

Supplementary Information

A Photolabile Linker for the Solid-Phase Synthesis of 4-Substituted NH-1,2,3-Triazoles

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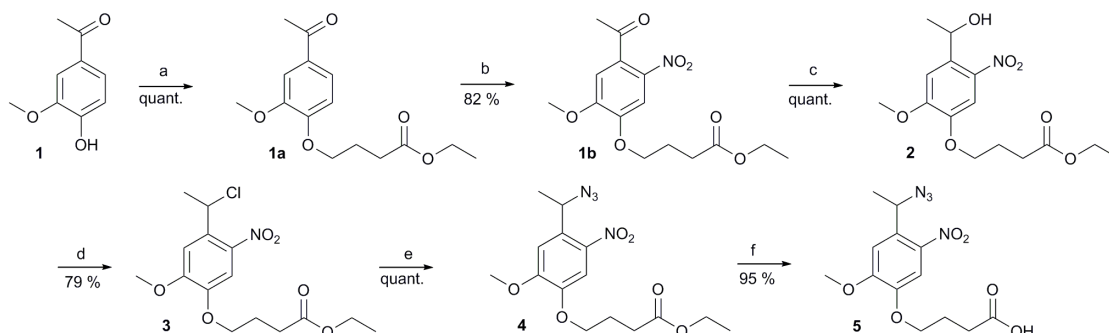
Abbreviations

NEM: *N*-ethyl morpholine; PEGA: polyethylene glycol dimethyl acrylamide; TBTU: *O*-(benzotriazol-1-yl)-*N,N,N,N*-tetramethyluronium tetrafluoroborate; NMP: *N*-methyl-2-pyrrolidone; PBS: phosphate buffered saline; HATU: *O*-(7-azabenzotriazol-1-yl)-*N,N,N,N*-tetramethyluronium hexafluorophosphate; Fmoc-Rink linker: 4-[(2,4-dimethoxyphenyl)(Fmoc-amino)methyl]phenoxyacetic acid.

General Methods

All reagents used were commercially available. All solvents were of HPLC grade. Solid-phase synthesis was carried out using plastic-syringe techniques. Flat-bottomed PE-syringes were fitted with PPE filters, Teflon® tubing and valves, which allow suction to be applied to the syringes. Acid-mediated release of products was carried out in glass vials. Products were analyzed using a Waters Alliance 2695 HPLC system using Symmetry® C-18 column 3.5 μm, 4.6 x 75 mm, column temp; 25 °C, (1 ml/min.) with detection at 215 nm and 254 nm using a diode array detector. Eluent A (0.1% TFA in H₂O) and eluent B (0.1% TFA in acetonitrile) was used with a linear gradient (100% A to 100% B) with a run time of 13 min. Analytical LC-MS analysis was performed on a Waters AQUITY UPLC system equipped with PDA and SQD MS detector; column: AQUITY UPLC BEH C18 1.7μm, 2.1 x 50mm; column temp: 65 °C; solvent A: 0.1% formic acid (aq); solvent B: 0.1% formic acid (acetonitrile); gradient: 5% B to 100% B in 2.4 min, hold for 0.1 min, total run-time ca. 2.6 min. ¹H and ¹³C NMR 300 MHz spectra were recorded on a Varian Mercury 300 BB spectrometer at room temperature. All NMR spectra were recorded using CDCl₃ or DMSO-*d*₆ as solvents.

Linker Synthesis



Ethyl 4-(4-Acetyl-2-methoxyphenoxy)butanoate (1a).¹

To a solution of acetovanillone **1** (35.5 g, 0.21 mol) in DMF (120 mL) was added K₂CO₃ (44.3 g, 0.32 mol) and ethyl 4-bromobutyrate (31.0 mL, 0.21 mol). The mixture was stirred for 16 hours at rt, then heated for 3 hours at 50 °C. After filtration the solution was partitioned between EtOAc and H₂O. The organic phase was washed several times with H₂O to remove the DMF. The organic phase was dried (MgSO₄), filtered and the solvent was removed by rotary evaporation to afford 58.9 g of **1a** (quant.) as a white solid.

¹H NMR (300 MHz, CDCl₃): δ = 1.23 (t, *J* = 7.3 Hz, 3 H), 2.17 (pentet, *J* = 7.3 Hz, 2 H), 2.52 (t, *J* = 7.3 Hz, 2 H), 2.53 (s, 3 H), 3.88 (s, 3 H), 3.87-4.17 (m, 4 H), 6.87 (d, *J* = 8.4 Hz, 1 H), 7.47-7.53 (m, 2 H); ¹³C NMR (75.5 MHz, CDCl₃): δ = 14.4, 24.5, 26.5, 30.8, 56.2, 60.7, 68.0, 110.6, 111.4, 123.4, 130.7, 149.5, 152.8, 173.3, 196.7; UPLC/MS (ESI) *m/z* 281.3 [M+H]⁺.

