DESIGN, SYNTHESIS, AND ANTI-TUMOR ACTIVITIES OF BENZOPOLYSULFANE COMPOUNDS THAT MIMICS A TUNICATE-DERIVED NATURAL PRODUCT

by

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Abstract

Design, Synthesis, and Anti-tumor Activities of Benzopolysulfane Compounds which Mimic Tunicate Derived Natural Products

by

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Although benzopolysulfanes display an impressive array of biological activities their therapeutic potential has not been evaluated. Because polysulfanes may have unusual mechanisms of action, they are likely to be active against drug resistant pathogens. However, polysulfanes are challenging compounds to prepare, and usually have very poor water-solubility. New synthetic methods and studies on solubility and cell-directed delivery are needed to explore the therapeutic potential of this novel class of compounds. This thesis describes (1) synthesis and biological activities of benzopolysulfane conjugates namely, PEGylated benzopoylsulfanes; and (2) mechanistic aspects on mode of introduction of sulfur atom to the benzene core;

Benzopolysulfanes, 4-$CH_3(OCH_2CH_2)_3NHC(O)$-C_6H_4-1,2-S_x (x = 3-7, and 9) were synthesized with a PEG group attached through an amide bond and examined for water solubility, antitumor activity, and propensity to equilibrate and desulfurate. LCMS and HPLC data show the PEG pentasulfane ring structure predominates, and the tri-, tetra-, hexa-, hepta-, and nonasulfanes were present at very low concentrations. The presence of the PEG group improved water solubility by 50-fold compared to the

unsubstituted benzopolysulfanes, $C_6H_4S_x$ (x = 3, 5, and 7), based on intrinsic solubility measurements. Polysulfur linkages in the PEG compounds decomposed in the presence of ethanethiol or hydroxide ion. The PEG pentathiepin desulfurated rapidly and an S_3 transfer reaction was observed in the presence of norbornene. No S_2 transfer reaction was observed with 2,3-dimethylbutadiene. The antitumor activities of the PEG-substituted benzopolysulfanes, 4-$CH_3(OCH_2CH_2)_3NHC(O)$-C_6H_4-1,2-S_x (x = 3-7, and 9), (mixtures were analyzed against four human tumor cell lines PC3 (prostate), DU145 (prostate), MDA-MB-231 (breast), and Jurkat (T-cell leukemia). The PEG conjugated polysulfanes had IC_{50} values 1.2-5.8 times lower than the parent "unsubstituted" benzopolysulfanes. Complete cell killing was observed for the PEG polysulfanes with 4 μM for PC3 and DU145 cells, and with 12 μM for MDA-MB-231 cells. The results suggest that solubilization of the polysulfur linkage is a key parameter to the success of these compounds as drug leads.

A mechanism is proposed for the formation of cyclic 5,6,7,8,9-pentathiabenzo-cycloheptene-1,2-diol, from the reaction of *o*-benzoquinone with reduced elemental sulfur, H_2S_x. 1,6-Conjugate addition to the quinone is favored over 1,4-conjugate addition. Hydrogen bonding to the quinone oxygen enhances the nucleophilicity of H_2S_x by facilitating the removal of the S-H proton. We propose that initially formed 3-polysulfidobenzene-diol intermediates are oxidized to their corresponding quinones, and closure of the polysulfur ring subsequently takes place at the C3-C4 bond leading to cyclic 5,6,7,8,9-pentathiabenzo- cycloheptene-1,2-diol. A possible mechanism for the formation of pentasulfur linkage in cyclic 5,6,7,8,9-pentathiabenzo- cycloheptene-1,2-diol, which found in a number of natural products, is discussed.

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To My Parents

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LIST OF SYMBOLS AND ABBREVIATIONS

Å	angstrom
Ac	acetyl
Ac_2O	acetic anhydride
B3P86	Becke-3 Parameters Density Functional
brine	saturated aqueous sodium chloride solution
br	broad
^oC	degree Celsius
calcd	calculated
COSY	Correlation Spectroscopy
kcal	kilocalorie
^{13}C NMR	carbon-13 nuclear magnetic resonance
δ	chemical shift in ppm
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
DCC	*N,N'*-Dicyclohexylcarbodiimide
DIAD	Diisopropyl azodicarboxylate
DMAP	4-(Dimethylamino)pyridine
DFT	Density Functional Theory
DMF	N,N-dimethylformamide
DLD-1	human colorectal adenocarcinoma cells
ED_{50}	Effective Dose 50
ESI	Electrospray Ionization

Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	Ethanol
eq	equivalent
g	gram
GC	gas chromatography
GCMS	gas chromatography mass spectroscopy
h	hour
^1H NMR	proton nuclear magnetic resonance
HeLa 33	Henrietta Lacks (uterine cell variety) 33
HPLC	high pressure liquid chromatography
HMBC	Heteronuclear Multiple Bond Correlation
HRMS	High Resolution Mass Spectrometry
Hz	hertz
IC_{50}	50% inhibitory concentrations
ID	Internal Diameter
IR	infra-red spectroscopy
J	coupling constant
L	liter
LCMS	Liquid chromatography–mass spectrometry
LD_{50}	lethal dose 50
m	multiplet
MeOH	methanol

mg	milligram
min	minute
mL	milliliter
MM2	Molecular Mechanics
mmol	millimole
MS	Mass Spectrometry
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
NOE	Nuclear Overhauser Enhancement
PCM	polarized continuum model
PEG	Polyethylene Glycol
*p*H	potential of hydrogen
Ph_2O	Diphenyl ether
*p*Ka	ionization constant
PKC	Protein Kinase C
ppm	parts per million
q	quartet
R_f	Retention factor
RT	room temperature
s	singlet
t	triplet
TBAF	tetrabuthyl ammonium fluoride
THF	tetrahydrofuran

TLC	thin layer chromatography
TMEDA	tetramethyl ethylenediamine
TOF	Time of flight
TS	transition state
UV	ultra-violet spectroscopy

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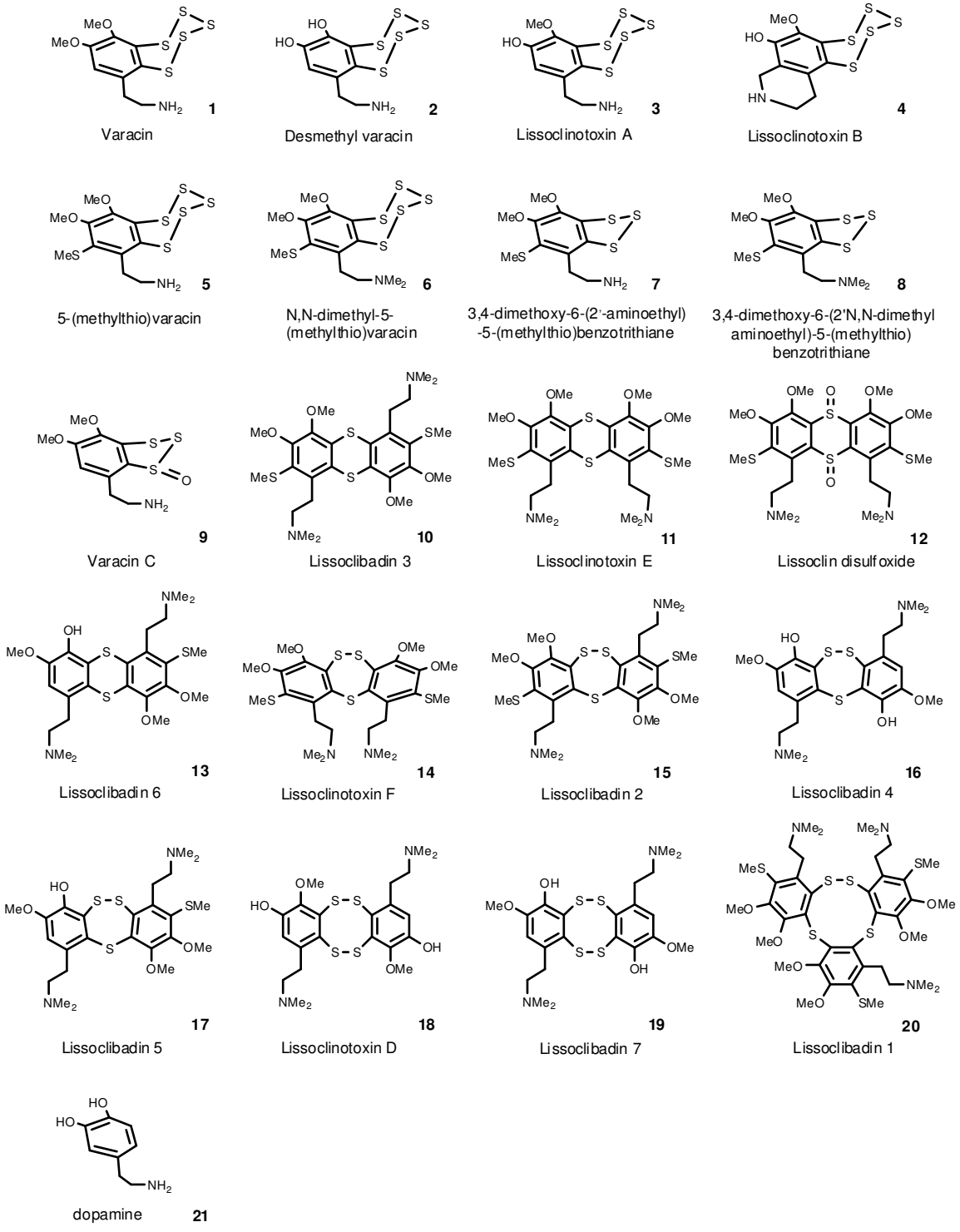
Chapter 1. Overview

1.1 Sulfur Containing Natural Products (Natural Polysulfanes)

Sulfur containing natural products (polysulfanes) arising from marine-based organisms represent a relatively untapped resource of potential therapeutic compounds especially for cancer. Natural polysulfanes were isolated and characterized fairly recently. The first natural benzopolysulfane was isolated in 1991 by Ireland and co-workers. In last two decades about 20 natural polysulfanes were isolated from organic extracts of marine tunicate samples.[1-13] Varacin (**1**), desmethyl varacin (**2**), lissoclinotoxin A (**3**), lissoclinotoxin B (**4**), 5-(methylthio)varacin (**5**), N,N-dimethyl-5-(methylthio)-varacin (**6**), 3,4-dimethoxy -6-(2'-aminoethyl) -5-(methylthio) benzotrithiane (**7**), 3,4-dimethoxy-6-(2' N,N-dimethylaminoethyl)-5-(methylthio)benzotrithiane (**8**), varacin C (**9**), lissoclinbadin 3 (**10**), lissoclinotoxin E (**11**), lissoclin disulfoxide (**12**), lissoclinbadin 6 (**13**), lissoclinotoxin F (**14**), lissoclinbadin 2 (**15**), lissoclinbadin 4 (**16**), lissoclinbadin 5 (**17**), lissoclinotoxin D (**18**), lissoclinbadin 7 (**19**) and lissoclinbadin 1 (**20**) (Scheme 1) are secondary metabolites that have obtained from tunicate samples.[1-13] Naturally occurring polysulfanes **1-20** arise from tunicates of the genera *Lissoclinum*, *Aplydium*, *Eudistoma*, and *Polycitor* their closely associated cyanobacteria.[25-27, 30]



Scheme 1. Naturally Occurring Polysulfanes and Dopamine.

1.1.1 Chemical Architecture of Natural Benzopolysulfanes.

The benzopolysulfanes listed in Scheme 1 show interesting and unusual structural features compared to other natural metabolites reported in the literature to date. These benozoplysulfanes **1-20** possess a common structural feature with a dopamine core (**21**) and an unusual polysulfur ring or sulfur containing heterocyclic rings. Some naturally occurring polysulfanes exist as dimers (**10-19**) and trimers (**20**).[7,12,13] Although spectroscopic techniques such as mass spectrometry and 2D-NMR have been utilized, the structural assignments are often not straight forward.

Lissoclinotoxin E **11** was isolated by Ireland and co-workers in 2003.[11] Cis-trans confirmation was assigned based on Monte Carlo computations with MM2 force field. Theoretical calculations suggested that the *trans* isomer is preferred over *cis* isomer by 0.4 kcal/mol, thus lissoclinotoxin E **11** was suggested to be the *trans* isomer. In 2005, Namikoshi and co-workers isolated Lissoclibadin 3 **10**, which had identical molecular weight (570) and formula ($C_{26}H_{38}N_2O_4S_4$) of **11**. NMR study by Namikoshi and co-workers showed that previous computational results on compound **11** are inconsistent with experimental results. In addition to the difference in ^{1}H NMR and ^{13}C NMR between *cis* and *trans* isomers compound **10** showed an NOE correlation between an OMe and NMe_2 in the NOESY spectrum. Thus, the orientation of two aromatic amine units in **10** was, therefore, deduced as *trans*.[12]

Determination of the number of sulfur atoms in natural *o*-benzopolysulfanes is not trivial because of the bond dissociation energy (BDE) of an S-S bond is low, 25-30 kcal/mol, which leads to easy bond breakage and equilibration processes. For example,

lissoclinotoxin A **3**, showed a prominent MS peak at m/z 261 led researchers to conclude that lissoclinotoxin A was a trisulfane, even though small peaks corresponding to five sulfur (m/z 325) and seven sulfur atoms (m/z 389) were present.[2] In 1994, it was suggested that the m/z 261 fragment peak had been mistaken for the parent peak, and the structure assignment of lissoclintoxin A was revised to be a pentasulfane possessing 5 sulfur atoms.[5] The existence of lissoclintoxin A as the heptasulfane had not been suggested even though there was mass evidence from the m/z 389 peak.

Benzopolysulfanes have unstable polysulfur rings which tend to different polysulfur ring structures in solution. A facile equilibration takes place between tri-, penta-, and heptasulfanes (*o*-$C_6H_4S_3$, *o*-$C_6H_4S_5$, and *o*-$C_6H_4S_7$) in CH_2Cl_2 with a ratio of 49:45:6 % respectively.[31] In 2004, a density functional theory (DFT) study revealed an interesting stability alternation pattern in benzopolysulfanes,o-$C_6H_4S_x$ (x = 1-8).[32] The odd-membered o-$C_6H_4S_x$ rings (except x = 1 which suffers from ring strain) have enhanced conformational stability compared to the even-membered rings. Thus, *o*-$C_6H_4S_3$, *o*-$C_6H_4S_5$, and *o*-$C_6H_4S_7$ were predicted to be the most stable in the series.

1.1.2 Bioactivity of Natural Benzopolysulanes

Natural benzopolysulfanes possess bioactivities against cancer, fungi, bacteria, and leukemia. Natural benzopolysulfanes shows high nM and low μM cytotoxicity against cancer cells. For example, Ireland and co-workers[1] discovered that varacin **1** exhibited cytotoxic activity toward human colon cancer HCT 116 was promising; it possessed an IC_{90} value of 0.05 μg/mL, which is ~100 times more active than 5-

fluorouracil, which is considered as a potent antitumor agent. It was suggested that the bioactivity of varacin **1** was derived from DNA damage because of an observed difference in toxicity toward the CHO cell line EM9 (chlorodeoxyuridine sensitive). The bioactivities of the naturally occurring (marine) polysulfanes **1**-**20** reported to date are presented in Table 1.[1-13] The tabulation of bioactivities reveals that marine invertebrates produces a remarkable variety of polysulfanes, which possess antimicrobial and antitumor activity.

Despite remarkable bioactivity surprisingly, the biological target(s) of benzopolysulfanes are still unknown. Biological studies with CHO and V79 cells implicate DNA as the target,[1,39] but DNA adducts have not yet been observed. Faulkner and coworkers showed compounds **5-8** inhibited PKC.[4] Benzopolysulfanes may be susceptible to attack by cellular thiols, such as glutathione or cellular thiols.

Bioactivity of these polysulfanes may arise from -SS_xS- linkage. Sulfur is known as a pharmaceutical agent. Human use for elemental sulfur as a scabicide and veterinary use as antiseptic and parasitical, and elemental sulfur constitutes 98% of a dusting fungicide used on a variety of fruit. Even though some literature refers to elemental sulfur as "highly toxic", its LD_{50} in rats is only ~6 g/Kg.[34,35] Better bioactivity benefits of sulfur may be gained by introduction of -SS_xS- linkage into a water-soluble organic molecule such as dopamine.

Table 1. Biological Activity of Natural Polysulfnes

Compound	Year	Bioactivity	Structure Purity
Varacin, **1**	1991	Antifungal activity against Candida albicans; cytotoxicity toward human colon cancer HCT 116 with IC_{90} of 0.05 µg/mL; 1.5 differential toxicity toward the CHO cell line EM9 vs BR1[1]	Possibility of mixture of compounds with S3 & S5.[15] But tandem MS study argues it is pentasulfane.[1] Free amine decomposes the S ring. At physiological pH this may exist in protonated form.
Desmethyl Varacin, **2**	1994	Selectivity inhibited PKC with an IC50 of 33.0 µg/mL and PKA >25 µg/mL[4]	
Lissoclinotoxin A, **3**	1991	Moderate activity against fungi & yeast; [5] C. albicans 40 ug/disk (27 mm)[7]. Toxicity towards L1210 leukemia cell line with an IC50 of 1 µg/mL. Antimalarial activity toward *Plasmodium falciparum*[5]	Pentathiepin structure was ascertained by UV data. Acetylation of this compound led mixture of compounds.[5]
Lissoclinotoxin B, **4**	1994	Toxic against Gram(+) and Gram (-) bacteria strains.[8]	
5-(methylthio) varacin and 3,4-dimethoxy-6-(2'-aminoethyl)-5-(methylthio) benzotrithiane **5** + **7**	1994	Selectively inhibited PKC with an IC50 of 0.3 µg/mL and PKA 25 µg/mL[4]	Inseparable 2:3 mixture of **5** and **7**[4]
N,N-dimethyl-5-(methylthio) varacin, **6**	1994	Selectively inhibited PKC with an IC50 of 3.0 µg/mL and PKA >50 µg/mL[4], Mildly antimicrobial against B. subtilis, S. aureus (100ug/disk) and C. albicans. And shows no selectivity in NCI 60 cell line.	Separable from its trithiane. [4]

Compound	Year	Bioactivity	Structure Purity
3,4-dimethoxy-6-(2'-N,N-dimethylaminoethyl)-5-(methylthio) benzotrithiane, **8**	1994	Selectively inhibited PKC with an IC50 of 1.3 µg/mL and PKA >50 µg/mL Mildly antimicrobial against B. subtilis, S. aureus (100ug/disk) and C. albicans. And shows no selectivity in NCI 60 cell line. [4]	Separable from its pentathiepin.[4]
Varacin C, **9**	1995	Cytotoxic activity against *C. albicans* and *Bacillus subtilis*; gave acid promoted DNA cleaving activity[11]	-[a]
Lissoclinobadin 3, **10**	2005	Displayed IC_{50} 1.8 µg/mL against the T-47D human breast carcinoma cell line and cytotoxicity against the MDA-MB-231 human breast carcinoma cell line with IC_{50} value 2.0 µg/mL[10]	-
Lissoclinotoxin E, **11**	2003	Displayed IC_{50} 2.3 µg/mL against the MDA-MB-468S human breast carcinoma cell line and cytotoxicity against the MDA-MB-435S human breast carcinoma cell line with IC_{50} value2.1 µg/mL[10]	-
Lissoclindisulfoxide, **12**	2003	Was inactive at 50 µg/mL against MDA-MB-435S human breast carcinoma cell line[10]	-
Lissoclinobadin 6, **13**	2007	Antimicrobial activity against S. cerevisiae (7.8 mm), S. aureus (13.8 mm) and E. coli (10.8 mm) inhibition zone at 50 µg/disk[30]	-
Lissoclinotoxin F, **14**	2003	Displayed IC_{50} 1.5 µg/mL against the MDA-MB-468S human breast carcinoma cell line and cytotoxicity against the MDA-MB-435S human breast carcinoma cell line with IC_{50} value 4.2 µg/mL[10]	-

Compound	Year	Bioactivity	Structure Purity
Lissoclinobadin 2, **15**	2005	IC_{50} value of 0.12 µg/mL against HL-60 leukemia cell line and 0.08 µg/mL against HCT116 colon cancer cell line[12]	-
Lissoclinobadin 4, **16**	2007	Antimicrobial activity against S. aureus (13.1 mm) and E. coli (15.7 mm) inhibition zone at 50 µg/disk[30]	-
Lissoclinobadin 5, **17**	2007	Antimicrobial activity against S. cerevisiae (6.2mm), S. aureus (15.8 mm) and E. coli (11.6 mm) inhibition zone at 50 µg/disk[30]	-
Lissoclinotoxin D, **18**	1994	Activity against C. albicans; gave zones of inhibition with loadings of 6.5 mm/disk. 40 ug/disk (19mm), 10 ug/disk (15mm)[8]	-
Lissoclinobadin 7, **19**	2007	Antimicrobial activity against S. cerevisiae (16.8mm), S. aureus (13.1mm) and E. coli (9.9 mm) inhibition zone at 50 µg/disk[30]	-
Lissoclinobadin 1, **20**	2005	IC_{50} value of 0.3 µg/mL against MDA-MB-231 breast cancer cell line and 0.7 µg/mL against HCT116 colon cancer cell line[43]	-

[a.] Purity data not available

1.1.3 Origin of Sulfur Atoms in Natural Benzopolysulfanes

Although benzopolysulfanes have been isolated from marine invertebrates the underling mechanism of incorporation of sulfur atoms into dopamine is largely unknown. Possible ingredients for the synthesis of benzopolysulfanes could be dopamine and elemental sulfur. Marine sponges and tunicates are primitive animals without a nervous

system; dopamine is therefore not produced as a neurotransmitter. Instead dopamine has been proposed as a grazer deterrent, although this constitutes a speculated function. Dopamine has been isolated in a relatively high yield (0.0022% of wet weight) from a marine sponge.[24] Dopamine from marine invertebrates may be produced by symbiotic microorganisms, such as cyanobacteria.[24-27]

Tunicates are filter-feeding animals that can uptake colloidal organic and inorganic particles from the ocean water. Filter-feeding sulfur sponges harbor significant quantities of elemental sulfur. Some marine environments contain inorganic S_8, pyrite and related particles.

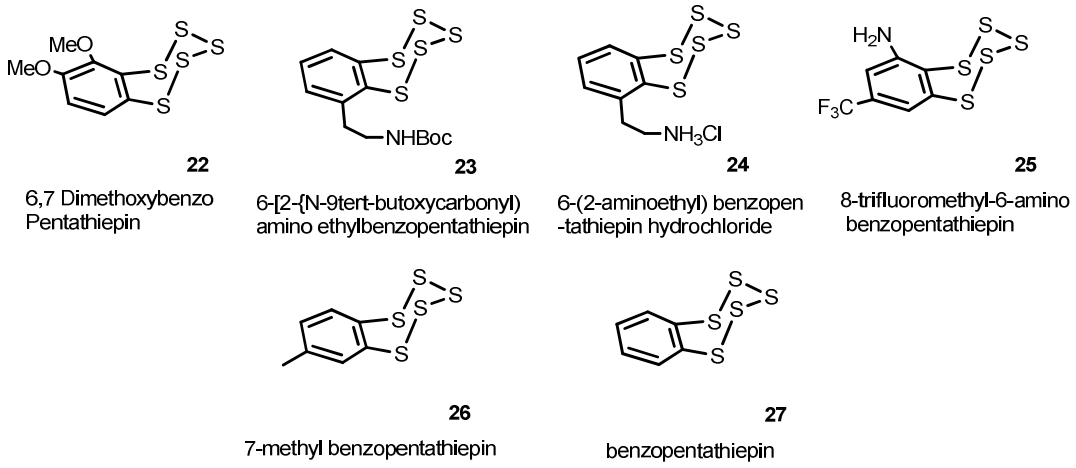
With the most common of chemical ingredients, elemental sulfur and dopamine, we suggest that an ascidian has developed a means to protect itself from predators using in essence chemical warfare.[1-13] Some organisms use simple molecules for defense and protection.[18] For example, ants use formic acid and millipedes use hydrogen cyanide.[18] Others organisms use more complicated defense molecules, such as intricate protein toxins produced by spiders, or the high-temperature quinoid compounds from a bombardier beetle.[19]

There is little data on chemical defense molecules of tunicates although some alkaloids have been implicated.[20-23] However, the polysulfanes shown in Scheme 1 are little appreciated in this regard, and may serve as a potent defense against predator fish and flatworms or pathogenic bacteria and fungi—since polysulfanes possess biocidal properties (e.g., antifungal and antibacterial activity).

Two reactions of sulfur and dopamine may relate to the biomimetic principles that underlie how benzopentathiepins arise biosynthetically. First, benzopentathiepins may

arise from a two-electron transfer reaction of reduced elemental sulfur, H_2S_x, with dopamine-*o*-quinone. Second, benzopentathiepins may arise from a one-electron oxidation of dopamine followed by a reaction with neutral S_8. We have examined the viability of the first reaction in the laboratory generation of cyclic 5,6,7,8,9-benzopentathiepin-1,2-diol using a *o*-benzoquinone—H_2S_x reaction. This is discussed in the third chapter of the thesis.

1.2 Unnatural Benzopolysulfanes



Scheme 2. Unnatural benzopolysulfanes.

Synthetic analogs of natural benzopolysulfanes (Scheme 2) have been synthesized and their biological activities have been reported. In 1995, Sato and co-workers synthesized compounds **22-24** and determined their biological activity against HeLa-S_3 cells.[36] For example, 6-(2-aminoethyl) benzopentathiepin hydrochloride **24** showed IC_{50} value of 0.26 µg/ml against HeLa-S_3 cells. Compound **25** can be regarded as a promising compound for the development of new low-toxic anticonvulsive agents with anxiolytic activity and agents improving the emotional state during Alzheimer's

disease.[37] Compound 7-methylbenzopentathiepin **26** was shown to be a potent thiol-dependent DNA-cleaving agent.[38]

1.3 Benzopolysulfanes as drug candidates

Even though natural polysulfanes possess impressive bioactivities against cancer, fungi, bacteria, and leukemia, these compounds have not been developed for clinical use. The number of research papers on mitomycins compared to marine polysulfanes is 25,000:50 but IC_{50} values against HeLa S3 cells for ethylamino-benzopentathiepin (0.26 μg/mL)[1] and mitomycin C (0.2-1.27 μg/mL). Only ~20 benzopolysulfanes have been synthesized to date due to the challenges these compounds present to synthesize. The polysulfur ring in these benzopolysulfane compounds is unstable and susceptible to under go nucleophilic attack. Benzopolysulfanes could be reductively activated in physiological conditions, but in vivo stability studies are needed to evaluate polysulfur ring liability to glutathione (GSH), protein thiols, etc.

1.4 Thiozone: A Possilbe Reactive Intermediate From Benzopolysulfanes

Information is limited on the intermediates involved in biochemical reactions of pentathiepins. 7-Methylbenzopentathiepin **26** cleaved plasmid DNA in the presence of thiol by a mechanism suggested to involve polysulfide ion.[38] Evidence suggested that the thiol-triggered DNA damage by **26** involved the conversion of molecular oxygen to oxygen radicals from a trace metal-dependent Fenton reaction.[38] Information on the

organic chemistry of pentathiepins may also help reveal the factors related to the bioactivity.

A density functional theoretical study showed a novel S_3-cleavage in the decomposition of pentathiepin.[40] Ring opening of pentathiepin leads to open chain polysulfur ion intermediate from which expulsion of triatominc sulfur S_3 is preferred over diatomic sulfur S_2 species. Another study shows that desulfuration of varacin **1** is promoted by side chain ammine group and energetically low lying process for S_3-loss.[41] Experimental evidence has been obtained for pentathiepin desulfuration via S_3-unit transfer by a trapping study with norbornene.

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Chapter 2. Synthesis, Characterization, Mechanism of Decomposition, and Antiproliferative Activity of a Class of PEGylated Benzopolysulfanes Structurally Similar to the Natural Product Varacin

2.1 Introduction

As stated in chapter 1, tunicates or their associated microorganisms produce benzopolysulfanes, such as varacin (**1**), lissoclinotoxin A (**2**), and *N,N*-dimethyl-5-(methylthio)varacin (**3**) (Scheme 1).[1-6] Unnatural benzopolysulfanes have also been synthesized, e.g., 6-(2-aminoethyl)benzopentathiepin (**4**)[7] and the parent benzopentathiepin (**5B**)[8,9] (alphabetical labels will given to some polysulfanes, as will be elaborated on below).

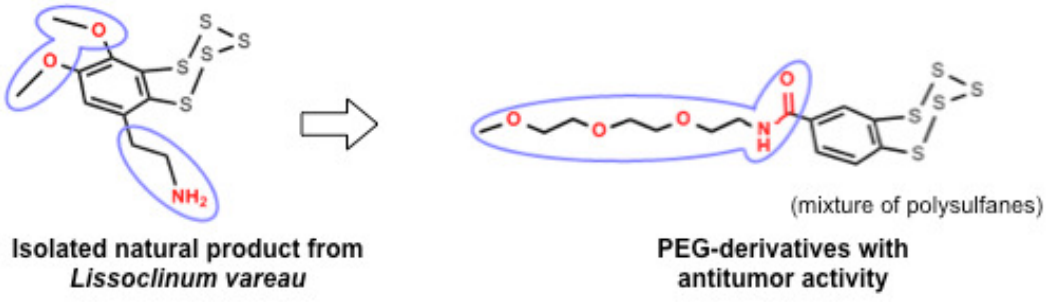
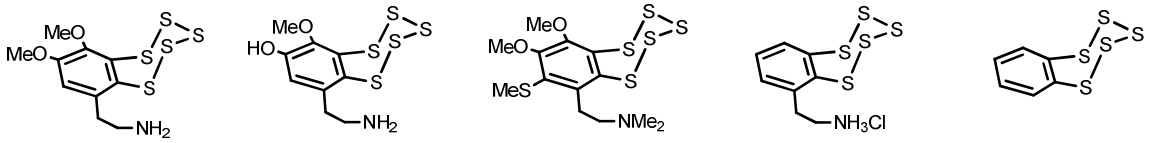


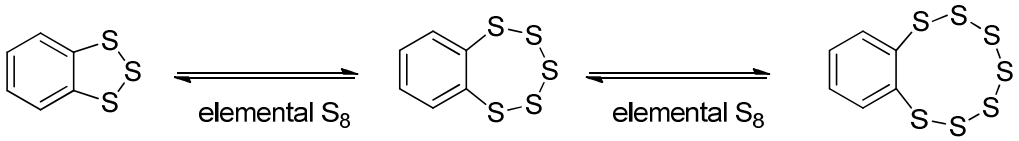
Figure 1. Natural product varacin and synthetic PEGylated benzopentasufane



Varacin **1** | Lissoclinotoxin A **2** | N,N-dimethyl-5-(methylthio)varacin **3** | 6-(2-aminoethyl) beznopentathiepin **4** | Parent benzopentasulfane **5B**

Scheme 1. Natural and Unnatural Benzopolysulfanes

Benzopolysulfanes have not been studied widely in the context of drug discovery due to the instability of the polysulfur ring. Even determining the number of sulfur atoms in *o*-benzopolysulfanes has not been a trivial task.[11-13] MS fragmentation patterns can be difficult to interpret; for example, the structure of the natural product lissoclinotoxin A was assigned first as a trisulfane[3] and later revised as a pentasulfane.[11] In 2007, the synthesis and purification of parent *o*-$C_6H_4S_5$ (**5B**) revealed an equilibration involving elemental sulfur, S_8 (Scheme 2).[12] A facile equilibration took place between the pentasulfane and the tri-, and heptasulfanes (*o*-$C_6H_4S_3$ and *o*-$C_6H_4S_7$): the ratio of **5A**:**B**:**C** was 49:45:6 in CH_2Cl_2 over 1-3 days. In **5**, the labels A, B, and C correspond to compounds with 3, 5, and 7 sulfur atoms, respectively. Analysis of GC/MS retention times revealed that **5A** and **5B** differed by 12.3 min, while the retention times of **5B** and **5C** differed by 12.6 min (Table 1).



5A **5B** **5C**

Scheme 2. Equilibration Between Tri-, Penta-, and Heptasulfanes

Table 1. GC/MS Detection of the Parent Benzopolysulfanes 5A-C[a]

Compound	No. of S atoms	MS t_R (min)[b]	Molecular Weights	
			calcd	found[c]
o-$C_6H_4S_3$ **5A**	3	14.2	172	172
o-$C_6H_4S_5$ **5B**	5	26.5	236	236
o-$C_6H_4S_7$ **5C**	7	39.1	300	300

[a] Ref. 12. See also Ref. 13. [b] GCMS retention time. [c] Low-resolution GCMS. Support for the GC/MS peak assignments came from spectroscopic comparisons of **5A**-**C**, which were independently synthesized and examined shortly after their purification, i.e., before equilibration was pronounced, which took 1-3 days.

Because natural benzopolysulfanes are in short supply and their stability is difficult to assess, we synthesized benzopolysulfanes with a short-chain PEG, in which the benzene ring and PEG group replaced the naturally occurring dopamine core **1**-**3**. The aims of the present work were to determine (1) whether benzopolysulfanes could be synthesized with a PEG side group, (2) whether the pentasulfur species predominates, (3) whether the polysulfur linkage(s) are unstable to medium effects, (4) whether the PEGylated benzopolysulfanes decompose at different rates and transfer sulfur to norbornene and butadiene traps, (5) the extent the PEG group enhances water solubility, (6) whether the polysulfur ring is essential for bioactivity, and (7) whether enhanced benzopolysulfane water solubility is correlated with an enhanced pharmacological activity against human tumor cells. We synthesized a mixture of PEG-benzopolysulfane conjugates 4-$CH_3(OCH_2CH_2)_3NHC(O)$-C_6H_4-1,2-S_x (x = 3-7 and 9) **6A**-**F** and explored their stability, and activity in a variety of tumor cell lines [PC3 (prostate), DU145

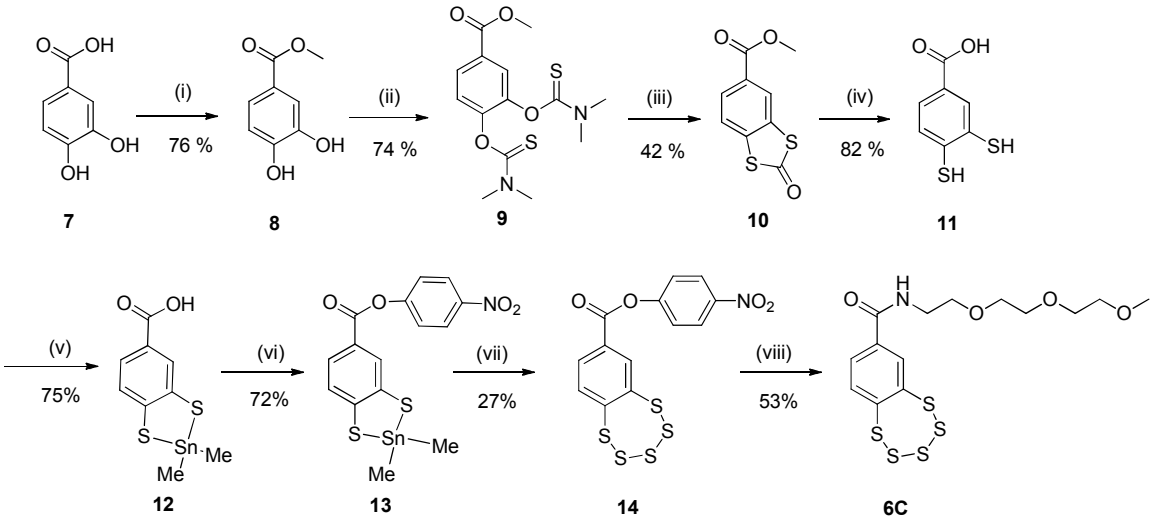
(prostate), MDA-MB-231 (breast), and Jurkat (T-cell leukemia)]. In **6**, the labels A-E and F correspond to compounds with 3-7 and 9 sulfur atoms, respectively. The octasulfane species was not detected.

2.2 Results and Discussion

2.2.1 Synthesis and Characterization.

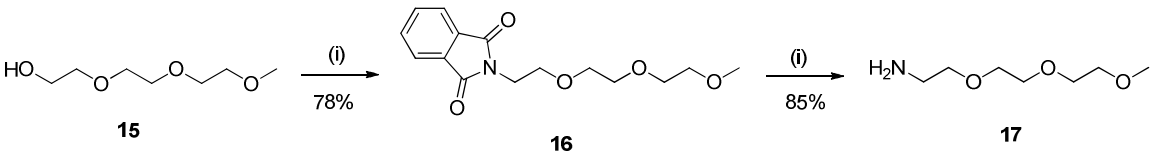
Pentasulfane **6C** was synthesized as the major constituent of a mixture of polysulfanes 4-$CH_3(OCH_2CH_2)_3NHC(O)$-C_6H_4-1,2-S_x (x = 3-7 and 9) in 8 steps and 1.5% overall yield. A procedure developed by Liénard et al.[14] was used for the conversion of 3,4-dihydroxybenzoic acid (**7**) to 3,4-disulfuranylbenzoic acid (**11**) (steps i-iv, Scheme 3). Dithiastannole-5-carboxylate anion **12** was generated under basic conditions by the reaction of dimethyltin chloride with **11** using a modified procedure by Sato et al.[15] Stannole **12** reacted with *p*-nitrophenol, DCC, and DMAP to yield 4-nitrophenyl ester stannole **13** in 72% yield. Stannole **13** reacted with disulfur dichloride giving 4-nitrophenyl ester benzopentasulfane (**14**) in 27% yield. Amino-terminated poly(ethylene glycol) (**17**) was prepared in 2 steps by the method of Dombi et al.[16] (Scheme 4) and PEGylated to benzopentasulfane **14** at the 7-position of the benzene ring. It is possible that other cyclic polysulfanes related to benzopentasulfane **14** were formed in ~1-10% yields, but this was not determined. The resulting mixture was purified by column chromatography to afford **6C** in 93% purity. In the ^{1}H NMR, ^{13}C NMR, COSY, HMBC, and HSQC spectra, the benzene and PEG portions of the structure were confirmed (Supporting Information). For example, in COSY NMR, a strong ^3J correlation was found between C9-H and C8-H, and between N-H and C13-H, and a

weak ^4J-W correlation found between C8-H and C6-H (Scheme 5). The HMBC and HSQC NMR data further bolstered the structural assignment of the non-sulfur portion of **6C**. LC/MS data indicated that **6C** contained 5 sulfur atoms (HRMS calcd for $C_{14}H_{19}NO_4S_5$ = 424.9918, found 424.9926).



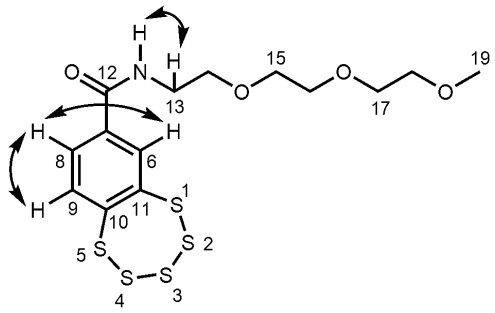
Scheme 3[a]. Synthesis of PEG Conjugated Benzopentasulfane 6C

[a] Reagents and conditions: (i) HCl (cat.), MeOH, reflux 80°C, 7 h; (ii) $Me_2NC(=S)Cl$, DABCO, DMF, RT, 30 min; (iii) Ph_2O, 230 °C, 40 min; (iv) (a) aqueous NaOH, under N_2, 70°C, 4 h, (b) 1N HCl; (v) (a) Me_2SnCl_2, KOH, EtOH, water, (b) 1N HCl; (vi) *p*-nitro phenol, DCC, DMAP, CH_2Cl_2, 1 d; (vii) S_2Cl_2, CH_2Cl_2, 0 °C, 30 min, then warmed to RT, 24 h; (viii) H_2N-$(CH_2O)_3$-CH_3 **17** (Scheme 4), THF, RT, 12 h.



Scheme 4[a,b] Synthesis of Amino-Terminated Poly(ethylene glycol)

[a] Ref. 16. [b] Reagents and conditions: (i) phthalimide, PPh_3, DIAD, THF, RT, 12 h; (ii) (a) N_2H_4, EtOH, 100 °C, 5 h; (b) HCl (cat.), 100 °C, 1 h.

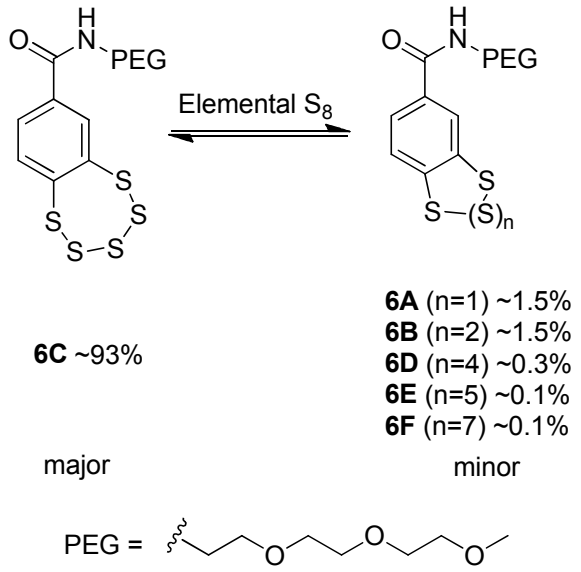


Scheme 5. COSY NMR Couplings of Benzopentasulfane 6C

2.2.2 Lability of the Pentasulfur Ring

Polysulfanes are challenging structures to study because of their instability. Few reports on benzopolysulfanes describe the distribution of the polysulfanes, or their equilibration, and often incorrectly assume the pentathiepin to be the sole compound present.[12] We found that over a 12-24 hour period **6C** equilibrates S_8 and forms low concentrations of structurally related polysulfanes in aqueous methanol at room temperature (Scheme 6). Table 2 lists the polysulfane masses and retention times to compare the number of sulfur atoms in the compounds. The LCMS spectra of polysulfanes **6A**-**F** contained peaks spaced by ~0.7 min per additional sulfur atom (Figure 2). For example, a minor amount of a sulfur-rich compound was assigned as nonasulfane **6F** (HRMS calcd for $C_{14}H_{19}NO_4S_9$ = 552.8800, found 552.8788). The ratio of **6A**:**B**:**C**:**D:E:F** was 1.5:1.5:93:0.3:0.1:0.1 by HPLC (methanol/water), and there was ~2% uncharacterized material and ~1.5% of elemental sulfur. The moderate solubility of the elemental sulfur in methanol/water suggested it to be a residue or colloid particles of the orthorhombic cyclo-S_8 form, but neither the monoclinic S_8 ring form (usually found at ~95 °C), nor the polymeric, oligomeric, or amorphous forms (insoluble materials).[17-19] The overlaid-ion extracted LCMS of **6** in Figure 2 displayed a series of polysulfanes,

analogous to that seen for parent **5** by SIM-GCMS.[12] Preference for odd-membered ring compounds was observed experimentally for the parent system *o*-$C_6H_4S_x$ (x = 3, 5, 7) in CH_2Cl_2.[12] Gas-phase DFT calculations showed the odd-membered *o*-$C_6H_4S_x$ rings to be conformationally stable with gauche adjacent lone-pair electron interactions, whereas eclipsing lone-pair electron interactions occurred in the even-numbered cases.[13] It may be noted that Nakayama et al. also observed odd-membered ring products (namely, the trithiolane and penathiepan) in the reaction of elemental sulfur with a benzobarrelene compound and an acenaphthylene compound.[20,21] However, the preference for odd-membered polysulfur rings does not apply to the PEG benzopolysulfanes, which is likely because the equilibrium is at an early stage and has not been reached in aqueous methanol, even after 24 h. The effect of solvent or HPLC conditions on the equilibrium of **6A**-**F** and S_8 was investigated next.



Scheme 6. Equilibration of Pentasulfane 6C with the Minor Amounts of Tri-, Tetra-, Hexa-, Hepta- and Nonasulfanes in Methanol/Water (1:1 vol/vol)

Table 2. Mass Spectrometry Data for PEGylated Benzopolysulfanes 6A-F

Compound	Formula	Retention time[a] (min)	Calculated mass	Experimental mass[c] $[(M+H^+)-H^+]$[b]	Error (ppm)
6A	$C_{14}H_{19}NO_4S_3$	4.6	361.0476	361.0478	0.62
6B	$C_{14}H_{19}NO_4S_4$	5.6	393.0197	393.0193	-1.00
6C	$C_{14}H_{19}NO_4S_5$	6.5	424.9918	424.9926	1.99
6D	$C_{14}H_{19}NO_4S_6$	7.1	456.9638	456.9636	-0.60
6E	$C_{14}H_{19}NO_4S_7$	7.8	488.9359	488.9354	-1.08
6F	$C_{14}H_{19}NO_4S_9$	9.2	552.8800	552.8787	-2.34

[a] LCMS retention time. Chromatography was performed on a SB-C18 3.5 µm column using water containing 0.1% formic acid and 5 mM ammonium formate (solvent A) and methanol containing 0.1% formic acid and 5 mM ammonium formate (solvent B) at a flow rate 0.5 mL/min. The gradient program was as follows 15-85% B (0-13 min) 85% B (13-15 min) 85-15% B (1 min). [b] The experimental exact mass is calculated by the subtraction of a proton (H^+; 1.00728 Da) to the measured *m/z* value the $[M+H^+]$ ions for the molecular formulas of interest.

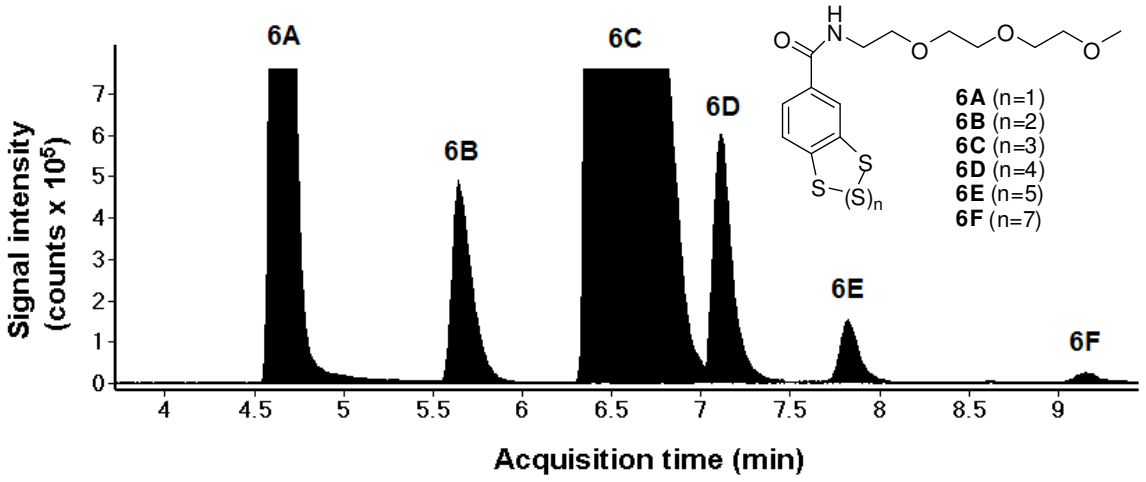


Figure 2. Ion extracted [M+H$^+$] LCMS spectra of compounds **6A**-**F**. Chromatograms were normalized to the largest peak **6C**. The "counts" on the Y-axis are equal to the number of ions detected.

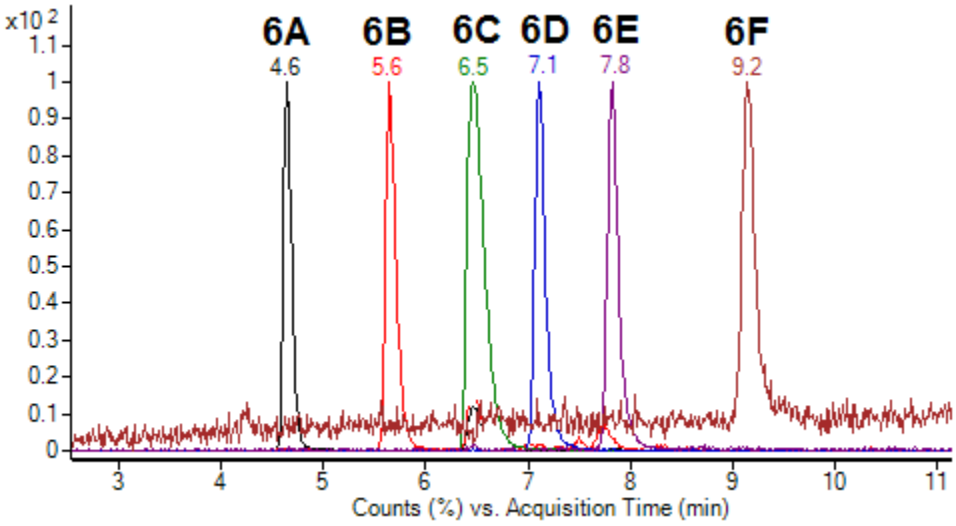


Figure 3. Ion extracted [M+H$^+$] LCMS spectra of compounds **6A**-**F**. Chromatograms were normalized to the largest in each chromatogram. The "counts" on the Y-axis are equal to the number of ions detected.

2.2.3 Influence of Solvent on Equilibration of Polysulfanes 6A-F

To better understand the equilibration between **6A**-**F** and S_8, the HPLC elution methods were modified. When eluting at 1:1 methanol:water, the ratio of **6A**:**B**:**C**:**D:E:F**:S_8 was 2.2:1.8:93.5:0.4:0.3:0.2:1.6 (Method 1, Table 3). Increasing the methanol concentration in water produces more pronounced polysulfane equilibration. When eluting at 85:15 methanol:water, the ratio of **6A**:**B**:**C**:**D:E:F**:S_8 was 5.3:5.7:79.0:3.4:1.2:0.1:5.3 (Method 5, Table 3). The HPLC measurements do not ensure that thermodynamic conditions have been reached. Equilibrium at a new condition is established in 1-3 days in CH_2Cl_2,[12] and probably much longer in methanol/water. Our results are similar with observations of 7-methylbenzopolysulfane equilibrations enhanced in polar solvents with higher solvation compared to nonpolar solvents.[22] Elemental S_8 also involves equilibration with S_6 and S_7, which was more pronounced in polar than nonpolar organic solvents,[23] and sulfur-reactive solvents such as pyridine or other amines which dissolve elemental sulfur readily.[18] The enhanced equilibration in methanol compared to methanol/water is likely a result of the increase in the solubility of S_8, which is pivotal to the reversible addition reactions leading to the ring compounds **6A**-**F**.

Table 3. Polysulfane Distribution at different HPLC Solvent Elution Conditions[a]

Method[b]	Ratio of MeOH-H_2O (V/V)	Polysulfanes and Elemental Sulfur						
		6A	**6B**	**6C**	**6D**	**6E**	**6F**	**S_8**
1	50-50	2.2	1.8	93.5	0.4	0.3	0.2	1.6
2	60-40	2.5	2.1	94.7	-	0.6	-	-
3	75-25	4.3	3.2	84.6	1.5	0.9	0.5	3.4
4	80-20	4.7	4.8	83.6	1.0	0.3	0.4	5.1
5	85-15	5.3	5.7	79.0	3.4	1.2	0.1	5.3

[a] HPLC analysis at 254 nm at room temperature with a flow rate of 1 mL/min in water methanol mixtures. [b] HPLC methods: Method 1 consisted of a gradient of methanol from 10% to 90% over 53 min and maintained for 2 min before reverting to 10% methanol over 1 min. Method 2 consisted of 60% methanol run isocratically over 91 min. Method 3 consisted of a gradient of methanol from 60% to 95% over 30 min, which was maintained for 10 min before reverting to 15% methanol over 5 min. Method 4 consisted of a gradient of methanol from 15% to 95% over 30 min, which was maintained for 15 min before reverting to 15% methanol over 3 min. Method 5 consisted of a gradient of methanol from 15% to 95% over 15 min, which was maintained for 15 min before reverting to 15% methanol over 2 min. Method 6 consisted of water (0.1% formic acid and 5 mM ammonium formate in water) and methanol (0.1% formic acid and 5 mM ammonium formate in MeOH) with a gradient of methanol from 15% to 85% over 13 min, which was maintained for 2 min before reverting to 15% methanol over 1 min.

