Reversible lysine-targeted probes reveal residence time-based kinase selectivity

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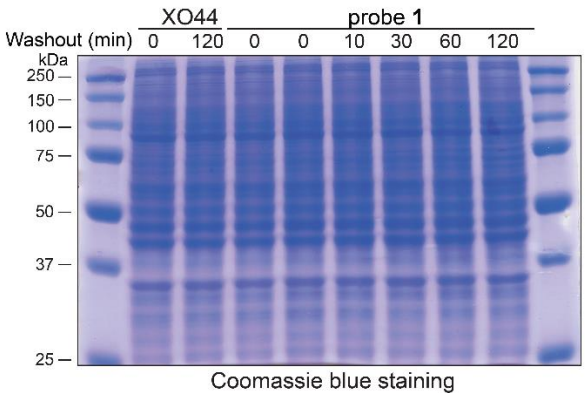
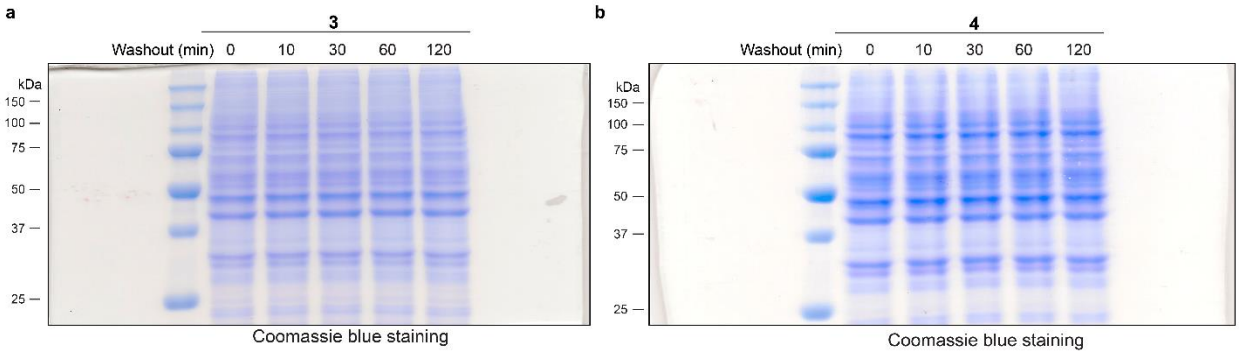
Supplementary Note



Supplementary Figure 1. SDS-PAGE gel from Figure 1b, stained with Coomassie blue. Data are representative of two independent experiments.



Supplementary Figure 2. Coomassie blue stained gels from Figure 2b (**a**) and Figure 2c (**b**). Data are representative of two independent experiments.

Supplementary Table 1 Data collection and refinement statistics (molecular replacement)

	AURKA-probe **3** complex
PDB Code	**7FIC**
Data collection	
Space group	$P3_221$
Cell dimensions□□	
a, *b*, *c* (Å)	87.95, 87.95, 76.82
α, β, γ (°)	90.0, 90.0, 120.0
Resolution (Å)	76.17-2.32 (2.40-2.32) *
R_{merge}	0.049 (2.109)
$I / \sigma I$	25.48 (1.40)
Completeness (%)	99.8 (99.7)
Redundancy	19.5 (20.2)
Refinement	
Resolution (Å)	76.17-2.32
No. reflections	296747
R_{work} / R_{free}	0.2355 / 0.2735
No. atoms	
Protein	1990
Ligand/ion	39
Water	6
B-factors (Å^2)	
Protein	90.26
Ligand/ion	90.09
Water	84.77
R.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.31

*Highest-resolution shell is shown in parentheses.

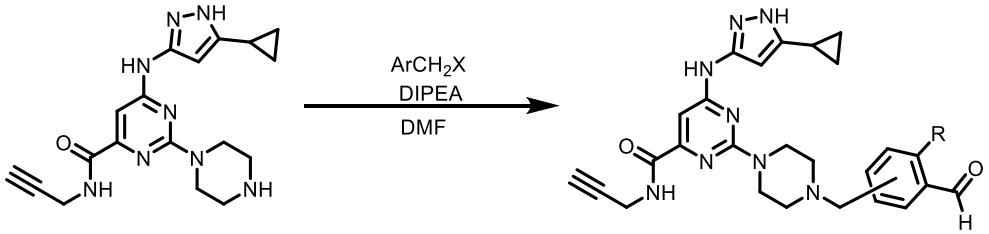
Supplementary Table 2. Plasma concentrations of probe **3** (related to Figure 5). Mice were injected subcutaneously with salicylaldehyde probe **3**. At the indicated times, the mice were euthanized and plasma and spleens were harvested. We chose individual samples (mouse spleens) for the TMT10 chemoproteomic analysis based on the probe **3** plasma concentrations measured for each mouse/time point, so that we could test whether kinase engagement correlated with probe **3** plasma concentrations across all time points. Spleens from the lower dose group (25 mg/kg) were used for the t=1 h time point, because the plasma concentrations of **3** (~1,000 ng/mL) closely matched the 50 mg/kg dose group at t=7 h (~1,000 ng/mL). Higher or equivalent levels of kinase engagement in spleens from t=7 h, relative to t=1 h and t=3 h, indicates prolonged probe-kinase engagement (despite decreased plasma concentration), as observed for AURKA, AURKB, and SGK3 (Figure 5c,d).

	Plasma Concentration (ng/mL)		
	1 h	3 h	7 h
vehicle (n=3)			BLQ BLQ BLQ
3 25 mg/kg (n=2)	1090 1170		
3 50 mg/kg (n=5)		4500 5450 5310	
			884 1150

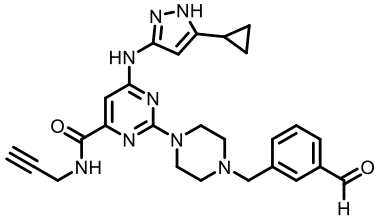
BLQ = Below limit of quantification

Supplementary Note

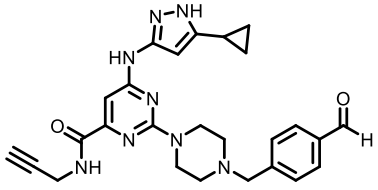
NMR spectra were recorded on a 400 MHz Varian spectrometer or a 500 MHz Bruker spectrometer. Chemical shifts are reported as parts per million (ppm) from an internal tetramethylsilane standard or solvent reference. LC−MS was performed on a Waters LC system coupled with Xevo G2-XS Q-Tof Mass Spectrometer with a flow rate of 0.2 mL/min using an Xterra MS C18 column (Waters) with water-acetonitrile gradient containing 0.1% formic acid. All solvents were of ACS chemical grade (Sigma Aldrich) and used without further purification. Commercially available reagents were used without further purification. Analytical thin-layer chromatography was performed with silica gel 60 F254 glass plates (Merck). Silica gel chromatography was performed with CombiFlash (TELEDYNE ISO).



Scheme 1. Chemical synthesis of probes **1**-**4**

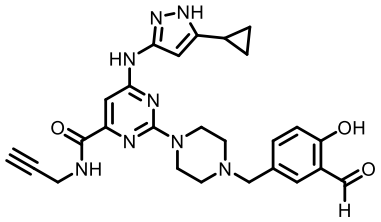


Probe **1**. To a mixture of 6-((5-cyclopropyl-1H-pyrazol-3-yl)amino)-2-(piperazin-1-yl)-N-(prop-2-yn-1-yl)pyrimidine-4-carboxamide[1] (40 mg, 0.083 mmol) and 3-(bromomethyl)benzaldehyde (95%, 22 mg, 0.104 mmol) in DMF (1 mL) was added *N,N*-diisopropylethylamine (58 μL, 0.33 mmol). The reaction was stirred at RT for 30 min, and then the solvents were evaporated *in vacuo*. The resulting residue was dissolved in EtOAc (20 mL), washed with sat. aqueous $NaHCO_3$ (20 mL), brine (20 mL) and dried over Na_2SO_4. Probe **1** (9.2 mg, 0.019 mmol, 23% yield) was obtained as a white solid after silica gel chromatography (50-100% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.43 (s, 1H), 7.98 (t, *J* = 5.0 Hz, 1H), 7.89 (s, 1H), 7.81 (d, *J* = 7.3 Hz, 1H), 7.66 (d, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 1H), 6.87 (s, 1H), 6.13 (s, 1H), 4.32 – 4.09 (m, 2H), 3.83 (s, 4H), 3.64 (s, 2H), 2.54 (s, 4H), 2.26 (s, 1H), 2.17 (s, 1H), 1.95 – 1.76 (m, 1H), 1.03 – 0.85 (m, 2H), 0.81 – 0.59 (m, 2H). HRMS calculated *m/z* for C26H29N8O2 (M+ H^+): 485.2408; found: 485.2415.

