Synthesis and Evaluation of Organic Sources of Reactive Sulfur Species

A thesis

Submitted in partial fulfillment of the requirements

of the degree of

Doctor of Philosophy

By

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ID: 20093042

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Dedicated to My Beloved Family…

भारतीय विज्ञान शिक्षा एवं अनुसंधान संस्थान, पुणे
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CERTIFICATE

Certified that the work incorporated in the thesis entitled *"**Synthesis and Evaluation of Organic Sources of Reactive Sulfur Species"*** submitted by Mr. Satish R. Malwal was carried out by the candidate at the Indian Institute of Science Education and Research (IISER), Pune, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other university or institution.

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DECLARATION

I declare that, this written submission represents my ideas in my own words and where others ideas have been included; I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea / data / fact/ source in my submission. I understand that violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

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.....Satish

CONTENTS

Abbreviations

Ar	Aryl
ACN	Acetonitrile
Bn	Benzyl
Bu	Butyl
^{t}Bu	Tertiary butyl
bs	Broad singlet
Calcd.	Calculated
Cat.	Catalytic
Compd.	Compound
d	Doublet
DEAD	Diethyl azodicarboxylate
DCM	Dichloromethane
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dd	doublet of doublet
ESI	Electrospray ionization
EtOAc	Ethyl acetate
FTIR	Fourier transform infrared spectroscopy
g	Gram
µg	microgram
h	Hour
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
Hz	Hertz
J	Spin coupling constant
LAH	Lithium Aluminium Hydride
m	Multiplet
m/z	mass/charge
mg	Milligram

min	Minutes
mL	Milliliters
mV	mili Volts
mM	Millimolar
mmol	Millimoles
mAU	mili Arbitrary unit
Max.	Maximum
MeOH	Methanol
Me	Methyl
MeCN	Acetonitrile
MS	Mass Spectroscopy
MSH	Mycothiol
MIC	Minimum Inhibitory Concentration
mV	mili Volts
mM	Millimolar
MALDI-TOF	Matrix-Assisted Laser Desorption /Ionization–Time of flight
Mtb	Mycobacterium tuberculosis
µL	Microliter
µM	Micromolar
µS	Micro Siemens
m p	Melting Point
m	multiplate
N	Normal
NADH	Nicotinamide adenine dinucleotide
NBS	*N*-Bromosuccinamide
NMO	*N*-Methylmorpholine N-oxide
NMR	Nuclear Magnetic Resonance
PB	Phosphate buffer
PE	Pet ether
ppm	Parts per million
Py	Pyridine
q	Quartet
RM	Reaction mixture

RT	Room temperature
SM	Starting material
s	Singlet
THF	Tetrahydrofuran
UV	Ultra Violet

Abstract

The thesis entitled *"Synthesis and Evaluation of Organic Sources of Reactive Sulfur Species"* comprises of four main chapters.

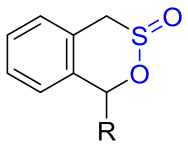
Chapter 1: Introduction: **Sulfur Dioxide (SO_2), A Reactive Sulfur Species (RSS)**

Sulfur dioxide (SO_2) is an environmental pollutant that is also produced during metabolism of sulfur containing amino acids. SO_2 is one of the possible products of oxidation of hydrogen sulfide (H_2S), which is produced inside the cell in order to mediate cellular processes. The vasodilatory effects of SO_2 in animal models are well documented suggesting possible signaling roles for this gas as well. At elevated levels, however, SO_2 is known to cause biomacromolecular damage and cell death. Together these aspects of SO_2 are responsible for this gas to be termed as a reactive sulfur species (RSS).

In contrast, SO_2 is used in the food industry as a preservative and an anti-bacterial agent. In order to better understand the roles of this gaseous entity, reliable sources of SO_2 are necessary. Further, controlled generation of SO_2 might have applications in developing novel therapeutic agents as well. Biological studies have thus far relied on gaseous SO_2 or a complex formulation of inorganic sulfites and both methods are not suitable for exploration of therapeutic applications. Here, we discuss the various small molecule-based strategies towards generating SO_2 under physiological conditions in a spatiotemporally controlled manner.

Chapter 2: Thermally Activated SO_2 Donors: Design and Synthesis of Benzosultines as SO_2 Donors

In this chapter, we present a strategy for controlled and efficient generation of SO_2 under physiological conditions in a non-enzymatic manner using heat as trigger. Benzosultine **1** (R = H, Figure 1) undergo a retro Diels-Alder reaction to produce a 1,3-diene, which then reacts with a dienophile to produce a cycloadduct. The byproduct of the cycloreversion reaction is presumably SO_2. The challenge, however, is to be able to conduct the retro Diels-Alder reaction at physiological temperature of 37 °C. We postulated that systematic structural modifications on the carbon atom adjacent to oxygen atom

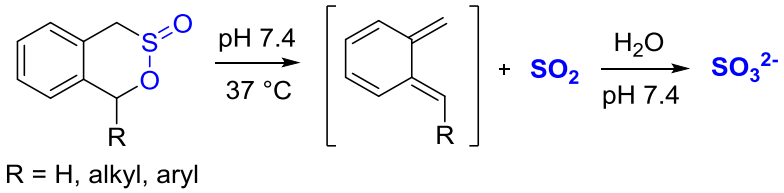


R = H, alkyl, aryl

Figure 1. Proposed benzosultine scaffold.

would lead to organic SO_2 donor at 37 °C (Figure 1). Benzosultines **1**, R = H; **2**, R = Me and **3**, R = Ph (Figure 1) were synthesized starting from phthalide.

HPLC analyses of acetonitrile solutions of **1**, **2** and **3** at 70 °C were carried out to study thermal stability of these compounds. The observed rate of decomposition of benzosultine **3** was greater than the rate of decomposition of bezosultine **2** and **1** suggesting that extended conjugation assisted cycloreversion. Our results are also similar to a previous report of Diels-Alder reactions of **1-3** with maleic anhydride at 80 °C, showing a similar trend in reaction rates i.e. **3** > **2** > **1**.



Scheme 1. Decomposition of benzosultines to generate SO_2.

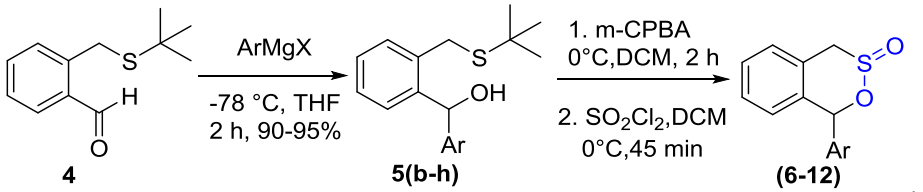
Due to possible use of benzosultines as SO_2 donors in physiological media, the stability study of benzosultines **1-3** was carried out at 37 °C in pH 7.4 buffer. The benzosultine **3** was nearly completely decomposed, whereas negligible amount of benzosultine **1** and **2** was decomposed after 3 h. Hence introduction of phenyl ring at 1-position of benzosultine **1** increases the rate of cycloreversion in organic as well as aqueous media. The ability of **3** to produce SO_2 under physiological conditions at 37 °C was evaluated by monitoring the formation of sulfite using an ion chromatograph (IC) attached with a conductivity detector (Scheme 1). The maximum SO_2 yield of 89% with half-life of 39 min for generation of SO_2 was obtained for benzosultine **3**.

As **3** was an efficient SO_2 donor, we evaluated the ability of this compound to mimic SO_2 under biological assay conditions. In the presence of metal ions such as Cu(II), SO_2 produces radical intermediates such as $SO_3^{\bullet}$, $SO_4^{\bullet-}$ and $HSO_5^{\bullet-}$, all are known to cause oxidative damage to biomacromolecules including DNA. A pBR322 supercoiled plasmid cleavage assay of **3** in presence of equimolar amount of Cu(II), showed SO_2-like biological activity.

In order to modulate the rate of generation of SO_2, benzosultines **6-12** were prepared by treating various freshly prepared Grignard reagents with aldehyde **4** (Table 1).

Decomposition profiles of **6-12** at 37 °C in pH 7.4 buffer revealed predictable rates for decomposition and half-lives for SO_2 generation ranged from 10-68 min (Table 2).

Table 1. Preparation of benzosultines **6-12**.



Entry	Ar	Compd No.	Compd No.	Yield (%)
1	4-FPh	**5b**	**6**	94
2	4-ClPh	**5c**	**7**	71
3	4-CNPh	**5d**	**8**	93
4	4-MePh	**5e**	**9**	97
5	3-OMePh	**5f**	**10**	87
6	3-CNPh	**5g**	**11**	92
7	2-NO_2Ph	**5h**	**12**	70

Table 2. Decomposition of sultines **3**, **6-12** prepared in this study in pH 7.4 at 37 °C.

Entries	Compd	decomp (%)[a]	k_{dec} (min^{-1})[b]	% SO_2[c]	$t_{1/2}$ (min)[d]	Max SO_2 yield (%)
1	**3**	37	0.0140	35	39	89
2	**6**	61	0.0244	60	14	83
3	**7**	38	0.0140	36	34	79
4	**8**	38	0.0093	37	38	76
5	**9**	70	0.0336	64	13	80
6	**10**	37	0.0104	25	56	73
7	**11**	36	0.0069	23	68	59
8	**12**	80	0.0500	71	10	81

[a]Determined by HPLC analysis. [b]Determined by kinetic analysis of decomposition of sultine. [c]Determined as sulfite by an ion chromatograph attached with a conductivity detector. [d]Determined by kinetic analysis of formation of SO_2 as sulfite.

In order to understand electronic effects on decomposition, a Hammett plot using rate constants of decomposition of 3- and 4-aryl substituted sultines was constructed (Figure 2). A nearly linear relationship between $\log(k_x/k_H)$ and substituent constant (σ) was found suggested a predictable mechanism of decomposition for this scaffold (Figure 2). The slope, ρ was found as -0.6 implying a weak electronic effect on the reaction center wherein an electron donating group accelerated the cycloreversion.

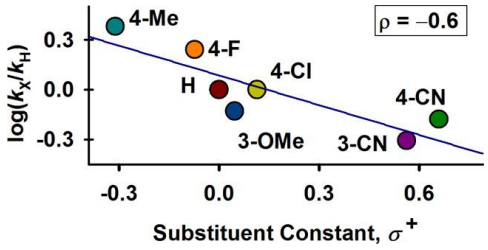


Figure 2. Hammett plot of rate constants for decomposition of benzosultines in pH 7.4 buffer at 37 °C. Linear regression analysis yielded ρ as -0.6 ($R^2 = 0.75$).

In conclusion, a strategy for controlled and efficient generation of SO_2 under physiological condition in non-enzymatic manner was presented. 1-Aryl-benzosultines are found to be labile at 37 °C and decompose in buffer to produce SO_2 with half-lives ranging between 10 – 68 min. The benzosultine **3** gave 89% of SO_2 and a half-life of 39 min and shows SO_2-like biological activity in a DNA cleavage assay.

Chapter 3: Section A: Design, Synthesis and Evaluation of Thiol Activated Sources of Sulfur Dioxide (SO_2)

We next prepared various 2,4-dinitrophenylsulfonamides and evaluated the potential of these compounds to act as thiol activated sources of sulfur dioxide under physiological condition. The presence of a trigger should provide a handle for controlled SO_2 generation and such compounds might be well suited to study the antibacterial properties of SO_2.

We considered 2,4-dinitrosulfonamides as potential SO_2 donors (Figure 3). The 2,4-dinitrophenylsulfonyl group has been used for thiol sensing applications. Here, a fluorescent amine is protected with 2,4-dinitrobenzenesulfonyl chloride (DNsCl) to form the non emissive probe. Upon reaction with a thiol, this compound undergoes cleavage to generate the amine and sulfur dioxide as a byproduct. Applications of this probe in live cell imaging

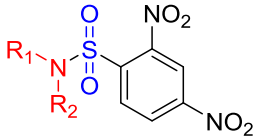


Figure 3. 2,4-Dinitrophenylsulfonamide.

suggest that sulfonamides are cell permeable and react with biological thiols at physiological pH 7.4. As biological thiols are present at low millimolar concentration inside cells, we decided to explore the possibility of using thiol as trigger.

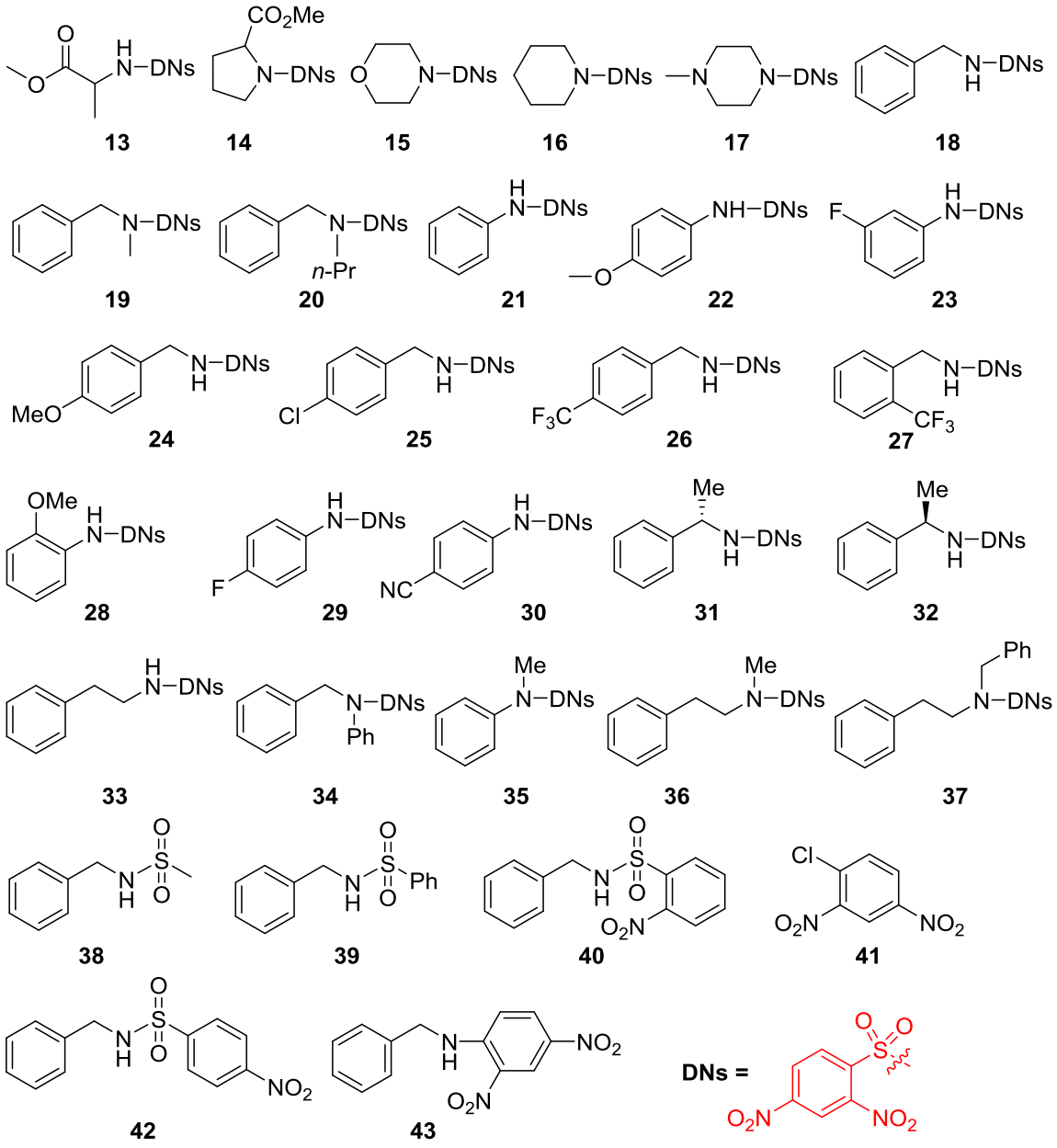


Figure 4. Library of 2,4-dinitrobenzenesulfonamides prepared.

Starting from a range of primary or secondary amines and 2,4-dinitrobenzenesulfonyl chloride, DNsCl, a series of 2,4-dinitrobenzenesulfonamides were prepared (Figure 4). The ability of 2,4-dinitrobenzenesulfonamides to act as SO_2 donors under physiological conditions by treating with cysteine or glutathione was evaluated.

We treated compounds **13-23** with cysteine in pH 7.4 buffer, and after 30 min SO_2 quantification was done as sulfite (SO_3^{2-}) by using ion chromatograph equipped with conductivity detector. All compounds tested were found to be excellent sources of SO_2 with maximum yields of SO_2 ranging from 79 to 100%, except aniline derivatives **21** and **23**. These results suggest that pK_{aH} of amine could affect the observed SO_2 yields. Several structural analogues **24-37** were prepared and SO_2 yields of cysteine mediated decomposition after 5 min and 30 min were recorded, it was found that decomposition profiles depended on pK_{aH} of the amine bearing the DNs group (Figure 5). The kinetics of decomposition of some of the sulfonamides revealed that, higher the pK_{aH} of amine, lower the $t_{1/2}$ for reaction and faster the reaction.

Having established that DNs derivatives were SO_2 donors, we tested the role of the nitro group. None of the compounds **38-43** were found to generate SO_2 when reacted with cysteine. Thus, presence of 2,4-dinitrobenzene sulfonyl group is required for sulfonamide in order to act as SO_2 donor.

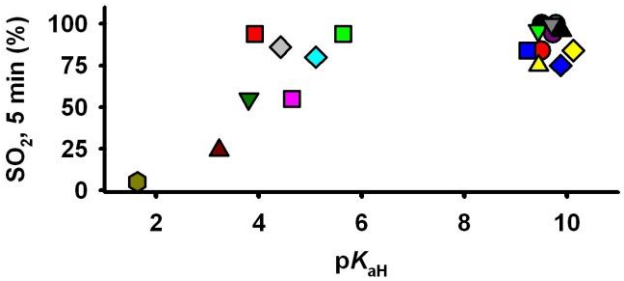


Figure 5. Relationship between SO_2 yields after 5 min and pK_{aH} of the amine.

Next, the possible use for these compounds as antimicrobials was evaluated. The target disease chosen was tuberculosis (TB). The 2,4-dinitrobenzenesulfonamides were screened for their antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}R_v$ and minimum inhibitory concentrations (MICs) were determined by our collaborators. The best *Mtb* inhibitor in this series was the benzylamine derivative **18** with MIC of 0.05 μg/mL (0.15 μM), which was better than the MIC of isoniazid (0.05 μg/mL, 0.37 μM) determined under similar conditions. Along with benzylamine derivative **18**, some of the compounds **16**, **19**, **24**–**26**, **33**, **36** and **37** had potent *Mtb* inhibitory activities with MICs < 1 μg/mL; better than those of ethambutol and pyrizinamide, both clinically used tuberculosis drugs, evaluated under similar assay conditions. The propensity of the compound to react with thiols and generate SO_2 in the test-tube appeared to dictate antimycobacterial activity.

In conclusion, we prepared various 2,4-dinitrophenylsulfonamides as a thiol activated sources of sulfur dioxide under physiological condition having controlled rate of

SO_2 generation, with antimycobacterial activity. The observed SO_2 yields depend upon the pK_{aH} of amine present in sulfonamide.

Chapter 3: Section B: Synthesis and Evaluation of 1,2-Cyclic Sulfite Diesters as SO_2 Donors

In this strategy, we prepared a series of 1,2-cyclic sulfite diesters and evaluated them as sulfur dioxide donors under physiological conditions.

1,2-cyclic sulfite diesters undergo nucleophilic substitution with various nucleophiles such as LiCl, NaN_3 and $(CH_3OOC)_2CH^-Na^+$ at one of the activated carbon rather than sulfite sulfur atom, to give an alcohol (Figure 6). This reaction would produce sulfite monoester as the intermediate and decomposition of this intermediate will presumably give SO_2 as a byproduct (Figure 6).

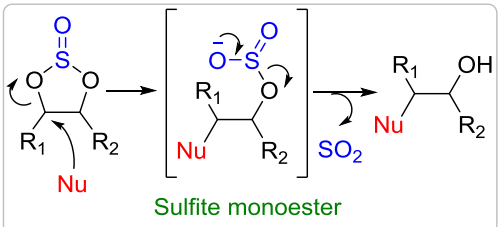
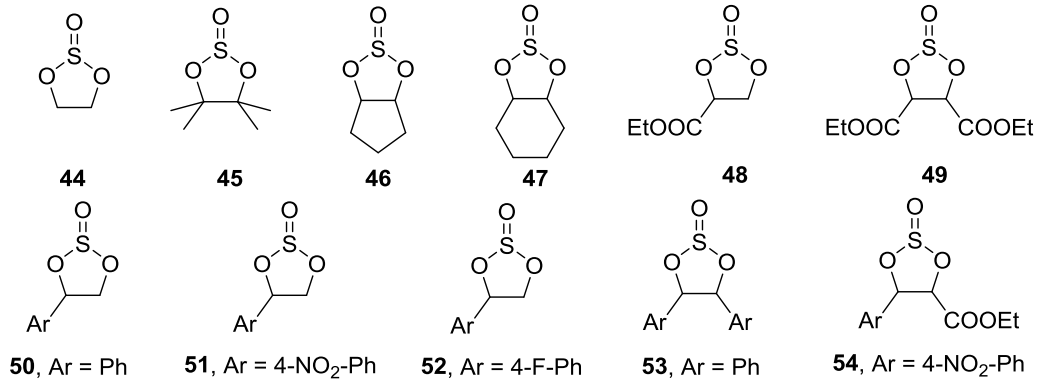


Figure 6. Reaction of sulfite diesters with nucleophile.



44 **45** **46** **47** **48** **49**

50, Ar = Ph **51**, Ar = 4-NO_2-Ph **52**, Ar = 4-F-Ph **53**, Ar = Ph **54**, Ar = 4-NO_2-Ph

Figure 7. 1,2-Cyclic sulfite diesters prepared in this study.

We hypothesized that modulating substituents R_1 and R_2 would enable us to tune the rate of substitution by a nucleophile. We prepared a series 1,2-cyclic sulfite diesters by $SOCl_2$ mediated cyclization of 1,2-diols (Figure 7). We evaluated cyclic diesters as SO_2 donors by incubating at 37 °C in pH 7.4 buffer for 30 min and SO_2 was quantified as sulfite (SO_3^{2-}) using ion chromatography.

All sulfite diesters studied underwent nucleophilic displacement by water in pH 7.4 and SO_2 yields ranged from 2 to 98% after 30 min. It appears that the substituent present on the carbons bearing the sulfite group affected the rate of decomposition of cyclic sulfite diesters and as a consequence, SO_2 yields. The pinacol derivative **45** gave negligible amount of SO_2, whereas ethylene glycol derivative **44** gave 43% yield after 30 min. Due to nearly similar pK_a values of pinacol and ethylene glycol, it is unlikely that their leaving group ability is very different. These results suggest that in case of pinacol derivative, **45**,

steric hinderance was slowing the rate of displacement. Thus, it appears that SO_2 generation involves a displacement at the carbon bearing the sulfite functional group. When an electron withdrawing group was present on the activated carbons of sulfite diester, nearly quantitative SO_2 yields were obtained, suggesting that electron withdrawing group increases the rate of displacement (Figure 7, Compd **48** and **49**).

In case of sulfite diesters **50-54** having phenyl substituent present on activated carbon atoms, gave SO_2 yields ranging from 68-98%, all higher than yield of SO_2 from the ethylene glycol derivative **44**, suggesting that phenyl substituent increases the rate of displacement. Several mechanistic paths considered for decomposition of sulfite diester are described in chapter 3B.

In conclusion, we prepared various 1,2-cyclic sulfite diesters as SO_2 donors under physiological conditions, with controlled rate of generation. The rate of donation depends upon the substituent present on carbon atoms bearing the sulfite functional group.

Chapter 4: Benzosulfones as Photochemically Activated Sulfur Dioxide (SO_2) Donors

A series of benzosulfones undergoing photolysis to generate SO_2 in physiological conditions was prepared.

Targeted drug delivery using light as an external stimulus plays an essential role in the treatment of life-threatening diseases, including cancers. We decided to explore the possibility of using light as trigger for localized delivery of SO_2 under physiological conditions. Here, benzosulfones were considered as candidates for photochemically activated SO_2 donors. Various benzosulfones were synthesized from respective benzosultines (Figure 8).

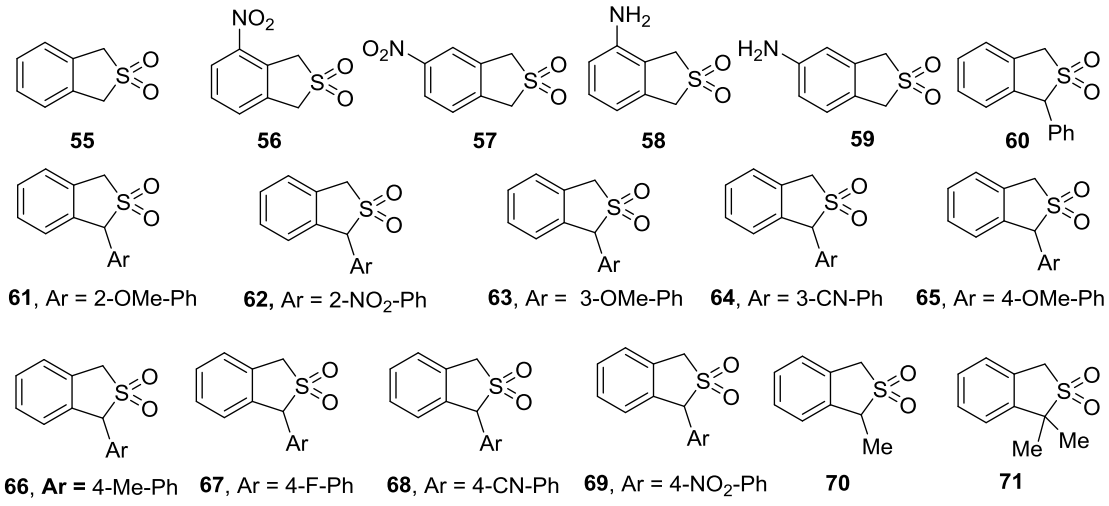


Figure 8. Benzosulfones synthesized for photochemical activation.

Benzosulfones were exposed to photolysis conditions in pH 7.4 buffer and % SO_2 generated was quantified as a sulfate by ion chromatography. All compounds studied were found to be photolabile and SO_2 yields ranged from 12 to 92% after 10 min of exposure to light. It appears that the substituent present affected the rate of decomposition of benzosulfones and as a consequence, SO_2 yields. All benzosulfones gave nearly quantitative yields of SO_2 after 60 min, except **56** and **57** (Figure 8). These results suggest that, when an electron withdrawing nitro group was present on aromatic ring, slows down the rate of decomposition and thus, decreases the SO_2 yields. When an electron donating amino group was present on the aromatic ring, increases the rates of decomposition, giving nearly quantitative SO_2 yields (Figure 8, Compd. **58** and **59**). Introduction of a phenyl ring at the α–position of sulfone resulted in increased rate of decomposition possibly due to extended conjugation and nearly quantitative SO_2 yields were obtained after 60 min (Figure 8, Compd. **60**-**69**).

In conclusion, we report a series of benzosulfones that undergo photolysis to generate SO_2 in physiological conditions. The yields of SO_2 can be modulated by altering substituents pattern.

* Compound numbers in thesis are different than abstract.

List of Publications

1. Benzosultines as Sulfur Dioxide (SO_2) Donors. Malwal, S. R.; Gudem, M.; Hazra, A.; Chakrapani, H. *Org. Lett.* **2013,** *15,* 1116-1119.

2. Design, Synthesis, and Evaluation of Thiol-Activated Sources of Sulfur Dioxide (SO_2) as Antimycobacterial Agents. Malwal, S. R.; Dharmarajan, S.; Yogeeswari, P.; Konkimalla, V. B.; Chakrapani, H. *J. Med. Chem.* **2012**, 55, 553-557. (Highlighted in Chemical and Engineering News)

3. Synthesis and Antimycobacterial Activity of Prodrugs of Sulfur Dioxide (SO_2). Malwal, S. R.; Dharmarajan, S.; Yogeeswari, P.; Chakrapani, H. *Bioorg. Med. Chem. Lett.* **2012**, 22, 3603-3606.

4. Synthesis and Evaluation of 1,2-Cyclic Sulfite Diesters as SO_2 Donors. Malwal, S. R.; Andhalkar A. S.; Chakrapani, H. (Manuscript under preparation)

5. Benzosulfones as Photochemically Activated Sulfur Dioxide (SO_2) Donors. Malwal, S. R.; Chakrapani, H. (Manuscript under preparation)

Patent Application:

Thiol Mediated/Activated Prodrugs of Sulfur Dioxide (SO_2) Having Anti-bacterial Activity. Appl. No.: **US 2014/01212111 A1;** Chakrapani, H.; Malwal S. R.

Chapter 1

Introduction: Sulfur Dioxide (SO_2), A Reactive Sulfur Species (RSS)

Chapter 1: Introduction: Sulfur Dioxide (SO_2), A Reactive Sulfur Species (RSS)

1.1 Reactive species: Reactive species are chemically reactive molecules containing N, O and S. These can be classified into three categories (Figure 1). These are produced endogenously during normal cell function as a response to various stimuli.

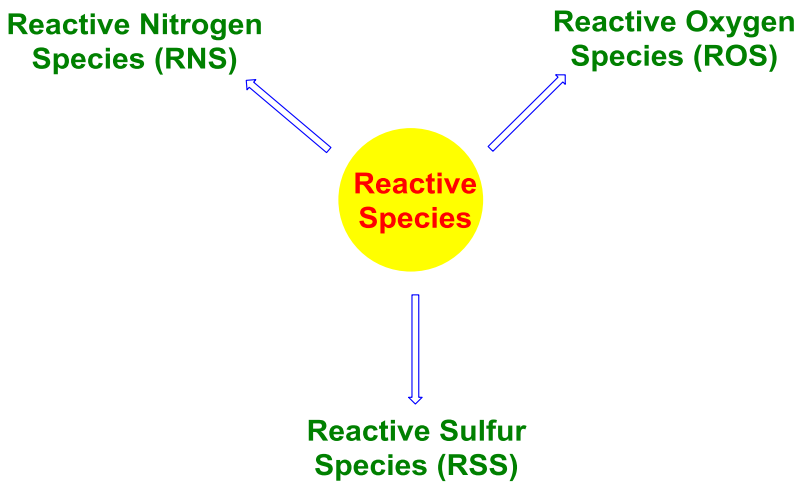
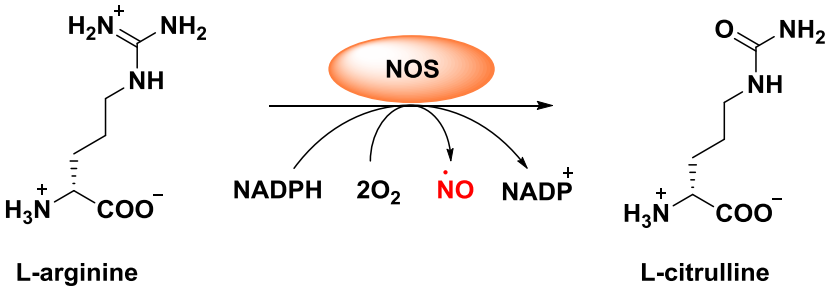


Figure 1. Reactive species classes.

Reactive species are smaller in size, freely diffusible across cell membrane, and mediate various cellular processes such as cell signalling. At normal concentrations, they are non-toxic and beneficial. At elevated concentrations, they cause damage to macromolecules such as DNA damage, protein modification and lipid peroxidation. Thus, resulting in toxicity at elevated levels. Reactive species are associated with various diseases including cancer, cardiovascular diseases and Alzheimer disease, at elevated levels.

1.2 Reactive nitrogen species (RNS): They are chemically reactive molecules containing a nitrogen atom. In reactive nitrogen species, nitric oxide (·NO) is prototypical reactive nitrogen species. Nitric oxide (NO) is formed during metabolism of L-arginine to L-citrulline, via the enzyme nitric oxide synthase (Figure 2).[1-3] Nitric oxide once produced can act as a signal translating agent in various physiological processes, including neurotransmission, defence mechanisms and blood pressure regulation.[4-8]



NOS: Nitric oxide synthase

Figure 2. Endogenous formation of nitric oxide (NO).

At elevated concentrations, nitric oxide is harmful to cells.[9] The deleterious effects of RNS are reduced by non-enzymatic and enzymatic antioxidants. Such cellular defences are useful as they are involved in the neutralization of free radicals. Thus, providing protection for various cell components against oxidative damage.

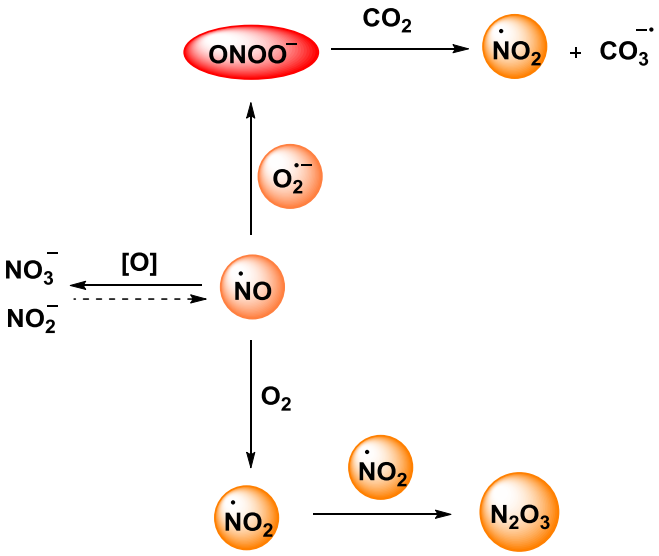


Figure 3. Reactive nitrogen species formation from NO.

Peroxynitrite: Reaction of nitric oxide (·NO) with superoxide ($O_2^{\cdot-}$) forms highly reactive peroxynitrite ($ONOO^-$).[10,11] Peroxynitrite ($ONOO^-$) can react with carbon dioxide (CO_2) molecules to form peroxycarbonate and additional types of RNS. These RNS include nitrogen dioxide ($^{\cdot}NO_2$), which in turn can be converted to dinitrogen trioxide (N_2O_3) (Figure 3).[12] $ONOO^-$ exist in another form, peroxynitrous acid (ONOOH; p*K*a = 6.8), both are in equilibrium with each other. Peroxynitrite, being highly reactive species, reacts with various biological targets and components of the cell. These targets

include DNA bases, thiols, amino acid residues, lipids and low-molecular weight antioxidants.[12,13]

1.3 Reactive oxygen species (ROS): Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen atom. Superoxide ($O_2^{\bullet-}$), is considered as a primary reactive oxygen species (ROS), formed mainly during mitochondrial electron transport chain and from NADPH oxidase (NOXs) enzyme complexes. Mitochondria contain various other enzymes like NADPH oxidase, xanthine oxidase, nitric oxide synthase and peroxisomal constituents that generate $O_2^{\bullet-}$. Superoxide formed, dismutated to hydrogen peroxide (H_2O_2) and oxygen by superoxide dismutase (SOD) as shown in figure 4.[14,15]

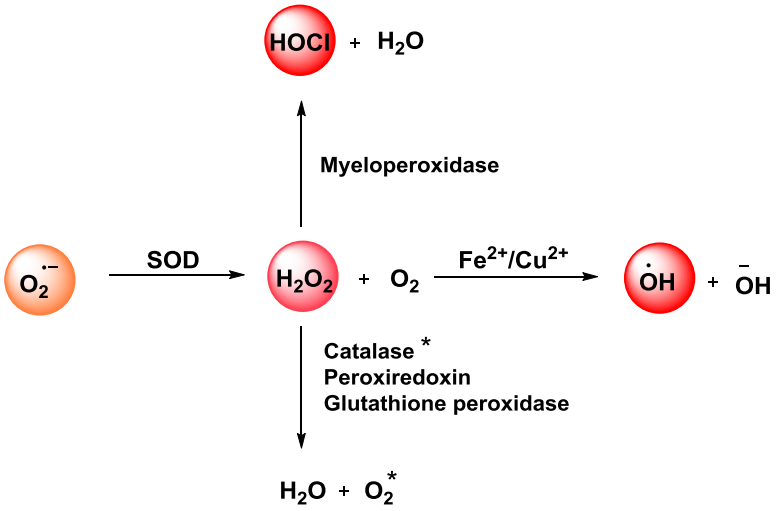


Figure 4. Formation and transformation of biologically relevant reactive oxygen species.

The enzymes such as glutathione peroxidases, peroxiredoxins and catalases, metabolize H_2O_2 to H_2O and O_2.[16] Hydrogen peroxide reacts slowly or not at all with most biological molecules, including low-molecular-weight antioxidants. However, H_2O_2 can react with trace metal ions (Fe^{2+} or Cu^{+}) via Fenton or Haber−Weiss reaction to generate hydroxyl radical ($^{\bullet}OH$) (Figure 4 and 5). In phagosomes, H_2O_2 involved in the generation of hypochlorous acid (HOCl) and water by acting as a substrate for myeloid peroxidase.

The hydroxyl radical ($^{\bullet}OH$) being a strong oxidizer reacts with various cellular components such as protein, DNA and lipid biomolecules, resulting in its shorter half-life (~1 ns).[17] In healthy cells, because of H_2O_2 metabolism and tightly regulated concentration of metal ions the rate of formation of $^{\bullet}OH$ is low. ROS depending upon its concentration can be either deleterious or beneficial.[9] ROS are involved in cellular defence against infectious agents, in cell signalling pathways and at lower levels in mitosis.[18]

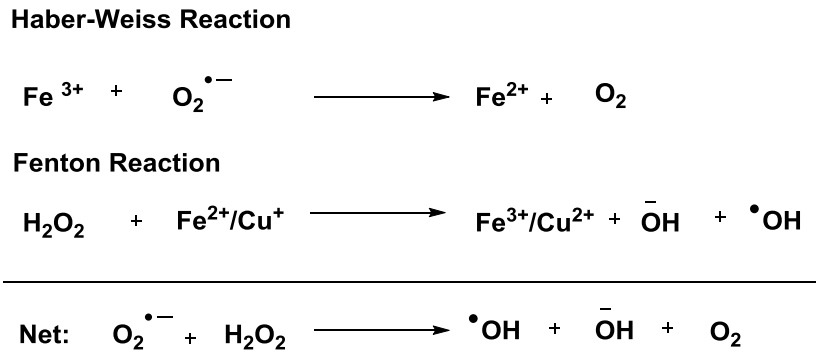
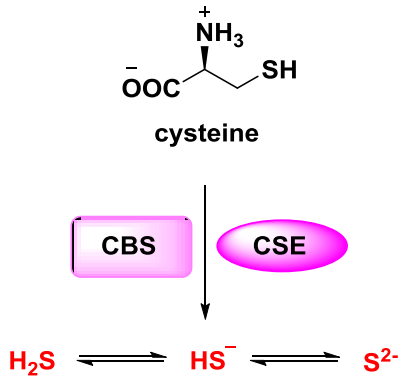


Figure 5. Haber-Wiess and Fenton reaction.

At elevated levels, ROS induce damage to various cell components, such as nucleic acids, proteins, membranes and lipids.[18] The damaging effects of ROS are reduced by non-enzymatic as well as enzymatic antioxidant.[19] Although oxidative damage caused by ROS, is associated with various diseases including cancer, even after the presence of the cellular antioxidant defence system.[20]

1.4 Reactive sulfur species (RSS): These are chemically active molecules containing reactive sulfur atom. Hydrogen sulfide (H_2S) is a prototypical species. H_2S is formed during metabolism of sulfur containing amino acid (SAA), by enzymes cystathionine β-synthase (CBS), and cystathionine γ-lyase (CSE) (Figure 6). H_2S functions as a gasotransmitter to control various biological processes such as secretion of hormone or chemical messenger of cell, which binds to a receptor on the same cell (autocrine), cell-cell signalling to induce changes in nearby cells (paracrine).[21]



CBS: Cystathionine β-synthase and **CSE:** Cystathionine γ-lyase

Figure 6. Endogenous formation of hydrogen sulfide.

Hydrogen sulfide along with NO and CO termed as a gasotransmitter. As a small molecule, H_2S rapidly travels through the cell membrane and performs various biological functions from cytotoxic to cytoprotective effects (Figure 7).[22-26]

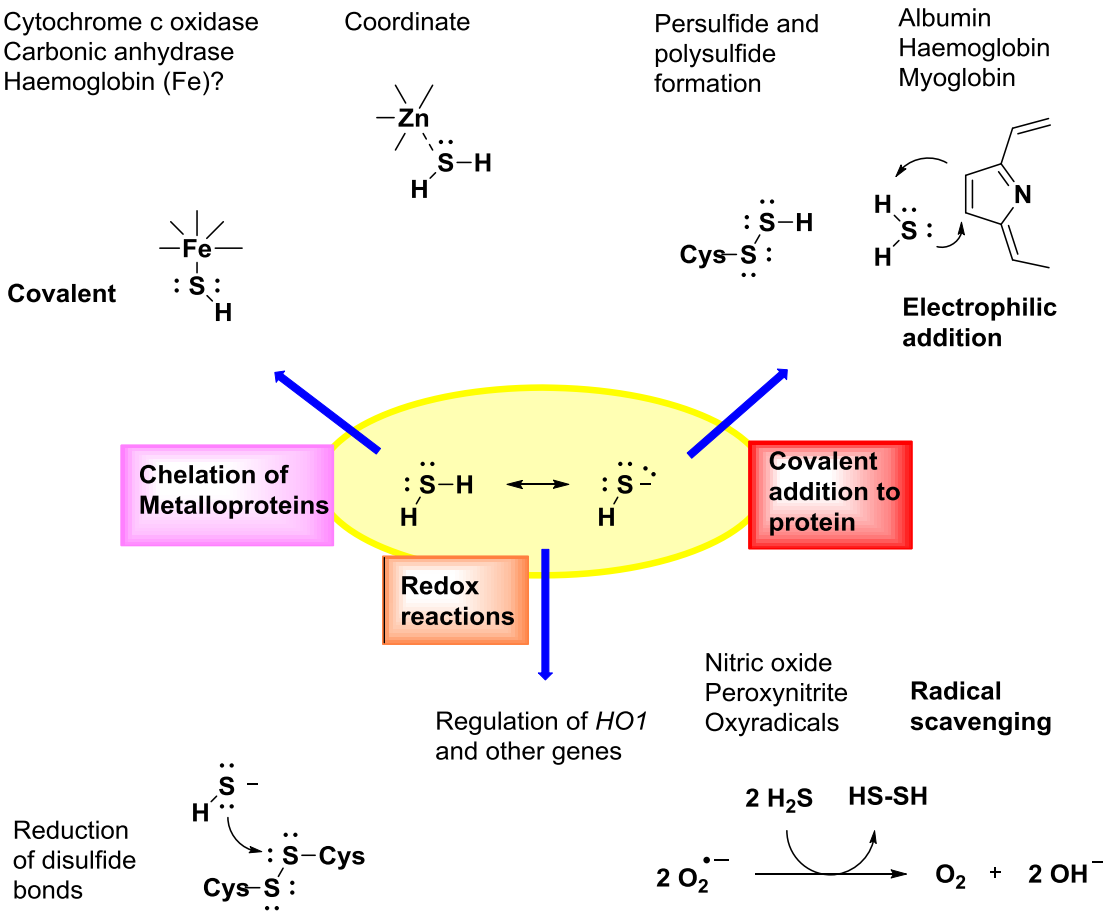


Figure 7. Some biological effects of H_2S in mammalian cells.

