Synthesis and Water Solubility of Novel Fullerene Bisadduct Derivatives

Susanna Bosi,[a] Luigi Feruglio,[a] Dragana Milic,[a] and Maurizio Prato*[a]

Keywords: Fullerenes / Cycloadditions / Synthetic methods

The synthesis of four classes of bisadduct derivatives of C_{60} is reported. The solubility of the new compounds in aqueous solvents is enhanced and is among the highest ever reported for fullerene derivatives.

Introduction

Many 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 due to the specific shape and dimensions of the C_{60} sphere, which seems to be perfectly accommodated by the hydrophobic cavity of the enzyme active site.[2] The ability of fullerenes to accept electrons and to capture free radical species has also been exploited for the inhibition of redox enzymes such as nitric oxide synthase (NOS) and for the direct neutralization of the excess of reactive oxygen species (ROS), thus providing a possible therapeutic approach for some neurodegenerative disorders.[3] Another interesting feature is that fullerenes can be easily excited by visible light, and the properties of the excited states could produce useful applications in medicinal chemistry. In fact, fullerenes are able to generate singlet oxygen in high yields by energy transfer from the fullerene triplet excited state to molecular oxygen, resulting in promising compounds with antitumor activity. However, the low solubility of fullerenes in aqueous media is a serious obstacle for their biological application. Several strategies have been developed to overcome the natural hydrophobicity of fullerenes: (a) organic functionalization of the spheroid, which allows the synthesis of numerous derivatives with improved solubility in physiological media (e.g. fullerenol, a polyhydroxylated fullerene with an excellent solubility in water[4] or the dendrofullerene synthesized by Brettreich and Hirsch,[5] which possesses a big dendrimeric water-soluble side chain), (b) suspension and/or aggregation mixtures of fullerenes (e.g. by adding small quantities of water/tetrahydrofuran into saturated aromatic solutions of fullerenes[6]) and (c) the preparation of water-soluble supramolecular complexes with macrocyclic host systems such as calixarenes,[7] surfactants, cyclodextrines[8] or artificial lipid membranes.[9]

The chemical functionalization of the fullerene sphere produces a large number of different derivatives that combine the desirable properties of C_{60} with the solubilizing power of the side chains. One of the most versatile addition reactions is the [1,3]-dipolar cycloaddition of azomethine ylides.[10] This reaction takes place in the presence of an aldeheyde and an α-amino acid in refluxing toluene and produces the so-called fulleropyrrolidines, in which the nitrogen and/or the carbon atom in the pyrrolidine ring can be variously functionalized.

However, even with very polar functionalizing chains, the water solubility is often very low, except in a few cases.[11] This very low solubility may be due to aggregation, in which the spheres tend to stick together in micelle-like aggregates.[12] In principle, one way to avoid aggregation and to increase solubility is to produce polyadducts of C_{60}.[13] Although isolation and characterization of such adducts is a very difficult task, many examples have been reported.[14] In the case of fulleropyrrolidines, bisadducts are produced using excess of amino acid and aldehyde and can give rise up to eight regioisomers with symmetrical pyrrolidines (Figure 1).[15a]

The different isomers generated in bisadditions are called *cis* or *trans*, depending on whether the two addends are on the same or opposite hemispheres, respectively, according to the nomenclature introduced by Hirsch.[15b] They can also be divided into three symmetry classes: (i) C_S with one plane of symmetry (*cis*-1, *cis*-2, *trans*-4, *equatorial*), (ii) C_2 with one axis (*trans*-2 and *trans*-3) and (iii) D_{2h} with three axes and three planes (*trans*-1). These symmetries define the number of signals in the ^{13}C NMR spectrum and are therefore diagnostic for the structure. A few complete series of bisadducts have been successfully isolated and characterized on the basis of their spectroscopic and analytical properties.[15] In some cases, the distribution of regioisomers of the bisadducts has been qualitatively correlated with the co-

[a] Dipartimento di Scienze Farmaceutiche, Università di Trieste, Piazzale Europa, 1, 34127 Trieste, Italy
Fax: (internat.) +39-040-52572
E-mail: prato@units.it

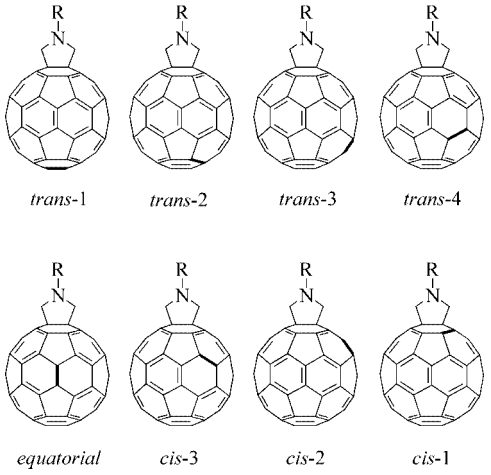


Figure 1. Nomenclature of possible bisadducts

efficients of the frontier orbitals in the corresponding monoadducts: amongst the eight isomers, *equatorial* and *trans*-3 bisadducts are generally formed preferentially, while *trans*-1 is always the least favoured.[15b]

In the present work, we have functionalized C_{60} with polar fragments, such as oligoethylene glycol chains, with positively or negatively charged groups. The aim is the following: the polar chains, distributed more or less homogeneously around the spheroid, should greatly improve the solubility. Furthermore, the presence of negative or positive charges should create a repulsive effect among the spheres, thus preventing aggregation in aqueous solutions.

Results and Discussion

The synthesis of four classes of new water-soluble bisadduct derivatives has been performed (Figure 2). The first family includes bis(pyrrolidines) that possess two terminal ammonium groups, linked to the pyrrolidine core by a long polar chain (Figure 2, a). The second class is similar to the first, but the pyrrolidine nitrogen is now methylated, so that these compounds carry four positive charges, two of them very close to the C_{60} core (Figure 2, b). The third group of compounds is characterized by a pyrrolidine nucleus and a cyclopropane ring (Figure 2, c). The pyrrolidine ring is functionalized with the same polar group as the first family, whereas the cyclopropane ring carries two triethylene glycol chains, able to greatly improve the solubility in polar solvents. The fourth class is similar to the first, but here, carboxylic acid functions (rather than positive ammonium salts) were introduced at the end of the chain (Figure 2, d), because they might exhibit different interactions with biological targets.

In all cases, the separation of the many isomers was difficult and time consuming because of their slight difference in polarity. The best way of purification was based on flash chromatography followed by HPLC. Yields were typically very low (in the range 5−10% relative to the starting material C_{60}) and the main product formed was always the mono adduct (ca. 30−40%). In addition, the formation of polyadducts always took place. The identification of the structures of all the bisadducts relied mainly on the comparison of their visible absorption spectra with those of reference compounds (in agreement with ref.[15b]), rather than standard spectroscopic means.

