ChemComm

Water-soluble [60]fullerene–cationic homooxacalix[3]arene complex which is applicable to the photocleavage of DNA

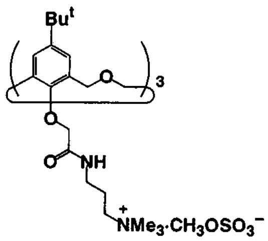
Atsushi Ikeda,[a] Tsukasa Hatano,[a] Masaru Kawaguchi,[a] Hikaru Suenaga[b] and Seiji Shinkai*[a]

[a] *Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka 812-8581, Japan. E-mail: seijitcm@mbox.nc.kyushu-u.ac.jp*
[b] *Fukuoka Industrial Technology Center, 1465-5 Aikawa, Kurume Fukuoka 839-0861, Japan. E-mail: suenaga@bfri01.fitc.pref.fukuoka.jp*

Received (in Cambridge, UK) 14th May 1999, Accepted 14th June 1999

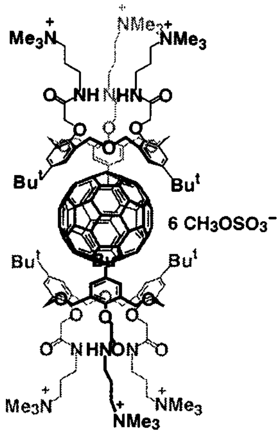
A new water-soluble, cationic homooxacalix[3]arene 1 was synthesized: this homooxacalix[3]arene solubilized [60]fullerene into water and the [60]fullerene–homooxacalix[3]arene complex acted as an efficient DNA photocleavage reagent.

The ready availability of [60]fullerene and its homologues has increasingly invited exploration of their outstanding new physical and chemical properties. Among them, one of the most important applications is medicinal use: for example, they act as a singlet oxygen ($^1O_2$) photosensitizer[1,2] to cleave DNA[3–8] and as an inhibitor to suppress HIV protease activity.[9,10] In spite of their high potentials, however, applications have been very limited because of their poor water solubility. Several lines of effort have so far been devoted toward compensation for this drawback, for example, by introduction of water-soluble substituents,[3–6] mixing with water-soluble polymers,[7,11] and solubilization in γ-cyclodextrin (γ-CD)[12] and water-soluble calix[8]arenes.[13] We considered that among these methods, the host–guest method should be superior to others because (i) one can directly use 'unmodified [60]fullerene' which can generate $^1O_2$ more efficiently than other [60]fullerene derivatives,[14] (ii) one can suppress undesired photo-dimerization of [60]fullerene and (iii) dioxygen or substrates can collide with [60]fullerene more efficiently. Recently, we found that homooxacalix[3]arene and its derivatives can include [60]fullerene in their cavities.[15–18] This finding suggests that if a cationic homooxacalix[3]arene can solubilize [60]fullerene into water *via* host–guest interactions, the resultant [60]fullerene–homooxa[3]arene complex should be readily bound to DNA and might act as a DNA photocleavage reagent therein. With these objects in mind, we designed a new water-soluble cationic homooxacalix[3]arene **1** with a cone conformation.



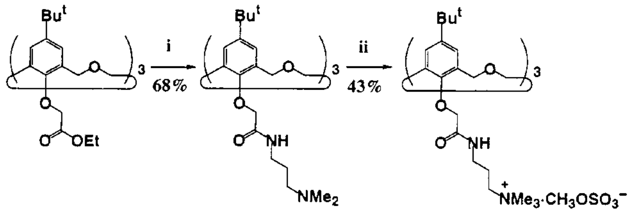
1

C$_{60}$



1−C$_{60}$ complex

Water-soluble calixarene **1** was synthesized according to Scheme 1 and identified by ^1H NMR, FT-IR and ESI-TOF MS spectroscopic evidence and elemental analysis.† The ^1H NMR data are consistent with a cone conformation.



