

Supporting Information for

Details of The Structure Determination of the Sulfated Steroids

PSDS and PADS—New Components of the Sea Lamprey

(*Petromyzon marinus*) Migratory Pheromone

*Thomas R. Hoye, Vadims Dvornikovs, Jared M. Fine, Kari R. Anderson, Christopher S. Jeffrey,
David C. Muddiman, Feng Shao, Peter W. Sorensen, and Jizhou Wang*

Department of Chemistry, 207 Pleasant Street, SE, University of Minnesota, Minneapolis, MN 55455; Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul, MN 55108; W.M. Keck FT-ICR MS Laboratory, Department of Chemistry, North Carolina State University, Raleigh, NC 27695.

Contents	page #
Contents of Supporting Information	S2
Instrumentation and data acquisition	S3
Natural Petromyzosterol Disulfate (PSDS, 2) NMR data; Table S1	S4
Natural Petromyzonamine Disulfate (PADS, 3) NMR data; Table S2	S5
Synthetic 24- <i>epi</i> -PADS (9) vs. PADS (3) NMR data; Table S3	S6
Mosher ester analyses for C24-carbinols 14 and (24R)- 14	S7-S10
Preparation and characterization data for amines 11 and cis-11	S10-S11
Preparation and characterization data for amine 12	S11-S12
References for Supporting Information	S13
Copies of ¹ H NMR spectra for 1-4 , 6 , 9 , 11 , cis-11 , 12 , 14 , 15 , 16 , (24S ,MTPA- <i>S</i>)-IV, (24S ,MTPA- <i>R</i>)-IV, (24R ,MTPA- <i>S</i>)-IV, and (24R ,MTPA- <i>R</i>)-IV	S14-S29
¹³ C NMR spectra for 3 , 9 , 11 , 12 , 15 , and 16	S30-S35

Instrumentation and data acquisition

ESI-MS and ESI-MS/MS measurements were performed on an ion trap mass spectrometer. It was operated in the negative ion mode with a spray voltage of 5 kV, sheath gas was 99% pure nitrogen at 60 psi, and sheath fluid was 50:50 20 mM triethylamine: acetonitrile [v/v]. For MS/MS, 30% collision energy was used. High resolution MS was performed in the negative ion mode with an ESI-source and a hybrid quadrupole-TOF detector. Ions of taurocholic acid were used for instrument calibration. MALDI-TOF MS was performed in the positive ion mode on an instrument equipped with an oMALDI source and a hybrid quadrupole-TOF detector. The matrix was α -cyano-4-hydroxycinnamic acid (CCA). FT-ICR mass spectra were obtained using an ESI-FT-ICR mass spectrometer equipped with an actively shielded 9.4 Tesla superconducting magnet. The ESI source was modified to accept a heated metal capillary and fitted with a custom made dual ESI source. Direct ESI infusion of the sample and internal standard was accomplished using two Harvard syringe pumps model PHD 2000 at a rate of 500 nL/sec through a 25- μ L Hamilton syringe. The potential on the electrospray emitters was approximately -2.0 kV for negative-ion electrospray. Emitter tips were 360 μ m o.d. \times 50 μ m i.d. tapered to 15 μ m \pm 1.0 i.d. tip. All spectra were acquired with 2048 k points at an ADC rate of 1 MHz and zero-filled four times prior to fast Fourier transformation to the frequency domain

^1H NMR spectra were recorded at 500 or 800 MHz in CD_3OD (referenced to the center line of residual CHD_2OD peak at δ 3.31 ppm for ^1H or CD_3OD at 49.15 ppm for ^{13}C) or CDCl_3 (referenced to TMS at δ = 0.00 ppm for ^1H or CDCl_3 at 77.23 ppm for ^{13}C). Coupling constants, J are reported in hertz. ^{13}C chemical shifts for natural **PADS** and **PSDS** samples were taken from HMQC and/or HMBC crosspeaks; for all other samples from the 1D ^{13}C NMR spectrum.

Natural Petromyzosterol Disulfate (PSDS, 2) NMR Data

Isolation of natural PSDS (**2**) is described elsewhere.^{1,2} The NMR data for **2** in Table S1 are also reported elsewhere.¹(in "Supplementary Methods")

Table S1.^a ¹H and ¹³C NMR assignments for natural PSDS (**2**) (CD₃OD, 800 MHz).

#	¹ H	<i>J</i> (Hz)	¹³ C
1β	1.86, m		38.1
1α	1.14, m		
2β	1.63, m ^b		29.8
2α	2.07, m ^b		
3α	4.14, dddd	11.4, 11.4, 4.8, 4.8	79.6
4β	2.35, br dd	ca. 13, 13	40.1
4α	2.54, ddd	13.2, 4.8, 2.2	
5			141.3
6	5.39, br ddd	5, 2, 2	123.1
7β	1.98, dddd	17.4, 5.2, 5.2, 3.1	32.6
7α	1.60, m		
8β	1.52, dddd	11, 11, 6	33.1
9α	1.38, m		44.9
10			37.2
11β	1.66, m ^b		29.5
11α	1.75, m ^b		
12β	3.99, dd	2.9, 2.9	73.7
13			46.9
14α	1.60, m		49.1
15β	1.11, m ^b		24.7
15α	1.60, m ^b		
16β	1.72, m ^b		29.4
16α	1.28, m ^b		
17α	1.86, m		47.6
18	0.76, s		13.1
19	1.03, s		19.2
20	2.05, m		41.8
21	1.09, d	6.7	20.2
22	5.34, dd	15.1, 9.0	141.2
23	5.21, dd	15.2, 9.2	126.2
24	2.12, m		49.6
24¹	3.95, d	6.5	70.6
25	1.86, m		29.0
26	0.87, d	6.9	18.3
27	0.93, d	6.8	21.2

^a ¹H and ¹³C chemical shift assignments were made on the basis of COSY, HMQC and HMBC analyses. ¹H chemical shift values (δ) for resonances designated as "m" were taken from COSY or HMQC cross peaks; those having assigned multiplicity (e.g., ddd) were taken directly from the 1D ¹H spectrum. ¹³C chemical shift values were taken from HMQC and HMBC cross peaks.

^b Assignments of these geminal protons may be interchanged.

Natural Petromyzonamine Disulfate (PADS, 3) NMR data

Isolation^{1,2} of natural PADS (3), as well as the NMR data in Table S2,¹(in “Supplementary Methods”) is described elsewhere.

Table S2.^a ¹H and ¹³C NMR assignments for natural PADS (3) (CD₃OD, 800 MHz).

#	free base		partially protonated		fully protonated	
	¹ H	¹³ C	¹ H		¹ H	
1β	1.73, m ^c	38.2	^b		1.85, m ^c	
1α	1.06, m ^c		1.09, m		1.14, m ^c	
2β	1.29, m ^c		^b		1.51, m ^c	
2α	1.74, m ^c				1.94, m ^c	
3α	2.50, dddd (11.3, 11.3, 4.2, 4.2)		2.74, m		3.08, m	
4β	1.11, m ^c	35.2	^b		1.35, m ^c	
4α	1.50, m ^c		^b		1.63, m ^c	
5α	1.76, m		^b		1.82, m	
6β	1.32, m	34.2	1.34, m		1.38, m	
6α	2.12, ddd (14.0, 3.2, 3.2)		2.14, m		2.16, m	
7β	4.45, ddd (2.3, 2.3, 2.3)		4.45, ddd (2.5, 2.5, 2.5)		4.45, ddd (2.6, 2.6, 2.6)	
8β	1.52, m	31.9	1.52, m		1.53, m	
9α	1.25, m	47.4	^b		^b	
10		36.9				
11β	1.34, m ^c	21.9	^b		1.35, m ^c	
11α	1.53, m ^c		^b		1.53, m ^c	
12β	1.96, ddd (12.6, 3.2, 3.2)		^b		1.98, m	
12α	1.12, m		1.13, m		1.14, m	
13		43.7				
14α	1.48, m	51.0	^b		^b	
15β	1.03, m	24.2	^b		1.05, dddd (11.9, 11.9, 11.9, 6.1)	
15α	2.07, m ^c		^b		^b	
16β	1.89, m ^c	28.9	^b		^b	
16α	1.31, m ^c		^b		^b	
17α	1.16, m	57.2	1.15, m		1.15, m	
18	0.69, s	12.2	0.69, s		0.70, s	
19	0.84, s	11.7	0.85, s		0.88, s	
20	1.43, m	37.0	1.43, m		1.43, m	
21	0.95, d (6.6)	19.1	0.95, d (6.8)		0.95, d (6.8)	
22	1.16, m	31.9	^b		1.15, m	
	1.52, m		^b		1.53, m	
23	1.55, m	27.8	1.54, m		1.55, m	
	1.72, m		1.72, m		1.71, m	
24	4.11, ddd (5.4, 5.4, 5.4)	85.7	4.11, ddd (5.3, 5.3, 5.3)		4.12, ddd (5.3, 5.3, 5.3)	
25	2.04, m	31.7	2.03, m		2.02, m	
26	0.95, d (6.6)	18.2	0.93, d (6.9)		0.93, d (6.9)	
27	0.93, d (7.1)	18.2	0.95, d (6.8)		0.95, d (6.8)	
1'	2.54, dt (11.8, 7.1)	44.3	2.74, m, 2H		2.99, m, 2H	
	2.58, dt (11.8, 7.1)					
2'	1.73, pent (6.9)	27.6	1.80, pent (7.0)		1.90, m	
3'	3.31, m	41.1	3.34, m		3.39, m	
4'	3.46, t (7.1)	48.4	3.47, t (7.1)		3.50, t (7.4)	
5'	2.05, pent (7.4)	18.7	2.06, pent (7.4)		2.09, pent (7.6)	
6'	2.38, t (8.2)	31.9	2.39, t (8.1)		2.42, t (8.1)	
7'		177.5				

^a ¹H and ¹³C chemical shift assignments were made on the basis of COSY, HMQC and HMBC analyses. ¹H chemical shift values (δ) for resonances designated as “m” were taken from COSY or HMQC cross peaks; those having assigned multiplicity (e.g., ddd) were taken directly from the 1D ¹H spectrum. ¹³C chemical shift values were taken from HMQC and HMBC cross peaks.

^b The quality of the COSY data set was insufficient to permit identification with confidence.

^c Assignments of these geminal protons may be interchanged

Table S3.^a ¹H and ¹³C NMR assignments for 24*epi*-PADS (**9**) (fully protonated form; CD₃OD, 500 and 75 MHz) in comparison with PADS (**3**).

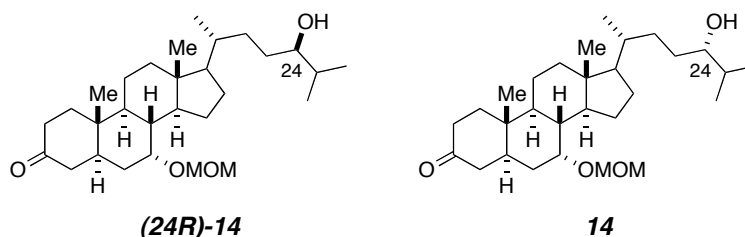
	24 <i>epi</i> -PADS (9, fully protonated)			Synthetic PADS (3, fully protonated)		
No.	H α	H β	¹³ C	H α	H β	¹³ C
1	1.12 (m)	1.85 (m)	37.7	1.15 (m)	1.87 (m)	37.8
2	1.98 (m)	1.52 (m)	^h	1.98 (m)	1.52 (m)	^h
3	3.11 (dddd, 11.8, 11.8, 4.3, 4.3)		58.8	3.11 (dddd, 11.7, 11.7, 4.5, 4.5)		58.8
4	^h	1.40 (m)	^h	1.64 (m)	1.40 (m)	31.88 ^e
5	1.81 (m)		38.7	1.82 (m)		38.8
6	2.16 (ddd, 14, 3, 3)	1.38 (m)	34.0	2.16 (ddd, 14.0, 3.0, 3.0)	1.38 (m)	34.0
7	4.45 (br s)		78.1	4.45 (ddd, 2.5, 2.5, 2.5)		78.1
8	1.53 (m)		41.1	1.53 (m)		41.1 ^f
9	1.27 (m)		47.5	1.27 (m)		47.5
10						
11	1.53 (m)	^h	^h	1.53 (m)	1.33 (m)	22.2
12	1.13 (m)	1.95 (m)	40.8	1.13 (m)	1.97 (m)	41.0 ^f
13						
14	1.50 (m)		51.3	1.48 (m)		51.3
15	2.01 (m)	1.03 (m)	24.5	2.03 (m)	1.05 (dddd, 11.8, 11.8, 11.8, 5.9)	24.5
16	1.29 (m)	1.90 (m)	29.3	1.31 (m)	1.9 (m)	29.2
17	1.17 (m)		57.4	1.17 (m)		57.5
18	0.69 (s, 3H)		12.4	0.69 (s, 3H)		12.5
19	0.85 (s, 3H)		11.9	0.88 (s, 3H)		11.9
20	1.41 (m)		37.2	1.41 (m)		37.2
21	0.95 (d, 6,5, 3H)		19.0 ^{+g}	0.94 ⁺ (d, 6.5, 3H)		19.0 ^c
22	^h	^h	^h	1.19(m) ⁱ	1.52(m) ⁱ	31.93 ^e
23	1.57 (m) ⁱ	1.67 ⁱ	28.3	1.72(m) ⁱ	1.56(m) ⁱ	28.1
24	4.12 (ddd, 5.9, 5.9, 4.1)		85.9	4.11 (ddd, 5.9, 5.1, 5.1)		86.0
25	2.02 (m)		31.7	2.02 (m)		32.0 ^e
26	0.91 (d, 6.8, 3H)		17.6	0.93 (d, 6.9, 3H)		18.52 ^c
27	0.96 (d, 6.7. 3H)		19.0 ^{-g}	0.94 ⁻ (d, 6.9, 3H)		18.52 ^c
1'	3.01 (dt, 12.5, 5.2)	2.99 (dt, 12.5, 5.3)	43.2	3.02 (dt, 12.5, 7.4)	2.98 (dt, 12.5, 7.3)	43.3
2'	1.93 (pent, 7.0, 2H)		25.5	1.92 (pent, 7.6, 2H)		25.5
3'	3.41 (ddd, 14.2, 7.7, 7.7)	3.39 (ddd, 14.2, 7.4, 7.4)	40.6	3.42 (ddd, 14.5, 6.9, 6.9) ^c	3.38 (ddd, 14.5, 6.4, 6.4) ^c	40.6
4'	3.50 (t, 7.0, 2H)		49.0	3.50 (t, 7.4, 2H)		49.0
5'	2.09 (pent, 7.6, 2H)		19.4 ^g	2.09 (pent, 8, 2H)		19.4 ^c
6'	2.42 (t, 8, 2H)		31.7	2.42 (t, 8.2, 2H)		32.2 ^e
7'			178.9			178.9

^a ¹H and ¹³C chemical shift assignments were made on the basis of COSY and HMQC analyses. ¹H chemical shift values (δ) for resonances designated as “m” were taken from COSY cross peaks; those having assigned multiplicity (e.g., ddd) were taken directly from the 1D ¹H spectrum. ¹³C chemical shift values were taken from the 1D ¹³C NMR spectrum.

^{b,c,d,e,f,g} Assignments may be interchanged.

