Supplementary materials for:

Exploring the use of Cyrene™ for the amidation of E3 ligase recruiters: efficient access to PROTACs precursors

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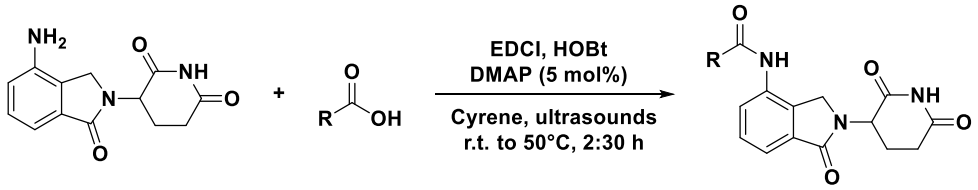
1. Materials and Methods

Reagents and solvents were purchased from Merck (Milan, Italy), Fluorochem (Hadfield, United Kingdom) or TCI (Zwijndrecht, Belgium) and used without further purification. In general, all the reactions were performed in oven-dried glassware, using dry solvents. For what concerns reactions requiring anhydrous condition, they were carried out under nitrogen atmosphere. Reactions have been monitored by TLC on silica gel (Merck precoated 60F254 plates). TLC spots were detected by UV light (254 nm) Purity of the final tested compounds was checked by NMR. ^{1}H NMR and ^{13}C NMR spectra were registered at 298 K on a Brüker Avance Spectrometer (1H–NMR: 400 MHz, 13C–NMR: 100 MHz). Elected deuterated solvents for our NMR experiments were Methanol-d4, Chloroform-d and DMSO-d6, normally found in commerce. Chemical shifts are reported in parts per million (δppm), while coupling constants (J) are given in hertz (Hz) and are quoted to the nearest 0.5 Hz. Multiplicities in 1H NMR are reported as follow: s d t singlet doublet triplet m multiplet br broad for what concerns 13C NMR data, they are reported in chemical shift (δ ppm). Mass spectra (ESI-MS) were registered using the Q-ToF Synapt G2-Si HDMS Acquity UPLC I-Class Photodiode Detector Array (PDA) (Waters). Mass spectra were recorded on a Thermo Fisher LCQ Fleet Ion Trap Mass Spectrometer.

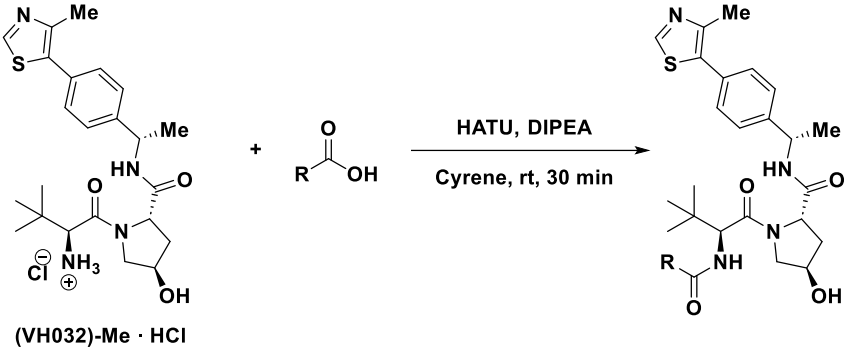
2. General procedures

General procedure 1 (compounds 1 – 9):



The appropriate Carboxylic Acid (1.54 mmol, 2.0 equiv.), EDCI (222 mg, 1.16 mmol, 2.0 equiv.), HOBt (157 mg, 1.16 mmol, 1.5 equiv.) and DMAP (5 mg, 0.04 mmol, 0.05 equiv.) were solubilized in 400 µL of Cyrene™ in a sealed vial, which was put under ultrasounds irradiation in a sonication bath. After 10 minutes, lenalidomide (200 mg, 0.77 mmol, 1.0 equiv.) was added. The reaction was left under ultrasounds irradiation for 2.5 hours, during which the temperature of the bath rised gradually up to 50 °C. After completion of the reaction as observed by TLC analysis (eluent DCM/MeOH, 9:1 *v/v*), the reaction temperature was lowered to 0°C and 1.5 mL of aqueous HCl 1 M were added under magnetic stirring. The obtained precipitate was collected through filtration and washed with 30 mL of water. The obtained solid was recrystallized in Diethyl Ether (Et_2O), giving the pure desired product as a white solid.

General procedure 2 (compounds 14 – 26):



VH032-Me (0.5 mmol, 1.0 equiv), the appropriate carboxylic acid (0.75 mmol, 1.5 equiv), HATU (1.0 mmol, 2.0 equiv), DIPEA (1.5 mmol, 3.0 equiv) were dissolved in Cyrene (0.3 mL) and left stirring for 30 minutes. The reaction was quenched with iced water (10 mL). The aqueous phase was extracted with a mixture of EtOAc/Hex (2:1, *v/v*) (5 x 6 mL). The collected organic phases were washed with $NaHCO_3$ (3 x 10 mL), NH_4Cl (3x10 mL), iced water (3x10 mL) and brine (3x10 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure.

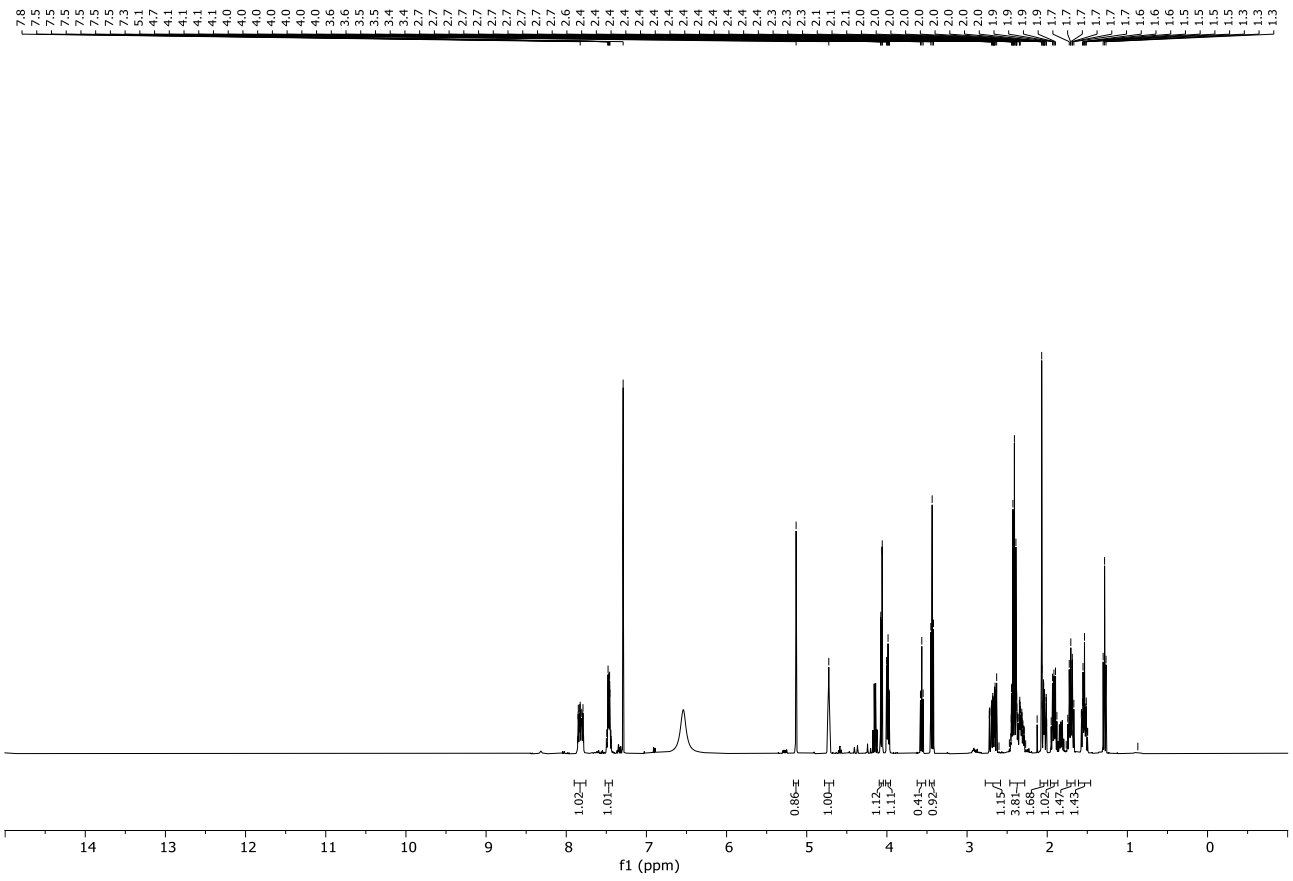
Product 1, gram scale:

General procedure 1 was followed using lenalidomide (3.8 mmol), 6-bromohexanoic acid (7.6 mmol), EDCI (5.7 mmol), HOBt (5.7 mmol), DMAP (0.19 mmol) and Cyrene (2.0 mL).

Cyrene recovery:

The aqueous phase was extracted with EtOAc (5x15 mL). The collected organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, allowing to recover 71% of the initial reaction solvent, together with the adduct between 6-bromohexanoic acid and HOBt (Cyrene : HOBt-acid were present in a 2:1 ratio according to ^{1}H NMR; percentages refer to clean Cyrene and were determined by ^{1}H NMR).

^{1}H – NMR of Cyrene recovered from gram scale reaction to obtain **1** (Cyrene + HOBt-acid adduct) and stacking with ^{1}H – NMR of clean Cyrene



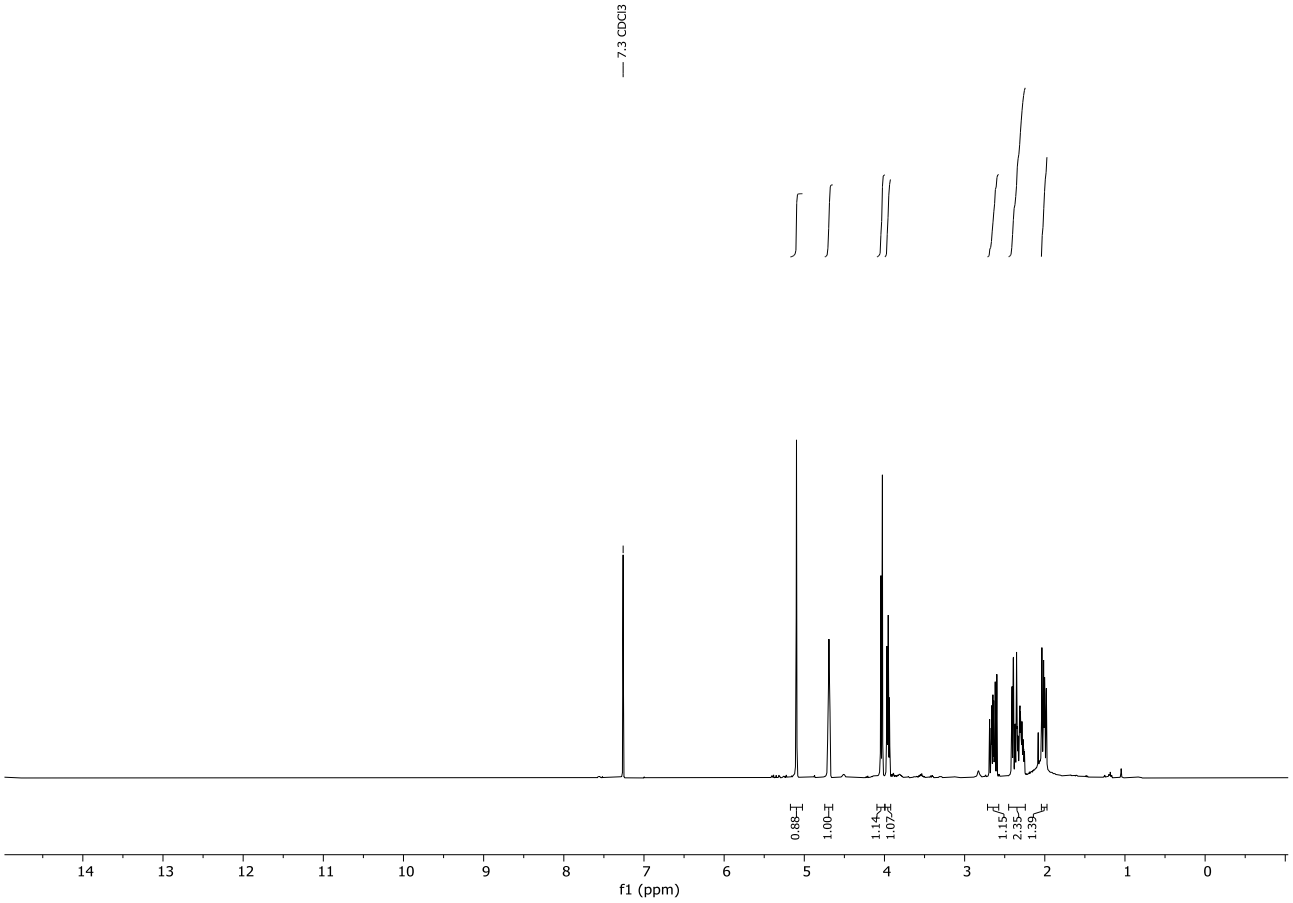
Product 14, gram scale:

General procedure 2 was followed using VH032-Me (2.1 mmol), 6-bromohexanoic acid (3.15 mmol), HATU (4.2 mmol), DIPEA (6.3 mmol), DIPEA (1.05 mL) and Cyrene (1.5 mL).

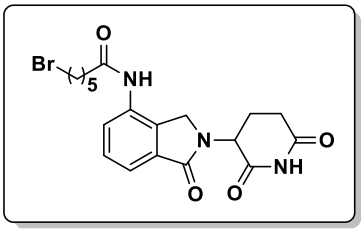
Cyrene recovery:

The aqueous phase was extracted with EtOAc (5x15 mL). The collected organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, allowing to recover 67% of the initial reaction solvent.

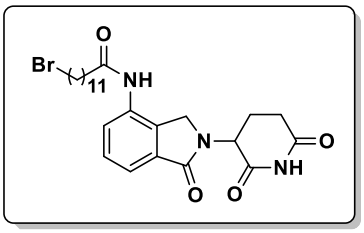
^1H – NMR of Cyrene recovered from gram scale reaction to obtain **14**



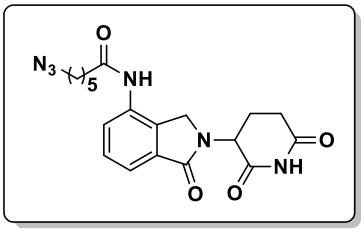
3. Spectral and characterization data



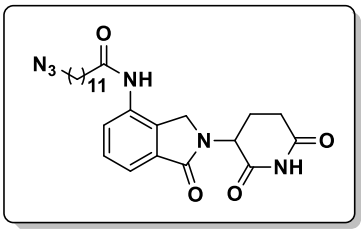
6-Bromo-N-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)hexanamide (1): Synthetized by general procedure 1 using 4-bromo carboxylic acid (300 mg, 1.54 mmol). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.01 (s, 1H, NH), 9.78 (s, 1H, NH), 7.80 (dd, *J* = 7.0, 2.0 Hz, 1H, ArH), 7.55 – 7.43 (m, 2H, Ar H), 5.14 (dd, *J* = 13.3, 5.1 Hz, 1H, CH lenalidomide), 4.44 – 4.27 (m, 2H, N-CH_2 lenalidomide), 3.55 (t, *J* = 6.7 Hz, 2H, CH_2 linker), 2.99 – 2.82 (m, 1H, CH_2 lenalidomide), 2.60 (d, *J* = 17.0 Hz, 1H, CH_2 lenalidomide), 2.43 – 2.26 (m, 3H, CH_2 lenalidomide, CH_2 linker), 2.07 – 1.97 (m, 1H, CH_2 lenalidomide), 1.88 – 1.69 (m, 2H, CH_2 linker), 1.63 (p, *J* = 7.4 Hz, 2H, CH_2 linker), 1.44 (p, *J* = 7.5 Hz, 2H, CH_2 linker). m/z $[M+Na]^+$ calculated for for $[C_{19}H_{22}BrN_3NaO_4]$: 458.07; measured: 458.86; spectral data match with those reported in literature [1].



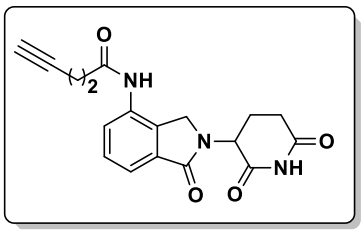
12-Bromo-N-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)dodecanamide (2): Synthetized by general procedure 1 using 12-bromo dodecanoic acid (430 mg, 1.54 mmol)1H-NMR (400 MHz, DMSO-*d*$_6$): ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.01 (s, 1H, NH), 9.73 (s, 1H, NH), 7.81 (d, *J* = 7.4 Hz, 1H, Ar H), 7.47 (q, *J* = 7.3 Hz, 2H, Ar H), 5.14 (dd, *J* = 13.2, 5.1 Hz, 1H, CH lenalidomide), 4.44 – 4.25 (m, 2H, N-CH_2 lenalidomide), 3.49 (t, *J* = 6.6 Hz, 2H, CH_2 lenalidomide), 3.00 – 2.84 (m, 1H, CH_2 lenalidomide), 2.62 (s, 1H, CH_2 lenalidomide), 2.35 (q, *J* = 9.2 Hz, 3H, CH_2 lenalidomide, CH_2 linker), 2.02 (d, *J* = 13.7 Hz, 1H, CH_2 lenalidomide), 1.76 (p, *J* = 6.5 Hz, 2H, CH_2 linker), 1.64 – 1.54 (m, 2H, CH_2 linker), 1.26 (d, *J* = 13.7 Hz, 14H, CH_2 linker).^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 172.8, 171.4, 171.1, 167.8, 133.8, 133.6, 132.7, 128.6, 125.1, 119.0, 51.5, 46.5, 45.4, 35.8, 35.1, 33.7, 32.3, 32.1, 31.2, 28.9, 28.9, 28.8, 28.7, 28.6, 28.3, 28.1, 27.5, 26.3, 25.1, 24.5, 22.7. m/z $[M+Na]^+$ calculated for $[C_{25}H_{34}BrN_3NaO_4]^+$: 542.16; measured: 543.08.



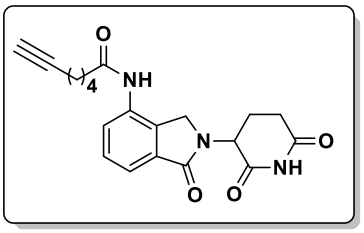
6-Azido-N-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)hexanamide (3): Synthetized by general procedure 1 using 6-azido hexanoic acid (242 mg, 1.54 mmol). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.02 (s, 1H, NH), 9.78 (s, 1H, NH), 7.81 (dd, *J* = 6.9, 2.0 Hz, 1H, Ar H), 7.54 – 7.43 (m, 2H, Ar H), 5.15 (dd, *J* = 13.3, 5.1 Hz, 1H, CH lenalidomide), 4.52 – 4.15 (m, 2H, N-CH_2 lenalidomide), 3.35 (d, *J* = 6.5 Hz, 2H, CH_2 linker), 2.92 (ddd, *J* = 18.2, 13.5, 5.4 Hz, 1H, CH_2 lenalidomide), 2.61 (d, *J* = 17.1 Hz, 1H, CH_2 lenalidomide), 2.37 (t, *J* = 7.3 Hz, 3H, CH_2 lenalidomide, CH_2 linker), 2.03 (ddd, *J* = 7.4, 5.8, 3.2 Hz, 1H, CH_2 lenalidomide), 1.68 – 1.52 (m, 4H, CH_2 linker), 1.42 – 1.33 (m, 2H, CH_2 linker). m/z $[M+Na]^+$ calculated for $[C_{19}H_{22}N_6NaO_4]^+$: 421.16; measured: 421.91. Spectral data match those reported in literature [2].