Hydrogen sulfide at micromolar concentration shows cytoprotective effects in *in vitro* studies, typically generated from Na_2S or NaSH, by neutralizing various reactive species such as oxyradicals, peroxynitrite, hypochlorous acid and homocysteine.[24,27-29] At elevated concentration, H_2S is involved in the generation of free radicals and oxidants. Thus showing various cytotoxic effects such as glutathione depletion, initiation of mitochondrial cell death pathways, intracellular iron release and calcium mobilization.[24,28-39] H_2S can also influence an up regulation of cytoprotective genes including haem oxygenase 1 (HO1) in pulmonary smooth muscle cells, in rat nasal tissues *in vivo* and in macrophages *in vitro*. Hydrogen sulfide in *in vitro* studies shows smooth-muscle relaxation effect on isolated organs (in the stomach or in blood vessels). At high concentrations

reduces the metabolic rate of the affected cell or organ.[40,41] Sulfide can form a covalent adduct with proteins and is involved in formation of persulfide and polysulfide. It also reacts with haemoglobin to form sulfhemoglobin and myoglobin to form sulfamyoglobin. Sulfhemoglobin has been detected in animals and humans after exposure to high doses of sulfide.[42] Hydrogen sulfide is also involved in chelation of metalloproteines and reduction of disulfide bonds.

1.5 Formation of reactive sulfur species *in vitro* and *in vivo*:

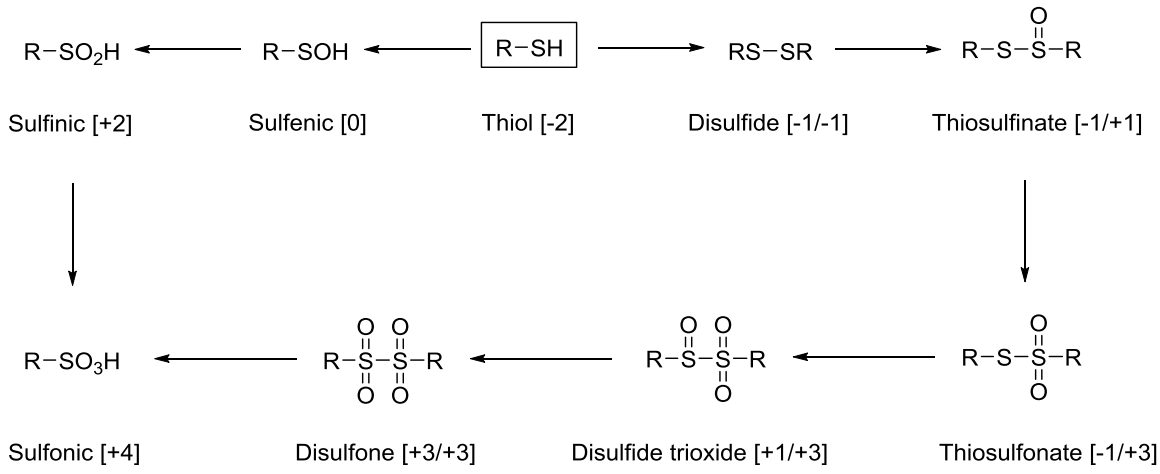


Figure 8. Biological oxidation states of cysteine.

Various oxidation products containing sulfur such as, thiosulfinates, sulfenic acids, disulfides and S-nitrosothiols were formed by oxidation of proteins and low molecular weight nonprotein thiols (Figure 8). Each transformation induces redox conversions that involve oxidation of other thiols similar to reactive oxygen and reactive nitrogen species. These chemically reactive forms of cysteine can be categorized as reactive sulfur species.[43,44]

Sulfur can exist in various oxidation states (Figure 8) starting from -2 (sulfides and thiols such as glutathione) to +6 (sulfates such as heparin sulfate) and possibly including fractional oxidation states, under physiological conditions.[45-49] Thiosulfinate, thiyl radical, sulfenic acid, thiosulfonate, disulfide and sulfide species have higher reactivity towards thiols as compare to other reactive sulfur species formed (Figure 9).

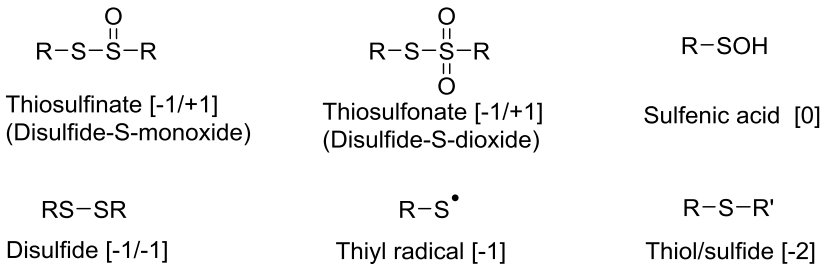


Figure 9. Reactive sulfur species formed in physiological conditions.

Sulfur-Centred radicals: Cysteine undergoes one-electron oxidation to form a highly reactive sulfur-centred radical under physiological conditions (Table 1).[50,51] Thiyl radicals (RS·) can be formed by hydrogen donation, enzymatic oxidation and reaction with ROS. Several other sulfur-centred radicals can be formed *in vitro* and *in vivo.* Many of these are highly unstable and observed by ESR in frozen solutions at low temperatures.[50,52,53]

Table 1. Sulfur-centred free radical species implicated in physiological processes

Radical species	Chemical formula	Radical centre (oxidation state)
Thiyl radical	$RS^{\cdot}$	S (– 1)
Sulfide radical cation	$(RSR)^{\cdot+}$	S (– 1)
Disulfide radical cation	$(R_2S\therefore SR_2)^{\cdot+}$	S (– 1.5)
Perthiyl radical	$RSS^{\cdot}$	S (0)
Sulfinyl radical	$RSO^{\cdot}$	S (+1)
Sulfonyl radical	$RS(O)_2^{\cdot}$	S (+3)
Sulfur trioxide radical anion	$SO_3^{\cdot-}$	S (+5)
Sulfur pentoxide radical anion	$SO_5^{\cdot-}$	O (0)
Thiyl peroxyl radical	$RSOO^{\cdot}$	O (0)
Sulfonyl peroxyl radical	$RS(O)_2OO^{\cdot}$	O (0)

Disulfides and disulfide-S-oxides: Disulfides are redox-active under physiological conditions with redox potentials around – 400 mV.[54] As a consequence, cellular disulfides cause oxidative damage when present at elevated concentrations and contributes towards oxidative stress. It has recently been postulated that an additional important pathway for the generation of RSS may arise from the oxidation of thiols and disulfides to disulfide-*S*-oxides.[55,56] The oxidation of GSH with hydrogen peroxide, singlet oxygen and S-nitrosoglutathione (SNG) *in vitro* leads to formation of disulfide, glutathione disulfide-*S*-

monoxide [GS(O)SG] and disulfide-S-dioxide [$GS(O)_2SG$]. These thiosulfinates [GS(O)SG] and thiosulfonates [$GS(O)_2SG$] can be considered as 'activated' disulfides that resemble the redox behaviour of strongly oxidising disulfides rather than of sulfinates or sulfonates.

Sulfur-based acids: Reduction of disulfide-S-oxides by thiols leads to the formation of sulfenic acids. Cysteine sulfenic acid is found in a number of proteins and enzymes, amongst them are NADH peroxidase, NADH oxidase and several human peroxiredoxins.[48,57,58] The formation of sulfenic acids *in vivo* is difficult to assess due to their high reactivity and consequent instability. In proteins and enzymes, sulfenic acids are formed by the direct oxidation of cysteine thiols with peroxides, peroxynitrite and nitric oxide.[59,60] Sulfenic acids react with thiols to form disulfides and with oxygen to form the more stable sulfinic acids.

1.6 Endogenous generation of SO_2:

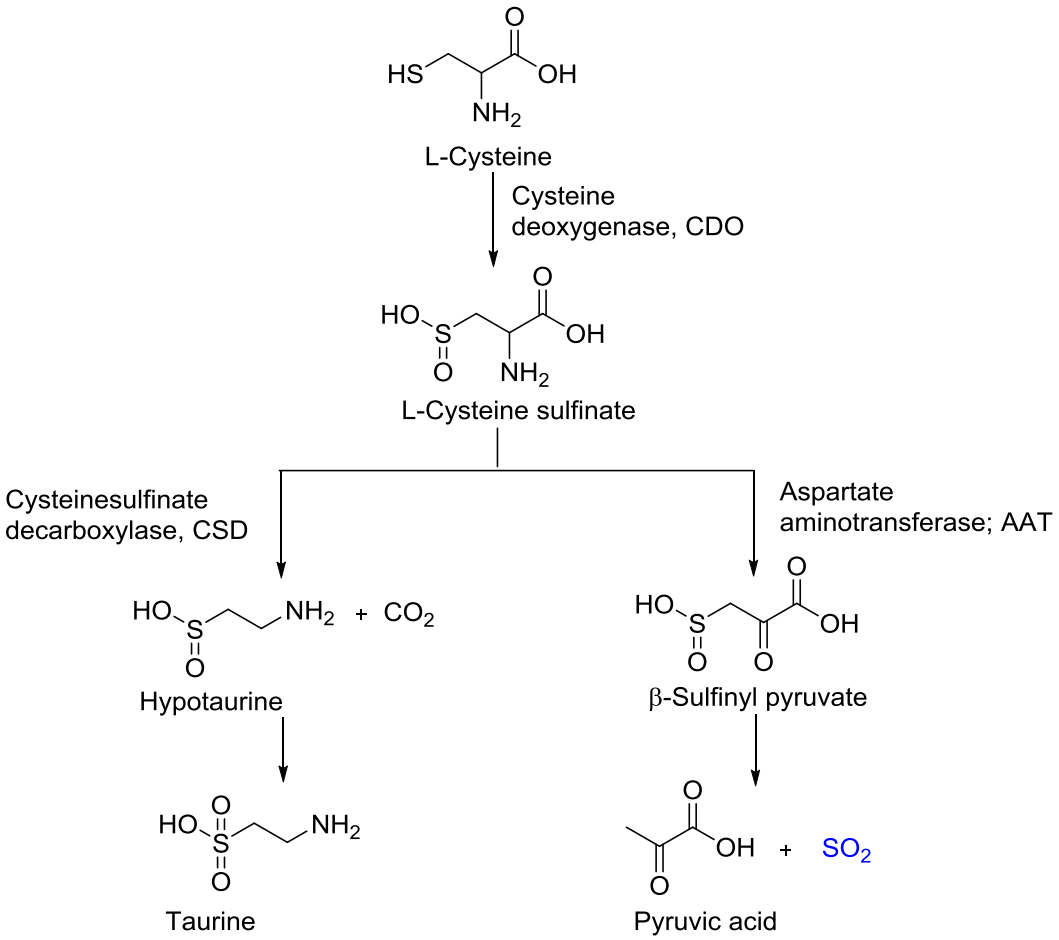


Figure 10. Endogenous generation of sulfur dioxide (SO_2).

SO_2 can be produced endogenously from the metabolism of sulfur containing amino acids (SAAs).[61] L-cysteine is a vital precursor for the endogenous generation of SO_2 (Figure 10).[61,62] L-cysteine is oxidized to L-cysteinesulfinate by enzyme cysteine dioxygenase (CDO). L-cysteinesulfinate is subsequently transaminated to form β-sulfinylpyruvate catalyzed by aspartate aminotransferase (AAT). β-sulfinylpyruvate decomposes spontaneously to pyruvate and SO_2.[62] Some of the SO_2 is hydrated to sulfite, which is subsequently oxidized by sulfite oxidase (Suox) to sulfate. But some of the SO_2 can exist in certain cellular compartments in the gaseous form thus evading this reaction.[63] Another pathway for L-cysteinesulfinate metabolism involves decarboxylation to CO_2 and hypotaurine by cysteinesulfinate decarboxylase. Hypotaurine formed is oxidized to taurine.[62,64] SO_2 is one of the oxidation products of H_2S produced inside the cell (Figure 11).[65-68]

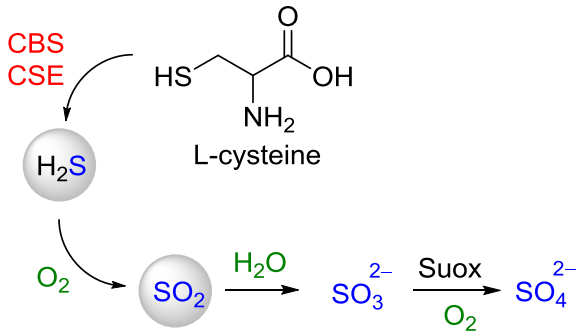


Figure 11. Endogenous production of SO_2 by oxidation of H_2S.

1.7 Characteristics of reactive species: Reactive species such as NO and H_2S are gaseous in nature and are produced during normal cell function in healthy cells (Figure 12).

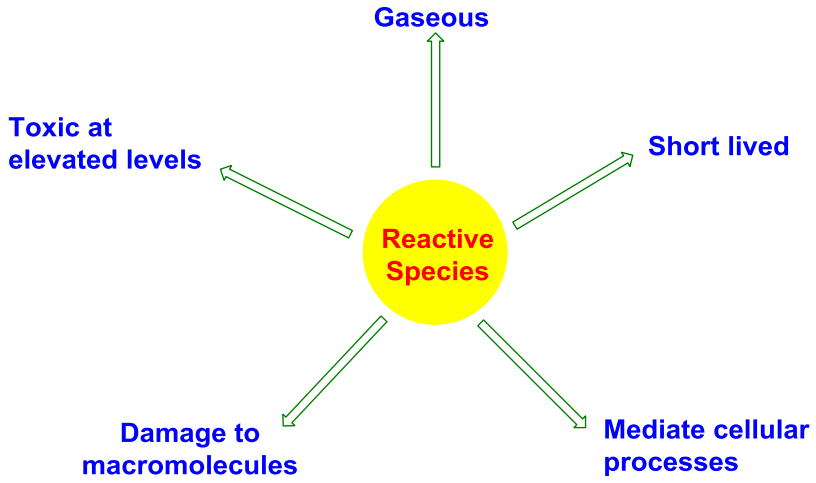


Figure 12. Characteristics of biological reactive species.

They are short-lived as nitric oxide and superoxide have a free unpaired electron. They are highly reactive, once formed spontaneously react with various targets. Hydrogen sulfide has a higher lifetime as compared to NO and $O_2^{\bullet-}$.

1.8 Characteristics of endogenously produced sulfur dioxide (SO_2): The synthetic chemical applications of sulfur dioxide are well documented in literature.[69] Similar to other reactive species, sulfur dioxide is gaseous in nature with short half-life. SO_2 once produced, forms sulfite (SO_3^{2-}) as a hydration product. Sulfite (SO_3^{2-}) in the presence of various metal ions form reactive sulfoxyl radicals $SO_3^{\bullet-}$, $SO_4^{\bullet-}$, and $SO_5^{\bullet-}$.[70]

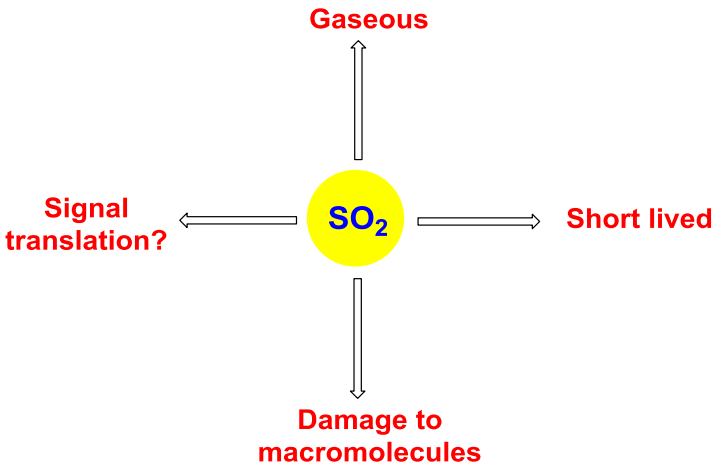


Figure 13. Characteristics of endogenously produced sulfur dioxide.

These radicals further cause damage to various macromolecules such as DNA damage along with protein modification and lipid peroxidation. SO_2 is hence toxic at elevated levels (Figure 14). SO_2 is endogenously produced is not in question. However, whether SO_2 produced as a signal transmitting agent has not been established. SO_2 shows the vasodilatory effect and lowering of blood pressure in mice.[11,71,72] Due to these characteristics SO_2 can be considered as a reactive sulfur species (RSS).

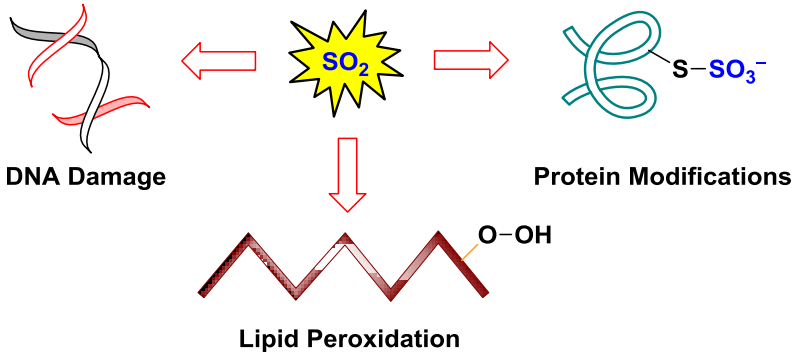
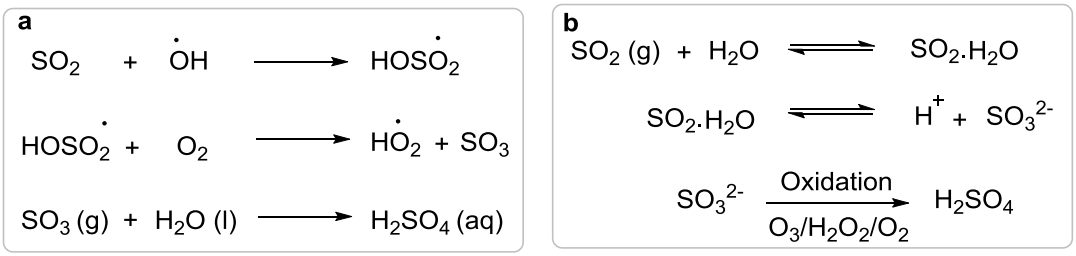


Figure 14. Biomacromolecular damage caused by SO_2.

1.9 Environmental aspects of sulfur dioxide (SO_2): Sulfur dioxide is a colorless dense gas with a pungent smell. Sulfur dioxide enters atmosphere naturally via volcano eruption. H_2S derived from the eruption is rapidly oxidized to SO_2 after entering the atmosphere. H_2S formed by biological decay of organic matter and is oxidized to SO_2 in the atmosphere. Sulfur dioxide enters the atmosphere via man made activities, such as combustion of coal and other fossil fuels.[73] In addition, industrial activities that process material and mineral ores containing sulfur, generate SO_2.

The biogeochemical cycle has SO_2 as a major member playing a critical role in maintaining the balance of environmental sulfur.[74] Once entered into the atmosphere, SO_2 is involved in acid rain and the formation of photochemical smog with harmful effect on living organisms.[75] Acid rain, where rainfall becomes so acidic by atmospheric pollution, is caused mainly by the emission of sulfur dioxide and nitrogen oxide. After a series of reactions on SO_2, sulfuric acid (H_2SO_4) is formed.



Scheme 1. Mechanism of acid rain formation.

In the atmosphere, SO_2 is oxidized by hydroxyl radical to sulfuric acid (Scheme 1a). In the clouds, SO_2 reacts with liquid water droplets to form SO_3^{2-}, which is then oxidized to sulfuric acid by O_3 or H_2O_2 or O_2 present in the atmosphere (Scheme 1b).[76] Inhaled SO_2 is hydrated to form its derivatives, bisulfite, HSO_3^- and sulfite, SO_3^{2-} (1:3 M/M in neutral fluid).[71] Exposure to sulfur dioxide is related with respiratory and cardiovascular diseases. Exposure to SO_2 have linked with many respiratory diseases such as lung cancer,[77] when the SO_2 concentration exceeds 0.6 mg/m^3. Sulfur dioxide exposure is toxic to the respiratory system and related to the increase in the severity of asthma.[78-80] Inhalation of SO_2 can cause oxidative as well as DNA damage in different organs of rat and mice, particularly in lung, heart and brain.[71,81] Also, SO_2 could affect blood pressure in rats.[82]

1.10 DNA damage by sulfur dioxide: As discussed earlier, inhaled SO_2 is hydrated to its derivatives sulfite and bisulfite. The sulfoxyl radicals $SO_3^{\bullet-}$, $SO_4^{\bullet-}$ and $SO_5^{\bullet-}$, are formed by the autoxidation of sulfite further causes oxidative damage to biomacromolecules (Figure 15).[83,84] Sulfite is the hydration product of SO_2, is also used as a preservative in food and pharmaceutical products.

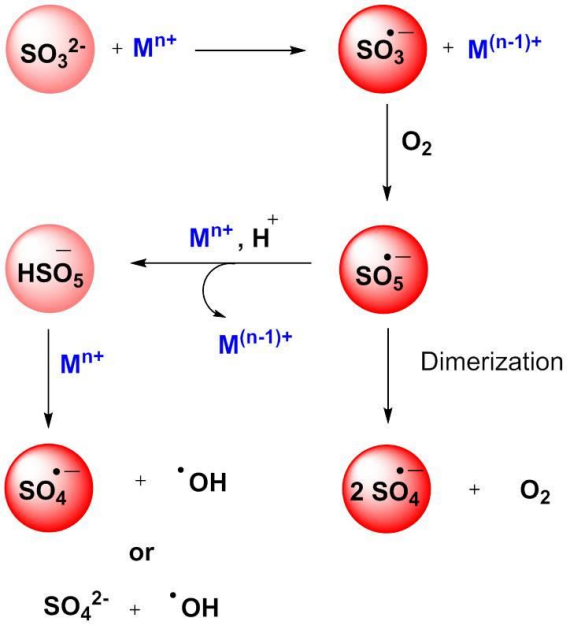


Figure 15. Mechanistic scheme proposed for metal catalyzed sulfite autoxidation.

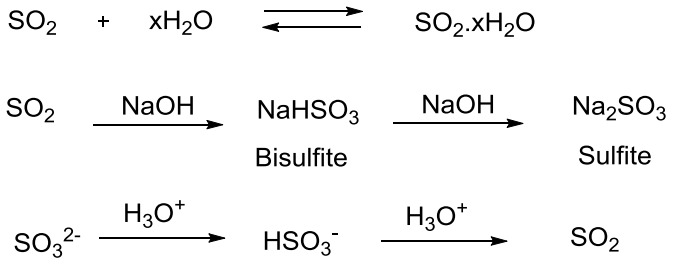
First sulfite radical $SO_3^{\bullet-}$, is formed by transition metal catalyzed autoxidation of SO_3^{2-}. In presence of molecular oxygen it is converted into $SO_5^{\bullet-}$. Sulfate radical $SO_4^{\bullet-}$, is formed by two pathways. In first pathway $HSO_5^{\bullet-}$ formed by one electron reduction of transition metal, followed by metal mediated conversion to $SO_4^{\bullet-}$. In the second pathway, dimerization of $SO_5^{\bullet-}$ to form $2SO_4^{\bullet-}$ and O_2. Thus, in DNA damage resulting from sulfoxyl radicals, it is not clear which radical is responsible for DNA damage.

Some metal ions such as Cu(II) and some Ni(II) complexes are involved in decomposition of HSO_5^- to sulfate ion and hydroxyl radical.[85,86] Out of sulfoxyl radicals formed, $SO_4^{\bullet-}$ is the more effective oxidant having a potential in the range of 2.5 - 3.1 V, whereas $SO_5^{\bullet-}$ and $SO_3^{\bullet-}$ have potentials between 0.63-1.1 V.[87] Hence, SO_2 itself might be well tolerated but in the presence of metal ion, it forms free radicals that can damage vital cellular components. DNA damage occurring from autoxidation of sulfite by transition metal ions such as, Cu(II),[88,89] Cr(VI),[90] Fe(III),[88,89,91] Mn(II),[89,92,93] Co(II),[89,94] and Ni(II),[94] has been reported.

1.11 SO_2 in food technology: In contrast, despite its well-documented toxic effects, SO_2 is used as a preservative in the form of sulfite and bisulfite in food and pharmaceutical industry.[74] SO_2 also used as an antimicrobial and antioxidant in wine making,[95] is not harmful at lower concentration. The anions sulfite and bisulfite, in which sulfur is present in same oxidation state +IV, are used as a preservative in food products.[96,97] These observations suggest that sulfur dioxide and its hydrated forms, sulfites, are well tolerated by humans at low concentration and have anti-microbial properties.

Sulfite oxidase (SO) is an enzyme present in the mitochondria of eukaryotes, which carries out the oxidation of sulfite to sulfate. Sulfite oxidase has molybdopterin as a cofactor and a heme group. In all organisms, this enzyme is involved in detoxification of sulfite (a strong nucleophilic and reducing compound) and catalyses oxidation to mild form sulfate.[98] SO also detoxifies the sulfite derived from exogenous sources, such as sulfiting agents used in pharmaceuticals and as preservatives in food.[99,100]

1.12 SO_2 in wine making: Romans first time used SO_2 in wine making, by burning sulfur containing candles inside the empty vessels used for wine storage, for keeping wine fresh and free of a vinegar smell. Sulfur dioxide (SO_2) until used in the science of wine making as an antimicrobial agent.



Scheme 2. Sulfite existence in basic and acidic pH.

During wine making, sulfites are used to inhibit microbial growth prior to yeast addition for fermentation.[101] Under aqueous basic conditions, SO_2 is converted to bisulfite (HSO_3^-) and sulfite (SO_3^{2-}). In acidic conditions, sulfite reverts back to sulfur dioxide (Scheme 2). The amount and time point of SO_2 addition is important and depends on the type of wine to which it is being added. Also, yeast produces about 10 ppm of SO_2 during fermentation. Thus, even without any added sulfites, wine may naturally contain SO_2. Potassium metabisulfite ($K_2S_2O_5$) is used as a stable, powdered source of sulfur dioxide in most of the wineries and is 57.6% available SO_2 by weight.[101] Some wineries prefer to use

an aqueous solution of SO_2 by passing a stream of SO_2 in water. The liquid is typically 5% to 10% SO_2 by weight. After dissolution in water, it forms sulfite and bisulfite. Since wine is acidic (pH 3 to 4), molecular SO_2 and HSO_3^- mostly forms the composition (99.99 % at pH 3.4). Dissolved oxygen in wine can react with phenolic compounds to give brownish shade to wine and also forms acetaldehyde. Sulfur dioxide prevents oxidation by binding with precursors involved in oxidation. Also, SO_2 binds with the products of oxidation. It has been known that SO_2 acts as an antimicrobial agent against a wide variety of microorganisms. The possible mechanism for antimicrobial activity of SO_2 may involve crossing of the cell membrane by SO_2 molecule to inhibit enzymes and proteins present inside bacterial cell.[101]

1.13 Organic sources of reactive species:

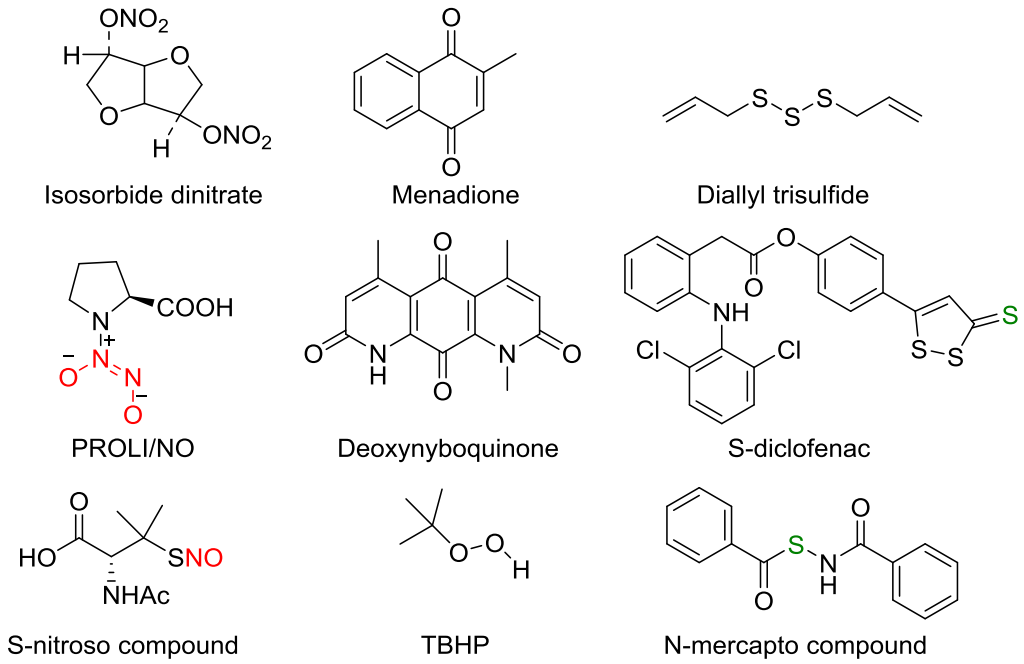


Figure 16. Organic sources of NO, ROS and H_2S under physiological condition.

In order to study the precise biological role of reactive species, donors which will generate reactive species in a controlled manner under physiological conditions, having good bioavailability are of importance. Several organic sources of reactive species are used for biological studies in order to understand their precise biological role or to treat medical conditions (Figure 16).

Nitric oxide donors: Isosorbide dinitrate (ISDN) is a nitric oxide donor used as a vasodilator for treating chest pain and lowering of blood pressure.[102] ISDN is also used

for treating congestive heart failure.[103,104] ISDN requires a sulfhydryl group for NO generation. Also PROLI/NO is a diazeniumdiolate based prodrug of NO, which generates NO by hydrolysis upon dissolution in the buffer.[103] S-nitroso compounds are also used as NO donors, which react with thiol to give nitric oxide.[104-106]

Reactive oxygen species donors: Menadione a synthetic analog of vitamin K and tert-butylhydroperoxide has been used as a source of ROS. Deoxynyboquinone (DNQ), a natural product reported as a potent anticancer compound (IC_{50}, 6 - 210 nM), induces death of cancer cells via ROS generation.[107] DNQ undergoes one-electron reduction to form semiquinones. The quinone can be regenerated in the presence of oxygen, forming superoxide.[108]

Hydrogen sulfide donors: Allicin (diallyl thiosulfinate) from garlic (*Allium sativum*), is a naturally occurring hydrogen sulfide donor, which decomposes in water to give a mixture of compounds. Diallyl disulfide and diallyl trisulfide (DATS, Figure 16) are effective H_2S donors, shows vasodilatory effects on rat aortas.[109] H_2S donors when attached to clinically effective drugs, shows additional beneficiary effects. Anethole trithione hydroxide (ADT-OH, Figure 16), a dithiolethione, is frequently used H_2S donor. This molecule has been combined with numerous nonsteroidal anti-inflammatory drugs such as diclofenac.[110,111]

Sources of sulfur dioxide (SO_2): In order to study the biological role of sulfur dioxide (SO_2) we have to rely on gaseous SO_2 or complex formulations of bisulfite and sulfite. Both of these have poor bioavailability and spatio temporal control is difficult to achieve.[78,112,113] Hence in order to understand the precise biological role of SO_2 reliable organic sources of SO_2 are necessary. These SO_2 donors upon activation by a suitable trigger should generate SO_2 under physiological condition.

1.14 Characteristics of ideal source of sulfur dioxide: For a compound to act as a SO_2 donor, it should be a stable solid, easy to handle and store. Under suitable physiological conditions, this donor must dissociate to produce SO_2 (Figure 17).

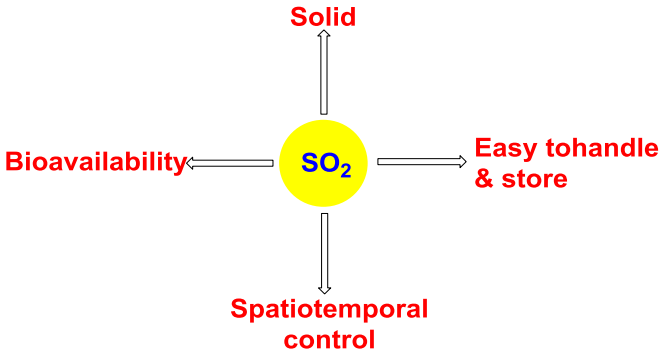


Figure 17. Characteristics of ideal source of SO_2.

1.15 Scaffolds to be used for SO_2 donation: A compound in order to act as a source of sulfur dioxide should have SO_2 present in its structure. Among various possibilities, sulfinic group (A), which is flanked by N or O or C and sulfone group (B), which is flanked by N or C (Figure 18) were considered as potential sources of SO_2.

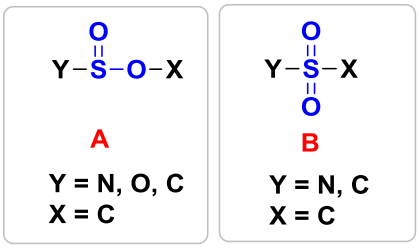


Figure 18. Functional group used for SO_2 generation.

Cyclic sulfinate ester, sulfonamides, sulfite diesters and sulfones were selected for this study. Systematic structural modifications will be carried out such that compounds prepared will generate sulfur dioxide (SO_2) by using suitable trigger, in physiological condition with the spatio temporal control.

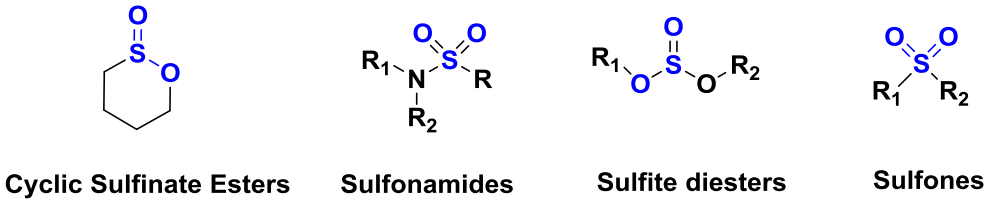


Figure 19. Scafolds to be used for SO_2 generation.

1.16 Strategies to be used for generation of sulfur dioxide:

We proposed to use three different strategies for SO_2 generation (Figure 20).

1) Thermally activated SO_2 donors

2) Nucleophile activated SO_2 donors

3) Photochemically activated SO_2 donors

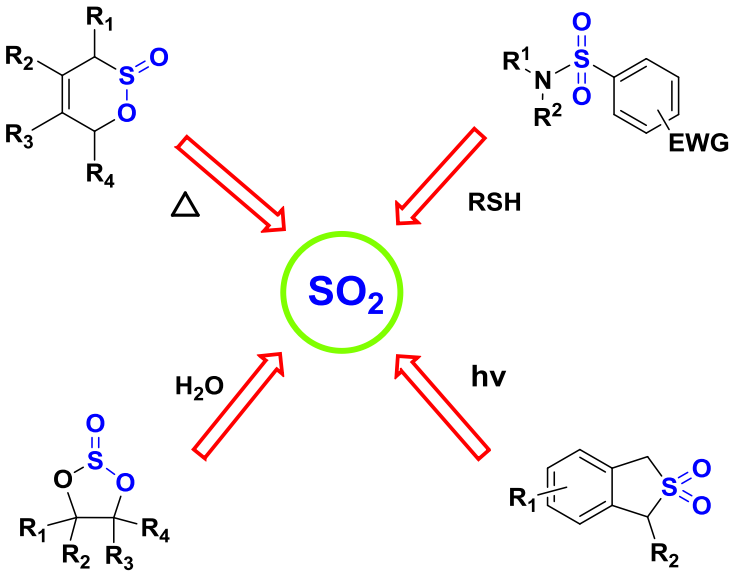


Figure 20. Strategies for SO_2 generation.

1.17 Strategy 1. Thermal Activation:

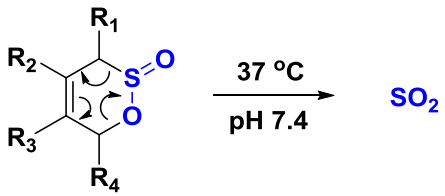


Figure 21. Thermal Activation (Sultine).

In this strategy cyclic sulfinate ester (Figure 21), will be used as a scaffold. Substituents on the ring will be varied such that corresponding cyclic sulfinate ester will be stable at room temperature. Upon the dissolution in buffer, this sulfinate ester will undergo retro Diels-Alder reaction to give SO_2. The major challenge is to be able to modulate reactivity of the sultine in order to achieve SO_2 generation under physiological temperature of 37 °C. In this strategy, heat will be used as a trigger. This strategy is discussed in Chapter 2.

1.18 Strategy 2. Nucleophile activation: This strategy is divided in to two sections.

Section A: Thiol activated sulfur dioxide donors: In this strategy, sulfonamides were prepared from primary or secondary amines having different substituents, by treating with 2,4-dinitrobenzenesulfonyl chloride. The 2,4-dinitrobenzenesulfonamides will be treated with biological thiols such as, L-cysteine and glutathione in pH 7.4 buffer. The

sulfonamides will undergo aromatic nucleophilic substitution by thiolate in pH 7.4 buffer to give sulfur dioxide and by products (amine and thiol adduct). This strategy can be used for intracellular studies.

Figure 22. Thiol activation.

The major challenge is to be able to modulate reactivity of the sulfonamides in such a way that, they should react selectively with thiols in pH 7.4 to generate SO_2 in a controlled manner. The sulfonamides should have higher bioavailability for controlled release of SO_2 inside cell. This strategy is discussed in section A of Chapter 3.

Section B: 1,2- Cyclic sulfite diesters as SO_2 donors: In this strategy, 1,2-cyclic sulfite diesters with different substituents present on carbon atom α to oxygen, will be prepared. Nucleophilic attack by water molecule on one of the carbon bearing sulfite functional group takes place to give sulfur dioxide and corresponding 1,2-diol (Figure 23). This strategy is discussed in section B of Chapter 3.

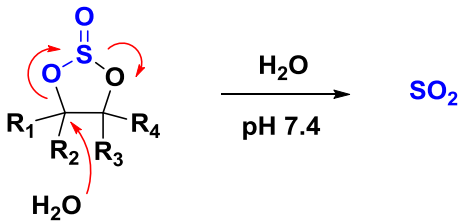


Figure 23. 1,2- cyclic sulfite diesters as SO_2 donors.

1.19 Strategy 3. Photochemically activated SO_2 donors: In this strategy, sulfones with different substituents present on α-position and aromatic part will be prepared (Figure 24).

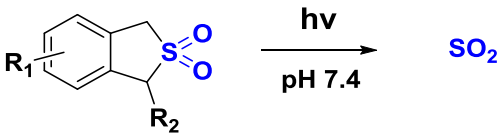


Figure 24. Benzosulfones as SO_2 donors.

Above sulfones will be subjected to photolysis under physiological condition, and their SO_2 donating ability will be evaluated. This strategy is discussed in Chapter 4.

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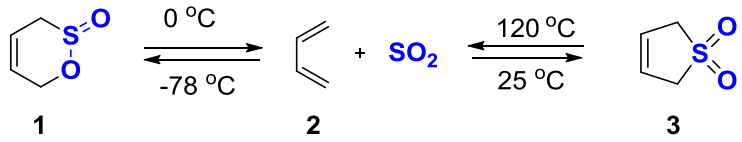
Chapter 2

Thermally Activated SO_2 Donors: Design and Synthesis of Benzosultines as SO_2 Donors

Chapter 2: Thermally Activated SO_2 Donors: Design and Synthesis of Benzosultines as SO_2 Donors

2.1 Introduction: Jung and co-workers reported that 3,6-dihydro-1,2-oxathiine 2-oxide **1**, (sultine) is unstable at 0 °C, undergoes cycloreversion to give SO_2 and a 1,3-diene **2** (Scheme 1). Chelotropic addition of SO_2 to 1,3-diene produces sulfone **3**. This sulfone is stable at room temperature. The same decomposition products are obtained from sulfone **3** at 120 °C (Scheme 1).



Scheme 1. Retro Diels-Alder reaction of sultine **1** and sulfone **3**.

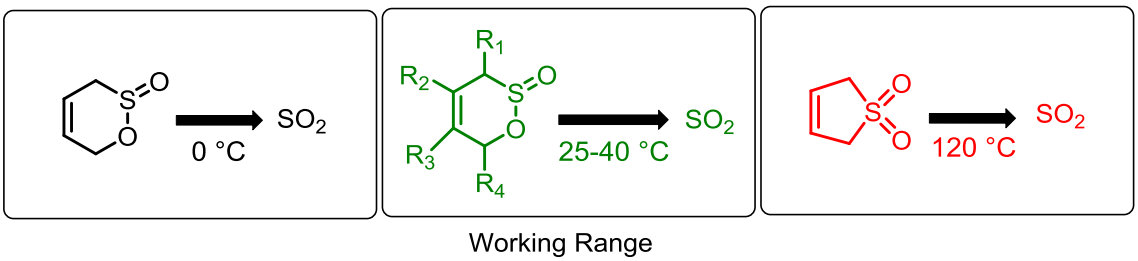
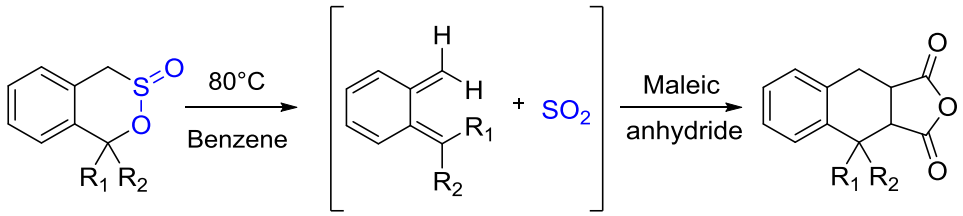


Figure 1. Cycloreversion temperatures of sultine and sulfone.

To increase the stability of sultine at room temperature, benzofused sultines (benzosultines) **4**, **5** and **6** were considered (Scheme 2). Benzosultine **4**, was stable up to 80 °C and undergoes Diels-Alder reaction with maleic anhydride to give corresponding Diels-Alder adduct (Scheme 2).[1] The relative rate of reaction is 1.9, when R_1 = -CH_3 and R_2 = H. When a phenyl substituent is introduced, the relative rate increased from 1.9 to 200 (R_1 = Ph and R_2 = H) with respect to benzosultine **4**. The rate of cycloreversion depends upon the



4, $R_1 = R_2$ = H, Relative rate : 1.0
5, R_1 = CH_3; R_2 = H, Relative rate : 1.9
6, R_1= Ph; R_2 = H, Relative rate : 200

4a, $R_1 = R_2$ = H,
5a, R_1 = CH_3; R_2 = H,
6a, R_1= Ph; R_2 = H

Scheme 2. Cycloaddition reaction of benzosultines.

substituent present on carbon adjacent to oxygen atom. Thus, modulating substituents provides opportunity for altering rates of cycloreversion

Benzosultines, in order to act as organic SO_2 donors, must undergo cycloreversion at physiological temperature of 37 °C (Figure 1). We postulated that systematic structural modifications on the carbon atom adjacent to oxygen atom would lead to organic SO_2 donors at physiological temperature of 37 °C (Figure 2).