Bis(fulleropyrrolidines) with oligoethylene side chains were synthesized starting from C_{60}, paraformaldehyde and amino acid **1** (Scheme 1).[16] This reaction led to the monoadduct **2**, bisadducts **3**−**6** and also some trisadduct derivatives. Most of the monoadduct and the excess C_{60} could be removed by flash chromatography. The Boc-protected products were separated by flash chromatography on silica gel, using toluene/EtOAc as the eluent.

The bisadducts were further purified by direct phase HPLC, with toluene/2-propanol (9:1) as eluent. They could then be identified and fully characterized. However, we could only isolate the *trans*-2, *trans*-3, *trans*-4 and *equatorial* isomers in a clean form, because the purification of the *cis* isomers was difficult due to their similar polarity and the *trans*-1 was formed in very small quantity (typically 1−2%). In any case, the *trans* isomers should be much more interesting in terms of solubility in water because the groups are far apart and should allow a better dispersion. To obtain

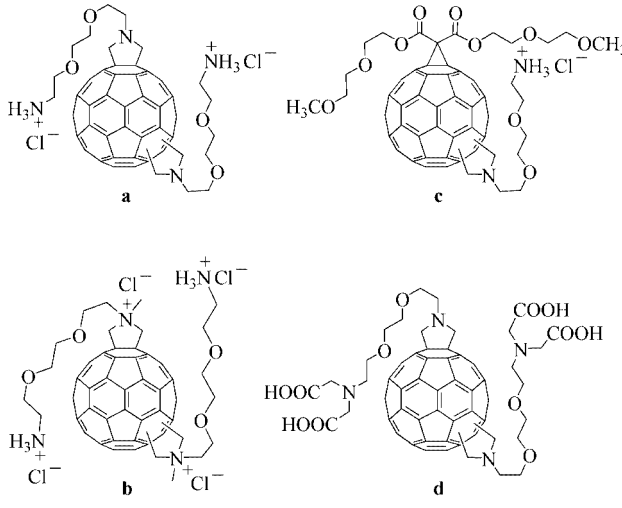
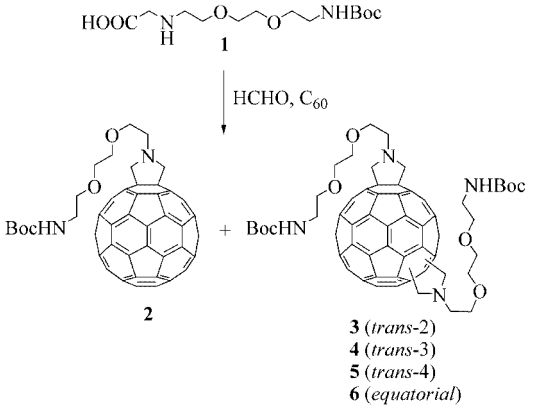
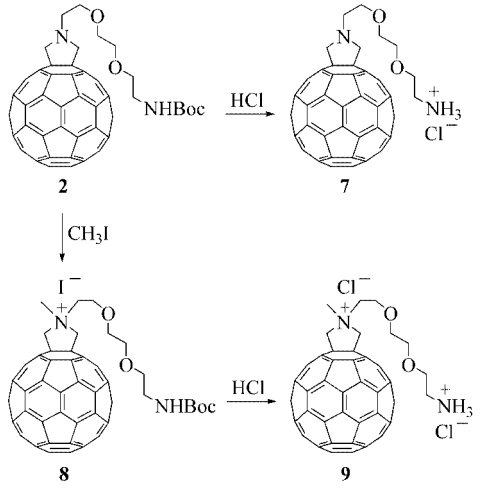


Figure 2. Structure of all compounds synthesized

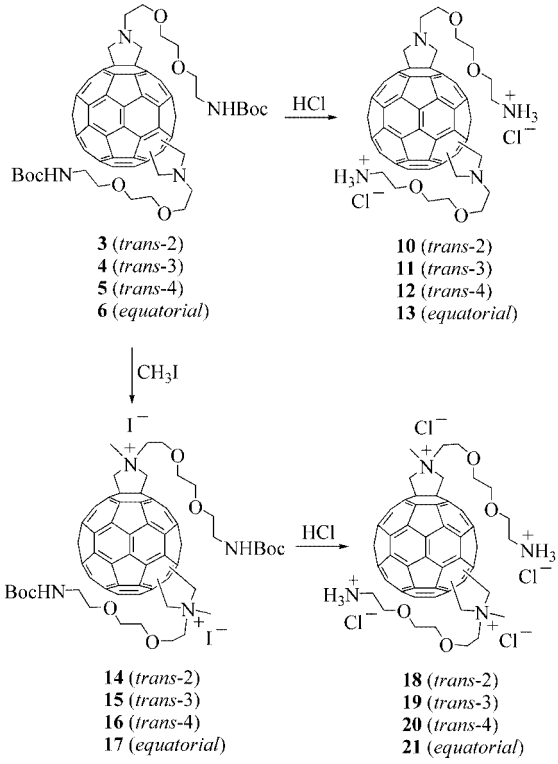


Scheme 1

deprotected monoadduct **7** and bisadducts **10**−**13**, compounds **2** and **3**−**6** were treated with gaseous HCl (Schemes 2 and 3). Alternatively, the same starting materials were first methylated at the pyrrolidine nitrogen with methyl iodide, to give **8** and **14**−**17**, whereas **9** and **18**−**21** were obtained, in turn, by treatment with HCl gas.



Scheme 2



Scheme 3

Two groups of derivatives were therefore obtained, containing two (**10**−**13**) and four (**18**−**21**) positive charges. The monoadduct salts **7** and **9**, and bisadduct salts **10**−**13** are only sparingly soluble in water, whereas the tetrakis salts **18**−**21** exhibit a very high solubility. However, whereas**10**−**13** are single diastereoisomers, **18**−**21** are mixtures of diastereoisomers. This is because, after alkylation, the two pyrrolidine nitrogen atoms become chiral, giving rise to diastereoisomeric mixtures. Characterization by NMR spectroscopy was therefore hampered, but their purity was checked by electrospray mass spectrometry (ES-MS) and reverse-phase HPLC coupled with a diode array detector. An example of a typical HPLC trace is shown in Figure 3.

