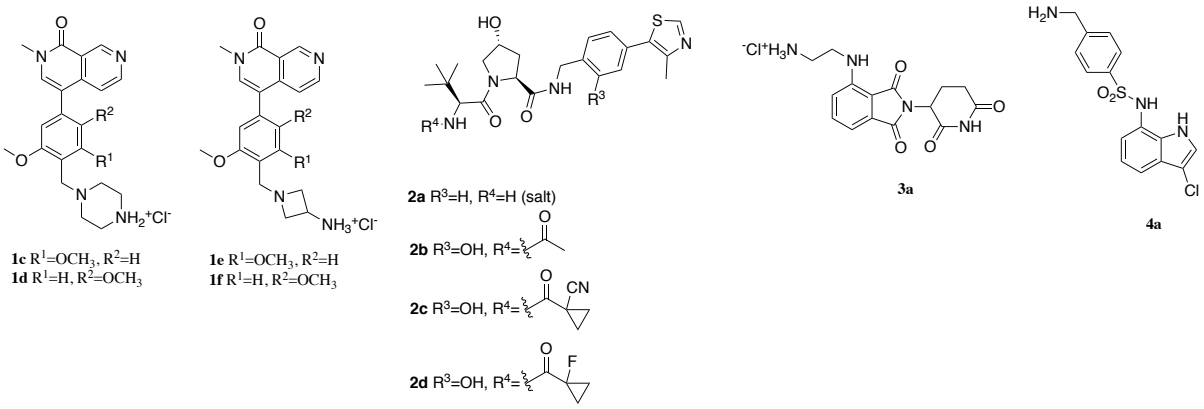
Supporting Information

Iterative design and optimization of initially inactive Proteolysis Targeting Chimeras (PROTACs) identify VZ185 as a potent, fast and selective von Hippel-Lindau (VHL)-based dual degrader probe of BRD9 and BRD7

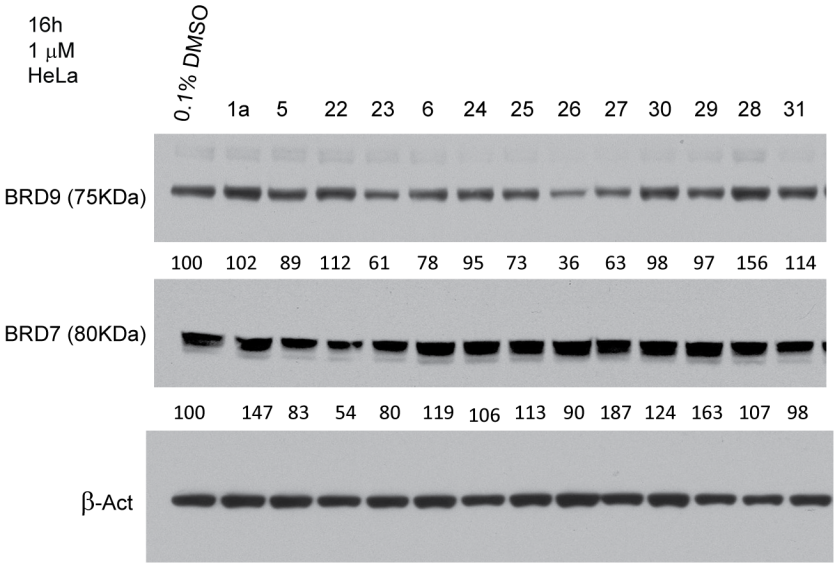
Vittoria Zoppi[1,2], Scott J. Hughes[1], Chiara Maniaci[1,3†], Andrea Testa[1], Teresa Gmaschitz[4], Corinna Wieshofer[4], Manfred Koegl[4], Kristin M. Riching[5], Danette L. Daniels[5], Andrea Spallarossa[2], Alessio Ciulli[1]*

[1] Division of Biological Chemistry and Drug Discovery, School of Life Sciences, James Black Centre, Dow Street, University of Dundee, DD1 5EH, Dundee, United Kingdom
[2] Dipartimento di Farmacia, Sezione di Chimica del Farmaco e del Prodotto Cosmetico, Viale Benedetto XV 3, 16132 Genova, Italy
[3] Medical Research Council Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, James Black Centre, Dow Street, Dundee, Scotland, DD1 5EH, United Kingdom
[4] Boehringer Ingelheim RCV GmbH & Co KG, 1221, Vienna, Austria
[5] Promega Corporation, 2800 Woods Hollow Road, Madison, Wisconsin 53711, United States

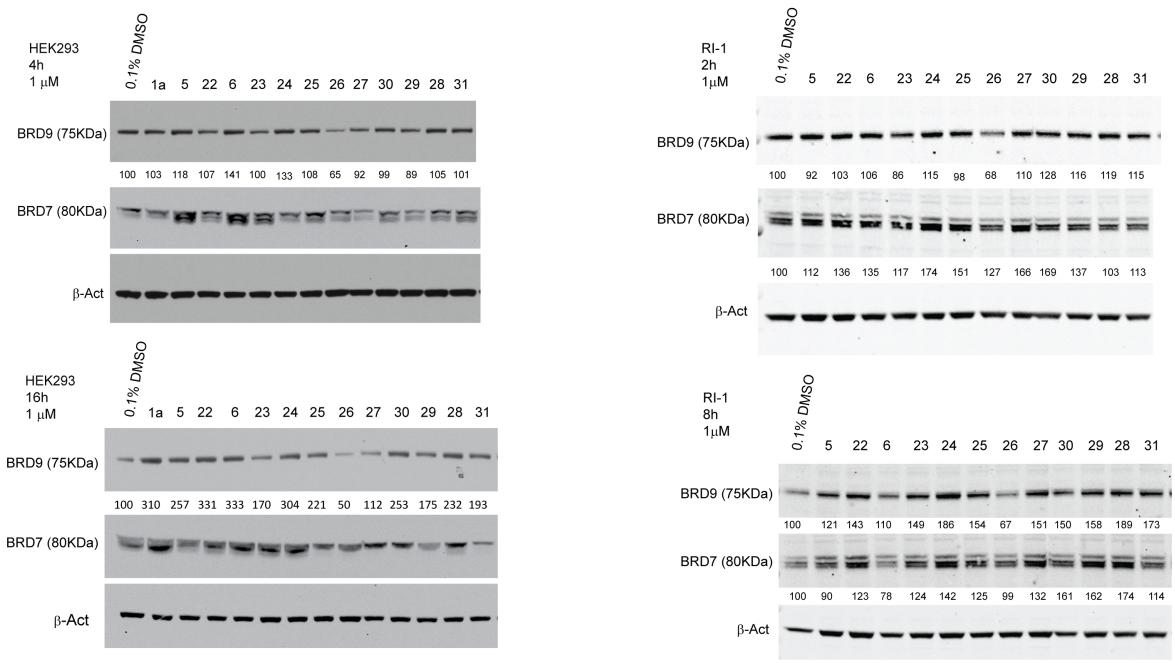
Supporting Figures and Tables



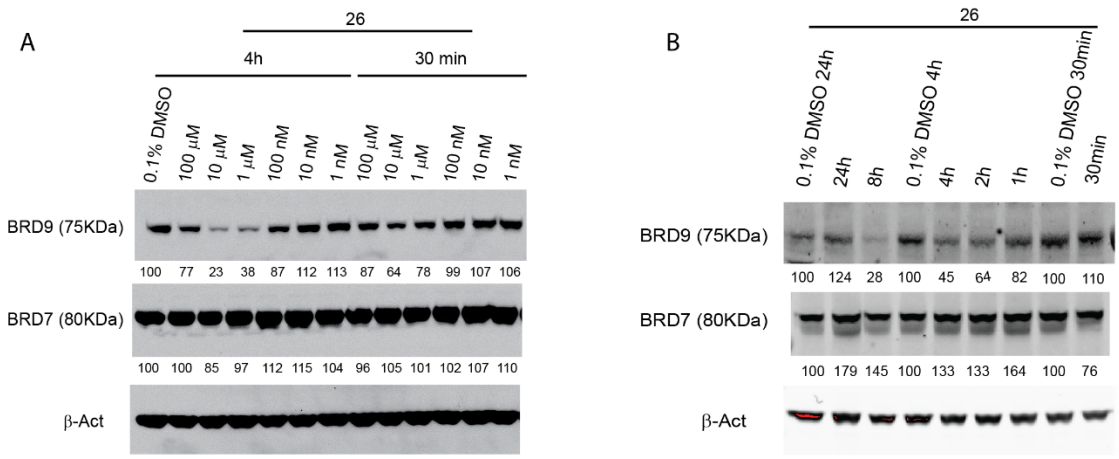
Supporting Figure S1. Chemical structure of **1c-f**, **2a-d**, **3a**, **4a**.



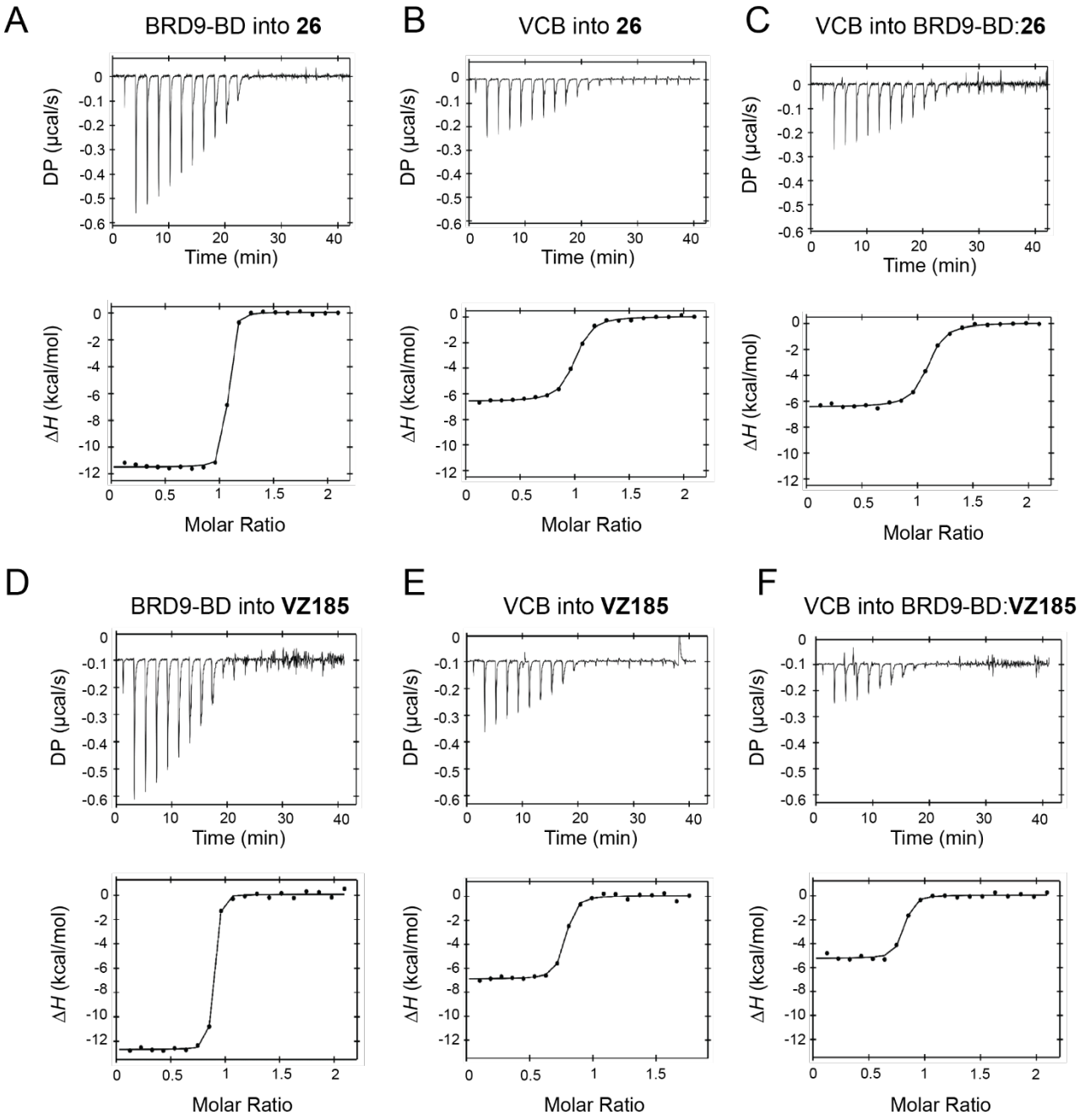
Supporting Figure S2. Screening of compounds **1a**, **5**, **6** and second generation of degraders. Western-blot analysis of BRD9, BRD7 and β-actin after treatment of HeLa cells with 1 μM of compounds at 16 h before harvesting. Intensity values are quantified as described in the experimental section.



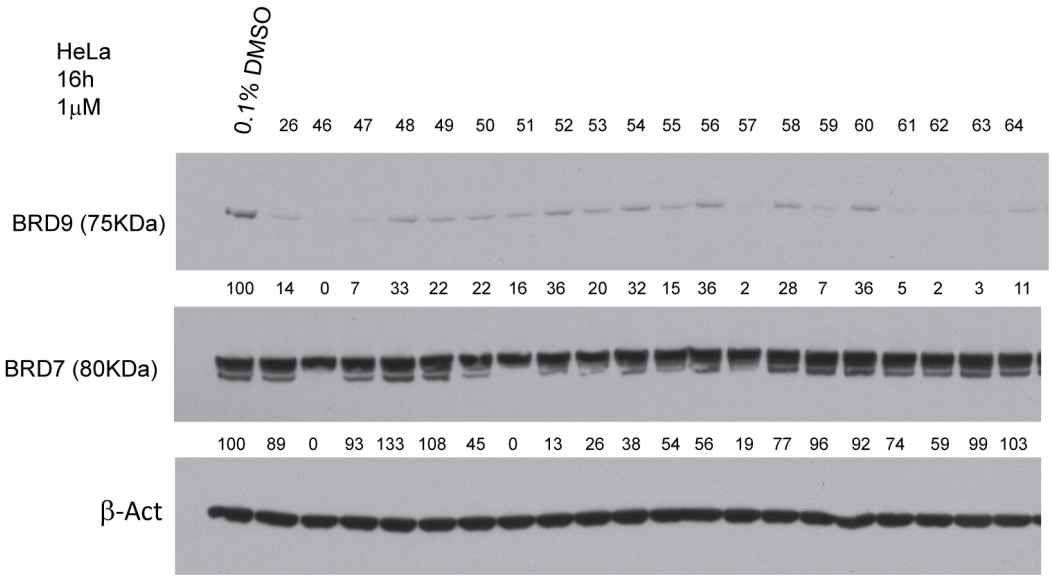
Supporting Figure S3. Screening of compounds **1a**, **5**, **6** and second generation of degraders. Western-blot analysis (on the left) of BRD9, BRD7 and β-actin after treatment of Hek293 cells with 1 μM of compounds at 4 h and 16 h before harvesting. Western-blot analysis (on the right) of BRD9, BRD7 and β-actin after treatment of RI-1 cells with 1 μM of compounds at 2 h and 8 h before harvesting. Intensity values are quantified as described in the experimental section.



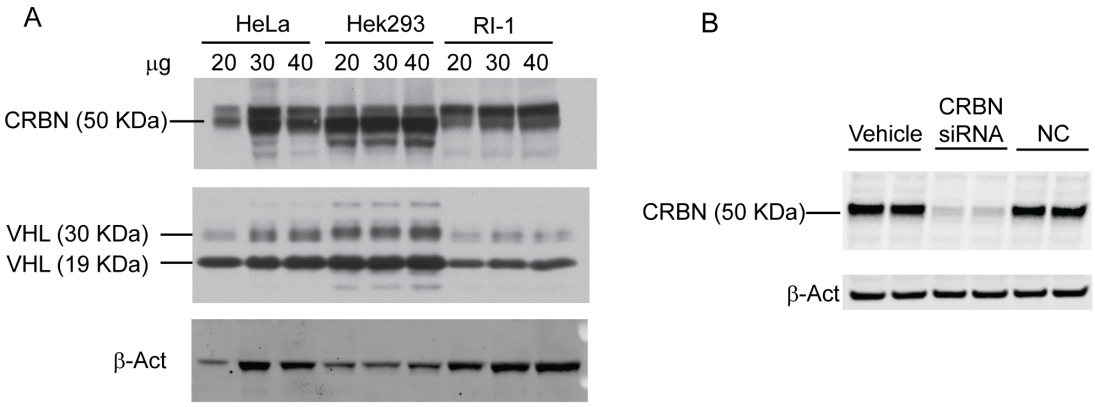
Supporting Figure S4. A) Concentration-dependency experiment of compound **26**; Western-blot analysis of BRD9, BRD7 and β-actin after treatment of HeLa cells with six different concentrations at 4 h and 30 min before harvesting. B) Time-dependency experiment of compound **26**. Western-blot analysis of BRD9, BRD7 and β-actin after treatment of HeLa cells with 1 μM of compound at different time points before harvesting. Intensity values are quantified as described in the experimental section.



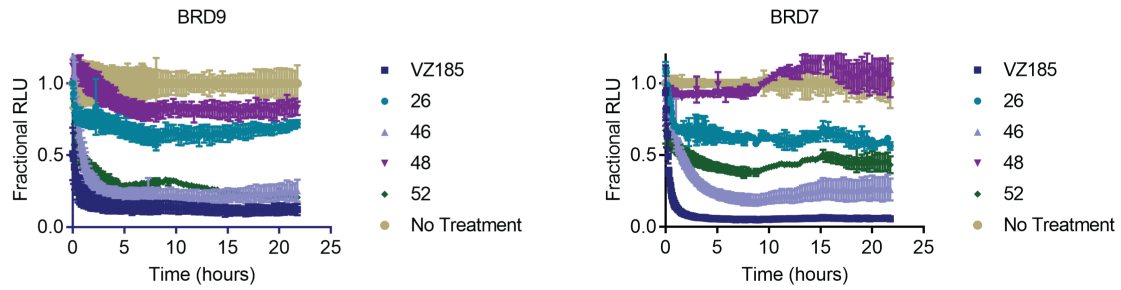
Supporting Figure S5. Measuring cooperativities for compound **26** and **VZ185** by isothermal titration calorimetry; A) BRD9-BD into **26** alone; B) VCB into **26** alone; C) VCB into BRD9-BD:**26** binary complex; D) BRD9-BD into **VZ185** alone; E) VCB into **VZ185** alone; F) VCB into BRD9-BD:**VZ185** binary complex.



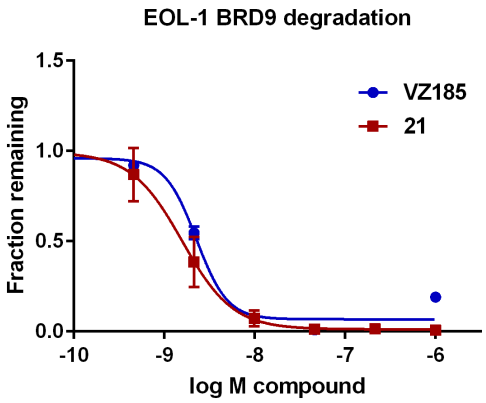
Supporting Figure S6. Screening of compound **26** and third generation of degraders. Western-blot analysis of BRD9, BRD7 and β-actin after treatment of HeLa cells with 1 μM of compounds at 16 h before harvesting. Intensity values are quantified as described in the experimental section.



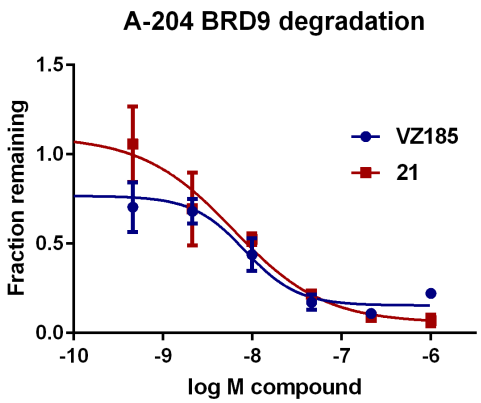
Supporting Figure S7. A) Western-blot analysis of CRBN and VHL expression protein levels in HeLa, Hek293 and RI-1 cells. 20, 30 and 40 µg of soluble protein fraction was tested for each cell line. B) Western-blot analysis of CRBN and β-Actin after transfection of HeLa cells with 20 nM of siRNA targeting CRBN, negative control (N.C.) siRNA, and vehicle control (1 x siRNA buffer) for 48 h. The bands of the VHL antibody were siRNA validated as shown in Maniaci et al.[1]



Supporting Figure S8. Degradation profile across of concentration series indicated compound using continual luminescent reading of CRISRP/Cas9 endogenously tagged HiBiT-BRD7 or HiBiT-BRD9 in HEK293 cells. Error bars are expressed as SEM taken from n=3 experiments.

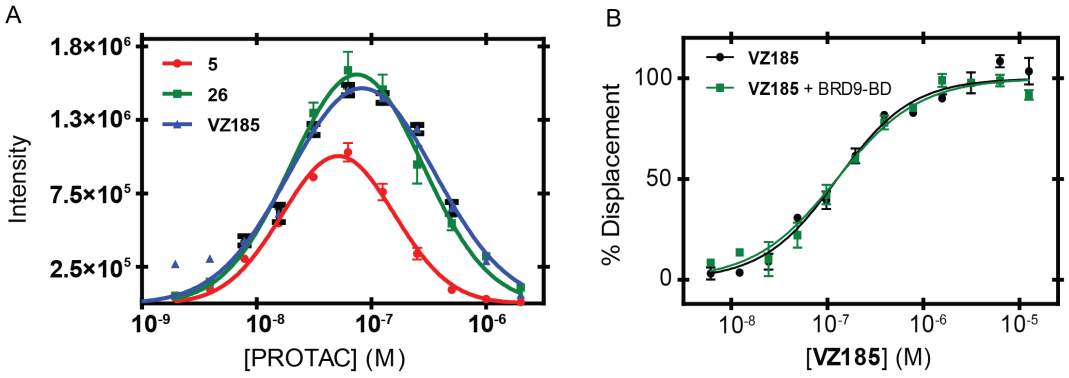


	21	VZ185
IC50	1.573e-009	2.259e-009

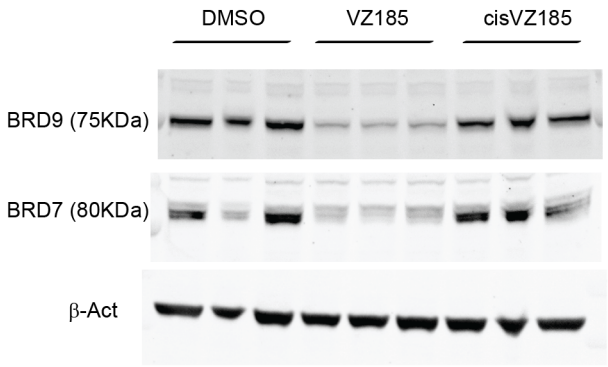


	VZ185	21
IC50	8.314e-009	6.117e-009

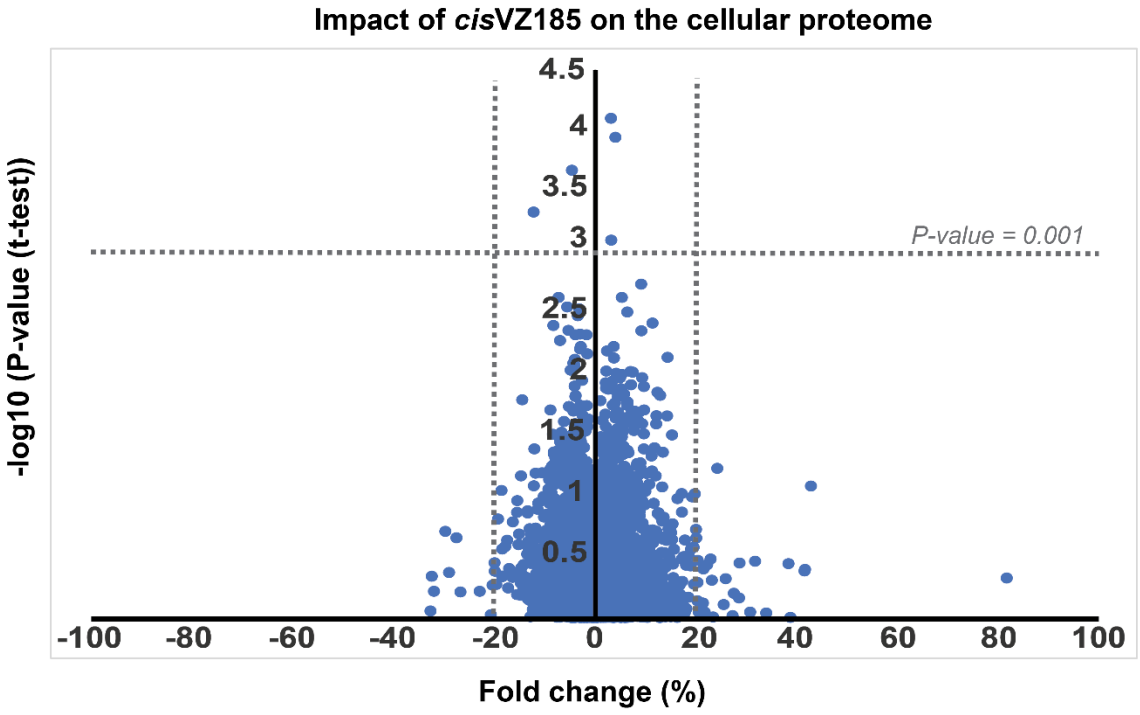
Supporting Figure S9. Concentration-dependency evaluation of **VZ185** and **dBRD9** activity in EOL-1and A-204 cells. After 18 h treatment with the desired compound protein levels were determined on a WES capillary electrophoresis instrument (Proteinsimple) using a BRD9 antibody (Bethyl A303-781A) and a GAPDH antibody (Abcam #ab9485) for normalization.



Supporting Figure S10. Measuring cooperativities and ternary complex formation for **VZ185**. (A) AlphaLISA assay showing the relative amount of ternary complex formed by **5**, **26**, and **VZ185**; (B) Fluorescence polarization data showing the absence of cooperativity with **VZ185**.

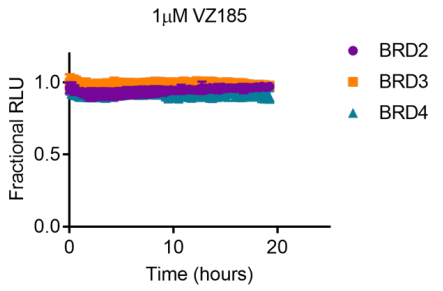


Supporting Figure S11. Proteomics experiments. Western-blot analysis of BRD9, BRD7 and β-actin after treatment of RI-1 cells in three replicates with **VZ185** (100 nM), ***cis*VZ185** (100 nM) and DMSO as control for 4 h before harvesting.

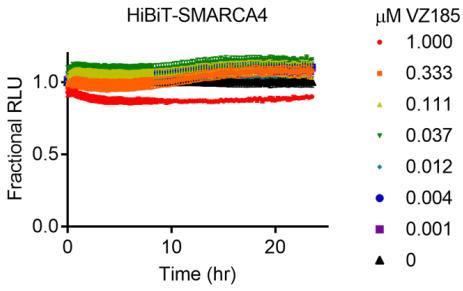


Supporting Figure S12. Impact of ***cis*VZ185** (100 nM for 4 h) on the cellular proteome. Data plotted as fold change (%) versus -log10 of P-value (t-test) for a total of 6273 proteins. For quantification see experimental section.

A



B



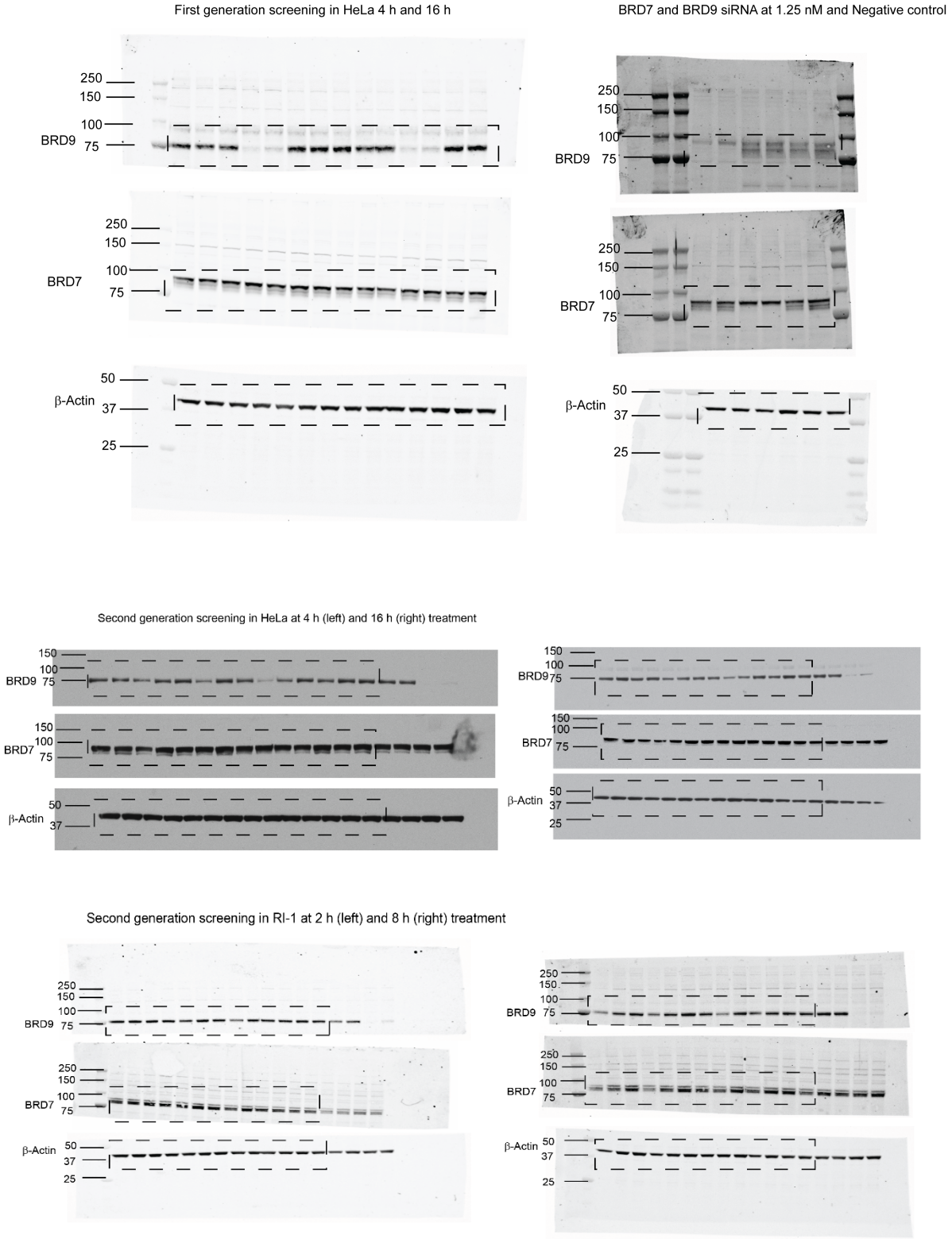
C

	Guide Sequence	Donor Sequence
Nterm SMARCA-4	gtctggagtggacatcttca	ctcgcccttggtcatgaaccccagactgaccaggactgtcttccagcaggaggccactgtctgcagctcccgtgaagatgGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCtccactccagacccaccccctgggcggaactcctcggccaggtccttccccgggccctggcccttcccctggagccatgct

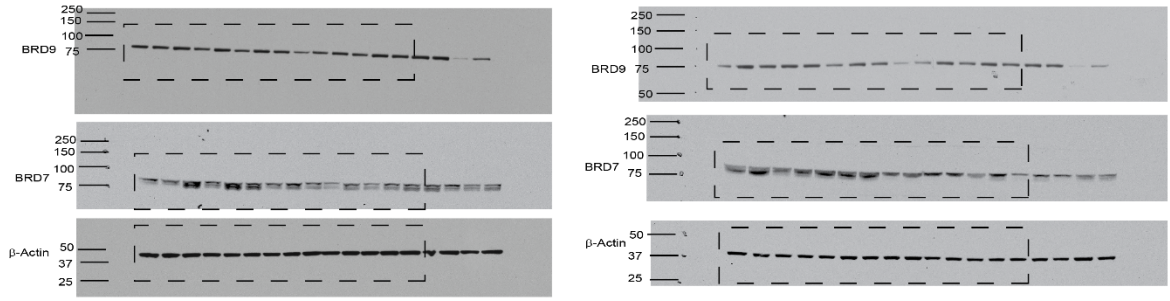
Supporting Figure S13. Live cell kinetic analysis of endogenously-tagged BRD2/3/4 and SMARCA4 proteins in HEK293 cells expressing LgBiT. A) Protein level profile of HiBiT-BRD2, HiBiT-BRD3, and HiBiT-BRD4 after treatment VZ185 (1 μM) using continual luminescent reading for 24 hours. B) Protein level profile of HiBiT-SMARCA4 after treatment of a concentration series of VZ185 using continual luminescent reading for 24 hours. Error bars are expressed as SD of the mean (n=3) of a representative experiment. C) Sequences of guide and donors (HiBiT sequence in red) used for HiBiT-SMARCA4 knock-in via CRISPR/Cas9. For HiBiT-BRD2, HiBiT-BRD3, and HiBiT-BRD4 see ref. [2]

Supporting Figure S14

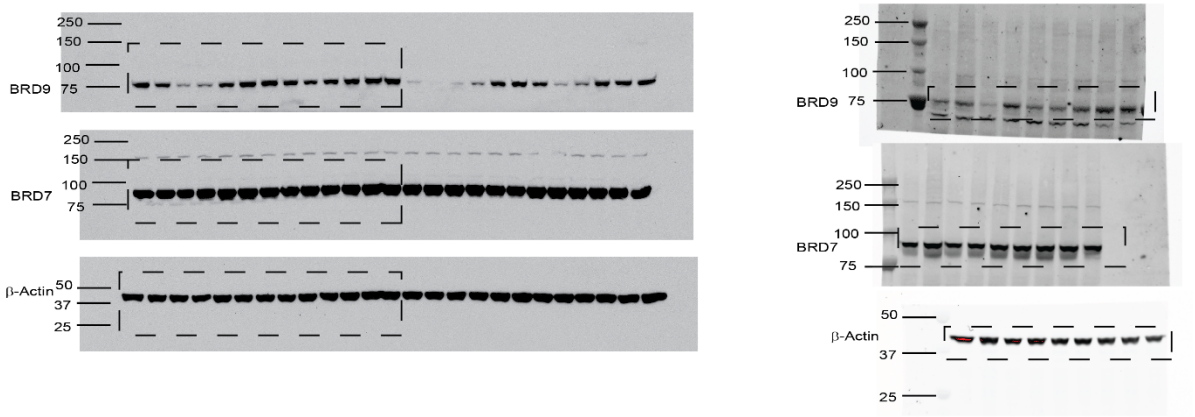
Original blots



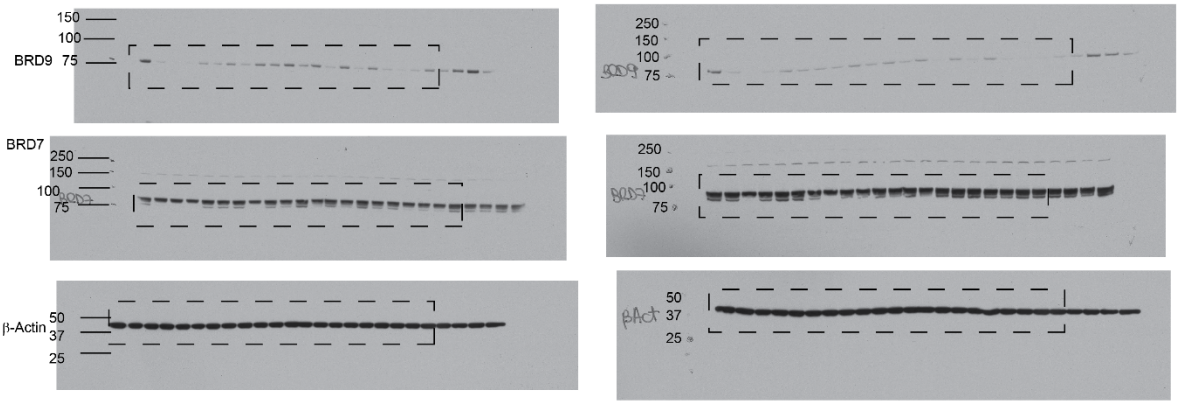
Second generation screening in Hek293 at 4 h (left) and 16 h (right) treatment



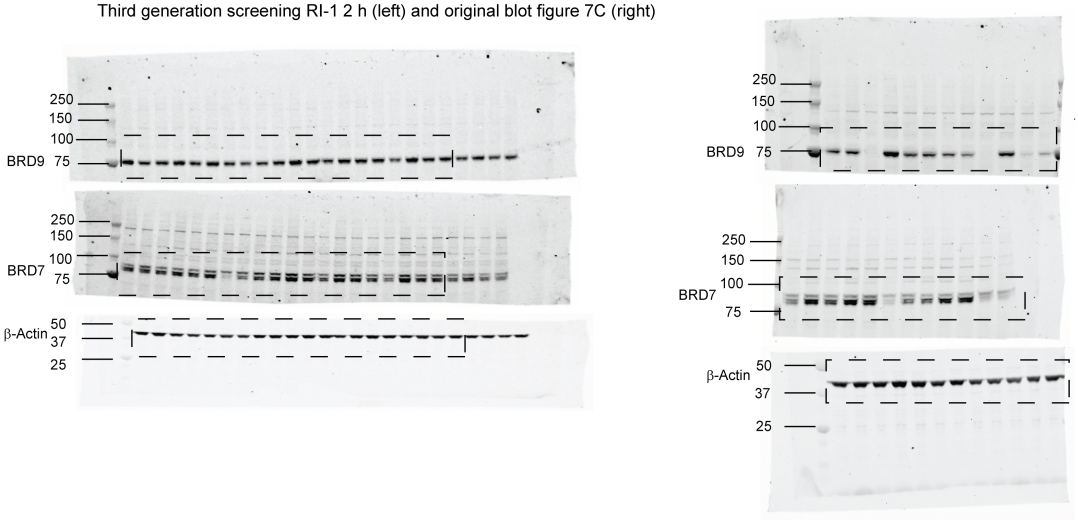
Concentration- (left) and time-dependency (right) experiment of **26** in HeLa



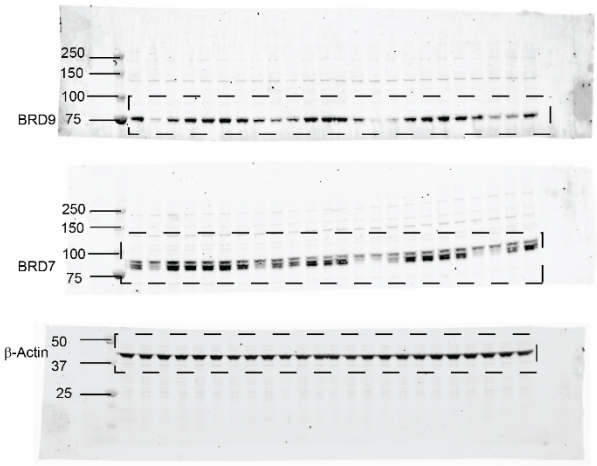
Third generation screening HeLa 4 h (left) and 16 h (right)



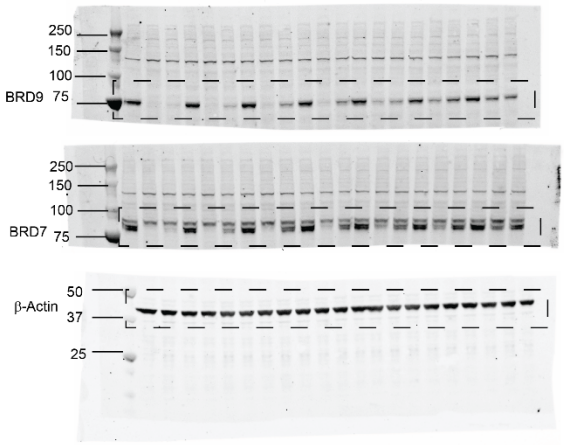
Third generation screening RI-1 2 h (left) and original blot figure 7C (right)



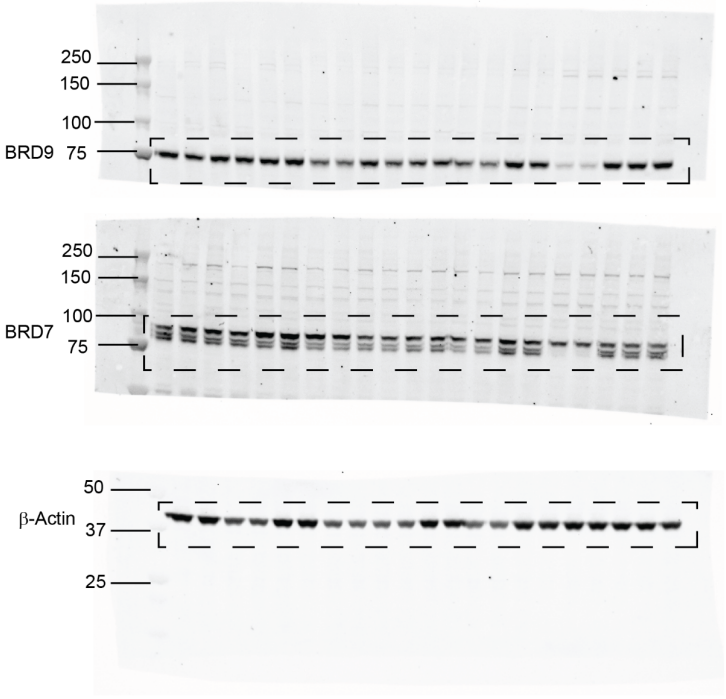
Concentration-dependency evaluation of **46** and **VZ185** in RI-1



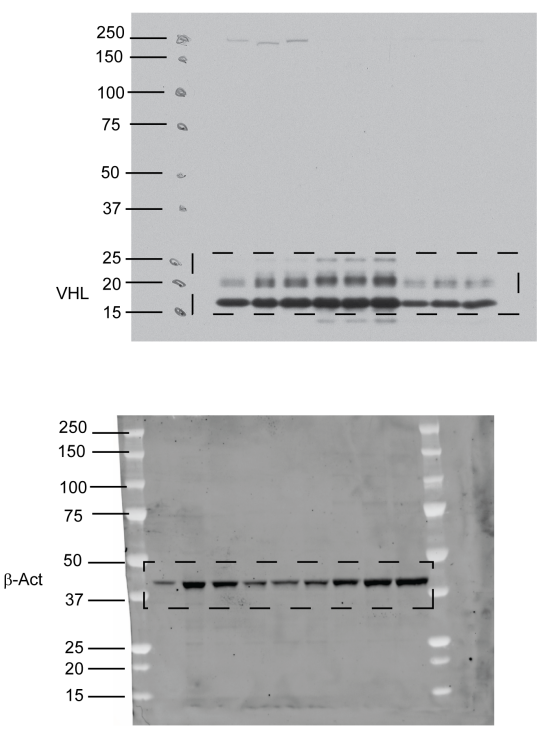
Time-dependency evaluation of **VZ185** in RI-1

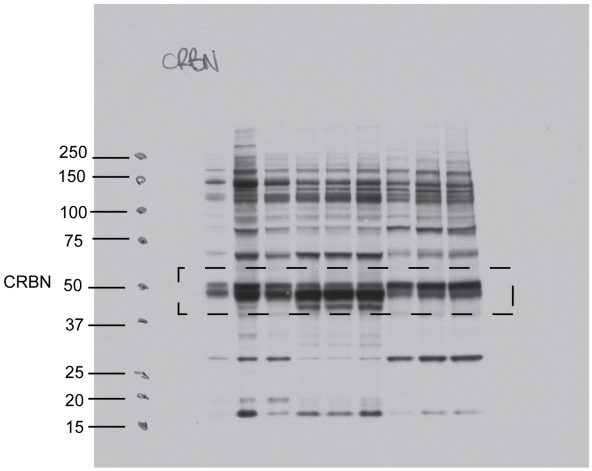


Mechanistic evaluation of VZ185



VHL and CRBN protein levels in HeLa, Hek293 and RI-1 cells





CRBN siRNA in HeLa

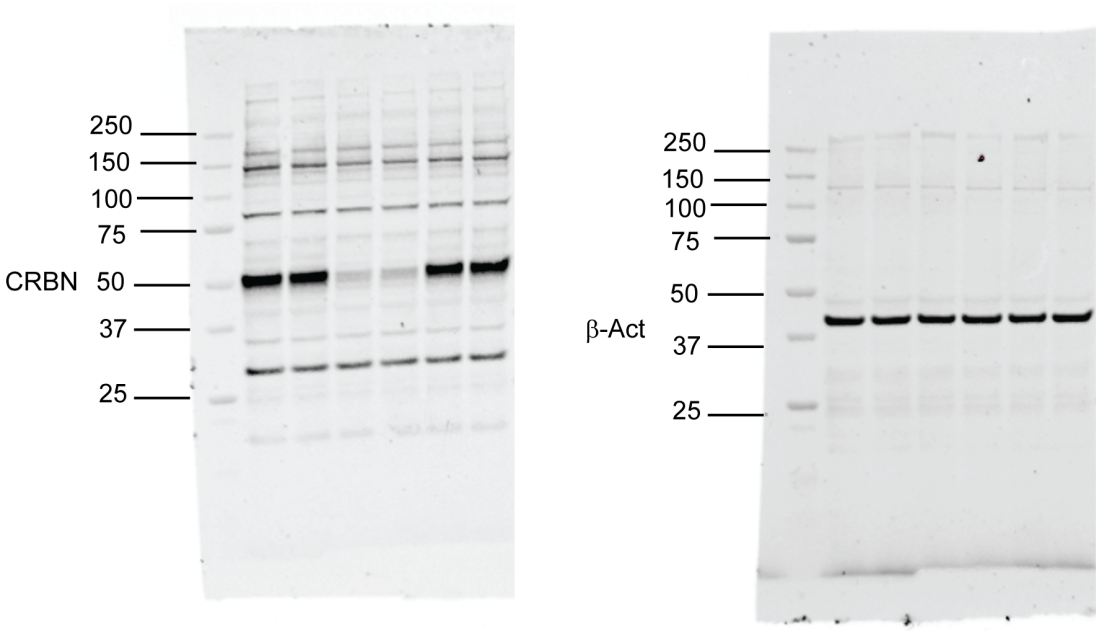


Table S1. Thermodynamic parameters of formation of binary and ternary complexes between VCB, BRD9 bromodomains and BRD9 degraders measured by isothermal titration calorimetry

Syringe	Cell	N	Kd (nM)	ΔH (kcal/mol)	ΔG (kcal/mol)	–TΔS (kcal/mol)	α	# rep
BRD9-BD	**26**	1.00 ± 0.03	8.8 ± 0.9	–11.4 ± 0.1	–11.0 ± 0.1	0.44 ± 0.05		2
	VZ185	0.77 ± 0.07	5.1 ± 0.6	–12.8 ± 0.1	–11.35 ± 0.05	1.485 ± 0.005		2
VCB	**26**	0.99 ± 0.03	87 ± 5	–6.56 ± 0.09	–9.64 ± 0.03	–3.1 ± 0.1		2
	VZ185	0.73 ± 0.01	26 ± 9	–7.04 ± 0.08	–10.4 ± 0.2	–3.4 ± 0.1		2
	Brd9-BD:**26**	0.99 ± 0.05	83 ± 2	–6.4 ± 0.2	–9.7 ± 0.2	–3.3 ± 0.2	1.05	2
	Brd9-BD:**VZ185**	0.73 ± 0.2	27 ± 2	–5.1 ± 0.1	–10.33 ± 0.08	–5.3 ± 0.1	0.97	3

Table S2. Crystallographic data collection and refinement statistics.

	BRD9-BD:**5** (6HM0)
Data collection	
Space group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	38.8 59.8 60.3
α, β, γ (°)	90, 108.8, 90
Resolution (Å)	41.3 – 2.40 (2.49–2.40)*
No. unique reflections	10196 (1066)
R_{merge}	8.4 (15.7)
$I/\sigma(I)$	18.9 (13.1)
$CC_{1/2}$	99.5 (98.0)
Completeness (%)	98.5 (99.9)
Redundancy	4.2 (4.3)
Refinement	
Resolution (Å)	41.3 – 2.4
R_{work} (%)	21.6
R_{free}	25.1
No. atoms	
Protein	1826
Ligand	66
Water	68
B factors	
Protein	23.2
Ligand	21.8
Water	23.4
R.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.202

PK measurements

Methods

Plasma stability

Test compound (10 μM) was incubated in pre-warmed plasma at 37 °C (that is buffered to pH 7.4 in ratio of 70:30 plasma to buffer). Immediately, at time zero, then at 30, 60, 120, and 180 min, a 50 μL aliquot of the incubation mixture was removed and mixed with 200 μL acetonitrile containing Donepezil as the internal standard (50 ng/ml) to stop the reaction. The samples were centrifuged to sediment the precipitated protein and the plates then sealed prior to UPLC-MS/MS analysis using a Xevo TQ-S Micro (Waters Corporation, USA).

XLfit (IDBS, UK) was used to calculate the exponential decay and consequently the rate constant (k) from the ratio of peak area of test compound to internal standard at each time point. The half-life was calculated for each test compound from the rate by using the following calculation:

$$t_{1/2} = 0.693/k$$

Parallel Artificial Membrane Permeability Assay (PAMPA)

PAMPA was performed using a 96-well pre-coated BD Gentest™ PAMPA plate (BD Biosciences, U.K.). Each well was divided into two chambers; donor and acceptor, separated by a lipid-oil-lipid tri-layer constructed in a porous filter. The effective permeability, P_e, of the compound was measured at pH 7.4. Stock solutions (5 mM) of the compound were prepared in DMSO. The compound was then further diluted to 10 μM in phosphate buffered saline at pH 7.4. The final DMSO concentration did not exceed 5% v/v. The compound dissolved in phosphate buffered saline was then added to the donor side of the membrane and phosphate buffered saline without compound was added to the acceptor side. The PAMPA plate was left at room temperature for 5 h. After which time, an aliquot (100 μL) was then

removed from both acceptor and donor compartments and mixed with acetonitrile (80 μL) containing an internal standard. The samples were centrifuged (10 min, 5 ºC, 3270 *g*) to sediment precipitated protein and sealed prior to UPLC-MS/MS analysis using a Xevo TQ-S Micro (Waters Corp, USA). P_e was calculated as shown in the below equation:

$$P_e \text{ (nm s}^{-1}) = \frac{10^7 \times -\ln\left[1 - C_A\,(t) \,/\, C_{equi}\right]}{A \times \left(\frac{1}{V_D} + \frac{1}{V_A}\right) \times t}$$

Where:

$C_A(t)$ = peak area of compound present in acceptor well at time t = 18000 s

$C_{equi} = [C_D(t) \times V_D + C_{A(t)} \times V_A] / (V_D + V_A)$

A = filter area

V_D = donor well volume

V_A = acceptor well volume

t = incubation time

$C_D(t)$ = peak area of compound present in donor well at time t = 18000 s

Recovery of compound from donor and acceptor wells was calculated and data was only accepted when recovery exceeded 70%.

