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Fullerene Unsymmetrical Bis-Adducts as Models for Novel Peptidomimetics

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Two classes of unsymmetrical, orthogonally protected bis-fulleropyrrolidine amino acids have been prepared as models for fullerene-based peptidomimetics with the carbon sphere inserted into the peptide backbone. Two successive [3+2] cycloadditions of azomethine ylides (thermally generated from formaldehyde and the corresponding orthogonally protected glycino-amines and -acids) to C_{60} afforded NHFmoc/CO_2tBu and NHBoc/CO_2Me fulleropyrrolidine couples, offering the possibility of selective deprotection under both acidic and basic conditions. In both classes of unsymmetrical bis-adducts, the distribution of all the *trans* (t_1–t_4) and *equatorial* (e' and e'') isomers was quite similar except for the t_3 NHBoc/CO_2Me compound, which rapidly decomposed during chromatography. All compounds were characterized by UV/Vis, ^{1}H and ^{13}C NMR spectroscopy, and mass spectrometry.

Introduction

The unique structural and physicochemical features of C_{60} have stimulated extensive research with a view to finding applications in biomedical fields.[1] In fact, the fullerene sphere fits almost perfectly into the hydrophobic pocket of HIV protease modifying its activity.[2] As a result of the ability of C_{60} to accept up to six electrons, fullero derivatives possess excellent radical scavenging ability,[3] acting as antioxidants,[4] membrane lipid peroxidation suppressors,[5] and cell oxidative stress defenders. The generation of singlet oxygen upon photo-oxidation of fullerenes, consequent DNA cleavage, and pro-oxidative activity make them attractive tools for photodynamic cancer therapy.[6] However, the bioavailability of C_{60} is severely limited by extremely low solubility in water and biological media. Two basic strategies for overcoming this obstacle include a noncovalent encapsulation of C_{60} into polar carriers[7] and, more often, its covalent modification.[8] Although C_{60} mono-adducts generally exhibit much higher solubility in all solvents than pristine fullerene, the carbon sphere subunit is still too big to be completely solubilized by the new addends. As a consequence, in polar media C_{60} derivatives tend to aggregate and form micelles.[9] Apart from the reduced solubility, this phenomenon changes the physicochemical properties of fullerene derivatives. Better covering of the carbon sphere and consequent decreased micellar aggregation can be achieved by introducing two or more addends. The relative positions of the addends in C_{60} bis-adducts are depicted in Figure 1. The first covalently modified [6,6]-double bond is labeled as the 1,2-site. For a second attack on a [6,6]-double bond nine different sites are available: Three from the same side of the sphere (*cis*, c_n, n = 1–3), four from the opposite side (*trans*, t_n, n = 1–4), and two orthogonally directed (*equatorials*, e' and e''). With different addends the formation of nine regioisomeric bis-adducts is possible, whereas with identical addends eight isomers should be considered because attack at both e' and e'' positions leads to the same products.

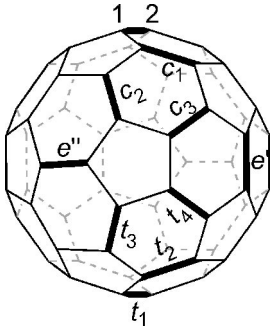

Figure 1. Relative positions of the addends in C_{60} bis-adducts. Double bonds have been omitted for clarity.

The synthesis of fullero-amino acids[10] and their incorporation into peptides[11] has attracted much interest as a result of physicochemical studies as well as their biological applications. So far the fullero subunit has been incorporated into peptides as hydrophobic side-chains (Figure 2, a), with no examples of the replacement of C_α with C_{60} having been found. Besides the improvement in solubility, the introduction of a second addend on to C_{60} leads to the possible incorporation of the fullerene amino acid into the pep-

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tide backbone. Up to nine bis-regioisomers with different and strictly defined geometries (from linear to various V-shaped forms, Figure 2, b) may allow the influence of a rigid structural subunit on the peptidomimetic conformation, properties, and biological behavior to be studied.

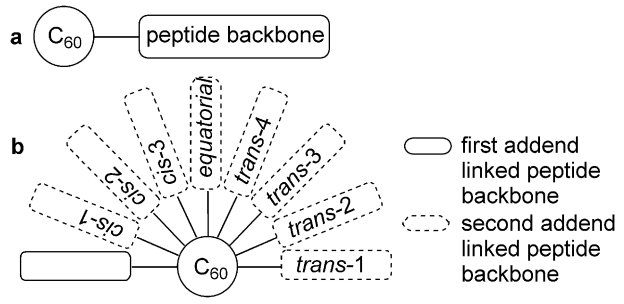


Figure 2. Fulleropeptides containing C_{60} as either a side-chain (a) or a backbone constituent (b).

Following this conceptual challenge, we present herein the synthesis of two classes of fulleropyrrolidine (Fp) bis-adducts containing orthogonally protected amino and carboxylic groups serving as models that could be used in the synthesis of novel fullero-peptidomimetics with the carbon spheroid inserted into the peptide backbone.

Results and Discussion

Synthetic Strategy

Owing to the spatial proximity of the *cis*-positioned addends and the expected micellar aggregation of these isomers, we concentrated our efforts on the isolation, characterization, and distribution of *equatorial* and *trans* unsymmetrical bis-adducts that contain terminal amino and carboxy groups. For use in peptide synthesis, these functional subunits should be orthogonally protected, avoiding at the same time the use of the Cbz or benzyl ester groups due to the sensitivity of fullerene to catalytic hydrogenation. Considering all of these factors, we designed the synthesis of NHFmoc/CO_2tBu and NHBoc/CO_2Me fulleropyrrolidine couples, allowing for the possibility of selective deprotection under both acidic and basic conditions. The synthesis of fulleropyrrolidines (Fp) involves the [3+2] cycloaddition of an azomethine ylide (thermally generated from an aldehyde and the corresponding *N*-substituted glycine) to C_{60} (Figure 3). To avoid the formation of a new stereocenter, formaldehyde was chosen, whereas unsymmetrical bis-adduct formation was achieved by two successive 1,3-dipolar cycloaddition reactions using the corresponding orthogonally protected glycino-*N*-alkylamines and glycino-*N*-alkyl acids. As acid-sensitive carboxy and amino protecting groups, *tert*-butyl ester and Boc were chosen, with methyl ester and Fmoc selected as base-sensitive protecting groups.