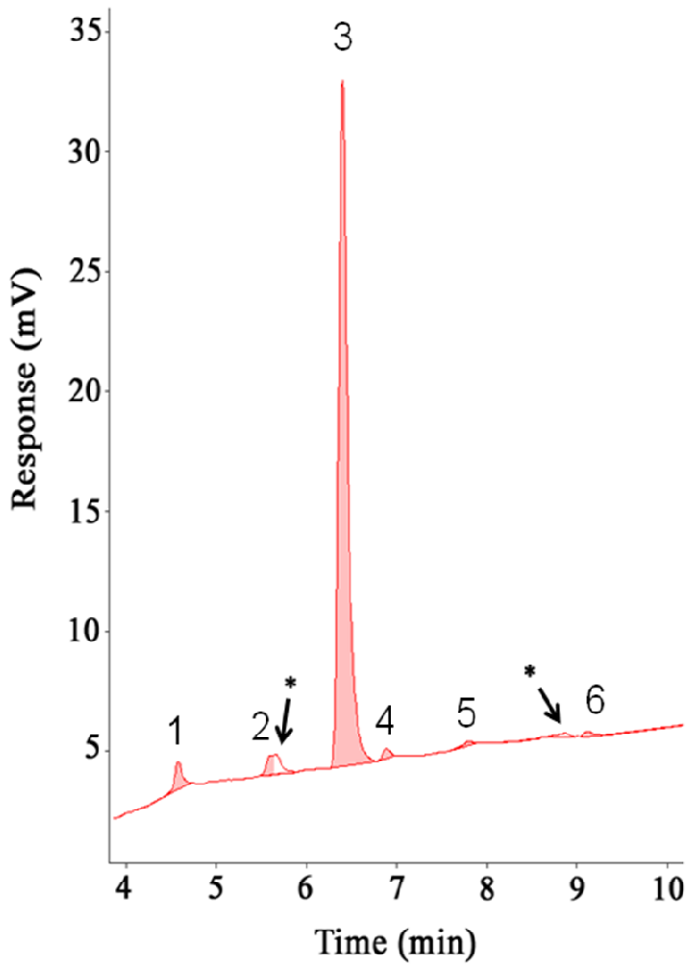


Figure 4. HPLC chromatogram of polysulfanes; peaks include, 1, **6A**; 2, **6B**; 3, **6C**; 4, **6D**; 5, **6E**; 6, **6F**. Elemental sulfur S_8 elutes in the 11-13 min range. Peaks marked with an asterisk belong to unidentified substances.

2.2.4 Desulfuration of 6A-F in the Presence of Nucleophiles and Trapping Agents

The PEGylated benzopolysulfanes **6A-F** decomposed in the presence of NaOH or ethanethiol (Table 4), which led to an increase in elemental S_8 and uncharacterized products, such as oligomers and/or polymers of desulfurated PEGylated benzopolysulfanes. The reaction of hydroxide ion with benzopolysulfanes **6A**-**F** was comparatively slower than with ethanethiol and could be monitored by HPLC. As can be seen in Table 4, the decomposition rate of pentasulfane **6C** was rapid while the

concentrations of the other benzopolysulfanes were barely reduced. Determining whether the concentration of tetrasulfane **6B** increased or decreased was not possible because it eluted along with an unidentified compound (X in Table 4) and the two peaks could not be de-convoluted. The data suggested that pentasulfane **6C** desulfurated faster compared to other polysulfanes, which led us to trapping studies to analyze the sulfur-transfer reaction with butadiene and norbornene traps.

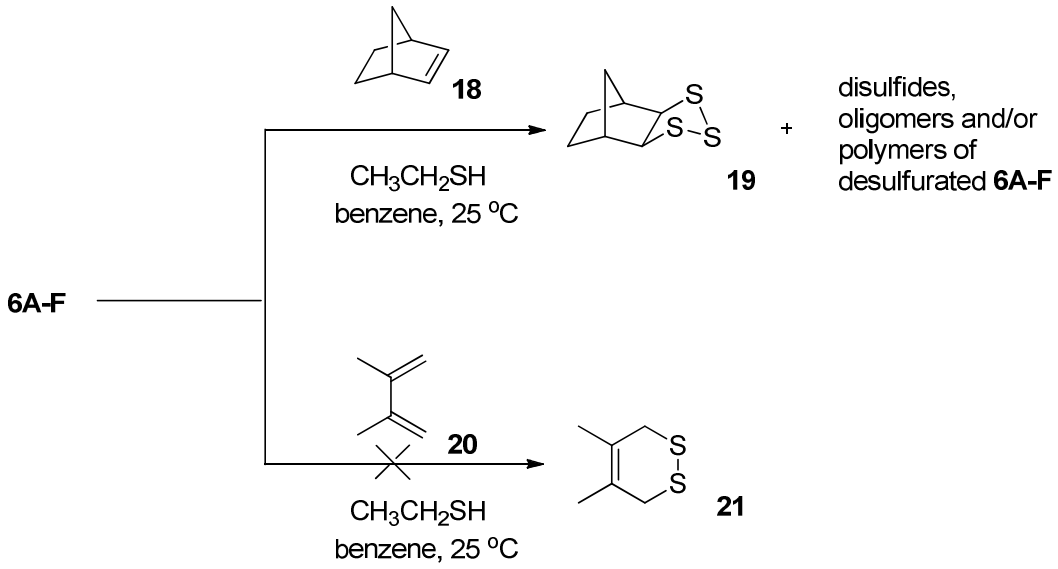
Table 4. Decomposition of PEGylated Benzopolysulfanes 6A-F as a Function of Hydroxide Ion Equivalents Added[a]

Hydroxide ion equivalents	Internal standard	Polysulfanes and elemental sulfur distribution[b]						
		6A	**6B+X**[c]	**6C**	**6D**	**6E**	**6F**	**S_8**
0.25	100	10.9	11.2	362.4	2.3	3.0	2.1	12.3
0.5	100	13.2	19.6	289.2	2.1	2.8	3.7	23.1
0.75	100	14.2	38.1	222.9	1.8	4.7	3.8	26.7
1.0	100	14.2	64.9	163.9	1.5	1.6	3.1	32.4
1.0[d]	100	14.9	85.5	113.6	0.7	2.2	-	3.1

[a] Reaction of benzopolysulfanes **6A**-**F** (2.5 mM) and hydroxide ion in the presence of internal standard acetanilide (0.25 mM) in methanol/water (99.9:0.1 vol/vol). [b] Polysulfane ratios were determined by HPLC monitoring at 254 nm at room temperature with the flow rate 1 mL/min using method 4 (Table 3). HPLC analysis was carried out after 1 hr of equilibration between benzopolysulfanes **6A**-**F** and hydroxide ion. [c] X is unidentified compound that was eluted with **6B**. [d] Analysis was done after 24 hrs.

The reaction of benzopolysulfanes **6A**-**F** with ethanethiol and norbornene (**18**) led to the formation of norbornenetrithiolane (**19**) and desulfurated or polymerized PEGylated benzopolysulfanes (Scheme 7). Unlike **6C**, the concentrations of **6A**, **6B**, and **6D**-**F** did not change significantly over the course of the trapping experiment. Thus, we

propose that **6C** is the most reactive polysulfane and responsible for the S_3-transfer to norbornene (Figure 5). Interestingly, the decomposition of benzopolysulfanes **6A**-**F** did not show an S_2 transfer reaction. The sulfuration of 2,3-dimethylbutadiene (**20**) by **6A**-**F** with ethanethiol did not yield the disulfide product **21**.



Scheme 7. PEGylated Benzopolysulfanes 6A-F as Sulfur-Transfer Reagents to Norbornene but not to 2,3-Dimethylbutadiene

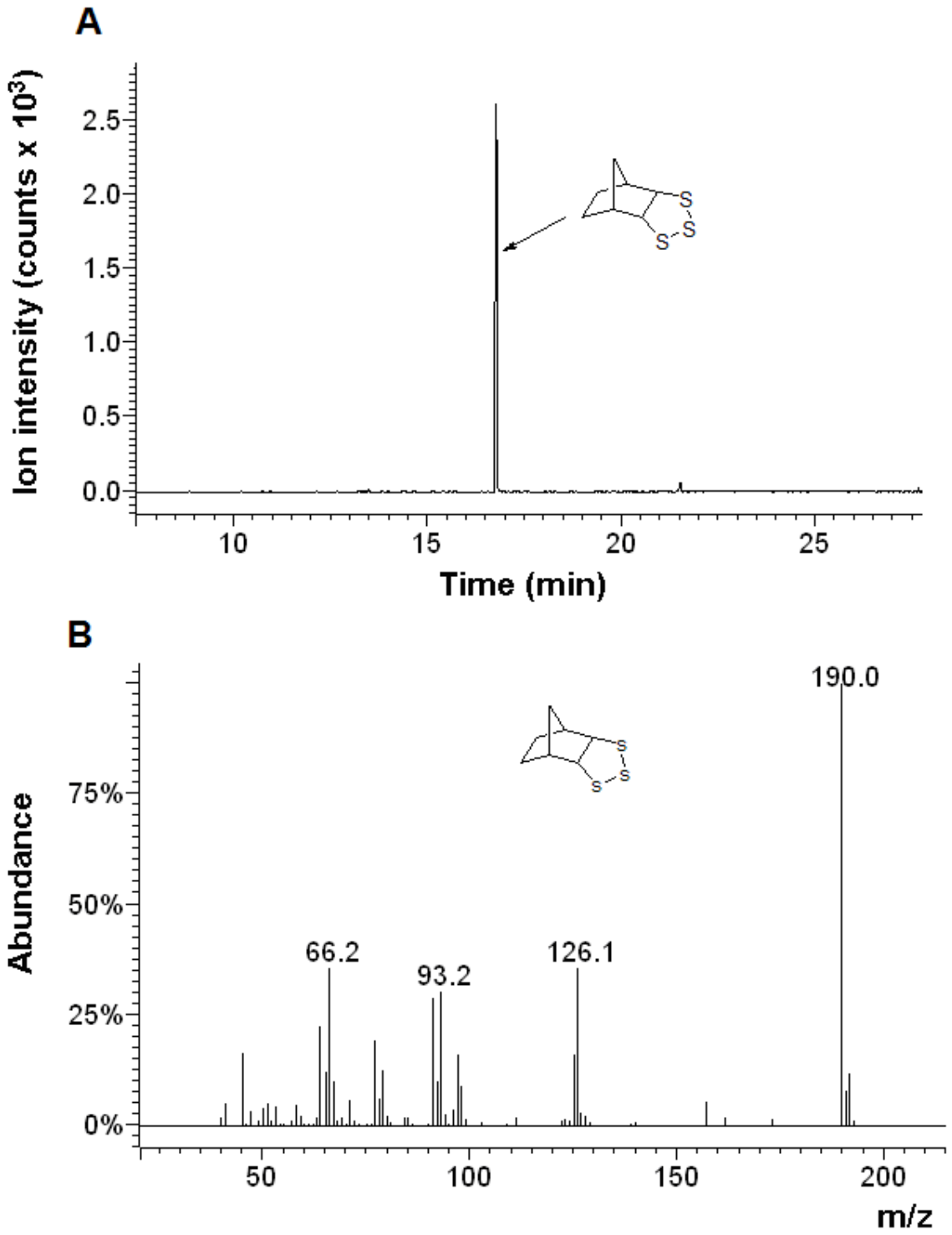
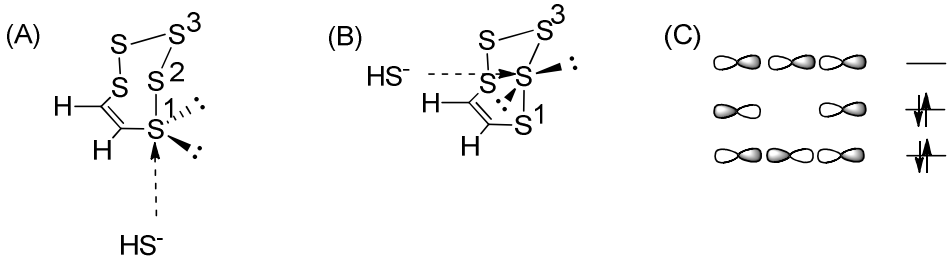


Figure 5. (A) Extracted ion chromatogram of trithiane **19** formed in the reaction of **6A**-**F** and norbonene in the presence of ethanethiol, Rt 16.75 min. (B) MS spectrum of trithiane **19**, Rt 16.75 min.

2.2.5 Mechanism for the Triatomic Sulfur-Transfer of Benzopentasulfane

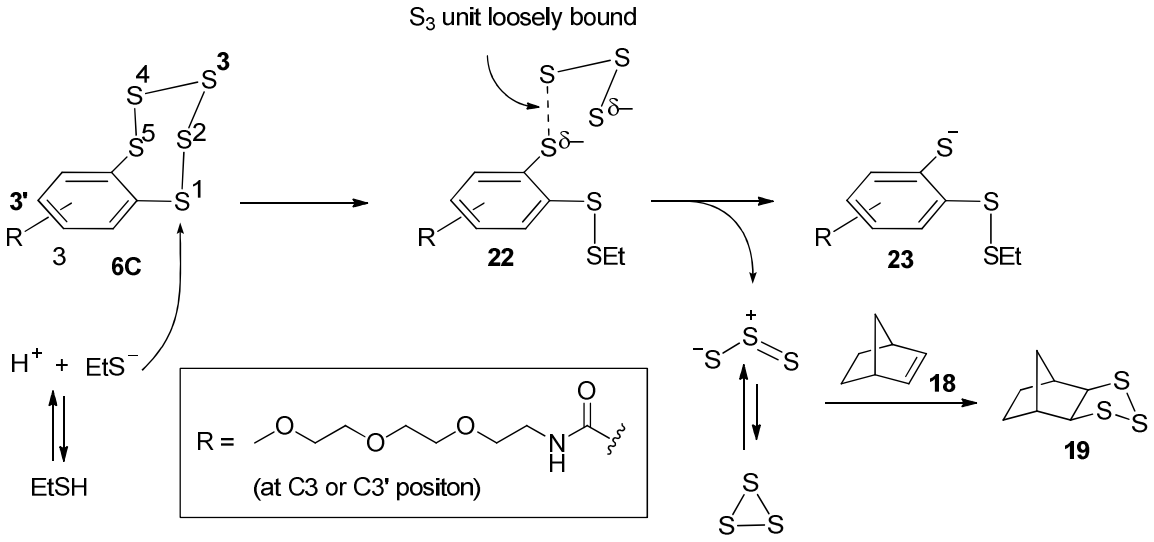
Attack of thiolate nucleophile to benzopentasulfane and subsequent loss of S_2 or S_3 units from benzopentasulfane was previously studied. A previous DFT study showed that attack of the HS^- nucleophile at the S1 position (Scheme 8A) of a pentasulfane was preferred (activation energy of 1.9 kcal/mol) compared to attack at the S2 possition (Scheme 8B) (activation energy of 4.5 kcal/mol).[24] The apical attack of nucleophile at S1 position lead to 3-center-4-electron bond which intern provide a model for S_N2 transition state. 3-center-4-electron bonding molecular orbitals is shown in Scheme 8C. Bachrach and co-workers also showed 2-5 kcal/mol preference for attack of thiolate ion on terminal sulfur of a trisulfide a linear acyclic compound. [36]



Scheme 8. Possible Pathways of Nucleophilic Acttak on Benzopentasulfane.

A possible mechanism for the desulfuration PEGylated pentathiepin **6C** is shown in Scheme 9, which begins with an apical attack of ethanethiolate ion to the sulfur atom (S1) adjoining the aryl ring. The resulting open-chain polysulfide anion (**22**) has the potential for thiozone (S_3) elimination driven by the delocalization of the negative charge

in the remaining carbon-sulfur fragment (**23**)[24,25] followed by thiozonation of norbornene. *Ab initio* calculations predict the open C_{2v} zwitterionic form[26] of thiozone S_3 to be energetically preferred to the cyclic D_{3h} form.[27,28]



Scheme 9. Potential Mechanism for the Triatomic Sulfur-Transfer of Benzopentasulfane 6C

2.2.6 Intrinsic Solubilities.

Elemental S_8 suffers from poor solubility.[19,29,30] We investigated the intrinsic solubilities of elemental S_8, **5A**-**C**, and **6A**-**F** (Table 5). Aliquots of methanol or water were added to 1-3 mg sample quantities and the solutions were vortexed (2 min) and stirred (10-15 min) at room temperature. As expected, the presence of the PEG group at the 7-position of **6A**-**F** significantly increased their solubilities. By comparison to **6A**-**F**, elemental sulfur S_8 and **5A**-**C** had ~50-fold lower solubility. Our aqueous solubility measurements are consistent with previous values for elemental sulfur S_8 reported in the

literature, namely, the solubility of S_8 in water was reported to be 0.4 μg/mL.[29] The solubility of S_8 in ethanol was reported to be 0.51 mg/mL.[30]

Table 5. Experimental Solubility Values

Reagent	Solvent	
	Methanol (mg/mL)	**Water (μg/mL)**
Elemental sulfur, S_8	0.33±0.03	0.14±0.02
o-Benzopolysulfanes **5A**-**C** (97%)	0.37±0.02	0.18±0.02
PEGylated benzopolysulfanes **6A**-**F** (93%)	19.00±0.05	8.70±0.25

[a] Equilibrium time of 24 h was used in the solubility study. [b] Measurements were conducted three times and the solubility value was averaged.

2.2.7 Antiproliferative Activities of Polysulfanes.

We examined the effects of 1,2-benzenedithiol, *o*-benzopolysulfanes **5A**-**C**, and PEGylated benzopolysulfanes **6A**-**F** on the proliferation of several cancer cell lines. Their bioactivities are shown in Table 6 and Figure 3. 1,2-Benzenedithiol was nontoxic; it stimulated the growth of PC3 cells, and had a minimal effect on the proliferation of MDA-MB-231 cells. However, at concentrations of 10 μM and above, it inhibited the proliferation of DU145 cells by ~40%. In a MTS assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium in the presence of phenazine methosulfate),[31] 1,2-benzenedithiol was also not effective in inhibiting Jurkat cell growth (IC_{50} of 60 μM). The requirement of the polysulfur ring was also observed by Molinski et al.[5] in the natural lissoclinotoxin A system when judged against the corresponding benzenedithiol compound.

Parent polysulfanes **5A**-**C** had a moderate antiproliferative effect on MDA-MB-231 cells, inhibiting the proliferation with an IC_{50} value of 28 μM. PC3 cell proliferation was inhibited with an IC_{50} of 11 μM with complete cell killing at 20 μM. DU145 and Jurkat cells were sensitive to parent polysulfanes **5A**-**C**; 6 μM killed 100% of the DU145 cells, and the IC_{50} was 0.5 μM for Jurkat cells. PEGylated benzopolysulfanes **6A**-**F** were the most potent of the three compound classes for the four cell lines examined, and they were toxic for all four cell lines. Complete cell killing was observed for **6A**-**F** with 4 μM for PC3 and DU145 cells, and with 12 μM for MDA-MB-231 cells. The PEG compounds **6A**-**F** were cytotoxic in Jurkat cells with an IC_{50} of 0.4 μM.

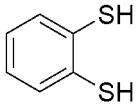
The data in Table 6 indicate that the polysulfur ring is significant in the enhancement of antiproliferative activity. The data also demonstrate that the PEG group leads to an increase in the antiproliferative effect. The PEGylated benzopolysulfanes **6A**-**F** have higher water solubility compared to the parent polysulfanes **5A**-**C**, and produced the highest growth inhibition. It was previously reported that lissoclinotoxin A **2** was cytotoxic against L1210 leukemia cells (IC_{50} of 3.1 μM) and *N,N*-dimethylvaracin **3** was cytotoxic against MDA-MB-231 cells (IC_{50} of 3.6 μM). Interestingly, varacin **1** showed greater cytotoxicity in HCT-116 colon cancer cells (IC_{90} of 0.15 μM).

2.2.8 Potential of Benzopolysulfanes as Drug Candidates.

Even though high nanomolar and low micromolar antiproliferative IC_{50} values for benzopolysulfanes were mentioned in the Introduction[1-7,10] and the above results for **6A**-**F** appear promising, *in vivo* stability and deliverability studies are needed to evaluate benzopolysulfane lability to glutathione, protein thiols, etc. Like some other drugs,

benzopolysulfanes are reductively activated. Our *in vitro* studies showed that ethanethiol-induced desulfuration of **6C** in aqueous methanol took ~2 min, but in benzene took 4 h, suggesting greater thiol ionization/nucleophilicity increases the polysulfane lability. Thus, hydrophobic or mild acidic conditions which maintain the less reactive thiol form will preserve **6C**, but in the presence of thiolate ion **6C** decomposes rapidly. In the absence of thiol and thiolate ion, the solvent effect is in the opposite direction, benzopolysulfane equilibria in organic solvents such as CH_2Cl_2 was more facile than in methanol or methanol/water, driven by solvation of elemental sulfur and the benzopolysulfanes (vide supra). Lastly, although a DNA cleaving study of 7-methylbenzopentathiepin suggested a metal- and oxygen-dependent Fenton pathway,[32,33] more work is needed to evaluate a potential anaerobic S_3-transfer pathway that may underlie the antitumor activity.

Table 6. Inhibition of Cancer Cell Gowth by 1,2-Benzenedithiol, *o*-Benzopolysulfan -es 5A-C, and PEGylated Benzopolysulfanes 6A-F

Compound	IC_{50} μM[a]			
	PC-3	**DU145**	**MDA-MB-231**	**Jurkat**
 1,2-Benzenedithiol	No effect	>30	>30	60
o-Benzopolysulfanes **5A**-**C** (97% purity)	10.5 (20)[b]	4.9 (6)[b]	27	0.5
PEGylated benzopolysulfanes **6A**-**F** (~98% total purity)	1.8 (4)[b]	3 (4)[b]	5.5 (12)[b]	0.4

[a] IC_{50} values were obtained for 24 h incubations with Jurkat cells and 48 h for PC3, DU145 and MDA-MB-231 cells. [b] Concentrations that resulted in 100% cell kill are shown in parentheses. Experiments were conducted in 96 well plates with 8 replicates for each concentration (0-30 μM) of the indicated compounds.

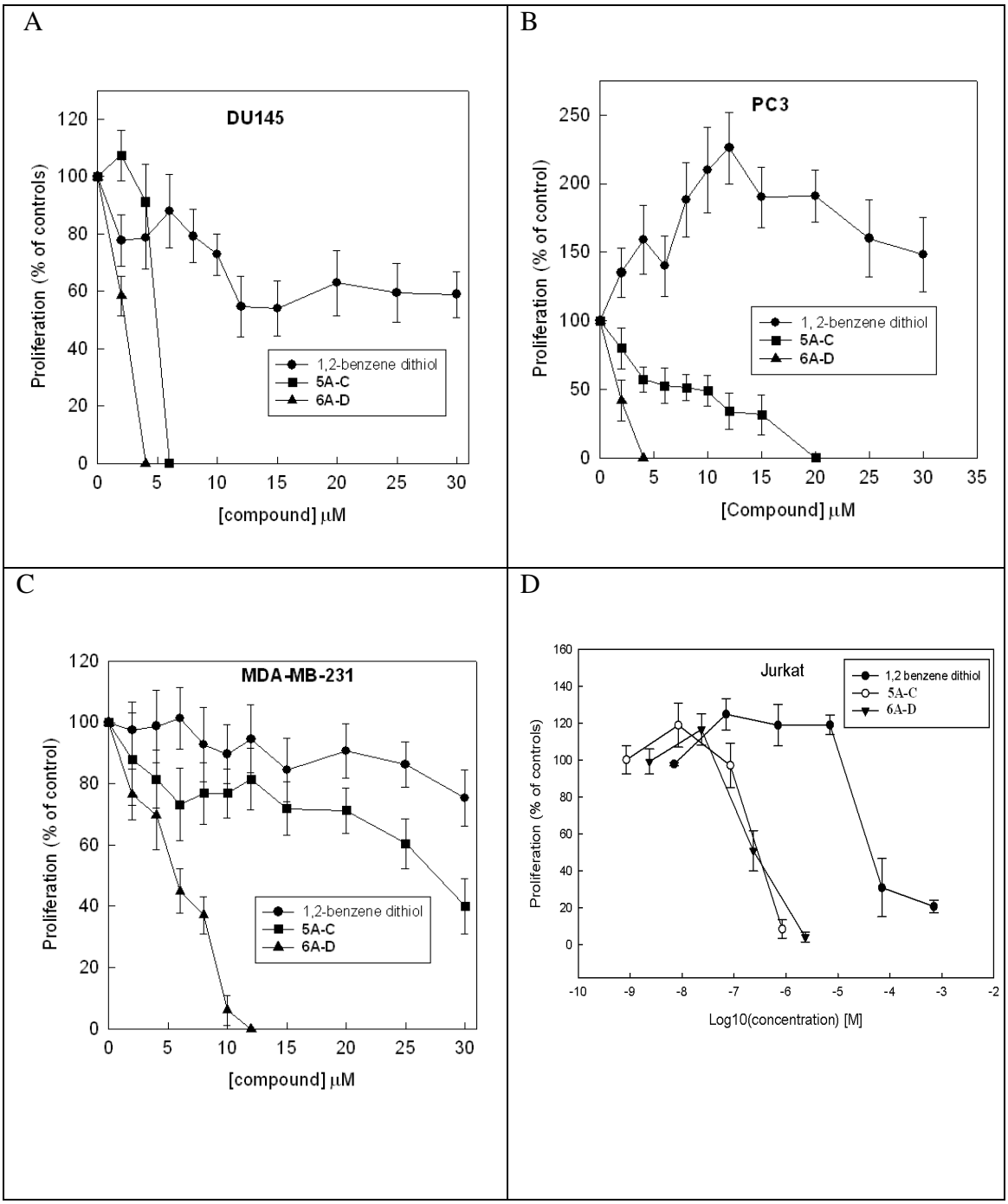


Figure 6. Effect of 1,2-benzenedithiol, *o*-benzopolysulfanes **5A**-**C**, and PEGylated benzopolysulfanes **6A**-**F** on the growth of human cancer cell lines. Prostate cancer cell lines DU145 and PC3 cells, breast cancer cell line MDA-MB-231 and Jurkat cells, a leukemia cell line, were grown in 96-well plates. When the cells were in exponential growth they were incubated with different concentrations of 1,2-benzenedithiol, **5A**-**C**, or **6A**-**F** for 48 h (DU145, PC3, MDA-MB-231) or 72 h (Jurkat). The increase in cell numbers relative to controls were determined by the CyQuant assay (A-C) and by the MTS assay (D) as described in the Experimental Section. The values were expressed as

the increase in cell numbers relative to controls without any compound. Data are the mean ± SE (n = 6-8).

2.2.9 Conclusion

The following conclusions can be made (1) the synthesis of benzopolysulfanes **6A**-**F** was accomplished with attached PEG groups of 160 Da molecular weight via an amide linkage in 1.5% overall yield. (2) Pentathiepin **6C** was the main product, but even after its purification, benzopolysulfanes **6A**, **6B**, **6D**-**F** formed in very low concentrations. (3) The pentasulfur linkage of **6C** was sensitive to the solvent composition in the HPLC experiments. Methanol rich elution conditions reduced the ratio of **6C** relative to the other polysulfanes. (4) Thiol-initiated reactions of **6A**-**F** led to an S_3-transfer to norbornene, but no S_2-transfer was observed to 2,3-dimethylbutadiene. (5) While the cause of the poor solubility of benzopolysulfanes is the polysulfur linkage itself, a key issue in the present study was the enhanced water solubility the PEG group provided, which did not reduce the prevalence of the pentasulfur ring species. (6) The results confirm the requirement of the polysulfur ring for low micromolar antiproliferative IC_{50} values, 1,2-benzenedithiol showed little or no antiproliferative activity. (7) The PEG polysulfanes **6A**-**F** were more water soluble and more active against four cancer cell lines than the parent polysulfanes **5A**-**C** suggesting that enhanced solubilization of benzopolysulfanes holds promise for advancing these compounds as drug candidates.

2.3 Experimental Section

2.3.1 General Aspects

3,4-Dihydroxybenzoic acid, *N,N*-dimethylthiocarbamoyl chloride, DABCO, sodium hydroxide, potassium hydroxide, ethanethiol, acetanilide, formic acid, ammonium formate, dimethyltin chloride, *p*-nitrophenol, DCC, S_2Cl_2, triethylene glycol monomethyl ether, phthalimide, triphenylphosphine, DIAD, hydrazine monohydrate, elemental sulfur (S_8), norbornene, 2,3-dimethylbutadiene, DMAP, sodium sulfate (anhydrous), magnesium sulfate (anhydrous), NaCl, DMF, Na_2CO_3, $NaHCO_3$, THF, $CHCl_3$, CH_2Cl_2, methanol, ethanol, ethyl acetate, hydrochloric acid (12 M), diphenyl ether, benzene, acetone-d_6, $CDCl_3$, CD_3CN, CD_3OD, and hexanes were used as received without further purification. Purification of product mixtures was carried out by column chromatography using silica gel with 40-60Å particle size. TLC was carried out using silica gel 60F 254 TLC-plates. Proton NMR data were acquired at 400 MHz and ^{13}C NMR data were acquired at 100.6 MHz. HRMS, GCMS, HPLC, and melting point data were collected.

2.3.2 HPLC Instrumentation and Analysis.

The HPLC instrument was equipped with an autosampler and diode array detector. The C18 column was 150 mm × 3.9 mm in size. The flow rate was 1 mL/min, and the injection volume was 50 µL. The mobile phase consisted of methanol and water. Compounds were detected by UV at 254 nm at room temperature.

2.3.3 LCMS Instrumentation and Analysis.

Liquid chromatography mass spectral data were collected on an Agilent Technologies G6220A high-resolution TOF mass spectrometer attached to an Agilent

Technologies 1200 series HPLC system equipped with an autosampler, diode array detector, and a binary pump. Samples were ionized by electrospray ionization in positive mode. Chromatography was performed on a Zorbax 2.1 × 30 mm SB-C18 3.5 μm column using water containing 0.1% formic acid and 5 mM ammonium formate (solvent A) and Methanol containing 0.1% formic acid and 5 mM ammonium formate (solvent B) at a flow rate 0.5 ml/min. The gradient program was as follows 15-85% B (0-13 min) 85% B (2 min) 85-15% B (1 min). The temperature of the column was held at 30 ºC for the entire analysis. Instrument parameters were as follows; Fragmentor = 125 V; drying gas temperature = 300 ºC; drying gas flow 12 L/min; nebulizer pressure = 40 psi; and capillary voltage = 2000 V. Data were collected with the instrument set to low mass range (100-1700 *m/z*) under high-resolution conditions at 4 GHz, and data were stored as both centroid and profile mode. The mass spectra were collected over a range of 100-1600 m/z at 1 spectra/s. The reference masses used were purine with $(M+H^+)^+$ ion at 121.05087 m/z and HP-922 with ion at 922.00980 m/z. They were infused into the spray chamber using Agilent's calibrant delivery system. The instrument was controlled with Agilent MassHunter Workstation Acquisition Software, and data were analyzed suing Agilent MassHunter Workstation Qualitative Analysis Software (Agilent Technologies, Santa Clara, CA).

2.3.4. GCMS Instrumentation and Analysis.

GCMS samples were ionized using the EI auto mode. The capillary column was a VF-5ms 30 m × 0.25 mm (ID DF=0.25). The solvent delay was set to 3 min. Temperature program was as follows; 80 ºC (0-5 min), 80-250 ºC (5-22 min, at a rate 10

ºC/min) and 250 ºC (22-28 min). Total run was 28 min. The instrument parameters were set as follows: Injector temperature 200 ºC, column flow rate 1 mL/min. Data were collected with the instrument set to mass range 40-650 *m/z*. The instrument was controlled with Varian MS Workstation Software Version 6.9.

2.3.5 Equilibration and Solubility Determinations.

To determine the extent of polysulfane equilibration, a 6.4 mg sample of dry **6A**-**F** was dissolved in 3 mL methanol or methanol/water mixtures by stirring for 2 min at room temperature. Then 0.2 mL was placed in a vial and diluted to 1 mL with methanol. The solution was stirred for 1 min, in which the concentration of each sample was 0.42 mg/mL. Finally, 50-µL of the solution was injected into the HPLC auto-sampler and quantitated by the absorption signal at 254 nm. The solubilities of elemental S_8, **5A**-**C**, or **6A**-**F** were determined by adding 10-50 µL aliquots of methanol or water to 1-3 mg of the compounds. The solutions were vortexed for 2 min, and then stirred 10-15 min at room temperature. Polysulfane decompositions were also conducted in the presence of sodium hydroxide. To a solution of polysulfanes **6A-F** (2.5 mM) and internal standard, acetanilide, (0.25 mM) was added NaOH (0.625, 1.25, 1.875 or 2.5 mM) in 0.1 mL methanol. After certain periods of time, the reaction was analyzed by HPLC by injecting 15 µL of the sample.

2.3.6 Sulfur Transfer and Trapping Studies.

Trapping studies were carried out in 0.2 mL benzene solution. To solution of PEGylated benzopenthasulfanes **6A-F** (2.3 mM) and norbornene or 2,3-dimethylbutadiene (2.3 mM)

was added ethanethiol (2.3 mM). The reaction mixture was vortexed for two minutes and analyzed in GCMS by injecting 1 μL of the sample. Both scan and single ion mode (SIM) analyses were conducted on the sample.

2.3.7. Cell Proliferation Assays.

The cell lines were grown from frozen stocks originally obtained from the American Type Culture Collection. The prostate cancer cell lines PC3 and DU145 cells were grown in F12K and DMEM, respectively. MDA-MB-231 breast cancer cells were grown in DMEM. All the media were supplemented with 10% FBS and penicillin /streptomycin. Cell proliferation was assessed by a cell proliferation assay as previously described.[34] Briefly, equal numbers of cells were distributed in 96-well plates and the cells were incubated at 37 °C in a 5% CO_2 incubator until they were in log phase growth. The medium was removed, and replaced with a growth medium containing the sulfur-containing compounds (0-30 μM). After 48 h incubation, the media was removed and the plates were frozen at -80 °C. The assay was performed by warming the plates to rt and followed by the addition of the reagent to the wells. The fluorescence was measured with a plate reader (excitation/emission, 485/530 nm).

The cytotoxicity assay was conducted as follows: Cells were cultured in RPMI-1640 medium with 10% fetal bovine serum and penicillin-streptomycin (100 U/mL and 100 μg/mL, respectively) and maintained in a 37 °C humidified 5% CO_2 incubator. On the day before the drug treatment, cells were plated onto each well of the 96-well plate at 2,000 cells/well (200 μL of the medium per well). After 24 h, cells were treated with different concentrations of the sulfur-containing compounds and incubated for 72 h.

After the incubation, cell growth was evaluated using a 96-titer solution cell proliferation assay. UV absorption (490 nm) of each well was quantified with a microplate reader.

2.3.8 Coumputational Details.

To shed light into the structural features of pentathiepin reactions with thiol (MeSH) density functional thoeretical (DFT) calculations were performed. Geometries were optimized by means of DFT calculations with the exchange-correlation of B3P86 along with the 6-311+G(2d) basis set.

2.3.9 Synthetic Procedures and Characterization of 6-14.

N-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[d][1,2,3]trithiole-5-carboxamide (6A). HPLC (150 mm × 3.9 mm C18 column, 90% acetonitrile in water, flow rate 1 mL/min): t_R = 20.3 min. LCMS: t_R = 4.6 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_3$ = 361.0476, found 361.0478.

N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[e][1,2,3,4]tetrathiine-6-carboxamide (6B). HPLC (150 mm × 3.9 mm C18 column, 90% acetonitrile in water, flow rate 1 mL/min): t_R = 22.6 min. LCMS: t_R = 5.6 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_4$ = 393.0197, found 393.0193.

N-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5]pentathiepine-7-carboxamide (6C). Yield 9.6 mg (53%). A solution of 3,6,9-trioxadecylamine **17** (13.8 mg, 0.0847 mmol) in 1.5 mL of THF was added to a solution of 4-nitrophenyl benzo[f][1,2,3,4,5]pentathiepine-7-carboxylate **14** (17 mg, 0.0423 mmol) in 2.5 mL of THF. The reaction mixture was stirred under argon atmosphere overnight. The solvent

was evaporated and the residue was dissolved in 30 mL of CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ solution (3 × 30 mL), 1 M HCl (3 × 30 mL), and water (3 × 30 mL). The organic solvent was evaporated and the crude product was chromatographed ($CHCl_3$/MeOH, 10:1) to yield 9.6 mg of **6C** (93% purity), R_f = 0.57, ^{1}H NMR ($CHCl_3$, 400 MHz): δ 8.30 (d, *J*=1.9 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.78 (dd, *J* = 7.90, 1.9 Hz, 1H), 7.18 (br s, 1H), 3.66 (m, 10H), 3.54 (m, 2H), 3.32 (s, 3H); ^{13}C NMR ($CHCl_3$, 100 MHz): δ 162.4, 155.2, 150.3, 145.8, 1145.0, 137.3, 136.3, 131.5, 130.2, 125.4, 122.5; HPLC (150 mm × 3.9 mm C18 column, 90% acetonitrile in water, flow rate 1 mL/min): t_R = 28.7 min. LCMS: t_R = 6.5 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_5$ = 424.9918, found 424.9926.

N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[g][1,2,3,4,5,6]hexathiocine-8-carboxamide (6D). HPLC (150 mm × 3.9 mm C18 column, acetonitrile/water 1:9 to 9:1 over 53 min, flow rate at 1 mL/min): t_R = 30.0 min. LCMS: t_R = 7.1 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_6$ = 456.9638, found 456.9636.

N-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[h][1,2,3,4,5,6,7]heptathionine-9-carboxamide (6E). HPLC (150 mm × 3.9 mm C18 column, acetonitrile/water 1:9 to 9:1 over 53 min, flow rate at 1 mL/min): t_R = 36.0 min. LCMS: t_R = 7.8 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_7$ = 488.9359, found 488.9354.

N-(2-(2-(2-ethoxyethoxy)ethoxy)ethyl)benzo[j][1,2,3,4,5,6,7,8,9]nonathiacycloundecine-11-carboxamide (6F). HPLC (150 mm × 3.9 mm C18 column, acetonitrile/water 1:9 to 9:1 over 53 min, flow rate 1 mL/min): t_R = 40.8 min. LCMS: t_R = 9.2 min; HRMS (+ESI) calcd for $C_{14}H_{19}NO_4S_9$ = 552.8800, found 552.8788. The

identification of peak **6F** at 9.2 min in the spectrum is complicated because the former is a shoulder in the chromatogram of **3A**. Peak **6F** has been assigned based on the extracted ion chromatogram feature of the software.

Methyl 3,4-dihydroxybenzoate (8). Yield 1.018 g (76%). 3,4-Dihydroxybenzoic acid **7** (1.0 g, 6.49 mmol) was dissolved in 40 mL MeOH. A catalytic amount of concentrated HCl (5 drops) was added to the methanol solution, which was then refluxed overnight at 80 °C. Water (300 mL) was added, and the mixture extracted with EtOAc/hexane (60:40) and dried over anhydrous $MgSO_4$. The solvent was removed affording a light brown solid (mp 137-139 ºC). ^{1}H NMR (acetone-d_6) δ 3.81 (s, 3H), 6.90 (d, J = 8.3 Hz, 1H), 7.45 (dd, J = 8.3, J = 2.0 Hz, 1H), 7.50 (d, J =2.0 Hz, 1H), 8.47 (br s, 2H); ^{13}C NMR (acetone-d_6) δ 51.9, 115.8, 117.2, 123.0, 123.4, 145.6, 150.8, 167.1; HRMS (+ESI) calcd for $C_8H_8O_4$ = 168.0423, found 168.0423.

Methyl 3,4-bis[(dimethylamino carbothioyl)oxy]benzoate (9). Yield 0.546 g (74 %). Methyl-3,4-dihydroxybenzoate **8** (738 mg, 4.39 mmol), DABCO (17.6 mmol), and *N,N*-dimethylthiocarbamoyl chloride (2.17 g, 17.6 mmol) were stirred in DMF (10 mL) for 30 min. The white solid that formed was dissolved in 50 mL of water. The product was extracted with EtOAc (3 × 30 mL), and dried over anhydrous $MgSO_4$. The solvent was removed affording a yellow-green oil. Benzoate **9** was obtained by flash chromatography (EtOAc/hexanes, 2:3); R_f = 0.4 or via recrystallization in 95% EtOH as white crystals (mp 110-112 °C). ^{1}H NMR ($CDCl_3$) δ 8.01 (dd, J = 8.4, J = 2.0 Hz, 1H), 7.85 (d, J = 2.0, 1H), 7.25 (d, J =8.4, 1H), 3.90 (s, 3H), 3.43 (s, 3H), 3.30 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 186.6, 186.1, 165.6, 149.4, 145.6, 128.7, 128.1, 125.9, 124.4, 52.3, 43.4, 43.3, 38.9, 38.8; HRMS (+ESI) calcd for $C_{14}H_{18}O_4S_2N_2$ calcd 342.0708, found 342.0708;

(Lit.:[14] ^{1}H NMR ($CDCl_3$) δ 8.00 (dd, *J* = 8.5, *J* = 2.0 Hz, 1H), 7.86 (d, *J* = 2.0 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 3.90 (S, 3H), 3.43, 3.42, 3.30, 3.29, 3.30 (4 × br s, 4 × 3H); ^{13}C NMR ($CDCl_3$) δ 186.4, 186.0, 165.6, 149.3, 145.5, 128.7, 128.2, 125.9, 124.4, 52.3, 43.4, 43.3, 38.9).

Methyl 2-oxo-1,3-benzodithiole-5-carboxylate (10). Yield 0.079 g (40%). Methyl 3,4-bis([dimethylamino-carbothioyl]oxy)benzoate **9** (300 mg, 0.876 mmol) was heated at 240 ºC in 10 mL of diphenyl ether for 30 min. The reaction mixture was purified by flash chromatography using a gradient of EtOAc/hexane (2:98 to 40:60) to afford 79 mg (40%) of **10** as a light brown solid. R_f = 0.75 (EtOAc/hexane 1:2), mp 140-142ºC. ^{1}H NMR ($CDCl_3$) δ 3.95,(s, 3H), 7.57 (d, *J* = 8.5, 1H), 7.98 (dd, *J* = 8.5, *J* = 2.0 Hz, 1H), 8.18 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 52.5, 122.9, 124.1, 128.2, 129.2, 133.0. 138.0. 165.7, 188.8); MS (EI): *m/z* = 226 (M), 198 (M-CO), 167 (M-CO_2Me) and 139 (M- CO_2Me - CO); Molecular peak ($C_9H_6O_3S_2$, M=226) isotopic ratio (calcd %, found %): [M+1] 227 (11.5, 11.4), [M+2] 228 (9.0, 9.8), and [M+3] 229 (1.2, 1.5); HRMS (+ESI) calcd for $C_9H_6O_3S_2$ = 225.9758, found 225.9757. (Lit.:[14] ($CDCl_3$) ^{1}H NMR δ 3.95 (s, 3H), 7.57 (d, *J* =8.5 Hz, 1H), 7.98 (dd, , *J* = 8.5, *J* = 2.0 Hz, 1H), 8.18 (d, *J* =2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 52.6, 122.9, 124.1, 127.8, 129.1, 133.0, 137.9, 165.7, 189.0).

3,4-Disulfanylbenzoic acid (11). Yield 0.52 mg (82%). An aqueous solution of NaOH (1 N, 3 mL) was added to **10** (79 mg, 0.35 mmol). The resulting mixture was heated at 70 ºC under nitrogen atmosphere for 4 h. The reaction mixture was cooled to room temperature and acidified with HCl (1 N). The white precipitate was collected by filtration, washed several times with H_2O and dried overnight to afford **11**. m.p. 218-220

ºC. ^{1}H NMR (CD_3CN): δ 9.50 (br s, 1H), 7.98 (d, *J* = 1.8 Hz, 1H), 7.66 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 1H), 4.34 (br, s, 2H). (CD_3OD): δ 8.07 (d, *J* = 1.8 Hz, 1H), 7.73 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), ^{13}C NMR (CD_3OD): δ 168.9, 140.4, 132.9, 131.5, 130.5, 129.5, 128.4 ppm. HRMS (-ESI): calcd for $C_7H_6O_2S_2$ = 185.9809, found 185.9811. (Lit.:[14] (CD_3OD): δ 8.03 (d, *J* = 2 Hz, 1H), 7.70 (dd, *J* = 8, 2 Hz, 1H), 7.47 (d, *J* = 8 Hz, 1H), 4.05 (s, 1H), 3.67 (s, 1H). ^{13}C NMR (CD_3OD) δ 167.9, 139.4, 131.9, 130.4, 129.5, 128.5, 127.4 ppm.

2,2-Dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylic acid (12). Compound **12** was synthesized using a modified literature procedure by Lee et al.[35] To an ethanolic solution (10 mL) of **11** (116 mg, 0.623 mmol) was added an ethanolic solution of NaOH (5 mL, 0.2 M). After the mixture was stirred at rt for 15 min, an aqueous solution (5 mL) of dimethyltin chloride (273 mg, 1.24 mmol) was added. The solution was stirred for 1.5 h, acidified with 1N HCl and extracted with CH_2Cl_2 (30 mL × 3). The resultant organic layers were combined and washed with water and brine solution. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed affording 155 mg (75%) of **12.** ^{1}H NMR (CD_3CN) 7.95 (d, *J*=1.6 Hz, 1H), 7.45 (d, *J*=8.1 Hz, 1H), 7.40 (dd, *J*=8.1, 1.6 Hz, 1H), 0.96 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 166.5, 146.5, 139.9, 129.7, 129.0, 125.1, 124.1 4.76 ppm; HRMS (+ESI) calcd for $C_9H_{10}O_2S_2Sn$ = 325.9170, found 325.9171.

4-Nitrophenyl 2,2-dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylate (13). Yield 149 mg (72%). Compound **12** (155 mg, 0.465 mmol) was dissolved in dry CH_2Cl_2 (5 mL). To this solution was added a catalytic amount of DMAP, with stirring for 5 min. DCC (113 mg, 0.51 mmol) was added. After the solution was stirred for 10 min, *p*-nitrophenol (138 mg, 0.93 mmol) was added, and the solution was stirred overnight and

30 mL of CH_2Cl_2 was added. The white precipitate of urea was removed by filtration. The organic layer was washed with concentrated citric acid solution, saturated aqueous $NaHCO_3$ solution, water, and the organic layer was dried over anhydrous $MgSO_4$. The solvent was removed affording crude compound **13**. The crude mixture was purified by column chromatography using EtOAc/hexanes (2:3) to afford **10;** R_f = 0.54, mp 198-199 ºC; ^{1}H NMR ($CDCl_3$) δ 8.33 (d, *J* = 8.3 Hz, 2H), 8.30 (d, *J* = 1.6 Hz, 1H), 7.67 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 7.39 (d, *J* = 8.3 Hz, 2H), 1.09 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 163.6, 155.9, 147.4, 145.4, 139.7, 131.2, 130.0, 125.6, 125.2, 124.4, 122.6, 3.2 ppm; HRMS (-ESI) calcd for $C_{15}H_{13}NO_4S_2Sn$ = 446.9334, found 446.9324.

4-Nitrophenyl benzo[f][1,2,3,4,5]pentathiepine-7-carboxylate (14). Yield 9.5 mg (27%). Compound **14** was synthesized using a modification of the procedure of Sato et al.[15] To a solution of **10** (40 mg, 0.088 mmol, in 7 mL of dry CH_2Cl_2) at 0 ºC was added dropwise a solution of S_2Cl_2 (23.8 mg 0.176 mmol in 3 mL of dry CH_2Cl_2) at 0 ºC. The reaction mixture was allowed to reach rt and stirred for 24 hr. After 15 mL of CH_2Cl_2 was added, the reaction mixture was washed with water (20 mL × 3). The organic layer was dried with anhydrous $MgSO_4$ and evaporated. The product was purified by column chromatography using EtOAc/hexanes (1:4) to afford **14**; R_f = 0.44 ($CHCl_3$/hexanes, 1:2) ^{1}H NMR ($CHCl_3$) δ 8.66 (d, *J* = 1.5 Hz, 1H), 8.36 (d, *J* = 8.1 Hz, 2H), 8.13 (dd, *J* = 8.1, 1.5 Hz, 1H), 8.02 (d, *J* = 8.1 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 162.4, 155.2, 150.3, 145.8, 1145.0, 137.3, 136.3, 131.5, 130.2, 125.4, 122.5.

N-(3,6,9-Trioxadecyl)phthalimide (16). Yield 1.15 g (78%). Phthalimide (0.882 g, 6.0 mmol) and PPh_3 (1.58 g, 6.0 mmol) were dried for 1 h under vacuum and

then was placed under argon for 1 h, dissolved in THF (25 mL), and treated with triethyleneglycol monomethylether (0.8 mL, 5.0 mmol). After 15 min, DIAD (1.18 mL, 6.0 mmol) was added dropwise at rt, resulting in slight warming of solution. After 12 h at rt, the reaction was quenched by addition of EtOH (15 mL). The solvent was evaporated in vacuo and the residue was dried under reduced pressure for 1 h. The residue was treated with PE/EtOAc (1:1, 10 mL) and stirred at 40 ºC for 1 h. The white residue was removed by filtration and washed with the same solvent mixture (10 mL). The filtrate was evaporated to dryness and the crude product was chromatographed on silica gel yielding the titled compound as colorless oil. R_f = 0.53 (EtOAc/hexanes, 2:1) ^{1}H NMR ($CDCl_3$) δ 7.83 (m, 2H), 7.70 (m, 2H), 3.89 (t, *J* = 6.0 Hz, 2H), 3.73(t, *J* = 6.0 Hz, 2H), 3.56-3.65 (m, 6H), 3.46 (m, 2H), 3.32 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 168.2, 133.9, 132.3, 123.2, 71.9, 70.6, 70.5, 70.2, 67.9, 59.0, 37.4; EI-MS m/z 293, 234, 218, 204, 190, 174, 59 HRMS (+ESI) calcd for $C_{15}H_{19}NO_5$ = 293.1263, found 293.1261. (Lit.:[16] ^{1}H NMR ($CDCl_3$) δ 7.77-7.81 (m, 2H), 7.65-7.69 (m, 2H), 3.83-3.84 (t, *J* = 5.5 Hz, 2H), 3.67-3.71 (t, *J* = 5.5 Hz, 2H), 3.51-3.62 (m, 6H), 3.28 (s, 1H); ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 168.1, 133.8, 132.0, 123.1, 71. 7, 70.39, 70.37, 69.9, 67.7, 58.9, 37.1 ppm. EI-MS (70 ev, 105 ºC): m/z (%) = 293, 234, 218, 204, 190, 174, 59.) Note: δ 3.28 (s, **1H**) should be **3H**.

3,6,9-Trioxadecylamine (17). Yield 0.390 mg, (85%). Compound **16** (1.15 g, 3.91 mmol) was dissolved in EtOH (20 mL) and treated with hydrazine monohydrate (231 µL, 4.46 mmol). The resulting mixture was refluxed at 100 ºC for 5 h whereupon a white precipitate formed. The slurry was allowed to cool and then was treated with conc. HCl (1.2 mL), followed by refluxing again for 1 h. The slurry was allowed to cool to rt

and the white solid was removed by filtration. The filtrate was evaporated in vacuo, the residue was dissolved in H_2O (30 mL), and the solution brought to pH 11 with 1 N NaOH. The aqueous phase was washed with saturated aqueous NaCl solution, and the product was extracted with CH_2Cl_2 (8 x 30 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated to yield the titled compound as colorless oil. ^{1}H NMR ($CDCl_3$): δ 3.65 (m, 6H), 3.60 (dd, *J* = 6.4, 5.4 Hz, 2H), 3.51 (t, *J* = 5.1 Hz, 2H), 3.39 (s, 3H); 2.87 (br s, 2H); ^{13}C NMR ($CDCl_3$): δ 73.5, 71.9, 70.6, 70.5, 70.3, 59.0, 41.8; HRMS (+ESI) calcd for $C_7H_{17}NO_3$ = 163.1208, found 163.1209. (Lit.:[16] ^{13}C NMR ($CDCl_3$): δ 41.6, 58.9, 70.1, 70.4, 70. 5, 71.8, 73.2.

