DOI: 10.1002/chem.200501481

Photoaddition of N-Substituted Piperazines to C_{60}: An Efficient Approach to the Synthesis of Water-Soluble Fullerene Derivatives

Olesya A. Troshina,[a] Pavel A. Troshin,*[a] Alexander S. Peregudov,[b] Viacheslav I. Kozlovski,[c] and Rimma N. Lyubovskaya[a]

Dedicated to Professor Roger Taylor

Abstract: An oxidative radical photoaddition of mono N-substituted piperazines to [60]fullerene was systematically investigated. Reactions of C_{60} with piperazines bearing bulky electron-withdrawing groups (2-pyridyl, 2-pyrimidinyl) were found to be the most selective and yielded C_{60}(amine)$_4$O as major products along with small amounts of C_{60}(amine)$_2$. In contrast, interactions of fullerene with *N*-methylpiperazine and *N*-(*tert*-butoxycarbonyl)piperazine were found to have low selectivity due to different side reactions. Tetraaminofullerene derivative C_{60}(*N*-(2-pyridyl)piperazine)$_4$O was found to react readily with organic and inorganic acids to yield highly water-soluble salts (solubility approximately 150 mg mL^{-1}). In contrast, C_{60}(*N*-(2-pyrimidinyl)piperazine)$_4$O undergoes hydrolysis under the same conditions and results in a complex mixture of compounds with an average composition of C_{60}(*N*-(2-pyrimidinyl)piperazine)$_2$(OH)$_2$O. Radical photoaddition of *N*-(2-pyridyl)piperazine to fullerene derivatives can be used as a facile route for their transformation into water-soluble compounds. Two model fullerene cycloadducts (a methanofullerene and a pyrrolidinofullerene) were easily converted into mixtures of regioisomers of A = C_{60}(*N*-(2-pyridyl)piperazine)$_4$O (A = cyclic addend) that give highly water-soluble salts under acid treatment.

Keywords: amination · amines · fullerenes · photochemistry

Introduction

Water-soluble fullerene derivatives exhibit a range of exciting biological properties, and are potentially useful for the development of novel drugs.[1–4] Some water-soluble derivatives of C_{60} inhibit quite efficiently enzymes of human immunodeficiency and hepatitis C viruses.[5–7] Several groups have reported the application of fullerene derivatives for photodynamic therapy of cancer[8,9] and as antiproliferative agents.[10] Implication of a "radical sponge" effect of water-soluble fullerenes is promising with respect to design of advanced neuroprotectors.[11] Finally, some fullerene derivatives exhibited bacteriostatic activity[10,12] and can be utilized as contrast agents in X-ray and magnetic resonance imaging.[13,14]

A serious drawback for the development of the biological chemistry of fullerenes is the strongly hydrophobic nature of the fullerene cage. It is necessary to attach at least four to six ionic groups or 24 to 30 hydroxyls to the carbon cage to obtain "truly" water-soluble fullerene derivatives that possess solubility in water above 1 mg mL^{-1}. There are no more than 10 to 15 of such fullerene derivatives with well-defined composition and structure synthesized so far.[1–4] Most of them have five to six organic addends that cover almost the whole fullerene surface, making them less than promising for biological applications.[15–17] Two other derivatives of C_{60} have three malonic acid residues arranged at one fullerene semisphere to give C_3 and D_3 isomers of C_{60}[C(COOH)$_2$]$_3$.[18] The only examples of "truly" water-soluble fullerene deriva-

[a] Dr. O. A. Troshina, Dr. P. A. Troshin, Prof. R. N. Lyubovskaya
Institute of Problems of Chemical Physics
Russian Academy of Sciences, Semenov Prospect 5
Chernogolovka, Moscow region, 142432 (Russia)
Fax: (+7) 496-522-18-52
E-mail: troshin@cat.icp.ac.ru

[b] Prof. A. S. Peregudov
A. N. Nesmeyanov Institute of Organoelement Compounds
1 Vaviliva St. 28, B-334, Moscow, 119991 (Russia)

[c] Dr. V. I. Kozlovski
Institute for Energy Problems of Chemical Physics Russian Academy of Science (Branch) Semenov Prospect 1/10
Chernogolovka, Moscow region, 142432 (Russia)

Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

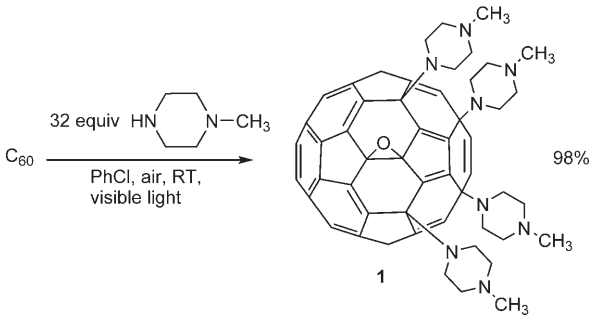
tives bearing one organic addend are compounds with dendrimeric appendages synthesized from corresponding dendrimeric malonates.[19] The most promising is a symmetrical dendro [60]fullerene which has 18 carboxylic groups, producing a solubility of ~30 mg mL^{-1} at pH 7.4 and ~250 mg mL^{-1} at pH 10.[20]

The cycloaddition reactions have relatively low potential for the synthesis of "truly" water-soluble fullerene derivatives, while highly selective radical additions can provide facile synthetic routes to water-soluble compounds. This strategy was exemplified by a three-stage synthesis of fullerene derivatives bearing five glycoside moieties based on five-fold radical addition of a ArMgBr/CuBr•SMe$_2$ reagent to C_{60}.[21] Here we explored the potential of the radical addition of secondary amines to C_{60} for the synthesis of individual aminofullerene derivatives and their subsequent conversion into water-soluble salts.

Results and Discussion

A reaction of C_{60} with amines was among the first discovered for fullerenes. Initially it was claimed to be nuchleophilic and to yield simple addition products $C_{60}H_n(NR_2)_n$.[22] However, no obvious evidence was obtained for the presence of hydrogen atoms attached to the carbon cage in the fullerene derivatives formed.[23] In contrast, a number of reports indicated unambiguous formation of aminofullerene derivatives $C_{60}(NR_2)_n$ under the same conditions.[24,25] Moreover, conduction of these reactions in air under irradiation by visible light resulted in increased product yields and decreased reaction times in comparison with the syntheses under an argon atmosphere whilst heating at reflux.[26–28]

However, a number of individual fullerene derivatives prepared by photochemical addition of amines to C_{60} is quite limited. A range of *N*,*N*′-disubstituted ethylendiamines afford piperazinofullerenes with moderate to high yields;[24,26,27] *N*,*N*′-dimethylpropylendiamine yields a mixture of a corresponding cyclic 1,4-addition product and a cagelike fullerene dimer.[29] Reactions of C_{60} with such cyclic secondary amines as morpholine and piperidine yielded fullerene dimers [1,4-C_{60}amine]$_2$ with low solubility, corresponding 1,4-diaminofullerenes C_{60}(amine)$_2$ and tetraaminofullerenes C_{60}[amine]$_4$O were formed as byproducts with very low yields.[25] Formation of 10 pure tetraaminofullerenes C_{60}-[amine]$_4$O as final products of a photochemical reaction of C_{60} with secondary amines has been claimed previously.[30] However, the NMR spectroscopic data and isolation procedure was provided only for *N*-methylpiperazine adduct **1**, which was obtained in an outstanding 98% yield (Scheme 1).



