Efficient solid-phase synthesis of fullero-peptides using Merrifield strategy

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Electronic Supplementary Information

General. All reagents and solvents were obtained from commercial suppliers and used without further purification. Compound **1** was synthesized as reported previously.[1] *t*-Boc-Phe-OCH_2-PAM (0.72 mmol/g) and *t*-Boc-Ala-OCH_2-PAM (0.77 mmol/g) resins were obtained from Applied Biosystems (Foster City, CA). Amino acids were purchased from NeoMPS (Strasbourg, France). Boc-L-Glu-OFm was purchased from Bachem (Bubendorf, Switzerland) Peptides were synthesized on a semi-automated synthesizer working under nitrogen flow until the introduction of the Boc-L-Fgu-OH residue.[2] The synthesis was continued manually in 5 ml fritted syringes. RP-HPLC analyses were carried out on a Macherey-Nagel C_4 column (5 μm, 150 × 4.6 mm) using a linear gradient of A: 0.1% TFA in water and B: 0.08% TFA in acetonitrile, 0-100% B in 20 min at 1.2 mL/min flow rate. Chromatograms were recorded on a Varian ProStar 330 photodiode array detector. RP-HPLC purifications were carried out on a Macherey-Nagel C_4 column (7 μm, 250 × 10 mm) using a linear gradient of A: 0.1% TFA in water and B: 0.08% TFA in acetonitrile, 30-100% B in 30 min at 6.0 mL/min flow rate. Chromatograms were recorded at 230 nm. MALDI-tof mass analysis was performed on a linear Protein-tof Bruker instrument using α-cyano-4-hydroxycinnamic as a matrix.

Abbreviations. Symbols and abbreviations for amino acids and peptides are in accord with the recommendations of the IUPAC-IUB Commission on Nomenclature (*J. Biol. Chem.* 1972, *247*, 977). Other abbreviations used are: Boc, *tert*-butyloxycarbonyl; BOP, benzotriazole-1-yl-oxy-tris-(dimethylmino)-phosphonium hexafluorophosphate; *t*Bu, *tert*-butyl; Bzl, benzyl; DBU, 1,8-diazabicyclo[5,4,0]undecen-7-ene; DIEA, diisopropylethylamine; EDC×HCl, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride; Fgu, fulleropyrrolidino-glutamic acid; Fm, 9*H*-fluoren-9-ylmethyl; HOBt, 1-hydroxybenzotriazole; MALDI-tof, Matrix Assisted Laser Desorption Ionization time-of-flight; TFA, trifluoroacetic acid; TMSOTf, trimethylsilyl trifluoromethanesulfonate.

Boc-L-fulleropyrrolidino-glutamic acid fluorenylmethyl ester (2). Boc-L-Glu-OFm (49 mg, 0.116 mmol) was solubilised in DCM (1 ml) in the presence of HOBt (18 mg, 0.116 mmol) and EDC×HCl (24 mg, 0.128 mmol). After stirring at room temperature for 15 min, the solution was added to the fullerene derivative **1** (53 mg, 0.058 mmol) previously neutralised with 2,4,6-collidine (76 μl, 0.58 mmol) in a 1:1 mixture of DMF/DCM (8 ml). After 30 min, the initial brown suspension became a solution. The mixture was stirred at room temperature for 32 h. The solvent was evaporated and the compound was purified by silica gel chromatography (eluant: toluene/ethyl acetate 2:1), precipitated by DCM/MeOH, washed with diethyl ether and dried. $C_{88}H_{35}N_3O_5$ (MW 1214.24). Yield: 82%. FT-IR: 3314, 1716, 1654,

1513 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.78-7.76 (m, 2H), 7.60-7.53 (m, 2H), 7.45-7.29 (m, 4H), 7.02 (m, 1H), 5.42 (d, J=7.8 Hz, 1H), 4.59 (m, 1H), 4.54-4.33 (m, 6H), 4.18 (t, J=6.4 Hz, 1H), 3.86-3.66 (m, 2H), 3.32-3.13 (m, 2H), 2.34-2.16 (m, 2H), 2.13-2.02 (m, 1H), 1.95-1.83 (m, 1H), 1.41 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 172.22, 172.16, 155.77, 154.77, 147.33, 146.26, 146.08, 145.94, 145.64, 145.40, 145.30, 144.55, 143.56, 143.20, 143.12, 142.65, 142.21, 142.06, 141.89, 141.43, 141.38, 140.18, 140.12, 136.03, 128.011, 127.40, 127.31, 125.11, 124.94, 120.24, 120.22, 80.18, 70.56, 67.66, 67.02, 53.56, 52.93, 46.82, 38.15, 32.54, 29.74, 28.38. UV-Vis (DCM): λ_{max} 300, 324, 432, 700. MALDI-tof: *m/z* 1159.81 [M-*t*Bu^+], 1215.01 [M+H^+], 1237.09 [M+Na^+], 1253.29 [M+K^+].

