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C_{60} Pyrrolidine Bis-carboxylic Acid Derivative as a Versatile Precursor for Biocompatible Fullerenes

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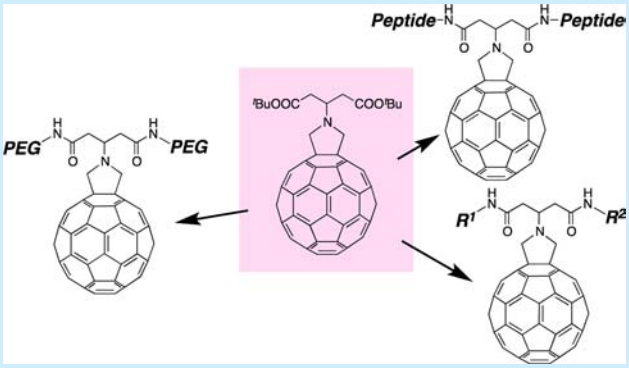
Supporting Information

ABSTRACT: A C_{60} Prato derivative with bis-^{t}Bu ester was prepared as a stable and convenient scaffold for the development of fullerene derivatives such as water-soluble C_{60}–PEG conjugates, fulleropeptides *via* solid phase synthesis, and bis-functionalized C_{60}.



Since the initial discovery of buckminsterfullerene,[1] enormous numbers of studies on fullerene-based materials have been reported.[2−10] For the development of fullerene biomaterials, chemical functionalization of fullerenes is often used to enhance their properties by the addition of moieties that increase their water solubility or to promote interactions with biomolecules such as DNA. Such chemical functionalizations can also be employed for the development of new classes of materials including donor–acceptor dyads and supramolecular assemblies. For the preparation of those materials, simple fullerene derivatives that can be used as a versatile platform for further elaboration are in high demand.

The Prato reaction,[11] a 1,3-dipolar cycloaddition of fullerenes, has been widely used in the derivatization of fullerenes. The features of this reaction include easy access to the starting materials (aldehydes and amino acids), good yields, and chemical stability of the fulleropyrrolidine adducts. By taking advantage of the photosensitivity and metal encapsulation of fullerenes, we are working on the preparation of biocompatible fullerene materials in combination with a nontoxic polymer, PVP (poly(vinylpyrrolidone)), by complexation[12] or chemical attachment.[13,14] For the attachment of water-soluble or biorelevant moieties to the fullerene core, it is important to develop a versatile and easily handled C_{60} derivative as a scaffold molecule. In the present study, we report the synthesis of a C_{60} fulleropyrrolidine bis-carboxylic acid derivative and its applications to a variety of C_{60} derivatives.

A glycine derivative **4** with two *tert*-butyl ester groups was synthesized by reductive amination of **1** and **2** with $NaBH_3CN$, followed by deprotection of the benzyl group (Figure 1). The Prato reaction of **4** and formaldehyde with C_{60} was complete within 1 h, as monitored by HPLC (Figures 2 and S9), to provide fulleropyrrolidine **5** in a sufficient yield (38%).

