# The Ugi four-component reaction enables expedient synthesis and comparison of photoaffinity probes

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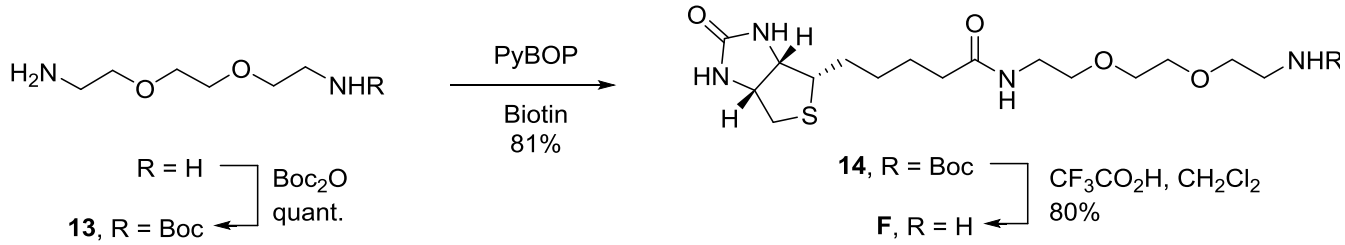
# Experimental procedures

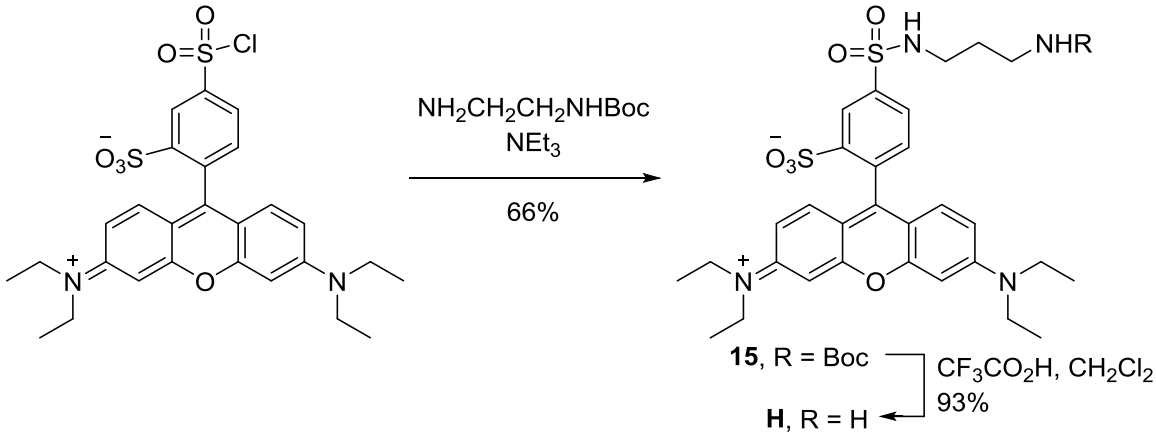
## Materials and methods

Reagents and solvents were obtained, unless otherwise stated, from Sigma Aldrich UK, Alfa Aesar or Acros. Bicyclononyne-succinimidyl ester (BCN-OSu) was from SynAffix B.V., Nijmegen, The Netherlands. Solvents were removed under reduced pressure using a Buchi™ rotary evaporator. Water was purified using an Elix® UV-10 system. Analytical thin layer chromatography (TLC) was carried out using Merck silica gel 60 F254 aluminium supported thin layer chromatography sheets. Visualisation was by absorption of UV light ($\lambda_{max}$ 254 nm) and/or staining by $KMnO_4$ stain. Column chromatography was carried out using a Biotage SP1 automated flash column chromatography platform, eluting with solvents under a positive pressure of compressed air. Melting points were determined using a Leica Galen III hot stage melting point apparatus and microscope. Infrared spectra were obtained as thin films. The spectra were recorded on a Bruker Tensor 27 spectrometer and a representative number of absorption maxima are reported in wavenumbers ($\nu_{max}$). $^{1}$H NMR spectra were recorded on a Bruker DPX400 (400 MHz) using $CDCl_3$ (unless indicated otherwise) as a reference for internal deuterium lock. The chemical shift data for each signal are given as $\delta_H$ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ(TMS) = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); t (triplet); q (quartet); dd (doublet of doublets) or m (multiplet). The number of protons (n) for a given resonance signal is indicated by nH. Coupling constants (*J*) are expressed in Hz and are recorded to the nearest 0.5 Hz. *J*-values for the same couplings are averaged in each spectrum and reported to the nearest 0.5 Hz. $^{13}$C NMR spectra were recorded on a Bruker AV500 (125.8 MHz) spectrometer using the PENDANT or DEPT Q pulse sequences with broadband proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where $\delta_C$ (TMS) = 0.00 ppm. $^{19}$F NMR spectra were recorded on a Bruker AV500 and chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to $CFCl_3$ where $\delta_F$ ($CFCl_3$) = 0.00 ppm, $^{1}$H, $^{13}$C and $^{19}$F spectra were assigned using 2D NMR experiments including COSY and HSQC, and by comparison with related compounds. The presence of conformational isomers (rotamers) was verified by variable temperature $^{1}$H NMR experiments, which demonstrated coalescence of the peaks for the conformational isomers at elevated temperatures. Integrated values for rotamers are approximate. Mass spectra were acquired on an Agilent technologies 6120 quadrupole liquid chromatography mass spectrometer using electrospray ionization, operating in positive or negative mode, from methanolic solutions. *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses. High resolution mass spectra (HRMS) were recorded using Bruker MicroTOF internally calibrated with polyalanine.

## Reaction schemes




**Scheme S1**

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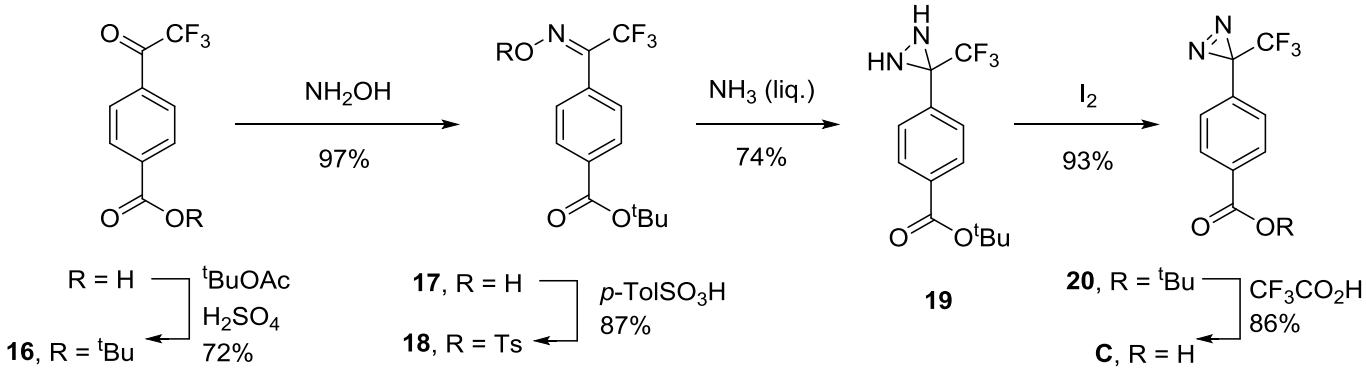


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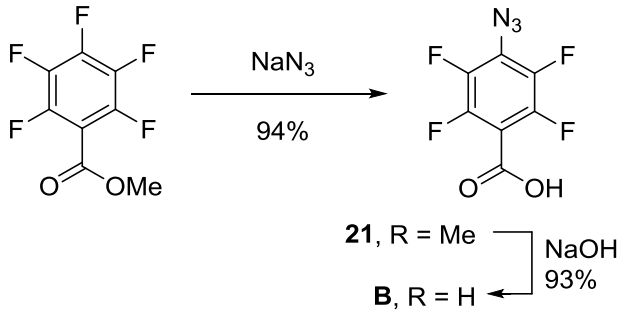
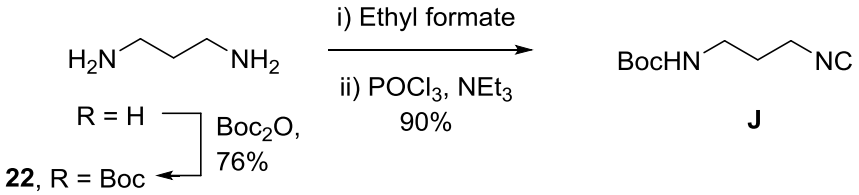
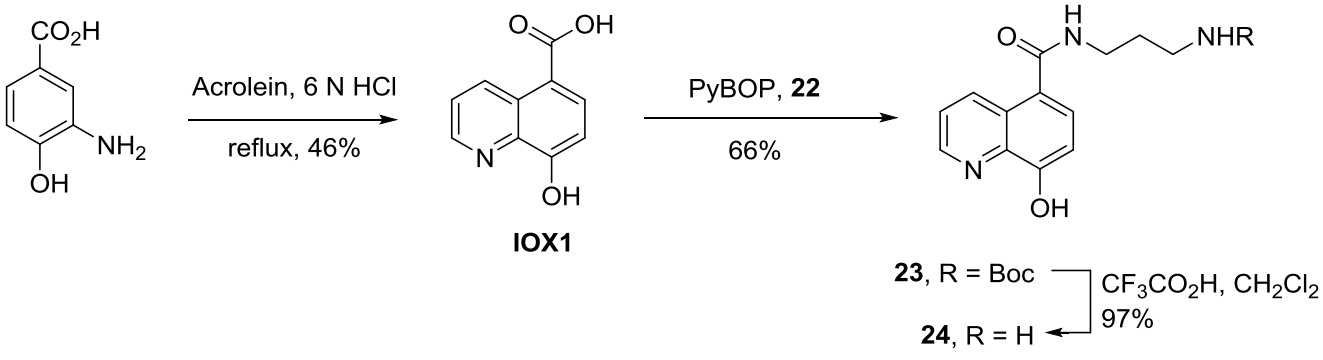



**Scheme S4**




**Scheme S5**




**Scheme S6**

**General procedure A - Ugi reaction**

The isocyanide (1 equiv.) was added to the carboxylic acid (1 equiv.), amine (1 equiv.) and paraformaldehyde (1.5 equiv.) in ethanol (4 mL $mmol^{-1}$) and the resulting mixture was heated in the microwave (20 min, 100 °C). The volatiles were evaporated under reduced pressure and the residue purified by column chromatography (eluent 20-100% $CH_2Cl_2$ / methanol / $NH_3$ (aq) – 90:10:1 in $CH_2Cl_2$).

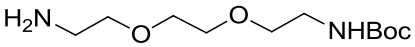
**General procedure B – Ester hydrolysis of Ugi products**

Sodium hydroxide (1.5 equiv.) in water (2 mL $mmol^{-1}$) was added to a solution of the ethyl ester (1 equiv.) in methanol (5 mL $mmol^{-1}$) and stirred for 12 h. Water (50 mL $mmol^{-1}$) was added and washed with $CH_2Cl_2$ (2 x 50 mL $mmol^{-1}$). The aqueous layer was acidified to pH 1 with 1M HCl and extracted with $CHCl_3$ / isopropylalcohol (4:1) (3 x 50 mL $mmol^{-1}$). Combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the desired acid.

**General procedure C - Amide coupling of inhibitor and photoreactive scaffold**

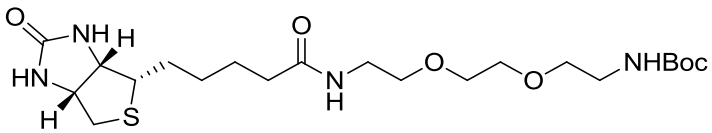
*O*-Benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (1 equiv.) was added to a solution of carboxylic acid (1 equiv.) and triethylamine (2 equiv.) in DMF (50 mL $mmol^{-1}$). The resulting mixture was stirred for 15 min before being added dropwise to a solution of amine (1 equiv.) and triethylamine (1 equiv.) in DMF (50 mL $mmol^{-1}$). After stirring for 12 h the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (eluent 0-15% methanol in $CH_2Cl_2$) to afford the desired product.

*tert*-Butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate,[1] 13




Di-*tert*-butyldicarbonate (1.26 g, 5.78 mmol) in $CHCl_3$ (80 mL) was added dropwise to a solution of 2,2'-(ethylenedioxy)-bis-(ethylamine) (8.00 mL, 54.0 mmol) in $CHCl_3$ (80 mL) at 0 °C. The reaction was allowed to warm to room temperature and stirred overnight. The solvent was evaporated, water (80 mL) added and extracted with $CH_2Cl_2$ (3 x 60 mL). Combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford **13** as a colourless oil (1.42 g, 99%); $\delta_H$ (400 MHz, $CDCl_3$) 1.45 (9 H, s, $C(CH_3)_3$), 2.89 (2 H, t, J=5.0 Hz, $NH_2CH_2$), 3.32 (2 H, m, J=14.5 Hz, $CH_2NHBoc$), 3.48–3.58 (4 H, m, 2 x $NCH_2CH_2O$), 3.62 (4 H, s, $OCH_2CH_2O$); LRMS: *m/z* ($ESI^+$) 271 ($[M+Na]^+$, 5%), 249 ($[M+H]^+$, 100%).

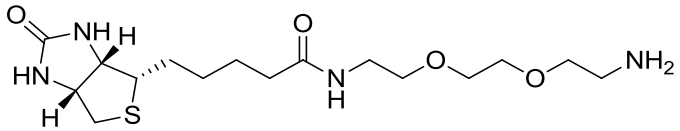
*tert*-Butyl (2-(2-(2-(5-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido) ethoxy)ethoxy)ethyl)carbamate,[1] 14




HBTU (2.41 g, 6.36 mmol) was added to a solution of D-biotin (1.43 g, 6.36 mmol) and diisopropylethyl amine (DIPEA, 1.15 mL, 6.94 mmol) in DMF (50 mL) and stirred for 20 min before being added dropwise to a solution of **13** (1.05 g, 4.24 mmol) in DMF (50 mL). The reaction mixture was stirred for 2 h at room temperature after which time the solvent was removed *in vacuo*. Purification by flash column chromatography (eluent $CH_2Cl_2$ / methanol, 95:5) afforded **14** (1.62 g, 81%) as a colourless gum; $\delta_H$ (400 MHz, $CD_3OD$) 1.32–1.53 (11 H, m, $C(CH_3)_3$, $SCHCH_2CH_2$), 1.54–1.84 (4 H, m, $SCHCH_2CH_2CH_2$), 2.23 (2 H, t, J=7.0 Hz, $NC(O)CH_2$), 2.72 (1 H, d, J=12.5 Hz, SCH*H*), 2.94 (1 H, dd, J=12.5, 4.0 Hz, SC*H*H), 3.23 (3 H, t, J=5.5 Hz,

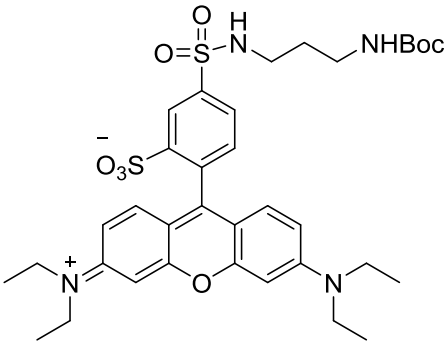
SC*H*), 3.34–3.42 (4 H, m, NC$H_2$C$H_2$O), 3.48–3.59 (4 H, m, 2 x NC$H_2$C$H_2$O), 3.62 (4 H, s, OC$H_2$C$H_2$O), 4.31–4.35 (1 H, m, NC(O)NHC*H*), 4.50–4.54 (1 H, m, C*H*NHC(O)N); LRMS: *m/z* (ESI$^+$) 497 ([M+Na]$^+$, 100%).

*N*-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)-5-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide,[1] F



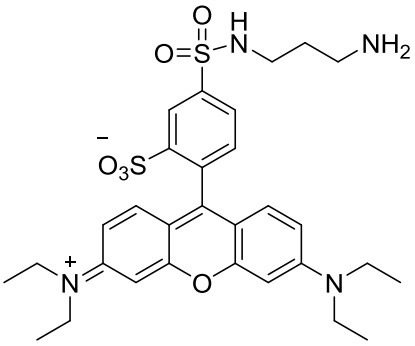
Trifluoroacetic acid (5 mL) was added to a solution of **14** (2.11g, 4.45 mmol) in $CH_2Cl_2$ (25 mL) and the resulting mixture was stirred overnight. The solvent was removed *in vacuo* and "azeotroped" with $CH_2Cl_2$ (3 x 25 mL). The resulting trifluoroacetic acid salt was dissolved in methanol and loaded onto an strong cation exchange chromatography column (TELOS® SCX SPE tube) eluting with methanol (5 column volumes) followed by methanol / $NH_3$ (aq) 9:1 (5 column volumes). The basic eluent was concentrated *in vacuo* to afford **F** as a colourless gum (1.37 g, 80%); $\delta_H$ (400 MHz, $CD_3OD$) 1.46 (2 H, q, J=7.5 Hz, SCHCH$_2$C$H_2$), 1.53–1.83 (4 H, m, SCHC$H_2$CH$_2$C$H_2$), 2.23 (2 H, t, J=7.5 Hz, NC(O)C$H_2$), 2.72 (1 H, d, J=12.5 Hz, SCH*H*), 2.87 (2 H, t, J=5.5 Hz, NH$_2$C$H_2$), 2.94 (1 H, dd, J=12., 4.9 Hz, SCH*H*), 3.17–3.26 (1 H, m, SC*H*), 3.38 (2 H, t, J=5.5 Hz, C(O)NHC$H_2$), 3.52–3.60 (4 H, m, 2 x NC$H_2$C$H_2$O), 3.65 (4 H, s, OC$H_2$C$H_2$O), 4.32 (1 H, m, C*H*NHC(O)N), 4.51 (1 H, m, NC(O)NHC*H*); LRMS: *m/z* (ESI$^+$) 375 ([M+H]$^+$, 100%).

2-(3,6-Bis(diethylamino)xanthylium-9-yl)-5-(*N*-(3-((*tert*-butoxycarbonyl)amino)propyl)sulfamoyl)benzenesulfonate, 15



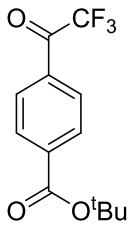
Triethylamine (36 µL, 0.26 mmol) was added to a solution of lissamine rhodamine B sulfonyl chloride (100 mg, 0.18 mmol) in $CHCl_3$ (5 mL). *tert*-Butyl (3-aminopropyl)carbamate was added and the resulting solution stirred for 16 h. The reaction mixture was extracted with $H_2O$ (6 x 25 mL) and concentrated *in vacuo*. Purification by flash column chromatography (eluent 0 – 5 % methanol in dichloromethane) afforded **15** as a dark purple solid (85 mg, 66%); mp > 350 °C; $\nu_{max}$ (thin film) 2982, 2922, 2850, 1739, 1592; $\delta_H$ (500 MHz, DMSO-$d_6$) 1.22 (12 H, t, *J*=7.0 Hz, 4 x CH$_2$C$H_3$), 1.37 (9 H, s, C(C$H_3$)$_3$), 1.58 (2 H, quin, *J*=7.0 Hz, C$H_2$CH$_2$NHBoc), 2.86, 2.95 (2 H, 2 x q, *J*=7.0 Hz, C$H_2$CH$_2$C$H_2$NHBoc), 3.60 - 3.72 (8 H, m, 4 x C$H_2$CH$_3$), 6.84 (1 H, br. s. N*H*), 6.95 (2 H, d, J=2.5 Hz, 2 x C(Ar)*H*), 6.98 (2 H, d, J=9.5 Hz, 2 x C(Ar)*H*), 7.05 (2 H, dd, J=9.5, 2.5 Hz, 2 x C(Ar)*H*), 7.48 (1 H, d, J=8.0 Hz, C(Ar)*H*), 7.89 - 7.91 (1 H, m, N*H*), 7.93 (1 H, dd, J=8.0, 2.0 Hz, C(Ar)*H*), 8.42 (1 H, d, J=2.0 Hz, C(Ar)*H*); $\delta_C$ (126 MHz, DMSO-$d_6$) 12.5 (CH$_2$*C*H$_3$), 28.2 (C(*C*H$_3$)$_3$), 29.8 (CH$_2$*C*H$_2$CH$_2$), 37.4, 40.5 (*C*H$_2$CH$_2$*C*H$_2$), 45.2 (*C*H$_2$CH$_3$), 77.5 (*C*(CH$_3$)$_3$), 95.4, 113.5, 113.6, 125.7, 126.5, 130.6, 132.7, 133.0, 141.4, 148.0, 155.0, 155.6, 157.1, 157.5 (aromatic and carbonyl carbons); HRMS (ESI$^+$) $C_{35}H_{46}N_4NaO_8S_2^+$ ([M+Na]$^+$) calculated 737.2649; found 737.2656.