Ethyl 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoate (1b).¹

A solution of ketoester **1a** (10.0 g, 35.7 mmol) in 30 mL acetic anhydride was slowly added to a solution of 70% HNO₃ (200 mL) and acetic anhydride (40 mL) at 0 °C. After stirring for 3 h the reaction mixture was poured into ice-cooled water. The precipitate was immediately collected by filtration (we found that leaving the mixture for longer time reduced the yield due to hydrolysis of the ester). The precipitate was washed extensively with water before being dried under vacuum to afford 10.8 g of **1b** (82%) as a pale yellow solid.

¹H NMR (300 MHz, CDCl₃): δ = 1.23 (t, *J* = 7.3 Hz, 3 H), 2.10 (pentet, *J* = 7.3 Hz, 2 H), 2.50 (t, *J* = 7.1 Hz, 2 H), 2.51 (s, 3 H), 3.83 (s, 3 H), 4.03 (t, *J* = 7.3 Hz, 2 H), 4.10 (q, *J* = 7.1 Hz, 2 H), 6.95 (s, 1 H), 7.57 (s, 1 H); ¹³C NMR (75.5 MHz, CDCl₃): δ = 14.3, 24.3, 28.2, 30.4, 55.7, 59.6, 68.4, 108.6, 110.7, 132.8, 138.2, 148.8, 154.3, 173.2, 198.7; UPLC/MS (ESI) *m/z* 326.3 [M+H]⁺.

Ethyl 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoate (2).¹

¹ The reported syntheses of **1a**, **1b** and **2** herein were adapted from: C. P. Holmes, *J. Org. Chem.* 1997, **62**, 2370.

To a solution of **1b** (4.00 g, 12.3 mmol) in 300 mL MeOH at 0 °C was slowly added NaBH₄ (1.2 g; 31.7 mmol) in portions. After complete addition, the mixture was allowed to reach rt. The reaction was complete after 3 h (as judged by TLC). The reaction was quenched by addition of 200 mL of sat. NH₄Cl (aq). The reaction was extracted with EtOAc (400 mL), washed with water (2 × 300 mL) and brine (300 mL). The organic phase was dried (MgSO₄), filtered and the solvent was removed by rotary evaporation to give 4.0 g of **2** (quant.) as a pale yellow solid.

¹H-NMR (300 MHz, CDCl₃): δ = 1.20 (t, *J* = 7.2 Hz, 3 H), 1.48 (d, *J* = 7.0 Hz, 3 H), 2.10 (pentet, *J* = 7.0 Hz, 2 H), 2.47 (t, *J* = 7.1 Hz, 2 H), 3.90 (s, 3 H), 4.03 (t, *J* = 7.0 Hz, 2 H), 4.08 (q, *J* = 7.1 Hz, 2H), 5.48 (q, *J* = 7.0 Hz, 1 H), 7.23 (s, 1 H), 7.50 (s, 1 H); ¹³C NMR (75.5 MHz, CDCl₃): δ = 14.9, 23.9, 27.8, 30.0, 56.0, 61.6, 68.1, 108.5, 109.8, 137.8, 138.5, 147.7, 153.8, 174.6; UPLC/MS (ESI) *m/z* 328.4 [M+H]⁺, 310.3 [M-OH]⁺.

Ethyl 4-(4-(1-chloroethyl)-2-methoxy-5-nitrophenoxy)butanoate (3):

To a solution of **2** (4.0 g, 12.3 mmol) in 60 mL CH₂Cl₂ at 0 °C was added thionyl chloride (20 mL). The reaction was allowed to reach rt. The reaction was complete after 2 h (as judged by TLC). The reaction was evaporated to dryness and co-evaporated several times with toluene (3 × 20 mL). The residue was passed through a short plug of silica using EtOAc/heptane (1:1) as the eluent. Evaporation of the solvent gave 3.6 g of **3** (79%) as a pale yellow solid.

