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A new route to fullerene substituted phenylalanine derivatives†

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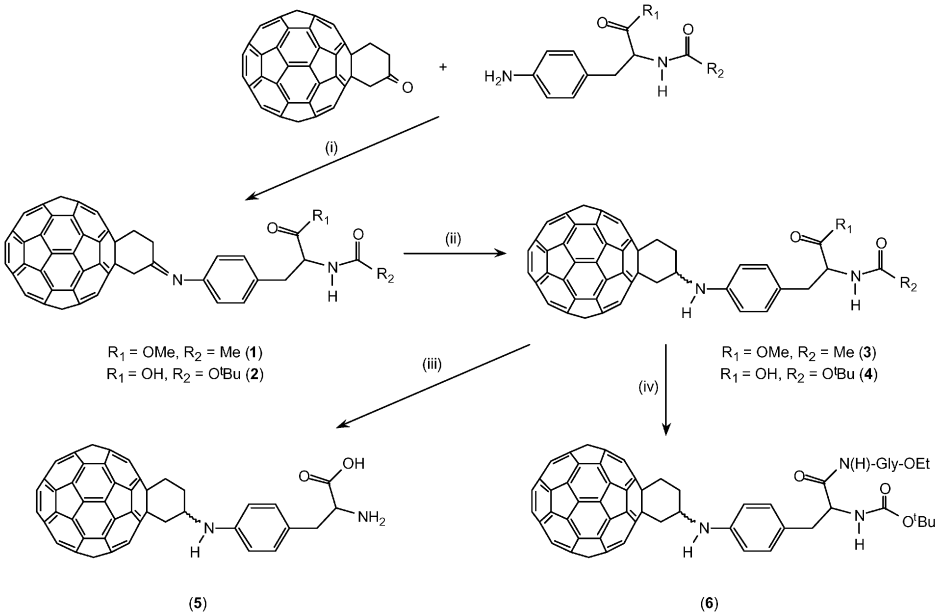
A series of fullerene substituted phenylalanine derivatives have been prepared by the condensation of 1,2-(4′-oxocyclohexano)fullerene with ester or Boc protected (4-amino)phenylalanine, $H_2NC_6H_4CH_2CH(COR_1)(NHCOR_2)$ (where R_1 = OMe, R_2 = Me; R_1 = OH, R_2 = Me, O^{t}Bu). Conversion of the imine to the corresponding amine is achieved by di-acid catalyzed hydroboration. Reaction of the *N*-Ac amino ester with BBr_3 led to the formation of the parent amino acid, while the Boc-protected derivative readily undergoes coupling with NH_2–Gly–OEt. The reduction of the imine is not accompanied by hydroboration of the fullerene cage.

The incorporation of fullerene into macromolecular structures of biological importance has been an area of growing interest over the past decade. C_{60} is well known for its unique hydrophobic nature and other physicochemical properties that offer potential applications in medicinal chemistry.[1,2] The hydrophobic nature of fullerenes makes them interesting pharmacophores in biologically active molecules, especially where hydrophobic interactions are important, *e.g.*, with receptors and membranes.[1] Thus, the functionalization of C_{60} as an amino acid analog and its incorporation into a peptide is desirable, especially with regard to targeted interactions. To date several approaches have been taken towards fullerene-based amino acids. The simplest approach has involved the reaction of an amino acid with C_{60};[3] however, in these derivatives only the carboxylic acid functional group remains available for reaction, limiting subsequent incorporation into peptides. Truly bi-functional fullerene substituted amino acids (those in which the carboxylic acid and amine functionality are available for reaction) are those limited to those derived from glycine,[4] proline,[5] glutamic acid,[6] and lysine.[7] All of these compounds are derived from aliphatic amino acids and commonly employ ester or amide linkages. In order to extend the range of fullerene-amino acids we have investigated the synthesis of an arene-based amino acid, in particular a phenylalanine (Phe) derivative. As an additional requirement a stable (non-hydrolysable) linkage is required (*e.g.*, an amine *versus* an amide or ester) to enable solid-phase synthesis of fulleropeptides. Our initial studies in this area are described herein.