Scheme 1 *Reagents and conditions*: i, *N*,*N*-dimethylpropane-1,3-diamine, DMAP, NaH, THF–EtOH; ii, Me_2SO_4, THF.

Firstly, the critical aggregate concentration (CAC) of **1** was estimated by surface tension (Wilhelmy method). As shown in Fig. 1, the surface tension of **1** in pure water abruptly decreased at *ca.* 3 mmol dm^{-3}. One can consider, therefore, that compound **1** forms aggregates above this concentration. In the subsequent experiments, we set the concentration of **1** to 0.50 mmol dm^{-3}, where **1** exists discretely in aqueous solution.

[60]Fullerene (solid) was extracted into water containing **1** by sonication (10 min) followed by stirring (3 days).‡ After centrifugation (10 000 rpm, 15 min, 20 °C), the solution was characterized by UV–VIS absorption and ^{13}C NMR spectroscopic methods. The UV–VIS absorption spectrum of the **1**–[60]fullerene complex shows absorption maxima at 264, 341 and 438 nm assignable to solubilized [60]fullerene (Fig. 2). The

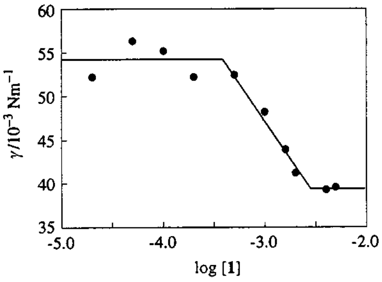


Fig. 1 Plot of surface tension *vs.* concentration of **1** at 25 °C.

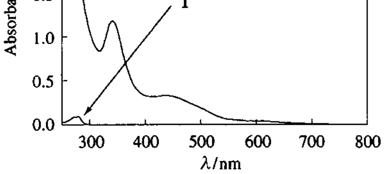
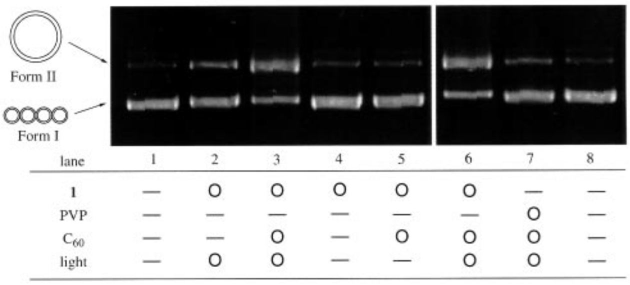


Fig. 2 Absorption spectra of **1** and **1**–[60]fullerene complex in water at 25 °C: [**1**] = 0.50 mmol dm^{-3}.



lane	1	2	3	4	5	6	7	8
1	—	○	○	○	○	○	—	—
PVP	—	—	—	—	—	—	○	—
C_{60}	—	—	○	—	○	○	○	—
light	—	○	○	—	—	○	○	—

Fig. 3 Agarose gel electrophoretic patterns of DNA nicked by **1**–[60]fullerene complex. The reaction samples contained 93 μmol dm^{-3} of ColE1 supercoiled plasmid. Lanes 1 and 8: no chemicals in distilled water. Lane 2 and 4: 25 μmol dm^{-3} of **1**. Lanes 3, 5 and 6: 25 μmol dm^{-3} of **1** and 12 μmol dm^{-3} of [60]fullerene. Lane 7: 0.9 wt% PVP and 12 μmol dm^{-3} of [60]fullerene. Lanes 1–3 and 6–8: incubated under visible light irradiation at a distance of 10 cm by a 140 W Xe lamp (Sumita LS-140UV) at 25 °C for 6 h. Lanes 4 and 5: incubated in the dark for 6 h. After the addition of 10 mm^3 of 10% SDS solution and loading buffer (Wako) in this order, electrophoresis was performed using 0.9% agarose gel. The gel was stained with 0.3 μg dm^{-3} ethidium bromide and visualized on a UV transilluminator.

