Aza-dibenzocyclooctynes for fast and efficient enzyme PEGylation via copper-free (3+2) dipolar cycloaddition

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Supporting Information

General experimental

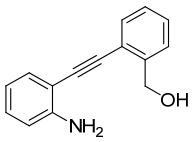
Unless stated otherwise all chemicals were obtained from commercial sources and used without further purification. If no further details are given the reaction was performed under ambient atmosphere and temperature. Analytical thin layer chromatography (TLC) was performed on silica gel-coated plates (*Merck* 60 F254) with the indicated solvent mixture, visualization was done using ultraviolet (UV) irradiation (λ = 254 nm) and/or staining with $KMnO_4$. Purification by column chromatography was carried out using *Silicycle* silica gel (0.040 - 0.063 mm, and ca. 6 nm pore diameter). The water used in the enzyme functionalization was deionised using a Labconco Water Pro PS purification system. The PBS-buffer was a 150 mM NaCl and 50 mM NaH_2PO_4 solution that was adjusted to pH 8. THF and CH_2Cl_2 were dried over an activated alumina column using an MBraun SPS800 solvent purification system. Et_3N was distilled under N_2-atmosphere from CaH_2.

Infrared Spectroscopy (IR spectroscopy): IR spectra were recorded on an ATI Matson Genesis Series FTIR spectrometer fitted with an ATR cell. The vibrations (ν) are given in cm^{-1}.

Nuclear Magnetic Resonance Spectroscopy (NMR spectroscopy): ^{1}H-NMR spectra were recorded on a *Varian Inova 400* (400 MHz) for room temperature measurements and a *Varian Inova 500* (500 MHz) spectrometer was used for low temperature measurements. ^{13}C-NMR spectra were recorded on a *Bruker DMX300* (75 MHz) spectrometer. Unless stated otherwise all spectra were taken at ambient temperature. ^{1}H-NMR chemical shifts (δ) are reported in parts per million (ppm) relative to a residual proton peak of the solvent, δ = 3.31 for CD_3OD and δ = 7.26 for $CDCl_3$. Broad peaks are indicated by the addition of br. Coupling constants are reported as a *J*-value in Hertz (Hz). In case of rotamers the spectrum was taken at lower temperature to freeze the compound in its two rotamer states, causing separate peaks for each rotamer. In these cases shifts, coupling constants and integrals are given of each separate peak. ^{13}C-NMR chemical shifts (δ) are reported in ppm relative to CD_3OD (δ = 49.0) or $CDCl_3$ (δ = 77.0). If rotamers are observed in the spectrum, the minor rotamer peaks are labelled with *.

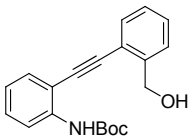
Mass Spectrometry (MS): High Resolution Mass Analyses were performed using Electrospray Ionization on a JEOL AccuToF.

Synthesis



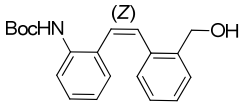
{2-[(2-Aminophenyl)ethynyl]phenyl}methanol (1)

2-Iodobenzylalcohol (4.0 g, 17.1 mmol), $Pd(PPh_3)_2Cl_2$ (239 mg, 0.34 mmol) and CuI (32.4 mg, 0.17 mmol) were added to a flame-dried Schlenk flask. The flask was evacuated and refilled with an N_2/H_2-mixture (3:2) three times. Dry THF (40 mL) and dry Et_3N (3.8 mL, 27.4 mmol) were bubbled through with an N_2/H_2-mixture (3:2) for 10 minutes and subsequently added to the mixture. Hereupon 2-ethynylaniline (2.5 mL, 24 mmol) was added and the mixture was stirred for 4 hours under an N_2/H_2-atmosphere. The mixture was diluted with CH_2Cl_2 (60 mL) and washed with H_2O (50 mL). The H_2O layer was extracted with CH_2Cl_2 (2 × 50 mL). The organic layers were combined and washed with H_2O (2 × 100 mL) and brine (100 mL) and subsequently dried over $MgSO_4$. The crude product was purified by gradient column chromatography (EtOAc/*n*-heptane, 1:4 to 1:2) to obtain the product as a white solid (3.8 g, 99%). R_F = 0.2 (EtOAc/*n*-heptane, 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.55 (dd, *J* = 7.0, 1.8 Hz, 1H), 7.44 (dd, *J* = 7.3, 1.4 Hz, 1H), 7.37-7.29 (m, 3H), 7.15 (ddt, *J* = 7.4, 1.6, 0.8 Hz, 1H), 6.74-6.70 (m, 2H), 4.89 (s, 2H), 4.43 (br s, 2H), 2.00 (br s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 148.2, 141.8, 132.1, 132.0, 130.0, 128.5, 127.8 (2C), 122.1, 117.8, 114.4, 107.6, 92.1, 91.1, 64.4. FT-IR ν_{max} film: 3412, 2993, 1735, 1584, 1523, 1446, 1230, 1148, 763 (cm^{-1}). HRMS (ESI+) *m/z* calcd for $C_{15}H_{14}NO$ $[M+H]^+$ 224.1075, found 224.1083.



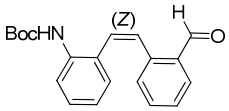
***tert*-Butyl (2-{[2-(hydroxymethyl)phenyl]ethynyl}phenyl)carbamate (2)**

Compound **1** (1.12 g, 5 mmol) was dissolved in THF (5 mL) and Boc_2O (1.09 g, 5 mmol) was added. The reaction was heated to 70 ºC in a sealed tube and stirred for 2 days. The mixture was diluted with CH_2Cl_2 (50 mL), washed with H_2O (60 mL) and the H_2O layer was extracted with CH_2Cl_2 (40 mL). The organic layers were combined and washed with H_2O (2 × 60 mL) and brine (60 mL) and then dried over $MgSO_4$. The crude product was purified by column chromatography (EtOAc/*n*-heptane, 1:4). The product was obtained as a white solid (1.34 g, 83%). R_F = 0.3 (EtOAc/*n*-heptane, 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, *J* = 7.4 Hz, 1H), 7.88 (br s, 1H), 7.58 (dd, *J* = 7.3, 1.7 Hz, 1H), 7.48-7.44 (m, 2H), 7.39-7.31 (m, 3H), 7.01 (dt, *J* = 7.6, 1.1 Hz, 1H), 4.92 (d, *J* = 4.8 Hz, 2H), 2.37 (br s, 1H), 1.57 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 152.6, 141.5, 140.0, 132.2, 131.4, 129.8, 128.8, 128.2, 127.9, 122.2, 121.9, 118.0, 111.4, 93.8, 89.4, 81.1, 64.3, 28.3 (3C). FT-IR ν_{max} film (cm^{-1}): 3403, 2697, 1731, 1584, 1506, 1446, 1152, 750, 603. HRMS (ESI+) *m/z* calcd for $C_{20}H_{21}NNaO_3$ $[M+Na]^+$ 346.1419, found 346.1406.