CHI Log D determination

Using a fast gradient, reverse-phase HPLC method to determine the chromatographic hydrophobicity index (CHI) was determined as described by Camurri and Zaramella (ref. [3]) and Valko *et al*. (ref. [4]). Briefly, by plotting the retention time of a set of reference

compounds against known CHI values. The CHI value of test compounds was then calculated according to their retention time.

Test compounds were prepared as 0.25 mM solutions in 50:50 acetonitrile:water and analysed by reversed-phase HPLC-UV (wavelength 254 nm) using a ACE Excel 3 SuperC18, 3 μm, 50 x 2.1 mm column with a gradient of aqueous phase (10 mM ammonium acetate, pH 7.4) and mobile phase (acetonitrile).

Intrinsic clearance (Cli) experiments

Test compound (0.5 μM) was incubated with female CD1 mouse or human (mixed gender) liver microsomes (Xenotech LLC™; 0.5 mg/mL 50mM potassium phosphate buffer, pH7.4) and the reaction initiated with addition of excess NADPH (8 mg/mL 50 mM potassium phosphate buffer, pH7.4). Immediately, at time zero, then at 3, 6, 9, 15 and 30 minutes an aliquot (50 μL) of the incubation mixture was removed and mixed with acetonitrile (100 μL) to stop the reaction. Internal standard was added to all samples, the samples centrifuged to sediment precipitated protein and the plates then sealed prior to UPLCMSMS analysis using a Xevo TQ-S Micro (Waters corporation, USA).

XLfit (IDBS, UK) was used to calculate the exponential decay and consequently the rate constant (k) from the ratio of peak area of test compound to internal standard at each timepoint. The rate of intrinsic clearance (CLi) of each test compound was then calculated using the following calculation:

$$CLi(mL/min/g\ liver) = k \times V \times Microsomal\ protein\ yield$$

Where V (mL/mg protein) is the incubation volume/mg protein added and microsomal protein yield is taken as 52.5 mg protein/ g liver. Verapamil (0.5 μM) was used as a positive control to confirm acceptable assay performance.

Aqueous Solubility

The aqueous solubility of the test compounds was measured using laser nephelometry. Compounds were subject to serial dilution from 10 mM to 0.5 mM in DMSO. An aliquot was then mixed with MilliQ water to obtain an aqueous dilution plate with a final concentration range of 250 – 12 μM, with a final DMSO concentration of 2.5%. Triplicate aliquots were transferred to a flat-bottomed polystyrene plate which was immediately read on the NEPHELOstar (BMG Lab Technologies). The amount of laser scatter caused by insoluble particulates (relative nephelometry units, RNU) was plotted against compound concentration using a segmental regression fit, with the point of inflection being quoted as the compounds aqueous solubility (μM).

Table S3. In vitro PK and physicochemical properties of **26**, **46**, **VZ185** and ***cis*VZ185**

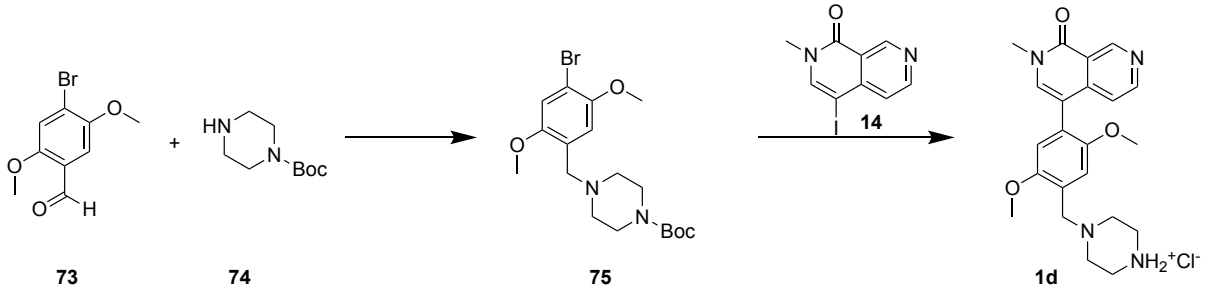
			26	**46**	**VZ185**	***cis*VZ185**
MWt			1041.25	997.20	995.23	995.23
Aqueous Solubility (Kinetic)	μM		n.d.	n.d.	85	79
CHI LogD (pH7.4)			2.0	2.0	2.3	2.4
CLogP calculated			4.0053	4.1409	5.0144	5.0144
Microsomal stability	mL/min/g liver	Mouse	n.d.	n.d.	1.2	2.4
		Human	n.d.	n.d.	3.8	8.1
Plasma stability	T 1/2 (mins)	Mouse	n.d.	n.d.	>180	>180
		Human	n.d.	n.d.	>180	>180
PAMPA pH 7.4	Pe (nm/s)		0.01	0.02	0.01	0.36
	HIGH/MED/LOW		LOW	LOW	LOW	LOW
	% Recovery		94	88	70	87
TPSA calculated			196.4	187.17	177.94	177.94

n.d.: not determined

Synthetic procedures

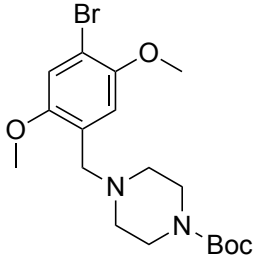
General method H: To NaHDMS 1M in THF (2.5 eq), glycol (5 eq) was added at 0 °C under nitrogen. The reaction mixture is stirred at 0 °C for 45 min. Then bromide (1 eq) and DMF (0.5 M) were added and the mixture was heated in microwave at 130 °C for 2h. The solvent was removed in vacuum. A 5% solution of $NaHSO_4$ was added and the reaction mixture was extracted with DCM. The organic phases were combined, dried over $MgSO_4$, filtered and evaporated to dryness. The crude was purified by flash column chromatography using a gradient from 0% to 5-10% of MeOH in DCM to yield the desired compound.

Synthesis compounds 1d-f.



73 74 75 1d

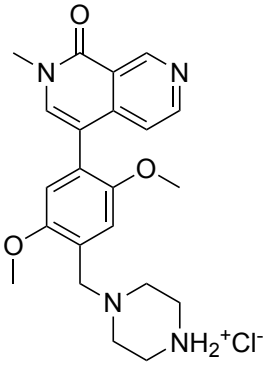
***tert*-butyl 4-(4-bromo-2,5-dimethoxybenzyl)piperazine-1-carboxylate (75).**



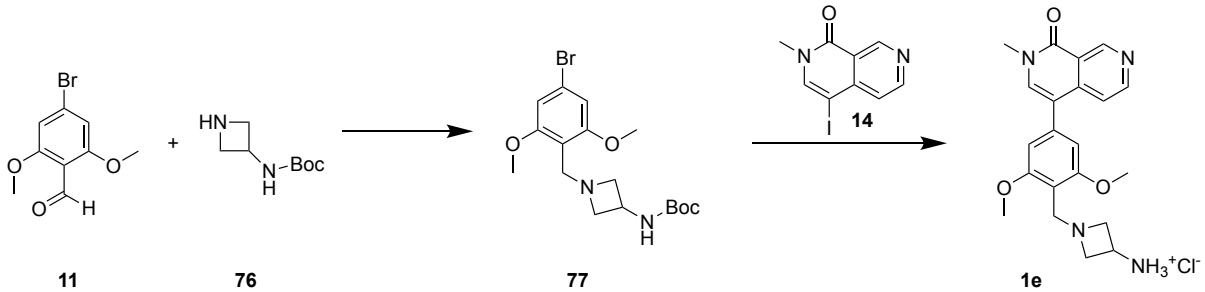
Following general method C, compound **75** was obtained from 4-bromo-2,5-dimethoxybenzaldehyde **73** and boc-piperazine **74** (both commercially available). The reaction was quenched with saturated solution of $NaHCO_3$, extracted with DCM, washed with water and brine. The organic phases were combined, dried over $MgSO_4$, filtered and

evaporated to dryness to give the desired compound without any further purification as sticky oil. Yield: 123 mg, 72%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.01 (s, 1H), 6.99 (s, 1H), 3.82 (s, 3H), 3.73 (s, 3H), 3.48 (s, 2H), 3.41 (t, *J* = 4.7 Hz, 4H), 2.39 (t, *J* = 4.3 Hz, 4H), 1.42 (s, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 154.9, 152.3, 150.1, 126.5, 116.2, 114.5, 109.9, 79.6, 57.0, 56.4, 55.7, 52.9, 31.0, 28.5. MS *m/z* calcd for $C_{18}H_{27}BrN_2O_4$ 414.12, found 415.2 [M + H^+].

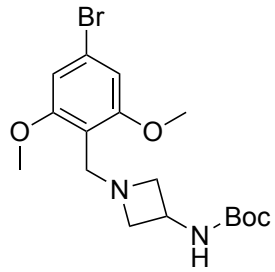
4-(2,5-dimethoxy-4-(piperazin-1-ylmethyl)phenyl)-2-methyl-2,7-naphthyridin-1(2*H*)-one (1d).



Following general method B, compound **1d** was obtained from compound **14** and **75** as brown powder. Yield: 72.3 mg, 55%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.65 (s, 1H), 8.71 (d, *J* = 6.2 Hz, 1H), 8.18 (s, 1H), 7.81 (d, *J* = 6.7 Hz, 1H), 7.54 (s, 1H), 7.24 (s, 1H), 3.99 (s, 3H), 3.88 - 3.64 (m, 16H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 163.2, 156.4, 153.6, 152.7, 151.3, 150.5, 144.1, 127.6, 124.1, 121.5, 120.3, 116.3, 115.7, 115.5, 56.9, 56.6, 56.4, 53.8, 37.4, 30.7. MS *m/z* calcd for $C_{22}H_{26}N_4O_3$ 394.2, found 395.3 [M + H^+].

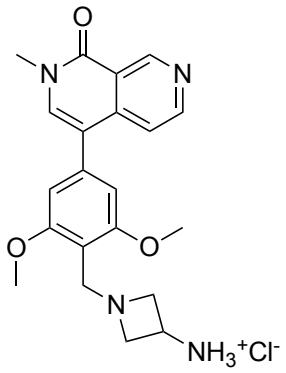


***tert*-butyl (1-(4-bromo-2,6-dimethoxybenzyl)azetidin-3-yl)carbamate (77).**



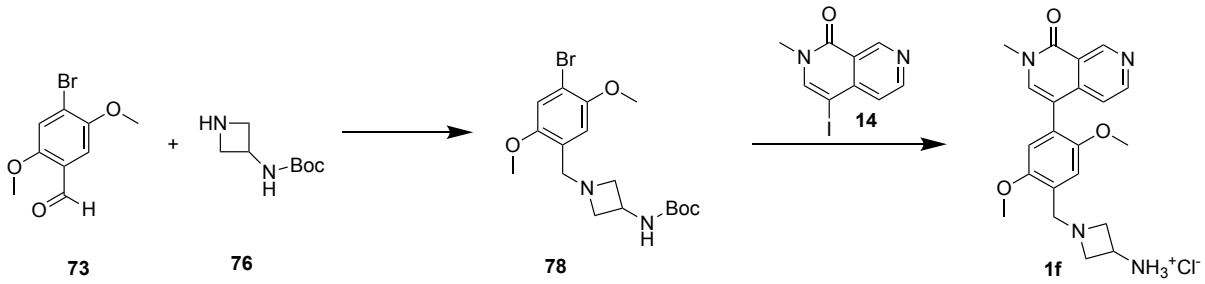
Following general method C, compound **77** was obtained from 4-bromo-2,6-dimethoxybenzaldehyde **11** and 3-(Boc-amino)azetidine **76** (both commercially available). The reaction was quenched with saturated solution of $NaHCO_3$, extracted with DCM, washed with water and brine. The organic phases were combined, dried over $MgSO_4$, filtered and evaporated to dryness to give the desired compound without any further purification as sticky oil. Yield: 120 mg, 78%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.72 (s, 2H), 4.33 (s, 1H), 3.87 - 3.80 (m, 10H), 3.57 (s, 2H), 1.40 (s, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 159.6, 155.3, 124.1, 107.9, 103.3, 79.8, 59.7, 56.2, 46.0, 41.1, 28.5. MS *m/z* calcd for $C_{17}H_{25}BrN_2O_4$ 400.1, found 401.1 [M + H^+].

4-(4-((3-aminoazetidin-1-yl)methyl)-3,5-dimethoxyphenyl)-2-methyl-2,7-naphthyridin-1(2*H*)-one (1e).

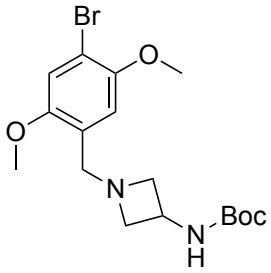


Following general method A, compound **1e** was obtained from compound **14** and **77** as brown powder. Yield: 77 mg, 50%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.67 (s, 1H), 8.76 (dd, *J*

= 0.6, 6.7 Hz, 1H), 8.27 (s, 1H), 8.20 (d, *J* = 5.5 Hz, 1H), 6.94 (s, 2H), 4.70 - 4.36 (m, 7H), 4.03 (s, 6H), 3.80 (s, 3H); ^{13}C-NMR (101 MHz, MeOD) δ: 161.3, 160.9, 149.0, 146.2, 145.8, 140.8, 139.2, 123.0, 122.9, 117.7, 106.9, 106.6, 58.9, 58.1, 57.0, 40.1, 37.9. MS *m/z* calcd for $C_{21}H_{24}N_4O_3$ 380.18, found 381.3 [M + H$^+$].

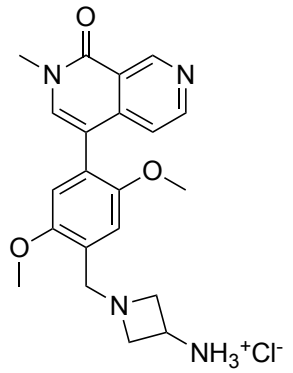


***tert*-butyl (1-(4-bromo-2,5-dimethoxybenzyl)azetidin-3-yl)carbamate (78).**



Following general method C, compound **78** was obtained from 4-bromo-2,5-dimethoxybenzaldehyde **73** and 3-(Boc-amino)azetidine **76** (both commercially available). The reaction was quenched with saturated solution of $NaHCO_3$, extracted with DCM, washed with water and brine. The organic phases were combined, dried over $MgSO_4$, filtered and evaporated to dryness to give the desired compound without any further purification as sticky oil. Yield: 150 mg, 72%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.02 (s, 1H), 6.88 (s, 1H), 4.38 - 4.31 (m, 1H), 3.86 (s, 3H), 3.77 (s, 3H), 3.73 - 3.66 (m, 4H), 2.91 (s, 2H), 1.43 (s, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 155.1, 151.7, 150.2, 115.9, 113.8, 109.9, 62.5, 57.1, 57.0, 56.2, 41.9, 31.1, 28.5. MS *m/z* calcd for $C_{17}H_{25}BrN_2O_4$ 400.1, found 401.1 [M + H$^+$].

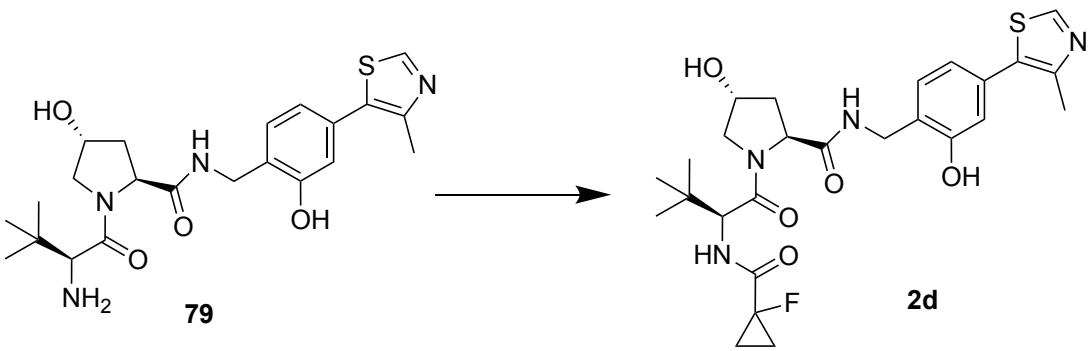
4-(4-((3-aminoazetidin-1-yl)methyl)-2,5-dimethoxyphenyl)-2-methyl-2,7-naphthyridin-1(2*H*)-one (1f).



Following general method A, compound **1f** was obtained from compound **78** and **14** as brown powder. Yield: 55.5 mg, 60%.^{1}H-NMR (400 MHz, MeOD) δ: 9.51 (s, 1H), 8.56 (d, *J* = 6.7 Hz, 1H), 7.99 (s, 1H), 7.56 (d, *J* = 6.4 Hz, 1H), 7.31 (s, 1H), 7.06 (s, 1H), 4.60 - 4.29 (m, 7H), 3.86 (s, 3H), 3.68 (s, 3H), 3.64 (s, 3H); ^{13}C-NMR (101 MHz, MeOD) δ: 160.2, 152.2, 151.4, 147.2, 145.0, 144.4, 140.1, 124.7, 122.1, 118.8, 115.4, 115.1, 114.6, 113.2, 55.6, 55.4, 49.0, 46.5, 42.6, 36.4. MS *m/z* calcd for $C_{21}H_{24}N_4O_3$ 380.18, found 381.3 [M + H$^+$].

Synthesis compound 2d.

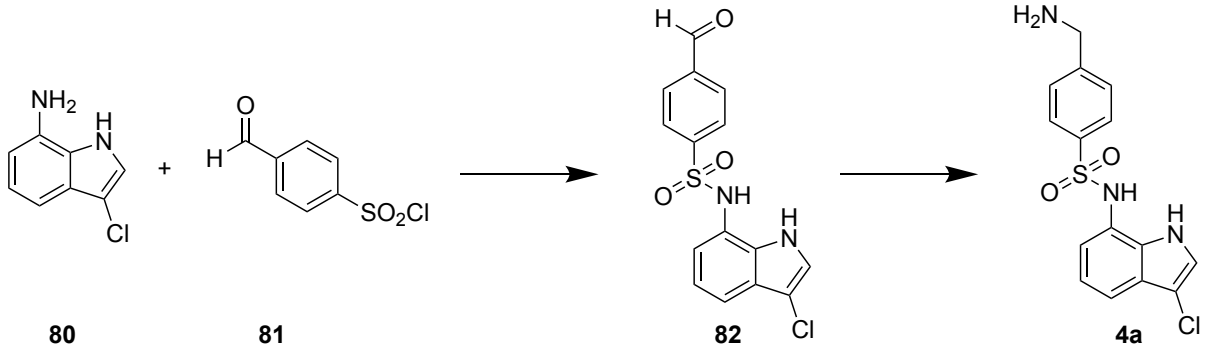
(2S,4R)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (2d, VHL4).



79 **2d**

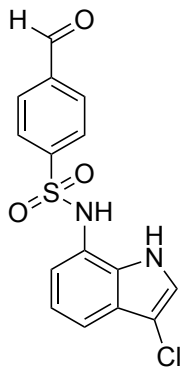
Following general method B, compound **2d** was obtained from compound **79** (synthetized accordingly to literature[1]) and 1-fluorocyclopropane-1-carboxylic acid. The crude was purified by flash column chromatography using a gradient from 0% to 20% of methanol in DCM, affording **2d**. Yield: 123 mg, 57%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.66 (s, 1H), 8.00 (t, *J* = 6.5 Hz, 1H), 7.11 (d, *J* = 7.7 Hz, 1H), 6.98 (d, *J* = 1.5 Hz, 1H), 6.97 - 6.93 (m, 1H), 6.88 (dd, *J* = 1.9, 7.8 Hz, 1H), 4.75 (t, *J* = 7.8 Hz, 1H), 4.55 - 4.49 (m, 2H), 4.42 (d, *J* = 8.5 Hz, 1H), 4.14 (dd, *J* = 5.5, 14.7 Hz, 1H), 4.01 (d, *J* = 11.7 Hz, 1H), 3.57 (dd, *J* = 3.7, 11.4 Hz, 1H), 2.57 - 2.46 (m, 4H), 2.10 - 2.04 (m, 1H), 1.35 - 1.25 (m, 4H), 0.85 (s, 10H); ^{13}C-NMR (125MHz, $CDCl_3$) δ: 172.6, 171.5, 170.5, 155.6, 150.3, 148.5, 133.6, 131.5, 123.8, 120.9, 118.6, 70.1, 57. 8, 56.6, 40.0, 35.0, 26.1, 20.6 16.23, 13.8. MS *m/z* calcd for $C_{26}H_{33}FN_4O_5S$ 532.22, found 533.3 [M + H^+].

Synthesis compound 4a.



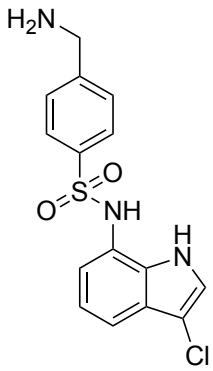
80 **81** **82** **4a**

***N*-(3-chloro-1*H*-indol-7-yl)-4-formylbenzenesulfonamide (82).**



To compound 3-chloro-1*H*-indol-7-amine **73** (synthetized as reported in literature[5]) in ethyl acetate, 4-formylbenzenesulfonyl chloride **81** (1.1 eq) and pyridine (2 eq) were added. The reaction mixture was stirred at rt for 3 h, diluted with ethyl acetate and washed successively with 1 N aqueous HCl, H_2O, saturated aqueous solution of $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and evaporated in vacuum. The crude was purified by flash column chromatography using a gradient from 10% to 60% of ethyl acetate in heptane to obtain compound **82**. Yield: 104.5 mg, 62%. ^{1}H-NMR (400 MHz, DMSO) δ: 11.11 (s, 1NH), 10.22 (d, *J* = 2.2 Hz, 1NH), 10.03 (s, 1COH), 8.02 (d, *J* = 8.6 Hz, 2H), 7.93 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 2.6 Hz, 1H), 7.28 (dd, *J* = 0.6, 8.0 Hz, 1H), 6.95 (t, *J* = 7.8 Hz, 1H), 6.77 - 6.74 (m, 1H); ^{13}C-NMR (101 MHz, DMSO) δ: 192.6, 144.2, 138.9, 130.2, 129.7, 127.8, 126.4, 123.3, 121.6, 120.2, 116.8, 115.4, 103.8. MS *m/z* calcd for $C_{15}H_{11}ClN_2O_3S$ 334.02, found 335.1 [M + H^+].

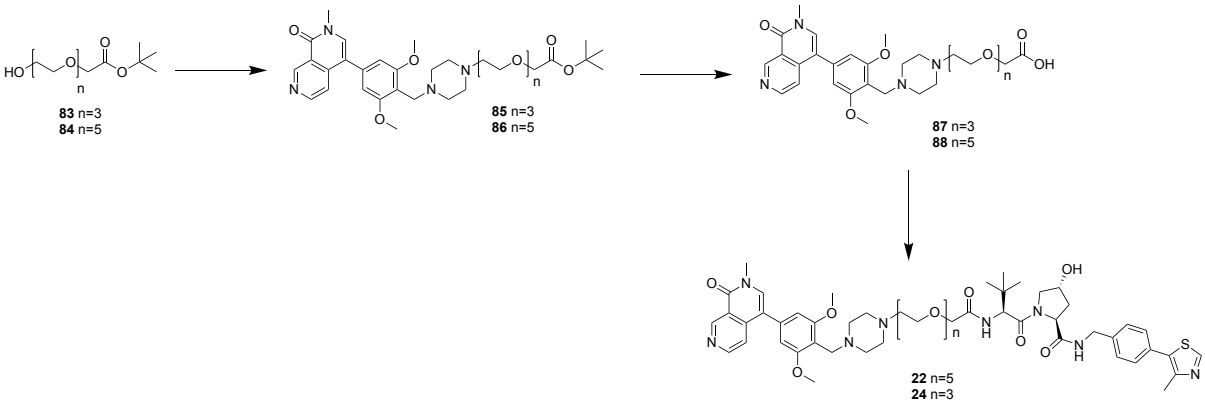
4-(aminomethyl)-*N*-(3-chloro-1*H*-indol-7-yl)benzenesulfonamide (4a).



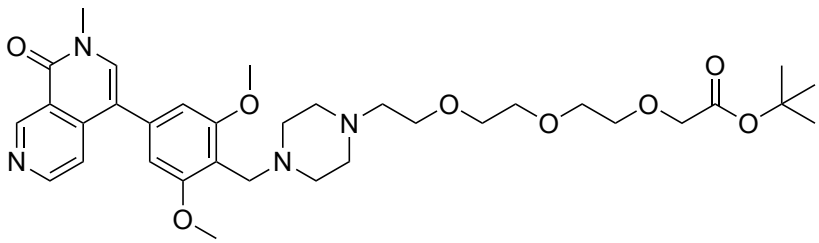
Compound **82** (0.33 mmol, 1 eq) was dissolved in a saturated solution of NH_4OAc in EtOH (6.6 mL), prepared as follow: 25ml of EtOH were heated to boil then NH_4OAc was added until saturation, then NH_3 30% (2.64 mL) was added. After 15 min, $NaBCNH_3$ (0.09mmol, 3 eq) was added and the reaction mixture was heated at 100 °C for 15 min. The solvent was removed in vacuum and the crude was dissolved in DCM/MeOH, washed successively with saturated aqueous solution of $NaHCO_3$ and 15% aqueous NaOH was added up to pH 8. The

solvent was evaporated in vacuum to give the corresponding crude, which was purified by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid to obtain compound **4a**. Yield: 42 mg, 35%. ^{1}H-NMR (400 MHz, MeOD) δ: 8.58 (d, *J* = 2.8 Hz, 1NH), 7.79 (d, *J* = 8.5 Hz, 2H), 7.55 (d, *J* = 8.1 Hz, 2H), 7.36 (dd, *J* = 0.7, 8.0 Hz, 1H), 7.29 (d, *J* = 2.1 Hz, 1H), 6.92 (t, *J* = 7.8 Hz, 1H), 6.75 - 6.70 (m, 1H), 4.14 (s, 2H); ^{13}C-NMR (101 MHz, MeOD) δ: 141.0, 140.9, 131.8, 130.2, 129.1, 128.3, 123.3, 122.8, 120.9, 119.1, 117.0, 106.3, 43.9. MS *m/z* calcd for $C_{15}H_{14}ClN_3O_2S$ 335.05, found 336.1 [M + H^+].

Synthesis of 22 and 24.



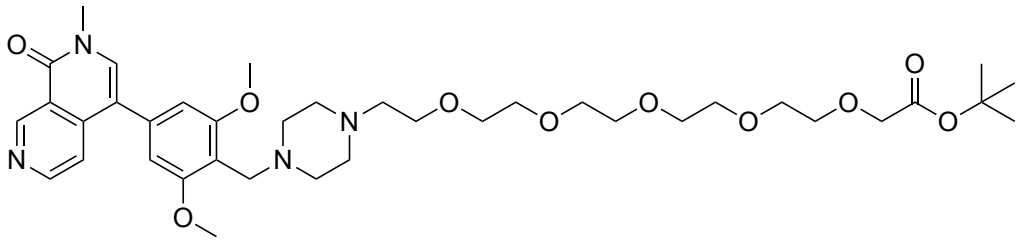
***tert*-butyl 2-(2-(2-(2-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)acetate (85).**



Following general method D, from compound **83** and **1c** compound **85** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of ammonia as white powder. Yield: 11.0 mg, 21%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.55 (s, 1H), 8.70 (d, *J* = 5.7 Hz, 1H), 7.76 (s, 1H), 7.65 (d, *J* = 6.5 Hz, 1H), 6.78 (s, 2H),

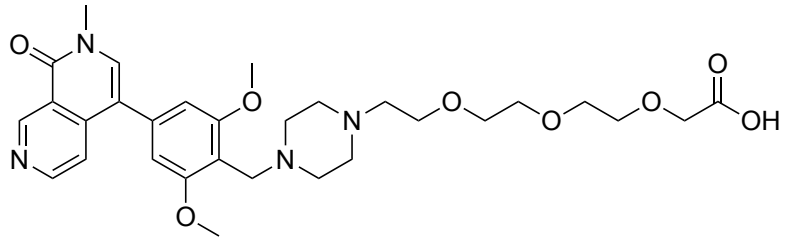
4.06 (s, 2H), 3.91 (s, 6H), 3.82 (s, 2H), 3.74 - 3.63 (m, 14H), 2.73 - 2.62 (m, 10H), 1.51 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 170.2, 161.7, 159.6, 150.3, 149.8, 142.3, 137.4, 135.9, 120.3, 118.0, 117.9, 111.9, 105.1, 81.3, 70.3, 70.2, 70.1, 70.0, 68.4, 68.2, 57.2, 54.9, 52.6, 51.9, 36.0, 26.9. MS *m/z* calcd for $C_{34}H_{48}N_4O_8$ 640.35, found 641.4[M + H^+].

***tert*-butyl 17-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)-3,6,9,12,15-pentaoxaheptadecanoate (86).**



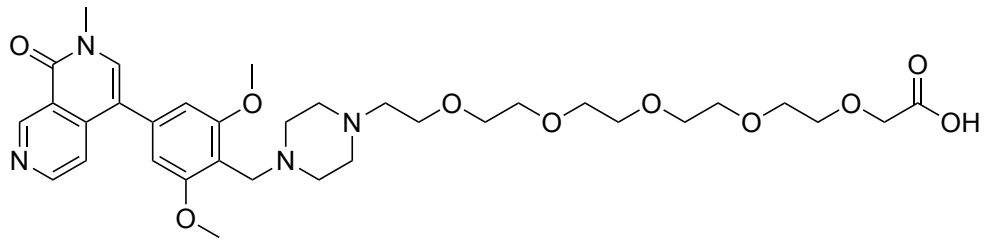
Following general method D, from compound **84** and **1c** compound **86** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of ammonia as white powder. Yield: 24.6 mg, 73%. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 9.68 (d, *J* = 0.9 Hz, 1H), 8.69 (d, *J* = 5.6 Hz, 1H), 7.42 (dd, *J* = 0.8, 5.6 Hz, 1H), 7.26 (s, 1H), 6.52 (s, 2H), 3.99 (s, 2H), 3.80 (s, 6H), 3.72 - 3.57 (m, 23H), 2.67 - 2.53 (m, 10H), 1.45 (s, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 170.0, 161.8, 160.0, 152.0, 151.2, 142.1, 136.1, 135.6, 120.8, 118.4, 117.8, 113.7, 105.7, 81.8, 71.0, 70.9, 70.6, 69.3, 69.2, 58.0, 56.2, 53.9, 52.5, 48.9, 37.4, 28.4. MS *m/z* calcd for $C_{38}H_{56}N_4O_{10}$ 728.40, found 729.42 [M + H^+].

2-(2-(2-(2-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)acetic acid (87).



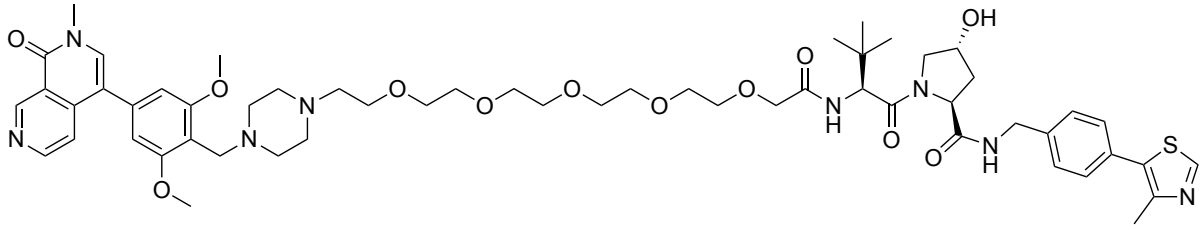
A mixture of compound **85** (0.017 mmol), TFA (0.5ml) and DCM (0.5ml) was stirred at rt for 3 h. Then the solvent was evaporated; the crude was dried under high pressure overnight and used directly without any further purification. Quantitative yield. MS *m/z* calcd for $C_{30}H_{40}N_4O_8$ 584.28, found 585.3[M + H^+].

17-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)-3,6,9,12,15-pentaoxaheptadecanoic acid (88).



A mixture of compound **86** (0.034 mmol), TFA (0.5ml) and DCM (0.5ml) was stirred at rt for 3 h. Then the solvent was evaporated; the crude was dried under high pressure overnight and used directly without any further purification. Quantitative yield. MS *m/z* calcd for $C_{34}H_{48}N_4O_{10}$ 672.34, found 673.36 [M + H^+].

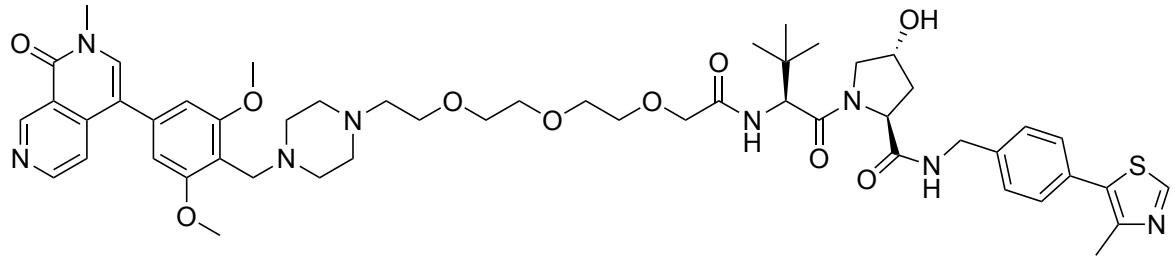
(2*S*,4*R*)-1-((*S*)-2-(*tert*-butyl)-20-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)-4-oxo-6,9,12,15,18-pentaoxa-3-azaicosanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (22).



Following general method B, compound **22** was obtained after reaction between **88** and **2a** (synthetized accordingly to literature[6]). The crude was purified by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid to obtain **22** as white powder. Yield: 12.8 mg, 35%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.43 (s, 1H), 8.81 (s, 1H), 8.59

(d, *J* = 5.6 Hz, 1H), 7.66 (s, 1H), 7.54 (d, *J* = 5.6 Hz, 1H), 7.37 (q, *J* = 9.3 Hz, 4H), 6.72 (s, 2H), 4.63 (s, 1H), 4.53 - 4.27 (m, 4H), 4.03 - 3.94 (m, 4H), 3.84 - 3.71 (m, 8H), 3.66 - 3.52 (m, 21H), 2.89 - 2.68 (m, 10H), 2.41 (s, 3H), 2.20 - 2.13 (m, 1H), 2.09 - 1.97 (m, 1H), 0.98 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.4, 172.0, 171.7, 170.1, 163.0, 161.0, 152.8, 151.7, 151.2, 149.0, 143.5, 140.3, 139.0, 138.5, 133.4, 131.5, 130.5, 130.4, 129.5, 129.0, 121.7, 119.2, 119.1, 106.6, 72.3, 71.7, 71.6, 71.5, 71.4, 71.1, 71.0, 69.0, 68.9, 60.8, 58.1, 56.6, 52.8, 43.7, 39.0, 37.4, 37.1, 27.0, 15.9. HRMS *m/z* calcd for $C_{56}H_{76}N_8O_{12}S$ 1084.53, found 1085.5578 [M + H^+].

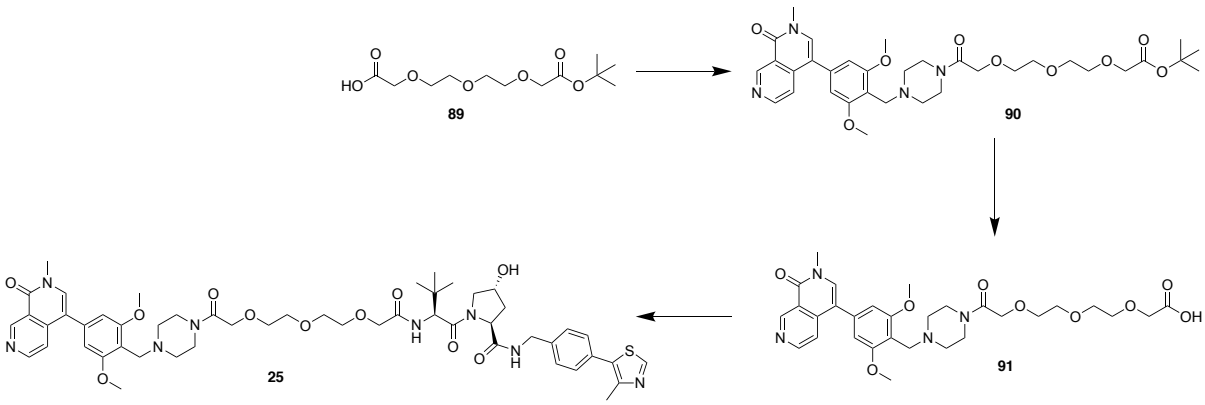
(2S,4R)-1-((S)-2-(tert-butyl)-14-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)-4-oxo-6,9,12-trioxa-3-azatetradecanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (24).



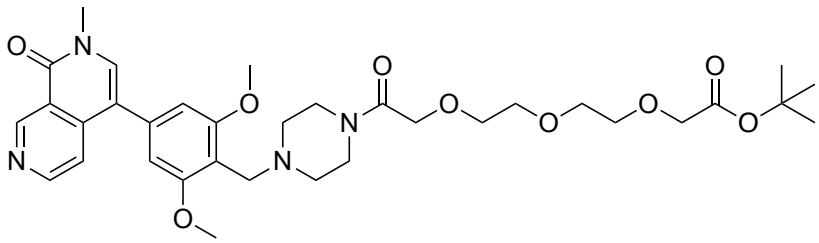
Following general method B, compound **24** was obtained after reaction between **87** and **2a** (synthetized accordingly to literature[6]). The crude was purified by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid to obtain **24** as white powder. Yield: 9.7 mg, 57%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.55 (s, 1H), 8.91 (s, 1H), 8.70 (d, *J*=5.9 Hz, 1H), 7.75 (s, 1H), 7.65 (d, *J*=6.1 Hz, 1H), 7.51 - 7.43 (m, 4H), 6.77 (s, 2H), 4.73 (s, 1H), 4.63 - 4.51 (m, 3H), 4.38 (d, *J*=15.4 Hz, 1H), 4.07 (d, *J*=3.7 Hz, 2H), 3.89 (s, 6H), 3.79 (s, 2H), 3.75 - 3.61 (m, 14H), 2.66 - 2.56 (m, 9H), 2.50 (s, 3H), 2.29 - 2.23 (m, 1H), 2.15 - 2.07 (m, 1H); ^{13}C-NMR (101 MHz, MeOD) δ:173.0, 170.7, 170.2, 161.7, 159.6, 151.4, 150.3, 149.8, 147.7, 142.3, 138.9, 137.4, 135.8, 132.0, 130.1, 129.1, 129.0, 128.1,

127.6, 120.3, 118.0, 117.9, 112.1, 105.1, 71.0, 70.2, 70.1, 69.7, 69.6, 68.2, 59.4, 57.2, 56.7, 54.9, 52.7, 52.0, 42.3, 37.6, 36.0, 35.7, 25.6, 14.5. HRMS *m/z* calcd for $C_{52}H_{68}N_8O_{10}S$ 996.48, found 997.5002 [M + H^+].

Synthesis compound 25.



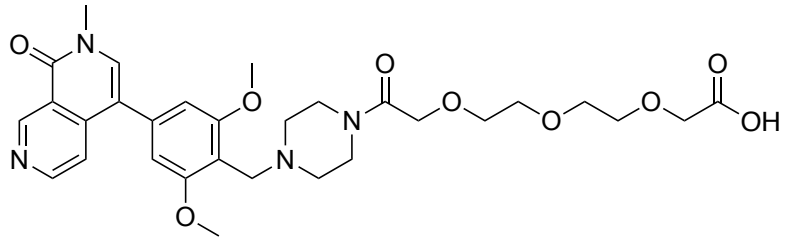
tert-butyl 2-(2-(2-(2-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)-2-oxoethoxy)ethoxy)ethoxy)acetate (90).



Following general method B, compound **90** was obtained from **89** (synthetized as previously reported[7]) and **1c** after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of ammonia as white powder. Yield: 30.7 mg, 57%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.51 (s, 1H), 8.68 (d, *J* = 5.8 Hz, 1H), 7.74 (s, 1H), 7.64 (d, *J* = 6.1 Hz, 1H), 6.78 (s, 2H), 4.26 (s, 2H), 4.04 (s, 2H), 3.90 (s, 6H), 3.83 (s, 2H), 3.71 - 3.65 (m, 14H), 3.64 - 3.56 (m, 4H), 2.70 - 2.60 (m, 4H), 1.48 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 170.1, 168.6, 161.6, 159.6, 150.3, 149.8, 142.2, 137.5, 136.1, 120.3, 117.9, 111.7, 105.1, 81.3, 70.5,

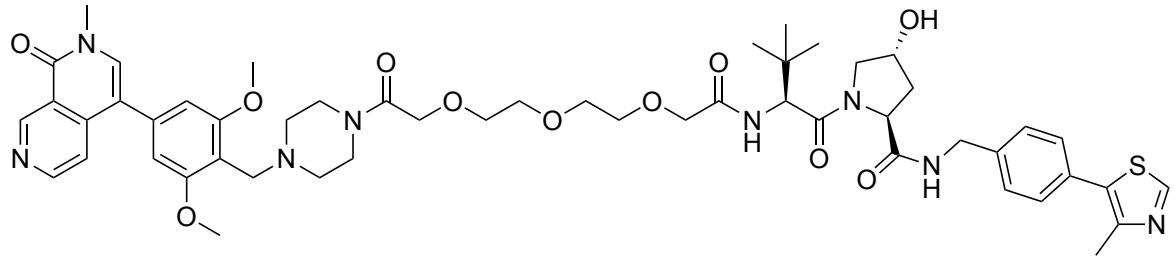
70.3, 70.3, 70.1, 70.1, 69.5, 68.4, 55.0, 52.6, 52.1, 44.2, 41.0, 36.0, 27.0. MS *m/z* calcd for $C_{34}H_{46}N_4O_9$ 654.33, found 655.3[M + H^+].

2-(2-(2-(2-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)-2-oxoethoxy)ethoxy)ethoxy)acetic acid (91).



A mixture of compound **90** (0.047 mmol), TFA (1 ml) and DCM (1 ml) was stirred at rt for 3 h. Then the solvent was evaporated; the crude was dried under high pressure overnight and used directly without any further purification. Quantitative yield. MS *m/z* calcd for $C_{30}H_{38}N_4O_9$ 598.26, found 599.3 [M + H^+].

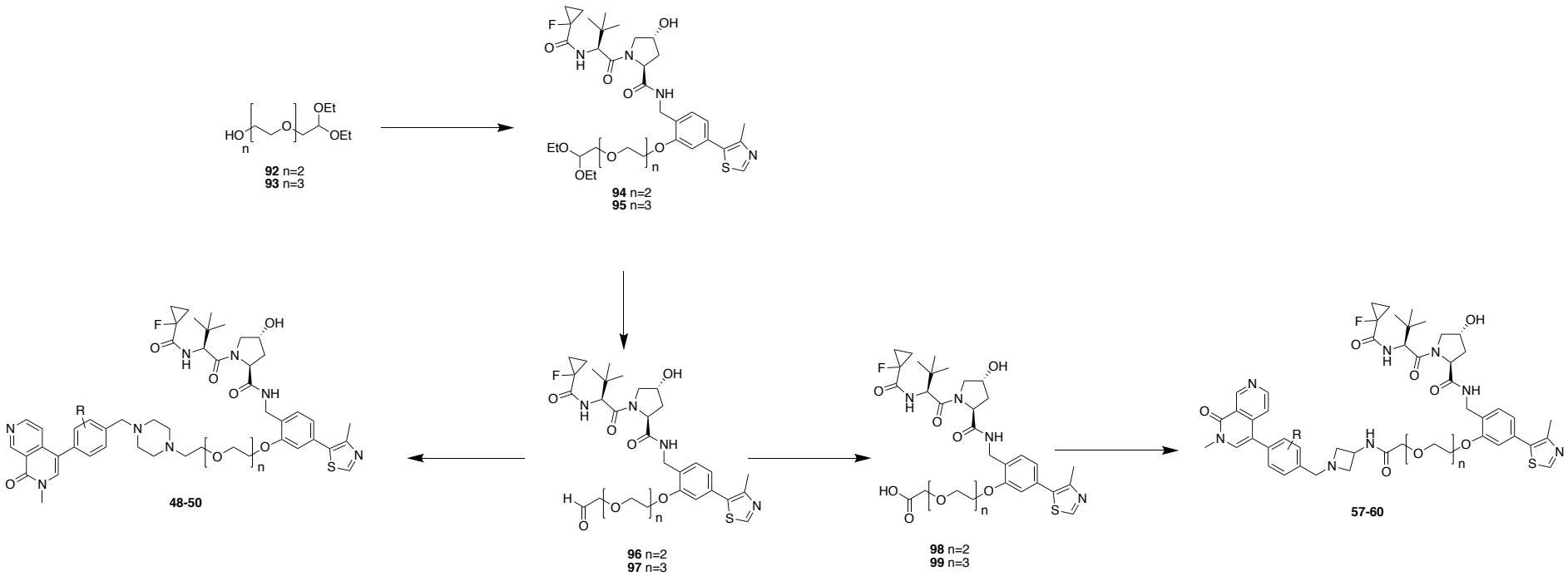
(2S,4R)-1-((S)-2-(tert-butyl)-14-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)-4,14-dioxo-6,9,12-trioxa-3-azatetradecanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (25).



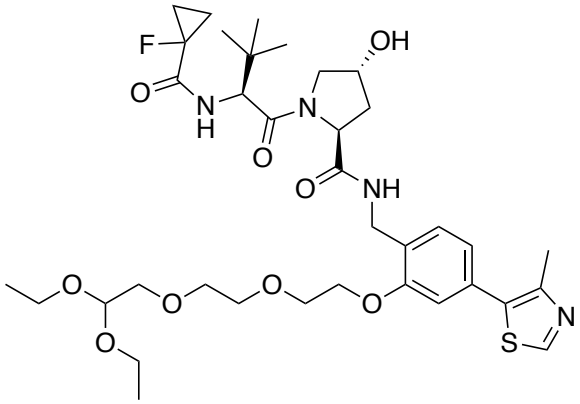
Following general method B, compound **25** was obtained after reaction between **91** and **2a** (synthetized accordingly to literature[6]). The crude was purified by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid to obtain **25** as white powder. Yield: 9.5 mg, 35%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.54 (s, 1H), 8.90 (s, 1H), 8.69

(d, J = 5.8 Hz, 1H), 7.75 (s, 1H), 7.65 (d, J = 5.8 Hz, 1H), 7.51 - 7.43 (m, 4H), 6.77 (s, 2H), 4.74 (s, 1H), 4.63 - 4.36 (m, 4H), 4.26 (d, J = 2.1 Hz, 2H), 4.08 (d, J = 6.4 Hz, 2H), 3.90 (s, 7H), 3.79 (s, 2H), 3.74 - 3.70 (m, 12H), 3.63 - 3.52 (m, 4H), 2.65 - 2.57 (m, 4H), 2.50 (s, 3H), 2.29 - 2.22 (m, 1H), 2.16 - 2.08 (m, 1H), 1.08 (s, 10H); ^{13}C-NMR (101 MHz, MeOD) δ: 173.0, 170.7, 170.2, 168.7, 161.7, 159.5, 151.4, 150.3, 149.8, 147.7, 142.2, 138.9, 137.5, 135.9, 132.0, 130.1, 129.1, 129.0, 127.6, 120.3, 118.0, 117.9, 112.1, 105.1, 70.9, 70.3, 70.2, 70.1, 69.7, 69.7, 69.5, 59.4, 56.7, 55.0, 52.6, 52.2, 44.3, 42.3, 41.2, 37.6, 36.0, 35.8, 25.6, 14.5. HRMS m/z calcd for $C_{52}H_{66}N_8O_{11}S$ 1010.46, found 1011.4801 [M + H^+].

Synthesis compounds 48-50 and 57-60.

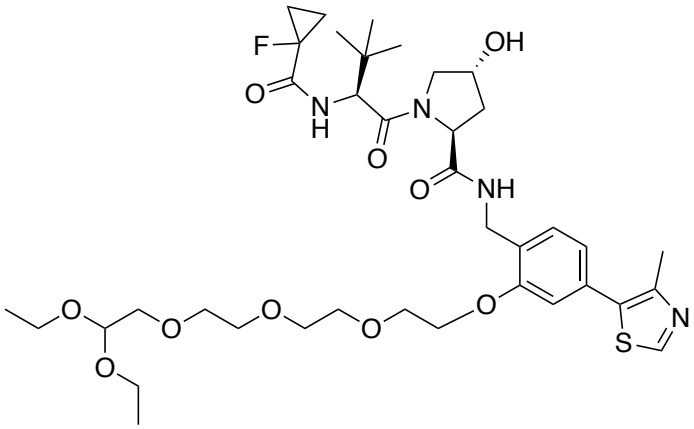


(2S,4R)-N-(2-(2-(2-(2,2-diethoxyethoxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (94).



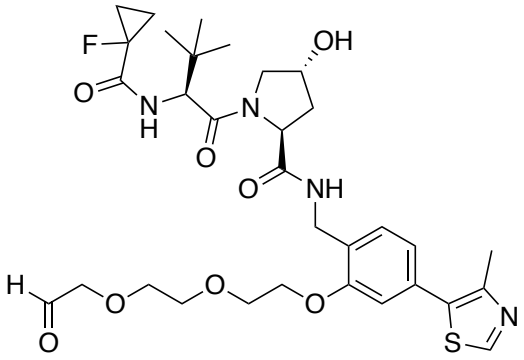
Following general method E, compound **94** was obtained from 2-(2-(2,2-diethoxyethoxy)ethoxy)ethan-1-ol **92** (synthetized accordingly to literature[8]) and **2d** as white powder. Yield: 87 mg, 79%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.66 (s, 1H), 7.31 (d, *J* = 7.7 Hz, 1H), 6.95 (dd, *J* = 1.6, 7.6 Hz, 1H), 6.88 (d, *J* = 1.4 Hz, 1H), 4.67 - 4.59 (m, 2H), 4.55 - 4.44 (m, 4H), 4.23 - 4.12 (m, 2H), 3.95 - 3.84 (m, 3H), 3.76 - 3.60 (m, 6H), 3.58 - 3.48 (m, 5H), 2.50 (s, 3H), 2.43 - 2.34 (m, 1H), 2.15 - 2.06 (m, 1H), 1.34 - 1.23 (m, 4H), 1.18 (t, *J* = 7.1 Hz, 6H), 0.93 (s, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 170.8, 170.7, 170.2, 170.0, 156.9, 150.4, 148.6, 132.4, 131.8, 129.9, 126.9, 122.1, 112.9, 101.2, 79.5, 71.8, 71.0, 70.8, 70.3, 69.7, 68.0, 62.5, 62.4, 58.7, 57.5, 56.7, 39.2, 36.5, 35.7, 26.4, 16.2, 15.4, 13.9, 13.8, 13.7. MS *m/z* calcd for $C_{36}H_{53}FN_4O_9S$ 736.35, found 737.3 [M + H^+].

