Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information

Oxathiazole-2-one Derivative of Bortezomib: Synthesis, Stability and Proteasome Inhibition Activity

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General Methods

All chemicals were purchased from Sigma Aldrich unless otherwise noted. Anhydrous solvents and other reagents were purchased and used without further purification. The enantiomers of **5** were each purchased from Bachem, Inc. Analtech silica gel plates (60 F_{254}) were used for analytical TLC, and Analtech preparative TLC plates (UV 254, 2000 μm) were used for purification. 200-400 Mesh silica gel was used in column chromatography. NMR spectra were recorded on a Varian-Gemini 400 magnetic resonance spectrometer. ^{1}H NMR spectra are recorded in parts per million (ppm) relative to the peak of $CDCl_3$, (7.24 ppm) or DMSO-d_6 (2.50ppm). ^{13}C spectra were recorded relative to the central peak of the $CDCl_3$ triplet (77.0 ppm) or the DMSO-d_6 septet (39.7 ppm). Multiplicities are described using the abbreviation s, singlet; d, doublet, t, triplet; q, quartet; m, multiplet. High-resolution mass spectra were recorded at the Georgia Institute of Technology mass spectrometry facility in Atlanta, Georgia.

Synthesis

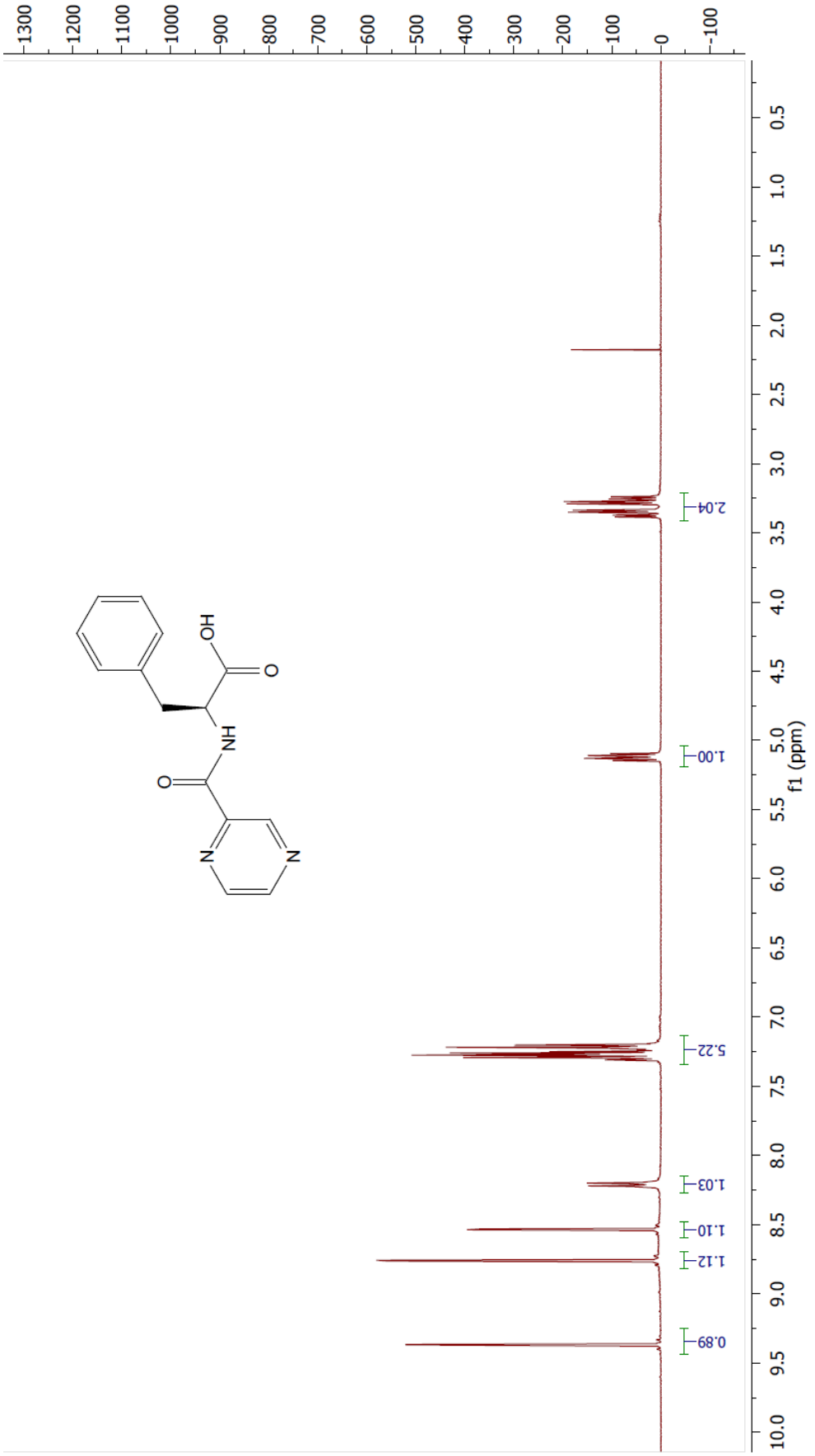
N-(2-pyrazinylcarbonyl)-L-Phenylalanine (4). Pyrzine-2-carboxylic acid **2** (4g, 32.23mmol) was coupled with the methyl ester hydrochloride of L-phenylalanine **3** (6.81g, 31.6mmol), using 11.7g (36.34mmol) either O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (TBTU), 16.5mL (94.8mmol) of N,N-Diisopropylethylamine (DIPEA) in 10mL DMF and 190mL DCM. Reaction ran at room temperature overnight. It oil was then diluted with DCM and workup commenced by washing with 1N HCl, saturated sodium bicarbonate, water and brine. Pure product was obtained, after separating and drying the organic phase with sodium sulfate and concentrating in vacuo, as yellow tinted (8.37g, 93% yield). This was then dissolved in acetone with 1 equivalent of NaOH and stirred at ambient temperature for 3-4 hours, then cooled to 0°C, acidified with 1N HCl causing precipitation of the carboxylic acid **4**, which was collected by filtration, and vacuum dried to give pure product as a white solid, 86% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.31 (2H, ddd, J = 6.0, 14.0, 20.5 Hz), 5.12 (1H, ddd, J = 5.5, 6.4, 8.2 Hz), 7.16 – 7.33 (5H, m), 8.21 (1H, d, J = 8.1 Hz), 8.54 (1H, dd, J = 1.5, 2.5 Hz), 8.76 (1H, d, J = 2.5 Hz), 9.37 (1H, d, J = 1.5 Hz) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ 175.29, 163.04, 147.52, 144.35, 144.17, 143.18, 135.68, 129.52, 128.96, 127.55, 53.43, 37.87.

