Pergamon

Tetrahedron Letters 39 (1998) 2731–2734

TETRAHEDRON LETTERS

A Highly Water-Soluble Dendro[60]fullerene

Michael Brettreich and Andreas Hirsch*

Institut für Organische Chemie der Universität Erlangen-Nürnberg, Henkestr. 42, D-91054 Erlangen

Received 2 February 1998; accepted 26 February 1998

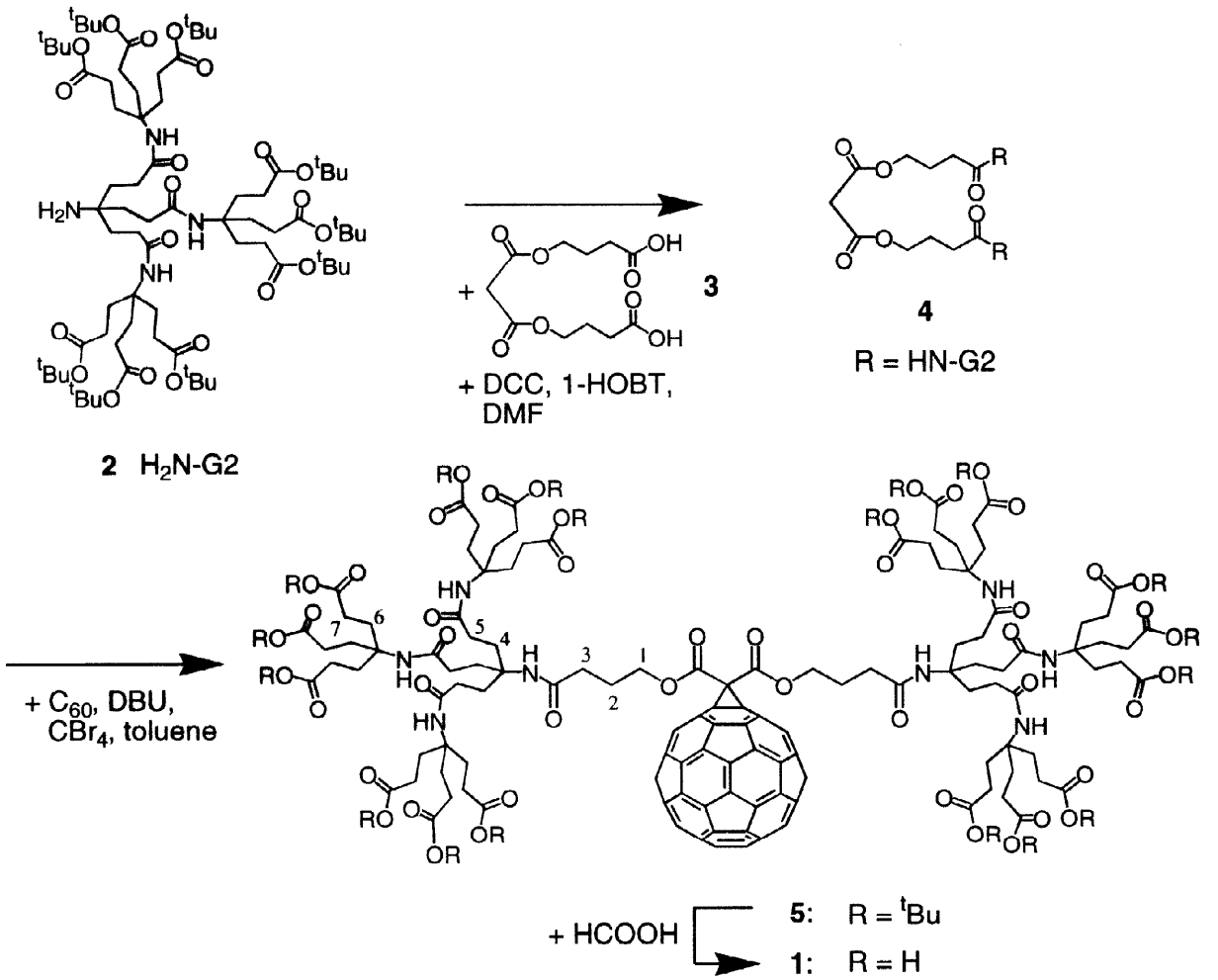
Abstract: The nucleophilic cyclopropanation of C_{60} with a second generation bis(polyamide)-malonate dendrimer and the subsequent deprotection of the terminal *t*-butyl groups provides a dendro[60]fullerene with 18 carboxylic groups in the periphery. This dendritic monoadduct of C_{60} is highly water-soluble.