(2S,4R)-N-(2-((11-ethoxy-3,6,9,12-tetraoxatetradecyl)oxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (95).



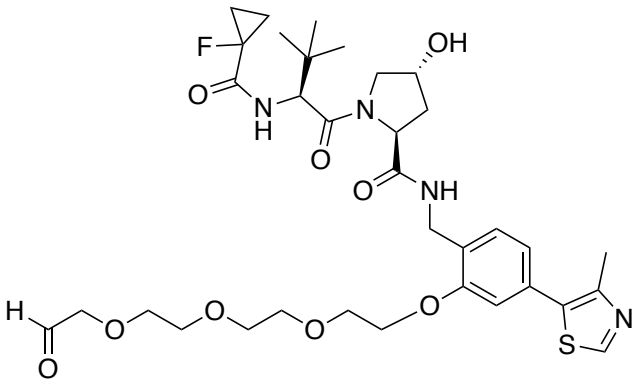
Following the general method E, compound **95** was obtained 11-ethoxy-3,6,9,12-tetraoxatetradecan-1-ol **90** (synthetized accordingly to literature[8]) and **2d** as white powder. Yield: 59 mg, 53%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.66 (s, 1H), 7.32 (d, *J* = 7.8 Hz, 1H), 6.96 (dd, *J* = 1.6, 7.6 Hz, 1H), 6.88 (d, *J* = 1.6 Hz, 1H), 4.67 - 4.59 (m, 2H), 4.56 - 4.43 (m, 4H), 4.24 - 4.14 (m, 2H), 3.95 - 3.85 (m, 3H), 3.79 - 3.60 (m, 10H), 3.58 - 3.50 (m, 5H), 2.51 (s, 3H), 2.42 - 2.34 (m, 1H), 2.14 - 2.07 (m, 1H), 1.35 - 1.26 (m, 4H), 1.18 (dt, *J* = 1.2, 7.0 Hz, 6H), 0.94 (s, 9H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 170.8, 170.2, 170.0, 156.9, 150.4, 148.6, 132.4, 131.8, 129.9, 126.9, 122.1, 112.9, 101.2, 79.5, 71.8, 70.9, 70.8, 70.6, 70.5, 70.3, 69.7, 68.0, 62.4, 58.8, 57.5, 56.6, 39.2, 36.5, 35.7, 26.4, 16.2, 15.4, 13.7. MS *m/z* calcd for $C_{38}H_{57}FN_4O_{10}S$ 780.38, found 781.4 [M + H^+].

(2S,4R)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)-2-(2-(2-(2-oxoethoxy)ethoxy)ethoxy)benzyl)pyrrolidine-2-carboxamide (96).



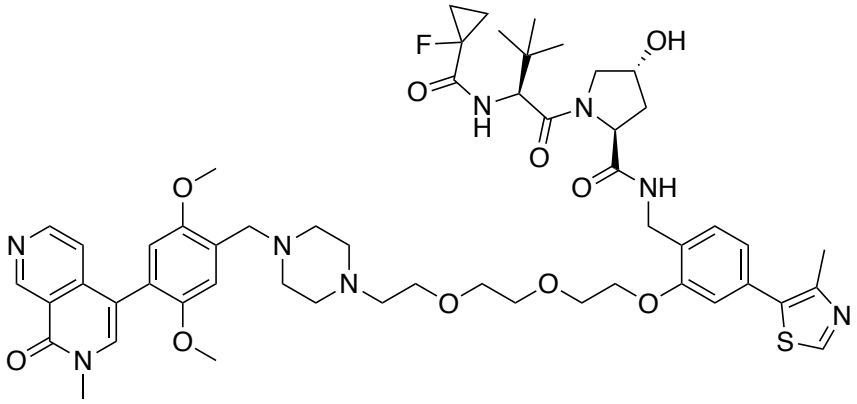
Following general method F, compound **96** was obtained from **94** (0.06mmol) and directly used in the next step without any further purification. MS *m/z* calcd for $C_{32}H_{43}FN_4O_8S$ 662.28, found 663.3 [M + H^+].

(2S,4R)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)-2-(2-(2-(2-(2-oxoethoxy)ethoxy)ethoxy)ethoxy)benzyl)pyrrolidine-2-carboxamide (97).



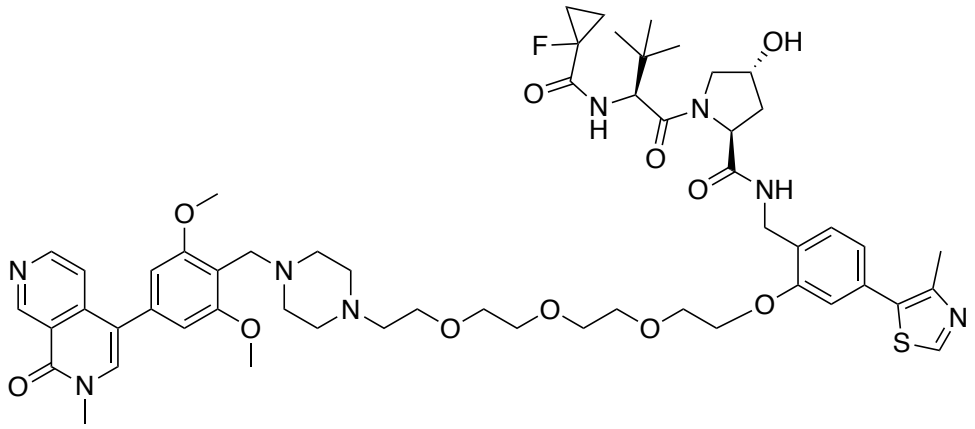
Following general method F, compound **97** was obtained from **95** (0.04mmol) and directly used in the next step without any further purification. MS *m/z* calcd for $C_{34}H_{47}FN_4O_9S$ 706.3, found 707.3 [M + H^+].

(2S,4R)-N-(2-(2-(2-(2-(4-(2,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (48).



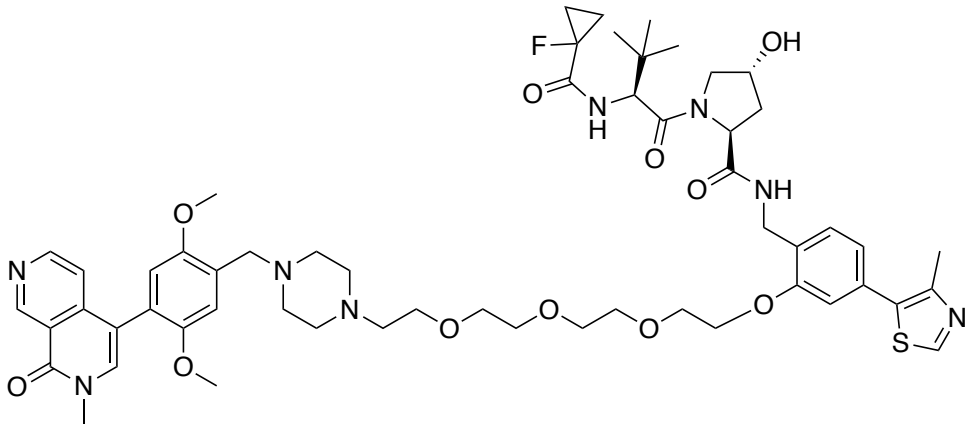
Following general method C, from **96** (0.03mmol) and **1d** (0.03mmol) compound **48** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid as white powder. Yield: 2.5 mg, 8%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.52 (s, 1H), 8.91 (s, 1H), 8.61 (d, *J* = 5.8 Hz, 1H), 7.64 (s, 1H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.19 (d, *J* = 5.6 Hz, 2H), 7.11 - 7.06 (m, 2H), 7.02 (s, 1H), 4.79 (s, 1H), 4.67 (t, *J* = 8.1 Hz, 1H), 4.56 - 4.44 (m, 3H), 4.32 - 4.25 (m, 2H), 3.99 - 3.81 (m, 12H), 3.72 - 3.71 (m, 9H), 3.24 - 2.94 (m, 10H), 2.52 (s, 3H), 2.32 - 2.26 (m, 1H), 2.16 - 2.09 (m, 1H), 1.42 - 1.30 (m, 4H), 1.07 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.3, 171.7, 166.3, 163.2, 157.9, 153.7, 152.9, 151.4, 150.5, 149.2, 144.0, 139.3, 133.4, 133.0, 129.8, 128.3, 123.0, 121.5, 120.2, 116.0, 115.9, 115.6, 113.7, 78.1, 71.8, 71.4, 71.0, 70.8, 69.4, 60.9, 58.7, 58.2, 57.5, 56.7, 56.5, 56.1, 53.0, 51.5, 39.4, 39.0, 37.4, 37.3, 26.9, 16.0, 14.1, 14.0, 13.9. HRMS *m/z* calcd for $C_{54}H_{69}FN_8O_{10}S$ 1040.48, found 1041.5090 [M + H^+].

(2S,4R)-N-(2-(2-(2-(2-(2-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (49).



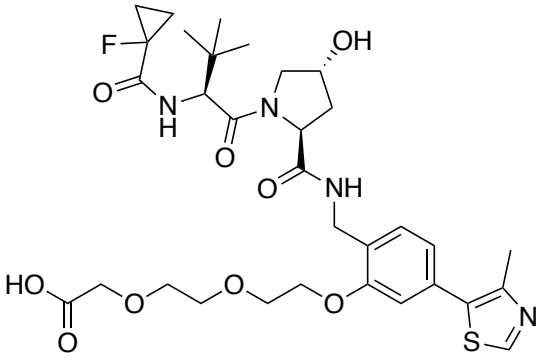
Following general method C, from **97** (0.02mmol) and **1c** (0.02mmol) compound **49** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid as white powder. Yield: 1.1 mg, 5%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.54 (d, *J* = 0.7 Hz, 1H), 8.90 (s, 1H), 8.70 (d, *J* = 5.5 Hz, 1H), 7.75 (s, 1H), 7.64 (d, *J* = 5.8 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.09 - 7.03 (m, 2H), 6.78 (s, 2H), 4.77 (s, 1H), 4.69 - 4.64 (m, 1H), 4.55 - 4.42 (m, 3H), 4.29 - 4.25 (m, 2H), 3.95 (t, *J* = 4.0 Hz, 2H), 3.91 - 3.84 (m, 9H), 3.80 - 3.61 (m, 14H), 2.77 - 2.64 (m, 10H), 2.52 (s, 3H), 2.31 - 2.23 (m, 1H), 2.16 - 2.08 (m, 1H), 1.42 - 1.29 (m, 4H), 1.07 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.2, 171.7, 163.1, 161.0, 158.1, 152.8, 151.7, 151.2, 149.2, 143.6, 138.8, 133.5, 132.9, 130.1, 128.4, 122.9, 121.7, 119.3, 113.9, 106.6, 80.3, 78.0, 71.9, 71.6, 71.4, 71.0, 70.9, 69.4, 60.8, 58.7, 58.5, 58.1, 56.4, 53.8, 53.2, 39.5, 38.9, 37.4, 37.3, 26.9, 16.0, 14.1, 14.0, 13.9. HRMS *m/z* calcd for $C_{56}H_{73}FN_8O_{11}S$ 1084.51, found 1085.5380 [M + H^+].

(2S,4R)-N-(2-(2-(2-(2-(2-(4-(2,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (50).



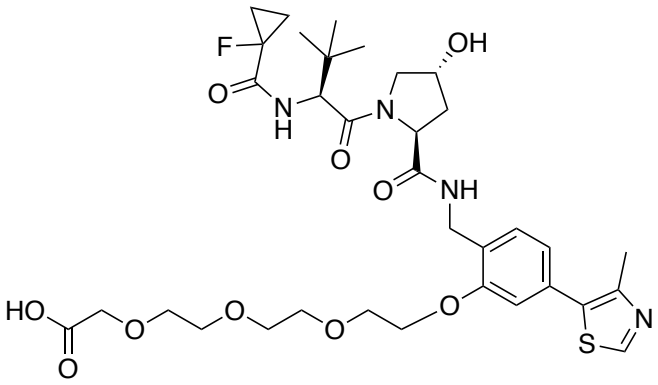
Following general method C, from **97** (0.02mmol) and **1d** (0.02mmol) compound **50** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid as white powder. Yield: 1.2 mg, 6%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.51 (d, *J* = 0.7 Hz, 1H), 8.90 (s, 1H), 8.62 (d, *J* = 5.6 Hz, 1H), 7.64 (s, 1H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.21 (dd, *J* = 0.7, 5.8 Hz, 1H), 7.18 (s, 1H), 7.09 - 7.04 (m, 2H), 6.97 (s, 1H), 4.79 (s, 1H), 4.66 (t, *J* = 8.5 Hz, 1H), 4.56 - 4.42 (m, 3H), 4.29 - 4.25 (m, 2H), 3.97 - 3.93 (m, 2H), 3.91 - 3.61 (m, 23H), 2.87 - 2.63 (m, 10H), 2.53 (s, 3H), 2.31 - 2.24 (m, 1H), 2.16 - 2.09 (m, 1H), 1.42 - 1.29 (m, 4H), 1.07 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.2, 171.7, 163.3, 158.1, 153.7, 152.8, 152.7, 151.3, 150.5, 149.2, 144.2, 139.2, 133.5, 132.9, 130.1, 130.0, 128.5, 127.9, 124.0, 122.9, 120.2, 116.4, 115.8, 115.7, 113.9, 71.9, 71.8, 71.6, 71.4, 71.0, 70.9, 69.6, 69.4, 60.8, 58.7, 58.5, 58.1, 56.8, 56.7, 56.5, 54.3, 53.5, 39.5, 38.9, 37.4, 37.3, 26.9, 16.0, 14.1, 14.0, 13.9. HRMS *m/z* calcd for $C_{56}H_{73}FN_8O_{11}S$ 1084.51, found 1085. 5423 [M + H^+].

2-(2-(2-(2-(((2S,4R)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)ethoxy)ethoxy)acetic acid (98)



Following general method G, compound **98** was obtained from compound **96** (0.06mmol) and used in the next step without any further purification. Quantitative yield. MS *m/z* calcd for $C_{32}H_{43}FN_4O_9S$ 678.77, found 679.3 [M + H^+].

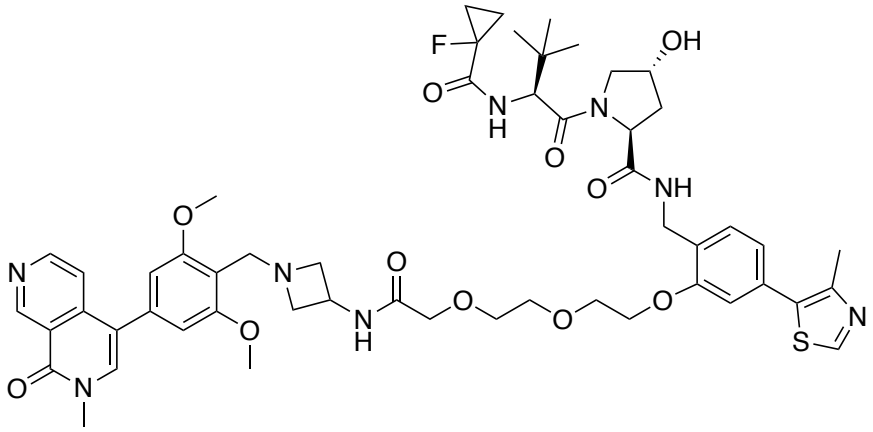
2-(2-(2-(2-(2-(((2S,4R)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)ethoxy)ethoxy)ethoxy)acetic acid (99).



Following general method G, compound **99** was obtained from compound **97** (0.04mmol) and used in the next step without any further purification. Quantitative yield. MS *m/z* calcd for $C_{34}H_{47}FN_4O_{10}S$ 722.30, found 723.3 [M + H^+].

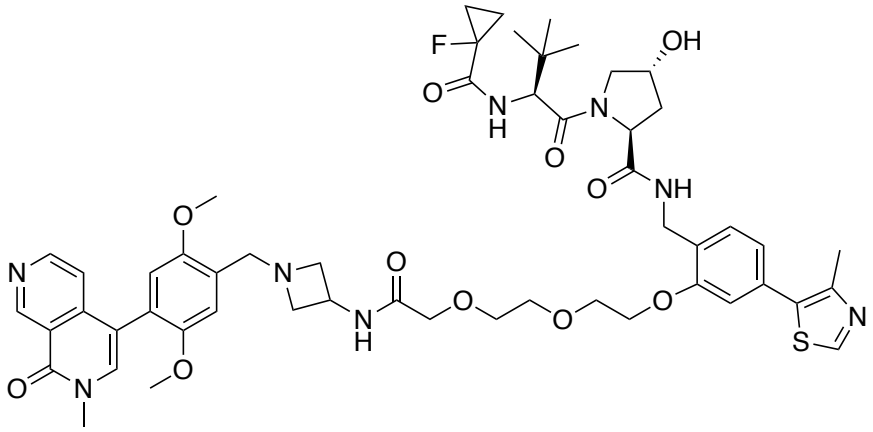
(2S,4R)-N-(2-(2-(2-(2-((1-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)azetidin-3-yl)amino)-2-oxoethoxy)ethoxy)ethoxy)-4-(4-

methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (57).



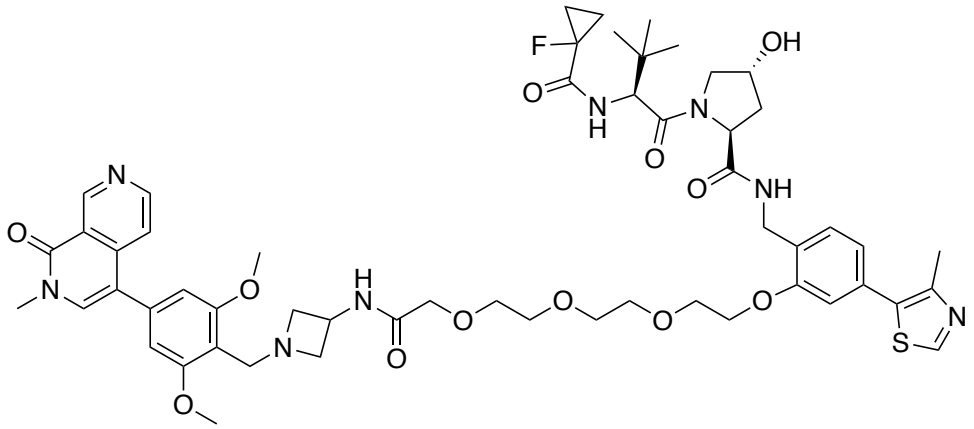
Following general method B, from **98** (0.03mmol) and **1e** (0.03mmol) compound **57** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of ammonia as white powder. Yield: 4.2 mg, 13%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.56 (d, *J* = 0.4 Hz, 1H), 8.91 (s, 1H), 8.71 (d, *J* = 5.8 Hz, 1H), 7.79 (s, 1H), 7.63 (d, *J* = 5.8 Hz, 1H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 1.3 Hz, 1H), 7.07 (dd, *J* = 1.6, 7.9 Hz, 1H), 6.87 (s, 2H), 4.78 (s, 1H), 4.71 - 4.64 (m, 2H), 4.56 - 4.30 (m, 11H), 4.11 (s, 2H), 4.01 - 3.97 (m, 8H), 3.92 - 3.79 (m, 6H), 3.73 (s, 3H), 2.52 (s, 3H), 2.31 - 2.25 (m, 1H), 2.16 - 2.09 (m, 1H), 1.42 - 1.30 (m, 4H), 1.08 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.3, 173.3, 171.7, 166.4, 163.0, 160.7, 157.9, 152.9, 151.8, 151.3, 149.2, 143.3, 140.4, 139.2, 133.4, 133.0, 129.9, 128.3, 123.0, 121.7, 119.1, 118.6, 113.7, 106.8, 80.3, 78.0, 71.9, 71.7, 71.2, 71.0, 70.9, 69.3, 60.9, 60.8, 58.7, 58.2, 56.9, 39.4, 39.0, 37.5, 37.3, 30.7, 26.9, 15.9, 14.1, 14.0, 13.9. HRMS *m/z* calcd for $C_{53}H_{65}FN_8O_{11}S$ 1040.45, found 1041.4976 [M + H^+].

(2S,4R)-N-(2-(2-(2-(2-((1-(2,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)azetidin-3-yl)amino)-2-oxoethoxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (58).



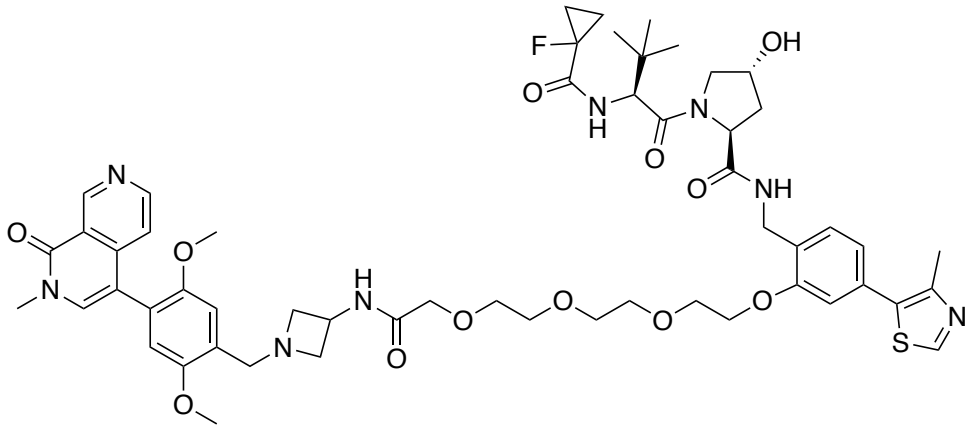
Following general method B, from **98** (0.03mmol) and **1f** (0.03mmol) compound **58** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of ammonia as white powder. Yield: 3.5 mg, 11%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.52 (d, *J* = 0.7 Hz, 1H), 8.91 (s, 1H), 8.62 (d, *J* = 5.7 Hz, 1H), 7.66 (s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.20 (s, 1H), 7.17 (dd, *J* = 0.8, 5.7 Hz, 1H), 7.12 - 7.06 (m, 3H), 5.53 (s, 1OH), 4.79 (d, *J* = 8.9 Hz, 1H), 4.69 - 4.64 (m, 2H), 4.56 - 4.41 (m, 5H), 4.34 - 4.27 (m, 4H), 4.23 - 4.17 (m, 2H), 4.11 (s, 2H), 4.00 (dd, *J* = 3.8, 5.2 Hz, 2H), 3.92 (s, 3H), 3.88 - 3.79 (m, 6H), 3.75 (s, 3H), 3.71 (s, 3H), 2.52 (s, 3H), 2.31 - 2.25 (m, 1H), 2.16 - 2.09 (m, 1H), 1.44 - 1.31 (m, 4H), 1.08 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.3, 171.8, 163.3, 157.9, 153.4, 153.0, 152.9, 151.4, 150.6, 149.2, 143.9, 139.5, 133.5, 133.0, 129.9, 128.3, 123.0, 121.5, 120.1, 116.0, 115.9, 115.7, 113.8, 78.1, 71.9, 71.7, 71.2, 71.0, 70.9, 69.3, 61.0, 60.8, 58.7, 58.2, 56.8, 56.6, 56.1, 49.9, 40.8, 39.4, 39.0, 37.4, 37.3, 26.9, 15.9, 14.1, 14.0, 13.9. HRMS *m/z* calcd for $C_{53}H_{65}FN_8O_{11}S$ 1040.45, found 1041.4680 [M + H^+].

(2S,4R)-N-(2-(2-(2-(2-(2-((1-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)azetidin-3-yl)amino)-2-oxoethoxy)ethoxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (59).



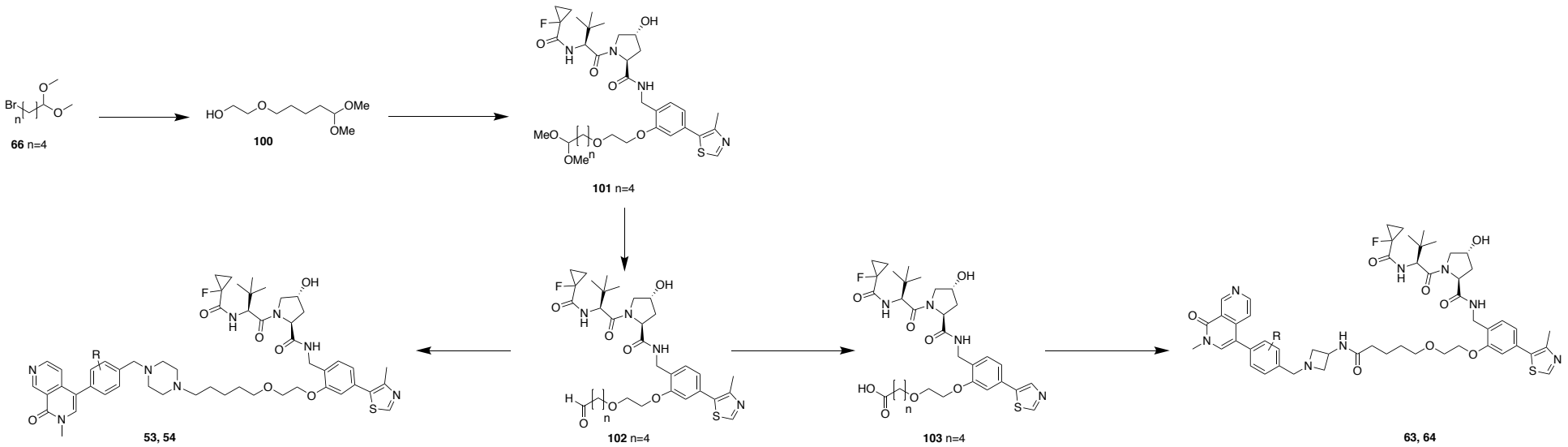
Following general method B, from **99** (0.02mmol) and **1e** (0.02mmol) compound **59** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of ammonia as white powder. Yield: 3.7 mg, 17%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.46 (d, *J* = 0.7 Hz, 1H), 8.80 (s, 1H), 8.61 (d, *J* = 5.8 Hz, 1H), 7.68 (s, 1H), 7.52 (dd, *J* = 0.6, 5.8 Hz, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 6.98 (d, *J* = 1.5 Hz, 1H), 6.95 (dd, *J* = 1.5, 7.8 Hz, 1H), 6.76 (s, 2H), 4.69 (s, 1H), 4.64 - 4.54 (m, 2H), 4.46 - 4.23 (m, 10H), 4.18 (dd, *J* = 3.3, 5.2 Hz, 2H), 3.97 (s, 2H), 3.87 (s, 6H), 3.82 - 3.71 (m, 4H), 3.69 - 3.63 (m, 10H), 2.40 (s, 3H), 2.21 - 2.15 (m, 1H), 2.06 - 1.98 (m, 1H), 1.33 - 1.19 (m, 4H), 0.97 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.3, 173.3, 171.7, 166.8, 163.0, 160.7, 157.9, 152.9, 151.8, 151.3, 149.1, 143.3, 140.4, 139.2, 133.4, 132.9, 129.8, 128.3, 122.9, 121.7, 119.0, 118.6, 113.7, 106.8, 80.3, 71.9, 71.7, 71.4, 71.2, 71.0, 70.8, 69.4, 61.0, 60.8, 58.7, 58.2, 56.9, 40.4, 39.4, 39.0, 37.5, 37.3, 26.9, 16.0, 14.1, 14.0, 13.9. HRMS *m/z* calcd for $C_{55}H_{69}FN_8O_{12}S$ 1084.47, found 1085. 5317 [M + H^+].

(2S,4R)-N-(2-(2-(2-(2-(2-((1-(2,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)azetidin-3-yl)amino)-2-oxoethoxy)ethoxy)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (60).

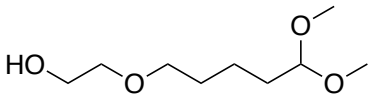


Following general method B, from **99** (0.02mmol) and **1f** (0.02mmol) compound **60** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of ammonia as white powder. Yield: 4.2 mg, 19%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.42 (s, 1H), 8.80 (s, 1H), 8.51 (d, *J* = 5.7 Hz, 1H), 7.56 (s, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.11 (s, 1H), 7.06 (dd, *J* = 0.5, 5.7 Hz, 1H), 7.01 - 6.98 (m, 2H), 6.96 (dd, *J* = 1.5, 7.7 Hz, 1H), 4.68 (s, 1H), 4.61 - 4.54 (m, 2H), 4.45 - 4.17 (m, 10H), 3.98 (s, 2H), 3.86 (dd, *J* = 3.8, 5.3 Hz, 2H), 3.83 - 3.78 (m, 5H), 3.76 - 3.71 (m, 3H), 3.69 - 3.65 (m, 9H), 3.61 (s, 3H), 2.41 (s, 3H), 2.21 - 2.15 (m, 1H), 2.06 - 1.99 (m, 1H), 1.34 - 1.19 (m, 4H), 0.97 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.3, 173.4, 171.7, 171.6, 171.3, 166.5, 163.2, 157.9, 153.4, 153.0, 152.9, 151.4, 150.6, 149.2, 143.8, 139.5, 133.4, 132.9, 129.9, 128.3, 127.2, 122.9, 121.5, 120.8, 120.0, 116.1, 115.5, 113.7, 80.3, 78.0, 71.9, 71.7, 71.4, 71.2, 71.0, 70.8, 69.4, 60.9, 60.8, 58.7, 58.2, 56.8, 56.6, 55.8, 40.7, 39.4, 39.0, 37.4, 37.3, 26.9, 16.0, 14.1, 14.0, 13.9. HRMS *m/z* calcd for $C_{55}H_{69}FN_8O_{12}S$ 1084.47, found 1085.5013 [M + H^+].

Synthesis compounds 53, 54, 63 and 64.

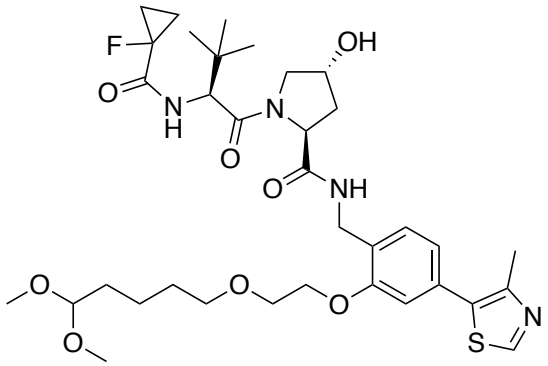


2-((5,5-dimethoxypentyl)oxy)ethan-1-ol (100).



Following general method H, compound **100** was obtained after reaction between ethylene glycol and 5-bromo-1,1-dimethoxypentane (**66**) as an oil. Yield: 66 mg, 36%. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 4.30 (t, *J* = 5.8 Hz, 1H), 3.65 (t, *J* = 4.6 Hz, 2H), 3.46 (t, *J* = 4.7 Hz, 2H), 3.41 (t, *J* = 6.6 Hz, 2H), 3.25 (s, 6H), 1.58 - 1.52 (m, 4H), 1.38 - 1.29 (m, 2H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 104.4, 71.9, 71.1, 61.8, 52.7, 32.3, 29.4, 21.2. MS *m/z* calcd for $C_9H_{20}O_4$ 192.14.

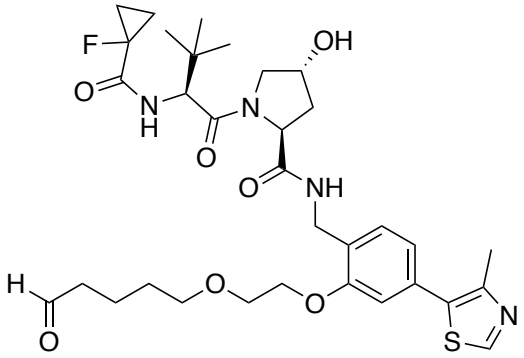
(2S,4R)-N-(2-(2-((5,5-dimethoxypentyl)oxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (101).



Following general method E, compound **101** was obtained from compounds **100** and **2d** as white powder. Yield: 64.7 mg, 70%. ^{1}H-NMR (400 MHz, MeOD) δ: 8.91 (s, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.10 (d, *J* = 1.6 Hz, 1H), 7.06 (dd, *J* = 1.6, 7.7 Hz, 1H), 4.79 (s, 1H), 4.65 (d, *J* = 7.6 Hz, 1H), 4.57 - 4.42 (m, 3H), 4.38 (t, *J* = 5.6 Hz, 1H), 4.29 - 4.26 (m, 2H), 3.91 - 3.84 (m, 4H), 3.62 (t, *J* = 6.3 Hz, 2H), 3.33 (s, 6H), 2.54 (s, 3H), 2.30 - 2.22 (m, 1H), 2.18 - 2.11 (m, 1H), 1.71 - 1.60 (m, 4H), 1.50 - 1.44 (m, 2H), 1.39 - 1.30 (m, 4H), 1.08 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.2, 171.7, 158.1, 133.5, 132.9, 131.3, 129.9, 129.2, 128.4,

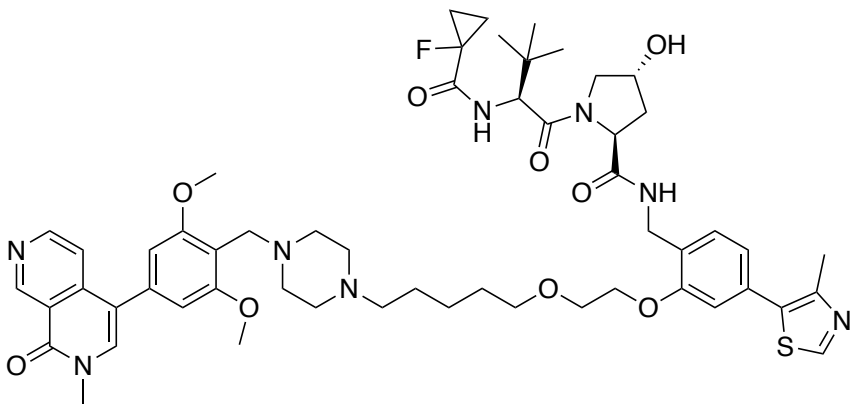
122.8, 113.8, 106.2, 80.3, 72.3, 71.1, 70.5, 69.4, 60.8, 58.7, 58.1, 53.4, 39.4, 38.9, 37.3, 33.4, 30.5, 26.9, 22.3, 15.9, 14.0. MS *m/z* calcd for $C_{35}H_{51}FN_4O_8S$ 706.34, found 707.3 [M + H^+].

(2S,4R)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)-2-(2-((5-oxopentyl)oxy)ethoxy)benzyl)pyrrolidine-2-carboxamide (102).



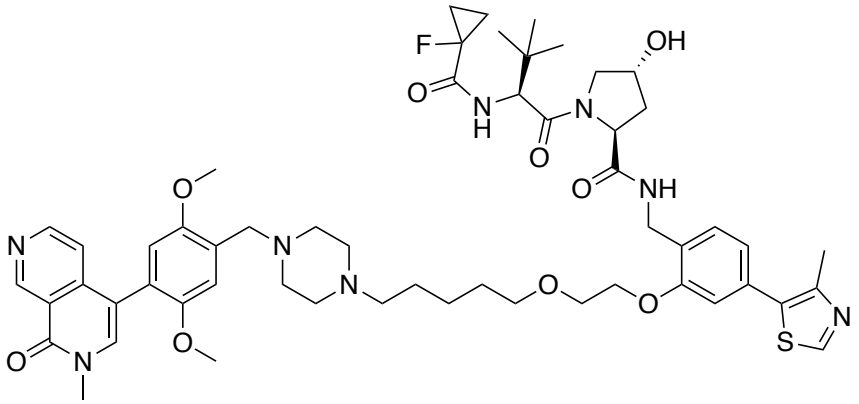
Following general method F, compound **102** was obtained from **100** (0.08mmol) and directly used in the next step without any further purification. MS *m/z* calcd for $C_{33}H_{45}FN_4O_7S$ 660.30, found 661.3 [M + H^+].

(2S,4R)-N-(2-(2-((5-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)pentyl)oxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (53).



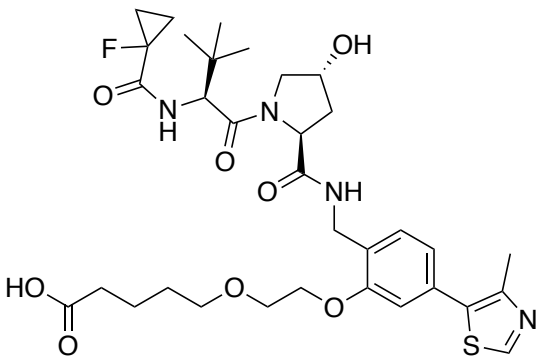
Following general method C, from **102** (0.015mmol) and **1c** (0.015mmol) compound **53** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid as white powder. Yield: 3.7 mg, 24%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.56 (d, *J* = 0.5 Hz, 1H), 8.92 (s, 1H), 8.70 (d, *J* = 5.7 Hz, 1H), 7.78 (s, 1H), 7.64 (dd, *J* = 0.6, 5.7 Hz, 1H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.11 - 7.05 (m, 2H), 6.85 (s, 2H), 4.79 (d, *J* = 9.2 Hz, 1H), 4.66 (t, *J* = 8.2 Hz, 1H), 4.56 - 4.43 (m, 3H), 4.29 - 4.26 (m, 2H), 4.18 (s, 2H), 3.95 (s, 6H), 3.92 - 3.81 (m, 4H), 3.74 (s, 3H), 3.65 (t, *J* = 6.2 Hz, 2H), 3.18 - 2.90 (m, 8H), 2.78 (s, 2H), 2.53 (s, 3H), 2.31 - 2.25 (m, 1H), 2.18 - 2.11 (m, 1H), 1.74 - 1.64 (m, 4H), 1.53 - 1.29 (m, 6H), 1.08 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.2, 171.7, 163.0, 161.0, 158.0, 152.9, 151.8, 151.2, 149.2, 143.5, 139.1, 133.5, 132.9, 129.8, 128.3, 122.8, 121.7, 119.1, 118.9, 113.8, 106.7, 80.3, 72.1, 71.0, 70.5, 69.4, 60.8, 58.8, 58.2, 56.7, 52.2, 51.8, 39.4, 39.0, 37.4, 37.3, 30.3, 26.9, 24.7, 15.9, 14.1, 14.0, 13.9. HRMS *m/z* calcd for $C_{55}H_{71}FN_8O_9S$ 1038.50, found 1039.5288 [M + H^+].

(2S,4R)-N-(2-(2-((5-(4-(2,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)pentyl)oxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (54).



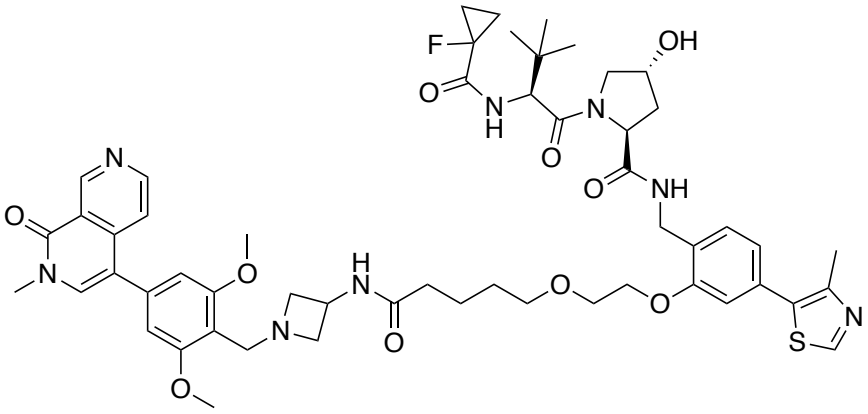
Following general method C, from **102** (0.015mmol) and **1d** (0.015mmol) compound **54** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid as white powder. Yield: 1.8 mg, 11%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.57 (s, 1H), 8.93 (s, 1H), 8.64 (d, *J* = 6.1 Hz, 1H), 7.80 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 6.0 Hz, 1H), 7.23 (s, 1H), 7.11 - 7.08 (m, 2H), 7.06 (s, 1H), 4.79 (d, *J* = 10.4 Hz, 1H), 4.66 (t, *J* = 8.2 Hz, 1H), 4.55 - 4.44 (m, 3H), 4.29 - 4.27 (m, 2H), 3.95 - 3.81 (m, 9H), 3.75 - 3.64 (m, 8H), 3.18 - 2.90 (m, 10H), 2.53 (s, 3H), 2.32 - 2.26 (m, 1H), 2.18 - 2.11 (m, 1H), 1.81 - 1.69 (m, 4H), 1.57 - 1.29 (m, 6H), 1.09 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.3, 171.8, 162.7, 161.2, 158.0, 156.5, 153.7, 152.9, 152.8, 149.5, 149.1, 147.1, 145.8, 141.7, 133.5, 129.7, 128.3, 122.9, 121.9, 121.3, 115.9, 115.6, 113.7, 80.3, 72.0, 71.0, 70.6, 69.4, 60.9, 58.7, 58.2, 58.0, 56.8, 56.5, 56.1, 52.6, 51.2, 43.4, 39.4, 39.0, 37.5, 37.3, 30.1, 26.9, 25.2, 24.6, 15.9, 14.1, 14.0. HRMS *m/z* calcd for $C_{55}H_{71}FN_8O_9S$ 1038.50, found 1039.5305 [M + H^+].

5-(2-(2-(((2S,4R)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)ethoxy)pentanoic acid (103).



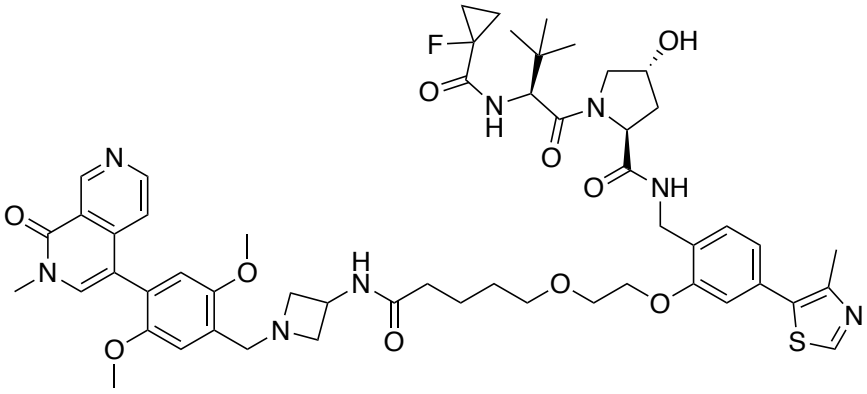
Following general method G, compound **103** was obtained from compound **102** (0.04mmol) and was used in the next step without any further purification. Quantitative yield. MS *m/z* calcd for $C_{33}H_{45}FN_4O_8S$ 676.29, found 677.3 [M + H^+].

(2S,4R)-N-(2-(2-((5-((1-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)azetidin-3-yl)amino)-5-oxopentyl)oxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (63).



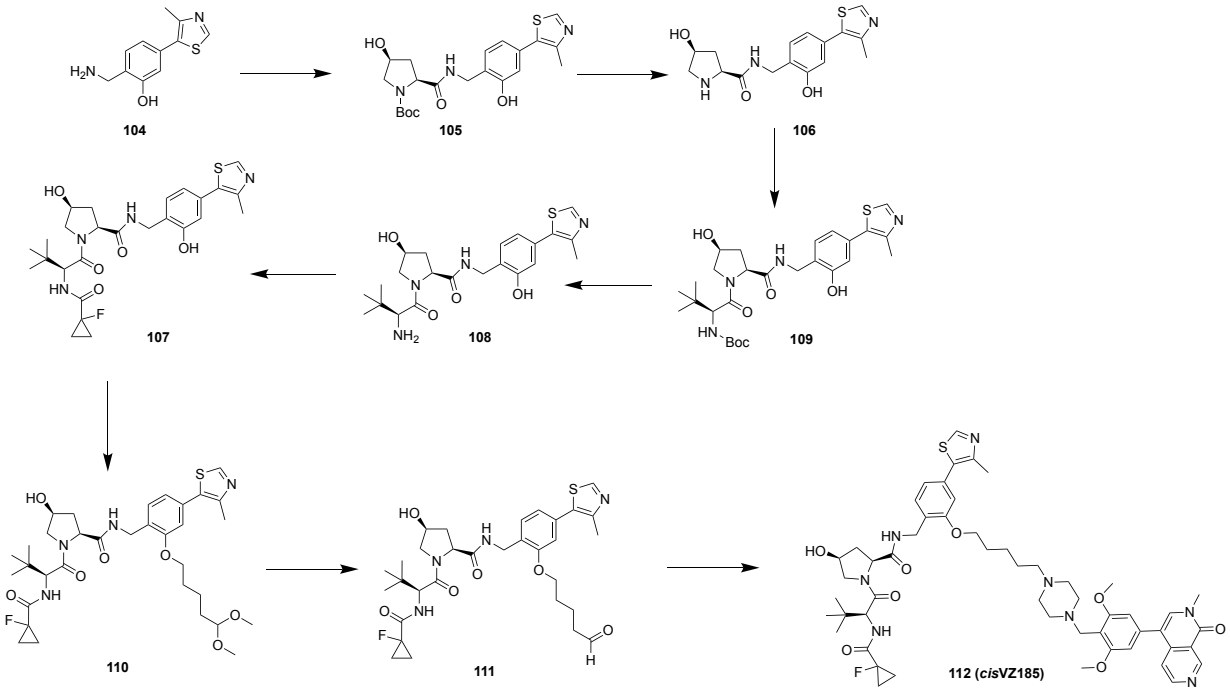
Following general method B, from **103** (0.02mmol) and **1e** (0.02mmol) compound **63** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid as white powder. Yield: 1.6 mg, 7%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.55 (d, *J* = 0.7 Hz, 1H), 8.90 (s, 1H), 8.71 (d, *J* = 5.8 Hz, 1H), 7.77 (s, 1H), 7.64 (dd, *J* = 0.6, 5.7 Hz, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.08 (d, *J* = 1.4 Hz, 1H), 7.05 (dd, *J* = 1.5, 7.8 Hz, 1H), 6.83 (s, 2H), 4.79 (s, 1H), 4.66 (t, *J* = 8.2 Hz, 1H), 4.55 - 4.43 (m, 4H), 4.29 - 4.20 (m, 4H), 4.08 (s, 2H), 3.95 - 3.62 (m, 17H), 2.52 (s, 3H), 2.30 - 2.23 (m, 3H), 2.14 (t, *J* = 13.3 Hz, 1H), 1.78 - 1.64 (m, 4H), 1.45 - 1.31 (m, 4H), 1.08 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 176.1, 174.2, 171.7, 163.0, 160.7, 158.0, 152.8, 151.8, 151.3, 149.1, 143.5, 139.0, 133.5, 132.9, 129.9, 128.3, 122.8, 121.7, 119.2, 119.0, 113.8, 106.7, 80.3, 72.1, 71.1, 70.5, 69.4, 61.3, 60.8, 58.7, 58.2, 56.6, 41.1, 39.4, 39.0, 37.4, 37.3, 36.5, 30.2, 29.5, 26.9, 23.5, 15.9, 14.1, 14.0, 13.9. HRMS *m/z* calcd for $C_{54}H_{67}FN_8O_{10}S$ 1038.47, found 1039.3641 [M + H^+].

(2S,4R)-N-(2-(2-((5-((1-(2,5-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)azetidin-3-yl)amino)-5-oxopentyl)oxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (64).

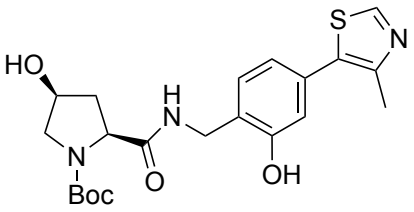


Following general method B, from **103** (0.02mmol) and **1f** (0.02mmol) compound **64** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid as white powder. Yield: 2.1 mg, 10%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.52 (d, *J* = 0.7 Hz, 1H), 8.91 (s, 1H), 8.62 (d, *J* = 5.8 Hz, 1H), 7.64 (s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.19 (dd, *J* = 0.6, 5.7 Hz, 1H), 7.14 (s, 1H), 7.09 (d, *J* = 1.4 Hz, 1H), 7.06 (dd, *J* = 1.6, 7.8 Hz, 1H), 7.01 (s, 1H), 4.78 (s, 1H), 4.66 (t, *J* = 8.4 Hz, 1H), 4.56 - 4.43 (m, 5H), 4.28 - 4.24 (m, 2H), 4.06 - 3.96 (m, 4H), 3.91 - 3.81 (m, 8H), 3.74 (s, 3H), 3.71 (s, 3H), 3.64 (t, *J* = 6.1 Hz, 2H), 2.52 (s, 3H), 2.31 - 2.24 (m, 3H), 2.20 - 2.11 (m, 1H), 1.79 - 1.64 (m, 4H), 1.45 - 1.31 (m, 4H), 1.07 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.2, 171.7, 163.3, 158.0, 153.1, 152.8, 151.4, 150.6, 149.1, 144.0, 139.3, 133.4, 132.9, 129.9, 128.3, 122.8, 121.5, 120.3, 120.2, 116.1, 115.7, 115.1, 113.8, 80.5, 78.0, 72.1, 71.1, 70.5, 69.4, 61.7, 60.8, 60.2, 58.7, 58.2, 56.6, 56.5, 41.4, 39.4, 39.0, 37.4, 37.3, 36.6, 30.2, 29.5, 26.9, 23.6, 15.9, 14.1. HRMS *m/z* calcd for $C_{54}H_{67}FN_8O_{10}S$ 1038.47, found 1039.3611 [M + H^+].

Synthesis of compound *cis*VZ185.



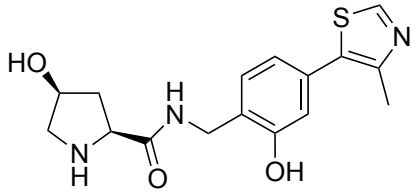
***tert*-butyl(2*S*,4*S*)-4-hydroxy-2-((2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamoyl) pyrrolidine-1-carboxylate (105)**



Following general method B, from (2-(aminomethyl)-5-(4-methylthiazol-5-yl)phenol (**104**, synthetized accordingly to literature[2]) and *N*-Boc-cis-4-hydroxy-L-proline, compound **105** was purified by flash column chromatography using a gradient from 0% to 20% of methanol in DCM. Yield: 66%, 200 mg. ^{1}H-NMR (400 MHz, MeOD) δ: 8.89 (s, 1H), 7.36 (d, *J* = 7.5 Hz, 1H), 6.97 - 6.93 (m, 2H), 4.54 - 4.48 (m, 1H), 4.43 - 4.34 (m, 2H), 4.29 - 4.25 (m, 1H), 3.81 - 3.73 (m, 1H), 3.63 - 3.58 (m, 1H), 3.51 - 3.47 (m, 1H), 2.52 (s, 3H), 2.50 - 2.44 (m, 1H), 2.09 - 2.04 (m, 1H), 1.32 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 176.1, 156.9, 152.7,

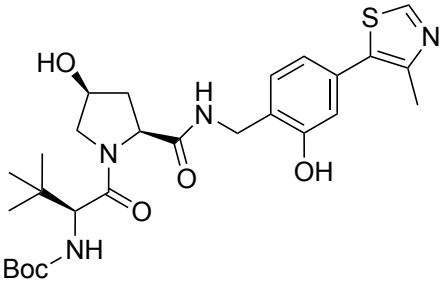
151.6, 133.2, 131.5, 125.9, 121.3, 117.2, 81.7, 70.3, 60.9, 55.7, 43.7, 39.6, 28.4, 13.1. MS *m/z* calcd for $C_{21}H_{27}N_3O_5S$ 433.52, found 434.2 [M + H^+].

