Stereoselective Synthesis of C_{60}-Based Cyclopropane Amino Acids

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Since the preparation of [60]fullerene in multigram scale in 1990, a huge amount of modified fullerenes have been synthesized.[1] However, the number of chiral C_{60} derivatives prepared is markedly lower[2] due to the spherical geometry of the [60]fullerene molecule.

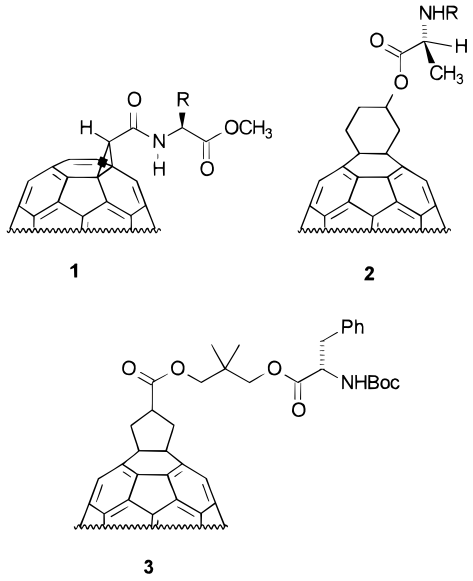
Chiral monoadducts of C_{60} have been achieved from chiral organic addends by using (i) asymmetric[3] or (ii) C_2-symmetric[4] molecules. Inherently asymmetric bisadducts of C_{60} with C_2-symmetry have been also reported,[5] as well as the first inherently asymmetrically cage-opened C_{60} derivative.[6]

Among the different chiral addends linked to the C_{60} core, amino acids have played a very important role and several fullerene-based amino acids (**1**−**3**) have been synthesized by different groups. The final goal of these systems are mainly focused on the potential incorporation of these nonnatural C_{60}-based amino acids in peptide analogues.[7]

In most of the C_{60}-based amino acids prepared so far, either the amino or the carboxyl groups are engaged in the covalent linkage to the C_{60}-containing moiety. In this communication we describe the first fullerene derivatives bearing enantiomerically pure cyclopropane amino acids in which both amino and carboxyl groups are suitably protected.

Cyclopropane amino acids constitute an interesting type of compounds that include naturally occurring products such as *allo*-coronamic and *allo*-norcoronamic acids which play important roles in some metabolic routes in plants. Moreover, methanologues of proteinogenic amino acids have been successfully incorporated into conformationally constrained, biologically active, peptide surrogates.[8]

Chart 1. Representative Examples of C_{60}-Based Amino Acids



Some of us have previously developed a highly versatile, stereocontrolled, and efficient methodology to prepare a variety of cyclopropane amino acids from homochiral aminopentenoates as precursors.[9] Thus, methyl (1*S*,2*R*)-(−)-1-*(N-tert*-butoxycarbonylamino*)*-2-formylcyclopropane-1-carboxylate (**4**) was prepared in five steps from D-glyceraldehyde acetonide (80% overall yield) and the appropriately substituted phosphonate endowed with the amino acid functional group, according to the method previously reported in the literature.[9]

Covalently attachment of **4** to [60]fullerene was carried out as depicted in Scheme 1. Fulleropyrrolidine bearing the cyclopropane amino acid (**6**) was prepared by 1,3-dipolar cycloaddition of the in situ generated azomethine ylide (**5**) to C_{60} by refluxing in toluene for 27 h, in 25% yield (48% based on consumed C_{60}), according to Prato's procedure.[10] (Scheme 1).

Interestingly, the cyclization to afford the pyrrolidine ring, with concomitant generation of a new stereogenic center at C-2 position, was highly diastereoselective. A 86:14 mixture of diastereomers was obtained as deduced from the ^{1}H NMR spectrum by integration of the absorption peaks for the N-Me group which appear at 3.0 ppm (major) and 3.5 ppm (minor).