Scheme 1.

Reactions of C_{60} with *N*-methylpiperazine and morpholine: We precisely reproduced a procedure reported for the synthesis of **1** and obtained a complex mixture of aminofullerene derivatives. This mixture was analyzed by ESI-MS, revealing the presence of molecular ions of protonated compounds C_{60}[*N*-methylpiperazine]$_3$OH (M1), C_{60}[*N*-methylpiperazine]$_5$OH (M2), and C_{60}[*N*-methylpiperazine]$_4$O (M3) in addition to a number of other species (Figure 1a). A change of the light source from a 60 W incandescent bulb to a 15 W Hg medical UV tube and variation of the reagent ratio (C_{60} per amine from 1:5 to 1:100), reaction time (from 5 h to 10 d), and solvent (chlorobenzene, 1,2-dichlorobenzene, benzene and 1,2,4-trichlorobenzene) did not significantly affect the product composition. Moreover, a reaction of C_{60} with morpholine also yielded similar products C_{60}[morf]$_3$OH (M′1), C_{60}[morf]$_4$O (M′2), and C_{60}[morf]$_5$OH (M′3) as was deduced from the ESI mass spectrum (Figure 1b).

The NMR spectra of the crude fullerene *N*-methylpiperazine product also confirmed the presence of several components in the sample (Figure 2). The ^{1}H NMR spectrum exhibited at least five overlapped signals at $\delta = 2.27$–2.43 ppm, corresponding to N–CH_3 fragments in contrast to two equal lines at $\delta = 2.38$ and 2.42 ppm reported for **1**.[21] The ^{13}C NMR spectrum was collected on an 100 MHz instrument within 24 h from 100 mg of the crude product dissolved in 0.7 mL of CS_2/C_6D_{12}. A set of 20–30 signals corresponding to the *N*-methylpiperazine addends of different fullerene derivatives was observed in the $\delta = 45$–60 ppm region; a broad hump formed by overlapped sp^2 fullerene carbon signals was observed in the typical $\delta = 125$–155 ppm range.

Thus, the spectroscopic data indicated quite low selectivity for the photochemical addition of *N*-methylpiperazine and morpholine to C_{60}. We examined the possibility of separating the fullerene derivatives bearing *N*-methylpiperazine addends by column chromatography on silica and alumina. These compounds underwent very strong absorption at the stationary phases and could be eluted from the column only by CH_2Cl_2/pyridine, CH_2Cl_2/Et_3N, CH_2Cl_2/CH_3COOH, or similar mixtures. Such separation procedures resulted in hydrolysis of the aminofullerenes affording fullerenol-like species (C_{60}(amine)$_x$(OH)$_y$O$_z$, $x = \sim 1$–2, $y = \sim 2$–5, $z = \sim 0$–1). These species were confirmed by IR spectroscopy and chemical analysis. Similar hydrolysis reactions slowly occurred when crude C_{60}-*N*-methylpiperazine product was dissolved in an aqueous acid (HCl, CH_3COOH, CF_3COOH) at room temperature. Heating of aqueous acid solutions of these aminofullerenes above 50 °C (particularly on a rotary evaporator) resulted in instantaneous precipitation of C_{60}(*N*-methylpiperazine)$_x$(OH)$_y$O$_z$ (Scheme 2).

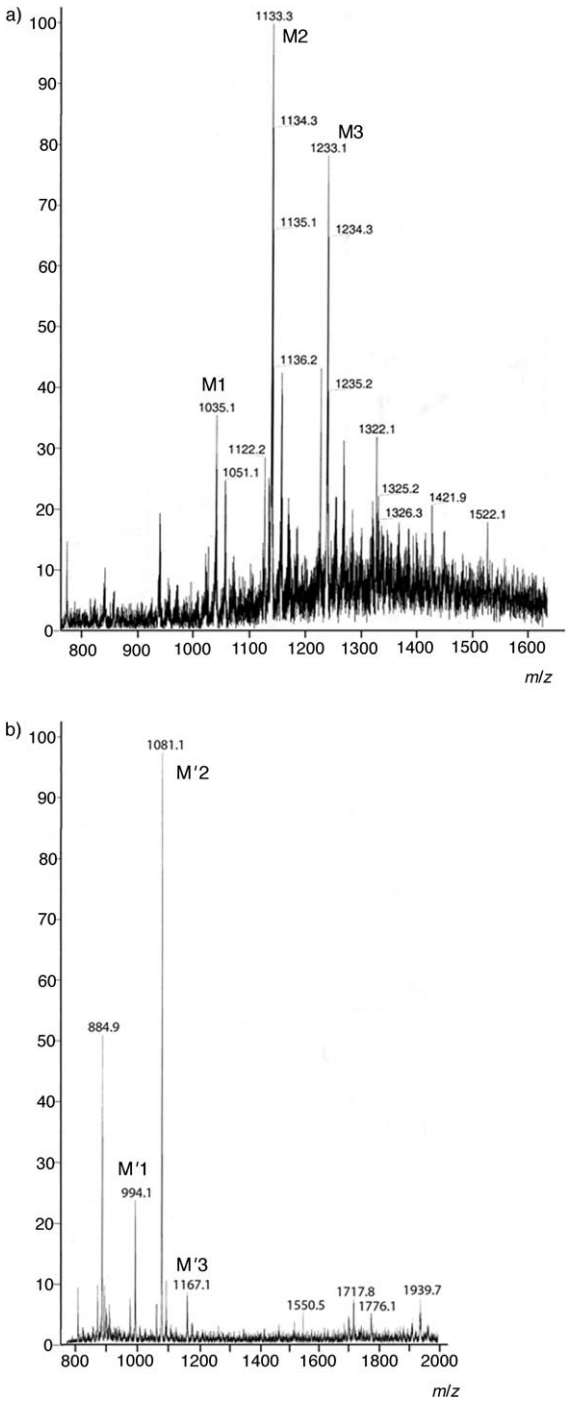


Figure 1. ESI mass spectra of *N*-methylpiperazine-C_{60} (a) and morpholine-C_{60} (b) crude products.