¹H-NMR (300 MHz, CDCl₃): δ = 1.20 (t, *J* = 7.2 Hz, 3H), 1.80 (d, *J* = 7.1 Hz, 3 H), 2.10 (pentet, *J* = 7.0 Hz, 2 H), 2.47 (t, *J* = 7.1 Hz, 2 H), 3.92 (s, 3 H), 4.03-4.13 (m, 4 H), 5.86 (q, *J* = 7.1 Hz, 1 H), 7.23 (s, 1 H), 7.43 (s, 1 H); ¹³C NMR (75.5 MHz, CDCl₃): δ = 14.5, 24.4, 27.6, 30.8, 54.0, 56.6, 60.8, 68.5, 108.7, 110.6, 133.0, 140.0, 147.8, 154.0, 173.1; UPLC/MS (ESI) *m/z* 346.4 [M+H]⁺, 310.3 [M-Cl]⁺.

Ethyl 4-(4-(1-azidoethyl)-2-methoxy-5-nitrophenoxy)butanoate (4)

Ethyl 4-(4-(1-chloroethyl)-2-methoxy-5-nitrophenoxy)butanoate (**3**) (2.0 g, 5.7 mmol) and sodium azide (1.1 g, 11.4 mmol) were dissolved in DMF (57 ml) and the reaction mixture was heated to 60 °C for 12 hours. Upon cooling to rt, the reaction was quenched by addition of water (100 ml). The mixture was extracted with EtOAc (200 ml) and the organic phase was washed with water (3 × 150 mL) and brine (150 ml), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*, to yield 1.9 g of **4** (95%) as a yellow solid.

¹H-NMR (300 MHz, CDCl₃): δ = 1.2 (t, *J* = 7.1 Hz, 3 H), 1.5 (d, *J* = 7.1 Hz, 3 H), 2.12 (pentet, *J* = 7.0 Hz, 2 H), 2.47 (t, *J* = 7.1 Hz, 2 H), 3.91 (s, 3 H), 4.05 (t, *J* = 7.0 Hz, 2 H), 4.10 (q, *J* = 7.0 Hz, 2 H), 5.49 (q, *J* = 7.0 Hz, 1 H), 7.20 (s, 1 H), 7.57 (s, 1 H); ¹³C NMR (75.5 MHz, CDCl₃): δ = 14.4, 22.4, 24.4, 30.8, 56.7, 68.5, 109.3, 109.4, 132.4, 140.0, 147.6, 154.4, 173.0; UPLC/MS (ESI) *m/z* 343.2 [M+H]⁺, 310.2 [M-N₃]⁺.

4-(4-(1-Azidoethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (5)

Ethyl 4-(4-(1-azidoethyl)-2-methoxy-5-nitrophenoxy)butanoate (**4**) (1.9 g, 5.4 mmol) was dissolved in THF (50 ml). Lithium hydroxide (1.8 g, 27.0 mmol) and water (15 ml) were added and the reaction mixture was stirred for 12 hours. The reaction was quenched by addition of 1 M HCl (100 ml). The reaction mixture was extracted with EtOAc (100 ml) and the organic phase was washed with brine (100 ml), dried over anhydrous Na₂SO₄ and concentrated *in vacuo* to give 1.6 g of **5** (93%) as a yellow crystalline compound.

$^1\text{H-NMR}$ (300MHz, CDCl_3): δ = 1.5 (d, J = 7.1 Hz, 3 H), 1.90 (pentet, J = 7.1 Hz, 2 H), 2.30 (t, J = 7.1 Hz, 2 H), 3.90 (s, 3 H), 4.05 (t, J = 7.1 Hz, 2 H), 5.42 (q, J = 7.1 Hz, 1 H), 7.13 (s, 1 H), 7.53 (s, 1 H); UPLC/MS (ESI) m/z 325.2 $[\text{M}+\text{H}]^+$, 310.3 $[\text{M}-\text{N}_3]^+$.

Solid-Phase Synthesis

Attachment of Fmoc-Rink linker to amino-functionalized PEG_{A800} beads

Fmoc-Rink linker (3 eq.), NEM (4 equiv.) and TBTU (2.88 equiv.) were mixed in DMF, and shaken for 5 min at rt. The solution was then added to amino-functionalized PEG_{A800} beads pre-swelled in DMF and allowed to react for 2 hours, followed by washing with DMF (\times 6).

Removal of the Fmoc protecting group was accomplished with 20% piperidine in DMF for 5 min. After washing twice with DMF, the deprotection procedure was repeated, now with a reaction time of 30 min. The support was finally washed with DMF (\times 8).

Attachment of azido-linker (5) to Rink linker-functionalized PEG_{A800} beads

4-(4-(1-azidoethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (**5**) was dissolved in DMF, and NEM (4 equiv.) followed by TBTU (2.88 equiv.) were added. The mixture was shaken for 5 min at room temperature before being added to Rink linker-functionalized PEG_{A800} beads pre-swelled in DMF. The mixture was shaken for 2 h at room temperature. The support was washed with DMF (\times 6), MeOH (\times 6) and CH_2Cl_2 (\times 6) before being lyophilized.