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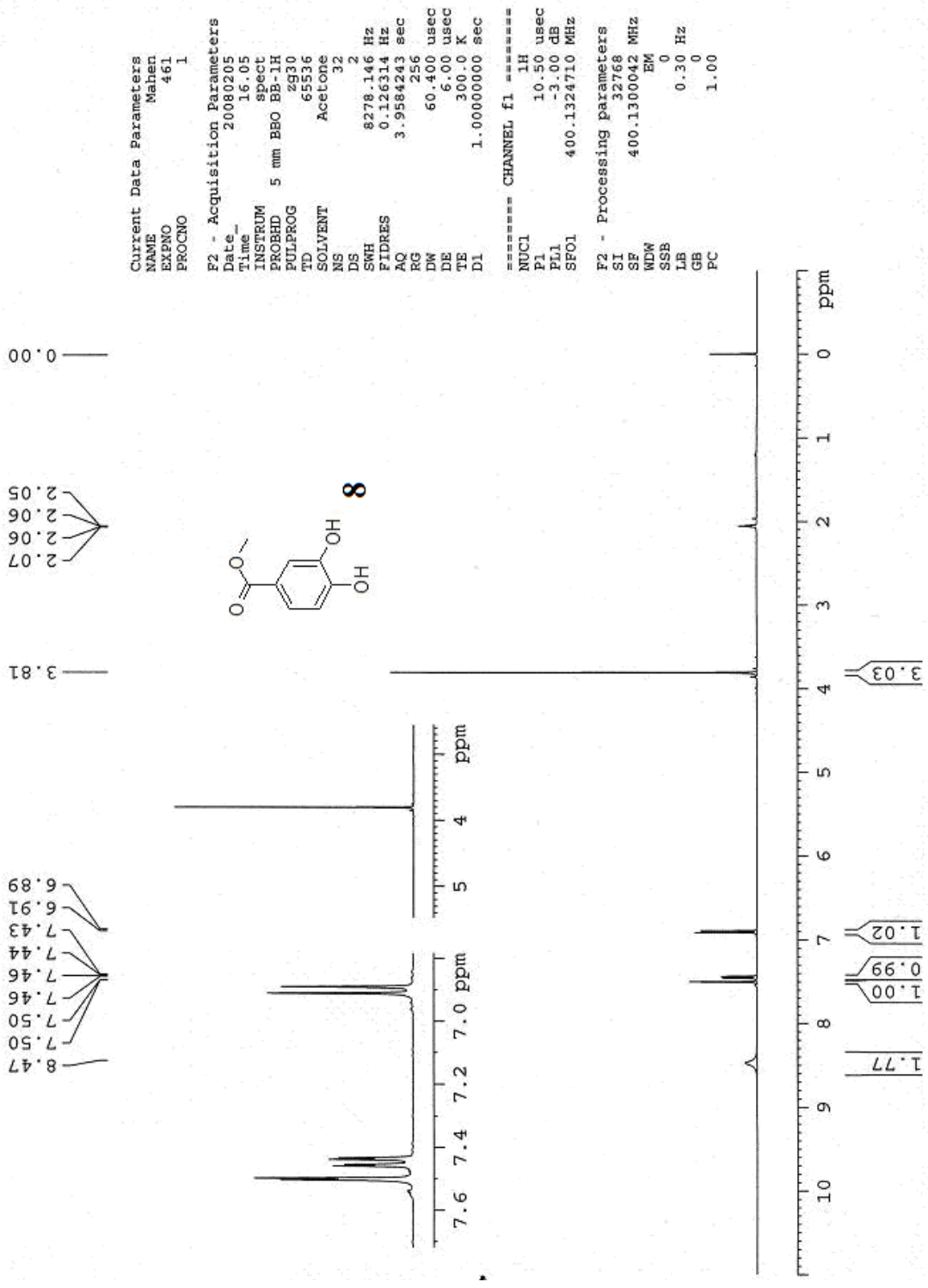


Figure 7. ^{1}H NMR of 3,4-dihydroxybenzoic acid methyl ester– acetone-d_6.

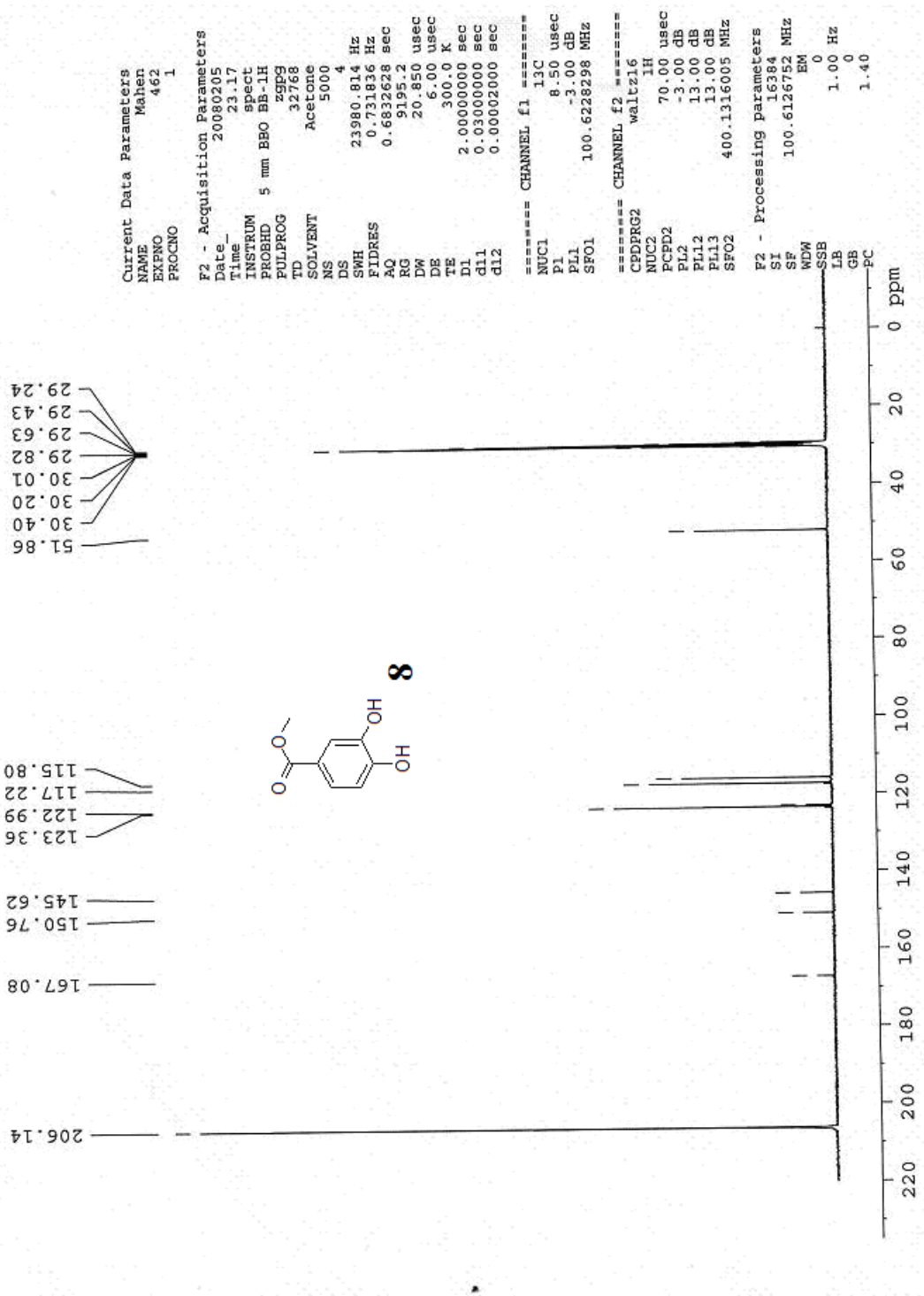


Figure 8. ^{13}C NMR of 3,4-dihydroxybenzoic acid methyl ester– acetone-d_6.

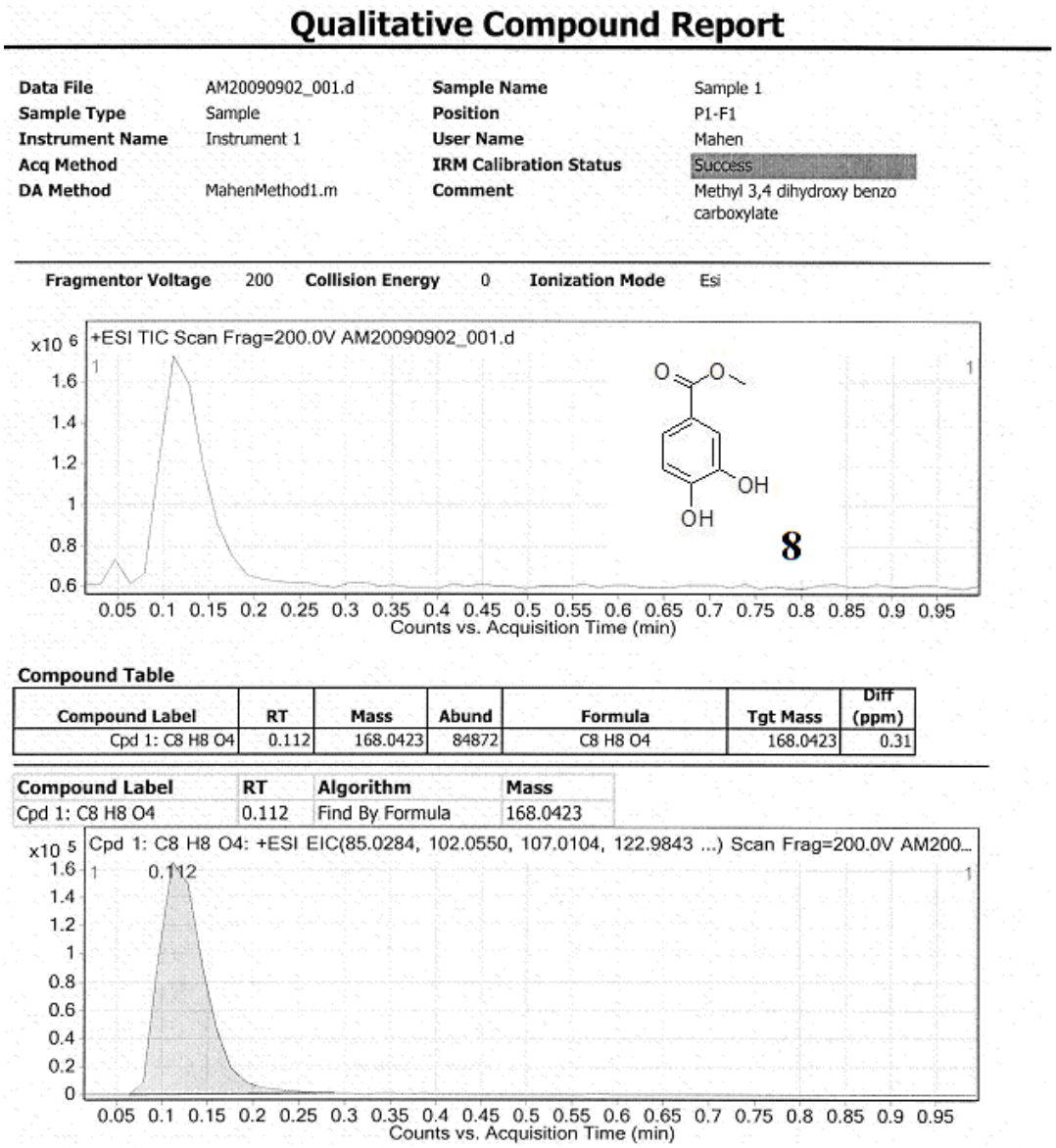
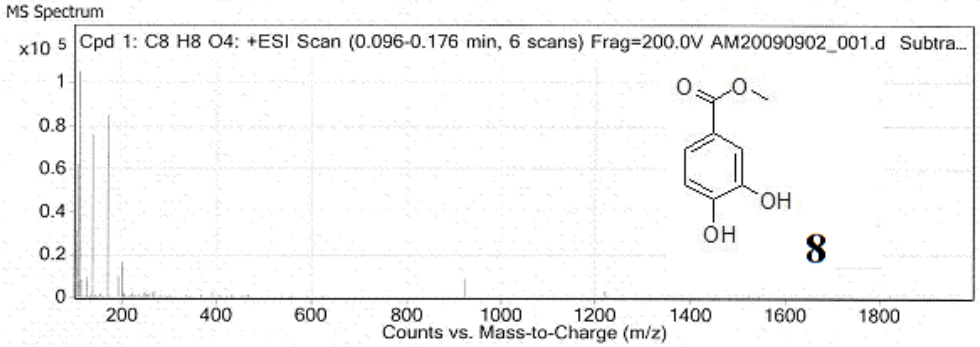


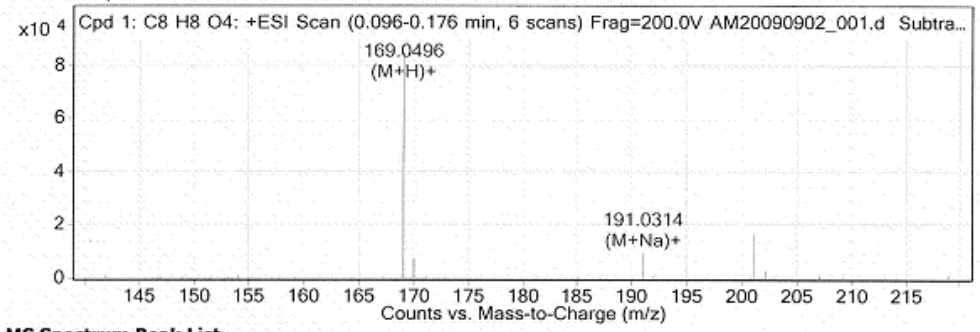
Figure 9. HRMS of 3,4-dihydroxybenzoic acid methyl ester.

Qualitative Compound Report

MS Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
105.0449				31352		
106.0478				2441		
107.0493				61806		
108.0513				5545		
109.0296				7255		
110.0363				105208		
111.0411				8409		
169.0496	169.0495	0.31	1	84872	C8 H9 O4	(M+H)+
170.053	170.0529	0.17	1	7472	C8 H9 O4	(M+H)+
191.0314	191.0315	-0.47	1	9551	C8 H8 Na O4	(M+Na)+

--- End Of Report ---

Figure 10. HRMS of 3,4-dihydroxybenzoic acid methyl ester.

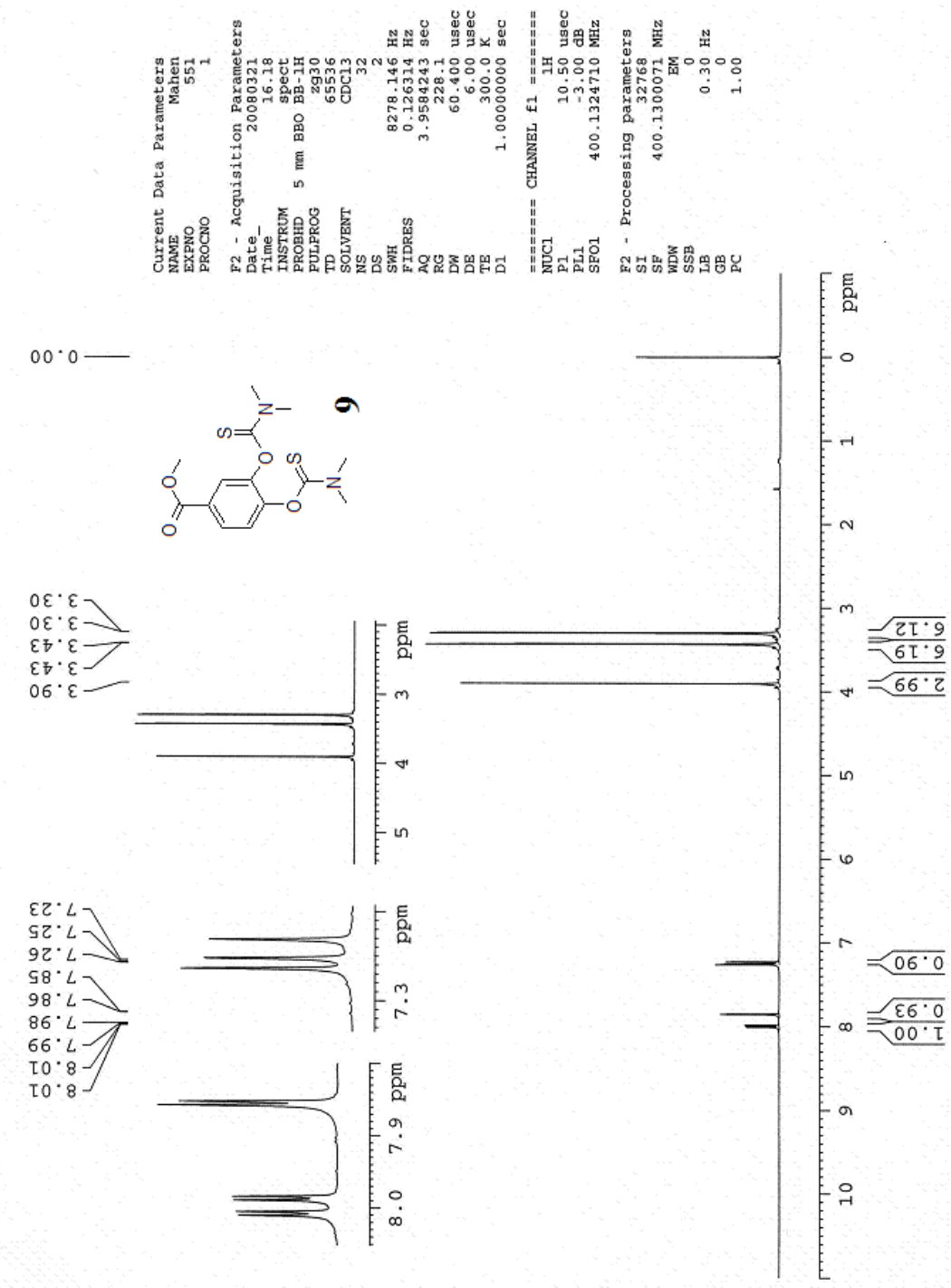


Figure 11. ^{1}H NMR of methyl-3,4-bis{[dimethylamino)carbothioyl]oxy}benzoate – $CDCl_3$.

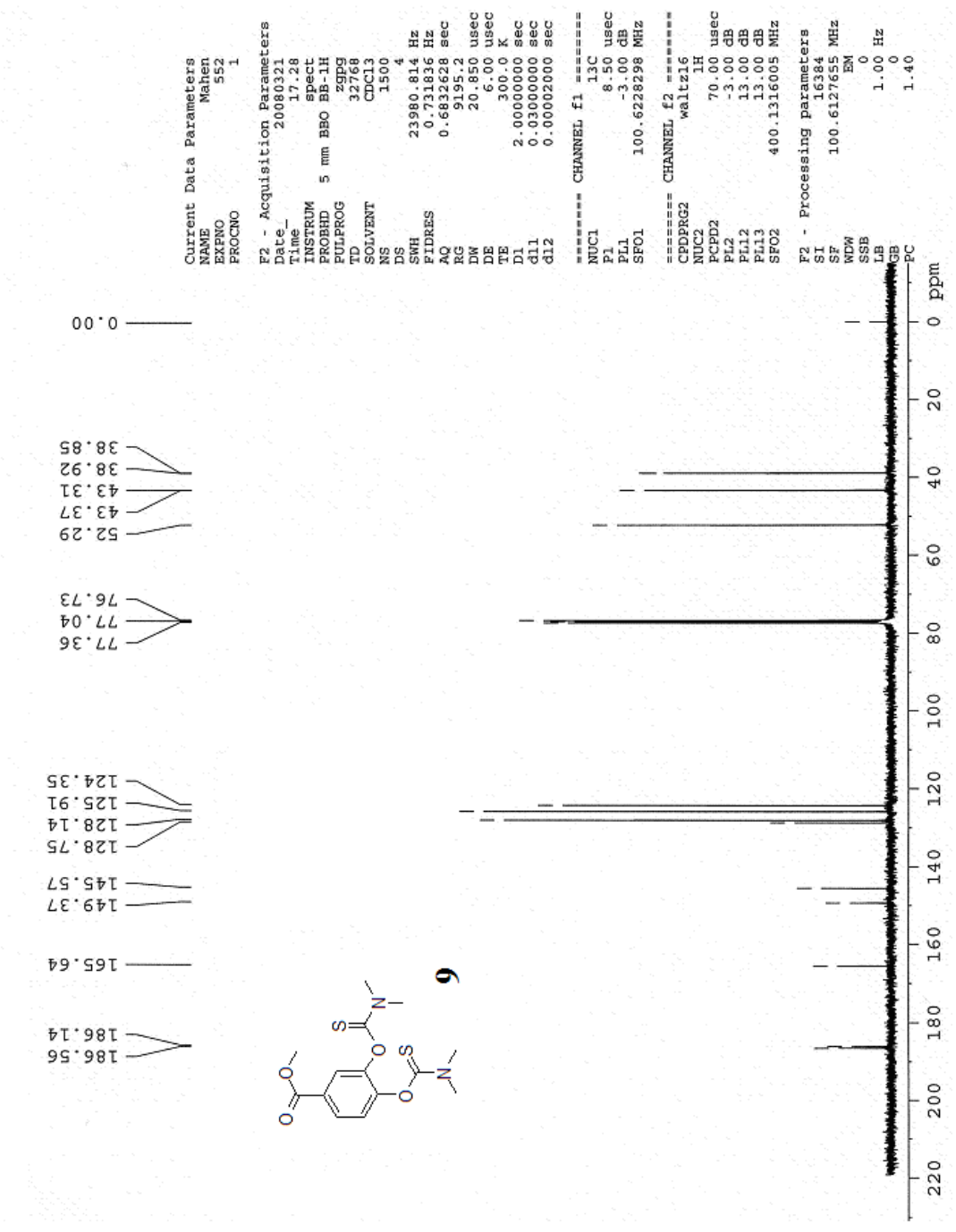


Figure 12. ^{13}C NMR of methyl-3,4-bis{[dimethylamino)carbothioyl]oxy}benzoate - $CDCl_3$.

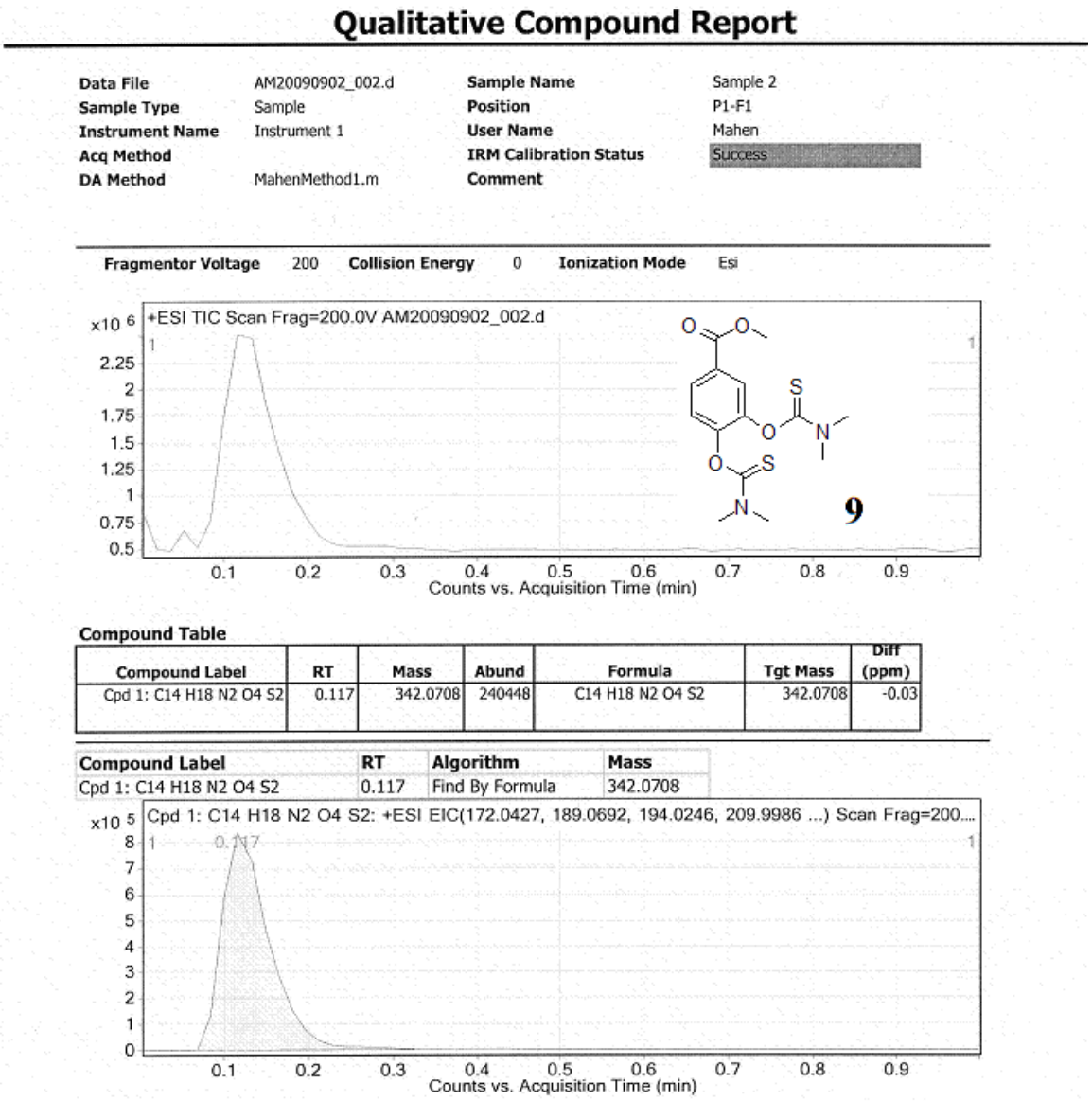
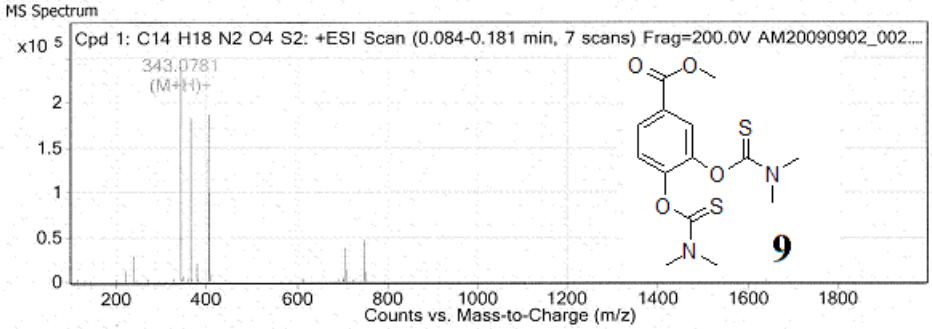


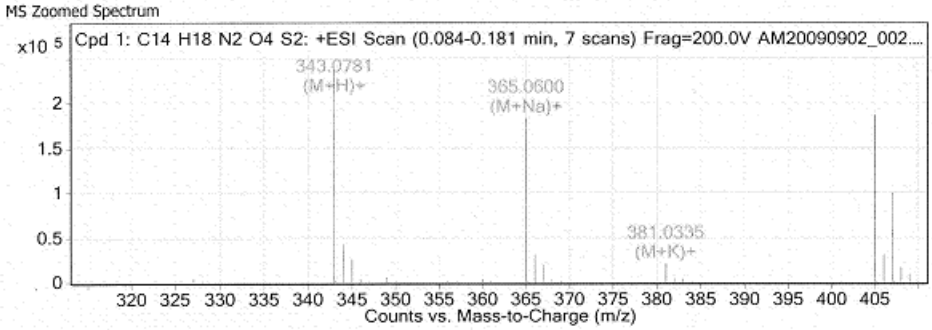
Figure 13. HRMS of methyl-3,4-bis{[dimethylamino)carbothioyl]oxy}benzoate.

Qualitative Compound Report

MS Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

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343.0781	343.0781	0.03		240448	C14 H19 N2 O4 S2	(M+H)+
344.0809	344.0809	0.17		42901	C14 H19 N2 O4 S2	(M+H)+
345.0758	345.0758	0.04		25743	C14 H19 N2 O4 S2	(M+H)+
346.0777	346.0779	-0.8		4196	C14 H19 N2 O4 S2	(M+H)+
360.1042	360.1046	-1.04	1	4505	C14 H22 N3 O4 S2	(M+NH4)+
365.06	365.06	-0.14	1	183559	C14 H18 N2 Na O4 S2	(M+Na)+
366.0624	366.0628	-1.03	1	31429	C14 H18 N2 Na O4 S2	(M+Na)+
367.0574	367.0577	-0.91	1	19764	C14 H18 N2 Na O4 S2	(M+Na)+
381.0335	381.034	-1.12	1	21248	C14 H18 K N2 O4 S2	(M+K)+
383.0314	383.0318	-1.19	1	4132	C14 H18 K N2 O4 S2	(M+K)+

--- End Of Report ---

Figure 14. HRMS of methyl-3,4-bis{[dimethylamino)carbothioyl]oxy}benzoate.

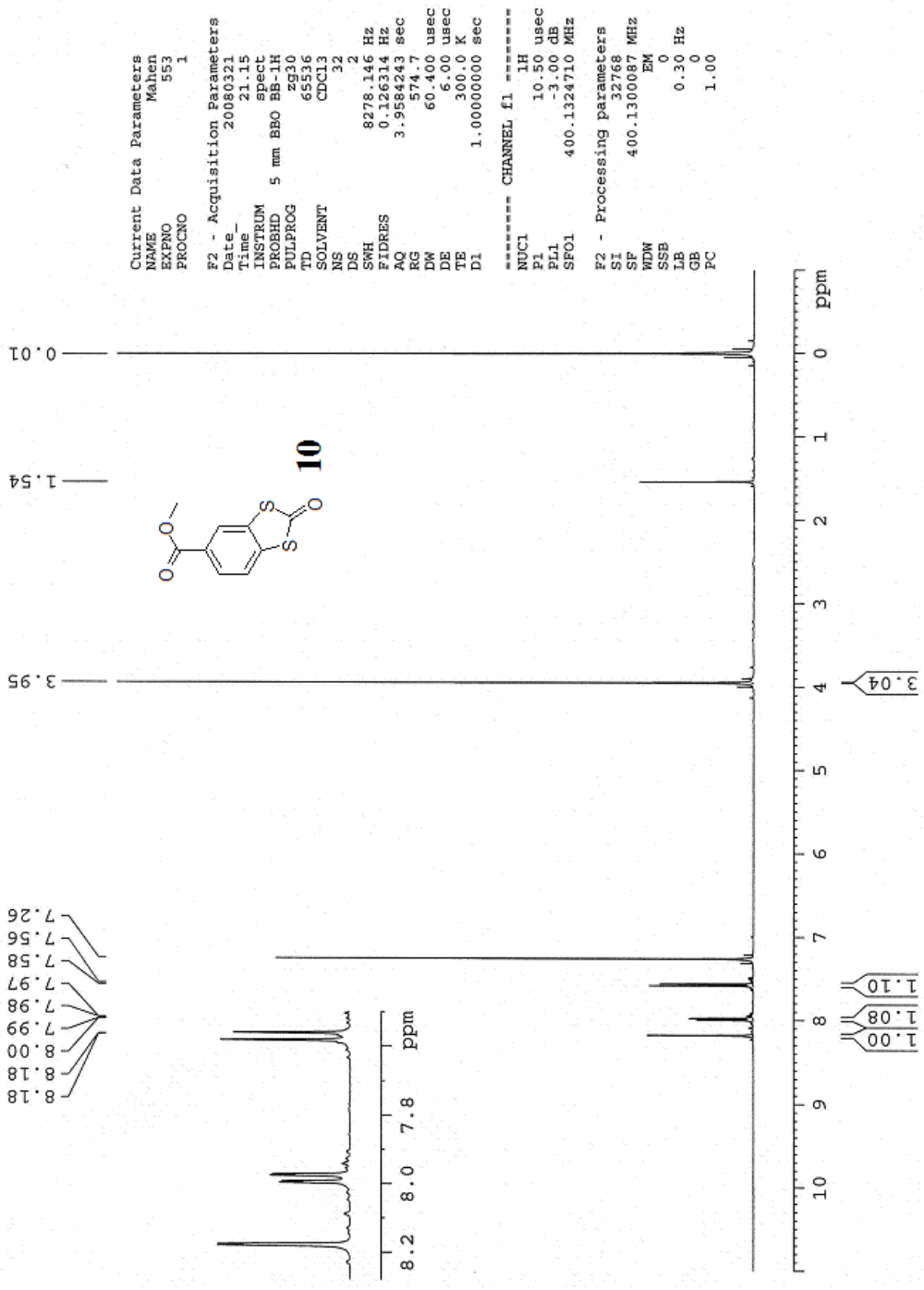


Figure 15. ^{1}H NMR of methyl-2-oxo-1,3-benzodithiole-5-carboxylate - $CDCl_3$.

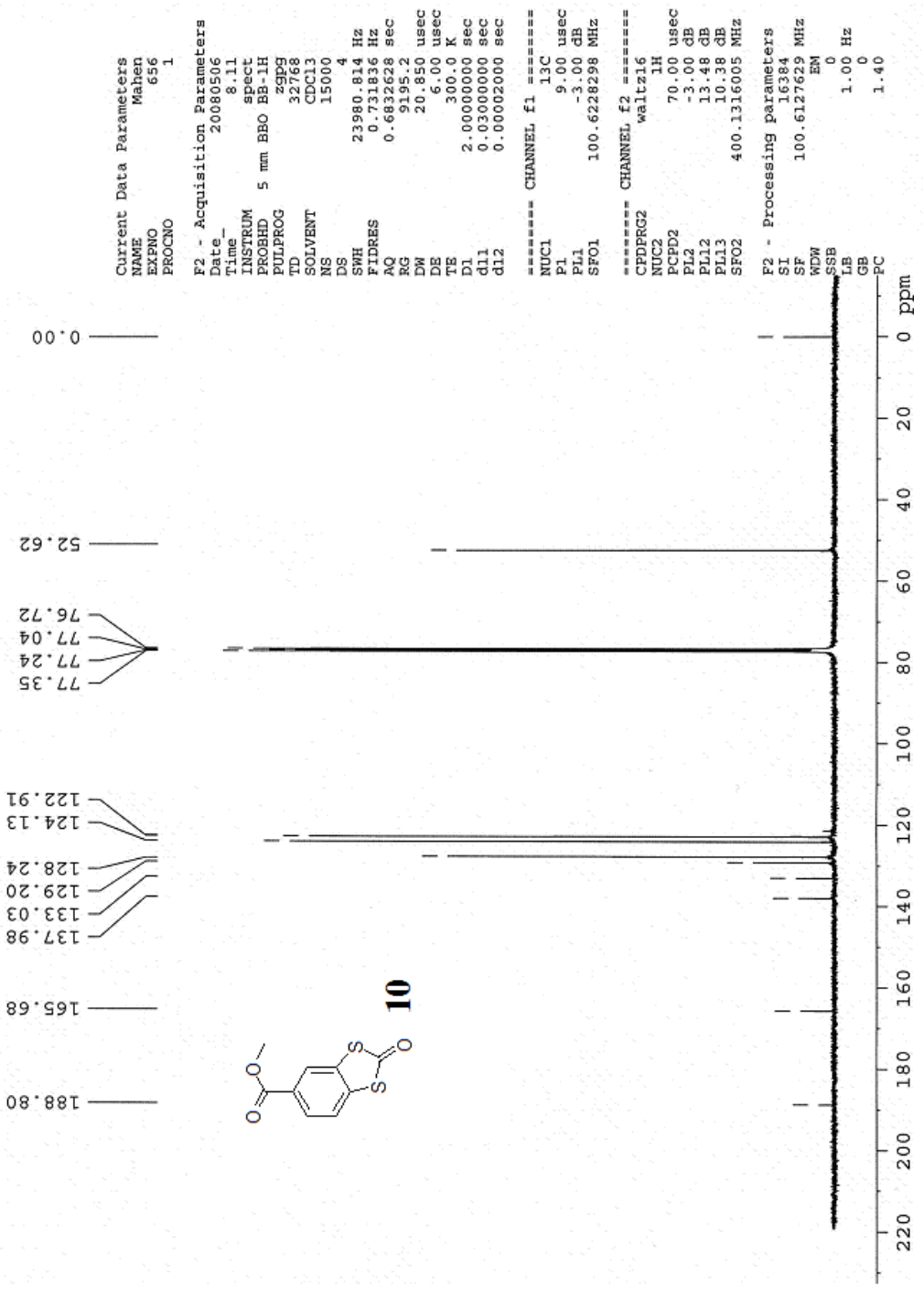


Figure 16. ^{13}C NMR of methyl-2-oxo-1,3-benzodithiole-5-carboxylate - $CDCl_3$.

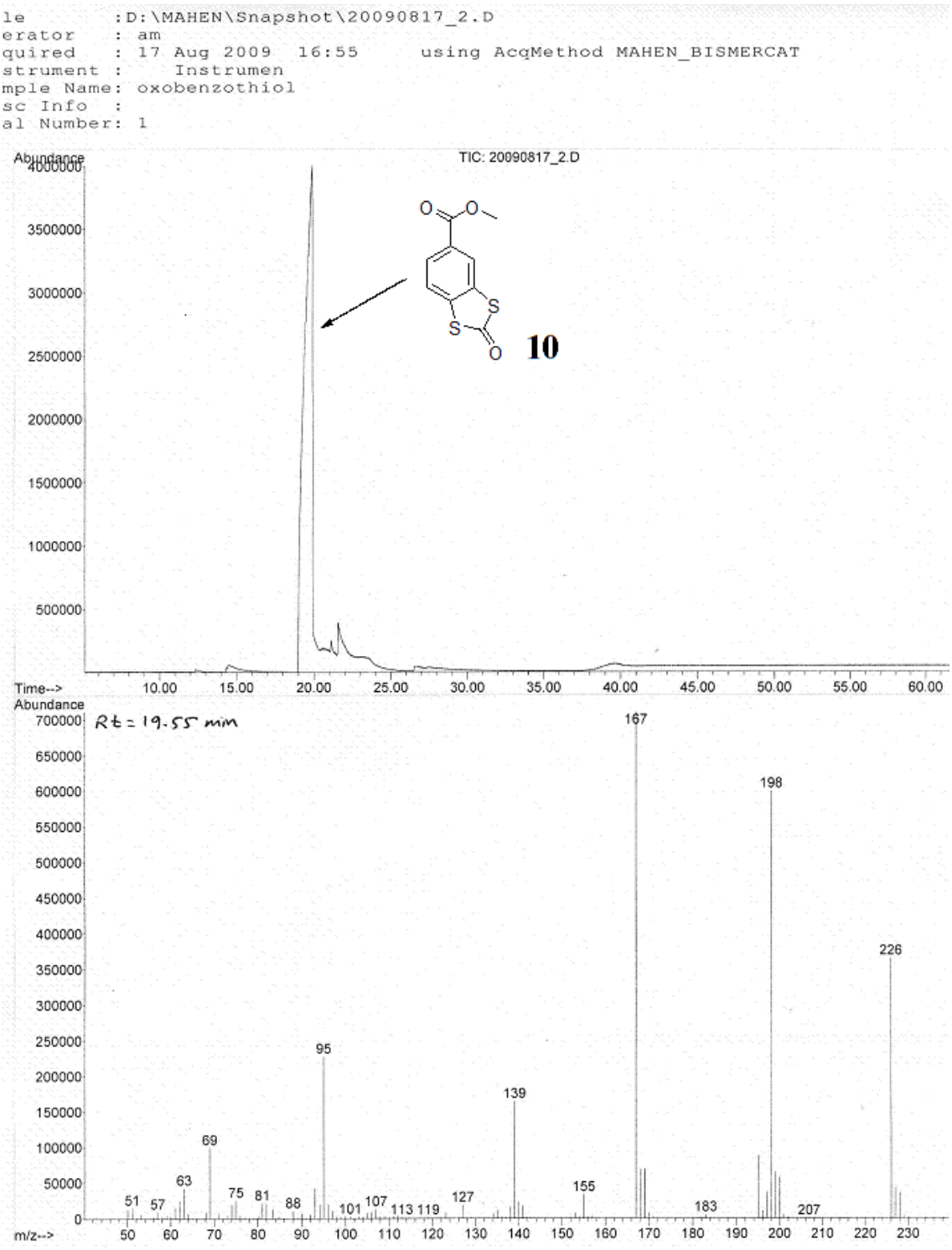
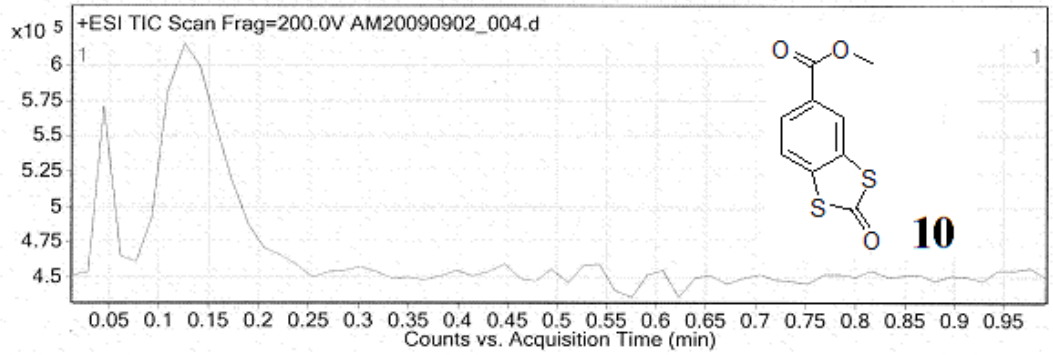


Figure 17. GC/MS of methyl-2-oxo-1,3-benzodithiole-5-carboxylate (Rt = 19.55 min).

Qualitative Compound Report

Data File	AM20090902_004.d	**Sample Name**	Sample 3
Sample Type	Sample	**Position**	P1-F1
Instrument Name	Instrument 1	**User Name**	Mahen
Acq Method		**IRM Calibration Status**	Success
DA Method	MahenMethod1.m	**Comment**	

Fragmentor Voltage 200 **Collision Energy** 0 **Ionization Mode** Esi



Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
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Compound Label	RT	Algorithm	Mass
Cpd 1: C9 H6 O3 S2	0.125	Find By Formula	225.9757

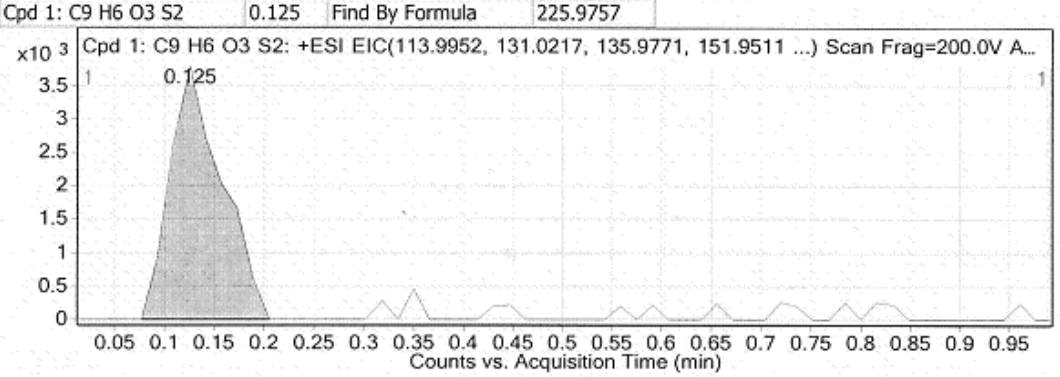
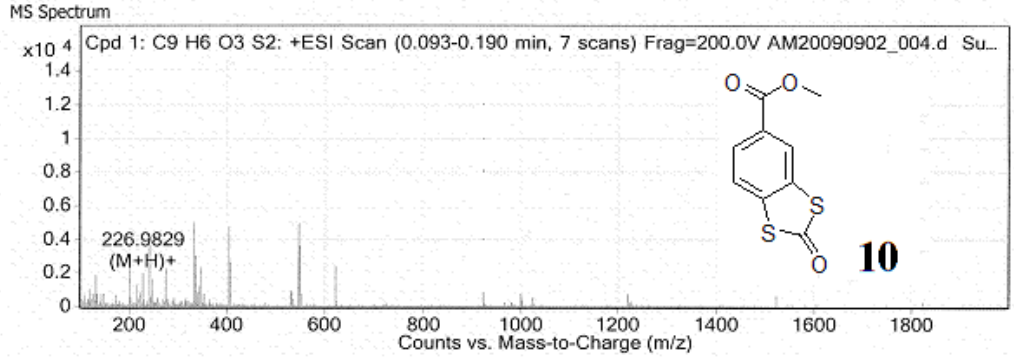


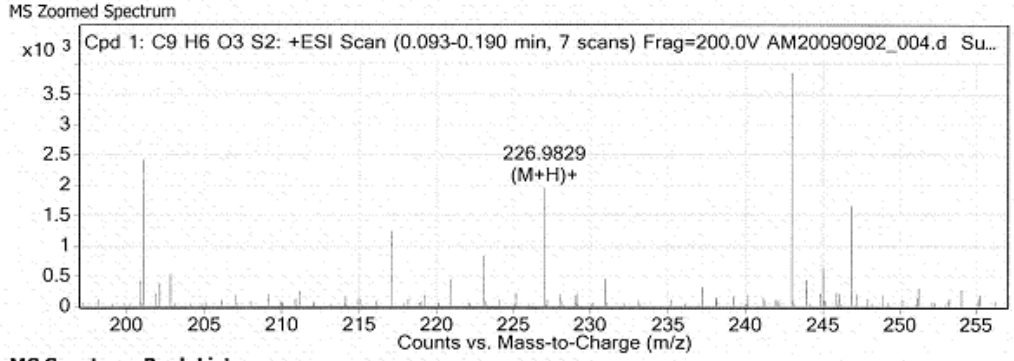
Figure 18. HRMS of methyl-2-oxo-1,3-benzodithiole-5-carboxylate.

Qualitative Compound Report

MS Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
226.9829	226.9831	-0.73	1	1949	C9 H7 O3 S2	(M+H)+
227.9902	227.986	18.84	1	201	C9 H7 O3 S2	(M+H)+
228.9859	228.98	25.56	1	167	C9 H7 O3 S2	(M+H)+
922.0097				857		
922.7779				105		
923.0126				097		
924.0144				160		
924.2575				174		
924.882				101		
925.8959				118		

--- End Of Report ---

Figure 19. HRMS of methyl-2-oxo-1,3-benzodithiole-5-carboxylate.

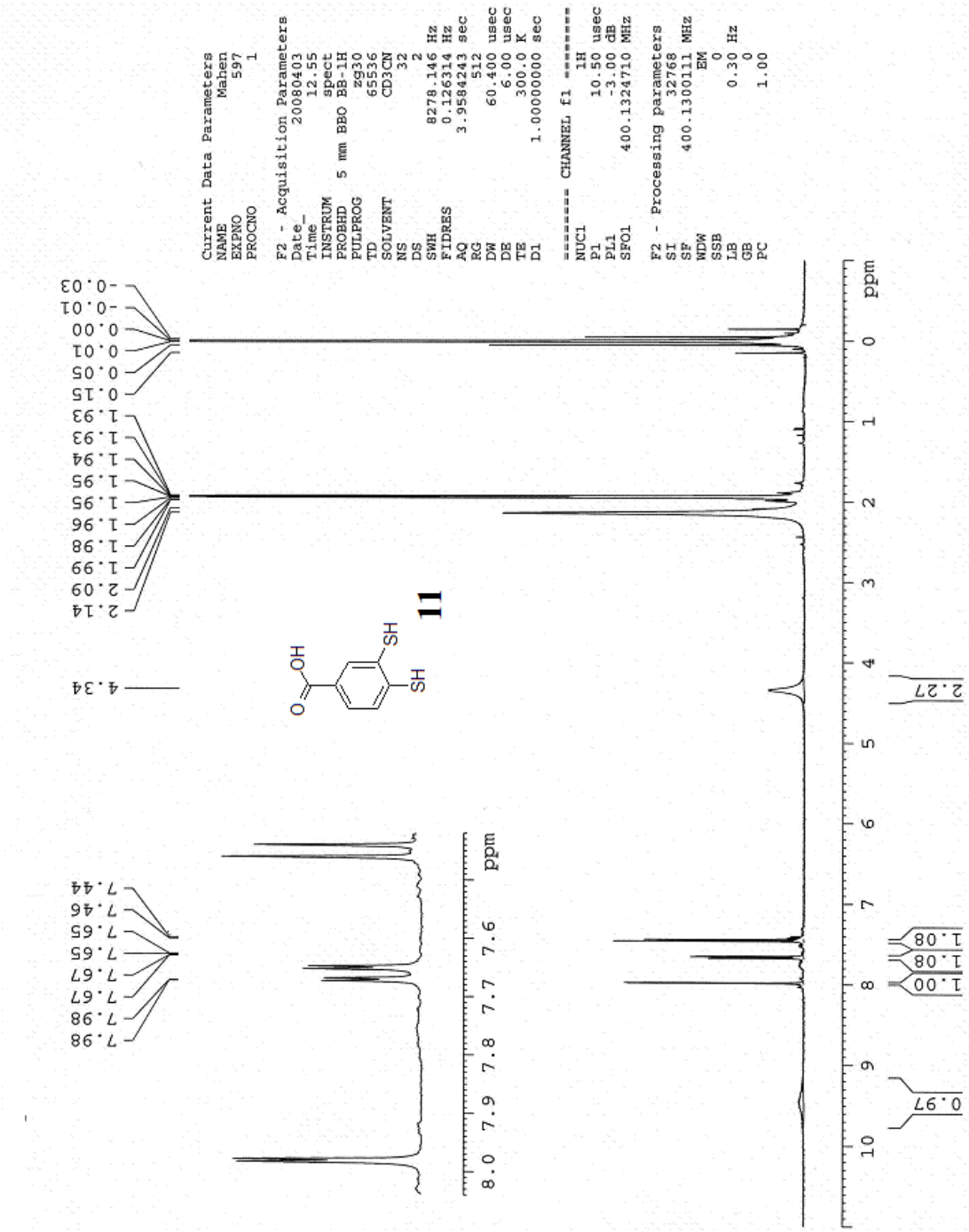


Figure 20. ^{1}H NMR of 3.4- disulfanylbenzoic acid - CD_3CN.

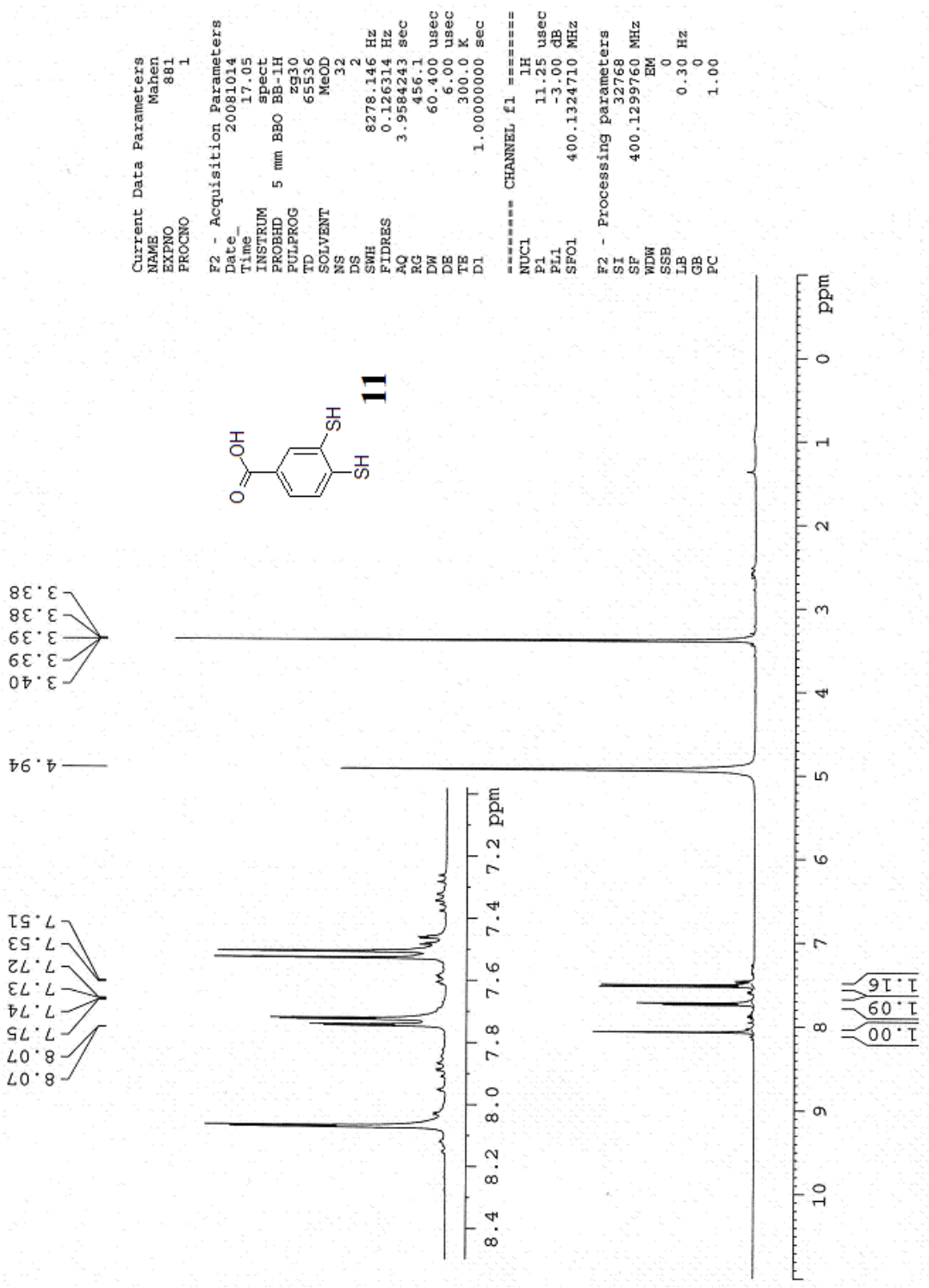


Figure 21. ^{1}H NMR of 3.4- disulfanylbenzoic acid - CD_3OD.

