Supporting Information

Stereochemical Course of Cobalamin-dependent Radical SAM Methylation by TokK and ThnK

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General Materials and Methods

DNA modifying enzymes and cloning reagents were purchased from New England Biolabs (Ipswich, MA) or Thermo Fisher Scientific (Waltham, MA). Protein purification and all methylation assays were carried out in a Coy (Grass Lake, MI) anaerobic chamber. All reagents were purchased and used without further purification unless otherwise indicated. Anhydrous solvents were either purchased or dried using an LC Technology Solutions (Salisbury, MA) SPBT-1 solvent purification system. Silica gel chromatography was performed using Sorbtech Silica Gel (60 Å, 40-75mm particle size) or RediSep Rf disposable flash columns (60 Å, 40-63 μm irregular particle size) on a Teledyne ISCO (Lincoln, NE) CombiFlash EZ Prep. Preparative high-performance liquid chromatography (HPLC) was carried out on the same instrument outfitted with a Phenomenex (Torrance, CA) Luna 10μ C18(2) 100 Å column (250 × 21.20 mm ID). All NMR spectra were recorded on a Bruker (Billerica, MA) UltraShield 300 or 400 MHz Avance spectrometer. Ultra-performance liquid chromatography-high resolution mass spectrometry (UPLC-HRMS) analyses were done using a Waters (Milford, MA) Acquity/Xevo-G2 at the Johns Hopkins University Department of Chemistry Mass Spectrometry Facility. Chromatographic separations were carried out on a Waters Acquity BEH UPLC column (ethylene-bridged hybrid C18 stationary phase, 2.1 mm × 350 mm, 1.7 um) with HRMS detection using an electrospray ionization (ESI) ion source in positive mode.

Expression and Purification of ThnK and TokK

The proteins used in this work were expressed and purified as previously described.[1]

Carbapenam Hydrogen Abstraction Assays

Assays with both TokK and ThnK were conducted in triplicate anaerobically in the dark at room temperature and contained 100 mM HEPES pH 7.5, 200 mM KCl, 1 mM SAM, 1 mM methyl viologen, 2 mM NADPH, 1 mM substrate [**1**, **21a** (98.0% D), or **21b** (98.7% D)], and 100 μM enzyme. After 90 min, a 20 μL aliquot was diluted with 80 μL water containing 125 uM phenylalanine (internal standard, 100 uM final concentration) and filtered through a 10 kDa MWCO Amicon ultrafiltration device. The filtrate was analyzed for product formation by UPLC-HRMS. Mobile phase (contained 0.1% formic acid, flow rate 0.3 mL/min): 0-1 min 100% water, 1-7.5 min gradient from 0-80% ACN, 7.5-8.4 min isocratic 80% ACN, 8.4-10 min 100% water.

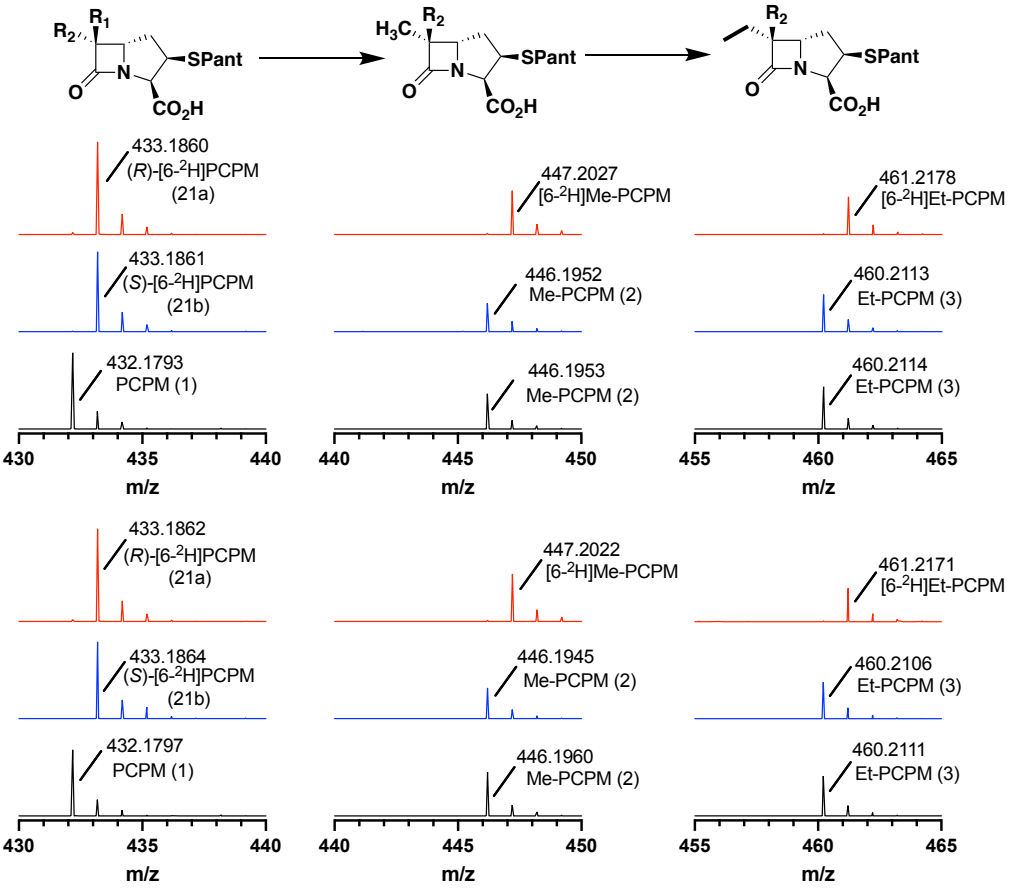
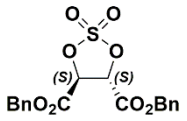
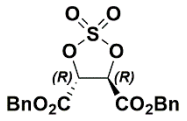


Figure S1. ESI mass spectra of substrate and reaction products of **A** TokK and **B** ThnK. Red traces are from reactions with (R)-[6-^{2}H]PCPM (**21a**), blue traces are from reactions with (S)-[6-^{2}H]PCPM (**21b**), black traces are from reactions with unlabeled PCPM (**1**). Calculated masses are: PCPM, 432.1799; [6-$^{2}H_{1}$]PCPM, 433.1862; Me-PCPM, 446.1955; [6-$^{2}H_{1}$]Me-PCPM, 447.2018; Et-PCPM, 460.2112, [6-$^{2}H_{1}$]Et-PCPM, 461.2175.

Synthetic Procedures

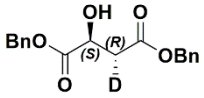


8a



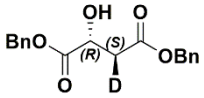
8b

Dibenzyl (4*S*,5*S*)-1,3,2-dioxathiolane-4,5-dicarboxylate 2,2-dioxide (8a) and Dibenzyl (4*R*,5*R*)-1,3,2-dioxathiolane-4,5-dicarboxylate 2,2-dioxide (8b): Prepared according to an established procedure. Spectral data were consistent with those reported.[2]



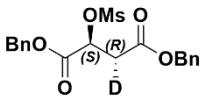
9a

Dibenzyl (2*S*,3*R*)-2-hydroxysuccinate-3-*d* (9a): Cyclic sulfate **8a** (6.43 g, 16.4 mmol) was placed under Argon and dissolved in 35 mL of anhydrous dimethyl acetamide. Sodium borodeuteride (686 mg, 16.4 mmol, 99% D) was added in a single portion, and the reaction mixture was stirred at room temperature for 1 h. Diethyl ether (175 mL) was added, followed by the slow addition of 20% aqueous sulfuric acid (175 mL) and ammonium sulfate (10.8 g, 82 mmol). The bi-phasic mixture was then stirred vigorously for 24 h. The phases were then separated, and the aqueous phase was extracted with diethyl ether (3 × 75 mL). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (8/2 → 7/3 hexanes/ethyl acetate) yielded **9a** (4.28 g, 83%) as a clear oil. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 7.37-7.27 (m, 10H), 5.17 (s, 2H), 5.09 (s, 2H), 4.52 (d, *J* = 6.1 Hz, 1H), 2.82 (d, *J* = 6.1 Hz, 1H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 173.4, 170.4, 135.6, 135.1, 128.9, 128.8, 128.8, 128.6, 128.6, 128.6, 68.0, 67.5, 67.0, 38.6 (t, *J* = 19.9Hz); **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{18}H_{17}DO_5$ 338.1109, found 338.1109.



9b

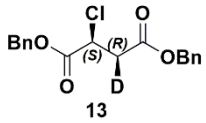
Dibenzyl (2*R*,3*S*)-2-hydroxysuccinate-3-*d* (9b): An identical procedure was performed as above on cyclic sulfate **8b**, which yielded the product enantiomer **9b** in similar yield. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 7.37-7.27 (m, 10H), 5.17 (s, 2H), 5.09 (s, 2H), 4.52 (d, *J* = 6.1 Hz, 1H), 2.82 (d, *J* = 6.1 Hz, 1H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 173.4, 170.4, 135.6, 135.1, 128.9, 128.8, 128.8, 128.6, 128.6, 128.6, 68.0, 67.5, 67.0, 38.6 (t, *J* = 19.9Hz); **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{18}H_{17}DO_5$ 338.1109, found 338.1112.



10

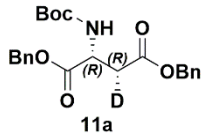
Dibenzyl (2*S*,3*R*)-2-((methylsulfonyl)oxy)succinate-3-*d* (10): Compound **9a** (2.85 g, 9.05 mmol) was placed under Argon and dissolved in 50 mL of anhydrous dichloromethane. The solution was cooled to 0 °C and methanesulfonyl chloride (1.53 mL, 19.9 mmol) was added, followed by the dropwise addition of *N,N*-diisopropylethylamine (3.15 mL, 18.1 mmol). The reaction was stirred at 0 °C for 1 h, then warmed to room temperature and stirred for an additional 30 min. The mixture was washed with 50 mL of water and

50 mL of a 10% aqueous solution of potassium bisulfate. The aqueous phase was extracted with dichloromethane (2 × 50 mL) and the combined organics were then washed with 50 mL of saturated brine, dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (8/2 → 7/3 hexanes/ethyl acetate) yielded **10** (3.38 g, 95%) as a colorless oil. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 7.38-7.29 (m, 10H), 5.40 (d, *J* = 7.9 Hz, 1H), 5.18 (ABq, *J* = 12.1 Hz, 2H), 5.12 (ABq, *J* = 12.1 Hz, 2H), 3.05 (s, 3H), 2.98 (d, *J* = 7.9 Hz, 1H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 168.6, 167.9, 135.3, 134.7, 129.0, 128.9, 128.8, 128.8, 128.7, 128.6, 74.0, 68.3, 67.5, 39.2, 36.9 (t, *J* = 20.0 Hz); **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{19}H_{19}DO_7S$ 416.0885, found 416.0887.



13

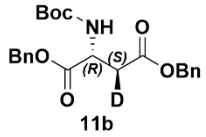
Dibenzyl (2*S*,3*R*)-2-chlorosuccinate-3-*d* (13): Compound **9b** (1.72 g, 5.46 mmol) was dissolved in 30 mL of chloroform and cooled to 0 °C. Pyridine (0.53 mL, 6.55 mmol) was added, followed by the dropwise addition of thionyl chloride (0.48 mL, 6.55 mmol). The reaction mixture was stirred at this temperature for 10 min, warmed to room temperature and then heated to 60 °C and stirred for 4 h until consumption of starting material was observed by thin layer chromatography. After cooling to room temperature, the reaction was diluted with 30 mL of dichloromethane and washed with 30 mL of a saturated aqueous solution of sodium bicarbonate. The aqueous phase was back-extracted with 15 mL of dichloromethane and the combined organics were washed with 30 mL of saturated brine, dried over anhydrous sodium sulfate and concentrated. The crude oil was purified by flash chromatography (9/1 → 8/2 hexanes/ethyl acetate), which yielded **13** (1.72 g, 95%) as a colorless oil. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 7.38-7.29 (m, 10H), 5.17 (ABq, *J* = 12.3 Hz, 2H), 5.11 (s, 2H), 4.68 (d, *J* = 7.5 Hz, 1H), 3.18 (d, *J* = 7.5 Hz, 1H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 169.2, 168.6, 135.4, 135.1, 128.9, 128.8, 128.8, 128.7, 128.6, 128.5, 68.2, 67.2, 51.5, 39.5 (t, *J* = 20.5 Hz); **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{18}H_{16}DClO_4$ 356.0770, found 356.0771.



11a

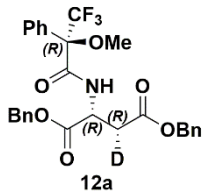
Dibenzyl (R)-(*tert*-butoxycarbonyl)-D-aspartate-3-*d* (11a): Compound **10** (3.38 g, 8.6 mmol) was dissolved in 50 mL of dimethyl sulfoxide, followed by the addition of sodium azide (839 mg, 12.9 mmol). The reaction was stirred at room temperature for 18 h, then diluted with 150 mL of water and 150 mL of diethyl ether and stirred vigorously until the layers resolved. The phases were separated, and the organic phase was washed with water (3 × 50 mL). The combined aqueous phase was then back-extracted with diethyl ether (3 × 50 mL). The combined organics were washed with 50 mL of a saturated brine solution, dried over anhydrous sodium sulfate and concentrated. The crude azide product was then dissolved in 90 mL of 1,4-dioxane and 45 mL of water and cooled to 0 °C. Anhydrous tin(II) chloride (5.88 g, 31.0 mmol) was added and the reaction mixture was stirred at this temperature for 1 h, then warmed to room temperature and stirred for an additional 1 h. The reaction was then cooled back to 0 °C and sodium bicarbonate (17.2 g, 194 mmol) was carefully added in portions followed by di-*tert*-butyl dicarbonate (8.44 g, 38.7 mmol). The solution was slowly warmed to room temperature and stirred for 18 h. Ethyl acetate (100 mL) was added and the reaction mixture was filtered through a pad of silica gel and Celite, which was washed with additional ethyl acetate. The filtrate was then washed with 100 mL of saturated brine, the organic layer was dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (9/1 → 8/2 hexanes/ethyl acetate) yielded **11a** (2.0 g, 55%) as a clear oil that solidified on standing. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 7.36-7.26 (m, 10H), 5.48 (br. d, *J* = 8.4 Hz, 1H), 5.11 (s, 2H), 5.05 (s, 2H) 4.60 (dd, *J* = 8.4, 4.8 Hz, 1H), 2.84 (d, *J* = 4.8 Hz, 1H), 1.41 (s, 9H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 171.1, 171.0, 155.6,

135.5, 135.4, 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 80.3, 67.6, 67.0, 50.2, 36.8 (t, J =20.1 Hz), 28.5; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{23}H_{26}DNO_6$ 437.1792, found 437.1791.



