Supporting information

Cyanide Antidotes for Mass Casualties: Water-Soluble Salts of the Dithiane (Sulfanegen) from 3-Mercaptopyruvate for Intramuscular Administration

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Animals

Male ND-4 Swiss-Webster mice weighing 25-34 g were purchased from Harlan Labs, Indianapolis, IN. These mice were group housed in 17in x 10in x 8in polycarbonate cages and allowed ad lib access to Harlan Teklad #8604 rodent chow (Madison, WI) and water. The cages containing 8 mice per cage were placed in a temperature-controlled animal facilities barrier room (21-22 ^{o}C) with 12:12, L:D photoperiods (lights on at 0700). A week of adaptation was allowed in this new environment before experiments commenced. Experiments were approved and conducted in accordance with guidelines of the University of Minnesota and Department of Veterans Affairs Medical Center IACUC committees.

Statistical analysis

Data was analyzed by a one-factor ANOVA with Scheffe post hoc for comparison of treatment means. Data was represented as values ± S.E. with a p-value of <0.05 considered statistically significant. A simple regression determined dose-response relationships.

Mouse Model

The righting reflex recovery paradigm of Crankshaw et al, [Tox. Letters 175(2007) 111-117] was used in the mouse studies. Briefly the test is as follows: Mice are pretreated with a dose of sodium cyanide (4.8 mg/kg) intraperitoneally (i.p.) which induces a state of immobility for approximately 1 h. The animal is tested for recovery of righting reflex by placement on top of a suspended screen. The screen is then inverted and the mouse given 1 min to climb over on to the screen's upper surface. The endpoint is reached when the mouse reaches the screen's upper surface.

Chemistry

General Methods.

^{1}H and ^{13}C NMR spectra were obtained on a Varian 600 MHz spectrometer in the Center for Drug Design, University of Minnesota, Minneapolis, MN, except for the time studies which were performed on a Varian 200 MHz spectrometer. All chemical shifts are referenced to residual undeuterated solvent. Data of proton spectra are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m)], coupling constants [Hz], and integration). Carbon spectra (150 MHz) were recorded with complete proton decoupling and the chemical shifts are reported in ppm (C) relative to solvent resonance as internal standard except those in D_2O where C_6D_6 or CD_3OD contained inside a coaxial insert was referenced as an external standard. X-Ray structure determinations were performed by Victor G. Young, Jr. of the X-Ray Crystallographic Laboratory, University of Minnesota, Minneapolis, MN. Mass spectra were recorded on an Agilent G1960-6090 TOF mass spectrometer. Compound purity at 95% or greater was determined by microanalyses performed by Atlantic Microlab, Inc., Atlanta, GA, or by MHW Laboratories, Phoenix AZ. Unless stated otherwise, all the reagents were purchased from commercial sources and used without additional purification.

Procedure for the preparation of 2,5-dihydroxy-1,4-dithiane-2,5-dicarboxylic acid (6). A solution of disodium 2,5-dihydroxy-1,4-dithiane-2,5-dicarboxylic acid tetrahydrate (0.25 g, 0.70 mmol) in H_2O (2.5 mL) was applied to a column of ion-exchange resin Dowex 50WX8-200 (H^+;

4 mL, 7 meq), and was eluted with H_2O until the eluate tested negative for the presence of dithiane by $KMnO_4$ stain on TLC silica plate. The resulting solution was then lyophilized to yield a white solid.

Procedure for Preparation of salts 7a, c-f

Sulfanegen salts **7a**, **7c-7f** were prepared by addition of a 1.0 M solution of the desired amine (2 equiv.) in H_2O to the solution obtained immediately upon eluting 2,5-dihydroxy-1,4-dithiane-2,5-dicarboxlic acid (**6**), followed by lyophilization to constant weight to yield white solids.

Procedure for preparation of 2,5-Dihydroxy-1,4-dithiane-2,5-dicarboxylic acid, bis-((3*R*,4*R*,5*S*)-3-Amino-6-(hydroxymethyl)-oxane-2,4,5-triol) sesquihydrate salt (7b). Compound **7b** was prepared by elution of a solution of glucosamine hydrochloride (2 equiv.) through a column of ion-exchange resin Dowex 1X8-200 (HCO_3^-; 10 equiv.) and immediately into the solution of 2,5-dihydroxy-1,4-dithiane-2,5-dicarboxylic acid until the eluate tested negative for the presence of glucosamine by $KMnO_4$ stain on TLC silica plate. The resulting solution was lyophilized to constant weight to yield a white solid.

Characterization of compounds 6, 7a-f

2,5-dihydroxy-1,4-dithiane-2,5-dicarboxylic acid (6). Yield 99%. ^{1}H NMR (600 MHz, D_2O): δ 2.94 (s, monomer), 3.02 (2H, d, *J* = 14.4 Hz, major isomer), 3.22 (2H, d, *J* = 14.4 Hz, minor isomer), 3.64 (2H, d, *J* = 14.4 Hz, minor isomer), 3.91 (2H, d, *J* = 14.4 Hz). ^{13}C NMR (150 MHz, CD_3OD as external reference): δ 35.5, 76.3, 175.0. Anal. Calcd for $C_6H_8O_6S_2$: C, 30.00; H, 3.36; S, 26.69. Found: C, 30.12; H, 3.44; S, 26.48.

2,5-Dihydroxy-1,4-dithiane-2,5-dicarboxylic acid, bis-((*2R,3R,4R,5S*)-6-methylaminohexane-1,2,3,4,5-pentol) dihydrate salt (7a). Yield 99%. ^{1}H NMR (600 MHz, D_2O): δ 2.80 (6H, s), 2.87 (2H, d, *J* = 14.4 Hz, major isomer), 3.11 (2H, d, *J* = 14.4 Hz, minor isomer), 3.19-3.28 (4H, m), 3.58 (2H, d, *J* = 14.4 Hz, minor isomer), 3.67-3.70 (4H, m), 3.77-3.80 (2H, m), 3.83-3.86 (4H, m), 3.88 (2H, d, *J* = 14.4 Hz, major isomer), 4.12-4.15 (2H, m). ^{13}C NMR (150 MHz, C_6D_6 as external reference): δ 33.7, 36.7, 51.8, 63.4, 68.8, 71.2, 71.4, 71.6, 77.8, 177.6. Anal. Calcd for $C_{20}H_{42}N_2O_{16}S_2 \cdot 2H_2O$: C, 36.03; H, 6.95; N, 4.20; S, 9.62. Found: C, 35.82; H, 6.96; N, 4.27; S, 9.58. mp: 119-120 ^{o}C (dec.).

2,5-Dihydroxy-1,4-dithiane-2,5-dicarboxylic acid, bis-((3*R*,4*R*,5*S*)-3-Amino-6-(hydroxymethyl)-oxane-2,4,5-triol) sesquihydrate salt (7b). Yield 99%. ^{1}H NMR (600 MHz, D_2O): δ 2.87 (2H, d, *J* = 14.4 Hz, major isomer), 3.03 (1H, m), 3.11 (2H, d, *J* = 14.4 Hz, minor isomer), 3.33 (1H, m), 3.48-3.55 (3H, m), 3.59 (2H, d, *J* = 14.4 Hz, minor isomer), 3.71 (1H, m), 3.78-3.82 (2H, m), 3.85 (2H, d, *J* = 14.4 Hz, major isomer), 3.89-3.95 (4H, m), 4.96 (1H, d, *J* = 7.8 Hz), 5.47 (1H, d, *J* = 3.0 Hz). ^{13}C NMR (150 MHz, C_6D_6 as external reference): δ 36.6, 55.0, 57.4, 61.1, 70.4, 72.3, 72.7, 73.5, 76.9, 77.8, 85.0, 89.9, 93.5, 174.7, 177.6. Anal. Calcd for $C_{18}H_{34}N_2O_{16}S_2 \cdot 1.5H_2O$: C, 34.56; H, 5.96; N, 4.48; S, 10.25. Found: C, 34.50; H, 6.00; N, 4.39; S, 10.25. mp: 126-128 ^{o}C.

2,5-Dihydroxy-1,4-dithiane-2,5-dicarboxylic acid, bis-(2-aminoethanol) dodranhydrate salt (7c). Yield 99%. ^{1}H NMR (600 MHz, D_2O): δ 2.87 (2H, d, *J* = 14.4 Hz, major isomer), 3.16 (2H, d, *J* = 14.4 Hz, minor isomer), 3.16 (4H, t, *J* = 5.4 Hz), 3.58 (2H, d, *J* = 14.4 Hz, minor isomer),

3.85 (4H, t, *J* = 5.4 Hz), 3.88 (2H, d, *J* = 14.4 Hz, minor isomer). ^{13}C NMR (150 MHz, C_6D_6 as external reference): δ 36.7, 42.0, 58.3, 77.8, 187.1. Anal. Calcd for $C_{10}H_{22}N_2O_7S_2 \cdot ¾\ H_2O$: C, 31.95; H, 6.30; N, 7.45; S, 17.06. Found: C, 31.95; H, 6.24; N, 7.38; S, 17.23. mp: 73-75 ^oC.

2,5-Dihydroxy-1,4-dithiane-2,5-dicarboxylic acid, bis-(2,2'-iminodiethanol) salt (7d). Yield 99%. ^{1}H NMR (600 MHz, D_2O): δ 2.85 (2H, d, *J* = 14.4 Hz, major isomer), 3.10 (2H, d, *J* = 14.4 Hz, minor isomer), 3.28 (8H, t, *J* = 5.4 Hz), 3.59 (2H, d, *J* = 14.4 Hz, minor isomer), 3.88 (2H, d, *J* = 14.4 Hz, minor isomer), 3.90 (8H, t, *J* = 5.4 Hz). ^{13}C NMR (150 MHz, C_6D_6 as external reference): δ 36.7, 49.6, 57.2, 77.8, 177.6. Anal. Calcd for $C_{10}H_{30}N_2O_{10}S_2$: C, 37.32; H, 6.71; N, 6.22; S, 14.23. Found: C, 37.53; H, 6.77; N, 6.24; S, 14.18. Elucidation of structure via X-Ray crystallography. Crystals were grown from MeOH/Et_2O. mp: 104-105 ^oC.

2,5-Dihydroxy-1,4-dithiane-2,5-dicarboxylic acid, bis-(2,2',2"-nitrilotriethanol) salt (7e). Yield 99%. ^{1}H NMR (600 MHz, D_2O): δ 2.86 (2H, d, *J* = 14.4 Hz, major isomer), 3.09 (2H, d, *J* = 14.4 Hz, minor isomer), 3.51 (12H, t, *J* = 5.4 Hz), 3.58 (2H, d, *J* = 14.4 Hz, minor isomer), 3.87 (2H, d, *J* = 14.4 Hz), 3.98 (12H, t, *J* = 5.4 Hz). ^{13}C NMR (150 MHz, CD_3OD as external reference): δ 36.8, 55.9, 56.2, 77.9, 177.7. Anal. Calcd for $C_{18}H_{38}N_2O_{12}S_2$: C, 40.14; H, 7.11; N, 5.20; S, 11.91. Found: C, 39.84; H, 7.18; N, 5.32; S, 11.63. Elucidation of structure via X-Ray crystallography. Crystals were grown from THF/acetone. Mp: 122-123 ^oC.

2,5-Dihydroxy-1,4-dithiane-2,5-dicarboxylic acid, bis-(2-amino-2-hydroxymethyl-propane-1,3-diol) salt (7f). Yield 99%. ^{1}H NMR (600 MHz, D_2O): δ 2.86 (2H, d, *J* = 14.4 Hz, major isomer), 3.10 (2H, d, *J* = 14.4 Hz, minor isomer), 3.58 (2H, d, *J* = 14.4 Hz, minor isomer), 3.75 (12H, s), 3.87 (2H, d, *J* = 14.4 Hz). ^{13}C NMR (150 MHz, CD_3OD as external reference): δ 36.8, 60.2, 62.3, 77.9, 177.8. Anal. Calcd for $C_{14}H_{30}N_2O_{12}S_2$: C, 34.85; H, 6.27; N, 5.81; S, 13.29. Found: C, 34.74; H, 6.40; N, 5.73; S, 13.21. mp: 125-127 ^oC.

References

(1) Nagasawa, H. T.; Goon, D. J. W.; Crankshaw, D. L.; Vince, R.; Patterson, S. E. Novel, orally effective cyanide antidotes. *J. Med. Chem.* **2007,** *50,* 6462-6464.

2,5-Dihydroxy-1,4-dithiane-2,5-dicarboxylic acid, bis-(2,2'-iminodiethanol) salt (7d).CRYSTAL STRUCTURE REPORT

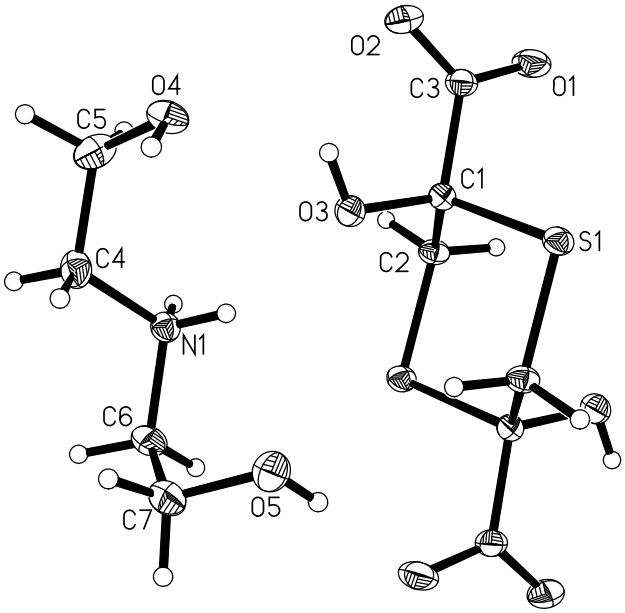
$C_{14}\ H_{30}\ N_2\ O_{10}\ S_2$

or

$2[C_4\ H_{12}\ N\ O_2]^+\ [C_6\ H_6\ O_6\ S]^{2-}$

Report prepared for:

A. Monteil / Prof. S. Patterson



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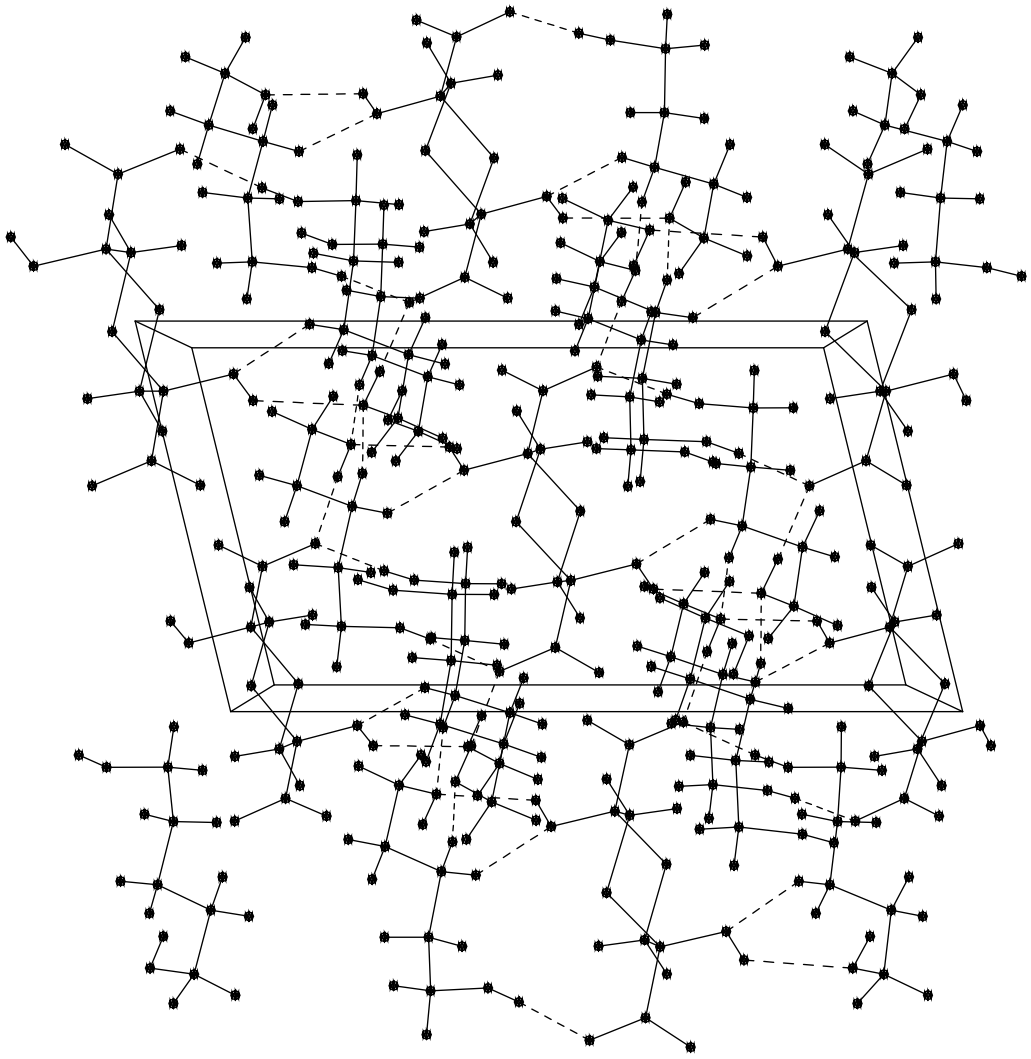

Data collection

A crystal (approximate dimensions 0.34x 0.20 x 0.18mm^3) was placed onto the tip of a 0.1 mm diameter glass capillary and mounted on a CCD area detector diffractometer for a data collection at173(2) K.[1] A preliminary set of cell constants was calculated from reflections harvested from three sets of 20 frames. These initial sets of frames were oriented such that orthogonal wedges of reciprocal space were surveyed. This produced initial orientation matrices determined from 58 reflections. The data collection was carried out using MoKα radiation (graphite monochromator) with a frame time of 45 seconds and a detector distance of 4.8 cm. A randomly oriented region of reciprocal space was surveyed to the extent of one hemisphere and to a resolution of 0.77 Å. Four major sections of frames were collected with 0.30º steps in ω at four different ϕ settings and a detector position of -28º in 2θ. The intensity data were corrected for absorption and decay (SADABS).[2] Final cell constants were calculated from 2978 strong reflections from the actual data collection after integration (SAINT).[3] Please refer to Table 1 for additional crystal and refinement information.