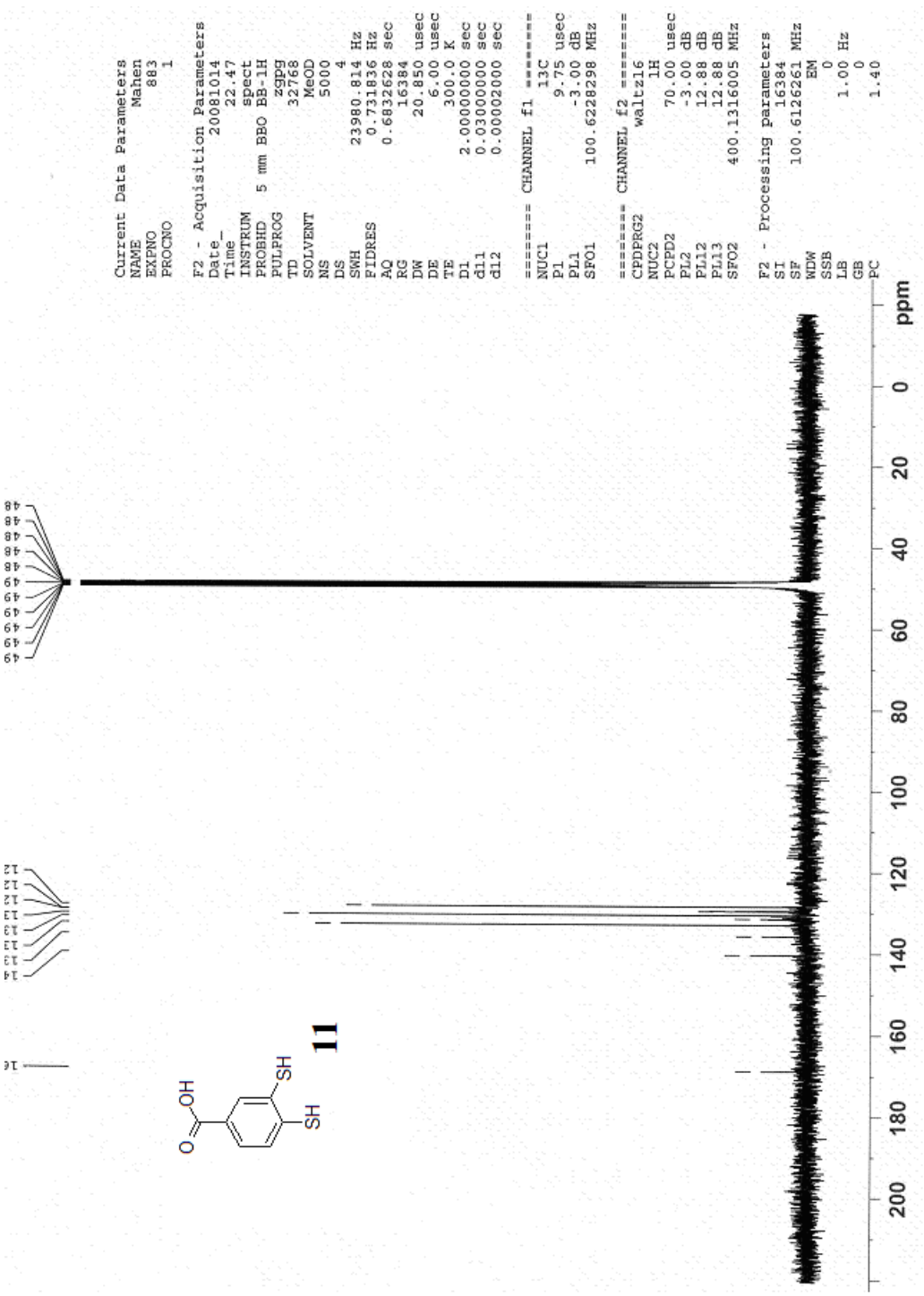
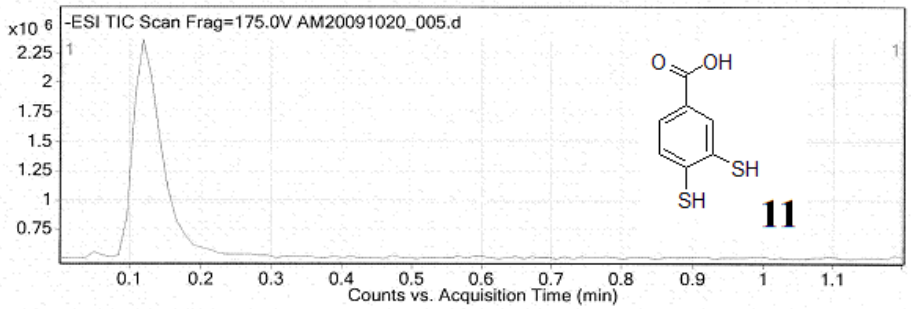


Figure 22. ^{13}C NMR of 3,4- disulfanylbenzoic acid - CD_3OD.

Qualitative Compound Report

Data File	AM20091020_005.d	**Sample Name**	Sample 1
Sample Type	Sample	**Position**	P1-F5
Instrument Name	Instrument 1	**User Name**	
Acq Method		**IRM Calibration Status**	Success
DA Method	MahenMethod1.m	**Comment**	EM=185.9809, CF=C7H6O2S2

Fragmentor Voltage 175 **Collision Energy** 0 **Ionization Mode** Esi



Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C7 H6 O2 S2	0.119	185.9811	35053	C7 H6 O2 S2	185.9809	0.98

Compound Label	RT	Algorithm	Mass
Cpd 1: C7 H6 O2 S2	0.119	Find By Formula	185.9811

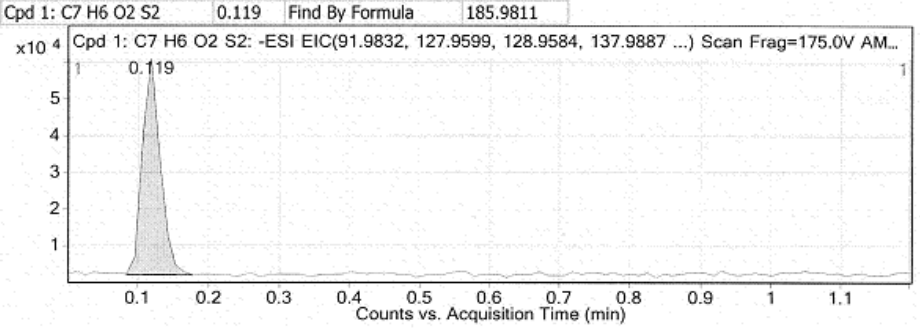
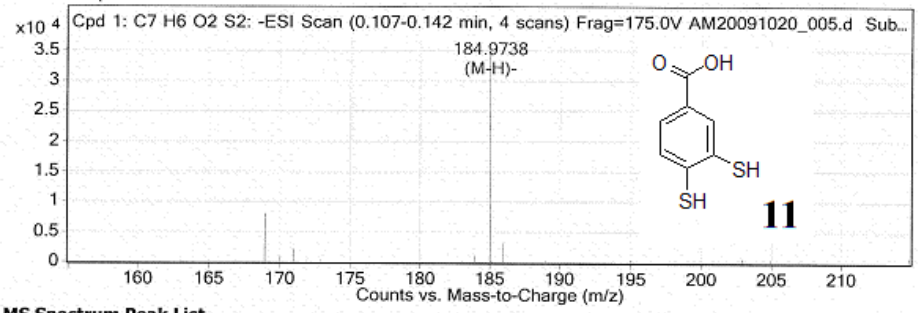


Figure 23. HRMS of 3,4- disulfanylbenzoic acid.

Qualitative Compound Report

MS Zoomed Spectrum



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
184.9738	184.9736	0.99	1	35053	C7 H5 O2 S2	(M-H)-
425.8635				15324		
430.861				505097		
431.0516				7314		
431.1046				4824		
431.8634				87070		
432.859				329559		
433.8614				55297		
434.8569				56000		
435.8594				10457		

--- End Of Report ---

Figure 24. HRMS of 3,4- disulfanylbenzoic acid.

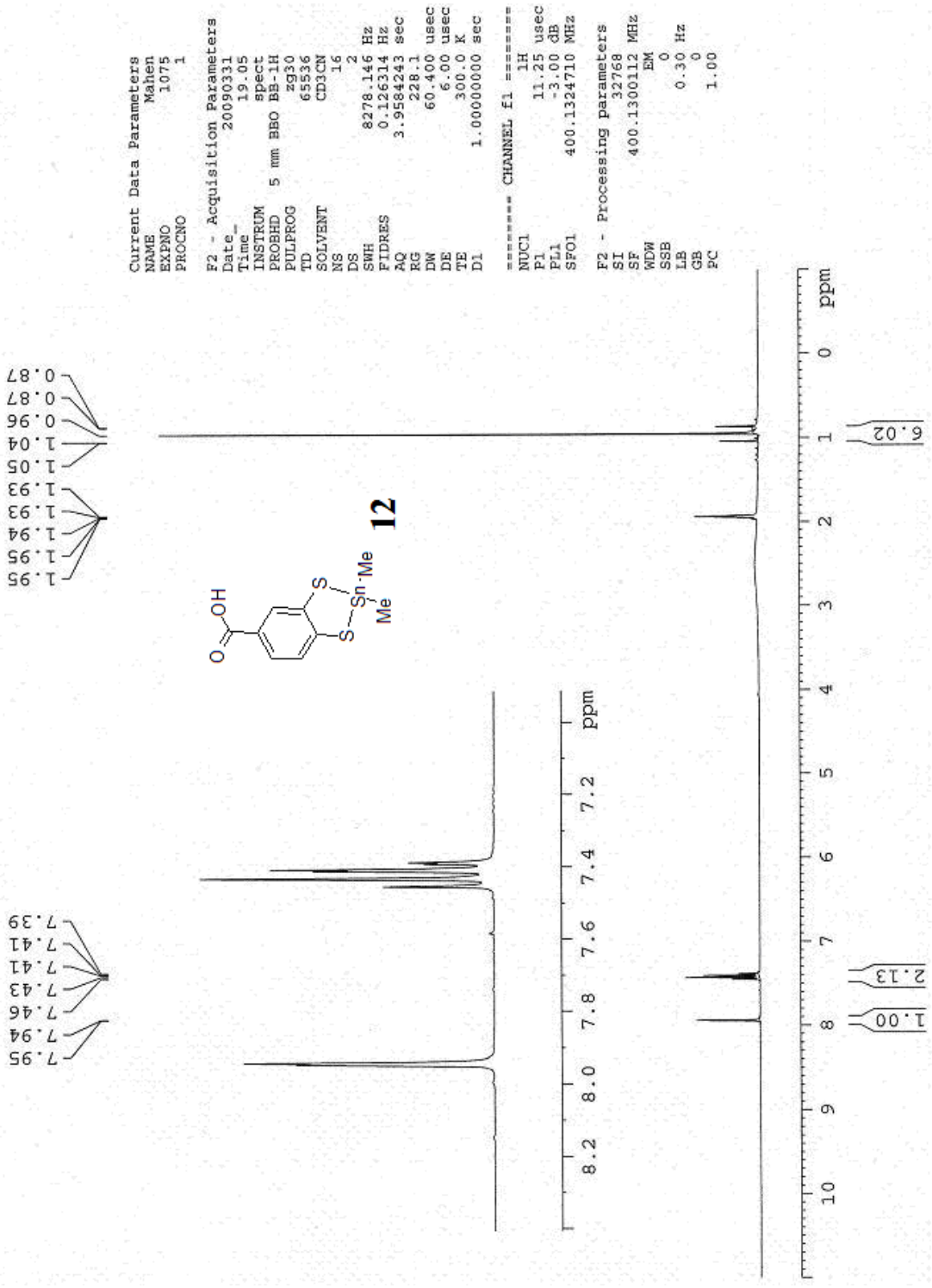


Figure 25. ^{1}H NMR of 2,2-dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylic acid - CD_3CN.

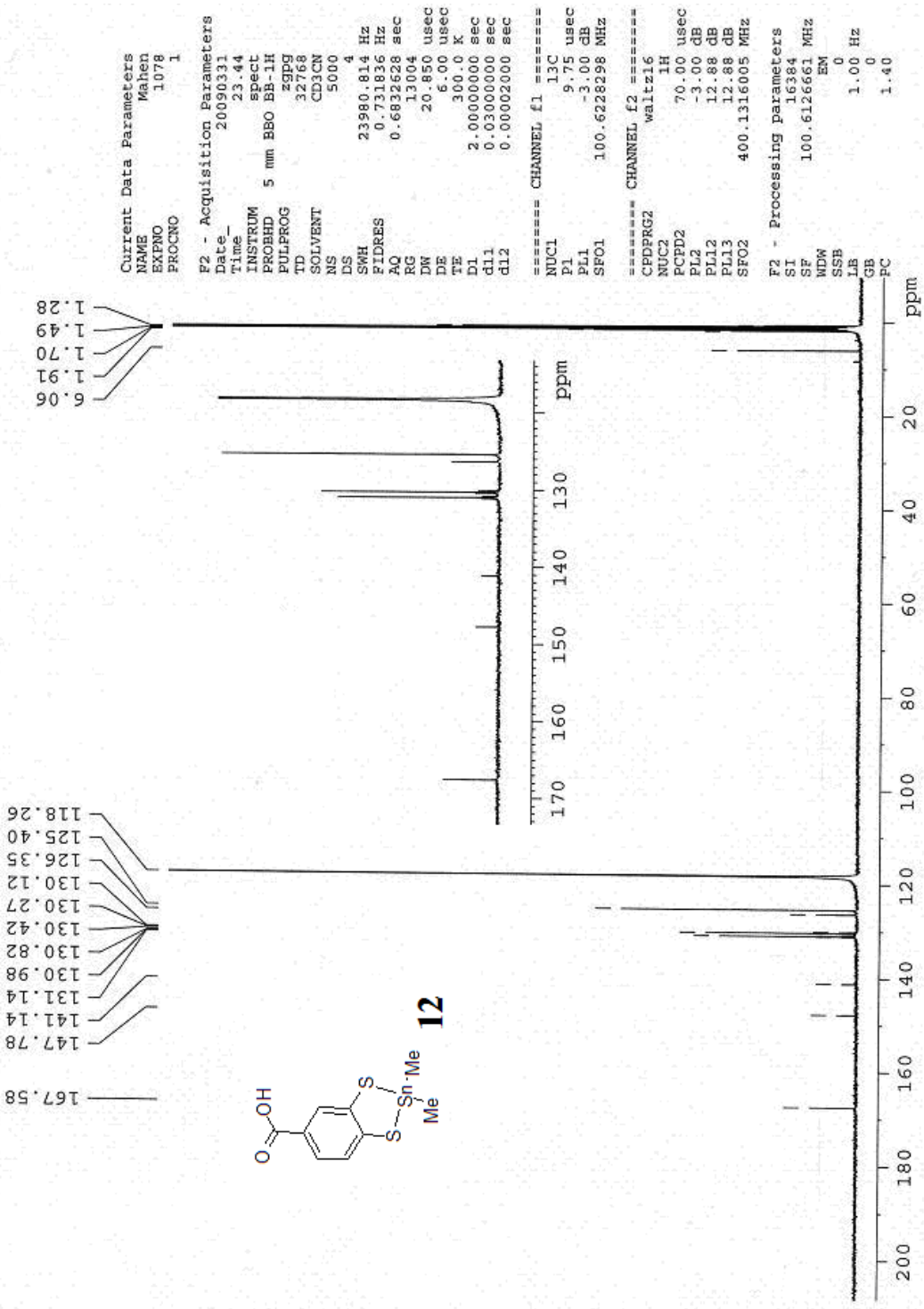


Figure 26. ^{13}C NMR of 2,2-dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylic acid - CD_3CN.

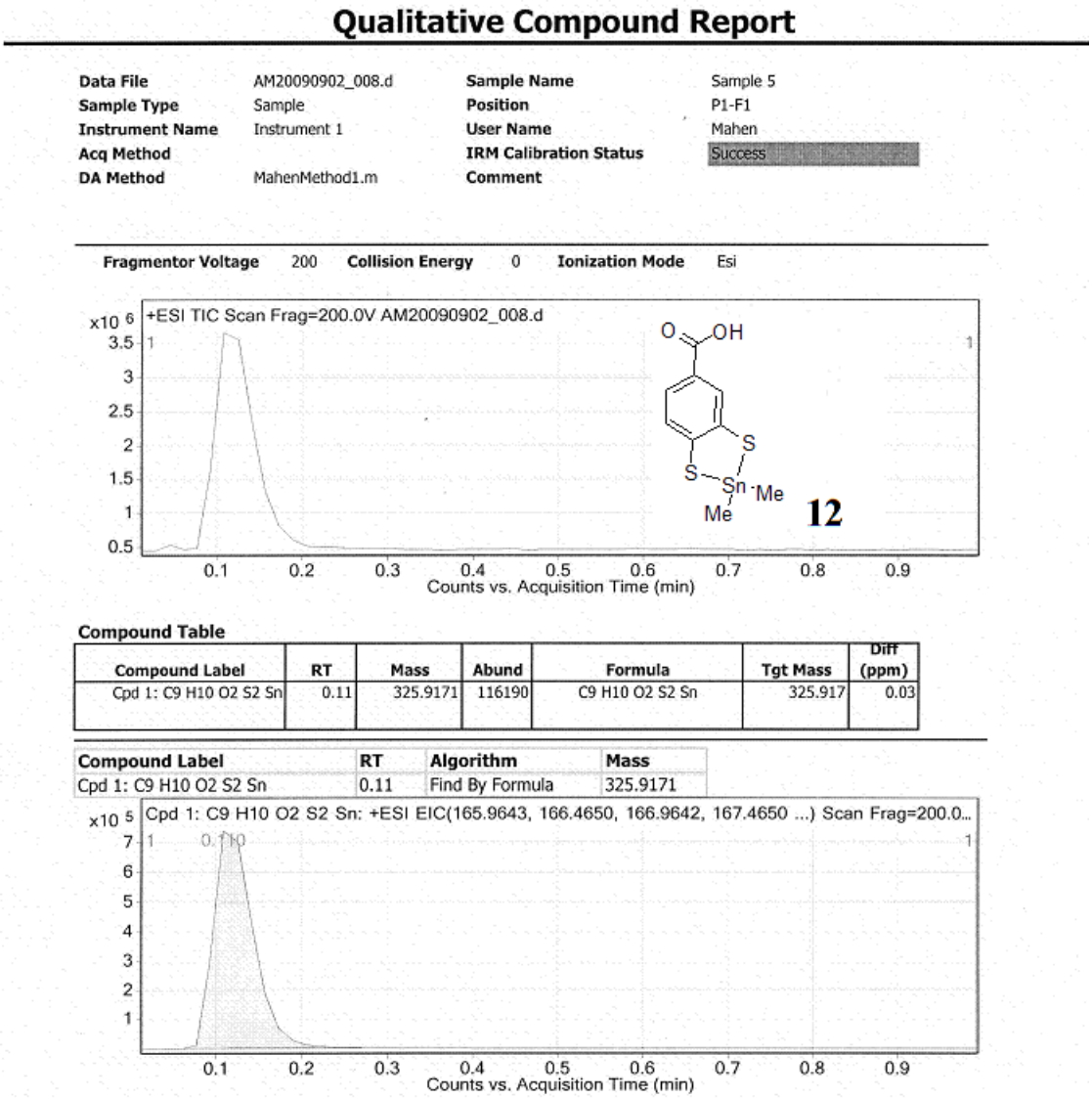
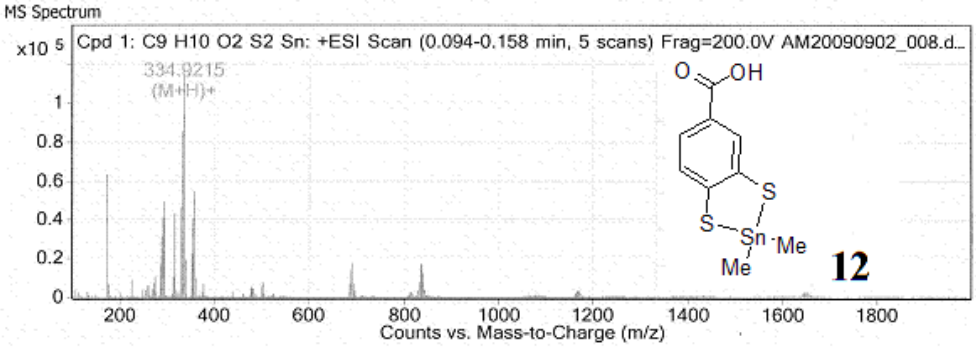


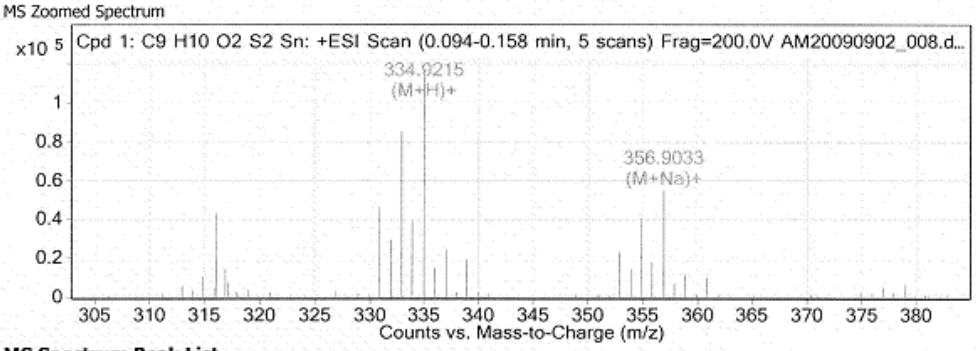
Figure 27. HRMS of 2,2-dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylic acid.

Qualitative Compound Report

MS Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
330.9209				46559		
331.9229				29457		
332.9212	332.9211	0.43		85534	C9 H11 O2 S2 Sn	(M+H)+
333.9229	333.9228	0.25		39409	C9 H11 O2 S2 Sn	(M+H)+
334.9215	334.9215	-0.05		116190	C9 H11 O2 S2 Sn	(M+H)+
336.921	336.921	-0.19		24528	C9 H11 O2 S2 Sn	(M+H)+
338.9245				19389		
354.9029	354.903	-0.32	1	40601	C9 H10 Na O2 S2 Sn	(M+Na)+
355.9048	355.9048	0	1	18120	C9 H10 Na O2 S2 Sn	(M+Na)+
356.9033	356.9035	-0.56	1	54781	C9 H10 Na O2 S2 Sn	(M+Na)+

--- End Of Report ---

Figure 28. HRMS of 2,2-dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylic acid.

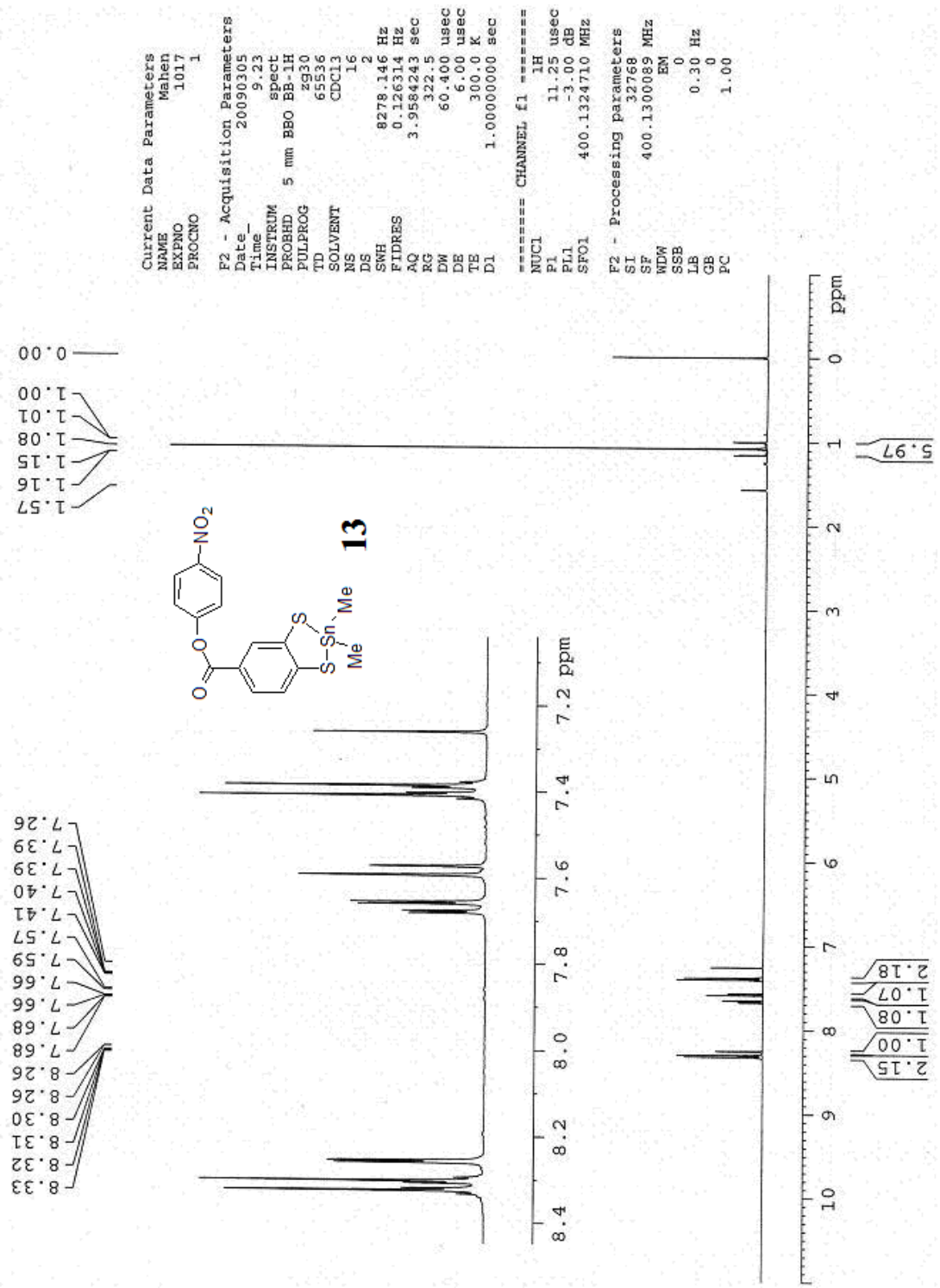


Figure 29. ^{1}H NMR of 4-nitrophenyl-2,2-dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylate – $CDCl_3$.

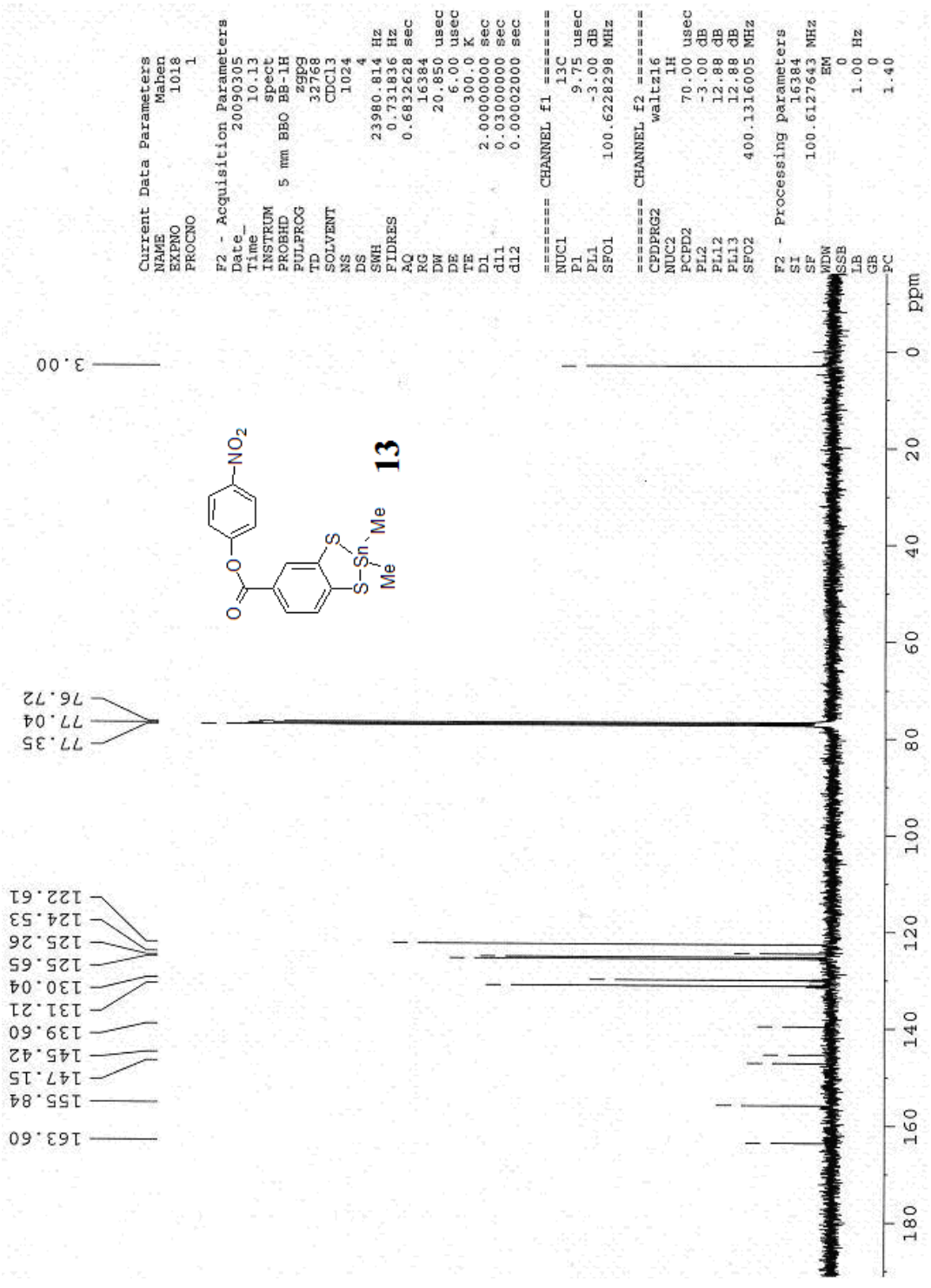


Figure 30. ^{13}C NMR of 4-nitrophenyl-2,2-dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylate – $CDCl_3$.

Qualitative Compound Report

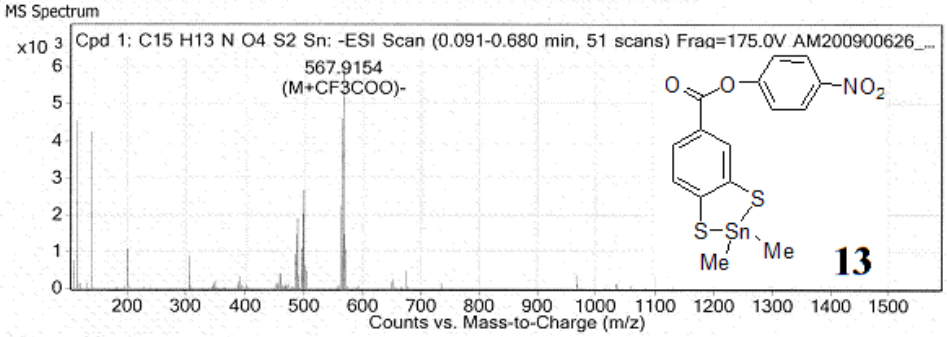
Data File	AM200900626_002 low and high mass.d	**Sample Name**	Sn-PNP
Sample Type	Sample	**Position**	P1-F3
Instrument Name	Instrument 1	**User Name**	Mahen
Acq Method		**IRM Calibration Status**	Success
DA Method	HCEmpirical1.m	**Comment**	FI

Compound Table

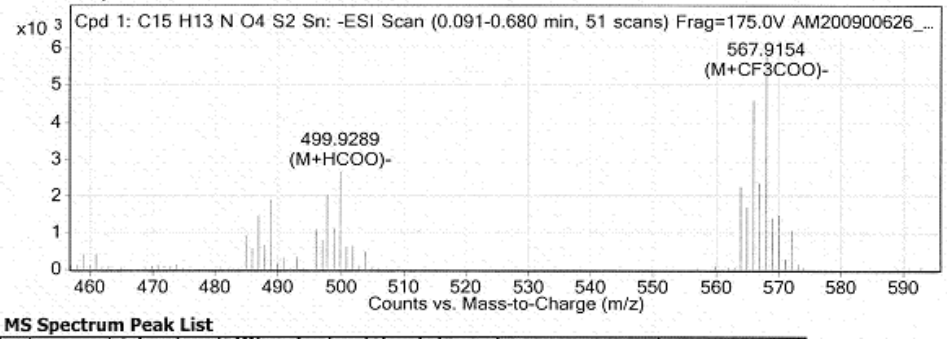
Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C15 H13 N O4 S2 Sn	0.115	446.9324	5940	C15 H13 N O4 S2 Sn	446.9334	-2.34

Compound Label	RT	Algorithm	Mass
Cpd 1: C15 H13 N O4 S2 Sn	0.115	Find By Formula	446.9324

MS Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
486.9047	486.9014	6.94	1	1491	C15 H13 Cl N O4 S2 Sn	(M+Cl)-
487.907	487.8993	15.79	1	688	C15 H13 Cl N O4 S2 Sn	(M+Cl)-
488.905	488.9009	8.38	1	1882	C15 H13 Cl N O4 S2 Sn	(M+Cl)-
489.9082	489.8995	17.65	1	174	C15 H13 Cl N O4 S2 Sn	(M+Cl)-

Figure 31. HRMS of 4-nitrophenyl-2,2-dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylate.

Qualitative Compound Report

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
490.907	490.9009	12.29	1	309	C15 H13 Cl N O4 S2 Sn	(M+Cl)-
491.9263	491.8983	56.82	1	37	C15 H13 Cl N O4 S2 Sn	(M+Cl)-
497.9285	497.9285	0	1	2017	C16 H14 N O6 S2 Sn	(M+HCOO)-
498.9304	498.9304	0.18	1	1134	C16 H14 N O6 S2 Sn	(M+HCOO)-
499.9289	499.929	-0.27	1	2675	C16 H14 N O6 S2 Sn	(M+HCOO)-
500.9315	500.9313	0.4	1	603	C16 H14 N O6 S2 Sn	(M+HCOO)-
501.9287	501.9288	-0.34	1	637	C16 H14 N O6 S2 Sn	(M+HCOO)-
502.9311	502.9315	-0.66	1	106	C16 H14 N O6 S2 Sn	(M+HCOO)-
562.9158				59		
563.9157				2238		
564.9176				1698		
565.914	565.9159	-3.48		4588	C17 H13 F3 N O6 S2 Sn	(M+CF3COO)-
566.1206				10		
566.917	566.9178	-1.32		2344	C17 H13 F3 N O6 S2 Sn	(M+CF3COO)-
567.0853				5		
567.9154	567.9164	-1.81		5940	C17 H13 F3 N O6 S2 Sn	(M+CF3COO)-
568.0776				10		
568.2491				5		
568.2786				5		
568.9174	568.9187	-2.2		1429	C17 H13 F3 N O6 S2 Sn	(M+CF3COO)-
569.1225				10		
569.9155	569.9163	-1.38		1484	C17 H13 F3 N O6 S2 Sn	(M+CF3COO)-
570.9175	570.9189	-2.44		293	C17 H13 F3 N O6 S2 Sn	(M+CF3COO)-
571.9193	571.919	0.54		1048	C17 H13 F3 N O6 S2 Sn	(M+CF3COO)-
572.9221	572.922	0.14	1	160	C17 H13 F3 N O6 S2 Sn	(M+CF3COO)-
573.9193	573.9177	2.75	1	68	C17 H13 F3 N O6 S2 Sn	(M+CF3COO)-

--- End Of Report ---

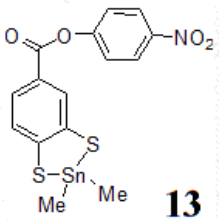


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Figure 32. HRMS of 4-nitrophenyl-2,2-dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylate.

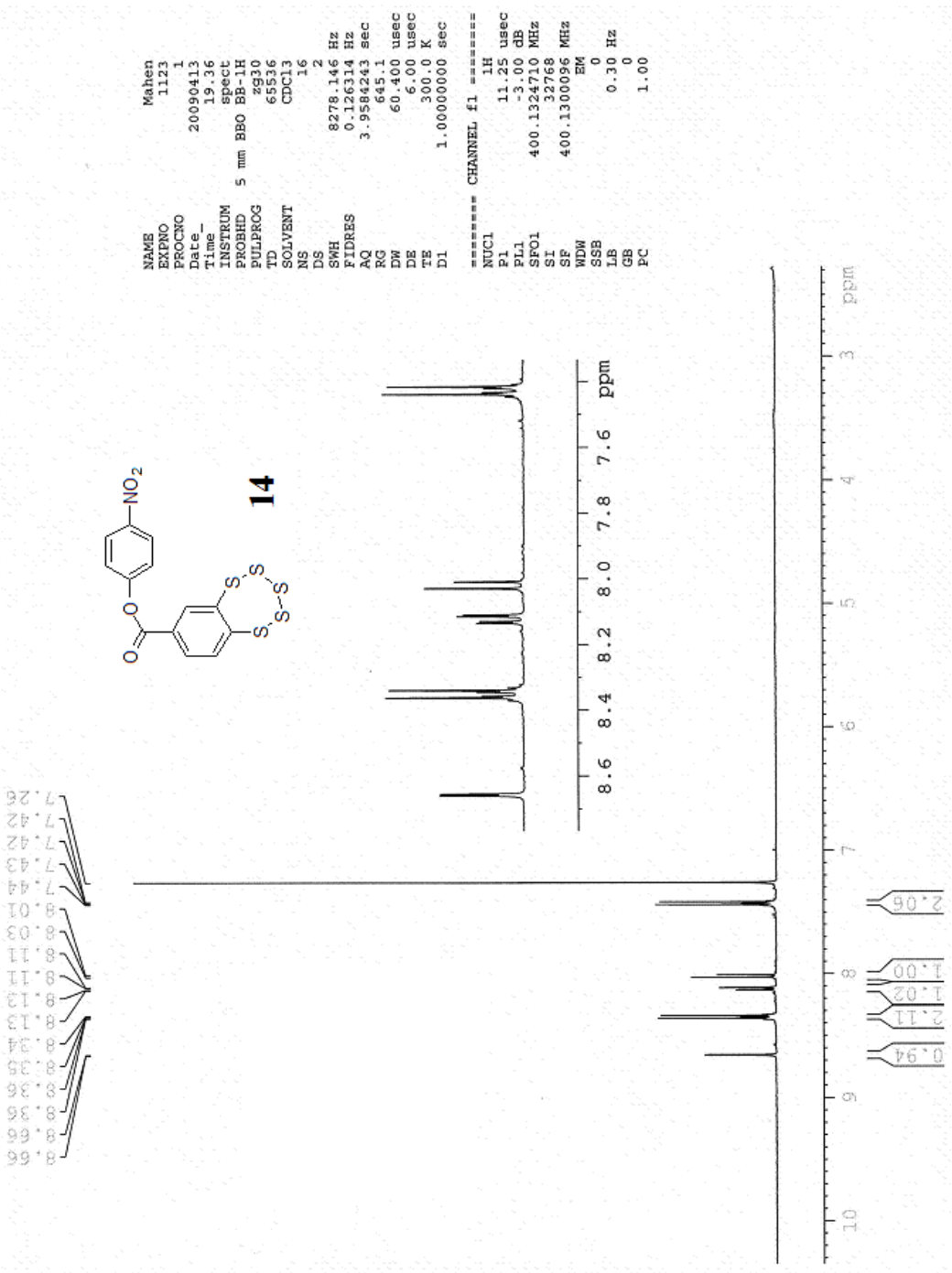


Figure 33. ^{1}H of NMR 4-nitrophenylbenzo[f][1,2,3,4,5]pentathiepine-7-carboxylate - $CDCl_3$.

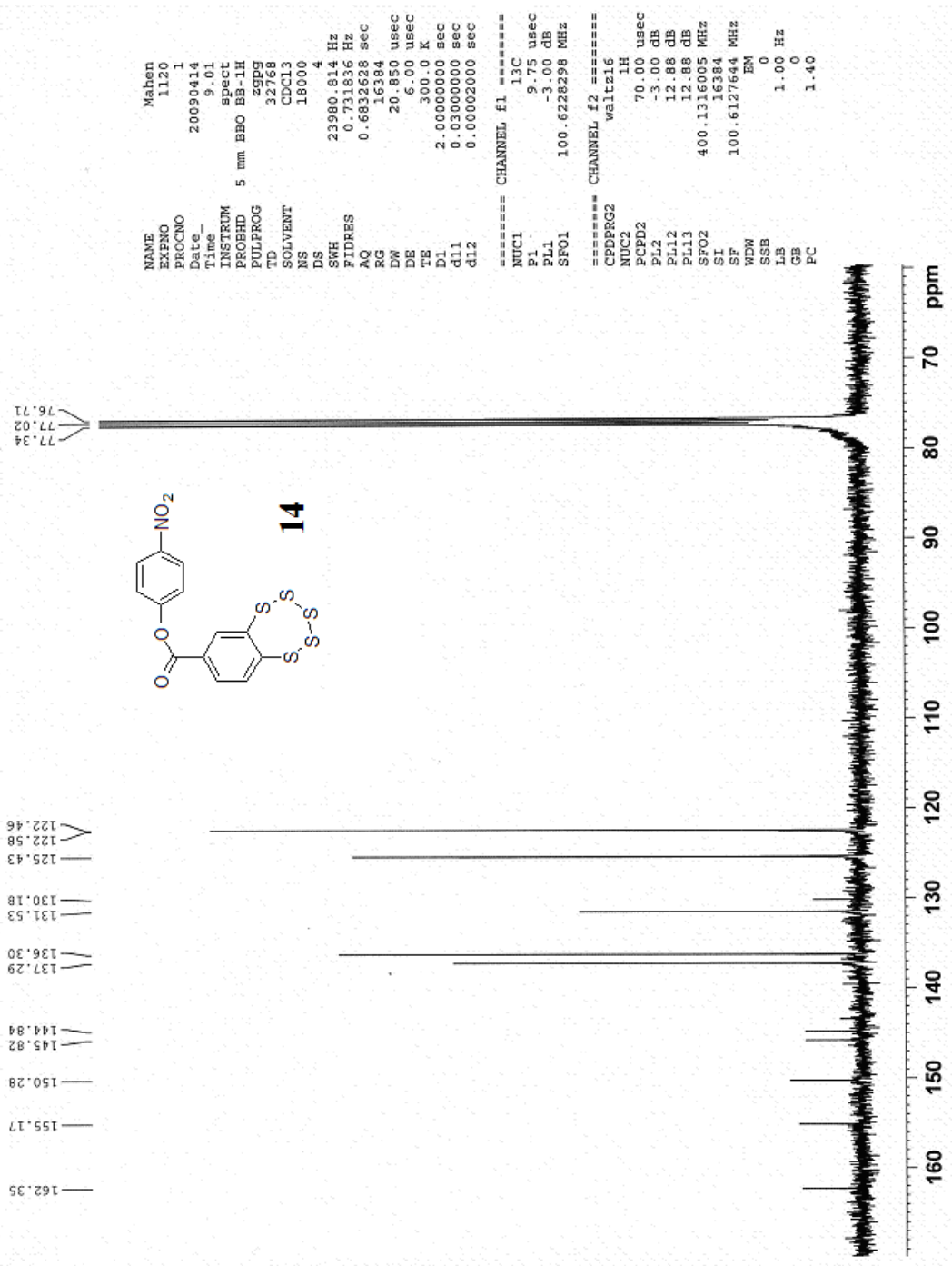


Figure 34. ^{13}C NMR of 4-nitrophenylbenzo[f][1,2,3,4,5]pentathiepine-7-carboxylate - $CDCl_3$.

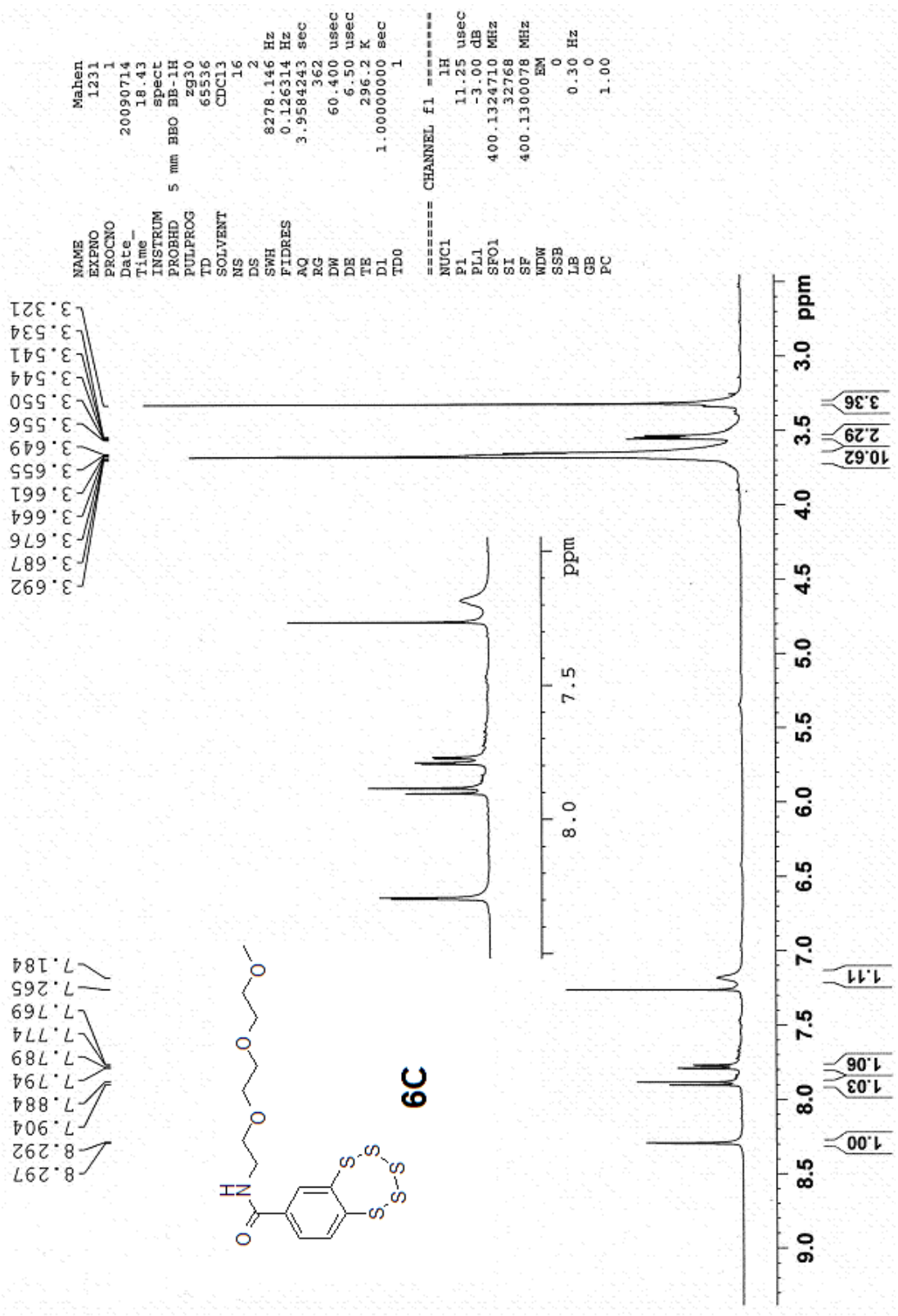


Figure 35. ^{1}H NMR of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5]pentathiepine-7-carboxamide - $CDCl_3$.

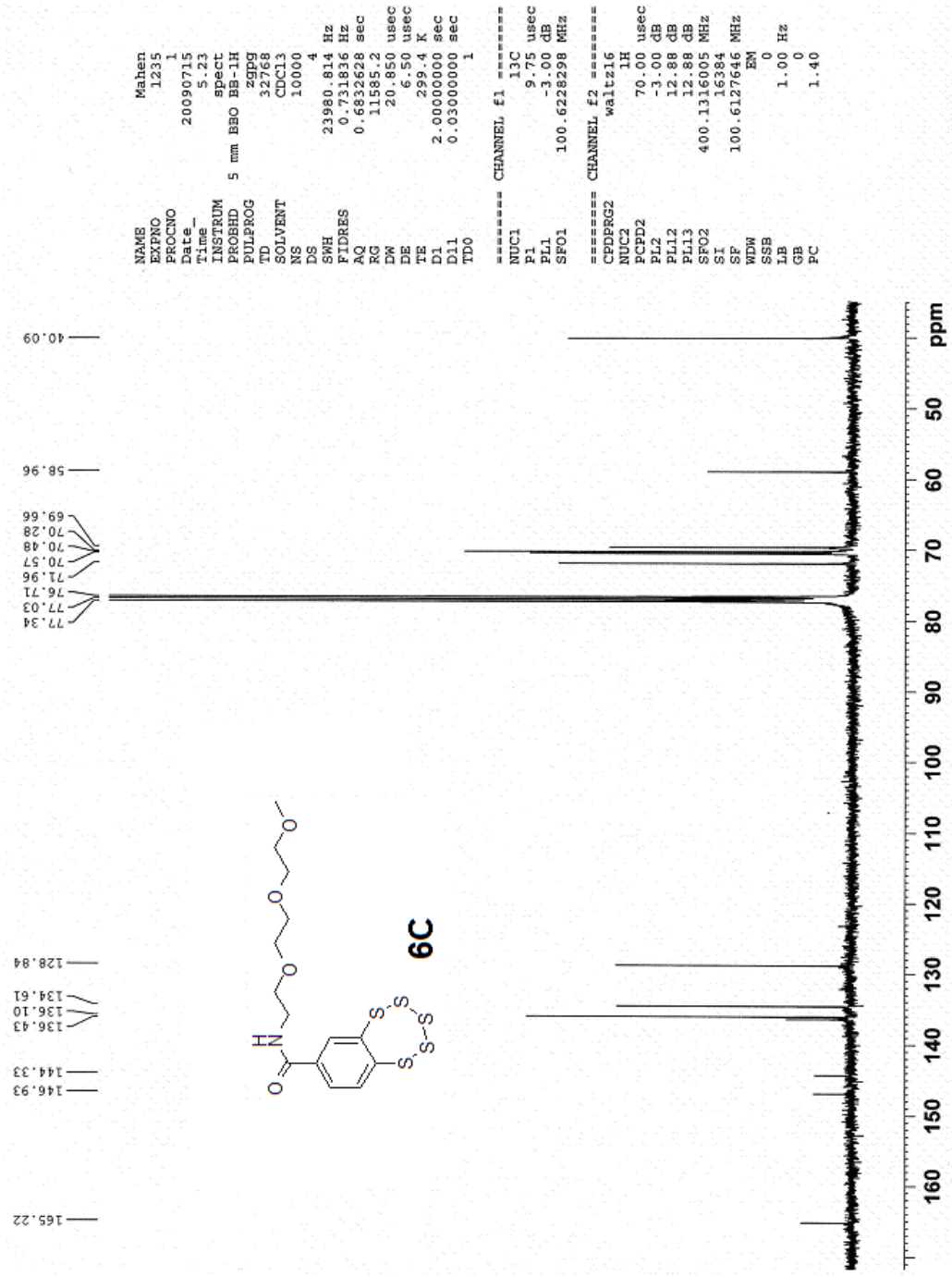


Figure 36. ^{13}C NMR of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5]pentathiepine-7-carboxamide - $CDCl_3$.