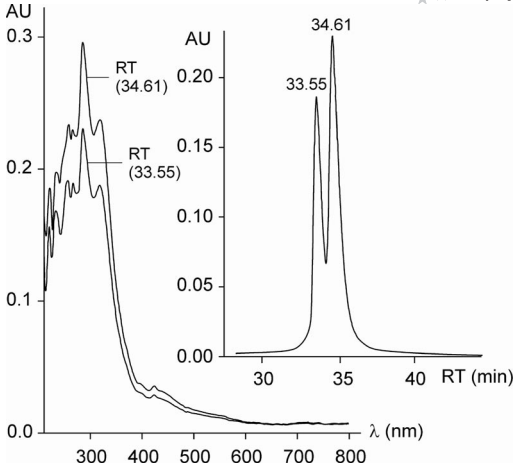
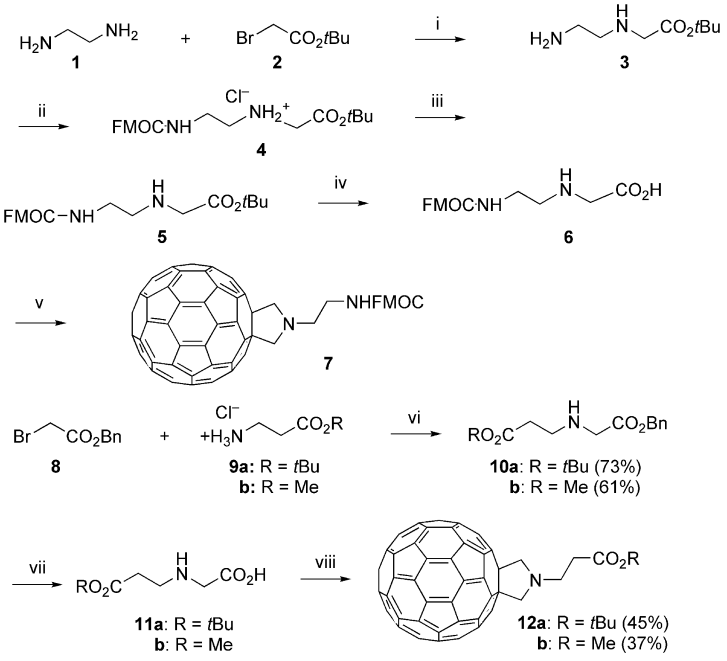


Figure 3. HPLC chromatogram (right) of the *equatorial* isomers (*e′* and *e″*) and their UV spectra (left).

Synthesis of Monofulleropyrrolidines Bearing Protected Amino and Carboxylic Groups

The synthesis of the protected mono-adducts is outlined in Scheme 1. Mono-*N*-alkylation of 1,2-ethanediamine (**1**) by *tert*-butyl bromoacetate (**2**) and subsequent protection of the primary amino group gave the hydrochloride salt of *N*-alkylated *tert*-butyl glycinate **4**. Liberation of the free base followed by acidic ester hydrolysis afforded the amino acid (AA) **6** and subsequent 1,3-dipolar cycloaddition to C_{60} provided *N*-Fmoc-protected Fp **7**. The yield of compound **7** was unexpectedly low, 3% under standard reaction conditions (molar ratio AA/HCHO/C_{60} = 1:5:1, reflux in toluene for 1 h). The same yield was achieved by prolonging the reaction time to 13 h and no product decomposition was observed. [To examine the stability of fulleropyrrolidine **7** under the applied conditions, the compound was heated at reflux in toluene and no changes were observed after 6 h (TLC).] No improvement was attained by changing the solvent; in tetrachloroethane the reaction proceeded much more slowly than in toluene with the same amount of compound **7** formed. Use of an excess of the amino acid **6** (3 mol equiv. with respect to C_{60}) resulted in a slightly better yield (5%). The effect of organic base on Fp formation was also examined. Thus, in the presence of triethylamine (TEA, molar ratio AA/:TEA/HCHO/C_{60} = 1:2:5:1, reflux 1.5 h) a complex mixture was formed, whereas with pyridine under the same reaction conditions a yield of 9% of the Fmoc-protected Fp amine **7** was achieved.

Fulleropyrrolidines **12** bearing side-chains with protected carboxylic groups were synthesized as outlined in Scheme 1. *N*-Alkylation of β-alanine esters **9** with benzyl bromoacetate (BBA, **8**) followed by hydrogenation of the benzyl esters **10** afforded the glycino acids **11**. Subsequent azomethine ylide formation in the presence of formaldehyde and [3+2] cycloaddition to C_{60} proceeded smoothly to afford the corresponding Fp acids **12a** and **12b** in yields of 45 and 37%, respectively.



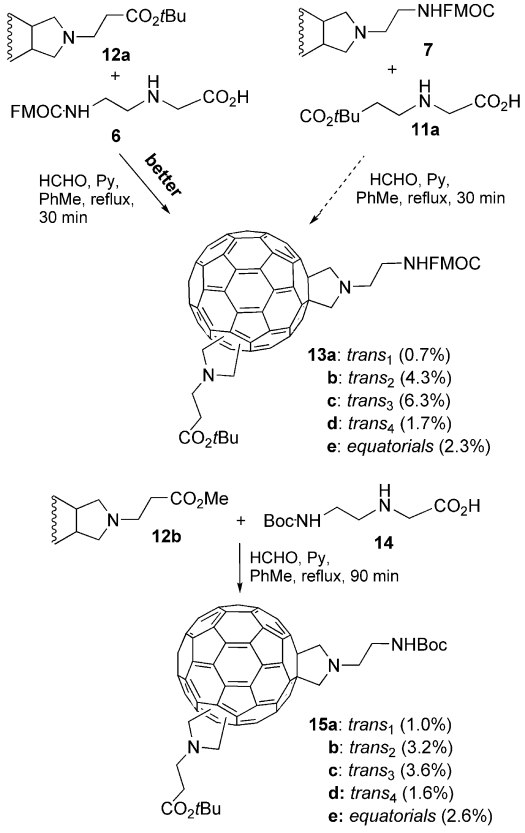
Scheme 1. Synthesis of mono-fulleropyrrolidines.

Synthesis of Unsymmetrical Bis-Fulleropyrrolidines

The two possible reactions for the preparation of the Fmoc/CO_2tBu couples **13a**–**e**, which differ in the order of introduction of the addends, were performed successfully. Heating of *tert*-butyl Fp ester **12a**, AA **6**, Py, and HCHO (molar ratio 1:1:2:5) at reflux in toluene for 75 min produced a mixture of bis-adducts **13a**–**e** with no tris-adducts observed (Scheme 2), whereas twice the amount of amino acid resulted in a reduction of the reaction time to 30 min. The same products were obtained by the complementary reaction of protected Fp amine **7**, AA **11a**, and HCHO (Scheme 2) for 30 min at reflux. Owing to the low yield of Fmoc-protected fulleroamine **7**, fullero-amino acids **13a**–**e** were synthesized by heating a toluene solution of fullero ester **12a**, Fmoc-protected glycino-amine **6**, pyridine, and paraformaldehyde (mol ratio 1:2:2:5) at reflux for 30 min. Flash chromatography (FC) on fine silica gel afforded pure products in the following order of elution: unreacted mono-Fp, $trans_1$, $trans_2$, $trans_3$, $trans_4$, and *equatorials*.

Both the *e′* and *e′′* bis-adducts showed the same adsorption affinity to silica during FC and TLC, whereas HPLC analysis showed two peaks with identical UV/Vis spectra (Figure 3).

By analogy, 1,3-dipolar cycloaddition of Boc-protected glycino-amine **14**[12] to Fp-ester **12b** led to the second class of orthogonally protected fullero-amino acids **15a**–**e**, separated by FC in the same order of elution as **13a**–**e**. In both classes of orthogonally protected fullero-amino acids (**13** and **15**), the product distribution was quite similar (Fig-



Scheme 2. Synthesis of unsymmetrical bis-fulleropyrrolidines.

ure 4), except for the t_3 isomers, the difference arising here because compound **15c** decomposed rapidly during chromatography.

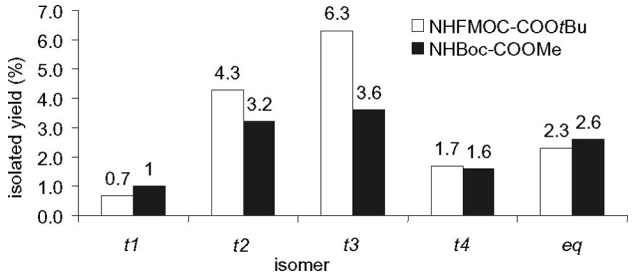


Figure 4. Distribution of bis-adducts based on the yields of isolated compounds.

