Enzymatic Deprotection of the Cephalosporin 3' Acetoxy Group Using *Candida antarctica* Lipase B

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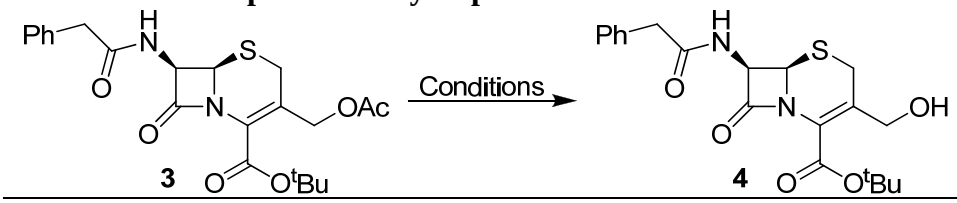
Materials and Methods

Anhydrous tetrahydrofuran and anhydrous toluene were obtained as AcroSeal solvents, anhydrous DCM was distilled over calcium hydride. All chemicals were commercially available and used without further purification; 7-ACA and Lipase acrylic resin from *Candida antarctica* (L-4777), were purchased from Aldrich. ^{1}H NMR were run on a 300 or 500 MHz NMR and ^{13}C NMR were run on a 125 MHz NMR in DMSO-*d*6 or $CDCl_3$ (with TMS as an internal standard); the shifts are reported in parts per million (ppm), and the coupling constants (J) are in hertz. IRs were obtained on an FT-IR spectrometer and reported as cm^{-1}. Mass spectrometry data was obtained on a MicroMass Quattro LC Triple Quadrupole Mass Spectrometer by direct infusion using electrospray ionization in the positive ion mode at the Center for Environmental Science and Technology at the University of Notre Dame or on a Micro TOF II by direct infusion using electrospray ionization in the positive ion mode at the Notre Dame Mass Spectrometry and Proteomics Facility; the samples were doped with 0.001 M NaI in isopropanol. Melting points were obtained on a capillary melting point apparatus and were uncorrected. The deacetylation reactions were performed in an incubated shaker. Reactions were monitored with thin layer chromatography (TLC) on aluminum-backed 0.2 mm silica gel 60 F_{254}. Flash chromatography was performed using silica gel 60 (230-400 mesh).

Attempted Acetoxy Deprotection Reactions

Table S1. Attempted Acetoxy Deprotection Reactions

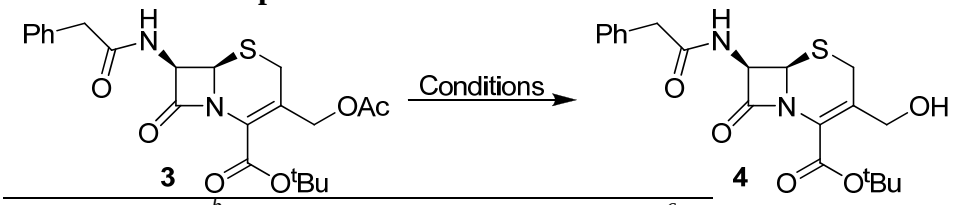


Entry	Conditions	Solvent	Time	Temp.(˚C)	Results	Δ^3:Δ^2
1[a]	K_2CO_3	CH_3OH	45min	room temp	19.7%	Δ^2
2	K_2CO_3	CH_3OH	3hr	<0	N/A[b]	1:5
3[c]	LiOH	CH_3OH	1.5hr	-25	N/A[b]	1:2
4	LiOH	THF/H_2O	4min	-5	N/A[b]	1:4
5	NaOH	THF/H_2O	4min	-10	N/A[b]	1:1
6	KOH	THF/H_2O	4min	-10	N/A[b]	1:1
7	CsOH	THF/H_2O	4min	-5	N/A[b]	1:1.3
8	$(Bu)_4NOH$	THF/H_2O	4min	-5	N/A[b]	1:7
9	KCN	CH_3OH	5.5hr	room temp	Trace	Δ^2
11	HCl	CH_3OH	O/N[d]	room temp	Total Deprotection[e]	Δ^3
12	HCl	CH_3OH	7hr	room temp	Trace	Δ^3
13	HCl[f]	CH_3OH	5days	room temp	Total Deprotection[e]	Δ^3
14[g]	$((Bu)_3SnO)_2$	THF	O/N[d]	room temp	No Reaction	N/A

[a] Decreased yield after 2h; [b] Product was not isolated due to unfavorable isomerization determined by ^{1}H NMR spectroscopy; [c] Ring opening after 4h; [d] Overnight; [e]Both the acetoxy and *t*-butyl groups were removed; [f]Addition over time; [g]Ring opening was seen upon refluxing.

CAL B Lot Comparisons

TABLE S2. Comparison of Different Lots of CAL B[a]



Entry	CAL B[b]	Additive	% Conversion[c]
1	A	*n*-butanol	48%
2	B	*n*-butanol	6%
3	C	*n*-butanol	5%
4	A	*s*-butanol	85%
5	B	*s*-butanol	11%
6	C	*s*-butanol	18%
7	A	*s*-butanol, MS[d]	93%
8	B	*s*-butanol, MS[d]	34%
9	C	*s*-butanol, MS[d]	38%

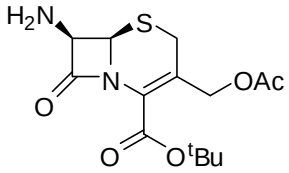
[a]All reactions were performed in an incubated shaker with 50 mass % CAL B, at 50 °C, in 9:1 hexanes/THF, for 4 nights; [b]lot A: 074K0651 (0.32% loss on drying), lot B: 047K1672 (1% loss on drying), lot C: 067K3522 (loss on drying not available); [c]% Conversions determined using ^{1}H NMR spectroscopy; [d]4 Å molecular sieves.

TABLE S3. Optimization of Deacetylation Using CAL B lot 047K1672[a]

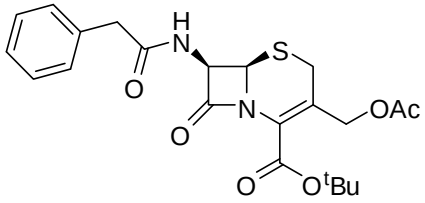
Entry	CAL B[b]	4 Å Molecular Sieves[b]	Time[c]	% Conversion[d]
1	50	500	4	85
2	50	100	4	81
3	50	500	8	93
4	100	500	4	93
5	100	500	8	96

[a]All reactions were performed in an incubated shaker with CAL B lot 047K1672 and *s*-butanol, at 50 °C, in 9:1 hexanes/anhydrous THF; [b]mass % based on amount of **3**; [c]days, [d]Conversions determined using ^{1}H NMR spectroscopy.

Experimental Procedures and Characterization Data

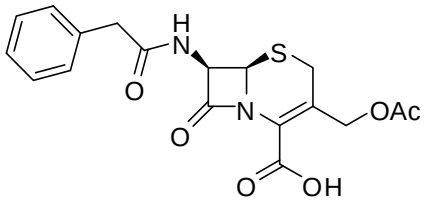


(6R,7R)-*tert*-Butyl 3-(acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (2). 7-Aminocephalosporanic acid, **1** (5.0912 g, 18.699 mmol), was suspended in *t*-butyl acetate (300 mL) in a sealed tube. p*ara*-Toluene sulfonic acid (3.9129 g, 20.571 mmol) was added and the suspension was stirred for 15 min. Sulfuric acid (5.7 mL, 102.66 mmol) was added over 15 min. Following the addition, the flask was sealed and stirred 3-6 h, until mostly homogenous. The sealed flask was opened slowly, and the reaction was quenched with saturated $NaHCO_3$ solution, until the reaction was slightly basic. The *t*-butyl acetate layer was removed and the aqueous layer was extracted with ethyl acetate. The organic extracts were combined, dried over Na_2SO_4, filtered by gravity, the solvent was removed *in vacuo*, and the yellow solid was dried under vacuum. The material was purified using a purification system and a column from Analogix, SEPRA Si 50, 90g. The product was eluted using a gradient from 10% EtOAc in DCM to 20% EtOAc in DCM to give **2** as a light yellow solid (3.4556g, 56%). This purification step is not necessary and the subsequent reaction does not suffer from using this material crude. mp 95-98 °C (lit. 114-115 °C)[1]; R_f 0.13 (5:1 DCM/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.53 (s, 9H), 1.88 (bs, 2H), 2.07 (s, 3H), 3.34 (d, J=18.3, 1H), 3.54 (d, J=18, 1H), 4.74 (overlapping d, J=5.1, 1H), 4.77 (overlapping d, J=12.9, 1H), 4.93 (d, J=5.1, 1H), 5.02 (d, J=13.2, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.8, 26.0, 27.7, 58.7, 63.2, 63.7, 83.6, 122.3, 127.7, 160.7, 168.4, 170.6; ESI-MS *m/z* 351.5 $[M+Na]^+$.

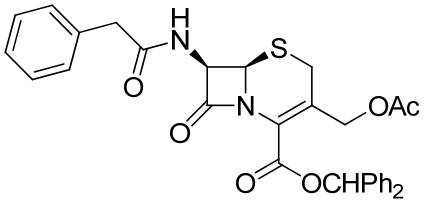


(**6R,7R)-*tert*-Butyl 3-(acetoxymethyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (3).** Compound **2** (3.4426 g, 10.483 mmol) was dissolved in CH_2Cl_2 (100 mL) and saturated $NaHCO_3$ solution (100 mL) was added. Phenylacetyl chloride (2.8 mL, 21.173 mmol) was added and the reaction was stirred vigorously, at room temperature, for 1 h. The aqueous portion was removed, and the organic layer was washed with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$, filtered by gravity, and the solvent was removed *in vacuo*. The material was purified using a purification system and a column from Analogix, SEPRA Si 50, 90g. The product was eluted using a gradient from 100% CH_2Cl_2 to 10% EtOAc in CH_2Cl_2. Compound **3** was isolated as a white solid (2.788 g, 60%). mp 156.5-158 ˚C (d) (lit. 148-150 °C)[2]; R_f 0.6 (5:1 CH_2Cl_2/EtOAc); IR (thin film) 1782, 1744, 1720, 1666; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.51 (s, 9H), 2.07 (s, 3H), 3.31 (d, J=18.5, 1H), 3.51 (d, J=18.5, 1H), 3.58-3.66 (overlapping d, 2H), 4.76 (d, J=13, 1H), 4.91 (d, J=5, 1H), 5.08 (d, J=13, 1H), 5.82 (dd, J=4.5, 9, 1H), 6.64 (d, J=9, 1H), 7.24-7.31 (m, 3H), 7.31-7.36 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.9, 26.5 27.9, 43.3, 57.4, 59.2, 63.2, 83.9, 123.8, 127.3, 127.6,

129.1, 129.5, 134.0, 160.4, 164.7, 170.7, 171.5; HRMS (ESI-TOF) Calcd for $C_{22}H_{26}N_2NaO_6S$, 469.1404; Found, 469.1378.

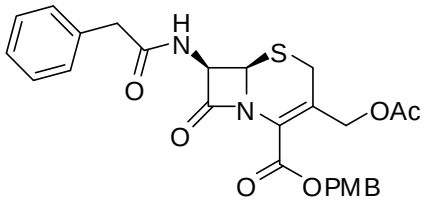


(6R,7R)-3-(Acetoxymethyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (5). 7-ACA, **1**, (1.0216 mg, 11.256 mmol) was dissolved in saturated $NaHCO_3$ solution (25 mL) and acetone (9 mL) was added. Phenylacetyl chloride (1.5 mL, 11.343 mmol) was added and the reaction became light yellow. The reaction was stirred at room temperature for 16 h. Methylene chloride (40 mL) was added and the reaction mixture was acidified using 6 N HCl to pH 2. The organic layer was removed and the aqueous portion was extracted again with CH_2Cl_2; the organic extracts were combined, washed with brine, dried over $MgSO_4$, gravity filtered, and the solvent was removed *in vacuo* giving a light yellow/off-white solid. This material was resuspended in ether and stirred for 8 hours to remove excess phenylacetic acid; the product was isolated through vacuum filtration as an off-white solid (804.4 mg, 55%). mp 154-156 °C (d) (lit 168-171 °C (d))[3]; R_f 0.67 (RP, 1:1 water/CH_3CN); ^{1}H NMR (500 MHz, DMSO-*d*6): δ 2.03 (s, 3H), 3.49 (overlapping d, J=17, 1H), 3.49 (overlapping d, J=14.5, 1H), 3.57 (d, J=14, 1H), 3.62 (d, J=18, 1H), 4.69 (d, J=12.5, 1H), 5.00 (d, J=12.5, 1H), 5.08 (d, J=5, 1H), 5.69 (dd, J=5, 8, 1H), 7.20-7.25 (m, 1H), 7.25-7.32 (m, 4H), 9.13 (d, J=8, 1H); ^{13}C NMR (125 MHz, DMSO-*d*6): δ 20.6, 25.6, 41.6, 57.5, 59.1, 62.8, 123.3, 126.4, 126.5, 128.3, 129.0, 135.8, 162.9, 164.8, 170.3, 171.0; HRMS (ESI-TOF) Calcd for $C_{18}H_{18}N_2NaO_6S$, 413.0774; Found, 413.0778.

