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Supporting Information

Discovery of histone deacetylase 3 (HDAC3)-specific PROTACs

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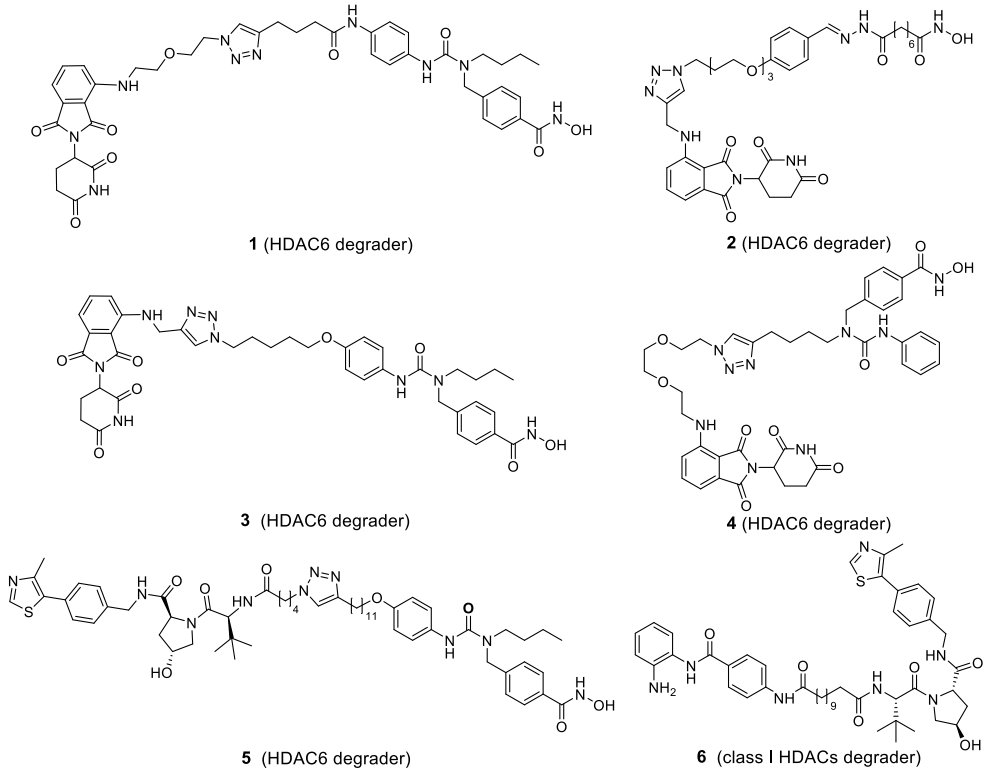


Figure S1. Representative PROTACs targeting HDAC6 or Class I HDACs.

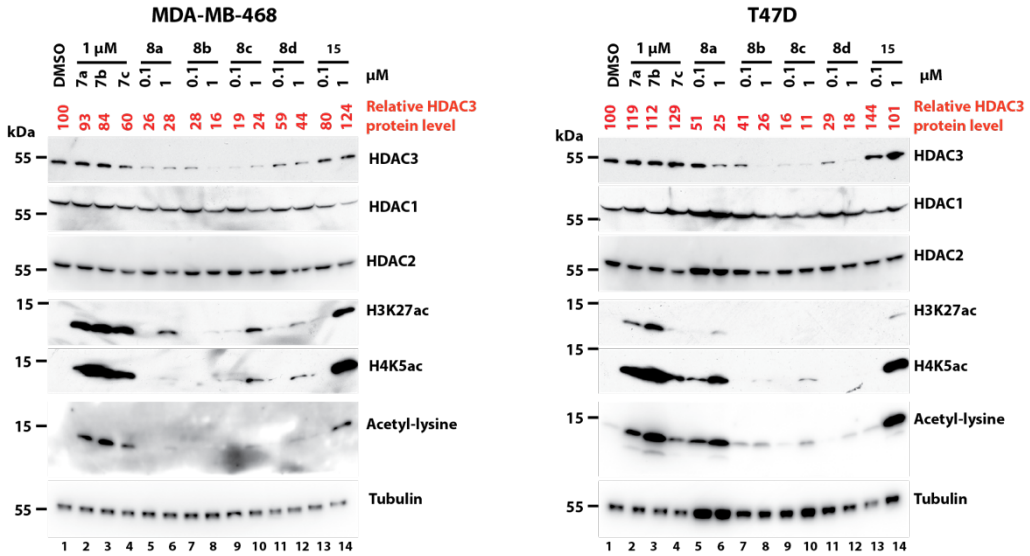
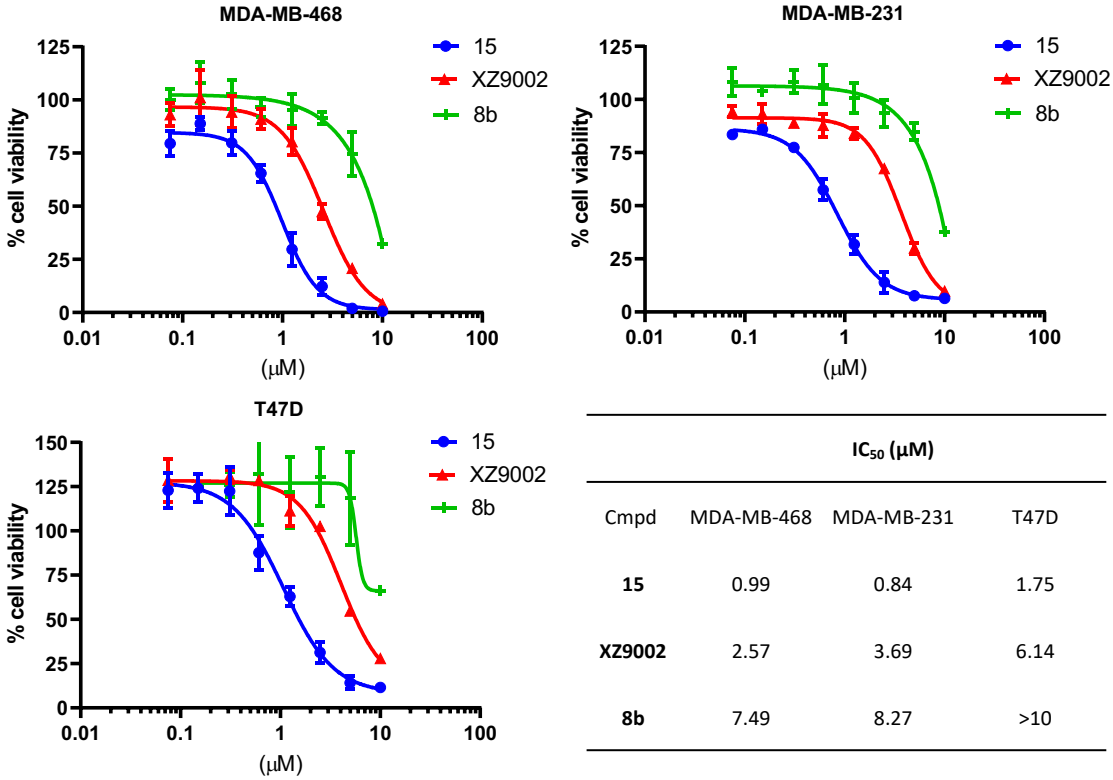


Figure S2. Western blot analysis of HDAC degradation in MDA-MB-468 and T47D cells. The culture of the triple-negative breast cancer cell line MDA-MB-468 and the ER+ breast cancer cell line T47D was exposed to vehicle control (DMSO) and the indicated compounds at specified concentrations for 14 h. Cells were lysed for Western blotting with antibodies against the indicated proteins. Antibodies against acetylated histones at specific sites (H3K27 and H4K5) and acetyl-lysine were used to assess levels of histone acetylation. Levels of HDAC3 were normalized against that of tubulin and % of HDAC3 protein levels in cells treated with the indicated compounds relative to that in DMSO control are shown.