Structure solution and refinement

The structure was solved using Bruker SHELXTL[4] and refined using Bruker SHELXTL.[4] The space group $P2_1/n$ was determined based on systematic absences and intensity statistics. A direct-methods solution was calculated which provided most non-hydrogen atoms from the E-map. Full-matrix least squares / difference Fourier cycles were performed which located the remaining non-hydrogen atoms. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were placed in ideal positions and refined as riding atoms with relative isotropic displacement parameters. The final full matrix least squares refinement converged to $R1 = 0.0338$ and $wR2 = 0.0896$ (F^2, obs. data).

Structure description

The structure is the one suggested, except for the charges of the resultant cation and dianion. The acid groups lose protons to the amine groups to form a classical organic salt. The dianion lies on a crystallographic inversion center so ½ of this group is unique. Included in the asymmetric unit is one diethanolammine cation. The five protons are involved in unique hydrogen bonds.

Data collection and structure solution were conducted at the X-Ray Crystallographic Laboratory, 192 Kolthoff Hall, Department of Chemistry, University of Minnesota. All calculations were performed using Pentium computers using the current SHELXTL suite of programs.

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[1] SMART V5.054, Bruker Analytical X-ray Systems, Madison, WI (2001).

[2] An empirical correction for absorption anisotropy, R. Blessing, *Acta Cryst.* **A51**, 33-38(1995).

[3] SAINT+ V7.34A, Bruker Analytical X-Ray Systems, Madison, WI (2003).

[4] SHELXTL V6.14, Bruker Analytical X-Ray Systems, Madison, WI (2000).

Some equations of interest:

$$R_{int} = \Sigma|F_o^2 - \langle F_o^2 \rangle| \,/\, \Sigma|F_o^2|$$

$$R_1 = \Sigma||F_o| - |F_c|| \,/\, \Sigma|F_o|$$

$$wR2 = [\Sigma[w(F_o^2 - F_c^2)^2] \,/\, \Sigma[w(F_o^2)^2]]^{1/2}$$

$$\text{where } w = q \,/\, [\sigma^2(F_o^2) + (a*P)^2 + b*P + d + e*\sin(\theta)]$$

$$\text{GooF} = S = [\Sigma[w(F_o^2 - F_c^2)^2] \,/\, (n-p)]^{1/2}$$

Table 1. Crystal data and structure refinement for 09192a.

Identification code	09192a	
Empirical formula	$C_{14}\ H_{30}\ N_2\ O1_0\ S_2$	
Formula weight	450.52	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/n$	
Unit cell dimensions	a = 7.7641(8) Å	α = 90°
	b = 9.542(1) Å	β = 103.727(1)°
	c = 14.1303(14) Å	γ = 90°
Volume	1016.94(18) $Å^3$	
Z	2	
Density (calculated)	1.471 Mg/m^3	
Absorption coefficient	0.316 mm^{-1}	
F(000)	480	
Crystal color, morphology	Colorless, Prism	
Crystal size	0.34 x 0.20 x 0.18 mm^3	
Theta range for data collection	2.60 to 27.50°	
Index ranges	$-10 \leq h \leq 9,0 \leq k \leq 12,0 \leq l \leq 18$	
Reflections collected	7216	
Independent reflections	2316 [R(int) = 0.0341]	

Observed reflections	1925
Completeness to theta = 27.50°	99.3%
Absorption correction	Multi-scan
Max. and min. transmission	0.9453 and 0.9001
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2316 / 0 / 130
Goodness-of-fit on F^2	1.062
Final *R* indices [*I*>2sigma(*I*)]	*R*1 = 0.0338, *wR*2 = 0.0836
R indices (all data)	*R*1 = 0.0439, *wR*2 = 0.0896
Largest diff. peak and hole	0.495 and -0.253 e.Å^{-3}

Table 2. Atomic coordinates(x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 09192a. U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U_{eq}
S1	4854(1)	6688(1)	4507(1)	16(1)
C1	3248(2)	5825(2)	5089(1)	14(1)
C2	3167(2)	4251(2)	4885(1)	15(1)
C3	1455(2)	6538(2)	4628(1)	18(1)
O1	795(2)	6300(1)	3736(1)	26(1)
O2	826(2)	7297(1)	5173(1)	24(1)
O3	3705(2)	6047(1)	6102(1)	21(1)
N1	4735(2)	4407(1)	7842(1)	16(1)
C4	4153(2)	5219(2)	8617(1)	22(1)
C5	2559(2)	6137(2)	8233(1)	24(1)
O4	2947(2)	7372(1)	7754(1)	20(1)
C6	6390(2)	3575(2)	8224(1)	22(1)
C7	8017(2)	4501(2)	8431(1)	27(1)
O5	8098(2)	5324(1)	7610(1)	24(1)

Table 3. Bond lengths [Å] and angles [°] for 09192a.

S(1)-C(2)#1	1.8096(16)	C(4)-C(5)	1.509(2)
S(1)-C(1)	1.8427(15)	C(4)-H(4B)	0.9900
C(1)-O(3)	1.4071(18)	C(4)-H(4C)	0.9900
C(1)-C(2)	1.528(2)	C(5)-O(4)	1.427(2)
C(1)-C(3)	1.547(2)	C(5)-H(5B)	0.9900
C(2)-S(1)#1	1.8096(16)	C(5)-H(5C)	0.9900
C(2)-H(2A)	0.9900	O(4)-H(4A)	0.8400
C(2)-H(2B)	0.9900	C(6)-C(7)	1.512(2)
C(3)-O(2)	1.238(2)	C(6)-H(6A)	0.9900
C(3)-O(1)	1.263(2)	C(6)-H(6B)	0.9900
O(3)-H(3A)	0.8400	C(7)-O(5)	1.414(2)
N(1)-C(4)	1.496(2)	C(7)-H(7A)	0.9900
N(1)-C(6)	1.498(2)	C(7)-H(7B)	0.9900
N(1)-H(1A)	0.9200	O(5)-H(5A)	0.8400
N(1)-H(1B)	0.9200		

C(2)#1-S(1)-C(1)	98.94(7)	C(2)-C(1)-S(1)	110.91(10)
O(3)-C(1)-C(2)	109.24(12)	C(3)-C(1)-S(1)	104.61(10)
O(3)-C(1)-C(3)	110.30(12)	C(1)-C(2)-S(1)#1	113.89(11)
C(2)-C(1)-C(3)	111.10(13)	C(1)-C(2)-H(2A)	108.8
O(3)-C(1)-S(1)	110.62(10)	S(1)#1-C(2)-H(2A)	108.8

C(1)-C(2)-H(2B)	108.8	C(4)-C(5)-H(5C)	108.8
S(1)#1-C(2)-H(2B)	108.8	H(5B)-C(5)-H(5C)	107.7
H(2A)-C(2)-H(2B)	107.7	C(5)-O(4)-H(4A)	109.5
O(2)-C(3)-O(1)	126.78(15)	N(1)-C(6)-C(7)	111.45(14)
O(2)-C(3)-C(1)	116.30(14)	N(1)-C(6)-H(6A)	109.3
O(1)-C(3)-C(1)	116.92(13)	C(7)-C(6)-H(6A)	109.3
C(1)-O(3)-H(3A)	109.5	N(1)-C(6)-H(6B)	109.3
C(4)-N(1)-C(6)	113.15(13)	C(7)-C(6)-H(6B)	109.3
C(4)-N(1)-H(1A)	108.9	H(6A)-C(6)-H(6B)	108.0
C(6)-N(1)-H(1A)	108.9	O(5)-C(7)-C(6)	111.34(14)
C(4)-N(1)-H(1B)	108.9	O(5)-C(7)-H(7A)	109.4
C(6)-N(1)-H(1B)	108.9	C(6)-C(7)-H(7A)	109.4
H(1A)-N(1)-H(1B)	107.8	O(5)-C(7)-H(7B)	109.4
N(1)-C(4)-C(5)	113.70(14)	C(6)-C(7)-H(7B)	109.4
N(1)-C(4)-H(4B)	108.8	H(7A)-C(7)-H(7B)	108.0
C(5)-C(4)-H(4B)	108.8	C(7)-O(5)-H(5A)	109.5
N(1)-C(4)-H(4C)	108.8		
C(5)-C(4)-H(4C)	108.8		
H(4B)-C(4)-H(4C)	107.7		
O(4)-C(5)-C(4)	113.73(14)		
O(4)-C(5)-H(5B)	108.8		
C(4)-C(5)-H(5B)	108.8		
O(4)-C(5)-H(5C)	108.8		

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,-y+1,-z+1

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$)for 09192a. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2 a^{*2} U_{11} + ... + 2\ h\ k\ a^*\ b^*\ U_{12}\ ]$

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
S1	16(1)	13(1)	19(1)	3(1)	4(1)	1(1)
C1	15(1)	15(1)	13(1)	1(1)	3(1)	2(1)
C2	12(1)	14(1)	19(1)	1(1)	1(1)	-1(1)
C3	14(1)	17(1)	22(1)	4(1)	4(1)	2(1)
O1	20(1)	34(1)	20(1)	-2(1)	-1(1)	11(1)
O2	22(1)	25(1)	26(1)	0(1)	8(1)	9(1)
O3	25(1)	23(1)	14(1)	-1(1)	5(1)	9(1)
N1	17(1)	13(1)	16(1)	0(1)	3(1)	-1(1)
C4	32(1)	17(1)	17(1)	0(1)	7(1)	3(1)
C5	22(1)	19(1)	35(1)	-1(1)	13(1)	0(1)
O4	18(1)	16(1)	23(1)	-2(1)	-2(1)	0(1)
C6	22(1)	19(1)	23(1)	6(1)	4(1)	6(1)
C7	20(1)	38(1)	21(1)	5(1)	1(1)	3(1)
O5	24(1)	24(1)	24(1)	0(1)	8(1)	-2(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for 09192a.

	x	y	z	U(eq)
H2A	2967	4102	4174	18
H2B	2139	3852	5093	18
H3A	3048	6671	6247	31
H1A	4926	5019	7375	19
H1B	3839	3807	7549	19
H4B	5149	5814	8963	26
H4C	3871	4553	9096	26
H5B	1653	5586	7771	29
H5C	2044	6414	8783	29
H4A	3849	7764	8099	30
H6A	6506	2853	7740	26
H6B	6304	3090	8830	26
H7A	7993	5123	8988	32
H7B	9092	3909	8613	32
H5A	8372	4814	7184	36

Table 6. Torsion angles [°] for 09192a.

C2#1-S1-C1-O3	61.31(11)
C2#1-S1-C1-C2	-60.06(13)
C2#1-S1-C1-C3	-179.93(10)
O3-C1-C2-S1#1	-52.76(15)
C3-C1-C2-S1#1	-174.66(10)
S1-C1-C2-S1#1	69.43(13)
O3-C1-C3-O2	7.9(2)
C2-C1-C3-O2	129.17(15)
S1-C1-C3-O2	-111.10(14)
O3-C1-C3-O1	-172.90(14)
C2-C1-C3-O1	-51.61(19)
S1-C1-C3-O1	68.12(16)
C6-N1-C4-C5	176.13(14)
N1-C4-C5-O4	-75.05(19)
C4-N1-C6-C7	-76.19(17)
N1-C6-C7-O5	-52.99(19)

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,-y+1,-z+1

Table 7. Hydrogen bonds for 09192a [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O3-H3A...O2	0.84	2.10	2.5979(16)	117.9
N1-H1B...O4#2	0.92	1.92	2.8257(18)	166.2
N1-H1A...O3	0.92	2.07	2.8640(18)	143.7
O4-H4A...O1#3	0.84	1.80	2.6391(16)	174.5
O5-H5A...O1#1	0.84	1.91	2.7454(17)	175.0

Symmetry transformations used to generate equivalent atoms:

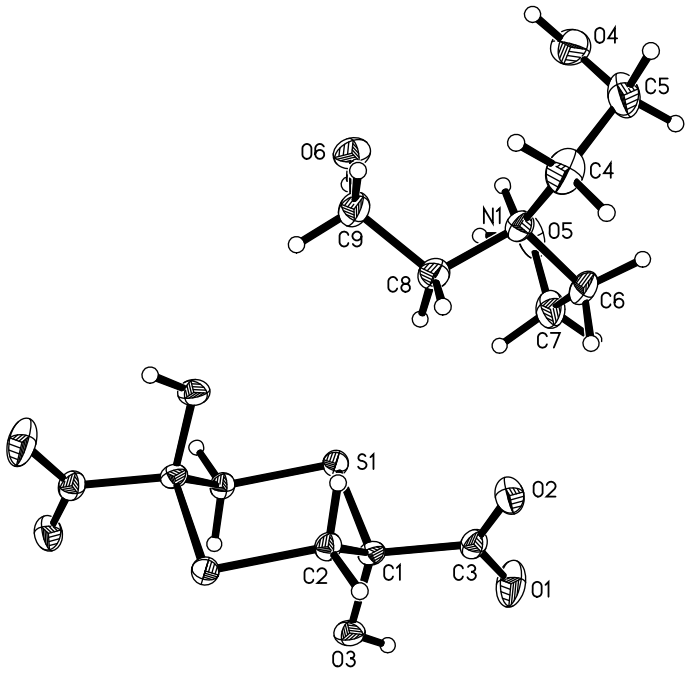
#1 -x+1,-y+1,-z+1 #2 -x+1/2,y-1/2,-z+3/2 #3 x+1/2,-y+3/2,z+1/2

2,5-Dihydroxy-1,4-dithiane-2,5-dicarboxylic acid, bis-(2,2',2"-nitrilotriethanol) salt (7e).
CRYSTAL STRUCTURE REPORT

$C_9 H_{19} N O_6 S$

Report prepared for:

A. Monteil / Prof. S. Patterson



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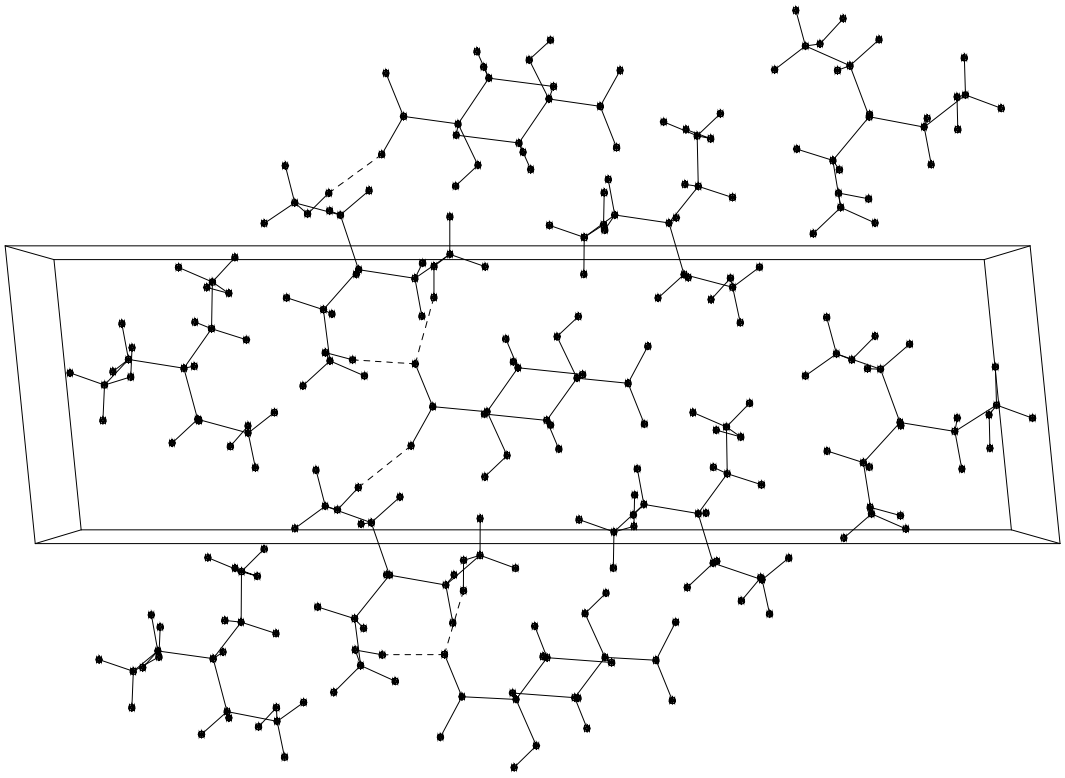


Data collection

A crystal (approximate dimensions 0.40x 0.20 x 0.08mm^3) was placed onto the tip of a 0.1 mm diameter glass capillary and mounted on a CCD area detector diffractometer for a data collection at173(2) K.[1] A preliminary set of cell constants was calculated from reflections harvested from three sets of 20 frames. These initial sets of frames were oriented such that orthogonal wedges of reciprocal space were surveyed. This produced initial orientation matrices determined from 49 reflections. The data collection was carried out using MoKα radiation (graphite monochromator) with a frame time of 30 seconds and a detector distance of 4.8 cm. A randomly oriented region of reciprocal space was surveyed to the extent of one sphere and to a resolution of 0.77 Å. Four major sections of frames were collected with 0.30º steps in ω at four different ϕ settings and a detector position of -28º in 2θ. The intensity data were corrected for absorption and decay (SADABS).[2] Final cell constants were calculated from 2934 strong reflections from the actual data collection after integration (SAINT).[3] Please refer to Table 1 for additional crystal and refinement information.

