

Supporting Information

Discovery of a First-in-class Protein Arginine Methyltransferase 6 (PRMT6) Covalent Inhibitor

Yudao Shen,^{†,#} Fengling Li,^{§,#} Magdalena M. Szewczyk,^{§,#} Levon Halabelian,[§] Kwang-su Park,[†] Irene Chau,[§] Aiping Dong,[§] Hong Zeng,[§] He Chen,[†] Fanye Meng,[†] Dalia Barsyte-Lovejoy,[§] Cheryl H. Arrowsmith,^{§,‡} Peter J. Brown,[§] Jing Liu,^{†,*} Masoud Vedadi,^{§,£,*} and Jian Jin^{†,*}

[†]Mount Sinai Center for Therapeutics Discovery, Departments of Pharmacological Sciences and Oncological Sciences, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States

[§]Structural Genomics Consortium, University of Toronto, Toronto, ON M5G 1L7, Canada

[£]Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario M5S 1A8, Canada

[‡]Princess Margaret Cancer Centre and Department of Medical Biophysics, University of Toronto, Toronto, Ontario M5G 2M9, Canada

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Table S1. Crystallography Data collection and refinement statistics of PRMT6-compound 4 (MS117) complex

	PRMT6_MS117 (PDB 6P7I)
Data collection	
Space group	$P 2_1$
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	78.09, 135.13, 83.21
α , β , γ (°)	90.00, 98.01, 90.00
Resolution (Å)	50.0-2.00 (2.03-2.00)*
R_{sym} or R_{merge}	0.093 (0.927)
$I / \sigma I$	26.6 (1.7)
Completeness (%)	99.9 (98.0)
Redundancy	7.0 (5.9)
Refinement	
Resolution (Å)	50.00-2.00
No. reflections	111464
$R_{\text{work}} / R_{\text{free}}$	0.184/0.222
No. atoms	
Protein	10666
Inhibitor MS117	106
SAH	104
Water	767
<i>B</i> -factors	
Protein	36.0
Inhibitor MS117	33.7
SAH	27.2
Water	41.6
R.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.422

*Values in parentheses are for highest-resolution shell.

Table S2. Selectivity of compound **4** against 33 methyltransferases

Mtases	MS117 (Activity %)	
	10 μ M	50 μ M
PRMT6	2	1
PRMT1	3	2
PRMT3	7	2
PRMT4	9	2
PRMT5	102	69
PRMT7	95	12
PRMT8	4	2
PRMT9	108	90
G9a	94	85
GLP	88	80
SUV39H1	81	5
SUV39H2	87	59
SETDB1	108	93
PRDM9	91	71
SETD7	89	10
SETD8	101	101
MLL1	96	92
MLL3	100	100
SUV420H1	107	77
SUV420H2	100	77
PRC2	85	52
SETD2	93	81
SMYD2	92	90
SMYD3	101	96
BCDIN3D	100	100
DNMT1	94	94
DNMT3A/3L	65	30
DNMT3B/3L	105	53
NSD1	109	105
NSD2	116	117
NSD3	122	120
Ash1L	103	99
DOT1L	95	103

CLUSTAL 0(1.2.4) multiple sequence alignment

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SP|099873|PRMT1_HUMAN -----
SP|P55345|PRMT2_HUMAN -----M-ATS----- 4
SP|060678|PRMT3_HUMAN MC-----SLASGATGGRGAVENEE-DLPELSDSGDEAAWEDED-----DADL 41
SP|086X55|CARM1_HUMAN MAAAAAAGVPGAG--GAGSA---VP--GGA----- 23
SP|014744|PRMT5_HUMAN MA---AMAVGGAGGSRVSSGRDLCVPEIADTLGAVAKQGFDFLCHPVFHPFRKRFIEQ 57
SP|09NV4|PRMT7_HUMAN -----
SP|09NR22|PRMT8_HUMAN -----
SP|06P2P2|PRMT9_HUMAN MSNSRPRSRRDAGGGAAGRDE-LVSRSL----- 29

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SP|099873|PRMT1_HUMAN -----
SP|P55345|PRMT2_HUMAN -----GDCPRSE-----SQGEEPAECSEAG--LLOEG---VQ 31
SP|060678|PRMT3_HUMAN PHGKQOTPLFCN---RLFT-----SAEETFSHCKSEHFNIDSMVHKH 82
SP|086X55|CARM1_HUMAN ---GPCATVSVFPGARLLT---IG-----DANGEIORHAEQQA-LRLEV---RA 62
SP|014744|PRMT5_HUMAN PAKNRPGPOTRSDLLLSGRDWNLTIVGKLSPIRPSKVEKIRNSEEAM-M-----LQ 109
SP|09NV4|PRMT7_HUMAN -----
SP|09NR22|PRMT8_HUMAN -----
SP|06P2P2|PRMT9_HUMAN -----QSAEHCLGVQ-----DF 41

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SP|099873|PRMT1_HUMAN -----
SP|P55345|PRMT2_HUMAN PEEFVAI----- 38
SP|060678|PRMT3_HUMAN GLEFYGYIKLINFIR-LKNPTVEY-----MNSIYNVPWKEEYL-KPVLEDDL 131
SP|086X55|CARM1_HUMAN GPDSAGI----- 69
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SP|09NR22|PRMT8_HUMAN -----
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SP|086X55|CARM1_HUMAN ---ALYSHEDV-CVFKCSVRETECSRVGKQSF-----ITLTKGNS- 106
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SP|09NV4|PRMT7_HUMAN -----
SP|09NR22|PRMT8_HUMAN -----MGMKHSRCLLLRRKMAE---NAAESTEVNSPPSOPP- 34
SP|06P2P2|PRMT9_HUMAN ---GCYEQALE-LFP---DDEVICNSM-----GE- 109

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SP|09NR22|PRMT8_HUMAN -----QPVVPAKPVQCVHHVS-----TOPSCPR- 58
SP|06P2P2|PRMT9_HUMAN ---HLFRMG-----FRDEAAGYF-----124

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SP|099873|PRMT1_HUMAN --AESSEKPNADMSTKDYFY---D-SYAHFGI-----HEEMLDEKVRTLYRNSM 80
SP|P55345|PRMT2_HUMAN --KHVEYDPEDTWQDEEYF---G-SYGTLLK-----HLEMLADQPRTKYHSVI 129
SP|060678|PRMT3_HUMAN ---STSVIADLQDEDEGVYF---S-SYGHYGI-----HEEMLKDERTESYRDFI 247
SP|086X55|CARM1_HUMAN ---VFSERTEESSAVQYF---Q-YLYLSQ-----QNMMDVYRTGTQRAI 176
SP|014744|PRMT5_HUMAN ---LQNRPPPN---AYELFAKGYEYDLSPLQPLMDNESQTYEVFEKDPKIKYSQYQAI 341
SP|09NV4|PRMT7_HUMAN -----ANPTT---GSVENLEDEHYD-----YHQETARSSYADMHLKDRNRYKQGI 52
SP|09NR22|PRMT8_HUMAN --GKMSKLNPEMSTRDYFY---D-SYAHFGI-----HEEMLDEKVRTLYRNSM 103
SP|06P2P2|PRMT9_HUMAN --HKAVKLNPFDSADKENFY-RVANLWVERW-----HFIMLNDTKRNTIYNAI 170
: : : * : * :

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SP|P55345|PRMT2_HUMAN LQNK- ---ESLTDKVLVDVCGGTGILSIF---CAHYARPRVYVEASE--MAQHT 176
SP|060678|PRMT3_HUMAN YQNP- ---HIFKDKVLDVCGGTGILSMF---AAKAG-AKVLGVDOSE--ILYQA 293
SP|086X55|CARM1_HUMAN LQNH- ---TDFKDKVLDVCGCGSGLSFF---AAQAG-ARKTYAVEAST--MAQHA 222
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SP|09NR22|PRMT8_HUMAN YHNK- ---HVFKDKVLDVCGSGTGLSMF---AAKAG-AKVFGEICSS--ISDYS 149
SP|06P2P2|PRMT9_HUMAN -QKA- ---VCLGSKVLDIGAGTGILSMF---AKKAG-AHSVYACELSKTYELA 216
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SP|P55345|PRMT2_HUMAN GOLVLONGFADITVYQKVEDVLP-----EKVDVIVSEWMGTCLLFEFMIESLYAR 230
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SP|014744|PRMT5_HUMAN LENWQFEWGSQVTVSSDMREWAP-----EKADIIIVSELLGSAFDELSP-CLDGA 453
SP|09NV4|PRMT7_HUMAN VKIVEKNGFSKIKVINKHSTEVTVGPEGDMPCRAMILVTELFDELIGEGALPSYEHAAH 163
SP|09NR22|PRMT8_HUMAN EKIKANHLNDIITIFGKVEEVELP---V---EKVDIIISEWMGYCLFYESMLNTLYAR 204
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SP |014744| PRMT5_HUMAN ---RDPMDNNRYCTLEFPVEVNTVLHGAFYFETVLYQD-----TTLIRPET 571
SP |09NV44| PRMT7_HUMAN FSKQVSS---SAACHSRRFELTSGRAQVLSWMDIEMDPEGKIKCTMAPFAHSIDPE- 306
SP |09NR22| PRMT8_HUMAN IYTVKTEE---LSFTSAFCLQIQNDYVHALVYFNIEFT-K---CHK-KKGFSTAPD- 329
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SP |09NV44| PRMT7_HUMAN EMQWRDHWMCQVYFLPOEE--PVVQGSALYLVAHDDYD-----C----- 343
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SP |014744| PRMT5_HUMAN -----WYEWAVTA-----PVCSAIHNPGRSYTI----- 635
SP |09NV44| PRMT7_HUMAN -----VMYSLQRTSPKNERVRQMRPVCDCAHLWNRPRFGEINDQDRDRYVQALRTVL 399
SP |09NR22| PRMT8_HUMAN -----VDLDFKG-----QLCETSVND-----YKM-R----- 394
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SP |060678| PRMT3_HUMAN -----
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SP |09NV44| PRMT7_HUMAN KPDSVCLCV--SDGSLLSVLAHHLGVEQ-----VFTV-----ESSAASH 436
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SP |099873| PRMT1_HUMAN -----
SP |P55345| PRMT2_HUMAN -----
SP |060678| PRMT3_HUMAN -----
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SP |09NV44| PRMT7_HUMAN K-----LLRKFKAN-----HLEDKINIEKRPPELLTNEQLGR-----KVSLLLGE 478
SP |09NR22| PRMT8_HUMAN -----
SP |06P2P2| PRMT9_HUMAN NTVQNILEPFVYLDVS-EG-----FVSLPVITAGTLGQVKPYSS-----VEKDQRIAL 619

