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Synthesis and Characterization of Water-Soluble Amino Fullerene Derivatives

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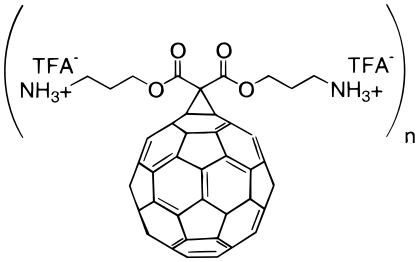
ABSTRACT

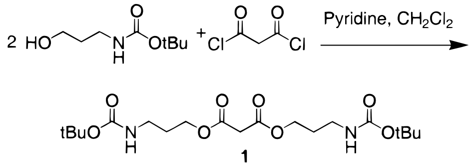


A series of amino-substituted methanofullerene derivatives were prepared by mono-, tris-, and hexa-Bingel–Hirsch reactions using an *N*-protected malonate derivative. Upon scission of the protecting groups, the *e,e,e*-tris- and octahedral T_h hexa-methano amino fullerenes were found to be among the most water-soluble fullerene derivatives yet prepared. ^{3}He NMR data on corresponding adducts of ^{3}He@C_{60} helped verify the assigned structures. UV spectral studies confirmed the influence of aggregation on solubility of these adducts in water.

Recently, several fullerene derivatives have been shown to possess significant biological activity.[1,2] The extraordinary chemical and physical properties of C_{60} and its derivatives confer activity of these compounds as potential therapeutic agents against HIV[3] and neurodegenerative diseases[4] and as agents for DNA cleavage.[5] Most recently, certain C_{60} derivatives have been shown to be excellent inhibitors of nitric oxide synthase.[6] Potential applications of fullerenes in medicine is enhanced by the finding that most fullerene derivatives have unusually low toxicity compared with typical drugs.[7] However, the insolubility of fullerenes in aqueous media has slowed the realization of these biological applications. The history of water-soluble fullerene derivatives is limited to only a few compounds, most of which contain carboxylic acids as the solubilizing group. Herein, we report the synthesis of a series of amino-substituted methanofullerenes, two of which exhibit pronounced water solubility.

Our main objective was to prepare simple fullerene derivatives containing polar groups to enhance their water solubility. A series of derivatives containing an increasing number of malonate appendages with polar groups on the ends were investigated. The reliable Bingel−Hirsch addition of malonates to C_{60} was used since it allowed the introduction of two polar headgroups for every malonate attached. The malonate reagent **1** was prepared as in Scheme 1 by treatment

Scheme 1. Synthesis of Malonate **1**



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of 5 g (2 equiv) of *tert*-butyl *N*-(3-hydroxypropyl)carbamate in 250 mL of dry methylene chloride with 1.97 g (1 equiv) of distilled malonyl chloride and 2.21 g (2 equiv) of pyridine. Following purification by column chromatography on silica gel using 1:1 hexane/ethyl acetate as the eluant, 3.20 g (54.7%) of malonate **1** was obtained ($R_f = 0.41$, silica gel, 1:1 EtOAc:hexane): ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.85 (s, 1H), 4.19 (t, 4H), 3.37 (s, 2H), 3.17 (q, 4H), 1.82 (m, 4H), 1.41 (s, 18H); CI-MS calcd for $C_{19}H_{34}N_2O_8$ [M + NH_3] 419, found 419; ^{13}C NMR (200 MHz, $CDCl_3$ δ 166.9, 156.3, 79.7, 63.6, 42.0, 37.9, 29.5, 28.5 ppm).

Preparation of the C_{60} monoadduct **2a** proceeded smoothly according to the general procedure of Hirsch and coworkers.[8] To that end, 500 mg (1 equiv) of C_{60} and 331 mg (1.5 equiv) of CBr_4 were dissolved in 300 mL of toluene by sonication, followed by the addition of 418 mg (1.5 equiv) of malonate **1** and 0.3 mL (3 equiv) of DBU. The reaction was complete in 30 min. After conventional workup, the crude reaction mixture was chromatographed on a silica gel column using toluene as eluent to remove unreacted C_{60}, followed by 10:1 toluene/ethyl acetate to yield, after evaporation and drying, 334.8 mg (51.8%) of the desired monoadduct **2a** ($R_f = 0.09$, silica gel, 100:5 toluene:ethyl acetate): ^{1}H NMR (200 MHz $CDCl_3$) δ 4.90 (bs), 4.57 (t), 3.32 (q), 2.06 (m), 1.46 (s); FAB-MS calcd for $C_{79}H_{32}N_2O_8$ 1137.129, found 1137.4). The synthesis of **2a** was repeated using ^{3}He@C_{60} to give the corresponding ^{3}He@**2a**, which showed a sharp singlet at δ −8.045 ppm in its ^{3}He NMR spectrum, which is in the expected range for a monomethano adduct across a 6,6 bond of C_{60}.[9]