Structure solution and refinement

The structure was solved using Bruker SHELXTL[4] and refined using Bruker SHELXTL.[4] The space group $P2_1/n$ was determined based on systematic absences and intensity statistics. A direct-methods solution was calculated which provided most non-hydrogen atoms from the E-map. Full-matrix least squares / difference Fourier cycles were performed which located the remaining non-hydrogen atoms. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were placed in ideal positions and refined as riding atoms with relative isotropic displacement parameters. The final full matrix least squares refinement converged to $R1 = 0.0339$ and $wR2 = 0.0886$ (F^2, obs. data).

Structure description

The structure is the one suggested. There is one-half dianion per asymmetric unit along with one triethanolammonium cation. This is similar to the other two recent results.

Data collection and structure solution were conducted at the X-Ray Crystallographic Laboratory, 192 Kolthoff Hall, Department of Chemistry, University of Minnesota. All calculations were

performed using Pentium computers using the current SHELXTL suite of programs. All publications arising from this report MUST either 1) include Victor G. Young, Jr. as a coauthor or 2) acknowledge Victor G. Young, Jr.and the X-Ray Crystallographic Laboratory.

[1] SMART V5.054, Bruker Analytical X-ray Systems, Madison, WI (2001).

[2] An empirical correction for absorption anisotropy, R. Blessing, *Acta Cryst.* **A51**, 33-38(1995).

[3] SAINT+ V6.45, Bruker Analytical X-Ray Systems, Madison, WI (2003).

[4] SHELXTL V6.14, Bruker Analytical X-Ray Systems, Madison, WI (2000).

Some equations of interest:

$$R_{int} = \Sigma|F_o^2 - \langle F_o^2 \rangle| \,/\, \Sigma|F_o^2|$$

$$R_1 = \Sigma||F_o| - |F_c|| \,/\, \Sigma|F_o|$$

$$wR2 = [\Sigma[w(F_o^2 - F_c^2)^2] \,/\, \Sigma[w(F_o^2)^2]]^{1/2}$$

$$\text{where } w = q \,/\, [\sigma^2(F_o^2) + (a*P)^2 + b*P + d + e*\sin(\theta)]$$

$$\text{GooF} = S = [\Sigma[w(F_o^2 - F_c^2)^2] \,/\, (n-p)]^{1/2}$$

Table 1. Crystal data and structure refinement for 09198a.

Identification code	09198a	
Empirical formula	$C_9 H_{19} N O_6 S$	
Formula weight	269.31	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/n$	
Unit cell dimensions	a = 7.9344(9) Å	α = 90°
	b = 5.7430(6) Å	β = 95.749(2)°
	c = 27.174(3) Å	γ = 90°
Volume	1232.0(2) Å^3	
Z	4	
Density (calculated)	1.452 Mg/m^3	
Absorption coefficient	0.280 mm^{-1}	
F(000)	576	
Crystal color, morphology	Colorless, Plate	
Crystal size	0.40 x 0.20 x 0.08 mm^3	
Theta range for data collection	1.51 to 27.51°	
Index ranges	$-10 \leq h \leq 10,0 \leq k \leq 7,0 \leq l \leq 35$	
Reflections collected	14674	
Independent reflections	2825 [R(int) = 0.0364]	

Observed reflections	2341
Completeness to theta = 27.51°	99.5%
Absorption correction	Multi-scan
Max. and min. transmission	0.978 and 0.935
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2825 / 0 / 158
Goodness-of-fit on F^2	1.047
Final *R* indices [*I*>2sigma(*I*)]	*R*1 = 0.0339, *wR*2 = 0.0827
R indices (all data)	*R*1 = 0.0448, *wR*2 = 0.0886
Largest diff. peak and hole	0.458 and -0.323 e.Å^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 09198a. U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U_{eq}
S1	5697(1)	6656(1)	5523(1)	18(1)
C1	5592(2)	3447(3)	5478(1)	16(1)
C2	4075(2)	2731(3)	5121(1)	16(1)
C3	5410(2)	2599(3)	6013(1)	19(1)
O1	6755(2)	2172(3)	6265(1)	37(1)
O2	3940(2)	2422(2)	6144(1)	27(1)
O3	7096(1)	2551(2)	5318(1)	21(1)
N1	907(2)	7544(2)	6536(1)	20(1)
C4	-839(2)	6853(3)	6646(1)	34(1)
C5	-1326(2)	8197(4)	7094(1)	42(1)
O4	-1109(2)	10610(3)	7031(1)	40(1)
C6	2249(2)	6431(3)	6887(1)	27(1)
C7	3890(2)	7767(4)	6901(1)	33(1)
O5	3584(2)	10156(2)	6985(1)	37(1)
C8	1207(2)	7135(3)	6005(1)	26(1)
C9	338(2)	8947(3)	5664(1)	26(1)
O6	636(2)	11245(2)	5847(1)	27(1)

Table 3. Bond lengths [Å] and angles [°] for 09198a.

S(1)-C(2)#1	1.8122(15)	C(5)-O(4)	1.409(3)
S(1)-C(1)	1.8484(15)	C(5)-H(5B)	0.9900
C(1)-O(3)	1.4084(18)	C(5)-H(5C)	0.9900
C(1)-C(2)	1.525(2)	O(4)-H(4A)	0.8400
C(1)-C(3)	1.553(2)	C(6)-C(7)	1.509(3)
C(2)-S(1)#1	1.8122(15)	C(6)-H(6B)	0.9900
C(2)-H(2A)	0.9900	C(6)-H(6C)	0.9900
C(2)-H(2B)	0.9900	C(7)-O(5)	1.416(3)
C(3)-O(1)	1.233(2)	C(7)-H(7A)	0.9900
C(3)-O(2)	1.2565(19)	C(7)-H(7B)	0.9900
O(3)-H(3A)	0.8400	O(5)-H(5A)	0.8400
N(1)-C(6)	1.499(2)	C(8)-C(9)	1.513(2)
N(1)-C(4)	1.500(2)	C(8)-H(8A)	0.9900
N(1)-C(8)	1.502(2)	C(8)-H(8B)	0.9900
N(1)-H(1A)	0.9300	C(9)-O(6)	1.422(2)
C(4)-C(5)	1.524(3)	C(9)-H(9A)	0.9900
C(4)-H(4B)	0.9900	C(9)-H(9B)	0.9900
C(4)-H(4C)	0.9900	O(6)-H(6A)	0.8400
C(2)#1-S(1)-C(1)	97.93(7)	O(3)-C(1)-C(3)	109.67(12)
O(3)-C(1)-C(2)	110.07(12)	C(2)-C(1)-C(3)	111.71(12)

O(3)-C(1)-S(1)	110.51(10)	C(5)-C(4)-H(4C)	109.7
C(2)-C(1)-S(1)	109.79(10)	H(4B)-C(4)-H(4C)	108.2
C(3)-C(1)-S(1)	104.98(10)	O(4)-C(5)-C(4)	110.96(16)
C(1)-C(2)-S(1)#1	114.46(10)	O(4)-C(5)-H(5B)	109.4
C(1)-C(2)-H(2A)	108.6	C(4)-C(5)-H(5B)	109.4
S(1)#1-C(2)-H(2A)	108.6	O(4)-C(5)-H(5C)	109.4
C(1)-C(2)-H(2B)	108.6	C(4)-C(5)-H(5C)	109.4
S(1)#1-C(2)-H(2B)	108.6	H(5B)-C(5)-H(5C)	108.0
H(2A)-C(2)-H(2B)	107.6	C(5)-O(4)-H(4A)	109.5
O(1)-C(3)-O(2)	127.11(15)	N(1)-C(6)-C(7)	110.83(14)
O(1)-C(3)-C(1)	115.15(14)	N(1)-C(6)-H(6B)	109.5
O(2)-C(3)-C(1)	117.75(13)	C(7)-C(6)-H(6B)	109.5
C(1)-O(3)-H(3A)	109.5	N(1)-C(6)-H(6C)	109.5
C(6)-N(1)-C(4)	111.82(13)	C(7)-C(6)-H(6C)	109.5
C(6)-N(1)-C(8)	111.87(13)	H(6B)-C(6)-H(6C)	108.1
C(4)-N(1)-C(8)	112.84(13)	O(5)-C(7)-C(6)	109.59(15)
C(6)-N(1)-H(1A)	106.6	O(5)-C(7)-H(7A)	109.8
C(4)-N(1)-H(1A)	106.6	C(6)-C(7)-H(7A)	109.8
C(8)-N(1)-H(1A)	106.6	O(5)-C(7)-H(7B)	109.8
N(1)-C(4)-C(5)	109.81(15)	C(6)-C(7)-H(7B)	109.8
N(1)-C(4)-H(4B)	109.7	H(7A)-C(7)-H(7B)	108.2
C(5)-C(4)-H(4B)	109.7	C(7)-O(5)-H(5A)	109.5
N(1)-C(4)-H(4C)	109.7	N(1)-C(8)-C(9)	111.95(14)

N(1)-C(8)-H(8A)	109.2
C(9)-C(8)-H(8A)	109.2
N(1)-C(8)-H(8B)	109.2
C(9)-C(8)-H(8B)	109.2
H(8A)-C(8)-H(8B)	107.9
O(6)-C(9)-C(8)	111.96(13)
O(6)-C(9)-H(9A)	109.2
C(8)-C(9)-H(9A)	109.2
O(6)-C(9)-H(9B)	109.2
C(8)-C(9)-H(9B)	109.2
H(9A)-C(9)-H(9B)	107.9
C(9)-O(6)-H(6A)	109.5

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,-y+1,-z+1

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$)for 09198a. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2 a^{*2} U_{11} + ... + 2\ h\ k\ a^*\ b^*\ U_{12}\ ]$

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
S1	23(1)	14(1)	16(1)	-2(1)	0(1)	-1(1)
C1	18(1)	13(1)	19(1)	0(1)	1(1)	1(1)
C2	19(1)	13(1)	17(1)	1(1)	1(1)	-2(1)
C3	24(1)	15(1)	19(1)	1(1)	0(1)	-2(1)
O1	29(1)	54(1)	27(1)	16(1)	-6(1)	-2(1)
O2	26(1)	33(1)	21(1)	3(1)	5(1)	-2(1)
O3	18(1)	22(1)	23(1)	-2(1)	0(1)	5(1)
N1	23(1)	16(1)	20(1)	2(1)	0(1)	-1(1)
C4	24(1)	35(1)	40(1)	8(1)	-3(1)	-11(1)
C5	23(1)	75(2)	29(1)	18(1)	4(1)	4(1)
O4	37(1)	56(1)	25(1)	-3(1)	-6(1)	17(1)
C6	29(1)	27(1)	24(1)	9(1)	-2(1)	6(1)
C7	24(1)	51(1)	23(1)	10(1)	-1(1)	4(1)
O5	46(1)	45(1)	19(1)	-3(1)	7(1)	-19(1)
C8	37(1)	22(1)	20(1)	-2(1)	0(1)	7(1)
C9	29(1)	25(1)	22(1)	2(1)	-3(1)	2(1)
O6	24(1)	20(1)	38(1)	2(1)	5(1)	0(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for 09198a.

	x	y	z	U(eq)
H2A	3057	3550	5215	20
H2B	3883	1039	5158	20
H3A	7826	2397	5561	31
H1A	994	9141	6588	24
H4B	-871	5160	6713	40
H4C	-1661	7191	6356	40
H5B	-2524	7870	7142	50
H5C	-615	7670	7393	50
H4A	-1844	11105	6813	59
H6B	2434	4809	6781	32
H6C	1866	6385	7222	32
H7A	4708	7151	7168	39
H7B	4385	7575	6583	39
H5A	3819	10936	6740	55
H8A	780	5571	5903	32
H8B	2440	7169	5975	32
H9A	756	8815	5334	31

H9B	-897	8642	5625	31
H6A	1682	11507	5882	41

Table 6. Torsion angles [°] for 09198a.

C2#1-S1-C1-O3	61.24(11)
C2#1-S1-C1-C2	-60.37(12)
C2#1-S1-C1-C3	179.41(10)
O3-C1-C2-S1#1	-50.82(15)
C3-C1-C2-S1#1	-172.92(10)
S1-C1-C2-S1#1	71.05(12)
O3-C1-C3-O1	27.36(19)
C2-C1-C3-O1	149.68(15)
S1-C1-C3-O1	-91.39(15)
O3-C1-C3-O2	-152.68(14)
C2-C1-C3-O2	-30.35(19)
S1-C1-C3-O2	88.58(15)
C6-N1-C4-C5	76.94(19)
C8-N1-C4-C5	-155.90(15)
N1-C4-C5-O4	52.7(2)
C4-N1-C6-C7	-158.90(15)
C8-N1-C6-C7	73.42(18)
N1-C6-C7-O5	51.16(19)
C6-N1-C8-C9	-157.40(15)
C4-N1-C8-C9	75.46(19)
N1-C8-C9-O6	47.2(2)

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,-y+1,-z+1

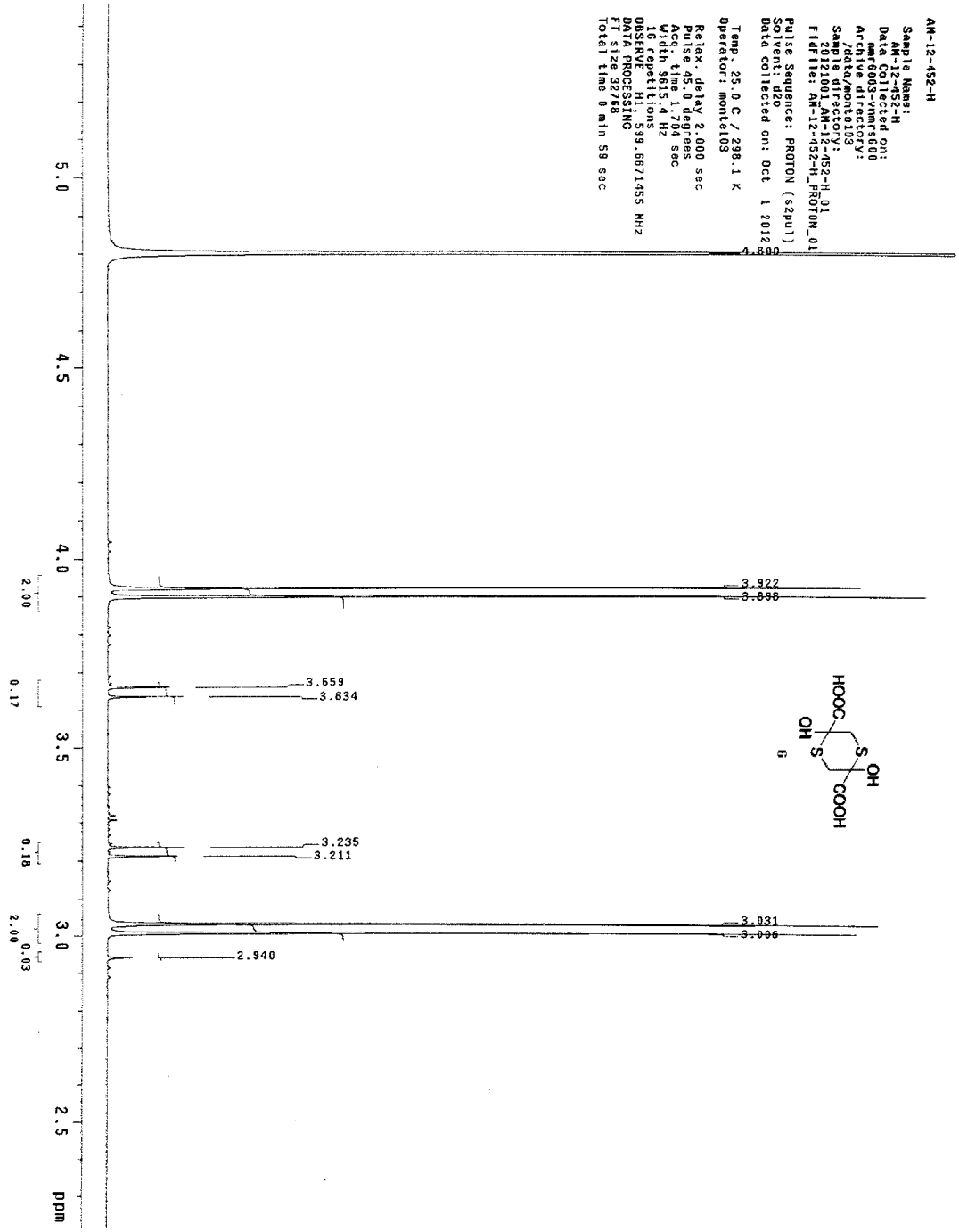
Table 7. Hydrogen bonds for 09198a [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O3-H3A...O6#2	0.84	2.38	3.1167(16)	146.8
N1-H1A...O4	0.93	2.31	2.811(2)	113.2
N1-H1A...O5	0.93	2.30	2.7805(19)	111.8
N1-H1A...O6	0.93	2.34	2.8248(18)	112.1
O4-H4A...O1#3	0.84	1.87	2.7042(17)	171.9
O5-H5A...O2#4	0.84	1.84	2.6704(17)	168.7
O6-H6A...O2#4	0.84	1.93	2.7490(18)	163.6