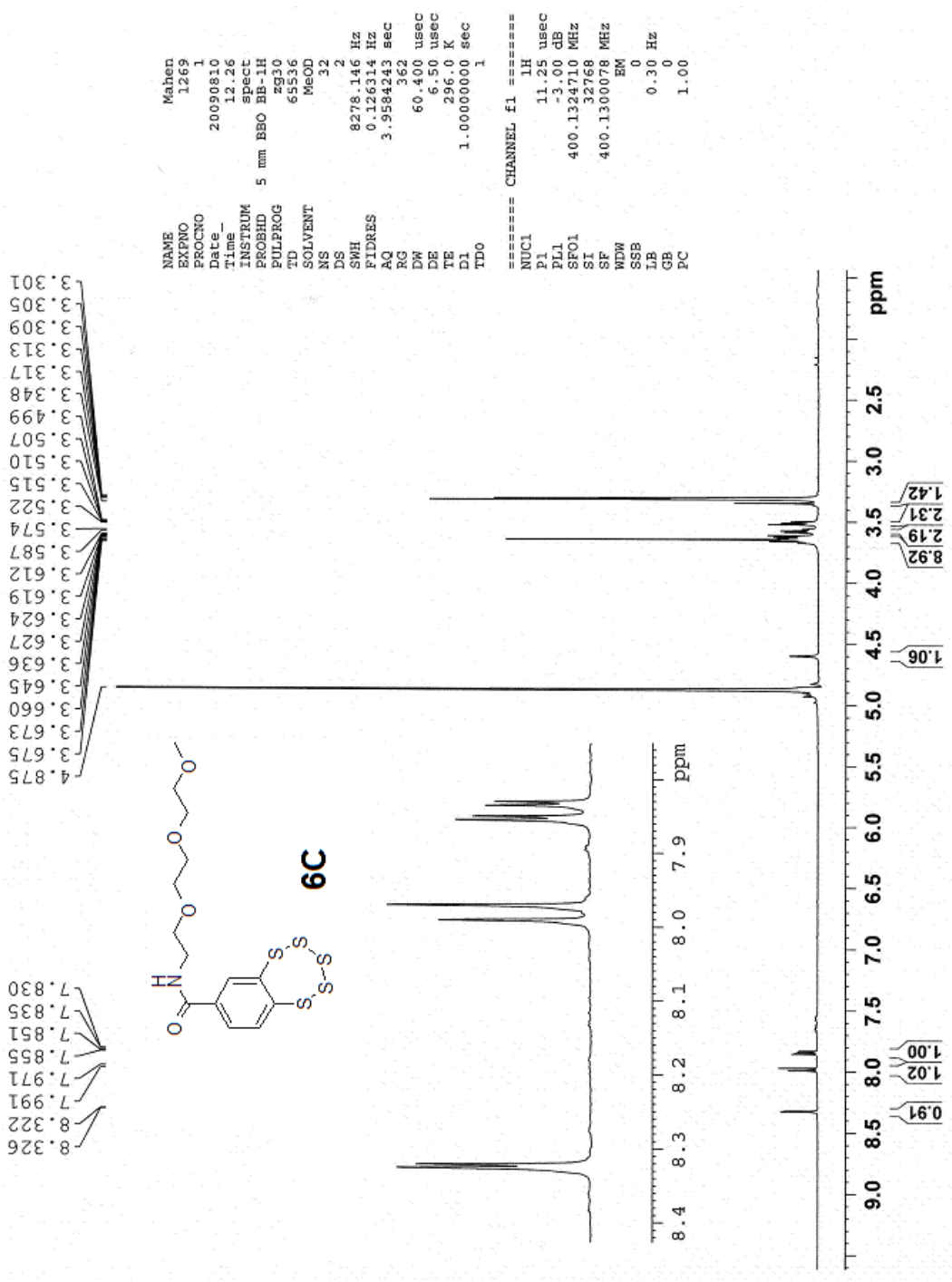


Figure 37. ^{1}H NMR of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5]pentathiepine-7-carboxamide – CD_3OD.

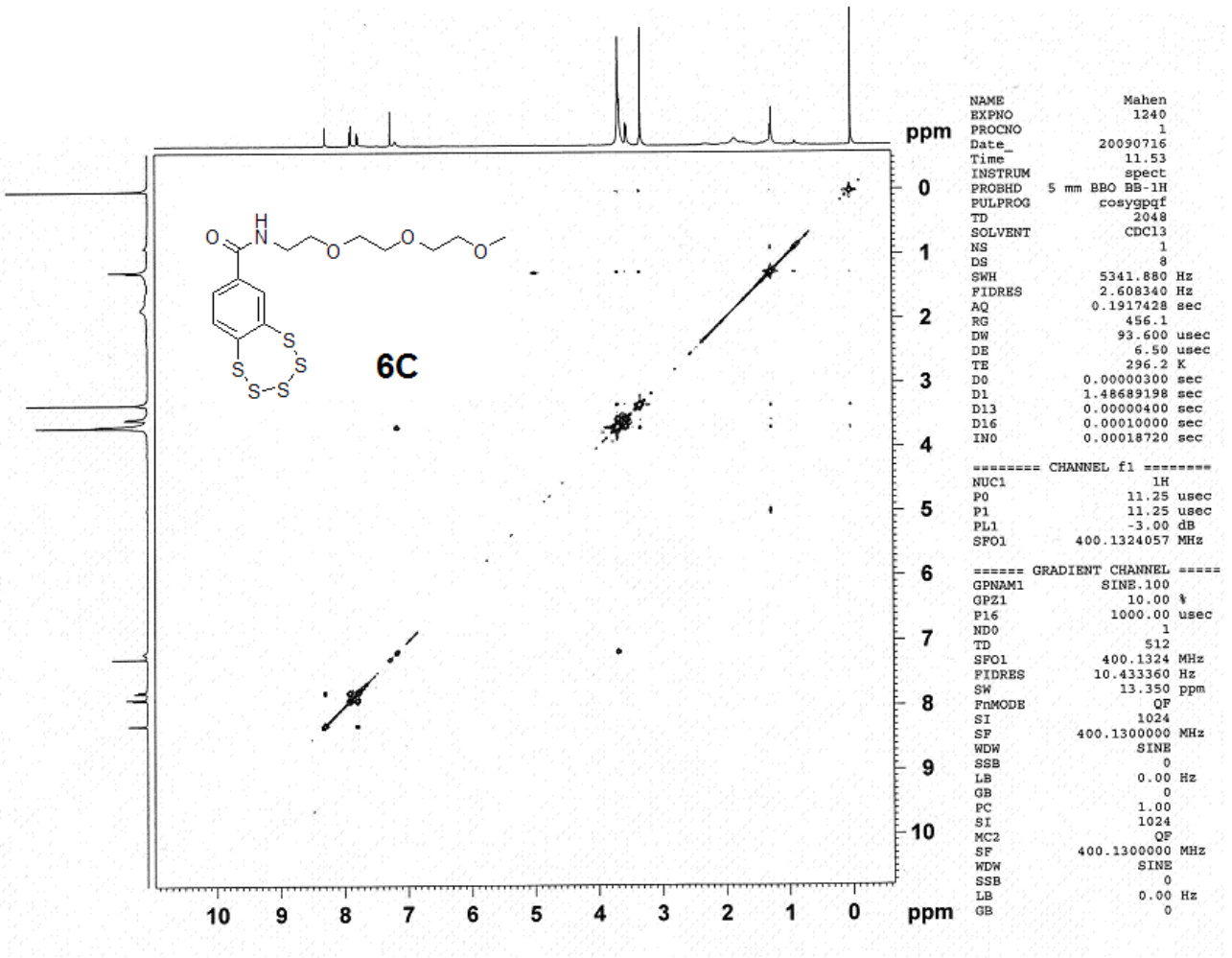


Figure 38. COSY NMR of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5]pentathiepine-7-carboxamide - $CDCl_3$

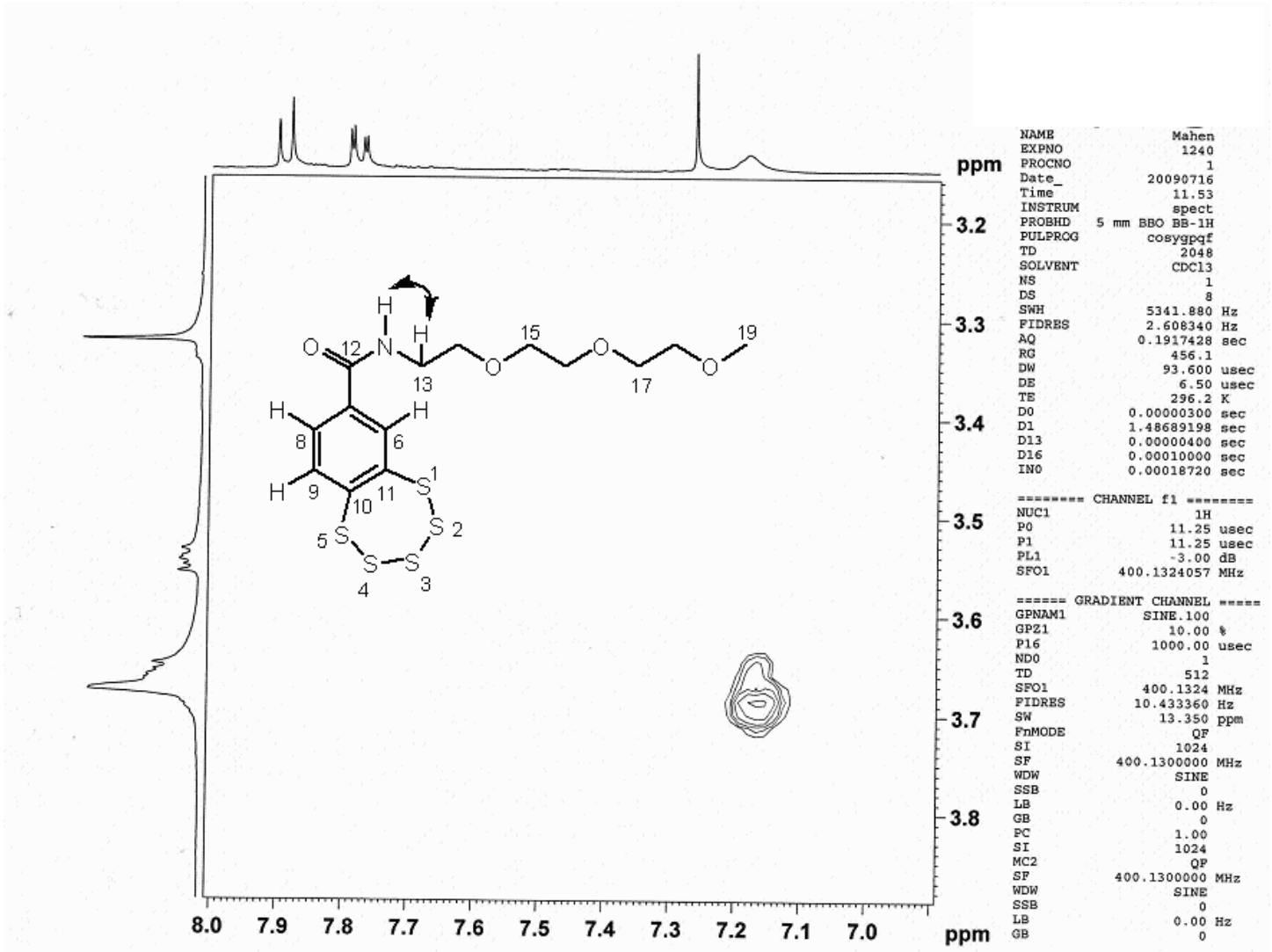


Figure 39. COSY NMR of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5]pentathiepine-7-carboxamide - $CDCl_3$.

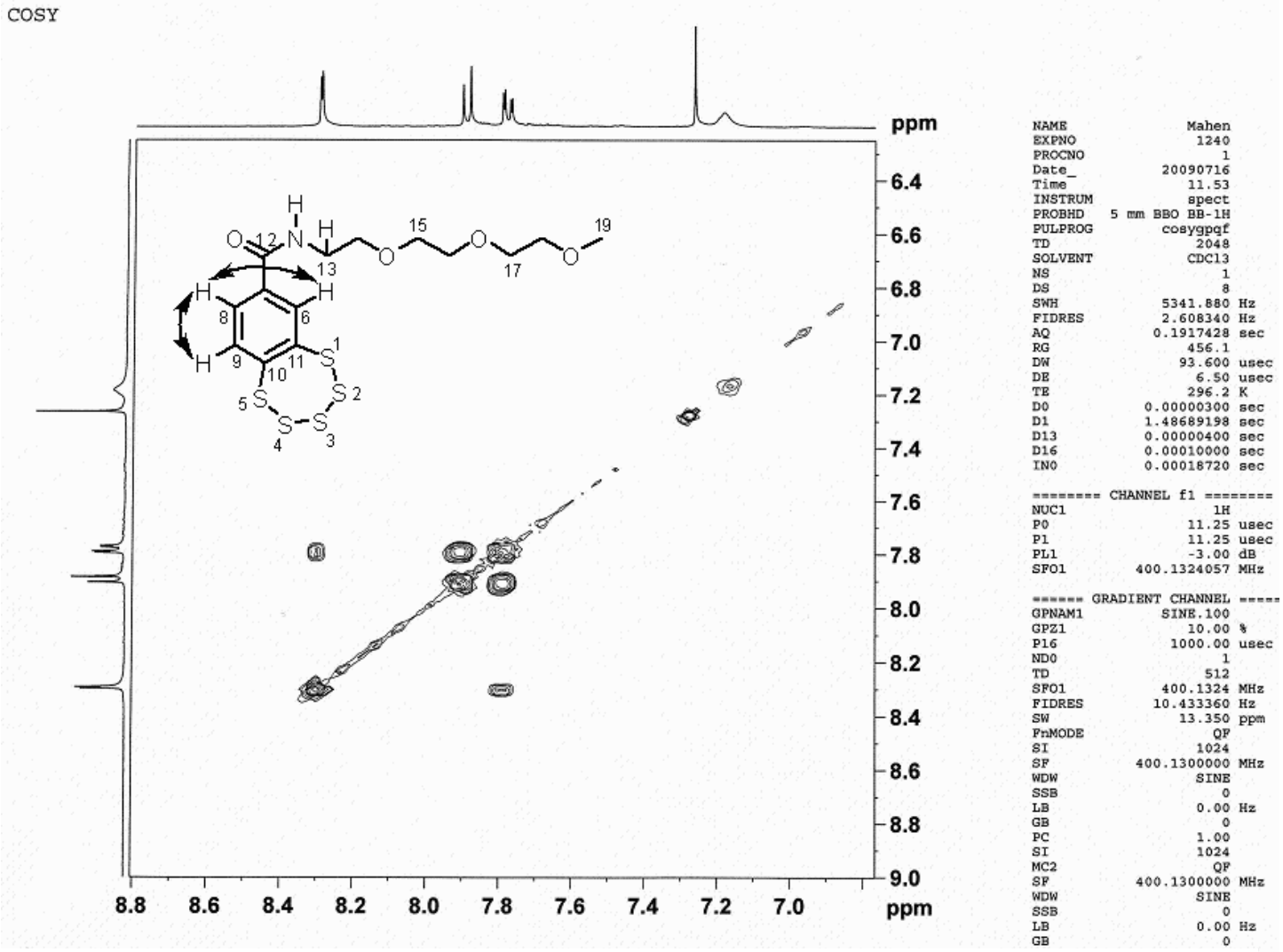


Figure 40. COSY NMR of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5]pentathiepine-7-carboxamide - $CDCl_3$.

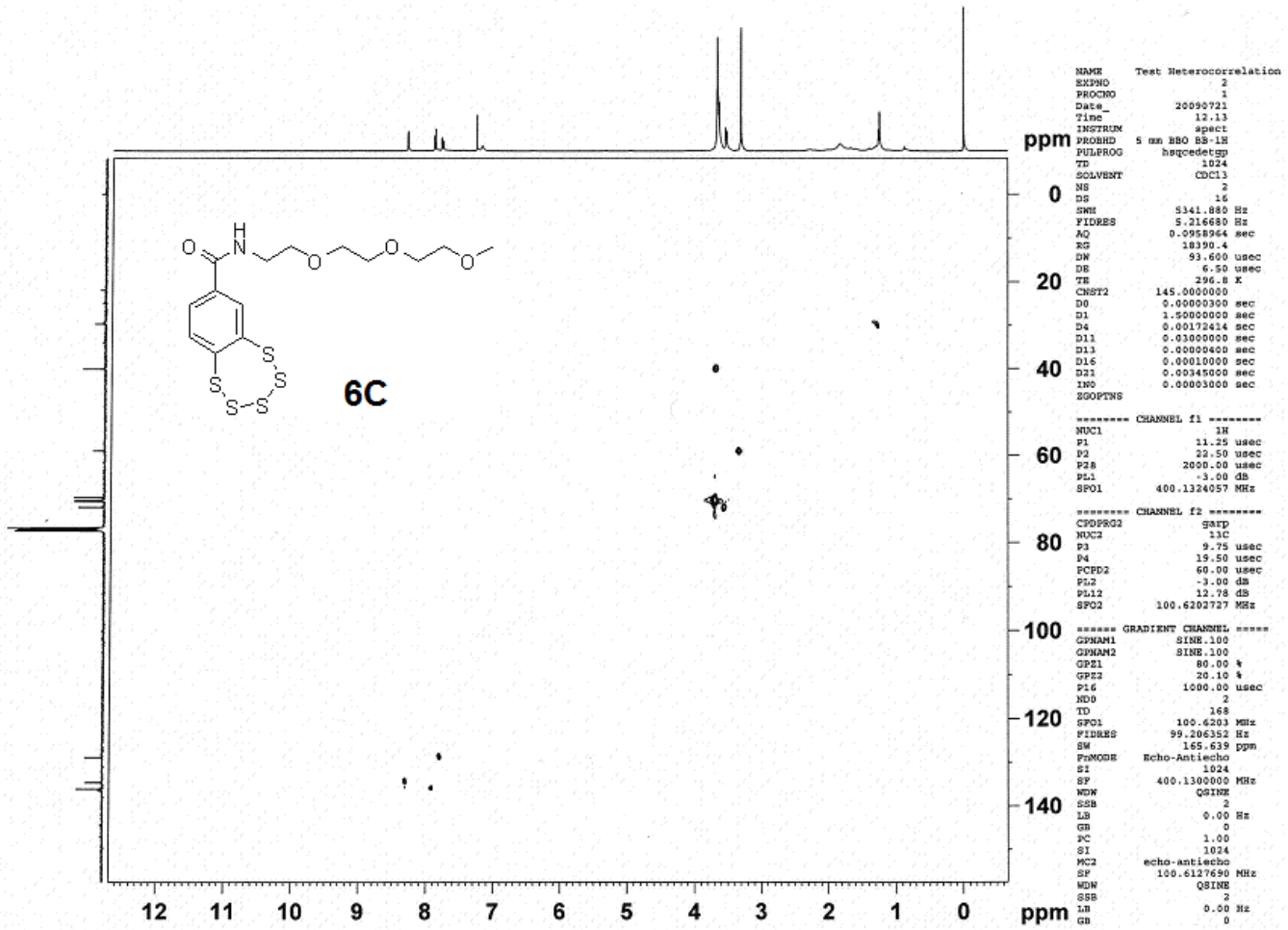


Figure 41. HSQC NMR of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5]pentathiepine-7-carboxamide - $CDCl_3$.

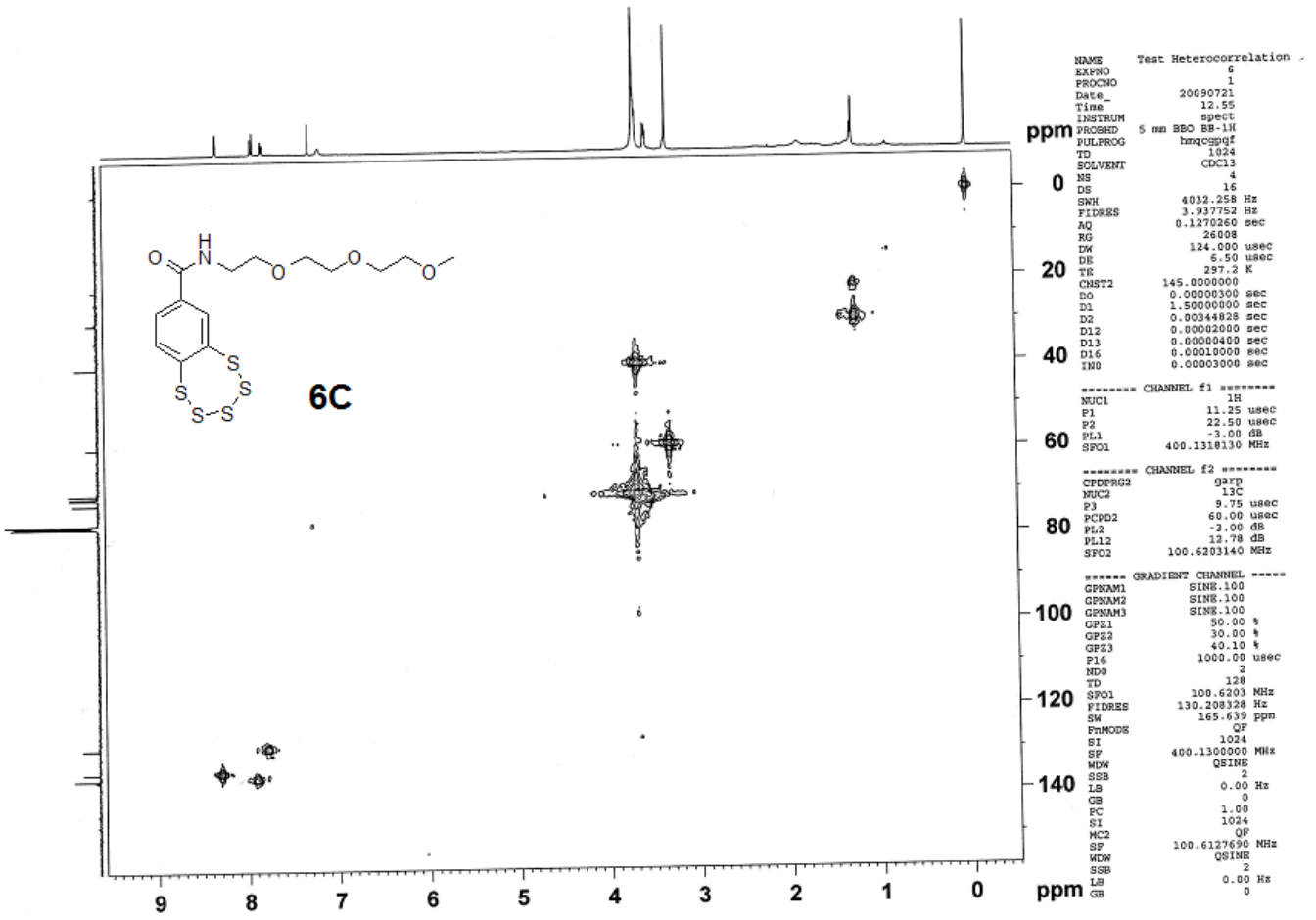


Figure 42. HMBC NMR of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5]pentathiepine-7-carboxamide- $CDCl_3$.

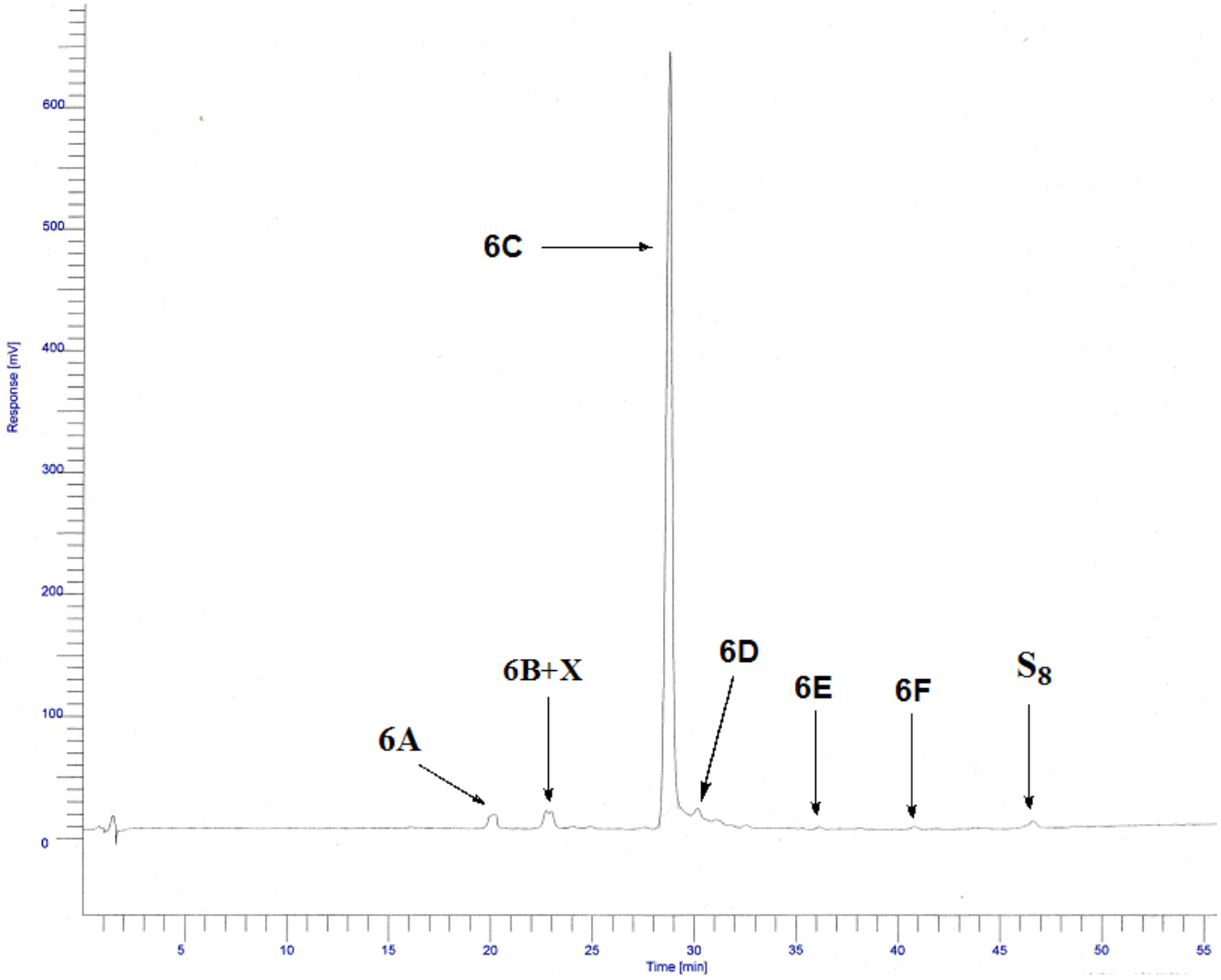


Figure 43. HPLC of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5]pentathiepine-7-carboxamide.

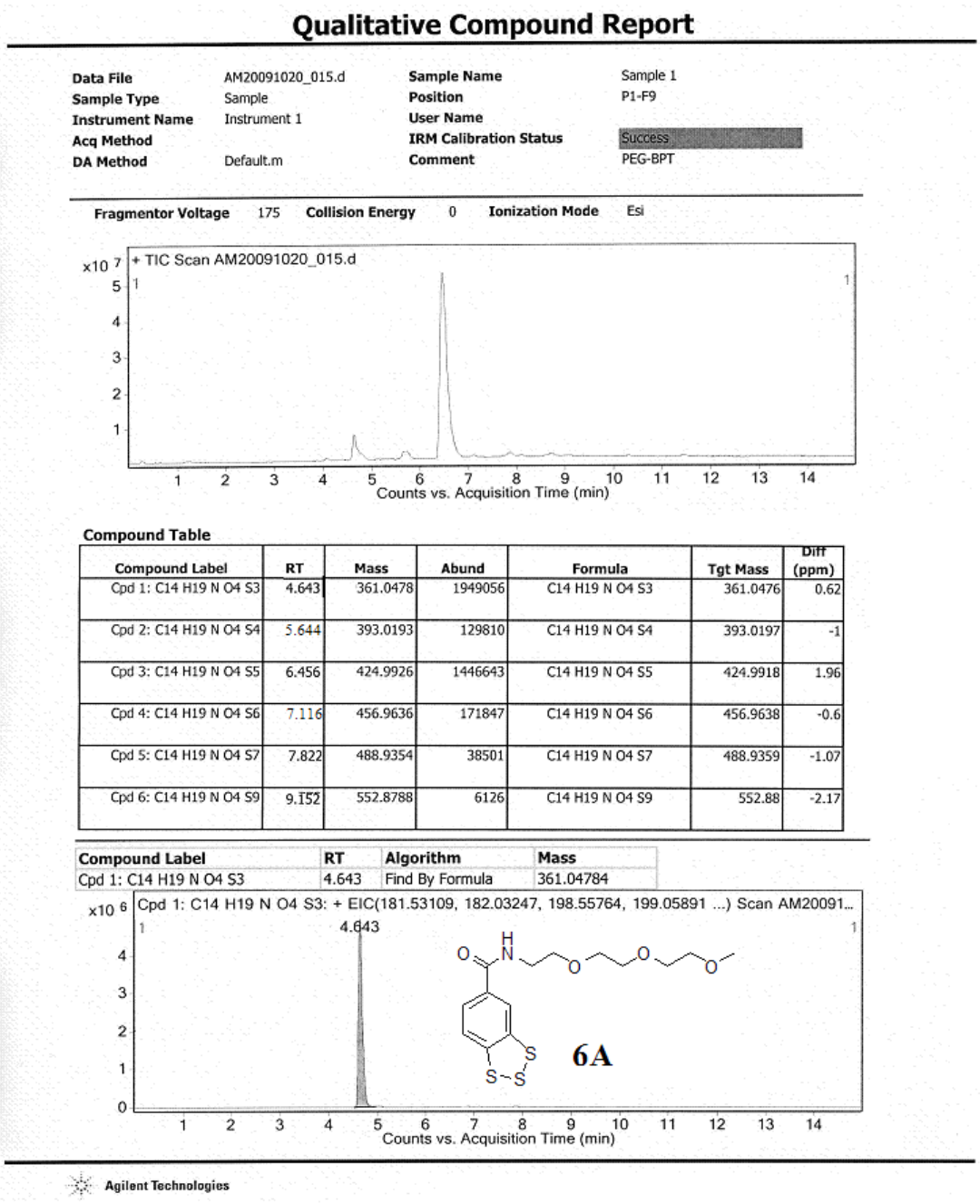
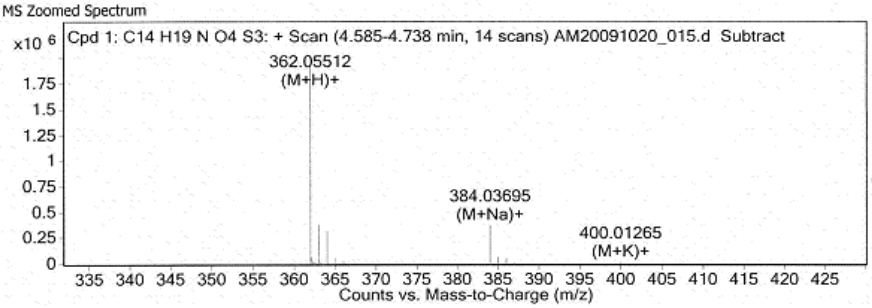


Figure 44. HRMS of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[d] [1,2,3]trithiole-5-carboxamide.

Qualitative Compound Report

MS Zoomed Spectrum



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
362.05512	362.0549	0.62		1949056	C14 H20 N O4 S3	(M+H)+
362.22313				59205		
362.30833				21866		
363.05794	363.05765	0.79		380735	C14 H20 N O4 S3	(M+H)+
364.05233	364.0521	0.63		317984	C14 H20 N O4 S3	(M+H)+
365.05446	365.0544	0.17		50422	C14 H20 N O4 S3	(M+H)+
384.03695	384.03684	0.29	1	374101	C14 H19 N Na O4 S3	(M+Na)+
385.03935	385.0396	-0.64	1	65545	C14 H19 N Na O4 S3	(M+Na)+
386.03369	386.03404	-0.9	1	54866	C14 H19 N Na O4 S3	(M+Na)+
400.01265	400.01078	4.68	1	6284	C14 H19 K N O4 S3	(M+K)+

Agilent Technologies

Figure 45. HRMS of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[d] [1,2,3] trithiole-5-carboxamide.

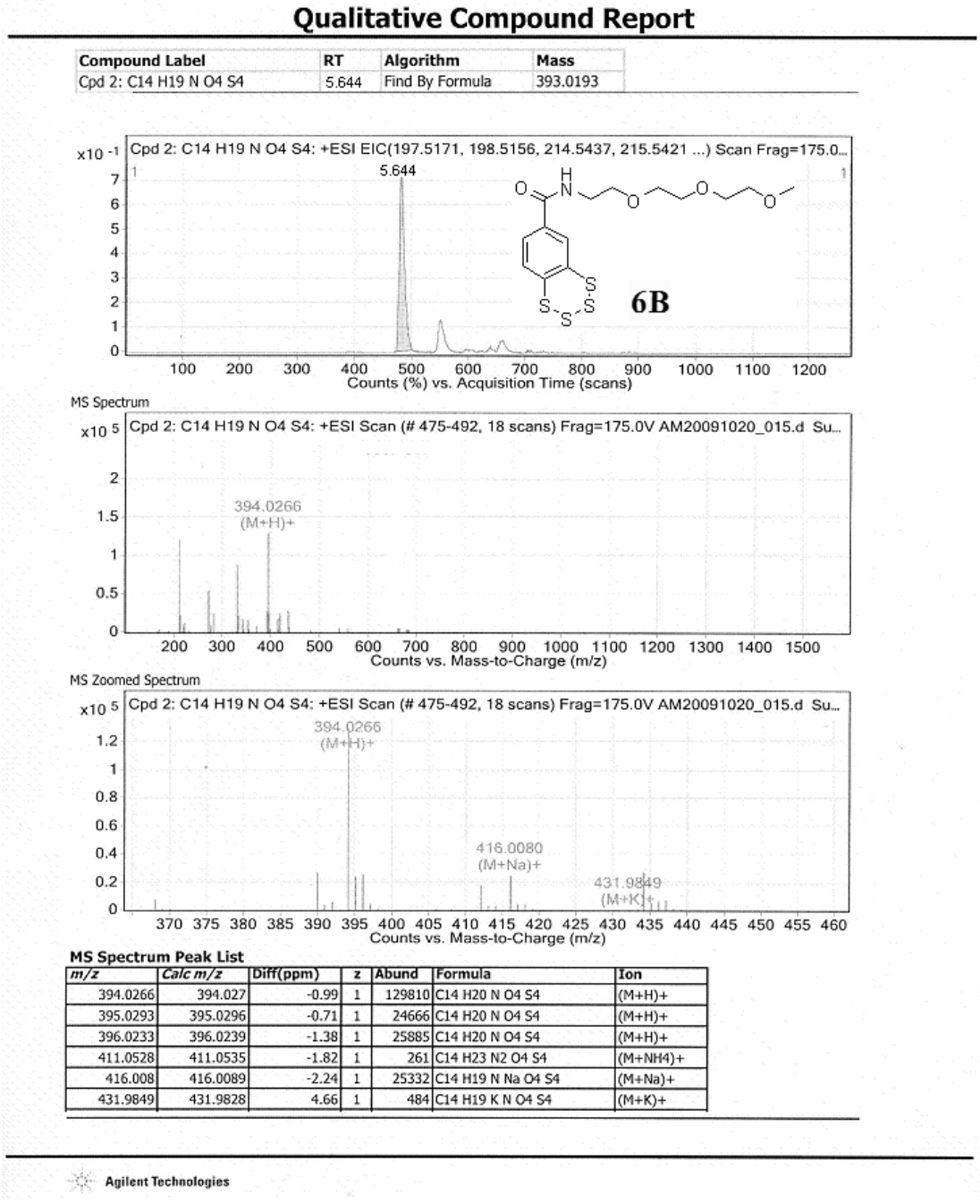


Figure 46. HRMS of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[e][1,2,3,4] tetrathiine-6-carboxamide

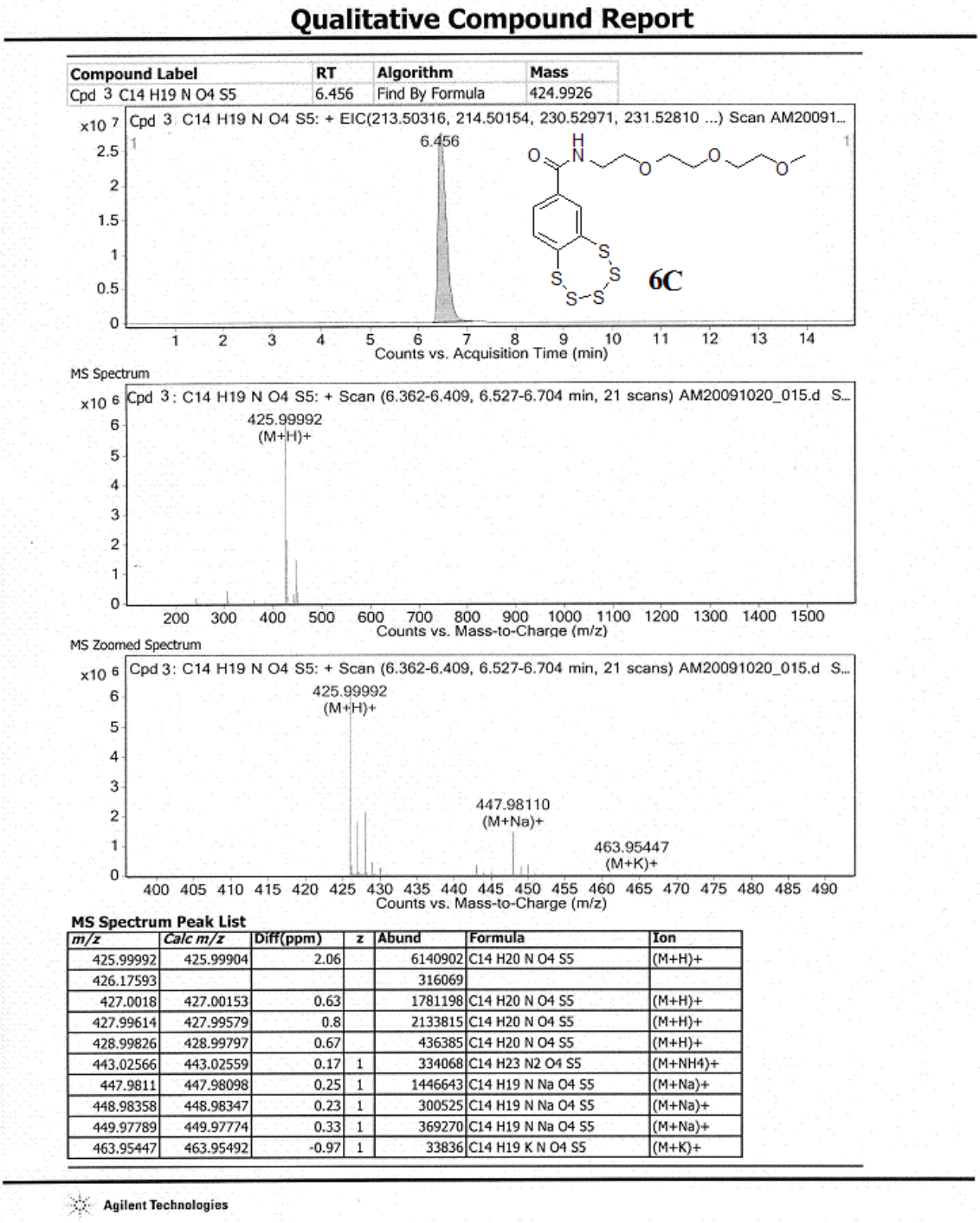


Figure 47. HRMS of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5] pentathiepine-7-carboxamide

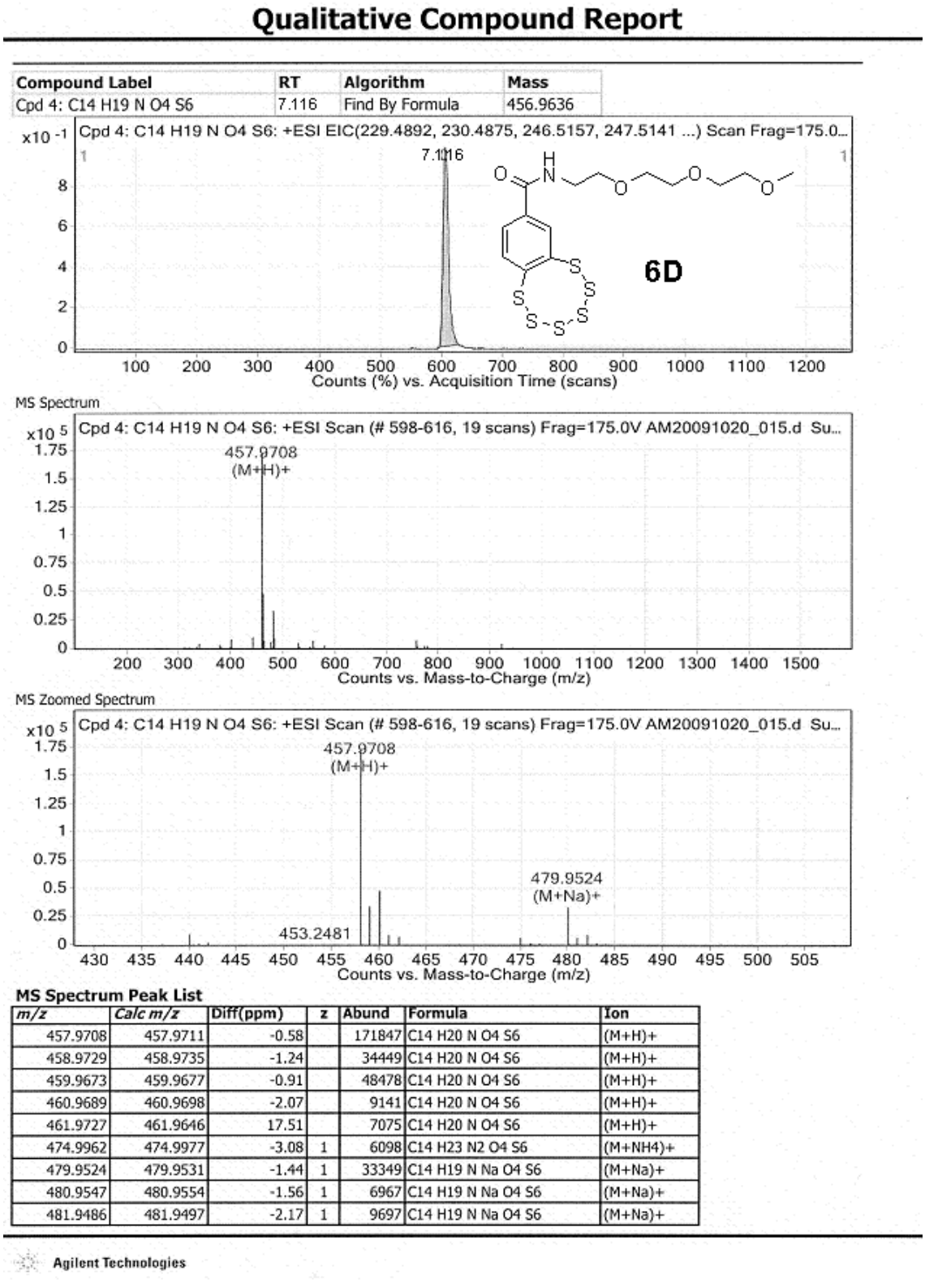


Figure 48. HRMS of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[g][1,2,3,4,5,6] hexathiocine-8-carboxamide

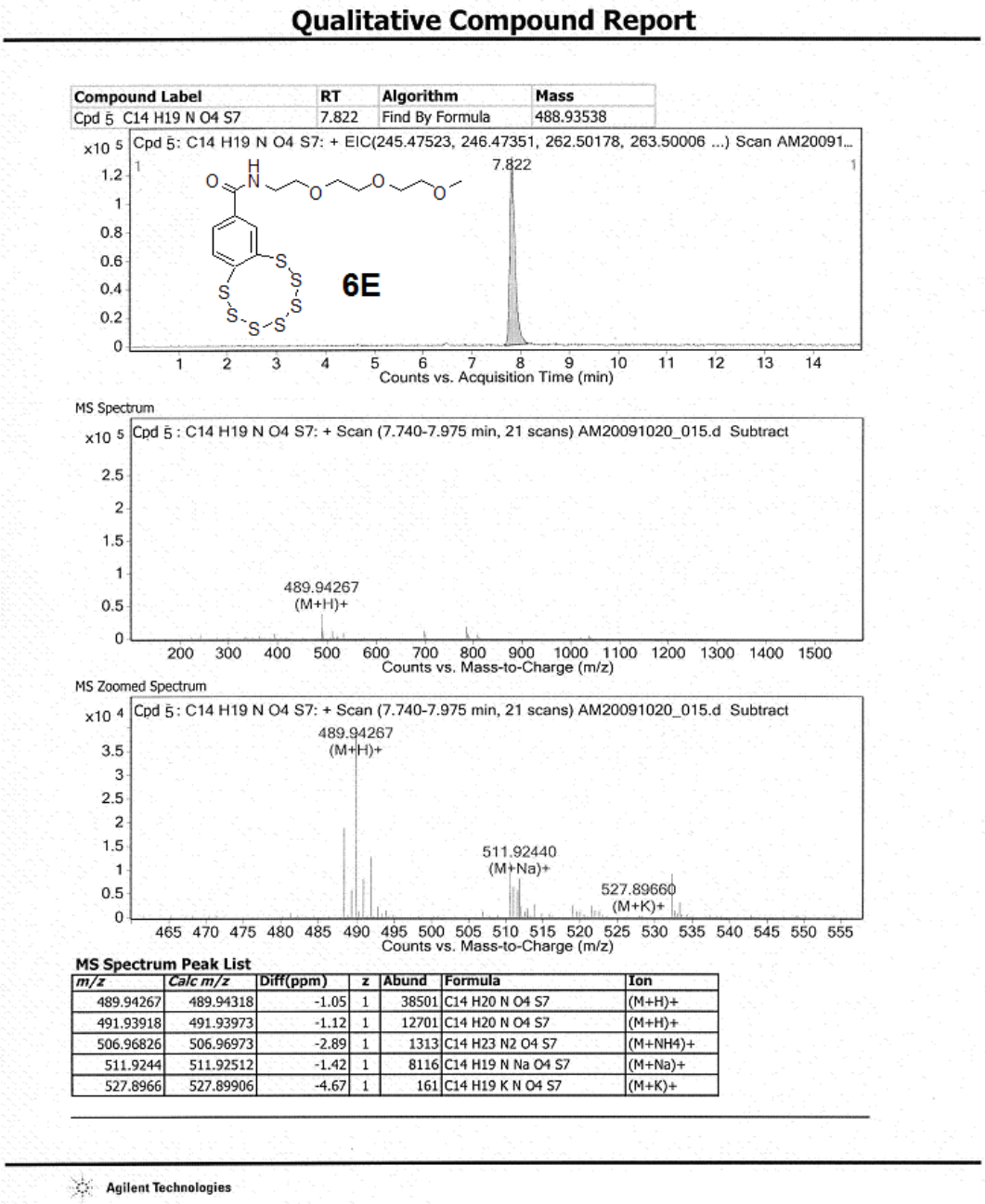
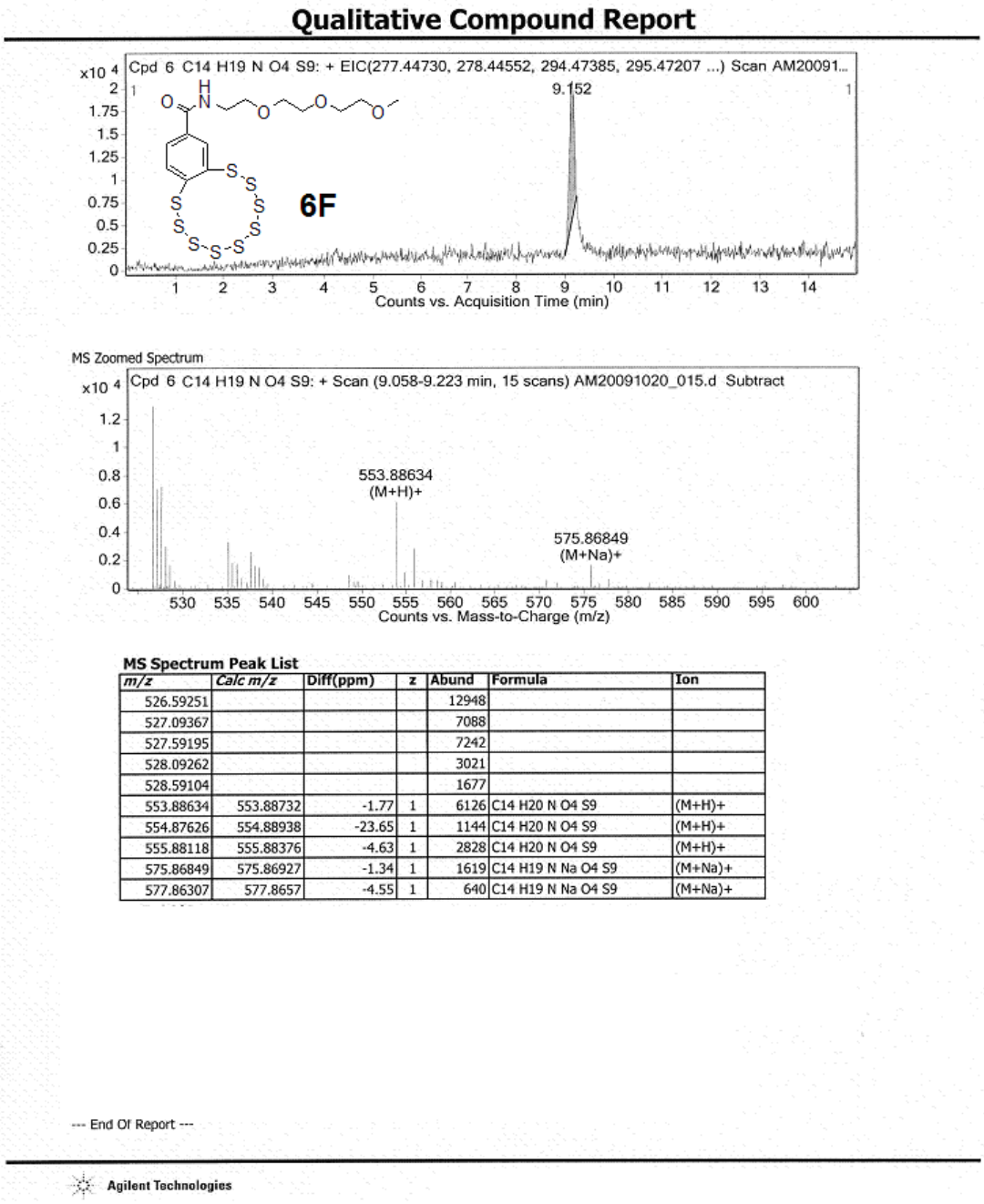


Figure 49. HRMS of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[h] [1,2,3,4,5,6,7] heptathionine-9-carboxamide.