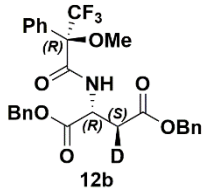
11b

Dibenzyl (*S*)-(tert-butoxycarbonyl)-D-aspartate-3-*d* (11b): An identical procedure to the above was employed using **13** (1.72 g, 5.17 mmol) which yielded **11b** (698 mg, 33%). **^{1}H NMR** (400 MHz; $CHCl_3$): δ 7.36-7.26 (m, 10H), 5.48 (br. d, J = 8.4 Hz, 1H), 5.11 (s, 2H), 5.05 (s, 2H) 4.60 (dd, J = 8.4, 4.8 Hz, 1H), 3.02 (d, J = 4.8 Hz, 1H), 1.41 (s, 9H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 171.1, 170.9, 155.5, 135.6, 135.4, 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 80.3, 67.6, 66.9, 50.2, 36.8 (t, J =20.6 Hz), 28.4; **HRMS** (ESI) *m/z*: $[M+Na]^+$ calculated $C_{23}H_{26}DNO_6$ 437.1792, found 437.1791



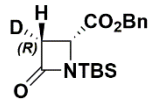
12a

Dibenzyl (R)-((*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)-D-aspartate-3-*d* (12a): Compound **11a** (23 mg, 0.055 mmol) was dissolved in 2 mL of a 5% solution of trifluoroacetic acid in dichloromethane and stirred at room temperature for 2 h. The solution was concentrated and residual trifluoroacetic acid was removed under high vacuum overnight. The crude material was placed under Argon and dissolved in 2 mL anhydrous dichloromethane. A catalytic quantity of 4-(dimethylamino)pyridine (~1 mg) was added, followed by triethylamine (23 µL, 0.166 mmol). The reaction mixture was stirred at room temperature for 10 min, then (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (14.5 µL, 0.078 mmol) was added. After stirring at room temperature for 2 h, the reaction was quenched by the addition of 5 mL of water. The solution was extracted with dichloromethane (3 × 5 mL) and the combined organics were washed with 5 mL of saturated brine. The organic phase was dried over anhydrous sodium sulfate and concentrated. Purification by flash chromatography (9/1 hexanes/ethyl acetate) yielded **12a** (12 mg, 41%) as a colorless oil. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 7.56 (d, J = 8.3 Hz, 1H), 7.47 (d, J = 7.2 Hz, 2H), 7.37-7.25 (m, 11H), 7.24-7.20 (m, 2H), 5.14 (ABq, J = 12.5 Hz, 2H), 4.95 (ABq, J = 12.2 Hz, 2H), 4.92 (dd, J = 8.3 Hz, 4.5 Hz, 1H), 3.39 (d, J = 1.5 Hz, 3H), 2.84, (d, J = 4.5 Hz, 1H); **^{19}F NMR** (282 MHz; $CDCl_3$): δ -68.25; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{28}H_{25}DF_3NO_6$ 531.1848, found 531.1856.



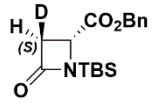
12b

Dibenzyl (*S*)-((*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)-D-aspartate-3-*d* (12b): An identical procedure to the above was employed using **11b** (21 mg, 0.051 mmol) which yielded **12b** (21 mg, 77%)**.** **^{1}H NMR** (400 MHz; $CHCl_3$): δ 7.56 (d, J = 8.3 Hz, 1H), 7.47 (d, J = 7.2 Hz, 2H), 7.37-7.25 (m, 11H), 7.24-7.20 (m, 2H), 5.14 (ABq, J = 12.5 Hz, 2H), 4.95 (ABq, J = 12.2 Hz, 2H), 4.92 (dd, J = 8.3 Hz, 4.5 Hz, 1H), 3.39 (d, J = 1.5 Hz, 3H), 3.06, (d, J = 4.5 Hz, 1H); **^{19}F NMR** (282 MHz; $CDCl_3$): δ -68.25; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{28}H_{25}DF_3NO_6$ 531.1848 , found 531.1854.



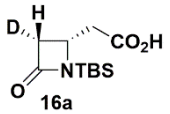
14a

Benzyl (2*R*,3*R*)-1-(*tert*-butyldimethylsilyl)-4-oxoazetidine-2-carboxylate-3-*d* (14a): Compound **11a** (1.034 g, 2.5 mmol) was dissolved in 20 mL of a 10% solution of trifluoroacetic acid in dichloromethane and stirred at room temperature for 2 h. The solution was concentrated, dissolved in 30 mL of dichloromethane and 30 mL of a 1 M aqueous solution of potassium carbonate was added, and the mixture was stirred vigorously for 45 min. The phases were separated, and the aqueous phase was extracted with dichloromethane (3 × 20 mL). The combined organics were dried over anhydrous sodium sulfate and concentrated. The crude amine was placed under Argon and dissolved in 30 mL of anhydrous dichloromethane. *tert*-Butyldimethylsilyl (TBS) trifluoromethanesulfonate (0.63 mL, 2.75 mmol) was added, followed by triethylamine (0.77 mL, 5.5 mmol) and the reaction mixture was stirred at room temperature for 4 h. After cooling to 0 °C, the reaction was quenched by the addition of 50 mL of a saturated aqueous solution of ammonium chloride. The phases were separated and extracted with 30 mL of dichloromethane . The combined organics were washed with 30 mL of a saturated aqueous solution of sodium bicarbonate and 30 mL of saturated brine, dried over anhydrous sodium sulfate and concentrated to afford a red-orange oil. The crude, silyl amine was placed under Argon, dissolved in 35 mL of anhydrous diethyl ether and cooled to 0 °C. *tert*-Butylmagnesium chloride (2.0 mL, 4 mmol, 2.0M in diethyl ether) was added dropwise and the reaction was allowed to warm to room temperature slowly over 16 h. The reaction mixture was then diluted with 35 mL of ethyl actetate and washed with 35 mL of a saturated aqueous solution of ammonium chloride. The aqueous phase was back-extracted with 30 mL of ethyl acetate, the combined organics were washed with 30 mL of saturated brine, dried over anhydrous sodium sulfate and concentrated under vacuum. Purification by flash chromatography (8/2 hexanes/ethyl acetate) yielded **14a** (600 mg, 75%) as a pale, yellow oil. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 7.38-7.30 (m, 5H), 5.17 (s, 2H), 4.04 (d, J = 6.0 Hz, 1H), 3.29 (d, J = 6.0 Hz, 1H), 0.91 (s, 9H), 0.22 (s, 3H), 0.04 (s, 3H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 172.2, 171.0, 135.2, 128.9, 128.9, 67.5, 48.9, 43.9 (t, J = 21.4 Hz), 26.3, 18.7, -5.8, -6.2; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{17}H_{24}DNO_3Si$ 321.1739, found 321.1739.



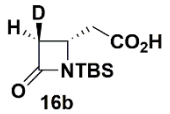
14b

Benzyl (2R,3S)-1-(*tert*-butyldimethylsilyl)-4-oxoazetidine-2-carboxylate-3-d (14b): An identical procedure to the above was used starting from **11b** to prepare **14b** in similar yield. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 7.38-7.30 (m, 5H), 5.16 (s, 2H), 4.04 (d, J = 2.8 Hz, 1H), 3.03 (d, J = 2.8 Hz, 1H), 0.91 (s, 9H), 0.22 (s, 3H), 0.04 (s, 3H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 172.2, 171.0, 135.2, 128.9, 128.8, 67.5, 48.9, 43.9 (t, J = 21.7 Hz), 26.3, 18.7, -5.8, -6.2; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{17}H_{24}DNO_3Si$ 321.1739, found 321.1739



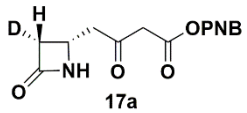
16a

2-((2*R*,3*R*)-1-(*tert*-butyldimethylsilyl)-4-oxoazetidin-2-yl-3-*d*)acetic acid (16a): To **14a** (1.03 g, 3.22 mmol) in 30 mL of ethyl acetate was added 10% palladium on carbon (60 mg). The solution was purged with hydrogen gas and stirred under 1 atm of hydrogen at room temperature for 3 h. The reaction mixture was then filtered through Celite, washed with ethyl acetate and concentrated to yield **15a** (740 mg, quantitative) in sufficient purity to be used directly. A flask containing **15a** (740 mg, 3.22 mmol) was flushed with Argon and dissolved in 32 mL anhydrous tetrahydrofuran. The solution was cooled to 0 °C and triethylamine (0.540 mL, 3.86 mmol) was added, followed by isobutyl chloroformate (0.502 mL, 3.86 mmol). The solution was then stirred at 0 °C under Argon for 30 min. An ethereal solution of diazomethane was freshly prepared from 33.3 mmol of Diazald and added by pipette to the reaction at 0 °C. The reaction was stirred at this temperature for 2.5 h, then warmed to room temperature and stirred for an additional 1 h. Acetic acid was added to quench excess diazomethane, the reaction was diluted with 50 mL of ethyl acetate and washed with 50 mL of a saturated aqueous solution of sodium bicarbonate, 50 mL of a saturated aqeous solution of ammonium chloride and 50 mL of saturated brine. The organic layer was dried over anhydrous sodium sulfate, concentrated under vacuum and then purified by flash chromatography (6/4 hexanes/ethyl acetate) to yield the intermediate diazoketone (600 mg, 2.36 mmol) as a yellow solid. The diazoketone was placed under Argon and dissolved in 48 mL of peroxide-free tetrahydrofuran and 12 mL of water. The solution was irradiated with a 400W HP-Hg lamp through Pyrex for 18 h, after which the starting material was completely consumed and the yellow solution had become colorless. The reaction was quenched by the addition of 50 mL of 0.1 N hydrochloric acid and the product was extracted with ethyl acetate (3 × 50 mL). The combined organics were dried over anhydrous sodium sulfate and purified by flash chromatography (7/3 heptane/ethyl acetate 1% acetic acid) to yield **16a** (380 mg, 48% overall) as a colorless solid. **^{1}H NMR** (400 MHz; $CHCl_3$): δ, 3.87 (ddd, J = 10.0, 5.2, 3.8 Hz, 1H), 3.28 (d, J = 5.2 Hz, 1H), 2.70 (ABX, J_{AB} = 16.0 Hz, J_{AX} = 10.0 Hz, J_{BX} = 3.8 Hz, 2H), 0.94 (s, 9H), 0.23 (s, 3H), 0.21 (s, 3H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 175.1, 173.1, 45.9, 44.7 (t, J = 20.4 Hz), 40.6, 26.4, 18.6, -5.3, -5.6; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{11}H_{20}DNO_3Si$ 245.1426, found 245.1422.



16b

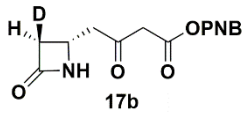
2-((2*S*,3*R*)-1-(*tert*-butyldimethylsilyl)-4-oxoazetidin-2-yl-3-*d*)acetic acid (16b): Compound **14b** (685 mg, 2.14 mmol) was subjected to an identical procedure to prepare the diastereomer **16b** (295 mg, 56% overall) as a colorless solid. **^{1}H NMR** (400 MHz; $CHCl_3$): δ, 3.87 (ddd, J = 10.0, 3.8, 2.7 Hz, 1H), 2.77 (d, J = 2.7 Hz, 1H), 2.69 (ABX, J_{AB} = 16.0 Hz, J_{AX} = 10.0 Hz, J_{BX} = 3.8 Hz, 2H), 0.93 (s, 9H), 0.23 (s, 3H), 0.20 (s, 3H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 175.2, 173.0, 45.8, 44.8 (t, J = 21.1 Hz), 40.6, 26.4, 18.6, -5.3, -5.6; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{11}H_{20}DNO_3Si$ 245.1426, found 245.1428.



17a

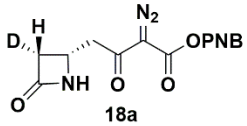
4-Nitrobenzyl 3-oxo-4-((2*S*,3*R*)-4-oxoazetidin-2-yl-3-*d*)butanoate (17a): Following chain extension of **16a** (400 mg, 1.64 mmol) as previously described,[3] the *tert*-butyldimethylsilyl (TBS) group was removed by stirring in tetrahydrofuran (33 mL) at 0 °C with tetrabutyl ammonium fluoride (1 M in tetrahydrofuran, 1.05 mL, 1.05 mmol) and acetic acid (0.27 mL, 8.2 mmol) for 30 min. The solution was then diluted with ethyl acetate (30 mL) and washed with water (2 × 20 mL) and 20 mL of saturated brine. The organic fraction was dried over anhydrous sodium sulfate and concentrated. The resulting oil was run through a plug of silica and eluted with 50% ethyl acetate/hexanes. Fractions containing the desired product were combined

and concentrated to give **17a** as a clear oil (220 mg, 1.40 mmol, 85%). **^{1}H NMR** (400 MHz; $CHCl_3$): δ 8.21 (d, J = 8.8 Hz, 2H), 7.50 (d, J = 8.8 Hz, 2H), 6.12 (br. s, 1H), 5.25 (s, 2H), 3.94 (ddd, J = 9.0, 5.1, 4.3 Hz, 1H), 3.55 (s, 2H), 3.15-3.10 (m, 1H), 2.91 (ABX, J_{AB} = 18.3 Hz, J_{AX} = 9.0 Hz, J_{BX} = 4.3 Hz, 2H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 200.7, 167.1, 166.5, 148.08, 142.4, 128.8, 124.1, 66.0, 49.2, 48.4, 43.4 (t, J = 21.4 Hz), 43.0; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{14}H_{13}DN_2O_6$ 308.0987, found 308.0989.



