SUPPORTING INFORMATION

Preparation, Gram-Negative Antibacterial Activity, and Hydrolytic Stability of Novel Siderophore-Conjugated Monocarbam Diols

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Contents of Supporting Information

Methods used in determining efficacy of monocarbam analogs and comparators in preclinical infection models; method used in determining PPB and hydrolytic stability of described compounds; additional statistical analysis regarding cumulative % of

susceptibility for organisms appearing in Table 4; in addition to methods used to prepare and characterize all study compounds.

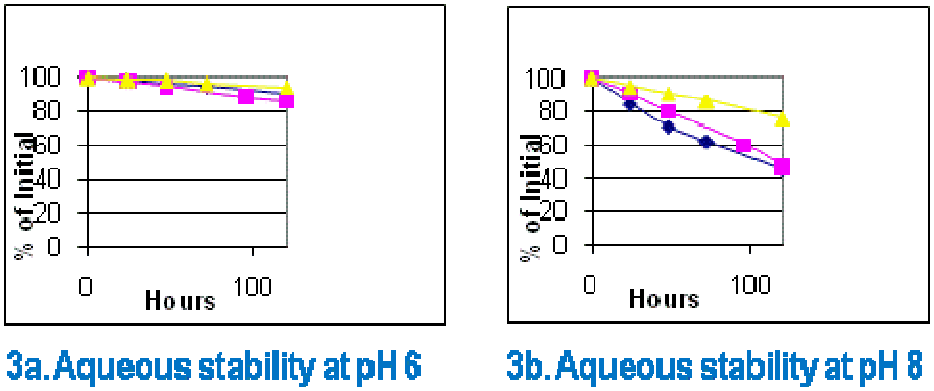
***In vivo* Infection models.** The fluoroquinolone and imipenem-sensitive clinical isolate *Pseudomonas aeruginosa* 1091-05 was used for *in vivo* evaluation of monocarbam analogs in systemic and acute respiratory models of infection. All *in vivo* procedures were reviewed and approved by the Institutional Animal Care and Use Committee.

Acute systemic infection model. CF-1 female mice (Charles River Laboratories) were infected intraperitoneally with *Pseudomonas aeruginosa* 1091-05 in 3% Brewer's yeast. Monocarbam analog doses were prepared in 10% DMSO/90% sterile water and dosed subcutaneously at 0.5 and 4 hours post-infection. Animal survivorship was assessed for 4 days following bacterial challenge and PD_{50}s were determined from non-linear regression analysis of the data using GraphPad Prism v 3.02.

Respiratory tract infection model. C3H/HeN female mice (Charles River Laboratories) were rendered transiently neutropenic by two oral doses of cyclophosphamide monohydrate prepared in sterile water. The first dose (150 mg/kg in 0.2 mL) was administered 4 days prior to infection, and the second dose (100 mg/kg in 0.2 mL) was administered 1 day prior to infection. For bacterial challenge, animals were anesthetized with isofluorane and infected intranasally (IN) by placing 40µl of bacterial suspension in brain heart infusion broth onto the external nares. Mice were held in a vertical position until the droplet was completely inhaled. Monocarbam analog doses were prepared in acetate buffer pH 4.2 and dosed subcutaneously at 4 hours post-infection plus 2 days of twice per day (BID) therapy (5 doses total). Animal survivorship

was assessed for 10 days following bacterial challenge and PD_{50}s were determined from non-linear regression analysis of the data using GraphPad Prism v 3.02.

Determination of hydrolytic stability. Solutions were prepared by weighing approximately 4 mg of sample into a scintillation vial and dissolving the sample in 8 mL of appropriate buffer (20 mM phosphate at specified pH; final concentration ~0.5 mg/mL). Approximately half of the resulting solution was transferred to a second scintillation vial and stored in an oven at 37 ^{o}C. At each time point, a portion of the sample stored at room temperature and at 37 ^{o}C was removed from the scintillation vials and transferred to an HPLC vial for analysis (gradient: 2% to 80% acetonitrile in 0.2% formic acid (aq) over 24 minutes; column HSS-T32.1 x 100 mm, 1.8 m; uv detection).

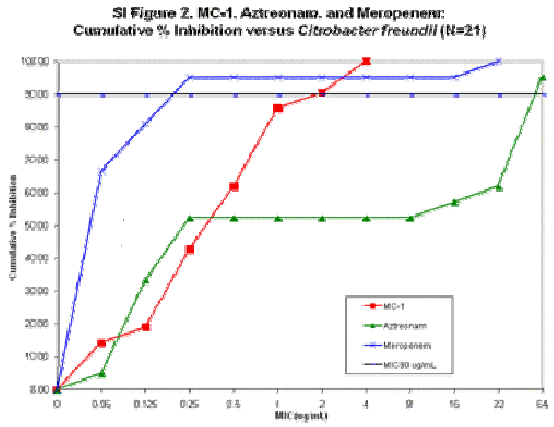


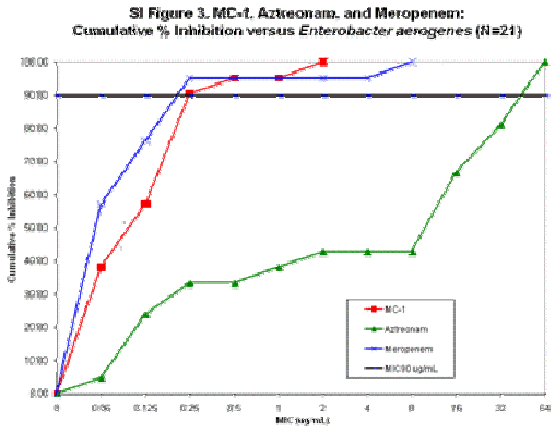
SI Figure 1. Relative aqueous degradation rates and dependence on pH as seen at 37 ^{o}C for **1** Blue; **31** Yellow; and Meropenem Pink

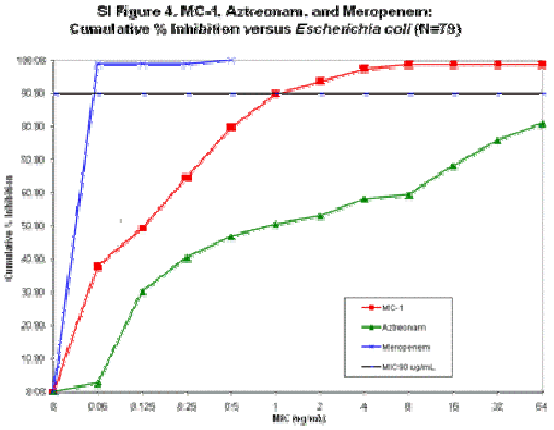
Assessment of plasma protein binding (PPB). Fraction unbound (fu) was determined in mouse plasma utilizing a 96-well Teflon equilibrium dialysis unit. Plasma and buffer loaded with drug at 1 µM (n = 6 replicates per concentration), were added to opposing chambers of each well and incubated for 6 hr at room temperature (room temperature

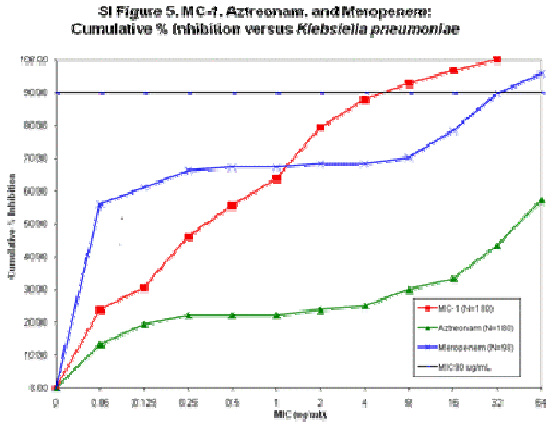
used due to drug hydrolysis at physiologic temperatures). Drug concentrations in plasma and buffer were determined by LC/MS/MS. Fraction unbound was calculated as the concentration in the buffer side divided by the total concentration in the plasma side.

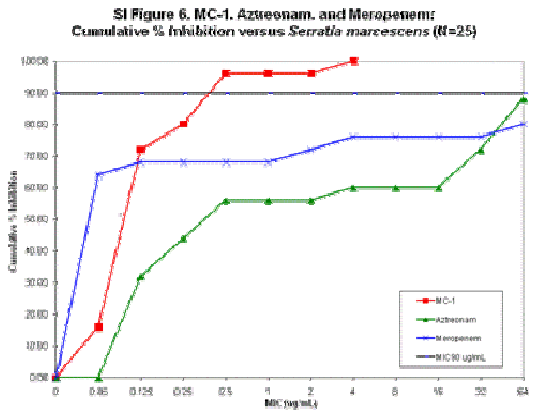
Cumulative % of susceptibility data for organisms appearing in Table 4.

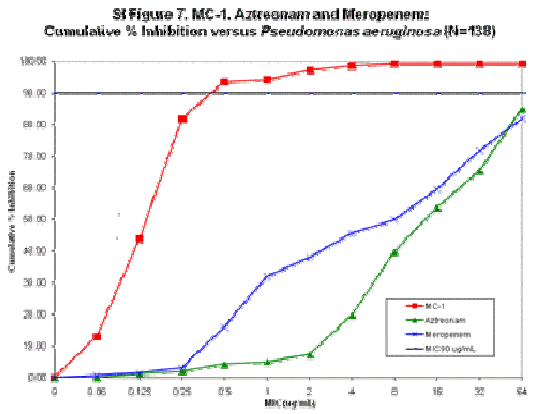


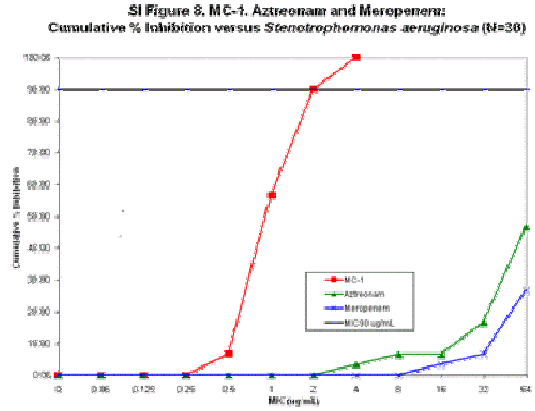


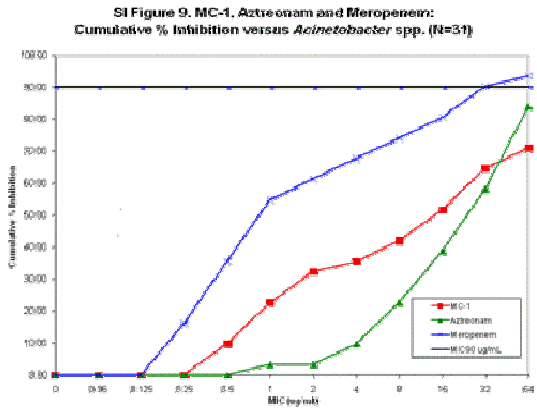












Compound Preparation and Characterization. Reagents and solvents were obtained from commercial sources unless otherwise noted. All reactions were run under nitrogen unless otherwise noted. Routine ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova 400 MHz spectrometer unless otherwise specified. Low resolution mass spectral data was collected using a Waters Micromass ZMD (electrospray ionization, chromatography on a Varian Polaris 5 C-18 column with acetonitrile and 0.1% formic acid aqueous gradient eluant). Compounds were determined to be ≥90% pure by HPLC: 5% to 30% acetonitrile (0.1% formic acid) in water (0.1% formic acid); UV 220 nm.

5-(Benzyloxy)-4-oxo-4H-pyran-2-carboxylic acid (**6**). 5-Hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one (300 g, 2.11 mol) was dissolved in methanol (9 L) and treated with potassium carbonate (439 g, 3.18 mol), followed by slow addition of benzyl

chloride (433 g, 3.42 mol). The reaction mixture was stirred at 65°C for 8 hours. After cooling to room temperature, it was stirred for an additional 16 hours, then concentrated *in vacuo* to a thick paste. This residue was cooled to 10°C and diluted with ice water, resulting in a precipitate that was collected by filtration and dried to afford 325 g, 1.40 mol, 66%. ^{1}H NMR (400 MHz) (DMSO-d_6) δ 4.29 (s, 2H), 4.94 (s, 2H), 6.32 (s, 1H), 7.33-7.42 (m, 5H), 8.17 (s, 1H). Next 64.6 g (0.646 mol) of this material in water (90 mL) was cooled to -5°C and treated drop-wise with concentrated sulfuric acid (56 mL). This was diluted with additional water (40 mL), and then added drop-wise to a cold (-5°C) solution of **C1** (100 g, 0.43 mol) in acetone (4.5 L). The reaction mixture was stirred at 20°C for 3 hours and then filtered through a pad of Celite. Concentration of the filtrate provided a residue, which was washed with hexane to provide **6**. Yield: 80 g, 0.325 mol, 76%. ^{1}H NMR (400 MHz) (DMSO-d_6) δ 4.97 (s, 2H), 6.93 (s, 1H), 7.34-7.42 (m, 5H), 8.37 (s, 1H).

5-(Benzyloxy)-4-oxo-1,4-dihydropyridine-2-carboxylic acid (**7**). A mixture of **6** (100 g, 0.406 mol) and aqueous ammonium hydroxide solution (25%, 1 L) was stirred in an autoclave for 1 hour, and then heated at 83°C for 7 hours at atmospheric pressure. After cooling slowly over about 18 hours, the reaction mixture was acidified to pH 3 with concentrated hydrochloric acid. The resulting precipitate was collected by filtration, washed with water, and dissolved in saturated aqueous sodium bicarbonate solution. The solution was washed with dichloromethane, then acidified with concentrated hydrochloride acid. The resulting solid was collected by filtration, washed with water and dried at 50°C to provide **7**. Yield: 85 g, 0.347 mol, 85%. ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 5.17 (s, 2H), 7.17 (br s, 1H), 7.33-7.49 (m, 7H).

Benzyl 4,5-bis(benzyloxy)pyridine-2-carboxylate (**8**). Benzyl chloride (105.6 mL, 0.918 mol) was added to a solution of **7** (90 g, 0.367 mol) in dimethylformamide (1.25 L). Potassium carbonate (124.8 g, 0.903 mol) was added, and the mixture was stirred at 80°C for 16 hours. After cooling to room temperature, the reaction was treated with ice water, and the resulting solid was collected by filtration and purified by silica gel chromatography to afford **8**. Yield: 50 g, 0.118 mol, 32%. LCMS *m/z*: 426 (M+1). ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 5.32 (s, 6H), 7.33-7.46 (m, 15H), 7.76 (s, 1H), 8.37 (s, 1H).

4,5-Bis(benzyloxy)pyridine-2-carbohydrazide (**9**). Hydrazine monohydrate (47.5 mL, 978 mmol) was added drop-wise over 10 minutes to a suspension of **8** (20 g, 47.0 mmol) in methanol (100 mL). The resulting mixture was heated to 65°C for 2 hours, then cooled to room temperature and filtered under vacuum. The collected solids were washed with methanol to provide **9** as a white solid. Yield: 15.4 g, 44.1 mmol, 94%. LCMS *m/z*: 350.1 (M+1). ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 4.47 (d, J = 4.6 Hz, 2H), 5.30 (s, 2H), 5.32 (s, 2H), 7.31-7.48 (m, 10H), 7.67 (s, 1H), 8.23 (s, 1H), 9.65 (t, J = 4.5 Hz, 1H).

5-[4,5-Bis(benzyloxy)pyridin-2-yl]-1,3,4-oxadiazol-2(3H)-one (**10**). Carbonyl diimidazole (97%, 2.87 g, 17.2 mmol) was added to a suspension of **9** (5.00 g, 14.3 mmol) in tetrahydrofuran (75 mL). The reaction mixture was stirred at room temperature for 3 hours, during which time the white suspension became a homogeneous solution, and then a white suspension. The solid was collected by filtration and washed with tetrahydrofuran (3 x 5 mL) to provide **10** as a white solid. Yield: 4.92 g, 13.1 mmol,

92%. LCMS *m/z*: 376.1 (M+1). ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 5.31 (s, 2H), 5.33 (s, 2H), 7.32-7.48 (m, 10H), 7.56 (s, 1H), 8.38 (s, 1H), 12.64 (br s, 1H).

5-[4,5-Bis(benzyloxy)pyridin-2-yl]-4-[(2R)-2,3-dihydroxypropyl]-2,4-dihydro-3H-1,2,4-triazol-3-one (**12**). (2*R*)-3-Aminopropane-1,2-diol (**11**, 0.291 g, 3.19 mmol) was added to a suspension of **10** (1.0 g, 2.66 mmol) in tetrahydrofuran (50 mL), and the mixture was heated to 60°C for 20 hours. After cooling to room temperature, the suspension was filtered, and the solid was washed with tetrahydrofuran (3 x 5 mL) to afford 1.07 g of carbozate intermediate as the white solid. LCMS *m/z*: 467.2 (M+1). ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 2.93 (m, 1H), 3.19 (m, 1H), 3.27 (m, 2H), 3.44 (m, 1H), 4.53 (t, *J* = 5.8 Hz, 1H), 4.77 (d, *J* = 4.8 Hz, 1H), 5.33 (s, 4H), 6.31 (t, *J* = 5.5 Hz, 1H), 7.31-7.48 (m, 10H), 7.69 (s, 1H), 8.01 (br s, 1H), 8.28 (s, 1H), 10.04 (br s, 1H). A solution of this intermediate (3.00 g, 6.43 mmol) in aqueous potassium hydroxide (1.6 M, 40.2 mL, 64.3 mmol) was heated at 100°C for 13 hours, after which it was cooled to 0°C, diluted with water (100 mL) and acidified to pH 7 with concentrated hydrochloric acid. The resulting solid was filtered and washed with water (3 x 10 mL) to afford **12** (2.66 g), contaminated with about 30% of the hydrolysis product 4,5-bis(benzyloxy)pyridine-2-carboxylic acid (carried forward without additional purification). LCMS *m/z*: 449.2 (M+1) and 336.1 (M+1 for the hydrolysis product). ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 3.28 (m, 2H), 3.70 (m, 1H), 4.05 (dd, half of ABX pattern, *J* = 13.7, 5.0 Hz, 1H), 4.12 (dd, half of ABX pattern, *J* = 13.7, 8.0 Hz, 1H), 4.61 (v br s, 1H), 5.01 (br s, 1H), 5.28 (s, 2H), 5.31 (s, 2H), 7.32-7.48 (m, 10H), 7.58 (s, 1H), 8.32 (s, 1H), 12.03 (br s, 1H). Selected peaks for hydrolysis product: 5.29 (s), 7.70 (s), 8.28 (s).

(3S)-3-Aminoazetidin-2-one (**14**). Benzyl [(3*S*)-2-oxoazetidin-3-yl]carbamate (**13**, 13.37 g, 60.7 mmol) was mixed with degassed ethanol (500 mL) and toluene (125 mL). For synthesis of **13**, see M.J. Miller *et al*., *Tetrahedron*, **1983**, *39*, 2571-2575, and M.S. Lall *et al*., *Journal of Organic Chemistry* **2002**, *67*, 1536-1547. The reaction mixture was sonicated until all the solids dissolved, then purged with nitrogen. Palladium on carbon (10%, 4.45 g) was added, and the reaction mixture was hydrogenated on a Parr shaker for 1 hour at 15 psi. The palladium was removed by filtration through Celite under nitrogen, and rinsed with degassed ethanol. The filtrate, containing **14**, was carried directly into the coupling reaction with **15**, Step 4B. Yield: assumed quantitative. Material from a similar experiment was concentrated to dryness to obtain NMR data: ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 2.12 (br s, 2H), 2.78 (dd, J = 5.1, 2.3 Hz, 1H), 3.31 (dd, J = 5.3, 5.3 Hz, 1H), 3.97 (m, 1H), 7.69 (br s, 1H).

tert-Butyl 2-({[(1Z)-1-{2-[(tert-butoxycarbonyl)amino]-1,3-thiazol-4-yl}-2-oxo-2-{[(3S)-2-oxoazetidin-3-yl]amino}ethylidene]amino}oxy)-2-methylpropanoate (**16**). 1-Hydroxypyrrolidine-2,5-dione (*N*-hydroxysuccinimide, 8.84 g, 76.8 mmol) was added to a suspension of (2*Z*)-{2-[(*tert*-butoxycarbonyl)amino]-1,3-thiazol-4-yl}[(2-*tert*-butoxy-1,1-dimethyl-2-oxoethoxy)imino]acetic acid (prepared by the method of K. Yamawaki *et al*., *Bioorganic and Medicinal Chemistry* **2007**, *15*, 6716-6732) (30 g, 70 mmol) in dichloromethane (400 mL). The mixture was cooled to 0°C, *N,N'*-dicyclohexylcarbodiimide (97%, 15.6 g, 73.3 mmol) was added, and the reaction was stirred at 0°C for 30 minutes and then at room temperature for 3 hours. The mixture was filtered through Celite and concentrated *in vacuo* to afford **15** as a white solid. Yield: 36.17 g, 68.7 mmol, 98%. LCMS *m/z*: 527.2 (M+1). ^{1}H NMR (400 MHz) ($CDCl_3$) δ:

1.43 (s, 9H), 1.54 (s, 9H), 1.61 (s, 6H), 2.91 (br s, 4H), 7.50 (s, 1H), 8.31 (br s, 1H). A solution of **14** (5.23 g, 60.7 mmol) in ethanol/toluene (900 mL, solution obtained in Step 4) was treated with compound **15** (26.6 g, 50.6 mmol), and the reaction mixture was slowly concentrated under reduced pressure, over the course of an hour, to one-third of its original volume. The resulting suspension was stirred at 35°C under nitrogen for about 18 hours. Removal of solvent *in vacuo* afforded a crude product, which was dried under vacuum for 30 minutes. The resulting solids were partitioned between 1:1 ethyl acetate/tetrahydrofuran (1 L) and aqueous sodium bicarbonate solution (500 mL). Additional water was required to dissolve solids observed during the separation. The aqueous layer was extracted with 1:1 ethyl acetate/tetrahydrofuran (2 x 300 mL), and the combined organic layers were filtered and concentrated *in vacuo*. The crude solid was triturated with 3:2 ethyl acetate/heptane (60 mL) for 30 minutes, and the solids were collected by filtration, rinsing with heptane, to provide **16** as a white solid. Yield: 22.08 g, 44.4 mmol, 88%. LCMS *m/z*: 498.6 (M+1). ^{1}H NMR (400 MHz, CD_3OD) δ: 1.47 (s, 9H), 1.52 (s, 6H), 1.54 (s, 9H), 3.39 (dd, *J* = 5.7, 2.5 Hz, 1H), 3.65 (dd, *J* = 5.5, 5.5 Hz, 1H), 5.10 (dd, *J* = 5.3, 2.5 Hz, 1H), 7.34 (s, 1H).

tert-Butyl 2-({[(1Z)-2-({(3S)-1-[({3-[4,5-bis(benzyloxy)pyridin-2-yl]-4-[(2R)-2,3-dihydroxypropyl]-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}sulfonyl)carbamoyl]-2-oxoazetidin-3-yl}amino)-1-{2-[(tert-butoxycarbonyl)amino]-1,3-thiazol-4-yl}-2-oxoethylidene]amino}oxy)-2-methylpropanoate (**17**). A mixture of **12** (4.00 g, 8.92 mmol) in tetrahydrofuran (35 mL) was treated with 2,2,2-trifluoro-*N*-methyl-*N*-(trimethylsilyl)acetamide (MSTFA, 98%, 10.2 mL, 53.7 mmol). After 45 minutes of stirring, the light yellow milky mixture was concentrated *in vacuo* at 60°C for 1 hour,

then dried under vacuum at 60°C for 1.5 hours. In a separate flask, a suspension of **16** (4.88 g, 9.81 mmol) in dichloromethane (32 mL) was cooled to 0°C, treated drop-wise with chlorosulfonyl isocyanate (95%, 0.929 mL, 10.7 mmol) and allowed to stir for 30 minutes under ice-cooling. The material derived from **12** was dissolved in tetrahydrofuran (8 mL), cooled to 0°C. The ice-cooled reaction mixture of **16** was then transferred into this solution via cannula. After stirring at 0°C for 1 hour, then at room temperature for 1.5 hours, the reaction mixture was quenched with methanol (5 mL), stirred for 10 minutes and concentrated *in vacuo*. The residue was purified by silica gel chromatography (Gradient: 40-100% ethyl acetate in heptane, then 0-12% methanol in ethyl acetate) to afford **17** as a solid. Yield: 3.85 g, 3.66 mmol, 41%. LCMS *m/z*: 1051.4 (M+1). ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 1.38 (s, 9H), 1.39 (s, 6H), 1.46 (s, 9H), 3.3 (obscured by HOD signal), 3.66 (m, 1H), 3.70 (dd, J = 6.3, 6.3 Hz, 1H), 4.00-4.13 (m, 2H), 4.56 (m, 1H), 4.93 (m, 2H), 5.29 (s, 2H), 5.30 (s, 2H), 7.25 (s, 1H), 7.31-7.50 (m, 10H), 7.57 (s, 1H), 8.35 (s, 1H), 9.02 (d, J = 8.5 Hz, 1H), 11.84 (br s, 1H).

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-({(3S)-1-[({4-[(2R)-2,3-dihydroxypropyl]-3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}sulfonyl)carbamoyl]-2-oxoazetidin-3-yl}amino)-2-oxoethylidene]amino}oxy)-2-methylpropanoic acid, disodium salt (**1**). A solution of **17** (0.460 g, 0.438 mmol) in tetrahydrofuran (10 mL) and acetic acid (0.1 mL) was degassed and flushed with nitrogen (3x) and treated with Pd black (134 mg). The mixture was hydrogenated using a Parr shaker under 36 psi hydrogen at room temperature for 4 hours (reaction complete by LCMS). The sample was filtered through acid washed cellulose powder and washed with THF to give a pale red filtrate, which was concentrated to

dryness in vacuo affording 0.382 g (100%) of the debenzylated intermediate as a red solid. LCMS *m/z*: 871.8 (M+1). ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 1.39 (s, 9H), 1.40 (s, 6H), 1.46 (s, 9H), 3.29 (m, 2H), 3.39 (dd, *J* = 6.3, 3.3 Hz, 1H), 3.65 (HOD lump obscures signal), 3.71 (m, 1H, estimated), 3.94 (m, 2H, estimated), 4.92 (m, 1H), 7.26 (s, 1H), 7.39 (s, 1H), 8.02 (s, 1H), 9.01 (d, *J* = 8.0 Hz, 1H), 11.82 (br s, 1H). Trifluoroacetic acid (13 mL) was added to a cooled (0^{o}C) solution of this material (2.54 g, 2.91 mmol) in 13 mL of dichloromethane. The reaction mixture was stirred at room temperature for 2 hours and then transfered slowly via a teflon cannula to another round bottom flask containing 186 mL of a 2:1 mixture of heptane/methyl-t-butyl ether (MTBE) resulting in a fine precipitate. The solids were collected, washed with heptane/MTBE (2:1) and dried in vacuo affording 1.82 g (88%) of the crude trifluoroacetic acid salt of **1** as a rose colored solid. A portion of this material (2.42 g) was then purified by reverse phase chromatography using an Isco Rf Chromatography system employing a RediSep Rf C18 column (130 g), loading the crude trifluoroacetic acid salt as a solution in dimethylsulfoxide (1.5 mL) in two batches. The gradient was 5% to 30% water (0.1% Formic acid)/acetonitrile (0.1% Formic acid). The product came off the column at 15-18% acetonitrile. The fractions were pooled and the solvent was removed under reduced pressure affording 0.847 g (35%) of material as a white solid. The solid was sonicated in methanol (4 times) and solvent was removed (done to remove formic acid). The ^{1}H NMR confirms the free-form product with a minimal amount of formic acid. LCMS *m/z*: 715.0 (M+1). ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 1.42 (s, 3H), 1.43 (s, 3H), 3.28 (m, 2H), 3.38 (dd, *J* = 6.3, 3.4 Hz, 1H), 3.65 (m, 1H), 3.70 (m, 1H), 3.95 (br d, *J* = 6.5 Hz, 2H), 4.91 (m, 1H), 6.79 (s, 1H), 7.36 (s, 1H), 8.01 (s, 1H), 9.03 (d, *J* = 8.3 Hz, 1H). To a

slurry of 1.20 g (1.65 mmol) of the free-form acid in 30 mL of deionized water at 0°C was slowly added 0.277 g (3.30 mmol) of sodium bicarbonate dissolved in 6 mL of deionized water (solids completely dissolve upon addition of the sodium bicarbonate solution). The resulting solution was then frozen and lyophilized affording 1.12 g of the disodium salt of **1** as a light pink lyophile. LCMS *m/z*: 715.6 (M+1). ^{1}H NMR (500 MHz) (D_2O) δ HOD: 1.31 (s, 3H), 1.32 (s, 3H), 3.44 (dd, ½ ABX, *J* = 12.1 Hz, 4.8 Hz, 1H), 3.48 (dd, ½ ABX, *J* = 11.8 Hz, 4.0 Hz, 1H), 3.65 (dd, *J* = 7.3 Hz, 3.3 Hz, 1H), 3.73 – 3.92 (m, 3H), 4.90 (dd, *J* = 3.2 Hz, 3.2 Hz, 1H), 6.79 (s, 1H), 6.97 (s, 1H), 7.72 (s, 1H).

Compounds **18** through **30** were prepared in an analogous manner to that described for **1**. Also see WO 2010070523 A1 for additional experimentals. Diagnostic ^{1}H NMR (400 MHz) peaks and LRMS data (M+1) for **18** through **30** are as follows:

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-{[(3S)-1-({[3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4-(2,2,2-trifluoroethyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl]sulfonyl}carbamoyl)-2-oxoazetidin-3-yl]amino}-2-oxoethylidene]amino}oxy)-2-methylpropanoic acid (**18**). LCMS *m/z*: 723.1 (M+1). ^{1}H NMR (400 MHz) (DMSO-*d6*) δ: 1.41 (s, 3H), 1.41 (s, 3H), 3.36 (dd, *J* = 6.2, 3.2 Hz, 1H), 3.69 (dd, *J* = 6.2, 6.2 Hz, 1H), 4.91 (m, 1H), 5.11 (m, 2H), 6.76 (s,1H), 7.37 (s, 1H), 8.00 (s, 1H), 9.01 (d, *J* = 8.3Hz, 1H).

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-{[(3S)-1-({[4-(3,3-dimethylbutyl)-3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]sulfonyl}carbamoyl)-2-oxoazetidin-3-yl]amino}-2-oxoethylidene]amino}oxy)-2-

methylpropanoic acid (**19**). LRMS *m/z*: 725.2 (M+1). ^{1}H NMR (400 MHz) (DMSO-*d6*) δ: 0.89 (s, 9H), 1.40 (m, 2H), 1.43 (s, 3H), 1.43 (s, 3H), 3.37 (dd, *J* = 6.4, 3.1 Hz, 1H), 3.70 (dd, *J* = 6.4, 6.4 Hz, 1H), 4.01 (m, 2H), 4.92 (m, 1H), 6.80 (s, 1H), 7.99 (s, 1H), 9.05 (br d, *J* = 8.8 Hz, 1H).

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-({(3S)-1-[({4-[2-(diethylamino)ethyl]-3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}sulfonyl)carbamoyl]-2-oxoazetidin-3-yl}amino)-2-oxoethylidene]amino}oxy)-2-methylpropanoic acid (**20**). LRMS *m/z*: 740.5 (M+1). ^{1}H NMR (400 MHz) (CD_3OD) δ: 1.27-1.35 (m, assumed 6H), 1.55 (s, 3H), 1.56 (s, 3H), 3.39 (m, 4H), 3.59 (dd, *J* = 6.6, 6.6 Hz, 1H), 3.66 (t, *J* = 5.4 Hz, 2H), 3.92(dd, *J* = 6.6, 6.6 Hz, 1H), 4.51 (t, *J* = 5.4 Hz, 2H), 5.34 (m, 1H), 6.89 (s, 1H), 7.50 (s, 1H), 8.05 (s, 1H).

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-{[(3S)-1-({[3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-4-(3-hydroxypropyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]sulfonyl}carbamoyl)-2-oxoazetidin-3-yl]amino}-2-oxoethylidene]amino}oxy)-2-methylpropanoic acid (**21**). LRMS *m/z*: 699.0 (M+1). ^{1}H NMR (500 MHz) (DMSO-*d6*) δ: 1.44 (s, 3H), 1.44 (s, 3H), 1.67 (m, 2H), 3.35 (t, *J* = 6.3 Hz, 2H), 3.38 (dd, *J* = 6.5, 3.3 Hz, 1H), 3.70 (dd, *J* = 6.4, 6.4 Hz, 1H), 4.01 (m, 2H), 4.92 (m, 1H), 6.84 (s, 1H), 7.35 (s, 1H), 8.01 (s, 1H), 9.07 (d, *J* = 8.5 Hz, 1H).

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-{[(3S)-1-({[4-(2-hydroxy-2-methylpropyl)-3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]sulfonyl}carbamoyl)-2-oxoazetidin-3-yl]amino}-2-oxoethylidene] amino}oxy)-2-methylpropanoic acid (**22**). LRMS *m/z*: 713.2 (M+1). ^{1}H NMR (400 MHz) (DMSO-*d6*) δ: 1.00 (s, 6H), 1.42 (s, 6H), 3.37 (m, 1H), 3.69 (dd, *J* = 6.2, 6.2 Hz, 1H),

3.96 (s, 2H), 4.91 (m, 1H), 6.75 (s, 1H), 7.31 (s, 1H), 7.98 (s, 1H), 8.98 (d, *J* = 8.6 Hz, 1H).

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-({(3S)-1-[({3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-4-[(5-methylisoxazol-3-yl)methyl]-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}sulfonyl)carbamoyl]-2-oxoazetidin-3-yl}amino)-2-oxoethylidene]amino}oxy)-2-methylpropanoic acid (**23**). LRMS *m/z*: 736.1 (M+1). ^{1}H NMR (400 MHz) (DMSO-*d6*) δ: 1.42 (s, 6H), 2.30 (s, 3H), 3.38 (m,1H), 3.42-3.74 (m, asume 1 H, obscured by water peak), 4.92 (m, 1H), 5.35 (s, 2H), 6.05 (s, 1H), 6.78 (s, 1H), 7.35 (s, 1H), 7.93 (s, 1H), 9.01 (d, *J* = 8.0 Hz, 1H).

(S,Z)-2-(1-(2-Aminothiazol-4-yl)-2-(1-(4-((1,5-dimethyl-1H-pyrazol-3-yl)methyl)-3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-ylsulfonylcarbamoyl)-2-oxoazetidin-3-ylamino)-2-oxoethylideneaminooxy)-2-methylpropanoic acid (**24**). LCMS *m/z*: 748.9 (M+1). ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 1.41 (s, 3H), 1.41 (s, 3H), 2.39 (s, 1H), 3.55 (s, 3H), 3.67 (m, 1H), 3.91 (dd, *J* = 6.3, 6.3 Hz, 1H), 5.05 (m, 1H), 5.58 (s, 2H), 6.73 (s, 1H), 7.26 (s, 1H), 7.34 (br s, 1H), 7.80 (s, 1H), 9.10 (d, *J* = 8.0 Hz, 1H), 11.85 (s, 1H).

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-({(3S)-1-[({3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-4-[(2S)-2-hydroxypropyl]-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}sulfonyl)carbamoyl]-2-oxoazetidin-3-yl}amino)-2-oxoethylidene]amino}oxy)-2-methylpropanoic acid, disodium salt (**25**). LCMS *m/z*: 699.6 (M+1). ^{1}H NMR (500 MHz) (D_2O-d_6) δ: 1.01(d, *J* = 8.5 Hz, 3H), 1.32 (d, *J* = 6.0 Hz, 6H), 3.61 – 3.70 (m, 2.5H), 3.77 (dd, ½ ABX, *J* = 18.5 Hz, 4.0 Hz, 0.5H), 3.88 (t, *J* = 8.0 Hz, 1H), 4.90 (dd, *J* = 7.5 Hz, 4.5 Hz, 1H), 6.80 (s, 1H), 6.93 (s, 1H), 7.71 (s, 1H).

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-({(3S)-1-[({4-[(2S)-2,3-dihydroxypropyl]-3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}sulfonyl)carbamoyl]-2-oxoazetidin-3-yl}amino)-2-oxoethylidene] amino}oxy)-2-methylpropanoic acid, disodium salt (**26**). LCMS *m/z*: 715.2 (M+1). ^{1}H NMR (500 MHz) (D_2O) δ: 1.40 (s, 3H), 1.42 (s, 3H), 3.49 (dd, *J* = 12.2, 4.9 Hz, 1H), 3.57 (dd, *J* = 12.2, 3.7 Hz, 1H), 3.74 (m, 1H), 3.88 (m, 1H), 3.98 (m, 3H), 5.03 (m, 1H), 6.90 (s, 1H), 7.02 (s, 1H), 7.80 (s, 1H).

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-({(3S)-1-[({3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-4-[(2R)-2-hydroxypropyl]-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl}sulfonyl)carbamoyl]-2-oxoazetidin-3-yl}amino)-2-oxoethylidene]amino}oxy)-2-methylpropanoic acid, disodium salt (**27**). LCMS *m/z*: 699.8 (M+1). ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 0.95 (d, *J* = 5.3 Hz, 3H), 1.41 (s, 3H), 1.49 (s, 3H), 3.30-3.40 (m, 1H, assumed; obscured by water peak) 3.82 (m, 1H), 3.97 (m, 3H), 5.11 (m, 1H), 6.78 (s, 1H), 7.19 (br s, 1H), 7.36 (s, 1H), 7.88 (s, 1H).

2-({[(1Z)-2-{[(3S)-1-({[4-(2-Amino-2-oxoethyl)-3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]sulfonyl}carbamoyl)-2-oxoazetidin-3-yl]amino}-1-(2-amino-1,3-thiazol-4-yl)-2-oxoethylidene]amino}oxy)-2-methylpropanoic acid, disodium salt (**28**). This compound was prepared from the corresponding nitrile of **12** (*2-(3-(4,5-bis(benzyloxy)pyridin-2-yl)-5-oxo-1H-1,2,4-triazol-4(5H)-yl)acetonitrile*). This material was coupled to **16** in an analogous manner to that described for **17** to afford the fully protected monocarbam (*tert*-butyl 2-({[(1*Z*)-2-({(3*S*)-1-[({3-[4,5-bis(benzyloxy)pyridin-2-yl]-4-(cyanomethyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}sulfonyl)carbamoyl]-2-oxoazetidin-3-yl}amino)-1-{2-[(*tert*-