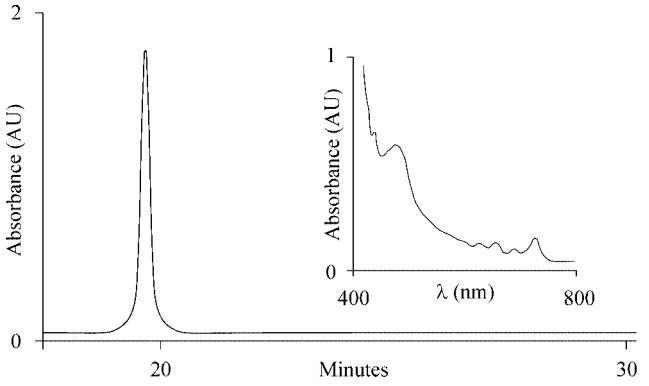


Figure 3. HPLC chromatogram (MeOH/water/acetic acid, 9:0.9:0.1) and UV spectrum (water) of **16**

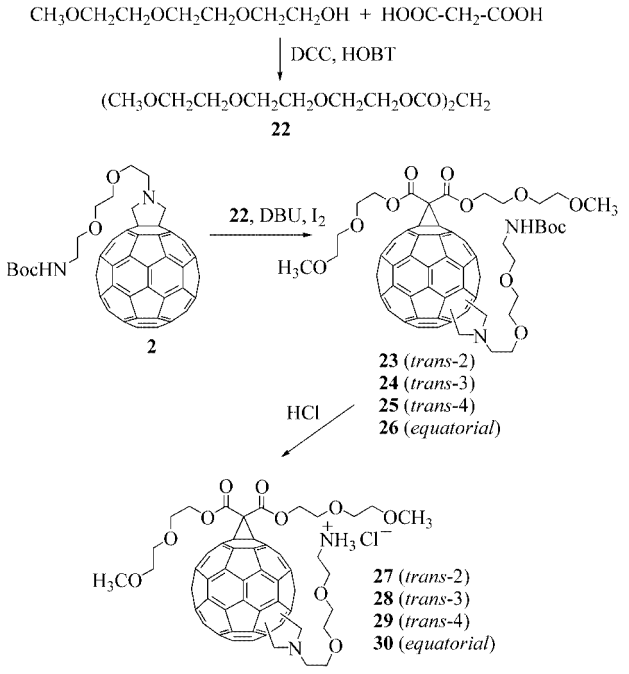
The solubility in water and PBS (phosphate buffered solution, pH 7.4) was determined by UV spectroscopy. Known amounts of all compounds were dissolved in water and/or PBS solutions to prepare the relative standard solutions. A linear calibration line was obtained. Saturated solutions of all compounds were then prepared and centrifuged to obtain clear solutions. After dilution, the solubility values were determined by interpolation using the calibration curve. For the bisadduct salts **10**−**13**, values of the order of 10^{-4} M in water were obtained, while the tetrakis salts **18**−**21** were about 100 times more soluble (Table 1).

Table 1. The solubility of synthesized compounds in water and PBS

Compound	Solubility (M) Water	PBS[a]
7, **9**	$< 1 \times 10^{-4}$	−
10−**13**	1×10^{-4}	−
18−**21**	2×10^{-2}	3×10^{-2}
27−**30**	2×10^{-2}	1.2×10^{-4}
36−**39**	3.3×10^{-4}	1×10^{-2}

[a] PBS − Phosphate buffered solution, pH 7.4.

The family of derivatives **27**−**30** is characterized by a decreased number of positive charges on the spheroid surface, but a good hydrophilicity is maintained with the help of neutral polar chains. These derivatives were synthesized in the following manner. Compound **2** was functionalized with malonic ester derivative **22** in the presence of DBU and iodine, by the Bingel−Hirsch cyclopropanation reaction,[17] to give the adducts **23**−**26**. The Boc protecting group was then cleaved with hydrochloric acid, leading to the formation of the respective ammonium salts **27**−**30** (Scheme 4).



Scheme 4

The compounds **27**−**30** are stereochemically homogeneous (since no chiral centers are formed, other than the intrinsic chiral centres of the C_{60} spheroid in some of the addition patterns). All these derivatives are very soluble in water and polar solvents, such as methanol. Their ^{1}H NMR spectra, however, give very broad peaks, both in methanol and water, so the purity was again checked by ES-MS and reverse-phase HPLC (Figure 4). The resulting solubility values in water for these compounds are of the order of 10^{-2} M in pure water and 10^{-4} M in PBS (see Table 1).

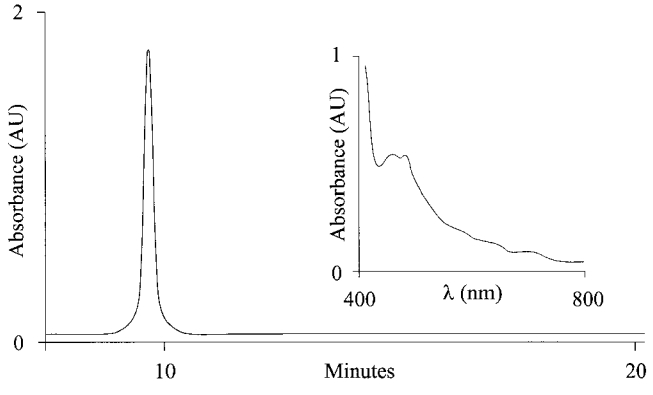
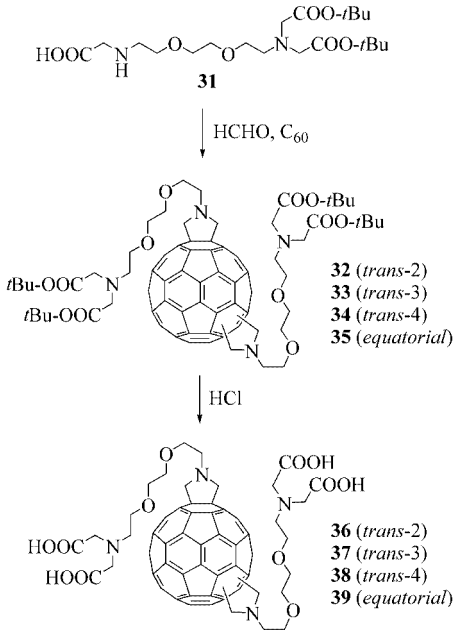


Figure 4. HPLC chromatogram (MeOH/water/acetic acid, 9:0.9:0.1) and UV spectrum (water) of **30**

In the last class of fullerene derivatives (Figure 2, d), negatively charged groups replace the positively charged groups on the polar side chains; in fact, each chain bears two carboxylic acid groups. The amino acid **31**[3a] was treated with paraformaldehyde in the cycloaddition reaction, providing compounds **32**−**35**. Even in this case, it was possible to purify only *trans*-2, -3, -4 and the *equatorial* isomers by flash column chromatography and by HPLC. The Boc protecting groups were removed with HCl as before, giving compounds **36**−**39** (Scheme 5).