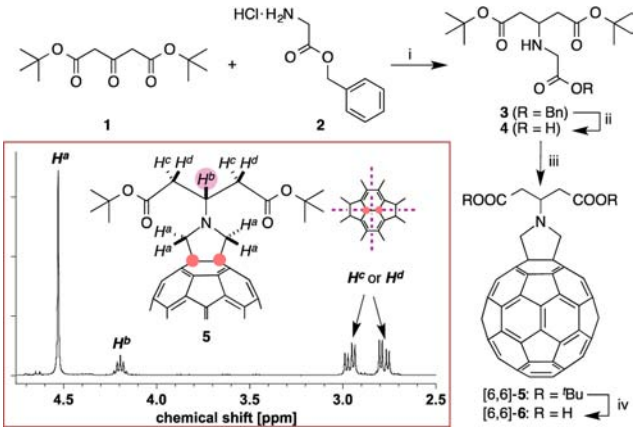


Figure 1. Synthesis of glycine derivative **4** and Prato reaction with C_{60} (inset: ^{1}H NMR of Prato adduct **5** in $CDCl_3$). *Reagents and conditions*: (i) $NaBH_3CN$, MeOH, rt, 48 h, 88%; (ii) H_2, Pd/C, EtOAc–MeOH, rt, 24 h, 98%; (iii) C_{60}, HCOH, toluene, refl., 1 h, 38%; (iv) TFA, $CHCl_3$, rt, 18 h, >99%. In the ^{1}H NMR, diastereotopic H^c and H^d appeared as magnetically nonequivalent protons. Due to the presence of two symmetry mirrors of [6,6]-adduct, four methylene protons in pyrrolidine appeared as one singlet (H^a).

Although a small amount of polyadducts was observed, the [6,6]-monoadduct was produced as a major product. The structure of adduct **5** was determined by ^{1}H and ^{13}C NMR. On the basis of the symmetry of the molecule (Figures 1 and S12), it was confirmed to be a [6,6]-adduct, the same as the other C_{60} fulleropyrrolidine derivatives reported previously. Adduct **5** was

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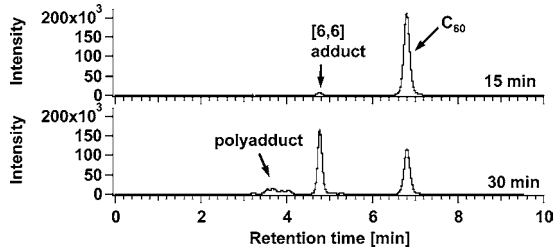

Figure 2. HPLC traces of Prato reaction of C_{60} to provide fulleropyrrolidine **5** ([6,6]-adduct) (Buckyprep-M 4.6 mm × 250 mm, toluene, 390 nm, 1 mL·min^{-1}).

highly soluble in most organic solvents and very stable against heating (at 180 °C for 4 h) without observing products from retro-cycloaddition (often observed in Diels–Alder adducts of C_{60}),[15,16] decarboxylation (can be observed in Hirsch–Bingel derivatives of C_{60}),[17,18] or rearrangement (often observed in azafulleroids of C_{60}[19] or Prato adducts of TNT-EMF).[20−22]

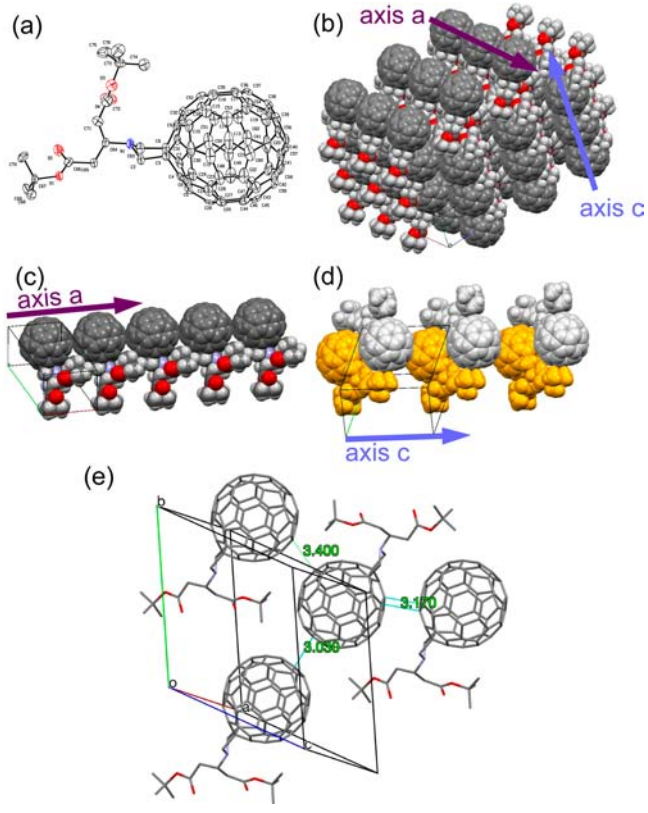

Figure 3. Crystal structure of Prato adduct **5**.