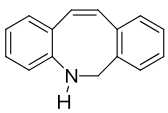
***tert*-Butyl (2-{(*Z*)-2-[2-(hydroxymethyl)phenyl]ethenyl}phenyl)carbamate (3)**

Compound **2** (990 mg, 3.1 mmol) was dissolved in MeOH (25 mL) followed by the addition of Pd/$BaSO_4$ (10%, 43 mg) and quinoline (43 µL, 0.31 mmol). The mixture was placed under an H_2-atmosphere for 1.5 hour. After completion of the reaction the mixture was filtered over Celite and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (EtOAc/*n*-heptane, 1:4) yielding compound **3** as a yellow solid (940 mg, 95%). R_F = 0.2 (EtOAc/*n*-heptane, 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.31 (d, *J* = 7.6 Hz, 1H), 7.26 (s, 1H), 7.21-7.16 (m, 3H), 7.04-6.94 (m, 4H), 6.70 (br s, 1H), 6.66 (d, *J* = 12.0 Hz, 1H), 4.75 (d, *J* = 6.4 Hz, 2H), 1.41 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 150.4, 138.4, 136.1, 135.6, 134.9, 131.4, 129.5 (2C), 129.0, 128.6, 128.0, 128.0 (2C), 126.2, 122.8, 80.8, 64.1, 28.2 (3C). FT-IR ν_{max} film (cm^{-1}): 3429, 2924, 1735, 1528, 1148, 603. HRMS (ESI+) *m/z* calcd for $C_{20}H_{23}NNaO_3$ $[M+Na]^+$ 348.1576, found 348.1557.



***tert*-Butyl {2-[(*Z*)-2-(2-formylphenyl)ethenyl]phenyl}carbamate (4)**

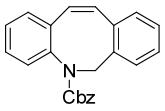
Compound **3** (940 mg, 2.9 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and placed under an Ar-atmosphere in a flame-dried flask. Subsequently, Dess-Martin periodinane (1.51 g, 3.5 mmol) and $NaHCO_3$ (0.80 g, 9.5 mmol) were added and the mixture was stirred for 40 minutes. The reaction was quenched with sat. aq. Na_2SO_3 (5 mL). The mixture was diluted with CH_2Cl_2 (20 mL), washed with sat. aq. $NaHCO_3$ (2 × 25 mL), H_2O (25 mL) and subsequently with brine (25 mL). Next, the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/*n*-heptane, 1:6) to obtain compound **4** as a yellow solid (847 mg, 90%). R_F = 0.35 (EtOAc/*n*-heptane, 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.23 (s, 1H), 7.88 (d, *J* = 8.3 Hz, 1H), 7.81 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.37 (dt, *J* = 7.5, 1.4 Hz, 1H), 7.32 (dt, *J* = 7.7, 1.7 Hz, 1H), 7.28 (d, *J* = 11.8 Hz, 1H), 7.18 (ddd, *J* = 8.3, 7.7, 1.7 Hz, 1H), 7.08 (d, *J* = 7.5 Hz, 1H), 6.99 (dd, *J* = 7.7, 1.6 Hz, 1H), 6.88 (dt, *J* = 7.5, 1.2 Hz, 1H), 6.78 (d, *J* = 11.9 Hz, 1H), 6.49 (br s, 1H), 1.44 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 192.1, 152.8, 152.4*, 138.6, 135.7*, 135.5, 134.8, 133.5, 133.4*, 131.7*, 131.1*, 130.8, 130.3, 129.6, 129.3*, 128.4*, 128.2, 128.0, 127.2, 126.8, 126.2*, 122.8, 120.0*, 119.8, 102.1, 80.3, 28.3 (3C). FT-IR ν_{max} film (cm^{-1}): 3382, 2958, 1731, 1684, 1515, 1450, 1243, 1139, 1057, 737. HRMS (ESI+) *m/z* calcd for $C_{20}H_{21}NNaO_3$ $[M+Na]^+$ 346.1419, found 346.1406.



5,6-Dihydrodibenzo[*b*,*f*]azocine (5)

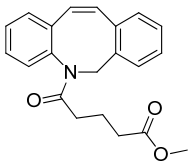
2 M HCl in EtOAc (25 mL) was added to compound **4** (847 mg, 2.6 mmol) and the mixture was stirred for 1 hour. Next, $NaBH_4$ (295 mg, 7.6 mmol) and H_2O (47 µL, 2.6 mmol) were added. After stirring overnight an additional portion of $NaBH_4$ (295 mg, 7.6 mmol) was added and after stirring for an additional hour the reaction

was quenched with H_2O (20 mL). The H_2O layer was extracted with EtOAc (2 × 20 mL). The organic layers were combined and washed with 0.2 M NaOH (2 × 40 mL), H_2O (2 × 40 mL) and brine (50 mL) and subsequently dried over $MgSO_4$. The solvents were evaporated under reduced pressure to afford compound **5** as a yellow solid (540 mg, 100%). R_F = 0.45 (EtOAc/*n*-heptane, 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.27-7.23 (m, 1H), 7.20-7.15 (m, 3H), 6.96 (dd, *J* = 7.8, 1.6 Hz, 1H), 6.88 (ddd, *J* = 8.0, 7.2, 1.6 Hz, 1H), 6.60 (ddd, *J* = 7.7, 7.2, 1.2 Hz, 1H), 6.54 (d, *J* = 13.0 Hz, 1H), 6.48-6.45 (m, 1H), 6.35 (d, *J* = 13.1 Hz, 1H), 4.58 (s, 2H), 4.29 (br s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 147.0, 139.2, 138.1, 134.7, 132.7, 130.1, 128.8, 127.9, 127.6, 127.4, 127.3, 121.8, 117.9, 117.7, 49.6. FT-IR ν_{max} film (cm^{-1}): 3391, 3049, 2932, 1502, 746, 620. HRMS (ESI+) *m/z* calcd for $C_{15}H_{14}N$ $[M+H]^+$ 208.1126, found 208.1107.