Determination of the Bis-Adduct Structures

The ES-MS spectra of all the isolated regioisomers **13a**–**e** gave a molecular ion peak of m/z = 1200 corresponding to the desired bis-adducts. Also, each compound of class **15** gave the molecular ion peak expected for the corresponding bis-adduct (m/z = 1036). The characteristic structure of the pyrrolidine signals resulted from the chemical and magnetic nonequivalence of the corresponding protons and is clearly observed in the ^{1}H NMR spectra of the NHBoc/CO_2Me-protected fullero-amino acids **15a**–**e**. The two singlets at δ = 4.77 and 4.73 ppm observed in the spectrum of **15a** are consistent with a D_{2h} symmetry of the *trans*$_1$ isomer (Figure 5, a). The diastereotopic methylene hydrogen atoms of the pyrrolidinic rings in other *trans* isomers showed pairs of AB quartets (mainly overlapped, see parts b–d in Figure 5). Equatorially oriented pyrrolidinic rings in compound **15e** gave pairs of singlets ($2\times CH_2$) and one AB quartet (two remaining CH_2 groups, Figure 5, e).

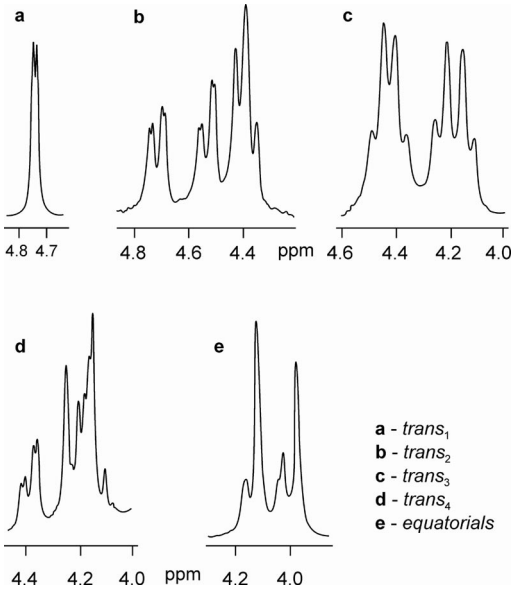


Figure 5. Pyrrolidinic signals in the ^{1}H NMR spectra of the bis-adducts **15a**–**e** (NHBoc/CO_2Me).

In class **13**, such fine structure partially overlapped with the multiplet belonging to the Fmoc benzylic hydrogen atom. The spatial arrangements of the addends were confirmed by characteristic substituent-independent UV/Vis spectra (Figure 6). The results obtained are in agreement with previous studies.[13]

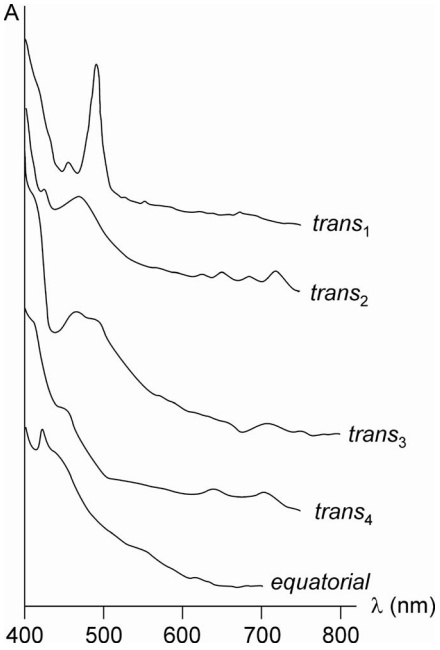


Figure 6. Part of the UV/Vis spectra of the bis-adducts **13a**–**e** and **15a**–**e**.

Both classes of compounds showed almost identical absorption behavior in the visible range (see Exp. Sect.).

Conclusions

We have described the synthesis and characterization of two classes of unsymmetrical fulleropyrrolidine bis-adducts containing orthogonally protected terminal amino and carboxy groups. The new compounds represent a model system for fullerene-based peptidomimetics with the carbon sphere inserted into the peptide backbone. As a result of the spatial proximity of the *cis* addends and consequent possible micellar aggregation, we were mainly interested in the isolation, characterization, and distribution of *equatorial* and *trans* isomers. Considering the requirements of peptide synthesis (orthogonally protected amino and carboxylic groups) and C_{60} reactivity (sensitivity to catalytic hydrogenation), NHFmoc/CO_2tBu and NHBoc/CO_2Me fulleropyrrolidine couples were designed as model compounds. The two target classes of compounds were prepared by two successive [3+2] cycloadditions of azomethyne ylides (thermally generated from formaldehyde and the corresponding orthogonally protected glycino-amines and -acids) to C_{60}. All compounds were characterized by UV/Vis, ^{1}H and ^{13}C NMR spectroscopy, and mass spectrometry. Because a low yield of the Fmoc-protected amino-fulleropyrrolidine (compound **7**) was obtained, the formation of a fulleropyrrolid-

ine ester and subsequent introduction of the NHFmoc addend was found to be a better way to prepare the NHFmoc/CO_2tBu bis-adduct In both classes of bis-adducts, the same relative polarity (eluting order) was observed by flash chromatography. The product distribution, based on the yield of the isolated compounds, was also found to be quite similar. Different yields of the t_3 isomers resulted from rapid decomposition of the NHBoc/CO_2Me structure (compound **15c**) on a silica column.

Experimental Section

Abbreviations: BBA – benzyl bromoacetate, DCM – dichloromethane, DIPEA – diisopropylethylamine, TBBA – *tert*-butyl bromoacetate, TEA – triethylamine, TFA – trifluoroacetic acid, pyrr – pyrrolydinic, Fp – fulleropyrrolidine.

General: FTIR spectra were recorded with a Jasco spectrophotometer FT/IR-200 using NaCl cells (for oils) or KBr powder (DRIFT system). UV spectra were recorded with a Perkin–Elmer Lambda 20 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini-200 spectrometer at 200 and 50 MHz, respectively, using TMS as the internal standard. Chemical shifts are given in parts per million (ppm) relative to that of tetramethylsilane. Mass spectra were recorded with a 7070H VG Microsomass (EI-MS) and PE SCIEX API 1 (ES-MS) spectrometers and compounds were dissolved in methanol unless otherwise noted. Yields of the azomethine ylide cycloaddition reactions are reported as absolute values without taking into account the recovery of the starting fullerene (30–40%). C_{60} was purchased from Bucky, USA (99.5%) and all other reagents and solvents were used as purchased from Fluka, Aldrich, J. T. Baker, and Cambridge Isotope Laboratories. The silica gel NM Kieselgel 60 (0.063–0.200 mm and 0.015–0.04 mm) used in column chromatography was obtained from Macherey–Nagel. The HPLC analysis was conducted with a Waters instrument with a direct phase Phenomenex Prodigy 5 μ silica 100 Å column.

