Pergamon

Tetrahedron Letters 43 (2002) 4321–4324

TETRAHEDRON LETTERS

# Water soluble supramolecular cyclotriveratrylene–[60]fullerene complexes with potential for biological applications

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Received 27 March 2002; revised 15 April 2002; accepted 23 April 2002

**Abstract**—Polybenzyl ether dendritic branches with peripheral triethyleneglycol chains have been attached to a cyclotriveratrylene core and the supramolecular complexes obtained from the resulting macrocyclic derivatives and $C_{60}$ were found to be water soluble. 

Several recent studies have shown that fullerenes exhibit interesting biological activities both in vitro and in vivo.[1] For example, a large number of fullerene derivatives are competitive inhibitors of the human immunodeficiency virus (HIV) protease thanks to the specific shape of the $C_{60}$ sphere which fits into the hydrophobic cavity of the enzyme active site.[2] The electron accepting ability of the fullerenes has also been exploited for the inhibition of redox enzymes such as nitric oxide synthase (NOS), thus providing a possible therapeutic approach for some neurodegenerative disorders.[3] It can also be mentioned that the easy excitation of the fullerenes by visible light and the special properties of the resulting excited-states allow other possibilities for applications in medicinal chemistry.[1] For example, fullerenes are able to generate efficiently cytotoxic singlet oxygen by energy transfer from the metastable fullerene triplet excited-state to molecular oxygen making them promising compounds for antitumor activities (photodynamic therapy).[1] However, the low solubility of fullerenes in aqueous media appears to be a major problem for their biological applications. Among the several strategies that have been developed to overcome the natural repulsion of fullerenes for water,[1] the preparation of water soluble supramolecular complexes with macrocyclic host systems such as γ-cyclodextrin or calixarene derivatives is of particular interest.[4] Effectively, they are easy to prepare and allow the solubilization of $C_{60}$ itself in the physiological media. As part of our research program on the supramolecular chemistry of fullerenes,[5] we now report the synthesis of new cyclotriveratrylene (CTV) derivatives with peripheral triethyleneglycol chains and show their ability to form water soluble supramolecular complexes with $C_{60}$. In the design of the CTV hosts **1** and **2** (Scheme 1), the polybenzyl ether dendritic type structure was chosen to provide an internal cavity capable of encapsulating totally the hydrophobic $C_{60}$ guest, thus preventing the fullerene–fullerene interactions and precipitation from the aqueous solutions.

The synthetic route leading to the CTV derivatives **1** and **2** is depicted in Scheme 1. Compounds **3**[6] and **4**[7] were prepared according to previously reported procedures. Reaction of the CTV derivative **4** (1 equiv.) with **3** (3.3 equiv.) in the presence of $K_2CO_3$ (10 equiv.) in refluxing acetone for 60 h afforded compound **1** in 84% yield. Compound **1** was characterized by $^1H$ and $^{13}C$ NMR spectroscopy and elemental analysis.[8] In the $^1H$ NMR spectrum recorded in $CDCl_3$, two distinct singlets are seen at $\delta$ 6.73 and 6.86 ppm for the CTV aromatic protons in full agreement with the $C_3$ symmetry of **1**. In addition, it can also be noted that the typical cone-shaped CTV conformation is retained in **1**. This is evidenced by the presence of the characteristic AX quartet for the methylene bridges,[7] in which the pseudo-axial hydrogens, sterically congested at the top of the cone, resonate ca. 1.2 ppm downfield with respect to their pseudo-equatorial counterparts. The structure of **1** is also confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, which displays the expected sodium molecular ion peak at $m/z$ 3284 (calcd for $C_{171}H_{246}O_{60}Na$: 3284.8).

