Supporting Information

for

Rational design and discovery of potent PROTAC degraders of ASK1: A targeted therapy in MASH

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Cytotoxicity Assay

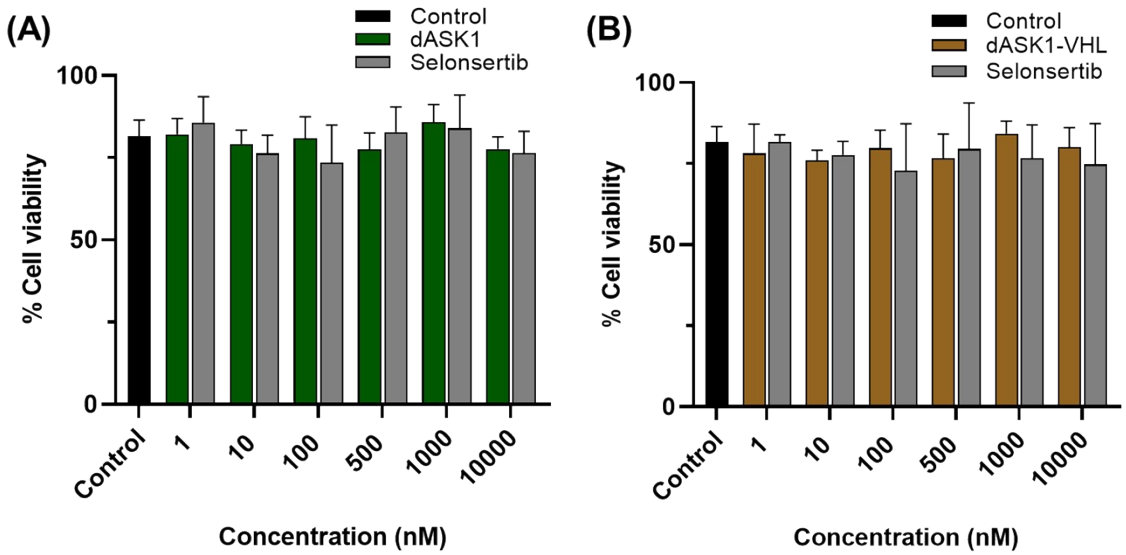


Figure S1. MTT assay for compounds (A) **dASK1 (35)** and (B) **dASK1-VHL (60)**.

Degradation of ASK1 via proteasome-dependent manner

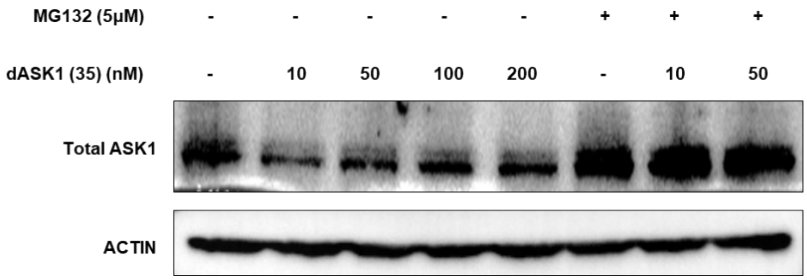


Figure S2. dASK1 (35) mediated degradation of ASK1 is proteasome-dependent. HepG2 cells were pre-treated for 30 min with 5 μM of MG132 followed by treatment with respective doses of **dASK1 (35)** for 8h. Total cell lysates were immunoblotted for ASK1 protein levels.

Table S1. Binding free energy of conformations with variable distance constraints in the linker

Protein complex minimized with an in-between distance of	Binding free energy (kcal/mol)
8 Å	-117.56
10 Å	-163.09
12 Å	-172.34
13 Å	-167.98
15 Å	-168.06
17 Å	-131.07

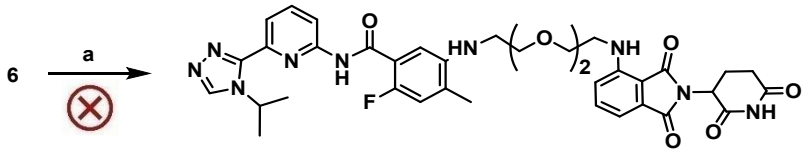


Table S2. The table depicts the reagents and conditions used for the failed reaction (Scheme 4): (a) **11**, different reagents and reaction conditions undertaken

Substrates	Catalysts	Ligands	Bases	Reaction conditions
Compound **6** + Compound **11**	$Pd_2(dba)_3$ (0.05-0.1 equiv)	± BINAP (0.1-0.5 equiv)	NaO^tBu	(i) Dry toluene, 110 ^{0}C, reactive vial, 18 h (ii) tert-butanol, 100 ^{0}C, 100 W, 1 h, µwave
	$Pd_2(dba)_3$ (0.05-0.1 equiv)	X-Phos (0.1-20 equiv)	K_2CO_3	Dioxane, 100 ^{0}C, overnight
	$Pd_2(dba)_3$ (0.05-20 equiv)	X-Phos (0.1-20 equiv)	K_2CO_3	DMF, 100 ^{0}C, 100 W, 1 h, µwave
	$Pd_2(dba)_3$ (0.05-20 equiv)	X-Phos (0.1-20 equiv)	K_2CO_3	tert-Butanol, 110 ^{0}C, 100 W, 1-2 h, µwave
	$Pd_2(dba)_3$ (0.05-20 equiv)	X-Phos (0.1-20 equiv)	Cs_2CO_3	DMF, 100 ^{0}C, 100 W, 1-2 h, µwave
	$Pd_2(dba)_3$ (0.05-20 equiv)	X-Phos (0.1-20 equiv)	NaO^tBu	DMF, 100 ^{0}C, 100 W, 1-2 h, µwave
	$Pd_2(dba)_3$ (0.05-20 equiv)	Xantphos (0.1-20 equiv)	K_2CO_3	DMF, 100 ^{0}C, 100 W, 1-2 h, µwave
	$Pd_2(dba)_3$ (0.05-30 equiv)	Xantphos (0.1-30 equiv)	LiHMDS	DMF, 100 ^{0}C, 100 W, 1-2 h, µwave
	$Pd_2(dba)_3$ (0.05-30 equiv)	X-Phos (0.1-30 equiv)	LiHMDS (1 M in THF)	DMF, 110 ^{0}C, 100 W, 1-2 h, µwave

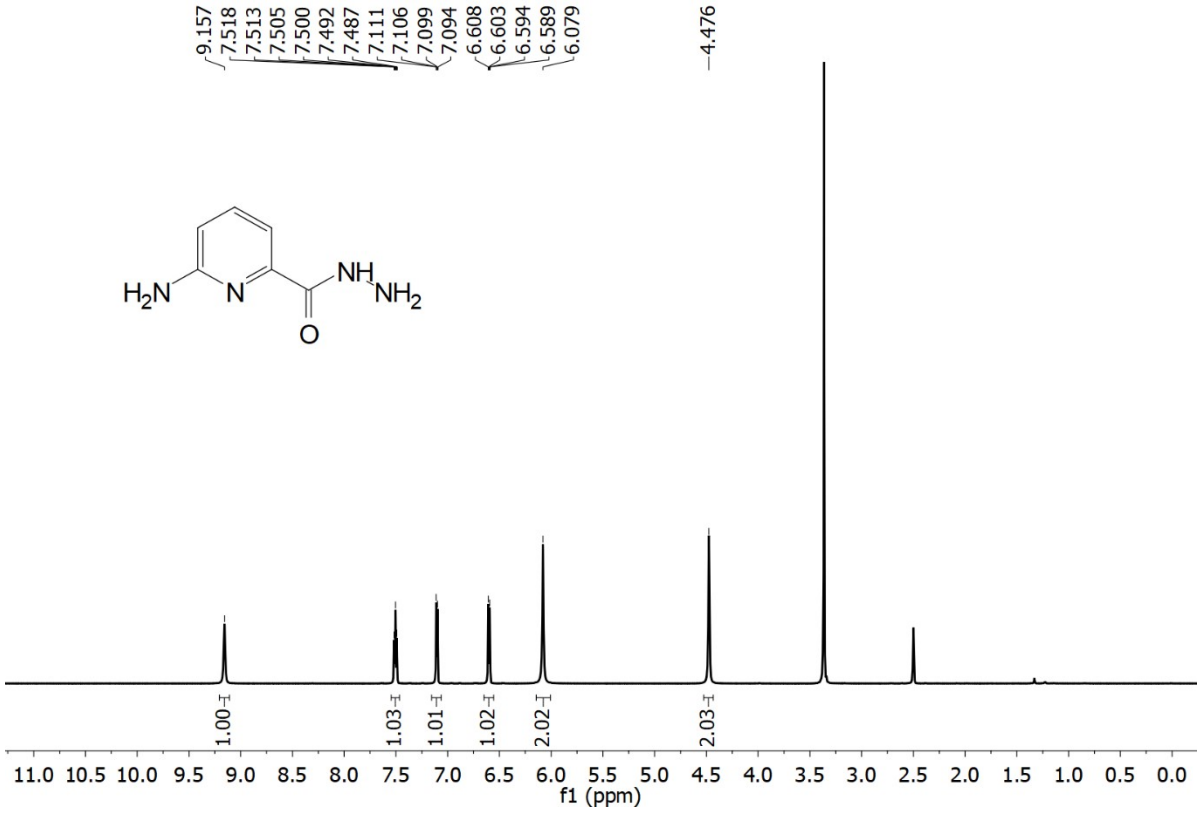
				Further heated to 150 ^{0}C
	$Pd_2(dba)_3$ (0.05-30 equiv)	Tri-o-tolyl phosphine	Et_3N	DMF, 100 ^{0}C, 100 W, 1-2 h, µwave
	$Pd_2(dba)_3$ (0.05-30 equiv)	X-Phos (0.1-10 equiv)	BuLi	Dry THF, 80 ^{0}C, 18 h
	Tetrakis(triphenylphosphine) palladium(0) (PdP_4)	X-Phos (0.1-30 equiv)	K_2CO_3	DMF, 110 ^{0}C, 100 W, 1-2 h, µwave
	$Pd(dppf)Cl_2.CH_2Cl_2$ (0.05-10 equiv)	-	Cs_2CO_3	DMF, 100 ^{0}C, 100 W, 1-2 h, µwave
	$Pd(dppf)Cl_2.CH_2Cl_2$ (0.05-20 equiv)	-	K_2CO_3	DMF, 100 ^{0}C, 100 W, 1-2 h, µwave

Table S3. Binding interactions from molecular docking

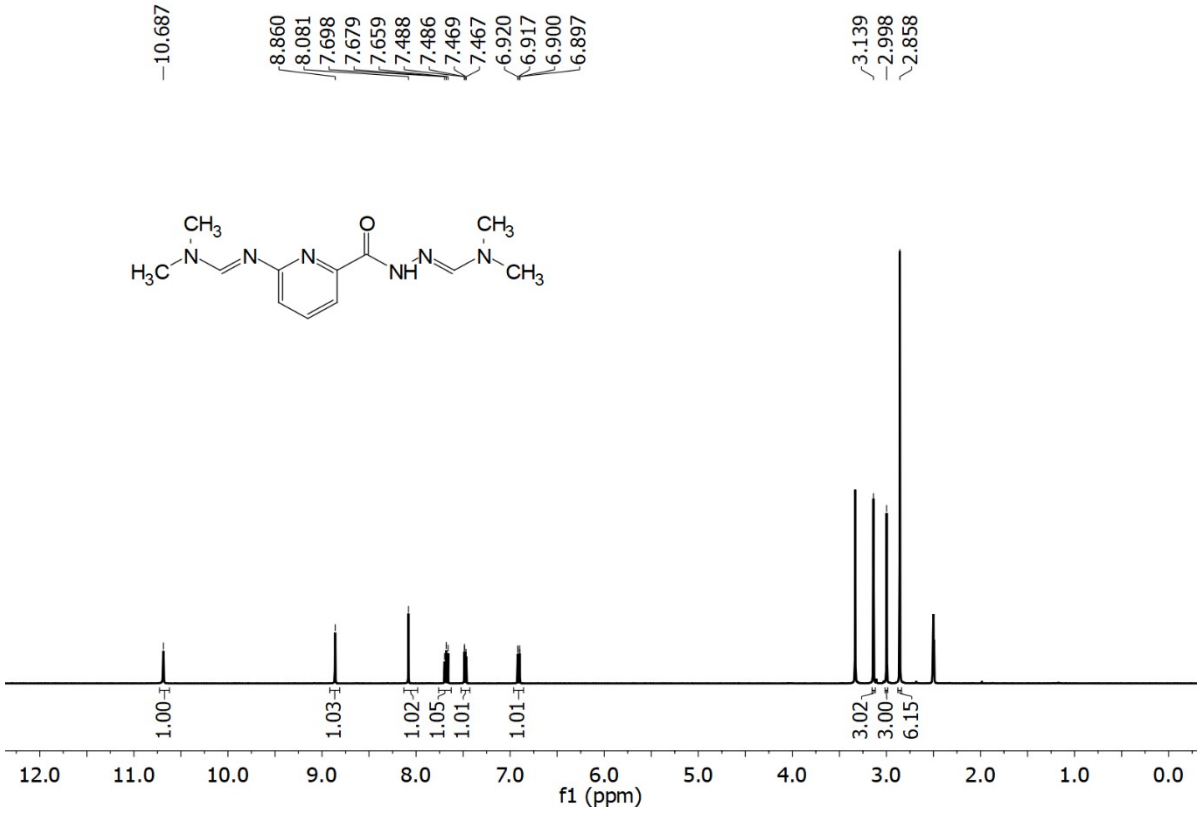
Protein	Residue	Residue Atom	Part of PROTAC	PROTAC atom	Interaction Type	Distance (Å)
CRBN	Asn 351	HD22	Thalidomide part	O5	Hydrogen Bond	1.851
CRBN	Trp 380	HN	Thalidomide part	O7	Hydrogen Bond	2.816
CRBN	Trp 386	HE1	Thalidomide part	O8	Hydrogen Bond	2.358
CRBN	His 378	O	Thalidomide part	H34	Hydrogen Bond	1.754
ASK1	Arg 705	HH21	Linker	O1	Hydrogen Bond	2.738
ASK1	Lys 709	HZ2	Linker	N6	Hydrogen Bond	2.931
ASK1	Val 757	HN	Selonsertib part	O4	Hydrogen Bond	1.709
ASK1	Gln 756	OE1	Selonsertib part	H15	Hydrogen Bond	2.782
ASK1	Asp 822	HN	Selonsertib part	N6	Hydrogen Bond	3.051
ASK1	Met 754	SD	Selonsertib part	Triazole pi	pi-Sulfur	5.147

NMR spectroscopic data

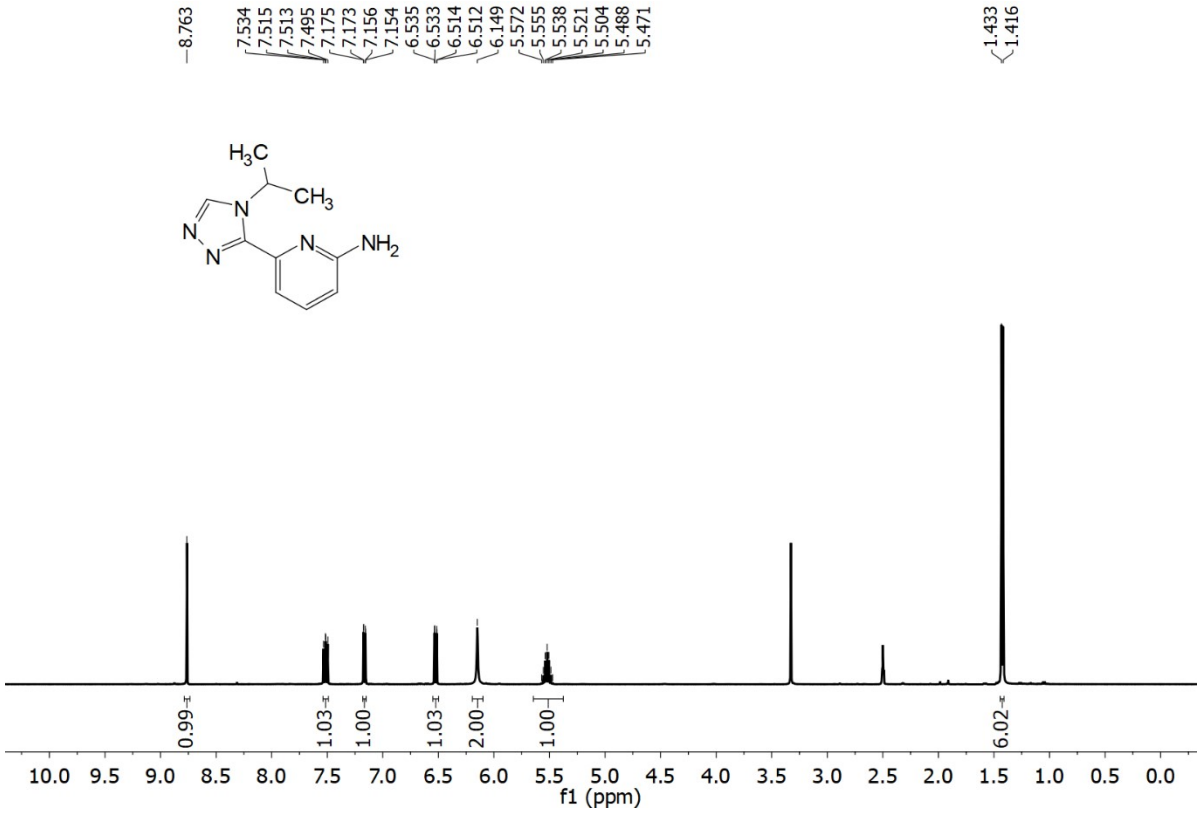
^{1}H NMR of compound **2** (400 MHz, DMSO-d_6):



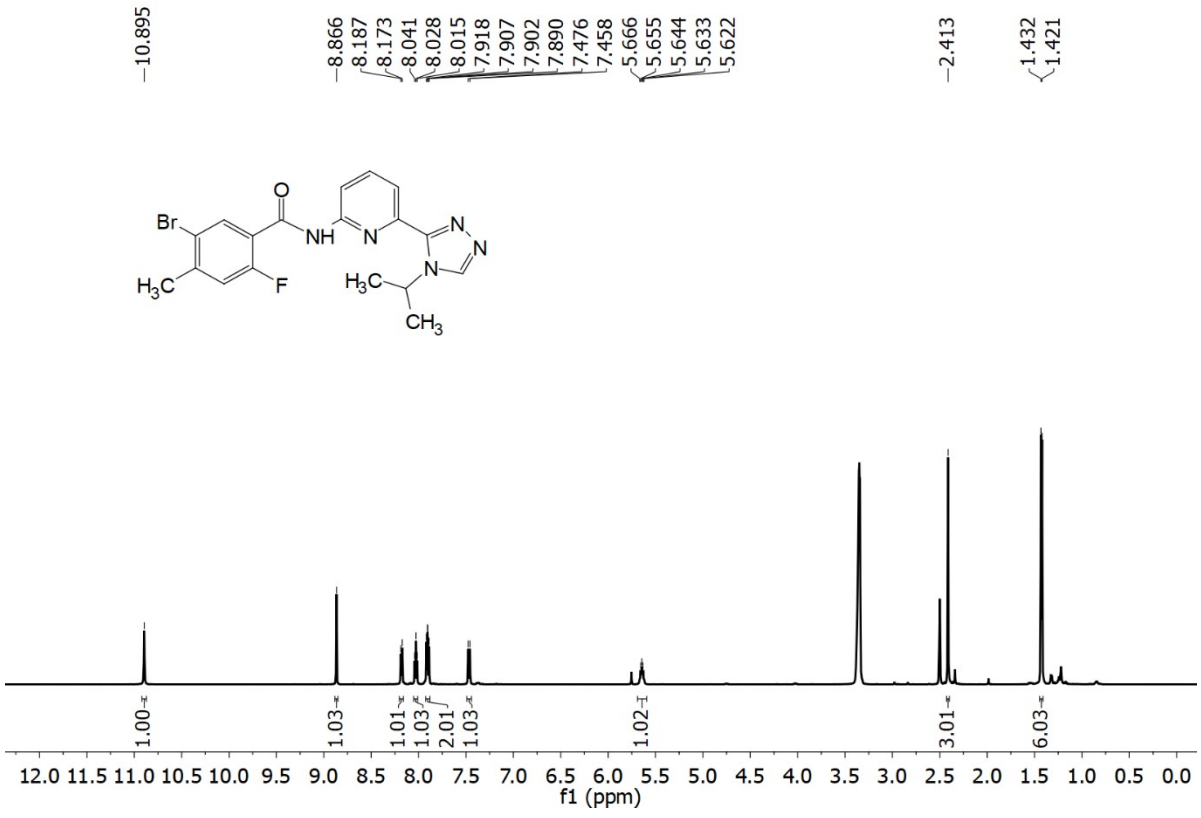
^{1}H NMR of compound **3** (400 MHz, DMSO-d_6):