The extraordinary radical-scavenger properties[1] of C_{60} are ideal prerequisites for a therapeutical use against several neurodegenerative diseases. This was shown in a landmark paper by Dugan et al.[2] demonstrating that fullerenes are able to act as neuroprotective agents *in vitro* and *in vivo*. To make fullerenes disposable for the organism it is necessary to synthesize water-soluble fullerene derivatives.[3] This is in principle possible *via* the covalent attachment of hydrophilic addends. Whereas hydrophilic polyadducts, like polyamino[1a] or polyhydroxyfullerenes[1b] are ill defined, most of the previously described 'water-soluble' monoadducts are only sparingly soluble in water, form suspensions or require a co-solvent. For their pharmaceutical investigations Dugan et al.[2] used the water-soluble trisadducts C_3-$C_{63}(COOH)_6$ and D_3-$C_{63}(COOH)_6$ which were synthesized for the first time in our group.[4] Compared to other hydrophilic fullerene derivatives the advantage of these tris malonic acid adducts of C_{60} is 1.) their high water-solubility, and at the same time their 2.) well-defined structure and 3.) their stability. However, since the radical scavenging ability and the loading capability of fullerenes decreases with the number of addends already bound[1] it is desirable to use highly water-soluble monoadducts instead of trisadducts for such neuroprotective applications. As a consequence, we decided to develop a dendrimer as addend[5] which contains a sufficient number of carboxylic groups in its periphery. With this strategy we were able to synthesize and characterize the monoadduct **1** which exhibits by far the highest water-solubility known to date.

We decided to use the second generation polyamide **2** (H_2N-G2)[6] as precursor dendron (*Scheme 1*). For these dendrimers we elaborated for the first time a convergent synthesis approach in order to avoid structural defects

0040-4039/98/$19.00
PII: S0040-4039(98)00491-2

with increasing dendritic generations.[7] The precursor dendron **2** was then subjected to a coupling reaction with the *adapter* molecule **3** leading to the didendro malonate **4** which is suitable for the direct nucleophilic cyclopropanation of C_{60} with malonates developed previously in our group.[8]



Scheme 1. Synthesis of the water-soluble dendro[60]fullerene **1**

For this purpose 3.9 g (2.71 mmol) of **2** was stirred for 24 h with 359 mg (1.3 mmol) of the malonate **3** in the presence of 559 mg (2.71 mmol) dicyclohexylcarbodiimide (DCC) and 366 mg (2.71 mmol) 1-hydroxybenzotriazole (1-HOBT) in DMF at room temperature. After chromatography on silica-gel the dendritic malonate **4** was obtained in 47 % yield (1.9 g, 0.61 mmol). The reaction of 1.9 g of **4** with 438.9 mg (0.61 mmol) of C_{60} in the presence of 102 mg (0.67 mmol) DBU and 202 mg (0.61 mmol) CBr_4 in toluene afforded the protected dendro[60]fullerene **5** in 29 % yield. **5** was purified with repeated flash chromatography on silica-gel using toluene then toluene : ethyl acetate (1:1) and finally ether : hexane (6:1) as eluents. Compound **5** is a red-brown amorphous solid which is very soluble in most organic solvents, e.g. ethyl acetate, chloroform, toluene and methanol. The deprotection of **5** was achieved by stirring the ester in formic acid for 12 hours at room

temperature. After removing the formic acid in vacuum the polycarboxylate **1** was obtained as a red-brown powder in quantitative yield. The new compounds **1**, **4** and **5** were completely characterized with ^{1}H NMR-, ^{13}C NMR-, IR-, and UV/Vis spectroscopy as well as by mass spectroscopy (FAB, ESI, MALDI-TOF).[9]

The polycarboxylate is soluble in water and methanol (red solution) and insoluble in the most organic solvents like toluene, THF, acetone, chloroform and DMF. Methanol is not a suitable solvent since it reacts slowly with **1** to give polymethyl esters. Due to the presence of 18 carboxy groups the water-solubility of **1** determined by the intensity of the 257 nm absorption of saturated solutions is very high. In a buffer-solution at pH 7.4 at least 34 mg of the acid is soluble. This corresponds to an amount of 8.7 mg C_{60} per mL water. In basic solution the solubility is much higher. An amount of at least 254 mg/mL of **1** is soluble at pH 10 which corresponds to an equivalent of 64.7 mg of C_{60} per mL.