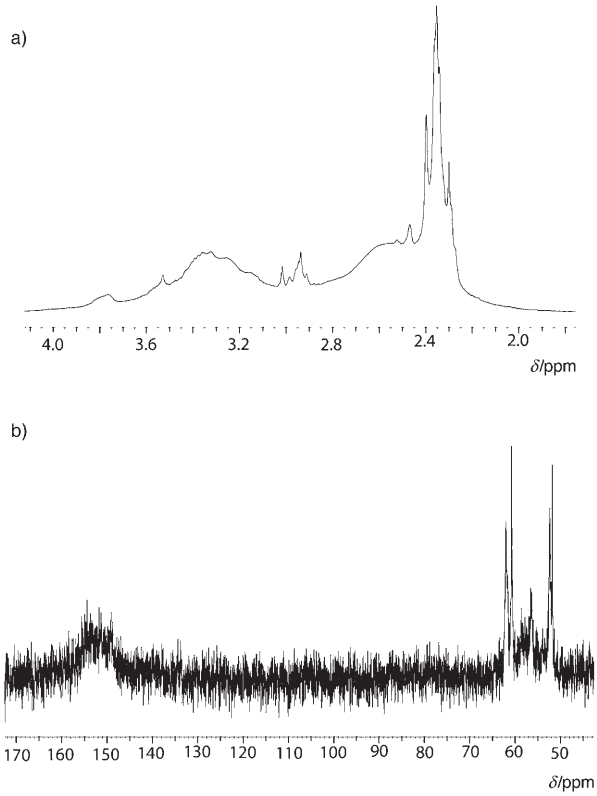
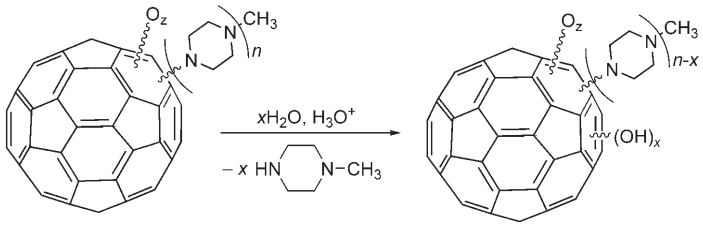


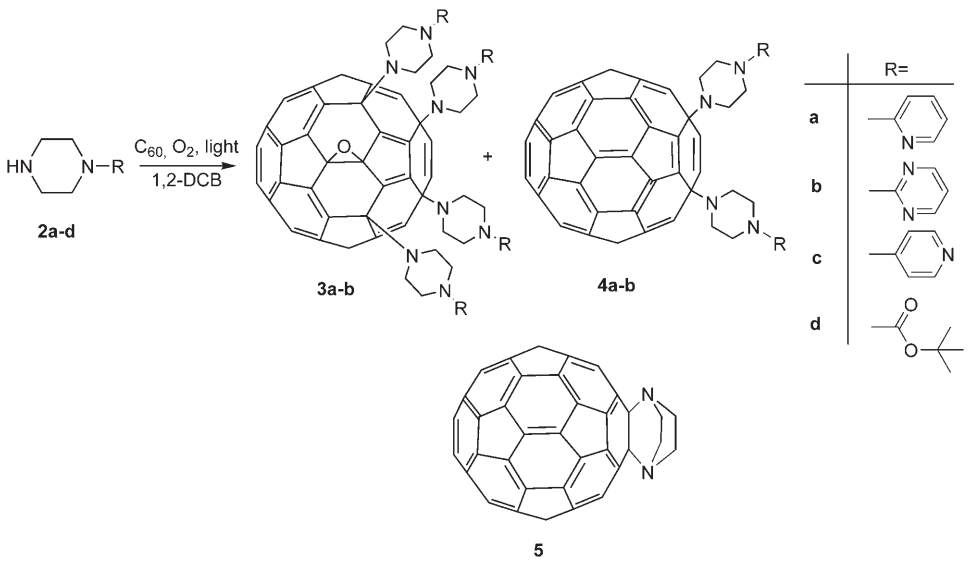
Figure 2. ^{1}H (a) and ^{13}C NMR (b) spectra of *N*-methylpiperazine-C_{60} crude product.



Scheme 2.

Reactions of C_{60} with piperazines 2a–d: Reactions of [60]fullerene with *N*-alkylpiperazines yielded complex mixtures of aminofullerenes that were quite labile towards hydrolysis and therefore are not promising for the preparation of water-soluble fullerene-based compounds. We applied less basic N-substituted piperazines **2a–d** bearing electron-withdrawing bulky groups as starting reagents for the derivatization of C_{60}. Indeed, reactions of **2a–b** with C_{60} were much more selective and afforded tetraaminofullerenenes **3a–b** and diaminofullerenes **4a–b**; these compounds were separated and purified by column chromatography (Scheme 3).

The reaction between fullerene and *N*-(2-pyridyl)piperazine proceeds smoothly and gives 50–70% yields of **3a** (Table 1). A combination of irradiation time, reagent ratio, and scale of the synthesis affects strongly product yields. Irradiation of the reaction mixture within just 10–14 h gives high product yields in the syntheses started from 100–150 mg of C_{60} and large excess of **2a** (20–30 equiv). However, both large-scale syntheses (500–1000 mg of C_{60}) and reactions with a lower excess of **2a** (10 equiv) require longer irradiation (40–50 h) to achieve satisfactory product yields. Yields of byproduct **4a** are generally low (1–5%) and it does not depend strongly on the time of irradiation. Interaction of C_{60} with *N*-(2-pyrimidinyl)piperazine exhibits much



Scheme 3.

Table 1. Experimental conditions and product yields for reactions of C_{60} with piperazines **2a–c**.

Piperazine	Amount of C_{60} [mg] (mmol)	Amount of piperazine [mg] (mmol)	*V* (DCB) [mL]	Mole ratio piperazine/C_{60}	*t* [h]	Yield **3a–c** [%]
2a	100 (0.139)	673 (4.129)	10	30.0	14	53
2a	100 (0.139)	428 (2.626)	10	18.9	30	47
2a	150 (0.208)	326 (2.000)	13	9.6	36	50
2a	200 (0.278)	453 (2.779)	17	10	20	20
2a	200 (0.278)	679.2 (4.167)	16	15	45	59
2a	500 (0.694)	1070 (6.564)	45	9.5	20	29
2a	500 (0.694)	1070 (6.564)	45	9.5	40	72
2a	1000 (1.389)	2300 (14.110)	90	10.0	30	16
2a	950 (1.389)	2152 (13.2)	80	9.5	80	71
2b	200 (0.278)	200 (1.220)	30	4.4	60	11
2b	500 (0.694)	1200 (7.317)	45	10.5	48	15
2b	200 (0.278)	600 (3.659)	20	13.2	24	17
2b	250 (0.347)	600 (3.659)	20	10.5	48	23
2c	106 (0.147)	478.5 (2.94)	20	20.0	24	80

lower selectivity, therefore a typical yield of **3b** is in the range of 15–20%. Compound **4b** was isolated as a byproduct with yields of 5–9%. A major part of the crude product was represented by a complex mixture of fullerene derivatives that could not be separated by column chromatography.

A reaction of C_{60} with piperazine **2c** gave a surprising result as it was accompanied by precipitation of a product with composition C_{60}(*N*-(4-pyridyl)piperazinyl)$_{4.0}$O (**3c**), as was determined by chemical analysis. It was completely insoluble in common organic solvents, preventing its spectroscopic characterization. In contrast to **2a–c**, piperazine **2d** undergoes partial degradation under the reaction conditions, resulting in the formation of piperazinofullerene **5** along with other products; this compound was synthesized previously by starting from unsubstituted piperazine and C_{60}.[24,27] A major part of the product was eluted from the column following **5** as a single fraction; however, this fraction consisted of a number of compounds as revealed from the ^{1}H and ^{13}C NMR spectra. We could not separate this mixture by repeatable chromatography on a silica-gel column with various eluent compositions. Most likely, photochemical cleavage of the *tert*-butoxycarbonyl group in piperazine **2c** results in formation of numerous fullerene derivatives containing both piperazine and *N*-(*tert*-butoxycarbonyl)piperazine unites attached to the carbon cage in different arrangements.