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SP |060678| PRMT3_HUMAN -----
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SP |09NV44| PRMT7_HUMAN -----
SP |09NR22| PRMT8_HUMAN DLIS--EANHFPKETLEFWLRHVEDESM LQRPKSOKLWSIIILDVIEPSGLIQEIMEK 677
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SP |096L8| PRMT6_HUMAN -----
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SP |060678| PRMT3_HUMAN -----
SP |086X55| CARM1_HUMAN -----
SP |014744| PRMT5_HUMAN -----
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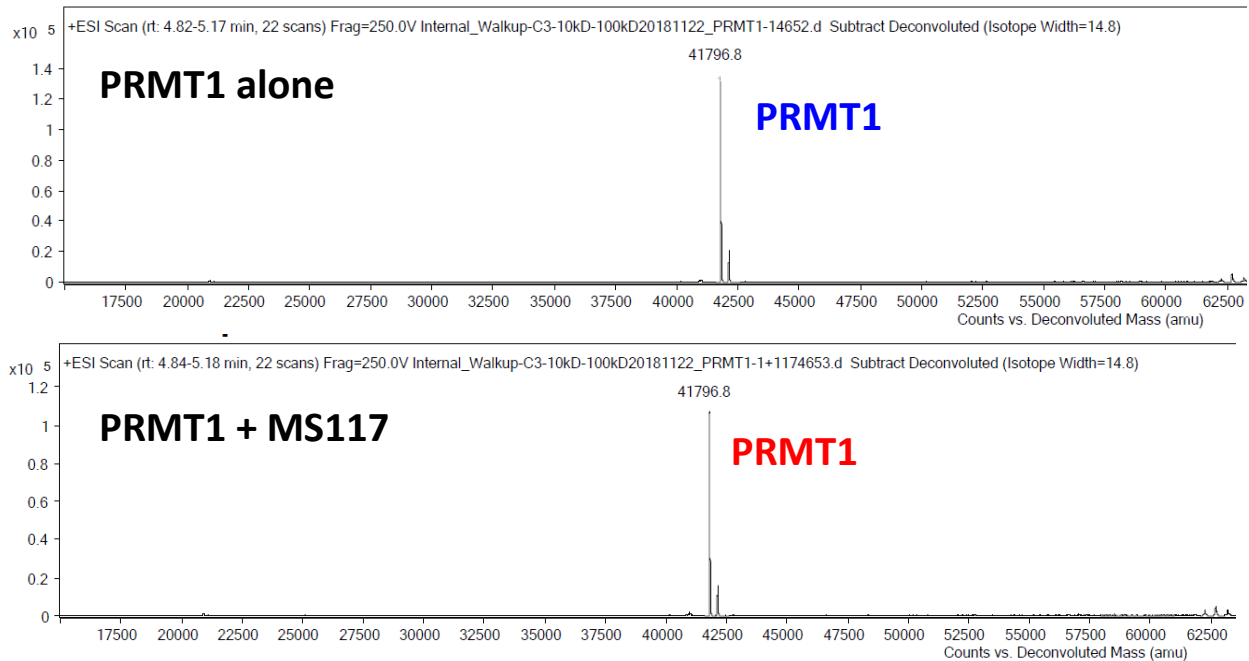
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SP |086X55| CARM1_HUMAN -----
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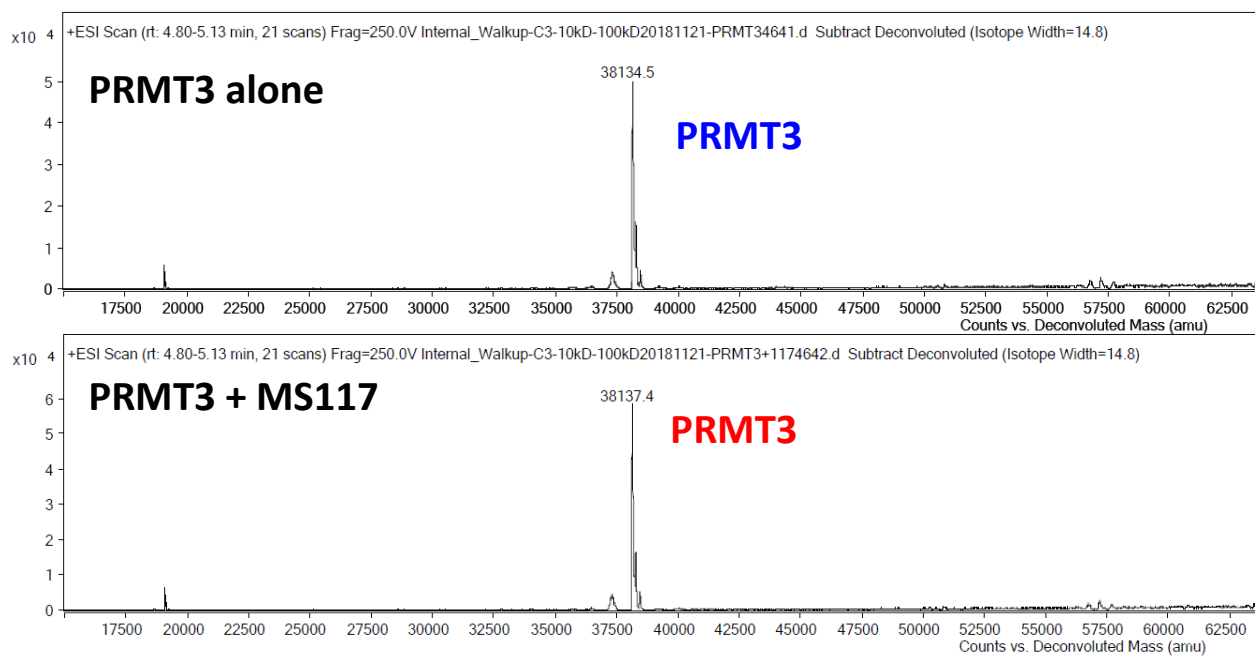
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SP |014744| PRMT5_HUMAN -----
SP |09NV44| PRMT7_HUMAN DTGDIIMEFRHADTPD----- 692
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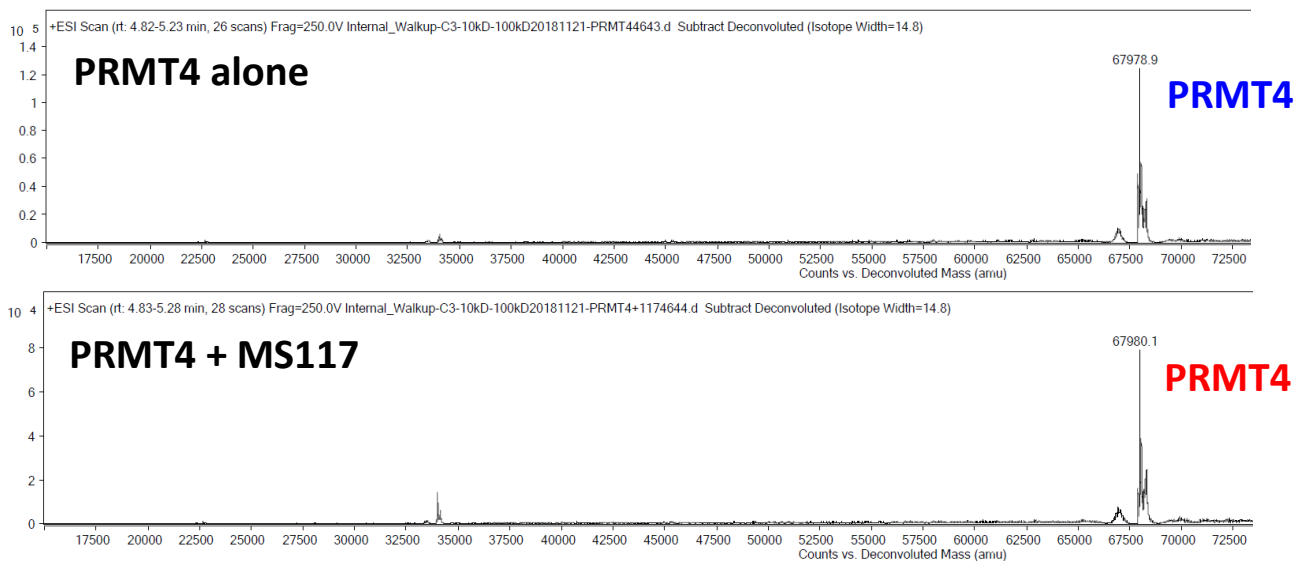
Supplementary Figure 1. Sequence alignment of 9 PRMTs. The alignment indicates Cys50 in PRMT6 is a unique cysteine residue to be targeted by an irreversible inhibitor.