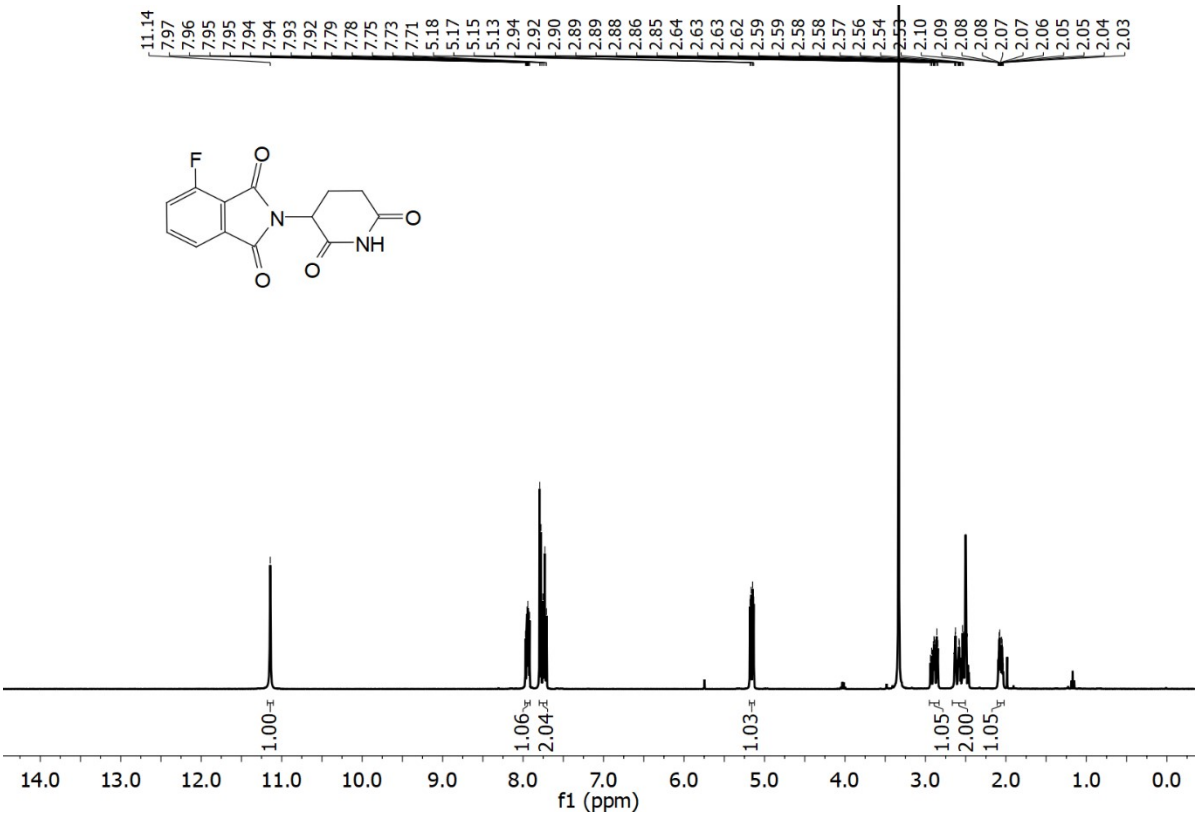
^{1}H NMR of compound **4** (400 MHz, DMSO-d_6):



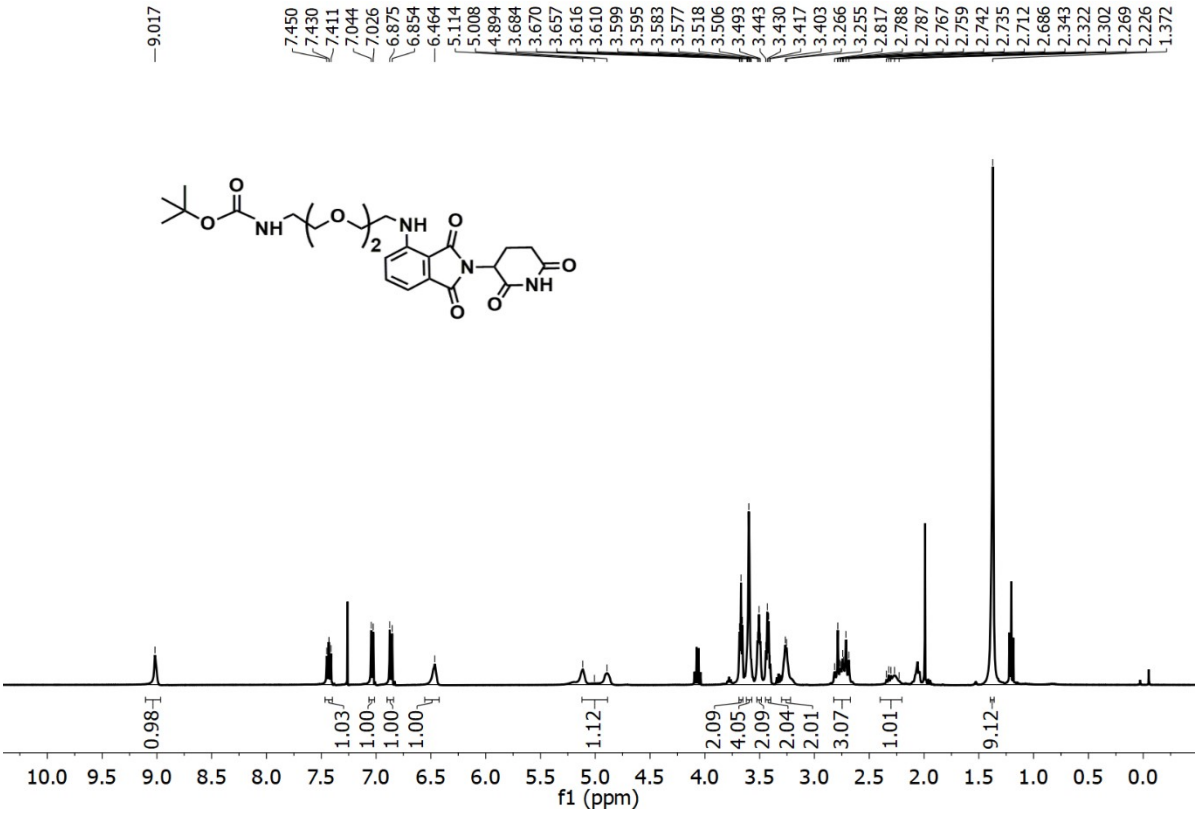
^{1}H NMR of compound **6** (600 MHz, DMSO-d_6):



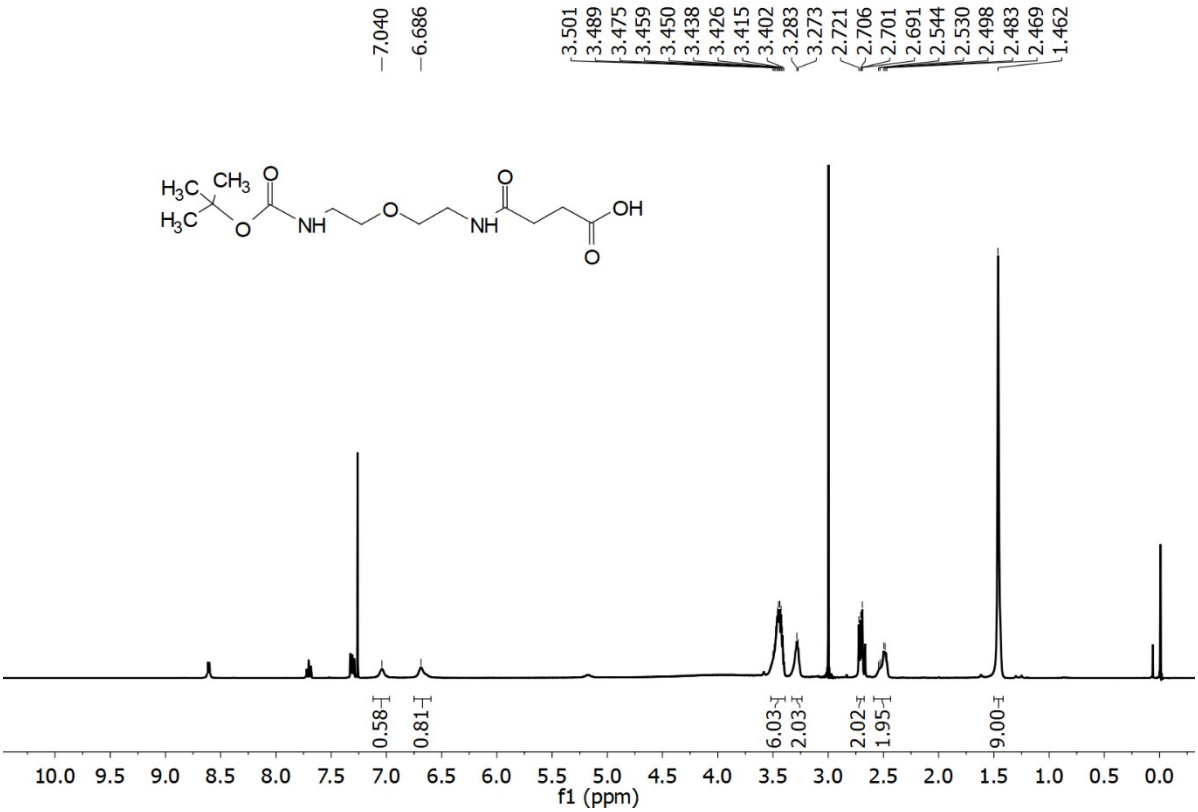
^{1}H NMR of compound **9** (400 MHz, DMSO-d_6):



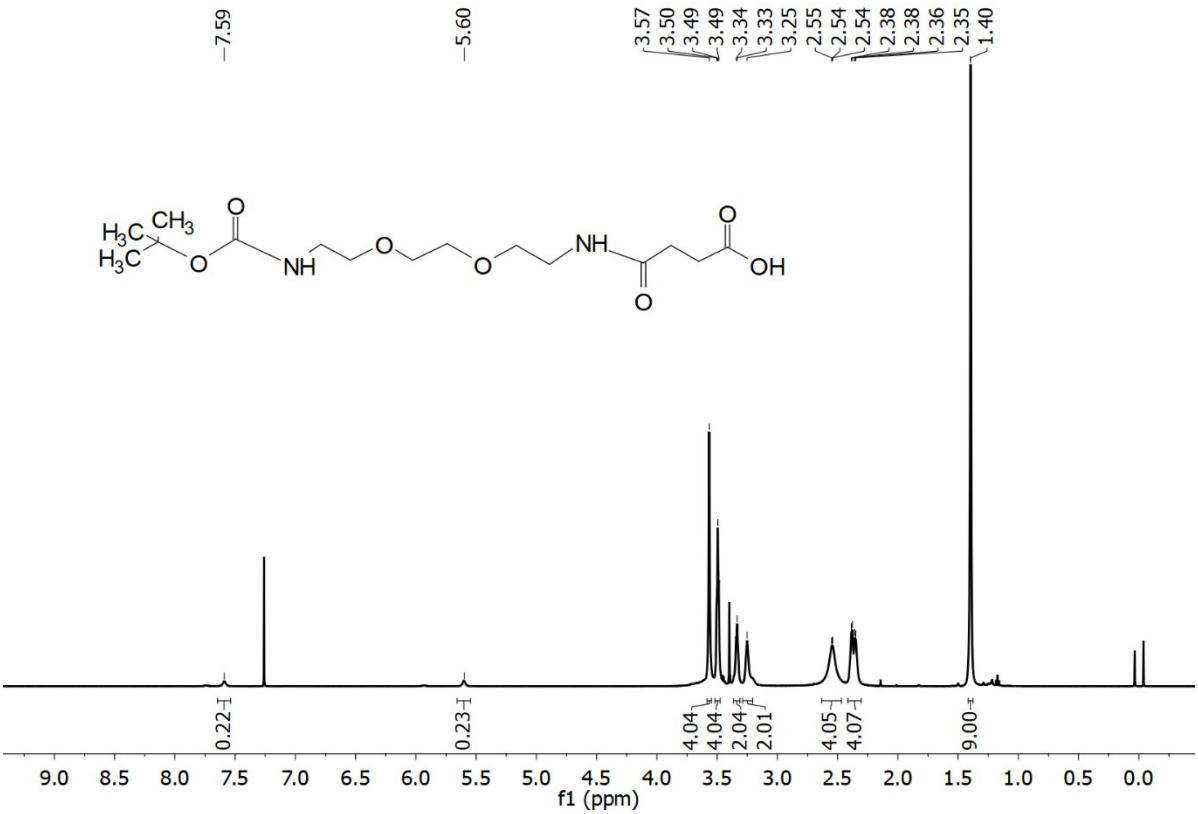
^{1}H NMR of compound **10** (400 MHz, Chloroform-*d*):



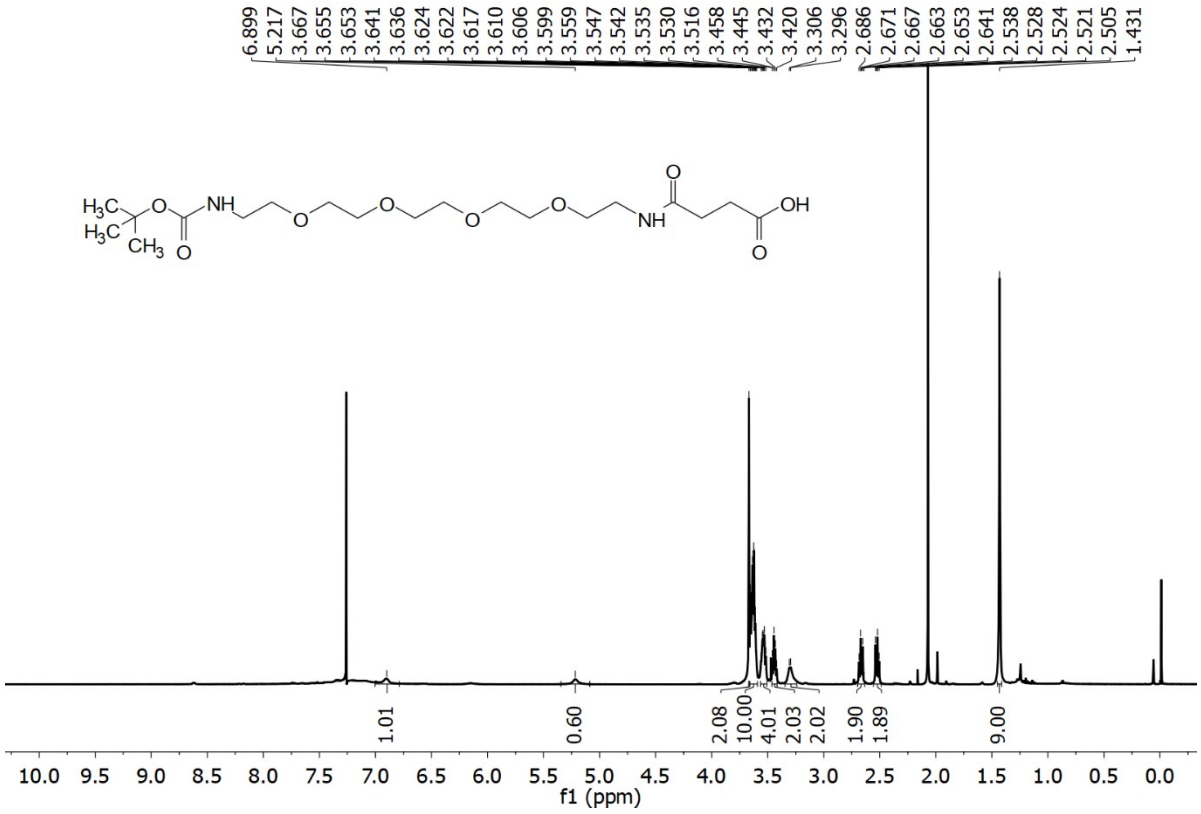
^{1}H NMR of compound **17** (400 MHz, Chloroform-*d*):



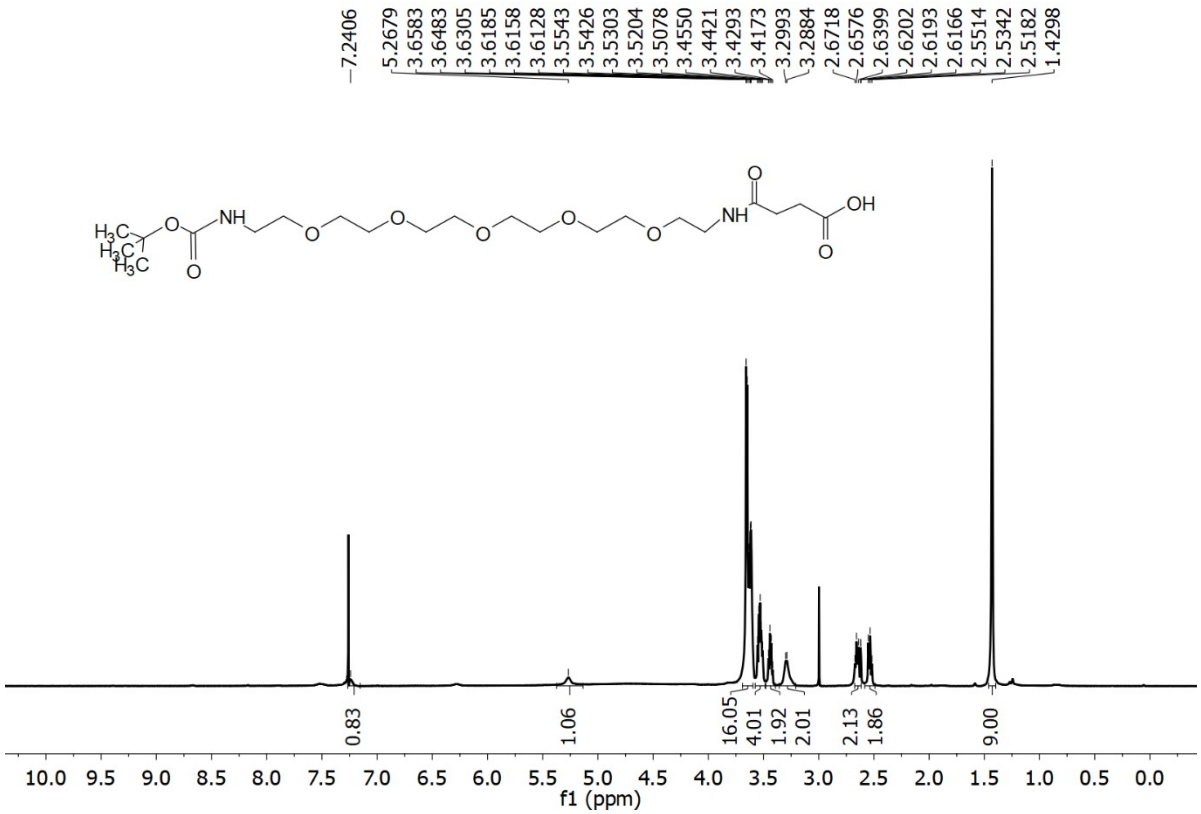
^{1}H NMR of compound **18** (400 MHz, Chloroform-*d*):



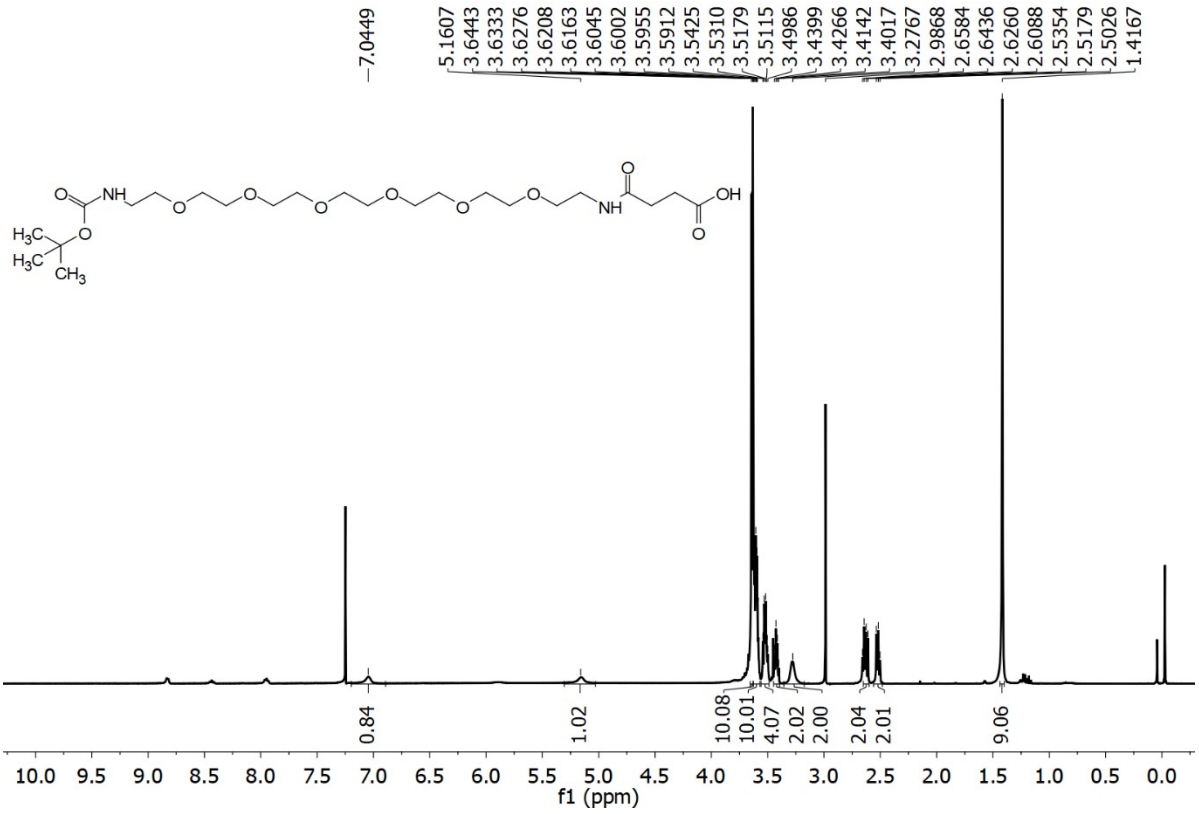
^{1}H NMR of compound **19** (400 MHz, Chloroform-*d*):



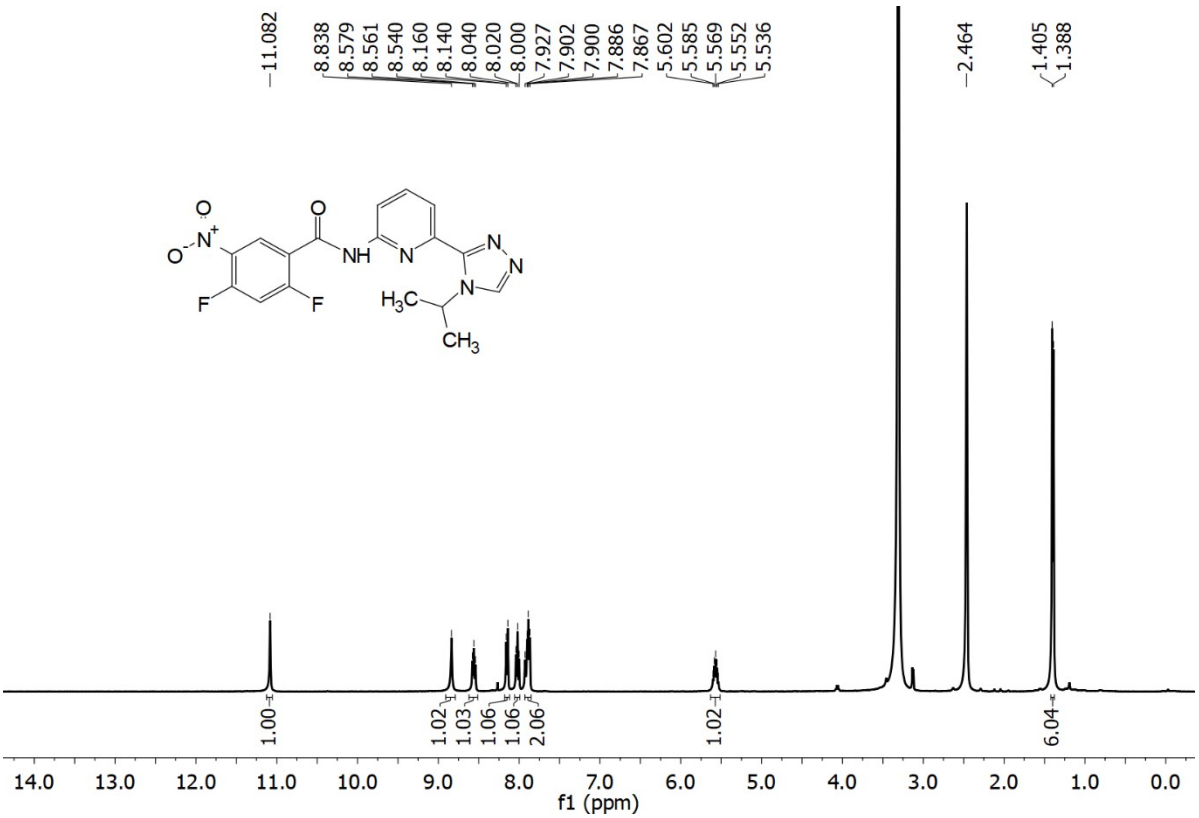
^{1}H NMR of compound **20** (400 MHz, Chloroform-*d*):