5-(*N*-(3-Aminopropyl)sulfamoyl)-2-(3,6-bis(diethylamino)xanthylium-9-yl)benzenesulfonate, H



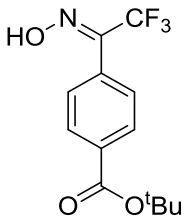
Trifluoroacetic acid (0.3 mL) was added to **15** (50 mg, 0.07 mmol) in $CH_2Cl_2$ (10 mL) and the resulting solution stirred for 16 h. The reaction mixture was concentrated *in vacuo* and purified by strong cation exchange chromatography (TELOS® SCX SPE tube, eluting with methanol, then 5% aqueous ammonia in methanol) to afford **H** as a dark purple solid (40 mg, 93%); mp > 350 °C; $\nu_{max}$ (thin film) 2984, 2919, 2850, 1720, 1602, 1592, 1496; $\delta_H$ (500 MHz, $CD_3OD$) 1.32 (12 H, t, *J*=7.0 Hz, 4 x $CH_2CH_3$), 1.81 (2 H, quin, *J*=7.0 Hz, $CH_2CH_2NH_2$), 2.88, 3.13 (2 H, 2 x t, *J*=7.0 Hz, $CH_2CH_2CH_2$), 3.70 (8 H, q, *J*=7.0 Hz, 4 x $CH_2CH_3$), 6.96 (2 H, d, *J*=2.5 Hz, 2 x C(Ar)*H*), 7.03 (2 H, dd, *J*=9.5, 2.5 Hz, 2 x C(Ar)*H*), 7.13 (2 H, d, *J*=9.5 Hz, 2 x C(Ar)*H*), 7.54 (1 H, d, *J*=8.0 Hz, C(Ar)*H*), 8.13 (1 H, dd, *J*=8.0, 2.0 Hz, C(Ar)*H*), 8.66 (1 H, d, *J*=2.0 Hz, C(Ar)*H*); $\delta_H$ (126 MHz, $CD_3OD$) 12.8 ($CH_2CH_3$), 32.1 ($CH_2CH_2CH_2$), 39.2, 41.6 ($CH_2CH_2CH_2$), 46.8 ($CH_2CH_3$), 97.0, 115.1, 115.3, 127.7, 129.3, 132.6, 133.6, 135.5, 143.9, 147.3, 157.2, 157.8, 159.4 (aromatic carbons); HRMS ($ESI^+$) $C_{30}H_{39}N_4O_6S_2^+$ ($[M+H]^+$) calculated 615.2306; found 615.2307.

*tert*-Butyl 4-(2,2,2-trifluoroacetyl)benzoate,[2] 16



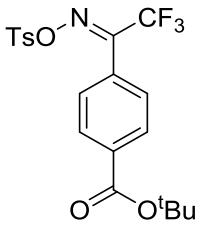
4-(2,2,2-Trifluoroacetyl)benzoic acid (1.0 g, 4.59 mmol) was added to *tert*-butyl acetate (4 mL) in a pressure vial. Concentrated sulfuric acid (0.04 mL, 0.37 mmol) was added and the vial sealed with a screw cap. The reaction mixture was stirred at room temperature for 48 h before being quenched by addition of sat. aqueous $NaHCO_3$ (50 mL) and extracted with $CH_2Cl_2$ (3 x 50 mL). Combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford **16** as a colourless oil (904 mg, 72%); $\nu_{max}$ (thin film) 2981, 2936 (C-H), 2360, 2341, 1717 (C=O), 1180, 1112 ($CF_3$); $\delta_H$ (400 MHz, $CDCl_3$) 1.61 (9 H, s, $C(CH_3)_3$), 8.14 (4 H, s, 4 x C(Ar)*H*); $\delta_C$ (126 MHz, $CDCl_3$) 28.1 ($C(CH_3)_3$), 82.4 ($C(CH_3)_3$), 116.5 (q, J=290.0 Hz, $CF_3$), 129.9 (4 x C(Ar)), 132.5, 137.9 (2 x C(ipso)), 164.2 ($CO_2$), 180.2 (q, J=35.5 Hz, $C(O)CF_3$); $\delta_F$ (470 MHz, $CDCl_3$) −71.6 ($CF_3$); LRMS: *m/z* ($FI^+$) 274 ($[M]^+$, 100%); HRMS ($FI^+$) $C_{13}H_{13}F_3O_3^+$ ($[M]^+$) calculated 274.0817; found 274.0815.

*tert*-Butyl 4-(2,2,2-trifluoro-1-(hydroxyimino)ethyl)benzoate,[2] 17



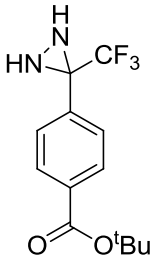

*tert*-Butyl 4-(2,2,2-trifluoroacetyl)benzoate, **16**, (904 mg, 3.30 mmol) in ethanol (5 mL) was added dropwise to a refluxing solution of hydroxylamine hydrochloride (1.01 g, 14.7 mmol) and NaOH (588 mg, 14.7 mmol) in ethanol (7 mL). The resulting solution was stirred at reflux for 16 h before being cooled to room temperature. Ethyl acetate (50 mL) was added and washed with 0.1 N aqueous HCl (2 x 20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to provide **17** (924 mg, 97%) as a colourless oil; $\nu_{max}$ (thin film) 3367 (O-H), 2982, 2936 (C-H), 2360, 2342, 1718 (C=O), 1182, 1115 ($CF_3$); $\delta_H$ (400 MHz, $CDCl_3$) 1.61 (9 H, s, C($CH_3$)$_3$), 7.57 (2 H, d, J=8.5 Hz, 2 x C(Ar)*H*), 8.10 (2 H, d, J=8.5 Hz, 2 x C(Ar)*H*); $\delta_C$ (126 MHz, $CDCl_3$) 28.1 (C($CH_3$)$_3$), 81.8 (*C*($CH_3$)$_3$), 120.4 (q, J=280.5 Hz, *C*$F_3$), 128.6 (2 x *C*(Ar)), 129.5 (2 x *C*(Ar)), 129.9, 133.7 (2 x *C*(ipso)), 147.2 (q, J=33.5 Hz, *C*$CF_3$), 165.0 (*C*$O_2$); $\delta_F$ (470 MHz, $CDCl_3$) −66.6 (C$F_3$); HRMS (ESI$^+$) $C_{13}H_{14}F_3NNaO_3^+$ ([M+Na]$^+$) calculated 312.0818; found 312.0813.

*tert*-Butyl 4-(2,2,2-trifluoro-1-((tosyloxy)imino)ethyl)benzoate,[2] 18



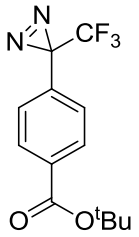

*p*-Toluenesulfonyl chloride (684 mg, 3.58 mmol) was added portionwise to a solution of *tert*-butyl 4-(2,2,2-trifluoro-1-(hydroxyimino)ethyl)benzoate **17** (900 mg, 3.11 mmol), *N*,*N*-dimethylpyridin-4-amine (38 mg, 0.31 mmol) and triethylamine (0.52 mL, 3.73 mmol) in $CH_2Cl_2$ (30 mL) at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed *in vacuo* and the residue dissolved in diethyl ether (60 mL). The organic solution was washed with water (3 x 10 mL) and brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford **18** (1.19 g, 87%) as a colourless oil, which was found to be a mixture of *E* and *Z* isomers; $\nu_{max}$ (thin film) 2980, 2934 (C-H), 2359, 1716 (C=O), 1195, 1180, 1118 ($CF_3$); $\delta_H$ (400 MHz, $CDCl_3$) 1.60, 1.61 (9 H, s, C($CH_3$)$_3$, *E* and *Z*-isomers), 2.47, 2.49 (3 H, s, C(Ar)C$H_3$, *E* and *Z* isomers), 7.33–7.53 (4 H, m, 4 x C(Tos)*H*), 7.83–8.21 (4 H, m, 4 x C(Ar)*H*); $\delta_F$ (470 MHz, $CDCl_3$) −66.8, −61.5 (C$F_3$); LRMS: *m/z* (FI$^+$) 443 ([M]$^+$, 100%); HRMS (FI$^+$) $C_{20}H_{10}NF_3O_5S^+$ ([M]$^+$) calculated 443.1014; found 443.1010.

*tert*-Butyl 4-(3-(trifluoromethyl)diaziridin-3-yl)benzoate,[2] 19



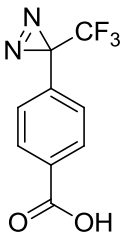
A solution of *tert*-butyl 4-(2,2,2-trifluoro-1-((tosyloxy)imino)ethyl)benzoate **18** (1.16 g, 2.61 mmol) in $CH_2Cl_2$ (6 mL) at −60 °C was added dropwise to liquid ammonia (1.5 mL) at −78 °C. The resulting mixture was allowed to warm to −60 °C and stirred for 12 h. The ammonia was allowed to evaporate and $CH_2Cl_2$ (10 mL) and water (15 mL) added. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography (eluent: 100% $CH_2Cl_2$) afforded **19** (585 mg, 74%) as a colourless oil; $\nu_{max}$ (thin film) 3251 (N-H), 2980, 2936 (C-H), 2361, 2342, 1712 (C=O), 1151, 1102 ($CF_3$); $\delta_H$ (400 MHz, $CDCl_3$) 1.60 (9 H, s, C(C*H*3)3), 7.68 (2 H, d, J=8.5 Hz, 2 x C(Ar)*H*), 8.04 (2 H, d, J=8.5 Hz, 2 x C(Ar)*H*); $\delta_C$ (126 MHz, $CDCl_3$) 28.1 (C(*C*$H_3$)$_3$), 57.8 (q, J=36.0 Hz, *C*$CF_3$), 81.6 (*C*($CH_3$)$_3$), 123.3 (q, J=280.0 Hz, *C*$F_3$), 128.0 (2 x *C*(Ar)), 129.8 (2 x *C*(Ar)), 133.7, 135.6 (2 x *C*(ipso)), 164.8 (*C*$O_2$); $\delta_F$ (470 MHz, $CDCl_3$) −74.8 (C*F*$_3$); HRMS ($ESI^+$) $C_{13}H_{15}F_3N_2NaO_2^+$ ($[M+Na]^+$) calculated 311.0978; found 311.0969.

*tert*-Butyl 4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzoate,[2] 20



*tert*-Butyl 4-(3-(trifluoromethyl)diaziridin-3-yl)benzoate **19** (565 mg, 1.96 mmol) and triethylamine (0.80 mL, 5.88 mmol) were added to $CH_2Cl_2$ (10 mL). Iodine (1.50 g, 5.88 mmol) was added portionwise until a brown colour persisted. The resulting mixture was stirred for 2 h and then washed with 1M aqueous NaOH (10 mL), water (10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography (eluent cyclohexane / ethyl acetate, 99:1) afforded **20** (524 mg, 93%) as a colourless oil; $\delta_H$ (200 MHz, $CDCl_3$) 1.60 (9 H, s, C(C*H*$_3$)$_3$), 7.23 (2 H, d, J=8.5 Hz, 2 x C(Ar)*H*), 8.01 (2 H, d, J=8.5 Hz, 2 x C(Ar)*H*); LRMS: *m/z* ($ESI^+$) 287 ($[M+H]^+$.

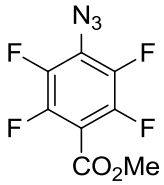
4-(3-(Trifluoromethyl)-3*H*-diazirin-3-yl)benzoic acid,[2] C



Trifluoroacetic acid (0.50 mL) was added to a solution of *tert*-butyl 4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzoate, **48**, (524 mg, 1.83 mmol) in $CH_2Cl_2$ (5 mL). The reaction mixture was stirred for 2 h before the

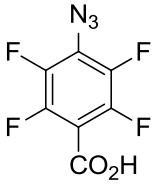
solvent was removed *in vacuo* and the residual trifluoroacetic acid azeotroped with $CH_2Cl_2$ (3 x 25 mL). $CH_2Cl_2$ (25 mL) and sat. aqueous $NaHCO_3$ (25 mL) were added. The layers were separated and the aqueous layer acidified to pH 3 with 1M aqueous HCl and extracted with $CH_2Cl_2$ (3 x 25 mL). Combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford **C** (364 mg, 86%) as a white foam; $\delta_H$ (400 MHz, $CDCl_3$) 7.30 (2 H, d, J=8.0 Hz, 2 x C(Ar)*H*), 8.15 (2 H, d, J=8.0 Hz, 2 x C(Ar)*H*); LRMS: *m/z* ($ESI^-$) 299 ($[M-H]^-$, 100%).

### Methyl 4-azido-2,3,5,6-tetrafluorobenzoate,[3] 21



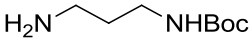

Sodium azide (0.65 g, 10.0 mmol) was added to a solution of methyl pentafluorobenzoate (1.50 g, 6.64 mmol) in acetone (12 mL) and water (4 mL). The reaction mixture was heated at reflux for 2 h and then cooled to room temperature before addition of water (30 mL) and diethyl ether (30 mL). The layers were separated and the aqueous layer extracted with diethyl ether (2 x 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford **21** (1.55 g, 94%) as a white solid; mp 53-55 °C {lit.[3] mp 54-55 °C}. $\delta_H$ (400 MHz, $CDCl_3$) 3.94 (3 H, s, $CO_2CH_3$); $\delta_C$ (101 MHz, $CDCl_3$) 53.10 ($CH_3$), 107.52 (t, J=16.0 Hz, $CN_3$), 123.35 ($CCO_2CH_3$), 138.61–142.06 (m, 2 x *C*F), 143.67–146.81 (m, 2 x *C*F), 159.69 ($CO_2CH_3$); $\delta_F$ (470 MHz, $CDCl_3$) −151.6 to −150.8 (2 F, m), −139.4 to −138.7 (2 F, m); LRMS: *m/z* ($ESI^+$) 250 ($[M+H]^+$).

### 4-Azido-2,3,5,6-tetrafluorobenzoic acid,[3] B




Methyl 4-azido-2,3,5,6-tetrafluorobenzoate (1.56 g, 6.27 mmol) was added to methanol (6 mL), 20% w/w aqueous NaOH (0.6 mL) and water (1.2 mL) and stirred overnight. 1M aqueous HCl (20 mL) was added and extracted with $CH_2Cl_2$ (3 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford **B** (1.37 g, 93%) as a white solid; mp 134-138 °C {lit.[3] mp 140-141°C}; $\delta_F$ (377 MHz, DMSO-d6) −151.6 to −151.5 (2 F, m), −141.4 to −141.2 (2 F, m); LRMS: *m/z* ($ESI^-$) 234 ($[M-H]^-$, 100%).

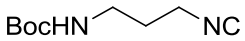
### *tert*-Butyl (3-aminopropyl)carbamate,[4] 22




A solution of di-*tert*-butyl dicarbonate (1.10 g, 5.05 mmol) in $CHCl_3$ (5 mL) was added dropwise over 30 min to a solution of propane-1,3-diamine (4.20 mL, 50 mmol) in $CHCl_3$ (10 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred for 12 h. The solvent was removed *in vacuo* and the residue dissolved in $CH_2Cl_2$ (10 mL), washed with water (30 mL), brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give **22** as a colourless oil (658 mg, 76%); $\delta_H$ (200 MHz, $CDCl_3$) 1.45 (9 H, s,

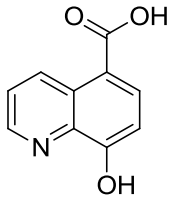
C(C$H_3$)$_3$), 1.62 (2 H, quin, *J*=6.5 Hz, NHCH$_2$C$H_2$), 2.77 (2 H, t, *J*=6.5 Hz, NH$_2$C$H_2$), 3.22 (2 H, q, *J*=6.5 Hz, C$H_2$NHBoc); LRMS: *m/z* (ESI$^+$) 175 ([M+H]$^+$).

### *tert*-Butyl (3-isocyanopropyl)carbamate, J



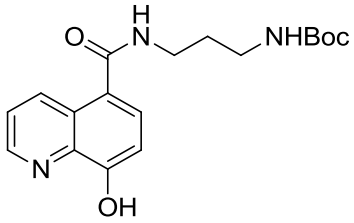

*tert*-Butyl (3-aminopropyl)carbamate, **22**, (1.00 g, 5.75 mmol) was dissolved in ethyl formate (6 mL) and heated at reflux for 18 h. The reaction mixture was cooled to room temperature and the solvent removed *in vacuo* to afford the crude *N*-(3-((*tert*-butoxycarbonyl)amino)propyl)formimidate as a pale yellow oil, which was used directly in the next step. Phosphoryl trichloride (0.59 mL, 6.35 mmol) in tetrahydrofuran (5 mL) was added dropwise over 30 min to the crude ethyl *N*-(3-((*tert*-butoxycarbonyl)amino)propyl)formimidate (5.75 mmol) and triethylamine (3.98 mL, 28.8 mmol) in tetrahydrofuran (35 mL) at 0 °C. The resulting mixture was stirred for 2 h at 0 °C and then at room temperature for 1 h. Water (50 mL) was added, the layers separated and the aqueous layer extracted with diethyl ether (3 x 50 mL). Combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was passed through a silica plug (eluent EtOAc / Hexane, 1:1) to afford **J** (947 mg, 90% over two steps) as a yellow oil; $\nu_{max}$ (thin film) 3338 (N-H), 2977, 2934 (C-H), 2149, (NC), 1686 (C=O); $\delta_H$ (200 MHz, $CDCl_3$) 1.45 (9 H, s, C(C$H_3$)$_3$), 1.79–2.02 (2 H, m, CH$_2$ C$H_2$CH$_2$), 3.28 (2 H, q, J=6.5 Hz, NHC$H_2$ CH$_2$CH$_2$), 3.47 (2 H, tt, J=6.5, 1.5 Hz, C$H_2$NC), 4.54–4.81 (1 H, s, N*H*); $\delta_C$ (101 MHz, $CDCl_3$) 28.3 (C(*C*H$_3$)$_3$), 77.1 (*C*(CH$_3$)$_3$, 30.0 (CH$_2$*C*H$_2$CH$_2$), 37.0, 39.0 (*C*H$_2$CH$_2$*C*H$_2$), 156.0 (CH$_2$N*C*), 161.6 (*C*O$_2$$^t$Bu); HRMS (FI$^+$) $C_9H_{16}N_2O_2^+$ ([M]$^+$) calculated 184.1212; found 184.1216.

### 8-Hydroxyquinoline-5-carboxylic acid,[5] IOX1



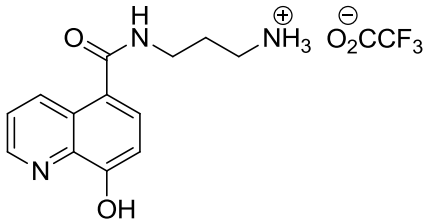

Acrolein (0.65 mL , 9.73 mmol) was added dropwise to a refluxing solution of 3-amino-4-hydroxybenzoic acid (1.00 g, 6.53 mmol) in 6N HCl (15 mL) and stirred at reflux for 2 h. The reaction mixture was cooled to room temperature, diluted with water (15 mL), adjusted to pH 8 with aqueous ammonia and filtered. The filtrate was acidified to pH 4 with acetic acid and the resulting precipitate isolated by filtration and dried under vacuum. Purification by reverse phase chromatography afforded **IOX1** as a pale brown powder (563 mg, 46%); mp 275-277 °C (decomposition) {lit.[6] mp 278-280 °C}; $\delta_H$ (200 MHz, DMSO-d6) 7.14 (1 H, d, J=8.0 Hz, C(Ar)*H*), 7.71 (1 H, dd, J=9.0, 4.0 Hz, C(Ar)*H*), 8.27 (1 H, d, J=8.0 Hz, C(Ar)*H*), 8.93 (1 H, m, C(Ar)*H*), 9.49 (1 H, dd, J=9.0, 1.0 Hz, C(Ar)*H*); LRMS: *m/z* (ESI$^+$) 190 ([M+H]$^+$).

*tert*-Butyl (3-(8-hydroxyquinoline-5-carboxamido)propyl)carbamate,[5] 23



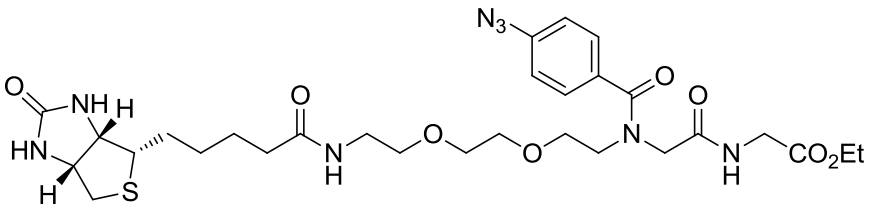
HBTU (441 mg, 1.16 mmol) was added to a solution of 8-hydroxyquinoline-5-carboxylic acid, **IOX1**, (200 mg, 1.06) and triethylamine (0.29 mL, 2.10 mmol) in DMF (10 mL) and stirred for 15 min before being added dropwise to a solution of *tert*-butyl (3-aminopropyl)carbamate (276 mg. 1.59 mmol) and triethylamine (0.29 mL, 2.10 mmol) in DMF (10 mL). The resulting mixture was stirred for 12 h before the solvent was removed *in vacuo* and the residue purified by column chromatography (eluent 2-15% methanol in $CH_2Cl_2$) to afford **23** (238 mg, 66%) as a white solid; mp 152-154 °C {lit.[6] mp 162-164 °C}; $\delta_H$ (200 MHz, $CDCl_3$) 1.43 (9 H, s, $C(CH_3)_3$), 1.75 (2 H, m, $CH_2CH_2CH_2$), 3.25 (2 H, m, $CH_2CH_2CH_2$), 3.53 (2 H, m, $CH_2CH_2CH_2$), 7.09 (1 H, d, J=7.5 Hz, C(Ar)*H*), 7.50 (1 H, dd, J=8.5, 4.0 Hz, C(Ar)*H*), 7.72 (1 H, d, J=7.5 Hz, C(Ar)*H*), 8.78 (1 H, dd, J=4.0, 1.5 Hz, C(Ar)*H*), 8.96 (1 H, app. d, J=8.5 Hz, C(Ar)*H*); LRMS: *m/z* ($ESI^+$) 346 ($[M+H]^+$).