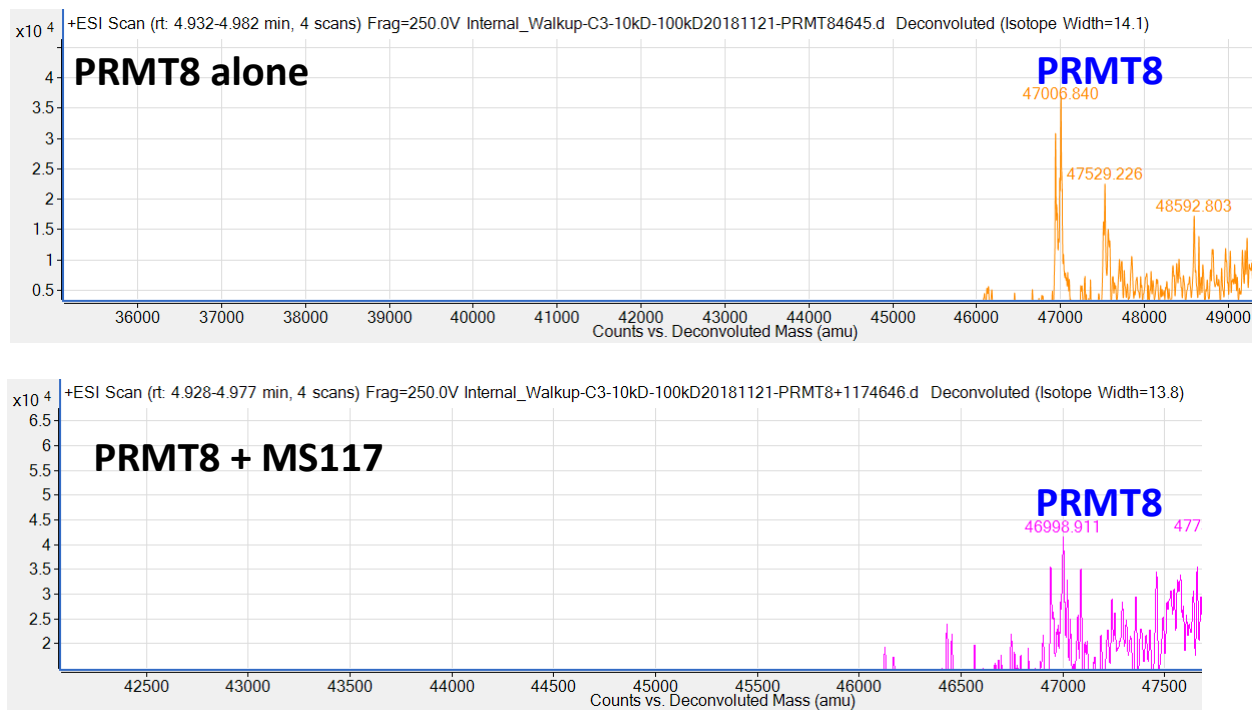
Supplementary Figure 2. MS assay results for PRMT1 incubated with compound 4 (MS117).



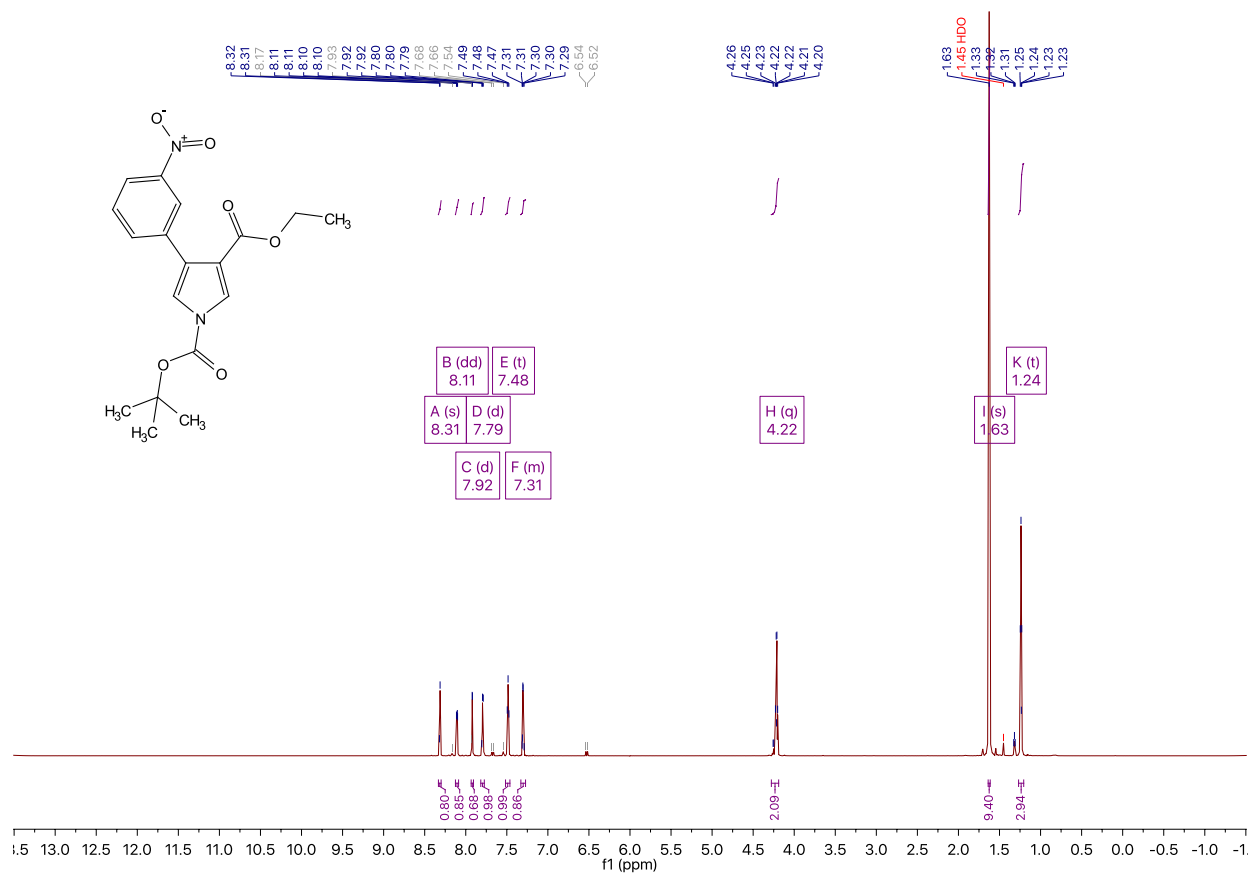
Supplementary Figure 3. MS assay results for PRMT3 incubated with compound 4 (MS117).



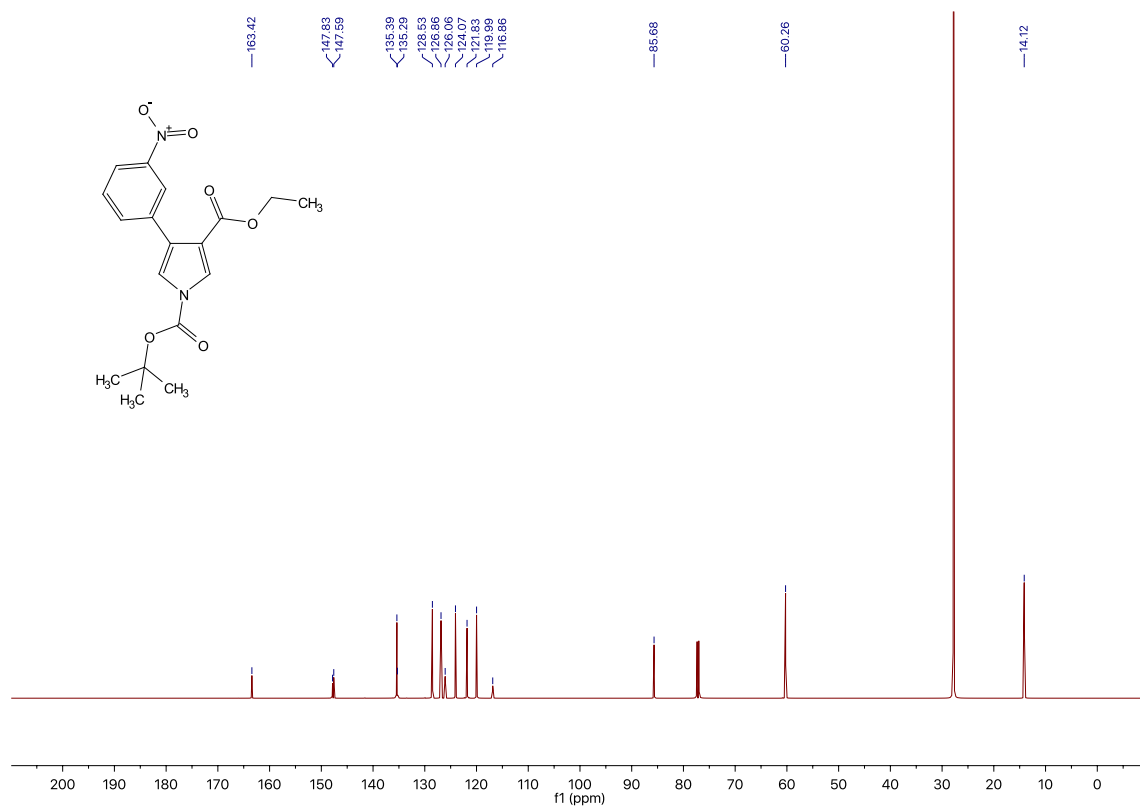
Supplementary Figure 4. MS assay results for PRMT4 incubated with compound 4 (MS117).



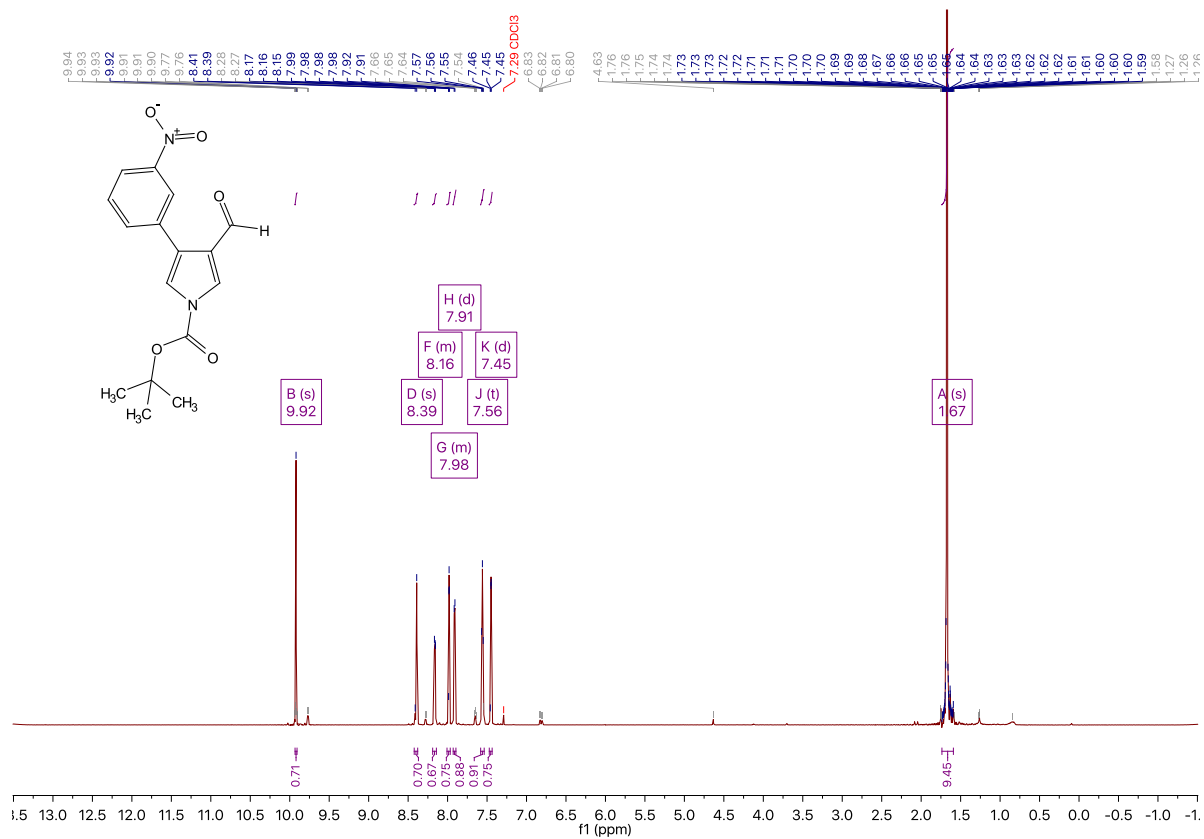
Supplementary Figure 5. MS assay results for PRMT8 incubated with compound 4 (MS117).