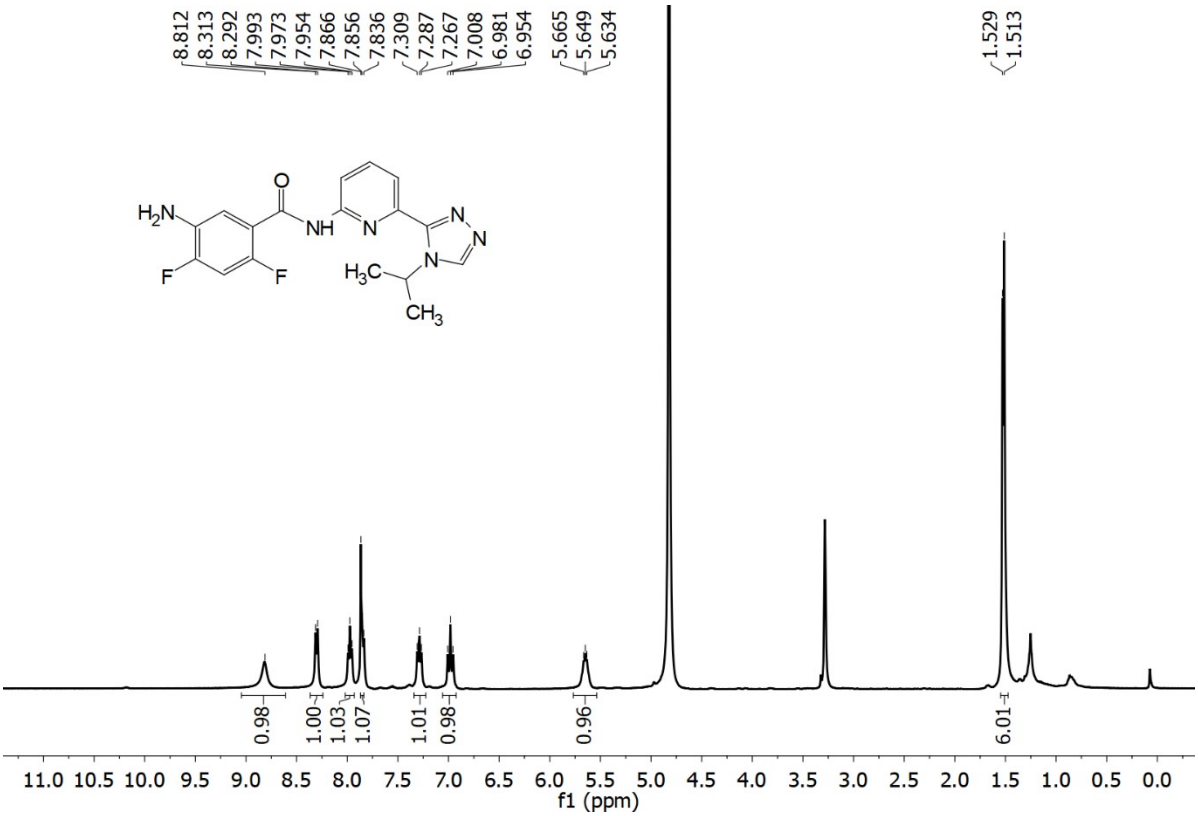
^{1}H NMR of compound **21** (400 MHz, Chloroform-*d*):



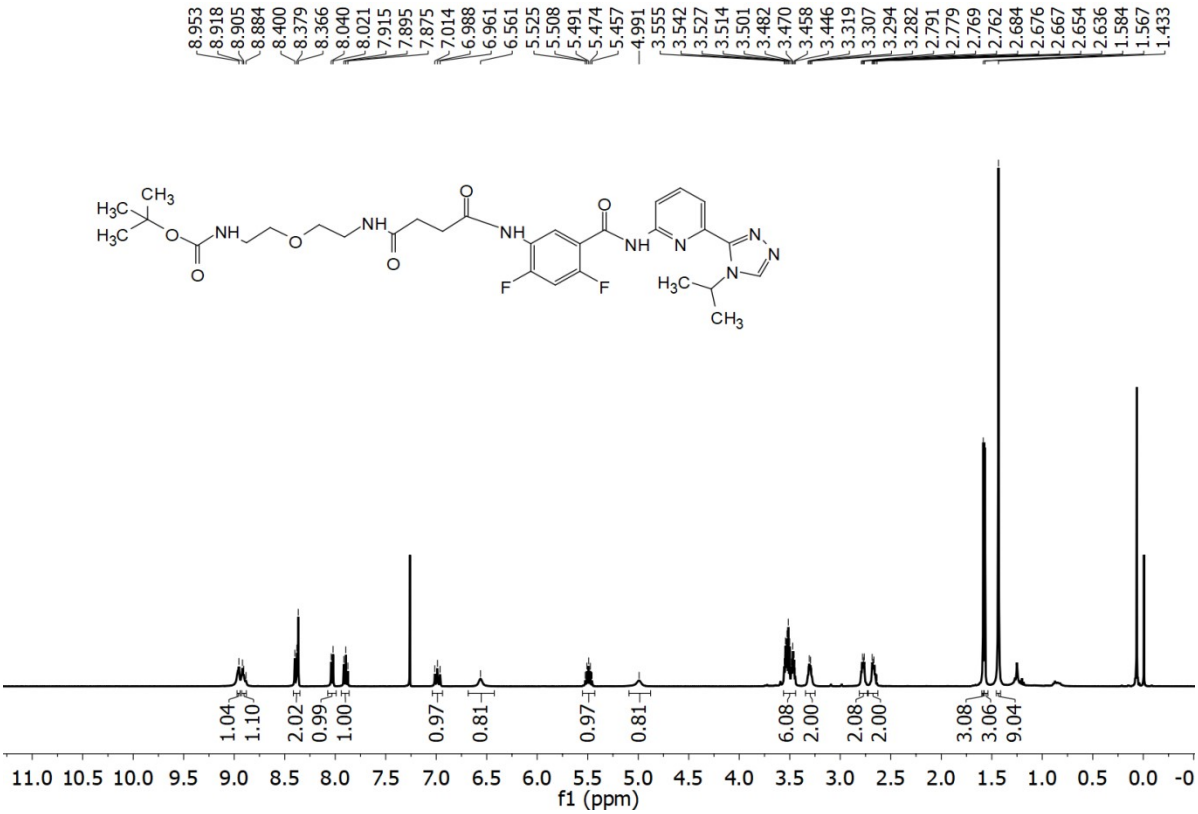
^{1}H NMR of compound **23** (400 MHz, DMSO-d_6):



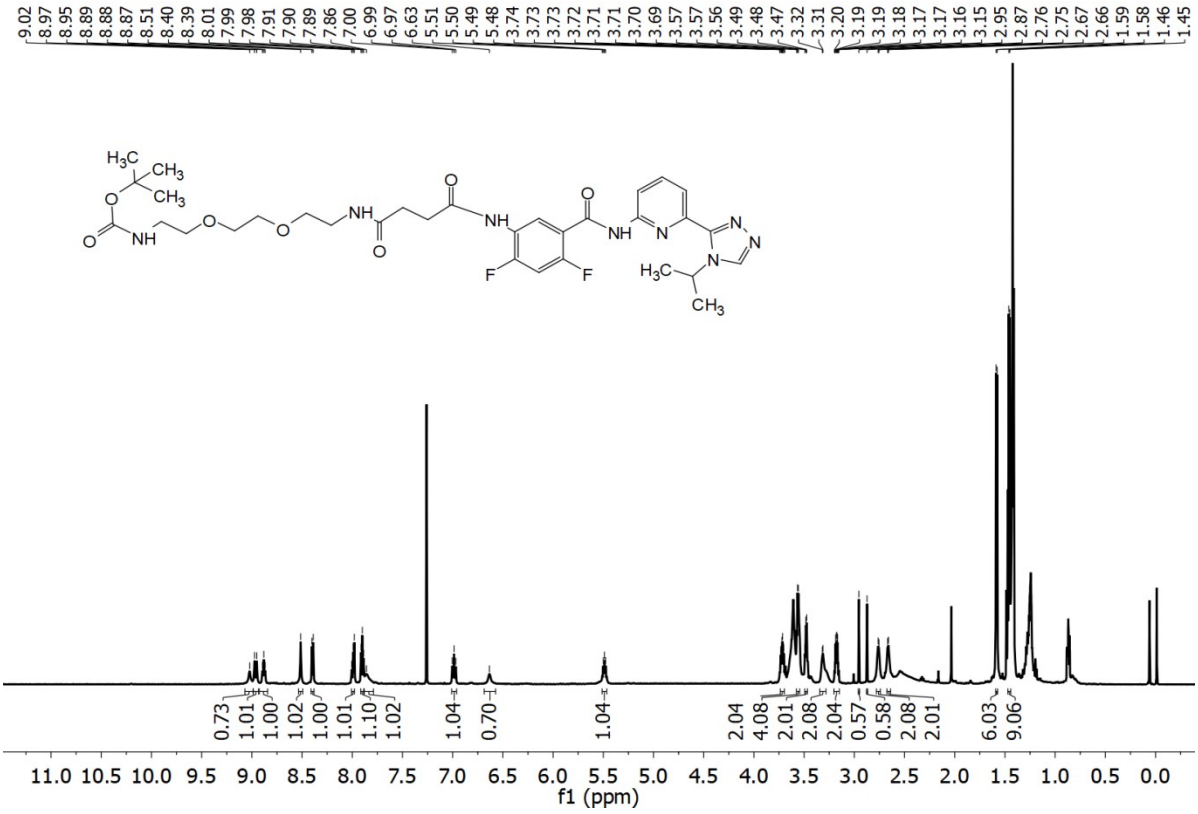
^{1}H NMR of compound **24** (400 MHz, Methanol-d_4):



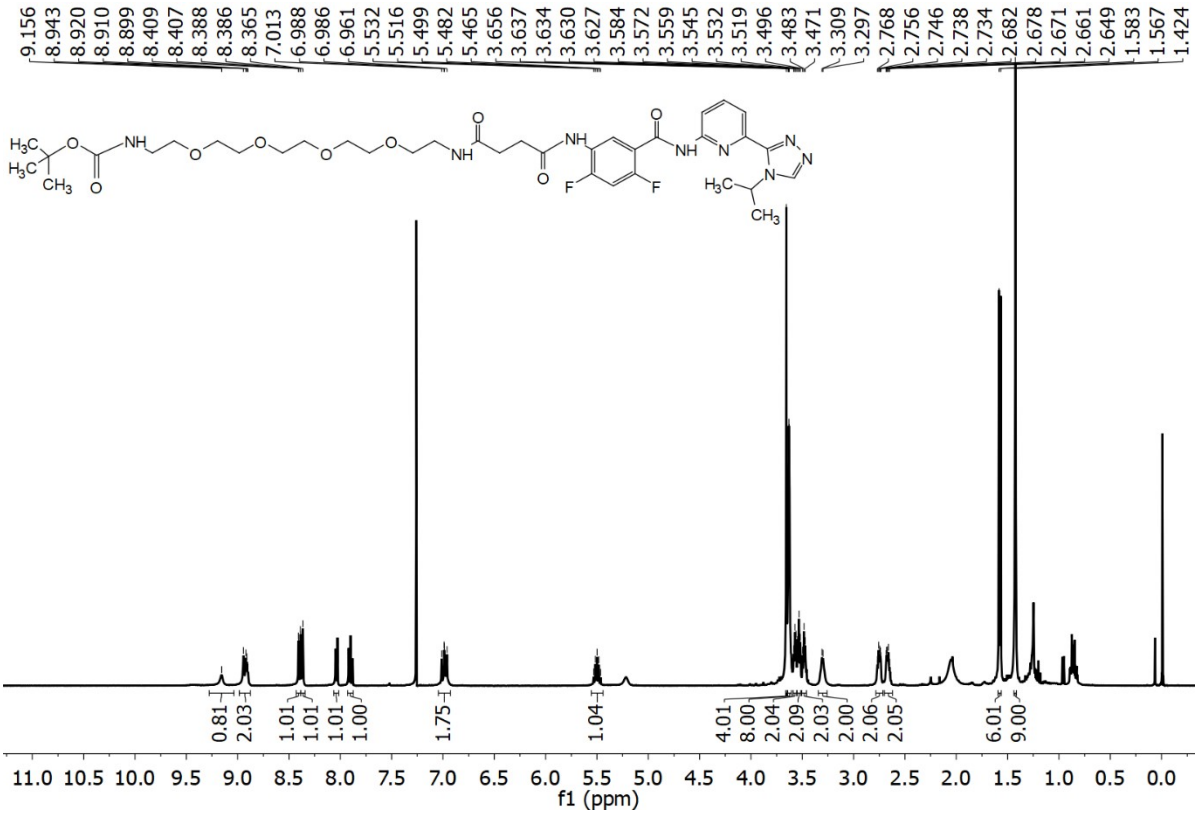
^{1}H NMR of compound **25** (400 MHz, Chloroform-*d*):



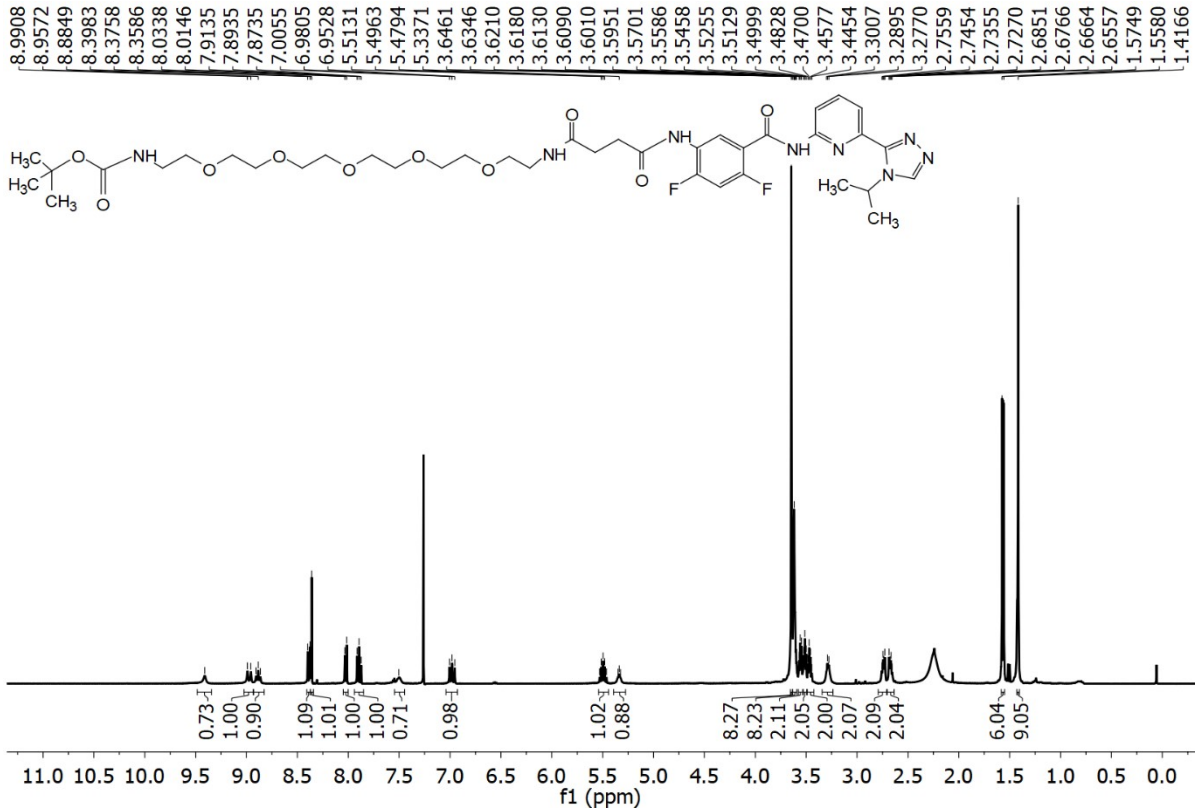
^{1}H NMR of compound **26** (400 MHz, Chloroform-*d*):



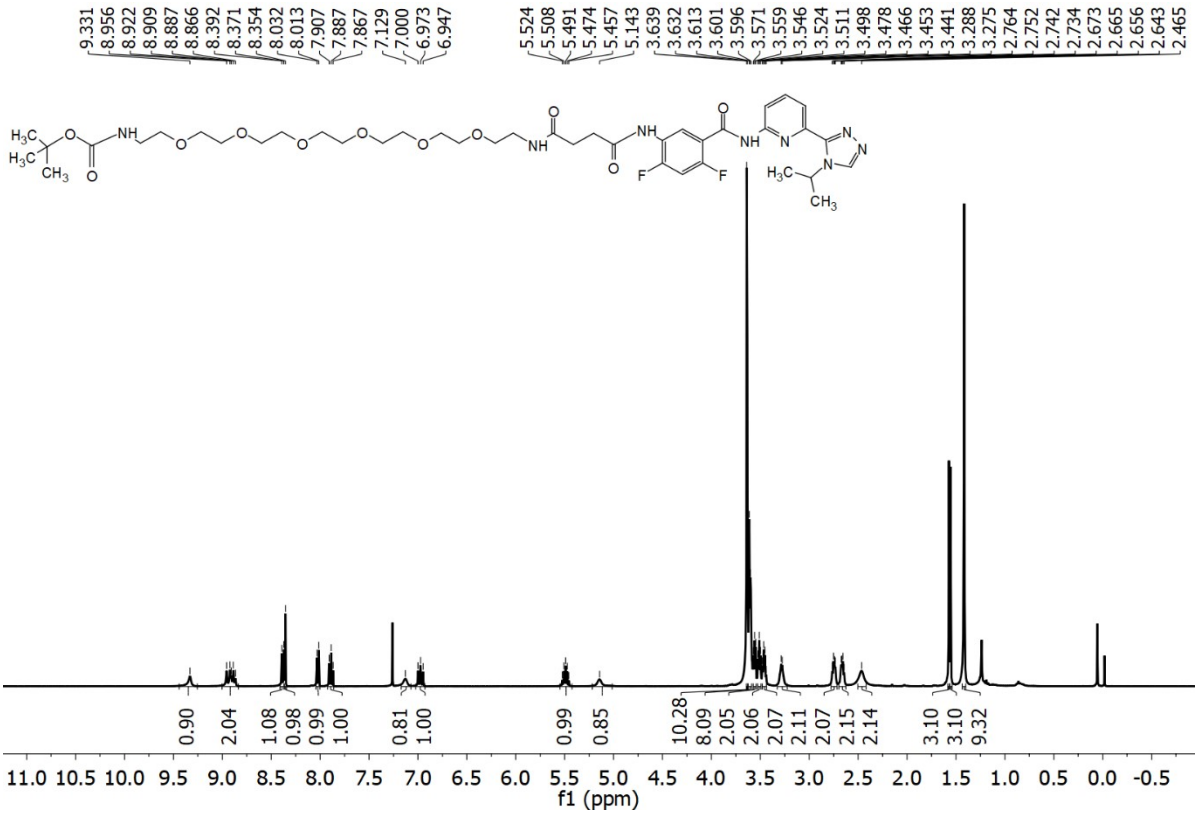
^{1}H NMR of compound **27** (400 MHz, Chloroform-*d*):



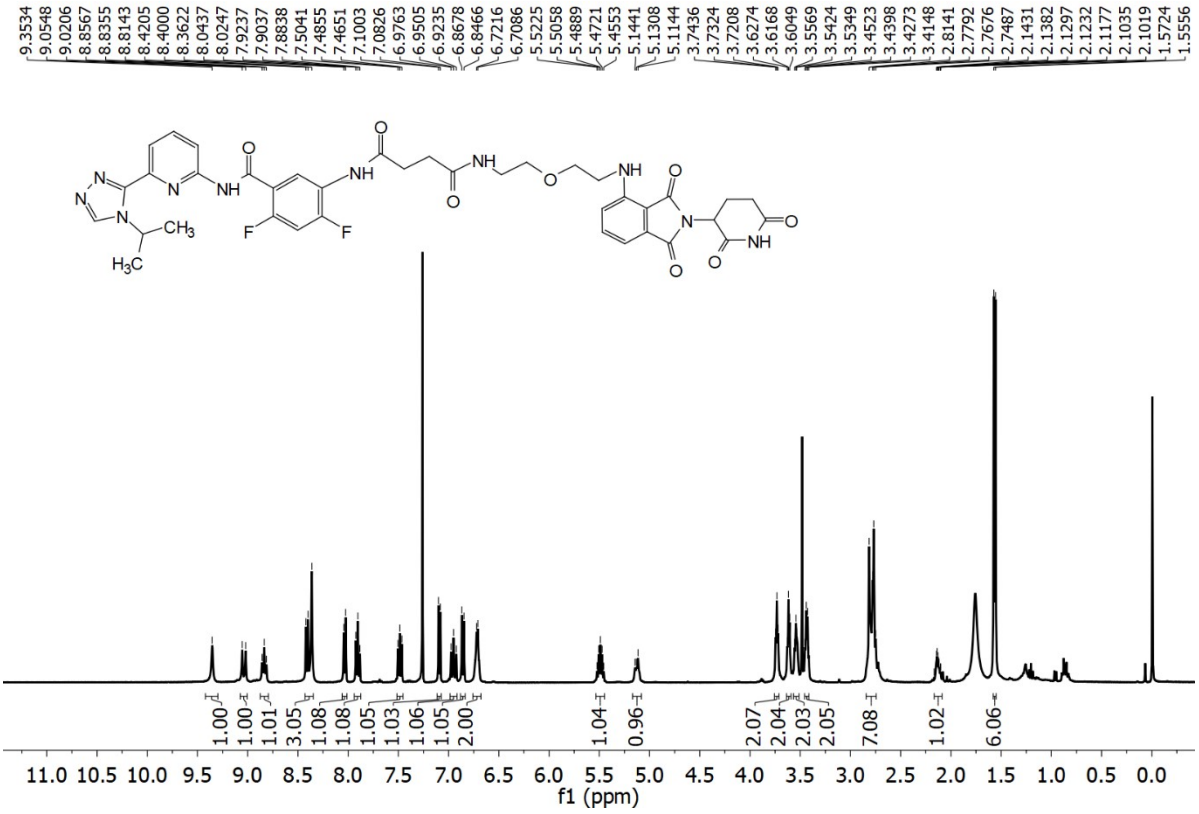
^{1}H NMR of compound **28** (400 MHz, Chloroform-*d*):