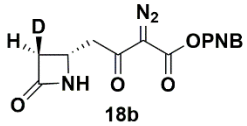
17b

4-Nitrobenzyl 3-oxo-4-((2*S*,3*S*)-4-oxoazetidin-2-yl-3-*d*)butanoate (17b): Compound **17b** (150 mg, 81%) was prepared as described above for **17a**. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 8.21 (d, J = 8.8 Hz, 2H), 7.50 (d, J = 8.8 Hz, 2H), 6.11 (br. s, 1H), 5.25 (s, 2H), 3.94 (ddd, J = 9.0, 4.3, 2.5 Hz, 1H), 3.55 (s, 2H), 2.91 (ABX, J_{AB} = 18.3 Hz, J_{AX} = 9.0 Hz, J_{BX} = 4.3 Hz, 2H), 2.58-2.55 (m, 1H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 200.7, 167.1, 166.5, 148.1, 142.4, 128.8, 124.1, 66.0, 49.2, 48.5, 43.4 (t, J = 21.9 Hz), 43.0; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{14}H_{13}DN_2O_6$ 308.0987, found 308.0985.



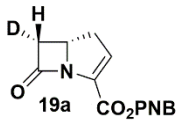
18a

4-Nitrobenzyl 2-diazo-3-oxo-4-((2*S*,3*R*)-4-oxoazetidin-2-yl-3-*d*)butanoate (18a): Compound **17a** was diazotized as described previously.[4] **^{1}H NMR** (400 MHz; $CHCl_3$): δ 8.22 (d, J = 8.9 Hz, 2H), 7.51 (d, J = 8.9 Hz, 2H), 6.11 (br. s, 1H), 5.33 (s, 2H), 3.96 (ddd, J = 9.0, 5.1, 4.3 Hz, 1H), 3.16 (ABX, J_{AB} = 18.0 Hz, J_{AX} = 9.0 Hz, J_{BX} = 4.3 Hz, 2H), 3.14-3.09 (m, 1H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 190.1, 167.3, 160.9, 148.3, 142.1, 129.0, 124.2, 76.2, 65.8, 45.9, 43.6, 43.3 (t, J = 21.4 Hz); **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{14}H_{11}DN_4O_6$ 334.0892, found 334.0890.



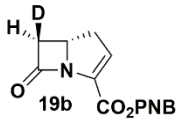
18b

4-Nitrobenzyl 2-diazo-3-oxo-4-((2*S*,3*S*)-4-oxoazetidin-2-yl-3-*d*)butanoate (18b): Compound **17b** was diazotized in the same manner as above. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 8.22 (d, J = 8.8 Hz, 2H), 7.51 (d, J = 8.8 Hz, 2H), 6.16 (br. s, 1H), 5.33 (s, 2H), 3.96 (ddd, J = 9.0, 4.3, 2.5 Hz, 1H), 3.17 (ABX, J_{AB} = 18.0 Hz, J_{AX} = 9.0 Hz, J_{BX} = 4.3 Hz, 2H), 2.65-2.61 (m, 1H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 190.1, 167.3, 160.8, 148.3, 142.1, 129.0, 124.2, 76.2, 65.8, 45.9, 43.6, 43.3 (t, J = 21.5 Hz); **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{14}H_{11}DN_4O_6$ 334.0892, found 334.0889.

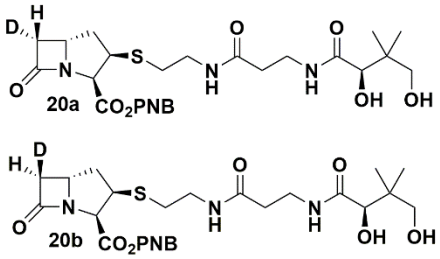


19a

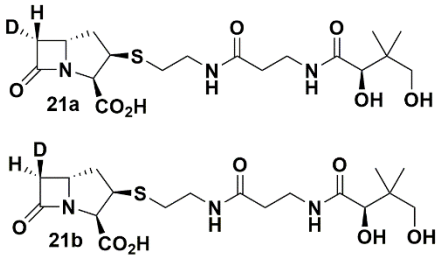
4-Nitrobenzyl (5*S*,6*R*)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate-6-*d* (19a). Compound **18a** was subjected to ring closure, reduction, mesylation, and elimination to give the *p*-nitrobenzyl protected carbapenem (**19a**) as previously described[3]. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 8.19 (d, J = 8.8 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 6.57 (br. t, J = 2.8 Hz, 1H), 5.34 (ABq, J = 13.7 Hz, 2H), 4.28 (ddd, J = 10.0, 8.1, 5.6 Hz, 1H), 3.51-3.46, (m, 1H), 2.86 ($ABX_{H5}Y_{H2}$, J_{AB} = 19.4 , J_{AX} = 10.0, J_{BX} = 8.1, J_{AY} = 3.2, J_{BY} = 2.5, plus long range coupling to Z_{H6} J_{AZ} = 0.8, J_{BZ} = 0.6 Hz, 2H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 177.0, 160.3, 147.9, 142.9, 135.2, 133.4, 128.4, 124.0, 65.6, 51.4, 45.6 (t, J = 21.5 Hz), 36.6; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{14}H_{11}DN_2O_5$ 290.0882, found 290.0879.



4-Nitrobenzyl (5*S*,6*S*)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate-6-*d* (19b). Prepared as descried above for **19a**. **^{1}H NMR** (400 MHz; $CHCl_3$): δ 8.18 (d, J = 8.8 Hz, 2H), 7.57 (d, J = 8.8 Hz, 2H), 6.56 (br. t, J = 2.8 Hz, 1H), 5.33 (ABq, J = 13.7 Hz, 2H), 4.28 (ddd, J = 10.0, 8.2, 3.3 Hz, 1H), 2.98-2.94 (m, 1H), 2.86 ($ABX_{H5}Y_{H2}$, J_{AB} = 19.4 Hz, J_{AX} = 10.0, J_{BX} = 8.2, J_{AY} = 3.1, J_{BY} = 2.6 Hz, 2H); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 177.0, 160.2, 147.9, 142.9, 135.1, 133.4, 128.4, 124.0, 65.6, 51.4, 45.6 (t, J = 21.5 Hz), 36.6; **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{14}H_{11}DN_2O_5$ 290.0882, found 290.0883.

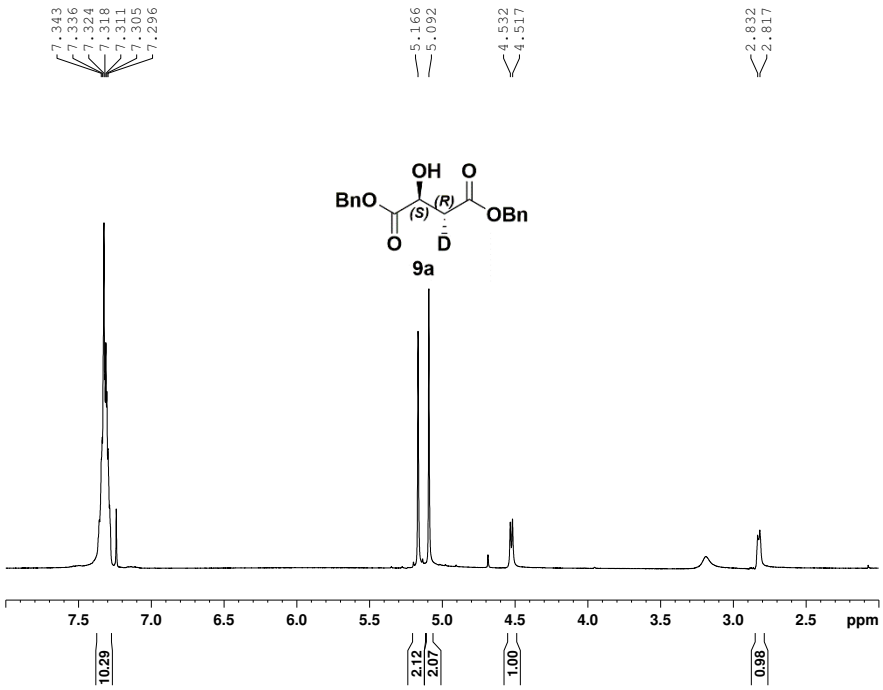


4-Nitrobenzyl (2*R*,3*R*,5*R*,6*R*)-3-((2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)-ethyl)thio)-7-oxo-1-azabicyclo[3.2.0]heptane-2-carboxylate-6-*d* (20a) and 4-nitrobenzyl (2*R*,3*R*,5*R*,6*S*)-3-((2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl)thio)-7-oxo-1-azabicyclo[3.2.0]heptane-2-carboxylate-6-*d*, (20b): Carbapenems **19a** and **19b** were subjected to β-addition by pantetheine as previously described.[5] C2 diastereomers were separated by preparative HPLC using a mobile phase of methanol and water containing 10 mM potassium phosphate (pH 6.65), 21 mL/min, gradient from 30-60% methanol over 15 min followed by 15 min of 60% methanol/water isocratic. The desired (2*R*, 3*R*)-isomer elutes second. Fractions containing the desired product **20a** or **20b** were combined, and methanol was removed *in vacuo*. **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{25}H_{33}DN_4O_9S$ 568.2182, found 568.2189 (**20a**) and 568.2191 (**20b**). **UPLC** (retention time) 5.88 min (**20a** and **20b**).

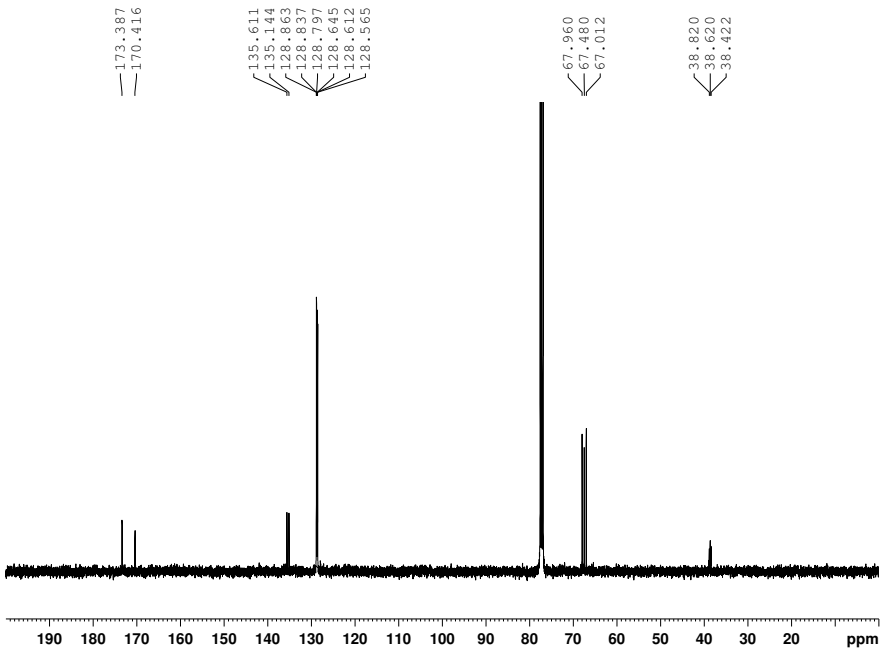


(2*R*,3*R*,5*R*,6*R*)-3-((2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl)thio)-7-oxo-1-azabicyclo[3.2.0]heptane-2-carboxylic-6-*d* acid ((*R*)-[6-^{2}H]PCPM, 21a) and (2*R*,3*R*,5*R*,6*S*)-3-((2-(3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethyl)thio)-7-oxo-1-azabicyclo[3.2.0]heptane-2-carboxylic-6-*d* acid ((*S*)-[6-^{2}H]PCPM, 21b): To the remaining buffered solutions (1 mL) of **20a** and **20b** was added tetrahydrofuran (1 mL) and 10% palladium on carbon. The mixture was placed in a pressure tube and was shaken in a Parr apparatus under 30 psi hydrogen for 1 h followed by centrifugation to separate the phases. The supernatant was then filtered through a 0.2 micron syringe filter to give buffered solutions of (*R*)- and (*S*)-[6-^{2}H]PCPM (**21a** and **21b**). Concentration to dryness led to nearly complete degradation of the carbapenam, so partial concentration was performed *in vacuo* at room temperature. Identity and purity were assessed by UPLC-HRMS. Concentration of resulting solutions was determined by UPLC-HRMS comparison to unlabeled PCPM. **HRMS** (ESI) *m/z*: $[M+H]^+$ calculated $C_{18}H_{28}DN_3O_7S$ 433.1862, found 433.1863 (**21a**) and 433.1863 (**21b**). **UPLC** (retention time) 4.24 min (**21a** and **21b**).