Synthetic Procedures

***tert*-Butyl *N*-(2-Aminoethyl)glycinate (3):** A solution of TBBA (**2**; 24.80 g, 18.8 mL, 0.13 mol, 0.11 mol-equiv.) in DCM (105 mL) was added dropwise over 5 h to a vigorously stirred solution of ethanediamine (**1**, 67.28 g, 75.0 mL, 1.12 mol) in DCM (525 mL) cooled in an ice bath. The reaction mixture was stirred at room temperature for 24 h, washed with water (3 × 120 mL), and aqueous wash-back-extracted with DCM (120 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and the solvents evaporated to dryness to leave the product **3** (15.68 g, 70%) as a pale-yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.21 (s, 2 H), 2.76–2.64 (m, 2 H), 2.64–2.52 (m, 2 H), 1.38 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.25, 80.43, 51.72, 51.10, 41.32, 27.63 ppm. IR (DRIFT, NaCl): $\tilde{\nu}$ = 3316, 2969, 1734, 1223, 1156 cm^{-1}. MS (EI): *m*/*z* = 175 [MH – *i*BuH]$^+$, 119 [MH]$^+$.

***tert*-Butyl *N*-(2-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}ethyl)-glycinate Hydrochloride (4):** A solution of Fmoc succinimide (9.21 g, 27.3 mmol, 0.95 mol-equiv.) in DCM (50 mL) was added dropwise over 3 h to a stirred solution of amino ester **3** (5.00 g, 28.7 mmol) and DIPEA (3.53 g, 4.7 mL, 27.3 mmol, 0.95 mol-equiv.) in DCM (200 mL), and stirring was continued at room temperature for 20 h. The reaction mixture was washed with 1 M HCl (5 × 35 mL) and brine (35 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuo to approx. 80 mL. After cooling overnight at –20 °C a white precipitate was obtained. Filtering and washing with DCM until a colorless filtrate was obtained gave the product **4** (10.36 g, 88%) as a white amorphous solid. ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 9.31 (s, 2 H), 7.89 (d, *J* = 7.0 Hz, 2 H), 7.67 (d, *J* = 7.0 Hz, 2 H), 7.52–7.15 (m, 4 H), 5.76 (s, 1 H), 4.40–4.26 (m, 2 H), 4.26–4.17 (m, 1 H), 3.88 (s, 2 H), 3.42–3.22 (m, 2 H), 3.08–2.90 (m, 2 H), 1.25 (s, 9 H) ppm. ^{13}C NMR (50 MHz, [D_6]DMSO): δ = 165.61, 156.20, 143.73, 140.68, 127.59, 127.04, 125.11, 120.12, 83.00, 68.23, 47.25, 46.64, 46.38, 36.61, 27.66 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 2970, 2850, 1735, 1640, 1440, 1295, 1162 cm^{-1}. MS (EI): *m*/*z* = 397 [M – *i*BuH]$^+$, 341 [M]$^+$.

***tert*-Butyl *N*-(2-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}ethyl)-glycinate (5):** A solution of the salt **4** (10.36 g, 24.0 mmol) in chloroform (500 mL) was washed with a satd. solution of $NaHCO_3$ (3 × 150 mL), dried with anhydrous $CaCl_2$, filtered, and the solvents evaporated to dryness. The product **5** (9.48 g, quantitative) was obtained as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.79 (d, *J* = 7.0 Hz, 2 H), 7.64 (d, *J* = 7.0 Hz, 2 H), 7.50–7.30 (m, 4 H), 5.48–5.32 (br. s, 1 H), 4.42 (d, *J* = 6.8 Hz, 2 H), 4.25 (t, *J* = 6.8 Hz, 1 H), 3.42–3.18 (m, 4 H), 2.79 (t, *J* = 5.4 Hz, 2 H), 1.50 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.65, 156.38, 143.88, 141.15, 127.51, 126.90, 124.98, 119.82, 81.31, 66.54, 51.18, 48.56, 47.22, 40.67, 28.08 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3521, 3443, 2909, 1738, 1640, 1443, 1290, 1158 cm^{-1}. MS (EI): *m*/*z* = 397 [MH – *i*BuH]$^+$, 341 [MH]$^+$.

***N*-(2-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}ethyl)glycine (6):** TFA (88 mL, 1.14 mol) was added to a stirred, ice-bath cooled solution of ester **5** (8.94 g, 22.6 mmol) in DCM (55 mL),. After 20 min a solution was warmed to room temperature and stirring was continued for an additional 5 h. The reaction mixture was evaporated to dryness and TFA was completely removed by co-evaporation with toluene. Crystallization from MeOH/Et_2O gave the acid **6** (5.16 g, 67%) as a white solid. M.p. 154–156 °C (MeOH/Et_2O). ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 7.89 (d, *J* = 7.2 Hz, 2 H), 7.69 (d, *J* = 7.0 Hz, 2 H), 7.61–7.45 (m, 1 H), 7.45–7.20 (m, 3 H), 4.35 (d, *J* = 6.4 Hz, 2 H), 4.26–4.18 (m, 1 H), 3.89 (s, 2 H), 3.31 (d, *J* = 5.0 Hz, 2 H), 3.12–2.95 (m, 2 H) ppm. ^{13}C NMR (50 MHz, [D_6]DMSO): δ = 168.10, 156.33, 143.80, 140.76, 127.66, 127.10, 125.12, 120.17, 65.68, 47.22, 46.74, 46.48, 36.76 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3303, 3004, 2806, 1694, 1540, 1269, 1145 cm^{-1}. MS (ES; MeOH): *m*/*z* = 364 [MH + Na]$^+$, 341 [MH]$^+$.

Fmoc-Protected Fulleroamine 7: A solution of amino acid **6** (283 mg, 0.83 mmol) and pyridine (134 μL, 1.66 mmol, 2 mol-equiv.) in MeOH (2 mL) was stirred at room temperature for 15 min and then added to a suspension of C_{60} (600 mg, 0.83 mmol) and paraformaldehyde (125 mg, 4.15 mmol, 5 mol-equiv.) in toluene (600 mL). The reaction mixture was heated at reflux over 1.5 h and the solvents evaporated to dryness. The residue was subjected to silica column chromatography using toluene and PhMe/EtOAc (97:3) as eluents (unreacted C_{60} and the product **7**, respectively). Precipitation with Et_2O from a highly concentrated DCM solution and drying under vacuum afforded Fmoc-protected fulleropyrrolidine **7** (998 mg, 9%) as a brown solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.78 (d, *J* = 8.0 Hz, 2 H), 7.68 (d, *J* = 6.0 Hz, 2 H), 7.33–7.29 (m, 4 H), 5.74–5.54 (br. s, 1 H), 4.60–4.43 (m, 2 H), 4.50 (s, 4 H), 4.34 (d, *J* = 6.0 Hz, 1 H), 3.89–3.71 (m, 2 H), 3.40–3.24 (m, 2 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 154.61, 147.25, 146.19, 146.02, 145.90, 145.59, 145.36, 145.23, 144.48, 143.89, 143.03, 142.58, 142.143, 142.00, 141.83, 141.29, 140.12, 136.09, 127.67, 127.03, 125.03, 119.97, 70.60, 67.60, 66.81, 65.86, 53.62, 47.37 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3420, 3004, 2950, 2788, 1720, 1540, 1150 cm^{-1}. MS (ES; MeOH/THF): *m*/*z* = 1030 [M – PhH]$^+$,

952 $[MH]^+$. UV/Vis (THF): λ = 242 (ε = 82000), 260 (103000), 300 (35000), 320 (32000), 435 (3500), 704 nm (990 $dm^3 mol^{-1} cm^{-1}$).