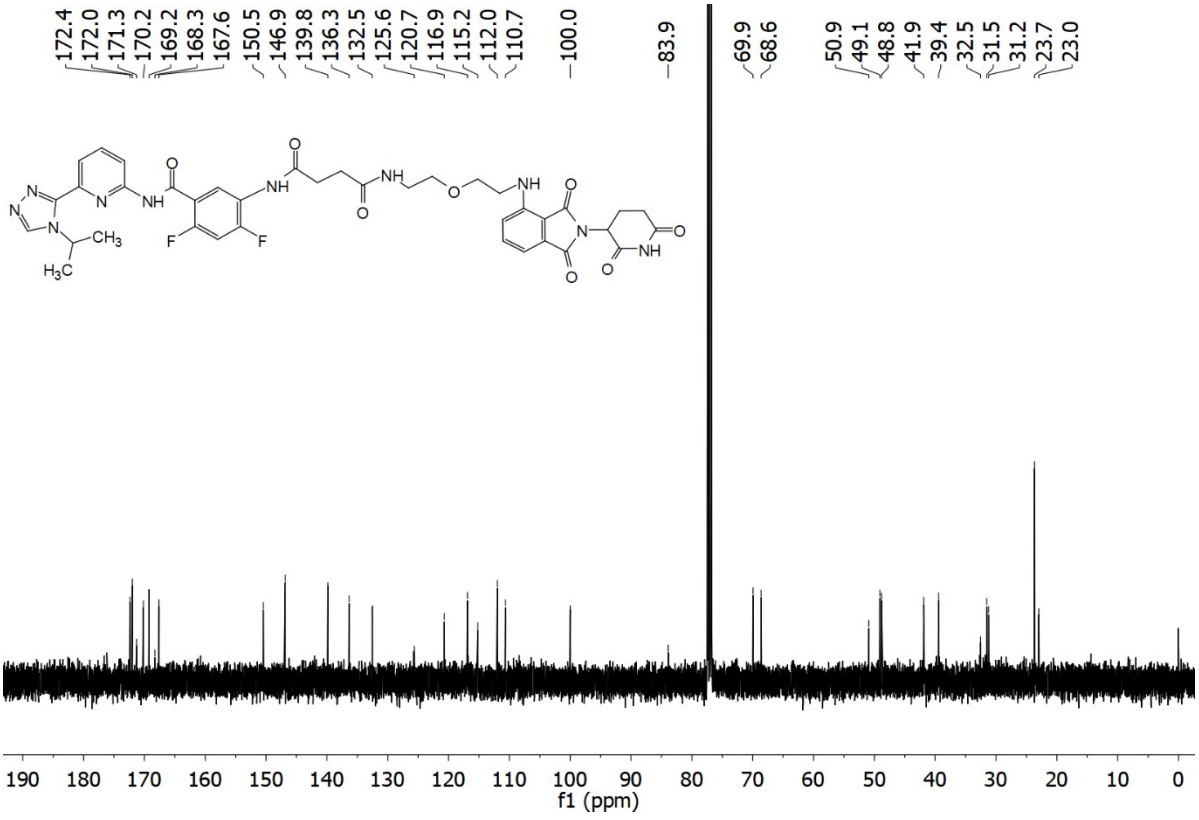
^{1}H NMR of compound **29** (400 MHz, Chloroform-*d*):



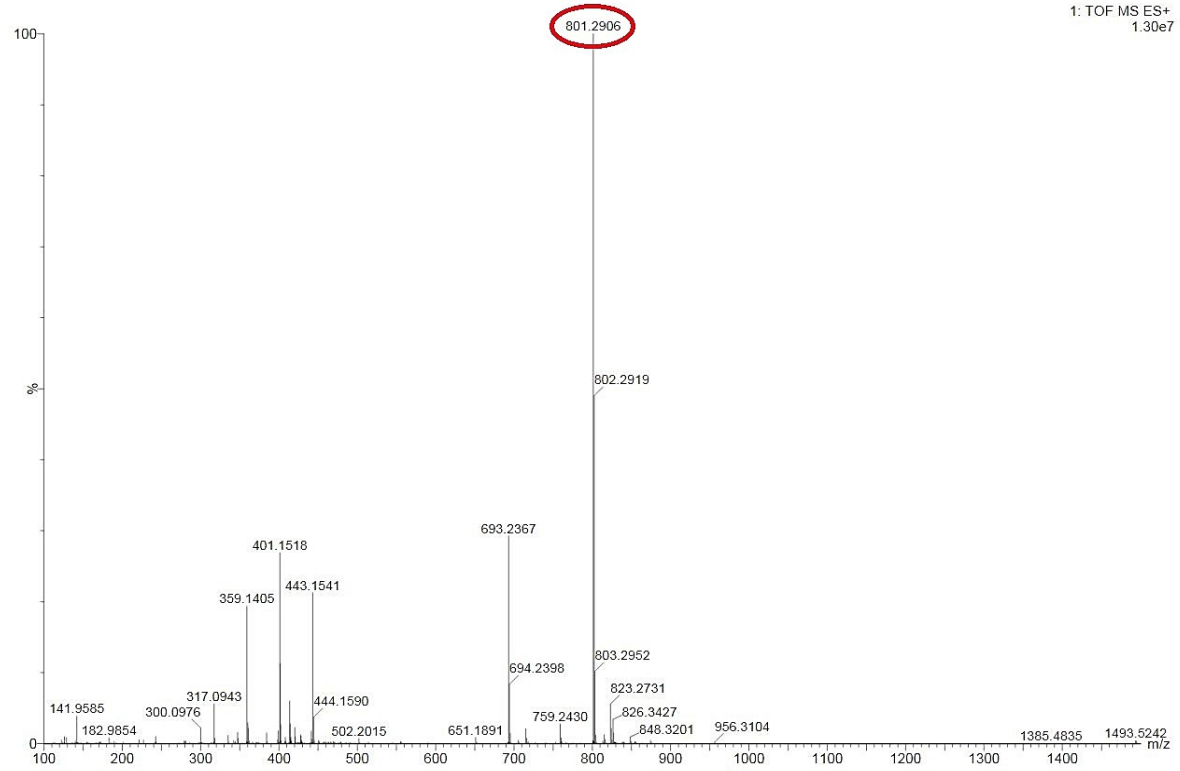
^{1}H NMR of compound **35** (400 MHz, Chloroform-*d*):



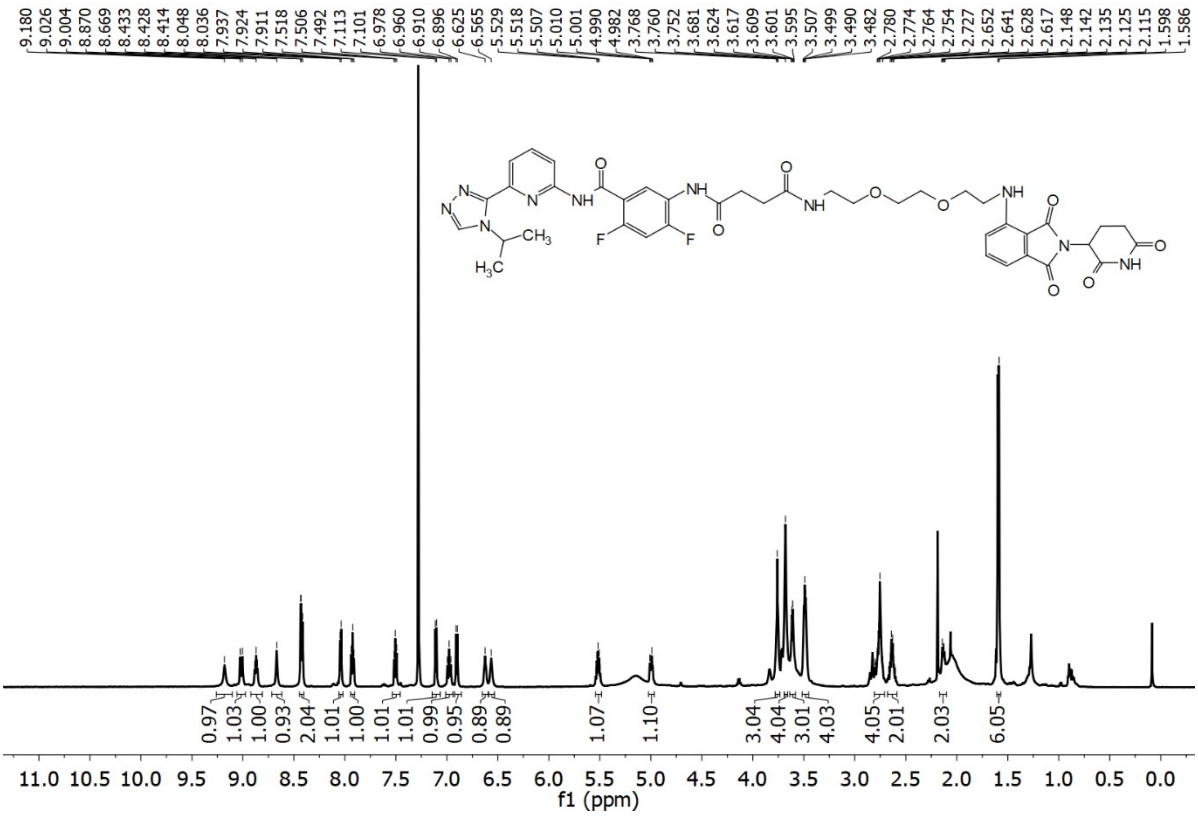
^{13}C NMR of compound **35** (101 MHz, Chloroform-*d*):



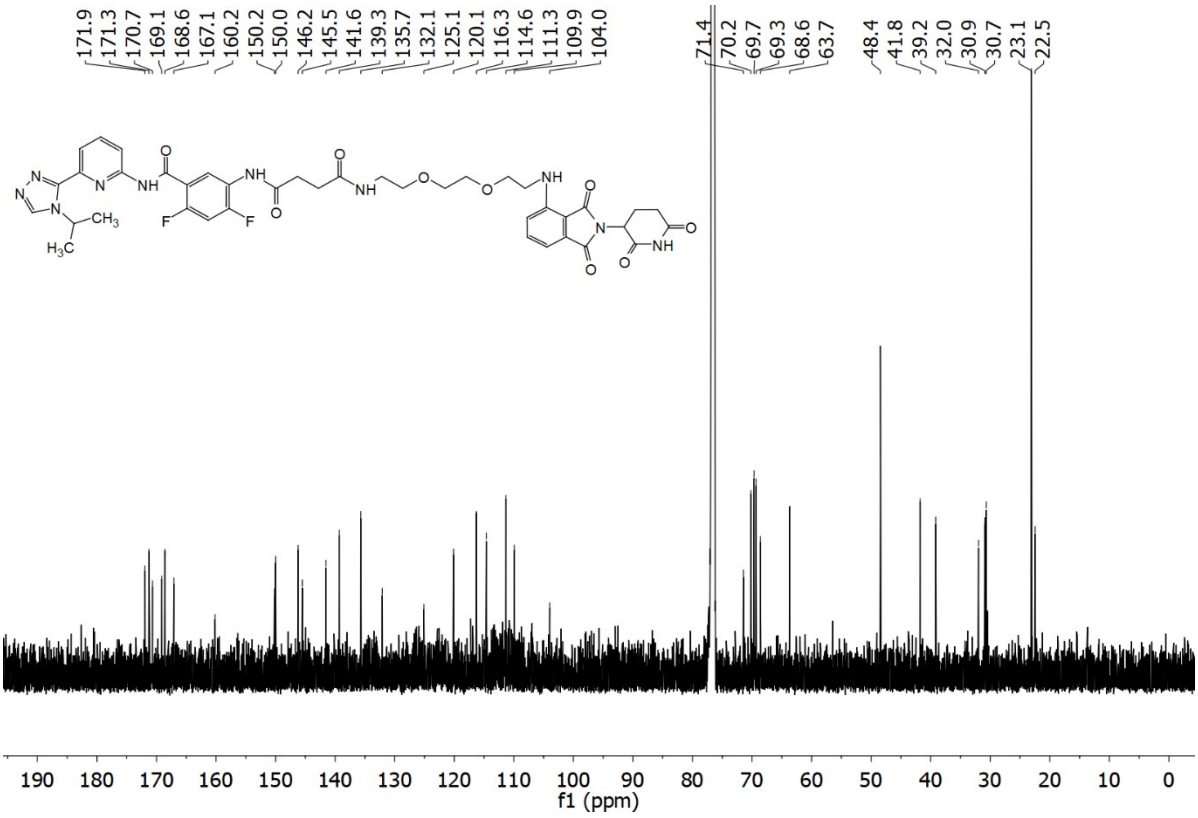
HR-MS spectra of compound **35**:



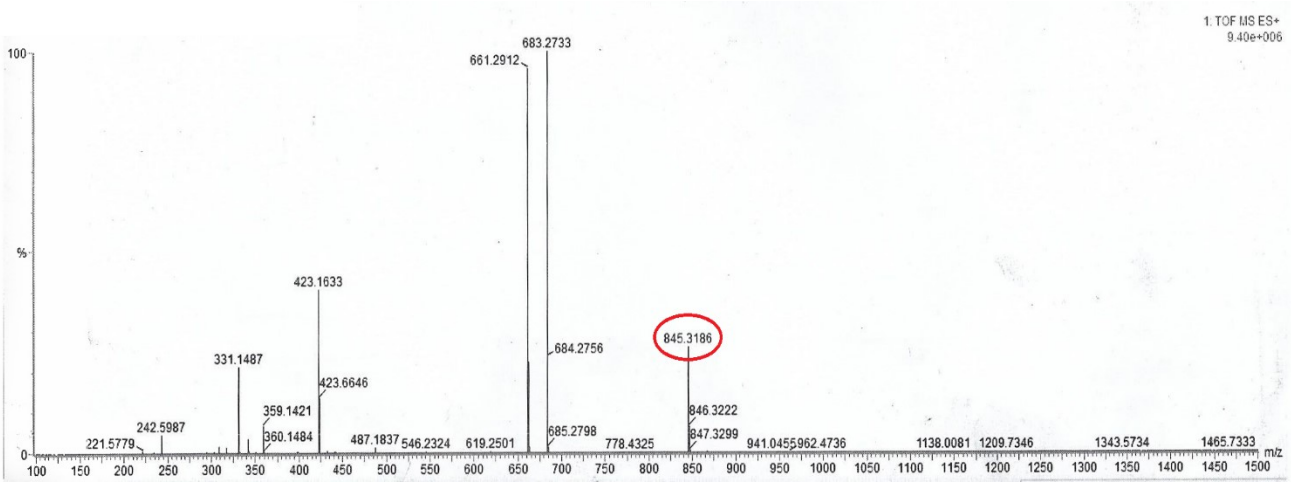
^{1}H NMR of compound **36** (400 MHz, Chloroform-*d*):



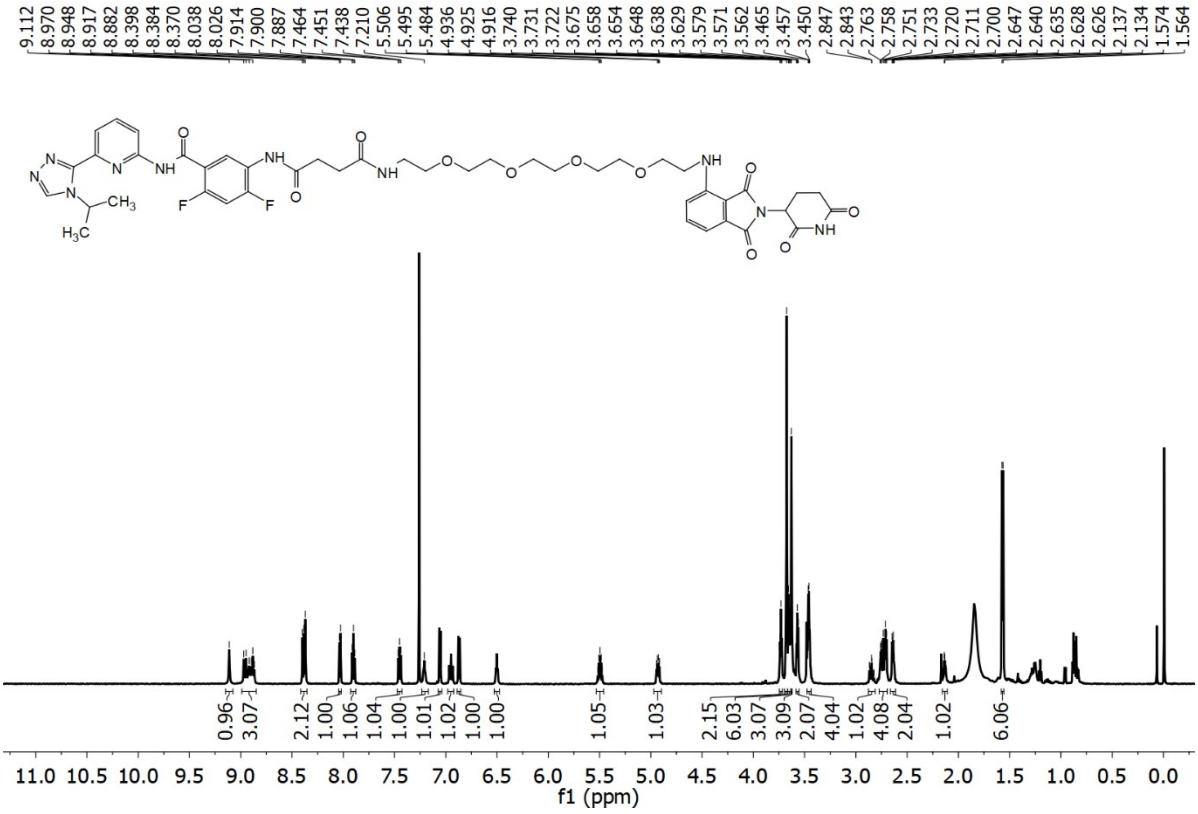
^{13}C NMR of compound **36** (101 MHz, Chloroform-*d*):



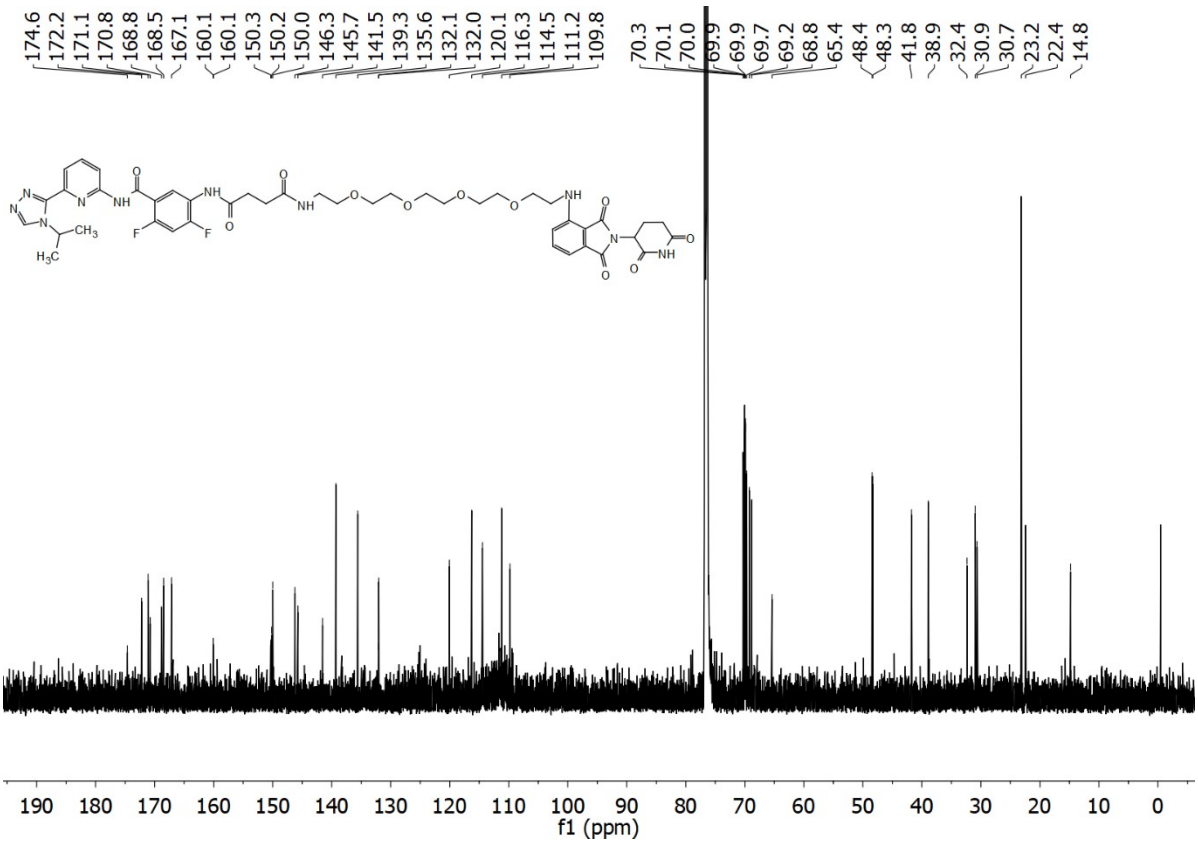
HR-MS spectra of compound **36**:



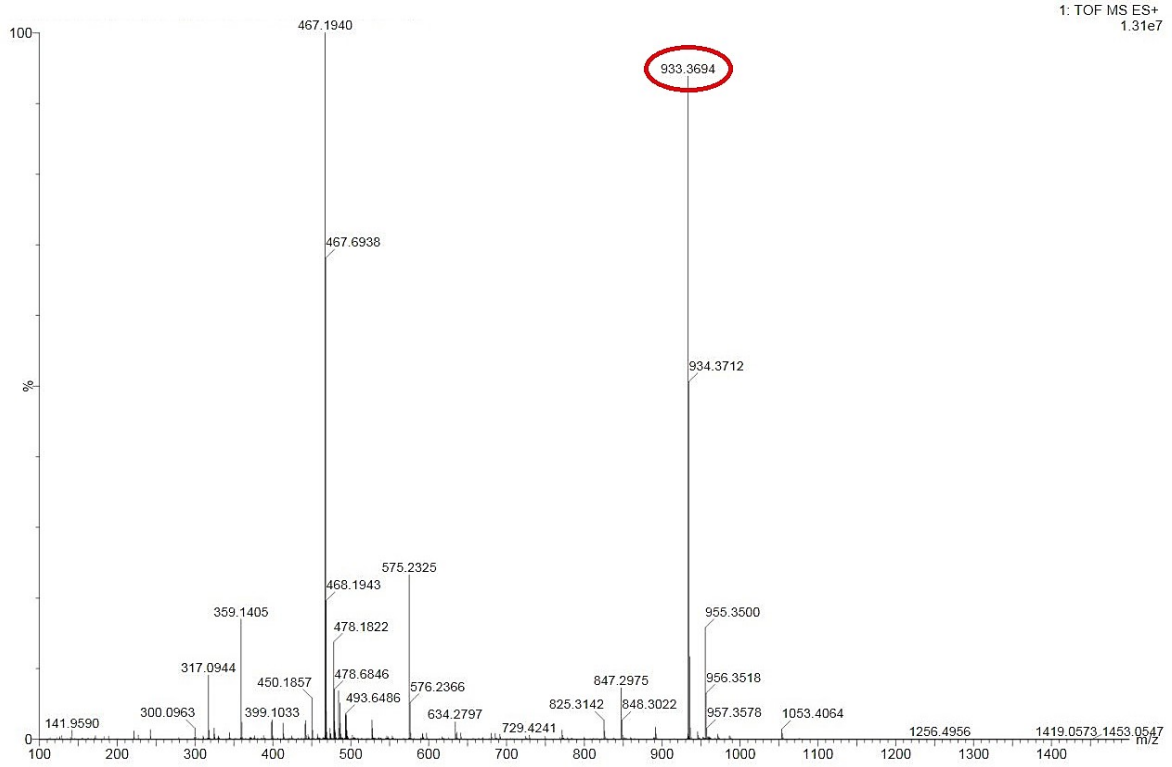
^{1}H NMR of compound **37** (400 MHz, Chloroform-*d*):



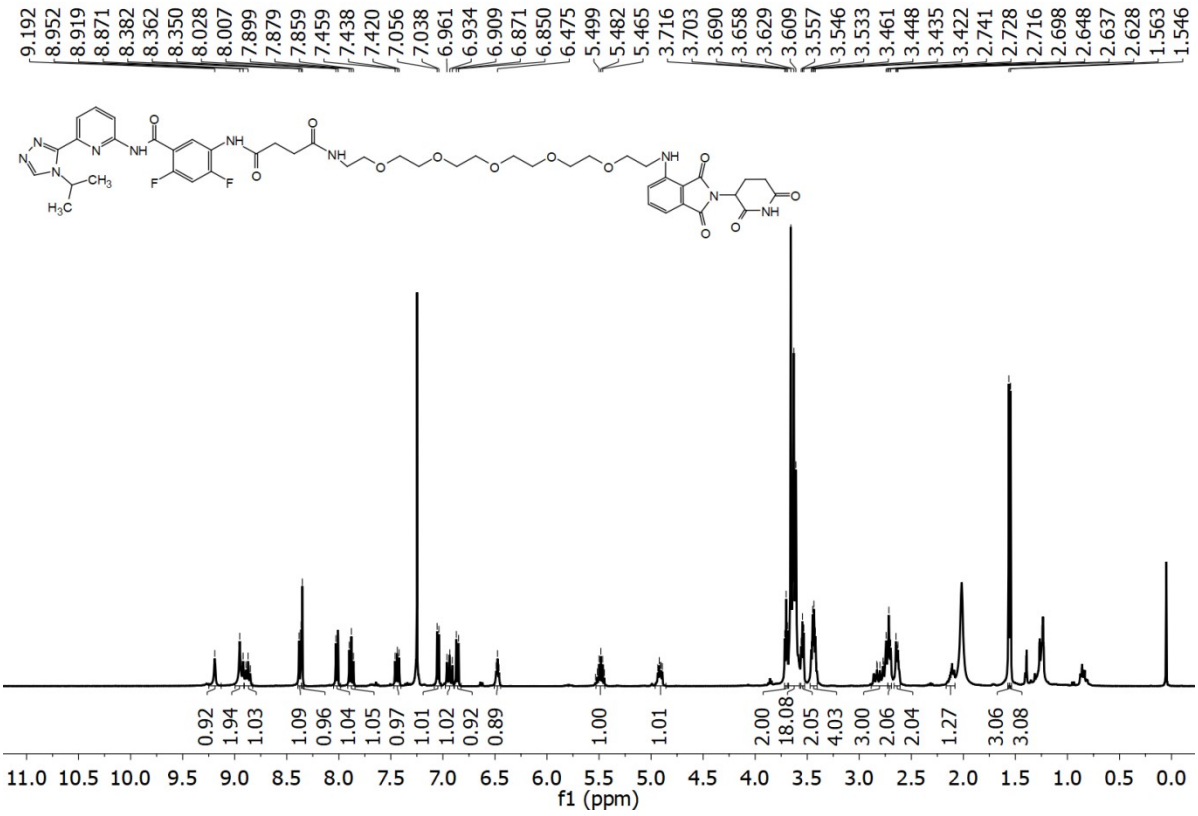
^{13}C NMR of compound **37** (101 MHz, Chloroform-*d*):



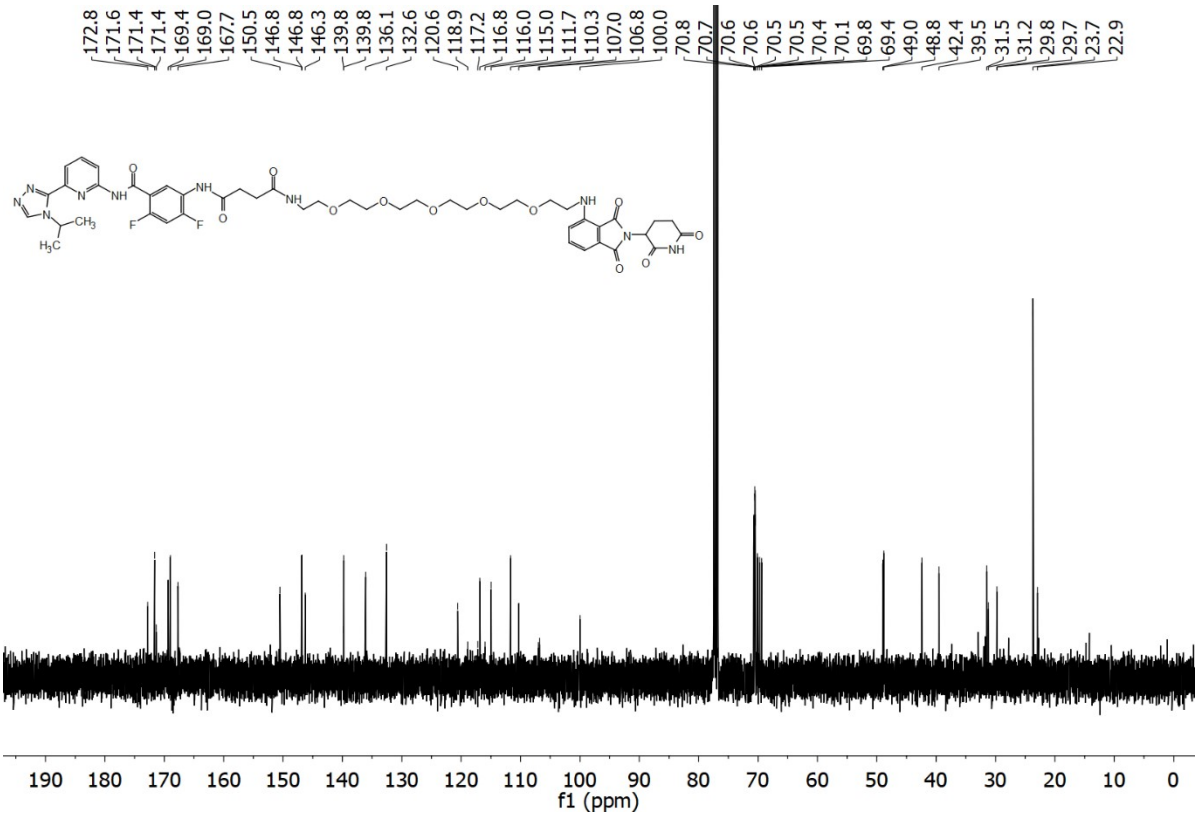
HR-MS spectra of compound **37**:



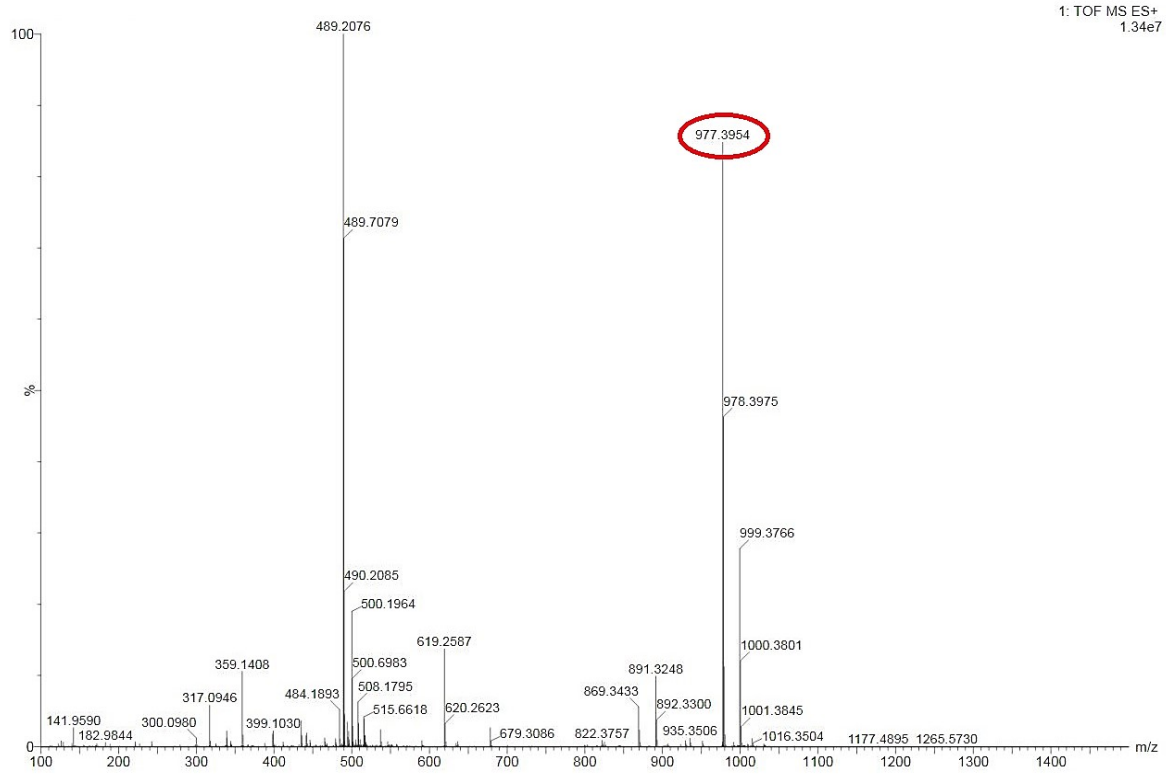
^{1}H NMR of compound **38** (400 MHz, Chloroform-*d*):



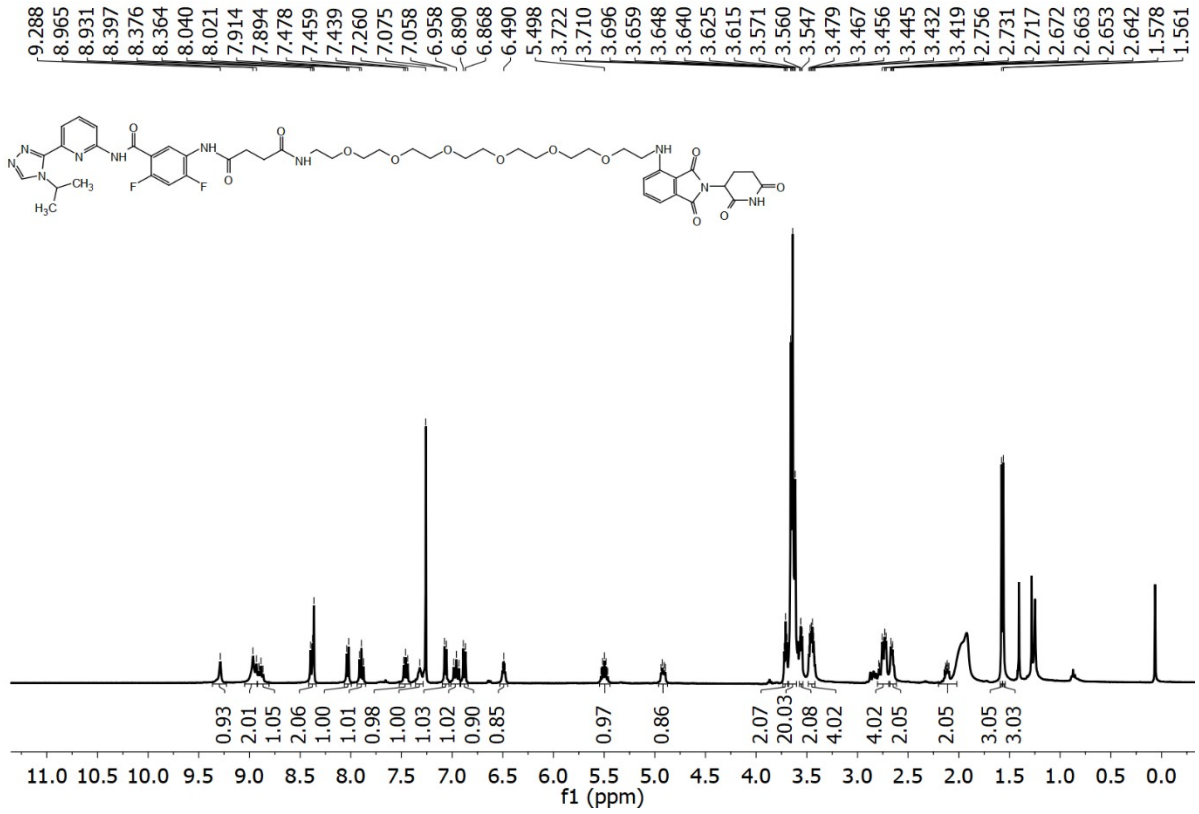
^{13}C NMR of compound **38** (101 MHz, Chloroform-*d*):



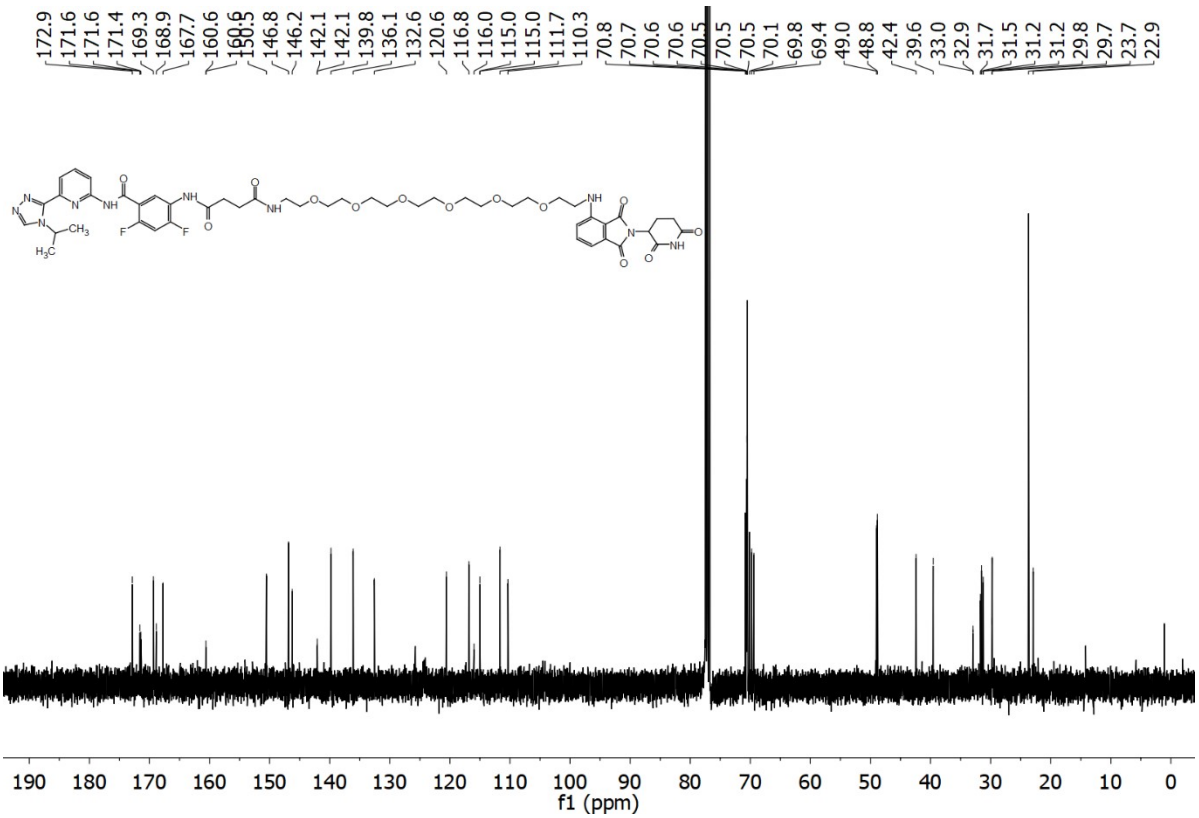
HR-MS spectra of compound **38**:



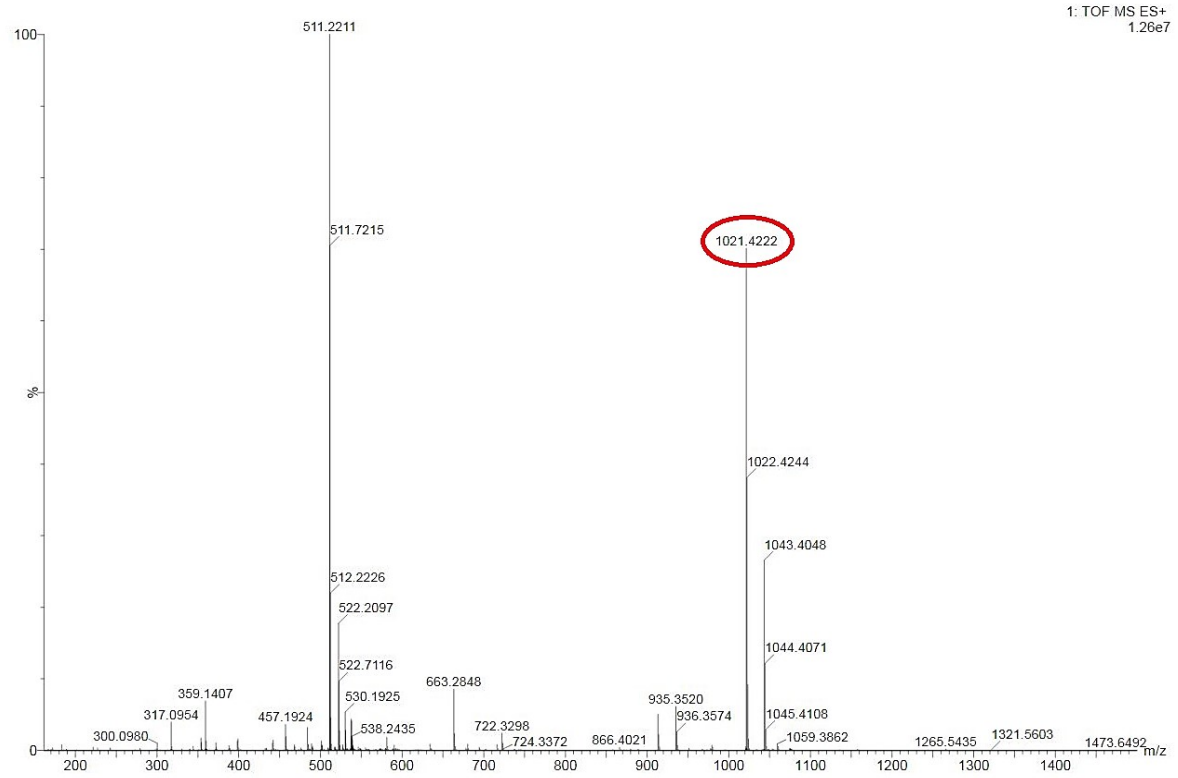
^{1}H NMR of compound **39** (400 MHz, Chloroform-*d*):