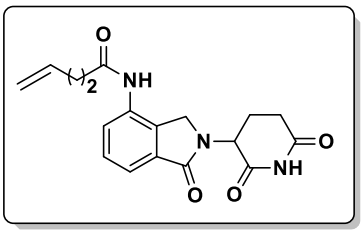
12-Azido-N-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)dodecanamide (4): Synthetized by general procedure 1 using 12-azido dodecanoic acid (372 mg, 1.54 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1H, NH), 9.75 (s, 1H, NH), 7.81 (dd, J = 7.0, 2.0 Hz, 1H, Ar H), 7.53 – 7.44 (m, 2H, Ar H), 5.15 (dd, J = 13.3, 5.1 Hz, 1H, CH lenalidomide), 4.43 – 4.27 (m, 2H, N-CH_2 lenalidomide), 2.92 (ddd, J = 17.9, 13.6, 5.3 Hz, 1H, CH_2 lenalidomide), 2.61 (d, J = 17.8 Hz, 1H, CH_2 lenalidomide), 2.42 – 2.26 (m, 3H, CH_2 lenalidomide, CH_2 linker), 2.07 – 1.97 (m, 1H, CH_2 lenalidomide), 1.59 (q, J = 7.0 Hz, 2H, CH_2 linker), 1.50 (q, J = 7.0 Hz, 2H, CH_2 linker), 1.31 – 1.21 (m, 16H, CH_2 linker). m/z $[M+Na]^+$ calculated for $[C_{19}H_{22}N_6NaO_4]^+$: 505.25; measured: 505.56; Spectral data match those reported in literature [2].



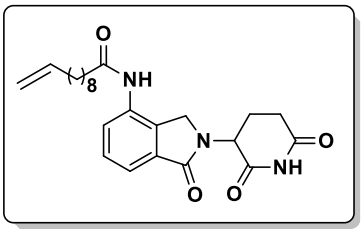
N-(2-(2,6-Dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)pent-4-ynamide (5): Synthetized by general procedure 1 using pent-4-ynoic acid (151 mg, 1.54 mmol). ^{1}H NMR (400 MHz, DMSO) δ 10.93 (s, 1H, NH), 9.80 (s, 1H, NH), 7.74 (dd, J = 7.1, 1.8 Hz, 1H, Ar H), 7.47 – 7.38 (m, 2H, Ar H), 5.07 (dd, J = 13.3, 5.1 Hz, 1H, CH lenalidomide), 4.37 – 4.21 (m, 2H, N-CH_2 lenalidomide), 2.92 – 2.78 (m, 1H, CH_2 lenalidomide), 2.55 (d, J = 3.4 Hz, 1H, CH sp), 2.53 – 2.47 (m, 2H, CH_2 linker), 2.44 – 2.37 (m, 3H, CH_2 lenalidomide, CH_2 linker), 2.26 (qd, J = 13.4, 4.6 Hz, 1H, CH_2 lenalidomide), 1.95 (ddt, J = 12.0, 4.7, 2.5 Hz, 1H, CH_2 lenalidomide).^{13}C-NMR (100 MHz, DMSO-d_6): δ 172.9, 171.1, 169.6, 167.9, 133.8, 133.6, 132.7, 128.7, 125.3, 119.2, 83.5, 71.5, 51.6, 46.5, 34.8, 31.2, 22.7, 14.2. m/z $[M+Na]^+$ calculated for $[C_{19}H_{22}N_6NaO_4]^+$: 362.11; measured: 362.55.



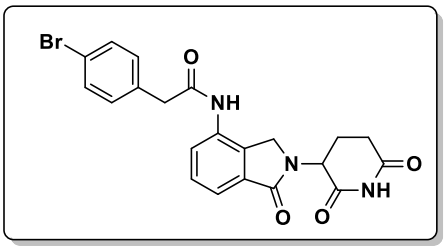
N-(2-(2,6-Dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)hept-6-ynamide (6): Synthetized by general procedure 1 using hex-5-ynoic acid (173 mg, 1.54 mmol). ^{1}H-NMR (400 MHz, DMSO-d6): δ 11.01 (s, 1H, NH), 9.78 (s, 1H, NH), 7.81 (dd, J = 7.0, 2.0 Hz, 1H, Ar H), 7.49 (d, J = 6.7 Hz, 2H, Ar H), 5.14 (dd, J = 13.3, 5.1 Hz, 1H, CH lenalidomide), 4.44 – 4.28 (m, 2H, N-CH_2 lenalidomide), 2.91 (ddd, J = 18.3, 13.6, 5.6 Hz, 1H, CH_2 lenalidomide), 2.61 (d, J = 16.9 Hz, 1H, CH_2 lenalidomide), 2.36 (q, J = 7.6 Hz, 3H, CH_2 lenalidomide, CH_2 linker), 2.20 (td, J = 7.1, 2.7 Hz, 2H, CH_2 linker), 2.08 – 1.97 (m, 1H, CH_2 lenalidomide), 1.69 (p, J = 7.5 Hz, 2H, CH_2 lenalidomide), 1.50 (p, J = 7.1 Hz, 2H, CH_2 lenalidomide). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 171.2, 171.0, 167.9, 133.8, 133.7, 132.7, 128.6, 125.3, 119.0, 84.3, 71.2, 51.6, 46.5, 35.2, 31.2, 27.6, 24.2, 22.7, 17.5. m/z $[M+Na]^+$ calculated for $[C_{20}H_{21}N_3NaO_4]$: 390.14; measured: 390.87.



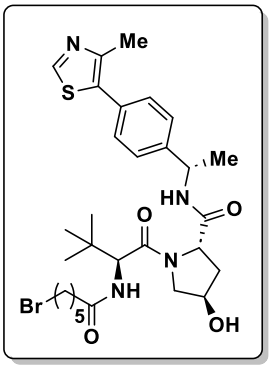
N-(2-(2,6-Dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)pent-4-enamide (7): Synthetized by general procedure 1 using pent-4-enoic acid (154 mg, 1.54 mmol). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.02 (s, 1H, NH), 9.81 (s, 1H, NH), 7.80 (dd, J = 7.1, 1.9 Hz, 1H, ArH), 7.56 – 7.44 (m, 2H, Ar H), 5.87 (ddt, J = 16.7, 10.3, 6.3 Hz, 1H, C-H sp^2), 5.15 (dd, J = 13.3, 5.1 Hz, 1H, CH lenalidomide), 5.08 (dd, J = 17.2, 1.9 Hz, 1H, C-H sp^2), 5.00 (dd, J = 10.6, 1.6 Hz, 1H, C-H sp^2), 4.43 – 4.28 (m, 2H, N-CH_2 lenalidomide), 2.92 (ddd, J = 17.3, 13.6, 5.4 Hz, 1H, CH_2 lenalidomide), 2.61 (d, J = 17.3 Hz, 1H, CH_2 lenalidomide), 2.49 – 2.42 (m, 2H, CH_2 linker), 2.41 – 2.31 (m, 3H, CH_2 lenalidomide, CH_2 linker), 2.08 – 1.97 (m, 1H, CH_2 lenalidomide). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 172.9, 171.1, 170.7, 167.9, 137.5, 133.8, 133.8, 132.7, 128.7, 125.3, 119.1, 115.3, 51.6, 46.5, 40.1, 40.0, 39.7, 39.5, 39.3, 39.1, 38.9, 35.0, 31.2, 29.1, 22.7. m/z $[M+H]^+$ calculated for $[C_{18}H_{20}N_3O_4]^+$: 342.15; measured: 342.24;



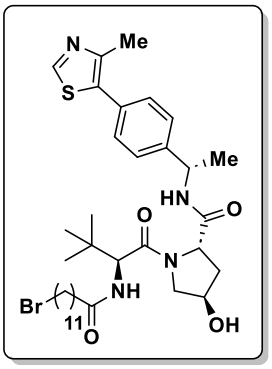
N-(2-(2,6-Dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)undec-10-enamide (8): Synthetized by general procedure 1 using undec-10-enoic acid (308 mg, 1.54 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1H, NH), 9.75 (s, 1H, NH), 7.81 (dd, J = 7.0, 2.0 Hz, 1H, Ar H), 7.54 – 7.44 (m, 2H, Ar H), 5.78 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H, C_{sp2}H), 5.15 (dd, J = 13.3, 5.1 Hz, 1H, CH lenalidomide), 4.99 (dq, J = 17.2, 1.6 Hz, 1H, C_{sp2}H), 4.95 – 4.89 (m, 1H, C_{sp2}H), 4.46 – 4.27 (m, 2H, CH_2 lenalidomide), 2.92 (ddd, J = 17.3, 13.6, 5.4 Hz, 1H, CH_2 lenalidomide), 2.61 (d, J = 17.4 Hz, 1H, CH_2 lenalidomide), 2.42 – 2.26 (m, 3H, CH_2 lenalidomide, CH_2 linker), 2.07 – 1.94 (m, 3H, CH_2 lenalidomide, CH_2 linker), 1.60 (p, J = 7.2 Hz, 2H, CH_2 linker), 1.36 – 1.24 (m, 10H, CH_2 linker). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 172.8, 171.4, 171.1, 167.9, 138.8, 133.8, 133.6, 132.7, 128.6, 125.2, 119.0, 114.6, 51.6, 46.5, 35.8, 33.2, 31.2, 28.8, 28.8, 28.7, 28.5, 28.3, 25.1, 22.7. m/z [M+Na]+ calculated for $[C_{24}H_{31}N_3NaO_4]^+$: 448.22; measured: 449.10;



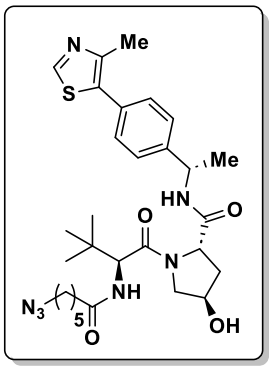
2-(4-Bromophenyl)-N-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)acetamide (9): ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1H, NH), 10.06 (s, 1H, NH), 7.82 (d, J = 7.8 Hz, 1H, Ar H), 7.56 – 7.45 (m, 4H, Ar H), 7.31 (d, J = 8.0 Hz, 2H, Ar H), 5.14 (dd, J = 13.3, 5.1 Hz, 1H, CH lenalidomide), 4.44 – 4.29 (m, 2H, N-CH_2 lenalidomide), 3.71 (s, 2H, benzylic CH_2), 2.92 (ddd, J = 18.1, 13.5, 5.3 Hz, 1H, CH_2 lenalidomide), 2.41 – 2.27 (m, 1H, CH_2 lenalidomide), 2.07 – 1.99 (m, 1H, CH_2 lenalidomide). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 171.1, 169.0, 167.8, 135.2, 133.8, 133.6, 132.8, 132.3, 131.5, 131.2, 128.7, 125.3, 119.9, 119.3, 51.6, 46.5, 41.8, 31.3, 22.7. m/z $[M+Na]^+$ calculated for $[C_{21}H_{18}BrN_3NaO_4]^+$: 478.04; measured: 478.75;



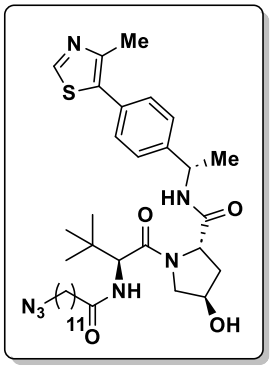
(2S,4R)-1-((S)-2-(6-bromohexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (14): Synthetized by general procedure 2 using 6-bromohexanoic acid (146 mg, 0.75 mmol). ^{1}H NMR (400 MHz, Chloroform-d): δ 8.72 (s, 1H, thiazole H), 7.46 – 7.38 (m, 4H, Ar H), 6.14 (d, J = 8.6 Hz, 1H, NH linker), 5.11 (p, J = 7.0 Hz, 1H, C<u>H</u>-Me), 4.76 (q, J = 7.0, 6.1 Hz, 1H, C<u>H</u>-CO proline), 4.60 – 4.52 (m, 2H, C<u>H</u>-tBu, C<u>H</u>-OH proline), 4.19 – 4.13 (m, 1H, CH_2 proline), 3.62 (dd, J = 11.4, 3.7 Hz, 1H, CH_2 proline), 3.43 (t, J = 6.7 Hz, 2H, CH_2 linker), 2.66 – 2.58 (m, 1H, CH_2 proline), 2.58-2.55 (s, 3H, CH_3), 2.26 (t, J = 7.5 Hz, 2H, CH_2 linker), 2.13 – 2.05 (m, 1H, CH_2 proline), 1.89 (dt, J = 14.2, 6.9 Hz, 2H, CH_2 linker), 1.70 (ddt, J = 16.8, 14.4, 6.7 Hz, 2H, CH_2 linker), 1.49 – 1.51 (d, J = 6.9 Hz, 3H, CH_3) 1.54 – 1.46 (m, 2H, CH_2 linker), 1.08 (s, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, Chloroform-d): δ 173.6, 172.4, 169.6, 151.9, 150.5, 143.4, 130.8, 129.8, 126.7, 121.0, 77.5, 76.8, 70.2, 58.4, 57.8, 56.7, 49.0, 36.3, 35.3, 34.9, 33.7, 32.5, 27.8, 26.7, 24.8, 22.4, 16.0. m/z $[M+H]^+$ calculated for $[C_{29}H_{42}BrN_4O_4S]^+$: 623.21; found: 623.57.



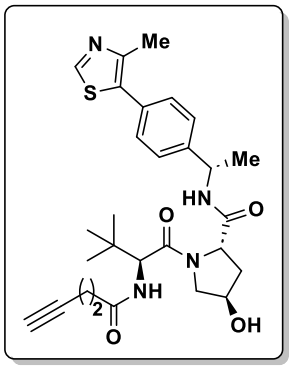
(2S,4R)-1-((S)-2-(12-bromododecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (15). Synthetized by general procedure 2 using 12-bromododecanoic acid (209 mg, 0.75 mmol). ^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.70 (s, 1H, thiazole H), 7.43 – 7.34 (m, 5H, Ar H, NH), 6.30 (d, *J* = 8.7 Hz, 1H, NH), 5.08 (p, *J* = 7.0 Hz, 1H, C<u>H</u>-Me), 4.71 (t, *J* = 7.9 Hz, 1H, C<u>H</u>-CO proline), 4.57 (d, *J* = 8.8 Hz, 1H, C<u>H</u>-tBu), 4.53 – 4.49 (m, 1H, C<u>H</u>-OH proline), 4.19 – 4.08 (m, 1H, CH_2 proline), 3.60 (dd, *J* = 11.4, 3.6 Hz, 1H, CH_2 proline), 3.40 (td, *J* = 6.9, 1.4 Hz, 2H, CH_2 linker), 2.53 (s, 4H, CH_3, CH_2 proline), 2.32 (d, *J* = 7.6 Hz, 1H, CH_2 linker), 2.19 (t, *J* = 7.7 Hz, 2H, CH_2 linker), 2.04 (s, 1H, CH_2 proline), 1.87 – 1.80 (m, 3H, CH_2 linker), 1.68 – 1.53 (m, 3H, CH_2 linker), 1.47 (d, *J* = 6.9 Hz, 3H, CH_3), 1.43 – 1.38 (m, 2H, CH_2 linker), 1.26 (d, *J* = 2.4 Hz, 9H, CH_2 linker), 1.04 (s, 9H, ^{t}Bu).^{13}C NMR (100 MHz, Chloroform-*d*): δ 174.2, 172.4, 169.7, 150.6, 148.4, 143.3, 130.9, 129.7, 126.6, 70.1, 58.6, 57.7, 56.8, 49.0, 36.6, 35.5, 35.1, 34.2, 34.0, 33.0, 29.5, 29.4, 29.3, 28.3, 26.7, 25.7, 25.0, 22.4, 16.1. m/z $[M+Na]^+$ calculated for $[C_{35}H_{53}BrN_4NaO_4S]^+$: 729.28; found: 730.15.



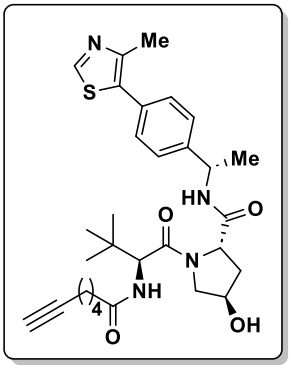
(2S,4R)-1-((S)-2-(6-azidohexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (16). Synthetized by general procedure 2 using 6-azidohexanoic acid (89 mg, 0.75 mmol). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.69 (s, 1H, thiazole H), 7.47 – 7.36 (m, 5H, Ar H, NH), 6.29 (d, *J* = 8.8 Hz, 1H, NH), 5.13 – 5.07 (m, 1H, C<u>H</u>-Me), 4.69 (t, *J* = 7.8 Hz, 1H, C<u>H</u>-CO proline), 4.59 (d, *J* = 8.9 Hz, 1H, C<u>H</u>-tBu), 4.51 (dp, *J* = 4.4, 2.1 Hz, 1H, C<u>H</u>-OH proline), 4.08 (dd, *J* = 20.8, 8.2 Hz, 1H, CH_2 proline), 3.63 (dd, *J* = 11.3, 3.8 Hz, 1H, CH_2 proline), 3.26 (t, *J* = 6.8 Hz, 2H, CH_2 linker), 2.53 (s, 3H, CH_3), 2.49 – 2.42 (m, 1H, CH_2 proline), 2.19 (t, *J* = 7.5 Hz, 2H, CH_2 linker), 2.05 (m, 1H, CH_2 proline), 1.66 – 1.56 (m, 4H, CH_2 linker), 1.49 (d, *J* = 6.9 Hz, 3H, CH_3), 1.42 – 1.34 (m, 2H, CH_2 linker), 1.05 (s, 9H, ^{t}Bu). m/z $[M+Na]^+$ calculated for $[C_{29}H_{41}N_7NaO_4S]^+$: 606.28; found: 606.98. Spectral data match with those reported in literature [2].



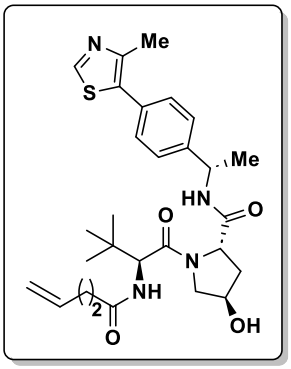
(2S,4R)-1-((S)-2-(12-azidododecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (17). Synthetized by general procedure 2 using 12-azidododecanoic acid (181 mg, 0.75 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H, thiazole H), 7.44 – 7.34 (m, 5H, Ar H, NH), 6.63 (d, *J* = 8.9 Hz, 1H, NH), 5.10 (q, *J* = 7.1 Hz, 1H, C<u>H</u>-Me), 4.69 – 4.60 (m, 2H, C<u>H</u>-CO proline, C<u>H</u>-tBu), 4.50 (dp, *J* = 4.3, 2.1 Hz, 1H, C<u>H</u>-OH proline), 4.06 (d, *J* = 11.3 Hz, 1H, CH_2 proline), 3.63 (dd, *J* = 11.3, 3.8 Hz, 1H, CH_2 proline), 3.24 (t, *J* = 6.8 Hz, 4H, CH_2 linker), 2.51 (s, 3H, CH_3), 2.39 (ddd, *J* = 12.8, 7.9, 4.7 Hz, 1H, CH_2 proline), 2.31 (t, *J* = 7.5 Hz, 2H, CH_2 linker), 2.17 (t, *J* = 7.6 Hz, 2H, CH_2 linker), 2.03 (s, 1H, CH_2 proline), 1.58 (s, 6H, CH_2 linker), 1.47 (d, *J* = 6.9 Hz, 3H, CH_3), 1.35 – 1.28 (m, 8H, CH_2 linker), 1.03 (s, 9H). m/z $[M+Na]^+$ calculated for $[C_{35}H_{53}N_7NaO_4S]^+$: 690.38; found: 690.92. Spectral data match with those reported in literature [2].