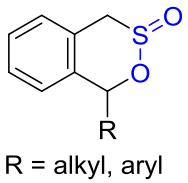


Figure 2. Proposed benzosultine scaffold.

Various benzosultines can be prepared from phthalide (Figure 3).[1] Benzosultine can be obtained by cyclization of sulfoxide which in turn can be obtained from thioether.

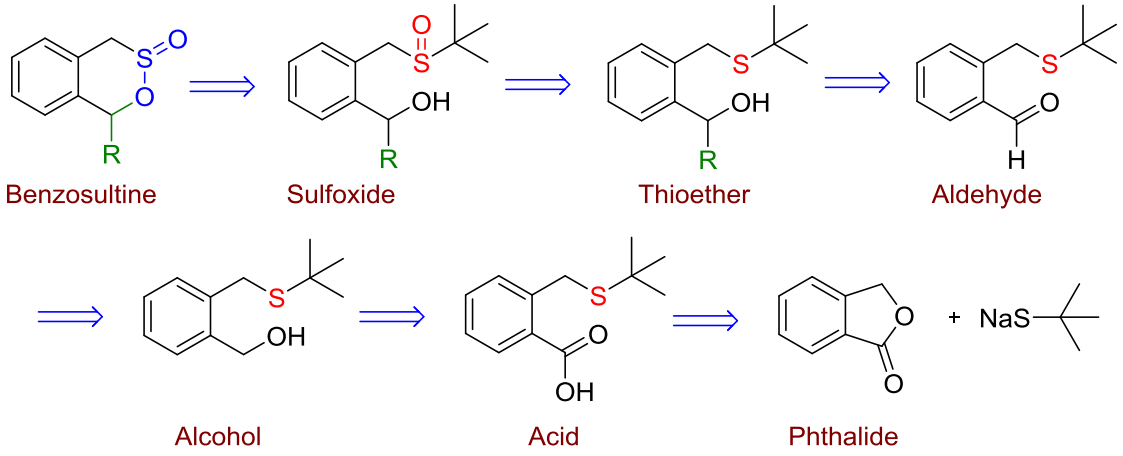
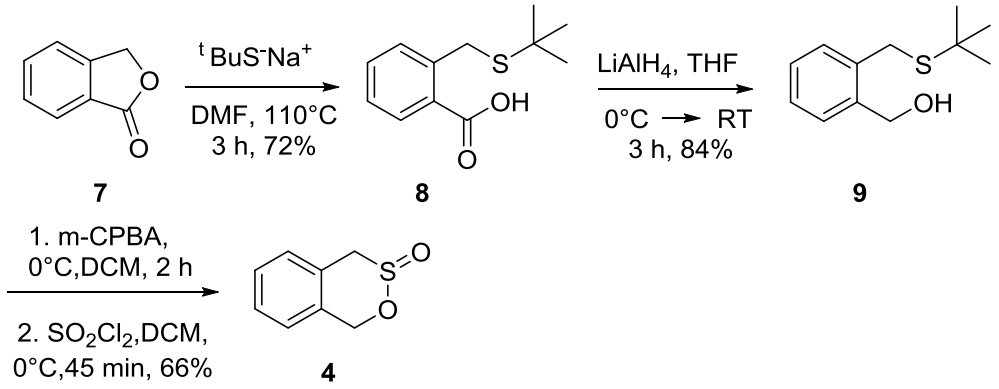


Figure 3. Retrosynthetic analysis of benzosultines.

The thioether having secondary alcohol functional group can be obtained from aldehyde by Grignard reaction. The aldehyde is obtained by the oxidation of alcohol, which in turn can be prepared from phthalide by nucleophilic ring opening by sodium *tert*-butyl thiolate.

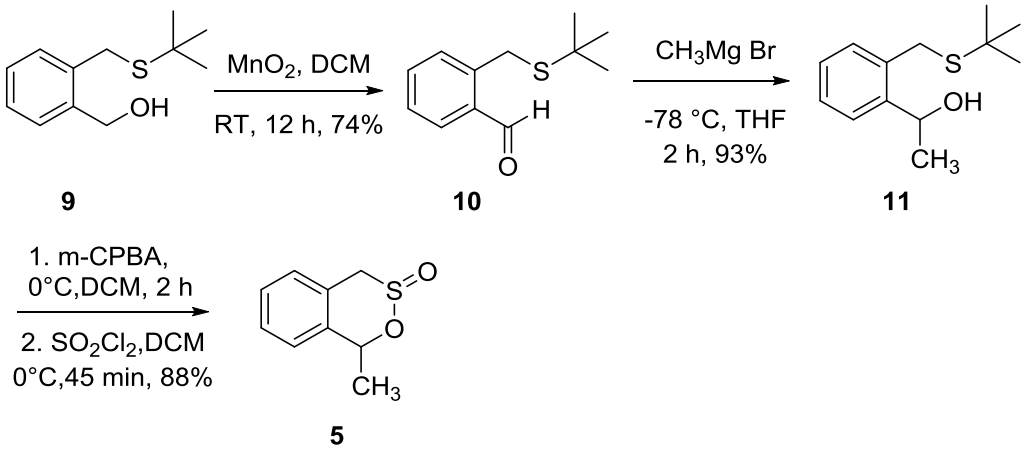
2.2 Results and discussion:

2.2.1 Synthesis of benzosultines:



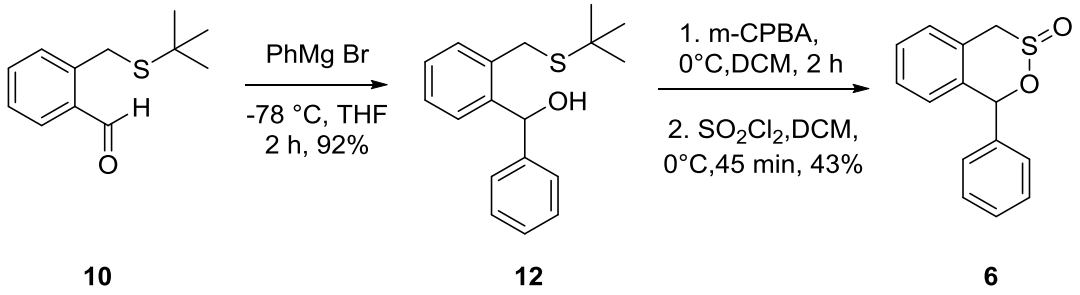
Scheme 3. Synthesis of benzosultine **4**.

First, phthalide **7** was treated with sodium 2-methylpropane-2-thiolate to give acid **8**, which was reduced to alcohol **9** by LAH.[2] Thioether was converted to sulfoxide by *m*-CPBA (meta-Chloroperoxybenzoic acid) oxidation[3] (0.9 eq.) from which benzosultine **4** was prepared in 66 % yield by cyclization with sulfuryl chloride (Scheme 3).[4]



Scheme 4. Synthesis of 1-methylbenzosultine **7**.

Oxidation of alcohol **9** to aldehyde **10** was done by mild oxidizing agent MnO_2.[27] The aldehyde **10** was reacted with MeMgBr to give secondary alcohol **11** (Scheme 4).[28] Treatment of **11** with *m*-CPBA forms sulfoxide, followed by SO_2Cl_2 mediated cyclization gives **5** as a single diastereomer in 88% yield (Scheme 4). Similarly, **6** was prepared by treating aldehyde **10** with PhMgBr followed by oxidation and cyclization to give **6** in 43% yield as a mixture of two diastereomer (Scheme 5).[5,6]



Scheme 5. Synthesis of phenyl benzosultine **6**.

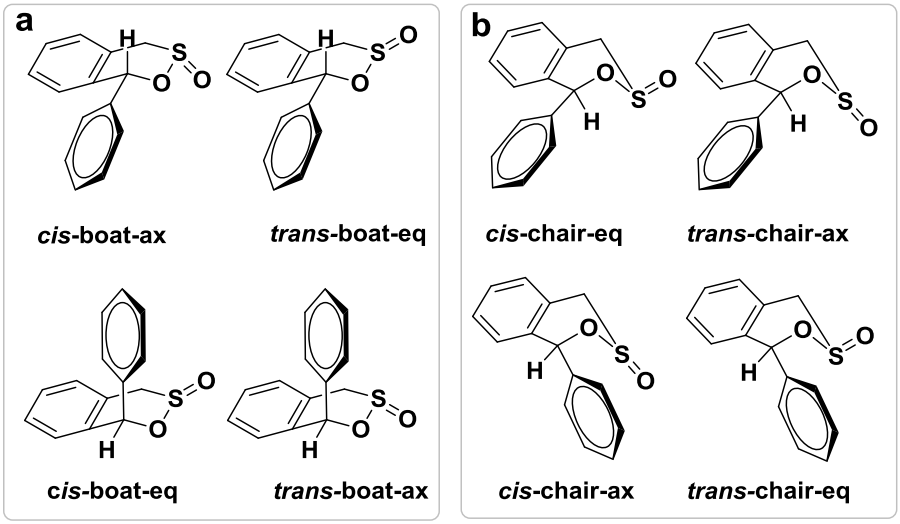


Figure 4. Possible isomers of **6**.

Benzosultine **6** can exist in eight possible conformations (Figure 4). The six-member sultine ring can form pseudoboat or pseudochair conformation (Figure 4a: pseudoboat, 4b; pseudochair). The notation "ax" (axial) or "eq" (equatorial) in the figure label, refers to the orientation of the S=O bond with respect to the plane of the ring. Isomers having a phenyl ring at axial position are likely to be unstable.

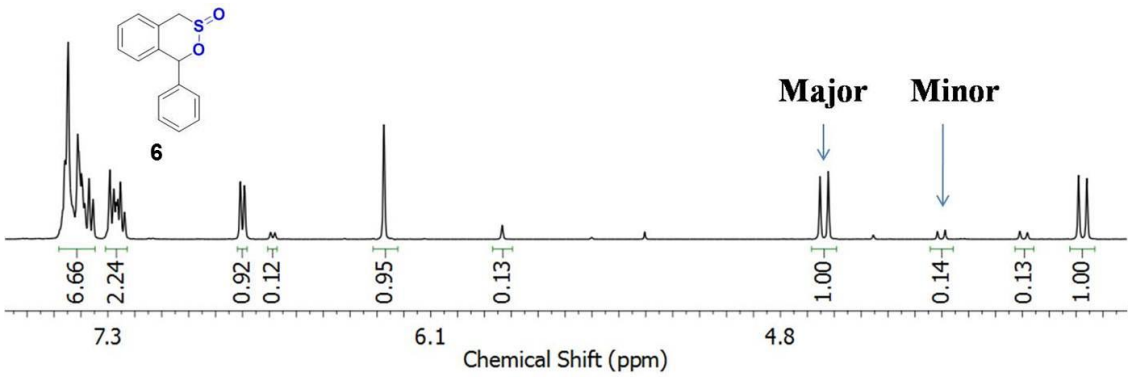


Figure 5. Portion of ^{1}H NMR spectrum of **6**.

^{1}H NMR analysis of **6** in $CDCl_3$ showed a mixture of products (12% minor isomer), tentatively assigned as a mixture of two isomers (Figure 5). HPLC analysis of **6** also showed two sets of peaks with a similar ratio (19% minor isomer) as shown in fig. 6.

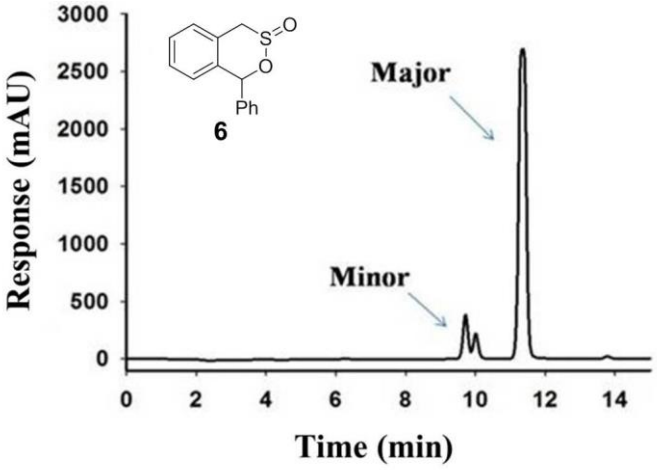


Figure 6. HPLC graph for **6**.

2.2.2 Decomposition of benzosultines at 70 °C in ACN:

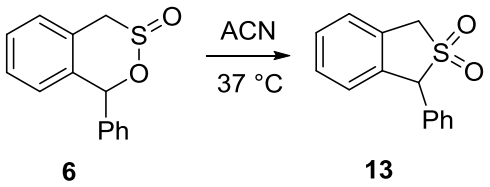
Next, HPLC analysis of acetonitrile solutions of **4**, **5** and **6** at 70 °C was carried out to study thermal stability of these compounds. The solutions of **4**, **5** and **6** in acetonitrile were heated at 70 °C for 3 h and followed by HPLC analysis for % compound left.

Table 1. HPLC analysis of decomposition of sultines conducted in acetonitrile at 70°C

Entry	Compd	% Remaining after 3 h
1	**4**	96
2	**5**	59
3	**6**	1

The analysis of the reaction mixture of **4** showed no significant decomposition (<5%) after 3 h. However, during this time period, **7** was 41% decomposed, while **6** was completely decomposed (Table 1). Furthermore, our results are in agreement with previous reports of Diels-Alder reactions of **4**, **5** and **6** with maleic anhydride at 80 °C, showing a similar trend in reaction rates i.e. **6** >> **5** > **4**.[1]

2.2.3 Decomposition of benzosultines at 37 °C in ACN: When a similar decomposition experiment was conducted at physiological temperature of 37 °C, **6** gradually decomposed (rate constant, 2.9×10^{-3} min^{-1}) during 24 h (Figure 7).



6 **13**

Scheme 6. Decomposition of **6** in ACN.

The sulfone **13** was found to be the major product (92 %) of decomposition of **6** in MeCN at 37 °C (Scheme 6). The rates of decomposition of **6** and formation of **13** were comparable in magnitude (rate constant = 4.5×10^{-3} min^{-1}). These results suggest that the intermediate diene is highly reactive and once formed rapidly reacts with SO_2 to produce the thermodynamically more stable sulfone **13**. Under the same conditions, the sulfone **13** was stable after 24 h suggesting that once formed, **13** does not revert to **6**. When a similar decomposition experiment was conducted with sultines **4** and **5**, HPLC analysis showed that **4** and **5** remained nearly unchanged for 24 h in MeCN.

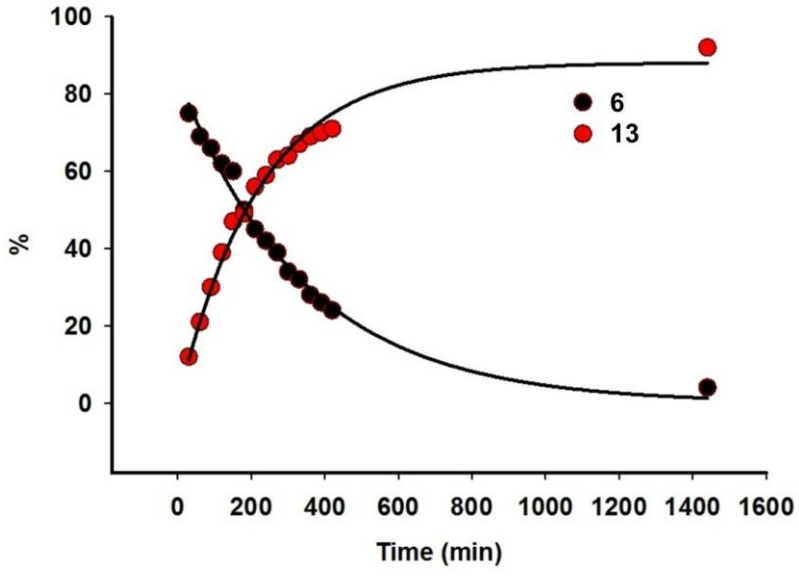
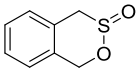
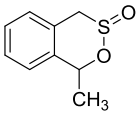
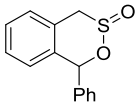


Figure 7. Decomposition study of **6** in acetonitrile at 37 °C.

2.2.4 Stability study of benzosultines at 37 °C in ACN: Due to possible use of benzosultines as SO_2 donors in physiological media, the stability study was carried out in pH 7.4 buffer.

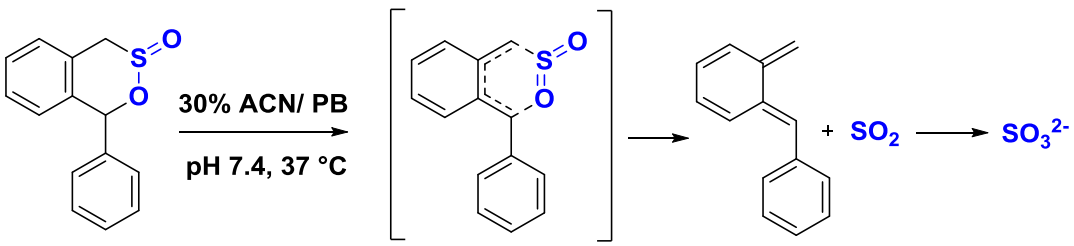
Stability study of **4**, **5** and **6** in phosphate buffer (pH 7.4) at 37 °C was conducted. The reaction was followed by HPLC and % compound decomposed (Table 2) was determined. After 3h incubation, less than 5 % of benzosultine **4** and methyl benzosultine **5** were decomposed (Table 1, entries 1 and 2), whereas 95% of **6** (Table 1, entry 3) was decomposed. These results are in agreement with the previous result.[1] Hence, introduction of phenyl ring at 1-position of benzosultine **4** increases the rate of cycloreversion in organic as well as aqueous media.

Table 2. HPLC study of decomposition of benzosultines

Entry	Compound	Structure	% Decomposed
1	**4**		< 5
2	**5**		< 5
3	**6**		95

The compounds were incubated for 3 h in pH 7.4 buffer at 37 °C and % by HPLC.

2.2.5 Quantification of SO_2 by Ion Chromatography: The ability of benzosultines to produce SO_2 under physiological conditions at 37 °C was evaluated by monitoring the formation of sulfite using an ion chromatograph attached with a conductivity detector in order to quantify SO_2 generated (Scheme 7).



Scheme 7. Decomposition of **6** in pH 7.4 phosphate buffer at 37 °C.

Sulfite analysis. Ion chromatography analysis: An ion chromatograph attached with a conductivity detector was used for sulfite analysis. The eluent used was 1 mM $NaHCO_3$/3.2 mM Na_2CO_3 with flow rate of 0.7 mL/min. Aliquots at appropriate time intervals were analyzed by IC. Maximum sulfur dioxide yield was calculated based on completion of the reaction with no further increase in sulfite formation (Figure 8).

2.2.6 Calibration curve for sulfite: A stock solution (100 mM) of sodium sulfite, Na_2SO_3 (Sigma-Aldrich) was prepared in dI water containing 1% paraformaldehyde. This stock solution was diluted to prepare 10, 40, 60, 80, 100, 120 and 160 µM sodium sulfite solutions. Injections of 20 µL were carried out to generate a calibration curve for sulfite. Linear correlation coefficient R^2 was found as 0.9999, and slope 0.0044 (Figure 9a).

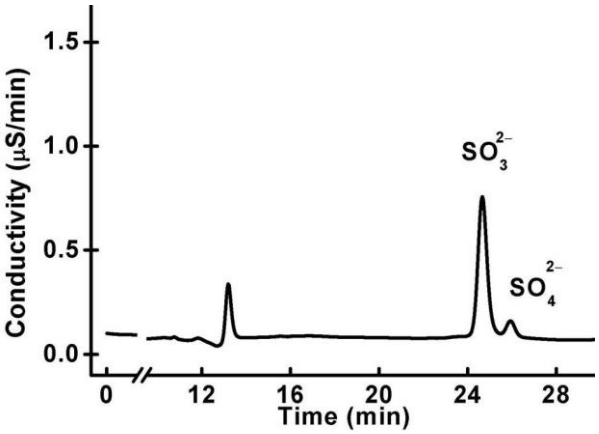


Figure 8. Ion chromatograph for sulfite.

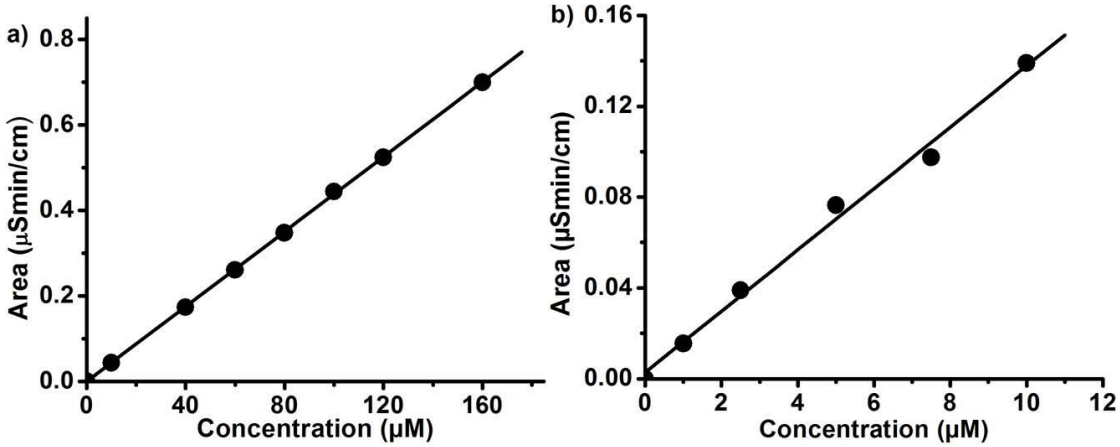


Figure 9. a) Calibration curve for sulfite, R^2= 0.9999; b) Calibration curve for sulfate, R^2= 0.9965.

2.2.7 Calibration curve for sulfate: Sulfite is susceptible to aerobic oxidation to form sulfate,[7,8] which was detected in small amounts in several instances during our study. In order to quantify sulfate, a calibration curve was generated by preparing a 10 mM solution of sulfate, which was diluted to prepare solutions of suitable concentrations for injections into the IC. As the levels of sulfate were below 5 μM, calibration curve was generated with concentrations below 10 μM, linear correlation coefficient R^2 was found as 0.9965 and slope 0.135 (Figure 9b).

2.2.8 Decomposition study of phenyl benzosultine 6 as SO_2 donor in pH 7.4 buffer:

The decomposition reaction of **6** was followed by HPLC for % compound consumed and by ion chromatography for sulfite quantification. During 30 min intervals, the reaction mixture was analyzed until complete consumption of **6** was observed (Figure 10).

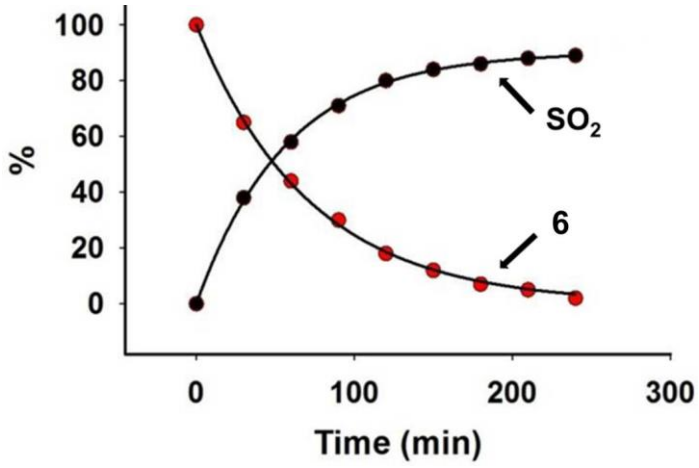


Figure 10. Decomposition of **6** in pH 7.4 phosphate buffer at 37 °C, R^2= 0.9995 for exponential rise to maximum and R^2= 0.9990 for exponential decay.

After 30 min, 35% yield of SO_2 was obtained and during this time, HPLC analysis showed 37% decomposition of **6**. The kinetics of decomposition of **6** gave a first order rate constant k_{dec}= 0.0176 min^{-1}. Our data indicated that the rate of retro Diels-Alder reaction of **6** was accelerated by approx. 6-fold in pH 7.4 in comparison with ACN (2.9×10^{-3} min^{-1}). Previous experimental and theoretical studies on retro Diels-Alder of anthracenedione indicate that stabilization of the activated complex by H-bonding played a major role in the observed acceleration of cycloreversion in water in comparison with an aprotic organic solvent.[9] Perhaps, a similar stabilization of transition states by H-bonding might help to explain our observed increase in rate of cycloreversion of **6** in aqueous media. The

maximum SO_2 yield of 89% with a half-life of 39 min for the generation of SO_2 was obtained.

2.2.9 DNA damage by SO_2 donor phenyl benzosultine 6: As **6** was an efficient SO_2 donor, we evaluated the ability of this compound to mimic SO_2 in physiological pH. In the presence of metal ions such as Cu(II), SO_2 produces radical intermediates such as $SO_3^{\bullet}$, $SO_4^{\bullet-}$ and $HSO_5^{\bullet-}$ which are known to cause oxidative damage to biomacromolecules including DNA (Figure 11).[10,11] A pBR322 supercoiled plasmid cleavage assay was used to measure the efficiency of nick induction by **6** in the presence of metal ions.

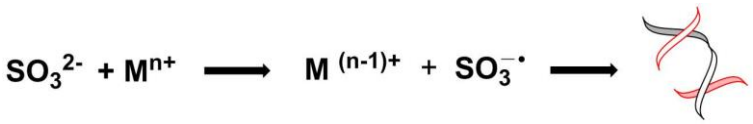


Figure 11. DNA damage by SO_2.

Circular DNA is represented by **I** and upon nicking the supercoiled DNA shows slower migration on a gel. Nicked DNA is represented as **II**. Incubation of supercoiled plasmid DNA with 100 μM of either Cu(II), **6** or an inorganic SO_2 source, sulfites (Na_2SO_3: $NaHSO_3$, 1:3) at 37 °C showed no significant increase in nick induction when compared with DMSO control (Figure 12).

Figure 12. DNA cleavage assay.

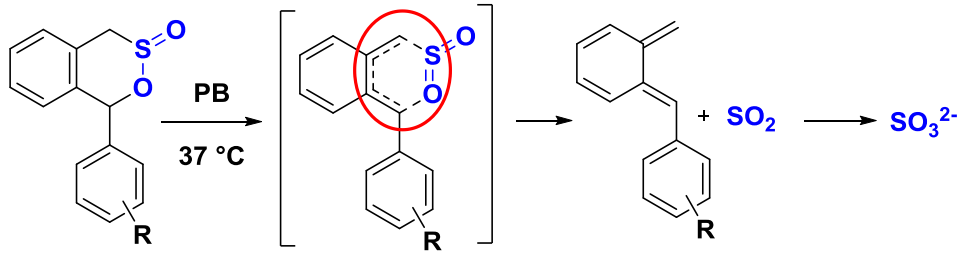
DNA damage profile for **6** in the presence of Cu(II) showed significant nick induction during 5 h (Figure 12). A nearly identical DNA damage profile was recorded in the presence of equimolar amounts of Cu(II) and sulfites. This observation is consistent with the intermediacy of oxidative species generated by S(IV) oxides in the presence of metal ions. No significant nick induction with **13** in the presence of Cu(II) was observed (data not shown), again consistent with our observation that **13** did not undergo any decomposition at 37 °C. Taken together, our results indicate that **6** is an efficient and reliable SO_2 donor under non-enzymatic and physiological conditions.

With following characteristics, it is envisaged that **6** can be used as an organic source of SO_2.

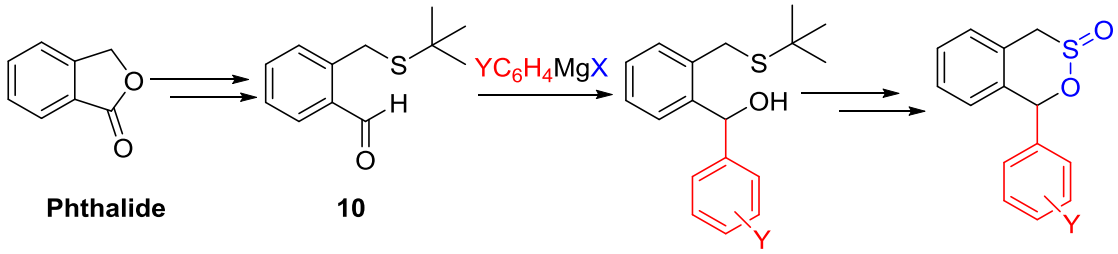
- Stable solid at room temperature
- Generates SO_2 upon dissolution in buffer at pH 7.4
- Half-life of SO_2 generation was 39 min (temporal control)
- Excellent SO_2 yield (89 %)
- Showed SO_2-like biological activity in a DNA damage assay

2.2.10 Synthesis of various benzosultines for modulating rate of generation of SO_2:

Next, we proposed that SO_2 generation could be controlled by varying substituents on phenyl ring (Schemes 8).



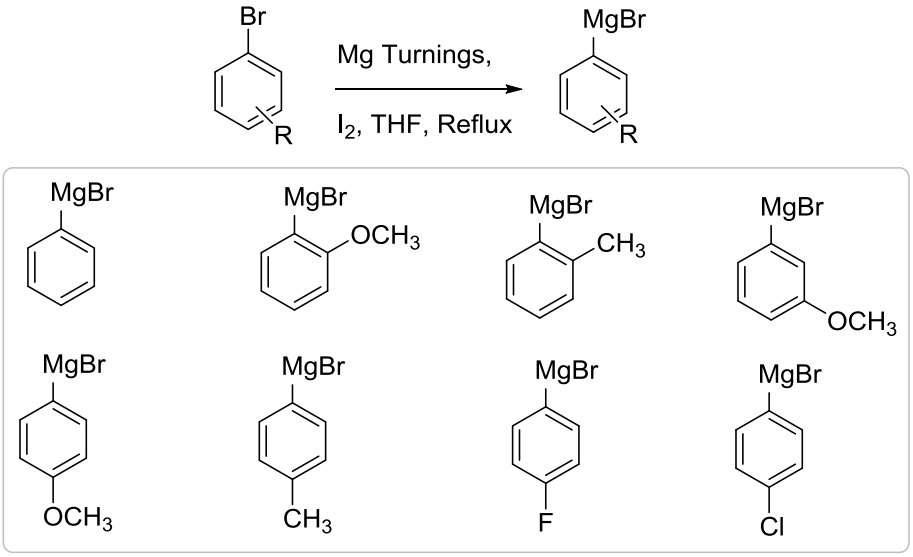
Scheme 8. Substituent effects on SO_2 generation by benzosultine.



Scheme 9. General scheme for synthesis of benzosultines.

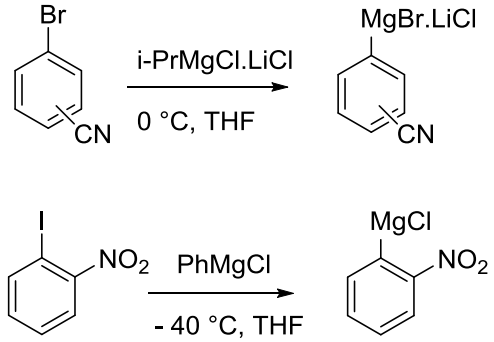
The substituent present on phenyl ring Y can be electron-donating or withdrawing group**.** These structural changes may lead to a change in rate of generation of SO_2 by respective benzosultine. Various benzosultines can be synthesized by following general scheme 9. As described above the aldehyde **10** can be prepared starting from phthalide (Scheme 9). Treatment of **10** with various Grignard reagents (YC_6H_4MgX) having different substituent present on phenyl ring, will give the corresponding alcohol. The thioether upon *m*-CPBA mediated oxidation will afford sulfoxide, followed by SO_2Cl_2 mediated cyclization to give benzosultine. Grignard reagents were freshly prepared by

treating aryl bromides with activated magnesium turnings and iodine in dry THF under refluxing conditions (Scheme 10).



Scheme 10. Preparation of Grignard reagents.

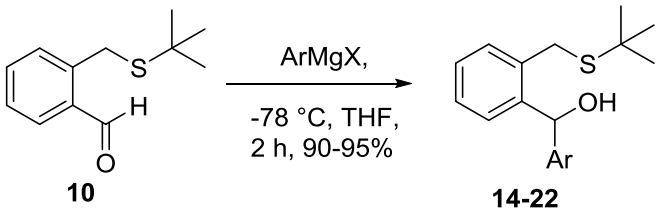
The Grignard reagents with an electron withdrawing group on the phenyl ring were prepared by treating 3-cyano bromobenzene and 4-cyano bromobenzene with i-PrMgCl.LiCl to afford respective Grignard reagents. The 2-nitro-phenyl magnesium chloride was prepared by treating phenyl magnesium chloride with iodo-2-nitrobenzene (Scheme 11).[12,13]



Scheme 11. Preparation of Grignard reagents with electron with drawing group.

Grignard reagents prepared, were reacted with 2-((tert-butylthio)methyl)benzaldehyde **10** to give alcohols **14-22** (Table 3).

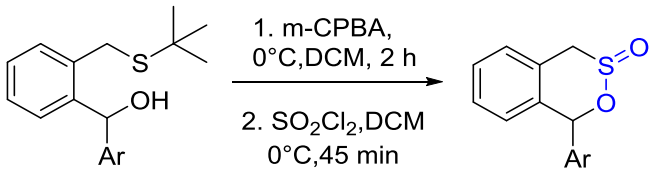
Table 3. Preparation of alcohols **14-22**



Entry	Ar	Compd No.	Yield (%)
1	4-FPh	**14**	94
2	4-ClPh	**15**	71
3	4-CNPh	**16**	93
4	4-MePh	**17**	97
5	4-OMePh	**18**	87
6	3-OMePh	**19**	92
7	3-CNPh	**20**	70
8	2-NO_2Ph	**21**	95
9	2-OMePh	**22**	90

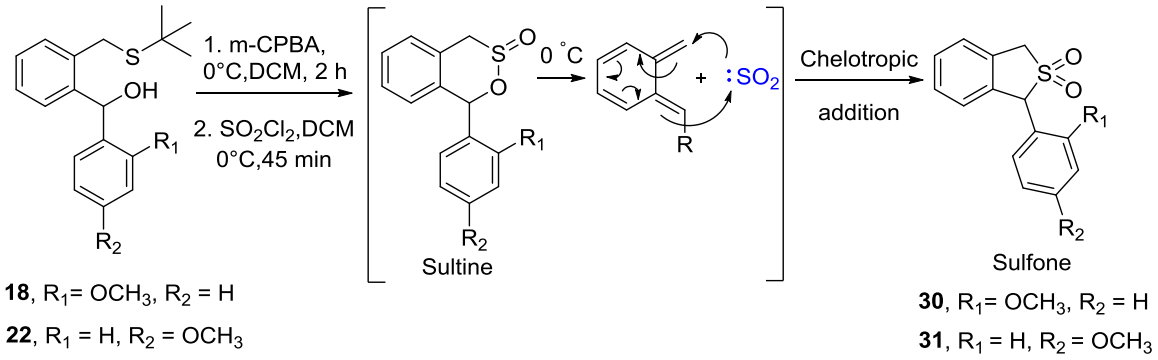
The different benzosultines **23-29**, were prepared by *m*-CPBA mediated oxidation of above alcohols followed by SO_2Cl_2 mediated cyclization (Table 4).

Table 4. Synthesis of benzosultines **23-29**



Entry	Ar	Compd No.	Yield (%)
1	4-FPh	**23**	42
2	4-ClPh	**24**	49
3	4-CNPh	**25**	51
4	4-MePh	**26**	38
5	3-OMePh	**27**	32
6	3-CNPh	**28**	38
7	2-NO_2Ph	**29**	35

While preparing benzosultine from alcohols **18** and **22** (Scheme 12), during SO_2Cl_2 mediated cyclization, corresponding sulfones **30** and **31**, were obtained as products. These results suggest that strong electron donating 2-OMe or 4-OMe substituents on the phenyl ring increases the rate of retro Diels-Alder reaction to give diene and SO_2 which subsequently undergoes chelotropic addition to give sulfones **30** and **31** (Scheme 12).



18, R_1= OCH_3, R_2 = H

22, R_1 = H, R_2 = OCH_3

30, R_1= OCH_3, R_2 = H

31, R_1 = H, R_2 = OCH_3

Scheme 12. Synthesis of 2-OMe and 4-OMe phenyl benzosultine.

2.2.11 Conversion of benzosultine to benzosulfone:

Solutions of benzosultines **6** and **23-29** in $CDCl_3$/ACN were converted to respective sulfone in quantitative yield upon standing at room temperature (Table 5).

Table 5. Formation of benzosulfones

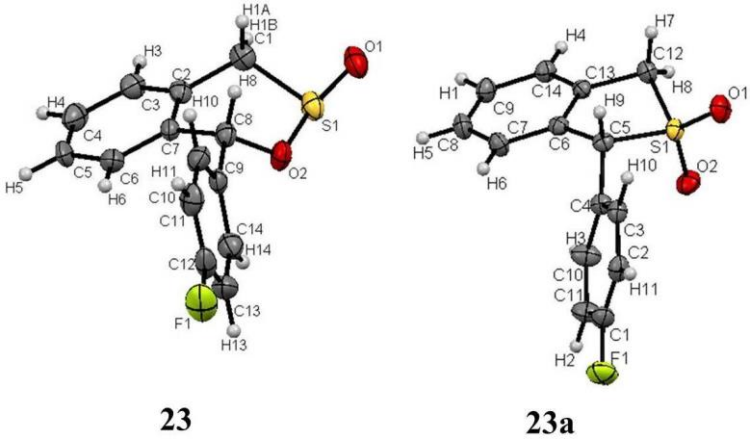
6 and **23-29** **13** and **23a-29a**

Entry	Compd No.	Ar
1	**13**	Ph
2	**23a**	4-FPh
3	**24a**	4-ClPh
4	**25a**	4-CNPh
5	**26a**	4-MePh
6	**27a**	3-OMePh
7	**28a**	3-CNPh
8	**29a**	2-NO_2Ph

All sulfones were obtained in quantitative yield, verified by NMR.

All sultines prepared (**6** and **23-29**) gave corresponding 1-aryl-benzosulfones (**13** and **23a-29a**) as a product suggesting the potential for efficient generation of SO_2 from these compounds (Table 5).

2.2.12 X-ray structure of 4F-phenyl benzosultine and benzosulfone:



23 **23a**

Figure 13. X-ray structure of **23** and **23a**.

X-ray diffraction analysis of the 4-fluorophenyl derivative **23** (Table 4, entry 1) showed that 4-F-phenyl ring is perpendicular to the plane of aryl and trans to sulfoxide bond (*trans*-boat-eq), suggesting a stable conformation (Figure 13). These results indicate that among the various conformers in figure 5, the *trans*-boat-eq is possibly dominant. Thus, varying electronics on 1-phenyl ring may change sterics and could affect stability of benzosultine. X-ray diffraction analysis of the sulfone **23a** (Figure 13) showed that the sulfonyl group was *cis* to the aryl ring, giving the least energetic conformation amongst the possible structures (Table 5, entry 2).

2.2.13 Decomposition study of benzosultine for determination of rate constants and maximum SO_2 yields: In pH 7.4, HPLC analysis of reaction mixtures revealed benzosultines **23-29** to be labile with 37-80% decomposition in 30 min (Table 6). Kinetic measurements of decomposition of these sultines were carried out and first order rate constants were determined. The half-lives of SO_2 generation during decomposition varied from 10-68 min (Table 6).

Table 6. Decomposition profiles of **6** and **23-29** prepared in this study in pH 7.4 at 37 °C.

Entry	Compd	decomp (%)[a]	k_{dec} (min^{-1})[b]	% SO_2[c]	$t_{1/2}$ (min)[d]	Max SO_2 yield (%)[e]
1	**6**	37	0.0140	35	39	89
2	**23**	61	0.0244	60	14	83
3	**24**	38	0.0140	36	34	79
4	**25**	38	0.0093	37	38	76
5	**26**	70	0.0336	64	13	80
6	**27**	37	0.0104	25	56	73
7	**28**	36	0.0069	23	68	59
8	**29**	80	0.0500	71	10	81

[a]Determined by HPLC analysis. [b]Determined by kinetic analysis of decomposition of sultine. [c]Determined as sulfite by an ion chromatograph attached with a conductivity detector. [d]Determined by kinetic analysis of formation of SO_2 as sulfite. [e]The highest yield of SO_2 produced during analysis.

2.2.14 Hammett plot of rate constants for decomposition of 6 and 23–29 in pH 7.4 at 37 °C:

In order to understand electronic effects on decomposition, a Hammett plot of rates of decomposition of 3 and 4-aryl substituted sultines was constructed (Figure 14). A nearly linear Hammett plot constructed using rate constants for decomposition of **6** and **23–29** (Table 6) and substituent constant σ+, suggested a predictable mechanism of decomposition for this scaffold. The slope ρ,was found as –0.6 implying a weak electronic effect on the reaction center wherein an electron donating group accelerated the cycloreversion.

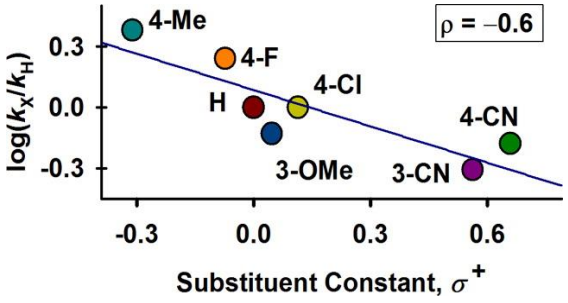


Figure 14. Hammett plot of rate constants for decomposition of **6** and **23–29** in pH 7.4 buffer at 37 °C. Linear regression analysis yielded ρ as −0.6 (R^2 = 0.75).

Although a Hammett plot for a similar retro Diels-Alder was not available for comparison, a previous report of Diels-Alder reaction of 1-aryl-1,3-butadienes with maleic anhydride at 35 °C found ρ as –0.621.[14] Both the sign and the magnitude of value of ρ were comparable with the Hammett slope determined in our present study suggesting a similar effect of varying electronics in the Diels-Alder of 1-aryl-1,3-butadienes and retro Diels-Alder of benzosultines. The negative ρ-value suggests strong electron donating group present on 1-aryl ring will increase the rate of cycloreversion and the resulting 1-aryl benzosultine will be unstable. This assumption was supported by the results, while preparing 1-aryl benzosultine with –OMe group present on 2 or 4 position of 1-phenyl ring, the corresponding sulfones were isolated as products at 0 °C instead of respective benzosultine (Scheme 12).

