Supporting Information

Synthesis and Development of Seven-Membered Constrained Cyclic Urea Based PSMA inhibitors

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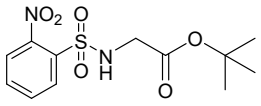
General Information.

All reagents were purchased as reagent grade and used without further purification. Grubbs catalyst® 1st generation, thiophenol, 3-bromo-1-propene, chlorodicyclohexyl borane (CBU), triethylamine (NEt_3), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), tetrabutylammonium iodide (TBAI), triphosgene (BTC) and 2-nitrobenzenesulfonyl chloride were purchased from Sigma-Aldrich (St. Louis, Missouri). *tert*-Butyl glycinate·HCl was purchased from GL Biochem (Shanghai, China). Di-*tert*-butyl *L*-glutamate·HCl and 1,4-dithiothreitol (DTT) were purchased from AK Scientific (Union City, CA). All reactions were performed under an oxygen-free atmosphere of nitrogen unless otherwise noted. Tetrahydrofuran (THF), dimethylformamide (DMF), and dichloromethane (CH_2Cl_2), were dried using an LC Technical SP-1 solvent purification system. Yields refer to chromatographically homogeneous materials. Reactions were monitored by thin-layer chromatography (TLC) carried out on E. Merck silica gel plates using UV light as the visualising agent with either ninhydrin or potassium permanganate solution as developing agents with heat application. Silica gel (60, 230−400 mesh) was used for flash column chromatography. Reactions performed at low temperatures were cooled either with and acetone/dry ice bath to reach -78 °C or an ice/water bath to reach 0 °C. NMR spectra were recorded at room temperature in $CDCl_3$ or D_2O solutions on either Bruker DRX 400 spectrometers operating at 400 MHz for ^1H nuclei and 100 MHz for ^{13}C nuclei or using a Bruker DRX 500 spectrometer operating at 500 MHz for ^1H nuclei and 125 MHz for ^{13}C nuclei. Chemical shifts are reported in parts per million (ppm) calibrated relative to: TMS (δ_H 0.00 ppm), $CDCl_3$ (δ_H 7.26 ppm, δ_C 77.2 ppm), or D_2O (δ_H 4.79 ppm). Multiplicities are reported as “s” (singlet), ‘br s’ (broad singlet), “d” (doublet), “dd” (doublet of doublets), “t” (triplet), “m” (multiplet), coupling constant (*J*, Hz), relative integral and structural assignment. ^{13}C NMR data were reported as position (δ), type and assignment of carbon resonance. Structural assignments were achieved with the aid of COSY, HSQC, HMBC and NOESY experiments where required. Infrared (IR) spectra were recorded as a thin film on a composite of zinc selenide and diamond crystal on a FT-IR system transform spectrometer expressed in wavenumbers (cm^{-1}). Optical rotations were measured with an automatic polarimeter using the sodium-D line (589 nm), with the concentration measured in grams per 100 mL. Microwave reactions were carried out on a Biotage® Initiator+ Microwave System with Robot Eight (Uppsala, Sweden) in sealed reaction vessels monitored by an external surface sensor with the required temperature maintained during the synthesis. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. High resolution mass spectra (HRMS) were obtained using the micrOTOF-Q II spectrometer operating at a nominal accelerating voltage of 70 eV. Semi-preparative RP-HPLC was performed on a Thermo Scientific (Waltham, MA) Dionex Ultimate 3000 HPLC equipped with a four channel UV Detector at 210, 225, 254 and 280 nm using an analytical XTerra® MS column (Waters (Milford, MA), C18, (5 μm; 4.6 × 150 mm) at a flow rate of 1 mL min^{-1}. A suitably adjusted gradient of 5% B to 95% B was used, where solvent A was 0.1% TFA in H_2O and B was 0.1% TFA in acetonitrile. LC-MS spectra were acquired using an Agilent Technologies (Santa Clara, CA) 1120 Compact LC equipped with an Agilent Technologies 6120 Quadrupole mass spectrometer. An analytical Agilent column (Santa Clara, CA), Agilent C3, (3.5 μm; 3.0 × 150 mm) was used at a flow rate of 0.3 mL min^{-1} using a linear gradient of 5% B to 95% B over 30 min, where solvent A was 0.1% formic acid in H_2O and B was 0.1% formic acid in acetonitrile.

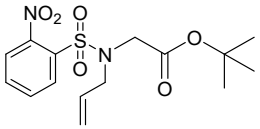
***tert*-Butyl ((2-nitrophenyl)sulfonyl)glycinate (11)**.



11

To a solution of *tert*-butyl glycinate·HCl (**7**) (2.0 g, 11.9 mmol) in CH_2Cl_2 (70 mL) at 0 °C was added triethylamine (2.4 mL, 17.5 mmol) and the resultant mixture was stirred at 0 °C for 5 min. 2-Nitrobenzenesulfonyl chloride (2.7 g, 12.0 mmol) was added in one portion and the suspension was stirred at 0 °C for 20 min then warmed to rt and stirred for 24 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ (20 mL), and the aqueous layer was further extracted with CH_2Cl_2 (3 × 15 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered then concentrated *in vacuo*. Purification of the crude residue by flash column chromatography (petroleum ether-EtOAc 6:1) afforded **11** (2.6 g, 69%) as a colourless solid; **^{1}H NMR** (400 MHz, $CDCl_3$, Me_4Si): δ 1.31 (9H, s, 3 × CH_3), 3.89-3.90 (2H, m, CH_2), 6.02 (1H, t, J = 5.4 Hz, NH), 7.69-7.76 (2H, m, 2 × Ar-H), 7.83-7.95 (1H, m, Ar-H), 8.06-8.13 (1H, m, Ar-H); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, Me_4Si): δ 27.9, 45.8, 83.1, 125.8, 130.7, 132.9, 133.7, 134.2, 148.1, 167.6. The spectroscopic data were in agreement with that reported in the literature.[1]

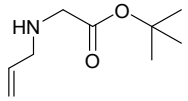
***tert*-Butyl *N*-allyl-*N*-((2-nitrophenyl)sulfonyl)glycinate (12)**.



12

To a solution of **11** (2.6 g, 8.21 mmol) in DMF (20 mL) at rt was added a solution of Cs_2CO_3 (10 g, 30.6 mmol) in 20 mL H_2O followed by addition of TBAI (11 mg, 3.10 × 10^{-5} mol). To the suspension was added 3-bromo-1-propene (1.4 mL, 16.4 mmol) dropwise and the reaction mixture was stirred for 24 h. The reaction was then quenched with saturated aqueous NH_4Cl (10 mL). The mixture was then extracted with Et_2O (2 × 10 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered then concentrated *in vacuo*. The crude residue was purified by flash column chromatography (petroleum ether-EtOAc 6:1) to afford **12** (2.4 g, 82%) as a pale yellow solid; **^{1}H NMR** (400 MHz, $CDCl_3$, Me_4Si): δ 1.37 (9H, s, 3 × CH_3), 4.04-4.05 (4H, m, 2 × CH_2), 5.19-5.24 (2H, m, CH_2=CH), 5.69-5.79 (1H, m, CH_2=CH), 7.61-7.64 (1H, m, Ar-H), 7.66-7.71 (2H, m, 2 × Ar-H), 8.07-8.12 (1H, m, Ar-H); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, Me_4Si): δ 28.0, 47.9, 51.1, 82.4, 120.2, 124.3, 131.1, 131.8, 132.2, 133.6, 133.9, 148.1, 167.8. The spectroscopic data were in agreement with that reported in the literature.[2]

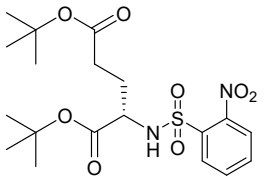
***tert*-Butyl allylglycinate (5)**.



5

To a solution of allyl **12** (1.4 g, 3.93 mmol) in DMF (25 mL) at 0 °C was added DBU (1.2 mL, 7.84 mmol) and the resultant mixture was stirred at 0 °C for 5 min. Thiophenol (1.0 mL, 9.75 mmol) was added to the mixture and stirred at 0 °C for 10 min then warmed to rt and stirred for an additional 3 h. The mixture was then diluted with EtOAc (15 mL), washed with H_2O (3 × 10 mL), aqueous brine and the combined organic layers dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo.* The crude product was purified by flash column chromatography (petroleum ether-diethyl ether 6:1) to afford **5** (0.49 g, 73%) as a pale yellow oil; **^{1}H NMR** (400 MHz, $CDCl_3$, Me_4Si): δ 1.42 (9H, s, 3 × CH_3), 3.20 (2H, dd, J = 6.0, 0.9 Hz, CH_2), 3.24 (2H, br s, CH_2), 5.04-5.16 (2H, m, CH_2=CH), 5.77-5.87 (1H, m, CH_2=CH); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, Me_4Si): δ 28.2, 50.9, 51.9, 81.1, 116.4, 136.4, 171.8. **HRMS** (ESI+) $[M+H]^+$ calcd: for $C_9H_{18}NO_2$: 172.1332; found: 172.1330. The ^{1}H NMR data was in agreement with that reported in the literature.[3]

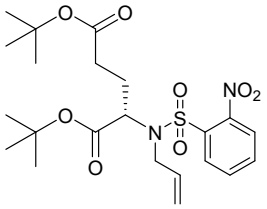
di-*tert*-Butyl ((2-nitrophenyl)sulfonyl)-L-glutamate (8).



8

To a solution of di-*tert*-butyl *L*-glutamate·HCl (**6**) (1.86 g, 6.28 mmol) in CH_2Cl_2 (70 mL) at 0 °C was added triethylamine (1.14 mL, 8.20 mmol) and the resultant mixture was stirred at 0 °C for 5 min. 2-Nitrobenzenesulfonyl chloride (1.1 g, 4.96 mmol) was added in one portion and the suspension was stirred at 0 °C for 20 min then warmed to rt and stirred for 24 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ (20 mL), and the aqueous layer was further extracted with CH_2Cl_2 (3 × 15 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered then concentrated *in vacuo*. Purification of the crude residue by flash column chromatography (petroleum ether-EtOAc 6:1) afforded **8** (2.0 g, 72%) as a colourless solid; **^{1}H NMR** (400 MHz, $CDCl_3$, Me_4Si): δ 1.21 (9H, s, 3 × CH_3), 1.47 (9H, s, 3 × CH_3), 1.83-1.92* (1H, m, CH_2-C(H_a)H_b), 2.09-2.18* (1H, m, CH_2-C(H_b)H_a), 2.42-2.46 (2H, m, CH_2), 4.09 (1H, br s, CH), 6.16 (1H, br s, NH), 7.68-7.73 (2H, m, 2 × Ar-H), 7.91-7.95 (1H, m, Ar-H), 8.05-8.09 (1H, m, Ar-H); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, Me_4Si): δ 27.8, 28.2, 28.6, 31.2, 57.0, 81.0, 82.9, 125.8, 130.7, 133.0, 133.7, 134.4, 148.0, 169.9, 171.9; **mp**: 98-105 °C; **$[\alpha]_D^{25}$** -14.2 (*c* 0.1, $CHCl_3$); **IR** ν_{max}(neat)/cm^{-1}: 2951, 1742, 1482, 1381, 1132, 1080, 720; **HRMS** (ESI+) $[M + Na]^+$ calcd: for $C_{19}H_{28}N_2NaO_8S$: 467.1459; found: 467.1462. *Represents diastereotopic glutamate protons. The ^{1}H NMR data was in agreement with that reported in the literature.[4]

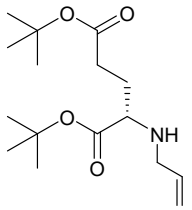
di-*tert*-Butyl *N*-allyl-*N*-((2-nitrophenyl)sulfonyl)-L-glutamate (9).



9

To a solution of **8** (2.0 g, 4.50 mmol) in DMF (20 mL) at rt was added a solution of Cs_2CO_3 (10 g, 30.6 mmol) in 20 mL H_2O followed by addition of TBAI (11 mg, 3.10 × 10^{-5} mol). To the suspension was added 3-bromo-1-propene (0.78 mL, 9.0 mmol) dropwise and the reaction mixture was stirred for 24 h. The reaction was then quenched with saturated aqueous NH_4Cl (10 mL). The mixture was then extracted with Et_2O (2 × 10 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered then concentrated *in vacuo*. The crude residue was purified by flash column chromatography (petroleum ether-EtOAc 4:1) to afford **9** (1.7 g, 78%) as a pale yellow solid; **^{1}H NMR** (400 MHz, $CDCl_3$, Me_4Si): δ 1.26 (9H, s, 3 × CH_3), 1.43 (9H, s, 3 × CH_3), 1.89-1.99* (1H, m, CH_2-C(H_a)H_b), 2.20-2.29* (1H, m, CH_2-C(H_b)H_a), 2.37-2.41 (2H, m, CH_2), 3.75-3.81 (1H, m, C(H)$_a$$H_b$-CH=$CH_2$), 4.13-4.19 (1H, m, C($H$)$_b$$H_a$-CH=$CH_2$), 4.56 (1H, dd, *J* = 10.4, 4.9 Hz, CH), 5.08-5.23 (2H, m, CH_2=CH), 5.88-5.98 (1H, m, CH_2=CH), 7.53-7.58 (1H, m, Ar-H), 7.62-7.71 (2H, m, 2 × Ar-H), 7.99-8.04 (1H, m, Ar-H); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, Me_4Si) δ 25.3, 27.8, 28.1, 31.8, 49.2, 60.6, 80.7, 82.4, 118.4, 124.0, 131.4, 131.6, 133.5, 133.6, 135.2, 148.2, 169.3, 171.9; **mp**: 153-162 °C; **$[\alpha]_D^{25}$** -30.2 (*c* 1.0, $CHCl_3$); **IR** ν_{max}(neat)/cm^{-1}: 2953, 2917, 2850, 1737, 1632, 1538, 1458, 1377, 1217, 1028, 803; **HRMS** (ESI+) $[M + Na]^+$ calcd: for $C_{22}H_{32}N_2NaO_8S$: 507.1772; found: 507.1786. *Represents diastereotopic glutamate protons

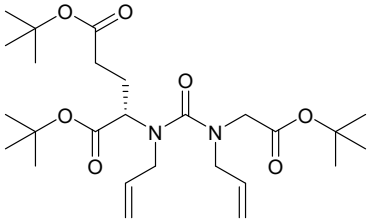
di-*tert*-Butyl *N*-allyl-L-glutamate (4).



4

To a solution of allyl **9** (1.22 g, 2.52 mmol) in DMF (30 mL) at 0 °C was added DBU (1.3 mL, 7.84 mmol) and the resultant mixture was stirred at 0 °C for 5 min. A solution of 1,4-dithiothreitol (0.96 g, 6.22 mmol) dissolved in DMF (6 mL) was added to the mixture and stirred at 0 °C for 10 min then warmed to rt and stirred for an additional 3 h. The mixture was then diluted with EtOAc (15 mL), washed with H_2O (3 × 10 mL), aqueous brine and the combined organic layers dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo.* The crude product was purified by flash column chromatography (petroleum ether-EtOAc 5:1) to afford **4** (0.65 g, 86%) as a pale yellow oil; **^{1}H NMR** (400 MHz, $CDCl_3$, Me_4Si): δ 1.42 (9H, s, 3 × CH_3), 1.45 (9H, s, 3 × CH_3), 1.72-1.92 (2H, m, CH_2), 2.26-2.38 (2H, m, CH_2), 3.05-3.10 (1H, m, C(H)$_a$H$_b$-CH=CH_2), 3.08-3.11 (1H, m, CH), 3.21-3.26 (1H, m, C(H)$_b$H$_a$-CH=CH_2), 5.03-5.18 (2H, m, CH_2=CH), 5.77-5.87 (1H, m, CH_2=CH); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, Me_4Si): δ 28.2, 28.8, 32.1, 50.8, 60.5, 80.3, 81.3, 116.3, 136.6, 172.6, 174.5; **[α]$_D^{25}$** -37.0 (*c* 0.6, $CHCl_3$); **IR** ν_{max}(neat)/cm^{-1}: 2963, 2845, 1650, 1422, 1317, 1211, 980, 770; **HRMS** (ESI+) $[M + H]^+$ calcd: for $C_{16}H_{30}NO_4$: 300.2169; found: 300.2150.

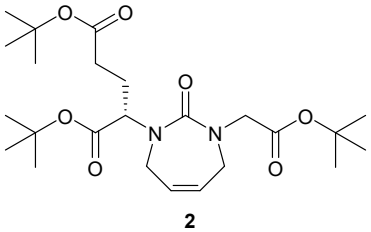
di-*tert*-Butyl *N*-allyl-*N*-(allyl(2-(*tert*-butoxy)-2-oxoethyl)carbamoyl)-L-glutamate (3).



3

To a stirred solution of glutamate **4** (200 mg, 0.67 mmol) in dry CH_2Cl_2 (7 mL) at rt was added triethylamine (0.32 mL, 2.31 mmol) and mixture was cooled to -78 °C. A solution of triphosgene (66 mg, 0.22 mmol) in anhydrous CH_2Cl_2 (3 mL) was added by slow dropwise addition and the mixture was stirred at −78 °C for 5 min, then the mixture was gradually warmed to rt and left stirring for a further 25 min. To the stirred mixture was added a mixture of glycinate **5** (100 mg, 0.58 mmol) in dry CH_2Cl_2 (1.6 mL) at rt followed by addition of triethylamine (86 µL, 0.61 mmol) and the reaction mixture stirred for a further 24 h. The reaction was quenched with aqueous NH_4OH (25% in H_2O); (1 mL) at rt and stirred 10 min. The resulting mixture was diluted with H_2O and acidified with aqueous NH_4Cl to pH 7–8, then extracted with CH_2Cl_2 (2 × 5 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo.* The crude residue was purified by flash column chromatography (petroleum ether-EtOAc 9:1) to afford urea **3** (230 mg, 70%) as a pale yellow oil; **^{1}H NMR** (400 MHz, $CDCl_3$, Me_4Si): δ 1.42 (9H, s, 3 × CH_3), 1.44 (9H, s, 3 × CH_3), 1.45 (9H, s, 3 × CH_3), 1.91-2.03* (1H, m, CH_2-C(H_a)H$_b$), 2.24-2.35* (3H, m, CH_2-C(H$_a$)H_b), 3.68-3.97 (7H, m), 5.13-5.29 (4H, m, 2 × CH_2=CH), 5.75-5.88 (2H, m, 2 × CH_2=CH); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, Me_4Si): δ 24.9, 28.1, 28.21, 28.24, 32.6, 49.7, 51.2, 52.3, 60.3, 80.4, 81.51, 81.54, 117.4, 117.7, 133.6, 134.5, 163.7, 169.4, 170.9, 172.6; **[α]$_D^{25}$** -21.2 (*c* 0.44, $CHCl_3$); **IR** ν_{max}(neat)/cm^{-1}: 2981, 2341, 2164, 1734, 1654, 1368, 1256, 1154, 849, 758; **HRMS** (ESI+) $[M + Na]^+$ calcd: for $C_{26}H_{44}N_2NaO_7$: 519.3041; found: 519.3022. *Represents diastereotopic glutamate protons

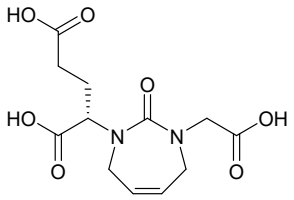
di-*tert*-Butyl (*S*)-2-(3-(2-(*tert*-butoxy)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-1,3-diazepin-1-yl)pentanedioate (2).



2

To urea **3** (50 mg, 0.10 mmol) under an inert argon atmosphere was added degassed anhydrous THF (16 mL) at a solution concentration of 6.3 mM at rt. To the resulting solution at approximately 5 min after addition of solvent was added Grubb's catalyst 1st generation (10-12 mg, 10 mol%, 1.0×10^{-5} mol) in degassed anhydrous THF (1 mL) via cannula addition followed by addition of chlorodicyclohexyl borane (1 M solution in hexane, 10 mol%) to make a final solution concentration of 5.9 mM, and the resultant mixture was heated under reflux at 75 °C for 16 h using a heating mantle. The mixture was cooled to rt, filtered with celite and then concentrated *in vacuo*. Purification of the crude residue by flash column chromatography (petroleum ether-EtOAc 9:1) afforded cyclic **2** (14 mg, 30%) as a pale yellow oil; **^{1}H NMR** (400 MHz, $CDCl_3$, Me_4Si): δ 1.43 (9H, s, 3 × CH_3), 1.44 (9H, s, 3 × CH_3), 1.46 (9H, s, 3 × CH_3), 1.86-1.96* (1H, m, CH_2-C(H_a)H_b), 2.16-2.25* (1H, m, CH_2-C(H_b)H_a), 2.29-2.33 (2H, m, CH_2), 3.69-3.85 (4H, m, 2 × CH_2), 3.90-3.98 (2H, m, CH_2), 4.34 (1H, dd, J = 10.4, 5.4 Hz, CH), 5.68-5.81 (2H, m, 2 × CH); **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$, Me_4Si): δ 24.9, 28.19, 28.24, 32.4, 45.4, 51.1, 53.5, 60.2, 80.5, 81.36, 81.45, 126.2, 126.5, 165.7, 169.6, 171.4, 172.5; **$[\alpha]_D^{25}$** -9.5 (*c* 0.28, $CHCl_3$); **IR** ν_{max}(neat)/cm^{-1}: 2988, 1744, 1449, 1375, 1245, 1048, 938, 848; **HRMS** (ESI+) $[M + Na]^+$ calcd: for $C_{24}H_{40}N_2NaO_7$: 491.2728; found 491.2727.*Represents diastereotopic glutamate protons.

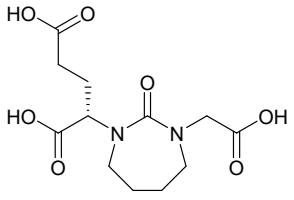
(*S*)-2-(3-(carboxymethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-1,3-diazepin-1-yl)pentanedioic acid (1a).



1a

To cyclic **2** (8 mg, 1.71×10^{-5} mol) was added TFA/CH_2Cl_2/H_2O (63.10:36.85:0.05, *v*/*v*/*v*, 0.044 mol/L) at rt for 7 h. The filtrate was partially concentrated under a gentle stream of N_2, then diluted with H_2O (4 mL) filtered on a C8 cartridge with H_2O (4 × 1 mL) and the collected aqueous fractions where combined and lyophilised. Crude mixture was further diluted in H_2O (2 mL) and was purified batchwise by semi-preparative RP-HPLC using Dionex Ultimate 3000 on a Xterra C18 column, using a linear gradient of 5% to 95% over 90 min (*ca* 1% B/min) with a flow rate of 1 mL/min. Fractions were collected at 0.2 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with correct *m*/*z* were combined and lyophilised to afford the *title compound* **1a** as a colourless oil (3.6 mg, 70%, t_R = 11.2 min, >99% purity as judged by RP-HPLC); ****^{1}H NMR** (500 MHz, D_2O): δ 2.02-2.09* (1H, m, CH_2-C(H_a)H_b), 2.24-2.31* (1H, m, CH_2-C(H_b)H_a), 2.42-2.53 (2H, m, CH_2), 3.81-4.05 (6H, m, 3 × CH_2), 4.42 (1H, dd, J = 11.5, 4.8 Hz, CH), 5.82-5.92 (2H, m, 2 × CH); **^{13}C{^{1}H} NMR** (125 MHz, D_2O): δ 23.6, 30.3, 45.9, 50.6, 52.4, 60.2, 125.7, 126.4, 166.5, 174.1, 175.6, 177.4; **$[\alpha]_D^{25}$** -60 (*c* 0.1, H_2O); **IR** ν_{max}(neat)/cm^{-1}: 2982, 2158, 1741, 1656, 1394, 1373, 1242, 1155, 1048, 939, 848; **HRMS** (ESI+) $[M + Na]^+$ calcd: for $C_{12}H_{16}N_2NaO_7$: 323.0850; found 323.0859.**Exchangeable carboxylic acid protons not detected in ^{1}H NMR but are accounted for in HRMS. *Represents diastereotopic glutamate protons

(*S*)-2-(3-(carboxymethyl)-2-oxo-1,3-diazepan-1-yl)pentanedioic acid (1b).