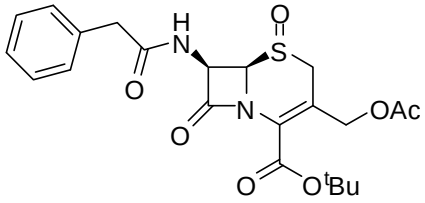


(6R,7R)-Benzhydryl 3-(hydroxymethyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (6). Diphenyldiazomethane[16b] (7.70 mmol) as a solution in CH_2Cl_2 (20 mL) was added to a suspension of compound **5** (1.0088 g, 2.584 mmol) in CH_2Cl_2 (5 mL) and immediately bubbling/frothing was seen which lasted a couple seconds. The reaction was stirred at room temperature, under argon, for 45 min; reaction completion was confirmed by TLC (RP 1:1 water/acetonitrile). Silica gel (~30 mL) was added into the reaction and stirred for 1 h until the purple color was gone, the mixture was yellow/tan in color. The solvent was removed *in vacuo* and the silica mixture was loaded onto a silica column for purification. The material was eluted with DCM and then 10% EtOAc in DCM. The solvent was removed *in vacuo* and the material was dried under vacuum to give **6** as an off-white solid/foam (1.1659 g, 81.1%). mp 143-144.5 °C; R_f 0.76 (5:1 DCM/EtOAc); IR (KBr pellet) 1783, 1739, 1712, 1651 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.99 (s, 3H), 3.27 (d, J=18.5, 1H), 3.44 (d, J=18.5, 1H), 3.54-3.63 (overlapping d, 2H), 4.74 (d, J=14, 1H), 4.90 (d, J=5, 1H), 5.00 (d, J=13.5, 1H), 5.84 (dd, J=5, 9, 1H), 6.55 (d, J=9, 1H), 6.90 (s, 1H), 7.20-7.35 (m, 13H), 7.38-7.42 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.8, 26.5, 43.3, 57.5, 59.2, 63.1, 79.9, 125.5,

127.1, 127.3, 127.7, 128.2, 128.3, 128.6, 128.7, 129.2, 129.6, 133.9, 139.1, 139.3, 160.7, 165.1, 170.6, 171.5; HRMS (ESI-TOF) Calcd for $C_{31}H_{28}N_2NaO_6S$, 579.1560; Found, 579.1558.

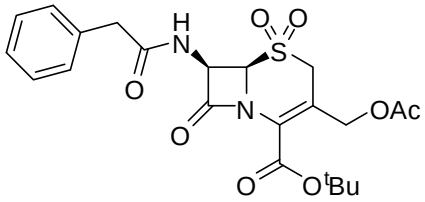


(6R,7R)-4-Methoxybenzyl 3-(acetoxymethyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (7). Sodium bromide (594.0 mg, 4.77 mmol) was added to a flame dried round bottom flask and suspended in anhydrous DMF (1 mL). *para*-Methoxybenzyl chloride (0.160 mL, 0.768 mmol) was added and immediatly a white precipitate formed. The reaction was stirred at room temperature under argon for 2 h. The reaction mixture was filtered under argon into a flame dried round bottom flask and the white solid was washed with 2 mL of DMF (anhydrous). Compound **5** (304.3 mg, 0.779 mmol) and $NaHCO_3$ (72.3 mg, 0.861 mmol) were added. The reaction immediately turned tan. The reaction was stirred at room temperature, under argon, overnight. The reaction mixture was diluted with EtOAc (5mL) and saturated $CaCl_2$ (5 mL) solution was added, which caused emulsions to form. The organic layer was removed and the aqueous layer was extracted with EtOAc. The organic extracts were combined and washed with saturated $CaCl_2$, water, saturated $NaHCO_3$ solution, water, and brine. The organic portion was dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo* to give 280 mg of a tan oil. The material was purified using a silica gel column chromatography to yield 228.9 mg, 57.5%. mp 112-115 °C; R_f 0.62 (5:1 DCM/EtOAc); IR (thin film) 1785, 1736, 1748, 1672 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.02 (s, 3H), 3.29 (d, J=18.5, 1H), 3.47 (d, J=18, 1H), 3.57 (s, 2H), 3.75 (s, 3H), 4.77 (d, J=13, 1H), 4.86 (d, J=5, 1H), 5.07 (d, J=13.5, 1H), 5.11-5.20 (overlapping d, 2H), 5.77 (dd, J=4.5, 9, 1H), 6.83-6.87 (m, 2H), 6.91 (d, J=9, 1H), 7.22-7.27 (m, 3H), 7.27-7.33 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.7, 26.4, 43.1, 55.2, 57.5, 59.2, 63.1, 68.1, 114.0, 125.5, 125.9, 126.7, 127.4, 128.9, 129.3, 130.6, 134.1, 159.9, 161.3, 164.9, 170.6, 171.5; HRMS (ESI-TOF) Calcd for $C_{26}H_{26}N_2NaO_7S$, 533.1353; Found, 533.1350.



(**6R,7R)-*tert*-Butyl 3-(acetoxymethyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5-oxide (8).** Cephalosporin **3** (1.0027 g, 2.246 mmol) was dissolved in anhydrous CH_2Cl_2 (25 mL) and cooled to -5 °C (internal temp) using a salt-ice bath and *m*CPBA (213.7 mg, 0.953 mmol) was added. A TLC was taken after 5 min, a small amount of the sulfone along with the sulfoxide had formed and some cephalosporin **3** remained. Another portion of *m*CPBA (213.6 mg, 0.953 mmol) was added at 8 min. After 5 min, another TLC confirmed that the reaction was complete. Saturated $NaHCO_3$ solution and CH_2Cl_2 were added with stirring, while the reaction remained at -5 °C. The aqueous layer was removed and the reaction mixture was further washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The material was purified using a silica column, loading with CH_2Cl_2, and eluting with 10-25% EtOAc in CH_2Cl_2. The sulfone eluted in 15% EtOAc in CH_2Cl_2 (19.0 mg). The sulfoxide eluted in 25% EtOAc in

CH_2Cl_2. The fractions were collected containing the sulfoxide. The solvent was removed *in vacuo* and the material dried under vacuum to give a yellowish glassy solid (743.9 mg, 69.2%). mp 143-147 °C (d); R_f 0.28 (5:1 DCM/EtOAc); IR (KBr) 1789, 1746, 1716, 1668, 1047 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.53 (s, 9H), 2.07 (s, 3H), 3.19 (d, J=18.5, 1H), 3.60 (overlapping d, 2H), 3.70 (d, J=18.5, 1H), 4.43 (d, J=5, 1H), 4.66 (d, J=13.5, 1H), 5.27 (d, J=13.5, 1H), 6.00 (dd, J=5, 10, 1H), 7.00 (d, J=10, 1H), 7.24-7.29 (m, 3H), 7.30-7.35 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.9, 27.8, 43.2, 45.9, 59.2, 63.3, 66.9, 84.4, 118.2, 127.2, 127.5, 129.0, 129.5, 134.1, 159.5, 164.3, 170.6, 171.6; HRMS (ESI-TOF) Calcd for $C_{22}H_{26}N_2NaO_7S$, 485.1353; Found, 485.1350.

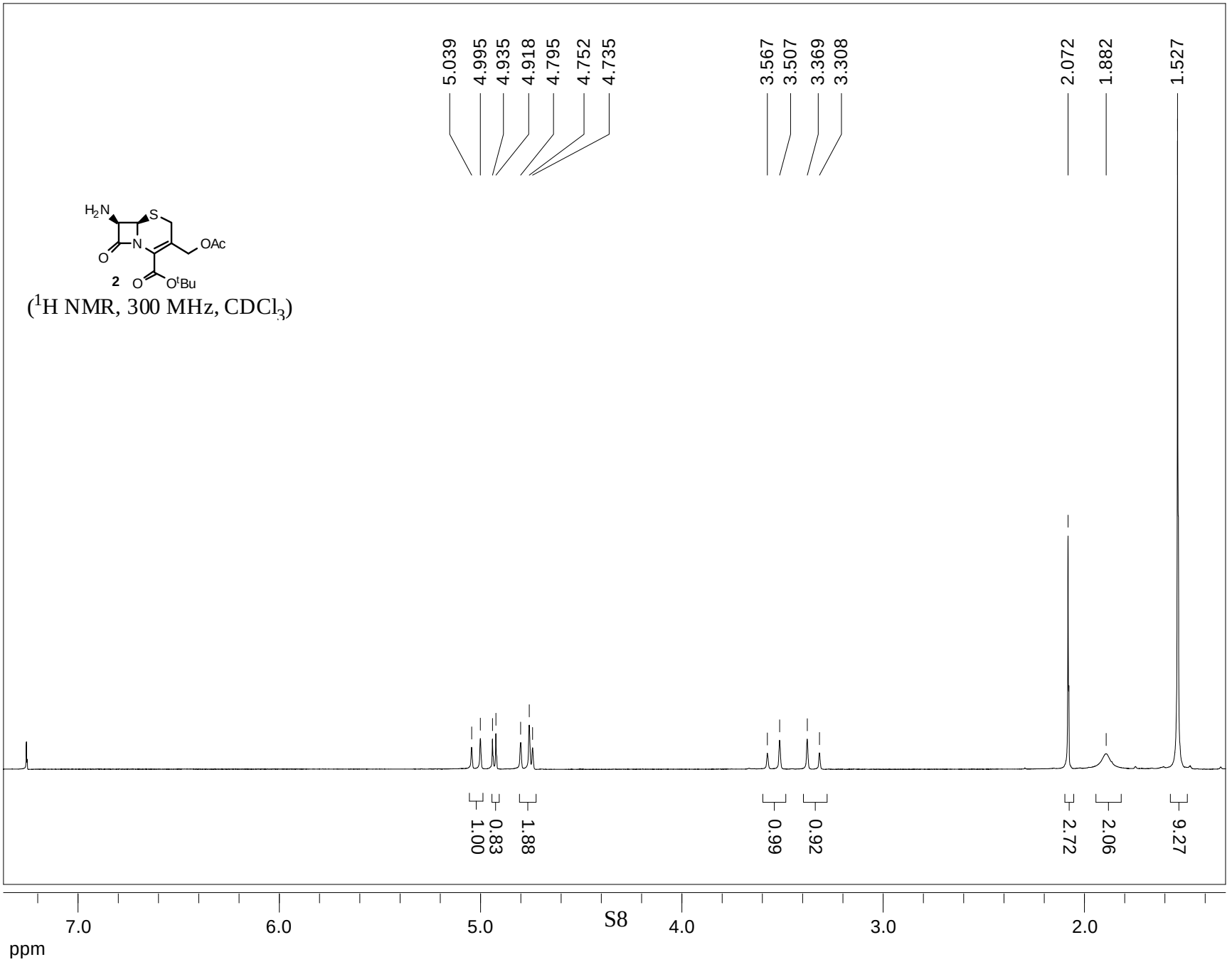


(6R,7R)-*tert*-Butyl 3-(acetoxymethyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate-5,5-dioxide (9). Cephalosporin **3** (302.2 mg, 0.677 mmol) was dissolved in CH_2Cl_2 (18 mL), cooled to 0 °C in an ice bath, and *m*CPBA (304.3 mg, 1.358 mmol) was added. The reaction was stirred at 0-5 °C for 2h. The reaction was diluted to 10 mL using CH_2Cl_2. The resulting solution was washed with saturated $NaHCO_3$ solution brine, dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The resulting material (361.1 mg) was purified using a silica column and eluting with 5-10% EtOAc in CH_2Cl_2 until the sulfone was obtained; the solvent was removed *in vacuo* to give an off-white foam/solid (268.0 mg, 85.6%). mp 72.5-79 °C; R_f 0.40 (5:1 DCM/EtOAc); IR (thin film) 1799, 1749, 1725, 1679, 1333, 1157 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.51 (s, 9H), 2.07 (s, 3H), 3.56-3.64 (overlapping d, 2H), 3.67 (d, J=19, 1H), 3.96 (d, J=18.5, 1H), 4.72 (d, J=14, 1H), 4.80 (d, J=5, 1H), 5.11 (d, J=14, 1H), 6.08 (dd, J=5, 10.5, 1H), 7.09 (d, J=10, 1H), 7.22-7.28 (m, 3H), 7.29-7.34 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.7, 27.7, 42.8, 51.4, 58.8, 61.9, 67.1, 84.9, 124.1, 125.8, 127.4, 128.9, 129.7, 133.6, 159.3, 164.1, 170.4, 171.4; HRMS (ESI-TOF) Calcd for $C_{22}H_{26}N_2NaO_8S$, 501.1302; Found, 501.1287.