Rubin and co-workers have shown that 1,2-(4′-oxocyclohexano)fullerene, prepared *via* the Diels–Alder reaction of C_{60} with 2-trimethylsiloxy-1,3-butadiene[8] is a versatile synthon for further substitution.[9] Amine substituted, protected D-phenylalanine undergoes a *p*-benzosulfonic acid catalyzed condensation reaction with 1,2-(4′-oxocyclohexano)fullerene to yield the appropriate imines, compounds **1** and **2** (Scheme 1). The formation of the imines is confirmed by MALDI-TOF mass spectrometry;‡ however, purification is hampered by their decomposition on a silica-gel column. Conversion of the imine to the corresponding amine (Scheme 1) was achieved by di-acid catalyzed BH_3(THF) reduction at −78 °C followed by conventional work up under basic conditions (KOH and $KHCO_3$ for compounds **3** and **4**, respectively).[10] It is worth noting that under similar reaction conditions it has been reported that hydroboration of C_{60} is facile.[11] Our present results suggest that the hydroboration of substituted fullerenes is disfavored or significantly retarded as compared to that of the imine. Irrespective, of the literature reports we see no evidence for hydroboration of the fullerene cage.§ Reaction of compound **3** with BBr_3 in CH_2Cl_2 leads to the formation of the parent deprotected amino acid.[12] When the reaction is quenched by careful dropwise addition of water, the solids stayed in between the interface of water and CH_2Cl_2. The solid was separated by centrifugation and washing with HCl (6 M) and water.

† Electronic supplementary information (ESI) available: details of the synthesis; ^{13}C NMR measurements; and additional MALDI-MS. See http://www.rsc.org/suppdata/cc/b4/b411118d/



Scheme 1 Synthesis of fullerene-based amino acids: (i) toluene, reflux; (ii) BH_3(THF), −78 °C; (iii) BBr_3/HCl, CH_2Cl_2; (iv) NH_2–Gly–OEt, HBTU, NEt_3.

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The parent amino acid (**5**), the ester and Boc protected derivatives (compounds **3** and **4**, respectively) have been characterized by NMR, UV-vis, and high resolution MALDI-TOF mass spectrometry.‡ The amine linkage in compounds **3–5** is stable to both acid and base hydrolysis. However, it is worth noting that in contrast to their chemical stability, the amine parent ion appears less stable under the operational condition of MALDI than the imines (Fig. 1). The UV-vis spectra of compounds **2**, **4**, and **5** are virtually identical and reproduce the spectra of other C_{60}-derivatives of the 1,9-dihydrofullerene-type (Fig. 2).[13] The NMR spectra of compound **5** was assigned from ^1H–^1H and ^1H–^{13}C COSY NMR experiments, while long range coupling was determined using ^1H–^{13}C HMBC. The introduction of the phenylalanine moiety increased the energy barrier of the inversion of the cyclohexyl ring. The ^1H NMR signals for the CH_2 groups of the cyclohexyl ring are well resolved even at room temperature. The two diastereomers, formed as a consequence of the achiral hydrogenation, are readily observed in the ^1H NMR at high field.‡

It is worth noting that the reaction of fullerene with 2-trimethylsiloxy-1,3-butadiene also produce the *bis*-adduct with two cyclohexyl ketone rings. We have shown that these can be reacted following the above procedure to produce a *bis*-phenyalanine derivative bridged with a fullerene linkage as identified from MALDI-MS (*m*/*z* = 1296). Therefore, it is possible to make di-amino acid fullerene that may function in a similar manner to the S–S unit between two cystines. We are presently investigating the purification and separation of the potential regioisomers.