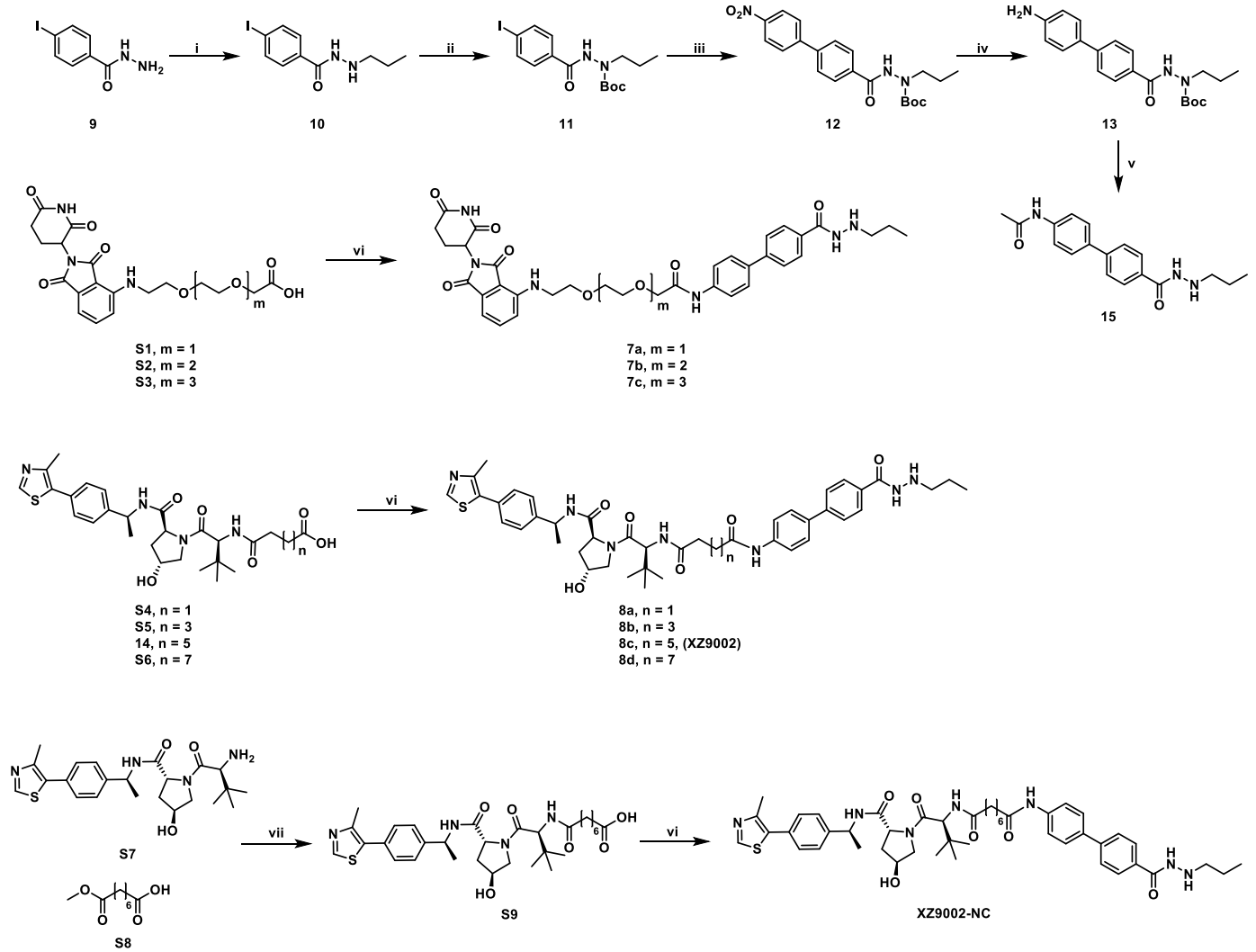
	IC_{50} (μM)		
Cmpd	MDA-MB-468	MDA-MB-231	T47D
15	0.99	0.84	1.75
XZ9002	2.57	3.69	6.14
8b	7.49	8.27	>10

Figure S3. XZ9002 potently inhibited cell viability in cancer cells. MDA-MB-468, MDA-MB-231, and T47D cells are treated with the indicated compounds at different concentrations for 72 h. Cell viability was assessed with CellTiter-Glo® reagents.

Table S1. In vitro inhibitory potencies of new PROTACs against HDAC1-3.

	IC_{50} (μM) [a]		
Compound	**HDAC3**	**HDAC1**	**HDAC2**
15	0.013±0.001	0.072±0.010	0.082±0.025
7a	0.043±0.018	0.082±0.025	0.410±0.014
7b	0.038±0.023	0.090±0.014	0.400±0.030
7c	0.072±0.015	0.125±0.035	0.450±0.070
8a	0.14±0.04	0.30±0.07	1.40±0.14
8b	0.32±0.03	0.36±0.06	1.65±0.07
XZ9002	0.35±0.07	0.65±0.07	1.55±0.07
8d	0.55±0.07	1.50±0.14	1.42±0.25

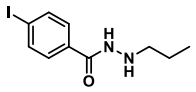
[a] Each value is the average of three independent assays ± standard deviation.



Scheme S1. Synthetic route for PROTACs 7a-c, 8a-d, and XZ9002-NC. *Reagents and conditions:* (i) a) Propionaldehyde, MeOH, THF; b) $NaBH_4$, MeOH, THF. (ii) Boc_2O, Et_3N, CH_2Cl_2. (iii) 4-Nitrophenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, toluene, EtOH, water, 90 °C. (iv) Pd/C, H_2, ethyl acetate. (v) a) Ac_2O, Et_3N, CH_2Cl_2; b) TFA, CH_2Cl_2. (vi) a) **13**, Et_3N, HATU, CH_2Cl_2; b) TFA, CH_2Cl_2. (vii) a) **S9**, HATU, Et_3N, CH_2Cl_2; b) LiOH, MeOH, water.

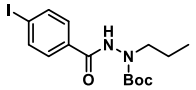
Experimental Section: Chemistry

General Methods. THF, CH_2Cl_2, and toluene were obtained via a solvent purification system by filtering through two columns packed with activated alumina and 4 Å molecular sieve, respectively. All other chemicals obtained from commercial sources were used without further purification. Flash chromatography was performed using silica gel (230–400 mesh) as the stationary phase. Reaction progress was monitored by thin-layer chromatography (silica-coated glass plates) and visualized by UV light, and/or by LC-MS. ^{1}H NMR spectra were recorded in $CDCl_3$, DMSO-d_6, or CD_3OD at 600 MHz. ^{13}C NMR spectrum was recorded in CD_3OD at 151 MHz. Chemical shifts δ are given in ppm using tetramethylsilane as an internal standard. Multiplicities of NMR signals are designated as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), and multiplet (m). All final compounds for biological testing were of ≥95.0% purity as analyzed by LC-MS, performed on an Advion AVANT LC system with the expression CMS using a Thermo Accucore™ Vanquish™ C18+ UHPLC Column (1.5 µm, 50 x 2.1 mm) at 40 °C. Gradient elution was used for UHPLC with a mobile phase of acetonitrile and water containing 0.1% formic acid. High resolution mass spectra (HRMS) were recorded on an Agilent 6230 Time-of-Flight (TOF) mass spectrometer.



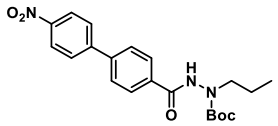
10

4-Iodo-*N*'-propylbenzohydrazide (10): A mixture of compound **9** (262 mg, 1.0 mmol) and propionaldehyde (87 mg, 1.5 mmol) in MeOH-THF (1:1, v/v, 10 mL) was stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the residue was dissolved in MeOH-THF (1:1, v/v, 10 mL). The solution was treated with $NaBH_4$ (60 mg, 1.6 mmol) and stirred for 30 min. Then it was diluted with water and extracted with ethyl acetate. The organic phase was washed with water x1, brine x1, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was used directly in the next step (270 mg, yield 89%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.4 Hz, 2H), 7.49 (d, *J* = 8.5 Hz, 2H), 2.93 (t, *J* = 7.2 Hz, 2H), 1.64 – 1.53 (m, 2H), 1.00 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI): m/z 305.0 $[M+H]^+$.



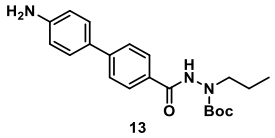
11

***tert*-Butyl 2-(4-iodobenzoyl)-1-propylhydrazine-1-carboxylate (11):** A mixture of compound **10** (100 mg, 0.32 mmol), Boc_2O (108 mg, 0.50 mmol), and Et_3N (140 µL, 1.0 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 16 h. Then it was diluted with water and extracted with CH_2Cl_2. The organic phase was washed with water x1, brine x1, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was further purified by column chromatography to afford the title compound (123 mg, yield 95%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.58 (s, 1H), 8.00 – 7.84 (m, 2H), 7.73 – 7.44 (m, 2H), 3.44 – 3.34 (m, 2H), 1.61 – 1.28 (m, 11H), 0.99 – 0.83 (m, 3H). LC-MS (ESI): m/z 405.1 $[M+H]^+$.