Compositions and structures of aminofullerenes **3a–b** were confirmed by ^{1}H and ^{13}C NMR spectroscopy, ESI-MS, and chemical analysis data. The NMR spectra for compounds **3a** and **4a** are shown in Figure 3. There are a total of 49 signals in the ^{13}C NMR spectrum, proving C_s symmetry of the molecule of **3a**; four resonances at $\delta = 70$–80 ppm correspond to sp^3 carbons of the fullerene cage bearing amine groups and epoxide oxygen.

The C_s-symmetrical structure of 1,4-diaminofullerene **4a** was confirmed by both its ^{1}H and ^{13}C NMR spectra. Unfortunately, it was not possible to obtain a MALDI-TOF mass spectrum of **4a** because of the facile elimination of addends from the fullerene cage under the measurements conditions (regardless of the matrix composition).

Suggested mechanism for the fullerene reaction with secondary amines: It is known that the first stage of interaction between amine and C_{60} is a photoinduced electron transfer that results in the formation of the radical pair $C_{60}^{-\bullet}$ $R_2NH^{+\bullet}$ **I**. (Scheme 4). Following recombination of the fullerene radical anion and amine radical cation affords zwitterionic intermediate **II**.[22] Next, **II** reacts with oxygen to give hydroperoxyde **III**. Similar hydroperoxydes were observed when anion $(CN)C_{60}^-$ was allowed contact with air;[31] there is also some analogy with the reaction of aliphatic R_3C^- with O_2.[32] Intermediate **III** can also react with amine to give zwitterion **IV**. Anionic species bearing hydroperoxide moieties are known to be unstable and undergo degradation by elimination of a hydroxyl anion.[33] In this case, elimination of OH^- should be accompanied by amine deprotonation and loss of

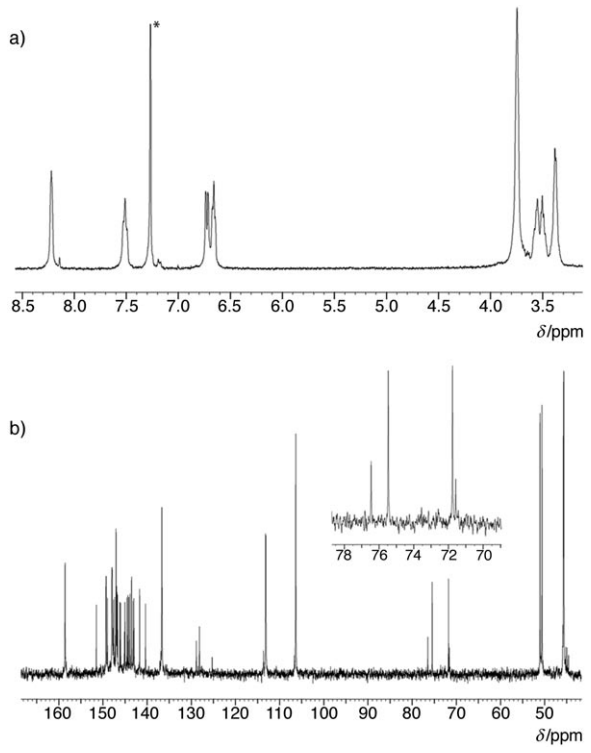
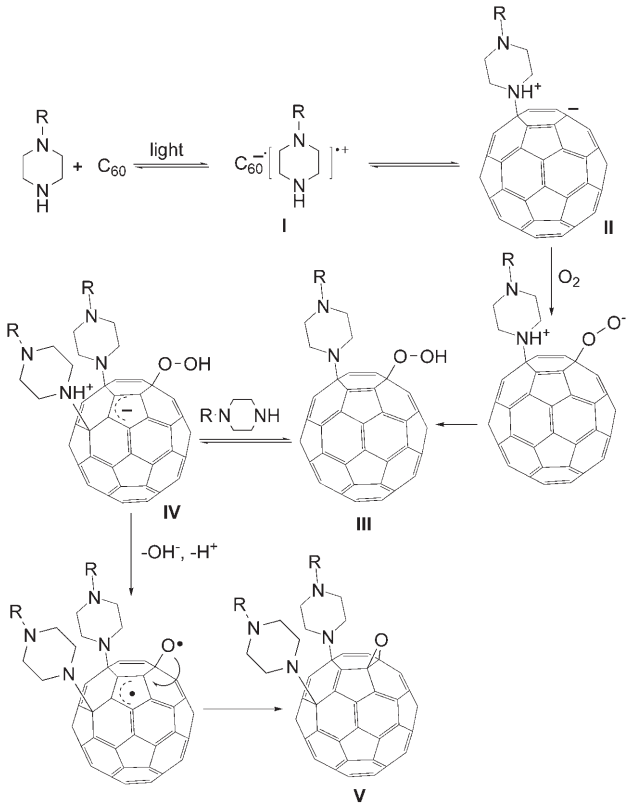


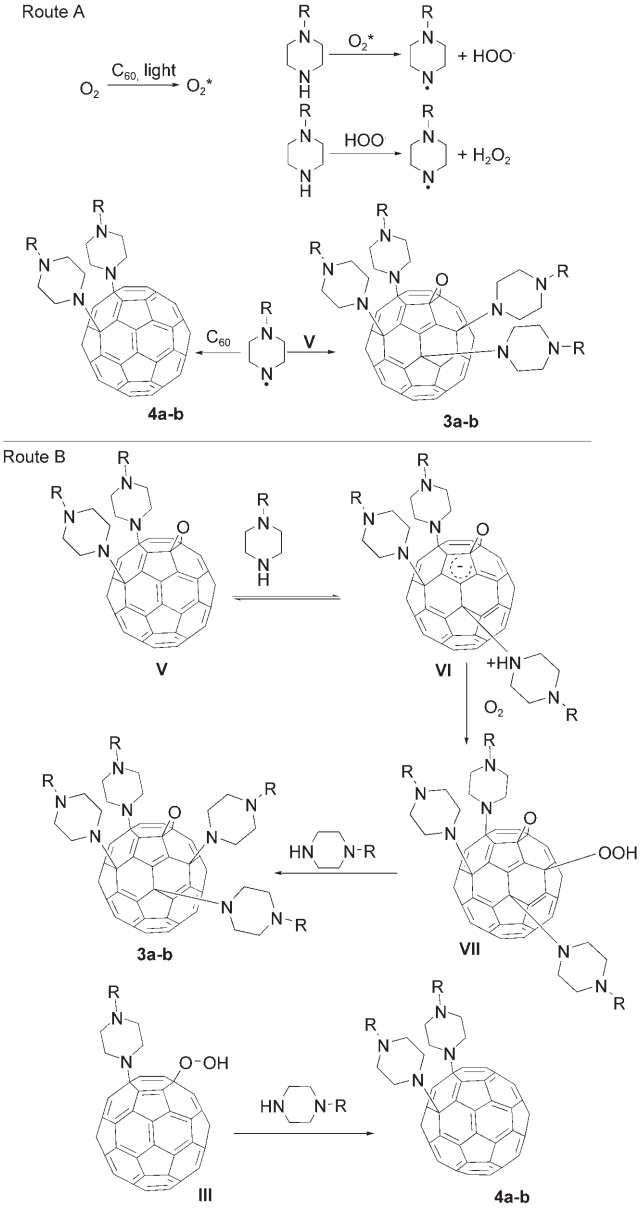
Figure 3. ^{1}H (a) and ^{13}C NMR (b) spectra of **3a**; * denotes $CHCl_3$ in $CDCl_3$.



