Supplementary information

2-(2-Nitrophenyl) propyl: A rapidly released photolabile COOH-protecting group for solid-phase peptide synthesis

Gang Wang,¹,² Tao Peng,³ Shouguo Zhang,² Junyi Wang,³ Xiaoxue Wen,² Haiyan Yan,² Liming Hu,²,† and Lin Wang²,³,†

¹ College of Life Science and Bio-engineering, Beijing University of Technology, Beijing, 100124, P.R. China
² Beijing institute of radiation medicine, Beijing, 100850, P.R. China
³ School of Basic Medical Sciences, Peking University, Beijing, 100191, P.R. China
† Corresponding author
‡ Co-corresponding author

1. GENERAL INFORMATION

Room temperature (r.t) refers to ambient temperature. Solid-phase synthesis was carried out manually in polypropylene syringe containing a polyethylene frit. All reagents were commercially available used without further purification. 1, 2-Dichloroethane was dried by 4Å molecular sieves. DMF and NMP were distilled from P₂O₅. Other commercial available solvents were used directly without any treatment.

Visualization of TLC was performed using UV light; Flash column chromatography was performed on silica gel (200-300 mesh). The ¹H NMR and ¹³C NMR were recorded by JEOL FT-NMR spectrometer at 400 and 150 MHz respectively.

High resolution mass spectrometry (HRMS) measurements were recorded using ESI-TOF by Agilent 1260-G6230A spectrometer.

Analytical high pressure liquid chromatography (HPLC) was carried out on an Agilent instrument (Agilent 1100), automatic injector, photodiode array detector and system controller (Empower login), performed with Venusil ASB C18 column (5 mm, 150 mm × 4.6 mm, Bonna-Agela Technologies, Tianjin, China). UV measurements were recorded at 220 nm.

Abbreviations: Boc, tert-butyloxycarbonyl; Cbz, benzzyloxycarbonyl; DCC,dicyclohexylcarbodiimide; DCM, dichloromethane; DMAP, 4-(N,N-dimethylamino) pyridine; DMF, N,N-dimethylformamide; DMSO, dimethylsulfoxide; ESI-TOF, electrospray ionization-time of flight technique; NMP, N-methyl-2-pyrrolidone; EtOAc, ethyl acetate; Et₂O, diethyl ether; Fmoc, 9-fluorenlymethoxycarbonyl; NMM, 4-methylmorpholine; Npp, 2-(2-nitrophenyl) propyl; HOBt, 1-hydroxy benzotriazole; HPLC, high-pressure liquid chromatography; NMR,
nuclear magnetic resonance; PPTS, pyridinium p-toluene sulfonate; TIS, tris-isopropylsilane; TBTU, O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate; tBu, tert-butyl; TFA, trifluoroacetic acid.

2. EXPERIMENTAL SECTION

2.1 General procedure for synthesis of Npp-OH (2)

To a solution of 1-ethyl-2-nitrobenzene (15.1 g, 100 mmol) and paraformaldehyde (3.0 g, 100 mmol) in DMSO (60 mL) was added 4 mL of Triton B (40% in MeOH). The solution turned red immediately, and the mixture was heated to 60°C until the reaction was completed. The mixture was concentrated under vacuum, then 200 mL of dilute hydrochloric acid was added, the dark-red oil was separated, and the aqueous layers were extracted with ethyl acetate (3 × 100 mL). The organic portion was washed with saturated brine (3 × 100 mL), combined with the dark-red oil, dried over MgSO₄. The solvent was removed under vacuum, and the crude product was purified by flash column chromatography (hexane: EtOAc = 3:1) to give 16.12 g (89 mmol, 89.0%) of 2- (2-nitrophenyl) propanol as a dark-red oil.

2.2 General esterification procedure

A solution of the Fmoc-L-amino acid or other carboxylic acid (10 mmol), DMAP (1 mmol), and the Npp-OH (10 mmol) in 50 mL of DCM was cooled with stirring in an ice bath. DCC (11 mmol) was added in small portions, and the reaction mixture was stirred at 0°C for 30 min and then at r.t for 2 h. The solution was filtered, the filtrate was concentrated to dryness under vacuum, and the residue was purified by flash column chromatography.

2.3 General TFA deprotection procedure

The tBu-protected Npp-ester (5 mmol) was dissolved in 80% TFA in DCM (20 mL) and stirred until the reaction was completed (30-60 min). The volatile components were removed under reduced pressure. The residue was purified by flash column chromatography to give the product as yellow oil or amorphous yellow solid.