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,-y+1,-z+1 #2 x+1,y-1,z #3 x-1,y+1,z

#4 x,y+1,z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16 repetitions
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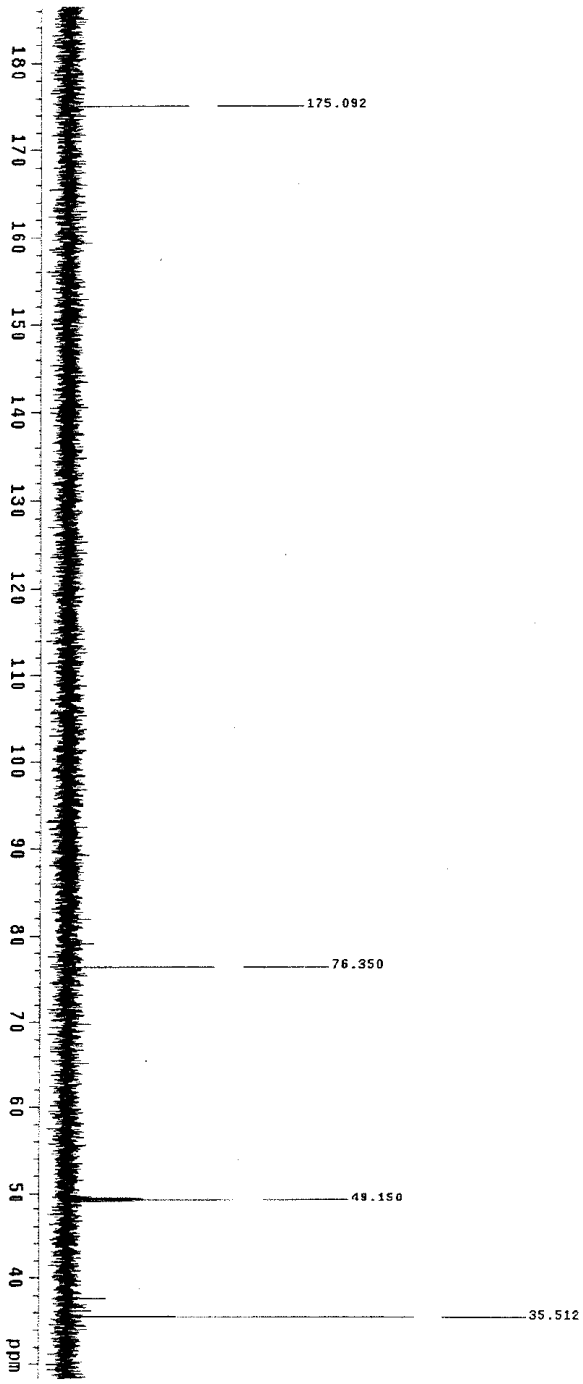
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Solvent: Cd3od
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Pulse 45.0 degrees
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256 repetitions
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DECOUPLE H1, 599.6710621 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
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S
OH
COOH
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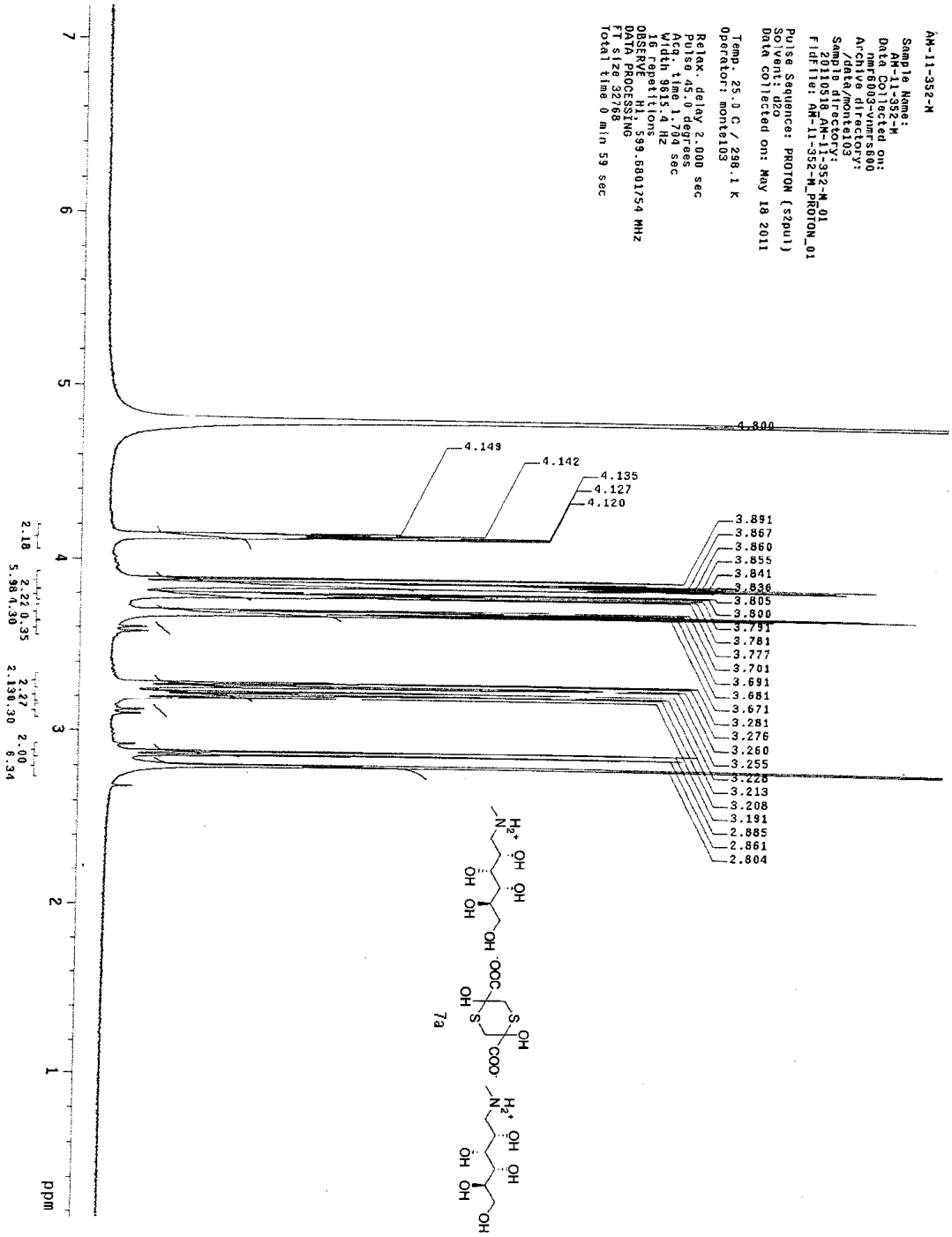
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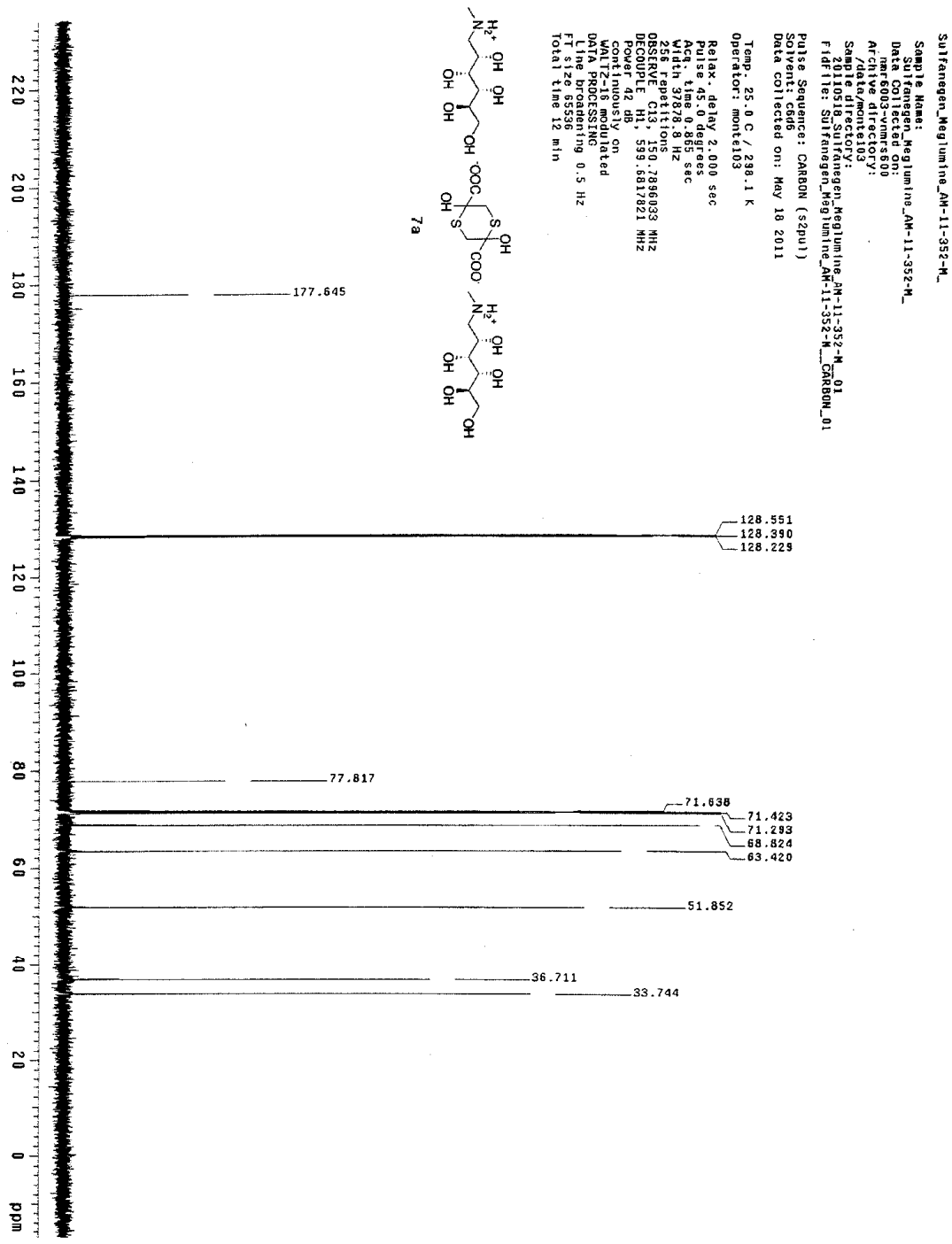
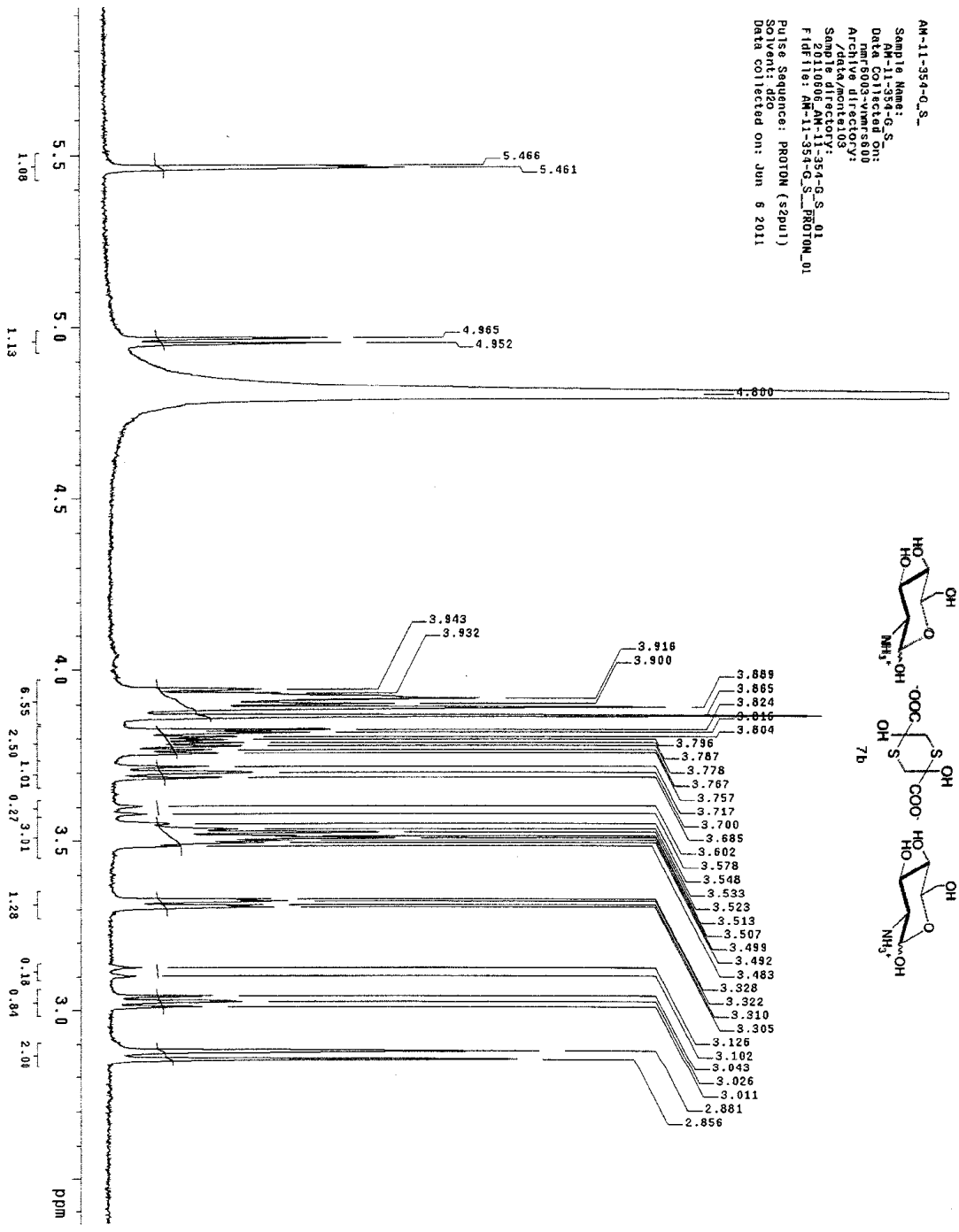
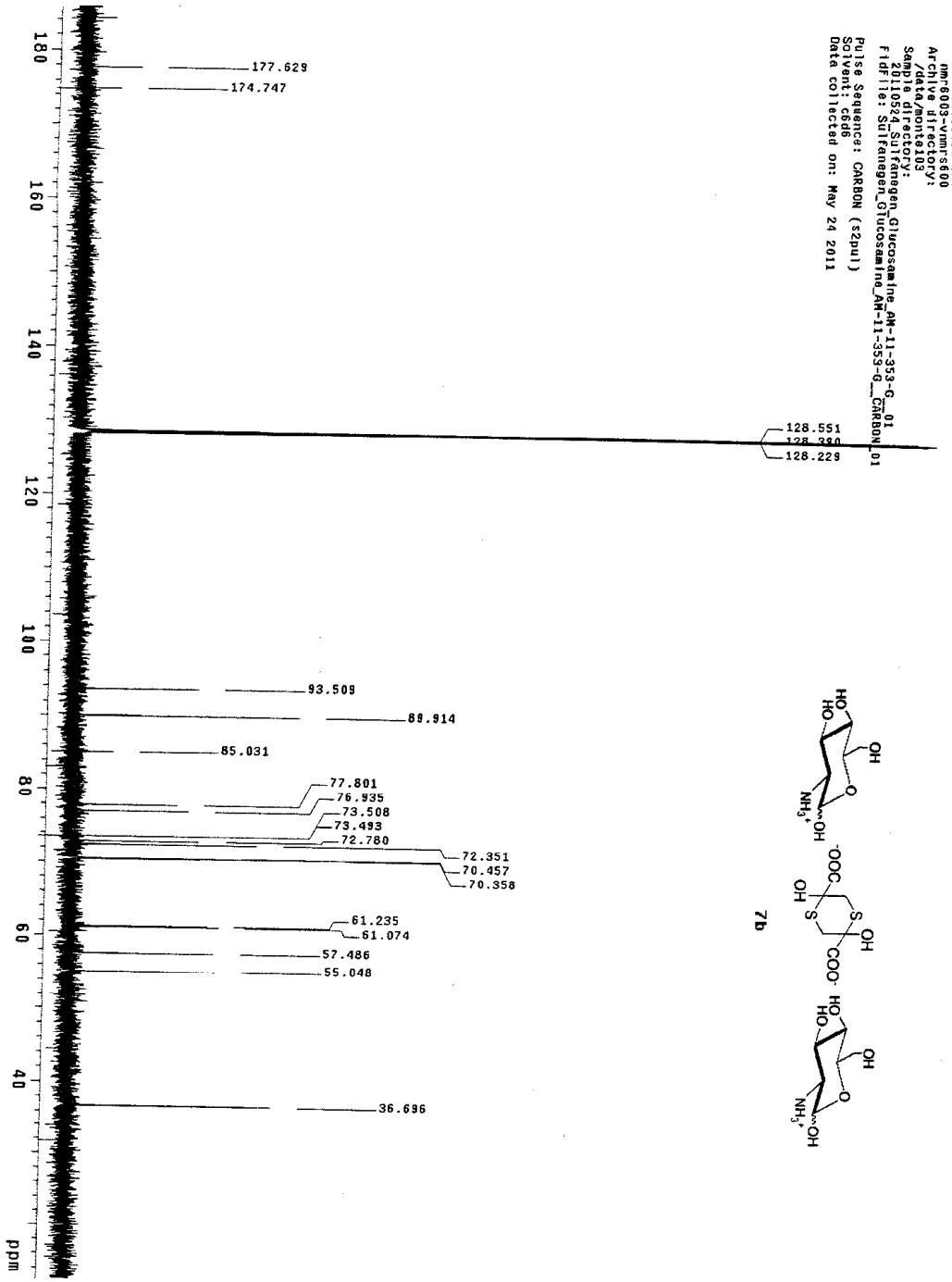
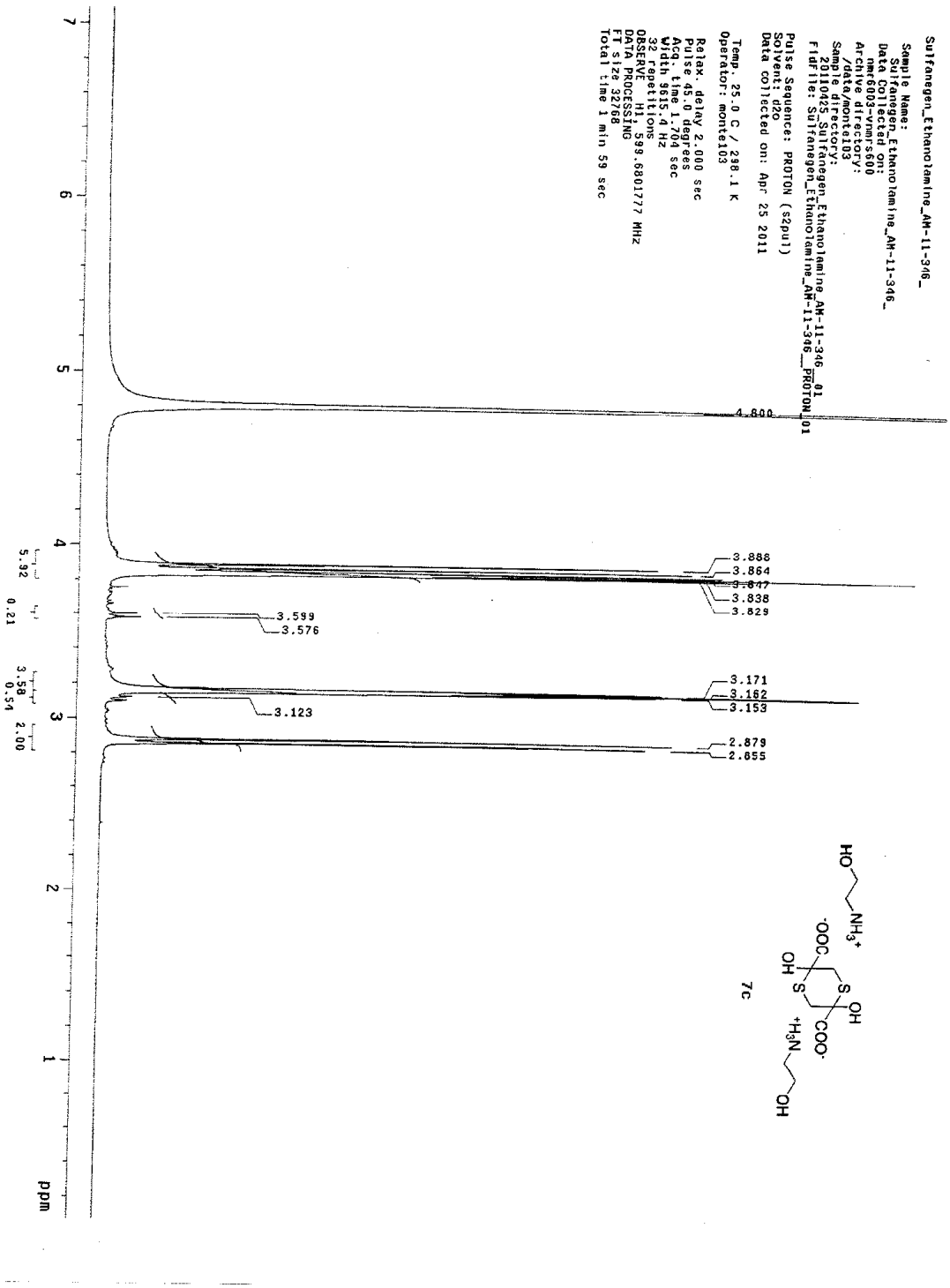
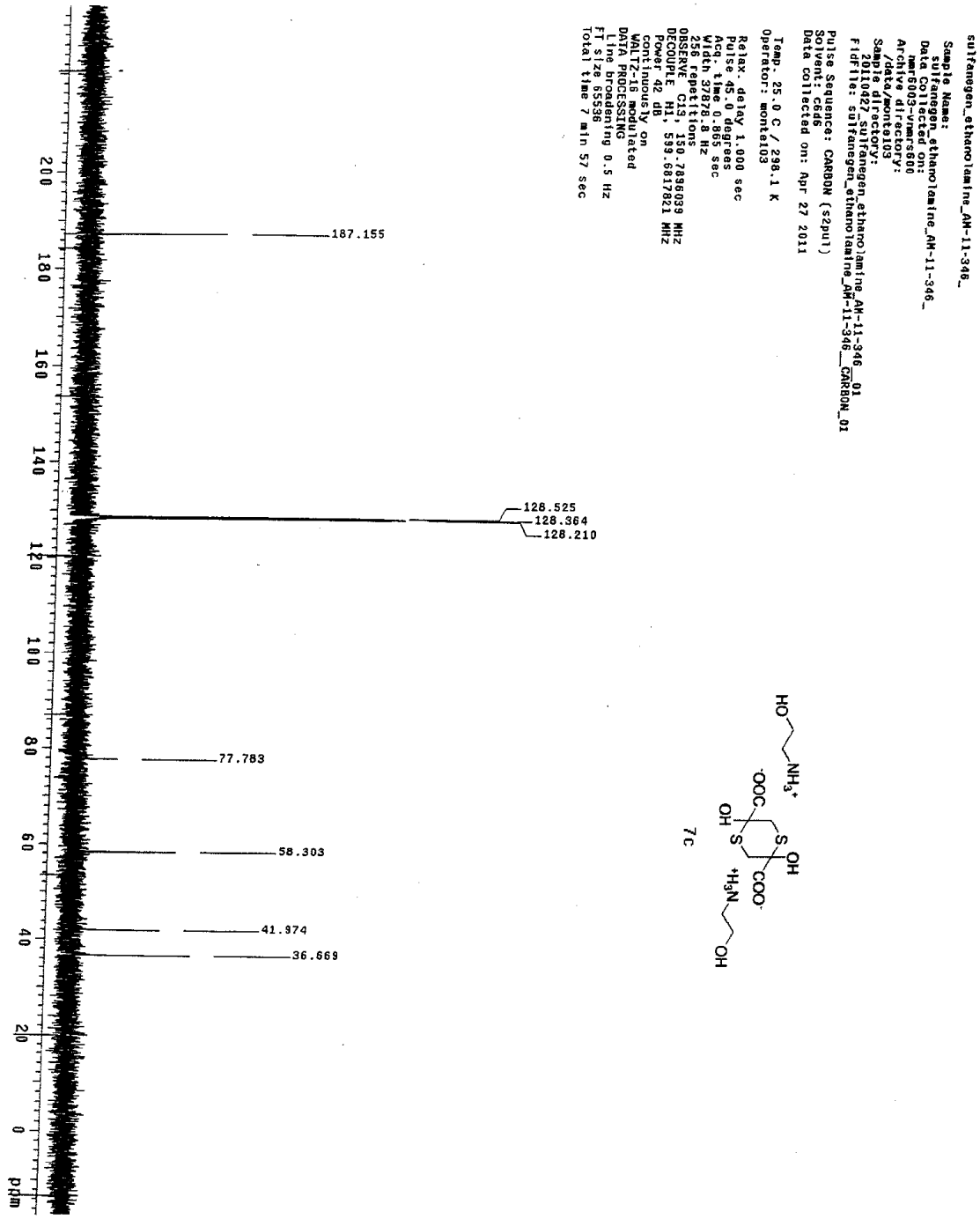
Pulse Sequence: PROTON (s2pul)
Solvent: d2o
Data collected on: May 18 2011
Temp. 25.0 C / 298.1 K
Operator: monte103
Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 9615.4 Hz
16 repetitions
OBSERVE H1, 599.6801754 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 59 sec
4.800
4.149
4.142
4.135
4.127
4.120
3.891
3.867
3.860
3.855
3.841
3.836
3.805
3.800
3.791
3.781
3.777
3.701
3.691
3.681
3.671
3.281
3.276
3.260
3.255
3.228
3.213
3.208
3.191
2.885
2.861
2.804
2.18
2.22 0.35
5.98 4.30
2.27
2.13 0.30
2.00
6.34
7a
ppm