2.3 Conclusions:

In conclusion, we prepared various 1,4-dihydro-2,3-benzoxathiin-3-oxides (benzosultines) as candidates for controlled and tunable generation of SO_2. 1-Aryl-benzosultines are found to be labile at 37 °C and decompose in pH 7.4 to produce SO_2 with half-lives ranging between 10 – 68 min. The maximum yield of 89% SO_2 for **6** was obtained. Finally, a supercoiled plasmid DNA cleavage assay demonstrates the suitability of **6** for applications as a source of SO_2 in biological studies. A strategy for controlled and efficient generation of SO_2 under physiological condition in a non-enzymatic manner was presented.

2.4 Experimental Section

General: All chemicals were purchased from commercial sources and used as received unless stated otherwise. Petroleum ether (PE) and ethyl acetate (EtOAc), for chromatography were distilled before use. THF was dried using a sodium wire and distilled before use. Dichloromethane (DCM) and acetonitrile (ACN) were pre-dried over calcium hydride and distilled under reduced pressure. Column chromatography was performed using Merck silica gel (60-120/100–200 mesh) as the solid support. ^{1}H and ^{13}C NMR spectra were recorded on aJEOL 400 MHz (or 100 MHz for 13C)/ Bruker 500 MHz (or 125 MHz for 13C) spectrometer using either residual solvent signals as an internal reference or with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. High-resolution mass spectra (HRMS)were obtained using a HRMS-ESI-Q-Time of Flight LC/MS (Synapt G2, Waters). For compounds containing Cl and F, the values reported are for ^{35}Cl, ^{19}F isotopes. Infrared spectra (IR) were obtained using NICOLET6700 FT-IR spectrophotometer using a KBr disc. Melting points were measured using a VEEGO melting point apparatus in open glass capillary and values reported are uncorrected. High performance liquid chromatography (HPLC) was performed on a Dionex ICS-3000 model with Phenomenex C-18 reverse phase column (250 × 4.6mm, 5 μm). Ion chromatography was conducted using a Metrohm 882 Compact IC plus instrument (Metrohm ASupp 5-250/4.0 mm, column). Unless otherwise specified, the general procedures were used for the synthesis of intermediates and products presented in this study.

General procedure A. The solution of the aryl halide (1 eq.) in dry THF (5 mL) was added dropwise to a 50 mL flask charged with activated magnesium turnings (1.1 eq.) and a crystal of iodine, in THF (15 mL) at room temperature (RT). The mixture was refluxed until magnesium turnings completely react. The flask was cooled to RT and the resulting Grignard reagent was used for the ensuing reaction. The alcohols **14**, **17-19** and **22** were prepared using Grignard reagents prepared according to this procedure.

General procedure B. Grignard reagent (prepared as above, 1.5 eq.) was added dropwise at -78 °C to a solution of **10** (1 eq.) in THF. The reaction mixture was stirred at −78 °C for 1 h and gradually warmed to RT, quenched with sat. aq. NH_4Cl solution (15 mL). The resulting solution was washed with ethyl acetate (3 × 25mL) and the combined organic

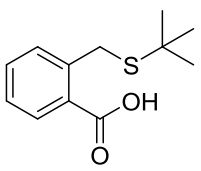
layer was dried (Na_2SO_4), filtered and the filtrate was concentrated on a rotary evaporator to get an oil. This crude was purified by silica gel column chromatography (EA/PE, 1:9) to yield the corresponding alcohol. The alcohols **11**, **12** and **14-22** were prepared according to this procedure.

General procedure C. To a solution of the thioether in THF, *m*-CPBA (0.95 equiv.) in THF (5 mL) was added dropwise at 0 °C, and the reaction mixture (RM) was stirred at 0 °C for 1 h. THF was evaporated using a rotary evaporator to get a white sticky mass. To this crude material, satd. $NaHCO_3$ solution (15 mL) was added the resulting mixture was washed with ethyl acetate (3×25 mL). The combined organic layer was dried on Na_2SO_4, filtered and the resulting filtrate was concentrated to get a sticky solid, which was used in the next step without further purification. The sulfoxides required for synthesis of **4-6** and **23-29** were prepared by oxidation of thioethers using this procedure.

General procedure D. To a solution of the sulfoxide in dry DCM (15 mL), sulfuryl chloride (1.5 equiv., in 5mL DCM) was added dropwise at 0 °C during 5 min. The reaction mixture was stirred at 0 °C for 45 min. To the RM, 10 mL of water was added and the resulting mixture was washed with DCM (2×15 mL). The combined DCM layer was dried on Na_2SO_4, filtered and the resulting filtrate was concentrated on a rotary evaporator (bath temperature maintained at 0-5 °C) to get a crude oil. The crude was then purified by silica gel column chromatography using EA/PE (1:4) as the eluant (Note: The eluant was cooled to 5-10°C before chromatography) to yield the corresponding sultine. The benzosultines **4-6** and **23-29** were prepared by this procedure.

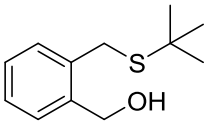
General procedure E. The solution of the sultine in $CDCl_3$ (NMR sample)/ACN was stirred at room temperature for24-48 h, to get the respective sulfone in a quantitative yield. The benzosulfones **13** and **23a-29a** were prepared by this procedure.

2-((*tert*-Butylthio)methyl)benzoic acid (8).[1] To a solution of phthalide (2.0 g, 14.9 mmol) in dry DMF (10mL), sodium 2-methylpropane-2-thiolate (1.67 g, 14.9 mmol) was added portionwise under N_2 atmosphere. The reaction mixture immediately turns pale yellow and was then heated at 110 °C. After 2 h, the RM was cooled gradually to RT and water (60 mL) was added, followed by acidification to pH 5-6 with 1N HCl. A white precipitate resulted, which was separated, and washed with ethyl acetate (2 × 25 mL). The combined organic layer

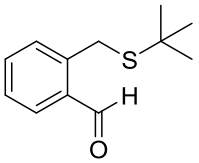


was dried on Na_2SO_4, filtered and the filtrate was concentrated to get a crude yellow oil, which was purified by silica gel column chromatography (EA/PE, 1:9) to give **8** (2.42 g, 72%) as a white solid: mp 90-91°C; FTIR (ν_{max}, cm^{-1}): 2958, 2640, 1685, 1571, 1456, 1402, 1269, 1158, 926; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.03 (d, *J* = 7.4 Hz, 1H), 7.47-7.51 (m, 2H), 7.36-7.29 (m, 1H), 4.25 (s, 2H), 1.37 (s, 9H); ^{13}C NMR ($CDCl_3$,100 MHz):δ 172.6, 141.1, 132.9, 131.9, 131.7, 128.5, 127.1, 43.2, 31.9, 30.9; HRMS (ESI-TOF): $C_{12}H_{16}O_2S[M+Na]^+$: 247.0769. Found $[M+Na]^+$: 247.0768.

(2-((*tert*-Butylthio)methyl)phenyl)methanol (9).[2] To powdered $LiAlH_4$ (0.75 g, 20 mmol), dry THF (20mL) was added at 0 °C under N_2 atmosphere. To the above suspension, a solution of 2-((tertbutylthio)methyl)benzoic acid **(8)** (11.1 mmol, 2.5 g) in dry THF (30 mL) was added dropwise. The resulting reaction mixture was stirred at RT for 3 h following which ethyl acetate (15 mL) was added at 0 °C, followed by con. HCl (3.37 mL). Vigorous bubbles were observed and a gray solid separated out. The organic layer was decanted and the gray mass was washed with another 20 mL ethyl acetate, and decanted. The resulting portions of organic layers were combined and dried (Na_2SO_4) and filtered. The filtrate was concentrated on a rotary evaporator to get a crude yellow oil, which was purified by silica gel column chromatography (EA/PE, 1:9) to give **9** (1.95 g, 84%) as a white solid: mp 44-45°C; FTIR (ν_{max}, cm^{-1}): 3327, 2952, 2356, 1453, 1370, 1163,1034; ^1H NMR (400 MHz, DMSO-d_6):δ 7.39 (d, *J* = 7.2 Hz, 1H), 7.26-7.15 (m, 3H), 5.14 (t, *J* = 5.4 Hz, 1H),4.65 (d, *J* = 5.4 Hz, 2H), 3.78 (s, 2H), 1.33 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 141.1, 135.3, 130.3,127.6, 127.3, 127.2, 60.7, 43.1, 31.0, 29.8; HRMS (ESI-TOF): $C_{12}H_{18}OS$ $[M+Na]^+$: 233.0979. Found $[M+ Na]^+$: 233.0976.

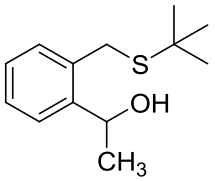


2-((*tert*-Butylthio)methyl)benzaldehyde (10).[3] To a solution of **9** (0.15 g, 0.66 mmol) in dry DCM (5 mL), MnO_2 (0.57 g, 6.6 mmol) was added portionwise under a N_2 atmosphere at 0 °C. The RM stirred at RT for 6 h. The reaction mixture diluted with DCM (2×10 mL) and filtered through a celite bed. The combined filtrate was dried on Na_2SO_4 and concentrated under reduced pressure to get a crude brown oil, which was purified by silica gel column chromatography (EA/PE, 0.5:9.5) to give **10** (0.11 g, 74%) as a colorless oil: FTIR (ν_{max}, cm^{-1}):2959, 2347, 1694, 1600, 1464, 1196; ^1H NMR ($CDCl_3$, 400MHz):δ 10.32 (s, 1H), 7.84 (dd, *J* = 1.3, 7.6

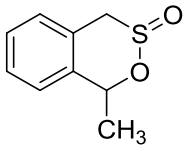


Hz,1H), 7.53-7.49 (m, 1H), 7.44-7.38 (m, 2H), 4.16 (s, 2H), 1.39 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 191.9,140.6, 133.3, 133.8, 131.5, 131.4, 127.7, 43.6, 30.8, 29.6; HRMS (ESI-TOF): $C_{12}H_{16}OS$ $[M+Na]^+$: 231.0820.Found $[M+Na]^+$: 231.0728.

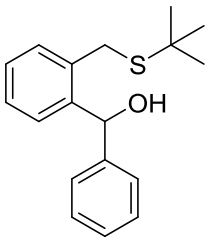
1-(2-((*tert*-Butylthio)methyl)phenyl)ethanol (11). Prepared according to General procedure **B**, starting from **10** (0.50 g, 2.64 mmol) to give **11** (0.54 g, 93%) as a colorless oil: FTIR (ν_{max}, cm^{-1}): 3411, 2967, 1458, 1364,1162, 1073, 1004; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (d, *J* = 7.8 Hz, 1H), 7.30-7.18 (m, 3H), 5.26 (q, *J* =6.4 Hz, 1H), 3.87 (d, *J* = 11.0 Hz, 1H), 3.81 (d, *J* = 11.0 Hz, 1H), 1.56 (d, *J* = 6.4 Hz, 3H), 1.40 (s, 9H); ^{13}CNMR (100 MHz, $CDCl_3$): δ 143.9, 134.2, 130.7, 127.9, 127.7, 125.9, 65.9, 43.4, 30.7, 30.5, 23.6; HRMS (ESITOF):MALDI-TOF MS (m/z): $C_{13}H_{20}OS$ $[M+K]^+$: 263.0872. Found $[M+K]^+$: 263.0347.



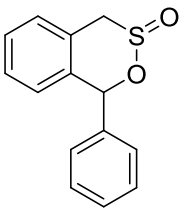
1-Methyl-1,4-dihydrobenzo-2,3-oxathiin-3-oxide (5). Prepared according to general procedure **D**. Starting from **11** (0.54 g, 2.41 mmol), **5** (0.40 g, 88% yield) was isolated as a white solid: mp 50-51 °C; FTIR (ν_{max},cm^{-1}): 1207, 1137, 1107, 1032, 1003; ^{1}H NMR (400 MHz, CDCl3): δ 7.39-7.35 (m, 3H), 7.26-7.23 (m, 1H),5.40 (q, *J* = 6.5 Hz, 1H), 4.56 (d, *J* = 15.2 Hz, 1H), 3.50 (d, *J* = 15.2 Hz, 1H), 1.82 (dd, *J* = 1.1, 6.6 Hz, 3H); ^{13}CNMR (100 MHz, $CDCl_3$): δ 139.0, 129.4, 128.5, 128.0, 127.3, 124.0, 69.2, 58.5, 18.6; HRMS (ESI-TOF):$C_9H_{10}O_2S$ $[M+H]^+$: 183.0480. Found $[M+H]^+$: 183.0479.



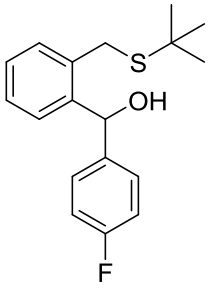
(2-((*tert*-Butylthio)methyl)phenyl)(phenyl)methanol (12). Prepared according to general procedure **B**, starting from **10** (1.00 g, 4.80 mmol) to give **12** (1.24 g, 92%) as a colorless oil: FTIR (ν_{max}, cm^{-1}): 3414, 3052,2958, 2346, 1693, 1458, 1374, 1170, 1018; ^{1}H NMR ($CDCl_3$, 400MHz):δ 7.33-7.14 (m, 9H), 6.15 (s, 1H), 3.74(d, *J* = 11.1 Hz, 1H), 3.68 (d, *J* = 11.1 Hz, 1H), 1.30 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ143.1, 142.4,135.0, 131.0, 128.6, 128.4, 128.0, 127.8, 127.4, 126.7, 72.8, 43.6, 30.8, 30.7; HRMS (ESI-TOF): $C_{18}H_{22}OS$ $[M+Na]^+$: 309.1289. Found $[M+Na]^+$: 309.1283.



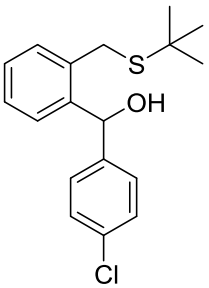
1-Phenyl-1,4-dihydrobenzo-2,3-oxathiin-3-oxide (6). Prepared according to general procedure **D**. Starting from **12** (0.50 g, 1.74 mmol), **6** (0.18 g, 43%) as white solid: mp 89-90 °C; FTIR (ν_{max}, cm^{-1}): 1492, 1484,1406, 1276, 1139, 1112, 1090, 1074; ^{1}H NMR recorded at 0 °C, showed that it was isomeric mixture with ratio1:0.14; For the major isomer: ^{1}H NMR (500 MHz, $CDCl_3$):δ 7.47-7.35 (m, 5H), 7.29-7.23 (m, 2H), 6.80 (d, *J* = 7.7 Hz, 1H), 6.27 (s, 1H), 4.64 (d, *J* = 15.4 Hz, 1H), 3.68 (d, *J* = 15.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ137.6, 136.7, 129.4, 129.0, 128.7, 128.6, 128.5, 127.7, 126.6, 126.5, 74.7, 57.5; HRMS (ESI-TOF): $C_{14}H_{12}O_2S[M+Na]^+$: 267.0456. Found $[M+Na]^+$: 267.0453.



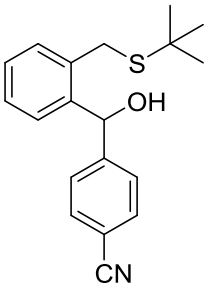
(2-((*tert*-Butylthio)methyl)phenyl)(4-fluorophenyl)methanol (14). Prepared according to general procedure **B** (Grignard reagent was prepared according procedure **A**), starting from **10** (0.9 g, 4.32 mmol) to give **14** (1.22 g,94%) as a colorless oil: FTIR (ν_{max}, cm^{-1}): 3421, 2962, 1603, 1508, 1458, 1364, 1223, 1157, 1014; ^{1}H NMR(400 MHz, $CDCl_3$): δ 7.37-7.22 (m, 6H), 7.05-7.00 (m, 2H), 6.20 (s, 1H), 3.80 (d, *J* = 11.1 Hz, 1H), 3.74 (d, *J* =11.1 Hz, 1H),1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.3, 160.9, 142.2, 138.8, 135.0, 131.1, 128.5 (d, *J*= 11.0 Hz), 128.3 (d, *J* = 13.0 Hz), 127.9, 115.2 (d, *J* = 21.0 Hz), 72.2, 43.6, 30.8, 30.7; HRMS (ESI-TOF):$C_{18}H_{21}FOS$ $[M+Na]^+$: 327.1195. Found $[M+Na]^+$: 327.1194.



(2-((*tert*-Butylthio)methyl)phenyl)(4-chlorophenyl)methanol (15). Prepared according to general procedure **B**, starting from **10** (0.5 g, 2.64 mmol) to give **15** (0.54 g, 71%) as a colorless oil: FTIR (ν_{max}, cm^{-1}): 3419,2960, 1488, 1457, 1364, 1163, 1089, 1013; 1H NMR (500 MHz, CDCl3): δ 7.34-7.29 (m, 5H), 7.25-7.19 (m,3H), 6.18 (d, *J* = 3.9 Hz, 1H), 3.80 (d, *J* = 8.9 Hz, 1H), 3.75 (d, *J* = 8.9 Hz, 1H), 3.16 (d, *J* = 4.1 Hz, 1H), 1.37 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 142.1, 141.6, 135.0, 133.0, 131.2, 128.8, 128.5, 128.3, 128.0, 127.9,72.2, 43.7, 30.9, 30.7; HRMS (ESI-TOF): $C_{18}H_{21}ClOS$ $[M+Na]^+$: 343.0899. Found $[M+Na]^+$: 343.0886.

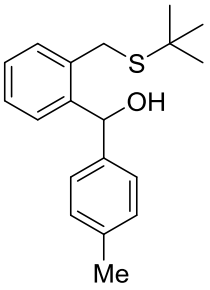


4-((2-((*tert*-Butylthio)methyl)phenyl)(hydroxy)methyl)benzonitrile (16).[13] To *i*-PrMgCl.LiCl (5.54 mL, 1.3M in THF, 7.21 mmol), 4-bromobenzonitrile (1.31 g, 7.21



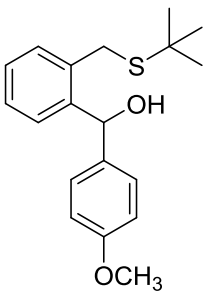
mmol, solution in 5 mL THF) was added drop wise using a syringe at 0 °C. The reaction mixture was stirred at 0 °C for 2 h. To the aforementioned solution, **10** was added (1.0 g, 4.80 mmol, solution in 15 mL THF) dropwise at -78 °C over 10 min. The RM was stirred at -78°C for 1.5 h, allowed to warm to RT, and quenched with sat. aqueous NH_4Cl solution. The aqueous phase was extracted with ethyl acetate (3 × 25 mL), dried on Na_2SO_4 and concentrated under reduced pressure to get a crude oil. The crude was purified by silica gel column chromatography (ethyl acetate/ pet ether, 1:9) to yield **16** (1.40 g, 93%), as a sticky brown oil: FTIR (ν_{max}, cm^{-1}): 3365, 2967, 2227, 1609, 1460, 1404, 1365, 1159, 1028;^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (d, *J* = 8.3 Hz, 2H), 7.54 (d, *J* = 8.2 Hz, 2H), 7.33-7.31 (m, 1H), 7.28-7.20(m, 2H), 7.08-7.06 (m, 1H), 6.23 (d, *J* = 3.1 Hz, 1H), 3.86 (d, *J* = 11.0 Hz, 1H), 3.77 (d, *J* = 11.0 Hz, 1H), 3.41(d, *J* = 3.9 Hz, 1H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.6, 141.5, 135.2, 132.1, 131.3, 129.1,128.7, 128.2, 127.3, 119.0, 110.9, 72.2, 43.9, 31.0, 30.7; HRMS (ESI-TOF): $C_{19}H_{21}NOS$ $[M+Na]^+$: 334.1242.Found $[M+Na]^+$: 334.1237.

(2-(*tert*-Butylthiomethyl)phenyl)(p-tolyl)methanol (17). Prepared according to general procedure **B**, (Grignard reagent was prepared according to procedure **A**). Starting from **10** (1.0 g, 4.80 mmol), **17** (0.54 g,97%) was isolated as a colorless oil: FTIR (ν_{max}, cm^{-1}): 3144, 2967, 1458, 1364, 1162, 1073, 1004; ^{1}H NMR (400MHz, $CDCl_3$): δ 7.33-7.21 (m, 6H), 7.15 (d, *J* = 8.0 Hz, 2H), 6.20 (d, *J* = 3.8 Hz, 1H), 3.79 (d, *J* = 11.1Hz, 1H), 3.81 (d, *J* = 11.1 Hz, 1H), 2.98 (d, *J* = 4.1 Hz, 1H), 2.34 (s, 3H), 1.37 (s, 9H); ^{13}C NMR (100 MHz,$CDCl_3$): δ 156.6, 141.7, 135.1, 131.3, 130.3, 128.6, 127.7, 127.6, 120.7, 110.4, 67.3, 55.4, 43.3, 30.7, 30.6;HRMS (ESI-TOF): $C_{19}H_{24}OS$ $[M+Na]^+$: 323.1446. Found $[M+Na]^+$: 323.1470.



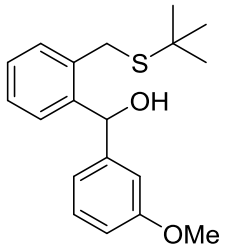
(2-((tert-butylthio)methyl)phenyl)(4-methoxyphenyl)methanol (18).

Prepared according to general procedure **B** (Grignard reagent was prepared according to procedure **A**) starting from **10** (1.0 g, 4.80 mmol), **18** (0.87 g, 87%) was isolated as a colorless oil: FTIR (ν_{max}, cm^{-1}): 3421, 2959, 2361, 1610, 1510, 1458, 1364, 1249, 1171, 1034; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34, (d, *J* = 5.6 Hz, 1H), 7.29-7.20 (m,

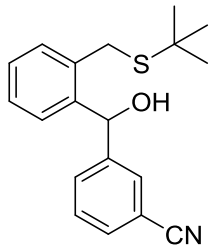


5H), 6.87 (d, *J* = 6.8 Hz, 2H), 6.19 (s, 1H), 3.80 (s, 3H), 3.77 (d, *J* = 8.9 Hz, 1H), 3.72 (d, *J* = 8.9 Hz, 1H), 2.90 (s, 1H), 1.36 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.9, 142.5, 130.9, 128.2, 128.1, 127.9, 127.7, 116.1, 114.8, 113.8, 72.4, 55.3, 43.5, 33.5, 33.7; HRMS (ESI-TOF): $C_{19}H_{24}O_2S$ $[M+Na]^+$: 339.1395. Found $[M+Na]^+$: 339.1392.

(2-((*tert*-Butylthio)methyl)phenyl)(3-methoxyphenyl)methanol (19). Prepared according to general procedure **B** (Grignard reagent was prepared according to procedure **A**) starting from **10** (1.1 g, 5.28 mmol), **19** (1.45 g, 87%) was isolated as a colorless oil: FTIR (ν_{max}, cm^{-1}): 3421, 2959, 1599, 1487, 1457, 1364, 1258,1159, 1025; ^{1}H NMR (400MHz, $CDCl_3$): δ 7.31-7.21 (m, 5H), 7.0 (s, 1H), 6.94 (d, *J* = 7.4 Hz, 1H), 6.82 (dd, *J*= 2.5, 8.1 Hz, 1H), 6.19 (d, *J* = 3.9 Hz, 1H), 3.83 (d, *J* = 11.1 Hz, 1H), 3.79 (s, 3H), 3.76 (d, *J* = 11.1 Hz, 1H),3.13 (d, *J* = 4.2 Hz, 1H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.7, 144.9, 142.3, 135.0, 131.0, 129.4,128.6, 128.1, 127.8, 119.1, 112.9, 112.1, 72.7, 55.3, 43.6, 30.8, 30.7; HRMS (ESI-TOF): $C_{19}H_{24}O_2S$ $[M+Na]^+$:339.1395. Found $[M+Na]^+$: 339.1400.

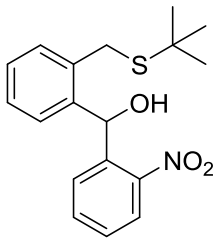


3-((2-((*tert*-Butylthio)methyl)phenyl)(hydroxy)methyl)benzonitrile (20).[13] Prepared according to procedure used for synthesis of **16**; starting from **10** (1.1 g, 5.28 mmol), **20** (1.13 g, 70% yield) was isolated as a brown oil: FTIR (ν_{max}, cm^{-1}): 3447, 2961, 2223, 1481, 1458, 1364, 1162, 1028; ^{1}H NMR (400MHz, $CDCl_3$): δ 7.74-7.73 (m, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 7.7 Hz, 1H), 7.46-7.42 (m, 1H), 7.33-7.31 (m, 1H), 7.29-7.21 (m, 2H), 7.11-7.09 (m, 1H), 6.21 (s, 1H), 3.85 (d, *J* = 11.0 Hz, 1H), 3.77 (d, *J* = 11.1 Hz, 1H), 1.39 (s, 9H);^{13}C NMR (100 MHz, $CDCl_3$): δ 144.8, 141.5, 131.3, 131.1, 130.9, 130.2, 129.1, 128.9, 128.6, 128.1, 119.0,112.3, 71.8, 43.9, 30.9, 30.7; HRMS (ESI-TOF): $C_{19}H_{21}NOS$ $[M+Na]^+$: 334.1242. Found $[M+Na]^+$: 334.1241.



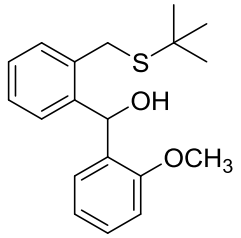
(2-((*tert*-Butylthio)methyl)phenyl)(2-nitrophenyl)methanol (21).[12] To a solution of 2-nitro-1-iodobenzene (1.79 g, 7.21 mmol) in dry THF (20 mL), PhMgCl (3.6 mL, 2.0 M in THF, 7.21 mmol) was added dropwise at -40 °C. The RM turns dark brown during addition and was then stirred at -40 °C for 15 min. The above solution was added to 2-

((tert-butylthio)methyl)benzaldehyde **10** (1.0 g, 4.80 mmol, solution in 15 mL THF) dropwise at -78 °C over 10 min. The RM was stirred at -78 °C for 1h and was gradually warmed to RT, quenched with satd. aq. NH_4Cl solution. The aqueous phase was extracted with ethyl acetate (3 × 25 mL), dried with Na_2SO_4 and concentrated under reduced pressure to get a crude oil, which was purified by silica gel column chromatography using EA/PE (1:9) as the eluant to produce **21** (1.51 g, 95% yield) as a faint yellow solid: mp93-94 °C; FTIR (ν_{max}, cm^{-1}): 3405, 2962, 1519, 1458, 1399, 1364, 1357, 1339, 1307, 1292, 1024; ^1H NMR (400MHz, $CDCl_3$): δ 8.10 (d, *J* = 7.8 Hz, 1H), 8.03 (dd, *J* = 1.1, 8.1 Hz, 1H), 7.76-7.72 (m, 1H), 7.52-7.47 (m, 1H),7.30 (d, *J* = 7.6 Hz, 1H), 7.21-7.17 (m, 1H), 7.14-7.10 (m, 1H), 6.82-6.81 (m, 2H), 4.26 (d, *J* = 11.4 Hz, 1H),4.11 (d, *J* = 2.9 Hz, 1H), 3.84 (d, *J* = 11.4 Hz, 1H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.4,141.3,138.2, 135.4, 133.5, 130.8, 129.1, 128.2, 128.0, 127.3, 125.0, 67.7, 44.1, 31.4, 30.7; MALDI-TOF MS (m/z):$C_{18}H_{21}NO_3S$ $[M+K]^+$: 370.0879. Found $[M+K]^+$: 370.0372



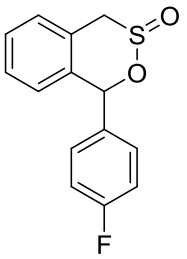
(2-(tert-butylthiomethyl)phenyl)(2-methoxyphenyl)methanol (22).

Prepared according to general procedure **B** (Grignard reagent was prepared according to procedure **A**) starting from **10** (1.0 g, 4.80 mmol), **22** (1.4 g, 93% yield) was isolated as a colorless oil: FTIR (ν_{max}, cm^{-1}): 3584, 2959, 1598, 1487, 1460, 1440, 1291, 1245, 1165, 101 6; ^1H NMR (400 MHz, $CDCl_3$): δ 7.32-7.25 (m, 4H), 7.21-7.19 (m, 2H), 6.98-6.94, (m, 1H), 6.87 (dd, *J* = 0.6, 8.2 Hz, 1H), 6.50 (s, 1H), 3.92 (d, *J* = 11.2 Hz, 1H), 3.77 (s, 3H), 3.75 (d, *J* = 12.0 Hz, 1H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.6, 141.7, 135.1, 131.2, 130.3, 128.6, 127.7, 127.6, 127.5, 120.7, 110.4, 67.3, 55.4, 43.3, 30.7, 30.6; HRMS (ESI-TOF): $C_{19}H_{24}O_2S$ $[M+Na]^+$: 339.1395. Found $[M+Na]^+$: 339.1384.



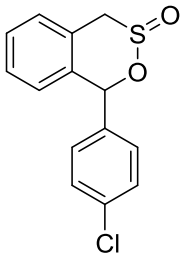
1-(4-Fluorophenyl)-1,4-dihydrobenzo-2,3-oxathiin-3-oxide (23).

Prepared according to general procedure **D.** Starting from **14** (1.24 g, 4.08 mmol), **23** (0.45 g, 42%) was isolated as a white solid: mp 86-87 °C; FTIR (ν_{max},cm^{-1}): 1607, 1512, 1482, 1458, 1284, 1156, 1124, 1108, 1087; ^1H NMR recorded at -10 °C, showed that it was isomeric mixture with ratio 1 : 0.16, for the major isomer; ^1H NMR (500 MHz, $CDCl_3$) : δ 7.40-

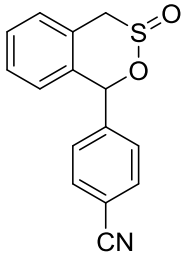


7.37 (m, 3H),7.34-7.25 (m, 2H), 7.15 (t, *J* = 8.5 Hz, 2H), 6.77 (d, *J* = 7.6 Hz, 1H), 6.29 (s, 1H), 4.66 (d, *J* = 15.3 Hz, 1H),3.67 (d, *J* = 15.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.9 (d, *J* = 197.3 Hz); 137.5, 132.7 (d, *J* = 2.5 Hz),130.5 (d, *J* = 6.6 Hz); 129.5, 128.7, 127.8, 126.6, 126.5, 115.9 (d, *J* = 17.3 Hz), 74.1, 57.6; HRMS (ESI-TOF):$C_{14}H_{11}FO_2S$ $[M+H]^+$: 263.0542. Found $[M+Na]^+$: 263.0541.

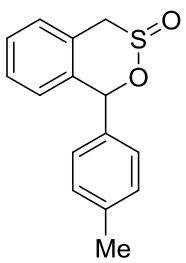
1-(4-Chlorophenyl)-1,4-dihydrobenzo-2,3-oxathiin-3-oxide (24). Prepared according to general procedure **D**. Starting from **15** (0.54 g, 1.68 mmol), **24** (0.23 g, 49%) was isolated as a white solid: mp 84-85 °C; FTIR (ν_{max},cm-1): 1488, 1458, 1365, 1289, 1087, 1012; ^{1}H NMR recorded at 0 °C, showed that it was isomeric mixture with ratio 1:0.18; For the major isomer: ^{1}H NMR (500 MHz, CDCl3): δ 7.42-7.30 (m, 5H), 7.29-7.30 (m, 2H),6.77 (d, *J* = 7.7 Hz, 1H), 6.24 (s, 1H), 4.60 (d, *J* = 15.4 Hz, 1H), 3.66 (d, *J* = 15.4 Hz, 1H); 13C NMR (125 MHz,CDCl3): δ 137.2, 135.5, 134.9, 129.9, 129.6, 128.9, 128.7, 127.8, 126.5, 126.4, 73.8, 57.5; HRMS (ESI-TOF):$C_{14}H_{11}ClO_2S$ $[M+H]^+$: 279.0247. Found $[M+H]^+$: 279.0252.



1-(4-Cyanophenyl)-1,4-dihydrobenzo-2,3-oxathiin-3-oxide (25). Prepared according to general procedure **D**, Starting from **16** (1.20 g, 3.66 mmol), **25** (0.50 g, 51%) was isolated as a white solid: mp 108-109 °C; FTIR (ν_{max}, cm^{-1}): 2230, 1483, 1457, 1105, 1087, 934; ^{1}H NMR recorded at -10 °C, showed that it was isomeric mixture with ratio 1 : 0.29 ; For the major isomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.1 Hz, 2H), 7.55(d, *J* = 8.1 Hz, 2H), 7.48-7.40 (m, 1H), 7.34-7.27 (m, 2H), 6.70 (d, *J* = 7.7 Hz, 1H), 6.30 (s, 1H), 4.69 (d, *J* =15.5 Hz, 1H), 3.71 (d, *J* = 15.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.9, 136.6, 132.6, 129.9, 129.8,129.1, 128.0, 126.5, 126.2, 118.5, 112.7, 73.7, 57.8; HRMS (ESI-TOF): $C_{15}H_{11}NO_2S$ $[M+H]^+$: 270.0589.Found $[M+H]^+$: 270.0586.

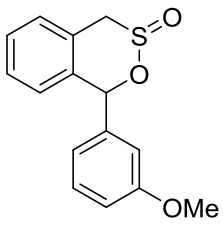


1-(*p*-Tolyl)-1,4-dihydrobenzo-2,3-oxathiin-3-oxide (26). Prepared according to general procedure **D**, starting from **17** (1.4 g, 4.66 mmol), **26** (0.46 g, 38%) was isolated as a white solid: mp 90-91 °C; FTIR (ν_{max}, cm^{-1}):1515, 1457, 1104, 1089, 912; ^{1}H NMR recorded at -20 °C, showed that it was isomeric mixture with ratio 1 :0.76; ^{1}H NMR (500 MHz, $CDCl_3$): for the major isomer: δ 7.45-7.24 (m, 7H), 6.82 (d, *J* = 7.6 Hz, 1H), 6.24 (s,1H),

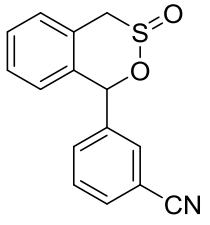


4.66 (d, *J* = 15.3 Hz, 1H), 3.67 (d, *J* = 15.3 Hz, 1H), 2.41 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.5,139.4, 139.2, 138.0, 136.2, 134.9, 134.0, 131.3, 129.8, 129.7, 129.4, 129.3, 128.8, 128.6, 128.0, 127.2, 127.0,126.9, 126.8, 124.6, 82.0, 75.1, 57.8, 55.6, 21.8, 21.7; MALDI-TOF MS (m/z): $C_{15}H_{14}O_2S$ $[M+K]^+$: 297.0352.Found $[M+K]^+$: 296.9847; Anal. Calcd. for $C_{15}H_{14}O_2S$: C, 69.74; H, 5.46; S, 12.41. Found: C, 70.12; H, 5.34;S, 12.54.

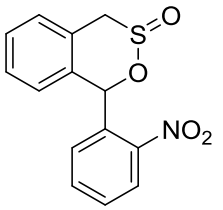
1-(3-Methoxyphenyl)-1,4-dihydrobenzo-2,3-oxathiin-3-oxide (27). Prepared according to general procedure **D**. Starting from **19** (1.36 g, 4.30 mmol), **27** (0.38 g, 32%) was isolated as a colorless oil: mp 80-81 °C; FTIR (ν_{max}, cm^{-1}): 1600, 1586, 1491, 1456, 1267, 1145, 1115, 1045; 1H NMR recorded at -20 °C, showed that it was a isomeric mixture with ratio 1:0.06; For the major isomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 (s, 2H), 7.29 (s,1H), 6.99-6.83 (m, 4H), 6.24 (s, 1H), 4.68 (d, *J* = 14.7 Hz, 1H), 3.82 (s, 3H), 3.68 (d, *J* = 14.6 Hz, 1H); ^{13}CNMR (125 MHz, $CDCl_3$): δ 159.8, 138.3, 138.0, 130.1, 129.7, 129.0, 128.1, 126.9, 121.0, 114.9, 113.8, 75.1,58.0, 55.6; HRMS (ESI-TOF): $C_{15}H_{14}O_3S$ $[M+H]+$: 275.0742. Found $[M+H]+$: 275.0749.



1-(3-Cyanophenyl)-1,4-dihydrobenzo-2,3-oxathiin-3-oxide (28). Prepared according to general procedure **D**. Starting from **20** (1.1 g, 3.53 mmol), **28** (0.37 g, 38% yield) was isolated as a white solid: mp 98-99 °C; FTIR (ν_{max}, cm^{-1}): 2232, 1484, 1457, 1110, 1091; ^{1}H NMR recorded at -20 °C showed that it was an isomeric mixture with ratio 1:0.36; ^{1}H NMR (500 MHz, $CDCl_3$), for the major isomer: δ 7.77-7.60 (m, 4H), 7.49-7.28 (m, 3H), 6.71 (d, *J* = 7.5 Hz, 1H), 6.29 (s, 1H), 4.69 (d, *J* = 15.5 Hz, 1H), 3.72 (d, *J* = 15.4 Hz, 1H); ^{13}C NMR (125 MHz,$CDCl_3$): δ 138.7, 136.7, 133.3, 133.0, 132.3, 130.1, 130.0, 129.4, 128.4, 126.7, 126.5, 118.7, 113.0, 73.8, 57.9;HRMS (ESI-TOF): $C_{15}H_{11}NO_2S$ $[M+H]+$: 270.0589. Found $[M+H]+$: 270.0584; Anal. Calcd. for $C_{15}H_{11}NO_2S$:C, 66.89; H, 4.12; N, 5.20; S, 11.91. Found: C, 67.04; H, 4.40; N, 5.10; S, 11.91.

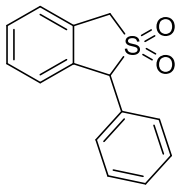


1-(2-Nitrophenyl)-1,4-dihydrobenzo-2,3-oxathiin-3-oxide (29). Prepared according to general procedure **D**. Starting from **21** (0.75 g, 2.26 mmol), **29** (0.23 g, 35%) was isolated as a faint brown solid: mp 101-102 °C; FTIR (ν_{max}, cm^{-1}): 1610, 1577, 1544, 1524, 1488,

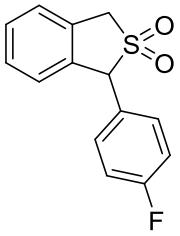


1456, 1347, 1293, 1113; 1H NMR recorded at -20 °C, showed that it was isomeric mixture with ratio 1:0.76: ^{1}H NMR (500 MHz, $CDCl_3$), for the major isomer: δ 8.14 (d, *J* =5.8 Hz, 1H), 7.81-7.25 (m, 6H), 6.64 (s, 1H), 6.49 (s, 1H), 4.31 (d, *J* = 13.4 Hz, 1H), 3.93 (d, *J* = 13.2 Hz, 1H);^{13}C NMR (125 MHz, $CDCl_3$): δ 148.9, 148.2, 139.2, 134.8, 134.7, 134.4, 134.2, 133.0, 132.6, 131.6, 130.4,130.1, 130.0, 129.7, 129.3, 128.9, 128.2, 127.2, 125.8, 125.1, 124.3, 124.1, 76.7, 71.3, 60.3, 55.7; MALDI-TOFMS (m/z): $C_{14}H_{11}NO_4S$ $[M+K]^+$: 328.0046. Found $[M+K]^+$: 328.0229; Anal. Calcd. for $C_{14}H_{11}NO_4S$: C, 58.12;H, 3.83; N, 4.84; S, 11.08. Found: C, 57.61; H, 3.70; N, 4.68; S, 11.01.

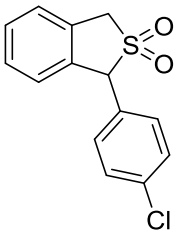
1-Phenyl-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (13). Prepared according to general procedure **E**. Starting from **6** (0.035 g, 0.143 mmol), **13** (0.035 g, quantitative yield) was isolated as a white solid: mp 116-117 °C; FTIR (ν_{max}, cm^{-1}): 3447, 2923, 2359, 1455, 1303, 1228, 1203, 1157, 1121, 1104; ^{1}H NMR (400MHz, $CDCl_3$): δ 7.45-7.35 (m, 6H), 7.28-7.25 (m, 2H), 7.14-7.12 (m, 1H), 5.48 (s, 1H), 4.43 (s, 2H); ^{13}C NMR(100 MHz, $CDCl_3$): δ 135.8, 131.0, 130.5, 130.3, 129.5, 129.2, 129.1, 129.0, 126.7, 126.0, 71.8, 55.2; HRMS (ESITOF):$C_{14}H_{12}O_2S$ $[M+Na]^+$: 267.0456. Found $[M+Na]^+$: 267.0462; Anal. Calcd. for $C_{14}H_{12}O_2S$: C, 68.83; H,4.95; S, 13.12. Found: C, 69.19; H, 4.84, 12.79.



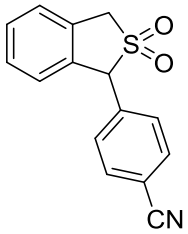
1-(4-Fluorophenyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (23a). Prepared according to general procedure **E**. Starting from **23** (0.035 g, 0.115 mmol), **23a** (0.035 g, quantitative yield) was isolated as a white solid: mp140-141 °C; FTIR (ν_{max}, cm^{-1}): 1605, 1508, 1459, 1313, 1293, 1226, 1198, 1155, 1136, 1095; ^{1}H NMR (400MHz, $CDCl_3$):δ 7.45-7.36 (m, 3H), 7.27-7.23 (m, 2H), 7.15-7.08 (m, 3H), 5.46 (s, 1H), 4.43 (s, 2H); ^{13}C NMR(100 MHz, $CDCl_3$): δ 163.5 (d, *J* = 247.6 Hz), 135.7, 132.4 (d, *J* = 8.4 Hz), 131.0, 129.3, 129.1, 126.6, 126.0,116.1 (d, *J* = 21.7 Hz), 70.9, 55.2; HRMS (ESI-TOF): $C_{14}H_{11}FO_2S$ $[M+H]^+$: 263.0542. Found $[M+H]^+$:263.0542; Anal. Calcd. for $C_{14}H_{11}FO_2S$: C, 64.11; H, 4.23; S, 12.22. Found: C, 64.19; H, 3.99, 11.89.



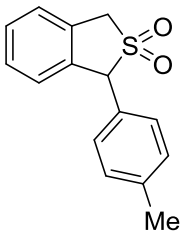
1-(4-Chlorophenyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (24a). Prepared according to general procedure **E**. Starting from **24** (0.035 g, 0.109 mmol), **24a** (0.035 g, quantitative yield) was isolated as a white solid: mp 94-95 °C; FTIR (ν_{max}, cm^{-1}): 1491, 1458, 1411, 1311, 1198, 1132, 1102, 1087; ^{1}H NMR (400MHz, $CDCl_3$): δ7.45-7.36 (m, 5H), 7.22-7.19 (m, 2H), 7.09 (d, *J* = 8.1 Hz, 1H), 5.45 (s, 1H), 4.43 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$):: δ 135.7, 135.4, 131.8, 131.0, 129.4, 129.3, 129.2, 128.7, 126.6, 126.0, 71.0, 55.3; HRMS (ESI-TOF):$C_{14}H_{11}ClO_2S$ $[M+H]^+$: 279.0247. Found $[M+H]^+$: 279.0247; Anal. Calcd. for $C_{14}H_{11}ClO_2S$: C, 60.32; H, 3.98;S, 11.50. Found: C, 60.64; H, 3.85; S, 11.06.