X-ray crystal structure analysis was employed to confirm the structure of **5** (Figure 3). In the crystal lattice, the hydrophobic C_{60} parts were located in line parallel to the axes *a* and *c* (Figure 3b–d; the shortest distance between C atoms of neighboring C_{60} all related by an inversion center are 3.03, 3.17, and 3.40 Å, Figure 3e). The distance of 3.03 Å is the shortest contact found between C_{60} moieties in known crystal structures (CSD database search version 5.34).

Adduct **5** was subjected to acidic deprotection of the two *tert*-butyl groups. In contrast to basic deprotection, which could often cause hydroxylation of the C_{60} core, the deprotection with TFA provided **6** in an excellent yield (99%) without affecting the C_{60} cage (Figure 4). Compound **6** was soluble in polar

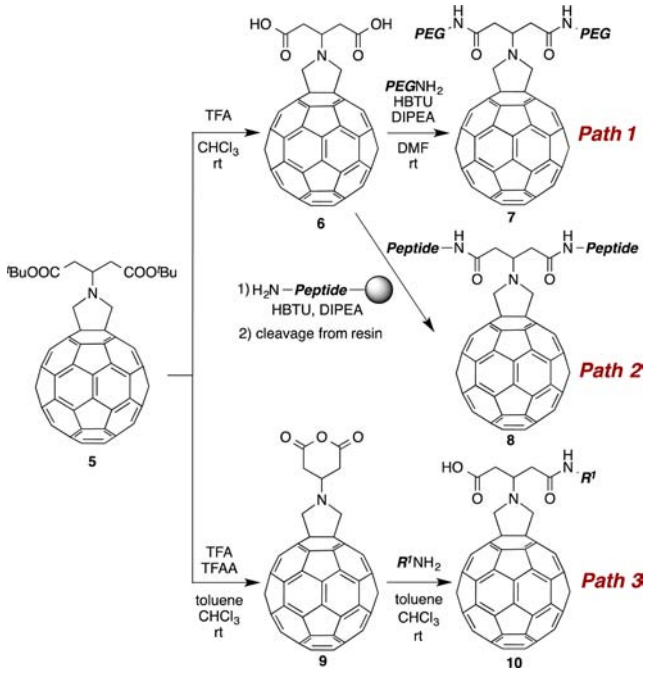

Figure 4. Production of functional C_{60} derivatives *via* Prato adduct **5** as a key intermediate.

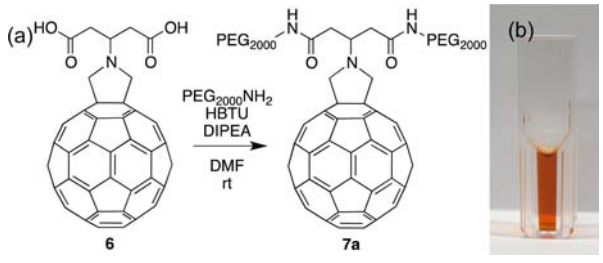

Figure 5. Addition of mPEG$_{2000}$–NH$_2$ to C_{60} bis-carboxylic acid **6** to provide water-soluble C_{60}–(mPEG$_{2000}$)$_2$ **7a** (a) and 1 mM aqueous solution of **7a** (b).

solvents such as pyridine and DMF and could be subjected to subsequent reactions (Figure 4, *Path 1*–*2* and Figure 5) to provide water-soluble C_{60} derivative **7** (*Path 1*) or C_{60}–peptide conjugates (fulleropeptide) **8** (Figure 4, *Path 2* and Figure 6). Alternatively, compound **5** lead to heterofunctionalized C_{60} derivative **10** through an acid anhydride intermediate **9** (Figure 4, *Path 3* and Figure 7).