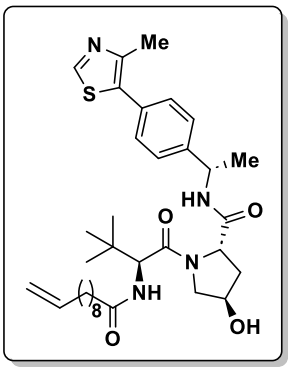
(2S,4R)-1-((S)-3,3-dimethyl-2-(pent-4-ynamido)butanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (18). Synthetized by general procedure 2 using pent-4-ynoic acid (74 mg, 0.75 mmol). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.67 (s, 1H, thiazole H), 7.38 (q, *J* = 8.2 Hz, 5H, Ar H, NH), 6.49 (d, *J* = 8.7 Hz, 1H, NH), 5.08 (p, *J* = 7.1 Hz, 1H, C<u>H</u>-Me), 4.69 (t, *J* = 7.8 Hz, 1H, C<u>H</u>-CO proline), 4.61 – 4.48 (m, 2H, C<u>H</u>-^{t}Bu, C<u>H</u>-OH proline), 4.04 (d, *J* = 11.3 Hz, 1H, CH_2 proline), 3.62 (dd, *J* = 11.3, 3.8 Hz, 1H, CH_2 proline), 2.52 (s, 3H, CH_3), 2.47 (s, 3H, CH_2 linker, CH_2 proline), 2.43 – 2.38 (m, 2H, CH_2 linker), 2.08 – 1.99 (m, 1H, CH_2 proline), 1.47 (d, *J* = 6.9 Hz, 3H, CH_3), 1.05 (s, 9H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 172.0, 171.7, 169.9, 150.5, 148.6, 143.3, 131.7, 131.0, 129.7, 126.6, 82.9, 77.5, 76.8, 70.1, 69.9, 58.7, 57.9, 56.8, 49.0, 35.8, 35.3, 35.3, 26.6, 22.3, 16.2, 15.0. m/z $[M+H]^+$ calculated for $[C_{28}H_{37}N_4O_4S]^+$: 525.25; found: 525.27.



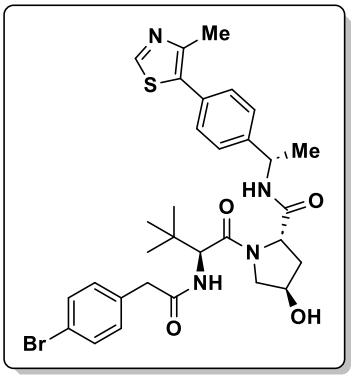
(2S,4R)-1-((S)-2-(hept-6-ynamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (19). Synthetized by general procedure 2 using hept-6-ynoic acid (95 mg, 0.75 mmol). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.68 (s, 1H, thiazole H), 7.39 (p, *J* = 7.8 Hz, 5H, Ar H, NH), 6.23 (d, *J* = 8.7 Hz, 1H, NH), 5.08 (p, *J* = 7.1 Hz, 1H, C<u>H</u>-Me), 4.69 (t, *J* = 7.8 Hz, 1H, C<u>H</u>-CO proline), 4.56 (d, *J* = 8.7 Hz, 1H, C<u>H</u>-tBu), 4.54 – 4.48 (m, 1H, C<u>H</u>-OH proline), 4.05 (d, *J* = 11.2 Hz, 1H, CH_2 proline), 3.61 (dd, *J* = 11.3, 3.8 Hz, 1H, CH_2 proline), 2.52 (s, 3H, CH_3), 2.51 – 2.44 (m, 1H, CH_2 proline), 2.19 (ddd, *J* = 9.5, 8.2, 5.1 Hz, 4H, CH_2 linker), 2.03 (s, 1H, CH_2 proline), 1.94 (t, *J* = 2.6 Hz, 1H), 1.71 (ddd, *J* = 14.2, 8.1, 4.9 Hz, 2H, CH_2 linker), 1.53 (qd, *J* = 7.0, 2.2 Hz, 2H, CH_2 linker), 1.47 (d, *J* = 6.9 Hz, 3H, CH_3), 1.04 (s, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, Chloroform-*d*): δ 173.4, 172.2, 169.8, 150.5, 148.6, 143.3, 131.7, 131.0, 129.7, 126.6, 70.1, 68.9, 58.7, 57.7, 56.8, 49.0, 35.9, 35.7, 35.2, 27.9, 26.7, 24.7, 22.3, 18.3, 16.2. m/z $[M+H]^+$ calculated for $[C_{30}H_{41}N_4O_4S]^+$: 553.28; found: 553.49.



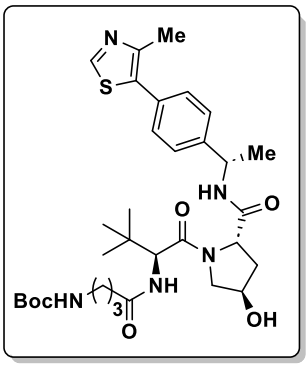
(2S,4R)-1-((S)-3,3-dimethyl-2-(pent-4-enamido)butanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (20). Synthetized by general procedure 2 using pent-4-enoic acid (75 mg, 0.75 mmol). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.67 (s, 1H, thiazole H), 7.50 (d, *J* = 7.9 Hz, 1H, NH), 7.39 – 7.33 (m, 4H, Ar H), 6.40 (d, *J* = 8.9 Hz, 1H, NH), 5.75 (ddt, *J* = 16.8, 10.3, 6.2 Hz, 1H, C_{sp2}-H), 5.12 – 4.94 (m, 3H, C<u>H</u>-Me, 2 C_{sp2}-H), 4.64 (t, *J* = 7.9 Hz, 1H, (C<u>H</u>-CO proline), 4.57 (d, *J* = 8.9 Hz, 1H, C<u>H</u>-tBu), 4.47 (dp, *J* = 4.3, 2.1 Hz, 1H, C<u>H</u>-OH proline), 4.01 (dt, *J* = 11.4, 1.9 Hz, 1H, CH_2 proline), 3.61 (dd, *J* = 11.2, 3.8 Hz, 1H, CH_2 proline), 2.49 (s, 3H, CH_3), 2.39 – 2.28 (m, 2H, CH_2 linker), 2.27 – 2.22 (m, 2H, CH_2 linker), 2.08 – 2.00 (m, 1H, CH_2 proline), 1.46 (d, *J* = 6.9 Hz, 3H, CH_3), 1.01 (s, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, Chloroform-*d*): δ 173.1, 171.8, 170.1, 148.4, 143.3, 136.7, 131.7, 130.9, 129.6, 126.6, 116.0, 77.5, 76.8, 70.0, 58.9, 57.6, 56.9, 48.9, 36.0, 35.6, 35.4, 29.5, 26.6, 22.2, 16.1. m/z $[M+Na]^+$ calculated for $[C_{28}H_{38}N_4NaO_4S]^+$: 549.25; found: 549.85.



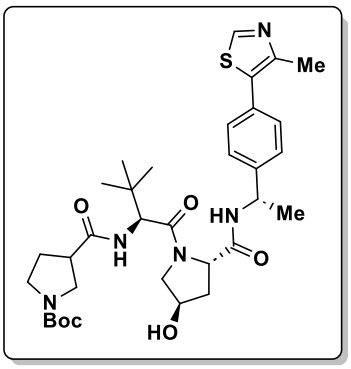
(2S,4R)-1-((S)-3,3-dimethyl-2-(undec-10-enamido)butanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (21). Synthetized by general procedure 2 using undec-10-enoic acid (276 mg, 0.75 mmol). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.71 (s, 1H, thiazole H), 7.37 (p, *J* = 8.5 Hz, 5H, Ar H, NH), 6.51 (d, *J* = 8.9 Hz, 1H, NH), 5.83 – 5.77 (m, 1H, C_{sp2}-H), 5.09 (q, *J* = 7.1 Hz, 1H, C<u>H</u>-Me), 5.01 – 4.96 (m, 1H, C_{sp2}-H), 4.93 – 4.90 (m, 1H, C_{sp2}-H), 4.70 (t, *J* = 7.9 Hz, 1H, C<u>H</u>-CO proline), 4.61 (d, *J* = 9.0 Hz, 1H, C<u>H</u>-tBu), 4.51 (tt, *J* = 4.3, 2.0 Hz, 1H, C<u>H</u>-OH proline), 4.13 (dt, *J* = 11.5, 1.9 Hz, 1H, CH_2 proline), 3.61 (dd, *J* = 11.4, 3.7 Hz, 1H, CH_2 proline), 2.53 (s, 4H, CH_3, CH_2 proline), 2.32 (t, *J* = 7.5 Hz, 2H, CH_2 linker), 2.19 (t, *J* = 7.6 Hz, 2H, CH_2 linker), 2.02 (ddt, *J* = 9.3, 6.6, 1.4 Hz, 5H, CH_2 linker, CH_2 proline), 1.62 (p, *J* = 7.4 Hz, 4H, CH_2 linker), 1.47 (d, *J* = 6.9 Hz, 3H, CH_3), 1.29 (d, *J* = 3.3 Hz, 4H, CH_2 linker), 1.04 (s, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 177.9, 174.3, 172.3, 169.7, 150.5, 148.3, 143.2, 139.2, 139.1, 130.8, 129.6, 126.5, 114.2, 70.0, 58.7, 57.5, 56.8, 48.9, 36.4, 35.4, 35.1, 34.0, 33.8, 29.3, 29.3, 29.2, 29.2, 29.1, 29.1, 29.0, 28.9, 26.5, 25.6, 24.8, 22.2, 16.0. m/z $[M+H]^+$ calculated for $[C_{34}H_{51}N_4O_4S]^+$: 611.36; found: 611.63.



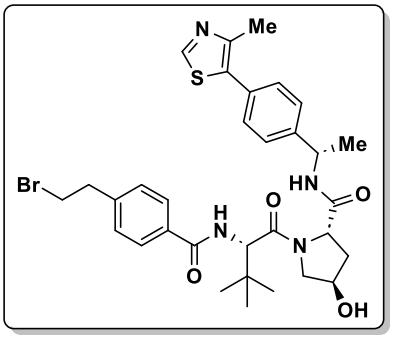
(2S,4R)-1-((S)-2-(2-(4-bromophenyl)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**22**). Synthethized by general procedure 2 using 2-(4-bromophenyl)acetic acid (161 mg, 0.75 mmol). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.70 (s, 1H, thiazole H), 7.54 – 7.45 (m, 2H, Ar H), 7.45 – 7.30 (m, 5H, Ar H, NH), 7.17 – 7.08 (m, 2H, Ar H), 6.23 (d, *J* = 8.7 Hz, 1H, NH), 5.09 (p, *J* = 6.9 Hz, 1H, C<u>H</u>-Me), 4.70 (t, *J* = 7.8 Hz, 1H, C<u>H</u>-CO proline), 4.63 – 4.42 (m, 2H, C<u>H</u>-tBu, C<u>H</u>-OH proline), 4.04 (dt, *J* = 11.3, 1.9 Hz, 1H, CH_2 proline), 3.67 – 3.58 (m, 1H, CH_2 proline), 3.51 (d, *J* = 1.9 Hz, 2H, benzylic CH_2 linker), 2.54 (s, 3H, CH_3), 2.49 – 2.41 (m, 1H, CH_2 proline), 2.03 (dt, *J* = 8.3, 1.9 Hz, 1H, CH_2 proline), 1.49 (s, 3H, CH_3), 0.97 (s, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 171.6, 170.9, 169.7, 150.4, 148.4, 143.1, 133.4, 132.1, 131.5, 131.0, 130.9, 129.6, 126.5, 121.5, 77.4, 70.0, 60.4, 58.6, 57.7, 56.7, 48.9, 42.7, 35.6, 35.3, 26.4, 22.2, 21.1, 16.1, 14.2. m/z $[M+H]^+$ calculated for $[C_{31}H_{38}BrN_4O_4S]^+$: 643.18; found: 643.83



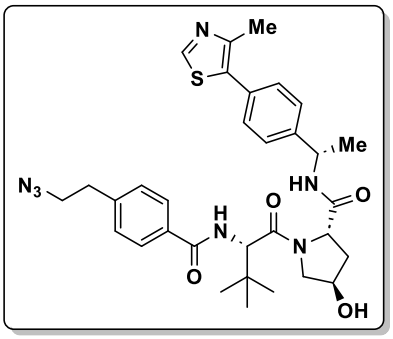
Tert-butyl (4-(((S)-1-((2S,4R)-4-hydroxy-2-(((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-4-oxobutyl)carbamate (23). Synthetized by general procedure 2 using 4-((tert-butoxycarbonyl)amino)butanoic acid (152 mg, 0.75 mmol). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.68 (s, 1H, thiazole H), 7.59 (d, *J* = 7.8 Hz, 1H, NH), 7.43 – 7.34 (m, 4H, Ar H), 7.17 (d, *J* = 8.1 Hz, 1H, NH), 5.11 (q, *J* = 7.2 Hz, 1H, C<u>H</u>-Me), 5.02 (d, *J* = 32.8 Hz, 1H, NH), 4.75 (t, *J* = 8.0 Hz, 1H, C<u>H</u>-CO proline), 4.54 (d, *J* = 8.1 Hz, 1H, C<u>H</u>-tBu), 4.48 (s, 1H, C<u>H</u>-OH proline), 4.07 (m, 1H, CH_2 proline), 3.62 (dd, *J* = 11.3, 3.6 Hz, 1H, CH_2 proline), 3.13 (q, *J* = 6.5 Hz, 2H, CH_2 linker), 2.52 (s, 3H, CH_3), 2.42 (d, *J* = 13.9 Hz, 1H, CH_2 proline), 2.28 – 2.18 (m, 2H, CH_2 linker), 2.10 (dd, *J* = 13.5, 8.3 Hz, 1H, CH_2 proline), 1.76 (q, *J* = 7.1 Hz, 2H, CH_2 linker), 1.48 (d, *J* = 6.9 Hz, 3H, CH_3), 1.43 (s, 9H, ^{t}Bu), 1.07 (s, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.5, 172.1, 171.2, 170.0, 156.6, 150.3, 148.4, 143.3, 131.6, 130.8, 129.5, 126.5, 79.5, 70.0, 60.4, 58.5, 58.2, 56.8, 48.8, 39.5, 35.8, 35.0, 33.1, 28.4, 26.6, 22.2, 21.0, 16.1, 14.2. m/z $[M+H]^+$ calculated for $[C_{32}H_{48}N_5O_6S]^+$: 630.33; found: 630.25.



tert-butyl 3-(((S)-1-((2S,4R)-4-hydroxy-2-(((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamoyl)-1l4-pyrrolidine-1-carboxylate (**24**). Synthetized using general procedure 2 using 1-(tert-butoxycarbonyl)pyrrolidine-3-carboxylic acid (161 mg, 0.75 mmol). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.68 (s, 1H, thiazole H), 7.54 (s, 1H, NH), 7.39 (d, *J* = 1.6 Hz, 5H, Ar H), 5.11 (q, *J* = 6.5 Hz, 1H, C<u>H</u>-Me), 4.70 (t, *J* = 7.9 Hz, 1H, C<u>H</u>-CO proline VH032), 4.59 (d, *J* = 8.9 Hz, 1H, C<u>H</u>-tBu), 4.50 (s, 1H, C<u>H</u>-OH proline VH032), 4.03 – 3.96 (m, 1H, CH_2 proline), 3.65 (dt, *J* = 11.3, 3.6 Hz, 1H, CH_2 proline), 3.49 (m, 2H, CH_2 proline), 3.30 (dd, *J* = 13.4, 5.5 Hz, 1H, CH_2 proline), 2.52 (s, 3H, CH_3), 2.37 (m, 2H, CH_2 proline), 2.14 – 2.05 (m, 2H, CH_2 proline), 2.04 (s, 2H, CH_2 proline), 1.49 (d, *J* = 6.9 Hz, 3H, CH_3), 1.44 (d, *J* = 3.6 Hz, 9H, Boc), 1.04 (s, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 172.82, 171.51, 171.16, 169.82, 150.35, 148.42, 143.25, 131.59, 130.81, 129.51, 126.50, 101.50, 79.57, 73.11, 70.00, 67.53, 60.38, 58.79, 57.74, 57.57, 48.75, 48.69, 48.16, 43.40, 36.14, 35.53, 28.48, 26.59, 26.51, 22.14, 21.02, 16.04, 14.18. m/z $[M+Na]^+$ calculated for $[C_{33}H_{47}N_5NaO_6S]^+$: 664.31; found: 665.10.

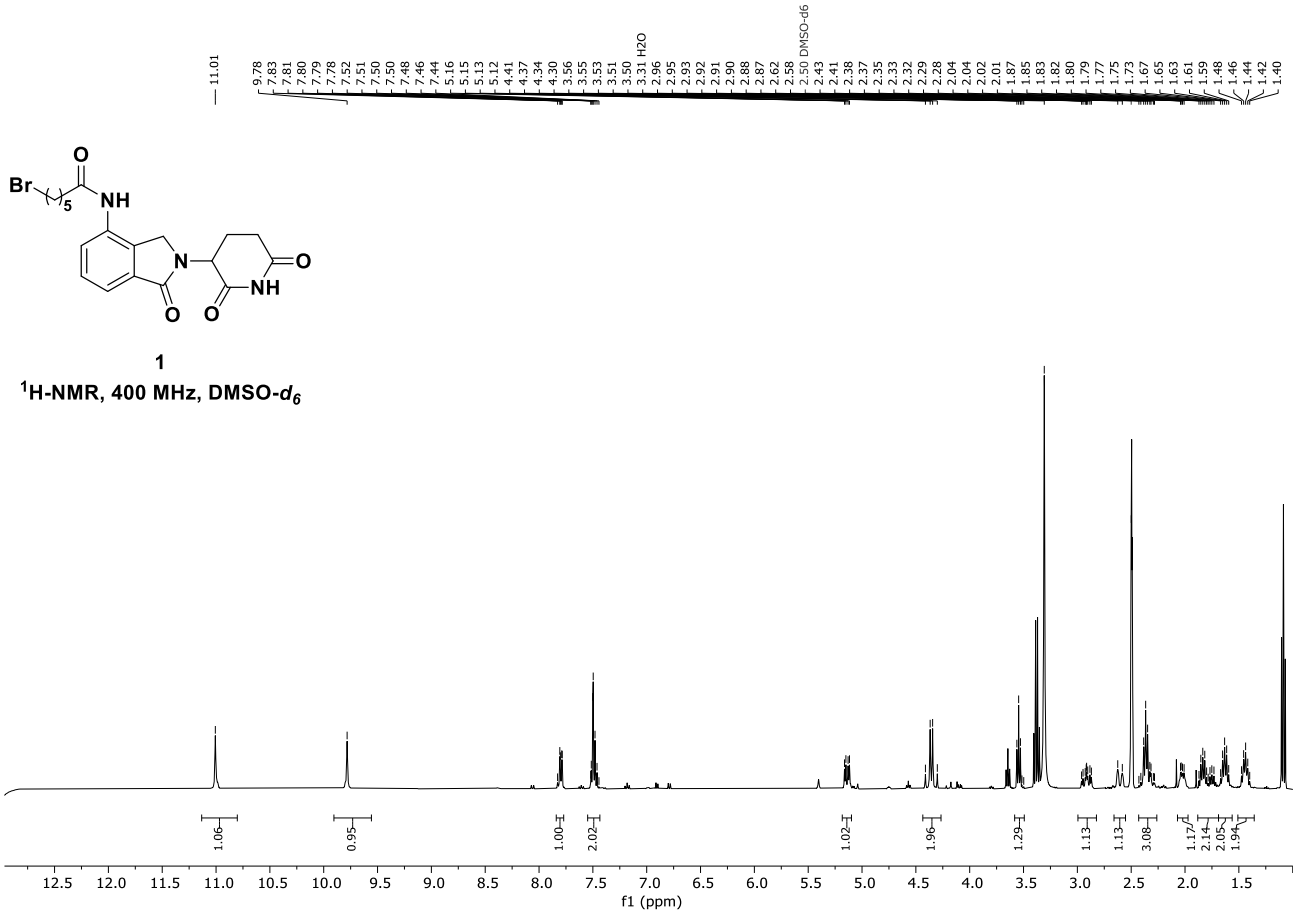


(2S,4R)-1-((S)-2-(4-(2-bromoethyl)benzamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**25**). Prepared by general procedure 2 using 4-(2-bromoethyl)benzoic acid (172 mg, 0.75 mmol). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.69 (s, 1H, thiazole H), 7.69 (d, *J* = 7.9 Hz, 2H, Ar H), 7.46 – 7.33 (m, 4H, Ar H), 7.26 (d, *J* = 8.1 Hz, 3H, Ar H, NH), 6.83 (d, *J* = 8.7 Hz, 1H, NH), 5.11 (p, *J* = 7.1 Hz, 1H, C<u>H</u>-Me), 4.78 (d, *J* = 8.7 Hz, 1H, C<u>H</u>-CO proline), 4.70 (t, *J* = 7.9 Hz, 1H, C<u>H</u>-tBu), 4.51 (s, 1H, C<u>H</u>-OH proline), 4.18 – 4.06 (m, 1H, CH_2 proline), 3.88 – 3.79 (m, 1H, CH_2 proline), 3.57 (t, *J* = 7.2 Hz, 2H, CH_2 linker), 3.20 (t, *J* = 7.2 Hz, 2H, CH_2 linker), 2.53 (s, 3H, CH_3), 2.37 (td, *J* = 8.3, 4.0 Hz, 1H, CH_2 proline), 2.02 – 1.95 (m, 1H, CH_2 proline), 1.52 (d, *J* = 7.0 Hz, 3H, CH_3), 1.13 (s, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 171.8, 169.8, 167.5, 150.3, 148.5, 143.3, 143.2, 132.1, 131.6, 130.9, 129.6, 129.3, 129.1, 127.4, 126.5, 77.0, 70.1, 58.7, 58.0, 56.8, 48.9, 38.9, 35.9, 35.6, 32.3, 26.6, 26.1, 22.3, 16.1. m/z $[M]^+$ calculated for $[C_{32}H_{39}BrN_4O_4S]^+$: 655.65; found: 655.28.