Benzyl dibenzo[*b*,*f*]azocine-5(6*H*)-carboxylate (6)

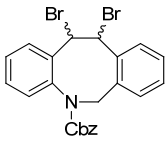
Compound **6** (100 mg, 0.48 mmol) was dissolved in CH_2Cl_2 (10 mL) after which Cbz-Cl (117.3 µL, 0.82 mmol) was added, followed by a solution of aq. Na_2CO_3 (2 mL, 0.29M). After 3 hours the reaction was quenched with H_2O (10 mL) and the mixture was extracted with CH_2Cl_2 (20 mL). The organic layer was washed with H_2O (3 × 20 mL) and brine (20 mL). Next, the organic layer was dried over $MgSO_4$ and subsequently concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/*n*-heptane, 1:9) to obtain compound **6** as a white solid (140 mg, 86%). R_F = 0.35 (EtOAc/*n*-heptane, 1:4). ^{1}H-NMR (400 MHz, 273 K, $CDCl_3$) δ: 7.37-7.27 (m, 5H), 7.25-7.18 (m, 7.45H), 7.11-7.06 (m, 0.55H), 6.71 (d, *J* = 13.5 Hz, 0.72H), 6.66 (d, *J* = 14.0 Hz, 0.28H), 6.58 (d, *J* = 13.9 Hz, 0.27H), 6.51 (d, *J* = 13.5 Hz, 0.72H), 5.10 (s, 0.68H), 5.07 (br s, 1.27H), 4.73 (br s, 2H). A clear ^{13}C-NMR spectrum could not be obtained as a result of a strong rotamer-effect. FT-IR ν_{max} film (cm^{-1}): 2967, 1692, 1489, 1403, 1299, 1242, 1035, 612. HRMS (ESI+) *m/z* calcd for $C_{23}H_{20}NO_2$ $[M+H]^+$ 342.1494, found 342.1477.



Methyl 5-dibenzo[*b*,*f*]azocin-5(6*H*)-yl-5-oxopentanoate (7)

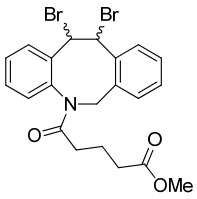
Compound **5** (500 mg, 2.4 mmol) was dissolved in dry CH_2Cl_2 (40 mL) and Et_3N (0.67 mL, 4.8 mmol) was added. The mixture was cooled to 0 °C whereupon methyl succinylchloride (0.51 mL, 3.6 mmol) was added. The reaction was stirred for 1.5 h, after which it was quenched with H_2O (10 mL). The mixture was diluted with CH_2Cl_2 (20 mL) and washed with 2 M NaOH (2 × 40 mL), 2 M HCl (2 × 40 mL), H_2O (2 × 40 mL) and brine (40 mL). Next, the organic layer was dried over $MgSO_4$ and subsequently concentrated *in vacuo*. The crude product was purified using column chromatography (EtOAc/*n*-heptane, 1:2) to obtain compound **7** as a yellow oil (760 mg, 94 %). R_F = 0.2 (EtOAc/*n*-heptane, 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.29-7.26 (m, 3H), 7.25-7.23 (m, 1H), 7.20-7.12 (m, 4H), 6.78 (d, *J* = 13.1 Hz, 1H), 6.58 (d, *J* = 13.1 Hz, 1H), 5.50 (d, *J* = 15.0 Hz, 1H), 4.22 (d, *J* = 15.0 Hz, 1H), 3.58 (s, 3H), 2.24-2.05 (m, 3H), 1.93-1.77 (m, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$), δ: 173.5, 171.7, 140.9, 136.3, 135.7, 134.7, 132.6, 132.0, 131.1, 130.3, 129.4, 128.1, 127.9, 127.3, 127.1, 126.9,

54.5, 51.3, 33.4, 33.0, 20.4. FT-IR ν_{max} film (cm^{-1}): 3308, 2941, 1740, 1658, 1446, 1234, 599. HRMS (ESI+) *m/z* calcd for $C_{21}H_{22}NO_3$ $[M+H]^+$ 336.1600, found 336.1597.



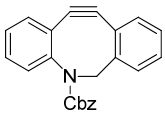
Benzyl 11,12-dibromo-11,12-dihydrodibenzo[*b*,*f*]azocine-5(6*H*)-carboxylate (8)