Scheme 5

For these products it was possible to obtain good NMR spectra in [D_4]MeOH (Figure 5). Their solubility in water was around 10^{-4} M, but in PBS solution, where the pH is slightly higher, it is 10^{-2} M (Table 1).

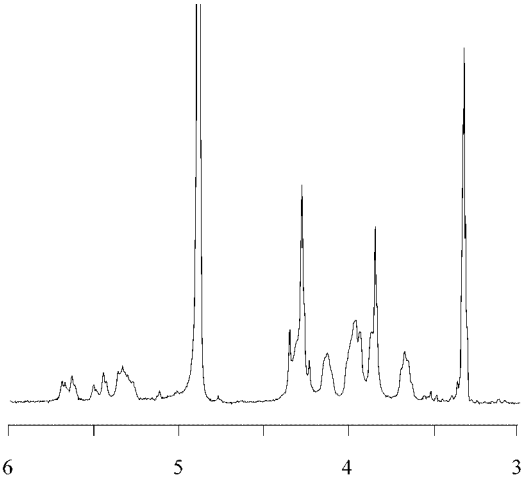


Figure 5. ^{1}H NMR spectrum (CD_3OD) of **38**

All the synthesized compounds are being considered for biological studies. In particular, their cytotoxicity, their anti-HIV activity and their prevention effect in case of glutamate-induced neurocytotoxicity are currently being investigated.

In conclusion, we have synthesized a number of new bisadduct derivatives of C_{60}, bearing positive or negative charges, that are particularly soluble in water or PBS and useful for exploitation in medicinal chemistry.

Experimental Section

General: FT-IR spectra were recorded with a Jasco spectrophotometer FT/IR-200 using NaCl cells (for oils) or KBr powder (DRIFT system). UV spectra were recorded with a Perkin−Elmer Lambda 20 spectrophotometer. ^{1}H and ^{13}C spectra were recorded with a Varian Gemini-200 spectrometer at 200 and 50 MHz, respectively using TMS as internal standard. Chemical shifts are given in parts per million (ppm) relative to that of tetramethylsilane. Mass spectra were taken with 7070H VG Microsomass (MS-EI) and PE SCIEX API 1 (MS-ES) spectrometers and compounds were dissolved in methanol unless otherwise noted. Yields in the azomethine ylide cycloadditions are reported as absolute values without taking into account C_{60} recovery (30−40% of the initial fullerene was routinely recovered). C_{60} was purchased from Bucky-USA (99.5%), and all other reagents and solvents were used as purchased from Fluka, Aldrich, J. T. Baker, and Cambridge Isotope Laboratories. The silica gel NM Kieselgel 60 (0.063−0.200 mm and 0.015−0.04 mm) used in column chromatography was obtained from Macherey−Nagel. Amino acids **1**[16] and **31**[3a] were prepared according to the literature.

The HPLC analysis was conducted with a Waters instrument with a Phenomenex Prodigy 5μ silica 100 A column for the direct phase purifications and with a Phenomenex Luna 5 μ C8(2) column for the reverse phase purifications.

General Procedures

Method A. Azomethine Ylide Cycloadditions: A solution of C_{60} (1.00 g, 1.4 mmol), paraformaldehyde (210 mg, 7.0 mmol) and amino acid (2.8 mmol) in toluene (500 mL) was refluxed for 2 h. After evaporation of the solvent, the residue was chromatographed on a silica gel (0.063−0.200 mm) column. Elution with toluene gave unchanged C_{60} and the mono adduct was eluted with PhMe/EtOAc (9:1). The mixture of all bisadducts was eluted with EtOAc and separated by flash column chromatography on fine SiO_2 (0.015−0.040 mm) using the solvents indicated below. Finally, each compound was purified by precipitation.

Method B. Boc Deprotection: Gaseous HCl was bubbled through a cooled suspension of the Boc derivative (0.1 mmol) in methanol (100 mL) for 20 minutes and the clear solution thus obtained stirred at room temperature for an additional two hours. After evaporation of the solvent, the product was precipitated from methanolic solution with diethyl ether. All the yields were quantitative.

Method C. Methylation: MeI (10 mL) was added to a solution of the fulleropyrrolidine (0.1 mmol) in chloroform (5 mL), and reaction mixture was kept at 80 °C in a screw-topped vial for 24 h. After evaporation of the solvent, the product was precipitated from methanolic solution with diethyl ether. All the yields were quantitative.

Fulleropyrrolidines 2−6 (Method A): The bisadducts were separated by eluting with following solvents: PhMe/EtOAc (9:1, *trans*-1), PhMe/EtOAc (8:2, *trans*-2), PhMe/EtOAc (7:3, *trans*-3), PhMe/EtOAc (7:3 + 1% *i*PrOH, *trans*-4), and PhMe/EtOAc (7:3 + 5% *i*PrOH, *equatorial*). Each compound was precipitated from CH_2Cl_2 solution with MeOH and purity was checked by HPLC on silica gel.

2: 799.1 mg (57.9%). The monoadduct was identical to a reference compound.[16]

3 (*trans*-2): 163 mg (4.62%). $C_{86}H_{52}N_4O_8$; *MW* = 1269.36. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 5.1 (br. s, 2 H), 4.75 (d, *J* = 9.52 Hz, 4 H), 4.5 (d, *J* = 9.52 Hz, 4 H), 4.4 (t, *J* = 6.1 Hz, 4 H), 4.09 (t, *J* = 5.86 Hz, 4 H), 3.86−3.64 (m, 8 H), 3.60 (t, *J* = 4.76 Hz, 4 H), 3.44−3.22 (m, 4 H), 1.45 (s, 18 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 159.07, 155.96, 153.38, 152.59, 148.43, 147.71, 147.14, 147.02, 146.41, 146.27, 146.17, 146.05, 145.69, 145.64, 145.33, 145.14, 144.21, 143.78, 143.68, 142.56, 142.39, 142.34, 141.55, 141.44, 139.58, 134.50, 133.74, 79.20, 70.83, 70.62, 69.54, 69.39, 68.82, 68.70, 54.36, 29.83, 28.67, 28.55, 28.46, 22.83 ppm ES-MS: *m*/*z* = 1268 [M$^+$]. IR (KBr): ν̃ = 3343, 2921, 1706, 1511, 1167 cm^{-1}. UV (THF): λ = 301, 430, 475, 654, 726 nm.

