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A highly water-soluble 2:1 β-cyclodextrin–fullerene conjugate

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A novel 2:1 (permethylated-β-cyclodextrin)–fullerene conjugate has been synthesised; highly soluble in cold water with formation of aggregates, it displays a negative solubility coefficient and has a partition coefficient between octanol and water (Log *P* = 1.58) in the suitable range for biological studies.

The study of the biological activity of fullerenes depends on the availability of water-soluble derivatives.[1] Such molecules have been obtained either by covalent addition of hydrophilic appendages or by complex formation with host molecules.[2] In both cases, it is advisable to use auxiliary compounds whose properties do not interfere with those of the fullerenes. Cyclodextrins (CD) (and their permethylated derivatives), known to be essentially non-toxic (at least β-CD),[3] are particularly attractive in this respect, and in fact have been used both as hydrophilic appendage[4,5] and as water-soluble carrier.[6–9]

These two types of derivatives possess different properties: contrary to the non-covalent 2:1 γ-CD–C_{60} complex,[6–8] the UV-Vis spectra of water solution of the 2:1 β-CD–C_{60} complex,[9] or of a 1:1 β-CD–C_{60} covalent conjugate[4] are typical of the presence of aggregates, also revealed by direct physical measurements.[10] Other 1:1 covalent conjugates (with α-, β- or γ-CD) have been prepared but are apparently less water-soluble.[11]

In the case of the 2:1 γ-CD–fullerene complex, different equilibria, including (1) and (2) (Scheme 1), may take place in solution, so that if some other substrate with sufficient affinity for γ-CD were present, the fullerene could be displaced and possibly precipitate. A covalent binding between the fullerene and the γ-CD would probably impede this displacement.

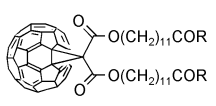
$$C_{60} + CD \rightleftharpoons (C_{60},CD) \qquad (1)$$

$$(C_{60},CD) + CD \rightleftharpoons (CD,C_{60},CD) \qquad (2)$$

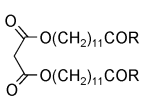
Scheme 1 Equilibria between γ-CD and C_{60} in water.

Because of the formation of aggregates, the equilibria of the β-CD–fullerene 2:1 complex are apparently more complicated. However, here again, a covalent link could stabilise the complex, an interesting possibility because of the easier availability of the β-CD.

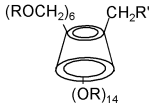
We have thus prepared the covalent conjugate **1a** in which the linker was expected to allow solvent-dependent equilibria between conformers such as **A**, **B** and **C** (Scheme 2). In water, **A** and **B** could form micelle-like aggregates, but if the CDs ensure sufficient hydrophilic protection, **C** could exist as a non-associated species.



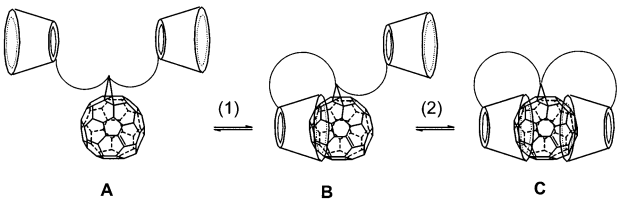
1a R= NH-PMCD
1b R= OBu^t



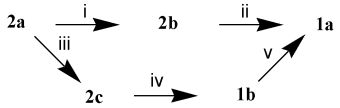
2a R=OH
2b R= NH-PMCD
2c R= OBu^t



3a R=H; R'=OTs
3b R=H; R'=N_3
3c R=Me; R'=N_3
3d R=Me; R'=NH_2



Scheme 2 Possible equilibria between conformers **A**, **B** and **C** of **1a**.



Scheme 3 *Reagents and conditions*: i, **3d**, 1-hydroxybenzotriazole (HOBt), CH_2Cl_2, DCC, rt, 48 h (89%); ii, C_{60}, toluene, CBr_4, DBU, rt, 14 h (30%); iii, ($COCl_2$, CH_2Cl_2, reflux, 36 h, solvent removal, then *t*-BuOH, Py (69%); iv, C_{60}, toluene, CBr_4, DBU, rt, 14 h (38%); v, TFA, CH_2Cl_2, rt, 4 h, solvent removal, then **3d**, DCC, HOBt, CH_2Cl_2, rt, 48 h (75%).

