Bisubstrate-type chemical probes identify GRP94 as a potential target of cytosine-containing adenosine analogs

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Supporting Information

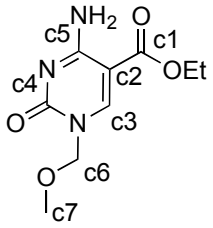
- Synthesis and characterization of compounds **6**-**17**.
- ^{1}H and ^{13}C NMR spectra of compounds **1**-**5**.
- Inhibition percentages of various DNMT, KHMTs and PRMTs for compounds **1**-**4**.
- Table of proteomic analysis result tables (Table SI-1 to SI-5) and volcano plot (Graph SI-1 to SI-5)

General procedure for synthesis of compound 6, 7 and 8

To a suspension of ethyl cytosine-5-carboxylate [6, 7] (200 mg; 1.09 mmol) and K_2CO_3 (301 mg; 2.18 mmol) in DMF (2 ml) was added the desired halide derivative (MOMCl (91 µl; 1.20 mmol); BOMCl (*tech.* 70%) (167 µl; 1.20 mmol) or 2-(Boc-amino)ethylbromide (270mg; 1.20 mmol)). The mixture was stirred overnight at room temperature. The mixture was filtrated, the solvent was removed and the residue was purified by silica gel flash chromatography using a linear gradient of ammonia 1N in methanol (0→10% MeOH/NH_3) in DCM to give the desired product.

***N*1-(methoxymethyl)-ethyl cytosine-5-carboxylate (6)**

Compound **6** was obtained as a white amorphous solid (191 mg; 0.90 mmol; 83%).



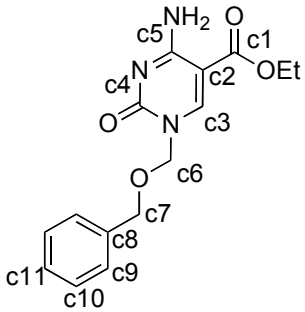
^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 8.57 (s, 1H, Hc3), 8.05 (s, 1H, HNH_2), 7.07 (s, 1H, HNH_2), 5.16 (s, 2H, Hc6), 4.27 (q, *J*=7.1Hz, 2H, HEt), 3.28 (s, 3H, Hc7), 1.30 (t, *J*=7.1Hz, 3H, HEt).

^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 164.9 (Cc1), 163.8 (Cc5), 154.30 (Cc4), 153.2 (Cc3), 95.5 (Cc2), 79.4 (Cc6), 61.1 (CEt), 56.7 (Cc7), 14.5 (CEt).

HRMS-ESI (m/z) calculated for $C_9H_{13}N_3Na_1O_4$ $[M+Na]^+$: 250.2092; found: 250.2079.

***N*1-(benzoxymethyl)-ethyl cytosine-5-carboxylate (7)**

Compound **7** was obtained as a white amorphous solid (224 mg; 0.74 mmol; 68%).



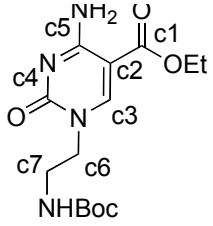
^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 8.67 (s, 1H, Hc3), 8.00 (s, 1H, HNH_2), 7.77 (s, 1H, HNH_2), 7.41-7.29 (m, 5H, Hc9 to Hc11), 5.28 (s, 2H, Hc6), 4.61 (s, 2H, Hc7), 4.27 (q, *J*=7.0Hz, 2H, HEt), 1.32 (t, *J*=7.0Hz, 3H, HEt).

^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 165.9 (Cc1), 164.0 (Cc5), 154.3 (Cc4), 153.0 (Cc3), 138.4 (Cc8), 128.7 (Cc10), 128.1 (Cc11), 127.9 (Cc9), 95.5 (Cc2), 78.4 (Cc6), 71.0 (Cc7), 61.0 (CEt), 14.9 (CEt).

HRMS-ESI (m/z) calculated for $C_{15}H_{17}N_3Na_1O_4$ $[M+Na]^+$: 326.1111; found: 326.1104.

***N*1-(2-((*tert*-butoxycarbonyl)amino)ethyl)-ethyl cytosine-5-carboxylate (8)**

Compound **8** was obtained as a white amorphous solid (218 mg; 0.67 mmol; 61%).



^{1}H NMR (500 MHz, DMSO-d_6) δ 8.29 (s, 1H, Hc3), 7.83 (d, *J* = 3.7 Hz, 1H, HNH), 7.57 (d, *J* = 3.6 Hz, 1H, HNH), 6.95 (t, *J* = 6.0 Hz, 1H, HNH), 4.24 (q, *J* = 7.1 Hz, 2H, HEt), 3.80 (t, *J* = 5.5 Hz, 2H, Hc6), 3.18 (q, *J* = 5.5 Hz, 2H, Hc7), 1.32 (s, 9H, HBoc), 1.29 (t, *J* = 7.1 Hz, 3H, HEt).

^{13}C NMR (125 MHz, DMSO-d_6) δ 165.1 (Cc1), 163.8 (Cc5), 156.1 (CBoc), 154.3 (Cc4), 153.7 (Cc3), 94.2 (Cc2), 78.2 (CBoc), 60.8 (CEt), 50.4 (Cc6), 38.7 (Cc7), 28.5 (CBoc), 14.7 (CEt).

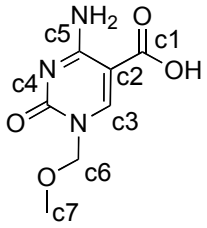
HRMS-ESI (m/z) calculated for $C_{14}H_{23}N_4O_5$ $[M+H]^+$: 327.1681; found: 327.1663.

General procedure for synthesis of compound 9, 10 and 11

Compound **6** (200 mg; 0.88 mmol), compound **7** (200 mg; 0.62 mmol) or compound **8** (200 mg; 0.61 mmol) was solubilised in a solution of NaOH 0.25 M in MeOH/water 1:1. The mixture was stirred overnight at room temperature and neutralised with concentrated HCl. The solvents were evaporated under vacuum and 500 µl of water was added to the mixture. After filtration the precipitate was wash with a minimum of MeOH and dried to afford the desired product.

***N*1-(methoxymethyl)-5-carboxycytosine (9)**

Compound **9** was obtained as a white amorphous solid (147 mg; 0.74 mmol; 84%).



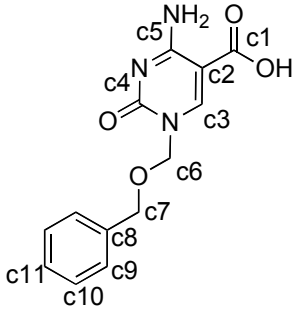
^{1}H NMR (500 MHz, DMSO-d_6) δ 9.24 (s, 1H, HNH2), 9.13 (s, 1H, HNH2), 8.84 (s, 1H, Hc3), 5.23 (s, 2H, Hc6), 3.33 (s, 3H, Hc7).

^{13}C NMR (125 MHz, $CDCl_3$) δ 165.1 (Cc5), 160.2 (Cc1), 154.9 (Cc3), 148.4 (Cc4), 96.5 (Cc2), 79.9 (Cc6), 57.7 (Cc7).

HRMS-ESI (m/z) calculated for $C_7H_9N_3Na_1O_4$ $[M+Na]^+$: 222.1552; found: 222.1550.

***N*1-(benzoxymethyl)-5-carboxycytosine (10)**

Compound **10** was obtained as a white amorphous solid (159 mg; 0.54 mmol; 87%).



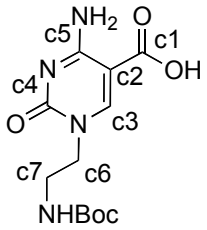
^{1}H NMR (500 MH, DMSO-d_6) δ 13.7 (brs, 1H, HCOOH), 9.57 (s, 1H, Hc3), 8.03 (s, 1H, HNH2), 7.92 (s, 1H, HNH2), 7.38-7.27 (m, 5H, Hc9 to c11), 5.30 (s, 2H, Hc6), 4.60 (s, 2H, Hc7).

^{13}C NMR (125 MHz, DMSO-d_6) δ 166.7 (Cc5), 164.1 (Cc1), 154.3 (Cc4), 153.3 (Cc3), 138.0 (Cc8), 128.7 (Cc10), 128.1 (Cc11), 128.0 (Cc9), 96.0 (Cc2), 78.2 (Cc6), 71.0 (Cc7).

HRMS-ESI (m/z) calculated for $C_{13}H_{13}N_3Na_1O_4$ $[M+Na]^+$: 298.0798; found: 298.0789.

***N*1-(2-((*tert*-butoxycarbonyl)amino)ethyl)-5-carbethoxycytosine (11)**

Compound **11** was obtained as a white amorphous solid (159 mg; 0.47 mmol; 76%).



^{1}H NMR (500 MHz, DMSO-d_6) δ 13.0 (s, 1H, HCOOH), 8.29 (s, 1H, Hc3), 7.82 (s, 2H, HNH), 6.94 (t, *J* = 6.0 Hz, 1H, HNH), 3.81 (t, *J* = 5.3 Hz, 2H, Hc6), 3.2 (q, *J* = 5.6 Hz, 2H, Hc7), 1.3 (s, 8H, HBoc).

^{13}C NMR (125 MHz, DMSO-d_6) δ 167.1 (Cc1), 164.2 (Cc5), 156.1 (CBoc), 154.5 (Cc4), 153.8 (Cc3), 94.7 (Cc2), 78.2 (CBoc), 50.1 (Cc6), 38.8 (Cc7), 28.5 (CBoc).

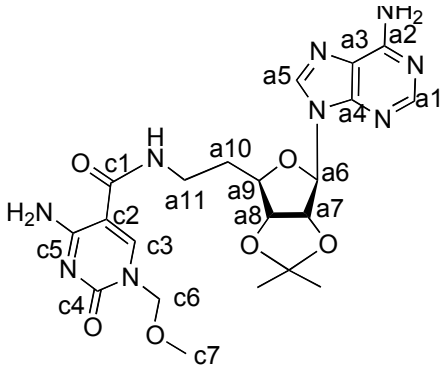
HRMS-ESI (m/z) calculated for $C_{12}H_{18}N_4NaO_5$ $[M+Na]^+$: 321.1169; found: 321.1158.

General procedure for synthesis of compounds 12, 13 and 14

To a suspension of compound **9**, **10** or **11** (0.41 mmol) and HATU (312 mg; 0.82 mmol) in DMF was added DiPEA (214 µl; 1.26 mmol). The mixture was stirred at room temperature for 5 min then 2',3'-*O*-isopropylidene-5'-deoxy-5'-aminomethyldenosine [8] was added to the mixture that was stirred again for 2 h. The solvent was removed and the residue was purified by silica gel flash chromatography using a linear gradient of ammonia 1 N in methanol (0→15% MeOH/NH_3) in DCM to give the desired product.

1-(methoxymethyl)-*N*-(2',3'-*O*-isopropylidene-(5'-deoxyadenosin-5'-yl)methyl)cytosine-5-carboxamide (12)

Compound **12** was obtained as a white amorphous solid (171 mg; 0.34 mmol; 83%).