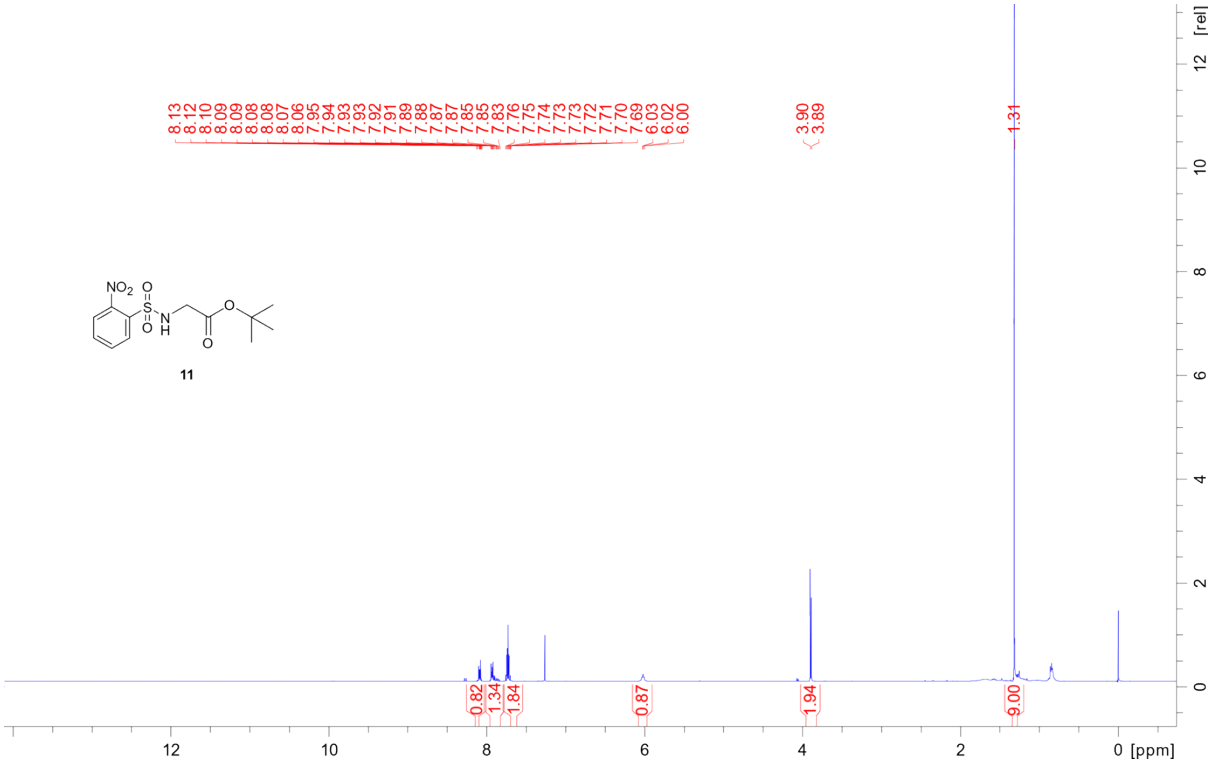


1b

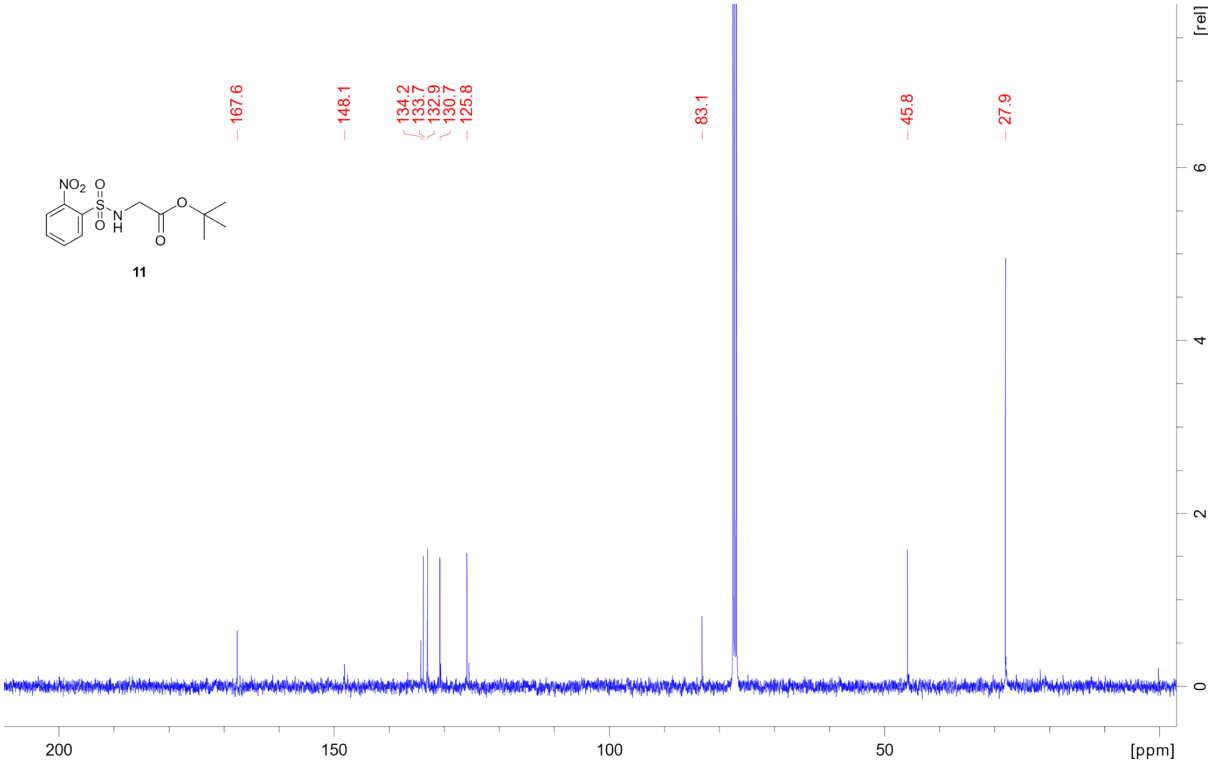
To cyclic **2** (18.5 mg, 3.94×10^{-5} mol) in EtOAc (2 mL) was added 10% Pd/C (0.5 mg, 3.9×10^{-6} mol) and the mixture stirred vigorously under H_2 (*ca*. 1 atm) for 16 h. The filtrate was then filtered by celite and concentrated *in vacuo* to give crude product (20 mg, 4.24×10^{-5} mol). Crude product **14** (20 mg, 4.24×10^{-5} mol) was then directly subjected to TFA/CH_2Cl_2/H_2O (63.10:36.85:0.05, *v*/*v*/*v*, 0.044 mol/L) at rt for 7 h. The filtrate was partially concentrated under a gentle stream of N_2, then diluted with H_2O (4 mL) filtered on a C8 cartridge with H_2O (4 × 1 mL) and the collected aqueous fractions where combined and lyophilised. Crude mixture was further diluted in H_2O (2 mL) and was purified batchwise by semi-preparative RP-HPLC using Dionex Ultimate 3000 on a Xterra C18 column, using a linear gradient of 5% to 95% over 90 min (*ca* 1% B/min) with a flow rate of 1 mL/min. Fractions were collected at 0.2 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with correct *m*/*z* were combined and lyophilised to afford the *title compound* **1b** as a colourless oil (3.5 mg, 30% yield over two steps, t_R = 11.5 min, >99% purity as judged by RP-HPLC); ****^{1}H NMR** (500 MHz, D_2O): δ 1.72-1.77 (4H, m, 2 × CH_2), 2.04-2.12* (1H, m, CH_2-C(H_a)H_b), 2.24-2.31*

(1H, m, CH_2-C(H_b)H_a), 2.47-2.58 (2H, m, CH_2), 3.30-3.39 (4H, m, 2 × CH_2), 3.98 (2H, ABq, $\Delta\delta_{AB}$ = 0.02, J = 17.8 Hz, CH_2), 4.33 (1H, dd, J = 10.3, 4.9 Hz, CH); **^{13}C{^{1}H} NMR** (125 MHz, D_2O): δ 23.7, 24.6, 25.2, 30.7, 48.3, 50.7, 51.6, 60.5, 164.6, 174.4, 175.8, 177.5; **IR** ν_{max}(neat)/cm^{-1}: 3368, 2954, 1736, 1645, 1562, 1437, 1223, 1158, 969, 848; **[α]$_D^{25}$** -51.3 (*c* 0.15, H_2O); **HRMS** (ESI+) $[M + H]^+$ calcd: for $C_{12}H_{19}N_2O_7$: 303.1187; found 303.1188.**Exchangeable carboxylic acid protons not detected in ^{1}H NMR but are accounted for in HRMS. *Represents diastereotopic glutamate protons

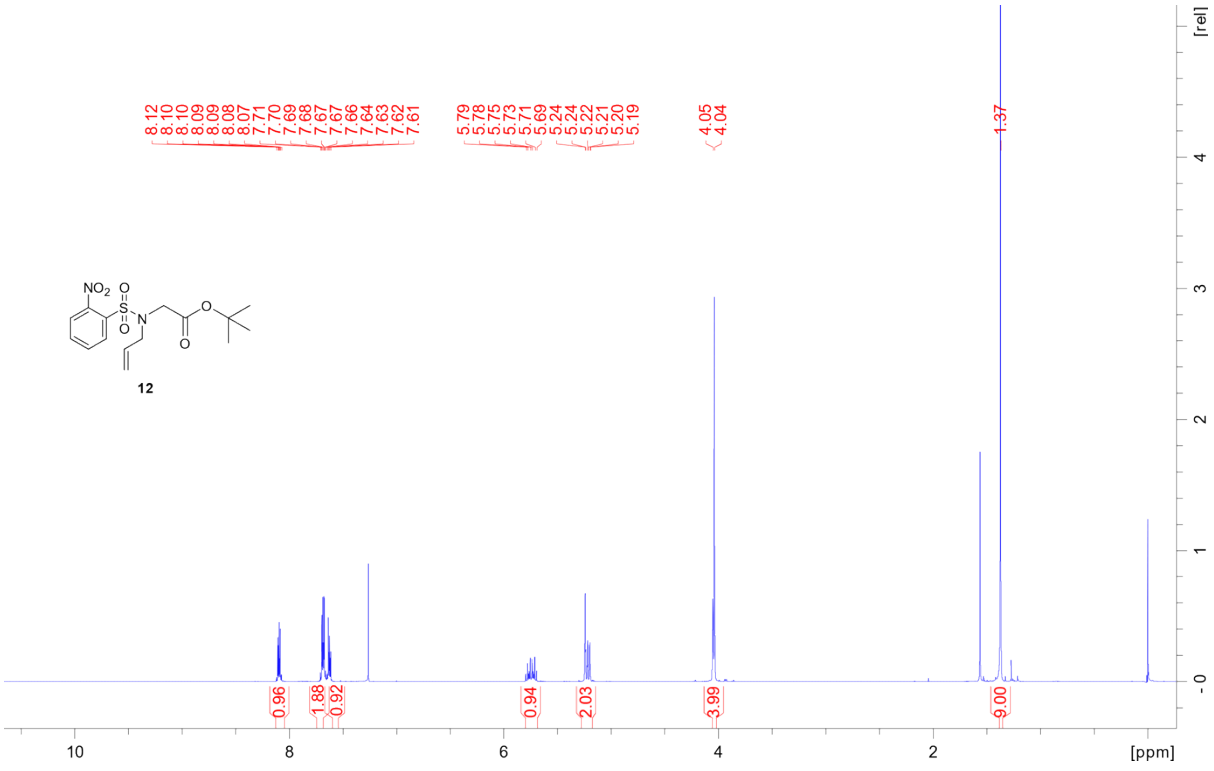
^{1}H NMR ($CDCl_3$, 400 MHz): **11** (The spectroscopic data were in agreement with that reported in the literature.[1])



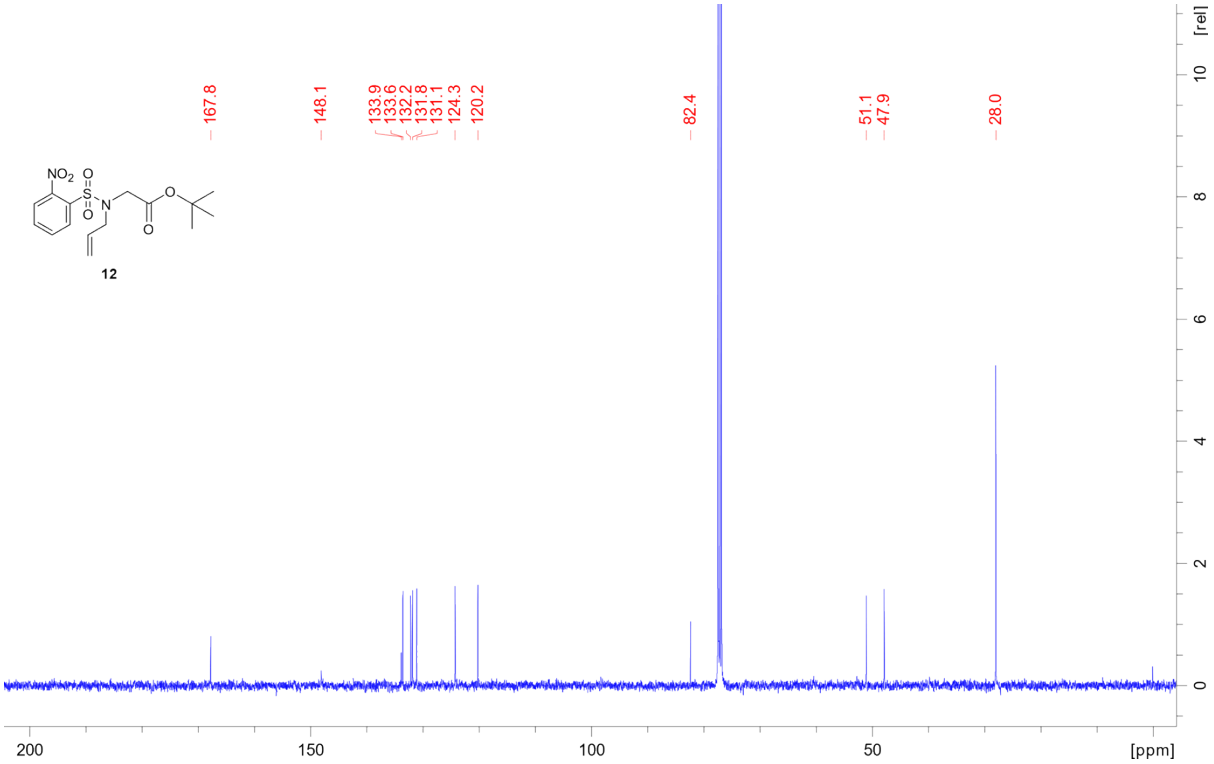
$^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): **11** (The spectroscopic data were in agreement with that reported in the literature.[1])



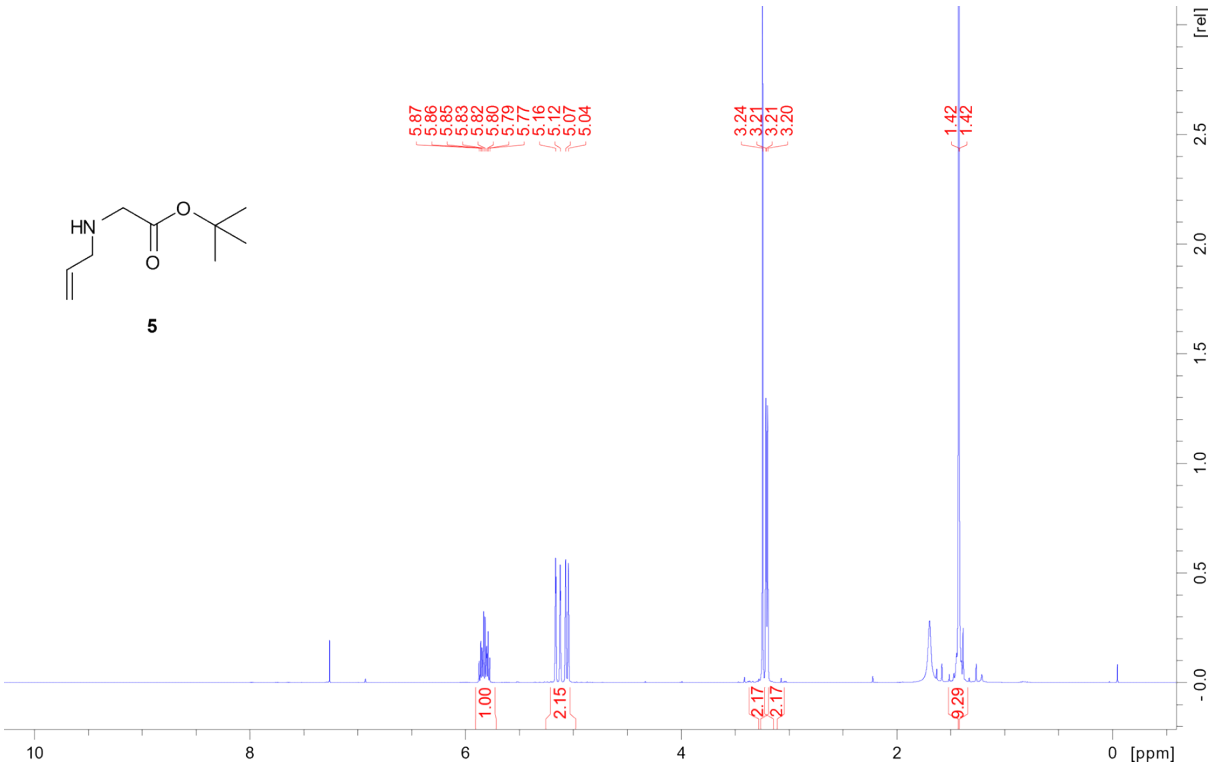
^{1}H NMR ($CDCl_3$, 400 MHz): **12** (The spectroscopic data were in agreement with that reported in the literature.[2])



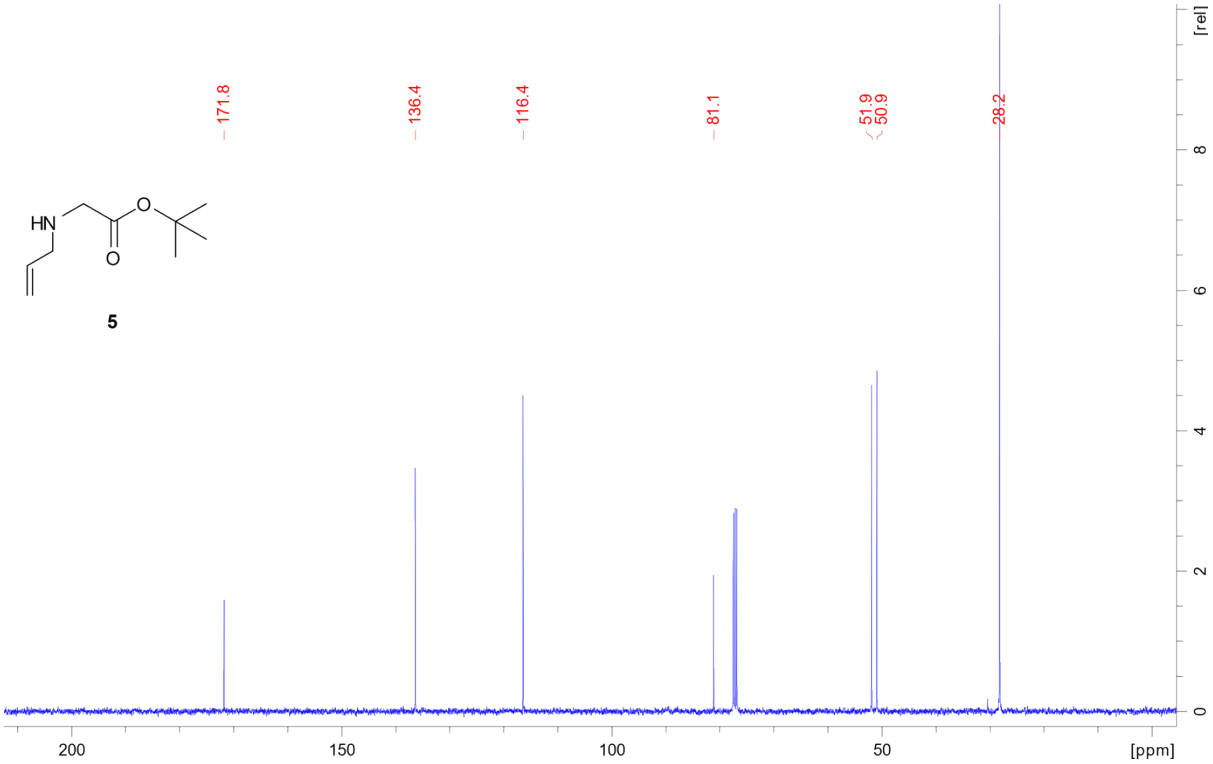
$^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): **12** (The spectroscopic data were in agreement with that reported in the literature.[2])



^{1}H NMR ($CDCl_3$, 400 MHz): **5** (The ^{1}H NMR data was in agreement with that reported in the literature.[3])



$^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): **5**



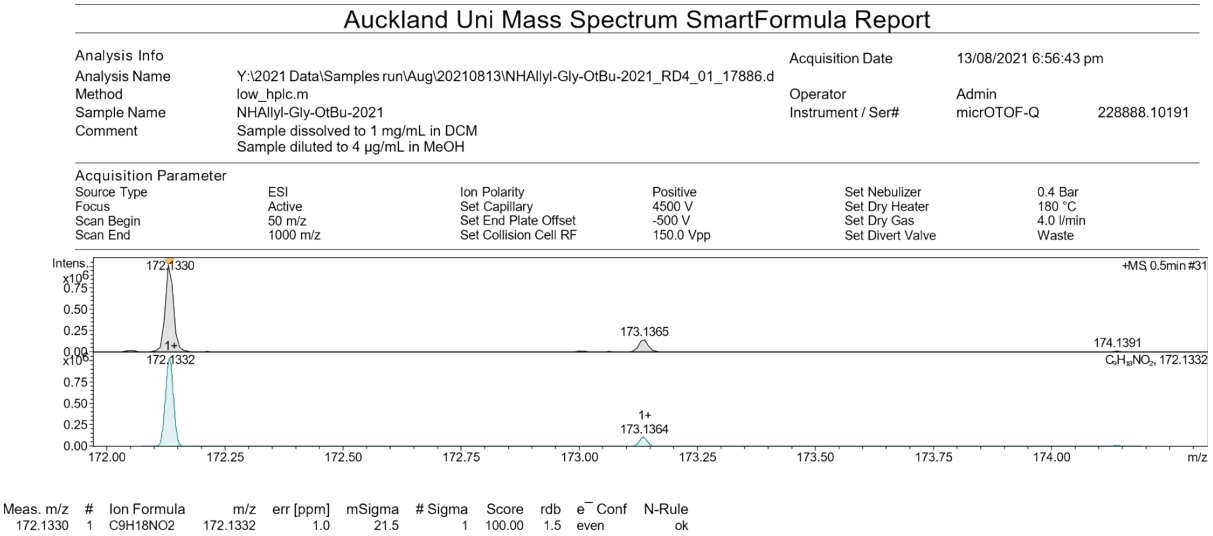
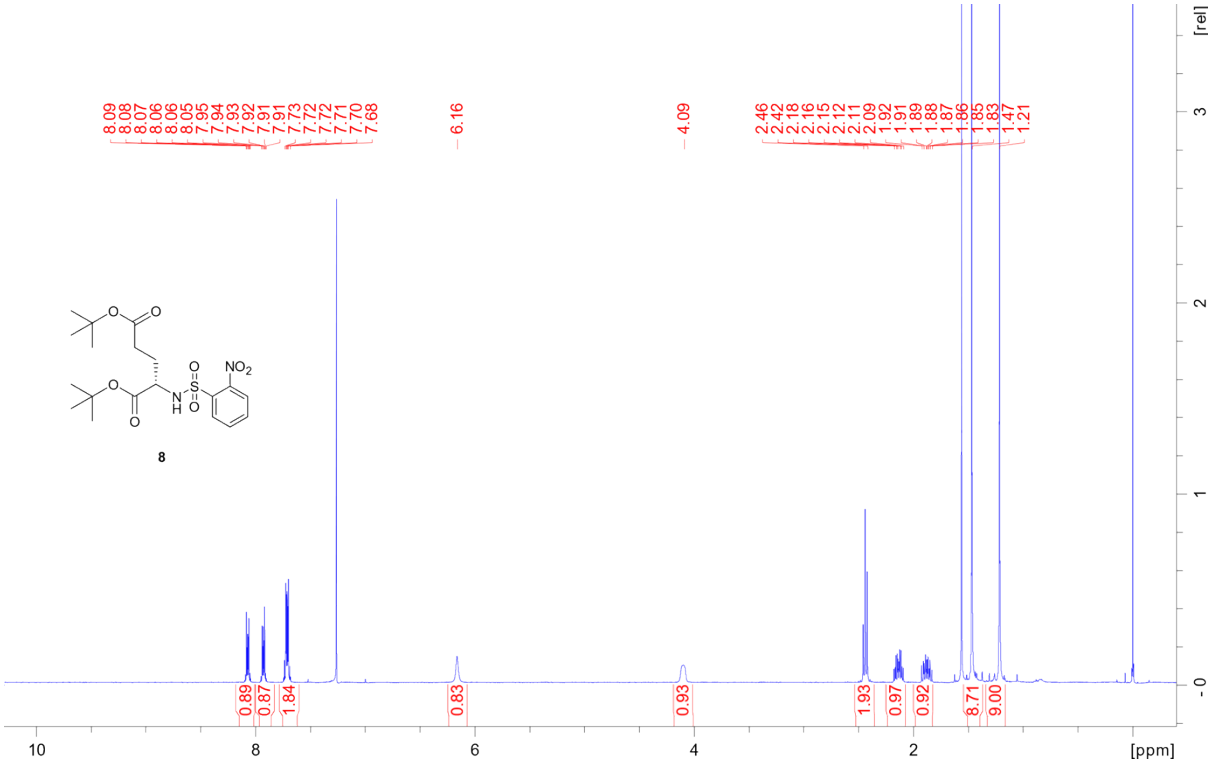
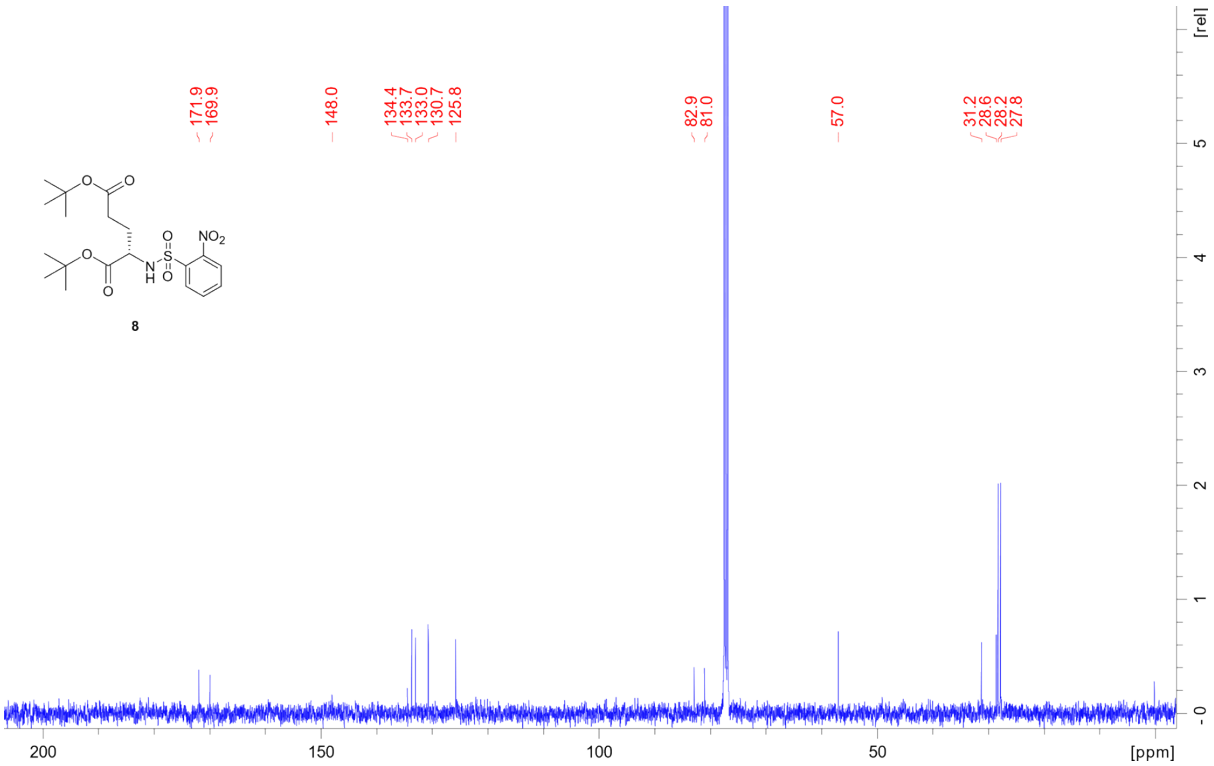