Bis-carboxylic acid **6** was subjected to an amide conjugation reaction with mPEG$_{2000}$–NH$_2$ using HBTU as a coupling reagent (*Path 1* in Figure 4 and Figure 5a). Using 3 equiv (1.5 equiv for each carboxylic acid) of PEG$_{2000}$–NH$_2$ provided **7a** in a good yield (78%). The structure of the C_{60}–PEG **7a** was confirmed by MALDI-TOF-MS and ^{1}H, ^{13}C NMR (Figures S28, S29, and S31). Compound **7a** was highly water-soluble (Figure 5b), forming particles with a diameter of about 10 nm in water (DLS; see Figure S27). The highly water-soluble **7a** (>3 mM) can potentially be a biocompatible fullerene material.

Bis-carboxylic acid **6** was subjected to the solid-phase coupling with a resin-supported peptide (*Path 2* in Figure 4 and Figure 6) to provide a fulleropeptide. Previous studies reported fulleropeptides with interesting bioactivities such as a higher cell penetration property than the corresponding peptides with even higher water solubility[23] and antimicrobial

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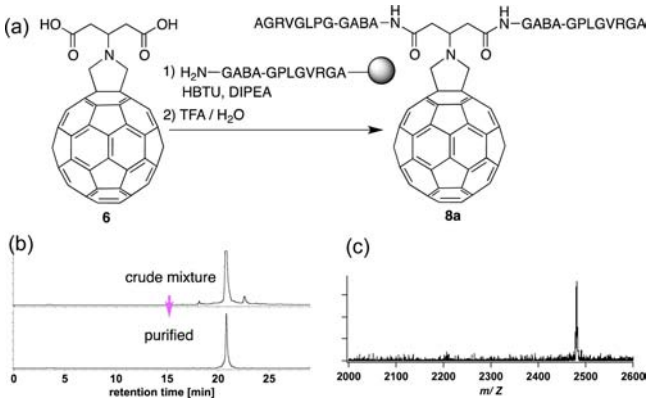

Figure 6. (a) Solid phase reaction of bis-carboxylic acid **6** to peptide-resin to provide a fulleropeptide **8a** (solid phase: chlorotrityl resin, peptide sequence: NH_2-GABA-GPLGVRGA-COO-resin). (b) HPLC trace of crude mixture of fulleropeptide cleaved from resin (top) and purified one (bottom) (column: C4, 4.6 mm × 250 mm, Vydac 214 MS protein, solvents: MeCN−H_2O (5:95 (0−5 min), gradient to 95:5 (5−15 min), 95:5 (15−27 min), gradient to 10:90 (27−29 min), detection: 280 nm). (c) MALDI-TOF-MS spectra of purified fulleropeptide (matrix: HCCA, m/z calcd for $C_{137}H_{132}N_{25}O_{22}$: 2479.00, found: 2479.00 M^+).

