Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 6569–6577

TETRAHEDRON

Synthesis of water soluble fulleropyrrolidines bearing biologically active arylpiperazines

Beatriz M. Illescas,[a] Roberto Martínez-Alvarez,[a] Javier Fernández-Gadea[b] and Nazario Martín[a,*]

[a]*Departamento de Química Orgánica, Facultad de Química, Universidad Complutense de Madrid, E-28040 Madrid, Spain*
[b]*Departamento de Investigación Básica, Janssen-Cilag S.A., C/Jarama s/n, Polígono Industrial, E-45007 Toledo, Spain*

Received 26 May 2003; revised 10 June 2003; accepted 26 June 2003

Abstract—New water soluble fulleropyrrolidines endowed with biologically active arylpiperazines have been synthesized by 1,3-dipolar cycloaddition of in situ generated azomethyne ylides to C_{60}. The substitution pattern on the pyrrolidine and piperazine rings ensures their solubility in a H_2O/DMSO (9:1) solvents mixture. The mass spectrometry study reveals a different fragmentation pathway for the fulleropyrrolidines depending upon the substituent on the pyrrolidine nitrogen atom, affording in all cases to differently substituted methanofullerenes. Preliminary biological tests reveal a moderate activity in vitro experiments.

1. Introduction

C_{60} and its chemically modified derivatives have shown promising biological activities in different fields both in vitro and in vivo.[1] Biological applications have included studies on the inhibition of HIV-protease,[2] photodynamic therapy,[3] site-selective DNA cleavage[4] and neuroprotection.[5] However, the low water solubility of fullerenes appears to be an important problem for biological use. In this sense, some methods have been studied to water solubilize fullerenes, including reduction of C_{60} or its derivatives to a water-soluble anion,[6] complexation of C_{60} in a host–guest fashion,[7] chemical modification of C_{60} with ionic organic groups[8] or covalent attachment of organic groups with non-ionic hydrophilic groups.[9] As an example of the latter approach, the introduction of polyether chains in the fullerene derivatives structure brings about the formation of water-soluble fulleropyrrolidines.[9a]

Following this methodology, we have recently reported the synthesis of new fulleropyrrolidines bearing 1,4-dihydropyridines as biologically active substituents, together with a polyether chain, thus enhancing the hydrophilic character of these molecules.[10]

Related to this study, we have now synthesized new fullerene derivatives in which the phenylpiperazine pharmacophore is covalently attached to C_{60}.

Arylpiperazine is a common fragment of many bioactive compounds which exhibit a variety of pharmacological effects (Fig. 1). Thus, the arylpiperazine ligand is present in compounds such as buspirone (**1**) or gepirone (**2**) which have agonist properties at 5-HT_{1A} receptors with high affinity for the 5-HT_{1A} binding sites.[11] Numerous studies have shown that even minor modifications in the chemical structure of arylpiperazine derivatives strongly affect their biological activity.[12] In this context, flesinoxan (**3**) and analogous[13] are described as selective 5-HT_{1A} vs dopamine D_2 receptor. Urapidil (**4**) and SL89.0591 (**5**)[14] are postsynaptic α-blockers with antihypertensive (**4**) and benign prostatic hypertrophy (**5**) activity respectively. Some pyridazinone derivatives (**6**) have been reported as adrenoceptor antagonists with high affinity for α_1-AR and selectivity towards α_2-AR[15] (Fig. 1).

In this work, we report the synthesis of new fulleropyrrolidines containing the arylpiperazine, arylpiperidine or arylmorpholine fragments as biologically active substituents. These derivatives have been prepared by 1,3-dipolar cycloaddition of the in situ generated azomethine ylides to C_{60}, by reaction of the corresponding aldehyde with [60]fullerene and sarcosine by following Prato's methodology.[16] In order to improve the water solubility of the fullerene derivatives we have also used *N*-(3,6,9-trioxadecyl)glycine (**11**) and L-tyrosine (**14**) as amino acids, thus

Keywords: fulleropyrrolidines; cycloaddition; arylpiperazines.
* Corresponding author. Tel.: +34-91-3944227; fax: +34-91-3944103; e-mail: nazmar@quim.ucm.es

0040–4020/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0040-4020(03)01017-2

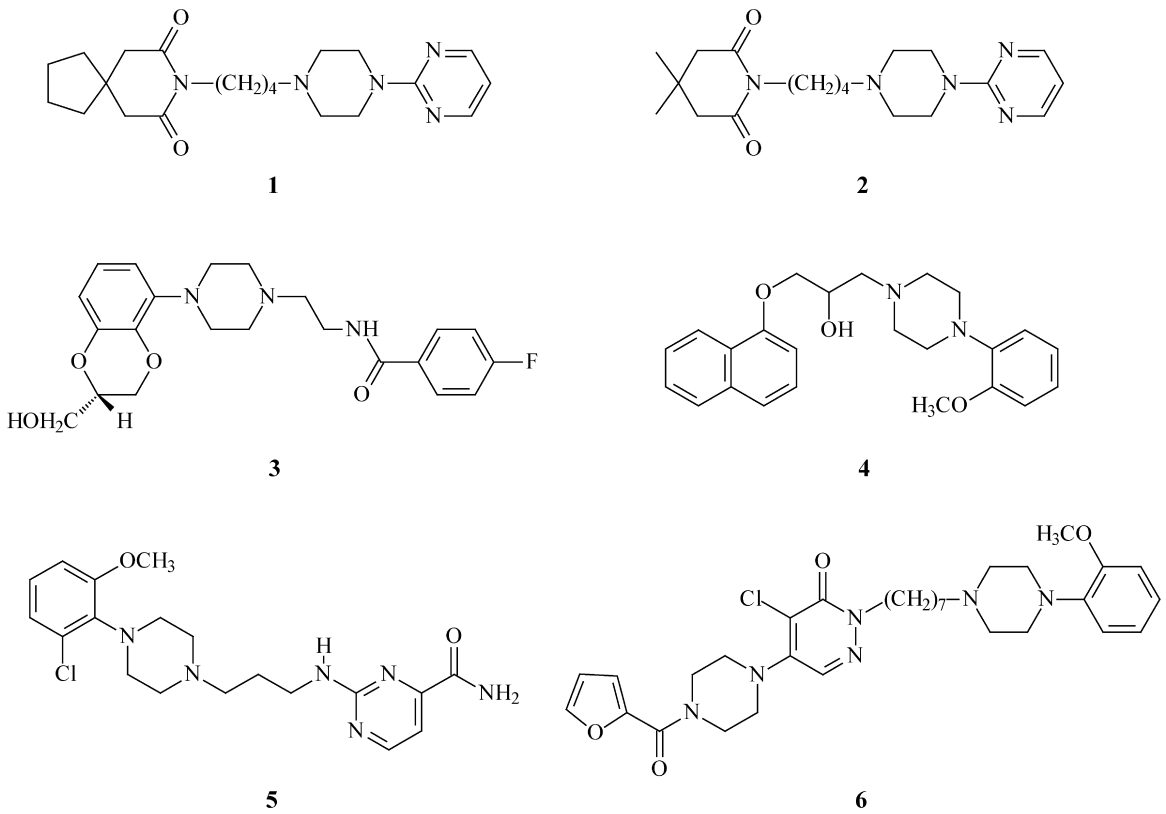


Figure 1. Pharmacologically active arylpiperazines.

obtaining structures in which C_{60} is covalently attached, on the one hand, to a pharmacophore fragment and, on the other hand, to an organic rest with non-ionic hydrophilic functionalities.