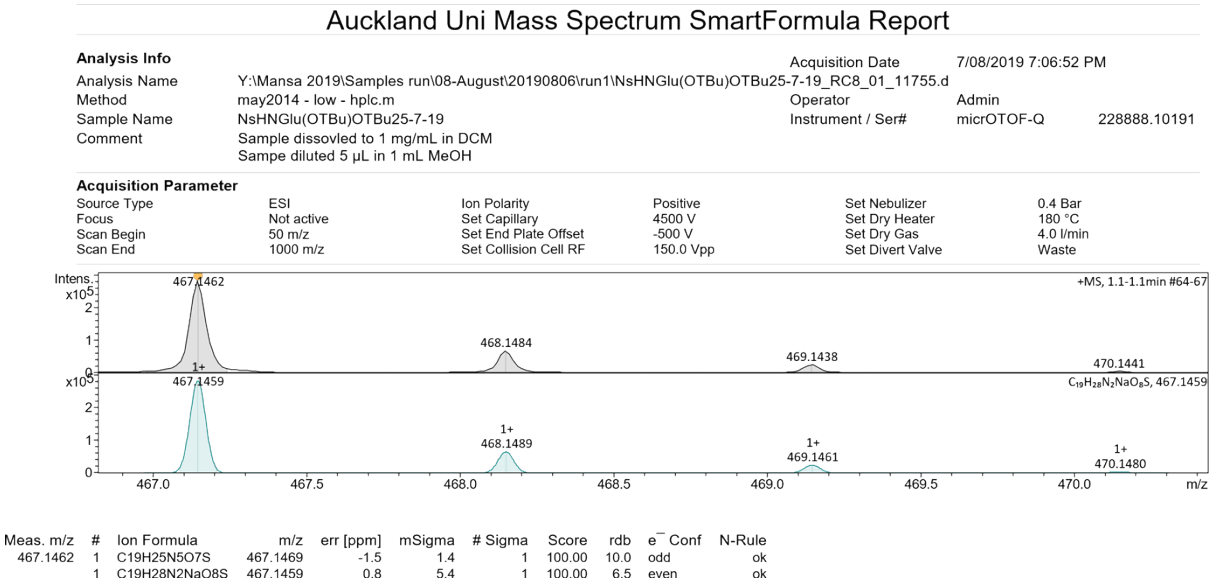
Figure S1: HRMS for **5**. HRMS (ESI): (*m/z* $[M + H]^+$ calcd: $C_9H_{18}NO_2^+$: 172.1332; found: 172.1330

^{1}H NMR ($CDCl_3$, 400 MHz): **8** The (^{1}H NMR data was in agreement with that reported in the literature.[4])



^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): **8**





Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
467.1462	1	C19H25N5O7S	467.1469	-1.5	1.4	1	100.00	10.0	odd	ok
	1	C19H28N2NaO8S	467.1459	0.8	5.4	1	100.00	6.5	even	ok

Figure S2: HRMS for **8**. HRMS (ESI): (*m/z* $[M + Na]^+$ calcd: $C_{19}H_{28}N_2NaO_8S^+$: 467.1459; found: 467.1462

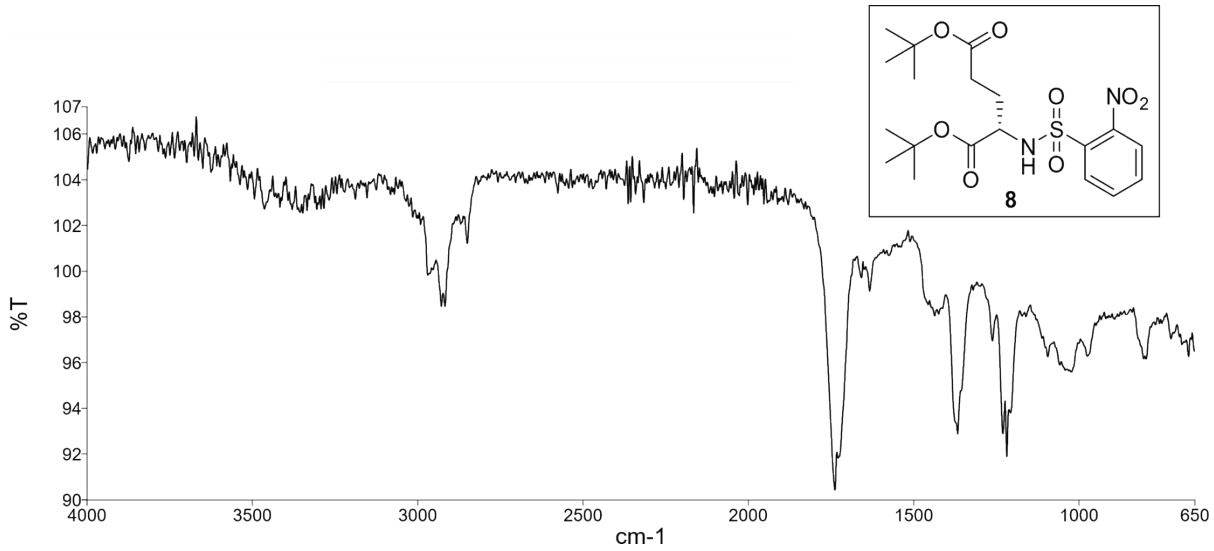
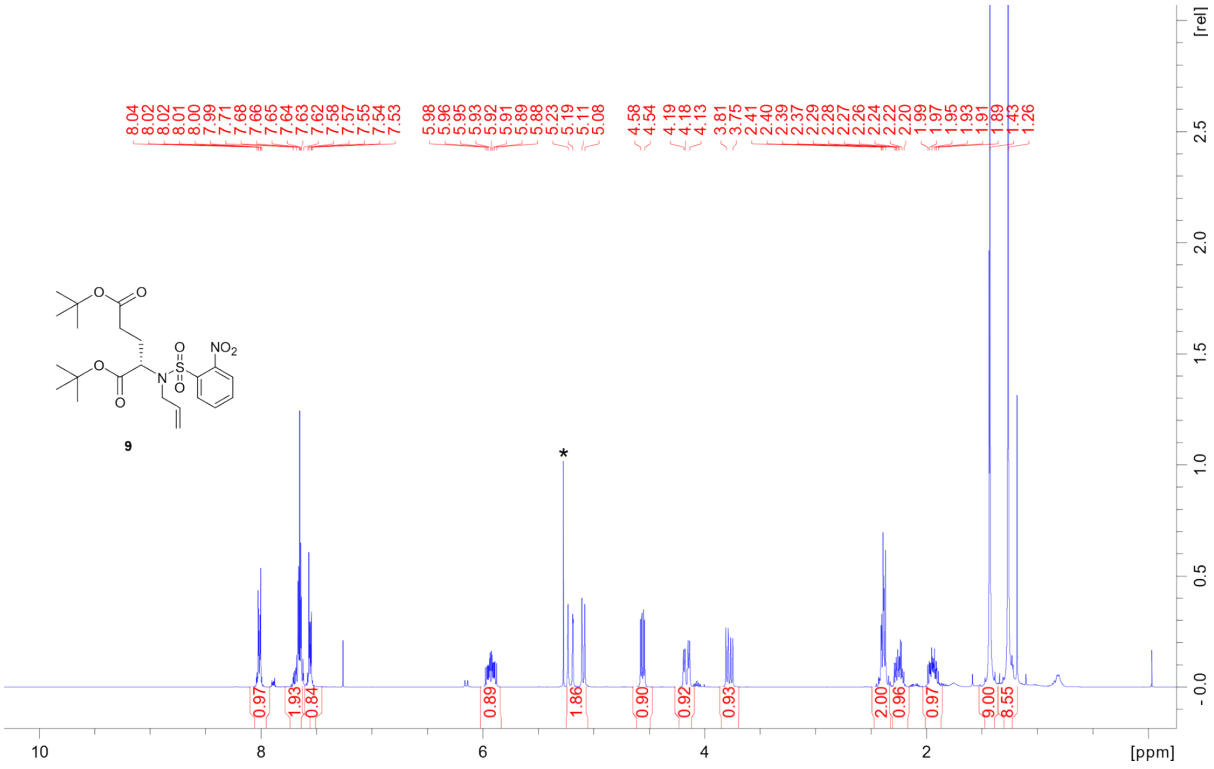
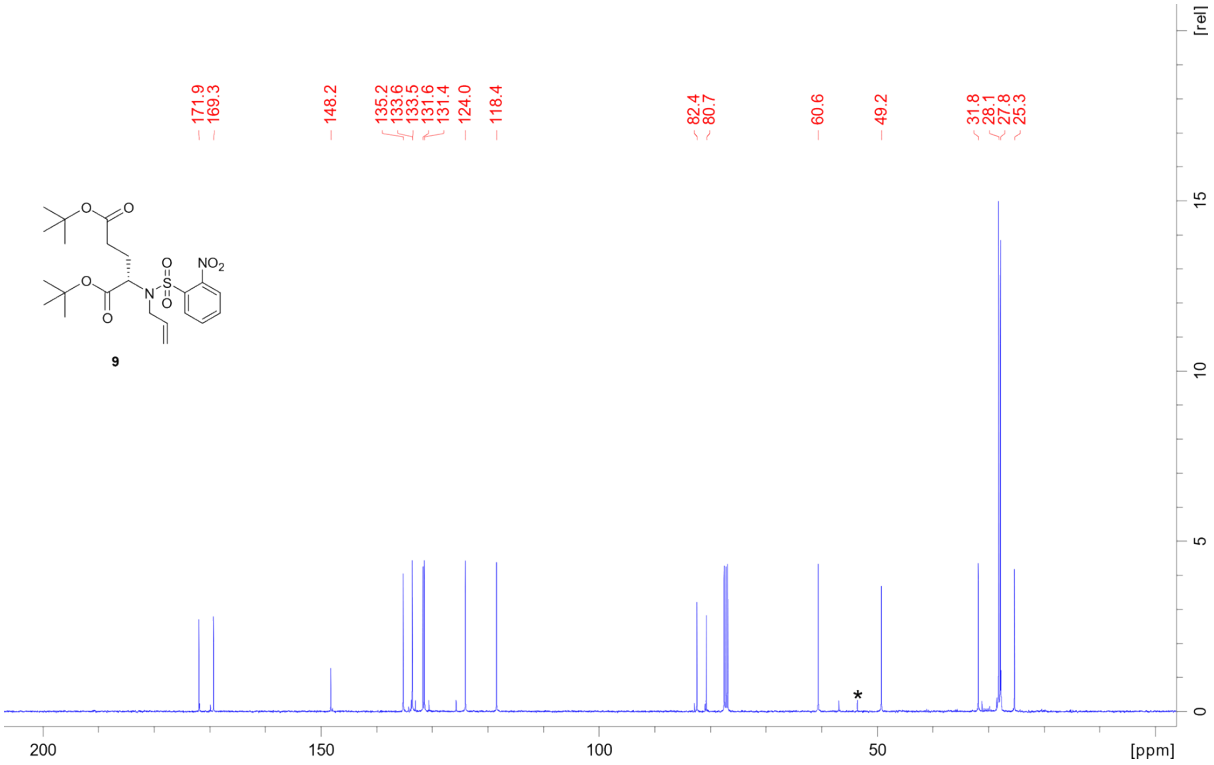


Figure SC3: IR spectra for **8**

^{1}H NMR ($CDCl_3$, 400 MHz): **9** (* corresponds to CH_2Cl_2 solvent peak)



^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): **9** (* corresponds to CH_2Cl_2 solvent peak)



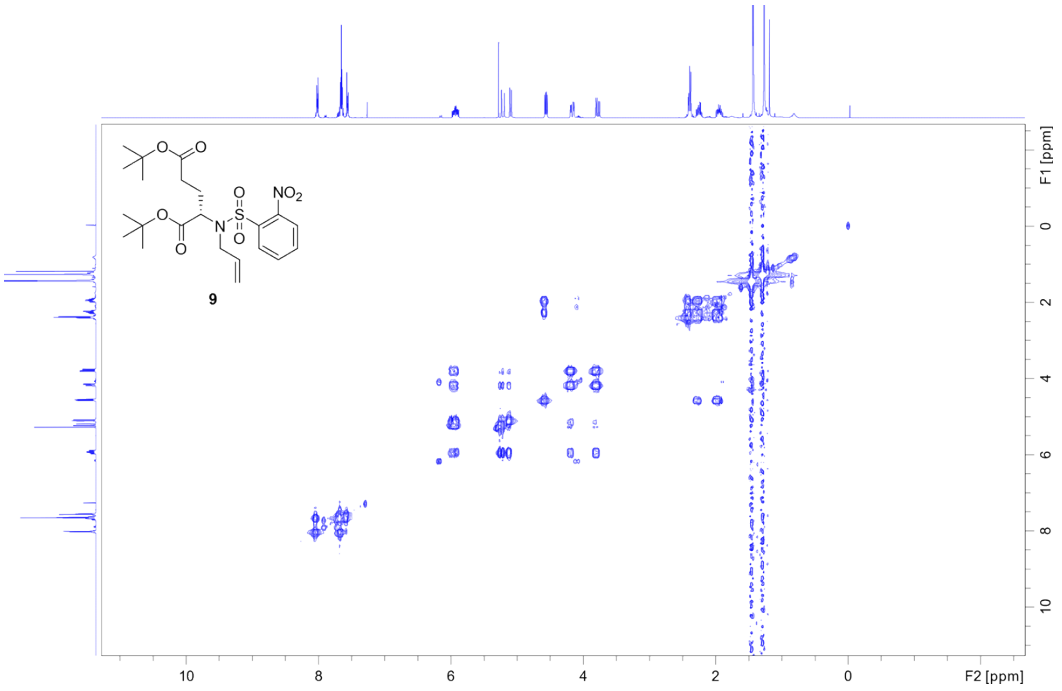


Figure S4. COSY spectrum for **9**

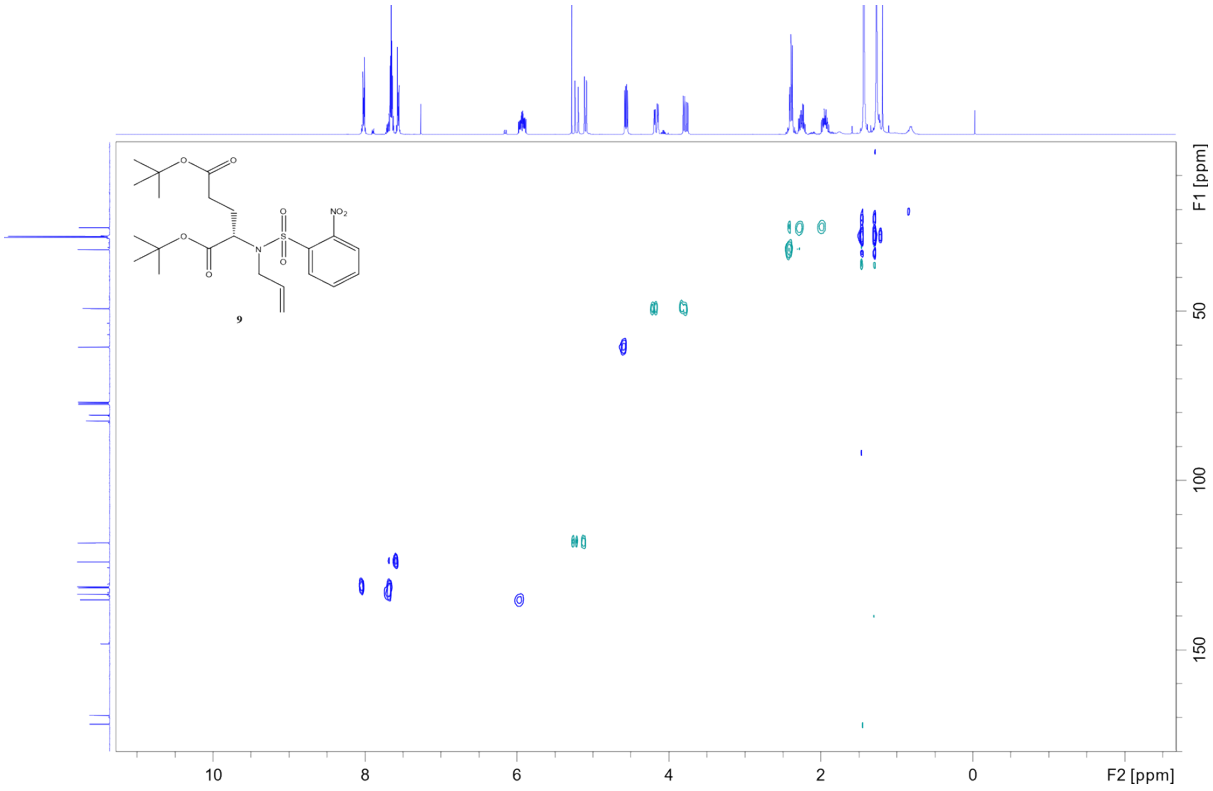


Figure S5. HSQC spectrum for **9**

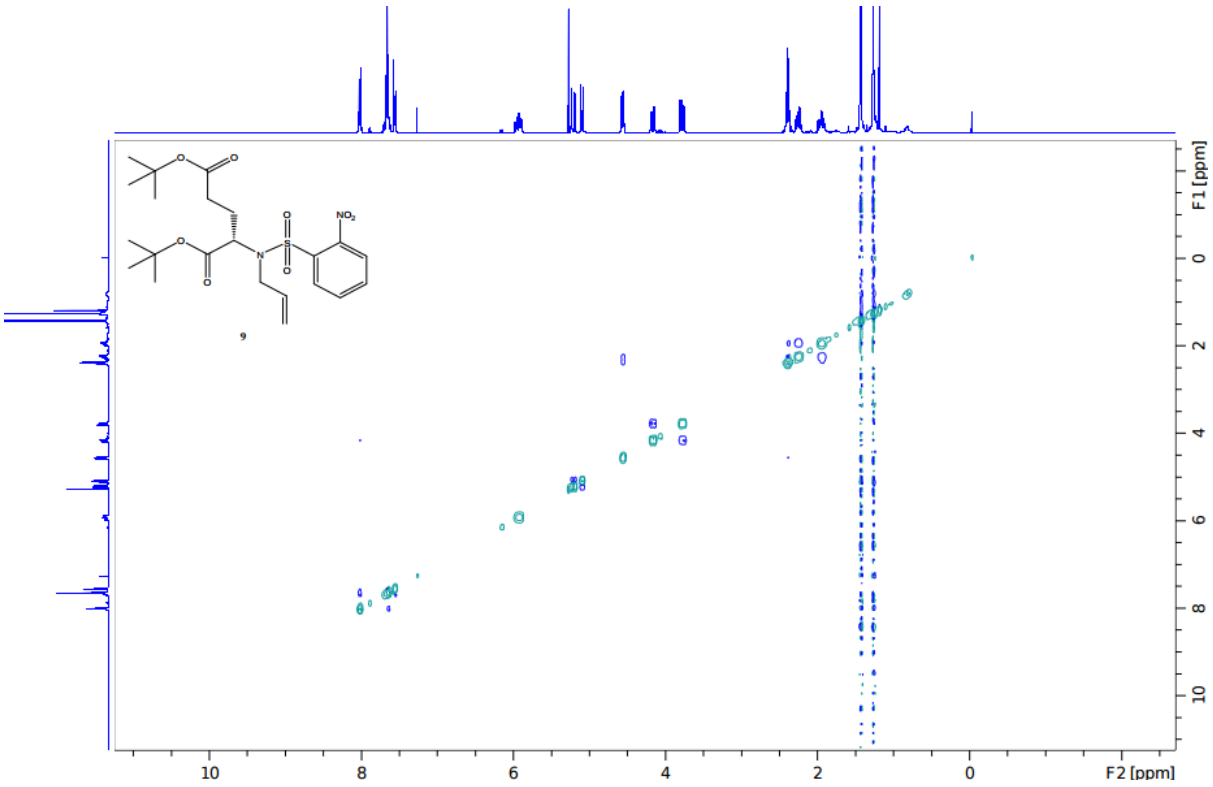
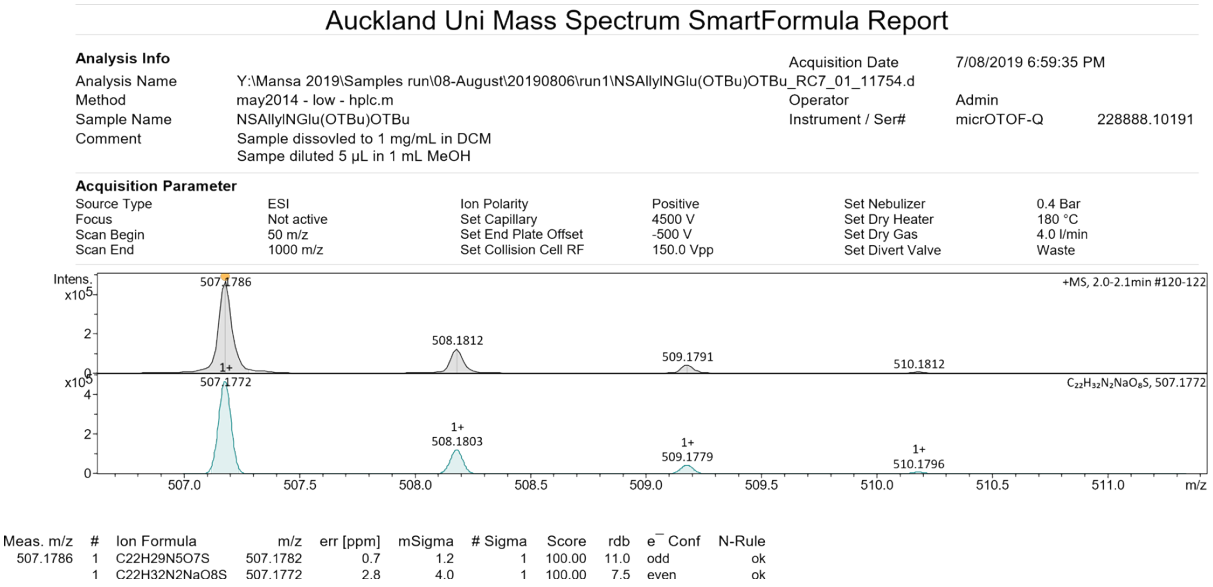


Figure S6. NOESY spectrum for **9**



Auckland Uni Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	Y:\Mansa 2019\Samples run\08-August\20190806\run1\NSAllylNGlu(OTBu)OTBu_RC7_01_11754.d	Acquisition Date	7/08/2019 6:59:35 PM
Method	may2014 - low - hplc.m	Operator	Admin
Sample Name	NSAllylNGlu(OTBu)OTBu	Instrument / Ser#	micrOTOF-Q 228888.10191
Comment	Sample dissovled to 1 mg/mL in DCM Sampe diluted 5 µL in 1 mL MeOH		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
507.1786	1	C22H29N5O7S	507.1782	0.7	1.2	1	100.00	11.0	odd	ok
	1	C22H32N2NaO8S	507.1772	2.8	4.0	1	100.00	7.5	even	ok

Figure S7: HRMS for **9**. HRMS (ESI): (*m/z* [M + Na]$^+$ calcd: $C_{22}H_{32}N_2O_8S_1Na^+$: 507.1772; found: 507.1786

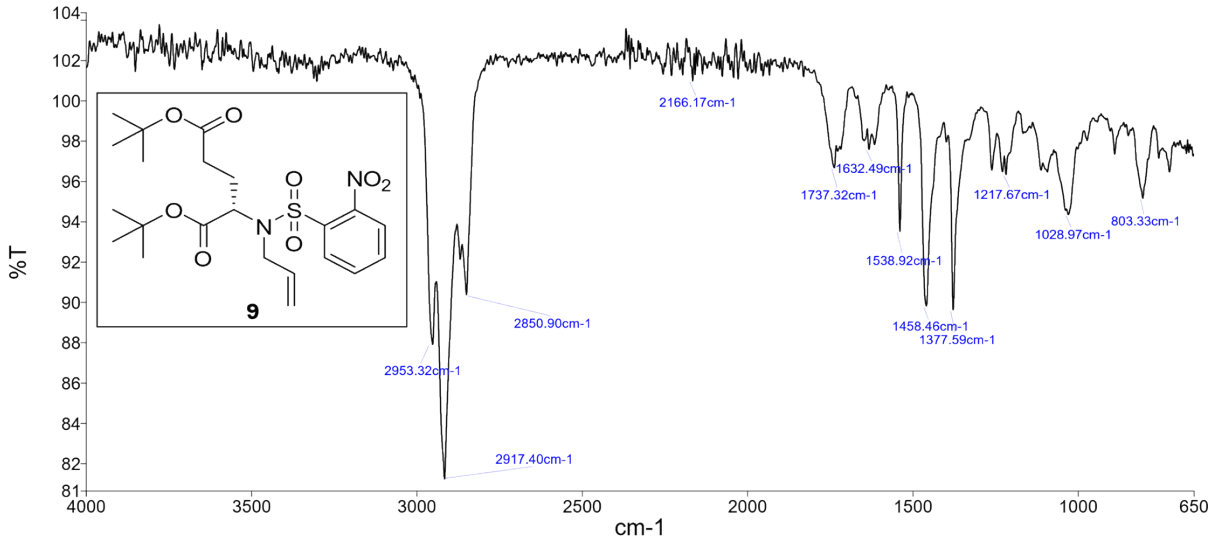
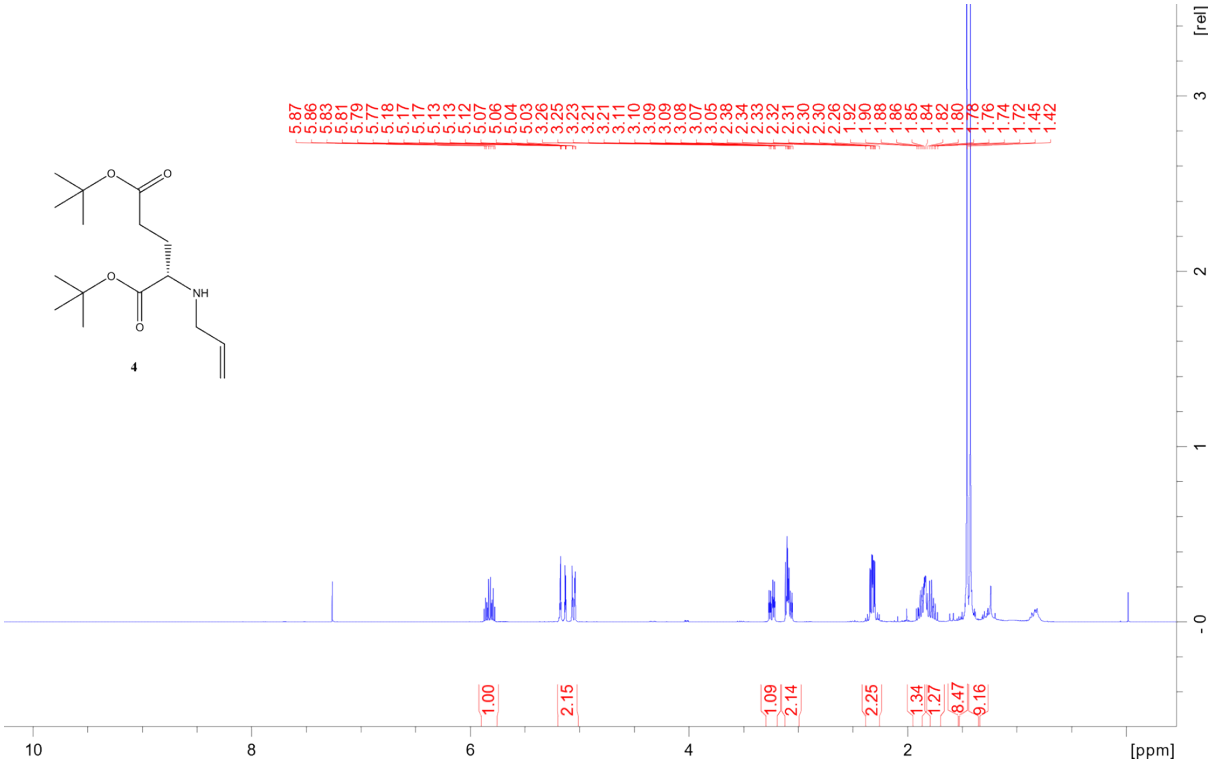


