Supporting Information

Design, synthesis, biological evaluation, and X-ray studies of HIV-1 protease inhibitors with modified P2'-ligands of Darunavir

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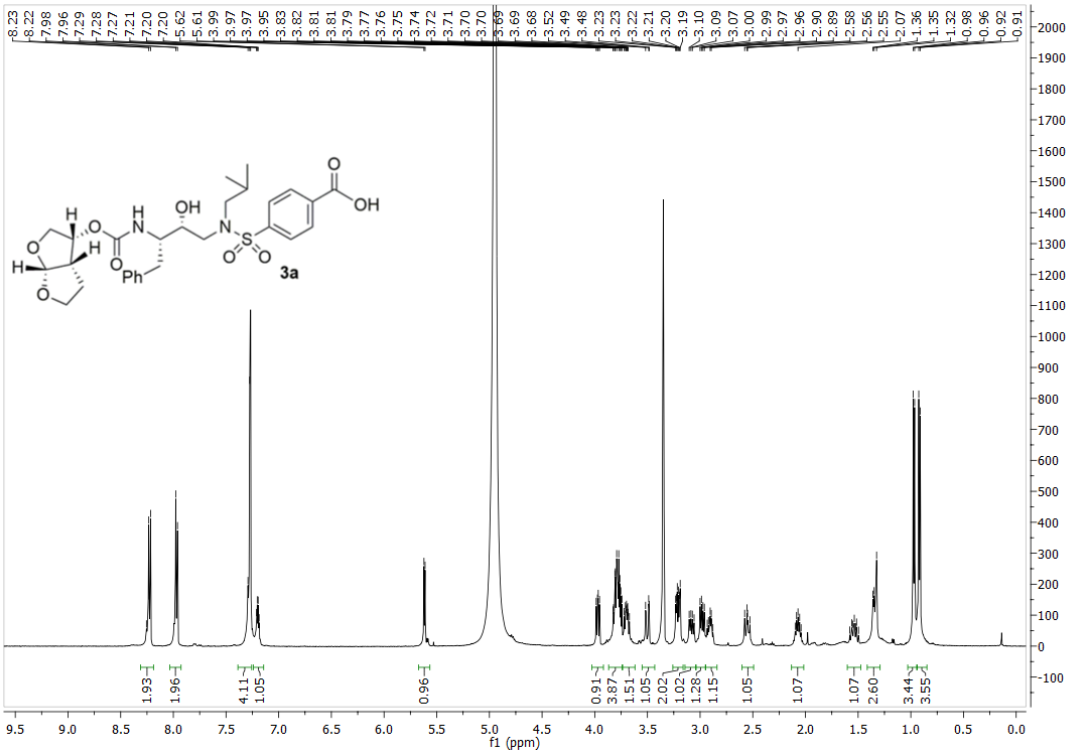
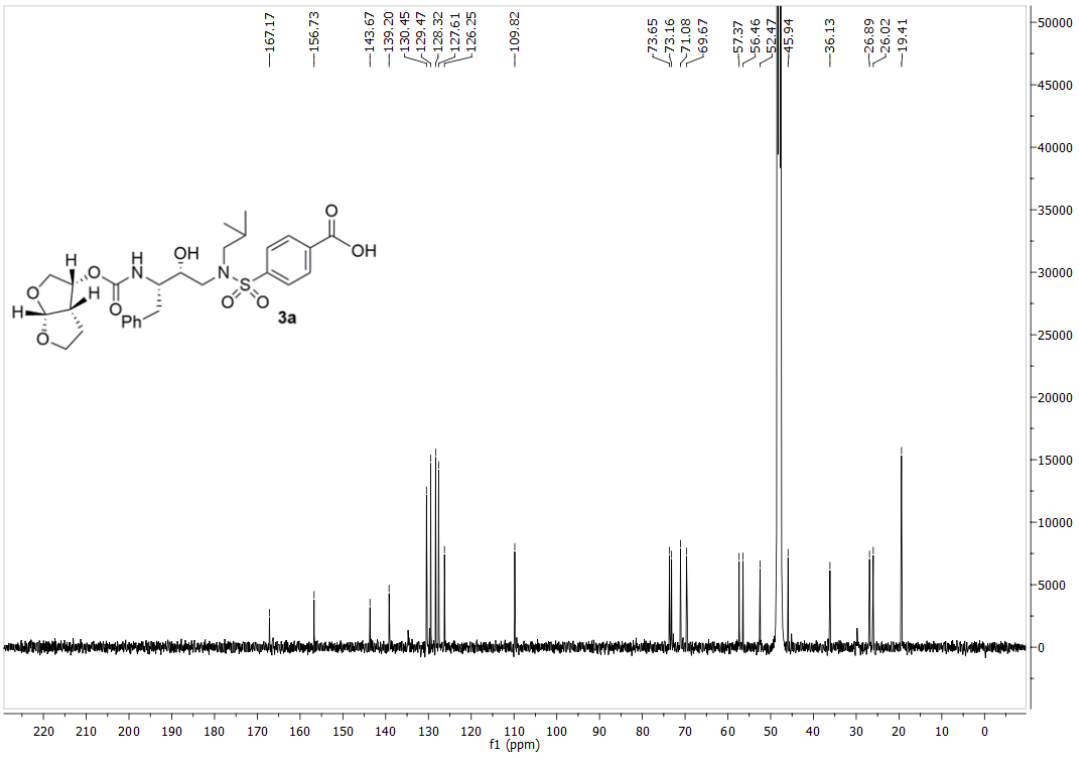
Experimental Section

All moisture sensitive reactions were carried out in an oven dried flask under argon atmosphere. All chemicals and reagents were purchased from commercial suppliers and used without further purification. Anhydrous solvents were obtained as follows: anhydrous tetrahydrofuran, diethyl ether, and benzene were distilled from sodium metal under argon. Anhydrous dichloromethane, toluene, methanol, and acetonitrile were dried via distillation from CaH_2 under argon. All other solvents were HPLC grade. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian INOVA300-1, Bruker Avance ARX-400 and Bruker DRX-500 spectrometers. NMR data are reported as: δ value (chemical shift, *J*-value (Hz), integration, where s = singlet, d = doublet, t = triplet, q = quartet, br = broad). Low resolution mass analyses were performed on a Agilent 1290 Infinity II spectrometer. High Resolution mass analyses were performed at the Purdue University Campus-wide Mass Spectrometry Center. TLC analysis was carried out with SiliCycle 60A-F_{254} plates. Flash chromatography was performed using SiliCycle 230-400 mesh, 60 Å pore diameter silica gel. HPLC analysis was performed on a Agilent 1260 Infinity instrument. All test inhibitors showed purity >95% by HPLC analysis.

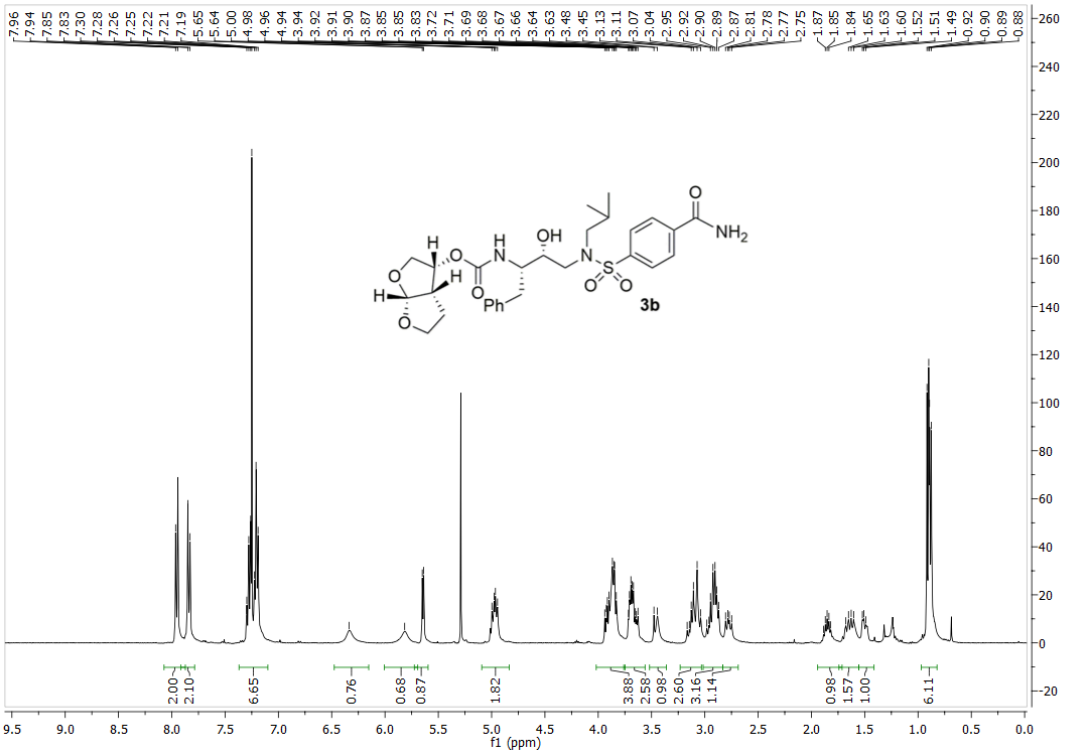
Compound **3a**: ^{1}H-NMR (500 MHz, CD_3OD)