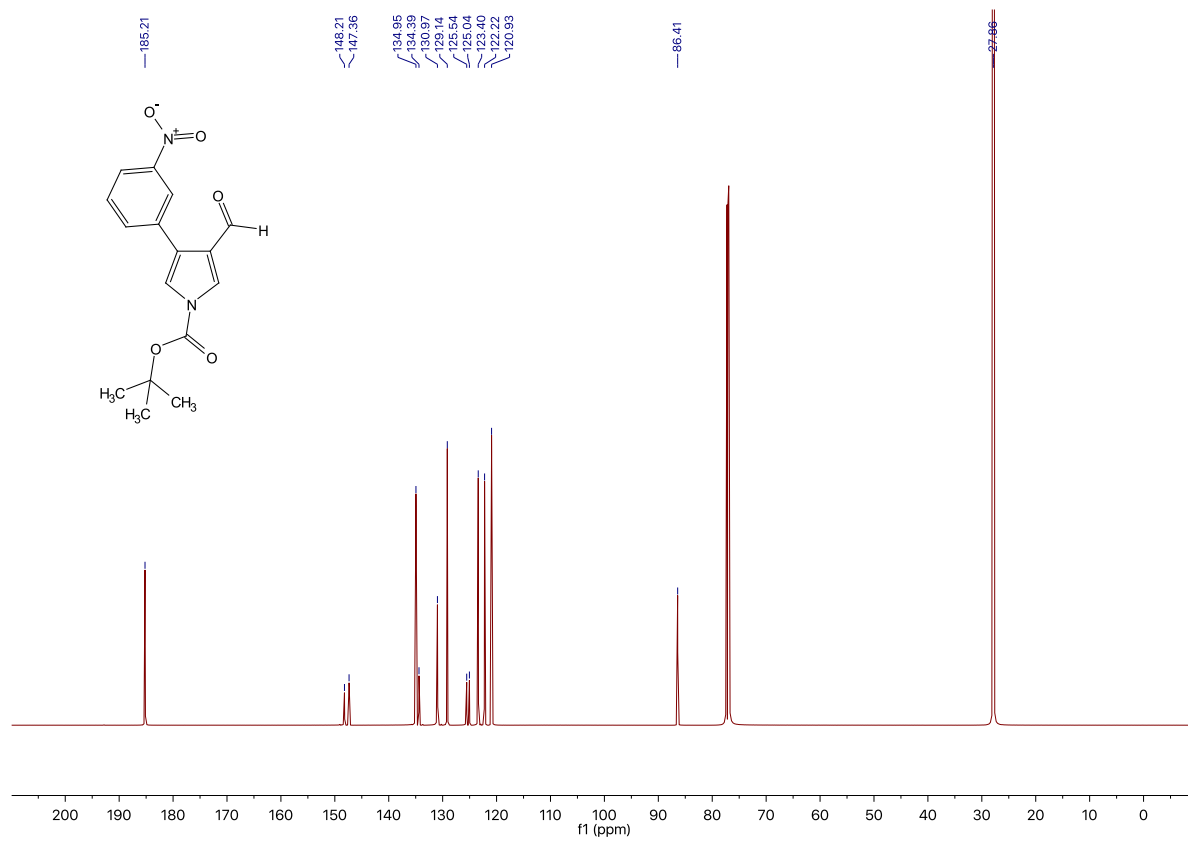
Supplementary Figure 6. ¹H NMR spectrum of compound 1.



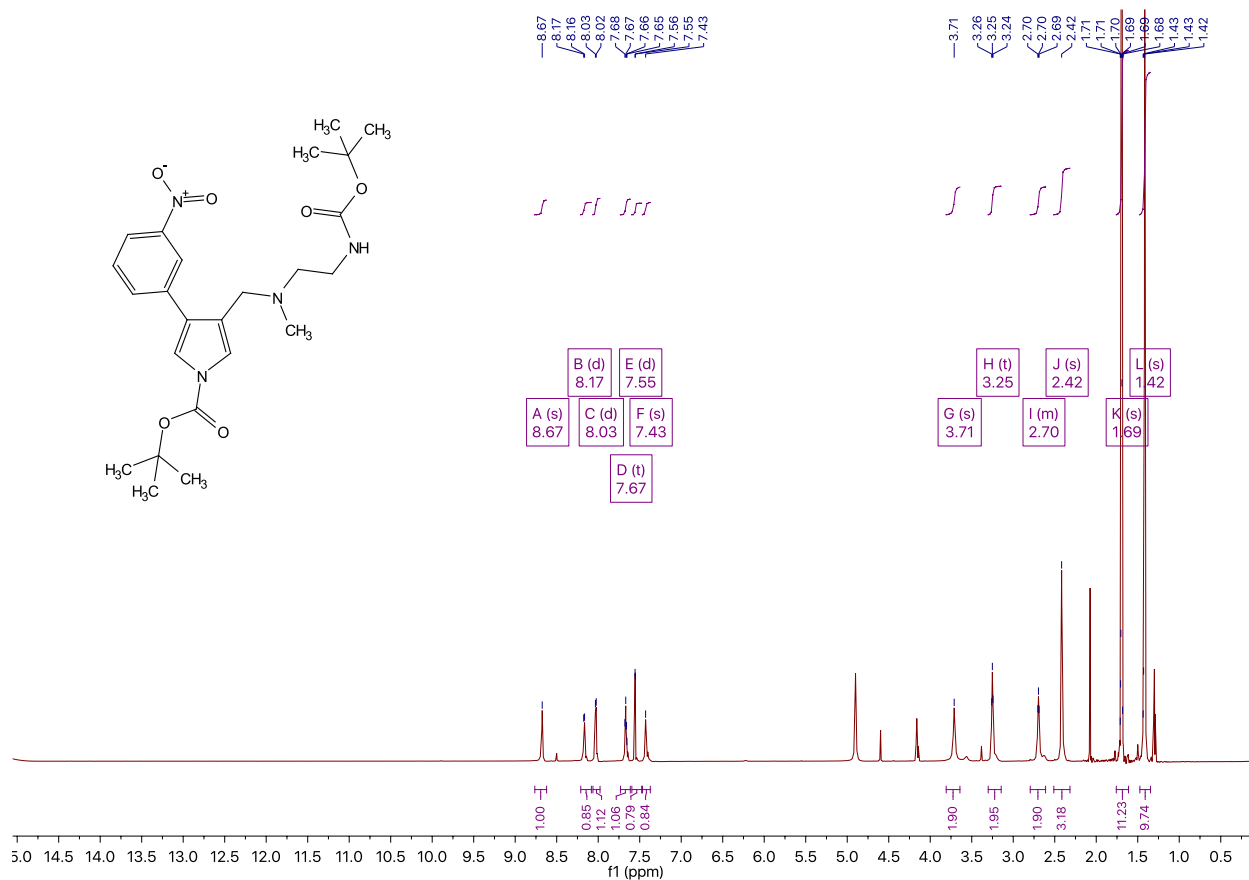
Supplementary Figure 7. ¹³C NMR spectrum of compound 1.



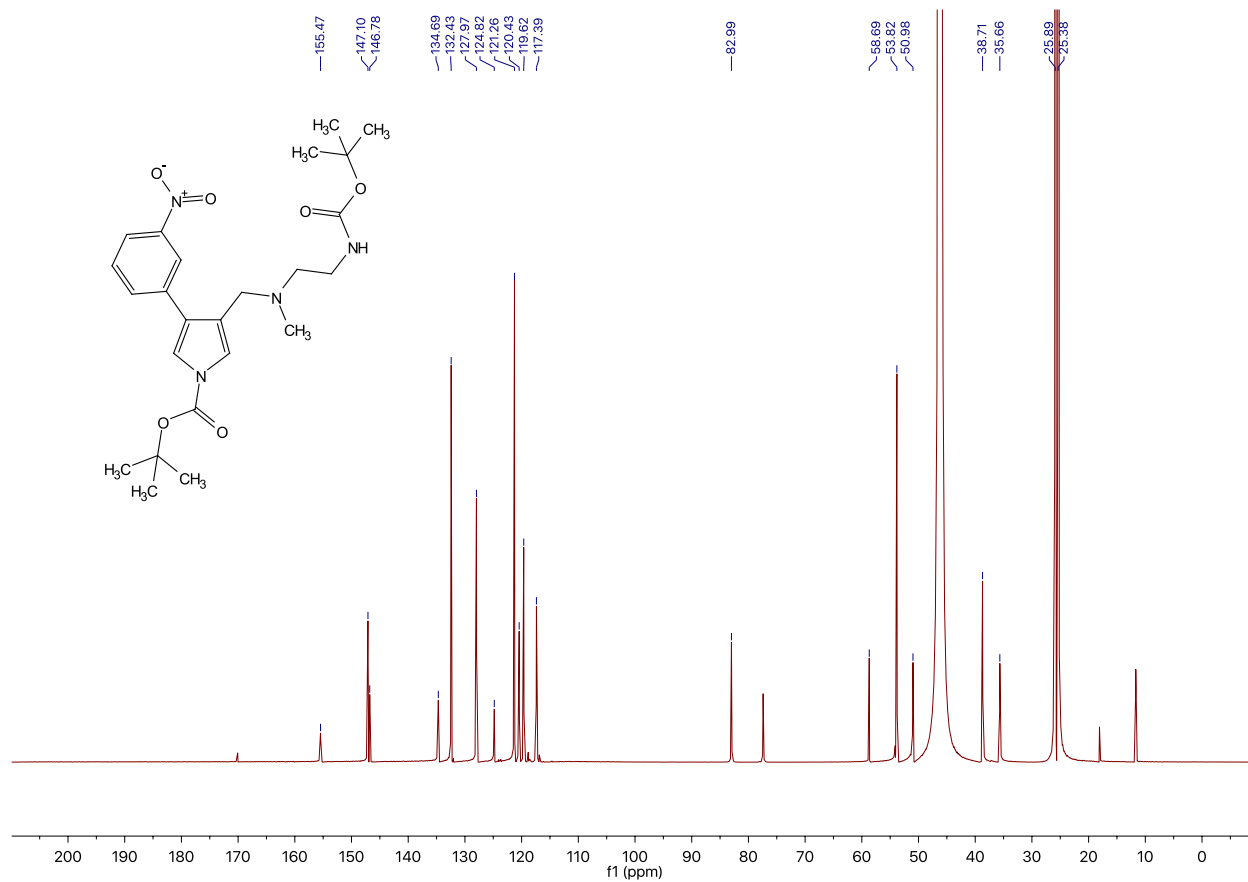
Supplementary Figure 8. ¹H NMR spectrum of compound 2.



