Synthesis of α-Amino Acid Derivatives of C_{60} from 1,9-(4-Hydroxycyclohexano)-buckminsterfullerene

Yi-Zhong An, Jamey L. Anderson, and Yves Rubin*

Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90024-1569

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Summary: A simple, high-yielding route to C_{60}-derivatives of biological importance is presented.

The unique reactivity of buckminsterfullerene (C_{60}) has resulted in an increasing exploration of its chemistry.[1] Recently, we reported the characterization and X-ray structures of two Diels–Alder adducts of C_{60}, of which one was further stabilized toward *retro*-Diels–Alder fragmentation by the presence of a substituted *o*-quinodimethane leaving group.[2,3] In fact, the Diels–Alder reaction of C_{60} with 1,3-dienes appears to give very stable adducts provided that the diene has limited substitution at its 1,4-positions.[4] In the present work, we report the application of the Diels–Alder functionalization to the preparation of the first α-amino acid derivatives of C_{60} (**3a**, **3b**, and **4a**) by esterification of alcohol **2** with *N*-(*tert*-butoxycarbonyl)-L-alanine (**5a**), *N*-(benzyloxycarbonyl)-L-alanine (**5b**), and α-benzyl *N*-(*tert*-butoxycarbonyl)-L-glutamate (**6**), respectively (Scheme I).[5] Alcohol **2**, namely 1,9-(4-hydroxycyclohexano)buckminsterfullerene,[6] is readily accessible in excellent yield by reduction of ketone **1**, obtained itself in good yield by Diels–Alder reaction of 2-[(trimethylsilyl)oxy]-1,3-butadiene with C_{60}. Compounds **1** and **2** bear the synthetically versatile ketone and alcohol functionalities which will also permit the preparation of a variety of other C_{60}-derivatives presenting interesting applications in photodynamic therapy,[7] protein design,[8] or materials science.[9] For biological activity studies, C_{60} derivatives have to be attached to hydrophilic groups to obtain solubility in water.[5,10] The deprotected amino acids **3c** (HCl salt) and especially **4b** are expected to be water-soluble while retaining the unusual redox[3b,11] and photophysical[12] properties of C_{60}. Large spherical hydrocarbon derivatives bearing polar functional groups

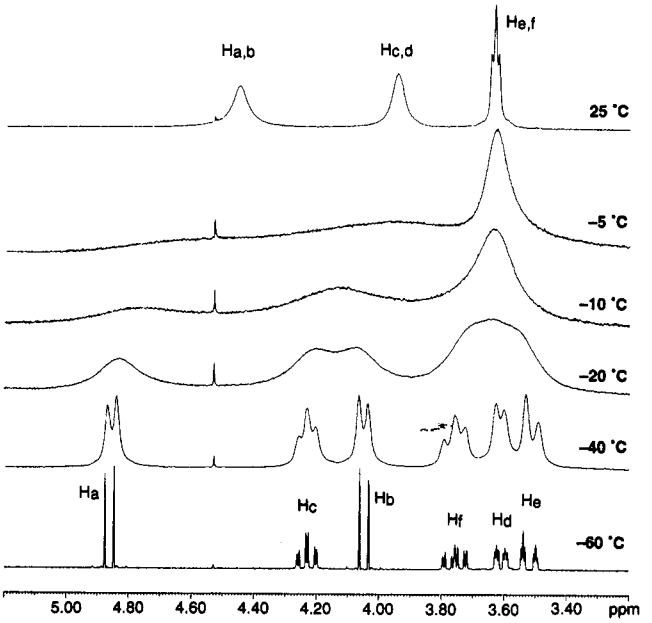
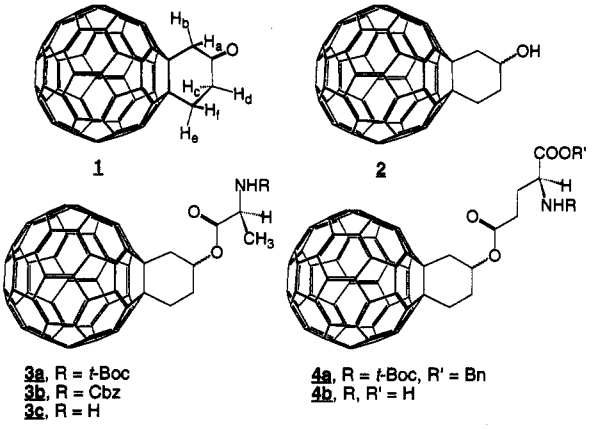


Figure 1. 500-MHz ^1H NMR spectrum of ketone **1** as a function of temperature. The resolution of the spectrum at −60 °C was slightly enhanced by Gaussian multiplication. The peak at 4.53 ppm is due to an impurity.