2.4 General procedure for anchoring threonine derivatives

To a solution of Fmoc-L-Thr (OH)-COONpp (4.65 g, 9.233 mmol, 3.81 equiv.) in dry 1,2-dichloroethane (20 mL) was added 2.002g of DHP resin (1.21 mmol/g, 2.002 g, 2.42 mmol, 1 equiv.), PPTS (1.21g, 4.84 mmol, 2 equiv.) and 4Å molecular sieves. The solution was heated at 80 °C for 20 h, and then the resin was filtered. The resin was then washed with DMF (3 × 1 min), CH₂Cl₂ (3 × 1 min), DMF (3 × 1 min) and MeOH (3 × 1 min). The mass of the resulting dry resin (4) was 2.760 g (62% loading). The loading was calculated to be 1.51 mmol.

2.5 General procedures for solid-phase reactions

The peptide was synthesized by using of 1.002 g of resin (4) (0.55 mmol) as starting material as followed (a): Fmoc deprotection: 20% piperidine in DMF (10 mL) 15 min; washings: DMF (3 × 1 min), CH₂Cl₂ (3 × 1 min), and DMF (3 × 1 min); (b): Peptide coupling conditions: HOBt (3 equiv.), TBTU (3 equiv.), Fmoc-amino acid (3 equiv.) and NMM (4 equiv.) in DMF (15 mL), 2 h; washings: DMF (3 × 1 min); the coupling reaction was monitored by the Kaiser test.

2.6 Removal of the Npp group

After the last Leu was loaded and the Fmoc group was removed, the resin-bound linear peptide(5) was transferred to a quartz bottle and suspended in 50 mL of DMF-MeOH (2:1, v/v). The Argon gas was bubbled for 10 min to remove the oxygen dissolved in the solvent. The mixture was then irradiated with the high pressure Hg lamp (λmax=365 nm, 250 W, power density=2.95 W/cm², irradiation area was calculated to be 4.9 cm²) with constant Argon bubbling. A reflux condenser was necessary because of the heat effect during the UV irradiation. After 15 min, the lamp was tuned off and the dark yellow solvent was replaced by fresh solvent mixtures of DMF-MeOH (2:1, v/v, 50 mL), followed continuous irradiation until the solvent was not getting yellow any more. This procedure usually needs 30 min in total. Finally the resin (6) was collected by filtration, washed with DMF (3 × 1 min), CH₂Cl₂ (3 × 1 min) and DMF (3 × 1 min). A microcleavage procedure was performed after irradiating for 15 min and 30 min respectively as described in 2.7.

2.7 General procedure for Microcleavage

...
Dry resin (5 mg) was treated with TFA/TIS/H₂O (95:2.5:2.5) for 1 h at r.t. The cleavage mixture was filtered and evaporated under vacuum, precipitated with Et₂O, centrifuged to give the pellet, which was dissolved in eluent system for HPLC analysis at 220 nm. (Figure 1)

2.8 Preparation of phakellistatin 13

The resin-bound linear peptide (6) was transferred into a reaction vessel and suspended in 15mL NMP, then TBTU (3 equiv.), HOBt (3 equiv.) and NMM (4 equiv.) were added sequentially, the vessel was capped and shaken at r.t until the Kaiser test was negative. Then the solvent was removed by filtration, and the beads were washed sequentially by DMF (3 × 1 min) and MeOH (3 × 1 min).

A heterogeneous mixture of TFA/H₂O/TIS (95:2.5:2.5 v/v/v) (10mL) were prepared and added to the reaction vessel. The vessel was capped, agitated for 60 min, and then filtered into 100 mL recovery flask. The beads were then sequentially washed and filtered into the flask with CH₂Cl₂ (10 mL) and CH₃OH (5 mL × 2). The resulting solution was concentrated in vacuum, after the iced ether was added, a white precipitate appeared soon, and the mixture was kept at 0°C for 30min. The precipitate was collected by centrifugation to give the crude product (315 mg) as a light yellow powder. The crude product was purified by flash column chromatography (CH₂Cl₂: MeOH=9:1, Rf: 0.82) and lyophilized to give the pure cyclic peptide (7) (106 mg, 33.7%) as a white fluffy solid. The cyclic peptide (7) was analysed by HPLC at 220 nm with 70% CH₃OH-H₂O (0.1% TFA) as eluent system (Figure 2). The structure was identified by HRMS (ESI-TOF): calculated for C₄₂H₅₄N₈O₈ [M+Na]+: 821.3957, Found [M+Na]+: 821.3962.