^h The quality of the 2D data set was insufficient to permit identification with confidence.

ⁱ H α and H β nomenclature lacks meaning for the side chain protons.

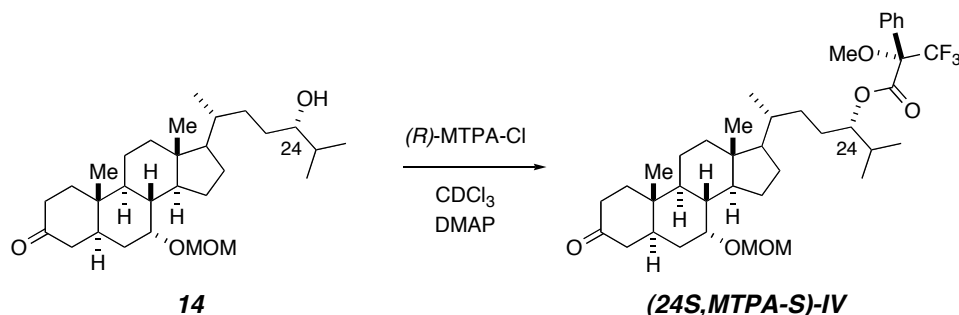
Mosher ester analyses for C24-carbinols³ (**(24R)**-**14** and **14**).General procedure for the preparation of Mosher esters **IV**:

R- or *S*-MTPA-Cl (5 μ L, 0.03 mmol) was added to a solution of **14** or **(24R)**-**14** (3 mg, 0.006 mmol) and DMAP (5 mg, 0.04 mmol) in CDCl₃ (0.3 mL) at room temperature and left overnight. The reaction mixture was concentrated under reduced pressure and the ester **IV** was purified directly by silica gel chromatography (5:1 Hexanes:EtOAc, *R_f* = 0.2). Values for $\Delta\delta$ were obtained from ¹H NMR and ¹H ¹H-COSY spectra. The *R*-MTPA-Cl gave the *S*-Mosher esters and the *S*-MTPA-Cl gave the *R*-Mosher esters.

Summary of Mosher ester data.

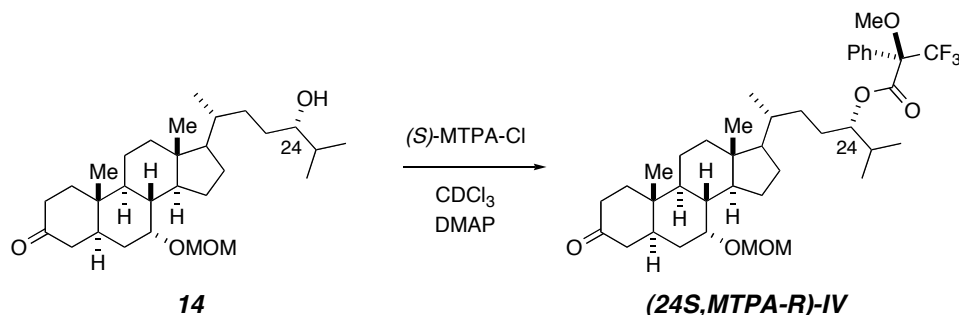
	δ_{MTPA-S}	δ_{MTPA-R}	$\Delta\delta$ ($\delta_S - \delta_R$)
24S	<p style="text-align: center;">(24S,MTPA-S)-IV</p>	<p style="text-align: center;">(24S,MTPA-R)-IV</p>	
24R	<p style="text-align: center;">(24R,MTPA-S)-IV</p>	<p style="text-align: center;">(24R,MTPA-R)-IV</p>	

(24S,MTPA-S)-IV [prepared from the reaction of **14** with (*R*)-MTPA-Cl]



^1H NMR (500 MHz, CDCl_3): δ 7.59-7.52 (m, 2H, ArH), 7.43-7.36 (m, 3H, ArH), 4.92 (dt, 1H, $J = 7.8, 4.6$ Hz, H24), 4.67 (d, 1H, $J = 7.0$ Hz, MOM- CH_aH_b), 4.60 (d, 1H, $J = 7.0$ Hz, MOM- CH_aH_b), 3.62 (br d, 1H, $J = 3, 3, 3$ Hz, H7), 3.54 (br s, 3H, MTPA-OMe), 3.36 (s, 3H, MOM-OMe), 2.38 (td, 1H, $J = 16.0, 6.6$ Hz), 2.31 (ddd, 1H, $J = 15.5, 5.0, 2$ Hz), 2.26 (app t, 1H, $J = 14.4$ Hz), 2.09-1.86 (m, 5H), 1.82-1.24 (m, 15 H), 1.24-1.00 (m, 5H), 1.004 (s, 3H, Me19), 0.91 (d, 3H, $J = 6.9$ Hz, Me26), 0.90 (d, 3H, $J = 6.9$ Hz, Me27), 0.87 (d, 3H, $J = 6.5$ Hz, Me21), and 0.64 (s, 3H, 18Me).

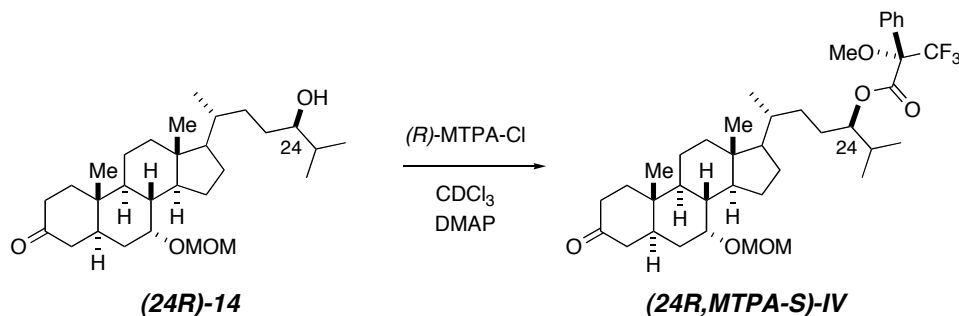
(24S,MTPA-R)-IV [prepared from the reaction of **14** with (*S*)-MTPA-Cl]



^1H NMR (500 MHz, CDCl_3): δ 7.61-7.54 (m, 2H, ArH), 7.44-7.36 (m, 3H, ArH), 4.93 (dt, 1H, $J = 9.5, 4.9$ Hz, H24), 4.68 (d, 1H, $J = 6.9$ Hz, MOM- CH_aH_b), 4.60 (d, 1H, $J = 6.9$ Hz, MOM- CH_aH_b), 3.62 (br ddd, 1H, $J = 3, 3, 3$ Hz, H7), 3.56 (br s, 3H, MTPA-OMe), 3.35 (s, 3H, MOM-OMe), 2.39 (dt, 1H, $J = 15.2, 6.5$ Hz), 2.32 (ddd, 1H, $J = 15.2, 5.5, 1.7$ Hz), 2.27 (app t, 1H, $J = 14.6$ Hz), 2.05 (m, 1H), 2.01 (ddd, 1H, $J = 13.2, 6.5, 2.2$ Hz), 1.95 (dt, 1H, $J = 13.0, 3.6$ Hz), 1.88 (d sept, 1H, $J = 4.5, 6.5$ Hz, H25), 1.78 (dddd, 1H, $J = 15.6, 9.4, 9.4, 6.2$ Hz), 1.73-1.29 (m,

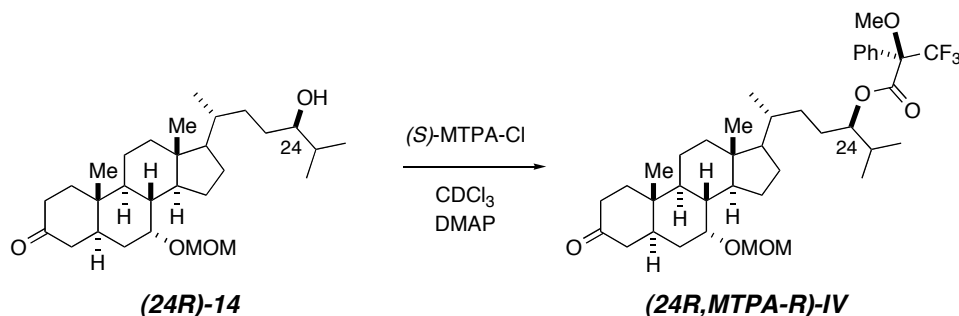
14H), 1.29-0.97 (m, 5H), 1.008 (s, 3H, Me19), 0.92 (d, 3H, $J = 6.7$ Hz, Me21), 0.85 (d, 3H, $J = 6.7$ Hz, Me26), 0.83 (d, 3H, $J = 6.7$ Hz, Me27), and 0.66 (s, 3H, Me18).

(24R,MTPA-S)-IV [prepared from the reaction of **(24R)-14** with (*R*)-MTPA-Cl]



^1H NMR (500 MHz, CDCl_3): δ 7.58-7.54 (m, 2H, ArH), 7.40-7.37 (m, 3H, ArH), 4.93 (ddd, 1H, $J = 8.7, 5.16, 3.8$ Hz, H24), 4.67 (d, 1H, $J = 6.8$ Hz, MOM- CH_aH_b), 4.60 (d, 1H, $J = 6.8$ Hz, MOM- CH_aH_b), 2.38 (td, 1H, $J = 15.7, 6.7$ Hz), 2.31 (ddd, 1H, $J = 15.5, 5.2, 2$ Hz), 2.26 (app t, 1H, $J = 14.4$ Hz), 2.09-1.98 (m, 5H), 1.97-1.86 (m), 1.77 (dddd, 1H, $J = 15.9, 9.6, 9.6, 6.2$ Hz), 1.72-1.29 (m, 14H), 1.23-0.99 (m, 5H), 1.011 (s, 3H, Me19), 0.89 (d, 3H, $J = 6.6$ Hz, Me21), 0.86 (d, 3H, $J = 7.0$ Hz, Me26), 0.84 (d, 3H, $J = 7.0$ Hz, Me27), and 0.66 (s, 3H, Me18).

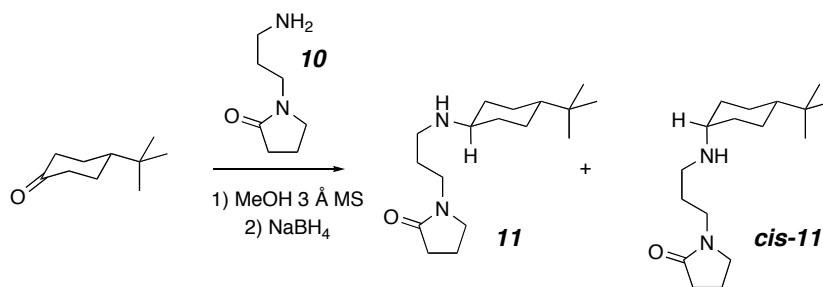
(24R,MTPA-R)-IV [prepared from the reaction of **(24R)-14** with (*S*)-MTPA-Cl]



^1H NMR (500 MHz, CDCl_3): δ 7.56 (m, 2H, ArH), 7.39 (m, 3H, ArH), 4.92 (ddd, 1H, $J = 8.4, 5.13, 3.6$ Hz, H24), 4.68 (d, 1H, $J = 6.9$ Hz, MOM- CH_aH_b), 4.60 (d, 1H, $J = 6.9$ Hz, MOM- CH_aH_b), 3.62 (br ddd, 1H, $J = 2, 2, 2$ Hz, H7), 3.55 (br s, 3H, MTPA-OMe), 3.36 (s, 3H, MOM-OMe), 2.38 (td, 1H, $J = 15.7, 6.4$ Hz), 2.31 (ddd, 1H, $J = 15.5, 5.4, 2$ Hz), 2.26 (app t, 1H, $J =$

14.2 Hz), 1.97-1.88 (m, 5H), 1.71-1.26 (m, 15H), 1.20-0.98 (m, 5 H), 1.007 (s, 3H, Me19), 0.91⁺ (d, 3H, $J = 6.8$ Hz, Me26), 0.91⁻ (d, 3H, $J = 6.8$ Hz, Me27), 0.84 (d, 3H, $J = 6.3$ Hz, Me21), and 0.64 (s, 3H, Me18).

Preparation and characterization data for amines **11** and **cis-11**



4-*tert*-Butylcyclohexanone (50 mg, 0.32 mmol) and 1-(3-aminopropyl)-pyrrolidin-2-one (commercial sample, 55 mg, 0.39 mmol) were dissolved in ethanol (0.6 mL) and 3Å molecular sieves were added. This mixture was capped and stirred at rt for 20 h, and sodium borohydride (15 mg, 0.39 mmol) was added. After one hour acetic acid (100 µL) was added and the resulting mixture was filtered through a bed of Celite[®]. The filtrate was evaporated and purified on silica gel (chloroform : ether : methanol : ammonium hydroxide conc. 60:60:9:1) to give in order of elution **cis-11** as a colorless film (13 mg, 14 %) and **11** as a white solid (61 mg, 68%).

11

mp: 63-66 °C; TLC: R_f (CHCl₃/MeOH/NH₄OH 80:18:2) = 0.53.

¹H NMR (500 MHz, CDCl₃): δ 3.39 (t, 2H, $J = 7.1$ Hz), 3.35 (t, 2H, 6.9 Hz), 3.07 (br s, 1H, NH), 2.64 (t, 2H, $J = 7.0$ Hz, H1'), 2.39 (t, 2H, 8.2 Hz, H6'), 2.38 (tt, 1H, $J = 11, 4$ Hz, H1), 2.03 (pent, 2H, $J = 7.5$ Hz, H5'), 1.98 (m, 2H), 1.75 (pent, 2H, $J = 7.0$ Hz, H2'), 0.96-1.11 (m, 5H), and 0.84 (s, 9H, Me₃C).

¹³C-NMR (75 MHz, CDCl₃): δ 175.4, 57.3, 47.8, 47.4, 43.8, 40.4, 33.5, 32.5, 31.2, 27.8, 27.6, 26.2, and 18.1.

IR (thin film): 3432, 3288 (shoulder), 2940, 2860, 1673, 1558, 1466, and 1426 cm⁻¹.

MS: ESI-MS calcd for C₁₇H₃₃N₂O (M+H⁺) 281.2587, found 281.2590.

cis-11

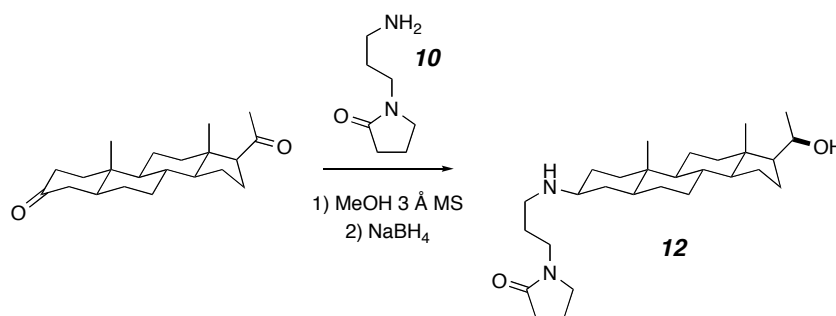
TLC: R_f (CHCl₃/MeOH/NH₄OH 80:18:2) = 0.60

¹H NMR (500 MHz, CDCl₃): δ 3.40 (t, 2H, J = 7.1 Hz), 3.35 (t, 2H, 7.1 Hz), 2.82 (tt, 1H, J = 3.1, 3.1 Hz, H1), 2.60 (br s, 1H, NH), 2.58 (t, 2H, J = 7.1 Hz, H1'), 2.38 (t, 2H, 8.1 Hz, H6'), 2.02 (pent, 2H, J = 7.6 Hz, H5'), 1.83 (br d, 2H, J = 14 Hz, H2eq/H6eq), 1.73 (pent, 2H, J = 7.1 Hz, H2'), 1.50 (br d, 2H, J ~ 13 Hz, H3eq/H5eq), 1.41 (dddd, 2H, J = 13.5, 13.5, 3.6, 3.6 Hz, H2ax/H6ax), 1.21 (dddd, 2H, J = 12.9, 12.9, 12.9, 3.2 Hz, H3ax/H5ax), 0.97 (tt, 1H, J = 12.0, 3.2 Hz, H4), and 0.84 (s, 9H, Me₃C).