^{1}H NMR (500 MHz, DMSO-d_6) δ 8.38-8.31 (m, 3H, Ha5 and Hc3 and HNH), 8.27 (brs, 1H, HNH), 8.17 (s, 1H, Ha1), 7.78 (s 1H, HNH), 7.35 (brs, 2H, HNH), 6.13 (d, *J*=2.9Hz, 1H, Ha6), 5.49 (dd,

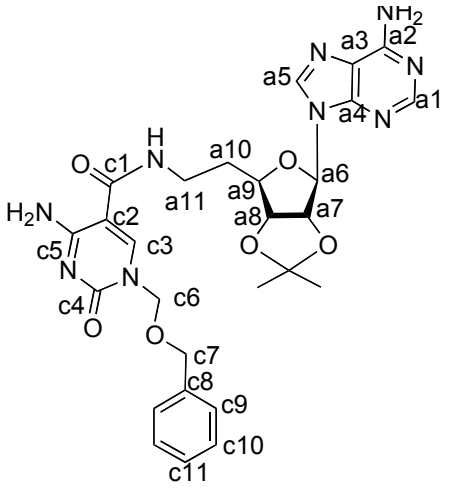
J=2.6, 6.3Hz, 1H, Ha7), 5.03 (s, 1H, Hc6), 4.91 (dd, *J*=3.5, 6.2Hz, 1H, Ha8), 4.21-4.15 (m, 1H, Ha9), 3.27 (s, 3H, Hc7), 3.23-3.13 (m, 2H, Ha11), 1.94-1.76 (m, 2H, Ha10), 1.53 (s, 3H, HiPr), 1.32 (s, 3H, HiPr).

^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 165.4 (Cc5), 164.5 (Cc1), 156.6 (Ca2), 154.5 (Cc4), 153.2 (Ca1), 149.4 (Ca4), 148.5 (Cc3), 140.4 (Ca5), 119.5 (Ca3), 113.9 (Cipr), 98.9 (Cc2), 88.9 (Ca6), 84.0 (Ca7), 83.9 (Ca8), 83.5 (Ca9), 79.6 (Cc6), 48.4 (Cc6), 56.7 (Cc7), 36.2 (Ca11), 33.2 (Ca10), 27.5 (CiPr), 25.7 (CiPr)

HRMS-ESI (m/z) calculated for $C_{21}H_{28}N_9O_6$ $[M+H]^+$: 502.2157; found: 502.2163.

1-(benzoxymethyl)-*N*-(2',3'-*O*-isopropylidene-(5'-deoxyadenosin-5'-yl)methyl)cytosine-5-carboxamide (13)

Compound **13** was obtained as a white amorphous solid (208 mg; 0.36 mmol; 88%).



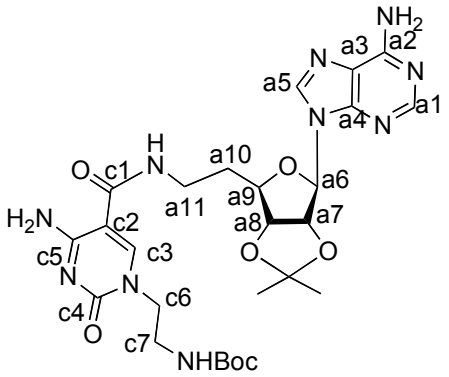
^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 8.39 (s, 1H, Hc3) and 8.35 (s, 1H, Ha5), 8.32 (t, 1H, *J*=5.4Hz, HNH), 8.28 (brs, 1H, HNH), 8.18 (s, 1H, Ha1), 7.80 (brs 1H, HNH), 7.38-7.26 (m, 7H, Hc9to10 and 2HNH), 6.13 (d, *J*=2.6Hz, 1H, Ha6), 5.50 (dd, *J*=2.6, 6.4Hz, 1H, Ha7), 5.19 (s, 1H, Hc6), 4.92 (dd, *J*=3.5, 6.4Hz, 1H, Ha8), 4.59 (s, 2H, Hc7), 4.21-4.15 (m, 1H, Ha9), 3.23-3.13 (m, 2H, Ha11), 1.95-1.77 (m, 2H, Ha10), 1.53 (s, 3H, HiPr), 1.32 (s, 3H, HiPr).

^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 165.4 (Cc5), 164.5 (Cc1), 156.6 (Ca2), 154.5 (Cc4), 153.2 (Ca1), 149.4 (Ca4), 148.6 (Cc3), 140.4 (Ca5), 138.0 (Cc8), 128.7 (Cc10), 128.1 (Cc11), 128.0 (Cc9), 119.6 (Ca3), 113.9 (Cipr), 99.0 (Cc2), 88.9 (Ca6), 84.0 (Ca7), 83.9 (Ca8), 83.5 (Ca9), 78.3 (Cc6), 71.0 (Cc7), 48.4 (Cc6), 56.5 (Cc7), 36.1 (Ca11), 33.2 (Ca10), 27.5 (CiPr), 25.7 (CiPr)

HRMS-ESI (m/z) calculated for $C_{27}H_{32}N_9O_6$ $[M+H]^+$: 578.2470; found: 578.2463.

1-(2-((*tert*-butoxycarbonyl)amino)ethyl)-*N*-(2',3'-*O*-isopropylidene-(5'-deoxyadenosin-5'-yl)methyl)cytosine-5-carboxamide (14)

Compound **14** was obtained as a white amorphous solid (174 mg; 0.29 mmol; 71%).

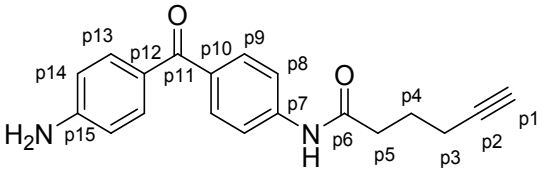


^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (s, 1H, Hc3), 8.22 (t, *J* = 5.4 Hz, 1H, HNH), 8.17 (s, 1H, Ha1), 8.15 (brs, 2H, Ha5 and HNH), 7.55 (brs, 1H, HNH), 7.35 (s, 2H, HNH), 6.94 (t, *J* = 5.8 Hz, 1H, HNH), 6.13 (d, *J* = 2.6 Hz, 1H, Ha6), 5.51 (dd, *J* = 6.3, 2.7 Hz, 1H, Ha7), 4.92 (dd, *J* = 6.3, 3.5 Hz, 1H, Ha8), 4.18 (ddd, *J* = 8.8, 5.4, 3.5 Hz, 1H, Ha9), 3.79 – 3.64 (m, 2H, Hc6), 3.17 (m, 4H, Hc7 and Ha11), 1.97-1.74 (m, 2H, Ha10), 1.54 (s, 3H, HiPr), 1.33 (s, 3H, HiPr), 1.32 (s, 9H, HBoc),

^{13}C NMR (125 MHz, DMSO-d_6) δ 165.7 (Cc5), 164.4 (Cc1), 156.6 (Ca2), 156.1 (CBoc), 154.5 (Cc4), 153.2 (Ca1), 149.4 (Ca4), 149.3 (Ca5), 140.3 (Cc3), 119.6 (Ca3), 113.9 (CiPr), 97.9 (Cc2), 88.9 (Ca6), 84.0 (Ca9), 83.9 (Ca8), 83.5 (Ca7), 78.3 (CBoc), 49.9 (Cc6), 49.0, 38.9 (Cc7), 36.1 (Ca11), 33.4 (Ca10), 28.6 (CBoc), 27.5 (CiPr), 25.7 (CiPr),

HRMS-ESI (m/z) calculated for $C_{26}H_{36}N_{10}O_7$ $[M+H]^+$: 601.2841; found: 601.2823.

***N*-(4-(4-aminobenzoyl)phenyl)hex-5-ynamide (15)**



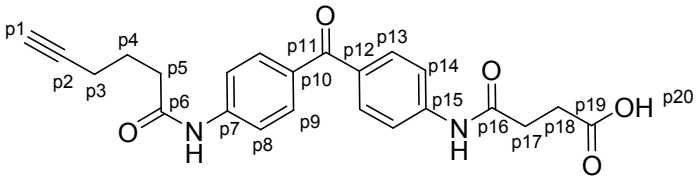
1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) (2.05 g; 5.3 mmol) was solubilized in DMA (10 ml) at RT under argon. To the solution was added 5-hexynoic acid (0.45 ml; 4.1 mmol) and *N*,*N*-diisopropylethylamine (DIPEA) (2 ml; 11.5 mmol). Reaction mixture was stirred for 15 min before addition of 4,4'-diaminobenzophenone (1 g; 4.9 mmol) and was then further stirred for 2 h at room temperature. The residue was diluted with ethyl acetate and washed with saturated $NaHCO_3$, 10% aqueous citric acid and dried over sodium sulfate. The solvent was removed and the residue was purified by silica gel flash chromatography using a linear gradient of methanol (0→20 % MeOH) in dichloromethane to obtain the title compound **15** as a yellow solid (926 mg; 3.0 mmol; 73%).

^{1}H NMR (500 MHz, DMSO) δ 10.22 (s, 1H, HNH), 7.71 (d, *J*=8.5 Hz, 2H, Hp8), 7.59 (d, *J*=8.6 Hz, 2H, Hp9), 7.50 (d, *J*=8.6 Hz, 2H, Hp13), 6.59 (d, *J*=8.6 Hz, 2H, Hp14), 6.09 (s, 2H, HNH), 2.83 (t, *J*=2.6 Hz, 1H, Hp1), 2.47 (t, *J*=7.4 Hz, 2H, Hp5), 2.24 (dt, *J*=2.6, 7.1Hz, 2H, Hp3), 1.77 (quint, *J*=7.3Hz, 2H, Hp4).

^{13}C NMR (125 MHz, DMSO) δ 192.4 (Cp11), 171 (Cp6), 153.4 (Cp15), 142 (Cp7), 133.2 (Cp10), 132.4 (Cp13), 130.2 (Cp9), 124.1 (Cp12), 118.1 (Cp8), 112.5 (Cp14), 83.9 (Cp2), 71.7 (Cp1), 35.2 (Cp5), 23.8 (Cp4), 17.3 (Cp3).

HRMS-ESI (m/z) calculated for $C_{19}H_{19}N_2O_2$ $[M+H]^+$: 307.1368; found: 307.1382.

4-((4-(4-(hex-5-ynamido)benzoyl)phenyl)amino)-4-oxobutanoic acid (16)



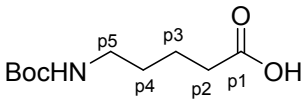
Succinic anhydride (100 mg, 1 mmol) was dissolved in 2 ml of dioxane and *N*-(4-(4-aminobenzoyl)phenyl)hex-5-ynamide (184 mg, 0.6 mmol) in 2 ml of dioxane was slowly added. The reaction mixture was heated to 80 °C for 4 h. The solvent was then removed and the residue was purified by silica gel flash chromatography using a linear gradient of methanol (0→20% MeOH) in dichloromethane to obtain the title compound **16** as a yellow solid (207 mg; 0.51 mmol; 85%).

^{1}H NMR (500 MHz, DMSO) δ 12.17 (brs, 1H, Hp20), 10.34 (s, 1H, NH), 10.30 (s, 1H, NH), 7.78-7.73 (m, 4H, Hp9, Hp13), 7.72-7.67 (m, 4H, Hp8, Hp14), 2.83 (t, *J*=2.5 Hz, 1H, Hp1), 2.62 (t, *J*=6.5 Hz, 2H, Hp17), 2.54 (t, *J*=6.5 Hz, 2H, Hp18), 2.48 (t, *J*=7.1 Hz, 2H, Hp5), 2.24 (dt, *J*=2.8, 6.9 Hz, 2H, Hp3), 1.78 (quint, *J*=7.4Hz, 2H, Hp4).