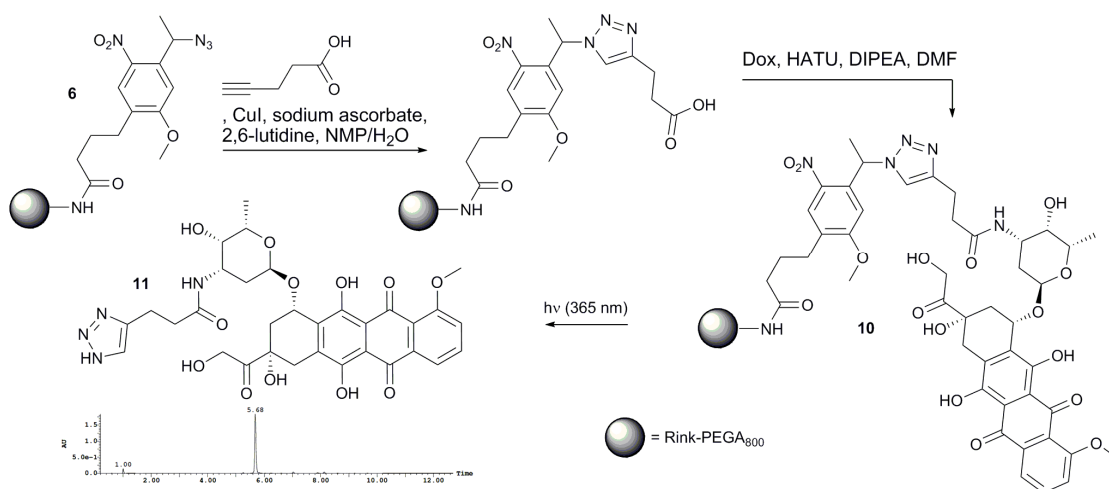
General procedure for Cu-catalyzed azide-alkyne cycloaddition (synthesis of 10a-m)

CuI (1 equiv.), sodium ascorbate (1 equiv.), and 2,6-lutidine (2 equiv.) were mixed in NMP/ H_2O (4:1) and shaken for 30 min before being added to pre-swelled azido-functionalized PEG_{A800} beads (**6**) followed by addition of the corresponding alkyne (2 equiv.). The reaction mixture was shaken at rt for 12h, filtered, washed with pyridine (\times 6), then with DMF (\times 6), water (\times 6), methanol (\times 6) and CH_2Cl_2 (\times 6) before being lyophilized.

Synthesis of 7n

Fmoc deprotection was accomplished as noted above.

Fmoc-phenylalanine (3 equiv.) was dissolved in DMF, then NEM (4 equiv.) and TBTU (2.88 equiv.) were added. The mixture was shaken for 5 min at room temperature before being added to the resin pre-swelled in DMF. The mixture was shaken for 2 h at room temperature. The support was washed with DMF (\times 6), MeOH (\times 6), CH_2Cl_2 (\times 6) before being lyophilized.



Synthesis of 10

CuI (1 equiv.), sodium ascorbate (1 equiv.), and 2,6-lutidine (2 equiv.) were mixed in NMP/H₂O (4:1) and shaken for 30 min before being added to azido-functionalized PEGA₈₀₀ beads (**6**) pre-swelled in DMF, followed by addition of pent-4-ynoic acid (2 equiv.). The reaction mixture was shaken at rt for 12 h, filtered, washed with 10 % DIPEA in DMF ($\times 2$), then with DMF ($\times 6$).

Doxorubicin (3 equiv.) was dissolved in DMF, and DIPEA (5 equiv.) and HATU (3 equiv.) were added. The mixture was shaken for 5 min at room temperature before being added to the resin pre-swelled in DMF. The mixture was shaken for 2 h at room temperature. The support was washed with DMF ($\times 6$), MeOH ($\times 6$), CH₂Cl₂ ($\times 6$) before being lyophilized.

General procedure for release of compounds from Rink linker

Release of the products from the Rink linker was achieved by treatment with TFA/CH₂Cl₂ (1:1) for 2 h at room temperature. The beads were filtered and washed with CH₃CN. The combined filtrates were used directly for analytically purposes.

Irradiation experiments

All irradiation experiments were conducted in a custom build ventilated cabinet equipped with an Omnilux UV-lamp 400W E40. Experiments were carried out in 1 mL Petri dishes with an opened top and irradiated from the top.