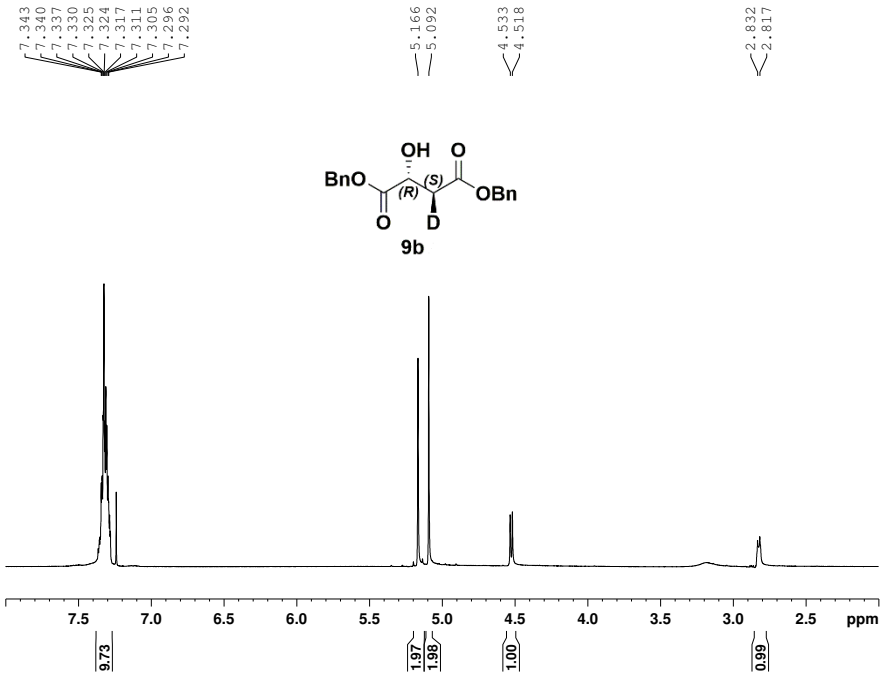
Spectral Data



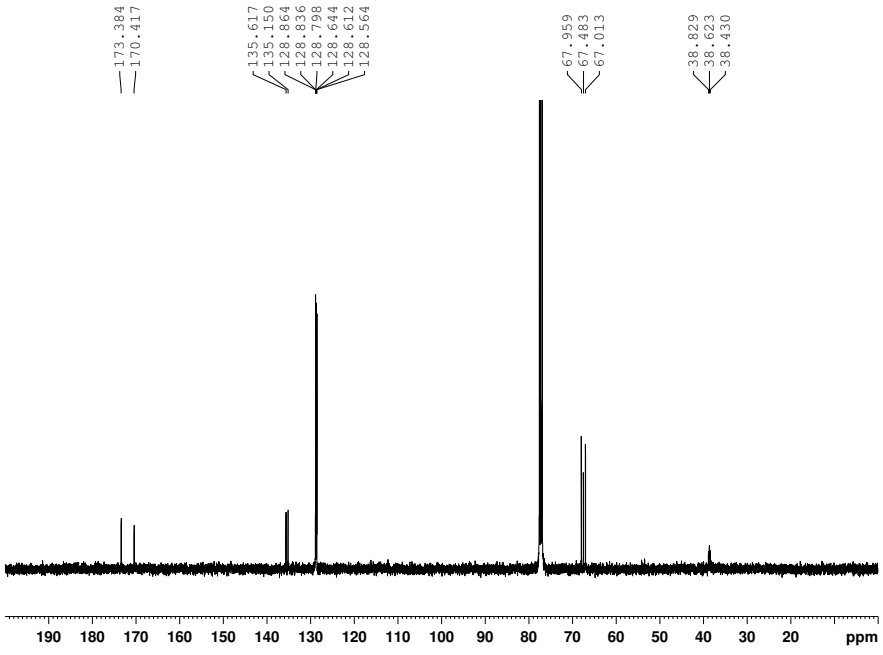
^{1}H NMR spectrum of **9a** ($CDCl_3$).



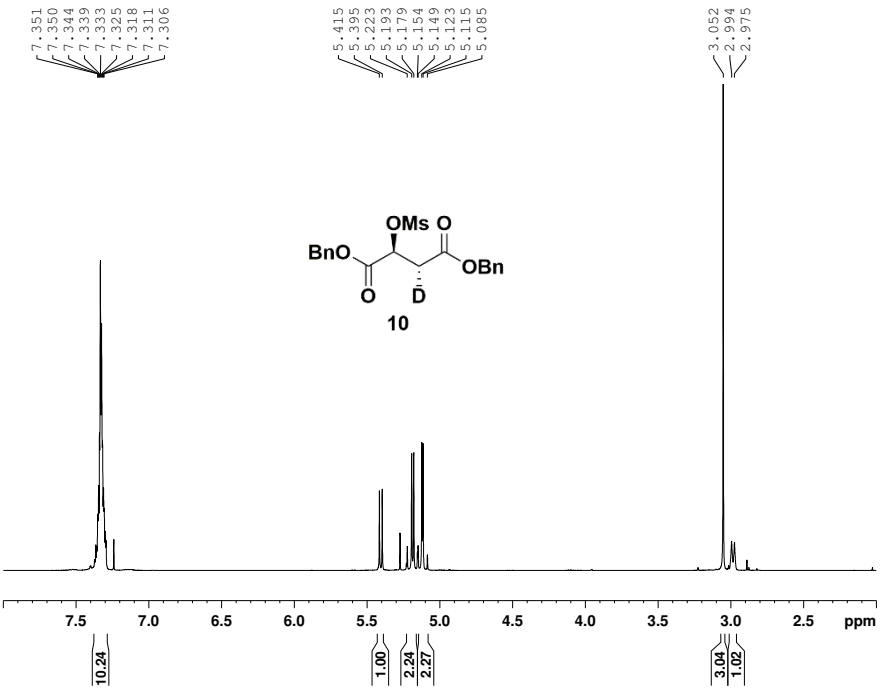
^{13}C NMR spectrum of **9a** ($CDCl_3$).



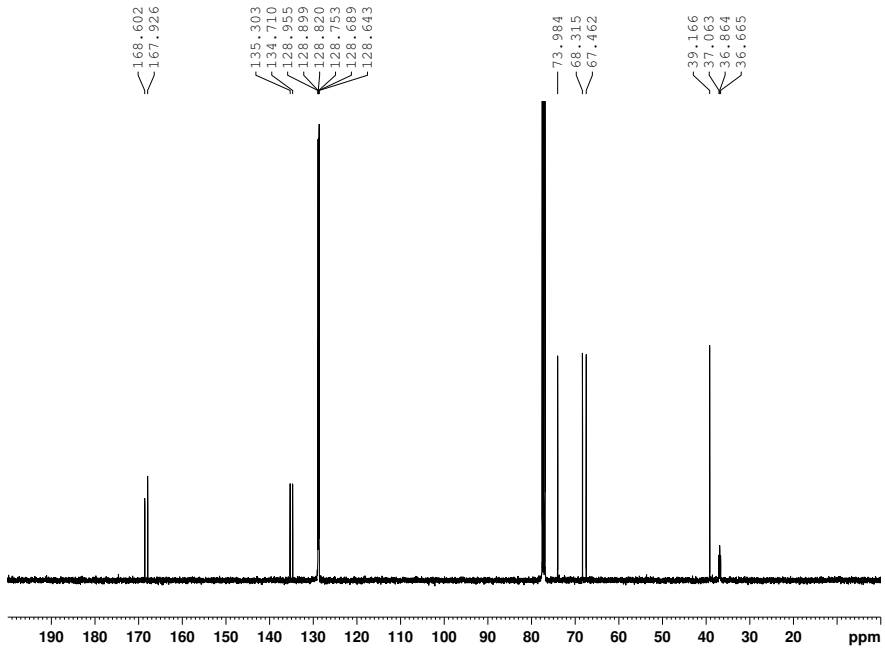
^{1}H NMR spectrum of **9b** ($CDCl_3$).



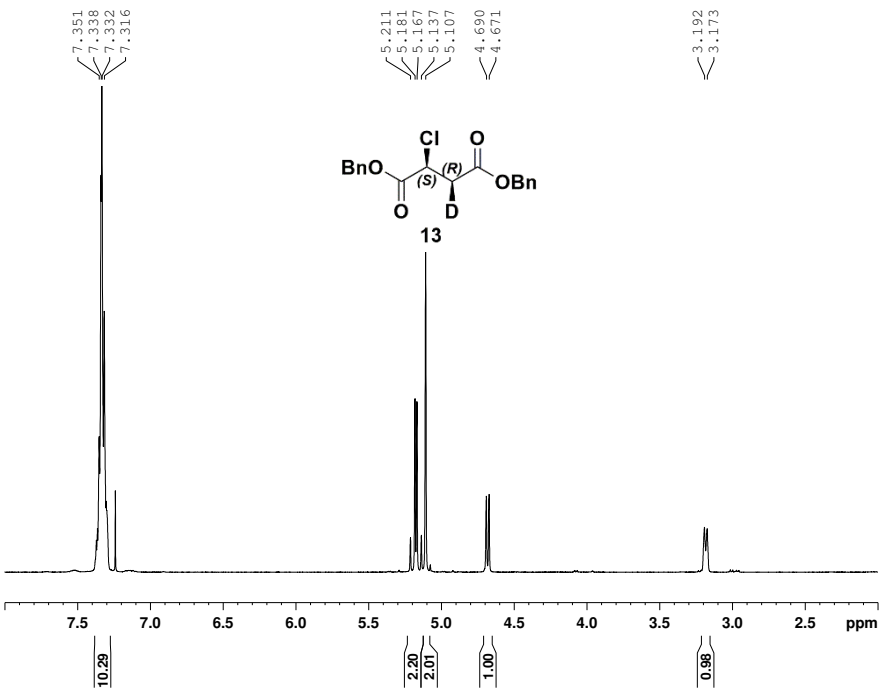
^{13}C NMR spectrum of **9b** ($CDCl_3$).



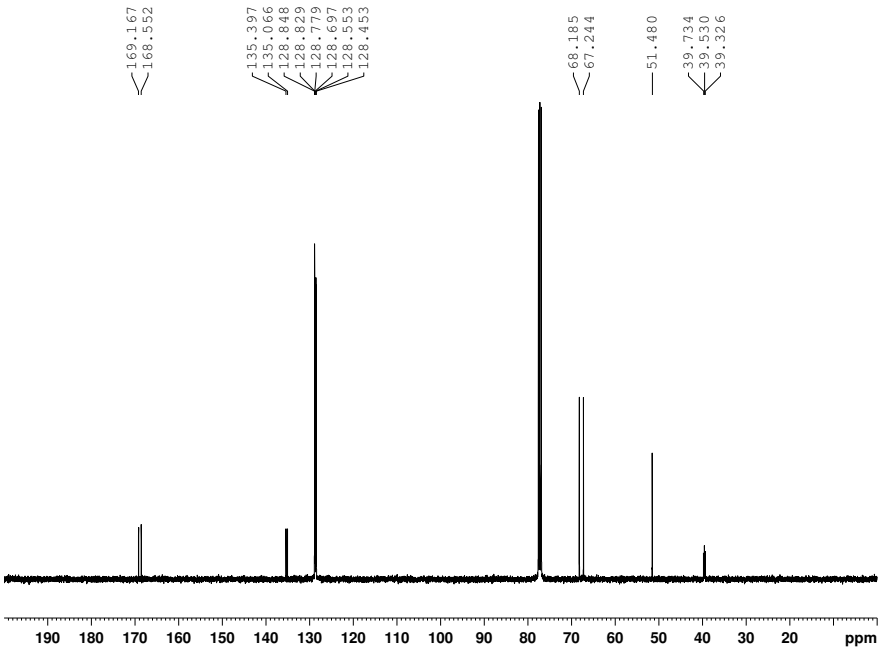
^{1}H NMR spectrum of **10** ($CDCl_3$).



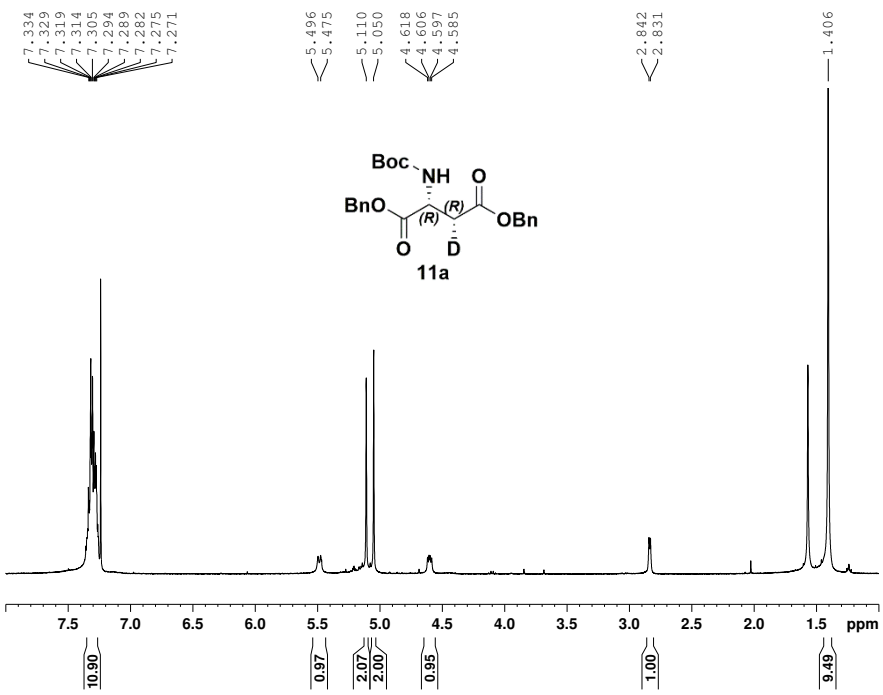
^{13}C NMR spectrum of **10** ($CDCl_3$).



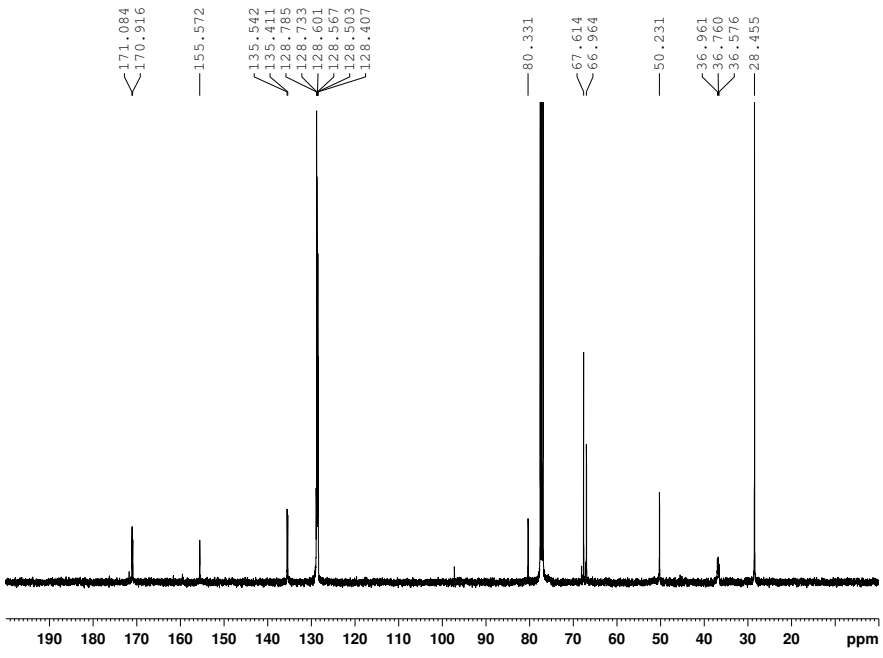
^{1}H NMR spectrum of **13** ($CDCl_3$).