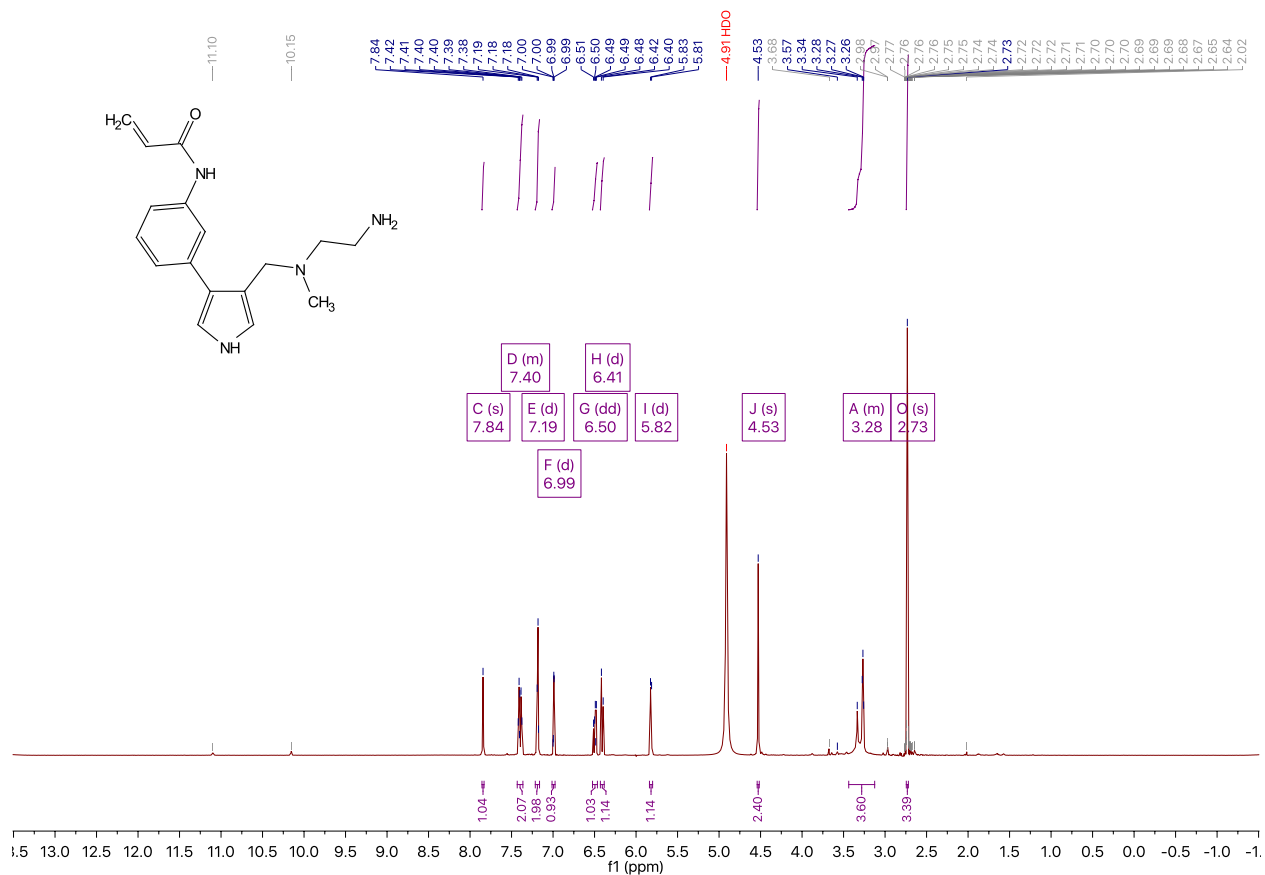
Supplementary Figure 9. ¹³CNMR spectrum of compound 2.



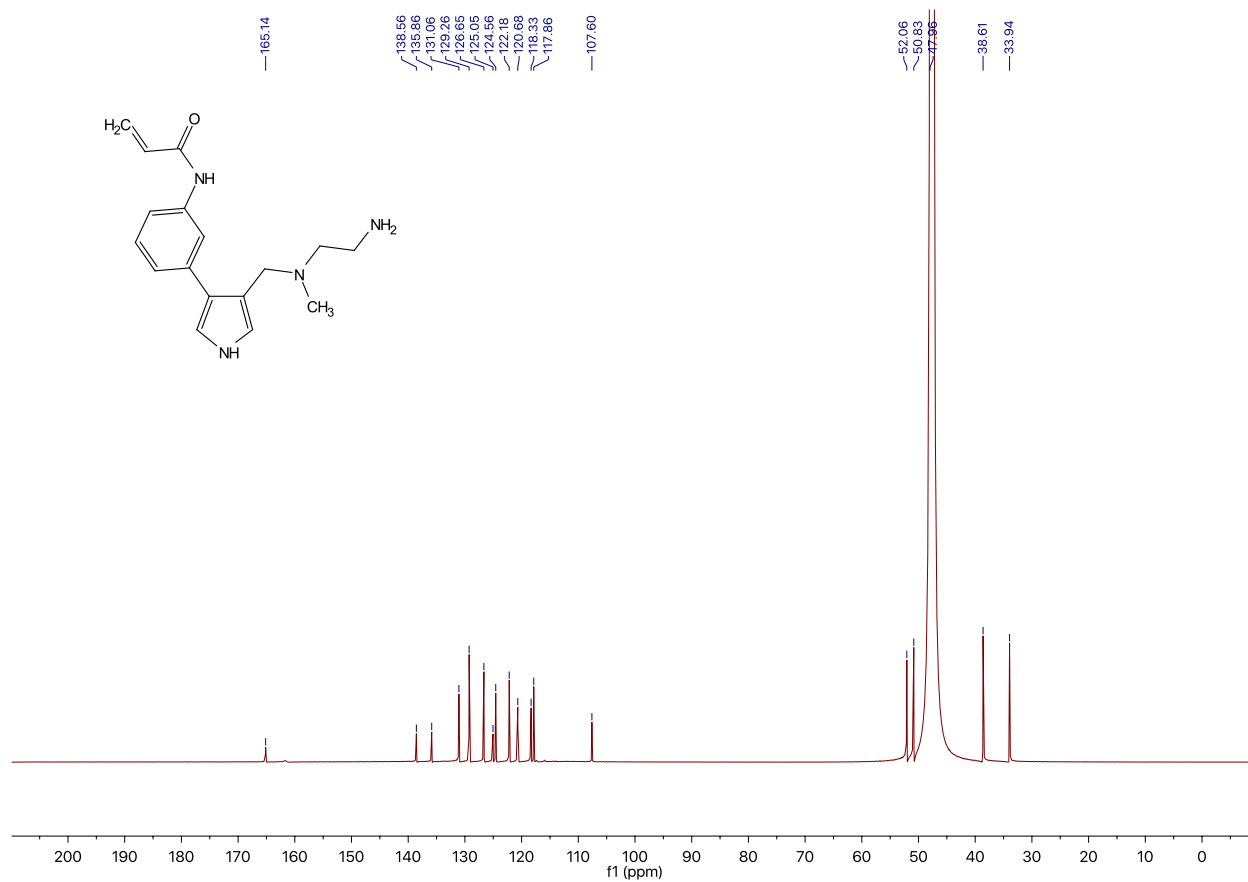
Supplementary Figure 10. ¹H NMR spectrum of compound 3.