^{13}C NMR (125 MHz, DMSO) δ 193.4 (Cp11), 173.8 (Cp19), 171.2 (Cp6), 170.8 (Cp16), 143.1 (Cp15), 143 (Cp7), 131.7 (Cp10 and Cp12), 130.9 (Cp9 and Cp13), 118.1 (Cp8 and Cp14), 83.9 (Cp2), 71.8 (Cp1), 35.2 (Cp5), 31.2 (Cp17), 28.7 (Cp18), 23.8 (Cp4), 17.4 (Cp3).

HRMS-ESI (m/z) calculated for $C_{23}H_{23}N_2O_5$ $[M+H]^+$: 407.1601; found: 407.1605.

5-(Boc-amino)pentanoic acid (17)



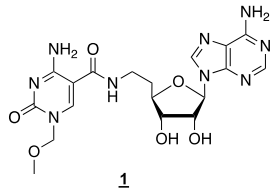
5-Aminopentanoic acid (0.981 g; 8.4 mmol) was solubilized in dioxane (20 ml). A solution of NaOH (0.34 g; 8.5 mmol) in water (10 ml) was added and the mixture was cooled down to 0 °C. Di-*tert*-butyl dicarbonate (2.4 g; 11.0 mmol) was then added and the reaction mixture was stirred at room temperature for 16 h. The solution was concentrated under reduced pressure. The basic residue was diluted in H_2O and washed with EtOAc. The aqueous phase was acidified to pH 1-2 with 1 N HCl, extracted with EtOAc and dried over sodium sulfate. The solvent was removed to afford the title compound **17** as a clear oil (1.58 g; 7.2 mmol; 87 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.59 (s, 1H, NH), 3.15-3.04 (m, 2H, Hp5), 2.35 (t, *J*=7.3 Hz, 2H, Hp2), 1.64 (quint, *J*=7.7 Hz, 2H, Hp3), 1.51 (quint, *J*=7.3 Hz, Hp4), 1.41 (s, 9H, HBoc)

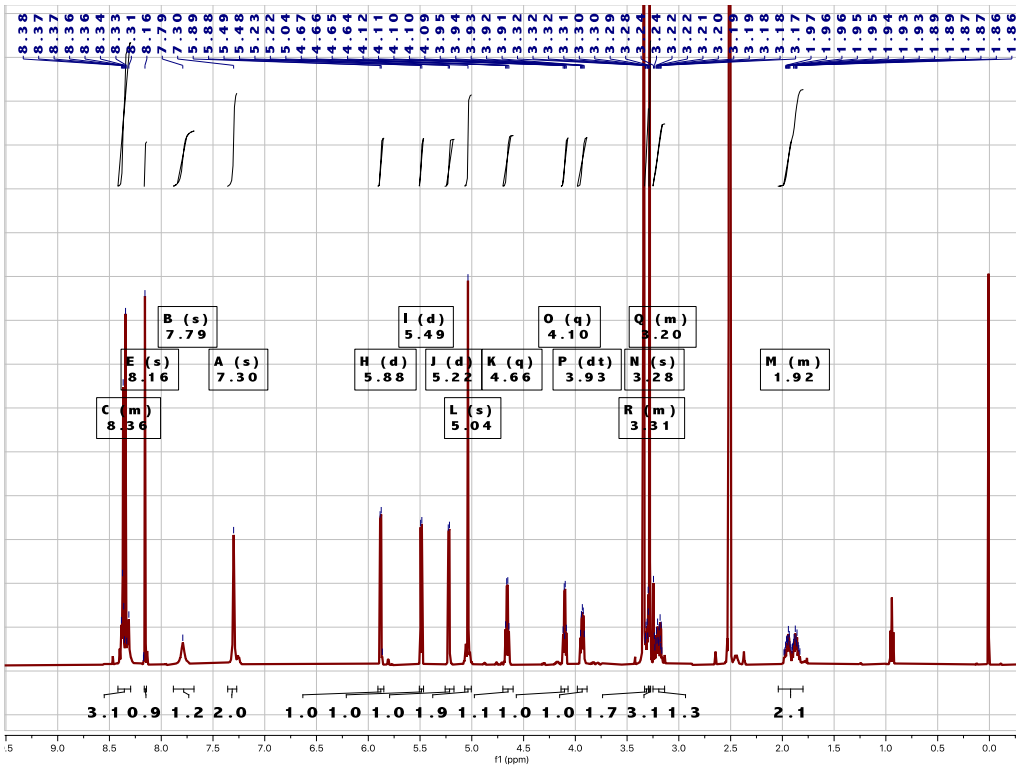
^{13}C NMR (125 MHz, $CDCl_3$) δ 178.9 (Cp1), 156.3 (CBoc), 79.5 (CBoc), 40.3 (Cp5), 33.7 (Cp2), 29.6 (Cp4), 28.6 (CBoc), 22 (Cp3).

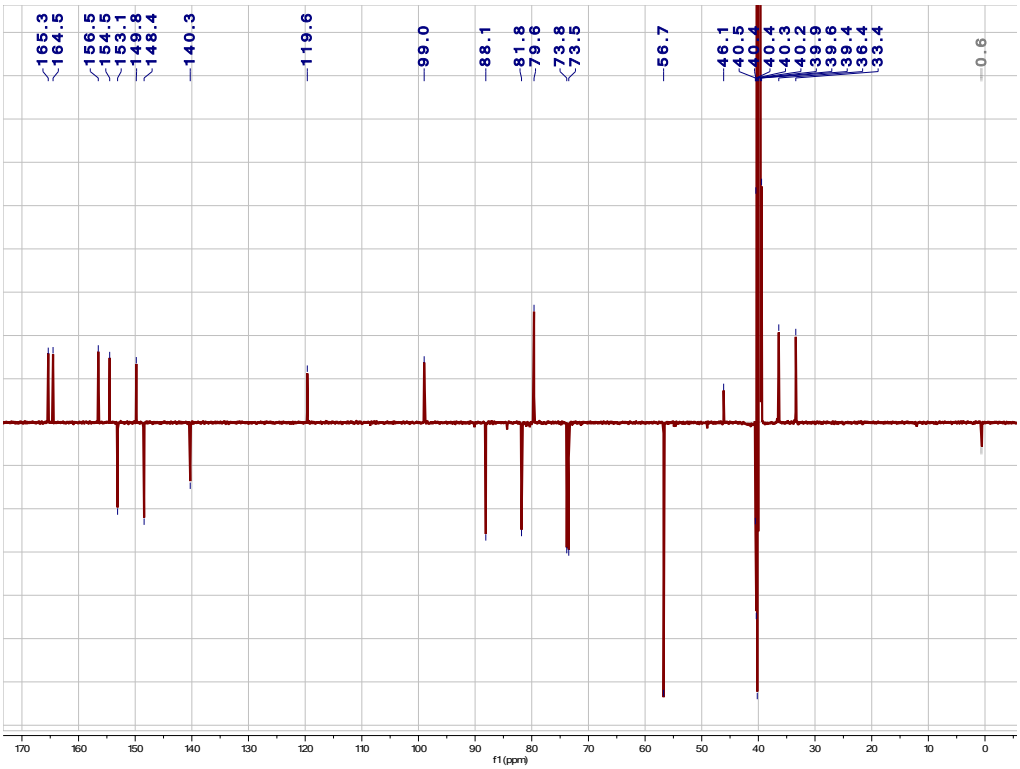
HRMS-ESI (m/z) calculated for $C_{10}H_{20}NO_4$ $[M+H]^+$: 218.1387; found: 218.1392.