Guldi et al.[10] reported that for water-soluble C_{60}-derivatives a considerable broadening of the UV/Vis bands especially the 425 nm absorption occurs. A similar phenomenon was observed for other water-soluble monoadducts of C_{60}.[3e,4] Without providing a quantitative analysis like measuring the cluster sizes it was concluded[10] that this is due to a pronounced aggregation behaviour. The water-soluble dendro fullerene **1**, however, exhibits a UV/Vis spectrum which is almost identical to that of its protected precursor **5** displaying the characteristic sharp absorption at 425 nm. To find out whether there is also aggregation involved in water solutions of **1** we carried out dynamic light scattering measurements, which provide a direct measure of the cluster size. Indeed, two types of clusters were detected with this technique, although the electronic absorption spectra of water-solutions of **1** are reminescent of a non-aggregated monoadduct. At pH = 8 21.5 % of **1** consists of small aggregates with an average hydrodynamic radius of 10.4 nm whereas the rest is organized in larger aggregates with an average hydrodynamic radius of 37.8 nm. At pH = 11 the amount of the smaller clusters is higher (30.0 %) and at the same time their hydrodynamic radius decreases to 5.0 nm whereas those of larger clusters (33.8 nm) is less affected by the pH change. We are currently investigating the question on the influence of the nature of the dendritic addends on the aggregation behaviour.

Acknowledgements: We thank the BMBF for financial support and Dr. M. Weißmüller from the Hoechst AG for the light scattering measurements.

References and Notes.

1. a) Krusic, P. J.; Wasserman, E.; Keizer, P. N.; Morton, J. R.; Preston, K. F. *Science* **1991**, *254*, 1183; b) Hirsch, A. *The Chemistry of the Fullerenes*, Thieme, Stuttgart 1994.
2. Dugan, L. L.; Turetsky, D. M.; Du, C.; Lobner, D.; Wheeler, M.; Almli, C. R.; Shen, C. K.-F.; Luh, T.-Y.; Choi, D. W.; Lin, T.-S. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 9434.
3. a) Wudl, F.; Hirsch, A.; Khemani, K.; Suzuki, T.; Allemand, P. M.; Koch, A.; Eckert, H.; Srdanov, G.; Webb, H. M. *ACS Symp. Ser.* **1992**, *481*, 161; b) Chiang, L. Y.; Wang, L.-Y.; Swirczewski, J. W.; Soled,

S.; Cameron, S. *J. Org. Chem.* **1994**, *59*, 3960; c) Sjibesma, R. P.; Srdanov, G.; Wudl, F.; Castoro, J. A.; Wilkins, C.; Friedman, S. H.; DeCamp, D. L.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6510; d) Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugiura, Y. *J. Am. Chem. Soc.* **1993**, *115*, 7918; e) Da Ros, T.; Prato, M.; Novello, F.; Maggini, M.; Banfi, E. *J. Org. Chem.* **1996**, *61*, 9070; f) Sun, Y.-P.; Liu, B.; Moton, D. K. *J. Chem. Soc., Chem. Comm.* **1996**, 2699; g) Boutorine, A. S.; Tokuyama, H.; Takasugi, M.; Isobe, H.; Nakamura, E.; Hélène, C. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2462; h) An, Y.-Z.; Chen, C.-H. B.; Anderson, J. L.; Sigman, D. S.; Foote, C.; Rubin, Y. *Tetrahedron* **1996**, *52*, 5179.