¹³C-NMR (75 MHz, CDCl₃): δ 175.2, 51.8, 48.4, 47.4, 44.4, 40.7, 32.7, 31.2, 30.7, 27.9, 27.7, 21.4, and 18.1.

IR (thin film): 3447, 2938, 2865, 1679, 1465, 1443, 1366, and 1288 cm⁻¹.

MS: ESI-MS calcd for C₁₇H₃₃N₂O (M+H⁺) 281.2587, found 281.2593.

Preparation and characterization data for amine 12

5 α -Pregnane-3,20-dione (commercial sample, 100 mg, 0.32 mmol) was added to methanol (5 mL) in a roundbottom flask followed by tetrahydrofuran (1 mL), 1-(3-aminopropyl)-pyrrolidin-2-one (**10**, 59 mg, 0.42 mmol) and 3 Å molecular sieves. This mixture was capped, stirred at rt for 20 h, placed under a N₂ atmosphere, and cooled to -78 °C. Sodium borohydride (100 mg, 2.63 mmol) was added and the mixture was stirred for 2 h at -78 °C and allowed to warm to rt. Acetic acid (630 μ L, 10.5 mmol) was added. The resulting mixture was filtered through a bed of Celite[®] and the filtrate was concentrated and the residue was purified on silica gel (chloroform : ether :

methanol : ammonium hydroxide conc. 60:60:9:1) to give **12** as pale yellow oil that solidified upon standing (80 mg, 57 %). Examination of the ^1H NMR spectrum of the crude reaction product from a similar experiment showed an ~10:1 mixture of **12** (major) and what was judged to be **3epi-12** (minor), having a 3-axial amino substituent.

12 (free base):

mp: 138-142 °C; TLC: R_f ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ 80:18:2) = 0.70.

$[\alpha]_D^{RT} = 0.75$ (c 2.8, CHCl_3).

^1H NMR (500 MHz, CD_3OD): δ 3.62 (dq, 1H, $J = 10, 6$ Hz, H20), 3.47 (t, 2H, $J = 7.1$ Hz, H4'), 3.34 (t, 2H, $J = 6.8$ Hz, H3'), 2.63 (t, 2H, $J = 7.2$ Hz, H1'), 2.54 (dddd, 1H, $J = 11.3, 11.3, 4.3, 4.3$ Hz, H3), 2.39 (t, 2H, $J = 8.1$ Hz, H6'), 2.13 (ddd, 1H, $J = 12.7, 3.3, 3.3$ Hz), 2.06 (pent, 2H, $J = 7.7$ Hz, H5'), 1.76 (pent, 2H, 7.1 Hz, H2'), 1.60-1.80 (m, 5H), 1.49-1.55 (m, 2H), 1.41 (dddd, 1H, $J = 11, 11, 11, 4$ Hz), 1.27-1.37 (m, 5H), 1.08-1.22 (m, 5H), 1.10 (d, 3H, $J = 6.2$ Hz, 21H₃), 0.89-1.08 (m, 3H), 0.84 (s, 3H), 0.75 (s, 3H), and 0.69 (ddd, 1H, $J = 12.4, 10.7, 4.1$ Hz).

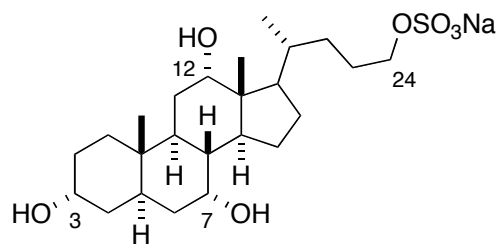
^{13}C -NMR (125 MHz, CD_3OD): δ 178.0, 71.0, 59.6, 58.5, 57.6, 56.2, 48.7, 46.8, 44.3, 43.9, 41.3, 41.2, 38.7, 37.2, 36.9, 35.6, 33.5, 32.1, 30.1, 29.0, 27.6, 27.0, 25.7, 24.0, 22.3, 19.0, and 13.0.

IR (thin film): 3452, 2926, 2862, 1667, 1464, 1448, 1375, 1291, and 1113 cm^{-1} .

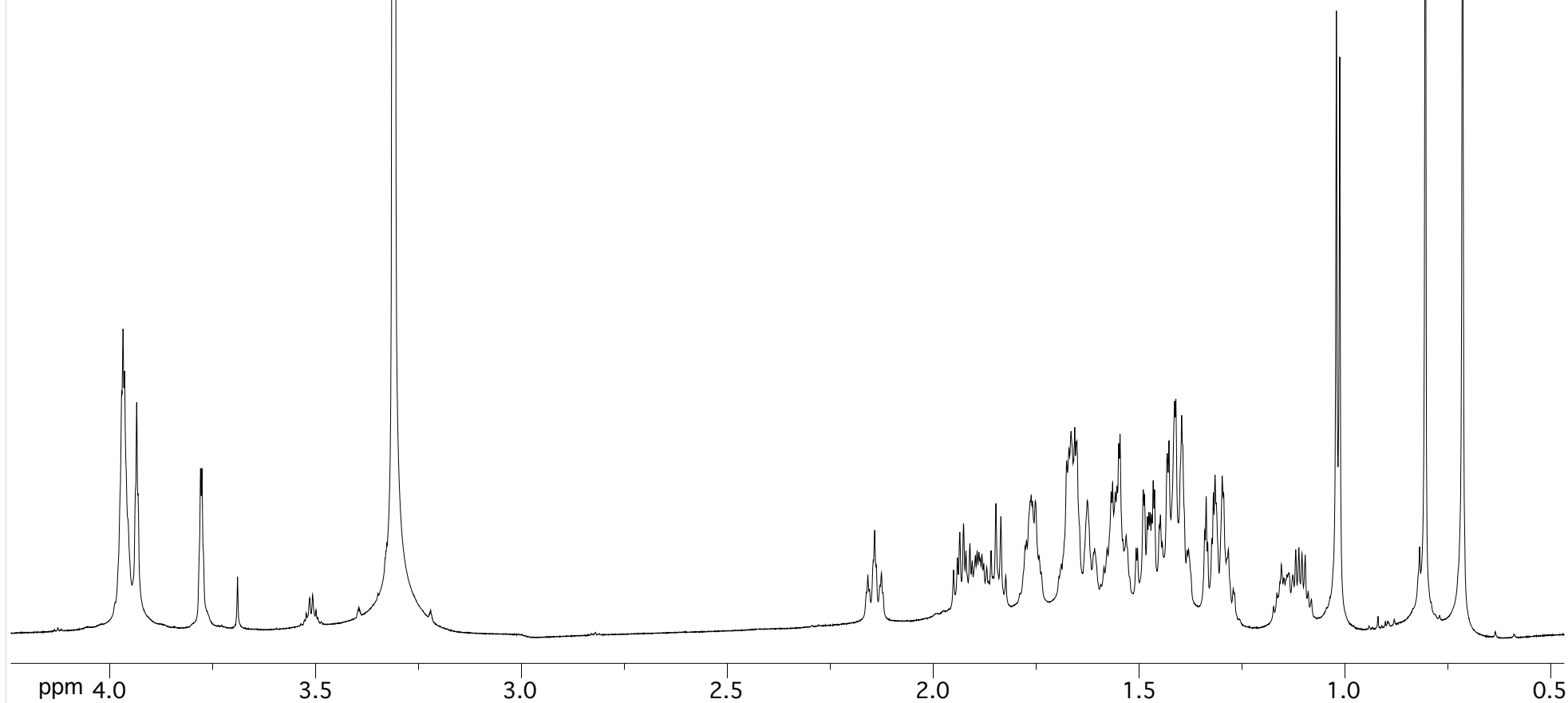
MS: ESI-MS calcd for $\text{C}_{28}\text{H}_{49}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}^+$) 445.3789, found 445.3790.

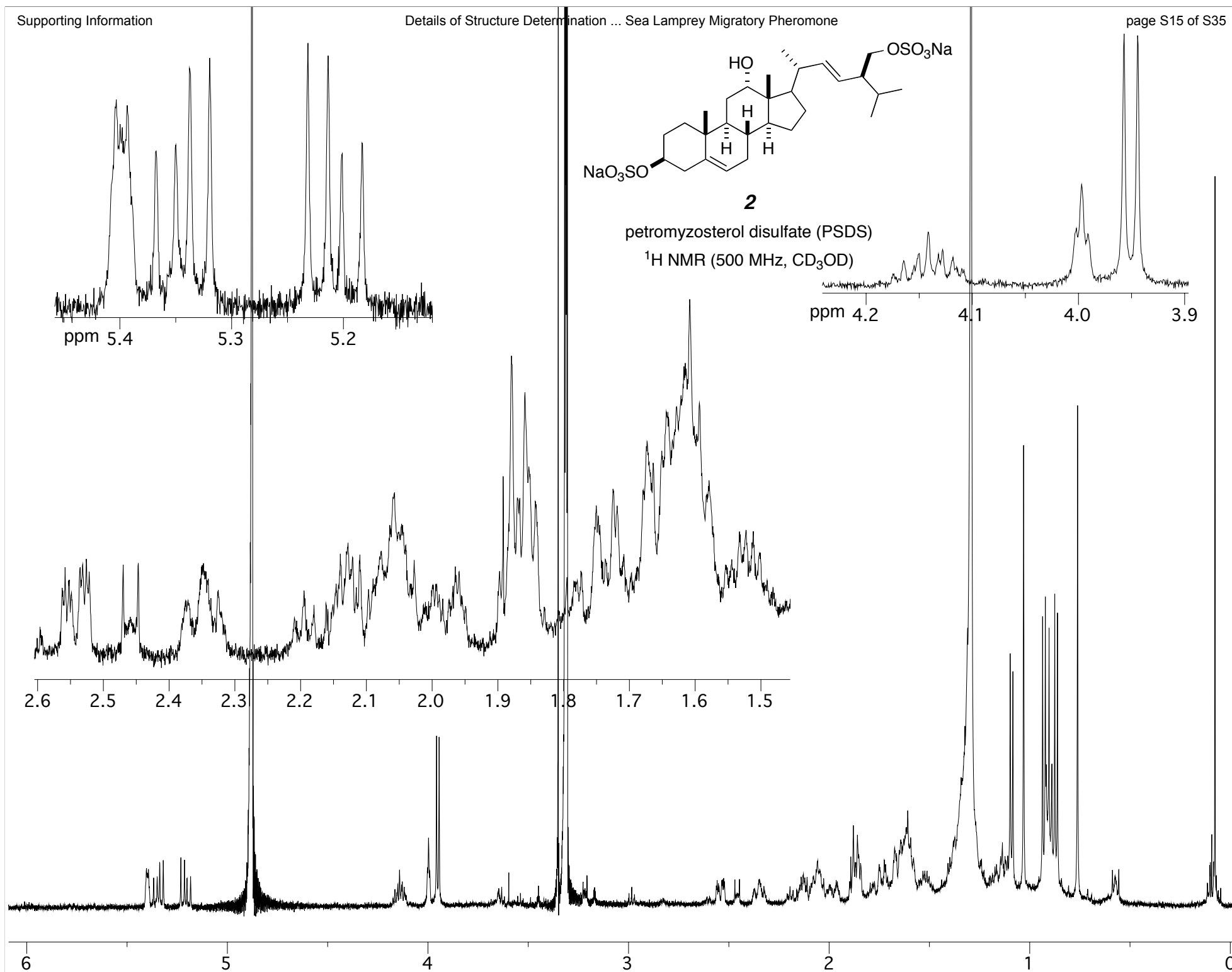
References for Supporting Information

- ¹ “Mixture of New Sulfated Steroids Functions as a Migratory Pheromone in the Sea Lamprey,” Sorensen, P. W.; Fine, J. M.; Dvornikovs, V.; Jeffrey, C. S.; Shao, F.; Wang, J.; Vrieze, L. A.; Anderson, K. R.; Hoye, T. R. *Nat. Chem. Biol.* **2005**, *1*, 324-328.
- ² “Isolation, Identification, and Biological Characterization of the Sea Lamprey (*Petromyzon marinus*) Migratory Pheromone,” Fine, J. M. Ph. D. Thesis, University of Minnesota, Minneapolis, MN, 2006.
- ³ “A Concise and Stereoselective Squalamine Synthesis,” Zhang, D.-H.; Cai, F.; Zhou, X.-D.; Zhou, W.-S. *Org. Lett.* **2003**, *5*, 3257-3259.