1-Benzyl 6-*tert*-Butyl 3-Azahexanedioate (10a): TEA (11.10 g, 110 mmol, 2 mol-equiv., 15.2 mL) was added to a suspension of β-alanine *tert*-butyl ester hydrochloride **9a** (10.00 g, 55 mmol) in dioxane (400 mL) and the reaction mixture was stirred at room temperature for 15 min. The suspension was then cooled in an ice bath and a solution of BBA (10.10 g, 44 mmol, 0.8 mol-equiv., 7.0 mL) in dioxane (100 mL) was added dropwise and the mixture stirred at room temperature for a further 24 h. After evaporation to dryness, the residue was diluted with water, extracted with EtOAc, dried with anhydrous Na_2SO_4, filtered, evaporated to dryness, and subjected to silica column flash chromatography. Elution with EtOAc gave the product **10a** (9.48 g, 73%) as a colorless oil, pure enough to be used in the next step. ^1H NMR (200 MHz, $CDCl_3$): δ = 7.36 (s, 5 H), 5.18 (s, 2 H), 3.45 (s, 2 H), 2.86 (t, J = 8.0 Hz, 2 H), 2.43 (t, J = 8.0 Hz, 2 H), 2.04 (br. s, 1 H), 1.46 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.97, 171.62, 135.48, 128.49, 128.26, 80.57, 66.50, 50.85, 44.92, 35.93, 28.09 ppm. IR (DRIFT, NaCl): $\tilde{\nu}$ = 3341, 2973, 2929, 1732, 1253, 1155 cm^{-1}. MS (ES; MeOH): m/z = 317 $[MH + Na]^+$, 294 $[MH]^+$.

1-Benzyl 6-Methyl 3-Azahexanedioate (10b): TEA (14.46 g, 144 mmol, 2 mol-equiv., 19.8 mL) was added to a suspension of β-alanine methyl ester hydrochloride **9b** (10.00 g, 72 mmol) in dioxane (500 mL) and the reaction mixture was stirred at room temperature for 15 min. A suspension was then cooled in an ice bath, a solution of BBA (11.48 g, 50 mmol, 0.7 mol-equiv., 7.9 mL) in dioxane (100 mL) was added dropwise, and the mixture was stirred at room temperature for a further 24 h. After evaporation to dryness, the residue was diluted with water, extracted with EtOAc, dried with anhydrous Na_2SO_4, filtered, evaporated to dryness and subjected to silica column flash chromatography. Elution with EtOAc gave the product **10b** (7.66 g, 61%) as a colorless oil, pure enough to be used in the next step. ^1H NMR (200 MHz, $CDCl_3$): δ = 7.23 (s, 5 H), 5.04 (s, 2 H), 5.55 (s, 3 H), 3.34 (s, 2 H), 2.78 (t, J = 6.4 Hz, 2 H), 2.38 (t, J = 6.4 Hz, 2 H), 1.92 (s, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.51, 171.79, 135.30, 128.32, 128.09, 66.32, 51.44, 50.58, 44.51, 34.42 ppm. IR (DRIFT, NaCl): $\tilde{\nu}$ = 3031, 2975, 2930, 1735, 1250, 1155 cm^{-1}. MS (ES; MeOH): m/z = 252 $[MH]^+$.

Hydrogenation of Benzyl Esters 10a,b to 11a,b: The catalyst (5% Pd/C, 10% w/w) was added to a solution of the benzyl ester (30 mmol) in MeOH (300 mL) and the suspension was hydrogenated at atmospheric pressure for 24 h. Filtration and subsequent evaporation to dryness gave the corresponding acid as a sticky oil in quantitative yield.

6-*tert*-Butyl Hydrogen 3-Azahexanedioate (11a): M.p. 199–201 °C (MeOH/AcOEt). ^1H NMR (200 MHz, D_2O): δ = 3.44 (s, 2 H), 3.12 (t, J = 6.6 Hz, 2 H), 2.58 (t, J = 6.6 Hz, 2 H), 1.28 (s, 9 H) ppm. ^{13}C NMR (50 MHz, D_2O): δ = 173.20, 172.79, 85.56, 51.20, 44.82, 33.32, 29.03 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3434, 3069, 2977, 2419, 1729, 1604, 1389, 1160 cm^{-1}. MS (ES; MeOH): m/z = 227 $[MH + Na]^+$, 204 $[MH]^+$.

6-Methyl Hydrogen 3-Azahexanedioate (11b): ^1H NMR (200 MHz, D_2O): δ = 3.70 (s, 3 H), 3.61 (s, 2 H), 3.32 (t, J = 6.6 Hz, 2 H), 2.83 (t, J = 6.6 Hz, 2 H) ppm. ^{13}C NMR (50 MHz, D_2O): δ = 175.96, 174.09, 55.85, 52.57, 46.06, 33.41 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3430, 2982, 2420, 1730, 1602, 1395, 1157 cm^{-1}. MS (ES; MeOH): m/z = 185 $[MH + Na]^+$, 162 $[MH]^+$.

Synthesis of the Fulleropyrrolidines 12: A suspension of amino acid **11** (1 mmol; 200 mg of **11a** or 160 mg of **11b**), paraformaldehyde (150 mg, 5 mmol), and C_{60} (720 mg, 1 mmol) in toluene (500 mL) was heated at reflux for 45 min, evaporated to dryness and purified by chromatography a on silica column. Unreacted C_{60} was eluted with toluene, whereas elution with PhMe/EtOAc (95:5) gave Fp esters **12a** and **12b**, further purified by precipitation with Et_2O from DCM solution.

Fp *tert*-Butyl Ester 12a: Yield 400 mg (45%). ^1H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 4.43 (s, 4 H), 3.39 (t, J = 7.0 Hz, 2 H), 2.86 (t, J = 7.0 Hz, 2 H), 1.54 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 171.22, 154.68, 147.12, 146.07, 145.85, 145.51, 145.25, 145.10, 144.37, 142.92, 142.45, 142.01, 141.90, 141.71, 139.99, 136.10, 80.65, 70.46, 67.62, 50.25, 35.40, 28.17 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3423, 2791, 1719, 1243, 1116 cm^{-1}. MS (ES; THF): m/z = 892 $[MH/2]^+$, 446 $[MH]^+$. UV/Vis (THF): λ = 317 (ε = 38000), 433 (4200), 704 nm (890 $dm^3 mol^{-1} cm^{-1}$).

Fp Methyl Ester 12b: Yield 310 mg (37%). ^1H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 4.45 (s, 4 H), 3.83 (s, 3 H), 3.43 (t, J = 7.2 Hz, 2 H), 2.98 (t, J = 7.2 Hz, 2 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 172.52 (CO), 154.77, 146.20, 146.01, 145.38, 144.51, 142.59, 142.17, 141.83, 140.12, 136.16, 70.57, 67.72, 51.05, 49.99, 34.04 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3433, 2790, 1722, 1245, 1115 cm^{-1}. MS (ES; THF): m/z = 850 $[MH/2]^+$, 425 $[MH]^+$. UV/Vis (THF): λ = 320 (ε = 50000), 430 (5000), 704 nm (1100 $dm^3 mol^{-1} cm^{-1}$).