(2*S*,4*S*)-4-hydroxy-*N*-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (106)



A mixture of **105** (0.46 mmol) and HCl 4N in dioxane (2 mL) in DCM (2 mL) was stirred at rt for 1.5 h. The solvents were evaporated and the product was freeze dried. Quantitative yield. ^{1}H-NMR (400 MHz, MeOD) δ: 9.93 (s, 1H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.07 - 7.04 (m, 2H), 4.61 - 4.56 (m, 1H), 4.56 - 4.53 (m, 2H), 4.48 - 4.43 (m, 1H), 3.81 - 3.73 (m, 1H), 3.43 - 3.41 (m, 1H), 2.71 - 2.62 (m, 4H), 2.25 - 2.19 (m, 1H); ^{13}C-NMR (101 MHz, MeOD) δ: 169.9, 157.3, 156.2, 152.2, 137.2, 131.3, 129.8, 127.8, 121.4, 116.6, 70.3, 60.0, 54.5, 39.9, 39.5, 13.4. MS *m/z* calcd for $C_{16}H_{19}N_3O_3S$ 333.41, found 334.2 [M + H^+].

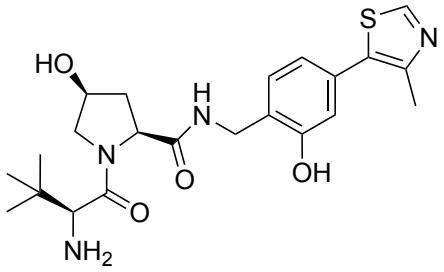
***tert*-butyl (1-((2*S*,4*S*)-4-hydroxy-2-((2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (107)**



Following general method B, from compound **106** and Boc-L-*tert*-leucine compound **107** was obtained and purified by flash column chromatography using a gradient from 0% to 20%

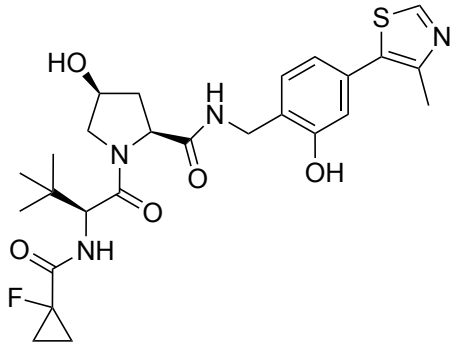
of methanol in DCM. Yield: 64%, 160 mg. ^{1}H-NMR (400 MHz, MeOD) δ: 8.88 (s, 1H), 7.38 (d, *J* = 7.7 Hz, 1H), 6.94 - 6.89 (m, 2H), 4.62 - 4.58 (m, 1H), 4.51 - 4.38 (m, 3H), 4.05 - 4.00 (m, 1H), 3.80 - 3.71 (m, 2H), 2.51 (s, 3H), 2.49 - 2.43 (m, 1H), 2.09 - 2.02 (m, 1H), 1.46 (s, 9H), 1.03 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.9, 173.1, 156.5, 152.6, 151.8, 148.6, 132.7, 130.5, 129.7, 125.8, 122.0, 121.3, 80.6, 71.5, 60.9, 60.1, 57.7, 43.7, 39.5, 37.5, 36.2, 28.6, 26.9, 13.1. MS *m/z* calcd for $C_{27}H_{38}N_4O_6S$ 546.25, found 547.3 [M + H^+].

(2*S*,4*S*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (108)



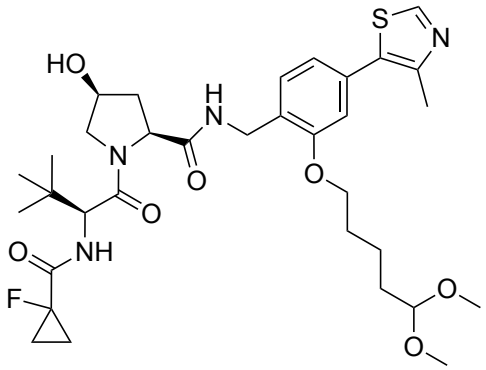
A mixture of **107** (0.3 mmol) and HCl 4N in dioxane (2 mL) in DCM (2 mL) was stirred at rt for 1.5 h. The solvents were evaporated and the product was freeze dried. Quantitative yield. ^{1}H-NMR (400 MHz, MeOD) δ: 9.93 (s, 1H), 7.51 (d, *J* = 7.7 Hz, 1H), 7.02 - 6.99 (m, 2H), 4.67 - 4.63 (m, 1H), 4.49 - 4.43 (m, 3H), 4.00 - 3.95 (m, 1H), 3.80 - 3.75 (m, 2H), 2.65 (s, 3H), 2.57 - 2.49 (m, 1H), 2.06 - 1.99 (m, 1H), 1.16 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.5, 168.7, 157.2, 156.2, 152.2, 131.0, 130.0, 129.4, 128.2, 122.2, 121.2, 116.5, 71.2, 61.0, 60.2, 57.2, 39.5, 37.9, 35.6, 26.7, 13.3. MS *m/z* calcd for $C_{22}H_{30}N_4O_4S$ 446.20, found 447.2 [M + H^+].

(2*S*,4*S*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (109)



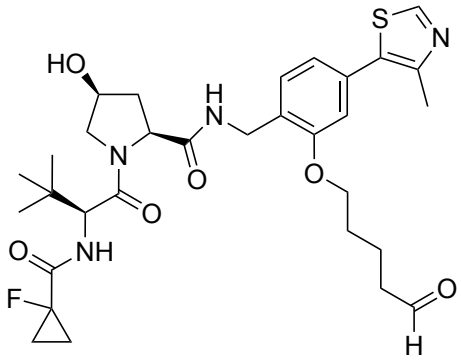
Following general method B, from compound **108** and 1-fluorocyclopropane-1-carboxylic acid, compound **109** was obtained and purified by flash column chromatography using a gradient from 0% to 20% of methanol in DCM. Yield: 65%, 72 mg. ^{1}H-NMR (400 MHz, MeOD) δ: 8.89 (s, 1H), 7.40 (d, *J* = 7.6 Hz, 1H), 6.95 - 6.92 (m, 2H), 4.71 (dd, *J* = 0.8, 9.0 Hz, 1H), 4.61 - 4.56 (m, 1H), 4.53 - 4.39 (m, 3H), 4.03 - 3.98 (m, 1H), 3.81 - 3.73 (m, 2H), 2.52 (s, 3H), 2.51 - 2.43 (m, 1H), 2.08 - 2.01 (m, 1H), 1.37 - 1.25 (m, 4H), 1.07 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.8, 171.9, 156.7, 152.7, 151.2, 148.8, 132.8, 130.6, 129.4, 125.9, 121.3, 116.7, 80.2, 77.9, 71.4, 61.0, 58.8, 57.7, 55.8, 43.8, 39.6, 37.7, 36.7, 18.7, 17.3, 15.9, 13.2. MS *m*/*z* calcd for $C_{26}H_{33}FN_4O_5S$ 532.63, found 533.3 [M + H^+].

(2*S*,4*S*)-*N*-(2-((5,5-dimethoxypentyl)oxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (110)



Following the same procedure applied for compound **68**, compound **110** was obtained from **109** and **66** as white powder. Yield: 61%, 48 mg. ^{1}H-NMR (400 MHz, MeOD) δ: 8.91 (s, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.04 - 7.02 (m, 2H), 4.72 (d, *J* = 8.8 Hz, 1H), 4.60 (dd, *J* = 4.3, 9.2 Hz, 1H), 4.56 - 4.41 (m, 4H), 4.12 (t, *J* = 6.2 Hz, 2H), 4.03 - 3.98 (m, 1H), 3.80 - 3.74 (m, 1H), 3.36 (s, 6H), 2.53 (s, 3H), 2.51 - 2.44 (m, 1H), 2.09 - 2.04 (m, 1H), 1.96 - 1.86 (m, 2H), 1.76 - 1.70 (m, 2H), 1.67 - 1.57 (m, 2H), 1.41 - 1.26 (m, 4H), 1.07 (s, 9H) ; ^{13}C-NMR (101 MHz, MeOD) δ: 174.7, 171.9, 158.0, 152.8, 149.1, 132.9, 129.8, 127.7, 122.4, 113.1, 106.1, 71.5, 69.2, 61.1, 58.7, 57.8, 53.4, 39.4, 37.7, 36.7, 33.4, 30.1, 26.9, 22.3, 15.9, 14.1, 14.0, 13.9. MS *m/z* calcd for $C_{33}H_{47}FN_4O_7S$ 662.31.

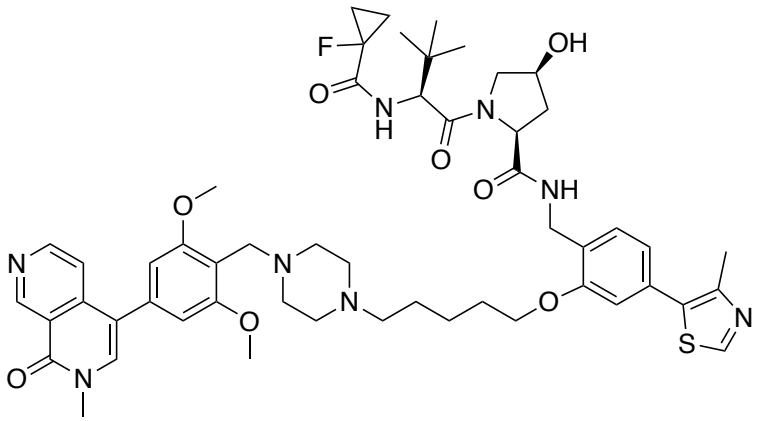
(2S,4S)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)-2-((5-oxopentyl)oxy)benzyl)pyrrolidine-2-carboxamide (111)



Following general method F, compound **111** was obtained from **110** (0.06 mmol) and directly used in the next step without any further purification. MS *m/z* calcd for $C_{31}H_{41}FN_4O_6S$ 616.27, found 617.4 [M + H^+].

(2S,4S)-N-(2-((5-(4-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)piperazin-1-yl)pentyl)oxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-

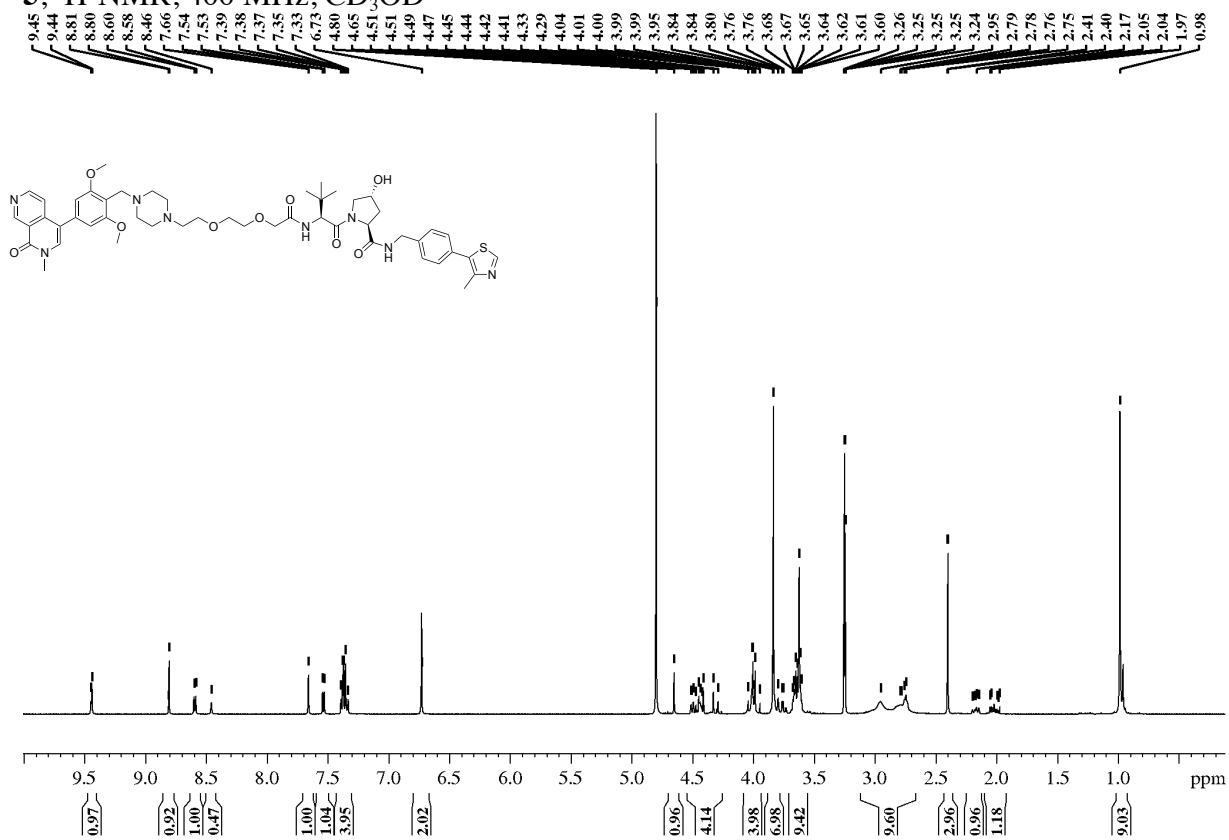
fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (112, *cis*VZ185).



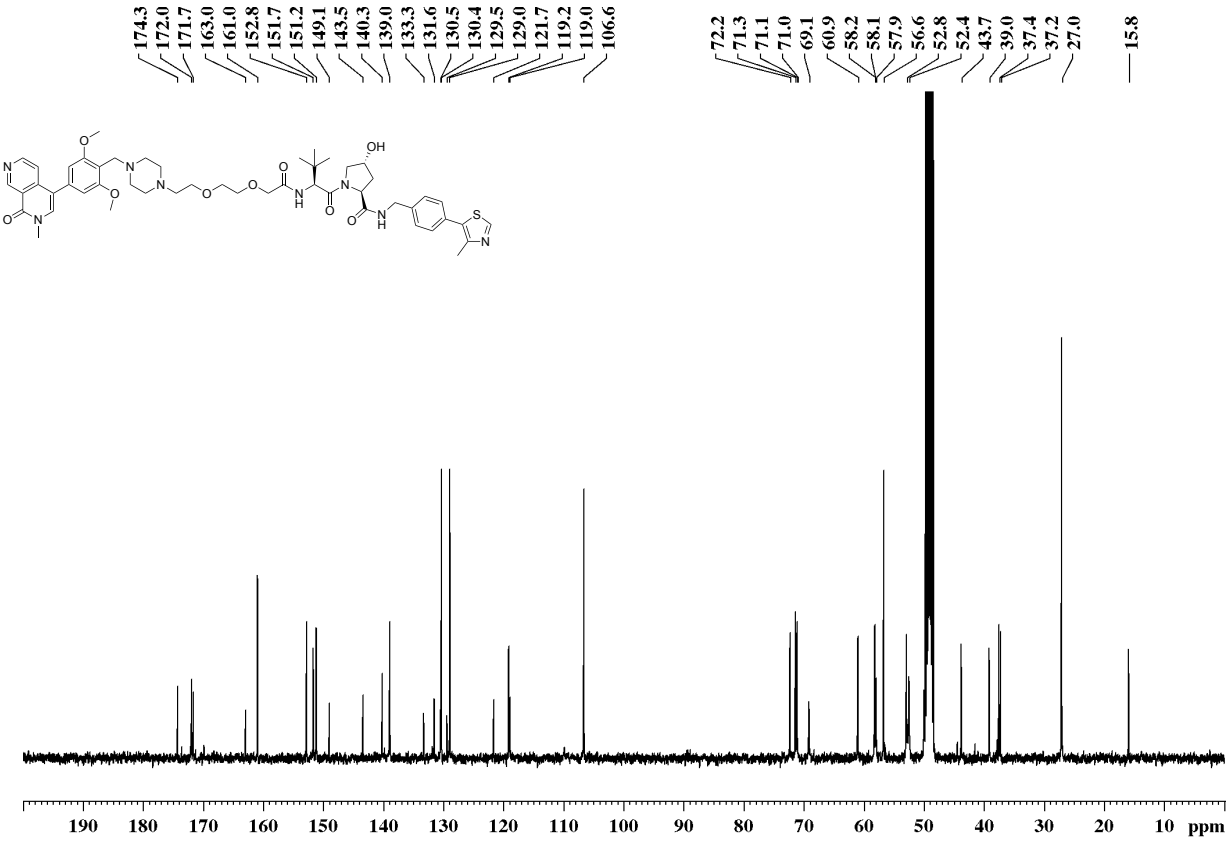
Following general method C, from **111** (0.06 mmol) and **1c** (0.06 mmol) compound **112** was obtained after purification by HPLC using a gradient of 5% to 95% v/v acetonitrile in 0.1% aqueous solution of formic acid as white powder. Yield: 4.5 mg, 7%. ^{1}H-NMR (400 MHz, MeOD) δ: 9.56 (s, 1H), 8.92 (s, 1H), 8.71 (d, *J* = 5.5 Hz, 1H), 7.76 (s, 1H), 7.65 (d, *J* = 5.7 Hz, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 7.05 - 7.02 (m, 2H), 6.80 (s, 2H), 4.71 (s, 1H), 4.60 (dd, *J* = 4.2, 9.2 Hz, 1H), 4.50 - 4.42 (m, 3H), 4.12 (t, *J* = 5.9 Hz, 2H), 4.02 - 3.86 (m, 10H), 3.74 (s, 3H), 2.84 - 2.44 (m, 14H), 2.05 (dt, *J* = 4.8, 8.9 Hz, 1H), 1.96 - 1.90 (m, 2H), 1.72 - 1.57 (m, 4H), 1.44 - 1.28 (m, 4H), 1.07 (s, 9H); ^{13}C-NMR (101 MHz, MeOD) δ: 174.7, 172.0, 163.1, 161.0, 158.1, 152.8, 151.7, 151.2, 149.1, 143.6, 138.9, 133.6, 133.0, 129.9, 127.7, 122.4, 121.7, 119.3, 113.1, 106.5, 80.3, 78.0, 71.5, 69.2, 61.2, 59.2, 58.7, 57.8, 56.4, 53.2, 53.0, 39.4, 37.8, 37.4, 36.7, 30.1, 26.9, 25.1, 15.9, 14.1, 14.0, 13.9. HRMS *m/z* calcd for $C_{53}H_{67}FN_8O_8S$ 994.48, found 995.5344 [M + H^+].

