A Novel [60]Fullerene Amino Acid for Use in Solid Phase Peptide Synthesis

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SUPPORTING INFORMATION

Synthesis of 4. To a toluene solution of C_{60} (500.0 mg, 0.69 mmol in 300 mL), 104.0 mg (3.47 mmol) of *para*formaldehyde and 150.0 mg (0.49 mmol) of **2** were added and the mixture was heated to reflux for 2 h. The product **3** was purified by chromatography (eluant toluene/ethyl acetate 99/1) and precipitated form dichloromethane/methanol (yield 42%). To a dichloromethane solution of **3** (83.3 mg, 0.10 mmol in 3 mL) 3 mL of trifluoroacetic acid was added and the mixture was stirred at rt for 3h. Then the solvent and the acid were removed in vacuo and the solid product **4** was washed with toluene. $C_{66}F_3H_{11}N_2O_2$ (MW 920.83), yield: 99% from **3** (86.4 mg, 0.10 mmol). DRIFT (KBr): cm^{-1} 2926, 1678, 1427, 1200, 836, 798, 725, 556. UV-Vis (THF): λ_{max} 255, 325, 431, 704. MS-ES (THF/MeOH 1/1): m/z 807 (MH^+). El. An. Calcd. for $C_{66}F_3H_{11}N_2O_2$: C, 78.92; H, 1.17; N, 2.71. Found: C, 76.10; H, 1.17; N, 2.54.

Synthesis of 1. A solution of N-Fmoc-L-glutamic acid α-*tert*-butil ester (92.8 mg, 0.22 mmol), HOBT (24.2 mg, 0.22 mmol) and EDC·HCl (41.7 mg, 0.22 mg) in 2.5 mL of dichloromethane was stirred for 15 min and then was added to a solution of **4** (100.0 mg, 0.11 mmol) and TEA (47 μL, 0.35 mmol). The mixture was stirred at rt for 12 h and then product **1** was purified by silica gel chromatography (eluant toluene/ethyl acetate 3/2) and precipitated by dichloromethane/ethyl

ether. $C_{88}H_{35}N_3O_5$ (MW 1214.28), yield: 73% (85.0 mg, 0.07 mmol). DRIFT (KBr): cm^{-1} 3306, 3063, 2974, 1723, 1655, 1155, 740, 527. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.77-7.69 (m, 2H), 7.61-7.49 (m, 2H), 7.42-7.25 (m, 4H), 7.18-7.06 (m, 1H), 5.65 (d, J=8.0 Hz, 1H), 4.54-4.32 (AB system, 4H) 4.35-4.01 (m, 4H), 3.96-3.76 (m, 1H), 3.74-3.35 (m, 1H), 3.44-3.25 (m, 1H), 3.20-3.08 (m, 1H), 2.46-2.15 (m, 2H), 2.10-1.96 (m, 2H), 1.42 (s, 9H). ^{13}C-NMR ($CDCl_3$): δ 172.1, 156.3, 154.4, 147.0, 145.9, 145.7, 145.6, 145.3, 145.0, 144.9, 144.2, 143.5, 143.2, 142.7, 142.3, 141.8, 141.7, 141.5, 140.9, 139.8, 136.7, 127.5, 128.8, 124.8, 119.8, 82.4, 70.3, 67.4, 66.9, 53.5, 46.8, 37.8, 32.6, 29.8, 29.5, 27.8. ES-MS (THF/MeOH 1/1): m/z 1215 (MH^+). UV-Vis (DMSO): λ_{max} 301, 326, 433, 706. El. An. Calcd. for $C_{88}H_{35}N_3O_5$: C, 87.05; H, 2.91; N, 3.46. Found: C, 83.40; H, 2.83; N, 3.38.

Synthesis of 5. Trifluoroacetic acid (5 mL) was added to a solution of **1** (30.0 mg, 0.02 mmol) in dichloromethane (5 mL). The solution was stirred for 2 h, then the solvent and the acid were removed in vacuo and the solid product **5** was washed with toluene. $C_{84}H_{27}N_3O_5$ (MW 1158.17), yield: 99% (28.1 mg, 0.02 mmol). DRIFT (KBr): cm^{-1} 3328, 3059, 2944, 1713, 1530, 736, 527. UV-Vis (THF) λ_{max} 326, 430, 704. ES-MS (THF/methanol 1/1): m/z 1159 (MH^+). MALDI-TOF: m/z 1159 (MH^+). El. An. Calcd. for $C_{84}H_{27}N_3O_5$: C, 81.11; H, 2.20; N, 3.30. Found: C, 82.20; H, 2.19; N, 3.39.

Synthesis of 6. The peptide synthesis was performed utilizing PS-PEG-PAL resin. The coupling reagents (TBTU and amino acid) were used in excess (6 x) with respect to resin loading, the coupling was performed twice for 30 min at rt in presence of DIPEA (10 x). Fmoc deprotection was performed using piperidine 20% in DMF 2 times for 10 min each. Yields for each coupling were calculated on the basis of UV-Vis analysis of liberated dibenzofulvene (FMOC deprotection) and were always higher than 90%. Peptide **6** was purified by HPLC-RP [semipreparative column Waters Symmetry C18 7 μm, 100 Å, 7.8 x 150 mm, CH_3CN-TFA (0.05%)/H_2O-TFA (0.05%) 30/70, 1 mL/min], with t_r 11 min. The fractions containing peptide **7** were collected and lyophilized. The compound was analyzed by electrospray mass spectrometry (see main text).

Synthesis of 7. The coupling between **5** and H_2N-(Gly-Orn)$_6$Gly-NH-PAL-PEG-PS (fully protected **6** bound to resin) was performed utilizing PyBOP as activating agent in presence of **5**

(3 x) solubilized in Magic Mix (DMF/DCM/NMP 1:1:1). The coupling was performed for 18 h at 45°C. The cleavage from the resin was performed utilizing TFA/TIPS/H_2O 95/2.5/2.5 for 2 h. UV-Vis analysis of the FMOC cleavage steps before and after fullerene coupling indicated an almost quantitative (>80% yield) for this reaction. The resulting mixture was purified by HPLC-RP [CH_3CN-TFA (0.05%)/H_2O-TFA (0.05%) 45/55, 1 mL/min], with t_r 16.9 min. The fractions containing peptide **7** were collected and lyophilized. The compound was analyzed by electrospray mass spectrometry (see main text).