Figure S8: IR spectra for **9**

^{1}H NMR ($CDCl_3$, 400 MHz): **4**



^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): **4**

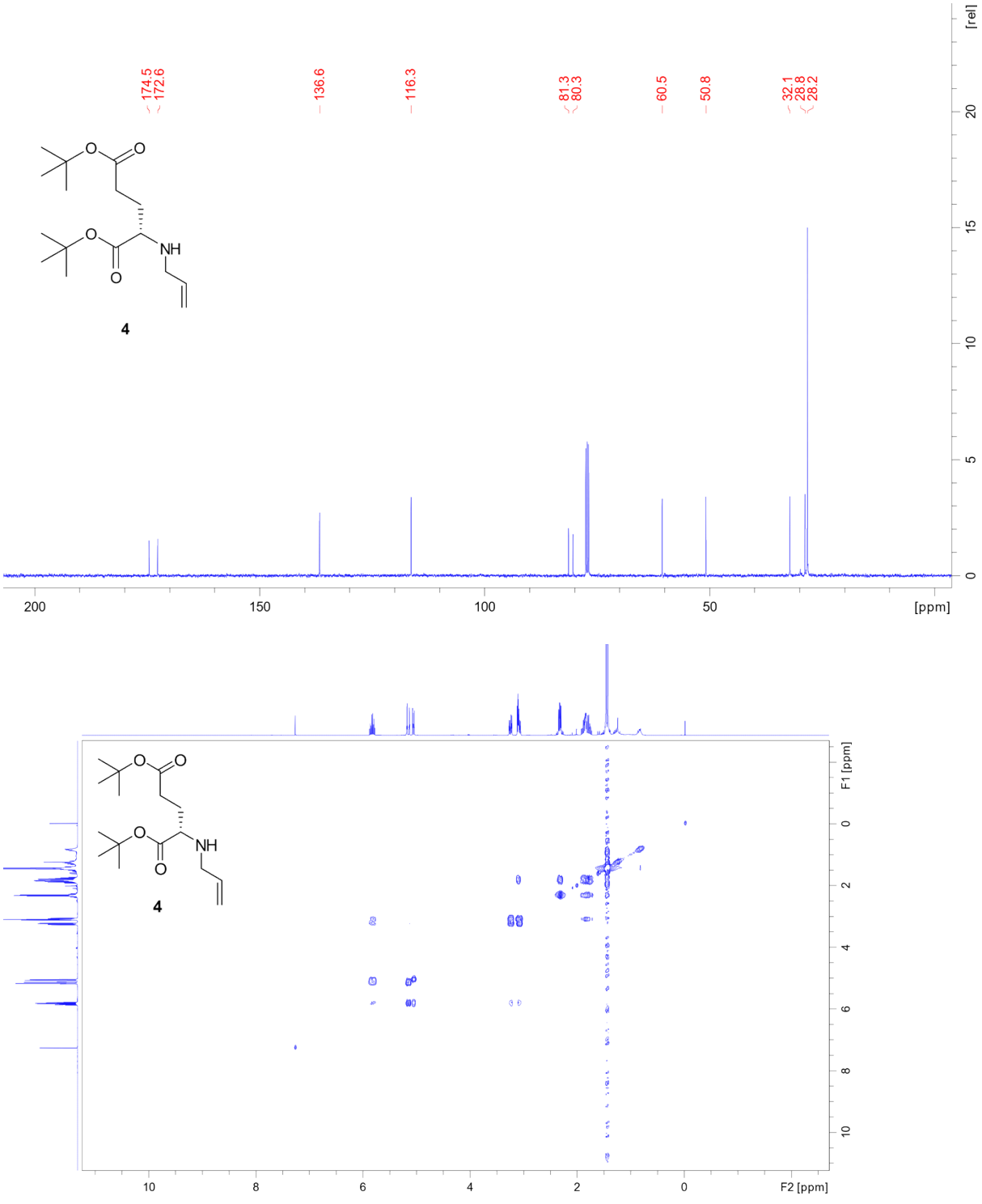


Figure S9. COSY spectrum for **4**

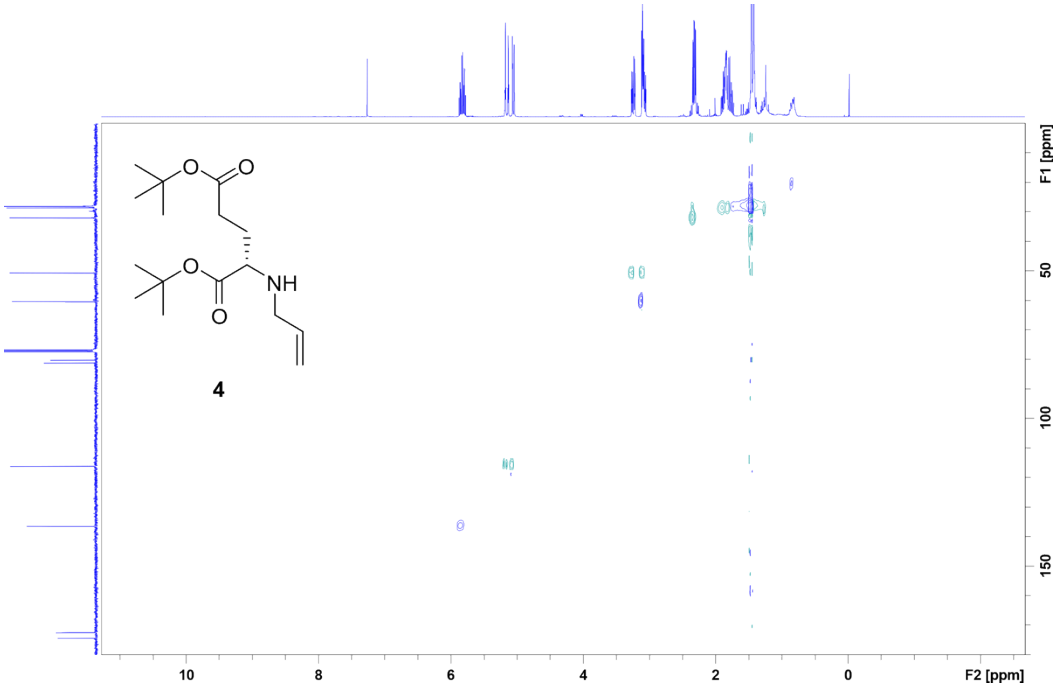


Figure S10. HSQC spectrum for **4**

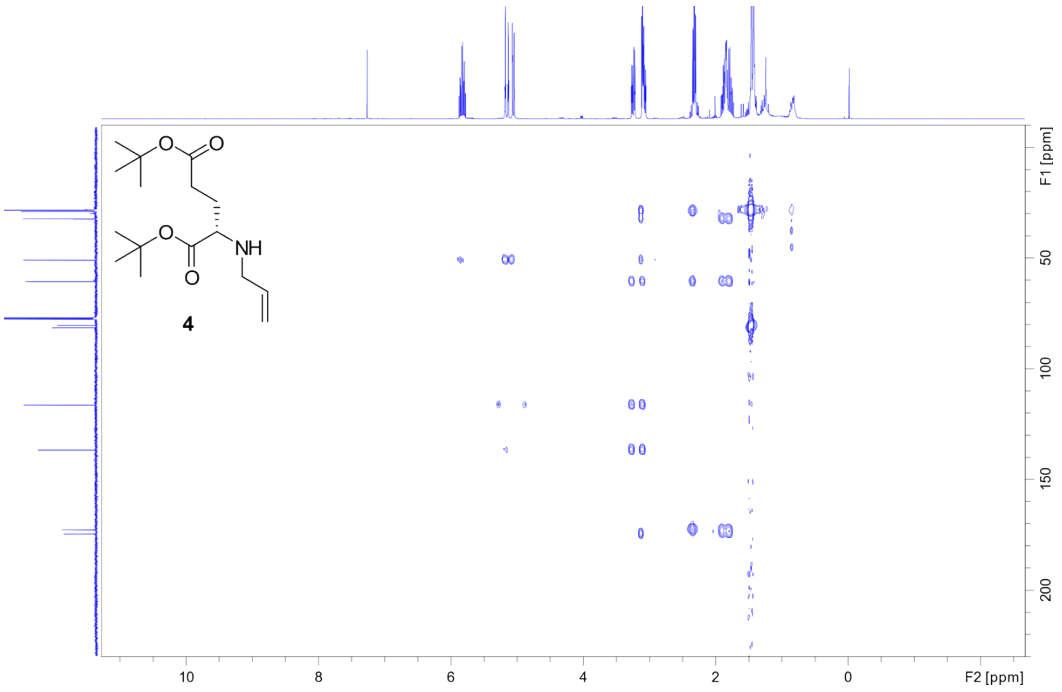


Figure S11. HMBC spectrum for **4**

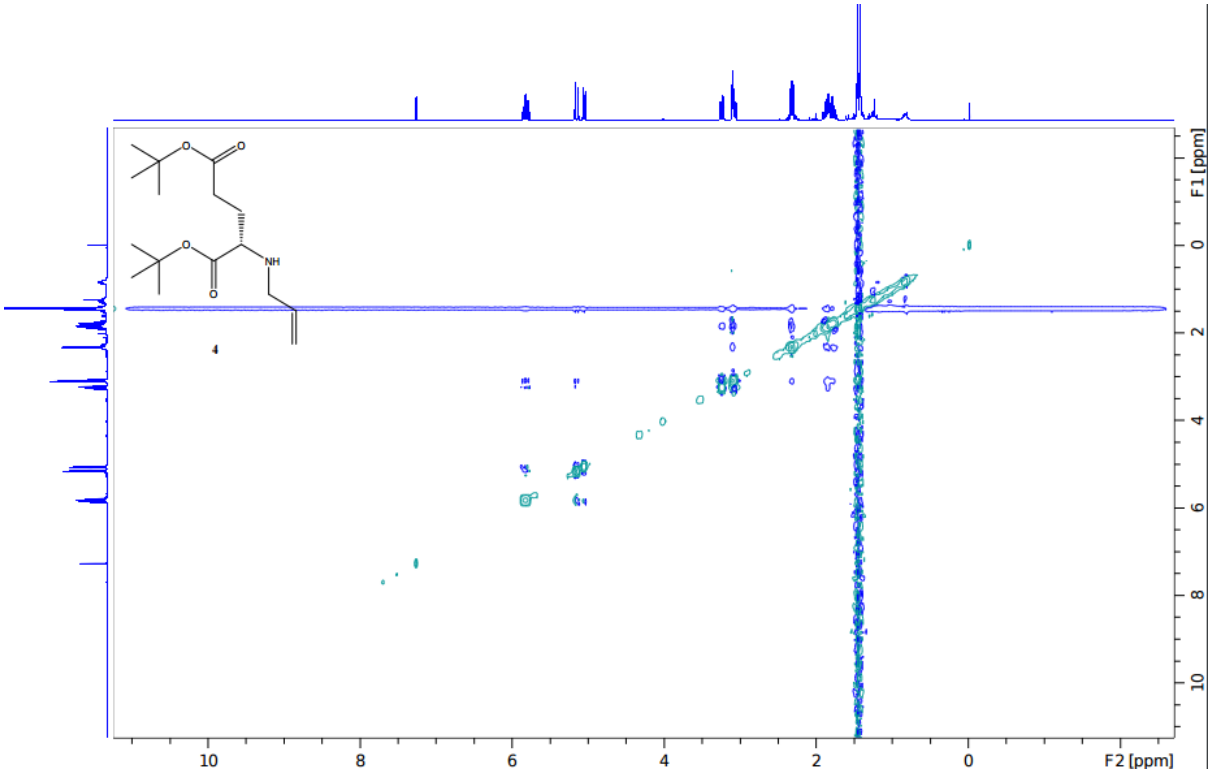


Figure S12. NOESY spectrum for **4**

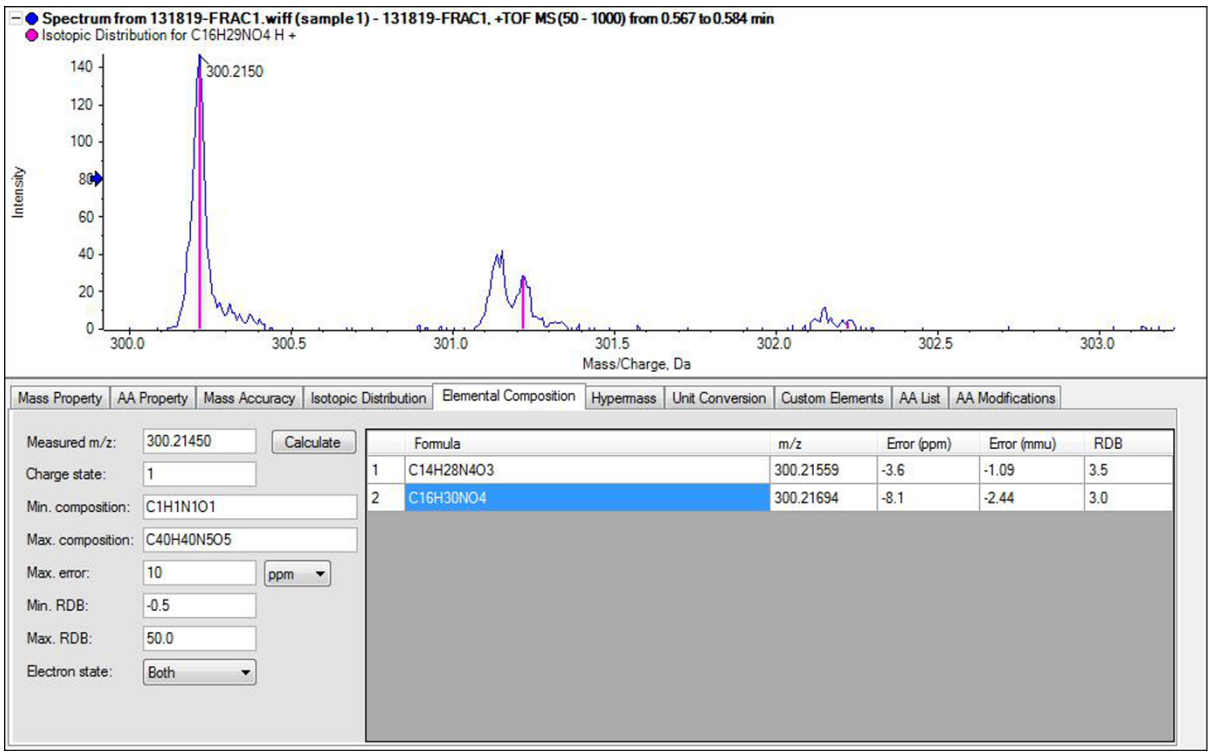


Figure S13: HRMS for **4**. HRMS (EI): (*m/z* $[M + H]^+$ calcd: $C_{16}H_{30}N_1O_4^+$: 300.2169; found: 300.2150

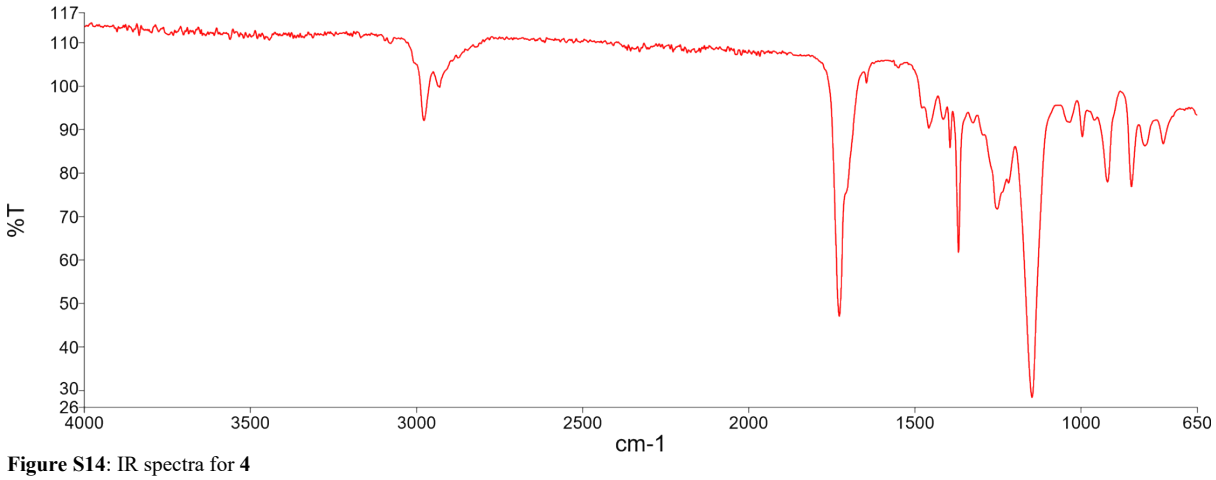
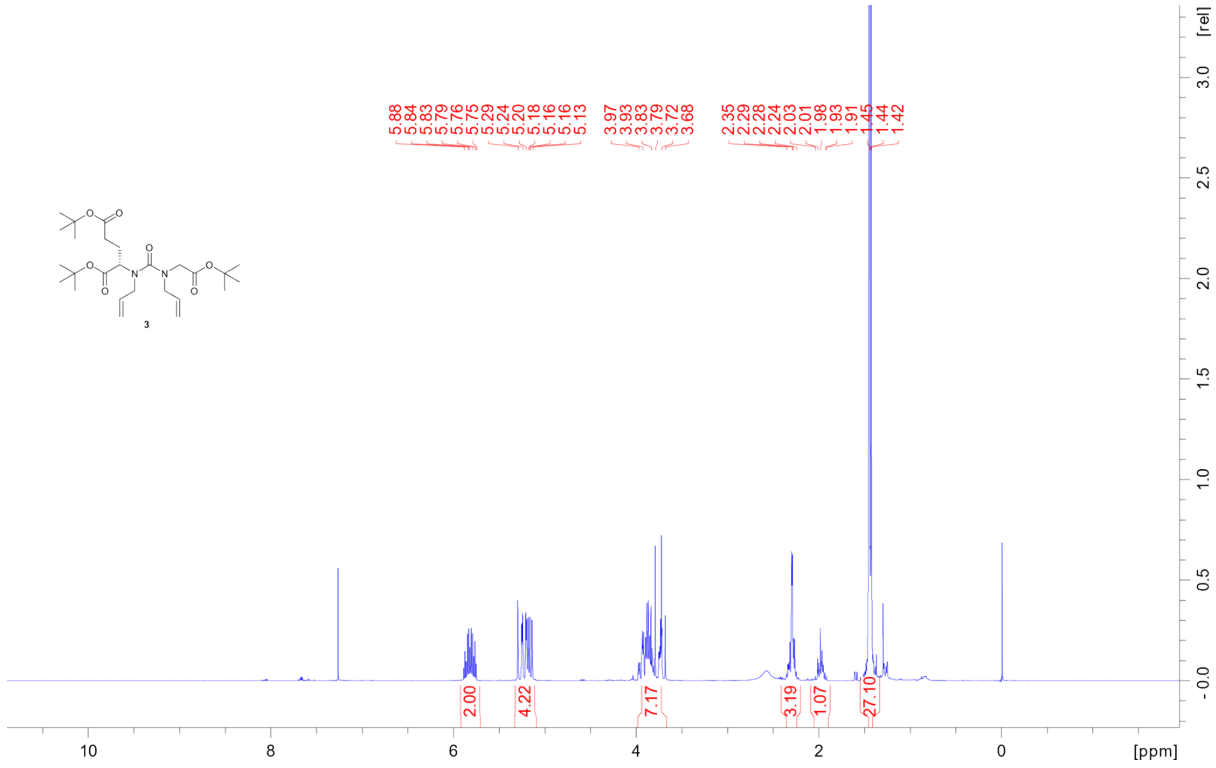