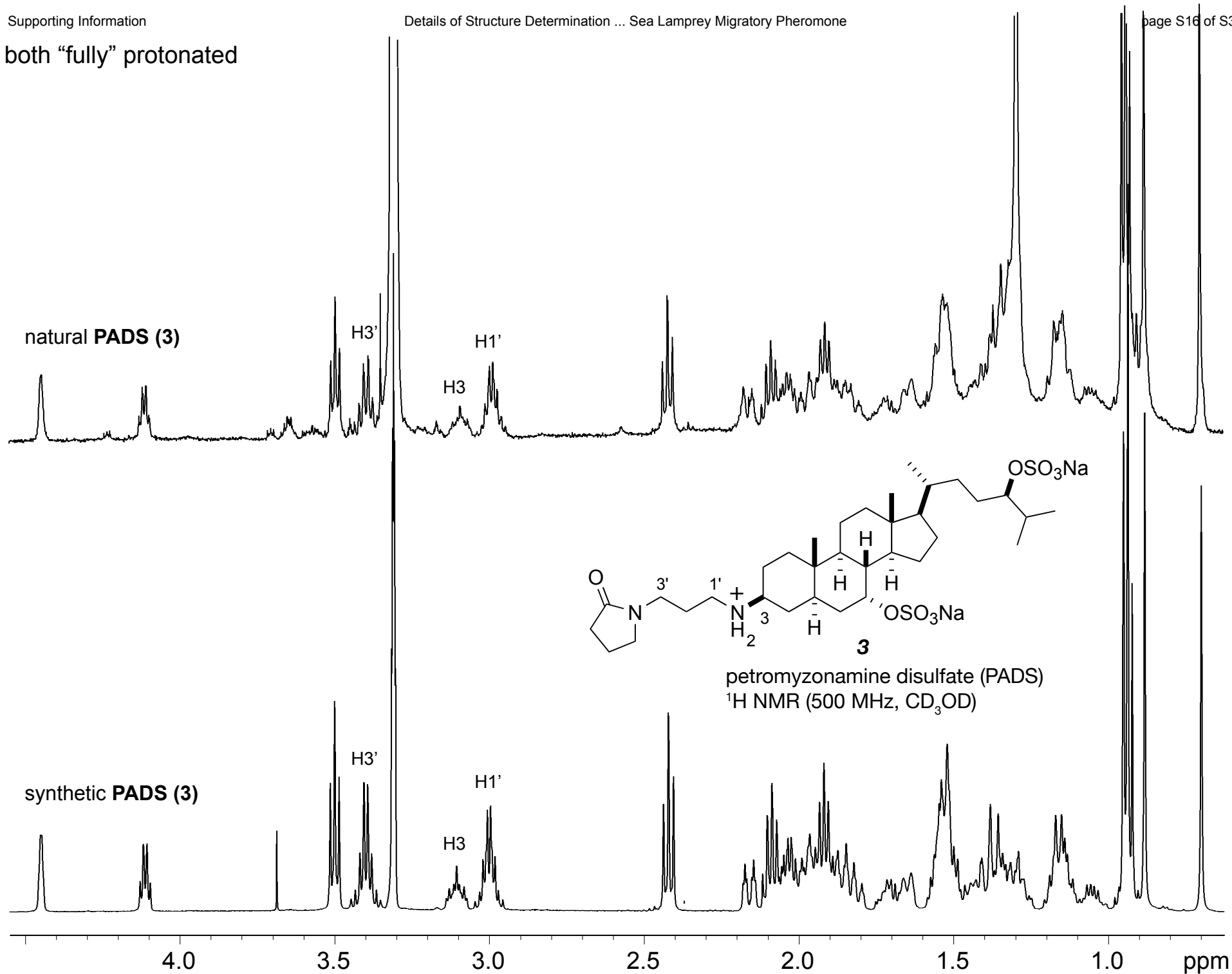
**1**

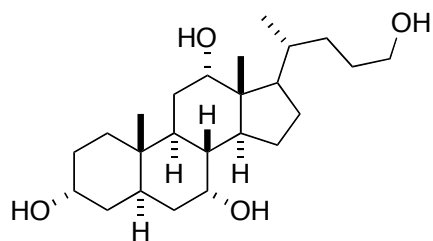
petromyzonol sulfate (PS)

¹H NMR (800 MHz, CD₃OD)

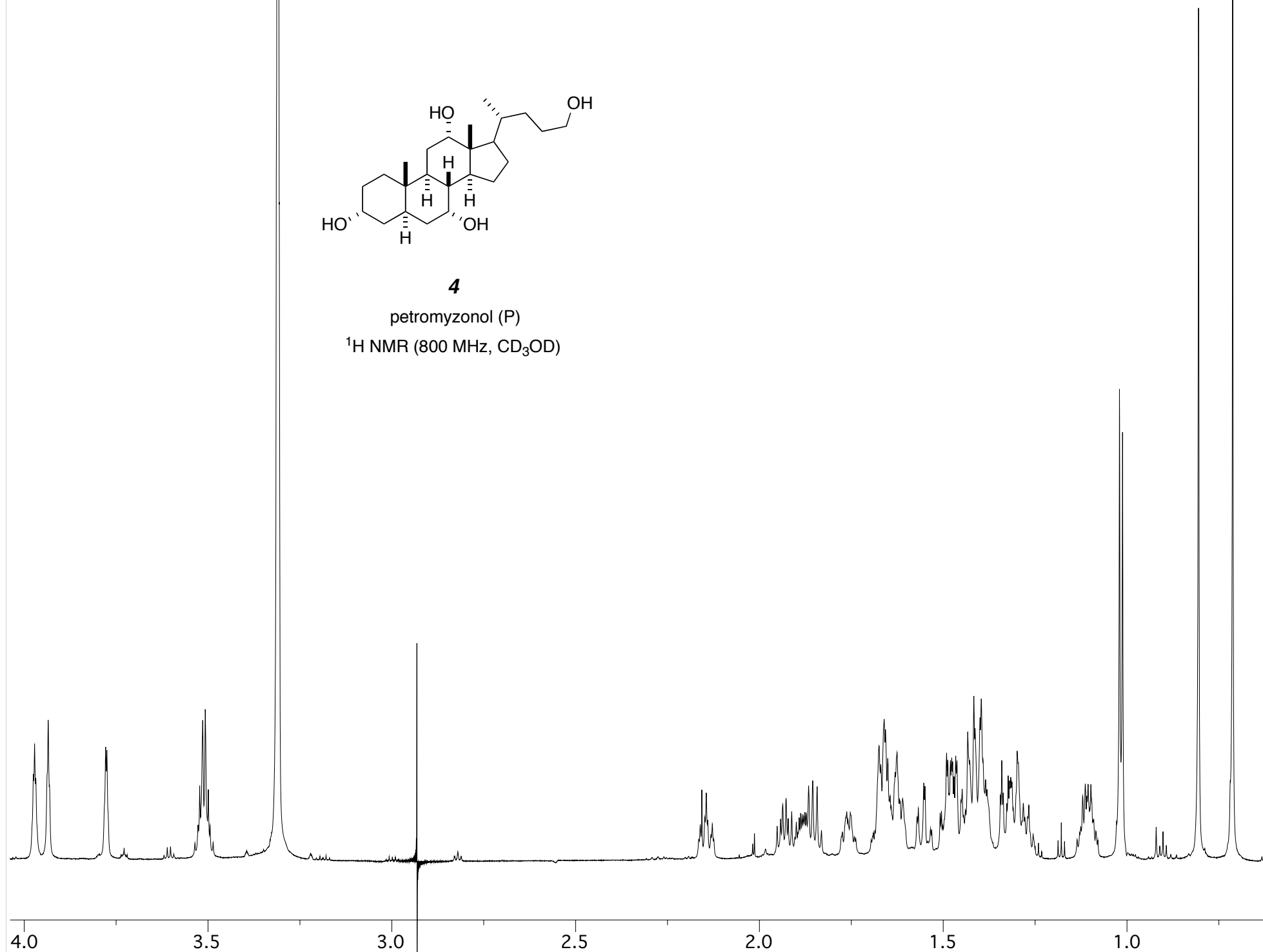


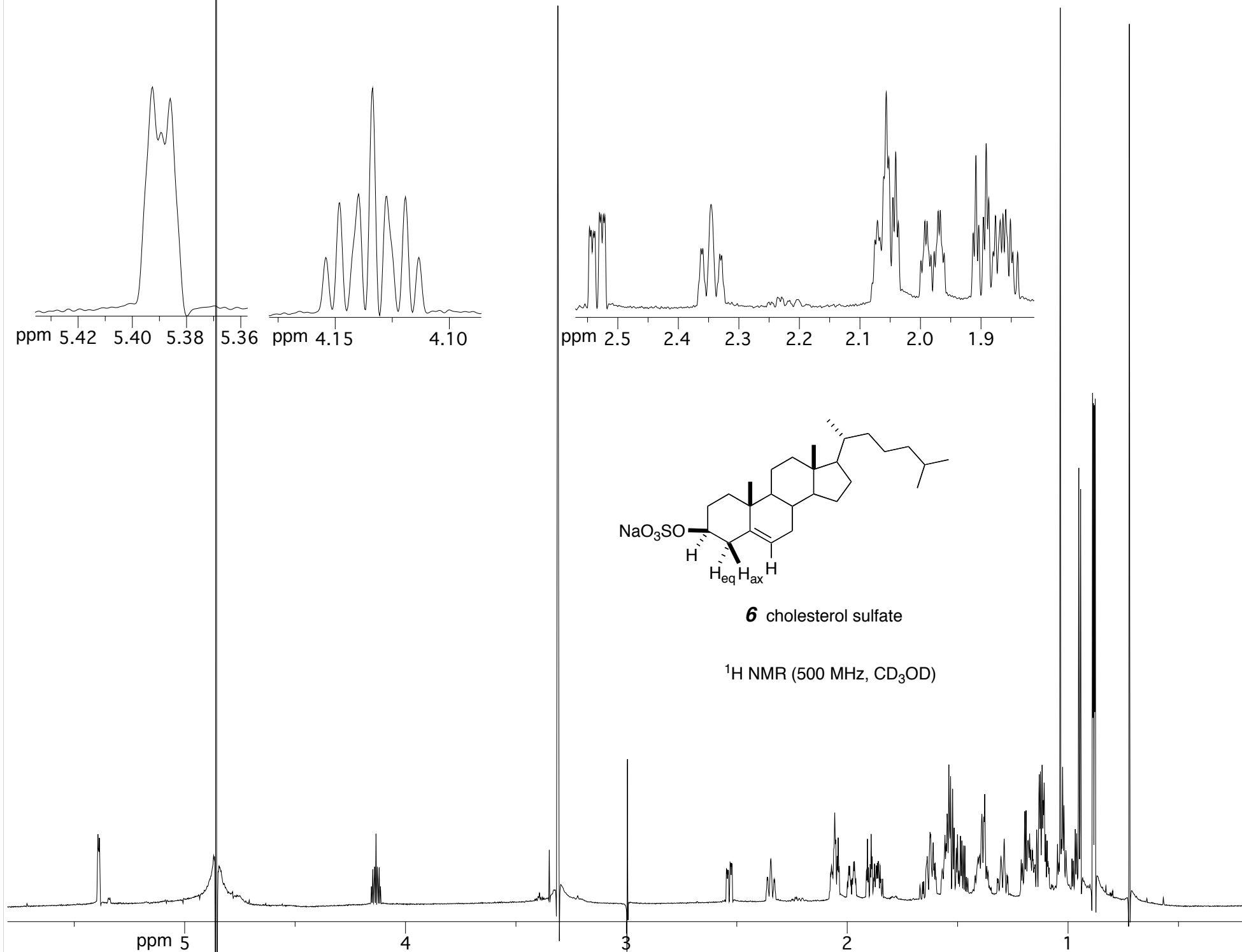
both "fully" protonated

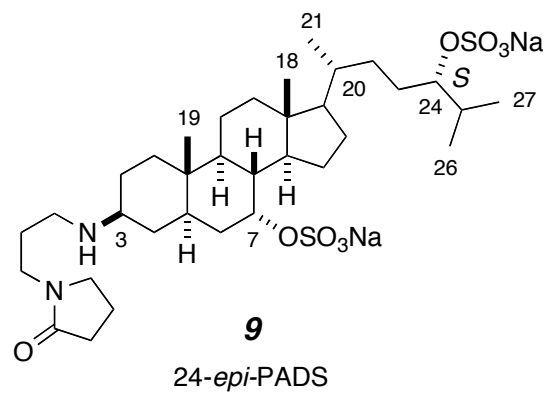


**4**

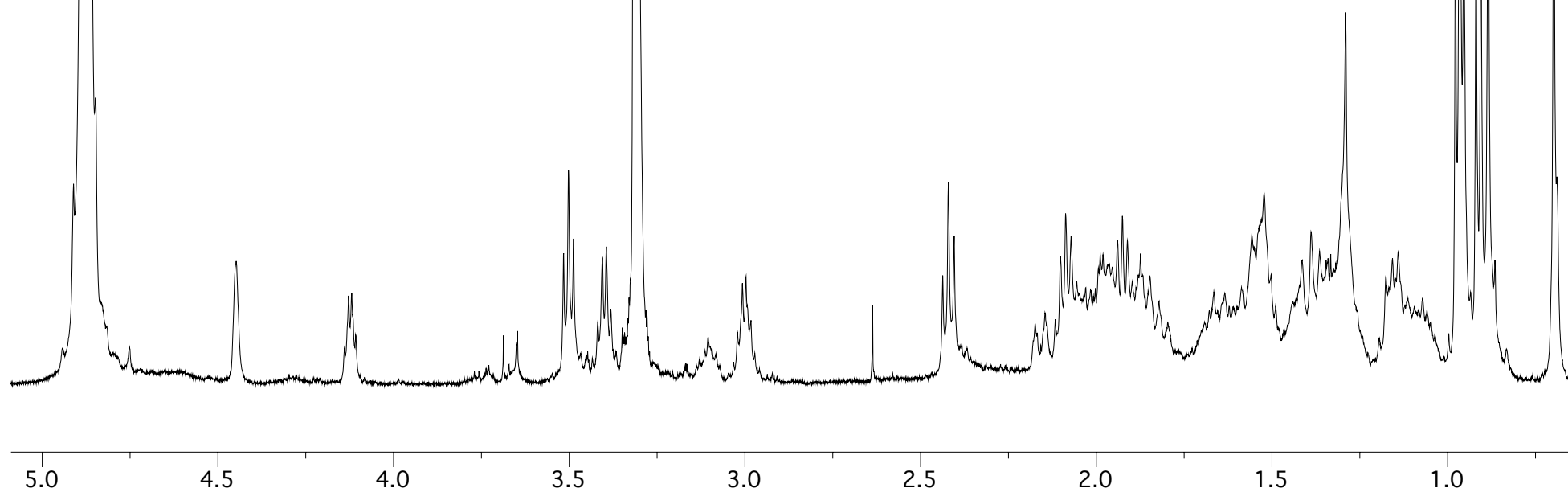
petromyzonol (P)

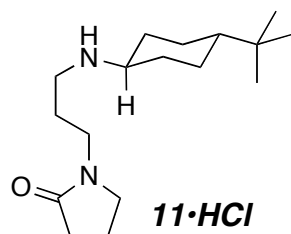
 ^1H NMR (800 MHz, CD_3OD)