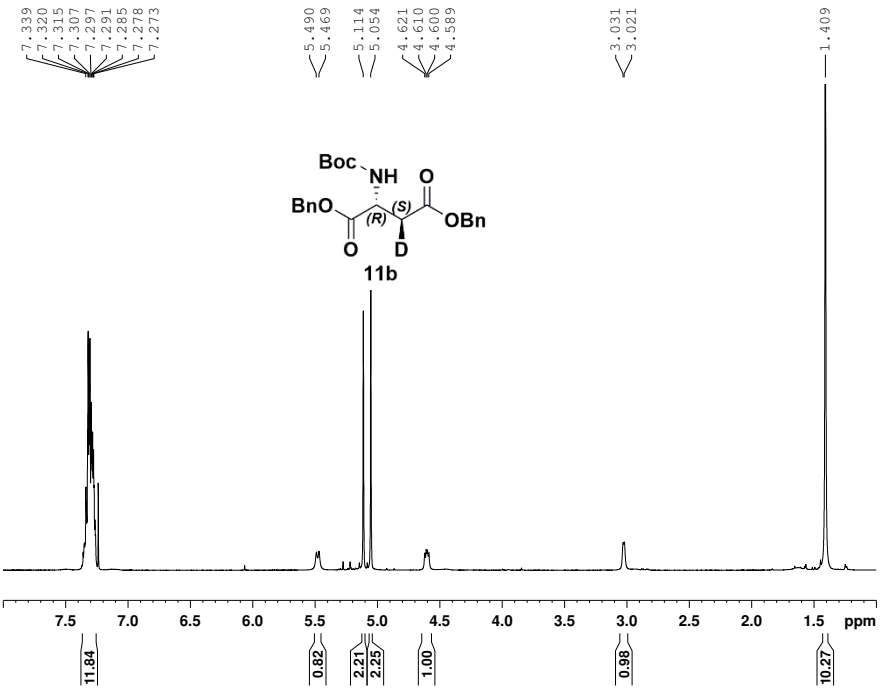
^{13}C NMR spectrum of **13** ($CDCl_3$).



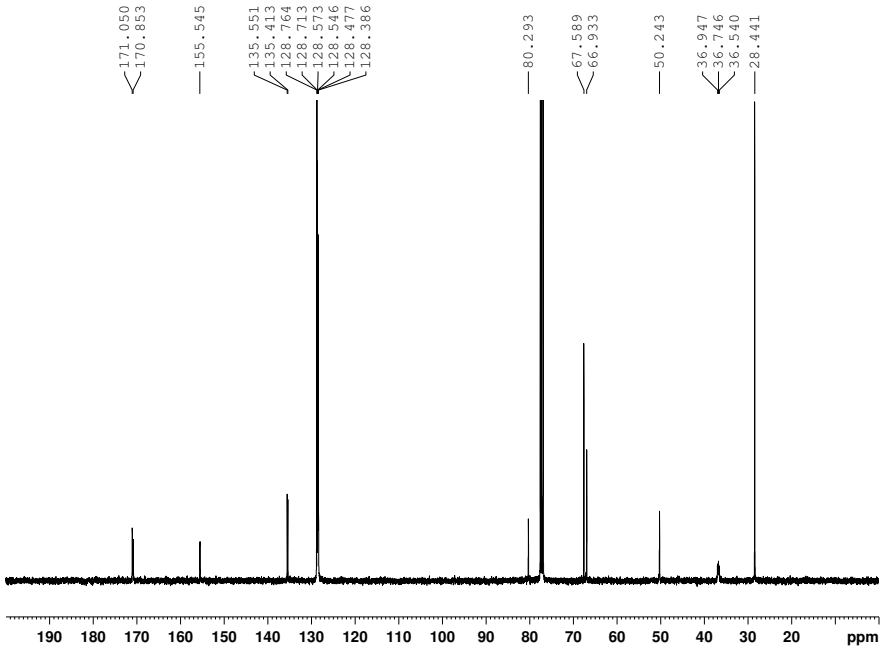
^{1}H NMR spectrum of **11a** ($CDCl_3$).



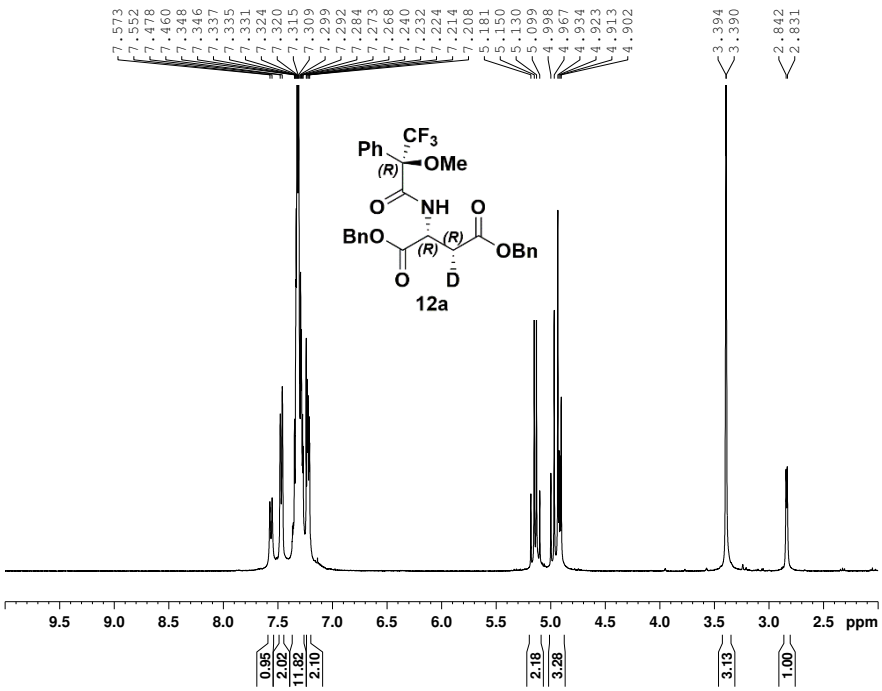
^{13}C NMR spectrum of **11a** ($CDCl_3$).



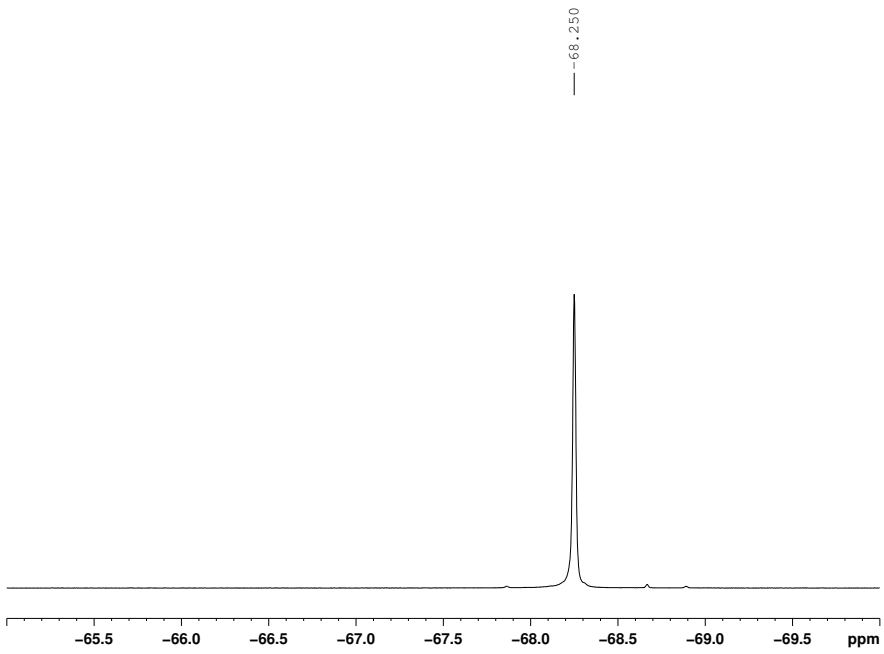
^{1}H NMR spectrum of **11b** ($CDCl_3$).



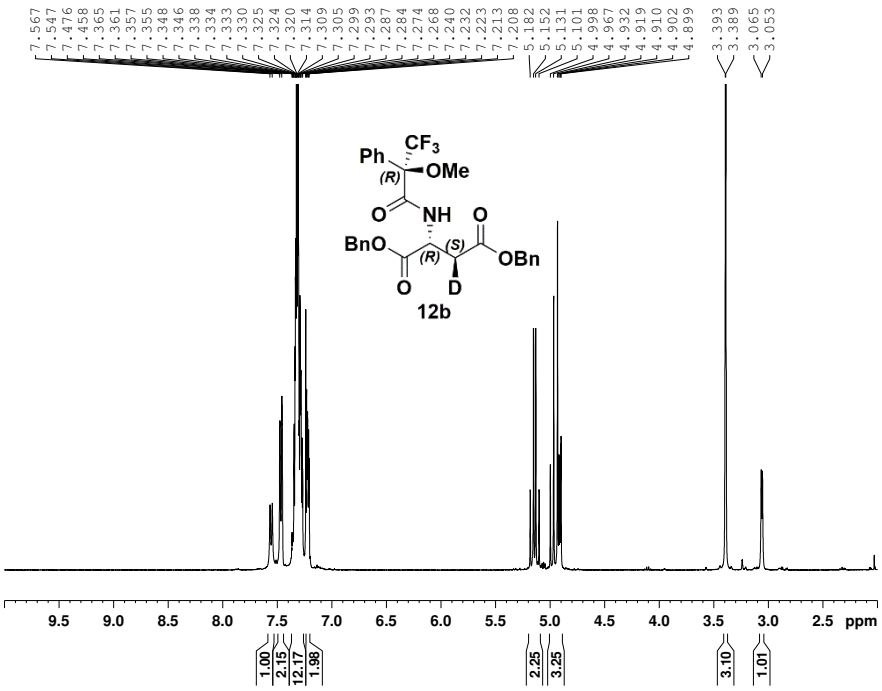
^{13}C NMR spectrum of **11b** ($CDCl_3$).



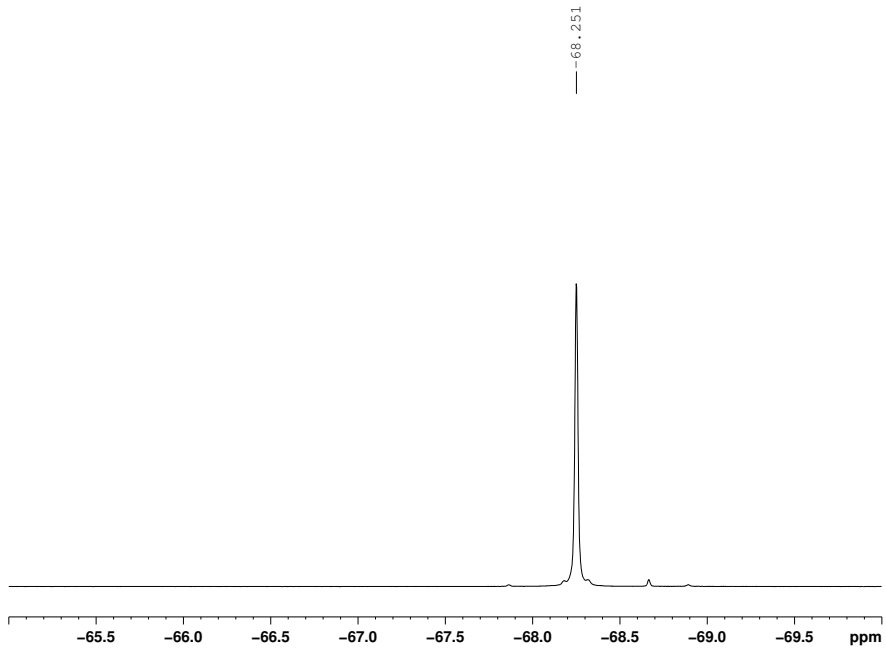
^{1}H NMR spectrum of **12a** ($CDCl_3$).



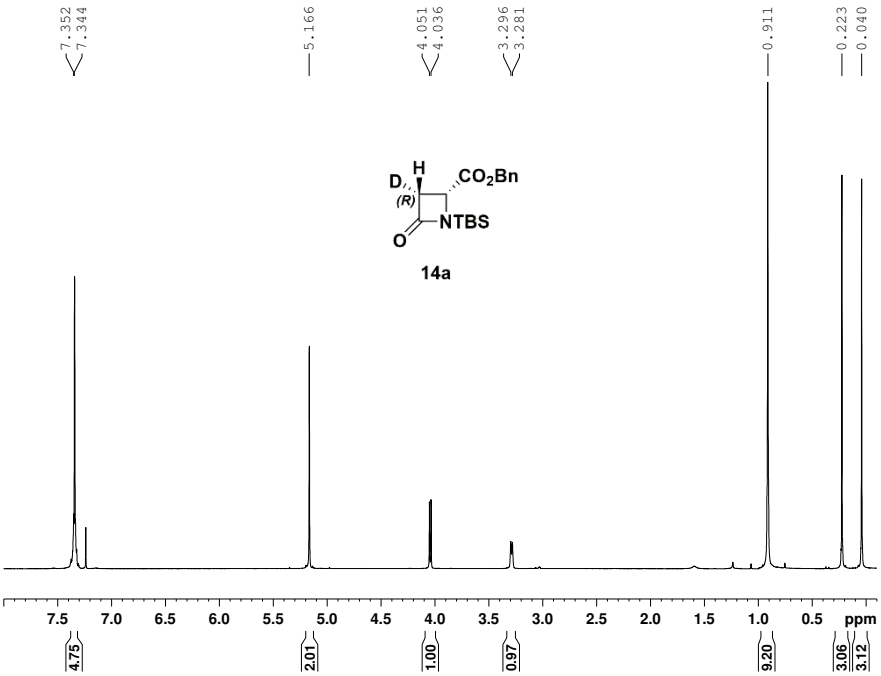
^{19}F NMR spectrum of **12a** ($CDCl_3$).