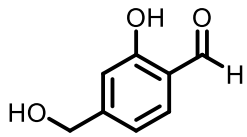


Probe **2**. To a mixture of 6-((5-cyclopropyl-1H-pyrazol-3-yl)amino)-2-(piperazin-1-yl)-N-(prop-2-yn-1-yl)pyrimidine-4-carboxamide (40 mg, 0.083 mmol) and 4-(bromomethyl)benzaldehyde (95%, 22 mg, 0.104 mmol) in DMF (1 mL) was added *N,N*-diisopropylethylamine (58 μL, 0.33 mmol). The reaction was stirred at RT for 30 min, and then the solvents were evaporated *in vacuo*. The resulting residue was dissolved in EtOAc (20 mL), washed with sat. aqueous $NaHCO_3$ (20 mL), brine (20 mL) and dried over Na_2SO_4. Probe **2** (10 mg, 0.021 mmol, 25% yield) was obtained as a white solid after silica gel chromatography (50-100% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.02 (s, 1H), 8.49 (s, 1H), 7.98 (t, *J* = 5.6 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 2H), 6.87 (s, 1H), 6.12 (s, 1H), 4.22

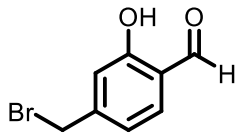
(dd, *J* = 5.6, 2.5 Hz, 2H), 3.92 – 3.75 (m, 4H), 3.63 (s, 2H), 2.62 – 2.39 (m, 4H), 2.26 (t, *J* = 2.5 Hz, 1H), 2.17 (s, 1H), 1.96 – 1.70 (m, 1H), 1.05 – 0.81 (m, 2H), 0.82 – 0.54 (m, 2H). HRMS calculated *m/z* for C26H29N8O2 (M+ H^+): 485.2408; found: 485.2431.



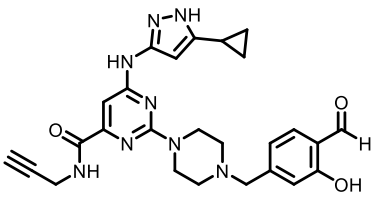
Probe **3**. To a mixture of 6-((5-cyclopropyl-1H-pyrazol-3-yl)amino)-2-(piperazin-1-yl)-N-(prop-2-yn-1-yl)pyrimidine-4-carboxamide (40 mg, 0.083 mmol) and and *N,N*-diisopropylethylamine (58 μL, 0.33 mmol) in DMF (0.5 mL) was added a solution of 5-(chloromethyl)-2-hydroxybenzaldehyde (9.94 mg, 0.058 mmol) in DMF (1 mL) dropwise at 4 ˚C over 45 min. The reaction was stirred at 4 ˚C for another 1 h, and then the solvents were evaporated *in vacuo*. The resulting residue was dissolved in EtOAc (20 mL), washed with sat. aqueous $NaHCO_3$ (20 mL), brine (20 mL) and dried over Na_2SO_4. Probe **3** (6.6 mg, 0.013 mmol, 23% yield) was obtained as a white solid after silica gel chromatography (50-100% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.98 (s, 1H), 9.91 (s, 1H), 8.26 (s, 1H), 7.96 (t, *J* = 5.6 Hz, 1H), 7.60 – 7.45 (m, 2H), 6.98 (d, *J* = 8.3 Hz, 1H), 6.88 (s, 1H), 6.10 (s, 1H), 4.22 (dd, *J* = 5.5, 2.3 Hz, 2H), 3.83 (s, 4H), 3.55 (s, 2H), 2.54 (s, 4H), 2.26 (t, *J* = 2.4 Hz, 1H), 2.18 (s, 1H), 1.96 – 1.79 (m, 1H), 1.01 – 0.88 (m, 2H), 0.79 – 0.65 (m, 2H). HRMS calculated *m/z* for C26H29N8O3 (M + H^+): 501.2357; found: 501.2368.



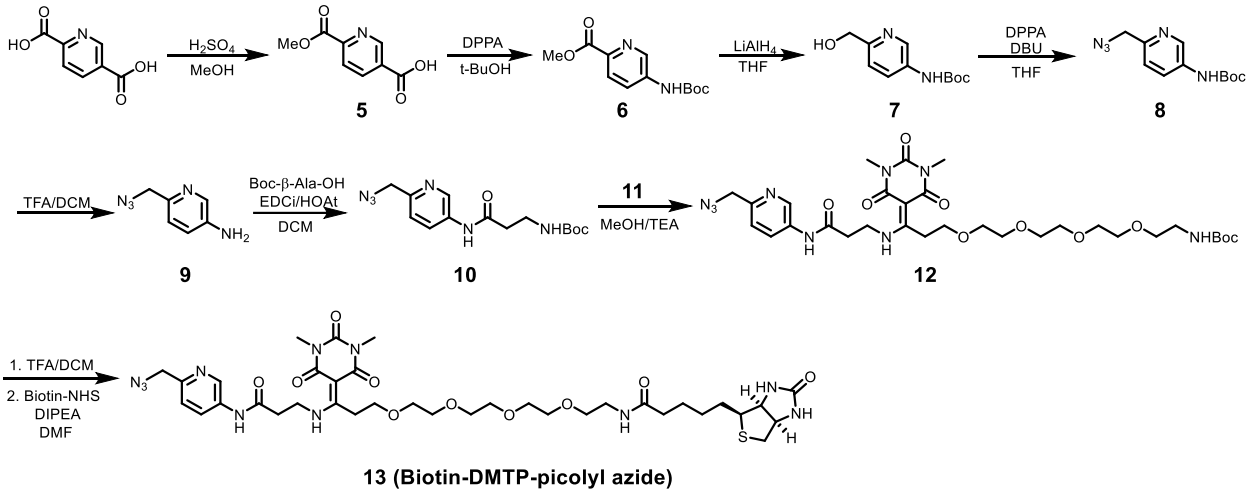
2-Hydroxy-4-(hydroxymethyl)benzaldehyde. 2-Hydroxy-4-(hydroxymethyl)benzaldehyde was prepared according to the reported procedure with some modifications.[2] Briefly, to a solution of 3-(hydroxymethyl)phenol (1g, 8.06 mmol), paraformaldehyde (1.64 g, 54.78 mmol) and $MgCl_2$ (1.15 g, 12.08 mmol) in anhydrous THF (50 mL) was added triethylamine (4.27 mL, 30.61 mmol). The reaction was stirred under reflux for 20 h. Then the reaction was cooled to RT, poured into 1 M HCl (100 mL), and extracted with EtOAc (50 mL x 3). The organic layers were pooled, and washed with brine (50 mL) and dried over Na_2SO_4. 2-Hydroxy-4-(hydroxymethyl)benzaldehyde (200 mg, 1.25 mmol, 16% yield) was obtained as a yellow solid after silica gel chromatography (10-60% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.06 (s, 1H), 9.85 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 6.99 (d, *J* = 7.9 Hz, 1H), 6.97 (s, 1H), 4.72 (s, 2H), 2.34 (s, 1H).