^{13}C NMR spectrum of the **1**–[60]fullerene complex shows a new peak (145.90 ppm) due to sp^2 carbon atoms of [60]fullerene. These results consistently support the view that [60]fullerene is solubilized into water by inclusion in **1**. We determined the concentration of solubilized [60]fullerene by elemental analysis of the dried sample obtained by evaporation of this solution. The analytical result shows that the concentration of [60]fullerene in the original aqueous solution was 0.24 mmol dm^{-3}. This indicates the ratio between **1** and [60]fullerene to be 2 : 1.§ From a dynamic light-scattering measurement (DLS: Otsuka Electronic DLS-7000), the particle size of the **1**–[60]fullerene complex was estimated to be 1.4 nm, which was comparable with the size of the **1**–[60]fullerene 1 : 2 complex (as simulated by a computational method). These results strongly support the view that the complex does not form large aggregates under the present experimental conditions.

The **1**–[60]fullerene complex was applied to the photocleavage of ColE1 supercoil plasmid. Under dark conditions, DNA was not cleaved even in the presence of these reagents (Fig. 3, lanes 4 and 5). Under visible light irradiation, the **1**–[60]fullerene complex clearly showed DNA-cleaving activity (lane 3). In lane 3, about 65% of supercoiled DNA (form I) was converted to nicked DNA (form II). On the other hand, compound **1** showed a weak DNA-cleaving activity by itself. We consider that 'cationic' **1** is bound to 'anionic' DNA to induce a conformational change in DNA, which facilitates the DNA photocleavage. For comparison, we carried out a control experiment using the poly(vinylpyrrolidone) (PVP)–[60]fullerene system.[7] The DNA-cleaving activity of the PVP–[60]fullerene system (lane 7) was obviously much lower than that of the **1**–[60]fullerene complex (lane 6) under visible light irradiation. Consequently, one can conclude that [60]fullerene included in 'cationic' **1** is transported onto 'anionic' DNA with the aid of electrostatic interactions and cleaves it with the aid of photoirradiation.

How was DNA cleaved by transported [60]fullerene? Foote *et al.*[19] proposed that two mechanisms have to be taken into consideration: type I is the photoinduced electron transfer from the guanine unit to [60]fullerene and type II is the reaction with $^1O_2$ photochemically generated by [60]fullerene. These two mechanisms can be distinguished by comparison of the results of the aerobic conditions with those of the anaerobic ones. We found that there is no significant difference between these two conditions: the yields of form II were 63% under the aerobic conditions, 63% under the anaerobic (N_2) conditions and 66% under the dioxygen-saturated conditions. The results suggest that the mechanism mainly operative in the present DNA photocleavage system is type I, which is basically in line with Foote's results.[19]

In conclusion, the present paper demonstrated that 'cationic' **1** can solubilize [60]fullerene into water and transport it to DNA. Hence, photocleavage can be readily accomplished therein using 'unmodified' [60]fullerene. To the best of our knowledge, this is one of a few successful examples in which 'unmodified' [60]fullerene is directly supplied to DNA. These findings imply, therefore, that the concepts cultivated in host–guest chemistry of [60]fullerene and its homologues are more fruitfully applied to medicinal chemistry.

Notes and references

† *Selected data* for **1**: δ_H(250 MHz, [$^2H_6$]DMSO) 8.11 (s, 3H, NH), 6.91 (s, 6H, ArH), 4.69 and 4.48 (each d, each 6H, $ArCH_2O$), 4.21 (s, 6H, OCH_2CO), 3.37 (s, 9H, CH_3OSO_3), 3.20–3.37 (m, 6H, NCH_2), 2.98–3.15 (m, 33H, NCH_3 and NCH_2), 1.93 (m, 6H, $CH_2CH_2CH_2$), 1.05 (s, 27H, Bu^t); *m/z* (ESI-TOF MS) 1270 ([M − $CH_3OSO_3^-$]$^+$).

