A light-responsive, self-immolative linker for controlled drug delivery via peptide- and protein-drug conjugates

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1. General methods and procedures

General Information

All chemicals were obtained from commercial supplier and used without further purification. All the solvents used for reactions were distilled under argon after drying over an appropriate drying agent. Peptide 20 - 24 were purchased from Shanghai Top-Peptide Biotechnology Co. Ltd. Expression and purification of histone H3-V35C was carried out as previously described\(^1\) Trastuzumab was purchased from Selleck (catalog NO. A2007) or prepared according to our previously published procedure.\(^2\) Goat antihuman antibody - Alexa 488 conjugate was purchased from Bioss. Cell Counting Kit-8 was obtained from Dojindo laboratories (Japan). Cell culture media and fetal bovine serum (FBS) were from Gibco.

Analytical thin-layer chromatography (TLC) was carried out on SiLiDa silica gel GF\(_{254}\) plates, using UV at 254 nm or staining with phosphomolybdic acid for visualization. Column chromatography was performed with normal phase silica gel (300 - 400 mesh). The preparative - reverse phase HPLC was conducted on FLEXA HP Series fitted with Venusil prepG C18 column 120 Å 10 μm 21.2 mm x 250 mm (Cat. No.: VX902520-A). The NMR spectra were recorded on a Bruker AV 400 spectrometer at 400 MHz (\(^1\)H NMR), 101 MHz (\(^{13}\)C NMR). Chemical shifts (ppm) were referenced to the residual proton signal of the solvent. Data are presented in the following space: chemical shift, multiplicity, coupling constant in hertz (Hz), and signal area integration in natural numbers. High resolution mass spectra (HRMS) were recorded on Waters Xevo G2-XS Q-TOF mass spectrometry. The mass of intact protein was obtained by deconvolution of the raw data using MaxEnt1 tool.

Reversed-phase (RP) UPLC were run in Waters Acquity® UPLC H-class system with PDA detector. Column I: ACQUITY UPLC BEH C18, 130 Å, 1.7 μm, 2.1 mm X 50 mm. Flow 0.4 mL/min at 35 °C. Column II: ACQUITY UPLC HSS C18, 300 Å, 1.7 μm, 2.1 mm X 100 mm. Flow 0.4 mL/min at 35 °C. Column III: ACQUITY UPLC protein BEH C4, 130 Å, 1.7 μm, 2.1 mm X 50 mm. Flow 0.4 mL/min at 60 °C. Buffer A: water. Buffer B: MeCN. Buffer C: 1% (v/v) formic acid in water.
Synthesis of 2. The known compound 1 was prepared according to the published method.\(^3\)

To a solution of 1 (5.5 g, 21.80 mmol, 1.0 equiv) in dry DMF (60.0 mL) under an Ar atmosphere was added imidazole (4.4 g, 65.64 mmol, 3.0 equiv) and TBDPSCI (9.0 g, 32.74 mmol, 1.5 equiv) at room temperature. The mixture was stirred for overnight. The solution was diluted with EtOAc and washed with distilled water. After drying over MgSO\(_4\), the organic phase was concentrated and used without further purification.

The above obtained product was dissolved in DCM (90.0 mL) and treated with a 25% solution of NaOMe in methanol (7.0 mL). After stirring for 1 h at room temperature, the entire mixture was loaded on a silica gel column and then purified by flash chromatography (EtOAc: PE = 1: 5) to give an anomeric mixture of 2 (7.2 g, 85% for 2 steps). Analytical samples of the single isomer were obtained by partial purification of the mixture.

Isomer 2a: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.65 - 7.62 (m, 4H), 7.44 - 7.38 (m, 6H), 5.12 (dd, \(J = 5.6, 2.0\) Hz, 1H), 4.51 - 4.47 (m, 1H), 4.10 - 4.07 (m, 1H), 3.42 (dd, \(J = 12.0, 2.8\) Hz, 1H), 3.33 (s, 3H), 3.11 (dd, \(J = 12.0, 4.4\) Hz, 1H), 2.19 (ddd, \(J = 14.0, 5.6, 4.8\) Hz, 1H), 2.04 (ddd, \(J = 14.0, 6.8, 2.4\) Hz, 1H), 1.06 (s, 9H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 135.7, 133.5, 133.5, 130.0, 129.9, 127.8, 127.8, 105.9, 88.4, 73.4, 63.4, 55.4, 43.1, 26.9, 19.0. HRMS (ESI): C\(_{22}\)H\(_{30}\)NaO\(_4\)Si, [M+Na]\(^+\) calc. 409.1811, found 409.1803.

Isomer 2b: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.67 - 7.64 (m, 4H), 7.44 - 7.38 (m, 6H), 4.91 (dd, \(J = 5.6, 2.4\) Hz, 1H), 4.21 - 4.17 (m, 1H), 4.01 - 3.98 (m, 1H), 3.57 (dd, \(J = 12.0, 2.8\) Hz, 1H), 3.38 (s, 3H), 3.23 (dd, \(J = 12.0, 4.4\) Hz, 1H), 2.17 - 2.09 (m, 1H), 1.91 (ddd, \(J = 13.6, 4.8, 2.4\) Hz, 1H), 1.07 (s, 9H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 135.8, 135.8, 133.7, 133.6, 129.9, 129.9, 127.8, 127.8, 104.6, 84.0, 72.0, 61.7, 55.1, 42.0, 26.9, 19.1. HRMS (ESI): C\(_{22}\)H\(_{30}\)NaO\(_4\)Si, [M+Na]\(^+\) calc. 409.1811, found 409.1803.
Synthesis of 3.

To a 0 °C solution of 2 (3.1 g, 8.03 mmol, 1.0 equiv) in dry THF (36.0 mL) under an Ar atmosphere was added NaH (817.0 mg, 20.32 mmol, 2.5 equiv). The mixture was stirred at 0 °C for 15 min, and then warmed to room temperature and stirred for an additional 30 min. Propargyl bromide (2.2 mL, 20.32 mmol, 2.5 equiv) was added and the solution was allowed to stir for 6 h before quenching with distilled water. The result mixture was diluted with EtOAc and washed with saturated NaCl. After drying over MgSO₄, the organic phase was concentrated, and the residue was chromatographed on silica gel (EtOAc: PE = 1:8) to obtain the compound 3 (3.3 g, 95%) as an oily, 1:1 mixture of isomers.