1-(4-Cyanoyphenyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (25a). Prepared according to general procedure **E**. Starting from **25** (0.035 g, 0.112 mmol), **25a** (0.035 g, quantitative yield) was isolated as a white solid: mp 132-133 °C; FTIR (ν_{max}, cm^{-1}): 2232, 1608, 1506, 1478, 1458, 1317, 1203, 1136, 1102; ^{1}H NMR (400MHz, $CDCl_3$): δ 7.72 (d, *J* = 8.1 Hz, 2H), 7.48-7.38 (m, 5H), 7.06 (d, *J* = 7.6 Hz, 1H), 5.52 (s, 1H), 4.50 (d, *J* =15.6 Hz, 1H), 4.45 (d, *J* = 15.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.7, 134.7, 132.6, 131.3, 131.0,129.7, 129.4, 126.5, 126.2, 118.3, 113.4, 71.2, 55.6; HRMS (ESI-TOF): $C_{15}H_{11}NO_2S$ $[M+H]^+$: 270.0589.Found $[M+H]^+$: 270.0588; Anal. Calcd. for $C_{15}H_{11}NO_2S$: C, 66.89; H, 4.12; N, 5.20; S, 11.91 Found: C, 67.25;H, 3.89; N, 4.89; S, 11.63.

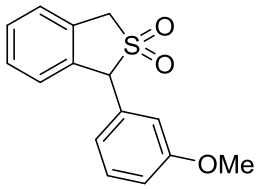


1-(p-Tolyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (26a). Prepared according to general procedure **E**. Starting from **26** (0.035 g, 0.116 mmol), **26a** (0.035 g, quantitative yield) was isolated as a white solid: mp 119-120 °C; FTIR (ν_{max}, cm^{-1}): 1316, 1194, 1175, 1135, 1087; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43-7.33 (m, 3H),7.23 (d, *J* = 8.0 Hz, 2H), 7.14-7.12 (m, 3H), 5.44 (s, 1H), 4.41 (s, 2H), 2.38 (s, 3H); ^{13}C NMR (100 MHz,$CDCl_3$): δ 139.5, 136.0, 131.1, 130.3, 129.7, 129.1, 129.0, 127.3, 126.7, 125.9, 71.6, 55.2, 21.3; HRMS (ESITOF):$C_{15}H_{14}O_2S$ $[M+Na]^+$: 281.0612. Found $[M+Na]^+$: 281.0576

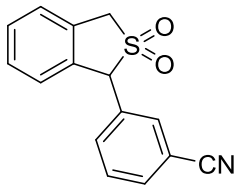


1-(3-Methoxyphenyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (27a). Prepared according to general procedure **E**. Starting from **27** (0.035 g, 0.115 mmol), **27a** (0.035 g,

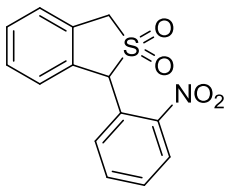
quantitative yield) was isolated as a white sticky solid: FTIR (ν_{max}, cm^{-1}): 1599, 1584, 1491, 1458, 1307, 1264, 1251, 1198; 1H NMR (400 MHz, CDCl3):δ 7.44-7.31 (m, 4H), 7.15 (d, *J* = 8.0 Hz, 1H), 6.96 (dd, *J* = 2.1, 8.2 Hz, 1H), 6.84 (d, *J* = 7.6 Hz, 1H), 6.77 (dd, *J* = 1.9, 2.0 Hz, 1H), 5.44 (s, 1H), 4.42 (s, 2H), 3.78 (s, 3H); 13C NMR (100 MHz, CDCl3): δ 159.9, 135.7,131.9, 131.0, 130.0, 129.2, 129.0, 126.8, 125.9, 122.8, 116.1, 114.9, 71.7, 55.4, 55.3; HRMS (ESI-TOF):$C_{15}H_{14}O_3S$ $[M+Na]^+$: 297.0561. Found $[M+Na]^+$: 297.0565.



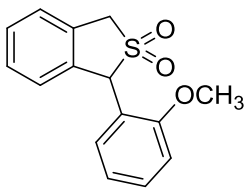
1-(3-Cyanoyphenyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (28a). Prepared according to general procedure **E**. Starting from **28** (0.035 g, 0.112 mmol), **28a** (0.035 g, quantitative yield) was isolated as a white sticky solid: FTIR (ν_{max}, cm^{-1}): 2231, 1482, 1319, 1197, 1132; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75-7.72 (m,1H), 7.57-7.40 (m, 6H), 7.08 (d, *J* = 8.1 Hz, 1H), 5.50 (s, 1H), 4.49 (d, *J* = 15.7 Hz, 1H), 4.45 (d, *J* = 15.7 Hz,1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.1, 134.6, 133.8, 133.0, 132.2, 131.0, 129.9, 129.7, 129.5, 126.4,126.2, 118.2, 113.4, 70.8, 55.4; HRMS (ESI-TOF): $C_{15}H_{11}NO_2S$ $[M+H]^+$: 270.0589. Found $[M+H]^+$: 270.0584.



1-(2-Nitrophenyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (29a). Prepared according to general procedure **E**. Starting from **29** (0.035 g, 0.105 mmol), **29a** (0.035 g, quantitative yield) was isolated as a brown solid: mp209-210 °C; FTIR (ν_{max}, cm^{-1}): 1522, 1342, 1315, 1198, 1147, 1097; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23-8.19(m, 1H), 7.59-7.55 (m, 2H), 7.50-7.41 (m, 3H), 7.17 (d, *J* = 7.4 Hz, 1H1), 7.00-6.96 (m, 1H), 6.53 (s, 1H), 4.45(d, *J* = 15.8 Hz, 1H), 4.39 (d, *J* = 15.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 134.8, 133.6, 132.5, 131.2,130.9, 130.1, 129.7, 129.5, 127.7, 127.0, 126.2, 125.8, 66.9, 55.5; HRMS (ESI-TOF): $C_{14}H_{11}NO_4S$ [M+K]+ :328.0046. Found [M+K]+ : 328.0040; Anal. Calcd. for $C_{14}H_{11}NO_4S$: C, 58.12; H, 3.83; N, 4.84; S, 11.08.Found: C, 58.30; H, 3.52; N, 4.60; S, 11.12.

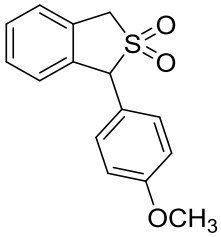


1-(2-methoxyphenyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (30). Formed as a product while preparing benzosultine from alcohol **22** according to general procedure D.



Starting from Starting from **22** (1.0 g, 3.16 mmol), **30** (0.58 g, 66%) was isolated as a white solid: mp 179-180 °C; FTIR (ν_{max}, cm^{-1}): 1601, 1587, 1493, 1471, 1306, 1297, 1252, 1203, 1135, 1104; ^1H NMR (400 MHz, $CDCl_3$): δ 7.42-7.34 (m, 4H), 7.14 (d, *J* = 7.0 Hz, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 6.93-6.89 (m, 1H), 6.79 (d, *J* = 7.1 Hz, 1H), 6.05 (s, 1H), 4.38 (s, 2H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.7, 135.9, 131.4, 130.6, 129.8, 129.0, 128.9, 126.8, 125.8, 120.8, 120.7, 111.2, 65.7, 56.0, 55.5; HRMS (ESI-TOF): $C_{15}H_{14}O_3S$ $[M+Na]^+$: 297.0561. Found $[M+Na]^+$: 297.0565; Anal. Calcd. for $C_{15}H_{14}O_3S$: C, 65.67; H, 5.14; S, 11.69. Found: C, 65.01; H, 4.81; S, 11.50.

1-(4-methoxyphenyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (31). Formed as a product while preparing benzosultine from alcohol **18** according to general procedure according to general procedure D. Starting from **18** (1.0 g, 3.16 mmol), **31** (0.266 g, 30%) was isolated as a white solid: mp 121-122 °C; FTIR (ν_{max}, cm^{-1}): 2932, 1610, 1512, 1456, 1317, 1272, 1252, 1194, 1178, 1134; ^1H NMR (400 MHz, $CDCl_3$): δ 7.43-7.37 (m, 3H), 7.19 (d, *J* = 8.7 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 1H), 6.95 (d, *J* = 11.6 Hz, 2H), 5.43 (s, 1H), 4.41 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5, 136.1, 131.7, 131.0, 129.1, 129.0, 126.7, 125.9, 121.9, 114.5, 71.2, 55.4, 55.0; HRMS (ESI-TOF): $C_{15}H_{14}O_3S$ $[M+Na]^+$: 297.0561. Found $[M+Na]^+$: 297.0566; Anal. Calcd. for $C_{15}H_{14}O_3S$: C, 65.67; H, 5.14; S, 11.69. Found: C, 65.18; H, 5.08; S, 11.12.



Decomposition of sultine in acetonitrile.

To 1 mL of a 10 mM stock solution of the compound in acetonitrile, 9 mL of acetonitrile was added to produce solution of final concentration 1 mM. This solution was analyzed by HPLC to determine amount of sultine remaining and sulfone formed. Analysis was carried out after 3h at 70 °C and after 3h at 37 °C to determine amount of compound remaining.

Sulfite analysis. Ion chromatography analysis: An ion chromatograph attached with a conductivity detector was used for sulfite analysis.1 mM $NaHCO_3$/3.2 mM Na_2CO_3 was the eluant and the flow rate was 0.7 mL/min. Using stock solutions of sulfite, a calibration curve was generated (R^2 = 0.9999). To 3 mL of 1 mM stock solution of compound in acetonitrile (21 or 24 mL) of phosphate buffer (pH = 7.4, 20 mM) and 3/0 mL of acetonitrlie was added (to make final volume 10/20%, ACN/PB), vortexed for 20 s. To this

mixture, 3 mL of 10 mM cysteine solution (pH7.4) was added and the reaction mixture was stirred at RT under inert atmosphere. Aliquots at appropriate time intervals were analyzed by IC. Maximum sulfur dioxide yield was calculated based on completion of the reaction with no further increase in sulfite formation.

Calibration curve for Sulfite: A stock solution (100 mM) of sodium sulfite, Na_2SO_3 (Sigma-Aldrich) was prepared in dI water containing 1% paraformaldehyde. This stock solution was diluted to prepare 10, 40, 60, 80, 100, 120 and 160 μM sodium sulfite solutions. Injections of 20 μL were carried out to generate a calibration curve for sulfite, R^2 was found as 0.9999, and slope 0.0044.

Calibration curve for Sulfate: Sulfite is susceptible to aerobic oxidation to form sulfate, which was detected in small amounts in several instances during our study. In order to quantify sulfate, a calibration curve was generated by preparing a 10 mM solution of sulfate, which was diluted to prepare solutions of suitable concentrations for injections into the IC. As the levels of sulfate were below 5 μM, calibration curve was generated with concentrations below 10 μM (Figure S3). R^2 was found as 0.9965 and slope 0.0135.

Kinetics of decomposition of sultine in pH 7.4 buffer.

To 3 mL of 1 mM stock solution of compound in acetonitrile, 6 (or 9) mL of acetonitrile, and 21 (or 18) mL of phosphate buffer (pH = 7.4, 20 mM) was added (final volume = 30 mL) as a 30 (or 40%) solution of acetonitrile in phosphate buffer. The reaction mixture was stirred at 37 °C under inert atmosphere. Aliquots at appropriate time intervals were analyzed by IC (to determine sulfite) and HPLC (to determine amount of starting material remaining). HPLC analysis: The eluant used was acetonitrile and water (1:1) and the flow rate was 1 mL/min (isocratic). The column temperature was maintained at 15 °C. Ion chromatography (IC) analysis: An ion chromatograph attached with a conductivity detector was used for sulfite analysis.1 mM $NaHCO_3$/3.2 mM Na_2CO_3 was the eluant and the flow rate was 0.7 mL/min. Using stock solutions of sulfite and sulfate, calibration curves were generated (R^2 = 0.9999 and 0.9965). Maximum sulfur dioxide yield was calculated based on completion of the reaction with no further increase in sulfite formation.

Supercoiled plasmid DNA cleavage: DNA cleavage was analyzed by the conversion of pBR322 circular DNA to open circular and/or linear forms. Briefly, pBR322 DNA (100 ng) was incubated with 100 μM of compounds **6** or **6b** as solutions in DMSO or an

aqueous solution of $NaHSO_3$:Na_2SO_3 (3:1 ratio) in the presence of 1 eq. of $Cu(OAc)_2$ in 10 mMTris–Cl (pH 8.5) and diluted with MOPS (pH 7.5, 10 mM) and NaCl (100 mM), to a final volume of 20 μL. The solutions were incubated at 37 °C. After 5 h incubation, the solutions were mixed with bromophenol blue (0.25 wt.%, 4 μL) and immediately subjected to electrophoresis on a 1.0% agarose gel with 1.0 μg/mL ethidium bromide in TAE (50 mL) using a horizontal slab gel apparatus containing TAE buffer medium for 60-90 min under constant 90 V. After electrophoresis, the DNA was visualized under GBOX Syngene UV-transilluminator, photographed using Genesnap-7.04.

X-ray data for 23: $C_{14}H_{11}FO_2S$; Compound **23** was crystallized from diethyl ether at –20 °C. A colorless rectangular shaped crystal with approximate dimensions 0.110 x 0.112 x 0.027 mm gave monoclinic crystal with space group P21/c; a = 14.639 (3) b = 8.3191 (19) c = 10.737 (3) Å, α = 90° β = 110.839(5) ° γ = 90°; V = 1222.1(5) Å^3; T = 100 K; Z = 4; ρ_{calc}= 1.426 Mgm^{-3}; $2\theta_{max}$= 50°; $MoK\alpha\lambda$ = 0.71073 Å. Fine-focus sealed tube source with graphite monochromator. R = 0.0422 (for 1687 reflection $I>2\sigma(I)$), wR= 0.0724 which was refined against $|F2|$ and S = 2.312 for 164 parameters and 2152 unique reflections. The structure was obtained by direct methods using SHELXS-97. All non-hydrogen atoms were refined isotropically. The hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they are bonded. μ = 0.267 mm^{-1}. **CCDC No.: 901688**

X-ray data for 23a: $C_{14}H_{11}FO_2S$; Compound **23a** was crystallized from diethyl ether at RT. A colorless rectangular shaped crystal with approximate dimensions 0.302 x 0.210 x 0.052 mm gave monoclinic crystal with space group P21/c; a = a=11.4383(13) b = b=11.6596(15) c = 9.4849(12) Å, α = 90° β = 104.523(3)° γ = 90°; V = 1224.5(3) Å^3; T = 296 K ; Z = 4; ρ_{calc}= 1.423 Mgm^{-3}; $2\theta_{max}$= 56.70°; $MoK\alpha\lambda$ = 0.71073 Å. Fine-focus sealed tube source with graphite monochromator. R = 0.0341 (for 2601 reflection $I>2\sigma(I)$), wR= 0.1042 which was refined against $|F2|$ and S = 1.491for 163 parameters and 3047 unique reflections. The structure was obtained by direct methods using SHELXS-97. All non-hydrogen atoms were refined isotropically. The hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they are bonded. μ = 0.267 mm^{-1}. **CCDC No.: 907013**