4 (*trans*-3): 291 mg (8.2%). $C_{86}H_{52}N_4O_8$; *MW* = 1269.36. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.08 (br. s, 2 H), 4.42 (dd, *J* = 9.52, 9.16 Hz, 4 H), 4.10 (dd, *J* = 14.1, 9.16 Hz, 4 H), 3.90 (t, *J* = 5.13 Hz, 4 H), 3.82−3.62 (m, 8 H), 3.58 (t, *J* = 5.49 Hz, 4 H), 3.38−3.20 (m, 4 H), 1.43 (s, 18 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 160.82, 158.41, 155.98, 155.78, 155.72, 155.06, 149.08, 148.90, 148.85, 148.27, 148.20, 146.64, 145.32, 145.23, 145.17, 144.93, 144.67, 143.97, 143.64, 142.55, 141.58, 141.50, 141.27, 141.04, 139.81, 136.43, 136.31, 135.54, 79.34, 70.59, 70.52, 70.50, 70.45, 69.92, 67.81, 29.84, 28.66, 28.57, 28.52, 28.47 ppm. ES-MS: *m*/*z* = 1268 [M$^+$]. IR (KBr): ν̃ = 3349, 2923, 1705, 1513, 1118 cm^{-1}. UV (THF): λ = 375, 418, 462, 490, 629, 722 nm.

5 (*trans*-4): 197 mg (5.6%). $C_{86}H_{52}N_4O_8$, *MW* = 1269.36. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.09 (br. s, 2 H), 4.40 (d, *J* = 8.46 Hz, 2 H), 4.23 (d, *J* = 8.46 Hz, 4 H), 4.20 (m, 4 H), 3.90 (t, *J* = 5.13 Hz, 4 H), 3.81−3.62 (m, 8 H), 3.56 (t, *J* = 5.13 Hz, 4 H) 3.38−3.26 (m, 8 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 156.00, 154.77, 152.73, 151.52, 150.85, 149.24, 148.29, 147.95, 147.65, 147.49,147.02, 146.25, 146.12, 145.53, 144.99, 144.67, 142.82, 142.63, 142.15, 141.77, 141.42, 141.25, 139.15, 138.59, 136.08, 135.52, 135.42, 131.19, 81.07, 79.33, 79.11, 70.57, 70.43, 69.68, 69.43, 69.41, 68.66, 68.63, 67.99, 54.32, 40.51, 32.07, 29.85, 29.70, 29.51, 28.61, 28.52, 22.85 ppm. ES-MS: *m*/*z* = 1268 [M$^+$]. IR (KBr): ν̃ = 3363, 2923, 1705, 1513, 1108 cm^{-1}. UV (THF): λ = 411, 454, 640, 708 nm.

6 (*equatorial*): 317.7 mg (9.2%). $C_{86}H_{52}N_4O_8$; *MW* = 1269.36. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.07 (br. s, 2 H), 4.25 (d, *J* = 5.56 Hz, 2 H), 4.15 (s, 2 H), 3.95 (d, *J* = 5.56 Hz, 2 H), 3.90 (t, *J* = 5.49 Hz, 4 H), 3.80−3.61 (m, 8 H), 3.61−3.51 (m, 4 H), 3.73−3.22, (m, 4 H), 3.22−3.07 (m, 4 H), 1.40 (s, 18 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 160.63, 159.13, 157.45, 155.99, 153.68, 153.14, 152.94, 152.74, 150.72, 148.87, 148.34, 148.05, 147.74, 147.22, 146.73, 145.73, 145.51, 145.16, 145.00, 144.66, 144.39, 143.69, 143.23, 142.29, 141.77, 140.68, 139.17, 138.68, 137.75, 137.49, 136.91, 136.08, 135.53, 132.90, 129.93, 128.64, 79.69, 79.33, 70.64, 70.56, 70.43, 70.35, 69.98, 69.83, 69.71, 69.66, 69.42, 68.50, 61.59, 56.51, 55.04, 45.38, 36.50, 32.56, 29.86, 29.81, 28.64, 28.59, 27.27, 26.39 ppm. ES-MS: *m*/*z* = 1268 [M$^+$]. IR (KBr): ν̃ = 3347, 2918, 1704, 1511, 1167 cm^{-1}. UV (THF): λ = 395, 421, 547, 619 nm.

Mono-Adduct Salt 7 (Method B): 93.4 mg. $C_{68}H_{19}ClN_2O_2$; *MW* = 931.34. ES-MS: *m*/*z* = 895 [M$^+$].

Compound 8 (Method C): 113.1 mg. $C_{74}H_{29}IN_2O_4$; *MW* = 1136.94. ES-MS: *m*/*z* = 1009 [M$^+$].

Fulleropyrrolidines 9−13 (Method B)

9 (Monoadduct): 94.3 mg. $C_{69}H_{22}Cl_2IN_2O_2$; *MW* = 1108.74. ES-MS: *m*/*z* = 912 [M^+], 455 [MH^{2+}/2].

10 (*trans*-2): 89.5 mg. $C_{76}H_{38}Cl_2N_4O_4$; *MW* = 1142.05. ES-MS: *m*/*z* = 1072 [MH^+], 536 [MH_2^{2+}/2].

11 (*trans*-3): 89.5 mg. $C_{76}H_{38}Cl_2N_4O_4$; *MW* = 1142.05. ES-MS: *m*/*z* = 1072 [MH^+], 536 [MH_2^{2+}/2].

12 (*trans*-4): 89.5 mg. $C_{76}H_{38}Cl_2N_4O_4$; *MW* = 1142.05. ES-MS: *m*/*z* = 1072 [MH^+], 536[MH_2^{2+}/2].

13 (*equatorial*): 89.5 mg. $C_{76}H_{38}Cl_2N_4O_4$; *MW* = 1142.05. ES-MS: *m*/*z* = 1072 [MH^+], 536[MH_2^{2+}/2].

Compounds 14−17 (Method C)

14 (*trans*-2): 121.0 mg. $C_{88}H_{58}I_2N_4O_8$; *MW* = 1553.23. ES-MS: *m*/*z* = 1300 [M^+], 649 [M^{2+}/2]

15 (*trans*-3): 120.5 mg. $C_{88}H_{58}I_2N_4O_8$; *MW* = 1553.23. ES-MS: *m*/*z* = 1300 [M^+], 649 [M^{2+}/2]

16 (*trans*-4): 121.0 mg. $C_{88}H_{58}I_2N_4O_8$; *MW* = 1553.23. ES-MS: *m*/*z* = 1300 [M^+], 649 [M^{2+}/2]

17 (*equatorial*): 121.0 mg. $C_{88}H_{58}I_2N_4O_8$, *MW* = 1553.23. ES-MS: *m*/*z* = 1300 [M^+], 649 [M^{2+}/2]

Compounds 18−21 (Method B)

18 (*trans*-2): 91.6 mg. $C_{78}H_{44}Cl_4N_4O_4$; *MW* = 1243.02. ES-MS: *m*/*z* = 1102 [M^+], 551 [M^{2+}/2], 275 [MH_2^{4+}/4].