*N*-(3-Aminopropyl)-8-hydroxyquinoline-5-carboxamide trifluoroacetic acid salt,[5] 24



Trifluoroacetic acid (0.4 mL) was added to *tert*-butyl (3-(8-hydroxyquinoline-5-carboxamido)propyl)carbamate, (**23**, 71 mg, 0.21 mmol) in $CH_2Cl_2$ (4 mL). The reaction mixture was stirred for 2 h before the solvent was removed *in vacuo* and the residual trifluoroacetic acid azeotroped with $CH_2Cl_2$ (3 x 25 mL) to afford **24** (72 mg, 97%) as a sticky brown oil; $\delta_H$ (400 MHz, $CD_3OD$) 2.07 (2 H, quin, J=7.0 Hz, $CH_2CH_2CH_2$), 3.13 (2 H, t, J=7.0 Hz, $CONHCH_2$), 3.61 (2 H, t, J=7.0 Hz, $CH_2NH_3^+$), 7.44 (1 H, d, J=8.0 Hz, C(Ar)*H*), 8.00–8.15 (2 H, m, C(Ar)*H*), 9.09 (1 H, d, J=4.5 Hz, C(Ar)*H*), 9.59 (1 H, d, J=8.5 Hz, C(Ar)*H*); LRMS: *m/z* ($ESI^+$) 246 ($[M+H]^+$).

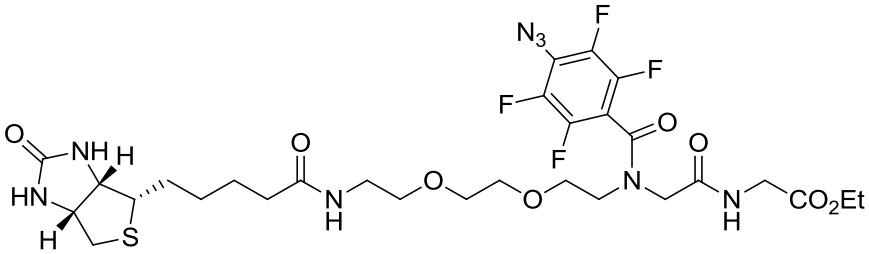
Ethyl 6-(4-azidobenzoyl)-4,16-dioxo-20-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-9,12-dioxa-3,6,15-triazaicosan-1-oate, 6a



According to general procedure A: 4-azidobenzoic acid (86 mg, 0.53 mmol), ethyl 2-isocyanoacetate (97 µL, 0.53 mmol), paraformaldehyde (24 mg, 0.80 mmol) and compound **F** (200 mg, 0.53 mmol) were reacted to afford **6a** (283 mg, 62%) as a colourless gum; $\nu_{max}$ (thin film) 3291 (N-H), 2929 (C-H), 2868 (C-H), 2127, 2097 ($N_3$), 1747 (C=O, ester), , 1690, 1644, 1604 (C=O, amides and urea); $\delta_H$ (500 MHz, $CD_3OD$) 1.30 (3 H,

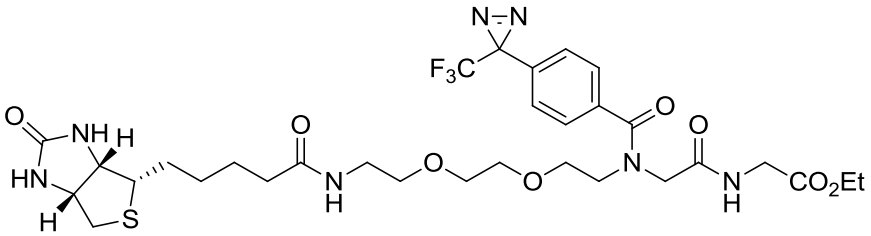
t, J=7.0 Hz, C*H*$_3$), 1.37–1.48 (2 H, m, C*H*$_2$CH$_2$CH$_2$C(O)N), 1.53–1.81 (4 H, m, SCHC*H*$_2$CH$_2$C*H*$_2$), 2.21 (2 H, t, J=7.5 Hz, CH$_2$C*H*$_2$C(O)N), 2.72 (1 H, d, J=12.5 Hz, SCH*H*), 2.94 (1 H, dd, J=12.5, 5.0 Hz, SC*H*H), 3.15–3.25 (1 H, m, SC*H*), 3.35–3.42 (2 H, m, C(O)NHC*H*$_2$CH$_2$O), 3.53–3.72 (8 H, m, C*H*$_2$OC*H*$_2$C*H*$_2$OC*H*$_2$), 3.72–3.86 (2 H, m, OCH$_2$C*H*$_2$NRR'), 3.96–4.07 (2 H, m, NHC*H*$_2$CO$_2$Et), 4.18, 4.33 (2 H, 2 x br. s, NC*H*$_2$C(O)NH, 2 rotamers), 4.22 (2 H, q, J=7.0 Hz, C(O)OC*H*$_2$), 4.28–4.32 (1 H, m, SCHRC*H*), 4.50 (1 H, dd, J=7.5, 5.0 Hz, SCH$_2$C*H*), 7.17 (2 H, d, J= 8.0 Hz, 2 x C(Ar)*H*), 7.51–7.67 (2 H, m, 2 x C(Ar)H). $\delta_C$ (126 MHz, CD$_3$OD) 14.58 (*C*H$_3$), 26.86, 29.51, 29.79 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 36.76 (CH$_2$*C*H$_2$CONH), 40.36 (C(O)NH*C*H$_2$CH$_2$O), 41.11 (S*C*H$_2$), 42.15 (*C*H$_2$CO$_2$Et), 48.2, 51.6 (OCH$_2$*C*H$_2$NRR', 2 rotamers), 50.1, 54.7 (NRR'*C*H$_2$C(O)N, 2 rotamers), 57.05 (S*C*HRR'), 61.65 (SCH$_2$*C*), 62.43 (*C*H$_2$CH$_3$), 63.39 (SCHR*C*), 69.36, 69.92, 70.72, 71.35, 71.46 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$, extra signals as a result of rotamers), 120.06, 120.25 (2 x *C*(Ar), 2 rotamers), 130.03, 130.52 (2 x *C*(Ar), 2 rotamers), 133.48 (*C*(Ar)), 143.26, 143.49 (2 x *C*(Ar), 2 rotamers), 166.11 (N*C*(O)N), 171.21, 171.37 (C*C*=O, 2 rotamers), 171.73 (C*C*=O), 174.20, 174.33 (C*C*=O, 2 rotamers), 176.19 (C*C*=O); HRMS (ESI$^+$) $C_{29}H_{42}N_8NaO_8S^+$ ([M+Na]$^+$) calculated 685.2739; found 685.2730.

Ethyl 6-(4-azido-2,3,5,6-tetrafluorobenzoyl)-4,16-dioxo-20-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-9,12-dioxa-3,6,15-triazaicosan-1-oate, 6b



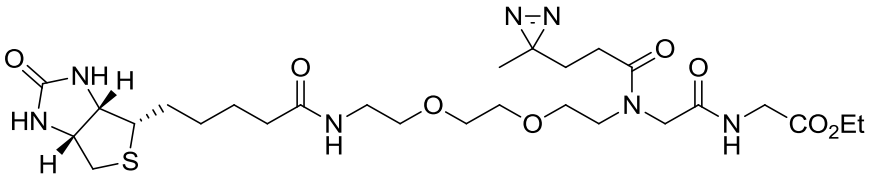
According to general procedure A: 4-azido-2,3,5,6-tetrafluorobenzoic acid (126 mg, 0.53 mmol), ethyl 2-isocyanoacetate (97 µL, 0.53 mmol), paraformaldehyde (24 mg, 0.80 mmol) and **F** (200 mg, 0.53 mmol) were reacted to afford **6b** (231 mg, 59%) as a colourless gum; $\nu_{max}$ (thin film) 3306 (N-H), 2929, 2868 (C-H), 2128 (N$_3$), 1745 (C=O ester), 1686, 1647 (C=O, amides and urea); $\delta_H$ (400 MHz, CD$_3$OD) 1.30 (3 H, t, J=7.5 Hz, C*H*$_3$), 1.45 (2 H, quin, J=7.5 Hz, C*H*$_2$CH$_2$CH$_2$C(O)N), 1.53–1.82 (4 H, m, SCHC*H*$_2$CH$_2$C*H*$_2$), 2.24 (2 H, t, J=7.0 Hz, CH$_2$C*H*$_2$C(O)N), 2.72 (1 H, d, J=12.5 Hz, SCH*H*), 2.94 (1 H, dd, J=12.5, 5.0 Hz, SC*H*H), 3.18–3.27 (1 H, m, SC*H*), 3.38 (2 H, t, J=5.0 Hz, C(O)NHC*H*$_2$CH$_2$O), 3.49–3.71 (8 H, m, C*H*$_2$OC*H*$_2$C*H*$_2$OC*H*$_2$), 3.73–3.86 (2 H, m, OCH$_2$C*H*$_2$NRR'), 3.90, 4.03 (2 H, 2 x s, NHC*H*$_2$CO$_2$Et, 2 rotamers), 4.14–4.26 (2 H, m, CO$_2$C*H*$_2$), 4.21, 4.38 (2 H, 2 x s, NC*H*$_2$C(O)NH, 2 rotamers),4.32 (1 H, dd, J=8.0, 4.5 Hz, SCHRC*H*), 4.47–4.55 (1 H, m, SCH$_2$C*H*); $\delta_C$ (126 MHz, CD$_3$OD) 14.5 (*C*H$_3$), 26.9, 29.5, 29.8 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 36.7 (CH$_2$*C*H$_2$CONH), 40.3 (C(O)NH*C*H$_2$CH$_2$O), 41.1 (S*C*H$_2$), 42.0, 42.3 (*C*H$_2$CO$_2$Et), 50.1, 51.5, 53.3, 54.8 (OCH$_2$*C*H$_2$NRR', 2 rotamers, NRR'*C*H$_2$C(O)N, 2 rotamers), 57.0 (S*C*HRR'), 61.7 (SCH$_2$*C*), 62.4 (*C*H$_2$CH$_3$), 63.4 (SCHR*C*), 69.2, 69.9, 70.3, 70.7, 71.2, 71.4, 71.4, 71.5 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$, extra signals as a result of rotamers), (aromatic signals not observed due to coupling to F), 166.1 (N*C*(O)N), 170.5, 170.6, 171.0, 171.3, 176.2 (4 x C*C*=O, extra signals as a result of rotamers). $\delta_F$ (470 MHz, CD$_3$OD) −153.0 to −152.9 (2 F, m, major rotamer), −143.1 to −142.9 (2 F, m, major rotamer); HRMS (ESI$^+$) $C_{29}H_{38}F_4N_8NaO_8S^+$ ([M+Na]$^+$) calculated 757.2362; found 757.2346.

Ethyl 4,16-dioxo-20-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzoyl)-9,12-dioxa-3,6,15-triazaicosan-1-oate, 6c



According to general procedure A: 4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzoic acid **C** (122 mg, 0.53 mmol), ethyl 2-isocyanoacetate (97 µL, 0.53 mmol), paraformaldehyde (24 mg, 0.80 mmol) and **F** (200 mg, 0.53 mmol) were reacted to afford **6c** (254 mg, 66%) as a colourless gum; $\nu_{max}$ (thin film) 3306 (N-H), 2928 (C-H), 1744 (C=O, ester), 1680, 1633 (C=O, amides and urea); $\delta_H$ (400 MHz, $CD_3OD$) 1.30 (3 H, t, J = 7.0 Hz, C$H_3$), 1.37–1.50 (2 H, m, C$H_2$CH$_2$CH$_2$C(O)N), 1.53–1.84 (4 H, m, SCHC$H_2$CH$_2$C$H_2$), 2.21 (2 H, t, J=7.0 Hz, CH$_2$C$H_2$C(O)N), 2.72 (1 H, d, J=12.5 Hz, SCH*H*), 2.93 (1 H, dd, J=12.5, 4.5 Hz, SCH*H*), 3.16–3.24 (1 H, m, SC*H*), 3.35–3.40 (2 H, m, C(O)NC$H_2$CH$_2$O), 3.48–3.71 (8 H, m, C$H_2$OC$H_2$C$H_2$OC$H_2$), 3.75–3.83 (2 H, m, OCH$_2$C$H_2$NRR'), 3.97, 4.04 (2 H, 2 x s, NHC$H_2$CO$_2$Et, 2 rotamers), 4.12, 4.35 (2 H, 2 x s, NC$H_2$C(O)NH, 2 rotamers), 4.22 (2 H, q, J=7.0 Hz, C$H_2$CH$_3$), 4.31 (1 H, dd, J=7.5, 4.5 Hz, SCHRC*H*), 4.50 (1 H, dd, J=7.5, 5.0 Hz, SCH$_2$C*H*), 7.32-7.37 (2 H, m, Hz, 2 x C(Ar)*H*), 7.53 - 7.73 (2 H, m, 2 x C(Ar)*H*); δC (126 MHz, CD3OD) 14.6 (*C*H$_3$), 26.9, 29.5, 29.8 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 29.5 (q, *J*=41.0 Hz, *C*N$_2$), 36.8 (CH$_2$*C*H$_2$C(O)NH), 40.4 (C(O)NH*C*H$_2$CH$_2$O), 41.1 (S*C*H$_2$), 42.1, 42.2 (*C*H$_2$CO$_2$Et, 2 rotamers), 48.1, 49.9, 51.5, 54.4 (OCH$_2$*C*H$_2$NRR', 2 rotamers, NRR'*C*H$_2$C(O)N, 2 rotamers), 57.0 (S*C*HRR'), 61.6 (SCH$_2$*C*), 62.4 (*C*H$_2$CH$_3$), 63.4 (SCHR*C*), 69.2, 69.9, 70.7, 71.3, 71.4 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$, extra signals as a result of rotamers), 123.5 (q, *J*=273.0 Hz, *C*F$_3$), 127.8, 128.0, 128.8, 129.3, 131.4, 131.7, 138.7, 138.8 (6 x C(Ar), extra signals as a result of rotamers), 166.1 (N*C*(O)N), 171.2, 171.4, 171.5, 173.6, 173.8, 176.2 (4 x C*C*=O extra signals as a result of rotamers); $\delta_F$ (470 MHz, $CD_3OD$) −66.8 (3F, s.); HRMS (ESI$^+$) $C_{31}H_{42}F_3N_7NaO_8S^+$ ([M+Na]$^+$) calculated 752.2660; found 752.2659.

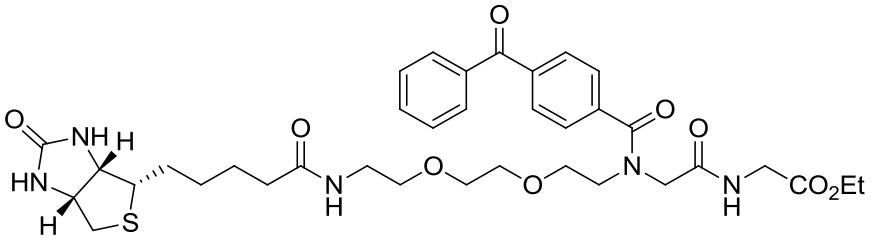
Ethyl 6-(3-(3-methyl-3H-diazirin-3-yl)propanoyl)-4,16-dioxo-20-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-9,12-dioxa-3,6,15-triazaicosan-1-oate, 6d



According to general procedure A: 3-(3-methyl-3*H*-diazirin-3-yl)propanoic acid, **E**, (67.8 mg, 0.53 mmol), ethyl 2-isocyanoacetate (97 µL, 0.53 mmol), paraformaldehyde (24 mg, 0.80 mmol) and **F** (200 mg, 0.53 mmol) were reacted to afford **6d** (208 mg, 63%) as a colourless gum; $\nu_{max}$ (thin film) 3304 (N-H), 2928 (C-H), 1746 (C=O, ester), 1697, 1636 (C=O, amides, urea); $\delta_H$ (400 MHz, $CD_3OD$) 1.03, 1.05 (3 H, 2 x s, C$H_3$, 2 rotamers), 1.29 (3 H, m, C$H_3$), 1.46 (2 H, quin, J=7.5 Hz, C$H_2$CH$_2$CH$_2$C(O)N), 1.54–1.82 (6 H, m, SCHC$H_2$CH$_2$C$H_2$, CN$_2$C$H_2$), 2.25 (2 H, t, J=7.5 Hz, CH$_2$C$H_2$C(O)N), 2.29, 2.45 (2 H, 2 x t, J=7.5 Hz, CN$_2$CH$_2$C$H_2$, 2 rotamers), 2.73 (1 H, d, J=12.5 Hz, SCH*H*), 2.95 (1 H, dd, J=12.5, 5.0 Hz, SCH*H*), 3.19–3.27 (1 H, m, SC*H*), 3.38 (2 H, t, J=5.0 Hz, C(O)NC$H_2$CH$_2$O), 3.50–3.72 (10 H, m, C$H_2$OC$H_2$C$H_2$OC$H_2$C$H_2$NRR'), 3.97, 4.01 (2 H, 2 x s, NHC$H_2$CO$_2$Et), 4.15, 4.26 (2 H, 2 x s, NC$H_2$C(O)NH, 2 rotamers), 4.16–4.24 (2 H, m, CO$_2$C$H_2$), 4.33 (1 H, dd, J=8.0, 4.5 Hz, SCHRC*H*), 4.51 (1 H, dd, J=8.0, 5.0 Hz, SCH$_2$C*H*); $\delta_C$ (126 MHz, $CD_3OD$) 14.5 (CH$_2$*C*H$_3$), 19.9, 20.0 (CN$_2$*C*H$_3$, 2 rotamers), 26.4, 26.5

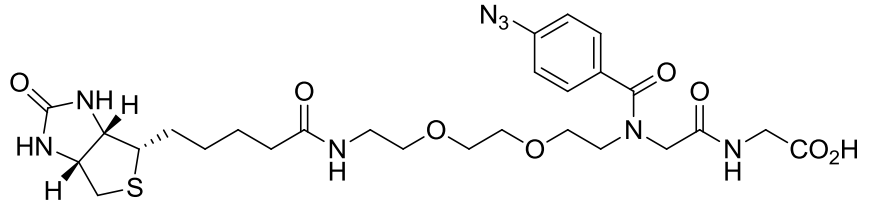
(*C*$H_2$CN$_2$, 2 rotamers), 26.9, 29.5, 29.8 (SCH*C*$H_2$*C*$H_2$*C*$H_2$), 28.2, 28.4 (*C*$H_2$CH$_2$CN$_2$), 30.8, 30.9 (*C*N$_2$, 2 rotamers), 36.8 (CH$_2$*C*$H_2$CONH), 40.3 (C(O)NH*C*$H_2$CH$_2$O), 41.1 (S*C*H$_2$), 42.1 (*C*$H_2$CO$_2$Et), 50.2, 50.7, 53.2, 54.9 (OCH$_2$*C*$H_2$NRR', 2 rotamers, NRR'*C*H$_2$C(O)N, 2 rotamers), 57.1 (S*C*HRR'), 61.7 (SCH$_2$*C*), 62.4 (*C*$H_2$CH$_3$), 63.4 (SCHR*C*), 69.8, 70.2, 70.7, 71.3, 71.4, 71.7 (*C*$H_2$O*C*$H_2$*C*$H_2$O*C*$H_2$, extra signals as a result of rotamers), 166.1 (N*C*(O)N), 171.2, 171.3, 171.9, 172.2, 175.1, 175.2, 176.2 (4 x C*C*=O, extra signals as a result of rotamers); HRMS (ESI$^+$) $C_{27}H_{45}N_7NaO_8S^+$ ([M+Na]$^+$) calculated 650.2943; found 650.2932.