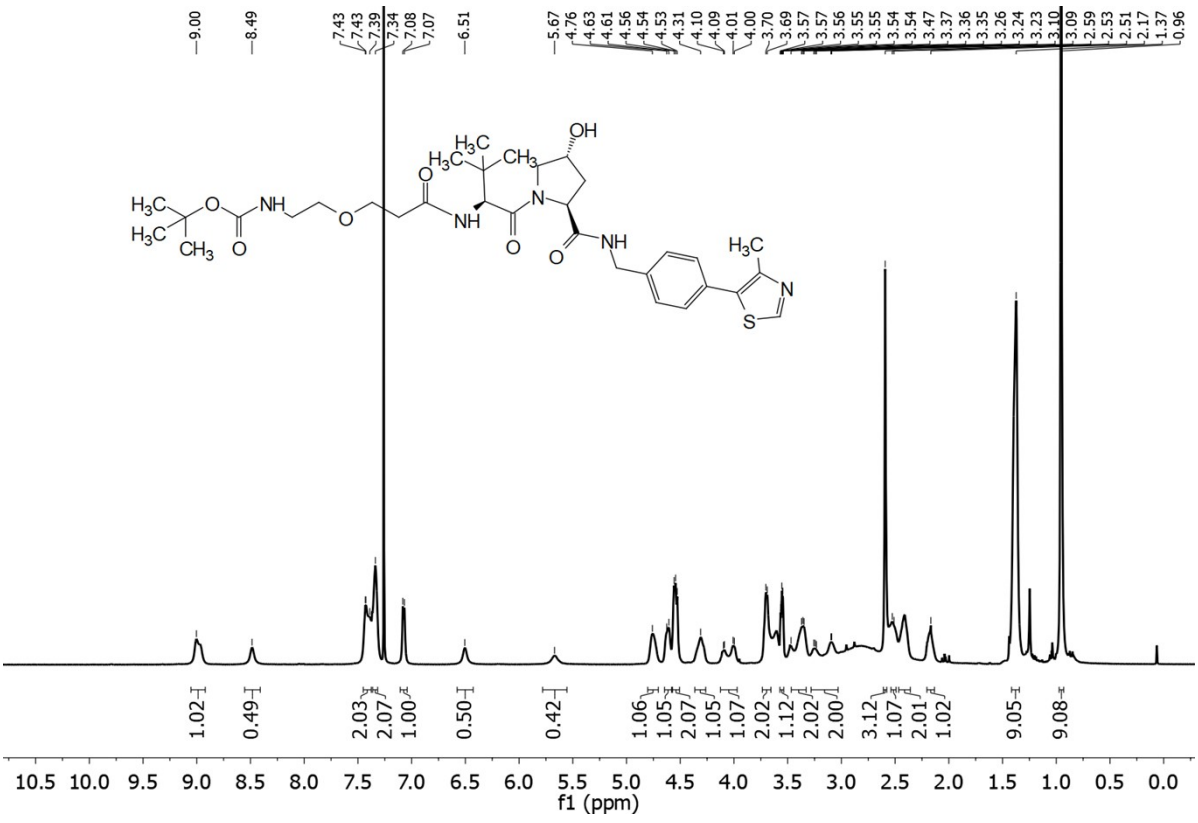
^{13}C NMR of compound **39** (101 MHz, Chloroform-*d*):



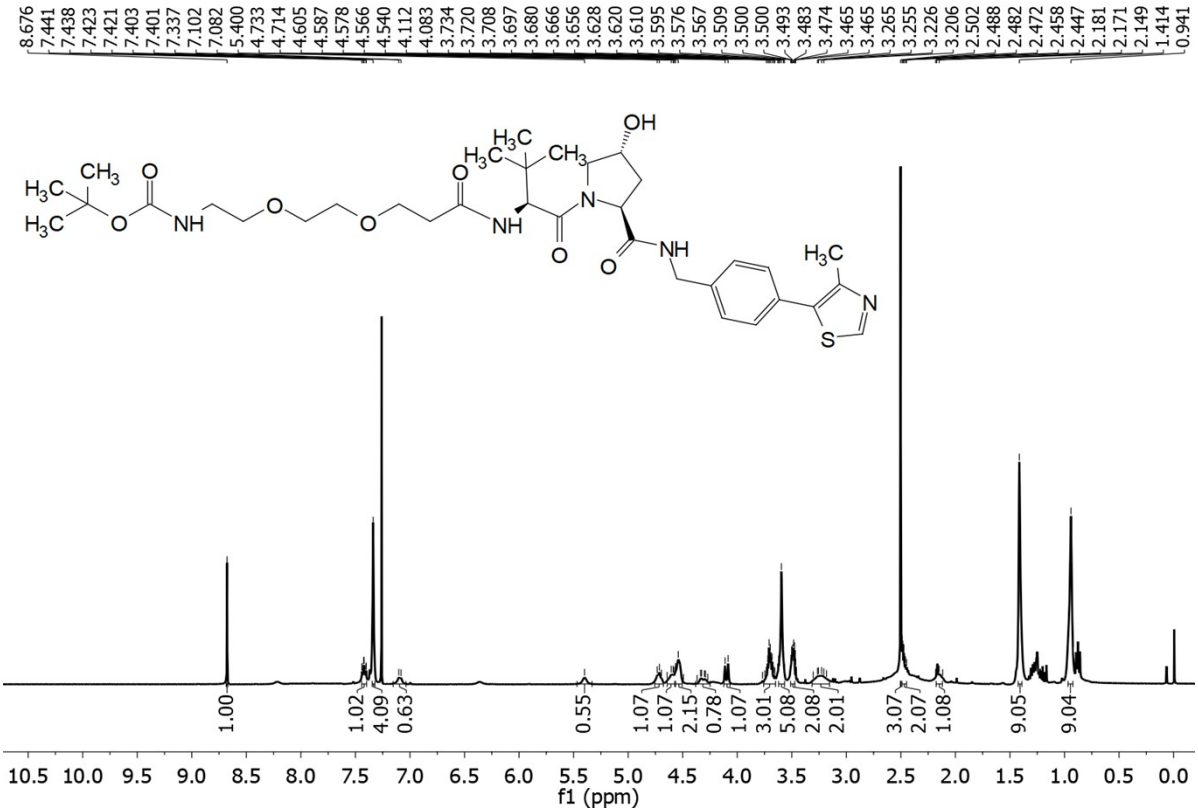
HR-MS spectra of compound **39**:



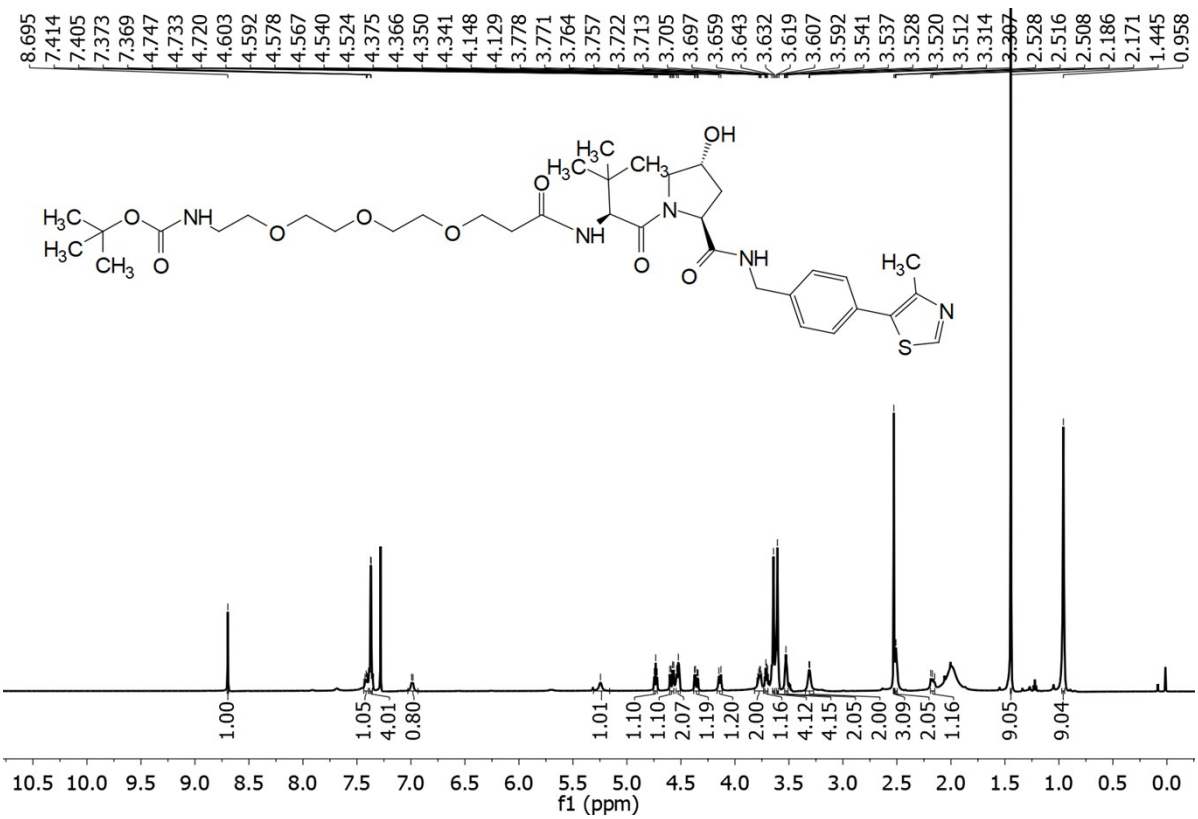
^{1}H NMR of compound **45** (400 MHz, Chloroform-*d*):



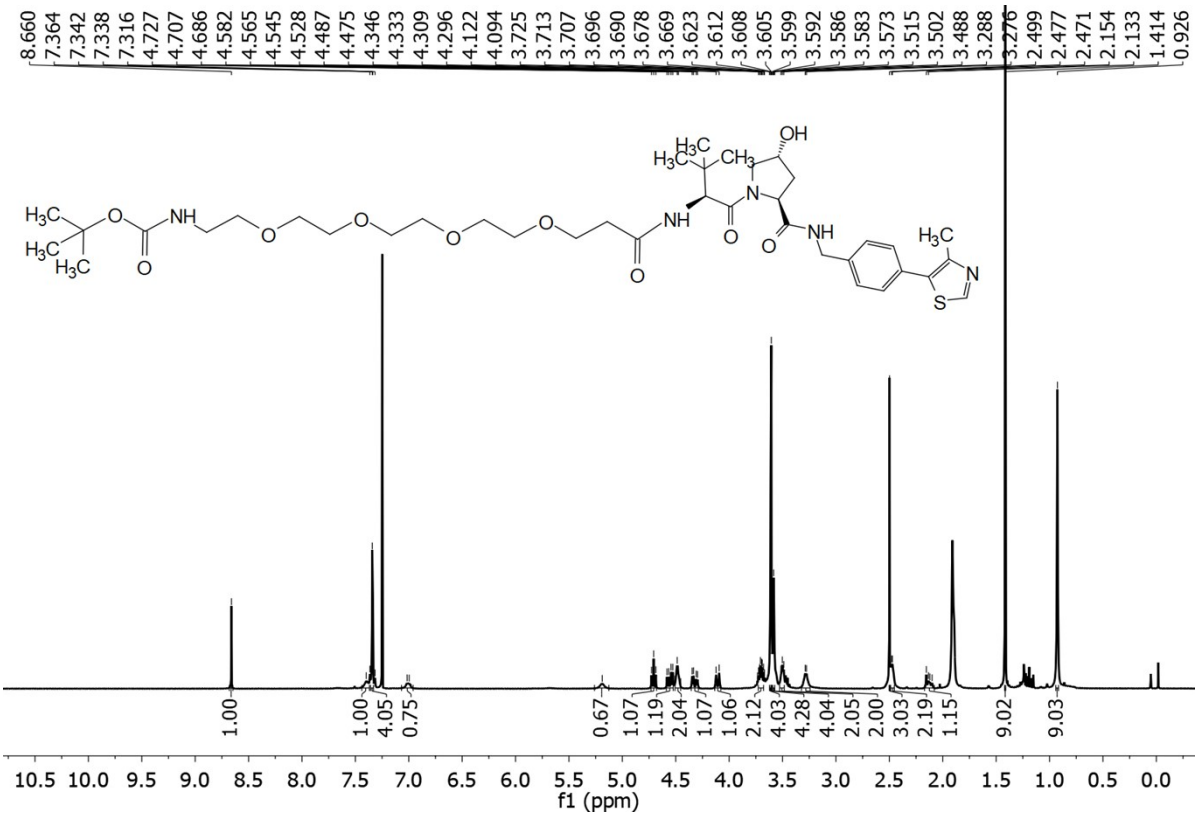
^{1}H NMR of compound **46** (400 MHz, Chloroform-*d*):



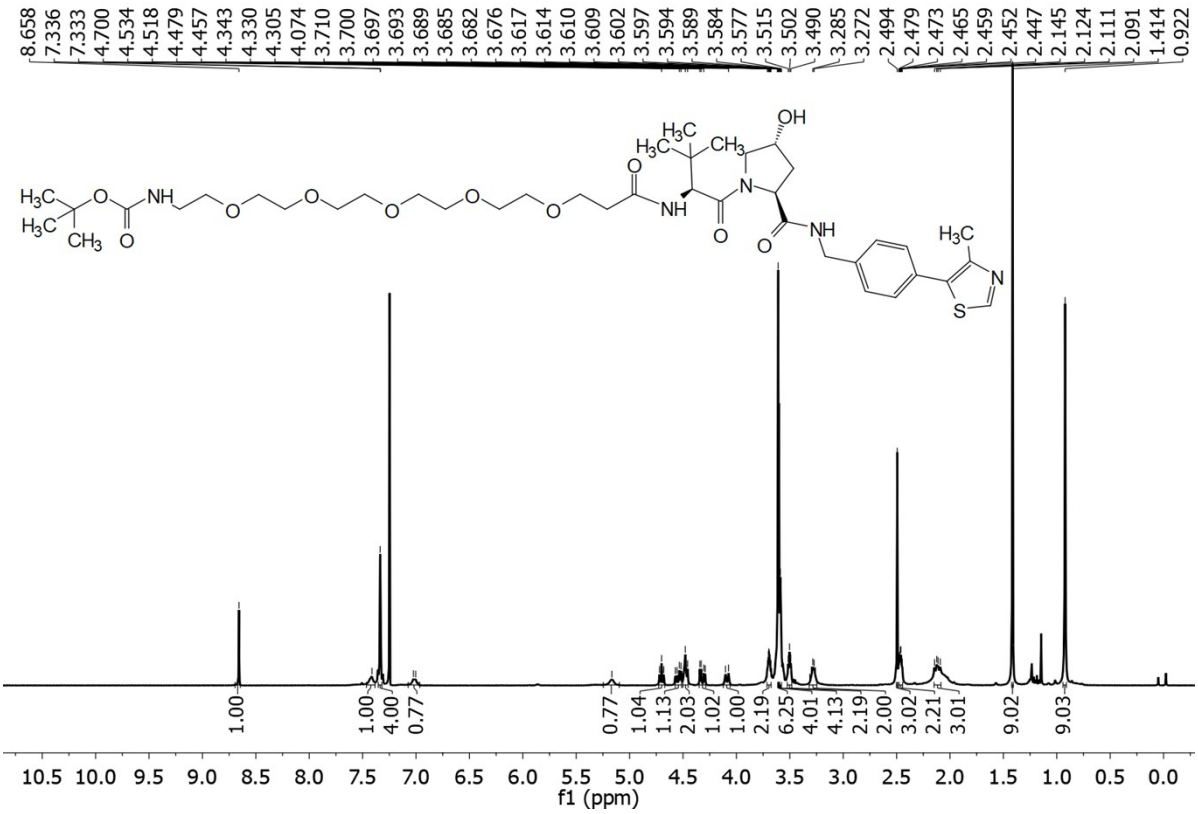
^{1}H NMR of compound **47** (400 MHz, Chloroform-*d*):



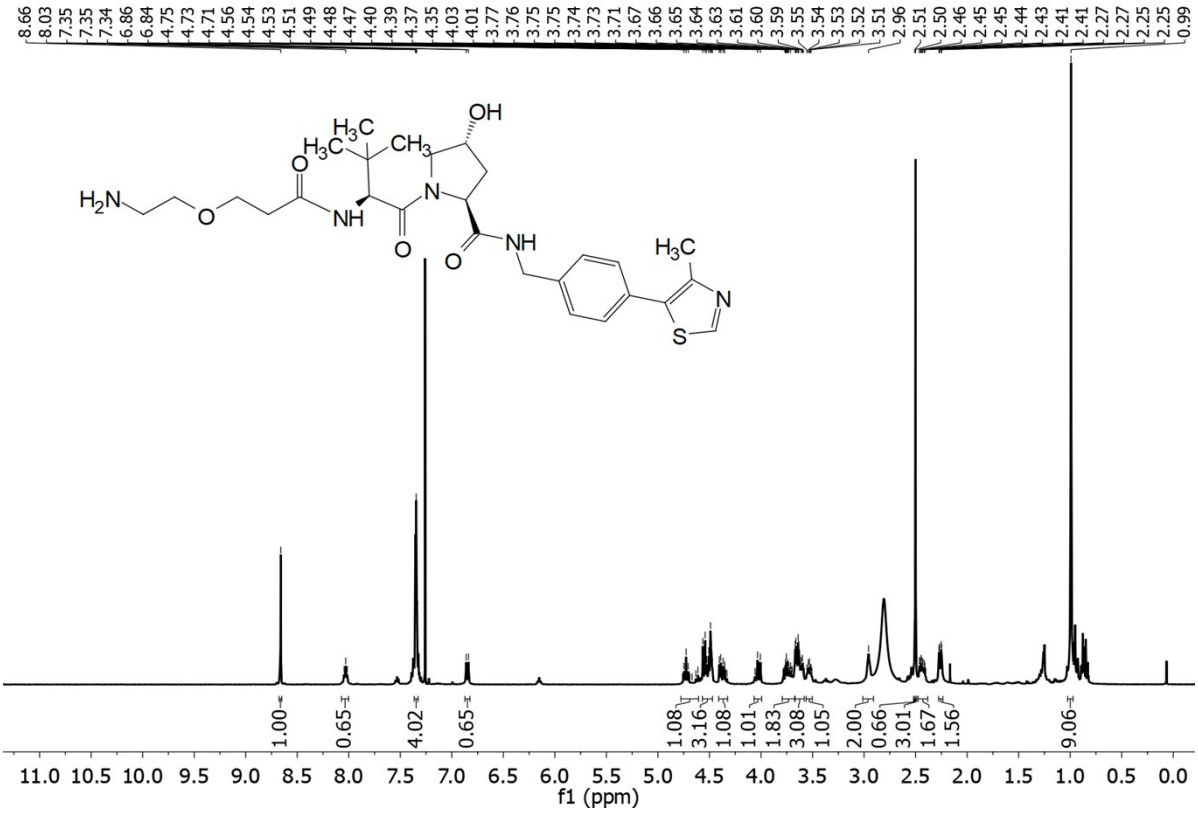
^{1}H NMR of compound **48** (400 MHz, Chloroform-*d*):



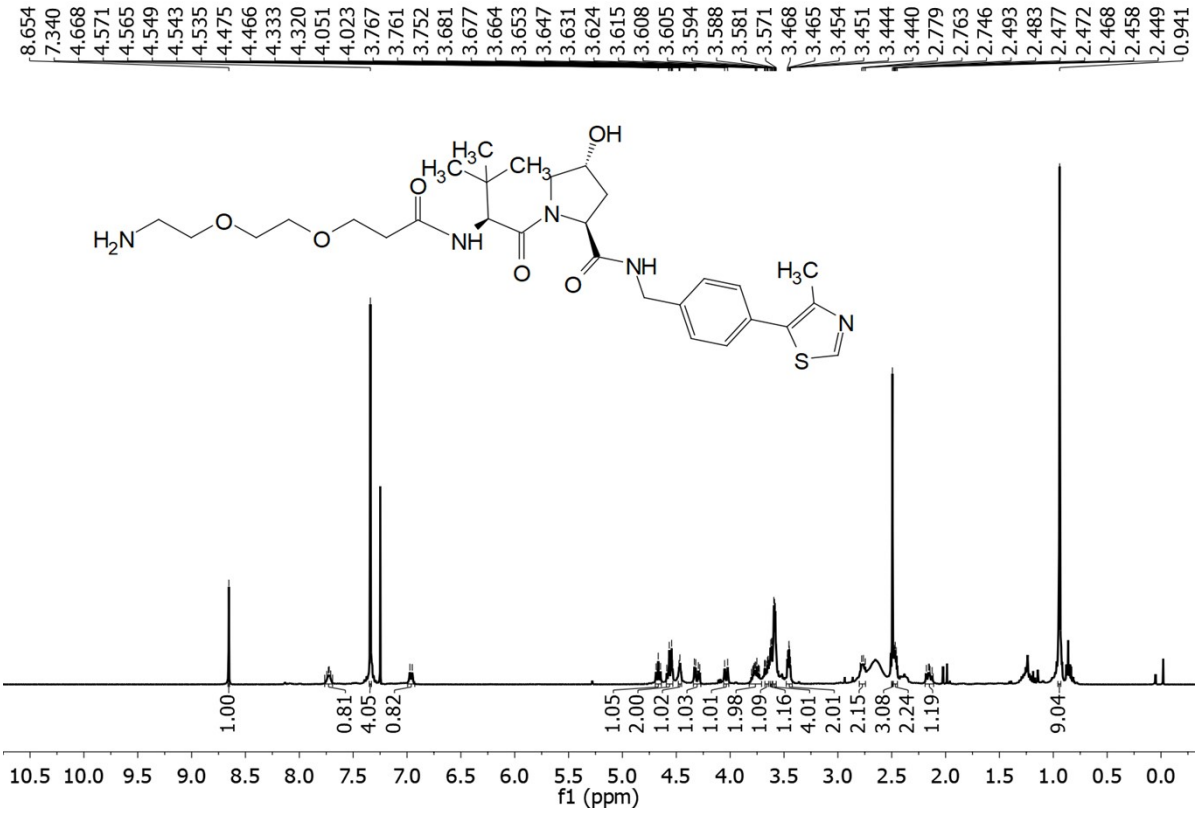
^{1}H NMR of compound **49** (400 MHz, Chloroform-*d*):