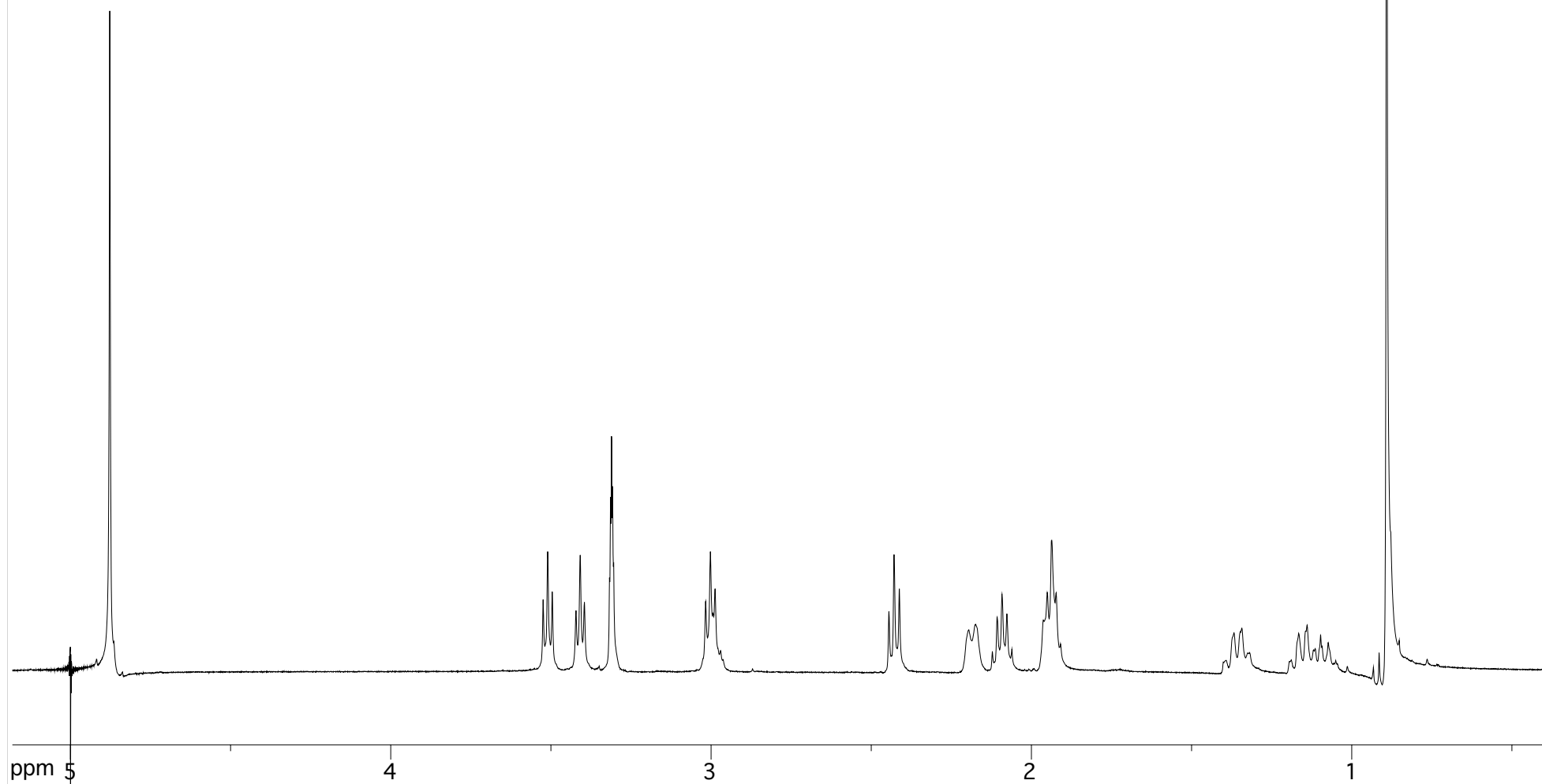


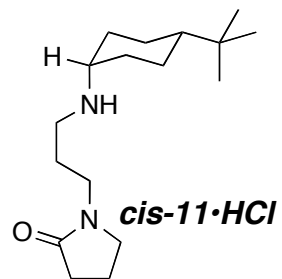
^1H NMR (CD_3OD , 500 MHz)



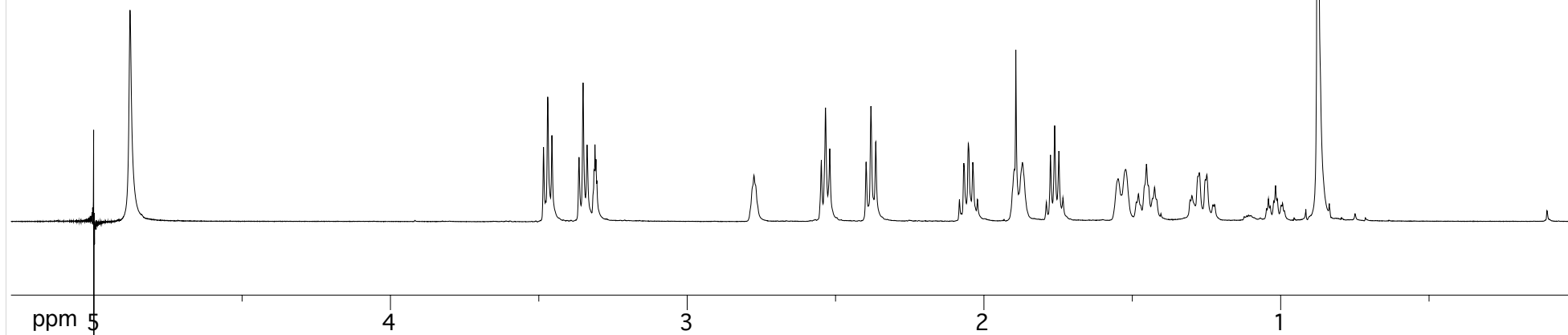


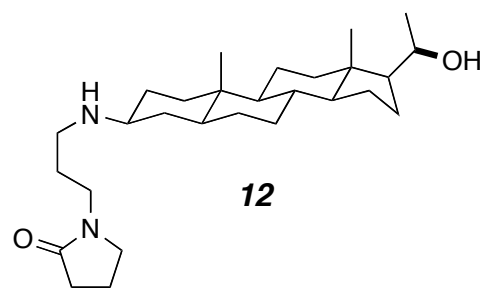
^1H NMR (CD_3OD , 500 MHz)



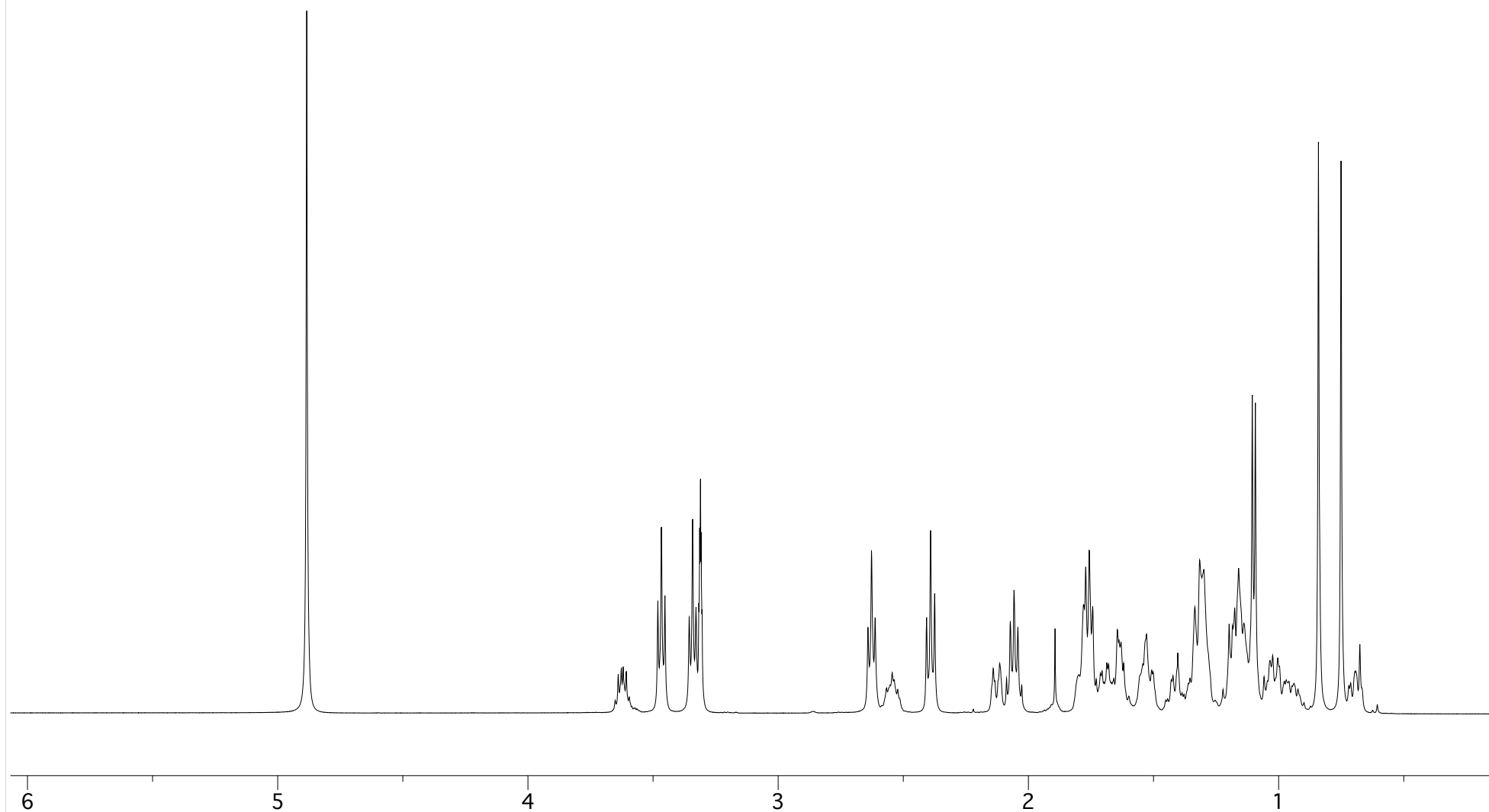


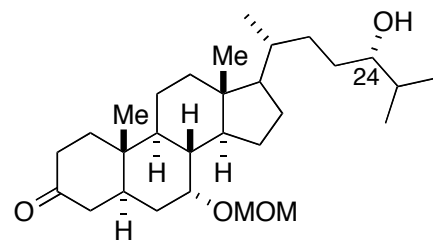
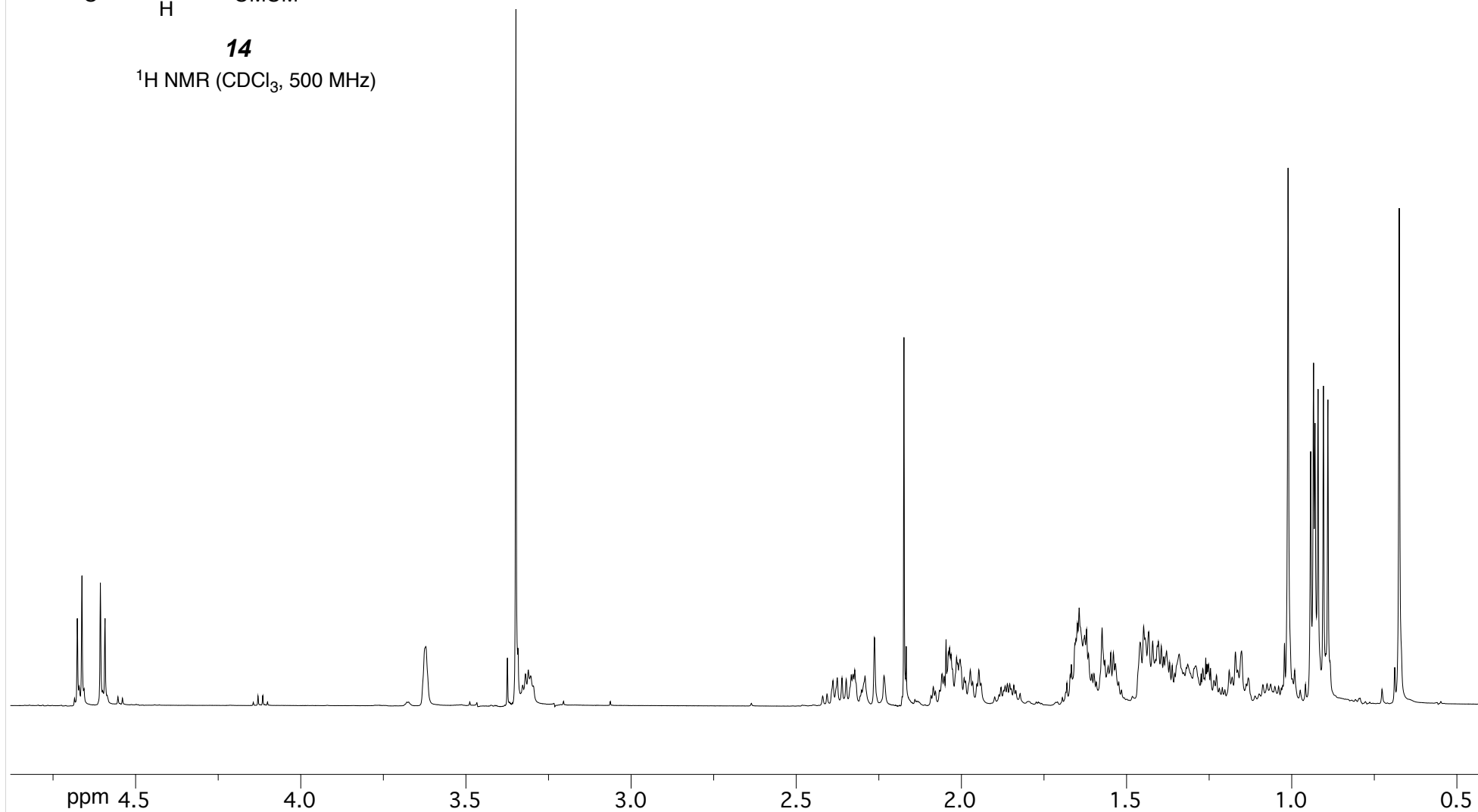
^1H NMR (CD_3OD , 500 MHz)

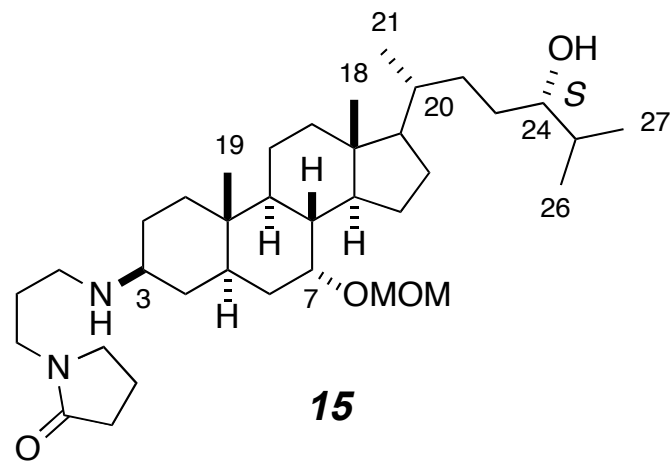




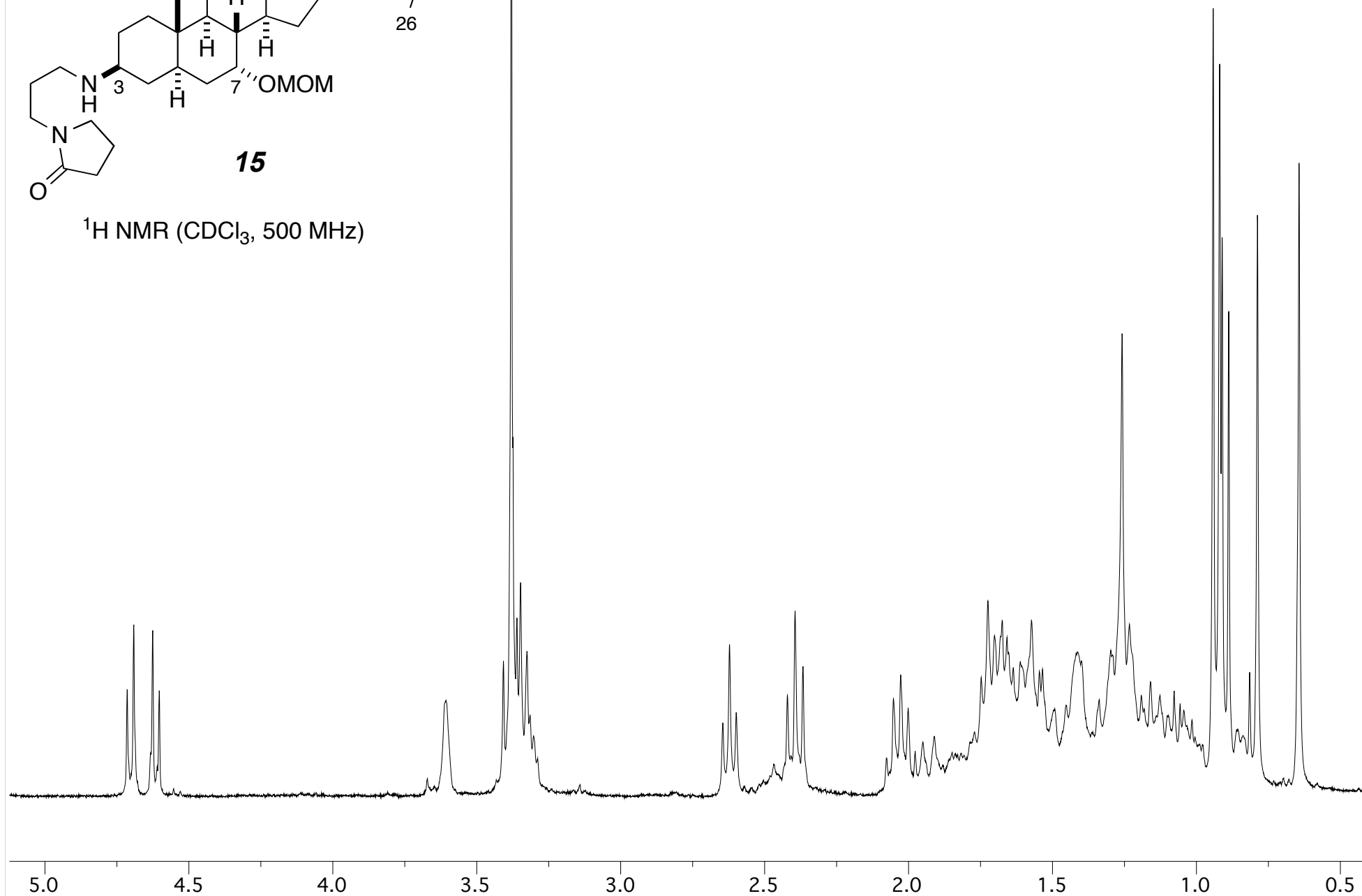
^1H NMR (CD_3OD , 500 MHz)