‡ By a similar method, [70]fullerene can be also solubilized into water by inclusion in **1**.

§ We determined the stoichiometry between **1** and [60]fullerene by the C/N ratio of the elemental analysis and then calculated the ε of the **1**–[60]fullerene mixture to be 4.87 × 10^4 dm^3 mol^{-1} cm^{-1} (341 nm) *via* the UV–VIS absorption spectrum (Calc. for $C_{63}H_{108}N_6O_{21}S_3\cdot 0.245C_{60}\cdot 3.5H_2O$: C, 57.6; H, 7.1; N, 5.2. Found C, 57.5; H, 6.6; N. 5.2%).

1 J. L. Arbogast, A. O. Darmanyan, C. S. Foote, Y. Rubin, F. N. Diederich, M. M. Alvarez, S. J. Anz and R. L. Whetten, *J. Phys. Chem.*, 1991, **95**, 11.
2 N. Martín, L. Sánchez, B. Illescas and I. Pérez, *Chem. Rev.*, 1998, **98**, 2527.
3 T. D. Ros and M. Prato, *Chem. Commun.*, 1999, 663.
4 Y.-Z. An, C.-H. B. Chen, J. L. Anderson, D. S. Sigman, C. S. Foote and Y. Rubin, *Tetrahedron*, 1996, **52**, 5179.
5 A. S. Boutorin, H. Tokuyama, M. Takasugi, H. Isobe, E. Nakamura and C. Hélène, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 2462.
6 E. Nakamura, H. Tokuyama, S. Yamago, T. Shiraki and Y. Sugiura, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 2143; H. Tokuyama, S. Yamago, E. Nakamura, T. Shiraki and Y. Sugiura, *J. Am. Chem. Soc.*, 1993, **115**, 7918.
7 Y. N. Yamakoshi, T. Yagami, S. Sueyoshi and N. Miyata, *J. Org. Chem.*, 1996, **61**, 7236.
8 N. Higashi, T. Inoue and M. Niwa, *Chem. Commun.*, 1997, 1507.
9 S. H. Friedman, D. L. DeCamp, R. P. Sijibesma, G. Srdanov, F. Wudl and G. L. Kenyon, *J. Am. Chem. Soc.*, 1993, **115**, 6506.
10 S. H. Friedman, P. S. Ganapathi, Y. Rubin and G. L. Kenyon, *J. Med. Chem.*, 1998, **41**, 2424.
11 Y. N. Yamakoshi, T. Yagami, K. Fukuhara, S. Sueyoshi and N. Miyata, *J. Chem. Soc., Chem. Commun.*, 1994, 517.
12 T. Anderson, K. Nilsson, M. Sundahl, G. Westman and O. Wennerström, *J. Chem. Soc., Chem. Commun.*, 1992, 604.
13 R. M. Williams and J. W. Verhoeven, *Recl. Trav. Chim. Pays-Bas*, 1992, **111**, 531.
14 T. Hamano, K. Okuda, T. Mashino, M. Hirobe, K. Arakane, A. Ryu, S. Mashiko and T. Nagano, *Chem. Commun.*, 1997, 21.
15 A. Ikeda, M. Yoshimura and S. Shinkai, *Tetrahedron Lett.*, 1997, **38**, 2107.
16 A. Ikeda, Y. Suzuki, M. Yoshimura and S. Shinkai, *Tetrahedron.*, 1998, **54**, 2497.
17 K. Tsubaki, K. Tanaka, T. Kinoshita and K. Fuji, *Chem. Commun.*, 1998, 895.
18 A. Ikeda, M. Yoshimura, H. Udzu, C. Fukuhara and S. Shinkai, *J. Am. Chem. Soc.*, 1999, **17**, 4291.
19 R. Bernstein, F. Prat and C. S. Foote, *J. Am. Chem. Soc.*, 1999, **121**, 464.

Communication 9/03872H