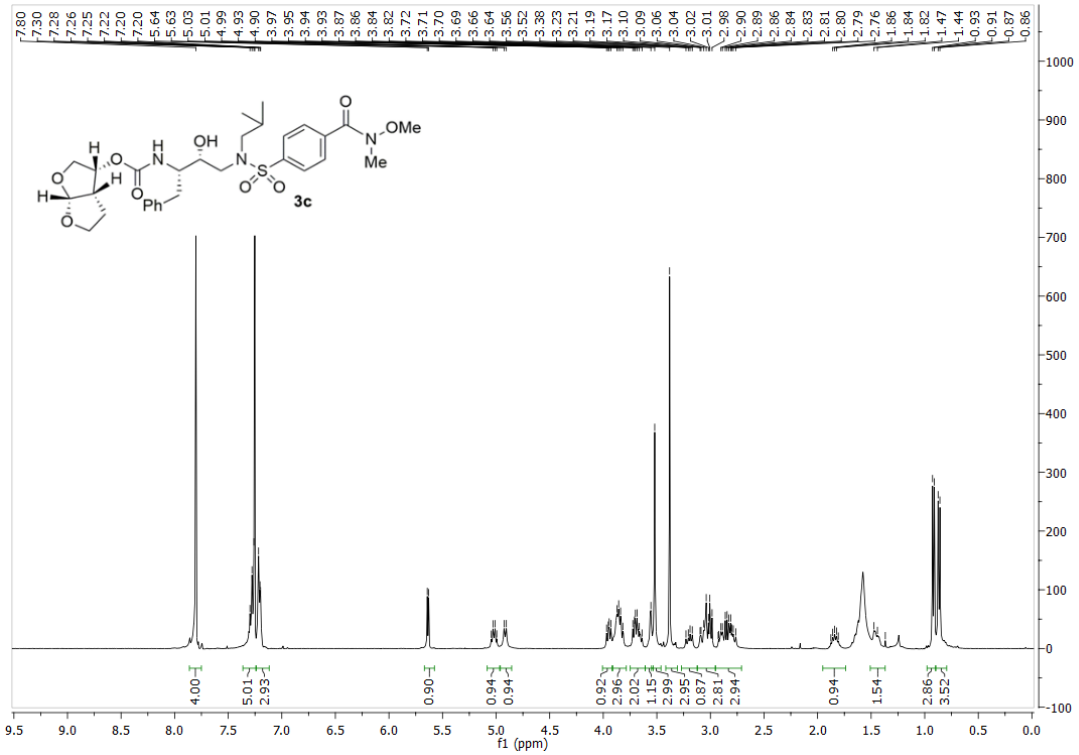
Compound **3a**: ^{13}C-NMR (125 MHz, CD_3OD)



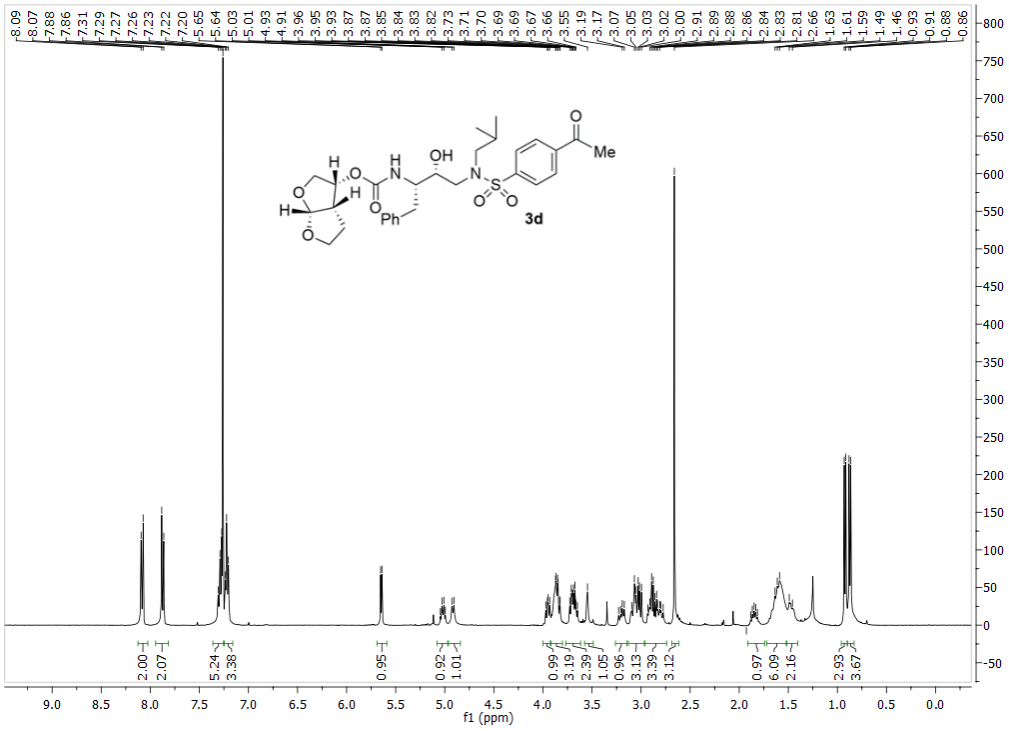
Compound **3b**: ^{1}H-NMR (400 MHz, $CDCl_3$)



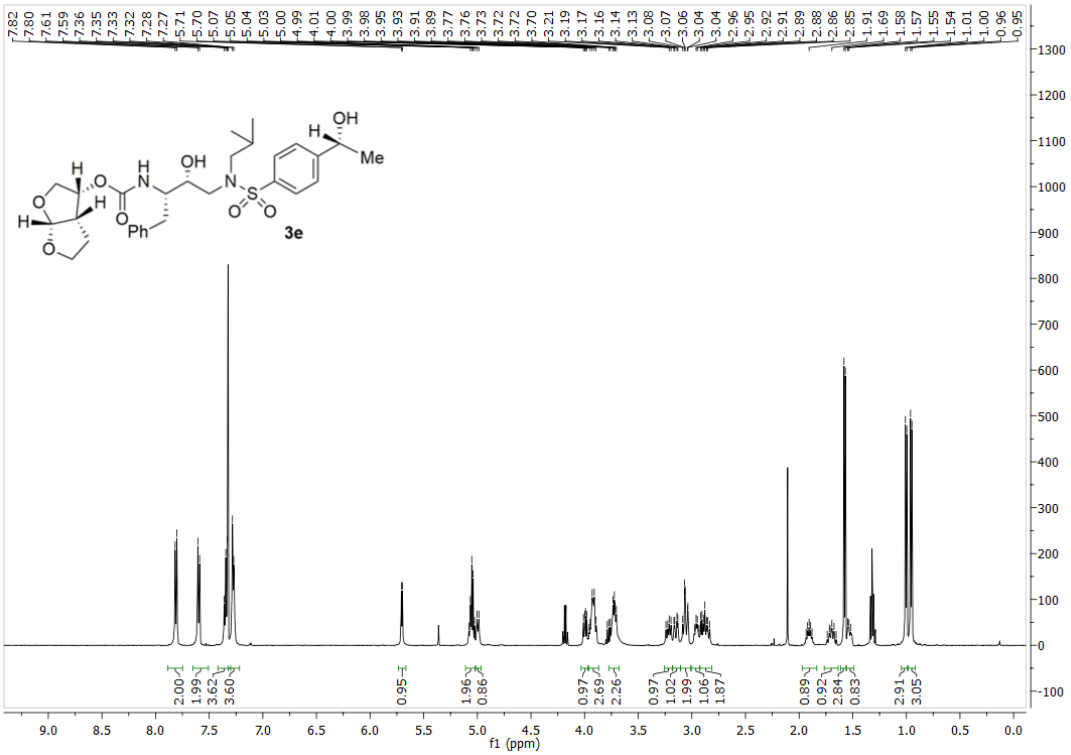
Compound **3c**: ^{1}H-NMR (400 MHz, $CDCl_3$)



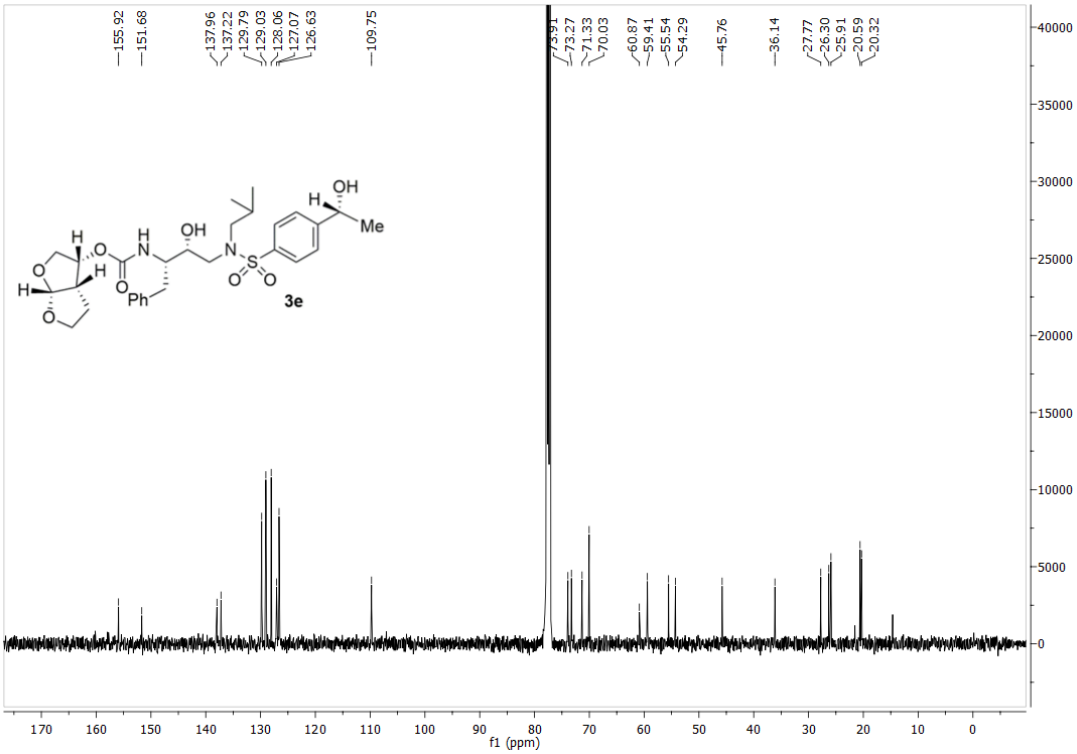
Compound **3d**: ^{1}H-NMR (400 MHz, $CDCl_3$)