^1H NMR (400 MHz, CDCl₃) δ 7.69 - 7.64 (m, 8H), 7.46 - 7.37 (m, 12H), 5.06 (dd, J = 5.2, 2.0 Hz, 1H), 4.93 (dd, J = 6.0, 2.4 Hz, 1H), 4.38 - 4.33 (m, 1H), 4.21 - 4.17 (m, 1H), 4.15 - 4.09 (m, 2H), 4.06 - 4.05 (m, 2H) 4.02 (dd, J = 4.8, 2.4 Hz, 1H), 3.53 (dd, J = 11.8, 2.8 Hz, 1H), 3.39 (s, 2H), 3.34 - 3.24 (m, 2H), 3.29 (s, 6H), 3.38 - 3.35 (m, 2H), 2.16 - 2.05 (m, 2H), 1.97 (ddd, J = 13.2, 6.8, 2.0 Hz, 1H), 1.86 (ddd, J = 14.0, 4.8, 2.4 Hz, 1H), 1.08 (s, 9H), 1.07 (s, 9H). ^13C NMR (101 MHz, CDCl₃) δ 135.9, 135.9, 135.8, 129.8, 129.8, 127.7, 127.7, 127.7, 105.4, 104.6, 85.2, 82.6, 79.6, 79.4, 74.6, 74.4, 73.8, 72.5, 71.4, 68.9, 58.4, 58.3, 55.1, 55.0, 41.9, 41.7, 26.9, 26.9, 19.1, 19.1. HRMS (ESI): C_{25}H_{32}NaO_4Si, [M+Na]^+ calc. 447.1968, found 447.1975.

Synthesis of 4.

BF₃·Et₂O (1.7 mL, 13.32 mmol, 2.2 equiv) was added dropwise to a solution of the propanedithiol (0.7 mL, 7.30 mmol, 1.2 equiv) and 3 (2.6 g, 6.06 mmol, 1.0 equiv) in dry DCM (25.0 mL) at 0 °C. After 15 min, the reaction mixture was quenched with saturated NaHCO₃ and the result organic phase was washed with saturated NaCl. After drying over MgSO₄. The organic solvent was
removed on vacuum and the residue was purified by silica gel column chromatography (EtOAc: PE = 1: 7) to give 4 (2.6 g, 85%) as a yellow oil.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 7.75 - 7.70 (m, 4H), 7.45 - 7.39 (m, 6H), 4.11 - 4.03 (m, 3H), 3.93 (dd, J = 10.0, 4.4 Hz, 1H), 3.85 - 3.80 (m, 1H), 3.51 (dd, J = 10.0, 4.0 Hz, 1H), 3.42 (dd, J = 9.6, 7.6 Hz, 1H), 2.71 - 2.63 (m, 3H), 2.46 - 2.41 (m, 1H), 2.39 (t, J = 2 Hz, 1H), 2.28 (d, J = 4 Hz, 1H), 2.09 - 2.02 (m, 1H), 2.00 - 1.96 (m, 1H), 1.87 - 1.72 (m, 2H), 1.09 (s, 9H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) δ 136.1, 136.1, 133.7, 133.2, 129.9, 129.8, 127.8, 127.7, 79.3, 74.8, 73.3, 71.5, 70.5, 58.4, 43.7, 38.5, 30.2, 29.6, 27.1, 25.8, 19.6. HRMS (ESI): C\(_{27}\)H\(_{35}\)O\(_3\)S\(_{2}\)Si, [M+Na]\(^+\) calc. 523.1773, found 523.1761.

**Synthesis of 5.**

To a -78 °C solution of (COCl\(_2\)) (1.3 mL, 15.28 mmol, 1.2 equiv) in CH\(_2\)Cl\(_2\) (25.0 mL) under an Ar atmosphere was dropwise added DMSO (2.1 mL, 29.18 mmol, 2.4 equiv). After 45 min, a solution of 4 (6.1 g, 12.18 mmol, 1.0 equiv) in CH\(_2\)Cl\(_2\) (25.0 mL) was added to the reaction mixture. After 45 min, TEA (8.4 mL, 60.88 mmol, 5.0 equiv) was added and the solution was allowed to stir for 30 min at -78 °C. The solution was warmed to room temperature and quenched with saturated NH\(_4\)Cl. The layers were separated and the aqueous phase was extracted with DCM. The combined organic phase were washed with saturated NaCl solution and dried with MgSO\(_4\). The solution was then concentrated and purified by column chromatography (EtOAc: PE = 1: 6) to afford 5 (5.2 g, 85%).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 7.65 - 7.64 (m, 4H), 7.47 - 7.37 (m, 6H), 4.53 (d, J = 18 Hz, 1H), 4.51 (t, J = 5.2 Hz, 1H), 4.33 (d, J = 18 Hz, 1H), 4.12 (d, J = 2.2 Hz, 2H), 3.95 (t, J = 7.6 Hz, 1H), 2.80 - 2.56 (m, 4H), 2.41 (t, J = 2.2 Hz, 1H), 2.36 - 2.27 (m, 1H), 2.05 - 1.95 (m, 2H), 1.88 - 1.80 (m, 1H), 1.13 (s, 9H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) δ 207.9, 135.9, 132.8, 132.3, 130.2, 128.0, 128.0, 78.8, 75.4, 75.2, 72.2, 58.1, 41.1, 39.9, 28.6, 28.5, 27.1, 25.5, 19.4. HRMS (ESI): C\(_{27}\)H\(_{35}\)O\(_3\)S\(_{2}\)Si, [M+H]\(^+\) calc. 499.1797, found 499.1800.
Synthesis of 7.

To a -10 ºC solution of 5 (727 mg, 1.46 mmol, 1.0 equiv) and (4,5-Dimethoxy-2-nitrophenyl)methanol (NvOH, 1.39 g, 6.52 mmol, 4.5 equiv) in a mixture of CH₃CN/CH₂Cl₂ (2:1, 42.0 mL) was added NBS (1.44 g, 8.09 mmol, 5.5 equiv). The reaction mixture was stirred for 1 h then warmed to room temperature and quenched with saturated Na₂S₂O₃. The layers were separated and the aqueous phase was extracted with ether. The combined organic layers were washed with saturated NaHCO₃, brine and dried over MgSO₄. After removing the solvent under reduced pressure the crude product was purified by silica gel flash chromatography (EtOAc: PE = 1:3) to give 6 (774.0 mg, 65%) as a mixture of isomers (yellow solid).