References

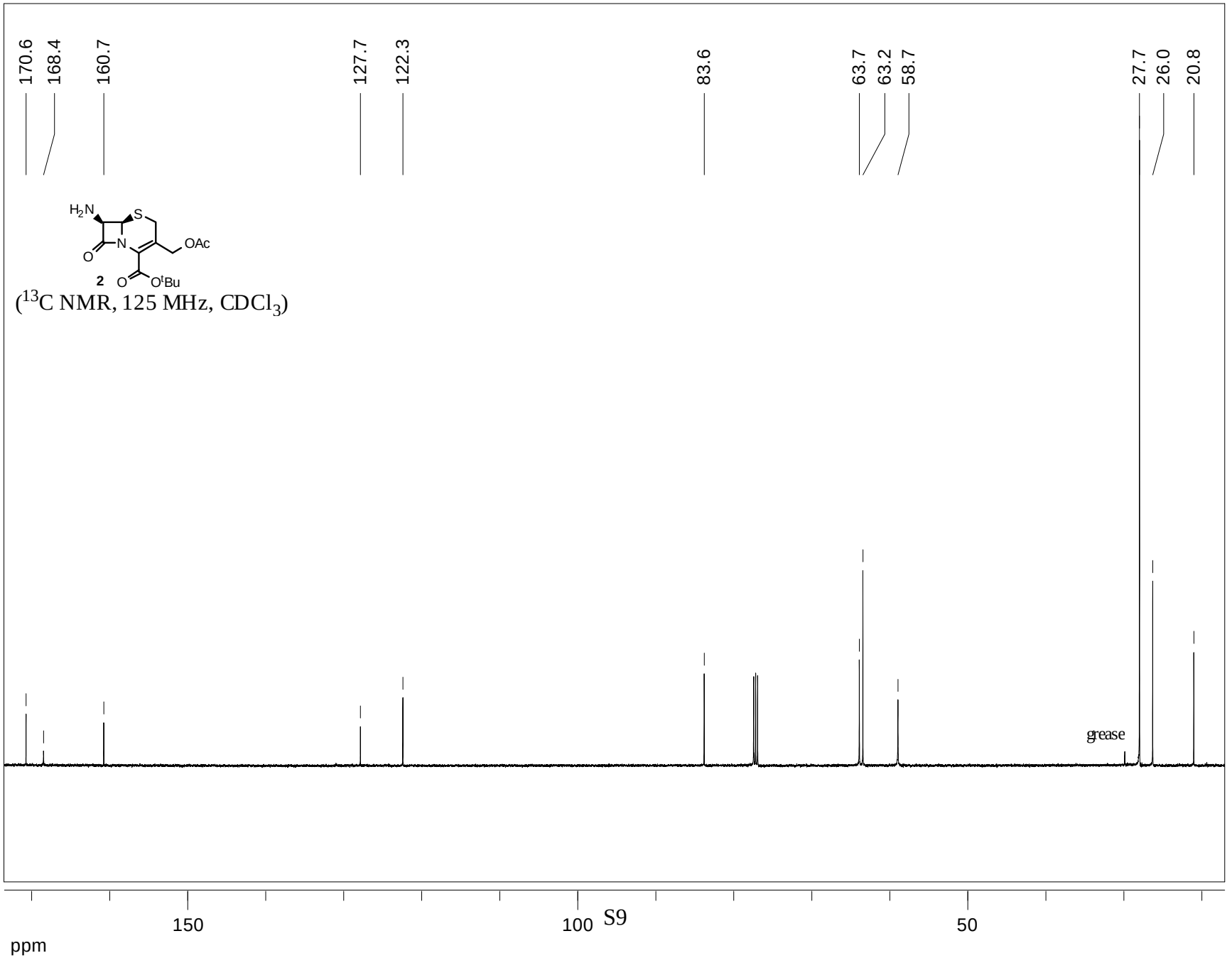
(1) Stedman, R. J. *J. Med. Chem*. **1966**, *9*, 444.
(2) Altman, J.; Karoly, E.; Maoz, N. *J. Med. Chem*. **1975**, *18*, 627.
(3) Keltjens, R.; Vadivel, S. K.; de Vroom, E.; Klunder, J. H.; Zwanenburg, B. *Eur. J. Org. Chem*. **2001**, *13*, 2529-2534.

^1H and ^{13}C NMR Spectra:



2

(^{1}H NMR, 300 MHz, $CDCl_3$)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168.4
160.7
127.7
122.3
83.6
63.7
63.2
58.7
27.7
26.0
20.8
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grease
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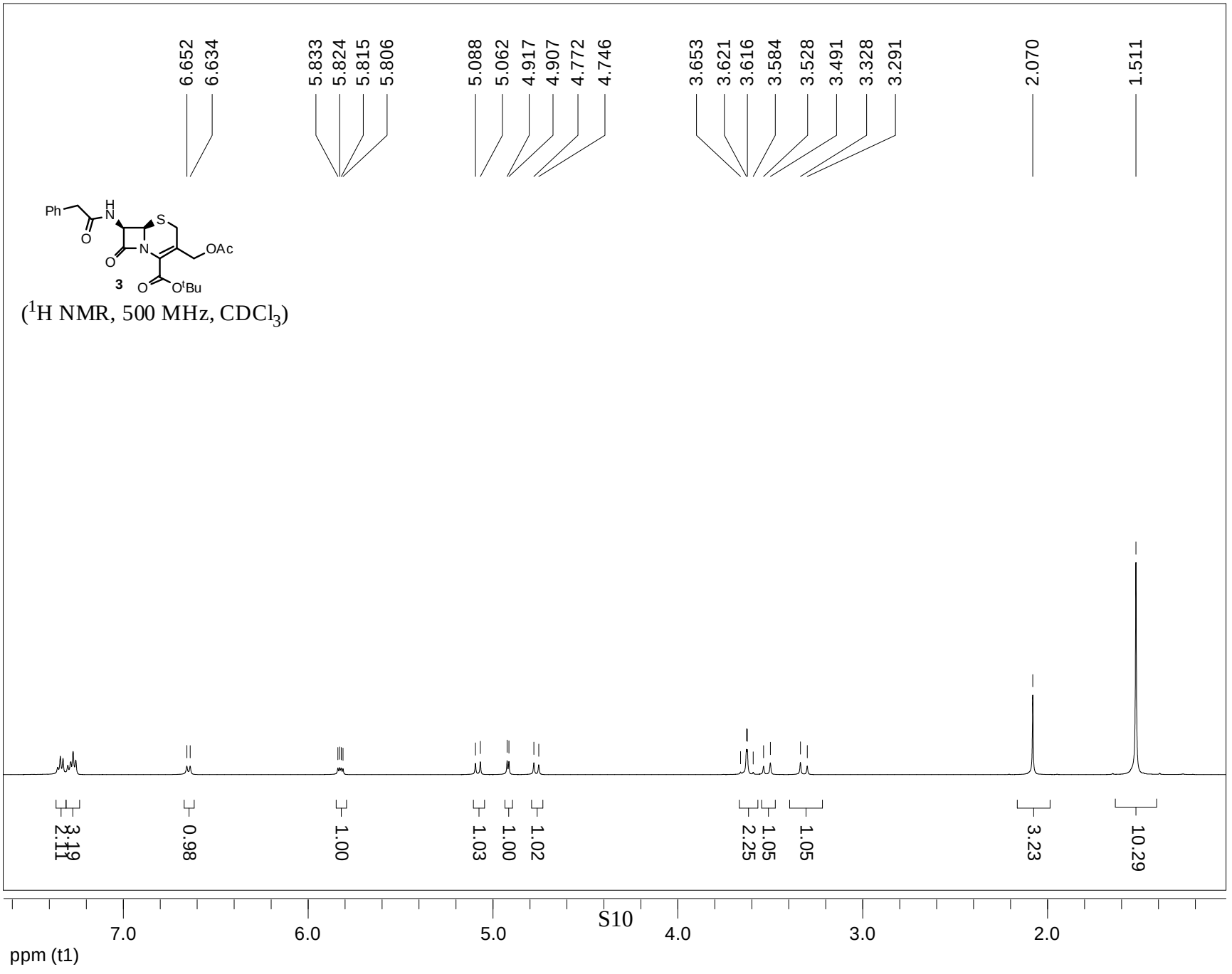
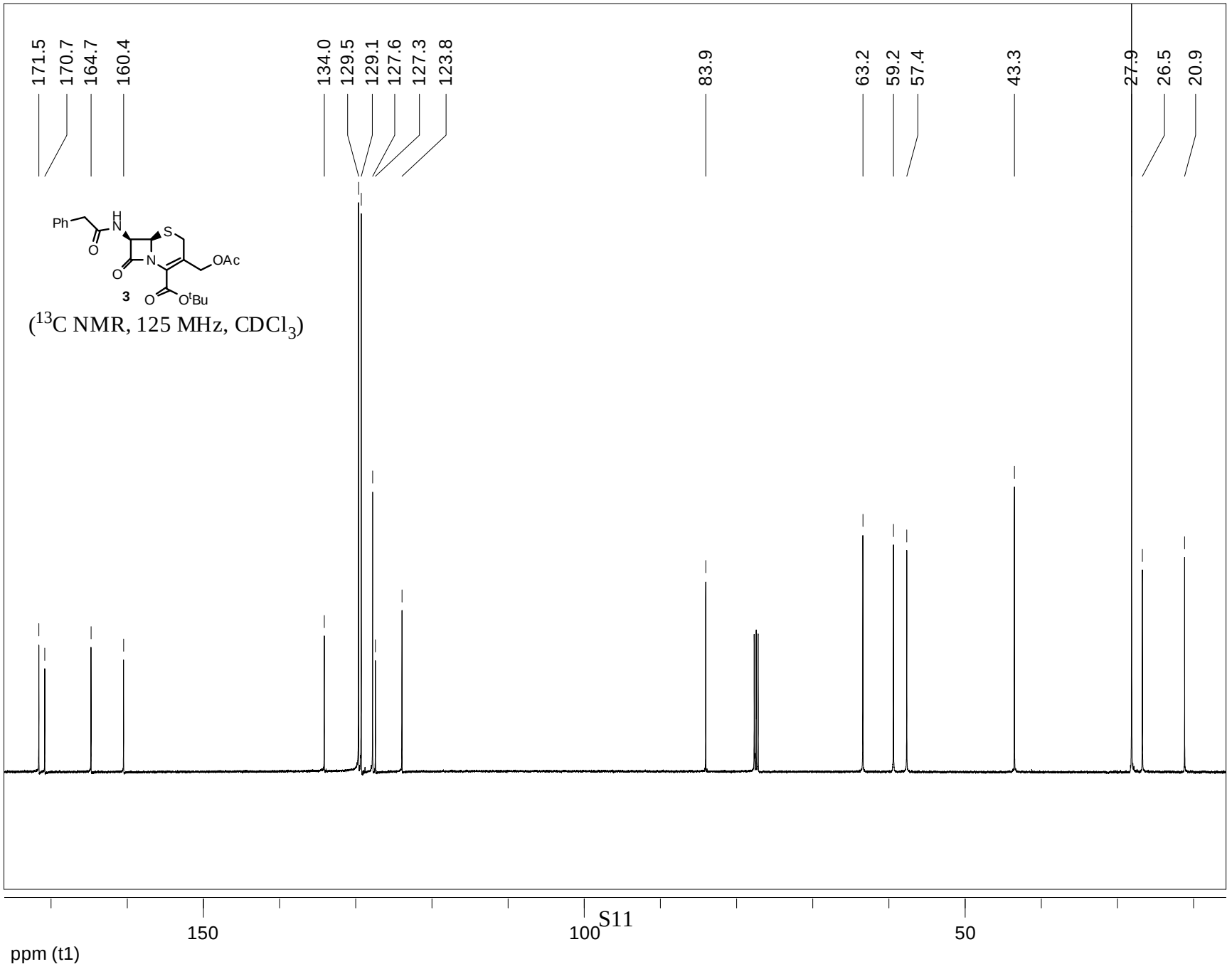
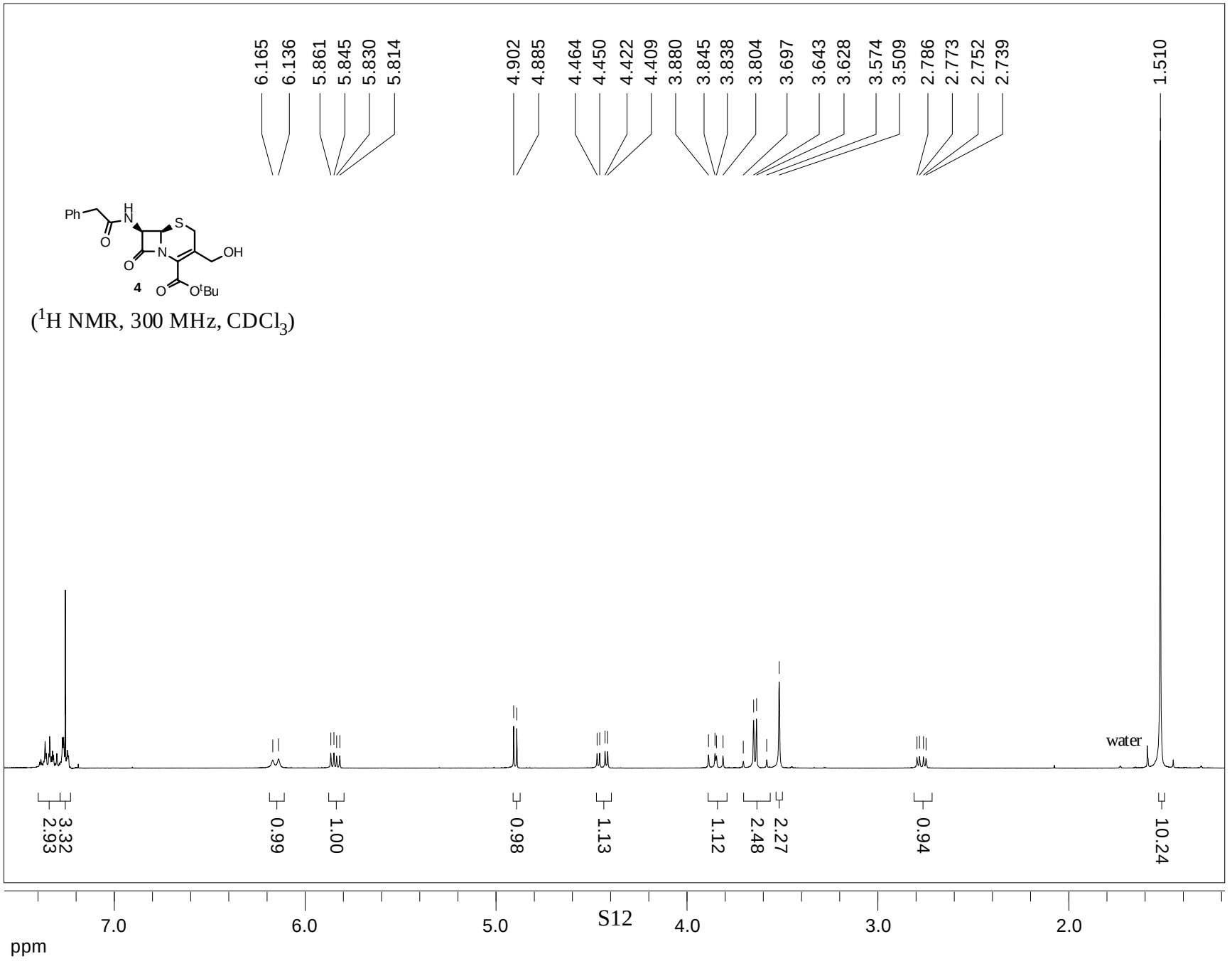
ppm



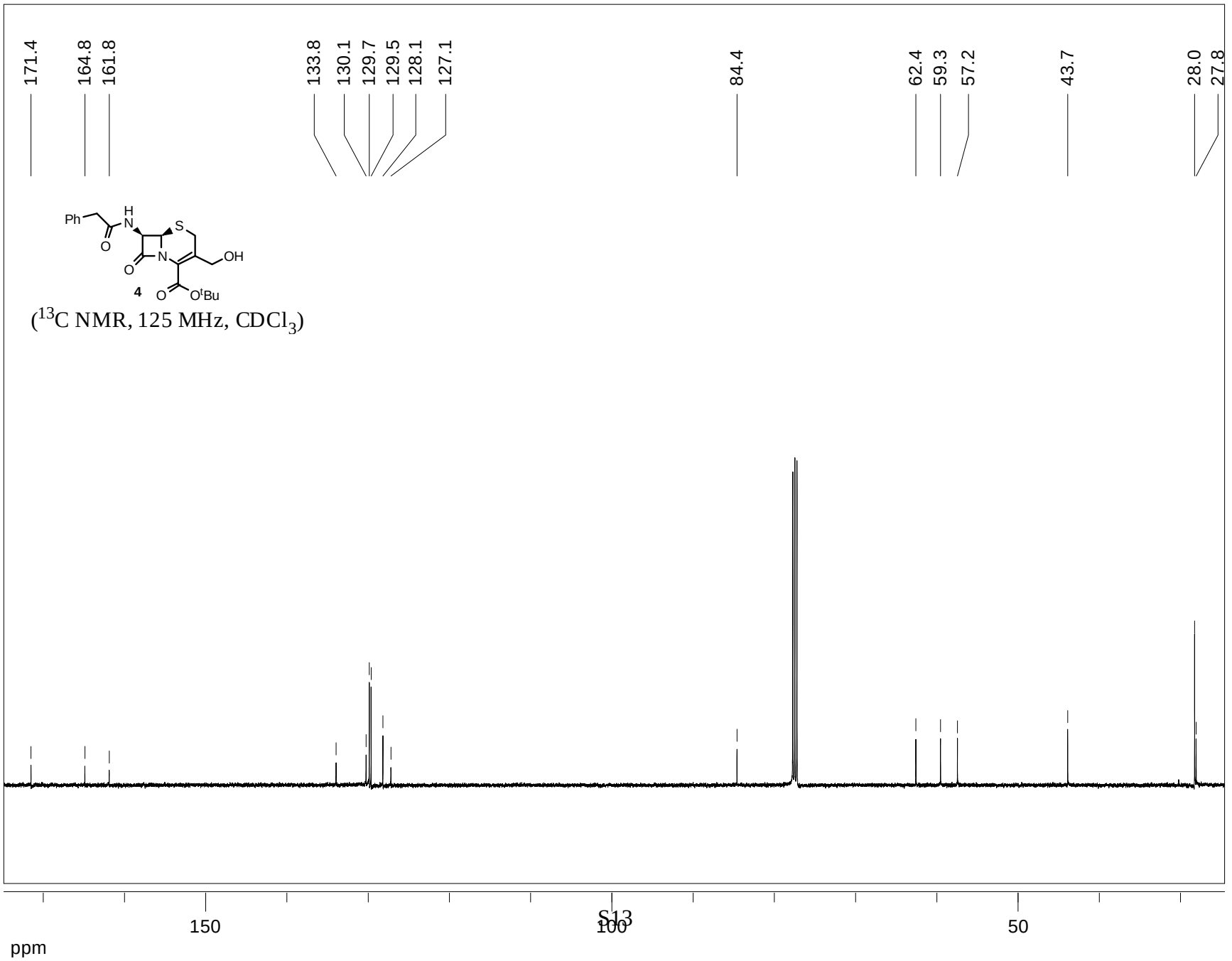
(^{1}H NMR, 500 MHz, $CDCl_3$)



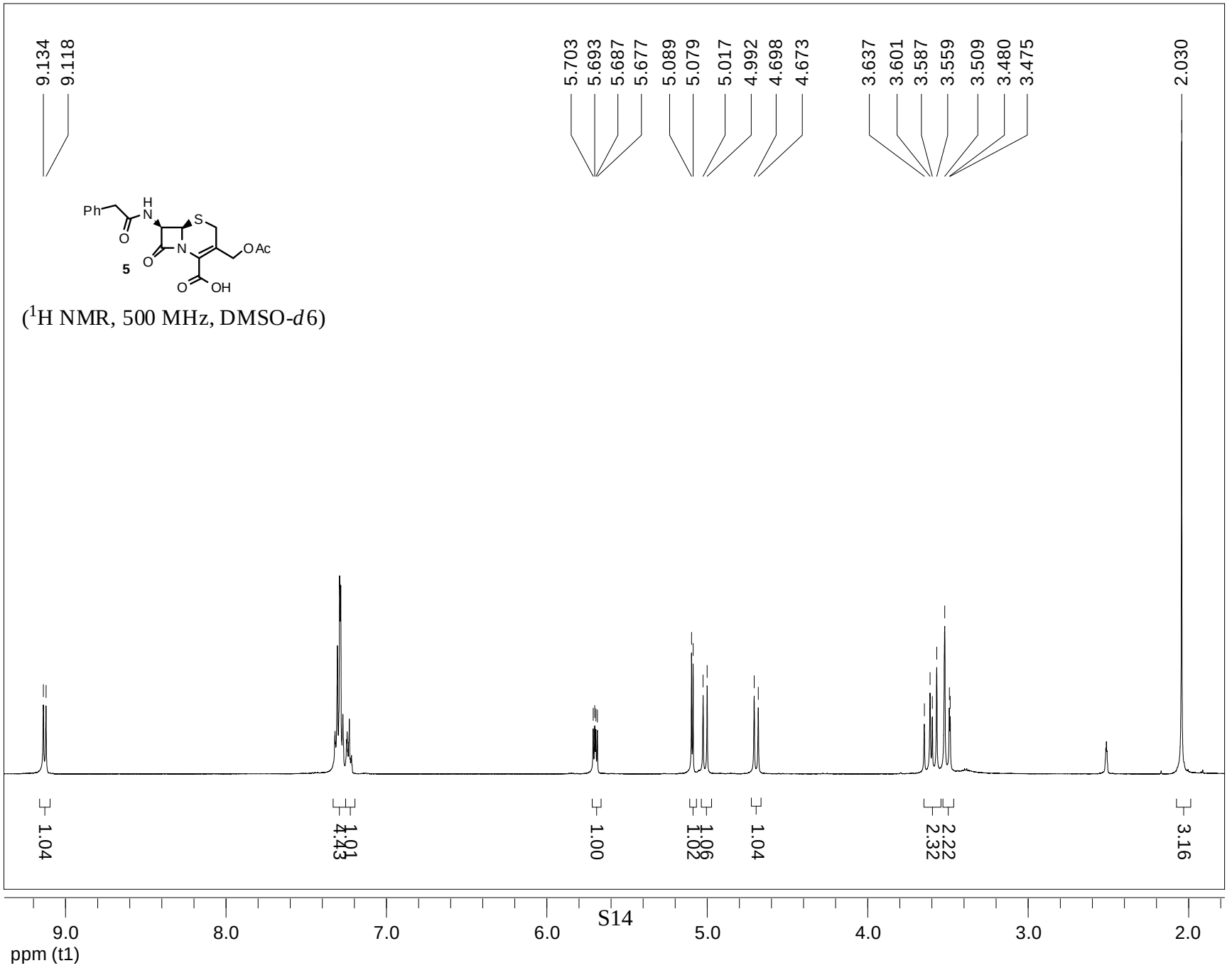
(^{13}C NMR, 125 MHz, $CDCl_3$)



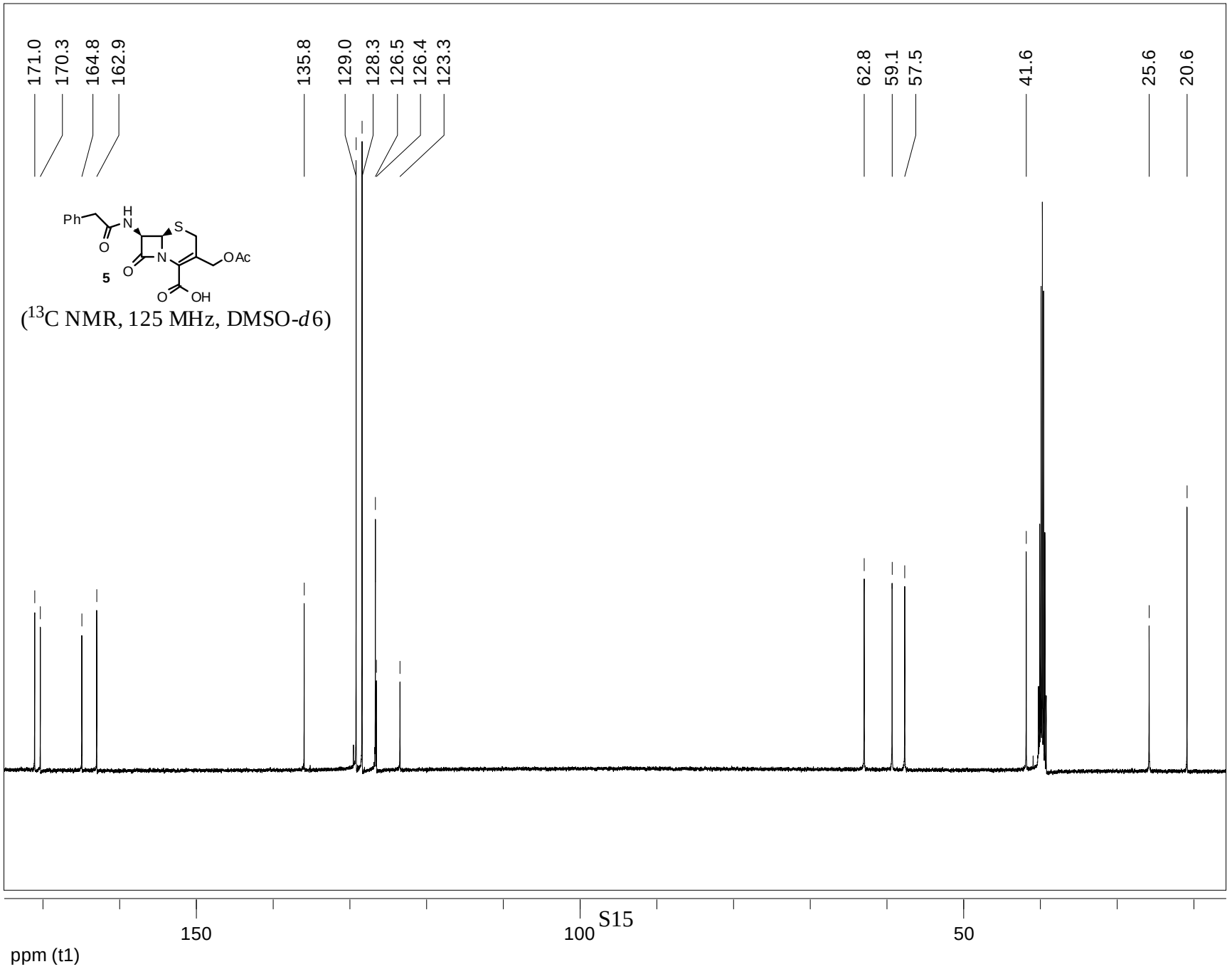
(^{1}H NMR, 300 MHz, $CDCl_3$)



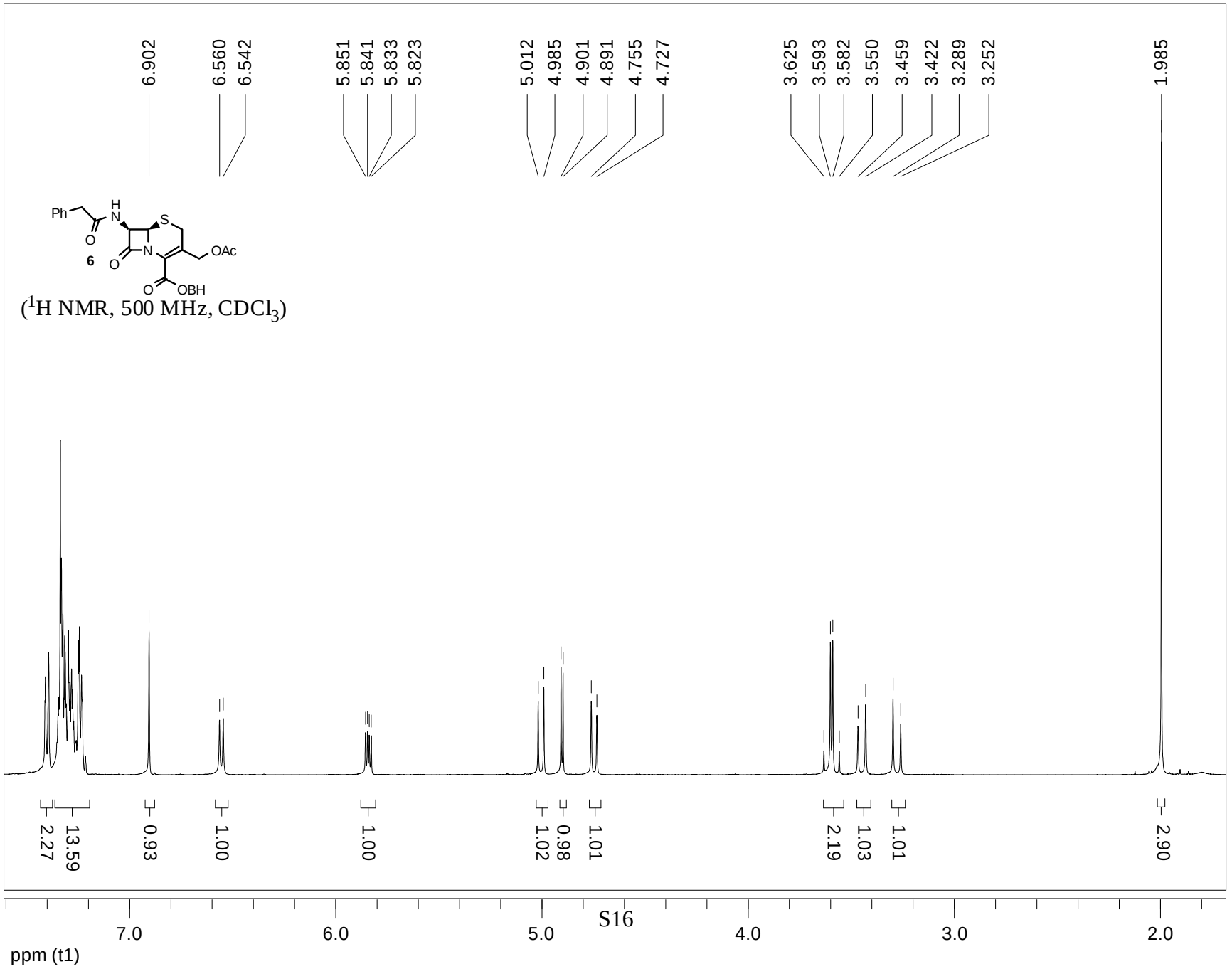
(^{13}C NMR, 125 MHz, $CDCl_3$)



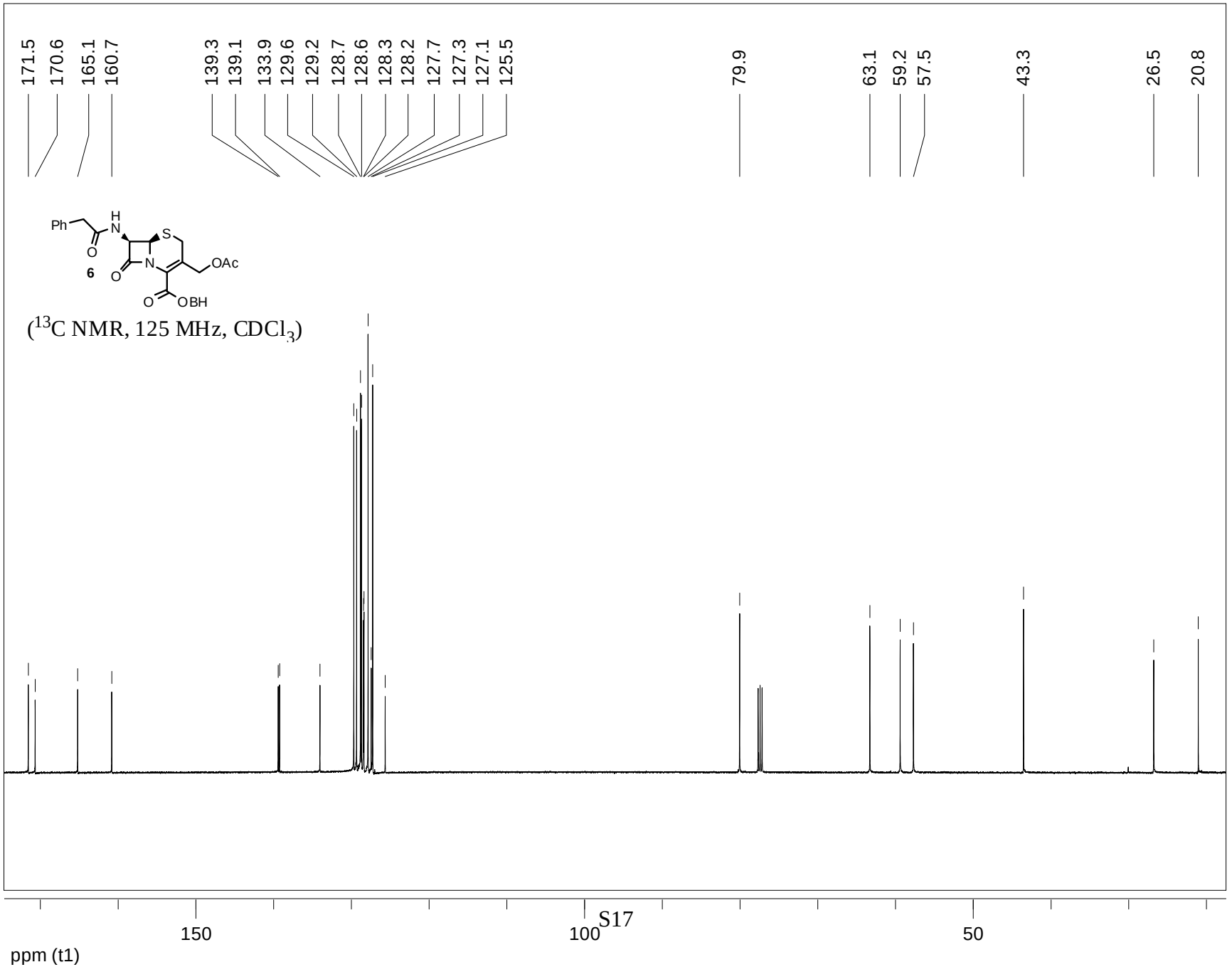
(^{1}H NMR, 500 MHz, DMSO-*d*6)

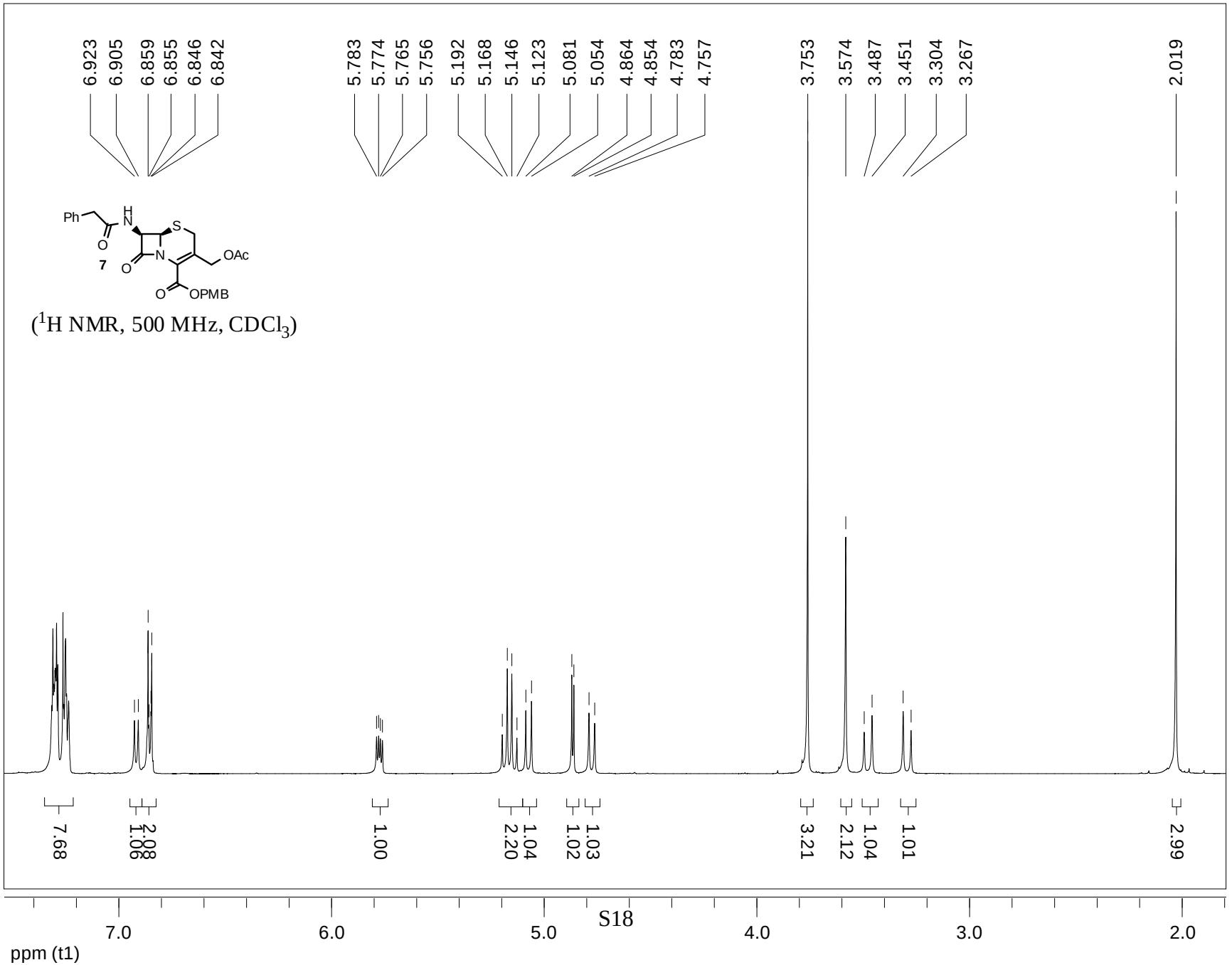


(^{13}C NMR, 125 MHz, DMSO-*d*6)

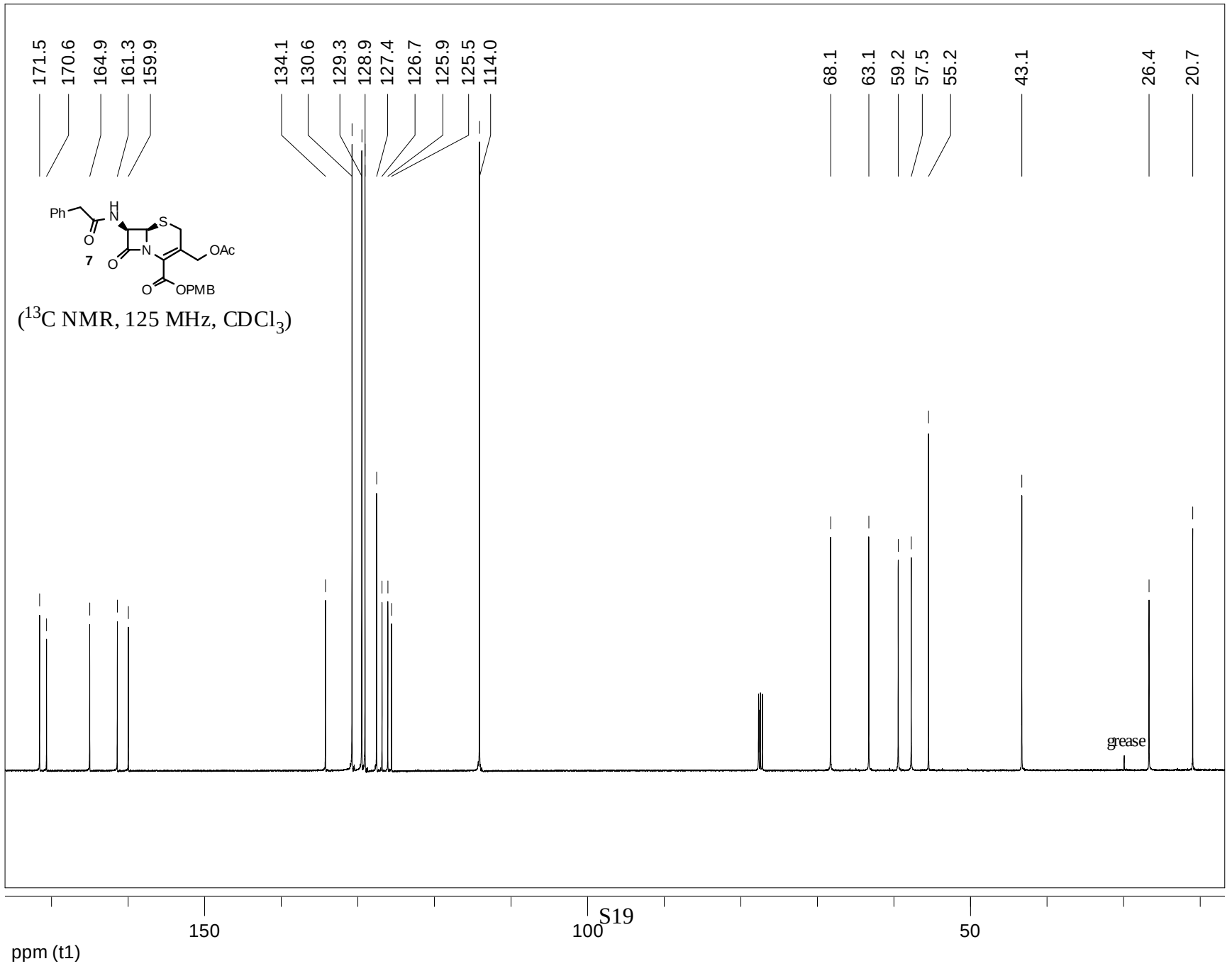


(^{1}H NMR, 500 MHz, $CDCl_3$)

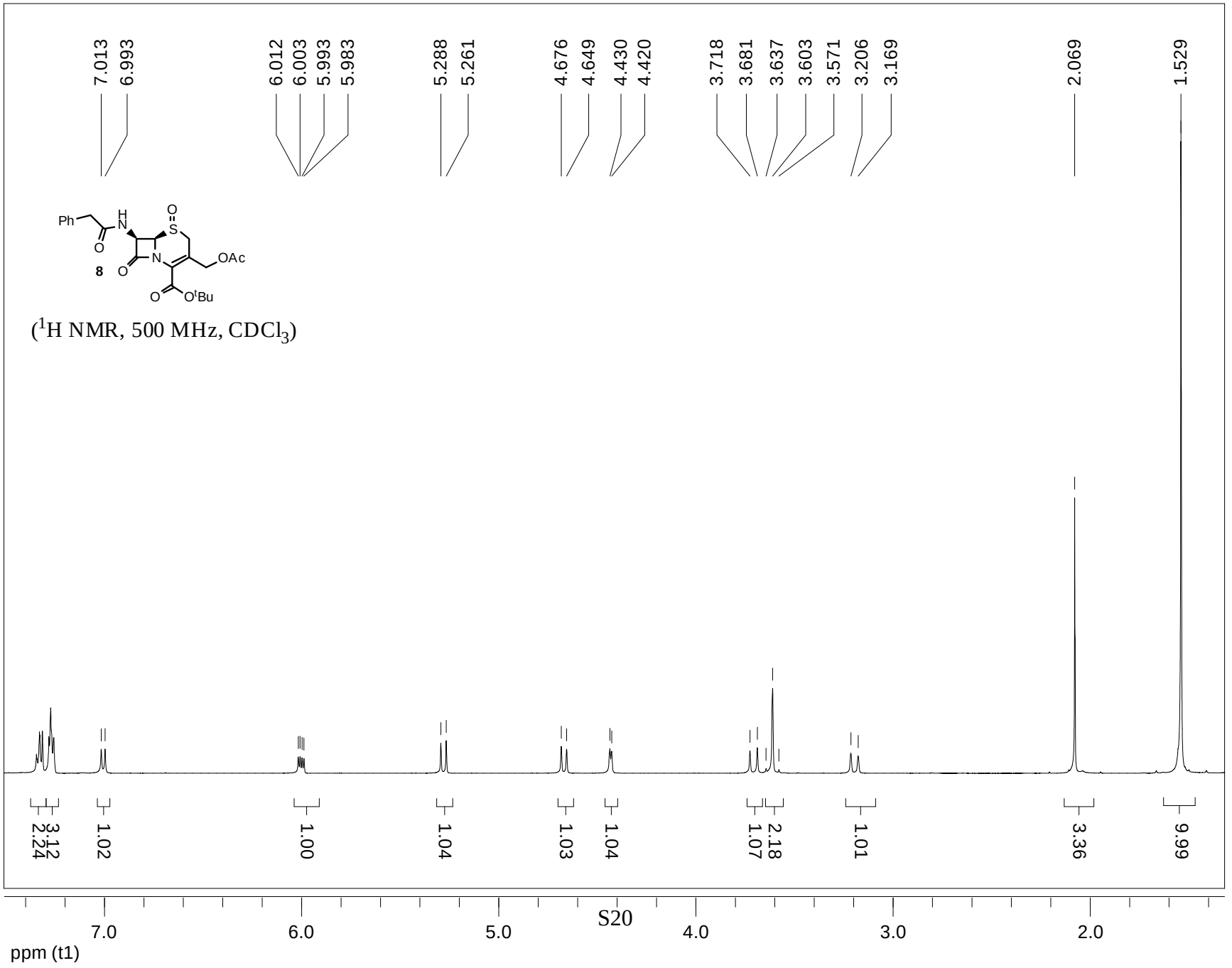

171.5
170.6
165.1
160.7
139.3
139.1
133.9
129.6
129.2
128.7
128.6
128.3
128.2
127.7
127.3
127.1
125.5
79.9
63.1
59.2
57.5
43.3
26.5
20.8
Ph
H
N
S
O
N
O
OAc
O
O
OBH
6
(^{13}C NMR, 125 MHz, $CDCl_3$)
150
100
50
ppm (t1)



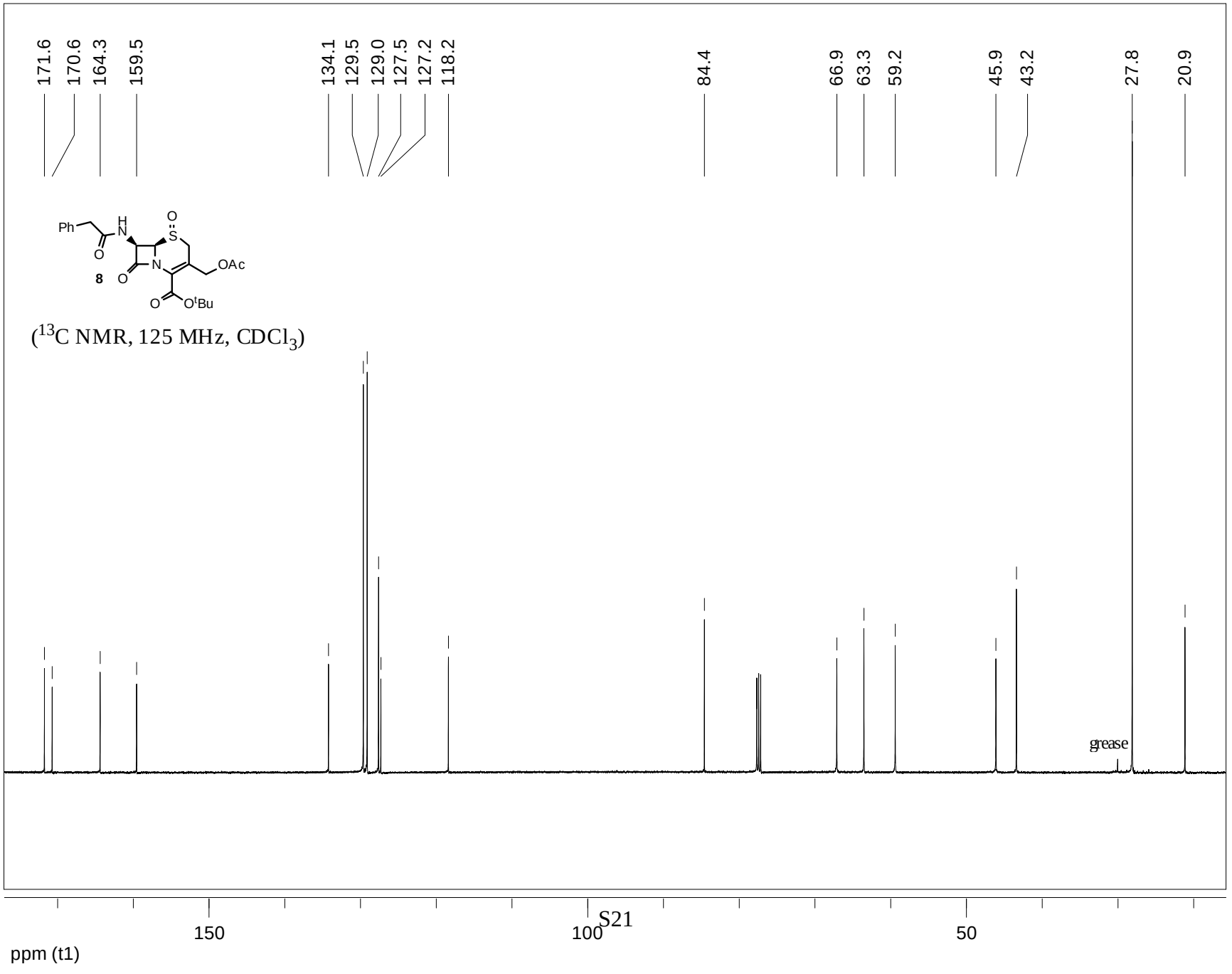
(^{1}H NMR, 500 MHz, $CDCl_3$)



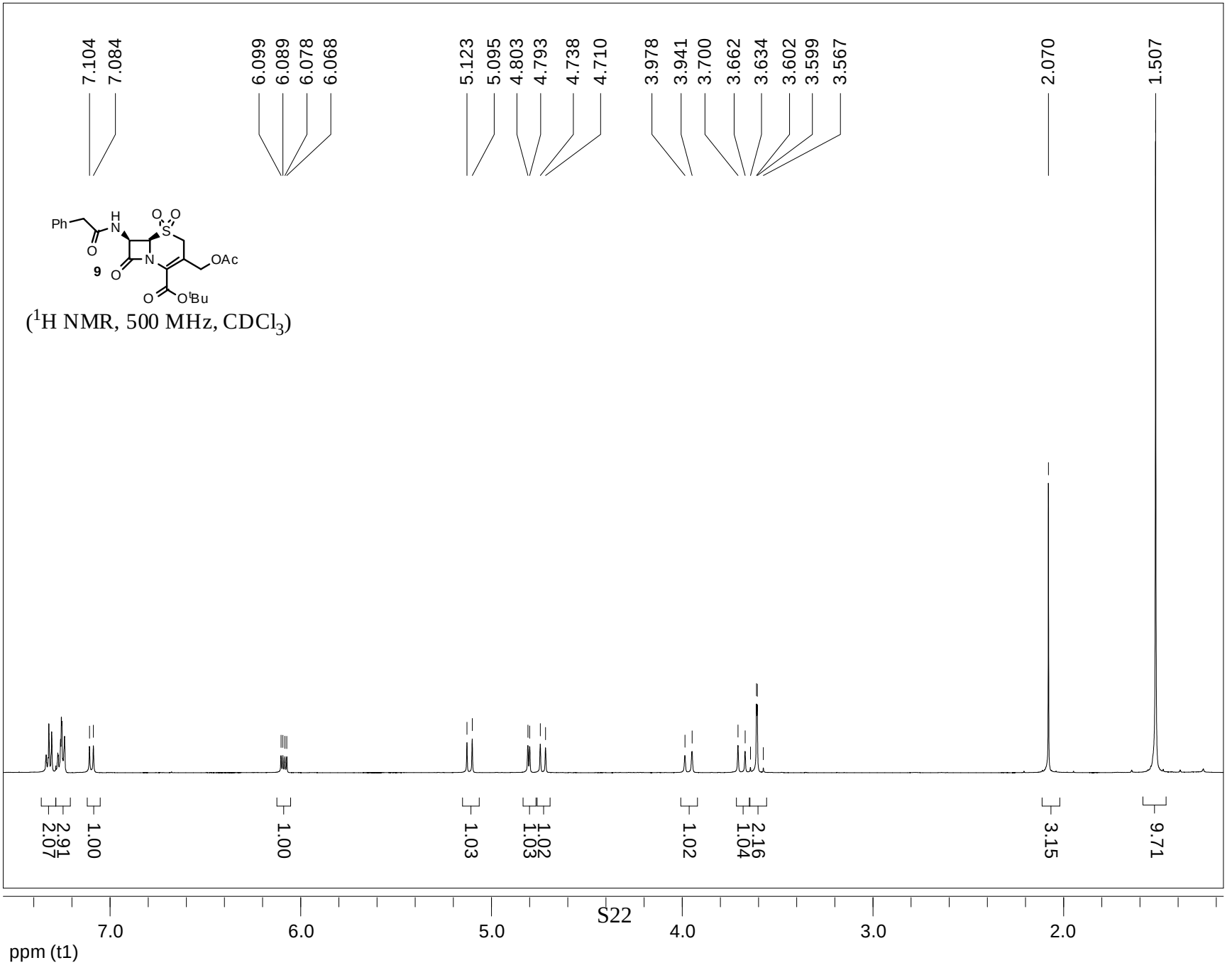
(^{13}C NMR, 125 MHz, $CDCl_3$)



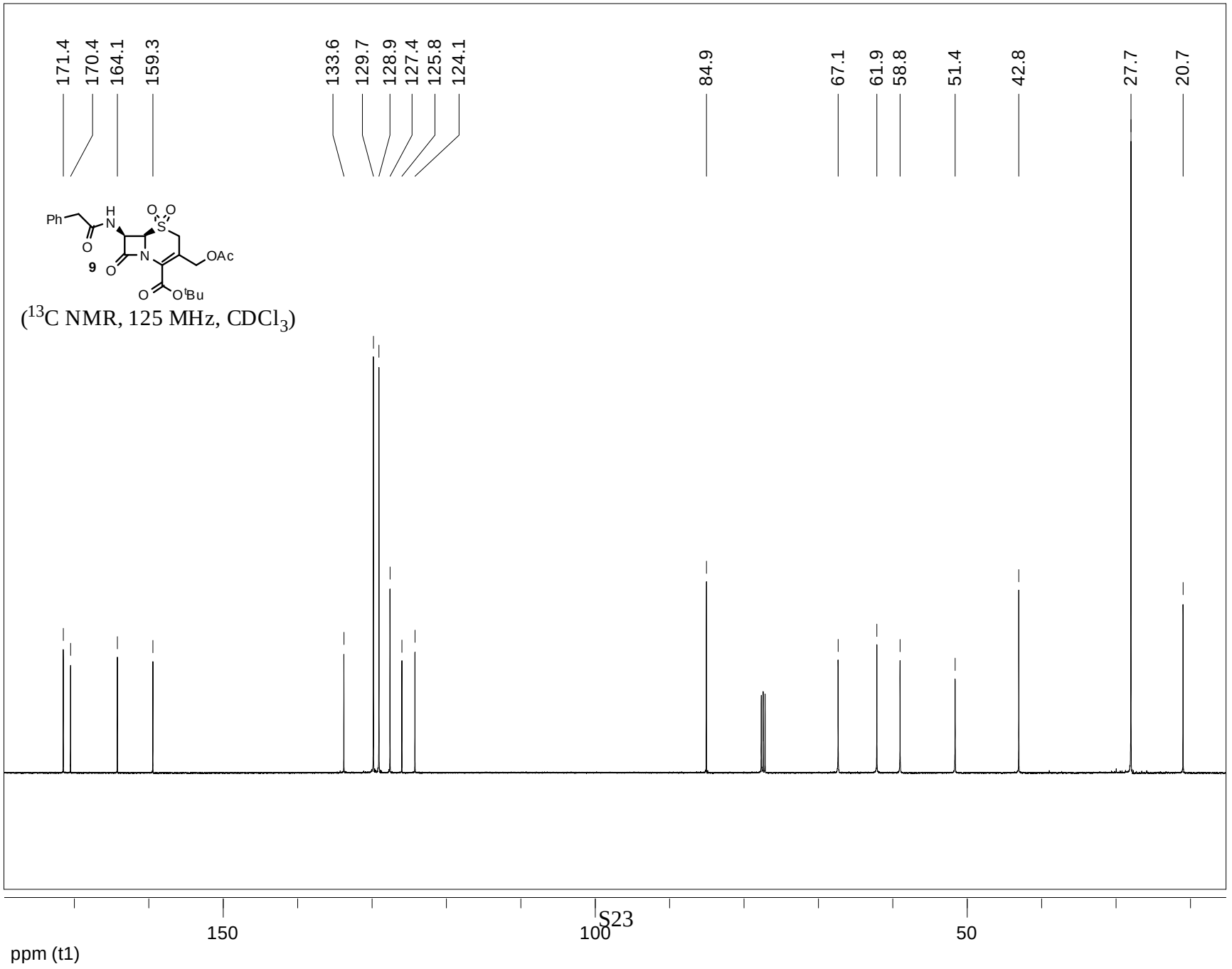
(^{1}H NMR, 500 MHz, $CDCl_3$)



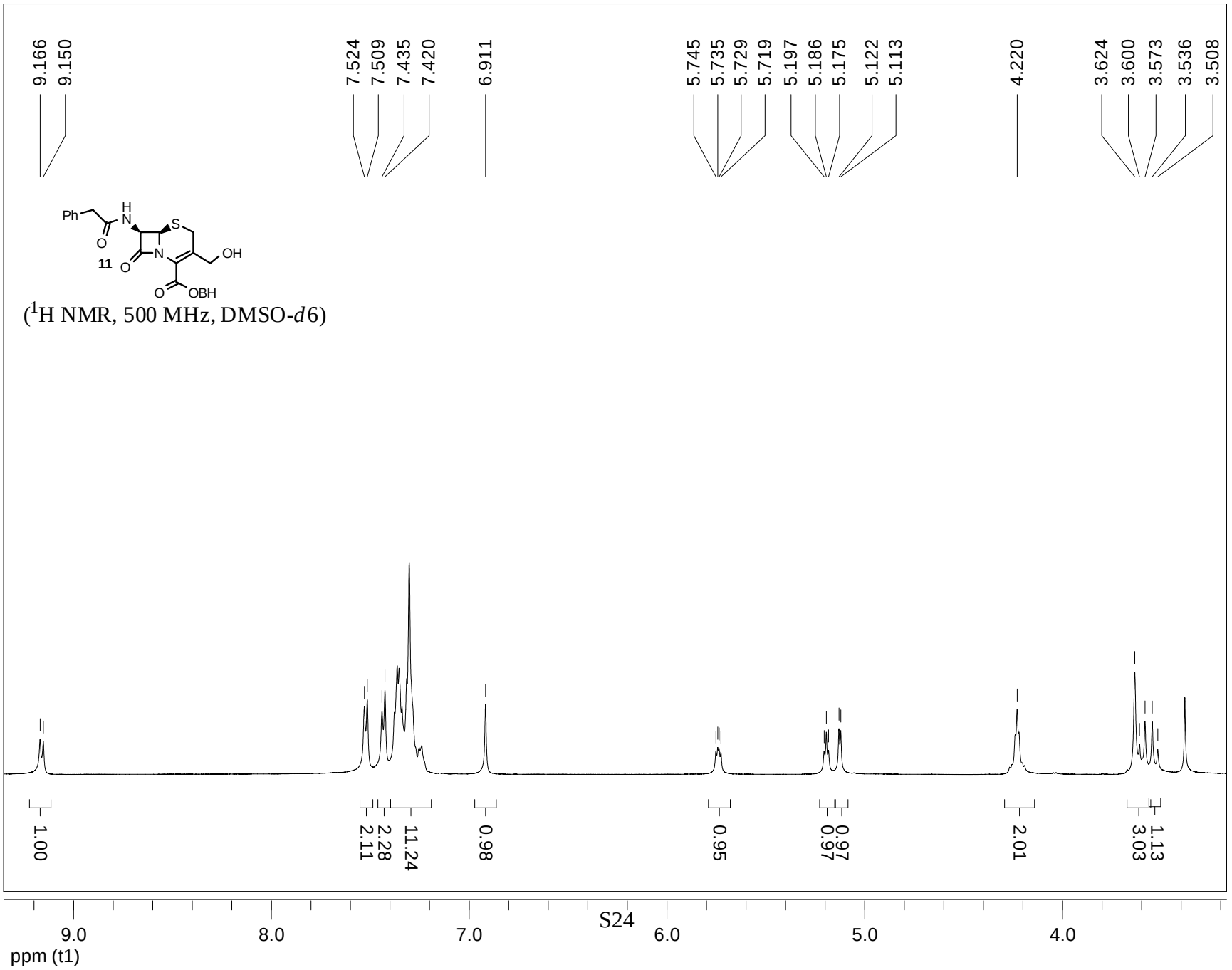
(^{13}C NMR, 125 MHz, $CDCl_3$)



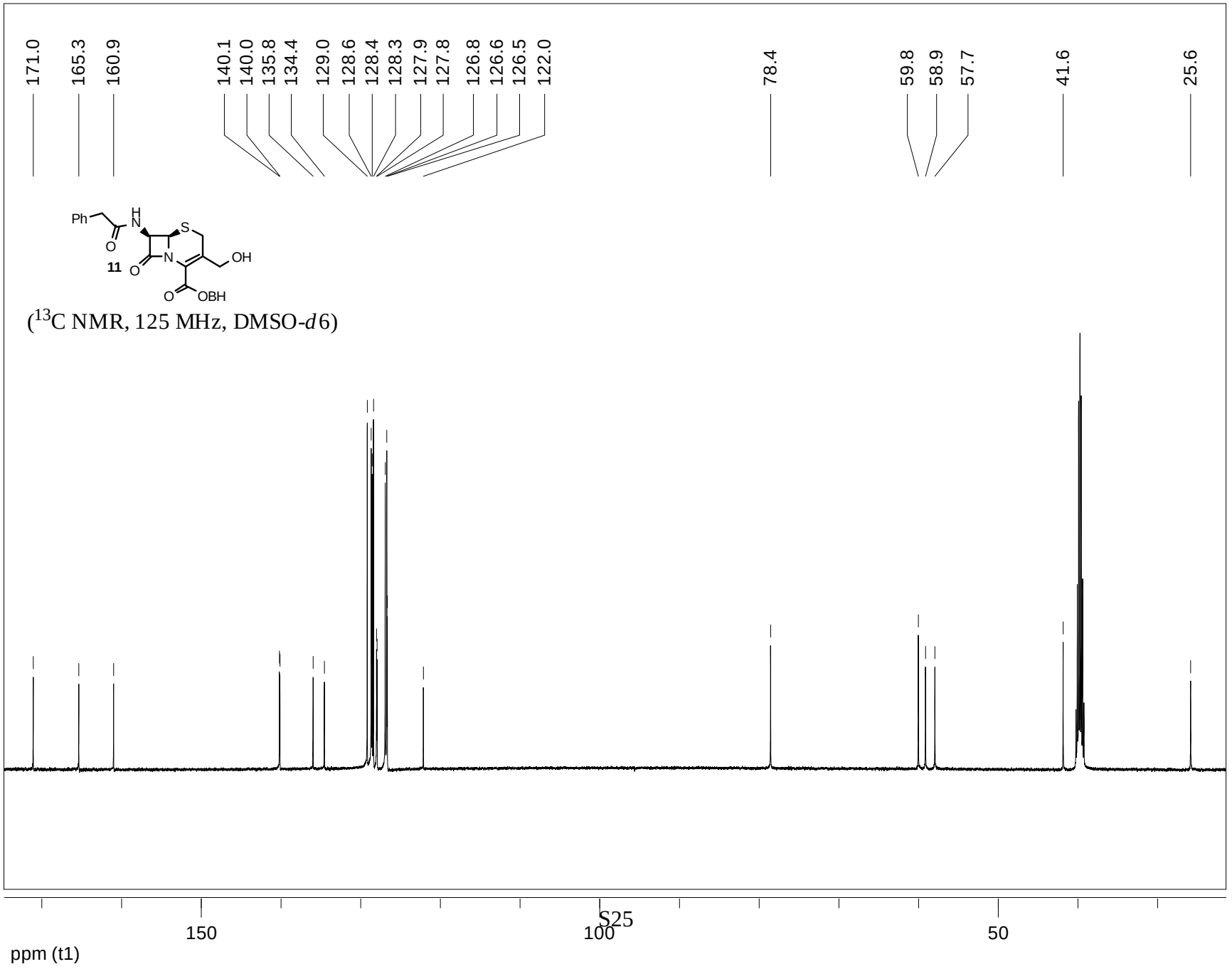
(^{1}H NMR, 500 MHz, $CDCl_3$)



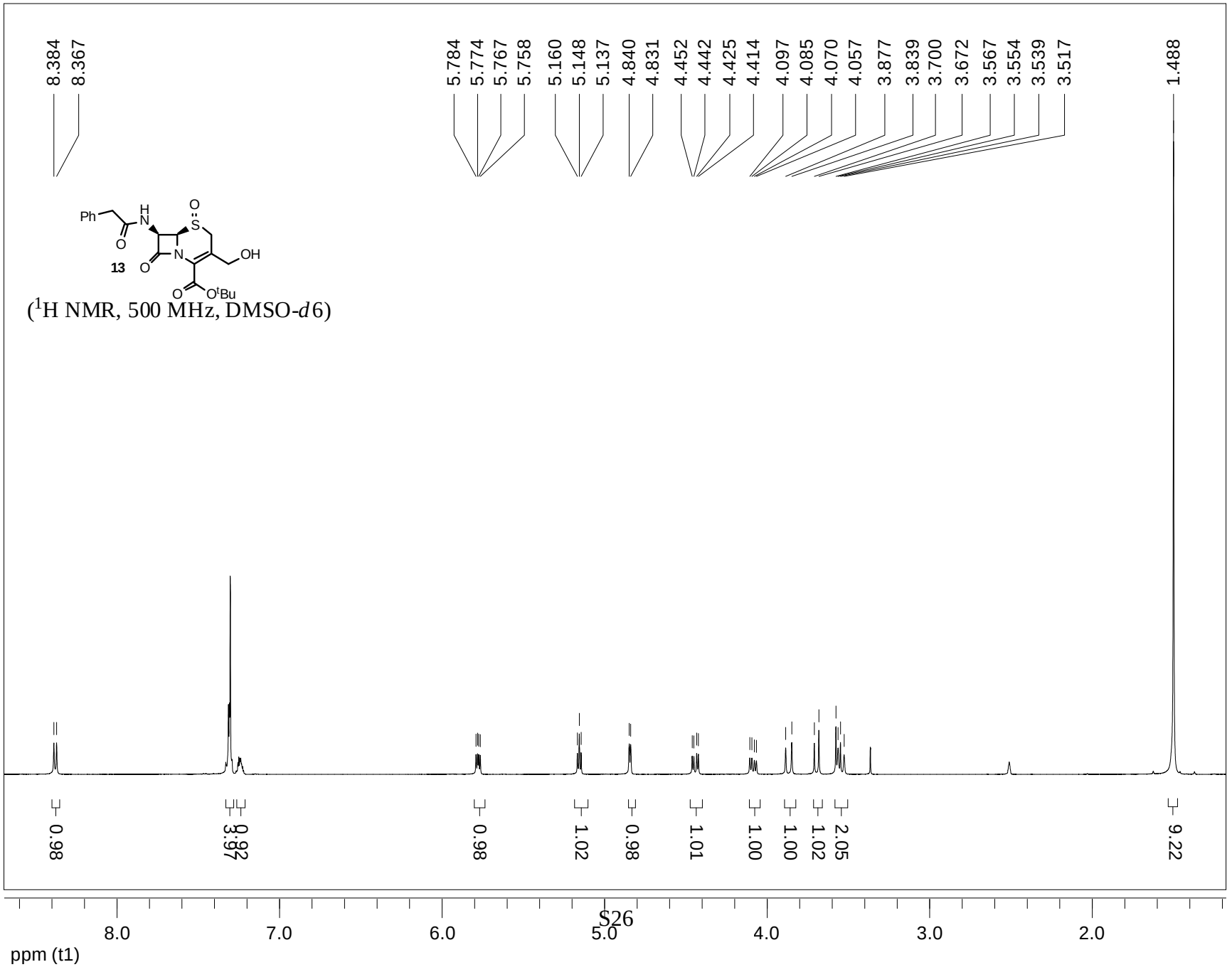
(^{13}C NMR, 125 MHz, $CDCl_3$)



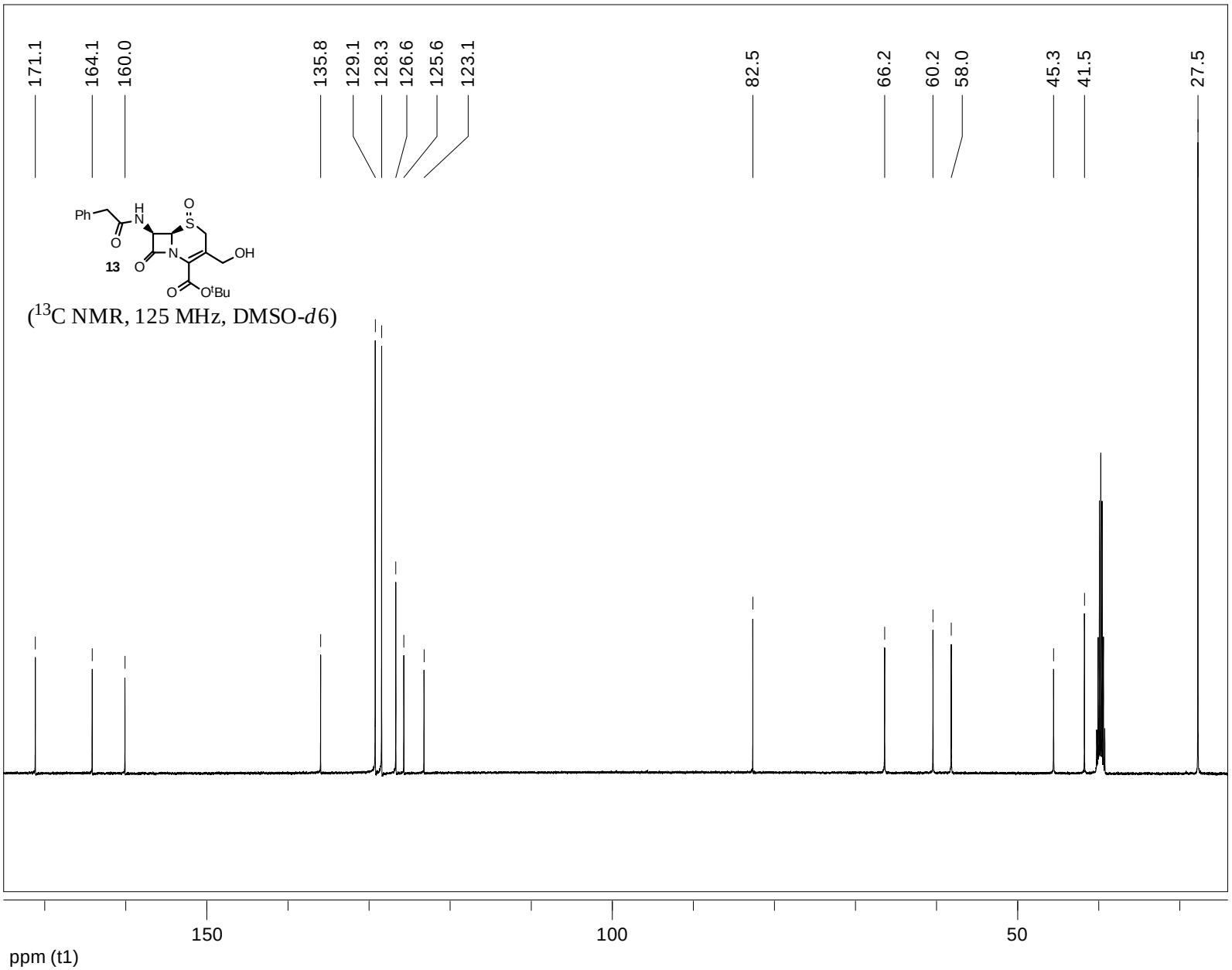
(^{1}H NMR, 500 MHz, DMSO-*d*6)



(^{13}C NMR, 125 MHz, DMSO-*d*6)

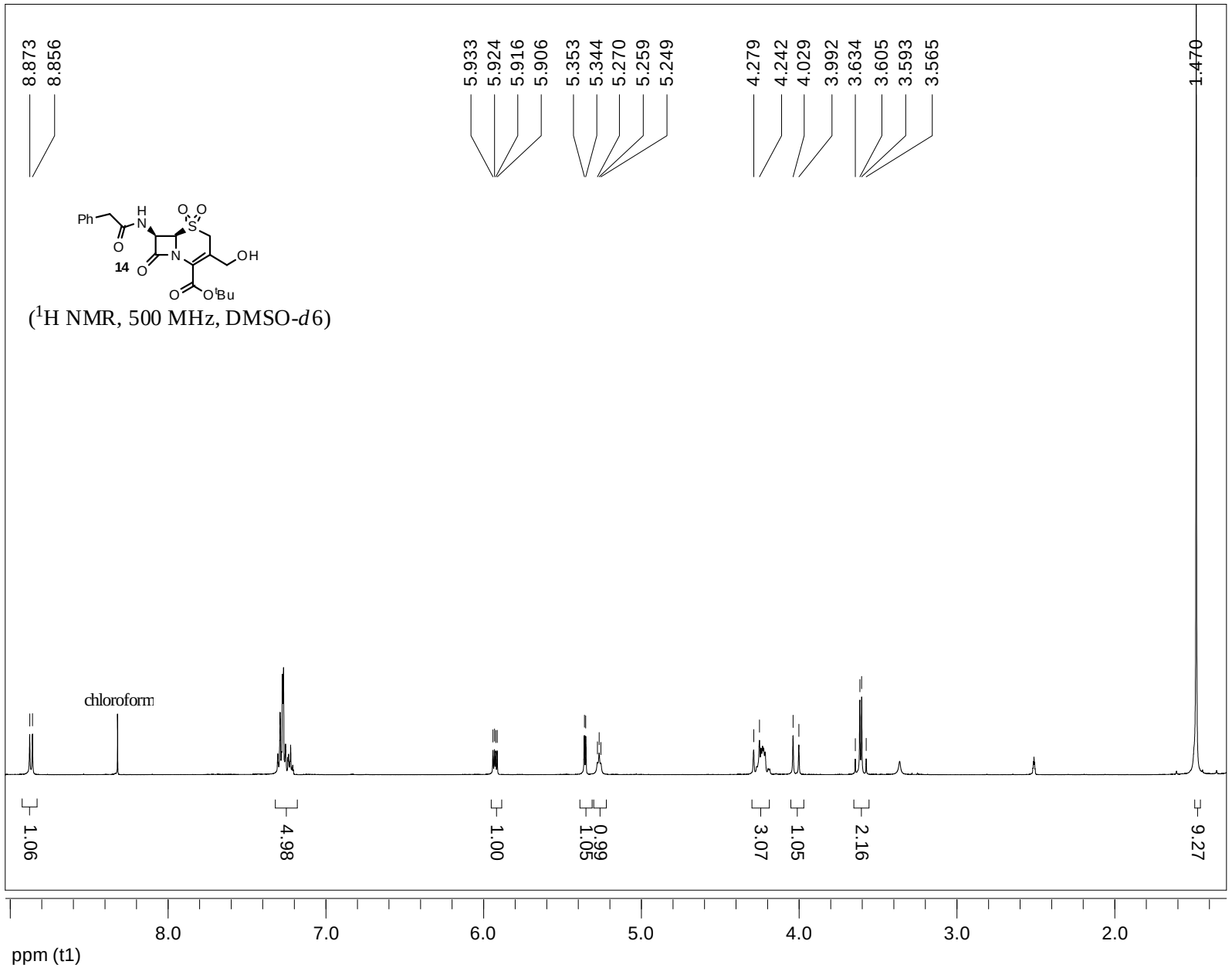


(^{1}H NMR, 500 MHz, DMSO-*d*6)



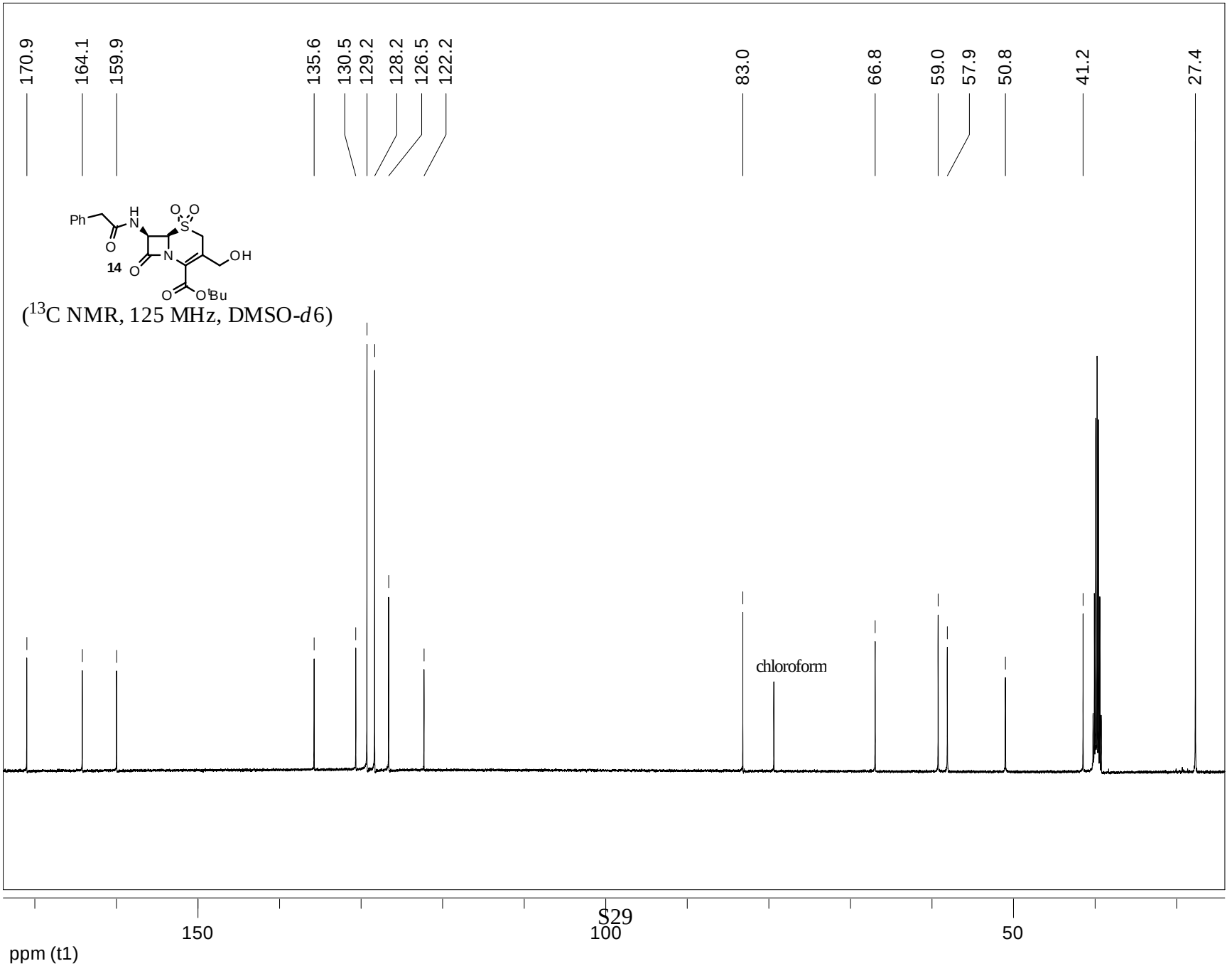
13

(^{13}C NMR, 125 MHz, DMSO-*d*6)



14

(^{1}H NMR, 500 MHz, DMSO-*d*6)



(^{13}C NMR, 125 MHz, DMSO-*d*6)