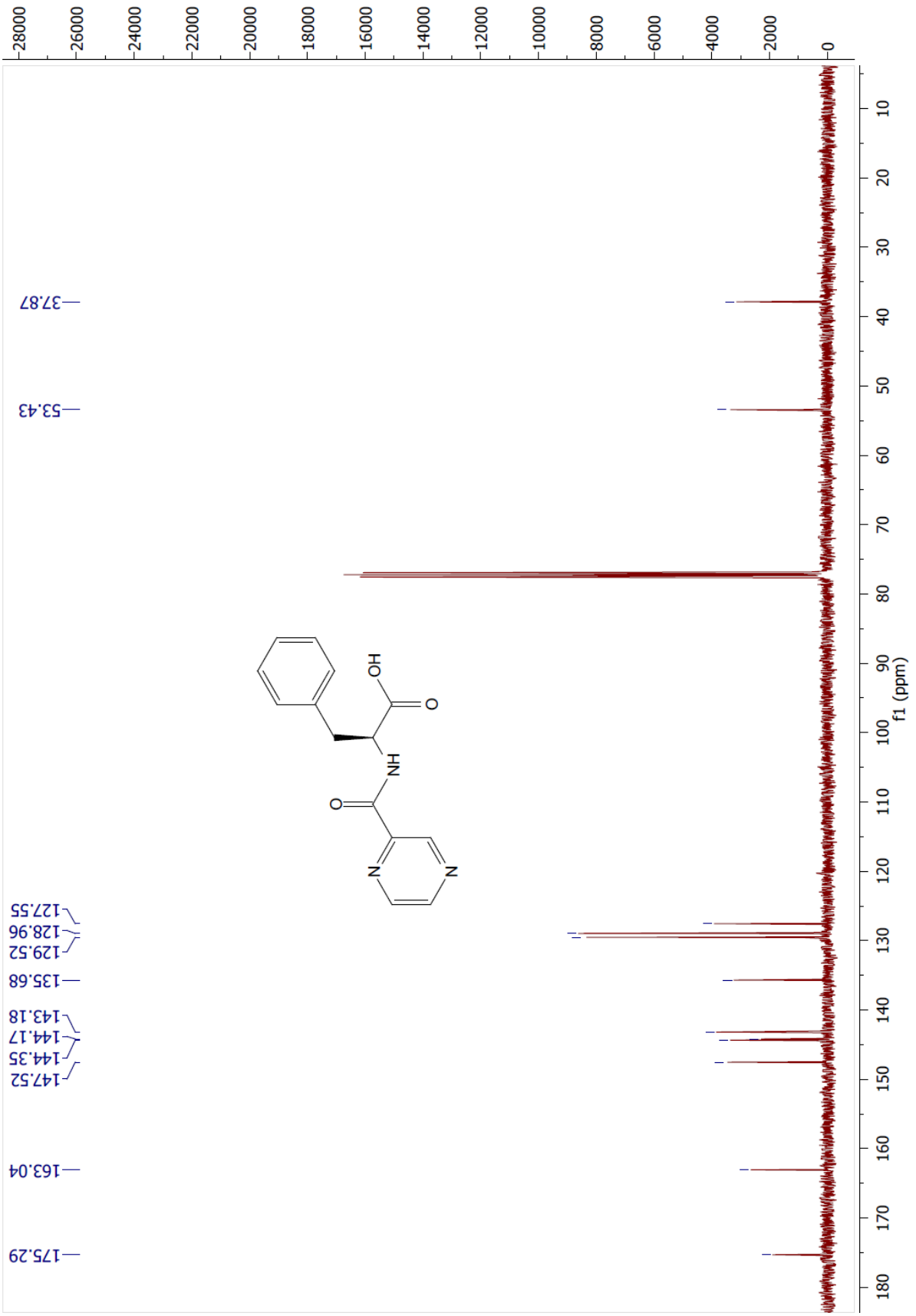
N-(2-pyrazinylcarbonyl)-L-Phenylalanine-L-Leucine amide (6a). Compound **4** (700mg, 2.58mmol), TBTU (911mg, 2.84mmol) and L-Leucine-amide **5a** (336mg, 2.58mmol) were dissolved in 10mL DCM at -5 °C. DIPEA (0.87mL, 4.98mmol) dissolved in 5mL DCM was slowly added drop-wise. Reaction was allowed to warm to room temperature while stirring overnight. Workup commenced by dilution with DCM followed by washing with 1N HCl, saturated sodium bicarbonate, and brine, then drying over sodium sulfate and concentrating *in vacuo*. Resulting solid was dissolved in minimal volume of hot methanol, precipitated upon addition of cold brine/water mixture, collected by filtration and dried under strongly reduced pressure to obtain pure product (751mg, 76% yield) as a white crystalline solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84 (6H, dd, J = 6.5, 18.0 Hz), 1.46 (2H, dd, J = 5.6, 11.6 Hz), 1.52 – 1.65 (1H, m), 3.10 (3H, ddd, J = 6.5, 13.8, 22.2 Hz), 4.27 (1H, td, J = 6.3, 8.7 Hz), 4.80 (1H, td, J = 4.6, 8.5 Hz), 6.97 – 7.04 (1H, m), 7.10 – 7.24 (5H, m), 7.33 (1H, s), 8.26 (1H, d, J = 8.3 Hz), 8.68 (1H, d, J = 8.4 Hz), 8.72 (1H, dd, J = 1.5, 2.5 Hz), 8.86 (1H, d, J = 2.5 Hz), 9.10 (1H, d, J = 1.5 Hz) ppm.