Two different routes led to **1a**† (Scheme 3), both starting from **2a**.†[12] 6^A-amino-6^A-deoxy-per(O-methyl)-β-cyclodextrin (NH_2-PMCD) **3d** was prepared in 28% yield from β-CD by a combination of published methods.[13] The methoxy groups were introduced in order not only to prevent side reactions but also to increase the solubility of the β-CD moiety, in spite of the negative solubility coefficient of the related permethylated β-CD;[14] functionalisations of C_{60} were effected by Hirsch-Bingel (HB) reactions.[15]

Soluble in toluene, methanol, ethanol, acetonitrile, dichloromethane and chloroform, **1a** may also be dissolved in water at 20 °C up to a limit of 90 mg mL^{-1}, one of the highest reported solubilities at pH 7.[1] Aggregates are present in water solutions: in dichloromethane, the expected UV-Vis spectrum of a methanofullerene is observed; in water, this spectrum is less resolved and a relative maximum at 430 nm is missing (Fig. 1), a sign of aggregate formation;[16,17] similarly, the NMR peaks are much broader in water than in chloroform. No induced circular dichroism could be detected in water or in dichloromethane, thus excluding an appreciable population of conformer **C** and

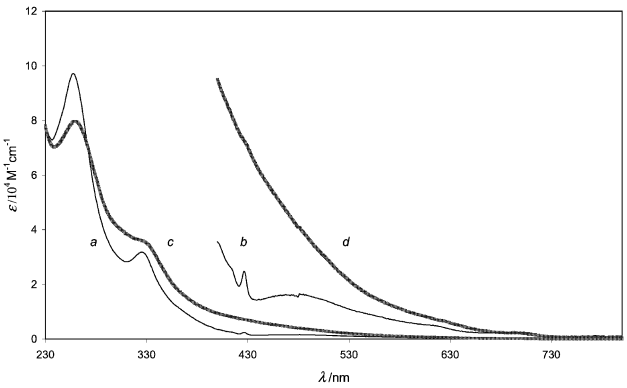


Fig. 1 Absorption spectra of **1a**: (*a*) 10^{-5} M in CH_2Cl_2; (*b*) same, ×10; (*c*) 10^{-5} M in water; (*d*) same, ×10.

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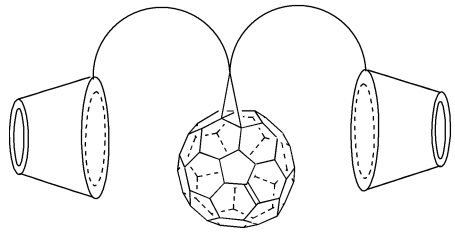
suggesting a predominance of the extended form **A** in these solutions. Like the related permethyl-β-CD, **1a** has a negative solubility coefficient in water: when heated, clear 10^{-3} M and 2.5×10^{-4} M solutions became turbid at 30 and 42 °C, respectively and returned to their original state after cooling. A measure of the partition coefficient between octanol and water gave Log P = 1.58,[18] in the range suitable for allowing penetration of cell membranes[19] or oral absorption.[20]

Thus, although both the high solubility in water at neutral pH and the convenient partition coefficient of **1a** make it well suited for biological studies, it may be desirable to eliminate the formation of aggregates in water. Since this phenomenon may be due to a poor steric fit or to a 'wrong' orientation of the β-CDs, it is possible that the γ-CD homologue of **1a** or β- or γ-CD conjugates such as **Z** be very soluble in water as well, but now without forming aggregates. Work along these lines is in progress.