2. Results and discussion

The formyl substituted arylpiperazine, piperidine or morpholine were prepared by reaction of 4-fluorbenzaldehyde with the chosen heterocyclic derivative in DMF and with potassium carbonate as the base.[17]

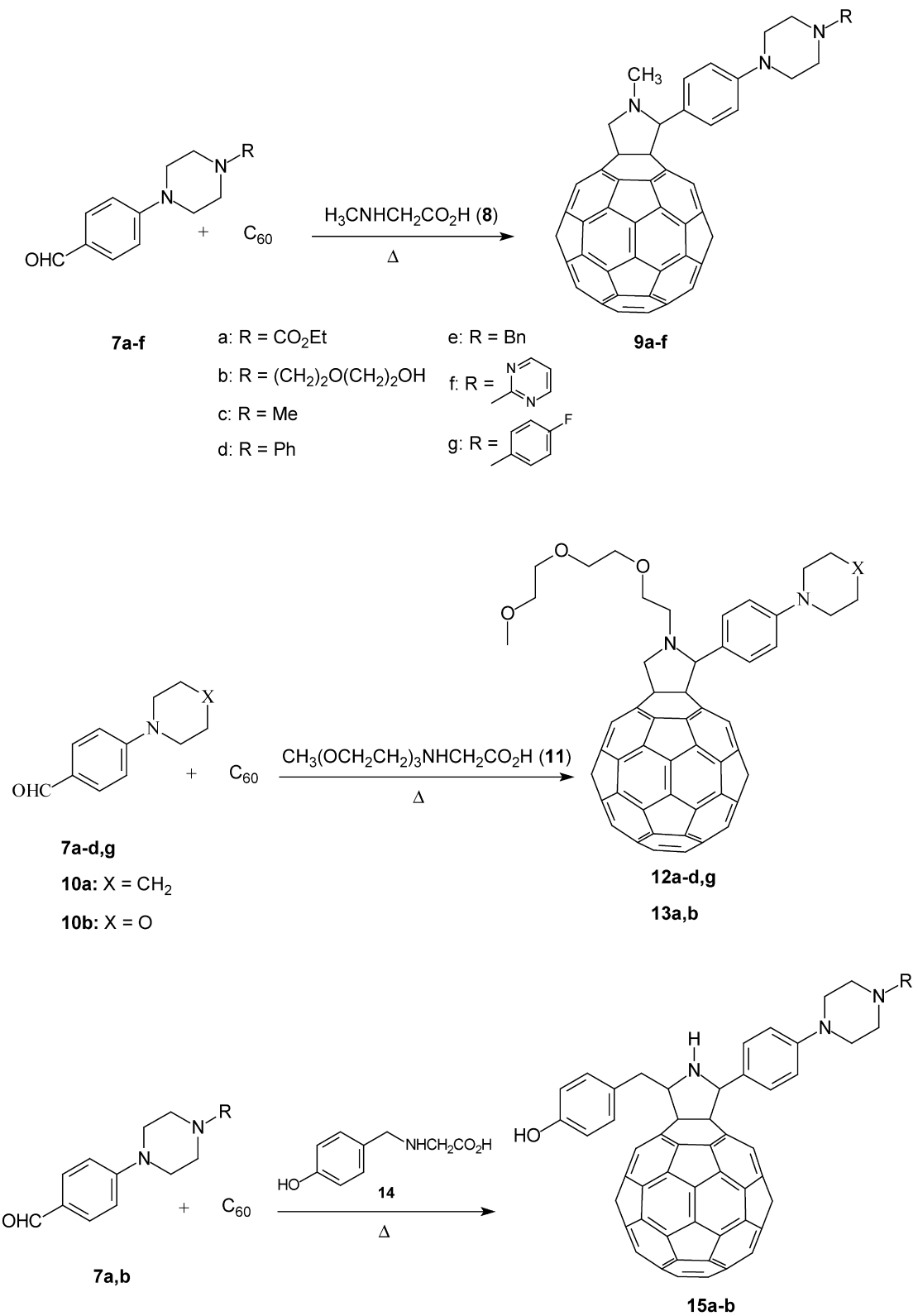
The desired fulleropyrrolidines were obtained by refluxing a mixture of C_{60}, the formyl derivative and the corresponding amino acid for a variable period of time (7–24 h) (Scheme 1). The solvent of the reaction was toluene in all cases except for the L-tyrosine derivatives (**15a**–**b**) which could not be prepared in this solvent probably due to the low solubility of the amino acid and, in turn, we used ODCB. The reaction was carried out with excess of the amino acid [C_{60}/R-CHO/aa 1:1:5 when aa is sarcosine and 1:1:3 when aa is *N*-(3,6,9-trioxadecylglycine) (**11**) or L-tyrosine (**14**)]. The target fulleropyrrolidines were obtained in general with moderate yields (see Section 3).

All these compounds were fully characterized and the spectroscopic data are in agreement with the proposed structures. Thus, the novel fulleropyrrolidines showed a typical weak absorption band at 430–431 nm in the UV–vis spectra as most dihydrofullerenes. The ^{1}H NMR spectra of fullerene derivatives **9**, **12** and **13** showed the presence of the pyrrolidine protons as two doublets and a singlet in the region between $\delta\sim5.21$–4.97 (d, $J\sim9.3$–9.9 Hz), $\delta\sim4.86$–5.11 (s), and $\delta\sim4.23$–4.28 (d, $J\sim9.3$–9.9 Hz). For compounds **15a**–**b**, the protons of the pyrrolidine ring appear at $\delta\sim5.63$–5.66 (s) and $\delta\sim4.93$–4.98 (m). The protons of the methylene group close to the tyrosine rest in these compounds are not magnetically equivalents and they appear as two multiplets at $\delta\sim3.99$ and $\delta\sim3.40$.

In the ^{13}C NMR spectra of new fullerene derivatives, the number of signals indicates the lack of symmetry in these compounds. These spectra show the pyrrolidine carbons around 82–83 and 66–69 ppm; the sp^3-hybridized carbons of the C_{60} moiety appear around 70–77 ppm and 67–70 ppm and the carbons of the piperazine ring appear as two to four signals at $\delta\sim43$–55 depending on the substitution pattern (Scheme 1).