Figure S14: IR spectra for **4**

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



$^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): **3**

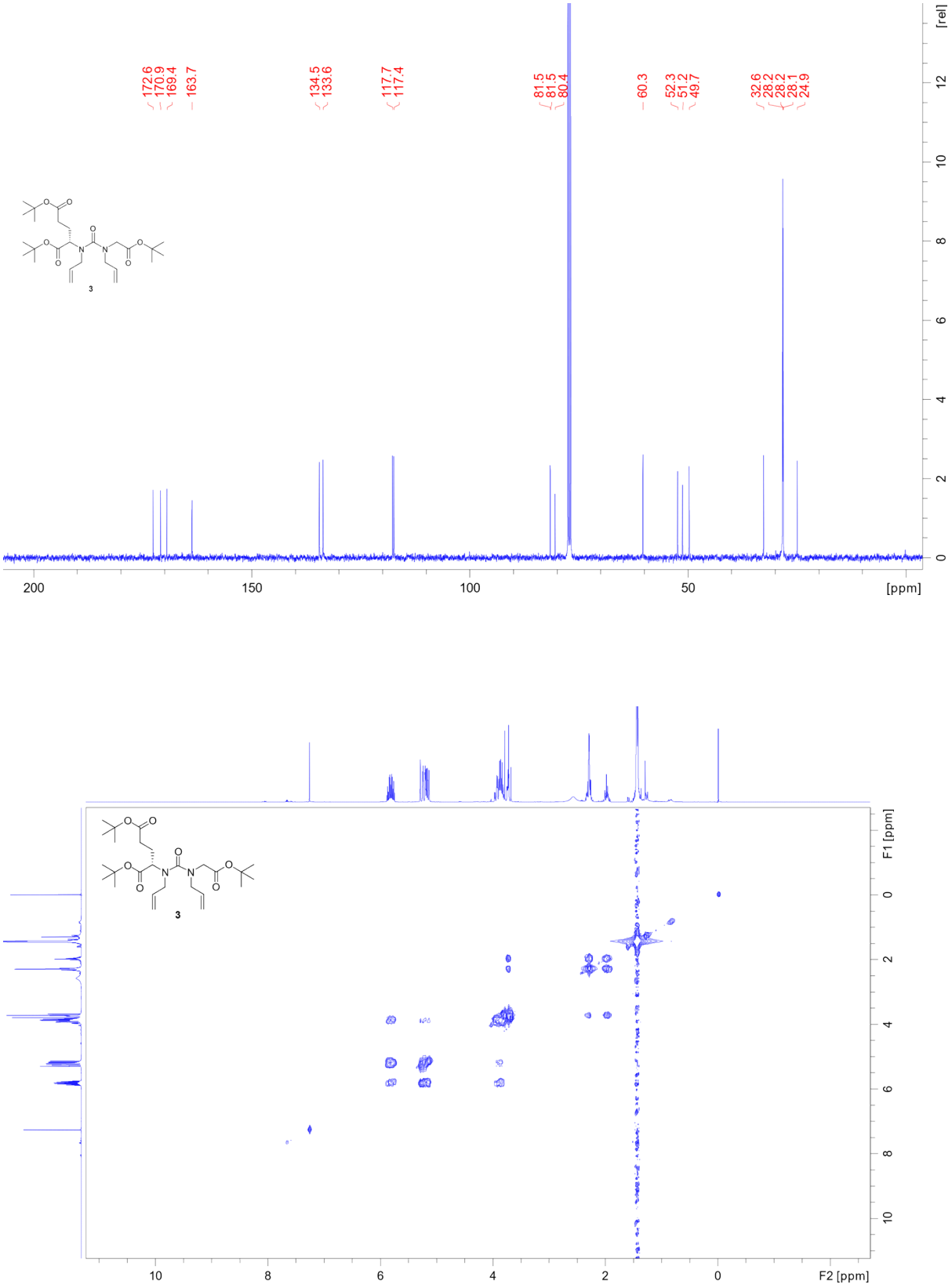


Figure S15. COSY spectrum for **3**

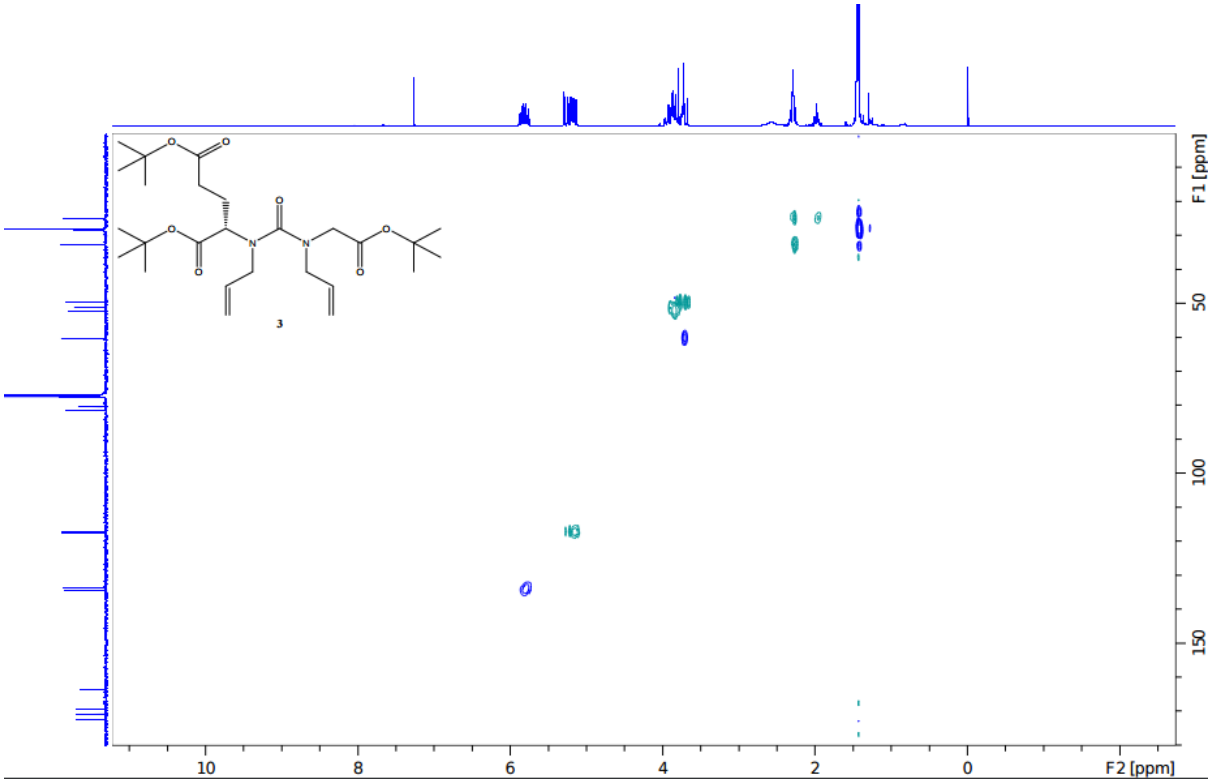


Figure S16. HSQC spectrum for **3**

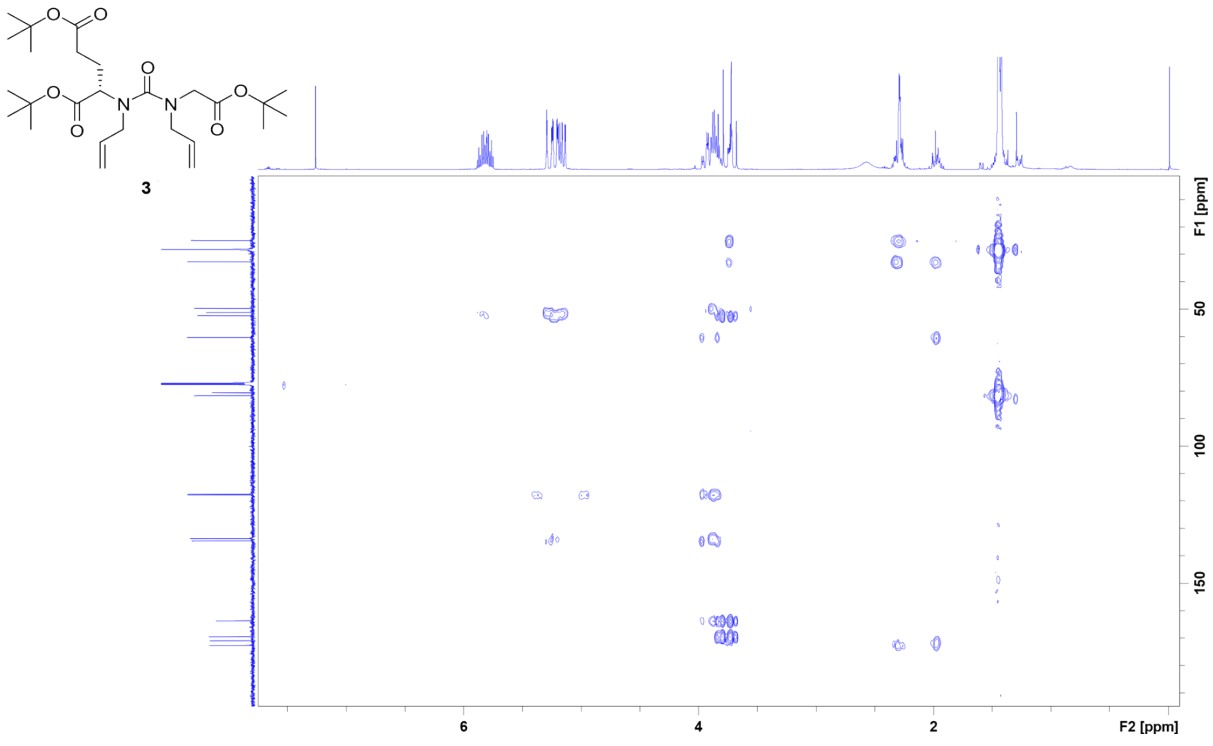


Figure S17. HMBC spectrum for **3**

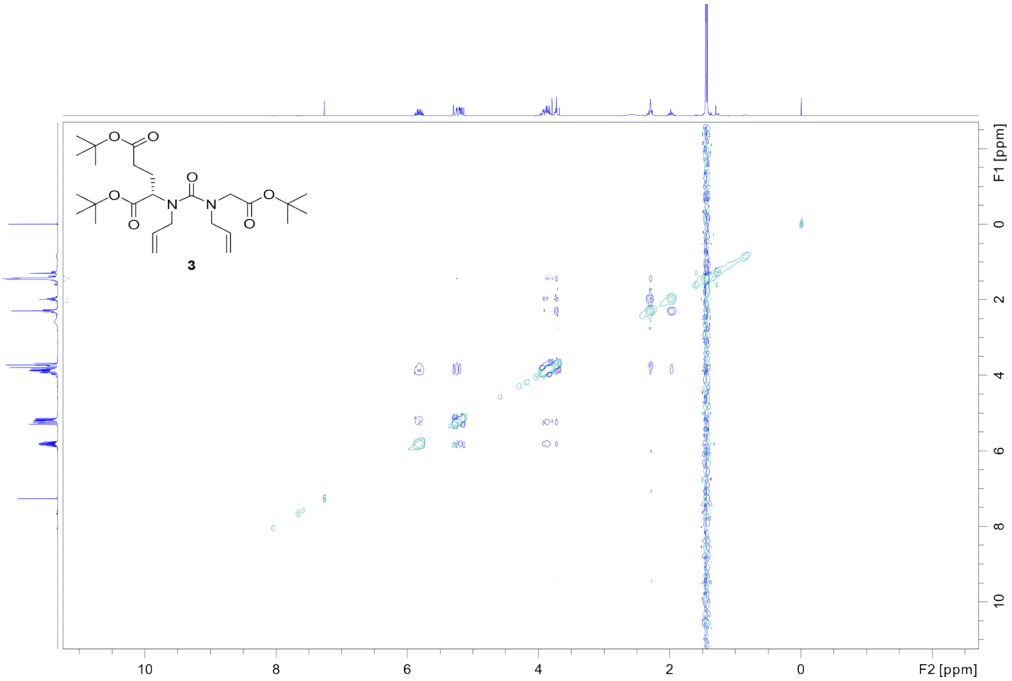


Figure S18. NOESY spectrum for **3**

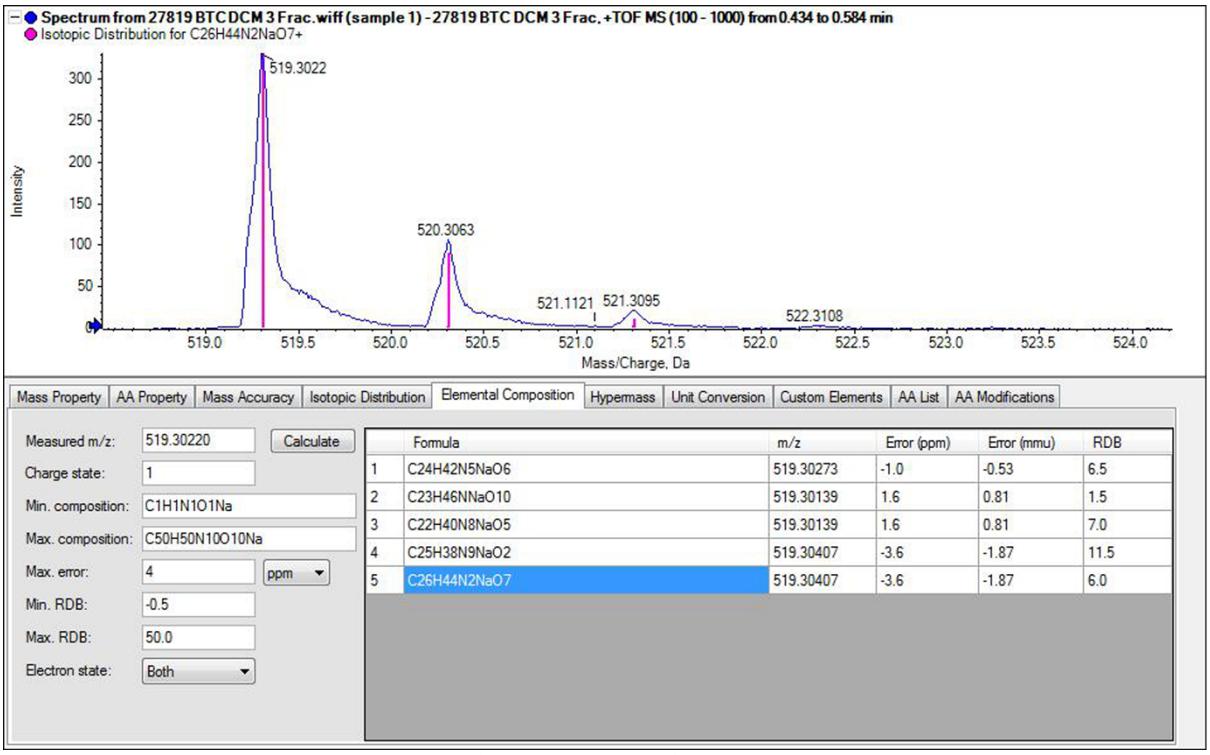


Figure S19: HRMS for **3**. HRMS (ESI): (*m/z* $[M + Na]^+$ calcd: $C_{26}H_{44}N_2NaO_7^+$: 519.3041; found: 519.3022

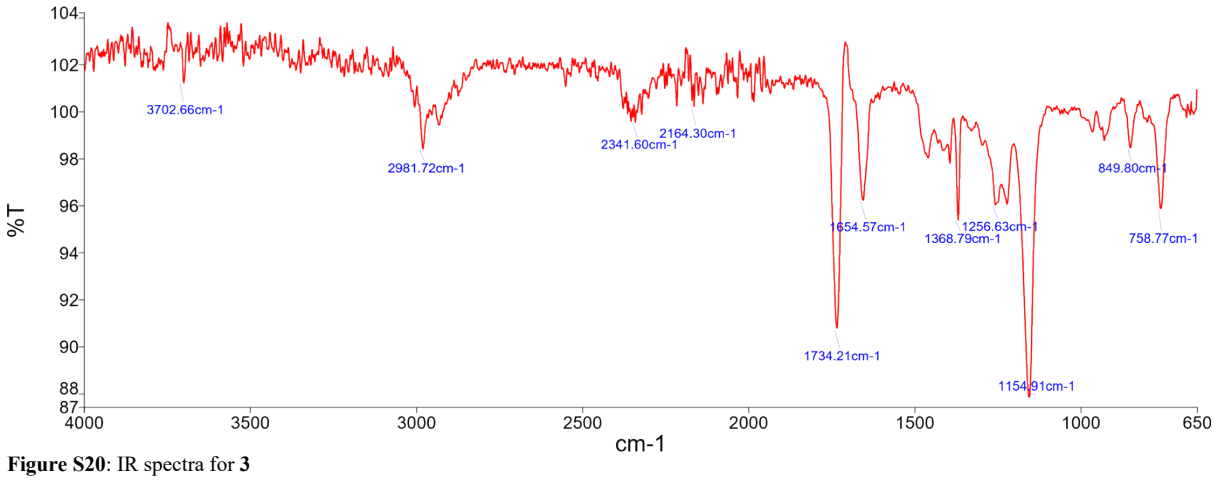
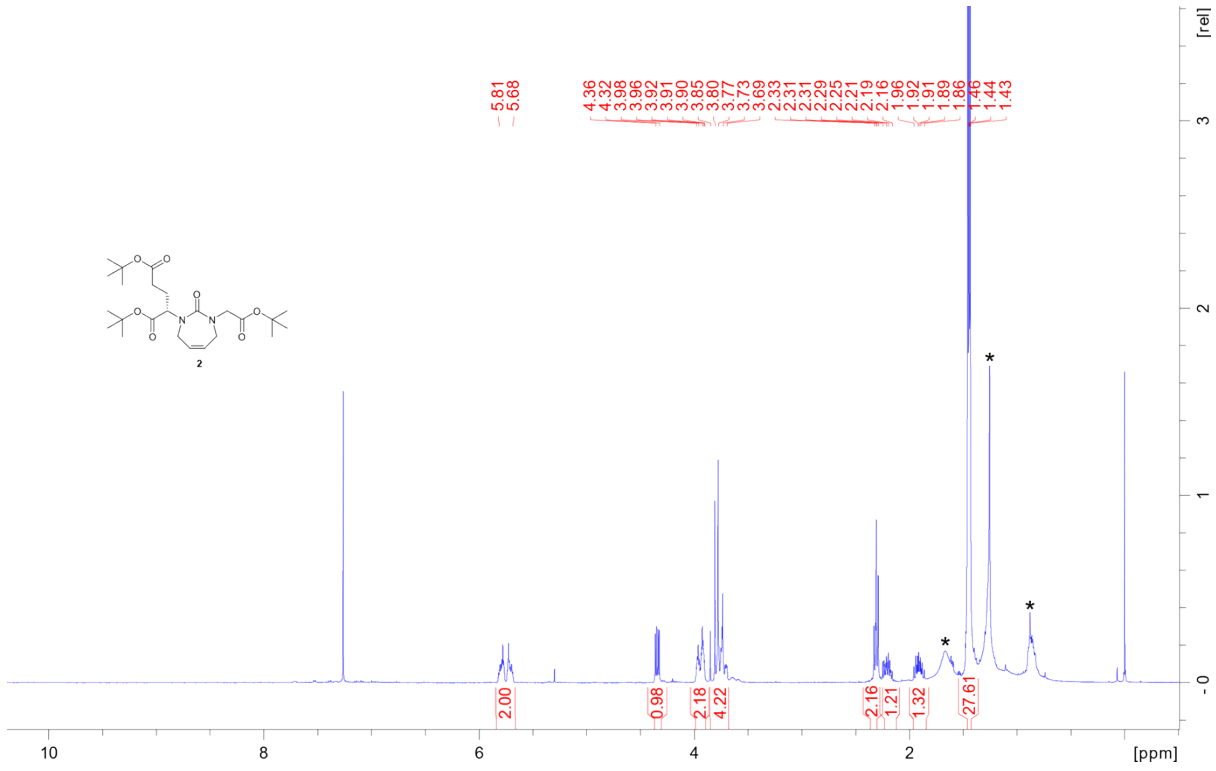
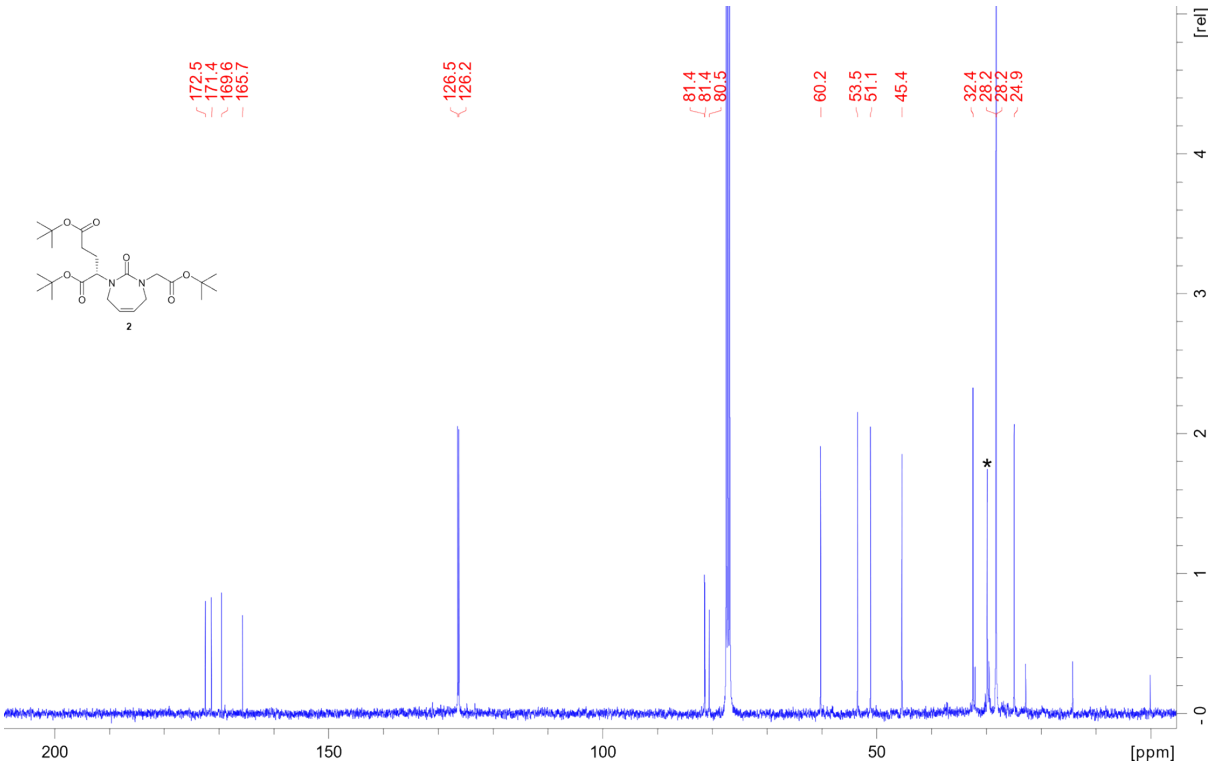


Figure S20: IR spectra for **3**

^{1}H NMR ($CDCl_3$, 400 MHz): **2** (* Corresponds to H_2O and ‘‘grease’’ peaks)



$^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz): **2** (* Corresponds to ‘‘grease’’ peak)