^{1}H and ^{13}C NMR of compound 1

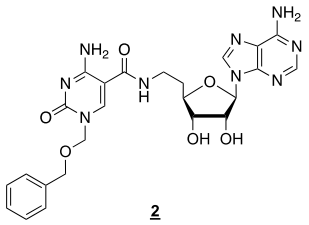


1

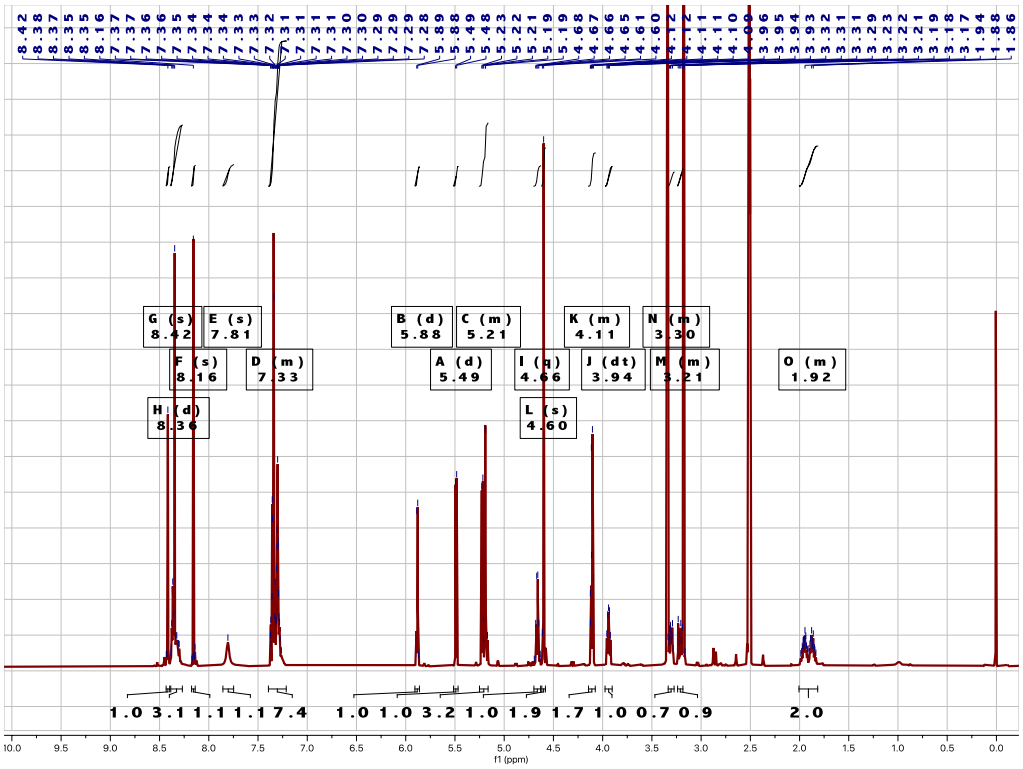


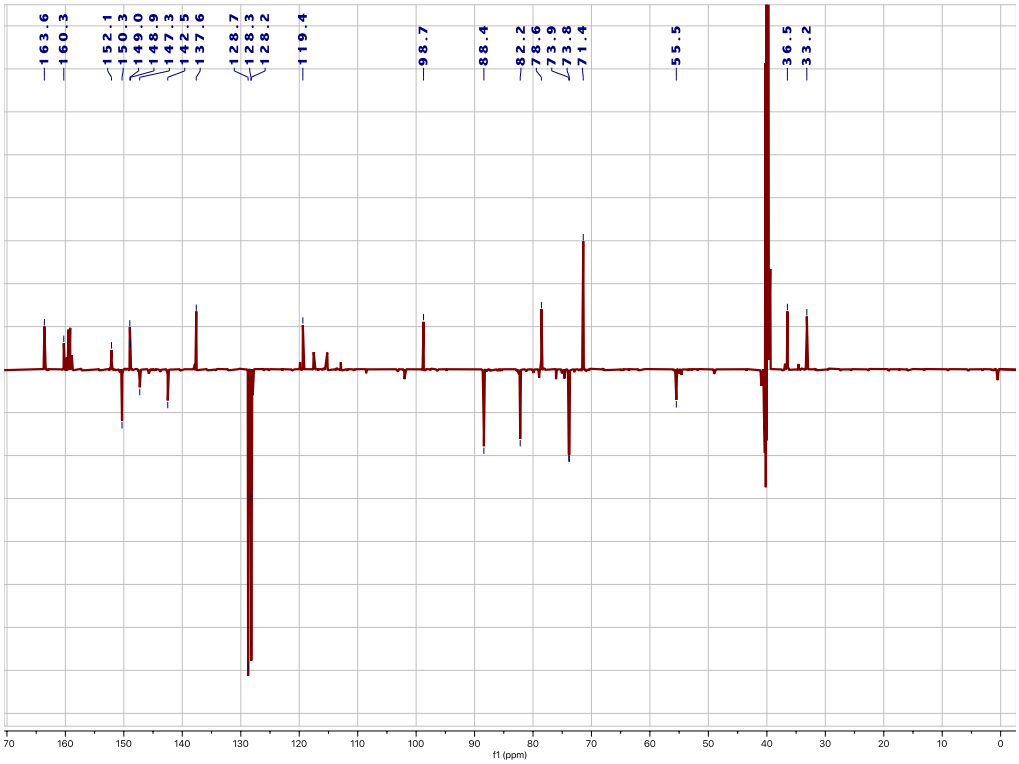


^{1}H and ^{13}C NMR of compound 2

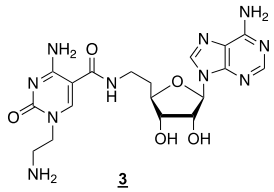


2





^{1}H and ^{13}C NMR of compound 3



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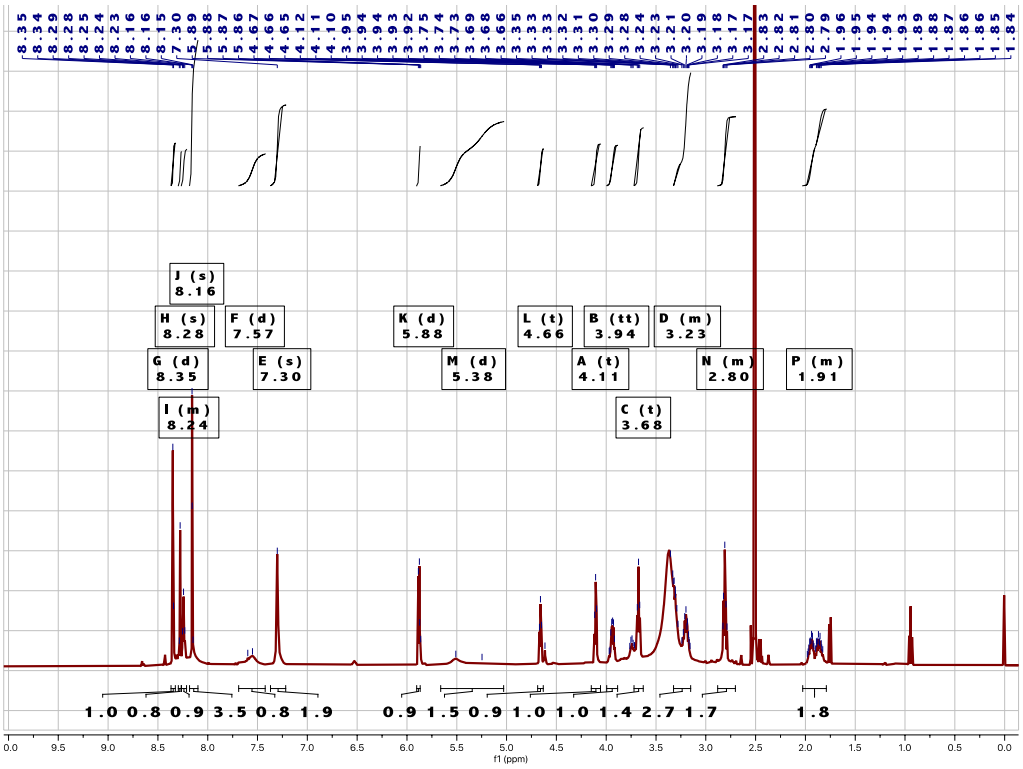
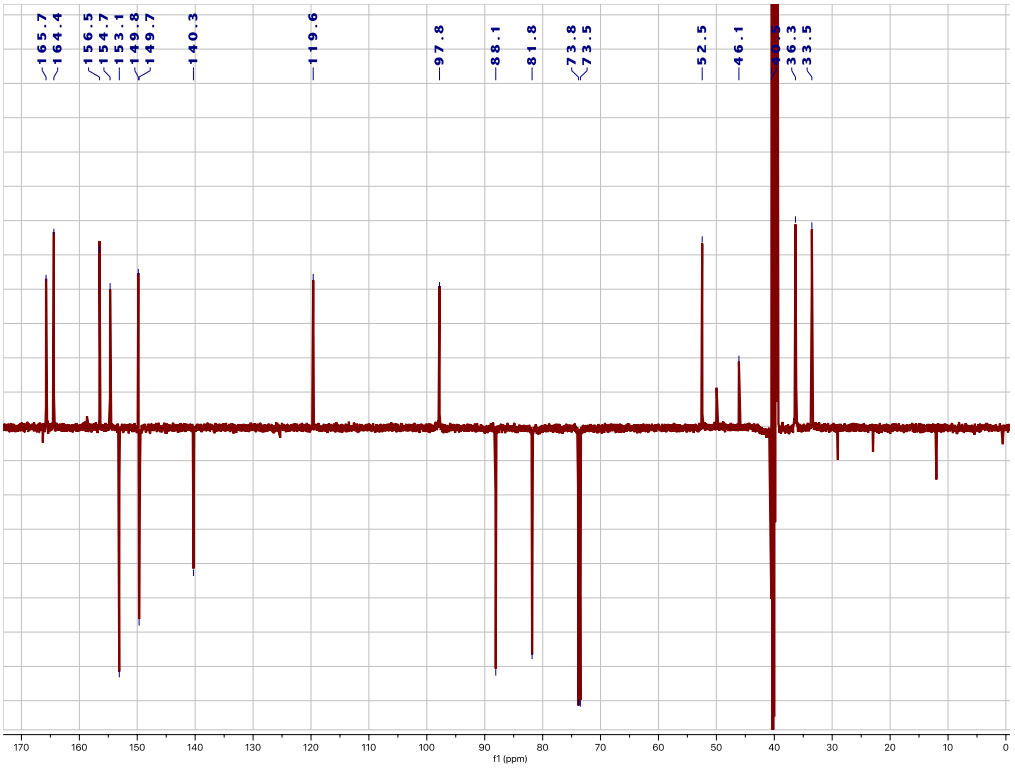
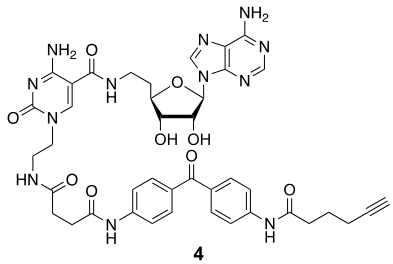
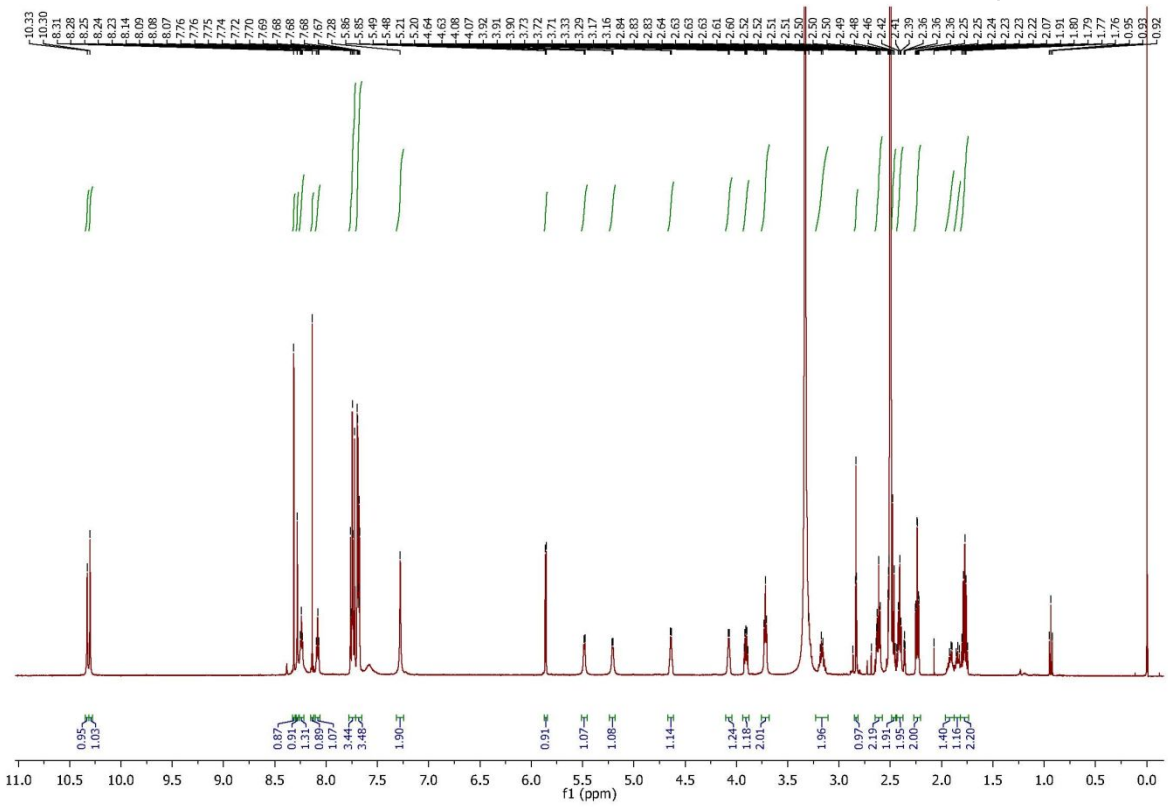