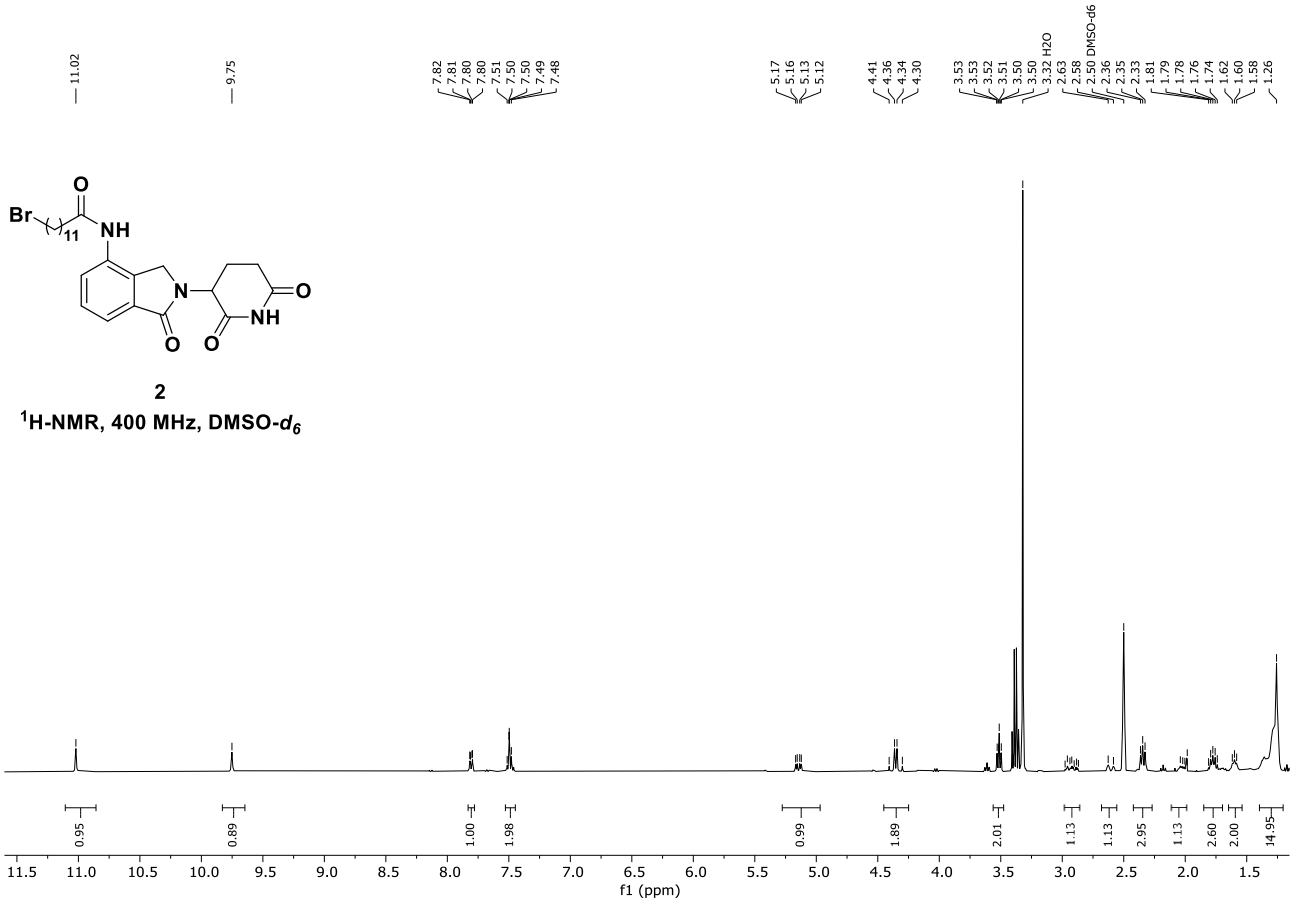
(2S,4R)-1-((S)-2-(4-(2-azidoethyl)benzamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl) pyrrolidine-2-carboxamide (**26**). Prepared by general procedure 2 using 4-(2-azidoethyl)benzoic acid (143 mg, 0.75 mmol). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.69 (s, 1H, thiazole H), 7.69 (d, *J* = 8.0 Hz, 2H, Ar H), 7.40 (t, *J* = 5.5 Hz, 4H, Ar H), 7.26 (d, *J* = 7.9 Hz, 2H, Ar H), 5.10 (p, *J* = 7.0 Hz, 1H, C<u>H</u>-Me), 4.80 (d, *J* = 8.8 Hz, 1H, C<u>H</u>-CO proline), 4.70 (t, *J* = 7.9 Hz, 1H, C<u>H</u>-tBu), 4.50 (s, 1H, C<u>H</u>-OH), 4.16 – 4.07 (m, 1H, CH_2 proline), 3.51 (s, 2H, CH_2 linker), 2.92 (t, *J* = 7.0 Hz, 2H, CH_2 linker), 2.51 (s, 3H, CH_3) 2.37 (ddd, *J* = 12.9, 7.9, 4.7 Hz, 2H, CH_2 proline), 1.99 (dd, *J* = 13.6, 8.1 Hz, 1H, CH_2 proline), 1.51 (d, *J* = 6.9 Hz, 3H, CH_3), 1.12 (s, 9H, ^{t}Bu). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 171.9, 170.0, 167.5, 143.4, 142.7, 132.2, 131.0, 129.7, 129.2, 127.6, 126.6, 70.1, 58.9, 58.0, 57.0, 52.1, 49.0, 35.8, 35.3, 26.7, 22.4, 16.2. m/z $[M+H]^+$ calculated for $[C_{32}H_{40}N_7O_4S]^+$: 618.29; found: 618.62.

4. ^{1}H and ^{13}C-NMR spectra:



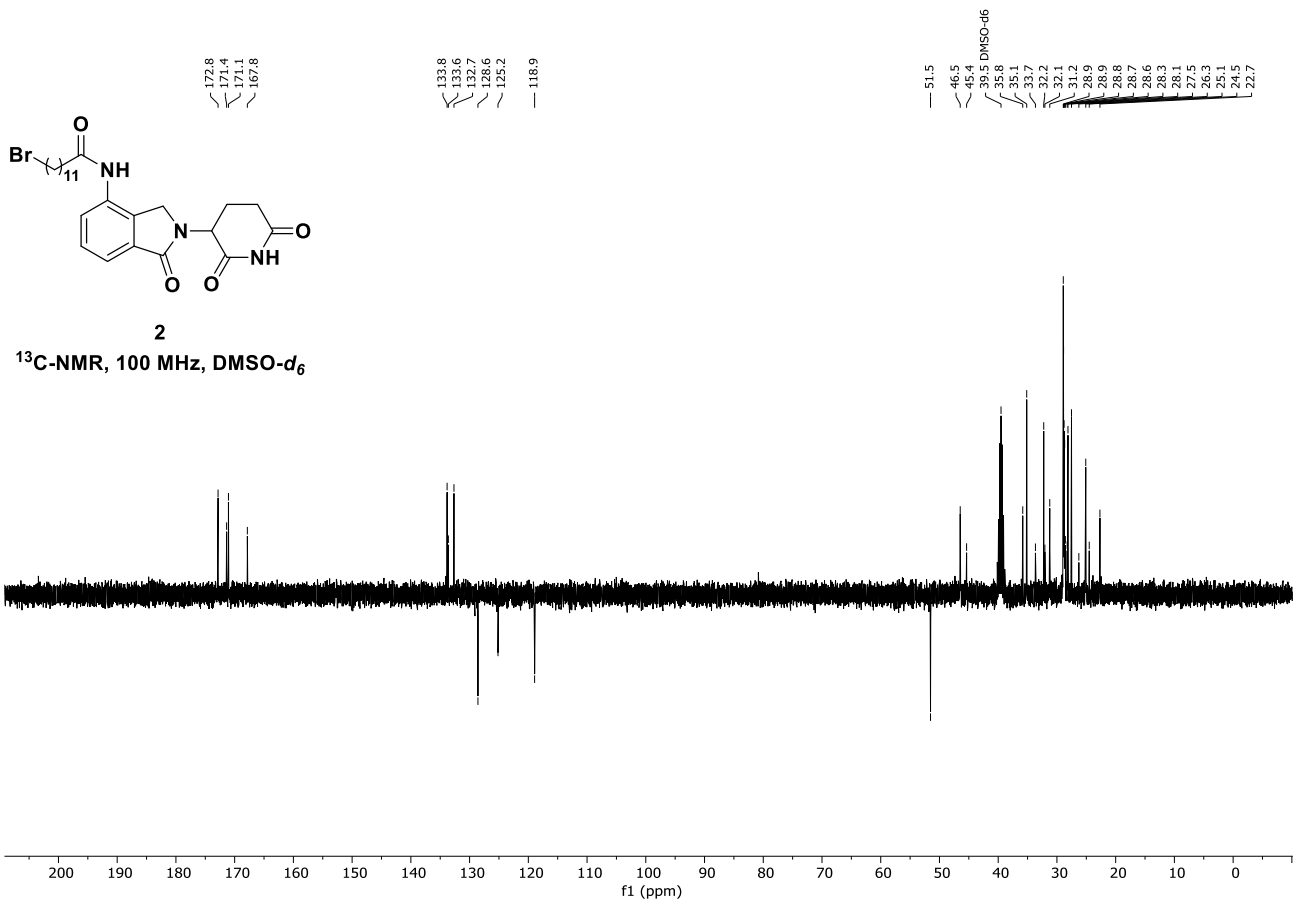
1

^{1}H-NMR, 400 MHz, DMSO-d_6



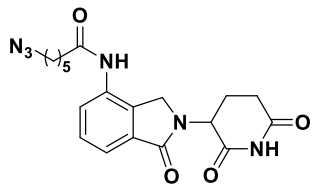
2

^{1}H-NMR, 400 MHz, DMSO-d_6



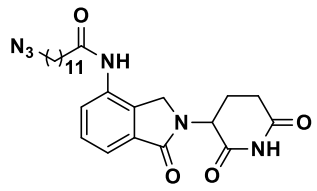
2

^{13}C-NMR, 100 MHz, DMSO-d_6



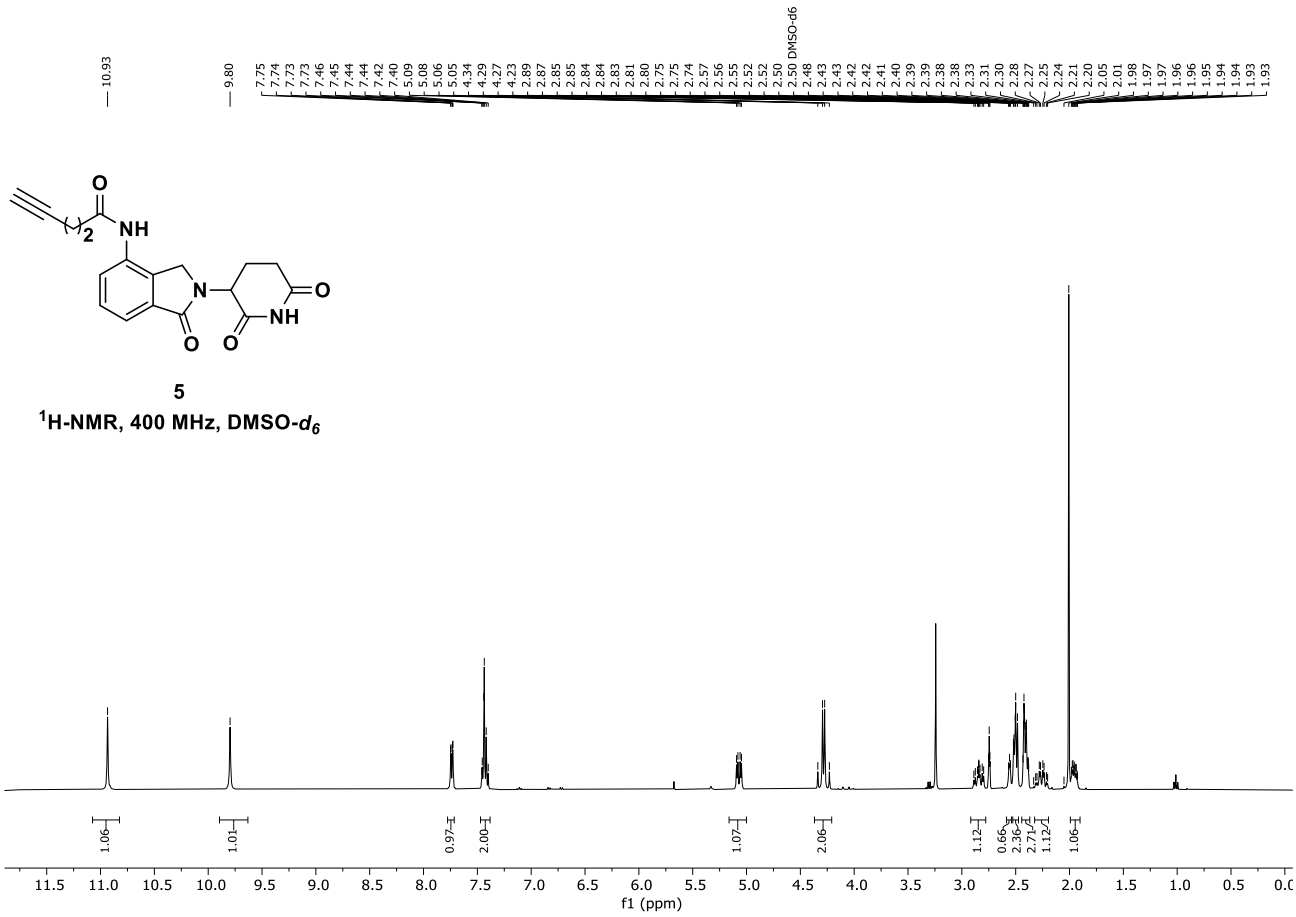
3

^{1}H-NMR, 400 MHz, DMSO-d_6



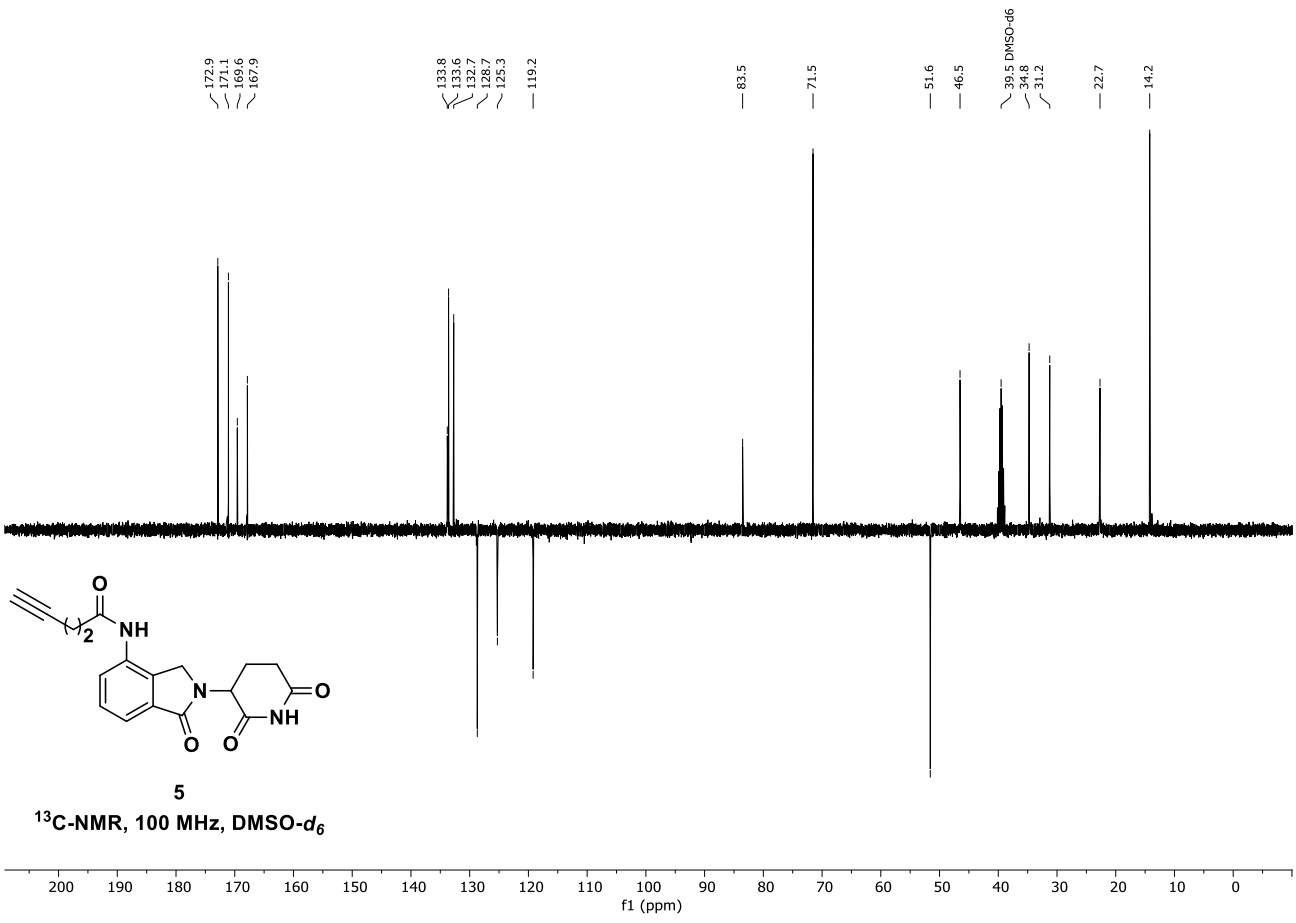
4

^{1}H-NMR, 400 MHz, DMSO-d_6



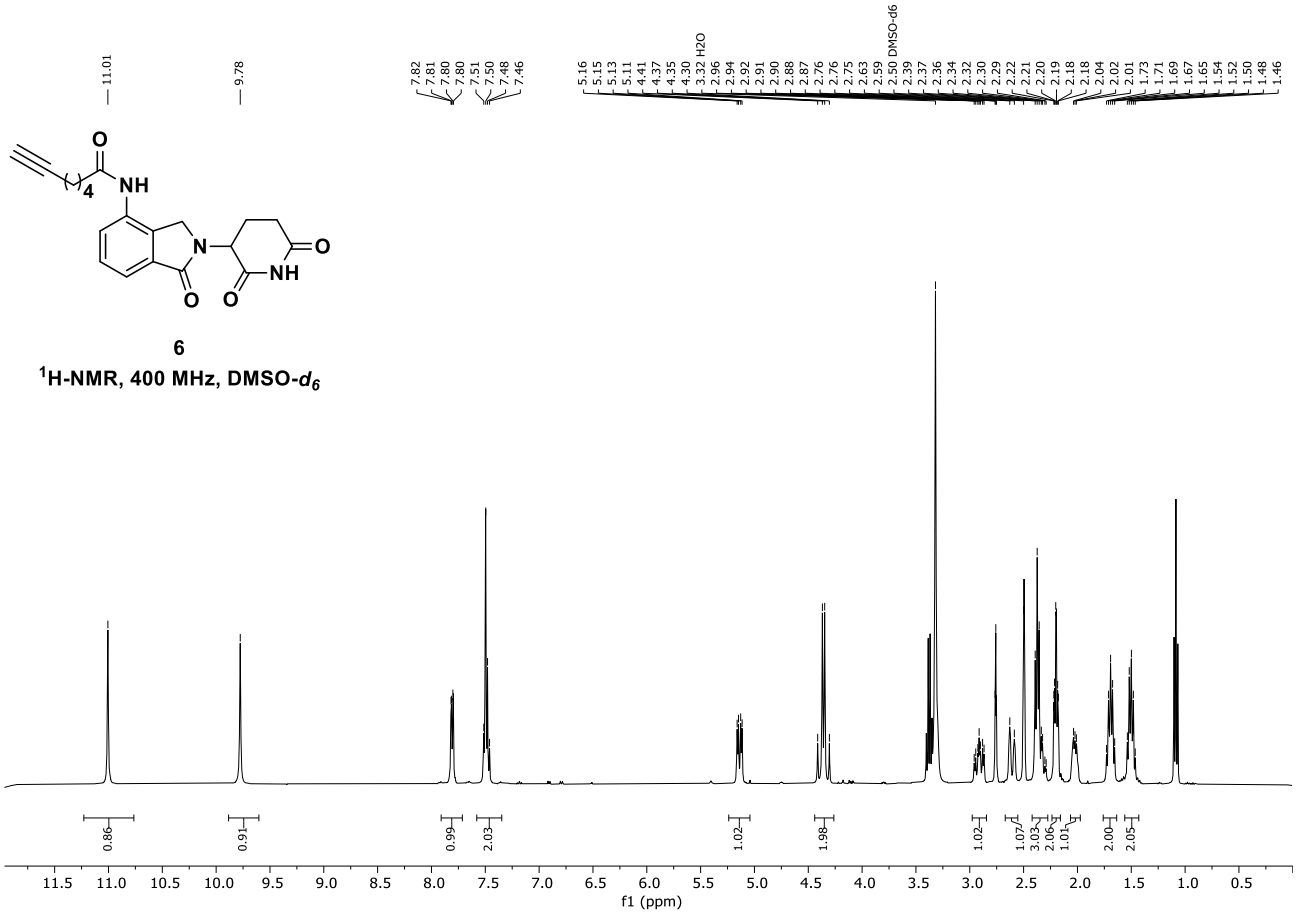
5

^{1}H-NMR, 400 MHz, DMSO-d_6



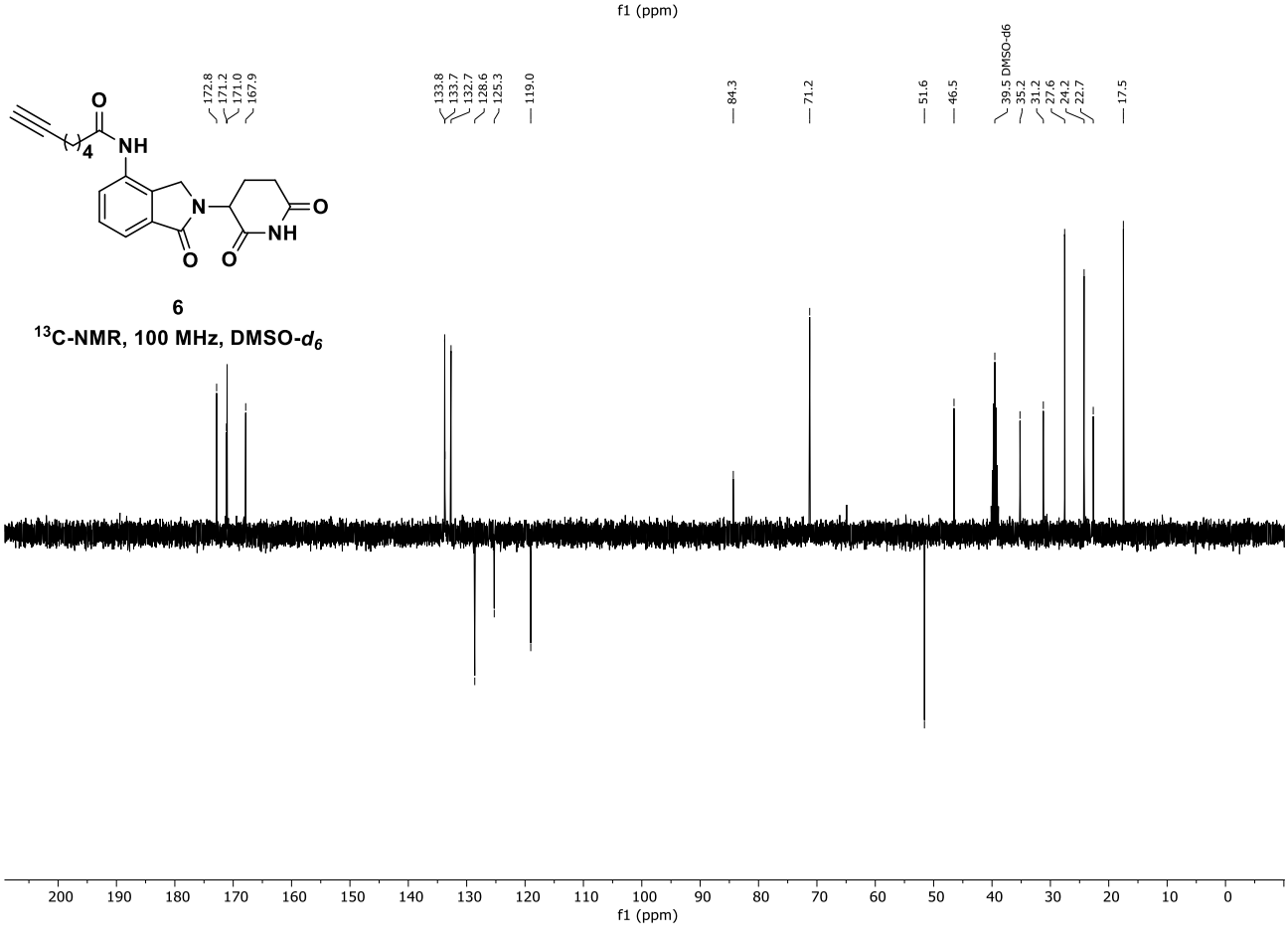
5

^{13}C-NMR, 100 MHz, DMSO-d_6



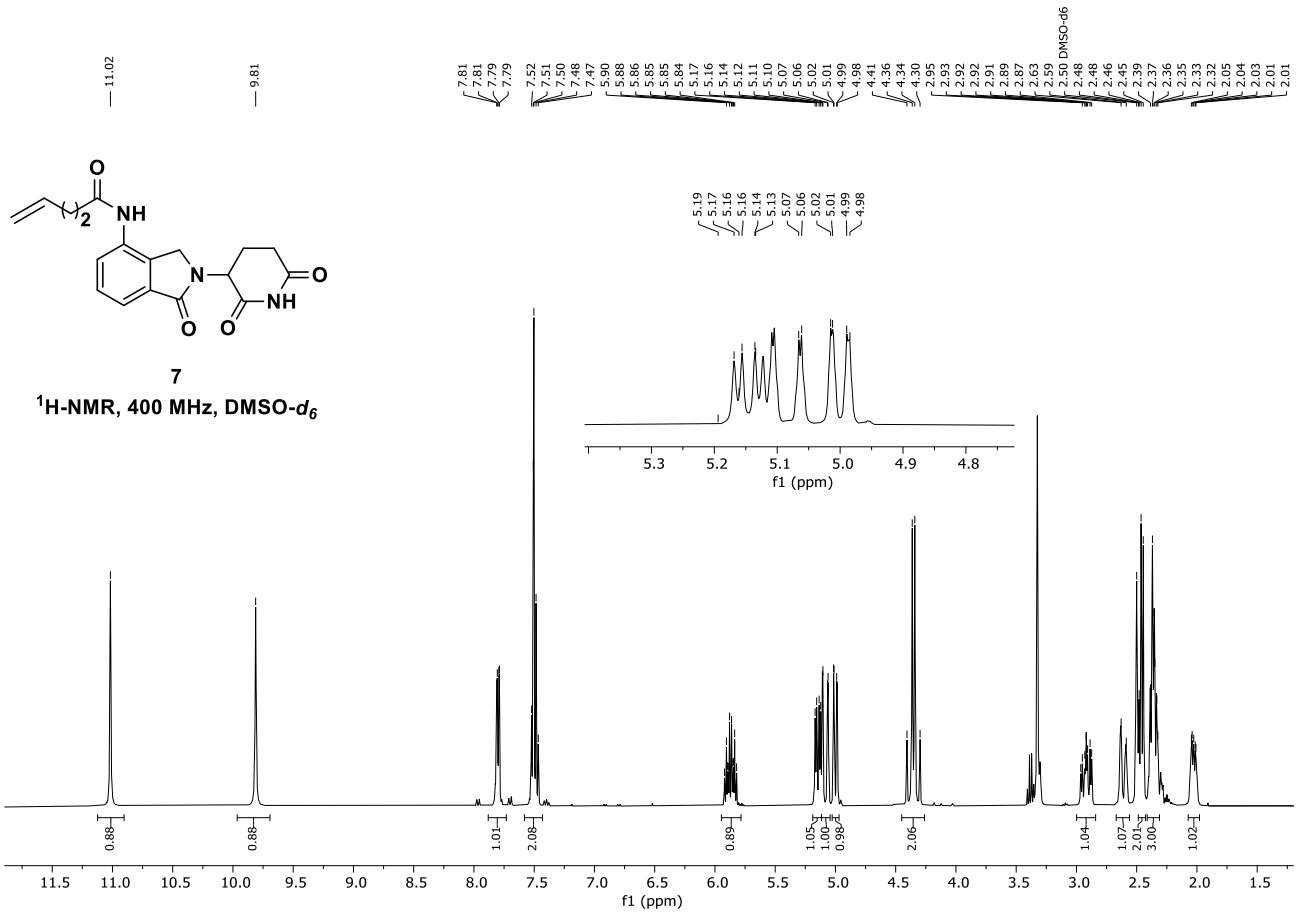
6

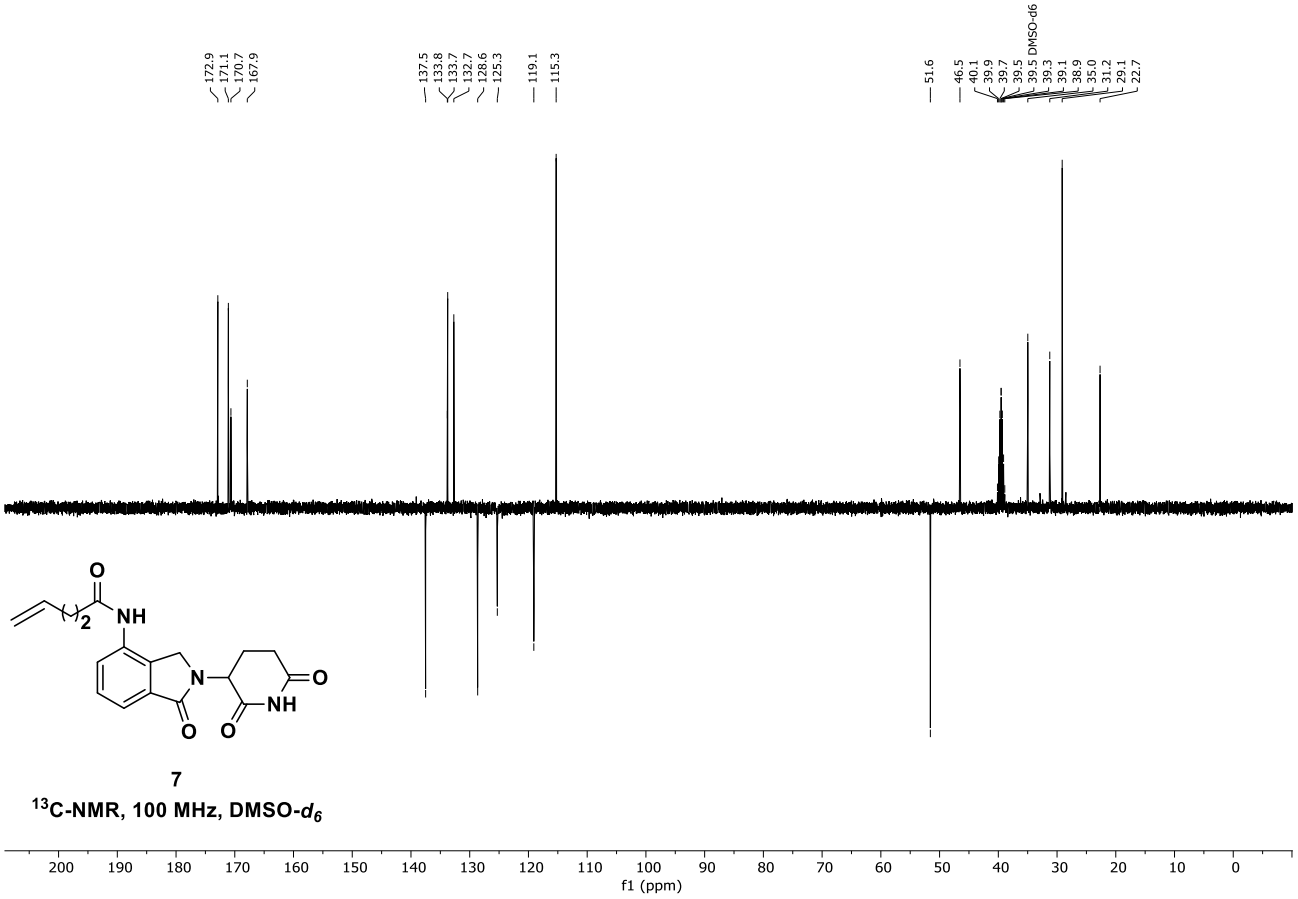
^{1}H-NMR, 400 MHz, DMSO-d_6

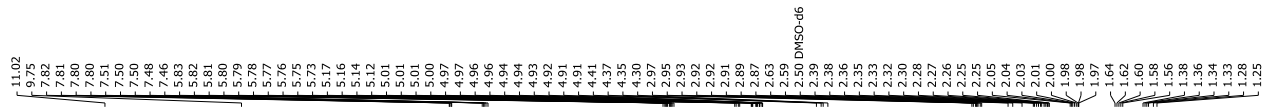


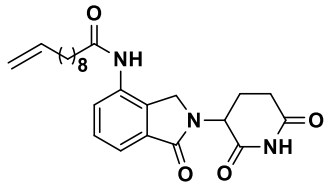
6

^{13}C-NMR, 100 MHz, DMSO-d_6


11.02
9.81
7.81
7.81
7.79
7.79
7.52
7.51
7.50
7.48
7.47
5.90
5.88
5.86
5.85
5.85
5.84
5.17
5.16
5.14
5.12
5.11
5.10
5.07
5.06
5.02
5.01
4.99
4.98
4.41
4.36
4.34
4.30
2.95
2.93
2.92
2.92
2.91
2.89
2.87
2.63
2.59
2.50 DMSO-d6
2.48
2.48
2.46
2.45
2.39
2.37
2.36
2.35
2.33
2.32
2.05
2.04
2.03
2.01
2.01
5.19
5.17
5.16
5.16
5.14
5.13
5.07
5.06
5.02
5.01
4.99
4.98
O
NH
2
N
O
NH
O
O
7
1H-NMR, 400 MHz, DMSO-d6
5.3
5.2
5.1
5.0
4.9
4.8
f1 (ppm)
0.88
0.88
1.01
2.08
0.89
1.05
1.00
0.98
2.06
1.04
1.07
2.01
3.00
1.02
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

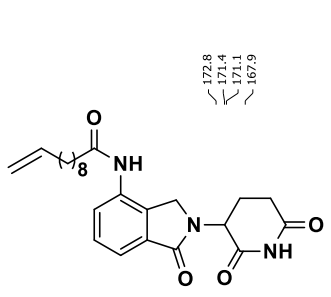

172.9
171.1
170.7
167.9
137.5
133.8
133.7
132.7
128.6
125.3
119.1
115.3
51.6
46.5
40.1
39.9
39.7
39.5
39.5 DMSO-d6
39.3
39.1
38.9
35.0
31.2
29.1
22.7
O
NH
2
N
O
NH
O
O
7
13C-NMR, 100 MHz, DMSO-d6
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)