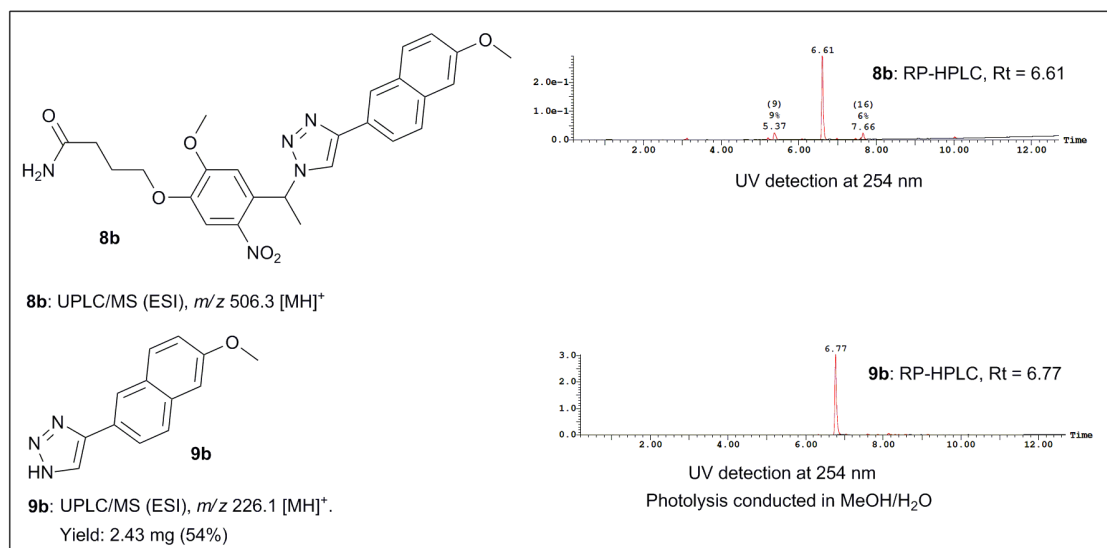
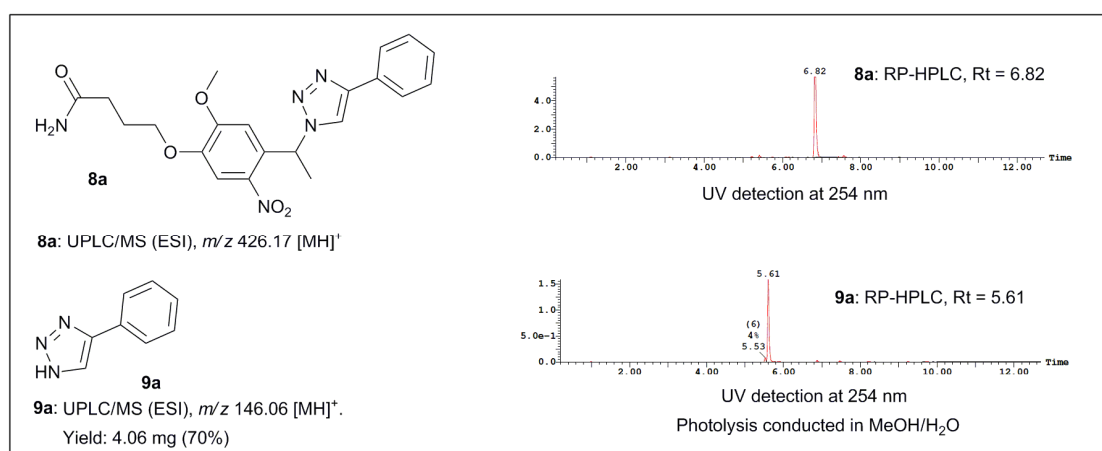


Irradiation times of 0.5-1 h were sufficient for substantial release of compounds (yields up to 50%), and 3h of irradiation time was generally sufficient for maximum release of material.

General procedure for release of compounds from photolabile linker in MeOH/H₂O
Resin sample (5-50 mg) was immersed in MeOH/H₂O (1:4, 500-2000 μ L) and irradiated for 0.5-3 h. The beads were filtered and washed with CH₃CN. The solvent were removed *in vacuo* and the crude products used directly for analytically purposes.

General procedure for release of compounds from photolabile linker in PBS.
The resin sample was pre-swelled in DMSO (25 μ L) and PBS (500 μ L) was added. Photolysis was conducted by irradiating the resin sample (~ 5 mg) for 0.5 h. The beads were filtered, and the crude filtrate was analyzed by RP-HPLC.

Crude RP-HPLC Data²



² For ¹H and ¹³C NMR data on released triazoles, consult: A. E. Cohrt, J. F. Jensen, T. E. Nielsen, *Org. Lett.* 2010, **12**, 5414.