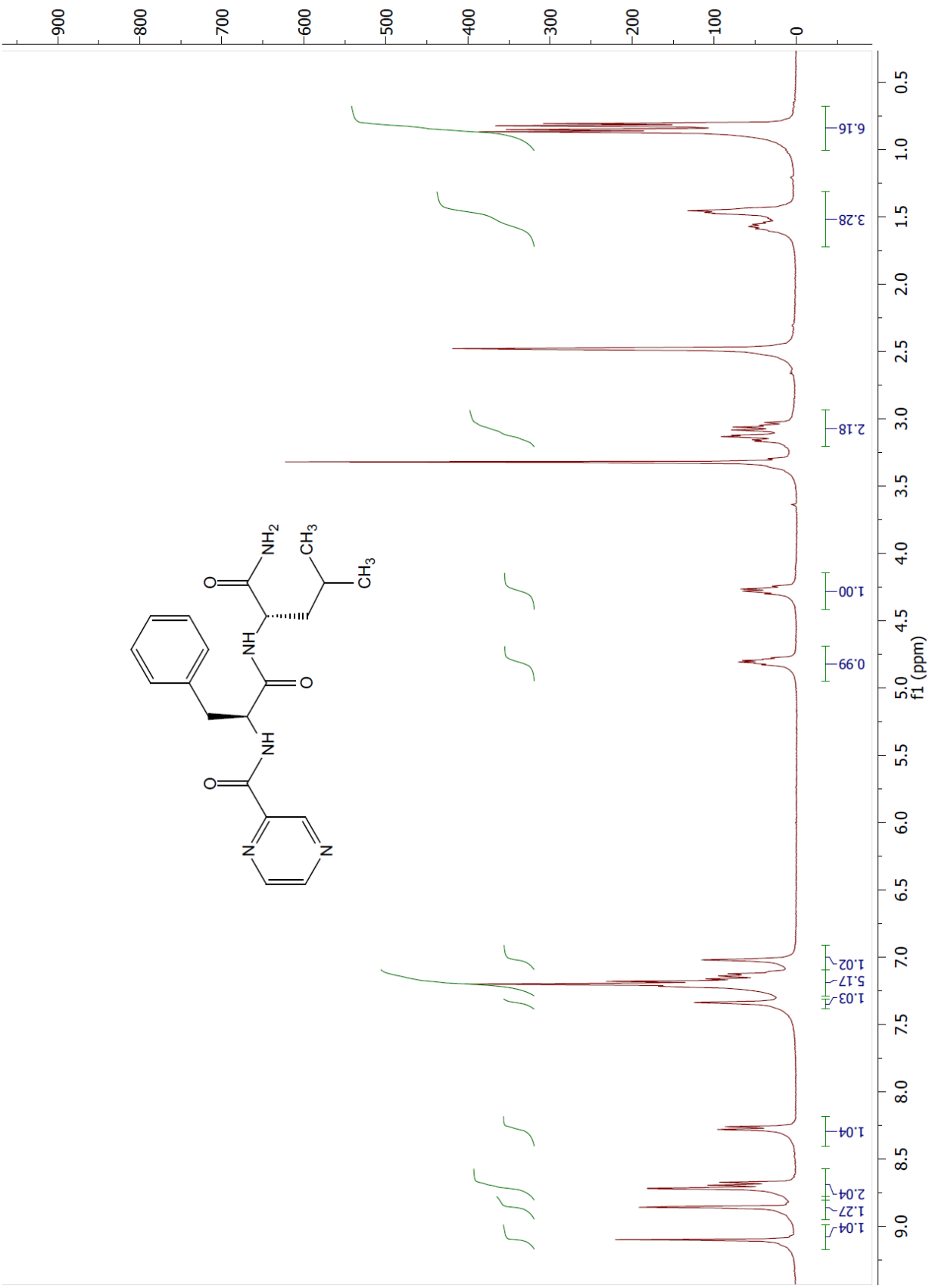
N-(2-pyrazinylcarbonyl)-L-Phenylalanine-D-Leucine amide (6b). Same as **6a** above, except with D-Leucine-amide **5b**. Product was obtained as a white, crystalline solid, 76% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.79 (6H, dd, J = 6.1, 16.8 Hz), 1.33 – 1.45 (3H, m), 3.07 (2H, qd, J = 7.0, 13.7 Hz), 4.16 (1H, dd, J = 7.9, 15.0 Hz), 4.83 (1H, td, J = 6.1, 8.1 Hz), 7.00 (1H, s), 7.11 – 7.24 (5H, m), 7.36 (1H, s), 8.37 (1H, d, J = 8.3 Hz), 8.68 – 8.76 (2H, m), 8.86 (1H, d, J = 2.5 Hz), 9.10 (1H, d, J = 1.5 Hz) ppm.