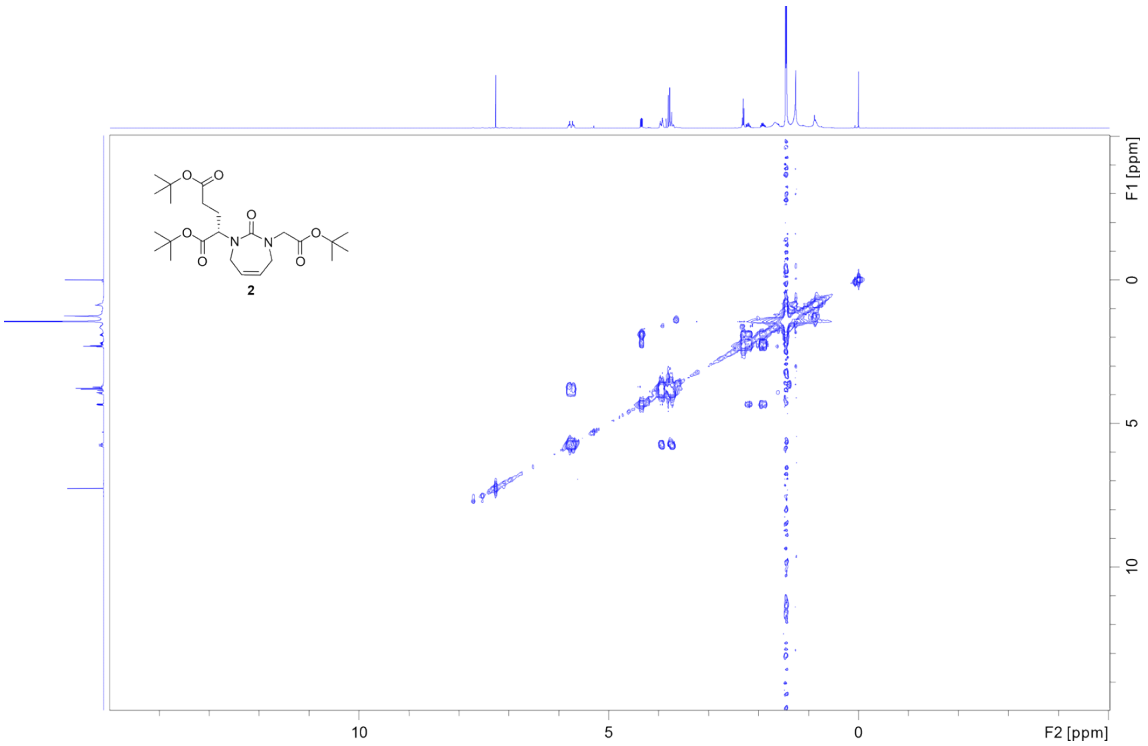


Figure S21. COSY spectrum for **2**

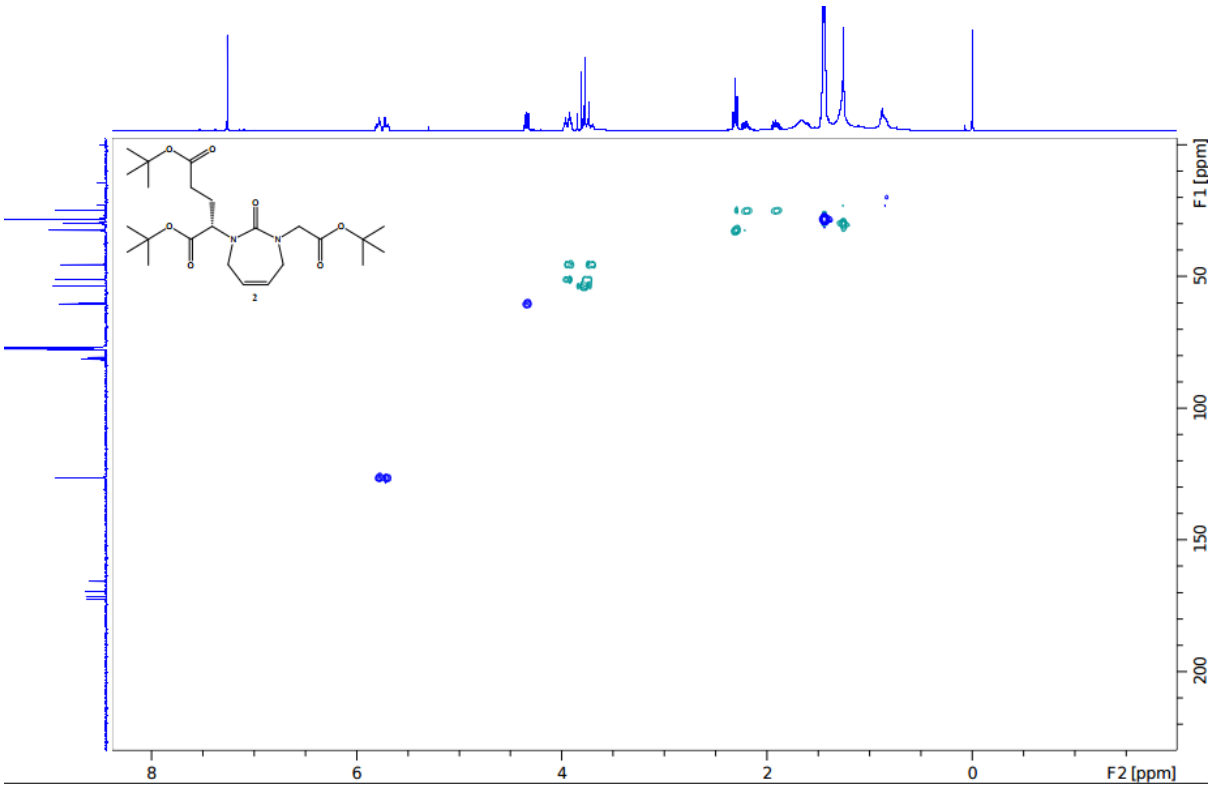


Figure S22. HSQC spectrum for **2**

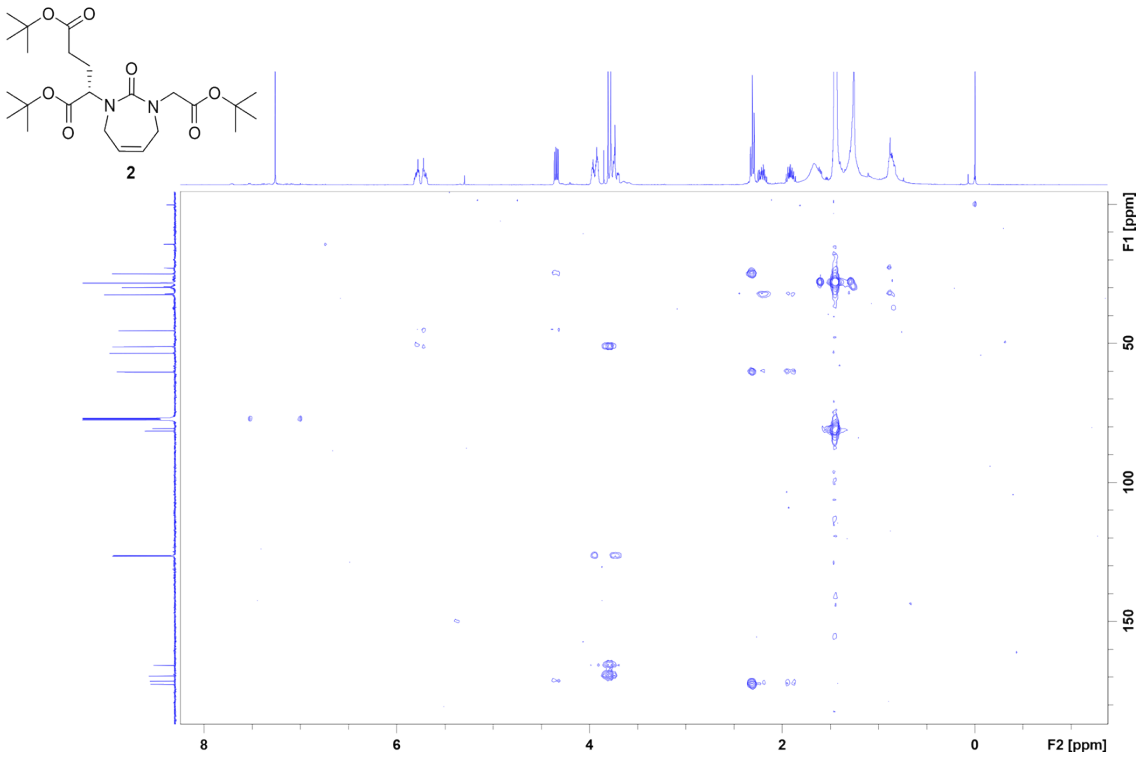


Figure S23. HMBC spectrum for **2**

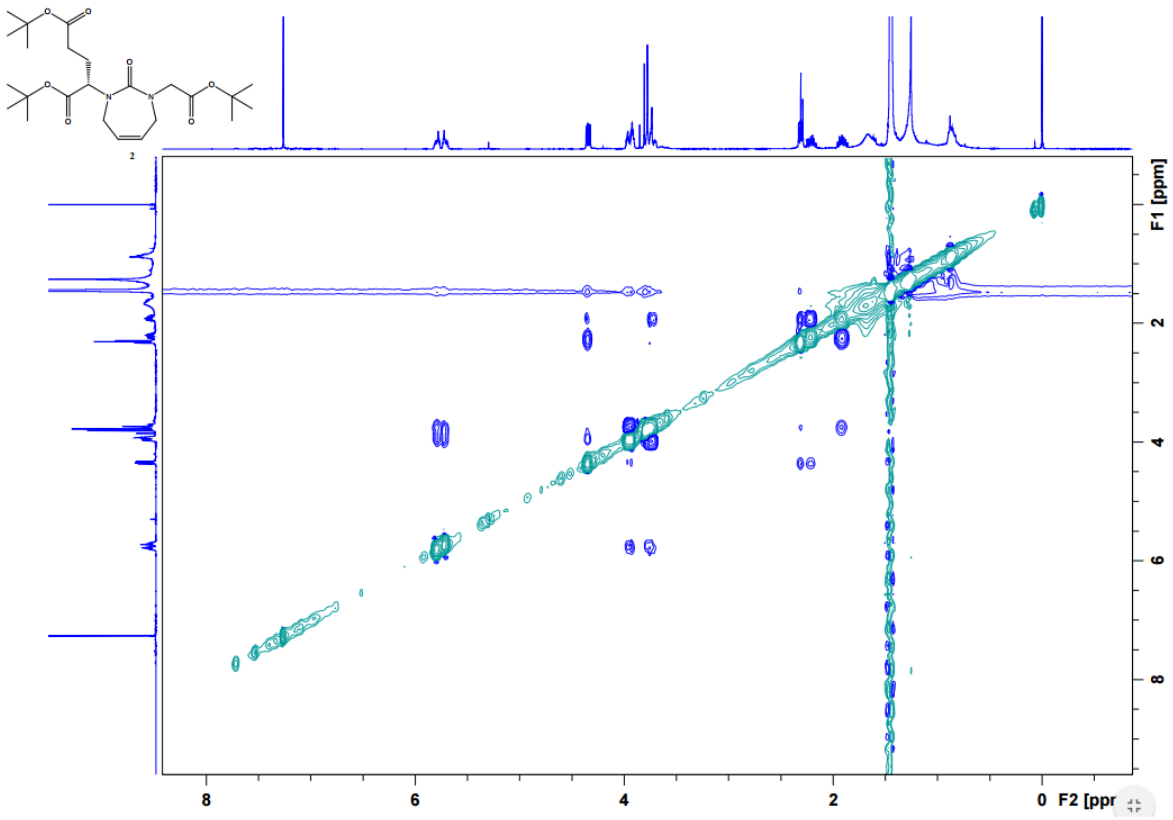


Figure S24. NOESY spectrum for **2**

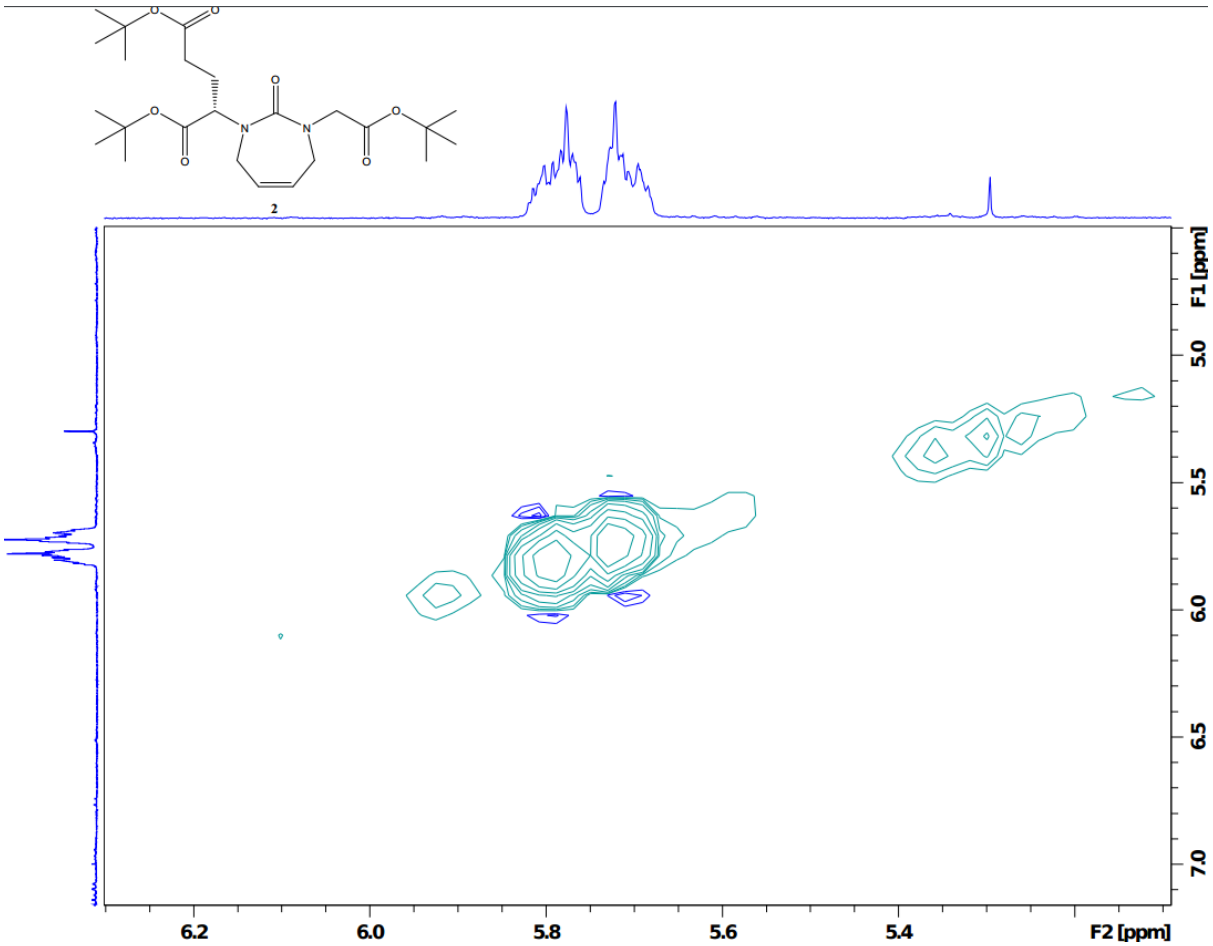


Figure 25. Expanded NOESY spectrum of compound **2** focusing on olefin region.

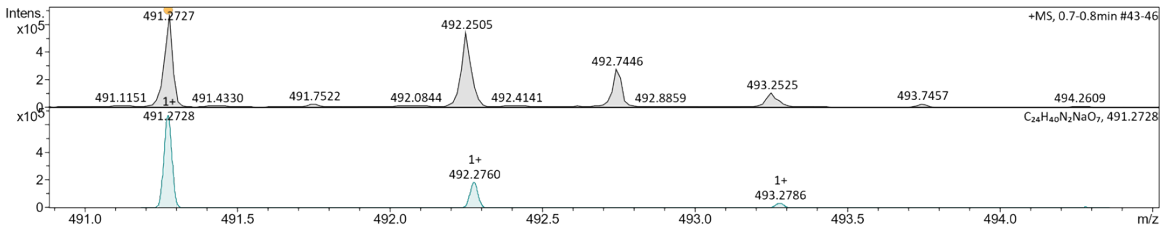
Auckland Uni Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	Y:\Mansa 2019\Samples run\October\191023\low\21-10-19-31-1-Grubbs type 1_RA1_01_12399.d	Acquisition Date	25/10/2019 2:11:31 PM
Method	low_hplc.m	Operator	Admin
Sample Name	21-10-19-31-1-Grubbs type 1	Instrument / Ser#	micrOTOF-Q 228888.10191
Comment	Sample dissolved to 1 mg/mL in DCM Sampe diluted 1.5 µL in 0.5 mL MeOH		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set Collision Cell RF	150.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
491.2727	1	C24H37N5O6	491.2738	-2.3	144.5	1	100.00	9.0	odd	ok
	1	C24H40N2NaO7	491.2728	0.2	139.4	1	100.00	5.5	even	ok

Figure S26: HRMS for **2**. HRMS (ESI): (*m/z* $[M + Na]^+$ calcd: $C_{24}H_{40}N_2NaO_7^+$: 491.2728; found: 491.2727

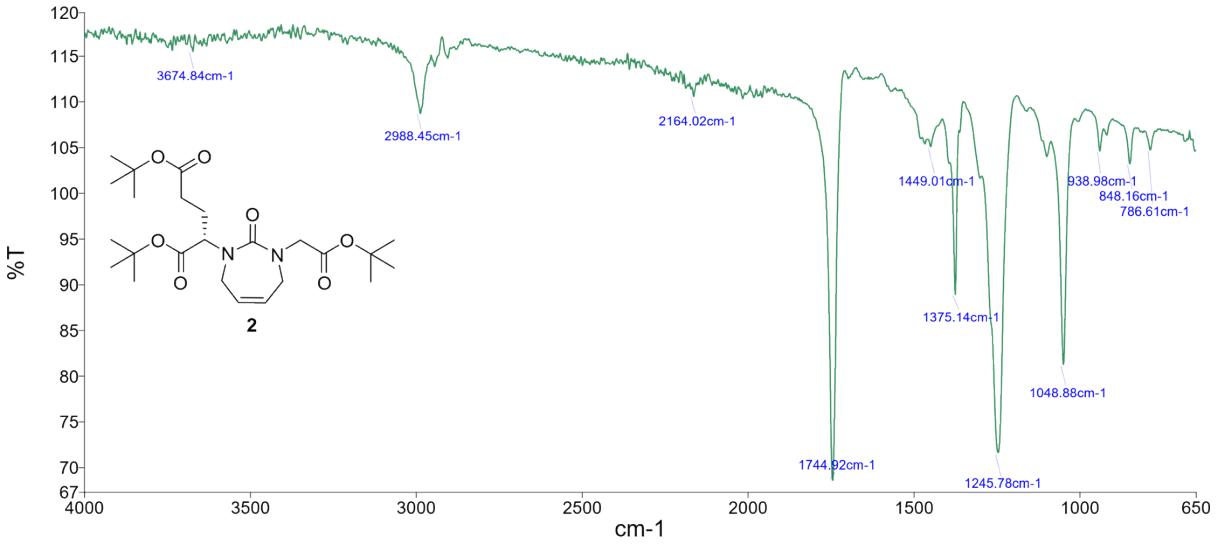
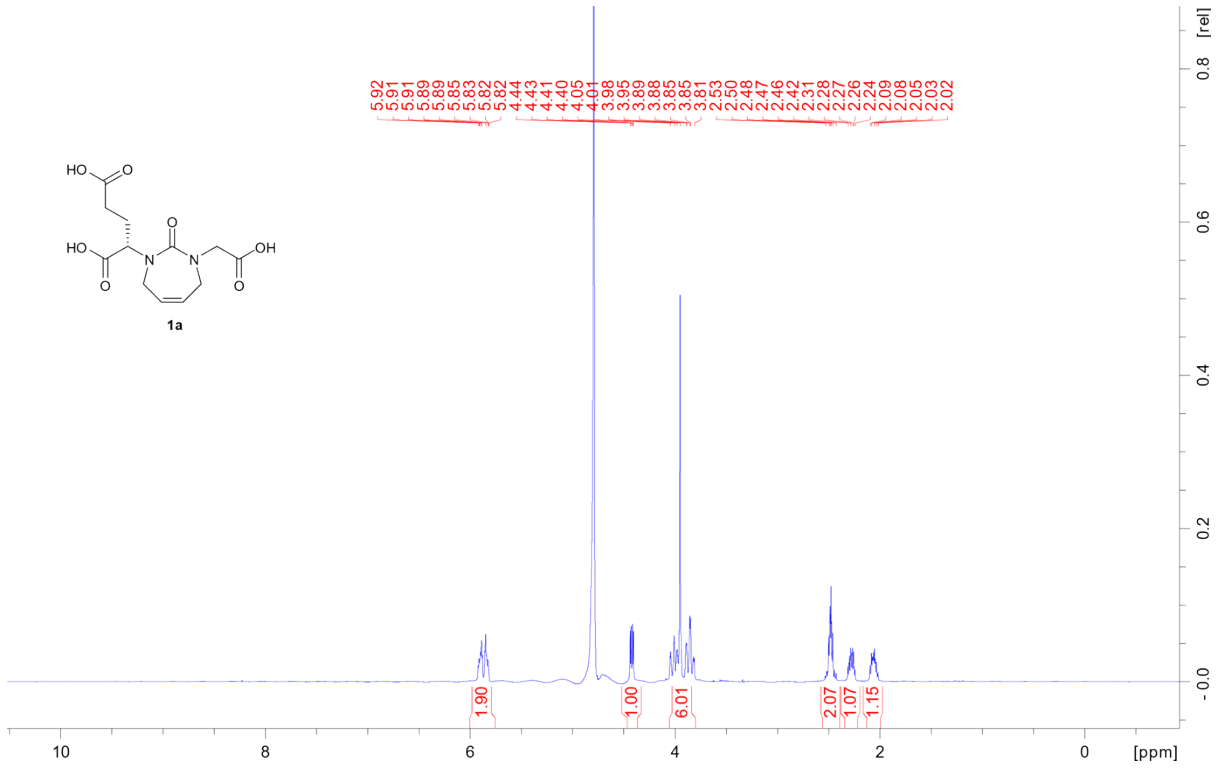
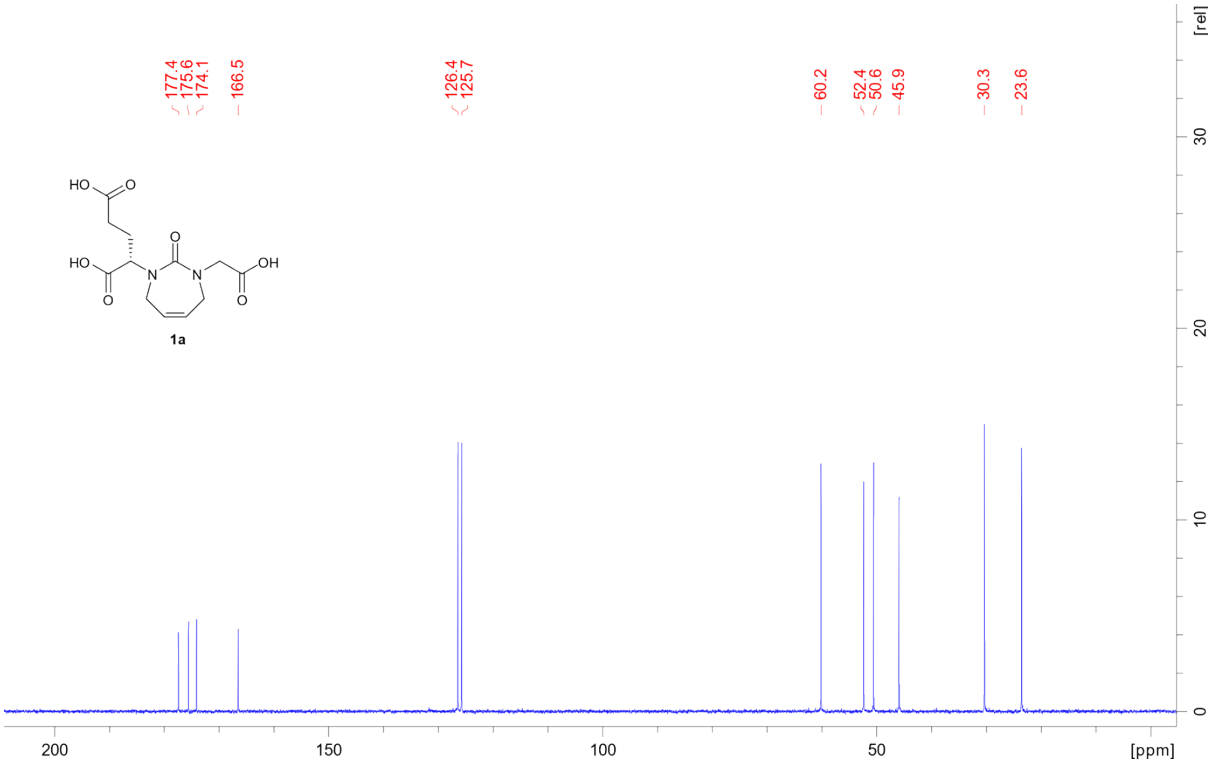


Figure S27: IR spectra for **2**

^{1}H NMR (D_2O, 500 MHz): **1a**



$^{13}C\{^{1}H\}$ NMR (D_2O, 125 MHz): **1a**



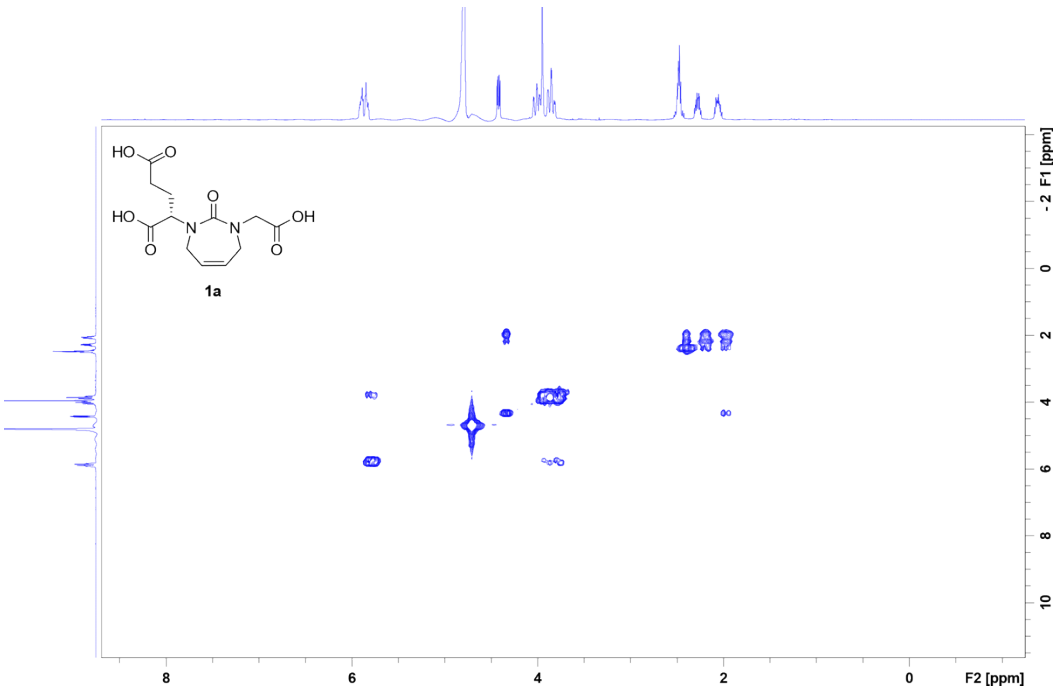


Figure S28. COSY spectrum for **1a**

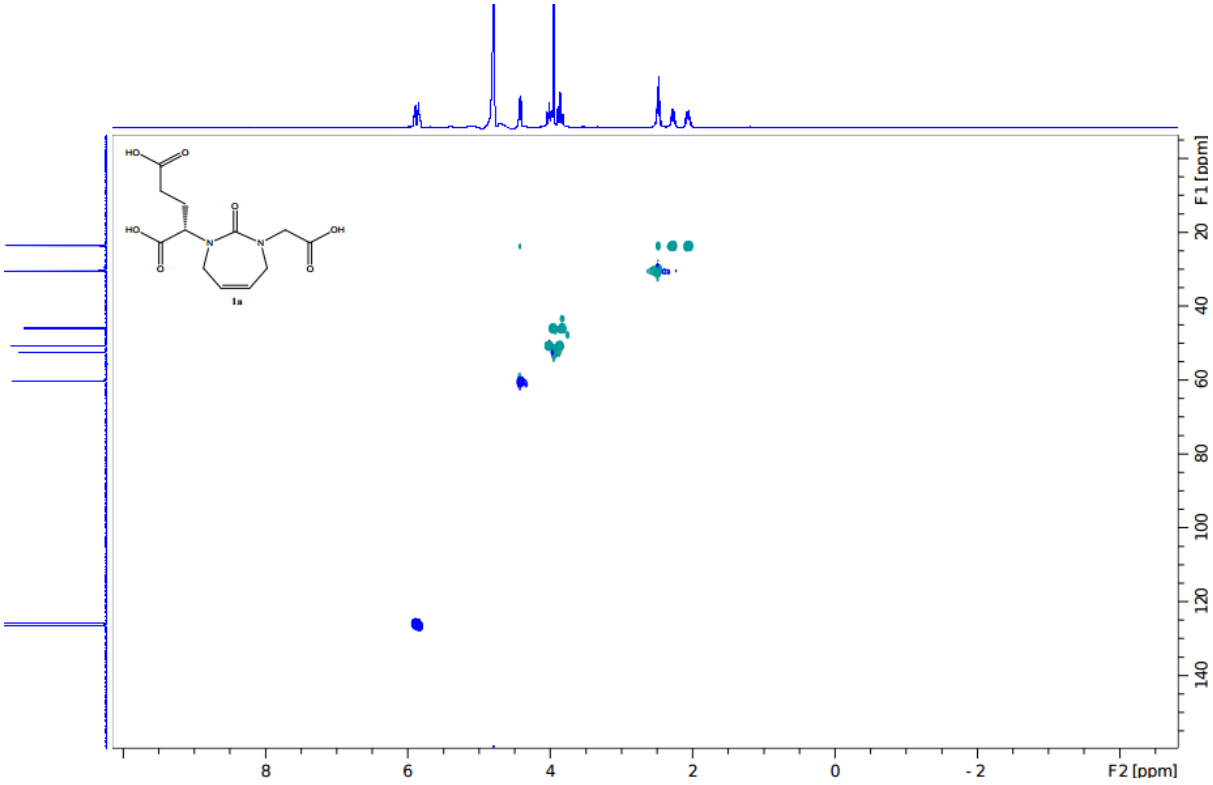


Figure S29. HSQC spectrum for **1a**

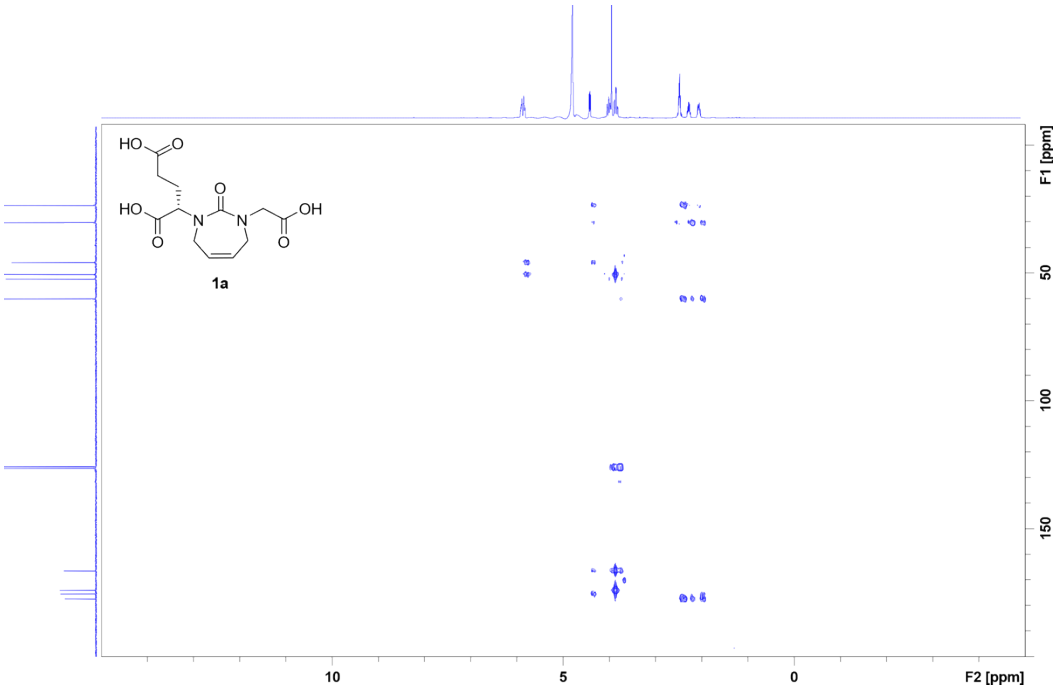


Figure S30. HMBC spectrum for **1a**

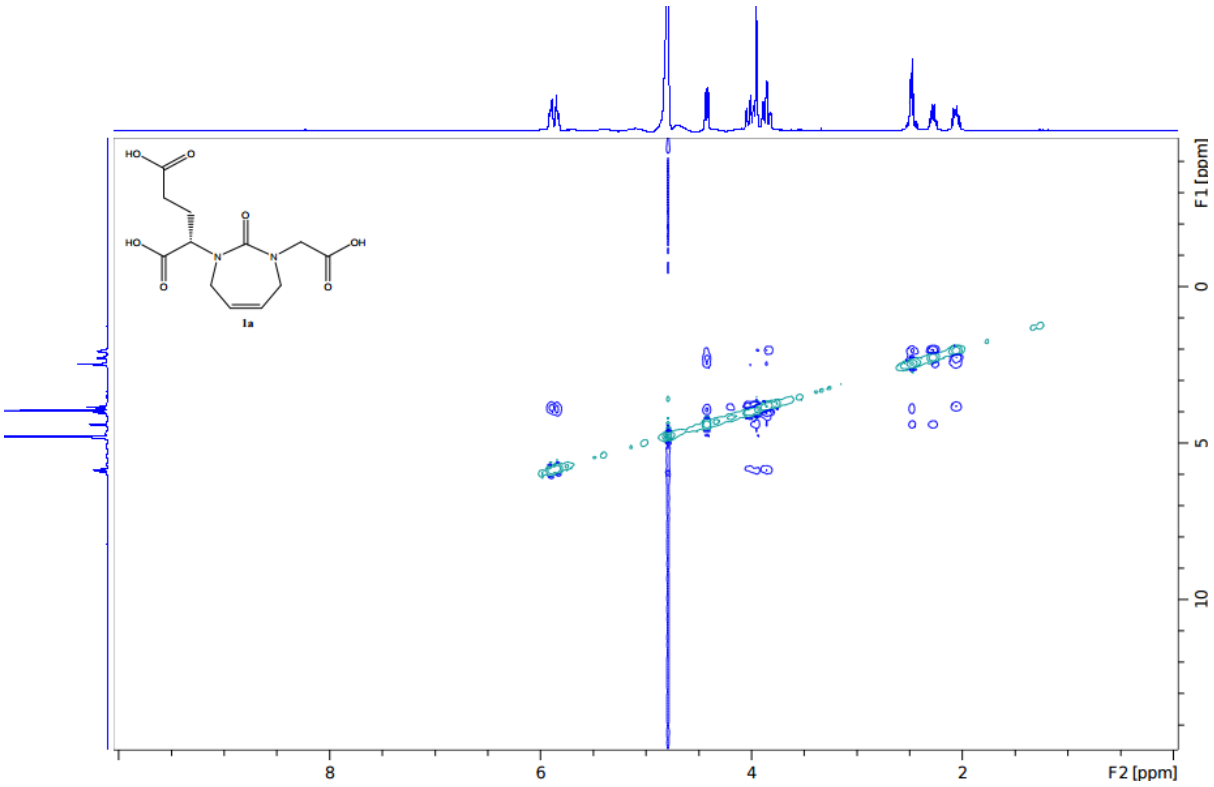


Figure S31. NOESY spectrum for **1a**

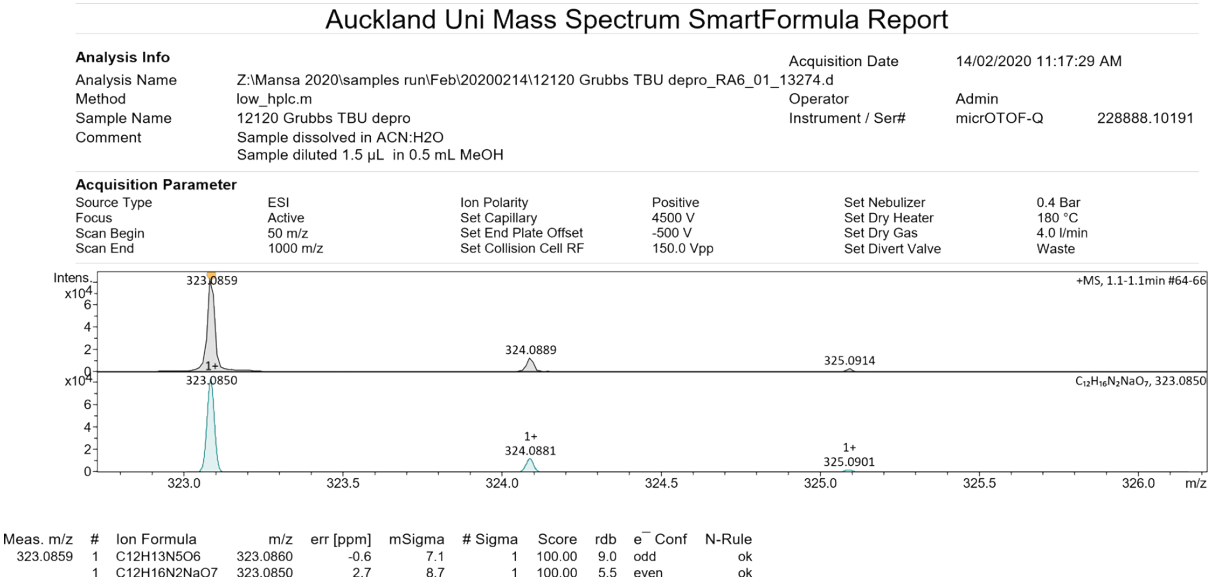


Figure S32: HRMS for **1a**. HRMS (ESI): (*m/z* $[M + Na]^+$ calcd: $C_{12}H_{16}N_2O_7Na^+$: 323.0850; found: 323.0859