![Figure 1](image1.jpg)

**Figure 1.** HPLC chromatograms before and after irradiation at different times with 40% CH₃CN-H₂O (0.1%TFA) as eluent system. (A): Before UV irradiation. (B): 15min after UV irradiation. (C): 30min after UV irradiation.

![Figure 2](image2.jpg)

**Figure 2.** HPLC chromatograms of phakellistatin 13 with 70% CH₃OH-H₂O (0.1%TFA) as eluent system.

3. CHARACTERIZATION DATA OF THE PRODUCTS
$^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 7.77 (dd, 1H, Ar-H), $\delta$ 7.63 (m, 2H, Ar-H), $\delta$ 7.43 (m, 1H), $\delta$ 4.77 (t, 1H, OH), $\delta$ 3.51 (m, 2H, CH$_2$), $\delta$ 3.19 (m, 1H, CH), $\delta$ 1.22 (d, 3H, CH$_3$)

$^{13}$C NMR (150 MHz, DMSO-d$_6$): $\delta$ 150.52, 138.11, 132.58, 128.48, 127.16, 123.37, 65.87, 36.22, 17.65.

HRMS (ESI-TOF) calculated for C$_9$H$_{11}$NO$_3$ [M+Na]$^+$$\sim$:204.0631, Found: [M+Na]$^+$$\sim$: 204.0627.

$^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 8.9 (d, 1H, Py-H), $\delta$ 8.80-8.79 (dd, 1H, Py-H), $\delta$ 8.18-8.15 (m, 1H, Ar-H), $\delta$ 7.85-7.80 (m, 2H, Ar-H), $\delta$ 7.71 (dd, 1H, Ar-H), $\delta$ 7.55 (m, 1H, Py-H), $\delta$ 7.49 (m, 1H, Ar-H), $\delta$ 4.56-4.45 (m, 2H, CH$_2$), $\delta$ 3.64 (m, 1H, CH), $\delta$ 1.39 (d, 3H, CH$_3$)

$^{13}$C NMR (150 MHz, DMSO-d$_6$): $\delta$ 164.36, 153.74, 150.23, 149.81, 136.70, 136.37, 132.94, 128.57, 127.94, 125.35, 123.90, 123.60, 68.85, 32.79, 17.53.

HRMS (ESI-TOF) calculated for C$_{15}$H$_{14}$N$_2$O$_4$ [M+1]$^+$$\sim$:287.1028, Found: [M+1]$^+$$\sim$:287.1026.

$^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 8.45 (dd, 1H, Ar-H), $\delta$ 8.20 (m, 1H, Ar), $\delta$ 7.85-7.65 (m, 4H, Ar), $\delta$ 7.50 (m, 1H, Ar), $\delta$ 4.52 (m, 2H, CH$_2$), $\delta$ 3.64 (m, 1H, CH), $\delta$ 1.37 (d, 3H, CH$_3$).

$^{13}$C NMR (150 MHz, DMSO-d$_6$): $\delta$ 162.97, 158.76, 156.09, 150.22, 144.30, 141.27, 136.70, 135.86, 132.96, 128.57, 128.00, 127.10, 126.44, 123.65, 119.59, 69.31, 32.81, 17.57.

HRMS (ESI-TOF) calculated for C$_{16}$H$_{13}$FN$_2$O$_6$ [M+Na]$^+$$\sim$:371.0651, Found: [M+Na]$^+$$\sim$:371.0650.

$^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 7.89 (d, 2H, Ar), $\delta$ 7.82 (d, 1H, NH), $\delta$ 7.76 (m, 1H, Ar), $\delta$ 7.66 (m, 4H, Ar), $\delta$ 7.48-7.40 (m, 3H, Ar), $\delta$ 7.33 (t, 2H, Ar), $\delta$ 4.32 (m, 1H, CH), $\delta$ 4.27 (d, 2H, CH$_2$), $\delta$ 4.22-4.15 (m, 2H, CH$_2$), $\delta$ 3.98 (m, 1H, CH), $\delta$ 3.46 (m, 1H, CH), $\delta$ 1.27 (dd, 3H, CH$_3$), $\delta$ 1.11 (dd, 3H, CH$_3$).