Compound **6** (140 mg, 0.41 mmol) was dissolved in dry CH_2Cl_2 (10 mL). The reaction mixture was cooled to 0 °C and a solution of Br_2 (22 µL, 0.41 mmol) in dry CH_2Cl_2 (3 mL) was added drop-wise. The reaction was stirred for 2 hours at 0 °C and subsequently quenched with sat. aq. Na_2SO_3 (15 mL). The product was extracted with CH_2Cl_2 (2 × 20 mL), the organic layers were combined and washed once again with sat. aq. Na_2SO_3 (2 × 20 mL), H_2O (30 mL) and brine (30 mL). The combined organic layers were subsequently dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by gradient column chromatography (EtOAc/*n*-heptane, 1:6 to 1:1) to obtain compound **8** as a brown solid (136 mg, 67%). R_F = 0.3 (EtOAc/*n*-heptane, 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, *J* = 7.7 Hz, 1H), 7.57-7.29 (m, 1H), 7.24-7.16 (m, 5H), 7.1-6.9 (m, 5H) 6.87 (d, *J* = 7.3 Hz, 1H), 6.04 (d, *J* = 9.7 Hz, 0.82H), 5.88 (d, *J* = 9.7 Hz, 0.17H), 5.65 (d, *J* = 14.4 Hz, 0.8H), 5.33-5.28 (m, 1.37H), 5.14 (d, *J* = 9.7 Hz, 0.82H), 5.08-5.03 (m, 1.22H), 4.31 (d, *J* = 14.4 Hz, 0.83H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 155.1, 138.3, 137.3, 136.4, 136.2, 132.7, 130.4, 130.1, 130.0, 129.7, 129.5, 129.4, 129.1, 128.8, 128.7, 128.2, 128.0, 127.7, 127.6, 67.4, 60.9*, 59.8, 56.5, 54.9*, 53.7, 52.9*. FT-IR ν_{max} film (cm^{-1}): 3075, 2915, 1688, 1502, 1394, 1342, 1282, 1203, 728. HRMS (ESI+) *m/z* calcd for $C_{23}H_{20}Br_2NO_2$ $[M+H]^+$ 499.9861, found 499.9848.



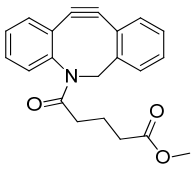
Methyl 5-(11,12-dibromo-11,12-dihydrodibenzo[*b*,*f*]azocin-5(6*H*)-yl)-5-oxopentanoate (9)

Compound **7** (500 mg, 1.5 mmol) was dissolved in dry CH_2Cl_2 (20 mL). The solution was then cooled to 0 ºC and a solution of Br_2 (77 µL, 1.5 mmol) in dry CH_2Cl_2 (1 mL) was added drop-wise. After stirring for 45 minutes at 0 ºC the reaction was quenched with sat. aq. Na_2SO_3 (5 mL). The mixture was diluted with CH_2Cl_2 and the organic layer was washed with sat. aq. Na_2SO_3 (3 × 10 mL), H_2O (2 × 15 mL) and brine (15 mL) and subsequently dried over $MgSO_4$. The crude product was purified by column chromatography (EtOAc/*n*-heptane, 1:3 to 1:2) to obtain compound **9** as a white solid (600 mg, 81%). A mixture of two stereoisomers was obtained in a ratio of 9:1. The major isomer (R_F = 0.25, EtOAc/*n*-heptane, 1:2) was separately isolated, the minor isomer (R_F = 0.3, EtOAc/*n*-heptane, 1:2). Analytical data of the major isomer are given. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.72 (d, *J* = 7.8 Hz, 1H), 7.25-7.12 (m, 3H), 7.08-7.02 (m, 2H), 6.98 (d, *J* = 7.6 Hz, 1H), 6.89 (d, *J* = 7.5 Hz, 1H), 5.92 (d, *J* = 9.9 Hz, 1H), 5.82 (d, *J* = 14.8 Hz, 1H), 5.15 (d, *J* = 9.9 Hz, 1H), 4.17 (d, *J* = 14.8 Hz, 1H), 3.62 (s, 3H), 2.44-2.81 (m, 3H), 2.23-2.15 (m, 1H), 2.09-1.94 (m, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 173.6, 172.7, 138.2, 137.1, 137.1, 132.9, 130.7 (2C), 130.5, 129.6, 129.4, 128.9, 128.9, 128.6, 60.1, 55.6, 52.5, 51.5, 34.8, 33.3, 20.4. FT-IR ν_{max} film (cm^{-1}): 2919, 1744, 1636, 1497, 1385, 1191, 655. HRMS (ESI+) *m/z* calcd for $C_{21}H_{22}Br_2N$ $[M+H]^+$ 493.9966, found 493.9960.



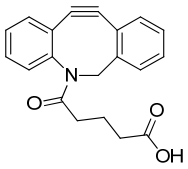
Benzyl 11,12-didehydrodibenzo[*b*,*f*]azocine-5(6*H*)-carboxylate (10)

Compound **8** (110 mg, 0.22 mmol) was placed in a flame-dried flask and dissolved in dry THF (5 mL). Next, a solution of KO^{t}Bu (77 mg, 0.66 mmol) in ^{t}BuOH (2 mL) was added drop-wise. The mixture was stirred overnight, whereupon an extra portion of KO^{t}Bu (77 mg, 0.66 mmol) was added. The reaction was stirred for an additional hour after which full conversion was observed. The reaction was cooled to 0 °C and subsequently quenched with H_2O (5 mL). The mixture was neutralised with 2 M HCl and the product was extracted with CH_2Cl_2 (2 × 20 mL). The organic layers were combined and subsequently washed with H_2O (3 × 30 mL) and brine (30 mL). After drying over $MgSO_4$ and concentrating, the crude product was purified by column chromatography (EtOAc/*n*-heptane, 1:6) to obtain **10** as a white solid (65 mg, 87%). R_F = 0.5 (EtOAc/*n*-heptane, 1:4). ^{1}H-NMR (400 MHz, 243 K, $CDCl_3$) δ: 7.69 (dd, *J* = 7.1, 1.6 Hz, 0.70H), 7.53 (dd, *J* = 8.1, 0.8 Hz, 0.36H), 7.44-7.30 (m, 9H), 7.26-7.12 (m, 3H), 5.29 (d, *J* = 12.6 Hz, 0.67H), 5.10 (d, *J* = 11.9 Hz, 0.32H), 5.00 (d, *J* = 14.2 Hz, 0.62H), 4.93 (d, *J* = 11.8 Hz, 0.29H), 4.88 (d, *J* = 14.4 Hz, 0.29H), 4.78 (d, *J* = 12.6 Hz, 0.64H), 3.93 (d, *J* = 14.5 Hz, 0.28H), 3.80 (d, *J* = 14.0 Hz, 0.67H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 155.6, 151.5, 147.2, 136.3, 132.0, 129.2, 128.5, 128.3, 128.1, 127.8, 127.7, 127.5, 126.9, 126.6, 125.5, 124.2, 121.2, 113.3, 109.1, 67.7*, 67.2, 55.8, 55.4*. FT-IR ν_{max} film (cm^{-1}): 3002, 2894, 1697, 1290, 1117, 840, 750. HRMS (ESI+) *m/z* calcd for $C_{23}H_{17}NNaO_2$ $[M+Na]^+$ 362.1157, found 362.1151.