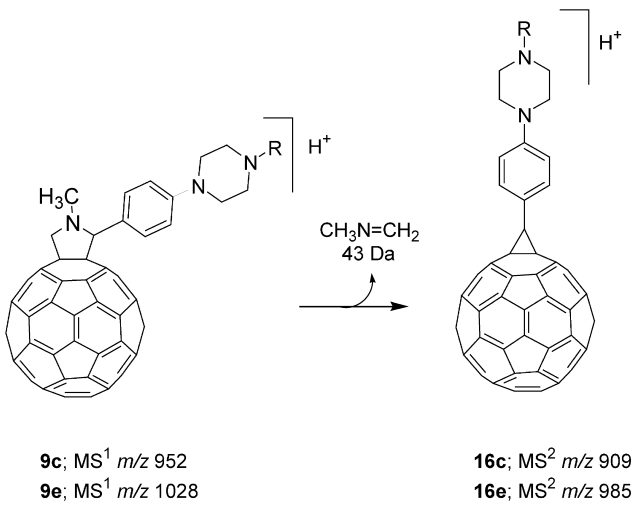
Unambiguous assignment of the sp^3 carbons of the molecule could be made for **12c** by HMQC and HMBC experiments. Thus, C-2 and C-6 pyrrolidine carbon atoms appear at $\delta\sim82$ and 68, respectively, and the Csp^3 of the C_{60} appear at $\delta\sim77$ and 69, being the latter the closer to the aryl substituted carbon of the pyrrolidine ring. The polyether chain gives five signals at 71–72 ppm together with the methoxy at $\delta\sim59$ and the methylene adjacent to the nitrogen of the pyrrolidine at $\delta\sim52$. Finally, the piperazine carbons appear as two signals at $\delta\sim55$ and 48 and the methyl group at $\delta\sim46$.

Mass spectra of new fulleropyrrolidines **9**, **12**, **13** and **15** were also in agreement with the proposed structures. In order to establish the fragmentation pathway for these compounds, we have recorded the mass spectra generated



Scheme 1.

by electrospray ionization (ESI) conditions. Strong evidences have been found for the general applicability of the ESI and nanospray ionization to the analysis of fullerene derivatives specially when the molecules present an amphiphilic nature.[18,19] In recent experiments,[20] it was noted that certain compounds undergo structural changes when electrosprayed providing evidence that the ESI source behaves as an electrolytic cell.[21] Thus, the electrochemical process involved in ESI has been used to obtain odd-electron molecular ions from electroactive C_{60}-TTF-C_{60}



9c; MS^1 *m/z* 952
9e; MS^1 *m/z* 1028

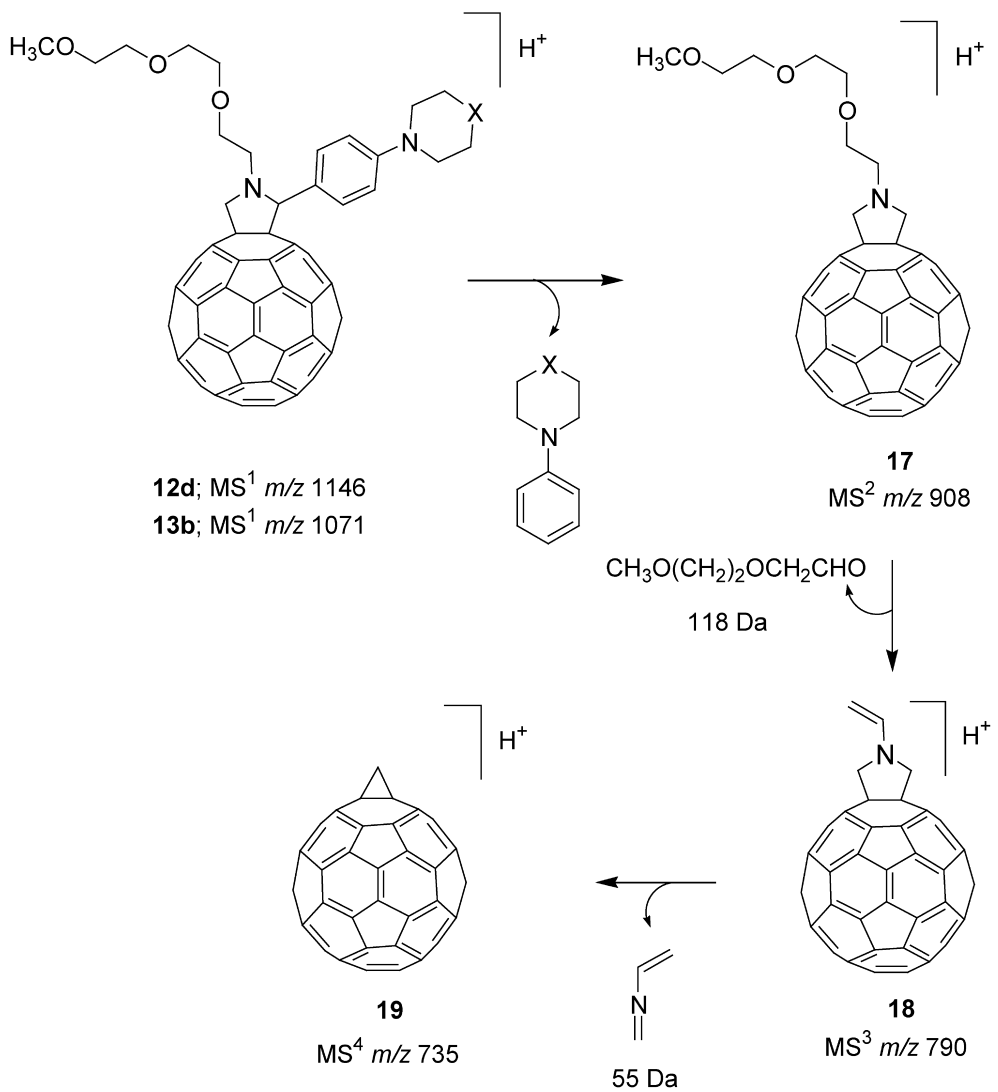
16c; MS^2 *m/z* 909
16e; MS^2 *m/z* 985

Scheme 2.

derivatives.[22] In other reported cases, the ESI spectra of fullerene derivatives were used to demonstrate the evidence of dimerized cation-radicals.[23]

The soft ionization carried out using ESI conditions applied to molecules **9**, **12**, **13** and **15** yields the corresponding molecular even-electron ions $[M+H]^+$ (see Section 3) with a very low range of internal energy. The double charged ion $[M+H]^{2+}$ was detected for **9c**. The difference with the above described fullerenes which form molecular odd-electron ions can be explained assuming a total different electrochemical behavior toward the ESI source. Moreover, the presence of nitrogen and/or oxygen atoms which can act as protonation centers, facilitates the ionization process forming the protonated molecular ions.

The second stage of fragmentation MS^2 reveals different processes depending on the substituents attached at the pyrrolidine ring. Thus, the corresponding protonated molecular ions of the *N*-methyl compounds such as **9** undergo the elimination of *N*-methyl-*N*-methyleneamine from the pyrrolidine ring affording a methanofullerene



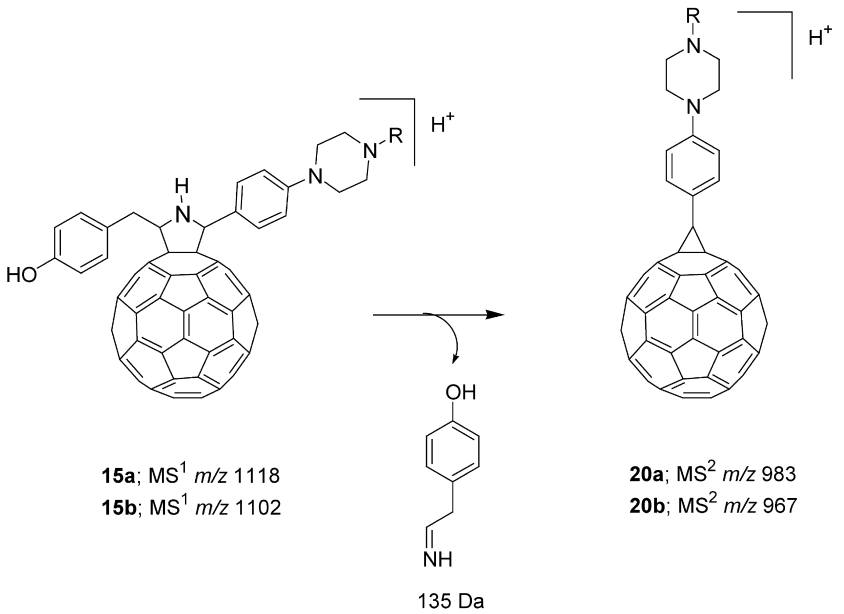
12d; MS^1 *m/z* 1146
13b; MS^1 *m/z* 1071

17
MS^2 *m/z* 908

19
MS^4 *m/z* 735

18
MS^3 *m/z* 790

Scheme 3.