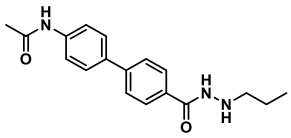
12

***tert*-Butyl 2-(4'-nitro-[1,1'-biphenyl]-4-carbonyl)-1-propylhydrazine-1-carboxylate (12):** A mixture of compound **11** (300 mg, 0.74 mmol), 4-nitrophenylboronic acid (150 mg, 0.90 mmol), $Pd(PPh_3)_4$ (40 mg, 0.03 mmol), and K_2CO_3 (204 mg, 1.48 mmol) was stirred in toluene-EtOH-water (9:1:1, v/v/v, 2 mL) at 90 °C under an argon atmosphere for 16 h. The reaction mixture was cooled to room temperature and poured into water. The resulting solution was extracted with ethyl acetate. The organic phase was washed with water x1, brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The residue was further purified by column chromatography to afford the title compound (243 mg, yield 82%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.35 (d, *J* = 8.8 Hz, 2H), 7.94 (d, *J* = 7.9 Hz, 2H), 7.81 – 7.76 (m, 2H), 7.76 – 7.62 (m, 2H), 3.61 (t, *J* = 7.2 Hz, 2H), 1.72 – 1.64 (m, 2H), 1.56 – 1.39 (m, 9H), 0.98 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI): m/z 400.1 $[M+H]^+$.



13

***tert*-Butyl 2-(4'-amino-[1,1'-biphenyl]-4-carbonyl)-1-propylhydrazine-1-carboxylate (13):** A mixture of compound **12** (243 mg, 0.61 mmol) and Pd/C (10% w/w, 30 mg) in ethyl acetate (20 mL) was stirred at room temperature for 2 h. After solid was removed by filtration, the filtrate was evaporated to dryness to afford the title compound (225 mg, 100% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90 – 7.69 (m, 3H), 7.66 – 7.59 (m, 2H), 7.50 – 7.45 (m, 2H), 6.81 – 6.77 (m, 2H), 3.83 (s, 2H), 3.60 (t, *J* = 7.2 Hz, 2H), 1.71 – 1.63 (m, 2H), 1.55 – 1.41 (m, 9H), 0.97 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI): m/z 370.2 $[M+H]^+$.

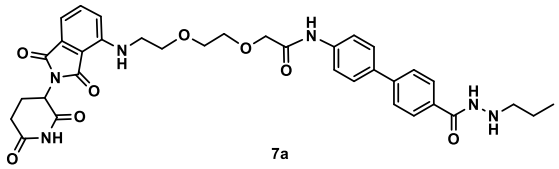


15

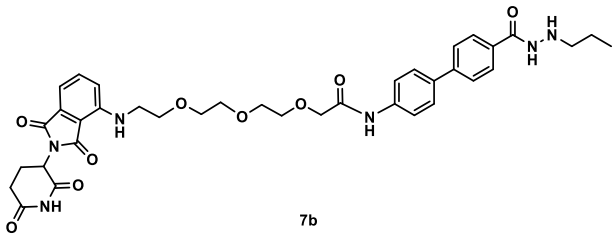
***N*-(4'-(2-Propylhydrazine-1-carbonyl)-[1,1'-biphenyl]-4-yl)acetamide (15):** A mixture of **13** (15 mg, 0.04 mmol), Ac_2O (4.6 µL, 0.05 mmol), and Et_3N (45 µL, 0.32 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature for 16 h. The mixture was poured into water and extracted with CH_2Cl_2. The combined organic layers were washed with NH_4Cl (aq.) × 1, brine × 1, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (2 mL) and treated with TFA (0.2 mL). The mixture was stirred at room temperature for 4 h and concentrated under reduced pressure. The crude product was purified by flash column chromatography to afford the title

compound (3.9 mg, yield 31%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.95 – 7.90 (m, 2H), 7.81 – 7.76 (m, 2H), 7.72 – 7.65 (m, 4H), 3.10 (t, *J* = 7.5 Hz, 2H), 2.17 (s, 3H), 1.78 – 1.67 (m, 2H), 1.06 (t, *J* = 7.5 Hz, 3H). LC-MS (ESI): m/z 312.1 $[M+H]^+$.

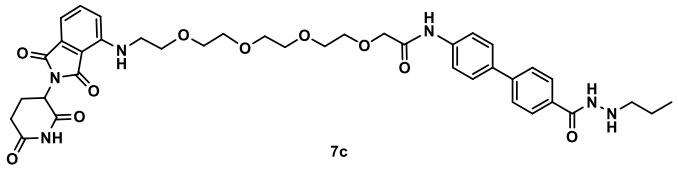
*General Method for Compounds **7a-c, 8a-d,** and **XZ9002-NC**.* Acids **S1-6** and **14** were prepared according to our previous publication.[1] A mixture of the corresponding acid (1.0 equiv.), amine (1.0 equiv.), HATU (1.05 equiv.) and Et_3N (5.0 equiv.) in CH_2Cl_2 was stirred at room temperature for 1 h. The mixture was poured into water and extracted with CH_2Cl_2. The combined organic layers were washed with NH_4Cl (aq.) × 1, brine × 1, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue was dissolved in CH_2Cl_2 and treated with TFA (20 equiv.). The mixture was stirred at room temperature for 4 h and concentrated under reduced pressure. The crude product was purified by flash column chromatography to afford the desired compound.



2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)-*N*-(4'-(2-propylhydrazine-1-carbonyl)-[1,1'-biphenyl]-4-yl)acetamide (7a): Following general method, compound **7a** was obtained from **S1** and **13** (6.9 mg, yield 38%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.96 (d, *J* = 8.4 Hz, 2H), 7.73 (d, *J* = 8.5 Hz, 2H), 7.62 (d, *J* = 8.6 Hz, 2H), 7.52 (d, *J* = 8.6 Hz, 2H), 7.48 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.05 (d, *J* = 8.6 Hz, 1H), 6.90 (d, *J* = 7.0 Hz, 1H), 4.96 – 4.91 (m, 1H), 4.18 (s, 2H), 3.92 – 3.79 (m, 6H), 3.59 – 3.54 (m, 2H), 3.32 – 3.29 (m, 2H), 2.80 – 2.54 (m, 3H), 2.01 – 1.96 (m, 1H), 1.86 – 1.79 (m, 2H), 1.10 (t, *J* = 7.5 Hz, 3H). LC-MS (ESI): m/z 671.3 $[M+H]^+$. HRMS *m/z* calcd for $C_{35}H_{39}N_6O_8$ $[M+H]^+$: 671.2824, found: 671.2848.

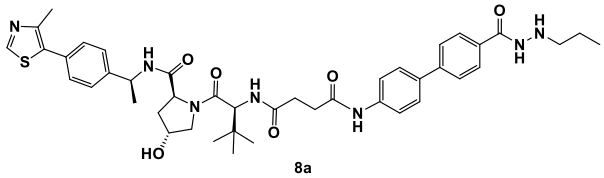


2-(2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)-*N*-(4'-(2-propylhydrazine-1-carbonyl)-[1,1'-biphenyl]-4-yl)acetamide (7b): Following general method, compound **7b** was obtained from **S2** and **13** (7.7 mg, yield 40%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.94 (d, *J* = 8.4 Hz, 2H), 7.76 – 7.70 (m, 4H), 7.63 – 7.57 (m, 2H), 7.44 (dd, *J* = 8.5, 7.1 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 6.94 (d, *J* = 7.0 Hz, 1H), 4.99 (dd, *J* = 12.8, 5.5 Hz, 1H), 4.16 (s, 2H), 3.84 – 3.69 (m, 10H), 3.44 (t, *J* = 5.2 Hz, 2H), 3.32 – 3.30 (m, 2H), 2.89 – 2.60 (m, 3H), 2.11 – 2.01 (m, 1H), 1.89 – 1.78 (m, 2H), 1.11 (t, *J* = 7.5 Hz, 3H). LC-MS (ESI): m/z 715.4 $[M+H]^+$. HRMS *m/z* calcd for $C_{37}H_{43}N_6O_9$ $[M+H]^+$: 715.3086, found: 715.3068.



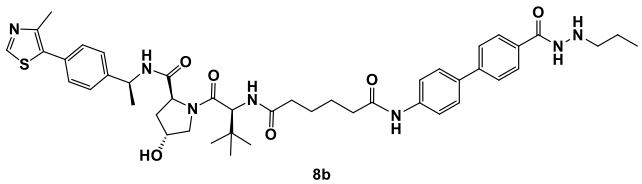
7c

14-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-*N*-(4'-(2-propylhydrazine-1-carbonyl)-[1,1'-biphenyl]-4-yl)-3,6,9,12-tetraoxatetradecanamide (7c): Following general method, compound **7c** was obtained from **S3** and **13** (6.8 mg, yield 33%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.94 (d, *J* = 8.5 Hz, 2H), 7.81 – 7.76 (m, 4H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.49 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.03 – 6.97 (m, 2H), 5.03 (dd, *J* = 12.8, 5.5 Hz, 1H), 4.16 (s, 2H), 3.81 – 3.61 (m, 14H), 3.43 (t, *J* = 5.3 Hz, 2H), 3.32 – 3.29 (m, 2H), 2.89 – 2.64 (m, 3H), 2.12 – 2.04 (m, 1H), 1.87 – 1.79 (m, 2H), 1.10 (t, *J* = 7.5 Hz, 3H). LC-MS (ESI): m/z 759.4 $[M+H]^+$. HRMS *m/z* calcd for $C_{39}H_{46}N_6O_{10}Na$ $[M+Na]^+$: 781.3168, found: 781.3183.