19 (*trans*-3): 92.0 mg. $C_{78}H_{44}Cl_4N_4O_4$; *MW* = 1243.02. ES-MS: *m*/*z* = 1102 [M^+], 551 [M^{2+}/2], 275 [MH_2^{4+}/4].

20 (*trans*-4): 91.5 mg. $C_{78}H_{44}Cl_4N_4O_4$; *MW* = 1243.02. ES-MS: *m*/*z* = 1102 [M^+], 551 [M^{2+}/2], 275 [MH_2^{4+}/4].

21 (*equatorial*): 92.0 mg. $C_{78}H_{44}Cl_4N_4O_4$; *MW* = 1243.02. ES-MS: *m*/*z* = 1102 [M^+], 551 [M^{2+}/2], 275 [MH_2^{4+}/4].

Malonic Diester 22: A solution of dimalonic acid (1.00 g, 9.6 mmol) and DCC (4.35 g, 21 mmol) in dichloromethane (100 mL) was stirred (with cooling in an ice bath) for 30 minutes. The ice bath was removed, triethylene glycol mono methyl ether (3.46 g, 21 mmol) and 4-(dimethylamino)pyridine (2.57 g, 21 mmol) added and the reaction was stirred for 4 h. The mixture was then filtered, washed with water and dried with anhydrous Na_2SO_4. After evaporation of the solvent, **22** (3.70 g, 97%) was obtained as yellow sticky oil. **22:** $C_{17}H_{30}O_{10}$; *MW* = = 394.41. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.24−4.15 (m, 4 H), 3.66−6.49 (m, 16 H), 3.49−3.40 (m, 4 H), 3.35 (s, 2 H), 3.28 (s, 6 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 166.41, 97.2, 71.92, 71.90, 70.58, 68.83, 64.60, 59.04, 41.30 ppm.

Compounds 23−26: DBU (200 μL) was added in small portions to a solution of **2** (200 mg, 0.2 mmol), **22** (17.2 mg, 0.22 mmol) and iodine (15.67 mg, 80.22 mmol) in *ortho*-dichlorobenzene (100 mL). After two hours the mixture was filtered and petroleum ether (50 mL) was added in order to decrease the polarity. The mixture was then purified on a silica gel column (eluent: PhMe/EtOAc, 9:1 to PhMe/EtOAc, 9:1 + 1% *i*PrOH). The products thus obtained were finally purified by precipitation from CH_2Cl_2 solution with hexane. The purity of each isomer was checked by HPLC on silica gel.

23 (*trans*-2): 34.2 mg (11.2%). $C_{90}H_{56}N_2O_{14}$; *MW* = 1389.41. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.10 (br. s, 1 H), 4.82 (t, *J* = 4.0 Hz, 2 H), 4.67 (d, *J* = 10.0 Hz, 2 H), 4.57−4.48 (m, 2 H), 4.46−4.42 (m, 2 H), 4.28, (t, *J* = 6.0 Hz, 2 H), 4.08−4.03 (m, 4 H), 3.89−3.46 (m, 24 H), 3.42−3.24 (m, 2 H), 3.37 (s, 3 H), 3.35 (s, 3 H), 1.43 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 164.13, 163.59, 158.27, 155.99, 153.87, 153.20, 148.51, 148.05, 147.22, 145.86, 146.49, 146.32, 146.21, 146.08, 145.48, 145.34, 145.24, 144.83, 144.65, 144.29, 144.05, 143.81, 143.62, 143.31, 143.01, 142.90, 142.78, 142.40, 141.99, 141.55, 141.23, 140.20, 139.84, 139.43, 137.91, 137.34, 137.19, 134.88, 134.48, 128.87, 79.36, 72.00, 71.39, 10.68, 70.48, 70.01, 69.06, 66.20, 59.22, 54.37, 48.57, 28.61 ppm. ES-MS (MeOH/THF): *m*/*z* = 1389 [MH^+]. IR (NaCl): ν̃ = 3340, 2290, 1708, 1513, 1119 cm^{-1}. UV (THF): λ = 301, 380, 403, 430, 474 nm.

24 (*trans*-3): 52.5 mg (17%). $C_{90}H_{56}N_2O_{14}$; *MW* = 1389.41. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.08 (br. s, 1 H), 4.65 (t, *J* = 4.0 Hz, 2 H), 4.56−4.38 (m, 4 H), 4.29 (q, *J* = 8.0 Hz, 4 H), 4.00 (t, *J* = 4.0 Hz, 2 H), 3.88 (t, *J* = 4.0 Hz, 2 H), 3.84−3.43 (m, 24 H), 3.41−3.24 (m, 2 H), 3.37 (s, 3 H), 3.34 (s, 3 H), 1.43 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 163.46, 157.02, 156.00, 155.81, 148.41, 148.07, 148.01, 147.89, 147.41, 147.24, 145.45, 146.37, 146.26, 146.13, 145.94, 145.76, 145.45, 145.27, 145.16, 145.02, 144.59, 144.46, 144.39, 144.30, 144.18, 143.87, 143.72, 143.38, 143.14, 143.07, 143.01, 142.53, 142.00, 141.79, 141.70, 141.45, 140.79, 139.94, 139.72, 139.43, 138.86, 138.00, 136.76, 135.93, 79.33, 72.00, 71.05, 70.82, 70.78, 70.72, 70.68, 70.45, 70.28, 68.91, 68.75, 68.60, 67.94, 66.27, 66.12, 59.17, 54.31, 50.94, 29.61 ppm. ES-MS (MeOH/THF): *m*/*z* = 1389 [MH^+], 1411 [M^+ + Na]. IR (NaCl): ν̃ = 3687, 2927, 1510, 1463, 1113 cm^{-1}. UV (THF): λ = 375, 418, 463 nm.

25 (*trans*-4): 12.8 mg (4.2%). $C_{90}H_{56}N_2O_{14}$; *MW* = 1389.41. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.06 (br. s, 1 H), 4.57 (t, *J* = 6.0 Hz, 2 H), 4.53−4.40 (m, 4 H), 4.40−4.23 (m, 4 H), 3.98 (t, *J* = 6.0 Hz, 2 H), 3.85 (t, *J* = 6.0 Hz, 2 H), 3.81−3.42 (m, 24 H), 3.36 (s, 3 H), 3.35 (s, 3 H), 3.39−3.21 (m, 2 H), 1.43 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 163.65, 155.99, 154.1, 153.9, 151.6, 151.7, 149.1, 148.9, 148.7, 145.9, 144.5, 143.4, 142.3, 142.1, 140.6, 140.5, 138.3, 137.8, 136.2, 136.0, 135.1, 132.4, 132.2, 130.9, 129.2, 128.8, 128.1, 72.0, 70.7, 70.4, 70.1, 68.8, 68.7, 68.2, 66.0, 59.2, 28.6 ppm. ES-MS (MeOH/THF): *m*/*z* = 1389 [MH^+]. IR (NaCl): ν̃ = 3730, 2938, 1738, 1510, 1443, 1108 cm^{-1}. UV (THF): λ = 413, 460 nm.