z

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Notes and references

† *Selected analytical data* **1a**: Calc. for $C_{211}H_{264}N_2O_{74}$, $7H_2O$: C 61.24, H 6.77, N 0.68; found: C 61.31, H 6.96, N: 0.57%. MS (FAB+, NaI) *m*/*z* : 4035 $[M + Na]^+$ (40%),1633. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.2-1.48 (m, 32H), 1.63 (br s, 4H), 1.85 (m, 4H), 2.18 (m, 4H), 3.16 (dd, J = 3.3, 9.5 Hz, 2H), 3.21 (m, 12 H), 3.38-3.46 (m, 40 H), 3.5-3.56 (m, 52 H), 3.58-3.70 (m, 70 H), 3.85 (m, 28 H), 4.5 (t, J = 6.5 Hz, 4H), 5.12 (t, J = 3.0 Hz, 4H), 5.16 (t, J = 2.9 Hz , 6H), 5.19 (br t, 4H), 6.02 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.84, 25.93, 25.53, 29.19, 29.36, 29.44, 29.55, 29.58, 36.84, 39.73, 58.26, 58.28, 58.31, 58.44, 58.56, 58.61, 58.91, 58.97, 58.98, 59.05, 59.32, 61.16, 61.24, 61.35, 61.39, 61.48, 61.59, 67.40, 69.83, 70.81, 70.92, 70.95, 71.07, 71.10, 71.15, 71.30, 71.47, 71.50, 71.61, 79.89, 79.94, 80.07, 80.23, 80.56, 80.70, 80.76, 81.32, 81.40, 81.52, 81.61, 81.72, 81.75, 81.92, 81.96, 82.06, 82.09, 98.55, 98.62, 98.72, 98.91, 99.00, 138.91, 140.89, 141.85, 142.14, 142.92, 142.96, 143.03, 143.82, 144.54, 144.59, 144.63, 144.82, 145.12, 145.20, 145.33, 163.66, 173.17.

1b: Calc. for $C_{95}H_{62}O_8$: C 85.69, H 4.69; found: C 84.11, H: 4.78%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.10-1.62 (m 54 H), 2.15 (t, J = 7.3Hz, 4H), 4.48 (t, J = 6.3 Hz, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 25.0, 25.9, 28.04, 28.49, 29.02, 29.15, 29.24, 29.41, 29.47, 35.5, 53.0, 67.3, 72.0, 79.8, 138.89, 140.82, 141.78, 142.08, 142.88, 142.89, 142.96, 143.76, 144.48, 144.55, 144.75, 145.06, 145.13, 145.25, 163.6, 173.2.

2a: Mp 71-73 °C.

2b: Calc. for $C_{151}H_{266}N_2O_{74}$: C 55.06, H 8.30, N 0.85; found: C 54.50, H 8.14, N 1.08%. MS (FAB+, NaI), *m*/*z*: 3315 (100%) $[M + Na]^+$.^{1}H NMR (400 MHz, $CDCl_3$): δ 1.2-1.35 (m, 32H), 1.61 (m, 4H), 2.16 (dd, J = 6.2, 6.4 Hz, 4H), 3.14 (dd, J = 3.3, 9.5 Hz, 2H), 3.18 (m, 12H), 3.35 (s, 2H), 3.39-3.43 (m, 40H), 3.48-3.53 (m, 58H), 3.55-3.70 (m, 68H), 3.73-3.91 (m, 28H), 4.11 (t, J = 6.7 Hz, 4H), 5.08(t, J = 2.8 Hz, 4H), 5.12 (t, J = 3.5 Hz, 6H), 5.16 (t, J = 4 Hz, 4H), 6.02 (br t, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.63, 25.73, 28.29, 29.06, 29.24, 29.29, 29.34, 29.39, 29.41, 36.72, 40.05, 58.16, 58.18, 58.20, 58.34, 58.47, 58.51, 58.80, 58.87, 58.88, 58.92, 58.95, 59.22, 61.05, 61.14, 61.25, 61.29, 61.38, 61.39, 61.48, 65.51, 69.74, 70.71, 70.81, 70.86, 71.36, 70.98, 71.01, 71.05, 71.21, 71.39, 79.76, 79.84, 79.86, 79.96, 80.10, 80.20, 80.45, 81.21, 81.30, 81.42, 81.51, 81.62, 81.66, 81.67, 81.82, 81.86, 81.92, 81.96, 82.00, 98.47, 98.50, 98.67, 98.81, 98.88, 98.92, 166.7, 173.12.

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