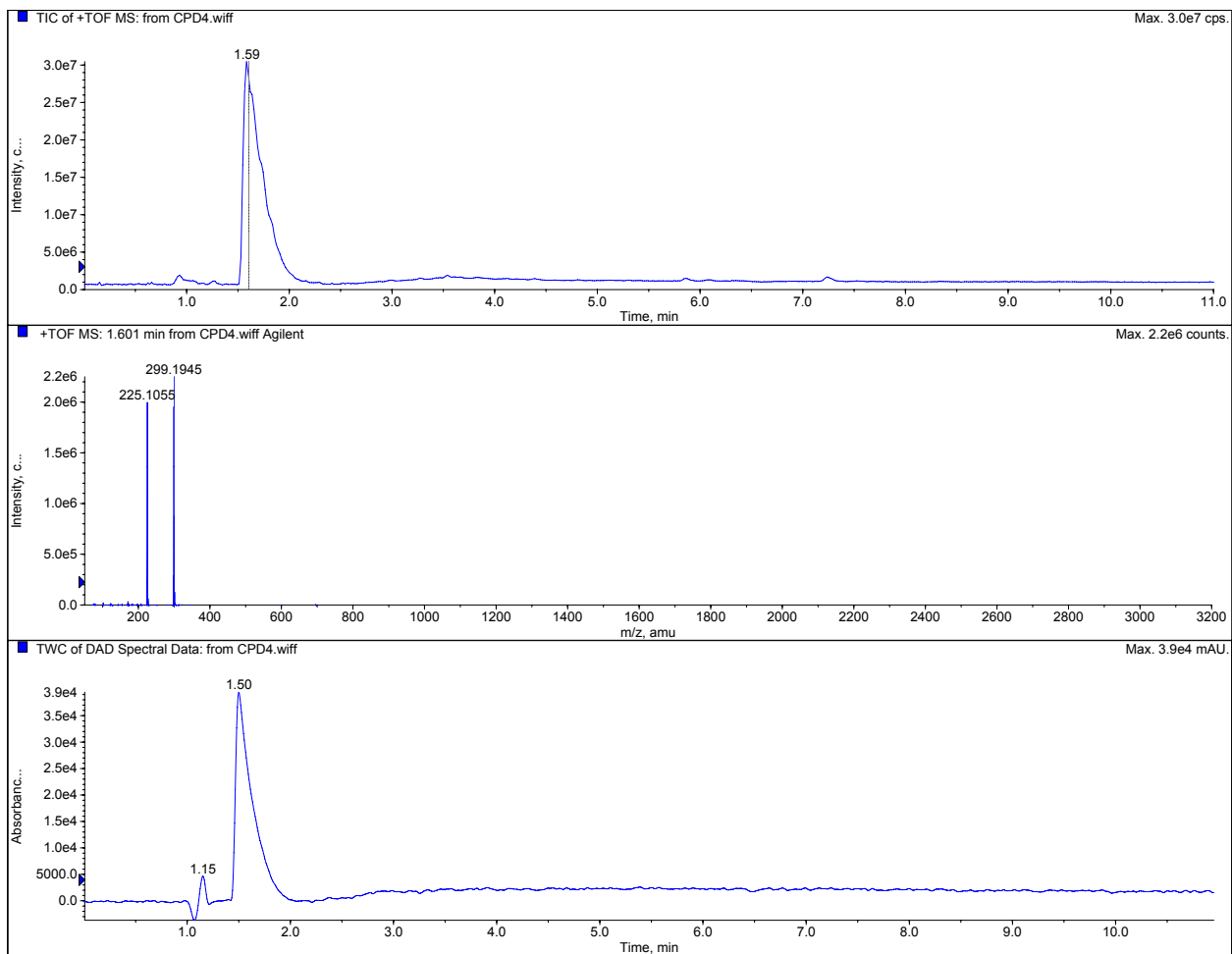
Supplementary Figure 11. ¹³CNMR spectrum of compound **3**.



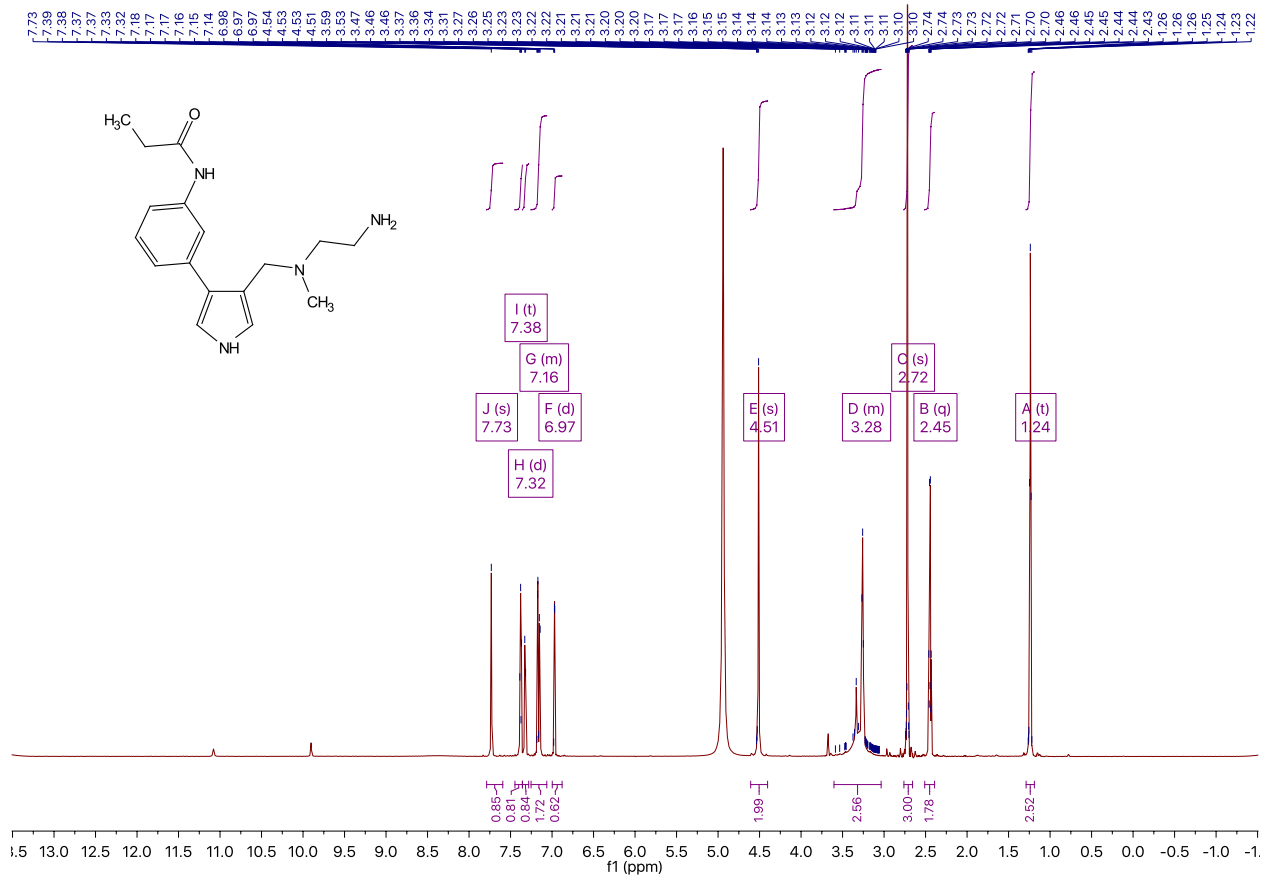
Supplementary Figure 12. ¹H NMR spectrum of compound 4.



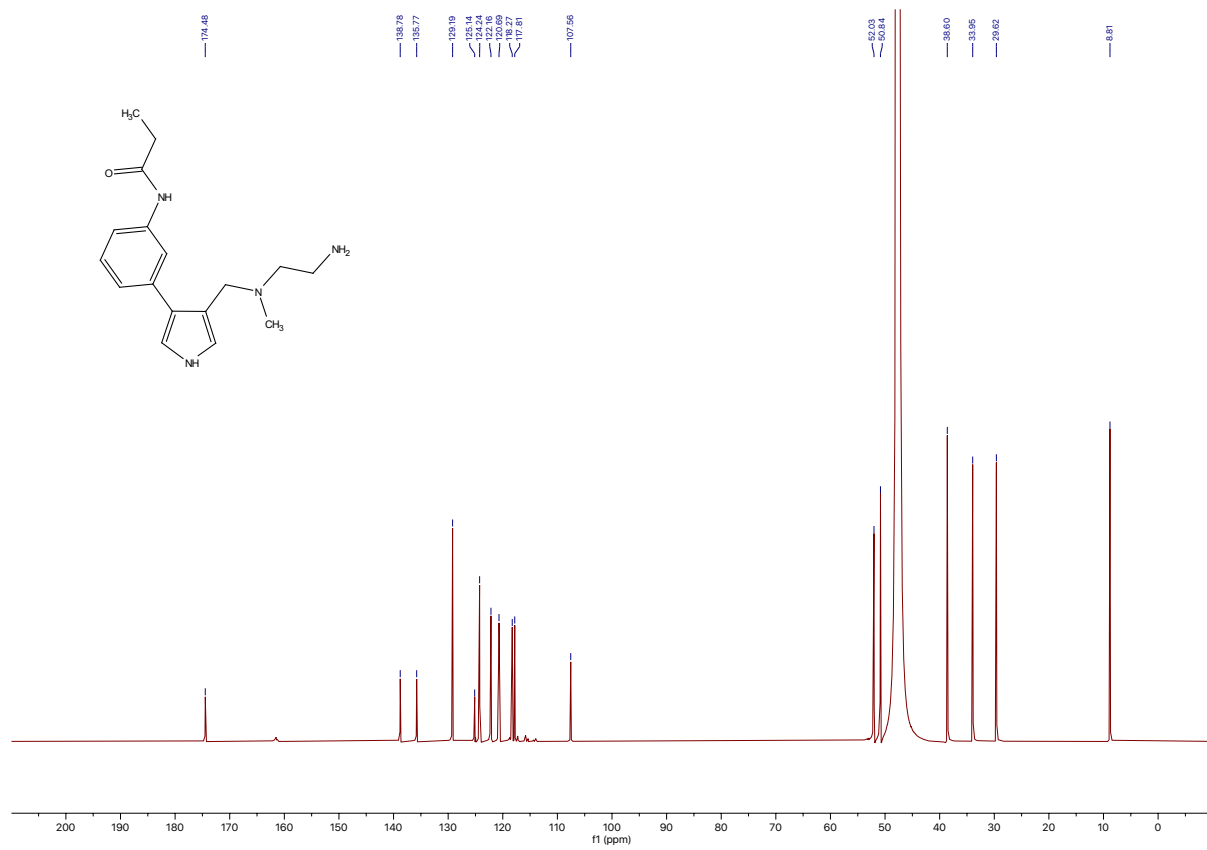
Supplementary Figure 13. ¹³CNMR spectrum of compound 4.