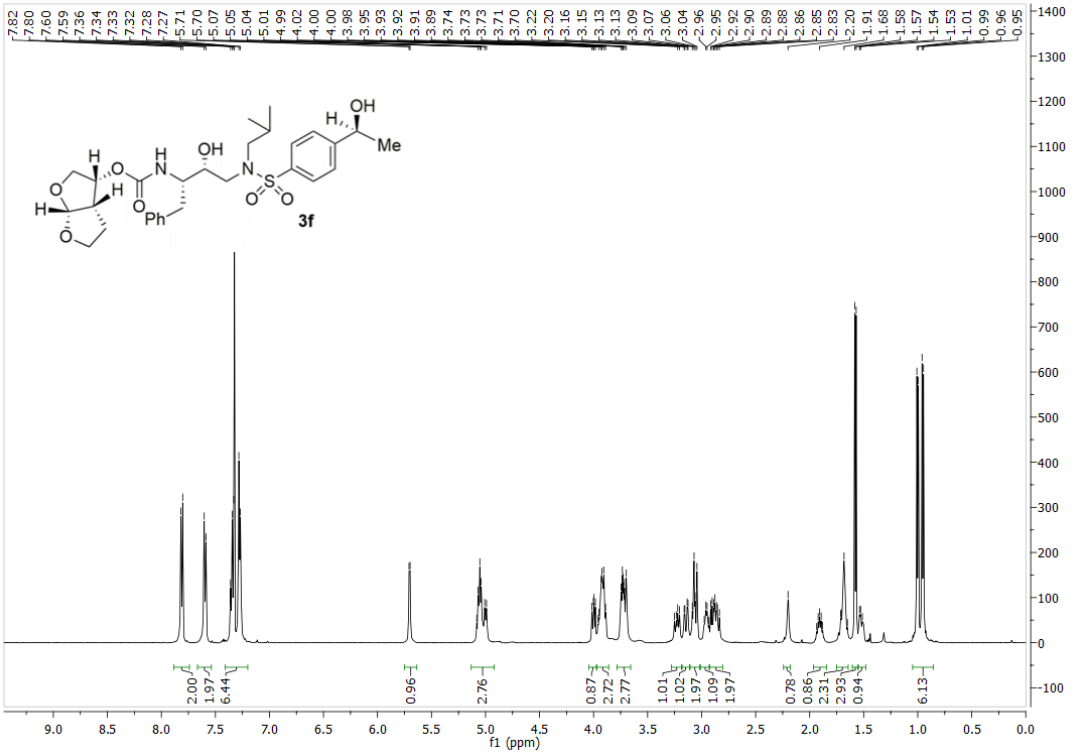
Compound **3e**: ^{1}H-NMR (500 MHz, $CDCl_3$)



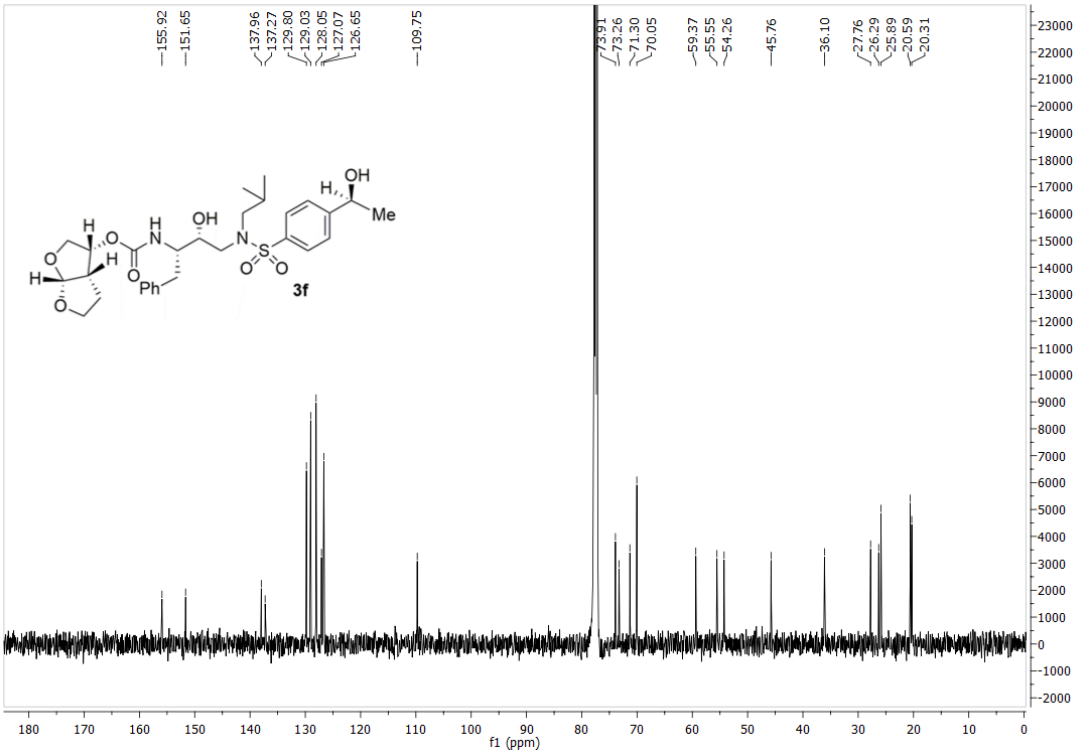
Compound **3e**: ^{13}C-NMR (125 MHz, $CDCl_3$)



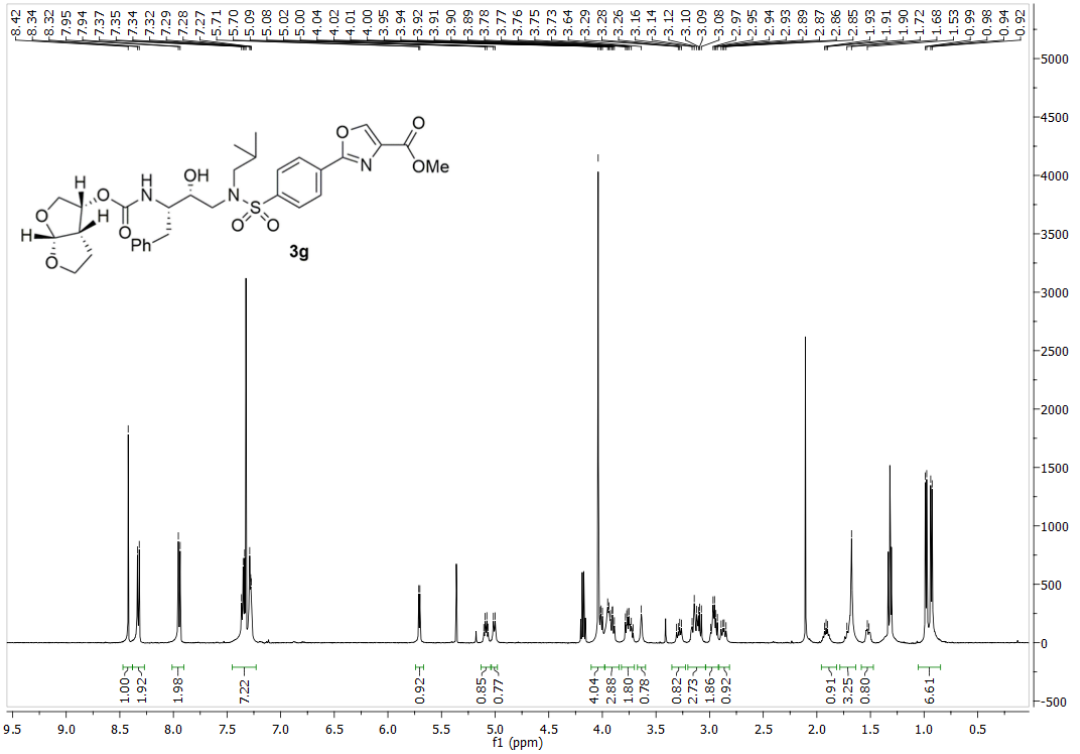
Compound **3f**: ^{1}H-NMR (500 MHz, $CDCl_3$)



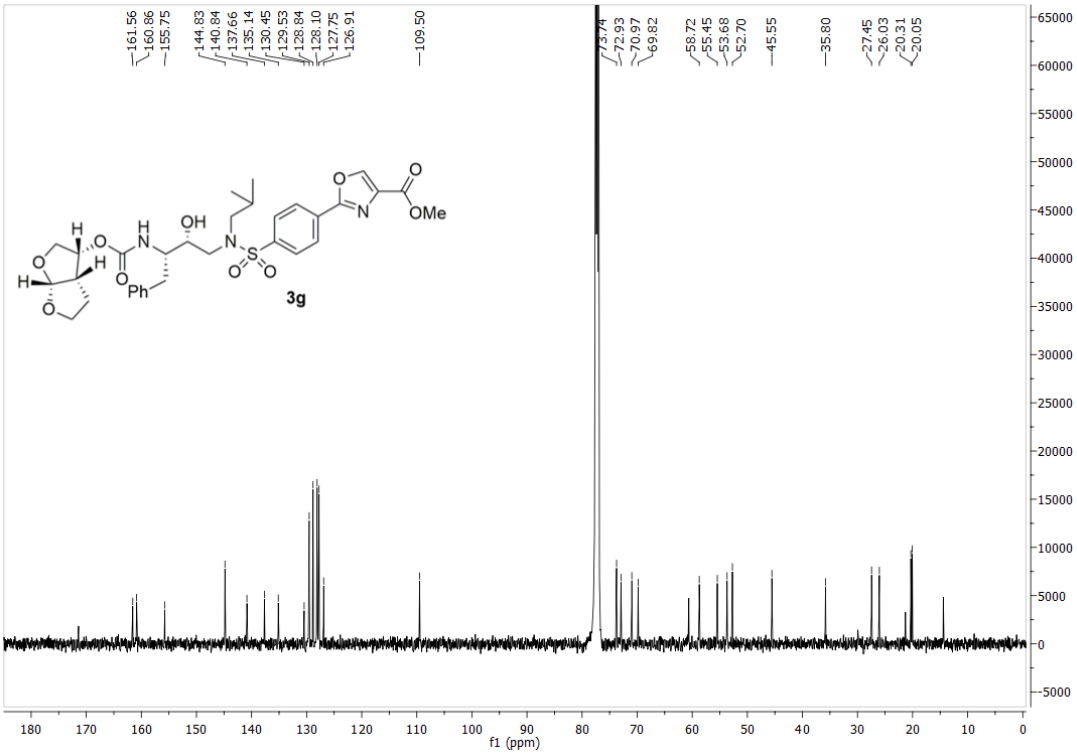
Compound **3f**: ^{13}C-NMR (125 MHz, $CDCl_3$)