The major isomer of compound **6** shows in the ^{1}H NMR spectrum the signals of the pyrrolidine protons at δ 4.82

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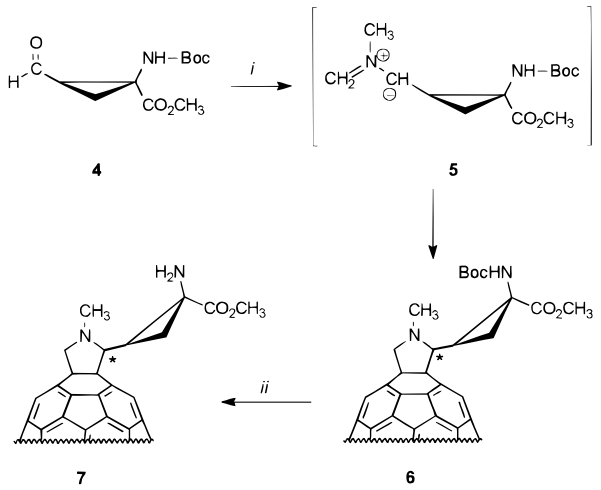
Scheme 1



i) $H_3C–NH–CH_2–CO_2H$, C_{60}, Toluene, Δ
ii) TMSI, $CHCl_3$

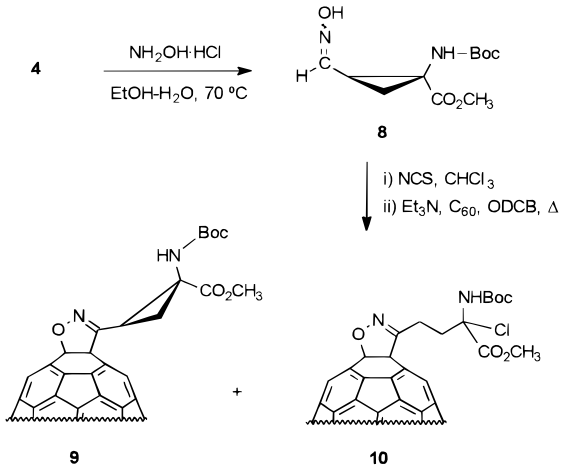
and 4.22 as doublets (J = 9.6 Hz; geminal hydrogens) and δ 3.87 (CH−N) as a doublet by coupling with the adjacent cyclopropane proton (J = 9.3 Hz).

It is well established that 1,3-dipolar cycloadditions occur at the 6,6-ring junction of the C_{60} framework. The ^{13}C NMR spectrum of **6** shows the presence of 62 signals for the major isomer which indicates the lack of symmetry in the molecule. The signals of the 6,6-ring junction and pyrrolidine carbons appear together with the remaining sp^3 carbons of the molecule at δ 28.35−81.01. The UV−vis spectrum shows the typical weak absorption band at 430 nm of dihydrofullerenes, thus confirming the [6,6]-closed character of the molecule.[11]

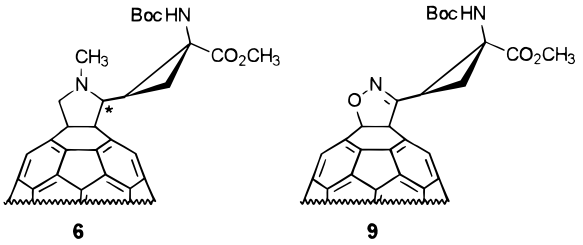
Deprotection of the amino group in compound **6** was carried out under mild conditions by treatment with trimethylsilyl iodide (TMSI) in chloroform at room temperature. After purification by flash chromatography (silica gel, toluene:ethyl acetate, 9:1), compound **7** was obtained in 89% yield as diastereomeric mixture. Pure major isomer could be isolated by careful flash chromatography {$[\alpha]_D$ + 7.1 (*c* 0.085, $CHCl_3$)}. The ^{1}H NMR spectrum of **7** clearly shows the absence of the *tert*-butyl moiety and the ^{13}C NMR spectrum, which presents 63 signals, shows the presence of the nine sp^3 carbons at δ 39.37−77.15, and the ester group at δ 176.28, in addition to 53 signals at δ 119.72−156.36 corresponding to the sp^2 carbons of the C_{60} moiety.