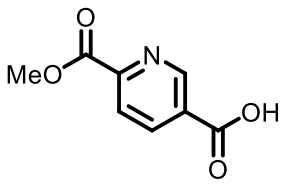
4-(Bromomethyl)-2-hydroxybenzaldehyde. 4-(Bromomethyl)-2-hydroxybenzaldehyde was prepared according to the reported procedure with some modifications.[3] Birefly, to a solution of 2-hydroxy-4-(hydroxymethyl)benzaldehyde (167 mg, 1.1 mmol) in toluene (4 mL) was added hydrobromic acid (48%, 1.5 mL, 13.26 mmol). The reaction was stirred under reflux for 3 h. Then the reaction was cooled to RT, and poured into water (50 mL), and extracted with EtOAc (25 mL x 3). The organic layers were pooled, washed with brine (50 mL) and dried over Na_2SO_4. 4-(Bromomethyl)-2-hydroxybenzaldehyde (152 mg, 0.71 mmol, 64% yield) was obtained as a blue solid after silica gel chromatography (0-100% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.05 (s, 1H), 9.89 (d, *J* = 0.5 Hz, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.05 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.02 (s, 1H), 4.42 (s, 2H).



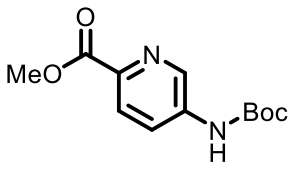
Probe **4**. To a mixture of 6-((5-cyclopropyl-1H-pyrazol-3-yl)amino)-2-(piperazin-1-yl)-N-(prop-2-yn-1-yl)pyrimidine-4-carboxamide (40 mg, 0.083 mmol) and and *N,N*-diisopropylethylamine (58 μL, 0.33 mmol) in DMF (0.5 mL) was added a solution of 4-(bromomethyl)-2-hydroxybenzaldehyde (15.22 mg, 0.071 mmol) in DMF (0.7 mL) dropwise at 4°C over 45 min. The reaction was stirred at 4 ˚C for another 30 min, and then the solvents were evaporated *in vacuo*. The resulting residue was dissolved in EtOAc (20 mL), washed with sat. aqueous $NaHCO_3$ (20 mL), brine (20 mL) and dried over Na_2SO_4. Probe **4** (18 mg, 0.036 mmol, 51% yield) was obtained as a white solid after silica gel chromatography (50-100% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.07 (s, 1H), 9.88 (s, 1H), 8.71 (s, 1H), 7.99 (t, *J* = 5.6 Hz, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.05 (d, *J* = 8.9 Hz, 2H), 6.85 (s, 1H), 6.13 (s, 1H), 4.22 (dd, *J* = 5.6, 2.5 Hz, 2H), 3.91 – 3.70 (m, 4H), 3.57 (s, 2H), 2.61 – 2.44 (m, 4H), 2.26 (t, *J* = 2.5 Hz, 1H), 2.17 (s, 1H), 1.93 – 1.76 (m, 1H), 0.99 – 0.83 (m, 2H), 0.78 – 0.56 (m, 2H). HRMS calculated *m/z* for C26H29N8O3 (M + H$^+$): 501.2357; found: 501.2372.



Scheme 2. Synthesis of biotin-DMTP-picolyl azide

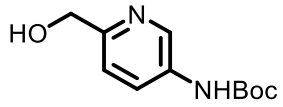


Compound 5. Following a literature procedure,[4] to a suspension of 2, 5-pyridinedicarboxylic acid (12.6 g, 75.4 mmol) in MeOH (150 mL) was added sulfuric acid (98%, 2.38 mL, 43.73 mmol). The reaction was stirred at 80 ˚C for 3 h. Then the reaction was cooled down to RT and poured into water (800 mL). The formed white solid was collected by filtration, washed with water, and then redissolved in DCM (200 mL), washed with sat. aqueous $NaHCO_3$ solution. The aqeous phase was separated and acidified to a pH of ~2 by adding 6 M HCl solution. The resulting white solid was collected and dried by vacuum overnight (Compound **5**, 5.38 g, 29.7 mmol, 39% yield). ^{1}H NMR (400 MHz, DMSO) δ 13.77 (s, 1H), 9.15 (d, *J* = 1.7 Hz, 1H), 8.44 (dd, *J* = 8.1, 2.1 Hz, 1H), 8.16 (d, *J* = 8.1 Hz, 1H), 3.91 (s, 3H).

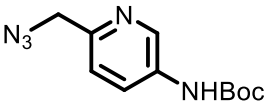


Compound 6. Following a literature procedure,[5] to a suspension of compound **5** (3 g, 16.56 mmol) in *t*-BuOH (30 mL) was added diphenylphosphoryl azide (DPPA, 3.57 mL, 16.56 mmol) and triethylamine (TEA, 2.31 mL, 16.46 mmol). The reaction was stirred at 80 ˚C overnight. Then the reaction was cooled down to RT, poured into EtOAc (200 mL), filtered though Celite. The filtrate was collected and

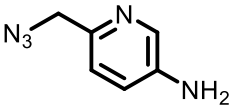
concentrated. The resulting residue was redissolved in EtOAc (150 mL), washed with 0.1 M HCl (100 mL), water (100 mL), and sat. aqeous $NaHCO_3$ solution (100 mL) sequentially. The organic phase was separated and dried over Na_2SO_4. Compound **6** (1.87 g, 7.41 mmol, 45% yield) was obtained as a yellow solid after silica gel chromatography (0-20% MeOH in DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 2.6 Hz, 1H), 8.25 – 8.16 (m, 1H), 8.11 (d, *J* = 8.6 Hz, 1H), 6.77 (s, 1H), 3.99 (s, 3H), 1.54 (s, 9H).



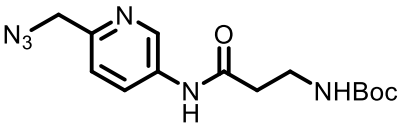
Compound 7. Following a literature procedure,[6] to a solution of compound **6** (1.06 g, 4.2 mmol) in anhydrous THF (20 mL) was added 1 M lithium aluminum hydride solution in THF (5.46 mL, 5.56 mmol) at 0 ˚C over 30 min. The reaction was warmed up to RT over 3 h. Then the reaction was cooled down to 0 ˚C and quenched by adding EtOAc (5 mL) and sat. aqeous Na_2SO_4 solution (5 mL) sequentially. The reaction solution was poured into EtOAc (100 mL). The organic phase was separated and dried over Na_2SO_4. Compound **7** (705 mg, 3.14 mmol, 75% yield) was obtained as a yellow liquid after silica gel chromatography (0-20% MeOH in DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, *J* = 2.5 Hz, 1H), 7.96 (d, *J* = 6.5 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 6.53 (s, 1H), 4.71 (s, 2H), 3.40 (s, 1H), 1.53 (s, 9H).



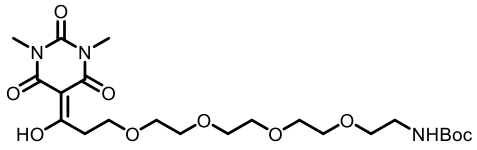
Compound 8. Following a literature procedure,[7] to a solution of compound **7** (300 mg, 1.34 mmol) in anhydrous THF (10 mL) were added DBU (408 μL, 2.68 mmol) and DPPA (588 μL, 2.68 mmol) at 0 ˚C over 3 min. The reaction was warmed up to RT and kept stirring for another 2 h. The reaction solution was poured into EtOAc (100 mL), and washed with water (50 mL). The organic phase was separated and dried over Na_2SO_4. Compound **8** (274 mg, 1.1 mmol, 82% yield) was obtained as a white solid after silica gel chromatography (0-60% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, *J* = 2.5 Hz, 1H), 8.03 (d, *J* = 7.0 Hz, 1H), 7.28 (d, *J* = 8.5 Hz, 1H), 6.56 (s, 1H), 4.42 (s, 2H), 1.53 (s, 9H).



