Synthesis and Characterization of the First Fullerene–Peptide

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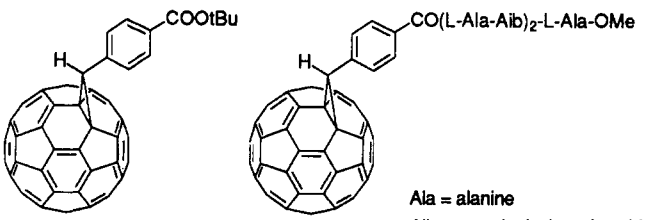
Summary: The synthesis and full characterization of a methanofullerene which contains a covalently linked pentapeptide is reported.

The C_{60} (buckminsterfullerene) functionalization is a very young research field, opened only recently by the availability of gram quantities of this new all-carbon molecule.[1] Several single and well characterized C_{60}-containing compounds have been reported:[2] the major problem of obtaining inseparable mixtures of multiple addition products has been partially solved by controlled additions of reactive dienes and 1,3-dipoles.[3] Most of the results obtained in this field are mainly concerned with the reactivity of C_{60} and the characterization of the addition products. Only very recently some attention has been given to the possibility of preparing suitable derivatives for the investigation of the biological activity of this novel system. Due to the particularly remarkable results obtained in the first experiments of pharmacological applications,[4] we have started a research program for the synthesis of C_{60} derivatives which contain potentially biologically active moieties. In this paper we report the preparation and the full characterization of the first peptide-containing fullerene derivative.

The goal of covalently linking a peptide to other molecular fragments is usually achieved by preparing compounds that can be attached to the partially protected peptide by means of some standard methods. Here the problem was to prepare a C_{60} derivative containing either a carboxylic acid or an amine derivative. Due to the reactivity of amines toward C_{60}[5] and the presumed instability of a fullerenic amine, we decided to prepare a fullerenecarboxylic acid. To this aim, [4-(*tert*-butoxycarbonyl)phenyl]diazomethane was allowed to react with C_{60} at ambient temperature, affording a crude mixture of isomeric products.[6] This mixture, purified over SiO_2 from unreacted C_{60}, was refluxed for 3 days in chlorobenzene, and the conversion to a single isomer was ascertained by ^{1}H-NMR spectroscopy. The methanofullerene structure of adduct **1** was confirmed by the ^{1}H–^{13}C coupling constant of the methine proton (J = 162.5 Hz, typical of cyclopropane rings[7]) and by the detection of an aliphatic resonance for the sp^3 carbons of the fullerene moiety (74.81 ppm).



1 **2**

The *tert*-butyl ester was hydrolyzed in dioxane with excess trifluoromethanesulfonic acid.[8] The precipitated

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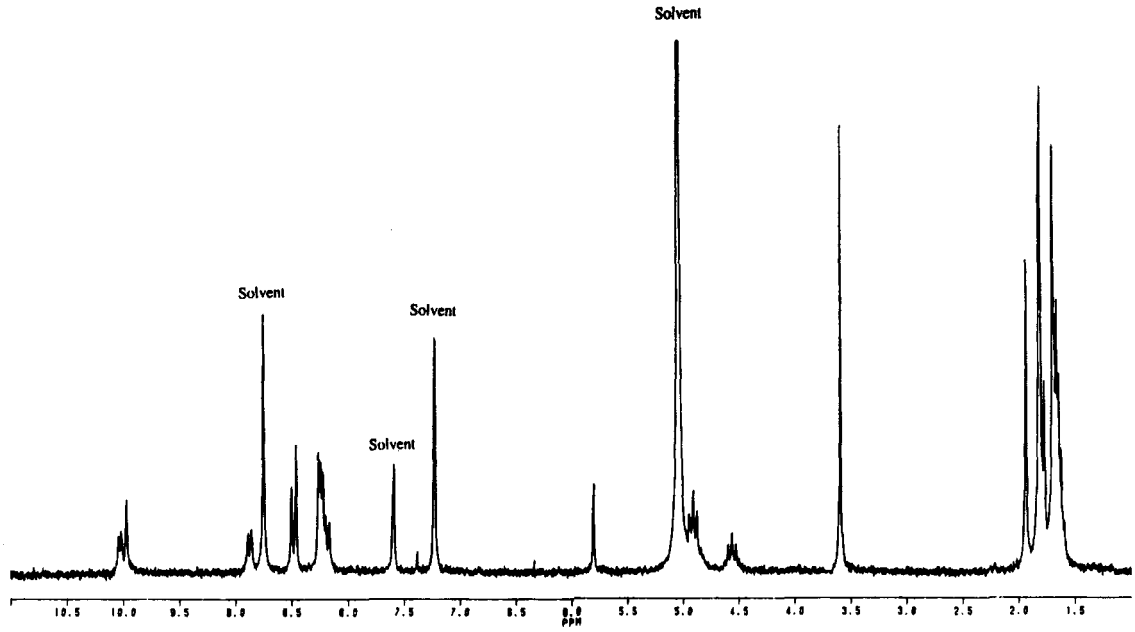