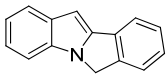
Methyl 5-(11,12-didehydrodibenzo[b,f]azocin-5(6H)-yl)-5-oxopentanoate (11)

Compound **9** (300 mg, 0.6 mmol) was placed in a flame-dried flask dissolved in dry THF (20 mL) and the solution was cooled to –40 ºC. Hereupon, 1 M KO^{t}Bu in THF (1.2 mL, 1.2 mmol) was added dropwise and the reaction was stirred at –40 ºC. The reaction was stirred for 1.5 hour at –40 ºC whereupon another portion of 1 M KO^{t}Bu in THF (0.3 mL, 0.3 mmol) was added dropwise. After an additional 30 minutes the reaction was completed and the mixture was poured into a large amount of H_2O (30 mL). Next, the product was extracted with CH_2Cl_2 (2 × 40 mL), the organic layers were combined and washed with H_2O (3 × 50 mL) and brine (50 mL) and dried over $MgSO_4$. The product was purified by gradient column chromatography (EtOAc/*n*-heptane, 1:3 to 1:2) to obtain **11** as a yellow oil (170 mg, 84%) R_F = 0.2 (EtOAc/*n*-heptane, 1:2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.69 (d, *J* = 7.4 Hz, 1H), 7.42-7.25 (m, 7H), 5.15 (d, *J* = 13.8 Hz, 1H), 3.66 (d, *J* = 13.8 Hz, 1H), 3.55 (s, 3H), 2.28 (ddd, *J* = 15.5, 7.5, 6.6 Hz, 1H), 2.10 (m, 2H), 1.91 (m, 2H), 1.74 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 173.5, 172.5, 151.7, 148.0, 132.3, 128.9, 128.3, 128.3, 128.0, 127.9, 127.7, 127.3, 127.1, 125.5, 123.0, 122.6, 115.1, 107.7,55.3, 51.4, 33.7, 32.8, 20.6. FT-IR ν_{max} film (cm^{-1}): 3274, 2924, 1727, 1666, 1390, 1243, 1161, 754. HRMS (ESI+) *m/z* calcd for $C_{21}H_{20}NNaO_3$ $[M+Na]^+$ 356.1263, found 356.1265.



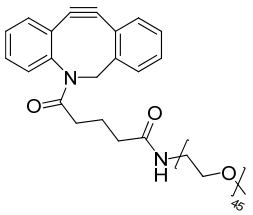
5-(11,12-Didehydrodibenzo[*b*,*f*]azocin-5(6*H*)-yl)-5-oxopentanoic acid (12)

Compound **11** (70 mg, 0.2 mmol) was dissolved in THF (3 mL) and a solution of LiOH (19 mg, 0.4 mmol) in H_2O (1 mL) was added dropwise. The reaction was stirred for 3 hours whereupon the reaction mixture was diluted with H_2O (10 mL) and basified to a pH of 14 with 2 M NaOH. The H_2O-layer was washed with CH_2Cl_2 (3 × 20 mL) and next acidified to a pH of 2 with 2 M HCl. The H_2O-layer was extracted with CH_2Cl_2 (3 × 20 mL), the organic layers were combined, washed with brine (40 mL) and dried over $MgSO_4$. The solvents were removed under reduced pressure to obtain compound **12** (62 mg, 92%) R_F = 0.4 (CH_2Cl_2/MeOH, 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 7.7 Hz, 1H), 7.42-7.27 (m, 5H) 7.21-7.00 (m, 2H), 5.16 (d, *J* = 13.8 Hz, 1H), 3.68 (d, *J* = 13.8 Hz, 1H), 2.42-2.27 (m, 1H), 2.15-2.10 (m, 2H), 2.00-1.95 (m, 1H), 1.76-1.69 (m, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 177.8, 172.8, 151.5, 147.9, 132.3, 128.9, 128.4, 128.3, 128.2, 127.8, 127.1, 125.5, 122.6, 115.1, 107.7, 97.5, 55.4, 33.5, 32.7, 20.3. FT-IR ν_{max} (cm^{-1}): 2933, 1740, 1628, 1398, 1187, 785, 612. HRMS (ESI+) *m/z* calcd for $C_{20}H_{17}NNaO_3$ $[M+Na]^+$ 342.1106, found 342.1105.



6*H*-isoindolo[2,1-*a*]indole[1] (13)

Compound **10** (10 mg, 0.03 mmol) was dissolved in HBr in AcOH (33 wt%, 1 mL). The mixture was stirred for 5 minutes whereupon the solvents were removed under reduced pressure. The crude product was purified by column chromatography (EtOAc/*n*-heptane, 1:6) to obtain (**12**) as a yellow solid (6 mg, 98%). R_F = 0.5 (EtOAc/*n*-heptane, 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.71 (d, *J* = 7.6 Hz, 1H), 7.66 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.36 (dd, *J* = 8.1, 0.8 Hz, 1H), 7.30 (dt, *J* = 7.5, 1.0 Hz, 1H), 7.19 (dt, *J* = 8.0, 1.1 Hz, 1H), 7.10 (dt, *J* = 7.9, 1.1 Hz, 1H), 6.63 (s, 1H), 5.07 (s, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 143.9, 141.7, 133.9, 133.0, 132.8, 128.1, 127.0, 123.5, 121.7, 121.5, 120.9, 119.6, 109.2, 91.2, 48.4. HRMS (ESI+) *m/z* calcd for $C_{15}H_{11}N$ $[M+H]^+$ 206.0970, found 209.0963.