2.5 Spectral Data

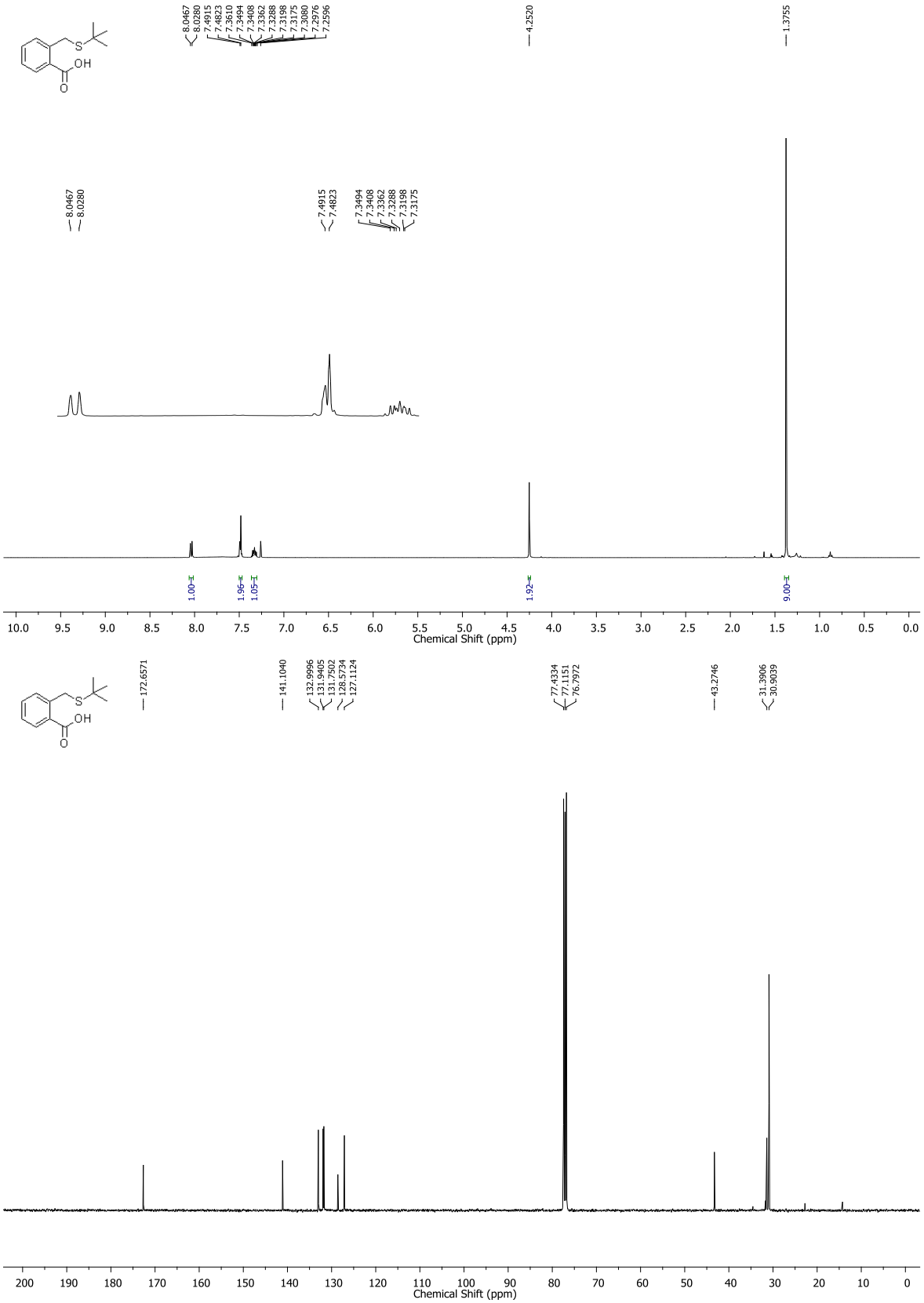


Figure S1. NMR spectra of **8**

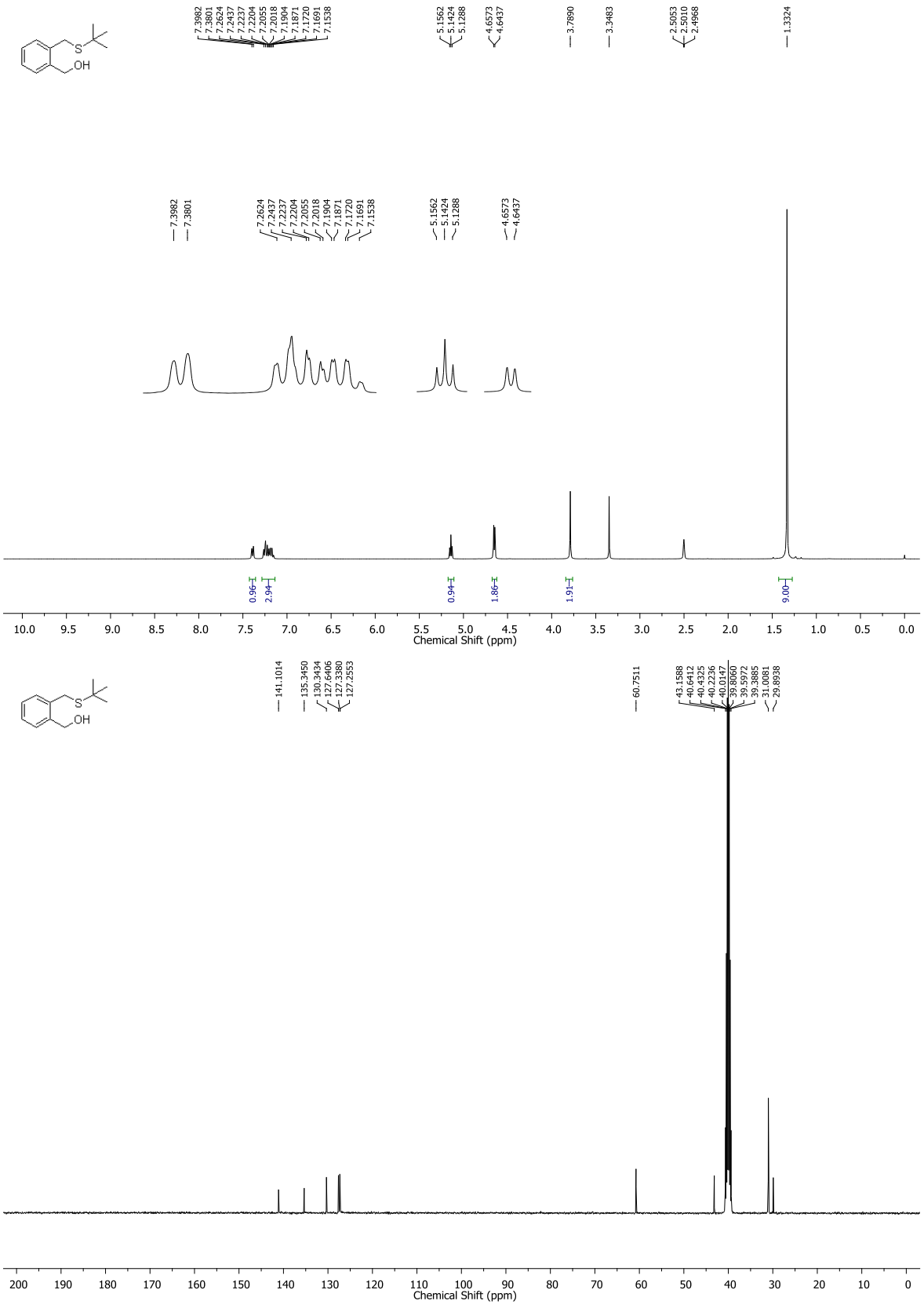


Figure S2. NMR spectra of **9**

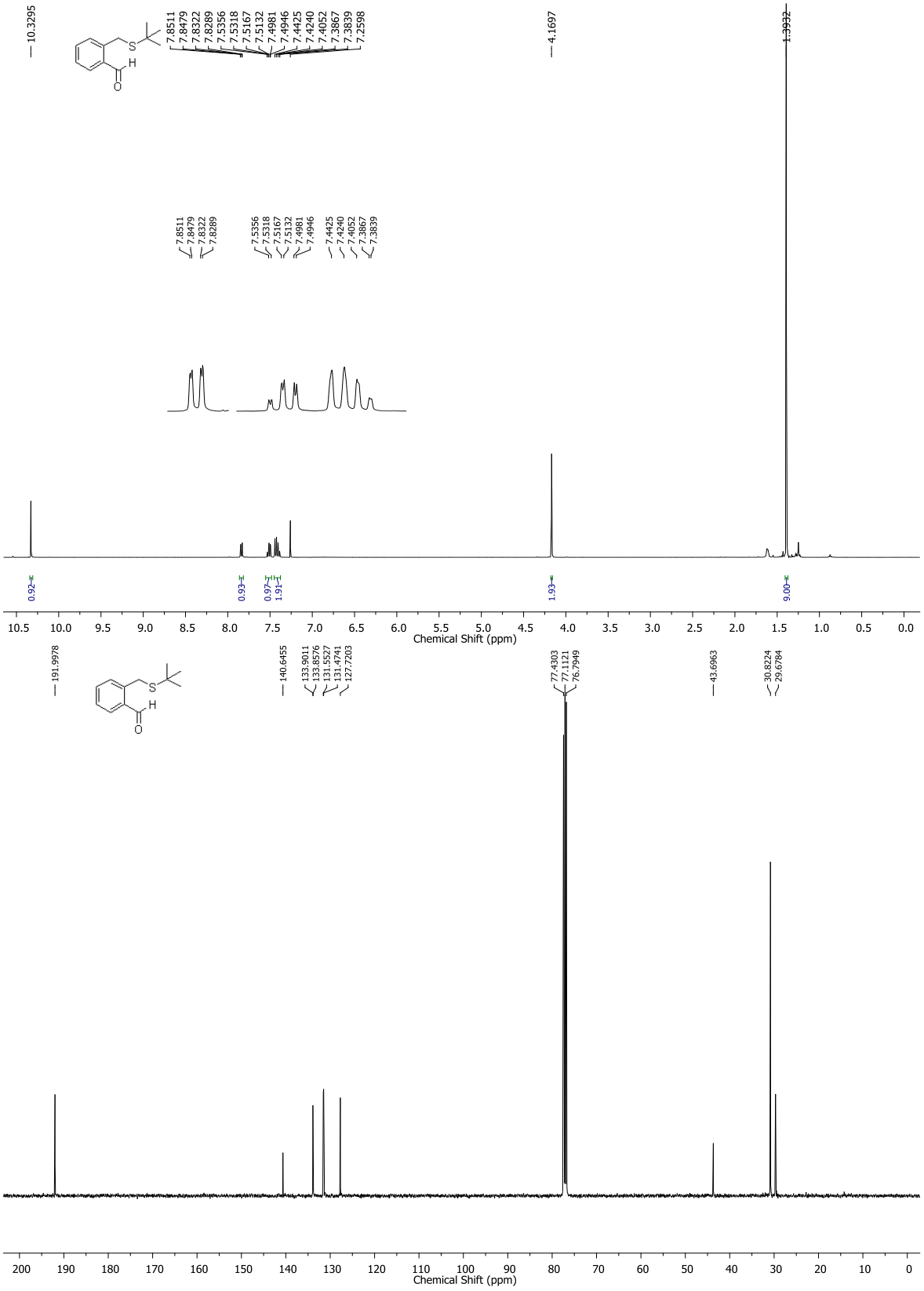


Figure S3. NMR spectra of **10**

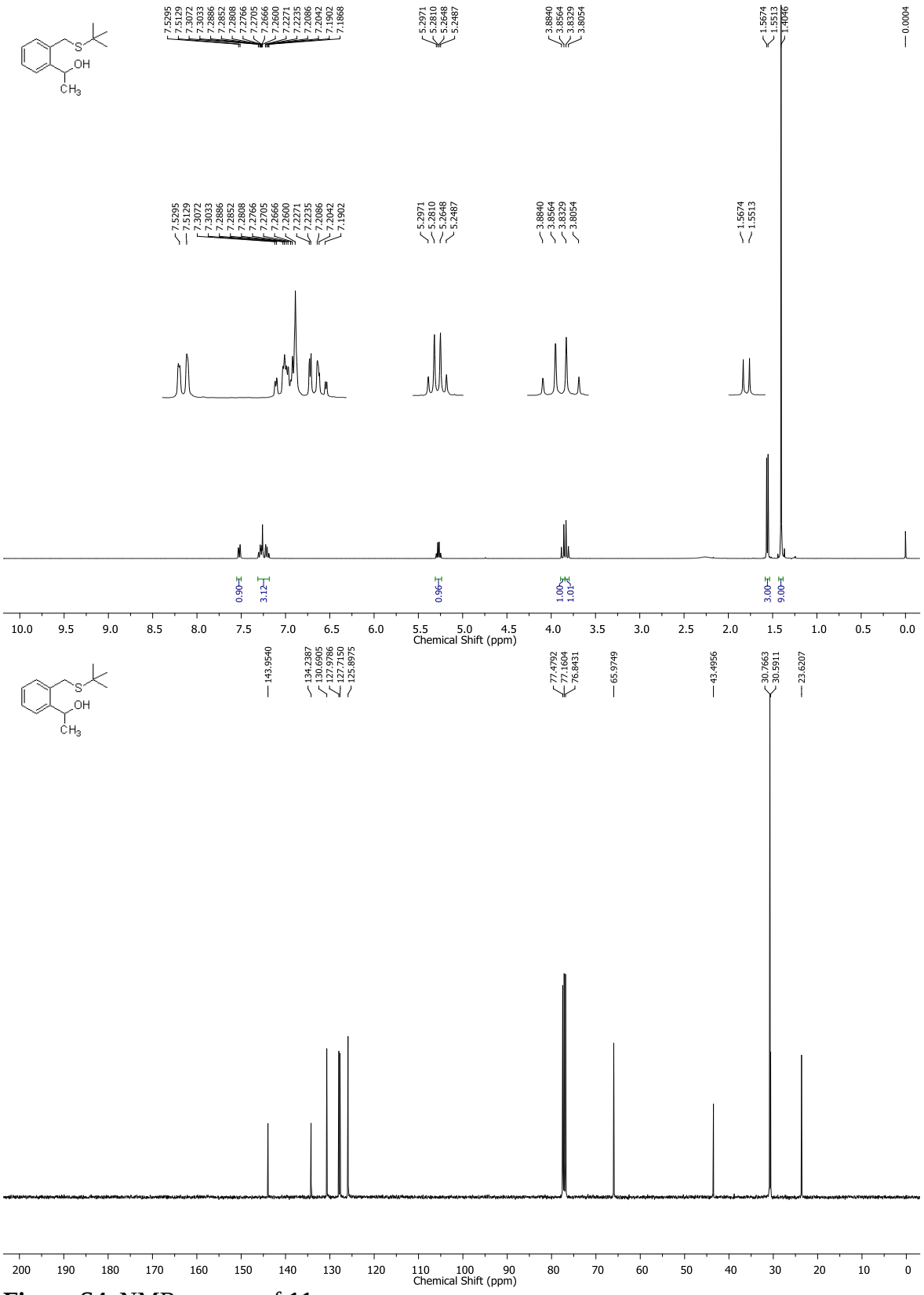


Figure S4. NMR spectra of **11**

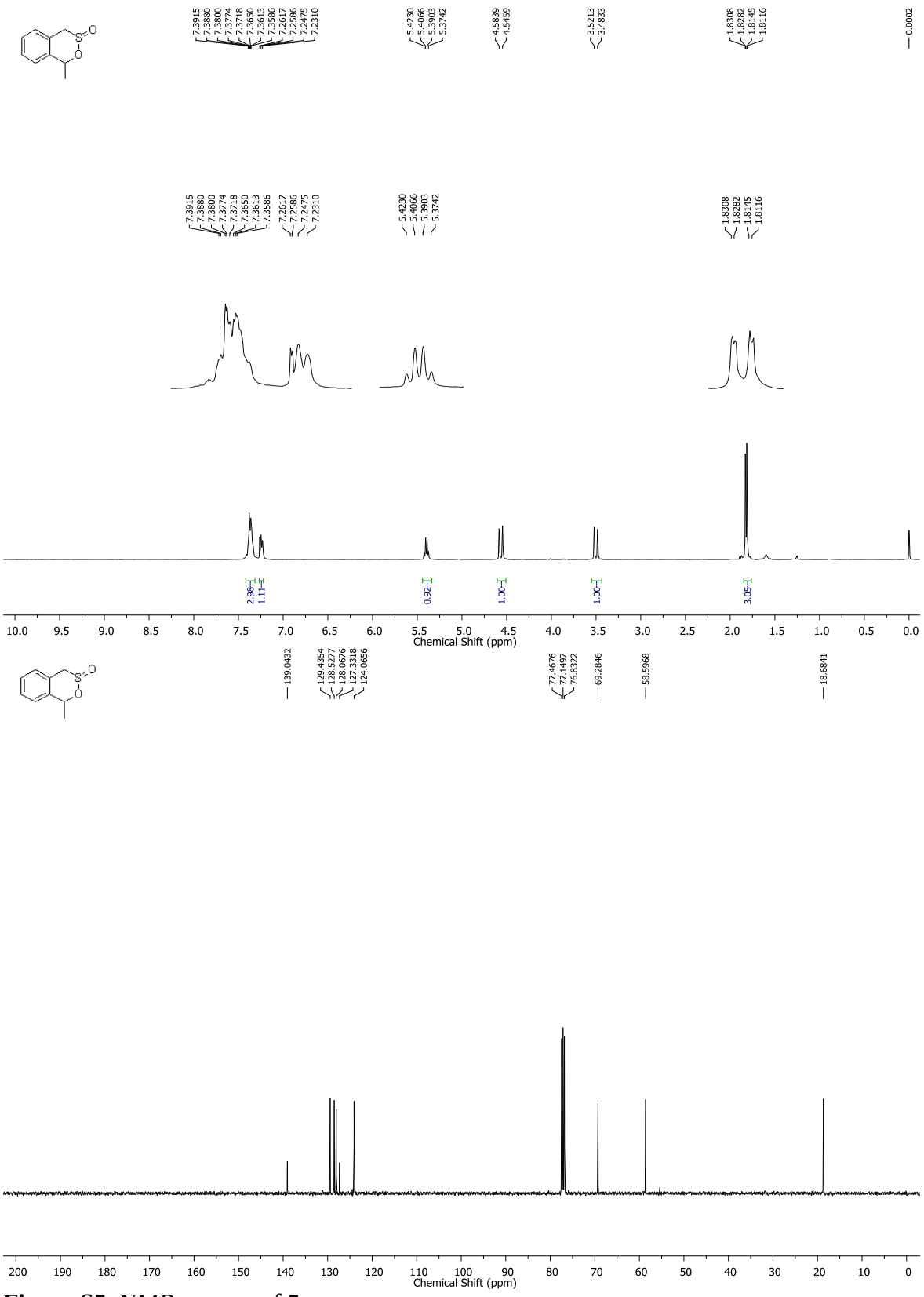


Figure S5. NMR spectra of **5**

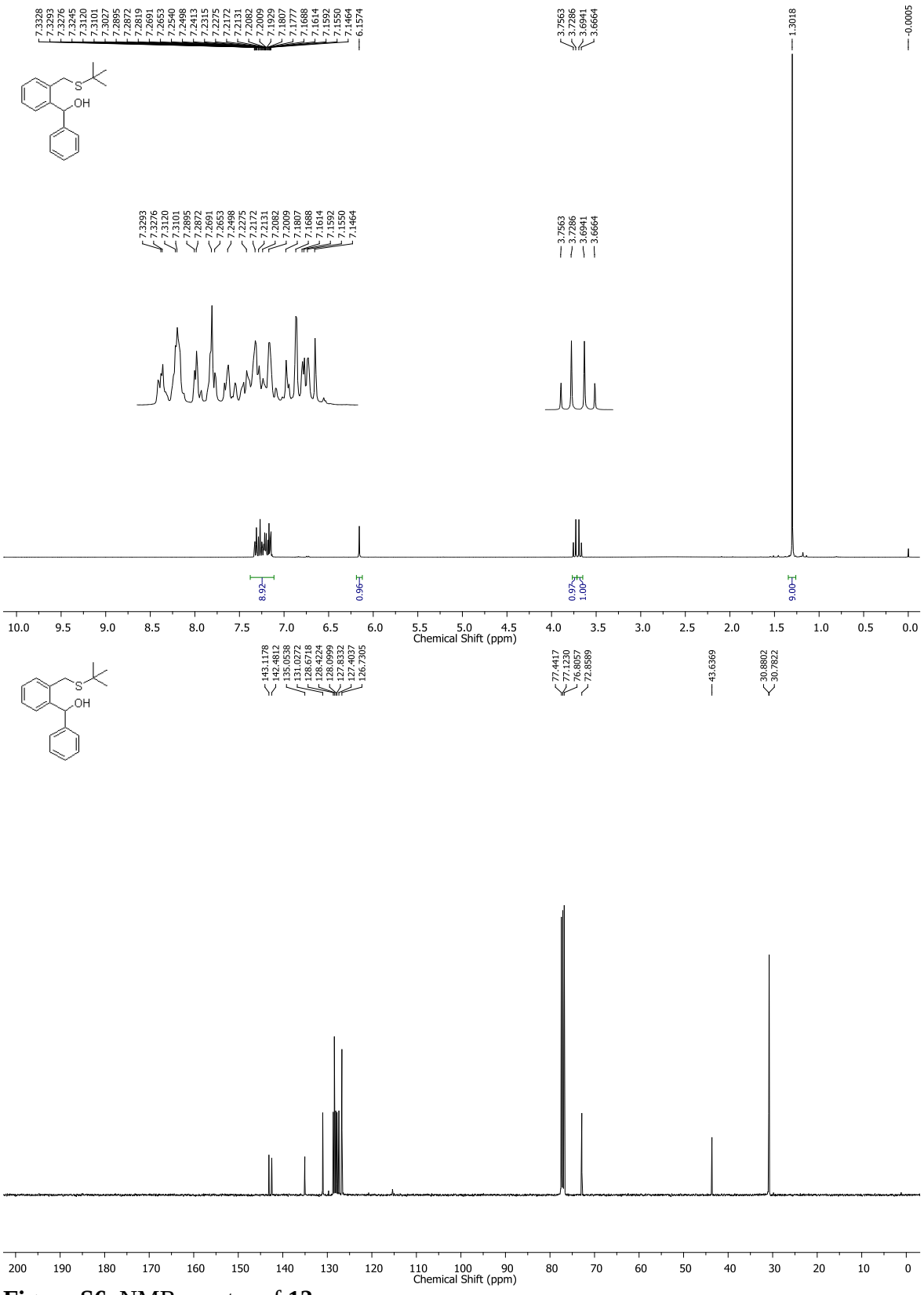


Figure S6. NMR spectra of **12**

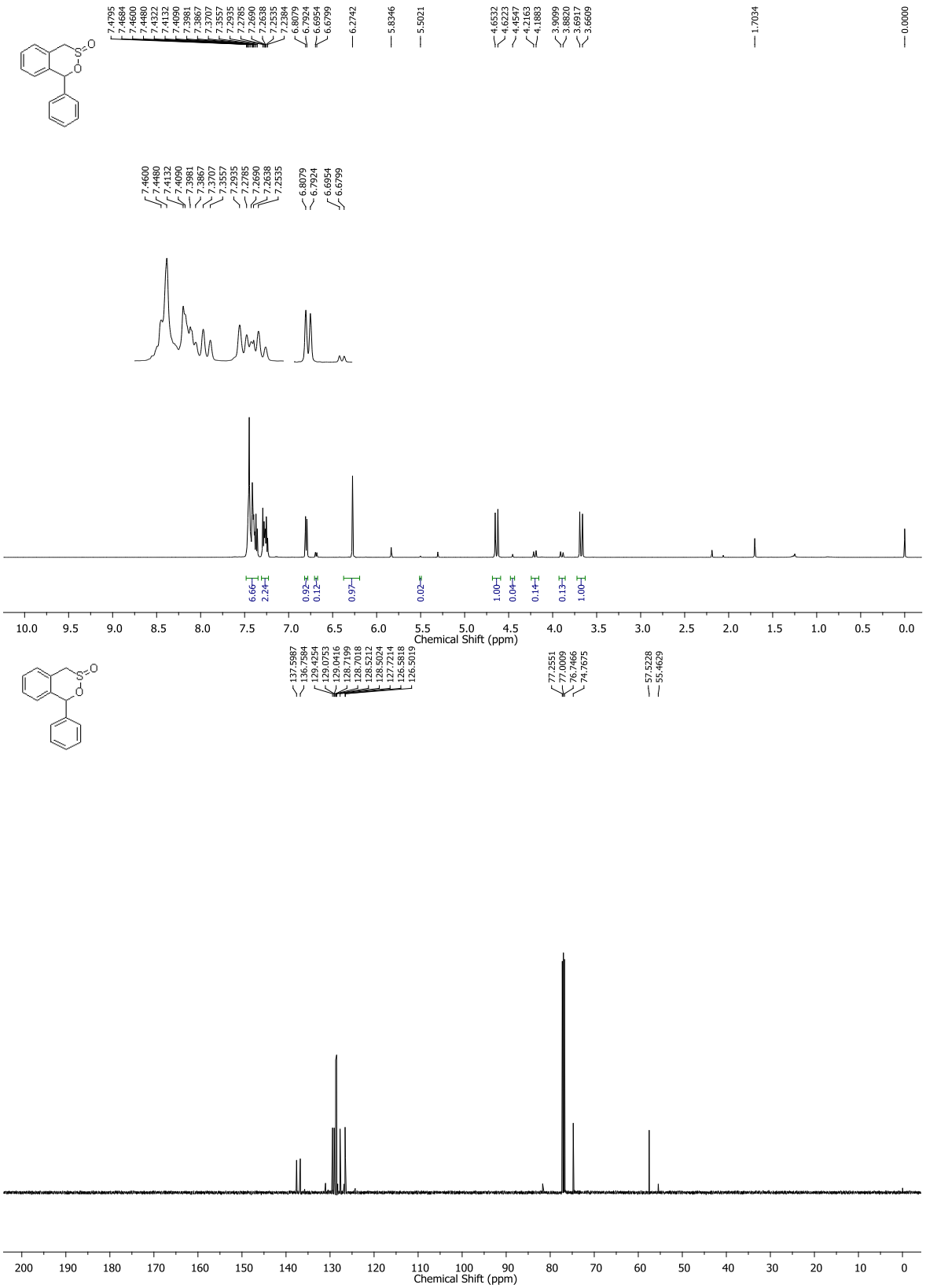


Figure S7. NMR spectra of **6**

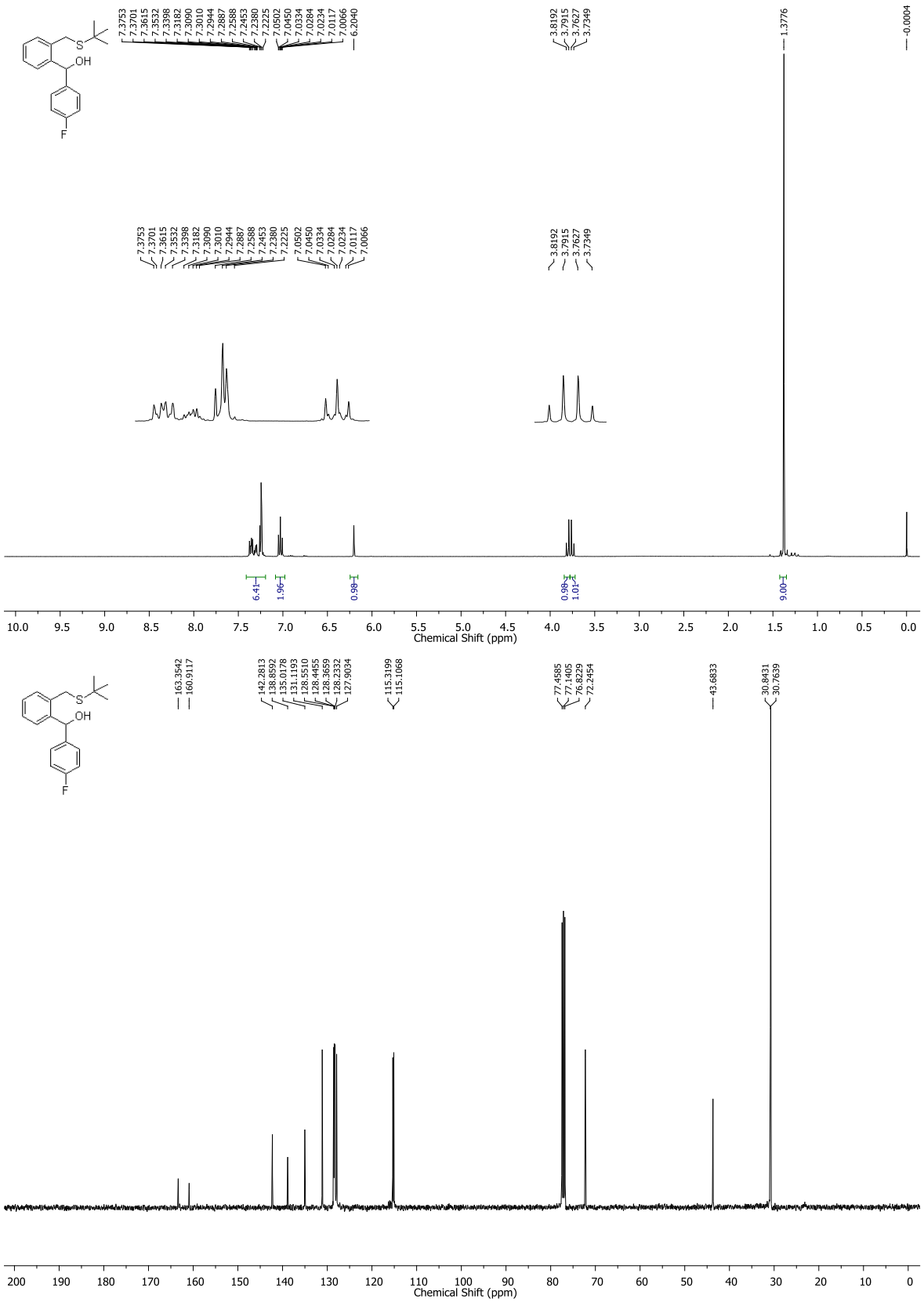


Figure S8. NMR spectra of **14**

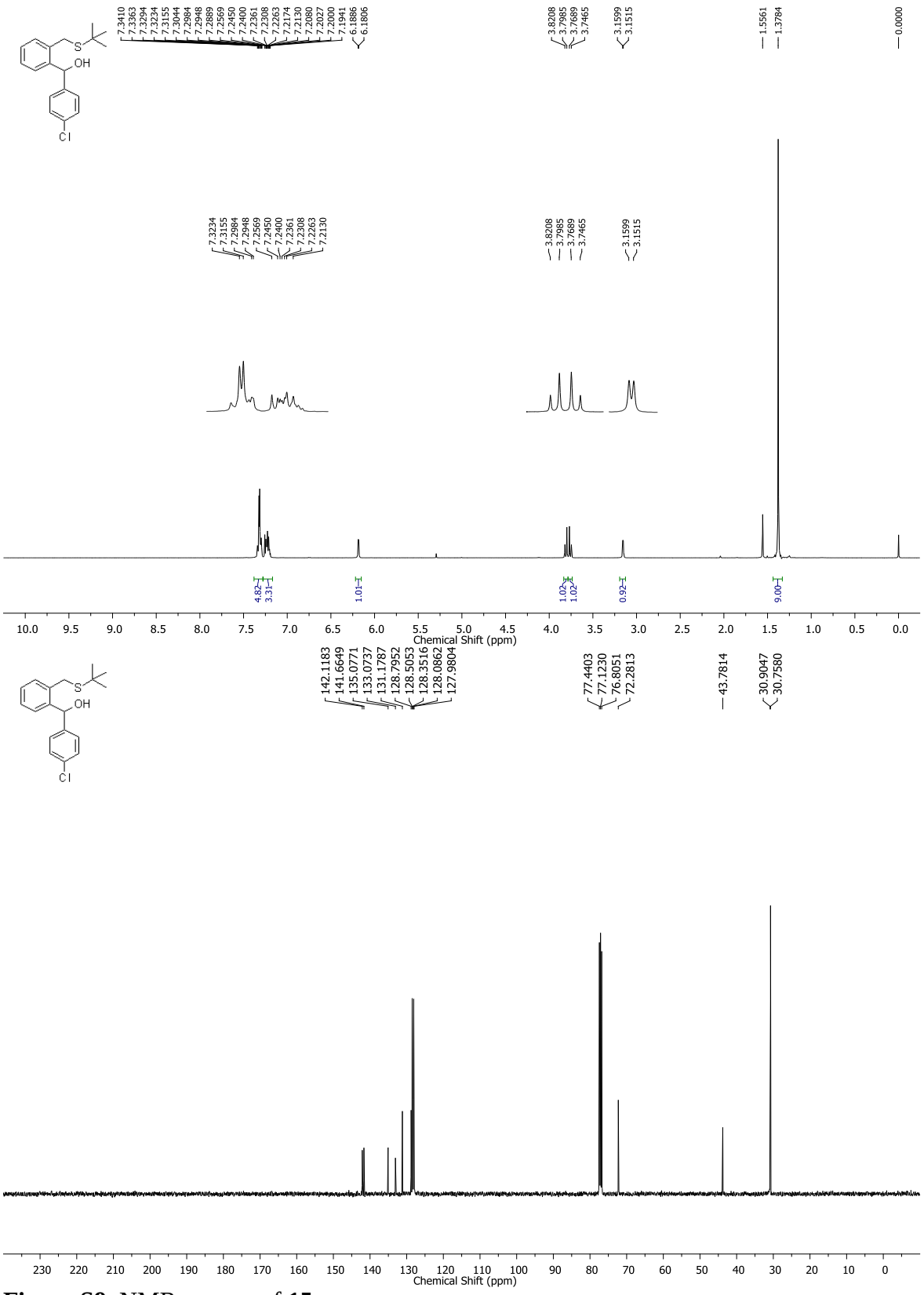


Figure S9. NMR spectra of **15**

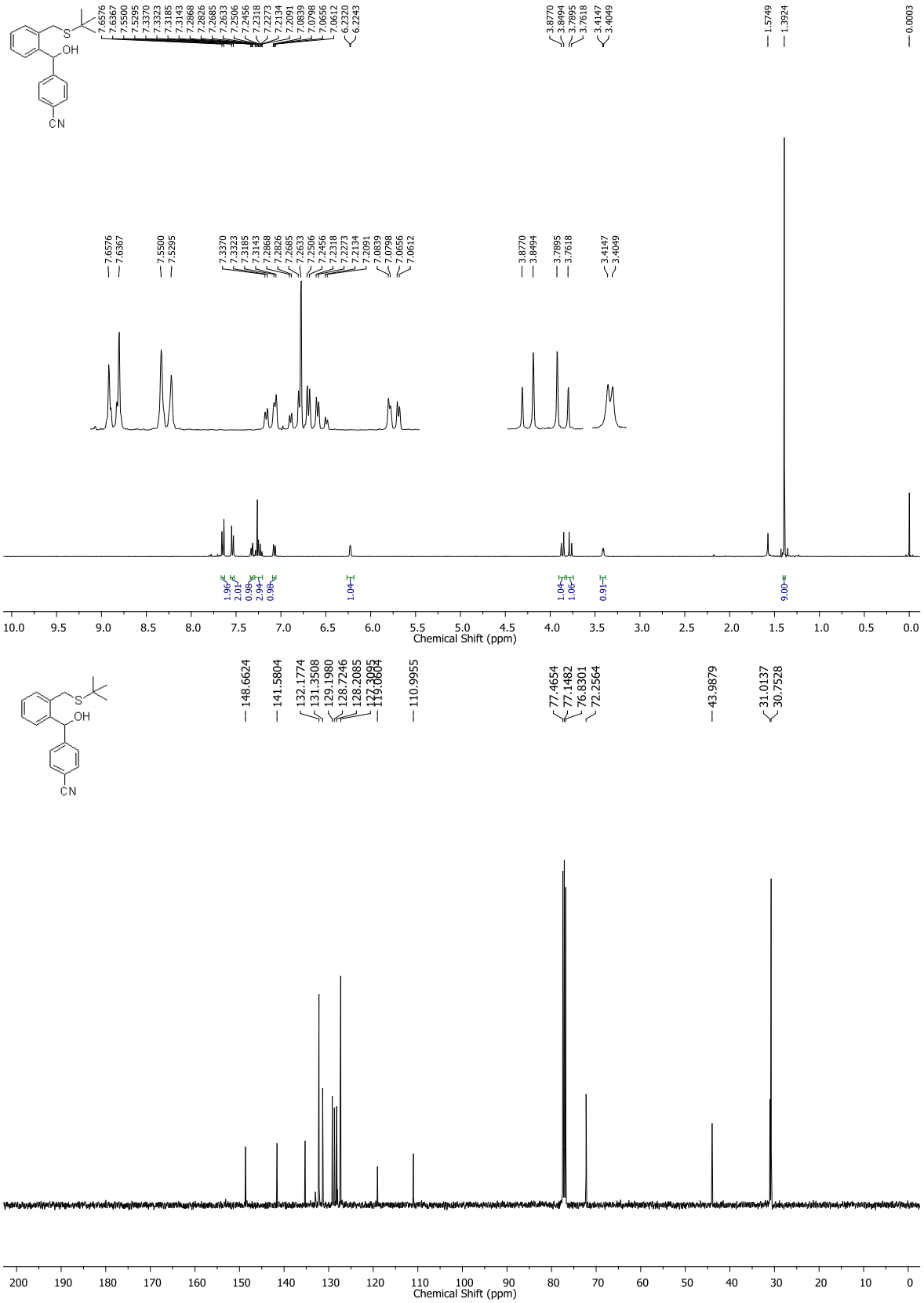


Figure S10. NMR spectra of **16**

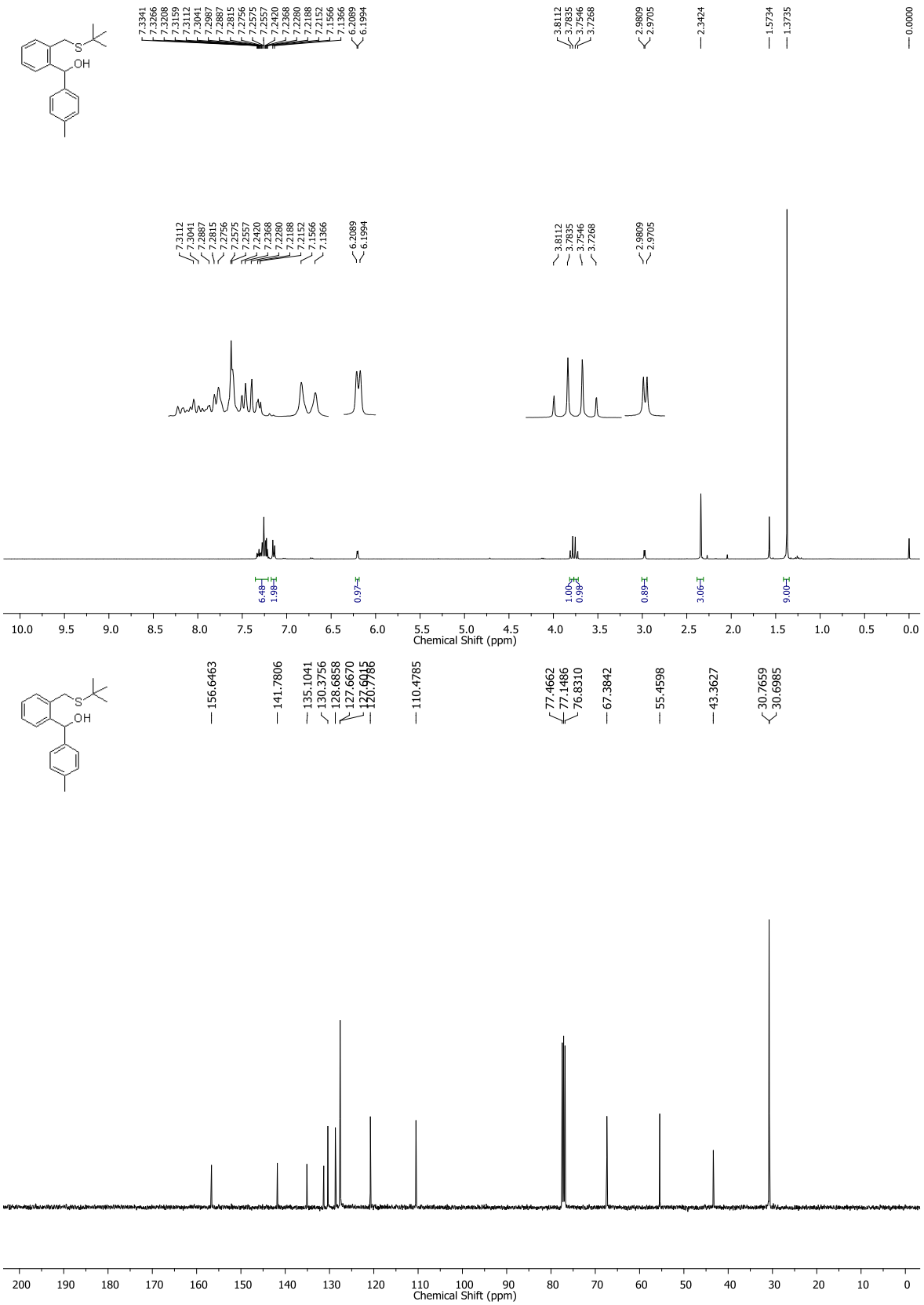


Figure S11. NMR spectra of **17**

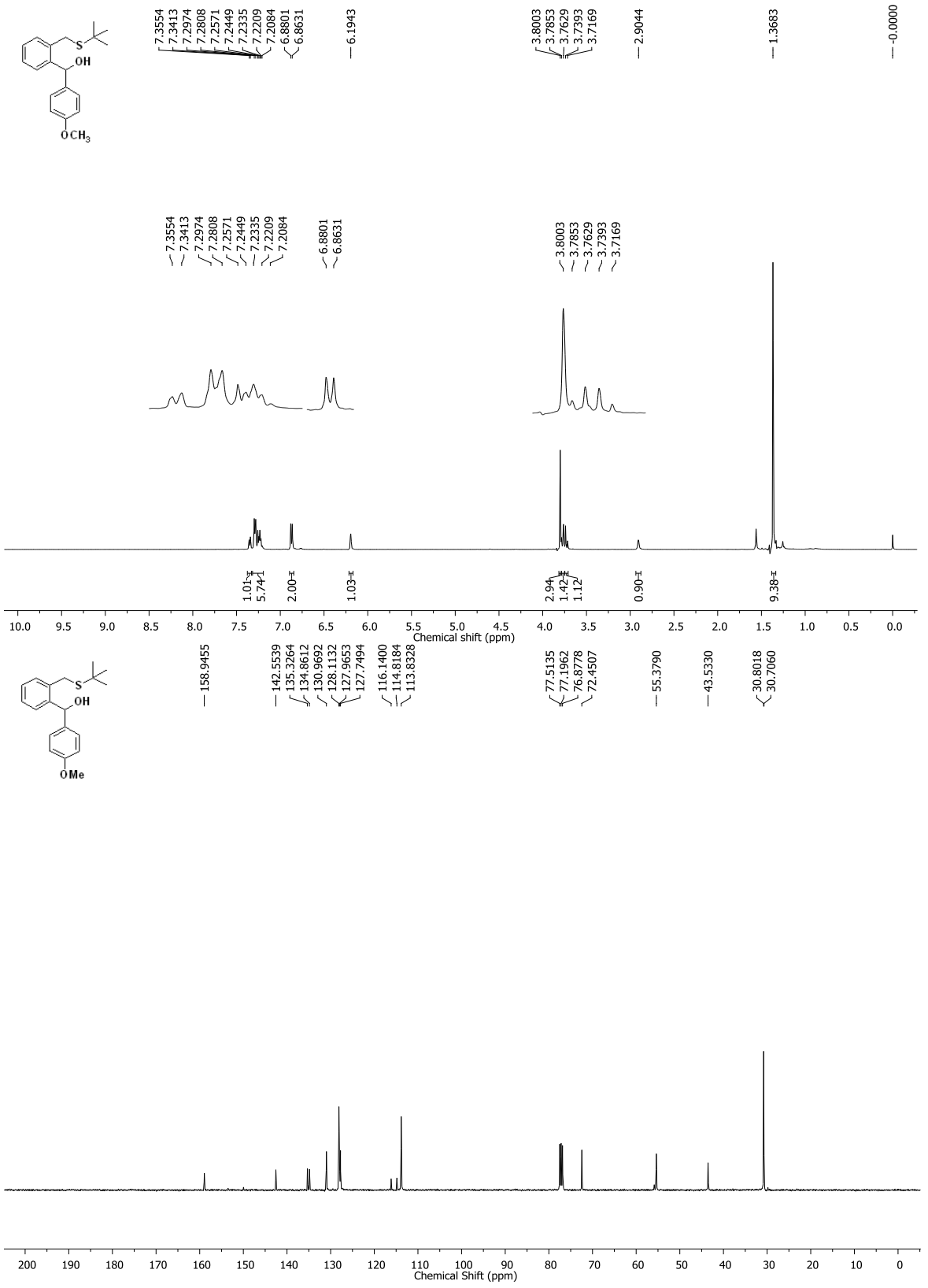


Figure S12. NMR spectra of **18**

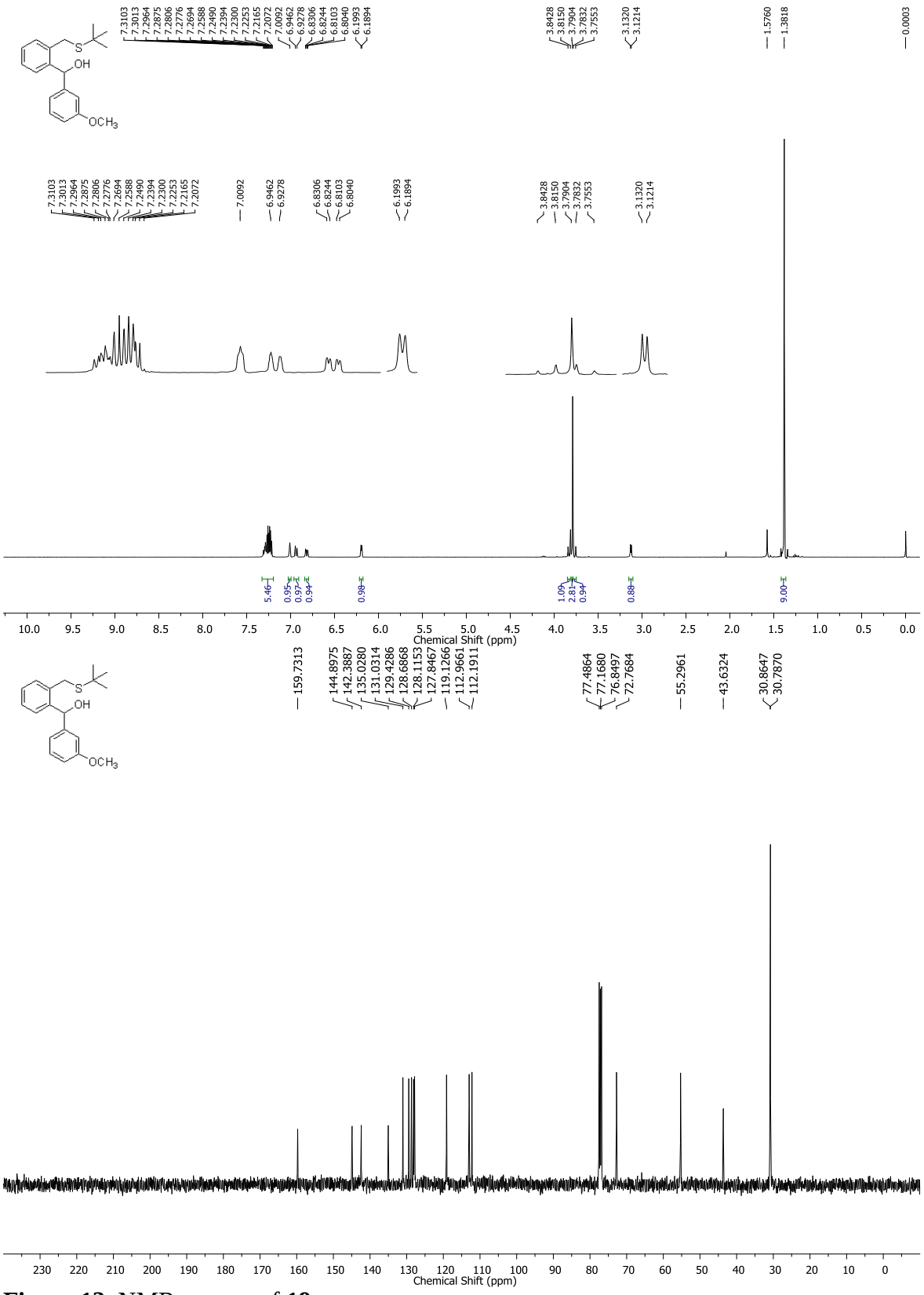


Figure 13. NMR spectra of **19**

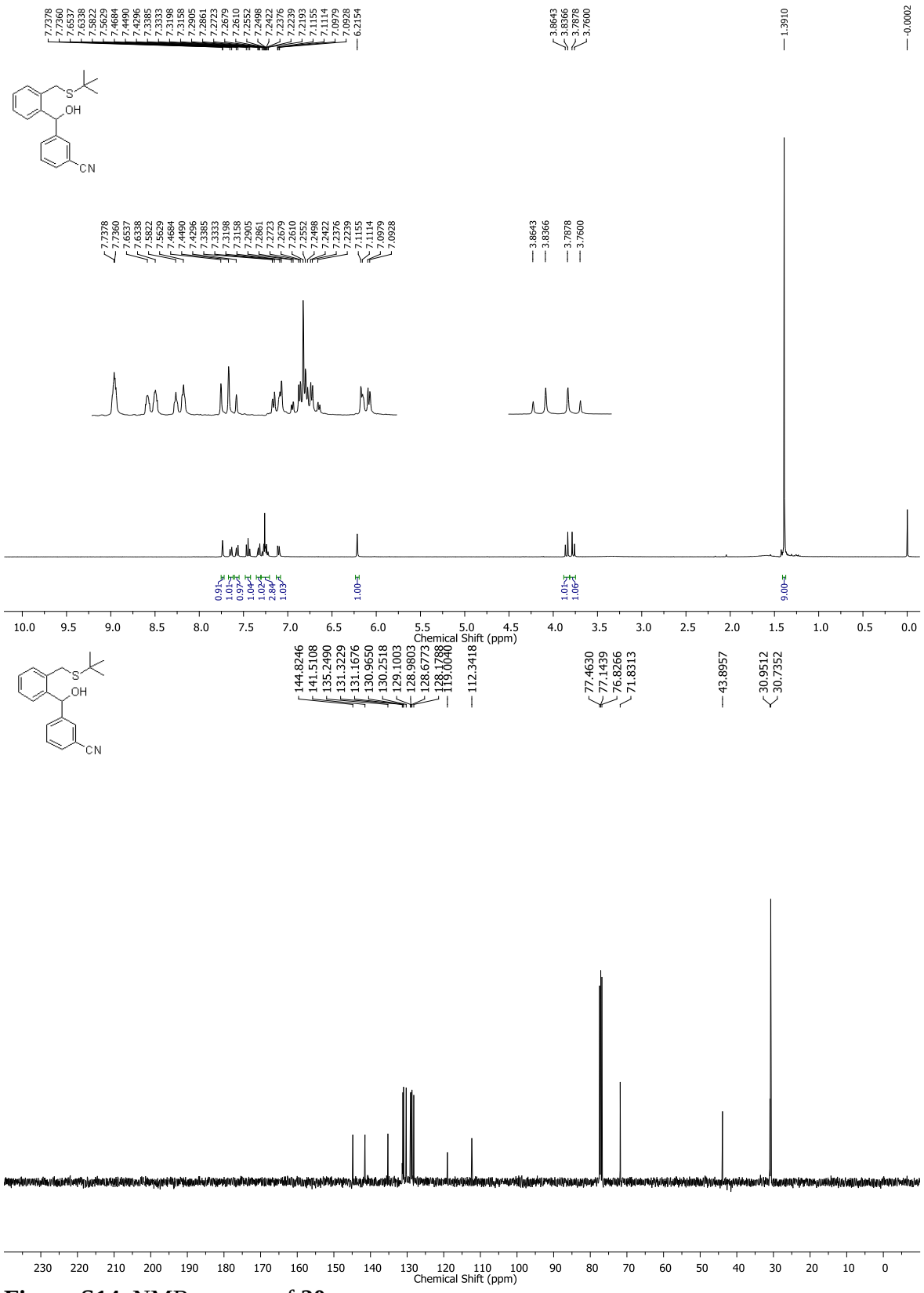