Ethyl 6-(4-benzoylbenzoyl)-4,16-dioxo-20-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-9,12-dioxa-3,6,15-triazaicosan-1-oate, 6e



According to general procedure A: 4-benzoylbenzoic acid (120 mg, 0.53 mmol), ethyl 2-isocyanoacetate (97 µL, 0.53 mmol), paraformaldehyde (24 mg, 0.80 mmol) and **F** (200 mg, 0.53 mmol) were reacted to afford **6e** (238 mg, 62%) as a colourless gum; $\nu_{max}$ (thin film) 3293 (N-H), 2931, 2870 (C-H), 1743 (C=O ester), 1687, 1648 (C=O, amides, urea and ketone); $\delta_H$ (400 MHz, CD$_3$OD) 1.30 (3 H, t, J=7.5 Hz, C*H*$_3$), 1.33–1.49 (2 H, m, C*H*$_2$CH$_2$CH$_2$C(O)N), 1.50–1.81 (4 H, m, SCHC*H*$_2$CH$_2$C*H*$_2$), 2.13–2.24 (2 H, m, CH$_2$C*H*$_2$C(O)N), 2.70 (1 H, d, J=12.5 Hz, SCH*H*), 2.91 (1 H, dd, J=12.5, 5.0 Hz, SCH*H*), 3.12–3.21 (1 H, m, SC*H*), 3.35–3.40 (2 H, m, C(O)NC*H*$_2$CH$_2$O), 3.51–3.75 (8 H, m, C*H*$_2$OC*H*$_2$C*H*$_2$OC*H*$_2$), 3.77–3.88 (2 H, m, OCH$_2$C*H*$_2$NRR'), 3.98, 4.05 (2 H, 2 x s, NHC*H*$_2$CO$_2$Et, 2 rotamers), 4.15–4.25 (2 H, m, CO$_2$C*H*$_2$), 4.20, 4.38 (2 H, 2 x s, NC*H*$_2$C(O)NH, 2 rotamers), 4.29 (1 H, dd, J=7.5, 4.5 Hz, SCHRC*H*), 4.44–4.52 (1 H, m, SCH$_2$C*H*), 7.43–8.06 (9 H, m, 9 x C(Ar)*H*); $\delta_C$ (126 MHz, CD$_3$OD) 14.7 (*C*H$_3$), 26.9, 29.5, 29.8 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 36.8 (CH$_2$*C*H$_2$CONH), 40.4 (C(O)NH*C*H$_2$CH$_2$O), 41.2 (S*C*H$_2$), 42.2, 42.3 (*C*H$_2$CO$_2$Et, 2 rotamers), 48.0, 50.0, 51.5, 54.4 (OCH$_2$*C*H$_2$NRR', 2 rotamers, NRR'*C*H$_2$C(O)N, 2 rotamers), 57.0 (S*C*HRR'), 61.6 (SCH$_2$*C*), 62.5 (*C*H$_2$CH$_3$), 63.3 (SCHR*C*), 69.3, 69.9, 70.8, 71.3, 71.5 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$, extra signals as a result of rotamers), 128.1, 128.6, 129.8, 131.2, 134.2, 138.4, 139.7, 139.9, 140.9, 141.1 (12 x C(Ar), extra signals as a result of rotamers), 166.0 (N*C*(O)N), 171.2, 171.5, 173.8, 173.9, 176.1 (4 x C*C*=O extra signals as a result of rotamers), 196.5 (Ar*C*(O)Ph); HRMS (ESI$^+$) $C_{36}H_{47}N_5NaO_9S^+$ ([M+Na]$^+$) calculated 748.2987; found 748.2964.

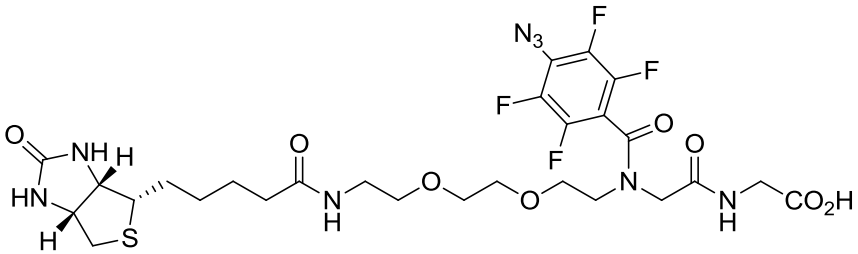
6-(4-Azidobenzoyl)-4,16-dioxo-20-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-9,12-dioxa-3,6,15-triazaicosan-1-oic acid, 12a



According to general procedure B: sodium hydroxide (15 mg, 0.38 mmol) and **6a** (166 mg (0.25 mmol) were reacted to afford **12a** (126 mg, 79%) as a colourless gum; $\nu_{max}$ (thin film) 3288 (N-H), 2924 (C-H), 2127, 2097 (N$_3$), 1680, 1624 (C=O, amides and urea), 1541 (C=C aromatic); $\delta_H$ (400 MHz, CD$_3$OD) 1.41 (2 H, m, C*H*$_2$CH$_2$CH$_2$C(O)N), 1.50–1.80 (4 H, m, SCHC*H*$_2$CH$_2$C*H*$_2$), 2.17–2.21(2 H, m, CH$_2$C*H*$_2$C(O)N), 2.71 (1 H, d,

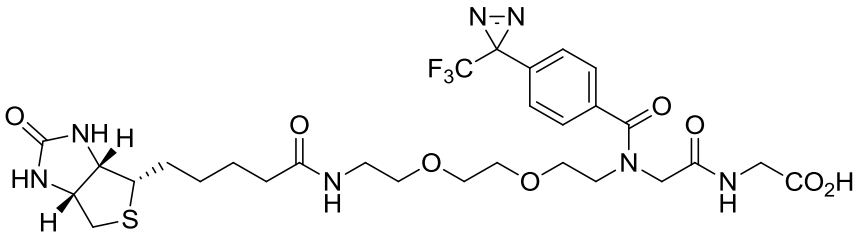
J=12.5 Hz, SCH*H*), 2.85–2.99 (1 H, m, SC*H*H), 3.14–3.23 (1 H, m, SC*H*), 3.34–3.40 (2 H, m, C(O)NC$H_2$C$H_2$O), 3.47–3.84 (10 H, m, C$H_2$OC$H_2$C$H_2$OC$H_2$C$H_2$NRR'), 3.93–4.02 (2 H, m, NHC$H_2$C(O)OH), 4.14, 4.31 (2 H, 2 x br. s., NC$H_2$C(O)NH, 2 rotamers), 4.27–4.34 (1 H, m, SCHRC*H*), 4.46–4.52 (1 H, m, SCH$_2$C*H*), 7.11–7.18 (2 H, m, 2 x C(Ar)*H*), 7.53 – 7.64 (2 H, m, 2 x C(Ar)*H*); $\delta_H$ (500 MHz, T = 363 K, DMSO-$d_6$) 1.31–1.42 (2 H, m. C$H_2$CH$_2$CH$_2$C(O)N), 1.44–1.73 (4 H, m, SCHC$H_2$CH$_2$C$H_2$), 2.10 (2 H, t, *J*=7.5 Hz, CH$_2$C$H_2$C(O)N), 2.63 (1 H, d, *J*=12.5 Hz, SCH*H*), 2.86 (1 H, dd, *J*=12.5, 5.0 Hz, SC*H*H), 3.10–3.17 (1 H, m, SC*H*), 3.23 (2 H, q, *J*=6.0 Hz, C(O)NHC$H_2$CH$_2$O), 3.45 (2 H, t, *J*=6.0 Hz, C(O)NHCH$_2$C$H_2$O), 3.53 (6 H, s, OC$H_2$C$H_2$OC$H_2$CH$_2$NRR'), 3.58–3.64 (2 H, m, CH$_2$C$H_2$NRR'), 3.82 (2 H, d, *J*=6.0 Hz, NHC$H_2$CO$_2$H), 4.07 (2 H, br. s., NC$H_2$C(O)NH), 4.15–4.18 (1 H, m, SCHRC*H*), 4.31–4.36 (1 H, m, SCH$_2$C*H*), 6.06 (1 H, br. s., N*H*), 6.07 (1 H, br. s., N*H*), 7.15 (2 H, d, *J*=8.5 Hz, 2 x C(Ar)*H*), 7.44 (1 H, br. s., N*H*), 7.49 (2 H, d, *J*=8.5 Hz, 2 x C(Ar)*H*), 7.97 (1 H, br. s., N*H*); $\delta_C$ (126 MHz, CD$_3$OD) 27.0, 29.6, 29.9 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 36.9 (CH$_2$*C*H$_2$CONH), 40.5 (C(O)NH*C*H$_2$CH$_2$O), 41.2 (S*C*H$_2$), 42.0 (*C*H$_2$CO$_2$H), 48.3, 51.8 (OCH$_2$C$H_2$NRR' 2 rotamers), 50.3, 54.9 (NC$H_2$C(O)NH, 2 rotamers), 57.1 (S*C*HRR'), 61.8 (SCH$_2$*C*), 63.5 (SCHR*C*), 69.4, 70.0, 70.8, 71.5, 71.6 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$, extra signals as a result of rotamers), 120.2, 120.3, 130.2, 130.6, 133.6, 143.4, 143.7 (6 x *C*(Ar), extra signals as a result of rotamers), 166.3 (NHC(O)NH), 171.8 (C*C*(O)N), 173.1, 174.4 (C*C*(O)N, 2 x rotamers), 176.4 (C*C*(O)N); HRMS (ESI$^+$) $C_{27}H_{38}N_8NaO_8S^+$ ([M+Na]$^+$) calculated 657.2426; found 657.2428.

6-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-4,16-dioxo-20-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-9,12-dioxa-3,6,15-triazaicosan-1-oic acid, 12b



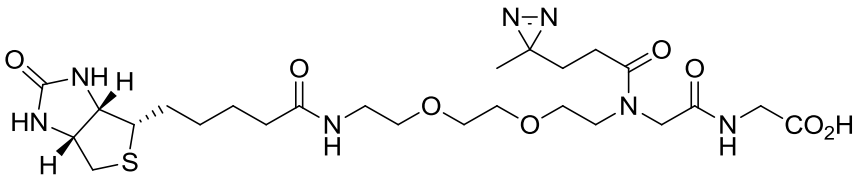

According to general procedure B: sodium hydroxide (12 mg, 0.30 mmol) and **6b** (143 mg, 0.19 mmol) were reacted to afford **12b** (114 mg, 85%) as a colourless gum; $\nu_{max}$ (thin film) 2929, 2868 (C-H), 2128, ($N_3$), 1646 (C=O, amides, urea, acid); $\delta_H$ (400 MHz, CD$_3$OD) 1.37–1.51 (2 H, m, C$H_2$CH$_2$CH$_2$C(O)N), 1.53–1.83 (4 H, m, SCHC$H_2$CH$_2$C$H_2$), 2.18–2.30 (2 H, m, CH$_2$C$H_2$C(O)N), 2.72 (1 H, d, J=12.5 Hz, SCH*H*), 2.94 (1 H, d, J=12.5 Hz, SC*H*H), 3.14–3.26 (1 H, m, SC*H*), 3.34–3.41 (2 H, m, C(O)NC$H_2$CH$_2$O), 3.49–3.69 (8 H, m, C$H_2$OC$H_2$C$H_2$OC$H_2$), 3.72–3.79, 3.79–3.85 (2 H, 2 x m, OCH$_2$C$H_2$NRR', 2 rotamers), 3.88, 4.01 (1 H, 2 x br. s., NHC$H_2$C(O)OH), 4.20, 4.37 (2 H, 2 x br. s., NC$H_2$C(O)NH, 2 rotamers), 4.28–4.35 (1 H, m, SCHRC*H*), 4.48–4.54 (1 H, m, SCH$_2$C*H*); $\delta_C$ (126 MHz, CD$_3$OD) 27.0, 29.6, 29.9 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 36.8 (CH$_2$*C*H$_2$CONH), 40.4 (C(O)NH*C*H$_2$CH$_2$O), 41.2 (C(O)NH*C*H$_2$CH$_2$O), 41.8 (S*C*H$_2$), 42.1 (*C*H$_2$CO$_2$H), 50.3, 51.6, 53.5 (OCH$_2$*C*H$_2$NRR', NRR'*C*H$_2$C(O)N, extra signals as a result of rotamers), 57.1 (S*C*HRR'), 61.8 (SCH$_2$*C*), 63.5 (SCHR*C*), 69.2, 69.9, 70.8, 71.3, 71.4, 71.6 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$, extra signals as a result of rotamers), 166.2 (N*C*(O)N), 170.4, 170.6, 172.7, 173.0, 176.3 (4 x C*C*=O, extra signals as a result of rotamers); $\delta_F$ (470 MHz, CD$_3$OD) -153.1 to -152.9, -152.8 to -152.6 (2 F, 2 x m, 2 rotamers), -143.4 to -143.2, -143.1 to -142.8 (2 F, 2 x m, 2 rotamers); HRMS (ESI$^+$) $C_{27}H_{34}F_4N_8NaO_8S^+$ ([M+Na]$^+$) calculated 729.2049; found 729.2062.

4,16-Dioxo-20-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-6-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzoyl)-9,12-dioxa-3,6,15-triazaicosan-1-oic acid, 12c



According to general procedure B: sodium hydroxide (20 mg, 0.50 mmol) and **6c** (208 mg, 0.29 mmol) were reacted to afford **12c** (206 mg, quantitative) as a colourless gum. $\nu_{max}$ (thin film) 3296 (N-H), 2930 (C-H), 1628 (C=O, amides, urea), 1151 ($CF_3$); $\delta_H$ (400 MHz, $CD_3OD$) 1.34–1.50 (2 H, m, C$H_2$CH$_2$CH$_2$C(O)N), 1.52–1.81 (4 H, m, SCHC$H_2$CH$_2$C$H_2$), 2.20 (2 H, t, J=7.0 Hz, CH$_2$C$H_2$C(O)N), 2.71 (1 H, d, J=13.0 Hz, SCH*H*), 2.86–2.98 (1 H, m, SC*H*H), 3.14–3.26 (1 H, m, SC*H*), 3.34–3.41 (2 H, m, C(O)NC$H_2$CH$_2$O), 3.51–3.69 (8 H, m, C$H_2$OC$H_2$C$H_2$OC$H_2$), 3.73–3.85 (2 H, m, OCH$_2$C$H_2$NRR'), 3.95, 4.02 (2 H, 2 x br. s., NHC$H_2$C(O)OH, 2 rotamers), 4.12, 4.35 (2 H, 2 x br. s., NC$H_2$C(O)NH, 2 rotamers), 4.27–4.33 (1 H, m, SCHRC*H*), 4.45–4.54 (1 H, m, SCH$_2$C*H*), 7.33–7.39 (2 H, m, 2 x C(Ar)*H*), 7.57–7.71 (2 H, m, 2 x C(Ar)*H*); $\delta_C$ (101 MHz, $CD_3OD$) 26.9, 29.5, 29.8 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 29.5 (app. d, *J*=39.9 Hz, *C*N$_2$), 36.8 (CH$_2$*C*H$_2$CONH), 40.4 (C(O)NH*C*H$_2$CH$_2$O), 41.2 (S*C*H$_2$), 42.0, 42.1 (*C*H$_2$CO$_2$H, 2 rotamers), 48.1, 51.6 (OCH$_2$*C*H$_2$NRR' 2 rotamers) 50.1, 54.5 (NC$H_2$C(O)NH, 2 rotamers), 57.1 (S*C*HRR'), 61.7 (SCH$_2$*C*), 63.4 (SCHR*C*), 69.2, 69.9, 70.7, 70.8, 71.3, 71.5 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$, extra signals as a result of rotamers), 123.2 (app. d, *J*=273.2 Hz, *C*F$_3$), 127.8, 128.0, 128.9, 129.4, 131.4, 131.7, 138.7, 138.9 (6 x C(Ar), extra signals as a result of rotamers), 166.1 (N*C*(O)N), 171.4, 172.9, 173.1, 173.6, 173.7, 176.2 (4 x C*C*=O extra signals as a result of rotamers); $\delta_F$ (470 MHz, $CD_3OD$) -66.9 (3 F, s.); HRMS (ESI$^+$) $C_{29}H_{39}F_3N_7O_8S^+$ ([M+H]$^+$) calculated 702.2527; found 702.2527.

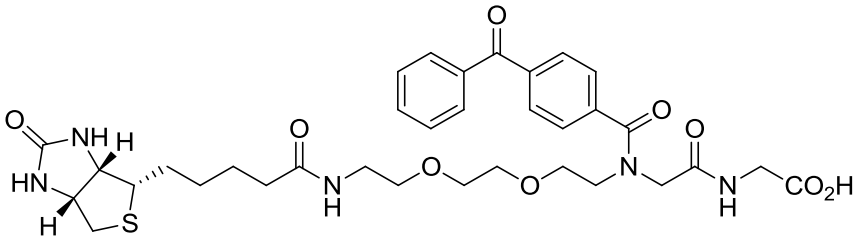
6-(3-(3-Methyl-3*H*-diazirin-3-yl)propanoyl)-4,16-dioxo-20-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-9,12-dioxa-3,6,15-triazaicosan-1-oic acid, 12d



According to general procedure B: sodium hydroxide (20 mg, 0.50 mmol) and **6d** (243 mg, 0.39 mmol) were reacted to afford **12d** (237 mg, 100%) as a colourless gum; $\nu_{max}$ (thin film) 3293 (N-H), 2928 (C-H), 1635 (C=O, amides, urea); $\delta_H$ (400 MHz, $CD_3OD$) 1.00–1.06 (3 H, m, C$H_3$), 1.38–1.52 (2 H, m, C$H_2$CH$_2$CH$_2$C(O)N), 1.53–1.82 (6 H, m, SCHC$H_2$CH$_2$C$H_2$, CN$_2$C$H_2$), 2.19–2.37 (2 H, m, CH$_2$C$H_2$C(O)N), 2.29, 2.44 (2 H, t, J=7.5 Hz, CN$_2$CH$_2$C$H_2$), 2.73 (1 H, d, J=12.5 Hz, SCH*H*), 2.90–3.00 (1 H, m, SC*H*H), 3.18–3.27 (1 H, m, SC*H*), 3.34–3.41 (2 H, m, C(O)NC$H_2$CH$_2$O), 3.50–3.72 (10 H, m, C$H_2$OC$H_2$C$H_2$OC$H_2$C$H_2$NRR'), 3.95, 3.99 (2 H, 2 x br. s., NHC$H_2$C(O)OH, 2 rotamers), 4.14, 4.25 (2 H, 2 x br. s., NC$H_2$C(O)NH, 2 rotamers), 4.30–4.37 (1 H, m, SCHRC*H*), 4.48–4.55 (1 H, m, SCH$_2$C*H*); $\delta_C$ (101 MHz, $CD_3OD$) δ ppm 19.8, 19.9 (CN$_2$*C*H$_3$, 2 rotamers), 26.3, 26.4 (*C*H$_2$CN$_2$, 2 rotamers), 26.7, 29.4, 29.6 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 28.1, 28.3 (*C*H$_2$CH$_2$CN$_2$), 30.6, 30.8 (*C*N$_2$), 36.6 (CH$_2$*C*H$_2$CONH), 40.2 (C(O)NH*C*H$_2$CH$_2$O), 41.0 (S*C*H$_2$), 41.7 (*C*H$_2$CO$_2$H), 50.0 (OCH$_2$*C*H$_2$NRR'), 50.6, 53.1 (NRR'*C*H$_2$C(O)N, 2 rotamers), 56.9 (S*C*HRR'), 61.5 (SCH$_2$*C*), 63.2 (SCHR*C*), 69.6, 70.0, 70.5, 70.6, 71.1, 71.2, 71.5

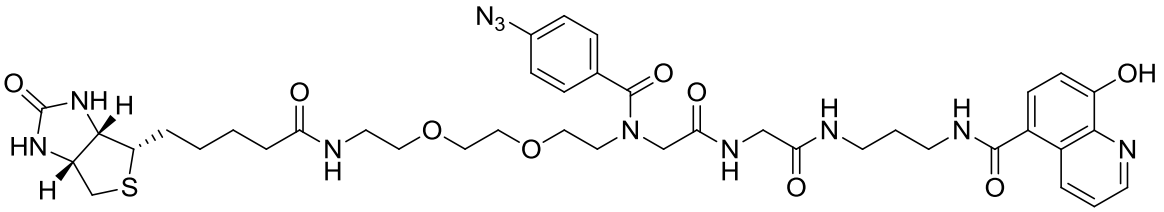
($CH_2OCH_2CH_2OCH_2$, extra signals as a result of rotamers), 165.9 (N*C*(O)N), 171.5, 171.8, 172.7, 172.8, 174.9, 174.9, 176.0 (4 x C*C*=O, extra signals as a result of rotamers); HRMS ($ESI^+$) $C_{25}H_{42}N_7O_8S^+$ ($[M+H]^+$) calculated 600.2810; found 600.2830.

6-(4-Benzoylbenzoyl)-4,16-dioxo-20-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-9,12-dioxa-3,6,15-triazaicosan-1-oic acid, 12e



According to general procedure B: sodium hydroxide (38 mg, 0.95 mmol) and **6e** (458 mg, 0.63 mmol) were reacted to afford **12e** (419 mg, 95%) as a colourless gum; $\nu_{max}$ (thin film) 3305 (N-H), 2929, 2868 (C-H), 1643 (C=O, amides, urea, ketone); $\delta_H$ (400 MHz, $CD_3OD$) 1.28–1.44 (2 H, m, C$H_2$CH$_2$CH$_2$C(O)N), 1.47–1.79 (4 H, m, SCHC$H_2$CH$_2$C$H_2$), 2.09–2.25 (2 H, m, CH$_2$C$H_2$C(O)N), 2.68 (1 H, d, J=12.5 Hz, SCH*H*), 2.81–2.95 (1 H, m, SC*H*H), 3.07–3.20 (1 H, m, SC*H*), 3.34–3.42 (2 H, m, C(O)NHC$H_2$CH$_2$O), 3.47–3.71 (8 H, m, C$H_2$OC$H_2$C$H_2$OC$H_2$), 3.75–3.86 (2 H, m, OCH$_2$C$H_2$NRR'), 3.97, 4.04 (2 H, 2 x br.s., NHC$H_2$C(O)OH, 2 rotamers), 4.20, 4.38 (2 H, 2 x br. s., NC$H_2$C(O)NH, 2 rotamers), 4.22–4.31 (1 H, m, SCHRC*H*), 4.42–4.52 (1 H, m, SCH$_2$C*H*), 7.43–8.03 (9 H, m, 9 x C(Ar)H); $\delta_C$ (101 MHz, $CD_3OD$) 26.9, 29.5, 29.8 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 36.8 (CH$_2$*C*H$_2$CONH), 40.4 (C(O)NH*C*H$_2$CH$_2$O), 41.2 (S*C*H$_2$), 42.1 (*C*H$_2$CO$_2$H), 48.0, 51.6 (OCH$_2$C*H*$_2$NRR' 2 rotamers) 50.1, 54.4 (NC$H_2$C(O)NH, 2 rotamers), 57.0 (S*C*HRR'), 61.6 (SCH$_2$*C*), 63.3 (SCHR*C*), 69.2, 69.8, 70.7, 71.3, 71.4 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$, extra signals as a result of rotamers), 128.1, 128.6, 129.8, 131.2, 134.2, 138.3, 139.6, 139.8, 140.9, 141.0 (12 x C(Ar), extra signals as a result of rotamers), 166.0 (N*C*(O)N), 171.3, 172.8, 173.0, 173.7, 173.9, 176.0, (4 x C*C*=O extra signals as a result of rotamers), 196.5 (Ar*C*(O)Ph); HRMS ($ESI^+$) $C_{34}H_{44}N_5O_9S^+$ ($[M+H]^+$) calculated 698.2854; found 698.2856.