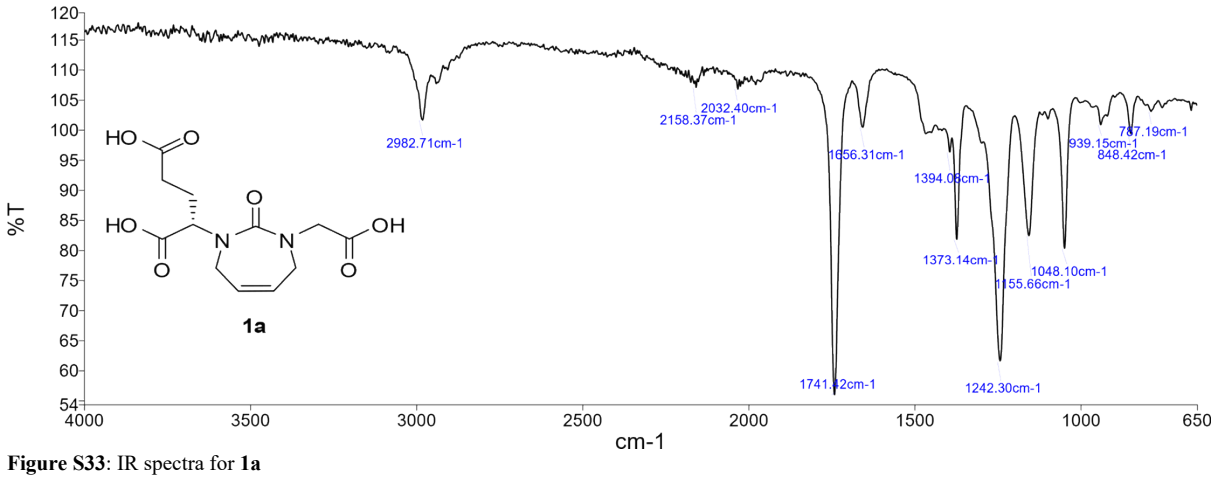


Figure S33: IR spectra for **1a**

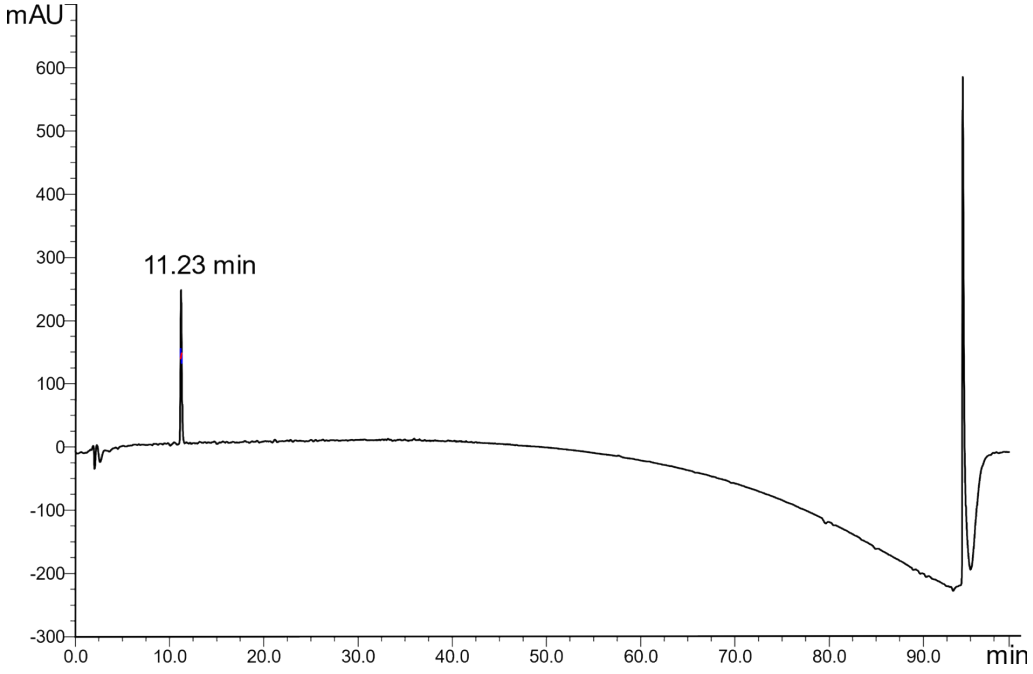


Figure S34: Analytical RP-HPLC chromatogram of purified **1a**, t_R = 11.23 min. Chromatographic separations were performed on a Thermo Scientific Dionex Ultimate 3000 HPLC using a XTerra® MS C-18 column (5 µm; 4.6 × 150 mm) and a linear gradient of 5-95% B in 90 min at room temperature, *ca*. 1% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*)

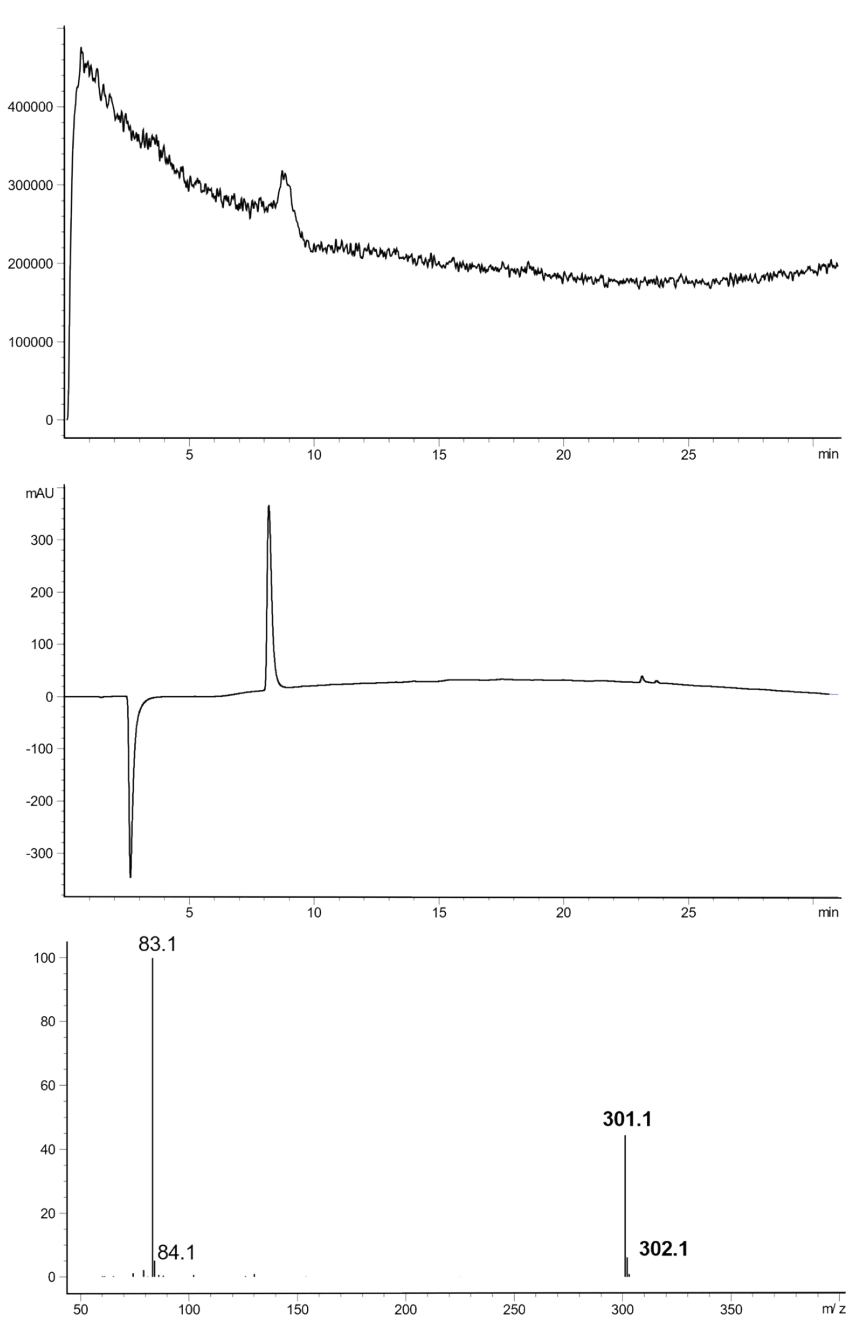


Figure S35: LC-MS profile of purified peptide **1a**; ion polarity positive. ESI-MS (*m/z* $[M + H]^+$ calcd: 301.1; found: 301.1)

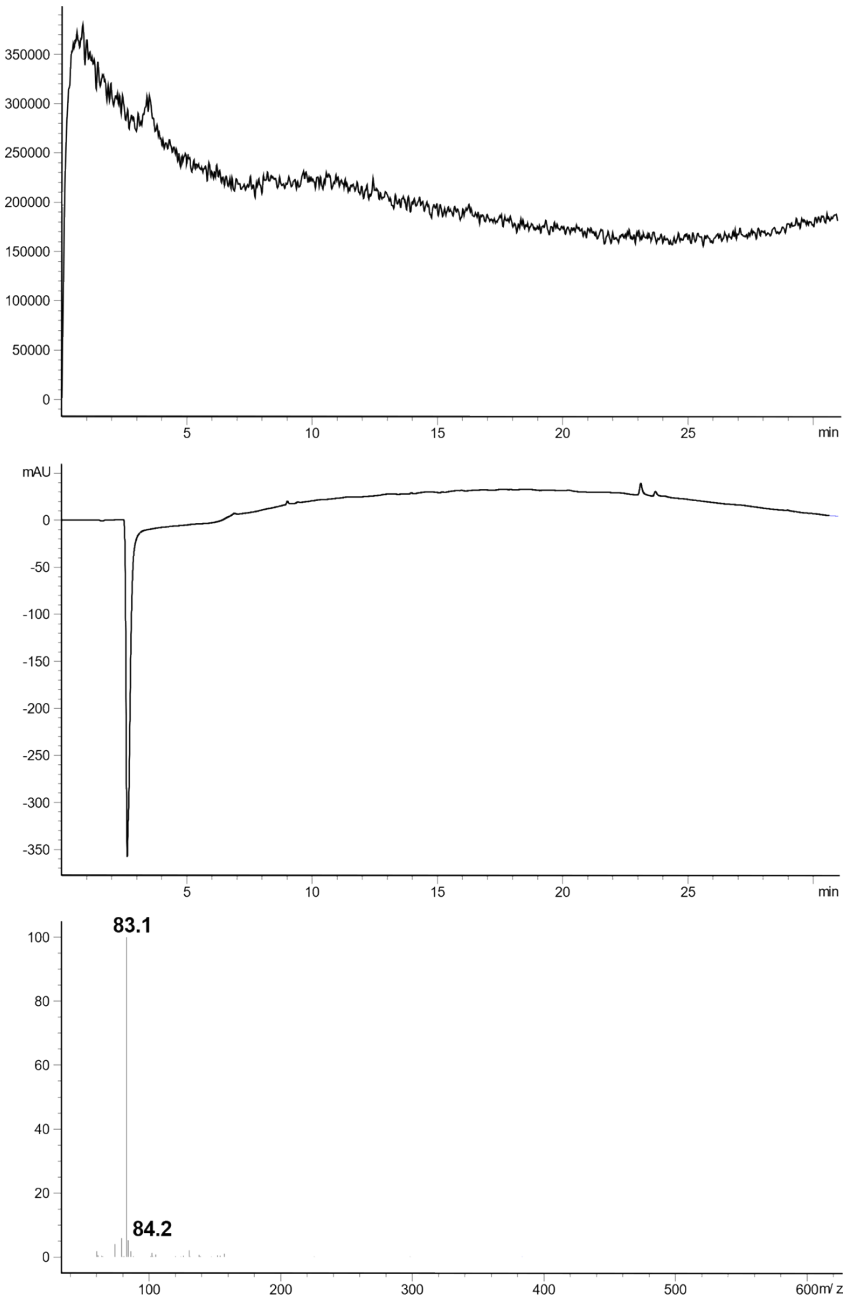
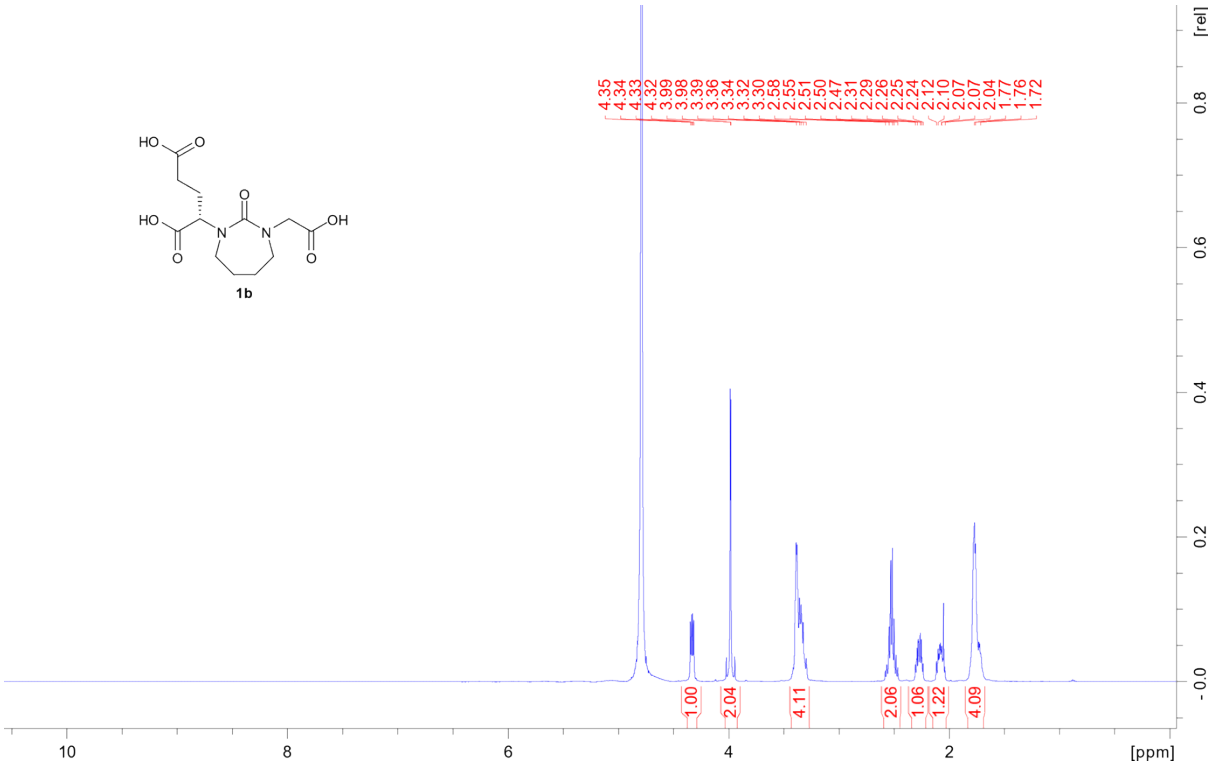
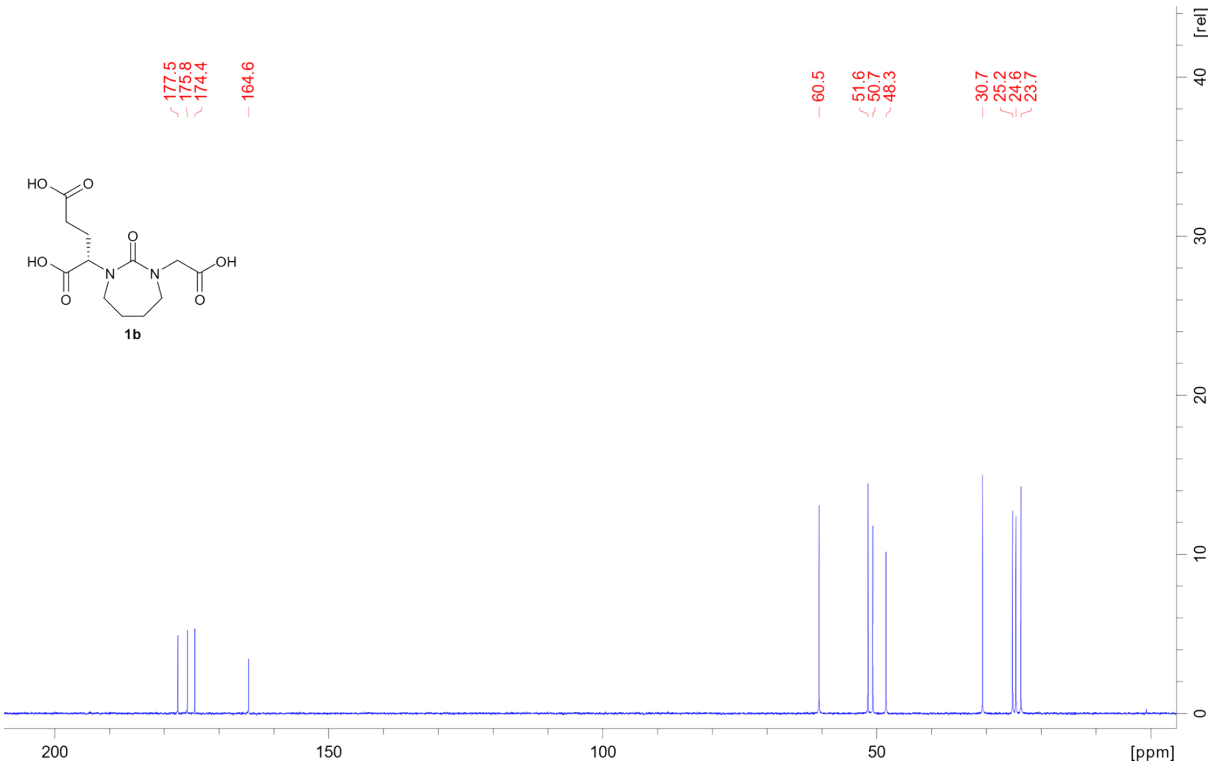


Figure S36: LC-MS profile of blank sample for sample **1a**; ion polarity positive