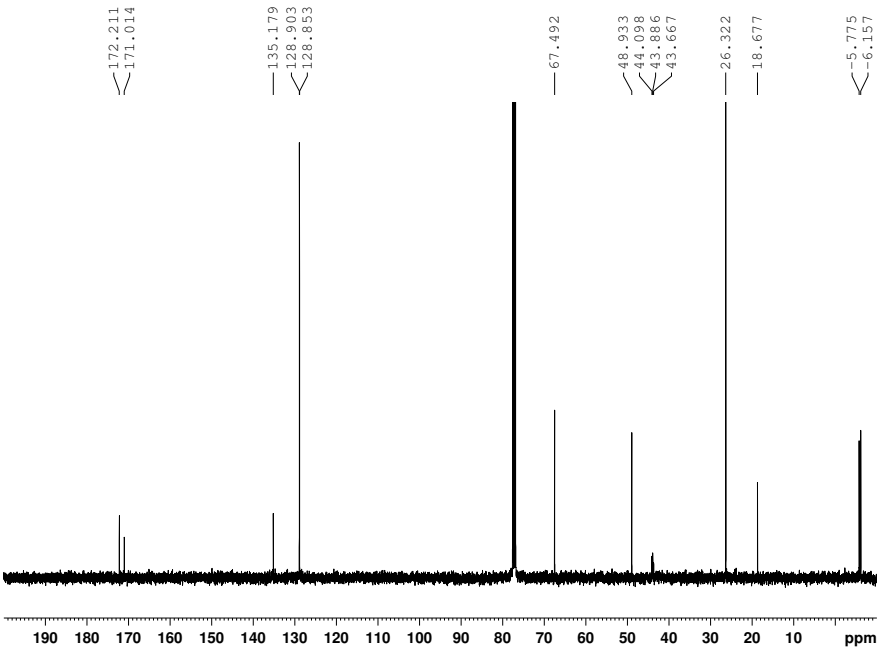
^{1}H NMR spectrum of **12b** ($CDCl_3$).



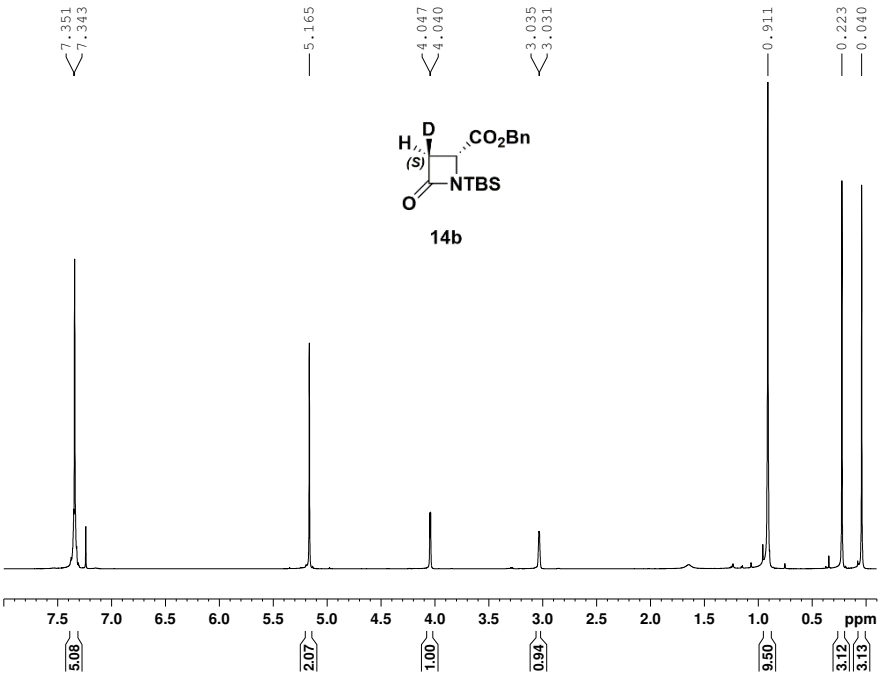
^{19}F NMR spectrum of **12b** ($CDCl_3$).



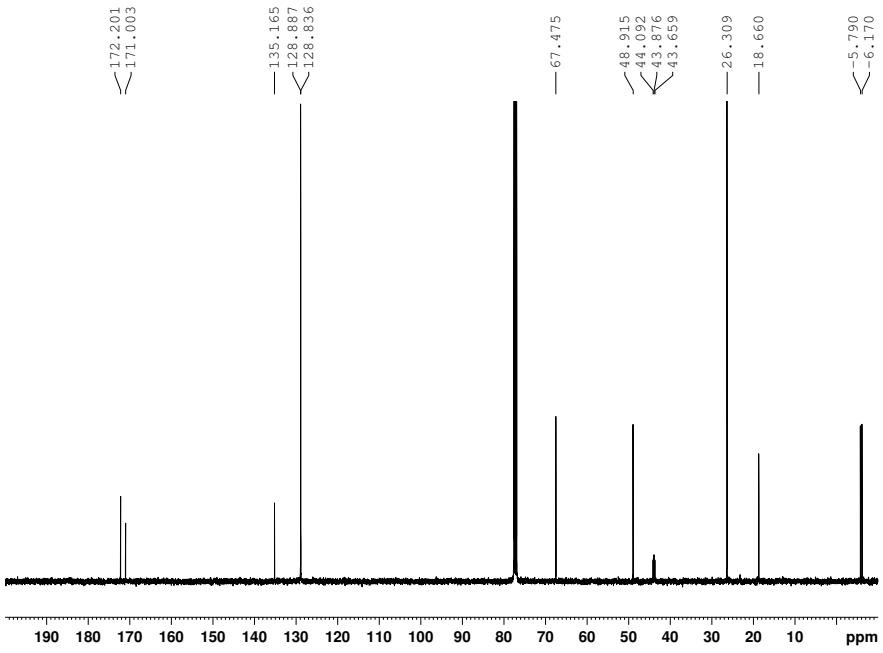
^{1}H NMR spectrum of **14a** ($CDCl_3$).



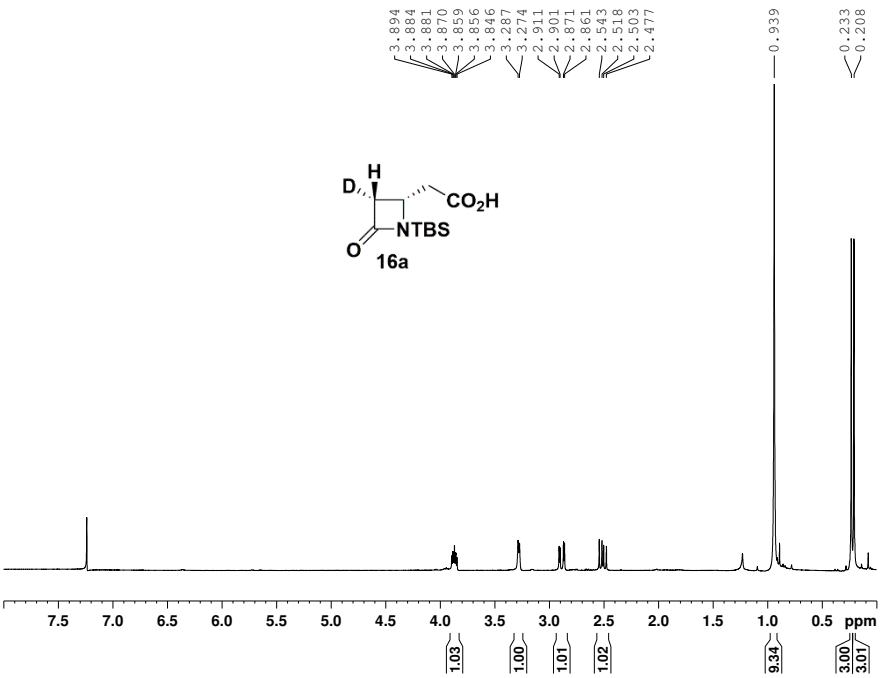
^{13}C NMR spectrum of **14a** ($CDCl_3$).



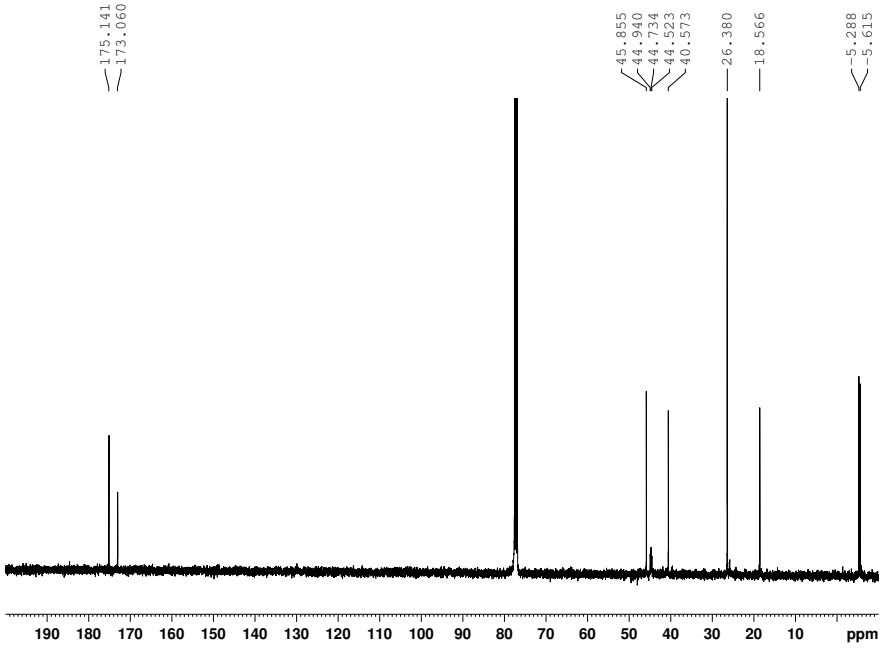
^{1}H NMR spectrum of **14b** ($CDCl_3$).



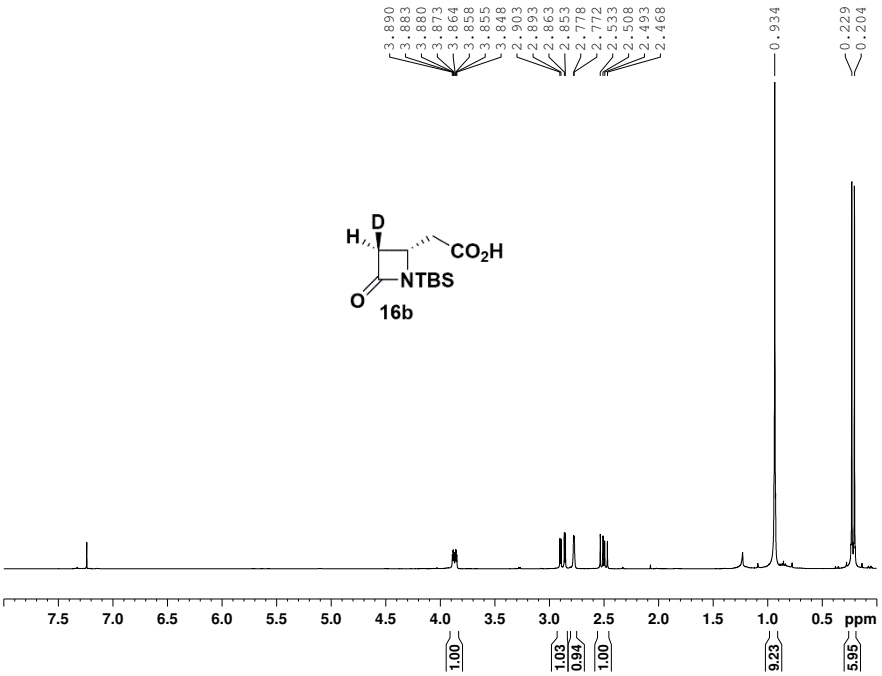
^{13}C NMR spectrum of **14b** ($CDCl_3$).



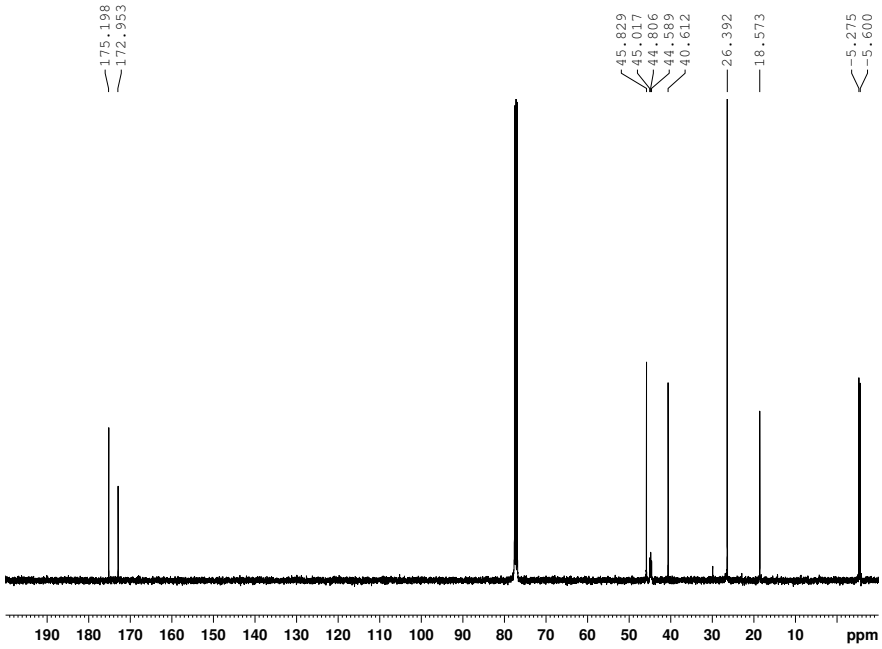
^{1}H NMR spectrum of **16a** ($CDCl_3$).



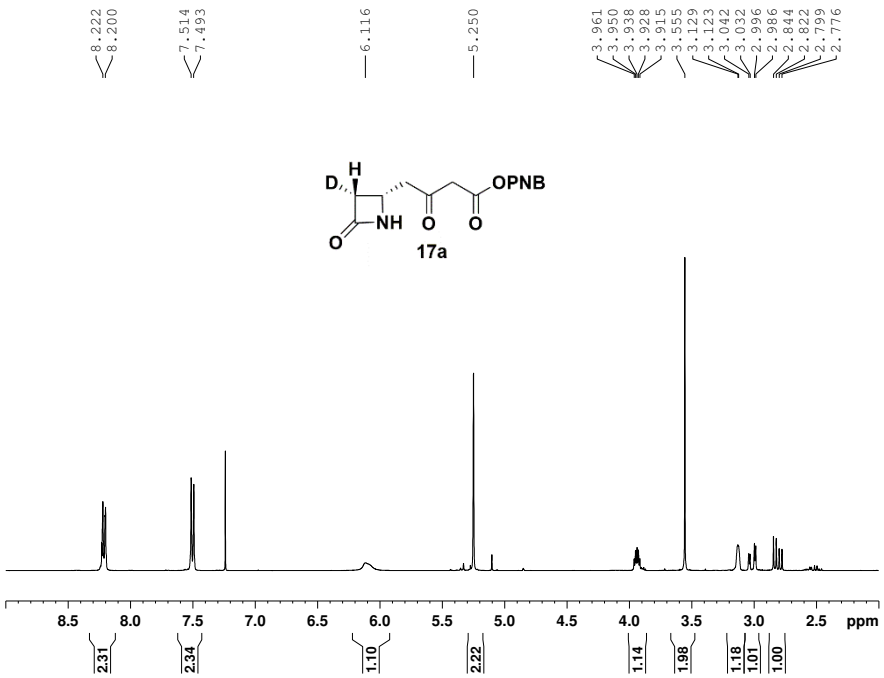
^{13}C NMR spectrum of **16a** ($CDCl_3$).



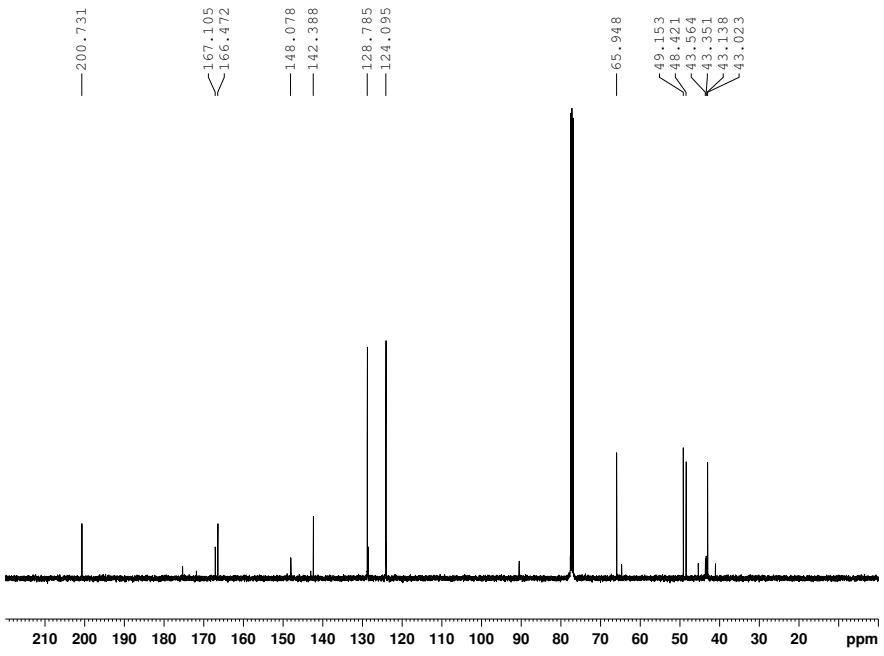
^{1}H NMR spectrum of **16b** ($CDCl_3$).



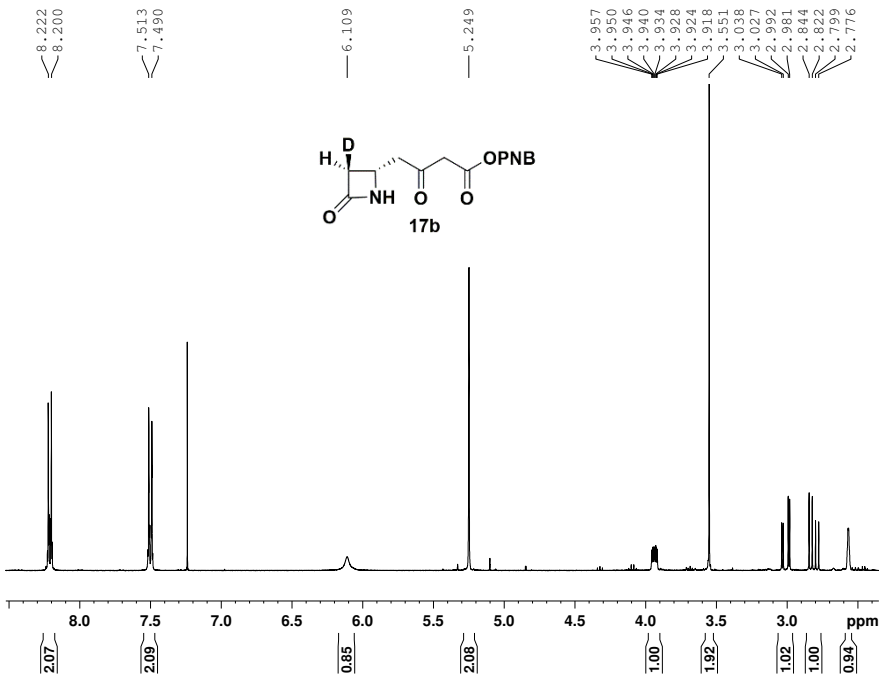
^{13}C NMR spectrum of **16b** ($CDCl_3$).



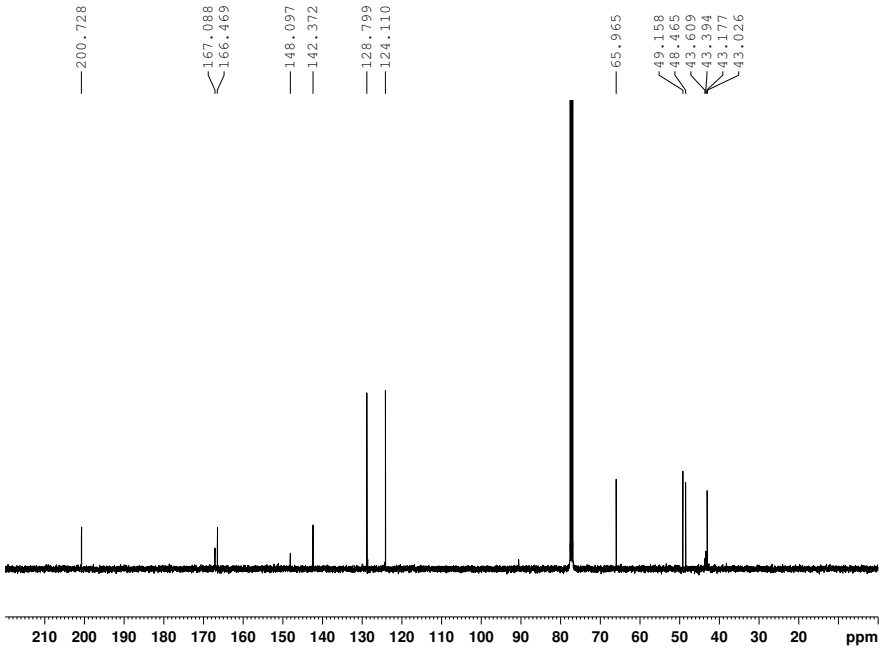
^{1}H NMR spectrum of **17a** ($CDCl_3$).



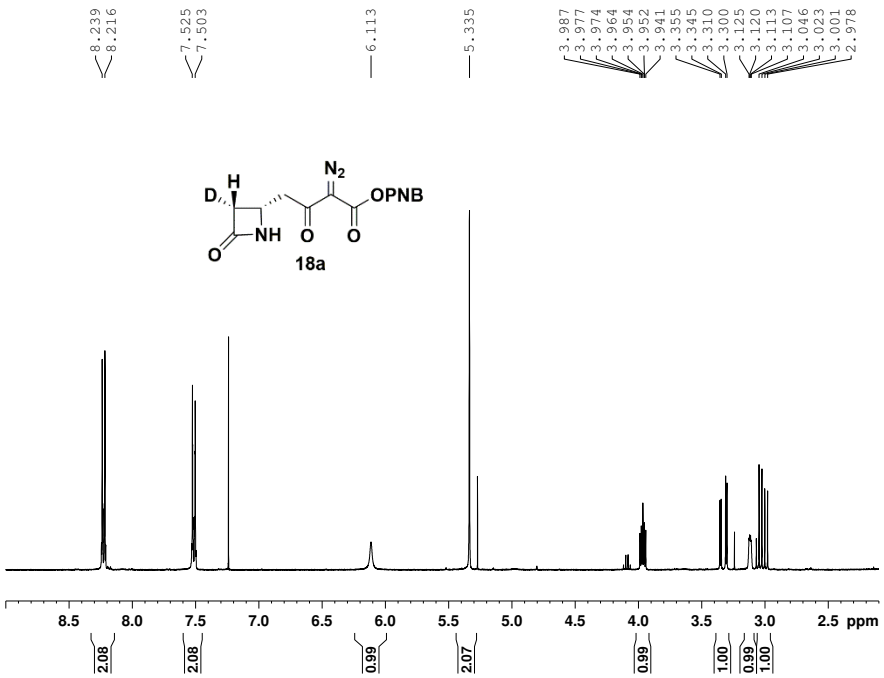
^{13}C NMR spectrum of **17a** ($CDCl_3$).



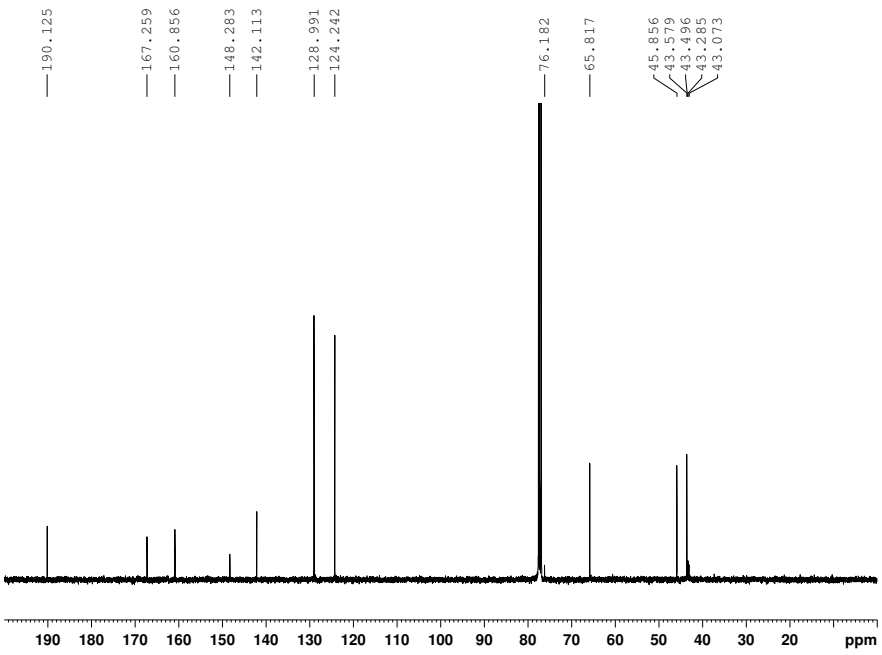
^{1}H NMR spectrum of **17b** ($CDCl_3$).



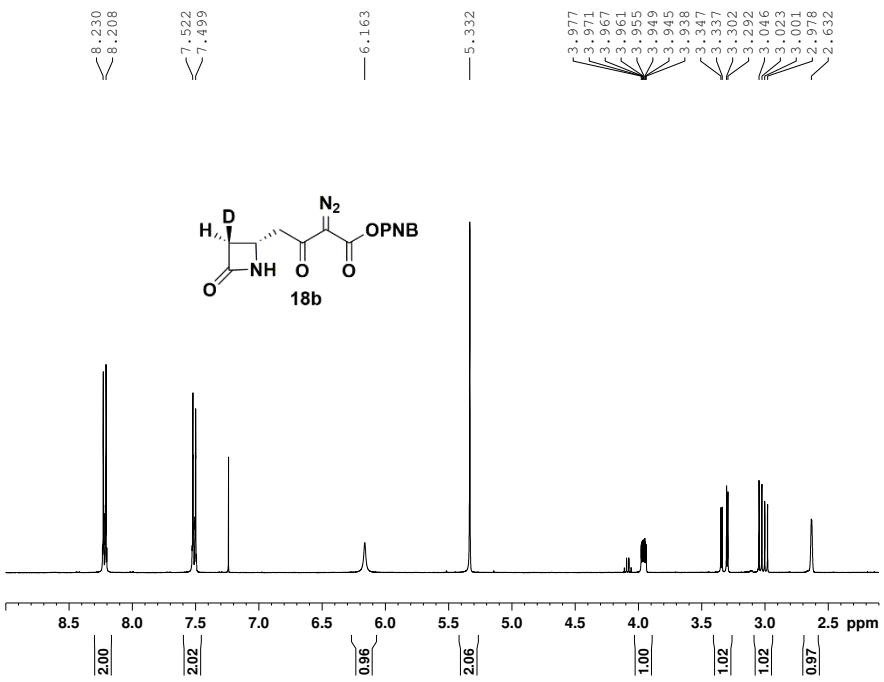
^{13}C NMR spectrum of **17b** ($CDCl_3$).



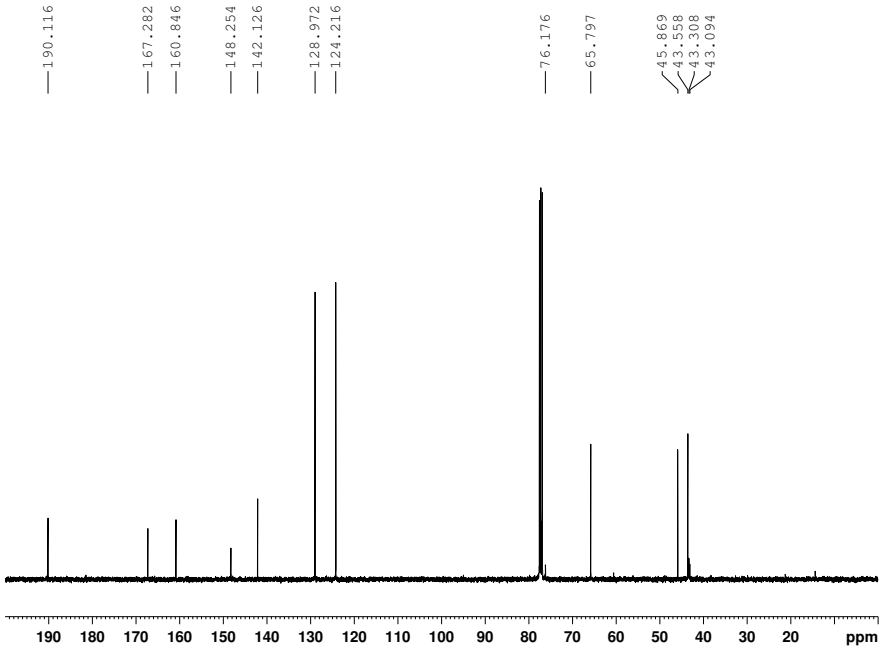
^{1}H NMR spectrum of **18a** ($CDCl_3$).



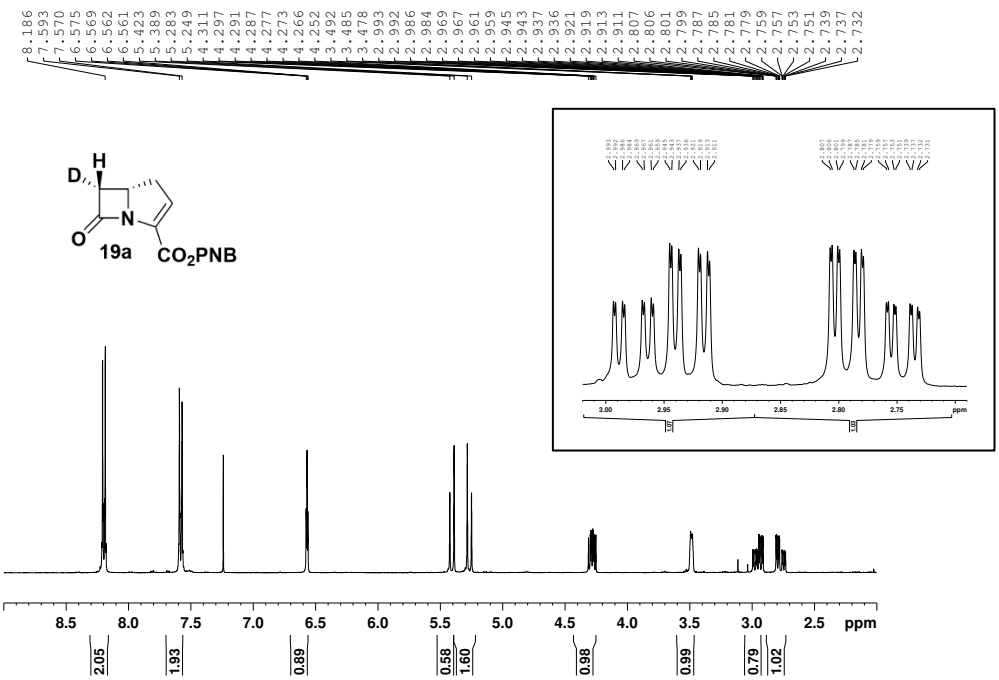
^{13}C NMR spectrum of **18a** ($CDCl_3$).



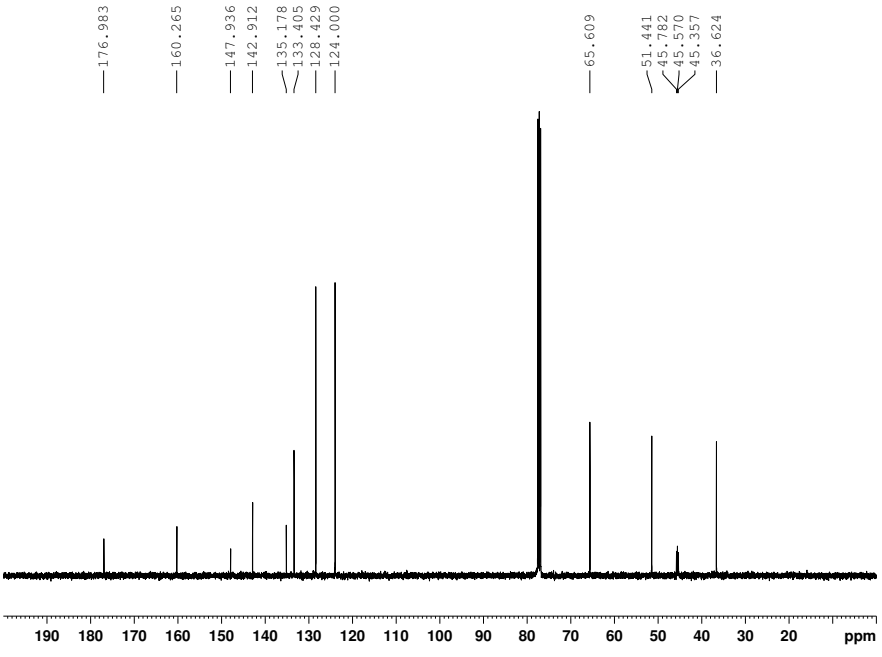
^{1}H NMR spectrum of **18b** ($CDCl_3$).



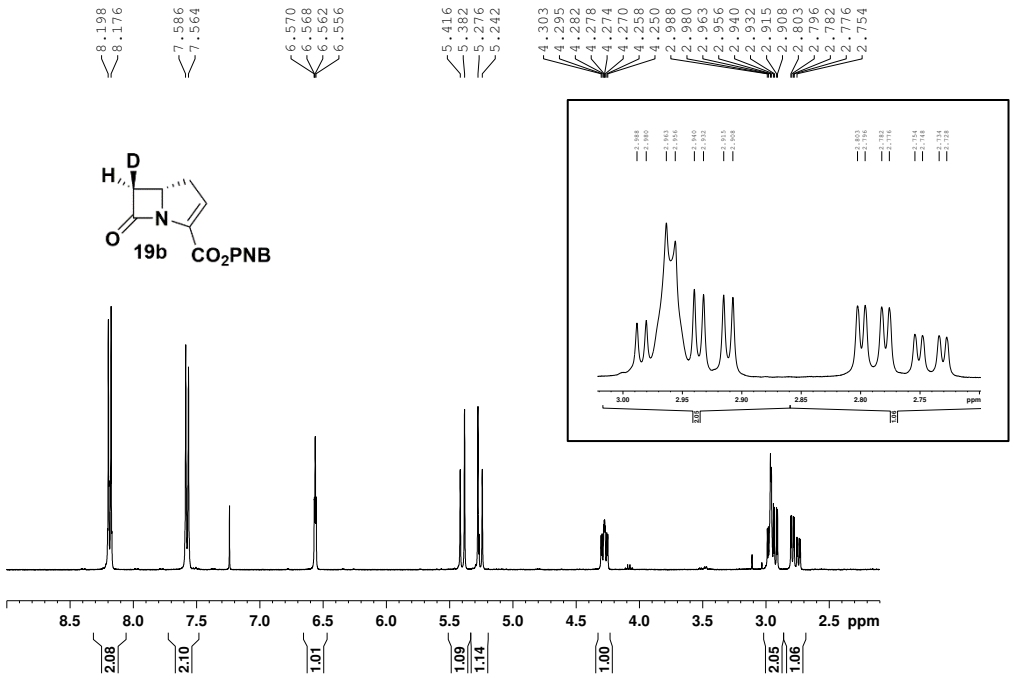
^{13}C NMR spectrum of **18b** ($CDCl_3$).



^{1}H NMR spectrum of **19a** ($CDCl_3$).



^{13}C NMR spectrum of **19a** ($CDCl_3$).



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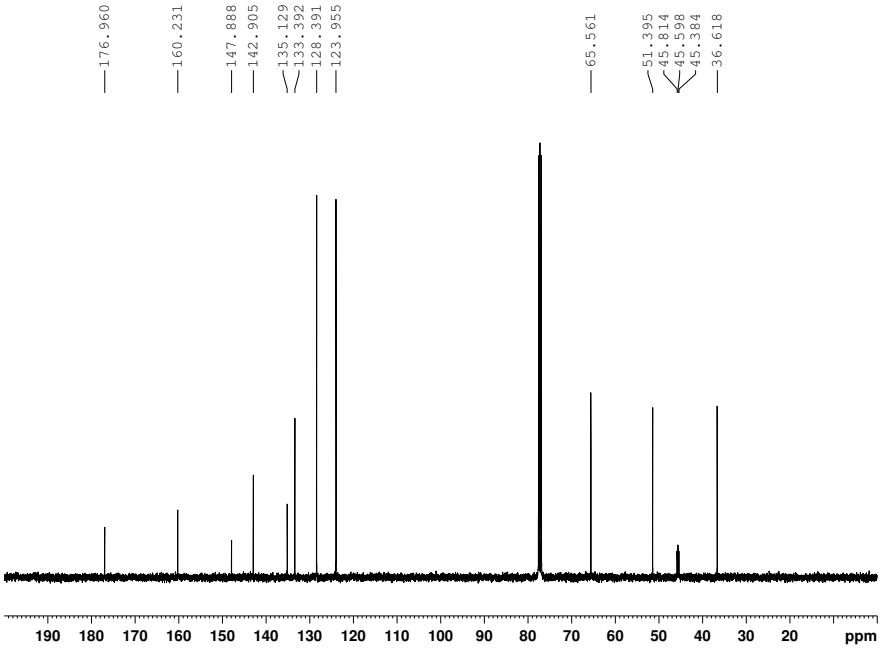
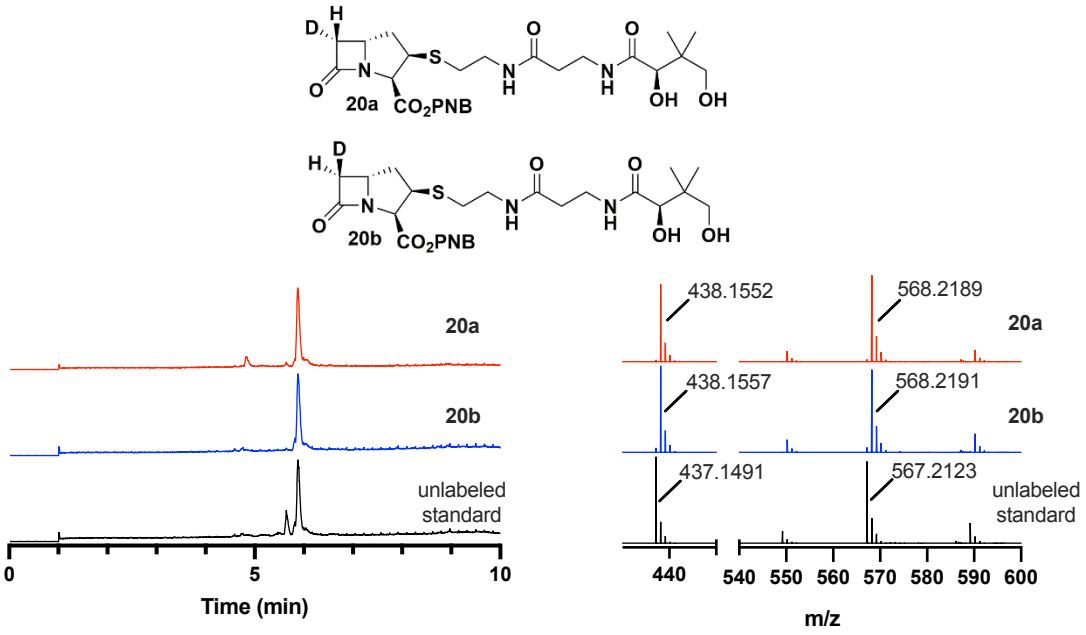
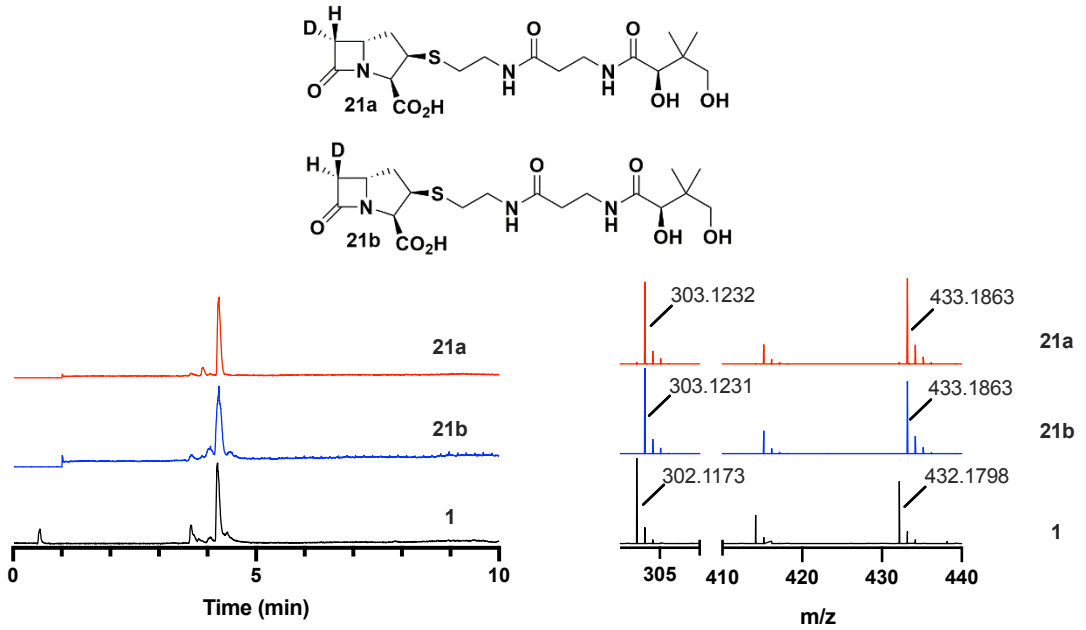


^{13}C NMR spectrum of **19b** ($CDCl_3$).



Total ion chromatograms (left) and ESI mass spectra (right) for **20a** (red), **20b** (blue) and unlabeled standard (black).



Total ion chromatograms (left) and ESI mass spectra (right) for **21a** (red), **21b** (blue) and unlabeled standard **1** (black).

References

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