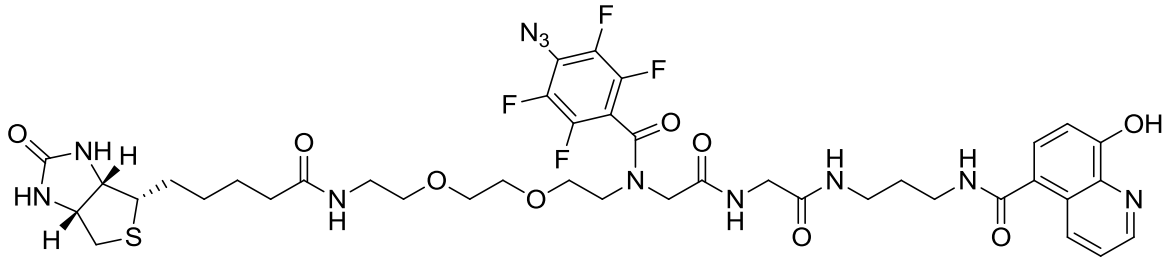
*N*-(10-(4-Azidobenzoyl)-5,8,20-trioxo-24-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-13,16-dioxa-4,7,10,19-tetraazatetracosyl)-8-hydroxyquinoline-5-carboxamide, 1



According to general procedure C: **12a** (65 mg, 0.10 mmol), HBTU (39 mg, 0.10 mmol) and **23** (37 mg, 0.10 mmol) were reacted to afford **1** (47 mg, 55%) as a pale yellow gum; $\nu_{max}$ (thin film) 3272 (N-H), 2932 (C-H), 2129, 2099 ($N_3$), 1662 (C=O, amides and urea); $\delta_H$ (400 MHz, $CD_3OD$) 1.35–1.43 (2 H, m, C$H_2$CH$_2$CH$_2$C(O)N), 1.50–1.77 (4 H, m, SCHC$H_2$CH$_2$C$H_2$), 1.80–1.92 (2 H, m, NCH$_2$C$H_2$CH$_2$N), 2.18 (2 H, t, J = 7.0 Hz, CH$_2$C$H_2$C(O)N), 2.70 (1 H, d, J=12.5 Hz, SCH*H*), 2.87–2.94 (1 H, m, SC*H*H), 3.12–3.21 (1 H, m, SC*H*), 3.35–3.80 (16 H, m,C$H_2$CH$_2$OC$H_2$C$H_2$OC$H_2$C$H_2$, NC$H_2$CH$_2$C$H_2$N), 3.90–3.97 (2 H, m, NHC$H_2$C(O)NH), 4.18–4.33 (3 H, m, NRR'C$H_2$C(O)NH, SCHRC*H*), 4.43–4.52 (1 H, m, SCH$_2$C*H*), 7.00 – 7.10 (3 H, m, 2 x C(Ar)*H*, C(quinoline ring)*H*), 7.49–7.54 (2 H, m, 2 x C(Ar)*H*), 7.57–7.65 (1 H, m,

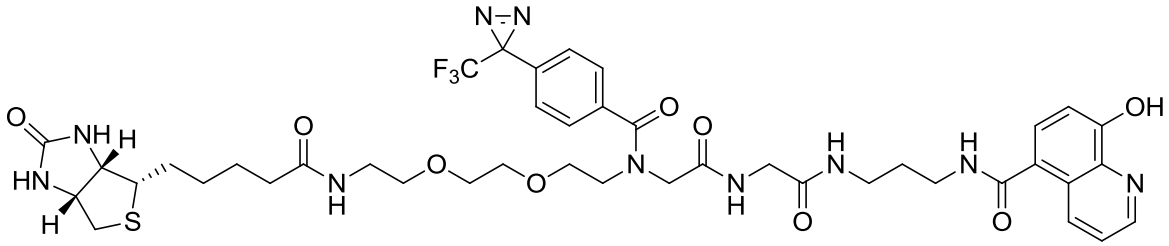
C(quinoline ring)*H*), 7.65–7.80 (1 H, m, C(quinoline ring)*H*), 8.82–8.89 (2 H, m, C(quinoline ring)*H*); $\delta_C$ (126 MHz, $CD_3OD$) 26.9, 29.5, 29.8 (SCH*C*$H_2$*C*$H_2$*C*$H_2$), 30.0 (NC$H_2$*C*$H_2$C$H_2$N), 36.8 (C$H_2$*C*$H_2$CONH), 37.6, 37.9 (N*C*$H_2$C$H_2$*C*$H_2$N), 40.3 (C(O)NH*C*$H_2$C$H_2$O), 41.1 (S*C*$H_2$), 43.9 (*C*$H_2$CONHC$H_2$C$H_2$), 48.0, 51.2, 52.0, 54.7 (OC$H_2$*C*$H_2$NRR', 2 rotamers, NRR'*C*$H_2$C(O)N, 2 rotamers), 57.0 (S*C*HRR'), 61.6 (SC$H_2$*C*), 63.4 (SCHR*C*), 69.7, 70.7, 71.3, 71.4 (*C*$H_2$O*C*$H_2$*C*$H_2$O*C*$H_2$), 110.9 (*C*(quinoline), 119.4, (*C*$N_3$), 123.7 *C*(quinoline), 128.2, 129.4 (2 x *C*(quinoline)), 130.4 (2 x *C*(Ar)H), 133.1 (2 x *C*(Ar)H), 136.2, 139.5 (2 x *C*(quinoline)), 143.3 (*C*(Ar)C(O)NRR'), 149.5, 156.6 (2 x *C*(quinoline)), 166.1 (NC(O)N), 171.1, 171.9, 172.1, 174.7, 176.2 (5 x C(O)N); HRMS ($ESI^+$) $C_{40}H_{51}N_{11}NaO_9S^+$ ($[M+Na]^+$) calculated 884.3484; found 884.3478.

*N*-(10-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-5,8,20-trioxo-24-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-13,16-dioxa-4,7,10,19-tetraazatetracosyl)-8-hydroxyquinoline-5-carboxamide, 2



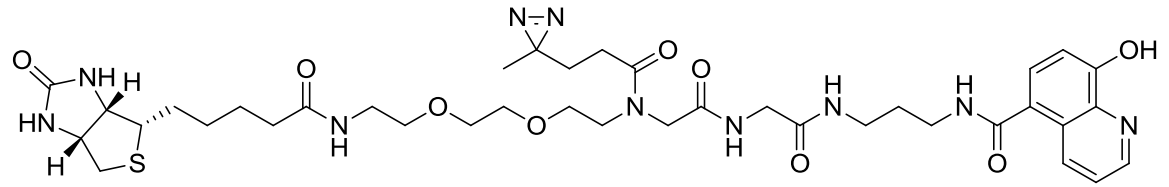
According to general procedure C: **12b** (73 mg, 0.10 mmol), HBTU (39 mg, 0.10 mmol) and **23** (37 mg, 0.10 mmol) were reacted to afford **2** (63 mg, 68%) as a pale yellow gum; $\nu_{max}$ (thin film) 3293 (N-H), 2931, 2865 (C-H), 2128, ($N_3$), 1638 (C=O, amides and urea); $\delta_H$ (400 MHz, $CD_3OD$) 1.36–1.49 (2 H, m, C*H*$_2$C$H_2$C$H_2$C(O)N), 1.50–1.91 (6 H, m, SCHC*H*$_2$C$H_2$C*H*$_2$, NC$H_2$C*H*$_2$C$H_2$N), 2.21 (2 H, t, J=7.0 Hz, C$H_2$C*H*$_2$C(O)N), 2.70 (1 H, d, J=12.5 Hz, SCH*H*), 2.92 (1 H, dd, J=12.5, 5.0 Hz, SC*H*H), 3.12–3.24 (1 H, m, SC*H*), 3.35–3.65 (14 H, m, C(O)NH(C*H*$_2$C*H*$_2$O)$_2$C*H*$_2$, NC*H*$_2$C$H_2$C*H*$_2$N), 3.67–3.99 (4 H, m, OC$H_2$C*H*$_2$NRR', NHC*H*$_2$C(O)NH), 4.23–4.36 (3 H, m, NRR'C*H*$_2$C(O)NH, SCHRC*H*), 4.44–4.53 (1 H, m, SC$H_2$C*H*), 7.07, 7.14 (1 H, 2 x d, J=8.0 Hz, C(quinoline ring)*H*, 2 rotamers), 7.60 (1 H, dd, J=9.0, 4.0 Hz), 7.70, 7.78 (1 H, d, J=8.0 Hz, C(quinoline ring)*H*, 2 rotamers), 8.76–8.90 (2 H, m, 2 x C(quinoline ring)*H*); $\delta_C$ (126 MHz, $CD_3OD$) 26.9, 29.5, 29.8 (SCH*C*$H_2$*C*$H_2$*C*$H_2$), 29.9, 30.3 (NC$H_2$*C*$H_2$C$H_2$N, 2 rotamers), 36.8 (C$H_2$*C*$H_2$CONH), 37.6, 37.9 (N*C*$H_2$C$H_2$*C*$H_2$N), 40.2, 40.4 (C(O)NH*C*$H_2$C$H_2$O, 2 rotamers), 41.1 (S*C*$H_2$), 43.7, 43.9 (*C*$H_2$CONHC$H_2$C$H_2$, 2 rotamers), 47.9, 51.3, 51.9, 53.6 (OC$H_2$*C*$H_2$NRR', 2 rotamers, NRR'*C*$H_2$C(O)N, 2 rotamers), 57.1 (S*C*HRR'), 61.6 (SC$H_2$*C*), 63.4 (SCHR*C*), 69.1, 69.8, 70.7, 71.1, 71.3, 71.5, 71.6 (*C*$H_2$O*C*$H_2$*C*$H_2$O*C*$H_2$ extra signals as a result of rotamers), 110.5 (*C*(quinoline)), 111.8 (m, *C*($N_3$)), 123.1 (m, *C*C(O)NRR'), 123.7, 125.2, 128.1, 128.3, 129.2, 129.4, 135.8, 139.7, 139.8 (6 x *C*(quinoline) extra signals as a result of rotamers), 141.0, 143.0, 145.1 (3 x m, 4 x *C*F), 149.7, 156.8 (2 x *C*(quinoline)), 166.1 (NC(O)N), 170.6, 170.9, 171.1, 171.2, 171.3, 171.7, 176.2 (5 x C(O)N, extra signals as a result of rotamers); $\delta_F$ (470 MHz, $CD_3OD$) -152.8 to -152.6 (2 F, m), -143.3 to -143.1, -143.1 to -142.9 (2 F, 2 x m, as a result of rotamers); HRMS ($ESI^+$) $C_{40}H_{47}F_4N_{11}NaO_9S^+$ ($[M+Na]^+$) calculated 956.3107; found 956.3092.

8-Hydroxy-*N*-(5,8,20-trioxo-24-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-10-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzoyl)-13,16-dioxa-4,7,10,19-tetraazatetracosyl)quinoline-5-carboxamide, 3



According to general procedure C: **12c** (130 mg, 0.19 mmol), HBTU (70 mg, 0.19 mmol) and **23** (68 mg, 0.19 mmol) were reacted to afford **3** (56 mg, 60%) as a pale yellow gum; $\nu_{max}$ (thin film) 3294 (N-H), 2932, 2871 (C-H), 2129, 2099 (azide), 1631 (C=O, amides and urea); $\delta_H$ (400 MHz, $CD_3OD$) 1.33–1.47 (2 H, m, C*H*$_2$CH$_2$CH$_2$C(O)N), 1.48–1.76 (4 H, m, SCHC*H*$_2$CH$_2$C*H*$_2$), 1.80–1.87 (2 H, m, NCH$_2$C*H*$_2$CH$_2$N), 2.18 (2 H, t, J=7.5 Hz, CH$_2$C*H*$_2$C(O)N), 2.70 (1 H, d, J=12.5 Hz, SCH*H*), 2.91 (1 H, dd, J=12.5, 4.5 Hz, SC*H*H), 3.13–3.20 (1 H, m, SC*H*), 3.35–3.79 (16 H, m, C*H*$_2$C*H*$_2$OC*H*$_2$C*H*$_2$OC*H*$_2$C*H*$_2$, NC*H*$_2$CH$_2$C*H*$_2$N), 3.90, 3.96 (2 H, 2 x br. s., NHC*H*$_2$C(O)NH, 2 rotamers), 4.17, 4.31 (2 H, br. s., NRR'C*H*$_2$C(O)NH, 2 rotamers), 4.25–4.29 (1 H, m, SCHRC*H*), 4.47–4.51 (1 H, m, SCH$_2$C*H*), 7.08, 7.12 (1 H, d, J=8.0 Hz, C(quinoline ring)*H*, 2 rotamers), 7.26–7.34 (2 H, m, 2 X C(Ar)*H*), 7.50–7.80 (4 H, m, 2 x C(Ar)*H*, 2 x C(quinoline ring)*H*), 8.75–8.89 (2 H, m, 2 x C(quinoline ring)*H*); $\delta_C$ (126 MHz, $CD_3OD$) 26.8, 29.5, 29.8 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 29.4 (q, *J*=42.0 Hz, *C*CF$_3$), 30.1 (NCH$_2$*C*H$_2$CH$_2$N), 36.8 (CH$_2$*C*H$_2$CONH), 37.7, 38.0 (N*C*H$_2$CH$_2$*C*H$_2$N), 40.3 (C(O)NH*C*H$_2$CH$_2$O), 41.1 (S*C*H$_2$), 43.6, 43.9 (*C*H$_2$CONHCH$_2$CH$_2$, 2 rotamers), 47.9, 50.9, 51.9, 54.5 (OCH$_2$*C*H$_2$NRR', 2 rotamers, NRR'*C*H$_2$C(O)N, 2 rotamers), 57.0 (S*C*HRR'), 61.6 (SCH$_2$*C*), 63.4 (SCHR*C*), 69.3, 69.8, 70.7, 71.3, 71.4 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$ extra signals as a result of rotamers), 110.6 (*C*(quinoline)), 123.5 (q, *J*=275.6 Hz, CF$_3$), 123.7 (*C*(quinoline)), 125.2 (*C*(quinoline)), 127.7, 127.9 (*C*(Ar), 2 rotamers), 128.2 (*C*(quinoline)), 128.7, 128.8 (*C*(Ar), 2 x rotamers), 129.3 (*C*(quinoline)), 131.5 (*C*(Ar)), 135.7 (*C*(quinoline)), 138.6, 138.8 (*C*(Ar), 2 rotamers), 139.8 (*C*(quinoline)), 149.7 (*C*(quinoline)), 156.8 (*C*(quinoline)), 166.1 (NC(O)N), 171.2, 171.4, 171.5, 171.8, 173.6, 174.0, 176.2 (5 x C(O)N, extra signals as a result of rotamers); $\delta_F$ (470 MHz, $CD_3OD$) -66.81 (3 F, s); HRMS (ESI$^+$) $C_{42}H_{51}F_3N_{10}NaO_9S^+$ ([M+Na]$^+$) calculated 951.3405; found 951.3408.

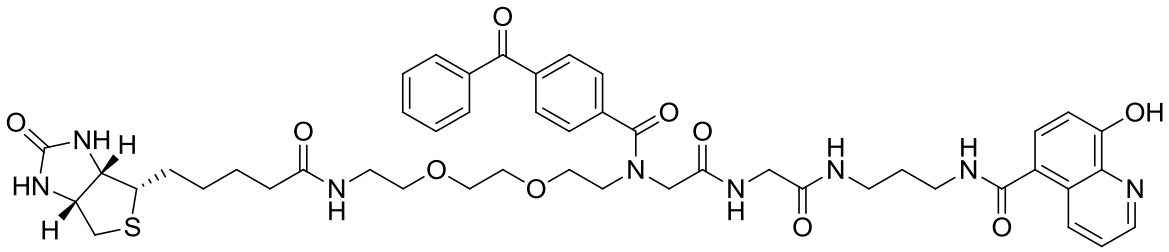
8-Hydroxy-*N*-(10-(3-(3-methyl-3*H*-diazirin-3-yl)propanoyl)-5,8,20-trioxo-24-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-13,16-dioxa-4,7,10,19-tetraazatetracosyl)quinoline-5-carboxamide, 4



According to general procedure C: **12d** (51 mg, 0.09 mmol), HBTU (32 mg, 0.09 mmol) and **23** (32 mg, 0.09 mmol) were reacted to afford **4** (43 mg, 58%) as a pale yellow gum; $\nu_{max}$ (thin film) 3277 (N-H), 2926, 2858 (C-H), 1635 (C=O, amides and urea); $\delta_H$ (400 MHz, $CD_3OD$) 0.94–1.02 (3 H, m, C*H*$_3$), 1.37–1.48 (2 H, m, C*H*$_2$CH$_2$CH$_2$C(O)N), 1.50–1.78 (6 H, m, SCHC*H*$_2$CH$_2$C*H*$_2$, CN$_2$C*H*$_2$), 1.80–1.94 (2 H, m, NCH$_2$C*H*$_2$CH$_2$N), 2.16–2.26 (2 H, m, CH$_2$C*H*$_2$C(O)N), 2.36–2.47 (1 H, m, CN$_2$CH$_2$C*H*$_2$), 2.70 (1 H, d, J=12.5 Hz, SCH*H*), 2.91 (1 H, dd, J=12.5, 5.0 Hz, SCH*H*), 3.12–3.26 (1 H, m, SC*H*), 3.35–3.71 (16 H, m,

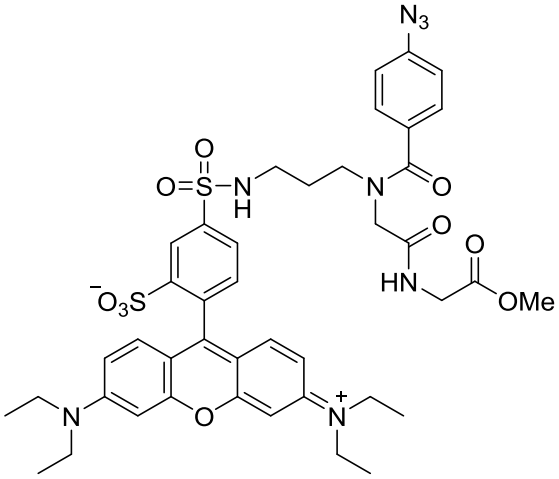
C*H*$_2$C*H*$_2$OC*H*$_2$C*H*$_2$OC*H*$_2$C*H*$_2$, NC*H*$_2$CH$_2$C*H*$_2$N), 3.84–3.98 (2 H, m, NHC*H*$_2$C(O)NH), 4.07, 4.29 (2 H, 2 x br. s., NRR'C*H*$_2$C(O)NH, 2 rotamers), 4.25–4.32 (1 H, m, SCHRC*H*), 4.42–4.55 (1 H, m, SCH$_2$C*H*); $\delta_C$ (126 MHz, CD$_3$OD) 19.9, 20.0 (CN$_2$CH$_3$, 2 rotamers), 26.4, 26.5 (*C*H$_2$CN$_2$, 2 rotamers), 26.9, 29.5, 29.8 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 28.3, 28.4 (*C*H$_2$CH$_2$CN$_2$), 30.1 (NCH$_2$*C*H$_2$CH$_2$N), 30.7, 30.8 (*C*N$_2$), 36.8 (CH$_2$*C*H$_2$CONH), 37.5, 37.7 (CH$_2$*C*H$_2$CONH, 2 rotamers), 37.9, 38.1 (N*C*H$_2$CH$_2$*C*H$_2$N, 2 rotamers), 40.2, 40.3 (C(O)NH*C*H$_2$CH$_2$O, 2 rotamers), 41.1 (S*C*H$_2$), 43.7, 43.8 (*C*H$_2$CONHCH$_2$CH$_2$, 2 rotamers), 47.9, 50.5, 51.9, 53.3 (OCH$_2$*C*H$_2$NRR', 2 rotamers, NRR'*C*H$_2$C(O)N, 2 rotamers), 57.0 (S*C*HRR'), 61.6 (SCH$_2$*C*), 63.4 (SCHR*C*), 69.8, 70.2, 70.7, 71.3, 71.7 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$ extra signal as a result of rotamers), 110.6, 123.7, 125.4, 128.2, 129.3, 135.8, 139.9, 149.7, 156.8 (9 x *C*(quinoline)), 166.1 (NC(O)N), 171.3, 171.4, 171.5, 171.9, 172.5, 175.1, 175.6, 176.2 (5 x C(O)N, extra signals as a result of rotamers); HRMS (ESI$^+$) $C_{38}H_{54}N_{10}NaO_9S^+$ ([M+Na]$^+$) calculated 849.3688; found 849.3679.

