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Synthesis of new C_{60} derivatives containing biologically active 4-aryl-1,4-dihydropyridines

Beatriz Illescas,[a] M. Angeles Martínez-Grau,[a] M. Luisa Torres,[a] Javier Fernández-Gadea[b] and Nazario Martín[a,*]

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

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Abstract—Water-soluble pyrrolidino[3,4:1,2][60]fullerene derivatives bearing the biologically active 1,4-dihydropyridine (1,4-DHP) system have been synthesized through different chemical approaches depending upon the linkage position of the fulleropyrrolidine to the 1,4-DHP ring.

Fullerenes and their derivatives have shown a broad range of promising biological activities,[1,2] especially in the fields of photodynamic therapy,[3] inhibition of HIV-proteasa,[4,5] neuroprotection[6] and apoptosis.[1,2] However, an important feature when dealing with fullerenes is the lack of solubility in polar solvents for their biological study. This problem can be overcome by means of chemical modification of fullerenes in such a way that they acquire solubility in polar media. In this regard, Prato et al.[7] have recently shown that the covalent attachment of polyether chains to [60]fullerene brings about the formation of water-soluble fulleropyrrolidines.

On the other hand, there is unabated interest in the study of 4-aryl-1,4-dihydropyridines (DHPs) as a consequence of their pharmacological activity as the most important class of the calcium channel modulators.[8] A big effort has been devoted to the synthesis of DHPs with different substituents[9] or heteroatoms,[10] thus enabling the definition of a structure–activity relationship for these types of compounds. The knowledge of stereochemical/conformational requirements for activity[11] makes it necessary the study of other analogues of the DHP ring.[12]

Here we report the synthesis of new fulleropyrrolidines (**7a–b**, **10**, **16a–b**) bearing DHPs as biologically active substituents related to the well-known nifedipine (3,5-dimethoxycarbonyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine). These fullerene derivatives have been synthesized by 1,3-dipolar cycloaddition of the in situ generated azomethine ylides to C_{60}, by reaction of the corresponding formyl substituted DHP with sarcosine and [60]fullerene. In order to improve the solubility of the new compounds in polar solvents, we have also synthesized the *N*-(3,6,9-trioxadecyl)fulleropyrrolidines, thus introducing a hydrophilic chain in the structures.

As there is not a general method for the preparation of formyl substituted 1,4-dihydropyridines (DHPs), it was necessary to employ a different synthetic approach depending on the position of the formyl group (Scheme 1).

Thus, the preparation of 3,5-diethoxycarbonyl-4-(4-formylphenyl)-2,6-dimethyl-1,4-dihydropyridine **6** was carried out in three synthetic steps, as depicted in Scheme 1. The Koevenagel condensation[13] of ethyl acetoacetate **1** and *p*-diethoxymethylbenzaldehyde **2** yielded compound **3** as a *Z*/*E* isomer mixture in a 1/1 proportion. Both isomers were separated by flash chromatography and the *Z*-isomer was assigned by comparison of the chemical shifts of the vinyl hydrogens with other α-acetylcinnamates.[14] Reaction of *Z*-**3** with ethyl 2-aminocrotonate gave the DHP **5** in good yield (82%). Acidic hydrolysis of **5** under smooth conditions led to the formyl substituted DHP **6**[15] in 89% yield.

The formyl-DHP **9** was obtained by reaction of benzylidenemalonaldehyde (**8**), which was in turn prepared in

