Light-Directed Maskless Synthesis of Peptide Arrays using Photolabile Amino acid Monomers

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Supporting Information

3,4-(Methylenedioxy)-ethylbenzene 2: 3,4-(Methylenedioxy)-acetophenone (1 g, 6.09 mmol) and hydrazine hydrate 55% (0.67 g, 21.51 mmol) was added to a solution of KOH (1.02 g, 18.39 mmol) in ethylene glycol (5 mL) at 60 ^{0}C, and heated for 2 h at 160-180 ^{0}C, and distilled at the same temperature. The distillate was decanted, the organic layer separated, and the aqueous layer extracted with ether (3 x 10 mL). The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated under vacuum to yield 92% of 2 (colorless oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ /ppm 6.63- 6.73 (m, 3H, Ar-H), 5.90 (s, 2H, OCH_2O), 2.56 (q, J=7.6 Hz, 2H, CH_2), 1.19 (t, J=7.4 Hz, 3H, CH_3); MS (EI^+) m/z: 150.1 (M^+)

3,4-(methylenedioxy)-6-nitrophenylethane 3: To a solution of **2** (0.75 g, 5 mmol) in CH_2Cl_2 (10 mL) under stirring at 0 ^{0}C was added a 40% aq. HNO_3 (5 mL) over a period of 15 min. The stirring was continued for ~1 h at room temperature. After disappearance of starting material (TLC), solution was poured over ice cold water (15 mL). The mixture was extracted with CH_2Cl_2 (3 x 25 mL), the organic layer was washed with 5% $NaHCO_3$ and water until pH 5.0, dried over Na_2SO_4 and concentrated at reduced pressure to yield 89% of **3** (light red oil), with no further purification required. ^{1}H NMR ($CDCl_3$ + CD_3COCD_3, 400 MHz): δ /ppm 7.36 (s, 1H, Ar-H), 6.65(s, 1H, Ar-H), 5.99 (s, 2H, OCH_2O), 2.79 (q, J=7.5 Hz, 2H, CH_2), 1.16 (t, J=7.6 Hz, 3H, CH_3); MS (CI^+) m/z: 195.1 (M^+), 196.1 ($M+H^+$), 213.2 ($M+NH_4^+$).

2-(3, 4-methylenedioxy-6-nitrophenyl)-propanol 4: Triton B (40% in MeOH, 3.65 mL, 8.03 mmol) was added to compound **3** (1.56 g, 8 mmol) and paraformaldehyde (2.43 g, 8.1 mmol), and the mixture was heated at reflux for 6 h. After concentration under vacuum, the reaction mixture was neutralized using 5% aqueous HCl. The mixture was extracted with ethyl acetate (3 × 10 mL), dried over Na_2SO_4 and concentrated at reduced pressure. The residue was purified by flash chromatography using hexane–ethyl acetate (4:1) to give compound **4** (96%, red oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ /ppm 7.28 (s, 1H, Ar-H), 6.86 (s, 1H, Ar-H), 6.06 (s, 2H, OCH_2O), 3.72 (m, 2H, CH_2), 3.59 (m, 1H, CH), 2.04 (br s, 1H, OH), 1.24 (d, *J*=6.8 Hz, 3H, CH_3); MS (CI^+) m/z: 226.1 ($M+H^+$), 243.1 ($M+NH_4^+$).

2-(3, 4-methylenedioxy-6-nitrophenyl)-propyloxycarbonyl Chloride 5: To a solution of **4** (1.35 g, 6 mmol) in anhydrous THF (5 mL) at 0°C, was added a solution of phosgene (20% in toluene, 4.76 mL, 9 mmol) over a period of 15 min with stirring under a nitrogen atmosphere. After 45 min, the ice bath was removed and stirring was continued at room temperature for 2 h. A stream of N_2 was then bubbled through the solution for 1 h to remove the excess phosgene, after which the mixture was evaporated to dryness under vacuum to give compound **5** (99%, brown oil). ^{1}H NMR ($CDCl_3$, 400 MHz): δ /ppm 7.39 (s, 1H, Ar-H), 6.83 (s, 1H, Ar-H), 6.10 (s, 2H, OCH_2O), 4.43 (d, J=6.0 Hz, 2H, CH_2), 1.34 (t, J=7.2 Hz, 3H, CH_3); MS (EI^+) m/z: 287.2 (M^+).

Typical procedure for the preparation of MNPPOC-protected amino acids 7: Na_2CO_3 (0.15g, 1.2 mmol) was added to the solution of L-amino acid (1 mmol) in 10 mL water/1,4-dioxane (1:1) at 0°C, followed by the dropwise addition of **5** (0.28 g, 0.97 mmol, in 1 mL THF) for 5 min. After 20 min the ice bath was removed and stirring was continued for 15–18 h. The reaction mixture was evaporated to dryness, 3 mL of water was added and the mixture was extracted with ethyl acetate (2 × 5 mL) to remove **5** or its hydrolysis product. The aqueous layer was acidified by addition of 5% HCl at 0°C and extracted with ethyl acetate (3 × 10 mL); the extracts were dried over Na_2SO_4 and concentrated at reduced pressure to give a glassy substance (Table 1) that, in most cases was essentially pure (free of by-products), based on spectroscopic measurements. Spectroscopic data for selected products:

7(b): ^{1}H NMR ($CDCl_3$, 400 MHz): δ /ppm 7.26 (s, 1H, Ar-H), 6.82 (s, 1H, Ar-H), 6.04 (s, 2H, OCH_2O), 5.37 (br d, 1H, NH), 4.13- 4.30 (m, 2H, CH_2), 4.09 (m, 1H, CH), 3.75 (m, 1H, CH), 1.38 (d, *J*=6.4 Hz, 3H, CH_3), 1.25 (d, *J*=6.8 Hz, 3H, CH_3); MS (EI^+) m/z: 340.1 (M^+).
7(c): ^{1}H NMR ($CDCl_3$, 400 MHz): δ /ppm 7.27 (s, 1H, Ar-H), 6.81 (s, 1H, Ar-H), 6.05 (s, 2H, OCH_2O), 5.12 (br d, 1H, NH), 4.12- 4.24 (m, 2H, CH_2), 4.08 (m, 1H, CH), 3.76 (m, 1H, CH), 1.63 (m, 2H, CH_2), 1.50 (m, 1H, CH), 1.25 (d, *J*=6.8 Hz, 3H, CH_3), 0.89 (d, *J*=6.8 Hz, 3H, CH_3); MS (CI^+) m/z: 383.1 ($M+H^+$), 400.3 ($M+NH_4^+$).
7(e): ^{1}H NMR ($CDCl_3$, 400 MHz): δ /ppm 7.29 (s, 1H, Ar-H), 6.83 (s, 1H, Ar-H), 6.06 (s, 2H, OCH_2O), 5.49 (br d, 1H, NH), 4.38 (m, 1H, CH), 4.12- 4.21 (m, 2H, CH_2), 3.74- 3.83 (m, 2H, CH_2), 3.53 (m, 1H, CH), 1.27 (d, *J*=6.8 Hz, 3H, CH_3), 1.13 (s, 9H, 3 x CH_3); MS (EI^+) m/z: 412.2 (M^+).

Array synthesis on glass slide: In a typical procedure to attach photolabile amino acids to amino-glass slide, MNPPOC-Ala was attached to the amino-glass slide mounted in a flow cell[1] by activation of the carboxyl groups of MNPPOC-Ala (8.8 mg, 0.03 mmol) in DMF (0.25 mL). After 20 minute, excess MNPPOC-Ala was flushed out with acetonitrile and specific areas (pixels) were irradiated at 365 nm in acetonitrile using the digital mirror device (DMD) for varying periods of time to remove the photolabile protecting group at specific locations. The amino groups exposed by this treatment were visualized by being covalently coupled to a BODIPY dye (100 μg) in DMF (0.25 mL) for 10 minute followed by scanning on a GenPix scanner (Figure 1). The similar experiment was repeated with NPPOC-Ala (Figure 2).

Synthesis of one to three layers of Ala was done to determine the per-step coupling efficiency. After first layer of Ala was coupled to the amino glass slide, the pixels corresponding to the second and third layers were irradiated, after which the second layer was coupled to the first. Similarly, a third layer of MNPPOC-Ala was added. Finally all of the Ala, Ala-Ala and Ala-Ala-Ala pixels were irradiated to remove MNPPOC groups, and the exposed amino groups were coupled to the fluorescent BODIPY dye (Figure 3). Similarly peptide YGGFL and PGGFL were synthesized. In all reactions coupling, capping and side-chain deprotection reactions were carried out according to standard peptide- synthesis procedures[2].

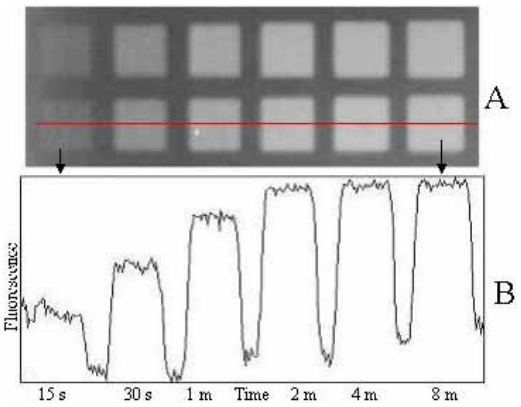

Figure 1. One layer of amino acid coupling to amino coated glass slide using MNPPOC-Ala. A is the scanned slide at 532 nm excitation after fluorescent dye treatment; the brightest group of pixels (~100 μm across) at the top are due to UV exposure at 8 minute, and the dimmest group at the bottom is due to 15 second UV exposure. B depicts the intensity of fluorescence at each time interval, indicating an optimum time (i.e., shortest effective time) of ~ 2 minute.