To a solution of 6 (540 mg, 0.66 mmol, 1 equiv) in THF (10.0 mL) was added 1.0 M solution of TBAF in THF (1.8 mL) at room temperature. After 2 h, the solution was concentrated on a rotary evaporator. The residue was purified by preparative reverse phase HPLC to give three isomers of 7 (Total yield 85%). RP HPLC conditions: C18, 10 μm, 21.2 mm X 250 mm. Flow 15 mL/min at rt. Buffer A: water. Buffer B: MeCN. 0 – 10 – 20 – 27 min, B: 5% – 40% – 70% – 70%.

Isomer 7a (209.5 mg, RT = 22 min): ¹H NMR (400 MHz, CDCl₃) δ 7.59 (s, 1H), 7.53 (s, 1H), 7.28 (s, 1H), 6.84 (s, 1H), 5.58 (dd, J = 5.6, 4.4 Hz, 1H), 4.99 (s, 2H), 4.93 (s, 2H), 4.56 - 4.54 (m, 1H), 4.24 - 4.21(m, 2H), 3.97 - 3.86 (m, 2H), 3.92 (s, 3H), 3.89 (s, 3H), 3.79 (s, 3H), 2.50 (t, J = 2.4 Hz, 1H), 2.46 - 2.41(m, 1H), 2.36 - 2.31 (m, 1H). ¹³C NMR (101 MHz, CDCl₃) δ 153.4, 153.2, 147.7, 147.2, 139.7, 138.7, 130.9, 128.6, 110.2, 109.8, 108.0, 107.4, 105.7, 78.8, 76.4, 75.8, 67.8, 66.8, 60.8, 58.7, 56.3, 56.2, 56.2, 56.1, 40.1. HRMS (ESI): C₂₆H₃₀N₂NaO₁₃, [M+Na]⁺ calc. 601.1646, found. 601.1643.

Isomer 7b (70.8 mg, RT = 23.5 min): ¹H NMR (400 MHz, CDCl₃) δ 7.67 (s, 1H), 7.65 (s, 1H), 7.25 (s, 1H), 7.15 (s, 1H), 5.33 (d, J = 4.8 Hz, 1H), 5.18 - 4.85 (m, 4H), 4.61 (dd, J = 9.6, 7.2 Hz, 1H),
4.15 (t, \(J = 2.4\) Hz, 2H), 3.96 - 3.91 (m, 2H), 3.97 (s, 3H), 3.96 (s, 3H), 3.92 (s, 6H), 2.45 - 2.41 (m, 2H), 2.24 - 2.17(m, 1H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 153.4, 153.2, 147.7, 147.2, 139.7, 138.7, 130.9, 128.6, 110.2, 109.8, 109.8, 108.0, 107.4, 105.7, 78.8, 76.4, 75.8, 67.8, 66.8, 60.8, 58.7, 56.3, 56.2, 56.1, 40.1. HRMS (ESI): C\(_{26}\)H\(_{30}\)N\(_2\)NaO\(_{13}\), [M + Na]\(^+\) calc. 601.1646, found. 601.1643.

Isomer 7c (43.8 mg, RT = 24.5 min): \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.71 (s, 1H), 7.68 (s, 1H), 7.24 (s, 1H), 7.23 (s, 1H), 5.41 (d, \(J = 5.6\) Hz, 1H), 5.19 - 5.06 (m, 2H), 4.97 - 4.92 (m, 2H), 4.33 (m, 1H), 4.21(d, \(J = 2.4\) Hz, 2H), 4.01 - 3.87 (m, 2H), 3.99 (s, 3H), 3.98 (s, 3H), 3.95 (s, 3H), 3.94 (s, 3H), 2.62 - 2.55 (m, 1H), 2.45 (t, \(J = 2.4\) Hz, 1H), 2.18 - 2.15 (m, 1H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 153.6, 153.5, 147.8, 147.6, 139.5, 139.4, 130.3, 129.6, 110.4, 110.3, 110.2, 108.0, 107.9, 105.2, 79.0, 75.4, 75.1, 67.2, 66.2, 61.0, 58.7, 56.5, 56.4, 56.3, 39.2. HRMS (ESI): C\(_{26}\)H\(_{30}\)N\(_2\)NaO\(_{13}\), [M + Na]\(^+\) calc. 601.1646, found. 601.1643.

**Synthesis of 8.**

![Diagram of synthesis](image)

To a solution of 7a (78.5 mg, 0.14 mmol, 1.0 equiv) in anhydrous THF (2.0 mL) under Ar atmosphere was added DIPEA (54.0 \(\mu\)L, 0.31 mmol, 2.2 equiv) and triphosgene (43.8 mg, 0.14 mmol, 1.0 equiv). The resulting mixture was stirred overnight at room temperature, then diluted with water, and extracted with CH\(_2\)Cl\(_2\). The organic layer was dried over anhydrous MgSO\(_4\), and concentrated to afford a yellow solid (90.0 mg, quant.) which is ready for the next reaction without further purification.