8a

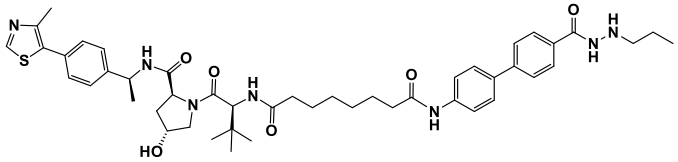
***N*^{1}-((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-*N*^{4}-(4'-(2-propylhydrazine-1-carbonyl)-[1,1'-biphenyl]-4-yl)succinimide (8a):** Following general method, compound **8a** was obtained from **S4** and **13** (6.1 mg, yield 28%). ^{1}H NMR (600 MHz, CD_3OD) δ 8.89 (s, 1H), 8.56 (d, *J* = 7.5 Hz, 1H), 8.00 (d, *J* = 8.9 Hz, 1H), 7.91 – 7.81 (m, 2H), 7.77 – 7.61 (m, 7H), 7.48 – 7.41 (m, 4H), 5.07 – 5.00 (m, 1H), 4.66 – 4.58 (m, 2H), 4.48 – 4.42 (m, 1H), 3.94 – 3.86 (m, 1H), 3.77 (dd, *J* = 11.1, 4.0 Hz, 1H), 3.01 – 2.83 (m, 2H), 2.76 – 2.63 (m, 4H), 2.49 (s, 3H), 2.25 – 2.17 (m, 1H), 2.01 – 1.94 (m, 1H), 1.67 – 1.57 (m, 2H), 1.53 (d, *J* = 7.0 Hz, 3H), 1.07 (s, 9H), 1.02 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI): m/z 796.4 $[M+H]^+$. HRMS *m/z* calcd for $C_{43}H_{54}N_7O_6S$ $[M+H]^+$: 796.3851, found: 796.3865.



8b

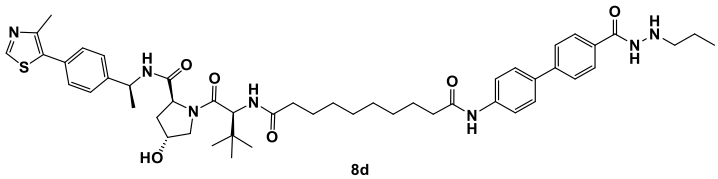
***N*^{1}-((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-*N*^{6}-(4'-(2-propylhydrazine-1-carbonyl)-[1,1'-biphenyl]-4-yl)adipamide (8b):** Following general method, compound **8b** was obtained from **S5** and **13** (3.8 mg, yield 17%).^{1}H NMR (600 MHz, CD_3OD) δ 8.88 (s, 1H), 8.55 (d, *J* = 7.5 Hz, 1H), 7.94 – 7.79 (m, 3H), 7.76 – 7.60 (m, 7H), 7.48 – 7.40 (m, 4H), 5.05 – 4.98 (m, 1H), 4.64 (d, *J* = 9.0 Hz, 1H), 4.62 – 4.57 (m, 1H), 4.48 – 4.42 (m, 1H), 3.94 – 3.89 (m, 1H), 3.77 (dd, *J* = 11.0, 4.0 Hz, 1H), 2.93 –

2.87 (m, 2H), 2.50 – 2.35 (m, 7H), 2.23 – 2.17 (m, 1H), 2.00 – 1.94 (m, 1H), 1.79 – 1.59 (m, 6H), 1.50 (d, *J* = 7.1 Hz, 3H), 1.09 – 1.02 (m, 12H). LC-MS (ESI): m/z 824.4 $[M+H]^+$. HRMS *m/z* calcd for $C_{45}H_{58}N_7O_6S$ $[M+H]^+$: 824.4164, found: 824.4154.



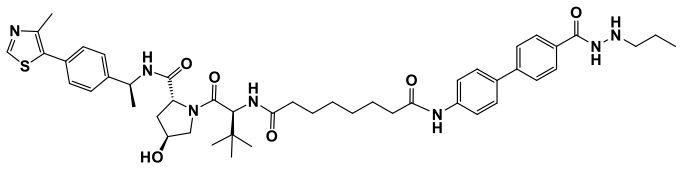
XZ9002 (8c)

***N*[1]-((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-*N*[8]-(4'-(2-propylhydrazine-1-carbonyl)-[1,1'-biphenyl]-4-yl)octanediamide (XZ9002):** Following general method, **XZ9002** was obtained from **14** and **13** (3.6 mg, yield 16%). ^{1}H NMR (600 MHz, CD_3OD) δ 8.88 (s, 1H), 7.90 – 7.81 (m, 3H), 7.76 – 7.64 (m, 6H), 7.46 – 7.41 (m, 4H), 5.04 – 4.97 (m, 1H), 4.68 – 4.60 (m, 2H), 4.48 – 4.43 (m, 1H), 3.95 – 3.88 (m, 1H), 3.77 (dd, *J* = 11.0, 4.0 Hz, 1H), 2.90 (t, *J* = 7.3 Hz, 2H), 2.49 (s, 3H), 2.45 – 2.40 (m, 2H), 2.37 – 2.27 (m, 2H), 2.25 – 2.19 (m, 1H), 2.00 – 1.93 (m, 1H), 1.79 – 1.71 (m, 2H), 1.69 – 1.59 (m, 4H), 1.50 (d, *J* = 7.0 Hz, 3H), 1.47 – 1.38 (m, 4H), 1.08 – 1.02 (m, 12H). ^{13}C NMR (151 MHz, CD_3OD) δ 176.0, 174.7, 173.2, 172.3, 168.6, 152.8, 149.1, 145.7, 145.1, 140.1, 136.6, 133.3, 132.6, 131.5, 130.5, 128.8, 128.4, 127.7, 127.6, 121.5, 71.0, 60.6, 59.1, 58.0, 54.7, 50.1, 38.8, 37.9, 36.5, 36.4, 29.9, 29.8, 27.1, 26.8, 26.7, 22.4, 22.0, 15.8, 11.9. LC-MS (ESI): m/z 852.6 $[M+H]^+$. HRMS *m/z* calcd for $C_{47}H_{62}N_7O_6S$ $[M+H]^+$: 852.4477, found: 852.4506.



8d

***N*[1]-((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-*N*[10]-(4'-(2-propylhydrazine-1-carbonyl)-[1,1'-biphenyl]-4-yl)decanediamide (8d):** Following general method, compound **8d** was obtained from **S6** and **13** (2.9 mg, yield 12%). ^{1}H NMR (600 MHz, CD_3OD) δ 8.89 (s, 1H), 7.91 – 7.86 (m, 2H), 7.82 (d, *J* = 9.0 Hz, 1H), 7.75 – 7.64 (m, 6H), 7.47 – 7.41 (m, 4H), 5.06 – 4.98 (m, 1H), 4.67 – 4.62 (m, 1H), 4.60 – 4.58 (m, 1H), 4.47 – 4.42 (m, 1H), 3.94 – 3.84 (m, 1H), 3.76 (dd, *J* = 11.0, 4.0 Hz, 1H), 2.90 (t, *J* = 7.3 Hz, 2H), 2.49 (s, 3H), 2.44 – 2.40 (m, 2H), 2.34 – 2.24 (m, 2H), 2.23 – 2.18 (m, 1H), 2.00 – 1.93 (m, 1H), 1.78 – 1.71 (m, 2H), 1.65 – 1.57 (m, 4H), 1.52 (d, *J* = 7.1 Hz, 3H), 1.43 – 1.34 (m, 8H), 1.07 – 1.02 (m, 12H). LC-MS (ESI): m/z 880.6 $[M+H]^+$. HRMS *m/z* calcd for $C_{49}H_{66}N_7O_6S$ $[M+H]^+$: 880.4790, found: 880.4809.



XZ9002-NC

***N*^{1}-((*S*)-1-((2*R*,4*S*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-*N*^{8}-(4'-(2-propylhydrazine-1-carbonyl)-[1,1'-biphenyl]-4-yl)octanediamide (XZ9002-NC):** Starting with **S7** and **S8**, compound **S9** was synthesized according to the reported method.[2] Following general method, **XZ9002-NC** was obtained from **S9** and **13** (3.2 mg, yield 16%). ^{1}H NMR (600 MHz, CD_3OD) δ 8.90 (s, 1H), 7.99 – 7.89 (m, 3H), 7.83 – 7.78 (m, 2H), 7.72 – 7.65 (m, 4H), 7.51 – 7.46 (m, 2H), 7.46 – 7.41 (m, 2H), 5.05 – 4.98 (m, 1H), 4.59 – 4.54 (m, 1H), 4.54 – 4.49 (m, 1H), 4.49 – 4.44 (m, 1H), 3.97 (dd, *J* = 10.8, 5.0 Hz, 1H), 3.70 (dd, *J* = 10.7, 3.1 Hz, 1H), 3.29 – 3.23 (m, 2H), 2.49 (s, 3H), 2.39 – 2.20 (m, 5H), 2.16 – 2.09 (m, 1H), 1.85 – 1.77 (m, 2H), 1.71 – 1.59 (m, 4H), 1.45 (d, *J* = 7.0 Hz, 3H), 1.41 – 1.35 (m, 4H), 1.11 – 1.05 (m, 12H). LC-MS (ESI): m/z852.5 $[M+H]^+$. HRMS *m/z* calcd for $C_{47}H_{62}N_7O_6S$ $[M+H]^+$: 852.4477, found: 852.4490.