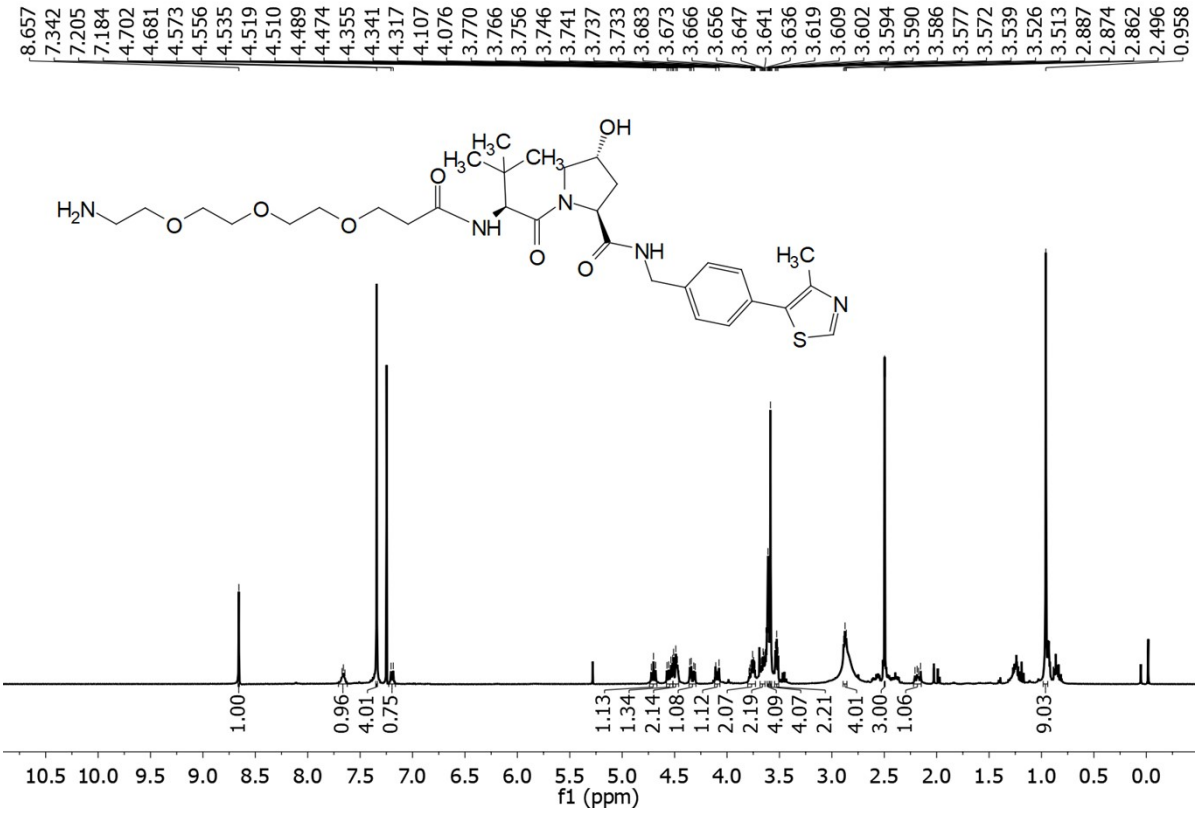
^{1}H NMR of compound **50** (400 MHz, Chloroform-*d*):



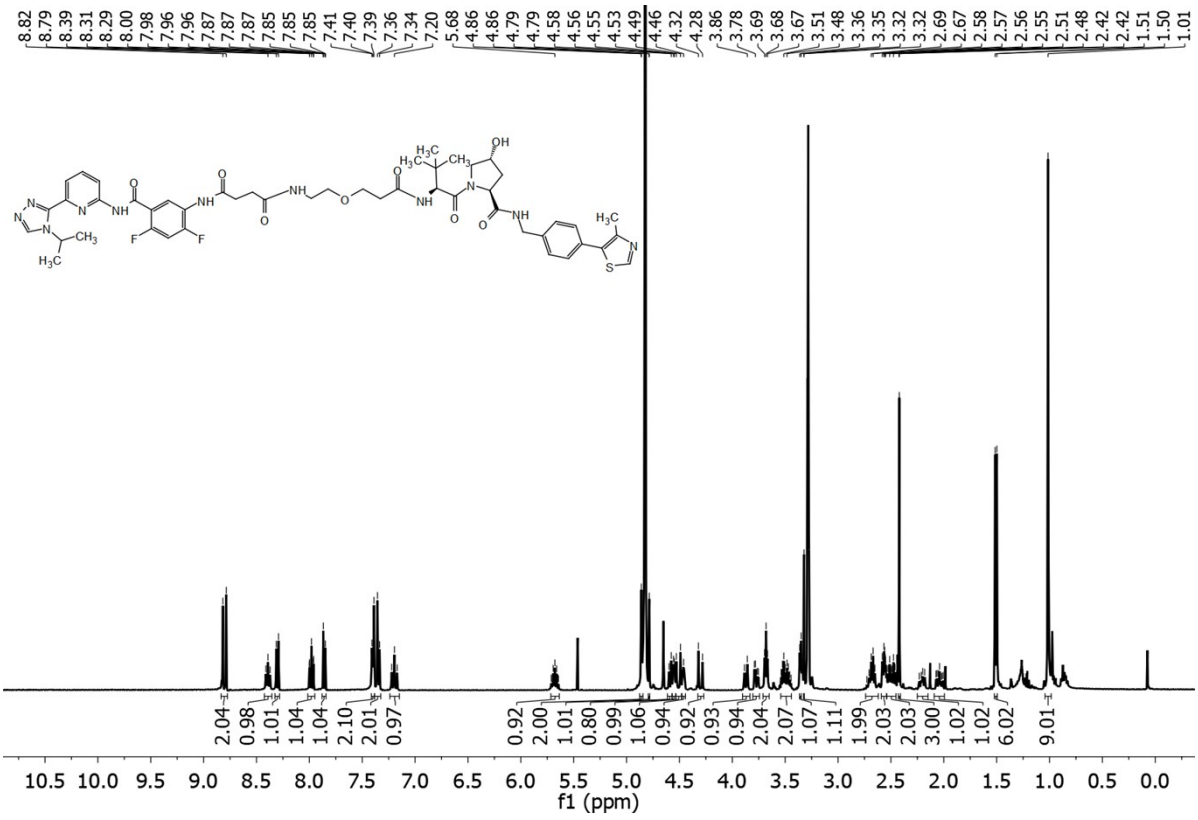
^{1}H NMR of compound **51** (400 MHz, Chloroform-*d*):



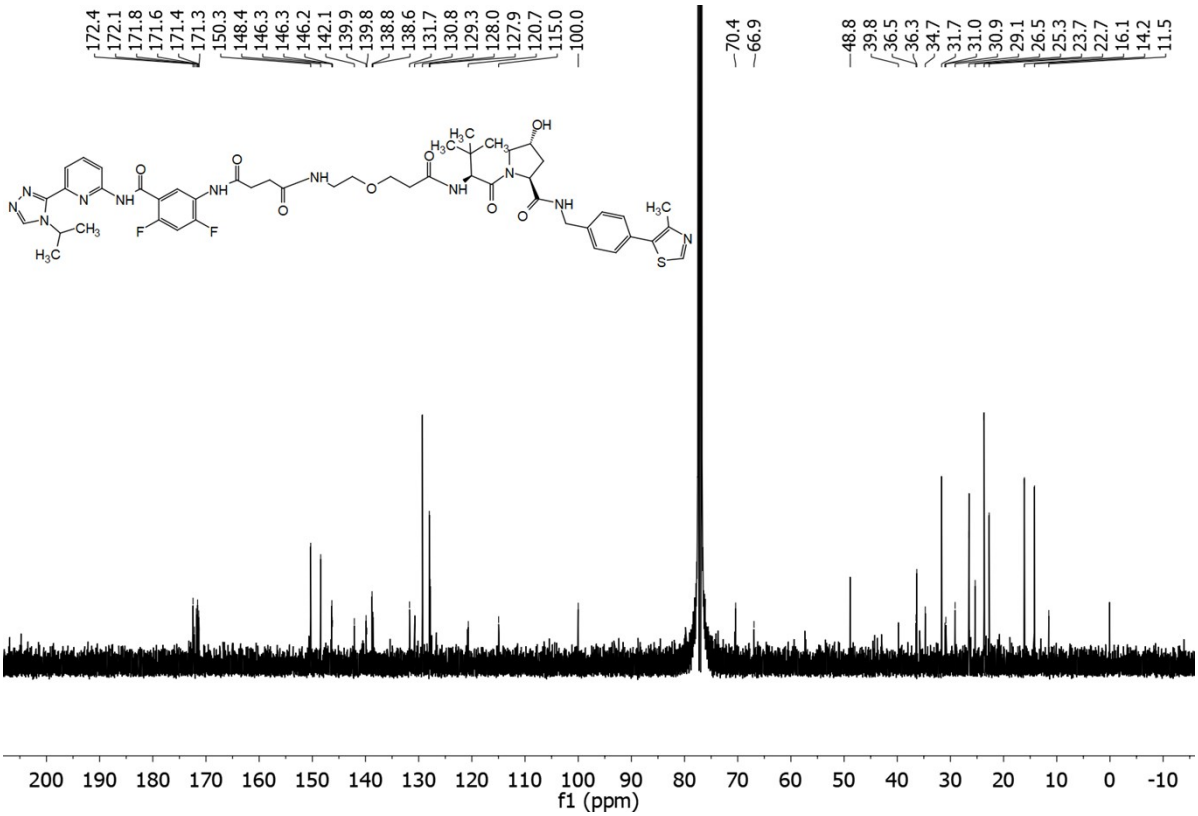
^{1}H NMR of compound **52** (400 MHz, Chloroform-*d*):



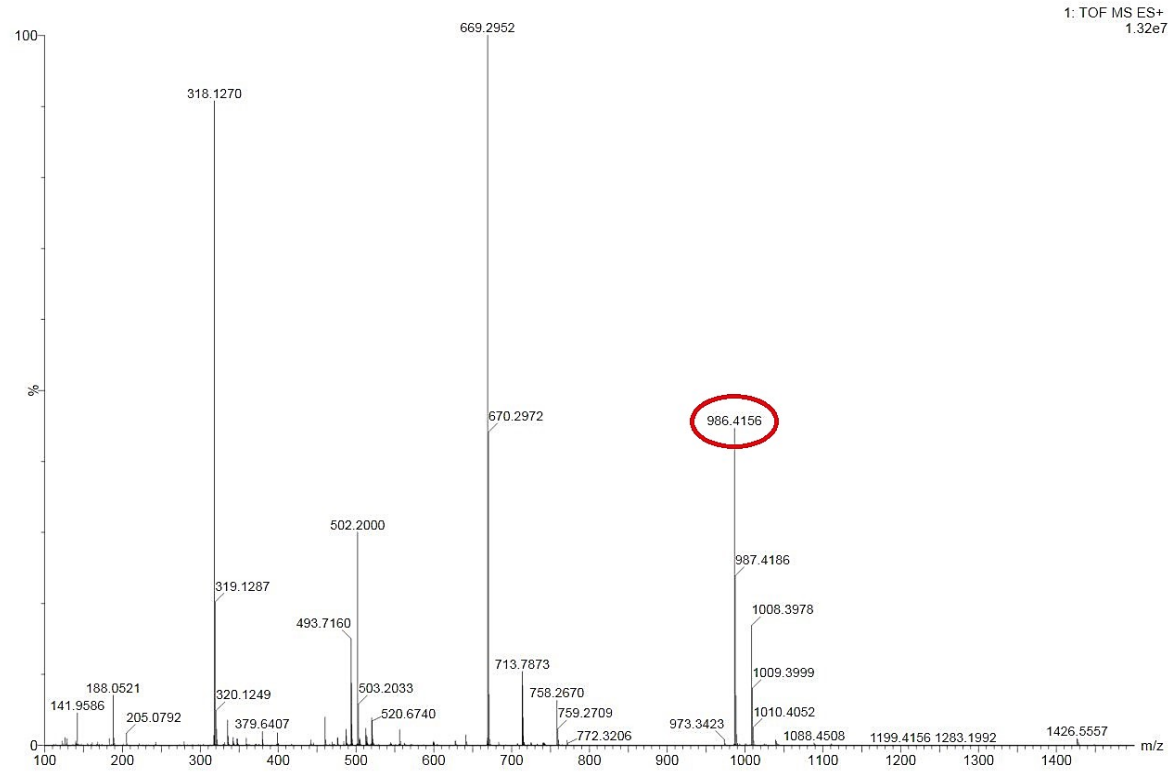
^{1}H NMR of compound **60** (400 MHz, Methanol-d_4):



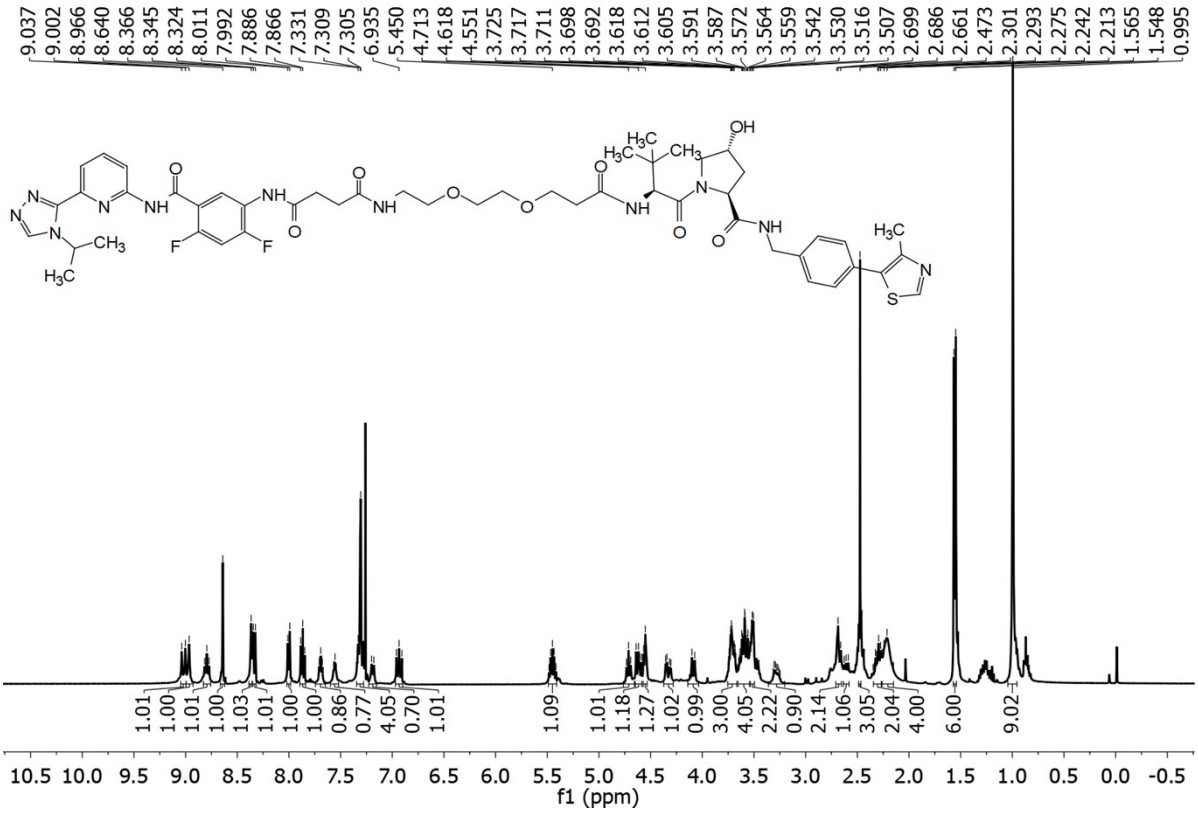
^{13}C NMR of compound **60** (101 MHz, Chloroform-*d*):



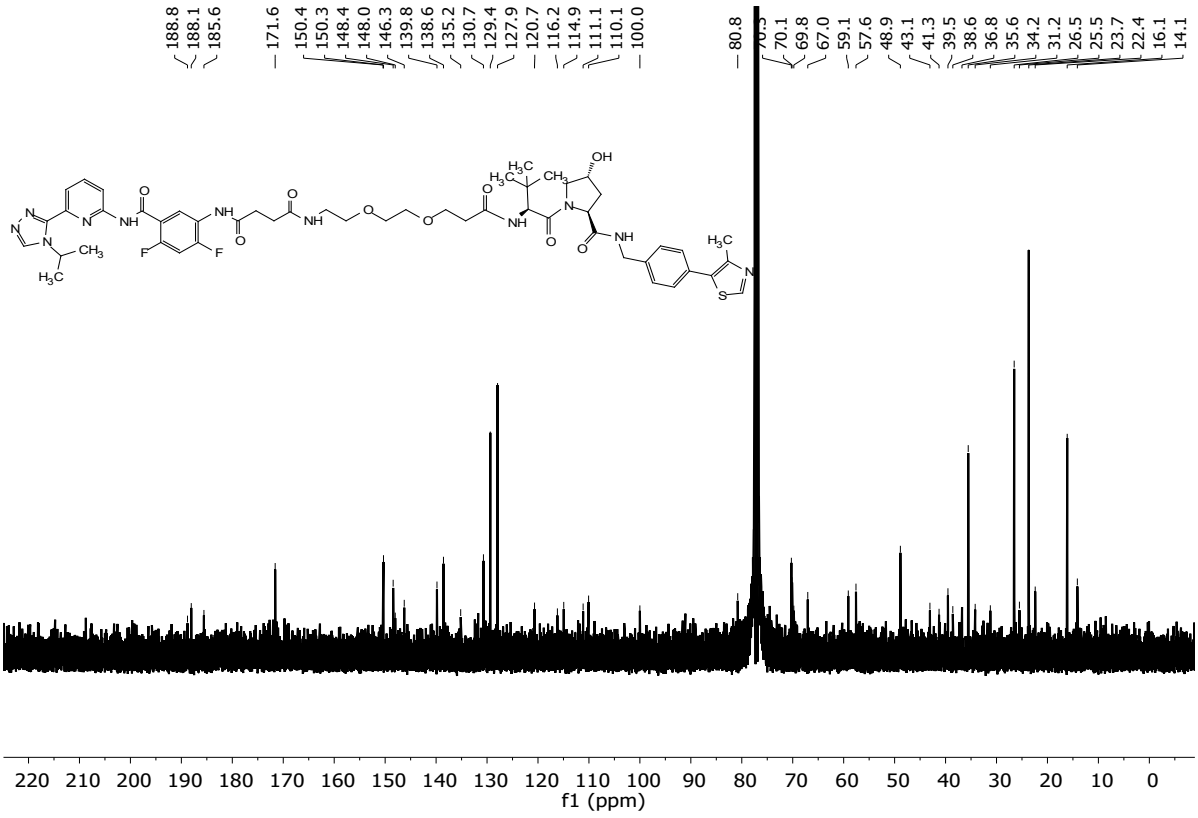
HR-MS spectra of compound **60**:



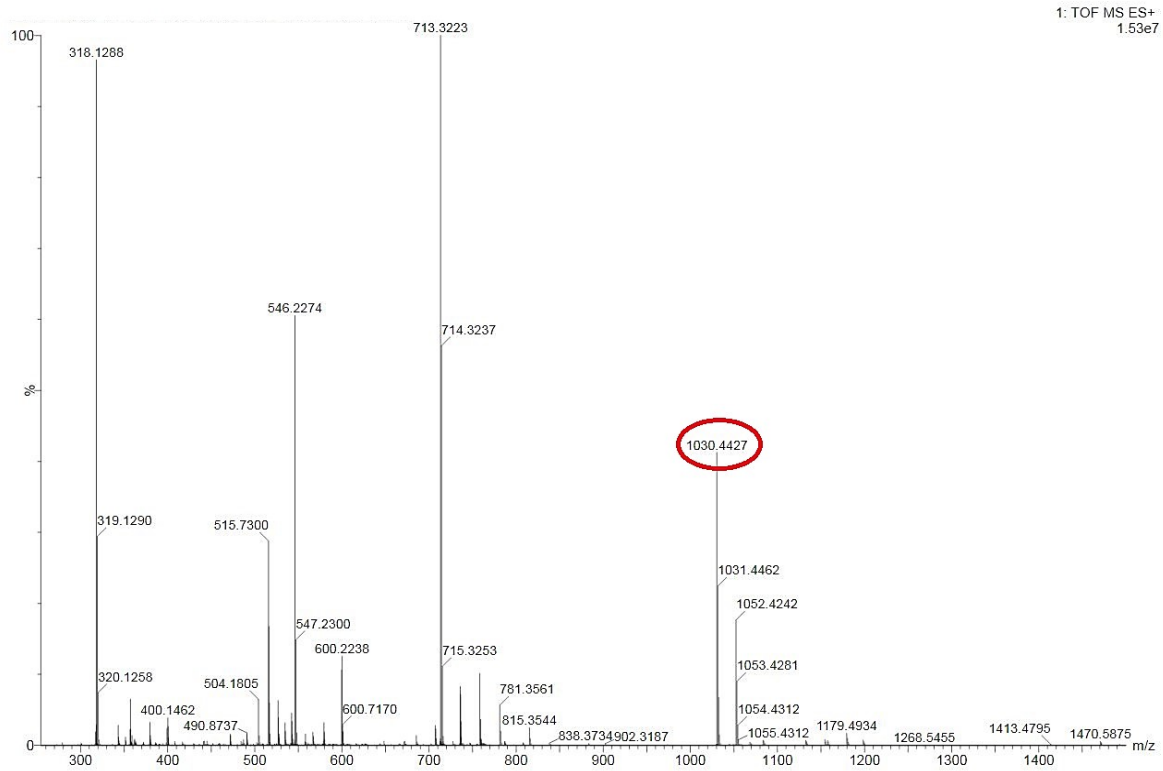
^{1}H NMR of compound **61** (400 MHz, Chloroform-*d*):