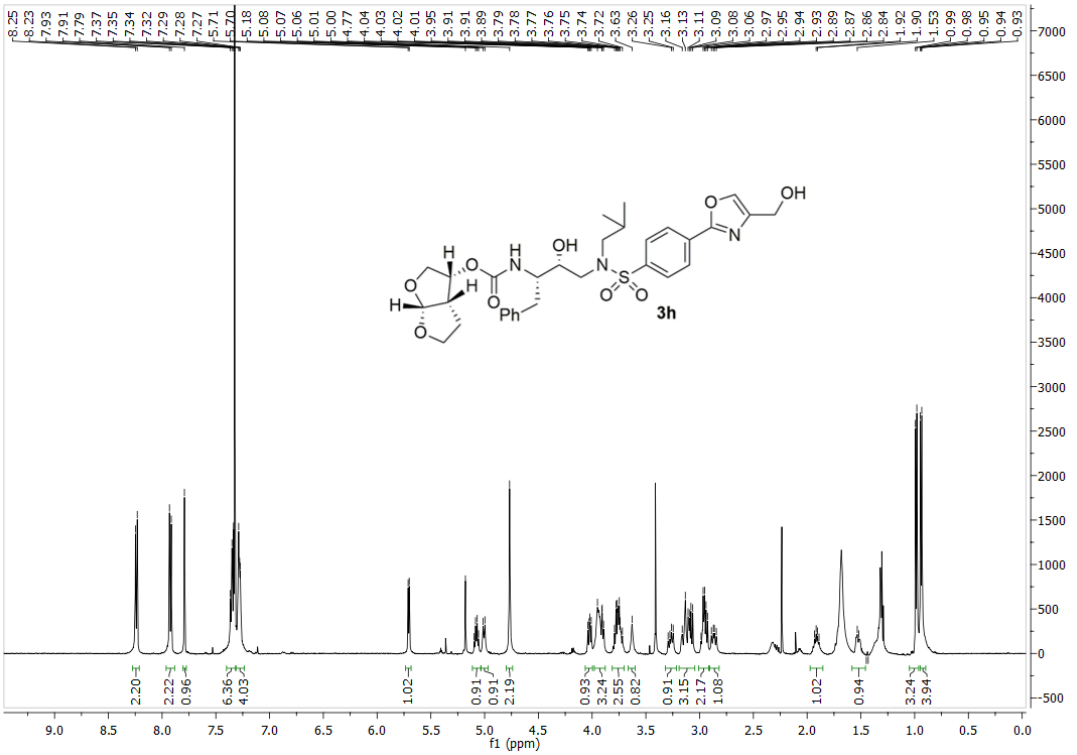
Compound **3g**: ^{1}H-NMR (500 MHz, $CDCl_3$)



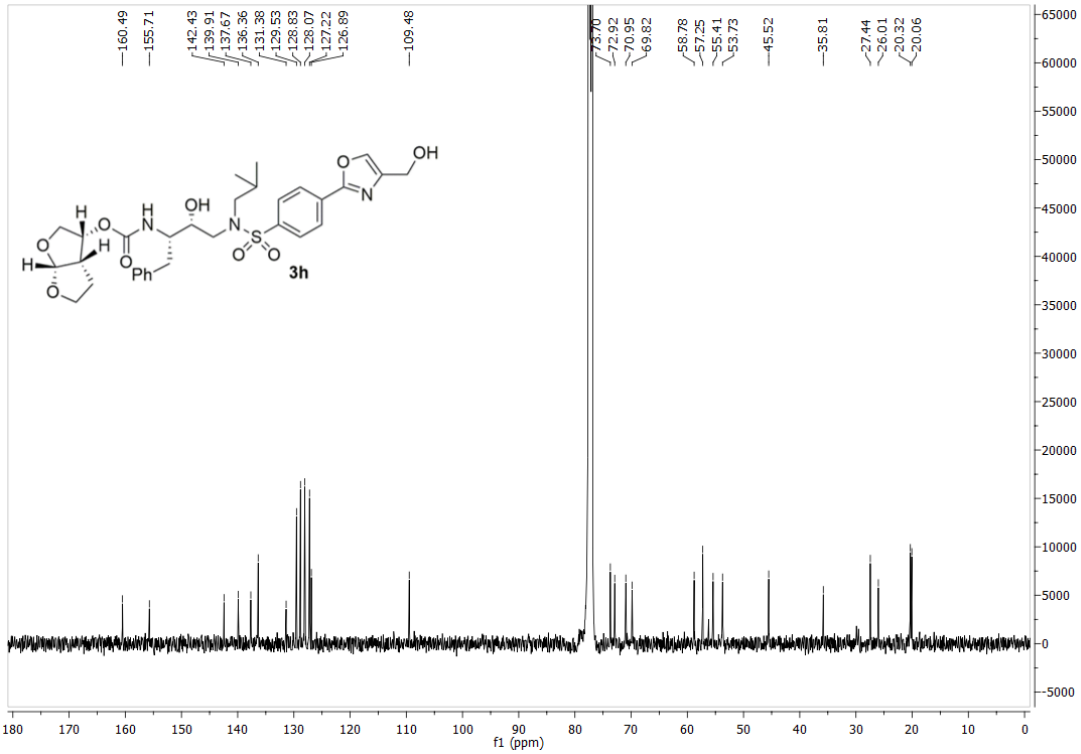
Compound **3g**: ^{13}C-NMR (125 MHz, $CDCl_3$)



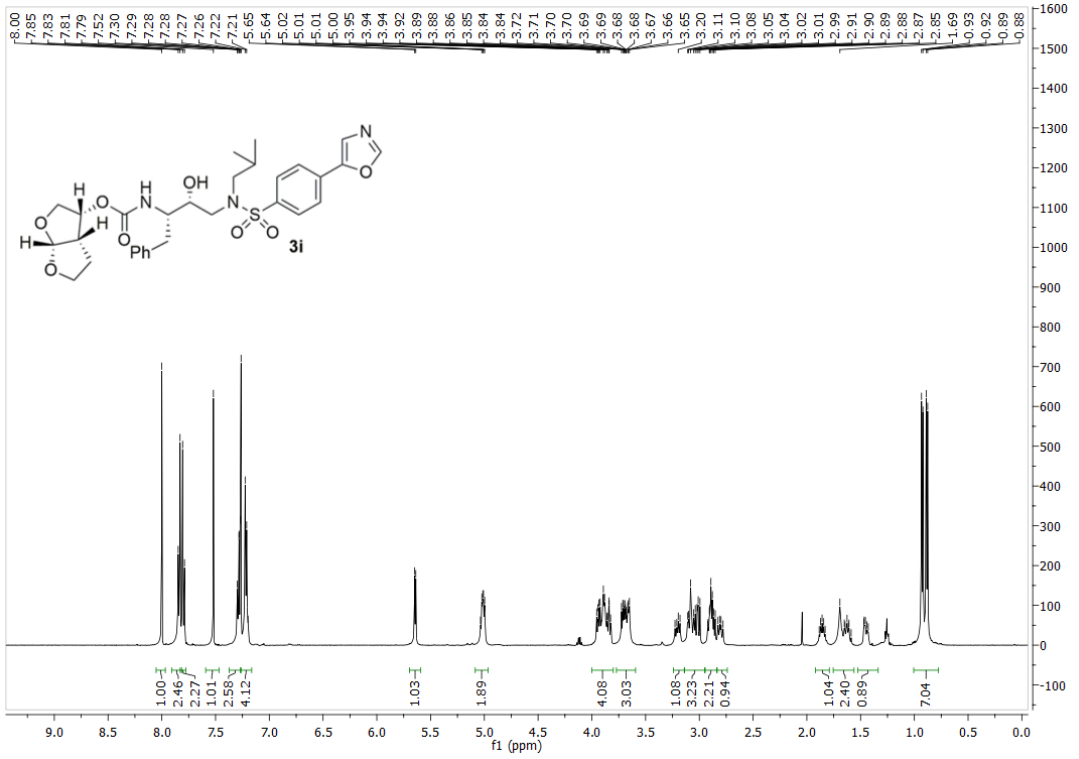
Compound **3h**: ^{1}H-NMR (500 MHz, $CDCl_3$)



