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Supporting Information

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Supporting Information

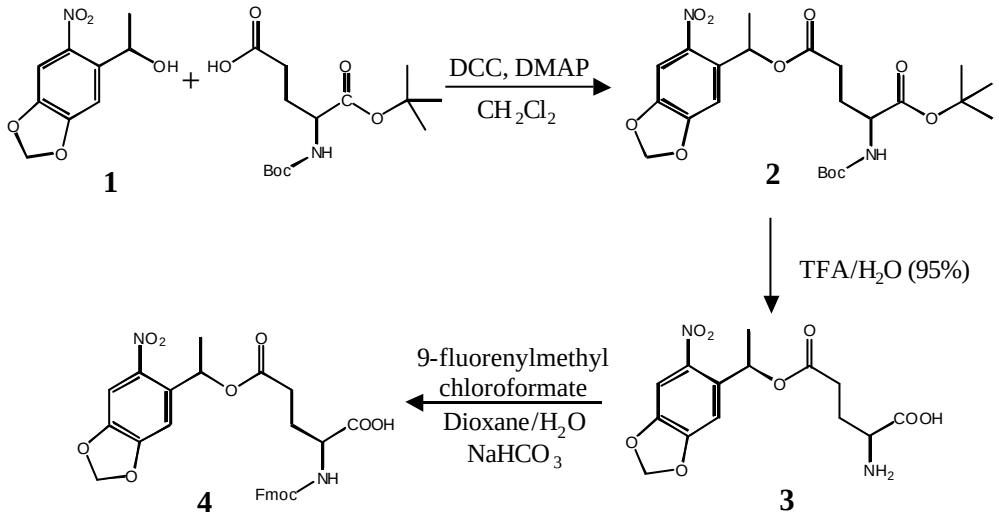
for

Manipulating Cell Migration and Proliferation with Light-Activated Polypeptide

Danielle S. Miller, Sara Chirayil, Haydn L. Ball, and Kevin J. Luebke*

Synthesis of Caged Glutamate for Solid-Phase Peptide Synthesis

N-(9-fFluorenylmethoxycarbonyl)-L-glutamic acid-γ-(R,S)-1-(3,4-(methylenedioxy)-6-nitrophenyl)ethyl ester (Compound **4**) was synthesized as shown in Scheme S1. (R,S)-1-(3,4-(methylenedioxy)-6-nitrophenyl)ethanol (**1**) was synthesized as described by McGall et al. [1]



Scheme S1

N-(tert-Butoxycarbonyl)-L-glutamic acid-α-tert-butyl-γ-(R,S)-1-(3,4-(methylenedioxy)-6-nitrophenyl)ethyl ester (**2**). A mixture of (R,S)-1-(3,4-(methylenedioxy)-6-nitrophenyl)ethanol (**1**) (2.11 g, 10 mmol), N-Boc-glutamate-α-t-butyl ester (3.03 g, 10 mmol), dicyclohexylcarbodiimide (2.06 g, 10 mmol), and dimethylaminopyridine (1.22 g, 10 mmol) was stirred in dichloromethane (100 mL) at room temperature under N_2 for 24 hours. The mixture was then

filtered, and the solvent was removed under reduced pressure. The residue was dissolved in a mini-mum quantity of dichloromethane, and purified by silica gel chromatography, eluting with hexane/ethyl acetate (2:1). Evaporation of solvent yielded the product as a pale brown vis-cous oil (4.6 g, 93%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41 (d, *J*=1.8 Hz, 1H), 6.97 (d, *J*=3.5 Hz, 1H), 6.31 (q, *J*=6.41 Hz, 1H), 6.07 (t, 2 Hz), 5.06 (d, *J*=7.0 Hz, 1H), 4.14 (t, *J*=4.4 Hz, 1H), 2.37 (m, 2H), 2.09 (m, 1H), 1.84 (m, 1H), 1.55 (dd, *J*=3.7, 2.8 Hz, 3H), 1.41 (s, 9H), 1.39 (s, 9H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 171.65, 171.58, 171.25, 155.42, 152.42, 147.15, 141.48, 135.31, 105.79, 105.06, 103.14, 82.08, 79.64, 68.49, 53.27, 30.33, 28.23, 27.88, 21.92. ESI MS (*m/z* $[M+H]^+$, found: 497.21, calculated mass: 496.21).

L-glutamic acid-g-(R,S)-1-(3,4-(methylenedioxy)-6-nitrophenyl)ethyl ester (**3**). Compound **2** (4.6 g, 9.27 mmol) was dissolved in 10 mL trifluoroacetic acid/water (95% *v/v*) and stirred at room temperature for six hours. The reaction mixture was diluted with 100 mL ice water and neutralized with saturated sodium carbonate solution. The resulting precipitate was washed with water and dried to yield the product as a white powder (2.4 g, 77%). ^{1}H NMR ([D_6]DMSO, 400 MHz) δ 7.58 (s, 1H), 7.18 (d, *J*=4.0 Hz, 1H), 6.23 (s, 2H), 6.9 (q, *J*=6.4 Hz, 1H), 3.18 (dd, *J*=6.59, 7.35 Hz, 1H), 2.49 (m, 2H), 1.85 (m, 2H), 1.54 (d, *J*=6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 172.43, 152.62, 147.79, 147.03, 141.20, 140.74, 106.66, 104.96, 103.83, 61.69, 55.62, 29.79, 25.36, 21.92. ESI MS (*m/z* $[M+H]^+$, found: 341.09, calculated mass: 340.09).