NMR Spectra

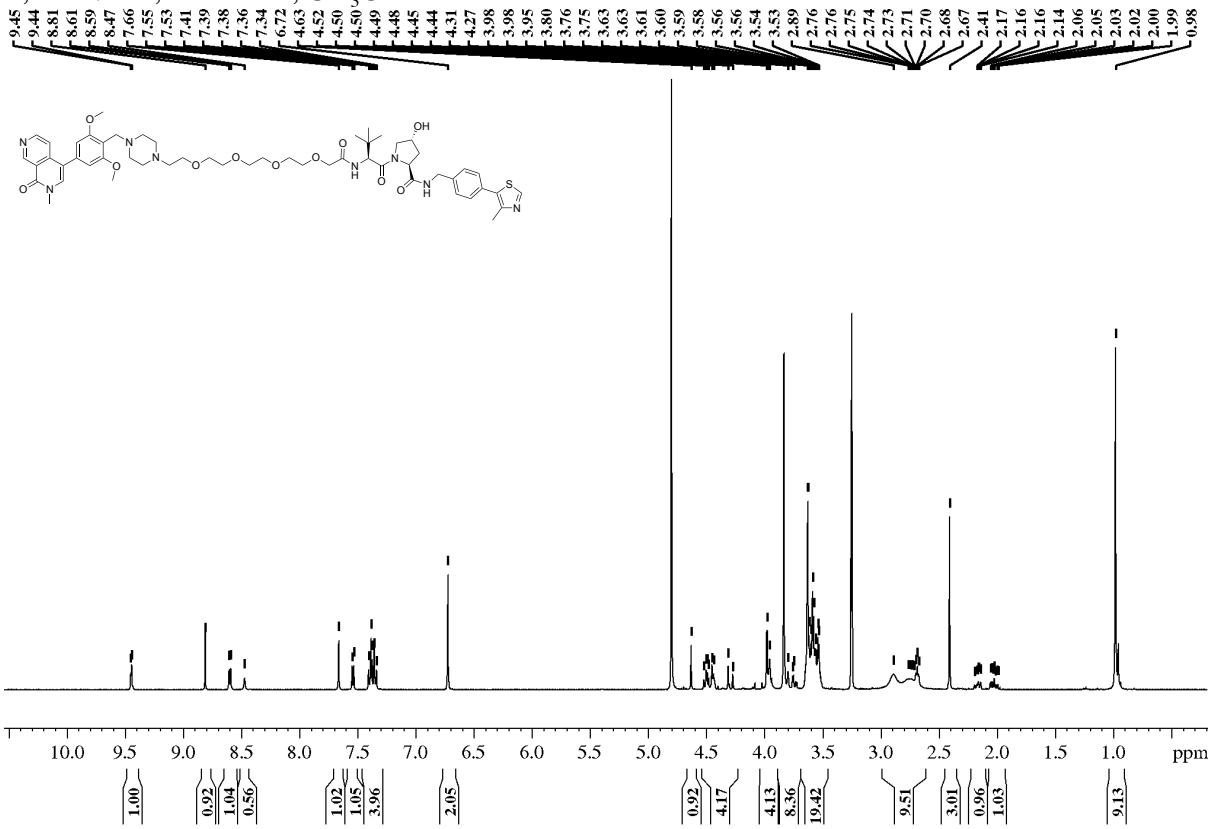
5, ^{1}H-NMR, 400 MHz, CD_3OD



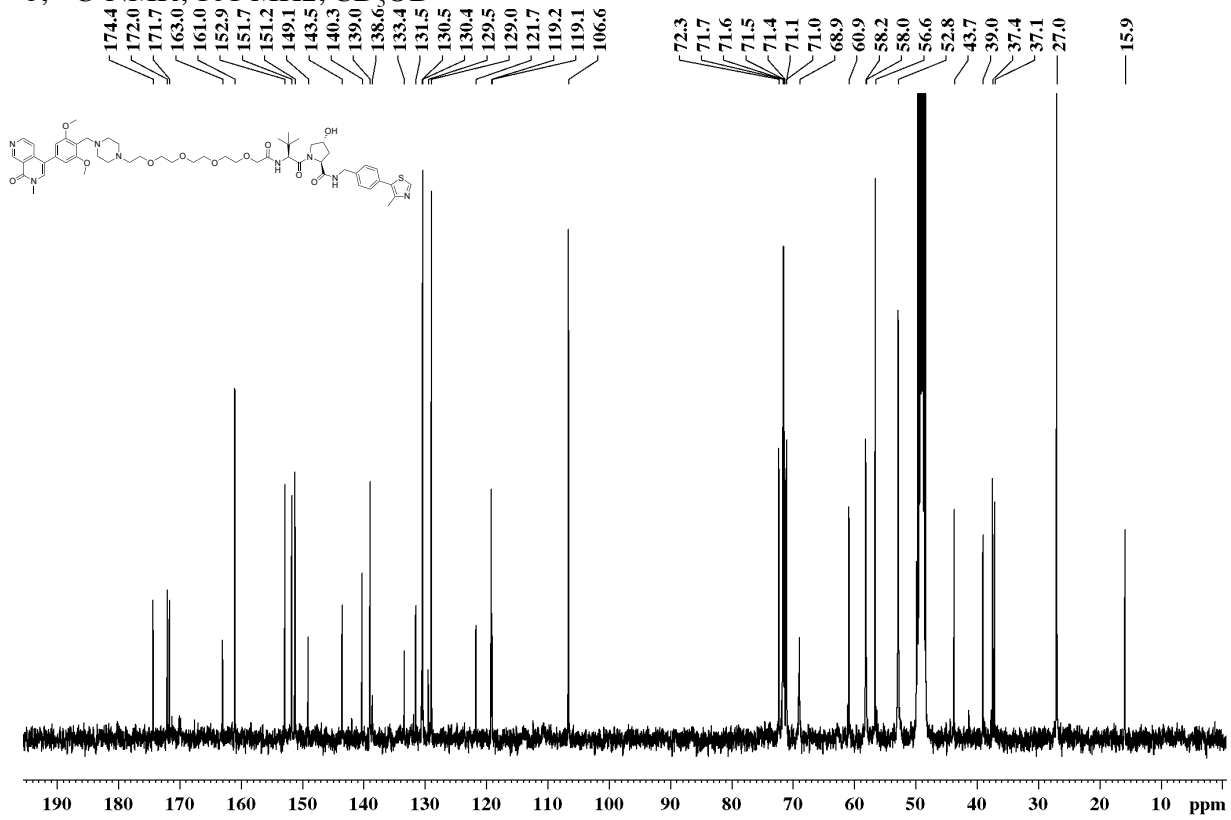
5, ^{13}C-NMR, 101 MHz, CD_3OD



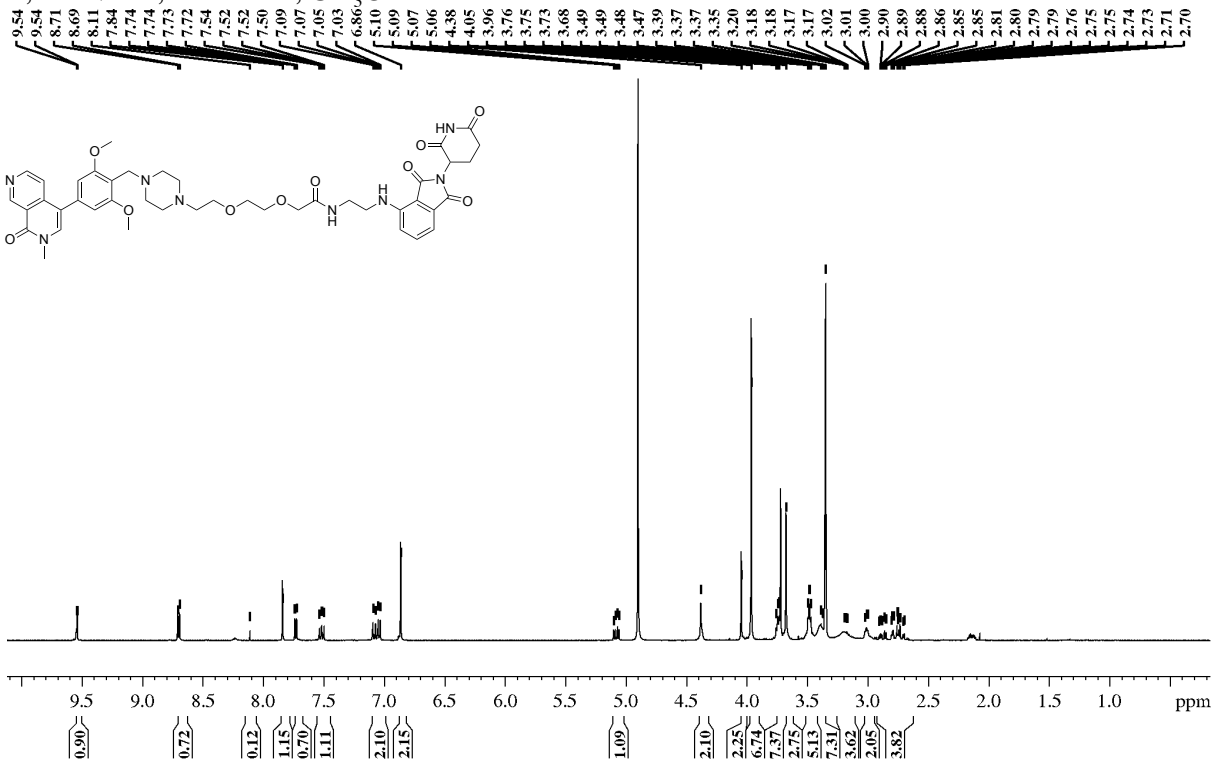
6, ^{1}H-NMR, 400 MHz, CD_3OD



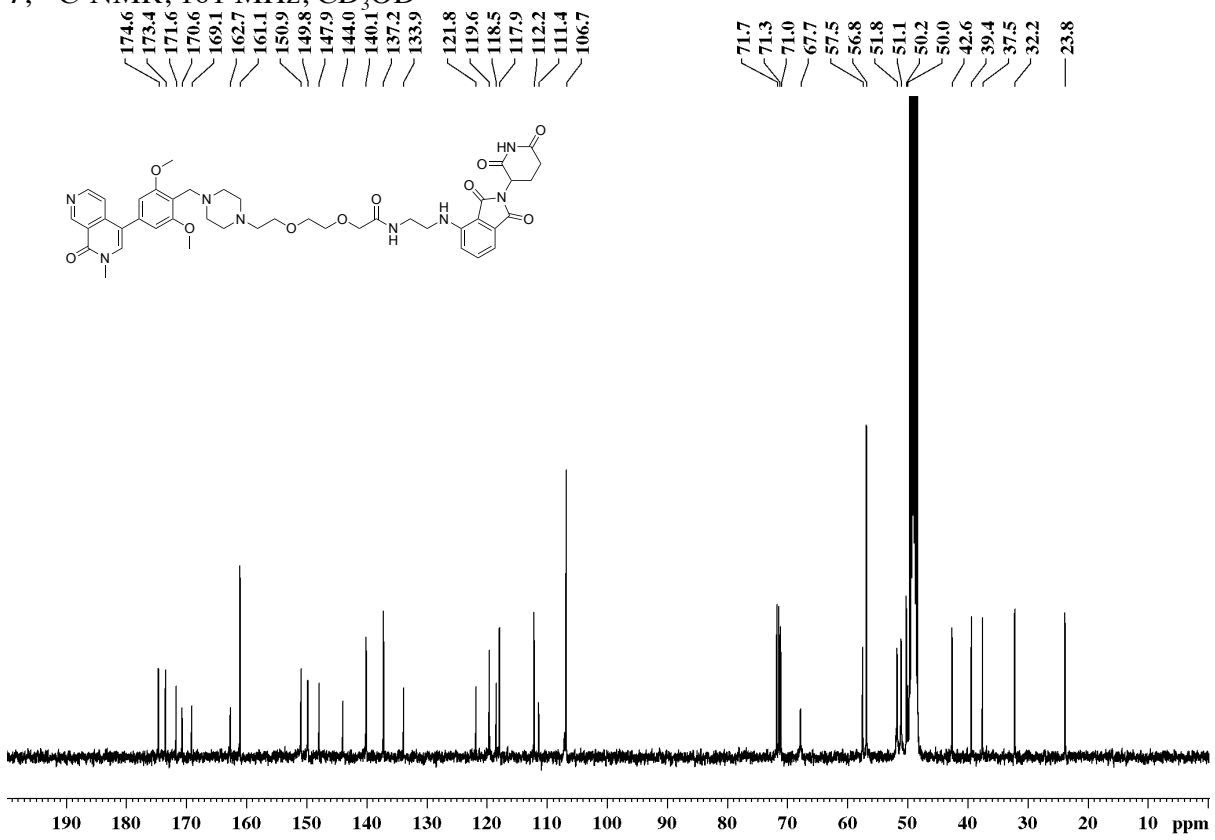
6, ^{13}C-NMR, 101 MHz, CD_3OD



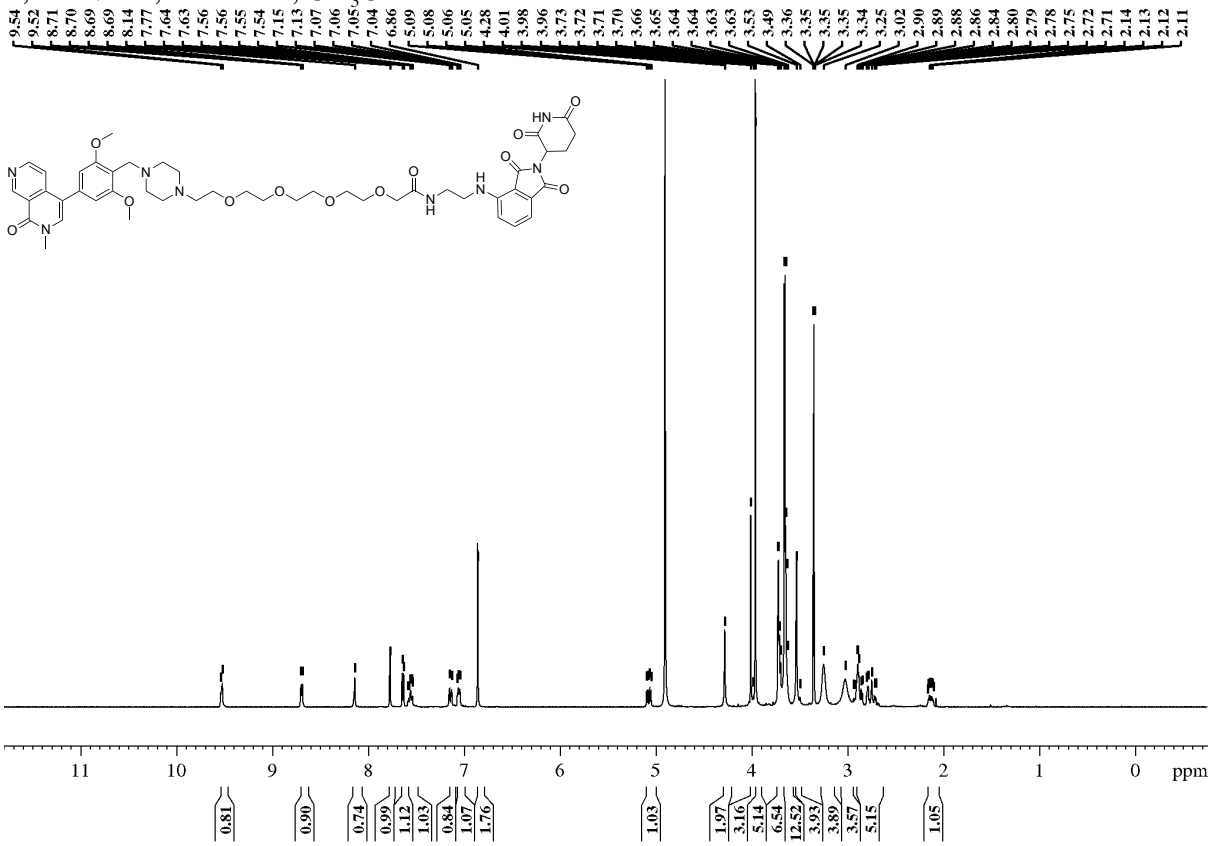
7, ^{1}H-NMR, 400 MHz, CD_3OD



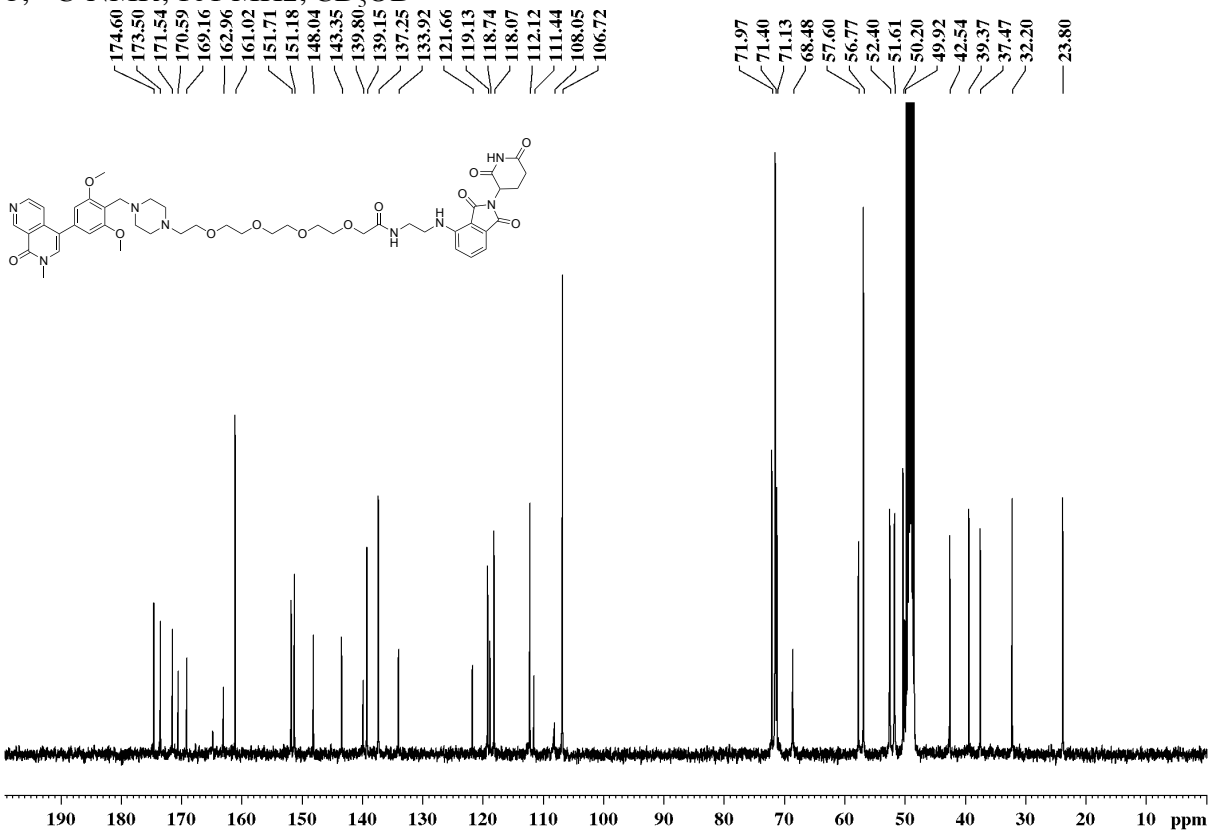
7, ^{13}C-NMR, 101 MHz, CD_3OD



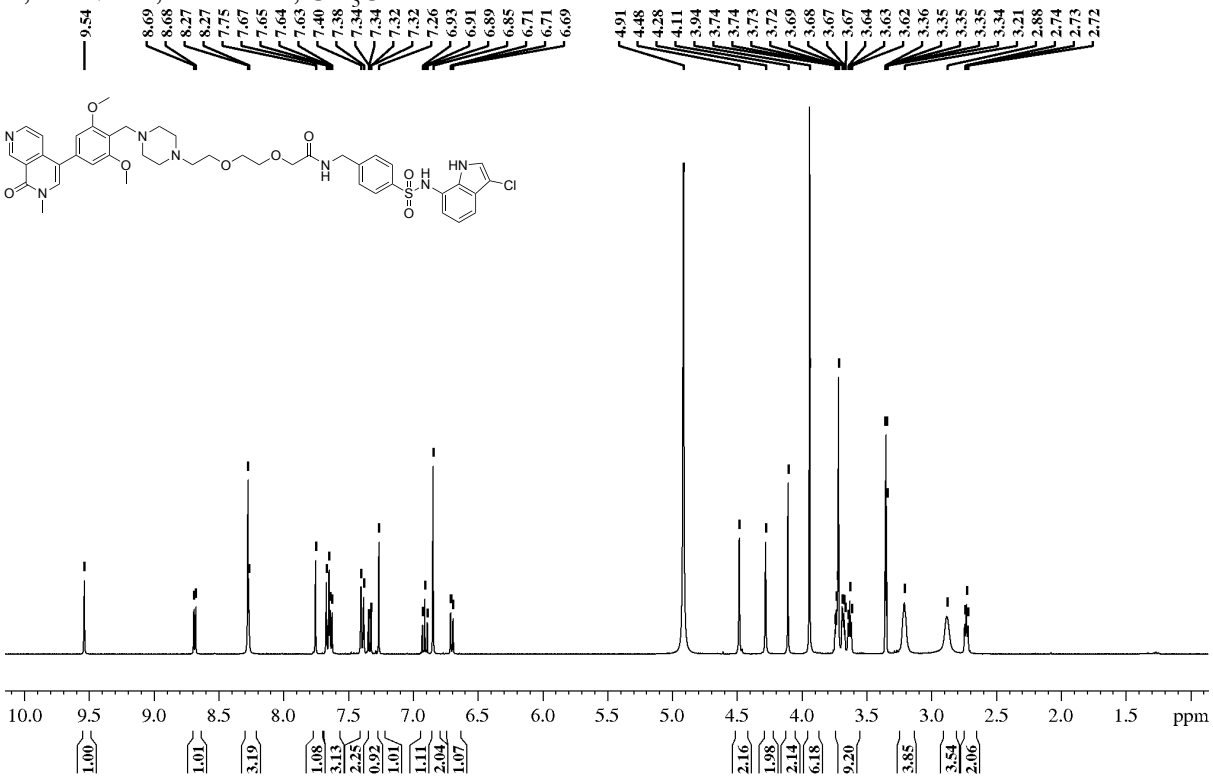
8, ^{1}H-NMR, 400 MHz, CD_3OD



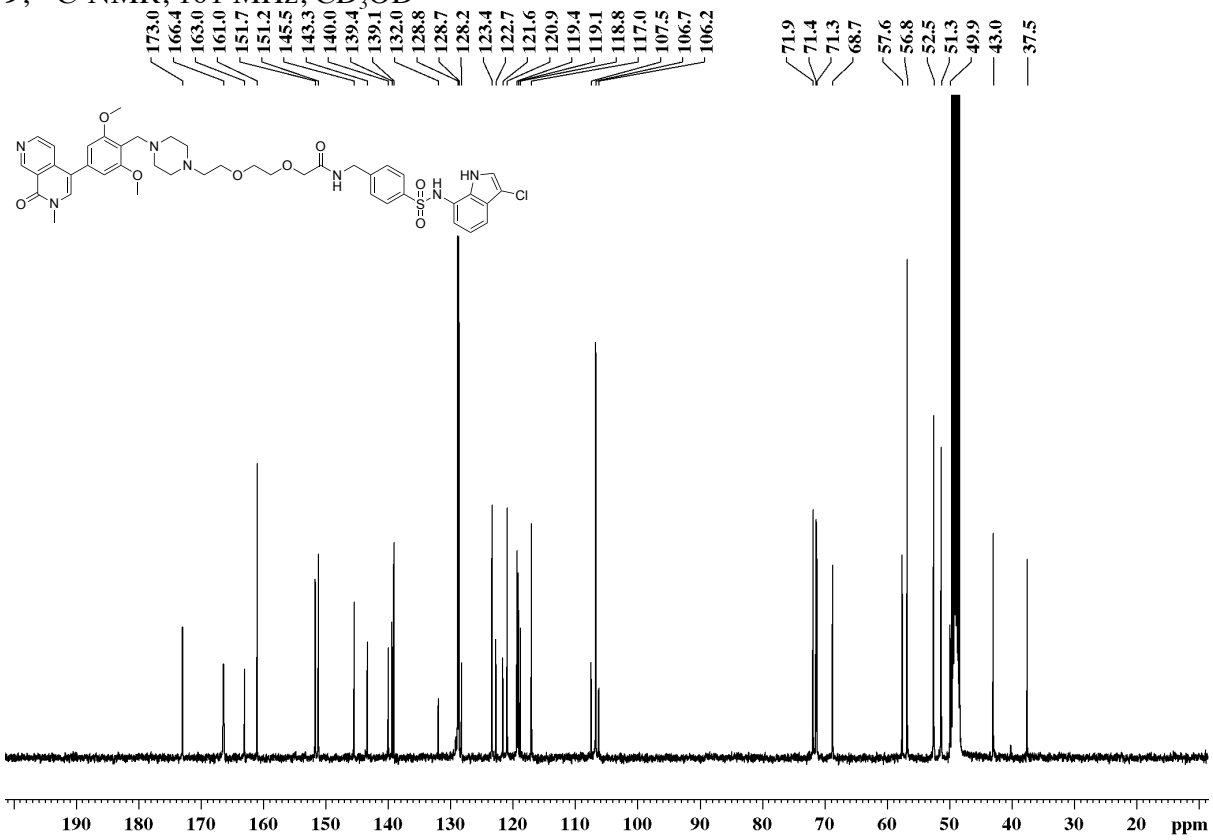
8, ^{13}C-NMR, 101 MHz, CD_3OD



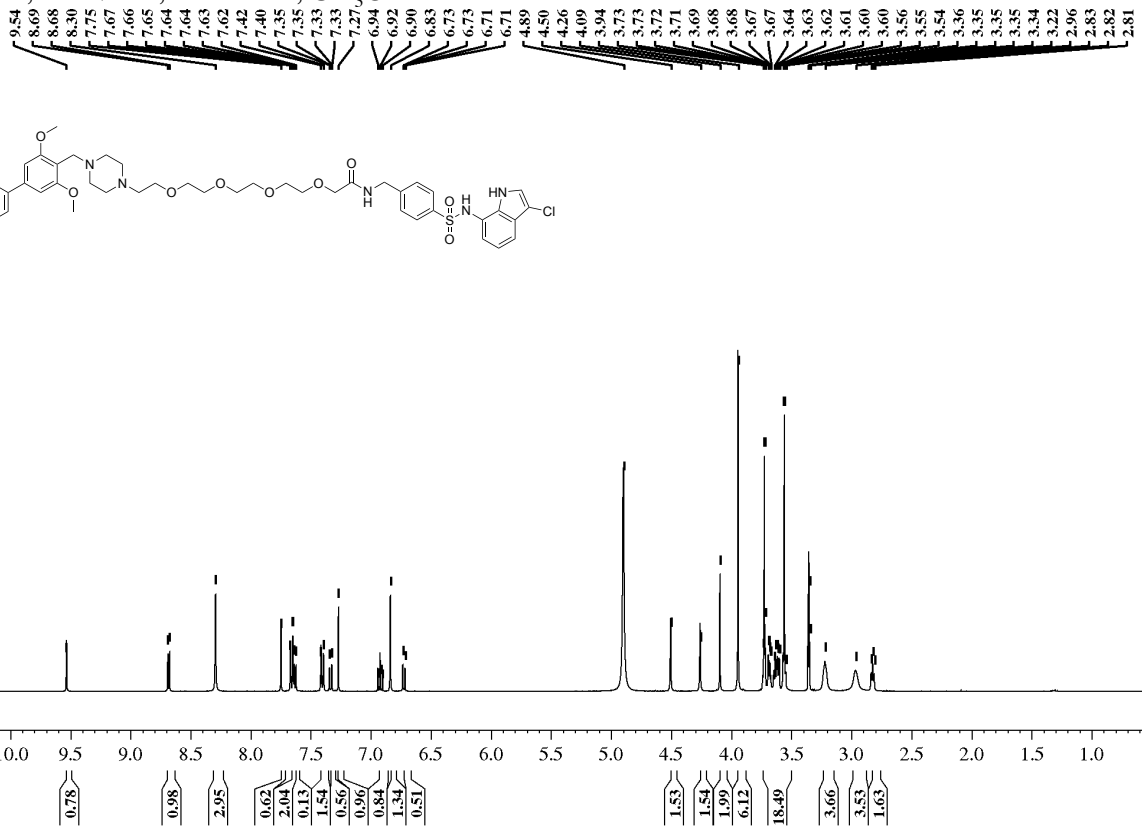
9, ^{1}H-NMR, 400 MHz, CD_3OD



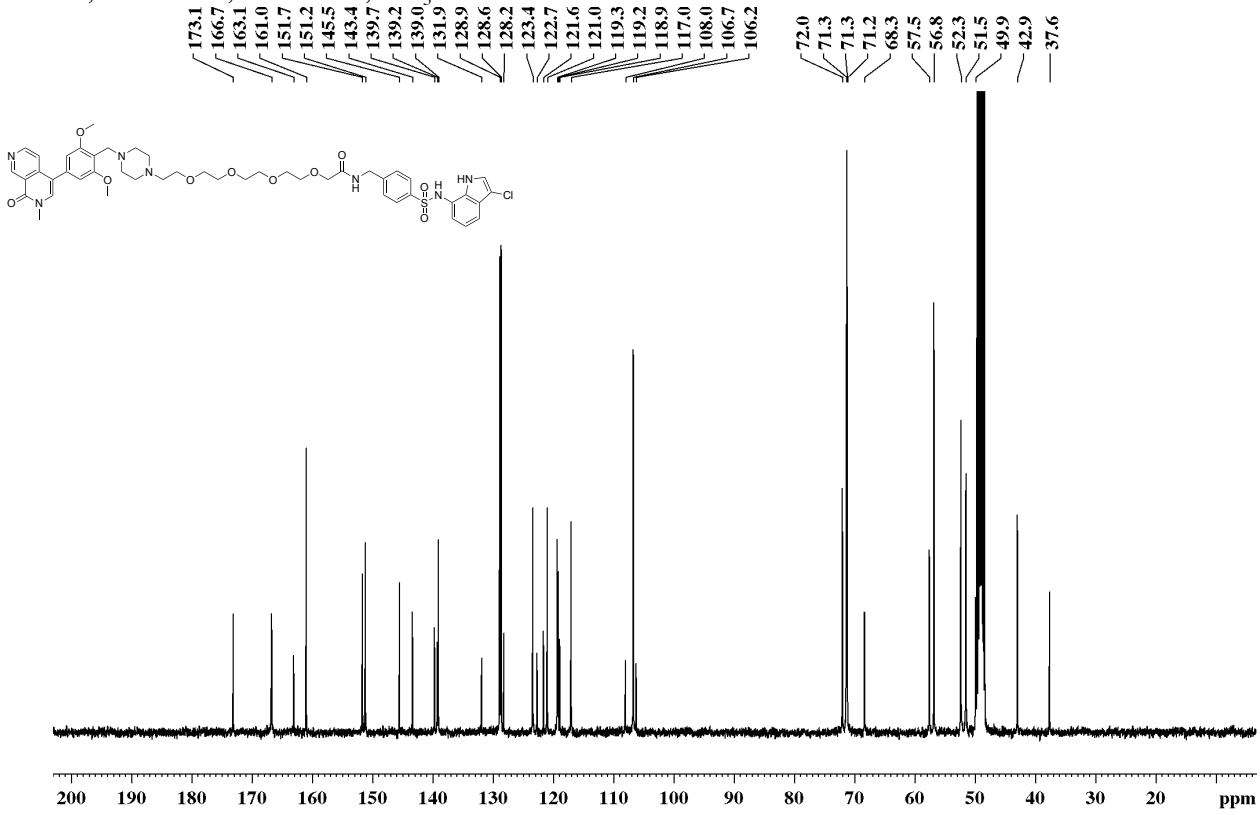
9, ^{13}C-NMR, 101 MHz, CD_3OD



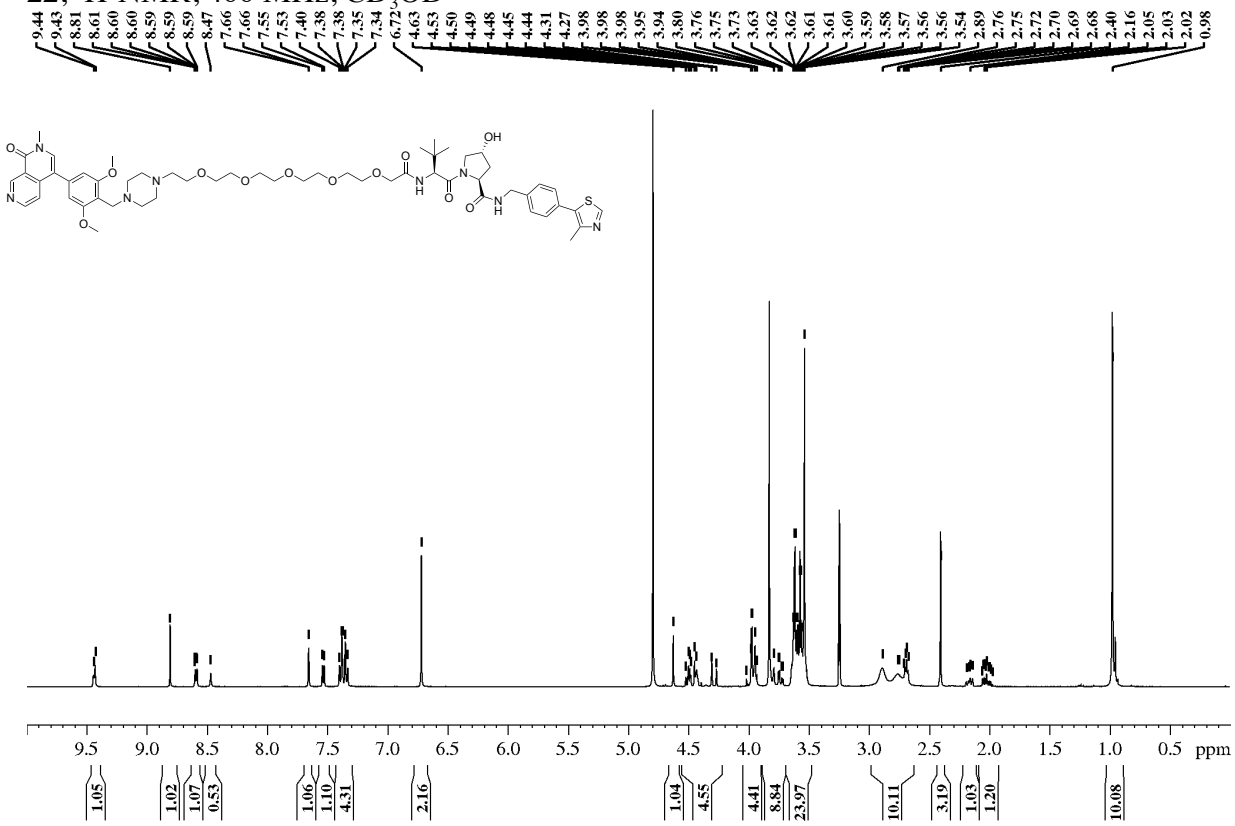
10, ^{1}H-NMR, 400 MHz, CD_3OD



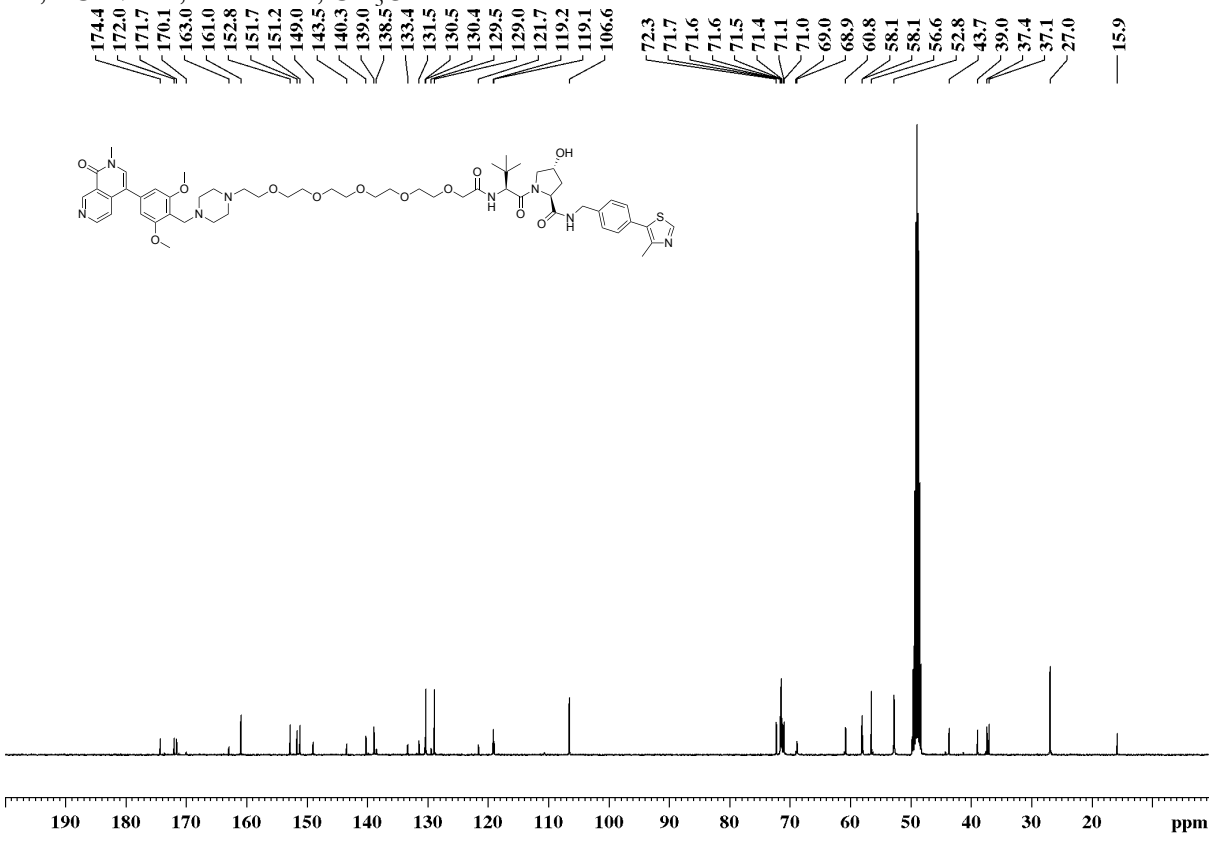
10, ^{13}C-NMR, 101 MHz, CD_3OD



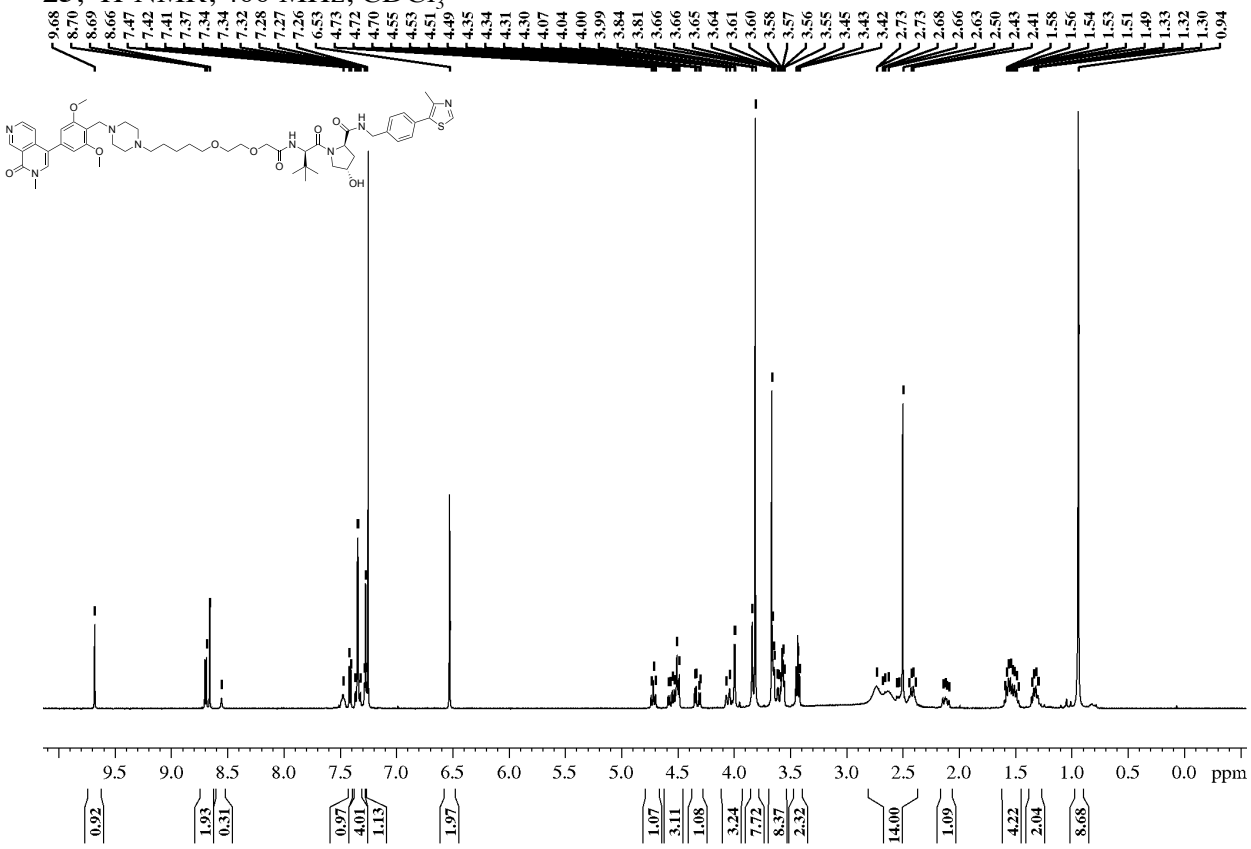
22, ^{1}H-NMR, 400 MHz, CD_3OD



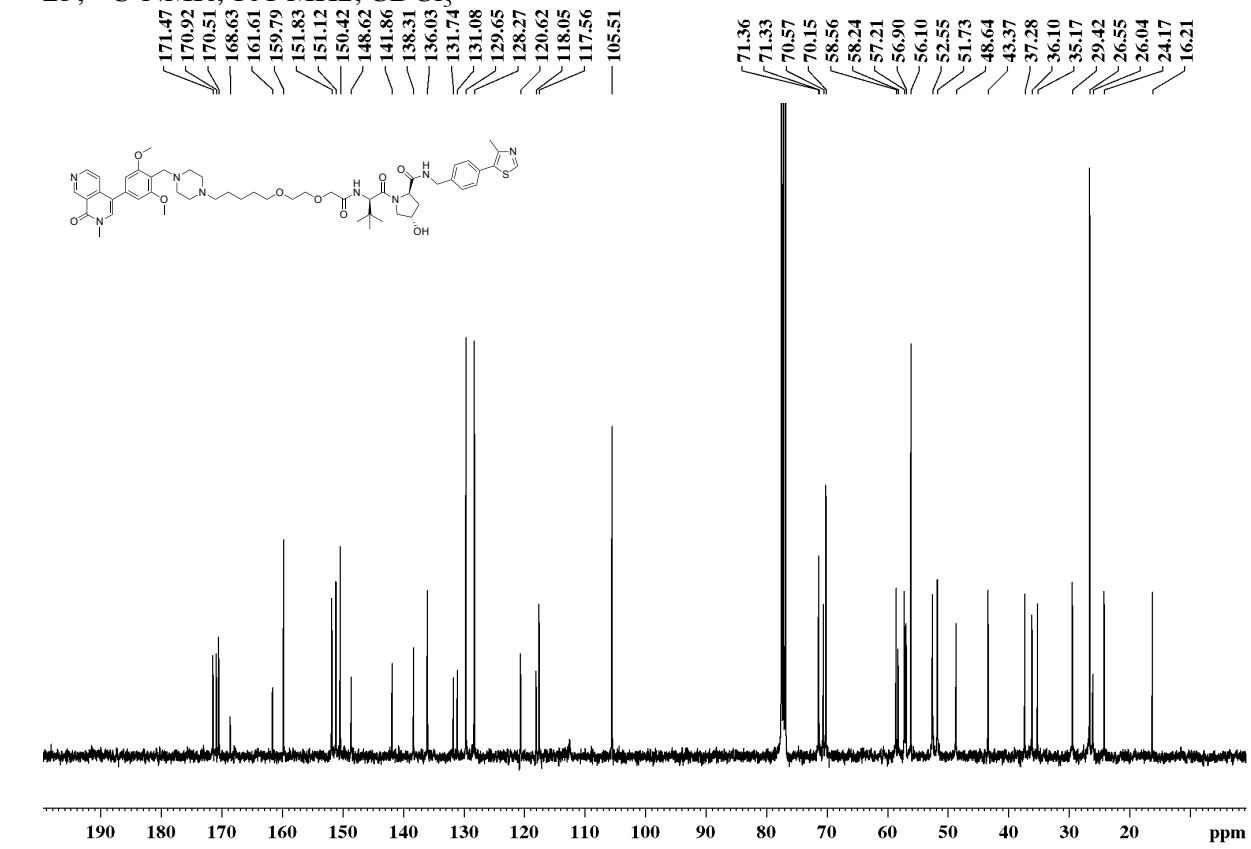
22, ^{13}C-NMR, 101 MHz, CD_3OD



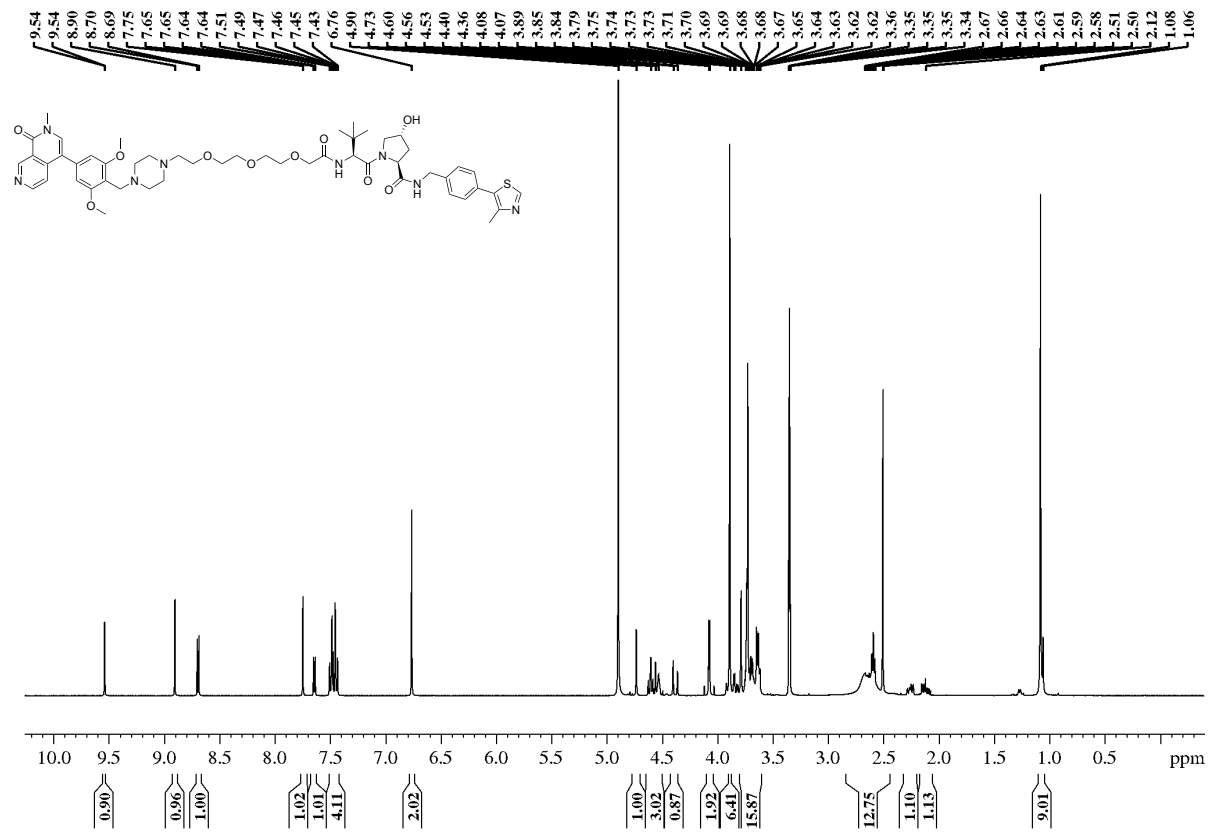