To prepare enantiomerically pure organofullerenes endowed with cyclopropane amino acids, we have carried out the synthesis of the novel isoxazolino[4′,5′:1,2][60]-fullerene (**9**) in which no new chiral center is formed from the cycloaddition reaction (Scheme 2). Thus, oxime derivative **8** was prepared from aldehyde **4** by treatment with hydroxylamine hydrochloride in hot ethanol−H_2O. Further reaction of **8** with *N*-chlorosuccinimide in chloroform for ca. 10 min followed by addition of Et_3N and stoichiometric C_{60} in *o*-dichlorobenzene (ODCB) and subsequent heating at 40−50 °C for 2 h affords, after purification by flash chromatography (silica gel, toluene, and toluene:ethyl acetate, 9:1), isoxazoline **9** in 8% yield

Scheme 2



(64% based on consumed C_{60}). The spectroscopic data of **9** clearly confirm the proposed structure. The ^{13}C NMR spectrum shows the presence of 53 signals, the ester carbonyl group appearing at δ 171.38 and the sp^3 carbons of the 6,6-ring junction at δ 77.21 and 80.69.



Compound **9** retains the configuration of aldehyde **4**, being the first enantiomerically pure C_{60}-based isoxazoline reported so far {$[\alpha]_D$ +2.1 (*c* 0.14, $CHCl_3$)}. Since cleavage of the isoxazoline ring has been recently reported in some isoxazolino[4′,5′:1,2][60]fullerenes,[12] these molecules are of interest as precursors to other functionalized cyclopropane amino acids containing fullerenes.

It is worth mentioning that when the synthesis of **9** was carried out with larger amounts of the starting oxime (120 mg), a second organofullerene was isolated as byproduct. Examination of its spectroscopic data suggests structure **10** for such a compound. Previously, in our laboratories, cyclopropane-ring-opening has been observed to occur under several conditions.[13]

In summary, we report herein the first synthesis of enantiomerically pure cyclopropane amino acids covalently attached to a fulleropyrrolidine or fulleroisoxazoline as new nonnatural C_{60}-based amino acids. Deprotection of the amino group has been easily achieved which opens up the way for further incorporation into peptide surrogates. Work is currently in progress in that way, as well as on the covalent linkage of other differently sized carbocyclic amino acids.

Experimental Section

General Details. Mass spectra were recorded operating at 30 kV by using a bombardment of Cs^+ ions and 2-NPOE or 3-NBA as matrix.

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All chromatography was performed using silica gel (70−230 mesh). All reagents were used as purchased unless otherwise stated. All solvents were dried according to standard procedures.

***N*-Methyl-2′{2[(1*S*,2*R*)-(−)-1-(*N*-*tert*-butoxycarbonylamino)-1-(methoxycarbonyl)cyclopropyl]}pyrrolidino[3′,4′:1,2][60]fullerene (6).** To a solution of C_{60} (150 mg, 0.21 mmol) in 60 mL of toluene were added methyl (1*S*,2*R*)-(−)-1-(*N*-*tert*-butoxycarbonylamino)-2-formylcyclopropanecarboxylate **4** (50 mg, 0.21 mmol) and *N*-methylglycine (93 mg, 0.21 mmol). After refluxing for 27 h, the solvent was removed under reduced pressure, and the solid residue thus obtained was purified by column chromatography over silica gel, using toluene/ethyl acetate 19/1 as eluent. Further purification was accomplished by washing the solid three times with hexane/methanol. A 25% yield (48% based on consumed C_{60}) (isomeric mixture) was obtained: FTIR (KBr) ν/cm^{-1} 3425, 2925, 1732, 1628, 528; ^{1}H NMR (major isomer) ($CDCl_3$, 200 MHz) δ 1.55 (9H, s), 2.18−2.32 (3H, m), 3.03 (3H, s), 3.81 (3H, s), 3.87 (1H, d, J = 9.3 Hz), 4.22 (1H, d, J = 9.6 Hz), 4.82 (1H, d, J = 9.6 Hz), 5.29 (1H, broad s); ^{13}C NMR (major isomer) ($CDCl_3$, 75 MHz) δ 28.35 (3C), 38.35, 39.17, 52.84, 52.88, 69.51, 70.02, 75.43, 75.54, 77.22, 81.01, 135.70, 136.07, 136.14, 137.33, 139.84, 139.99, 140.27, 140.33, 141.73, 141.75, 141.94, 142.03, 142.05, 142.13, 142.15, 142.17, 142.21, 142.23, 142.66, 142.68, 142.70, 142.74, 143.07, 143.18, 144.41, 144.63, 144.73, 145.28, 145.33, 145.36, 145.50, 145.55, 145.83, 146.01, 146.06, 146.11, 146.19, 146.25, 146.33, 146.38, 146.76, 146.97, 147.28, 147.30, 152.84, 153.27, 153.73, 156.09, 156.27, 156.59, 172.94; UV−vis ($CHCl_3$) λ_{max}/nm 256, 312, 430, 698; M. S. (EI) *m*/*z* (%) 1013 [(M + Na)$^+$, 16] 991 [(M$^+$ + 1), 13], 720 [C_{60}, 100]. HRMS: calculated for $C_{73}H_{23}N_2O_4$ [M + H]$^+$: 991.1658; found: 991.1610.