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

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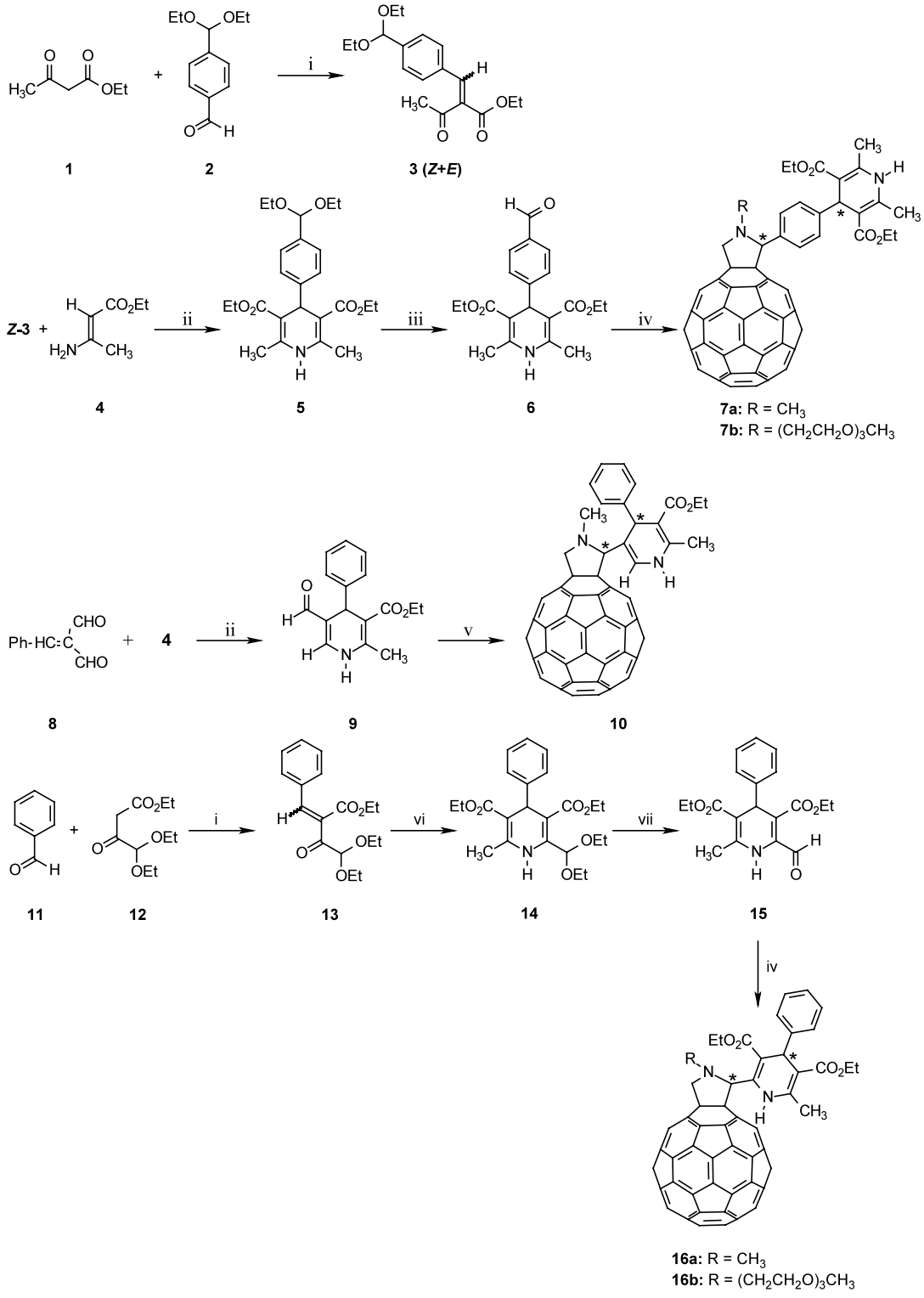

Scheme 1. *Reagents and conditions*: (i) Piperidine, toluene, Δ; (ii) EtOH, Δ; (iii) *p*TsOH, acetone; (iv) a: C_{60}, *N*-methylglycine, toluene, Δ, b: C_{60}, *N*-(3,6,9-trioxadecyl)glycine, ODCB, Δ; (v) C_{60}, *N*-methylglycine, toluene, Δ; (vi) **4**, EtOH, Δ; (vii) acetone, 6N HCl.

a multistep synthetic procedure,[16] with ethyl 2-aminocrotonate **4** in a low yield (10%).

Finally, the 2-formyl DHP **15** was prepared starting from the Knoevenagel condensation of ethyl γ-diethoxyacetoacetate[17] (**12**) with benzaldehyde (**11**), using piperidine as the catalyst. Compound **13** was employed in the next synthetic step without further purification yielding, on reaction with ethyl 2-aminocrotonate **4**, the formyl protected DHP **14**[18] in 28% yield. Acidic hydrolysis of the acetal group in **14** with 6N HCl afforded compound **15** in 79% yield.

The ^{1}H NMR spectra for the formyl substituted DHPs **6**, **9** and **15** show the signal for the aldehyde group at ~9.2–10.5 ppm. The amino group gives rise to a broad singlet at 5.7 ppm for **6**, 6.9 ppm for **15** and a broad doublet at 6.6 ppm for compound **9**. The signal for the proton (H-4) attached to the sp^3 carbon of the 1,4-dihydropyridine ring appears at δ ~5.0–5.1 as a singlet. For compound **9**, the hydrogen atom at C-6 of the DHP is observed as a doublet at 6.87 ppm with a coupling constant of 5.7 Hz.

The signals in the ^{13}C NMR spectra of the DHPs **6**, **9** and **15** are also in agreement with the proposed structures. Thus, the aldehyde group is observed around 183–192 ppm, the carbonyl group of the ester appears at δ ~167 and the C-4 of the DHP ring at δ ~37–42.

The target fulleropyrrolidines were synthesized by refluxing in toluene a mixture of the corresponding formyl substituted DHP (1 equiv.), C_{60} (1 equiv.) and sarcosine during a variable period of time (4 h for **7a**, 48 h for **10** and 24 h for **16a**). In order to increase the solubility of the fullerene derivatives in polar solvents, we also prepared the *N*-(3,6,9-trioxadecyl)glycine following the procedure previously reported by Prato et al.[7] It is worth mentioning that, with this amino acid, the best yields were obtained using ODCB instead of toluene as the solvent. For DHP **9**, no reaction was observed after refluxing in ODCB for 48 h when *N*-(3,6,9-trioxadecyl)glycine was employed instead of sarcosine.

