Supplementary Material (ESI) for Chemical Communications
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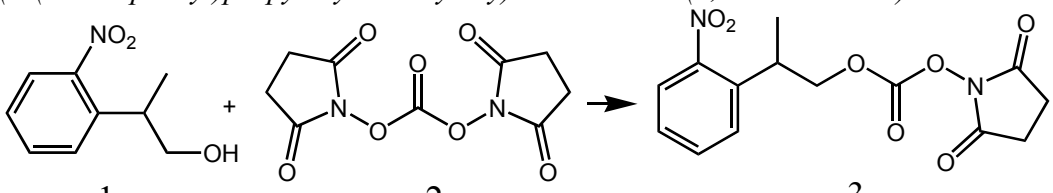
Supporting Material

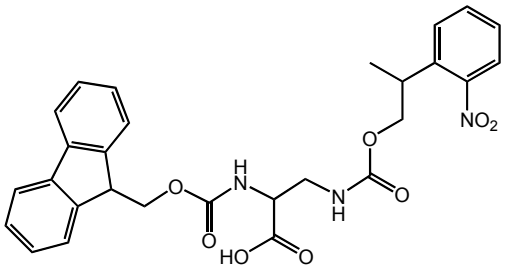
*N-(2-(2-nitrophenyl)propyloxycarbonyloxy) succinimide (**3**, NPPOC-NHS)*



1 2 3

2-(2-nitrophenyl)propanol (**1**, 1.81 g, 10 mmol)[1] and Di-(N-succinimidyl) carbonate (**2**, 2.82 g, 11 mmol) were dissolved in 50 ml anhydrous acetonitrile. Triethylamine (2 ml) was added and the mixture was stirred in the dark for 3 hours until the reaction was completed. After most of the solvent was removed under vacuum, ethyl acetate (50 ml) was added. The organic layer was washed with 10% citric acid solution (2 × 100 ml), water (2 × 100 ml), brine solution (100 ml) and dried over anhydrous sodium sulfate. Organic solvent was removed under vacuum and the residue was purified on silica with dichloromethane to give dark brown oily product **3**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20-7.90 (m, 4H), 4.52 (m, 2H), 3.80 (m, 1H), 2.81 (s, 4H), 1.43 (d, 3H, J=7.2 Hz). ESI-MS: $[M+H]^+$=323.15.

Fmoc-Dpr(NPPOC)-OH



Fmoc-Dpr-OH (1g, 3 mmol)[2] and sodium bicarbonate (0.5g) were dissolved in a mixture of water (30 ml) and DMF (50 ml). NPPOC-NHS (1g, 3 mmol) was added and the solution was stirred in the dark for 2 hours until the reaction was completed. The solvent was removed under vacuum and the residue was redissolved in 5% sodium bicarbonate solution. Covered by ethyl acetate, this solution was acidified to pH=2 by 40% phosphoric acid. The organic layer was then washed by water, brine solution, and dried over anhydrous sodium sulfate. The residue was purified on silica with methanol:DCM:TFA (5:95:0.1) to produce slight purple solid. MALDI-MS: $[M+Na]^+$=556.1.

Synthesis of Fmoc-Glu(O-2-PhiPr)-Dpr(ivDde)-Dpr(NPPOC)-Dpr(Mtt)-Gly-OH

The peptide was synthesized manually using a stepwise solid-phase procedure. In each coupling step, two-fold excess of protected amino acid was activated in DMF solution containing 1.9-fold of HBTU, HOBt and 2% DIPEA, and shaken with resin for one hour. Because ivDde group can migrate to α-amine during piperidine treatment of Fmoc-Dpr(ivDde)-peptidyl resin, the last coupling step was optimized as recommended

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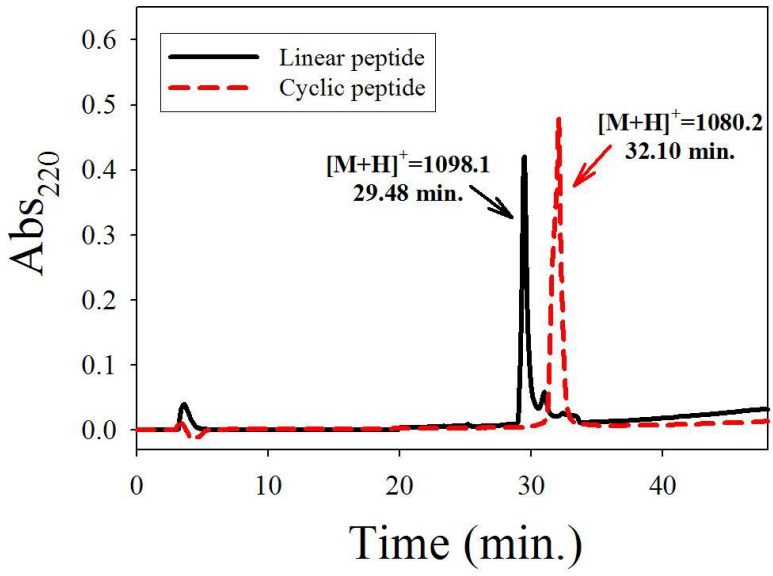
by the manufacturer, in which the Fmoc group was removed by morpholine:DMF (50:50) and coupling was achieved by PyBOP/DIPEA at -40 °C. A small amount of peptide was cleaved from the resin with TFA:water:TIS (95:2.5:2.5) and checked by analytic HPLC to assess the quality of synthesis. MALDI-MS: $[M+H]^+$=1098.1