The number of malonate substituents connected to the fullerene core was then increased to three by using 3.3 equiv of CBr_4, 3.3 equiv of malonate **1**, and 6.6 equiv of DBU. After workup, the crude residue was chromatographed on silica gel using 10:1 toluene/ethyl acetate followed by 2:1 toluene:ethyl acetate to give the *e,e,e* isomer of the tris malonate adduct **3a** as a red-orange solid in 10.4% yield (R_f = 0.25, silica gel, 2:1 toluene:ethyl acetate): FAB-MS calcd for $C_{117}H_{96}O_{48}N_{12}$ 1968, found 1969). The UV−vis absorption spectrum of **3a** was identical with that of the *e,e,e* Hirsch tris-ethyl ester.[10] The synthesis of **3a** was repeated with ^{3}He@C_{60} to give the corresponding ^{3}He@**3a** which showed a sharp singlet in its ^{3}He NMR spectrum at δ −11.966 ppm, which is in the expected position for an *e,e,e*-tris adduct to C_{60} (see Figure 1).[9d]

Next, we turned our attention to the synthesis of the octahedral (T_h) hexa-malonate derivative, **4b**, a derivative with 12 amino groups, which was expected to show even greater hydrophilicity. This symmetrical addition pattern is well-known from the work of Hirsch.[11] Thus, 10 equiv of CBr_4, 10 equiv of malonate **1**, and 20 equiv of DBU were subjected to the same reaction conditions as above. Following workup, the crude reaction mixture was separated on a silica gel column using 2:1 toluene/ethyl acetate and an orange-yellow band was isolated. This band was then rechromatographed on a preparative TLC plate using 2:1 toluene/ethyl acetate to yield **4a** as a yellow solid ($R_f = 0.42$, silica gel, 2:1 ethyl acetate:toluene); ^{13}C NMR (200 MHz, $CDCl_3$) δ 164.13, 156.44, 146.18, 141.51, 69.00, 65.28, 37.91, 29.23, 29.04; FAB-MS calcd for $C_{174}H_{192}O_{48}N_{12}$ 3216, found 3217.9). In the ^{13}C NMR spectrum, only three distinct signals appear at δ 69, 141, and 145 ppm, corresponding, respectively, to the three types of sp^3 and sp^2 carbon atoms remaining on the fullerene core. This spectrum compares well with the literature report of the analogous T_h-symmetrical hexa adduct of diethyl malonate to C_{60} reported by Hirsch (see Figure 2).[11] Further evidence supporting the octahedral symmetry of **4a** is its UV spectrum which is in excellent agreement with that reported for the Hirsch hexa-adduct.[10] The ^{3}He NMR spectrum of ^{3}He@**4a** showed a sharp singlet at δ −12.1 ppm, which is consistent with that reported for a T_h-symmetrical hexa-adduct.[9d]

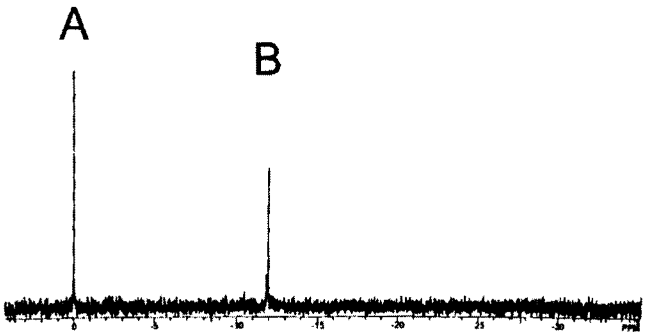

Figure 1. ^{3}He−NMR Spectrum of *e,e,e*-tris methanofullerene **3a**. Signal A is ^{3}He@C_{60} (0 ppm) and B is ^{3}He@**3a** (−11.966 ppm).

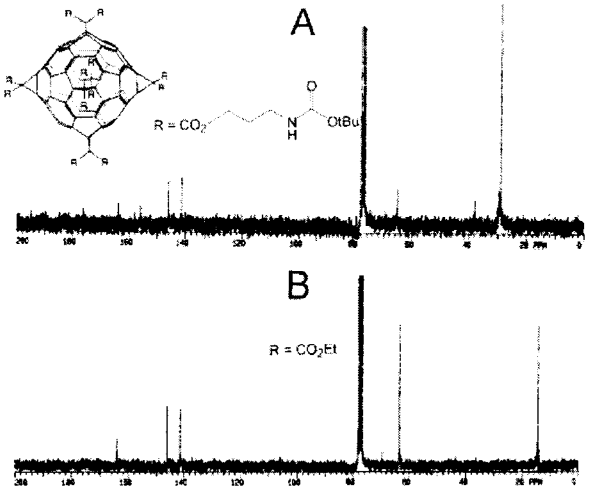

Figure 2. Comparison of ^{13}C NMR spectrum of hexa-adducts.