$^{13}$C NMR (150 MHz, DMSO-d$_6$): $\delta$ 173.13, 156.31, 150.60, 144.30, 141.27, 136.92, 133.45, 129.08, 128.37, 128.17, 127.61, 125.74, 124.22, 120.65, 68.21, 66.15, 49.90, 47.10, 33.43, 18.06, 17.18.
HRMS (ESI-TOF) calculated for C\textsubscript{27}H\textsubscript{26}N\textsubscript{2}O\textsubscript{6}[M+1]\textsuperscript{+}:475.1864, [M+Na]\textsuperscript{+}:497.1863,
Found:[M+1]\textsuperscript{+}:475.1861, [M+Na]\textsuperscript{+}:497.1862.

1\textsuperscript{H} NMR (400 MHz, DMSO-d\textsubscript{6}): \(\delta\) 7.89 (d, 2H, Ar), \(\delta\) 7.83 (d, 1H, NH), \(\delta\) 7.74-7.62 (m, 5H, Ar), \(\delta\) 7.49-7.40 (m, 3H, Ar), \(\delta\) 7.33 (t, 2H, Ar), \(\delta\) 4.91 (m, 1H, OH), \(\delta\) 4.31-4.19 (m, 5H, CH\textsubscript{2}, CH\textsubscript{3}), \(\delta\) 4.07 (m, 1H, CH), \(\delta\) 3.55 (m, 2H, CH\textsubscript{2}), \(\delta\) 3.46 (m, 1H, CH), \(\delta\) 1.27 (d, 3H, CH\textsubscript{3}).

1\textsuperscript{3}C NMR (150 MHz, DMSO-d\textsubscript{6}): \(\delta\) 170.56, 156.11, 149.96, 143.84, 140.79, 136.42, 132.97, 128.74, 127.89, 127.69, 127.12, 125.30, 123.83, 120.16, 68.07, 65.83, 61.13, 56.78, 46.61, 32.89, 17.67.

HRMS (ESI-TOF) calculated for C\textsubscript{27}H\textsubscript{26}N\textsubscript{2}O\textsubscript{7}[M+1]\textsuperscript{+}:491.1813, [M+Na]\textsuperscript{+}:513.1632,
Found:[M+1]\textsuperscript{+}:491.1815, [M+Na]\textsuperscript{+}:513.1635.

1\textsuperscript{H} NMR (400 MHz, DMSO-d\textsubscript{6}): \(\delta\) 12.49 (s, 1H, COOH), \(\delta\) 7.89 (d, 2H, Ar), \(\delta\) 7.82 (d, 1H, NH), \(\delta\) 7.76 (m, 1H, Ar), \(\delta\) 7.71-7.61 (m, 4H, Ar), \(\delta\) 7.48-7.40 (m, 3H, Ar), \(\delta\) 7.33 (t, 2H, Ar), \(\delta\) 4.33 (m, 1H, CH), \(\delta\) 4.26-4.18 (m, 5H, CH, CH\textsubscript{2}, CH\textsubscript{3}), \(\delta\) 3.45 (m, 1H, CH), \(\delta\) 2.62-2.51 (m, 2H, CH\textsubscript{2}), \(\delta\) 1.25 (dd, 3H, CH\textsubscript{3}).

1\textsuperscript{3}C NMR (150 MHz, DMSO-d\textsubscript{6}): \(\delta\) 171.42, 171.01, 155.85, 149.91, 143.79, 140.76, 136.34, 132.95, 128.72, 127.90, 127.69, 127.12, 125.31, 123.80, 120.14, 68.18, 66.35, 65.80, 60.21, 46.59, 32.79, 19.97, 17.55.

HRMS (ESI-TOF) calculated for C\textsubscript{28}H\textsubscript{28}N\textsubscript{2}O\textsubscript{8}[M+1]\textsuperscript{+}:505.1969, [M+Na]\textsuperscript{+}:527.1789,
Found:[M+1]\textsuperscript{+}:505.1971, [M+Na]\textsuperscript{+}:527.1789.