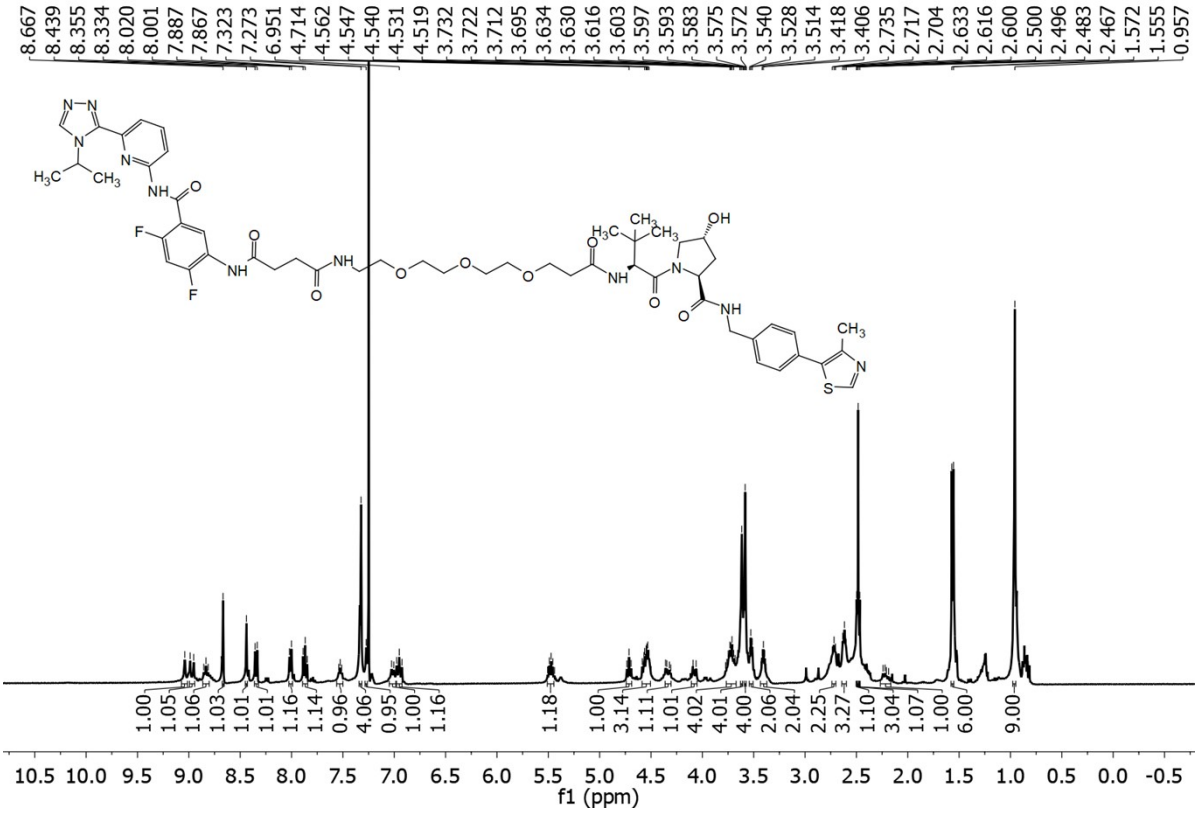
^{13}C NMR of compound **61** (101 MHz, Chloroform-*d*):



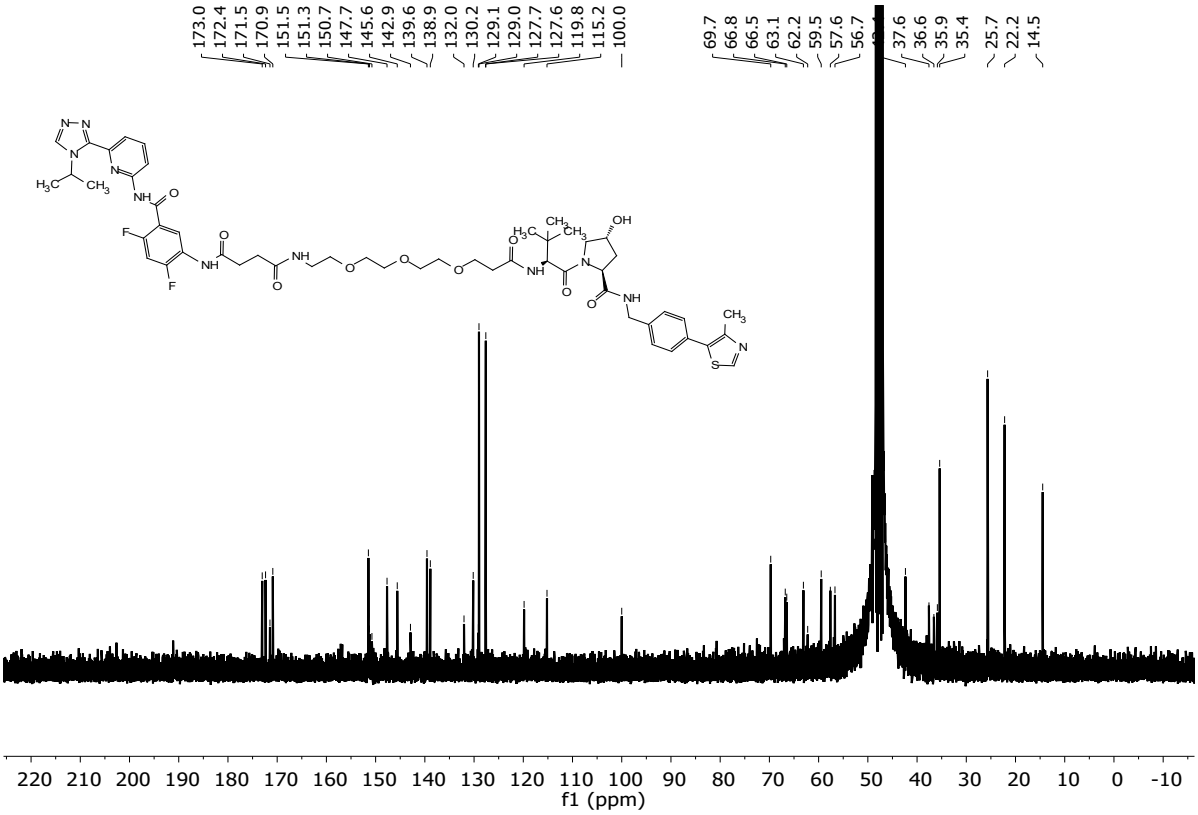
HR-MS spectra of compound **61**:



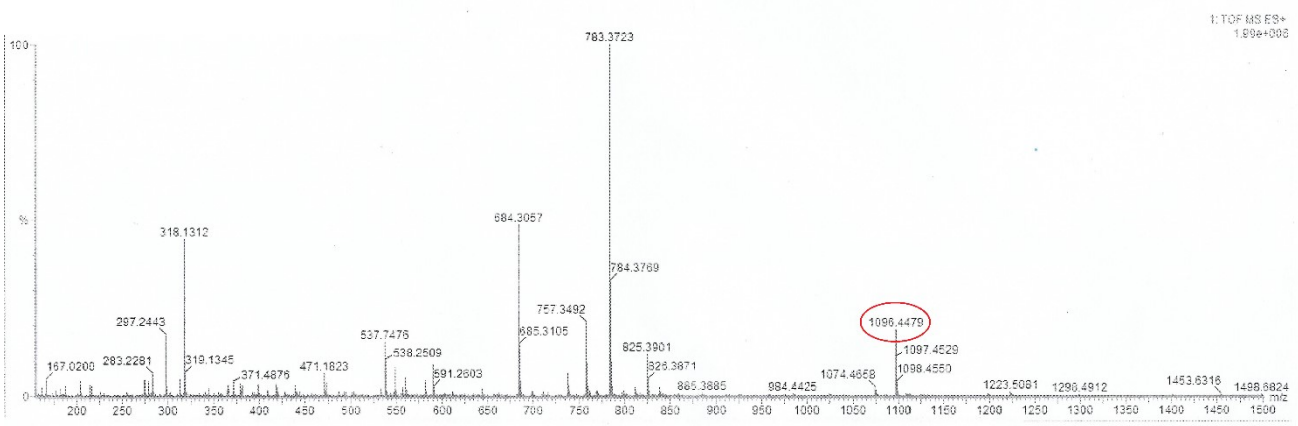
^{1}H NMR of compound **62** (400 MHz, Chloroform-*d*):



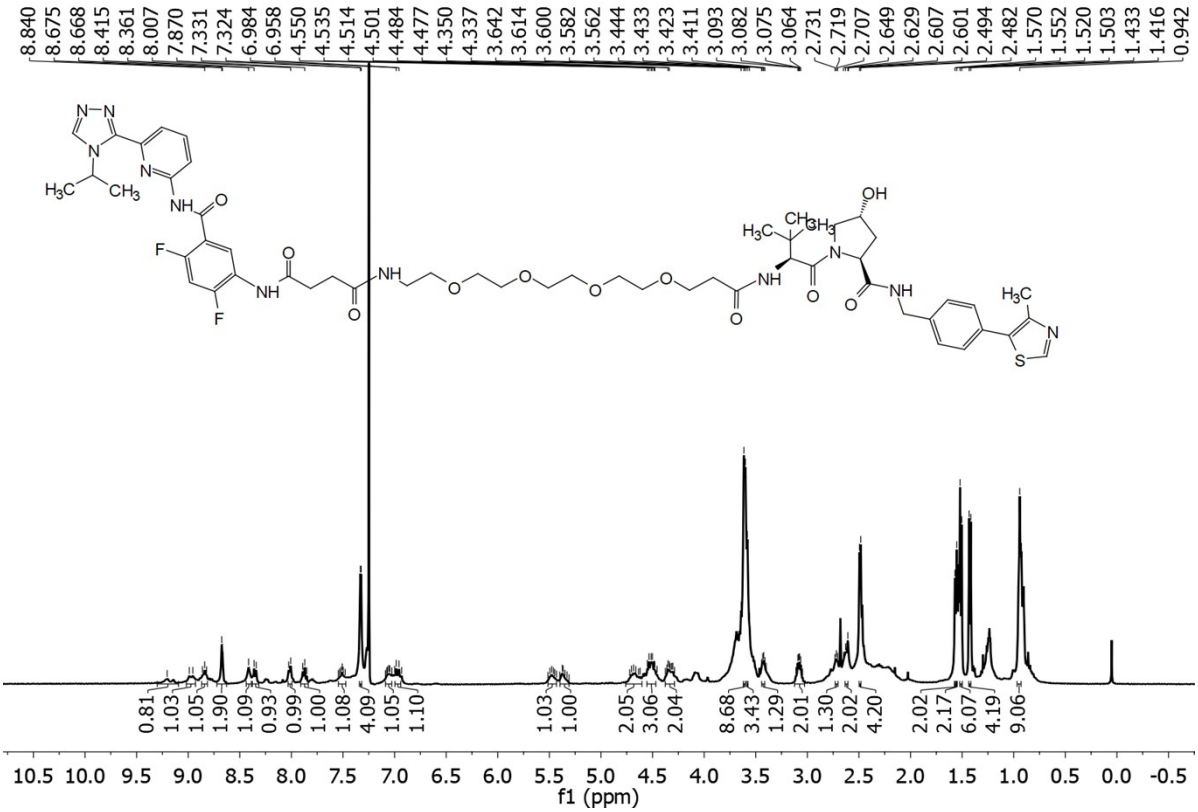
^{13}C NMR of compound **62** (101 MHz, Chloroform-*d*):



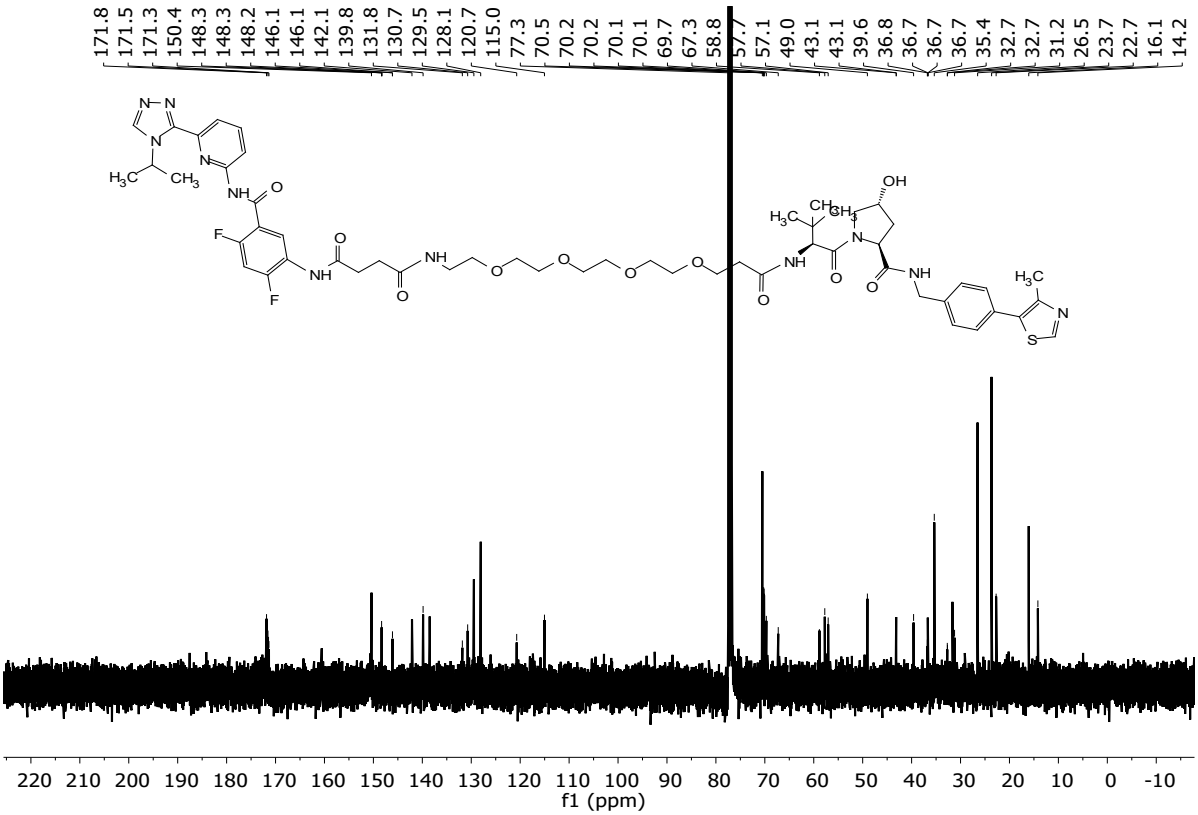
HR-MS spectra of compound **62**:



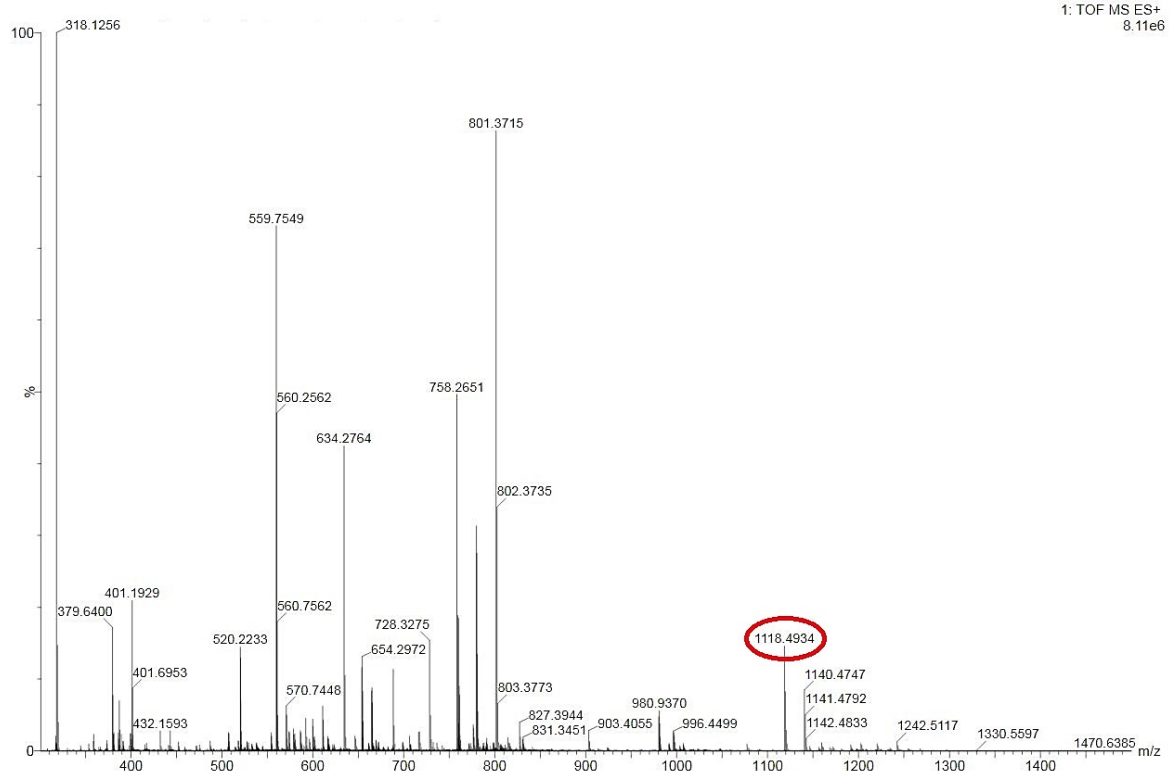
^{1}H NMR of compound **63** (400 MHz, Chloroform-*d*):



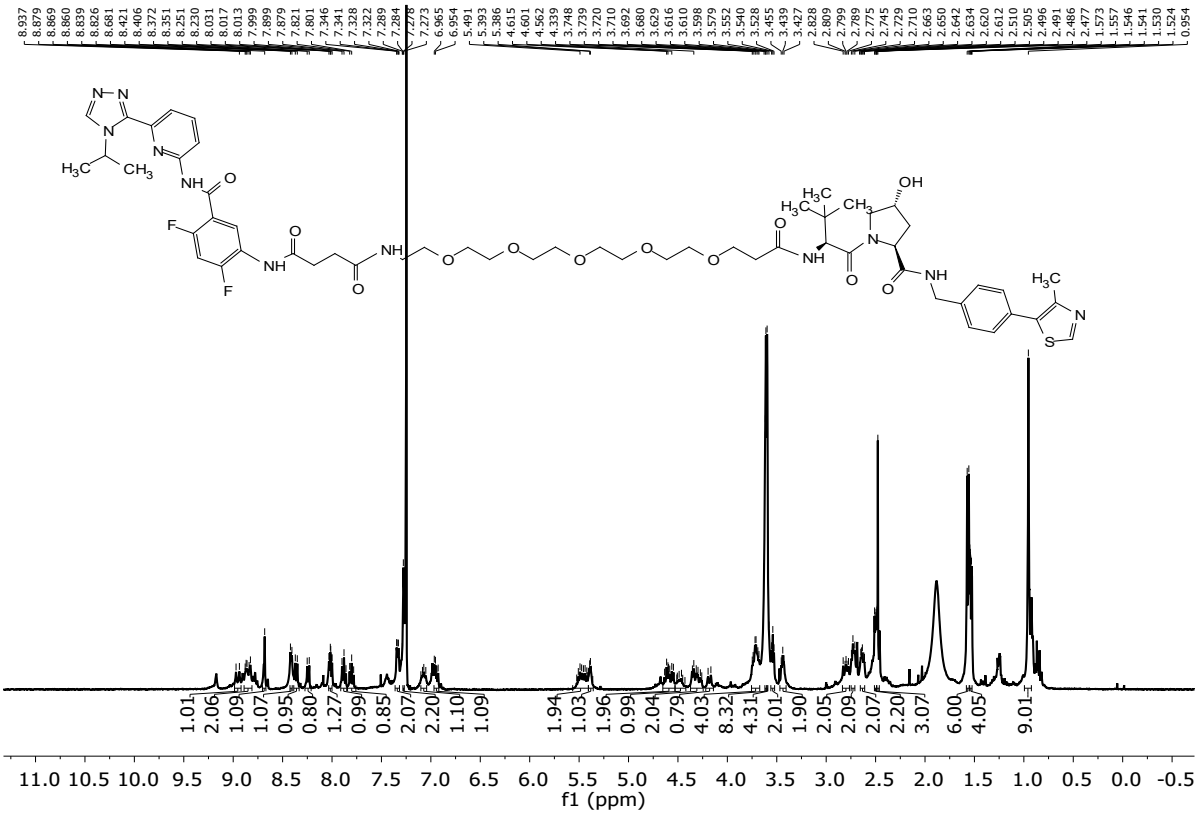
^{13}C NMR of compound **63** (101 MHz, Chloroform-*d*):



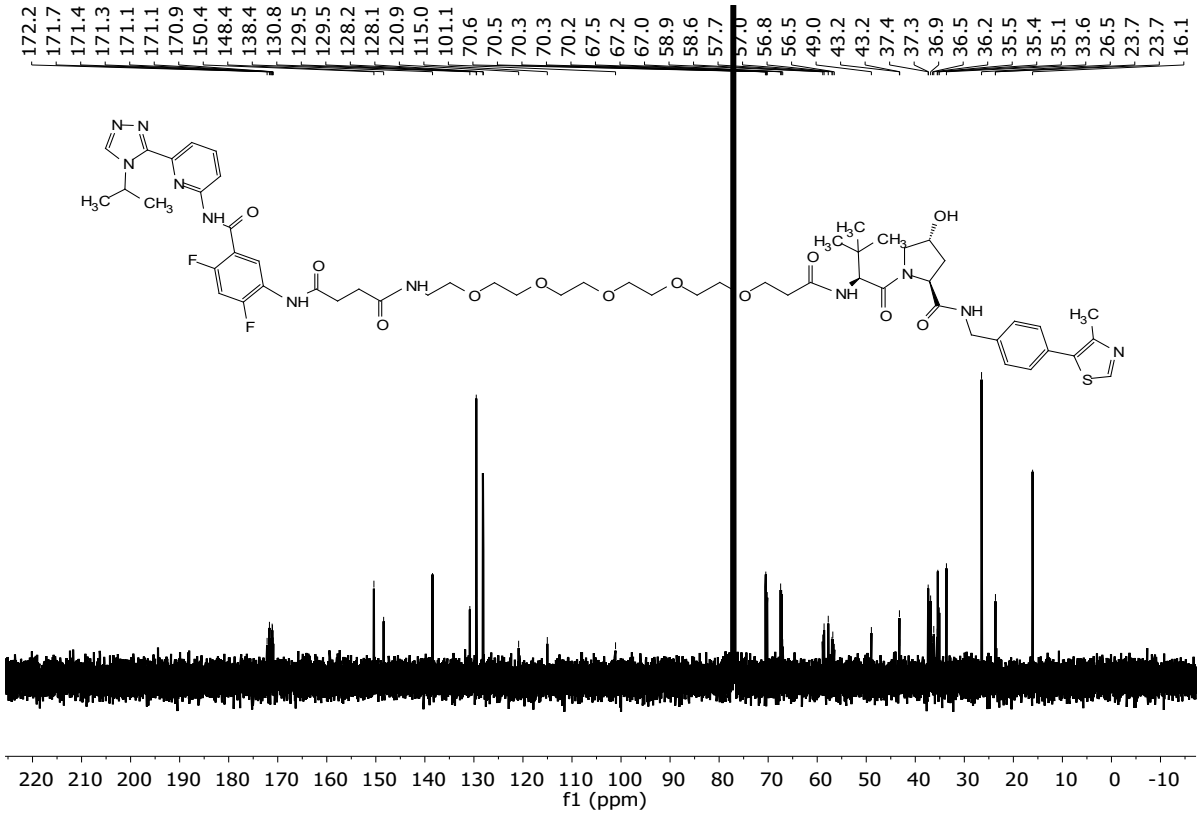
HR-MS spectra of compound **63**:



^{1}H NMR of compound **64** (400 MHz, Chloroform-*d*):



^{13}C NMR of compound **64** (101 MHz, Chloroform-*d*):



HR-MS spectra of compound **64**: