Supporting Information

Sphingosine Kinase 2 Inhibitors: Rigid Aliphatic Tail Derivatives Deliver Potent and Selective Analogs

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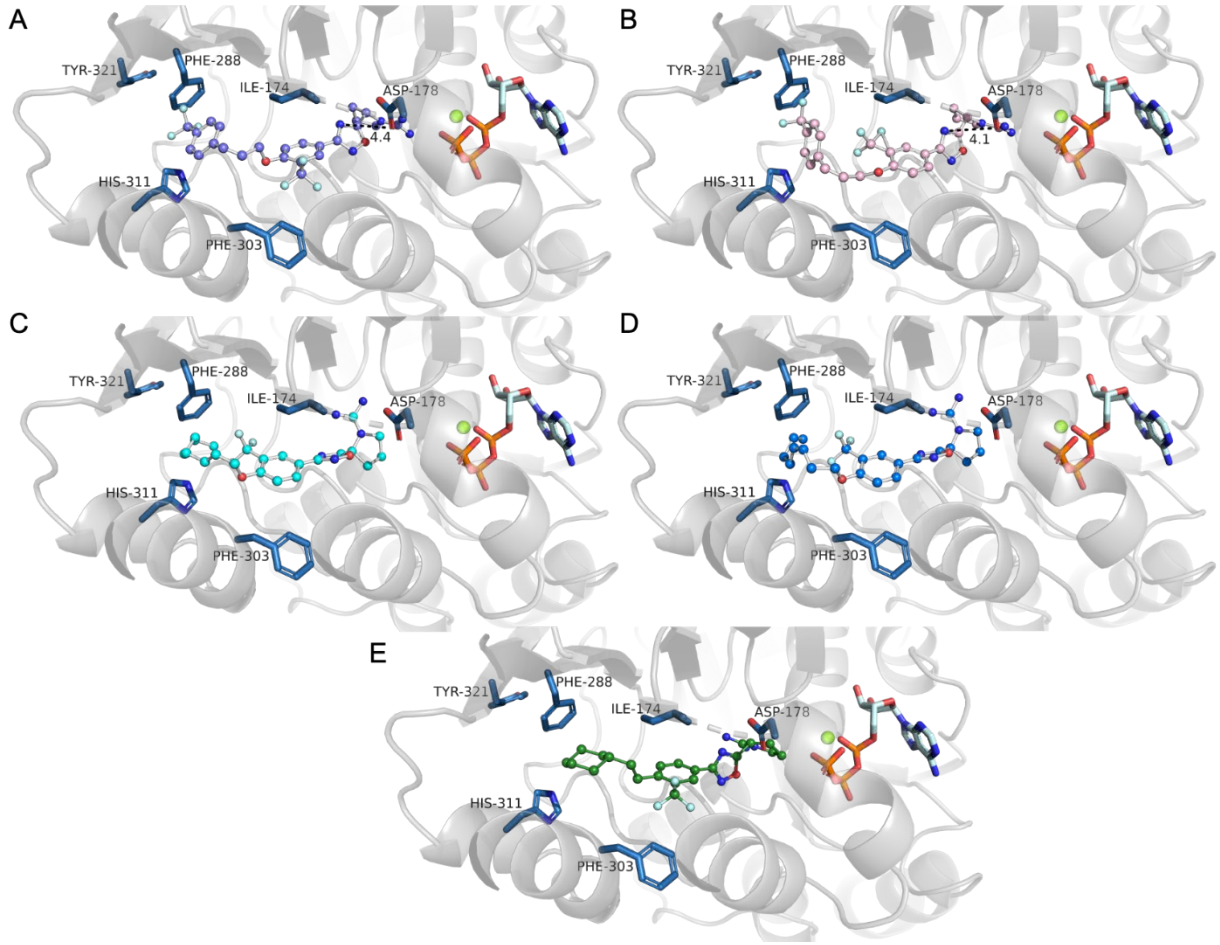
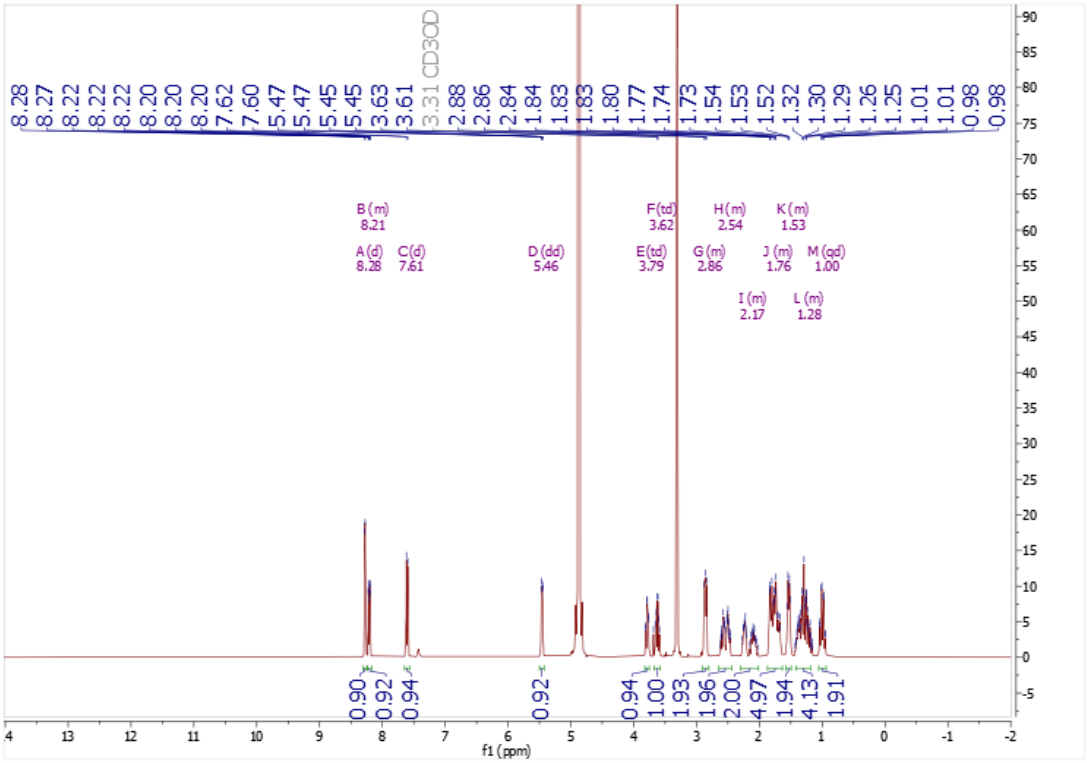
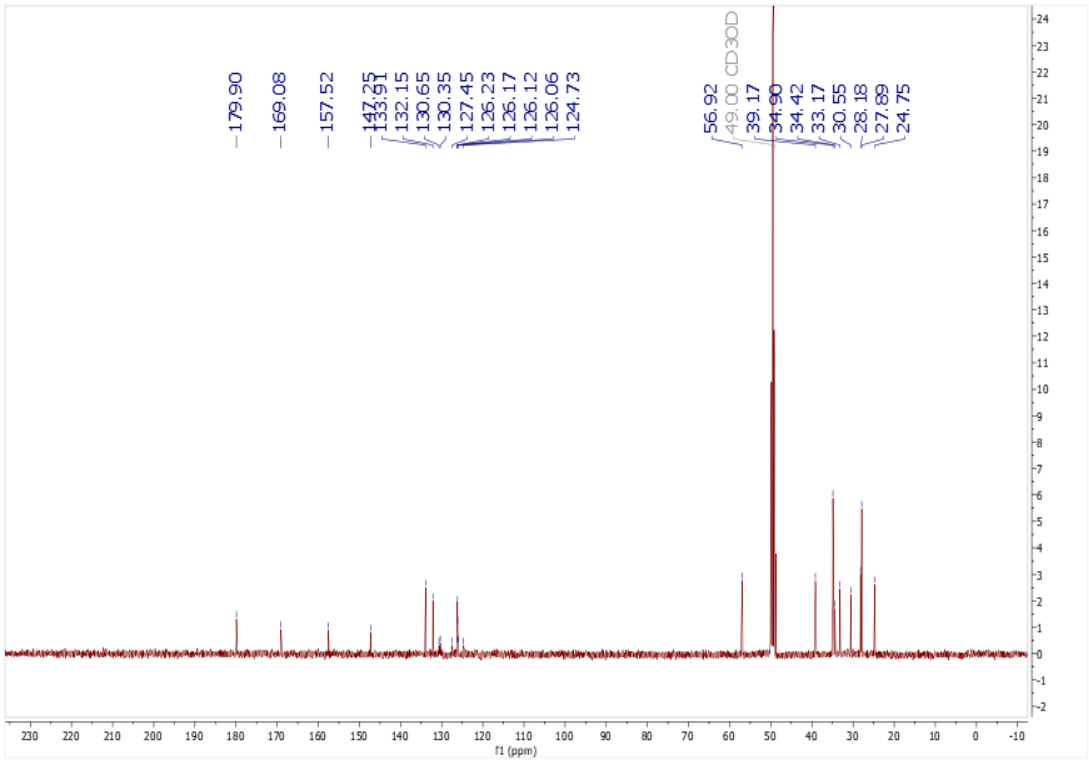


Figure S1. Molecular docking of top compounds in hSphK1 (PDB ID: 3VZB). Molecular docking of **23d** (blue, A) and **23e** (pink, B) in hSphK1 position the ligand towards the top of the binding cavity near ATP but are out of range for hydrogen bonding to the oxadiazole ring as observed in SphK2. Binding cavity steric hindrance and lack of inhibitor flexibility in alkyl tail analogs **26d** (cyan, C) and **26a** (blue, D) do not allow the utilization of the side pocket near Phe303. Compound **14c** (green, E) does not utilize the side pocket near Phe303 and is further distorted to position the guanidine group away from Asp178 due to steric hindrance of the trifluoromethyl group with Phe303. Black lines with measurements are shown for distance comparison to SphK2 results and are outside of the range for a hydrogen bond (A,B).

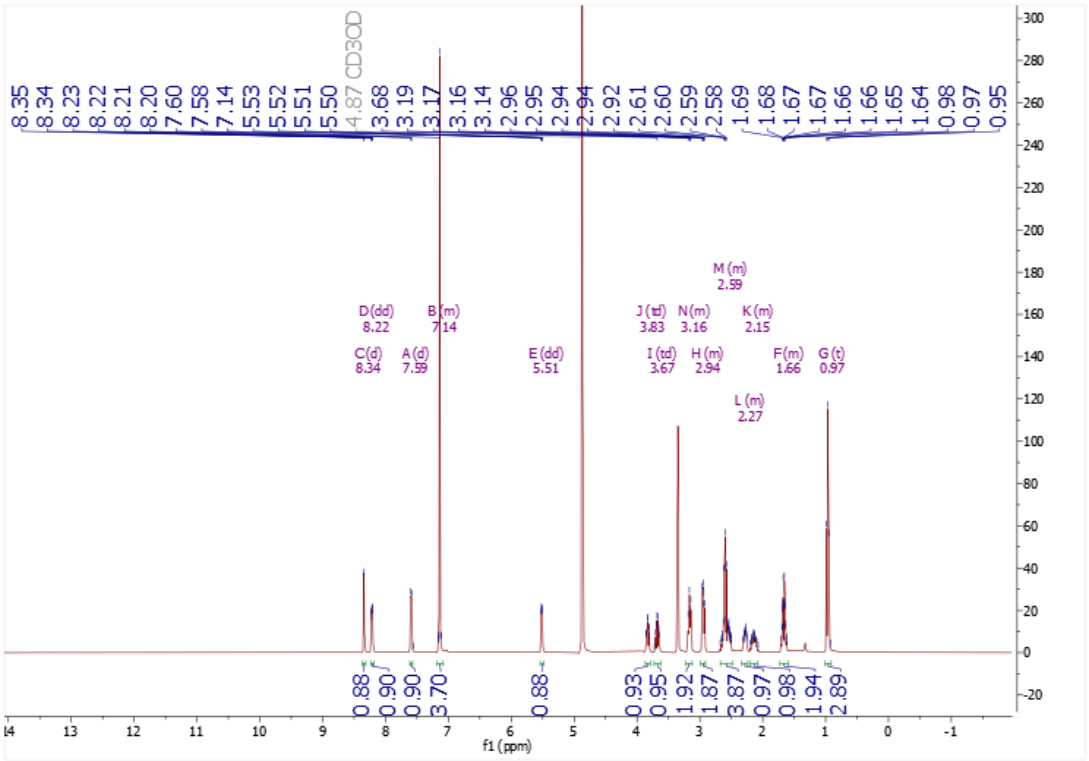
^{1}H NMR (400 MHz, CD_3OD) **14c**



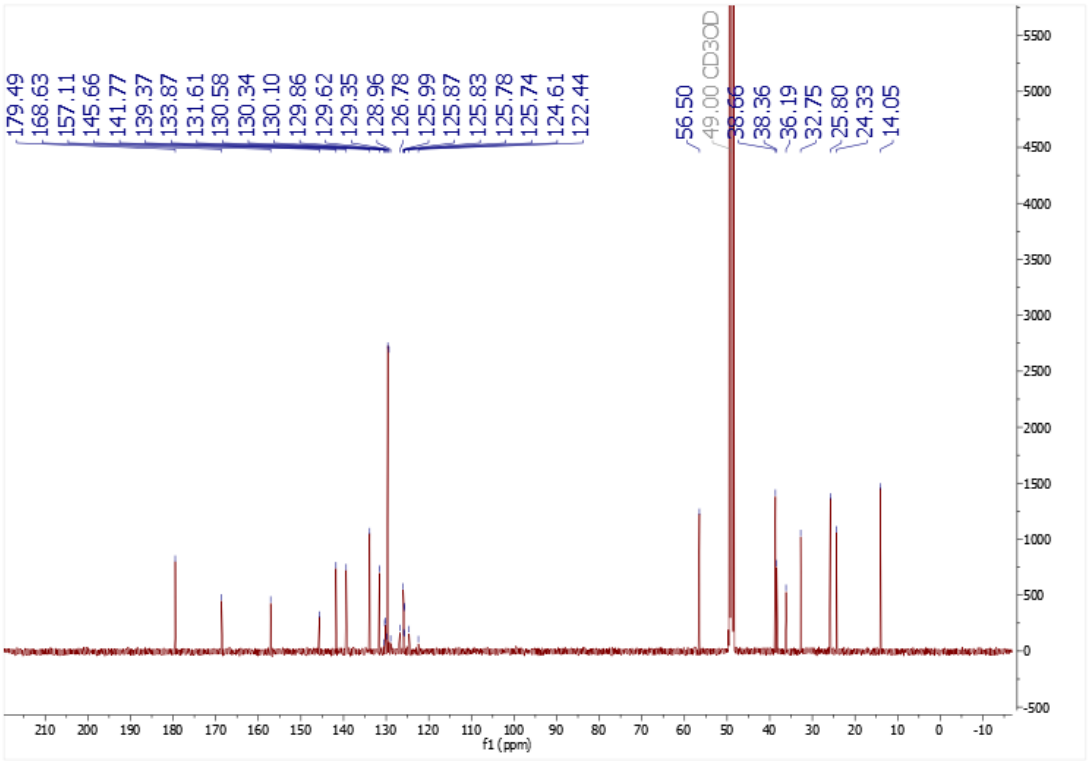
^{13}C NMR (101 MHz, CD_3OD) **14c**



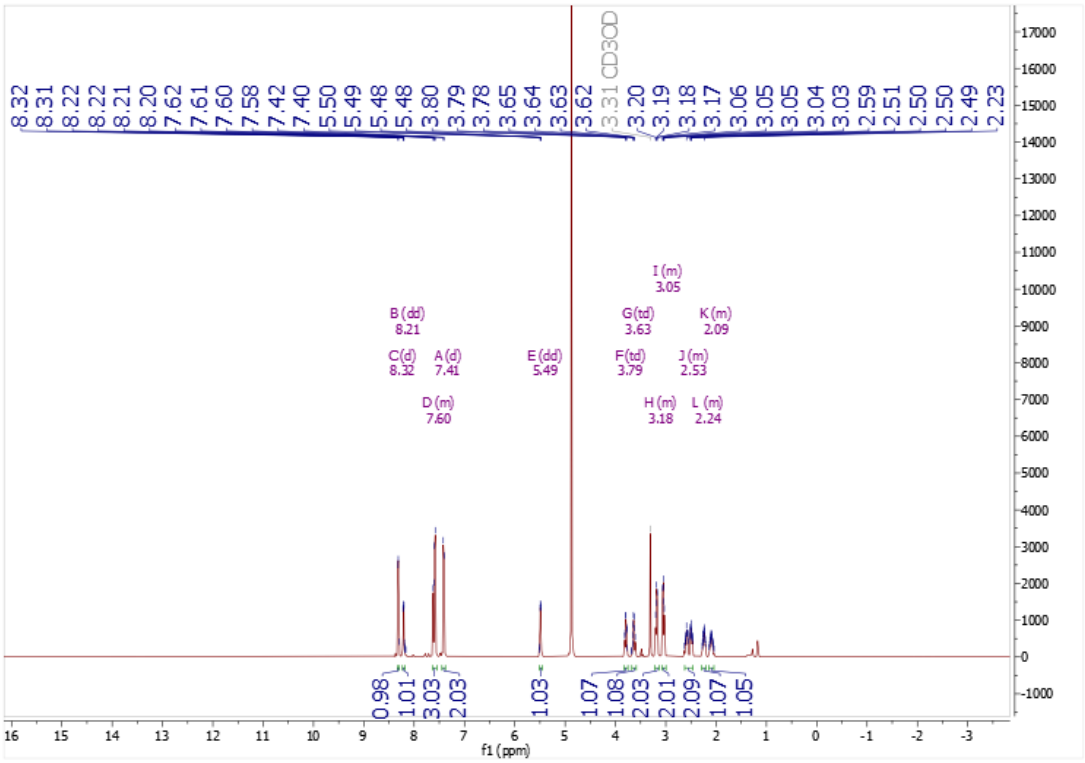
^{1}H NMR (400 MHz, CD_3OD) **14e**



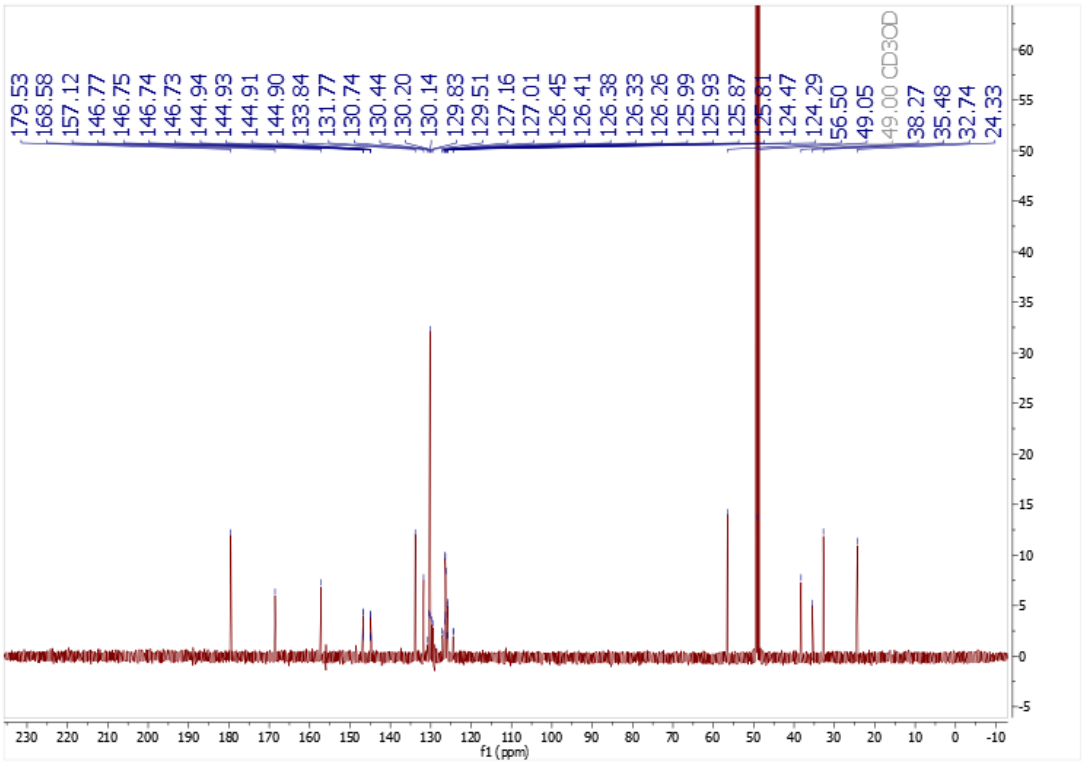
^{13}C NMR (126 MHz, CD_3OD) **14e**



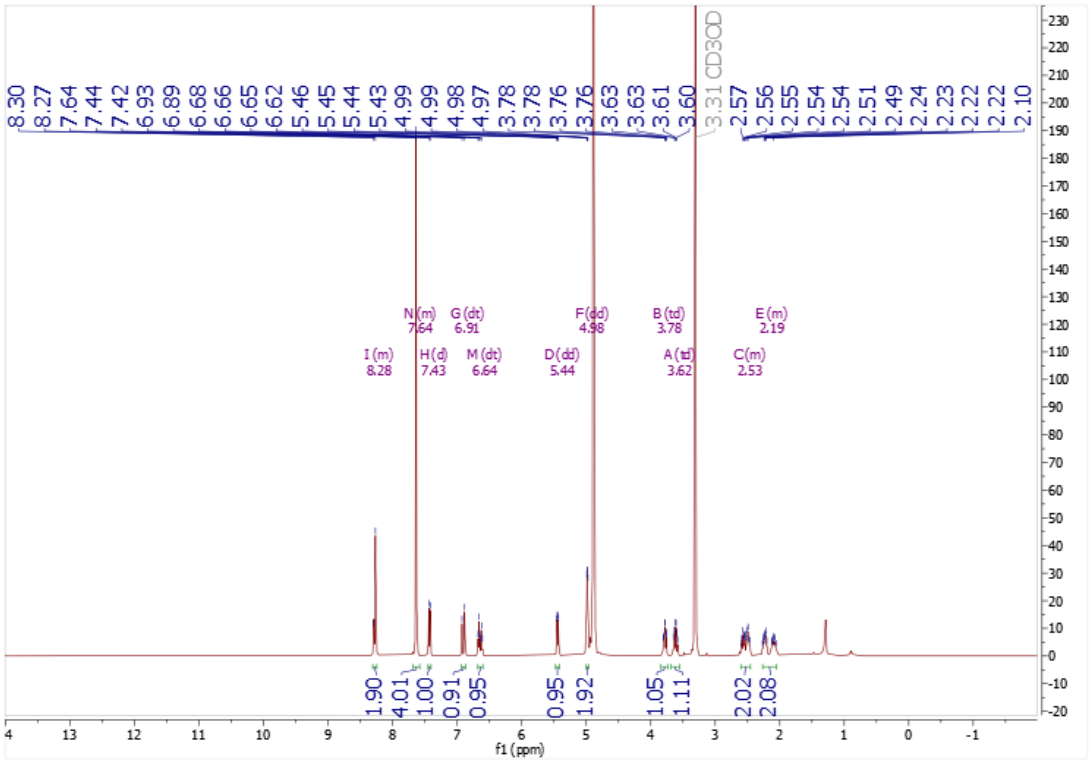
^{1}H NMR (500 MHz, CD_3OD) **14i**



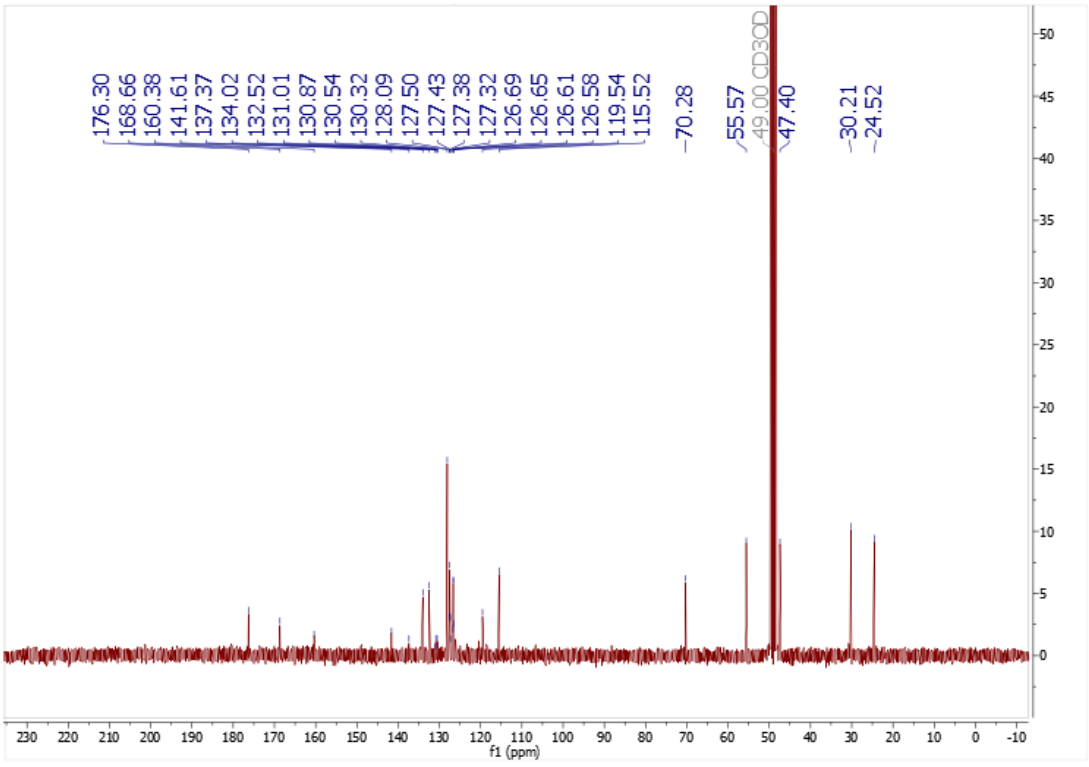
^{13}C NMR (101 MHz, CD_3OD) **14i**



^{1}H NMR (400 MHz, CD_3OD) **23d**

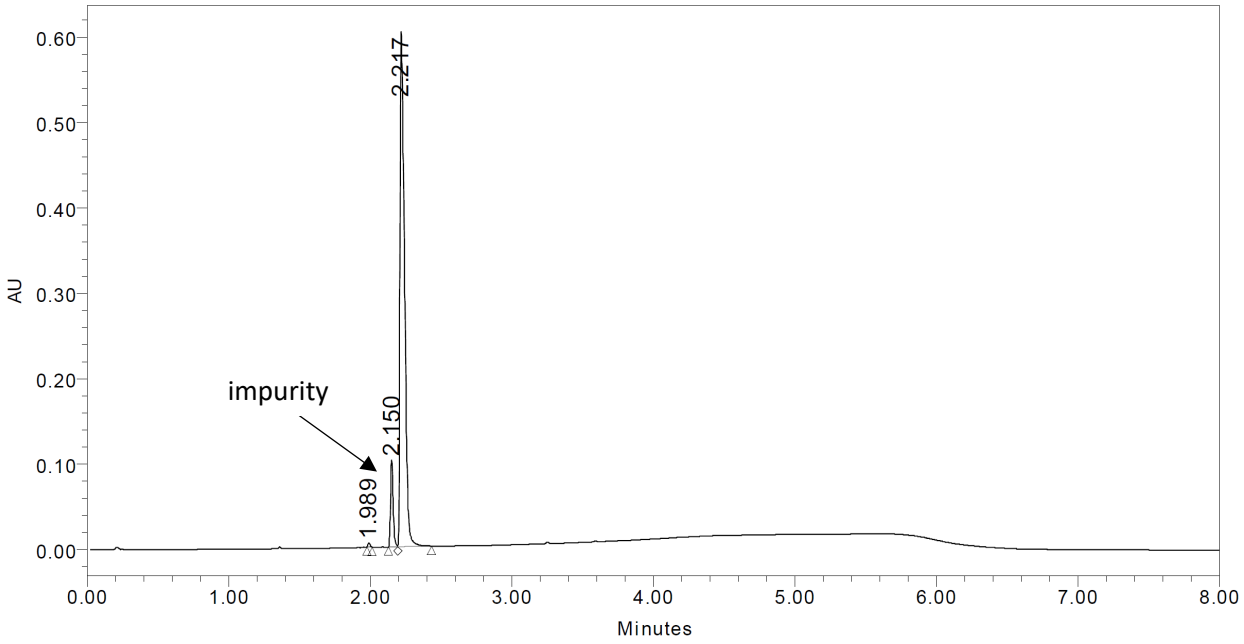


^{13}C NMR (101 MHz, CD_3OD) **23d**



UPLC of **14c**

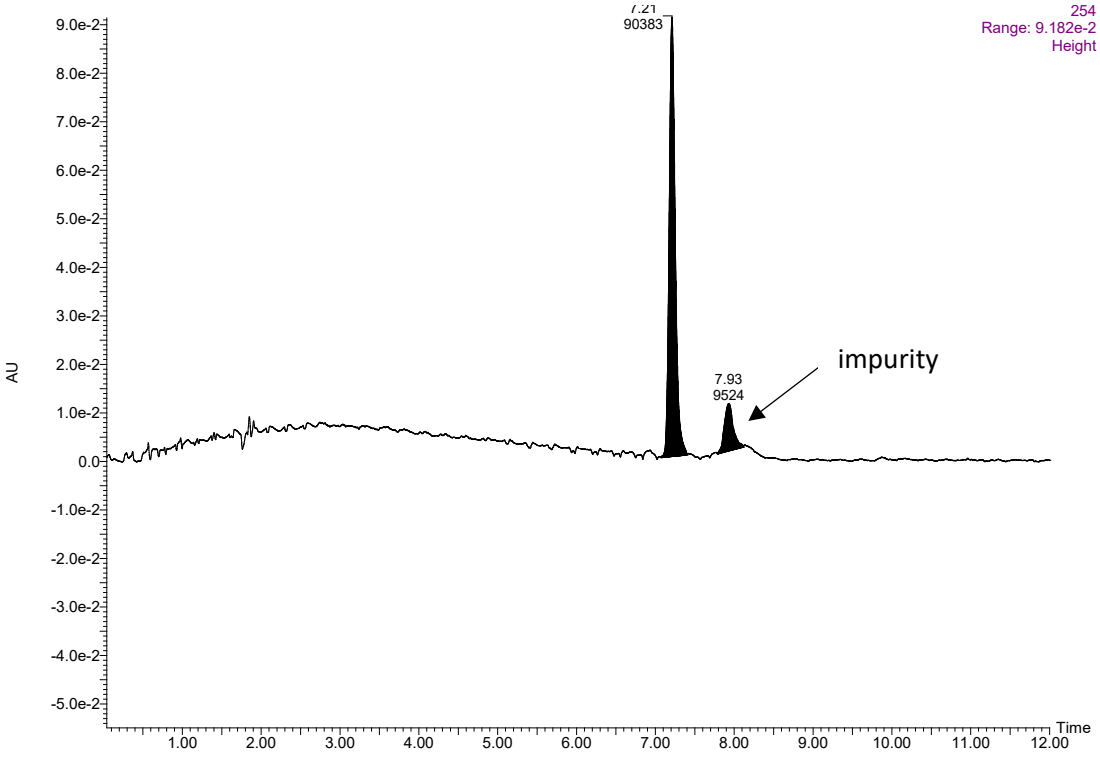
An approximately 6 year old sample of compound **14c** (**SLP9101555**) (stored at -20 C in DMSO) was rerun on Waters Acquity H Class UPLC. Conditions: Solvent A: Water (0.1% TFA); solvent B: acetonitrile (0.1% TFA); column: Acquity BEH C18 1.7 µm 2.1 x 50 mm; method: isocratic 60% A, 40% B from 0-3.50 min then linear gradient from 40-95% B by 5 minutes, return to 40% B by 6 minutes, then hold for 2 minutes at 60% A, 40% B; UV wavelength = 254 nm; flow rate: 0.613 mL/min. These studies indicate 91% purity.