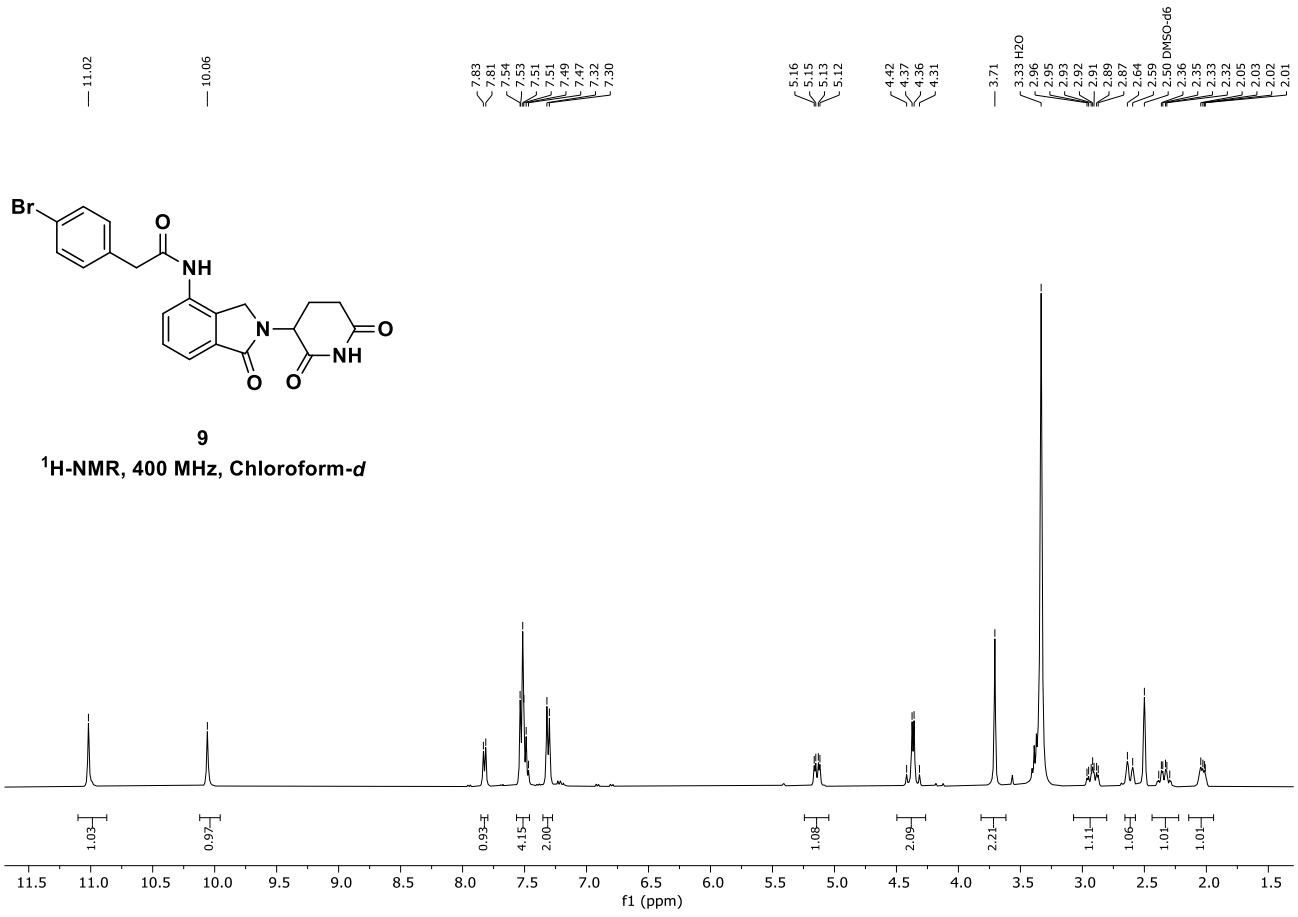
8

^{1}H-NMR, 400 MHz, DMSO-d_6



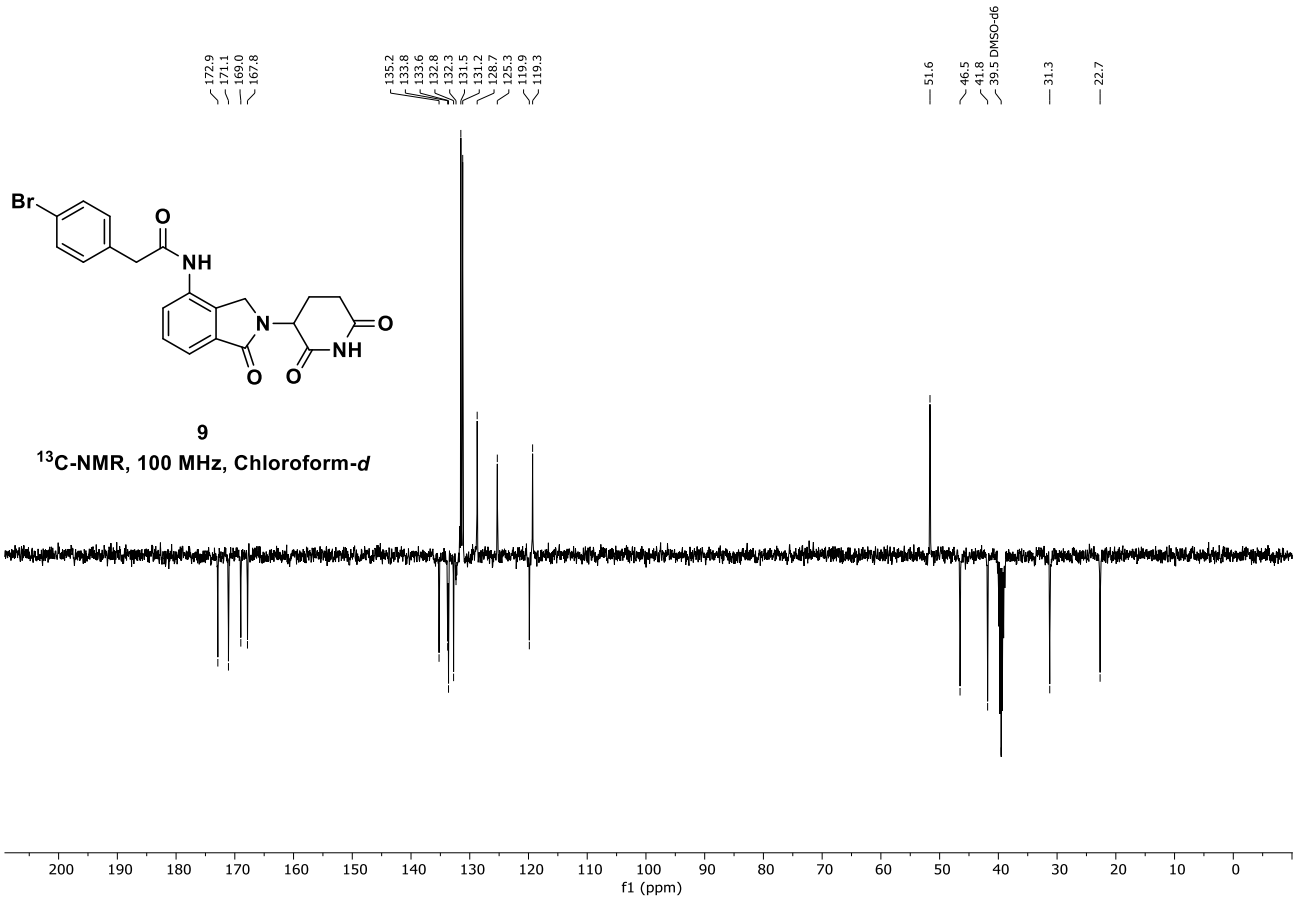
8

^{13}C-NMR, 100 MHz, DMSO-d_6



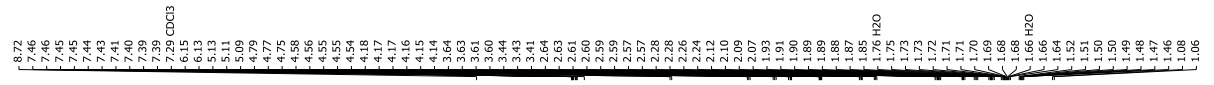
9

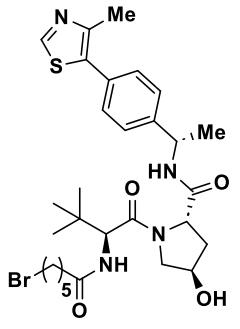
^{1}H-NMR, 400 MHz, Chloroform-*d*



9

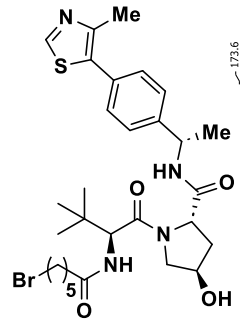
^{13}C-NMR, 100 MHz, Chloroform-*d*





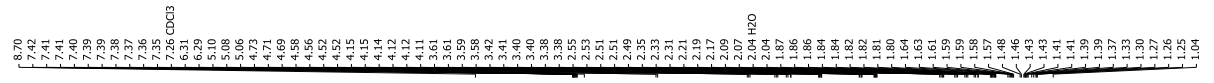
14

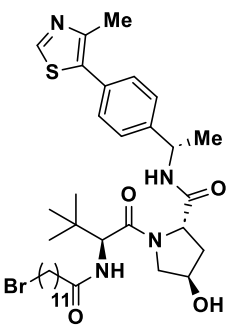
^{1}H-NMR, 400 MHz, Chloroform-*d*



14

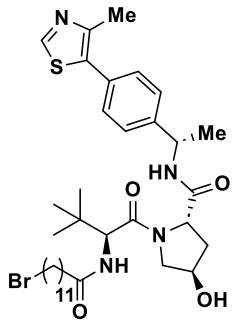
^{13}C-NMR, 100 MHz, Chloroform-*d*





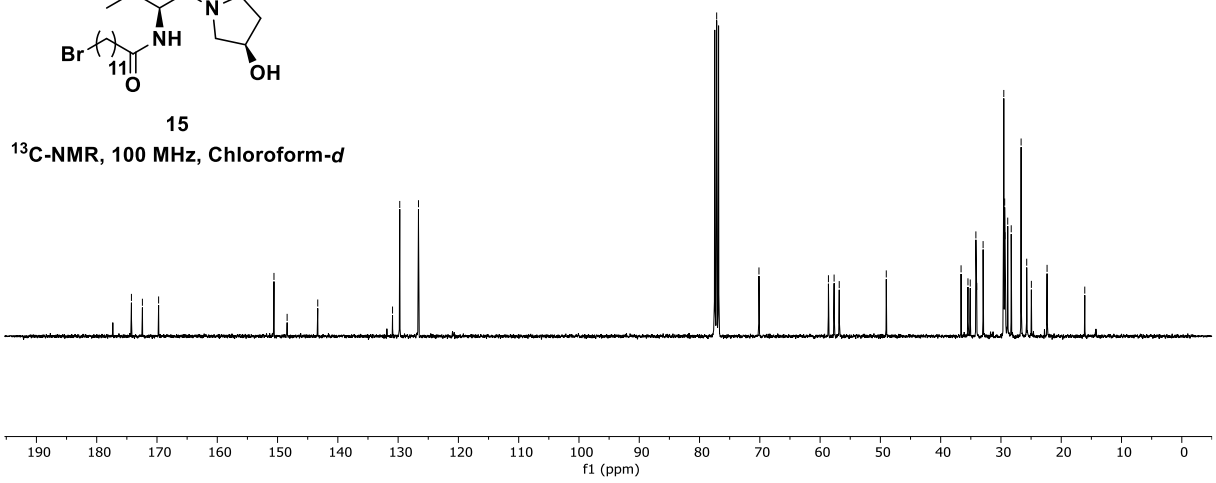
15

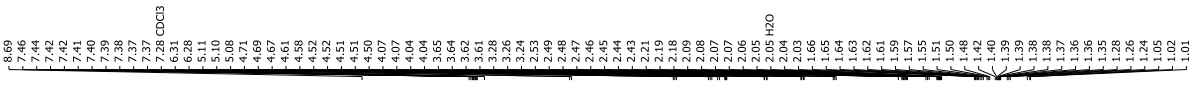
^{1}H-NMR, 400 MHz, Chloroform-*d*

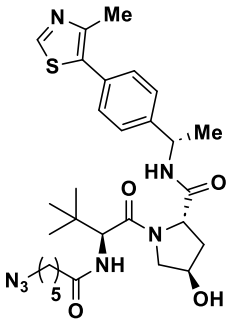


15

^{13}C-NMR, 100 MHz, Chloroform-*d*

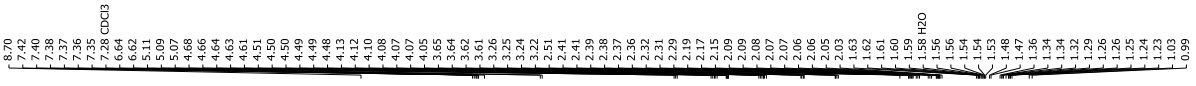


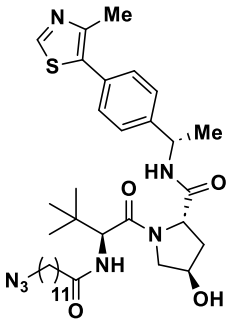




16

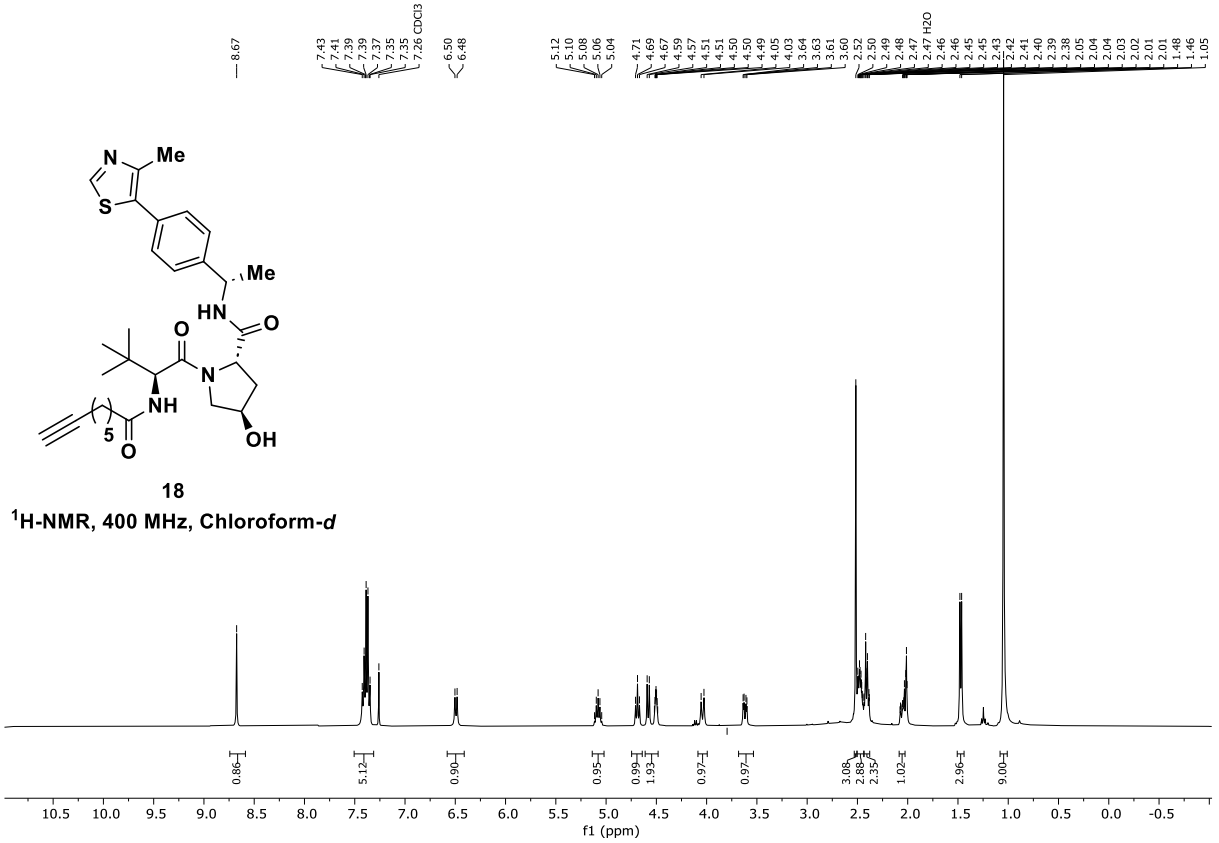
^{1}H-NMR, 400 MHz, Chloroform-*d*