Figure S14. NMR spectra of **20**

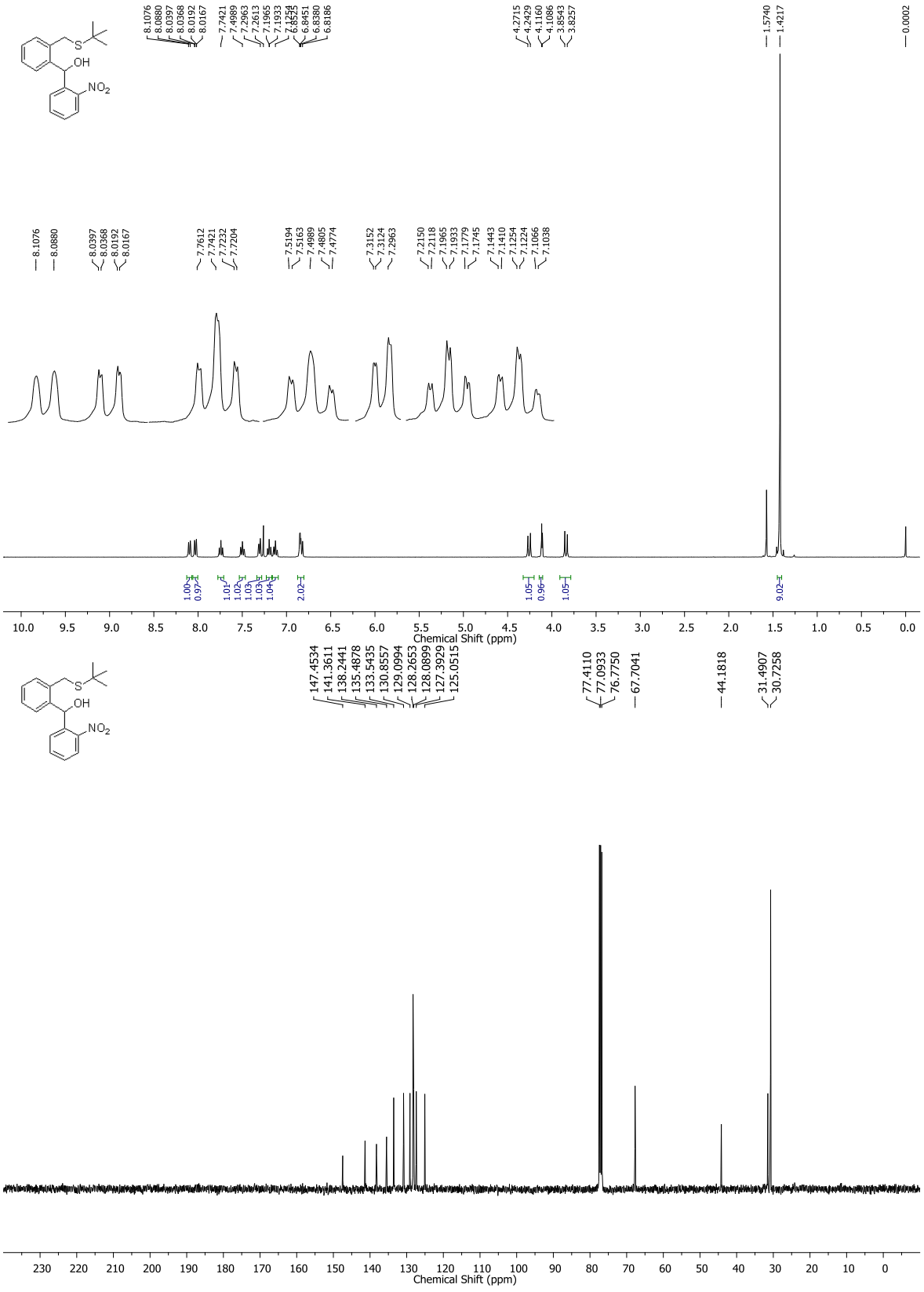


Figure S15. NMR spectra of **21**

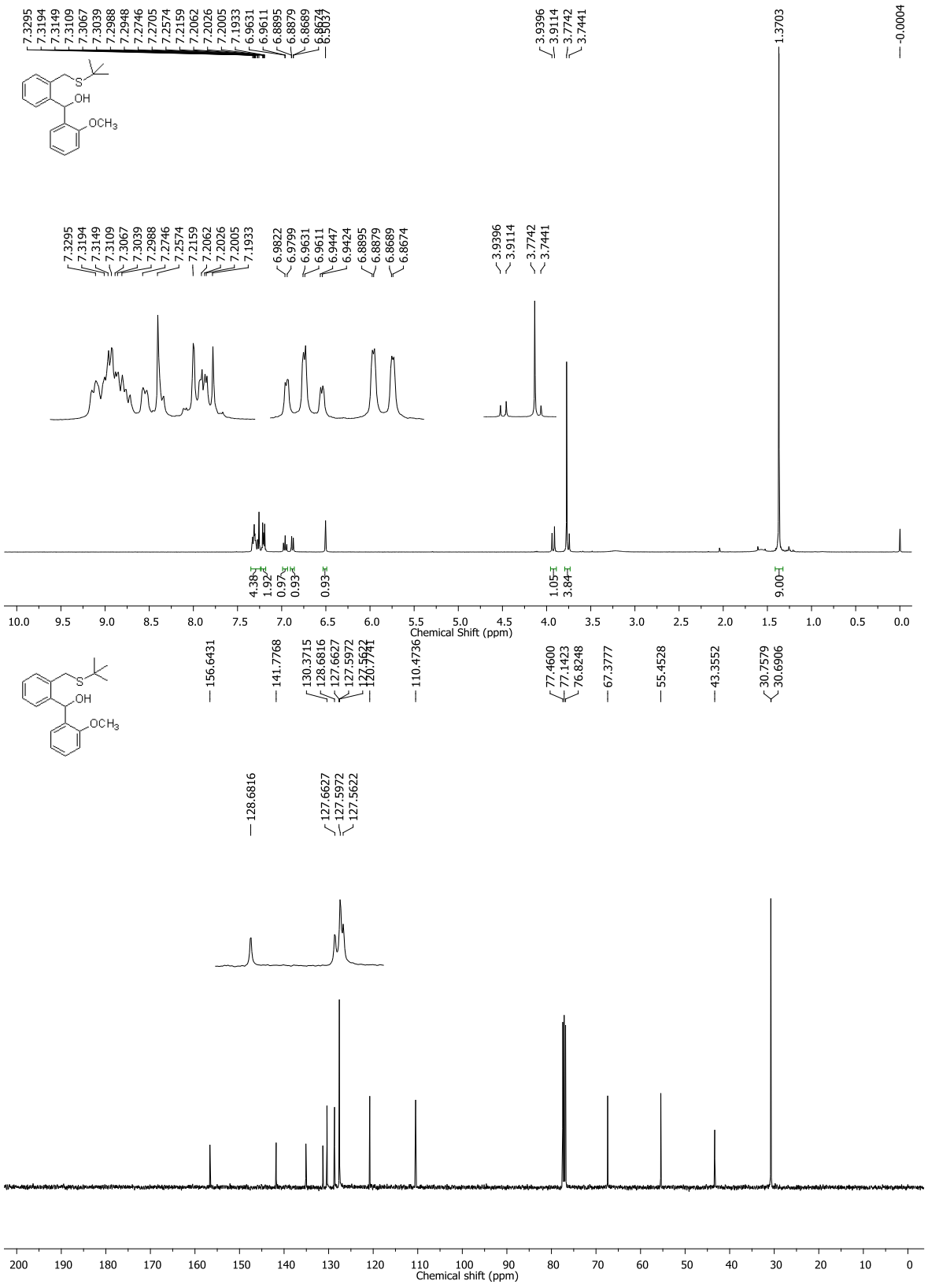


Figure S16. NMR spectra of **22**

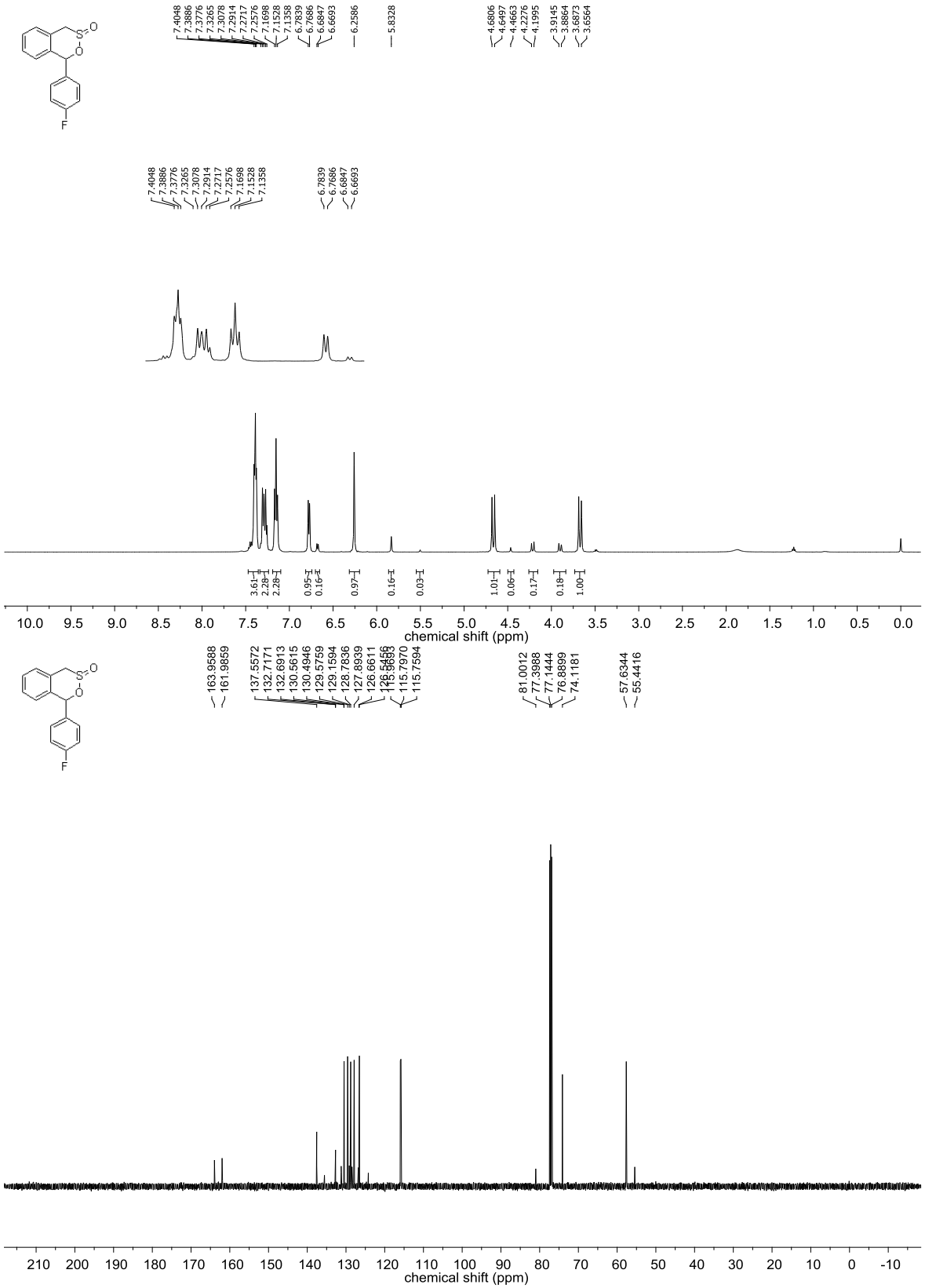


Figure S17. NMR spectra of **23**

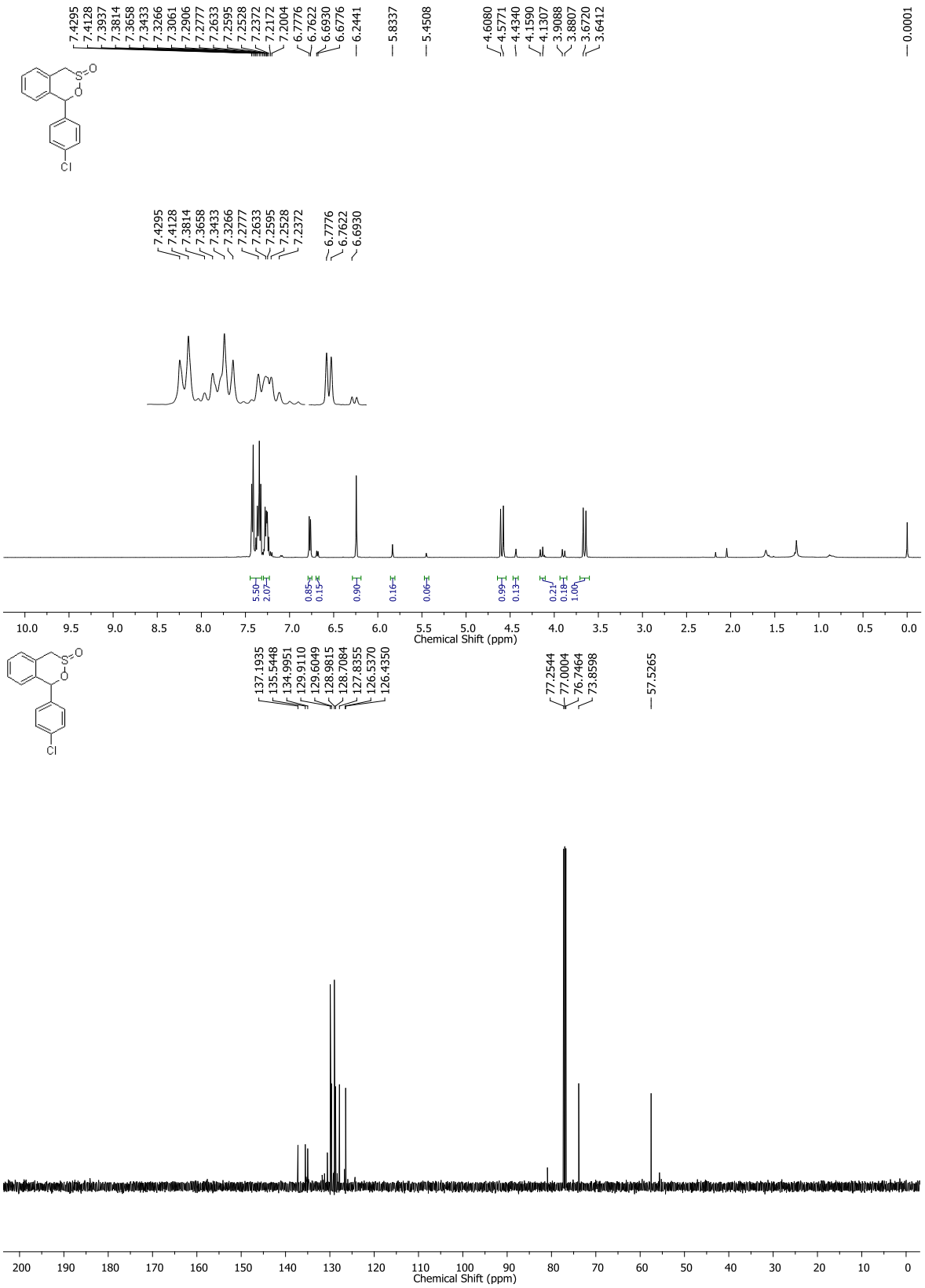


Figure S18. NMR spectra of **24**

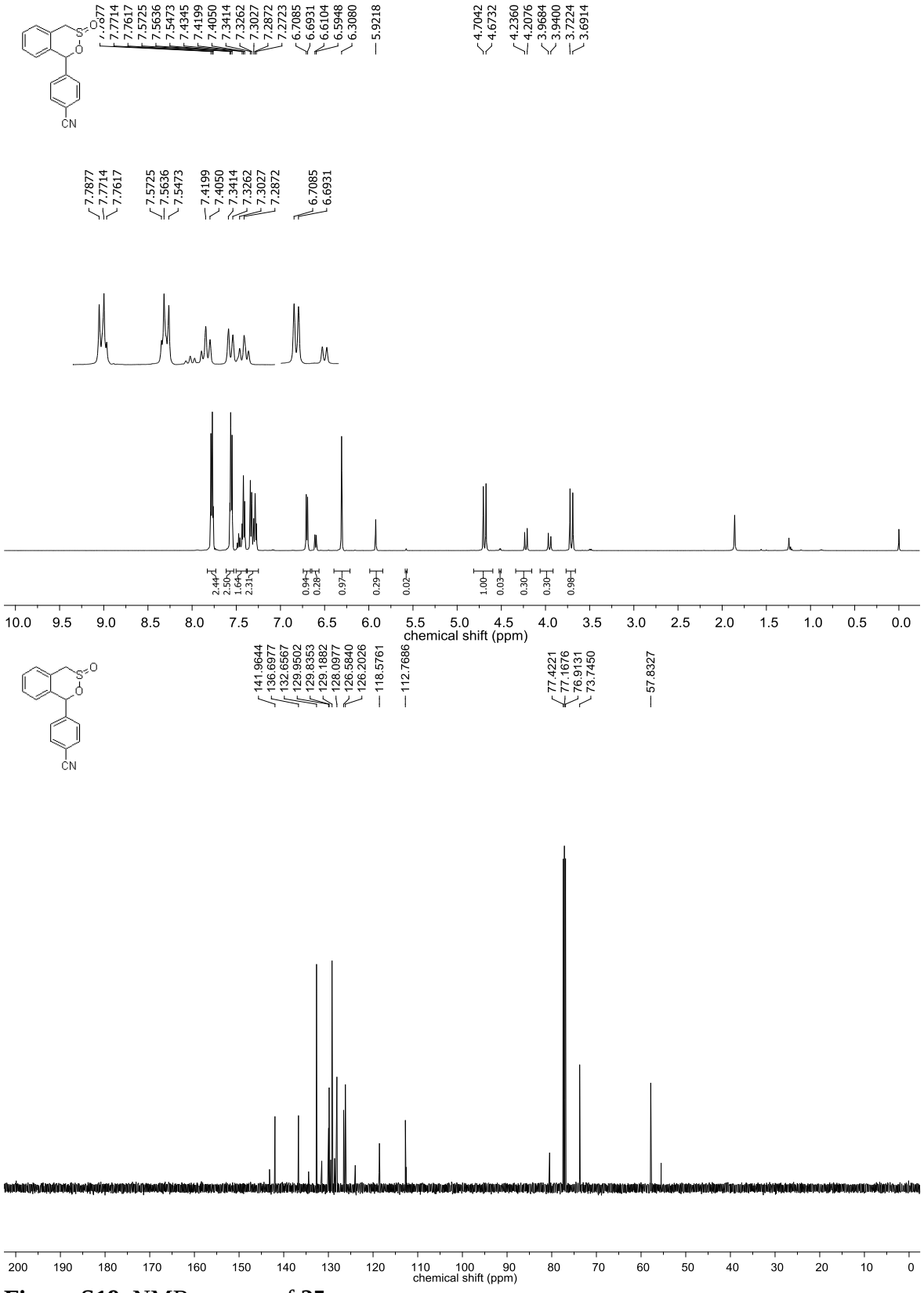


Figure S19. NMR spectra of **25**

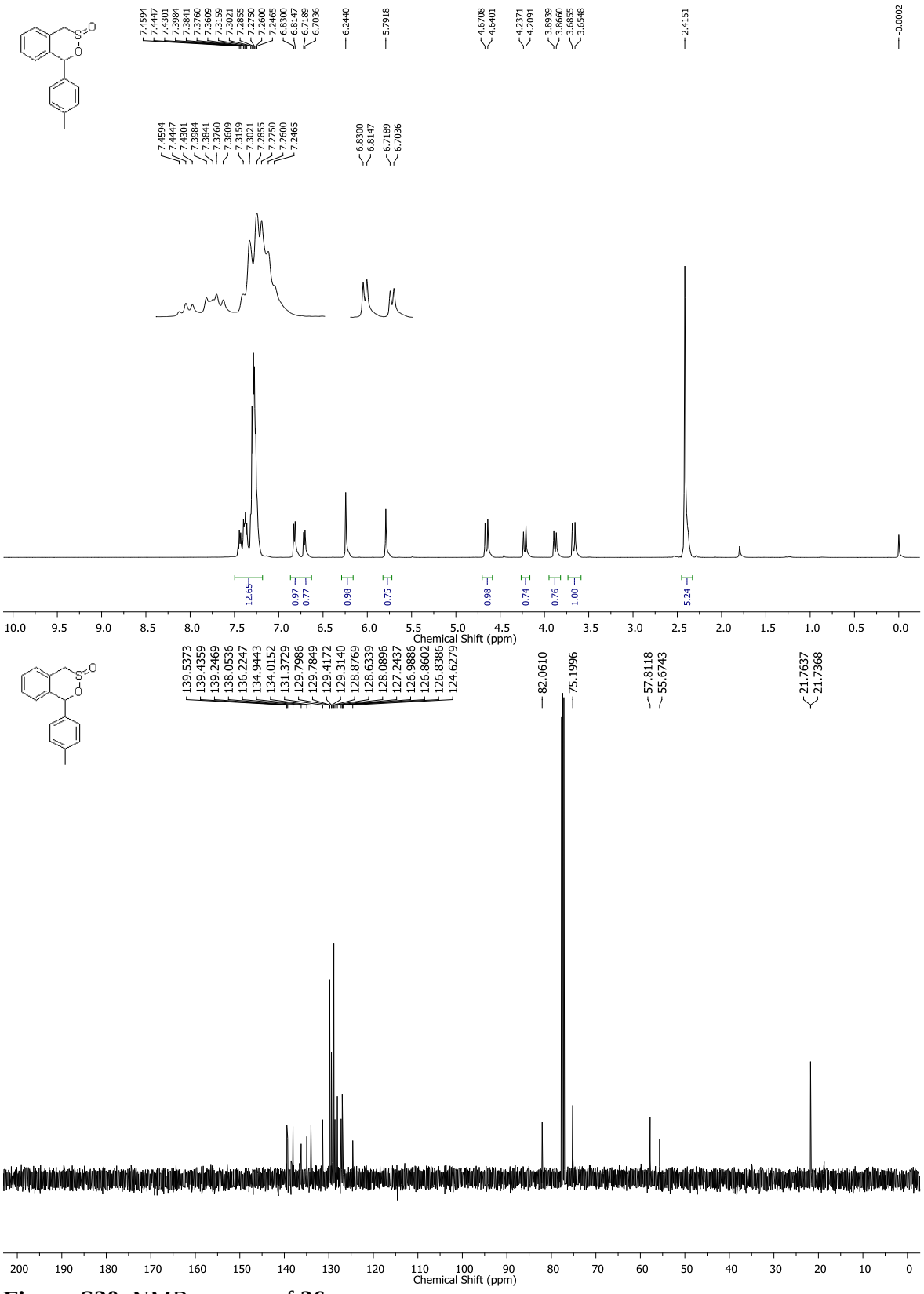


Figure S20. NMR spectra of **26**

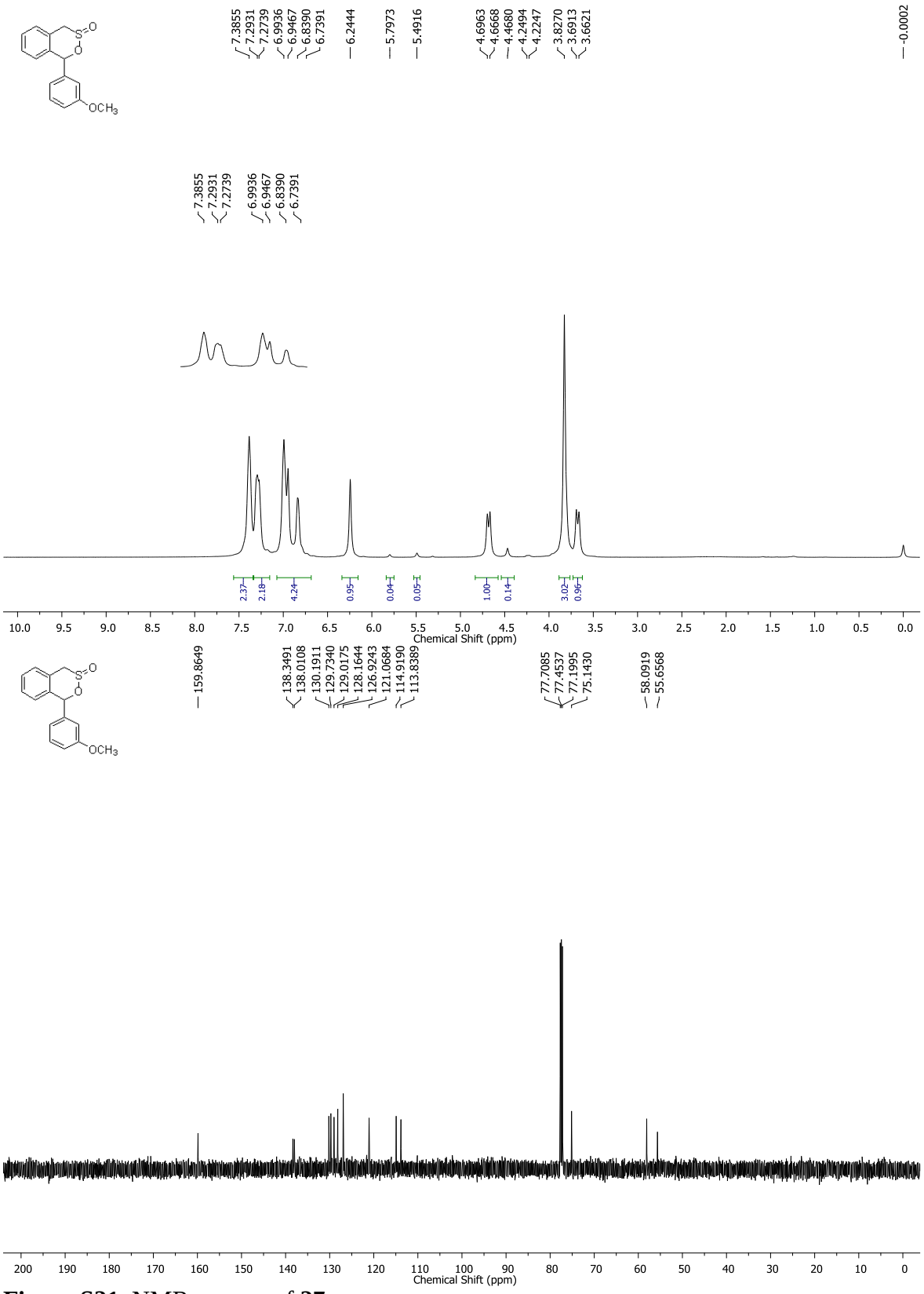


Figure S21. NMR spectra of **27**

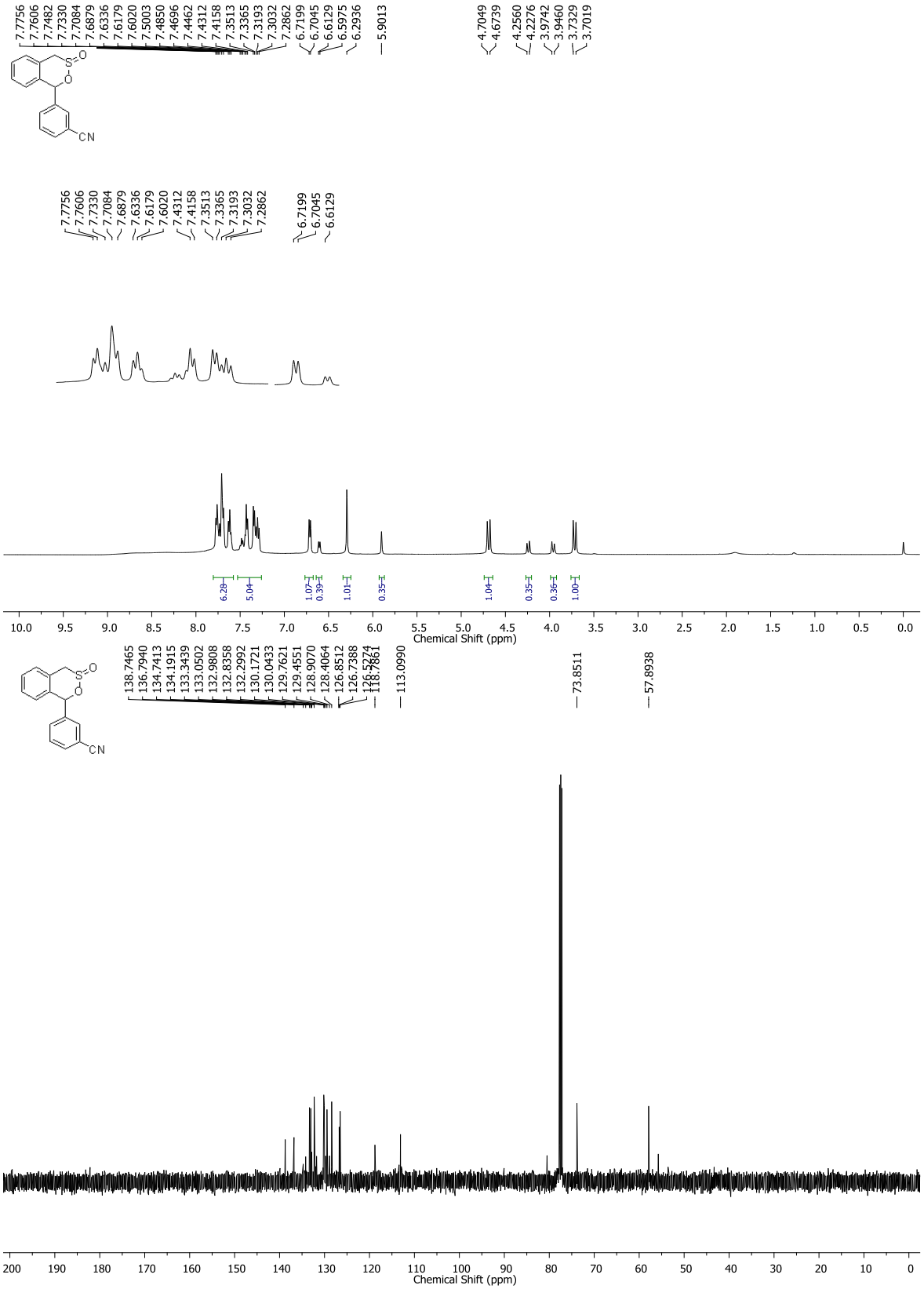


Figure S22. NMR spectra of **28**

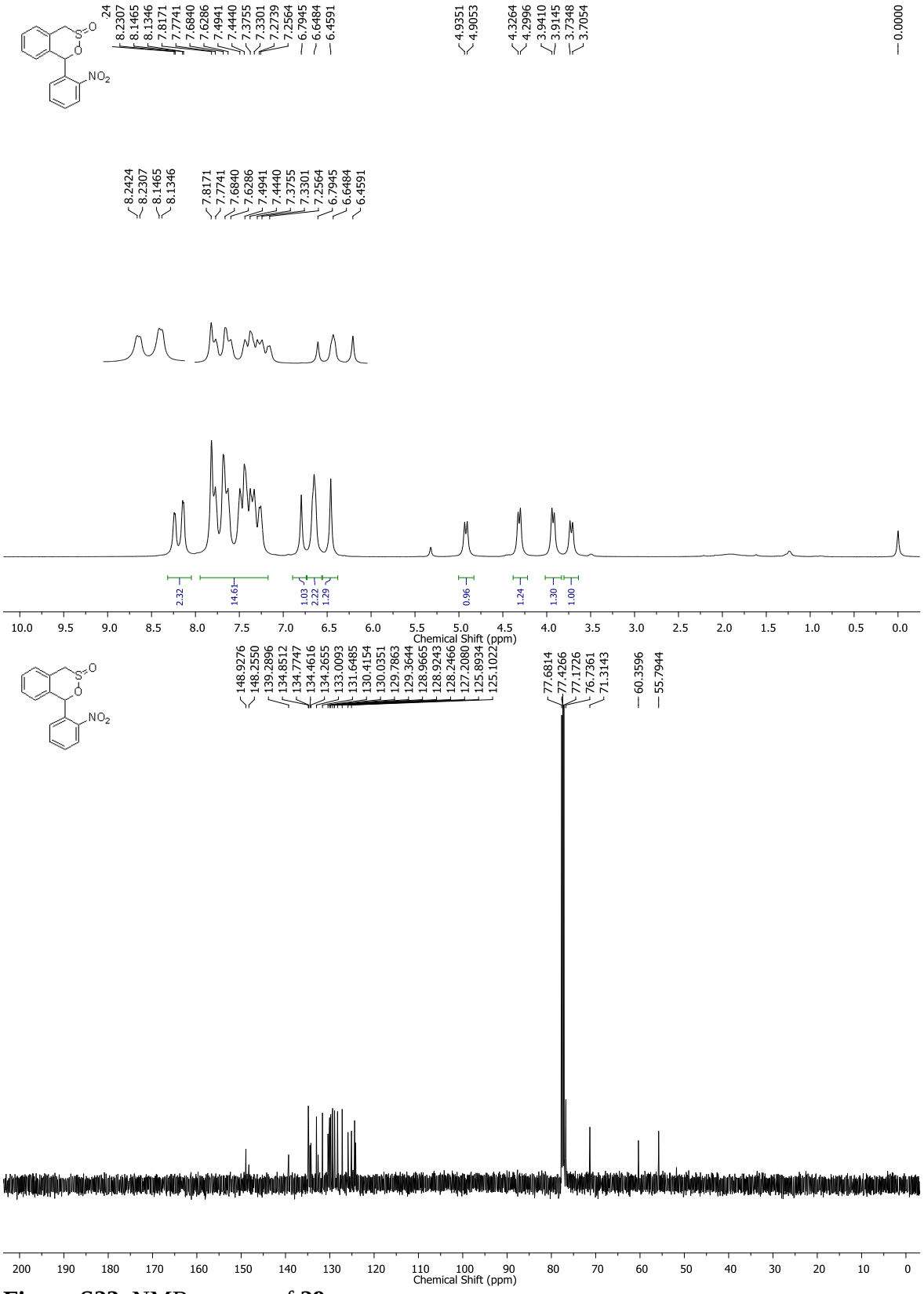
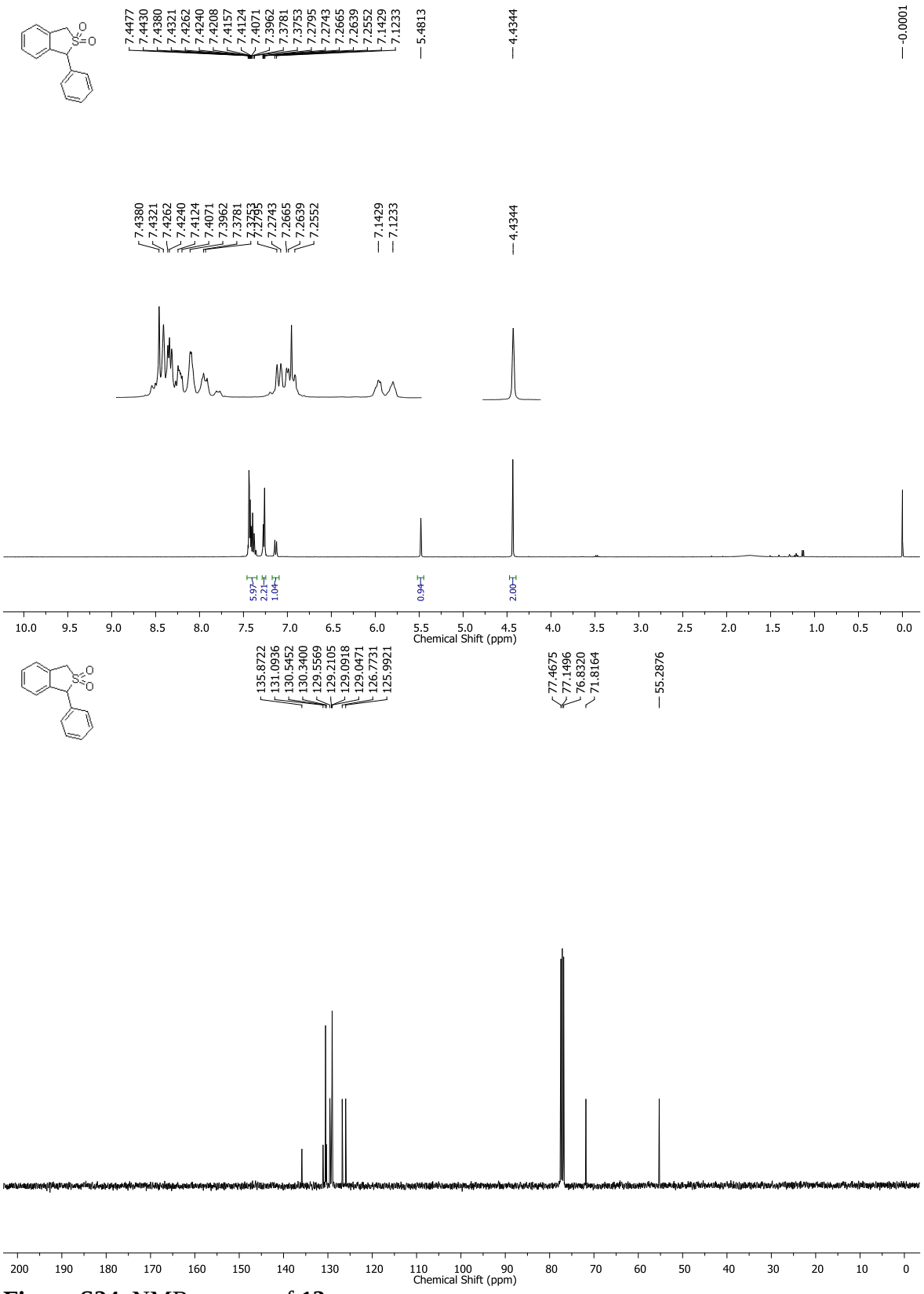


Figure S23. NMR spectra of **29**



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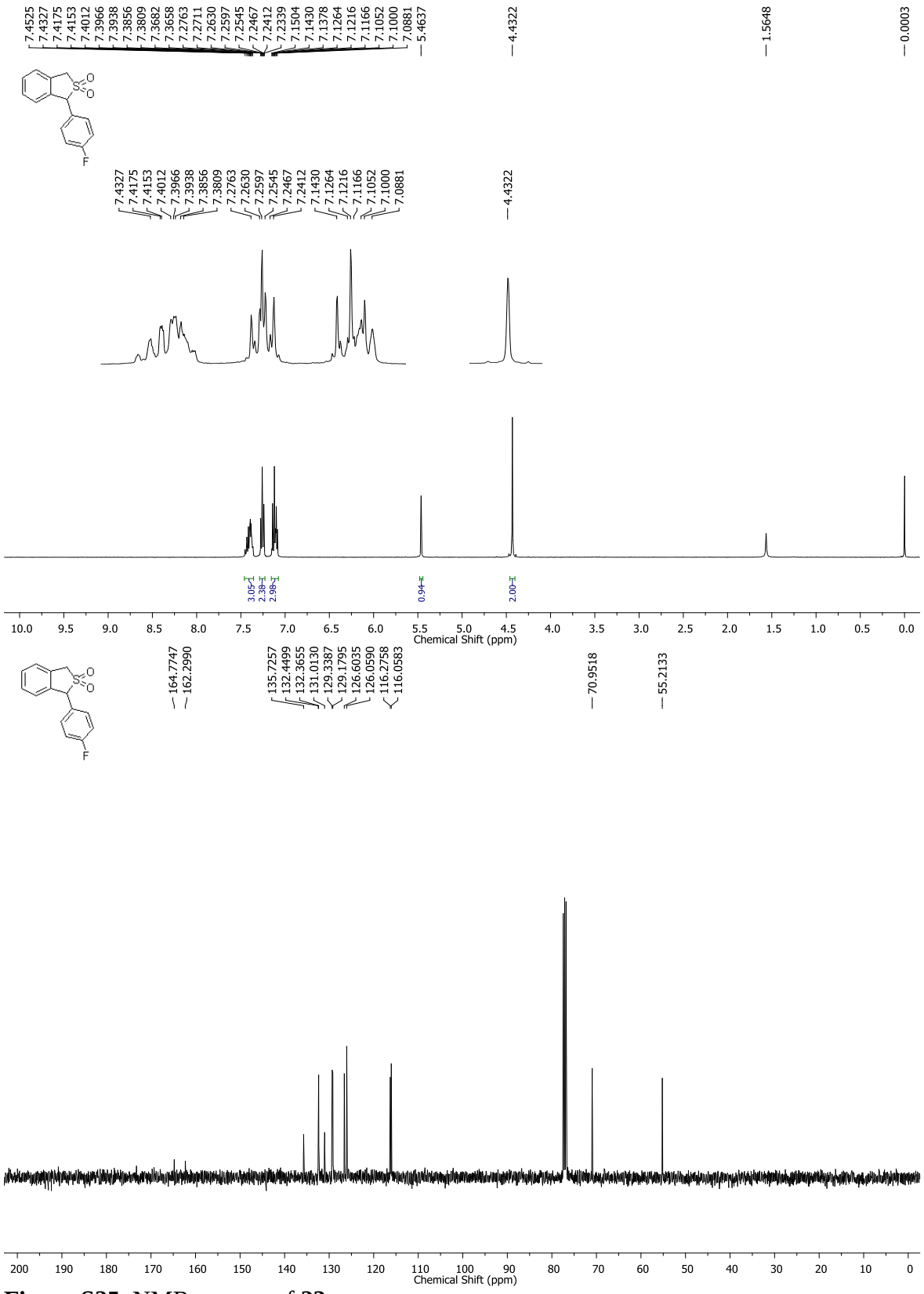
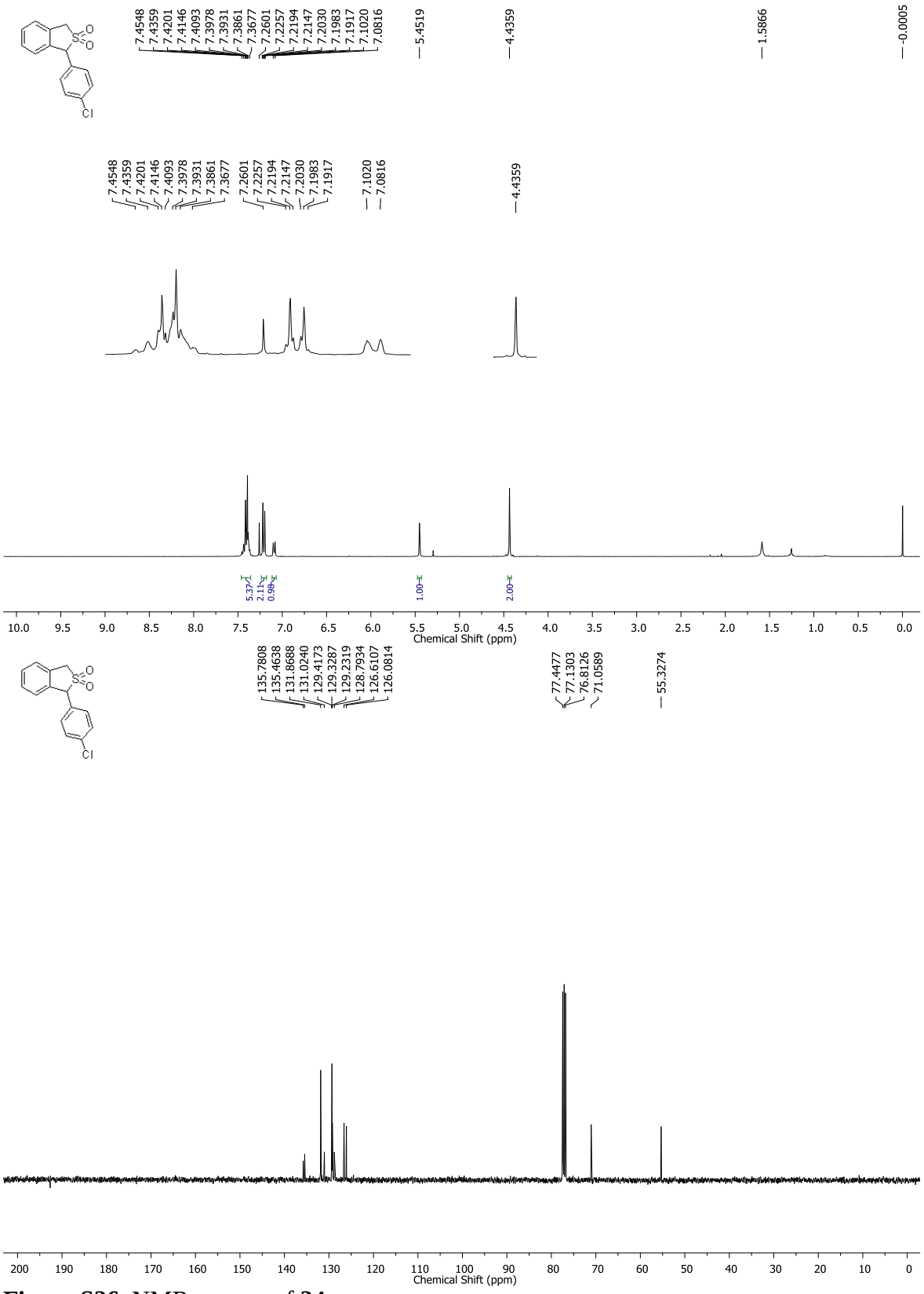


Figure S25. NMR spectra of **23a**



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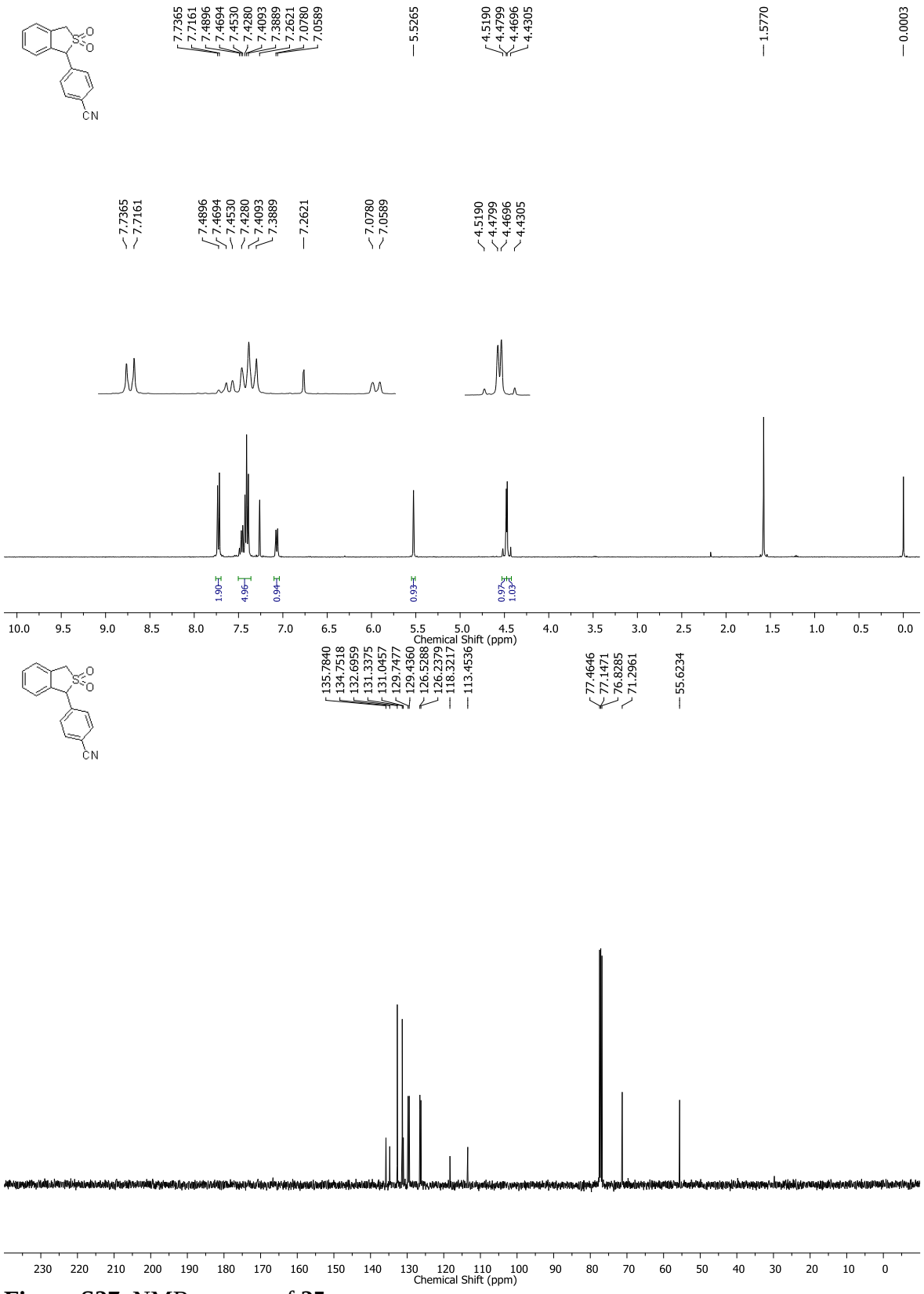