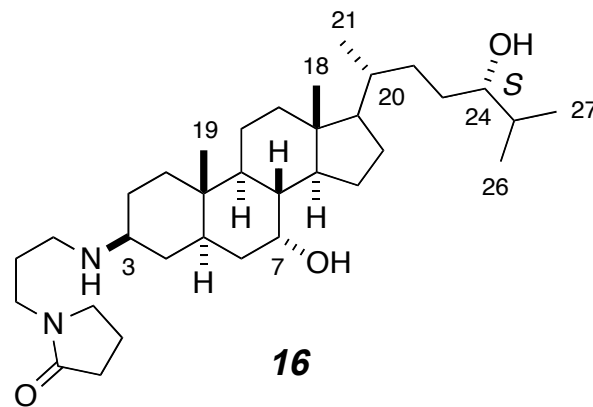


**14**¹H NMR (CDCl₃, 500 MHz)

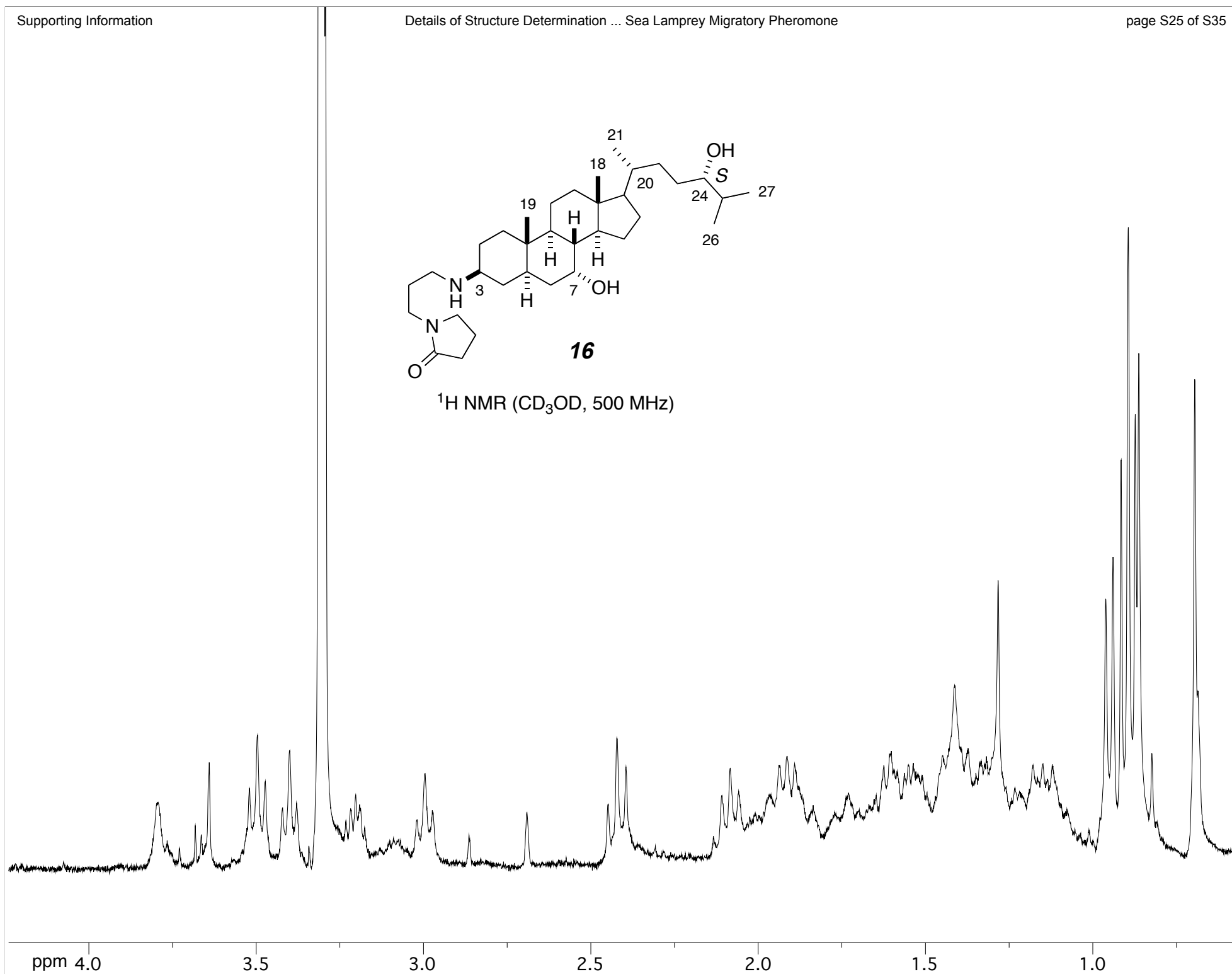


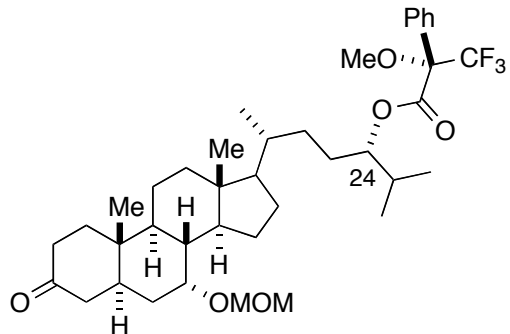
^1H NMR (CDCl_3 , 500 MHz)





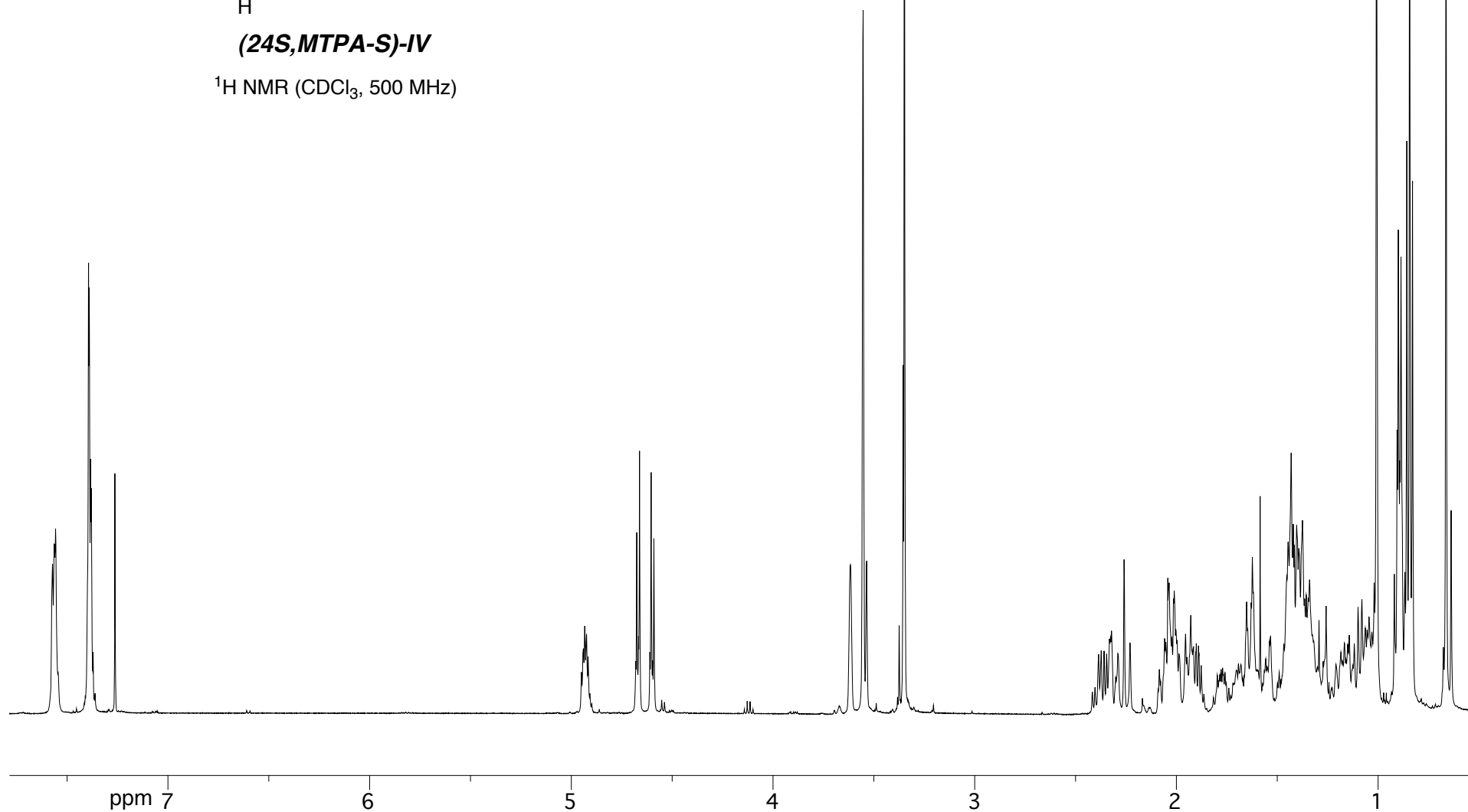
^1H NMR (CD_3OD , 500 MHz)

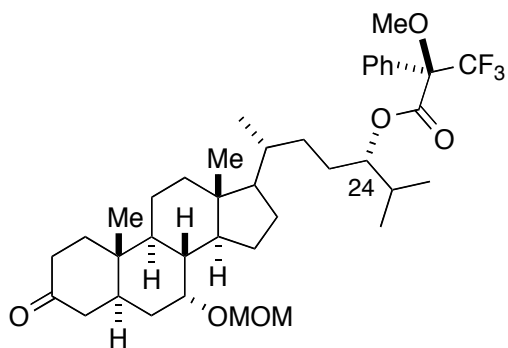
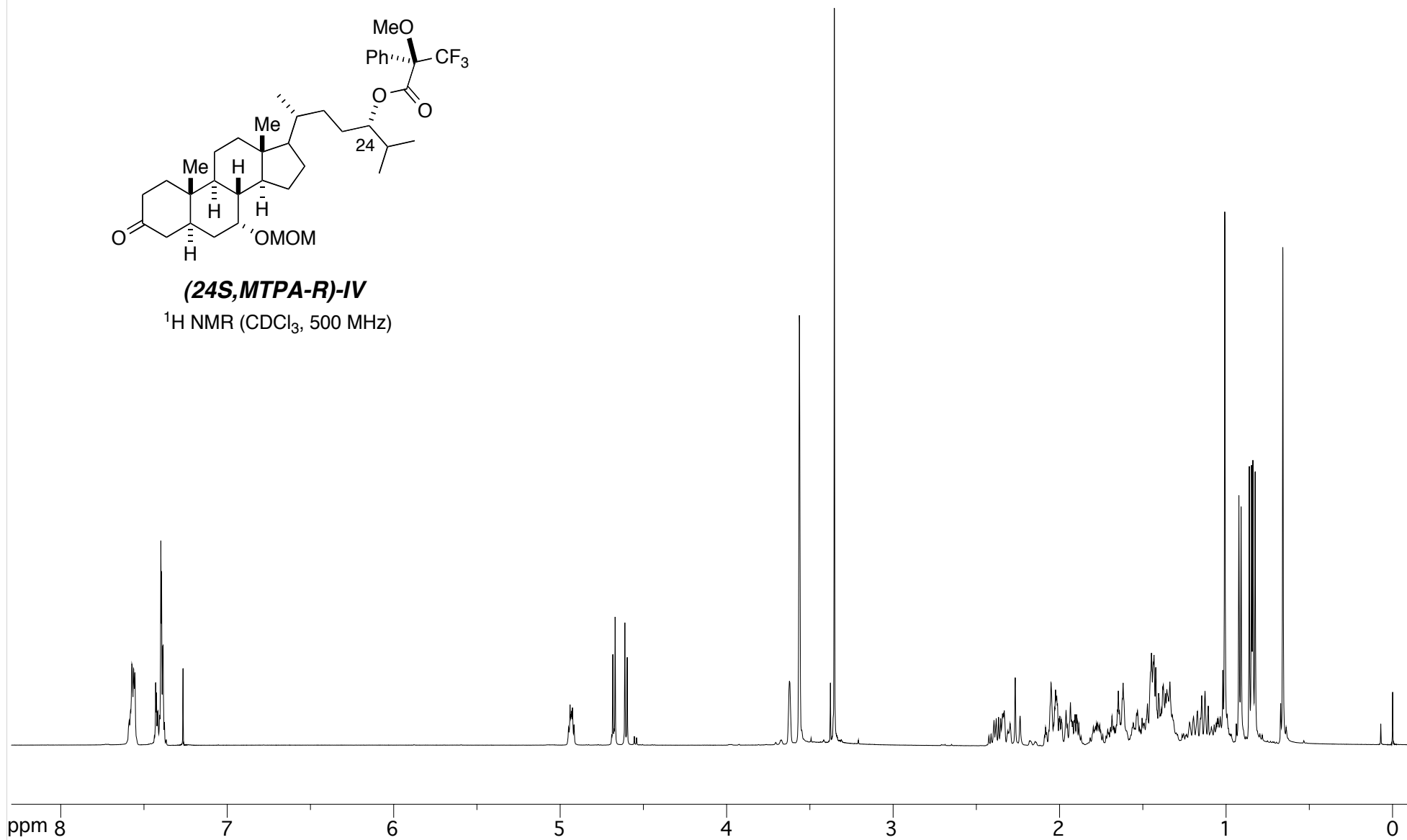


