbance (Fig. 1) observed for **1** ($\lambda_{\max}/(\log\epsilon)$) in water are 384.0 (4.04), 364.5 (4.07), 346.5 (3.90), and 330.0 (3.62) nm, while **2** only exhibits two clear absorbances at 253.5 (5.15) and 329.5 (4.43) nm under the same conditions. The disappearance of the corresponding characteristic bands of anthracene above 330 nm indicates that the conjugated system of anthracene is broken. In addition, a characteristic vibration band at 801 cm⁻¹ assigned to the C₆₀ unit is also observed in the FT-IR spectrum of **2** (see Supplementary data). Moreover, we observe that the ¹H NMR spectrum of **2** is significantly different from that of **1**. Seen from Figure 2, the ¹H NMR spectrum of **2** shows three sets of signals centered at 7.92, 7.59, and 5.98 ppm, respectively, assigned to the Ha, Hb, and Hc protons of the dihydroanthracene group in **2**, while the corresponding H1(8)/H4(5), H2(7)/H3(6), and H9 protons of the anthracene moiety in **1** are centered at 8.05, 7.46, and 8.40 ppm.

From the ¹³C NMR data of **2** (Fig. 3), we observe that, apart from the signals assigned to the methylene carbons (47.1 and 45.9 ppm), the β -CD skeleton carbons (60.6, 68.8, 72.5, 73.1, 73.8, 81.9, 82.3, and 102.7 ppm), the carbonyl carbon (172.3 ppm), the aromatic carbons of dihydroanthracene (127.5 and 128.6 ppm), and the unsaturated sp² carbons of C₆₀ (140–150 ppm), there appears a new signal at 70.7 ppm assigned to the saturated sp³ carbons. This signal indicates a

closed conformation of the C₆₀ unit in **2**, since the newly generated saturated carbons in the closed structures will resonate at a higher field than normal unsaturated sp² carbons of C₆₀ that usually appear in the range of δ 130–160 ppm.^{24,25,35}

The above information strongly supports the anticipated structure of **2**.

The conformation of the CD-C₆₀ conjugate **2** is investigated by circular dichroism spectroscopy, since it has been amply demonstrated that the inclusion of a chromophoric achiral guest in a chiral host such as CDs produces induced circular dichroism (ICD) signals at the absorbance wavelengths of the guest chromophore.^{36,37} From a comparative analysis based on the circular dichroism spectra of hosts **1** and **2** (see Supplementary data), we observe that the host **2** displays relatively weak ICD signals in the examined wavelength range, which indicates that the C₆₀ unit in **2** is not included in the CD cavity. This is expected, because β -CD is known to be too small to accommodate the fullerene sphere. The observed conformation will provide a C₆₀-capped CD cavity to selectively bind with certain biologic guests and thus enable the interaction between C₆₀ and a model substrate. It is noteworthy that the water-soluble CD-C₆₀ conjugate **2** shows a satisfactory DNA-cleavage ability under the visible-light irradiation at 298 K.^{38,39} As shown in Figure 4, both **1** and **2** show no DNA-cleavage ability in the absence of light (Fig. 4, lanes b and c, respectively). However, under the visible-light irradiation, CD-C₆₀ conjugate **2** can effectively cleave pGEX5X2 DNA (form I) into nicked DNA (form II) and linear DNA (Fig. 4, lane e, form III), and about 70% of supercoiled DNA is converted to nicked and linear DNA, which is close to the reported values (about 50–90%) by Yamakoshi et al.^{17c} In order to explore the DNA cleavage mechanism, the EPR spectrum⁴⁰ of **2** with 2,2,6,6-tetramethyl-4-piperidone (TEMP) is performed under the visible-light irradiation at 298 K. It is well documented that, when the singlet oxygen (¹O₂) is sensitized by the photoexcitation of fullerene, it can be detected by the EPR spin-trapping using a ¹O₂-trapping agent such as TEMP,^{17b,21,41} because TEMP can react with ¹O₂ to give a ¹O₂-adduct, TEMPO. In the present case, three characteristic EPR signals assigned to TEMPO are observed from a phosphate buffer aqueous solution (pH 7.4, containing 5% DMF) of **2**/O₂ system under the visible-light irradiation as shown in Figure 5, which indicates that ¹O₂ is generated. In the control experiments, neither **2** nor TEMP exhibits the appreciable EPR signals in the same condition. Moreover, CD-C₆₀ conjugate **2** shows no DNA cleavage ability in the absence of O₂. Therefore, we deduce that a singlet oxygen mechanism should be responsible for the DNA cleavage reaction. That is, the C₆₀ moiety in **2** may be located close to the guanosine position of DNA.^{16a} Under the visible-light irradiation, the singlet oxygen (¹O₂) is sensitized by the photoexcitation of C₆₀. Then, the sensitized singlet oxygen reacts with the guanosines of DNA by either [4+2] or [2+2] cycloaddition to the