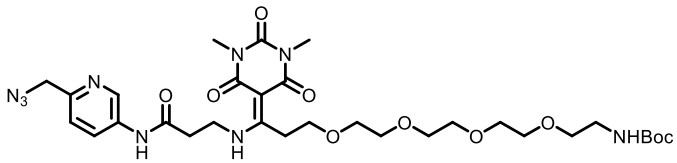
Compound 9. Following a literature procedure,[7] compound **8** (279 mg, 1.12 mmol) was dissolved in a mixture of DCM and TFA (1:1, 10 mL). The reaction was stirred at RT for 2 h, and then concentrated in vacuo. The resulting residue was dissolved in EtOAc (20 mL), washed with sat. aqueous $NaHCO_3$ (20 mL), brine (20 mL) and dried over Na_2SO_4. Compound **9** (120 mg, 0.80 mmol, 72% yield) was obtained as a brown solid after silica gel chromatography (0-100% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 2.8 Hz, 1H), 7.12 (d, *J* = 8.2 Hz, 1H), 6.99 (dd, *J* = 8.3, 2.8 Hz, 1H), 4.35 (s, 2H), 3.74 (s, 2H).



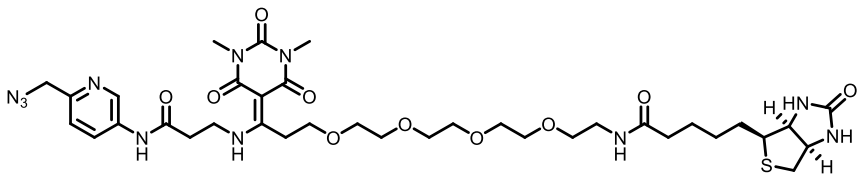
Compound 10. To a solution of β-Boc-Ala-OH in DCM (2 mL) were added EDCi hydrochloric acid (943 mg, 4.92 mmol), HOAt (335 mg, 2.46 mmol) and TEA (1.03 mL, 7.38 mmol). The mixture was kept stirring at RT for 2 h followed by adding compound **9** (367 mg, 2.46 mmol) in one portion. The reaction was kept stirring at RT for 24 h. Then the reaction solution was poured into EtOAc (100 mL), washed with 0.1 M HCl (50 mL), brine (50 mL), and dried over Na_2SO_4. Compound **10** (648 mg, 2.02 mmol, 82% yield) was obtained as a brown solid after silica gel chromatography (0-100% EtOAc in hexane). ^{1}H NMR (400 MHz, DMSO) δ 10.22 (s, 1H), 8.73 (d, *J* = 2.2 Hz, 1H), 8.07 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.38 (d, *J* = 8.4 Hz, 1H), 6.89 (t, *J* = 5.2 Hz, 1H), 4.44 (s, 2H), 3.24 (dd, *J* = 12.9, 6.9 Hz, 2H), 2.51 (t, *J* = 7.0 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, DMSO) δ 171.00, 156.50, 150.68, 141.38, 136.22, 127.69, 123.49, 78.58, 55.07, 37.63, 37.34, 29.15. HRMS calculated *m/z* for C14H21N6O3 ($M + H^+$): 321.1670; found: 321.1708.



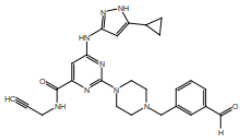
Compound 11. To a solution of 15-(Boc-amino)-4,7,10,13-tetraoxapentadecanoic acid (90 mg, 0.25 mmol) and 1,3-dimethylbarbituric acid (50 mg, 0.32 mmol) in DCM (3 mL) were added DMAP (39 mg, 0.32 mmol) and EDCi hydrochloric acid (52 mg, 0.27 mmol). The reaction was stirred at RT for 24 h and concentrated. Compound **11** (104 mg, 0.21 mmol, 84% yield) was obtained as colorless liquid after silica gel chromatography (0-20% MeOH in DCM). ^{1}H NMR (400 MHz, DMSO) δ 6.74 (t, *J* = 5.3 Hz, 1H), 3.75 (t, *J* = 6.4 Hz, 2H), 3.56 – 3.45 (m, 12H), 3.36 (dt, *J* = 13.9, 8.4 Hz, 4H), 3.20 (s, 6H), 3.13 – 2.99 (m, 2H), 2.55 – 2.47 (m, 1H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, DMSO) δ 196.27, 156.49, 150.85, 96.88, 80.10, 78.48, 73.27, 70.75, 70.70, 70.66, 70.60, 70.51, 70.43, 70.09, 67.10, 61.14, 37.37, 29.61, 29.14, 28.60. HRMS calculated *m/z* for C22H37N3NaO10 (M + Na^{+}): 526.2371; found: 526.2373.



Compound 12. Compound **10** (104 mg, 0.21 mmol) was dissolved in a mixture of DCM and TFA (1:1, 10 mL). The reaction was stirred at RT for 30 min, and then concentrated in vacuo. The resulting residue was redissolved in MeOH (5 mL), followed by adding compound **11** (132 mg, 0.41 mmol) and TEA (144 μL, 1.03 mmol). The reaction was stirred under reflux for 24 h. Then the reaction was cooled to RT and concentrated. Compound **12** (30 mg, 0.043 mmol, 21% yield) was obtained as colorless liquid after silica gel chromatography (0-100% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.08 (s, 1H), 9.21 (s, 1H), 8.57 (d, *J* = 2.2 Hz, 1H), 8.42 (d, *J* = 8.2 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 1H), 5.24 (s, 1H), 4.43 (s, 2H), 3.98 (q, *J* = 6.8 Hz, 2H), 3.85 (t, *J* = 5.1 Hz, 2H), 3.75 – 3.53 (m, 12H), 3.47 (t, J = 5.2 Hz, 4H), 3.32 (s, 3H), 3.29 (s, 3H), 3.21 (dd, *J* = 10.8, 5.3 Hz, 2H), 2.98 (t, *J* = 6.9 Hz, 2H), 1.43 (s, 9H). HRMS calculated *m/z* for C31H48N9O10 (M + Na^{+}): 706.3519; found: 706.3474.



Compound 13 (Biotin-DMTP-picolyl azide). Compound **12** (88 mg, 0.12 mmol) was dissolved in a mixture of DCM and TFA (1:1, 10 mL). The reaction was stirred at RT for 30 min, and then concentrated in vacuo. The resulting residue was redissolved in DMF (3 mL), followed by addition of biotin-NHS (97%, 48 mg, 0.047 mmol) and DIPEA (109 μL, 0.62 mmol). The reaction was stirred at RT for 3 h and then diluted with 50% formic acid in water (7 mL). Compound **13 (**Biotin-DMTP-picolyl azide) (48 mg, 0.05 mmol, 41% yield) was obtained as a white solid after HPLC purification (5-95% acetonitrile in water with 0.1% TFA). ^{1}H NMR (400 MHz, MeOD) δ 12.96 (s, 1H), 9.18 (d, *J* = 2.3 Hz, 1H), 8.35 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.81 (d, *J* = 8.7 Hz, 1H), 4.77 (s, 2H), 4.49 (dd, *J* = 7.8, 4.5 Hz, 1H), 4.30 (dd, *J* = 7.9, 4.5 Hz, 1H), 4.04 (t, *J* = 9.1 Hz, 2H), 3.83 (t, *J* = 5.8 Hz, 2H), 3.67 – 3.57 (m, 12H), 3.57 – 3.47 (m, 4H), 3.36 – 3.28 (m, 3H), 3.24 (s, 6H), 3.23 – 3.15 (m, 1H), 2.97 – 2.86 (m, 3H), 2.73 – 2.63 (m, 1H), 2.21 (t, *J* = 7.4 Hz, 2H), 1.81 – 1.51 (m, 4H), 1.50 – 1.31 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 174.89, 173.05, 171.20, 170.49, 169.87, 163.65, 151.53, 150.68, 140.89, 136.08, 128.40, 123.85, 89.62, 70.77, 70.73, 70.71, 70.67, 70.63, 70.47, 70.10, 69.51, 61.98, 60.14, 56.35, 54.76, 39.36, 36.56, 36.02, 30.96, 29.13, 28.97, 28.39, 26.38, 26.19. HRMS calculated *m/z* for C36H54N11O10S (M + H^{+}): 832.3770; found: 832.3796.