Docking Studies. The structures of HDAC inhibitors (HDACi) were generated with ChemDraw Professional 17.0. They were converted to 3D structures using Chem3D 17.0. in which they were subjected to energy minimization using the MM2 method. AutoDockTools (ADT) v.4.2.6 was used for ligand and protein (HDAC3 bound to human NCOR2 corepressor, PDB: 4A69)[3] preparation. The charge on zinc was manually modeled to +2. The grid size was set to 25 × 25 × 25 xyz points. The ligands were docked into receptor utilizing AutoDock Vina v.1.1.2.[4] Analyses of the docking results were visualized by PyMOL v.2.2.3.

Biological Methods.

In vitro HDAC activity assay. Purified HDAC1, HDAC2, and HDAC3 (in complex with the deacetylase activation domain of the human NCOR2 (amino acids 395–498)) were obtained from Enzo Life Sciences (HDAC1, catalog # BML-SE456-0050) and BPS Bioscience (HDAC2, catalog # 50002 and HDAC3, catalog # 50003), respectively. The enzyme activities were tested using the HDAC-Glo I/II reagents (Promega) according to the manufacturer's protocol as described previously.[5]

Cell Culture, viability assay, colony formation assay, and Western blotting. Cell lines were obtained from ATCC and subsequently authenticated. Cells were cultured with DMEM supplemented with 10% bovine calf serum, penicillin to 10 units/mL, and streptomycin to 10 µg/mL. For viability assays, 5,000 cells/well were seeded in 96-well plates. Compounds at a specified concentration or DMSO were added 24 h later. Viability assays were performed at 72 h after compound addition using the CellTiter-Glo reagents (Promega). For colony formation assay, cells (~1,000 cells/well) were seeded in a 24-well plate in quadruplicates. At 24 h after seeding, DMSO, XZ9002, or XZ9002-NC were added at a specified concentration. Medium was replaced with freshly prepared medium containing DMSO, XZ9002 or XZ9002-NC every five days until visible colonies appeared, typically in 15 to 20 days after initial cell seeding. Cells were fixed with 4% paraformaldehyde for 15 min and stained with 2% methylene blue in 20% ethanol for 30 min to 1 h. The cells were washed twice with distilled water and dried in air. For western blotting, cell cultures were exposed to compounds as indicated in relevant figures. Medium was removed from culture plate, and 1x passive lysis buffer was then added. The plates were frozen at -80°C overnight and thawed at room temperature. The total cell lysates were mixed with one filth volume of 6x SDS sample buffer, heated at 95°C for 5 min and cooled on ice. The lysates were cleared by centrifugation and then subjected to SDS-PAGE and Western blotting essentially as described.[6] Antibodies used for Western blotting include anti-HDAC3 (Abcam, Ab32369, 1:5,000 dilution), anti-HDAC1 (Sigma-Aldrich, H3284, 1:10,000 dilution), anti-HDAC2 (Santa Cruz Biotechnology, SC-7899, 1:10,000 dilution), anti-HDAC6 (Cell Signaling Technology, 7558, 1:1,000 dilution), anti-histone H3 acetylated at lysine 27 (H3K27ac, Cell Signaling Technology, 8173, 1:10,000 dilution), anti-histone H4 acetylated at lysine 5 (H4K5ac, Abcam, ab51997, 1:10,000 dilution), anti-acetylated lysine (Cell Signaling Technology, 9441, 1:10,000 dilution), anti-α-tubulin (Sigma-Aldrich, T5168, 1:20,000 dilution).

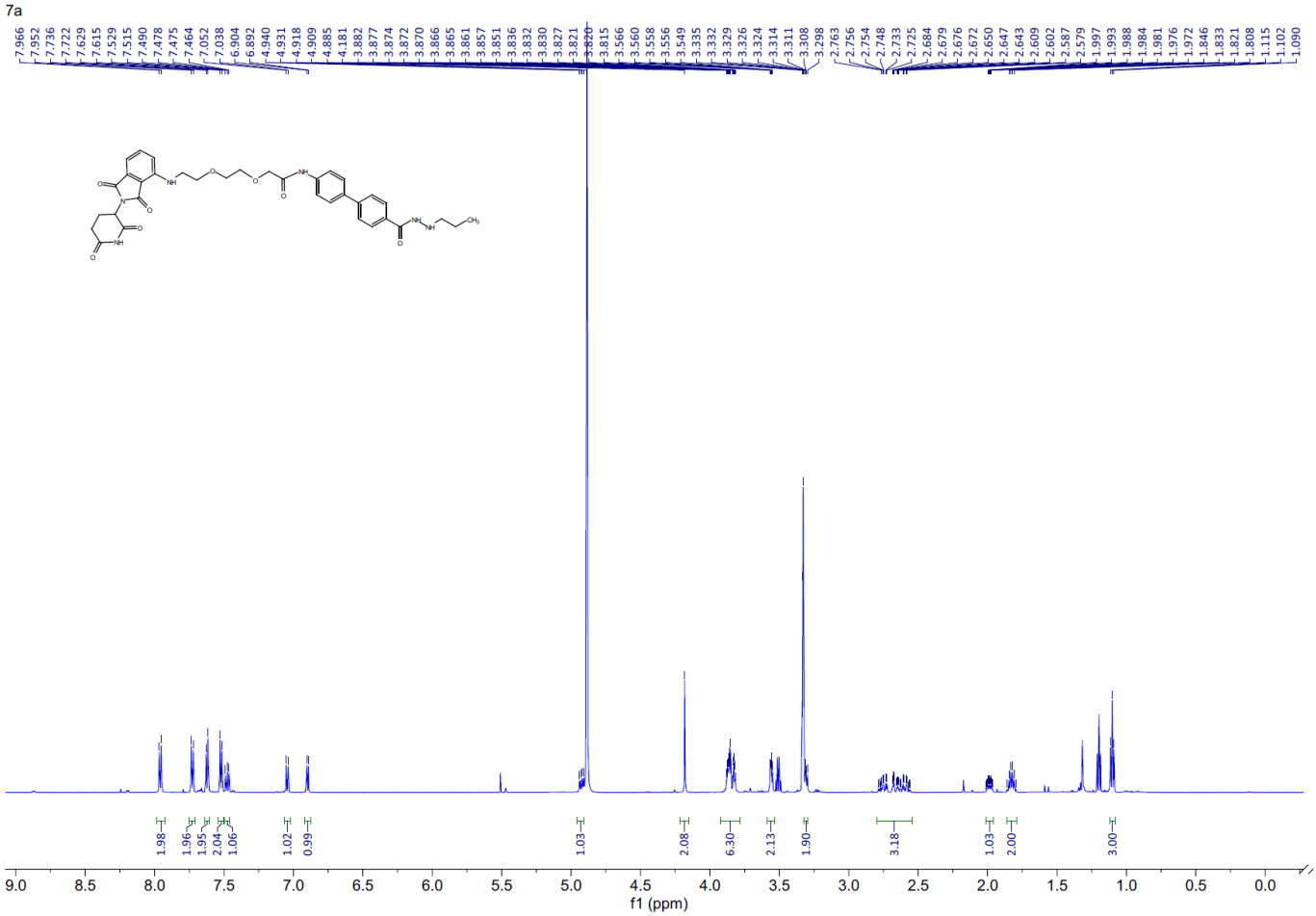


Figure S4. ^{1}H-NMR spectrum of compound **7a**.