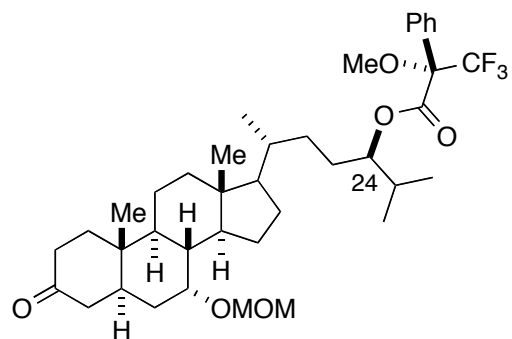


(24S, MTPA-S)-IV

^1H NMR (CDCl_3 , 500 MHz)

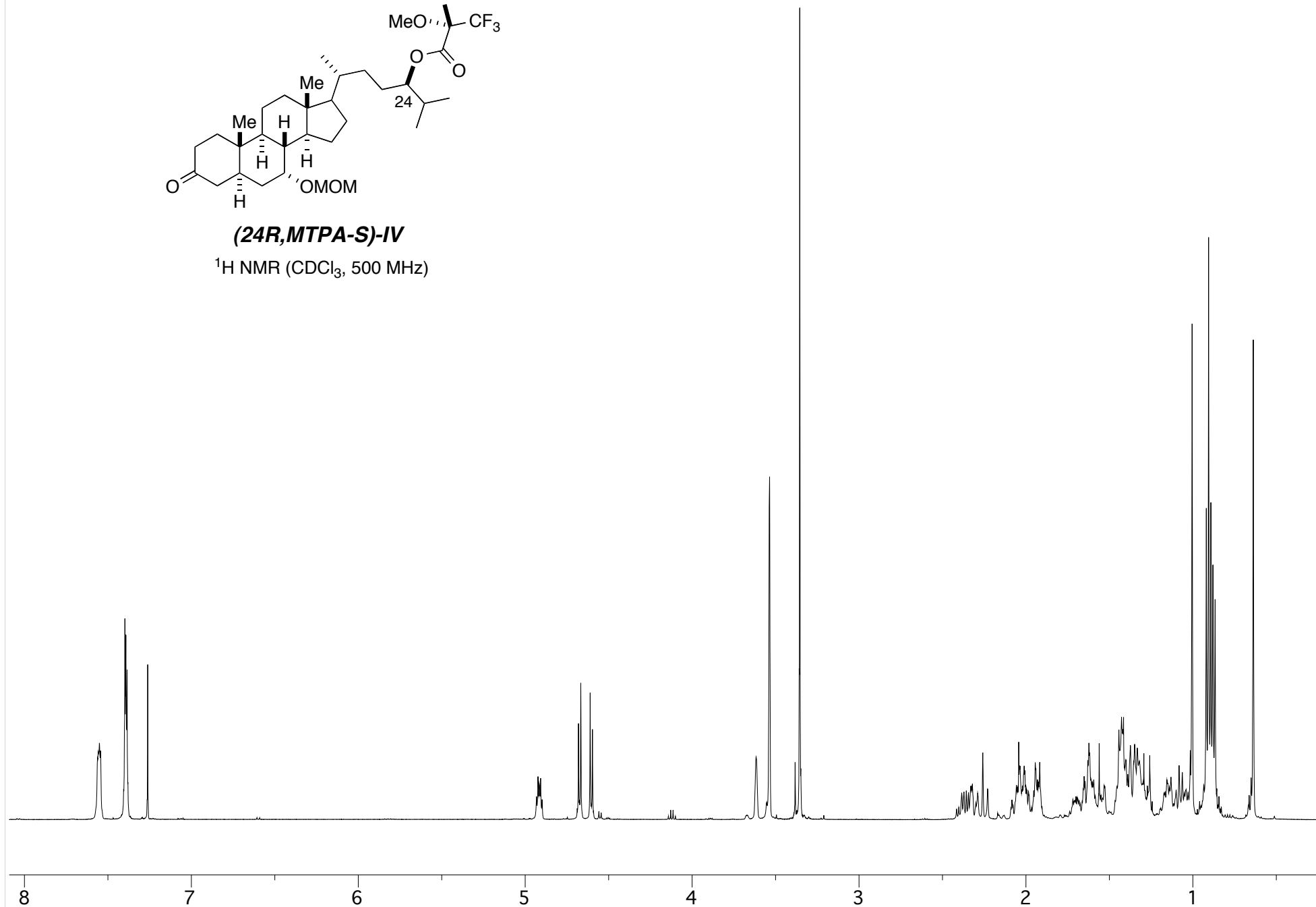


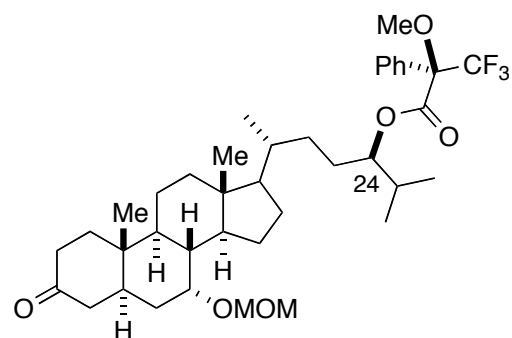
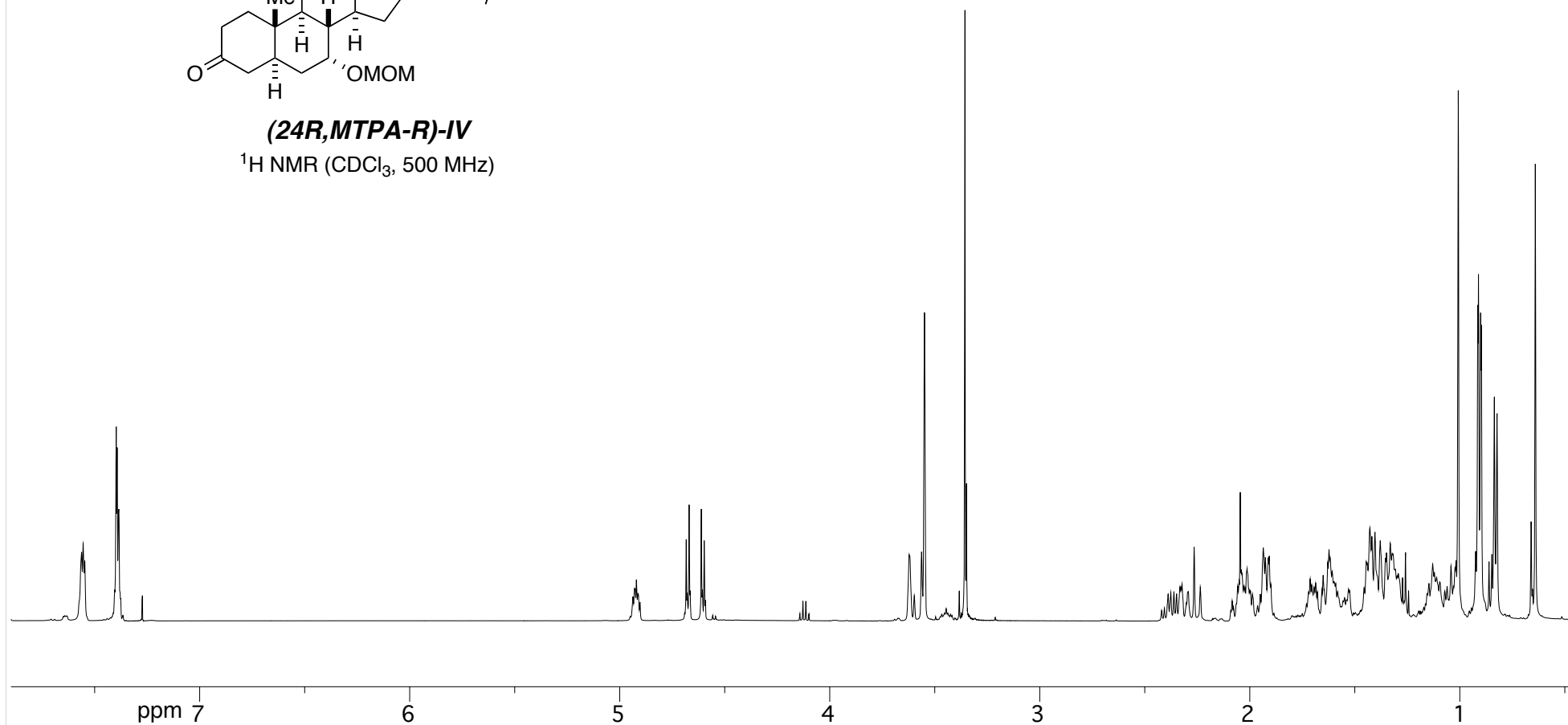
**(24S,MTPA-R)-IV**¹H NMR (CDCl₃, 500 MHz)

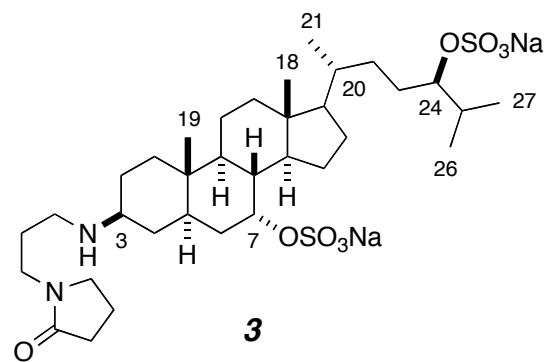


(24R,MTPA-S)-IV

^1H NMR (CDCl_3 , 500 MHz)

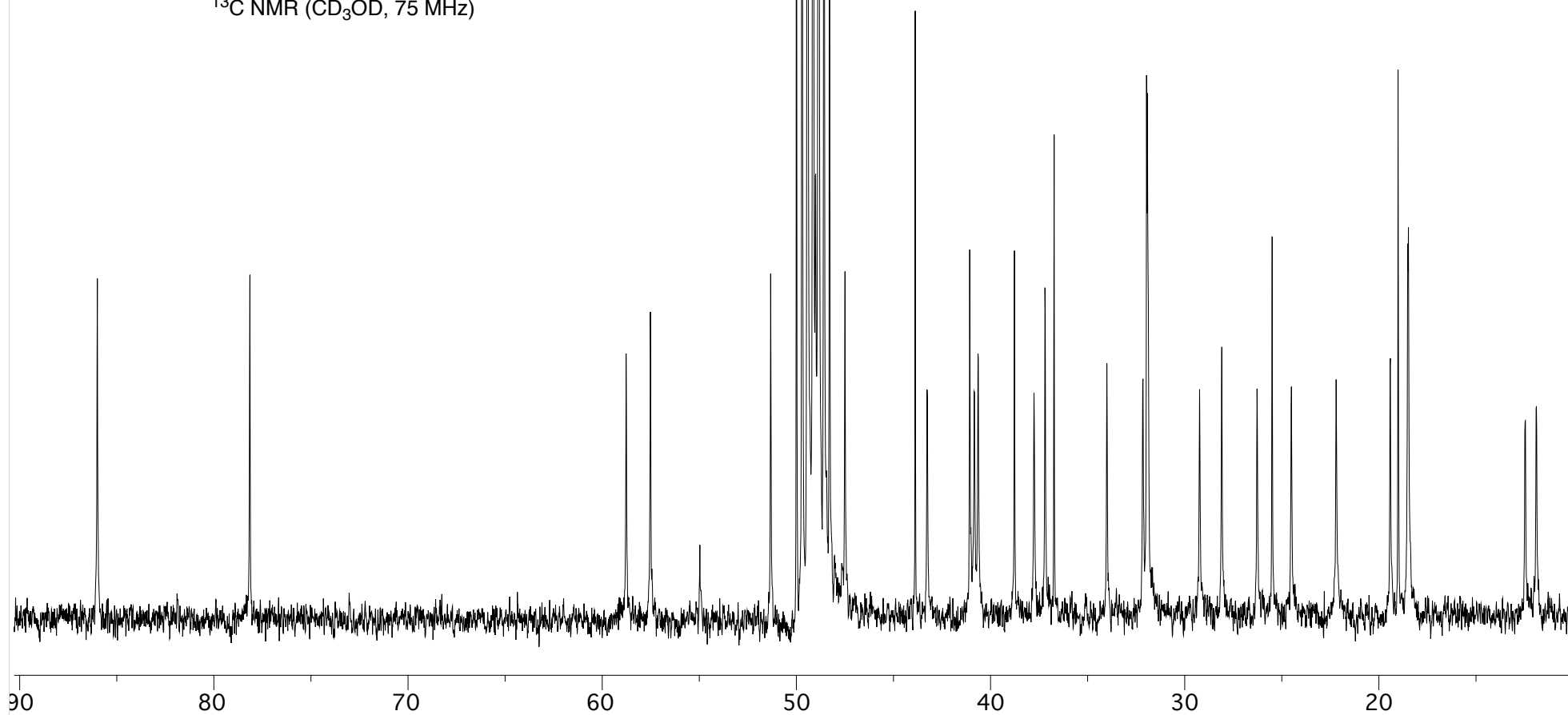
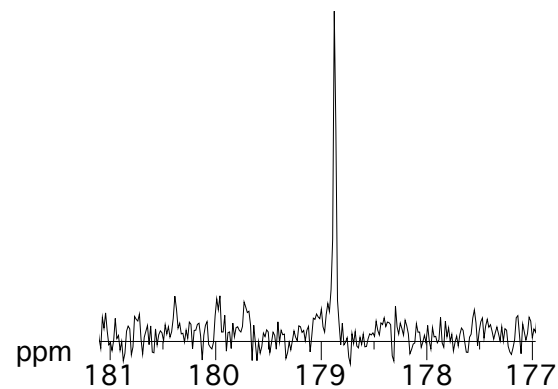