five-membered imidazole ring of the purine base and thus cleaves the DNA.⁴² Herein, the function of CD is to enhance the water-solubility of fullerene and thus enables the DNA cleavage reaction in aqueous solution. In conclusion, we have presented the preparation of a water-soluble CD-C₆₀ conjugate **2** using a simple synthetic route, which will be useful for the design of functional fullerene derivatives. Furthermore, the results obtained also show that the above water-soluble CD-C₆₀ conjugate can act as an efficient photodriven DNA-cleavage reagent, which may serve as a potential photo-active medicine precursor in the biochemical field. Further endeavors to explore the effect of the tether's length of the CD-C₆₀ conjugate are currently in progress.

Acknowledgements

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Supplementary data

Circular dichroism, ¹³C NMR, and FT-IR spectra of **1** and **2**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.01.181.

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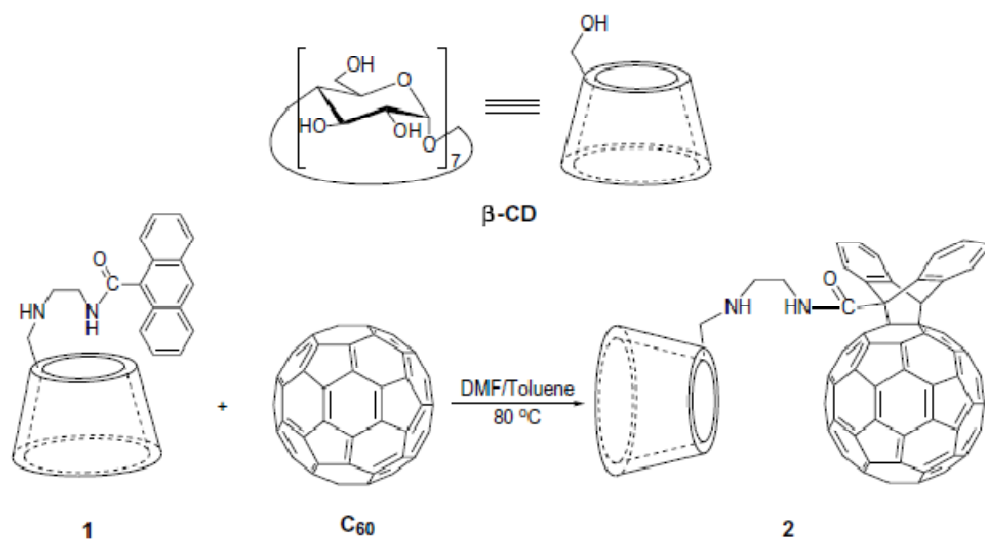
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33. Synthesis of **2**: A DMF solution (30 mL) of **1** (30 mg, 0.021 mmol) is added dropwise to a toluene solution (30 mL) of C₆₀ (20 mg, 0.028 mmol), and the resultant mixture is stirred at 80 °C for 48 h under nitrogen. The initial purple color of the solution becomes red as the reaction proceeds. Then the solvent is removed under a reduced pressure, and the precipitate formed is dried in vacuo. The precipitate is dissolved in water (30 mL), and hence the unconsumed C₆₀ is removed by filtration. The filtrate is purified on a Sephadex G-25 column with water as eluent. After the residue is dried in vacuo, a pure sample **2** (dark brown color) is obtained in 14% yield. UV–vis λ_{max} (H₂O, nm)/(log ϵ) 253.5 (5.15), 329.5 (4.43). ¹H NMR (300 MHz, DMSO-d₆ TMS, ppm): δ 2.73–2.90 (m, 4H), 3.35–3.62 (m), 4.45–4.62 (m), 4.82–4.84 (m, 7H), 5.66–5.94 (m, 14H), 6.04 (s, 1H), 7.55–7.67 (m, 4H), 7.92–7.97 (m, 4H). ¹³C NMR (300 MHz, DMSO-d₆ TMS, ppm): δ 172.3, 151.7, 147.9, 146.8, 145.9, 144.7, 144.1, 143.2, 141.9, 141.2, 140.1, 128.6, 127.5, 102.7, 82.3, 81.9, 73.8, 73.1, 72.5, 70.7, 68.8, 60.6, 47.1, 45.9. FT-IR (KBr, cm⁻¹): ν 3318, 2960, 2925, 1658, 1560, 1431, 1367, 1261,

1154, 1081, 1032, 944, 863, 801, 757, 704, 665, 608, 578, 527. Elemental analysis calcd for C₁₁H₈O₃N₂·4H₂O: C 65.75, H 4.27, N 1.29. Found: C 65.69, H 4.31, N 1.24.