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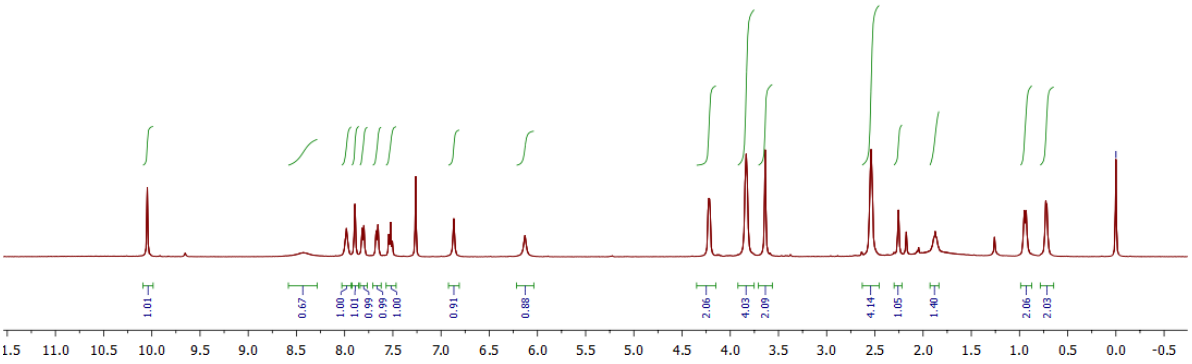

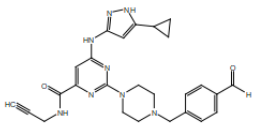

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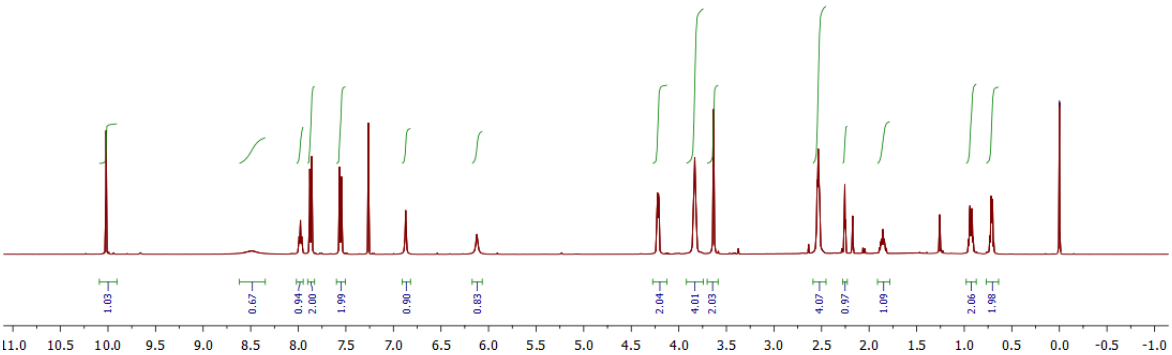

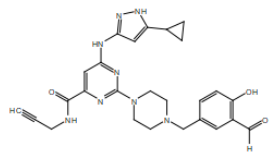

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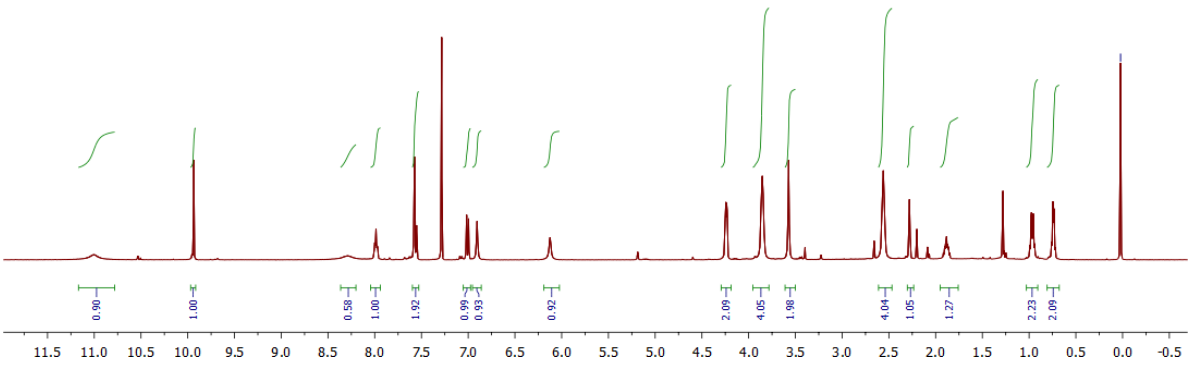
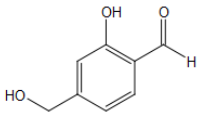
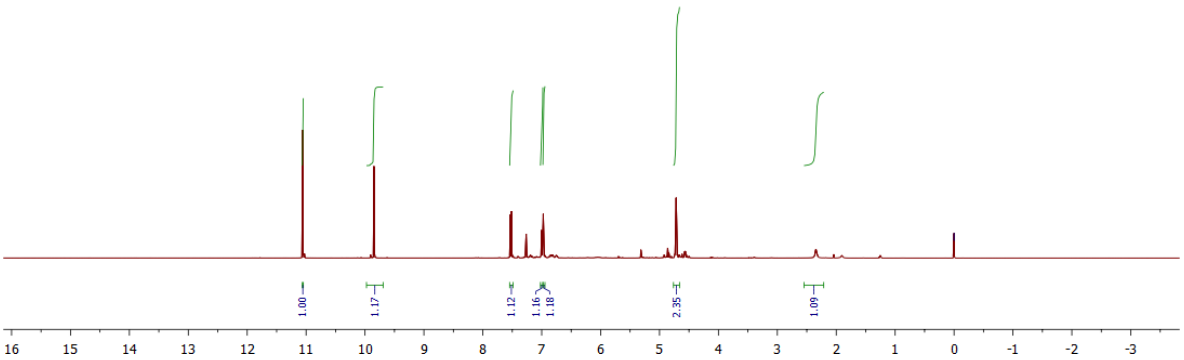
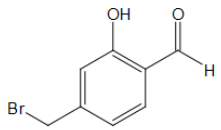