26 (*equatorial*): 47.3 mg (15.5%). $C_{90}H_{56}N_2O_{14}$; *MW* = 1389.41. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.06 (br. s, 1 H), 4.53−4.42 (m, 2 H), 4.35−4.22 (m, 2 H), 4.22−4.08 (m, 2 H), 3.93 (t, *J* = 6.0 Hz, 2 H), 3.82−3.65 (m, 8 H), 3.65−3.46 (m, 16 H), 3.35 (s, 6 H), 3.21 (t, *J* = 6.0 Hz, 2 H), 1.42 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 163.20, 155.95, 154.17, 154.04, 148.51, 148.42, 148.07, 147.38, 146.93, 146.55, 146.42, 146.32, 145.25, 145.14, 144.55, 144.32, 143.93, 143.83, 143.62, 143.00, 142.29, 142.16.141.60, 141.49, 141.06, 140.02, 137.26, 136.16, 130.93, 128.83, 79.32, 72.00, 71.08, 70.68, 70.41, 68.75, 68.46, 66.04, 59.18, 54.23, 28.60 ppm. ES-MS (MeOH/THF): *m*/*z* = 1389 [MH^+]. IR (NaCl): ν̃ = 3404, 2927, 1742, 1453, 1249, 1112 cm^{-1}. UV (THF): λ = 313, 396, 451 nm.

Compounds 27−30 (Method B): The purity of each isomer was checked by HPLC on a C18 stationary phase.

27 (*trans*-2): 9.4 mg. $C_{85}H_{49}ClN_2O_{12}$, *MW* = 1325.76. ES-MS: *m*/*z* = 1289 [MH^+], 645 [MH_2^{2+}/2].

28 (*trans*-3): 9.4 mg. $C_{85}H_{49}ClN_2O_{12}$, *MW* = 1325.76. ES-MS: *m*/*z* = 1289 [MH^+], 645 [MH_2^{2+}/2].

29 (*trans*-4): 9.3 mg. $C_{85}H_{49}ClN_2O_{12}$, *MW* = 1325.76. ES-MS: *m*/*z* = 1289 [MH^+], 645 [MH_2^{2+}/2].

30 (*equatorial*): 9.4 mg. $C_{85}H_{49}ClN_2O_{12}$, *MW* = 1325.76. ES-MS: *m*/*z* = 1289 [MH^+], 645 [MH_2^{2+}/2].

Compounds 32−35 (Method A): The separation of the bisadducts was performed on a fine silica gel column (eluent: PhMe/EtOAc, 9:1 to PhMe/EtOAc, 9:1 + 1% *i*PrOH. Each compound was precipitated from CH_2Cl_2 solution with hexane.

32 (*trans*-2): 36.0 mg (13.4%). $C_{100}H_{76}N_4O_{12}$; *MW* = 1525.69. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.73 (d, *J* = 10.25 Hz, 2 H), 4.54 (d, *J* = 9.16 Hz, 2 H), 4.40 (t, *J* = 7.32 Hz, 4 H), 4.07 (t, *J* = 5.13 Hz, 4 H), 3.89−3.60 (m, 12 H), 3.49 (s, 8 H), 3.37 (t, *J* = 5.13 Hz, 4 H), 2.97 (t, *J* = 6.23 Hz, 4 H), 1.40 (s, 36 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 170.83, 153.47, 153.27, 152.67, 148.50, 147.89, 147.77, 147.04, 146.43, 146.30, 146.07, 145.75, 145.65, 145.16, 144.23, 143.81, 143.73, 142.65, 142.59, 142.51, 142.41, 141.59, 141.46, 139.61, 134.53, 133.76, 133.17, 80.98, 70.74, 70.66, 69.60, 69.43, 56.84, 38.89, 29.84, 28.37 ppm. ES-MS: *m*/*z* = 1524 [M^+]. IR (KBr): ν̃ = 3729, 3616, 3450, 2920, 1736, 1460, 1146 cm^{-1}. UV (THF): λ = 300, 378, 430, 474 nm.

33 (*trans*-3): 62.0 mg (22.9%). $C_{100}H_{76}N_4O_{12}$; *MW* = 1525.69. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.45 (q, *J* = 8.79 Hz, 4 H), 4.08−4.28 (m, 4 H), 3.95 (t, *J* = 5.49 Hz, 4 H), 3.82−3.65 (m, 12 H), 3.51 (s, 8 H), 3.26 (t, *J* = 5.49 Hz, 4 H), 2.92 (t, *J* = 5.49 Hz, 4 H), 1.40 (s, 36 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 170.79, 158.47, 155.83, 155.77, 155.12, 152.21, 150.52, 149.62, 149.12, 149.06, 148.88, 148.28, 148.18, 146.67, 147.58, 145.31, 145.30, 145.26, 145.17, 144.96, 144.70, 143.97, 143.62, 142.54, 141.50, 141.26, 141.24, 141.06, 139.82, 136.45, 135.54, 80.97, 70.21, 69.93, 32.07, 29.85, 29.81, 28.36, 22.85 ppm. ES-MS: *m*/*z* = 1524 [MH^+]. IR (KBr): ν̃ = 3445, 2923, 2291, 2126, 1736, 1461, 1361, 1145 cm^{-1}. UV (THF): λ = 376, 399, 422, 468 nm.

34 (*trans*-4): 39.0 mg (14.8%). $C_{100}H_{76}N_4O_{12}$; *MW* = 1525.69. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.48−4.18 (m, 8 H), 3.95 (t, *J* = 5.49 Hz, 4 H), 3.80−3.58 (m, 12 H), 3.50 (s, 8 H), 3.21 (t, *J* = 4.13 Hz, 4 H), 2.95 (t, *J* = 5.49 Hz, 4 H), 1.41 (s, 36 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 170.79, 154.86, 153.88, 152.77, 151.55, 150.92, 149.25, 148.27, 147.98, 147.66, 147.54, 146.89, 146.23, 146.12, 146.10, 145.03, 144.68, 143.46, 142.86, 142.60, 142.15, 141.75, 141.40, 141.23, 141.13, 139.13, 135.44, 133.97, 131.19, 80.96, 69.70, 69.43, 32.07, 29.85, 29.78, 29.51, 28.37, 22.86 ppm. ES-MS: *m*/*z* = 1524 [MH^+]. IR (KBr): ν̃ = 3729, 3323, 2941, 1735, 1444, 1256, 1122 cm^{-1}. UV (THF): λ = 413, 460 nm.

