Orally Absorbed Derivatives of the β-Lactamase Inhibitor Avibactam. Design of Novel Prodrugs of Sulfate Containing Drugs

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Supporting Information

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GENERAL

All reagents were purchased from commercial suppliers and used without further purification. All solvents were reagent, or HPLC grade. Analytical TLC was performed on silica gel 60 F254 plates and visualized by UV if possible, or by staining with $KMnO_4$ dip, or phosphomolybdic acid in EtOH dip. Flash chromatography was carried out using an automated system with pre-packed silica columns. Yields refer to isolated yields of pure compounds. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer at ambient temperature. Chemical shifts are reported in parts per million (ppm) relative to deuterated solvent, or a TMS internal standard. Multiplicities are reported as follows: s = singlet; d = doublet, t = triplet; m = multiplet; br = broad; f = fine. High-resolution mass spectrometry was obtained on a Waters Xevo G2 QTOF with Acquity LC system. All final compounds are of ≥95% purity as assessed by ^{1}H and ^{13}C NMR, together with HPLC (20 minute method). Mass spectrometry and high-resolution mass-spectrometry (key compounds) were also used to assess final compounds.

LC-MS 6 minute method:

Autosampler: Finnigan Surveyor Autosampler Plus
MS Pump: Finnigan Surveyor MS Pump Plus
UV Detector: Finnigan Surveyor PDA Plus Detector
Mass Spectrometer: Finnigan LTQ
Ionization Method: APCI
Column: Phenomenex, Gemini 5µm C18 110Å, 50 x 3 mm
Solvent system:
Solvent A: water +0.1% formic acid
Solvent B: 90% acetonitrile / 10% water +0.1% formic acid

Gradient time table:

Time (min)	Flow (mL/min)	%A	%B
0.00	0.50	90.00	10.00
0.10	0.50	90.00	10.00
3.50	0.50	0.00	100.00
4.50	0.50	0.00	100.00
5.00	0.50	90.00	10.00
6.00	0.50	90.00	10.00

LC-MS 10 minute method:

Autosampler: Finnigan Surveyor Autosampler Plus
MS Pump: Finnigan Surveyor MS Pump Plus
UV Detector: Finnigan Surveyor PDA Plus Detector
Mass Spectrometer: Finnigan LTQ
Ionization Method: APCI
Column: Phenomenex, Gemini 5µm C18 110Å, 50 x 3 mm
Solvent system:
Solvent A: water +0.1% formic acid
Solvent B: 90% acetonitrile / 10% water +0.1% formic acid

Gradient time table:

Time (min)	Flow (mL/min)	%A	%B
0.00	0.50	90.00	10.00
0.10	0.50	90.00	10.00
7.50	0.50	0.00	100.00
8.50	0.50	0.00	100.00
9.00	0.50	90.00	10.00
10.00	0.50	90.00	10.00

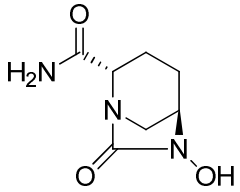
HPLC 20 minute method: Used to assess purity of final compounds

Pump: Varian ProStar Model 210
UV Detector: Varian ProStar (Photo Diode Array)
Column: Phenomenex, Gemini-NX 5µm C18 110Å, 150 x 4.60 mm
Solvent system:
Solvent A: water +0.1% trifluoroacetic acid
Solvent B: acetonitrile

Gradient time table:

Time (min)	**Flow (mL/min)**	**%A**	**%B**
Prerun	1.20	90.00	10.00
1.00	1.20	90.00	10.00
16.00	1.20	10.00	90.00
22.00	1.20	10.00	90.00
22.50	1.20	90.00	10.00

PREPARATION OF (2*S*,5*R*)-6-HYDROXY-7-OXO-1,6-DIAZABICYCLO[3.2.1]OCTANE-2-CARBOXAMIDE[1]



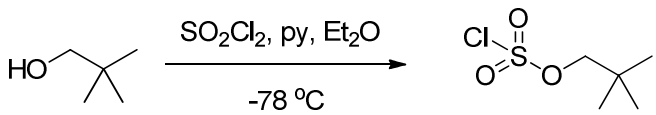
Reference is made to (*a*) Abe, T.; Okue, M.; Sakamaki, Y. Preparation of optically-active diazabicyclooctane derivative and method for manufacturing same. PCT WO 2012086241; *Chem. Abstr.* **2012**, *157*, 165634. (*b*) Lampilas, M.; Aszodi, J.; Rowlands, D. A.; Fromentin, C. Azabicyclic compounds, including 1,3-diazabicyclo[2.2.1]heptan-2-one and 1,6-diazabicyclo[3.2.1]octan-7-one derivatives, preparation thereof, and use as medicines, in particular as antibacterial agents. PCT WO 2002010172; *Chem. Abstr.* **2002**, *136*, 136397, together with related procedures from the patent literature[1]

A stirred mixture of (2*S*,5*R*)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide (550 mg, 2.0 mmol), palladium on carbon (10% by weight; 340 mg, 0.3 mmol) and MeOH (18 mL) was hydrogenated under 1 atm (balloon) until analysis by thin-layer chromatography (TLC) indicated completion of the reaction (approximately, 30 min; reaction monitored by TLC using MeOH / CH_2Cl_2 - 5:95 as eluent). The mixture was filtered through a pad of celite and the pad was rinsed thoroughly with MeOH (*ca.* 20 mL). The filtrate was concentrated under vacuum (water bath temperature not exceeding 25 °C) to give the product as a clear and colorless oil. The oil was dried under vacuum for 1 h, and the residue was used immediately in the next step without further purification. Yield assumed quantitative.

LC-MS: *m/z* = 186.0 $[M+H]^+$

PREPARATION OF COMPOUND (9)[2]

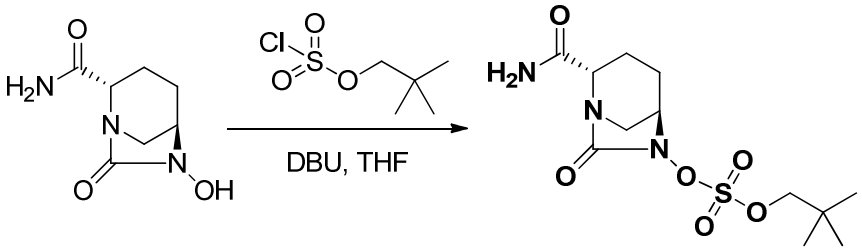
STEP 1: PREPARATION OF NEOPENTYL SULFUROCHLORIDATE



Reference is made to Simpson, L. S.; Widlanski, T. S. Comprehensive Approach to the Synthesis of Sulfate Esters. *J. Am. Chem. Soc.* **2006**, *128*, 1605-1610[2]

Sulfuryl chloride (0.11 mL, 1.4 mmol) in Et_2O (20 mL) was added dropwise to a stirred solution of 2,2-dimethylpropan-1-ol (0.21 g, 2.3 mmol) and pyridine (0.11 mL, 1.4 mmol) in Et_2O (5 mL) at -78 °C under an atmosphere of nitrogen. The mixture was stirred at -78 °C for 30 minutes, then filtered through a pad of Celite. The filtrate containing a solution of the desired product in Et_2O was used directly in the next step without further purification, or concentration.

STEP 2: PREPARATION OF (2*S*, 5*R*)-2-CARBAMOYL-7-OXO-1,6-DIAZABICYCLO[3.2.1]OCTAN-6-YL NEOPENTYL SULFATE (9)[2]



Reference is made to Simpson, L. S.; Widlanski, T. S. Comprehensive Approach to the Synthesis of Sulfate Esters. *J. Am. Chem. Soc.* **2006**, *128*, 1605-1610[2]

(2*S*, 5*R*)-6-hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide (0.20 g, 1.1 mmol) was suspended in THF (50 mL) and the resulting suspension was cooled to 0 °C under an atmosphere of nitrogen. 1,8-Diazabicyclo[5.4.0]undec-7-ene (177 μL, 1.2 mmol) was added slowly to the cooled solution, and the mixture stirred for 10 min. An solution of neopentyl sulfurochloridate (0.25 g, 1.3 mmol) in Et_2O was added to the reaction mixture in one portion. The mixture was stirred at 0 °C, and then allowed to warm slowly to ambient temp. and stirred overnight. The solution was decanted from the solid which had precipitated from the reaction mixture, and the solid was rinsed with EtOAc (x2). The combined organic layers were concentrated under vacuum, and the

resulting solid was dissolved in DCM and purified by column chromatography on silica gel (12 g column) using EtOAc / hexanes (1:9 to 7:3) as eluent to give a solid product. The solid was triturated with MTBE to give the product (142 mg). The filtrate was concentrated to give another aliquot of product (50 mg). The total yield = 53%.

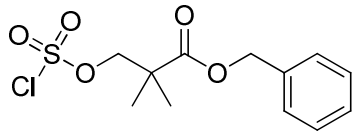
LC-MS: *m/z* = 335.95 $[M+H]^+$

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 6.48 (br. s, 1H), 5.57 (br. s, 1H), 4.48 (d, *J* = 8.7 Hz, 1H), 4.18 (d, *J* = 8.7 Hz, 2H), 4.05 (d, *J* = 6.9 Hz, 1H), 3.36-3.32 (m, 1H), 3.02 (d, *J* = 12.3 Hz, 1H), 2.46-2.41 (m, 1H), 2.19-2.14 (m, 1H), 1.99-1.82 (m, 2H), 1.01 (s, 9H)

^{13}C-NMR (300 MHz, $CDCl_3$): δ 171.2, 167.0, 85.2, 62.0, 60.2, 47.2, 32.0, 26.0, 20.8, 17.5

PREPARATION OF COMPOUND (10)

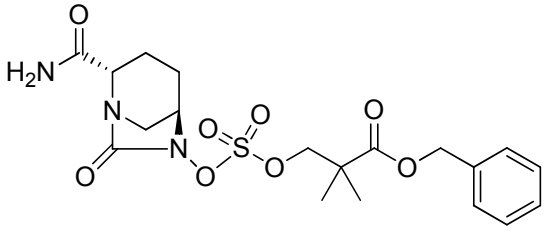
STEP 1: PREPARATION OF BENZYL 3-((CHLOROSULFONYL)OXY)-2,2-DIMETHYLPROPANOATE (8)



A solution of distilled sulfuryl chloride (0.77 mL, 10.6 mmol) in Et_2O (10 mL) was cooled to -78 °C under an atmosphere of argon. A solution of ethyl 3-hydroxy-2,2-dimethylpropanoate (Sigma-Aldrich; 2.0 g, 9.6 mmol) and pyridine (0.85 mL, 10.6 mmol) in Et_2O (2.0 mL) was then added dropwise over 1 h *via* a syringe. The syringe was rinsed with Et_2O with each rinse being added to the reaction mixture. The acetone / CO_2 bath was removed, and the mixture allowed to warm to rt, then stirred at rt for 30 min. Reaction was incomplete by TLC analysis (EtOAc / hexanes; 3:7), so re-cooled to -78 °C and added more SO_2Cl_2 (0.07 mL), then allowed to warm to rt, and stirred for an additional 1 h. Et_2O (5 mL) was added, and the mixture stirred for a few min, then filtered and the filtrate concentrated under vacuum to give the product (2.19 g, 75%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41-7.32 (m, 4H), 5.18 (s, 2H), 4.52 (s, 2H), 1.34 (s, 6H)

STEP 2: PREPARATION OF BENZYL 3-(((((2*S*,5*R*)-2-CARBAMOYL-7-OXO-1,6-DIAZABICYCLO[3.2.1]OCTAN-6-YL)OXY)SULFONYL)OXY)-2,2-DIMETHYLPROPANOATE (10)



(2*S*,5*R*)-6-hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide (370 mg, 2.0 mmol) was dissolved in THF (7.0 mL) and 1,3-dimethyltetrahydropyrimidin-2(1*H*)-one (2.0 mL), and the resulting solution was cooled to -78 °C under an atmosphere of argon. A solution of NaHMDS in THF (1M; 2.2 mL, 2.2 mmol) was added dropwise, and the mixture was stirred at -78 °C for 10 min. A solution of benzyl 3-((chlorosulfonyl)oxy)-2,2-dimethylpropanoate (674 mg, 2.2 mmol) in THF (1 mL) was then added quickly to

the reaction mixture via syringe. The syringe was rinsed with THF (3 x 0.5 mL), each rinse being added to the reaction mixture. After 10 min at -78°C, the reaction mixture was allowed to warm to rt and stirred at rt until judged complete by LC-MS and TLC analysis. EtOAc (20 mL) and saturated aqueous $NaHCO_3$ (20 mL) were added, and the organic and aqueous layers were partitioned. The organic layer was washed with saturated $NaHCO_3$ (20 mL), water (3 x 20 mL), brine (20 mL), dried (Na_2SO_4), and concentrated under vacuum to leave a crude residue. The residue was purified by column chromatography on silica gel using EtOAc / hexanes (1:9 to 1:0) as eluent to give the product (244 mg, 26%) as a solid.

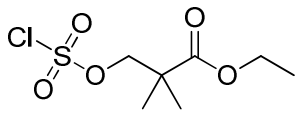
LC-MS: m/z = 456.2 $[M+H]^+$

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.39-7.28 (m, 4H), 6.49 (s, 1H), 5.84 (s, 1H), 5.20-5.11 (m, 2H), 4.74 (d, J = 9.0 Hz, 1H), 4.61 (d, J = 9.0 Hz, 1H), 4.15-4.14 (m, 1H), 4.04 (d, J = 6.9 Hz, 1H), 3.29-3.25 (m, 1H), 2.99 (d, J = 11.7 Hz, 1H), 2.45-2.38 (m, 1H), 2.17-2.10 (m, 1H), 1.99-1.78 (m, 2H), 1.30 (s, 3H), 1.29 (s, 3H)

^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.1, 171.1, 167.1, 135.7, 128.7, 128.4, 128.0, 80.3, 67.0, 62.0, 60.2, 47.2, 43.0, 22.2, 21.7, 20.8, 17.5

PREPARATION OF PRODRUG (14)

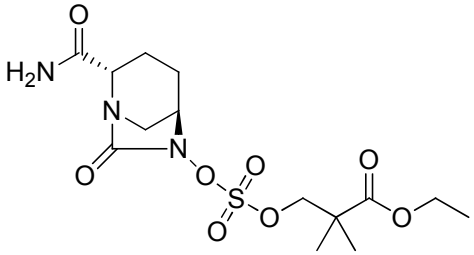
PREPARATION OF ETHYL 3-((CHLOROSULFONYL)OXY)-2,2-DIMETHYLPROPANOATE



A solution of distilled sulfuryl chloride (0.55 mL, 7.5 mmol) in Et_2O (10 mL) was cooled to -78 °C under an atmosphere of argon. A solution of ethyl 3-hydroxy-2,2-dimethylpropanoate (Sigma-Aldrich; 1.0 g, 6.8 mmol) and pyridine (0.55 mL, 6.8 mmol) in Et_2O (1.0 mL) was then added dropwise over 1 h via a syringe. The syringe was rinsed with Et_2O (3 x 1 mL), each rinse being added to the reaction mixture. The acetone / CO_2 bath was removed, and the mixture allowed to warm to rt, then stirred at rt for 4 h. Reaction was incomplete by TLC analysis (EtOAc / hexanes; 3:7), so re-cooled to -78 °C and added more SO_2Cl_2 (0.11 mL), then allowed to warm to rt, and stirred for an additional 2 h. The mixture was filtered, and the filtrate was concentrated under vacuum to give the product (yield assumed quantitative).

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.50 (s, 2H), 4.19 (q, J = 6.9 Hz, 2H), 1.31 (s, 6H), 1.28 (t, J = 6.9 Hz, 3H)

PREPARATION OF ETHYL 3-(((((2*S*,5*R*)-2-CARBAMOYL-7-OXO-1,6-DIAZABICYCLO[3.2.1]OCTAN-6-YL)OXY)SULFONYL)OXY)-2,2-DIMETHYLPROPANOATE (14)



(2*S*,5*R*)-6-hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide (370 mg, 2.0 mmol) was dissolved in THF (7.0 mL) and 1,3-dimethyltetrahydropyrimidin-2(1*H*)-one (3.0 mL), and the resulting solution was cooled to -78 °C under an atmosphere of argon. A solution of NaHMDS in THF (1M; 2.2 mL, 2.2 mmol) was added dropwise, and the mixture was stirred at -78 °C for 10 min. A solution of ethyl 3-((chlorosulfonyl)oxy)-2,2-dimethylpropanoate (538 mg, 2.2 mmol) in THF (1 mL) was then added quickly to the

reaction mixture via syringe. The syringe was rinsed with THF (3 x 0.5 mL), each rinse being added to the reaction mixture. After 10 min at -78°C, the reaction mixture was allowed to warm to rt and stirred at rt until judged complete by LC-MS and TLC analysis (*ca*. 2 h). EtOAc (20 mL) and saturated aqueous $NaHCO_3$ (20 mL) were added, and the organic and aqueous layers were partitioned. The organic layer was washed with saturated $NaHCO_3$ (20 mL), water (3 x 20 mL), brine (20 mL), dried (Na_2SO_4), and concentrated under vacuum to leave a crude residue. The residue was purified by column chromatography on silica gel using EtOAc / hexanes (1:9 to 1:0) as eluent to give the product (318 mg, 39%) as a solid.

LC-MS: *m/z* = 394.1 $[M+H]^+$

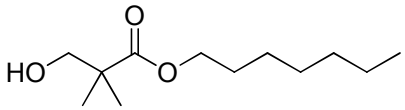
^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.50 (s, 1H), 5.78 (s, 1H), 4.71 (d, *J* = 8.7 Hz, 1H), 4.59 (d, *J* = 8.7 Hz, 1H), 4.22-4.12 (m, 3H), 4.05 (d, *J* = 6.9 Hz, 1H), 3.34-3.30 (m, 1H), 3.01 (d, *J* = 12.3 Hz, 1H), 2.46-2.40 (m, 1H), 2.18-2.12 (m, 1H), 2.00-1.79 (m, 2H), 1.28-1.24 (m, 9H)

^{13}C NMR (300 MHz, $CDCl_3$): δ 174.2, 171.2, 167.1, 80.5, 61.9, 61.4, 60.2, 47.2, 42.8, 22.2, 21.7, 20.8, 17.5, 14.2

HRMS (ESI): $[M+H]^+$ calc'd for $C_{14}H_{23}N_3O_8S$ *m/z* 394.1284, found 394.1279

PREPARATION OF PRODRUG (15)

STEP 1: PREPARATION OF HEPTYL 3-HYDROXY-2,2-DIMETHYLPROPANOATE[3]

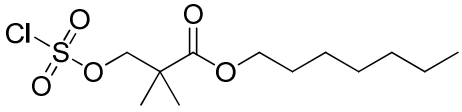


Reference is made to Schaper, U. A.; Bruns, K. Esters of 3-hydroxy-2,2-dimethylpropionic acid and their use as perfume components. German Patent Application DE 3045373; *Chem. Abstr.* **1982**, *97*, 162388.

A mixture of 3-hydroxy-2,2-dimethylpropionic acid (4.7 g, 40 mmol), 1-heptanol (70 mL) and concentrated sulfuric acid (or fuming sulfuric acid; 1 mL) was heated to 80 °C and stirred overnight. After allowing to cool, the mixture was concentrated under vacuum (high vacuum pump required) and the residue partitioned between EtOAc (100 mL) and saturated aqueous $NaHCO_3$ (100 mL). The aqueous was washed with H_2O (50 mL), saturated $NaHCO_3$ (50 mL) and brine (50 mL), then dried (Na_2SO_4), filtered and concentrated under vacuum to leave the product as an oil. The product was used directly in the next step without further purification.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.31 (t, J = 6.5 Hz, 2H), 3.77 (s, 2H), 1.87-1.81 (m, 2H), 1.53-1.50 (m, 8H), 1.41 (s, 6H), 1.12-1.08 (m, 3H)

STEP 2: PREPARATION OF HEPTYL 3-((CHLOROSULFONYL)OXY)-2,2-DIMETHYLPROPANOATE

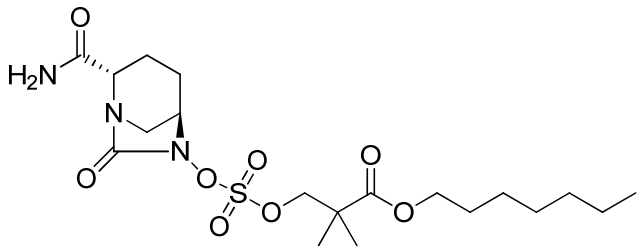


A solution of sulfuryl chloride (2.0 mL, 27.7 mmol) in Et_2O (40 mL) was cooled to -78 °C under an atmosphere of Ar. A solution of heptyl 3-hydroxy-2,2-dimethylpropanoate (3.0 g, 13.9 mmol) and pyridine (1.4 mL, 16.6 mmol) in Et_2O (15 mL) was added dropwise to the sulfuryl chloride solution over the course of 30 min. The flask was rinsed with Et_2O (3 x 5 mL) and the rinse added to the reaction mixture. The mixture was stirred at -78 °C until completion as monitored by TLC (30 min; EtOAc/hexanes 3:7). The precipitate was filtered, and the filtrate was concentrated under vacuum to afford heptyl

3-((chlorosulfonyl)oxy)-2,2-dimethylpropanoate (3.3 g, 75%). The mixture was stored at -78 °C and was used immediately for the next step without further purification.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.46 (s, 2H), 4.11-4.00 (m, 2H), 1.64-1.55 (m, 2H), 1.26-1.24 (m, 8H), 0.85-0.81 (m, 3H)

STEP 3: PREPARATION OF HEPTY 3-(((((2*S*,5*R*)-2-CARBAMOYL-7-OXO-1,6-DIAZABICYCLO[3.2.1]OCTAN-6-YL)OXY)SULFONYL)OXY)-2,2-DIMETHYLPROPANOATE (15)



(2*S*,5*R*)-6-hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide (673 mg) was dissolved in THF (35 mL) and 1,3-dimethyltetrahydropyrimidin-2(1*H*)-one (5 mL) and the resulting solution was cooled to -78 °C under an atmosphere of Ar. A 1.0 M solution of NaHMDS in THF (4.0 mL, 4.0 mmol) was added dropwise to the cooled solution and stirred for 20 min. Neat heptyl 3-((chlorosulfonyl)oxy)-2,2-dimethylpropanoate (1.3 g, 4.0 mmol) was added quickly to the reaction mixture. The syringe was rinsed with THF (3 x 3 mL) and the rinse was also added to the mixture. After 10 min, the reaction mixture was warmed to rt and stirred until judged complete by TLC and LC-MS. EtOAc (50 mL) and saturated aqueous $NaHCO_3$ (50 mL) were added to the mixture. The aqueous and organic layers were partitioned, and the organic layer was washed with saturated aqueous $NaHCO_3$ (50 mL), water (3 x 50 mL), brine (50 mL), then dried (Na_2SO_4), filtered and concentrated under vacuum to leave a crude residue. The residue was purified by column chromatography on silica gel using EtOAc/hexanes (1:9 to 1:0) as eluent, followed by purification using high-performance liquid chromatography to give the product (65 mg, 4%) as a solid.

LC-MS: 464.3 $[M+H]^+$

^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.48 (s, 1H), 5.71 (s, 1H), 4.71 (d, *J* = 9.6 Hz, 1H), 4.60 (d, *J* = 9.3 Hz, 1H), 4.18-4.04 (m, 4H), 3.34-3.29 (m, 1H), 3.02 (d, *J* = 11.7 Hz, 1H),

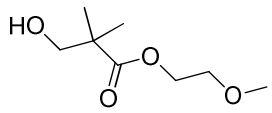
2.47-2.40 (m, 1H), 2.19-2.11 (m, 1H), 2.01-1.79 (m, 2H), 1.66-1.59 (m, 2H), 1.37-1.26 (m, 14H), 0.90-0.86 (m, 3H)

^{13}C-NMR (75 MHz, $CDCl_3$): δ 174.3, 171.1, 167.0, 80.5, 65.6, 62.0, 60.2, 47.2, 43.0, 31.8, 29.0, 28.6, 25.9, 22.7, 22.2, 21.8, 20.9, 17.6, 14.2

HRMS (ESI): $[M+H]^+$ calc'd for $C_{19}H_{33}N_3O_8S$ *m/z* 464.2067, found 464.2060

PREPARATION OF PRODRUG (16)

STEP 1: PREPARATION OF 2-METHOXYETHYL 3-HYDROXY-2,2-DIMETHYLPROPANOATE[4]

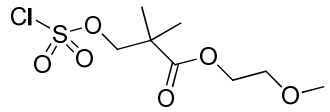


Reference is made to Subramanyam, C.; Bell, M. R. 2-saccharinylmethyl benzoates, their preparation and use for the treatment of degenerative diseases. US Patent Application US 5306818; *Chem. Abstr.* **1995**, *123*, 143928.