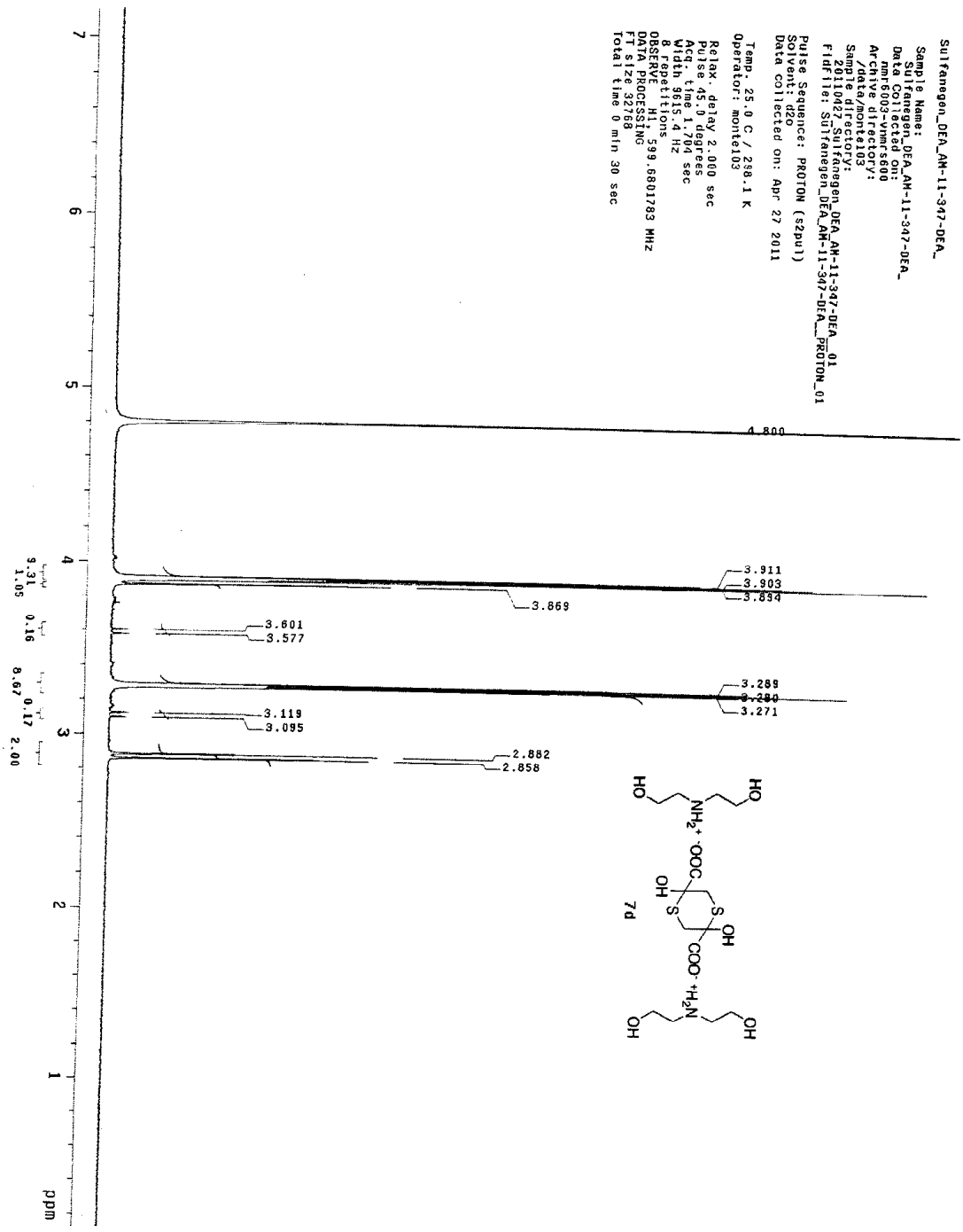

Sulfanegen_Meglumine_AM-11-352-M_
Sample Name:
Sulfanegen_Meglumine_AM-11-352-M_
Data Collected on:
nmr600-vnmrs600
Archive directory:
/data/monte103
Sample directory:
20110518_Sulfanegen_Meglumine_AM-11-352-M__01
FidFile: Sulfanegen_Meglumine_AM-11-352-M__CARBON_01
Pulse Sequence: CARBON (s2pul)
Solvent: cd3od
Data collected on: May 18 2011
Temp. 25.0 C / 298.1 K
Operator: monte103
Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 0.865 sec
Width 37878.8 Hz
256 repetitions
OBSERVE C13, 150.7896033 MHz
DECOUPLE H1, 599.6817821 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 12 min
7a
177.645
128.551
128.390
128.229
77.817
71.638
71.423
71.293
68.824
63.420
51.852
36.711
33.744
220
200
180
160
140
120
100
80
60
40
20
0
ppm

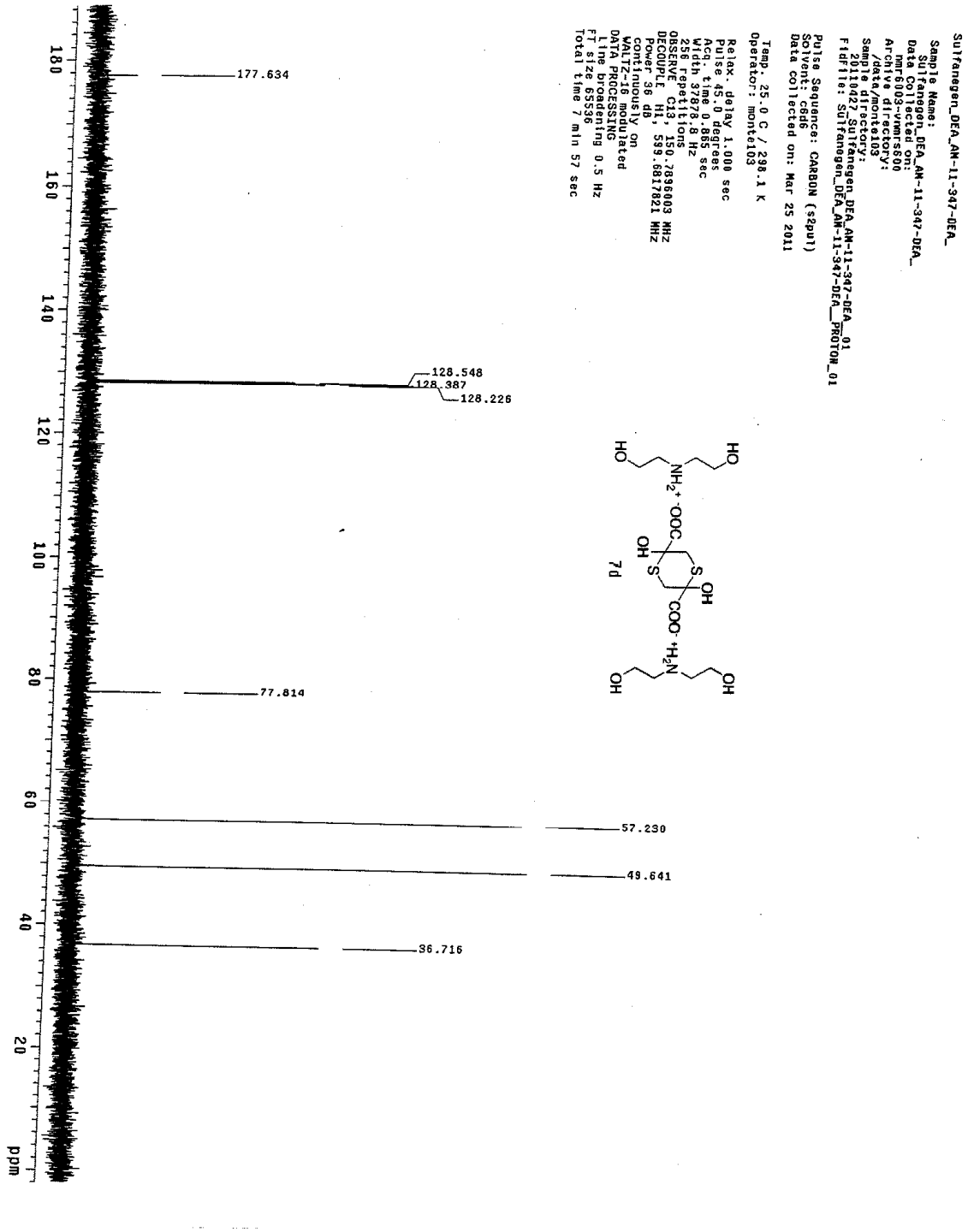

AM-11-354-G_S_
Sample Name: AM-11-354-G_S_
Data Collected on: nmr500-wmrs600
Archive directory: /data/monte103
Sample directory: 20110606_AM-11-354-G_S__01
FidFile: AM-11-354-G_S__PROTON_01
Pulse Sequence: PROTON (s2pul)
Solvent: d2o
Data collected on: Jun 6 2011
5.466
5.461
4.965
4.952
4.800
3.943
3.932
3.916
3.900
3.889
3.865
3.824
3.816
3.804
3.796
3.787
3.778
3.767
3.757
3.717
3.700
3.685
3.602
3.578
3.548
3.533
3.523
3.513
3.507
3.499
3.492
3.483
3.328
3.322
3.310
3.305
3.126
3.102
3.043
3.026
3.011
2.881
2.856
7b
ppm