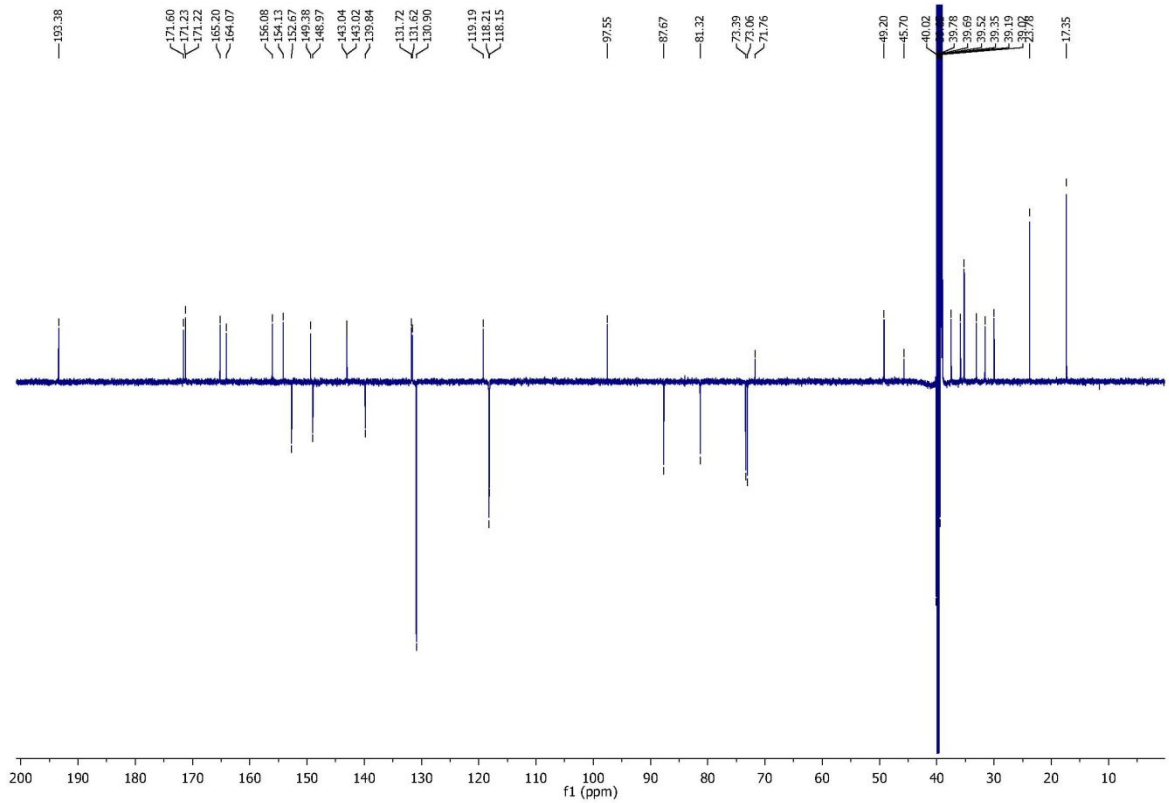


^{1}H and ^{13}C NMR of compound 4

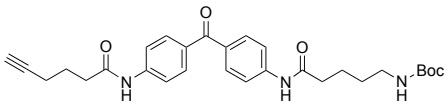


4

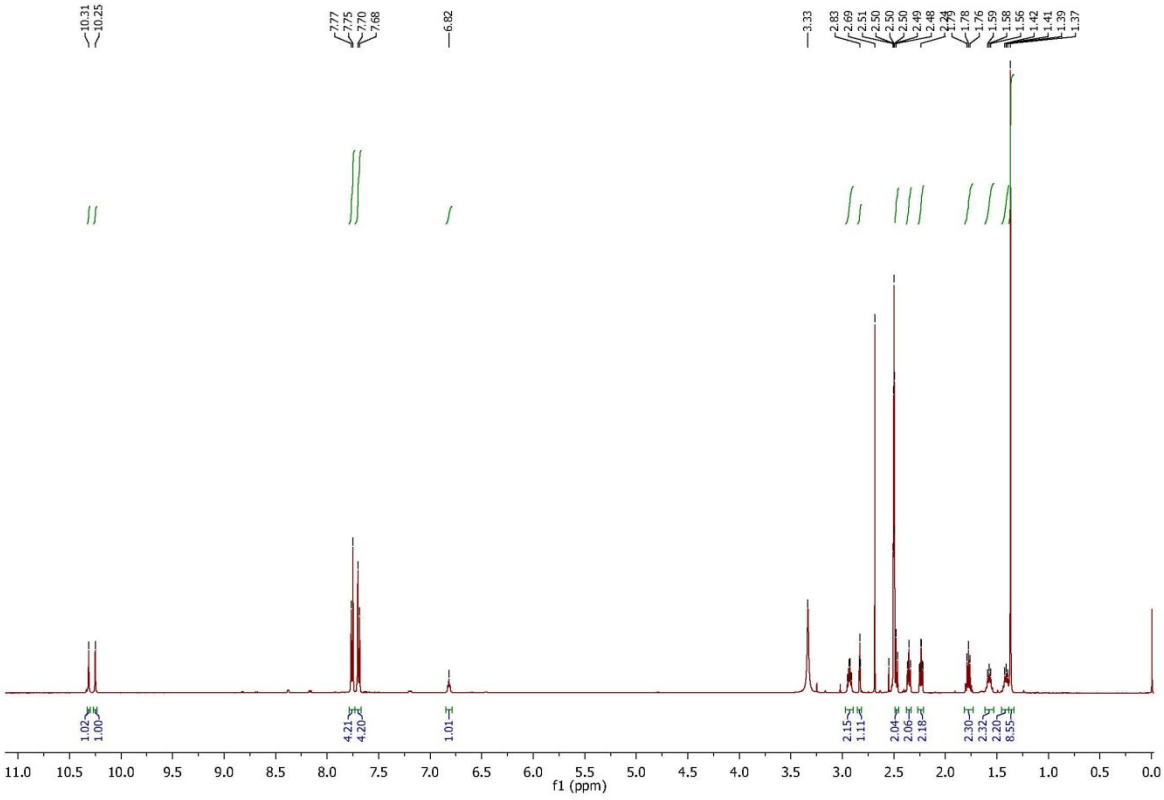


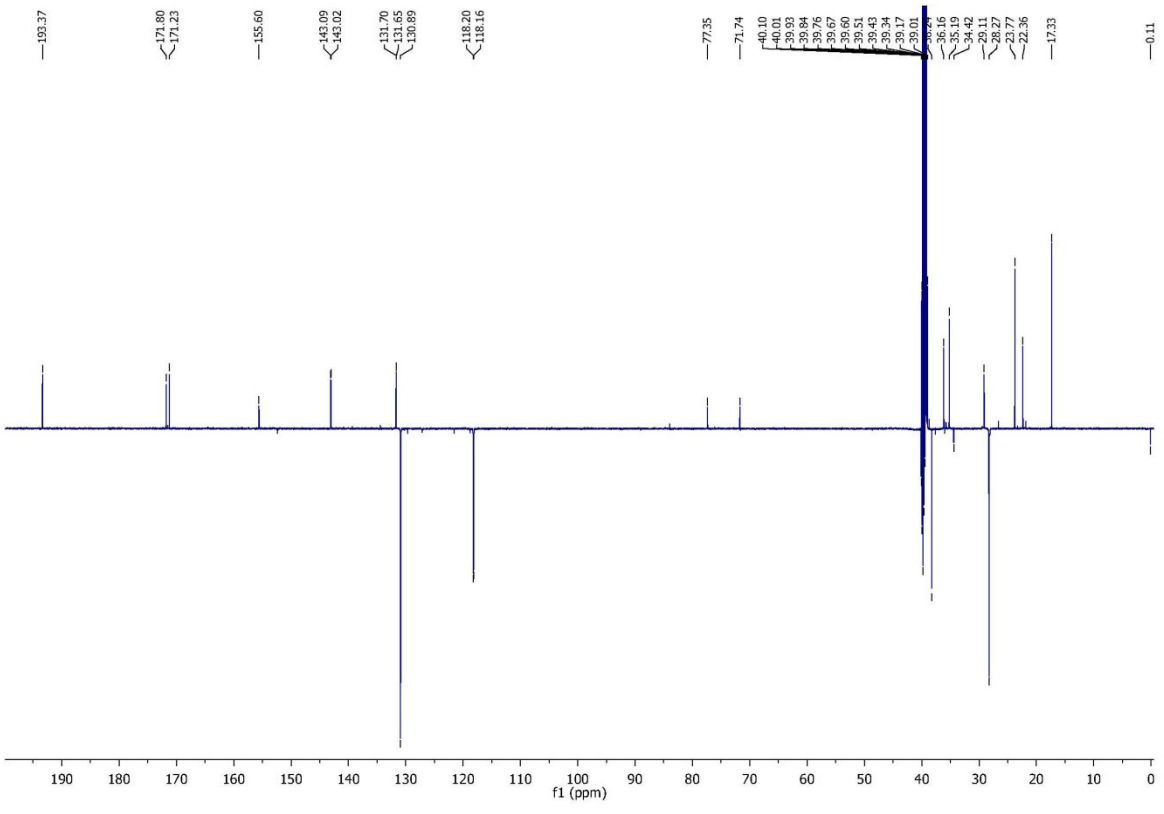


^{1}H and ^{13}C NMR of compound 5



5





Graph SI-1. Inhibition percentages of various DNMT, KHMTs and PRMTs with 10 µM of compound **1**, **2** or **4**

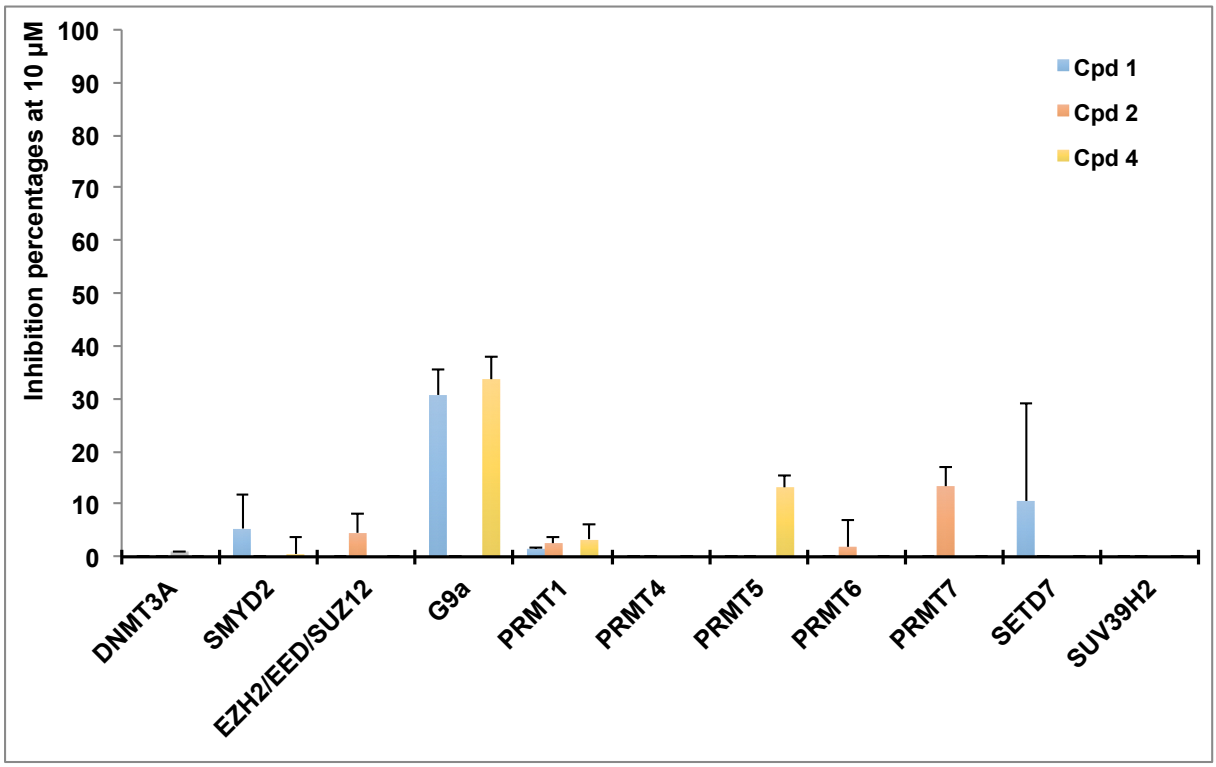


Table SI-1. Table of the 24 selected relevant proteins with significant enrichment profile after ABPP pull-down experiments with chemical probe **4** in KG-1 cell line versus control probe **5**. Ratio and Student's T-test difference data are the means of the triplicates.