Scheme 4.

a H_2O molecule. Finally, intermolecular recombination of radicals affords diaminofullerene C_{60}[amine]$_2$O **V**.

There are two possible ways for conversion of C_{60}-[amine]$_2$O to C_{60}[amine]$_4$O (Scheme 5). According to the



Scheme 5.

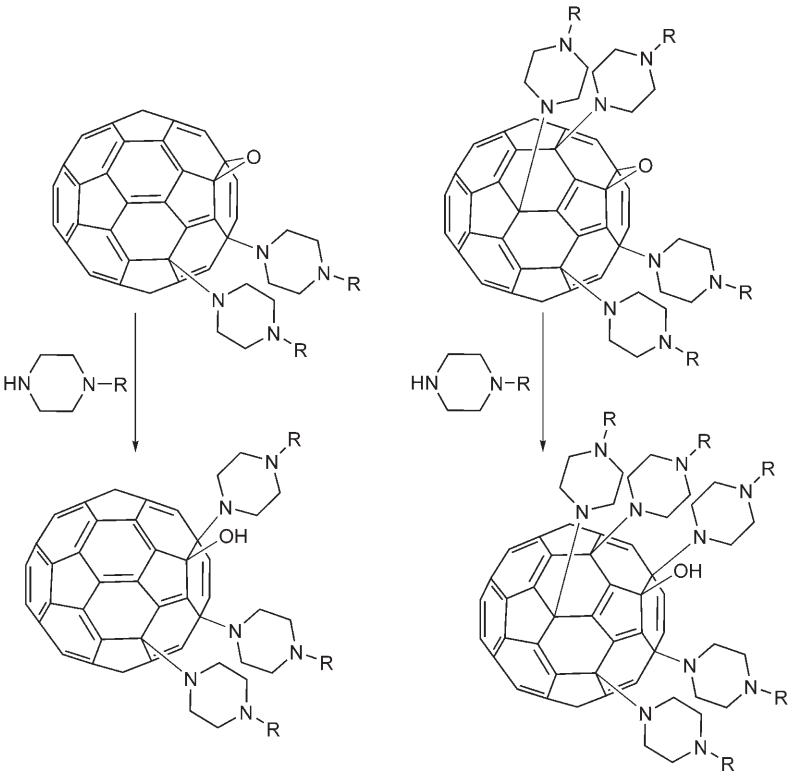
first one, fullerene-sensitized generation of singlet oxygen causes the oxidation of amine and the formation of amine radicals that undergo regioselective addition to the carbon cage yielding the final product **3a–b** (cyclopentadienyl mode).[34] If we assume that this pathway is true, formation of diaminofullerenes **4a–b** can be explained by addition of amine radicals to the parent fullerene C_{60}.

Alternatively, one more amine molecule can attack diaminofullerene **V** giving zwitterion **VI**; the latter reacts with oxygen yielding hydroperoxide **VII**. The conversion of **VII** to tetraaminofullerenes **3a–b** can be achieved only by a nu-

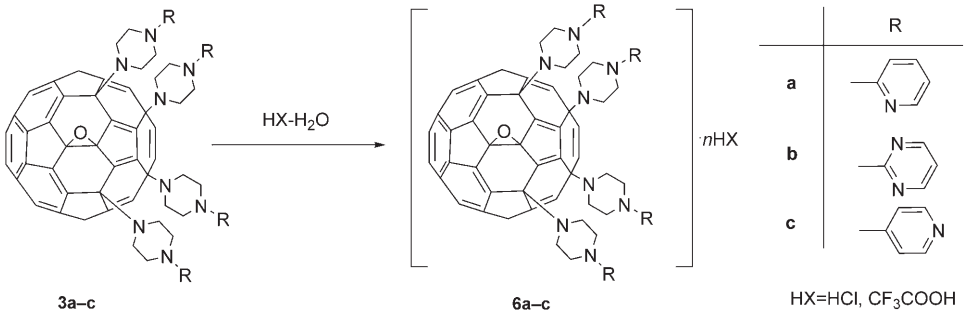
cleophilic substitution of an −OOH group with an amine residue. The substitution of the −OOH group in **III** by an amine is responsible for the formation of small amounts of diaminofullerenes **4a–b**.

It is important to note that hydrogen peroxide is formed as a side product in both routes. Indeed, H_2O_2 was observed when similar reactions were conducted in DMSO/chlorobenzene media.[35] Further investigations are required to establish which of the suggested reaction sequences is actually responsible for formation of cyclopentadienyl-type tetraaminofullerenes C_{60}[amine]$_4$O from C_{60} and secondary amines.

The conversion of **V** to **3a–b** proceeds quite rapidly as corresponding intermediates were not isolated even at low reaction times (1 h) when fullerene consumption was just about 10%. It was observed that the rate of the reaction between corresponding piperazine and [60]fullerene (the rate of fullerene consumption) correlates with the electron-withdrawing effect of the group attached to the piperazinyl nitrogen. Thus, *N*-methylpiperazine is the most active reagent; **2a** and **c** have moderate activity, while piperazines **2b** and **d** react with C_{60} very slowly. This trend fits well with the suggested mechanism as it involves such steps as nucleohphilic addition of amine to the double C=C bonds and probably nucleohphilic substitution of −OOH by an amine residue (route B, Scheme 5).



Scheme 6.



Scheme 7.

A nucleophilic addition of R_2NH to epoxide oxygen can also yield C_{60}[amine]$_3$OH and C_{60}[amine]$_5$OH from C_{60}[amine]$_2$O and C_{60}[amine]$_4$O, respectively (Scheme 6). These products were actually formed in the reactions of C_{60} with morpholine and *N*-methylpiperazine. However, nucleophilic epoxide ring opening becomes sterically suppressed when piperazines with bulky groups are used as starting reagents.