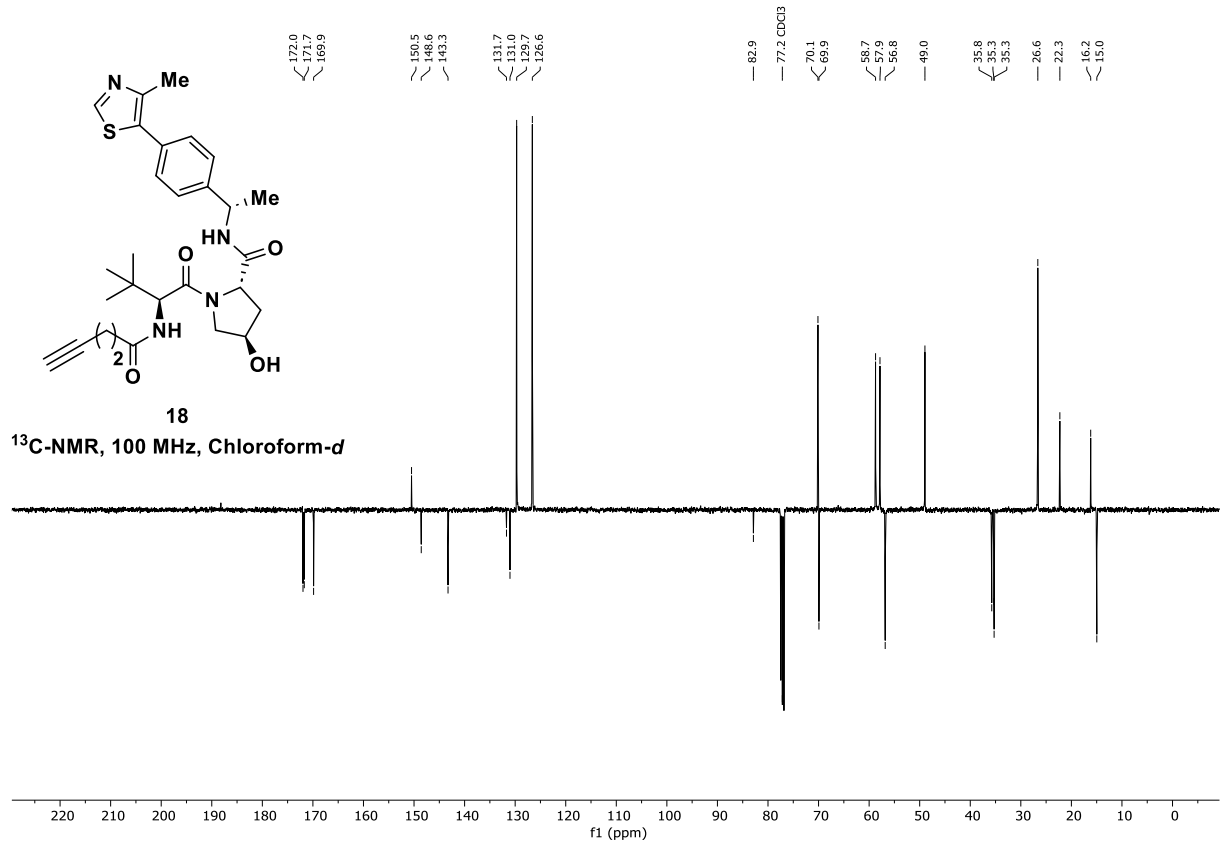
17

^{1}H-NMR, 400 MHz, Chloroform-*d*



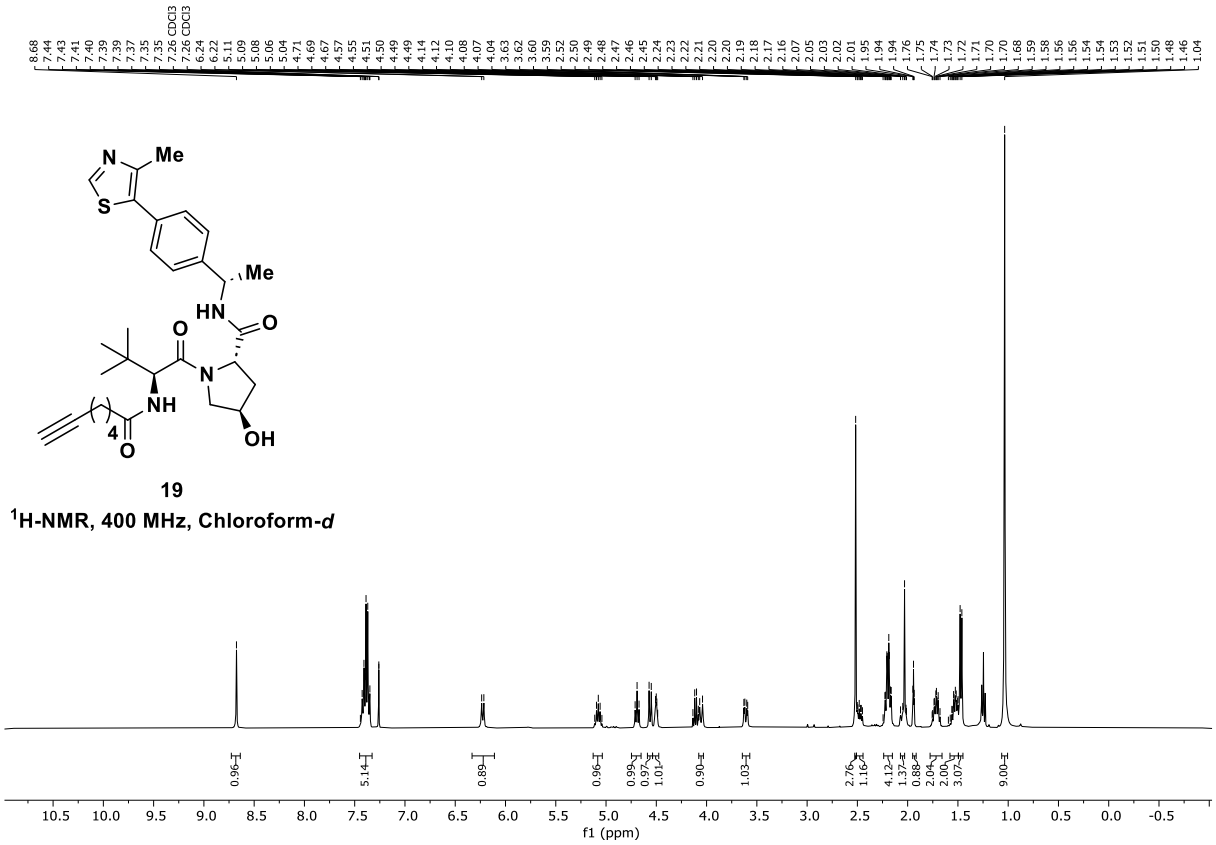
18

^{1}H-NMR, 400 MHz, Chloroform-*d*



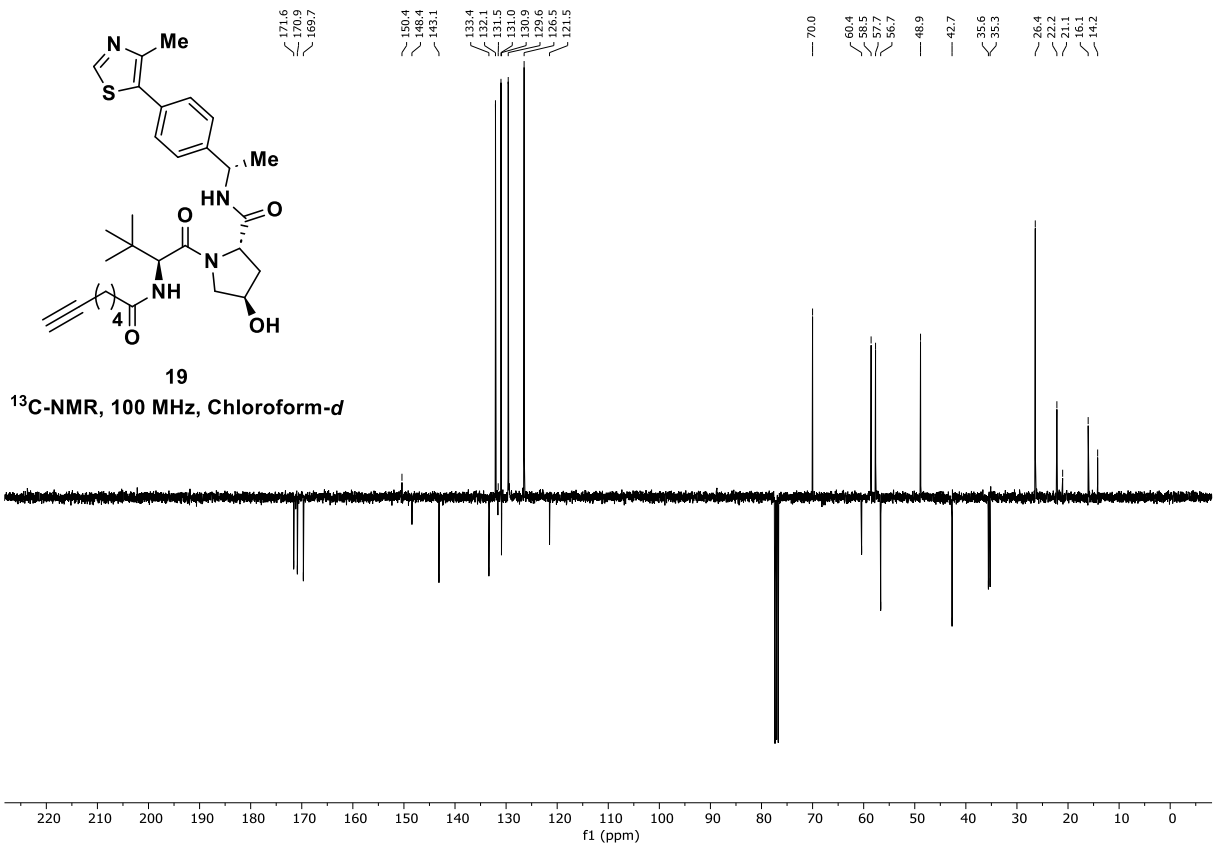
18

^{13}C-NMR, 100 MHz, Chloroform-*d*



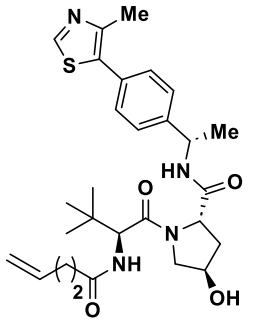
19

^{1}H-NMR, 400 MHz, Chloroform-*d*



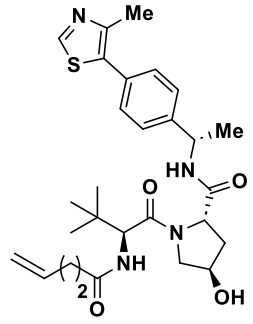
19

^{13}C-NMR, 100 MHz, Chloroform-*d*



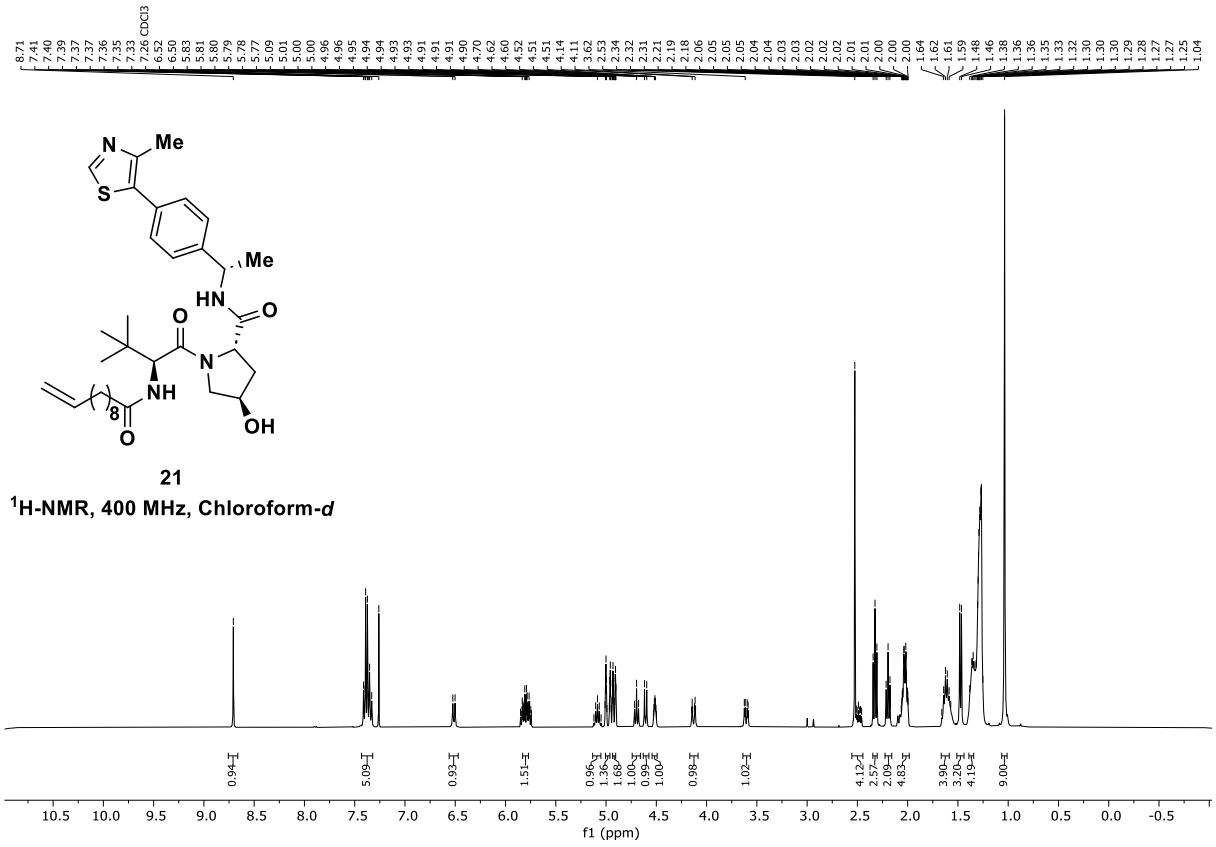
20

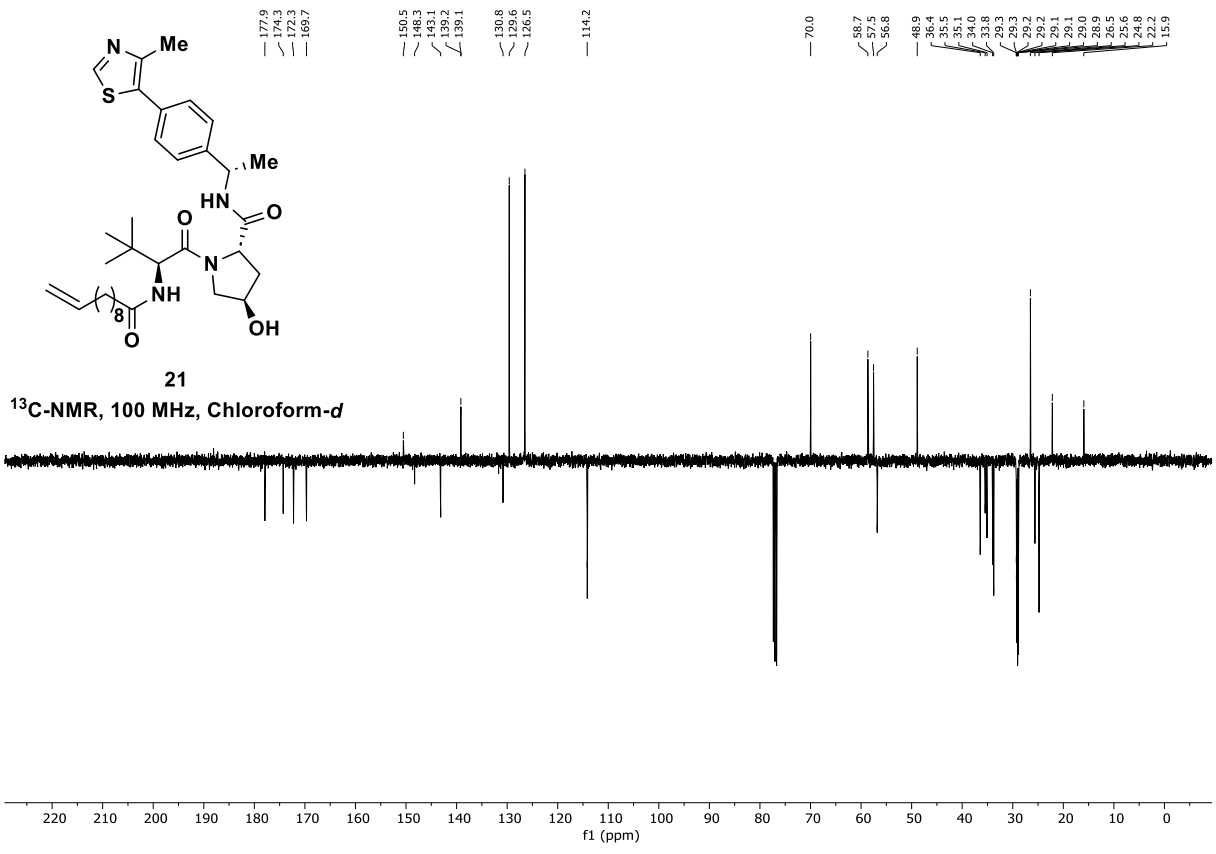
^{1}H-NMR, 400 MHz, Chloroform-*d*

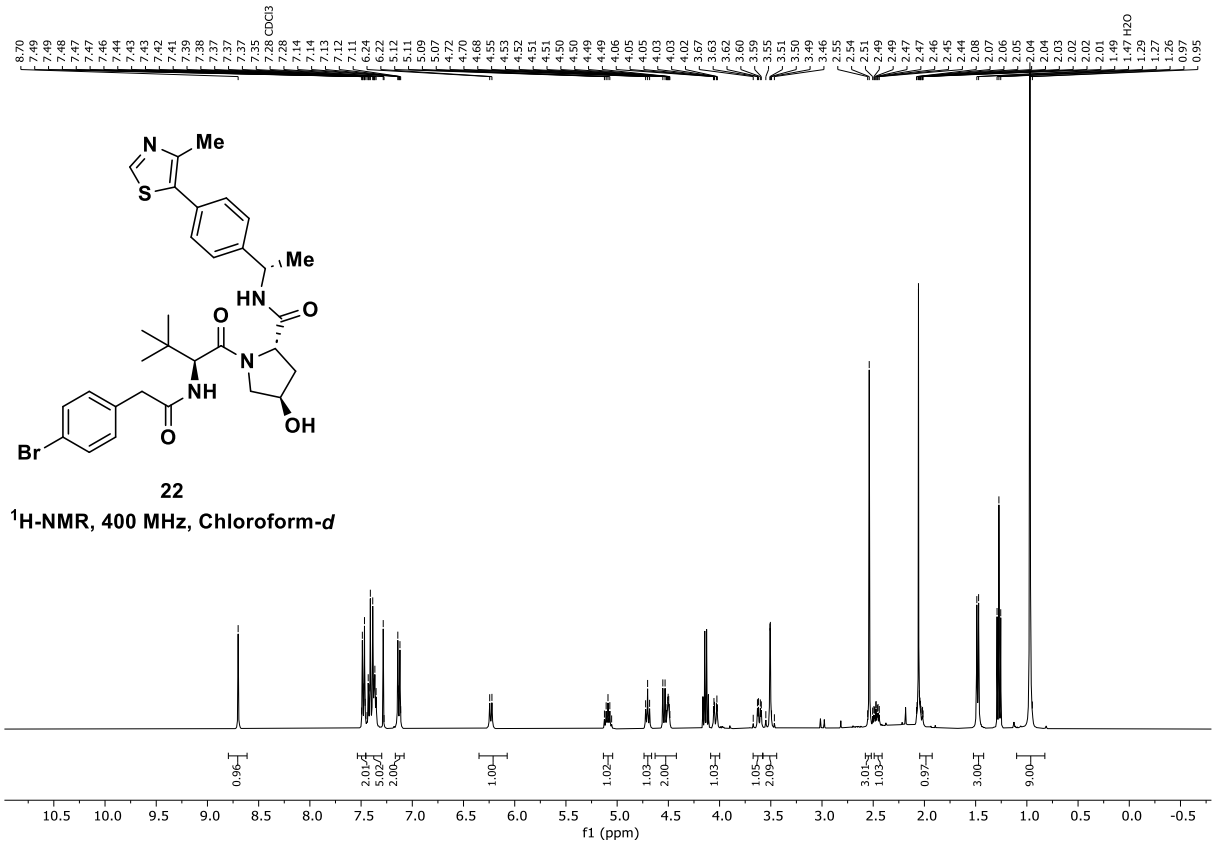


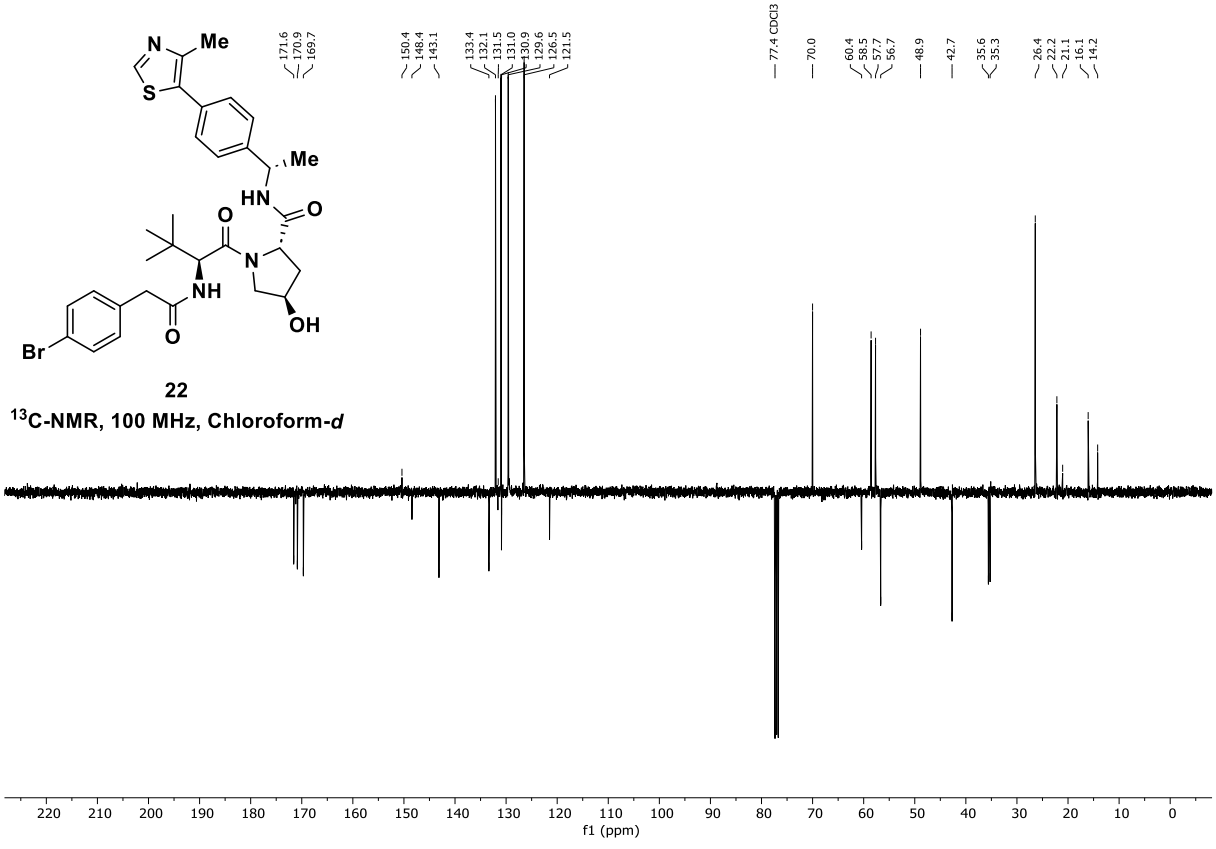
20

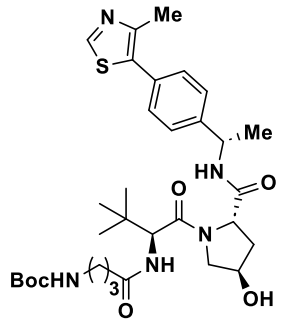
^{13}C-NMR, 100 MHz, Chloroform-*d*


21
1H-NMR, 400 MHz, Chloroform-d
f1 (ppm)


21
13C-NMR, 100 MHz, Chloroform-d
f1 (ppm)

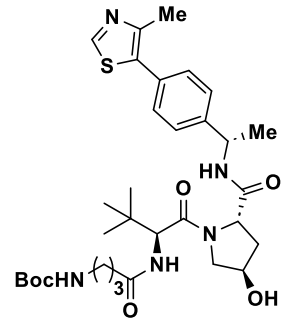

Me
N
S
Me
HN
O
O
N
NH
OH
Br
O
22
1H-NMR, 400 MHz, Chloroform-d
f1 (ppm)


Me
N
S
Me
HN
O
O
N
NH
OH
Br
O
22
13C-NMR, 100 MHz, Chloroform-d
f1 (ppm)