*N*-(10-(4-Benzoylbenzoyl)-5,8,20-trioxo-24-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-13,16-dioxa-4,7,10,19-tetraazatetracosyl)-8-hydroxyquinoline-5-carboxamide, 5



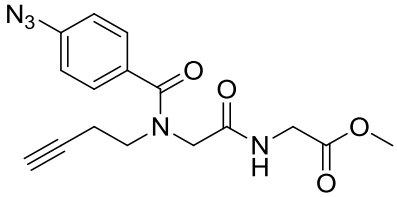
According to general procedure C: **12e** (57 mg, 0.08 mmol), HBTU (31 mg, 0.08 mmol) and **23** (29 mg, 0.08 mmol) were reacted to afford **5** (47 mg, 64%) as a pale yellow gum; $\nu_{max}$ (thin film) 3290 (N-H), 2936 (C-H), 1643 (C=O, amides and urea); $\delta_H$ (400 MHz, CD$_3$OD) 1.31–1.44 (2 H, m, C*H*$_2$CH$_2$CH$_2$C(O)N), 1.47–1.75 (4 H, m, SCHC*H*$_2$CH$_2$C*H*$_2$), 1.77–1.92 (2 H, m, NCH$_2$C*H*$_2$CH$_2$N), 2.17 (2 H, t, J=7.0 Hz, CH$_2$C*H*$_2$C(O)N), 2.68 (1 H, d, J=12.5 Hz, SCH*H*), 2.89 (1 H, dd, J=12.5, 5.0 Hz, SC*H*H), 3.10–3.18 (1 H, m, SC*H*), 3.35–3.82 (16 H, m, C*H*$_2$C*H*$_2$OC*H*$_2$C*H*$_2$OC*H*$_2$C*H*$_2$, NC*H*$_2$CH$_2$C*H*$_2$N), 3.88–4.01 (2 H, m, NHC*H*$_2$C(O)NH), 4.20–4.37 (3 H, m, NRR'C*H*$_2$C(O)NH, SCHRC*H*), 4.46 (1 H, dd, J=7.5, 5.0 Hz, SCH$_2$C*H*), 7.04–7.09 (1 H, m, C(quinoline ring)*H*), 7.47–7.84 (11 H, m, 9 x C(benzophenone)*H*, 2 x C(quinoline ring)*H*), 8.76–8.86 (2 H, m, 2 x C(quinoline ring)*H*); $\delta_C$ (126 MHz, CD$_3$OD) 26.8, 29.5, 29.8 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 30.1 (NCH$_2$*C*H$_2$CH$_2$N), 36.7 (CH$_2$*C*H$_2$CONH), 37.7, 38.0 (N*C*H$_2$CH$_2$*C*H$_2$N), 40.3 (C(O)NH*C*H$_2$CH$_2$O), 41.1 (S*C*H$_2$), 43.7, 43.9 (*C*H$_2$CONHCH$_2$CH$_2$, 2 rotamers), 47.9, 51.0, 51.9, 54.5 (OCH$_2$*C*H$_2$NRR', 2 rotamers, NRR'*C*H$_2$C(O)N, 2 rotamers), 57.0 (S*C*HRR'), 61.6 (SCH$_2$*C*), 63.4 (SCHR*C*), 69.3, 69.8, 70.7, 71.3, 71.5 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$ extra signals as a result of rotamers), 110.6 (*C*(quinoline)), 123.7 (*C*(quinoline)), 125.3 (*C*(quinoline)), 128.0 (*C*(benzophenone)), 128.2 (*C*(quinoline)), 128.5 (*C*(benzophenone)), 129.2 (*C*(quinoline)), 129.7 (*C*(benzophenone)), 131.1 (*C*(benzophenone)), 134.1 (*C*(benzophenone)), 135.7 (*C*(quinoline)), 138.4 (*C*(benzophenone)), 139.8, 139.9 (*C*(quinoline), *C*(benzophenone)), 140.7 (*C*(benzophenone)), 149.7 (*C*(quinoline)), 156.8 (*C*(quinoline)), 166.1 (NC(O)N), 171.2, 171.8, 171.9, 174.4, 176.2 (5 x C(O)N, extra signals as a result of rotamers), 197.5 (Ar*C*(O)Ph); HRMS (ESI$^+$) $C_{47}H_{56}N_8NaO_{10}S^+$ ([M+Na]$^+$) calculated 947.3732; found 947.3739.

5-(*N*-(3-(4-Azido-*N*-(2-((2-methoxy-2-oxoethyl)amino)-2-oxoethyl)benzamido)propyl)sulfamoyl)-2-(6-(diethylamino)-3-(diethyliminio)-3*H*-xanthen-9-yl)benzenesulfonate, 7



A solution of **H** (33 mg, 0.054 mmol), 4-azidobenzoic acid (8.8 mg, 0.054 mmol), paraformaldehyde (2.4 mg, 0.081 mmol) and methyl 2-isocyanoacetate (10 µL, 0.054 mmol) in methanol (1 mL) was heated in the microwave (100 °C, 20 min). The solvent was removed *in vacuo* and the resulting residue purified by flash column chromatography (eluent 0 – 10% methanol in dichloromethane) to afford **7** as a dark purple solid (27 mg, 56%); mp > 350 °C; $\nu_{max}$ (thin film) 3069, 2971, 2930 (C-H), 2110, 2102 ($N_3$), 1750, 1592. $\delta_H$ (500 MHz, $CD_3OD$-$d_4$) 1.31 (12 H, t, *J*=6.5 Hz, 4 x $CH_2CH_3$), 1.79 - 1.86, 1.88 - 1.97 (2 H, 2 x m, $CH_2CH_2CH_2$, 2 rotamers), 2.86 - 2.95, 3.09 - 3.16 (2 H, 2 x m, $CH_2CH_2CH_2$, 2 rotamers), 3.42 - 3.50, 3.55 - 3.62 (2 H, 2 x m, $CH_2CH_2CH_2$, 2 rotamers), 3.64 - 3.72 (8 H, m, 4 x $CH_2CH_3$), 3.97, 4.02 (2 H, 2 x br. s, $NCH_2C(O)$, 2 rotamers), 4.06, 4.27 (2 H, 2 x br. s, $NCH_2C(O)$, 2 rotamers), 6.88 - 8.72 (13 H, m, 13 x C(Ar)*H*). $\delta_C$ (126 MHz, $CD_3OD$) 12.8 ($CH_2CH_3$), 28.1, 29.6 ($CH_2CH_2CH_2$, 2 rotamers), 41.5, 42.0 ($CH_2CH_2CH_2$, 2 rotamers), 41.9 ($NCH_2C(O)$), 46.0 ($CH_2CH_2CH_2$), 46.8 ($CH_2CH_3$), 54.8 ($NCH_2C(O)$), 97.0, 115.1, 115.3, 119.9, 120.3, 127.8, 129.5, 130.0, 132.6, 133.8, 135.5, 143.3, 143.7, 146.0, 147.3, 157.2, 157.9, 159.4 (aromatic carbons), 169.5, 171.3, 171.8, 173.9, 174.2 (3 x C=O, 2 rotamers). HRMS ($ESI^+$) $C_{42}H_{48}N_8NaO_{10}S_2^+$ ($[M+Na]^+$) calculated 911.2827; found 911.2846.

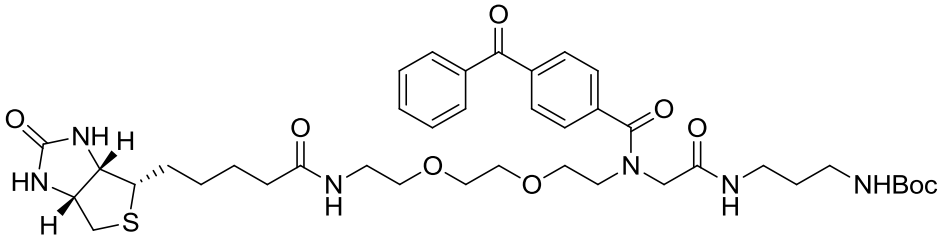
Methyl 2-(2-(4-azido-*N*-(but-3-yn-1-yl)benzamido)acetamido)acetate 8



A solution of but-3-yn-1-amine (43 µL, 0.52 mmol), 4-azidobenzoic acid (84 mg, 0.52 mmol), paraformaldehyde (23 mg, 0.77 mmol) and methyl 2-isocyanoacetate (47 µL, 0.52 mmol) in methanol (1.5 mL) was heated in the microwave (100 °C, 20 min). The solvent was removed *in vacuo* and the resulting residue purified by flash column chromatography (eluent 0 – 5 % methanol in dichloromethane) to afford **8** as a pale yellow gum (131 mg, 73%); $\nu_{max}$ (thin film) 3293 (N-H), 2928 (C-H), 2127, 2119 ($N_3$), 1747, 1625. $\delta_H$ (500 MHz, $CD_3OD$) 2.38 (1 H, t, *J*=2.5 Hz, $CH_2CCH$), 2.45 - 2.55, 2.58 - 2.69 (1 H, 2 x m, $CH_2CH_2N$), 3.53 - 3.60, 3.64 - 3.73 (1 H, 2 x m, $CH_2CH_2N$), 3.77 (3 H, s, $CH_3$), 3.96 - 4.00, 4.02 - 4.04 (1 H, m, 2 x $C(O)CH_2N$), 4.14 - 4.18, 4.28 - 4.36 (1 H, 2 x m, $C(O)CH_2N$), 7.10 - 7.25 (2 H, m, 2 x C(Ar)*H*), 7.47 - 7.63 (2 H, m, 2 x

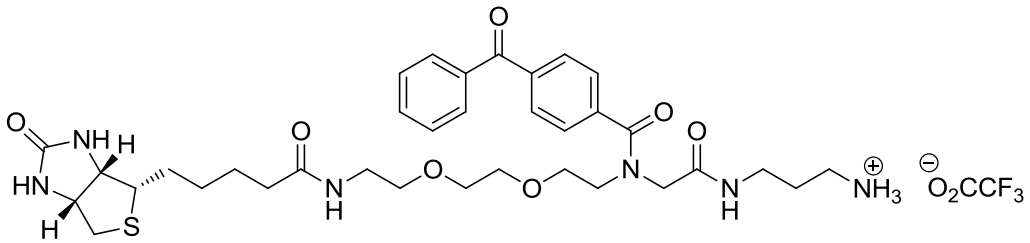
C(Ar)*H*). $\delta_H$ (126 MHz, $CD_3OD$) 17.5, 18.9 (*C*$H_2$C$H_2$N, 2 rotamers), 41.9 (C(O)*C*$H_2$N), 47.5, 50.2 (C$H_2$*C*$H_2$N), 49.0, 54.0 (C(O)*C*$H_2$N), 52.7 (*C*$H_3$), 71.3, 72.3 (C$H_2$*C*CH), 81.3, 82.2 (C$H_2$*C*CH), 120.2, 130.0, 130.1, 133.3, 143.5 (*C*(Ar)'s, rotamer peaks observed), 171.4, 171.6, 171.7, 174.0, 174.2 (3 x *C*=O, rotamer peaks observed); HRMS ($ESI^+$) $C_{16}H_{17}N_5NaO_4^+$ ($[M+Na]^+$) calculated 366.1173; found 366.1172.

*tert*-Butyl (7-(4-benzoylbenzoyl)-5,17-dioxo-21-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-10,13-dioxa-4,7,16-triazahenicosyl)carbamate 9




According to general procedure A: 4-benzoylbenzoic acid (160 mg, 0.71 mmol), *tert*-butyl (3-isocyanopropyl)carbamate (130 mg, 0.71 mmol), paraformaldehyde (32 mg, 1.06 mmol) and **F** (265 mg, 0.71 mmol) were reacted to afford **9** (334 mg, 59%) as a colourless gum; $\nu_{max}$ (thin film) 3306 (N-H), 2930, 2867 (C-H), 1691, 1648 (C=O, ketone, amides and urea); $\delta_H$ (400 MHz, $CD_3OD$) 1.32–1.50 (11 H, m, (C(C*H*$_3$)$_3$, SCHCH$_2$C*H*$_2$CH$_2$), 1.52–1.79 (6 H, m, SCHC*H*$_2$CH$_2$C*H*$_2$, BocNHCH$_2$C*H*$_2$), 2.10–2.25 (2 H, m, CH$_2$C*H*$_2$C(O)N), 2.69 (1 H, d, *J*=12.5 Hz, SCH*H*), 2.86–2.95 (1 H, m, SC*H*H), 2.97–3.34 (5 H, m, SC*H*, NHC*H*$_2$CH$_2$C*H*$_2$NH), 3.35–3.41 (2 H, m, C(O)NHC*H*$_2$CH$_2$O), 3.47–3.71 (8 H, m, C*H*$_2$OC*H*$_2$C*H*$_2$OC*H*$_2$), 3.74–3.86 (2 H, m, OCH$_2$C*H*$_2$NRR'), 4.12, 4.29 (2 H, 2 x s, NCH$_2$C(O)NH), 4.23–4.32 (1 H, m, SCHRC*H*), 4.42–4.52 (1 H, m, SCH$_2$C*H*), 7.47–7.89 (9 H, m, 9 x C(Ar)*H*); $\delta_C$ (101 MHz, $CD_3OD$) 25.9, 28.5, 28.8 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 27.0, 28.0 (C(*C*H$_3$)$_3$, 2 rotamers), 29.8, 30.0 (NHCH$_2$*C*H$_2$CH$_2$NH, 2 rotamers), 35.8 (CH$_2$*C*H$_2$CONH), 36.7, 36.9, 37.5, 37.8 (NH*C*H$_2$CH$_2$*C*H$_2$NH, 2 rotamers), 39.4 (C(O)NH*C*H$_2$CH$_2$O), 40.2 (S*C*H$_2$), 47.0, 50.7 (OCH$_2$C*H*$_2$NRR' 2 rotamers), 49.4, 53.4 (NC*H*$_2$C(O)NH, 2 rotamers), 56.1 (S*C*HRR'), 60.6 (SCH$_2$*C*), 62.4 (SCHR*C*), 68.7, 69.0, 69.8, 70.4, 70.6 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$, extra signals as a result of rotamers), 79.0 (*C*(CH$_3$)$_3$), 127.0 (*C*(Ar)), 127.7 (*C*(Ar)), 128.8 (*C*(Ar)), 130.2 (*C*(Ar)), 133.2 (*C*(Ar)), 137.4 (*C*(Ar)), 138.7, 138.8 (*C*(Ar), 2 rotamers), 140.1, 140.2 (*C*(Ar), 2 rotamers), 165.0 (NH*C*(O)NH), 169.7, 169.9 (C(O)NH, 2 rotamers), 172.7, 173.0 ((C(O)NH, 2 rotamers)), 175.0 (C(O)NH), 196.4, 196.5 (C*C*(O)C ); HRMS ($ESI^+$) $C_{40}H_{56}N_6NaO_9S^+$ ($[M+Na]^+$) calculated 819.3722; found 819.3697.

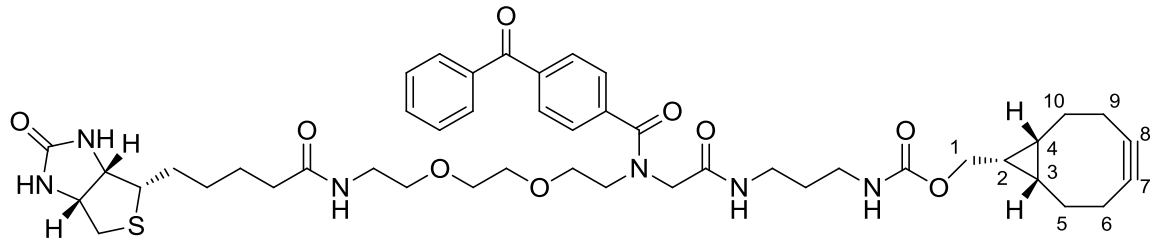
*N*-(2-((3-Aminopropyl)amino)-2-oxoethyl)-4-benzoyl-*N*-(2-(2-(2-(5-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)ethoxy)ethoxy)ethyl)benzamide, 10




Trifluoroacetic acid (0.6 mL) was added to **9** (334 mg, 0.420 mmol) in $CH_2Cl_2$ (5 mL) and the reaction mixture was stirred for 2 h. The solvent was removed *in vacuo* and the residual trifluoroacetic acid was azeotroped with $CH_2Cl_2$ (3 x 10 mL) to afford **10** (340 mg, quant.) as a colourless gum; $\nu_{max}$ (thin film) 3286 (N-H), 2931 (C-H), 1650 (C=O, ketone, amides and urea); $\delta_H$ (400 MHz, $CD_3OD$) 1.36–1.48 (2 H, m,

SCHCH$_2$C*H*$_2$CH$_2$), 1.52–1.77 (4 H, m, SCHC*H*$_2$CH$_2$C*H*$_2$), 1.79–1.96 (2 H, m, NHCH$_2$C*H*$_2$CH$_2$NH), 2.20 (2 H, t, *J*=7.5 Hz, CH$_2$C*H*$_2$C(O)N), 2.71 (1 H, d, *J*=13.0 Hz, SCH*H*), 2.89–3.07 (3 H, m, SC*H*H, C(O)N*H*$_2$CH$_2$CH$_2$CH$_2$), 3.14–3.24 (1 H, m, SC*H*), 3.35–3.46 (4 H, m, CH$_2$C*H*$_2$NH$_3^+$, C(O)NHC*H*$_2$CH$_2$O), 3.53–3.84 (10 H, m, C*H*$_2$OC*H*$_2$C*H*$_2$OC*H*$_2$C*H*$_2$NRR'), 4.15, 4.27 (2 H, 2 x s, NC*H*$_2$C(O)NH, 2 rotamers), 4.28–4.33 (1 H, m, SCHRC*H*), 4.50 (1 H, dd, *J*=7.3, 4.9 Hz, SCH$_2$C*H*), 7.53–7.90 (9 H, m, 9 H, m, 9 x C(Ar)*H*); $\delta_C$ (126 MHz, CD$_3$OD) 27.0, 29.6, 29.9 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 29.1 (NHCH$_2$*C*H$_2$CH$_2$NH), 36.9 (CH$_2$*C*H$_2$CONH), 37.0, 38.3 (NH*C*H$_2$CH$_2$*C*H$_2$NH), 40.4 (C(O)NH*C*H$_2$CH$_2$O), 41.2 (S*C*H$_2$), 48.1, 52.0 (OCH$_2$C*H*$_2$NRR', 2 rotamers), 50.7, 54.4 (NC*H*$_2$C(O)NH, 2 rotamers), 57.2 (S*C*HRR'), 61.8 (SCH$_2$*C*), 63.5 (SCHR*C*), 69.8, 70.2, 70.8, 71.5, 71.7 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$, extra signals as a result of rotamers), 128.0, 128.7, 129.8, 131.2, 134.3, 138.5, 140.1, 141.0 (12 x C(Ar)), 166.2, 172.2, 174.3, 176.3 (4 x *C*(O)N), 197.8 (CC(O)C); HRMS (ESI$^+$) $C_{35}H_{49}N_6O_9S^+$ ([M+H]$^+$) calculated 697.3368; found 697.3378.