***N*-Methyl-2′-{2-[(1*S*,2*R*)-(−)-1-amino-1-(methoxycarbonyl)-cyclopropyl]}pyrrolidino[3′,4′:1,2]-[60]fullerene (7).** To a solution of **6** (20 mg, 0.021 mmol) in dry chloroform was added Me_3SiI (0.1 mL, 0.70 mmol) dropwise under argon at room temperature. Precipitation of a solid is inmediately observed. After keeping the mixture 30 min with stirring, it was quenched with 3−4 equiv of methanol to 1 equiv of TMSI, stirring for 5 min more. The volatile components were removed under reduced pressure, and the solid residue thus obtained was purified by column chromatography over silica gel, using toluene/ethyl acetate 9/1 as eluent. Further purification was accomplished by washing the solid three times with hexane: 89% yield; FTIR (KBr) ν/cm^{-1} 3423, 1726, 1630, 574, 527; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.38 (1H, m), 1.77 (1H, m), 2.15 (1H, m), 2.96 (3H, s), 3.80 (3H, s), 4.18 (1H, d, J = 9.3 Hz), 4.20 (1H, d, J = 9.5 Hz), 4.79 (1H, d, J = 9.3 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.37, 52.49, 52.67, 69.58, 69.77, 74.18, 74.28, 75.28, 77.15, 119.72, 120.14, 120.56, 120.99, 135.83, 136.17, 136.29, 137.29, 139.76, 139.79, 140.25, 140.27, 141.52, 141.68, 141.74, 141.88, 142.05, 142.13, 142.18, 142.26, 142.64, 142.67, 142.70, 143.04, 143.17, 144.39, 144.45, 144.63, 144.75, 145.24, 145.28, 145.32, 145.41, 145.44, 145.48, 145.60, 145.90, 146.00, 146.02, 146.09, 146.16, 146.22, 146.32, 146.36, 146.53, 146.99, 147.22, 147.27, 147.31, 153.53, 153.78, 154.04, 156.36, 176.28; UV−vis ($CHCl_3$) λ_{max}/nm 256, 314, 428, 696; MS (EI$^+$) *m*/*z* (%) 913 [(M + Na)$^+$, 9], 891 [(M$^+$ + 1), 19], 720 (C_{60}, 100).

(1*S*,2*R*)-(−)-1-(*N*-*tert*-Butoxycarbonylamino)-1-(methoxycarbonyl)-2-formylcyclopropane Oxime (8). To a solution of aldehyde **4** (50 mg, 0.21 mmol) in 25 mL of ethanol at 70 °C was added a solution of hydroxylamine hydrochloride (409 mg, 5.88 mmol) in 5 mL of water. After refluxing for 30 min, the mixture was diluted with water and extracted three times with methylene dichloride. The organic extracts were dried over $MgSO_4$, and the solvent was removed under reduced pressure. Oxime **8** was obtained as an oil in quantitative yield and was used without further purification: FTIR (KBr) ν/cm^{-1} 3440, 3350, 2988, 1719, 1686, 1508; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (s, 9H), 2.57 (m, 1H), 3.73 (s, 3H), 3.83 (m, 1H), 5.31 (broad s, 1H), 5.71 (broad s, 1H), 6.42 (d, 1H, J = 7.5 Hz), 7.19 (d, 1H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.9, 28.2 (3C), 52.8, 77.2, 80.6, 148.1, 148.5, 155.9, 171.9. Anal. Calcd for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.04; N, 5.76. Found: C, 54.17; H, 6.99; N, 5.75.