^{1}H NMR (D_2O, 500 MHz): **1b**



^{13}C{^{1}H} NMR (D_2O, 125 MHz): **1b**



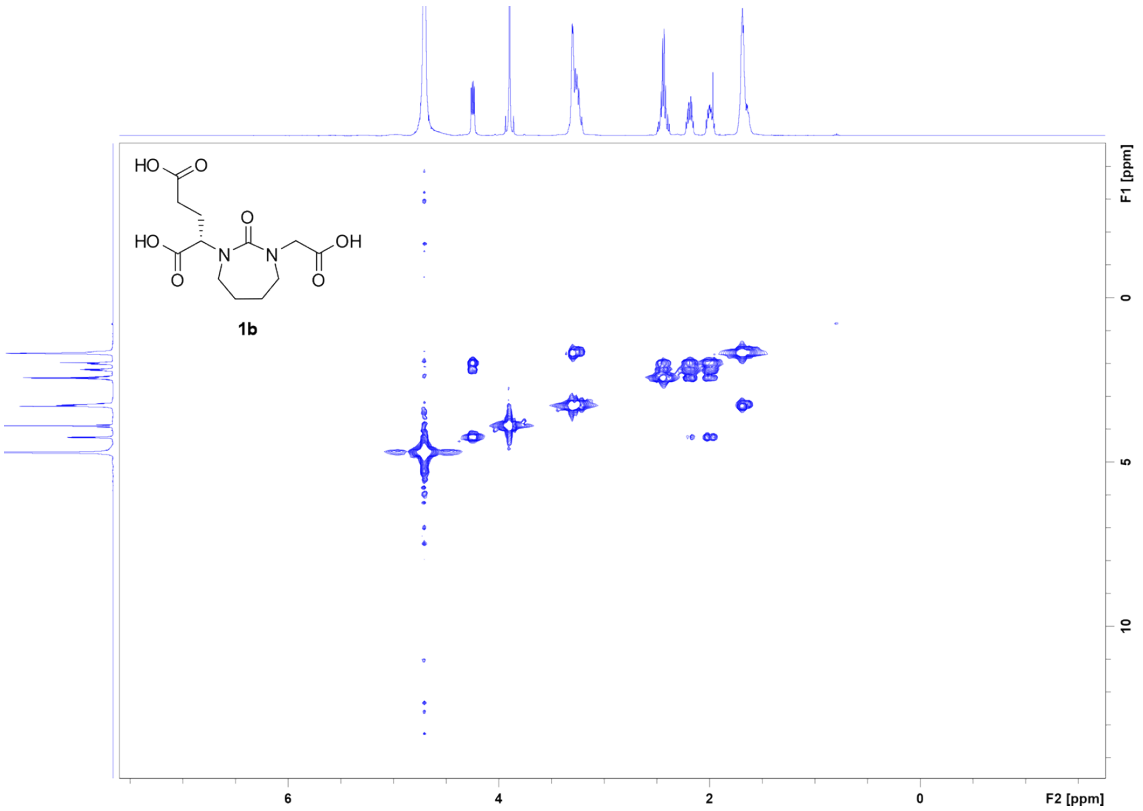


Figure S37. COSY spectrum for **1b**

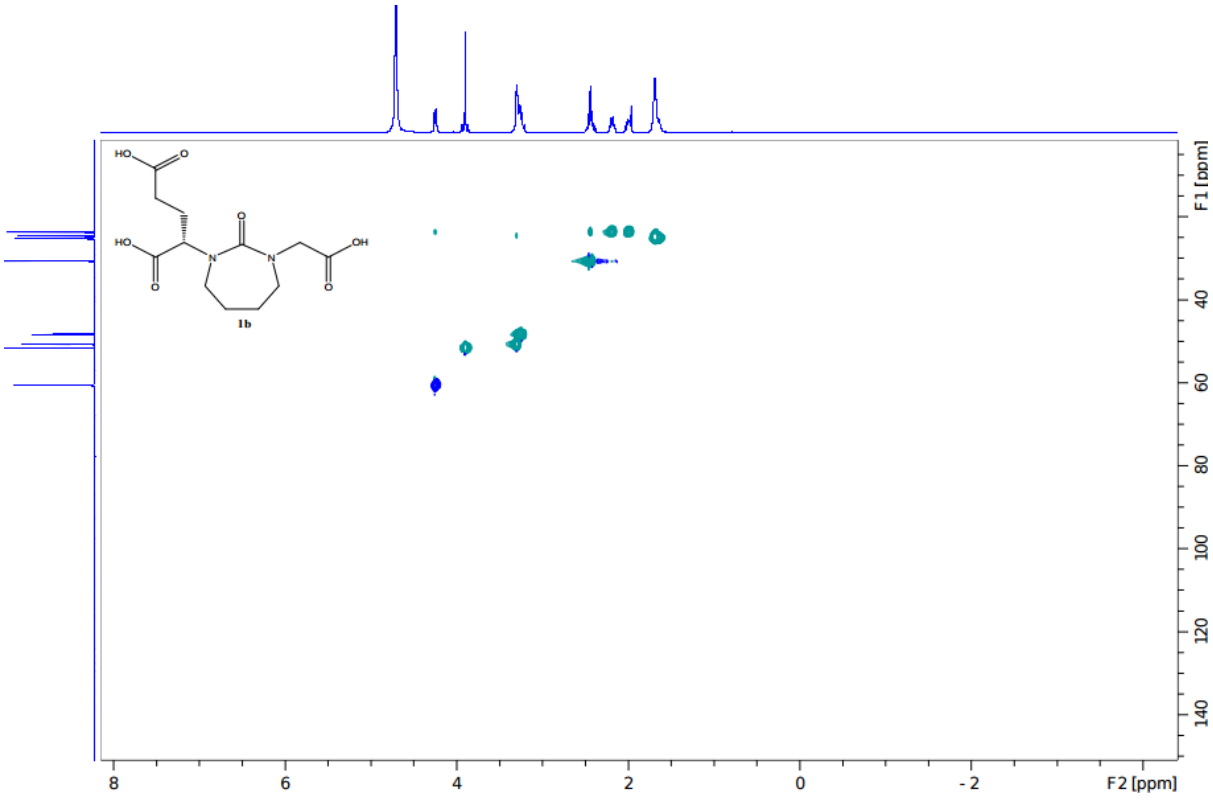


Figure S38. HSQC spectrum for **1b**

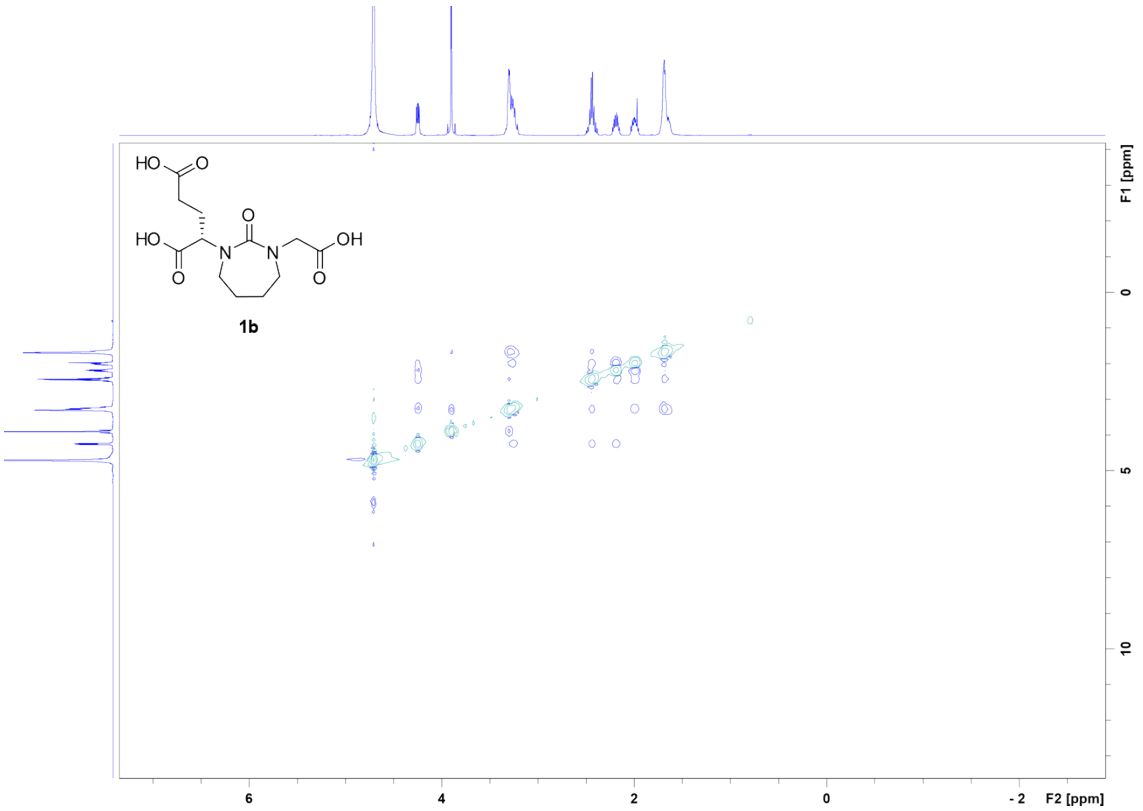
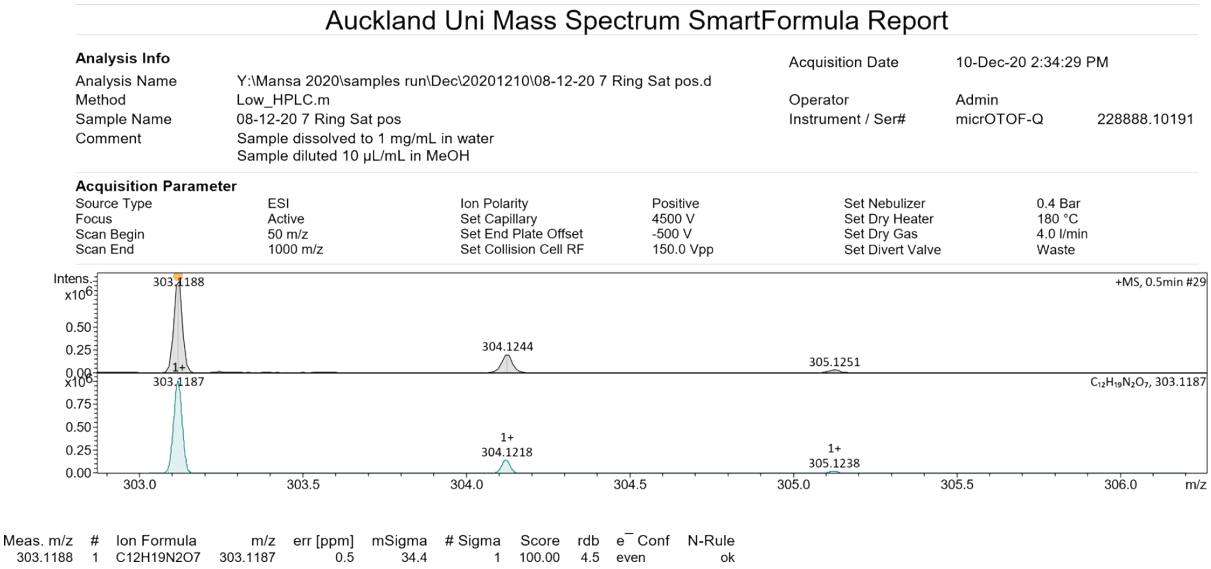


Figure S39. NOESY spectrum for **1b**



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
303.1188	1	C12H19N2O7	303.1187	0.5	34.4	1	100.00	4.5	even	ok

Figure S40: HRMS for **1b**. HRMS (ESI): (*m/z* $[M + H]^+$ calcd: $C_{12}H_{19}N_2O_7^+$: 303.1187; found: 303.1188

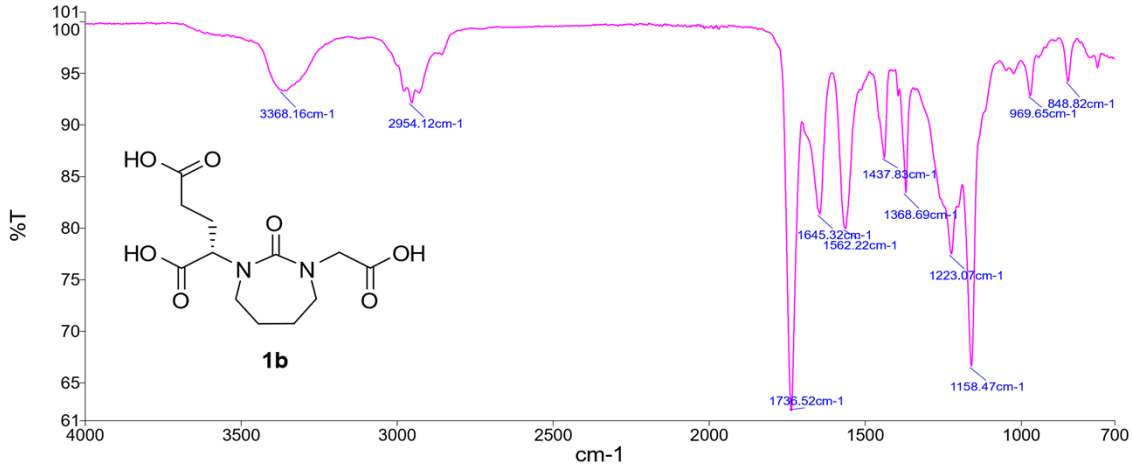


Figure S41: IR spectra for **1b**

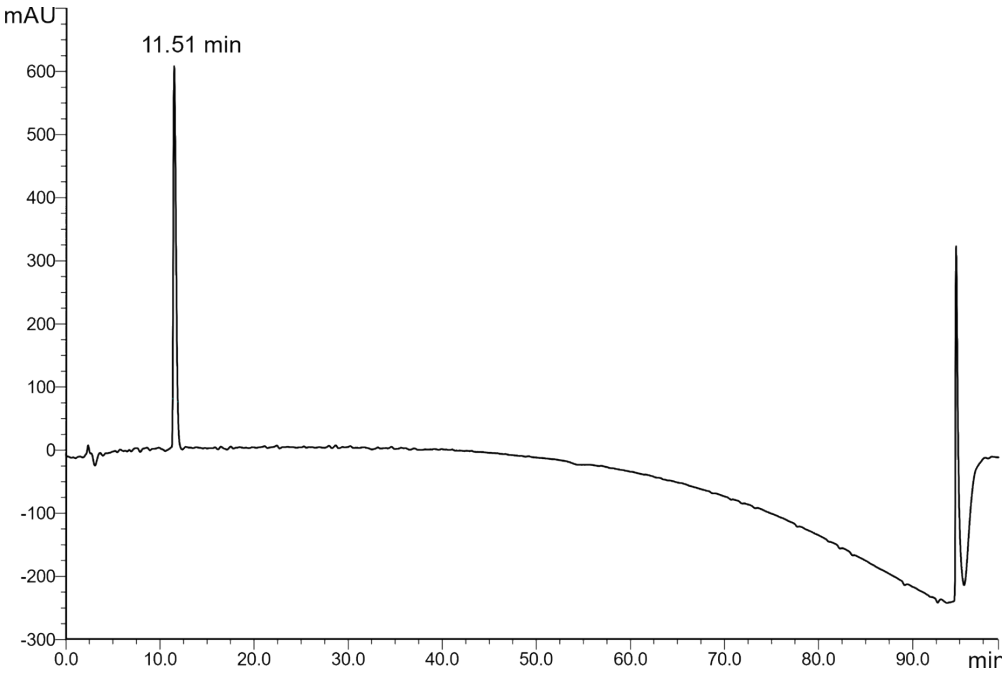


Figure S42: Analytical RP-HPLC chromatogram of purified **1b**, t_R = 11.51 min. Chromatographic separations were performed on a Thermo Scientific Dionex Ultimate 3000 HPLC using a XTerra® MS C-18 column (5 µm; 4.6 × 150 mm) and a linear gradient of 5-95% B in 90 min at room temperature, *ca*. 1% B per min at a flow rate of 1.0 mL/min. Buffer A: H_2O containing 0.1% TFA (*v/v*); Buffer B: acetonitrile containing 0.1% TFA (*v/v*)

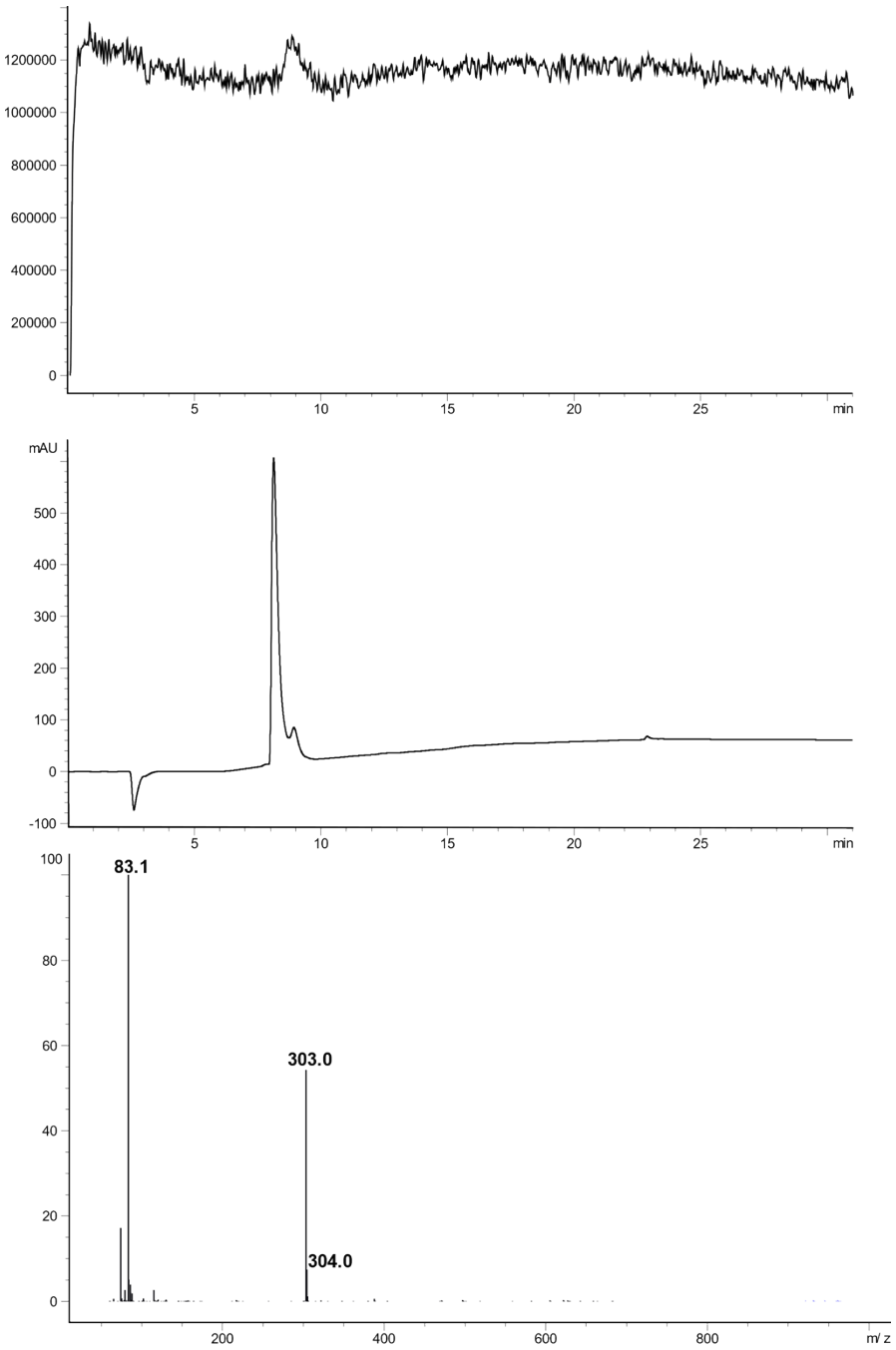


Figure S43: LC-MS profile of purified peptide **1b**; ion polarity positive. ESI-MS (*m/z* $[M + H]^+$ calcd: 302.8; found: 303.0)

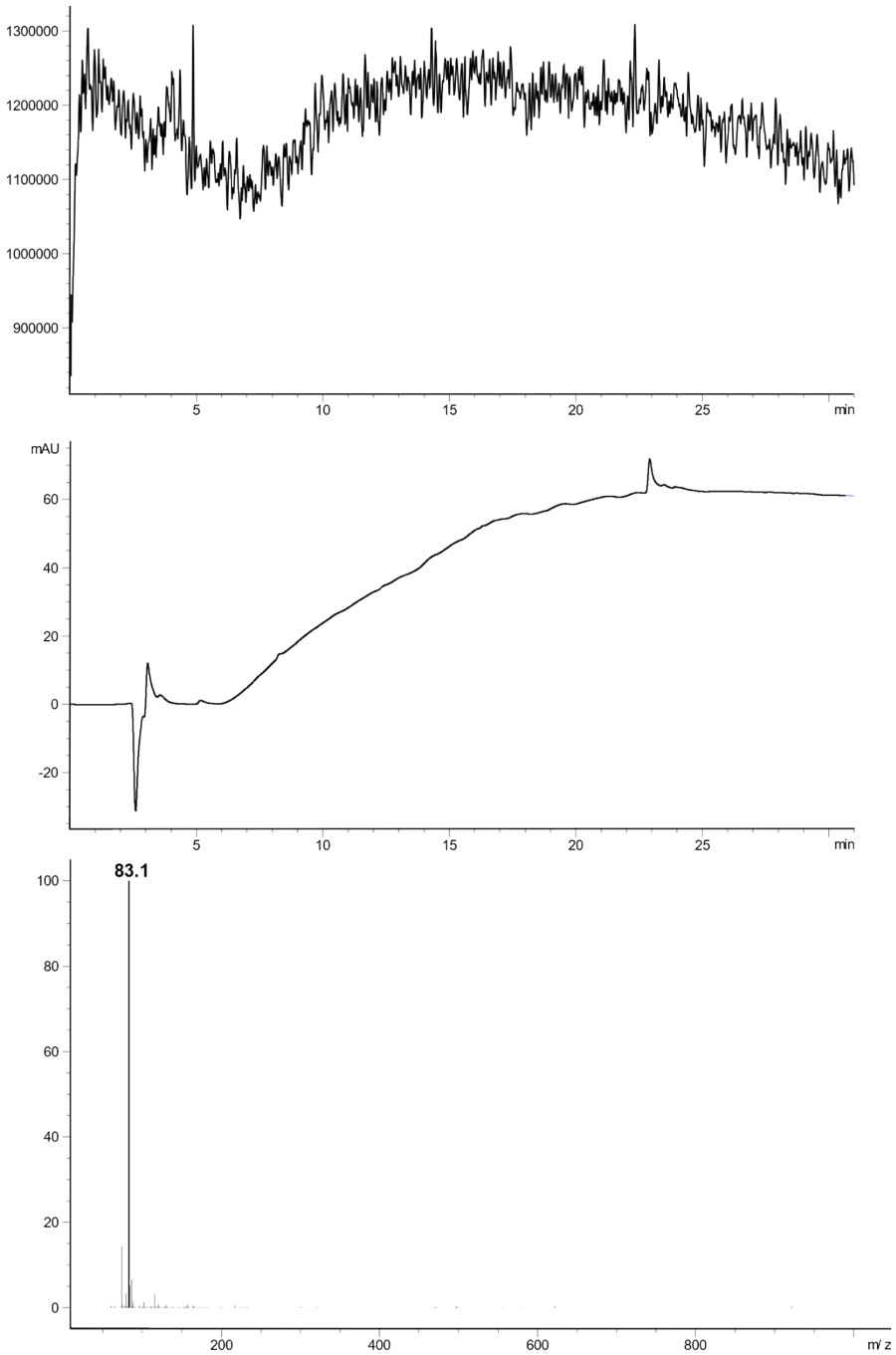


Figure S44: LC-MS profile of blank sample for sample **1b**; ion polarity positive

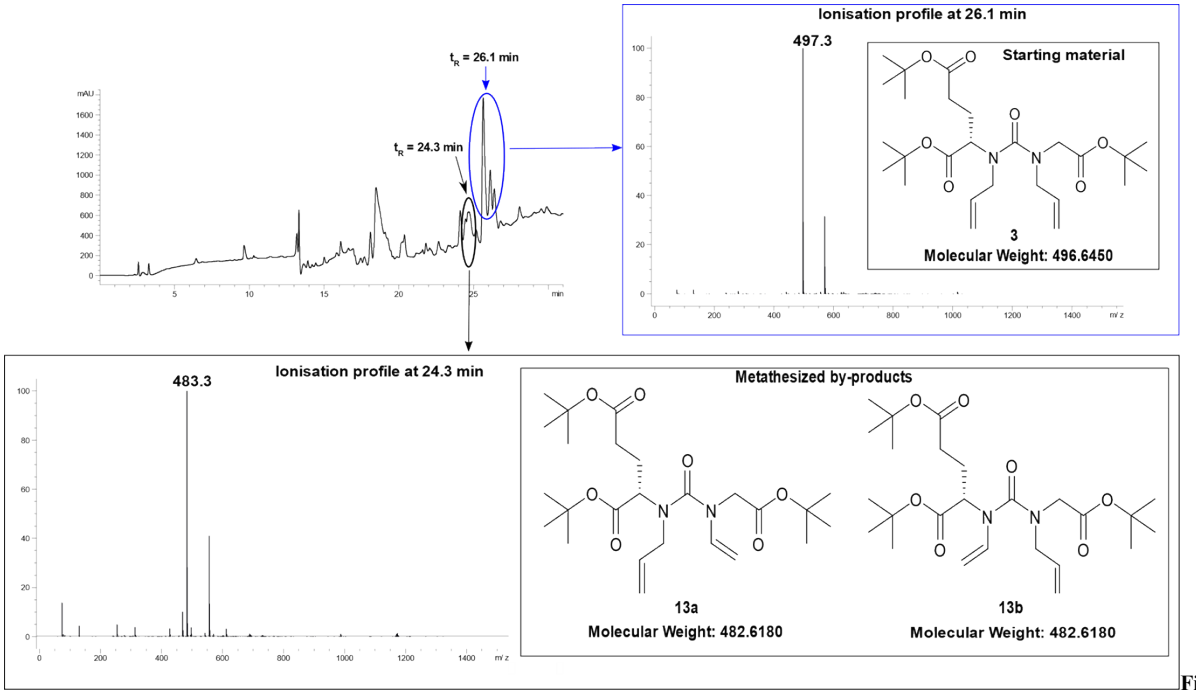


Figure S45: Crude LC-MS profile displaying methylene loss during attempted RCM on **3** affording by-products **13a** and **13b**. ESI-MS (*m/z* $[M + H]^+$ calcd: 483.6; found: 483.3)

Glutamate Carboxypeptidase II Inhibitor Screening Kit

Methods

Screening of PSMA inhibitors was completed using the Glutamate Carboxypeptidase II inhibitor screening kit (Cat# K440-100; BioVision, CA, USA). Inhibition of the human GCPII enzyme was indicated by a reduction in fluorescence over the 90 min time course. For inhibitor dose-response curves, inhibitors were serially diluted using the kit supplied assay buffer to give a final inhibitor concentration range of 10 pM to 100 nM for 2-PMPA (kit supplied control inhibitor) and 100 nM to 3 mM for the test inhibitors. Inhibitor assays were performed as per the manufacturer's instructions. Briefly, 10 µL diluted sample, assay buffer or water was added to a 96-well Spectraplate-MB (PerkinElmer Life and Analytical Sciences, MA, USA). A further 30 µL assay buffer was added to each well to give a total volume of 40 µL. Human GCPII enzyme was diluted 80-fold in assay buffer and 40 µL added to each well excluding the background control wells where assay buffer was added. The plate was incubated at 37 °C for 20 min. Following incubation, 20 µL reaction mix (13 µL assay buffer, 2 µL 1:100 diluted substrate, 2 µL enzyme mix, 2 µL developer and 1 µL PicoProbe) was added to each well to give a final volume of 100 µL. Fluorescence (Ex/Em = 535/587) was immediately measured at 37 °C for 90 min using a SpectraMax ID3 (Molecular devices, CA, USA) on the low PMT setting.

Data and statistical analysis

All data were plotted and analysed using GraphPad Prism 9.0 (GraphPad Software, CA, USA). Data points are the mean ± standard error of the mean (SEM) from 3 independent experiments, combined. Each independent experiment was performed with two technical replicates. The location of inhibitors and controls were randomised between independent experiments.

Time courses

Control (enzyme, water and background) condition time courses were baseline corrected to account for variation in the basal enzyme activity. The average value generated for each enzyme and background control replicates within an independent experiment was subtracted from all control values and plotted as the relative fluorescent units (RFU) over time (min). Inhibitor time courses were normalised to the maximal (90 min) and minimum (0 min) RFU of the enzyme control and expressed as a percentage of the enzyme control (100%).

Inhibitor dose-response curves

Inhibitor activity (IC_{50}) was determined by choosing two time points (45 and 60 min) on the linear range of the time course and calculating the slope (ΔRFU (R_{t2}-R_{t1}) /Δt (t_2-t_1)) (**Figure S45**). The relative activity of each inhibitor concentration was then calculated (Relative activity (%) = (Slope of inhibitor/Slope of enzyme control) × 100) and plotted to generate dose-response curves. Dose-response curves were fitted with a three-parameter inhibitor logistic equation to determine pIC_{50}. The bottom of the curve was fixed to 0 and data are expressed as a percentage of the

enzyme control (100%). IC_{50} values were averaged from each separate independent experiment to generate mean values ± SEM.

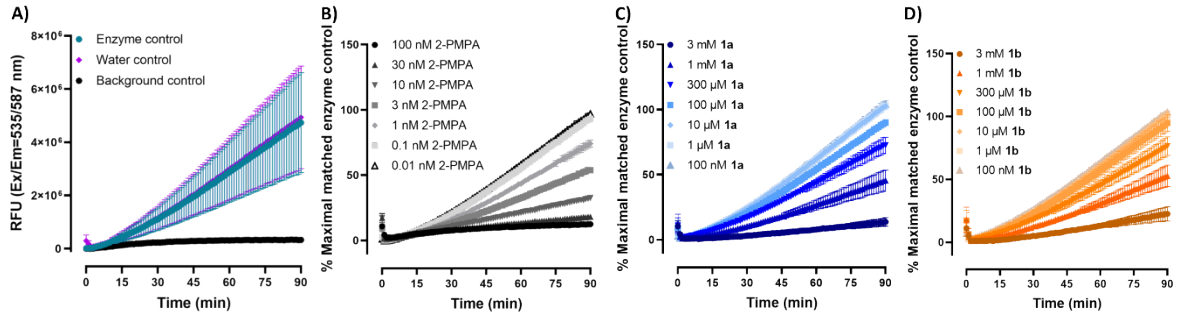


Figure S46. Time course of A) control conditions, B) 2-PMPA inhibitor activity, C) Compound **1a** inhibitor activity and D) compound **1b** inhibitor activity. Time courses were left as raw RFU (A) or normalised to the enzyme control (B-D) and plotted over time. Data points are the mean ± SEM from 3 independent experiments

References

(1) Sakaguchi, H.; Tokuyama, H.; Fukuyama, T. Stereocontrolled total synthesis of (–)-kainic acid. *Org. Lett.* **2007**, *9* (9), 1635–1638.

(2) Joy, S, T.; Arora, P. S. An optimal hydrogen-bond surrogate for α-helices. *Chem. Commun*. **2016**, *52* (33), 5738–5741.

(3) Pinsker, A.; Einsiedel, J.; Harterich, S.; Waibel, R.; Gmeiner, P. A highly efficient type 1 β-turn mimetic simulating an Asx-Pro-turn-like structure. *Org. Lett*. **2011**, *13*(13), 3502–3505.

(4) Huo, C.; Wang, C.; Zhao, M, Peng, S. Stereoselective synthesis of natural *N*-(1-deoxy-D-β-fructos-1-yl)-L-amino acids and their effect on lead decorporation. *Chem. Res. Toxicol*, **2004**. *17*(8), 1112–1120.