Bicyclo[6.1.0]non-4-yn-9-ylmethyl (7-(4-benzoylbenzoyl)-5,17-dioxo-21-((*S*,*S*,*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-10,13-dioxa-4,7,16-triazahenicosyl)carbamate, 11



Bicyclo[6.1.0]non-4-yn-9-yl (2,5-dioxopyrrolidin-1-yl) carbonate (25 mg, 0.086 mmol), **10** (70 mg, 0.086 mmol) and triethylamine (0.036 mL, 0.258 mmol) were dissolved in DMF (2 mL) and stirred for 12 h before the solvent was removed *in vacuo*. The residue was dissolved in $CH_3Cl$ (10 mL), washed with aqueous sat. $NaHCO_3$ (10 mL), water (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash column chromatography (eluent 20-100% $CH_2Cl_2$ / methanol / $NH_3$ (aq) – 90:10:1 in $CH_2Cl_2$) afforded **11** as a colourless gum (52 mg, 69%); $\nu_{max}$ (thin film) 3295 (N-H), 2925, 2859 (C-H), 1692, 1649 (C=O, ketone, amides and urea); $\delta_H$ (400 MHz, CD$_3$OD) 0.86–0.96 (2 H, m, C(3)*H*C(4)*H*), 1.33–1.47 (3 H, m, SCHCH$_2$C*H*$_2$CH$_2$, C(2)*H*), 1.48–1.79 (8 H, m, C(5)H*H*, C(10)H*H*, SCHC*H*$_2$CH$_2$C*H*$_2$, NHCH$_2$C*H*$_2$CH$_2$NH), 2.15–2.25 (8 H, m, CH$_2$C*H*$_2$C(O)N, C(9)*H*$_2$, C(6)*H*$_2$, C(5)*H*H, C(10)*H*H), 2.69 (1 H, d, *J*=12.5 Hz, SCH*H*), 2.92 (1 H, dd, *J*=5.0, 12.5 Hz, SC*H*H), 3.04–3.31 (5 H, m, SC*H*, NHC*H*$_2$CH$_2$C*H*$_2$NH), 3.36–3.40 (2 H, m, C(O)NHC*H*$_2$CH$_2$O), 3.52 – 3.85 (10 H, m, C*H*$_2$OC*H*$_2$C*H*$_2$OC*H*$_2$ C*H*$_2$NRR'), 4.05–4.33 (5 H, m, C(1)H$_2$, NCH$_2$C(O)NH, SCHRC*H*), 4.48 (1 H, m, SCH$_2$C*H*), 7.56–7.90 (9 H, m, 9 x C(Ar)*H*); $\delta_C$ (101 MHz, CD$_3$OD) 18.0 (C(1)), 20.4 (C(C(3), C(4)), 21.0 (C(5), C(10)), 25.9, 28.5, 28.8 (SCH*C*H$_2$*C*H$_2$*C*H$_2$), 29.2 (C(6), C(9)), 29.8 (NHCH$_2$*C*H$_2$CH$_2$NH), 35.8 (CH$_2$*C*H$_2$CONH), 36.6, 36.8, 37.9, 38.1 (NH*C*H$_2$CH$_2$*C*H$_2$NH, 2 rotamers), 39.4 (C(O)NH*C*H$_2$CH$_2$O), 40.1 (S*C*H$_2$), 47.0, 50.7 (OCH$_2$C*H*$_2$NRR' 2 rotamers), 49.4, 53.4 (NC*H*$_2$C(O)NH, 2 rotamers), 56.0 (S*C*HRR'), 60.6 (SCH$_2$*C*), 62.4 (SCHR*C*), 62.7 (C(1)), 68.7, 69.0, 69.7, 70.3, 70.5, 70.5 (*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$, extra signals as a result of rotamers), 98.6 (C(8), C(7)), 126.9, 127.6, 128.7, 130.1, 133.2, 137.4, 138.7, 140.1 (12 x C(Ar)), 158.3 (O*C*(O)NH), 165.0 (NH*C*(O)NH), 169.9, 172.8, 175.1 (3 X C*C*(O)N), 196.5 (C*C*(O)C); HRMS (ESI$^+$) $C_{46}H_{60}N_6NaO_9S^+$ ([M+Na]$^+$) calculated 895.4009; found 895.4053.

## Production of PHD2$_{181-426}$.

Human PHD2$_{181-426}$ (termed PHD2 throughout) was produced in *E. coli* BL21(DE3) and purified by cation exchange and size exclusion chromatography, as described.[7] Apo-PHD2 was obtained, after cation exchange chromatography, by incubation with EDTA.

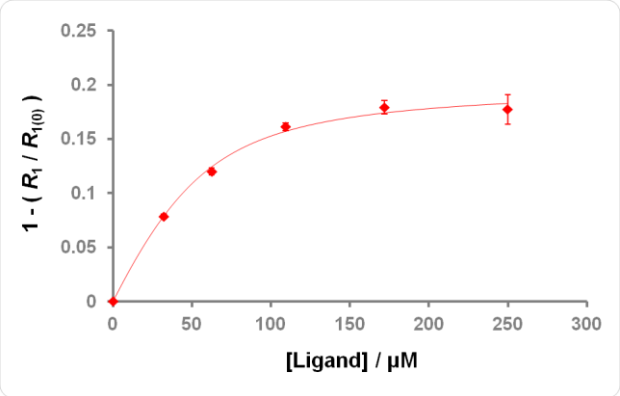
## $K_D$ determinations by NMR

Solvent water relaxation experiments were conducted using a Bruker Avance II 500 MHz spectrometer equipped with a 5 mm inverse $^{1}H/^{19}F(^{13}C)$ TXI probe. All experiments were conducted at 298 K. 3 mm MATCH tubes (Bruker) were used and the sample volume was 160 µL.

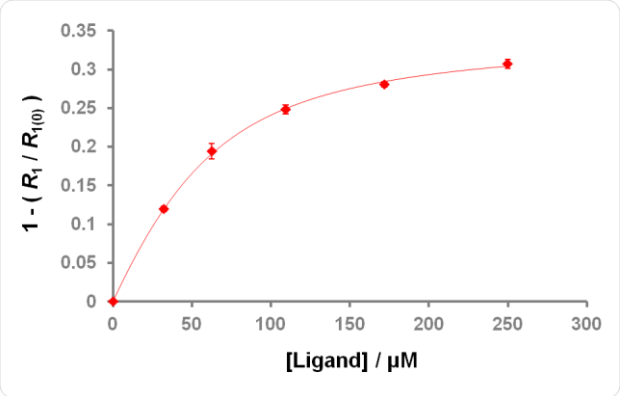
Samples contained 50 µM *apo*-PHD2$_{181-426}$, 50 µM $MnCl_2$, 125 mM NaCl, 0.02% $NaN_3$ and 50 mM Tris-D11 (pH 7.5) dissolved in 12.5% $H_2O$ and 87.5% $D_2O$. The titratant stock solutions of probes **1**-**5** (25 mM) were prepared in DMSO / DMSO-D6, which was then titrated directly into the NMR tube (typically 0.2 µL to 0.5 µL per addition) using a 1 µL plunger-in-needle syringe (SGE). Changes in the bulk water relaxation rate (1-$R_1/R_{1(0)}$, in which $R_1$ is the longitudinal relaxation rate of the bulk water in the presence of ligands and $R_{1(0)}$ is the longitudinal relaxation rate of the bulk water in the absence of ligands) was plotted against the titrated ligand concentrations to obtain $K_D$'s (Figure S1).

Experimental procedures were carried out as described.[8]

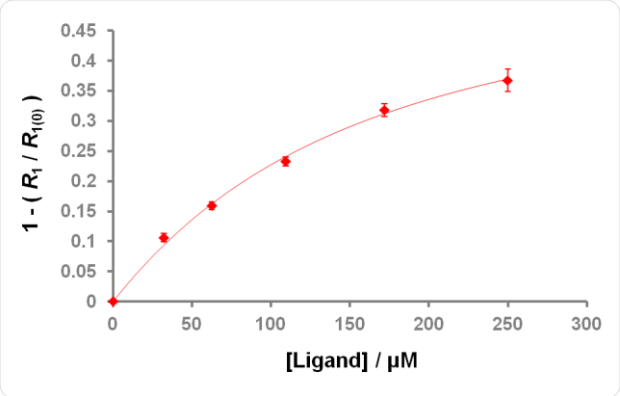
Compound **1,** $K_D$ = 19 µM



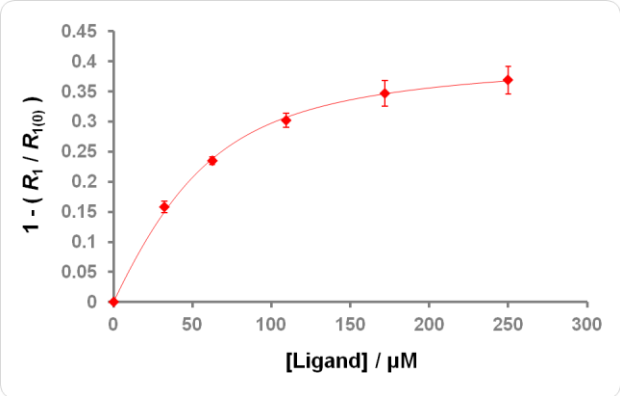

Compound **2**, $K_D$ = 28 µM




Compound **3,** $K_D$ = 126 µM




Compound **5**, $K_D$ = 25 µM




Compound **4**, $K_D$ = 8 µM

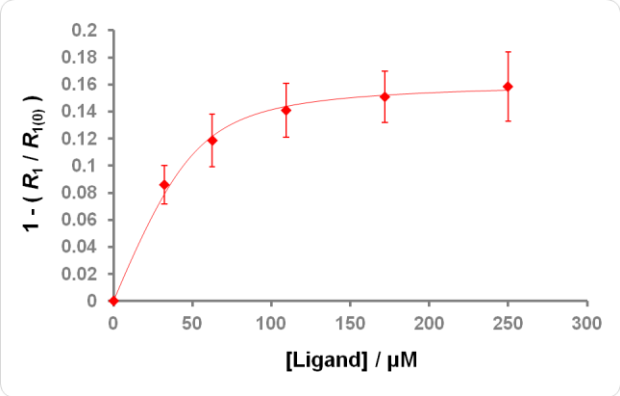



Figure S1. NMR-based $K_D$ value determinations with PHD2 and probes **1**-**5** using solvent water relaxation.[8] Data represent mean intensities of all repeats (n = 3), error bars show 1 standard deviation.

## Protein Electrospray Ionization Mass Spectrometry

Intact protein masses were recorded by liquid chromatography / mass spectrometry (LC/MS) using a 6530QTof (Agilent) single quadrupole mass spectrometer, interfaced with a Agilent 1100 liquid chromatography and sample handling system.

The protein sample (5 µM, 5 µL) was injected onto a Chromolith FastGradient RP-18 2x50mm Monolithic reverse phase HPLC column and eluted at 0.4 $mL.min^{-1}$ using a gradient system from Solvent A (water, 0.1 % (v/v) formic acid) to Solvent B (methanol, 0.1 % (v/v) formic acid) according to the following conditions:

| Time (Min) | %A | %B | Flow Rate ($mL.min^{-1}$) |
|---|---|---|---|
| 0:00 | 95 | 5 | 0.4 |
| 1:00 | 95 | 5 | 0.4 |
| 4 | 0 | 100 | 0.4 |
| 4.5 | 0 | 100 | 1 |
| 5 | 95 | 5 | 1 |
| 5.1 | 95 | 5 | 1 |

The eluent was injected directly into the mass spectrometer. The following MS parameters were used: Vcap 3500; Desolvation temperature – 350$^{o}$C; Drying gas flow – 10 $l.hour^{-1}$; Desolvation gas flow ($N_2$) – 20 $l.hour^{-1}$. Data acquisition was done in 2GHz standard high gain mode.

Sodium formate was used to calibrate the instrument. Spectra were processed using Mass Hunter Qualitative analysis™ B06.00 (Agilent) with the Maximum Entropy method (MaxEnt1) employed.

## PHD2 crosslinking time-course

PHD2 (5 µM), $MnCl_2$ (10 µM) and probe (at twice $K_D$) in Tris buffer (50 mM Tris, 100 mM NaCl [pH 7.5]) were incubated at room temperature for 20 min before being irradiated (310 nm or 350 nm, 4 °C) for the required time using a CaproBox™. Irradiation times longer than 5 min were carried out in 5 min pulses separated by 1 min intervals to prevent sample warming. Samples were analysed by liquid chromatography-mass spectrometry. Percentage crosslinking was calculated from the ratio of the heights of the modified and unmodified protein peaks in the deconvoluted mass spectrum and plotted against time (Figure 3 (310 nm), Figure S2 (350 nm)).

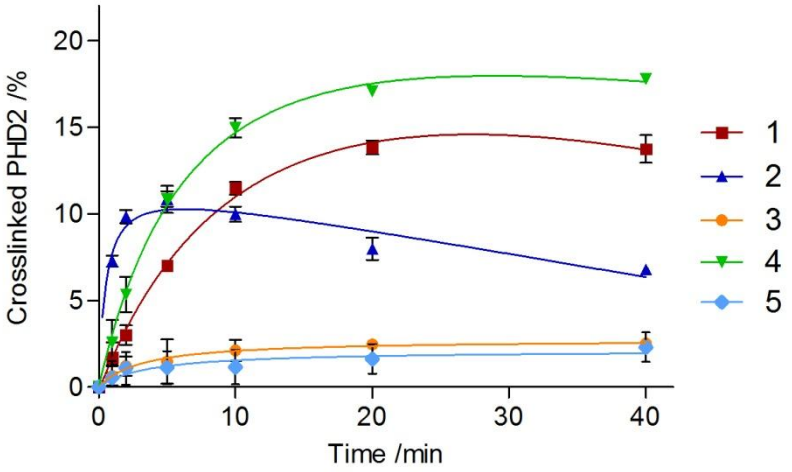



Figure S2. Percentage of crosslinked PHD2 upon irradiation (350 nm) in the presence of probes **1**-**5**, as determined by ESI mass spectrometry. Data represent mean intensities of all repeats (n = 3), error bars show 1 standard deviation.

**Time-course of PHD2 crosslinking at 310 nm**

Tables reporting the intensities of modified and unmodified PHD2 peaks in the deconvoluted mass spectra obtained after irradiation (310 nm) of PHD2 in the presence of $Mn^{II}$ and probes **1**-**5** (Table S1-5). The heights of the 20 most intense peaks in the range 25000 to 30000 Da were extracted and all others masses assigned a height of zero. Peaks corresponding to crosslinked protein were not observed without irradiation, at T = 0.

Table S1. Probe **1**. PHD2 mass = 27644; crosslinked PHD2 mass = 28477 (PHD2 + **1** – $N_2$)

| Time | Run A | | Run B | | Run C | | Mean | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| /min | PHD2 | PHD2+**1** | PHD2 | PHD2+**1** | PHD2 | PHD2+**1** | PHD2 | PHD2+**1** | % crosslinked |
| 1 | 77033599 | 6235938 | 86091948 | 6137505 | 92952424 | 5024645 | 85359324 | 5799362.7 | 6.4 |
| 2 | 62054543 | 11509134 | 63768391 | 11817925 | 76519094 | 10773525 | 67447343 | 11366861 | 14.4 |
| 5 | 47475799 | 15077139 | 49802718 | 15920622 | 58037197 | 16586288 | 51771905 | 15861350 | 23.5 |
| 10 | 42754510 | 15671272 | 44207674 | 16405514 | 50982483 | 18051443 | 45981556 | 16709410 | 26.7 |
| 20 | 40429389 | 15758190 | 38969835 | 15346531 | 43830170 | 16626102 | 41076465 | 15910274 | 27.9 |
| 40 | 32545083 | 13843349 | 33865921 | 14252383 | 37866671 | 15455552 | 34759225 | 14517095 | 29.5 |

Table S2. Probe **2**. PHD2 mass = 27644; crosslinked PHD2 mass = 28549 (PHD2 + **2** – $N_2$)

| Time | Run A | | Run B | | Run C | | Mean | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| /min | PHD2 | PHD2+**2** | PHD2 | PHD2+**2** | PHD2 | PHD2+**2** | PHD2 | PHD2+**2** | % crosslinked |
| 1 | 56643851 | 13971203 | 52506926 | 14961637 | 60697565 | 13672316 | 56616114 | 14201719 | 20.1 |
| 2 | 35633148 | 17127552 | 34700882 | 18524283 | 46621037 | 17767219 | 38985022 | 17806351 | 31.4 |
| 5 | 22270087 | 19016506 | 23018368 | 19664533 | 29986984 | 20553594 | 25091813 | 19744878 | 44.0 |
| 10 | 16980192 | 18344998 | 17229725 | 19916389 | 21890124 | 20185896 | 18700014 | 19482428 | 51.0 |
| 20 | 15382503 | 19281739 | 15527540 | 19566129 | 18284290 | 21788822 | 16398111 | 20212230 | 55.2 |
| 40 | 13044445 | 15568728 | 14210907 | 17694043 | 16158892 | 19939752 | 14471415 | 17734174 | 55.0 |

Table S3. Probe **3**. PHD2 mass = 27644; crosslinked PHD2 mass = 28544 (PHD2 + **3** – $N_2$)

| Time | Run A | | Run B | | Run C | | Mean | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| /min | PHD2 | PHD2+**3** | PHD2 | PHD2+**3** | PHD2 | PHD2+**3** | PHD2 | PHD2+**3** | % crosslinked |
| 1 | 19143505 | 0 | 21805689 | 0 | 25098962 | 0 | 22016052 | 0 | 0 |
| 2 | 35509258 | 0 | 28109778 | 0 | 22988892 | 0 | 28869309 | 0 | 0 |
| 5 | 32228532 | 105256 | 24105134 | 88544 | 31009060 | 51893 | 29114242 | 81897 | 0.3 |
| 10 | 26400919 | 365991 | 11922003 | 40403 | 27791368 | 484660 | 22038097 | 297018 | 1.3 |
| 20 | 6357273 | 117689 | 26519598 | 461854 | 22492697 | 312072 | 18456523 | 297205 | 1.6 |
| 40 | 16481329 | 529714 | 15075757 | 465833 | 16392535 | 558273 | 15983207 | 517940 | 3.1 |

Table S4. Probe **4**. PHD2 mass = 27644; crosslinked PHD2 mass = 28442 (PHD2 + **4** – $N_2$)

| Time | Run A | | Run B | | Run C | | Mean | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| /min | PHD2 | PHD2+**4** | PHD2 | PHD2+**4** | PHD2 | PHD2+**4** | PHD2 | PHD2+**4** | % crosslinked |
| 1 | 100190593 | | 1.12E+08 | 3848459 | 14444481 | 2473693 | 75682094 | 3161076 | 4.0 |
| 2 | 109111449 | 3197485 | 1.1E+08 | 4942616 | 33220718 | 1880214 | 84108238 | 3340105 | 3.8 |
| 5 | 105795831 | 8633412 | 1.12E+08 | 8343240 | 10963314 | 1484740 | 76242587 | 6153797.3 | 7.5 |
| 10 | 89324457 | 13975257 | 88341747 | 13418798 | 8676872 | 1576063 | 62114359 | 9656706 | 13.5 |
| 20 | 72411851 | 20942422 | 78626252 | 18317894 | 5855393 | 1154381 | 52297832 | 13471566 | 20.5 |
| 40 | 43179982 | 23748592 | 66365538 | 18384477 | 6426072 | 3525429 | 38657197 | 15219499 | 28.2 |

Table S5. Probe **5**. PHD2 mass = 27644; crosslinked PHD2 mass = 28568 (PHD2 + **5**)

| Time | Run A | | Run B | | Run C | | Mean | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| /min | PHD2 | PHD2+**5** | PHD2 | PHD2+**5** | PHD2 | PHD2+**5** | PHD2 | PHD2+**5** | % crosslinked |
| 1 | 53129492 | 0 | 70738596 | 0 | 73508064 | 0 | 65792051 | 0 | 0 |
| 2 | 49762652 | 1131233 | 58449698 | 0 | 86859203 | 0 | 65023851 | 377078 | 0.6 |
| 5 | 50739525 | 1874630 | 69624952 | 2299397 | 84257139 | 2262340 | 68207205 | 2145456 | 3.1 |
| 10 | 46016326 | 2726497 | 51489272 | 2327900 | 70608989 | 4245311 | 56038196 | 3099903 | 5.2 |
| 20 | 39085318 | 3744646 | 42142189 | 4253007 | 64292099 | 5037361 | 48506535 | 4345005 | 8.2 |
| 40 | 28560425 | 4105575 | 31158058 | 4030993 | 41794046 | 5623791 | 33837510 | 4586786 | 11.9 |

**Time-course of PHD2 crosslinking at 350 nm**

Tables reporting the intensities of modified and unmodified PHD2 peaks in the deconvoluted mass spectra obtained after irradiation (350 nm) of PHD2 in the presence of $Mn^{II}$ and probes **1**-**5** (Table S6-10). The heights of the 20 most intense peaks in the range 25000 to 30000 Da were extracted and all others masses assigned a height of zero. Peaks corresponding to crosslinked protein were not observed without irradiation, at T = 0.