Scheme 4.

derivative **16** (Scheme 2). The nature of the substituent R at the piperazine ring do not play any role in the fragmentation pathway. This intermediate which seems to be stable did not undergo further fragmentations.[24]

On the other hand, fulleropyrrolidines bearing an oligoether chain at the nitrogen atom (**12**, **13**) loss the aromatic substituent at the C2 of the pyrrolidine ring forming an intermediate cation (**17**) at *m*/*z* 908 (Scheme 3). This cation eliminates (2-methoxyethoxy)acetaldehyde (118 Da) affording a protonated *N*-ethylenefulleropyrrolidine **18** which finally affords the corresponding protonated methanofullerene **19** after elimination of a molecule of *N*-methylene-*N*-vinylamine (Scheme 3).

Fullerene derivatives with a *p*-hydroxybenzyl rest (**15**) eliminate a 4-(2-iminoethyl)phenol molecule affording a substituted methanofullerene **20** (Scheme 4).

The driving force for the most observed fragmentations of these fulleropyrrolidine derivatives seems to be the formation of a substituted methanofullerene bearing the aromatic substituent which enhances the stability of this intermediate. Compounds **12**, **13** undergo a different fragmentation mechanism. The oxygen atoms of the oligoether chain facilitate the protonation process and enhance the stability of the intermediate **17** allowing the loss of the aromatic substituent. Finally, an unsubstituted methanofullerene is postulated for the last step of fragmentation pathway. To the best of our knowledge, the ESI mass spectrometry has been used in fullerene chemistry for analytical purposes. We report herein for the first time the fragmentation pattern of arylpiperazine substituted fulleropyrrolidines under ESI conditions in which the fulleropyrrolidines are easily transformed into a protonated methanofullerene.

As we mentioned above, solubility of new fulleropyrrolidines in aqueous media is a prior matter. Thus, we performed solubility tests finding out that the differently substituted fulleropyrrolidines are reasonably soluble in a 9:1 ratio of H_2O/DMSO. A quantitative determination of the solubility of these compounds by spectrophotometric methods revealed that their solubility is in the range of $\sim 10^{-5}$ M. Thus, as a representative example, compound **15a** showed a solubility of 3.2×10^{-5} M in H_2O/DMSO (9/1).[25]

Preliminary biological tests show that compound **9a** exhibits a moderate antibacterial and antifungal activity in vitro and compounds **9c**–**e** a moderate cytostatic activity in vitro. Work is currently in progress to confirm the range of activity of this class of compounds and to evaluate the influence of the presence of the C_{60} moiety on the pharmacological effects of arylpiperazines.

3. Experimental

3.1. General

All ESMS experiments were carried out using an ESQUIRE-LC™ (Bruker Daltonics, Bremen, Germany) ion trap spectrometer. A syringe pump was used to deliver the fullerene derivatives solutions (chloroform: acetonitrile: formic acid 50:48:2 v/v/v). Nitrogen was used as nebulizer gas (flow: 4 L min^{-1}), the ionization potential was 5.0 kV and the chamber temperature 150°C. MS^n spectra were carried out with helium after isolation of the appropriate precursor using an isolation width of 0.4 *m*/*z*, fragmentation voltage amplitude of 0.60 V and fragmentation time of 40 ms.

3.1.1. Compound 9a. Yield: 28% (45% based on recovered C_{60}); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.66 (broad d, 2H), 6.95 (d, 2H, J=8.7 Hz), 4.98 (d, 1H, J=9.3 Hz), 4.87 (s, 1H), 4.25 (d, 1H, J=9.3 Hz), 4.16 (q, 2H, J=7.2 Hz), 3.65–3.61 (m, 4H), 3.20–3.17 (m, 4H), 2.8 (s, 3H), 1.30 (t, 3H, J=7.2 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 156.27, 155.17,

153.98, 153.59, 153.55, 150.87, 147.18, 147.17, 146.72, 146.40, 146.35, 146.20, 146.16, 146.10, 146.05, 146.02, 145.99, 145.83, 145.82, 145.67, 145.46, 145.43, 145.35, 145.25, 145.19, 145.16, 145.12, 145.11, 145.02, 144.60, 144.51, 144.29, 144.28, 143.04, 142.89, 142.58, 142.49, 142.46, 142.45, 142.17, 142.15, 142.06, 142.03, 141.99, 141.99, 141.93, 141.89, 141.86, 141.72, 141.58, 141.44, 140.07, 140.03, 139.82, 139.50, 136.69, 136.46, 135.67, 135.66, 130.11, 128.10, 116.17, 83.13, 69.91, 68.85, 67.87, 61.39, 48.87, 43.56, 39.92, 14.68; FTIR (KBr) ν (cm^{-1}) 2922, 2775, 1701, 1610, 1516, 1429, 1380, 1333, 1224, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 256, 431, 702; MS (ESI) *m/z* 1010 $[M+H]^+$.

3.1.2. Compound 9b. Yield: 21% (39% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.67 (broad s, 2H), 6.97 (d, 2H, *J*=9.3 Hz), 4.97 (d, 1H, *J*=9.3 Hz), 4.86 (s, 1H), 4.23 (d, 1H, *J*=9.3 Hz), 3.74–3.60 (m, 6H), 3.50 (s, 1H), 3.29–3.25 (m, 4H), 2.79 (s, 3H), 2.73–2.64 (m, 6H); ^{13}C NMR ($CDCl_3/CS_2$, 500 MHz) δ 156.39, 154.12, 153.76, 150.89, 147.25, 147.23, 146.84, 146.49, 146.46, 146.26, 146.20, 146.16, 146.08, 146.04, 145.88, 145.75, 145.50, 145.44, 145.42, 145.19, 145.09, 144.64, 144.61, 144.35, 143.08, 142.92, 142.61, 142.50, 142.22, 142.07, 141.98, 141.93, 141.83, 141.63, 141.50, 140.09, 140.05, 139.87, 139.65, 136.70, 136.54, 135.73, 135.68, 130.11, 127.69, 115.80, 83.26, 72.41, 69.97, 68.95, 67.45, 61.99, 57.87, 53.25, 48.41, 39.97, 29.73; FTIR (KBr) ν (cm^{-1}) 3431, 2920, 2850, 1655, 1610, 1514, 1429, 1124, 1032, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 261, 431, 702; MS (ESI) *m/z* 1026 $[M+H]^+$.