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38. Examination of DNA-cleavage ability: 10 μL of aqueous solution of DNA pGEX5X2 (0.51 $\mu\text{g } \mu\text{L}^{-1}$) is diluted by adding 90 μL of water. Then, 10 μL of aqueous solution of **2** (1.6×10^{-4} M), 12 μL of aqueous solution of DNA pGEX5X2 (0.051 $\mu\text{g } \mu\text{L}^{-1}$), and 8 μL of phosphate buffer (250 mM, pH 7.4) are mixed in a microtest tube under dark conditions to give the sample solution. Sample (20 μL) is incubated under irradiation with a 300 W reflector lamp for 1 h at 298 K, then mixed with 4 μL of 6 x loading buffer, and loaded onto a 1% agarose gel containing ethidium bromide (10 $\mu\text{g mL}^{-1}$). The gels are run at a constant voltage of 80 V for 2 h in TAE buffer, washed with distilled water, visualized under a UV transilluminator, and photographed using an instant camera.
39. NMR experimental results show that **2** do not decompose after the visible-light irradiation for 1 h.
40. EPR measurements: In a typical experiment of the EPR measurement for the detection of $^1\text{O}_2$, a phosphate buffer aqueous solution (pH 7.4, containing 5% DMF) of **2** (1.5×10^{-4} M) with TEMP (9.2×10^{-3} M) is introduced into an EPR sample tube. The sample is then irradiated with a 300 W reflector lamp for 2 min. The EPR spectrum is measured with a BRUKER EMX-6/1 spectrometer and recorded for 170 s at 298 K. The magnitude of the modulation is chosen to optimize the resolution and the signal-to-noise ratio (S/N) of the observed spectra.
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List of Scheme

Scheme 1. Synthetic route of CD-C₆₀ conjugate 2.



Scheme 1.

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- Figure 1. The UV–vis spectra of **1** (1.4×10^{-5} M) and **2** (1.2×10^{-5} M) in aqueous solution at 25 °C.
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- Figure 5. The EPR spectrum of a mixture of **2** (1.5×10^{-4} M) with TEMP (9.2×10^{-3} M) after the visible-light irradiation in an aqueous phosphate buffer solution (pH 7.4, containing 5% DMF) at 298 K.

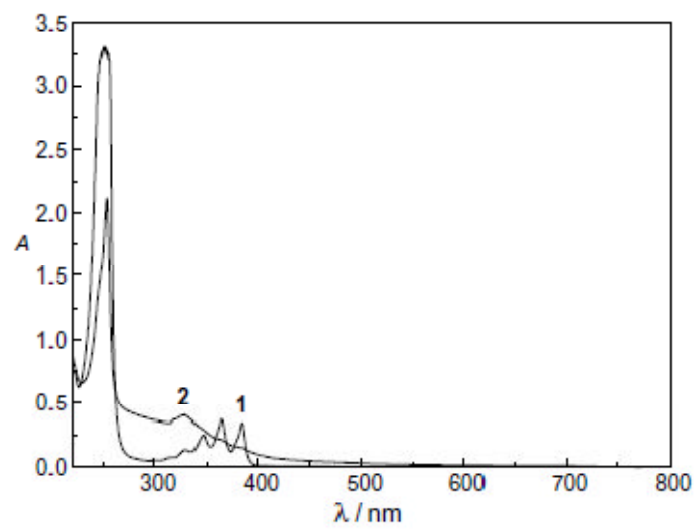


Figure 1.