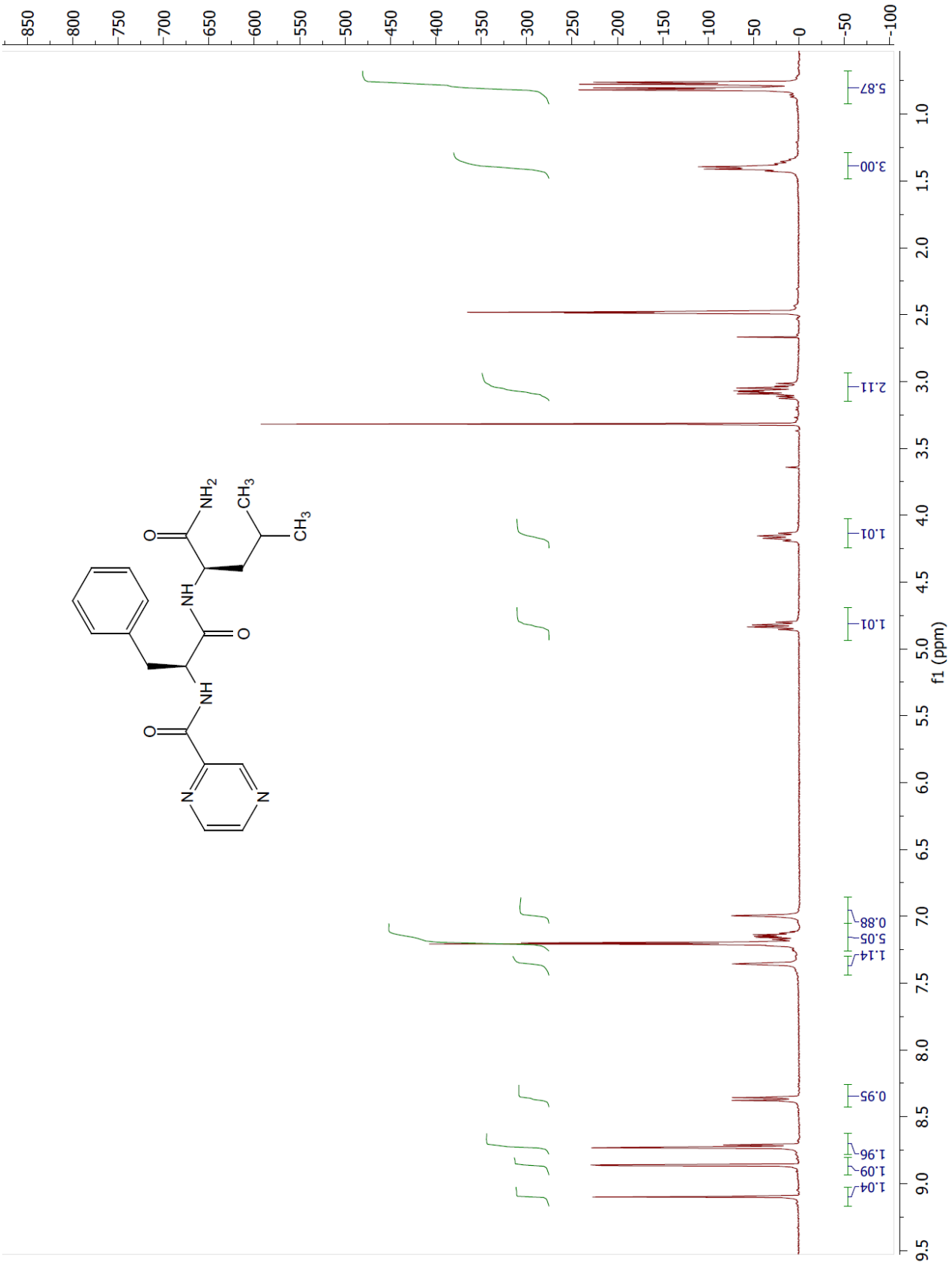
N-(2-pyrazinylcarbonyl)-L-Phenylalanine-L-Leucine-Oxathiazole-2-one (1). Amide **6a** (110mg, 0.287mmol) was dissolved in THF while stirring under argon. Chlorocarbonyl sulfonylchloride (0.04mL, 0.488mmol) was added and reaction was refluxed for 3 hours. Reaction was concentrated *in vacuo* and purified by preparative TLC with 4:3 hexane:ethyl acetate to obtain 99.5mg (79%) of **1** as a cream colored solid. >96% pure by HPLC, with 1.48 minute retention time using 100% Acetonitrile eluent. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (7H, dd, *J* = 3.9, 6.3 Hz), 1.44 – 1.58 (2H, m), 1.59 – 1.69 (2H, m), 3.21 (2H, ddd, *J* = 7.4, 13.7, 21.9 Hz), 4.85 (1H, dt, *J* = 7.4, 14.7 Hz), 4.97 (1H, dt, *J* = 7.3, 14.8 Hz), 6.25 (1H, d, *J* = 8.2 Hz), 7.19 – 7.34 (7H, m), 8.38 (1H, d, *J* = 8.0 Hz), 8.51 – 8.63 (1H, m), 8.78 (1H, d, *J* = 2.4 Hz), 9.36 (1H, d, *J* = 1.2 Hz) ppm. 2D COSY spectra shows appropriate proton coupling connectivity. ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.55, 170.41, 163.17, 160.24, 147.80, 144.32, 143.79, 142.97, 135.97, 129.37, 128.68, 127.27, 54.71, 47.88, 41.26, 38.76, 24.72, 22.44, 21.98. HRMS: $[C_{21}H_{23}N_5O_4S+Na]^+$ calculated: 464.1368, found 464.1359 m/z.