3.1.3. Compound 9c. Yield: 30% (47% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.67 (broad d, 2H), 6.98 (d, 2H, *J*=9 Hz), 4.98 (d, 1H, *J*=9.6 Hz), 4.87 (s, 1H), 4.24 (d, 1H, *J*=9.6 Hz), 3.28–3.24 (m, 4H), 2.79 (s, 3H), 2.60–2.57 (m, 4H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3/CS_2$, 500 MHz) δ 156.25, 154.72, 153.95, 153.64, 153.60, 150.85, 147.13, 147.10, 146.73, 146.39, 146.37, 146.15, 146.09, 146.05, 145.99, 145.96, 145.92, 145.77, 145.75, 145.63, 145.41, 145.38, 145.31, 145.19, 145.12, 145.10, 145.07, 144.97, 144.54, 144.50, 144.24, 144.22, 142.98, 142.83, 142.51, 142.44, 142.40, 142.14, 142.11, 142.00, 141.98, 141.95, 141.88, 141.82, 141.69, 141.52, 141.39, 140.01, 139.95, 139.79, 139.50, 137.54, 136.61, 136.45, 135.64, 135.58, 131.62, 129.98, 128.89, 128.10, 127.18, 125.19, 115.50, 113.44, 83.16, 69.86, 68.79, 54.98, 54.58, 48.47, 46.98, 46.01, 39.87; FTIR (KBr) ν (cm^{-1}) 2930, 2775, 1655, 1597, 1458, 1425, 1331, 1377, 1292, 1238, 824, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 261, 431, 704; MS (ESI) *m/z* 952 $[M+H]^+$, 476 $[M+H]^{2+}$.

3.1.4. Compound 9d. Yield: 19% (28% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.68 (broad d, 2H), 7.33–7.30 (m, 1H), 7.03–6.85 (m, 6H), 4.99 (d, 1H, *J*=9.6 Hz), 4.89 (s, 1H), 4.26 (d, 1H, *J*=9.6 Hz), 3.94–3.34 (m, 8H), 2.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 154.02, 153.69, 153.64, 151.01, 150.91, 147.69, 147.51, 147.26, 147.21, 147.17, 146.85, 146.78, 146.43, 146.27, 146.22, 146.17, 146.11, 146.04, 145.98, 145.84, 145.36, 145.18, 145.12, 144.55, 144.29, 143.05, 142.59, 142.53, 142.46, 142.18, 142.07, 142.01, 141.95, 141.87, 141.75, 141.58, 141.44, 140.09, 140.03, 139.84, 139.55, 138.02, 137.82, 136.74, 136.71, 136.50, 136.45, 135.69, 135.67, 130.12, 129.08, 127.67, 120.02, 116.23, 115.79, 83.24, 77.37, 69.94, 68.87, 49.32, 48.86, 39.91; FTIR (KBr) ν (cm^{-1}) 2920, 2850, 2777, 1601, 1500, 1333, 1230, 1186, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 234, 263, 431, 704; MS (ESI) *m/z* 1014 $[M+H]^+$.

3.1.5. Compound 9e. Yield: 25% (40% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.65 (broad d, 2H), 7.35–7.30 (m, 5H), 6.96 (d, 2H, *J*=8.7 Hz), 4.97 (d, 1H, *J*=9.6 Hz), 4.86 (s, 1H), 4.23 (d, 1H, *J*=9.6 Hz), 3.57 (s, 2H), 3.25–3.22 (m, 4H), 2.79 (s, 3H), 2.62–2.59 (m, 4H); ^{13}C NMR ($CDCl_3/CS_2$, 500 MHz) δ 156.16, 153.83, 153.54, 153.50, 150.87, 147.03, 146.99, 146.63, 146.30, 146.27, 146.05, 145.99, 145.95, 145.89, 145.87, 145.82, 145.67, 145.66, 145.54, 145.33, 145.27, 145.22, 145.20, 145.11, 145.02, 144.99, 144.97, 144.96, 144.87, 144.44, 144.42, 144.15, 144.13, 142.89, 142.74, 142.42, 142.35, 142.31, 142.05, 142.02, 141.91, 141.89, 141.86, 141.79, 141.78, 141.73, 141.61, 141.43, 141.31, 139.93, 139.87, 139.72, 139.41, 137.80, 136.51, 136.39, 135.56, 135.48, 129.84, 128.81, 128.08, 126.93, 126.83, 115.33, 83.06, 69.78, 68.66, 62.91, 52.97, 48.47, 39.78, 32.02, 29.82, 29.77, 29.49, 22.92, 14.25; FTIR (KBr) ν (cm^{-1}) 2920, 2850, 2775, 1653, 1558, 1508, 1489, 1458, 1228, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 257, 431, 703; MS (ESI) *m/z* 1028 $[M+H]^+$.

3.1.6. Compound 9f. Yield: 27% (42% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.29 (d, 2H, *J*=4.8 Hz), 7.66 (broad d, 2H), 6.96 (d, 2H, *J*=8.7 Hz), 6.48 (t, 1H, *J*=4.8 Hz), 4.97 (d, 1H, *J*=9.3 Hz), 4.87 (s, 1H), 4.25 (d, 1H, *J*=9.3 Hz), 3.99–3.96 (m, 4H), 3.29–3.25 (m, 4H), 2.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 161.13, 157.14, 156.06, 153.72, 153.42, 153.35, 150.86, 146.97, 146.94, 146.53, 146.20, 146.00, 145.94, 145.90, 145.85, 145.81, 145.77, 145.62, 145.61, 145.47, 145.29, 145.21, 145.18, 145.13, 145.07, 144.97, 144.94, 144.92, 144.82, 144.40, 144.36, 144.09, 144.08, 142.84, 142.70, 142.38, 142.31, 142.27, 142.26, 141.99, 141.96, 141.86, 141.84, 141.81, 141.74, 141.72, 141.67, 141.55, 141.38, 141.26, 139.90, 139.84, 139.68, 139.35, 136.49, 136.32, 135.49, 135.45, 129.89, 129.85, 127.31, 115.79, 109.80, 82.96, 77.13, 69.73, 68.59, 59.97, 48.61, 43.42, 39.73; FTIR (KBr) ν (cm^{-1}) 2920, 2849, 2775, 1611, 1582, 1547, 1516, 1447, 1364, 1231, 1178, 984, 797, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 266, 431, 704; MS (ESI) *m/z* 1016 $[M+H]^+$.