Boc-L-fulleropyrrolidino-glutamic acid (3). Compound **2** (44 mg, 3.62 μmol) was treated with a solution of 2% DBU (16 equiv) in DCM (4.4 ml) for 30 sec under sonication and immediately precipitated with diethyl ether. The solid was washed several times with diethyl ether and dried. Compound **3** was recovered as a salt of DBU. $C_{74}H_{25}N_3O_5$ (MW 1036.01). Yield: 99%. FT-IR: 3250, 1697, 1645, 1588 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.45 (m, 1H), 5.97 (d, J=5.6 Hz, 1H), 4.51 (s, 4H), 3.59-3.14 (m, 11H), 2.67-2.64 (m, 2H), 2.18-2.13 (m, 2H), 1.95-1.83 (m, 4H), 1.67-1.56 (m, 6H), 1.36 (s, 9H). ^{1}H-NMR (300 MHz, pyridine-d_5): δ 9.31 (m, 1H), 7.10 (d, J=6.3 Hz, 1H), 4.88 (m, 1H), 4.47 (s, 4H), 4.14-4.07 (m, 2H), 3.43-3.38 (m, 4H), 3.23-3.14 (m, 4H), 3.08-3.00 (m, 2H), 2.94-2.76 (m, 4H), 1.69-1.31 (m, 8H), 1.52 (s, 9H). ^{13}C-NMR (75 MHz, pyridine-d_5): δ 176.50, 174.30, 166.30, 156.71, 156.09, 147.72, 146.94, 146.70, 146.48, 146.37, 145.84, 145.72, 145.08, 143.53, 143.06, 142.89, 142.59, 142.34, 140.55, 136.97, 78.48, 71.54, 68.30, 56.38, 54.95, 54.00, 48.64, 39.46, 38.65, 34.55, 32.22, 32.04, 29.39, 29.03, 27.19, 24.62, 20.06. UV-Vis (DCM): λ_{max} 327, 324, 432, 700. MALDI-tof: *m/z* 982.53 [M-*t*Bu^+], 1037.28 [M+H^+].

Synthesis of peptide 4 and 5. Fullero-peptides **4** and **5** were prepared on 8 μmol of *t*-Boc-Ala-OCH_2-PAM (0.77 mmol/g) and *t*-Boc-Phe-OCH_2-PAM (0.72 mmol/g), respectively. Five-fold excess of each Boc-protected amino acid in DMF, activated with BOP/HOBt/DIEA at a 1:1:1:3 ratio was added. The coupling was repeated two times for 10 min. Boc deprotection was performed twice using pure TFA for 1 and 3 min, followed by extensive washings with DCM, *i*PrOH, DMF. Fullero-amino acid Fgu **3** was coupled using a three-fold excess in a 3 ml mixture of DCM/DMF/NMP (1:1:1), activated by addition of BOP/HOBt/DIEA in 1:1:1:3 ratio. The reaction was carried out for 6 h under gentle stirring. Completeness of coupling was confirmed by negative Kaiser test. After this step, an analytical amount of peptide **4** was cleaved from the resin and controlled by HPLC and mass

spectrometry (see below for the cleavage conditions). The introduction of the final N-terminal amino acids was performed in the same conditions described above. Cleavage of the final peptide from the resin was done using 3 ml of TFA:TMSOTf:*p*-cresol (325 μl/87 μl/42.5 mg) at room temperature for 15 h.[3] Fullero-peptides **4** and **5** were precipitated with 10 ml of cold diethyl ether, washed and lyophilised from 3 ml of H_2O/AcOH 9:1. RP-HPLC characterisation was carried out on a C_4 column using a linear gradient of A: 0.1% TFA in water and B: 0.08% TFA in acetonitrile, 0-100% B in 20 min at 1.2 mL/min flow rate. The elution times were 15.23 and 14.83 min for peptide **4** and **5**, respectively. Recovered: 17 and 18 mg of crude **4** and **5**, respectively. RP-HPLC purification afforded 1.1 mg (6.5%) of **4** and 2.2 (12%) of **5**. MALDI-tof (**4**) (MW 2649.91): *m/z* 2650.44 [$M+H^+$], 2672.28 [$M+Na^+$], 2689.38 [$M+K^+$]. MALDI-tof of (**5**) (MW 2630.87): *m/z* 2631.65 [$M+H^+$], 2653.90 [$M+Na^+$], 2670.47 [$M+K^+$].

References

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