N-*(9-fFluorenylmethoxycarbonyl)-L-glutamic acid-g-(R,S)-1-(3,4-(methylenedioxy)-6-nitrophenyl)ethyl ester* (**4**). To a stirred suspension of **3** (2.4 g, 1.1 mmol) in 160 mL dioxane: water (70% *v/v*) and 20 mL aqueous sodium bicarbonate solution (10% w/v) at 0 °C was added dropwise 9-fluorenylmethyl chloroformate (1.84 g, 7.1 mmol) in 20 mL dioxane:water (70% *v/v*) over 15 min. The reaction mixture was stirred at room temperature for 2 h, then neutralized with 1 N HCl. The precipitated oil was extracted with dichloromethane (2 x 100 mL), washed with water (2 x 100 mL), and dried over anhydrous sodium sulfate. The solvent was evaporated and the residue was purified by silica gel chromatography, eluting with dichloromethane:methanol (50:1) to yield the product as a pale yellow crystalline solid (3.4 g, 88%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.89 (d, *J*=7.5 Hz, 2H), 7.71 (d, *J*=7.0 Hz, 2H), 7.57 (s, 1H), 7.41 (t, *J*=7.3,7.5 Hz, 2H), 7.33 (t, *J*=7.3, 7.5 Hz, 2H), 7.17 (d, *J*=6.2 Hz, 1H), 6.21 (t, 5.5, 7.9 Hz, 2H), 6.11 (dd, *J*=6.0, 6.4 Hz, 1H), 4.29 (d, *J*=5.7 Hz, 2H), 4.24 (t, *J*=3.3, 6.8 Hz, 1H), 3.93 (m, 1H), 2.4 (m, 2H), 1.98 (m, 1H), 1.80 (m, 1H), 1.53 (d, *J*=6.4 Hz, 3H), 1.24 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 172.08, 156.55, 152.52, 147.28, 143.94, 143.76, 141.60, 135.28, 127.89, 127.24, 125.23, 120.12, 105.96, 105.28, 103.21, 68.96, 67.36, 53.63, 47.19, 31.76, 27.31, 22.09. ESI MS (*m/z* $[M+H]^+$, found: 563.32, calculated mass: 562.53).

Synthesis of caged polypeptide

Synthesis of caged EGF was carried out in a single linear synthesis on a 50 µmol scale starting with Fmoc arg(Pmc) Wang resin (0.5 mmol/g, 100-200 mesh). Automated coupling cycles were carried out on a Rainin Symphony peptide synthesizer. Activation of Fmoc amino acids was with HBTU/NMM, and two couplings per cycle were used throughout with 10-fold excess of activated amino acid. Each coupling step was allowed to proceed for 1 h. The Fmoc-protected photoactivatable glutamate was coupled manually using a 3-fold excess of activated amino acid. Fmoc removal was with 20% piperidine in DMF, 2 x 20 min, with the exception of the step following the photoactivatable glutamate, in which deprotection was limited to 1 min. The complete peptide was deprotected and cleaved from the resin with 2.5% ethanedithiol, 2.5 % thioanisole, 2.5 % phenol, and 5 % water in TFA. The crude peptide was precipitated from cold diethyl ether, dried, and purified by reversed-phase HPLC (C4 column). The final purified product was characterized by MALDI-MS. A typical yield of purified material was 5-10 mg.

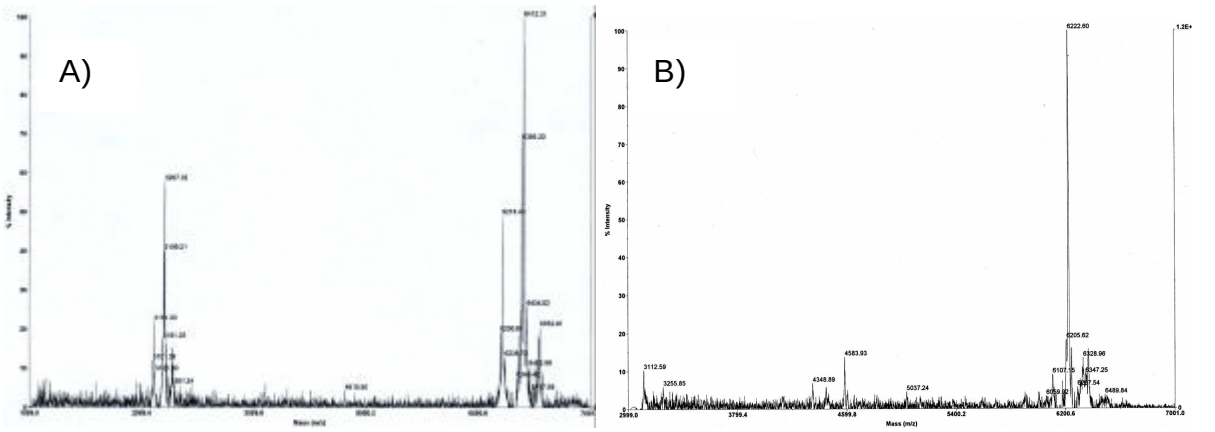


Figure S1. MALDI-MS characterization of caged polypeptide and photolysis product. A) MALDI-MS of caged polypeptide. Peaks at *m/z* 6412 and 3207 are $[M+H]^{+}$ and $[M+2H]^{+2}$, respectively for the caged material. Peaks at *m/z* 6218 and 3111 are due to photolysis of the caging group by the laser during MALDI. B) MALDI-MS of caged material after complete photolysis (10 half-lives) as described in the Experimental Section of the main text.

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[1] G. H. McGall, A. D. Barone, M. Diggelmann, S. P. A. Fodor, *J. Am Chem. Soc.* **1997**, *119*, 5081-5090.