Figure 1. ^{1}H-NMR spectrum of compound **2** in pyridine-d_5 (5 mM).

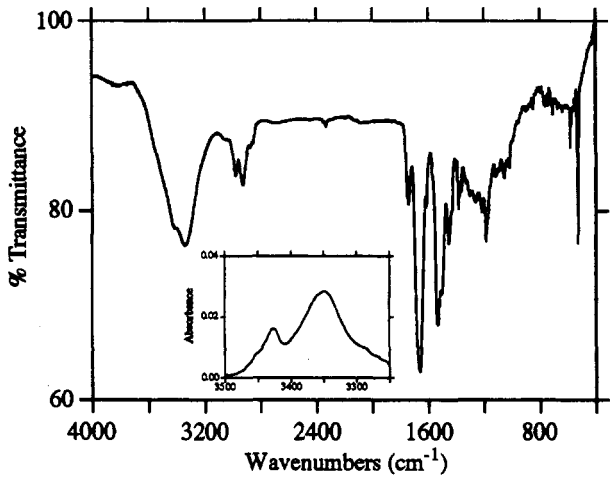

Figure 2. FT-IR spectrum of compound **2** (KBr). Inset: detailed N–H stretching region in $CDCl_3$ solution (0.05 mM).

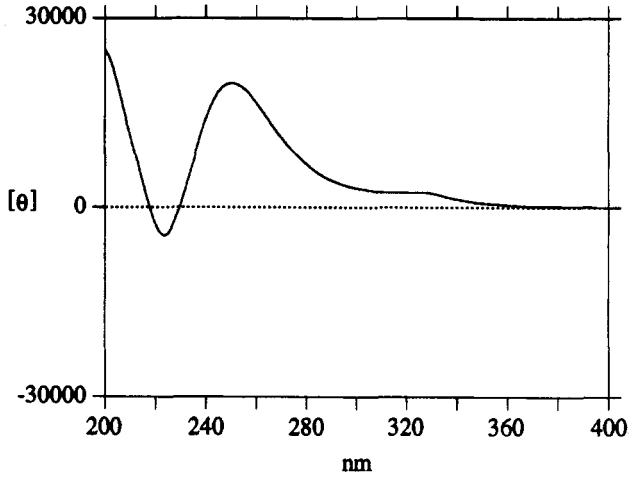

Figure 3. CD spectrum of compound **2** in 1-octanol (0.34 mM).

acid was centrifuged and washed several times with water and acetone and then dried in vacuo. Although insoluble in most solvents, the carboxylic acid could be dissolved in pyridine-d_5 for ^{1}H-NMR analysis, which showed the aromatic AA′BB′ system along with a singlet for the methine proton at 5.78 ppm. The acid was subsequently suspended in benzene and treated with an excess of oxalyl chloride. When all the suspension was dissolved, stirring was continued for 4 h. The solvent and the excess of oxalyl chloride were evaporated in vacuo and the residue was dissolved in anhydrous $CHCl_3$ and then treated with 1.5 equiv of the N-deprotected pentapeptide H-(L-Ala-Aib)$_2$-L-Ala-OMe (Ala = alanine; Aib = α-aminoisobutyric acid).[9] The main product was purified by flash chromatography affording the fullerene–peptide **2** in 31% yield.

The spectroscopic data show that compound **2** possesses both C_{60} and peptide properties. The ^{1}H-NMR spectrum Figure 1 shows *inter alia* that the ratio of Ala vs Aib residues is 3:2. This was confirmed by amino acid analysis. The presence of the methyl ester was evident by ^{1}H-NMR and by the 1740 cm^{-1} band in the IR absorption spectrum Figure 2.

The UV–vis spectra of compounds **1** and **2** are very similar to that of C_{60} even though they display the same loss of the C_{60} absorption fine structure above 450 nm typical of methanofullerenes.[6] The FAB mass spectrum shows a weak signal at m/z 1252 for the $[MH]^+$ ion (0.9 %), with the base peak at *m/z* 720 (C_{60}^+).