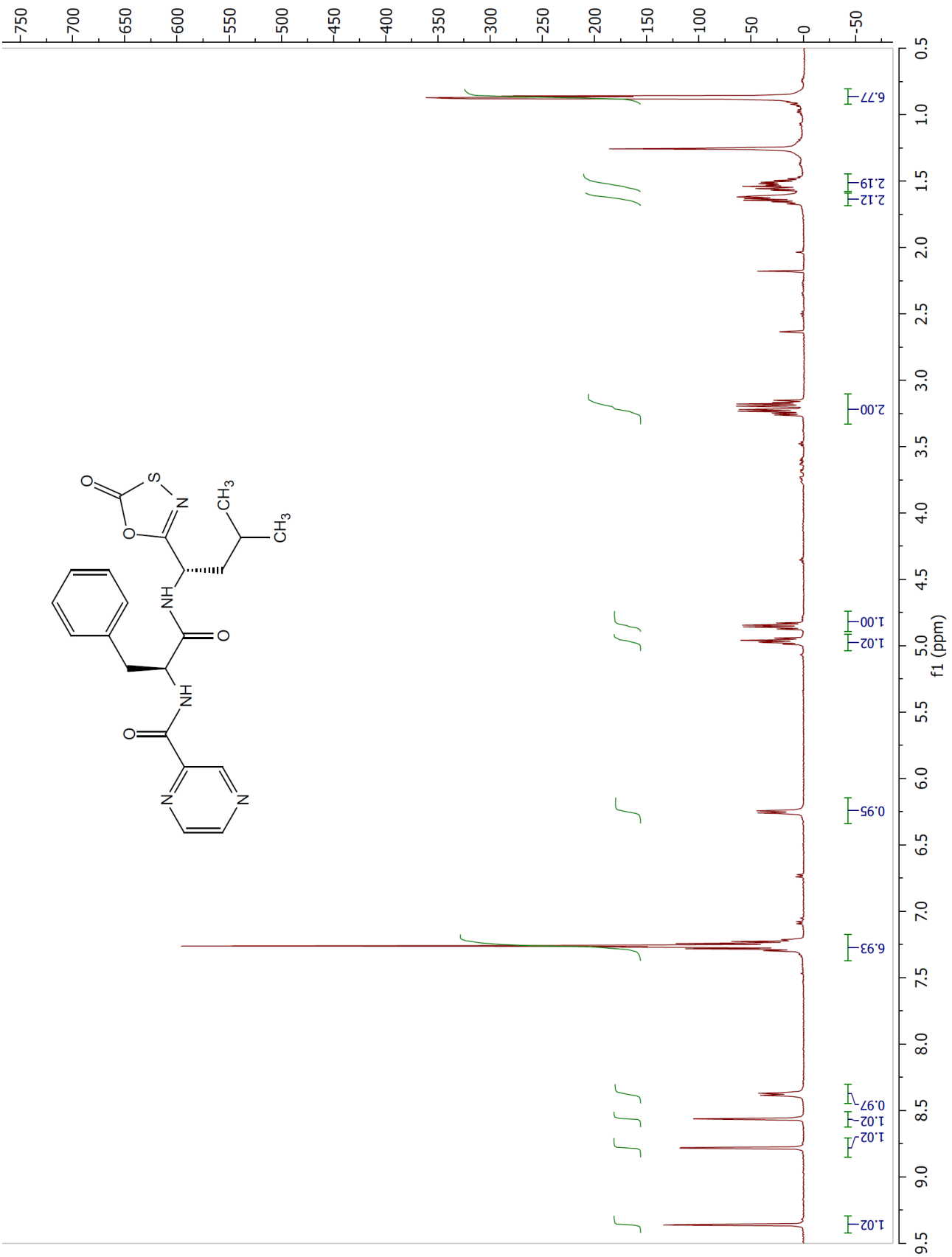
N-(2-pyrazinylcarbonyl)-L-Phenylalanine-L-Leucine-Oxathiazole-2-one (7). Same as **1** above. Amide **6b** (32mg, 0.0834mmol) was used to obtain 31.1mg (85%) of **7** as a cream colored solid. >95% pure by HPLC, with 1.48 minute retention time using 100% Acetonitrile eluent. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (6H, dd, *J* = 3.5, 6.5 Hz), 1.29 – 1.40 (1H, m), 1.40 – 1.48 (1H, m), 1.57 (1H, ddd, *J* = 5.9, 8.4, 14.0 Hz), 3.23 (2H, ddd, *J* = 7.3, 13.7, 21.9 Hz), 4.93 (1H, td, *J* = 5.9, 8.8 Hz), 4.99 (1H, td, *J* = 6.7, 8.2 Hz), 6.69 (1H, d, *J* = 8.4 Hz), 7.16 – 7.36 (5H, m), 8.41 (1H, d, *J* = 8.3 Hz), 8.50 – 8.58 (1H, m), 8.76 (1H, d, *J* = 2.4 Hz), 9.30 (1H, d, *J* = 1.3 Hz) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.57, 170.46, 163.29, 160.47, 147.76, 144.34, 143.84, 142.96, 136.22, 129.38, 128.88, 127.29, 54.75, 48.01, 41.29, 38.54, 24.51, 22.69, 21.82. HRMS: $[C_{21}H_{23}N_5O_4S+H]^+$ calculated: 442.1549, found: 442.1602 m/z.