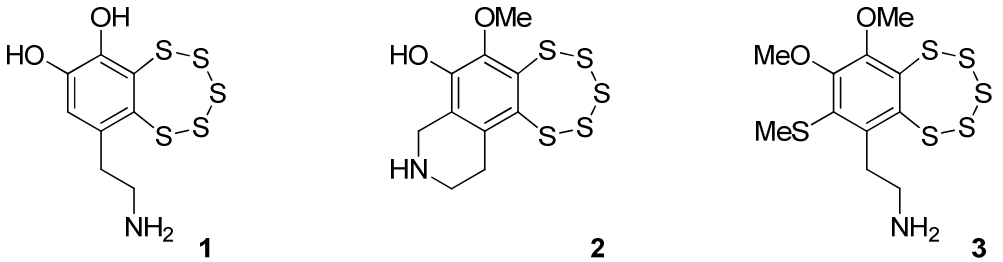
m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
526.59251				12948		
527.09367				7088		
527.59195				7242		
528.09262				3021		
528.59104				1677		
553.88634	553.88732	-1.77	1	6126	C14 H20 N O4 S9	(M+H)+
554.87626	554.88938	-23.65	1	1144	C14 H20 N O4 S9	(M+H)+
555.88118	555.88376	-4.63	1	2828	C14 H20 N O4 S9	(M+H)+
575.86849	575.86927	-1.34	1	1619	C14 H19 N Na O4 S9	(M+Na)+
577.86307	577.8657	-4.55	1	640	C14 H19 N Na O4 S9	(M+Na)+

Figure 50. HRMS of N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[j] [1,2,3,4,5,6,7,8,9] nonathiacycloundecine-11-carboxamide.

Chapter 3: Regioselective (Biomimetic) Synthesis of a Pentasulfane From *Ortho*-benzoquinone

3.1 Introduction

Desmethyl varacin (**1**), lissoclinotoxin B (**2**), and 5-(methylthio)varacin (**3**) represent a class of cytotoxic polysulfanes isolated from marine invertebrates (Scheme 1).[1-20] These compounds contain a dopamine core and a pentasulfur linkage. As indicated in chapter 1, although natural benzopolysulfanes have been isolated,[1-10] the biosynthetic origin and mechanism for introduction of the sulfur atoms into dopamine has not been examined. Two reactions of sulfur and dopamine may relate to the biomimetic principles that underlie how benzopentathiepins arise biosynthetically. First, benzopentathiepins may arise from a two-electron transfer reaction of reduced elemental sulfur, H_2S_x, with dopamine-*o*-quinone. Second, benzopentathiepins may arise from a one-electron oxidation of dopamine followed by a reaction with neutral S_8. We have examined the viability of the first reaction in the laboratory generation of cyclic 5,6,7,8,9-benzopentathiepin-1,2-diol (**4**) using a *o*-benzoquinone—H_2S_x reaction. Our work with catechol [*o*-$(HO)_2C_6H_4$] served as a model for dopamine. A discussion is presented on the reaction of o-quinone and H_2S_x with specific interest to the mechanism of formation of pentasulfane **4**.



Scheme 1. Natural Polysulfanes

3.2 Results and Discussion

3.2.1 Reaction of Quinone with H_2S_x.

The reaction of *o*-benzoquinone (180 mM) (generated in a reaction of Ag_2O with catechol in acetone)[21] with H_2S_x (3.9 M) (generated by sodium sulfide nonahydrate heated with elemental sulfur, and precipitated with Cl_3CCO_2H)[22] was conducted by stirring for 1 hr at room temperature. The red color of the quinone was rapidly converted to pale yellow on addition of H_2S_x. The reaction produced a series of products of high and low molecular weight. Precipitation of neutral elemental S_8 also accompanied the reaction. Polymeric and insoluble material was removed by filtration. GC/MS analysis revealed a large peak that is likely due to pentathiepin **4** (Figure 1). The GC/MS data is indicative of a pentathiepin [*m/z* = 64 (43), 96 (12), 110 (48), 142 (66), 204 (100), 268 (10)] since it displays a weak molecular ion peak *m/z* 268 [M^+] and a strong base peak at *m/z* 204 [M^+-2S] representing the loss of two sulfur atoms.[23] The M+2 and M+4 peaks caused by isotope ratios are as predicted. Another reaction product observed by GC/MS corresponded to 3-mercaptobenzene-1,2-diol (**5**). However, attempts to purify the raw mixture by preparative TLC and HPLC were unsuccessful due to the high polarity of the compounds. Therefore, the hydroxy and thiol groups of the products were protected by acetylation with addition of DMAP (0.18 mmol) and acetic anhydride (2.52 mmol) in ethyl acetate. Ten products could then be chromatographically separated through preparative TLC and HPLC and characterized as the corresponding di-, tri-, or tetraacetates of **4**-**12** using ^1H and ^{13}C NMR, and MS techniques (Scheme 2). Additional support for the structures assigned to **5** (x=1), **6** (x=1), **7**, and **8** came from their

independent synthesis by the reaction of *o*-benzoquinone (18 mmol) with thioacetic acid (26 mmol) as described in the Supporting Information Section.

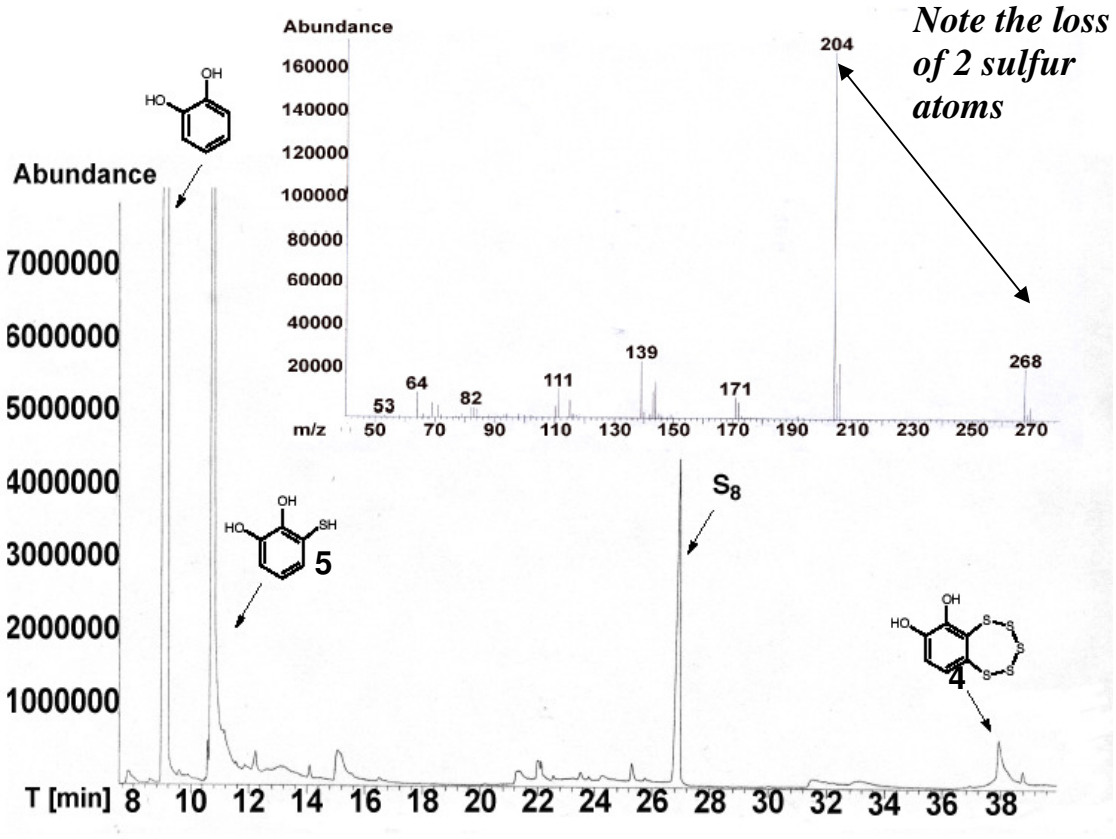
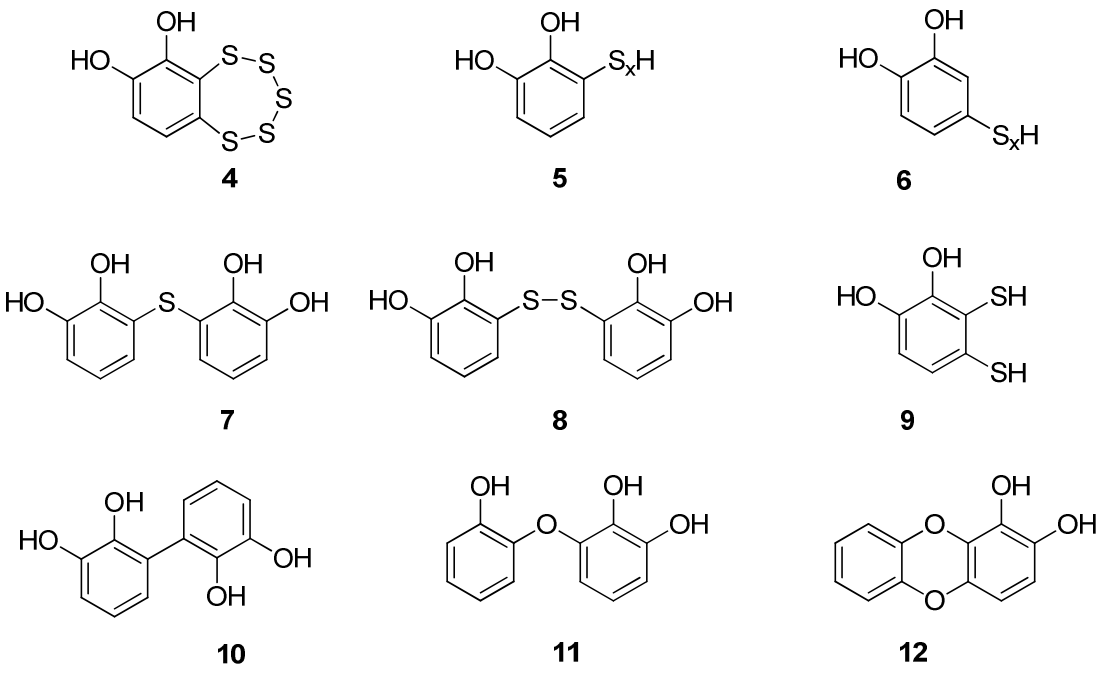


Figure 1. GC/MS trace of H_2S_x + *o*-benzoquinone crude reaction mixture.

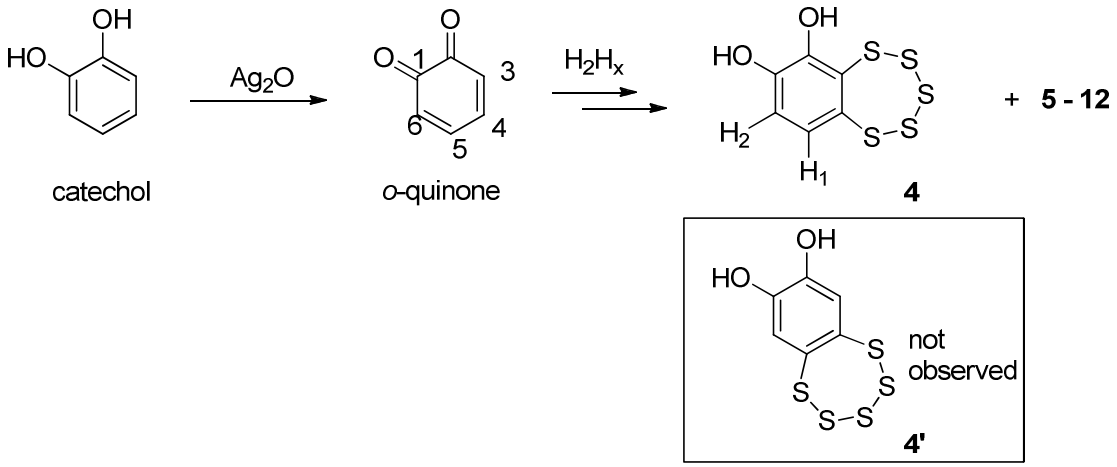


Scheme 2. Compounds 4-12 Detected as the Corresponding Di-, Tri-, or Tetraacetates

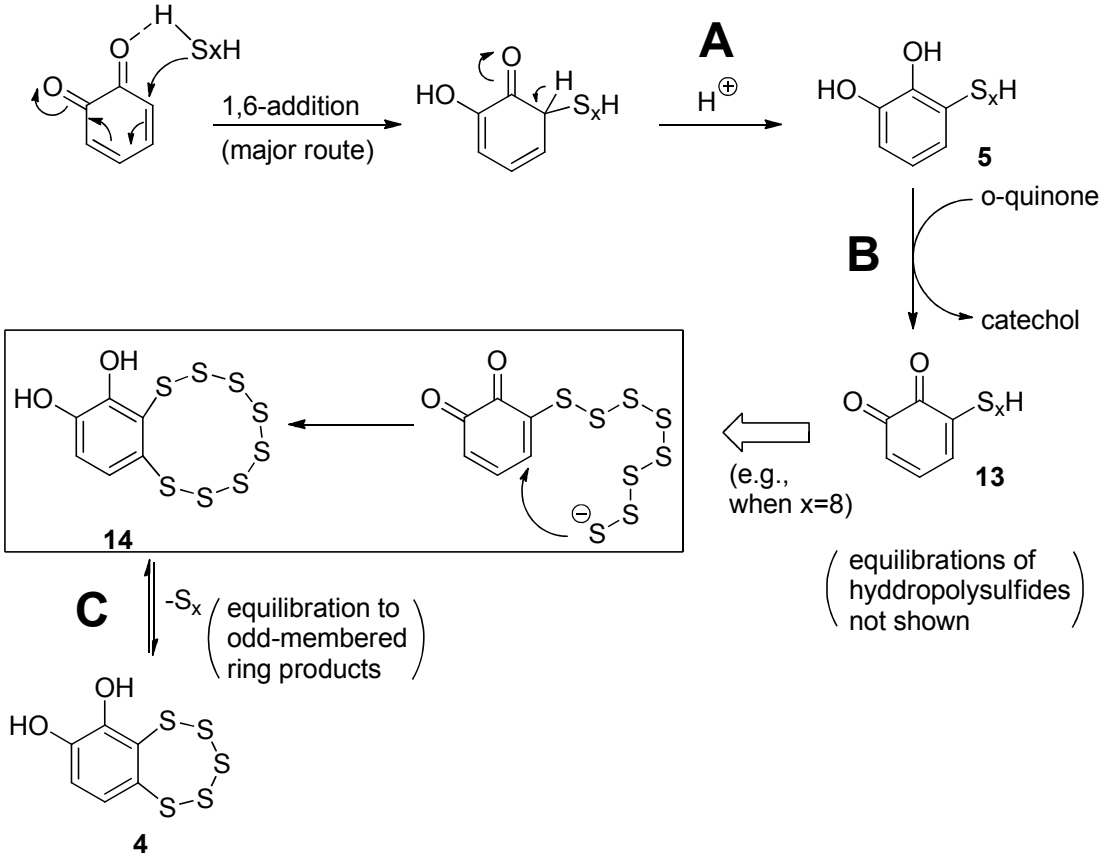
Of particular interest to us is the fact that **4** was formed in the quinone—H_2S_x reaction. The yield of **4** is low (3%), but it demonstrates the viability of the quinone—H_2S_x reaction in generating a benzopolysulfane. The quinone—H_2S_x reaction also gives by-products, namely 15% **5** (x=1), 5% **6** (x=1), 0.16% **7**, ~1% **8**, and ~2% **9**. Traces of known compounds **10**-**12** are observed (≤1%) (assigned on the basis of GC/MS analysis), which represent nucleophilic addition of catechol to the quinone. Acetylated **10**-**12** were obtained in higher yield (>5% total) in the absence of H_2S_x when quinone and catechol (1:1) were stirred in a 5% $NaHCO_3$ solution followed by a reaction with pyridine and acetic anhydride. Such nucleophilic additions to *o*-quinone are known.[21,26-30]

The NMR data pointed to diacetylated **4** not **4'** (Scheme 3). The ^1H NMR spectrum revealed two singlets corresponding to methyls of acetyl groups protecting two

OH groups adjacent to each other at 2.31 and 2.35 ppm and two doublets for H_1 and H_2 of the aromatic ring with δ=7.76 ppm $^3J_{13}$=8.4 Hz and δ=7.18 ppm $^3J_{13}$=8.4 Hz, respectively. The J_{13} coupling for **4** is comparable to that observed by Sato et al. for 6,7-dimethoxybenzopentathiepin (*J*=8.4 Hz, 1H, ArH).[31] The regioselective formation of the polysulfur ring at the C3-C4 bond of *o*-benzoquinone and the predominance of sulfur products arising from *o*-quinone 1,6-conjugate addition (e.g, **5**, **7**, and **8**) were not immediately understandable. This regiochemical discrimination for closure of the polysulfur ring at the C3-C4 bond leading to **4** rather than the C4-C5 bond leading to **4'** is interesting, which led us to consider the mechanism of formation.



Scheme 3. 1,2-Dihydroaxy Benzopentasulfane from Catechol



Scheme 4. Mechanism of Closure of the Polysulfide Ring 4

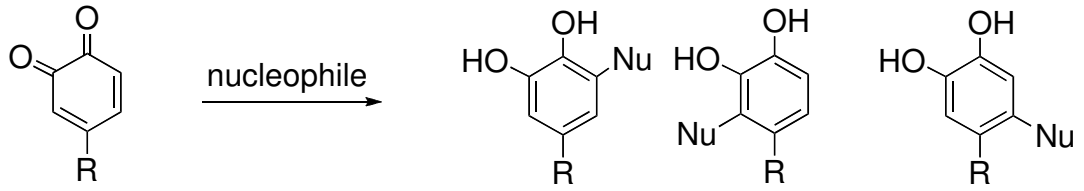
3.2.2 Mechanism of Formation of 4.

A route is proposed to reach **4** from *o*-quinone and reduced elemental sulfur. The experimental and theoretical evidence that support the mechanism outlined in Scheme 4, include the following:

(1) H_2S_x is more likely to react at C3 of *o*-quinone compared to C4 because of possible carbonyl H-bonding in the former, which enhances the nucleophilicity of H_2S_x (Table 1, reaction 1). As a consequence, 3- and 4-mercaptobenzene-1,2-diols [triacetylated **5** and **6** (x=1)] are found in the product mixtures in a 74:26 ratio. The

corresponding acetylated 3- and 4-hydrosulfidobenzene-1,2-diols [**5** and **6** ($x \geq 2$)] are assumed to be present, but are unstable to isolation.[32] The regioselectivity of the H_2S_x addition to *o*-quinone is in the same direction as the addition of thioacetic acid (reaction 2). Thioacetic acid was added to an acetone solution containing 0.3 equivalents of *o*-quinone, and indicated a preference for the 1,6-addition product to the 1,4-addition product (98:2). Similar biological examples exist where 1,6-addition processes are favored, such as the reaction of cysteine with dopamine-*o*-quinone in the formation of 5-*S*-cysteinyldopamine, which is relevant to the mechanism underlying *in vivo* neuronal degeneration[37-41] (Parkinson's disease) (Table 1, reaction 3 and 4).[42-45] In contrast, thioacetic acid in the presence of 5% $NaHCO_3$ (reaction 5), triazolium thiolate (reaction 6),[46] and thiourea (reactions 7 and 8)[47,48] readily undergo 1,4-addition reactions to quinones because these sulfur nucleophiles lack an ionizable S-H proton so that H-bonding to the quinone oxygen cannot take place.[49]

Table 1. Ratios of Catechol Products that Arise from Nucleophilic Addition to *o*-Benzoquinones

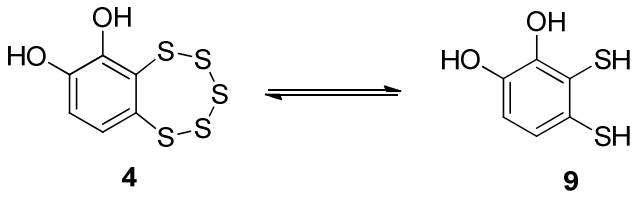


reaction	R	nucleophile	1,6-product(s)	1,4-product	Ref.
1	H	H_2S_x[a]	74	26	this work
2	H	$CH_3C(=O)SH$[b]	98	2	this work
3	$CH_2CH_2NH_2$	cysteine	~100	-	*d*
4	$CH_2CH_2NH_2$	*N*-acetylcysteine	99	-	*e*
5	H	$CH_3C(=O)S^{\ominus}$ [c]	-	~100	this work
6	H or CH_3	1,2,4-triazolium-3-thiolate	-	~100	*f*
7	CH_2CH_2NHAc	thiourea	-	~100	*g*
8	H or CH_3	thiourea	-	80-90	*h*

[a] NMR detection of products formed in acetone-d_6 at 25 °C after addition of H_2S_x to *o*-benzoquinone. [b] NMR detection of products formed in CD_3Cl at 25 °C after addition of thioacetic acid to *o*-benzoquinone. [c] NMR detection of products formed in acetone-d_6 at 25 °C after addition of H_2S_x and 5% aqueous $NaHCO_3$ to *o*-benzoquinone. [d] Relative ratios were not reported, however, the 5-S-cysteinyl conjugate of dopamine is the predominant product. Reference 44. [e] At pH 2 the

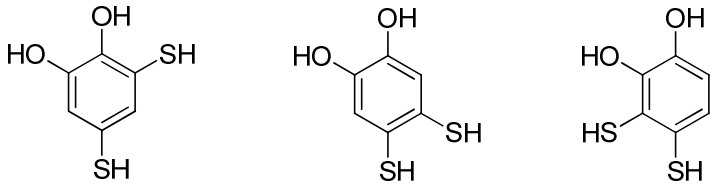
ratio of the two 1,6-products, 2-*S*-(*N*-acetylcysteinyl)dopamine to 5-*S*-(*N*-acetylcysteinyl)dopamine, is 10:89. A small amount of the di-1,6-adduct 2,5-*S,S'*-di(*N*-acetylcysteinyl)dopamine is also produced. Reference 45. A similar result is reported for reactions of *N*-acetyldopamine-*o*-quinone or *N*-β-alanyldopamine-*o*-quinone with *N*-acetylcysteine. Reference 47. [f] This mesoionic mercaptan adds to *o*-quinone and 4-methyl-*o*-quinone to form salt products. TsO^- is the counter salt. Reference 46. [g] Relative ratios were not reported, however, the 6-*S*-thiourea conjugate of *N*-acetyldopamine is the predominant product. A similar result is also reported for reactions of *N*-β-alanyldopamine-*o*-quinone with thiourea. Reference 47. [h] Based on isolated yields. The 1,6-products were not observed. Reference 48.

(2) 3,4-Dimercaptobenzene-1,2-diol **9** is present in the quinone—H_2S_x reaction mixture. However, other related dimercaptobenzene-1,2-diol isomers are not observed. 4,5-Dimercaptobenzene-1,2-diol (**15**), 3,5-dimercaptobenzene-1,2-diol (**16**), and 3,6-dimercaptobenzene-1,2-diol (**17**) are not found to within the experimental error for their detection, ~0.1 mM. The quinone—H_2S_x reaction contains a large excess of the H_2S_x, but does not show the production of the C3/C6 product **17**. A second 1,6-addition would have been expected with the anticipated regioselectivity to give **17**. Furthermore, we find that over the course of a few days the peak for **9** increases and concomitantly the magnitude of the peak for **4** decreases (Scheme 5), which is reminiscent of thiol—disulfide interconversions seen in many organic reactions and biological systems.[50] It is an important observation that **9** is derived from **4** in a decomposition process. Tri- and tetra-sulfur addition products of quinone and H_2S_x are also not detected.



4 **9**

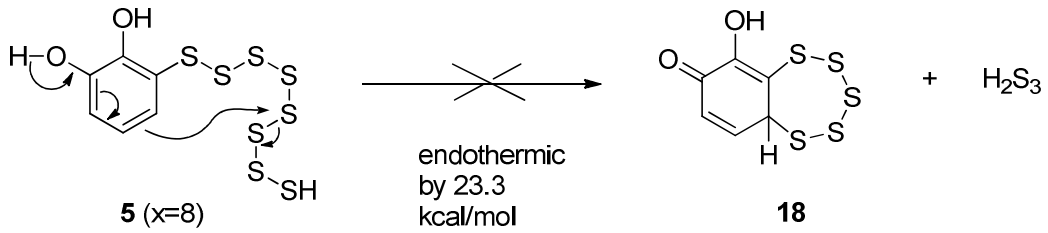
Scheme 5. Equilibration of 1,2 Dihydroxy Benzopentathiepin and Mercapto Catechol



15 **16** **17**

Scheme 6. Compounds Not Observed in the Reaction

(3) Control experiments show that there is no reaction between catechol and neutral S_8. Furthermore, a direct pathway to polysulfur ring closure of **5** (x=8) is predicted to be endothermic and probably irrelevant to the chemistry to form **4** based on energetic demands (Scheme 7). B3LYP/6-31G(d) calculations were used to predict the energetics of the alternative cyclization of **5**, where x = 8. We compared the energy difference of **5** (x=8) relative to 1-hydroxy-4a*H*-5,6,7,8,9-pentathiabenzocyclohepten-2-one (**18**) with H_2S_3. The control experiments with neutral S_8 and the computational results provide evidence that the new C-S bonds in **4**-**9** come only from reactions between quinones and reduced sulfur compounds.



Scheme 7. Unlikely Unimolecular Ring Closure Pathway

The above data taken together provide support for the mechanism in Scheme 4. H_2S_x incorporates regioselectively into *o*-benzoquinone to give 3-hydrosulfidobenzene-1,2-diols **5** (x=1-8). A second oxidation can then take place, where **5** is oxidized by unreacted *o*-benzoquinone producing **13** and catechol as a by-product (Scheme 4B). Unimolecular attack of the polysulfur chain ion onto the C4 of **13** should occur at some

point when the sulfur chain is sufficiently long, e.g, x=8. Formation of **4** is consistent with an oxidation of 3-polysulfidobenzene-diols with a dependence on the chain length of the 3-polysulfidoquinone thus leading to unimolecular ring closure. A subsequent equilibration between benzooctathiecin **14** and pentathiepin **4** (Scheme 4C) can be envisioned similar to one observed previously in an *o*-benzyne—S_8 reaction.[25] The odd-membered ortho benzofused polysulfane *o*-$(HO)_2C_6H_2S_x$ rings (except x = 1 which suffers from ring strain) are likely to possess enhanced conformational stability compared to the even-membered rings. Pentasulfane structures are quite common and often formed in polysulfur heterocycle syntheses.[51-62] Furthermore, the presence of nucleophiles can facilitate equilibria between **14** and stable **4**. Such nucleophile-dependent equilibria of polysulfane compounds[25,63-67] and difficulties in the isolation and purification of polysulfanes have been previously reported in the literature.[68,69]

An alternative mechanism that we deem unlikely is the bimolecular addition of H_2S_x with **5** (x=1-8) (followed by subsequent ring closure). The absence of **15**-**17** provides an argument for a unimolecular ring closure by 3-polysulfidoquinone with subsequent formation of **4**. The absence of **15**-**17** in the reaction is probably due to the lesser efficiency of a bimolecular reaction of H_2S_x with **13**. The mechanistic conclusions may be reasonable even though it is difficult to know exactly what is taking place due to the many equilibration processes and possible intermediate radicals that may be present in the formation of the polysulfanes. The quinone- H_2S_x reaction is reminiscent of thiol chemistry reported in the literature based on formation of sulfur-containing adducts of catechols,[38-42] although the present work describes the formation of polysulfanes. Catechol-amines can undergo autoxidation yielding semiquinones, quinones, protonated

superoxide ion, and H_2O_2 under aerobic conditions.[70-72] A competing 1,4-addition and radical addition reaction takes place in the reaction of HS^- with the *p*-quinone-containing natural product juglone, where the radical addition is favored at higher pH.[73] We have not investigated whether radical scavengers influence the *o*-quinone—H_2S_x reaction.

3.2.3 Conclusion.

The reaction of *o*-benzoquinone and H_2S_x leads to pentathiepin **4**, and several by-products. The proposed mechanism involves a unimolecular cyclization of 3-polysulfidoquinone intermediates **13**, e.g., where x=8. It is likely that the quinone—H_2S_x reaction can be used to synthesize natural product polysulfanes. We are currently exploring dopamine-*o*-quinone as an intermediate in an H_2S_x reaction to generate polysulfane **1**.

3.3 Experimental Section

3.3.1 General Aspects.

Reagents and solvents were available commercially [catechol, silver oxide, acetone, acetone-d_6, sodium sulfide nonahydrate, elemental sulfur (S_8), trichloroacetic acid, sodium sulfate (anhydrous), sodium periodate, carbon disulfide, TsOH, DMF, K_2CO_3, diethyl ether, THF, $CHCl_3$, MeOH, $NaHCO_3$, ethyl acetate, acetic anhydride, DMAP, sodium bicarbonate, hydrochloric acid (12 M), sulfuric acid (1 M), thioacetic acid, magnesium sulfate, ethanol, $CHCl_3$, $CDCl_3$, CH_3CN, and hexane] and used as received without further purification. Purification of product mixtures were carried out using silica gel 60F 254 TLC-plates and an HPLC instrument equipped with a C18

column. Proton NMR data acquired at 400 MHz and ^{13}C NMR data acquired at 100.6 MHz. HRMS, UV-visible, IR, and GC/MS data were collected. Examination of temperature programs were used in an effort to limit polysulfide decomposition on the GC/MS injection port.[74] Comparison of integrated peaks of the compounds provided relative percent yields in the ^{1}H NMR studies.

3.3.2 Experimental Procedures.

***Ortho*-benzoquinone** was generated by a literature method.[75] A solution of catechol (0.1g, 0.9 mmol) and Ag_2O (0.4 g, 1.8 mmol) in acetone (5 mL) was stirred for 10 min. Ag_2O was removed from the solution by gravity filtration. Silver is also presumably filtered out of the acetone solution since we do not see the formation of a silver mirror. In some cases, *o*-benzoquinone was generated by a different literature method,[76] where two solutions were stirred together [3 g (27 mmol) catechol in 150 mL water and 1.1 equivalents (6.2 g, 29 mmol) of $NaIO_4$ in 100 mL of water] and cooled to 0º C in an ice bath. *o*-Benzoquinone is stable for 3-4 hours according to ^{1}H NMR, therefore, it is used immediately after preparation. Inorganic polysulfanes H_2S_x were prepared by the modified method of Steudel.[77] Two grams of sodium sulfide nonahydrate was dissolved in 2 mL of water with 1 g of S_8 and heated to 100 °C over night. To the solution of polysulfanes [$NaS(S)_xSNa$] was added an aqueous solution of Cl_3CCO_2H (4 g in 3 mL of H_2O, 8 mM), which was stirred at 0 ^{o}C. Since the first dissociation constants (pK_1) of H_2S_x molecules in water at 20 °C range from 7.0 to 3.5,[78] H_2S_x precipitated as a yellow oil and was separated from the aqueous phase, and then dissolved in 1 mL of CS_2. With stirring, the CS_2 solution of H_2S_x was added drop wise to

the acetone solution containing 180 mM quinone, where the precipitation of elemental sulfur took place. Elemental sulfur (S_8) and other insoluble material were removed by gravity filtration. Acetone and CS_2 were also then removed by rotary evaporation leaving a light brown viscous liquid.

The acetylation reaction. The quinone—H_2S_x product mixture was dissolved in 3.6 mL ethyl acetate, followed by addition of 0.2 eq (0.006 g, 0.18 mmol) DMAP and (230 μL, 2.52 mmol) acetic anhydride. After stirring for 16 h, the mixture was diluted with 5 mL ethyl acetate, washed twice with 10 mL saturated $NaHCO_3$ and brine, dried with anhydrous Na_2SO_4, and then separated by preparative HPLC and/or preparative TLC.

The reaction of quinone with thioacetic acid was conducted via two different methods depending on the regioselectivity desired. Method 1: With stirring 3 g (27 mmol) catechol was dissolved in 150 mL water and the solution cooled in an ice bath. To this solution was added 1.1 equivalents (6.2 g, 29 mmol) of $NaIO_4$ in 100 mL of water. The dark red aqueous solution containing the *o*-benzoquinone was then extracted twice with CH_2Cl_2. Thioacetic acid (2 mL, 28 mmol) was dissolved in 100 mL of CH_2Cl_2 and added drop wise to *o*-benzoquinone (18 mmol) in 60 mL of CH_2Cl_2 and stirred for an additional 20 minutes. The organic layer is filtered and dried with sodium sulfate and CH_2Cl_2 is removed by rotary evaporation. Method 2: Thioacetic acid (2 mL, 28 mmol) was added to 100 mL 5% $NaHCO_3$ solution. Evolution of CO_2 was noted, as well as a solution color change from clear to light yellow upon dissolving of the thioacetic acid. The solution containing thioacetic acid was added to *o*-benzoquinone (18 mmol) in 60 mL acetone and stirred for 20 minutes. The resulting mixture was then acidified with 100

mL of 4 N HCl and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was dried with sodium sulfate and CH_2Cl_2 was removed by rotary evaporation. Adding of 2 g of the acetylated sulfur-containing catechols to 60 mL of 4 N NaOH, followed by refluxing under Ar for 20 minutes, led to their hydrolysis. The solution was allowed to cool to room temperature and then acidified with 4 N HCl. Extraction with CH_2Cl_2 (3 × 25 mL) followed by rotary evaporation yielded a red viscous oil (98% Yield).

Acetylated 10-12 were generated in a reaction of *o*-benzoquinone (1.0 g, 9.2 mmol), generated from Ag_2O with catechol (1.0 g, 9.0 mmol) in a solution of 5% $NaHCO_3$. The reaction was stirred for 20 minutes, and subsequently acidified with 100 mL of 4 N HCl. Extractions were conducted with CH_2Cl_2 (3 × 30 mL), followed by a reaction with pyridine and acetic anhydride.

3.3.3 Characterization of compounds 4-12.

5,6,7,8,9-Pentathiabenzocycloheptene-1,2-diol (4) was separated by preparative silica gel TLC R_f=0.5 EtOAc:hexanes (1:3). Mass spectrum (EI); *m/z* = 64 (43), 96 (12), 110 (48), 142 (66), 204 (100), 268 (10).

Acetic acid 2-acetoxy-5,6,7,8,9-pentathia-benzocyclohepten-1-yl ester or "diacetylated 4". Yield 0.0007 g. Silica gel TLC, R_f=0.4 (THF:hexanes, 1:3); ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H), 2.35 (s, 3H), 7.18 (d, 1H, *J* = 8.4 Hz), 7.76 (d, 1H, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 20.4, 20.6, 117.8, 124.8, 125.5, 133.7, 142.5, 144.5, 167.7, 167.8. UV (CH_2Cl_2) λ_{max} 232 nm. Mass spectrum (EI); *m/z* = 64 (23), 102 (10), 131 (21), 139 (15), 172 (6), 204 (100), 246 (34), 288 (26), 352 (0.9). Peaks at 246 and 204 represent the loss of two ketene CH_2=C=O fragments.

3-Mercaptobenzene-1,2-diol (5). ^{1}H NMR (acetone-d_6) δ 4.03 (s, 1H), 6.60 (t, *J* = 7.82 Hz, 1H), 6.66 (dd, *J* = 1.6, 7.8 Hz, 1H), 6.74 (dd, *J* = 1.6, 7.8 Hz, 1H), 7.76 (s, 1H), 8.50 (s, 1H).

Acetic acid 2-acetoxy-3-acetylsulfanyl-phenyl ester or "triacetyltated 5." Yield 0.0006 g. Triacetylated **5** was isolated from silica gel TLC R_f=0.42 (EtOAc:hexanes, 1:3) from the quinone—H_2S_x reaction or via recrystallization in chloroform as white crystals (mp 80-83 °C) from the quinone—thiolacetic acid reaction (Method 1, Supporting Information section). The ^{1}H NMR spectrum of triacetylated **5** shows three singlets corresponding to methyls of the acetyl groups on 2 OH and SH groups adjacent to each other at 2.27, 2.29 and 2.41 ppm respectively. ^{1}H NMR (acetone-d_6) δ 2.27 (s, 3H), 2.29 (s, 3H), 2.41 (s, 3H) 7.37-7.46 (m, 3H); ^{13}C NMR (acetone-d_6) δ 20.2, 20.3, 30.1, 124.6, 126.4, 134.5, 144.6, 168.1, 168.5, 192.0. IR (KBr) ν 1765, 1703, 1466, 1368, 1262, 1213, 1005 cm^{-1}. Mass spectrum (EI); *m/z* = 83 (2), 96 (1), 113 (2), 142 (100, M-3Ac), 184 (55, M-2Ac), 226 (27, M-Ac), 268 (8). HRMS calcd for $C_{12}H_{12}O_5S$ $(M + H)^+$ 269.0478, found 269.0476.

4-Mercaptobenzene-1,2-diol (6). ^{1}H NMR (acetone-d_6) δ 3.10 (s, 1H), 6.68 (dd, *J* = 2.1, 8.2 Hz, 1H), 6.73 (d, *J* = 8.2 Hz, 1H), 6.84 (d, *J* = 2.1 Hz, 1H), 8.03 (s, 1H), 7.93 (s, 1H).

Acetic acid 2-acetoxy-5-acetylsulfanyl-phenyl ester or "triacetylated 6" was prepared from the quinone—H_2S_x reaction and the quinone—thiolacetic acid reaction (Method 2). Purification was accomplished by column chromatography using EtOAc:hexanes (1:4). Yellow crystals were obtained: mp 79-82 °C. ^{1}H NMR (acetone-d_6) δ 2.47 (s, 3H), 2.48 (s, 3H), 2.62 (s, 3H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.51 (s, 1H), 7.54

(dd, J = 2.1, 8.4 Hz, 1H); ^{13}C NMR (acetone-d_6) δ 19.5, 19.5, 29.2, 124.0, 126.0, 129.3, 132.6, 142.4, 143.3, 168.0, 193.0. IR (KBr) ν 1773, 1752, 1703, 1491, 1364 cm^{-1}. Mass spectrum (EI); *m/z* = 268 (7), 226 (39, M-Ac), 184 (48, M-2Ac), 142 (100, M-3Ac). HRMS calcd for $C_{12}H_{12}O_5S$ $(M + H)^+$ 269.0478, found 269.0478.

Acetic acid 2-acetoxy-6-(2,3-diacetoxy-phenylsulfanyl)-phenyl ester "tetraacetylated 7." Yield 0.0012 g. Silica gel TLC R_f = 0.48 (2:3 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3H), 2.29 (s, 3H), 7.12-7.16 (m, 2H), 7.44 (dd, J = 8.0 Hz, J = 1.5 Hz, 1H). UV (CH_2Cl_2) λ_{max} 280 nm, 321 nm. Mass spectrum (EI); *m/z* = 110 (20), 142 (11), 250 (100, M-4Ac), 292 (60, M-3Ac), 334 (41, M-2Ac), 376 (19, M-Ac), 418 (12).

Acetic acid 2-acetoxy-3-(2,3-diacetoxy-phenyldisulfanyl)-phenyl ester or "tetraacetylated 8". Yield 0.0048 g. Silica gel TLC R_f =0.42 (2:3 EtOAc:hexanes); ^{1}H NMR (acetone-d_6) δ 2.28 (s, 6H), 2.30 (s, 6H), 7.25 (dd, J = 1.5, 7.3 Hz, 2H), 7.34 (t, J = 7.3 Hz, 2H), 7.51 (dd, J = 1.5, 7.3 Hz, 2H); ^{13}C NMR (acetone-d_6) 20.1, 20.5, 124.5, 127.8, 131.7, 142.0, 144.4, 168.2, 168.6. UV (CH_2Cl_2) λ_{max} 280 nm, 320 nm. Mass spectrum (EI); *m/z* = 83 (6), 111 (10), 142 (71), 184 (22), 282 (100, M-4Ac), 324 (60, M-3Ac), 366 (91, M-2Ac), 408 (29, M-Ac), 450 (41). HRMS calcd for $C_{20}H_{18}O_8S_2$ $(M + NH_4)^+$ 468.0781, found 468.0784.

3,4-Dimercapto-benzene-1,2-diol or "tetraacetylated 9." ^{1}H NMR 400 MHz (acetone-d_6) δ 2.30 (s, 3H), 2.31 (s, 3H), 2.42 (s, 3H), 2.42 (s, 3H), 7.22 (d, J = 8.7 Hz, 1H), 7.40 (d, J = 8.7 Hz, 1H). Mass spectrum (EI); *m/z* = 342 (3), 300 (44, M-Ac), 258 (55, M-2Ac), 216 (70, M-3Ac), 174 (100, M-4Ac).

Acetic acid 3,2',3'-triacetoxy-biphenyl-2-yl ester or "tetraacetylated 10. " Catechol acts as an ambident nucleophile in a reaction between the carbanion tautomer of catechol with *o*-quinone. Mass spectrum (EI); *m/z* = 386, 344 (M-Ac), 302 (M-2Ac), 260 (M-3Ac), 218 (M-4Ac).

Acetic acid 2-(3,4-diacetoxy-phenoxy)-phenyl ester or "triacetylated 11." Mass spectrum (EI); *m/z* = 344, 302 (M-Ac), 260 (M-2Ac), 218 (M-3Ac).

Acetic acid 1-acetoxy-dibenzo[1,4]dioxin-2-yl ester or "diacetylated 12." Mass spectrum (EI); *m/z* = 300, 258 (M-Ac), 216 (M-2Ac).

Description of the geometry of the stationary point of **5** (x=8) (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -3568.2238471

0 1

C	-3.39555400	0.82480700	0.17681900
C	-3.32644900	-0.31321100	-0.63816200
C	-4.14610500	-1.41933500	-0.34260600
C	-5.01968800	-1.38892600	0.73621500
C	-5.08879300	-0.24773100	1.54456500
C	-4.28172300	0.84917200	1.26779600
H	-5.77223300	-0.22596200	2.38743800
H	-5.64538600	-2.25382800	0.94752600
O	-2.47936700	-0.36315100	-1.69562900
H	-2.57560000	-1.23934400	-2.10939900

O	-3.99590800	-2.48163500	-1.20402200
H	-4.56937400	-3.21364600	-0.93179400
S	-2.43618300	2.27878300	-0.21816100
S	-0.56963200	1.97431400	0.70223100
S	0.77058700	1.16843200	-0.70379000
S	0.67580800	-0.93432700	-0.64567300
S	1.88393700	-1.62200500	0.92683800
S	3.86265100	-1.81527500	0.23167100
S	4.90951000	-0.08393800	0.79415000
S	4.81528500	1.29792000	-0.77562700
H	-4.32483800	1.73857400	1.88748700
H	3.64810100	1.90136800	-0.43803700

Description of the geometry of the stationary point of **19** (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -2372.4202041

0 1

C	-0.91333597	0.97251739	1.64888051
C	-0.35433597	-0.27848261	2.01288051
C	0.05366403	-1.16148261	1.02088051
C	-0.05733597	-0.84748261	-0.34511949
C	-0.62833597	0.38551739	-0.71511949
C	-1.08633597	1.28251739	0.29288051
S	-0.77233597	0.77751739	-2.46111949
S	0.75166403	2.18651739	-2.83111949
S	-0.16433597	4.06051739	-2.54811949
S	-0.47033597	4.16851739	-0.46211949
S	-1.88733597	2.84851739	-0.07011949
O	-1.29433597	1.87351739	2.65288051
O	-0.27233597	-0.52348261	3.38988051
H	0.46366403	-2.12748261	1.29188051
H	-1.12801495	1.45078479	3.47582307
H	0.29254027	-1.57197382	-1.05052654
H	-1.57557813	-0.07416471	-0.52452922

Description of the geometry of the stationary point of H_2S_3 (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -1195.7664858

S	-1.70392200	-0.38318900	-0.08585000
H	-1.88405200	-0.58399200	1.24215100
S	-0.00001600	0.83936400	0.00000300
S	1.70392000	-0.38317900	0.08584200
H	1.88434800	-0.58394300	-1.24207600

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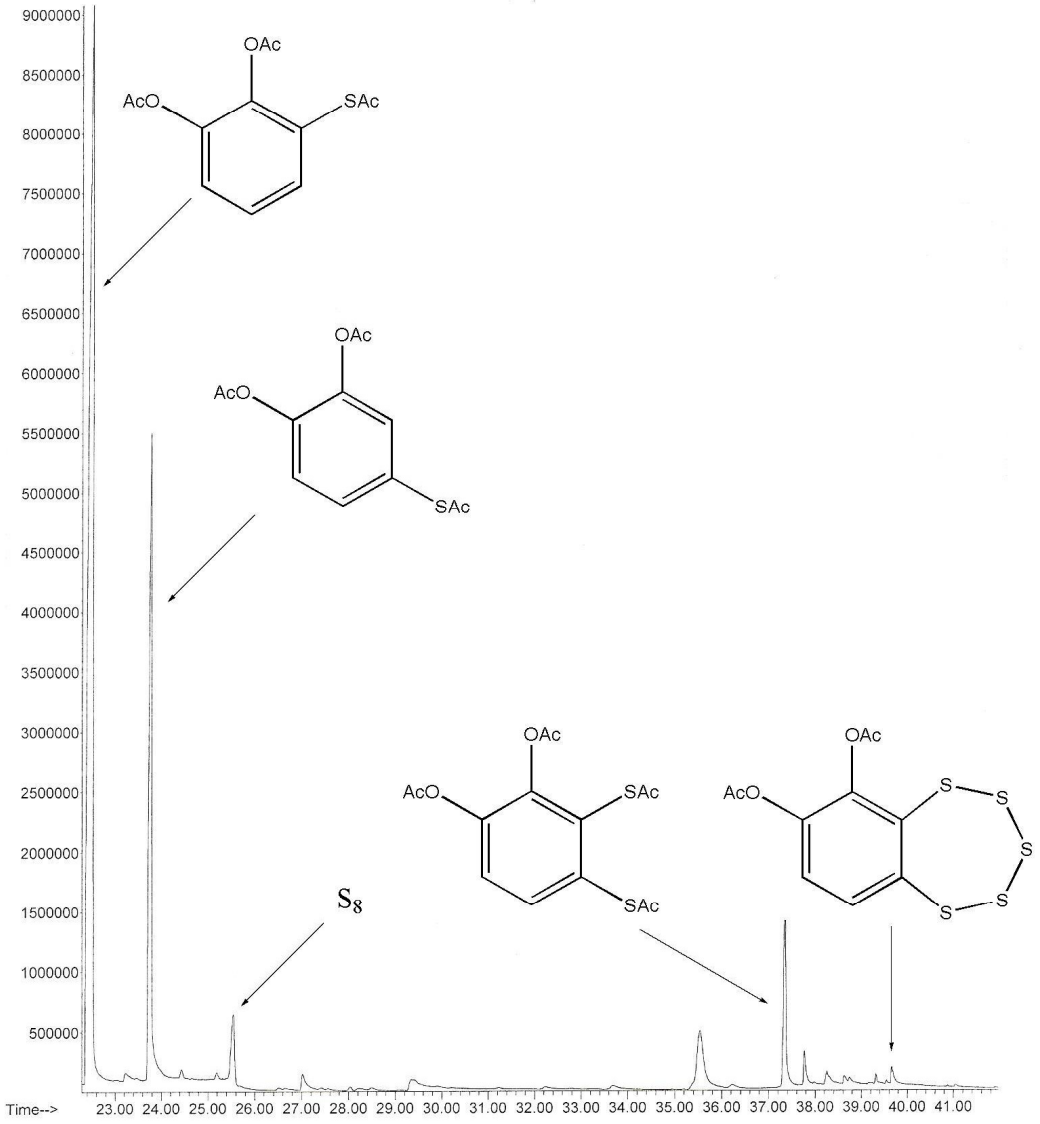

Figure 2. GC/MS trace of H_2S_x + *o*-benzoquinone acetylated reaction mixture.

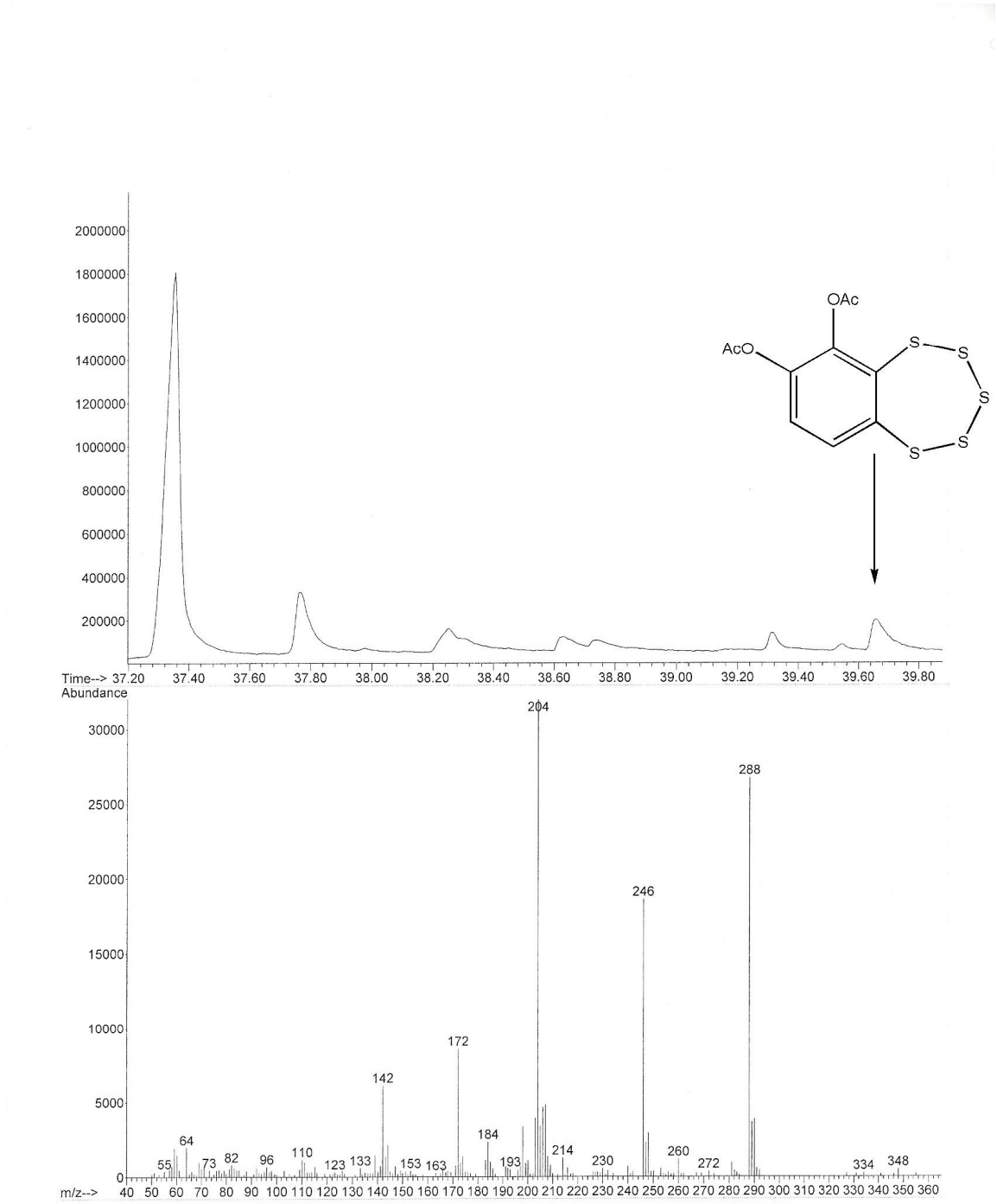

Figure 3. Mass spectrum for Acetic acid 2-acetoxy-5,6,7,8,9-pentathia-benzocyclohepten-1-yl ester or "diacetylated **4**."