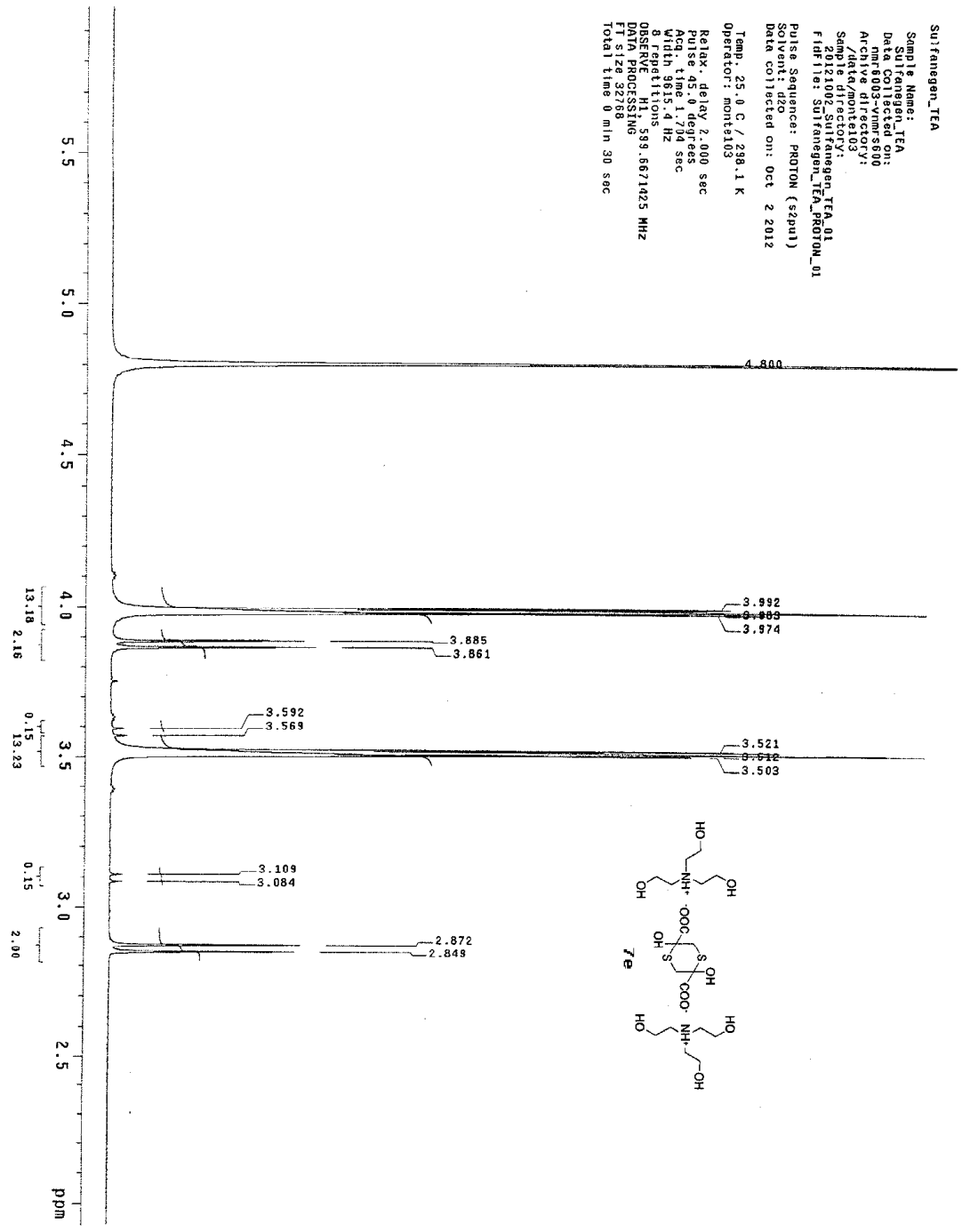

Sulfanegen_Glucosamine_AM-11-353-G_
Sample Name:
Sulfanegen_Glucosamine_AM-11-353-G_
Data Collected on:
nmr600-vnmrs600
Archive directory:
/data/monte103
Sample directory:
20110524_Sulfanegen_Glucosamine_AM-11-353-G_01
FidFile: Sulfanegen_Glucosamine_AM-11-353-G__CARBON_01
Pulse Sequence: CARBON (s2pul)
Solvent: c6d6
Data collected on: May 24 2011
177.629
174.747
128.551
128.229
93.509
88.914
85.031
77.801
76.935
73.508
73.493
72.780
72.351
70.457
70.358
61.235
61.074
57.486
55.048
36.696
ppm
7b

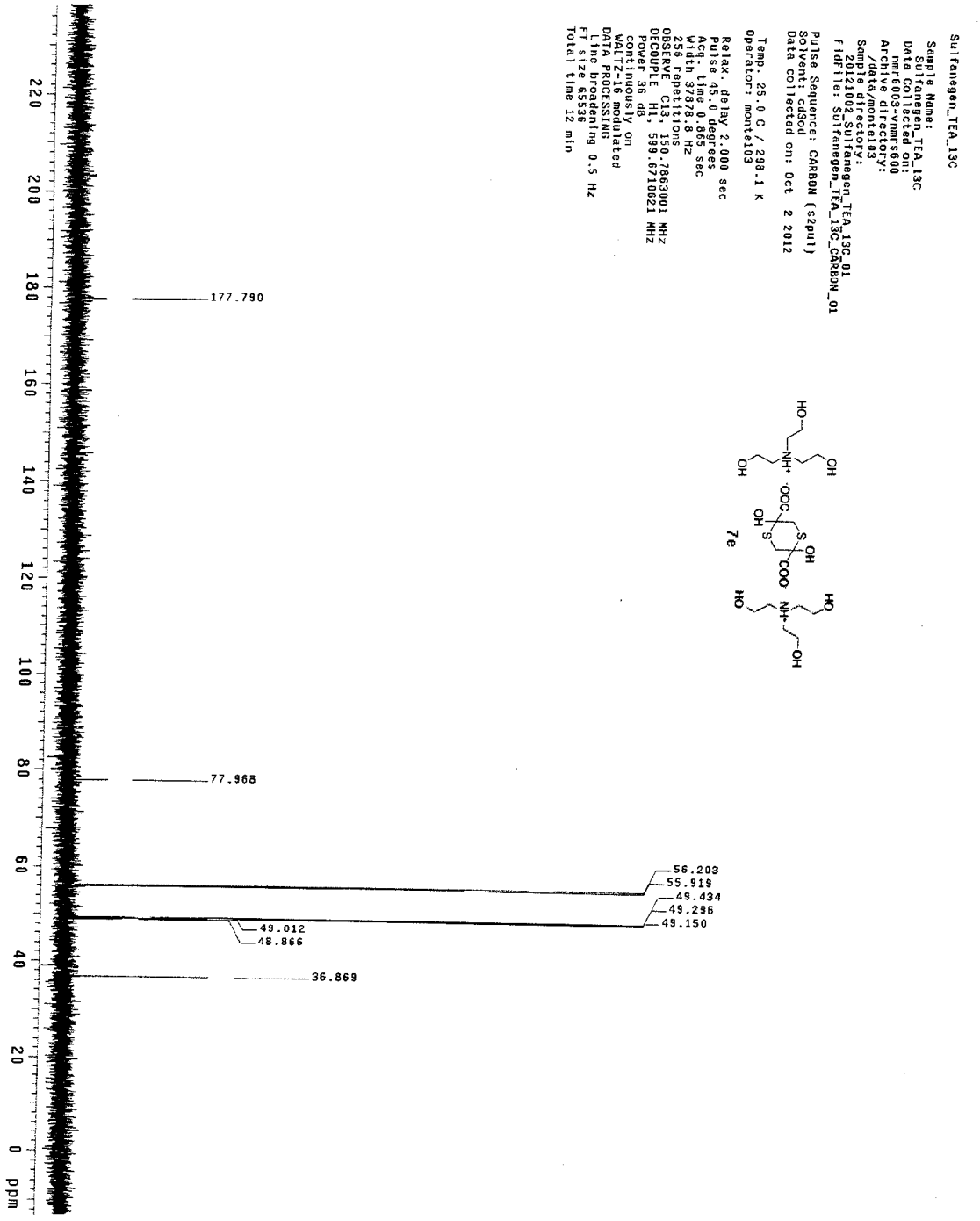

Sulfanegen_Ethanolamine_AM-11-346_
Sample Name:
Sulfanegen_Ethanolamine_AM-11-346_
Data Collected on:
nmr600-vnmrs600
Archive directory:
/data/monte103
Sample directory:
20110425_Sulfanegen_Ethanolamine_AM-11-346_01
Fidfile: Sulfanegen_Ethanolamine_AM-11-346_PROTON_01
Pulse Sequence: PROTON (s2pul)
Solvent: d2o
Data collected on: Apr 25 2011
Temp. 25.0 C / 298.1 K
Operator: monte103
Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 9615.4 Hz
32 repetitions
OBSERVE H1, 599.6801777 MHz
DATA PROCESSING
FT size 32768
Total time 1 min 59 sec
4.800
3.886
3.864
3.847
3.838
3.829
3.599
3.576
3.171
3.162
3.153
3.123
2.879
2.655
5.92
0.21
3.58
0.54
2.00
ppm
7c

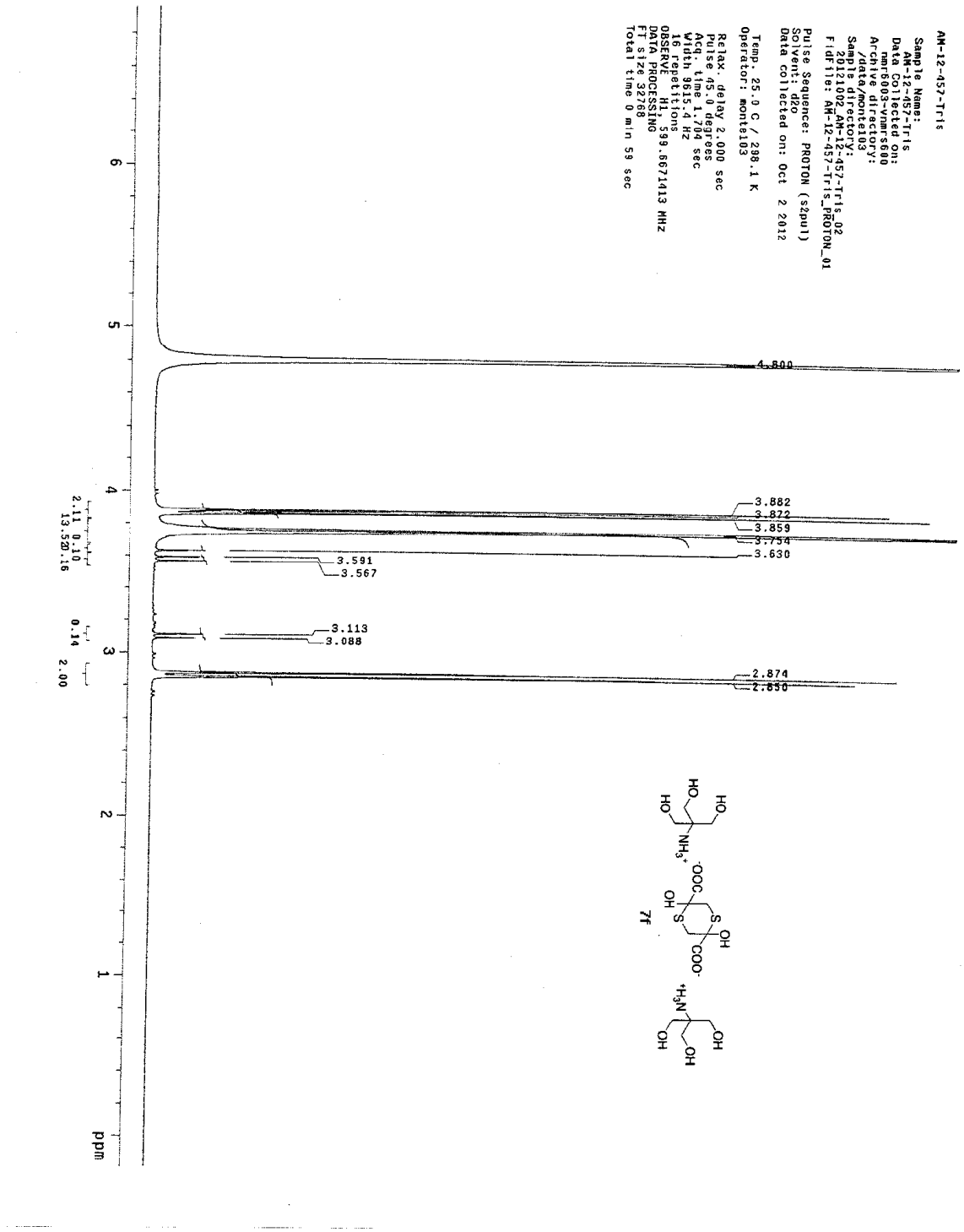

sulfanegen_ethanolamine_AM-11-346_
Sample Name:
sulfanegen_ethanolamine_AM-11-346_
Data Collected on:
nmr600-acted on:
Archive directory:
/data/monte103:
Sample directory:
20110427_sulfanegen_ethanolamine_AM-11-346_01
FidFile: sulfanegen_ethanolamine_AM-11-346_CARBON_01
Pulse Sequence: CARBON (s2pul)
Solvent: c6d6
Data collected on: Apr 27 2011
Temp. 25.0 C / 298.1 K
Operator: monte103
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.865 sec
Width 37878.8 Hz
256 repetitions
OBSERVE C13, 150.7896039 MHz
DECOUPLE H1, 599.6817821 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 7 min 57 sec
187.155
128.525
128.364
128.210
77.783
58.303
41.974
36.669
ppm
7c

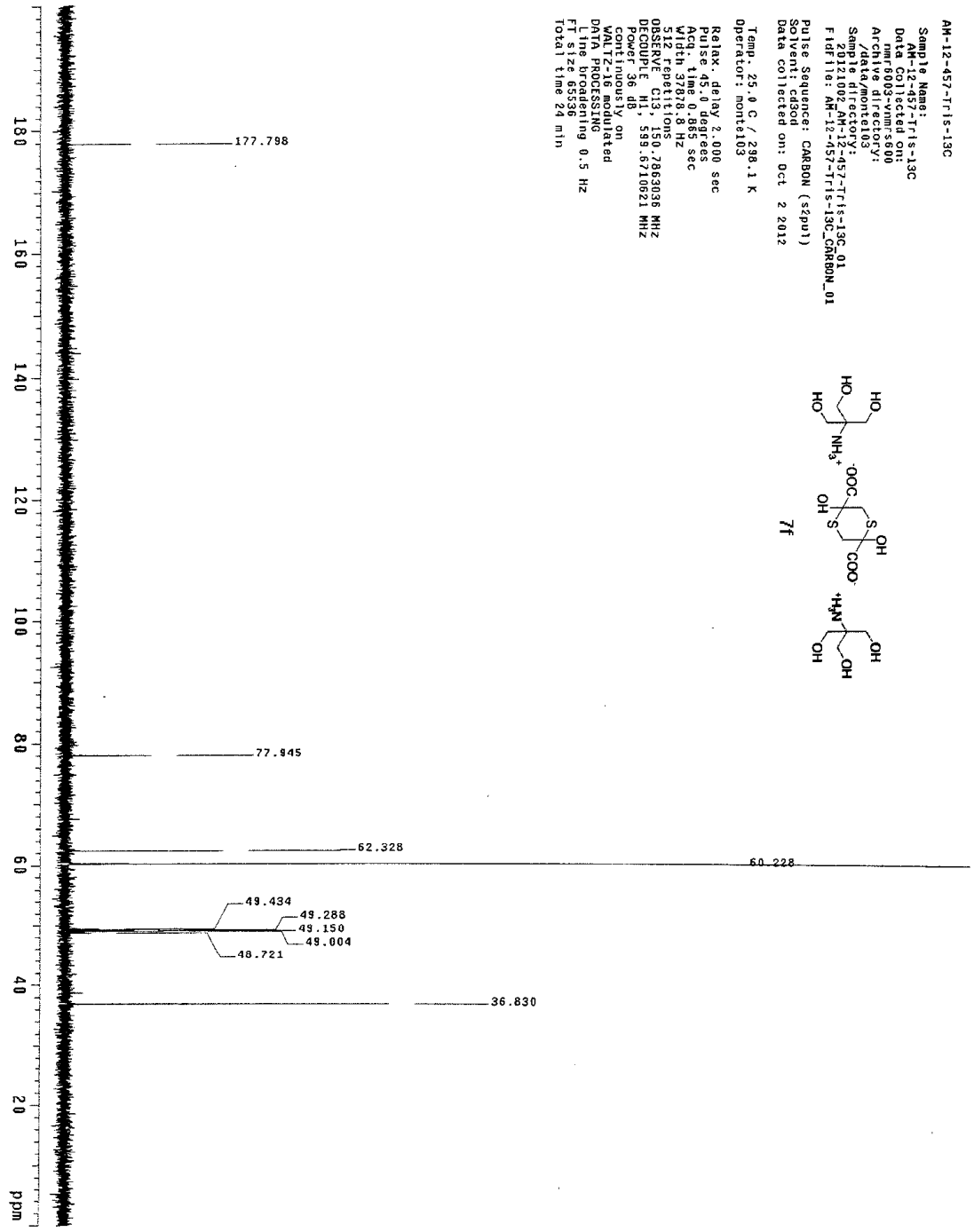

Sulfanegen_DEA_AM-11-347-DEA_
Sample Name:
Sulfanegen_DEA_AM-11-347-DEA_
Data Collected on:
nmr6003-vnmrs600
Archive directory:
/data/monte103
Sample directory:
20110427_Sulfanegen_DEA_AM-11-347-DEA_01
Fidfile: Sulfanegen_DEA_AM-11-347-DEA__PROTON_01
Pulse Sequence: PROTON (s2pul)
Solvent: d2o
Data collected on: Apr 27 2011
Temp. 25.0 C / 298.1 K
Operator: monte103
Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 9615.4 Hz
8 repetitions
OBSERVE H1, 599.6801783 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 30 sec
4.800
3.911
3.903
3.894
3.869
3.601
3.577
3.289
3.280
3.271
3.119
3.095
2.882
2.858
9.31
1.05
0.16
8.67
0.17
2.00
7d
ppm