Table S6. Probe **1.** PHD2 mass = 27644; crosslinked PHD2 mass = 28477 (PHD2 + **1** – $N_2$)

| Time | Run A | | Run B | | Run C | | Mean | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| /min | PHD2 | PHD2+**1** | PHD2 | PHD2+**1** | PHD2 | PHD2+**1** | PHD2 | PHD2+**1** | % crosslinked |
| 1 | 16812316 | 138888 | 14653830 | 385001 | 16513825 | 289956 | 15993324 | 271282 | 1.7 |
| 2 | 15116865 | 406061 | 13289616 | 502733 | 15536668 | 436422 | 14647716 | 448405 | 3.0 |
| 5 | 12295164 | 942147 | 12005202 | 924818 | 11756118 | 853344 | 12018828 | 906770 | 7.0 |
| 10 | 9057162 | 1140955 | 8749051 | 1121262 | 8497359 | 1145839 | 8767857 | 1136019 | 11.5 |
| 20 | 8317823 | 1290106 | 8163044 | 1310966 | 7646308 | 1268979 | 8042392 | 1290017 | 13.8 |
| 40 | 8126434 | 1196329 | 8126434 | 1196329 | 7837541 | 1308804 | 8030136 | 1233821 | 13.3 |

Table S7. Probe **2.** PHD2 mass = 27644; crosslinked PHD2 mass = 28549 (PHD2 + **2** – $N_2$)

| Time | Run A | | Run B | | Run C | | Mean | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| /min | PHD2 | PHD2+**2** | PHD2 | PHD2+**2** | PHD2 | PHD2+**2** | PHD2 | PHD2+**2** | % crosslinked |
| 1 | 8211903 | 609216 | 7923838 | 647520 | 8232816 | 651591 | 8122852 | 636109 | 7.3 |
| 2 | 7331316 | 761363 | 6709374 | 758952 | 7081139 | 779502 | 7040610 | 766606 | 9.8 |
| 5 | 7036098 | 825497 | 6458919 | 827412 | 6378676 | 766962 | 6624564 | 806624 | 10.9 |
| 10 | 7271772 | 782282 | 6511159 | 762414 | 6408374 | 692463 | 6730435 | 745720 | 10.0 |
| 20 | 7430880 | 608948 | 7509881 | 622196 | 6794139 | 649351 | 7244967 | 626832 | 8.0 |
| 40 | 7588098 | 547569 | 7665174 | 541088 | 7544155 | 570170 | 7599142 | 552942 | 6.8 |

Table S8. Probe **3.** PHD2 mass = 27644; crosslinked PHD2 mass = 28544 (PHD2 + **3** – $N_2$)

| Time | Run A | | Run B | | Run C | | Mean | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| /min | PHD2 | PHD2+**3** | PHD2 | PHD2+**3** | PHD2 | PHD2+**3** | PHD2 | PHD2+**3** | % crosslinked |
| 1 | 10768522 | 0 | 16753046 | 153197 | 17015629 | 180297 | 14845732 | 111165 | 0.7 |
| 2 | 9263327 | 56269 | 16549733 | 225892 | 16996976 | 292012 | 14270012 | 191391 | 1.3 |
| 5 | 2633086 | 0 | 16011537 | 357493 | 16058031 | 371896 | 11567551 | 243130 | 2.1 |
| 10 | 5549984 | 80017 | 14834270 | 359357 | 14483168 | 383260 | 11622474 | 274211 | 2.3 |
| 20 | 14600312 | 324469 | 14266145 | 386142 | 15317667 | 406746 | 14728041 | 372452 | 2.5 |
| 40 | 14238121 | 350429 | 13139176 | 354079 | 14804178 | 381159 | 14060492 | 361889 | 2.5 |

Table S9. Probe **4.** PHD2 mass = 27644; crosslinked PHD2 mass = 28442 (PHD2 + **4** – $N_2$)

| Time | Run A | | Run B | | Run C | | Mean | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| /min | PHD2 | PHD2+**4** | PHD2 | PHD2+**4** | PHD2 | PHD2+**4** | PHD2 | PHD2+**4** | % crosslinked |
| 1 | 17797984 | 212530 | 16794764 | 653236 | 17551066 | 507538 | 17381271 | 457768 | 2.6 |
| 2 | 16028543 | 815918 | 14522152 | 1012350 | 16707998 | 815921 | 15752898 | 881396 | 5.3 |
| 5 | 14149188 | 1680832 | 14974340 | 1984281 | 14885125 | 1690189 | 14669551 | 1785101 | 10.8 |
| 10 | 14021915 | 2478107 | 12884050 | 2364314 | 14523887 | 2443946 | 13809951 | 2428789 | 15.0 |
| 20 | 13589883 | 2787517 | 13134416 | 2676636 | 13126587 | 2744964 | 13283629 | 2736372 | 17.1 |
| 40 | 13277356 | 2848601 | 10908524 | 2335008 | 11668740 | 2574748 | 11951540 | 2586119 | 17.8 |

Table S10. Probe **5.** PHD2 mass = 27644; crosslinked PHD2 mass = 28568 (PHD2 + **5**)

| Time | Run A | | Run B | | Run C | | Mean | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| /min | PHD2 | PHD2+**5** | PHD2 | PHD2+**5** | PHD2 | PHD2+**5** | PHD2 | PHD2+**5** | % crosslinked |
| 1 | 1254876 | 0 | 16579720 | 0 | 17161193 | 286426 | 11665263 | 95475 | 0.8 |
| 2 | 1004346 | 0 | 17740185 | 282245 | 18919245 | 323304 | 12554592 | 201850 | 1.6 |
| 5 | 5824683 | 0 | 17316928 | 289503 | 16705638 | 291115 | 13282416 | 193539 | 1.4 |
| 10 | 4626984 | 0 | 16928664 | 310836 | 16263827 | 269568 | 12606492 | 193468 | 1.5 |
| 20 | 4745577 | 30752 | 15728825 | 354879 | 15923761 | 331767 | 12132721 | 239133 | 1.9 |
| 40 | 5000432 | 67017 | 14366518 | 417349 | 13528612 | 385231 | 10965187 | 289866 | 2.6 |

**PHD2 crosslinking controls**

Tables reporting the intensities of modified and unmodified PHD2 peaks in the deconvoluted mass spectra obtained after irradiation (310 nm, 20 min) of PHD2-$Mn^{II}$ in the presence either probes **1**-**5** with 200 µM **IOX1** (Table S11) or scaffolds **6a**-**e** (Table S12). The heights of the 20 most intense peaks in the range 25000 to 30000 Da were extracted and all others masses assigned a height of zero. No crosslinking was observed.

Table S11 Crosslinking of PHD2 with **1**-**5** in the presence of 200 µM **IOX1**

| Probe | PHD2 | Crosslinked PHD2 |
|---|---|---|
| **1** | 1035257 | 0 |
| **2** | 1547122 | 0 |
| **3** | 1530899 | 0 |
| **4** | 1326526 | 0 |
| **5** | 1326526 | 0 |

Table S12 Crosslinking of PHD2 with scaffolds **6a**-**e**

| Scaffold | PHD2 | Crosslinked PHD2 |
|---|---|---|
| **6a** | 2056724 | 0 |
| **6b** | 2134435 | 0 |
| **6c** | 2053258 | 0 |
| **6d** | 2010009 | 0 |
| **6e** | 1981418 | 0 |

**Mass spectrometric analysis of rate of probe activation and resulting products**

Mass spectrometric analysis of probes after irradiation (310 nm, 0.5, 2.5 and 10 min) indicates that the rate of activation of the probes increases in the order **4** < **1** < **3** < **2** (Figure S3). The rate of activation of **5** is unknown since intramolecular reactions and reaction with water afford the same mass as the parent compound. The masses of the species formed when activated probe molecules do not react with PHD2 gives an indication of the mechanisms of quenching. Probe **1** primarily reacts to give a species with a mass that corresponds to loss of $N_2$, which likely arises from intramolecular insertion of the nitrene into a C-H or X-H bond. The major peak in the mass spectrum of probe **2** after 10 min irradiation has a mass of **2** – $N_2$ + $H_2$, which may arise from reduction of the formed nitrene to give the amine. Probe **3** gives predominantly a species resulting from reaction of the carbene with $H_2O$, while in contrast, diazirine **4** appears to give exclusively a species resulting from intramolecular insertion of the carbene, likely αC-H insertion to give the alkene.[9]

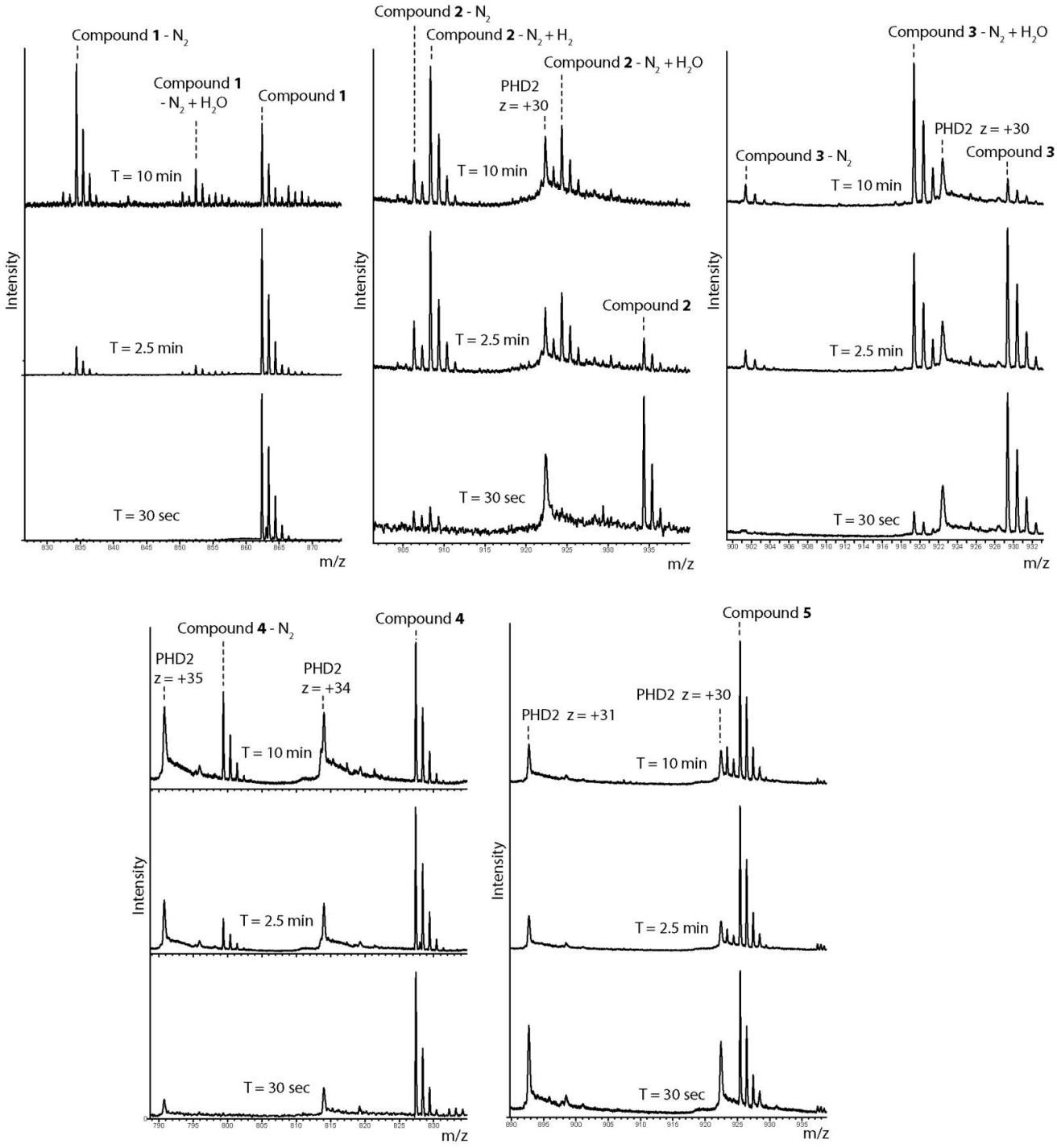



Figure S3. Mass spectrometric analysis of probes **1-5** after 0.5, 2.5 and 10 min irradiation (310 nm) in the presence of PHD2 and $Mn^{2+}$. Speactra for compound **1** are in the absence of PHD2 since **1** could not be detected in the presence of PHD2.

## Limited trypsin digests and MALDI MS[10]

PHD2 (20 µM), $Mn^{II}$ (20 µM) and probe (50 µM) in 15 mM $NH_4OAc$ (pH 7.5) were incubated at room temperature for 20 min before irradiation (310 nm, 12 min, 4 °C). Sequence grade modified trypsin (trypsin / PHD2 1:200 w/w) was added and the digest mixture incubated at room temperature (1 h) and then 37 °C (2 h). At regular intervals over a 3 h period, 2 µL samples were removed and quenched by addition of 4 µL quenching buffer ($H_2O$ / methanol / $HCO_2H$, 90:10:0.1). 1 µL sample was mixed with 1 µL sinapinic acid (SA) or α-cyano-4-hydroxycinnamic acid (CHCA) matrix (both Laser BioLabs) and pippetted onto a 96 spot MALDI target. Spots were allowed to air dry and analysed using a MALDI micro MX mass spectrometer (Waters) in linear positive ion mode with laser energy 220, pulse voltage 1250 V, detector voltage 2750 V and mass suppression 1000 Da. Data were analysed using MassLynx v4.0. Spectra were smoothed using the mean method (smooth windows = 200; number of smooths = 2).

## Trypsin digests and LC-MS/MS

PHD2 (20 µM), $MnCl_2$ (20 µM) and probe (50 µM) in Tris buffer (50 mM Tris, 100 mM NaCl [pH 7.5]) were incubated at room temperature for 20 min before being irradiated (310 nm, 12 min, 4 °C). The protein was subjected to reductive acetylation and trypsin digest according to standard procedures.[11]

The analysis of digested material was performed by LC-MS/MS using a Waters Q-Tof Premier mass spectrometer (Waters) coupled to a nano-UPLC system (NanoAcquity, Waters) using a reversed phase 75 µm x 250 mm column as previously described.[11]

Table S13. Crosslinked trypsin derived peptides observes in MS/MS analysis

| Peptide sequence | Expected Mass | Expected mass of peptide + 318 – $N_2$ | MS of crosslinked PHD2 Obs/charge/**Mr** |
|---|---|---|---|
| AMVACYPGNGTGYVR | **1614.73** | **2413.10** | 805.38/2+/**2413.11**<br>1207.56/3+/**2413.11** |
| AMVACYPGNGTGYVR + M. Ox. | **1630.72** | **2429.10** | 810.71/ 3+/**2429.12**<br>1215.58/ 2+/**2429.14** |
| TKAMVACYPGNGTGYVR | **1843.87** | **2642.24** | 661.56/4+/**2642.21**<br>881.77/3+/**2642.27** |

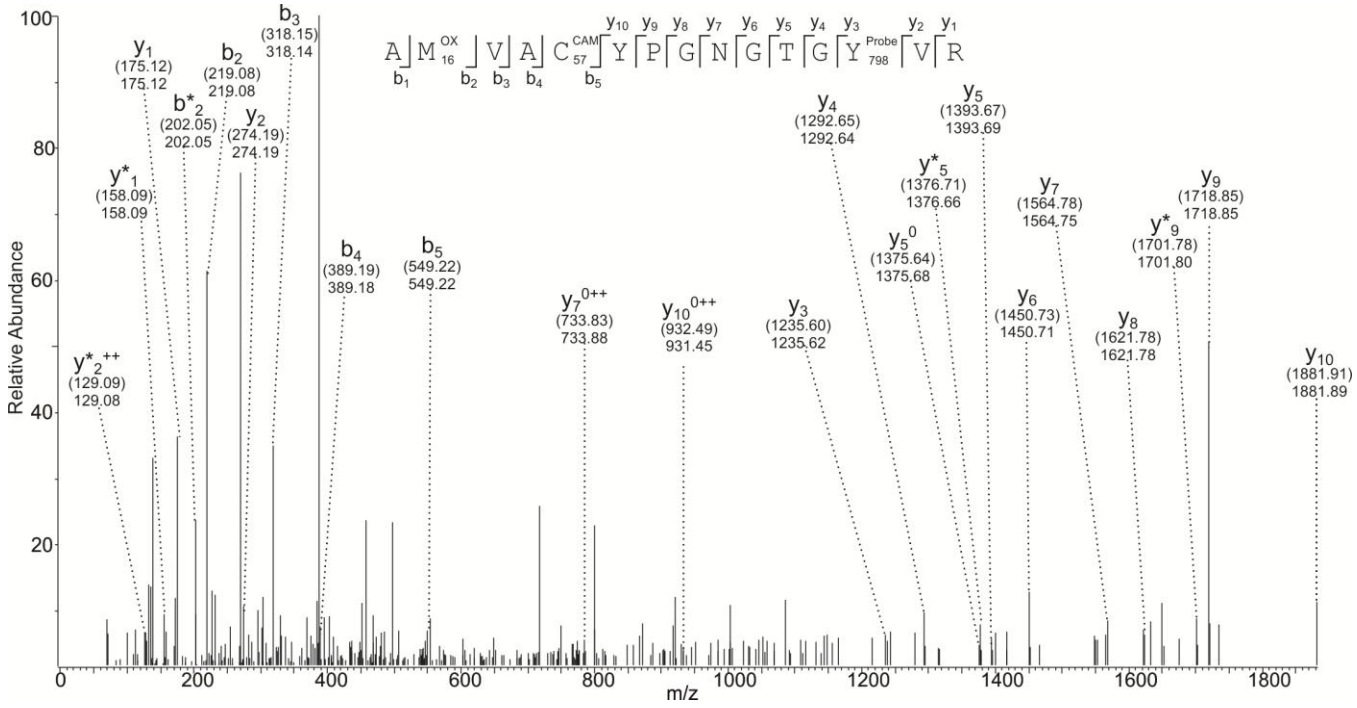



Figure S4. MS/MS analysis of trypsin derived peptide $_{298}$AMVACYPGNGTGYVR$_{312}$ crosslinked to **4**. Analysis by LC-MS/MS reveals a mass corresponding to compound **4** (– $N_2$) on residue $Y_{310}$ of PHD2. The MS/MS spectrum of the modified tryptic peptide $_{298}$AMVACYPGNGTGYVR$_{312}$ + **4** – $N_2$ $[M+2H]^{2+}$ 1215.58 Da (MW 2429.14 Da) is shown. Fragment ions are indicated as b and y ions. Loss of $NH_3$ is represented by *, loss of $H_2O$ by 0, and doubly charged ions by ++.

## Crystal structure analysis

The binding mode of probes **1**-**5** was modelled by manual docking of the selectivity function (derived from **IOX1**) with PHD2. Docking was guided by crystal structures of PHD2 in complex with a bidentate isoquinoline inhibitor {[(4-hydroxy-8-iodoisoquinolin-3-yl)carbonyl]amino}acetic acid (PDB ID: 2G1M), and the structurally related 2-oxoglutarate dependent histone demethylase KDM4A in complex with 4-carboxy-8-hydroxyquinoline (PDB ID: 4BIS) (**Figure S5**).

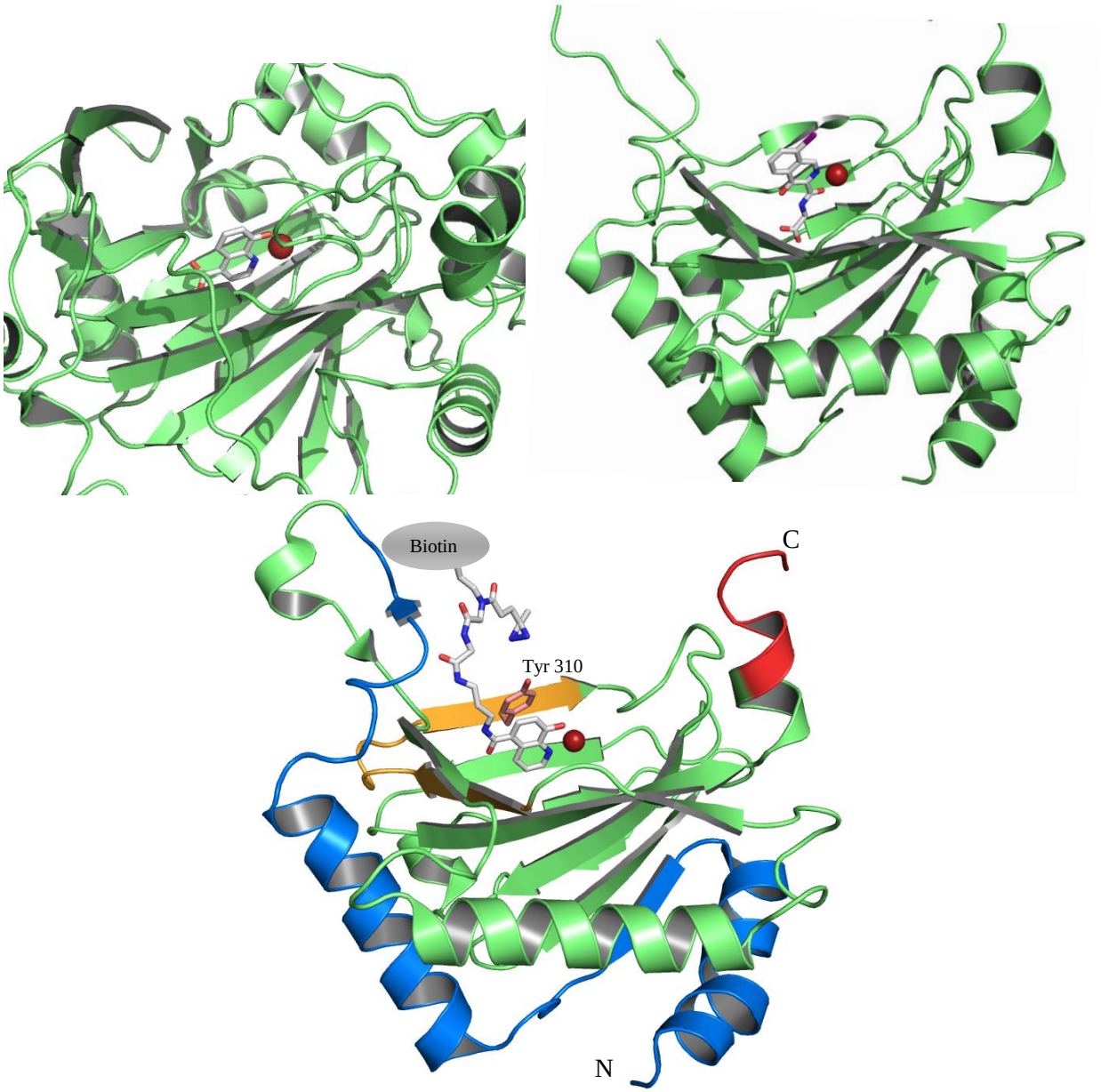



Figure S5. Top left: view derived from a crystal structure of KDM4A in complex with 4-carboxy-8-hydroxyquinoline (PDB ID: 4BIS), top right: view derived from a crystal structure of PHD2 in complex with a bidentate isoquinoline inhibitor (PDB ID: 2G1M). Below: View derived from a PHD2 crystal structure (PDB ID: 3HQU) docked with diazirine **4**.

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