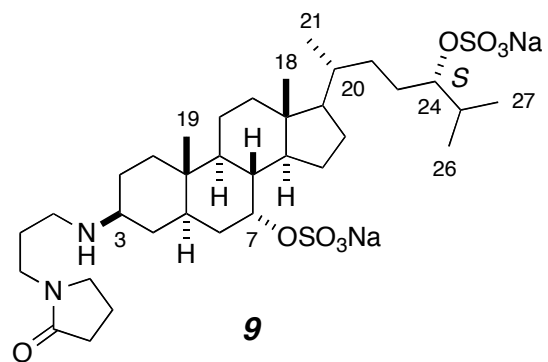
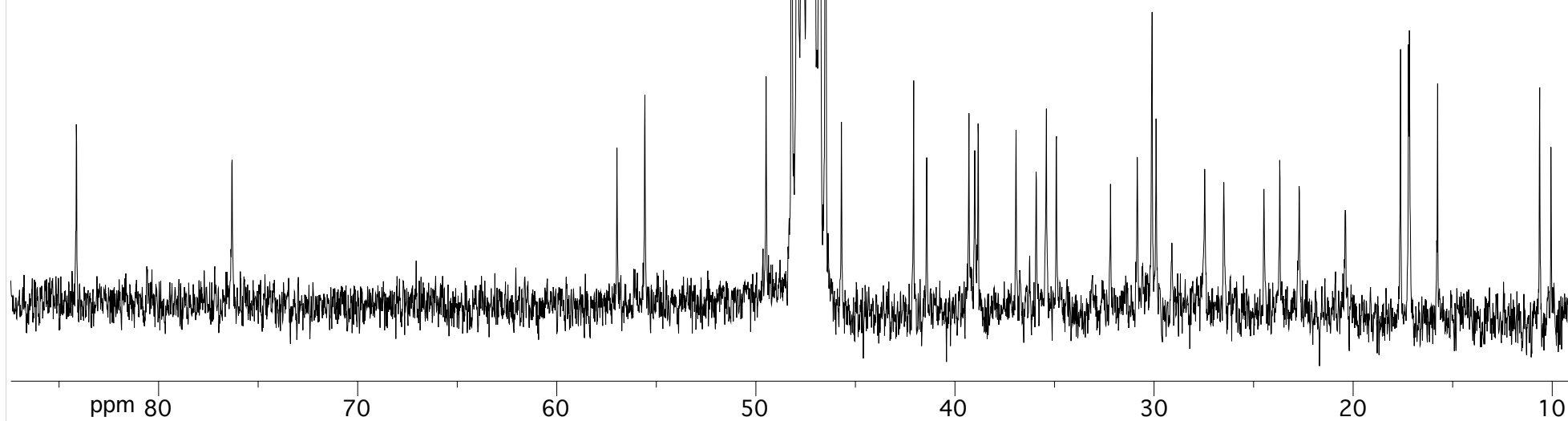
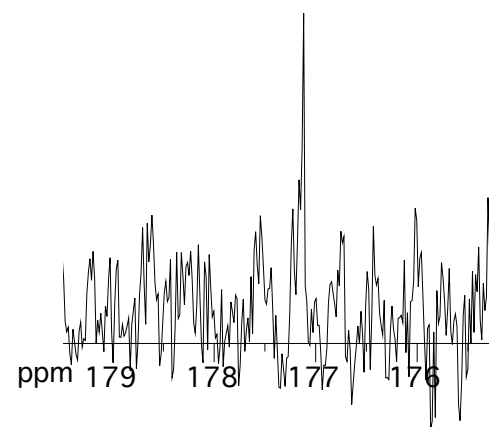
**(24R,MTPA-R)-IV**¹H NMR (CDCl₃, 500 MHz)

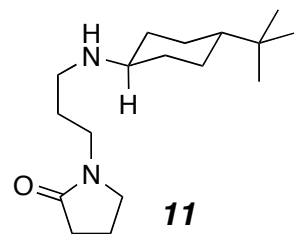


PADS protonated

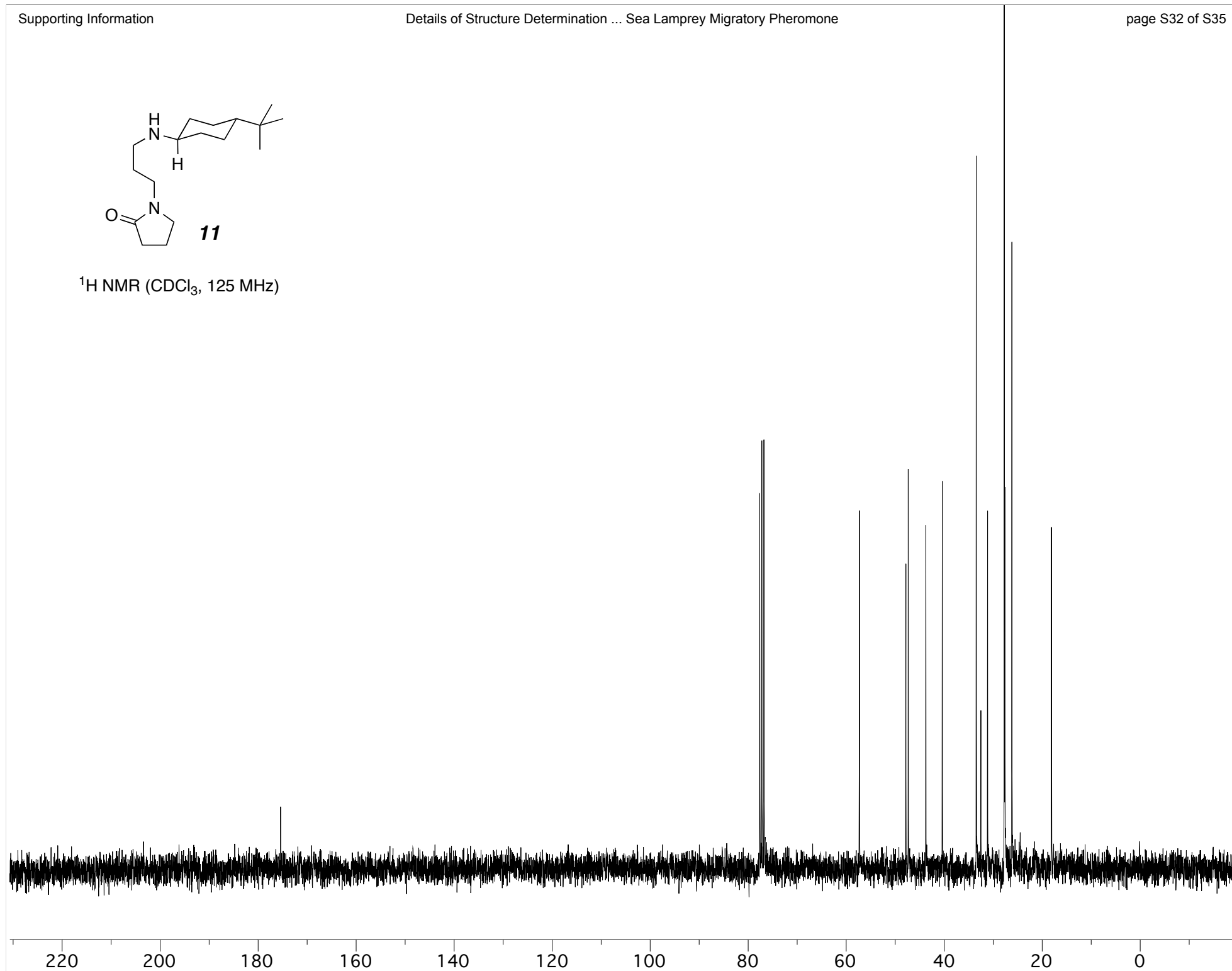
^{13}C NMR (CD_3OD , 75 MHz)

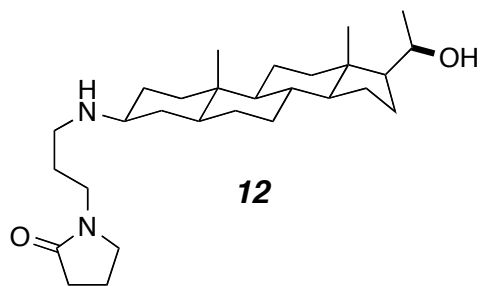


*24-epi-PADS protonated* ^{13}C NMR (CD_3OD , 75 MHz)

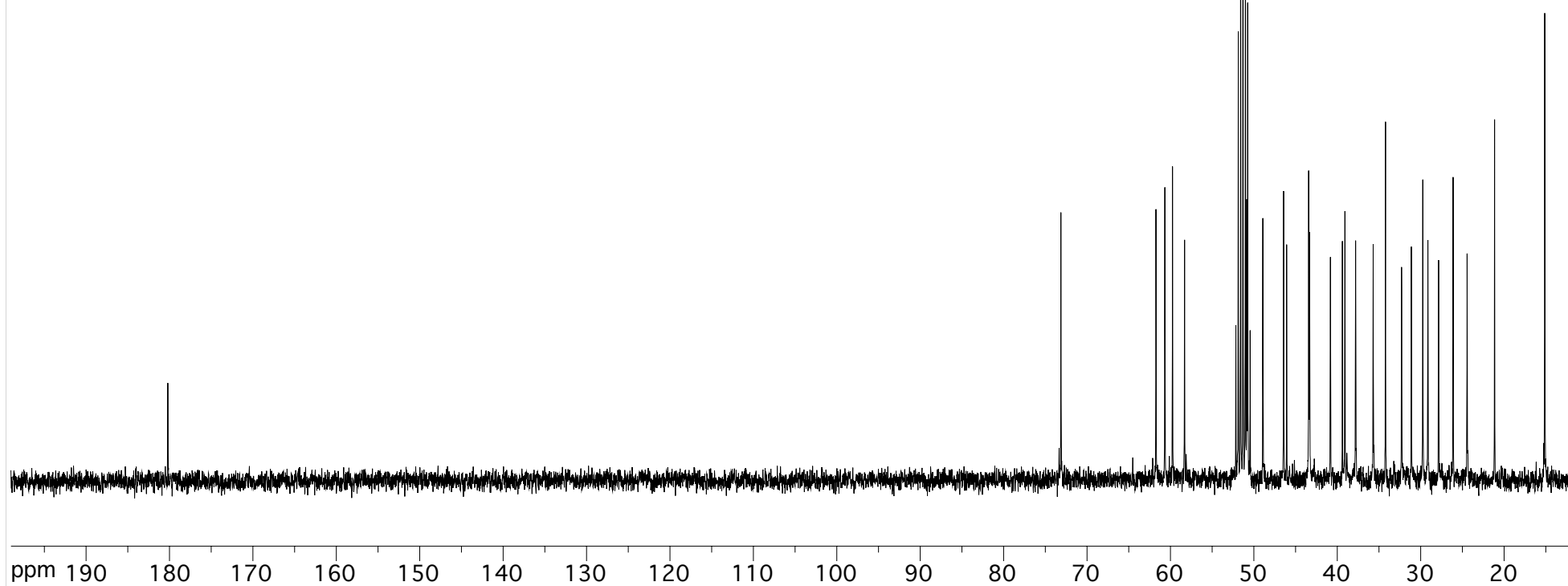


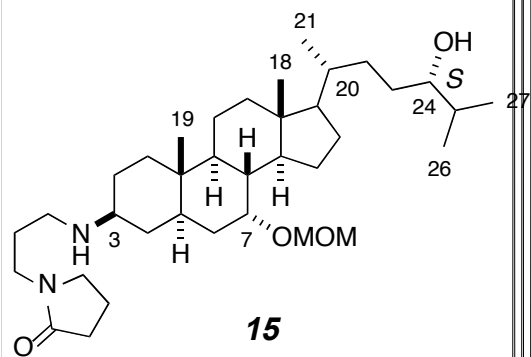
^1H NMR (CDCl_3 , 125 MHz)



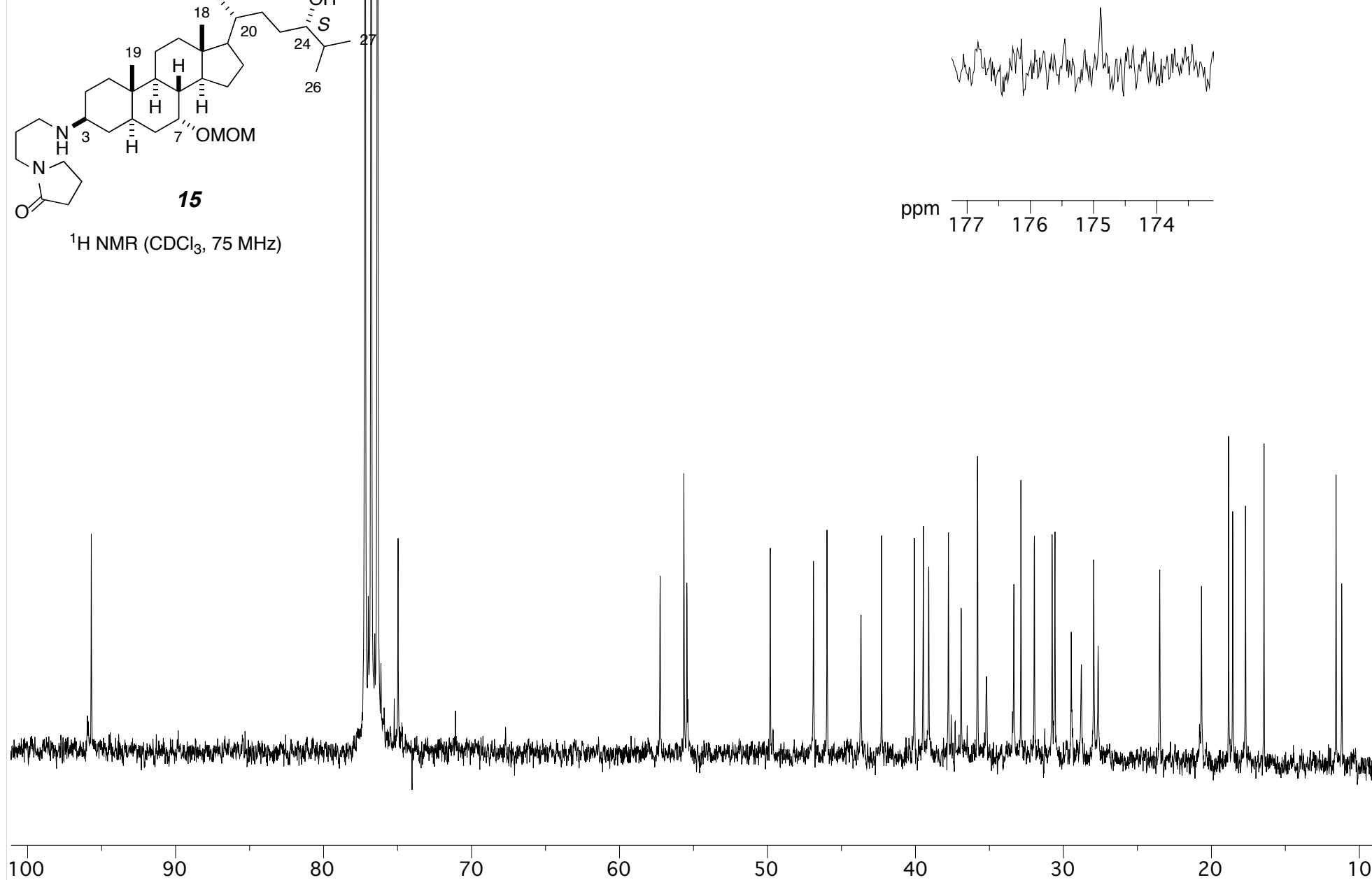


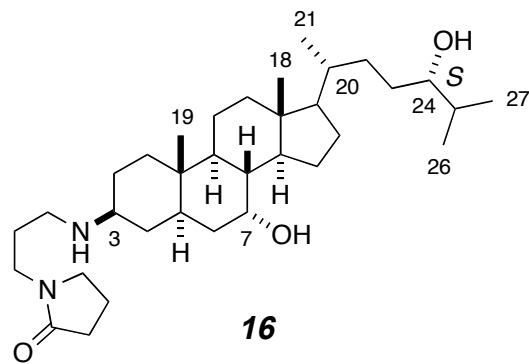
^{13}C NMR (CD_3OD , 125 MHz)





^1H NMR (CDCl_3 , 75 MHz)





^{13}C NMR (CD_3OD , 75 MHz)