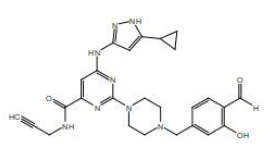


2-Hydroxy-4-(hydroxymethyl)benzaldehyde

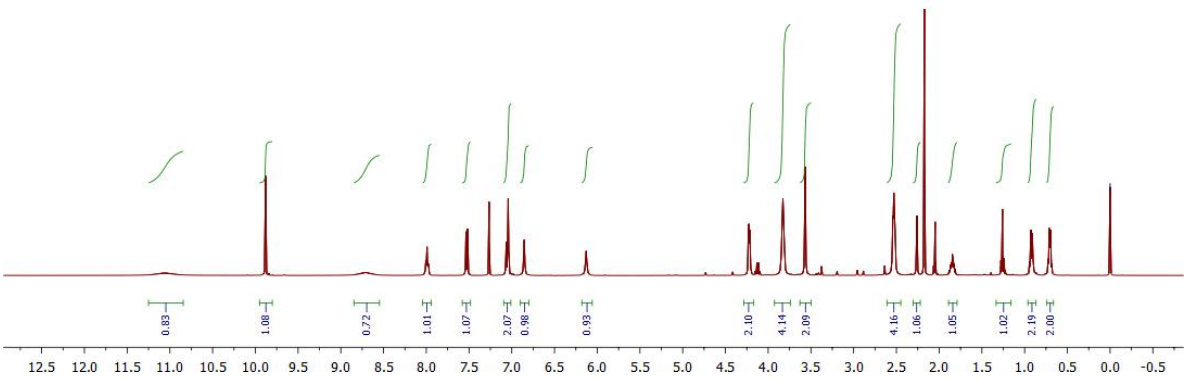


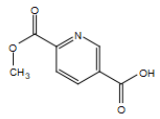


4-(Bromomethyl)-2-hydroxybenzaldehyde



Probe 4





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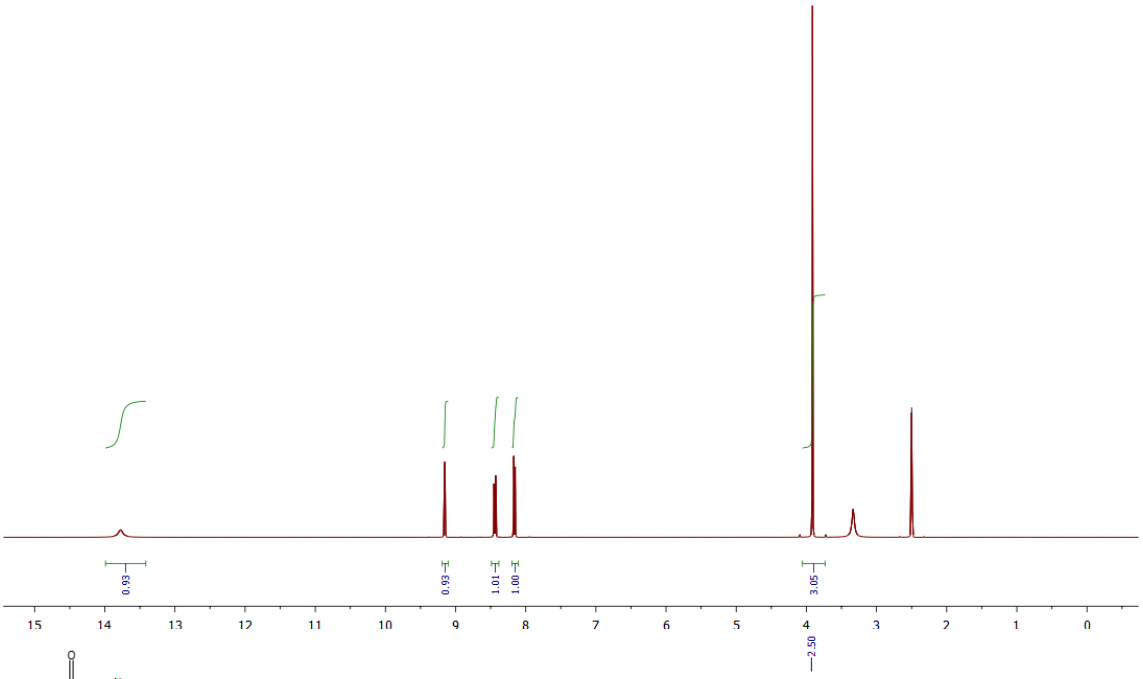

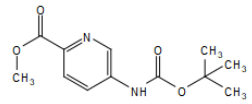

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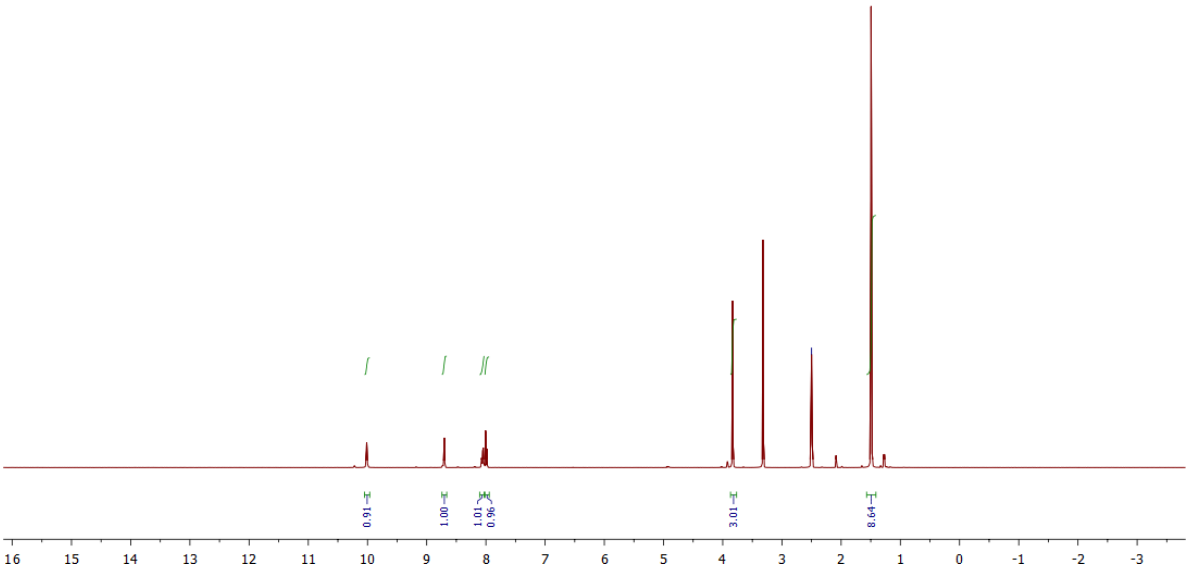

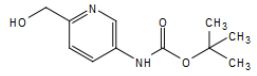
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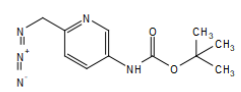
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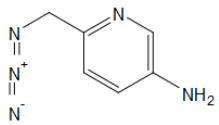

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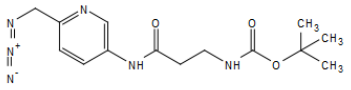

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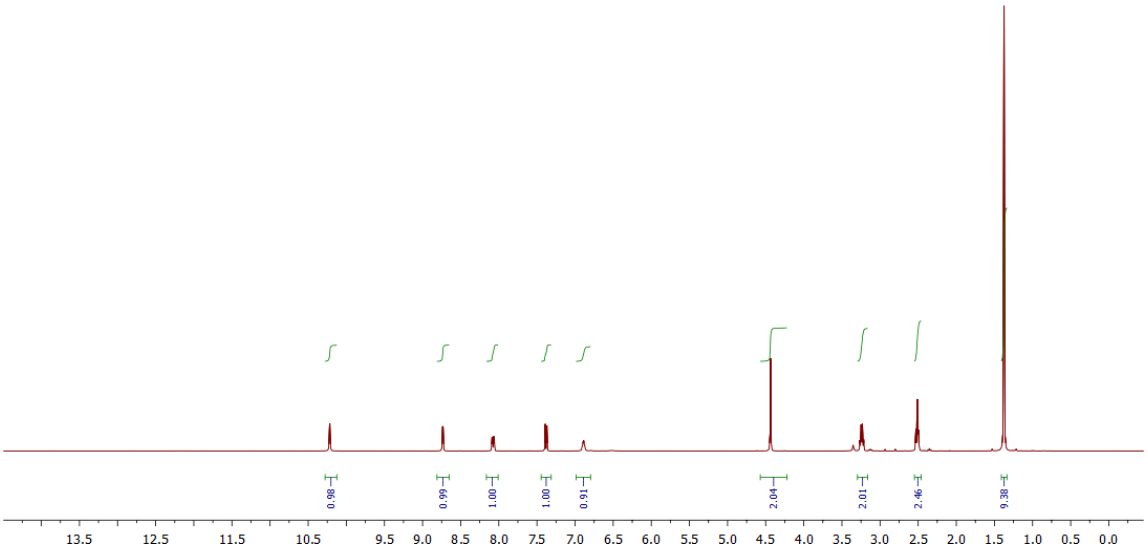