The above obtained product was dissolved in anhydrous DCM (3.0 mL), to which DIPEA (60.0 \(\mu\)L, 0.34 mmol, 2.0 equiv) and doxorubicin (98.4 mg, 0.17 mmol, 1.0 equiv) was added. The reaction
was stirred at room temperature for 4 h. After solvent removal, the residue was purified by column chromatography (MeOH: DCM = 1: 20) to give 8 (100.0 mg, 65%, red solid).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 13.96 (s, 1H), 13.20 (s, 1H), 8.00 (d, $J = 7.6$ Hz, 1H), 7.77 (t, $J = 8.0$ Hz, 1H), 7.58 (s, 1H), 7.49 (s, 1H), 7.37 (d, $J = 8.4$ Hz, 1H), 7.27 (s, 1H), 6.82 (s, 1H), 5.52 - 5.48 (m, 2H), 5.35 - 5.34 (m, 1H), 5.24 (d, $J = 8.8$ Hz, 1H), 4.96 - 4.91 (m, 4H), 4.75 (s, 2H), 4.54 (s, 1H), 4.17 - 4.12 (m, 2H), 4.10 (d, $J = 2$ Hz, 2H), 4.06 (s, 3H), 3.91 - 3.81 (m, 2H), 3.90 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.75 (s, 3H), 3.70 - 3.66 (m, 2H), 3.24 (d, $J = 18.8$ Hz, 1H), 2.97 (d, $J = 18.8$ Hz, 1H), 2.53 - 2.49 (m, 1H), 2.42 - 2.38 (m, 1H), 2.38 - 2.30 (m, 2H), 2.20 - 2.15 (m, 1H), 1.92 - 1.79 (m, 2H), 1.29 (d, $J = 6.4$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 213.8, 187.1, 186.6, 161.0, 156.1, 155.6, 154.3, 153.4, 153.2, 147.7, 147.1, 139.5, 138.5, 135.8, 135.4, 133.5, 133.4, 131.0, 128.6, 120.7, 119.8, 118.4, 111.6, 111.4, 110.0, 109.7, 108.6, 107.9, 107.3, 105.0, 100.6, 79.2, 75.3, 69.6, 69.6, 67.9, 67.2, 65.9, 65.5, 60.8, 58.5, 56.7, 56.3, 56.2, 56.1, 47.0, 38.8, 35.6, 34.0, 30.2, 16.8. HRMS (ESI): C$_{54}$H$_{57}$N$_3$NaO$_{25}$, [M+Na]$^+$ calc. 1170.3179, found. 1170.3206.

Synthesis of 9.

Solid cupric sulfate (2.2 mg, 9.0 $\mu$mol, 0.5 equiv) and sodium ascorbate (3.4 mg, 17.4 $\mu$mol, 1.0 equiv) were added to a stirred solution of 8 (20.3 mg, 17.4 $\mu$mol, 1.0 equiv) and the Mal-PEG3-N$_3$ (7.8 mg, 20.9 $\mu$mol, 1.2 equiv) in DMF (2 mL) and H$_2$O (1 mL) at room temperature. A color change from red to brown was observed as the reaction proceeded. After stirring overnight, analysis by LC-MS indicated a substantial amount of 9 formed. [NOTE: in some cases reaction may progress stalled, however, the reaction was driven to completion upon addition of further cupric sulfate and sodium ascorbate]. The reaction was diluted with DMF and purified directly by RP HPLC to afford 9.
as a dark red solid (12 mg, 45%). RP HPLC conditions: C18, 10 μm, 21.2 mm X 250 mm. Flow 15 mL/min at rt. Buffer A: water. Buffer B: MeCN. 0 – 18 min, B: 45% – 80%. Retention time of 9 is 10.5 min.

$^1$H NMR (400 MHz, CDCl$_3$) δ 13.99 (s, 1H), 13.26 (s, 1H), 8.03 (d, J = 7.6 Hz, 1H), 7.79 (t, J = 8.0 Hz, 1H), 7.72 (s, 1H), 7.58 (s, 1H), 7.49 (s, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.36 (s, 1H), 6.84 (s, 1H), 6.69 (s, 2H), 5.54 - 5.44 (m, 2H), 5.35 - 5.32 (m, 2H), 4.96 - 4.86 (m, 4H), 4.78 (s, 2H), 4.68 - 4.53 (m, 5H), 4.17 - 4.15 (m, 1H), 4.07 (s, 3H), 3.92 (s, 3H), 3.88 (s, 3H), 3.84 - 3.82 (m, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 3.73 - 3.70 (m, 2H), 3.65 - 3.50 (m, 10H), 3.42 - 3.40 (m, 2H), 3.30 - 3.26 (m, 1H), 3.16 - 3.02 (m, 2H), 2.52 - 2.49 (m, 3H), 2.38 - 2.34 (m, 3H), 2.22 - 1.85 (m, 5H), 1.34 - 1.32 (m, 3H). HRMS (ESI): C$_{69}$H$_{81}$N$_{8}$O$_{31}$, [M+H]$^+$ calc. 1517.5008, found. 1517.5007.

**Synthesis of 10.**

[8'] was prepared as described above. The obtained carbonochloridate [8'] (221.8 mg, 0.35 mmol, 1.0 equiv) was dissolved in anhydrous DCM (3.0 mL), to which DIPEA (132.0 μL, 0.76 mmol, 2.2 equiv), DMAP (3.9 mg, 0.035 mmol, 0.1 equiv) and pyrenemethanol (90.0 mg, 0.38 mmol, 1.1 equiv) was added. The reaction was stirred overnight at room temperature. After solvent removal, the residue was purified by column chromatography (EtOAc: PE = 1: 1) to give 10 (140.0 mg, 50%, yellow solid).

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.32 (d, J = 12 Hz, 1H), 8.24 - 8.20 (m, 3H), 8.17 (d, J = 12 Hz, 1H), 8.11 (s, 1H), 8.09 (s, 1H), 8.07 - 8.04 (m, 2H), 7.58 (s, 1H), 7.52 (s, 1H), 7.29 (s, 1H), 6.81 (s, 1H), 5.93 (s, 2H), 5.56 - 5.47 (m, 2H), 4.98 (s, 2H), 4.93 (s, 2H), 4.02 - 3.97 (m, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.77 (s, 3H), 3.67 (d, J = 8 Hz, 1H), 2.58 - 2.43 (m, 2H), 2.22 (s, 1H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 154.2, 153.5, 153.1, 147.8, 147.2, 139.7, 138.6, 132.1, 131.2, 130.8, 130.6, 129.6, 128.5, 128.3, 128.1, 128.0, 127.7, 127.3, 126.2, 125.7, 125.3, 124.9, 124.6, 122.7, 110.2,
UPLC-MS analysis of reactions between 8 and BnNH$_2$.

To a solution of 8 (4 mM in CH$_3$CN, 5 μL) was added BnNH$_2$ (4 μL, 20 eq., 100 mM stock solution in H$_2$O, adjusted to pH 7.0 with HOAc). The resulting mixture was irradiated with 365 nm for 3 min (7.0 mW cm$^{-2}$), followed by 5 min incubation at 37 °C, 3 μL of mixture was subjected UPLC-MS analysis. UPLC conditions (column II): 0 - 6.0 min, B 5-85%. Buffer A: water. Buffer B: MeCN.