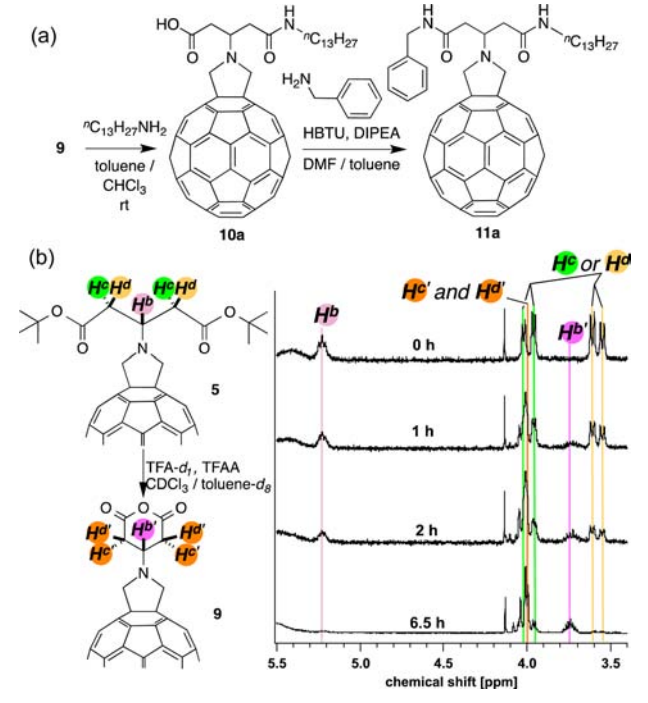

Figure 7. (a) Conjugation of two different amines to C_{60} *via* an acid anhydride **9**. Two distinct amines (^{n}tridecyl and benzyl groups were used as examples) were attached successfully to the C_{60} core by a stepwise procedure to provide heterofunctionalized C_{60} derivative **11a**. (b) *In situ* generation of acid anhydride **9** from the reaction of **5** and TFA/TFAA observed by ^{1}H NMR (in TFA-d_1, $CDCl_3$, and toluene-d_8).

activity to Gram-positive bacteria.[24] Previously, there were a few examples of fullerene derivatives that can be applied in solid-phase synthesis.[24−27] In this study, a typical peptide GABA-GPLGVRGA, prepared on a 2-chlorotrityl resin (3 equiv to **6**), was subjected to coupling with **6** using HBTU. After 1 h of reaction subsequent to the addition of **6** to a suspension of peptide resin, the solution, which was initially very dark, turned nearly colorless suggesting that conjugation of **6** to amino groups of GABA of the peptide on solid phase was successfully proceeding. The resulting resin was subjected to conditions for peptide cleavage from the solid support (TFA/H_2O), and the fulleropeptide **8a** was obtained without many byproducts, as shown in the HPLC trace of a crude mixture (Figure 6b top diagram). By further HPLC purification (Figure 6b bottom diagram) fulleropeptide **8a** was obtained in 64% yield and confirmed by MALDI-TOF-MS analysis (Figure 6c).

Bis-carboxylic acid ester **5** was converted to an acid anhydride **9** by simultaneous deprotection and dehydration of **5** in the presence of TFA and TFAA for the preparation of heterofunctionalized C_{60} derivatives (*Path 3* in Figure 4 and Figure 7a). The *in situ* generation of **9** was monitored by NMR, with a decrease of peak H^b in **5** and a simultaneous increase of $H^{b\prime}$ in **9** observed (Figure 7b). Although compound **9** was somewhat water-sensitive, it was stable enough for isolation and full characterization (stable at room temperature at least for 5 h under ambient conditions, Figures S37−S44). Compound **9** could not be prepared *via* acid **6**, since **6** was not soluble in toluene or chloroform. The addition of 5 equiv of tridecylamine to **9** allowed selective single amide conjugation without the need for coupling reagents to provide **10a** with one substituted moiety (tridecyl group) and one unsubstituted carboxylic acid. This remaining carboxylic acid was converted to the amide with benzylamine in the presence of a coupling reagent (HBTU) to provide a heterofunctionalized C_{60} derivative **11a** with two different side chains.

In this study, we reported a new, simple, and stable Prato derivative bearing two carboxylic acid groups. The Prato reaction of C_{60} with formaldehyde and a glycine derivative **4**, which were easily accessible using commercially available chemicals, proceeded in good yield. Under acidic conditions, ^{t}Bu deprotection proceeds smoothly without affecting the C_{60} core and efficiently provided a bis-acid derivative **6**. The Prato derivative **6** was stable compared to other C_{60} carboxylic acid derivatives, such as those derived by Bingel reactions.[17,18] By addition of an amine derivative or peptide, this Prato derivative was easily functionalized even under solid phase conditions and was shown potentially to provide various other biocompatible derivatives. In addition, the anhydride intermediate **9** was used to provide C_{60} derivatives bearing multiple functional moieties. These results demonstrate the versatility of the Prato derivative **5** for easy access to a variety of C_{60} functional derivatives including biocompatible ones.

■ ASSOCIATED CONTENT

Supporting Information

Spectral data of all new compounds and crystallographic data of **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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