Cyclization

Side-chain protection groups on Dpr(Mtt) and Glu(O-2-PhiPr) were removed by treating the resin with 1% TFA (TFA:TIS:DCM 1:2:97) for 2×30 minutes. The resin was washed with 5% DIPEA (v/v) in DCM thoroughly, dried, then treated with 5-fold excess of DIC and HOAt in DMF (2×24 hr). The cyclic peptide was cleaved from the resin with TFA:water (95:5) for two hours and purified by HPLC (See figure below as the overlapping HPLC chromatography of linear and cyclic peptide). MALDI-MS: $[M+H]^+$=1080.2



Exchange of Fmoc to NPPOC group and time-dependent deprotection of NPPOC on glass slides (UV at 20 mW/cm^2)

An amine-functionalized glass slide was assembled in a large plastic reaction chamber with a transparent window. Fmoc-glycine-OH (9 mg, 0.03 mmol) was dissolved in 0.5 ml DMF. HBTU (9.5 mg, 0.025 mmol), HOBt (6.8 mg, 0.05 mmol) and DIPEA (9 µl, 0.05 mmol) were dissolved in 0.5 ml DMF. The two solutions were mixed and incubated for 2 minutes before adding onto glass slide. The reaction was carried out at room temperature for 15 minutes and the glass slide was washed extensively with DMF and acetonitrile. The remaining amine groups on the glass slide was then capped by capping solution (acetic anhydride-lutidine-DMF 5:6:89) for 15 minutes. Piperidine (10% in DMF) was added for five minutes twice to remove the Fmoc group. The slide was then assembled in a small plastic reaction chamber, treated with mixture of NPPOC-NHS and DIPEA solution (40 mM NPPOC-NHS, 40 mM DIPEA in acetonitrile) for 15 minutes twice, washed and dried, then subjected to UV deprotection.

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After deprotecting different regions of the slide for various times with 365 nm UV irradiation, the glass slide was moved back to the original large plastic chamber. Activated 5(6)-carboxyltetramethylrhodamine (12 mM in DMF containing 10 mM HBTU, 20 mM HOBt, and 20 mM DIPEA) was added to label free amine groups on the surface of the glass slide.

Construction of a model cyclic peptide microarray containing 12288 features
The flowchart to construct a model cyclic peptide microarray containing 12288 features is listed as below.

1) Coating of the glass slide:
   Cyclic peptide (Fmoc-Glu(O-2-PhiPr)-Dpr(ivDde)-Dpr(NPPOC)-Dpr(Mtt)-Gly-OH, 11 μmol, 12 mg) was dissolved in 1 ml DMF and mixed with an equal amount of activator solution A (10 mM HBTU, 20 mM DIPEA, 20 mM HOBt in DMF). The mixture was added onto an amine-functionized glass slide and allowed to react for 15 minutes. The remaining groups was capped by reacting with an equal amount of Cap A (THF/2,6-lutidine/Ac_2O [8:1:1]) and Cap B (16% 1-methyl-imidazole in THF). The coated glass slide then was assembled in the reaction chamber of the synthesizer and the following steps from 2-19 were done automatically.
2) UV deprotection of NPPOC group:
   The glass slide was deprotected with UV irradiation through a "visual" mask containing checkboard pattern of 4096 features (Mask-A) in 1,4-dioxane for 5 minutes.
3) Coupling with biotin:
   Biotin (12 mM) was mixed with an equal amount of activation solution A and reacted with the glass slide for 15 minutes.
4) UV deprotection of remaining NPPOC group:
   The glass slide was deprotected with UV irradiation through the negative image of Mask-A in 1,4-dioxane for 5 minutes.
5) Capping:
   An equal amount of Cap A (THF/2,6-lutidine/Ac_2O [8:1:1]) and Cap B (16% 1-methyl-imidazole in THF) were mixed and reacted with the slide for 5 minutes.
6) Deprotection of Fmoc-group:
   Piperidine (10% in DMF) was reacted with the slide for 5 minutes, twice.
7) NPPOC-NHS conversion:
   NPPOC-NHS solution (40 mM in acetonitrile) was mixed with an equal amount of DIPEA solution (40 mM in acetonitrile) and reacted with the slide for 15 minutes, twice.
8) Capping:
   Same as Step-5
9) UV deprotection:
   Same as Step-2, but with a different Mask-B that contains 8192 features
10) Coupling with biotin:
    Same as Step-3
11) UV deprotection:
    Same as Step-2, but with the negative image of Mask-B
12) Capping:

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Same as Step-5

13) Hydrazine deprotection:
Hydrazine solution (1% in DMF) was reacted with the slide for 5 minutes, twice

14) NPPOC-NHS conversion:
Same as Step-7

15) Capping:
Same as Step-5

16) UV deprotection:
Same as Step-2, but with a different Mask-C that contains 12288 features

17) Coupling with biotin:
Same as Step-3

18) UV deprotection:
Same as Step-9, but with negative image of Mask-C

19) Capping:
Same as Step-5

20) Fluorescent labeling:
The glass slide was labeled with streptavidin-Alexa Fluor 647 conjugate in PBS buffer solution (10 mM NaH_2PO4, 150 mM NaCl, 1 mM EDTA, pH=7.4) for 30 minutes.

[1] K. R. Bhushan, C. DeLisi, R. A. Laursen, *Tetrahedron Letters* **2003**, *44*, 8585.
[2] L. H. Zhang, G. S. Kauffman, J. A. Pesti, J. G. Yin, *Journal of Organic Chemistry* **1997**, *62*, 6918.