4. Lamparth, I.; Hirsch, A. *J. Chem. Soc., Chem. Commun.* **1994**, 1727.
5. For water-insoluble dendritic fullerenes see for example: a) Nierengarten, J.-F.; Habicher, T.; Kessinger, R.; Cardullo, F.; Diederich, F.; Gramlich, V.; Gisselbrecht, J.-P.; Boudon, C.; Gross, M. *Helv. Chim. Acta.* **1997**, *80*, 2238; b) Camps, X.; Schönberger, H.; Hirsch A. *Chem. Eur. J.* **1997**, *3*, 561.
6. Newkome, G. R.; Nayak, A.; Behera, R. K.; Moorefield, C. N.; Baker, G. R. *J. Org. Chem.* **1992**, *57*, 358.
7. The convergent synthesis of **2** and its higher generation analogues will be reported in detail elsewhere.
8. Camps, X.; Hirsch, A. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1595.
9. Spectroscopic data for **4:** ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ (ppm) 1.43 (s, ^{t}Bu, 162 H), 1.94 (m, H-5, H-7, H-2, 52 H), 2.19 (m, H-4, H-6, H-3, 52 H), 3.44 (s, malonic CH_2, 2 H), 4.20 (t, H-1, 4 H), 6.05 (s, NH, 6 H), 7.43 (s, NH, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ (ppm) 24.46, 28.03, 29.72, 29.77, 31.51, 31.56, 32.84, 41.34, 57.36, 57.58, 64.68, 80.45, 166.94, 171.77, 172.61, 172.70; FAB-MS, m/z 3252 ($(M + Cs)^+$), 3143 ($(M + Na)^+$), 3120 (M^+); FT-IR (KBr) $\tilde{\nu}$/cm^{-1} 3333, 2978, 2935, 1734, 1656, 1539, 1458, 1421, 1392, 1368, 1317, 1256, 1215, 1157, 1033, 955, 921, 849, 758, 590, 462, 432. **5:** ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ (ppm) 1.43 (s, ^{t}Bu, 162 H), 1.95 (m, H-5, H-7, 48 H), 2.19 (m, H-4, H-6, H-2, 52 H), 2.36 (t, H-3, 4 H), 4.56 (t, H-1, 4 H), 6.13 (s, NH, 6 H), 7.53 (s, NH, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ (ppm) 24.40, 28.09, 29.75, 31.34, 31.55, 32.66, 52.34, 57.42, 57.693, 66.89, 71.56, 80.45, 138.97, 140.94, 141.89, 142.19, 142.95, 143.03, 143.85, 144.65, 144.84, 145.13, 145.17, 145.24, 145.38, 163.62, 171.44, 172.63, 172.75; FAB-MS, m/z 3971 ($(M + Cs)^+$); ESI-MS, m/z 1942 ($(M - H + Na)^{2+}$); FT-IR (KBr) $\tilde{\nu}$/cm^{-1} 3375, 2977, 2929, 1731, 1656, 1540, 1457, 1392, 1367, 1316, 1254, 1154, 1103, 955, 849, 758, 527; UV-Vis ($CHCl_3$) λ_{max} (ε) 258 (98000), 325 (34000), 425 nm (2800); **1:** ^{1}H NMR (400 MHz, D_2O + K_2CO_3, 25 °C) δ (ppm) 1.91 (m, H-5, H-7, 48 H), 2.13 (m, H-4, H-6, H-2, 52 H), 2.47 (t, H-3, 4 H), 4.78 (t, H-1, 4 H); ^{13}C NMR (100 MHz, D_2O + K_2CO_3, 25 °C) δ (ppm) 23.96, 30.95, 31.18, 31.64, 31.87, 32.06, 52.76, 58.21, 58.40, 67.62, 71.53, 138.56, 141.54, 141.79, 142.37, 143.26, 143.52, 143.58, 144.11, 144.47, 145.07, 145.17, 145.22, 145.26, 145.35, 145.60, 145.71, 165.79, 171.45, 174.51, 175.41, 182.78; MALDI-TOF, m/z 2830 (M^+); ESI-MS, m/z 1415 (M^{2+}); FT-IR (KBr) $\tilde{\nu}$/cm^{-1} 3344, 3074, 2925, 2854, 2625 (broad), 1712, 1654, 1542, 1458, 1414, 1268, 1231, 1206, 1103, 908, 814, 669, 527; UV-Vis (H_2O) λ_{max} (ε) 257 (103000), 325 (30000), 425 (2100);
10. Guldi, D. M. *Res. Chem. Intermed.* **1997**, *23*, 653.