23, ^{1}H-NMR, 400 MHz, $CDCl_3$



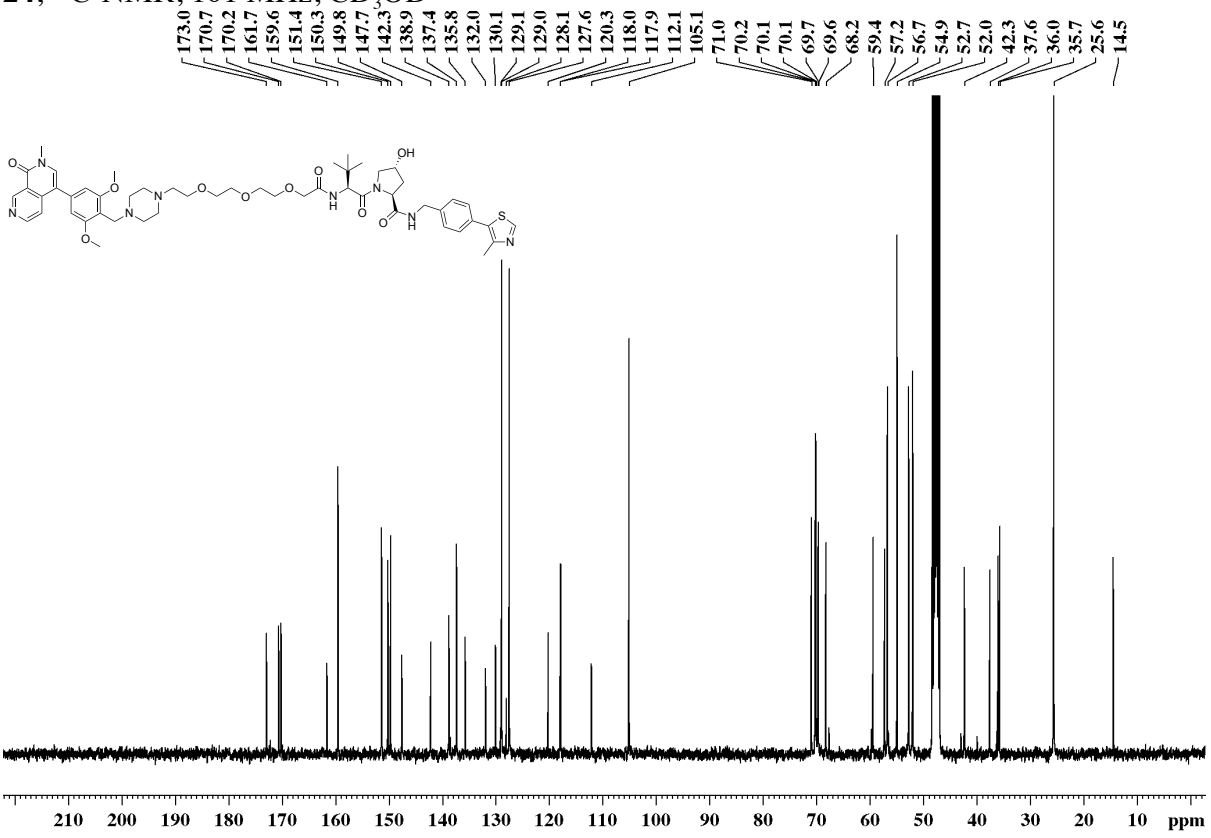
23, ^{13}C-NMR, 101 MHz, $CDCl_3$



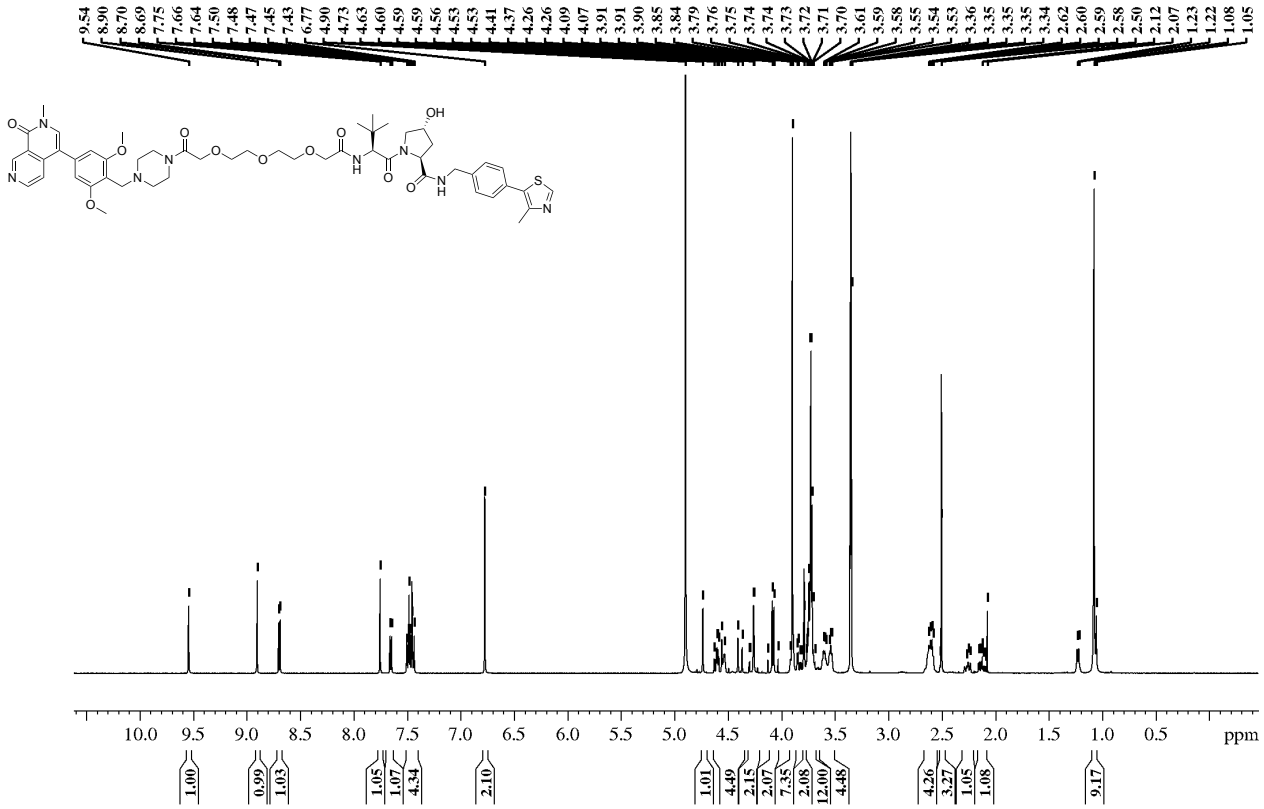
24, ^{1}H-NMR, 400 MHz, CD_3OD



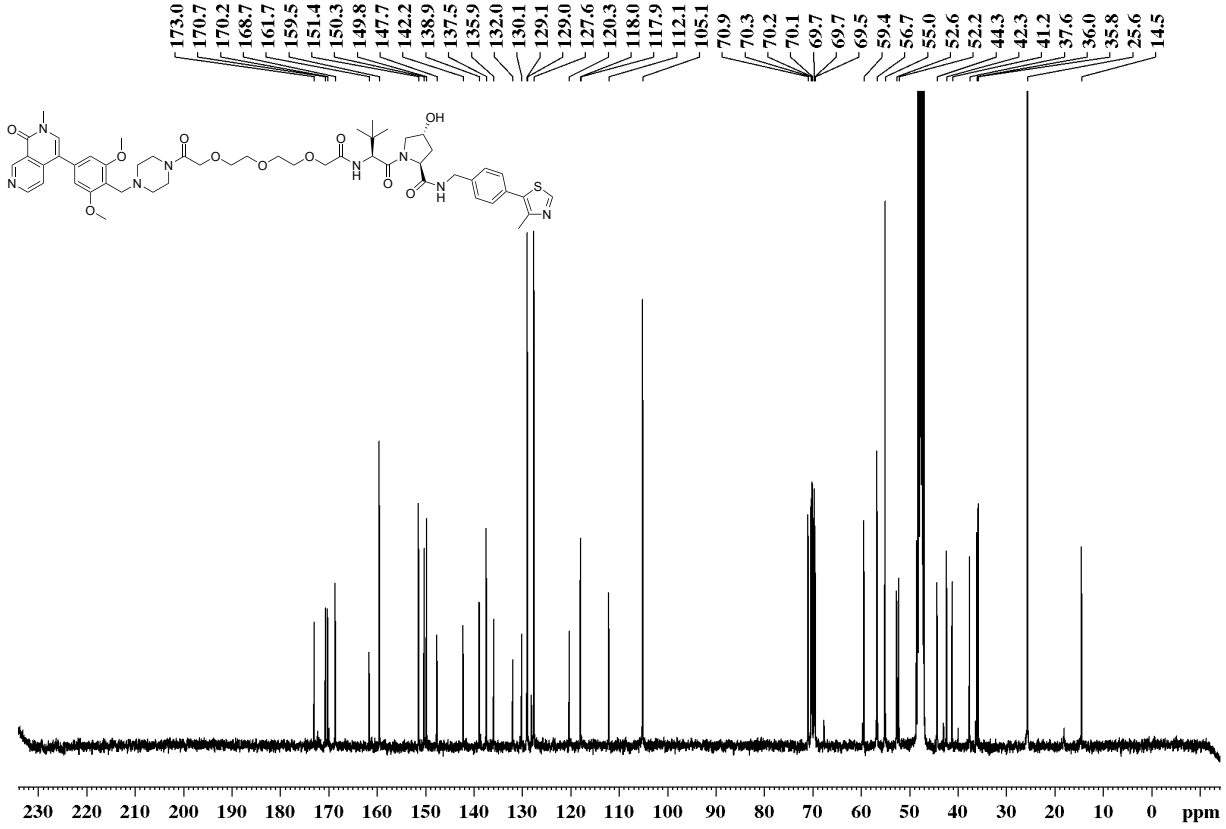
24, ^{13}C-NMR, 101 MHz, CD_3OD



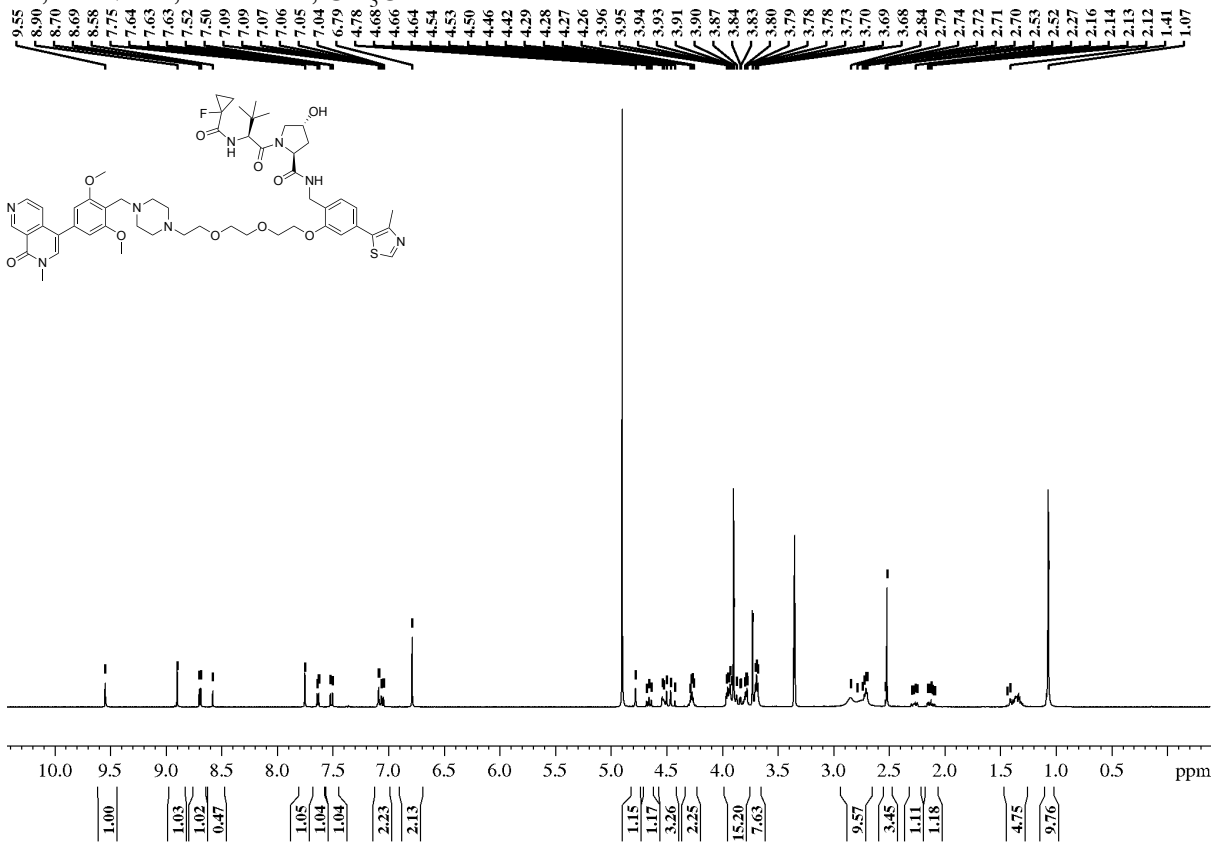
25, ^{1}H-NMR, 400 MHz, CD_3OD



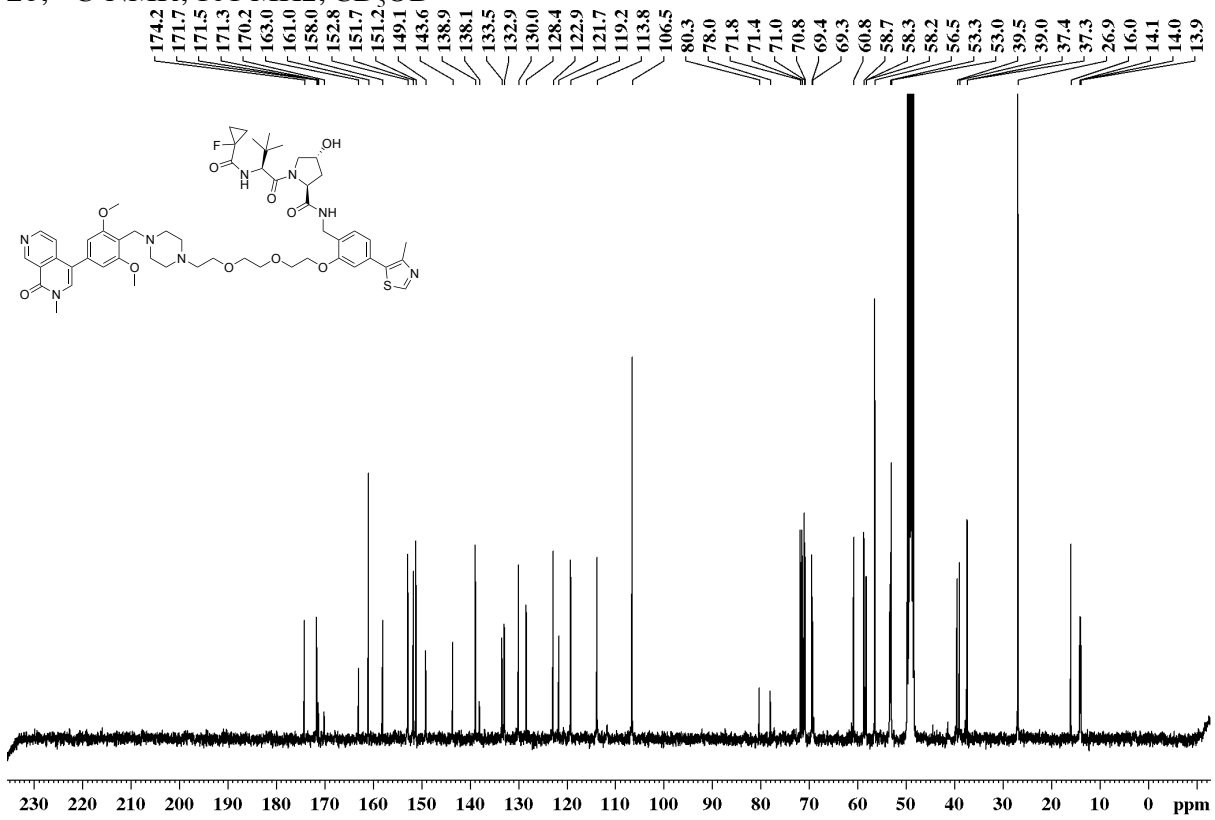
25,^{13}C-NMR, 101 MHz, CD_3OD



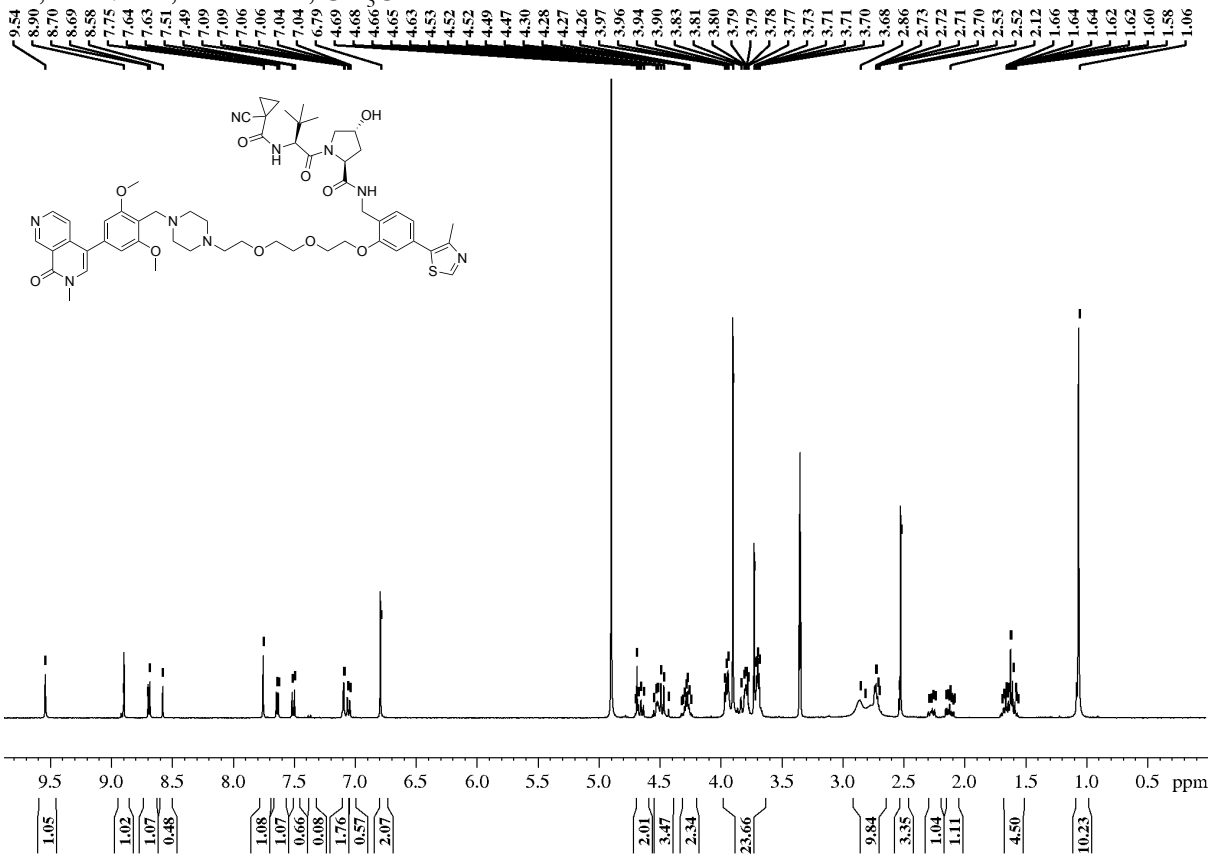
26, ^{1}H-NMR, 400 MHz, CD_3OD



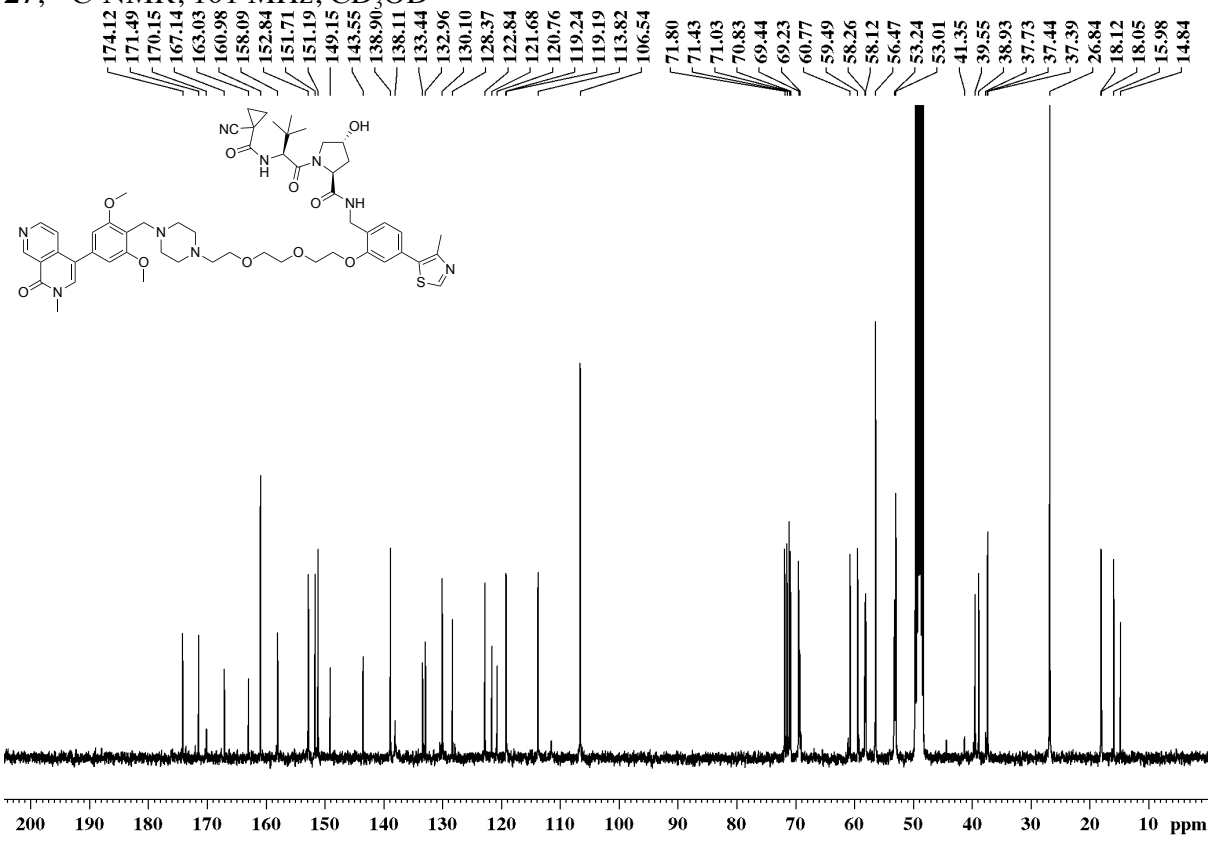
26, ^{13}C-NMR, 101 MHz, CD_3OD



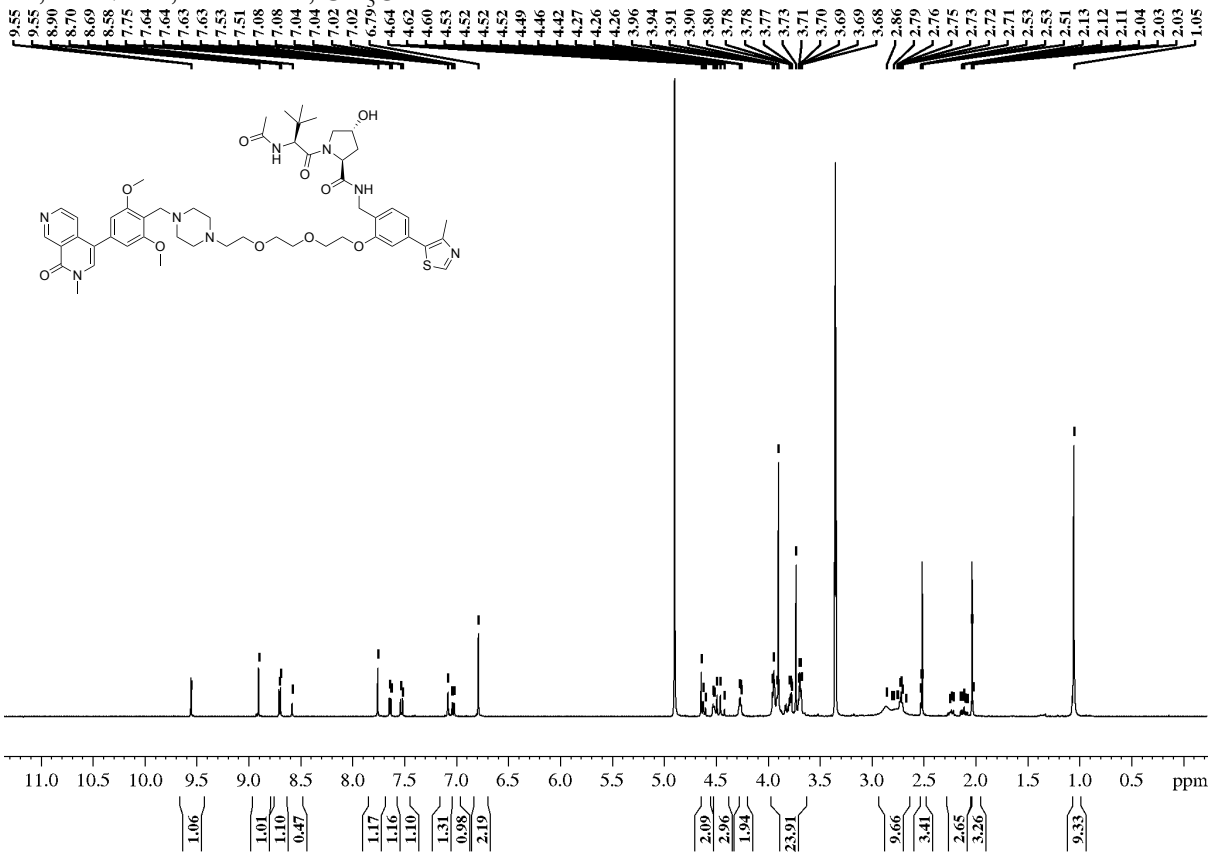
27, ^{1}H-NMR, 400 MHz, CD_3OD



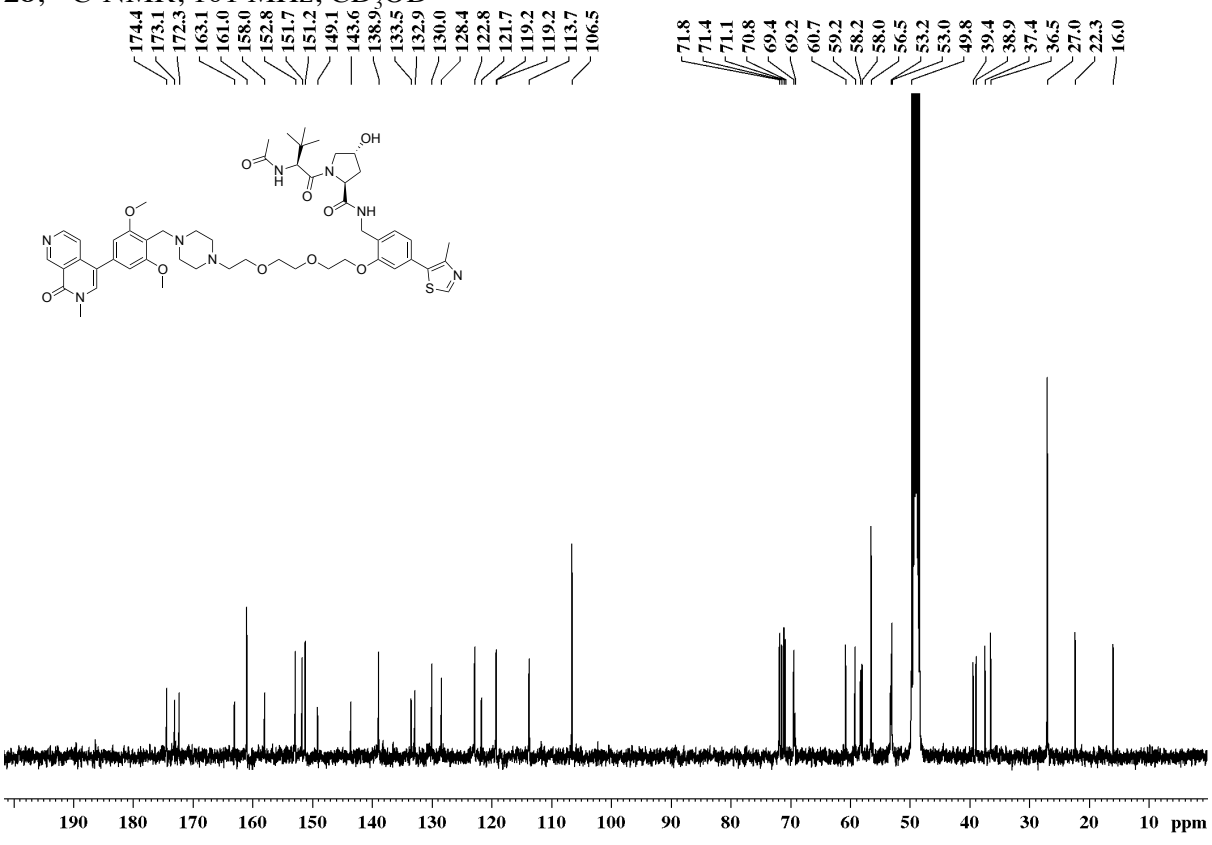
27, ^{13}C-NMR, 101 MHz, CD_3OD



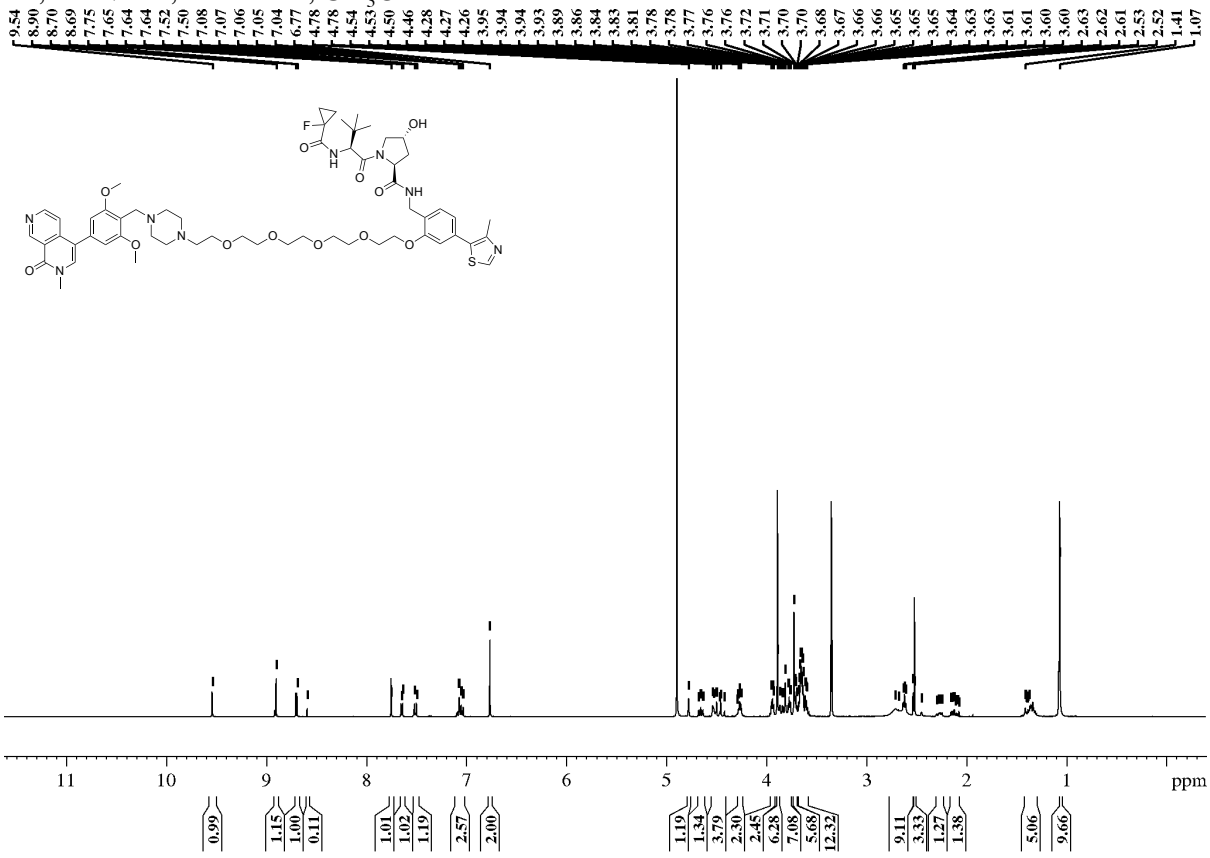
28, ^{1}H-NMR, 400 MHz, CD_3OD



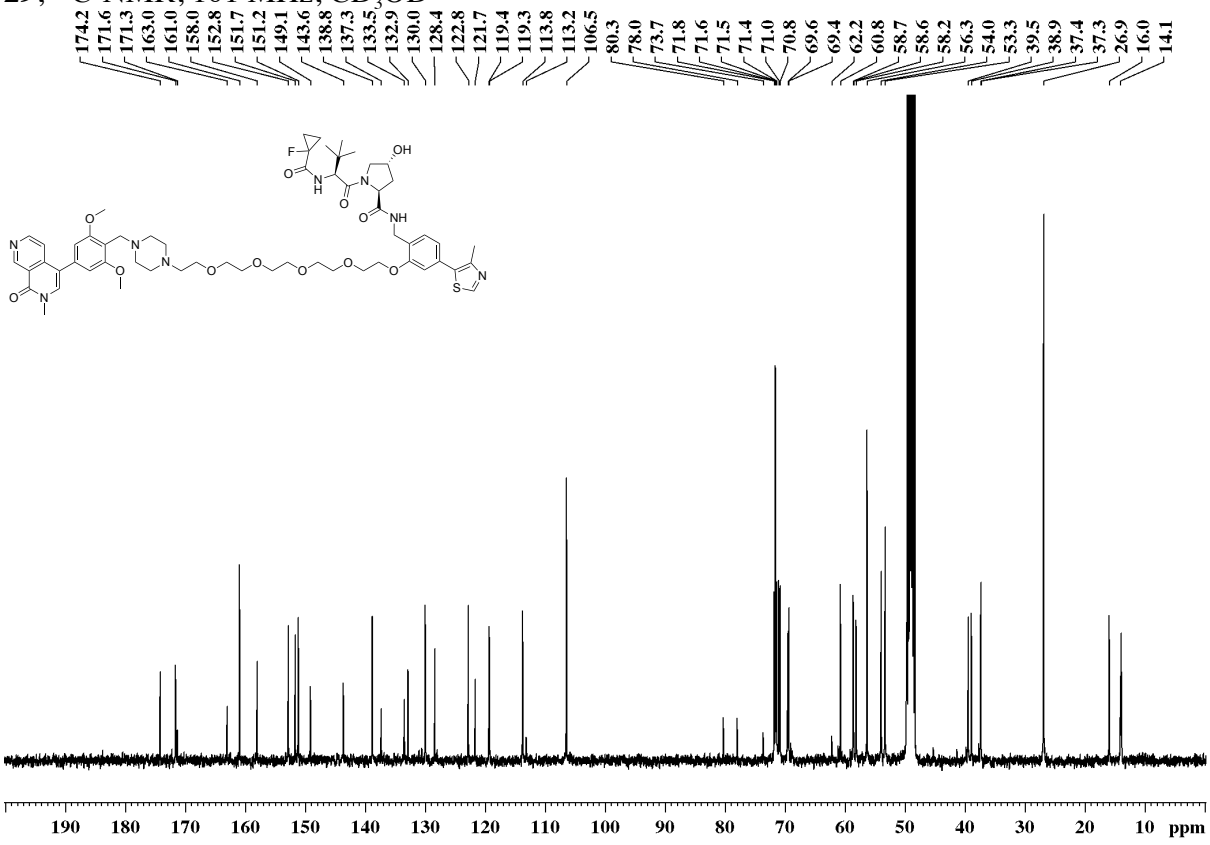
28, ^{13}C-NMR, 101 MHz, CD_3OD



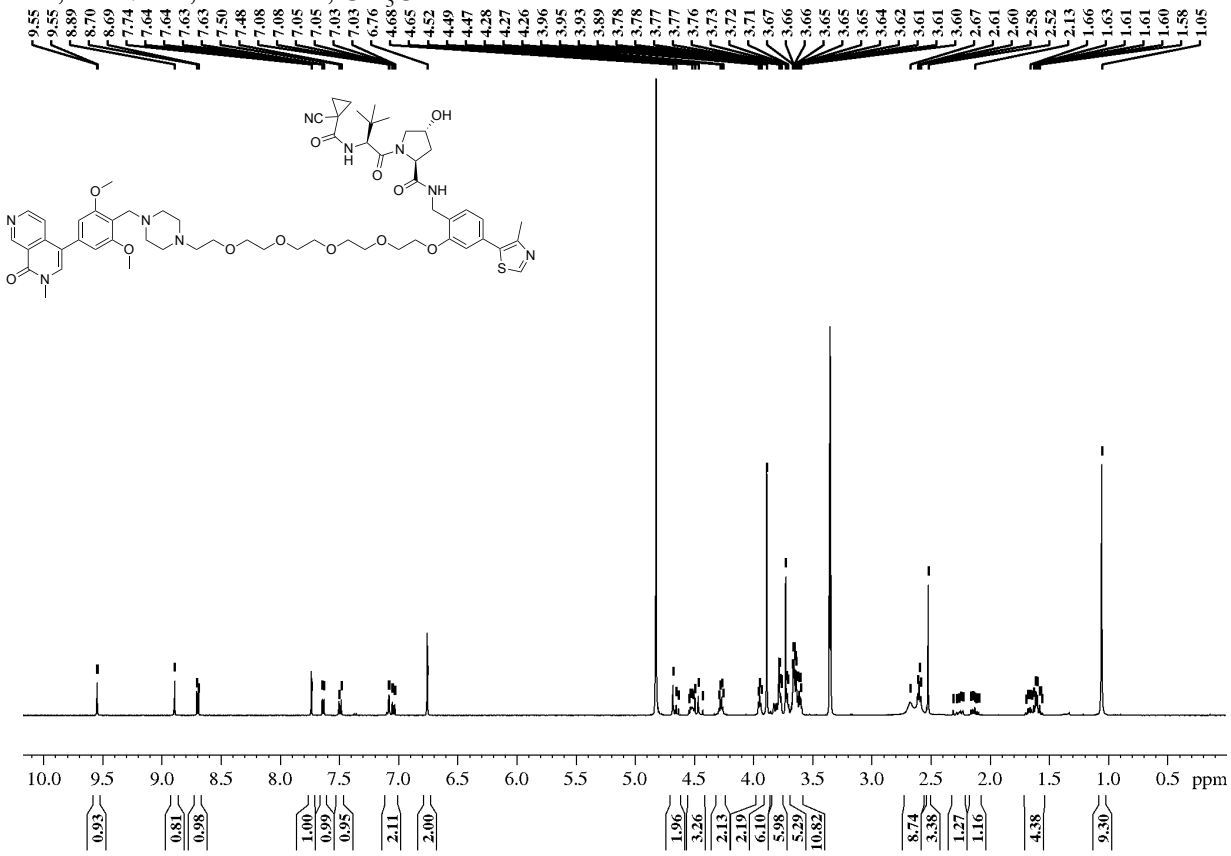
29, ^{1}H-NMR, 400 MHz, CD_3OD



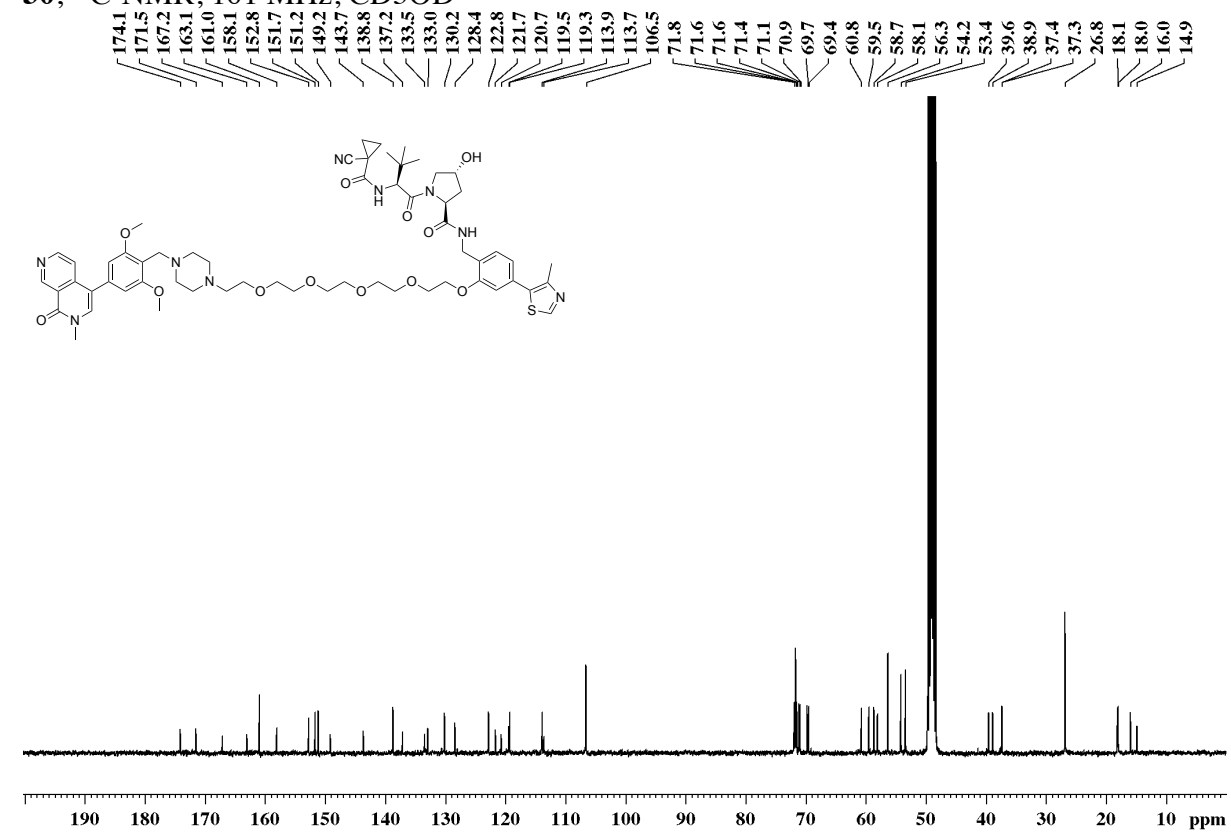
29, ^{13}C-NMR, 101 MHz, CD_3OD



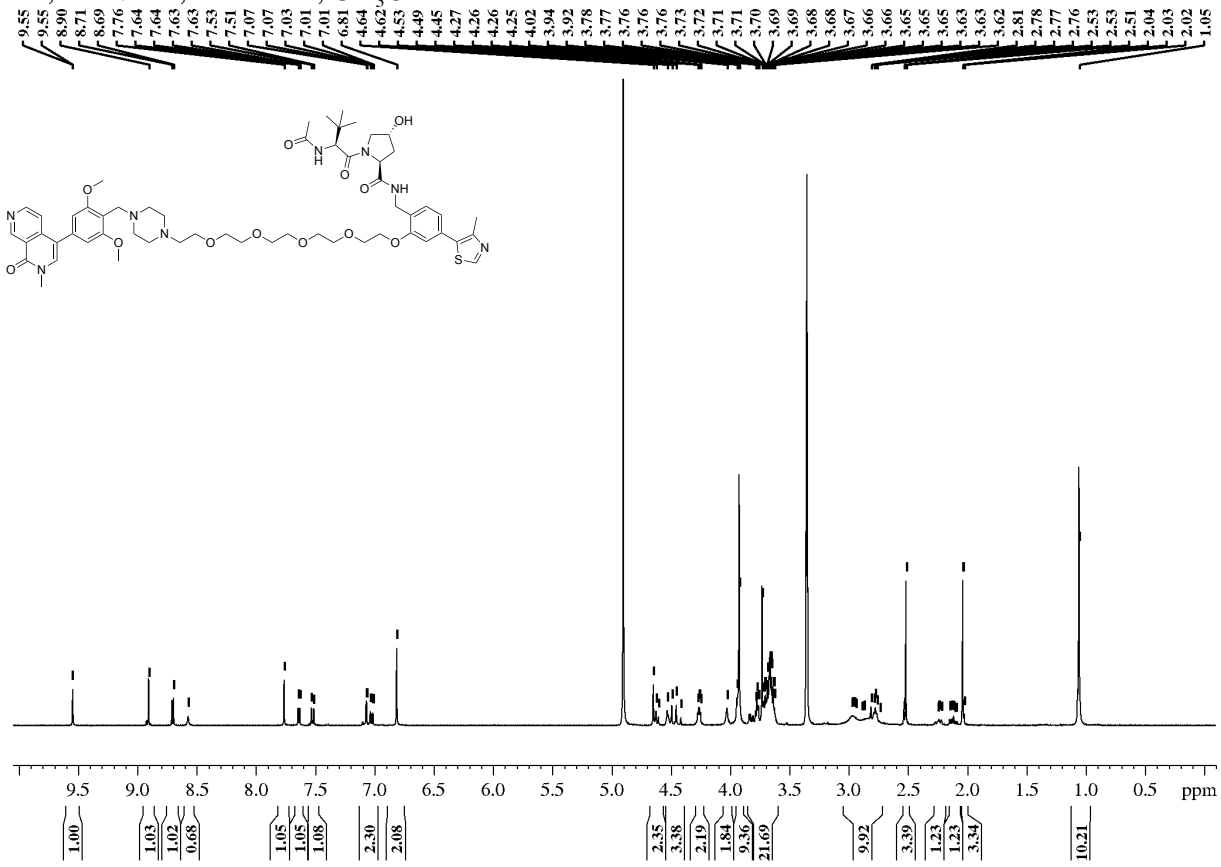
30, ^{1}H-NMR, 400 MHz, CD_3OD



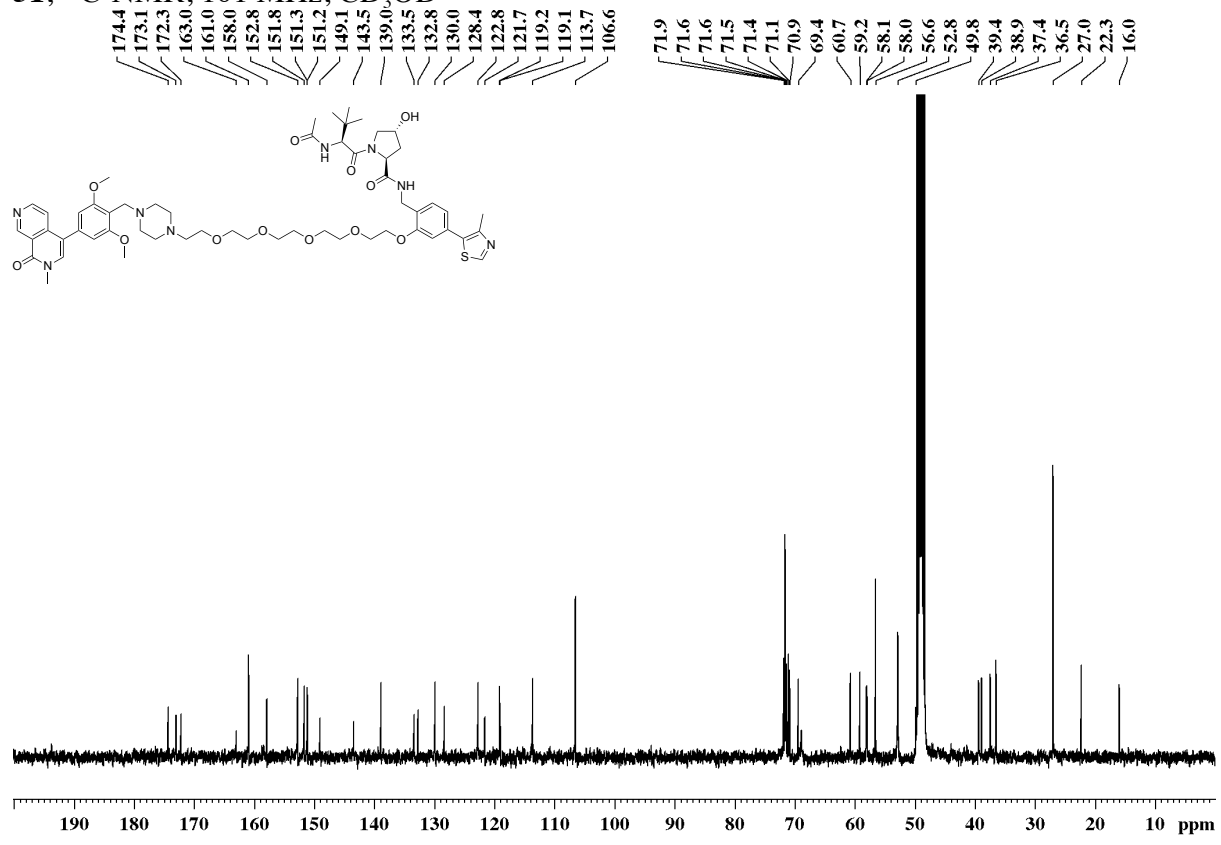