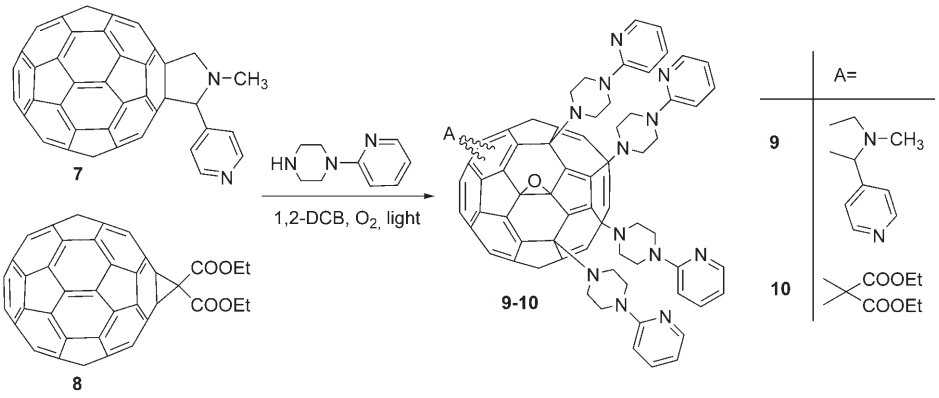
Conversion of tetraaminofullerenes C_{60}[amine]$_4$O into water-soluble salts: Tetraaminofulllerene derivatives C_{60}[amine]$_4$O **3a–c** are quite strong organic bases that afford corresponding salts **6a–c** (Scheme 7) by dissolving in organic (CF_3COOH) and inorganic acids (36% aqueous HCl). Isolation of **6a–c** from aqueous acid solutions by removal of acid and water in vacuum (on a rotary evaporator) at elevated temperatures was challenged by partial hydrolysis similar to that observed for *N*-methylpiperazine-fullerene derivatives. Therefore, these solutions were concentrated at room temperature within 1–2 weeks and substantial degradation did not occur for **6a,c**. Alternatively, removal of acid and water can be undertaken under vacuum at room temperature. Chemical analysis data for hydrochlorides **6a,c** (X = Cl) evidenced the presence of 7.78 and 7.10 HCl formula units on average per carbon cage, respectively. This corresponds to 0.65 and 0.59 HCl per nitrogen atom in **6**,**c** thus indicating that less basic nitrogen atoms attached to the fullerene cage do not undergo substantial protonation. The ^{1}H and

^{13}C NMR spectra of **6a**,**c** (in D_2O) were badly resolved, perhaps due to a statistical degree of protonation of amine groups in **6a**,**c**.

In contrast to **6a** and **6c**, concentration of the solution of **6b** was accompanied by relatively rapid (10–20 h) hydrolysis even at room temperature yielding an insoluble in water solid with composition C_{60}(*N*-(2-pyrimidinyl)piperazine)$_2$(OH)$_2$O·*n*HX (HX = HCl, CF_3COOH; $n = \sim 2$–3) as was determined by chemical analysis. This material was converted into the free base C_{60}(*N*-(2-pyrimidyl)piperazine)$_2$(OH)$_2$O by treatment with Et_3N in toluene; characterization of this product by ^1H and ^{13}C NMR spectroscopy evidenced that it is most probably a mixture of isomers. Thus, hydrolysis of aminofullerenes C_{60}[amine]$_4$O was proven to be nonregioselective.

The solubility of salts **6a** and **c** in water was estimated to be in the range of 150–200 mg mL^{-1} at pH ~ 7.0, enough for all kinds of biological investigations. We point out that these cationic compounds are among the most soluble in water fullerene derivatives; their preparation is straightforward and large quantities can be easily obtained (from grams to hundreds of grams).

Transformation of pyrrolidino- and methanofullerenes A = C_{60} into tetraaminofullerenes A = C_{60}[amine]$_4$O: We found that the photoaddition of N-substituted piperazines can be applied not only to parent C_{60} but also to some fullerene derivatives. In particular, pyrrolidinofullerene **7** and methanofullerene **8** readily react with *N*-(2-pyridyl)piperazine to yield the corresponding aminofullerenes A = C_{60}[amine]$_4$O (A is a cyclic addend) **9**–**10** as major products (Scheme 8).



Scheme 8.

The NMR spectra showed that this reaction is nonregioselective and yields mixtures of isomers of A = C_{60}[amine]$_4$O that could not be separated by column chromatography on silica. Nevertheless, addition of piperazine groups provides a facile route for the conversion of a range of fullerene derivatives into water-soluble compounds that can find applications, particularly for the design of specific targets for biological studies (Figure 4).

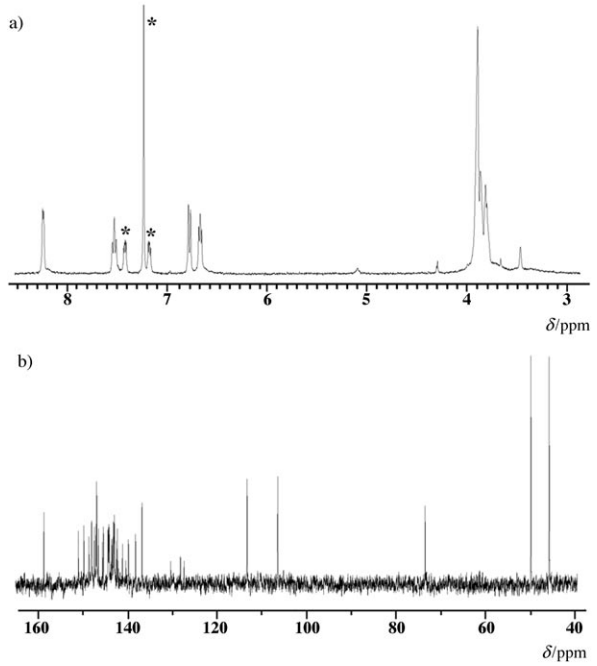


Figure 4. ^1H (a) and ^{13}C (b) NMR spectra of **4a**; * denotes $CHCl_3$ and signals of 1,2-DCB trapped in the lattice of solid **4a**.

Conclusions

A comparative study of the oxidative photoaddition of N-substituted piperazines to [60]fullerene revealed a different selectivity for these reactions depending on the reagent structure. Addition of *N*-methylpiperazine (and morpholine) resulted in complex product mixtures with C_{60}[amine]$_3$OH, C_{60}[amine]$_4$O, and C_{60}[amine]$_5$OH as predominant components. Reaction of C_{60} with *N*-(*tert*-butoxycarbonyl)piperazine also had a low selectivity because of photochemical deprotection of the starting reagent. On the contrary, *N*-(2-pyridyl)- and *N*-(2-pirimidinyl)-piperazines were found to be the most efficient reagents yielding C_{60}[amine]$_4$O as the major product. A mechanism of formation for C_{60}[amine]$_2$ and C_{60}[amine]$_4$O under photochemical conditions was suggested. It was shown that pyrrolidino- and methanofullerenes also undergo reactions with N-substituted piperazines to yield A = C_{60}[amine]$_4$O (A is a cyclic addend) as mixtures of regioisomers.

Tetraaminofullerenes C_{60}[amine]$_4$O and A = C_{60}[amine]$_4$O can be easily converted into highly water-soluble salts; this procedure is accompanied in some cases by nonselective hy-

drolysis with formation of insoluble in water fullerenol-like derivatives $C_{60}[amine]_{4-x}(OH)_xO{\cdot}nHX$ (HX = corresponding acid).

The simple and efficient syntheses of tetraaminofullerene derivatives $C_{60}[amine]_4O$ and $A = C_{60}[amine]_4O$ and their facile conversion into corresponding salts can be considered as a power approach to the preparation of novel highly water-soluble fullerene derivatives bearing cationic groups attached to the fullerene cage. Biological studies of these compounds may result in new breakthroughs in this field as previous studies were focused predominantly on water-soluble fullerenes bearing anionic COO^- units.