The CD spectrum of **2** Figure 3 shows that only a band (330 nm) is optically active above 300 nm, although weakly. This result is not surprising in view of the significant separation of the N-terminal Ala chiral C$^\alpha$ from the fullerene chromophoric group. Below 300 nm the CD is indicative of the overlapping of the classical doublet of the *para*-substituted benzamide chromophore (centered

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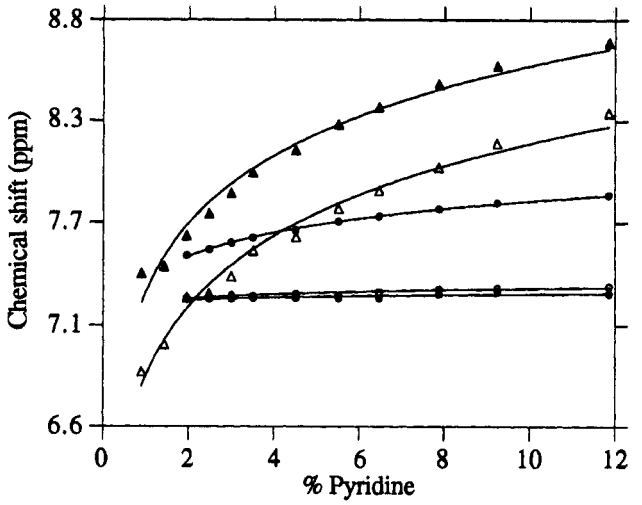

Figure 4. Plot of N–H chemical shifts in ^{1}H-NMR spectra of compound **2** (1.02 mM in $CDCl_3$) as a function of increasing percentage of pyridine-d_5(v/v): ▲ = L-Ala, △ = Aib, ● = L-Ala, ○ = Aib.

near 235 nm)[10] with the optically active bands arising from the fullerene absorption in the 260–215 nm region.[11]

A preliminary conformational analysis was carried out for the N-acylated-(L-Ala-Aib)$_2$-L-Ala-OMe pentapeptide **2** by FT-IR and ^{1}H-NMR. The FT-IR spectrum in $CDCl_3$ (concd 0.05 mM) in the 3500–3300 cm^{-1} (N–H stretching) region exhibits a weak band at 3427 cm^{-1} (free, solvated NH groups) followed by a more intense and broad band at 3349 cm^{-1} (H-bonded NH groups) (Figure 2, inset). At this high dilution it is reasonable to assume that the observed extensive H-bonding would not be related to self-association.[12]

Additional information was obtained by solvent titration of the peptide (amide) NH groups using ^{1}H-NMR. The delineation of inaccessible (or intramolecularly H-bonded) NH groups was performed with use of pyridine-d_5 dependency of NH proton chemical shifts in deuteriochloroform. Figure 4 clearly shows two classes of NH protons: (i) the first class (one Ala and one Aib) includes protons whose chemical shifts are remarkably sensitive to the addition of the H-bonding acceptor solvent pyridine-d_5; (ii) the second class (the three other NH protons) includes those displaying a behavior characteristic of shielded protons (very modest sensitivity of the chemical shifts to the solvent composition).

In view of all these observations it can be concluded that the ordered secondary structure predominantly adopted in solution by the fullerene–pentapeptide **2** is the 3_{10}-helix[13] rather than the α-helix, which, in particular, would require the NH protons involved in the intramolecular H-bonding to be only two (the Ala and Aib NH protons near the C-terminus) instead of three (the two Ala and the Aib residues near C-terminus) required by the 3_{10}-helix. These conformational conclusions appear to be in full agreement with the published data for a variety of short Aib-rich peptides.[14]

The present work clearly indicates that chemically and optically pure peptide-based fullerenes can be easily prepared. As a first step in this line of research, we exploited an Aib-rich peptide as these conformationally constrained compounds are currently being used as templates for molecular recognition studies[15] and, owing to their extremely high tendency to crystallize,[11,13] are promising candidates for an X-ray diffraction investigation. Attempts to obtain suitable crystals of **2** are in progress.

Although this work has been performed on a model peptide, it is reasonable to assume that the approach described here would be applicable to either peptides endowed with high biological activity and water-soluble peptides. Work along these directions is presently underway.

Supplementary Material Available: Experimental procedures and characterization of **1** and **2** (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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