butoxycarbonyl)amino]-1,3-thiazol-4-yl}-2-oxoethylidene]amino}oxy)-2-methylpropanoate) in 21% yield. LRMS *m/z*: 1016.5 (M+1). The benzyl deprotection was carried out in an analogous manner as described above affording crude *tert*-butyl 2-({[(1*Z*)-1-{2-[(*tert*-butoxycarbonyl)amino]-1,3-thiazol-4-yl}-2-{[(3*S*)-1-({[4-(cyanomethyl)-3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]sulfonyl}carbamoyl)-2-oxoazetidin-3-yl]amino}-2-oxoethylidene]amino}oxy)-2-methylpropanoate in 98% yield. However, during the subsequent trifluoroacetic acid deprotection step, the nitrile of this material was completely converted to the corresponding free form of amide **28**. The crude product from the trifluoroacetic acid deprotection step was dissolved in dimethyl sulfoxide to a concentration of 100 mg/mL, filtered, and purified by preparative HPLC (column: Waters Symmetry C8, 5 μm, 30 x 50 mm; Solvent A: 0.1% aqueous formic acid; Solvent B: 0.1% formic acid in acetonitrile. Gradient: 3% to 22% B). The fractions that pertained to the desired product were combined, cooled to -78°C and lyophilized to provide **28** free form in 12% yield. LCMS *m/z*: 698.9 (M+1). ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 1.38 (br s, 6H), 3.33 (m, 1H), 3.65 (m, 1H), 4.61 (s, 2H), 4.88 (m, 1H), 6.74 (br s, 1H), 7.03 (br s, 1H), 7.30 (s, 1H), 7.89 (s, 1H), 8.99 (d, J = 7.42 Hz, 1H). A solution of this material (78 mg, 0.11 mmol) in a mixture of acetonitrile (5 mL) and water (45 mL) was cooled to 0°C and sodium bicarbonate (18.8 mg, 0.224 mmol) was added. The mixture was vigorously stirred for ten minutes at 0°C. The suspension was then cooled to -78°C (using a dry ice / acetone bath) and lyophilized to afford 79 mg (0.106 mmol, 95%) of **28** disodium salt as a pink solid. ^{1}H NMR (400 MHz) (DMSO-d_6) δ: 1.42 (s, 3H), 1.50 (s, 3H), 3-3.5 ppm obscured by water peak, 3.78 (m, 1H), 4.57 (d, J=16.4 Hz, 1H), 4.72 (d, J

= 16.4 Hz, 1H), 5.15 (m, 1H), 6.78 (s, 1H), 6.99 (br. s, 1H), 7.18 (br s, 3H), 7.38 (br s, 1H), 7.41 (s, 1H), 7.81 (s, 1H).

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-{[(3S)-1-({[3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-4-(2-methoxyethyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]sulfonyl}carbamoyl)-2-oxoazetidin-3-yl]amino}-2-oxoethylidene]amino}oxy)-2-methylpropanoic acid, disodium salt (**29**). LRMS *m/z*: 699.2 (M+1). ^{1}H NMR (400 MHz) (D_20) δ HOD:1.42 (s, 3H), 1.50 (s, 3H), 3.16 (s, 3H), 3.32 (HOD, obscures region), 3.48 (m, 2H), 3.78 (dd, *J* = 6.3, 6.3 Hz, 1H), 4.18 (m, 2H), 5.14 (m, 1H), 6.79 (s, 1H), 7.19 (br s, 2H), 7.40 (s, 1H), 7.88 (s, 1H).

2-({[(1Z)-1-(2-Amino-1,3-thiazol-4-yl)-2-({(3S)-1-[({3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4-[(2S)-tetrahydrofuran-2-ylmethyl]-4,5-dihydro-1H-1,2,4-triazol-1-yl}sulfonyl)carbamoyl]-2-oxoazetidin-3-yl}amino)-2-oxoethylidene] amino}oxy)-2-methylpropanoic acid (**30**). LRMS *m/z*: 725.2 (M+1). ^{1}H NMR (400 MHz) (DMSO-*d6*) δ: 1.42 (br s, 6H), 3.38 (m, 1 H), 3.48 (t, *J* = 5.1, 2 H), 3.70 (dd, *J* = 6.2, 6.2, 2H), 3.97 (m, 2H), 4.13 (m, 1 H), 4.92 (m, 1 H), 6.82 (s, 1 H), 7.35 (s, 1 H), 8.01 (s, 1H), 9.04 (d, *J* = 8.0 Hz, 1H).

Compounds **31** and **32** were prepared in an analogous manner to that described for compounds **1** and **25** respectively starting with the C4-methylated congener of **13** prepared by the method of Woulfe, S.R. and Miller, M.J., *J. Org. Chem.,* **1986**, *51*, 3133.

*2-((Z)-1-(2-Aminothiazol-4-yl)-2-((2S,3S)-1-(4-((R)-2,3-dihydroxypropyl)-3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-

ylsulfonylcarbamoyl)-2-methyl-4-oxoazetidin-3-ylamino)-2-oxoethylideneaminooxy)-2-methylpropanoic acid disodium salt (**31**). LRMS *m/z*: 728.9 (M+1). ^{1}H NMR (400 MHz) (DMSO-*d6*) δ: 1.40 (s, 3H), 1.41 (s, 3H), 2.04 (s, 3H), 3.25 (dd, *J* = 8.0 Hz, *J* = 3.2 Hz, 1H), 3.75 – 3.80 (m, 2H), 3.90 (dd, *J* = 8.0 Hz, *J* = 12 Hz, 1H), 4.43 (dd, *J* = 8.0 Hz, *J* = 8.0 Hz, 1H), 6.78 (s, 1H), 7.35 (s, 1H), 7.98 (s, 1H), 9.03 (d, *J* = 8.0 Hz).

2-((Z)-1-(2-Aminothiazol-4-yl)-2-((2S,3S)-1-(3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-4-((S)-2-hydroxypropyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-ylsulfonylcarbamoyl)-2-methyl-4-oxoazetidin-3-ylamino)-2-oxoethylideneaminooxy)-2-methylpropanoic acid disodium salt (**32**). LRMS *m/z*: 713.8 (M+1). ^{1}H NMR (400 MHz) (DMSO-*d6*) δ: 0.93 (s, 3H), 0.94 (s, 3H), 1.39 (s, 3H), 2.04 (s, 3H), 3.74 – 3.83 (m, 2H), 4.43 (dd, *J* = 8.0 Hz, *J* = 3.2 Hz, 1H), 6.8 (s, 1H), 7.36 (s, 1H), 7.99 (s, 1H), 9.05 (d, *J* = 8.0 Hz).