KG-1 cell line																
Gene	Human Protein ID	Mol. weight (kDa)	Student's T-test Difference Probe **4** in KG-1_Boc Probe **5**	Probe **4** in KG-1/Boc Probe **5** ratio	MS/MS count Boc-probe **5**_1	MS/MS count Boc-probe **5**_2	MS/MS count Boc-probe **5**_3	MS/MS count Probe **4**_KG-1_1	MS/MS count Probe **4**_KG-1_2	MS/MS count Probe **4**_KG-1_3	LFQ intensity Boc-probe **5**_1	LFQ intensity Boc-probe **5**_2	LFQ intensity Boc-probe **5**_3	LFQ intensity Probe **4**_KG-1_1	LFQ intensity Probe **4**_KG-1_2	LFQ intensity Probe **4**_KG-1_3
SLC16A1	P53985|MOT1	53.944	8.32	318.91	0	0	0	8	1	5	11.77	13.05	16.06	21.92	20.88	23.04
HSD17B4	P51659|DHB4	79.685	7.17	143.56	0	2	0	26	9	19	14.55	16.71	15.53	23.47	21.07	23.74
SLC29A1	Q99808|S29A1	50.219	6.51	90.97	0	0	0	5	1	6	14.14	14.74	13.53	21.35	18.89	21.67
HADHA	P40939|ECHA	82.999	6.09	68.22	1	1	0	36	12	27	17.17	19.27	15.32	24.45	21.24	24.36
HSP90B1	P14625|ENPL	92.468	5.34	40.61	9	10	2	84	244	73	22.77	23.30	21.95	28.80	26.50	28.75
KDSR	Q06136|KDSR	36.187	4.63	24.79	0	0	0	5	2	1	13.14	14.18	14.10	19.59	18.05	17.68
HSD17B10	Q99714|HCD2	26.923	4.02	16.19	0	1	0	1	4	2	12.78	16.60	12.95	17.77	18.94	17.67
SLC2A3	P11169|GTR3;Q8TDB8|GTR14	53.924	4.00	16.01	0	0	0	4	2	2	15.94	15.36	15.26	19.45	19.62	19.49
NNT	Q13423|NNTM	113.89	3.37	10.36	1	1	1	14	1	13	18.71	18.71	18.95	22.59	21.47	22.43
HM13	Q8TCT9|HM13	41.488	3.16	8.91	0	0	0	5	6	1	15.89	15.58	15.63	18.75	19.85	17.97
ATP5C1	P36542|ATPG	32.996	3.13	8.74	0	0	0	6	0	0	19.13	18.11	17.41	22.19	20.74	21.11
SLC16A3	O15427|MOT4	49.469	2.97	7.83	0	0	0	4	1	4	16.66	17.02	17.00	19.54	19.84	20.21
NSDHL	Q15738|NSDHL	41.9	2.76	6.76	0	0	0	4	0	4	15.63	16.07	15.83	18.59	18.73	18.47
PMPCA	Q10713|MPPA	58.252	2.71	6.54	0	0	0	4	2	3	16.09	15.95	14.70	18.04	18.76	18.06
CPT1A	P50416|CPT1A	88.367	2.68	6.41	1	1	1	13	3	12	16.93	16.84	17.08	19.88	18.89	20.12
STT3A	P46977|STT3A	80.529	2.37	5.16	0	0	0	3	1	2	17.92	18.19	18.25	20.42	19.44	21.60
HSPD1	P10809|CH60	61.054	2.34	5.06	29	37	9	60	145	58	24.74	24.67	23.77	26.86	26.36	26.98
LEPROTL1	O95214|LERL1	14.428	2.28	4.86	0	0	0	0	3	3	11.57	11.39	11.46	12.21	14.55	14.51
SHMT2	P34897|GLYM	55.992	1.68	3.21	2	3	0	8	4	6	19.96	20.35	19.09	21.36	22.05	21.03
SDHA	P31040|SDHA	72.691	1.52	2.87	2	2	0	7	1	2	19.25	18.84	18.31	20.41	20.56	19.99
MRPL34	Q9BQ48|RM34	10.165	1.45	2.73	0	0	0	3	1	2	15.04	15.47	15.44	16.42	17.37	16.51
MBOAT7	Q96N66|MBOA7	52.764	1.36	2.56	1	1	0	2	3	3	16.64	16.69	16.47	17.86	18.34	17.67
SEC61A1	P61619|S61A1	52.264	1.14	2.21	3	2	0	2	5	3	19.24	19.26	18.96	20.11	20.71	20.07
SF3A3	Q12874|SF3A3	58.848	1.07	2.10	1	2	1	1	1	2	15.45	16.16	15.34	16.58	17.24	16.33

Graph SI-2. Volcano plot of the proteomic data obtained following pull-down ABPP experiments with active chemical probe **4** in MCF-7 cell line and inactive probe **5**.

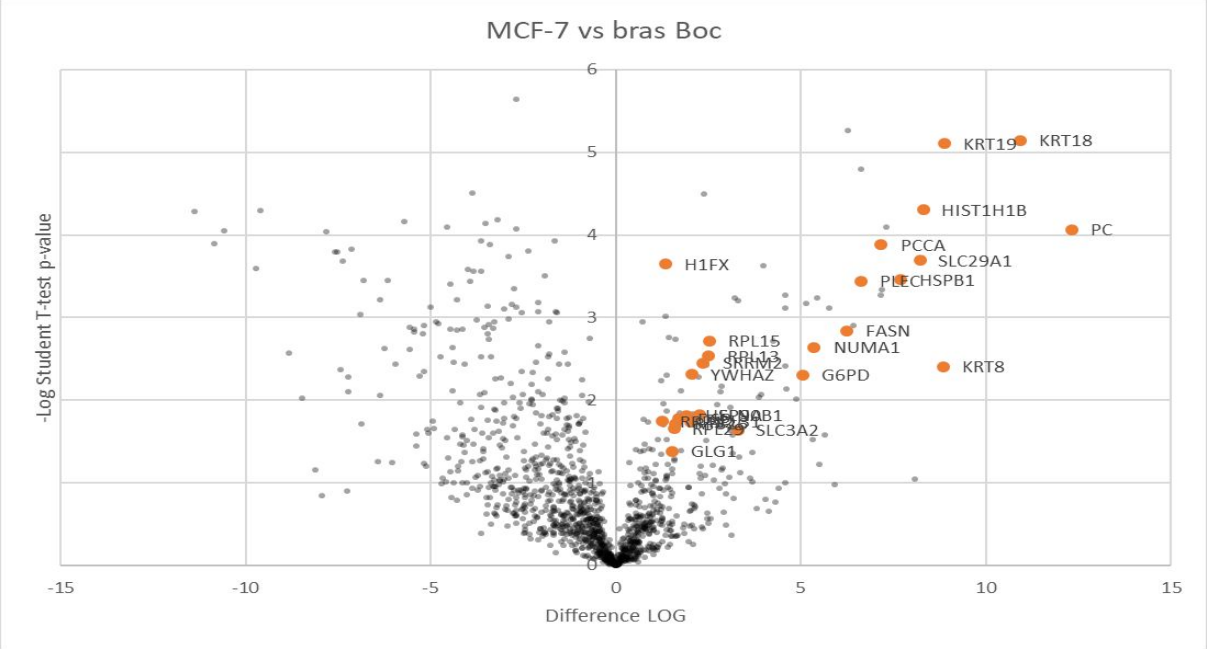


Table SI-2. Table of the 26 selected relevant proteins with significant enrichment profile after ABPP pull-down experiments with chemical probe **4** in MCF-7 cell line versus control probe **5**. Ratio and Student's T-test difference data are the means of the triplicates.

MCF-7 cell line																	
Gene	Human Protein ID	Mol. weight (kDa)	Student's T-test Difference Probe **4** in MCF-7_Boc Probe **5**	Probe **4** in MCF-7/Boc Probe **5** ratio	MS/MS count Boc-probe **5**_1	MS/MS count Boc-probe **5**_2	MS/MS count Boc-probe **5**_3	MS/MS count Probe **4**_MCF-7_1	MS/MS count Probe **4**_MCF-7_2	MS/MS count Probe **4**_MCF-7_3	LFQ intensity Boc-probe **5**_1	LFQ intensity Boc-probe **5**_2	LFQ intensity Boc-probe **5**_3	LFQ intensity Probe **4**_MCF-7_1	LFQ intensity Probe **4**_MCF-7_2	LFQ intensity Probe **4**_MCF-7_3
PC	P11498|PYC	129.63	12.33	5162.48	0	0	0	54	65	64	15.82	16.37	13.98	27.51	28.21	27.44
KRT18	P05783|K1C18	48.057	10.92	1940.25	0	0	0	2	4	9	12.89	12.71	12.25	23.05	23.45	24.11
KRT19	P08727|K1C19;CON__P08727	44.105	8.89	474.75	0	0	0	4	9	12	13.94	14.07	13.30	22.30	22.84	22.84
KRT8	P05787|K2C8;CON__P05787	53.704	8.84	458.88	0	0	0	12	17	23	18.86	15.42	13.93	24.49	25.05	25.19
HIST1H1B	P16401|H15	22.58	8.32	320.56	0	0	0	4	1	1	13.28	13.50	12.43	21.75	21.65	20.78
SLC29A1	Q99808|S29A1	50.219	8.24	301.73	0	0	0	2	3	9	14.14	14.74	13.53	21.46	22.35	23.30
HSPB1	P04792|HSPB1	22.782	7.71	208.93	0	0	0	2	3	4	14.85	15.87	13.55	22.28	22.64	22.46
PCCA	P05165|PCCA	80.058	7.17	144.15	0	0	0	22	33	26	19.14	18.71	19.29	25.97	27.12	25.58
PLEC	Q15149|PLEC	531.78	6.62	98.40	0	0	0	0	0	6	12.45	11.77	12.30	18.06	18.45	19.88
FASN	P49327|FAS	273.42	6.23	75.00	0	0	0	1	8	8	16.50	17.00	15.07	21.34	23.14	22.76
NUMA1	Q14980|NUMA1	238.26	5.37	41.23	0	0	0	3	1	3	13.13	15.25	13.10	19.11	18.71	19.76
G6PD	P11413|G6PD	59.256	5.06	33.34	0	0	0	0	1	5	12.90	15.65	13.99	18.61	19.08	20.03
SLC3A2	P08195|4F2	67.993	3.30	9.82	2	1	0	2	1	1	16.03	16.36	13.62	18.52	18.16	19.21
RPL15	P61313|RL15	24.146	2.54	5.80	9	7	7	9	13	14	23.50	23.30	24.42	26.28	26.19	26.35
RPL13	P26373|RL13	24.261	2.52	5.73	2	2	3	7	6	11	22.54	22.63	23.46	25.86	24.97	25.36
SRRM2	Q9UQ35|SRRM2	299.61	2.37	5.17	21	12	16	21	21	27	22.97	22.31	23.59	25.29	25.16	25.52
FLNA	P21333|FLNA	280.74	2.27	4.81	2	4	1	2	3	8	19.17	19.40	18.79	20.48	21.38	22.31
YWHAZ	P63104|1433Z	27.745	2.06	4.18	3	4	4	4	3	6	20.34	20.45	19.72	21.68	22.37	22.64
RPL31	P62899|RL31	14.463	2.02	4.05	4	5	10	3	5	5	21.67	21.83	22.55	24.57	24.40	23.14
HSP90B1	P14625|ENPL	92.468	1.92	3.78	9	10	2	6	8	22	22.77	23.30	21.95	24.12	24.62	25.04
DSP	P15924|DESP	331.77	1.69	3.23	5	1	2	1	1	3	17.58	16.47	17.41	18.70	18.50	19.34
RPS26	P62854|RS26;Q5JNZ5|RS26L	13.015	1.61	3.06	1	3	1	3	3	2	18.48	18.76	19.25	21.07	20.44	19.82
RPL29	P47914|RL29	17.752	1.57	2.98	4	2	1	1	1	1	21.79	21.38	22.74	23.83	23.33	23.46
GLG1	Q92896|GSLG1	134.55	1.53	2.89	2	1	1	0	1	1	13.90	12.93	14.09	14.84	14.76	15.91
H1FX	Q92522|H1X	22.487	1.33	2.52	1	1	1	1	1	1	18.69	19.01	18.74	20.19	20.09	20.17
RRP12	Q5JTH9|RRP12	143.7	1.24	2.37	2	2	3	0	0	1	16.90	17.08	17.88	18.59	18.32	18.68

Graph SI-3. Volcano plot of the proteomic data obtained following pull-down ABPP experiments with active chemical probe **4** in K-562 cell line and inactive probe **5**.