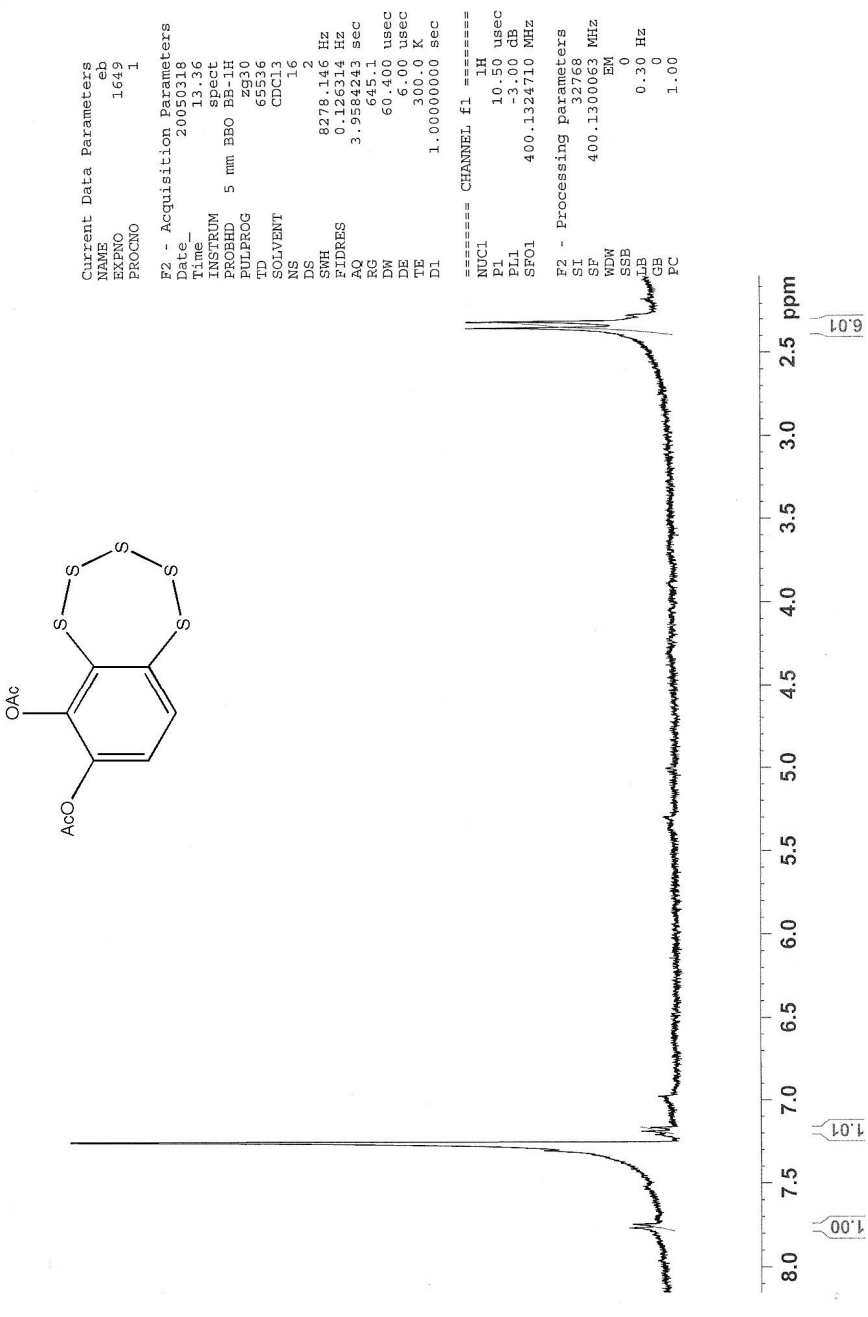

Figure 4. ^{1}H NMR of acetic acid 2-acetoxy-5,6,7,8,9-pentathia-benzocycloheptен-1-yl ester or "diacetylated **4**" in $CDCl_3$.

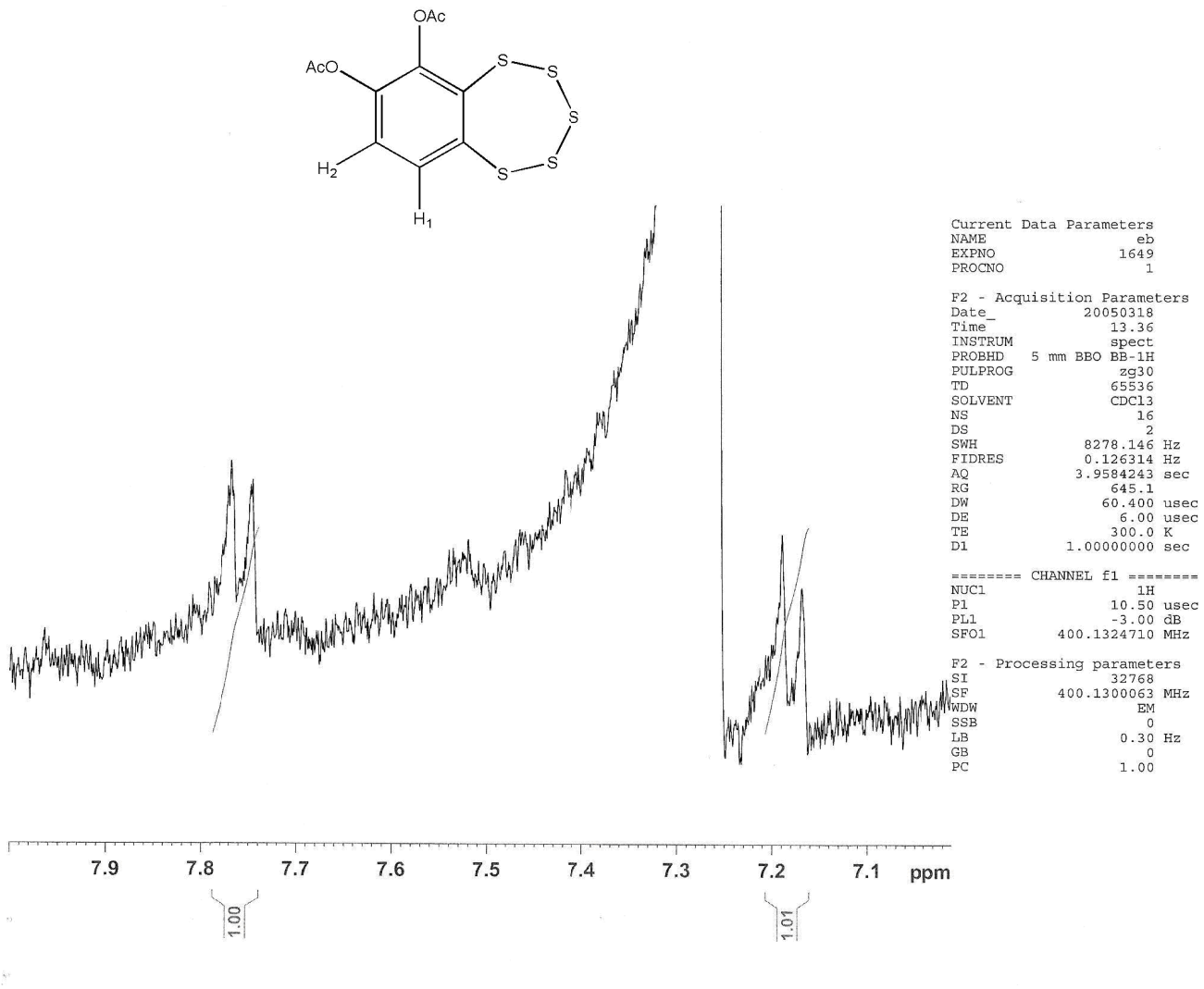

Figure 5. ^{1}H NMR of acetic acid 2-acetoxy-5,6,7,8,9-pentathia-benzocyclohepten-1-yl ester or "diacetylated **4**" in $CDCl_3$ expanded to show aromatic protons.

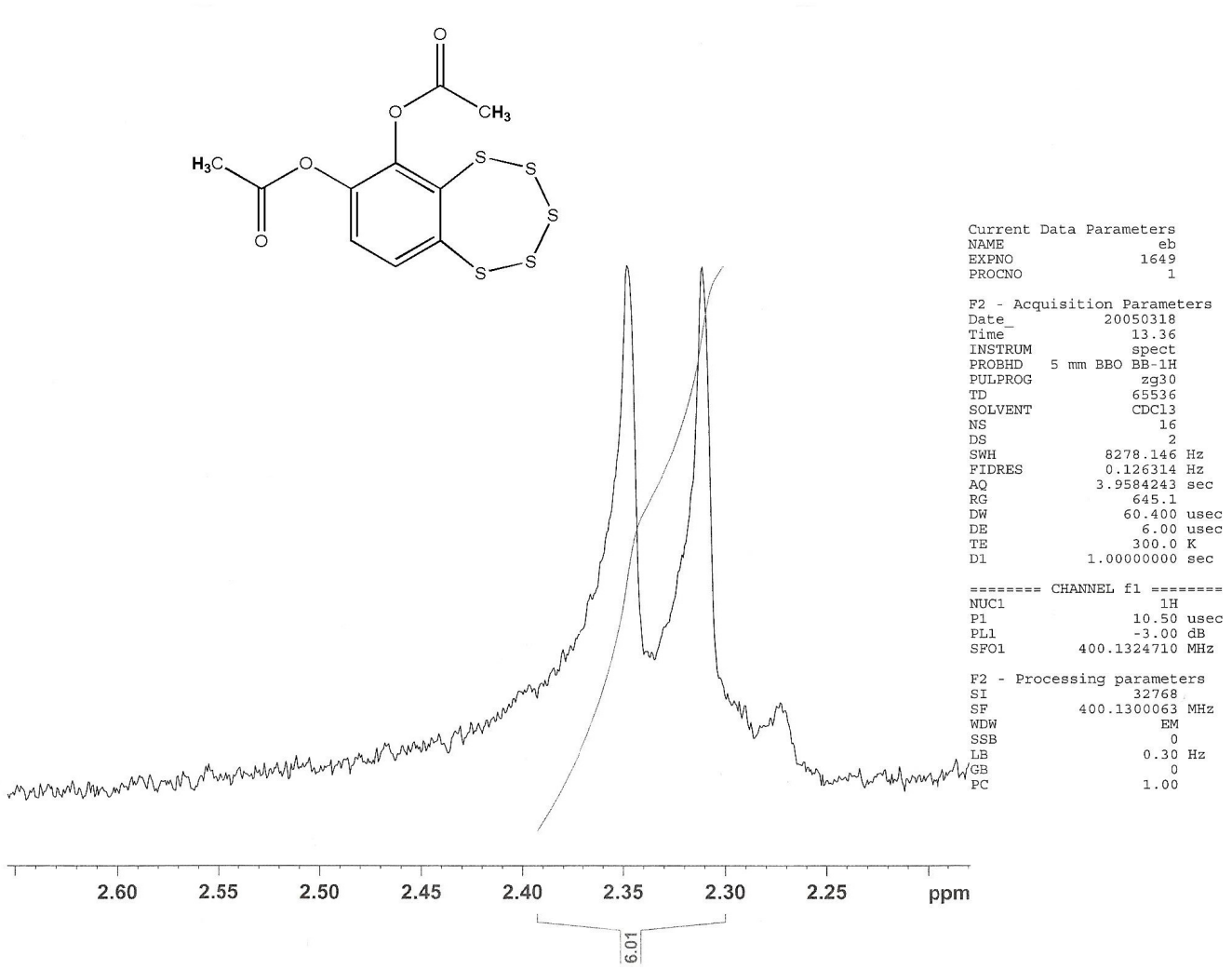

Figure 6. ^{1}H NMR of acetic acid 2-acetoxy-5,6,7,8,9-pentathia-benzocyclohepten-1-yl ester or "diacetylated **4**" in $CDCl_3$ expanded to show acetyl protons.

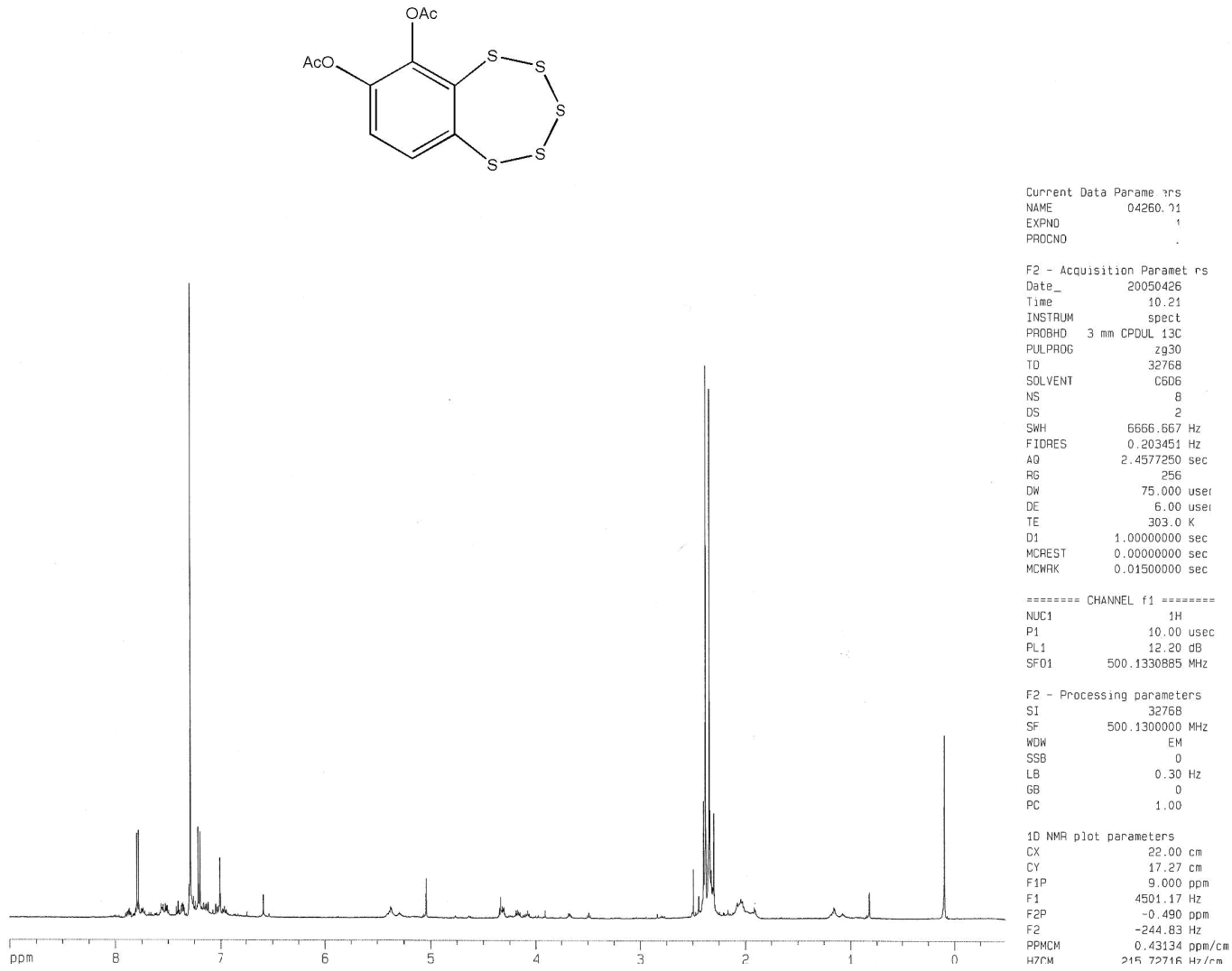

Figure 7. ^{1}H NMR of acetic acid 2-acetoxy-5,6,7,8,9-pentathia-benzocyclohepten-1-yl ester or "diacetylated **4**" in C_6D_6.

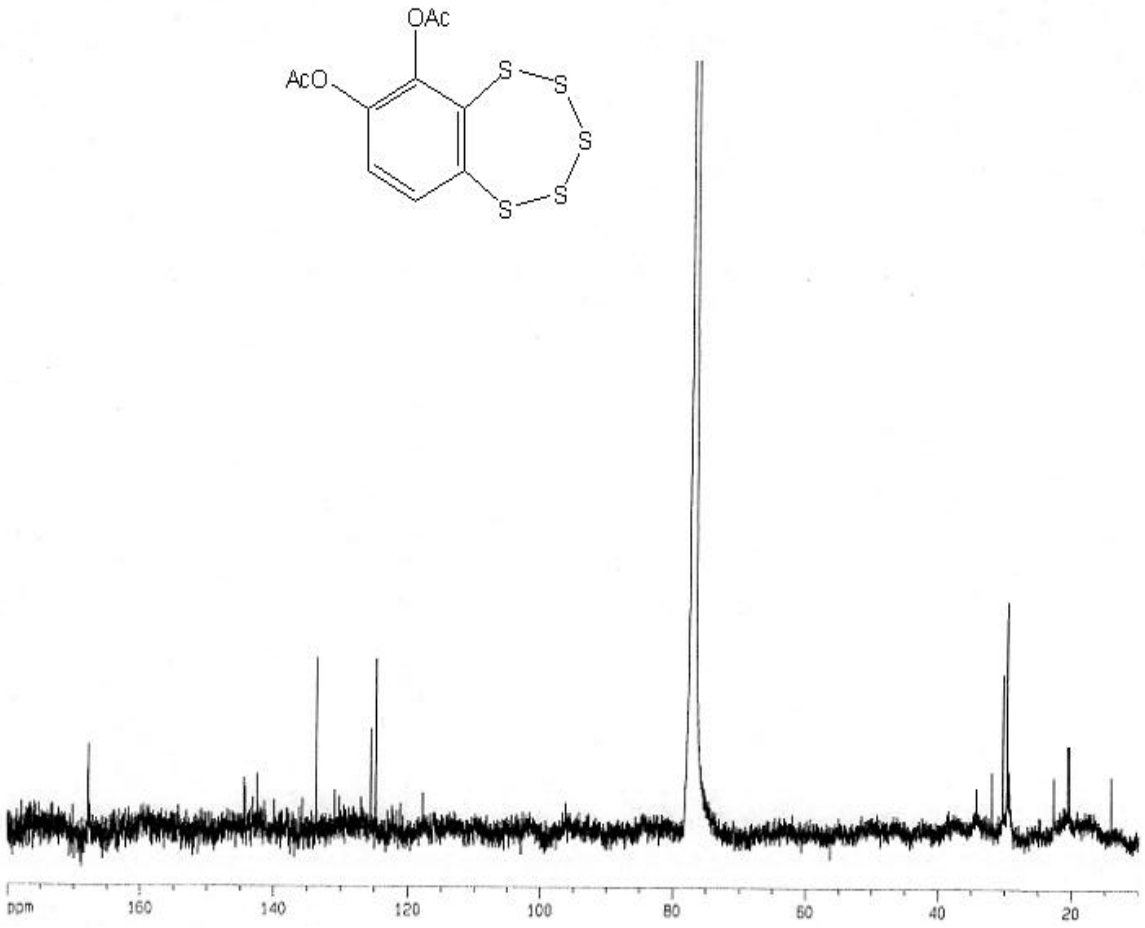

Figure 8. ^{13}C NMR of acetic acid 2-acetoxy-5,6,7,8,9-pentathiabenzocyclohepten-1-yl ester or "diacetylated **4**" in $CDCl_3$.

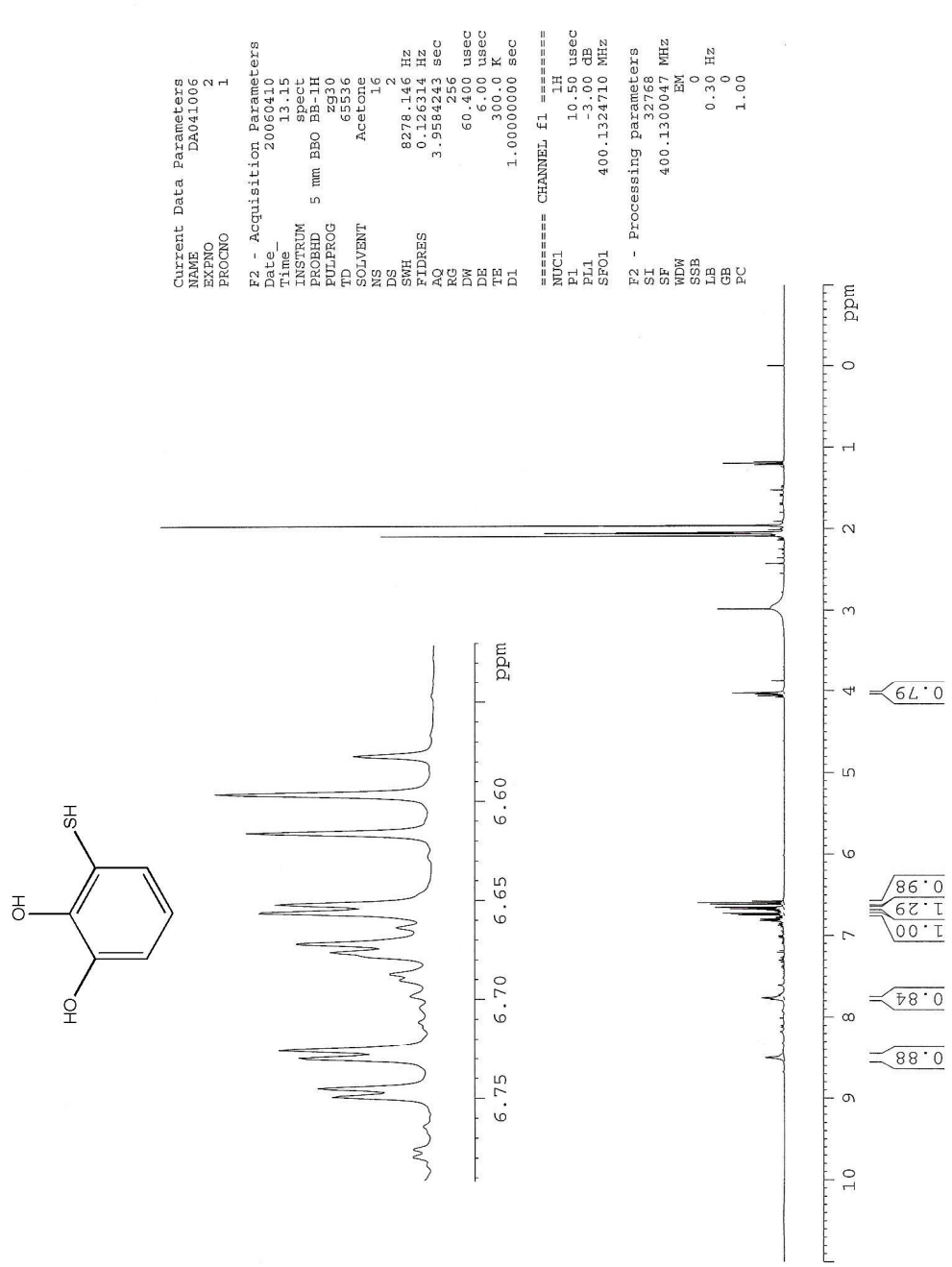

Figure 9. ^{1}H NMR of 3-mercaptocatechol, **5**, in acetone-d_6.

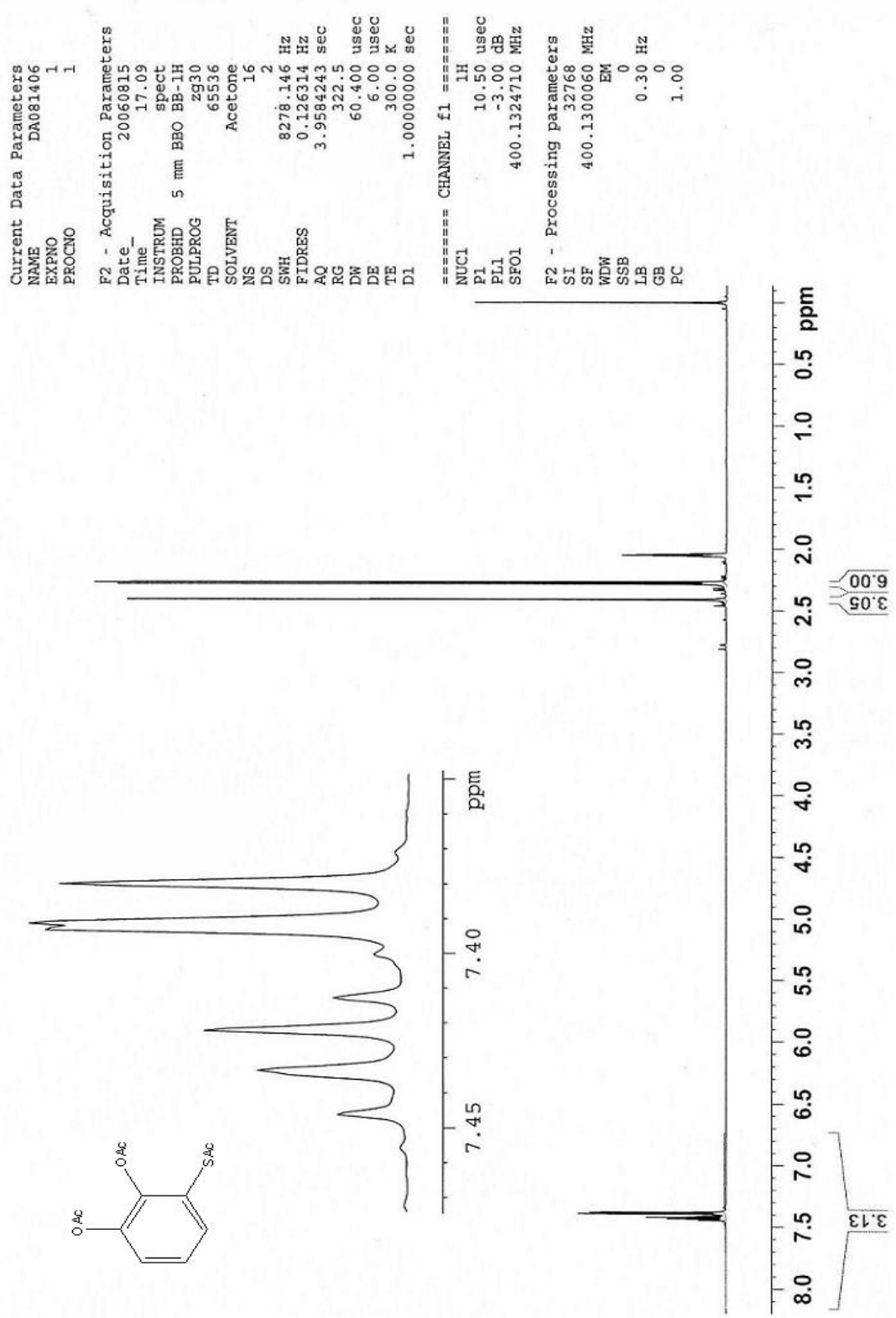

Figure 10. ^{1}H NMR of acetic acid 2-acetoxy-3-acetylsulfanyl-phenyl ester or "triacetylated **5**" in acetone-d_6.

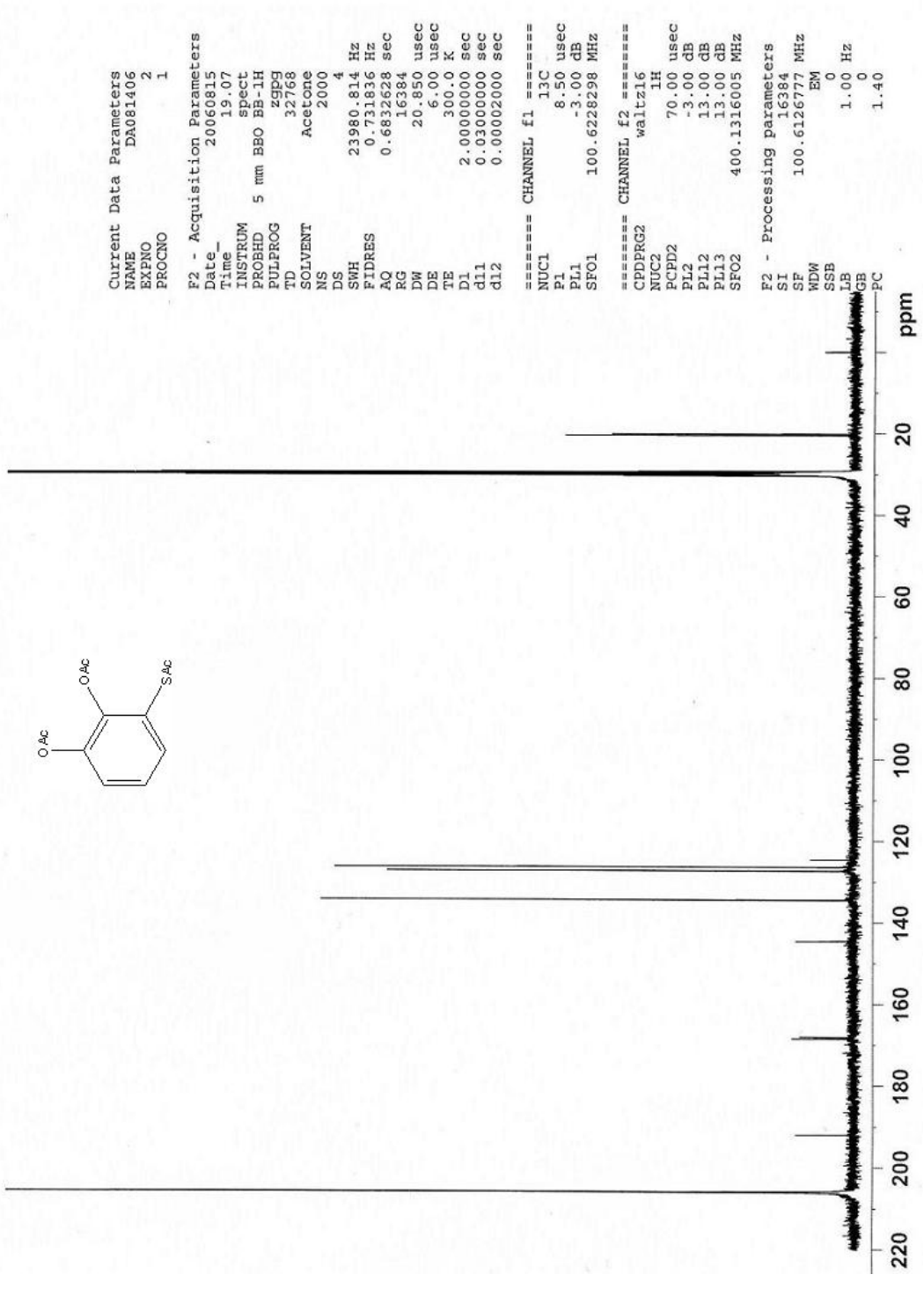

Figure 11. ^{13}C NMR of acetic acid 2-acetoxy-3-acetylsulfanyl-phenyl ester or "triacetylated **5**" in acetone-d_6.

Empirical Formula Confirmation Report

Sample Name: 3-mercaptocatechol Sample Location: P1-A1 Sample Id: Operator:
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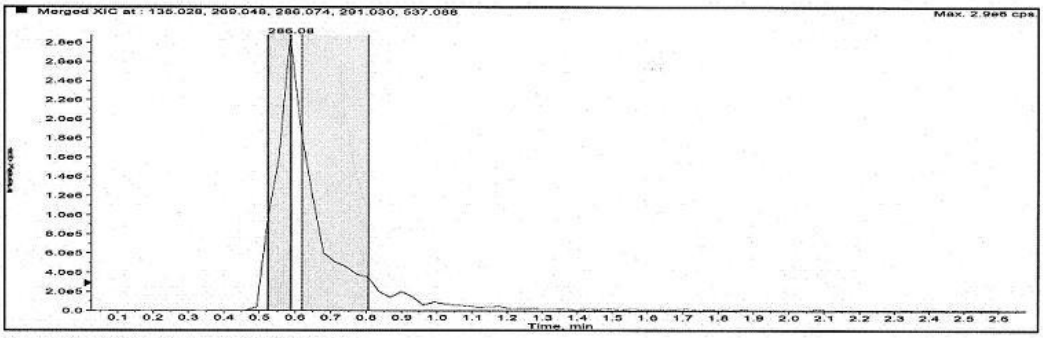
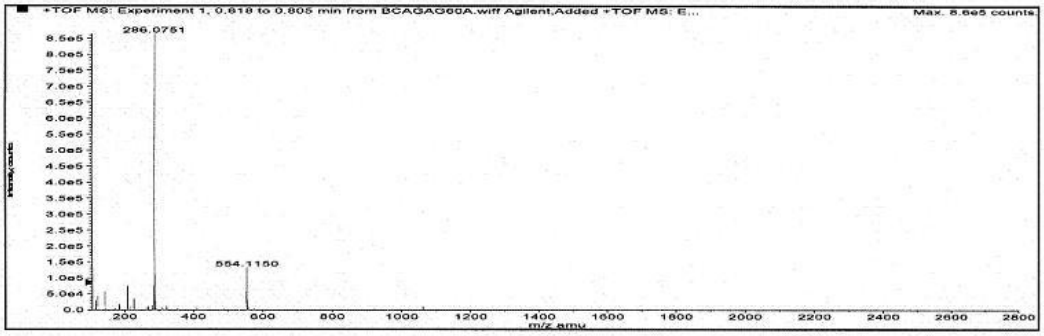
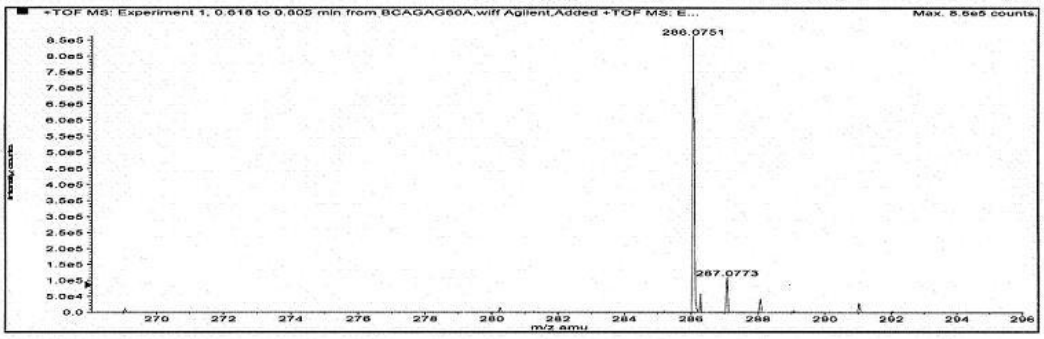
Method: D:\TOF_Data\damethods\efcHC4.anm\efc.xml



Merged XIC, Period# : 1 Experiment# : 1





Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C12H12O5S1	--	268.04054	0.59	1.91161 E7	--

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	11805.93	269.04782	269.04757	-0.25124	-0.93	--
[M+NH4]+	865896.91	286.07437	286.07514	0.77348	2.70	--
[M+Na]+	31548.26	291.02977	291.02974	-0.02675	-0.09	--

Figure 12. : HRMS of acetic acid 2-acetoxy-3-acetylsulfanyl-phenyl ester or "triacetylated **5**."

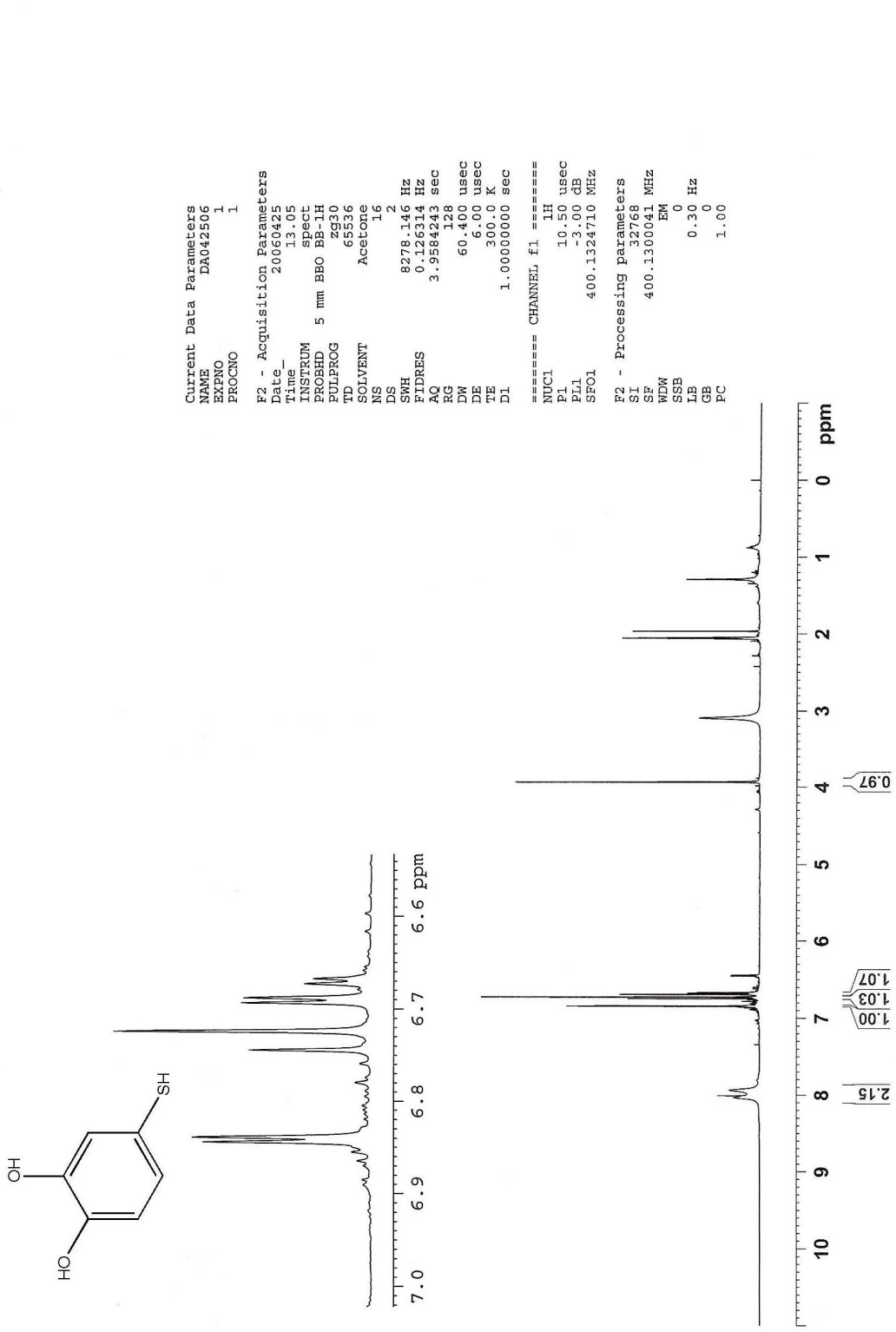


Figure 13. ^{1}H NMR of 4-Mercaptocatechol, **6**.

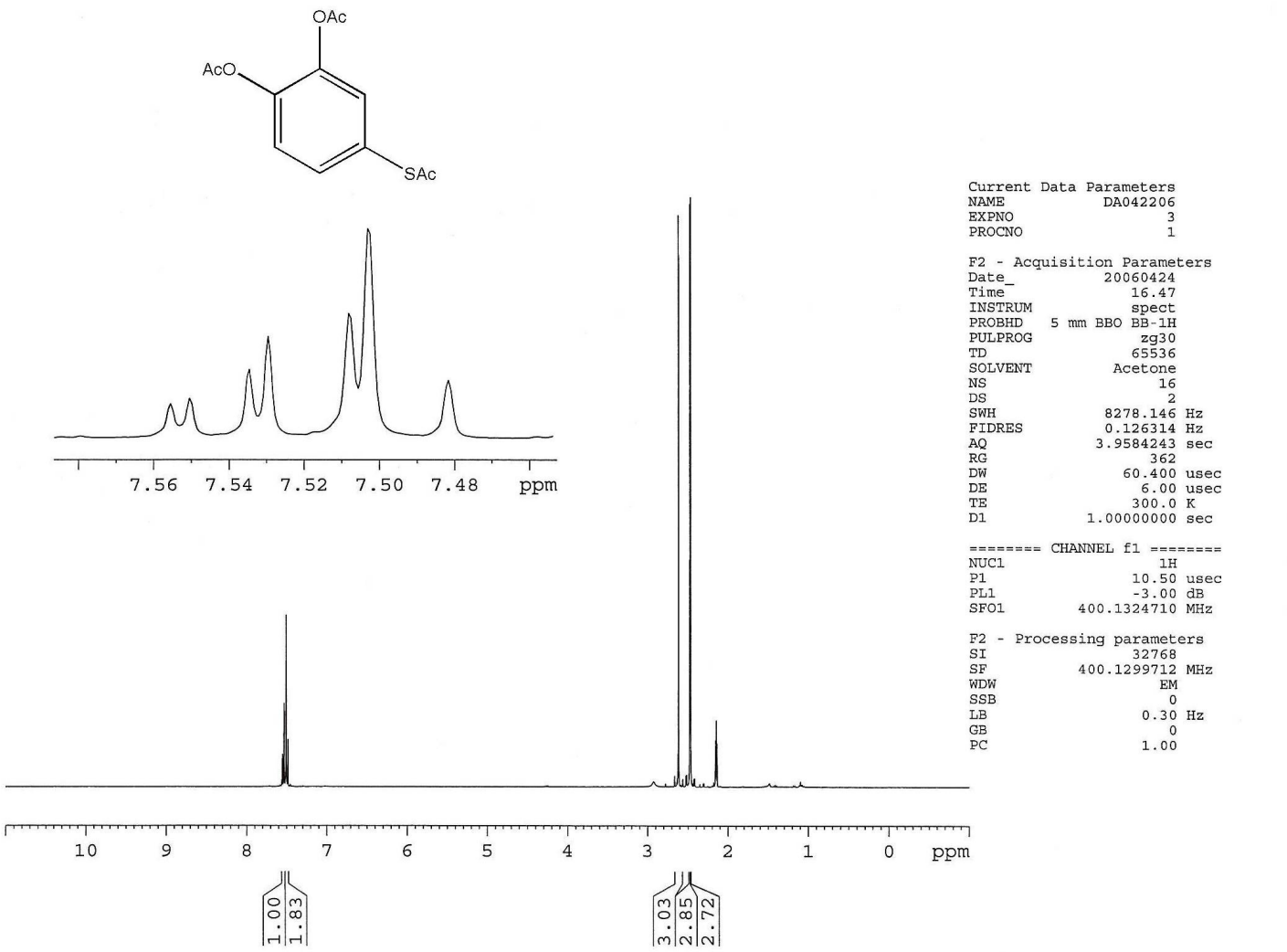


Figure 14. ^{1}H NMR of acetic acid 2-acetoxy-4-acetylsulfanyl-phenyl ester or "triacetylated **6**" in acetone-d_6.

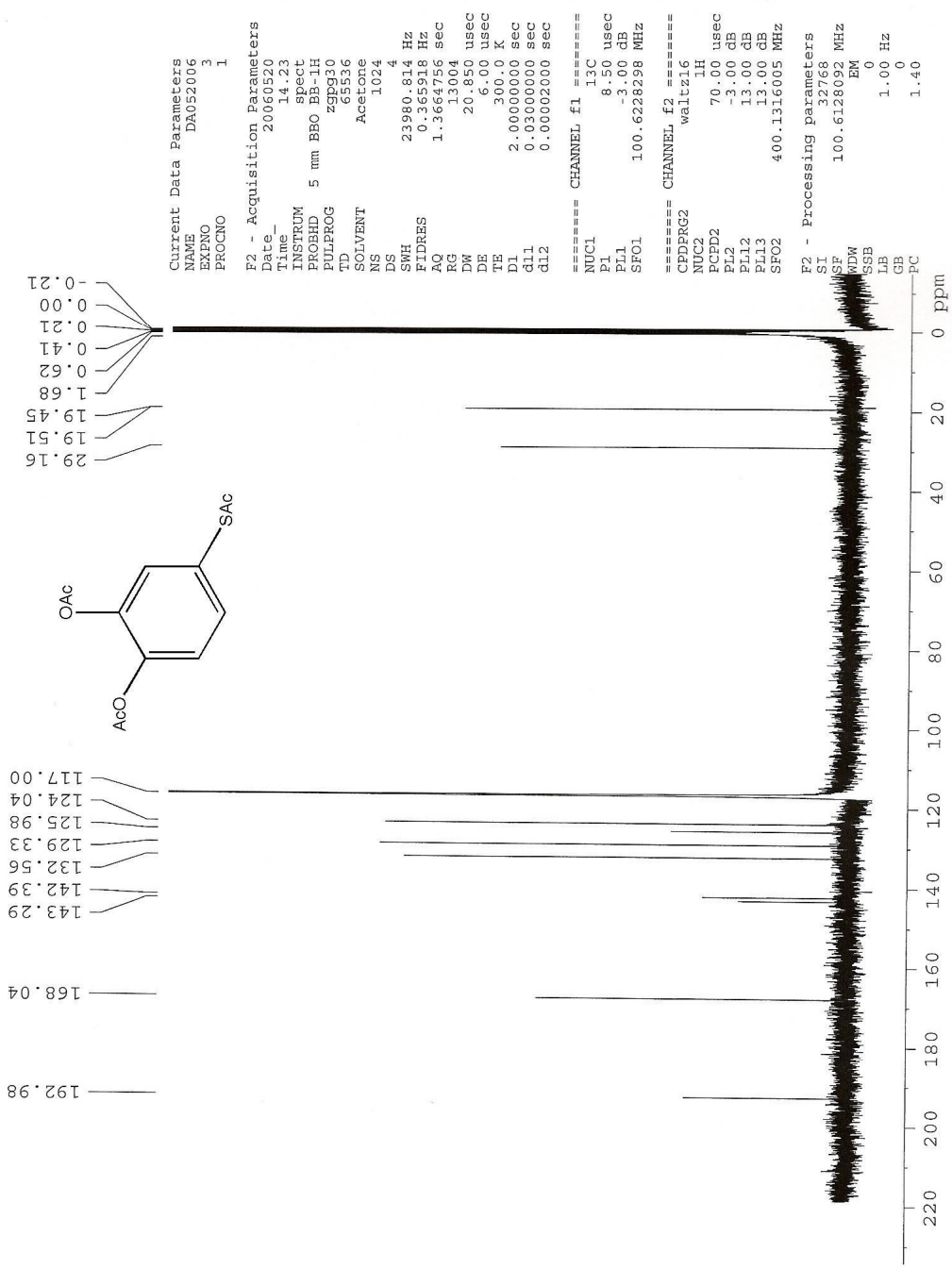


Figure 15. ^{13}C NMR of acetic acid 2-acetoxy-4-acetylsulfanyl-phenyl ester or "triacetylated **6**" in acetone-d_6.

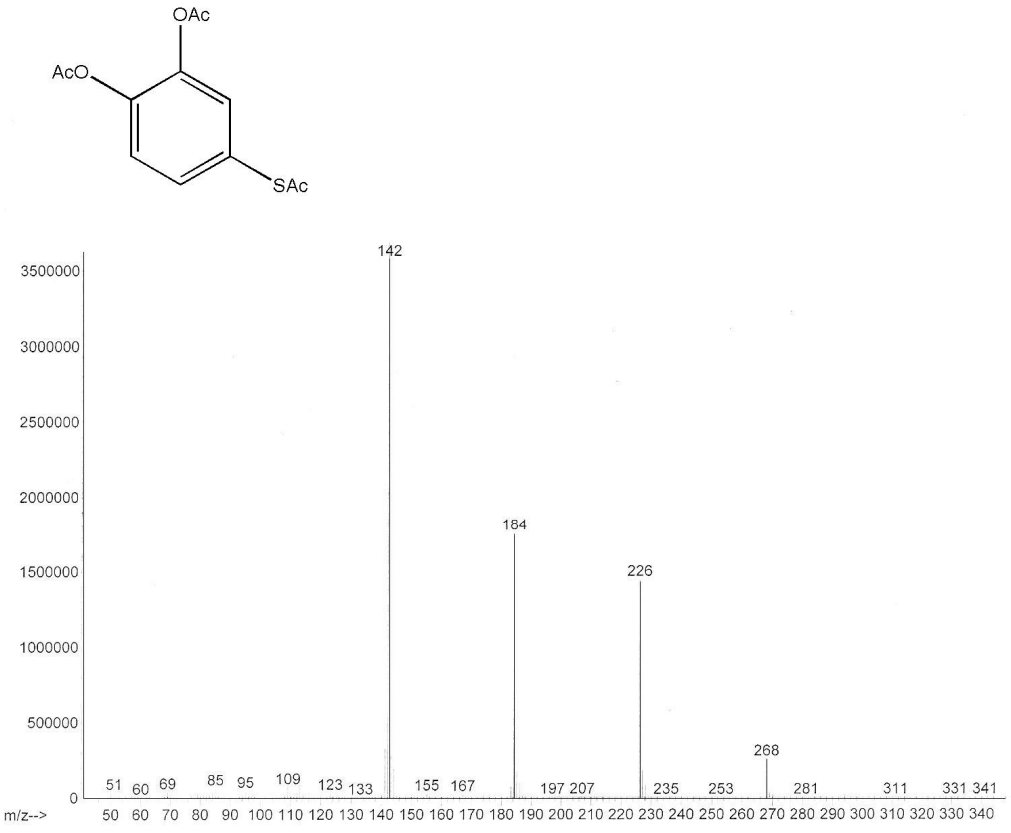


Figure 16. Mass spectrum of acetic acid 2-acetoxy-3-acetylsulfanyl-phenyl ester or "triacetylated **6**."

Empirical Formula Confirmation Report

Sample Name: 4-mercaptocatechol Sample Location: P1-A1 Sample Id: Operator:
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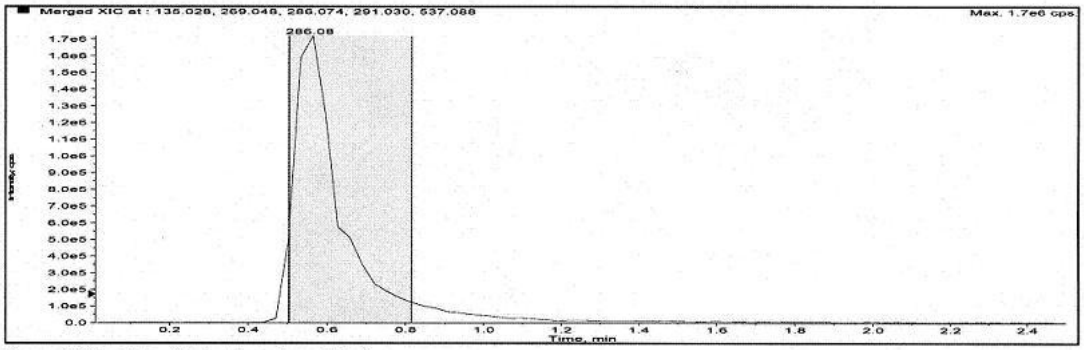
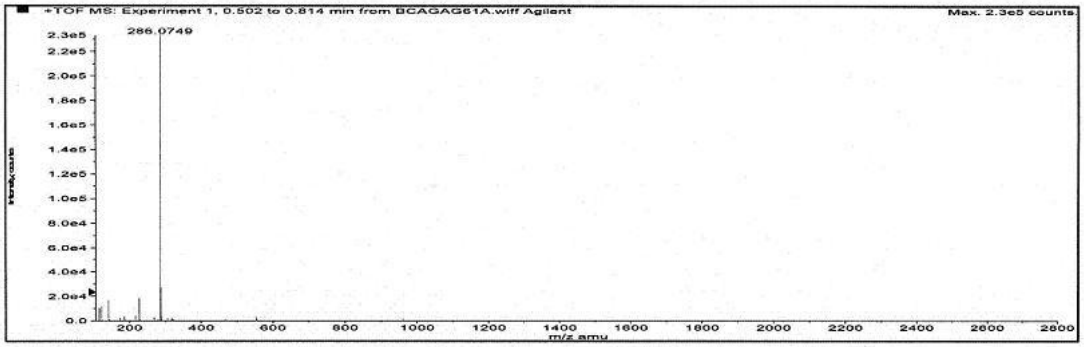
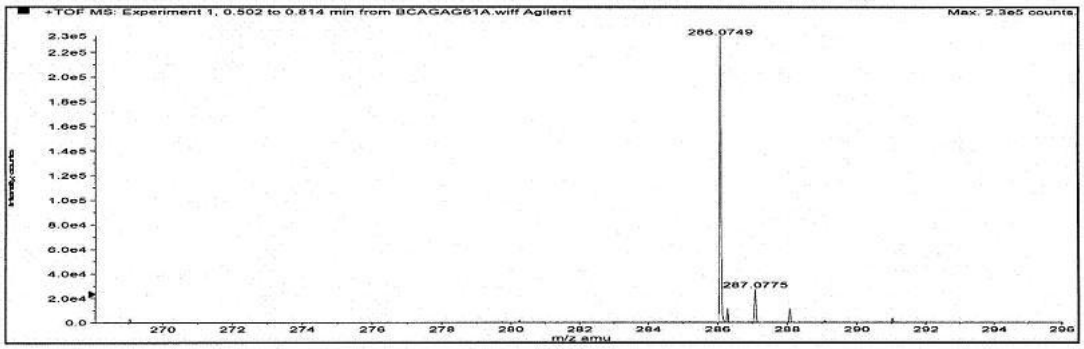


Merged XIC, Period# : 1 Experiment# : 1





Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C12H12O5S1	--	268.04054	0.56	1.40317 E7	--

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	2918.42	269.04782	269.04778	-0.04426	-0.16	--
[M+NH4]+	235211.82	286.07437	286.07491	0.53848	1.88	--
[M+Na]+	3593.43	291.02977	291.03020	0.43530	1.50	--

Figure 17. HRMS of acetic acid 2-acetoxy-3-acetylsulfanyl-phenyl ester or "triacetylated **6**."