Experimental Section

General: All commercially available solvents and reagents were purchased from Acros or Aldrich and used as received. NMR spectra were recorded from solutions in $CDCl_3$ (^1H) and $CS_2/[D_{12}]$cyclohexane 10:1 (^{13}C) on an AMX 400, Bruker (400 MHz-^1H) instrument with the solvent residual proton signal or tetramethylsilane (TMS) as a standard. Deuterated water D_2O was used as a solvent for spectroscopic characterization of salts **4a** and **c**. LCQ Deca XP (Thermo Finnigan) and custom-made high resolution ESI O-TOF MS[36] were used to obtain ESI mass spectra. Fullerene derivatives **7** and **8** were synthesized as previously described.[37,38]

General procedure for the reaction of C_{60} with piperazines 2a–d: Fullerene C_{60} was dissolved in 1,2-dichlorobenzene (DCB) within 2 h whilst stirring in air. After the addition, a solution of piperazine **2a–d** in 10 mL of 1,2-dichlorobenzene was added in one run to the fullerene solution. The resulting reagent mixture was stirred in air (in an opened Erlenmeyer flask) under irradiation from the top by 60 W incandescent light bulb. The course of the reaction was monitored by TLC and all syntheses were stopped when conversion of C_{60} was nearly complete. Reagent ratios and optimal reaction times are specified in Table 1. In the case of piperazine **2c**, almost complete precipitation of the material from the solution was observed.

When the reaction was accomplished (for piperazines **2a–b** and **d**), the reagent mixture was initially diluted by toluene (1:7 by volume) and then as much *n*-hexane as possible was added until the material began to precipitate. The resulting solution was filtered and poured onto a silica-gel column (purchased from Acros Organics, 30–75 μ, 90 Å). A small amount of unreacted fullerene was washed out from the column with toluene/hexane mixtures ~1:1. Elution by toluene/methanol mixtures resulted in the fractions of diaminofullerenes **4a–b** and then tetraaminofullerenes **3a–b**. Compound **5** was followed by mixture of unidentified products in the course of separation of the crude material obtained from piperazine **2d**.

General synthetic procedure for the preparation of aminofullerenes 9 and 10: Pyrrolidinofullerene **8** (100 mg, 0,117 mmol) or methanofullerene **9** (110 mg, 0.125 mmol) was dissolved in 1,2-dichlorobenzene (10 mL) and then piperazine **2a** (26.24 equiv, 535 mg, 3.28 mmol) was added. The reagent mixture was stirred in air under irradiation by a 60 W incandescent light bulb for 48 h. The workup and isolation of aminofullerenes **9** and **10** was carried out as described above for tetraaminofullerenes **3a–b**. No corresponding diaminofullerenes $A = C_{60}[amine]_2O$ were observed in the course of chromatographic separation.

General procedure for the preparation of 6a, 6c, and salts of aminofullerenes 9 and 10: The starting aminofullerene (0.5 g) was dissolved in aqueous HCl (30 mL, 36%) or in the same volume of CF_3COOH (98%) within 5 min whilst stirring at room temperature. The resulting solution was filtered through a glass filter under reduced pressure and the filtrate poured into a Petri dish. The later was introduced into a large desiccator filled with solid NaOH where it was stored until complete evaporation of the acid and water had occurred. The resultant solid was redissolved in water (10 mL) and precipitated by the addition of acetonitrile. The precipitate was isolated by centrifugation, washed three times with hexane, and dried in air. Typical product yields ranged between 0.45–0.55 g.

Compound 3a: Eluent: toluene/MeOH 99:1; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 3.25$–4.25 (br m, 16H), 6.65 (m, 3H), 6.81 (d, 1H), 7.47 (t, 1H), 7.66 (t, 1H), 8.17 (d, 1H), 8.27 ppm (d, 1H); ^{13}C NMR (100 MHz, CS_2/C_6D_{12} 10:1): $\delta = 45.33$, 45.65, 46.48, 47.86, 97.87, 100.06, 106.40, 106.5, 113.2, 113.3, 125.27, 127.39, 130.46, 130.80, 130.88, 131.20, 131.85, 131.95, 133.83, 134.64, 142.51, 143.75, 143.82, 143.91, 144.12, 144.63, 144.78, 144.82, 146.45, 147.42, 147.76, 147.91, 148.00, 148.08, 148.27, 148.62, 148.79, 149.28, 149.97, 150.05, 150.48, 150.64, 151.21, 151.26, 153.20, 155.77, 158.66, 158.89, 164.07 ppm; ESI-MS (CH_2Cl_2/HCOOH): calcd (%): 462.476, 693.21, 1385.414; found: 462.506 (10) $[M+3H]^{3+}$, 693.256 (100) $[M+2H]^{2+}$, 1385.473 (25) $[M+H]^+$.

Compound 3b: Eluent: toluene/MeOH 98.5:1.5; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 3.25$–4.25 (br m, 16H), 6.52 (t, 2H), 6.58 (t, 2H), 8.35 (d, 1H), 8.44 ppm (d, 1H); ^{13}C NMR (100 MHz, CS_2/C_6D_{12} 10:1): $\delta = 43.34$, 43.44, 44.17, 44.22, 50.73, 51.17, 71.54, 71.81, 75.50, 76.43, 109.72, 109.77, 110.48, 128.18, 140.38, 141.70, 142.94, 143.17, 143.49, 143.53, 143.85, 144.12, 144.37, 144.56, 145.01, 145.99, 146.17, 146.69, 146.83, 146.90, 147.04, 147.29, 147.62, 147.69, 148.98, 149.24, 149.28, 151.52, 157.10, 160.96, 161.19 ppm.

Compound 4a: Eluent: toluene/MeOH 99.2:0.8; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 3.40$–4.20 (br m; 8H), 6.70 (t, 1H), 6.81 (d, 1H), 7.56 (t, 1H), 8.27 ppm (d, 1H); ^{13}C NMR (100 MHz, CS_2-C_6D_{12} 10:1): $\delta = 45.82$, 49.89, 73.52, 106.43, 113.29, 127.36, 128.17, 130.36, 136.75, 138.20, 139.78, 140.38, 141.11, 142.09, 142.25, 142.53, 142.97, 143.03, 143.16, 143.41, 143.50, 144.06, 144.16, 144.22, 144.32, 144.39, 145.37, 145.60, 146.47, 146.88, 147.00, 147.35, 148.00, 148.61, 149.75, 150.98, 158.67 ppm.

Compound 4b: Eluent: toluene/MeOH 99:1; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 3.72$–4.07 (br m, 8H), 4.22 (br s, 8H), 6.56 (t, 2H), 8.39 ppm (d, 4H); ^{13}C NMR (100 MHz, CS_2/C_6D_{12} 10:1): $\delta = 44.31$, 50.02, 73.57, 109.85, 138.19, 139.78, 140.35, 141.10, 142.09, 142.25, 142.53, 142.97, 143.01, 143.17, 143.41, 143.50, 143.69, 144.05, 144.16, 144.22, 144.31, 145.36, 145.59, 146.47, 146.87, 146.99, 147.34, 148.60, 149.76, 150.99, 157.08, 157.16, 157.25, 161.27 ppm.