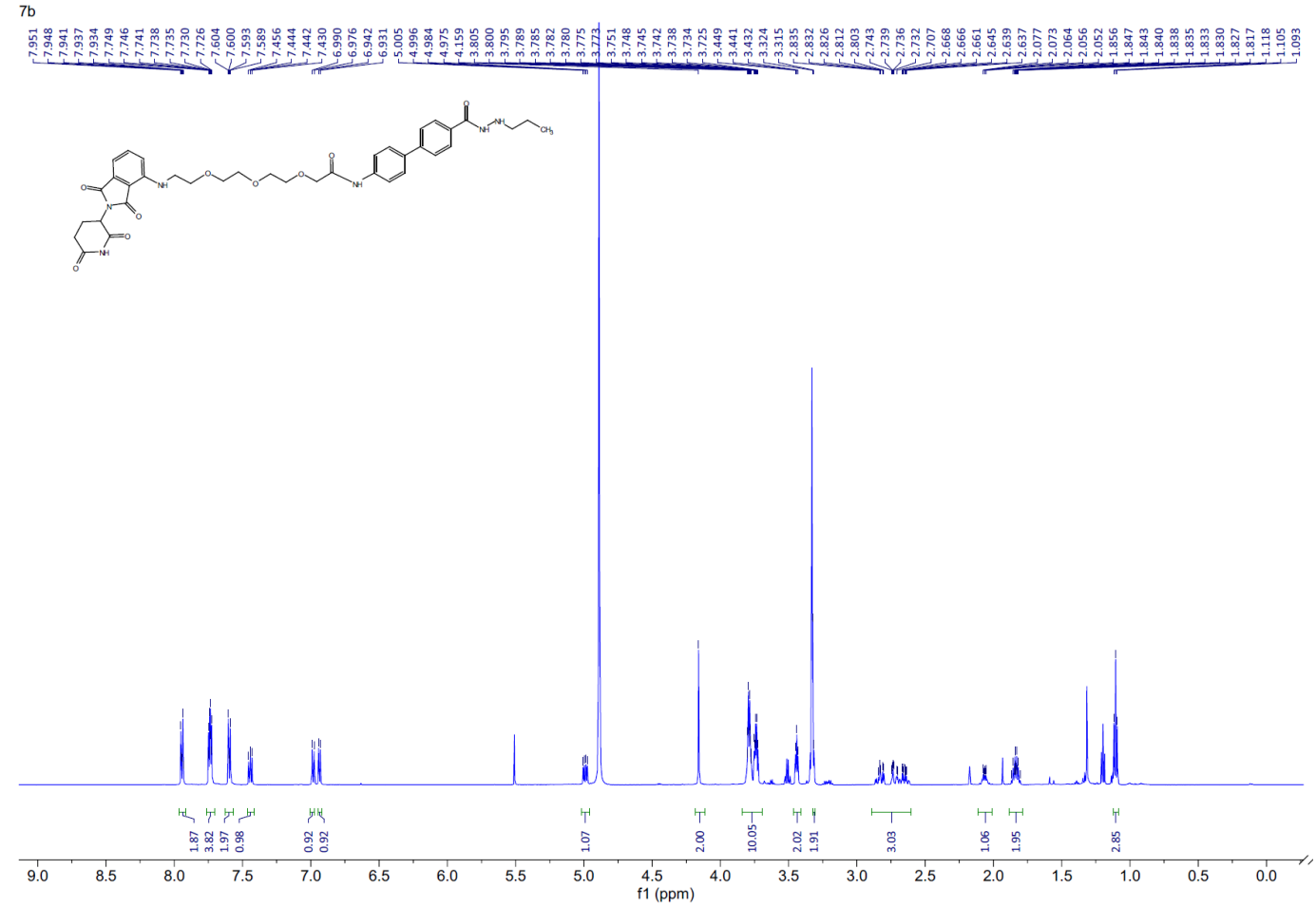


Figure S5. ^{1}H-NMR spectrum of compound **7b**.

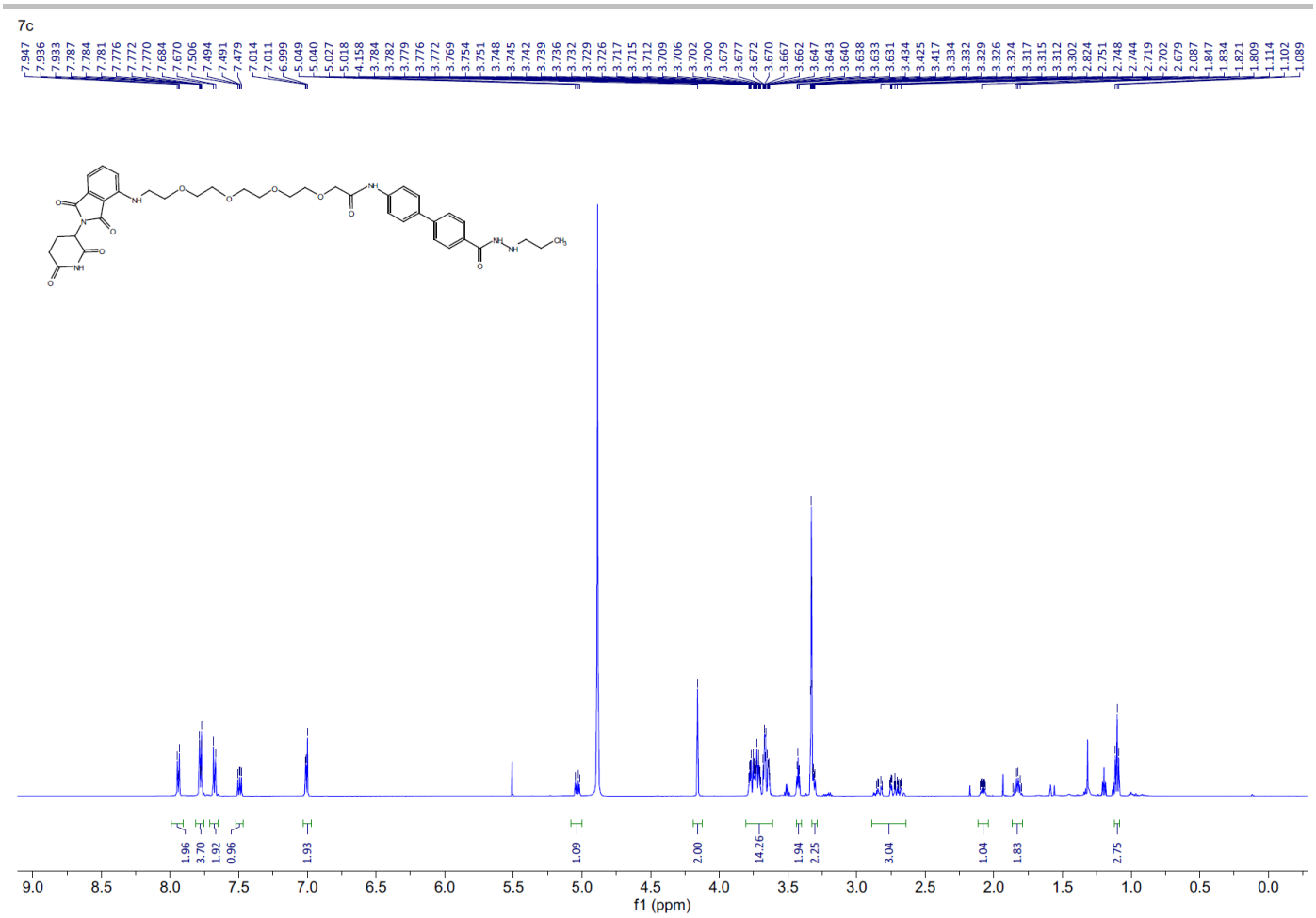


Figure S6. ^{1}H-NMR spectrum of compound **7c**.