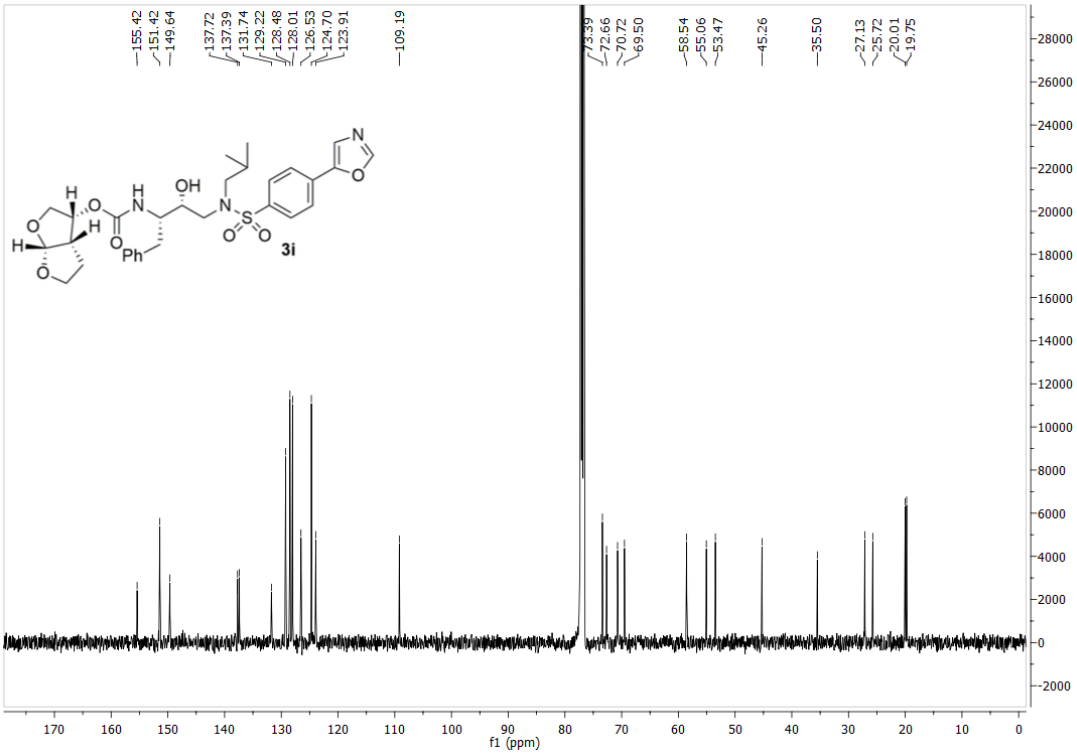
Compound **3h**: ^{13}C-NMR (125 MHz, $CDCl_3$)



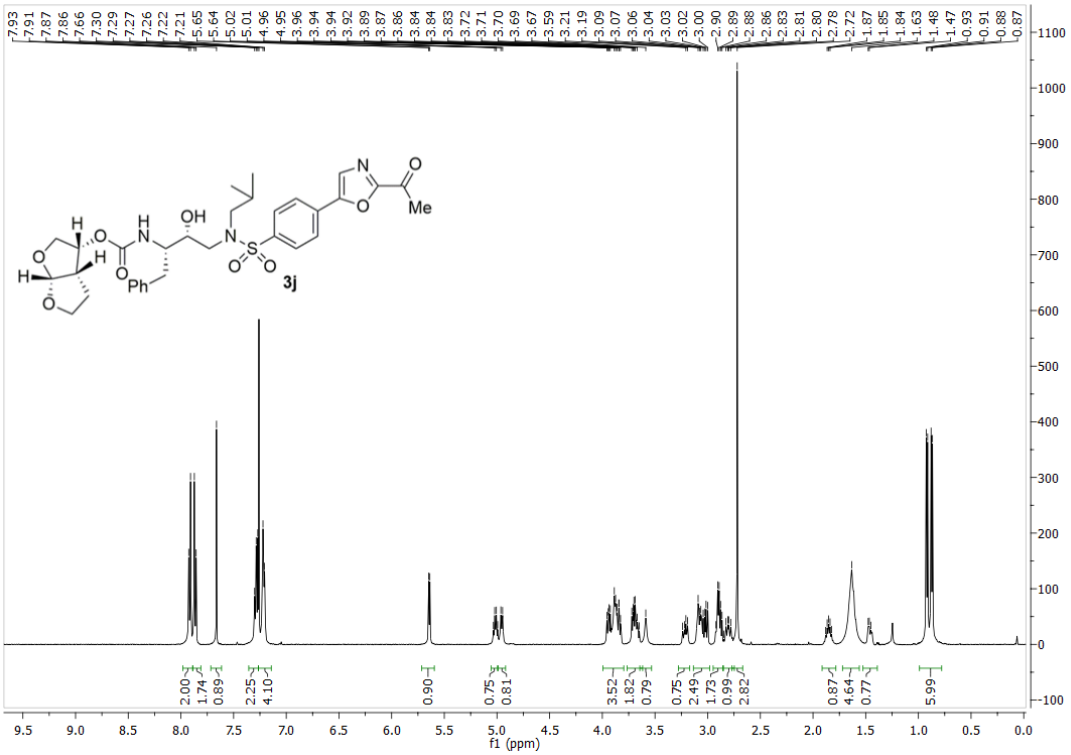
Compound **3i**: ^{1}H-NMR (500 MHz, $CDCl_3$)



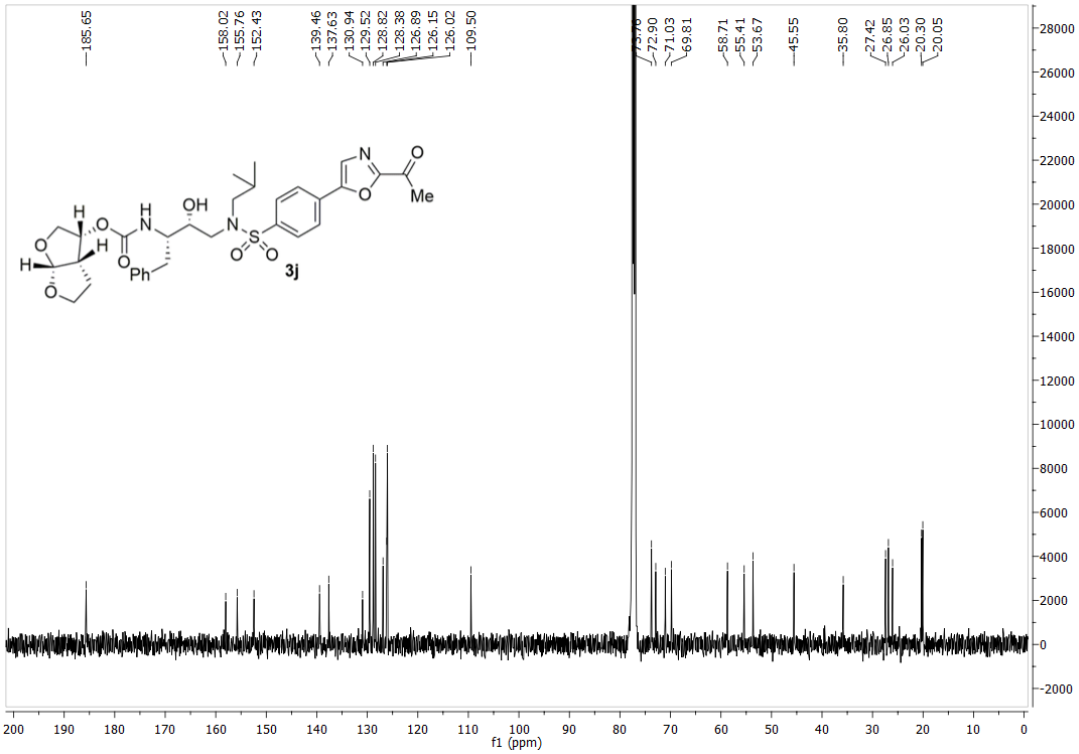
Compound **3i**: ^{13}C-NMR (125 MHz, $CDCl_3$)



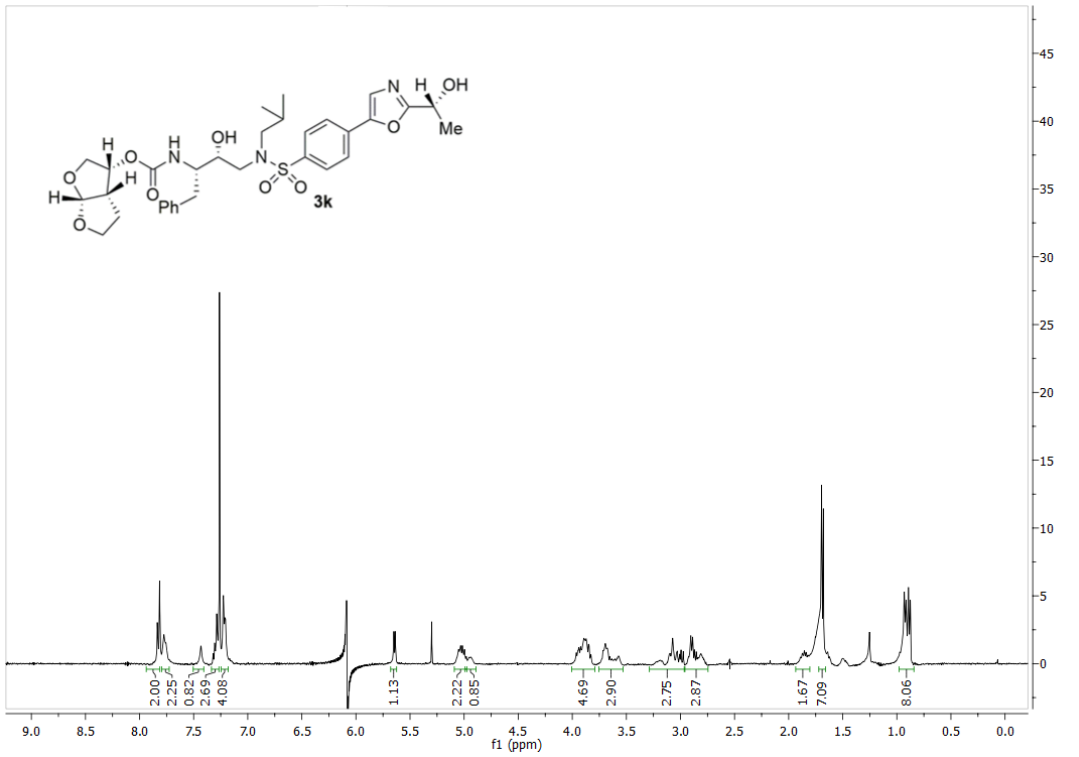
Compound **3j**: ^{1}H-NMR (500 MHz, $CDCl_3$)