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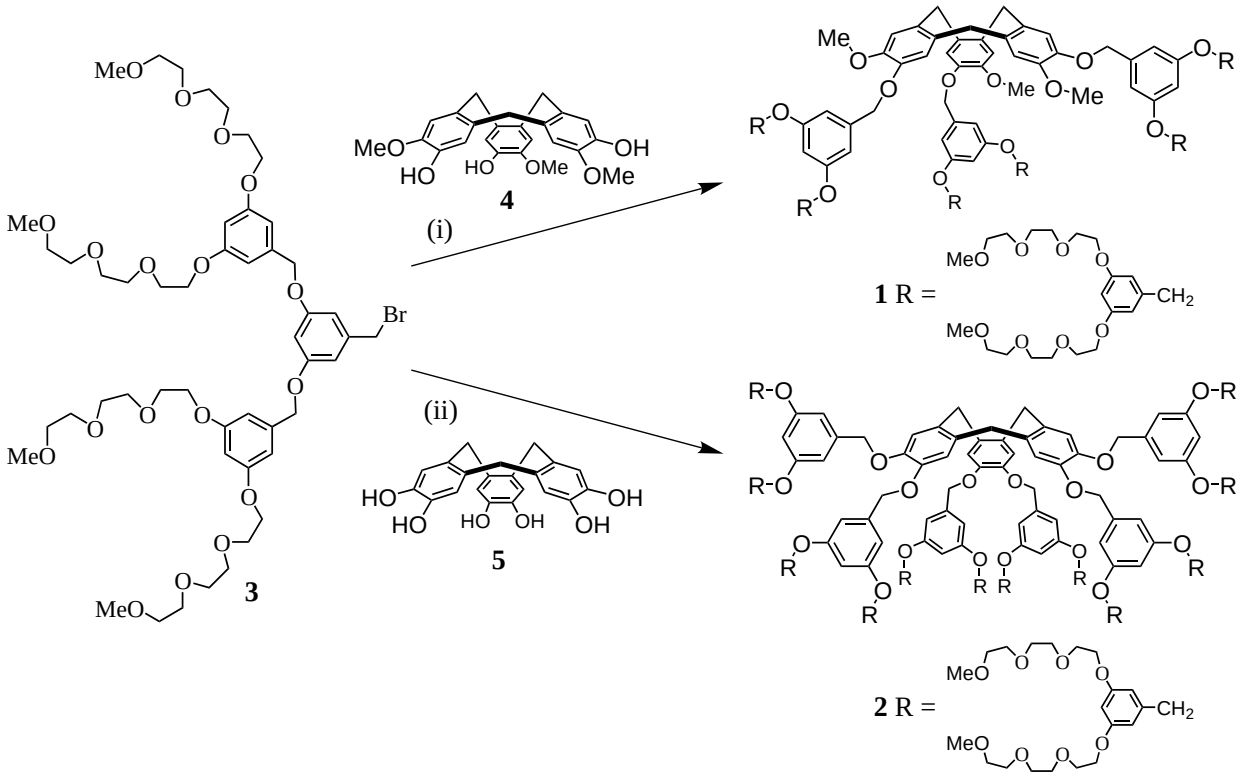
0040-4039/02/$ - see front matter 
PII: S0040-4039(02)00793-1




**Scheme 1.** *Reagents and conditions*: (i) $K_2CO_3$, acetone, △ (84%); (ii) $K_2CO_3$, acetone, △ (88%).

Reaction of **3** with the commercially available hexaphenol **5** in the presence of $K_2CO_3$ gave CTV derivative **2** in 88% yield. The $^1H$ and $^{13}C$ NMR spectra of compound **2** are in full agreement with the proposed formulation.[8] In particular, only one singlet is observed for the CTV aromatic protons ($\delta$ 6.92 ppm) in the $^1H$ NMR spectrum as expected owing to the $C_{3v}$ symmetry of CTV derivative **2**. The mass spectrum also confirmed the structure of **2**, with no peaks corresponding to partially substituted CTV derivatives being observed. The MALDI-TOF-MS of **2** displays the sodium–molecular ion complex at $m/z$ 6095.5 (calcd for $C_{315}H_{462}O_{114}Na$: 6096.0) as the base peak and provides clear evidence for the monodispersity of **2**.

The formation of host–guest complexes between $C_{60}$ and the CTV derivatives **1**–**2** was first studied in benzene solutions.[9] Continuous changes were observed in the UV–vis spectra upon successive additions of the host to the fullerene solutions (Fig. 1). Specifically, each new addition of **1** or **2** to the $C_{60}$ solution in benzene led to an increase in the absorption in the whole visible

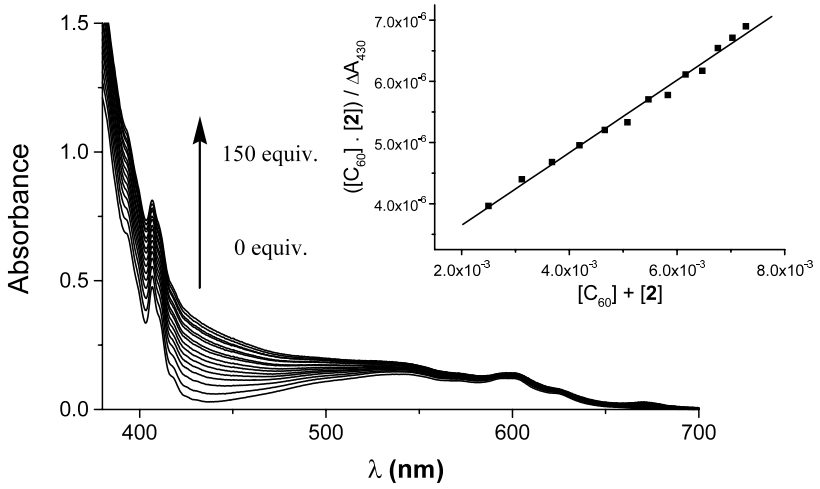



**Figure 1.** Changes in the absorption spectra of $C_{60}$ benzene solutions ($[C_{60}]=0.146$ mM) containing increasing amounts of **2** at 298 K; the inset shows the Benesi–Hildebrand plot for determining the association constant between $C_{60}$ and **2** via UV–vis titration.[9]

region with the most pronounced effect was at approximately 430 nm. These spectral changes are not attributable to the added CTV derivative, which does not absorb in this spectral region. Indeed, they are an indication of fullerene complexation, as previously reported in the literature for fullerene containing supramolecular adducts.[10] Assuming the formation of solvated 1:1 complexes in dilute solutions,[11] treatment of the titration data with the Benesi–Hildebrand equation gave the association constant ($K_A$) values: 130±20 $M^{-1}$ for **1** and 190±20 $M^{-1}$ for **2** at 298 K. The binding behavior observed in benzene solutions for both **1** and **2** and the $K_A$ values are in good agreement with those of other examples of substituted CTV derivatives already reported.[5,10]

Slow evaporation of $C_6H_6$ solutions of $C_{60}$ and CTV derivative **1** or **2** afforded the corresponding supramolecular complexes. For both **1** and **2**, only the 2:1 host–guest ratio gave homogeneous samples after complete evaporation of the solvent suggesting the formation of supramolecules in which the $C_{60}$ guest is encapsulated within the cavity of two host molecules. The latter observation is in good agreement with the previously reported liquid crystalline supramolecular complex of $C_{60}$ with a CTV derivative[5b] where effectively such a 2:1 complex has been obtained in the solid state. Whereas the supramolecular complex prepared with CTV **1** and $C_{60}$ is only poorly soluble in polar solvents, the brown glassy compound obtained from CTV **2** and $C_{60}$ can be easily dissolved in water or methanol. The UV–vis spectrum of a water solution of [$C_{60}$.(**2**)$_2$] is shown in Fig. 2. The solubility of [$C_{60}$.(**2**)$_2$] in water (ca. 3 mmol/l) was estimated from the absorbance of a solution prepared by dilution of a saturated water solution of the supramolecular complex. The $^1$H NMR spectrum of compound [$C_{60}$.(**2**)$_2$] in $CD_3OD$ is similar to the spectrum of the corresponding ligand **2** recorded in the same solvent, but with some chemical shift changes. The largest shifts are actually observed for the aromatic protons suggesting that the $C_{60}$ guest is in the space present around the aromatic moieties. The NMR characterization of [$C_{60}$.(**2**)$_2$] also

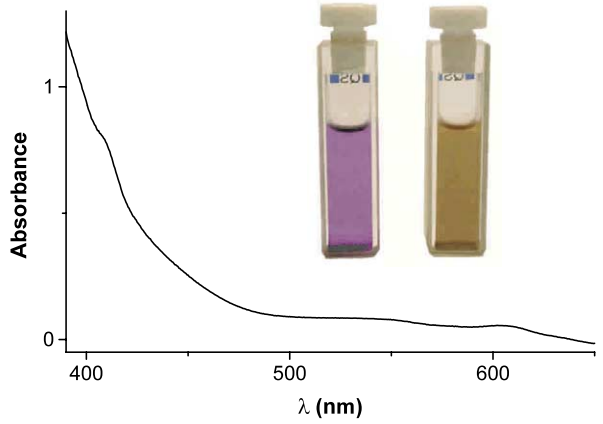



**Figure 2.** Absorption spectra of [$C_{60}$.(**2**)$_2$] in water; the inset shows the magenta solution of $C_{60}$ in benzene (left) and the brown solution of [$C_{60}$.(**2**)$_2$] in water (right).

reveals the homogeneity of the sample and the absence of uncomplexed ligand. The latter observations are in good agreement with the formation of complexes in which the $C_{60}$ is encapsulated within the cavity of two CTV molecules.