For light-triggered decomposition of 8 in the absence of BnNH$_2$, a solution of 8 (4 mM in CH$_3$CN/H$_2$O (v/v = 5/4)) was exposed to 365 nm irradiation (7.0 mW cm$^{-2}$) for 3 min, followed by incubation at 37 °C for 0.5 h. 3 μL of mixture was subjected UPLC-MS analysis as described.

UPLC-MS analysis of reactions between 8 and BnNH$_2$ in the presence of NaBH$_3$CN.

To a solution of 8 (4 mM in CH$_3$CN, 5 μL) was added a solution of BnNH$_2$ (4 μL, 20 eq., 100 mM stock solution in H$_2$O, adjusted to pH 7.0 with HOAc), NaBH$_3$CN (0.8 μL, 20 eq., 500 mM stock solution in H$_2$O). The final reaction conditions are: 1 mM 8, 20 mM BnNH$_2$, 20 mM NaBH$_3$CN. The resulting mixture was exposed to light irradiation (365 nm, 7.0 mW cm$^{-2}$) for 3 min followed by incubation at 37 °C for 30 min, 3 μL of mixture was subjected UPLC-MS analysis. UPLC conditions (column II): 0 - 6.0 min, B 5 - 85%. Buffer A: water. Buffer B: MeCN.

UPLC-MS analysis of reactions between 10 and BnNH$_2$.

A solution of 10 (4 mM in dry CH$_3$CN, total 9 μL) was exposed to light irradiation (365 nm, 7.0 mW cm$^{-2}$) for 3 min followed by addition of a solution of BnNH$_2$ (4 μL, 20 eq., 100 mM stock solution in H$_2$O, adjusted to pH 7.0 with HOAc). After incubation at 37 °C for 5 min, 3 μL of mixture was subjected UPLC-MS analysis. UPLC condition (column II): 0 - 10.0 min, B 5 - 100%.

It is known that photolysis occurs for pyrene-containing molecules in aqueous solution, but they are stable in nonprotonic solvents upon photo-irradiation.$^{4,6}$ Hence, photodecaging of 10 was carried out in pure CH$_3$CN to prevent the photolysis of the pyrene moiety, then BnNH$_2$ in aqueous solution was added followed by incubation in dark.

109.8, 108.6, 108.0, 107.3, 80.3, 78.6, 75.2, 68.5, 68.1, 65.7, 58.5, 56.3, 56.2, 56.1, 38.5, 29.7. HRMS (ESI): C$_{44}$H$_{40}$N$_2$NaO$_{15}$, [M+Na]$^+$ calc. 859.2326, found 859.2369.
UPLC-MS analysis of light-triggered DOX release from peptide-PC4AP-DOX conjugates.

A mixture of 9 (1.1 mM) and peptide (1.0 mM) was incubated in phosphate buffer (20 mM, pH 7.5) at 37 °C for 1 h. The resulting mixture was exposed to UV light for 3 min. Aliquots (3 μL) were taken at appropriate intervals and analyzed UPLC-MS. UPLC conditions (column II): 0 - 6 min, B 5 - 85%. Buffer A: water. Buffer B: MeCN.

Preparation of H3-PC4AP-DOX (36)

Protein (H3-V35C) sequence:
ARTKQ TARKS TGKPR ATKAP ATGGC35 KKPHR YRPGT VALREI RRYQK STELL IRKLP FQRLV REIAQ DFKTD LRFQS SAVMA LQEAS EAYLV ALFED TNLAA IHAKR VTIMP KDIQL ARRIR GERA

Calculated Mass: 15243 Da

9 (33.5 μL, 1.5 mM in DMF) was added to a solution of H3-V35C (160 μL, 300 μM) in HEPES buffer (20 mM, pH 7.5). The reaction mixture was incubated at 37 °C for 1 h. 2 μL of the mixture was taken and analyzed by UPLC-MS. Excess 9 and salts were removed by PD Minitrap G-25 column (GE Healthcare) according to spin protocol. UPLC established that there was <0.5% unconjugated 9 remaining in the sample. UPLC conditions (column III): 0-7.5 min, B 2-80%, C 10% keep constant. The mass of intact protein was obtained by deconvolution of the raw data using MaxEnt1 tool.

UPLC-MS analysis of the stability and light-triggered decomposition of H3-PC4AP-DOX conjugate 36

The H3-PC4AP-DOX 36 was dissolved in phosphate buffer (20 mM, pH 7.5) to afford a 50 μM solution (total volume 50 μL). The sample was incubated at 37 °C for 2 h. 10 μL aliquot was analyzed by UPLC-MS. UPLC conditions: (column III): 0 - 7.5 min, B 2 - 80%, C 10% keep constant. UV detection at 484 nm. The mass of intact protein was obtained by deconvolution of the raw data using MaxEnt1 tool.

UPLC-MS analysis of light-triggered decomposition of conjugate 36 was carried out following the same procedure except that 365 nm irradiation (7.0 mW cm⁻², 3 min) was applied before the incubation.
SDS-PAGE analysis of the kinetics of DOX release from H3-PC4AP-DOX (36) upon light irradiation.

40 μL of 36 (45 μM) conjugates in phosphate buffer (20 mM, pH 7.5) was irradiated with 365 nm UV light (7.0 mW cm⁻²) for 5 min followed by incubation at 37 ºC in dark. 3 μL of mixture were taken at appropriate intervals, quenched by adding NaBH₃CN (final concentration 20 mM), and analyzed by 15% SDS PAGE. As a control, unirradiated sample was operated in the same manner. Fluorescence intensity of each band was quantified using GE Typhoon Gel Imaging Scanner with excitation at 532 nm and emission at 570 nm. Then the gels were stained by comassie blue.