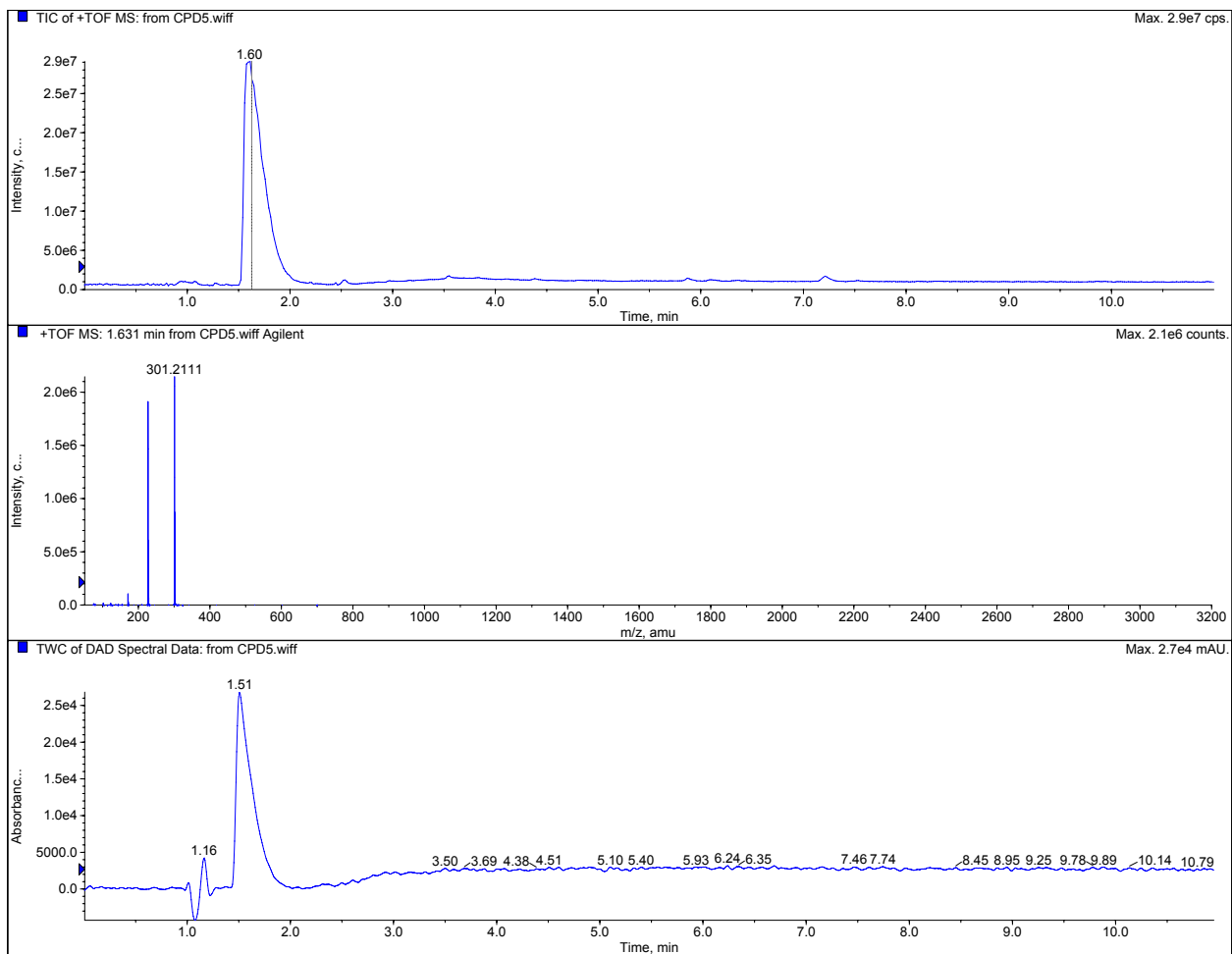
Supplementary Figure S14. HPLC-HRMS spectrum of compound 4.



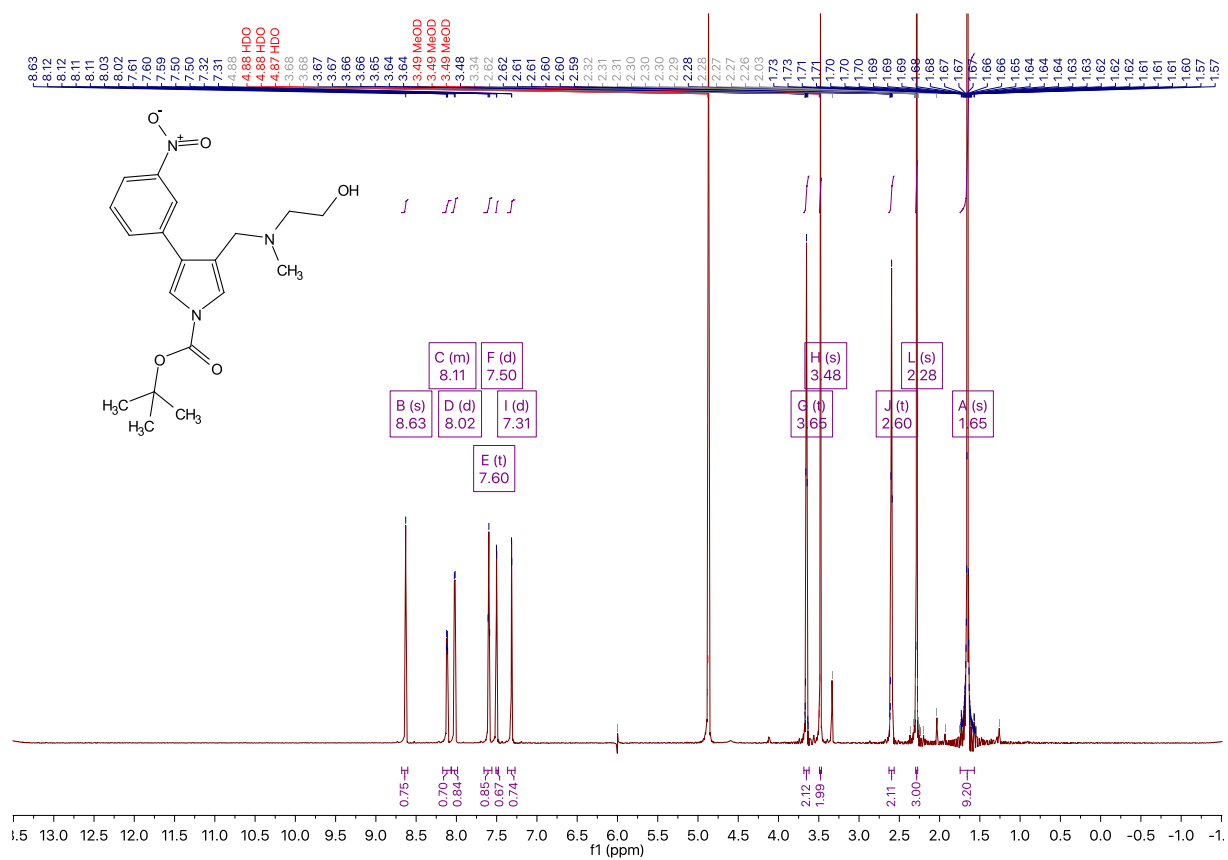
Supplementary Figure 15. ¹H NMR spectrum of compound 5.



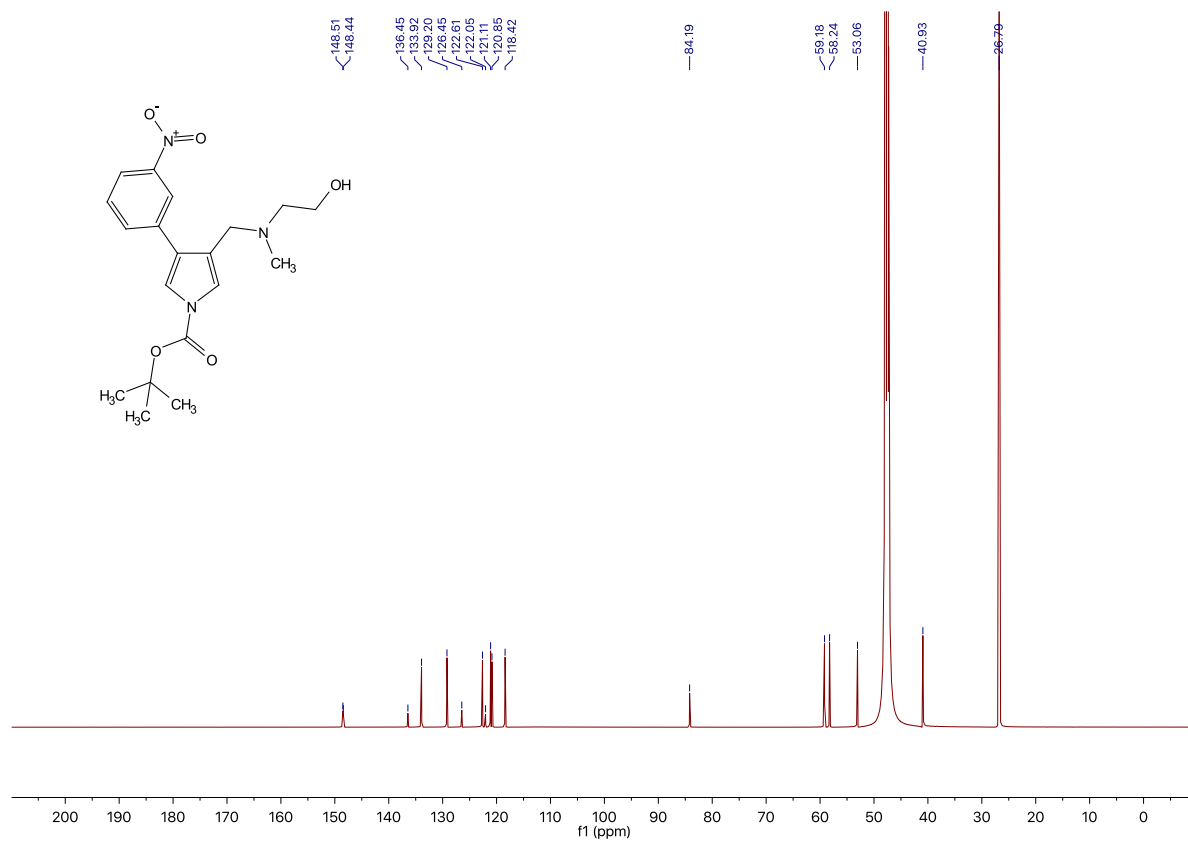
Supplementary Figure 16. ¹³CNMR spectrum of compound 5.