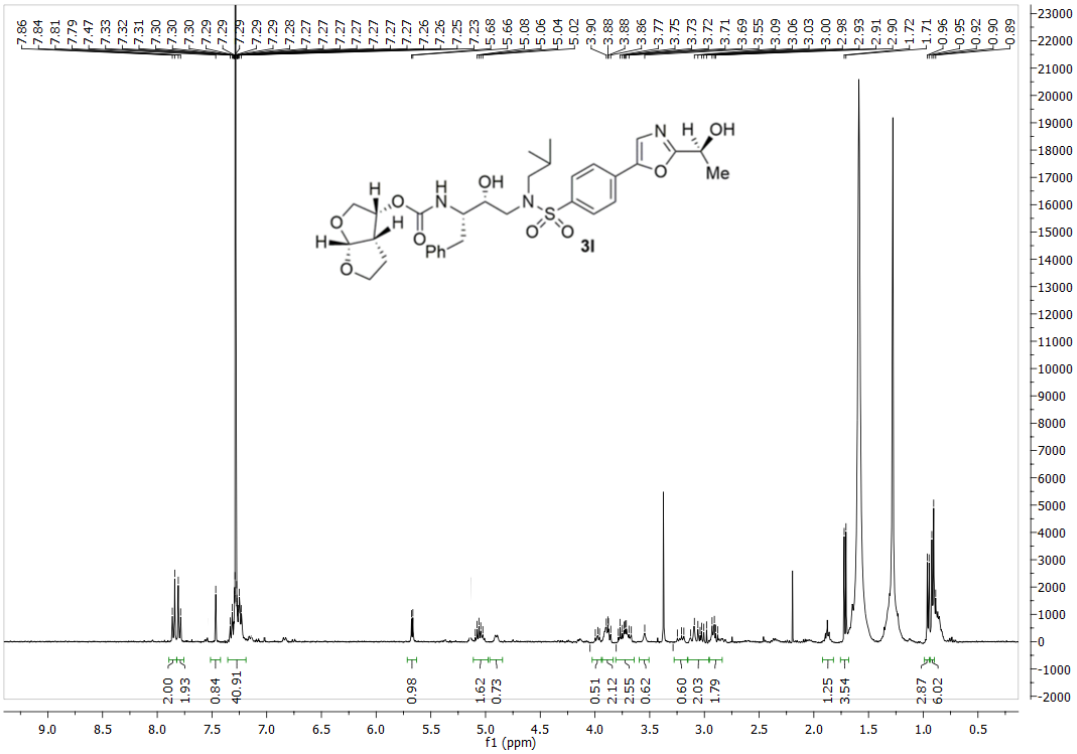
Compound **3j**: ^{13}C-NMR (125 MHz, $CDCl_3$)



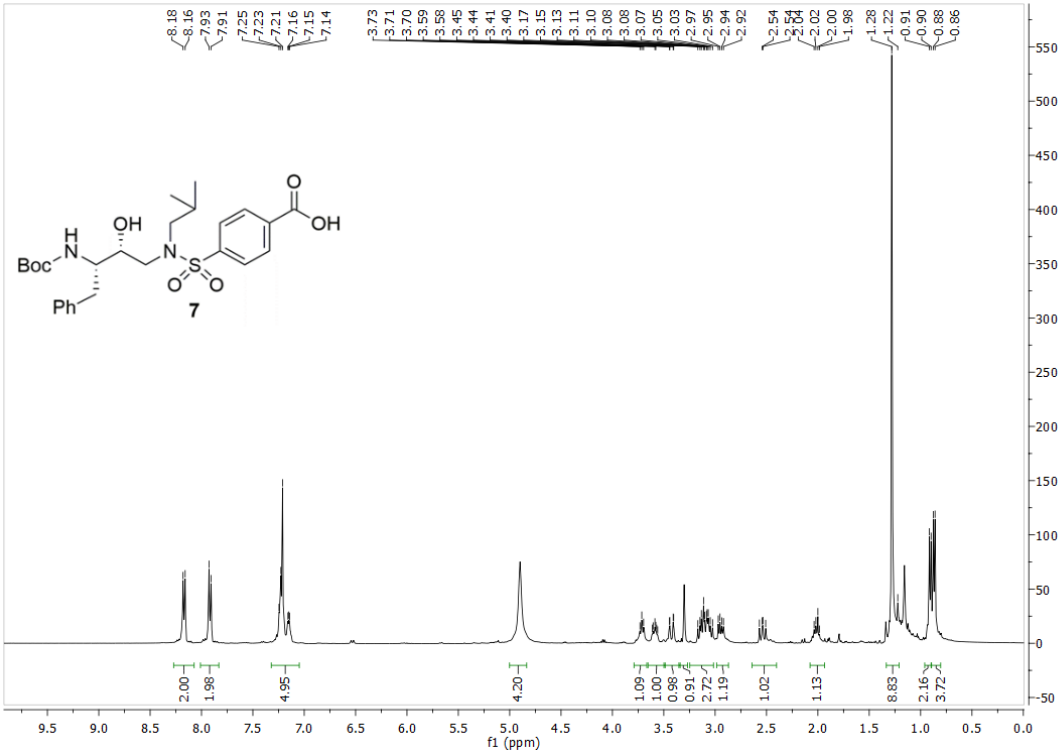
Compound **3k**: ^{1}H-NMR (500 MHz, $CDCl_3$)



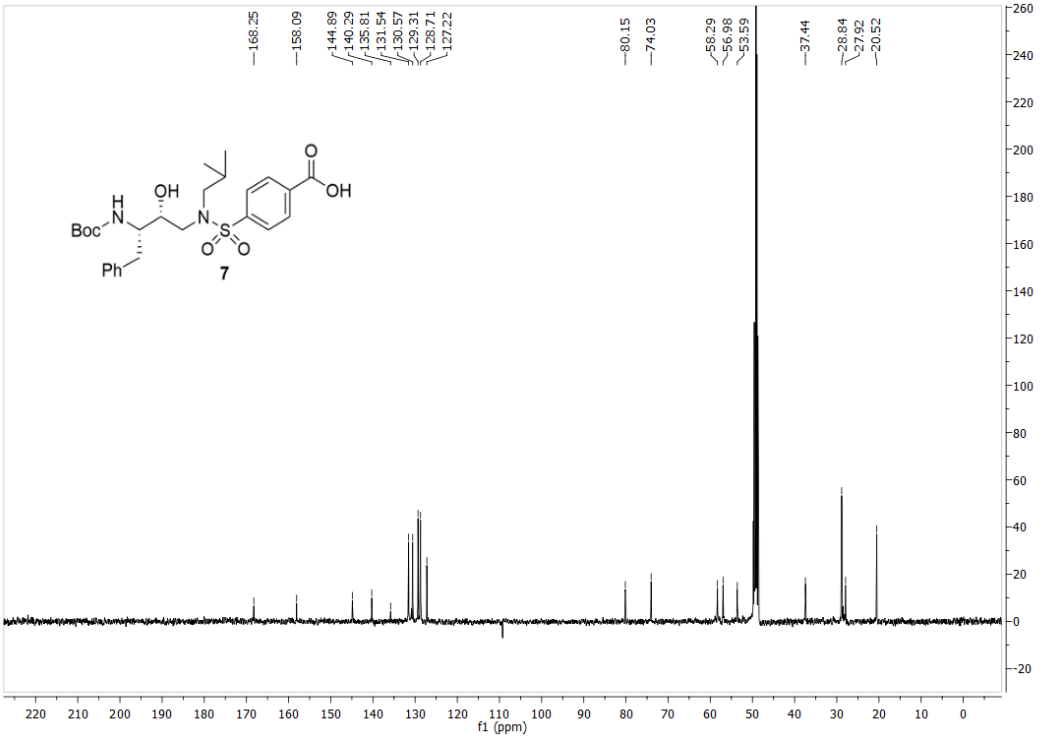
Compound **3l**: ^{1}H-NMR (400 MHz, $CDCl_3$)



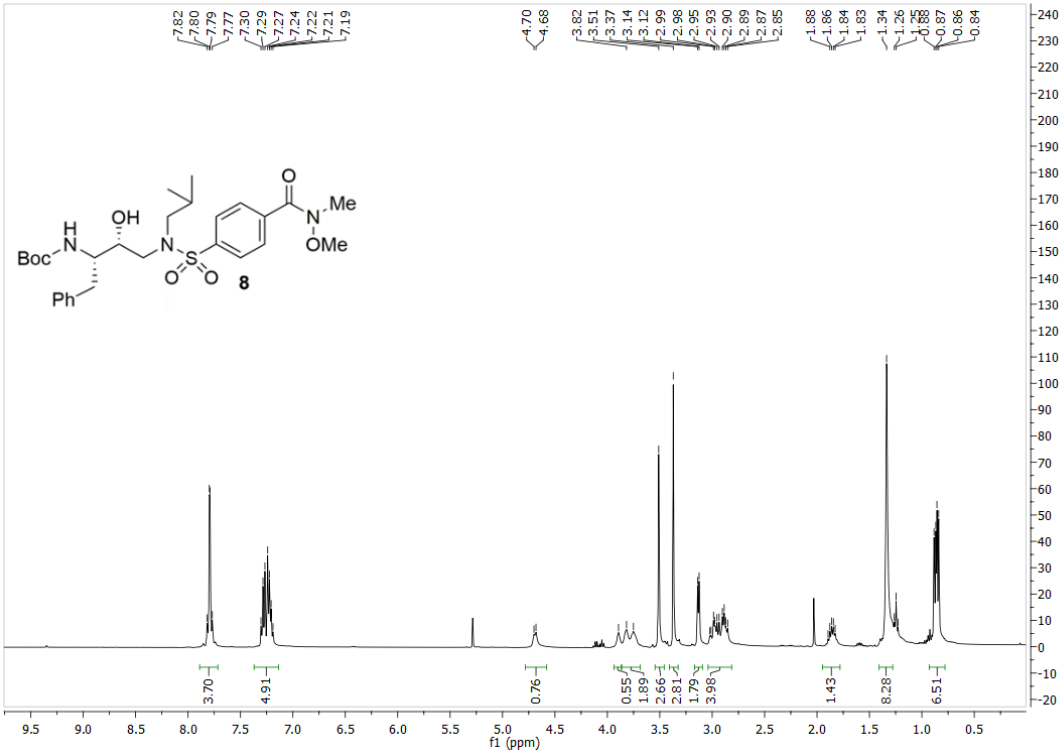
Compound **7**: ^{1}H-NMR (400 MHz, CD_3OD)