3-Hydroxy-2,2-dimethylpropanoic acid (1.2 g, 10.3 mmol) and Cs_2CO_3 (3.4 g, 10.4 mmol) were suspended in DMF (25 mL) at rt, then 2-bromoethyl methyl ether (1.0 mL, 10.4 mmol) was added. The resulting mixture was stirred at 70 ^oC overnight. After cooling, the mixture was filtered through a pad of Celite. The filtrate was diluted with EtOAc (150 mL), and the mixture washed with water (3 x 100 mL) and brine, then dried (Na_2SO_4), filtered and concentrated to leave a crude residue. The residue was purified by column chromatography on silica gel using EtOAc/hexanes (1:4 to 4:1) as eluent to give the product (1.3 g, crude weight) as an oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.28 (t, J = 4.8 Hz, 2H), 3.62-3.55 (m, 4H), 3.38 (s, 3H), 2.65 (t, J = 6.0 Hz, 1H), 1.21 (s, 6H)

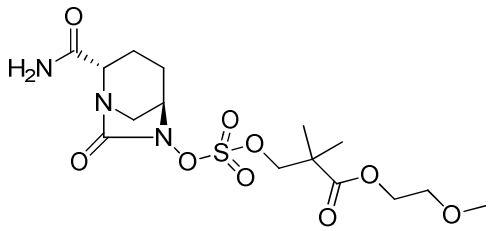
STEP 2: PREPARATION OF 2-METHOXYETHYL 3-((CHLOROSULFONYL)OXY)-2,2-DIMETHYLPROPANOATE



A solution of freshly distilled sulfuryl chloride (0.2 mL, 2.8 mmol) in Et_2O (7.000 ml) was cooled to -78 °C under an atmosphere of Ar. A solution of 2-methoxyethyl 3-hydroxy-2,2-dimethylpropanoate (0.48 g, 2.7 mmol) and pyridine (0.24 mL, 3.0 mmol) in Et_2O (1 mL) was added dropwise to the sulfuryl chloride solution over the course of 11 min. The flask was rinsed with Et_2O (3 x 1 mL) which was also added to the reaction mixture. The mixture was stirred at -78 °C until completion (monitored by TLC, EtOAc/hexanes, 3:7, 30 min). The precipitate was filtered, and the filtrate was concentrated under vacuum to afford the product (0.5 g, 67%) as an oil, which was used

directly in the next step without further purification [Note: ^{1}HNMR indicated desired product with residue of pyridine and starting material].

STEP 3: PREPARATION OF 2-METHOXYETHYL 3-(((((2*S*,5*R*)-2-CARBAMOYL-7-OXO-1,6-DIAZABICYCLO[3.2.1]OCTAN-6-YL)OXY)SULFONYL)OXY)-2,2-DIMETHYLPROPANOATE (16)



(2*S*,5*R*)-6-hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide (162 mg, 0.9 mmol) was dissolved in THF (2.5 mL) and 1,3-dimethyltetrahydropyrimidin-2(1*H*)-one (0.3 mL), and the resulting solution was cooled to -78 °C under an atmosphere of Ar. A 1.0 M solution of NaHMDS in THF (1.0 mL, 1.0 mmol) was added dropwise to the cooled solution and the mixture stirred for 10 min. 2-methoxyethyl 3-((chlorosulfonyl)oxy)-2,2-dimethylpropanoate (0.3 g, 1.1 mmol) in THF (2 mL) was added quickly to the reaction mixture. After 10 min at -78 °C, the mixture was allowed to warm to rt and stirred for 30 min. The mixture was diluted with EtOAc (40 mL) and water. The aqueous and organic layers were partitioned, and the organic layer was washed with water (3 x 20 mL), and brine (50 mL), then dried (Na_2SO_4), filtered and concentrated under vacuum to leave a crude residue. The crude residue was purified by column chromatography on silica gel (4g column) using EtOAc / hexanes (3:7 to 1:0) as eluent to give an impure solid. The product was dissolved in Et_2O (20 mL) with the aid of sonication, and precipitated with hexanes. The resulting solid was filtered, and dried under vacuum to leave the product (72 mg, 19%) as a solid.

LCMS: *m/z* = 424.3 $[M+H]^+$

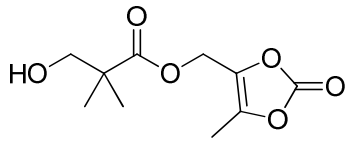
^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.48 (br. s, 1H), 5.56 (br. s, 1H), 4.72 (d, *J* = 8.7 Hz, 1H), 4.62 (d, *J* = 8.7 Hz, 1H), 4.33-4.22 (m, 2H), 4.17 (br. s, 1H), 4.05 (d, *J* = 6.9 Hz, 1H), 3.60 (t, *J* = 4.6 Hz, 2H), 3.38 (s, 3H), 3.33 (d, *J* = 11.1 Hz, 1H), 3.02 (d, *J* = 12.0 Hz, 1H), 2.46-2.41 (m, 1H), 2.18-2.13 (m, 1H), 1.98-1.84 (m, 2H), 1.31 (s, 3H), 1.29 (s, 3H)

^{13}C-NMR (75 MHz, $CDCl_3$), δ 174.1, 170.8, 166.9, 80.2, 70.2, 64.1, 61.8, 60.0, 59.0, 47.1, 42.9, 22.1, 21.6, 20.7, 17.4

HRMS (ESI): $[M+H]^+$ calc'd for $C_{15}H_{25}N_3O_9S$ *m/z* 424.1389, found 424.1382

PREPARATION OF PRODRUG (17)

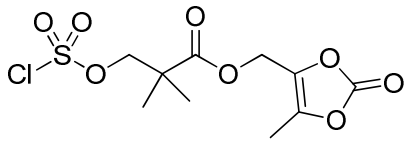
STEP 1: PREPARATION OF (5-METHYL-2-OXO-1,3-DIOXOL-4-YL)METHYL 3-HYDROXY-2,2-DIMETHYLPROPANOATE



To a stirred solution of 3-hydroxy-2,2-dimethylpropanoic acid (4.0 g, 33.9 mmol) and K_2CO_3 (4.68 g, 33.9 mmol) in DMF (45 mL) at 0 °C was added 4-(chloromethyl)-5-methyl-1,3-dioxol-2-one (5.03 g, 33.9 mmol) in DMF (5 mL) dropwise over 1 h. The reaction was stirred at rt for overnight. The reaction mixture was diluted with EtOAc and washed with water and brine. The organic layer was dried with anhydrous Na_2SO_4, filtered, and concentrated under vacuum to give a crude residue. The residue was purified by silica gel column chromatography using EtOAc / hexane (1:4 to 2:3) as eluent to give the product as a yellow liquid (1.6 g, yield 21%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.86 (s, 2H), 3.58 (s, 2H), 2.18 (s, 3H), 1.20 (s, 6H)

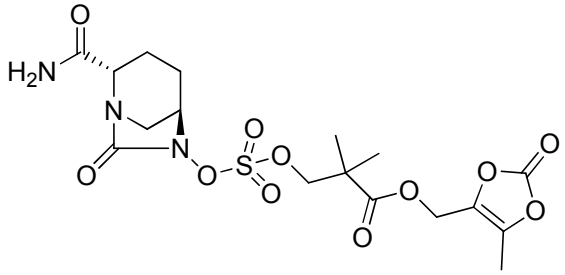
STEP 2: PREPARATION OF (5-METHYL-2-OXO-1,3-DIOXOL-4-YL)METHYL 3-((CHLOROSULFONYL)OXY)-2,2-DIMETHYLPROPANOATE



A solution of distilled sulfuryl chloride (0.61 mL, 7.53 mmol) in Et_2O (15 mL) was cooled to -78 °C under nitrogen. A solution of (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 3-hydroxy-2,2-dimethylpropanoate (1.48 g, 6.43 mmol) in Et_2O (1 mL) was added. Subsequently, a solution of pyridine (0.55 mL, 6.86 mmol) in Et_2O (1 mL) was added over a period of 1 h. The reaction was stirred at -78 °C for 1 h. After the mixture was filtered, the filtrate was concentrated under vacuum to give the product as a yellow oil (1.6 g, yield 76%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.90 (s, 2H), 4.49 (s, 2H), 2.19 (s, 3H), 1.33 (s, 6H)

STEP 3: PREPARATION OF (5-METHYL-2-OXO-1,3-DIOXOL-4-YL)METHYL 3-(((((2S,5R)-2-CARBAMOYL-7-OXO-1,6-DIAZABICYCLO[3.2.1]OCTAN-6-YL)OXY)SULFONYL)OXY)-2,2-DIMETHYLPROPANOATE (17)



(2*S*,5*R*)-6-Hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide (0.22 g, 1.2 mmol) was dissolved in THF (10 mL) and HMPA (0.5 mL), and the resulting stirred solution was cooled to -78 °C under an atmosphere of argon. A solution of NaHMDS, 1.0 M in THF (1.3 mL, 1.3 mmol) was added to the mixture, and the mixture stirred for 10 min. A solution of (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 3-((chlorosulfonyl)oxy)-2,2-dimethylpropanoate (0.42 g, 1.3 mmol) in THF (5 x 1 mL) was added quickly to the reaction mixture. After 10 min stirring at -78 °C, the mixture was allowed to warm to rt and stirred for 1 h. The mixture was cooled to 0 °C and quenched with H_2O and diluted with EtOAc (40 mL). The aqueous and organic layers were separated, and the organic layer was washed with brine, dried (Na_2SO_4), and concentrated under vacuum. The residue was purified by preparative HPLC to give the desired product (189 mg, 34%) as a solid.

LCMS: *m/z* = 478.1 $[M+H]^+$

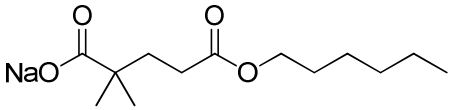
^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.68 (br. s, 1H), 5.74 (br. s, 1H), 4.95-4.79 (m, 3H), 4.50 (d, *J* = 9.3 Hz, 1H), 4.14 (br. s, 1H), 4.03 (d, *J* = 7.2 Hz, 1H), 3.32 (d, *J* = 12.3 Hz, 1H), 3.02 (d, *J* = 12.3 Hz, 1H), 2.45-2.39 (m, 1H), 2.17-2.09 (m, 4H), 1.98-1.79 (m, 2H), 1.30 (s, 3H), 1.29 (s, 3H)

^{13}C-NMR (75 MHz, $CDCl_3$): δ177.6, 171.0, 167.0, 152.2, 140.5, 133.2, 80.0, 61.8, 60.2, 54.4, 47.0, 43.0, 21.8, 21.7, 20.7, 17.5, 9.3

HRMS (ESI): $[M+H]^+$ calc'd for $C_{17}H_{23}N_3O_{11}S$ *m/z* 478.1132 found 411.1128

PREPARATION OF PRODRUG (18)

STEP 1: PREPARATION OF SODIUM 5-(HEXYLOXY)-2,2-DIMETHYL-5-OXOPENTANOATE[5]



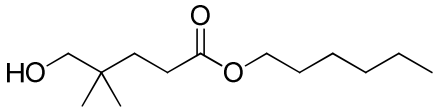
Reference is made to Kong, X.; Levens, N.; Bouzide, A.; Ciblat, S.; Frenette, R.; Renaud, J. Methods, compounds, and compositions for delivering 1,3-propanedisulfonic acid. PCT WO 2011017800; *Chem. Abstr.* **2011**, *154*, 259094[5]

To a solution of 2,2-dimethylglutaric anhydride (5.0 g, 35.2 mmol) in 1-hexanol (50 mL) was added a solution of sodium hexan-1-olate (5.4 g, 43.5 mmol) in 1-hexanol. After 20 h of stirring, the solvent was removed under vacuum, and the resulting solid was suspended in diethyl ether (80 mL). The mixture was filtered and the solid was washed with diethyl ether (2 x 40 mL). The solid was dried under high vacuum to afford the product (3.84 g, 41%) as a solid.

^{1}H-NMR (300 MHz, D_2O): δ 4.14 (t, J = 6.5 Hz, 2H), 2.38-2.33 (m, 2H), 1.82-1.77 (m, 2H), 1.75-1.63 (m, 2H), 1.43-1.28 (m, 6H), 1.12 (s, 6H), 0.92-0.88 (m, 3H)

The spectrum revealed that the product was contaminated with a small amount of an unidentified substance.

STEP 2: PREPARATION OF HEXYL 5-HYDROXY-4,4-DIMETHYLPENTANOATE[5]

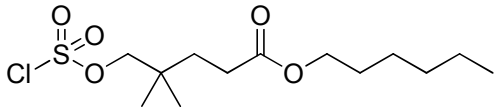


Reference is made to Kong, X.; Levens, N.; Bouzide, A.; Ciblat, S.; Frenette, R.; Renaud, J. Methods, compounds, and compositions for delivering 1,3-propanedisulfonic acid. PCT WO 2011017800; *Chem. Abstr.* **2011**, *154*, 259094[5]

To a suspension of sodium 5-(hexyloxy)-2,2-dimethyl-5-oxopentanoate (3.84 g, 14.4 mmol) in a mixture of THF (31 mL) and DMF (10 mL) was added isopropyl chloroformate, 1.0M in toluene (21.6 mL, 21.6 mmol) at 0° C and stirred for 10 min. After 3.3 h of stirring at room temperature, the solution was cooled to 0 °C and sodium borohydride (0.98 g, 28.8 mmol) was added. The mixture was stirred for 20 min then MeOH (5.2 mL) was added to the solution (reaction monitored by TLC using

2:8 EtOAc / hexanes as eluent). After 15 min a few drops of triethylamine were added. After another 15 min of stirring, EtOAc (25 mL) and a solution of saturated aqueous NH_4Cl was added (25 mL). The organic and aqueous layers were separated, and the aqueous layer was extracted with EtOAc (2 x 40 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using EtOAc / hexanes modified with 0.1% Et_3N (5:95 to 3:7) to give the product (2.16 g, 65%) as a colorless oil. One drop of Et_3N was added to suppress lactonization.

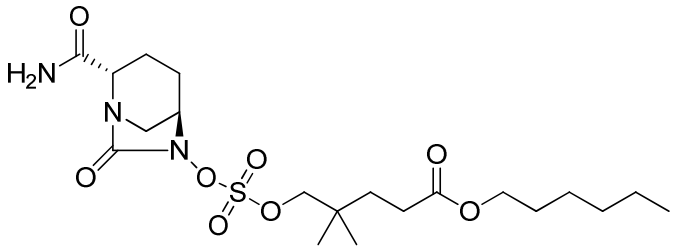
STEP 3: PREPARATION OF HEXYL 5-((CHLOROSULFONYL)OXY)-4,4-DIMETHYLPENTANOATE



A solution of sulfuryl chloride (0.38 mL, 5.2 mmol) in Et_2O (8.5 mL) was cooled to -78 °C under an atmosphere of nitrogen. A solution of hexyl 5-hydroxy-4,4-dimethylpentanoate (0.60 g, 2.6 mmol) and pyridine (0.42 mL, 5.2 mmol) in Et_2O (8.5 mL) was added dropwise to the sulfuryl chloride solution over the course of 10 min. The syringe was rinsed with Et_2O (3x 1 mL) and this was also added to the mixture. The mixture was stirred for 4.5 h (reaction monitored by TLC using 2:8 EtOAc / hexanes as eluent). The solids were filtered off and the solvent was concentrated *in vacuo* to give the product as a colorless oil - yield assumed quantitative. To this was added 3 mL of THF and the solution was stored at -78 °C. This was used in the next step without further purification.

See next page for Step 4

STEP 4: PREPARATION OF HEXYL 5-(((((2*S*,5*R*)-2-CARBAMOYL-7-OXO-1,6-DIAZABICYCLO[3.2.1]OCTAN-6-YL)OXY)SULFONYL)OXY)-4,4-DIMETHYLPENTANOATE (18)



(2*S*,5*R*)-6-hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide (370 mg, 2.0 mmol) was dissolved in tetrahydrofuran (19 mL) and HMPA (0.8 mL), and the resulting solution was cooled to -78 ^{o}C under an atmosphere of argon. A solution of NaHMDS, 1.0 M in THF (2.2 mL, 2.2 mmol) was added dropwise to the cooled solution and stirred for 10 min. A solution of hexyl 5-((chlorosulfonyl)oxy)-4,4-dimethylpentanoate (0.72 g, 2.2 mmol) in THF (3 mL) was cooled to -78 °C and quickly added to the reaction mixture. The flask containing the sulfating reagent was rinsed with THF (3 x 1 mL), while the flask temperature was maintained at -78 °C, and this was added quickly to the reaction mixture. After stirring for 10 min the mixture was warmed to rt and stirred overnight. The reaction was quenched with saturated $NaHCO_3$ (40 mL) and extracted with EtOAc (40 mL). The organic layer was concentrated, and the oily residue partitioned between H_2O (40 mL) and EtOAc (40 mL). The organic layer was washed with brine, dried ($MgSO_4$), filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel using EtOAc / hexanes as eluent (1:9 to 8:2) to give the product (421 mg, 44%) as a solid.

LC-MS: *m/z* = 478.0 $[M+H]^+$

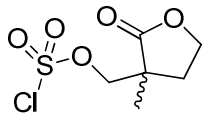
^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.48 (s, 1H), 5.59 (s, 1H), 4.51 (d, *J* = 8.7 Hz, 1H), 4.22-4.18 (m, 2H), 4.08-4.04 (m, 3H), 3.36-3.32 (m, 1H), 3.02 (d, *J* = 12.6 Hz, 1H), 2.47-2.41 (m, 1H), 2.33-2.28 (m, 2H), 2.18-2.13 (m, 1H), 2.01-1.79 (m, 2H), 1.72-1.59 (m, 4H), 1.35-1.31 (m, 6H), 0.99 (s, 6H), 0.91-0.87 (m, 3H)

^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.6, 170.9, 167.1, 83.5, 64.9, 62.0, 60.2, 47.3, 34.3, 33.3, 31.6, 29.3, 28.7, 25.7, 23.6, 23.3, 22.7, 20.9, 17.6, 14.1

HRMS (ESI): $[M+H]^+$ calc'd for $C_{20}H_{35}N_3O_8S$ *m/z* 478.2223, found 478.2202

PREPARATION OF PRODRUG (19)

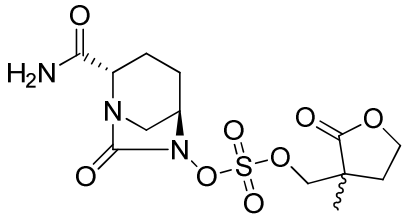
STEP 1: PREPARATION OF *R*/*S*-(3-METHYL-2-OXOTETRAHYDROFURAN-3-YL)METHYL SULFOCHLORIDATE[6]



Reference is made to Soengas, R. G.; Estevez, A. M. Convenient procedure for the indium-mediated hydroxymethylation of active bromo compounds: transformation of ketones into α-hydroxymethyl nitroalkanes. *Synlett* **2010**, 2625-2627[6]

Pyridine (0.28 mL, 3.5 mmol) was added to a stirred mixture of 3-(hydroxymethyl)-3-methyldihydrofuran-2(3*H*)-one[6] (0.30 g, 2.3 mmol) and Et_2O (8 mL) under an atmosphere of argon. The solution was cooled to -78 °C and sulfuryl chloride (0.28 mL, 3.5 mmol) in Et_2O (3 mL) was slowly added at -78 °C. The mixture was stirred at -78 °C for 1 h and then warmed to room temperature, and stirred for 1 h. The reaction mixture was filtered to remove the pyridine salt, and the filtrate was concentrated under vacuum to give an oil, that was used directly in the next step without further purification (yield assumed quantitative).

STEP 2: PREPARATION OF (2*S*,5*R*)-2-CARBAMOYL-7-OXO-1,6-DIAZABICYCLO[3.2.1]OCTAN-6-YL ((3-METHYL-2-OXOTETRAHYDROFURAN-3-YL)METHYL) SULFATE (19)



To a stirred mixture of (2*S*,5*R*)-6-hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide (0.24 g, 1.3 mmol) in THF (20 mL) under an atmosphere of argon was added several drops of 1,3-dimethyltetrahydropyrimidin-2(1*H*)-one. The mixture was cooled to -78 °C and stirred for 10 min, then a solution of NaHMDS, 1.0M in THF (1.4 mL, 1.4 mmol) was added dropwise. The mixture was stirred at -78 °C for 8 min, then (3-methyl-2-oxotetrahydrofuran-3-yl)methyl sulfochloridate (0.30 g, 1.3 mmol) in THF (30

mL) was added at -78 °C. The mixture was stirred at -78 °C for 10 min, then allowed to warm to room temperature and stirred overnight. The mixture was diluted with EtOAc and saturated sodium bicarbonate solution. The aqueous and organic layers were separated, and the organic layer was washed with water, dried (Na_2SO_4), and concentrated under vacuum. The residue was purified by column chromatography on silica gel using EtOAc / hexanes (0: 1 to 1:0) as eluent to give a solid (150 mg). NMR indicated a trace impurity, which was removed by trituration with EtOAc to give the product (35 mg) as a solid.

Note: In the ^{13}C spectrum there is a noticeable doubling of peaks reflecting the fact that compound **19** is a mixture of diastereomers.

LC-MS: 378.2 $[M+H]^+$

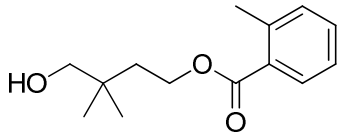
^1H-NMR (300 MHz, d_6-DMSO): δ 7.55 (s, 1H), 7.40 (s, 1H), 4.69 (dd, J = 2.4, 9.3 Hz, 1H), 4.57 (d, J = 9.9 Hz, 1H), 4.32 (t, J = 6.5 Hz, 2H), 4.11 (s, 1H), 3.92 (d, J = 6.6 Hz, 1H), 3.19 (s, 2H), 2.40-2.31 (m, 1H), 2.15-1.66 (m, 5H), 1.23 (s, 3H)

^{13}C-NMR (75 MHz, d_6-DMSO): δ 177.9, 177.8, 170.6, 170.6, 168.2, 168.1, 78.0, 77.5, 65.2, 61.3, 60.8, 60.7, 45.85, 45.8, 42.8, 30.8, 30.7, 20.4, 18.7, 18.5

HRMS (ESI): $[M+H]^+$ calc'd for $C_{13}H_{19}N_3O_8S$ *m/z* 378.0971, found 378.0982

PREPARATION OF PRODRUG (20)

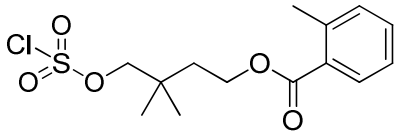
STEP 1: PREPARATION OF 4-HYDROXY-3,3-DIMETHYLBUTYL 2-METHYLBENZOATE



To a stirred solution of 2,2-dimethylbutane-1,4-diol (0.80 g, 6.8 mmol) in pyridine (5 mL) at *ca*. 0 °C (ice bath) under an atmosphere of argon, was added toluoyl chloride (0.89 mL, 6.8 mmol), was added dropwise. The reaction mixture was allowed to gradually warm to rt and the mixture was stirred for 4h. The mixture was concentrated under vacuum and suspended in EtOAc, then filtered and the filter cake washed with EtOAc. The filtrate was concentrated under vacuum and the residue purified by column chromatography on silica gel using EtOAc / hexanes (0:1 to 3:7) as eluent to give the desired product (0.7 g, 44%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.88 (d, *J* = 8.4 Hz, 1H), 7.40 (t, *J* = 7.1 Hz, 1H), 7.26-7.24 (m, 2H), 4.38 (t, *J* = 7.3 Hz, 2H), 3.41 (s, 3H), 2.60 (s, 3H), 1.78 (t, *J* = 7.5 Hz, 2H), 0.98 (s, 6H)

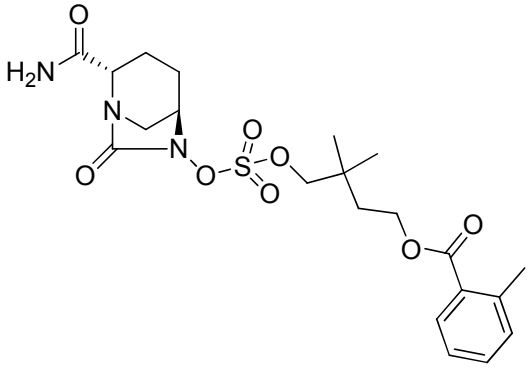
STEP 2: PREPARATION OF 4-((CHLOROSULFONYL)OXY)-3,3-DIMETHYLBUTYL 2-METHYLBENZOATE



A solution of freshly distilled sulfuryl chloride (96 μL, 1.3 mmol) in Et_2O (0.8 mL) was cooled to -78 °C under an atmosphere of argon. A solution of 4-hydroxy-3,3-dimethylbutyl 2-methylbenzoate (0.31 g, 1.3 mmol) and pyridine (106 μL, 1.3 mmol) in Et_2O (1.1 mL) was added dropwise to the sulfuryl chloride solution over the course of 15 min. The flask was rinsed with Et_2O (2 x 20 mL), which was added to the reaction mixture. The mixture was stirred at -78 °C for 10 min then allowed to warm to rt and stirred for 30 min. The mixture was filtered, and the filtrate was used immediately in the next step without further purification.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.89 (d, *J* = 8.1 Hz, 1H), 7.41-7.39 (m,1H), 7.26-7.25 (m, 2H), 4.41-4.35 (m, 2H), 4.28 (s, 2H), 2.61 (s, 3H), 1.87 (t, *J* = 7.2 Hz, 2H), 1.13 (s, 6H)

STEP 3: PREPARATION OF 5-(((((2*S*,5*R*)-2-CARBAMOYL-7-OXO-1,6-DIAZABICYCLO[3.2.1]OCTAN-6-YL)OXY)SULFONYL)OXY)-4,4-DIMETHYLPENTYL 2-METHYLBENZOATE (20)



(2*S*,5*R*)-6-Hydroxy-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxamide (0.22 g, 1.2 mmol) was dissolved in THF (10 mL) and HMPA (0.5 mL), and the resulting stirred solution was cooled to -78 °C under an atmosphere of argon. A solution of NaHMDS, 1.0 M in THF (1.2 mL, 1.2 mmol) was added to the mixture, and the mixture stirred for 10 min. A solution of 4-((chlorosulfonyl)oxy)-3,3-dimethylbutyl 2-methylbenzoate (0.42 g, 1.3 mmol) in Et_2O (20 mL) was added quickly to the reaction mixture. After 10 min stirring at -78 °C, the mixture was allowed to warm to rt and stirred for 1 h. The mixture was cooled to 0 °C and quenched with H_2O and diluted with EtOAc. The aqueous and organic layers were separated, and the organic layer was washed with brine, dried (Na_2SO_4), and concentrated under vacuum. The residue was purified by column chromatography on silica gel using EtOAc / hexanes (0:1 to 7:3) as eluent to give the product (231 mg, 40%) as a solid.

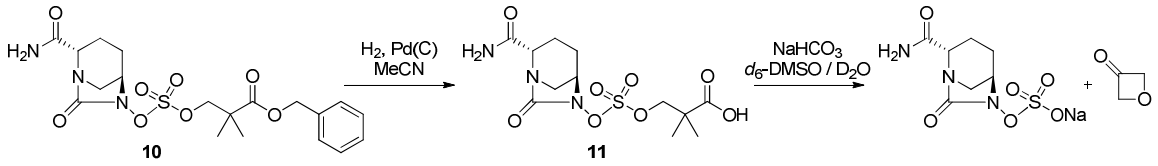
LCMS: *m/z* = 484.06 $[M+1]^+$

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.90 (d, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.26-7.24 (m, 2H), 6.44 (br. s, 1H), 5.53 (br. s, 1H), 4.60 (d, *J* = 8.7 Hz, 1H), 4.35 (t, *J* = 7.1 Hz, 2H), 4.28 (d, *J* = 8.7 Hz, 1H), 4.17 (br. s, 1H), 4.03 (d, *J* = 7.2 Hz, 1H), 3.33 (d, *J* = 12.3 Hz, 1H), 2.99 (d, *J* = 11.7 Hz, 1H), 2.60 (s, 3H), 2.47-2.40 (m, 1H), 2.18-2.14 (m, 1H), 1.95-1.82 (m, 4H), 1.10 (s, 6H)

^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.0, 167.5, 167.1, 140.5, 132.2, 131.9, 130.7, 129.5, 125.9, 83.7, 62.0, 61.2, 60.2, 47.2, 36.9, 34.0, 24.1, 23.8, 21.9, 20.8, 17.5

HRMS (ESI): $[M+H]^+$ calc'd for $C_{21}H_{29}N_3O_8S$ *m/z* 484.1754, found 484.1750

RELEASE OF PIVALOLACTONE AND AVIBACTAM FROM COMPOUND (10)



A stock solution of $NaHCO_3$ (7.9 mg, 94 μmol) dissolved in D_2O was prepared.

To a 4 dram vial was added benzyl 3-(((((2*S*, 5*R*)-2-carbamoyl-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl)oxy)sulfonyl)oxy)-2,2-dimethylpropanoate (**10**) (11.1 mg, 24 μmol), and this was dissolved in MeCN (1.11 mL). The mixture was degassed and purged with nitrogen. To this was added palladium on carbon (10% Pd by weight; 2.7 mg), and the mixture was degassed and purged with hydrogen (3 cycles). The mixture was stirred under nitrogen at rt for 80 min, filtered through a 0.2 μm PTFE syringe filter, concentrated, and dissolved in d_6-DMSO (0.5 mL). This solution was analyzed by ^{1}H-NMR 10 min after the hydrogenation was completed. The mixture was then treated with 25 μL of the $NaHCO_3$ solution in D_2O (*ca.* 0.97 equiv. of $NaHCO_3$) to mimic physiological conditions. The mixture was then analyzed by ^{1}H-NMR immediately after adding the $NaHCO_3$ solution (t = 30 seconds).

A stock solution of pivalolactone in d_6-DMSO was also prepared by dissolving 28.3 mg of pivalolactone in 600 μL of d_6-DMSO (concentration = 47.2 μg/μL) and was stored at -20 °C until use. To confirm the presence of pivalolactone after treatment with $NaHCO_3$, the ^{1}H-NMR was re-run, but with the sample spiked with 50 μL of a pivalolactone stock solution. The following Figures detail the ^{1}H-NMR spectra.

Figure S1. Spectrum of compound (**10**) in d_6-DMSO.

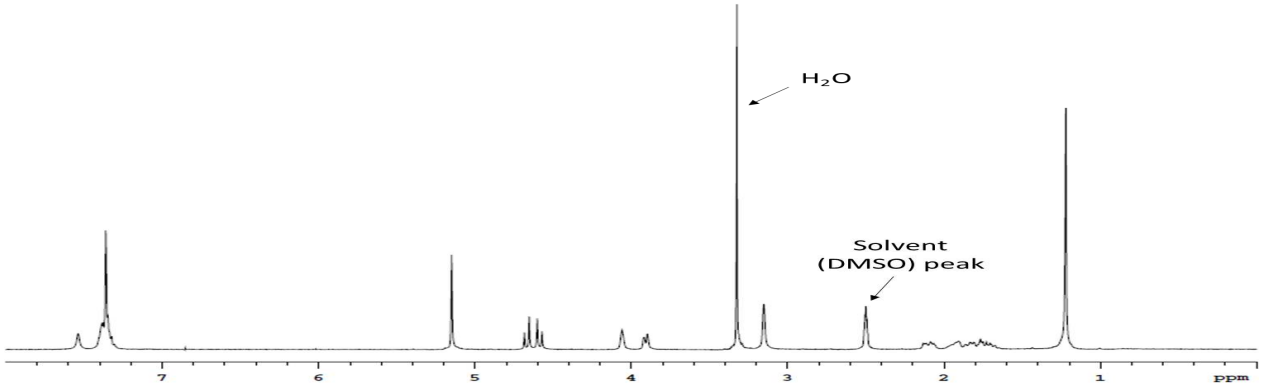
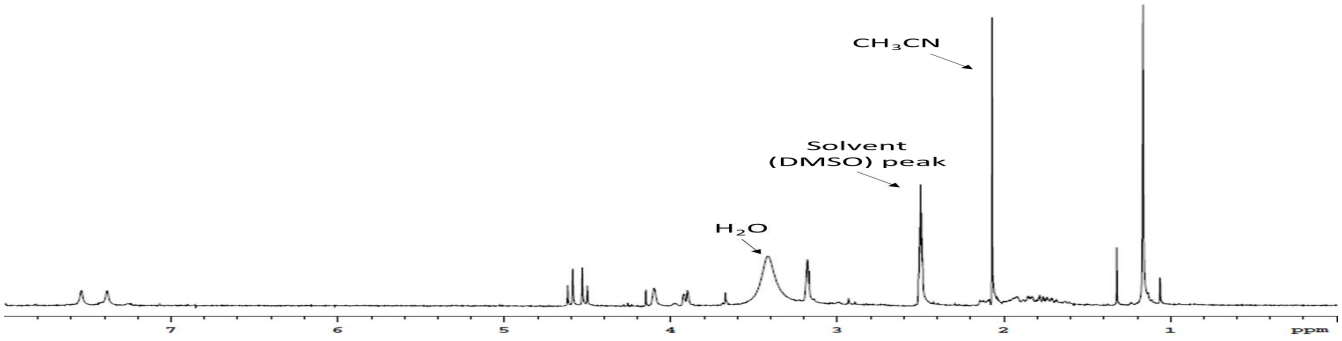
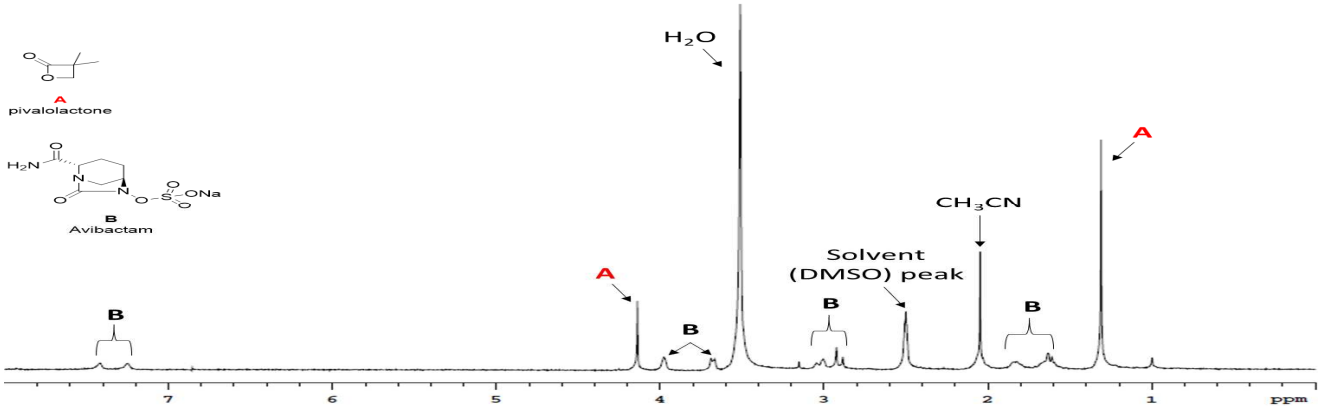


Figure S2.

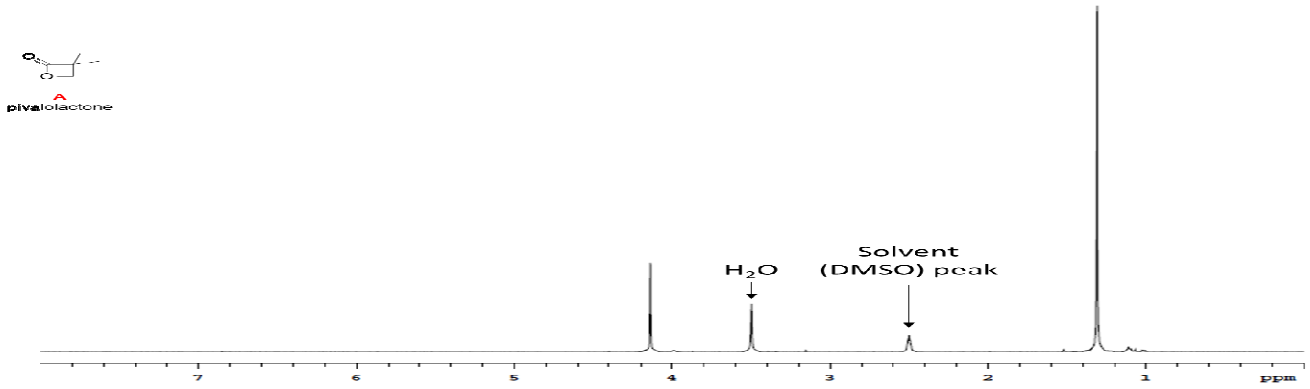
A. ^{1}H-NMR spectrum after hydrogenation in d_6-DMSO (residual CH_3CN - solvent for the hydrogenation - also present).



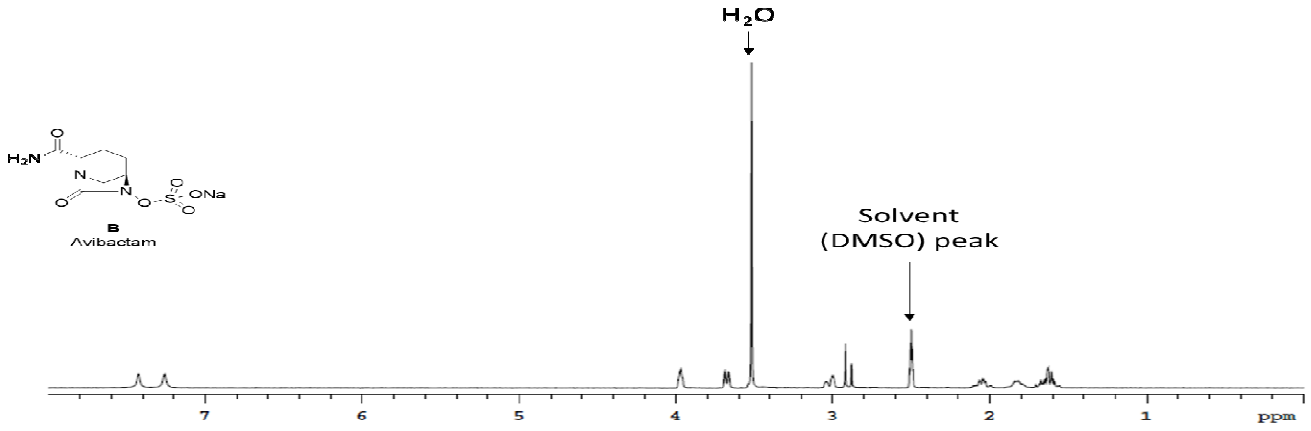
B. ^{1}H-NMR spectrum of mixture after treatment with $NaHCO_3$ in d_6-DMSO / D_2O (95:5). The spectrum reveals the release of pivalolactone and Avibactam from the acid (**11**).



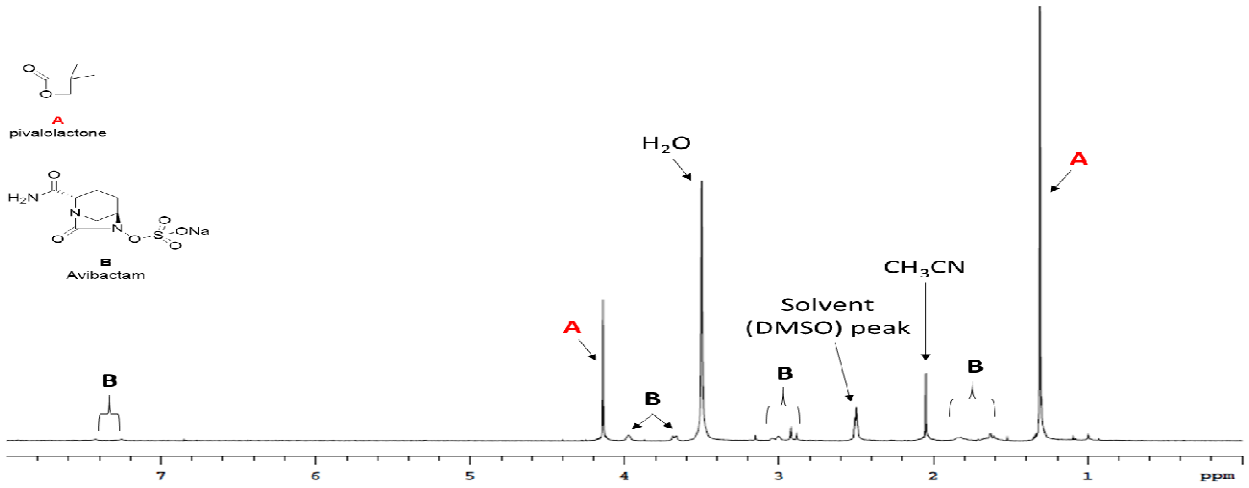
C. ^{1}H-NMR spectrum of pivalolactone in d_6-DMSO : D_2O (95:5).



D. ^{1}H-NMR spectrum of Avibactam in d_6-DMSO : D_2O (95:5).

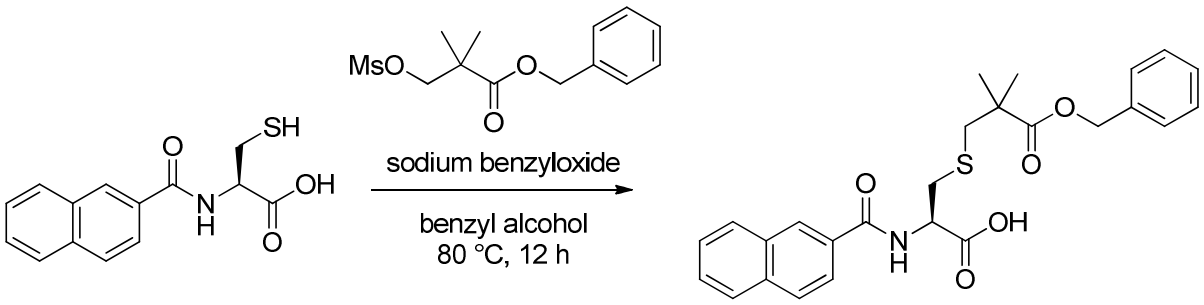


E. ^{1}H-NMR spectrum of mixture after treatment with $NaHCO_3$, then spiked with additional pivalolactone.



REACTION OF COMPOUND (10) WITH AN *N*-ACYLCYSTEINE (MIMIC OF A BIOLOGICAL NUCLEOPHILE)

PREPARATION OF (*R*)-2-(2-NAPHTHAMIDO)-3-((3-(BENZYLOXY)-2,2-DIMETHYL-3-OXOPROPYL)THIO)PROPANOIC ACID - USED AS AN AUTHENTIC STANDARD



Reference is made to Katrizky, A. R.; Tala, S. R.; Abo-Dya, N. E.; Gyanda, K.; El-Gendy, B. E-D. M.; Abdel-Samii, Z. K.; Steel, P. J. Selective synthesis and structural elucidation of *S*-acyl- and *N*-acylcysteines. *J. Org. Chem.* **2009,** *74,* 7165-7167[7]

To a solution of benzyl alcohol (2.3 mL) was added a 1.0 M solution of sodium phenylmethanolate (1.8 mL, 1.8 mmol) and the solution was degassed. To this was added (*R*)-2-(2-naphthamido)-3-mercaptopropanoic acid[7] (0.50 g, 1.8 mmol) followed by benzyl 2,2-dimethyl-3-((methylsulfonyl)oxy)propanoate (255 mg, 0.9 mmol). The suspension was stirred at 80 °C for 12 h. The reaction was quenched with water (10 mL), acidified with 1 N HCl (20 mL), extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried (Na_2SO_4), and concentrated under vacuum to leave a crude oil. The oil was purified by column chromatography on silica gel (9:1 to 1:1 ethyl acetate / hexanes) and the product obtained was purified by preparative reverse-phase HPLC (2:8 to 9:1 acetonitrile / H_2O + 0.1% formic acid) to give the title compound (132 mg) as an oil after drying *in vacuo* at 40 °C overnight.

LC-MS: 465.0 $[M-H]^-$

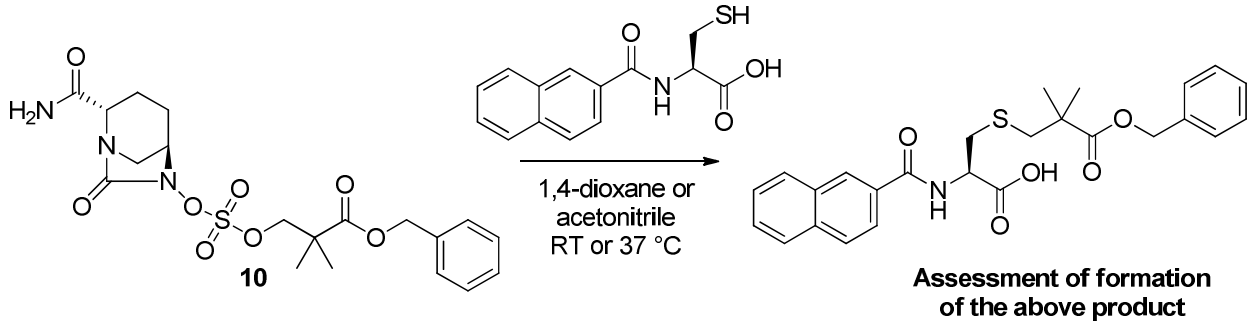
^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.38 (s, 1H), 7.92-7.84 (m, 4H), 7.60-7.50 (m, 2H), 7.36-7.24 (m, 6H), 5.10 (s, 2H), 5.06-5.00 (m, 1H), 3.17 (d, *J* = 5.4 Hz, 2H), 2.93-2.82 (m, 2H), 1.26 (s, 3H), 1.25 (s, 3H)

^{13}C-NMR (75 MHz, $CDCl_3$): δ 176.5, 173.6, 168.2, 135.9, 135.1, 132.7, 130.4, 129.2, 128.7, 128.6, 128.3, 128.11, 128.1, 127.9, 127.0, 123.7, 66.8, 53.0, 44.4, 43.5, 35.6, 25.1, 24.8

^{1}H-NMR (300 MHz, d_6-DMSO): δ 8.88 (d, J = 8.1 Hz, 1H), 8.48 (s, 1H), 8.03-7.92 (m, 4H), 7.65-7.56 (m, 2H), 7.33-7.24 (m, 5H), 5.06 (s, 2H), 4.64-4.56 (m, 1H), 3.08-2.92 (m, 2H), 2.84 (s, 2H), 1.19 (s, 3H), 1.18 (s, 3H)

^{13}C-NMR (75 MHz, d_6-DMSO): δ 175.5, 172.1, 166.4, 136.2, 134.2, 132.1, 131.2, 128.9, 128.4, 127.92, 127.9, 127.7, 127.65, 127.6, 126.8, 124.2, 65.7, 53.1, 43.6, 42.2, 34.6, 24.5, 24.3

REACTION OF COMPOUND (10) WITH (*R*)-2-(2-NAPHTHAMIDO)-3-MERCAPTOPROPANOIC ACID: ASSESSMENT OF FORMATION OF (*R*)-2-(2-NAPHTHAMIDO)-3-((3-(BENZYLOXY)-2,2-DIMETHYL-3-OXOPROPYL)THIO)PROPANOIC ACID BY HPLC AND LC-MS



Benzyl 3-(((((2*S*,5*R*)-2-carbamoyl-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl)oxy)sulfonyl)oxy)-2,2-dimethylpropanoate (6 mg, 13 μmol) and (*R*)-2-(2-naphthamido)-3-mercaptopropanoic acid (4 mg, 15 μmol) were dissolved in a degassed anhydrous solvent [1,4-dioxane (stabilized with 2-5 ppm BHT) or acetonitrile] under an inert atmosphere of nitrogen. The mixture was degassed again and stirred at ambient temperature for 48h, or 37 °C for 24h. From this mixture 200 μL aliquots were removed and 20 μL were sampled by HPLC (10-90% ACN / H_2O + 0.1% TFA added to the aqueous solvent) at t = 0, 1h, 2h, 4h, 8h, and 24h (and 48h for reaction at ambient temperature). Another 20 μL sample of the t = 24h sample was analyzed by LC-MS [10-100% (solvent A: 9:1 acetonitrile-water + 0.1% formic acid)/(solvent B: water + 0.1% formic acid)].

Conclusions:

No (*R*)-2-(2-naphthamido)-3-((3-(benzyloxy)-2,2-dimethyl-3-oxopropyl)thio)propanoic acid was observed in the sample by HPLC, or LC-MS at any timepoint up to t = 24h (ambient, or 37 °C).

No (*R*)-2-(2-naphthamido)-3-((3-(benzyloxy)-2,2-dimethyl-3-oxopropyl)thio)propanoic acid was detected by HPLC, or LC-MS, after 48h in the sample stirred at ambient temperature.

Note 1: A small amount of dissulfide (dimerization of starting sulfide - (*R*)-2-(2-naphthamido)-3-mercaptopropanoic acid) was observed to have formed over the course of the experiment by HPLC and LC-MS.

Note 2: The 24h and 48h timepoints were separately co-injected with an authentic sample of (*R*)-2-(2-naphthamido)-3-((3-(benzyloxy)-2,2-dimethyl-3-oxopropyl)thio)propanoic acid (concentration = 30 μM), to make sure no suppression of ionization occurred in the samples analyzed by LC-MS.

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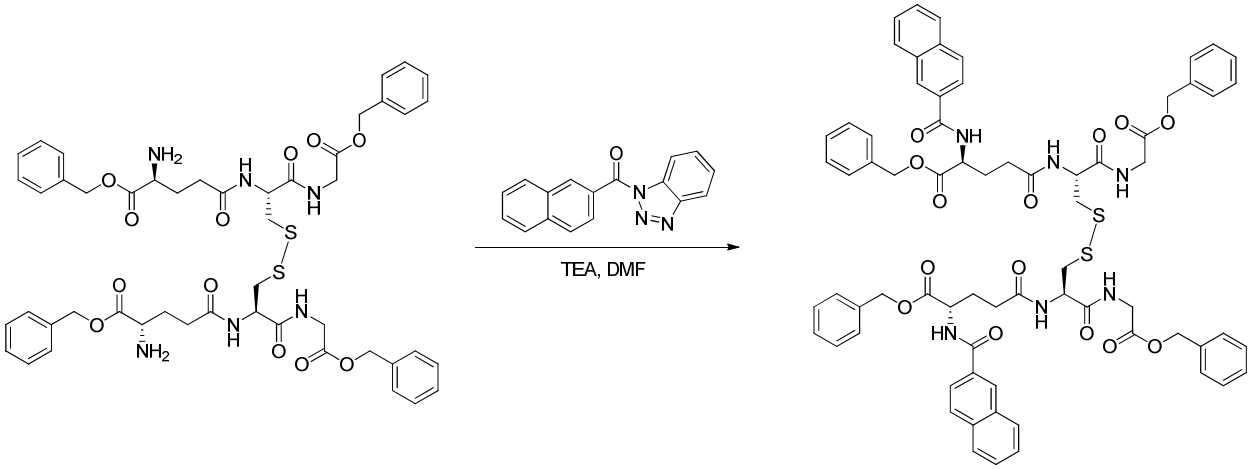
REACTION OF COMPOUND (10) WITH AN *N*-ACYLGLUTATHIONE (MIMIC OF A BIOLOGICAL NUCLEOPHILE)

PREPARATION OF (*S*)-BENZYL 2-(2-NAPHTHAMIDO)-5-(((*R*)-11,11-DIMETHYL-3,6,12-TRIOXO-1,14-DIPHENYL-2,13-DIOXA-9-THIA-5-AZATETRADECAN-7-YL)AMINO)-5-OXOPENTANOATE - USED AS AN AUTHENTIC STANDARD

STEP 1: PREPARATION OF (3*S*, 8*R*, 13*R*, 18*S*)-BENZYL 18-(2-NAPHTHAMIDO)-8,13-BIS((2-(BENZYLOXY)-2-OXOETHYL)CARBAMOYL)-3-((BENZYLOXY)CARBONYL)-1-(NAPHTHALEN-2-YL)-1,6,15-TRIOXO-10,11-DITHIA-2,7,14-TRIAZANONADECAN-19-OATE



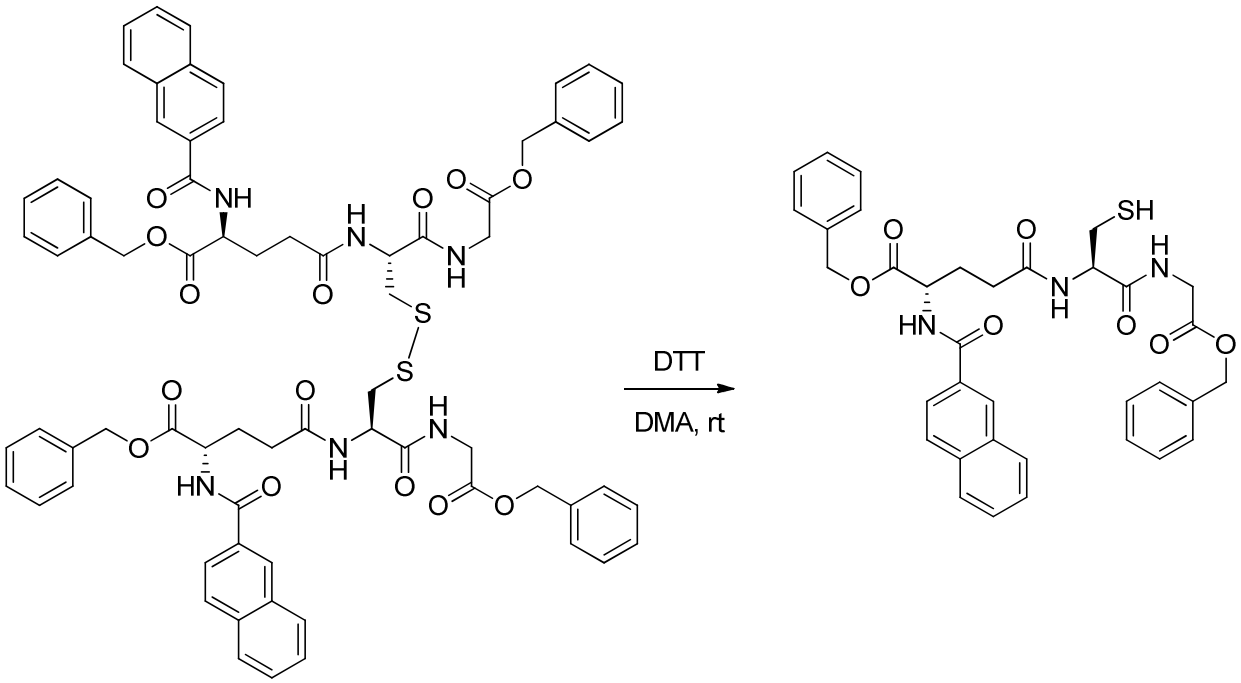
Reference is made to Katrizky, A. R.; Tala, S. R.; Abo-Dya, N. E.; Gyanda, K.; El-Gendy, B. E-D. M.; Abdel-Samii, Z. K.; Steel, P. J. Selective synthesis and structural elucidation of *S*-acyl- and *N*-acylcysteines. *J. Org. Chem.* **2009,** *74,* 7165-7167[7] and to Gatterdam, V.; Stoess, T.; Menge, C.; Heckel, A.; Tampé, R. Caged glutathione – triggering protein interaction by light. *Angew. Chem., Int. Ed.* **2012**, *51*, 3960-3963[8]

To a solution of (2*S*,2'*S*)-dibenzyl-5,5'-(((7*R*,12*R*)-3,6,13,16-tetraoxo-1,18-diphenyl-2,17-dioxa-9,10-dithia-5,14-diazaoctadecane-7,12-diyl)bis(azanediyl))bis(2-amino-5-oxopentanoate)[8] (2.13 g, 2.2 mmol) and (1*H*-benzo[d][1,2,3]triazol-1-yl)(naphthalen-2-yl)methanone (1.19 g, 4.4 mmol) in DMF (40 mL) was added Et_3N (0.61 mL, 4.4 mmol). The mixture was stirred at rt overnight. The solvent was concentrated at 50 °C and the residue triturated with EtOAc (100 mL). The solids were collected by filtration, triturated with EtOAc (2 x 50 mL), and air dried to give the product (2.44 g, 81%) as a solid, which was used in the next step without further purification.

^{1}H-NMR (300 MHz, d_6-DMSO): δ 9.02 (d, J = 6.6 Hz, 1H), 8.51 (m, 2H), 8.29 (d, J = 7.5 Hz, 1H), 8.01-7.97 (m, 4H), 7.61 (m, 2H), 7.35-7.34 (m, 10H), 5.16 (s, 2H), 5.10 (s, 2H), 4.60 (m, 2H), 3.89-3.88 (fd, J = 4.2 Hz, 2H), 3.12-3.08 (m, 1H), 2.88-2.80 (m, 1H), 2.387-2.393 (m, 2H), 2.19-2.08 (m, 2H).

^{13}C-NMR (75 MHz, d_6-DMSO): δ 171.8, 171.8, 170.7, 169.4, 166.8, 136.0, 135.8, 134.3, 132.1, 131.1, 128.9, 128.4, 128.1, 128.0, 127.9, 127.9, 127.7, 126.8, 124.3, 65.9, 52.8, 51.6, 40.9, 31.8, 26.3

STEP 2: PREPARATION OF (*S*)-BENZYL 2-(2-NAPHTHAMIDO)-5-(((*R*)-1-((2-(BENZYLOXY)-2-OXOETHYL)AMINO)-3-MERCAPTO-1-OXOPROPAN-2-YL)AMINO)-5-OXOPENTANOATE



To a degassed suspension of (3*S*, 8*R*, 13*R*, 18*S*)-benzyl 18-(2-naphthamido)-8,13-bis((2-(benzyloxy)-2-oxoethyl)carbamoyl)-3-((benzyloxy)carbonyl)-1-(naphthalen-2-yl)-1,6,15-trioxo-10,11-dithia-2,7,14-triazanonadecan-19-oate (1.33 g, 0.9 mmol) in dimethylacetamide (20 mL) was added (2*S*, 3*S*)-1,4-dimercaptobutane-2,3-diol (271 mg, 1.8 mmol). The mixture was degassed again and stirred at rt. After 15 h of stirring a further aliquot of (2*S*, 3*S*)-1,4-dimercaptobutane-2,3-diol (271 mg, 1.8 mmol) was added again. After stirring for 26 h the mixture was diluted with H_2O (200 mL) and the

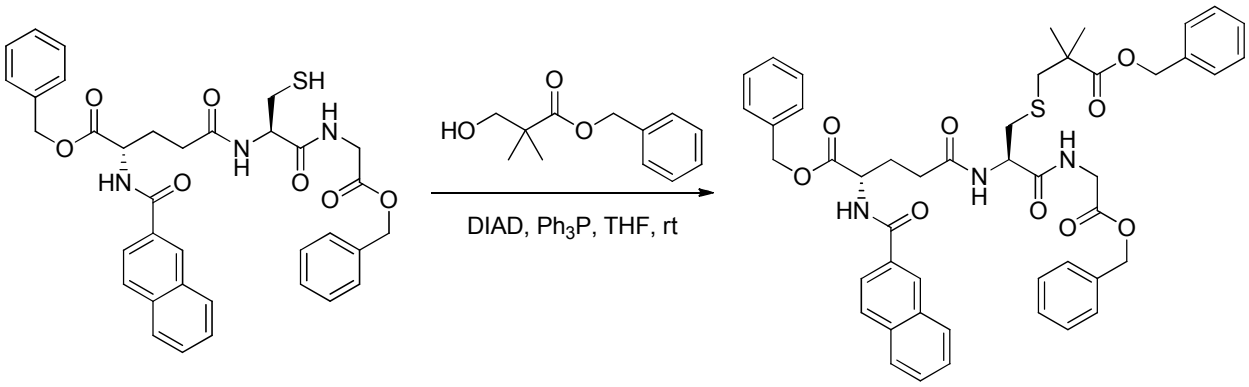
precipitate was collected by filtration. The solids were rinsed with H_2O (2 x 50 mL) and the filter cake was dried *in vacuo* at 60 °C for 1 h. The crude material was suspended in EtOAc (80 mL), washed with H_2O (6 x 50 mL, after which all the solids dissolved), brine (50 mL), dried (Na_2SO_4), and concentrated under vacuum. The residue was purified by column chromatography on silica gel using MeOH / DCM + 0.1% formic acid (0-1% MeOH / DCM + 0.1% formic acid) to give the product (0.83 g, 72%) as a solid, which was dried *in vacuo* at 45 °C.

LC-MS: 642.22 $[M+H]^+$, 687.18 $[M+HCO_2H]^+$

^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.99 (d, *J* = 7.2 Hz, 1H), 8.49-8.45 (m, 2H), 8.14 (d, *J* = 8.4 Hz, 1H), 8.03-7.93 (m, 4H), 7.64-7.56 (m, 2H), 7.38-7.27 (m, 10H), 5.16 (s, 2H), 5.09 (s, 2H), 4.56-4.53 (m, 1H), 4.47-4.40 (m, 1H), 3.89-3.86 (m, 2H), 2.80-2.70 (m, 1H), 2.67-2.57 (m, 1H), 2.38 (t, *J* = 7.5 Hz, 2H), 2.27-2.15 (m, 2H), 2.09-1.99 (m, 1H)

^{13}C-NMR (75 MHz, DMSO-d_6): δ 171.9, 171.7, 170.5, 169.5, 166.8, 136.0, 135.8, 134.3, 132.1, 131.1, 128.8, 128.4, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.66, 126.8, 124.3, 65.9, 54.8, 52.6, 40.8, 31.6, 26.2

STEP 3: PREPARATION OF (*S*)-BENZYL 2-(2-NAPHTHAMIDO)-5-(((*R*)-11,11-DIMETHYL-3,6,12-TRIOXO-1,14-DIPHENYL-2,13-DIOXA-9-THIA-5-AZATETRADECAN-7-YL)AMINO)-5-OXOPENTANOATE[9]



Reference is made to Lepore, S. D.; He, Y. Use of sonication for the coupling of sterically hindered substrates in the phenolic Mitsunobu reaction. *J. Org. Chem.* **2003,** *21*, 8261-8263[9]

To a 2 mL crimp seal vial was added benzyl 3-hydroxy-2,2-dimethylpropanoate (85 mg, 0.4 mmol) and triphenylphosphine (105 mg, 0.4 mmol) in tetrahydrofuran (104 uL). The mixture was lowered into a sonicating bath (42 kHz, Branson 3510) and sonicated for several minutes to allow for mixing under an inert atmosphere of nitrogen. While sonicating diisopropyl azodicarboxylate, DIAD (79 μl, 0.4 mmol) was added to form a viscous, orange solution. After 15 min the mixture was analyzed by TLC (1:4 EtOAc / hexanes) which revealed that the alcohol had been completely consumed. The mixture was sonicated for another 15 min, then (*S*)-benzyl 2-(2-naphthamido)-5-(((*R*)-1-((2-(benzyloxy)-2-oxoethyl)amino)-3-mercapto-1-oxopropan-2-yl)amino)-5-oxopentanoate (200 mg, 0.3 mmol) was added in one portion. The mixture was diluted with 0.5 mL of THF after sonicating for 30 min (note: this was done to further immerse the thiol in solvent). The mixture was sonicated overnight (note: the next morning, it was discovered that the solvent had evaporated). The mixture was diluted with 2 mL of THF and 1 mL of DMF. The light orange solution was degassed then set in the sonicating bath again. After sonicating for 1 h and 40 min, the mixture was concentrated giving an oil, that was dry-loaded on to silica gel and purified by column chromatography on silica gel using MeOH / DCM (1:0 to 4:96) as eluent to give a crude product, The product was re-purified by preparative-HPLC (50-90% ACN / H_2O+0.1% formic acid) to give the product (26 mg, 10%) as a solid.

LC-MS: *m/z* = 832.2 $[M+H]^+$, 877.2 $[M+HCO_2H]^+$, 876.0 $[M+HCO_2]^-$

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.37 (s, 1H), 7.93-7.83 (m, 4H), 7.65 (d, *J* = 6.9 Hz, 1H), 7.58-7.49 (m, 2H), 7.38-7.27 (m, 13H), 6.98-6.93 (m, 2H), 5.27-5.17 (m, 2H), 5.09 (s, 4H), 4.95-4.89 (m, 1H), 4.57 (q, *J* = 6.9 Hz, 1H), 3.97-3.81 (m, 2H), 2.99-2.87 (m, 2H), 2.78-2.70 (m, 2H), 2.46-2.19 (m, 4H), 1.23 (s, 6H)

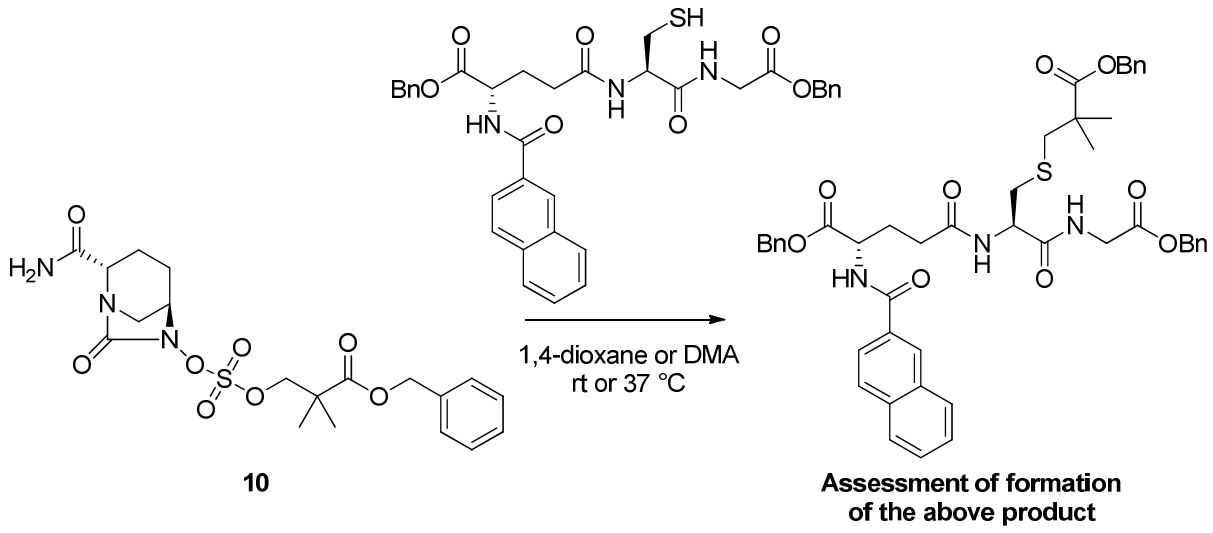
^{13}C-NMR (75 MHz, $CDCl_3$): δ 176.7, 172.8, 172.1, 170.5, 169.3, 167.6, 136.1, 135.4, 135.2, 135.0, 132.7, 130.9, 129.2, 128.8, 128.7, 128.6, 128.6, 128.5, 128.2, 128.1, 127.9, 127.8, 126.9, 123.8, 67.6, 67.3, 66.7, 52.9, 52.7, 44.2, 42.8, 41.5, 35.3, 32.4, 27.7, 25.3, 24.8

^{1}H-NMR (300 MHz, d_6-DMSO): δ 9.02 (d, *J* = 7.2 Hz, 1H), 8.56-8.51 (m, 2H), 8.19 (d, *J* = 9.0 Hz, 1H), 8.04-7.95 (m, 4H), 7.65-7.58 (m, 2H), 7.39-7.27 (m, 15H), 5.17 (s, 2H),

5.11 (s, 2H), 5.08 (s, 2H), 4.61-4.45 (m, 2H), 3.89 (d, J = 5.7 Hz, 2H), 2.85-2.77 (m, 3H), 2.64-2.56 (m, 1H), 2.40-2.35 (m, 2H), 2.22-2.02 (m, 2H), 1.15 (s, 6H)

^{13}C-NMR (75 MHz, d_6-DMSO): δ 175.4, 171.8, 171.5, 170.8, 169.4, 166.7, 136.2, 136.0, 135.8, 134.2, 132.0, 131.1, 128.8, 128.4, 128.0, 127.9, 127.9, 127.8, 127.6, 127.5, 126.7, 124.3, 65.9, 65.8, 65.6, 52.7, 52.4, 43.6, 42.0, 40.8, 35.8, 31.7, 26.3, 24.3, 24.2

REACTION OF COMPOUND (10) WITH (*S*)-BENZYL 2-(2-NAPHTHAMIDO)-5-(((*R*)-1-((2-(BENZYLOXY)-2-OXOETHYL)AMINO)-3-MERCAPTO-1-OXOPROPAN-2-YL)AMINO)-5-OXOPENTANOATE: ASSESSMENT OF FORMATION OF (*S*)-BENZYL 2-(2-NAPHTHAMIDO)-5-(((*R*)-11,11-DIMETHYL-3,6,12-TRIOXO-1,14-DIPHENYL-2,13-DIOXA-9-THIA-5-AZATETRADECAN-7-YL)AMINO)-5-OXOPENTANOATE BY HPLC AND LC-MS



10 **Assessment of formation of the above product**

To a 5 mL crimp-seal vial was added benzyl 3-(((((2*S*, 5*R*)-2-carbamoyl-7-oxo-1,6-diazabicyclo[3.2.1]octan-6-yl)oxy)sulfonyl)oxy)-2,2-dimethylpropanoate (6 mg, 13 μmol) and (*S*)-benzyl 2-(2-naphthamido)-5-(((*R*)-1-((2-(benzyloxy)-2-oxoethyl)amino)-3-mercapto-1-oxopropan-2-yl)amino)-5-oxopentanoate (9 mg, 14 μmol), which were dissolved in degassed 1,4-dioxane (4 mL, stabilized with 2-5 ppm BHT), or DMA, under an atmosphere of nitrogen. The mixture was thoroughly degassed again, and stirred at ambient temperature or 37 °C. From this mixture *ca.* 200 μL aliquots were sampled by HPLC (10-90% ACN / H_2O+0.1% TFA added to water; 20 μL injection) at t = 0, 1h, 2h, 4h, 8h, and 24h. The t = 24h undiluted sample was analyzed by LC-MS [30-100%

(solvent 1: 9:1 ACN / H_2O + 0.1% formic acid; solvent 2: H_2O + 0.1% formic acid); 20 μL injection].

Conclusions:

No (*S*)-benzyl 2-(2-naphthamido)-5-(((*R*)-11,11-dimethyl-3,6,12-trioxo-1,14-diphenyl-2,13-dioxa-9-thia-5-azatetradecan-7-yl)amino)-5-oxopentanoate was observed in the sample by HPLC, or LC-MS, at any timepoint up to t = 24h (ambient, or 37 °C).

Note 1: The 24h timepoints were separately co-injected with an authentic sample of (*S*)-benzyl 2-(2-naphthamido)-5-(((*R*)-11,11-dimethyl-3,6,12-trioxo-1,14-diphenyl-2,13-dioxa-9-thia-5-azatetradecan-7-yl)amino)-5-oxopentanoate (concentration = 30 μM), to make sure no suppression of ionization occurred in the samples analyzed by LC-MS.

STATEMENT REGARDING ANIMAL EXPERIMENTATION

Statement adapted from Eurofins for rat PK:

Male Sprague-Dawley (SD) rats weighing 250 to 300 g were provided by BioLasco Taiwan (under Charles River Laboratories License). Space allocation for three animals was 47 x 25 x 21 cm. All animals were maintained in a controlled temperature (20 - 24oC) and humidity (30% - 70%) environment with 12 hours light/dark cycles in Eurofins Panlabs Taiwan, Ltd. Laboratory. Free access to standard lab diet [MFG (Oriental Yeast Co., Ltd., Japan)] and autoclaved tap water were granted. All aspects of this work including housing, experimentation, and animal disposal were performed in general accordance with the "Guide for the Care and Use of Laboratory Animals: Eighth Edition" (National Academies Press, Washington, D.C., 2011)[10] in our AAALAC-accredited laboratory animal facility.[11] In addition, the animal care and use protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Eurofins Panlabs Taiwan, Ltd.

Note: AAALAC stands for The Association for Assessment and Accreditation of Laboratory Animal Care, International.

Statement adapted from Charles River Laboratories for dog / monkey PK:

Studies will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (Code of Federal Regulations, Title 9), the Public Health Service Policy on Humane Care and Use of Laboratory Animals from the Office of Laboratory Animal Welfare, and the Guide for the Care and Use of Laboratory Animals from the National Research Council.[10] The protocol and any amendments or procedures involving the care or use of animals will be reviewed and approved by the Testing Facility Institutional Animal Care and Use Committee (IUCAC) before the initiation of such procedures.

The Testing Facility's attending veterinarian is responsible for implementation of programs for the evaluation of the health status of study animals, the recommendation of treatment for health conditions, the evaluation of response to treatment, as well as the diagnosis of pain or distress. The veterinary staff will communicate to the Study Director signs of animal illness, injury, pain and distress, and recommendations on treatment of the animal(s) and/or alteration of study procedures. In nonemergency situations, decisions

regarding the study animals, including treatments, alterations in study design, and/or justification of action(s) will be documented, and will be approved in advance by the Study Director, and in consultation with the Sponsor as appropriate. If euthanasia is deemed necessary, it will be in accordance with the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia and with the procedures outlined in the protocol. The Sponsor will be fully informed of any such events.

If an animal is determined by the veterinary staff to be in overt pain / distress, or appears moribund and is beyond the point where recovery appears reasonable, the animal will be euthanized for humane reasons in accordance with the AVMA Guidelines on Euthanasia.

By approving the protocol, the Sponsor affirms that there are no acceptable non-animal alternatives for this study, that this study is required by a relevant government regulatory agency(ies) and that it does not unnecessarily duplicate any previous experiments.

PK WITH PRODRUGS (14) TO (20)

For rat PK:

Formulation:

Avibactam sodium, a reference compound provided by Arixa Pharmaceuticals, Inc. was dissolved in phosphate buffered saline (PBS) (pH 7.5) at 0.4 mg/mL for intravenous (IV) injection. In addition, pro-drugs of Avibactam (supplied by Arixa Pharmaceuticals, Inc.) were formulated in 10% ethanol / 40% polyethylene glycol (PEG) 400 / 50% water for injection (WFI) (pH 6.5) at 1 mg/mL for oral (PO) administration. The dosing volumes were 5 mL/kg for IV and 10 mL/kg for PO.

Administrations:

Animal Dosing Design - In vivo PK, non-fasted animals

Avibactam, sodium: n = 2 animals / group

Prodrug animals: n = 3 animals / group

+ Control animal (for drug-free blood collection), n = 3 animals

Dosing levels:

IV 2 mg/kg

Oral 10 mg/kg

Time points (dog and monkey):

IV: 0 (predose), 5, 10, 30, 60, and 120 min

Oral: 0 (predose), 1, 2, 4, 6, 8 and 24 hr

Target for Bioanalysis:

Avibactam

Plasma Sample Collection from rats (serial sampling):

Blood aliquots (300-400 μL) were collected from jugular vein catheterized rats into tubes coated with lithium heparin. The tubes were mixed gently and kept on ice and then centrifuged at 2,500 × g for 15 minutes at 4°C, within 1 hour after collections. For animals in the control groups, blood was collected by cardiac puncture and the plasma was harvested and kept frozen at -70 °C until further analysis.

Quantitative Bioanalysis (Plasma):

The plasma samples were processed using acetonitrile precipitation and analyzed by LCMS/MS. A plasma calibration curve was generated. Aliquots of drug-free plasma were

spiked with the test substance at the specified concentration levels. The spiked plasma samples were processed together with the unknown plasma samples using the same procedure. The processed plasma samples were stored at -70 °C until receiving LC-MS/MS analysis, at which time peak areas were recorded, and the concentrations of the test substance in the unknown plasma samples were determined using the respective calibration curve. The reportable linear range of the assay was determined, along with the lower limit of quantitation (LLQ).

Pharmacokinetics:

Plots of plasma concentration of compound versus time are constructed. The fundamental pharmacokinetic parameters of compound after IV and PO dosing (AUClast, AUCINF, T1/2, Tmax, and Cmax) are obtained from the non-compartmental analysis (NCA) of the plasma data using WinNonlin.

For dog & monkey PK:

Formulation:

Avibactam sodium, a reference compound provided by Arixa Pharmaceuticals, Inc. was dissolved in phosphate buffered saline (PBS) (pH 7.5) at 2 mg/mL for intravenous (IV) injection. In addition, pro-drugs of Avibactam (supplied by Arixa Pharmaceuticals, Inc.) were formulated in 10% ethanol / 40% polyethylene glycol (PEG) 400 / 50% water for injection (WFI) (pH 6.5) at 2 mg/mL for oral (PO) administration. The dosing volumes were 5 mL/kg for IV and 10 mL/kg for PO.

Administrations:

Animal Dosing Design - In vivo PK, non-fasted animals

Avibactam, sodium: n = 2 animals / group (male beagle dogs & male cynomolgus monkey)

Prodrug animals: n = 2 animals / group (male beagle dogs & male cynomolgus monkey)

Dosing levels:

IV 10 mg/kg

Oral 20 mg/kg

Time points (dog and monkey):

predose, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hours postdosing (±5 minutes for all time points)

Target for Bioanalysis:

Avibactam

Plasma Sample Collection:

Blood will be collected from a jugular vein (dog) (note: cephalic or vein may be used if necessary), or femoral vein (monkey) (note: cephalic or saphenous vein may be used if necessary). Approximately 1 mL/time point; collection into chilled sampling tubes.

Sample Handling

K_2EDTA tubes will be placed deeply into an ice bath with both water and crushed ice prior to blood collection. Cryovials (1.2 mL - VWR, capped with O-rings) to be used for the prodrug aliquots (daughter tube – 2/animal/time point) will be prepared by adding 300 µL of chilled acetonitrile (HPLC grade, 100%) prior to blood collection. These daughter tubes are to be maintained on ice. Prior to sample collection, vials will be visually inspected to ensure all of the acetonitrile is still present and has not evaporated. Following collection, gently invert the tube. Within 2 minutes of collection, transfer 100 µL of whole blood into each daughter tube, each containing 300 µL of acetonitrile. Each vial with the blood/acetonitrile mixture will be vortexed for approximately 30 seconds and immediately frozen on dry ice and maintained frozen (-55°C to -85°C) until shipment for analysis.

Note: Samples quenched with acetonitrile may vary in appearance and may have noticeable clumping. This is expected as a normal variation.

The remaining 800 µL will be maintained in the original K_2EDTA tube and will be chilled (wet ice) after collection and within 15 minutes of collecting, will be centrifuged refrigerated for approximately 10 minutes at 2400-2700 rpm at approximately 4ºC. Following centrifugation, the maximum amount of plasma will be recovered and split into 2 approximately equal volume aliquots and transferred to a freezer set to maintain -55°C to -85°C.

Quantitative Bioanalysis (Plasma):

The plasma samples were processed using acetonitrile precipitation and analyzed by LCMS/MS. A plasma calibration curve was generated. Aliquots of drug-free plasma were spiked with the test substance at the specified concentration levels. The spiked plasma samples were processed together with the unknown plasma samples using the same

procedure. The processed plasma samples were stored at -70 °C until receiving LC-MS/MS analysis, at which time peak areas were recorded, and the concentrations of the test substance in the unknown plasma samples were determined using the respective calibration curve. The reportable linear range of the assay was determined, along with the lower limit of quantitation (LLQ).

Pharmacokinetics:

Plots of plasma concentration of compound versus time are constructed. The fundamental pharmacokinetic parameters of compound after IV and PO dosing (AUClast, AUCINF, T1/2, Tmax, and Cmax) are obtained from the non-compartmental analysis (NCA) of the plasma data using WinNonlin.

We thank Ritu Lal (consultant to Arixa Pharmaceuticals, Inc.) for overseeing the PK studies and calculating the oral bioavailability of prodrugs (**14**) to (**20**).

REFERENCES

1. (*a*) Abe, T.; Okue, M.; Sakamaki, Y. Preparation of optically-active diazabicyclooctane derivative and method for manufacturing same. PCT WO 2012086241; *Chem. Abstr.* **2012**, *157*, 165634. (b) Lampilas, M.; Aszodi, J.; Rowlands, D. A.; Fromentin, C. Azabicyclic compounds, including 1,3-diazabicyclo[2.2.1]heptan-2-one and 1,6-diazabicyclo[3.2.1]octan-7-one derivatives, preparation thereof, and use as medicines, in particular as antibacterial agents. PCT WO 2002010172; *Chem. Abstr.* **2002**, *136*, 136397.

2. Simpson, L. S.; Widlanski, T. S. Comprehensive approach to the synthesis of sulfate esters. *J. Am. Chem. Soc.* **2006**, *128*, 1605-1610.

3. Schaper, U. A.; Bruns, K. Esters of 3-hydroxy-2,2-dimethylpropionic acid and their use as perfume components. German Patent Application DE 3045373; *Chem. Abstr.* **1982**, *97*, 162388.

4. Subramanyam, C.; Bell, M. R. 2-saccharinylmethyl benzoates, their preparation and use for the treatment of degenerative diseases. US Patent Application US 5306818; *Chem. Abstr.* **1995**, *123*, 143928.

5. Kong, X.; Levens, N.; Bouzide, A.; Ciblat, S.; Frenette, R.; Renaud, J. Methods, compounds, and compositions for delivering 1,3-propanedisulfonic acid. PCT WO 2011017800; *Chem. Abstr.* **2011**, *154*, 259094.

6. Soengas, R. G.; Estevez, A. M. Convenient procedure for the indium-mediated hydroxymethylation of active bromo compounds: transformation of ketones into α-hydroxymethyl nitroalkanes. *Synlett* **2010**, 2625-2627.

7. Katrizky, A. R.; Tala, S. R.; Abo-Dya, N. E.; Gyanda, K.; El-Gendy, B. E-D. M.; Abdel-Samii, Z. K.; Steel, P. J. Selective synthesis and structural elucidation of *S*-acyl- and *N*-acylcysteines. *J. Org. Chem.* **2009,** *74,* 7165-7167.

8. Gatterdam, V.; Stoess, T.; Menge, C.; Heckel, A.; Tampé, R. Caged glutathione – triggering protein interaction by light. *Angew. Chem., Int. Ed.* **2012**, *51*, 3960-3963.

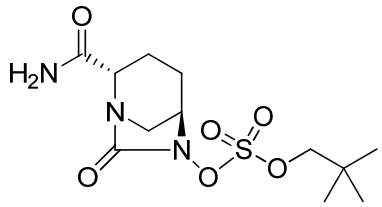
9. Lepore, S. D.; He, Y. Use of sonication for the coupling of sterically hindered substrates in the phenolic Mitsunobu reaction. *J. Org. Chem.* **2003,** *21*, 8261-8263.

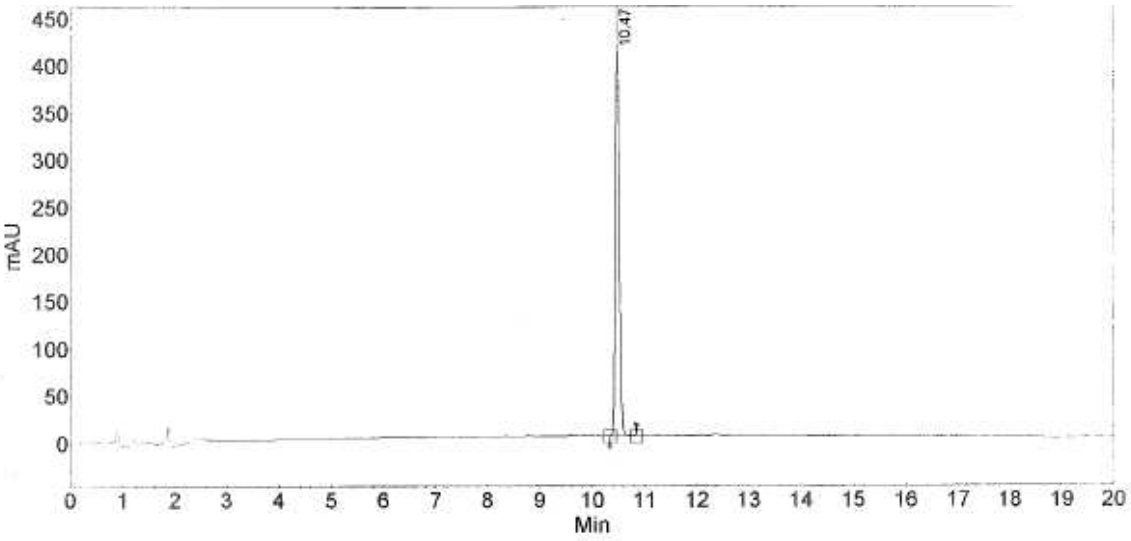
10. Guide for the Care and Use of Laboratory Animals: Eighth Edition; National Academies Press: Washington, D.C., 2011.

11. AAALAC stands for The Association for Assessment and Accreditation of Laboratory Animal Care, International.

DATA FOR FINAL COMPOUNDS

LC FOR COMPOUND (9)





Peak

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Total			411.8	2119.3	35.3	100.000

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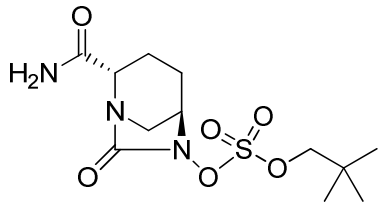

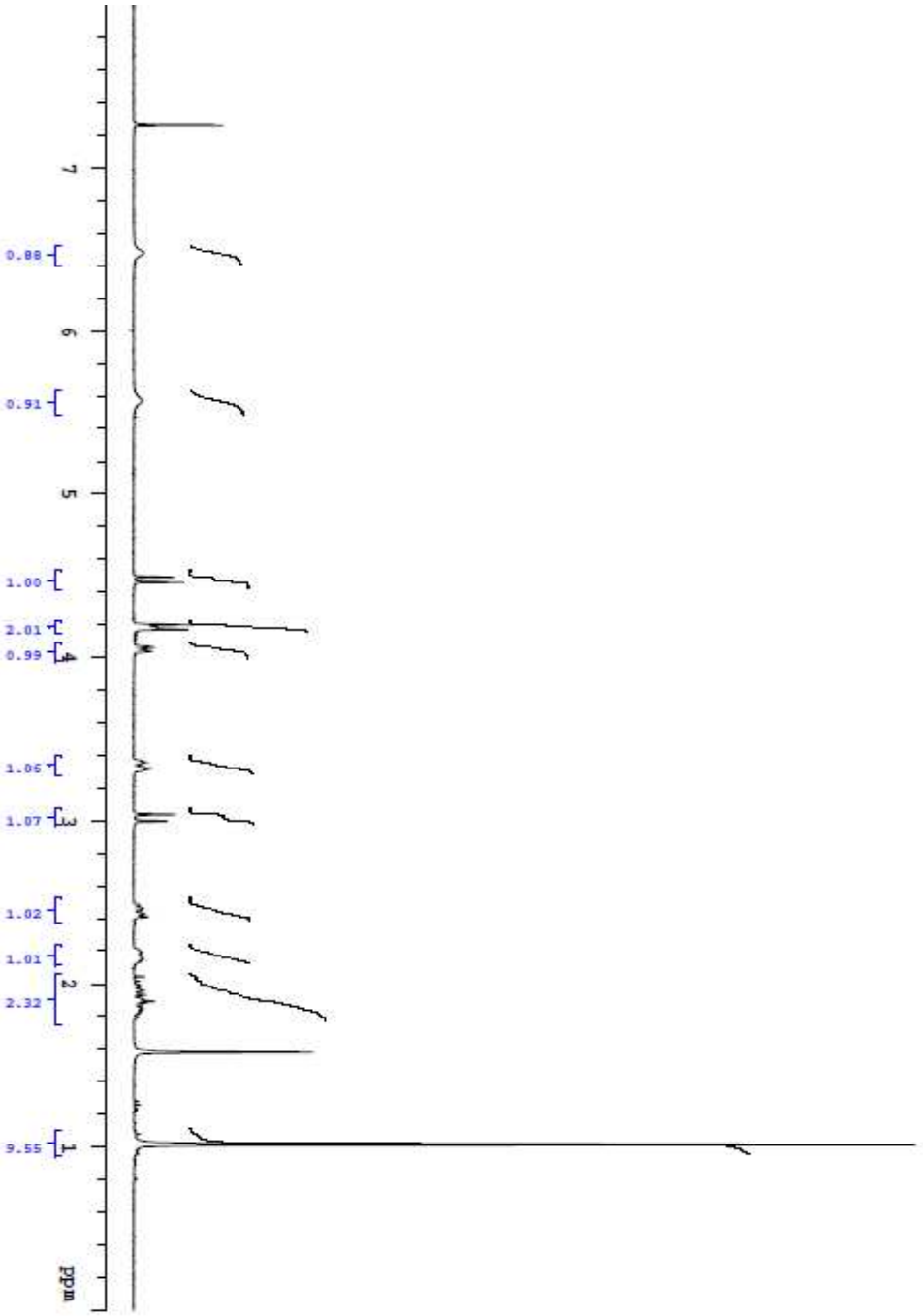

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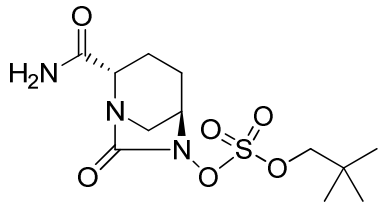

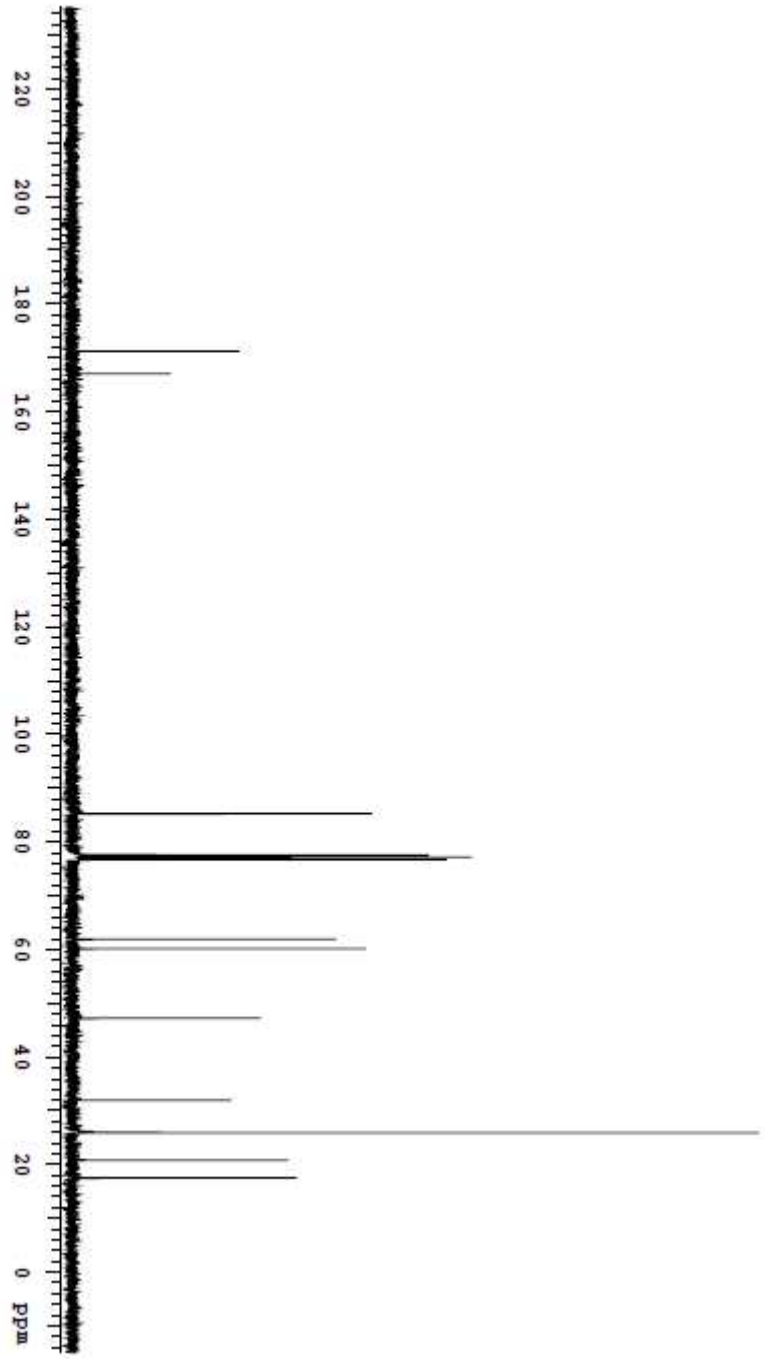

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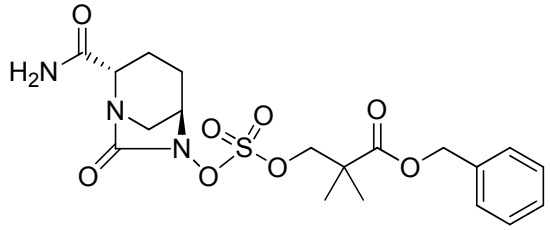

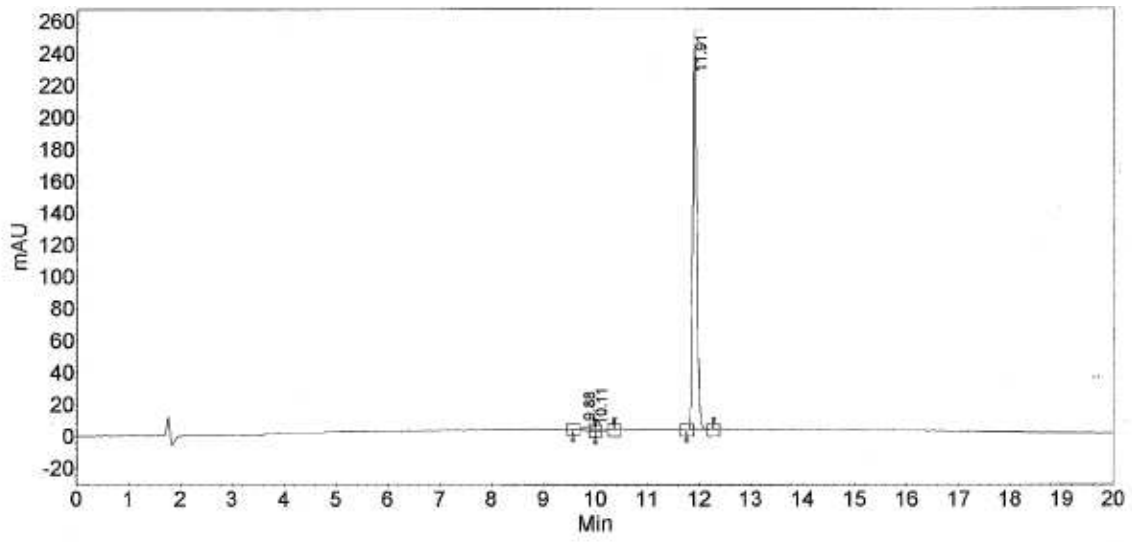

Peak

Index	Name	Time [Min]	Height [mAU]	Area [mAU.Sec]	Area [mAU.Min]	Area % [%]
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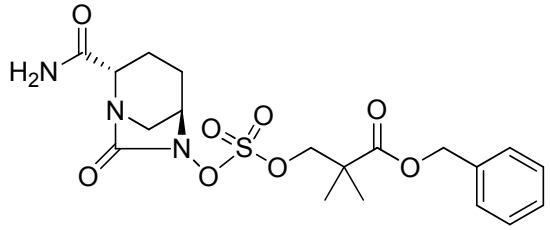
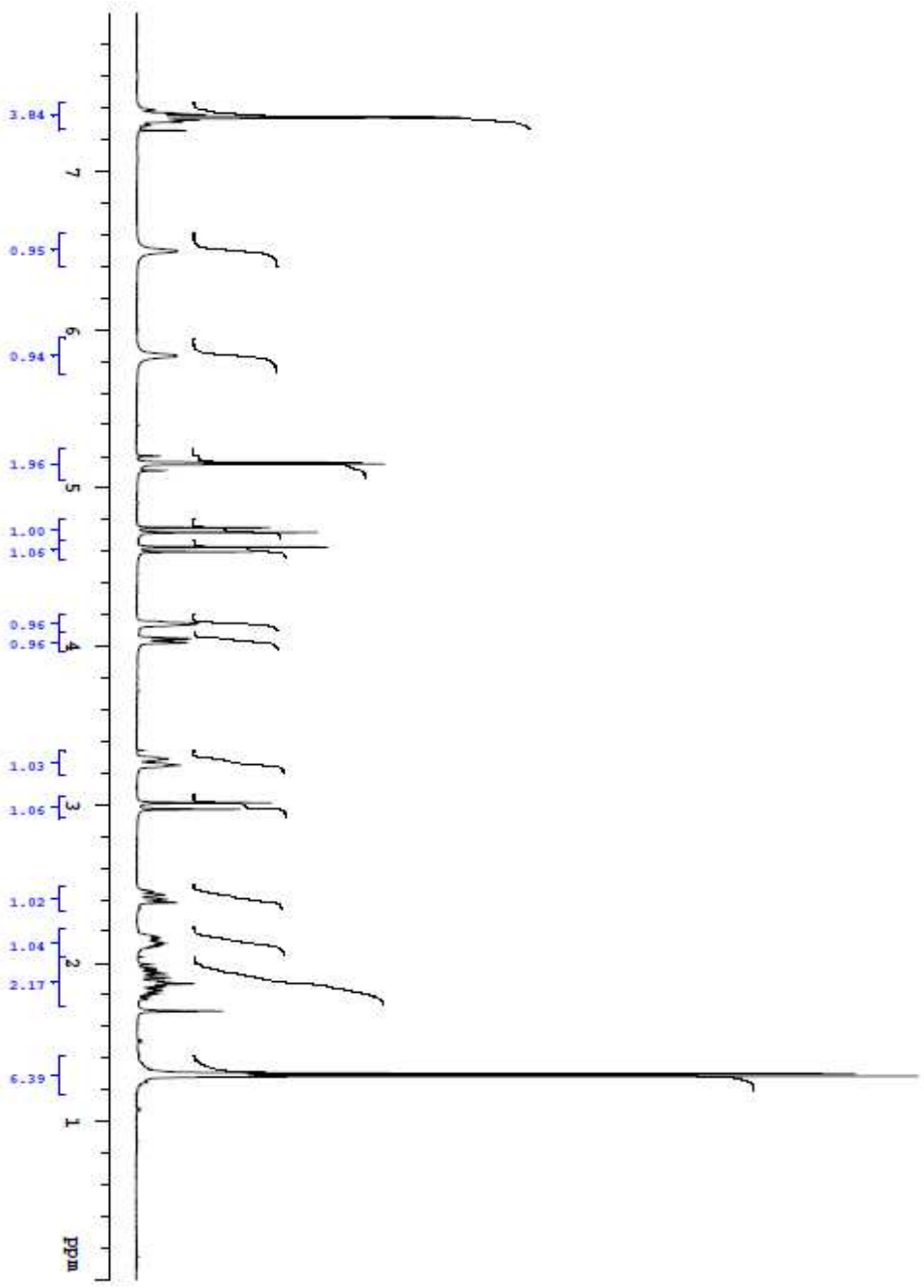
2	UNKNOWN	10.11	0.6	8.5	0.1	0.615
3	UNKNOWN	11.91	238.7	1345.3	22.4	97.122
Total			241.7	1385.1	23.1	100.000

^{1}H NMR FOR COMPOUND (10)





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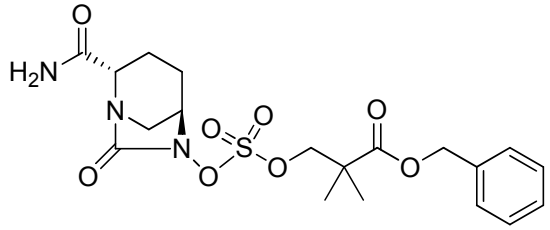

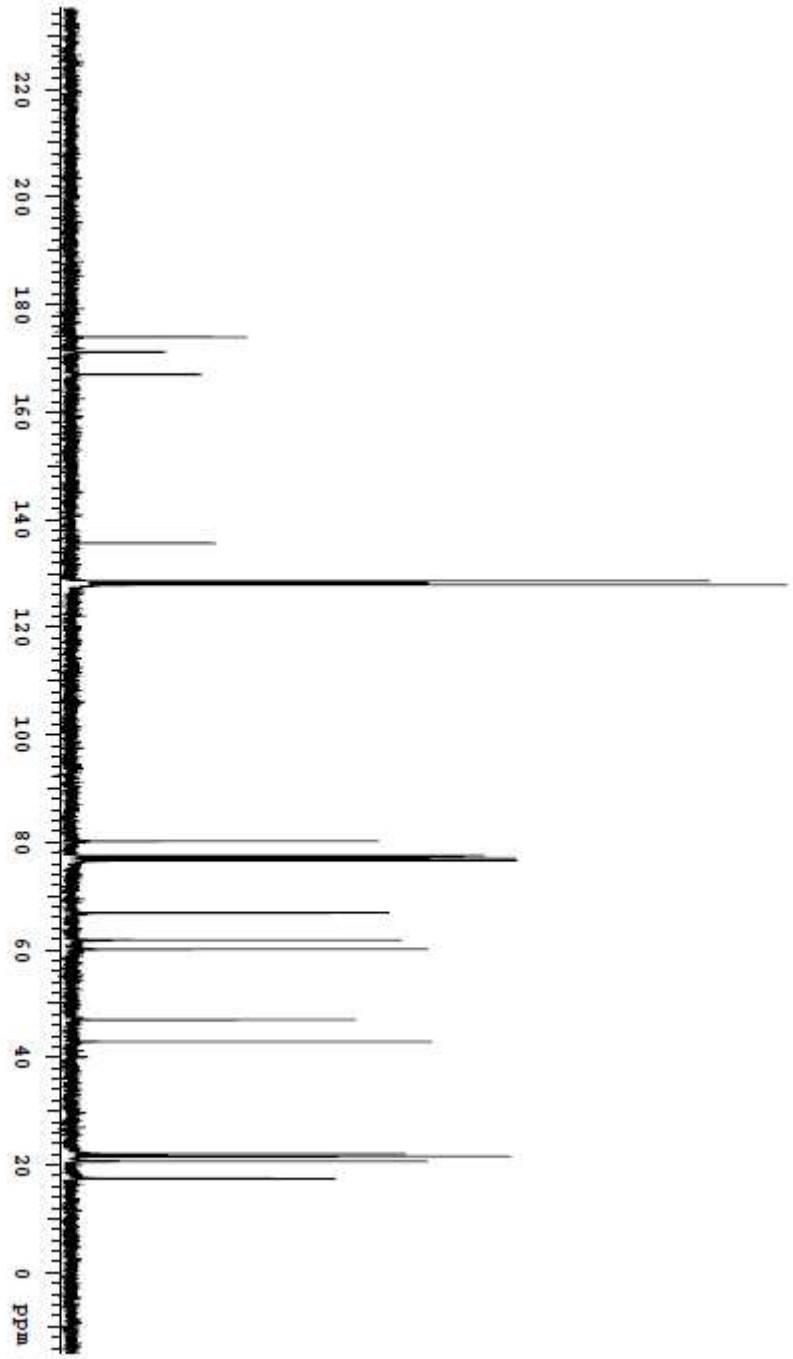

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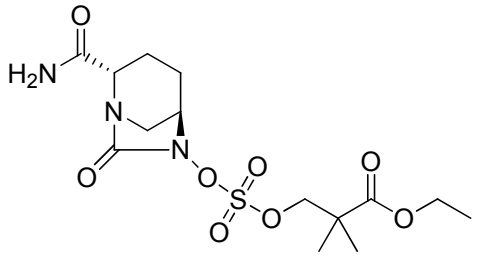

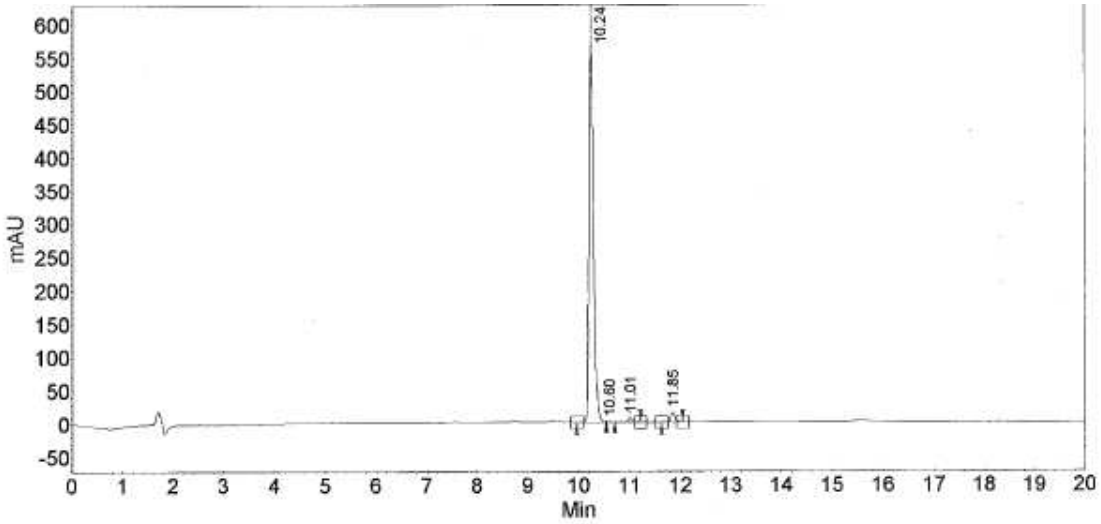

Peak

Index	Name	Time [Min]	Height [mAU]	Area [mAU Sec]	Area [mAU Min]	Area % [%]
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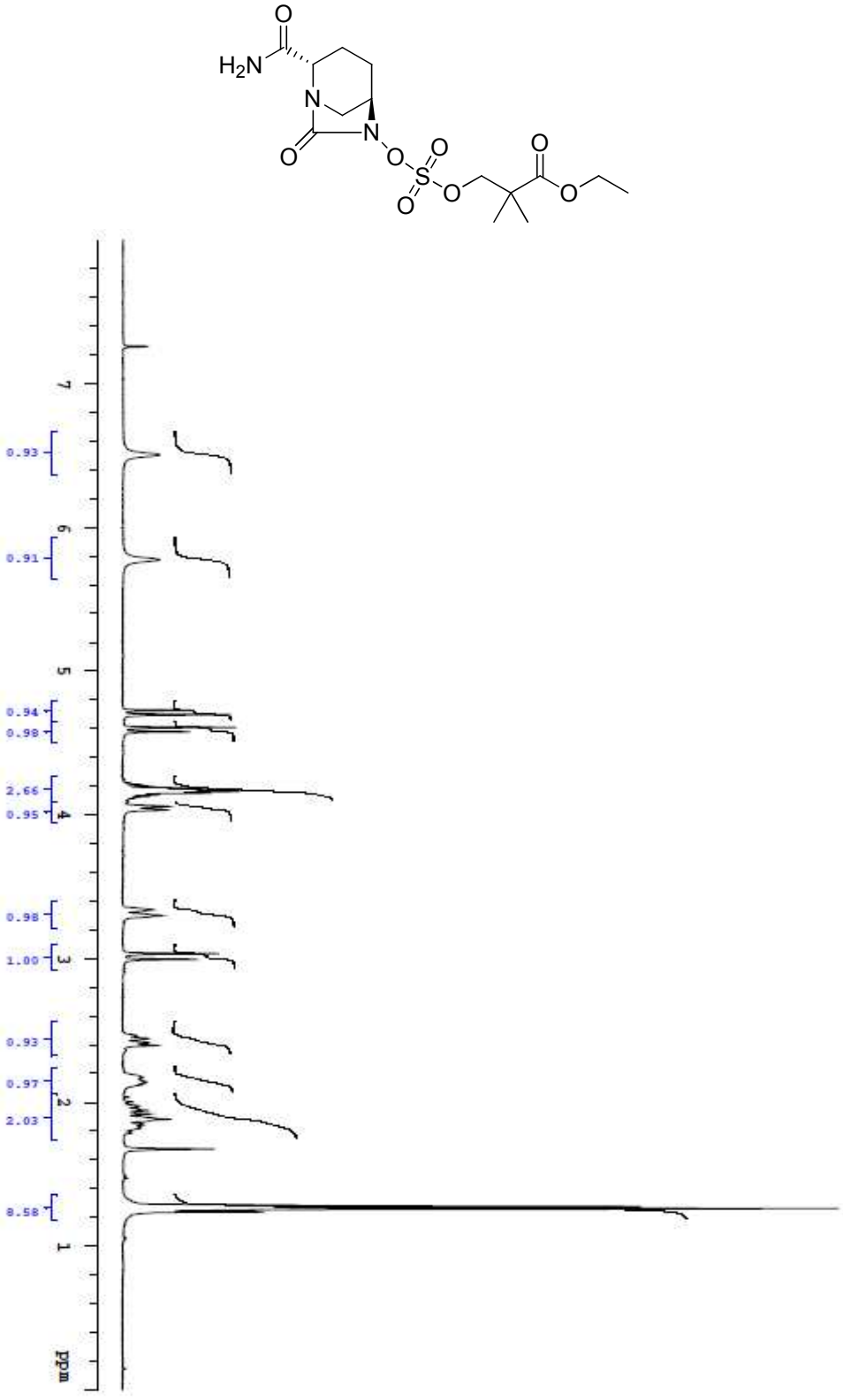
2	UNKNOWN	10.60	1.1	8.0	0.1	0.223
3	UNKNOWN	11.01	5.8	41.2	0.7	1.146
4	UNKNOWN	11.85	14.7	84.0	1.4	2.334
Total			590.0	3597.9	60.0	100.000

^{1}H NMR FOR PRODRUG (14)



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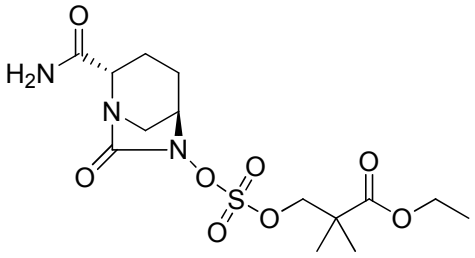
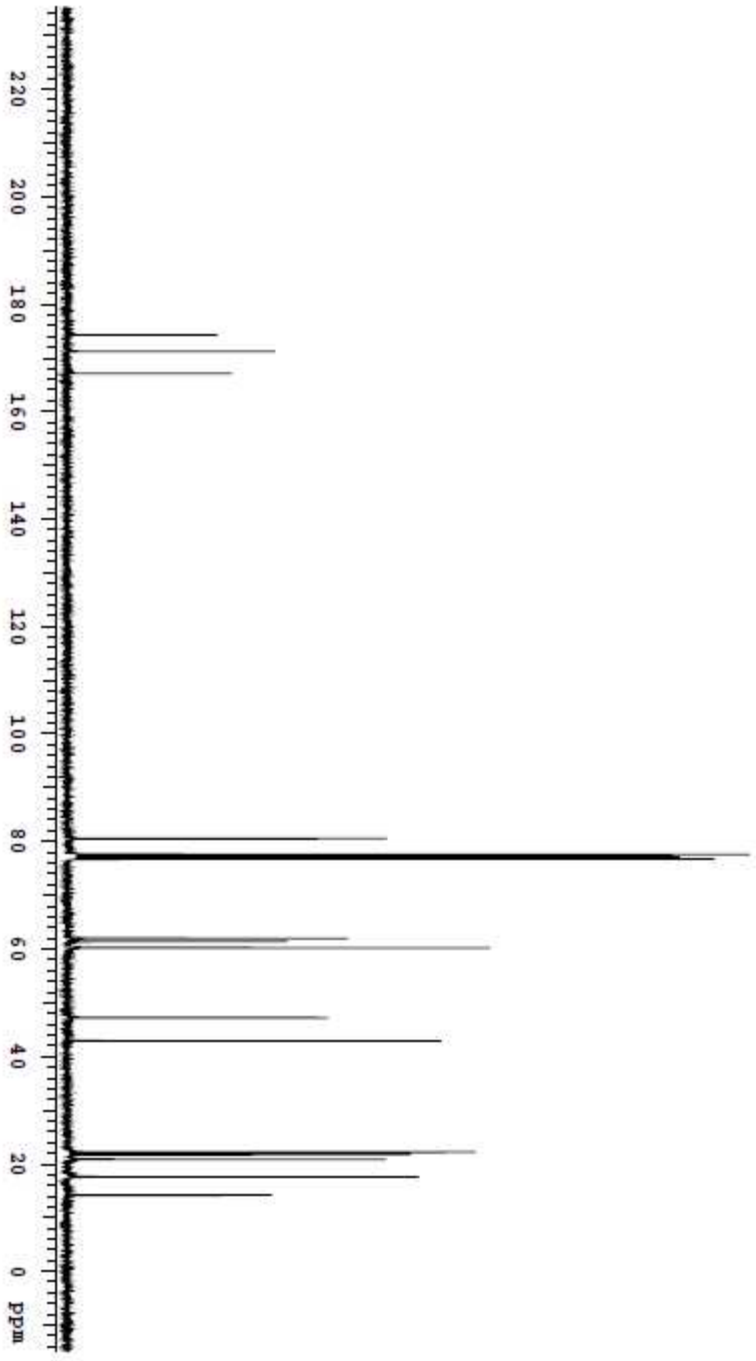
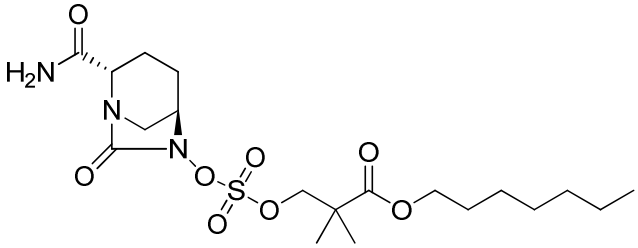
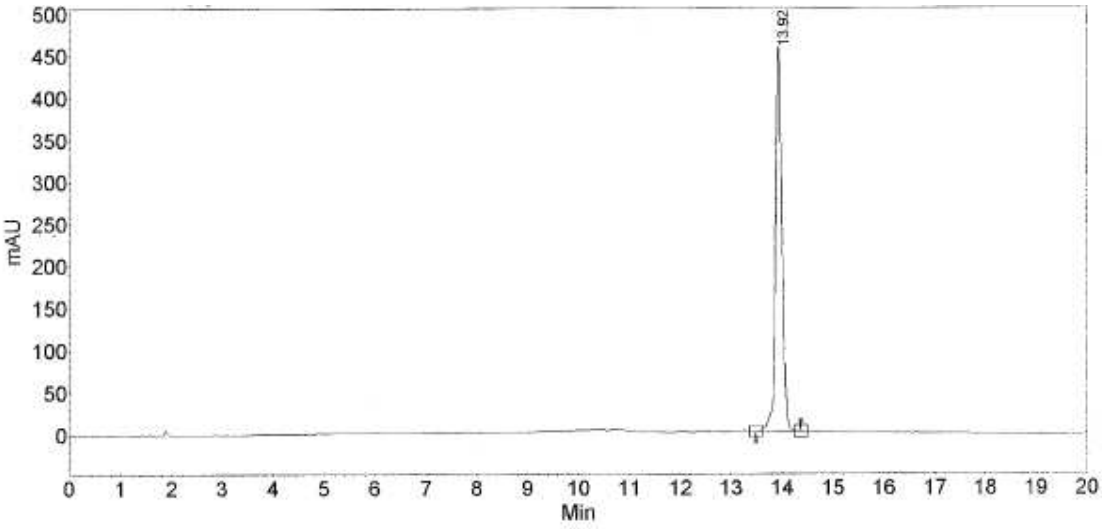




LC FOR PRODRUG (15)

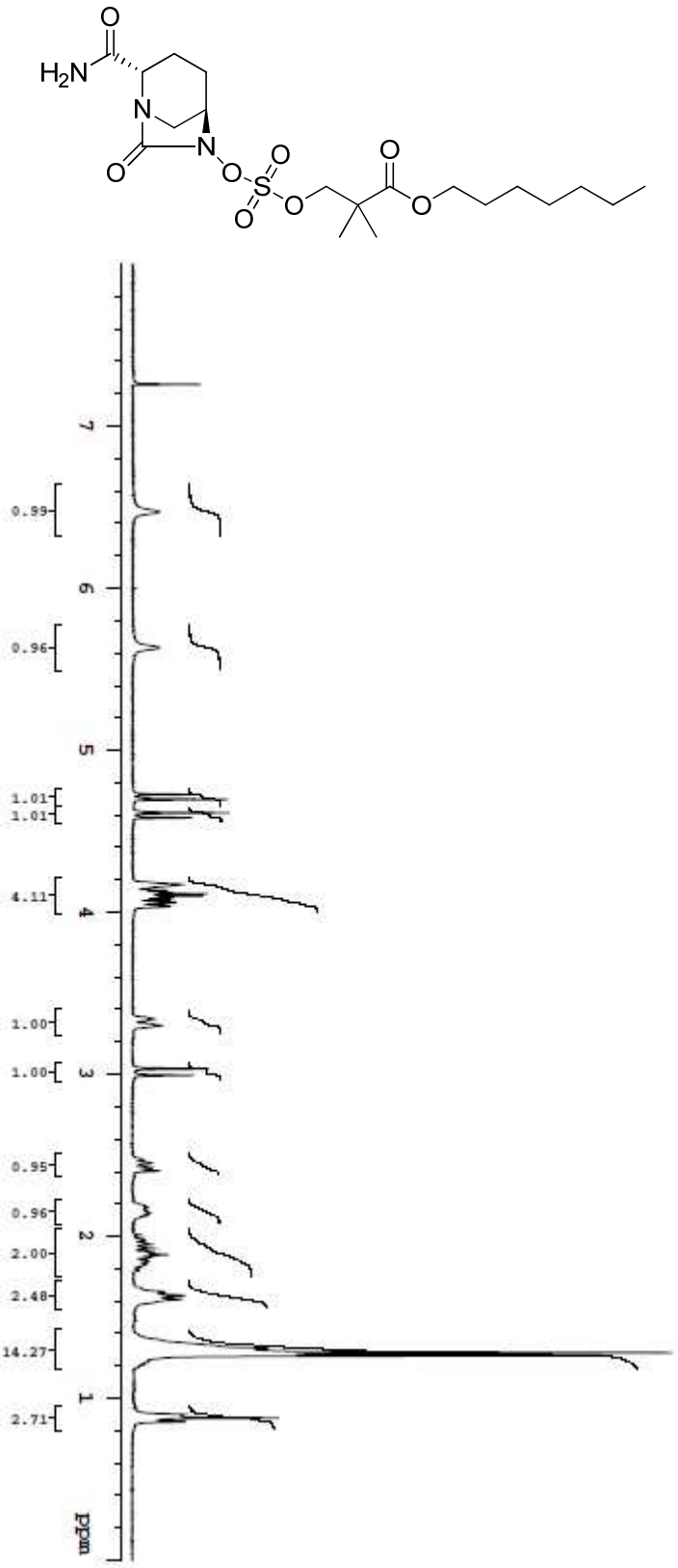




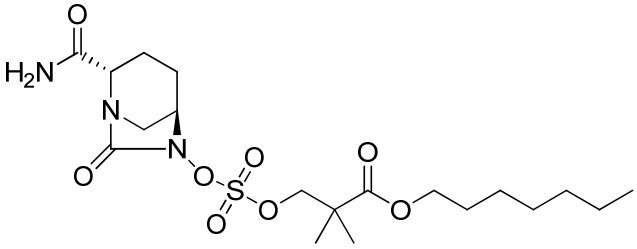
Peak

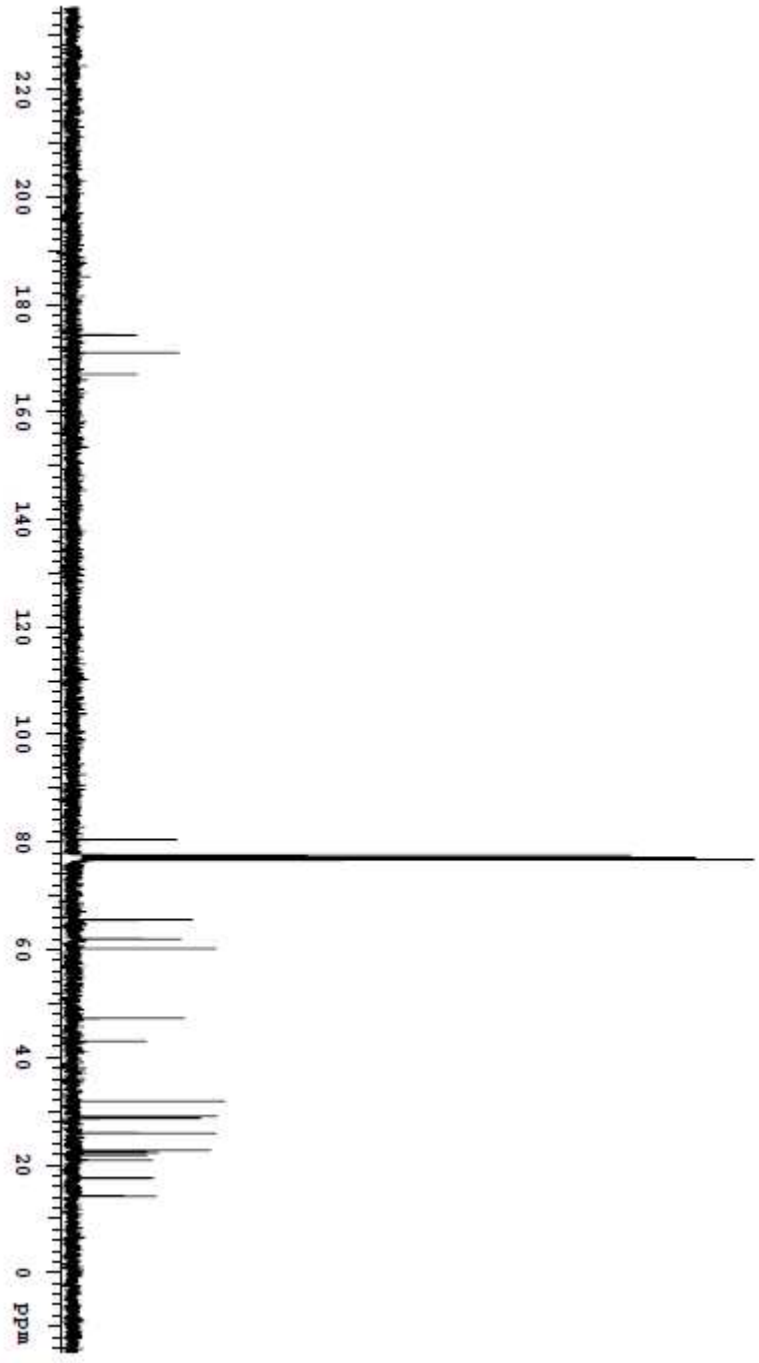
Index	Name	Time [Min]	Height [mAU]	Area [mAU Sec]	Area [mAU Min]	Area % [%]
1	UNKNOWN	13.92	456.1	3904.4	65.1	100.000
Total			456.1	3904.4	65.1	100.000

^{1}H NMR FOR PRODRUG (15)

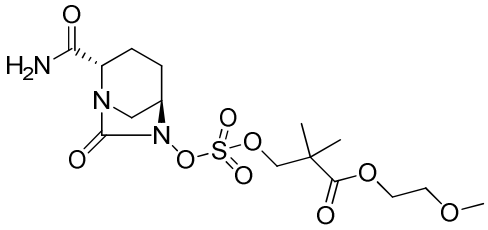


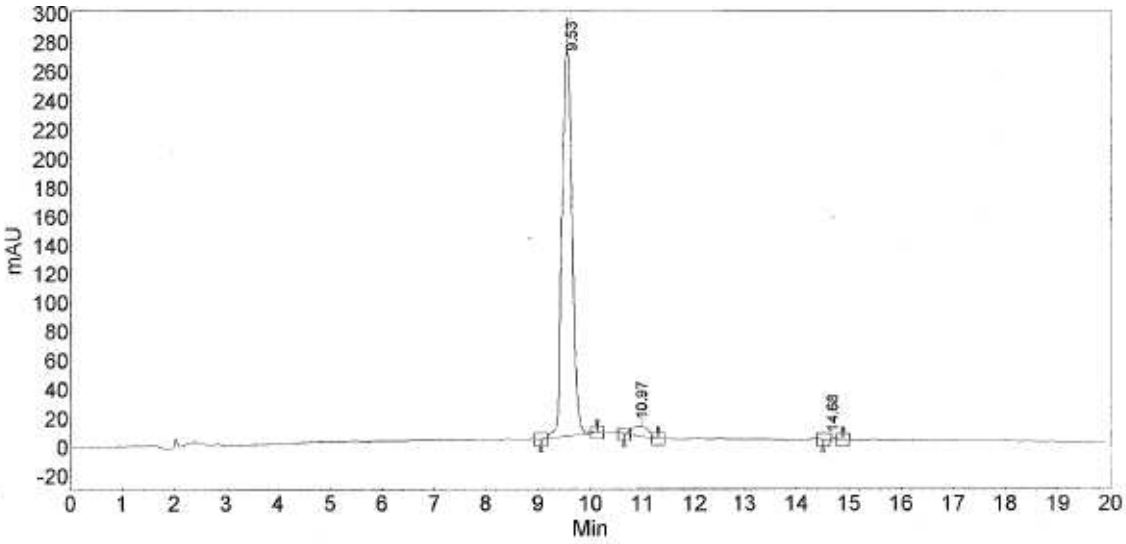
^{13}C NMR FOR PRODRUG (15)





LC FOR PRODRUG (16)

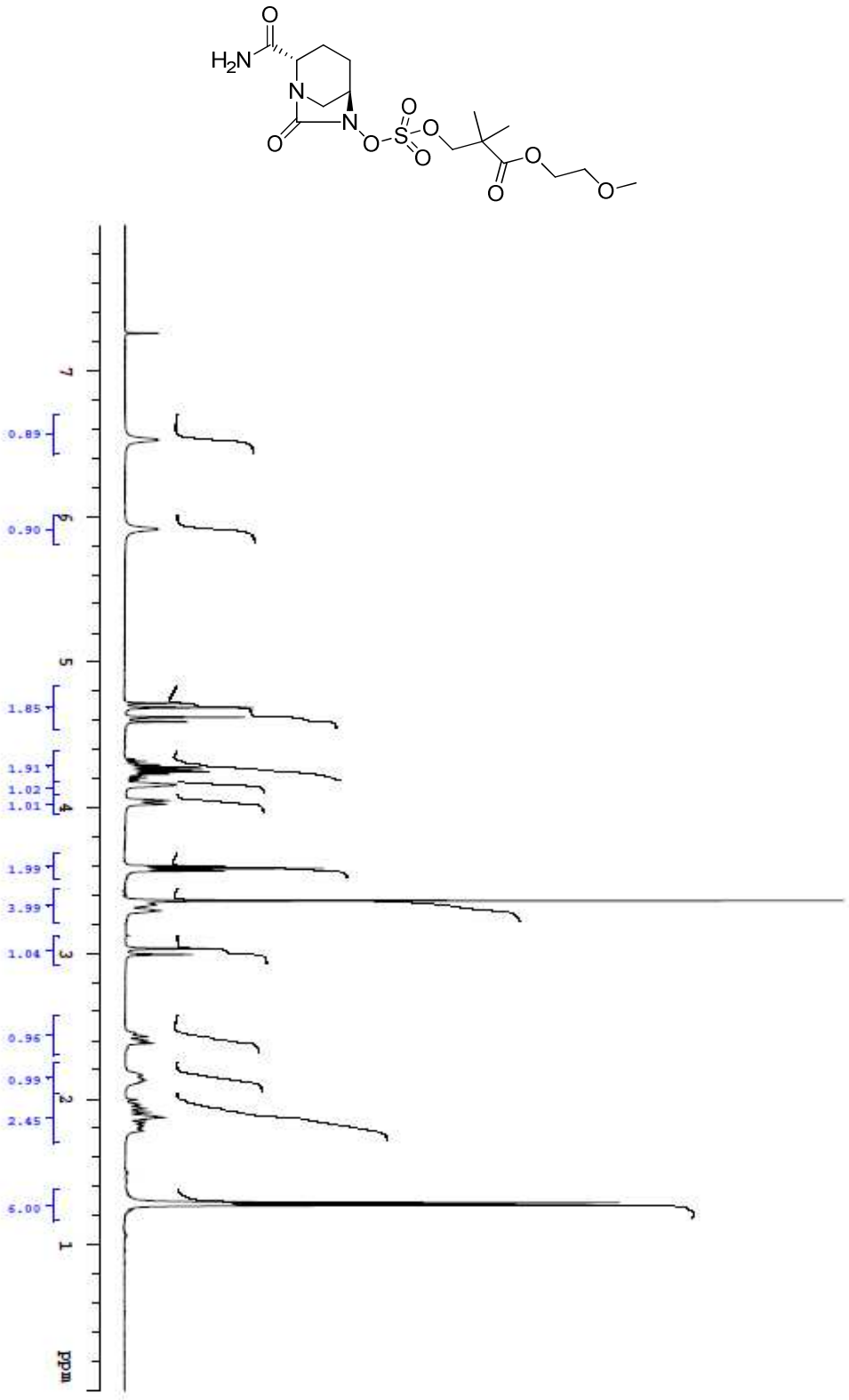




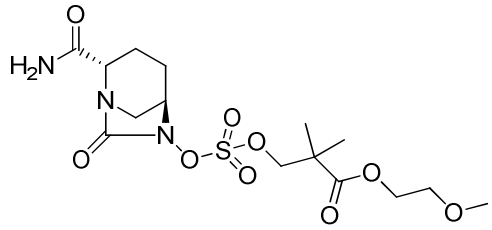
Peak

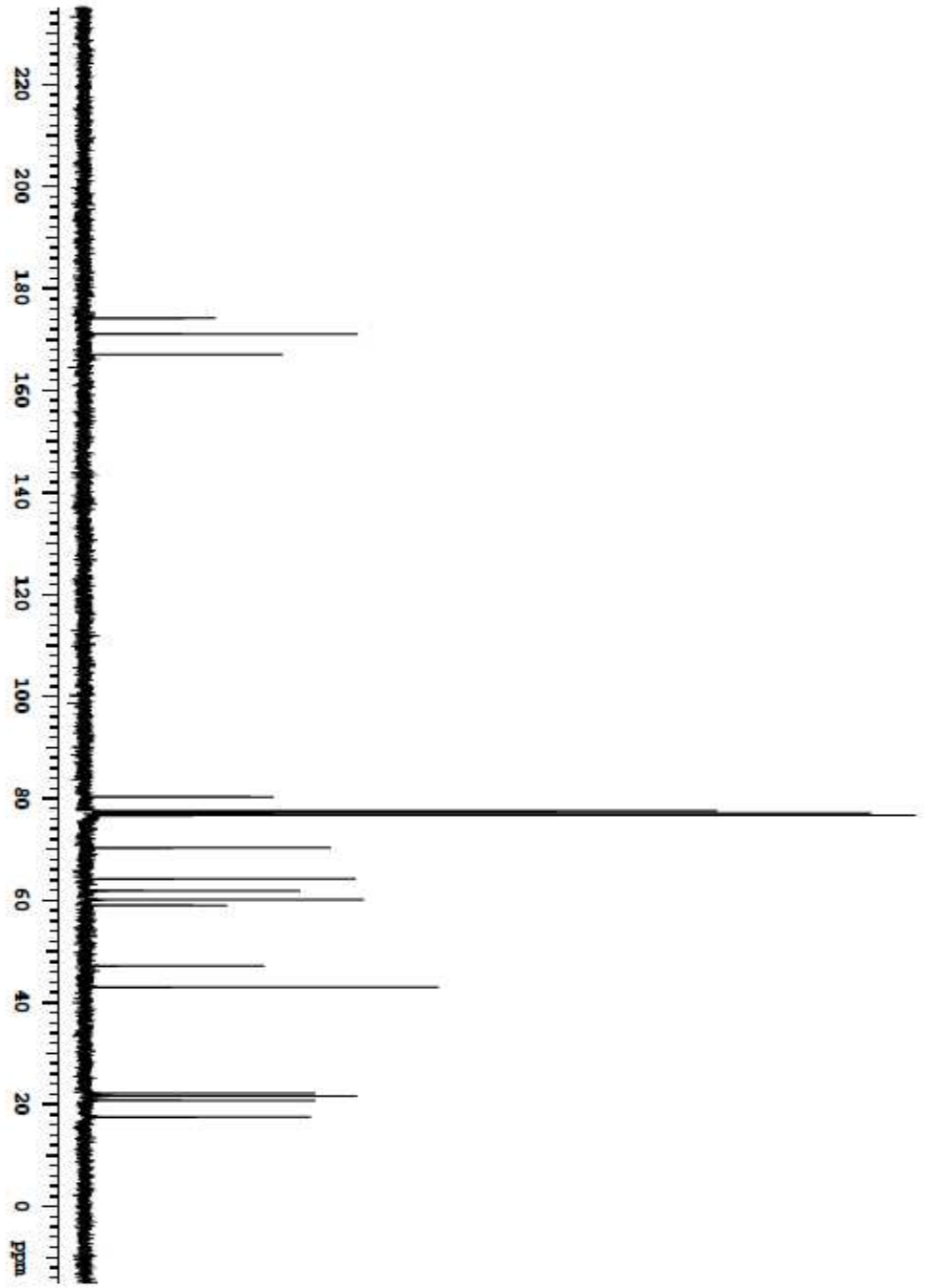
Index	Name	Time [Min]	Height [mAU]	Area [mAU.Sec]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	9.53	266.5	3617.7	60.3	95.390
2	UNKNOWN	10.97	7.1	161.7	2.7	4.263
3	UNKNOWN	14.68	1.6	13.2	0.2	0.347
Total			275.3	3792.6	63.2	100.000

^{1}H NMR FOR PRODRUG (16)

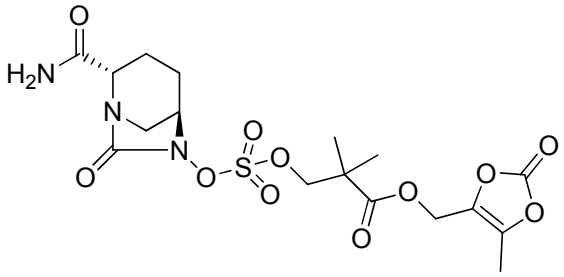


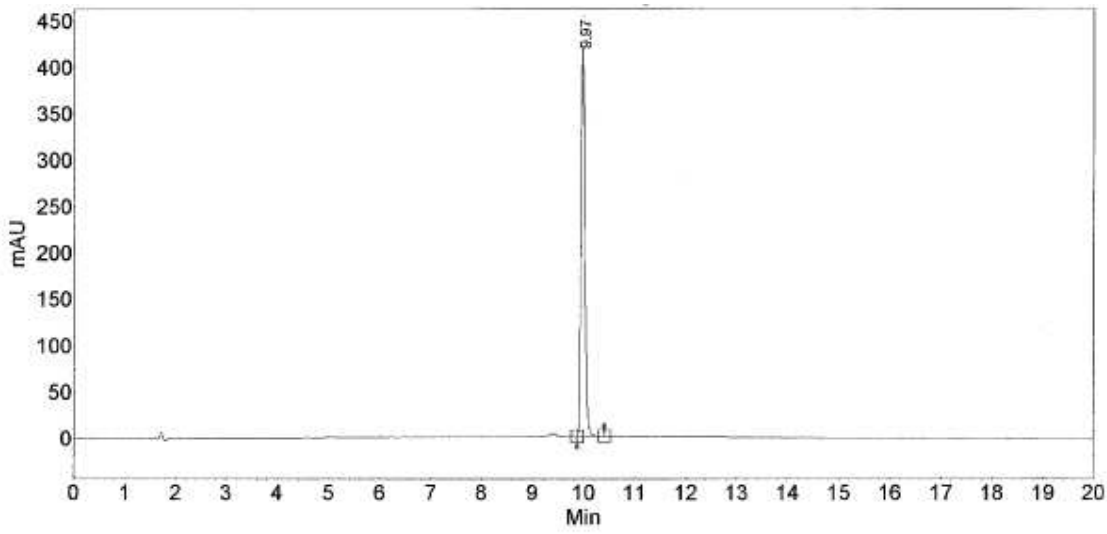
^{13}C NMR FOR PRODRUG (16)





LC FOR PRODRUG (17)

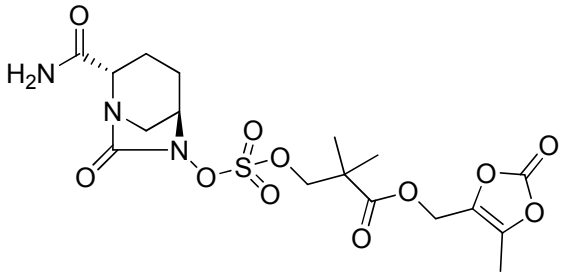


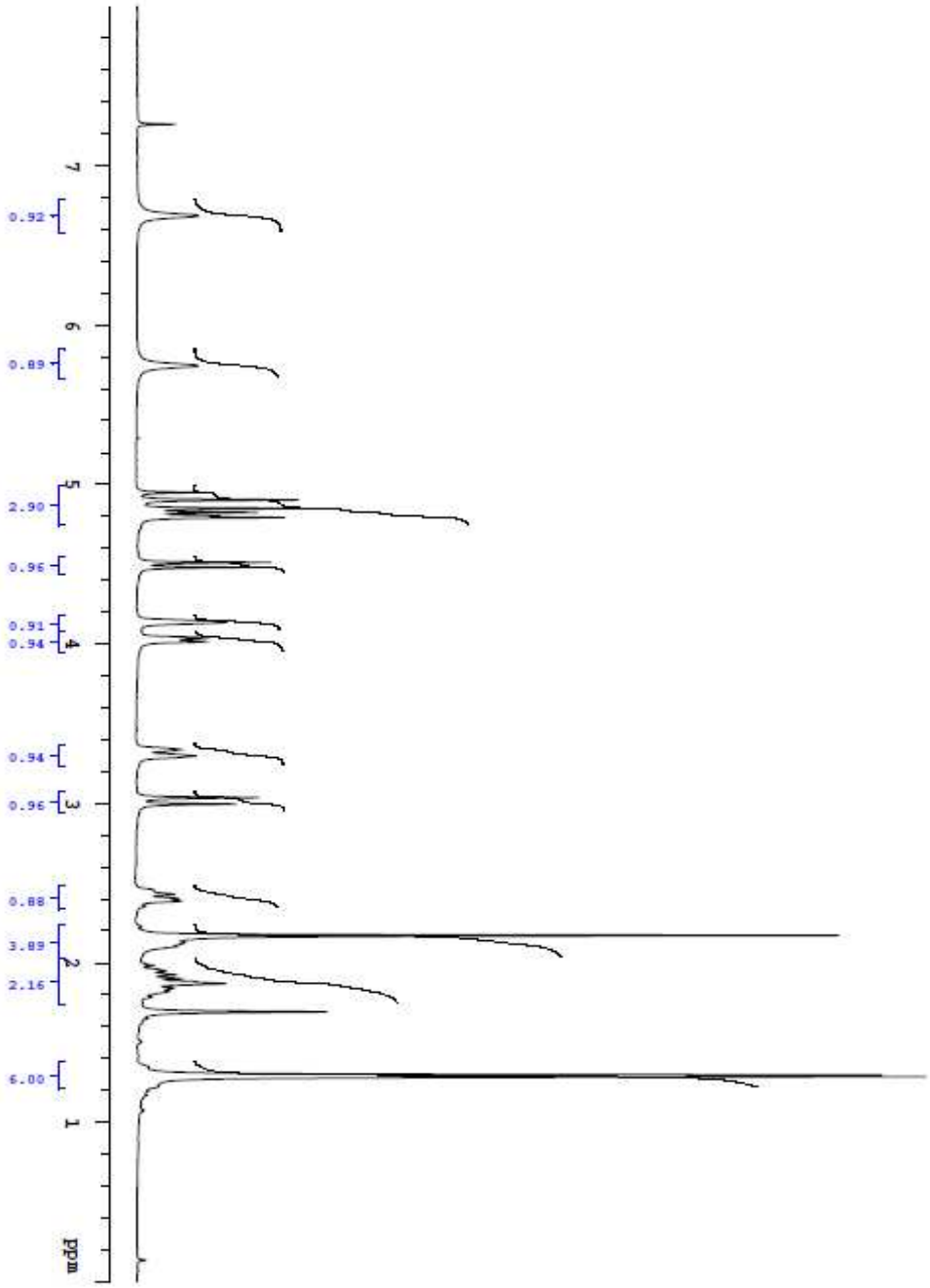


Peak

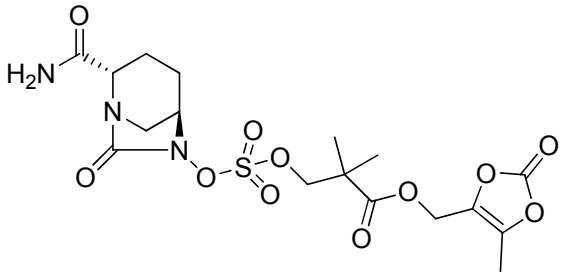
Index	Name	Time [Min]	Height [mAU]	Area [mAU.Sec]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	9.97	418.5	2210.6	36.8	100.000
Total			418.5	2210.6	36.8	100.000

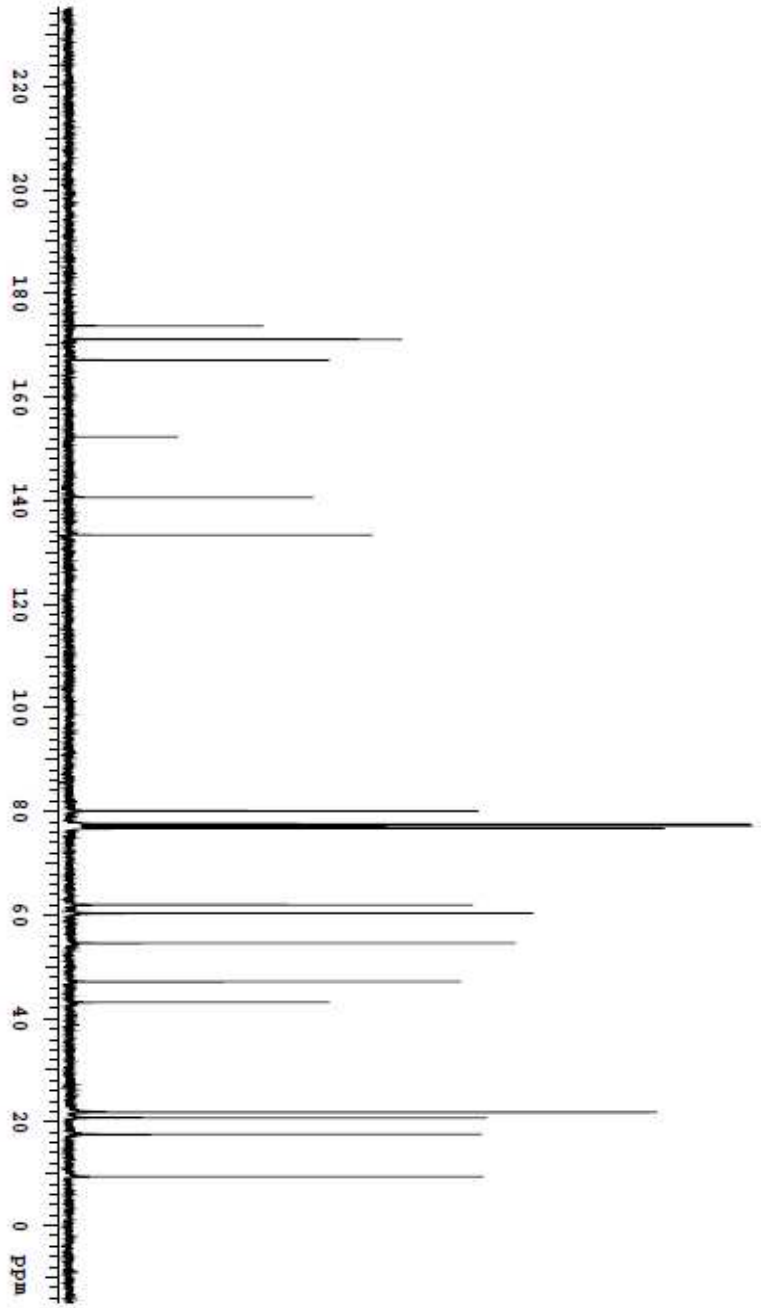
^{1}H NMR FOR PRODRUG (17)



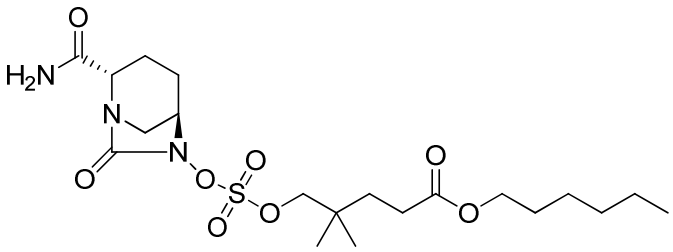


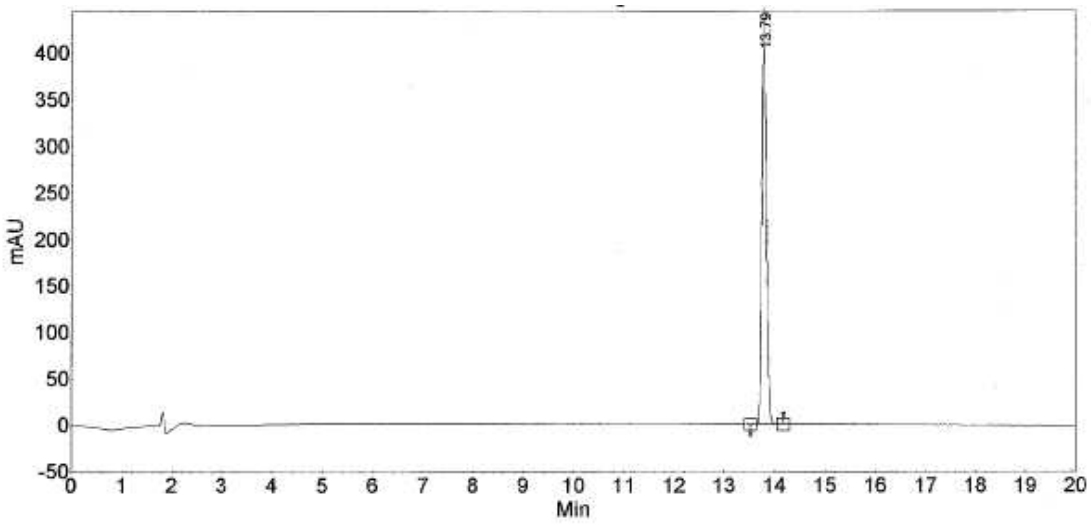
^{13}C NMR FOR PRODRUG (17)





LC FOR PRODRUG (18)

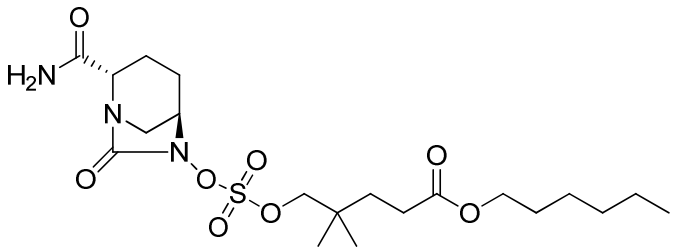


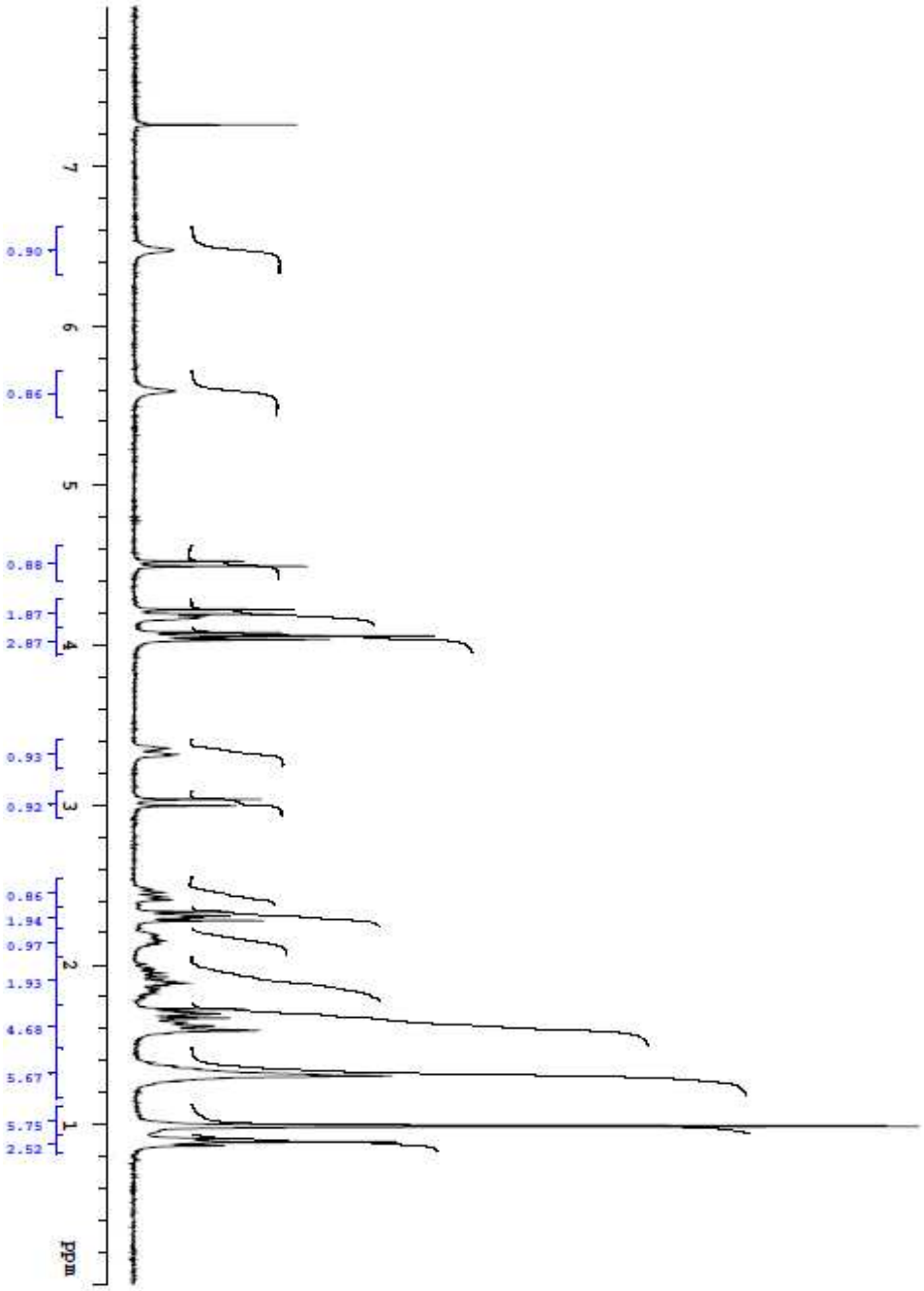


Peak

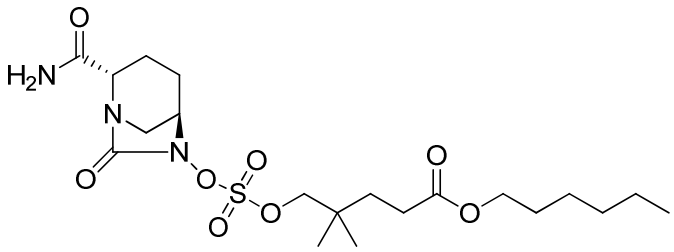
Index	Name	Time [Min]	Height [mAU]	Area [mAU.Sec]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	13.79	403.5	2560.1	42.7	100.000
Total			403.5	2560.1	42.7	100.000

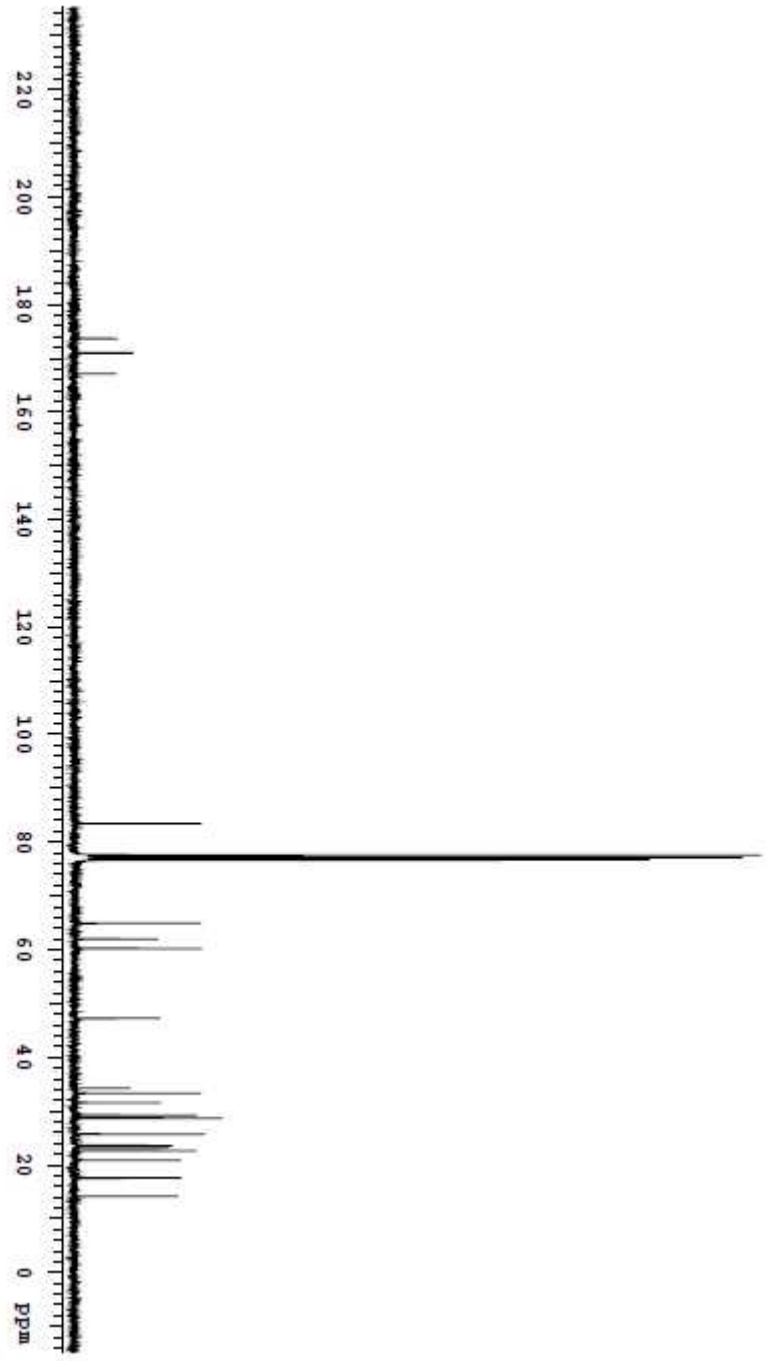
^{1}H NMR FOR PRODRUG (18)



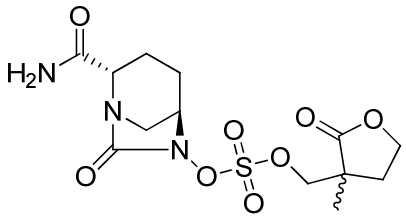


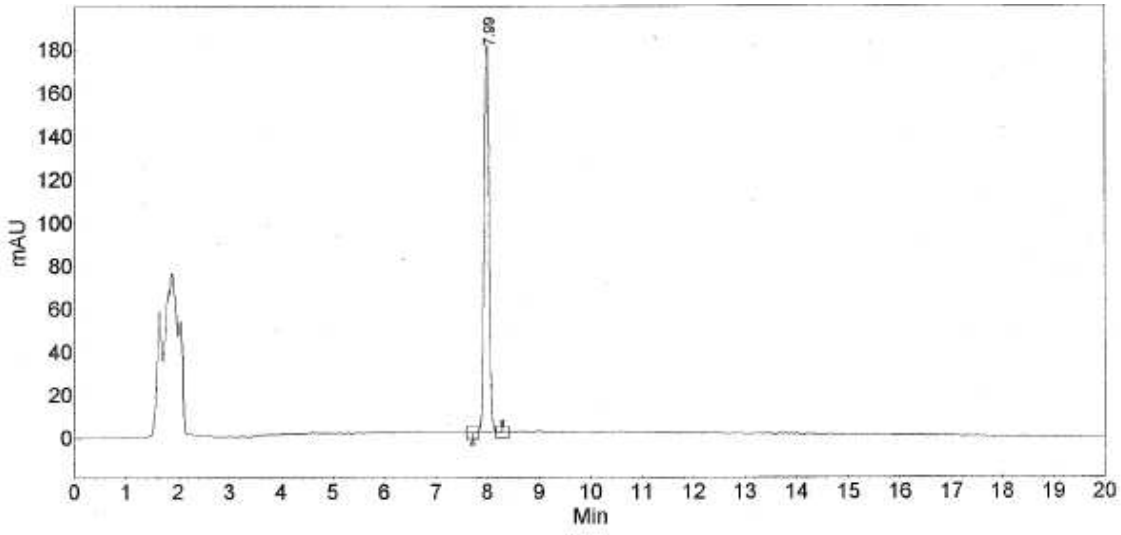
^{13}C NMR FOR PRODRUG (18)





LC FOR PRODRUG (19)

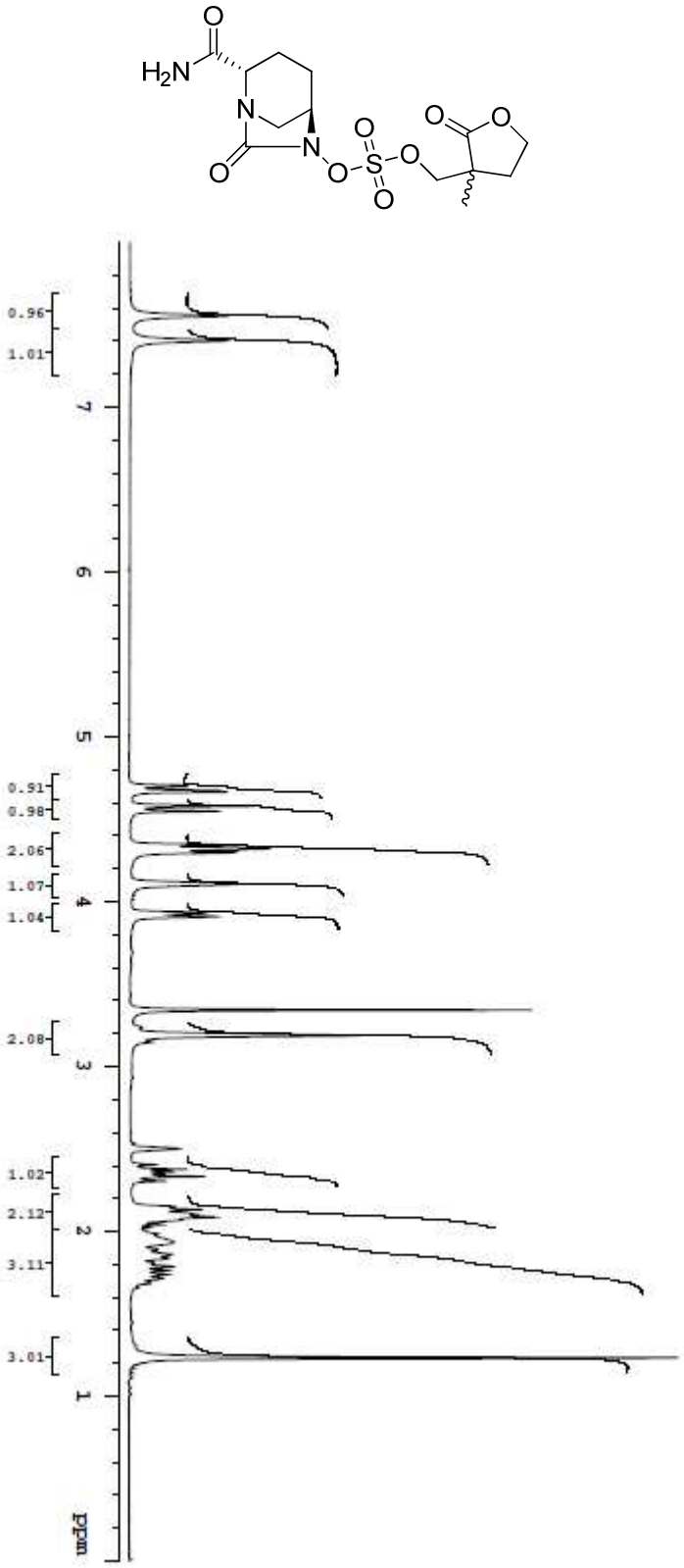




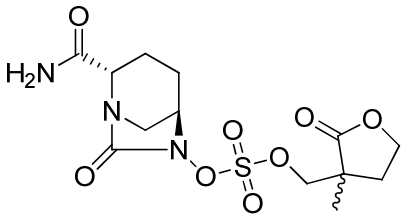
Peak

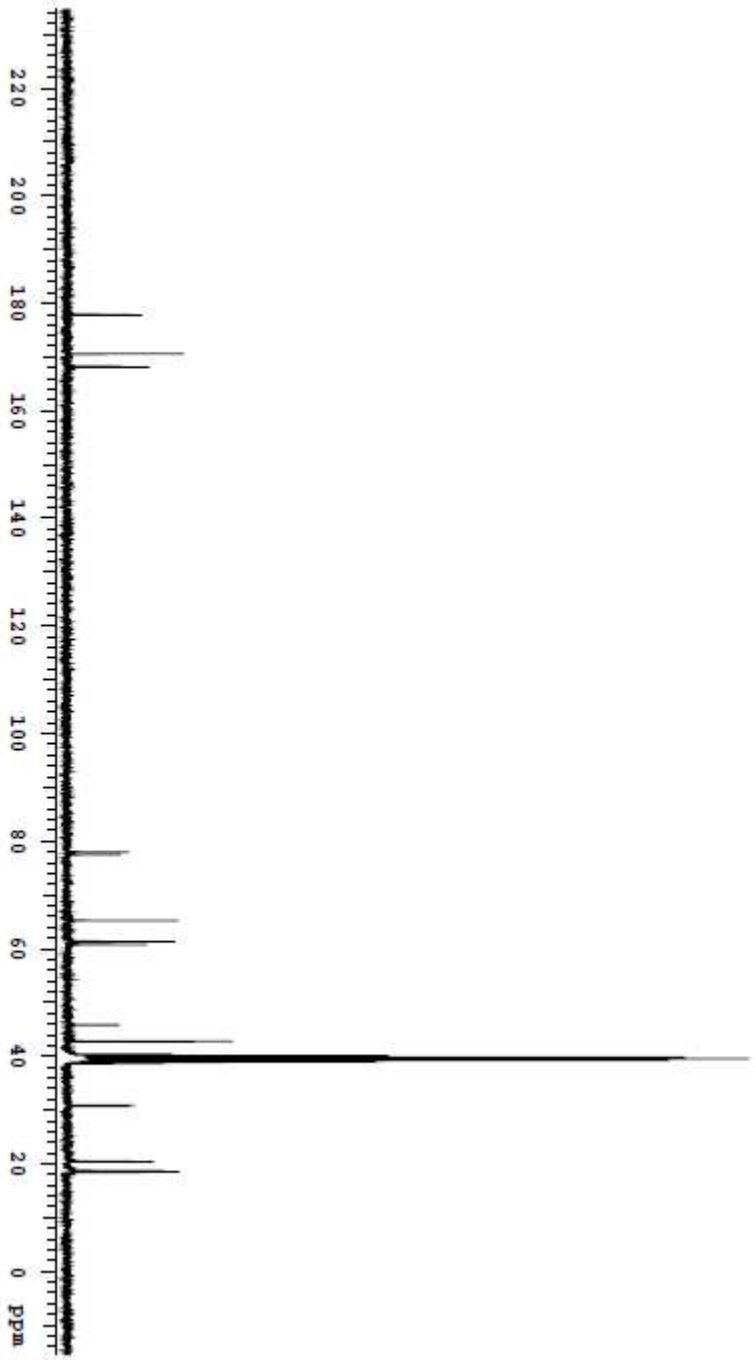
Index	Name	Time [Min]	Height [mAU]	Area [mAU*Sec]	Area [mAU*Min]	Area % [%]
1	UNKNOWN	7.99	179.4	1156.1	19.3	100.000
Total			179.4	1156.1	19.3	100.000

^{1}H NMR FOR PRODRUG (19)

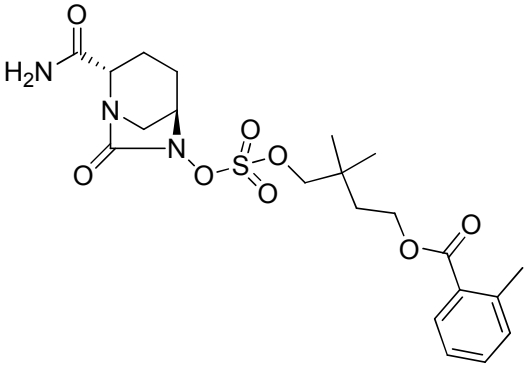


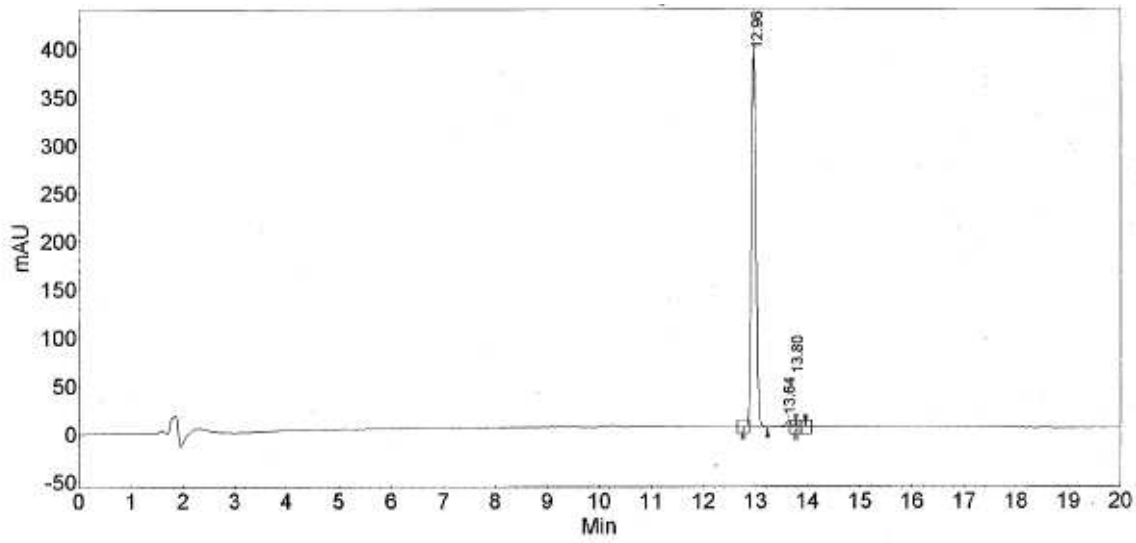
^{13}C NMR FOR PRODRUG (19)





LC FOR PRODRUG (20)

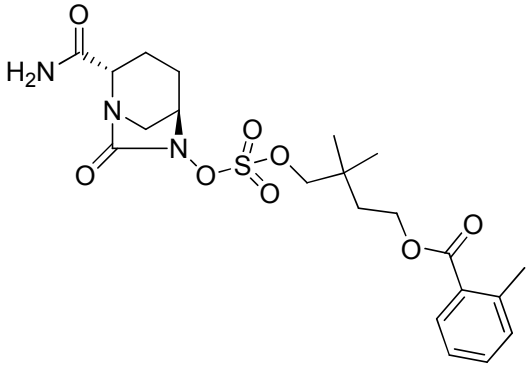


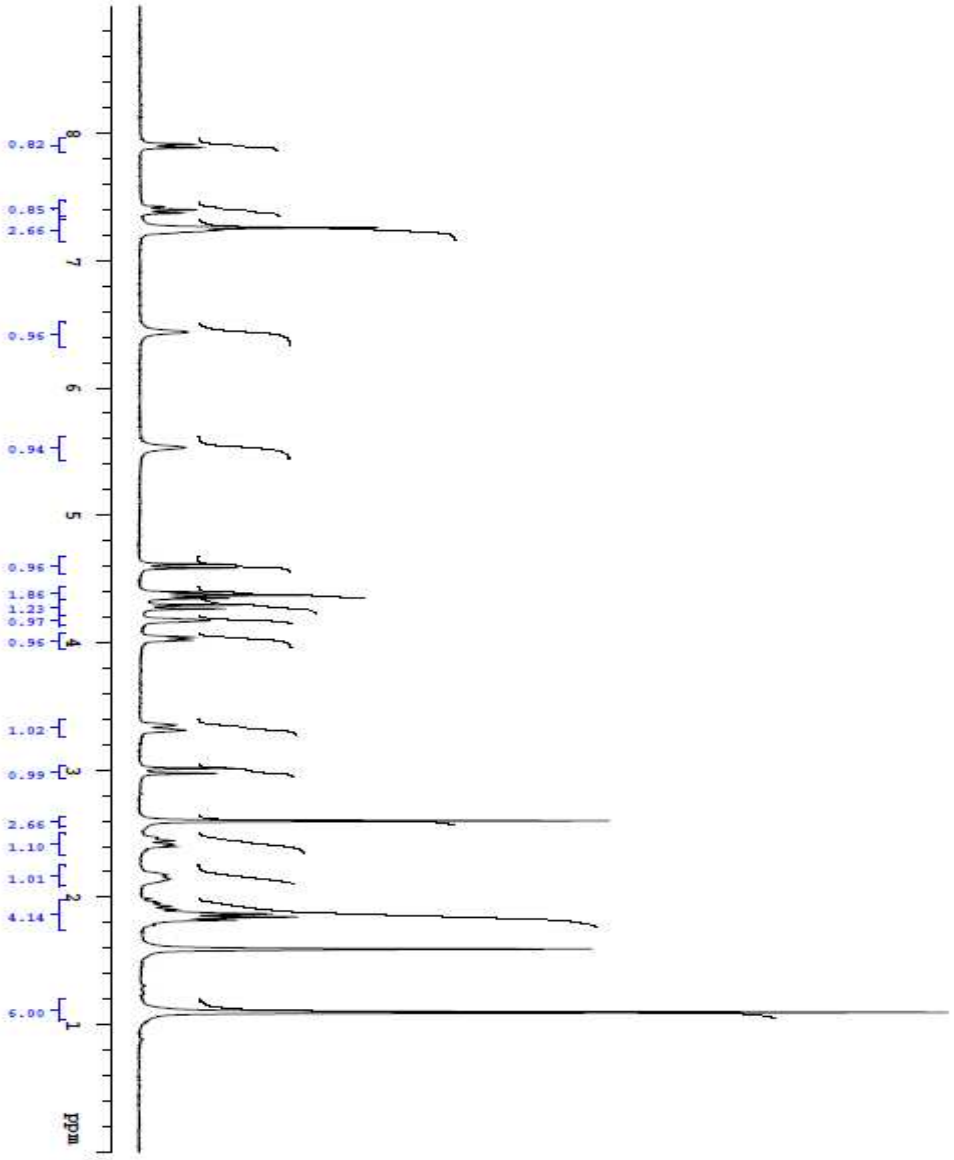


Peak

Index	Name	Time [Min]	Height [mAU]	Area [mAU.Sec]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	12.96	392.2	2279.5	38.0	97.808
2	UNKNOWN	13.64	6.7	48.0	0.8	2.059
3	UNKNOWN	13.80	0.5	3.1	0.1	0.133
Total			399.4	2330.5	38.8	100.000

^{1}H NMR FOR PRODRUG (20)





^{13}C NMR FOR PRODRUG (20)