The spectroscopic data for the novel fulleropyrrolidines (**7a–b**, **10**, **16a–b**) are in agreement with the proposed structures.[19] Thus, these compounds showed a typical weak absorption band at around 430 nm in the UV–vis spectra, similar to that of most dihydrofullerenes. The ^{1}H NMR spectra showed the presence of the pyrrolidine protons in the region between 4.2 and 5.3 ppm, as one singlet and two doublets with a coupling constant of ~9.3–9.9 Hz. The proton attached to the C-4 of the DHP ring gives rise to a singlet around ~5.0–5.3 ppm and the N–H appears as a broad singlet for **7a–b** and **16a–b** at δ ~5.5–6.4 and for compound **10** it is observed as a broad doublet with a coupling constant of 5.1 Hz. It is important to note that compound **10** is isolated as a diastereomeric mixture in 60:40 proportion which could not be separated by column chromatography. This diastereomeric mixture was not observed by TLC or ^{1}H NMR analysis for **7** and **16** due, probably, to the longer distance between both stereogenic centers.

A restricted rotation of phenyl substituents on the pyrrolidine ring has been described for phenylfulleropyrrolidine derivates.[20] We were able to observe this dynamic effect in compounds **7a–b** which showed broad signals for the *ortho* aromatic protons (δ = 7.60) close to the fullerene surface. In contrast, a sharp doublet (J = 5.1 Hz) was observed for the vinyl proton close to the C_{60} surface in compound **10**. This vinyl proton is slightly shifted (δ ~6.77) in comparison with its precursor **9** (δ = 6.87, J = 5.7 Hz), thus showing the influence of the close C_{60} surface. Finally, due to the dynamic behavior of the NH proton in compounds **16a–b** we were not able to observe the restricted rotation on them.

It is worth mentioning that the non-planar geometry of the 1,4-DHP ring could account for the different dynamic behavior observed in compounds **7a–b** bearing the phenyl ring adjacent to the pyrrolidine moiety and compounds **10** and **16a–b** in which the 1,4-DHP ring is directly linked to the fulleropyrrolidine.

^{13}C NMR spectra could be recorded for the more soluble compounds, that is, **7a–b** and **16b**. The number of signals of these spectra show the lack of symmetry in the compounds. The carbonyl carbon of the ester group appears at δ ~167 and the signals for the sp^3 carbons of the pyrrolidine ring and those at the 6,6-ring junction of the C_{60} cage are observed at δ ~81–84 and δ ~70–72, respectively.

In order to carry out a biological study of DHP derivatives (**7a–b**, **10**, **16a–b**) their solubility in polar solvents is an important aspect. In this sense, we performed solubility tests finding that the *N*-oligoether fulleropyrrolidines are quite soluble in a 9/1 ratio of H_2O/DMSO, thus paving the way for further biological evaluation.

In this regard, preliminary biological tests are in progress to determine the range of potential activity of this class of compounds. Work is currently directed to prepare other fullerene derivatives containing differently substituted 1,4-dihydropyridines covalently attached to the C_{60} core as promising candidates as calcium channel modulators.

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19. Selected spectroscopic data for compound **7b**:
IR FT (KBr) ν 3400, 2923, 2850, 1701, 1618, 1460, 1408, 1207, 527 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.60 (d, 2H, $J=7.83$ Hz), 7.26 (m, 2H), 5.57 (s, 1H), 5.22 (d, 1H, $J=9.6$ Hz), 5.09 (s, 1H), 4.90 (s, 1H), 4.27 (d, 1H, $J=9.6$ Hz), 3.97 (m, 6H), 3.72 (m, 6H), 3.54 (m, 2H), 3.36 (m, 4H), 2.84 (m, 1H), 2.33 (s, 6H), 1.14 (t, 3H, $J=7.1$ Hz), 1.03 (t, 3H, $J=7.3$ Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.95, 166.64, 166.55, 156.31, 156.04, 154.86, 154.06, 153.83, 152.94, 152.68, 147.87, 147.02, 146.63, 146.24, 146.07, 145.95, 145.88, 145.71, 145.66, 145.50, 145.26, 145.08, 145.02, 144.98, 144.90, 144.50, 144.45, 144.36, 144.28, 144.19, 144.12, 143.31, 143.23, 142.93, 142.87, 142.80, 142.74, 142.37, 142.32, 142.00, 141.83, 141.69, 141.48, 141.41, 140.00, 139.92, 139.85, 139.75, 139.57, 139.15, 138.99, 137.37, 136.89, 136.52, 136.37, 136.22, 135.73, 135.51, 135.42, 134.36, 128.52, 128.41, 126.56, 103.85, 82.14, 81.95, 71.90, 70.55, 70.26, 68.92, 67.61, 67.51, 60.95, 59.38, 59.32, 58.70, 52.13, 39.41, 29.81, 22.58, 19.27, 14.31, 14.10, 13.85; UV–vis ($CHCl_3$), λ_{max} 255, 310, 430, 695.
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