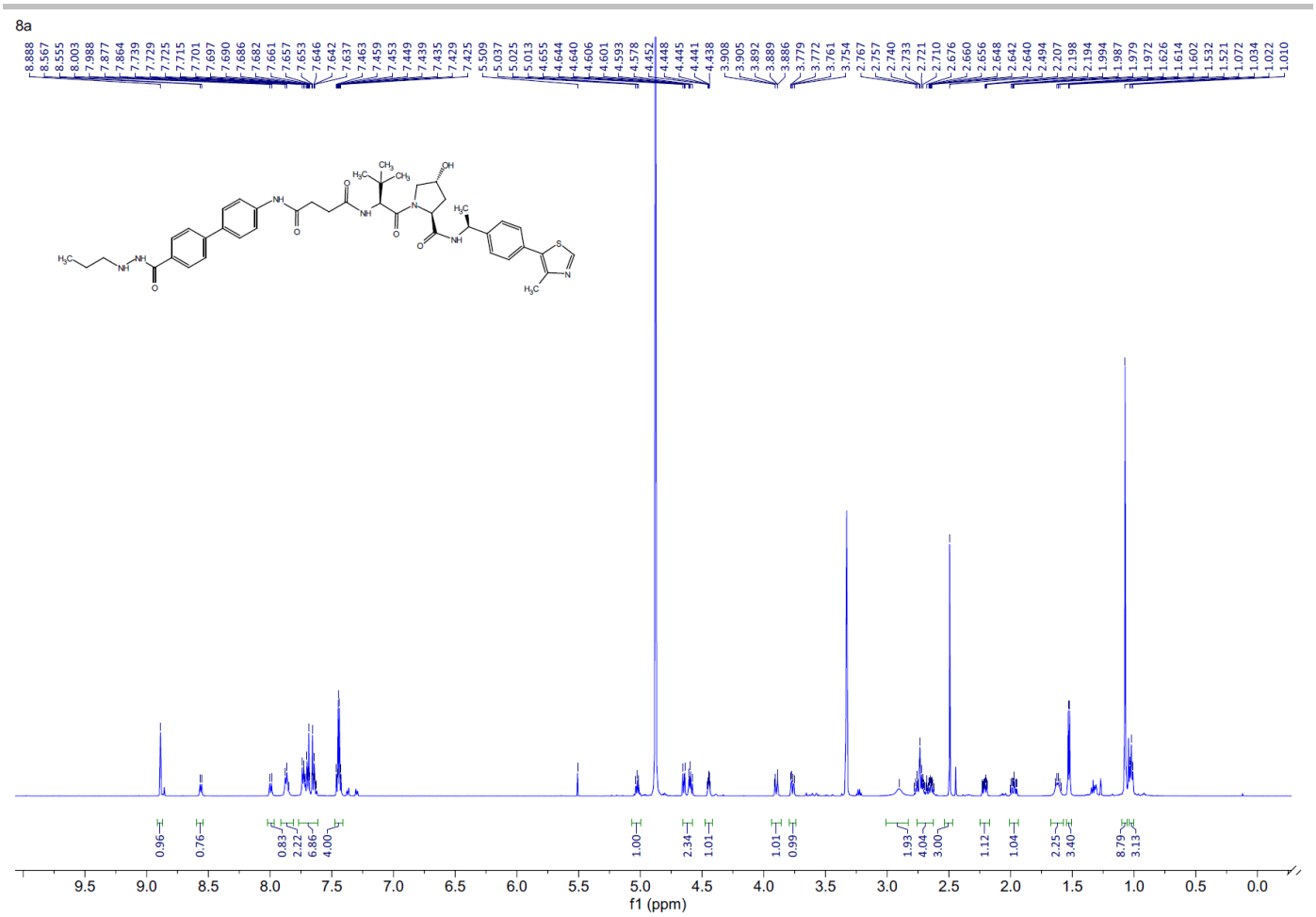


Figure S7. ^{1}H-NMR spectrum of compound **8a**.

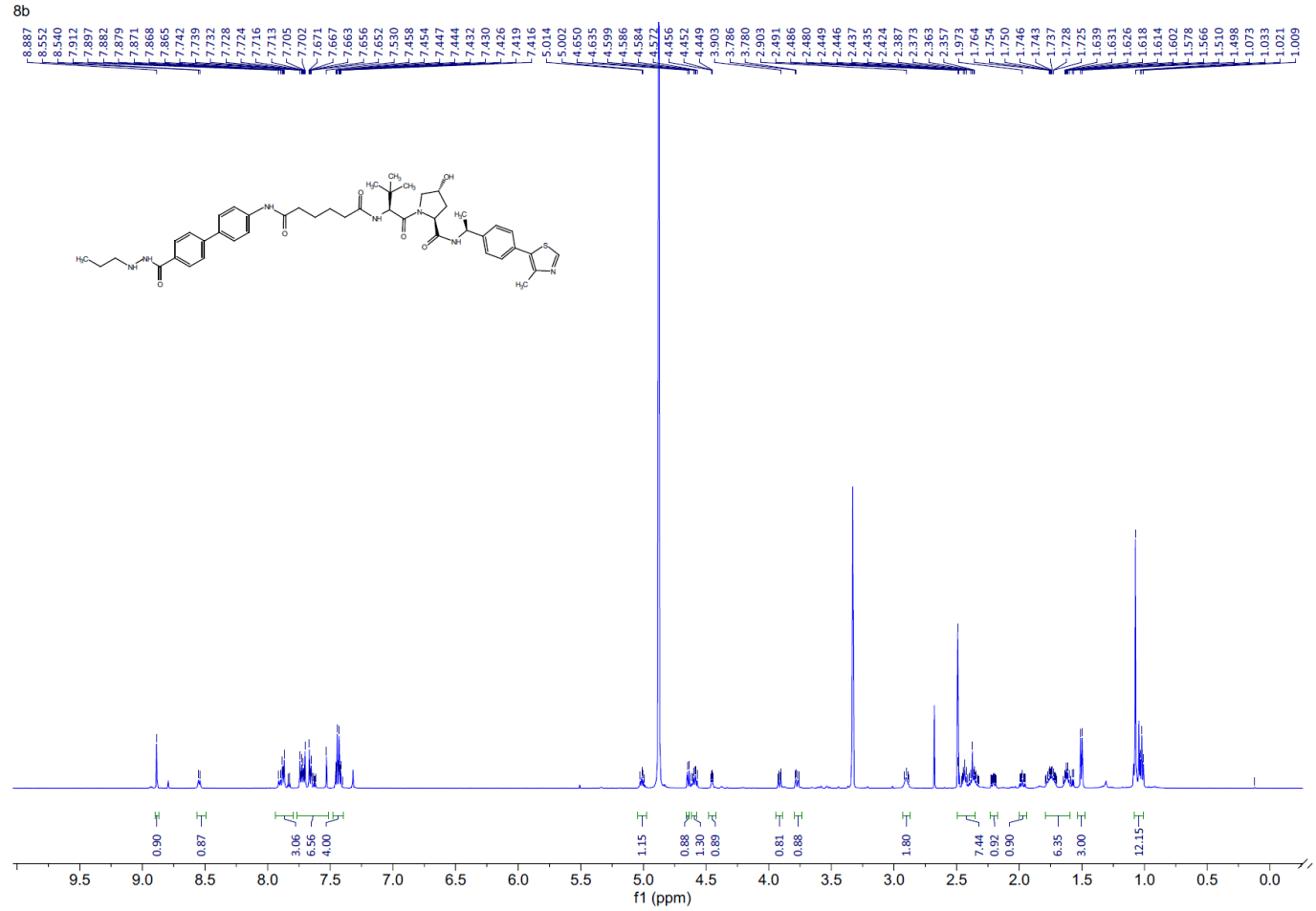


Figure S8. ^{1}H-NMR spectrum of compound **8b**.

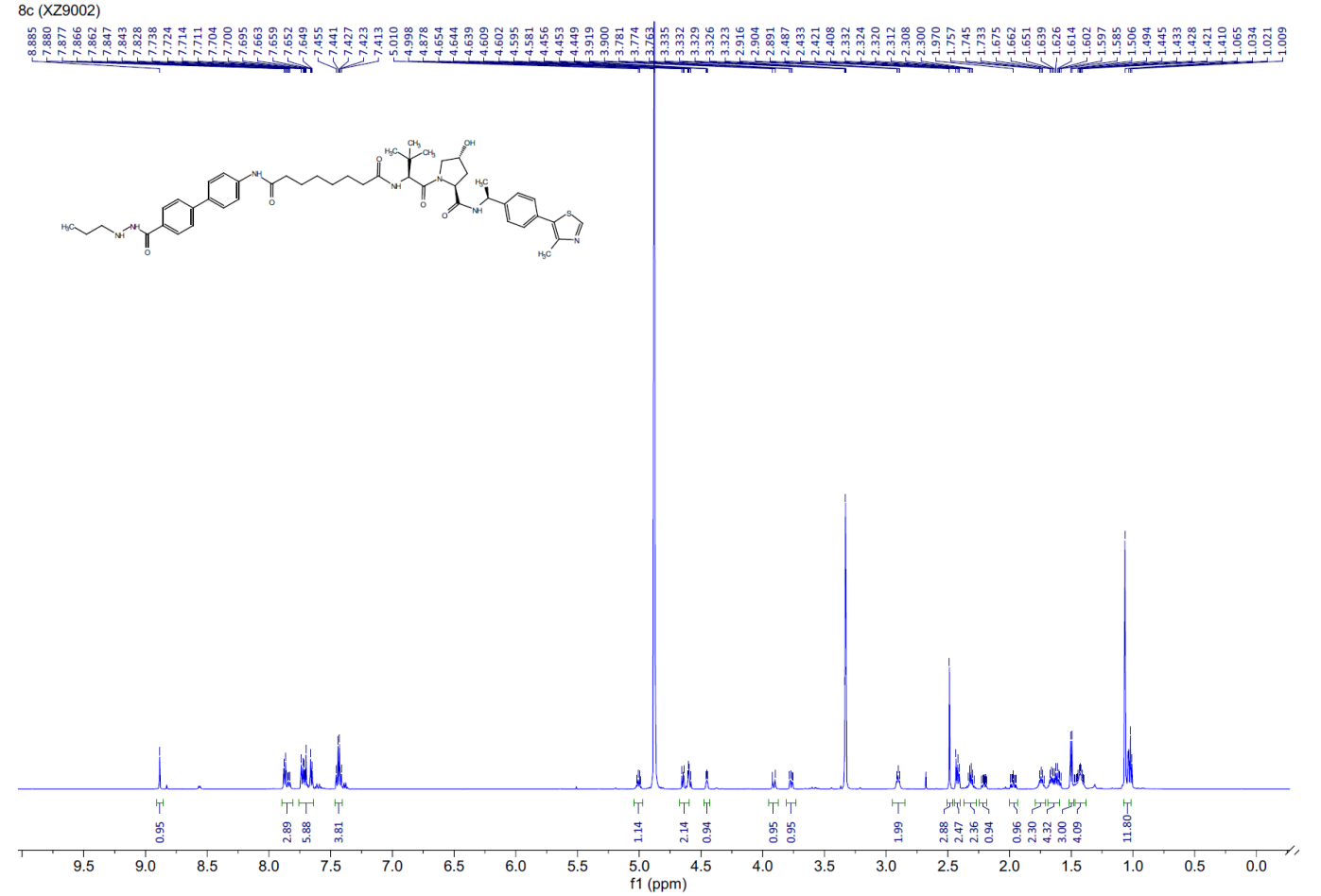


Figure S9. ^{1}H-NMR spectrum of compound **8c** (XZ9002).