NHFmoc-CO_2*t*Bu-Protected Bis-Adducts 13a–e: A solution of Fmoc-protected amino-glycinate **6** (0.76 g, 2.24 mmol, 2 mol-equiv.) in pyridine (2 mL) was added to a suspension of Fp *tert*-butyl ester **12a** (1.00 g, 1.12 mmol) and paraformaldehyde (0.17 g, 5.60 mmol, 5 mol-equiv.) in toluene (1 L) and the reaction mixture was heated at reflux for 1 h. After washing with water (3 × 300 mL), drying with anhydrous Na_2SO_4, filtering, and evaporation to dryness, the residue was subjected to column chromatography on SiO_2 (63–200 μm). Unreacted starting mono-Fp (298 mg, 30%) was recovered by elution with PhMe and PhMe/EtOAc (100:1), evaporation to dryness, and subsequent precipitation from a highly concentrated DCM solution with hexane. The bis-adducts were eluted with PhMe/EtOAc (1:1) and separated by dry column flash chromatography on fine silica (15–40 μm) using the following PhMe/EtOAc mixtures as eluents: 100:1.5 (*trans*$_1$), 100:2 (*trans*$_2$), 100:3 (*trans*$_3$), 100:3.5 (*trans*$_4$), and 100:4 (e' + e''). Precipitation of the separated bis-adducts from a highly concentrated DCM solution with hexane gave the products **13a**–**e** as brown solids. The purity of the compounds was checked by HPLC [SiO_2, PhMe/EtOAc (7:3) 1 mL/min, 320 nm].

13a (*trans*$_1$): Yield 9.2 mg (0.7%). ^1H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 7.87–7.60 (m, 4 H), 7.50–7.20 (m, 4 H), 5.81–5.64 (br. s, 1 H), 4.78 (s, 4 H), 4.75 (s, 4 H), 4.50 (d, J = 7.0 Hz, 2 H), 4.40–4.23 (m, 1 H), 3.97–3.80 (m, 2 H), 3.55 (t, J = 7.2 Hz, 2 H), 3.50–3.15 (m, 2 H), 2.98 (t, J = 7.2 Hz, 2 H), 1.62 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 171.13, 153.35, 153.09, 146.03, 145.96, 145.86, 145.63, 145.28, 145.00, 144.42, 144.32, 144.04, 143.77, 142.15, 141.63, 141.41, 141.20, 140.59, 136.39, 136.24, 127.58, 126.96, 124.94, 119.86, 80.51, 68.30, 67.82, 67.75, 66.69, 53.72, 50.34, 47.39, 35.57, 28.17 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3420, 2942, 2784, 1720, 1513, 1461, 1347, 1151, 747, 483, 454 cm^{-1}. MS [ES; THF/MeOH (1:1)]: m/z = 1200 $[MH]^+$. UV/Vis (THF): λ = 244 (ε = 130000), 263 (135000), 301 (45000), 317 (50000), 458 (900), 491 nm (4000 $dm^3 mol^{-1} cm^{-1}$).

13b (*trans*$_2$): Yield 58.3 mg (4.3%). ^1H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 7.82–7.60 (m, 4 H), 7.50–7.20 (m, 4 H), 5.80–5.67 (br. s, 1 H), 4.80–4.20 (m, 11 H), 3.90–3.70 (m, 2 H), 3.44 (t, J = 7.2 Hz, 2 H), 3.38–3.26 (m, 2 H), 2.93 (t, J = 7.2 Hz, 2 H), 1.60 (s, 9

H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 171.40, 158.76, 158.53, 156.39, 153.14, 153.00, 152.90, 152.71, 152.36, 152.13, 148.30, 147.60, 146.91, 146.31, 146.11, 145.96, 145.56, 145.06, 144.09, 143.82, 143.69, 143.54, 142.42, 142.28, 141.20, 139.50, 134.44, 133.85, 133.78, 127.61, 127.00, 125.01, 119.91, 80.73, 69.16, 68.99, 67.71, 67.59, 67.42, 66.75, 53.54, 50.28, 47.32, 35.49, 28.22 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3420, 2942, 2784, 1720, 1513, 1461, 1347, 1151, 747, 483, 454 cm^{-1}. MS [ES; THF/MeOH (1:1)]: *m*/*z* = 1200 [MH]$^+$. UV/Vis (THF): λ = 248 (ε = 131000), 263 (118000), 300 (63000), 429 (4900), 476 (4900), 625 (600), 654 (620), 689 (450), 723 nm (1300 dm^3 mol^{-1} cm^{-1}).

13c (*trans*$_3$): Yield 85.2 mg (6.3%). ^{1}H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 7.77 (d, *J* = 7.0 Hz, 2 H), 7.64 (d, *J* = 7.0 Hz, 2 H), 7.47–7.18 (m, 4 H), 5.65–5.53 (br. s, 1 H), 4.55–4.00 (m, 11 H), 3.80–3.63 (m, 2 H), 3.34 (t, *J* = 7.2 Hz, 2 H), 3.28–3.13 (m, 2 H), 2.83 (t, *J* = 7.2 Hz, 2 H), 1.53 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 171.46, 158.13, 157.55, 156.46, 155.52, 155.31, 154.86, 154.60, 149.01, 148.92, 148.65, 148.74, 148.13, 146.58, 146.50, 145.24, 145.11, 144.86, 144.72, 144.53, 143.86, 143.55, 142.48, 141.60, 141.38, 141.24, 141.01, 139.69, 136.39, 136.31, 135.56, 135.37, 134.48, 131.34, 130.51, 128.81, 128.53, 128.38, 128.15, 127.95, 127.83, 127.64, 127.01, 125.03, 119.94, 80.84, 70.31, 70.22, 69.84, 69.59, 67.61, 66.78, 53.51, 50.24, 47.32, 35.90, 28.24 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3420, 2942, 2784, 1720, 1513, 1461, 1347, 1151, 747, 483, 454 cm^{-1}. MS [ES; THF/MeOH (1:1)]: *m*/*z* = 1200 [MH]$^+$. UV/Vis (THF): λ = 285 (ε = 63000), 413 (5000), 465 (2700), 702 (270), 743 nm (140 dm^3 mol^{-1} cm^{-1}).

13d (*trans*$_4$): Yield 23.5 mg (1.7%). ^{1}H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 7.78 (d, *J* = 7.0 Hz, 2 H), 7.64 (d, *J* = 7.0 Hz, 2 H), 7.50–7.25 (m, 4 H), 5.60–5.50 (br. s, 1 H, NH), 4.55–3.95 (m, 11 H), 3.75–3.60 (m, 2 H), 3.28 (t, *J* = 7.2 Hz, 2 H), 3.22–3.07 (m, 2 H), 2.79 (t, *J* = 7.2 Hz, 2 H), 1.55 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 171.46, 154.52, 152.53, 151.33, 150.38, 148.21, 147.53, 146.02, 145.44, 144.90, 144.53, 143.83, 142.55, 142.02, 141.66, 141.22, 127.64, 127.02, 125.06, 119.94, 80.70, 69.31, 69.06, 67.96, 67.08, 66.88, 66.77, 53.70, 50.22, 47.31, 35.39, 28.24 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3420, 2942, 2784, 1720, 1513, 1461, 1347, 1151, 747, 483, 454 cm^{-1}. MS [ES; THF/MeOH (1:1)]: *m*/*z* = 1200 [MH]$^+$. UV/Vis (THF): λ = 242 (ε = 130000), 266 (116000), 300 (62000), 411 (1100), 449 (700), 642 (180), 709 nm (130 dm^3 mol^{-1} cm^{-1}).