Preparation of antibody drug conjugate 39

To a solution of trastuzumab (5 mg/mL in PBS (pH 7.4), 20 μL) was added a solution of TCEP (2.8 μL, 4 eq., 1 mM stock solution in H₂O). The resulting mixture was incubated at 37 ºC for 2 h to afford reduced trastuzumab followed by addition of 3.8 μl of 9 (8 eq., 1.5 mM stock solution in DMF). The reaction mixture was incubated at 37 ºC for 1 h. The antibody conjugate was purified by gel filtration (PD SpinTrap G-25, GE Healthcare, 28-9180-04) according to spin protocol. The number of conjugated DOX per mAb was determined to be 8 by UPLC-MS.⁷


To conjugate 39 (25 μL, 3 μM) was added 15 μL of human serum. The mixture was incubated at 37 ºC. Aliquots (8 μL) were taken at appropriate intervals, quenched by adding NaBH₃CN (final concentration 20 mM), and analyzed by reducing glycine-SDS-PAGE (12%) following standard lab procedures. The gel was run at constant voltage (120 V) for 1 h using Tris (25 mM) - Glycine (192 mM) - SDS (0.1%) as a running buffer. Fluorescence was visualized on GE Typhoon Gel Imaging Scanner (excitation at 532 nm and emission at 570 nm) prior to staining with Coomassie Blue.

SDS-PAGE analysis of DOX release from the ADC 39 in human serum upon light irradiation.

To conjugate 39 (30 μL, 3 μM) was added 18 μL of human serum. The mixture was irradiated with 365 nm UV light for 5 min followed by incubation at 37 ºC. Aliquots (7 μL) were taken at appropriate intervals, quenched by adding NaBH₃CN (final concentration 20 mM), and analyzed by reducing glycine-SDS-PAGE (12%) as described above.
Mammalian cell culture
SK-BR-3 (HER2 overexpression) and MCF-7 (HER2 negative) human breast cancer cell lines were purchased from Shanghai Zhong Qiao Xin Zhou Biotechnology Co. Ltd. All cell lines were cultured at 37 °C, 5% CO₂ in DMEM (Dulbecco’s modified Eagle’s medium) complemented with 10% fetal bovine serum and 1% penicillin - streptomycin.

Confocal microscopic analysis of cellular location of DOX and H3-PC4AP-DOX conjugate (36) in HeLa cells.
HeLa cells were seeded on TC-treated coverslips in 24-well plates at a density of 1.0 × 10⁵/well and cultured in an incubator at 37 °C and 5% CO₂ overnight. Media was removed and replaced with solutions of 10 μM DOX or 36 in complete growth media. After incubation for different intervals, the cells washed twice with PBS and fixed in 4% paraformaldehyde at rt for 15 min. Following fixation, nuclei were stained with DAPI at rt for 7 min. After extensively washing, cells were mounted with antifade mounting medium (Beyotime, P0126). The coverslips were sealed using nail varnish. Cell images were taken using a 60×oil immersion objective (NA 1.40) of a Nikon A1+ confocal microscope (Nikon, Tokyo, Japan). DAPI channel: excitation at 408 nm and emission with 425–475 nm bandpass filter. DOX channel: excitation at 561 nm and emission with 570–620 nm bandpass filter.

For confocal microscopic analysis of cellular location of H3-PC4AP-DOX conjugate (36) in HeLa cells upon light irradiation, cells were incubated with 36 (10 μM) in complete growth media for 3 h. After supernatant removal and replacement with fresh medium, cells were exposed to 365 nm irradiation (7.0 mW cm⁻²) for 5 minutes, followed by incubation at 37 °C for another 3 hours. Then, the cells were subjected to fixation, DAPI staining and confocal microscopic analysis as described.

In Vitro cytotoxicity of H3-PC4AP-DOX conjugate (36) to HeLa cells (CCK-8)
HeLa cells were seeded into 96-well plates at 3000 cells/well and allowed to adhere for 24 h. Media was then removed from the cells and replaced with the 36 conjugate diluted in DMEM at the indicated concentrations and incubated at 37 °C for 40 min. After media removal, the cells were thoroughly washed with PBS followed by addition of 100 μL of fresh DMEM to each well. Cells
were exposed to 365 nm light irradiation (7.0 mW cm\(^{-2}\)) for 7 min. Following a 48 h incubation period at 37 °C, 10 μL of CCK-8 in 100 μL DMEM was added to each well and incubated for 1 h at 37 °C. Absorbance at 450 nm was analyzed via a plate reader. Drug effects were expressed as % cell inhibition relative to the untreated sample.

**Confocal microscopic analysis of the binding of trastuzumab-(PC4AP)-DOX conjugate (39) to breast cancer cells.**

SK-BR-3 cells (HER2 positive) and MCF-7 cells (HER2 negative) were seeded on TC-treated coverslips in 24-well plates at a density of 1.0 × 10^5/well and cultured in an incubator at 37 °C and 5% CO\(_2\) overnight. Cells were rinsed with PBS (3 × 400 μL) and fixed with 4% paraformaldehyde in PBS (300 μL) at rt for 20 min. Then the fixed cells were washed with PBS buffer (3 × 400 μL) and blocked with 10% goat serum for 30 min.

After that, the cells were incubated with 500 nM of trastuzumab and 39 respectively for 30 min at rt, followed by incubation with goat antihuman antibody-Alexa488 conjugate (1:200) for another 30 min. After washing 3 times with PBS, cells were stained with DAPI (300 μL, 10 μg/mL) at rt for 7 min. After extensively washing, cells were mounted with antifade mounting medium. The coverslips were sealed using nail varnish. Cells were imaged with a Nikon A1+ confocal microscope. DAPI channel: excitation at 408 nm excitation and emission with 425–475 nm bandpass filter. AF488 channel: excitation at 486 nm and emission with 500–550 nm bandpass filter.

**Cytotoxicity of antibody-drug conjugate (39) against breast cancer cells**

The effect of antibody-drug conjugate 39 on the viability of SK-BR-3 cells (HER2 positive) and MCF-7 (HER2 negative) was assessed by CCK-8 assay. Free DOX and caped DOX 9 was used as positive and negative control respectively. SK-BR-3 and MCF7 cells were plated in 96-well plates at 7,000 and 3,000 cells/well in 100 μL medium, respectively. Cells were allowed to attach for 24 h at 37 °C and 5% CO\(_2\). Following incubation, medium was removed and 9, 39, DOX, at different concentrations in fresh medium were added. After incubation for 6 h in dark, media was replaced with fresh media followed by light irradiation as described above. Following a 48 h incubation period at 37 °C, 10 μL of CCK-8 in 100 μL DMEM was added to each well and incubated for 1 h at 37 °C.
37 °C. Absorbance at 450 nm was analyzed via a plate reader. Drug effects were expressed as % cell viability relative to the untreated sample. IC$_{50}$ values were calculated by fitting the concentration-dependent curves using the sigmoidal function.
2. Supplementary figures

(A) pH 7.5

(B) pH 5.2

Figure S1. UPLC analyses of the stability of 8 under physiologically relevant conditions.