In conclusion, the macrocyclic host systems **1** and **2** are able to form stable supramolecular assemblies with $C_{60}$ and the host–guest complex obtained from **2** and $C_{60}$ is water soluble thus making it an interesting candidate for biological applications. The excited-state properties of the supramolecular complexes are also under investigation, in particular to evaluate their potential for the production of cytotoxic singlet oxygen in aqueous media.

## Acknowledgements

This research was supported by the French Ministry of Research (*ACI Jeunes Chercheurs*). We further thank L. Oswald for technical help.

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8. Selected spectroscopic data for **1**: $^1$H NMR ($CDCl_3$, 200 MHz): 3.37 (s, 36H), 3.46 (d, $J$=14 Hz, 3H), 3.50–3.90

(m, 129H), 4.10 (t, $J=5$ Hz, 24 H), 4.70 (d, $J=14$ Hz, 3H), 4.93 (s, 12H), 5.01 (s, 6H), 6.44 (d, $J=2$ Hz, 6H), 6.51 (t, $J=2$ Hz, 3H), 6.58 (d, $J=2$ Hz, 12H), 6.68 (d, $J=2$ Hz, 6H), 6.73 (s, 3H), 6.86 (s, 3H); $^{13}$C NMR ($CDCl_3$, 50 MHz): 59.01, 67.46, 69.64, 70.53, 70.61, 70.78, 71.89, 101.06, 105.85, 106.11, 131.67, 132.68, 138.97, 140.12, 147.10, 148.43, 160.11; anal. calcd for $C_{171}H_{246}O_{60}\cdot H_2O$: C, 62.62; H, 7.62; found: C, 62.56; H, 7.57%; MALDI-TOF-MS: 3284 ([$M$+Na]$^+$, calcd for $C_{171}H_{246}NaO_{60}$: 3284.8). For **2**: $^1$H NMR ($CDCl_3$, 200 MHz): 3.35 (s, 72H), 3.50–3.80 (m, 291H), 4.01 (t, $J=$ 4Hz, 48H), 4.60–4.85 (m, 27H), 5.11 (broad, 12H), 6.38 (d, $J=2$ Hz, 12H), 6.41 (t, $J=2$Hz, 6H), 6.48 (d, $J=2$ Hz, 24H), 6.70 (t, $J=2$ Hz, 12H), 6.92 (s, 6H); $^{13}$C NMR ($CDCl_3$, 50 MHz): 58.97, 67.34, 69.56, 70.49, 70.55, 70.68, 71.86, 101.02, 106.03, 132.96, 139.01, 139.96, 147.92, 159.94; anal. calcd for $C_{315}H_{462}O_{114}$: C, 62.30; H, 7.67; found: C, 62.62; H, 7.79; MALDI-TOF-MS: 6095.5 ([$M$+Na]$^+$, calcd for $C_{315}H_{462}NaO_{114}$: 6096.0).

9. Binding studies were performed in benzene solution at 298±1 K. In a typical experiment, a 1 ml volume of a $C_{60}$ solution (0.146 mM) was placed in the sample cell. An aliquot of a stock solution of the host compound (typical concentration: 10–20 mM) was added to the sample cell and, after homogenization, the absorption spectrum was recorded. Additional aliquots of the host compound were added to the sample cell, and the spectrum was recorded after each addition. The association constants were calculated from the absorption intensity changes observed at 430 or 440 nm, compared to pure $C_{60}$ using the Benesi–Hildebrand equation (see Ref. 5c). All experiments were performed at least in triplicate.
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11. The binding isotherms provide a good fit for a 1:1 stoichiometry. The stoichiometry of the host–guest complexes obtained by crystallization between **1** or **2** and $C_{60}$ however is 2:1. This apparent contradictory host–guest ratio has also been observed in several recent examples of fullerene-based supramolecular assemblies and it is quite reasonable to have a different (lower) complexation ratio in dilute solution to that in the solid state. For examples, see: (a) Haino, T.; Yanase, M.; Fukazawa, Y. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 259–260; (b) Haino, T.; Yanase, M.; Fukazawa, Y. *Tetrahedron Lett.* **1997**, *38*, 3739–3742; (c) Mizyed, S.; Ashram, M.; Miller, D. O.; Georghiou, P. E. *J. Chem. Soc., Perkin Trans. 2* **2001**, 1916–1919.