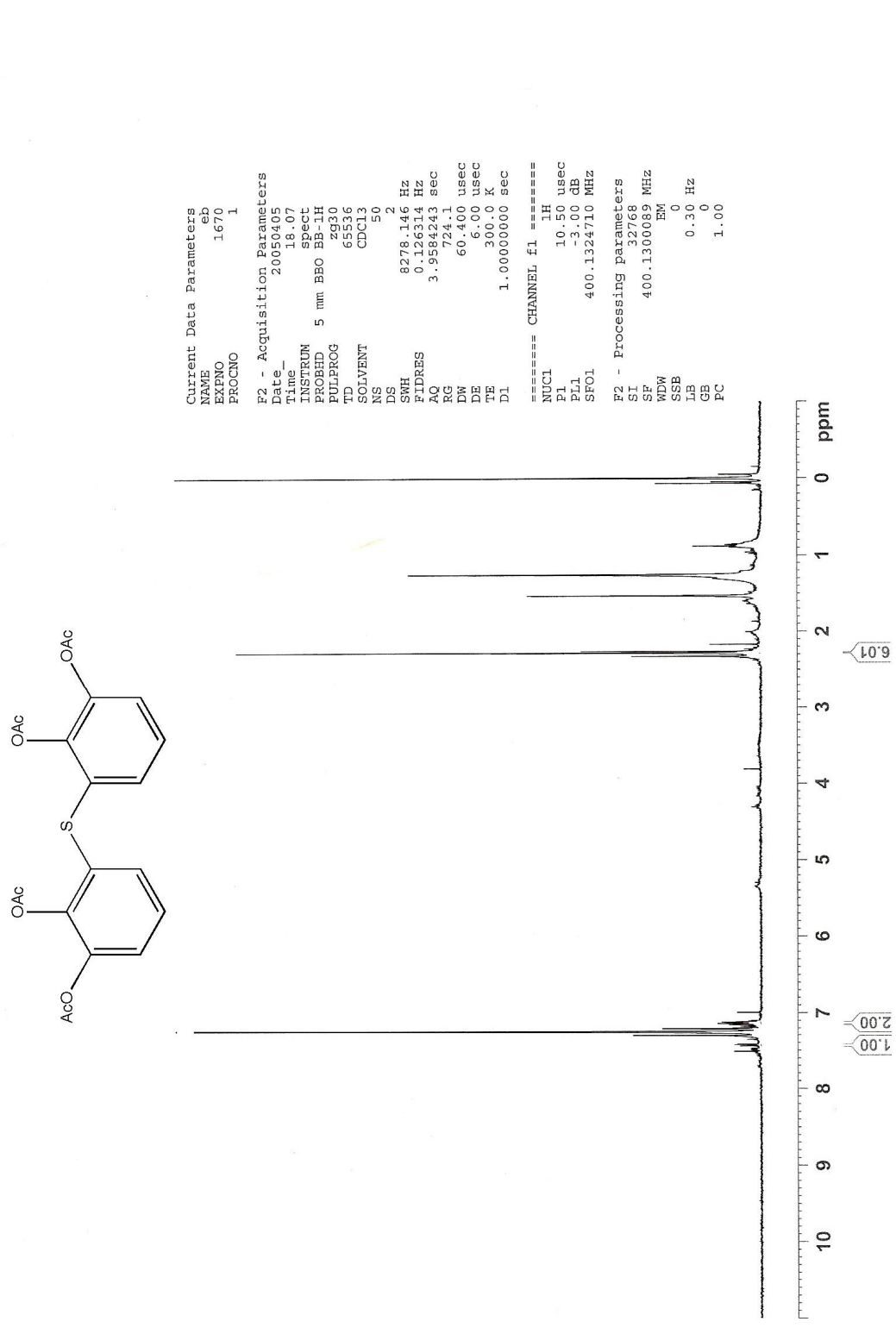


Figure 18. ^{1}H NMR of acetic acid 2-acetoxy-6-(2,3-diacetoxy-phenylsulfanyl)-phenyl ester or "tetraacetylated **7**" in $CDCl_3$.

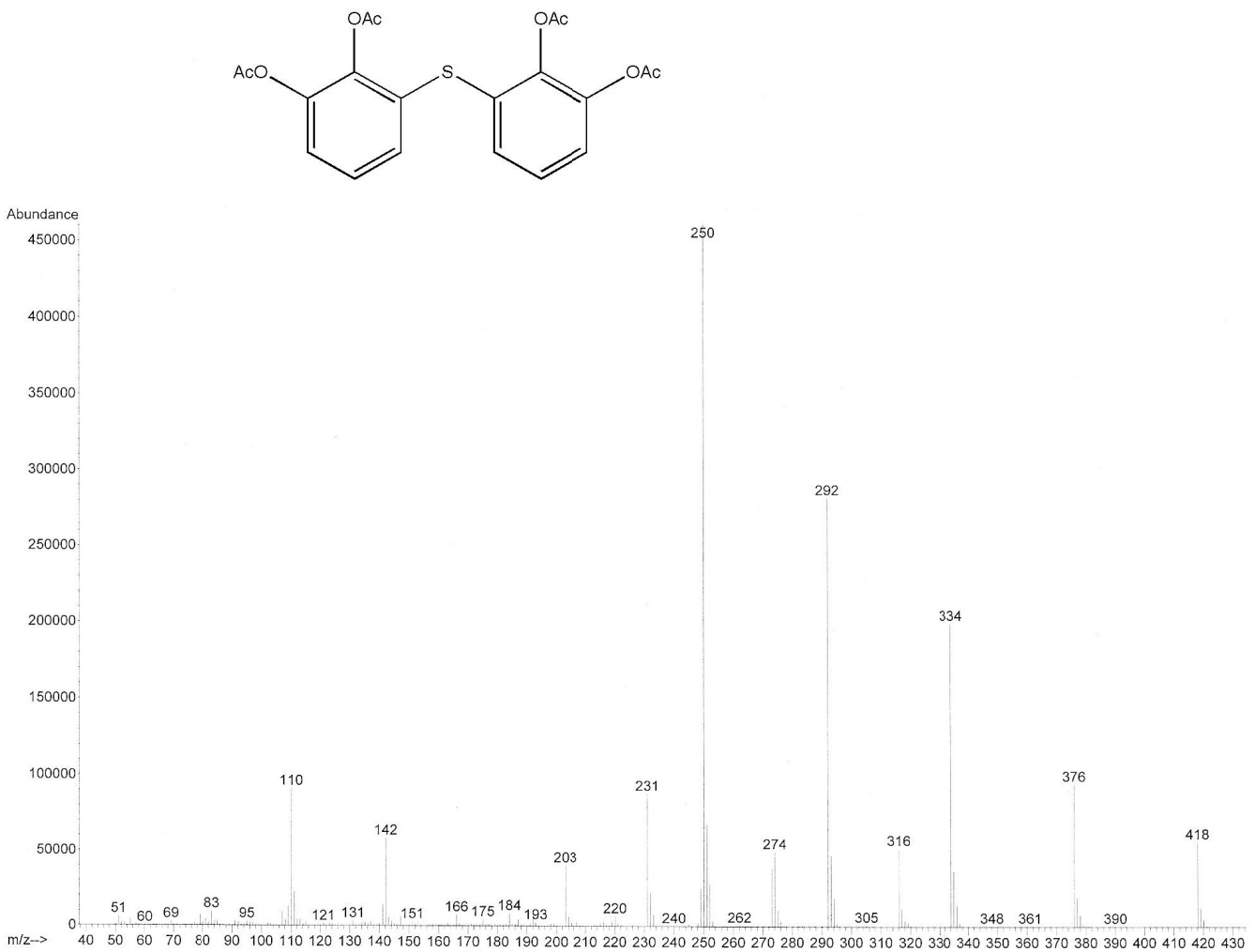


Figure 19. Mass spectrum of acetic acid 2-acetoxy-6-(2,3-diacetoxy-phenylsulfanyl)-phenyl ester or "tetraacetylated **7**."

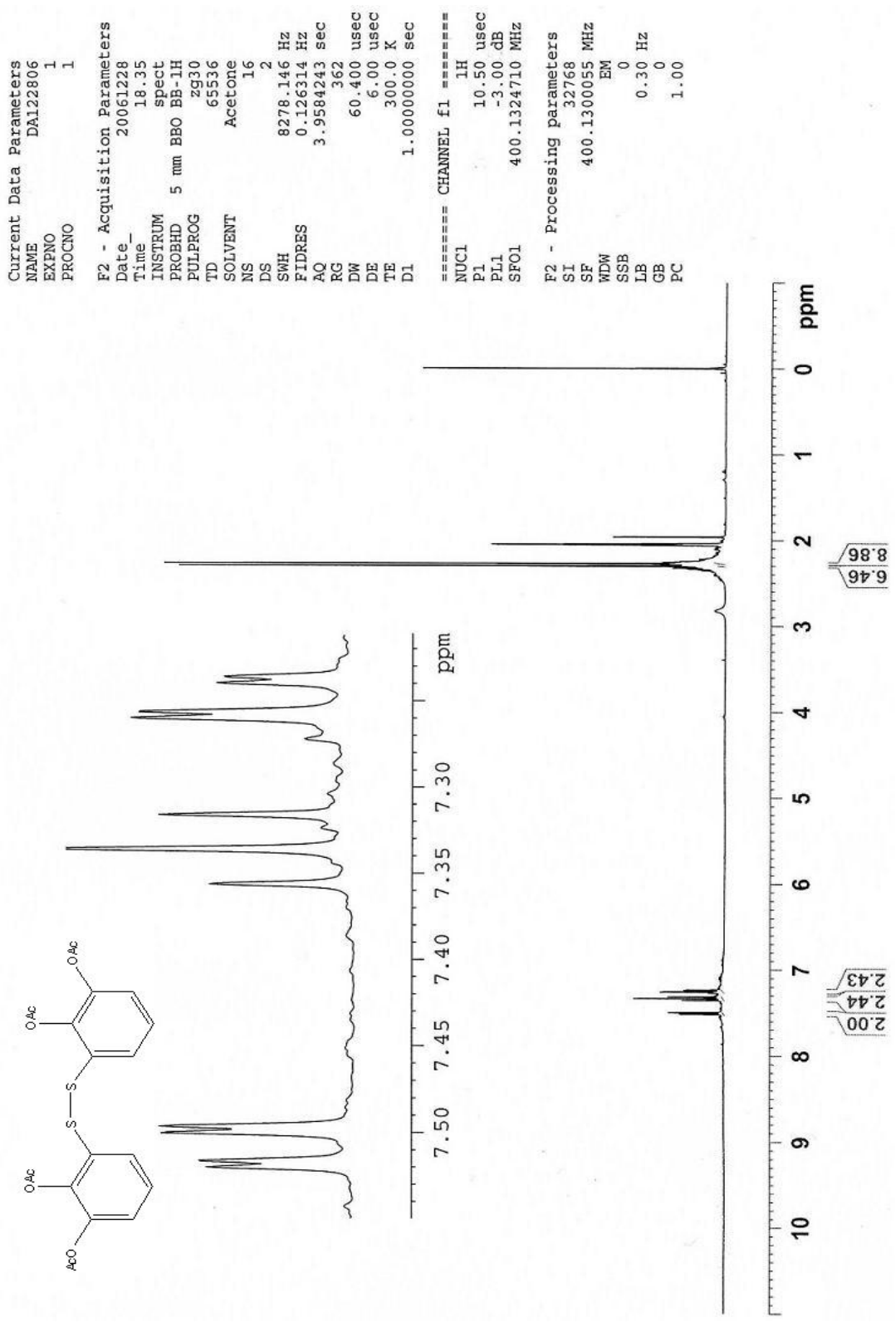


Figure 20. ^{1}H NMR of acetic acid 2-acetoxy-3-(2,3-diacetoxy-phenyldisulfanyl)-phenyl ester or "tetraacetylated **8**" in acetone-d_6.

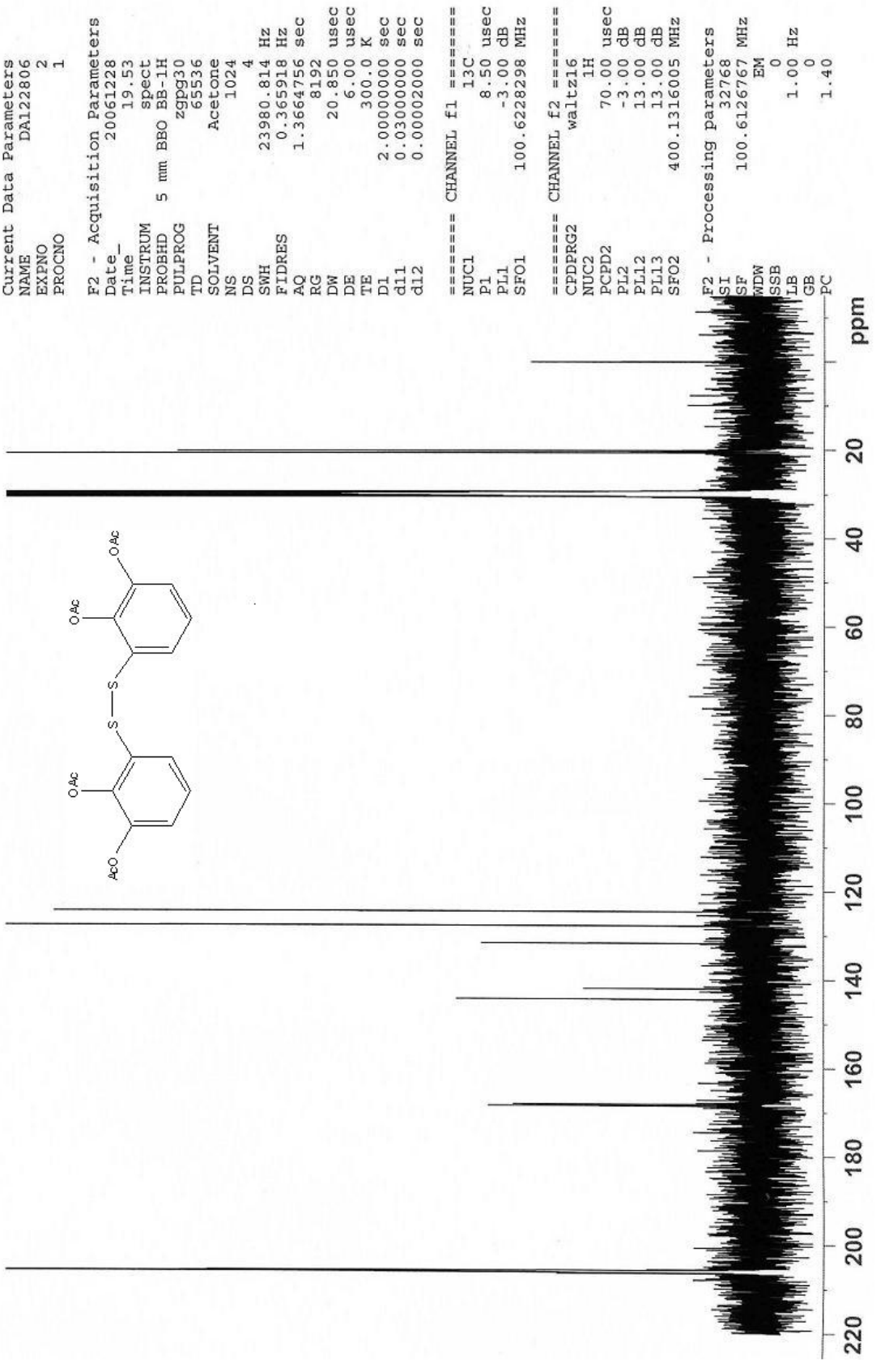


Figure 21. ^{13}C NMR of acetic acid 2-acetoxy-3-(2,3-diacetoxy-phenyldisulfanyl)-phenyl ester or "tetraacetylated **8**" in acetone-d_6.

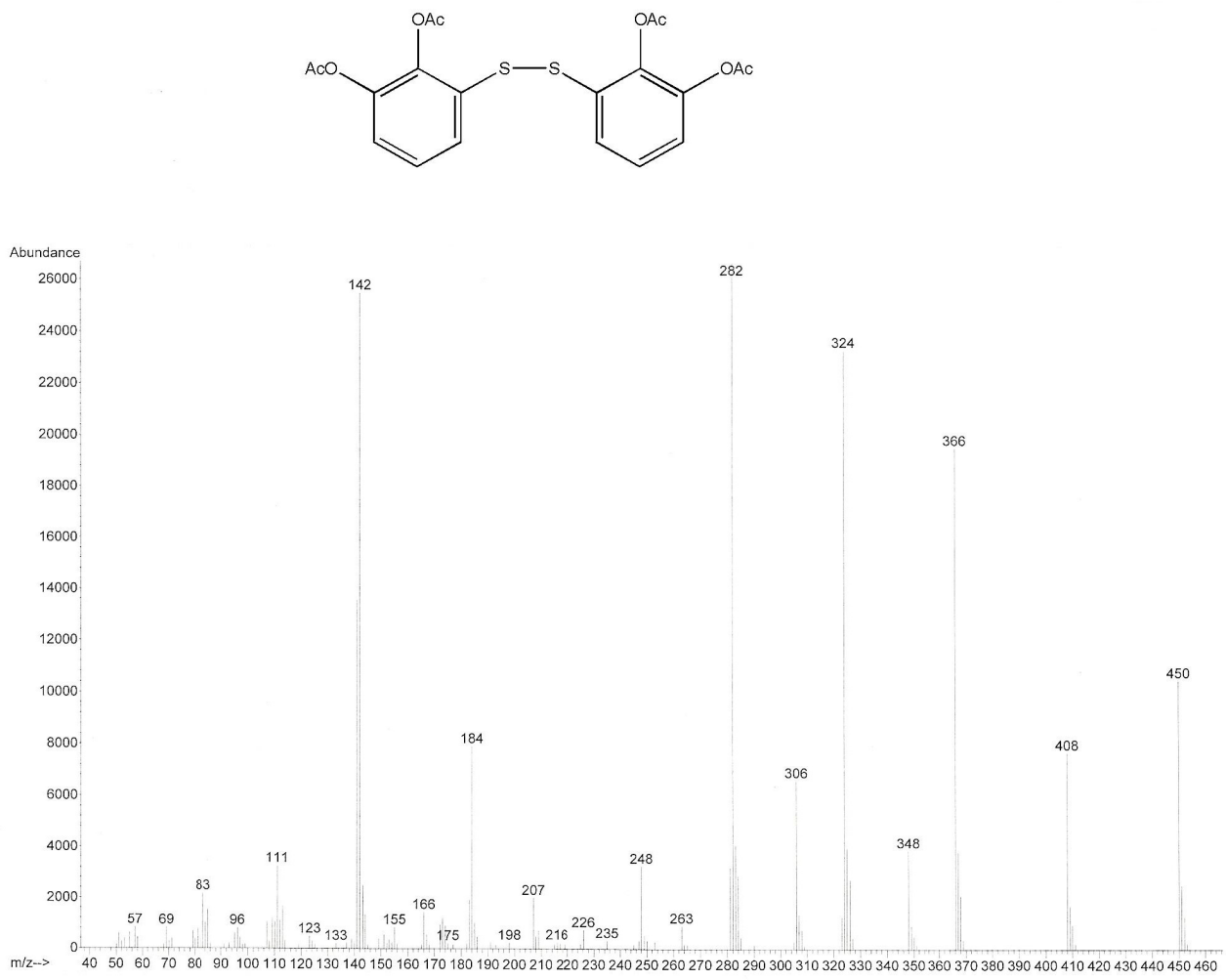
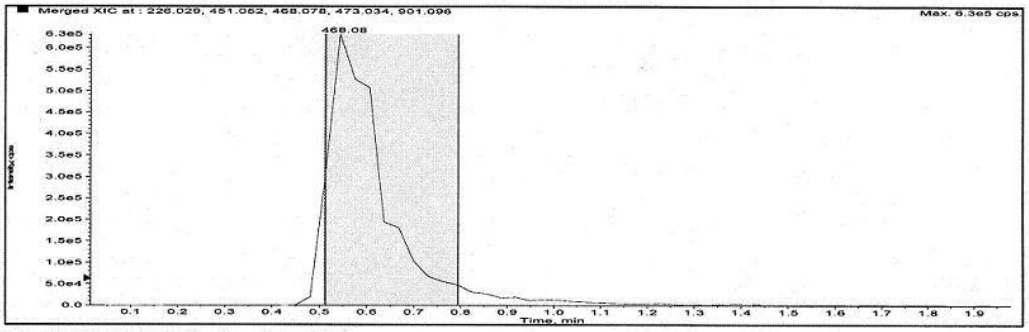


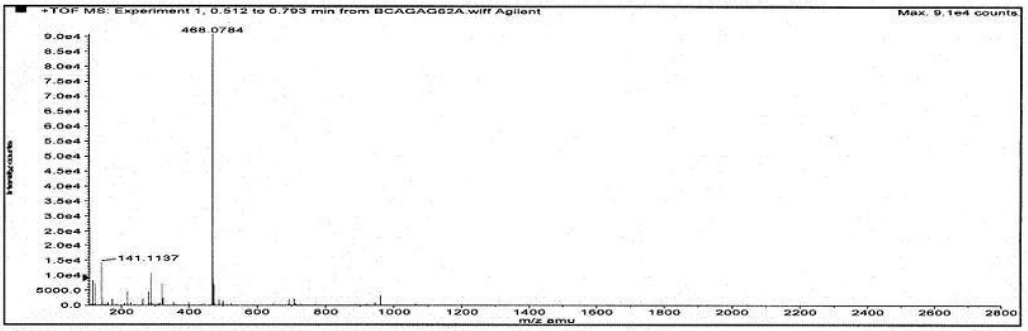
Figure 22. Mass spectrum of acetic acid 2-acetoxy-3-(2,3-diacetoxy-phenyldisulfanyl)-phenyl ester or "tetraacetylated

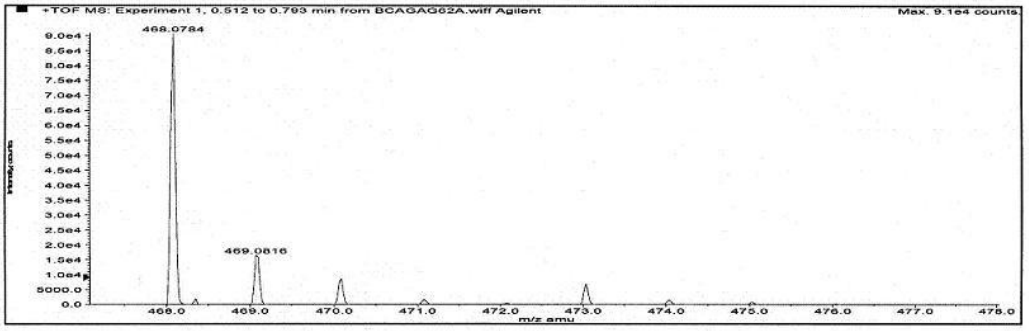
Empirical Formula Confirmation Report

Sample Name: **diphenyl disulfide** Sample Location: **P1-A1** Sample Id: Operator:
Data File Name: **D:\Data\060828\BCAGAG62A.wiff** Acq Time: **August 28 2006, 01:23:45 PM**
Method: **D:\TOF_Data\damethods\efcHC4.anm\efc.xml**



Merged XIC, Period# : 1 Experiment# : 1





Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C20H18O8S2	--	450.04431	0.55	5.02142 E6	--

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+NH4]+	91166.19	468.07813	468.07843	0.29991	0.64	--
[M+Na]+	6999.85	473.03353	473.03405	0.51776	1.09	--

calced measured

Figure 23. HRMS of acetic acid 2-acetoxy-3-(2,3-diacetoxy-phenyldisulfanyl)-phenyl ester or "tetraacetylated **8**."

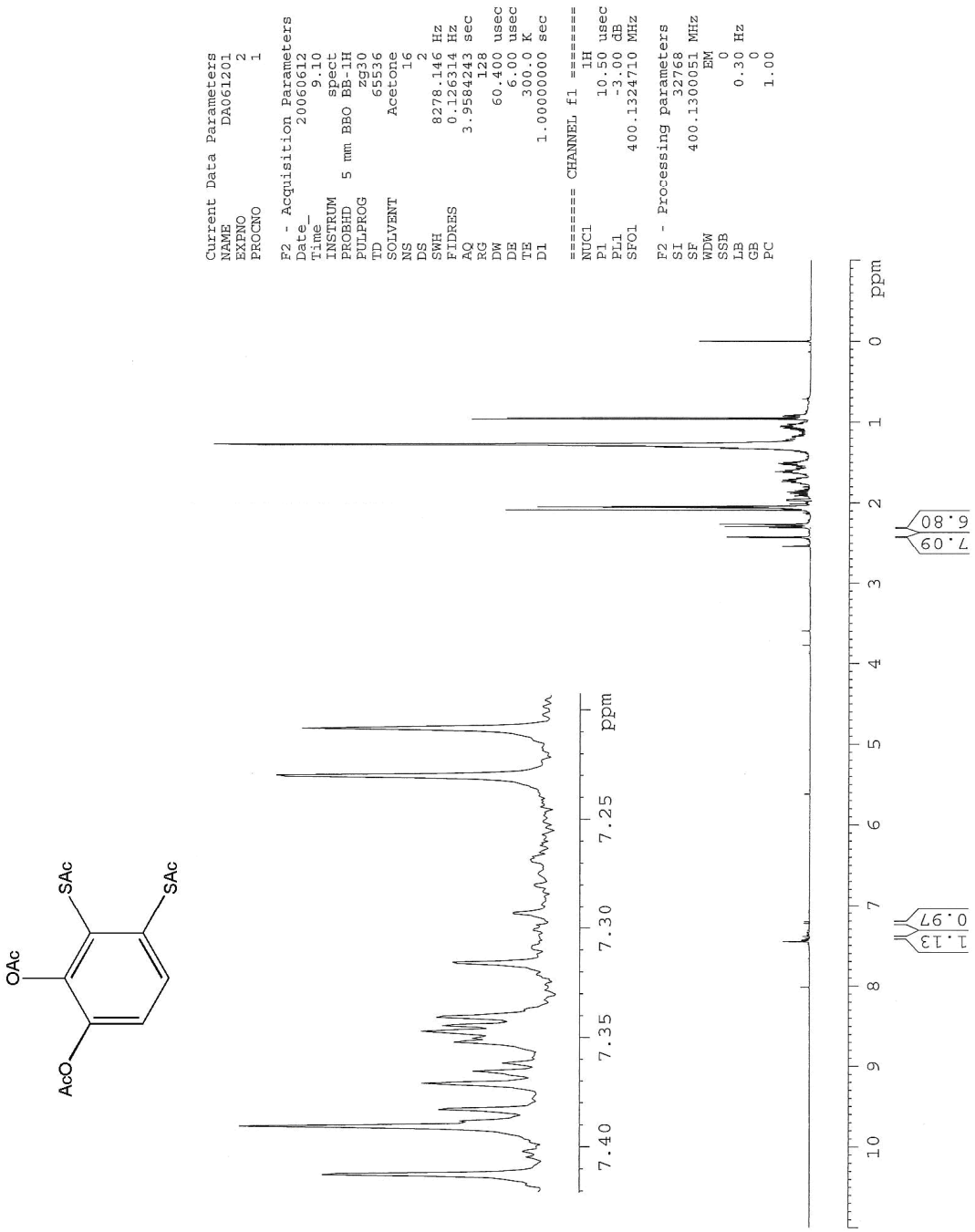


Figure 24. ^{1}H NMR for 3,4-dimercapto-benzene-1,2-diol or "tetraacetylated **9**."

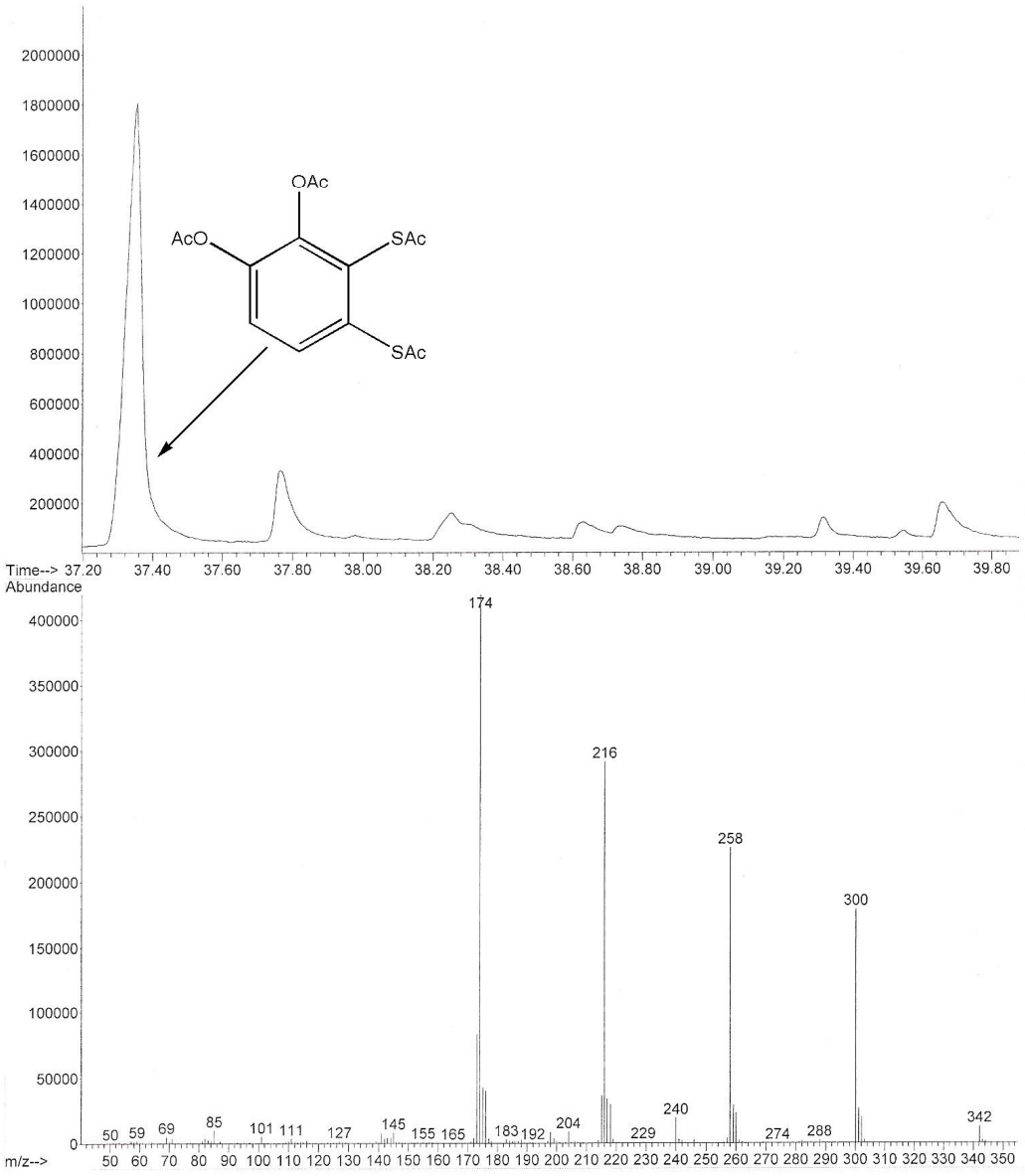


Figure 25. Mass spectrum of 3,4-dimercapto-benzene-1,2-diol or "tetraacetylated **9**."

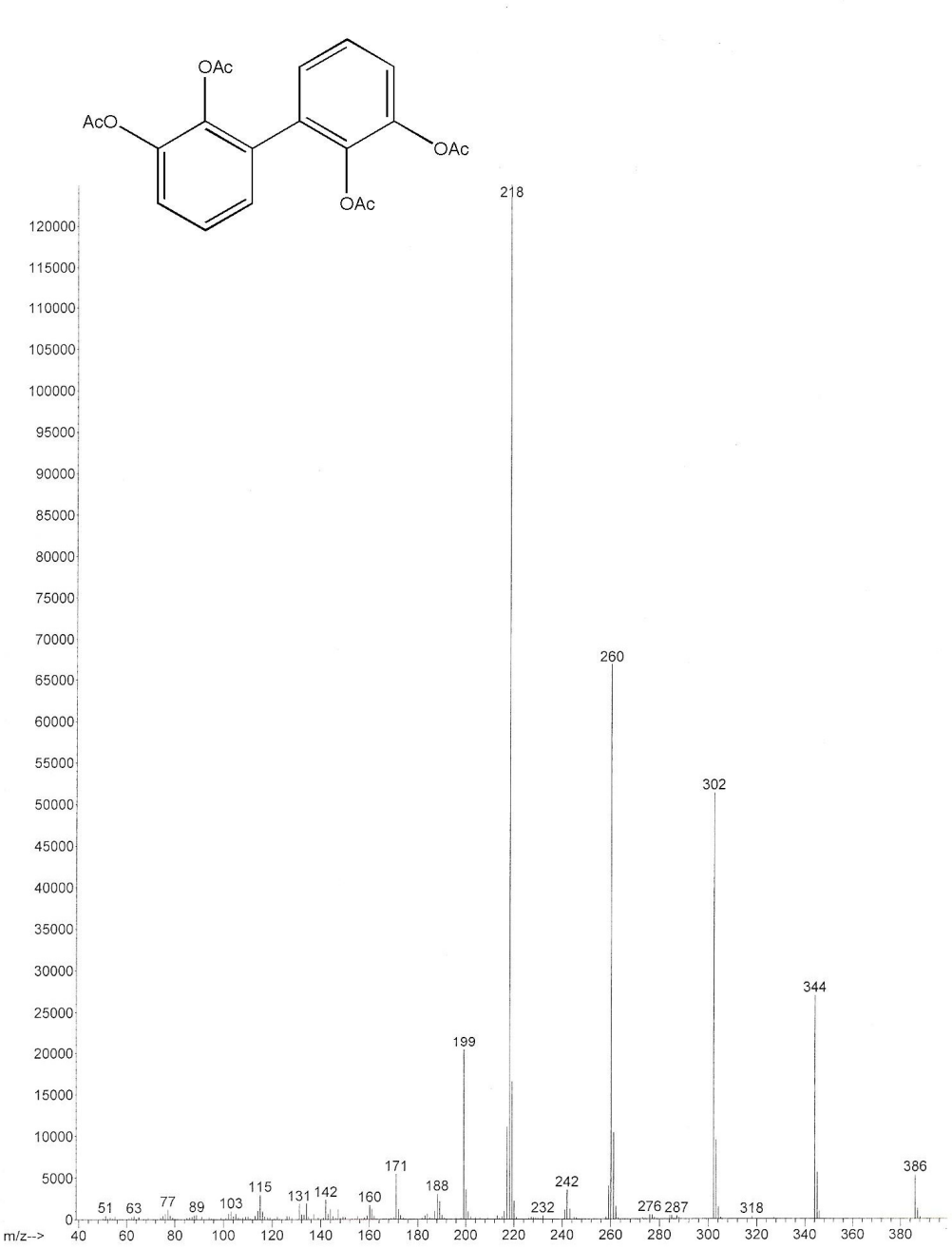
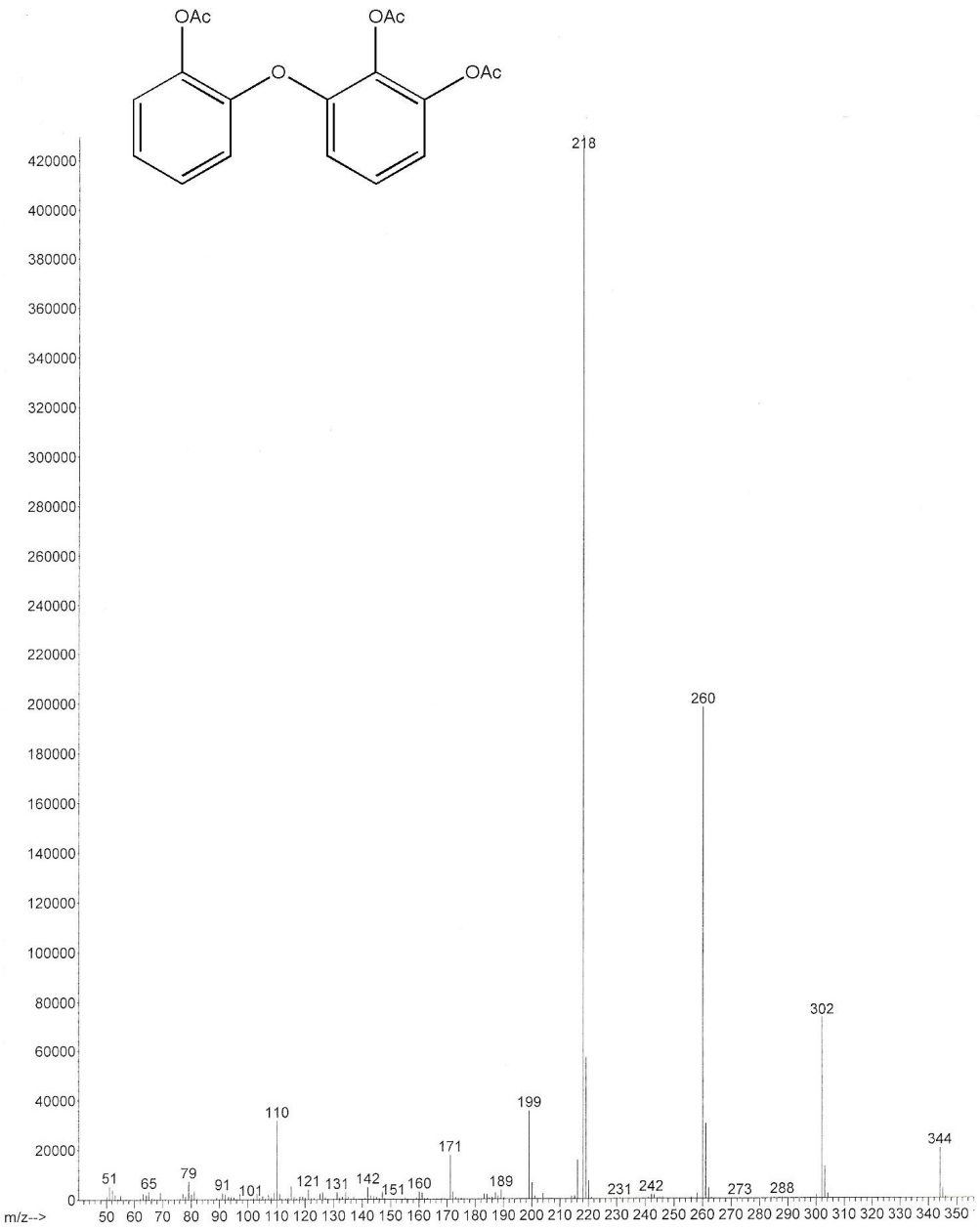


Figure 26. Mass spectrum of acetic acid 3,2',3'-triacetoxy-biphenyl-2-yl ester or "tetraacetylated **10**."

Figure 27. Mass spectrum of acetic acid 2-(3,4-diacetoxy-phenoxy)-phenyl ester or "triacetylated **11**."



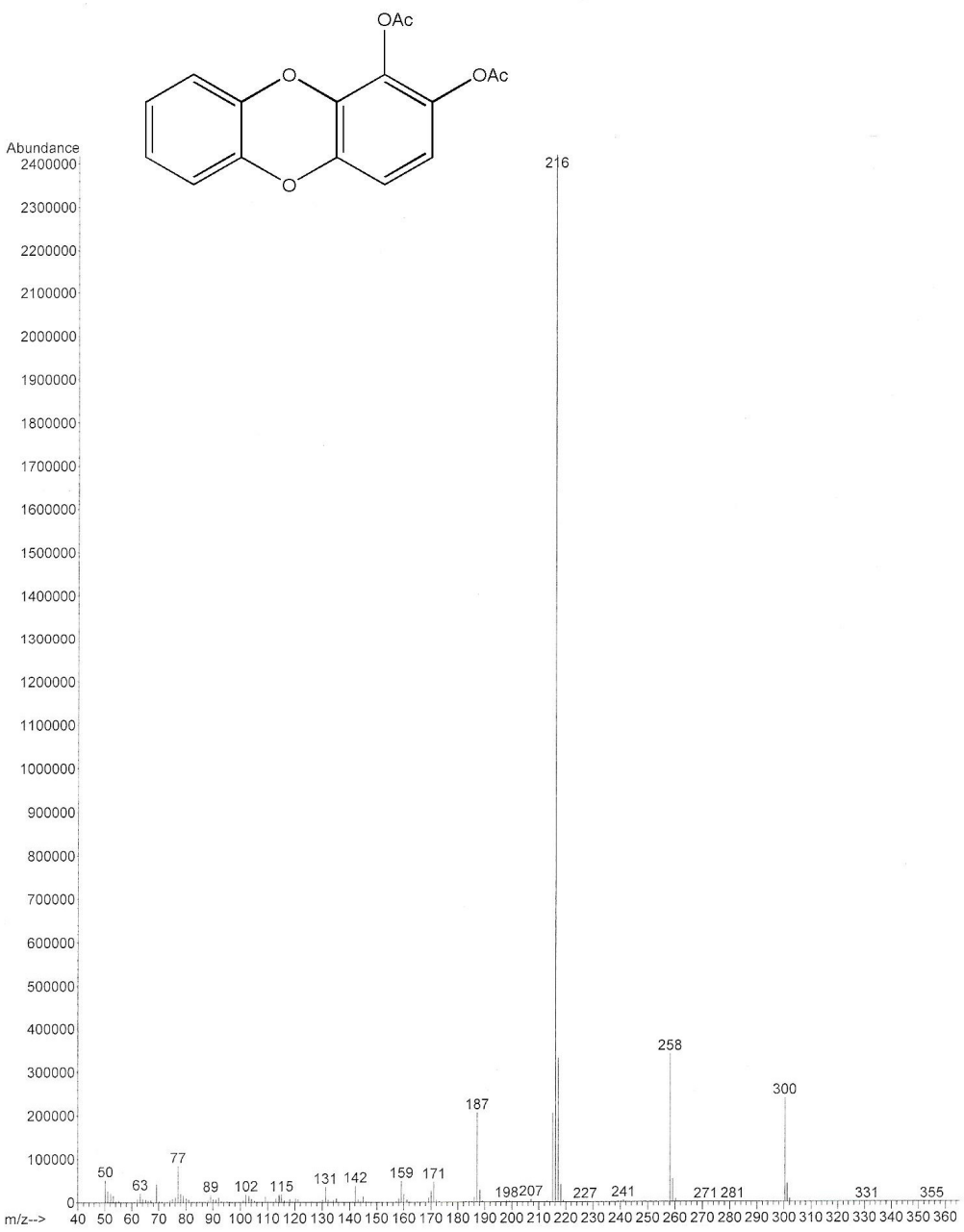


Figure 28. Mass spectrum of acetic acid 1-acetoxy-dibenzo[1,4]dioxin-2-yl ester or "diacetylated **12**."

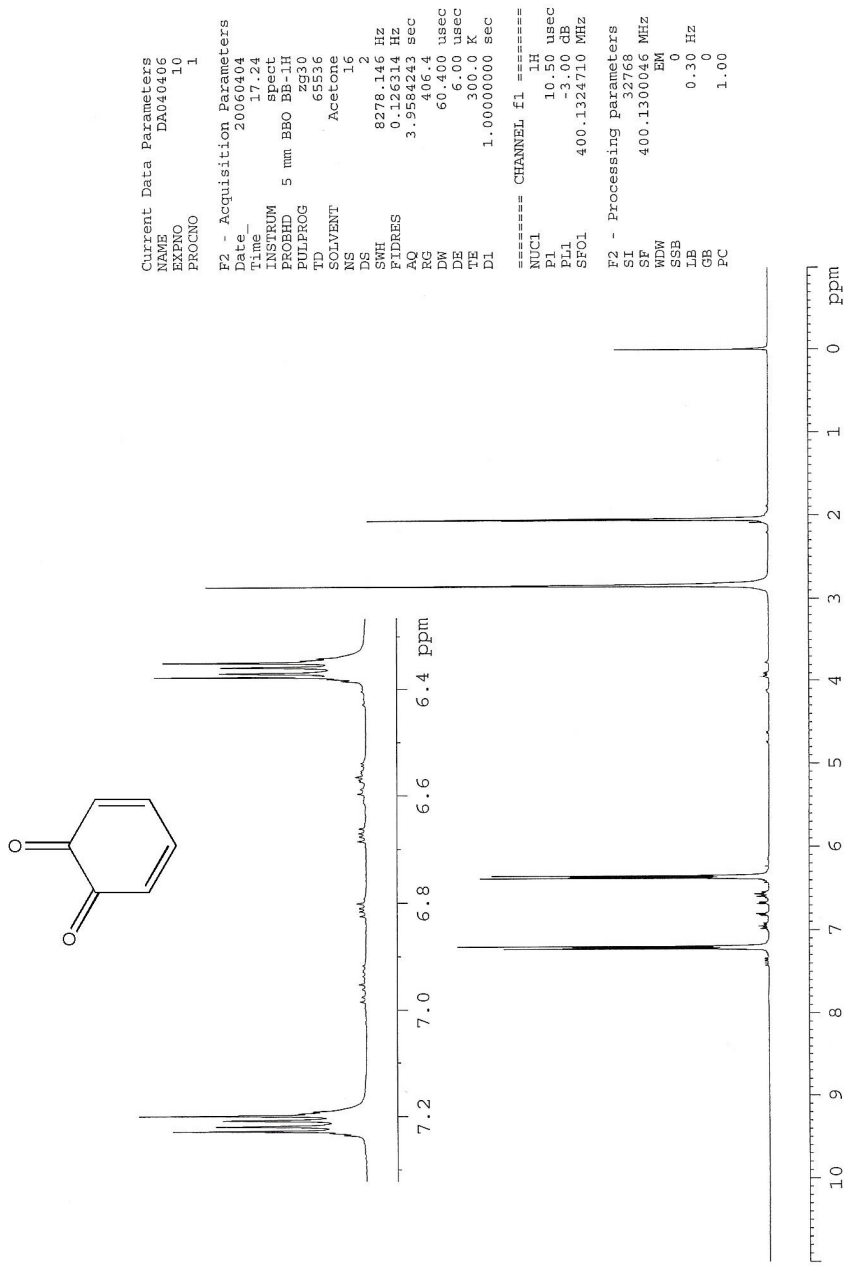
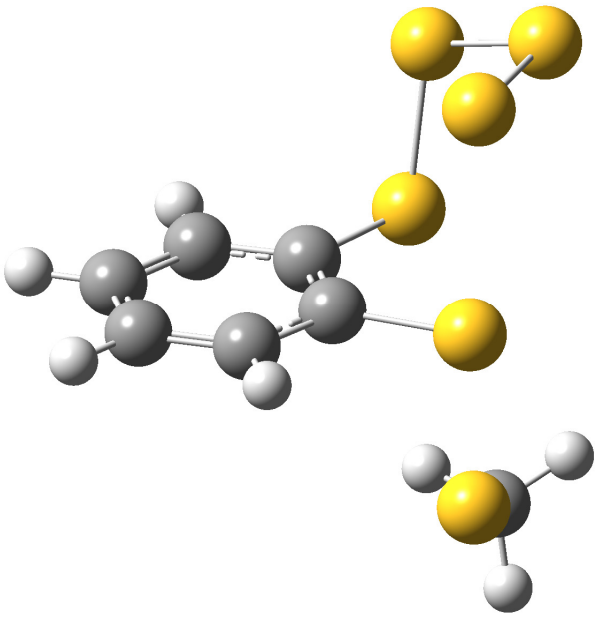


Figure 29. ^{1}H NMR of *o*-benzoquinone in acetone-d_6.

Appendix

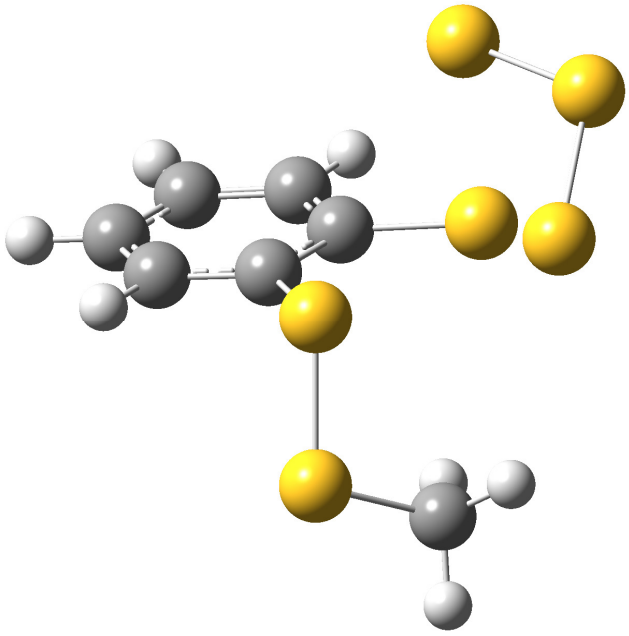


Optimized structure of benzopentathiepin and ethylthiolate ion

Description of the geometry of the stationary point of **5C** and thiomethylate ion (cartesian coordinates) and absolute energy in hartrees. B3P86/6-311+G(2d) gas phase (Charge=-1, spin state=1) -2663.012575

```
-1 1
 C                  1.45567200    3.17462200   -0.47043000
 C                  2.00625000    2.39610100   -1.47792500
 C                  1.76400800    1.03435700   -1.50405900
 C                  0.97360900    0.41664200   -0.53730800
 C                  0.40559300    1.20639400    0.47420000
 C                  0.66370900    2.57755900    0.49424900
 S                  0.75176400   -1.35952400   -0.64123900
 S                 -1.83874200   -0.89430400   -1.86081800
 S                 -2.95648800   -0.95569500   -0.15070500
```

S	−2.56699900	0.75331400	0.95170000
S	−0.62642400	0.54191800	1.76889000
H	1.63510900	4.24438300	−0.43896800
H	2.62019500	2.84962300	−2.24940100
H	2.18786700	0.42013700	−2.28942600
H	0.21865900	3.17357600	1.28257100
S	2.70706500	−1.88314800	0.14397900
C	2.47710600	−1.65417700	1.92692400
H	3.39001900	−1.99489100	2.41937500
H	2.31778100	−0.60354000	2.16882200
H	1.63186400	−2.23924000	2.28421100



Optimized structure of 23

Description of the geometry of the stationary point of **23** and thiomethylate ion (cartesian coordinates) and absolute energy in hartrees. B3P86/6-311+G(2d) gas phase (Charge=-1, spin state=1) -2663.020932

-1 1

C	0.26296000	2.77159600	-0.55269300
C	-1.06128400	3.17594100	-0.42914100
C	-1.99307800	2.28464800	0.06751800
C	-1.61960500	0.98918300	0.41874500
C	-0.28214600	0.57742700	0.29107700
C	0.65405800	1.49619300	-0.19110300
S	-2.85598300	-0.09487200	1.10764400
S	4.28091100	0.83088300	-0.19941500

S	3.69293700	-1.05506000	0.28838200
S	1.85212700	-1.55327600	-0.50376400
S	0.19586300	-1.05863300	0.77668100
H	1.01500500	3.46094300	-0.92168000
H	-1.36149600	4.18118700	-0.70520300
H	-3.03047600	2.57700500	0.18671300
H	1.70302400	1.21888500	-0.26917700
S	-3.93588200	-0.76905600	-0.52922200
C	-2.92447100	-2.13226100	-1.17052500
H	-3.48248300	-2.54907000	-2.01124800
H	-1.96063400	-1.76879700	-1.51677700
H	-2.78109900	-2.89627300	-0.41078100

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