3.1.7. Compound 12a. Yield: 7% (10% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.69 (broad d, 2H), 6.96 (d, 2H, *J*=8.1 Hz), 5.20 (d, 1H, *J*=9.6 Hz), 5.09 (s, 1H), 4.28 (d, 1H, *J*=9.6 Hz), 4.17 (q, 2H, *J*=7.5 Hz), 4.06–3.96 (m, 2H), 3.81–3.73 (m, 4H), 3.63–3.56 (m, 6H), 3.50–3.40 (m, 2H), 3.38 (s, 3H), 3.22–3.12 (m, 4H), 2.90–2.80 (m, 2H), 1.28 (t, 3H, *J*=7.5 Hz); ^{13}C NMR ($CDCl_3/CS_2$, 500 MHz) δ 147.37, 146.64, 146.57, 146.54, 146.52, 146.41, 146.38, 146.34, 146.27, 146.21, 146.18, 146.17, 146.15, 146.08, 145.99, 145.94, 145.93, 145.89, 145.74, 145.45, 145.42, 145.37, 145.36, 145.33, 145.31, 145.27, 145.05, 145.03, 144.79, 144.76, 144.32, 144.10, 143.15, 143.01, 142.95, 142.83, 142.72, 142.67, 142.62, 142.23, 142.09, 141.91, 141.49, 141.27, 140.96, 140.17, 140.13, 140.08, 139.95, 139.59, 139.56, 139.12, 82.24, 77.20, 72.02,

70.63, 61.72, 59.09, 52.69, 51.05, 49.82, 43.92, 42.95, 14.66; FTIR (KBr) ν (cm^{-1}) 2920, 2851, 1701, 1609, 1458, 1429, 1229, 1121, 766, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 236, 267, 431, 703; MS (ESI) *m/z* 1142 $[M+H]^+$.

3.1.8. Compound 12b. Yield: 21% (32% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.67 (broad d, 2H), 6.95 (d, 2H, *J*=8.7 Hz), 5.20 (d, 1H, *J*=9.9 Hz), 5.08 (s, 1H), 4.27 (d, 1H, *J*=9.9 Hz), 4.09–3.93 (m, 2H), 3.87–3.56 (m, 13H), 3.49–3.38 (m, 5H), 3.28–3.24 (m, 4H), 2.90–2.78 (m, 2H), 2.70–2.63 (m, 6H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 156.79, 154.43, 153.92, 153.25, 153.18, 150.86, 147.28, 146.92, 146.57, 146.50, 146.27, 146.22, 146.18, 146.10, 145.91, 145.76, 145.58, 145.55, 145.49, 145.43, 145.28, 145.23, 145.21, 145.20, 145.10, 144.74, 144.69, 144.63, 144.39, 143.12, 142.94, 142.63, 142.54, 142.52, 142.28, 142.10, 141.99, 141.92, 141.63, 141.50, 140.10, 140.03, 139.87, 139.61, 136.77, 136.59, 135.71, 130.44, 130.34, 130.15, 128.05, 115.95, 115.88, 115.84, 88.00, 82.07, 72.93, 72.42, 72.02, 71.92, 70.78, 70.68, 70.58, 70.39, 69.20, 67.67, 61.94, 61.74, 59.08, 59.02, 53.19, 51.97, 48.35; FTIR (KBr) ν (cm^{-1}) 3429, 2920, 2852, 1609, 1508, 1109, 1026, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 256, 431, 701; MS (ESI) *m/z* 1158 $[M+H]^+$.

3.1.9. Compound 12c. Yield: 16% (28% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.67 (broad d, 2H), 6.95 (d, 2H, *J*=7.8 Hz), 5.20 (d, 1H, *J*=9.6 Hz), 5.08 (s, 1H), 4.27 (d, 1H, *J*=9.6 Hz), 4.10–3.92 (m, 2H), 3.87–3.54 (m, 6H), 3.50–3.38 (m, 4H), 3.30–3.25 (m, 4H), 2.83–2.78 (m, 2H), 2.64–2.52 (m, 4H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 156.77, 154.40, 153.90, 153.88, 150.75, 147.27, 147.25, 146.90, 146.55, 146.51, 146.26, 146.22, 146.17, 146.11, 146.09, 146.05, 145.90, 145.87, 145.75, 145.57, 145.53, 145.48, 145.41, 145.27, 145.23, 145.19, 145.08, 144.69, 144.61, 144.38, 143.12, 142.95, 142.63, 142.54, 142.51, 142.28, 142.13, 142.08, 141.97, 141.92, 141.79, 141.63, 141.48, 140.10, 140.03, 139.85, 139.55, 136.77, 136.58, 135.73, 135.68, 130.37, 128.05, 115.87, 82.06, 76.58, 72.01, 70.77, 70.66, 70.61, 70.53, 69.19, 67.67, 59.06, 54.78, 51.97, 48.31, 45.67; FTIR (KBr) ν (cm^{-1}) 2920, 2851, 1611, 1514, 1425, 1244, 1109, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 237, 269, 431, 703; MS (ESI) *m/z* 1084 $[M+H]^+$.

3.1.10. Compound 12d. Yield: 24% (44% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.71 (broad d, 2H), 7.33–7.28 (m, 1H), 7.03–6.87 (m, 6H), 5.21 (d, 1H, *J*=9.6 Hz), 5.11 (s, 1H), 4.28 (d, 1H, *J*=9.6 Hz), 4.12–3.93 (m, 2H), 3.84–3.72 (m, 4H), 3.60–3.58 (m, 2H), 3.55–3.30 (m, 13H), 2.91–2.80 (m, 2H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 154.19, 153.70, 147.30, 146.78, 146.48, 146.28, 146.21, 146.13, 145.93, 145.78, 145.53, 145.32, 145.25, 145.14, 144.72, 144.61, 144.38, 143.14, 142.98, 142.67, 142.56, 142.26, 142.16, 142.10, 142.03, 141.92, 141.85, 141.65, 141.51, 140.13, 140.08, 139.90, 139.57, 136.96, 136.61, 135.73, 135.64, 130.59, 129.45, 116.41, 82.05, 72.02, 70.76, 70.68, 70.63, 69.11, 67.57, 59.10, 52.09, 49.58, 48.83, 48.47; FTIR (KBr) ν (cm^{-1}) 2920, 2853, 1597, 1500, 1448, 1383, 1227, 1109, 754, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 261, 431, 703; MS (ESI) *m/z* 1146 $[M+H]^+$.

3.1.11. Compound 12g. Yield: 29% (39% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.71 (broad d, 2H, *J*=4.8 Hz), 7.07 (m, 6H), 5.21 (d, 1H, *J*=9.5 Hz), 5.11 (s, 1H), 4.28 (d, 1H, *J*=9.5 Hz), 4.12–3.93 (m, 2H), 3.86–3.73 (m, 4H), 3.60–3.57 (m, 2H), 3.50–3.35 (m, 9H), 3.27–3.23 (m, 4H), 2.91–2.83 (m, 2H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 154.22, 153.65, 147.30, 146.78, 146.48, 146.28, 146.20, 146.12, 145.93, 145.78, 145.53, 145.47, 145.31, 145.26, 145.22, 145.13, 144.72, 144.59, 144.37, 143.14, 142.98, 142.67, 142.56, 142.26, 142.15, 142.08, 142.03, 141.98, 141.92, 141.83, 141.65, 141.50, 140.13, 140.07, 139.88, 139.56, 136.97, 136.88, 136.62, 135.73, 135.64, 130.87, 130.58, 128.79, 128.56, 128.49, 118.54, 115.89, 82.05, 72.01, 70.76, 70.67, 70.63, 69.12, 68.14, 67.56, 59.10, 52.08, 50.40, 48.80; FTIR (KBr) ν (cm^{-1}) 2920, 2851, 1609, 1560, 1508, 1448, 1229, 1109, 824, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 233, 260, 431, 704; MS (ESI) *m/z* 1164 $[M+H]^+$.