	RT	Area	% Area
1	1.989	4611	0.31
2	2.150	127904	8.67
3	2.217	1343183	91.02

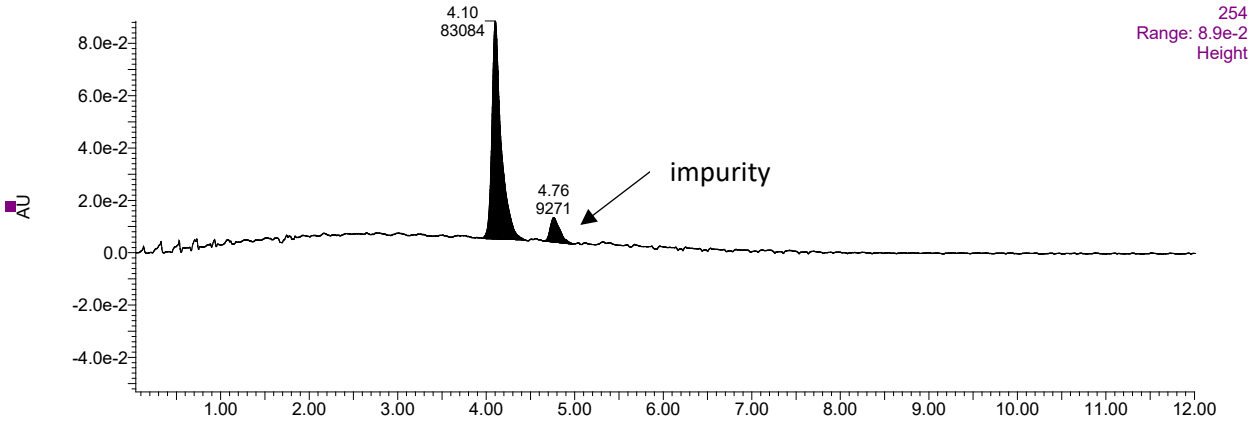
Chiral HPLC analysis: *Daicel IC column*

An approximately 6 year old sample of compound **14c** (**SLP9101555**) (stored at -20 C in DMSO) was run using a super critical fluid chromatography - Waters SFC. The chiral column used was *Daicel IC*, 250 x 4.6 mm, 5 µm. Method: flow rate of 3 ml/min; linear gradient from 80% CO_2, 20% (MeOH+0.5% Isopropylamine) to 60% CO_2, 40% (MeOH+0.5% Isopropylamine) from 0-5 min, then hold for 5 min.



Chiral HPLC analysis: *Daicel IA column*

An approximately 6 year old sample of compound **14c** (**SLP9101555**) (stored at -20 C in DMSO) was run using a super critical fluid chromatography - Waters SFC. The chiral column used was *Daicel IC*, 250 x 4.6 mm, 5 µm. Method: flow rate of 3 ml/min; linear gradient from 80% CO_2, 20% (MeOH+0.5% Isopropylamine) to 60% CO_2, 40% (MeOH+0.5% Isopropylamine) from 0-5 min, then hold for 5 min.



Using the two different chiral columns suggest that compound **14c** is a single enantiomer.