The carbamate protecting groups on **2a**, **3a**, and **4a** were removed using trifluoroacetic acid (see Scheme 2). Following

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(5) Ikeda, A.; Hatano, H.; Kawaguchi, M.; Suenaga, H.; Shinkai, S. *Chem. Commun.* **1999**, 1403−1404.

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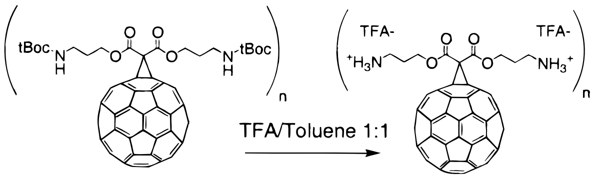
Scheme 2. Deprotection of tBoc-Protected Amines



2a n=1
3a n=3
4a n=6

2b n=1
3b n=3
4b n=6

deprotection, the water solubility of **2b**, **3b**, and **4b** was determined spectroscopically by UV analysis. Extinction coefficients for each adduct were determined by analysis of standard solutions. The corresponding extinction coefficient was then used to calculate the amount in mg/mL of each derivative that dissolved in water. To that end, saturated solutions of each adduct in water were prepared and allowed to stir for 36 h. Each solution was then centrifuged and a portion was analyzed by UV spectroscopy. Thus, 0.64 mg/mL of monoadduct **2b**, 152 mg/mL of tris adduct **3b**, and 418 mg/mL of hexa adduct **4b** were found to be soluble in water at room temperature.[12] Previously, Hirsch and Brettreich reported a polycarboxylated fullerene dendrimer with a water solubility of 254 mg/mL using a similar method.[13]

It has been known for some time that fullerene derivatives aggregate and this property might enhance their solubility. In 1995, Guldi and co-workers reported that evidence of clustering could be obtained from UV spectra, specifically, broadening of the bands is characteristic of aggregation.[14] To investigate whether aggregation was influencing the solubility of our compounds, UV studies were initiated.

The UV spectra of **2b**, **3b**, and **4b** were examined over a range of concentrations from 8 to 20 μM at 257 nm. Pronounced red shifts were observed as the concentration decreased for **2b**, **3b**, and **4b**. For a given absorbance, hypochromism on the order of 10−50% is observed in aggregates.[15] The UV spectrum of **3a** in methanol was compared to that of **3b** in water (see Figure 3). In water, the spectrum is characterized by broader bands compared to that in methanol.

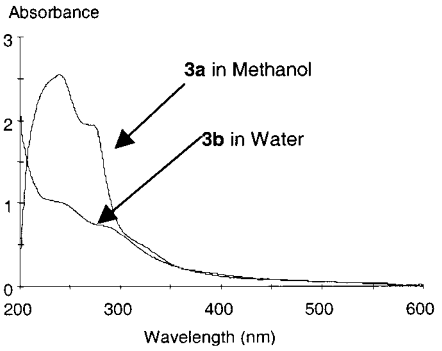


Figure 3. Comparison of UV spectra of tris-adducts **3a** in MeOH and **3b** in water.

Similarly, hexa-adducts **4a** and **4b** were also compared. The UV spectra were compared in methanol and water (see Figure 4), and a broadening of the bands in water was observed. Similar band broadening was observed when the organic solvent was THF. A table of values for λ_{max} and the corresponding extinction coefficients in both organic solvent (THF) and water for compounds **2**−**4a**,**b** are presented below (see Table 1).

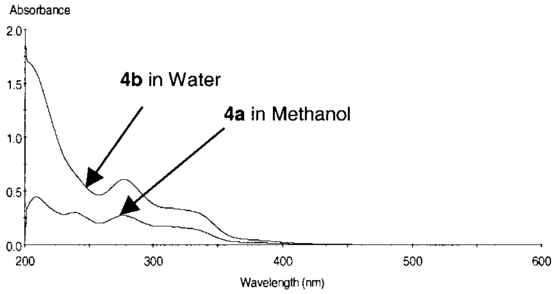


Figure 4. Comparison of UV spectra of hexa-adducts **4a** in MeOH and **4b** in water.

Table 1. λ_{max} and Values for ϵ for Compounds **2a**, **3a**, and **4a** in THF and **2b**, **3b**, and **4b** in Water

	λ_{max}	ϵ
2a	248	118052
	289	44789
	368	19842
2b	212	36750
	276	37600
	342	32850
3a	239	37062
	263	39125
	287	46200
3b	212	76000
	248	67250
	265	46900
4a	213	75000
	245	33950
	278	32000
	319	17550
	336	15700
4b	243	79500
	265	49400
	290	53600
	316	48250
	335	39600

In summary, a series of mono, tris, and hexa adducts to C_{60} of malonates with *N*-protected amino headgroups have been synthesized and characterized. Following removal of the protecting groups, the tris- and hexa-amino adducts were

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found to be highly water soluble. In addition to conventional spectroscopic methods, ^{3}He NMR was used to confirm the structures of these new compounds. Through UV studies, aggregation was found to affect the solubility of the mono, tris, and hexa adducts.

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