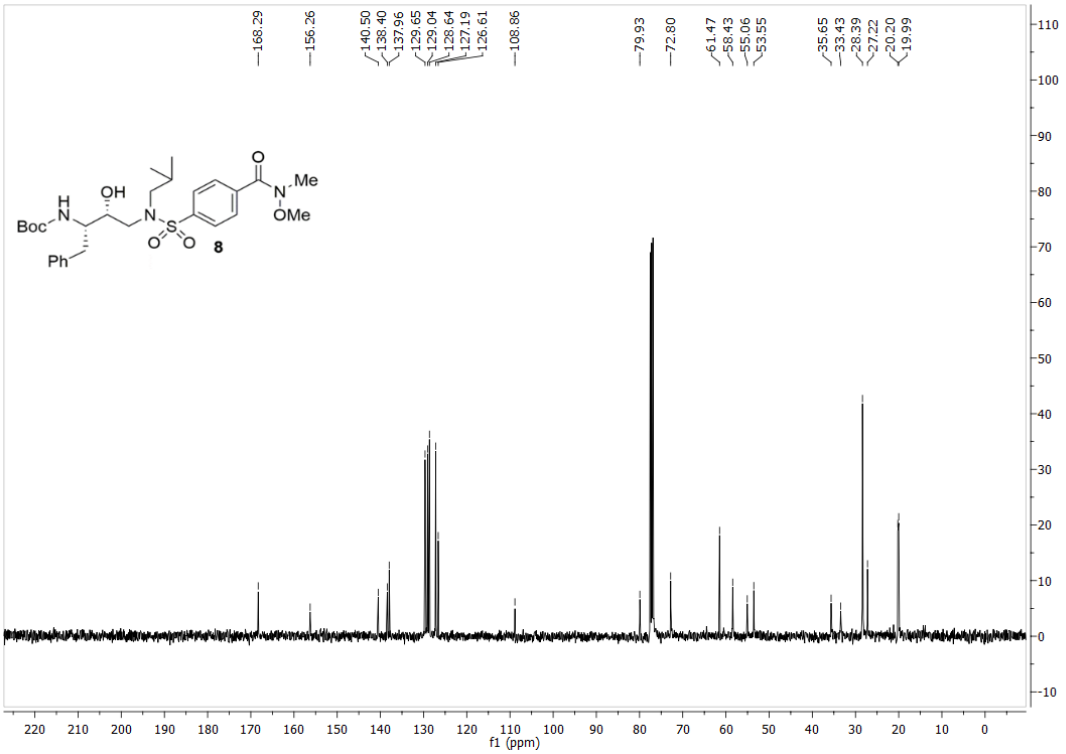
Compound **7**: ^{13}C-NMR (100 MHz, DMSO-d_6)



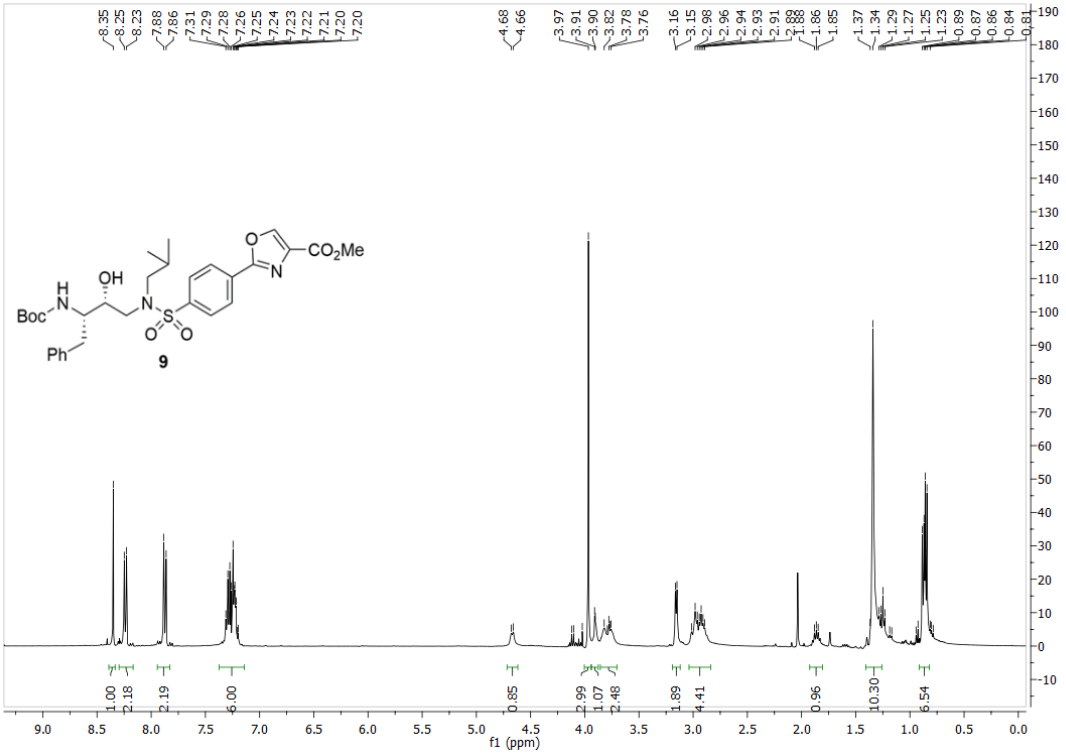
Compound **8**: ^{1}H-NMR (400 MHz, $CDCl_3$)



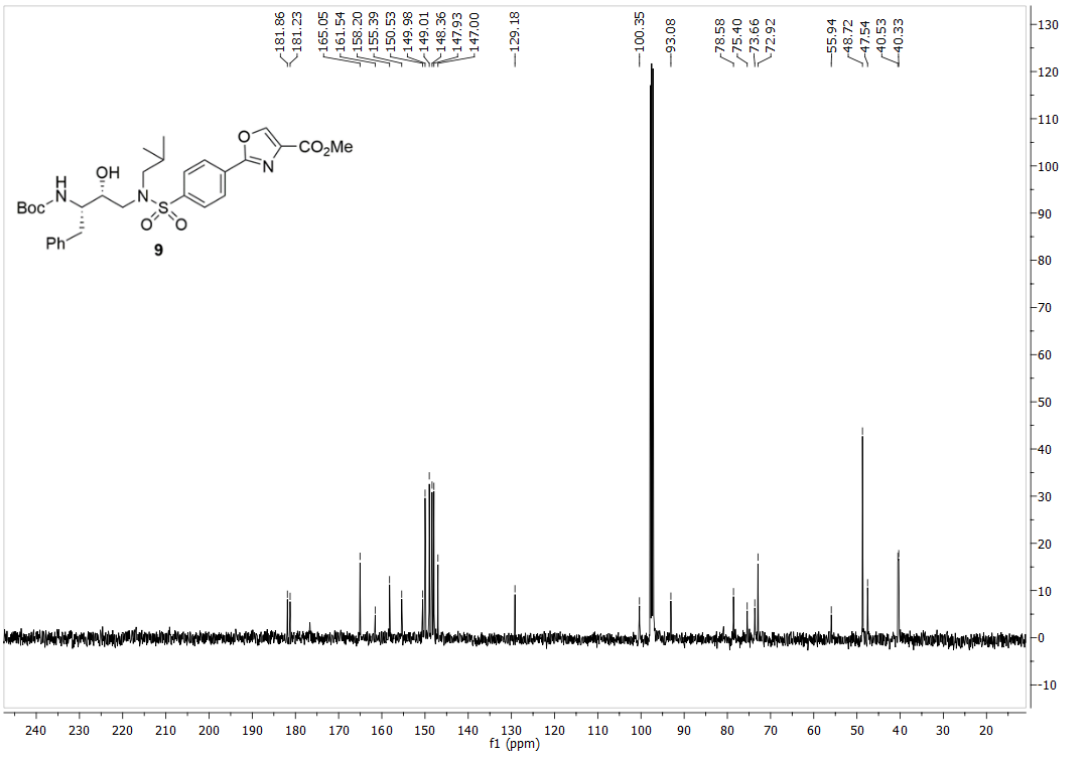
Compound **8**: ^{13}C-NMR (100 MHz, $CDCl_3$)