To test the reactivity of our fullerene amino acid, the coupling reaction of compound **4** with the ethoxy ester protected glycine (NH_2–Gly–OEt) was carried out in 4 : 1 DCM/DMF in the presence of HBTU and NEt_3 in a sonication bath for 2 h. The brown solid may be collected by precipitation with Et_2O. The parent ion of the final coupling product (Scheme 1, compound **6**) was observed by MALDI-MS (*m*/*z* = 1141), while the peak associated with compound **4** (*m*/*z* = 1056) was completely gone (see Fig. 1) indicating a high yield reaction.

In summary, a new route to fullerene-based phenyalanine has been established through the condensation reaction of 1,2-(4′-oxocyclohexano)fullerene with amines to form imine intermediates. The selective reduction of the imine (without effecting the fullerene) allows for the formation of a hydrolytically stable amine linkage.

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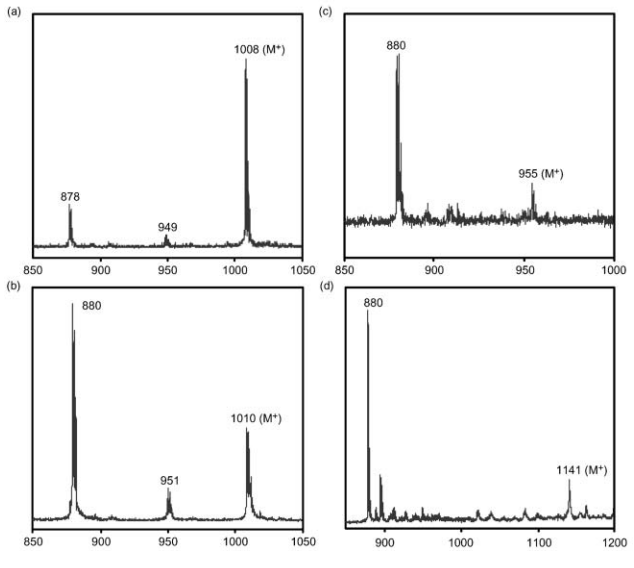

Fig. 1 MALDI-TOF MS of compounds **1** (a), **2** (b), **5** (c) and **6** (d).‡

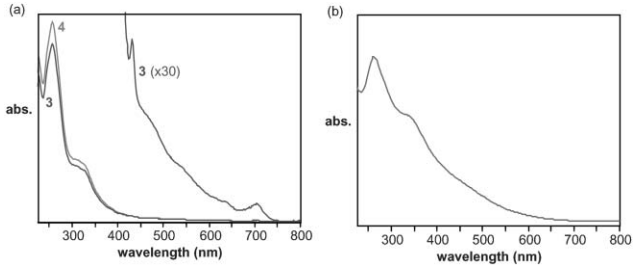

Fig. 2 UV-vis spectrum of (a) compounds **3** and **4** in CH_2Cl_2, and (b) compound **5** in H_2O.