Sulfanegen_DEA_AM-II-347-DEA_
Sample Name:
Sulfanegen_DEA_AM-II-347-DEA_
Data Collected on:
mmr6003-vnmrs600
Archive directory:
/data/monte103
Sample directory:
20110427_Sulfanegen_DEA_AM-II-347-DEA_01
FidFile: Sulfanegen_DEA_AM-II-347-DEA_PROTON_01
Pulse Sequence: CARBON (s2pul)
Solvent: cd3d
Data collected on: Mar 25 2011
Temp. 25.0 C / 298.1 K
Operator: monte103
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 0.885 sec
Width 37878.8 Hz
256 repetitions
OBSERVE C13, 150.7896003 MHz
DECOUPLE H1, 599.6817821 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 7 min 57 sec
177.634
128.548
128.387
128.226
77.814
57.230
49.641
36.716
ppm
7d


Sulfanegen_TEA
Sample Name:
Sulfanegen_TEA
Data Collected on:
nmr6003-vnmrs600
Archive directory:
/data/monte103
Sample directory:
20121002_Sulfanegen_TEA_01
Fidfile: Sulfanegen_TEA_PROTON_01
Pulse Sequence: PROTON (s2pul)
Solvent: d2o
Data collected on: Oct 2 2012
Temp. 25.0 C / 298.1 K
Operator: monte103
Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 9615.4 Hz
8 repetitions
OBSERVE H1, 599.6671425 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 30 sec
4.800
3.992
3.983
3.974
3.885
3.861
3.592
3.569
3.521
3.512
3.503
3.109
3.084
2.872
2.849
13.18
2.16
0.15
13.23
0.15
2.00
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
HO
NH+
OH
-OOC
OH
S
S
OH
COO-
Te


Sulfanegen_TEA_13C
Sample Name:
Sulfanegen_TEA_13C
Data collected on:
ionyx603.vnmrs600
Archive directory:
/data/monte103
Sample directory:
20121002_Sulfanegen_TEA_13C_01
FidFile: Sulfanegen_TEA_13C_CARBON_01
Pulse Sequence: CARBON (s2pul)
Solvent: cd3od
Data collected on: Oct 2 2012
Temp. 25.0 C / 298.1 K
Operator: monte103
Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 0.865 sec
Width 37878.8 Hz
256 repetitions
OBSERVE C13, 150.7863001 MHz
DECOUPLE H1, 599.6710621 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 12 min
7e
177.790
77.968
56.203
55.919
49.434
49.296
49.150
49.012
48.866
36.869
220
200
180
160
140
120
100
80
60
40
20
0
ppm


AM-12-457-Tris
Sample Name:
AM-12-457-Tris
Data collected on:
chem-601-vnmrs600
Archive directory:
/data/monte103
Sample directory:
20121002_AM-12-457-Tris_02
Fidfile: AM-12-457-Tris_PROTON_01
Pulse Sequence: PROTON (s2pul)
Solvent: d2o
Data collected on: Oct 2 2012
Temp. 25.0 C / 298.1 K
Operator: monte103
Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.704 sec
Width 9615.4 Hz
16 repetitions
OBSERVE H1, 599.6671413 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 59 sec
4.800
3.882
3.872
3.859
3.754
3.630
3.591
3.567
3.113
3.088
2.874
2.850
2.11
13.52
0.10
0.16
0.14
2.00
ppm
7f


AM-12-457-Tris-13C
Sample Name:
AM-12-457-Tris-13C
Data Collected on:
nmr600-vnmrs600
Archive directory:
/data/monte103
Sample directory:
AM-12-457-Tris-13C_01
FidFile: AM-12-457-Tris-13C_CARBON_01
Pulse Sequence: CARBON (s2pul)
Solvent: cd3od
Data collected on: Oct 2 2012
Temp. 25.0 C / 298.1 K
Operator: monte103
Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.365 sec
Width 37878.8 Hz
512 repetitions
OBSERVE C13, 150.7863036 MHz
DECOUPLE H1, 599.6710621 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 24 min
7f
177.798
77.945
62.328
60.228
49.434
49.288
49.150
49.004
48.721
36.830
ppm

NMR studies on Sulfanegen sodium (2)

1) <u>At physiological pH in Phosphate buffer</u>

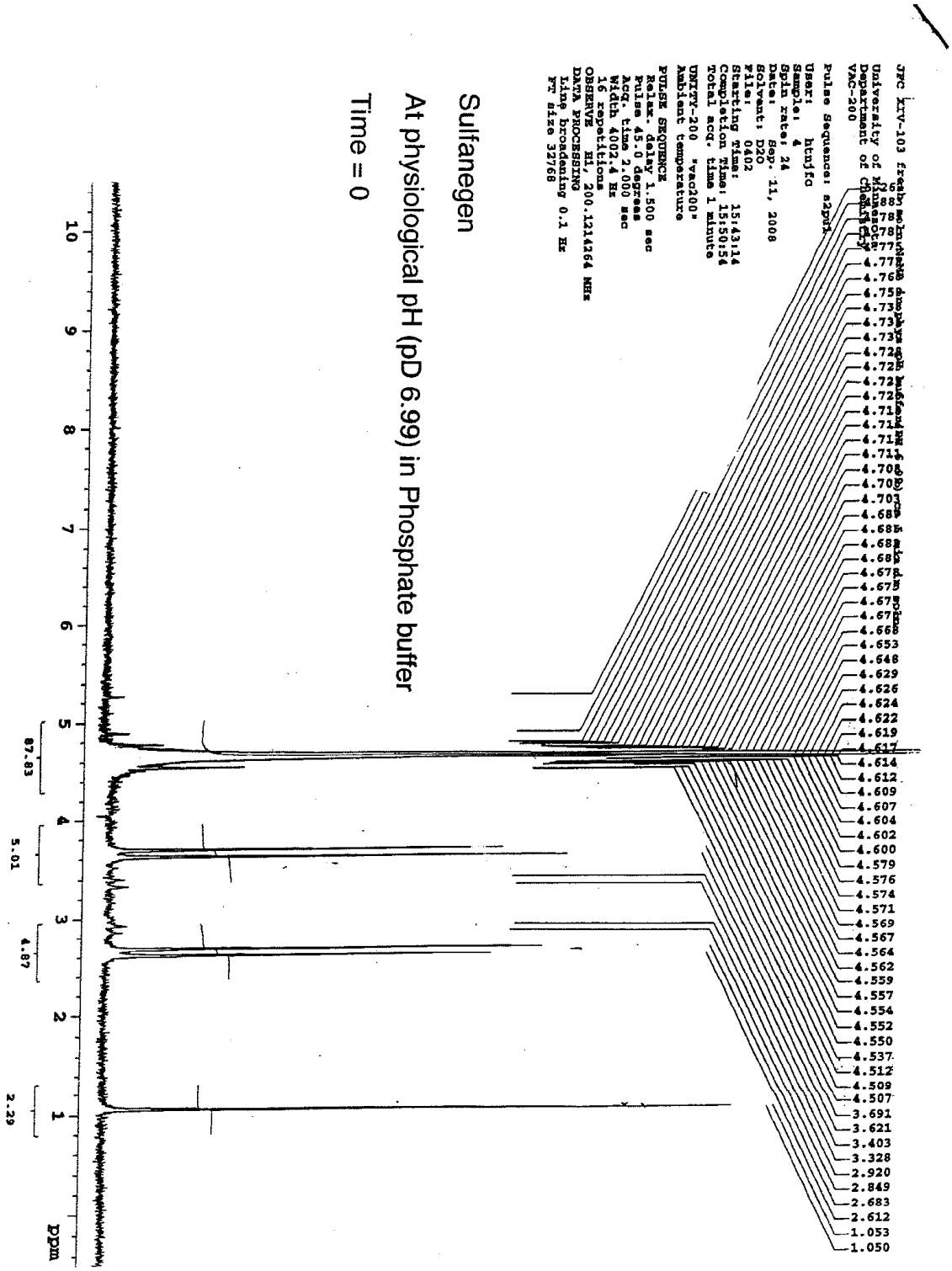
Phosphate buffer was made up by mixing solutions of 0.1 M dipotassium deuterophosphate and 0.1 M potassium dideuterophosphate in D_2O and adjusting the pD to 6.99 (pH 7.4). To ca 0.7 mL of this solution in an NMR tube was added 0.6 mg t-Butyl alcohol (0.008 mmole) as internal standard and 5.7 mg (0.032 mmole) Sodium mercaptopyruvate just before starting to run NMR spectra. This was run on a 200 MHz (Varian) autosampler machine.

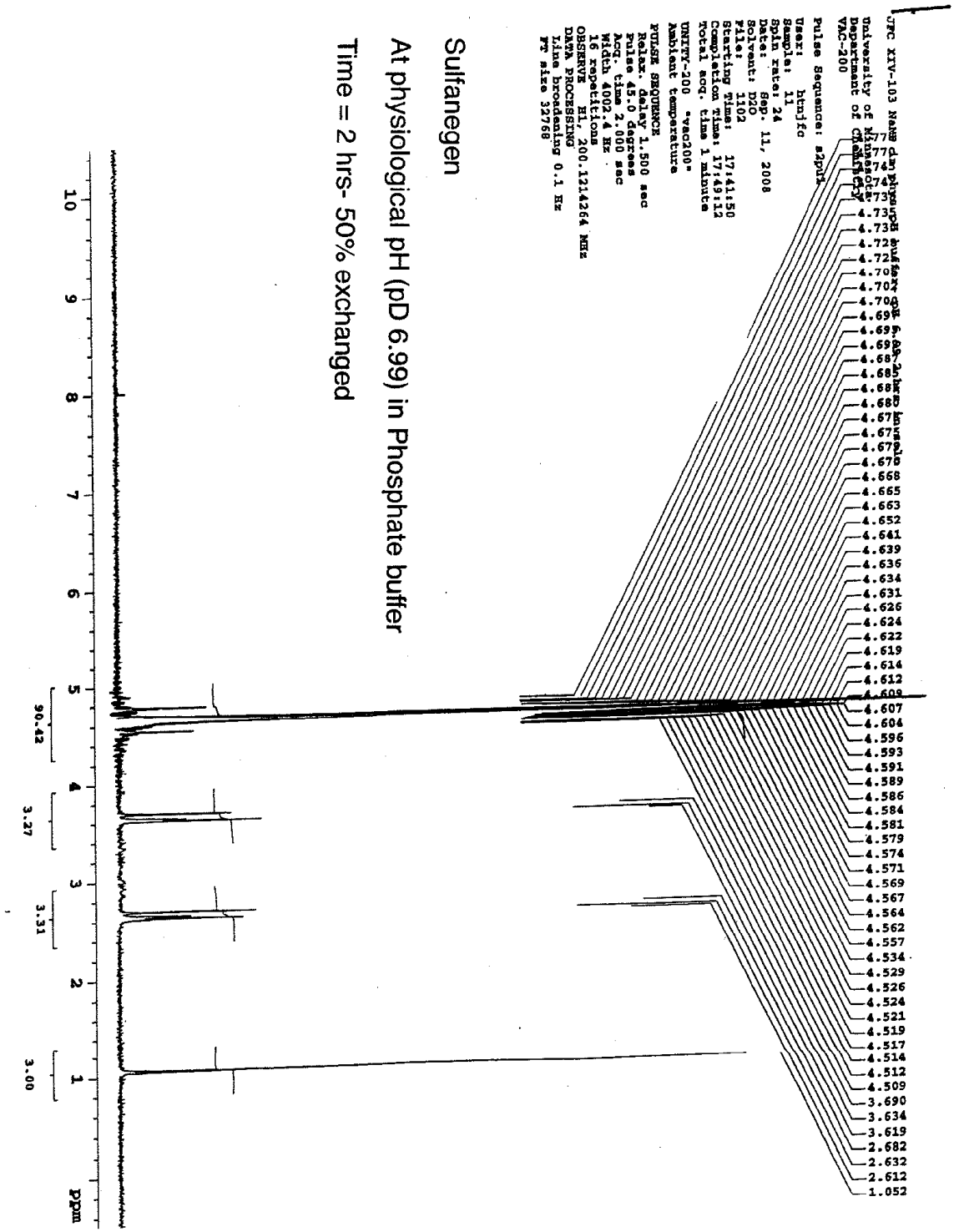
Time	Percent exchanged
0	0
15 min	11%
30 min	19%
45 min	27%
70 min	34%
2 hrs	50%
3 ½ hrs	67%