13e (*equatorials*): Yield 31.1 mg (2.3%). ^{1}H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 7.87–7.52 (m, 8 H), 7.50–7.18 (m, 8 H), 5.61–5.40 (br. s, 2 H), 4.60–4.37 (m, 4 H), 4.17–3.83 (m, 18 H), 3.72–3.55 (m, 4 H), 3.20 (t, *J* = 7.2 Hz, 4 H), 3.17–3.00 (m, 4 H), 2.73 (t, *J* = 7.2 Hz, 4 H), 1.53 (s, 9 H), 1.50 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 171.30, 158.89, 158.64, 156.32, 153.40, 152.86, 152.66, 149.65, 148.77, 147.93, 147.63, 147.10, 146.59, 146.52, 145.63, 145.07, 144.88, 144.53, 144.26, 143.79, 143.61, 143.05, 142.15, 141.77, 141.62, 141.51, 141.34, 141.24, 140.55, 139.04, 136.64, 135.51, 135.48, 127.67, 127.59, 127.02, 126.97, 124.96, 119.97, 119.88, 80.65, 69.65, 69.43, 69.29, 67.52, 67.23, 66.97, 66.72, 66.49, 53.70, 53.29, 50.21, 50.06, 47.28, 39.44, 35.29, 28.18 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3420, 2942, 2784, 1720, 1513, 1461, 1347, 1151, 747, 483, 454 cm^{-1}. MS [ES; THF/MeOH (1:1)]: *m*/*z* = 1200 [MH]$^+$. UV/Vis (THF): λ = 242 (ε = 102000), 253 (96000), 301 (44000), 316 (44000), 395 (7400), 423 nm (6800 dm^3 mol^{-1} cm^{-1}).

NHBoc-CO_2Me-Protected Bis-Adducts 15a–e: A suspension of the Fp methyl ester **12b** (874 mg, 1.03 mmol), paraformaldehyde (155 mg, 5.15 mmol, 5 mol-equiv.), and Boc-protected amino-glycinate **14**[12] (225 mg, 1.03 mmol) in toluene (500 mL) was heated at reflux for 1.5 h, evaporated to dryness, and purified by chromatography on SiO_2 (63–200 μm). Unreacted starting mono-Fp (243 mg, 28%) was isolated by elution with PhMe/EtOAc (100:1) and subsequent precipitation with hexane from highly concentrated DCM solution. The second fraction containing all the bis-adducts was obtained by elution with PhMe/EtOAc (1:1) and repurified by chromatography by DC-FC on fine SiO_2 (15–40 μm) using the following PhMe/EtOAc mixtures as eluents: 100:2 (*trans*$_1$), 100:3 (*trans*$_2$), 100:4 (*trans*$_3$), 100:5 (*trans*$_4$), and 100:6 (*e*′ + *e*″). Precipitation of the separated bis-adducts from a highly concentrated DCM solution with hexane gave the products **15a–e** as brown solids. The purity of the compounds was checked by HPLC (SiO_2, PhMe, 320 nm).

15a (*trans*$_1$): Yield 10.6 mg (1.0%) ^{1}H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 5.50–5.30 (br. s, 1 H), 4.74 (s, 4 H), 4.73 (s, 4 H), 3.88 (s, 3 H), 3.79 (br. q, *J* = 5.8 Hz, 2 H), 3.60 (t, *J* = 7.2 Hz, 2 H), 3.39 (t, *J* = 5.8 Hz, 2 H), 3.08 (t, *J* = 7.2 Hz, 2 H), 1.54 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 172.67, 153.43, 153.43, 147.60, 146.08, 145.39, 144.16, 142.26, 140.69, 136.41, 79.44, 68.48, 67.96, 67.89, 54.26, 51.98, 50.08, 34.20, 28.57 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3433, 3333, 2967, 1722, 1692, 1520, 1255, 1170, 1115 cm^{-1}. MS [ES; THF/MeOH (1:1)]: *m*/*z* = 1036 [MH]$^+$. UV/Vis (THF): λ = 285 (ε = 95000), 308 (50000), 320 (56000), 458 (1100), 492 nm (4200 dm^3 mol^{-1} cm^{-1}).

15b (*trans*$_2$): Yield 34.1 mg (3.2%). ^{1}H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 5.50–5.25 (br. s, 1 H), 4.70, 4.41 (ABq, *J* = 9 Hz, 4 H), 4.52, 4.37 (ABq, *J* = 9 Hz, 4 H), 3.87 (s, 3 H), 3.74 (br. q, *J* = 6.0 Hz, 2 H), 3.52 (t, *J* = 7.2 Hz, 2 H), 3.31 (t, *J* = 6.0 Hz, 2 H), 3.04 (t, *J* = 7.2 Hz, 2 H), 1.55 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 172.62, 158.74, 156.02, 153.12, 153.05, 152.94, 152.84, 152.34, 152.24, 148.33, 148.30, 147.63, 147.60, 147.02, 146.97, 146.36, 146.19, 146.17, 146.01, 145.59, 145.53, 145.26, 145.10, 144.14, 143.74, 143.61, 143.59, 142.51, 142.48, 142.41, 142.33, 141.47, 141.40, 139.53, 134.45, 133.70, 79.55, 69.27, 69.09, 67.75, 67.72, 67.59, 54.21, 51.99, 50.02, 34.05, 28.55 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3433, 3333, 2967, 1722, 1692, 1520, 1255, 1170, 1115 cm^{-1}. MS [ES; THF/MeOH (1:1)]: *m*/*z* = 1036 [MH]$^+$. UV/Vis (THF): λ = 272 (ε = 50000), 285 (120000), 311 (58000), 431 (5200), 476 (5400), 624 (800), 660 (930), 689 (750), 721 nm (1000 dm^3 mol^{-1} cm^{-1}).

15c (*trans*$_3$): Yield 38.3 mg (3.6%). ^{1}H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 5.40–5.15 (br. s, 1 H), 4.47, 4.38 (ABq, *J* = 9 Hz, 4 H), 4.22, 4.12 (ABq, *J* = 9 Hz, 4 H), 3.82 (s, 3 H), 3.67 (br. q, *J* = 5.0 Hz, 2 H), 3.39 (t, *J* = 7.0 Hz, 2 H), 3.18 (br. t, *J* = 5.0 Hz, 2 H), 2.94 (t, *J* = 7.0 Hz, 2 H), 1.51 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 172.47, 158.01, 155.87, 155.43, 155.37, 155.30, 154.71, 154.63, 148.98, 148.90, 148.78, 148.67, 148.14, 148.10, 146.52, 146.48, 145.20, 145.06, 144.77, 144.72, 144.50, 143.85, 143.51, 142.44, 141.51, 141.35, 141.16, 140.93, 139.68, 139.65, 137.50, 136.32, 135.46, 135.44, 128.47, 126.88, 79.42, 69.81, 69.57, 67.73, 67.66, 66.84, 54.13, 51.87, 49.92, 33.92, 28.48 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3433, 3333, 2967, 1722, 1692, 1520, 1255, 1170, 1115 cm^{-1}. MS [ES; THF/MeOH (1:1)]: *m*/*z* = 1036 [MH]$^+$. UV/Vis (THF): λ = 260 (ε = 52000), 274 (75000), 285 (110000), 320 (70000), 417 (6000), 467 (3500), 702 (450), 742 nm (220 dm^3 mol^{-1} cm^{-1}).