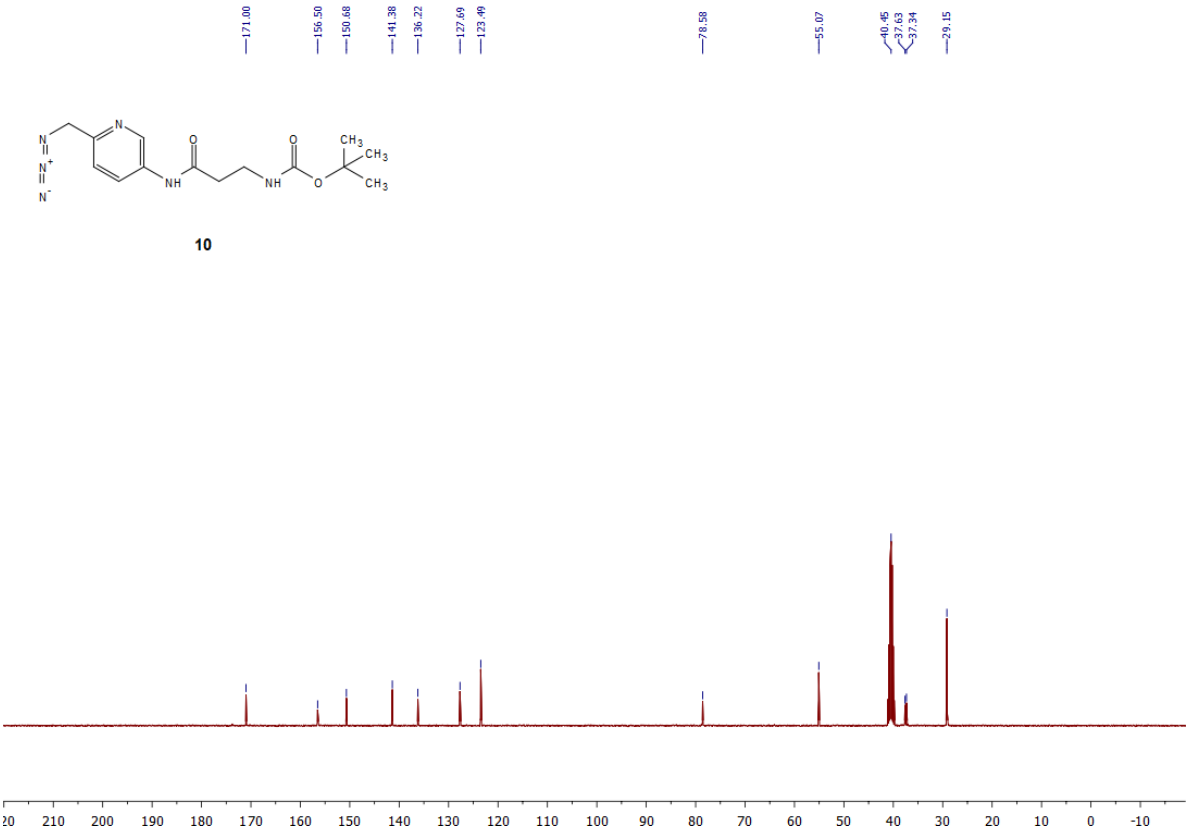
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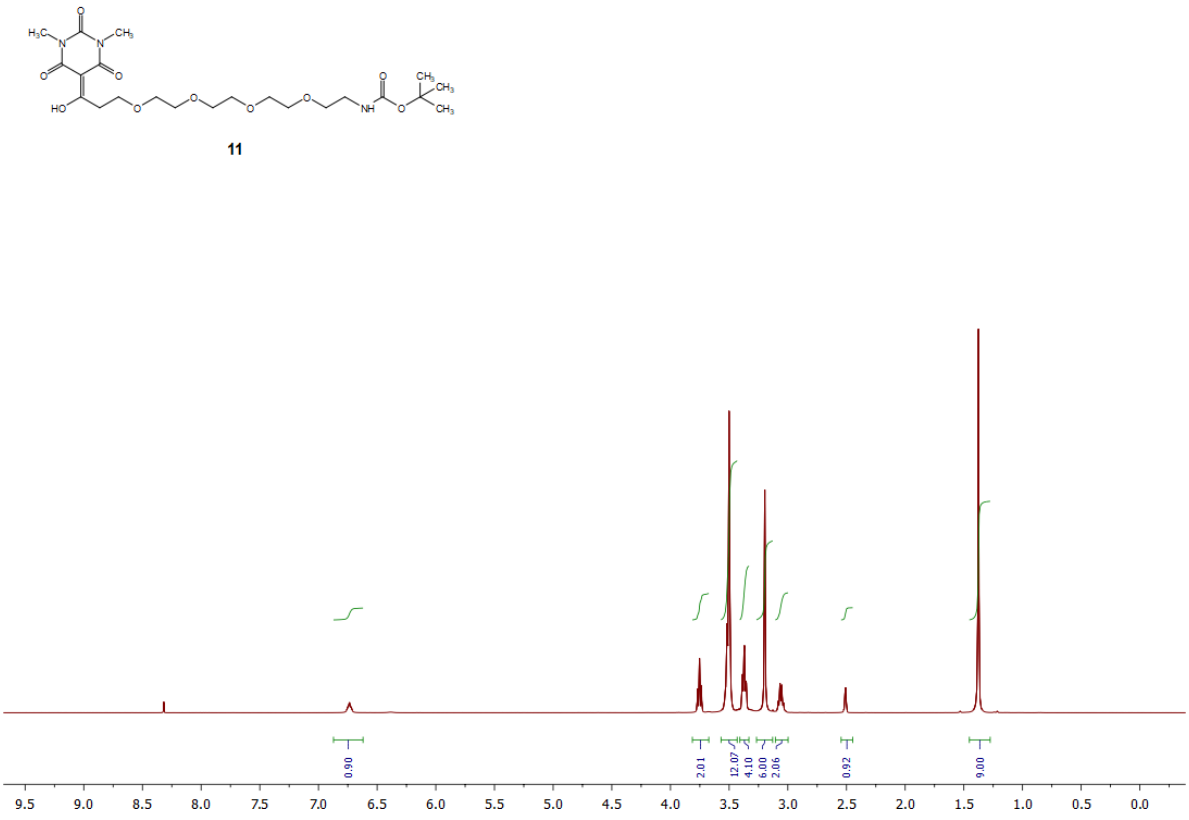
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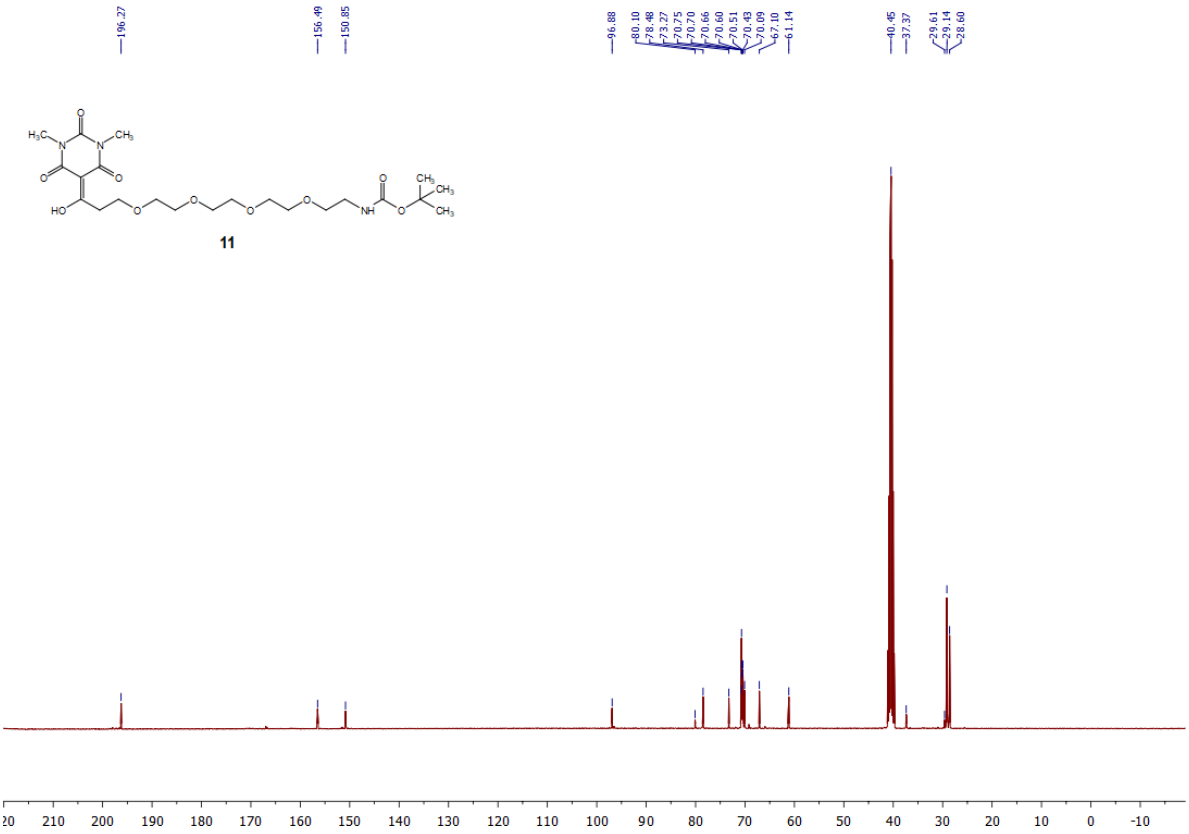
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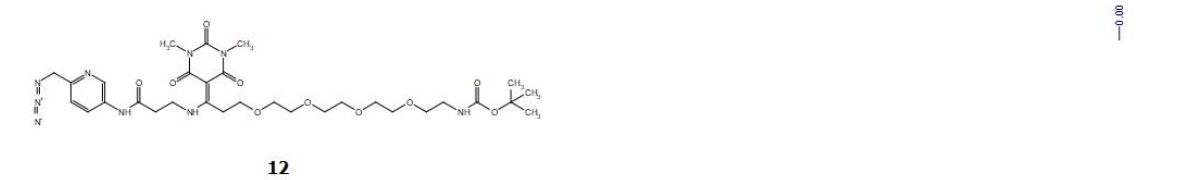