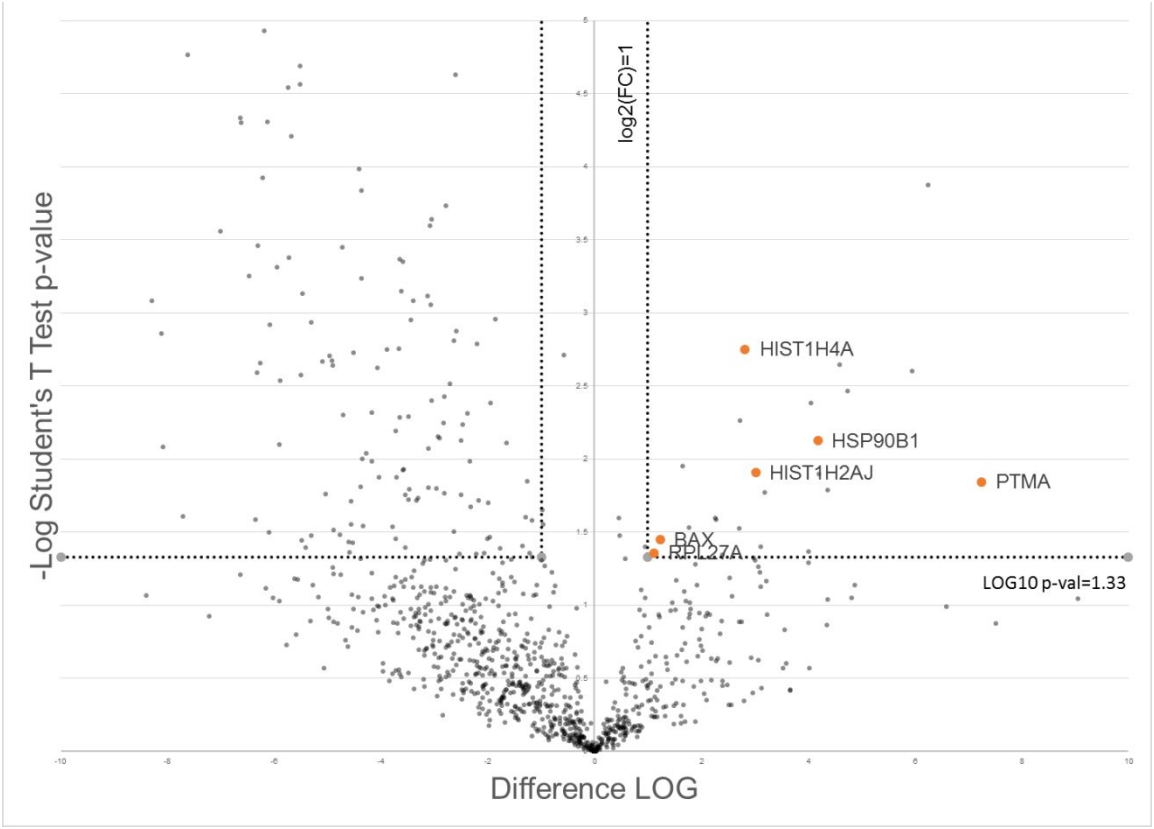


Table SI-3. Table of the 6 selected relevant proteins with significant enrichment profile after ABPP pull-down experiments with chemical probe **4** in K-562 cell line versus control probe **5**. Ratio and Student's T-test difference data are the means of the triplicates.

K-562 cell line																
Gene	Human Protein ID	Mol. weight (kDa)	Student's T-test Difference Probe **4** in K-562_Boc Probe **5**	Probe **4** in K-562/Boc Probe **5** ratio	MS/MS count Boc-probe **5**_1	MS/MS count Boc-probe **5**_2	MS/MS count Boc-probe **5**_3	MS/MS count Probe **4**_K-562_1	MS/MS count Probe **4**_K-562_2	MS/MS count Probe **4**_K-562_3	LFQ intensity Boc-probe **5**_1	LFQ intensity Boc-probe **5**_2	LFQ intensity Boc-probe **5**_3	LFQ intensity Probe **4**_K-562_1	LFQ intensity Probe **4**_K-562_2	LFQ intensity Probe **4**_K-562_3
PTMA	P06454|PTMA	12.203	7.25	152.49	0	0	0	0	25	0	12.93	12.76	12.36	17.83	23.39	18.59
HSP90B1	P14625|ENPL	92.468	4.19	18.22	9	10	2	34	19	25	22.77	23.30	21.95	28.34	26.20	26.04
BAX	Q07812|BAX	21.184	1.23	2.34	3	4	2	2	0	2	23.25	23.73	23.16	26.67	27.40	25.11
RPL27A	P46776|RL27A	16.561	1.12	2.17	0	1	1	1	1	5	24.71	25.01	25.90	28.13	28.15	27.77
HIST1H2AJ	Q99878|H2A;Q96KK5|H2A1H;Q	13.936	3.02	8.09	4	4	3	5	5	6	16.98	16.65	17.57	18.35	17.77	18.76
HIST1H4A	P62805|H4	11.367	2.81	7.02	6	8	9	16	8	39	20.84	21.22	22.01	22.52	22.76	22.15

Graph SI-4. Volcano plot of the proteomic data obtained following pull-down ABPP experiments with active chemical probe **4** in MOLM-13 cell line and inactive probe **5**.

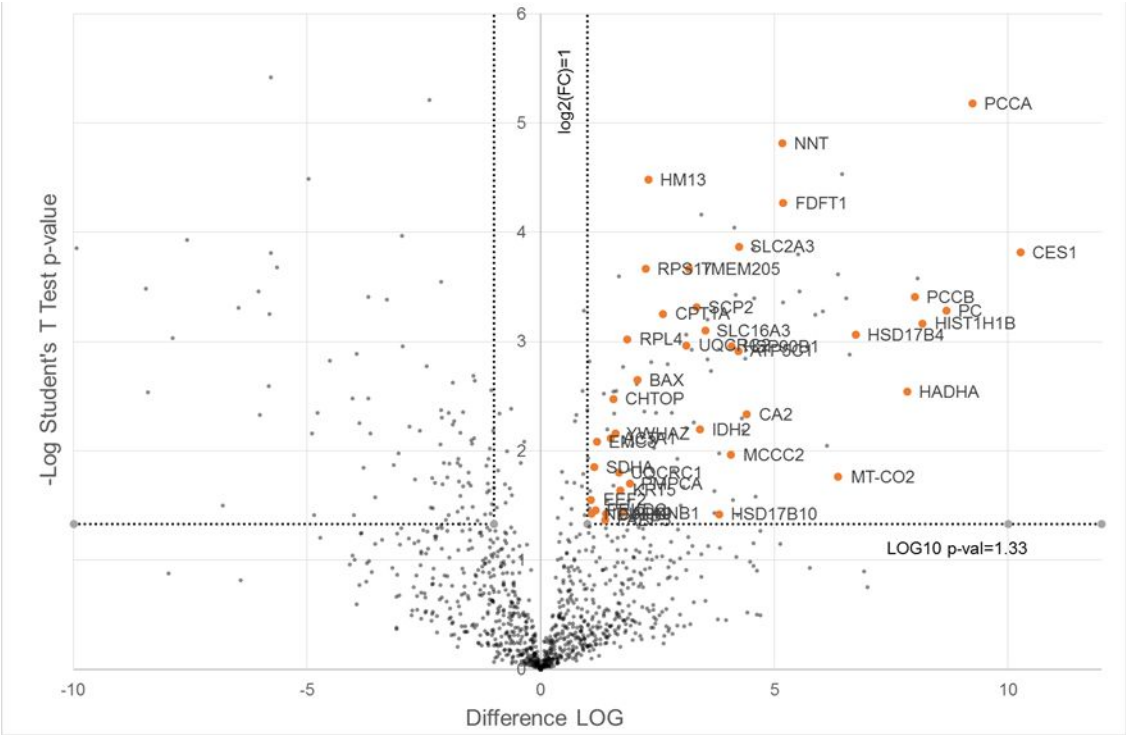


Table SI-4. Table of the 40 selected relevant proteins with significant enrichment profile after ABPP pull-down experiments with chemical probe **4** in MOLM-13 cell line versus control probe **5**. Ratio and Student's T-test difference data are the means of the triplicates.