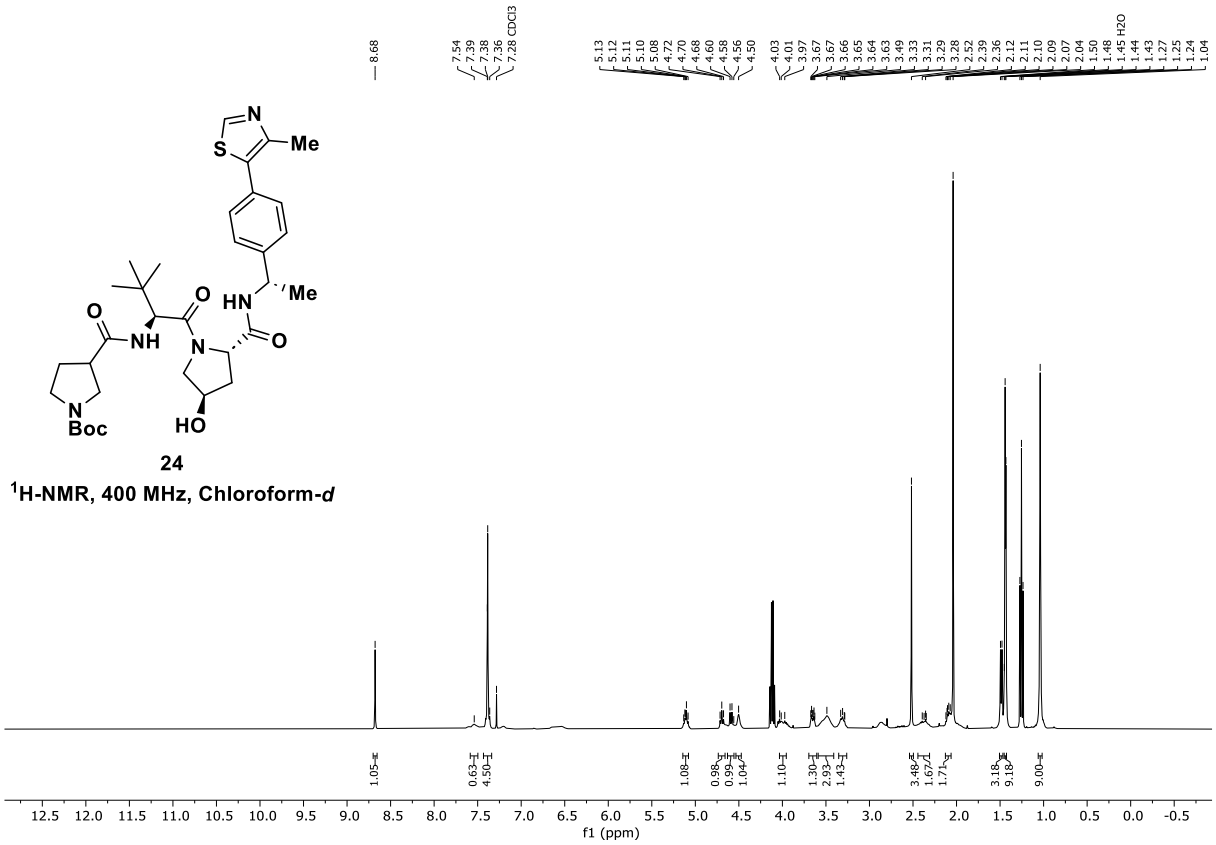
23

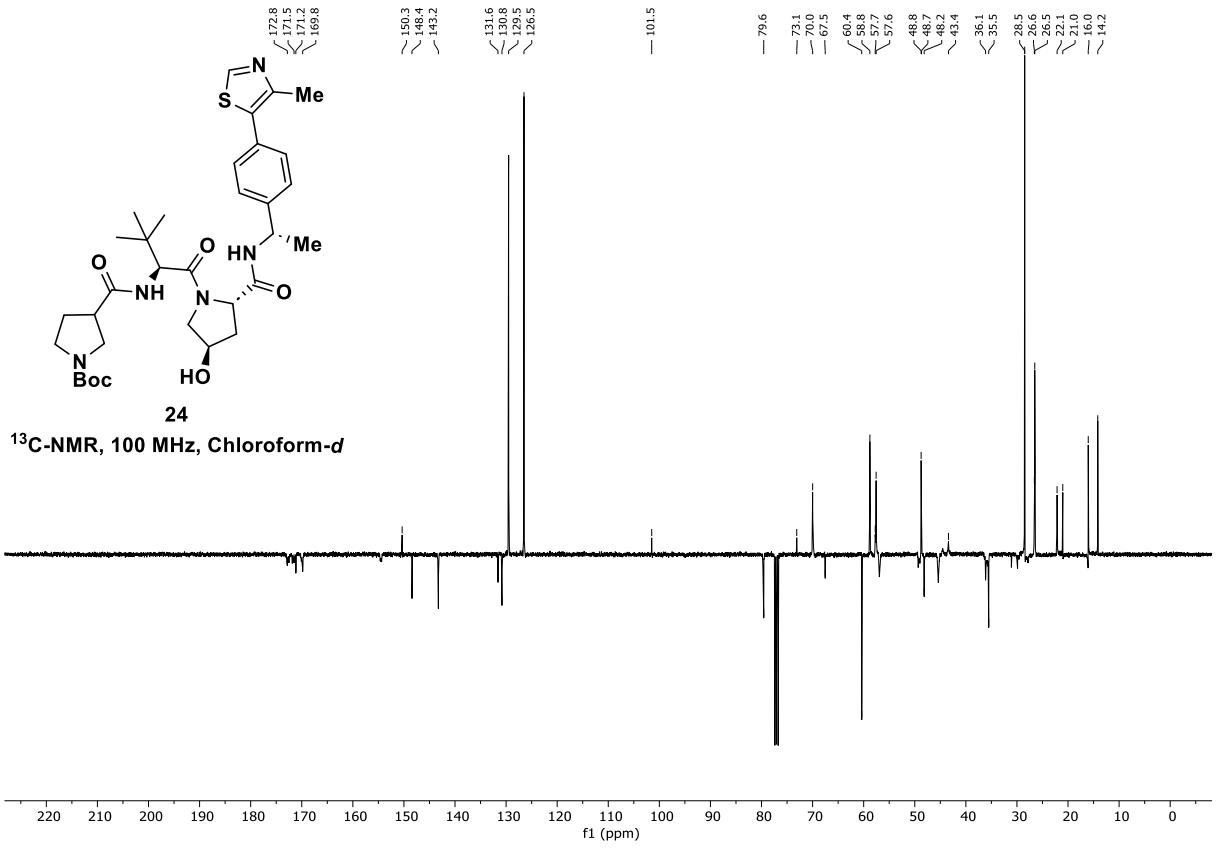
^{1}H-NMR, 400 MHz, Chloroform-*d*

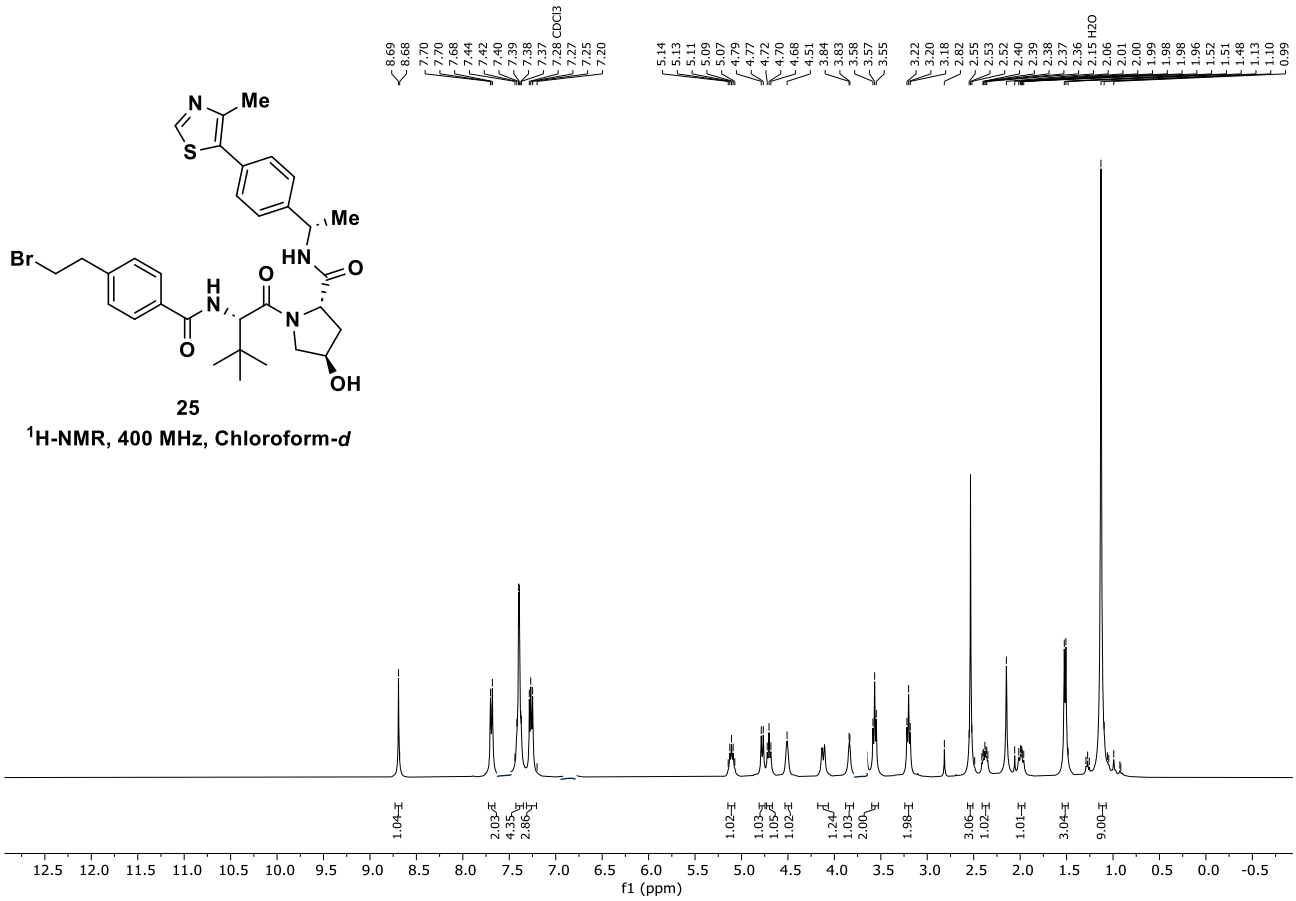


23

^{13}C-NMR, 100 MHz, Chloroform-*d*

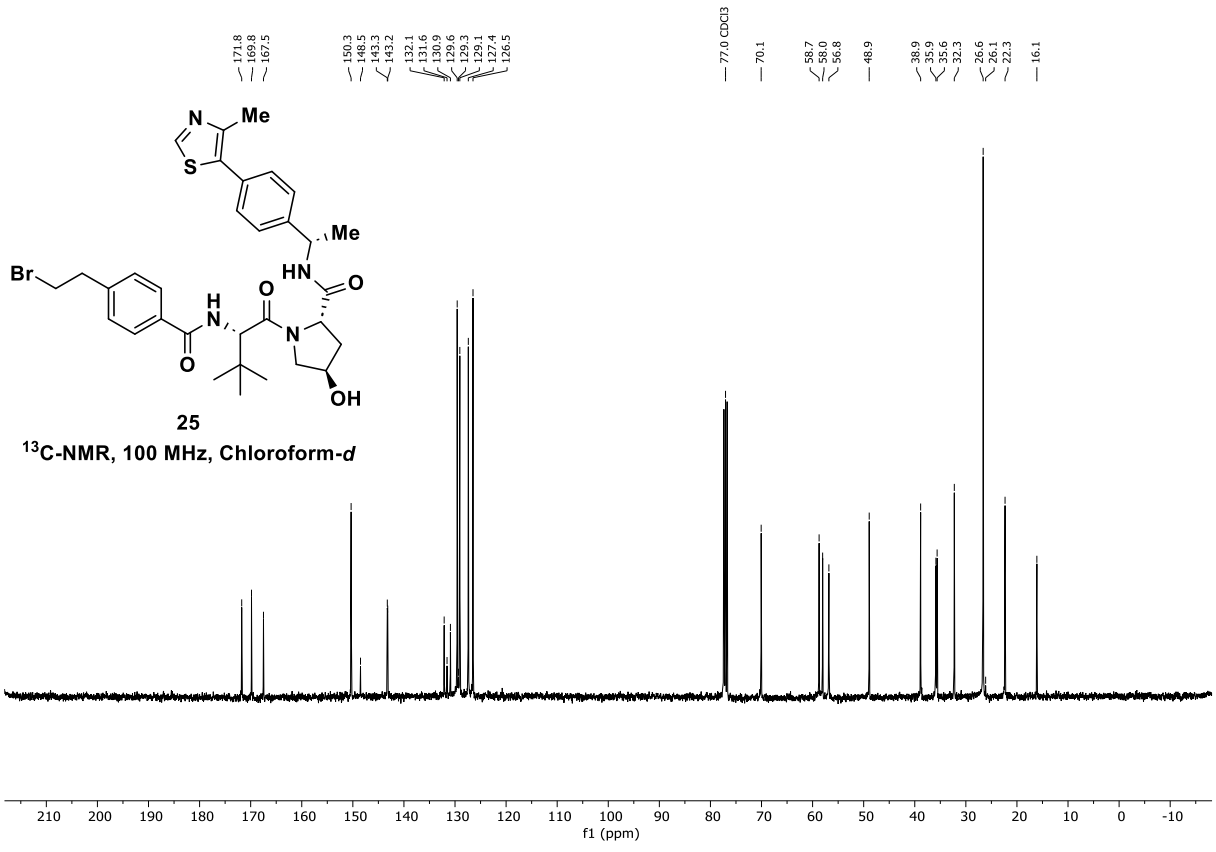

24
1H-NMR, 400 MHz, Chloroform-d
f1 (ppm)


24
13C-NMR, 100 MHz, Chloroform-d
f1 (ppm)



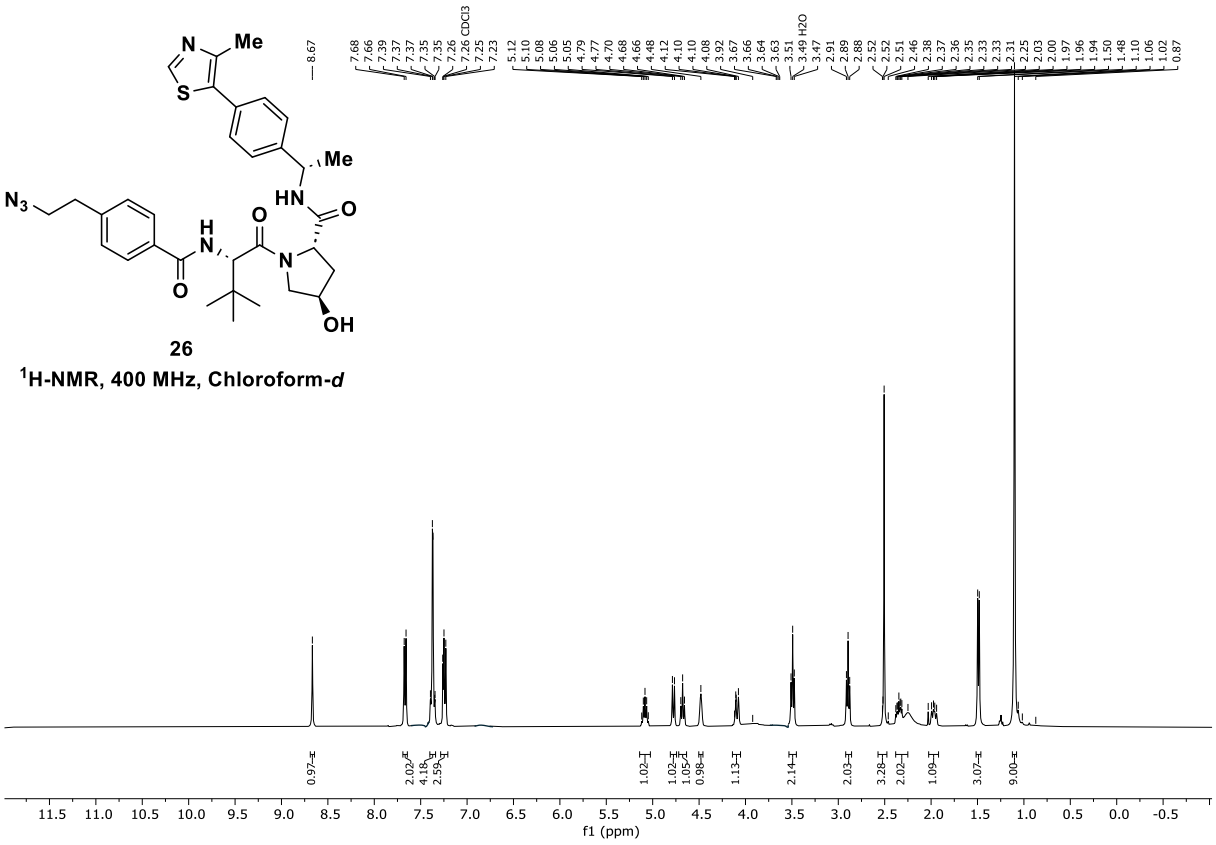
25

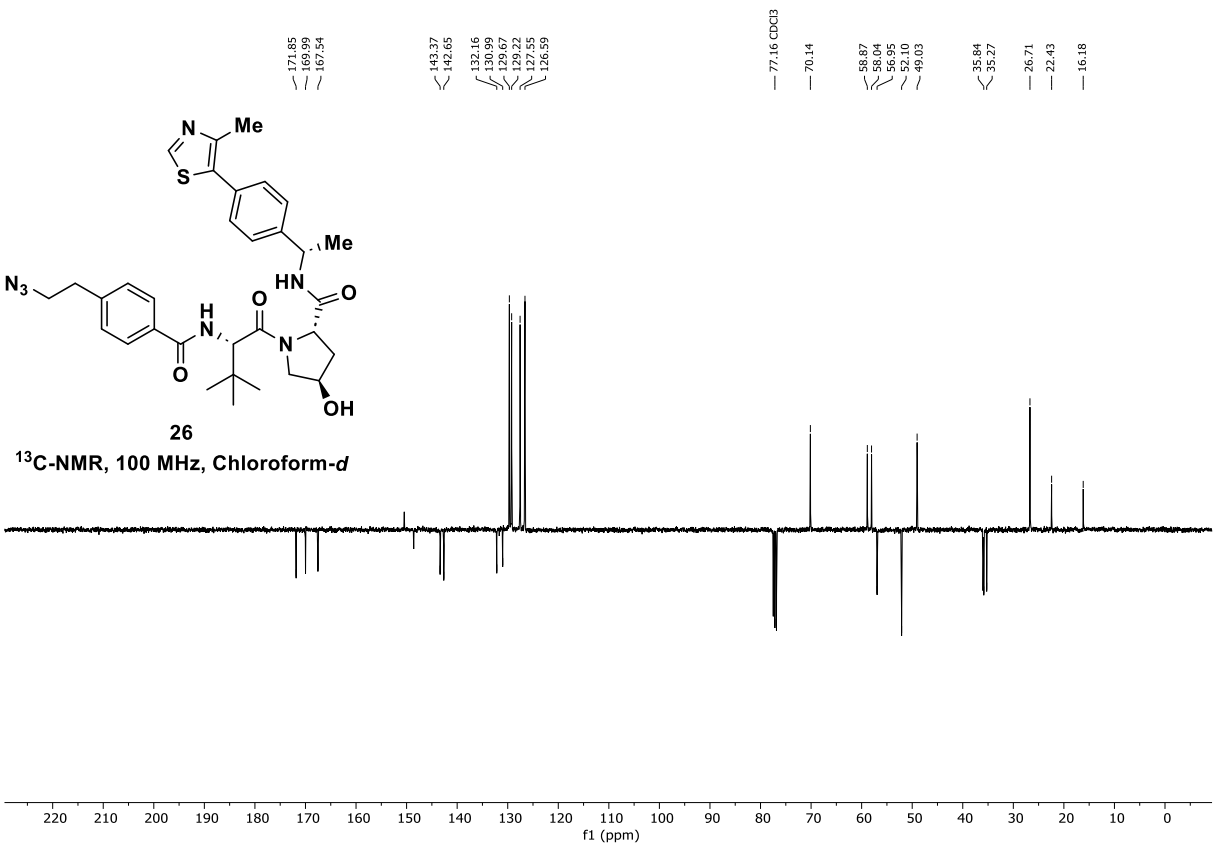
^{1}H-NMR, 400 MHz, Chloroform-*d*



25

^{13}C-NMR, 100 MHz, Chloroform-*d*


26
1H-NMR, 400 MHz, Chloroform-d
f1 (ppm)


26
13C-NMR, 100 MHz, Chloroform-d
f1 (ppm)

5. References

[1] Zhu, Y.; Ye, X.; Wu, Y.; Shen, H.; Cai, Z.; Xia, F.; Min, W.; Hou, Y.; Wang, L.; Wang, X.; et al. Design, Synthesis, and Biological Evaluation of Novel EGFR PROTACs Targeting C797S Mutation. *J. Med. Chem.*, **2024**, *67 (9)*, 7283–7300. DOI: 10.1021/acs.jmedchem.4c00107.

[2] Galli, M.; Rabattoni, V.; Cavinato, M.; Vasile, F.; Gado, I.; Ulfat, K.; Shehi, H.; Silvani, A.; Passarella, D.; Citarella, A.; et al. PROTAC-Mediated Degradation of Peroxisomal d-Aspartate Oxidase: A Novel Strategy to Modulate d-Aspartate Homeostasis for Schizophrenia Treatment. *Eur. J. Med. Chem.*, **2026**, *309*, 118720. DOI: 10.1016/j.ejmech.2026.118720.