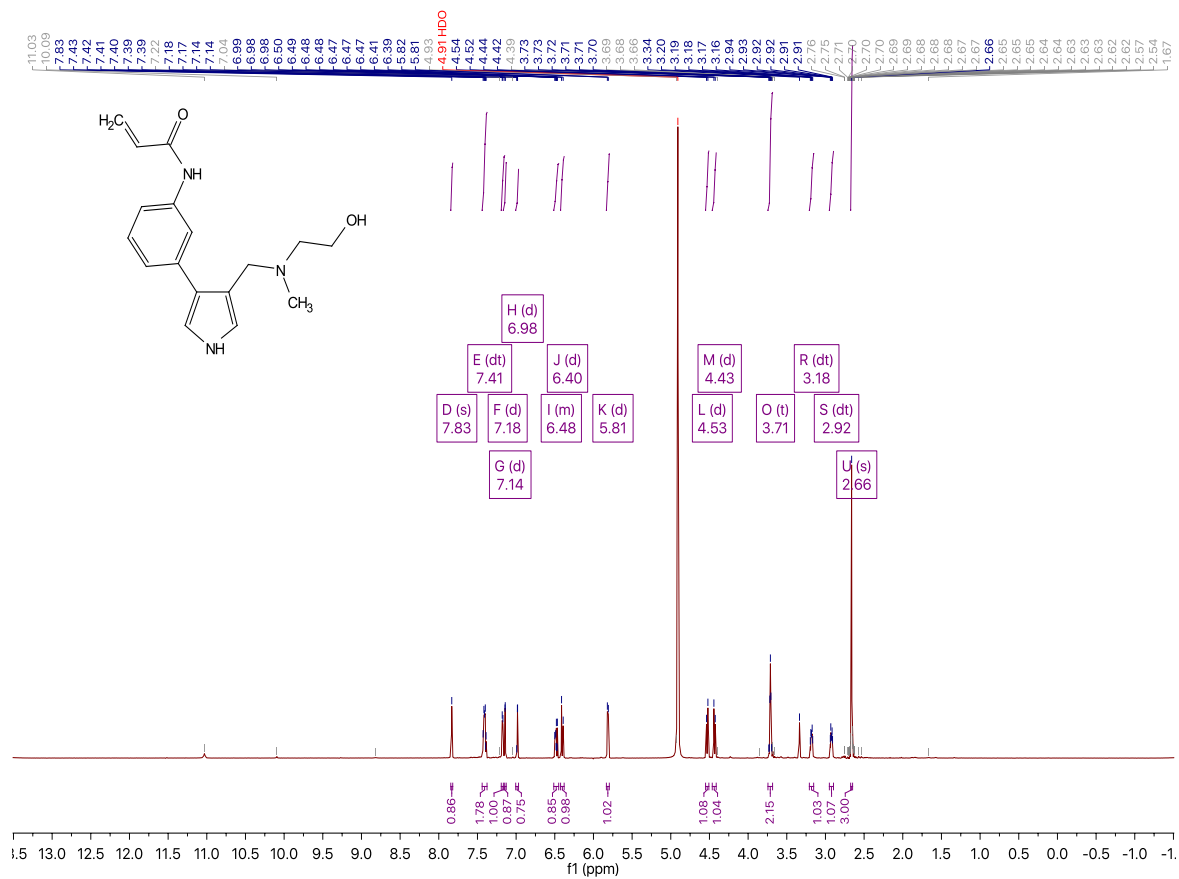
Supplementary Figure S17. HPLC-HRMS spectrum of compound 5.



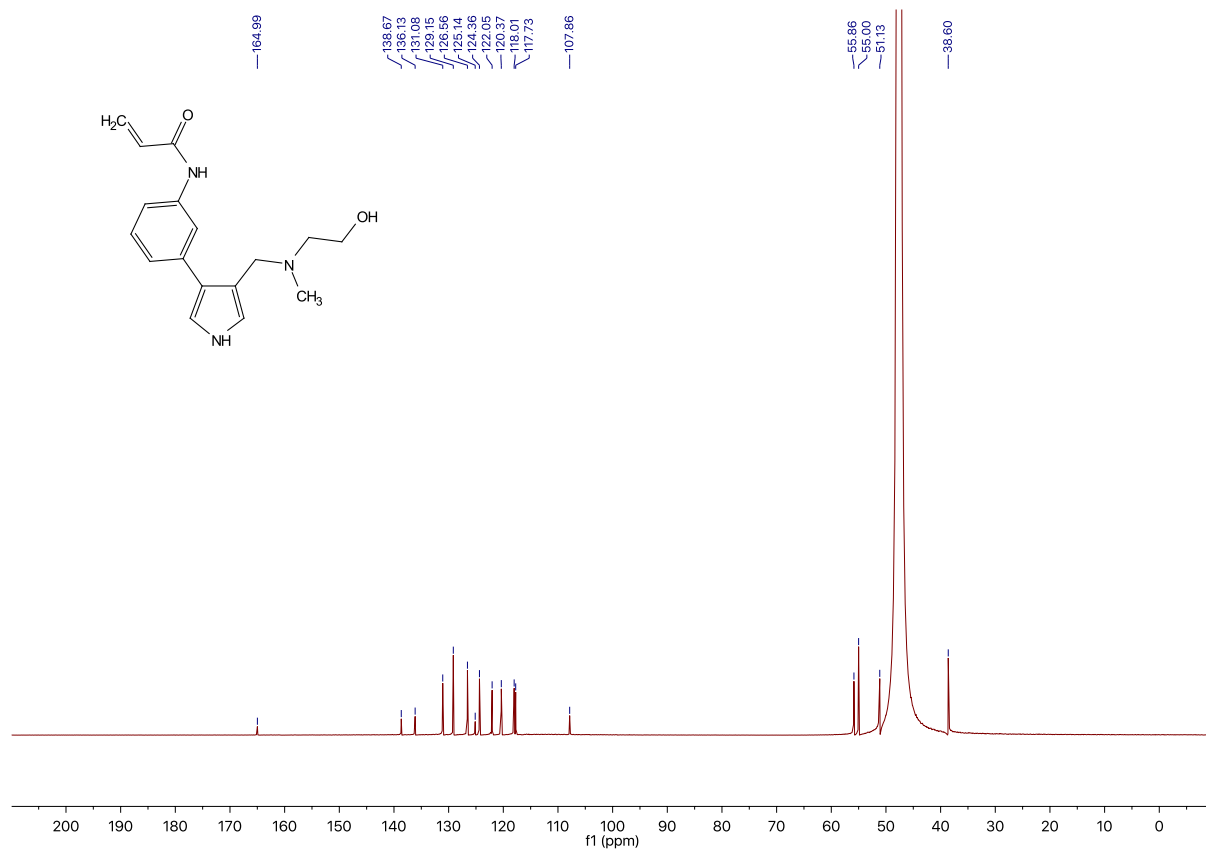
Supplementary Figure 18. ¹H NMR spectrum of compound 6.



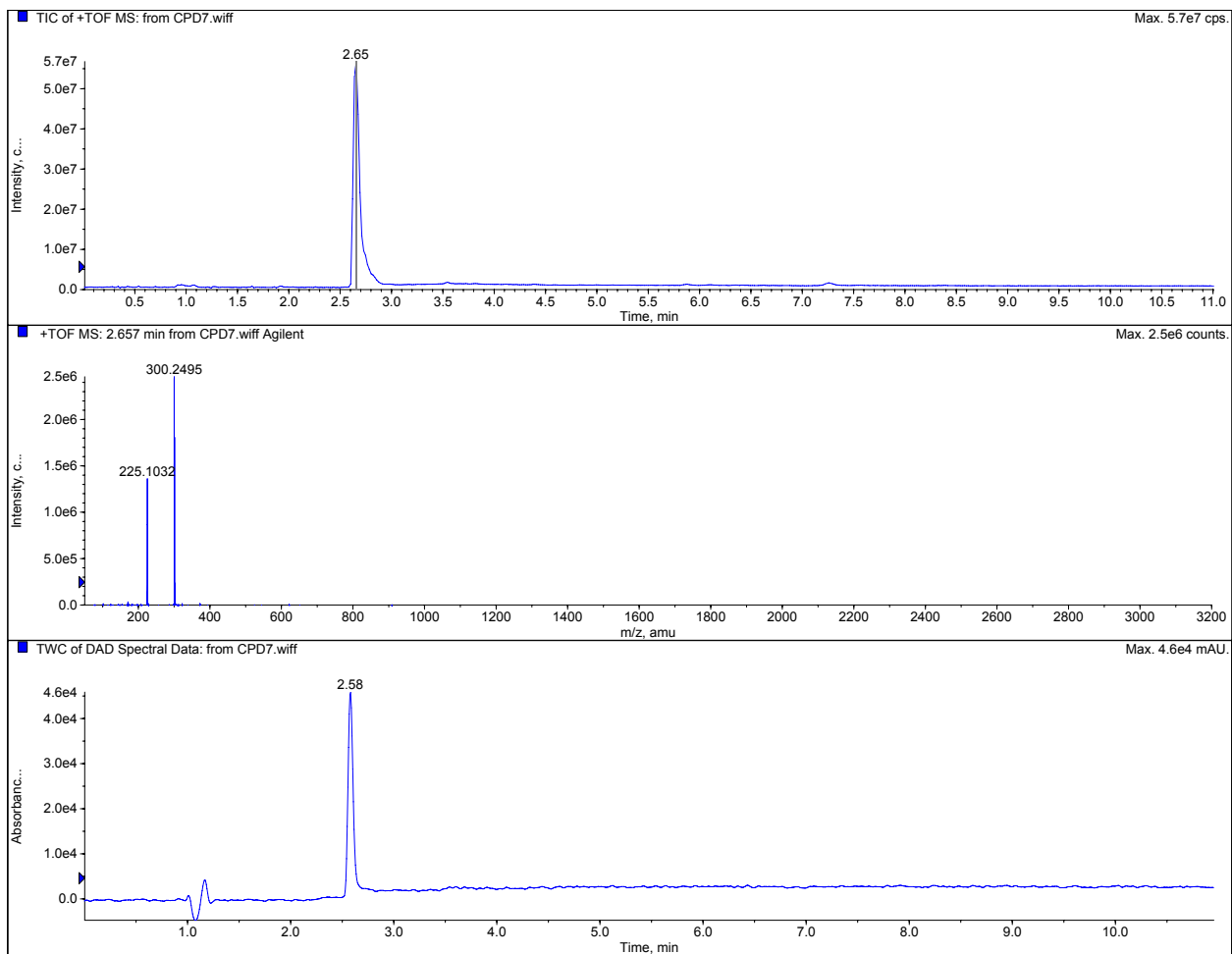
Supplementary Figure 19. ¹³CNMR spectrum of compound 6.



Supplementary Figure 20. ¹H NMR spectrum of compound 7.



Supplementary Figure 21. ¹³CNMR spectrum of compound 7.



Supplementary Figure S22. HPLC-HRMS spectrum of compound 7.