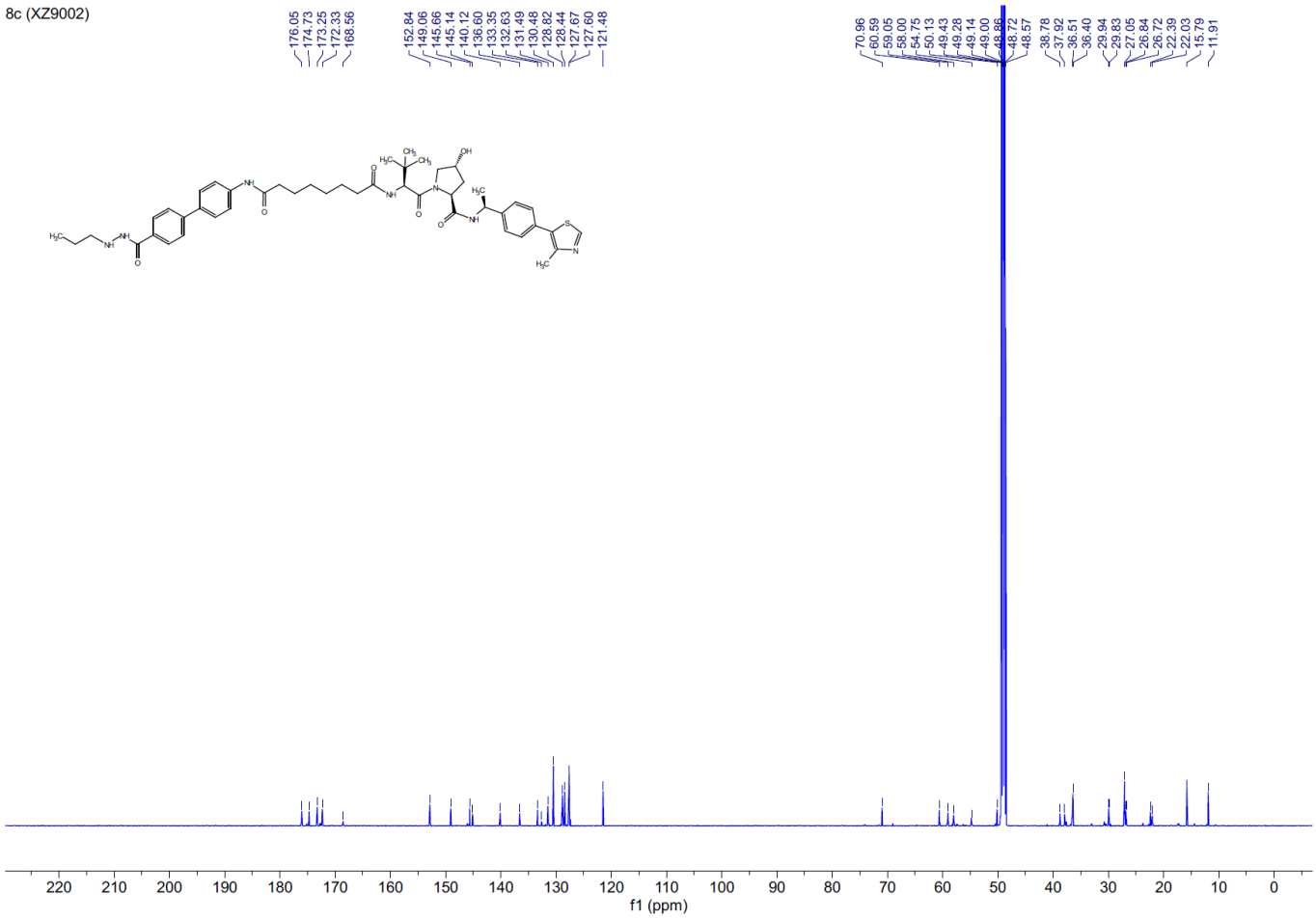


Figure S10. ^{13}C-NMR spectrum of compound **8c** (XZ9002).

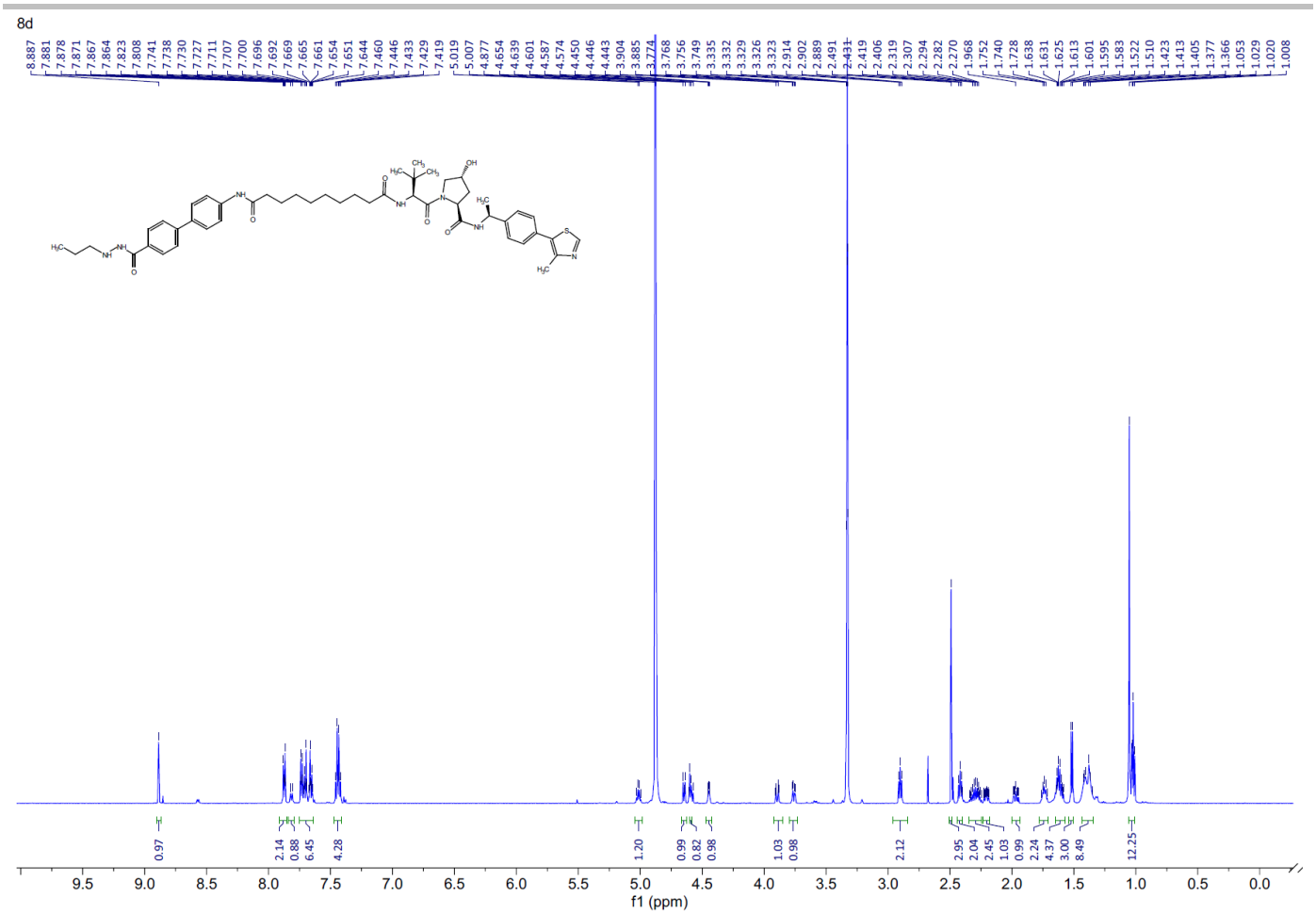


Figure S11. ^{1}H-NMR spectrum of compound **8d**.

References

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