30, ^{13}C-NMR, 101 MHz, CD3OD



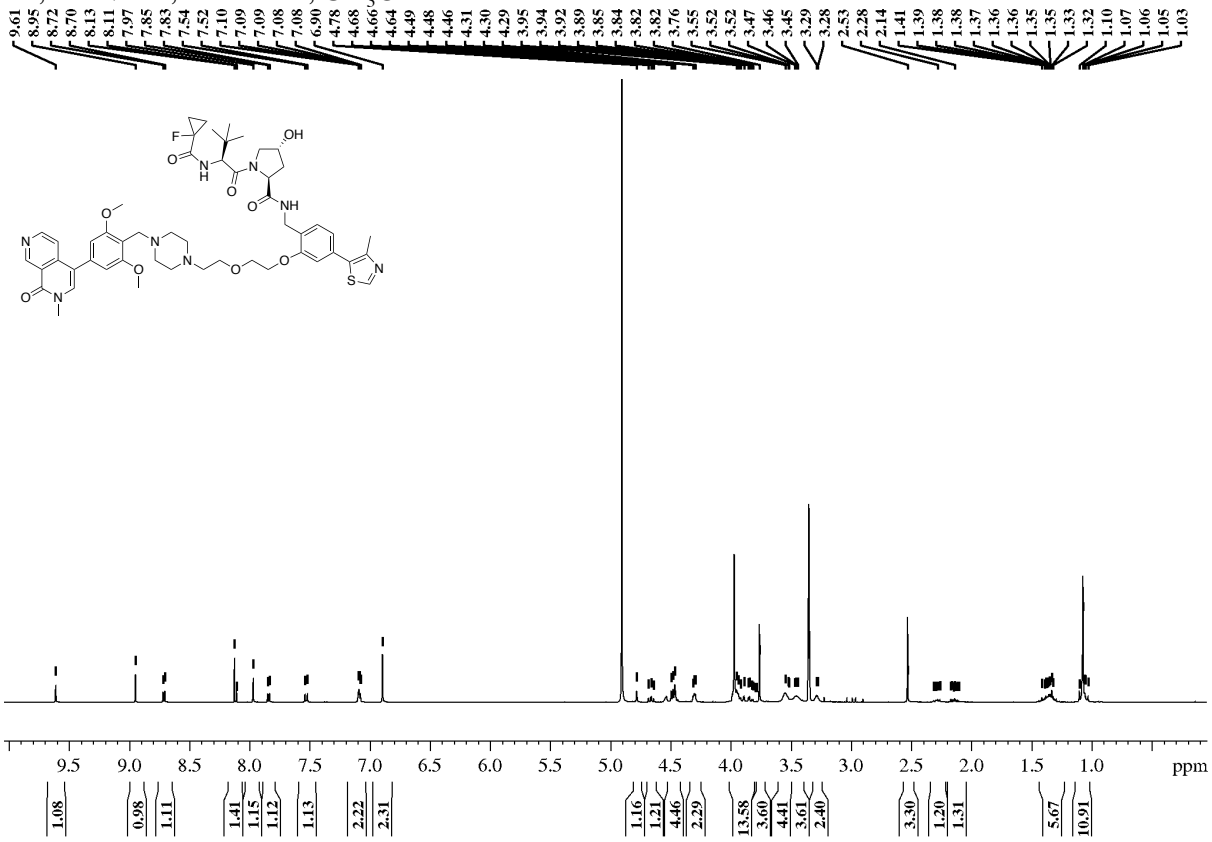
31, ^{1}H-NMR, 400 MHz, CD_3OD



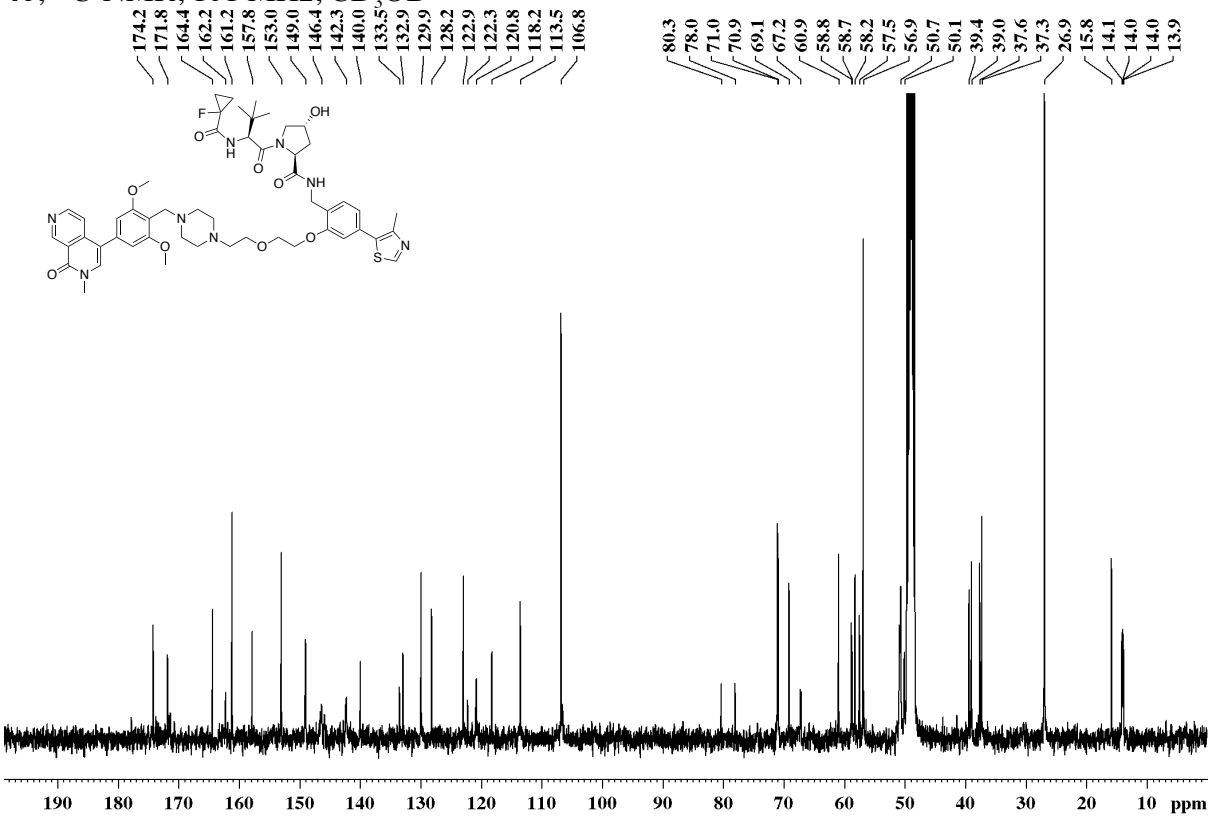
31, ^{13}C-NMR, 101 MHz, CD_3OD



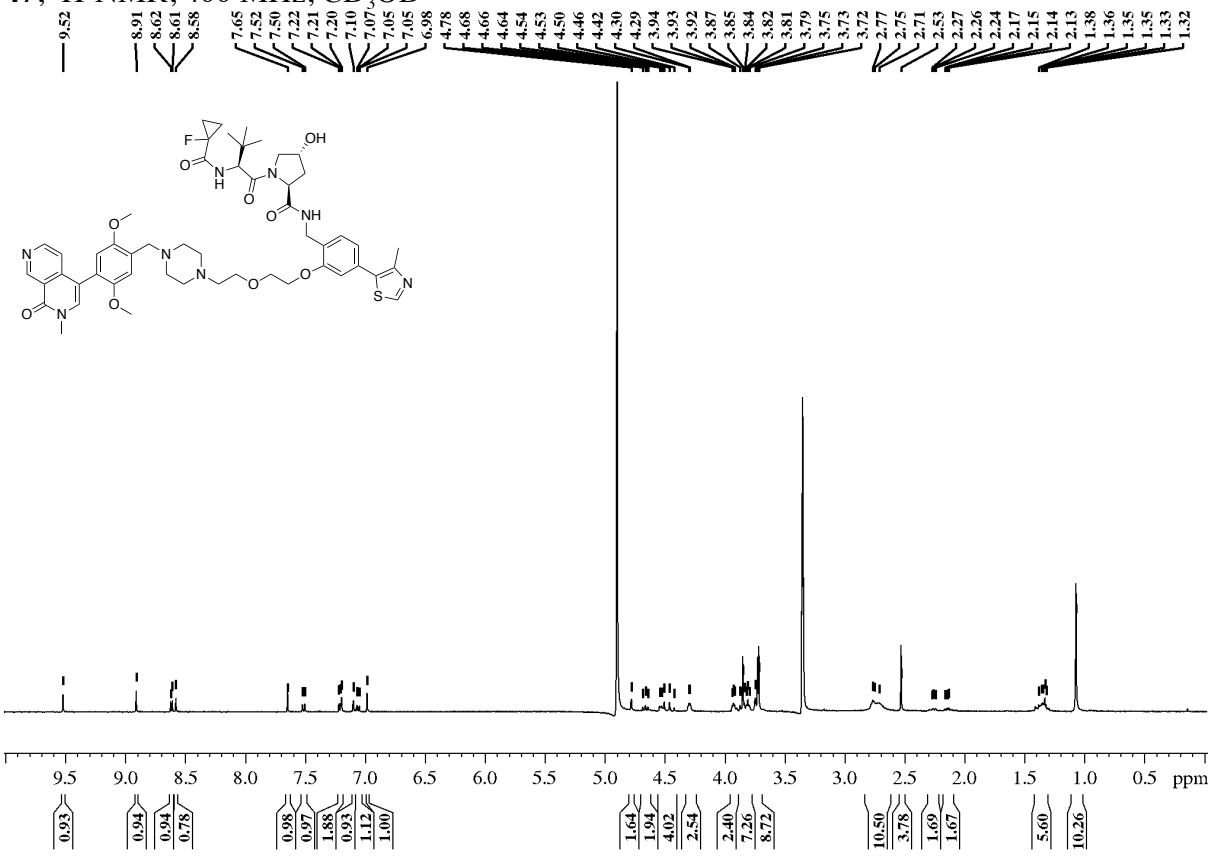
46, ^{1}H-NMR, 400 MHz, CD_3OD



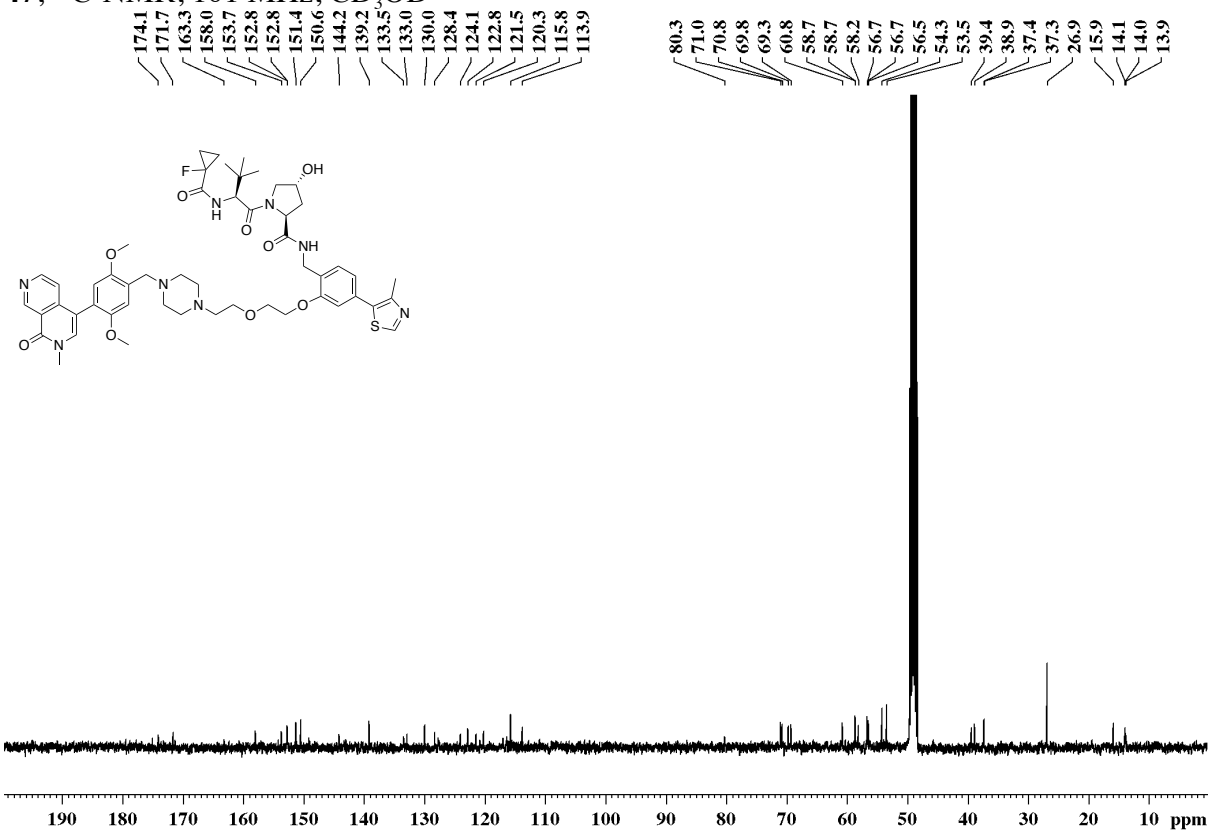
46, ^{13}C-NMR, 101 MHz, CD_3OD



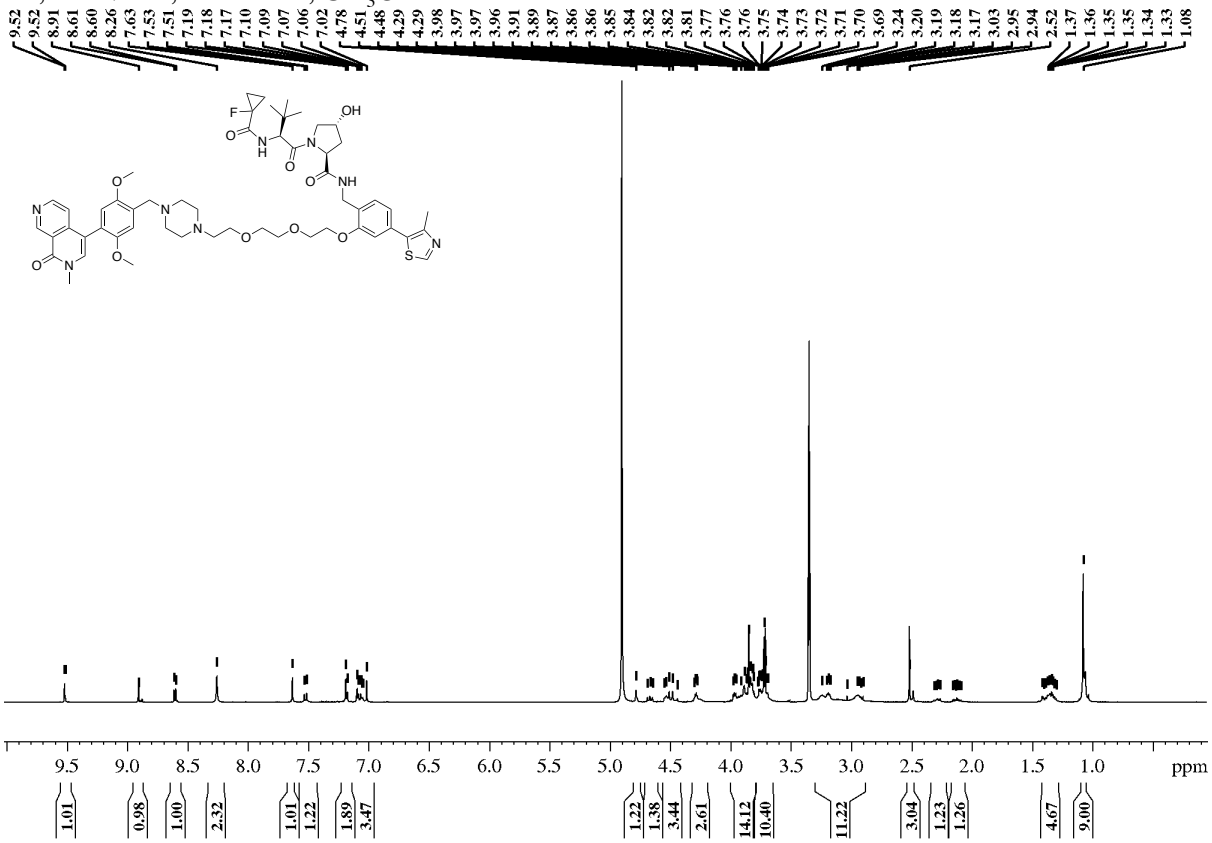
47, ^{1}H-NMR, 400 MHz, CD_3OD



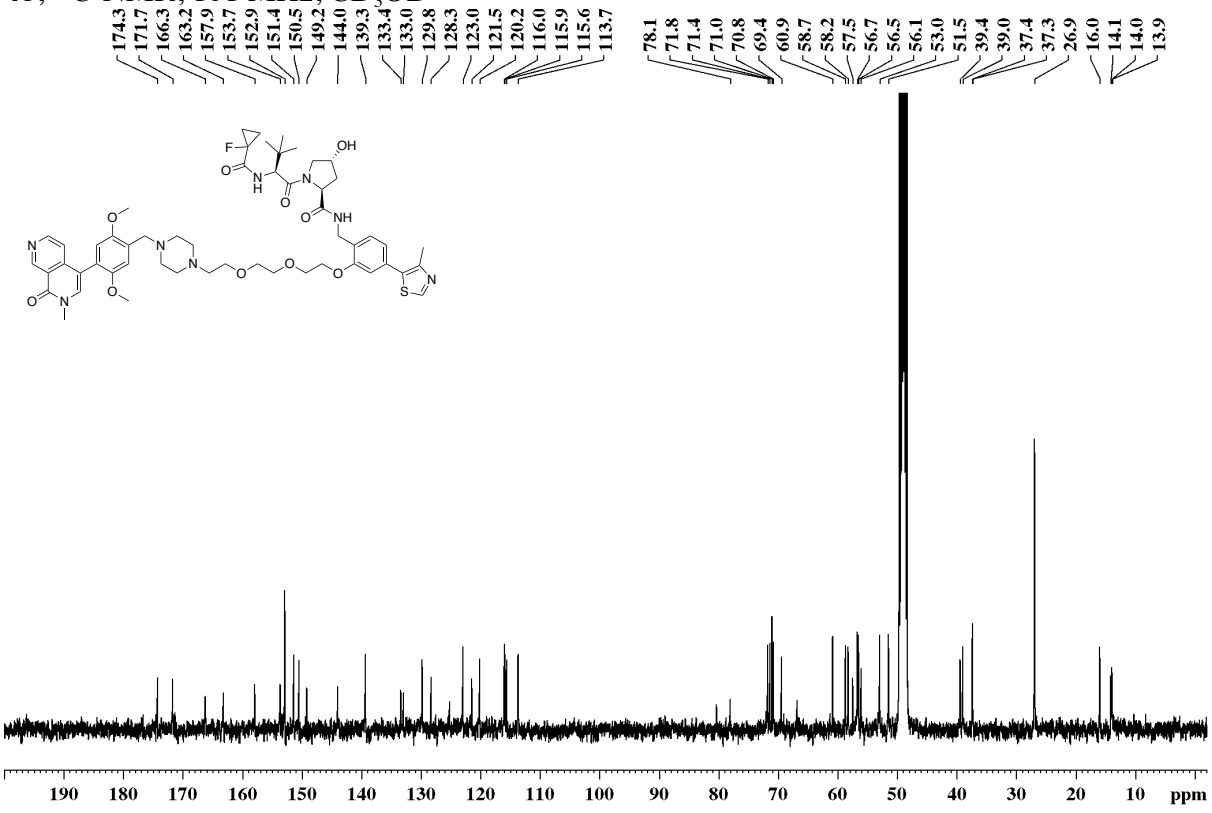
47, ^{13}C-NMR, 101 MHz, CD_3OD



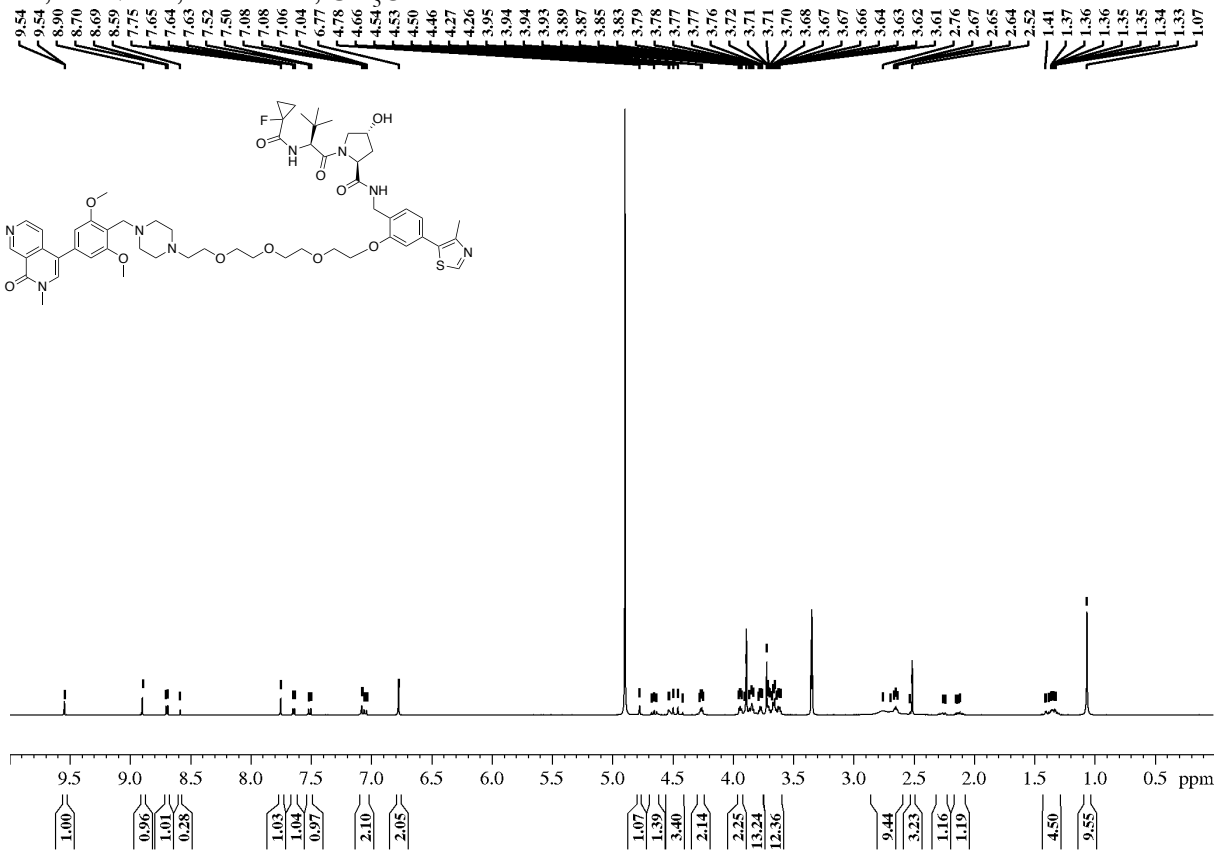
48, ^{1}H-NMR, 400 MHz, CD_3OD



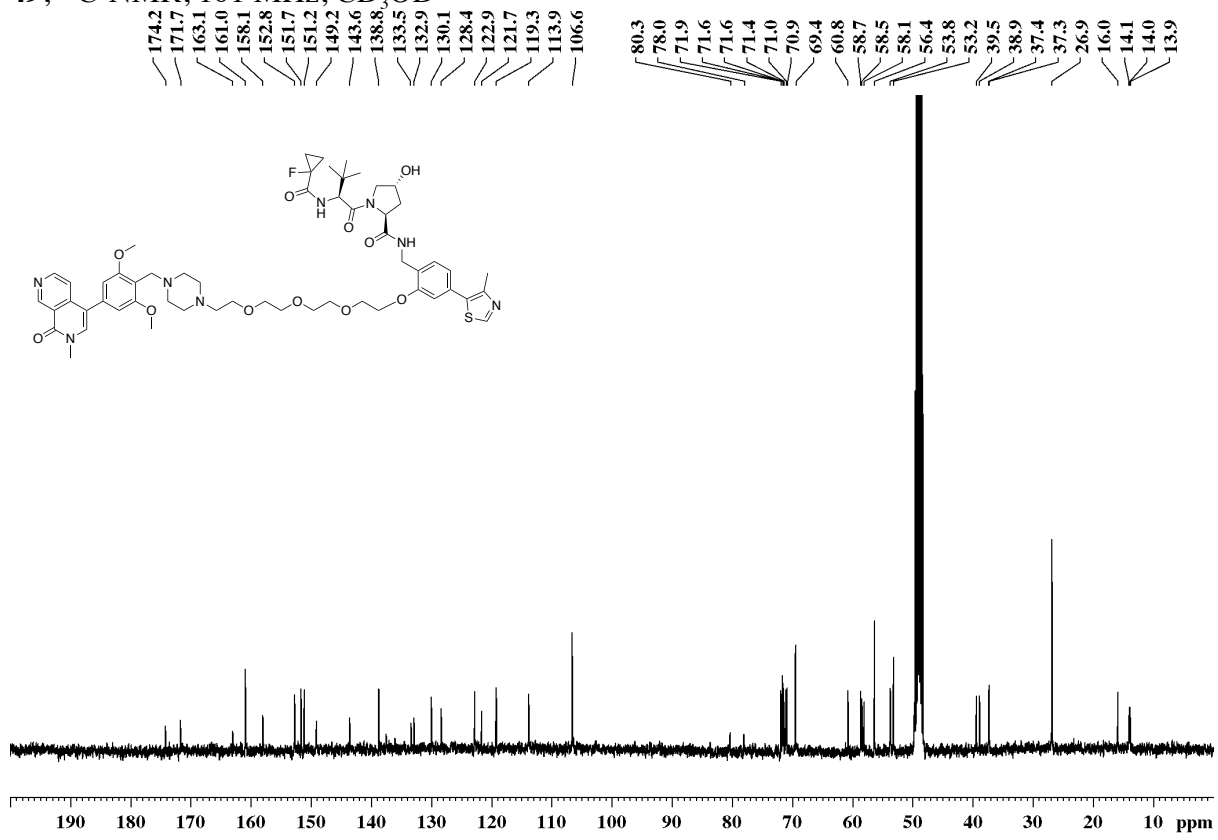
48, ^{13}C-NMR, 101 MHz, CD_3OD



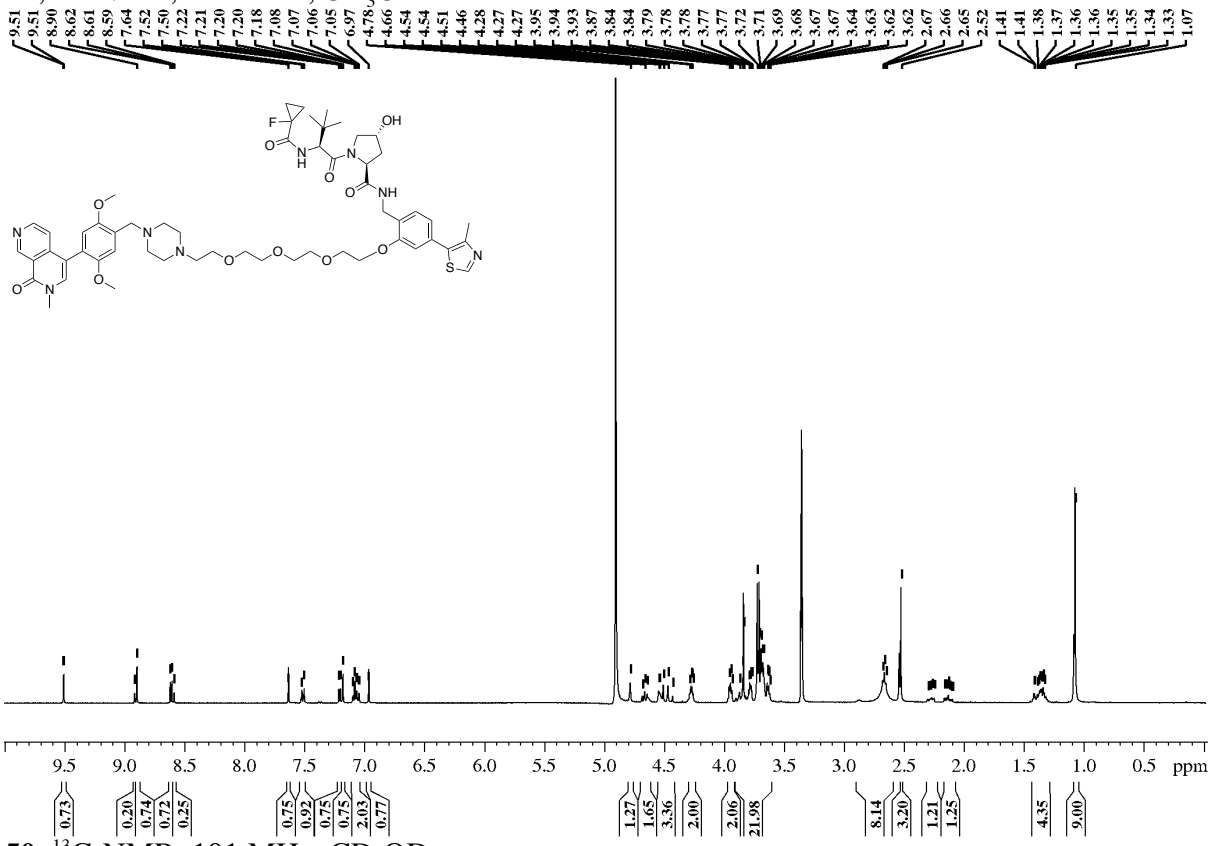
49, ^{1}H-NMR, 400 MHz, CD_3OD



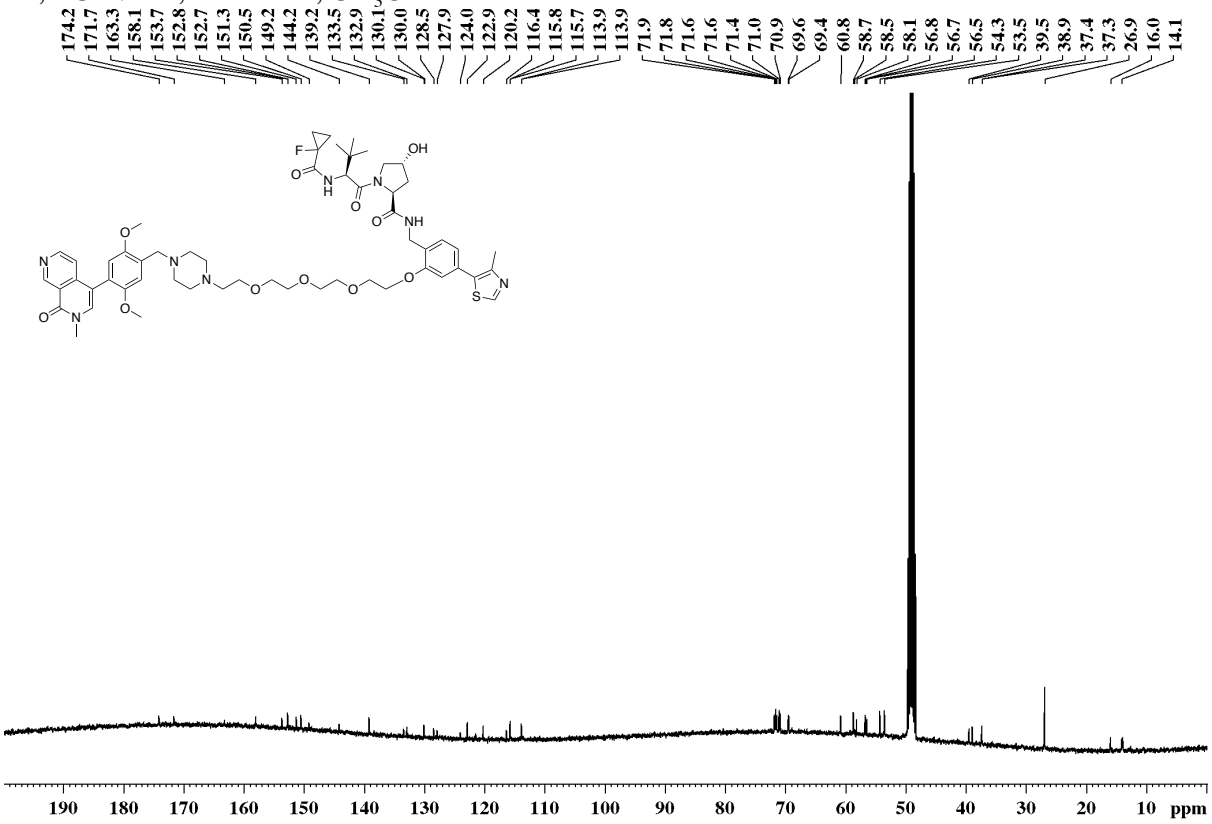
49, ^{13}C-NMR, 101 MHz, CD_3OD



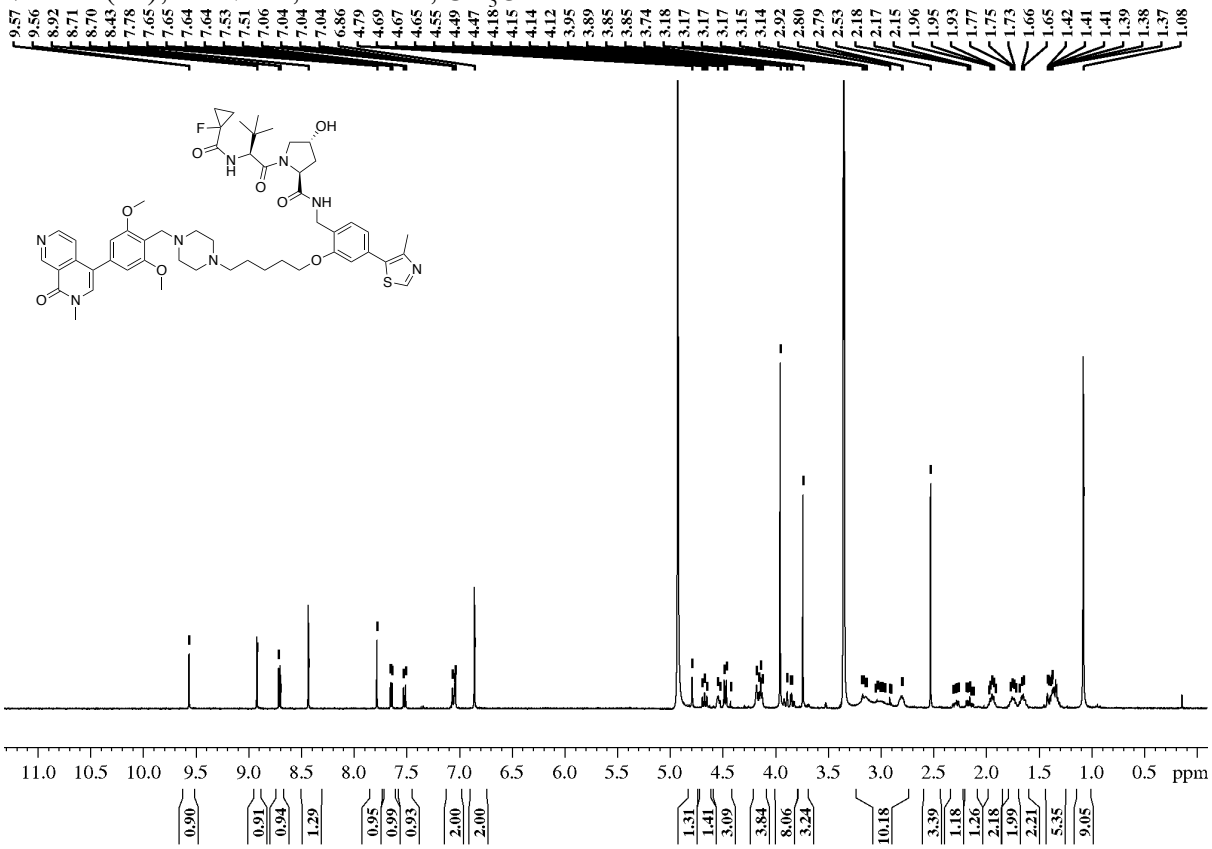
50, ^{1}H-NMR, 400 MHz, CD_3OD



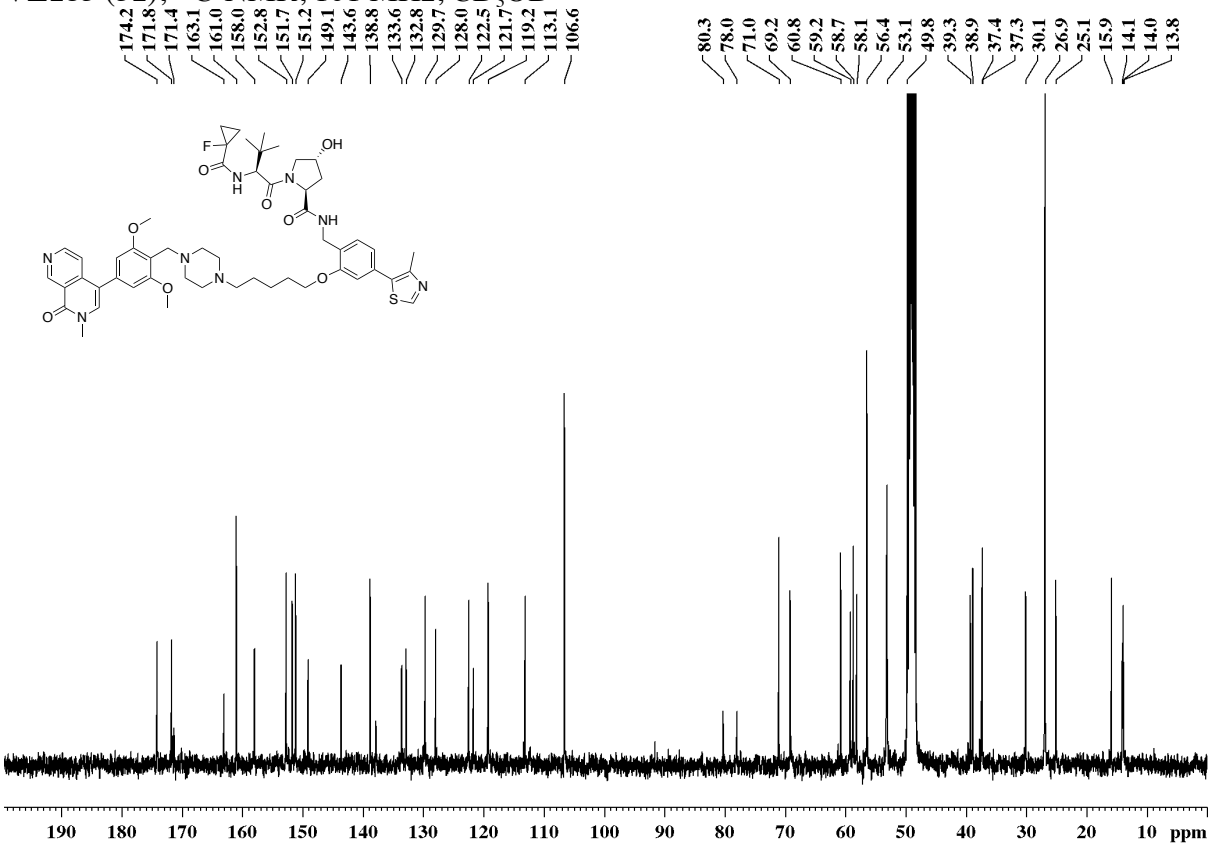
50, ^{13}C-NMR, 101 MHz, CD_3OD



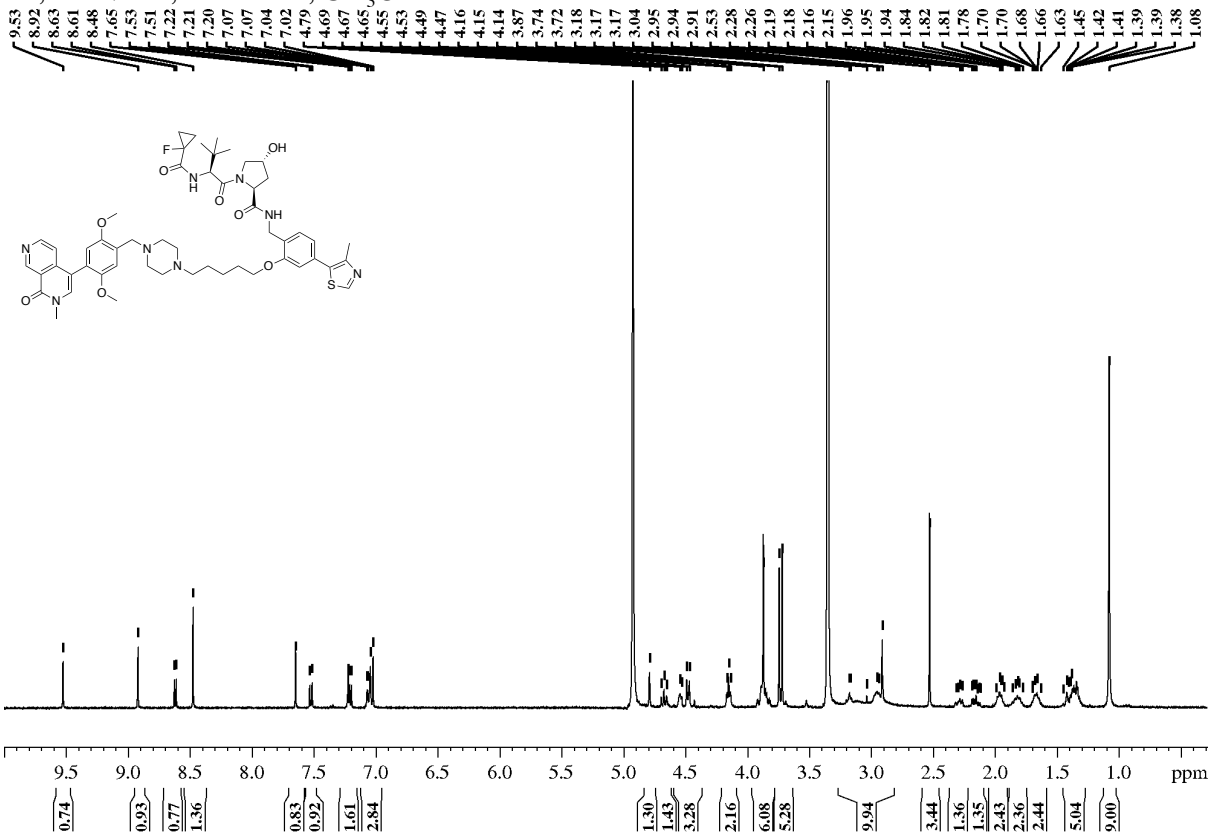
VZ185 (**51**), ^{1}H-NMR, 400 MHz, CD_3OD



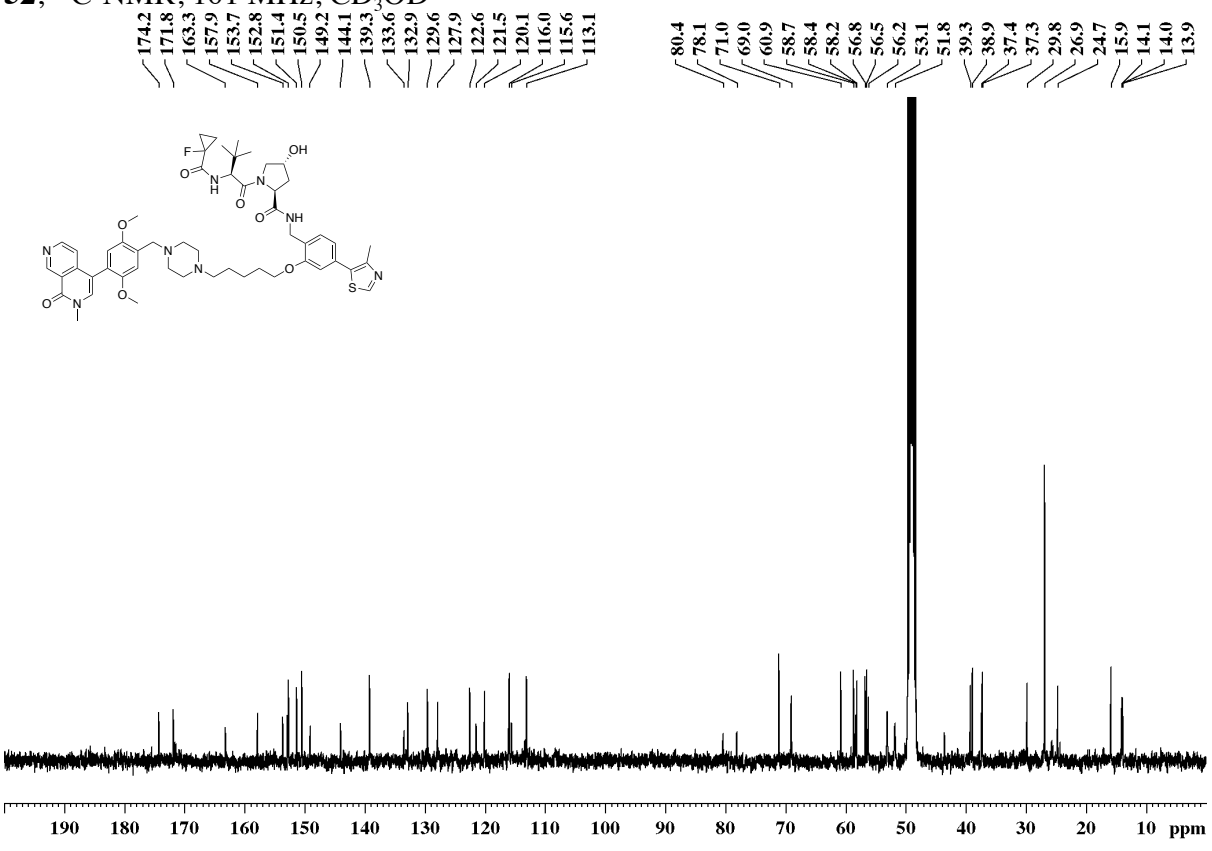
VZ185 (**51**), ^{13}C-NMR, 101 MHz, CD_3OD