Reaction conditions: A solution of 8 (final concentration 1 mM) in a appropriate buffer (sodium acetate, phosphate buffer were used for pH 5.2, 7.5 respectively, final concentration 20 mM, 100 mM NaCl) was incubated at 37 °C. 1 μL of reaction mixture was taken at appropriate intervals and analyzed by UPLC-MS. UPLC condition (column II): 0-6 min, B: 5-85%.
Figure S2. Kinetics of photodecaging of 9 as a function of irradiation time (365 nm, 7.0 mW cm$^{-2}$).

UV-Vis analysis: 9 (500 μM) was dissolved in CH$_3$CN: PBS (2:1) solution, and exposed to the light (365 nm, 7.0 mW cm$^{-2}$). A series of aliquots were collected as a function of irradiation time and analyzed by UV–Vis spectrometry.
(A) UPLC-MS analyses of the light-triggered decomposition of 8 in the presence or absence of BnNH₂.

(i) To a solution of 8 (4 mM in CH₃CN, 5 μL) was added a solution of BnNH₂ (4 μL, 20 eq., 100 mM stock solution in H₂O, adjusted to pH 7.0 with HOAc). The resulting mixture was incubated at 37 °C for 0.5 h, 3 μL of mixture was subjected UPLC-MS analysis.

(ii) A solution of 8 (4 mM in CH₃CN/H₂O [v/v = 5/4], total 9 μL) was exposed to light irradiation (365 nm, 7.0 mW cm⁻²) for 3 min followed by incubation at 37 °C for 0.5 h, 3 μL of mixture was subjected UPLC-MS analysis.

(iii) To a solution of 8 (4 mM in CH₃CN, 5 μL) was added a solution of BnNH₂ (4 μL, 20 eq., 100 mM stock solution in H₂O, adjusted to pH 7.0 with HOAc). The resulting mixture was exposed to light irradiation (365 nm, 7.0 mW cm⁻²) for 3 min followed by incubation at 37 °C for 5 min, 3 μL of mixture was subjected UPLC-MS analysis.

UPLC condition (column II): 0 - 6.0 min, B 5-85%.
(B) UPLC-MS analyses of the light-triggered decomposition of 8 in the presence of BnNH\textsubscript{2} and NaBH\textsubscript{3}CN.

(iv) To a solution of 8 (4 mM in CH\textsubscript{3}CN, 5 µL) was added a solution of BnNH\textsubscript{2} (4 µL, 20 eq., 100 mM stock solution in H\textsubscript{2}O, adjusted to pH 7.0 with HOAc), NaBH\textsubscript{3}CN (0.8 µL, 20 eq., 500 mM stock solution in H\textsubscript{2}O). The final reaction conditions are: 1 mM 8, 20 mM BnNH\textsubscript{2}, 20 mM NaBH\textsubscript{3}CN. The resulting mixture was exposed to light irradiation (365 nm, 7.0 mW cm\textsuperscript{-2}) for 3 min followed by incubation at 37 ºC for 30 min, 3 µL of mixture was subjected UPLC-MS analysis. UPLC conditions (column II): 0 - 6.0 min, B 5-85%. Buffer A: water. Buffer B: MeCN.

(v) Treatment of DOX with NaBH\textsubscript{3}CN produced reduced DOX (DOX\textsuperscript{R}). UPLC-MS analysis showed that DOX\textsuperscript{R} contains two more hydrogen atoms than DOX.

**Figure S3.** UPLC-MS analyses of the light-triggered decomposition of 8.
Figure S4. UPLC-MS analyses of the light-triggered decomposition of 10 in the presence or absence of BnNH₂.

(i) To a solution of 10 (4 mM in CH₃CN, 5 μL) was added a solution of BnNH₂ (4 μL, 20 eq., 100 mM stock solution in H₂O, adjusted to pH 7.0 with HOAc). The resulting mixture was incubated at 37 °C for 0.5 h, 3 μL of mixture was subjected UPLC-MS analysis. (ii) A solution of 10 (4 mM in dry CH₃CN, total 9 μL) was exposed to light irradiation (365 nm, 7.0 mW cm⁻²) for 3 min followed by incubation at 37 °C for 0.5 h, 3 μL of mixture was subjected UPLC-MS analysis. (iii) A solution of 10 (4 mM in dry CH₃CN, total 9 μL) was exposed to light irradiation (365 nm, 7.0 mW cm⁻²) for 3 min followed by addition of a solution of BnNH₂ (4 μL, 20 eq., 100 mM stock solution in H₂O, adjusted to pH 7.0 with HOAc). After incubation at 37 °C for 5 min, 3 μL of mixture was subjected UPLC-MS analysis. UPLC condition (column II): 0 - 10.0 min, B 5 - 100%.

Photolysis occurs for pyrene-containing molecules in aqueous solution, but they are stable in nonprotonic solvents upon photo-irradiation.⁴⁻⁶ Hence, photodecaging of 10 was carried out in dry CH₃CN to gave 19, then BnNH₂ in aqueous solution was added followed by incubation in dark.
**Figure S5.** UPLC-MS analyses of the light-triggered decomposition of peptide-PC4AP-DOX conjugates 25-29.

**Reaction conditions:** A mixture of 9 (1.1 mM) and peptide (20-24) (1.0 mM) was incubated in phosphate buffer (20 mM, pH 7.5) at 37 °C for 1 h. The resulting mixture was exposed to UV light for 3 min. Aliquots (3 μL) were taken at appropriate intervals and analyzed UPLC-MS. UPLC conditions (column II): 0 - 6 min, B 5 - 85%. Buffer A: water. Buffer B: MeCN.
Figure S6. Mass spectra of H3-V35C (A) and H3-PC4AP-DOX (B).
Figure S7. UPLC-MS analysis of photo-decomposition of H3-PC4AP-DOX (36). (A) UPLC profile. (B) MS spectrum of product with RT = 4.32 min. (C) MS spectrum of product with RT = 3.64 min.