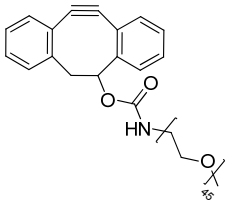


5-(11,12-Didehydrodibenzo[*b*,*f*]azocin-5(6*H*)-yl)-*N*-mPEG$_{2000}$-1-yl-5-oxopentanamide (15)

Compound **12** (10 mg, 0.031 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and cooled to 0 ºC. Subsequently, H_2N-PEG_{2000}-OMe (69 mg, 0.034 mmol), EDC·HCl (13 mg, 0.068 mmol) and DMAP (15 mg, 0.12 mmol) were added. The reaction was stirred for 30 minutes at 0 ºC, slowly warmed to room temperature and stirred overnight. Since the reaction did not go to completion another 0.2 equivalents of H_2N-PEG_{2000}-OMe (12 mg, 0.006 mmol) were added and the reaction was stirred for an additional day. The reaction was diluted with CH_2Cl_2 (15 mL) and washed with 2 M HCl (3 × 20 mL), H_2O (20 mL) and brine (20 mL) and dried over $MgSO_4$.

The crude product was purified by column chromatography (CH_2Cl_2/MeOH, 9:1) to obtain a mixture of **15** and H_2N-PEG_{2000}-OMe in a ratio of 9:1, which was determined by comparing the methoxy-signal with the aromatic signals in the ^{1}H-NMR spectrum. ^{1}H-NMR ($CDCl_3$, 400 MHz), δ: 7.70 (d, J = 6.4 Hz, 1H), 7.62-7.42 (m, 5H), 7.21-7.03 (m, 2H), 5.89 (br s, 1H), 5.16 (d, J = 13.8 Hz, 1H), 3.64 (s, 196 H), 3.60-3.54 (m, 4H), 3.49-3.46 (m, 4H), 3.38 (s, 3.3H), 2.31-2.24 (m, 1H), 2.04-1.83 (m, 3H), 1.76-1.70 (m, 2H).

The ESI-ToF spectrum showed a clear shift of the molecular weight distribution. In the double charge distribution a shift in molecular mass between starting material and product of 150.557 was observed, where a shift of 150.555 was expected.



11,12-Didehydro-5,6-dihydrodibenzo[*a*,*e*][8]annulen-5-yl mPEG$_{2000}$-1-ylcarbamate (16)

Carbonic acid 7,8-didehydro-1,2:5,6-dibenzocyclooctene-3-yl ester 4-nitrophenyl ester (4.9 mg, 0.0127 mmol) was dissolved in CH_2Cl_2 (300 µL) and Et_3N (5.2 µL, 0.0381 mmol) and H_2N-PEG_{2000}-OMe (33 mg, 0.0165 mmol) were added. The reaction was stirred overnight, diluted with CH_2Cl_2 (5 mL) and then quenched with H_2O (1 mL). The organic layer was washed with 1 M HCl (1 mL), H_2O (1 mL) and brine (1 mL) and dried over $MgSO_4$. The organic layer was concentrated *in vacuo* and the crude product was purified by column chromatography (CH_2Cl_2/MeOH, 9:1 → 5:1) to obtain a mixture of **15** and H_2N-PEG_{2000}-OMe in a ratio of 3:1. The ratio between starting material and product was determined by comparing the integral of the aromatic signals with the integral of the methoxy-peak in the ^{1}H-NMR spectrum. ^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.53-7.51 (m, 1H), 7.38-7.27 (m, 7H), 5.62 (br s, 1H), 5.50 (br s, 1H), 3.69 (br s, 256H), 3.48-3.45 (m, 2H), 3.42-3.39 (m, 2H), 3.38 (s, 4H), 3.21-3.16 (m, 1H), 2.96-2.88 (m, 1H).

The ESI-ToF spectrum showed a clear shift in the molecular weight distribution. In the double charge distribution a shift in molecular mass between starting material and product of 123.0643 was observed, where a shift of 123.0337 was expected.

Kinetic Experiments:

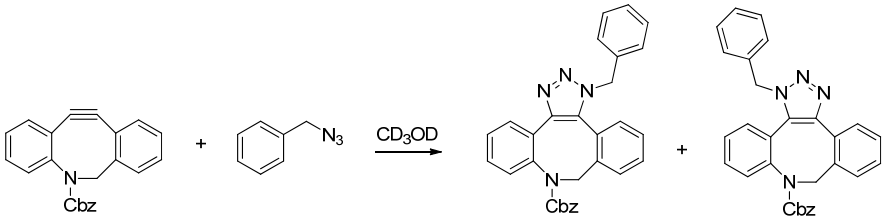
General procedure for kinetic experiments:

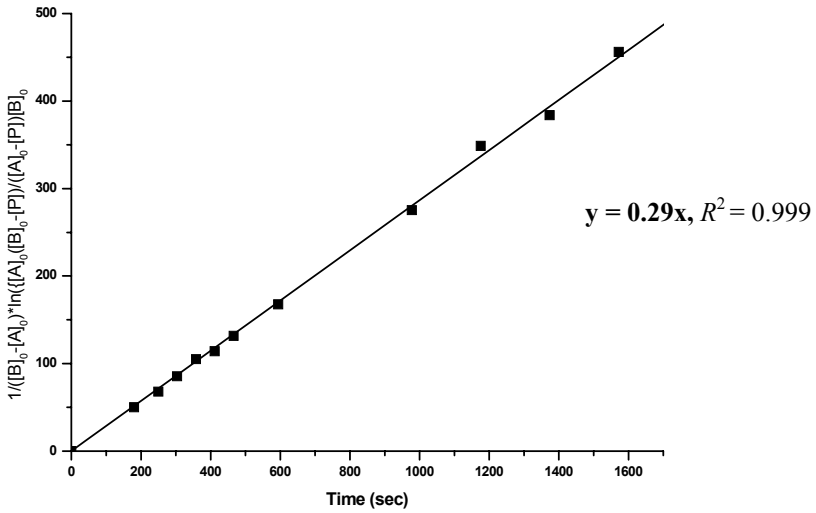
The alkyne (9.0 μmol) was dissolved in CD_3OD (250 μL). Next, 250 μL of a BnN_3 solution (36 mM in CD_3OD) was added to reach a final volume of 500 μL. ^{1}H-NMR spectra were taken during 30-45 minutes at preset time-intervals. The exact ratio between benzyl azide and alkyne was determined by comparison of the integrals of the aromatic signals and the benzylic protons of the alkyne. For the cycloaddition of **10** with benzyl azide, the kinetics were determined by comparing the decreasing signal of the benzylic protons of benzyl azide and the increasing signal of the same benzylic protons of the product. For the cycloaddition of **12** with benzyl azide, the decrease of the signal of one of the benzylic protons of **12** was used for determination of the kinetics, using the aromatic protons as standard. Both reactions gave two regioisomers, which in the kinetic experiments were integrated as a single product. From the conversion plots thus obtained the second order rate plots were calculated, by fitting the data to equation (1).