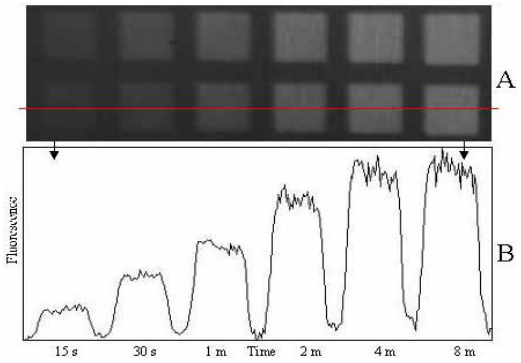

Figure 2. One layer of amino acid coupling to amino coated glass slide using NPPOC-Ala. A is the scanned slide at 532 nm excitation after fluorescent dye treatment; the brightest group of pixels (~100 μm across) at the top are due to UV exposure at 8 minute, and the dimmest group at the bottom is due to 15 second UV exposure. B depicts the intensity of fluorescence at each time interval, indicating an optimum time (i.e., shortest effective time) of ~ 4 minute.

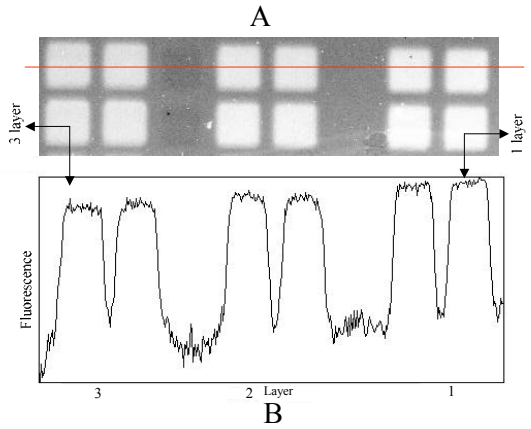

Figure 3. Synthesis (right to left) of mono- di- and tri-Ala using MNPPOC-Ala. A is the scanned slide at 532 nm excitation after the fluorescent dye treatment; B depicts the intensity of fluorescence at each layer; the intensity of the tri-Ala pixels is about 95% that of the mono-Ala pixels, indicating a stepwise yield of 98%.

Antibody binding assay: The glass slide bearing the peptides was washed with Tris/ NaCl (0.02 M Tris- HCl, 0.5 M NaCl, pH 7.5) and was then incubated for 1 h at room temperature in Tris/ NaCl containing 10% crude ovalbumin and 1% Tween-20 to block non-specific protein binding sites on the glass slide. The slide was washed twice with 0.05% Tween 20/ Tris/ NaCl, and was incubated for 2 h at room temperature with anti-endorphin antibody (1 μL) diluted in 250 μL Tween 20/ Tris/ NaCl. The slide was again washed twice with Tween 20/ Tris/ NaCl, after which it was treated with protein-A labeled with fluorescent BODIPY dye. The slide was again washed as before with Tween 20/ Tris/ NaCl and then with Tris/ NaCl.

In order to verify the presence of actual peptide on glass surface, Tyr-Gly-Gly-Phe-Leu-Aca was synthesized by derivetizing entire surface with Fmoc-Rink Linker followed by the above procedure of maskless photolithography on area 1 cm x 1 cm. The peptide was cleaved off at Rink Linker in acid from the glass slide and was finally analyzed by API-ES Mass spectrometry (Figure 4).

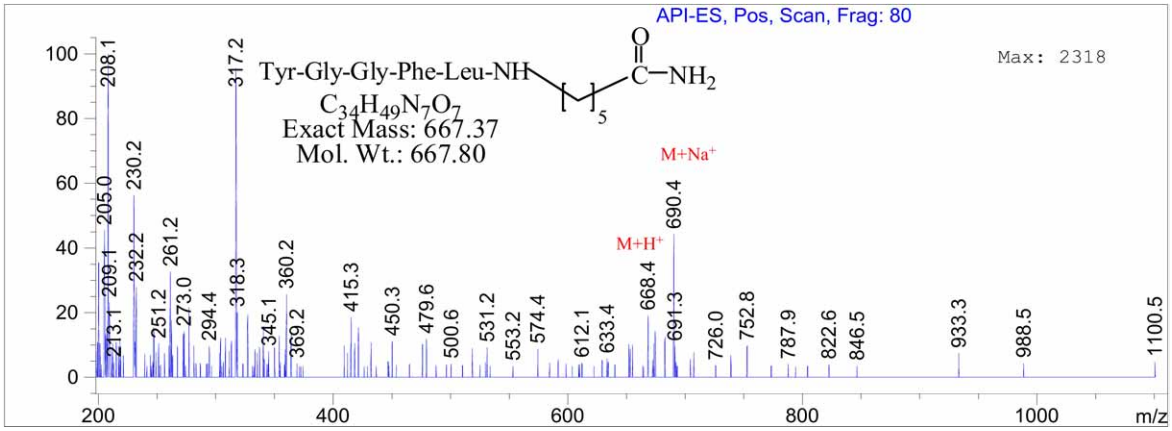

Figure 4. Positive mode API-ES mass spectra of cleaved off peptide.

References

[1] S. Singh-Gasson, R. D. Green, Y. Yue, C. Nelson, F. Blattner, M. R. Sussman and F. Cerrina, *Nat. Biotechnol*., 1999, **17**, 974-978.

[2] J. M. stewart, and J. D. Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Co., Rockford, IL, **1984**.