3′-{2-[(1*S*,2*R*)-(−)-1-(*N*-*tert*-butoxycarbonylamino)-1-(methoxycarbonyl)cyclopropyl]}isoxazolino[4′,5′:1,2][60]-fullerene (9). To a solution of NCS (29 mg, 0.22 mmol) and dry pyridine (0.1 mL) in 5 mL of dry chloroform was added oxime **8** (58 mg, 0.22 mmol) in one portion at 25 °C. The chlorination was usually over in ca. 10 min. A solution of C_{60} (160 mg, 0.22 mmol) in 15 mL of ODCB was added and the temperature raised to 40−50 °C. Triethylamine (22 mg, 0.22 mmol) in 3 mL of dry $CHCl_3$ was added dropwise. After 2 h at the same temperature, the solvent was removed under reduced pressure, and the solid residue thus obtained was purified by column chromatography over silica gel using toluene and toluene/ethyl acetate 9/1 as eluents. Further purification was accomplished by washing the solid three times with hexane/methanol. An 8% yield (64% based on consumed C_{60}) was obtained: FTIR (KBr) ν/cm^{-1} 3421, 1728, 1487, 527; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.57 (9H, s), 2.24 (2H, m), 3.31 (1H, m), 3.77 (3H, s), 5.57 (1H, broad s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.68 (3C), 24.97, 28.42, 41.70, 53.18, 77.21, 80.69, 98.57, 136.64, 136.96, 137.07, 137.23, 139.96, 140.11, 140.80, 140.95, 141.75, 141.91, 142.02, 142.07, 142.16, 142.19, 142.29, 142.34, 142.38, 142.42, 142.76, 142.81, 142.94, 143.79, 144.13, 144.17, 144.27, 144.70, 144.79, 145.11, 145.18, 145.22, 145.30, 145.54, 145.72, 145.88, 145.92, 145.96, 146.00, 146.25, 146.28, 146.35, 147.22, 147.87, 151.69, 155.68, 171.38; UV−vis ($CHCl_3$) λ_{max}/nm 258, 318, 428, 686; MS (EI$^+$) *m*/*z* (%) 999 [(M + Na)$^+$, 9], 720 (C_{60}, 100).

Carrying out the above reaction with larger amounts of reactants allowed us to isolate compound **10** in 3% yield [oxime **8** (121 mg, 0.47 mmol), pyridine (0.1 mL), NCS (63 mg, 0.47 mmol), C_{60} (416 mg, 0.58 mmol), triethylamine (50 mg, 0.50 mmol)] together with **9** in 22% yield (51% based on consumed C_{60}).

3′-[3-(*tert*-Butoxycarbonylamino)-3-chloro-3-(methoxycarbonyl)propyl]isoxazolino[4′,5′:1,2]-[60]fullerene (10): FTIR (KBr) ν/cm^{-1} 3443, 1730, 1630, 1263, 527; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.57 (9H, s), 3.40 (2H, t, J = 7.0 Hz), 3.79 (2H, t, J = 7.0 Hz), 3.99 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.22, 29.72, 35.75, 53.22, 77.20, 79.97, 98.53, 119.64, 120.06, 120.49, 125.89, 129.41, 136.75, 137.05, 140.09, 140.78, 141.80, 142.08, 142.19, 142.36, 142.78, 142.95, 144.09, 144.31, 144.35, 144.38, 144.75, 145.12, 145.19, 145.24, 145.35, 145.69, 145.88, 145.99, 146.22, 146.26, 146.35, 147.23, 147.81, 153.24, 153.73, 160.78, 161.63, 175.09; UV−vis ($CHCl_3$) λ_{max}/nm 260, 282, 318, 426, 686; MS (EI$^+$) *m*/*z* (%): 1013 [(M$^+$ + 1), 6], 720 (C_{60}, 100).

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