$$kt = \frac{1}{[B]_0 - [A]_0} \times \ln \frac{[A]_0([B]_0 - [P])}{([A]_0 - [P])[B]_0} \qquad (1)$$

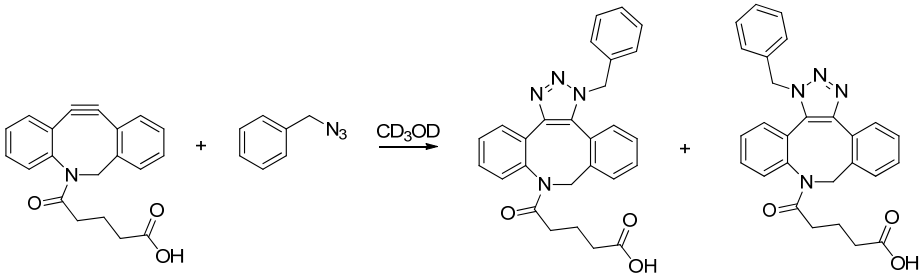
Herein, k = 2nd order rate constant ($M^{-1}s^{-1}$), t = reaction time (s), $[A]_0$ = the initial concentration of substrate A (mmol/mL), $[B]_0$ = the initial concentration of substrate B (mmol/mL) and $[P]$ = the concentration of the product (mmol/mL). For compound **10** this fit is shown in Graph S1 and for compound **12** the fit is shown in Graph S2. Both experiments were performed in triplicate giving identical plots and results.

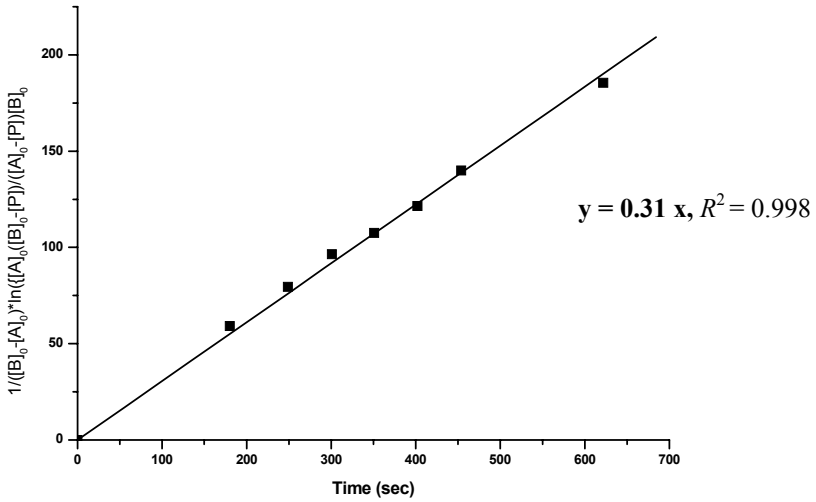
The formation of the presumed products was confirmed by mass spectrometry and NMR-spectroscopy.





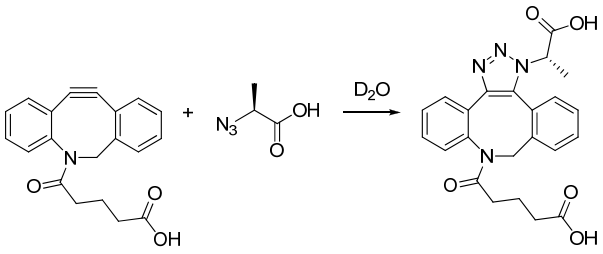
Graph S1. Logarithmic plot of the reaction of compound **10** with benzyl azide in CD_3OD. The experiment was performed in triplicate, with identical results (only one experiment shown).

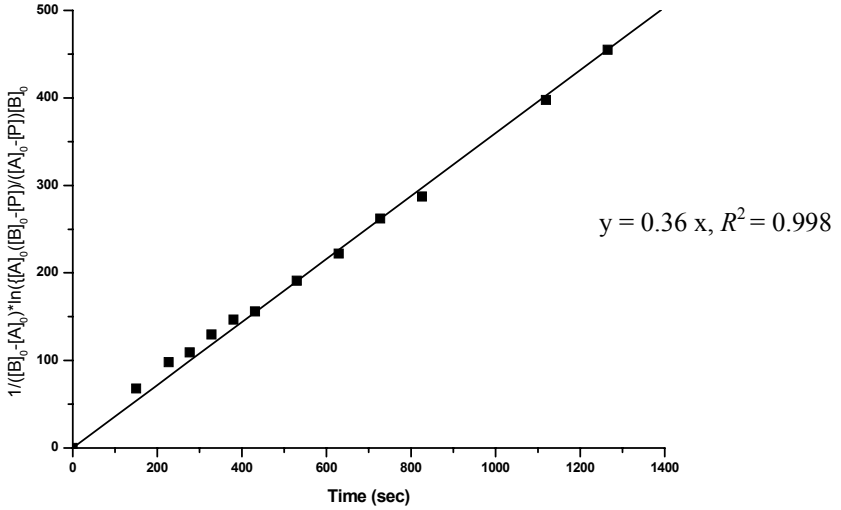




Graph S2. Logarithmic plot of the reaction of compound **12** with benzyl azide in CD_3OD. The experiment was performed in triplicate with identical results (only one experiment shown).