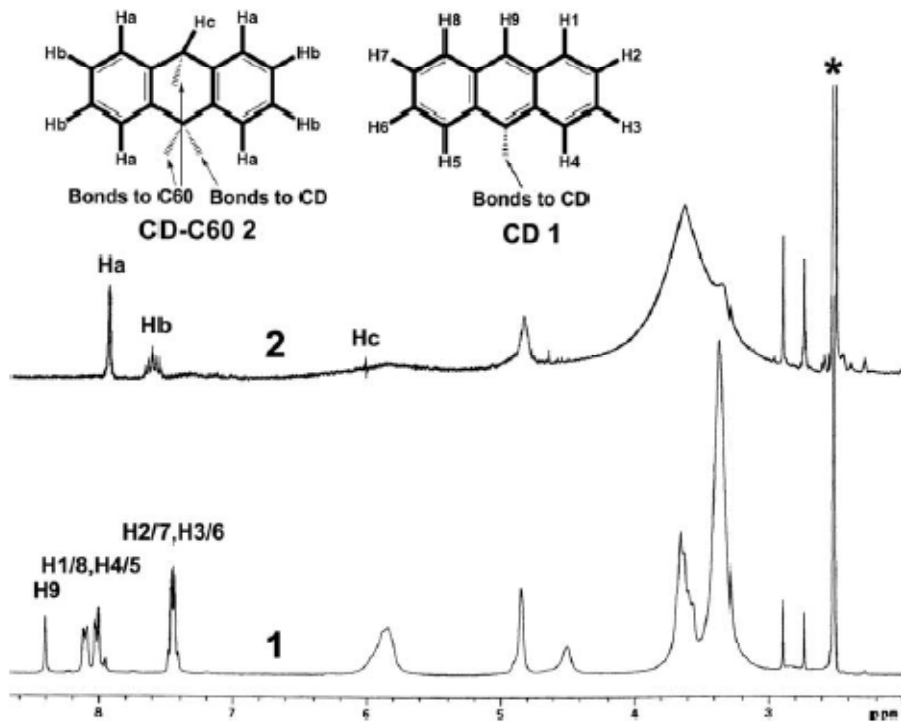


Figure 2.

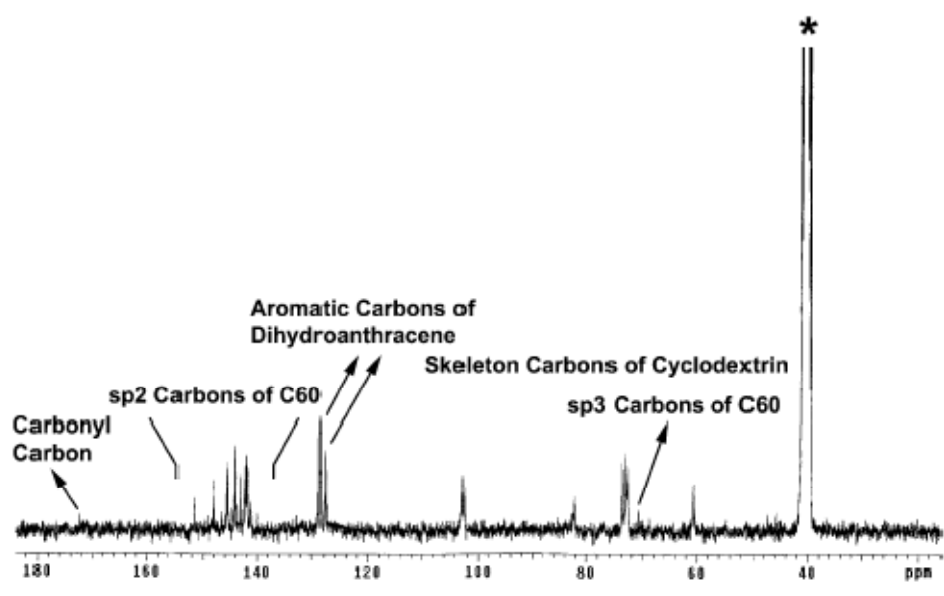


Figure 3.

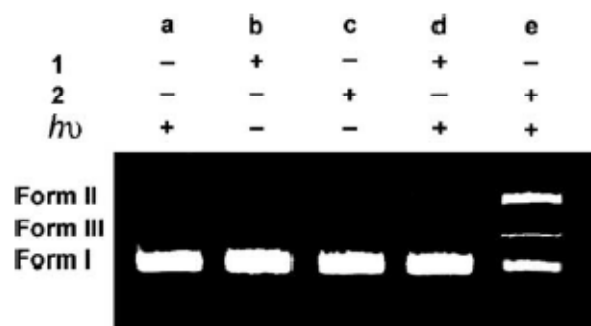


Figure 4.

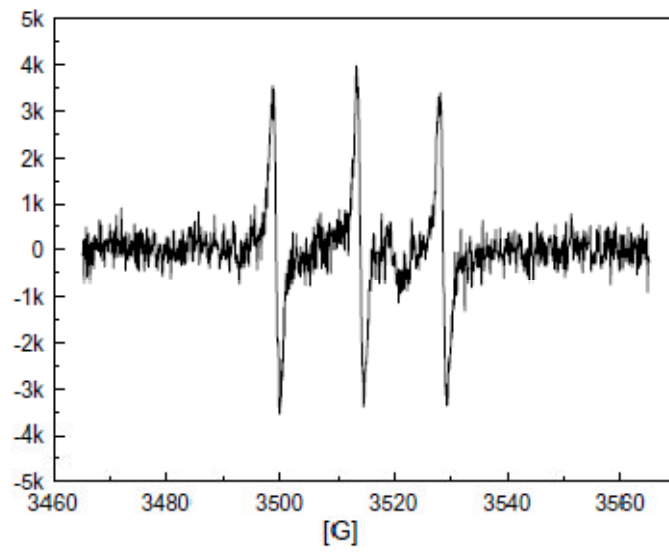


Figure 5.