Scheme I



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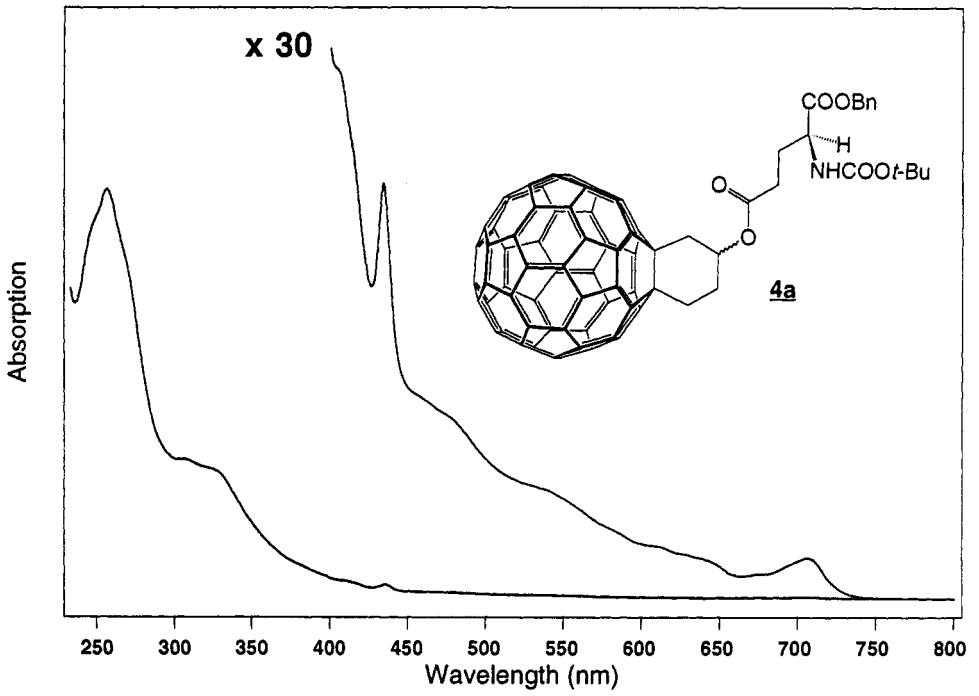

Figure 2. UV–vis spectrum of the amino acid derivative **4a** in CH_2Cl_2.

have gained increasing attention for their biological activity.[13] Of precedent are the adamantane (van der Waals radius, 7.4 Å; C_{60}, 10.7 Å) derivatives amantadine[13b,c] and rimantadine[13d] which are both potent antiviral agents.

Ketone **1** (59% yield) was obtained by reaction of C_{60} with 2-[(trimethylsilyl)oxy]-1,3-butadiene[14] in toluene at reflux, followed by hydrolysis of the uncharacterized intermediate silyl enol ether through flash chromatography on silica gel (CS_2, then toluene). Reduction of **1** with DIBAL-H (toluene, 20 °C) gave the racemic alcohol **2** in 93% yield. Esterification of **2** with the protected amino acids **5a**, **5b**, and **6** (DCC, DMAP, toluene, 20 °C), followed by flash chromatography (0–5% EtOAc in toluene), provided compounds **3a**, **3b**, and **4a** as ~1:1 mixtures of diastereomers in 89%, 52%, and 88% yield, respectively.[15]

At 25 °C, the 500-MHz ^{1}H NMR spectrum of **1** is characterized by three broad signals at 3.59 (t, H_e/H_f), 3.90 (s, H_c/H_d), and 4.41 ppm (s, H_a/H_b) for the three methylene groups (Figure 1). On cooling to −60 °C, the spectrum of **1** resolves into six multiplets at 3.54, 3.62, 3.77, 4.06, 4.24, and 4.89 ppm, suggesting that slow conformational inversion of the cyclohexanone ring occurs on the NMR time scale. The two signals for H_a and H_b coalesce at −5 ± 2 °C. The corresponding activation energy for ring inversion in **1** is calculated at 12.0 ± 0.2 kcal·mol^{-1},[16] which is much higher than that of cyclohexanone (4.1 kcal·mol^{-1}).[17] The origin of this high barrier seems to be related to that of the *o*-quinodimethane Diels–Alder adducts.[2a,3a]

The ^{1}H NMR spectrum of alcohol **2** shows seven well-resolved multiplets for the ring protons (2.68, 3.26, 3.35, 3.55, 3.68, 3.78, and 5.21 ppm) and a singlet at 1.99 ppm for the alcohol proton. Compared to cyclohexanone and cyclohexanol, the ^{1}H NMR resonances in compounds **1** and **2** show significant downfield shifts due to the deshielding effect of the C_{60} moiety.[5] The largest shift difference is observed between the axial hydrogen H_a in **1** and the corresponding hydrogen (H-2) in cyclohexanone ($\Delta\delta$ = 2.55 ppm). The ^{1}H NMR spectra of the α-amino acid derivatives **3a**, **3b**, and **4a** reproduce the features observed for alcohol **2** in addition to the respective absorptions expected for the amino acid side chains. Distinguishable diastereomers in approximately 1:1 ratios can be seen as judged by the presence of doubled resonances for $OC(CH_3)_3$ and N*H* in **3a** and **4a**, and $PhCH_2O$ and N*H* in **3b**. The ^{13}C NMR spectra of **1**, **2**, **3a**, and **4a** show resonances of the sp^3-hybridized carbons of C_{60} at 60–70 ppm, while the fullerene signals appears between 128 and 159 ppm.

The UV–vis spectra of compounds **1**, **2**, **3a**, **3b**, and **4a** are virtually identical and reproduce the spectra of other C_{60}-derivatives of the 1,9-dihydrofullerene type, including $C_{60}H_2$ (Figure 2).[2,18] Interestingly, a previously unreported low-energy absorption is observed in all these systems at ~705 nm (ϵ 250–400) and appears far to the red of the lowest energy absorption in C_{60} (620 nm). Although it is conceivable that this band could be due to a forbidden singlet–triplet absorption, this could not be confirmed through intensity enhancement of the band in EtI or PhI as solvents. On the other hand, photolysis experiments show that $^1O_2$ is formed *efficiently* in the presence of **2** (Φ = 0.84 at 532 nm and 0.72 at 355 nm in C_6D_6), which raises the exciting possibility of using these systems for photodynamic therapy, e.g., by attaching **2** to tumor-specific

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antibodies.[19,20] Further studies on the redox properties and the singlet oxygen production by the α-amino acid derivatives **3a–c** and **4a,b** are currently under way.

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Supplementary Material Available: Characterization data for **1**, **2**, **3a**, **3b** and **4a** (4 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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