15d (*trans*$_4$): Yield 17.0 mg (1.6%). ^{1}H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 5.35–5.10 (br. s, 1 H), 4.50–4.00 (m, 8 H), 3.82 (s, 3 H), 3.62 (q, *J* = 7.5 Hz, 2 H), 3.36 (t, *J* = 8.0 Hz, 2 H), 3.14 (t, *J* = 7.5 Hz, 2 H), 2.90 (t, *J* = 8.0 Hz, 2 H), 1.56 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 172.60, 158.50, 152.64, 151.40, 150.72, 148.25, 147.50, 146.05, 145.44, 144.88, 144.61, 142.57,

142.17, 141.75, 141.40, 141.22, 138.58, 136.10, 135.40, 131.32, 79.48, 69.75, 69.48, 67.70, 67.58, 67.20, 54.18, 51.95, 50.04, 34.09, 28.54 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3433, 3333, 2967, 1722, 1692, 1520, 1255, 1170, 1115 cm^{-1}. MS [ES; THF/MeOH (1:1)]: *m*/*z* = 1036 $[MH]^+$. UV/Vis (THF): λ = 220 (ε = 60000), 267 (87000), 286 (120000), 308 (94000), 414 (2000), 453 (1100), 640 (270), 705 nm (550 $dm^3 mol^{-1} cm^{-1}$).

15e (*equatorials*): Yield 27.7 mg (2.6%). ^1H NMR (200 MHz, $CDCl_3$ + CS_2): δ = 5.30–5.10 (br. s, 1 H, NH), 4.13, 3.95 (ABq, *J* = 9 Hz, 4 H, pyrr), 4.10 (s, 2 H), 3.96 (s, 2 H), 3.79 (s, 3 H), 3.78 (s, 3 H), 3.56 (br. q, *J* = 7.0 Hz, 4 H), 3.26 (t, *J* = 8.0 Hz, 4 H), 3.07 (br. t, *J* = 7.5 Hz, 4 H), 2.85 (t, *J* = 8.0 Hz, 4 H), 1.52 (s, 9 H), 1.48 (s, 9 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$ + CS_2): δ = 172.50, 158.80, 158.55, 156.55, 153.30, 152.82, 149.77, 148.82, 147.90, 147.50, 147.26, 146.65, 146.50, 145.67, 145.11, 144.92, 144.57, 144.30, 143.62, 143.10, 142.15, 141.72, 141.59, 141.47, 141.20, 139.12, 136.68, 135.52, 80.65, 69.65, 69.62, 69.43, 69.29, 67.52, 67.23, 66.97, 66.72, 66.49, 54.10, 53.89, 51.99, 51.75, 50.25, 50.11, 34.10, 33.90, 28.18 ppm. IR (DRIFT, KBr): $\tilde{\nu}$ = 3433, 3333, 2967, 1722, 1692, 1520, 1255, 1170, 1115 cm^{-1}. MS [ES; THF/MeOH (1:1)]: *m*/*z* = 1036 $[MH]^+$. UV/Vis (THF): λ = 231 (ε = 50000), 257 (62000), 285 (123000), 318 (63000), 423 nm (4800 $dm^3 mol^{-1} cm^{-1}$).

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[1] a) M. Satoh, I. Takayanagi, *J. Pharmacol. Sci.* **2006**, *100*, 513–518; b) A. W. Jensen, S. R. Wilson, D. I. Schuster, *Bioorg. Med. Chem.* **1996**, *4*, 767–769; c) S. Bosi, T. Da Ros, G. Spalluto, M. Prato, *Eur. J. Med. Chem.* **2003**, *38*, 913–923; d) T. Da Ros, M. Prato, *Chem. Commun.* **1999**, 663–669.

[2] a) R. Sijbesma, G. Srdanov, F. Wudl, J. A. Castoro, C. Wilkins, S. H. Friedman, D. L. DeCamp, G. L. Kenyon, *J. Am. Chem. Soc.* **1993**, *115*, 6510–6512; b) G. L. Marcorin, T. Da Ros, S. Castellano, G. Stefancich, I. Bonin, S. Miertus, M. Prato, *Org. Lett.* **2000**, *2*, 3955–3958.

[3] D. L. Kronholm, J. C. Hummelen, A. B. Sieval, US patent WO20050580002 A2, **2005**.

[4] M. S. Bjelakovic, D. M. Godjevac, D. R. Milic, *Carbon* **2007**, *45*, 2260–2265.

[5] I. C. Wang, L. A. Tai, D. D. Lee, P. P. Kanakamma, C. K. F. Shen, T. Y. Luh, C. H. Cheng, K. C. Hwang, *J. Med. Chem.* **1999**, *42*, 4614–4620.

[6] a) Y. Mikata, S. Takagi, M. Tanahashi, S. Ishii, M. Obata, Y. Miyamoto, K. Wakita, T. Nishisaka, T. Hirano, T. Ito, M. Hoshino, C. Ohtsuki, M. Tanihara, S. Yano, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3289–3292; b) C. Yu, T. Canteenwala, L. Y. Chiang, B. Wilson, K. Pritzker, *Synth. Met.* **2005**, *153*, 37–40.

[7] a) T. Kawauchi, J. Kumaki, A. Kitaura, K. Okoshi, H. Kusanagi, K. Kobayashi, T. Sugai, H. Shinohara, E. Yashima, *Angew. Chem. Int. Ed.* **2008**, *47*, 515–519; b) I. Ramakanth, A. Patnaik, *Carbon* **2008**, *46*, 692–698; c) T. Haino, M. Yanase, C. Fukunaga, Y. Fukazawa, *Tetrahedron* **2006**, *62*, 2025–2035; d) F. Cataldo, *Polym. Degr. Stab.* **2002**, *77*, 111–120.

[8] a) A. Hirsch, M. Brettreich, *Fullerenes*, Wiley-VCH, Weinheim, Germany, **2005**, pp. 49–289; b) A. Hirsch, *Top. Curr. Chem.* **1999**, *199*, 1–65; c) B. I. Kharisov, O. V. Kharissova, M. Jimenez Gomez, M. U. Ortiz Mendez, *Ind. Eng. Chem. Res.* **2009**, *48*, 545–571.

[9] a) V. Georgakilas, F. Pellarini, M. Prato, D. M. Guldi, M. Melle-Franco, F. Zerbetto, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5075–5080; b) D. M. Guldi, F. Zerbetto, V. Georgakilas, M. Prato, *Acc. Chem. Res.* **2005**, *38*, 38–43.

[10] a) J. Yang, L. B. Alemany, J. Driver, J. D. Hartgerink, A. R. Barron, *Chem. Eur. J.* **2007**, *13*, 2530–2545; b) L. A. Watanabe, M. P. I. Bhuiyan, B. Jose, T. Kato, N. Nishino, *Tetrahedron Lett.* **2004**, *45*, 7137–7140.

[11] a) A. Bianco, T. Da Ros, M. Prato, C. Toniolo, *J. Pept. Sci.* **2001**, *7*, 208–219; b) D. Pantarotto, A. Bianco, F. Pellarini, A. Tossi, A. Giangaspero, I. Zelezetsky, J.-P. Briand, M. Prato, *J. Am. Chem. Soc.* **2002**, *124*, 12543–12549; c) D. Pantarotto, N. Tagmatarchis, A. Bianco, M. Prato, *Mini-Rev. Med. Chem.* **2004**, *4*, 805–814.

[12] K. Kordatos, T. Da Ros, S. Bosi, E. Vazquez, M. Bergamin, C. Cusan, F. Pellarini, V. Tomberli, B. Baiti, D. Pantarotto, V. Georgakilas, G. Spalluto, M. Prato, *J. Org. Chem.* **2001**, *66*, 4915–4920.

[13] K. Kordatos, S. Bosi, T. Da Ros, A. Zambon, V. Lucchini, M. Prato, *J. Org. Chem.* **2001**, *66*, 2802–2808.

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