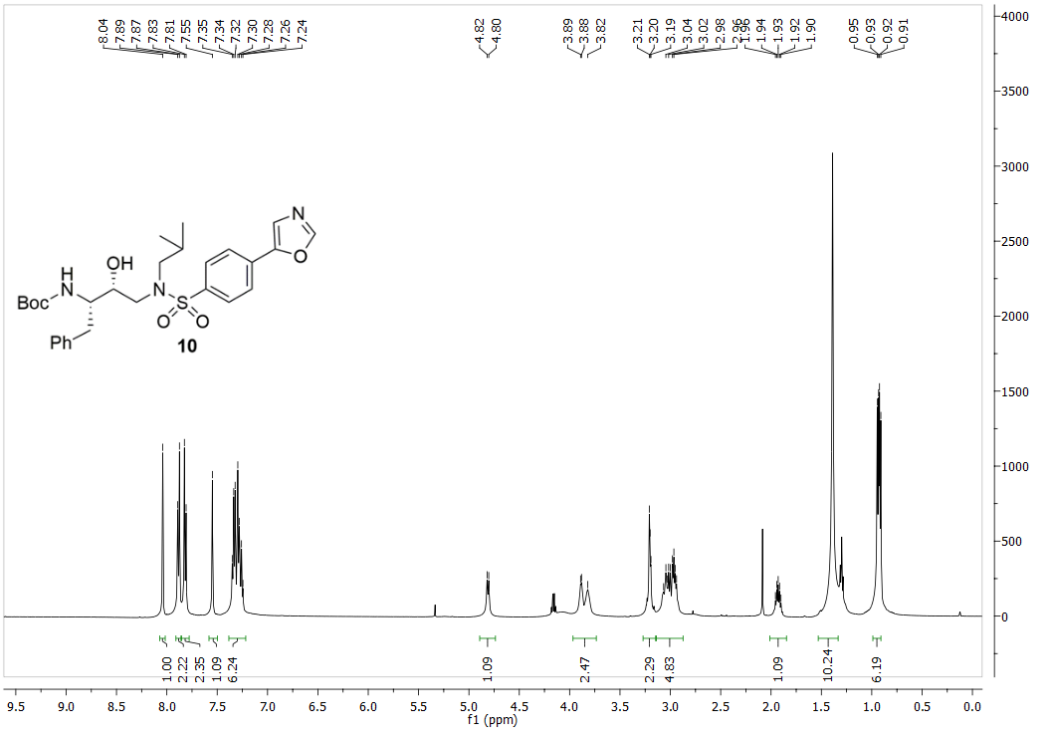
Compound **9**: ^{1}H-NMR (400 MHz, $CDCl_3$)



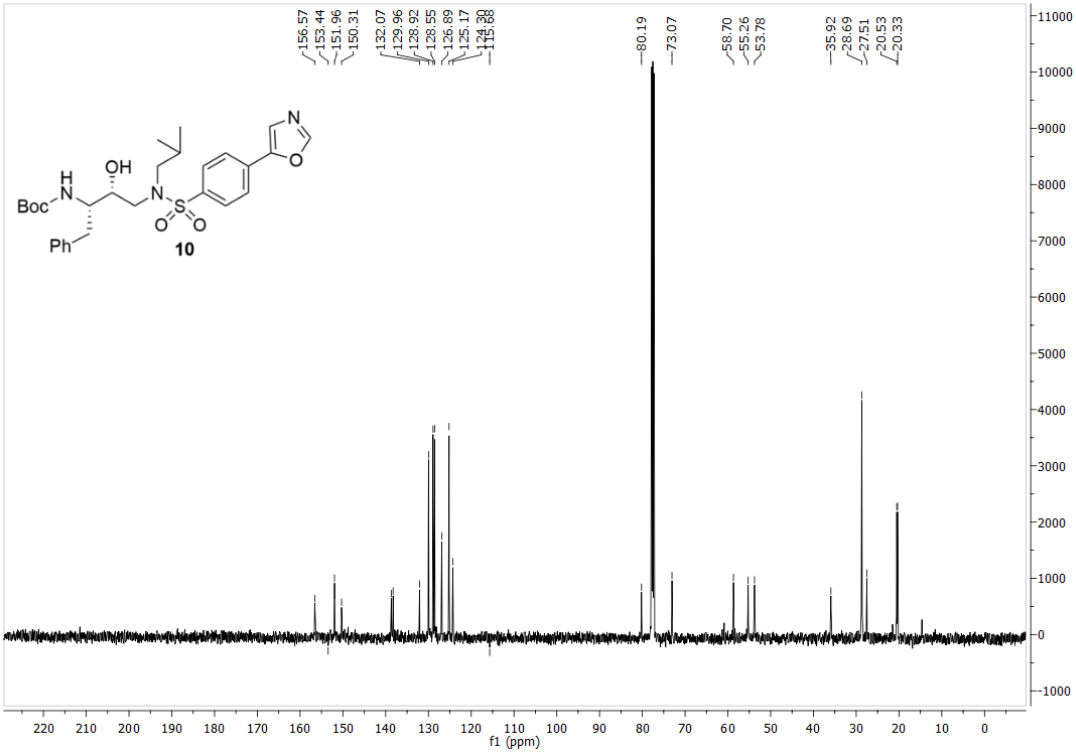
Compound **9**: ^{13}C-NMR (100 MHz, $CDCl_3$)



Compound **10**: ^{1}H-NMR (500 MHz, $CDCl_3$)



Compound **10**: ^{13}C-NMR (125 MHz, $CDCl_3$)



Compound **11**: ^{1}H-NMR (500 MHz, $CDCl_3$)

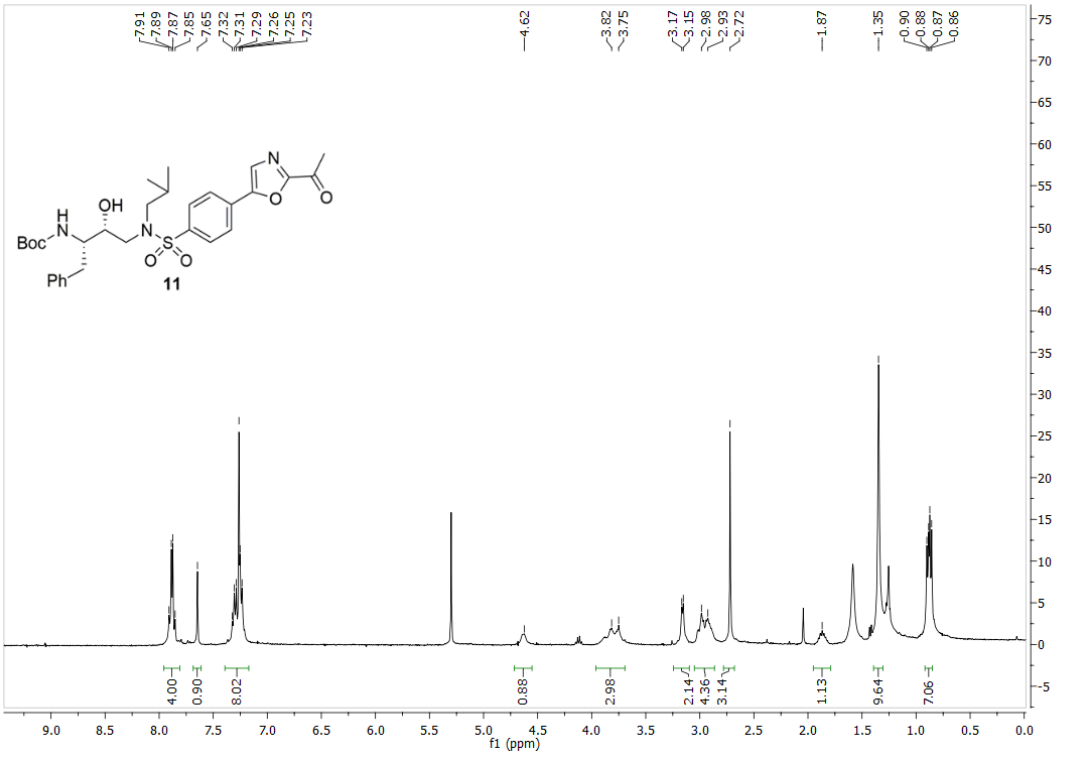


Table 1: Crystallographic Data Collection and Refinement Statistics

	WT-053-11A
Space group	$P2_12_12$
Unit cell dimensions: (Å)	
A	57.99
B	86.40
C	45.76
Resolution range (Å)	50-1.30
Unique reflections	53863
R_{merge} (%) overall (final shell)	7.8 (39.3)
I/σ(I) overall (final shell)	19.7 (2.1)
Completeness (%) overall (final shell)	94.2 (63.0)
Redundancy overall (final shell)	5.6 (2.1)
Refinement	
R (%)	15.4
R_{free} (%)	19.6
No. of solvent atoms (total occupancies)	234 (179.95)
RMS deviation from ideality	
Bonds (Å)	0.012
Angle distance (Å)	0.031
Wilson Plot B factor	11.3
Average B-factors ($Å^2$)	
Main-chain atoms	12.6
Side-chain atoms	18.5
Whole chain atoms	15.5
Inhibitor	15.5
Solvent	26.5

Cells, viruses, and antiviral agents. Human $CD4^+$ MT-2 cells were grown in RPMI-1640-based culture medium supplemented with 10% fetal calf serum (FCS: JRH Biosciences, Lenexa, MD), 50 unit/mL penicillin, and 100 μg/mL of kanamycin. The following HIV-1 viruses were employed for the drug susceptibility assay (see below): a laboratory HIV-1strain ($HIV\text{-}1_{LAI}$), a clinical HIV-1 strain isolated from drug-naive patients with AIDS ($HIV\text{-}1_{ERS104pre}$) (1), and six HIV-1 clinical isolates which were originally isolated from patients with AIDS, who had received 9 to 11 anti-HIV-1 drugs over the past 32 to 83 months, and were genotypically and phenotypically characterized as multi-PI-resistant HIV-1 variants (1, 2). All such primary HIV-1 strains were passaged once or twice in 3-day old phytohemagglutinin-activated peripheral blood mononuclear cells (PHA-PBM), and the culture supernatants were stored at –80 °C until use. Amprenavir (APV) was received as a gift from Glaxo-Wellcome, Research Triangle Park, NC. Darunavir (DRV) was synthesized as previously described (3).

1. Yoshimura, K., et al. *Proc. Natl. Acad. Sci.* USA **96**, 8675-8680 (1999).
2. Koh, Y., et al. *Antimicrob. Agents Chemother.* **53**, 987-996 (2009).
3. Koh Y, et al *J Mol Biol* **282**, 28709-28720 (2007)