1\textsuperscript{H} NMR (400 MHz, DMSO-d\textsubscript{6}): \(\delta\) 12.49 (s, 1H, COOH), \(\delta\) 7.89 (d, 2H, Ar), \(\delta\) 7.82 (d, 1H, NH), \(\delta\) 7.76 (m, 1H, Ar), \(\delta\) 7.71-7.61 (m, 4H, Ar), \(\delta\) 7.48-7.40 (m, 3H, Ar), \(\delta\) 7.33 (t, 2H, Ar), \(\delta\) 4.33 (m, 1H, CH), \(\delta\) 4.26-4.18 (m, 5H, CH, CH\textsubscript{2}, CH\textsubscript{3}), \(\delta\) 3.45 (m, 1H, CH), \(\delta\) 2.62-2.51 (m, 2H, CH\textsubscript{2}), \(\delta\) 1.25 (dd, 3H, CH\textsubscript{3}).

1\textsuperscript{3}C NMR (150 MHz, DMSO-d\textsubscript{6}): \(\delta\) 171.42, 171.01, 155.85, 149.91, 143.79, 140.76, 136.34, 132.95, 128.72, 127.90, 127.69, 127.12, 125.31, 123.80, 120.14, 68.18, 66.35, 65.80, 60.21, 46.59, 32.79, 19.97, 17.55.

HRMS (ESI-TOF) calculated for C\textsubscript{28}H\textsubscript{28}N\textsubscript{2}O\textsubscript{8}[M+1]\textsuperscript{+}:519.1762, [M+Na]\textsuperscript{+}:541.1581,
Found:[M+1]\textsuperscript{+}:519.1763, [M+Na]\textsuperscript{+}:541.1584.
**H NMR (400 MHz, DMSO-\textit{d}_6):** \(\delta\) 12.41 (s, 1H, COOH), \(\delta\) 7.90 (d, 2H, Ar), \(\delta\) 7.83-7.77 (m, 2H, NH, Ar), \(\delta\) 7.72-7.61 (m, 4H, Ar), \(\delta\) 7.47-7.40 (m, 3H, Ar), \(\delta\) 7.32(t, 2H, Ar), \(\delta\) 4.33-4.18 (m, 5H, CH, \(\text{CH}_2\), \(\text{CH}_3\)), \(\delta\) 4.01 (m, 1H, CH), \(\delta\) 3.47 (m, 1H, CH), \(\delta\) 2.26-2.16 (m, 2H, \(\text{CH}_2\)), \(\delta\) 1.88-1.65 (m, 2H, CH), \(\delta\) 1.27 (dd, 3H, \(\text{CH}_3\)).

**C NMR (150 MHz, DMSO-\textit{d}_6):** \(\delta\) 173.59, 171.84, 156.07, 149.93, 143.77, 140.72, 136.30, 132.94, 128.70, 127.87, 127.66, 127.08, 125.23, 123.82, 120.14, 68.03, 65.70, 53.04, 46.57, 32.88, 29.87, 25.79, 17.65.

HRMS (ESI-TOF) calculated for C\(_{29}\)H\(_{28}\)N\(_2\)O\(_8\) [M+1]\(^+\): 533.1918, Found: [M+1]\(^+\): 533.1917.

**H NMR (400 MHz, DMSO-\textit{d}_6):** \(\delta\) 9.25 (s, 1H, OH in phenol-), \(\delta\) 7.88 (d, 2H, Ar), \(\delta\) 7.85-7.78 (m, 2H, Ar, NH), \(\delta\) 7.70-7.76 (m, 4H, Ar), \(\delta\) 7.49-7.26 (m, 5H, Ar), \(\delta\) 7.00-6.93 (m, 2H, phenol-Ar), \(\delta\) 6.66-6.63 (m, 2H, phenol-Ar), \(\delta\) 4.30-4.14 (m, 5H, CH, \(\text{CH}_2\), \(\text{CH}_3\)), \(\delta\) 4.12-4.05 (m, 1H, CH), \(\delta\) 3.48 (m, 1H, OH), \(\delta\) 2.78-2.55 (m, 2H, \(\text{CH}_2\)), \(\delta\) 1.26 (dd, 3H, \(\text{CH}_3\)).

**C NMR (150 MHz, DMSO-\textit{d}_6):** \(\delta\) 170.81,156.07, 150.15, 143.75, 140.74, 136.39, 132.96, 130.02, 128.72, 128.61, 127.89, 127.68, 127.52, 127.45, 127.10, 125.31, 123.82, 123.73, 120.14, 115.09, 68.28, 65.74, 55.98, 46.59, 32.77, 17.53.

4. COPIES OF $^1$H- & $^{13}$C-NMR SPECTRUM OF THE PRODUCTS:
EtOAc
CH₂Cl₂