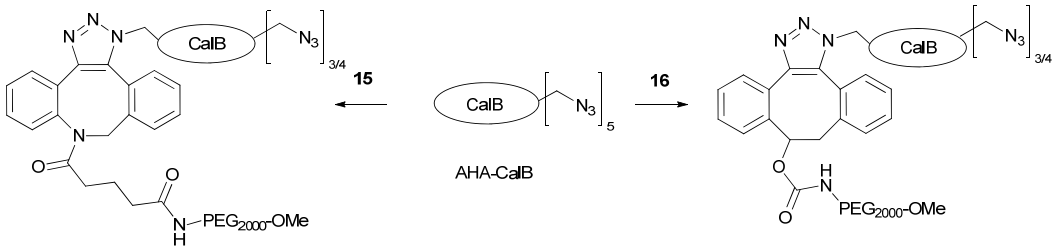
Procedure for the kinetic experiment in D_2O:
Compound **12** (2.87 mg, 0.009 mmol) was dissolved in 210 µL D_2O by the addition of 40 µL 2 M NaOH. To this a solution of (S)-2-azidopropanoic acid (0.87 mg, 0.0076 mmol) in D_2O (250 µL) was added. ^{1}H-NMR spectra were taken during 30 minutes at preset time-intervals. Graph S shows the kinetic plot of this reaction, which was made *vide supra*.





Graph S3. Logarithmic plot of the reaction of compound **12** with 2-azidopropanoic acid in D_2O under basic conditions.

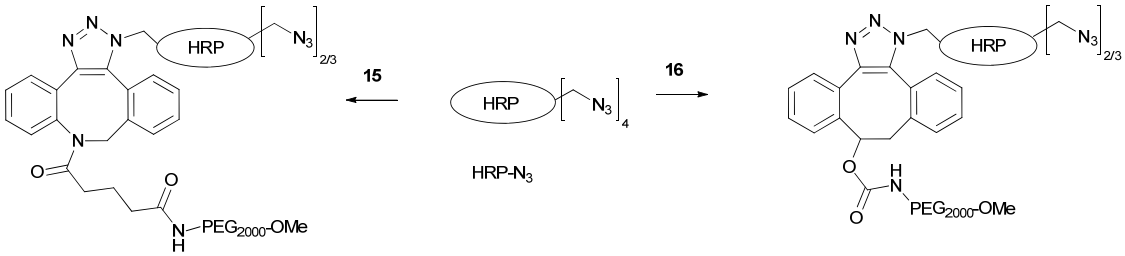
Enzyme functionalization



For the functionalization of AHA-CalB, reactions were performed with 8.27 µL of an AHA-CalB solution in PBS (1.26 mg/mL, 10.4 µg) which was further diluted with PBS to reach a final concentration of 1 µg/µL after addition of **15** or **16**. To these solutions, various amounts of **15** and **16** (1, 2 and 5 equivalents) were added. To this end, 4 different stock-solutions were prepared, namely, 0.55 mg/mL and 2.2 mg/mL of compound **15** and of compound **16**, respectively. The PEG-contamination was taken into account in the preparation of the stock-solutions; the weighed amounts of sample were corrected in order to obtain the right concentration of **15** and **16**. The amounts added of each of these stock-solutions are shown in Table S1. All reactions were shaken for 3 hours, after which the reactions were quenched by the addition of an excess benzyl azide (± 10 equiv) and subsequent shaking for 15 minutes. The conjugation was analysed using a 12% (w/v) polyacrylamide gel followed by Coomassie staining (see Figure 2).

Table S1. Amounts of compound **15** and **16** used in the ligation with AHA-CalB.

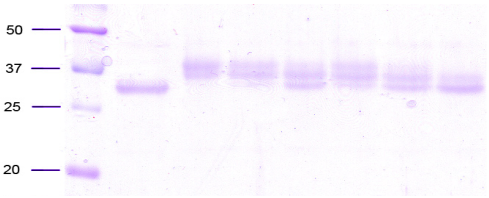
Entry	Substrate	Stock 0.55 mg/mL	Stock 2.2 mg/mL
1	**15** (1 equiv)	1.1 µL	x
2	**15** (2 equiv)	2.1 µL	x
3	**15** (5 equiv)	x	1.3 µL
4	**16** (1 equiv)	1.1 µL	x
5	**16** (2 equiv)	2.1 µL	x
6	**16** (5equiv)	x	1.3 µL



For the functionalization of HRP-N_3, reactions were performed with 6 µL of an HRP-N_3 solution in H_2O (2.5 mg/mL, 15 µg) which was further diluted with MilliQ water to reach a concentration of 1 µg/µL after addition of **15** or **16**. To the solutions, various amounts of **15** and **16** (1, 2 and 5 equivalents) were added. To this end, 4 different stock-solutions were prepared, namely, 0.55 mg/mL and 2.2 mg/mL of compound **15** and of compound **16,** respectively. The PEG-contamination was taken into account in the preparation of the stock-solutions; the weighed amounts of sample were corrected in order to obtain the right concentration of **15** and **16**. The amounts added of each of these stock-solutions are shown in Table S2. All reactions were shaken for 3 hours, after which the reactions were quenched by the addition of an excess benzylazide (± 10 equiv) and subsequent shaking for 15 minutes. The conjugation was analysed using a 12% (w/v) polyacrylamide gel followed by Coomassie staining (see Figure S2).

Table S2. Amounts of compound **15** and **16** used in the ligation with HRP-N_3.

Entry	Substrate	Stock 0.55 mg/mL	Stock 2.2 mg/mL
1	**15** (1 equiv)	1.6 µL	x
2	**15** (2 equiv)	x	0.8 µL
3	**15** (5 equiv)	x	2 µL
4	**16** (1 equiv)	1.6 µL	x
5	**16** (2 equiv)	x	0.8 µL
6	**16** (5 equiv)	x	2 µL



Lane	1	2	3	4	5	6	7
15	-	+	+	+	-	-	-
16	-	-	-	-	+	+	+
Equiv	-	5	2	1	5	2	1

Figure S1. The ligation of HRP-N_3 with different equivalents of compound **15** and **16** after 3 hours.

1 L. A. Crawford, N. C. Clemence, H. McNab,R. G. Tyas, *Org. Biomol. Chem.*, 2008, **6**, 2334-2339.