Notes and references

‡ Selected spectral data for **1**: IR $\nu_{N=C}$ = 1653 cm^{-1}; MALDI-MS *m*/*z* 1008 (M^+), 949 ($M^+ - CO_2Me$), 878 [$M^+ - CH(CO_2Me)(NHCOMe)$]. For **2**: MALDI-MS *m*/*z* 1052 (M^+), 996 ($M^+ - C_4H_8$), 878 [$M^+ - CH(CO_2H)(NHCO^tBu)$]. For **3**: MALDI-MS: *m*/*z* 1010 (M^+), 951 ($M^+ - CO_2Me$), 880 [$M^+ - CH(CO_2Me)(NHCOMe)$]; ^1H NMR (400 MHz, toluene-d_8/methanol-d_4): δ 8.07 [1H, dd, *J*(H–H) = 2.5 Hz, *J*(H–H) = 7.8 Hz, N–*H*], 7.24 [2H, d, *J*(H–H) = 8.4 Hz, *m*-C*H*], 6.92 [2H, d, *J*(H–H) = 8.4 Hz, *o*-C*H*], 5.78 [1H, d, *J*(H–H) = 7.6 Hz, N–*H*], 4.93 [1H, dt, *J*(H–H) = 5.9 Hz, *J*(H–H) = 7.8 Hz, α-C*H*], 4.80 [1H, m, C(*H*)N], 3.74 [1H, dt, *J*(H–H) = 4.2 Hz, *J*(H–H) = 13.2 Hz, CH_2], 3.65 [1H, dd, *J*(H–H) = 10.9 Hz, *J*(H–H) = 13.2 Hz, CH_2], 3.48 (3H, s, OCH_3), 3.39 (2H, m, CH_2), 3.16 [1H, dd, *J*(H–H) = 5.8 Hz, *J*(H–H) = 13.9 Hz, $C_6H_4CH_2$], 3.04 [1H, dd, *J*(H–H) = 8.3 Hz, *J*(H–H) = 13.7 Hz, $C_6H_4CH_2$], 2.95 (1H, m, CH_2), 2.56 (1H, m, CH_2), 1.93 [3H, d, *J*(H–H) = 2.8 Hz, C(O)CH_3]; λ_{max} (CH_2Cl_2, nm) 254, 301, 427, 700. For **4**: MALDI-MS: *m*/*z* = 1054 (M^+), 998 ($M^+ - C_4H_8$), 880 [$M^+ - CH(CO_2H)(NHCO^tBu)$]; ^1H NMR (400 MHz, toluene-d_8/methanol-d_4): δ 7.18 [2H, d, *J*(H–H) = 8.3 Hz, *m*-C*H*], 6.73 [2H, d, *J*(H–H) = 8.3 Hz, *o*-C*H*], 5.66 [1H, d, *J*(H–H) = 8.3 Hz, N–*H*], 4.71 [2H, m, α-C*H* and C(*H*)N], 3.57 [1H, dd, *J*(H–H) = 3.5 Hz, *J*(H–H) = 13.6 Hz, CH_2], 3.41 – 3.03 (5H, m, CH_2 and $C_6H_4CH_2$), 2.92 (1H, m, CH_2), 2.38 (1H, m, CH_2), 1.41 [9H, s, $C(CH_3)_3$]; λ_{max} (CH_2Cl_2, nm) 254, 300, 427, 700. For **5**: MALDI-MS *m*/*z* 955 (M^+); ^1H NMR (500 MHz, toluene-d_6/methanol-d_4) δ 7.25 [2H, d, *J*(H–H) = 8.4 Hz, *m*-C*H*], 6.90 [2H, d, *J*(H–H) = 8.4 Hz, *o*-C*H*], 4.83 [1H, dd, *J*(H–H) = 5.2 Hz, *J*(H–H) = 8.6 Hz, α-C*H*], 4.80 [1H, m, C(*H*)N], 3.76 [1H, dt, *J*(H–H) = 4.2 Hz, *J*(H–H) = 13.2 Hz, CH_2], 3.63 [1H, ddd, *J*(H–H) = 0.7 Hz, *J*(H–H) = 10.9 Hz, *J*(H–H) = 13.2 Hz, CH_2], 3.39 [1H, ddd, *J*(H–H) = 0.7 Hz, *J*(H–H) = 4.9 Hz, *J*(H–H) = 13.2 Hz, CH_2], 3.24 (1H, dd, *J*(H–H) = 5.2 Hz, *J*(H–H) = 14.1 Hz, $C_6H_4CH_2$], 3.04 [1H, dd, *J*(H–H) = 8.6 Hz, *J*(H–H) = 14.1 Hz, $C_6H_4CH_2$], 2.99 [1H, dddd, *J*(H–H) = 1.0 Hz, *J*(H–H) = 4.1 Hz, *J*(H–H) = 4.1 Hz, *J*(H–H) = 13.4 Hz, CH_2], 2.56 (1H, m, CH_2); λ_{max} (H_2O, nm) 247, 338, 470, 707.

§ Attempts to reduce the imine with Raney nickel resulted in hydrogenated fullerene, while Pd/C/H_2 is ineffective, and Wilkinson's catalyst forms an adduct with the fullerene.

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