11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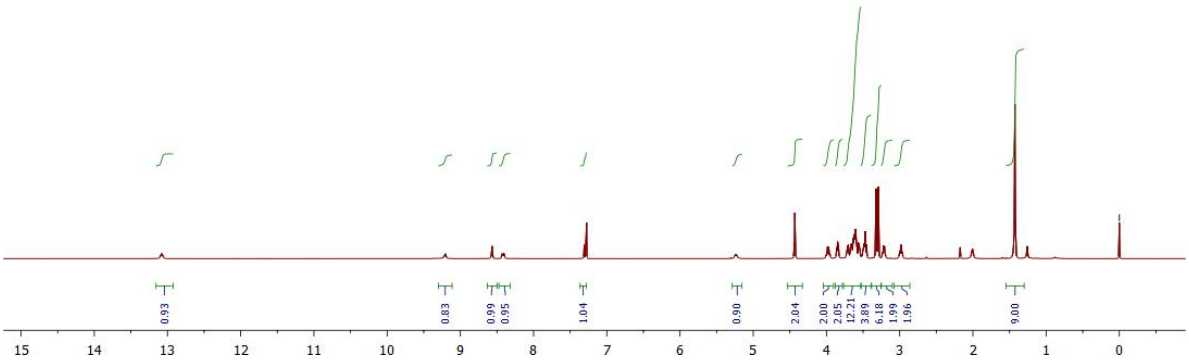
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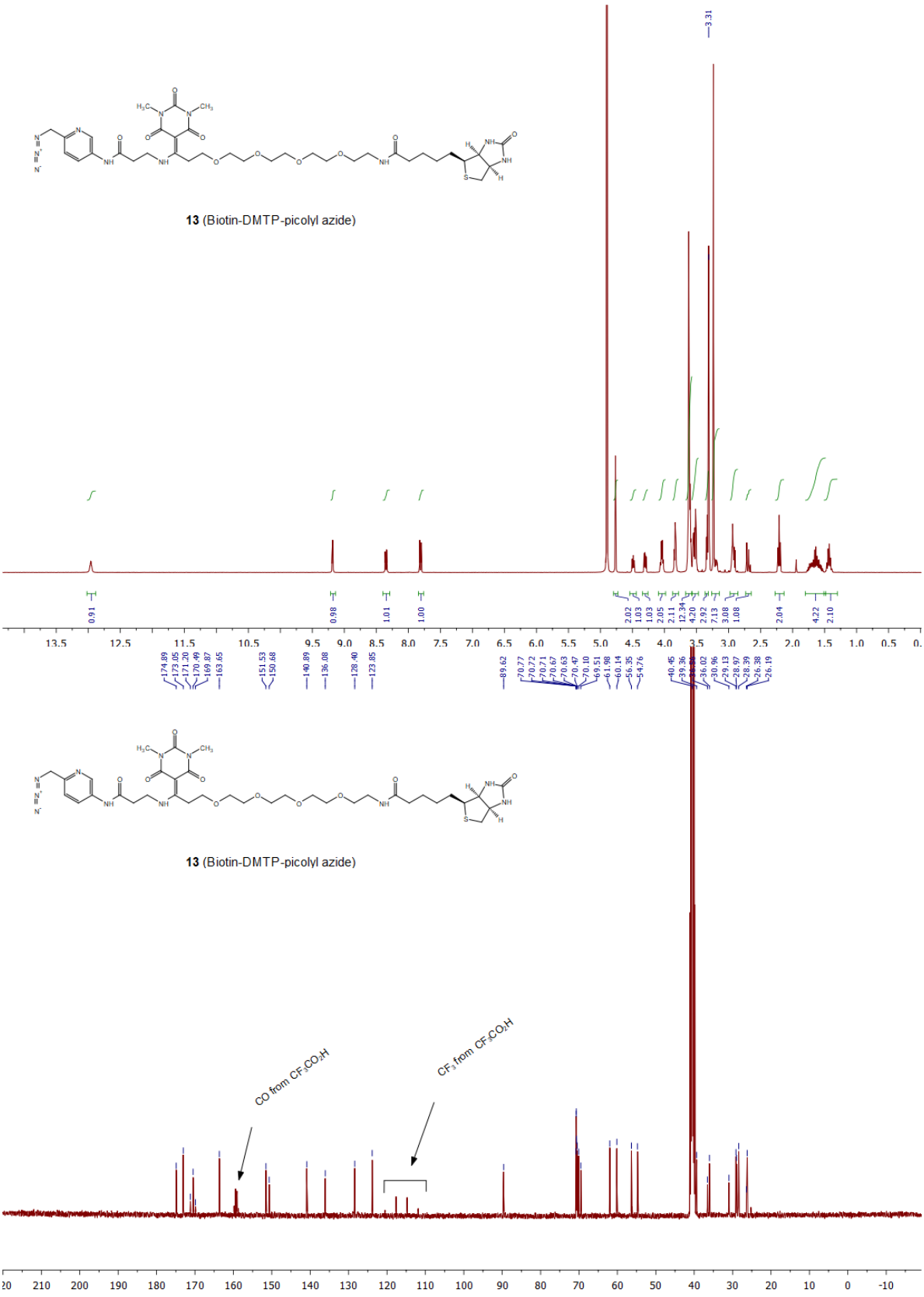
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26.19
13 (Biotin-DMTP-picolyl azide)
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CF3 from CF3CO2H

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