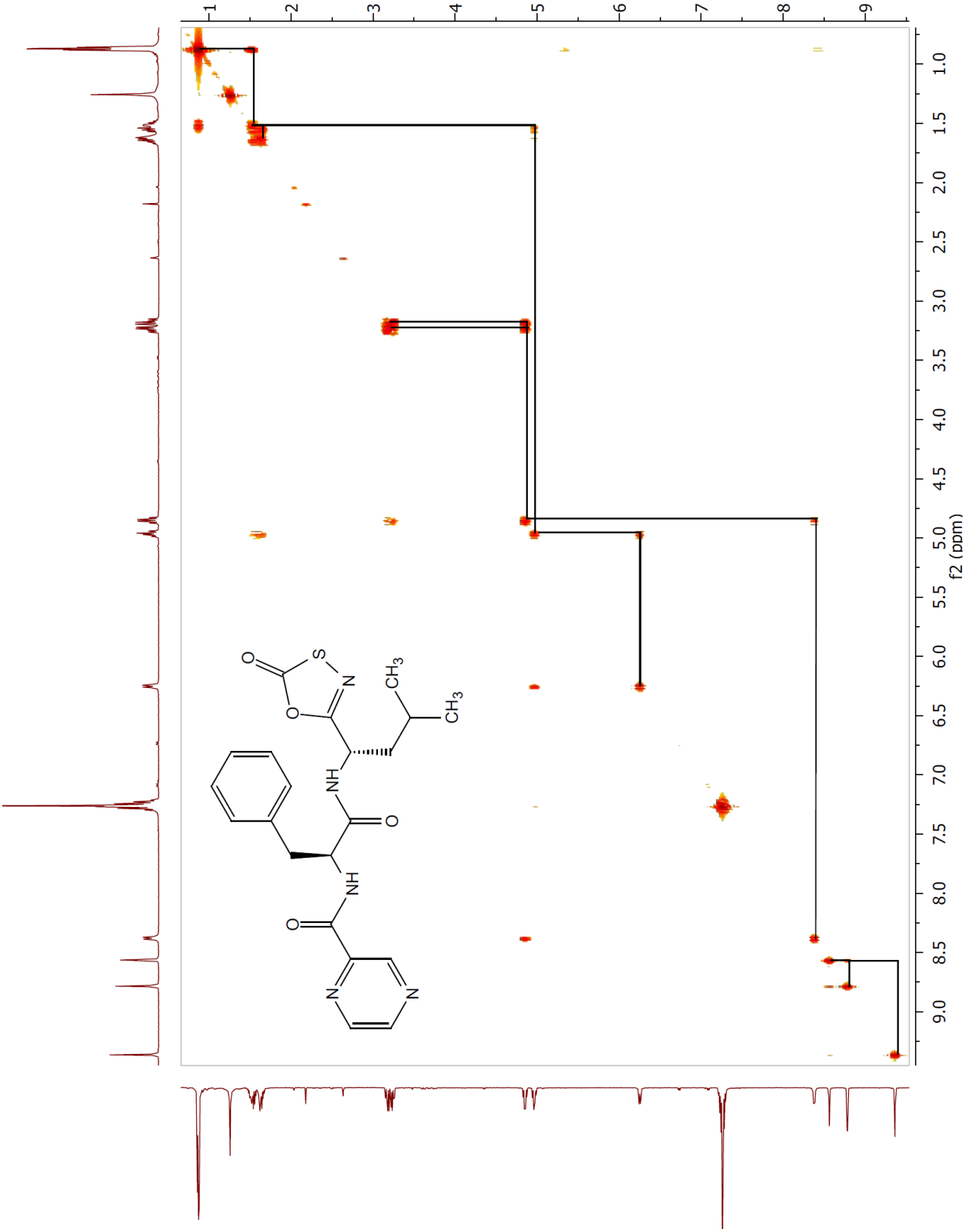


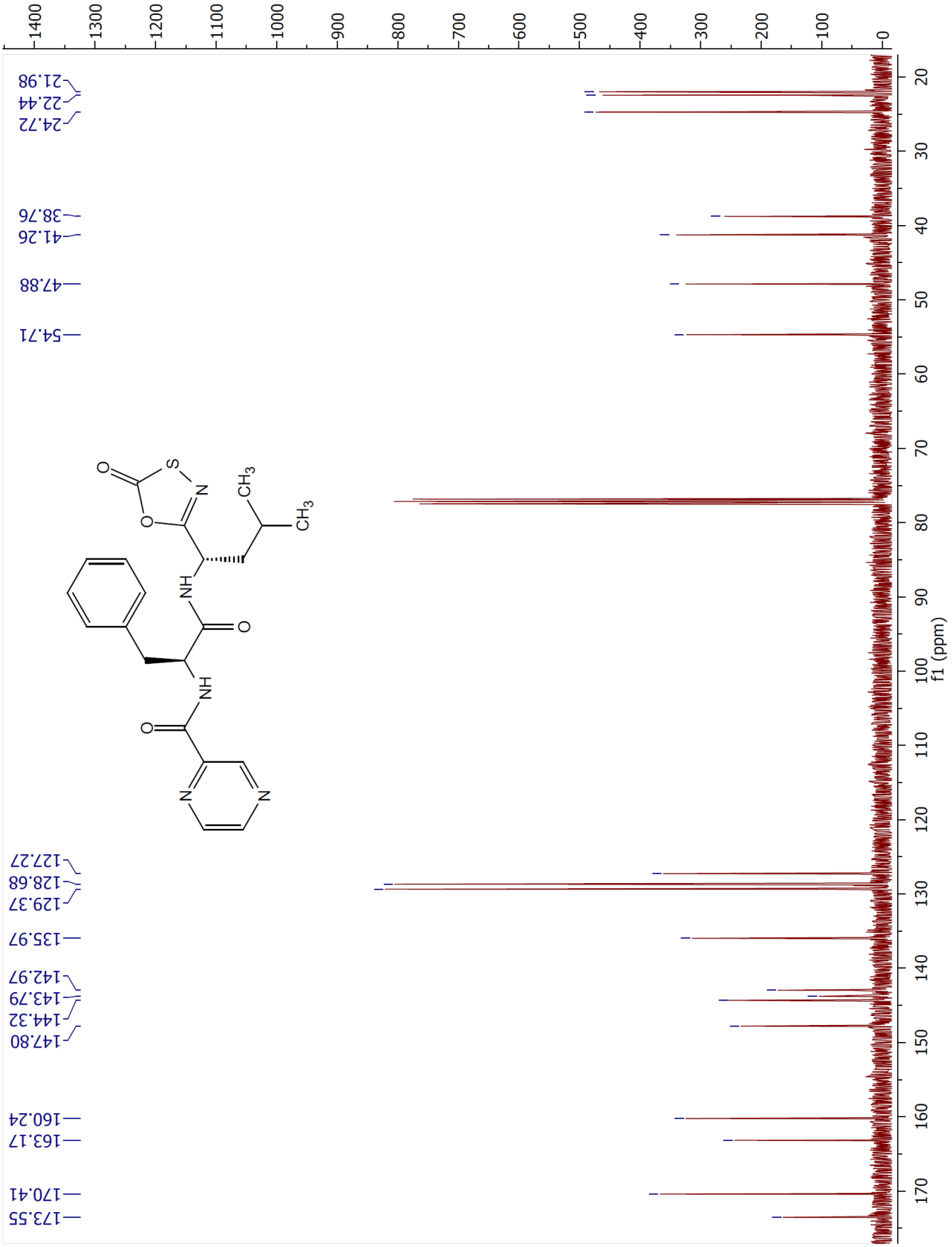


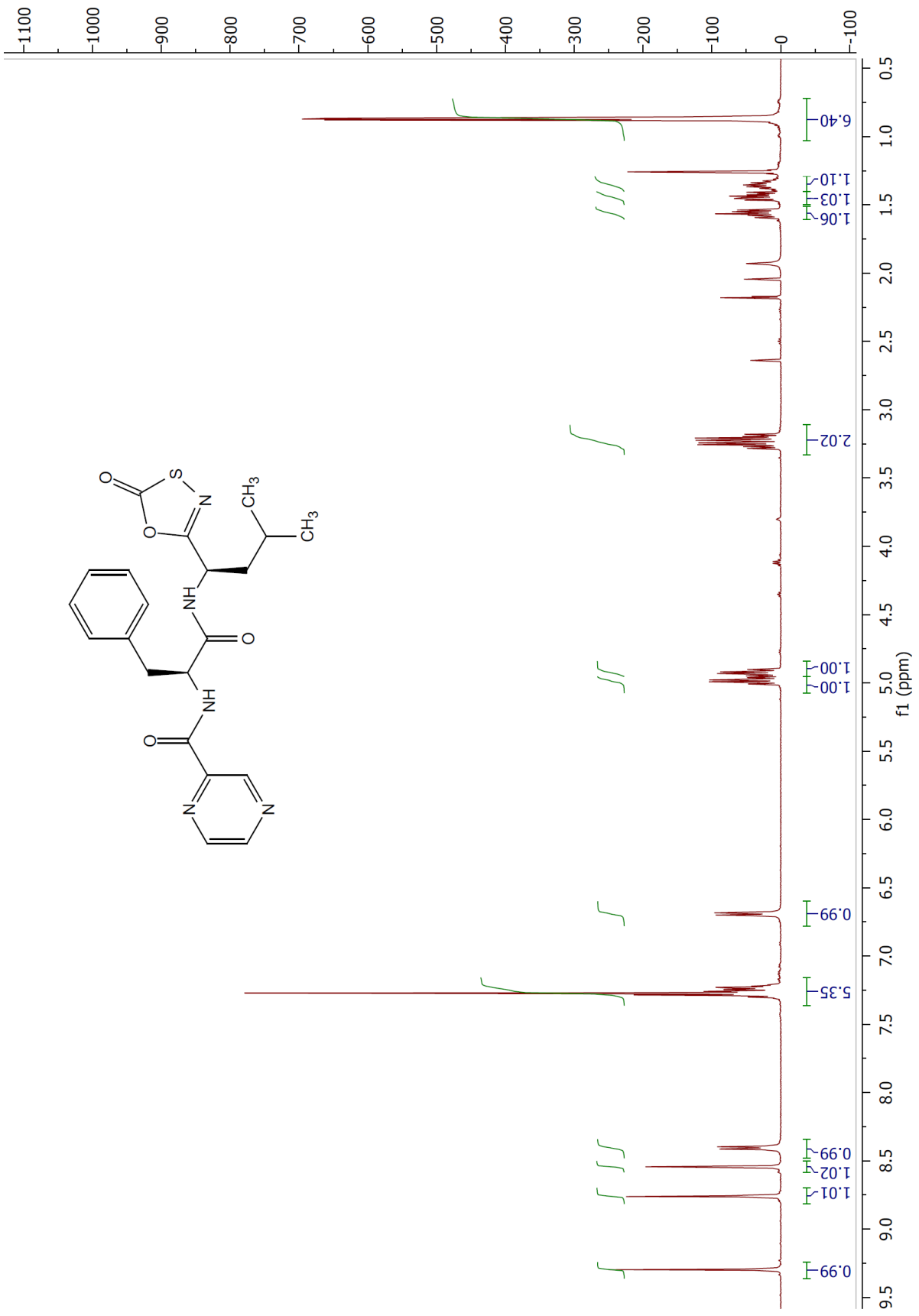


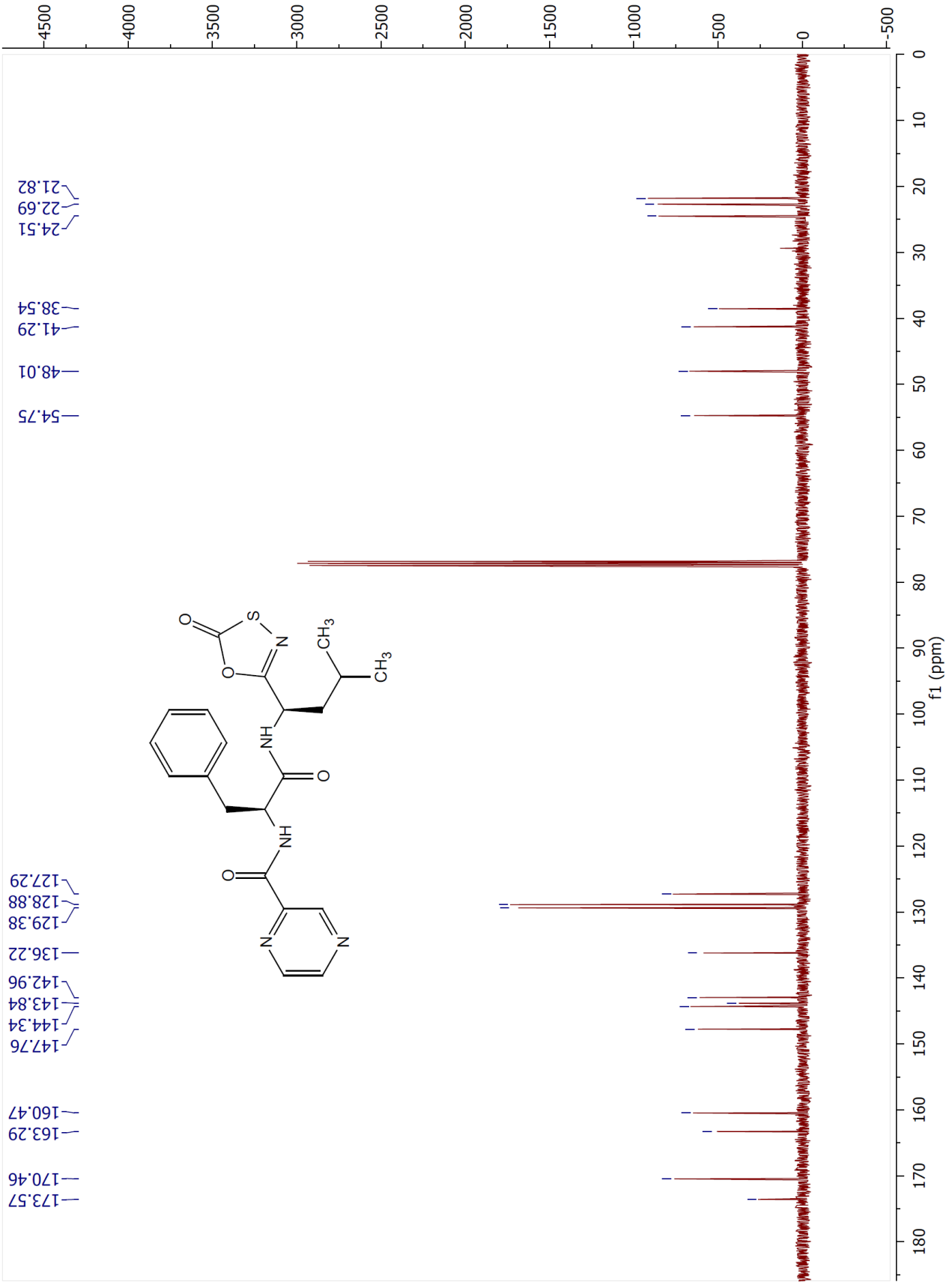




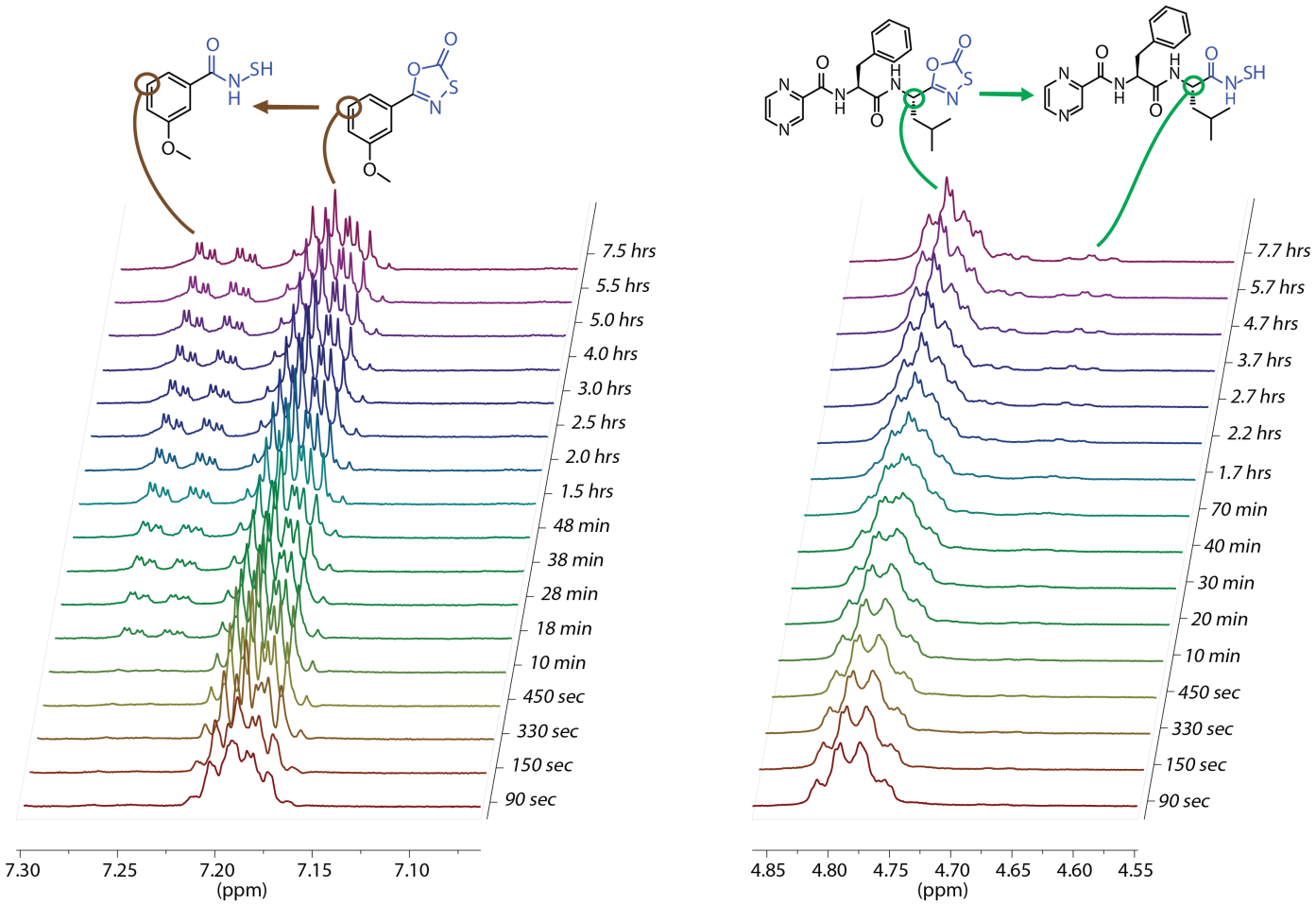








Kinetic ^{1}H-NMR Data



Monitoring the stability of Bort(L)-Oxathiazole-2-one **1** and HT1054 in deuterated water/DMSO mixture by ^{1}H-NMR over time revealed a half-life of 6.5 days and 20 hours, respectively. In each case the oxathiazole-2-one ring was hydrolytically cleaved to produce the thio-hydroxamic acid, and over many weeks the amide precursor emerges (not shown). Large excess of D_2O (100 fold molar excess) allowed us to calculate the half-life using linear regression analysis of the integrated pseudo-first order rate law. Concentrations at each time point were determined from integration of fixed peak regions, normalized as mole-fractions. Compounds were confirmed by NMR of pure hydrolysis products and corroborated using mass spectrometry.

Proteasome Inhibition Assay

Standard methods were used to test for the inhibition of proteasome activity, for both the human 20S proteasome and the *M. tuberculosis* 20SOG proteasome. Briefly, proteasome solutions were made to a final concentration of 3 nM (Mtb20SOG) and 10 μg/mL (H20S), with final Suc-LLVY-AMC substrate concentrations of 50 μM (Mtb20SOG) and 75 μM (H20S), having varying concentrations of inhibitor, to a total volume of 100 μL per well in black 96 well plates. Reactions were incubated for 30 minutes at 37 °C before addition of fluorogenic substrate, and was then monitored at 37 °C for 90 minutes with a Spectra MAX Gemini plate-reader, excitation at 360 nm and emission at 460 nm.