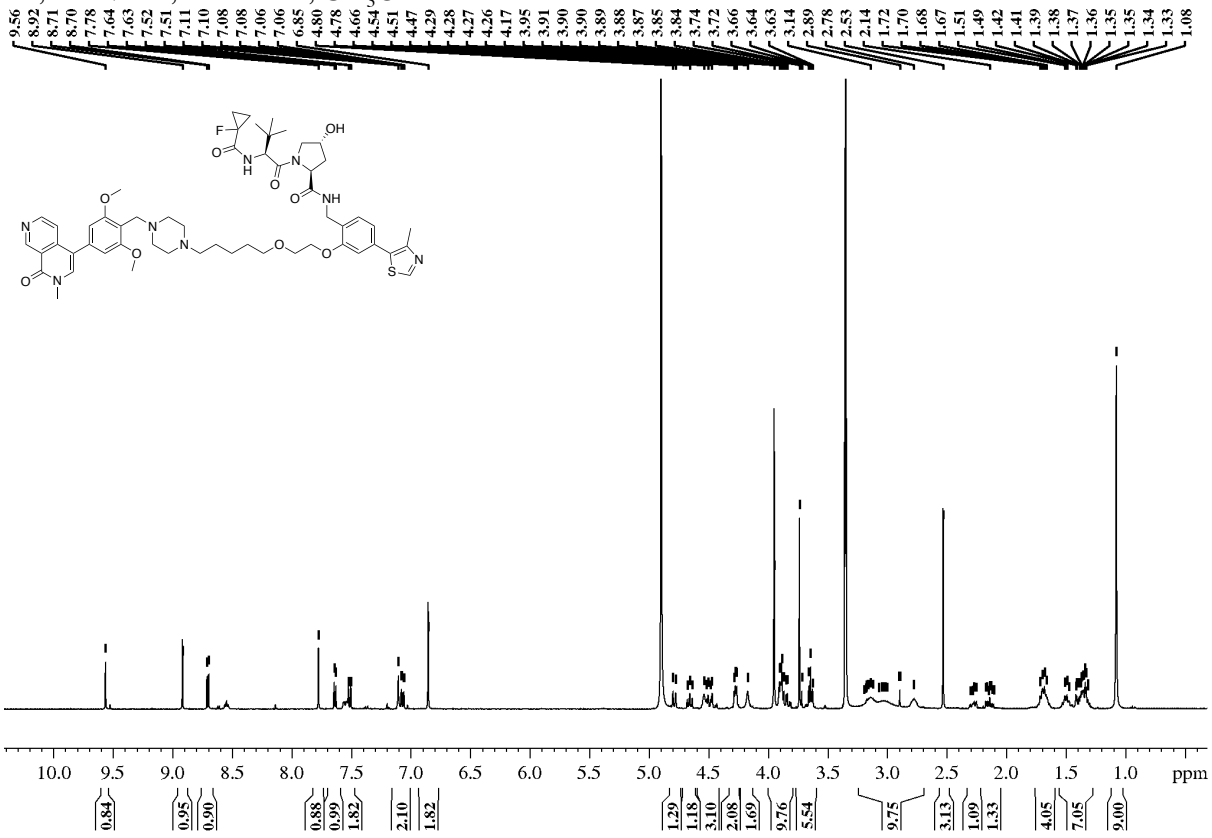
52, ^{1}H-NMR, 400 MHz, CD_3OD



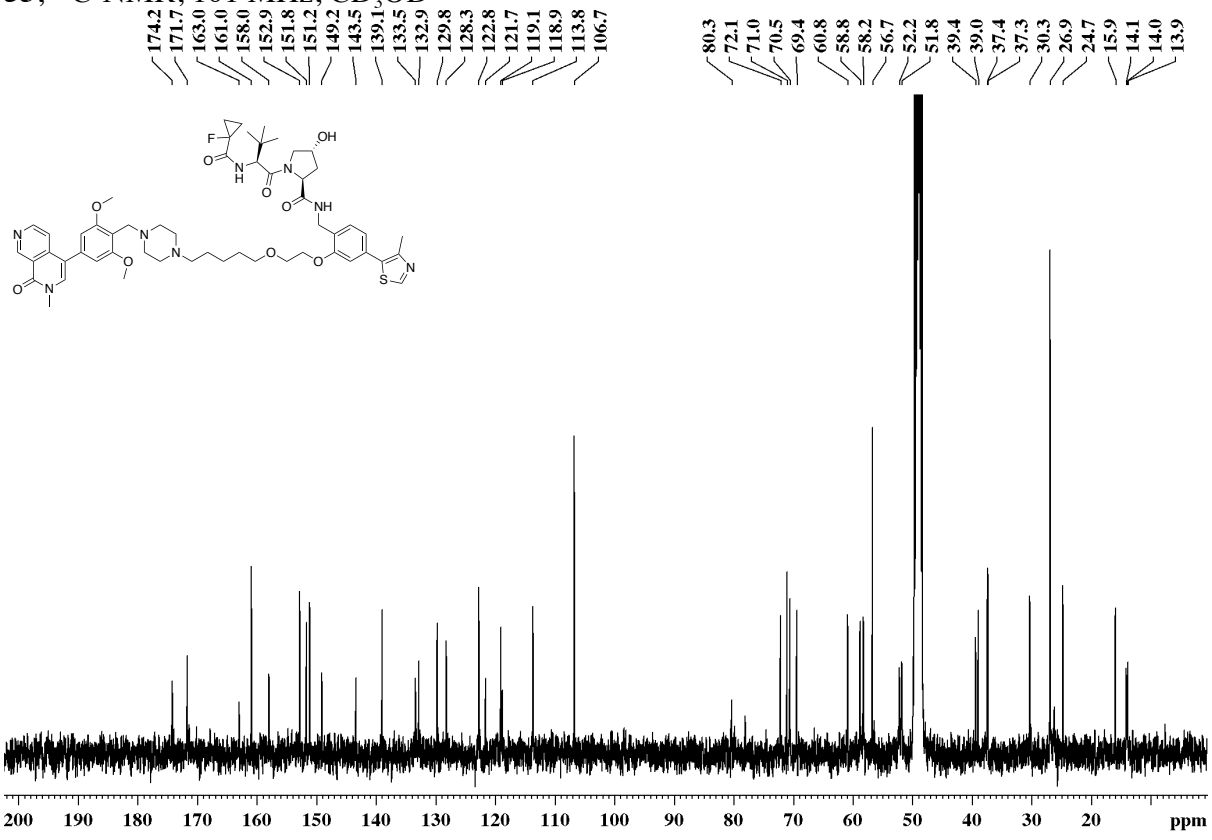
52, ^{13}C-NMR, 101 MHz, CD_3OD



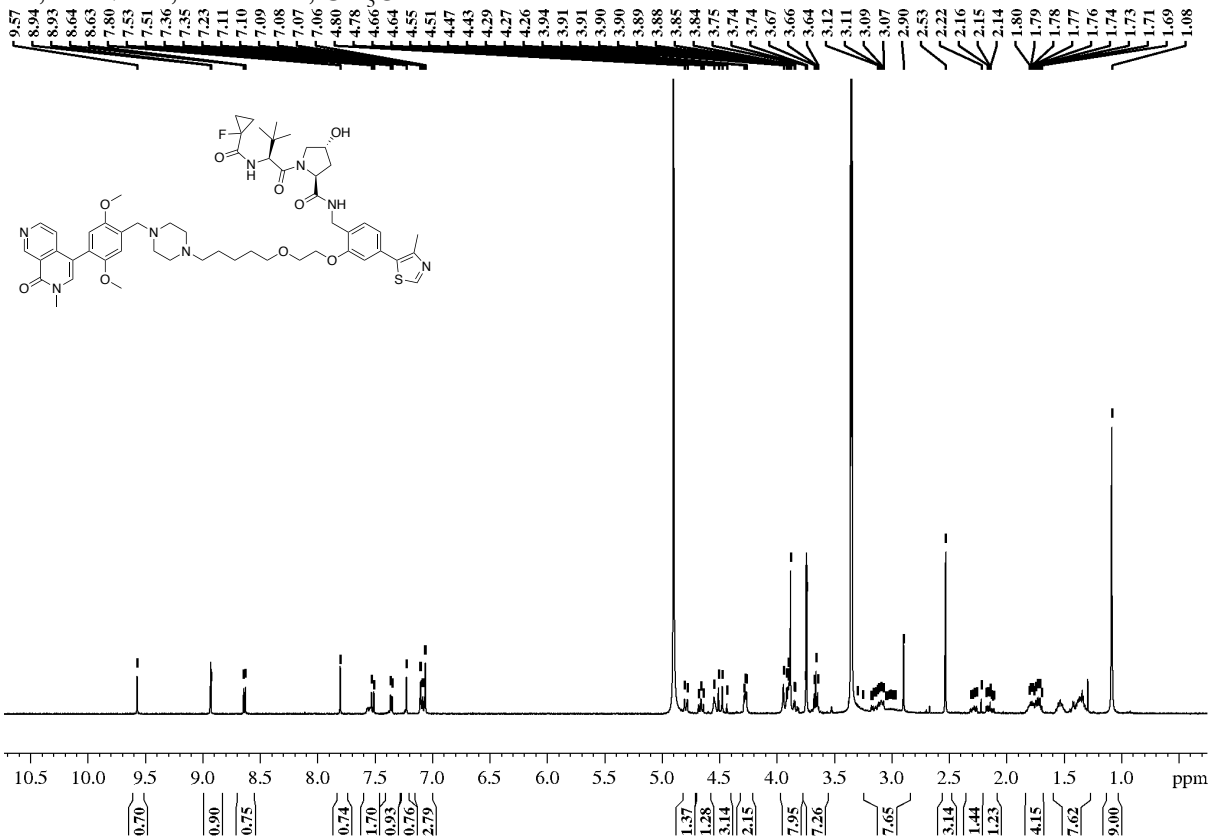
53, ^{1}H-NMR, 400 MHz, CD_3OD



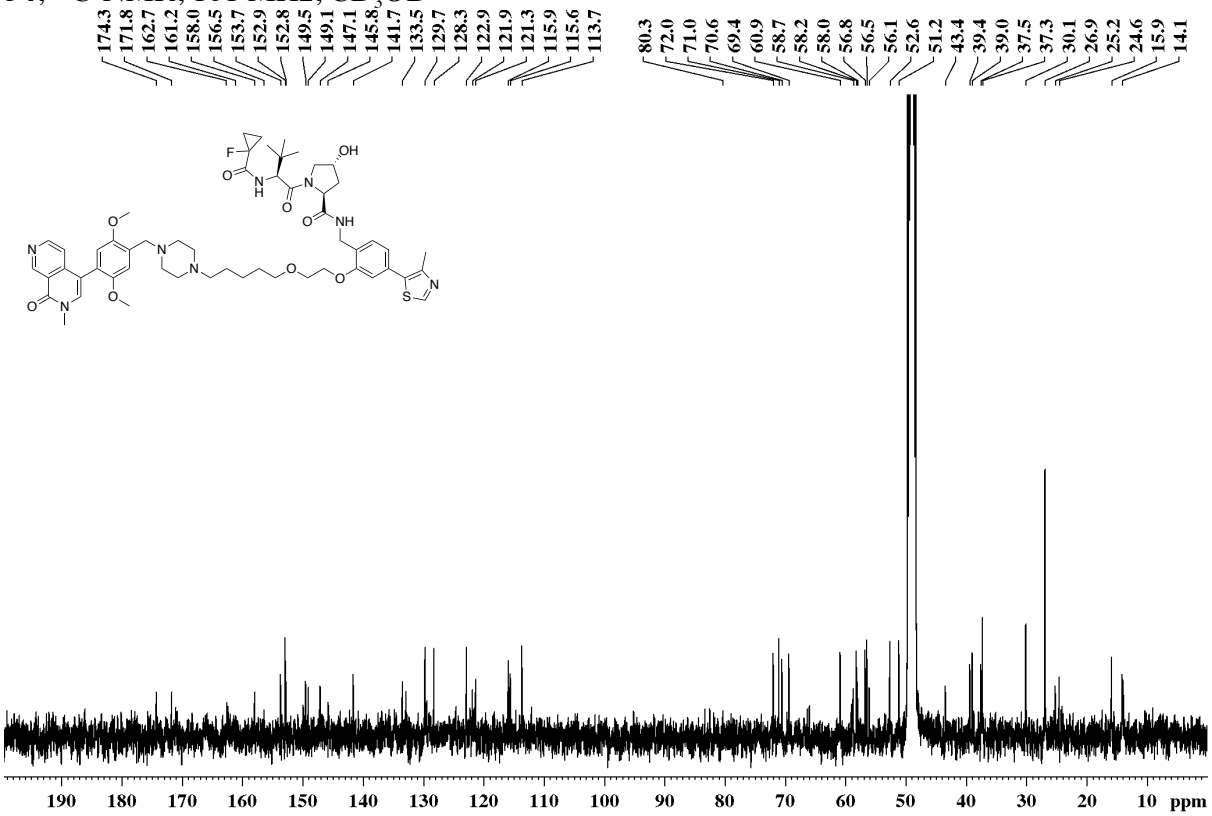
53, ^{13}C-NMR, 101 MHz, CD_3OD



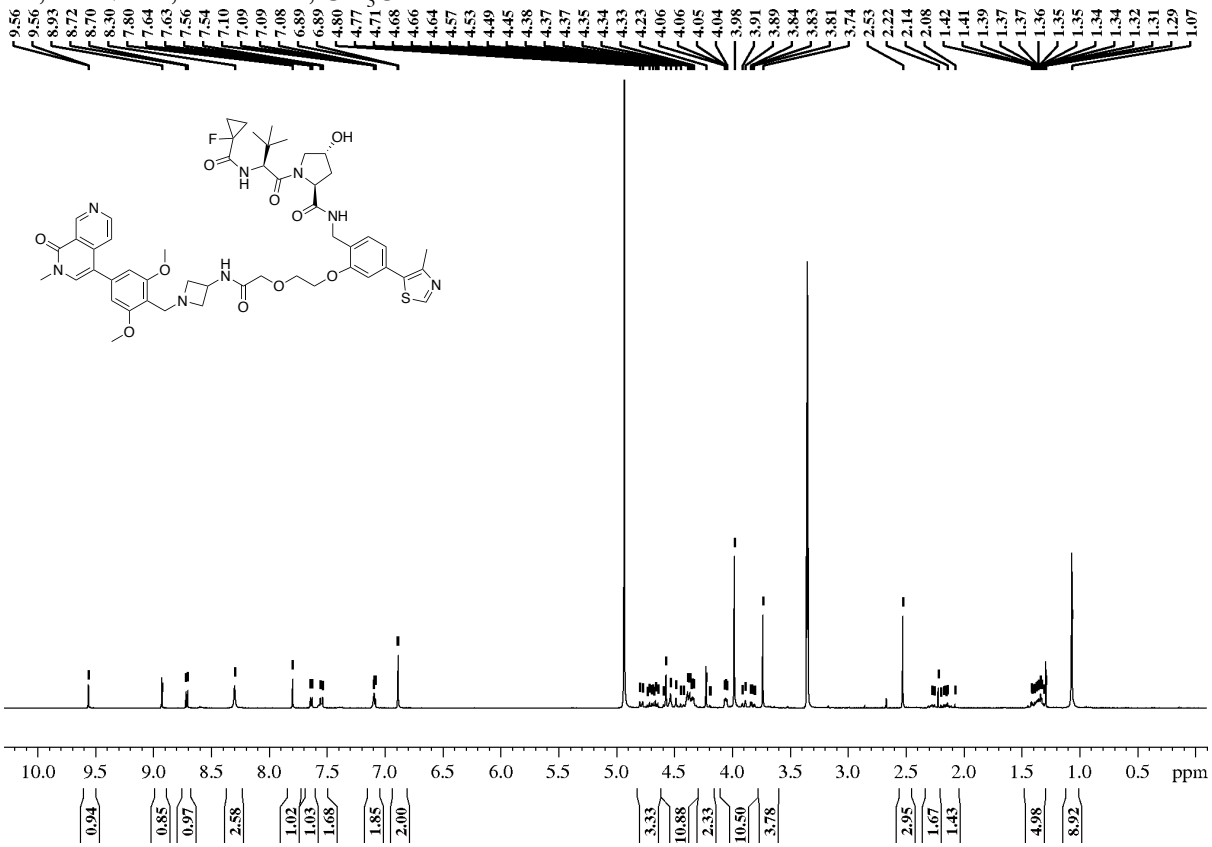
54, ^{1}H-NMR, 400 MHz, CD_3OD



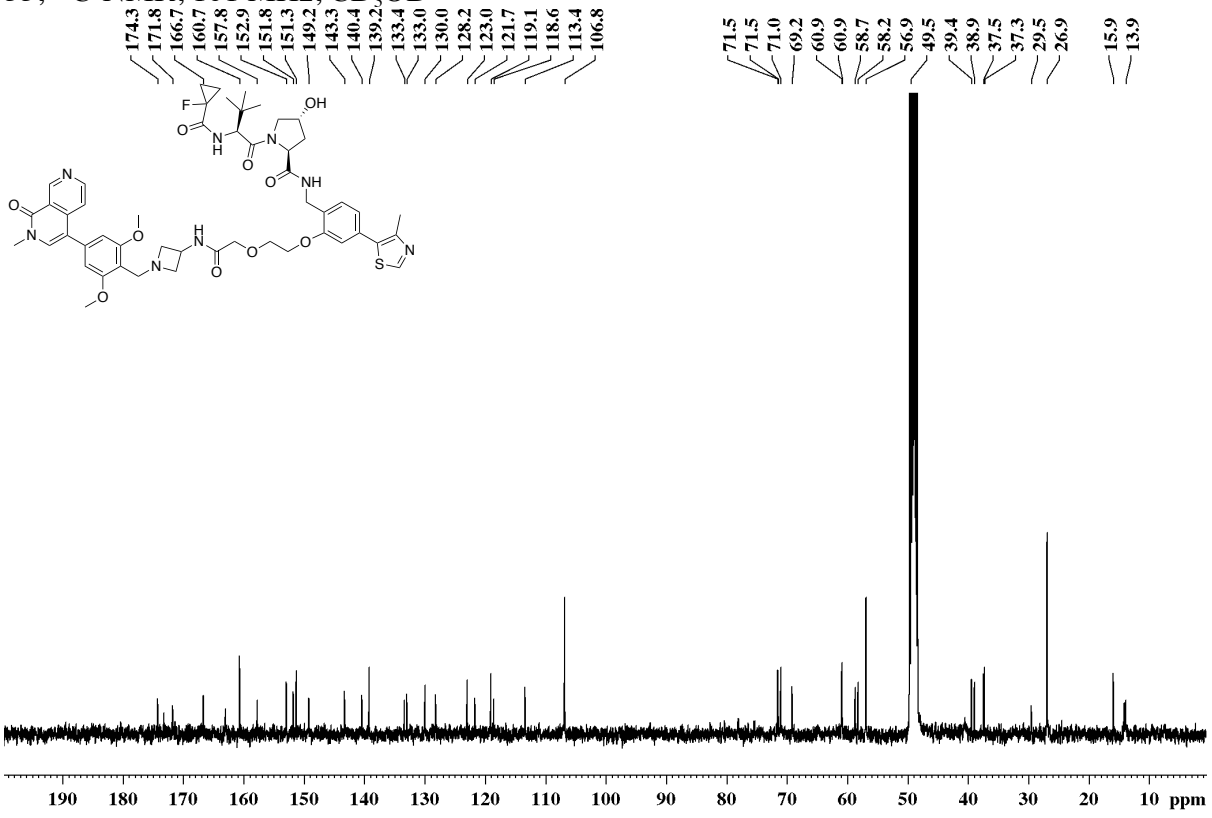
54, ^{13}C-NMR, 101 MHz, CD_3OD



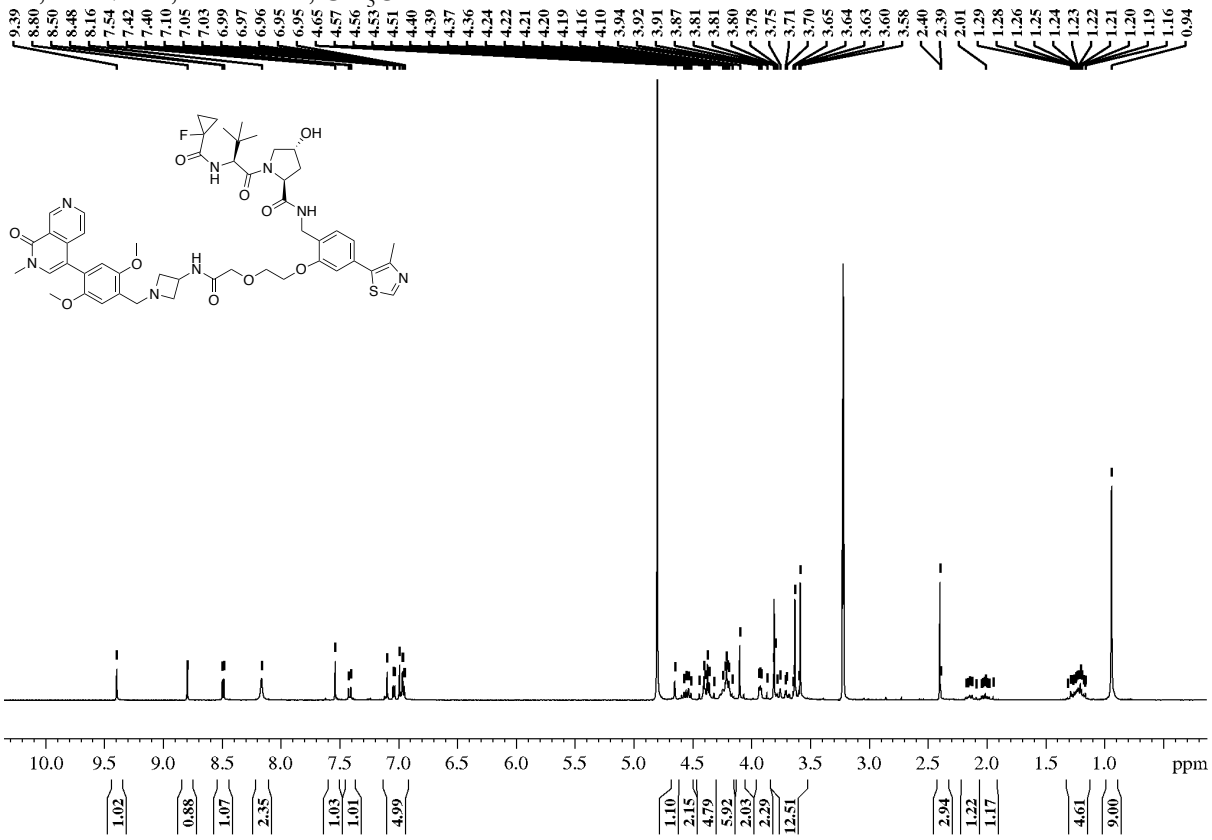
55, ^{1}H-NMR, 400 MHz, CD_3OD



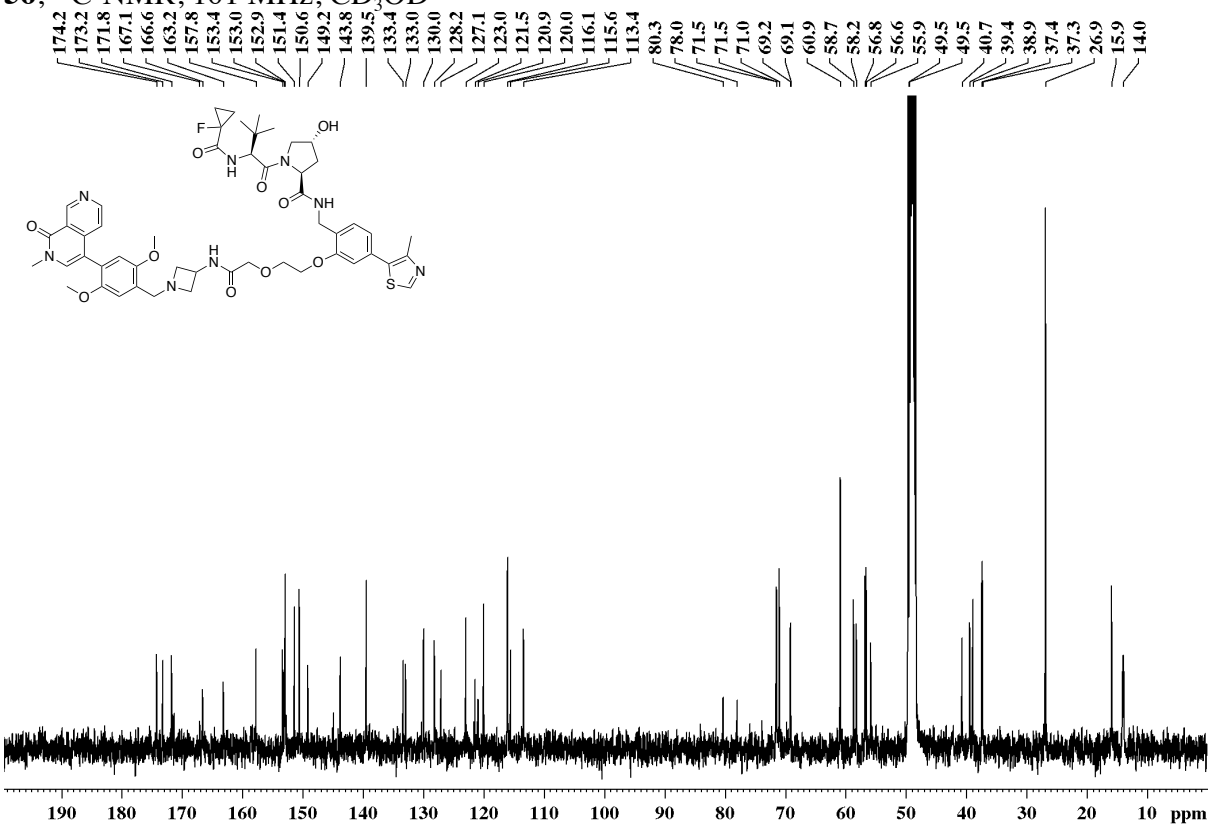
55, ^{13}C-NMR, 101 MHz, CD_3OD



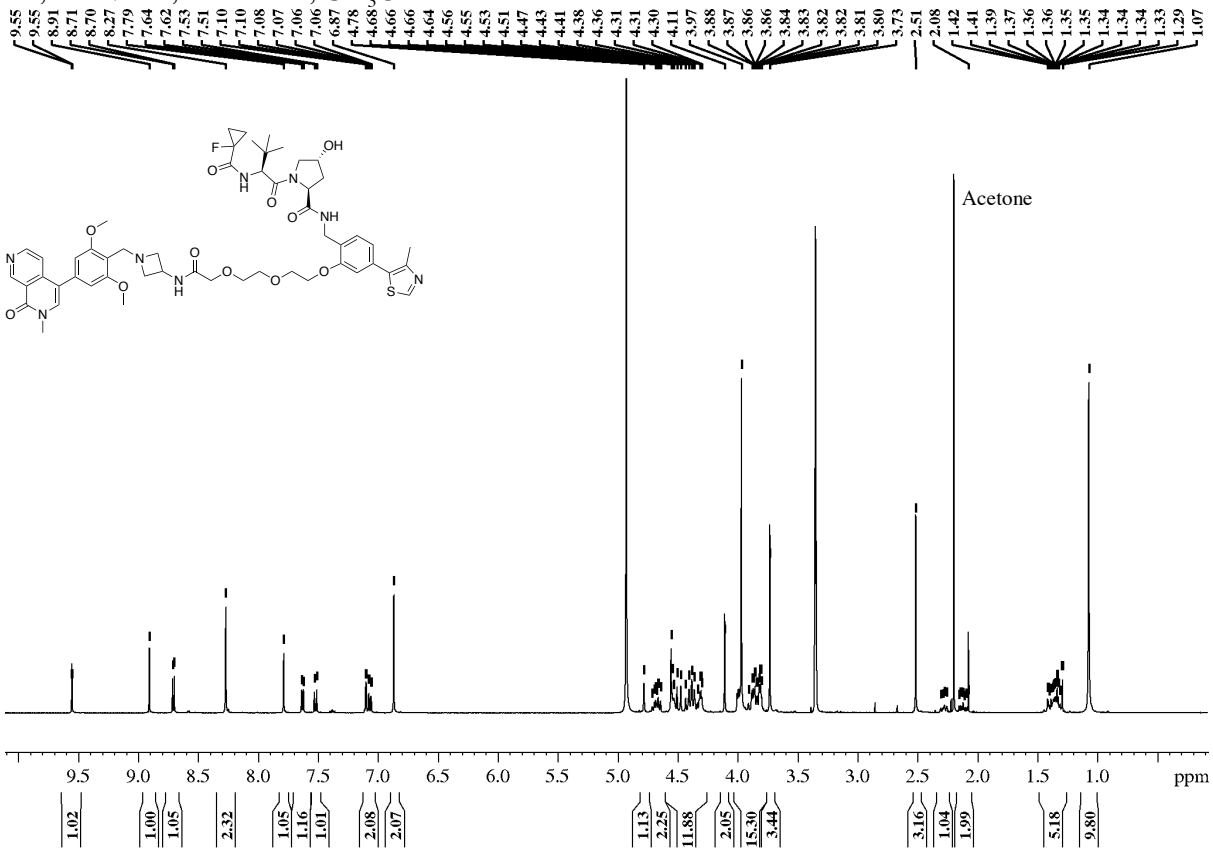
56, ^{1}H-NMR, 400 MHz, CD_3OD



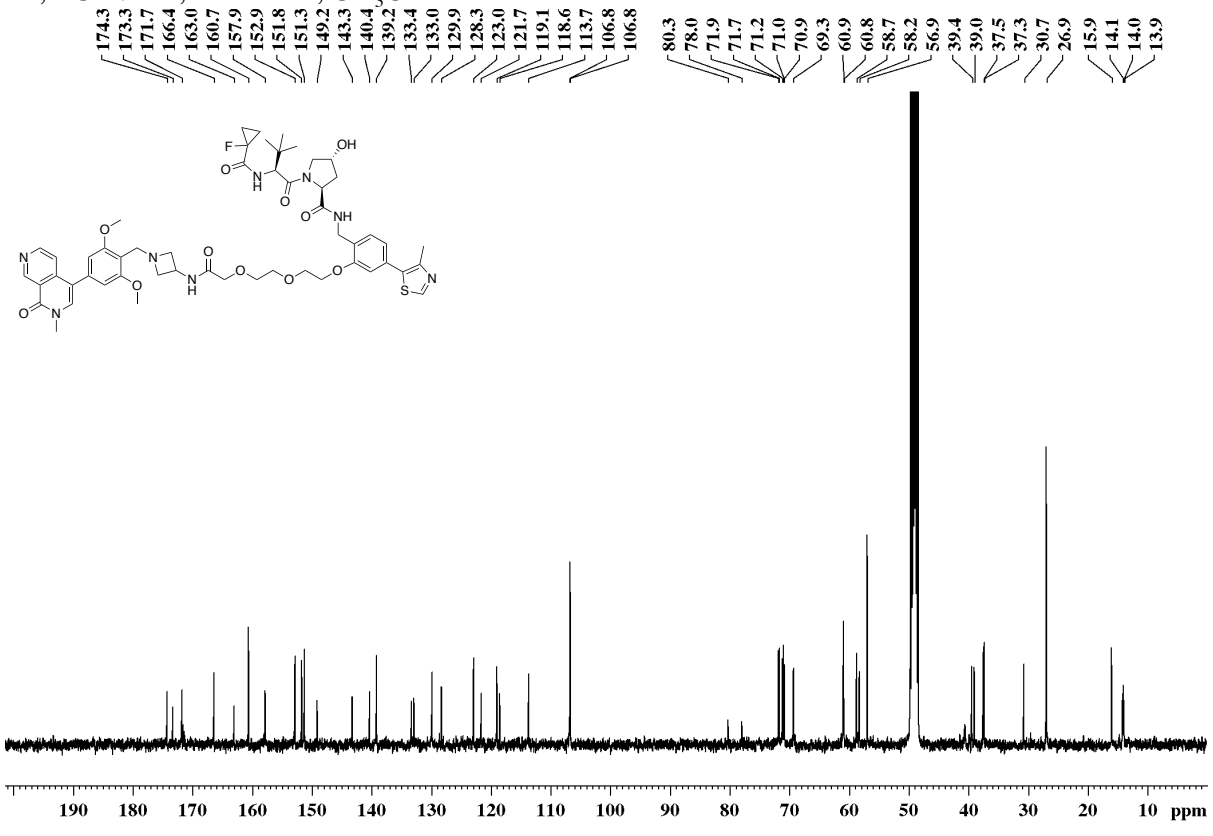
56, ^{13}C-NMR, 101 MHz, CD_3OD



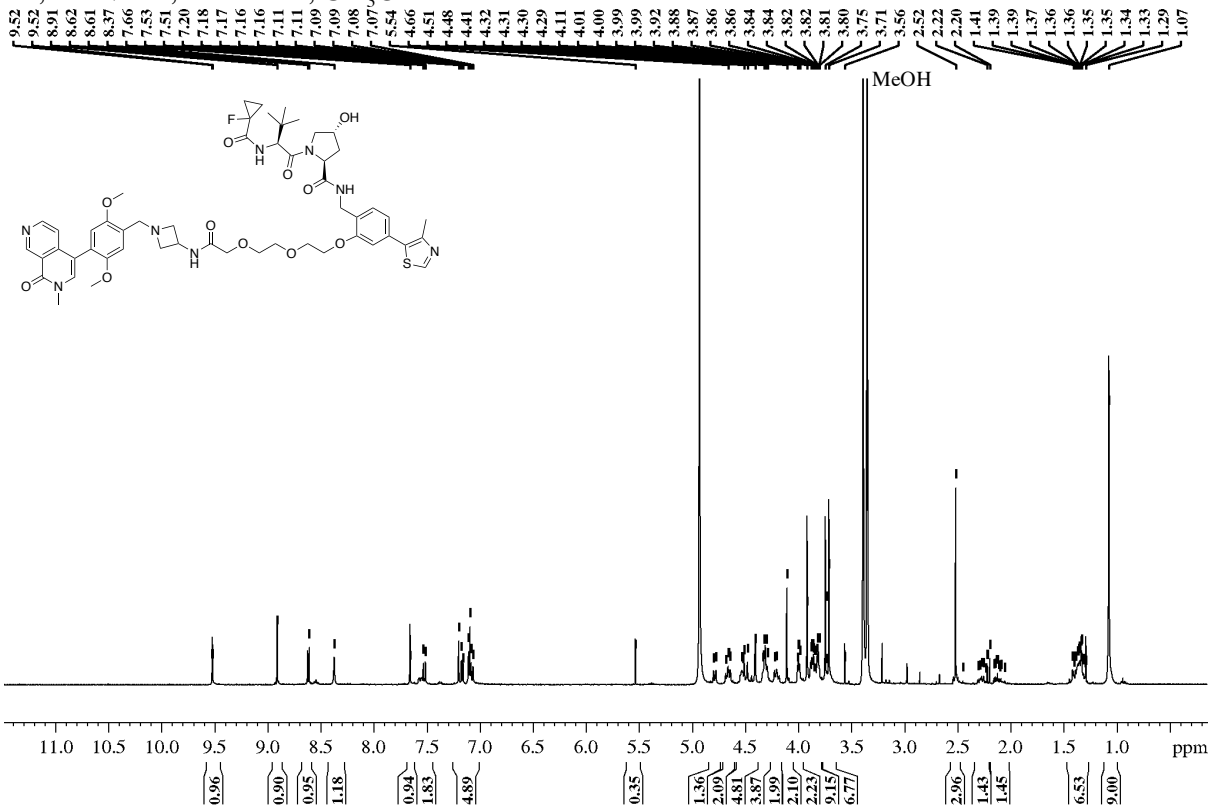
57, ^{1}H-NMR, 400 MHz, CD_3OD



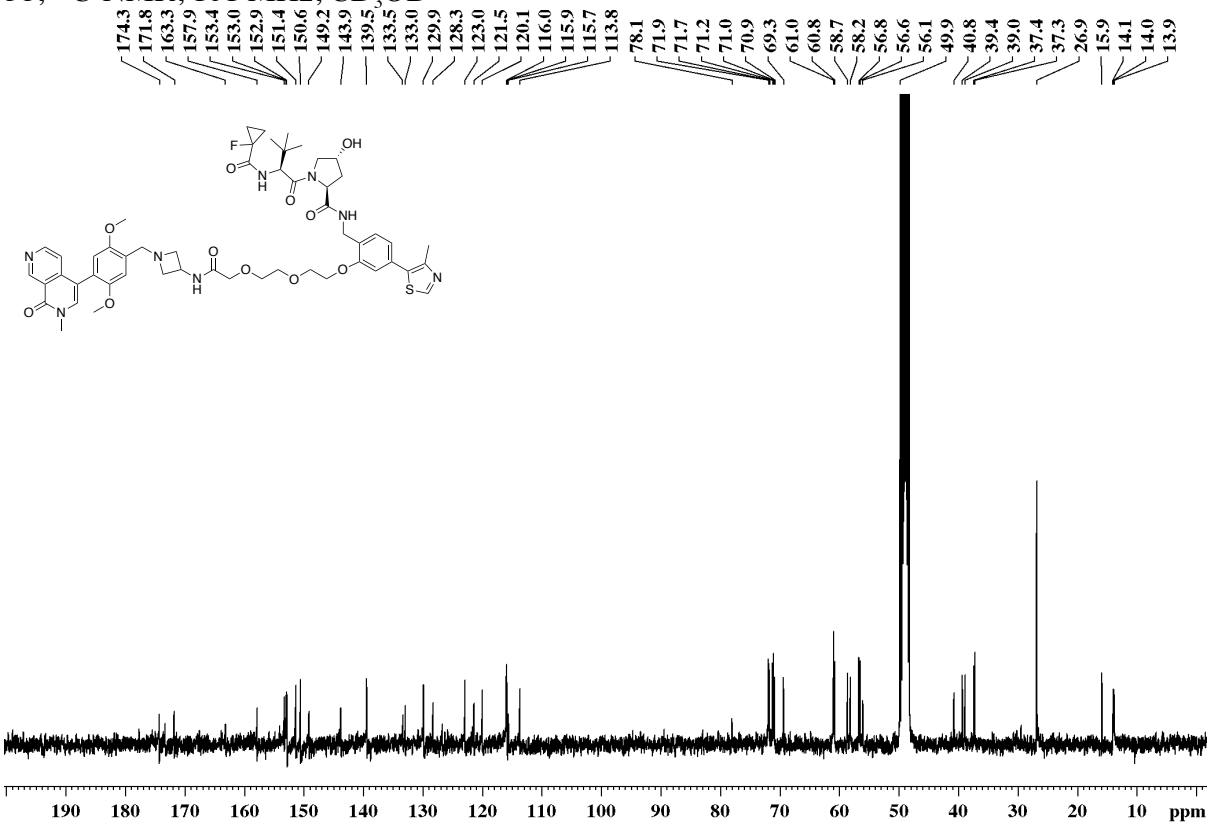
57, ^{13}C-NMR, 101 MHz, CD_3OD



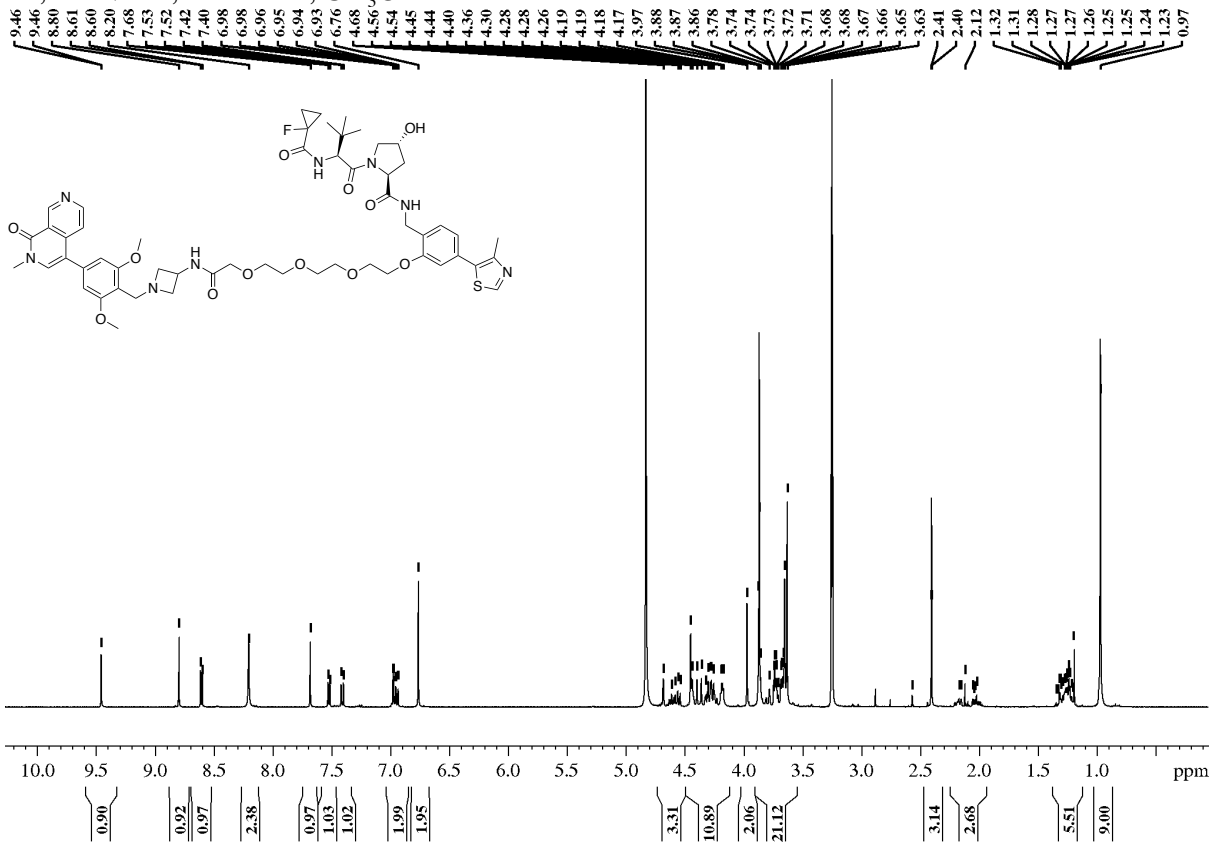
58, ^{1}H-NMR, 400 MHz, CD_3OD



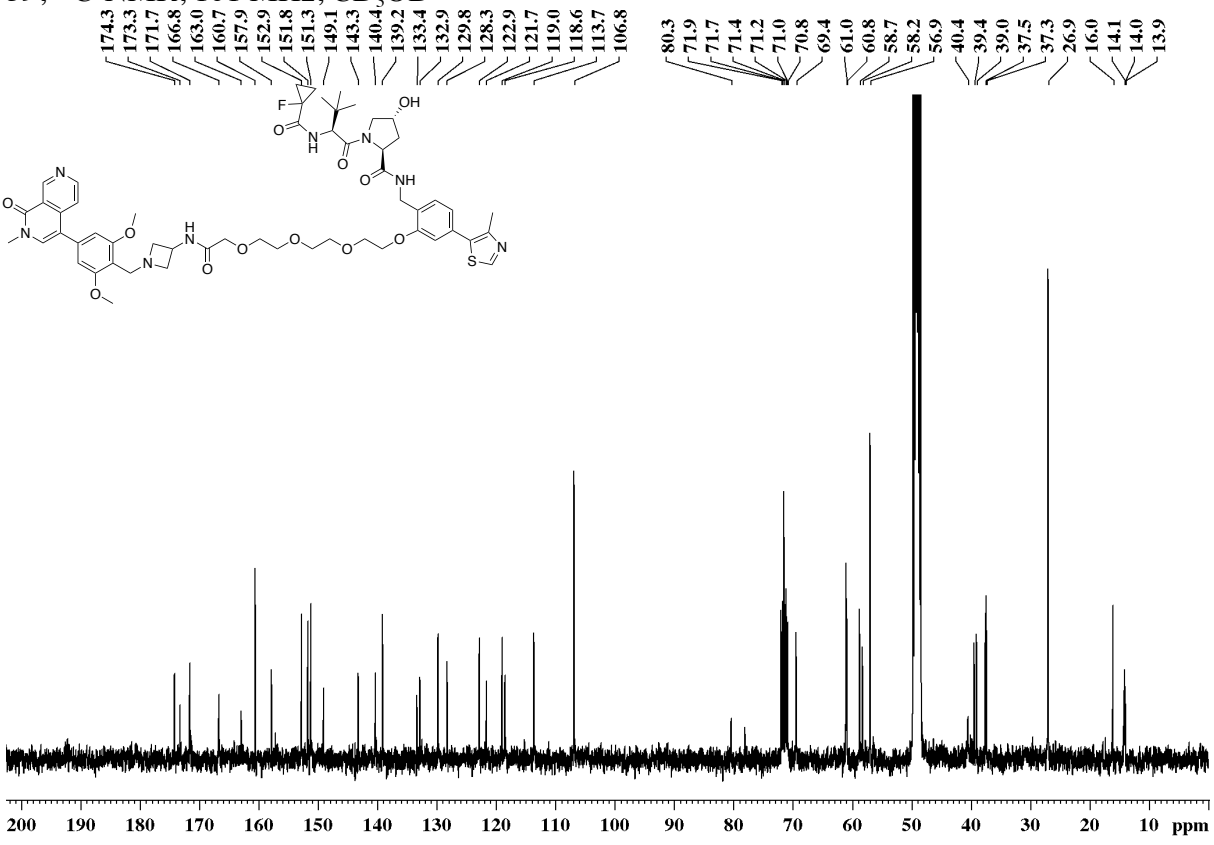
58, ^{13}C-NMR, 101 MHz, CD_3OD



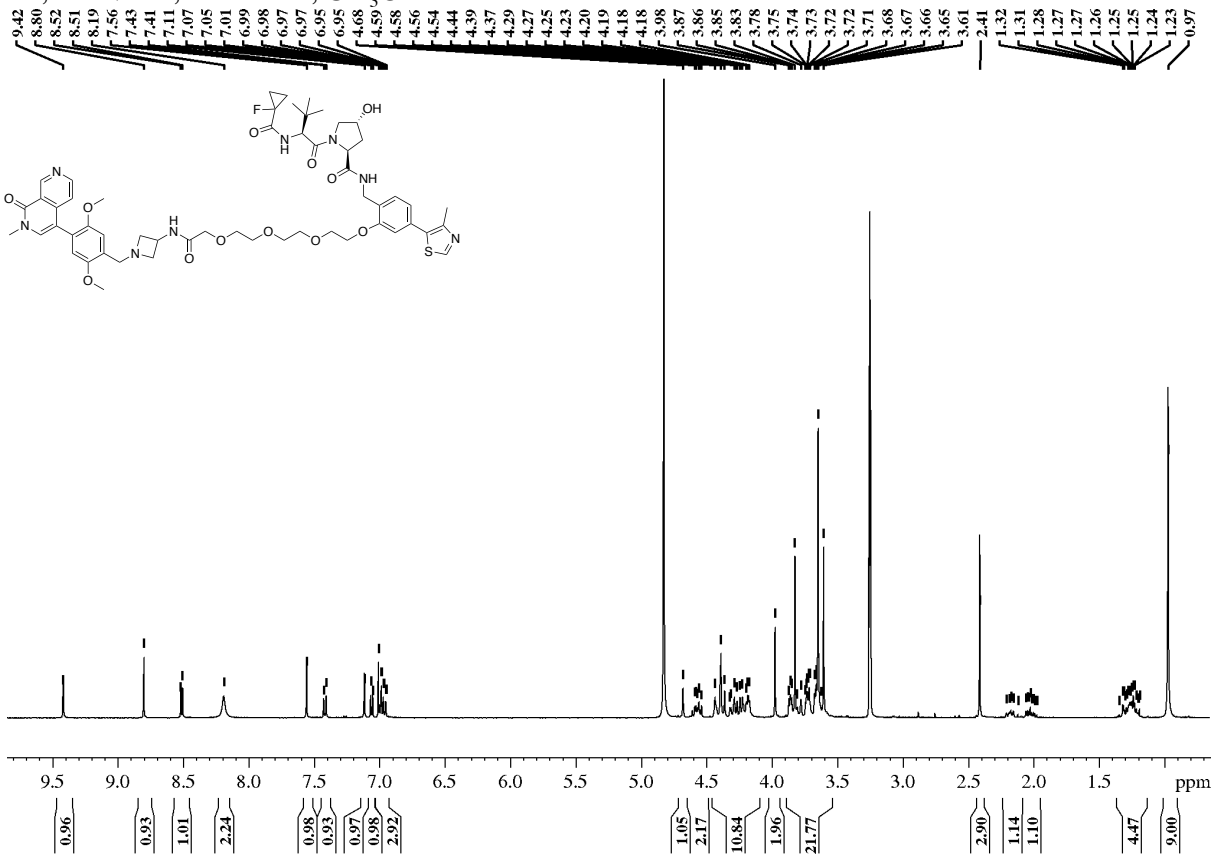
59, ^{1}H-NMR, 400 MHz, CD_3OD



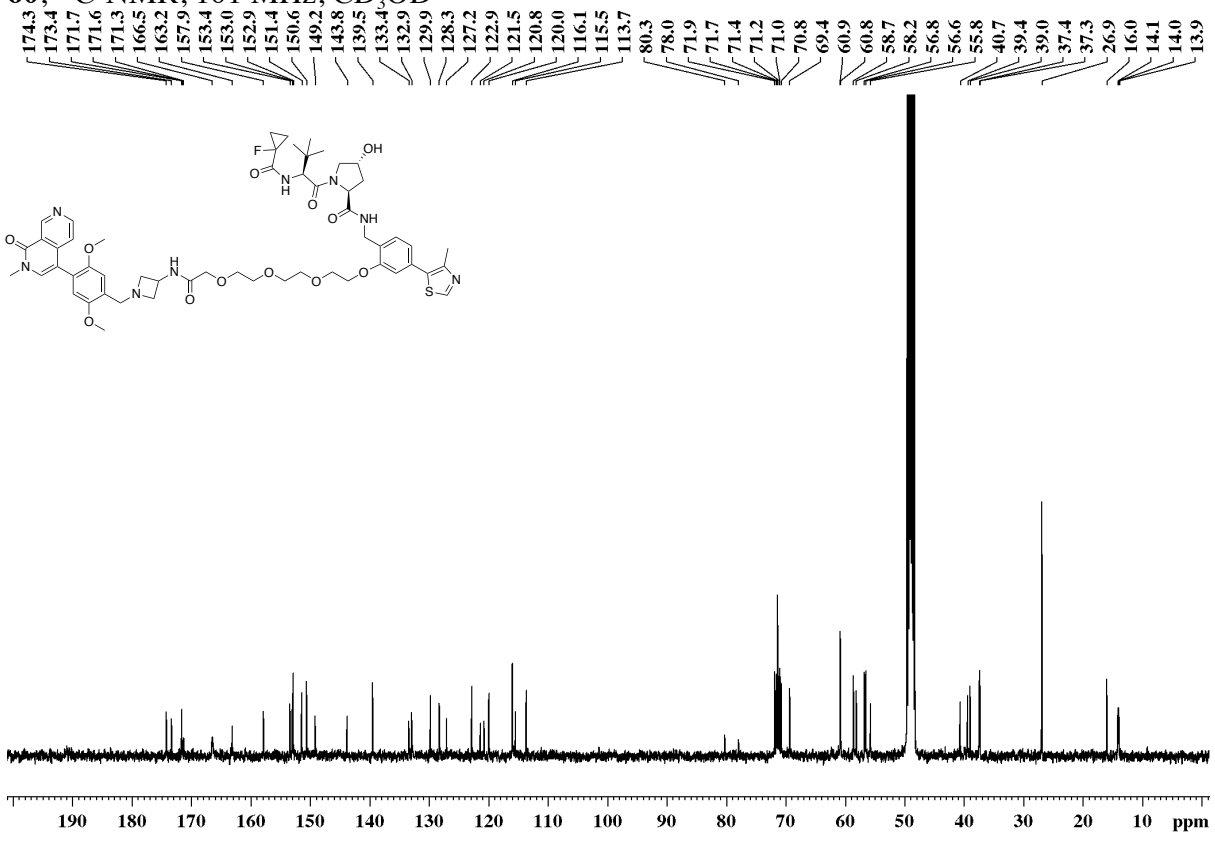
59, ^{13}C-NMR, 101 MHz, CD_3OD



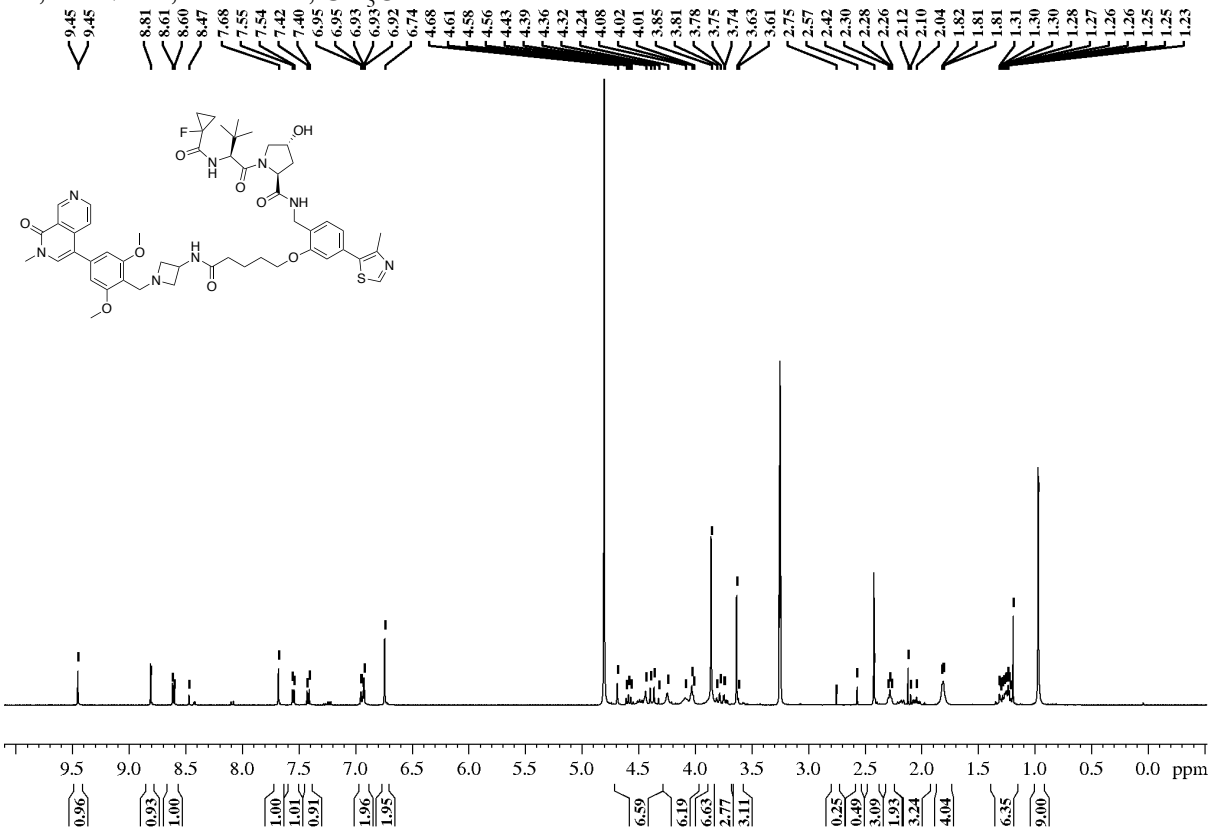
60, ^{1}H-NMR, 400 MHz, CD_3OD



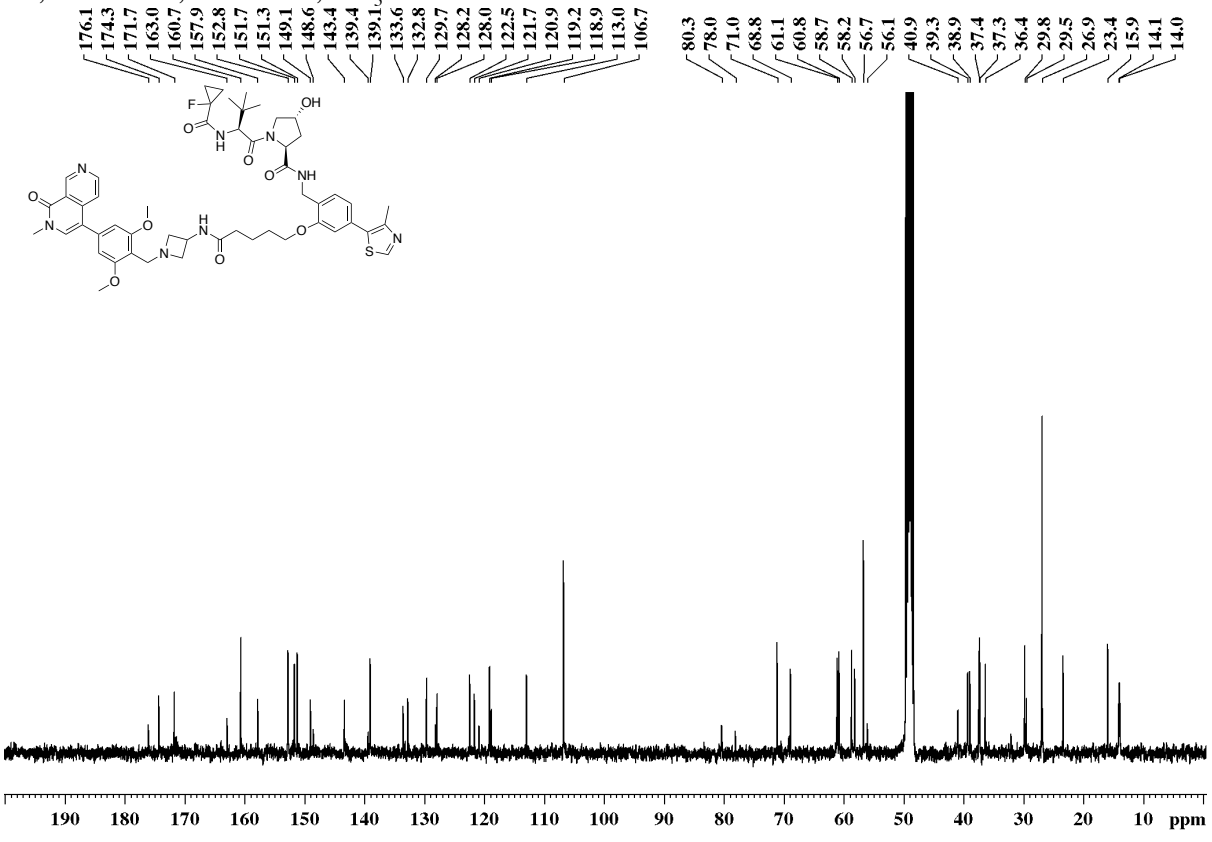
60, ^{13}C-NMR, 101 MHz, CD_3OD



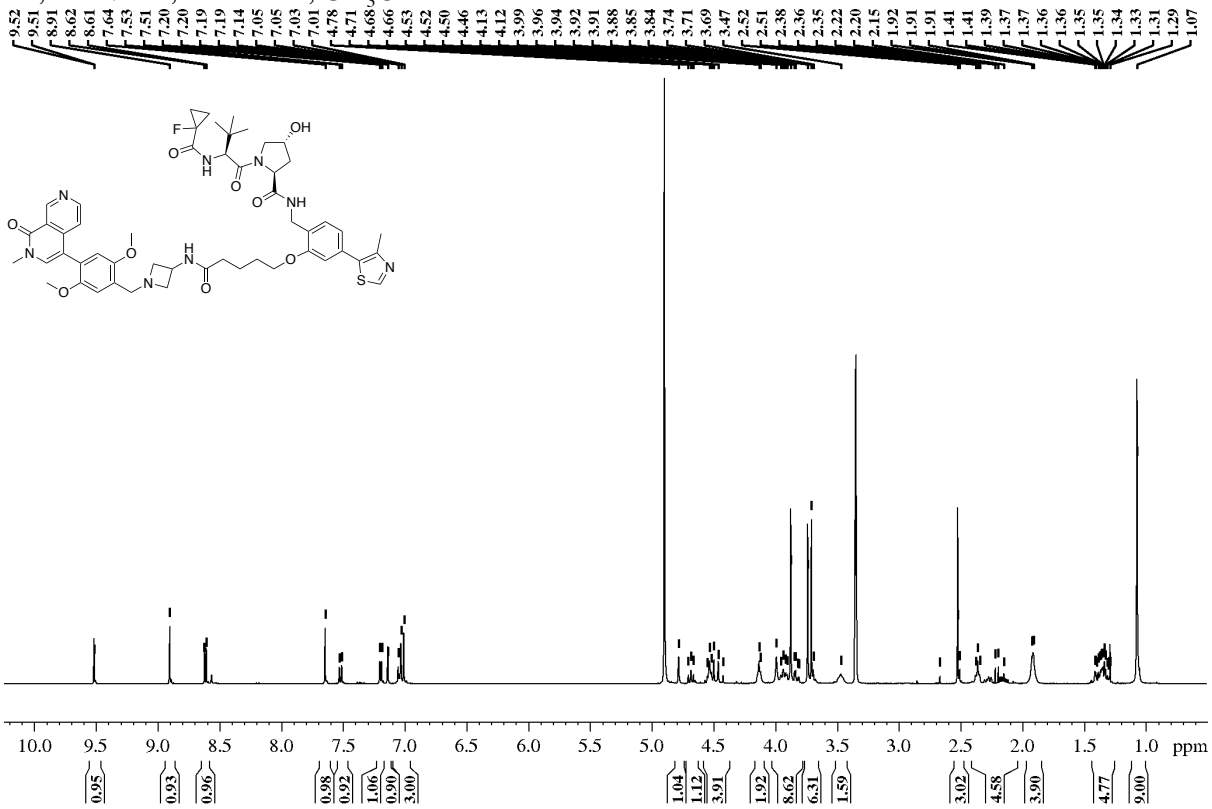
61, ^{1}H-NMR, 400 MHz, CD_3OD



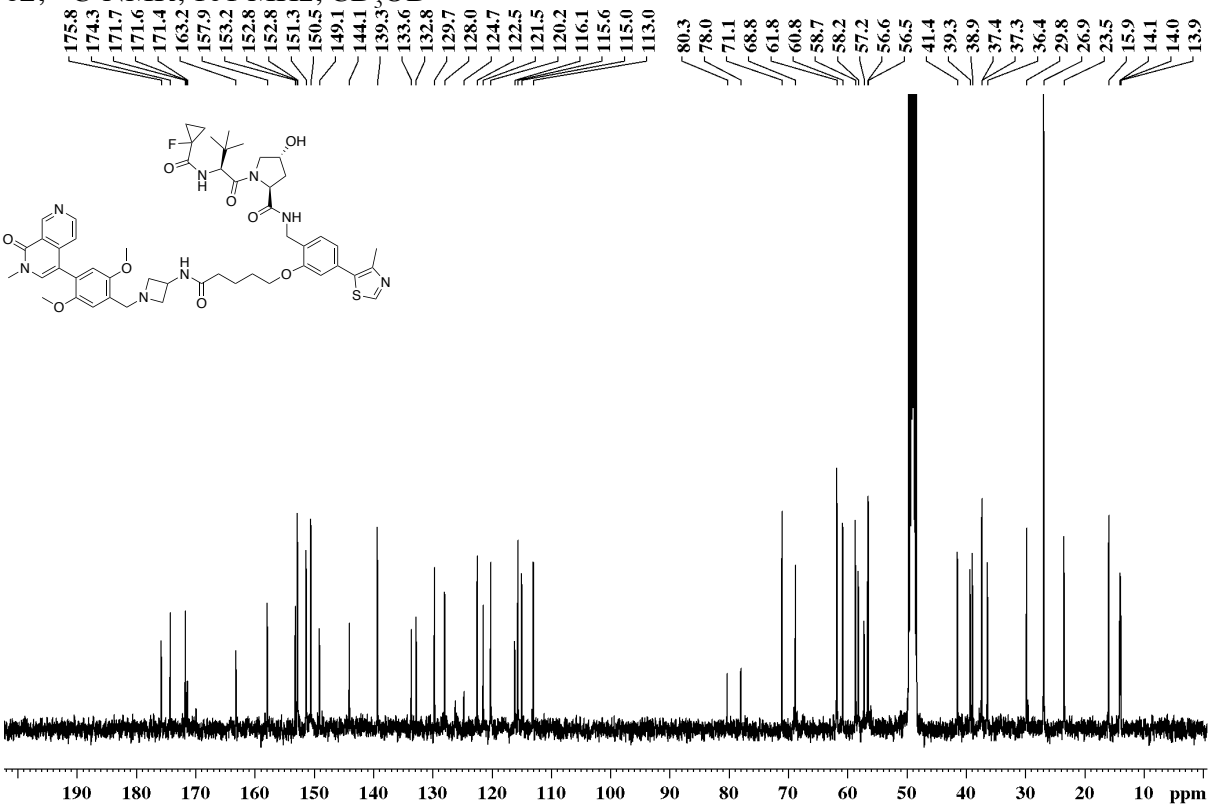
61, ^{13}C-NMR, 101 MHz, CD_3OD



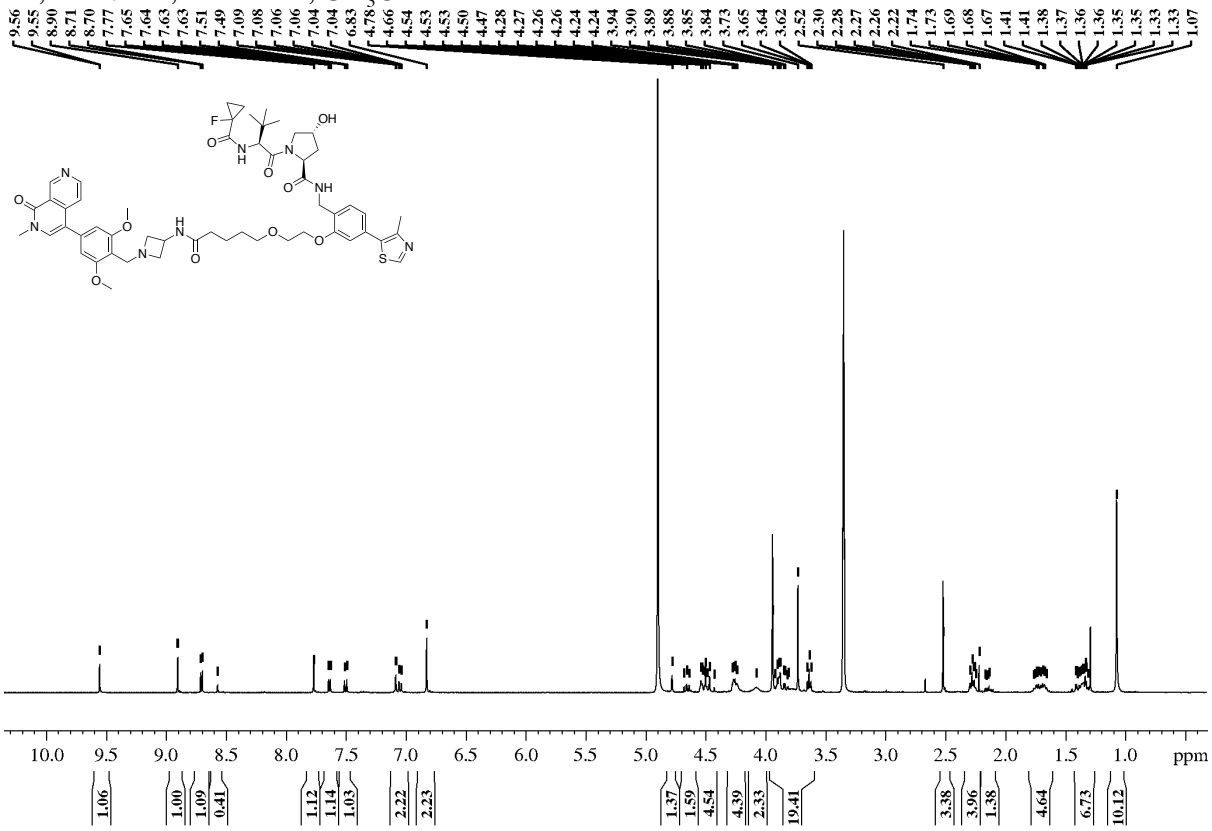
62, ^{1}H-NMR, 400 MHz, CD_3OD



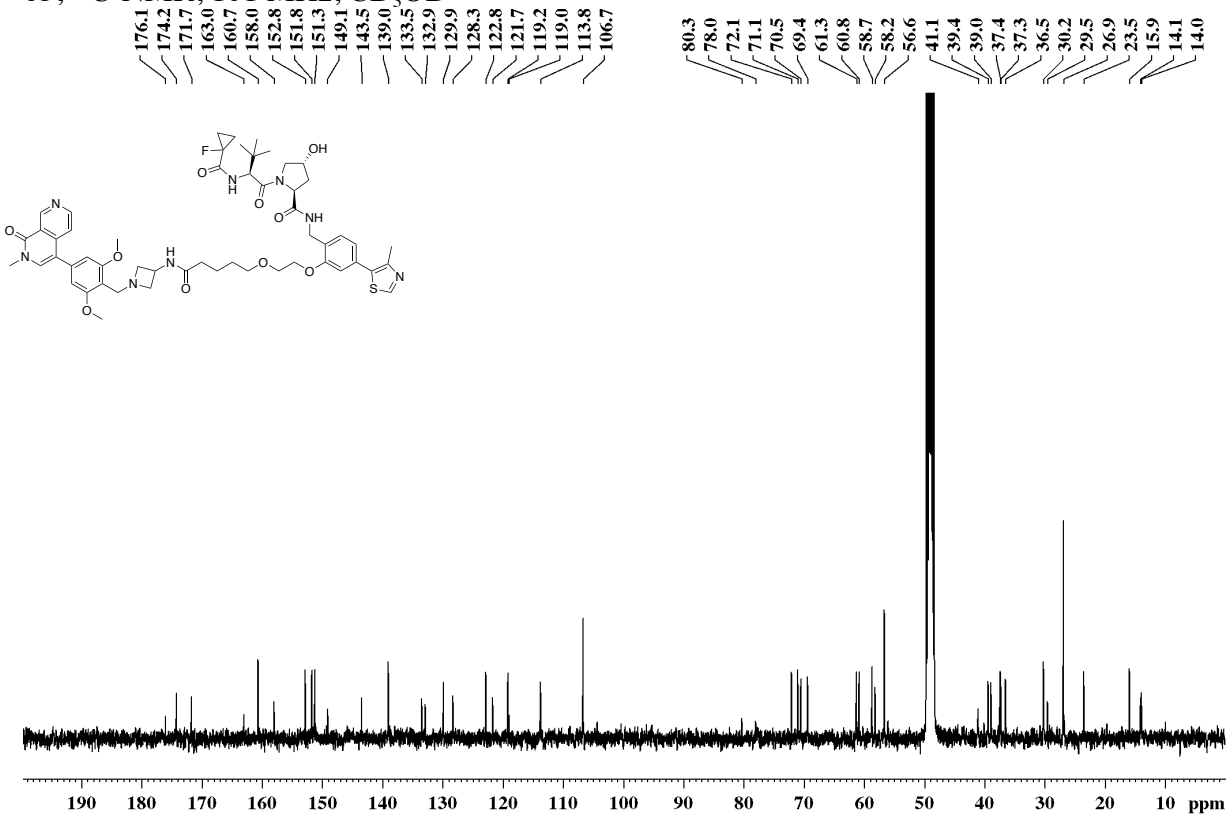
62, ^{13}C-NMR, 101 MHz, CD_3OD



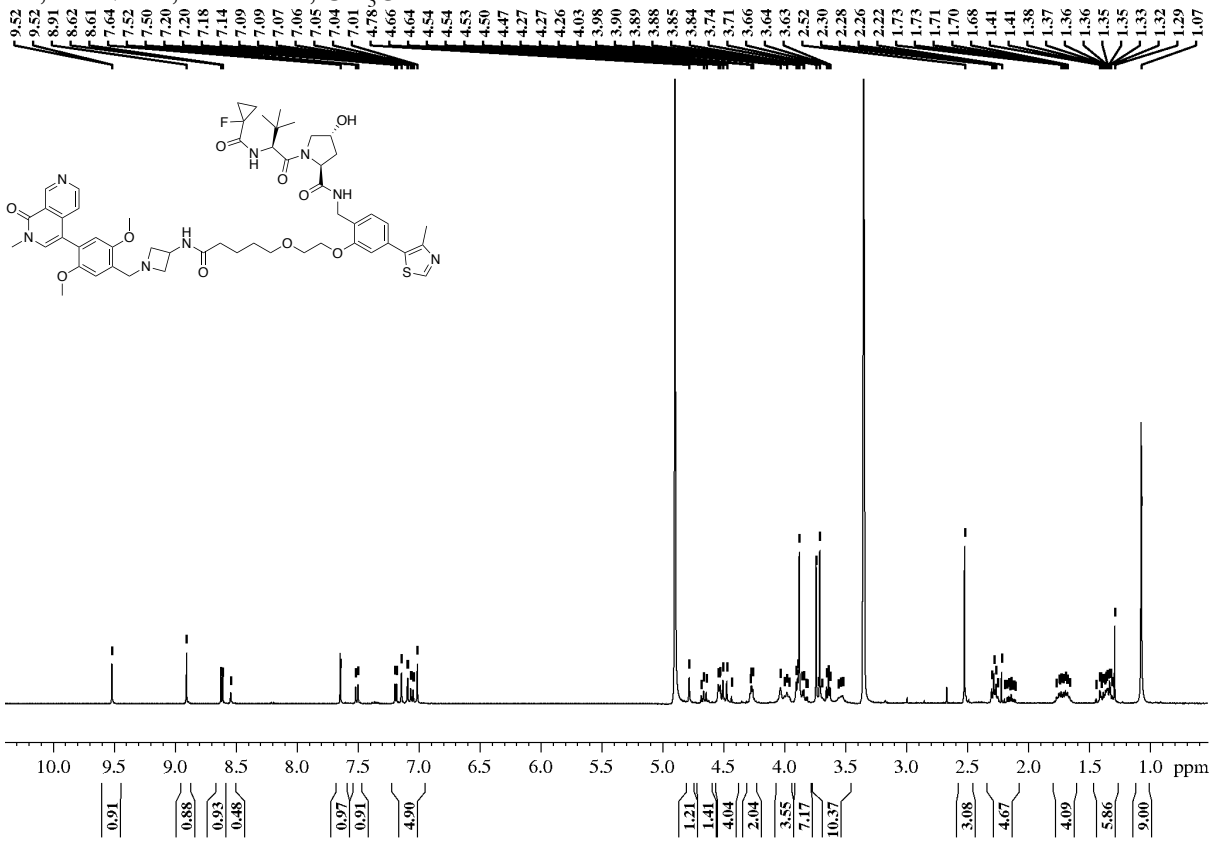
63, ^{1}H-NMR, 400 MHz, CD_3OD



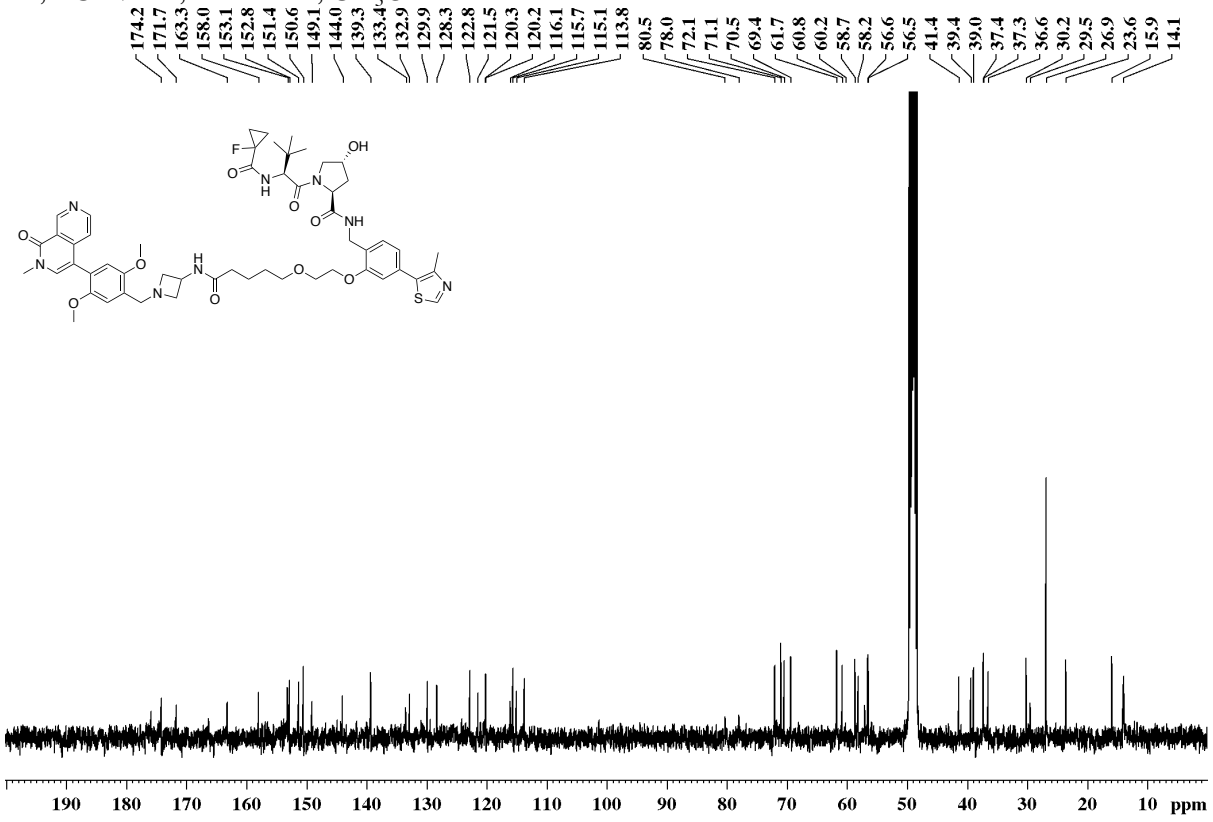
63, ^{13}C-NMR, 101 MHz, CD_3OD



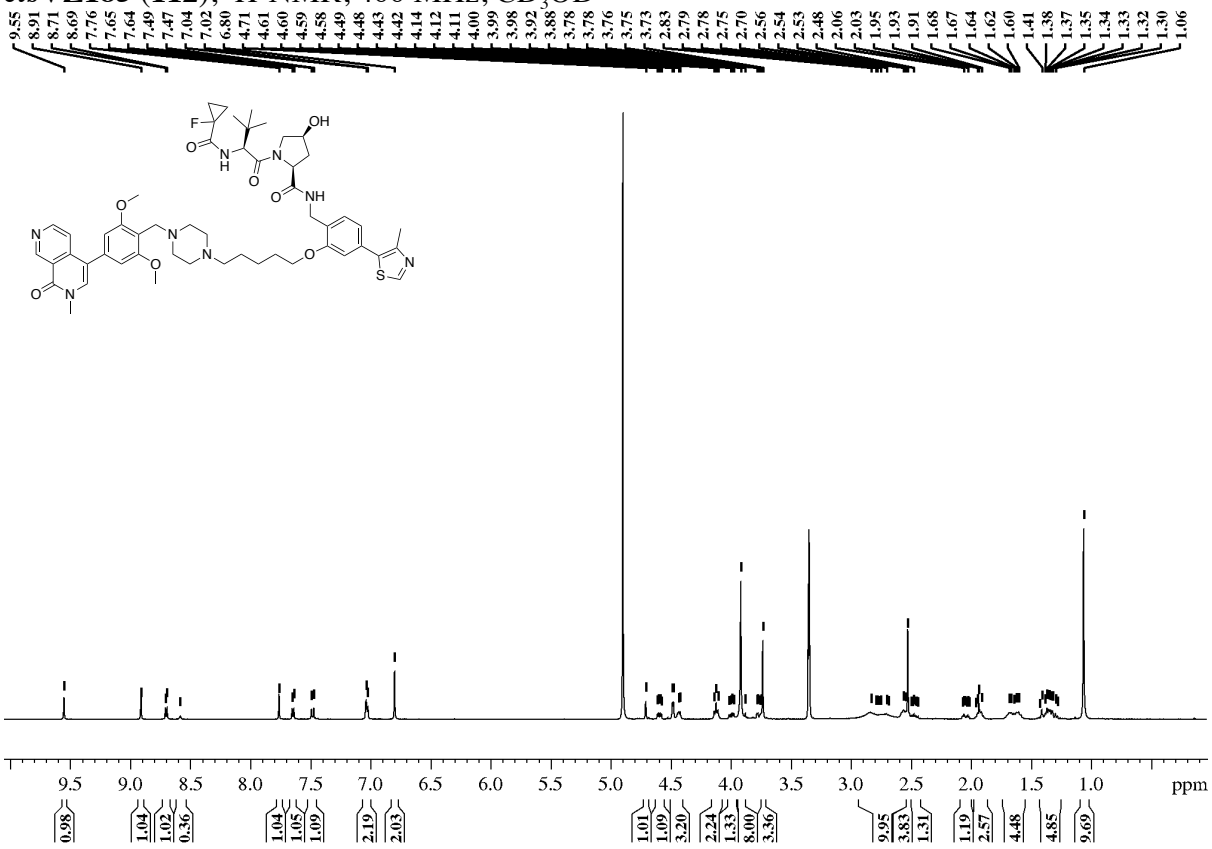
64, ^{1}H-NMR, 400 MHz, CD_3OD



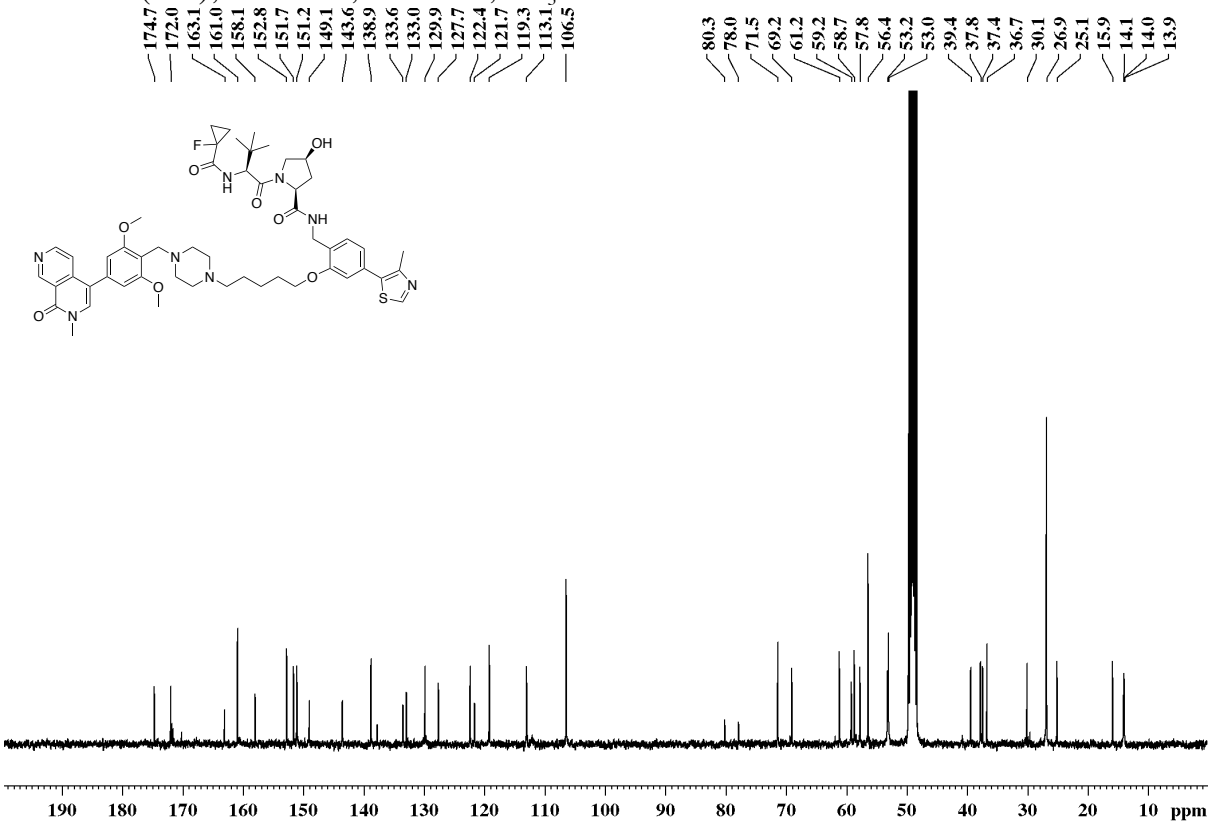
64, ^{13}C-NMR, 101 MHz, CD_3OD



***cis*VZ185 (112)**, ^{1}H-NMR, 400 MHz, CD_3OD



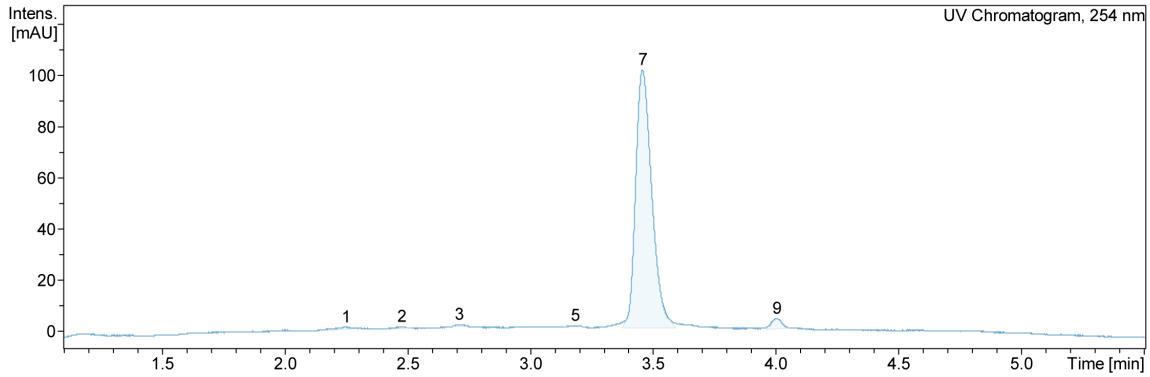
***cis*VZ185 (112)**, ^{13}C-NMR, 101 MHz, CD_3OD

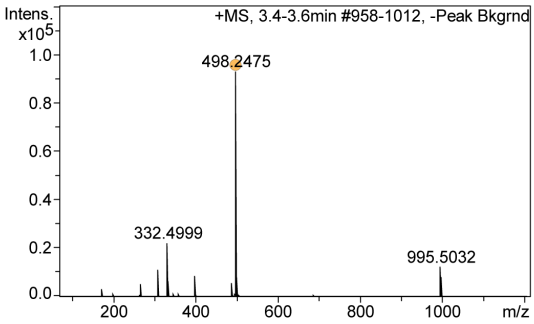


HPLC traces

HPLC trace of **VZ185** (**51**)

Method: 5-95% ACN/H_2O + 0.1% Formic Acid gradient over 8 min
Column: X-Bridge 50 mm x 2.1 mm x 3.5 µm

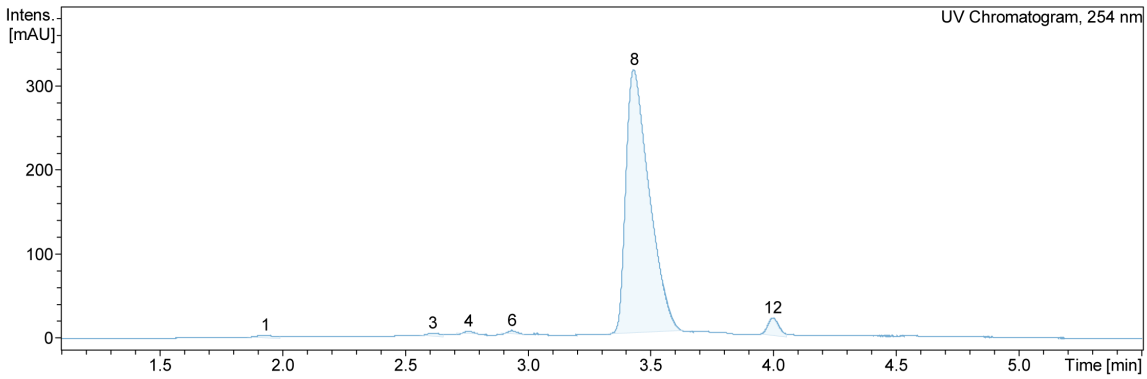


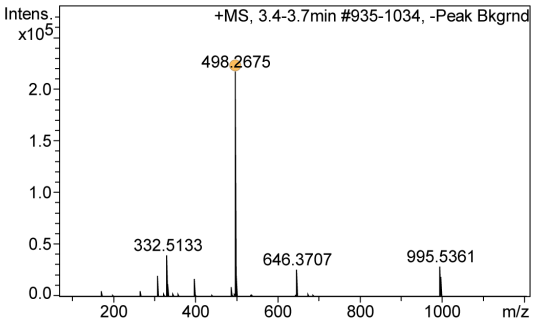


#	RT [min]	Area Frac. %	Chromatogram
1	2.2	1.0633	UV Chromatogram, 254 nm
2	2.5	0.5420	UV Chromatogram, 254 nm
3	2.7	0.4685	UV Chromatogram, 254 nm
5	3.2	0.1978	UV Chromatogram, 254 nm
7	3.5	95.2245	UV Chromatogram, 254 nm
9	4.0	2.5038	UV Chromatogram, 254 nm

HPLC trace of ***cis*VZ185 (112)**

Method: 5-95% ACN/H_2O + 0.1% Formic Acid gradient over 8 min
Column: X-Bridge 50 mm x 2.1 mm x 3.5 μm





#	RT [min]	Area Frac. %	Chromatogram
1	1.9	0.8485	UV Chromatogram, 254 nm
3	2.6	0.6530	UV Chromatogram, 254 nm
4	2.8	0.3365	UV Chromatogram, 254 nm
6	2.9	0.3722	UV Chromatogram, 254 nm
8	3.4	94.6813	UV Chromatogram, 254 nm
12	4.0	3.1084	UV Chromatogram, 254 nm

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