3.1.12. Compound 13a. Yield: 38% (56% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.65 (broad d, 2H), 6.96 (d, 2H, *J*=8.8 Hz), 5.20 (d, 1H, *J*=9.8 Hz), 5.08 (s, 1H), 4.27 (d, 1H, *J*=9.8 Hz), 4.09–3.94 (m, 2H), 3.85–3.73 (m, 4H), 3.60–3.57 (m, 2H), 3.48–3.38 (m, 5H), 3.19–3.16 (m, 4H), 2.88–2.82 (m, 2H), 1.76–1.67 (m, 4H), 1.62–1.54 (m, 2H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 156.83, 154.46, 154.06, 154.02, 152.02, 147.27, 147.24, 146.99, 146.62, 146.59, 146.27, 146.21, 146.17, 146.11, 146.09, 146.04, 145.90, 145.87, 145.77, 145.59, 145.55, 145.48, 145.41, 145.27, 145.23, 145.18, 145.08, 144.68, 144.64, 144.39, 144.37, 143.11, 142.94, 142.62, 142.53, 142.50, 142.50, 142.30, 142.28, 142.12, 142.09, 141.99, 141.98, 141.94, 141.80, 141.62, 141.48, 140.08, 140.01, 139.85, 139.57, 136.73, 136.62, 135.71, 135.69, 130.22, 127.07, 116.11, 82.17, 76.65, 72.02, 70.78, 70.68, 70.60, 69.20, 67.68, 59.0935, 51.97, 50.24, 25.83, 24.22; FTIR (KBr) ν (cm^{-1}) 2920, 2851, 1609, 1558, 1508, 1429, 1234, 1115, 1024, 766, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 234, 260, 431, 703; MS (ESI), *m/z* 1069 $[M+H]^+$.

3.1.13. Compound 13b. Yield: 28% (46% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.69 (broad d, 2H), 6.94 (d, 2H, *J*=8.7 Hz), 5.20 (d, 1H, *J*=9.6 Hz), 5.09 (s, 1H), 4.28 (d, 1H, *J*=9.6 Hz), 4.12–3.94 (m, 2H), 3.90–3.71 (m, 8H), 3.60–3.57 (m, 2H), 3.50–3.33 (m, 5H), 3.21–3.18 (m, 4H), 2.89–2.83; ^{13}C NMR ($CDCl_3/CS_2$, 500 MHz) δ 153.9481, 153.51, 153.22, 152.91, 147.31, 146.30, 146.21, 146.14, 145.93, 145.80, 145.58, 145.49, 145.32, 145.26, 145.17, 144.73, 144.57, 144.35, 143.13, 142.97, 142.66, 142.58, 142.19, 142.07, 142.00, 141.91, 141.63, 141.48, 140.12, 140.07, 139.89, 139.56, 137.11, 136.92, 136.82, 135.56, 132.35, 131.80, 130.89, 130.74, 128.82, 124.75, 124.44, 123.96, 119.07, 116.05, 115.75, 115.54, 115.40, 114.05, 113.45, 82.13, 72.00, 71.78, 70.65, 67.09, 66.49, 59.09, 52.12, 51.56, 49.31, 49.02, 47.29; FTIR (KBr) ν (cm^{-1}) 2918, 2851, 1653, 1609, 1516, 1508, 1458, 1338, 1228, 1120, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 261, 431, 704; MS (ESI) *m/z* 1071 $[M+H]^+$, 1093 $[M+Na]^+$.

3.1.14. Compound 15a. Yield: 20% (78% based on recovered C_{60}); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.74 (d, 2H, *J*=8.6 Hz), 7.47 (d, 2H, *J*=8.6 Hz), 6.96–6.89 (m, 4H),

5.66 (s, 1H), 4.98–4.94 (m, 1H), 4.17 (q, 2H, J=7.1 Hz), 3.98–3.93 (m, 1H), 3.65–3.60 (m, 4H), 3.49–3.41 (m, 2H), 3.18–3.12 (m, 4H), 1.28 (t, 3H, J=7.1 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 155.49, 154.64, 154.01, 153.84, 153.66, 153.51, 151.01, 150.74, 147.22, 147.19, 146.83, 146.41, 146.36, 146.33, 146.24, 146.21, 146.16, 146.09, 146.07, 145.91, 145.88, 145.70, 145.51, 145.51, 145.40, 145.35, 145.30, 145.29, 145.18, 145.17, 145.14, 144.63, 144.62, 144.37, 144.33, 143.19, 143.09, 142.98, 142.67, 142.63, 142.61, 142.57, 142.38, 142.22, 142.22, 142.19, 142.15, 142.08, 142.02, 142.01, 141.97, 141.86, 141.64, 141.52, 140.09, 139.94, 139.64, 139.61, 137.47, 136.71, 136.19, 135.71, 131.88, 131.21, 130.73, 130.38, 129.49, 129.19, 116.30, 115.80, 113.88, 99.58, 78.01, 74.90, 74.68, 72.41, 61.54, 49.09, 43.62, 43.57, 40.94, 38.87; FTIR (KBr) ν (cm^{-1}) 3414, 2924, 2760, 1701, 1676, 1655, 1611, 1597, 1516, 1437, 1383, 1225, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 265, 431, 69; MS (ESI) m/z 1102 $[M+H]^+$.

3.1.15. Compound 15b. Yield 35% (72% based on recovered C_{60}) ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.70 (d, 2H, J=8.8 Hz), 7.42 (d, 2H, J=8.4 Hz), 6.91–6.84 (m, 4H), 5.63 (s, 1H), 4.98–4.93 (m, 1H), 3.97–3.92 (m, 1H), 3.73–3.63 (m, 6H), 3.50–3.32 (m, 2H), 3.25–3.22 (m, 4H), 2.74–2.65 (m, 6H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 155.89, 155.37, 154.63, 154.33, 153.64, 152.23, 152.08, 150.95, 149.38, 147.25, 147.18, 146.91, 146.83, 146.30, 146.24, 146.18, 146.11, 146.05, 145.96, 145.85, 145.72, 145.48, 145.33, 145.25, 145.14, 145.10, 145.01, 144.64, 144.58, 144.41, 144.36, 144.28, 144.11, 144.03, 143.13, 142.75, 142.61, 142.29, 142.17, 141.97, 141.68, 141.59, 141.50, 140.34, 140.26, 140.05, 139.91, 139.84, 139.69, 139.68, 139.56, 139.48, 137.48, 136.04, 135.45, 131.13, 131.02, 130.77, 130.67, 130.33, 129.31, 128.54, 124.93, 115.81, 115.36, 114.76, 85.69, 74.85, 74.74, 72.47, 72.37, 71.27, 70.51, 70.20, 67.60, 67.48, 62.03, 61.88, 57.89, 53.2186, 53.07, 52.90, 48.57, 47.64, 46.89, 42.28, 38.89, 31.67, 29.75, 26.95, 19.31, 13.91; FTIR (KBr) ν (cm^{-1}) 3420, 2922, 2851, 1653, 1604, 1514, 1462, 1366, 1064, 527; UV–vis ($CHCl_3$), λ_{max} (nm) 234, 257, 430, 695; MS (ESI) m/z 1118 $[M+H]^+$.

Acknowledgements

Support of this work by MCYT of Spain (BQU-2002-00855) is gratefully acknowledged.