35 (*equatorial*): 22.0 mg (8.3%). $C_{100}H_{76}N_4O_{12}$; *MW* = 1525.69. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.20−3.94 (m, 8 H), 3.89, (t, *J* = 5.46 Hz, 4 H), 3.78−3.49 (m, 12 H), 3.46 (s, 8 H), 3.20−3.07 (m, 4 H), 3.00−2.86 (m, 4 H), 1.40 (s, 36 H) ppm. ES-MS: *m*/*z* = 1524 [MH^+]. IR (KBr): ν̃ = 3341, 2925, 1866, 1723, 1459, 1078 cm^{-1}. UV (THF): λ = 307, 396, 451 nm.

Compounds 36−39 (Method B, but using 0.01 mmol of starting material): ES-MS were taken in negative mode in methanol.

36 (*trans*-2): 8.5 mg. $C_{84}H_{44}N_4O_{12}$; *MW* = 1301.27; ES-MS: *m*/*z* = 1299 [$M - H^+$], 648 [$M - 2H^+$/2].

37 (*trans*-3): 8.4 mg. $C_{84}H_{44}N_4O_{12}$; *MW* = 1301.27; ES-MS: *m*/*z* = 1299 [M^{4-}], 648 [$M - 2H^+$/2].

38 (*trans*-4): 8.5 mg. $C_{84}H_{44}N_4O_{12}$; *MW* = 1301.27; ES-MS: *m*/*z* = 1299 [M^{4-}], 648 [$M - 2H^+$/2].

39 (*equatorial*): 8.5 mg. $C_{84}H_{44}N_4O_{12}$; *MW* = 1301.27; ES-MS: *m*/*z* = 1299 [M^{4-}], 648 [$M - 2H^+$/2].

Acknowledgments

This work was supported by the University of Trieste (Fondi 60%), by MIUR (PRIN 2001, prot. N. 2001063932) and by CNR (Agenzia 2000).

[1] [1a] N. Tagmatarchis, H. Shinoara, *Mini Rev. Med. Chem.* **2001**, *4*, 339−353. [1b] S. R. Wilson, *Fullerenes: Chemistry, Physics and Technology* **2000**, 437. [1c] T. Da Ros, M. Prato, *Chem. Commun.* **1999**, 663−669. [1d] A. W. Jensen, S. R. Wilson, D. I. Schuster, *Bioorg. Med. Chem.* **1996**, *4*, 767−779.

[2] [2a] G. L. Marcorin, T. Da Ros, S. Castellano, G. Stefancich, I. Bonin, S. Miertus, M. Prato, *Org. Lett.* **2000**, *2*, 3955−3958. [2b] S. H. Friedman, D. L. DeCamp, R. P. Sijbesma, G. Srdanov, F. Wudl, G. L. Kenyon, *J. Am. Chem. Soc.* **1993**, *115*, 6506−6512. [2c] S. H. Friedman, P. S. Ganapathi, Y. Rubin, G. L. Kenyon, *J. Med. Chem.* **1998**, *41*, 2424−2429.

[3] [3a] C. Cusan, T. Da Ros, G. Spalluto, S. Foley, J. M. Janot, P. Seta, C. Larroque, M. C. Tomasini, T. Antonelli, L. Ferraro, M. Prato, *Eur. J. Org. Chem.* **2002**, 17, 2928−2934. [3b] D. J. Wolff, D. Mialkowski, K. Richardson, S. R. Wilson, *Biochemistry* **2001**, *40*, 37−45. [3c] L. L. Dugan, M. Turetsky, C. Du, D. Lobner, M. Wheeler, C. R. Almli, C. K. F. Shen, T. Y. Luh, D. W. Choi, S. T. Lin, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 9434−9437.

[4] L. Y. Chiang, R. B. Upasani, J. W. Swirczewski, *J. Am. Chem. Soc.* **1992**, *14*, 10154−10157.

[5] M. Brettreich, A. Hirsch, *Tetrahedron Lett.* **1998**, *39*, 2731−2734.

[6] W. A. Scrivens, J. M. Tour, *J. Chem. Soc., Chem. Commun.* **1993**, 1207−1209.

[7] A. Ikeda, S. Nobukuni, H. Udzu, Z. L. Zhong, S. Shinkai, *Eur. J. Org. Chem.* **2000**, *19*, 3287−3293.

[8] [8a] S. Samal, K. E. Geckeler, *Chem. Commun.* **2000**, 1101−1102. [8b] S. Filippone, F. Heimann, A. Rassat, *Chem. Commun.* **2002**, *21*, 1508−1509.

[9] M. Hetzer, S. Bayerl, X. Camps, O. Vostrowsky, A. Hirsch, T. M. Bayerl, *Adv. Mater.* **1997**, *9*, 913−914.

[10] [10a] N. Tagmatarchis, M. Prato, *Synlett* **2003**, *6*, 768−779. [10b] M. Maggini, G. Scorrano, M. Prato, *J. Am. Chem. Soc.* **1993**, *115*, 9798−9799. [10c] M. Prato, M. Maggini, *Acc. Chem. Res.* **1998**, *31*, 519−526.

[11] T. Da Ros, M. Prato, F. Novello, M. Maggini, E. Banfi, *J. Org. Chem.* **1996**, *61*, 9070−9072.

[12] V. Georgakilas, F. Pellarini, M. Prato, D. M. Guldi, M. Melle-Franco, F. Zerbetto, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5075−5080.

[13] I. Lamparth, A. Hirsch, *J. Chem. Soc., Chem. Commun.* **1994**, 1727−1728.

[14] [14a] A. Hirsch, *Top. Curr. Chem.* **1999**, *199*, 1−65. [14b] F. Diederich, R. Kessinger, *Acc. Chem. Res.* **1999**, *32*, 537−545.

[15] [15a] K. Kordatos, S. Bosi, T. Da Ros, A. Zambon, V. Lucchini, M. Prato, *J. Org. Chem.* **2001**, *66*, 2802−2808. [15b] A. Hirsch, I. Lamparth, H. R. Karfunkel, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 437−438. [15c] Y. Nakamura, K. Okawa, M. Matsumoto, J. Nishimura, *Tetrahedron* **2000**, *56*, 5429−5434.

[16] K. Kordatos, T. Da Ros, S. Bosi, E. Vàzquez, M. Bergamin, C. Cusan, F. Pellarini, V. Tomberli, B. Baiti, D. Pantarotto, V. Georgakilas, G. Spalluto, M. Prato, *J. Org. Chem.* **2001**, *66*, 4915−4920.

[17] [17a] C. Bingel, *Chem. Ber.* **1993**, *126*, 1957−1959. [17b] A. Hirsch, *Synthesis* **1995**, *8*, 895−913.

Received August 4, 2003
Early View Article
Published Online October 23, 2003