**Reaction conditions:** The H3-PC4AP-DOX (36) was dissolved in phosphate buffer (20 mM, pH 7.5) to afford a 50 μM solution (total volume 50 μL) and irradiated with 365 nm UV light (7.0 mW cm⁻²) for 3 min. The sample was incubated for 2 h at 37°C. 10 μL aliquot was analyzed by UPLC-MS. UPLC conditions: (column III): 0-7.5 min, B 2-80%, C 10% keep constant. UV detection at 484 nm. The mass of the product with RT of 4.32 min was obtained by deconvolution of the raw data using MaxEnt1 tool.
**Figure S8.** 15% SDS-PAGE analysis of DOX release from H3-PC4AP-DOX (36). (A) Stability of 36 in phosphate buffer (20 mM, pH 7.5) in dark. (B) DOX release from 36 after 365 nm irradiation in phosphate buffer (20 mM, pH 7.5).

**Reaction conditions:** 40 μL of conjugate 36 (45 μM) in phosphate buffer (20 mM, pH 7.5) was irradiated with 365 nm UV light (7.0 mW cm⁻²) for 5 min followed by incubation at 37 °C in dark. 3 μL of mixture were taken at appropriate intervals, quenched by adding NaBH₃CN (final concentration 20 mM), and analyzed by 15% SDS PAGE. As a control, unirradiated sample was operated in the same manner.

Fluorescence intensity of each band was quantified using GE Typhoon Gel Imaging Scanner with excitation at 532 nm and emission at 570 nm. Then the gels were stained by commassie blue.
**Figure S9.** Fluorescence confocal microscopy analysis showing the cellular location of DOX in HeLa cells after treated with DOX and H3-PC4AP-DOX conjugate (36) respectively. No light irradiation was applied. The images were obtained by overlaying DAPI channel (408 nm excitation laser with 425–475 nm bandpass filter) with DOX channel (561 nm excitation laser with 570–620 nm bandpass filter).
(A) 10% SDS-PAGE analysis of trastuzumab

Lane 1: Protein ladder with the molecular weights (kDa).
Lane 2: Trastuzumab under non-reducing condition. The unreduced trastuzumab shows a molecular weight ~ 170 kDa in the SDS-PAGE, larger than its theoretical molecular weight (~ 150 kDa) due to glycosylation.
Lane 3: Trastuzumab under reducing condition. After treated with 50 mM DTT, the molecular weights of heavy chain and light chain are ~55 KDa and ~25 KDa respectively.

(B) MS of trastuzumab

(C) MS of trastuzumab- PC4AP-DOX (39)

Figure S10. Characterization of trastuzumab and trastuzumab-PC4AP-DOX (39).
Figure S11. Reducing glycine-SDS-PAGE analysis of the stability of ADC 39 in human serum.

**Reaction conditions:** To conjugate 39 (25 μL, 3 μM) was added 15 μL of human serum. The mixture was incubated at 37 °C. Aliquots (8 μL) were taken at appropriate intervals, quenched by adding NaBH₃CN (final concentration 20 mM), and analyzed by reducing glycine-SDS-PAGE (12%) following standard lab procedures. Fluorescence was visualized on GE Typhoon Gel Imaging Scanner (excitation at 532 nm and emission at 570 nm) prior to staining with Coomassie Blue.
Figure S12. SDS-PAGE analysis of DOX release from the ADC 39 in human serum upon light irradiation.

**Reaction conditions:** To conjugate 39 (30 μL, 3 μM) was added 18 μL of human serum. The mixture was irradiated with 365 nm UV light for 5 min followed by incubation at 37 °C. Aliquots (7 μL) were taken at appropriate intervals, quenched by adding NaBH₃CN (final concentration 20 mM), and analyzed by reducing glycine-SDS-PAGE (12%). Fluorescence was visualized on GE Typhoon Gel Imaging Scanner (excitation at 532 nm and emission at 570 nm) prior to staining with Coomassie Blue.
(A) SK-BR3 cells (HER2 positive)

![Fluorescence microscopy image of SK-BR3 cells](image)

(B) MCF-7 cells (HER2 negative)

![Fluorescence microscopy image of MCF-7 cells](image)

**Figure S13.** Fluorescence microscopy image of (A) SK-BR3 cells (HER2 positive) and (B) MCF-7 cells (HER2 negative) stained with 39 as a primary antibody and goat antihuman antibody-Alexa488 conjugate (Ab-AF488) as a secondary antibody. Cells were fixed before treatment with antibodies. Equivalent concentration of trastuzumab was used as positive control for binding on cell surface HER2. DAPI channel: 408 nm excitation laser with 425–475 nm bandpass filter. AF488 channel: 486 nm excitation laser with 500–550 nm bandpass filter.
3. NMR spectra of new compounds.

Figure S14. $^1$H (top) and $^{13}$C (bottom) NMR spectra of 2a.
Figure S15. $^1$H (top) and $^{13}$C (bottom) NMR spectra of 2b.
Figure S16. $^1$H (top) and $^{13}$C (bottom) NMR spectra of 3.
Figure S17. $^1$H (top) and $^{13}$C (bottom) NMR spectra of 4
Figure S18. $^1$H (top) and $^{13}$C (bottom) NMR spectra of 5
Figure S19. $^1$H (top) and $^{13}$C (bottom) NMR spectra of 7a
Figure S20. $^1$H (top) and $^{13}$C (bottom) NMR spectra of 7b
Figure S21. $^1$H (top) and $^{13}$C (bottom) NMR spectra of 7c
Figure S22. $^1$H (top) and $^{13}$C (bottom) NMR spectra of 8
Figure S23. $^1$H NMR spectra of 9
Figure S2. $^1$H NMR and $^{13}$C NMR spectra of 10
Figure S25. $^1$H (top) and $^{13}$C (bottom) NMR spectra of 12.
**Figure S26.** DEPT 135 NMR spectra of 12.

**Figure S27.** HSQC NMR spectrum of 12.
Figure S28. HMBC NMR spectrum of 12.

4. References