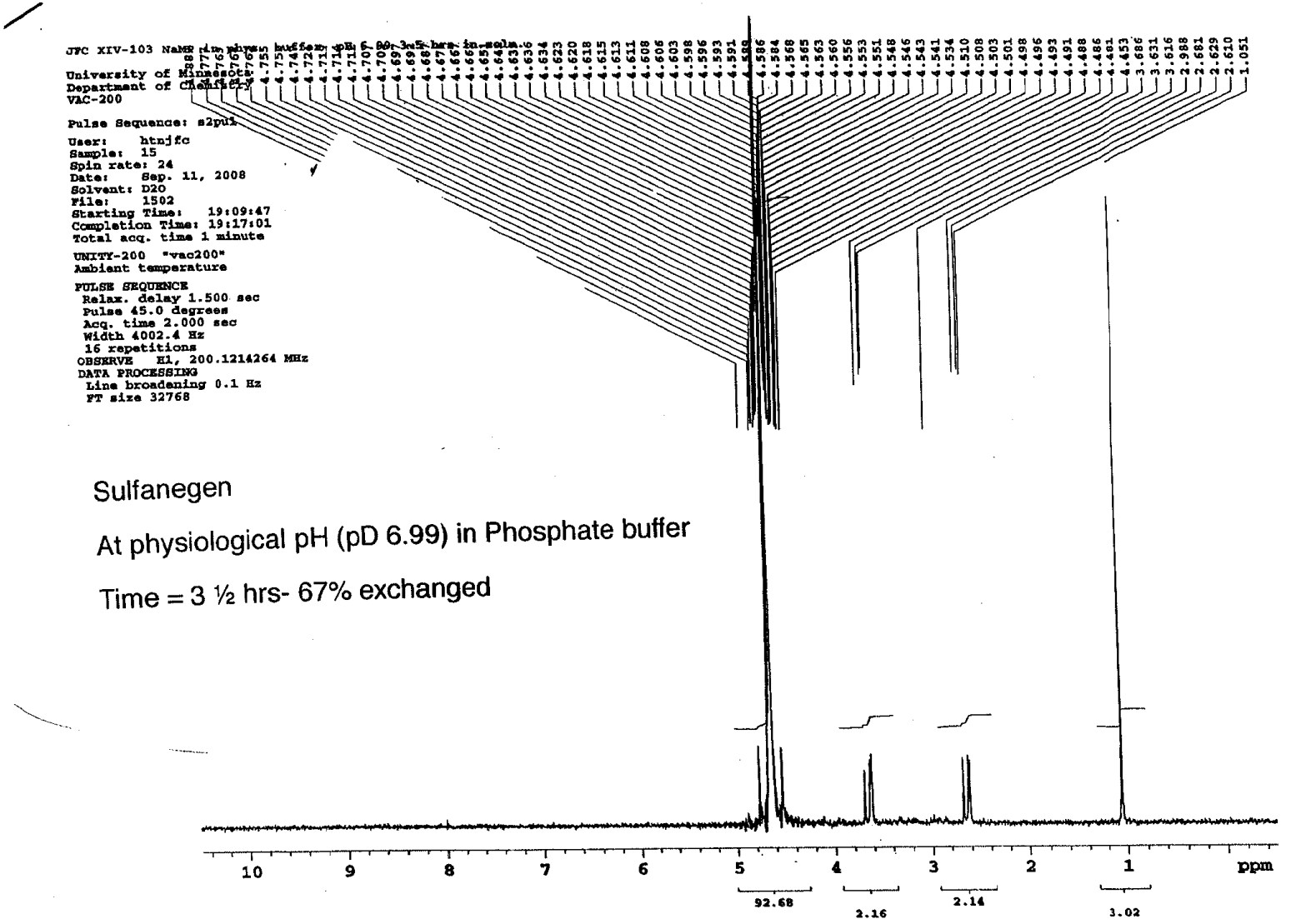
2) At pH ca 1.0

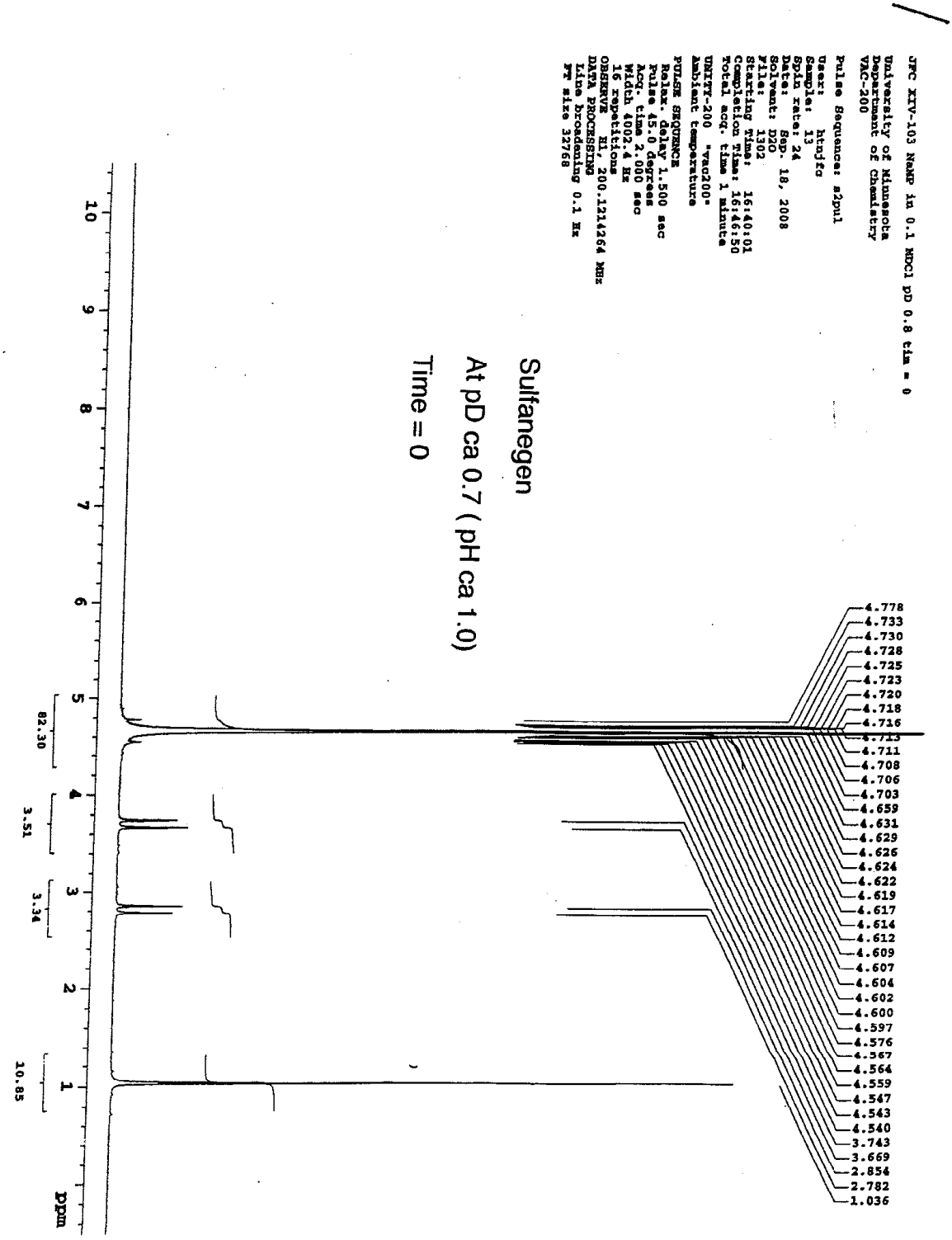
0.1M DCl (ca 0.7 mL) was placed in an NMR tube to which was added 0.8 mg (0.01 mmole) t-Butyl alcohol (as internal standard) and 5.3 mg (0.030 mmole) Sodium mercaptopyruvate immediately before starting to run spectra. This was run on a 200 MHz autosampler spectrometer (Varian) This time the protons did not exchange over 4 d, but the isomer ratio changed dramatically as the less abundant isomer went from a trace up to about 30% with a half-life of ca 1 hour. At 19 d 75% of the protons were exchanged.

Time	% smaller isomer
0	Trace
30 min	12%
60 min	15%
22 hrs	26%
4 days	30%


Sulfanegen
At physiological pH (pD 6.99) in Phosphate buffer
Time = 0
ppm


Sulfanegen
At physiological pH (pD 6.99) in Phosphate buffer
Time = 2 hrs- 50% exchanged
90.42
3.27
3.31
3.00
ppm


JFC XIV-103 NaMR in phys
University of Minnesota
Department of Chemistry
VAC-200
Pulse Sequence: s2pul
User: htnjfc
Sample: 15
Spin rate: 24
Date: Sep. 11, 2008
Solvent: D2O
File: 1502
Starting Time: 19:09:47
Completion Time: 19:17:01
Total acq. time 1 minute
UNITY-200 "vac200"
Ambient temperature
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 45.0 degrees
Acq. time 2.000 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1214264 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Sulfanegen
At physiological pH (pD 6.99) in Phosphate buffer
Time = 3 ½ hrs- 67% exchanged
10
9
8
7
6
5
4
3
2
1
ppm
92.68
2.16
2.14
3.02


JFC XIV-103 NaHP in 0.1 MDCl pD 0.8 tim = 0
University of Minnesota
Department of Chemistry
VAC-200
Pulse Sequence: s2pul
User: htnjfc
Sample: 13
Spin rate: 24
Date: Sep. 18, 2008
Solvent: D2O
File: 1302
Starting Time: 16:40:01
Completion Time: 16:46:50
Total acq. time 1 minute
UNITY-200 "vac200"
Ambient temperature
PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 45.0 degrees
Acq. time 2.000 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1214264 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Sulfanegen
At pD ca 0.7 (pH ca 1.0)
Time = 0
4.778
4.733
4.730
4.728
4.725
4.723
4.720
4.718
4.716
4.711
4.708
4.706
4.703
4.659
4.631
4.629
4.626
4.624
4.622
4.619
4.617
4.614
4.612
4.609
4.607
4.604
4.602
4.600
4.597
4.576
4.567
4.564
4.559
4.547
4.543
4.540
3.743
3.669
2.854
2.782
1.036
82.30
3.51
3.34
10.85
ppm

JFC XIV-103 NaMP in 0.1 M DCl-pD 0.8 time = 60 min

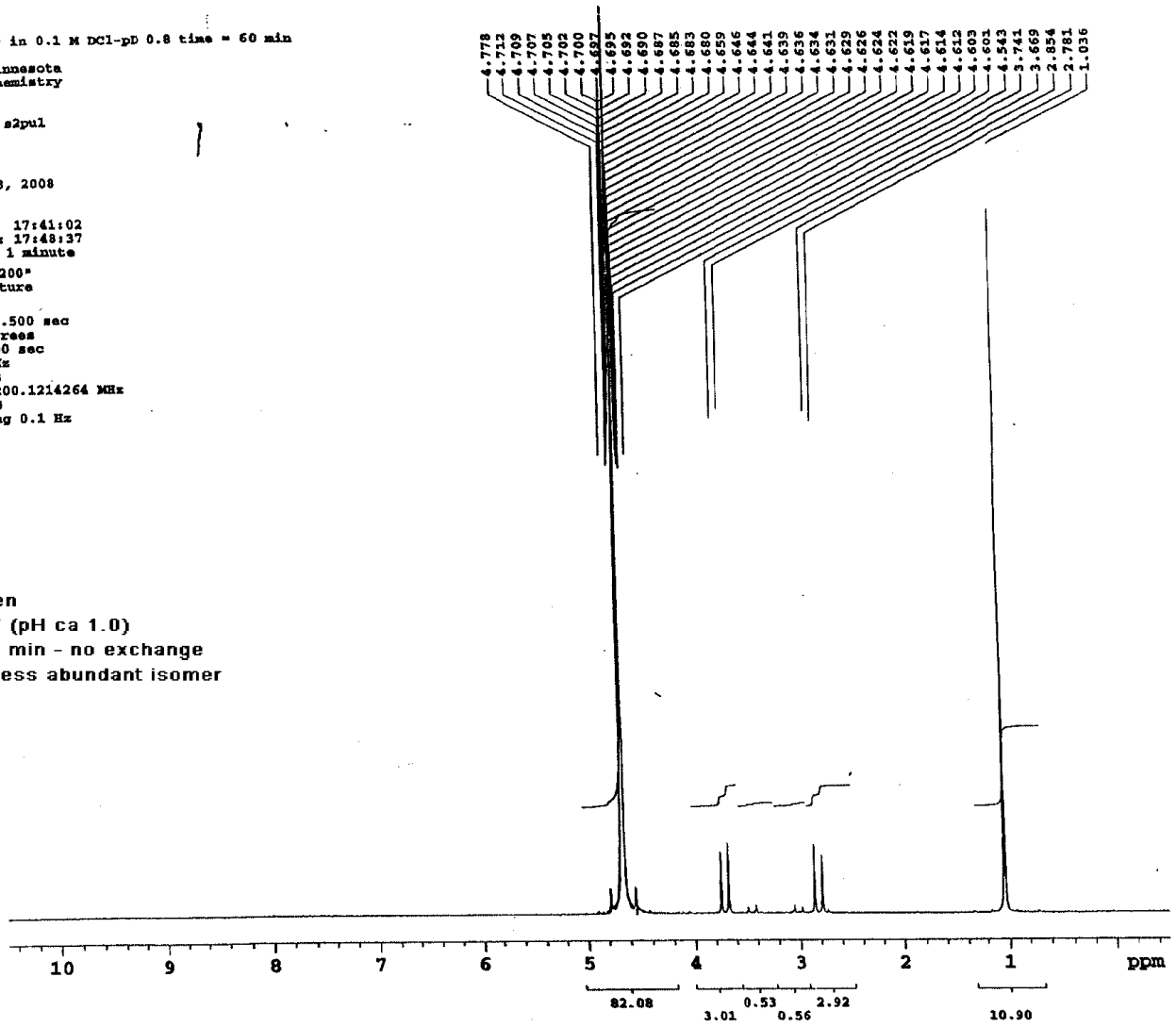
University of Minnesota
Department of Chemistry
VAC-200

Pulse Sequence: s2pul

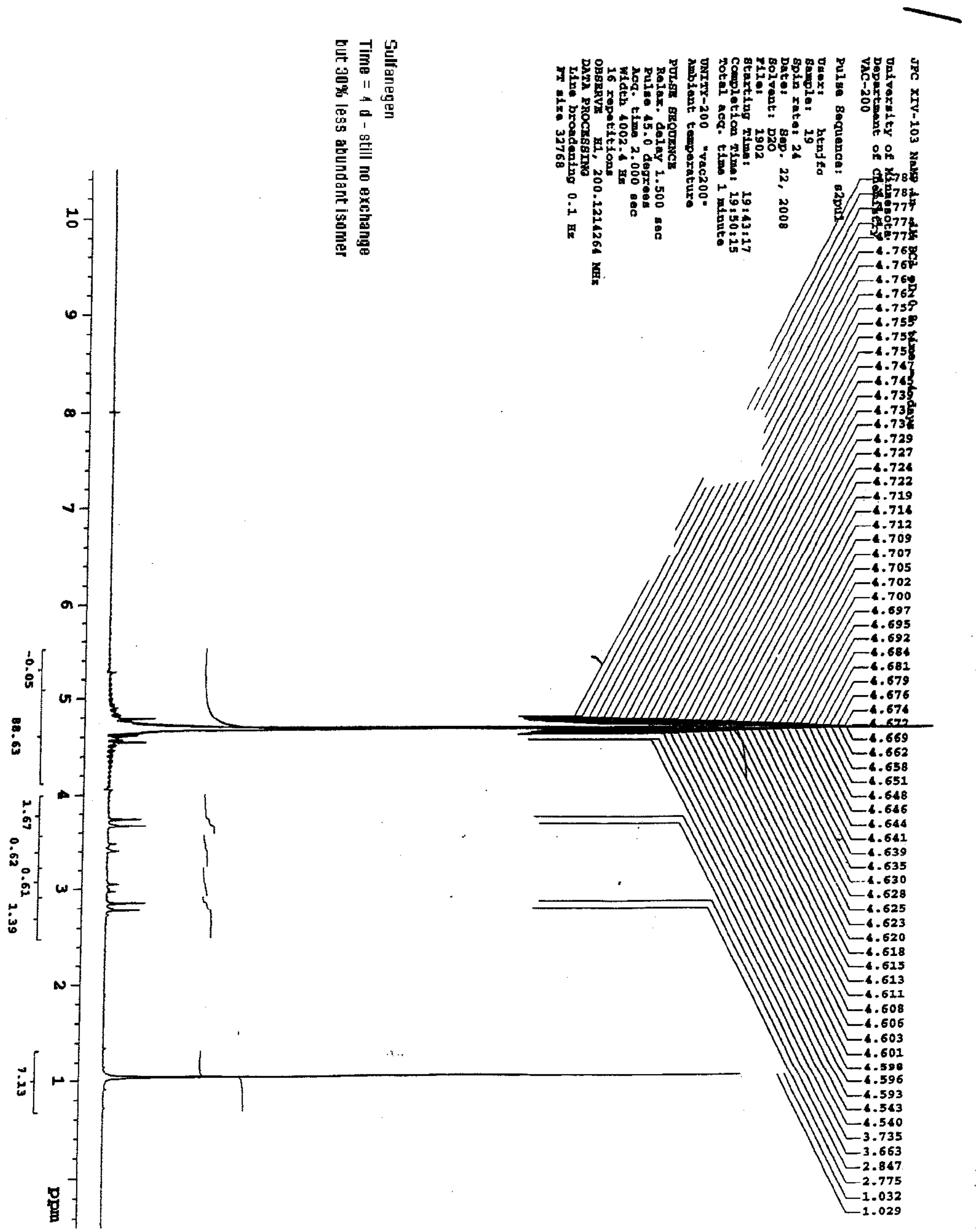
User: htnjfc
Sample: 16
Spin rate: 23
Date: Sep. 18, 2008
Solvent: D2O
File: 1602
Starting Time: 17:41:02
Completion Time: 17:48:37
Total acq. time 1 minute

UNITY-200 "vac200"
Ambient temperature

PULSE SEQUENCE
Relax. delay 1.500 sec
Pulse 45.0 degrees
Acq. time 2.000 sec
Width 4002.4 Hz
16 repetitions
OBSERVE H1, 200.1214264 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768



Sulfanegen
At pD 0.7 (pH ca 1.0)
time = 60 min - no exchange
but 15% less abundant isomer


Sulfanegen
Time = 4 d - still no exchange
but 30% less abundant isomer
ppm

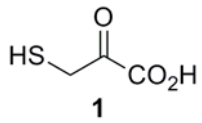


Figure 1. 3-Mercaptopyruvic acid (3-MP), the endogenous substrate for 3-MST

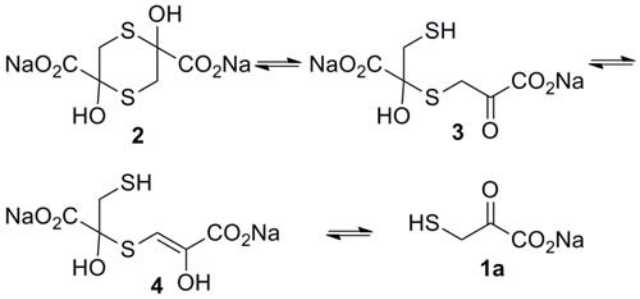
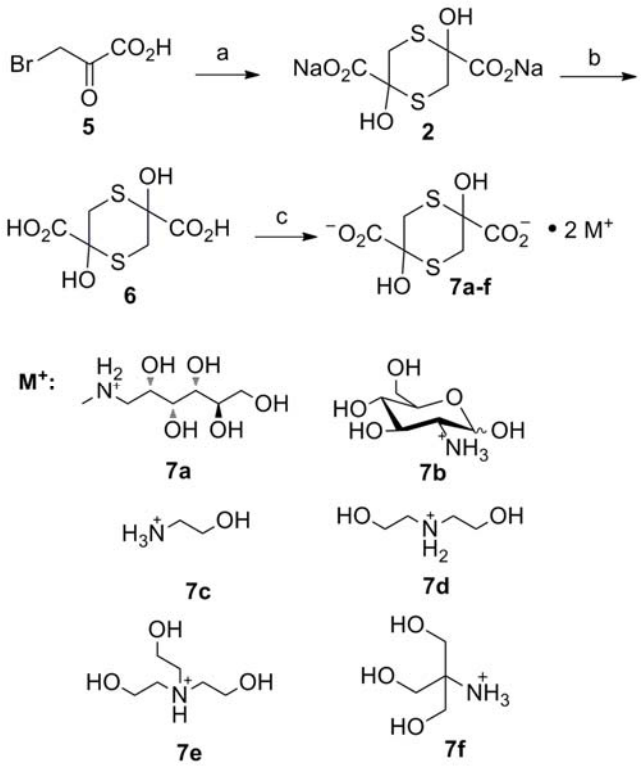


Figure 2. Chemical equilibria of dithiane **2**

Scheme 1. Synthesis of sulfanegen salts 7a-f



[a]Reagents and conditions: (a) 2 molar equivalents NaHS, ethanol, 0 °C; (b) Dowex-50WX8, H^+ form, 7 equivalents; (c) 2 equivalents of a biocompatible amine (M).