MOLM-13 cell line																
Gene	Human Protein ID	Mol. weight (kDa)	Student's T-test Difference Probe **4** in MOLM-13_Boc Probe **5**	Probe **4** in MOLM-13/Boc Probe **5** ratio	MS/MS count Boc-probe **5**_1	MS/MS count Boc-probe **5**_2	MS/MS count Boc-probe **5**_3	MS/MS count Probe **4**_MOLM-13_1	MS/MS count Probe **4**_MOLM-13_2	MS/MS count Probe **4**_MOLM-13_3	LFQ intensity Boc-probe **5**_1	LFQ intensity Boc-probe **5**_2	LFQ intensity Boc-probe **5**_3	LFQ intensity Probe **4**_MOLM-13_1	LFQ intensity Probe **4**_MOLM-13_2	LFQ intensity Probe **4**_MOLM-13_3
CES1	P23141|EST1	62.52	10.27	1230.50	0	0	0	6	3	4	12.50	10.88	13.20	22.67	21.94	22.76
PCCA	P05165|PCCA	80.058	9.23	600.93	0	0	0	53	55	48	19.14	18.71	19.29	27.80	28.49	28.56
PC	P11498|PYC	129.63	8.67	407.86	0	0	0	33	25	31	15.82	16.37	13.98	23.16	24.55	24.46
HIST1H1B	P16401|H15	22.58	8.17	287.34	0	0	0	11	4	1	13.28	13.50	12.43	22.70	21.03	19.97
PCCB	P05166|PCCB	58.215	8.00	256.39	0	0	0	5	6	4	13.58	11.43	11.70	19.69	20.57	20.46
HADHA	P40939|ECHA	82.999	8.84	228.70	1	1	0	33	22	25	17.17	19.27	15.32	25.55	24.33	25.41
HSD17B4	P51659|DHB4	79.685	6.74	106.86	0	2	0	22	7	8	14.55	16.71	15.53	22.90	21.51	22.59
MT-CO2	P00403|COX2	25.565	6.36	82.23	0	0	0	3	2	1	14.63	18.46	13.03	22.13	21.55	21.52
FDFT1	P37268|FDFT	48.115	5.19	36.46	0	0	0	3	1	2	12.82	12.32	13.19	17.89	17.77	18.22
NNT	Q13423|NNTM	113.89	5.16	35.77	1	1	1	26	11	15	18.71	18.71	18.95	24.21	23.58	24.06
CA2	P00918|CAH2	29.246	4.41	21.26	0	0	0	4	1	1	13.14	11.42	13.62	17.54	17.51	16.36
SLC2A3	P11169|GTR3;Q8TDB8|GTR14	53.924	4.24	18.85	0	0	0	4	5	4	15.94	15.36	15.26	19.80	19.38	20.09
ATP5C1	P36542|ATPG	32.996	4.22	18.68	0	0	0	3	4	4	19.13	18.11	17.41	22.33	22.29	22.70
HSP90B1	P14625|ENPL	92.468	4.08	16.87	9	10	2	41	30	33	22.77	23.30	21.95	26.72	26.27	27.26
MCCC2	Q9HCC0|MCCB	61.332	4.07	16.79	0	0	0	4	2	0	14.44	16.15	13.10	18.79	18.87	18.23
HSD17B10	Q99714|HCD2	26.923	3.82	14.08	0	1	0	2	2	3	12.78	16.60	12.95	18.12	17.87	17.78
SLC16A3	O15427|MOT4	49.469	3.52	11.44	0	0	0	3	2	2	16.66	17.02	17.00	21.05	19.78	20.39
IDH2	P48735|IDHP	50.909	3.41	10.60	0	0	0	3	5	3	16.61	14.80	14.58	18.64	18.94	18.64
SCP2	P22307|NLTP	58.993	3.33	10.03	2	1	1	4	4	4	18.04	18.27	18.47	22.08	21.05	21.64
TMEM205	Q6UW68|TM205	21.198	3.18	9.04	1	0	0	3	1	3	18.10	17.50	17.95	21.37	20.89	20.82
UQCRC2	P22695|QCR2	48.442	3.11	8.64	1	3	0	7	4	3	18.51	18.51	17.67	21.80	20.99	21.24
CPT1A	P50416|CPT1A	88.367	2.62	6.13	1	1	1	5	8	9	16.93	16.84	17.08	19.36	19.27	20.07
HM13	Q8TCT9|HM13	41.488	2.30	4.91	0	0	0	3	2	2	15.89	15.58	15.63	17.95	18.11	17.92
RPS17	P08708|RS17	15.55	2.24	4.73	3	5	2	5	2	2	18.91	19.06	19.28	21.58	21.10	21.29
BAX	Q07812|BAX	21.184	2.07	4.18	3	4	2	4	3	4	16.98	16.65	17.57	19.26	18.88	19.24
PMPCA	Q10713|MPPA	58.252	1.90	3.74	0	0	0	3	1	2	16.09	15.95	14.70	17.78	16.98	17.68
RPL4	P36578|RL4	47.697	1.85	3.60	8	5	3	11	13	9	21.92	21.74	22.35	23.66	24.03	23.87
PLXNB1	O43157|PLXB1	232.3	1.73	3.33	0	0	0	1	4	1	26.02	25.95	26.63	26.91	28.61	28.28
KRT5	P13647|K2C5;CON__P13647	62.378	1.71	3.26	0	0	0	1	4	1	20.45	19.97	20.59	21.26	22.12	22.76
UQCRC1	P31930|QCR1	52.645	1.66	3.17	1	2	0	3	3	2	19.04	18.67	17.85	20.60	19.85	20.11
YWHAZ	P63104|1433Z	27.745	1.60	3.03	3	4	4	7	6	6	20.34	20.45	19.72	22.17	21.68	21.44
CHTOP	Q9Y3Y2|CHTOP	26.396	1.55	2.92	2	2	2	3	2	1	16.81	16.39	17.13	18.16	18.24	18.57
ACTA1	P68133|ACTS;P68032|ACTC;P6	42.051	1.50	2.83	5	2	2	1	3	2	21.87	22.42	21.38	23.33	23.47	23.37
CAPG	P40121|CAPG	38.498	1.39	2.63	5	4	1	3	4	3	18.64	18.86	17.54	20.10	19.72	19.40
FABP5	Q01469|FABP5	15.164	1.38	2.60	1	1	2	5	2	0	17.34	17.57	17.81	19.79	18.84	18.23
EMC3	Q9P0I2|EMC3	29.952	1.20	2.29	0	0	0	5	1	1	13.59	13.23	13.98	14.58	14.84	14.98
PRKDC	P78527|PRKDC	469.08	1.16	2.24	0	1	0	1	5	2	19.01	19.86	19.89	20.29	20.89	21.07
SDHA	P31040|SDHA	72.691	1.14	2.21	2	2	0	2	5	2	19.25	18.84	18.31	19.94	19.99	19.90
NDUFA9	Q16795|NDUA9	42.509	1.08	2.12	3	2	1	6	4	2	20.22	20.11	20.61	21.91	21.48	20.81
EEF2	P13639|EF2	95.337	1.07	2.11	7	8	6	13	11	5	21.12	21.35	20.37	21.98	22.24	21.84

Graph SI-5. Volcano plot of the proteomic data obtained following pull-down ABPP experiments with active chemical probe **4** and inactive probe **5** in WM-266-4 cell line.

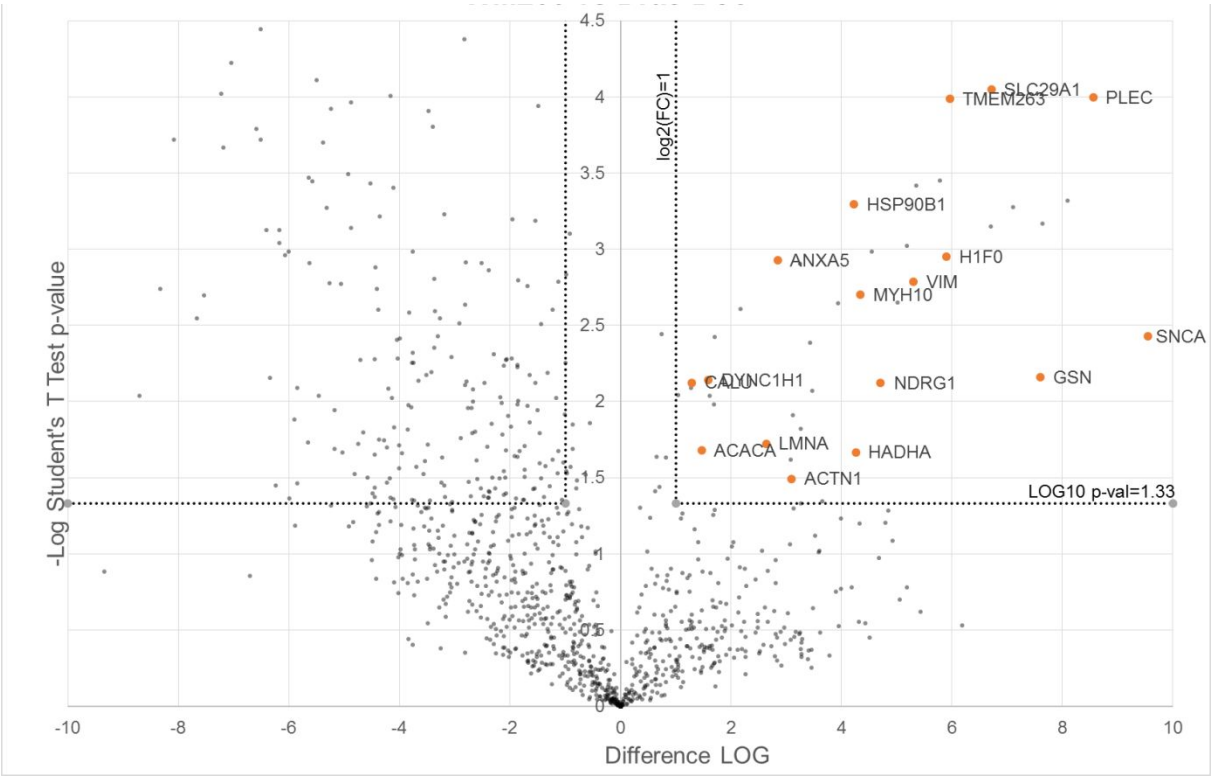


Table SI-5. Table of the 19 selected relevant proteins with significant enrichment profile after ABPP pull-down experiments with chemical probe **4** in WM-266-4 cell line versus control probe **5**. Ratio and Student's T-test difference data are the means of the triplicates.

WM-266-4 cell line																
Gene	Human Protein ID	Mol. weight (kDa)	Student's T-test Difference Probe **4** in WM-266-4_Boc Probe **5**	Probe **4** in WM-266-4/Boc Probe **5** ratio	MS/MS count Boc-probe **5**_1	MS/MS count Boc-probe **5**_2	MS/MS count Boc-probe **5**_3	MS/MS count Probe **4**_WM-266-4_1	MS/MS count Probe **4**_WM-266-4_2	MS/MS count Probe **4**_WM-266-4_3	LFQ intensity Boc-probe **5**_1	LFQ intensity Boc-probe **5**_2	LFQ intensity Boc-probe **5**_3	LFQ intensity Probe **4**_WM-266-4_1	LFQ intensity Probe **4**_WM-266-4_2	LFQ intensity Probe **4**_WM-266-4_3
PC	P11498|PYC	129.63	13.15	9116.51	0	0	0	97	97	72	15.82	16.37	13.98	28.78	28.70	28.14
SNCA	P37840|SYUA	14.46	9.54	746.09	0	0	0	12	0	1	12.52	11.87	15.03	25.17	21.59	21.29
PCCA	P05165|PCCA	80.058	9.15	568.88	0	0	0	45	54	46	19.14	18.71	19.29	28.93	27.88	27.80
PLEC	Q15149|PLEC	531.78	8.56	378.07	0	0	0	15	8	9	12.45	11.77	12.30	21.19	21.30	19.71
GSN	P06396|GELS_HUMAN;CON__Q	85.696	7.61	194.85	0	0	0	5	2	15	15.63	15.04	11.50	21.66	20.39	22.95
SLC29A1	Q99808|S29A1	50.279	6.72	105.60	0	0	0	2	5	3	14.14	14.74	13.53	21.15	21.02	20.39
TMEM263	Q8WUH6|TM263	11.748	5.96	62.26	0	0	0	2	2	4	13.13	13.27	13.40	19.67	19.55	18.48
H1F0	P07305|H10	20.863	5.90	59.58	0	0	0	2	3	1	12.36	12.17	14.02	17.97	19.08	19.19
VIM	P08670|VIME	53.651	5.30	39.43	3	2	0	18	11	26	19.18	18.95	17.67	24.48	22.87	24.36
NDRG1	Q92597|NDRG1	42.835	4.71	26.19	0	0	0	3	1	3	16.13	15.49	13.16	20.03	19.08	19.80
MYH10	P35580|MYH10	229	4.35	20.33	0	0	0	6	2	2	12.52	14.21	14.00	18.46	17.86	17.46
HADHA	P40939|ECHA	82.999	4.27	19.25	1	1	0	3	2	4	17.17	19.27	15.32	21.17	21.42	21.98
HSP90B1	P14625|ENPL	92.468	4.23	18.74	9	10	2	25	25	27	22.77	23.30	21.95	27.15	26.78	26.77
ACTN1	P12814|ACTN1	103.06	3.09	8.52	0	1	0	2	0	4	13.78	16.50	16.51	19.18	18.11	18.76
ANXA5	P08758|ANXA5	35.936	2.84	7.18	3	1	0	8	4	10	20.61	20.31	19.64	23.16	22.65	23.27
LMNA	P02545|LMNA	74.139	2.64	6.22	0	3	0	8	2	12	19.04	19.75	18.55	21.79	20.69	22.77
DYNC1H1	Q14204|DYHC1	532.4	1.59	3.02	0	2	0	5	1	1	17.24	17.44	17.92	19.59	19.02	18.77
ACACA	Q13085|ACACA	265.55	1.47	2.77	22	27	23	44	34	34	24.29	24.38	25.28	26.32	26.39	25.63
CALU	O43852|CALU	37.106	1.30	2.46	3	1	1	2	1	2	15.56	15.41	15.41	17.21	16.32	16.74