Compound 5: Eluent: toluene/MeOH 99.7:0.3; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 3.67$ (d, 4H), 4.63 ppm (d, 4H); ^{13}C NMR (100 MHz, CS_2/C_6D_{12} 10:1): $\delta = 47.54$, 78.46, 137.08, 139.96, 141.13, 141.96, 142.35, 142.74, 144.74, 145.37, 145.75, 146.12, 146.48, 152.20 ppm.

Compound 9: Eluent: toluene/MeOH 98:2; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 2.20$–5.16 (br m, 35H), 6.50–6.95 (br m, 8H), 7.36–7.73 (m, 6H), 8.11–8.38 (m, 4H), 8.47–9.00 ppm (br m, 2H); ESI-MS (CH_2Cl_2/HCCOH) (%): 760.30 (100) $[M+2H]^{2+}$, 768.30 (80) $[M+O+2H]^{2+}$, 1519.55 (10) $[M+H]^+$, 1535.55 (8) $[M+O+H]^+$.

Compound 10: Eluent: toluene/MeOH 98.7:1.3; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.14$–1.62 (m, 6H), 2.60–4.76 (br m, 36H), 6.67 (br s, 8H), 7.50 (br s, 4H), 8.20 ppm (br s, 4H).

Acknowledgements

This work was partially supported by the RFBR (Grant No. 04–03–32870_a), INTAS (Grant No. 04–83–3733), and the Russian Science Support Foundation.

[1] N. Tagmatarchis, H. Shinohara, *Med. Chem.* **2001**, *1*, 339.
[2] *Perspectives of Fullerene Nanotechnology* (Ed.: E. Osawa), Kluwer Academic, Dordrecht, **2001**.
[3] A. W. Jensen, S. R. Wilson, D. I. Shuster, *Bioorg. Med. Chem.* **1996**, *4*, 767.
[4] T. Da Ros, M. Prato, *Chem. Commun.* **1999**, 663.
[5] T. Mashino, K. Shimotohno, N. Ikegami, D. Nishikawa, K. Okuda, K. Takahashi, S. Nakamura, M. Mochizuki, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1107.

[6] S. Bosi, T. Da Ros, G. Spalluto, J. Balzarini, M. Prato, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4437.
[7] S. H. Friedman, P. S. Ganapathi, Y. Rubin, G. L. Kenyon, *J. Med. Chem.* **1998**, *41*, 2424.
[8] N. Nakajima, C. Nishi, F.-M. Li, Y. Ikada, *Fuller. Sci. Technol.* **1996**, *4*, 1.
[9] Y. Tabata, T. Ishii, T. Aoyama, R. Oki, Y. Hirano, O. Ogawa, Y. Ikada in *Perspectives of Fullerene Nanotechnology* (Ed.: E. Osawa), Kluwer Academic, Dordrecht, **2001**.
[10] T. Mashino, D. Nishikawa, K. Takanashi, N. Usui, T. Yamory, M. Seki, T. Endo, M. Mochizuki, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4395.
[11] L. L. Dugan, D. M. Turelsky, C. Du, D. Lobner, M. Wheeler, R. Almli, C. K. F. Shen, T. Y. Luh, D. Choi, T. S. Lin, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 9434.
[12] S. Bosi, T. Da Ros, S. Castellano, E. Bafni, *M. Prato, Bioorg. Med. Chem. Lett.* **2000**, *10*, 1043.
[13] T. Wharton, L. J. Wilson, *Bioorg. Med. Chem.* **2002**, *10*, 3545.
[14] M. Mikawa, H. Kato, M. Okumura, M. Narazaki, Y. Kanazawa, N. Miwa, H. Shinohara, *Bioconjugate Chem.* **2001**, *12*, 510.
[15] J. Cerar, J. Cerkovnik, J. Skerjanc, *J. Phys. Chem. B* **1998**, *102*, 7377.
[16] C. F. Richardson, D. I. Shuster, S. R. Wilson, *Org. Lett.* **2000**, *2*, 1011.
[17] T. Wharton, V. U. Kini, R. A. Mortis, L. J. Wilson, *Tetrahedron Lett.* **2001**, *42*, 5159.
[18] L. Lamparth, A. Hirsch, *J. Chem. Soc. Chem. Commun.* **1994**, *1727*.
[19] M. Braun, S. Atalick, D. M. Guldi, H. Lanig, M. Brettreich, S. Burghardt, M. Hatzimarinaki, E. Ravanelli, M. Prato, R. van Eldik, A. Hirsch, *Chem. Eur. J.* **2003**, *9*, 3867.
[20] M. Brettreich, A. Hirsch, *Tetrahedron Lett.* **1998**, *39*, 2731.
[21] H. Isobe, H. Mashima, H. Yurimitsu, E. Nakamura, *Org. Lett.* **2003**, *5*, 4461.
[22] A. Hirsch, Q. Li, F. Wudl, *Angew. Chem.* **1991**, *103*, 1339; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1309.
[23] G. P. Miller, J. M. Millar, B. Liang, S. Uldrich, J. E. Johnston, *J. Chem. Soc. Chem. Commun.* **1993**, *897*.
[24] K. D. Kampe, N. Egger, M. Vogel, *Angew. Chem.* **1993**, *105*, 1203–1205; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1174.
[25] G. Schick, K. D. Kampe, A. Hirsch, *J. Chem. Soc. Chem. Commun.* **1995**, *2023*.
[26] C. P. Butts, M. Jazdzyk, *Chem. Commun.* **2003**, 1530.
[27] N. X. Wang, *Tetrahedron* **2002**, *58*, 2377.
[28] C. P. Butts, R. W. A. Havenith, M. Jazdzyk, T. Drewello, S. Kotsiris, *Tetrahedron Lett.* **2003**, *44*, 3565.
[29] H. Isobe, A. Ohbayashi, M. Sawamura, E. Nakamura, *J. Am. Chem. Soc.* **2000**, *122*, 2669.
[30] H. Isobe, N. Tomita, E. Nakamura, *Org. Lett.* **2000**, *2*, 3663.
[31] A. A. Tuiman, R. N. Compton, *J. Phys. Chem. A* **1998**, *102*, 9791.
[32] M. Smith, J. March, *March's Advanced Organic Chemistry*, Wiley, Weinheim, **2001**, p. 795.
[33] M. Smith, J. March, "March's Advanced Organic Chemistry", Wiley, Weinheim, **2001**, p. 923.
[34] A. Hirsch, *Top. Cur. Chem.* **1999**, *199*, 1.
[35] H. Isobe, T. Tanaka, W. Nakanishi, L. Lemie′gre, E. Nakamura, *J. Org. Chem.* **2005**, *70*, 4826–4832.
[36] A. F. Dodonov, A. V. Loboda, V. I. Kozlovski, I. V. Soulimenkov, V. V. Raznikov, Zhou Zhen, T. Horwath and H. Wollnik, *Eur. J. Mass Spectrom.* **2000**, *6*, 481.
[37] C. Bingel, *Chem. Ber.* **1993**, *126*, 1957.
[38] T. Da Ros, M. Prato, D. Guldi, E. Alessio, M. Ruzzi, L. Pasimeny, *Chem. Commun.* **1999**, *635*.

Received: November 29, 2005
Revised: February 7, 2006
Published online: June 6, 2006