References

1. (a) Da Ros, T.; Prato, M. *Chem. Commun.* **1999**, 663–669. (b) Jensen, A. W.; Wilson, S. R.; Schuster, D. I. *Bioorg. Med. Chem.* **1996**, *4*, 767–779.
2. (a) Friedman, S. H.; DeCamp, D. L.; Sijbesma, R. P.; Srdanov, G.; Wudl, F.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6506–6508. (b) Sijbesma, R.; Srdanov, G.; Wudl, F.; Castoro, J. A.; Wilkins, C.; Friedman, S. H.; DeCamp, D. L.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6510–6512. (c) Friedman, S. H.; Ganapathi, P. S.; Rubin, Y.; Kenyon, G. L. *J. Med. Chem.* **1998**, *41*, 2424–2429.
3. Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugiura, Y. *J. Am. Chem. Soc.* **1993**, *115*, 7918–7919.
4. Boutorine, A. S.; Tokuyama, H.; Takasugi, M.; Isobe, H.; Nakamura, E.; Hélène, C. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2462.
5. (a) 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. (b) Hirsch, A.; Lamparth, I.; Karfunkel, H. R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 437.
6. (a) Wei, X.; Migfei, W.; Qi, L.; Xu, Z. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1389–1393. (b) Sawamura, M.; Nagahama, N.; Toganoh, M.; Hackler, U. E.; Isobe, H.; Nakamura, E.; Zhou, S.-Q.; Chu, B. *Chem. Lett.* **2000**, 1098–1099.
7. (a) Andersson, T.; Nilsson, K.; Sundahl, M.; Westman, G.; Wennerstrom, O. *J. Chem. Soc., Chem. Commun.* **1992**, 604–606. (b) Priyadarsini, K. I.; Mohan, H.; Tyagi, A. K.; Mittal, J. P. *J. Phys. Chem.* **1994**, *98*, 4756–4759. (c) Ikeda, A.; Hatano, T.; Kawaguchi, M.; Suenaga, H.; Shinkai, S. *Chem. Commun.* **1999**, 1403–1404. (d) Rio, Y.; Nierengarten, J.-F. *Tetrahedron Lett.* **2002**, *43*, 4321–4324.
8. (a) Brettreich, M.; Hirsch, A. *Tetrahedron Lett.* **1998**, *39*, 2731–2734. (b) Takenaka, S.; Yamashita, K.; Takagi, M.; Hatta, T.; Tsuge, O. *Chem. Lett.* **1999**, 321–322. (c) Richardson, C. F.; Schuster, D. I.; Wilson, S. R. *Org. Lett.* **2000**, *2*, 1011–1014.
9. (a) Da Ros, T.; Prato, M.; Novello, F.; Maggini, M.; Banfi, E. *J. Org. Chem.* **1996**, *61*, 9070–9072. (b) Rio, Y.; Nicoud, J.-F.; Rehspringer, J.-L.; Nierengarten, J.-F. *Tetrahedron Lett.* **2000**, *41*, 10207. (c) Wharton, T.; Kini, V. U.; Mortis, R. A.; Wilson, L. J. *Tetrahedron Lett.* **2001**, *42*, 5159–5162.
10. Illescas, B. M.; Martínez-Grau, M. A.; Torres, M. L.; Fernández-Gadea, J.; Martín, N. *Tetrahedron Lett.* **2002**, *43*, 4133–4136.
11. (a) Hibert, M. F.; Gittos, M. W.; Middlemiss, D. N.; Mir, A. K.; Fozard, J. R. *J. Med. Chem.* **1988**, *31*, 1087. (b) Misztal, S.; Bojarski, A.; Mackowiak, M.; Boksa, J.; Bieleeka, Z.; Mokrosz, J. L. *Med. Chem. Res.* **1992**, *2*, 82. (c) Chilmonczyk, Z.; Lcs, A.; Wozniakowska, A.; Cybulski, J.; Koziol, A.; Gdaniec, M. *J. Med. Chem.* **1995**, *38*, 1701.
12. Glennon, R. A.; Dukat, M.; Westkaemper, R. B. In *Psychopharmacology: The Fourth Generation of Progress*; CD-Rom Ed. Bloom, F. E., Kupfer, D. J., Eds.; Raven: New York, 1999.
13. Kuipers, W.; Kruse, C. G.; van Wijngaarden, I.; Standaar, P. J.; Tulp, M. Th. M.; Veldman, N.; Spek, A. L.; Ijzerman, A. P. *J. Med. Chem.* **1997**, *40*, 300.
14. Gerge, P.; Borg, F.; O'Connor, S. *Eur. J. Med. Chem.* **1995**, *30*, 299.
15. (a) Barbaro, R.; Betti, L.; Botta, M.; Corelli, F.; Giannaccini, G.; Maccari, L.; Manetti, F.; Strappaghetti, G.; Corsano, S. *J. Med. Chem.* **2001**, *44*, 2118. (b) Betti, L.; Botta, M.; Corelli, F.; Floridi, M.; Giannaccini, G.; Maccari, L.; Manetti, F.; Strappaghetti, G.; Tafi, A.; Corsano, A. *J. Med. Chem.* **2002**, *45*, 3603. (c) Betti, L.; Floridi, M.; Giannaccini, G.; Manetti, F.; Strappaghetti, G.; Tafi, A.; Botta, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 171–173.
16. Reviews: (a) Prato, M.; Maggini, M. *Acc. Chem. Res.* **1998**, *31*, 519. (b) Tagmatarchis, N.; Prato, M. *Synlet* **2003**, 768.
17. (a) Tanaka, A.; Terasawa, T.; Hagihara, H.; Sakuma, Y.; Ishibe, N.; Sawada, M.; Takasugi, H.; Tanaka, H. *J. Med. Chem.* **1998**, *41*, 2390. (b) Bader, H.; Hansen, A. R.; McCarty, F. J. *J. Org. Chem.* **1966**, *31*, 2319.

18. Barrow, M. P.; Feng, X.; Wallace, J. I.; Boltalina, O. V.; Taylor, R.; Derrick, P. J.; Drewello, T. *Chem. Phys. Lett.* **2000**, *330*, 267.
19. Rio, Y.; Accorsi, G.; Nierengarten, H.; Rehspringer, J.-L.; Hönerlage, B.; Kopitkovas, G.; Chugreev, A.; Van Dorsselaer, A.; Armaroli, N.; Nirengarten, J.-F. *New J. Chem.* **2002**, *9*, 1146.
20. Charbonnier, F.; Berthelot, L.; Rolando, C. *Anal. Chem.* **1999**, *71*, 1585.
21. Van Berkel, G. J. In *Electrospray Ionization Mass Spectrometry: Fundamentals, Instrumentation and Applications*; Cole, R. B., Ed.; Wiley: New York, 1997; pp 65–105 and references cited therein.
22. Rondeau, D.; Kreher, D.; Cariou, M.; Hudhomme, P.; Gorgues, A.; Richomme, P. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1708.
23. Rondeau, D.; Martineau, C.; Blanchard, P.; Roncali, J. *J. Mass Spectrom.* **2002**, *37*, 1081.
24. Fati, D.; Leeman, V.; Vasil'ev, Y. V.; Drewello, T.; Leyh, B.; Hungerbühler, H. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 1448.
25. The measured solubility is in the range of that determined for other related fulleropyrrolidines endowed with oligoether chains. See: Bosi, S.; Da Ros, T.; Castellano, S.; Banfi, E.; Prato, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 10431.