Figure S27. NMR spectra of **25a**

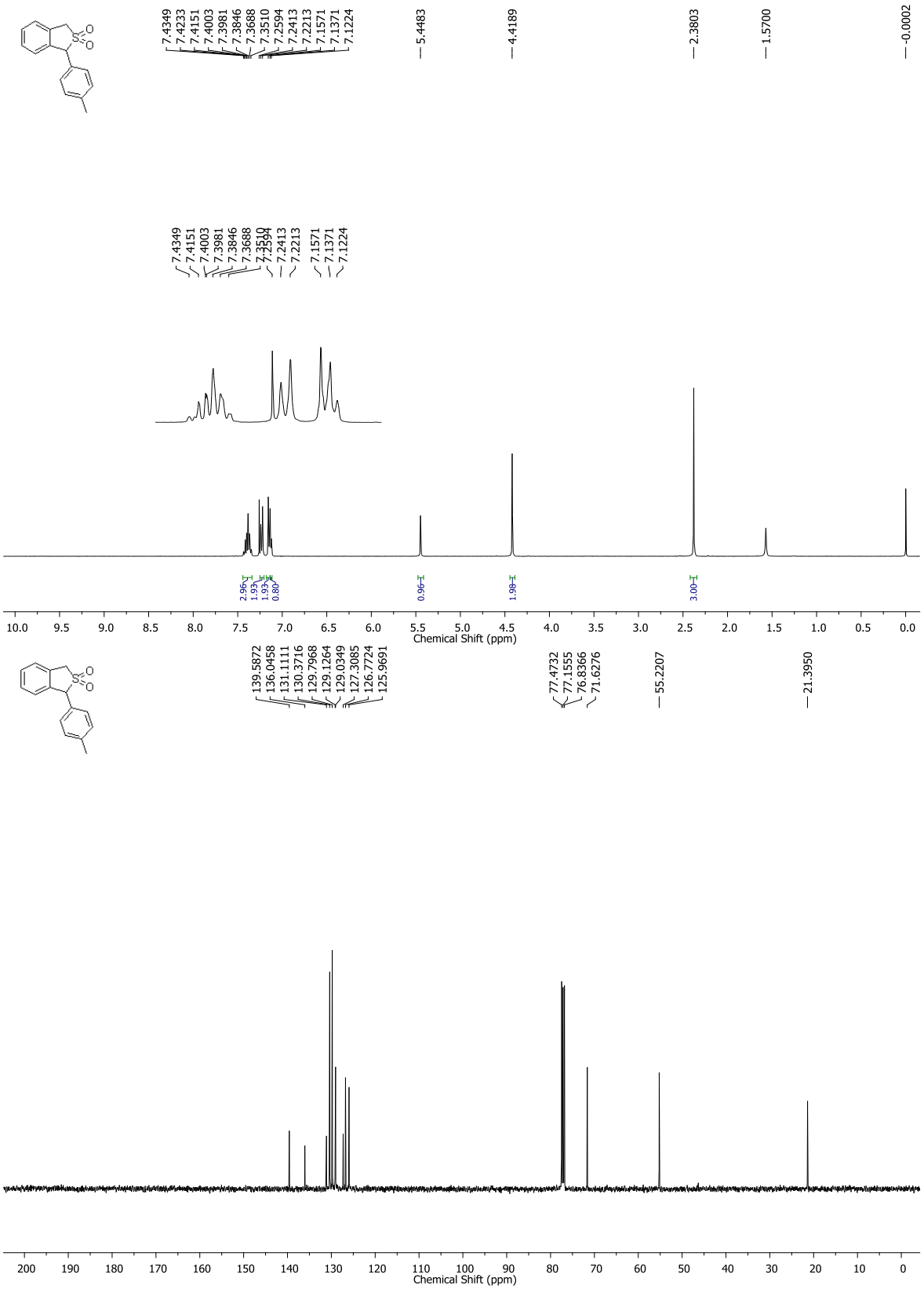

Figure S28. NMR spectra of **26a**

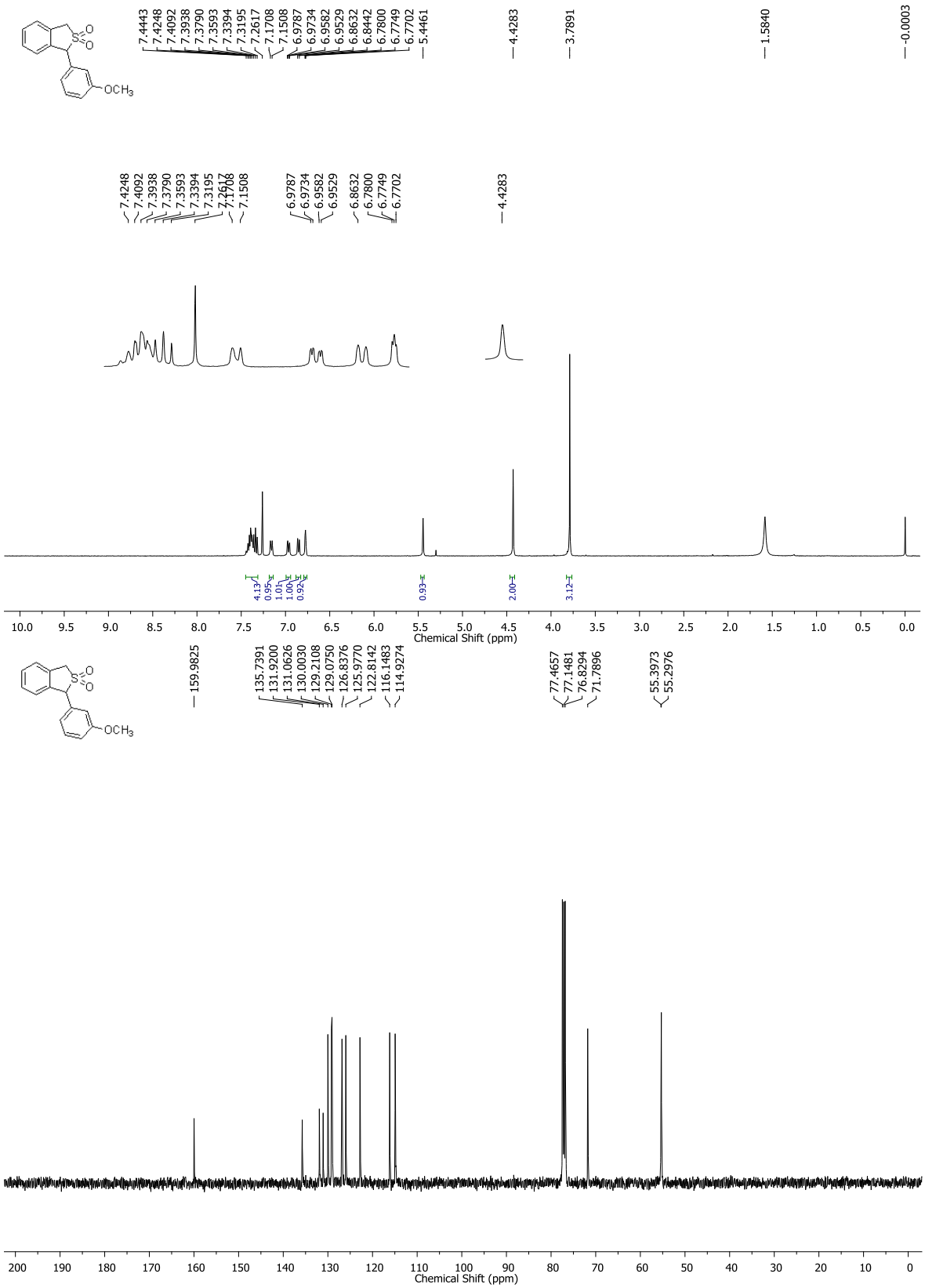

Figure S29. NMR spectra of **27a**

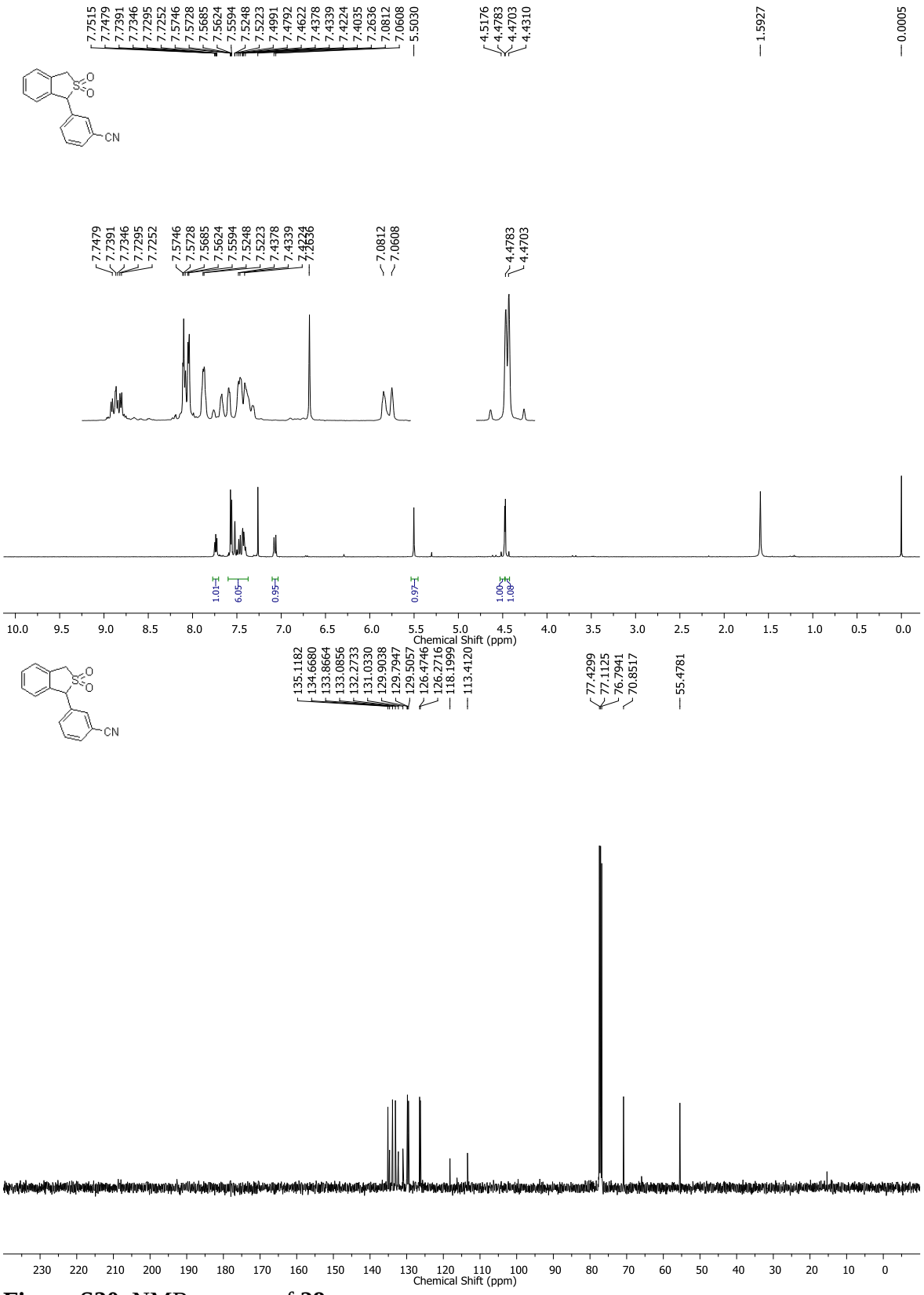

Figure S30. NMR spectra of **28a**

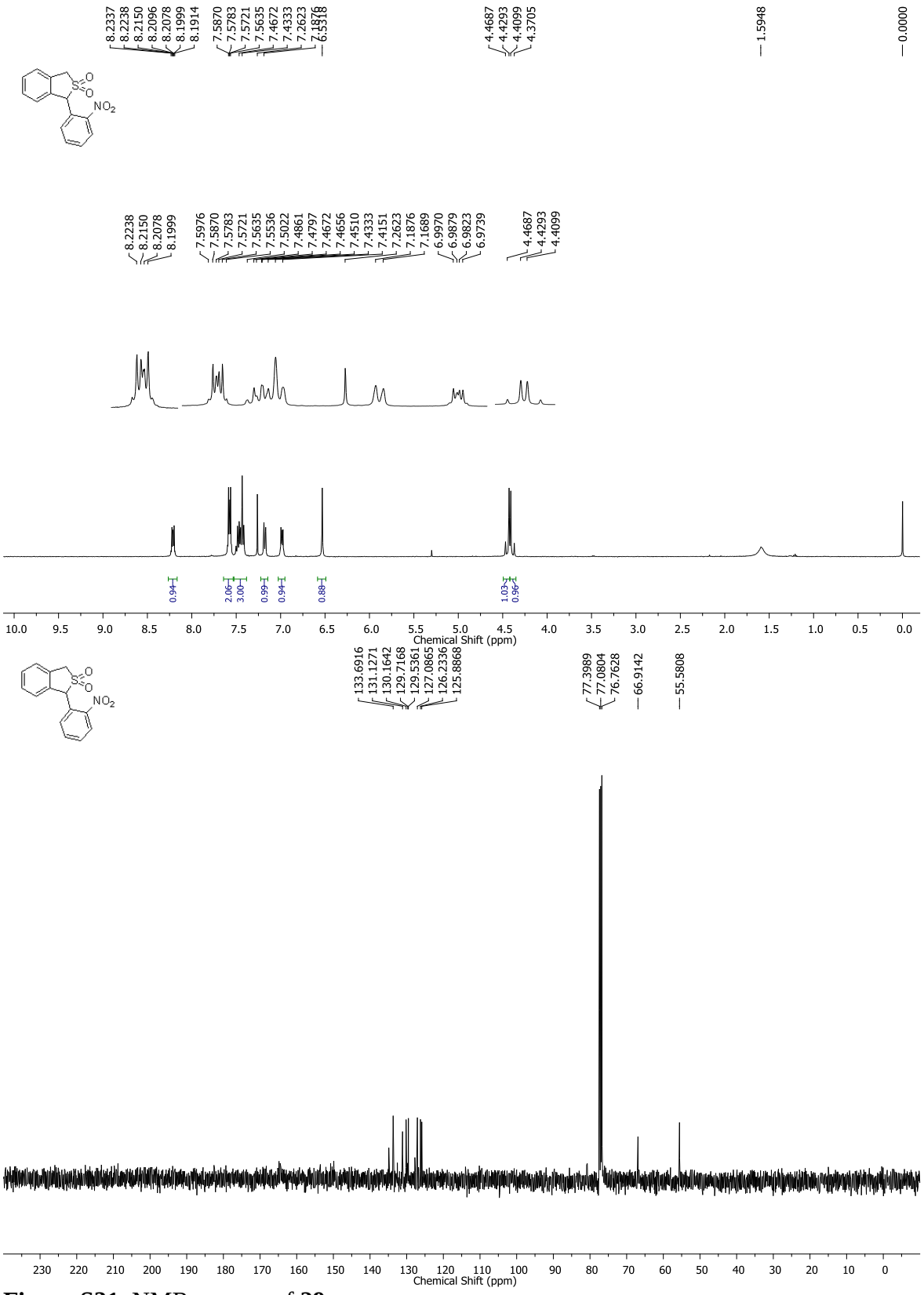

Figure S31. NMR spectra of **29a**

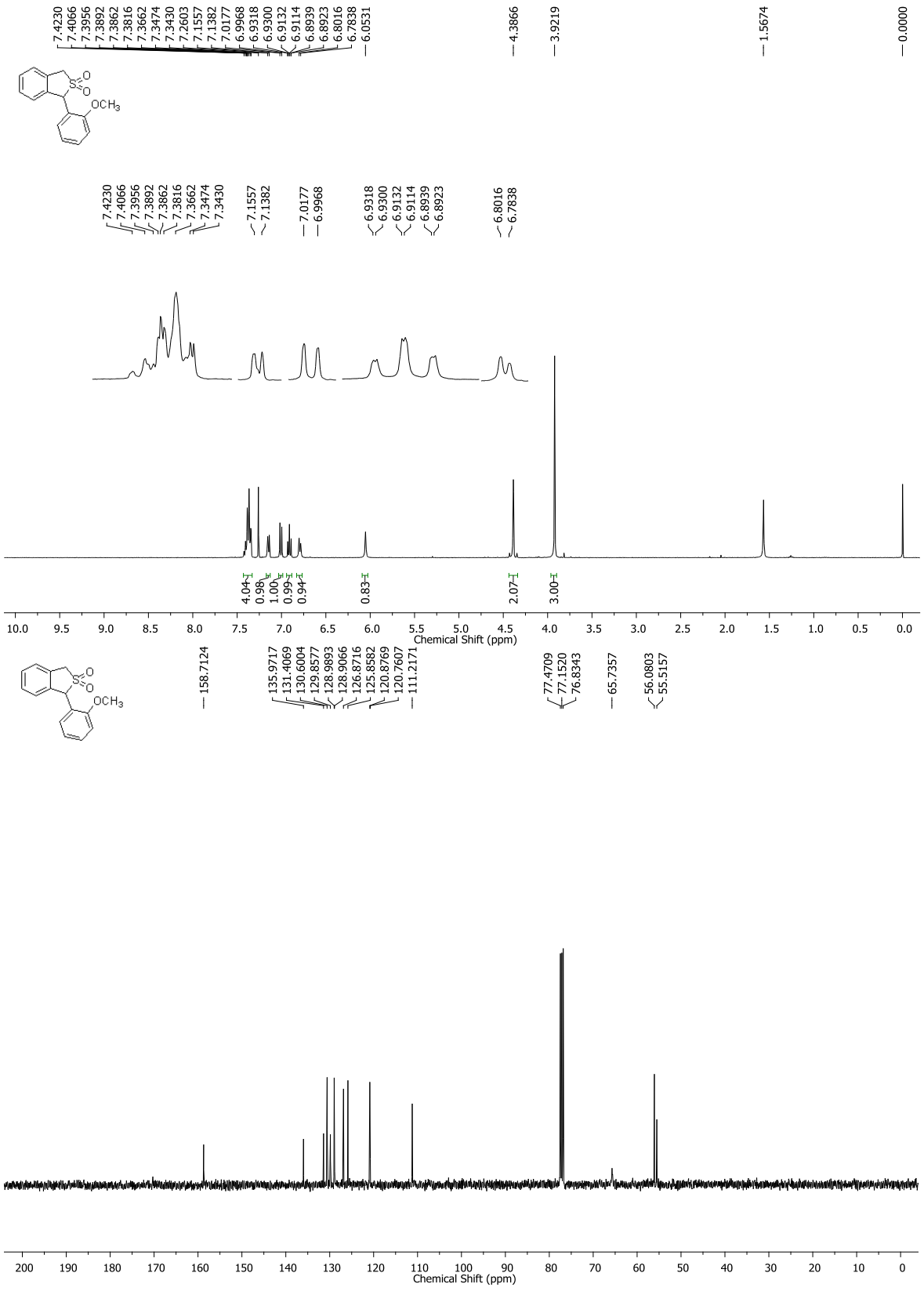

Figure S32. NMR spectra of **30**

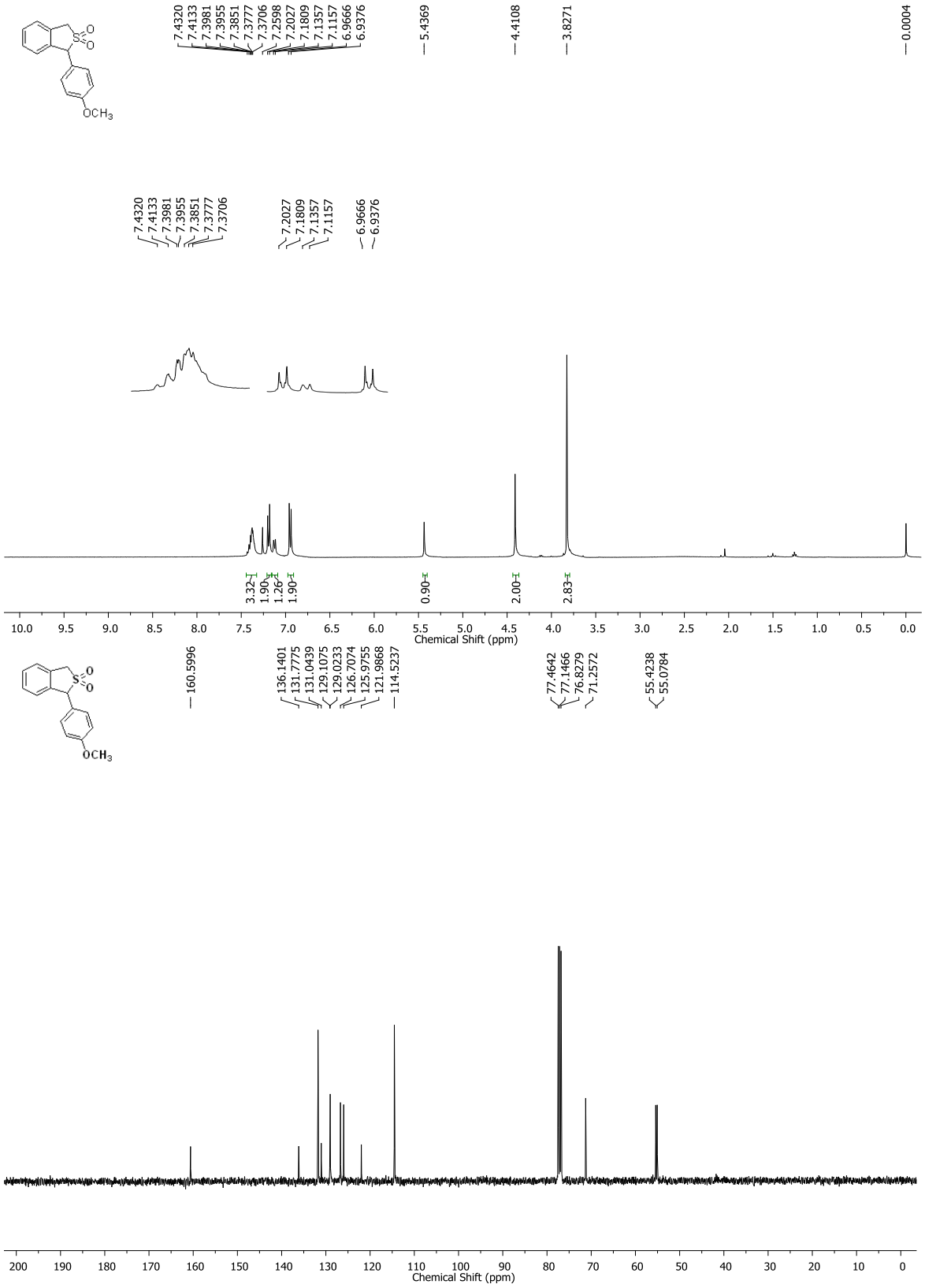

Figure S33. NMR spectra of **31**

2.6 References:

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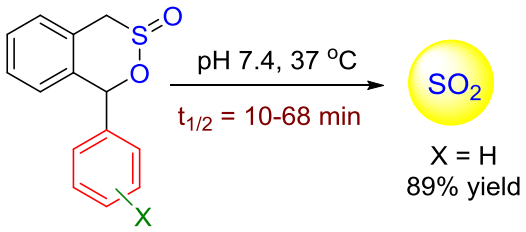
Chapter 3

Section A: Design, Synthesis and Evaluation of Thiol Activated Sources of Sulfur Dioxide (SO_2)

Chapter 3: Section A: Design, Synthesis and Evaluation of Thiol Activated Sources of Sulfur Dioxide (SO_2)

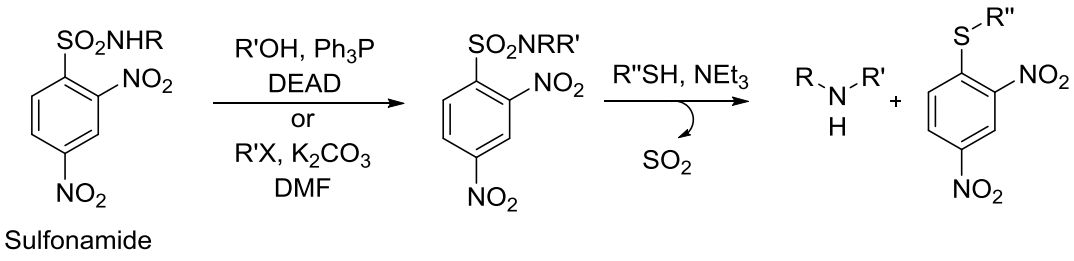
3A.1 Introduction: In Chapter 2, we prepared benzosultines as SO_2 donors (Scheme 1). Benzosultine showed similar activity comparable to that of standard source of SO_2 in DNA cleavage assay.[1]



Scheme 1. Benzosultines as temperature dependent sulfur dioxide (SO_2) donors.

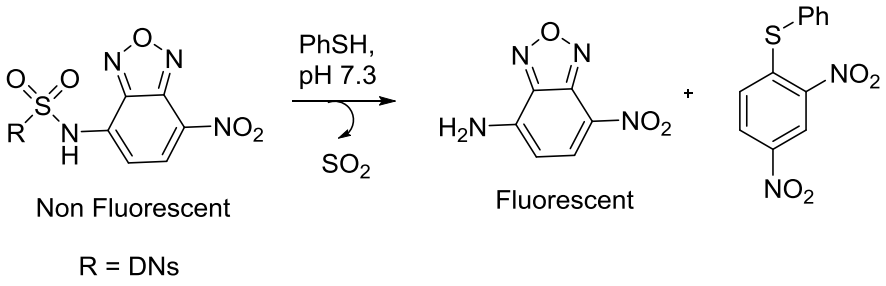
In the above strategy physiological temperature was used as a trigger to release SO_2. But benzosultine synthesis involves multiple steps and purification of last step is challenging. Benzosultine rearranges to corresponding sulfone in organic solvent upon standing at room temperature. Thus, stock solution of benzosultine must be used immediately after preparation. In order to overcome these limitations new organic sources of SO_2 with different trigger are necessary to understand biological role of SO_2.

Fukuyama and co-workers reported that sulfonamides prepared by reaction of primary amine with 2,4-dinitrobenzenesulfonyl chloride (DNsCl) can be used for synthesis of secondary amines (Scheme 2).[2,3] They performed Mitsunobu reaction using sulfonamide as nucleophile or alkylation of sulfonamide. Deprotection of 2,4-dinitrobenzenesulfonyl group (DNs group) by using various thiols in presence of mild base triethylamine in organic solvent gave corresponding secondary amine, a thioether and SO_2 (Scheme 2).



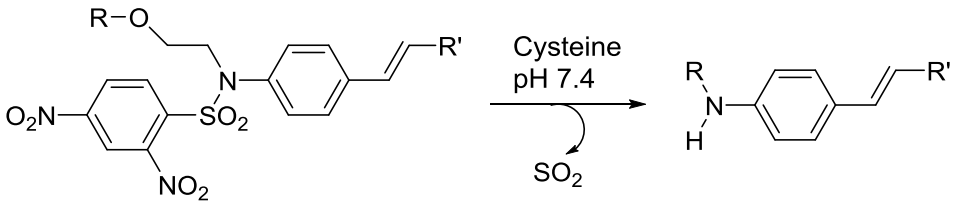
Scheme 2. Synthesis of secondary amine from 2,4-dinitrophenylsulfonamide.

This strategy was adapted by Wi *et al.* to prepare thiophenol sensing fluorescence turn on probe (Scheme 3).[4]



Scheme 3. 2,4-Dinitrophenylsulfonamide as thiophenol sensing probe.

The non emissive sulfonamide was prepared by treating fluorescent 7-nitrobenzo[c] [1,2,5]oxadiazol-4-amine (NBD amine) with 2,4-dinitrobenzenesulfonyl chloride (DNsCl), using triethylamine as a base. The phenolate generated at pH 7.3 cleaves DNs group to give fluorescent NBD amine and presumably SO_2 is released in the process. The same strategy was employed by Bouffard *et al.* to prepare thiol sensing fluorescence turn on probe (Scheme 4).[5]



Scheme 4. 2,4-Dinitrophenylsulfonamide as thiol sensing probe.

The 2,4-dinitrophenylsulfonamide react with biological thiols like cysteine and glutathione in physiological conditions. Application of this probe in live cell imaging was also shown suggesting that sulfonamides are cell permeable and react with biological thiols at physiological pH. As biological thiols are present at low millimolar concentration inside cells,[6,7] we decided to use thiol as trigger. Various 2,4-dinitrobenzenesulfonamides are prepared starting from primary or secondary amine and DNsCl (Figure 1). The ability of these sulfonamides to act as SO_2 donors under physiological conditions by treating with cysteine or glutathione will be evaluated.

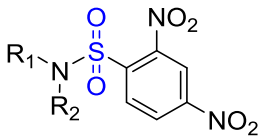
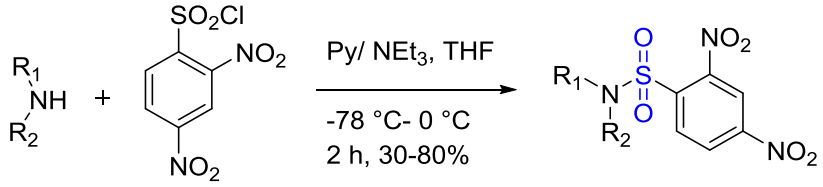


Figure 1. 2,4-Dinitrophenylsulfonamides scaffold.

3A.2 Results and discussion:

3A.2.1 Synthesis of 2,4-dinitrobenzenesulfonamides: We prepared various sulfonamides by reacting different amines with 2,4-dinitrobenzenesulfonyl chloride (DNsCl) in presence of triethylamine or pyridine as a base at -78°C (Scheme 5, Figure 2).[2,3]



Scheme 5. Synthesis of 2,4-dinitrobenzenesulfonamides.

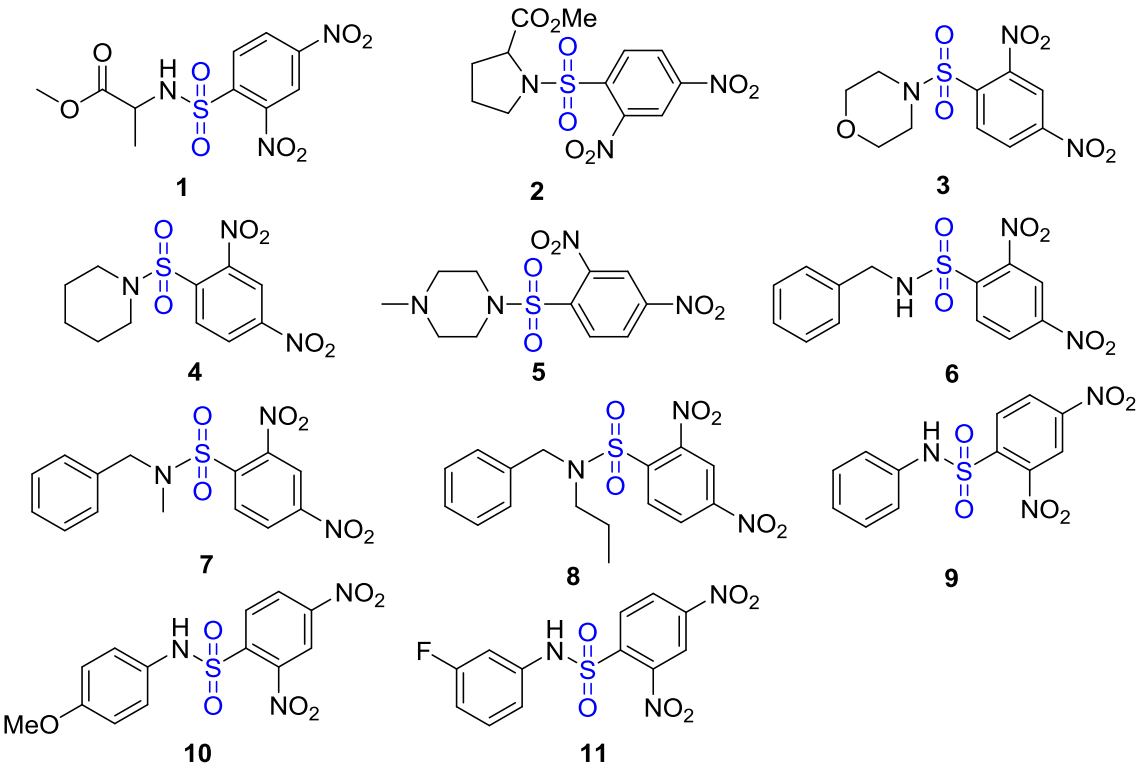
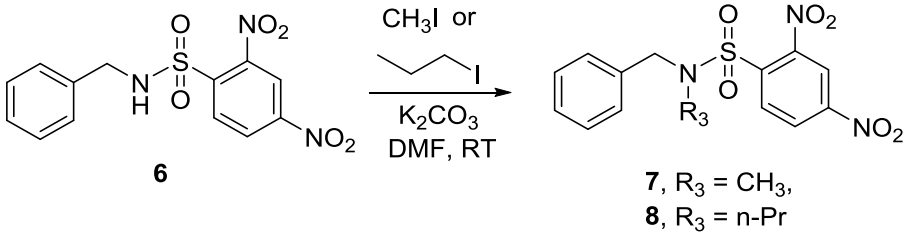


Figure 2. 2,4-Dinitrobenzenesulfonamides.

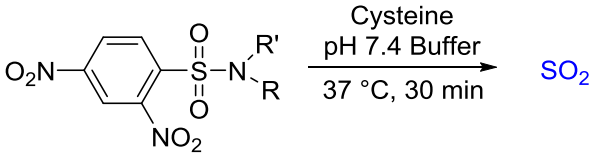
In order to study steric effects on SO_2 generation, compound **6** was alkylated to give compound **7** and **8**, by treating with alkyl halide and K_2CO_3 (Scheme 6).



Scheme 6. Alkylation of 2,4-dinitrobenzenesulfonamides.

3A.2.2 Cysteine-mediated decomposition of 2,4-dinitrobenzenesulfonamides in physiological condition to generate SO_2: Cysteine-mediated decomposition of compounds **1**−**11** in pH 7.4 buffer was carried out. SO_2 quantification was done as sulfite (SO_3^{2-}) by using ion chromatograph equipped with ion conductivity detector (Table 1).[8]

Table 1. Sulfur dioxide yields



Entry	Compd	% SO_2 (30 min)
1	**1**	93
2	**2**	83
3	**3**	96
4	**4**	91
5	**5**	88
6	**6**	100
7	**7**	100
8	**8**	88
9	**9**	55
10	**10**	79
11	**11**	24

100 µM of compound was treated with 1 mM cysteine in pH 7.4 phosphate buffer (30% ACN/PB) and SO_2 was quantified as SO_3^{2-} by ion chromatograph equipped with conductivity detector.

The compounds **1-5** were found to be excellent sources of SO_2 with maximum yields of SO_2 ranging from 83 to 96 % (Table 1, entry 1-5). The benzyl amine derivatives (Table 1, entries 6 and 7) gave 100% SO_2 after 30 minutes. The aniline derivative gave 55% SO_2 yield (Table 1, entry 9), whereas 4-methoxy aniline derivative **10** gave 79% yield after 30 minutes (Table 1, entry 10). These results suggest electron donating group on aniline increases the rate of reaction. The 3-F-aniline derivative gave 24% of SO_2 (Table 1, entry 11), implying mild electron withdrawing group slows down the rate of reaction.

3A.2.3 Kinetics of decomposition of 2,4-dinitrobenzenesulfonamides: The kinetics of decomposition of compound **8-11** was studied by following reaction after every 30 minutes (Figure 3). SO_2 release during cysteine-mediated decomposition of **8-11** followed pseudo-first-order kinetics with half-lives ranging from 4.6 to 63 min (Table 2).

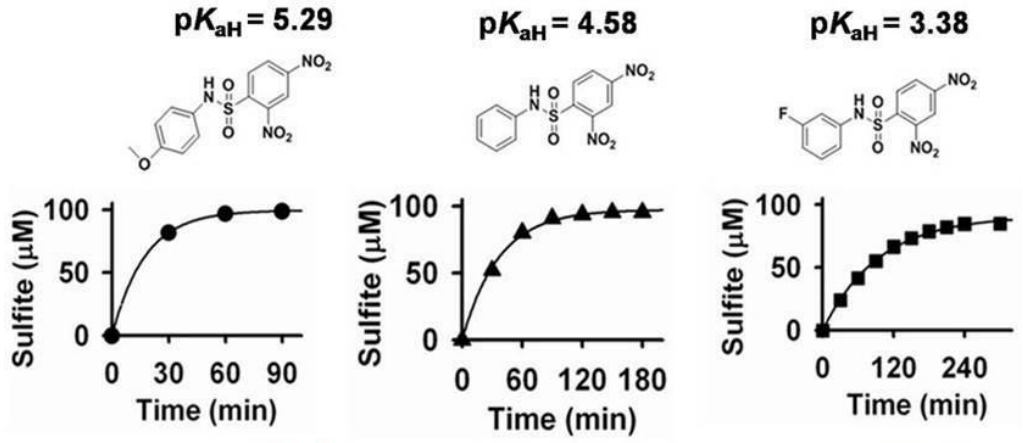


Figure 3. Kinetics of decomposition of 2,4-dinitrobenzenesulfonamides

Table 2. The maximum SO_2 yield, pK_{aH} of amines and $t_{1/2}$

Entry	Compd	k (min^{-1})[a]	$t_{1/2}$(min)[b]	Max. SO_2 yield (μM)[c]	pK_{aH} of amine[d]
1	**6**	*e*	2[f]	100	9.34
2	**7**	*e*	4[f]	100	9.58
3	**8**	0.1517	4.6	96	9.68
4	**9**	0.0273	25	94	4.64
5	**10**	0.0575	12	97	5.29
6	**11**	0.0106	63	86	3.38

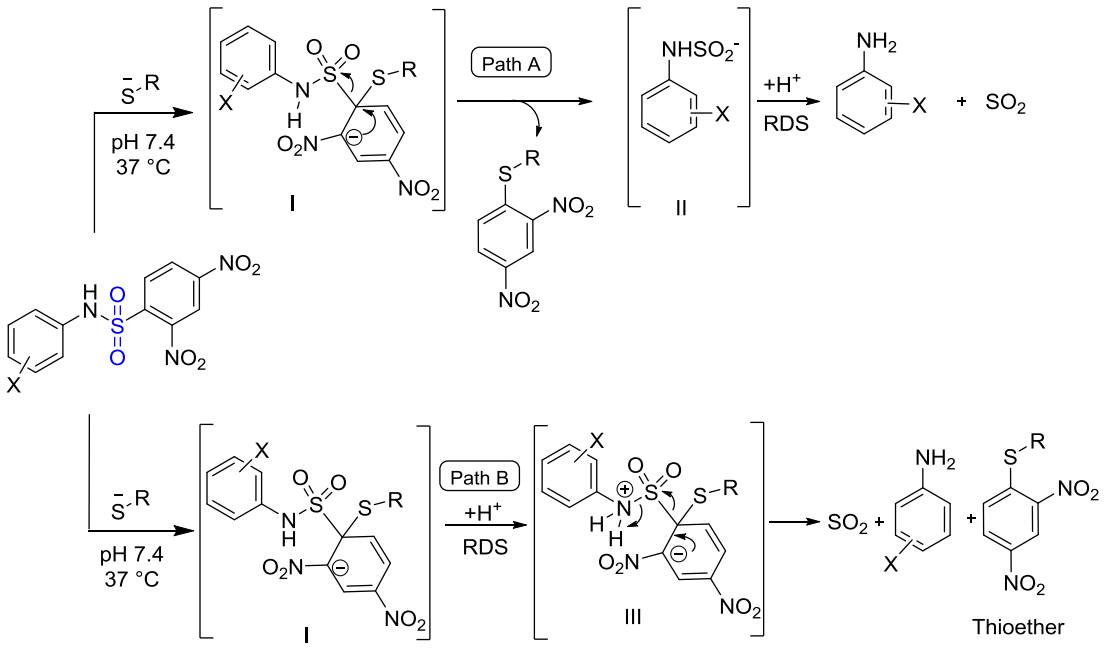
[a]Rate analysis of sulfite release from the compound (100 μM) in the presence of cysteine (10 equiv) in pH 7.4 phosphate buffer (20 mM). [b]Half-life estimated from rate constants. [c]Maximum amount of sulfur dioxide generated during the reaction with no further increase

in total sulfur dioxide; values reported are a sum of sulfite and sulfate (minor). HPLC analysis showed complete disappearance of 2,4- dinitrophenylsulfonamide. [d]Values for the amine without a DNs group. [e]Accurate rate constant could not be determined. [f]Approximate half-life estimated based on yields of sulfur dioxide.

We found the $t_{1/2}$ for 4-methoxy aniline derivative **10** is 12 min, for aniline derivative **9** is 25 min and for 3-F-aniline derivative is 63 min. For **6** and **7**, under the assay conditions, accurate determination of rate constant (k) for SO_2 formation was not possible; the $t_{1/2}$ for **6** was estimated to be 2 min; and for **7**, $t_{1/2}$ was 4 min (Table 2). These $t_{1/2}$ values can be correlated with pK_{aH} of amine, higher the pK_{aH} lower the $t_{1/2}$ for reaction and faster the reaction (Table 2, entry 1-6). In case of benzyl amine derivatives, the $t_{1/2}$ is 2 to 4 min, due to higher pK_{aH} of benzyl amines.

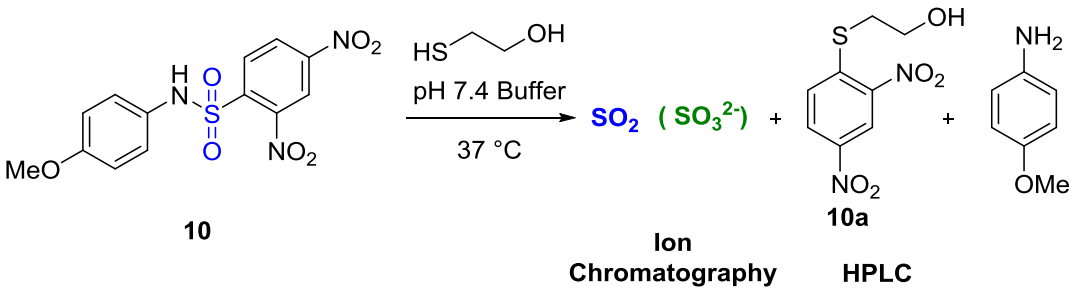
3A.2.4 Proposed mechanism for decomposition of 2,4-dinitrobenzenesulfonamides:

We proposed a possible mechanism for thiol-mediated sulfur dioxide release (Scheme 7). In path A, thiolate attack DNs group to produce intermediate **I**, which rearranges to give intermediate **II** with simultaneous release of thiol adduct (thioether). The intermediate **II** will rearrange to give SO_2 and amine by a slow decomposition step which involves protonation. Thus, rate of generation of SO_2 and thioether would be different.



Scheme 7. Proposed mechanisms of decomposition of 2,4-Dinitrobenzenesulfonamide to produce SO_2.

Path B is an alternative wherein nitrogen of intermediate **I** will undergo protonation, as decomposition of **I** will produce an anion (ArN^-), which may not have physiological relevance. It is likely that protonation of **I** would be the rate determining step to give **III**. The intermediate **III** will decompose to give SO_2 and thioether simultaneously along with the amine.



Scheme 8. Reaction of **10** with β-mercaptoethanol.

In order to understand which pathway was dominant, **10** was treated with β-mercaptoethanol (10 eq.) in pH 7.4 buffer at 37 °C. The reaction was followed by ion chromatography for SO_2 (SO_3^{2-}) generation and concurrently by HPLC for thioether, 2-((2,4-dinitrophenyl)thio) ethan-1-ol, **10a** (Scheme 8).

As discussed earlier, if path A has to occur, the rate of generation of sulfite and thioether is expected to be different. It is likely that the thioether **10a** will be generated before sulfite generation. In path B, simultaneous generation of sulfite and thioether is expected. Thus, rate of generation of sulfite and thioether will be similar. The kinetic study shows the rate of generation of sulfite and thioether **10a** is nearly similar (Figure 4). Thus, path B would be more favoured over path A.

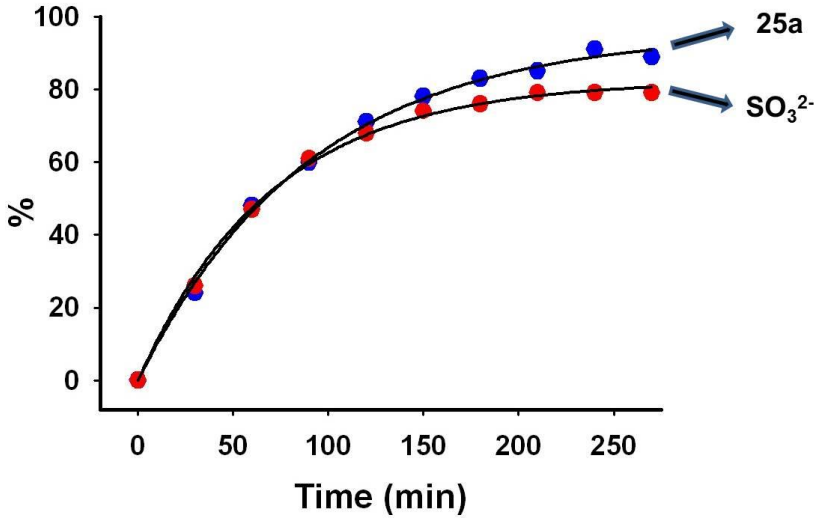


Figure 4. Time course of formation of **10a** and SO_3^{2-}.

3A.2.5 Synthesis and study of controlled compounds: Having established that DNs derivatives were SO_2 donors, we tested the role of the nitro group. To study the effect of removal of one or both the aryl nitro groups and replacement of the aryl ring with a methyl group, compounds **12-17** were prepared (Figure 5). None of the compounds were found to generate SO_2 when reacted with cysteine in pH 7.4 buffer at 37 °C. Thus, presence of 2,4-dinitrobenzene sulfonyl group is required for the sulfonamide in order to act as a SO_2 donor.

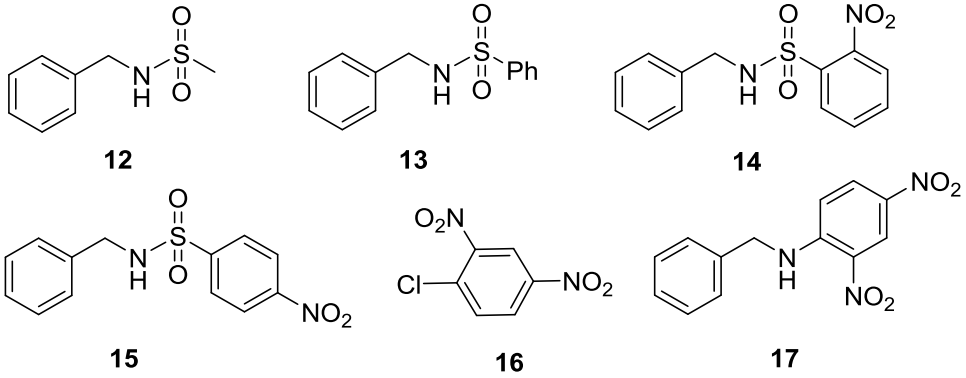


Figure 5. Control compounds synthesized for studying the effect of removal of one or both the aryl nitro groups and replacement of the aryl ring with a methyl group.

3A.2.6 Evaluation of SO_2 donors as antimycobacterial agents: Next, the possible use for these compounds as antimicrobial was evaluated. The target disease chosen was tuberculosis (TB) as it is a lethal infectious disease which is becoming a global threat. It affects millions of people each year with large number of fatalities.[9,10] Multidrug resistant (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are forms of tuberculosis caused by bacteria that are becoming resistant to some of the most effective anti-TB drugs. The attrition rate of new leads is becoming higher; hence there is an urgent need of newer strategies to target *Mtb*.

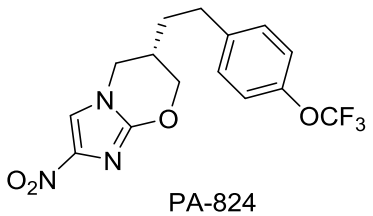


Figure 6. Structure of PA-824.

The nitro imidazole (6S)-2-nitro-6-[4-(trifluoromethoxy)benzyl]oxy-6,7-dihydro-5H-imidazo[2,1-b]-[1,3]oxazine (PA-824) is a lead tuberculosis drug candidate in phase III

clinical trials (Figure 6). PA-824 was recently demonstrated to be a deazaflavin-dependent nitroreductase-activated prodrug of reactive nitrogen species such as nitric oxide (NO).[10-12]

Table 3. Sulfur dioxide analysis during thiol-mediated decomposition, antimycobacterial activity, and calculated partition coefficients of 2,4-dinitrosulfonamides and related analogs.

Entry	Compd	SO_2 yield, 30 min (μM)[a]	MIC (μg mL^{-1})[b]	MIC (μM)	−*c*logP[c]
1	**1**	93	6.25	18.7	1.39
2	**2**	83	12.5	35	1.27
3	**3**	96	6.25	19.6	0.59
4	**4**	91	0.78	2.5	1.84
5	**5**	88	12.5	38	1.15
6	**6**	100	0.05	0.15	2.87
7	**7**	100	0.4	1.1	2.41
8	**8**	88	3.13	8.25	3.47
9	**9**	55	3.13	9.7	2.76
10	**10**	79	1.56	4.4	2.69
11	**11**	24	25	73	2.93
12	**12**	0	>50	>100	2.90
13	**13**	0	>50	>100	3.08
14	**14**	0	>50	>100	2.69
15	**15**	0	>50	>100	0.81
16	**16**	0	6.25	30.8	2.19
17	**17**	0	>100	>250	3.60
18	$BnNH_2$	0	6.25	30.8	2.19
19	BnNHMe	0	>100	>250	3.60
20	Isoniazid	-	0.05	0.37	0.67

[a]Sulfur dioxide as sulfite was quantified using an ion chromatograph equipped with a conductivity detector: yields are 30 min after treatment of compound (100 µM) with 10 eq. of cysteine in pH 7.4 phosphate buffer. In the absence of cysteine, sulfite could not be detected during several hours under the assay conditions. [b]Minimum inhibitory concentration (MIC) is the minimum concentration of the compound required to inhibit 99% of bacterial growth and was found against *Mycobacterium tuberculosis* $H_{37}R_v$ strain. [c]Calculated using Chembiodraw Ultra®. Data was provided by our collaborator Dr. Sriram from BITS Hyderabad, India.

The compounds **1−15** were screened for their antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}R_v$ and minimum inhibitory concentrations (MICs) were determined by our collaborators. In this assay, seven compounds were found to have MICs < 10 µg mL^{-1} (Table 3, entries 1−11). The 4-aniline derivative **11** showed MIC > 50 µM as it reacted slowly with $t_{1/2}$ of 63 min. Also control compounds (**12-15** and **17**) which are not source of SO_2 showed MIC > 50 µg mL^{-1}. These results clearly indicate a correlation between the analogues ability to release sulfur dioxide within 30 min and its *Mtb* inhibitory potency. The best *Mtb* inhibitor in this series was the benzylamine derivative **6** with MIC of 0.05 µg mL^{-1} (0.15 µM), which was better than the MIC of isoniazid (0.05 µg mL^{-1}, 0.37 µM) determined under similar conditions (Table 3). Hence, further studies conducted were focused on understanding the mechanisms of efficacy of this compound.

Reaction of compound **6** with a number of biologically relevant nucleophiles such as amino acids and nucleosides in pH 7.4 buffer was done, a nearly quantitative recovery was observed (HPLC analysis), suggesting that **6** was selectively reactive to thiols under physiological pH conditions.

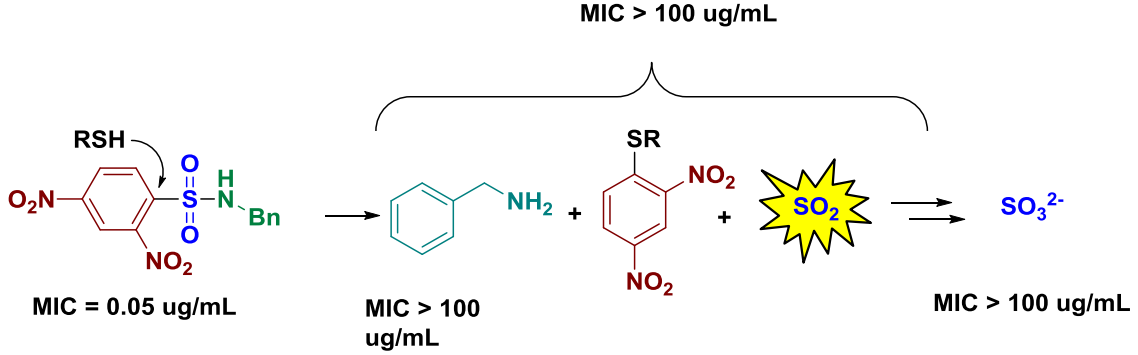


Figure 7. Decomposition products of **6**.

The decomposition of **6** after reaction with thiol generates sulfur dioxide, benzylamine, and the arylated thiol (Figure 7). Benzylamine, a decomposition product of **6** did not show a significant inhibition of growth of *Mtb* at 100 μg mL^{-1} (Table 3, entry 18); a similar observation for N-methylbenzylamine was recorded (Table 3, entry 19). Sodium sulfite was inactive against *Mtb* at 100 μg mL^{-1}. To understand if combinations of decomposition products were responsible for *Mtb* inhibitory activity, a mixture of cysteine and **6** in pH 7.4 buffer approximately 2 h post mixing was tested at 2 μg mL^{-1}; this mixture was found to be inactive against *Mtb*. These results demonstrated that intracellular formation of sulfur dioxide (and not sulfite) contributes to the *Mtb* inhibitory activity of **6**.

To test the selectivity of SO_2 inhibitory activity toward *Mtb*, a cell viability assay with **6** was conducted using human embryonic kidney 293 cells (HEK) cell lines in collaboration with Dr. Badireenath (NISER, Bhubaneswar). The IC_{50} for **6** was determined as 7 μM (See experimental section). Based on the MIC and IC_{50}, **6** was nearly 50-fold selective in inhibitory activity (SI = 47) toward *Mtb* over human embryonic kidney cells.

In addition to generation of SO_2, the reaction of **6** with thiols could also affect cellular redox balance by mycothiol depletion in *Mtb*. The possible role of thiol-depletion in the observed efficacy of **6** was determined by testing 1-chloro-2,4- dinitrobenzene (**16**, Figure 6) against *Mtb*; the MIC of **16** was found as 6.25 μg mL^{-1} (Table 3, entry 16). The analogue of **6** without a sulfonyl group **17** (Figure 5), which is unreactive towards thiols showed no *Mtb* inhibitory activity (Table 3, entry 17). These results support a role for thiol depletion and protein S-arylation as a contributor to the observed *Mtb* activity of **6**.

3A.2.7 Proposed Mechanism of *Mtb* Inhibition:

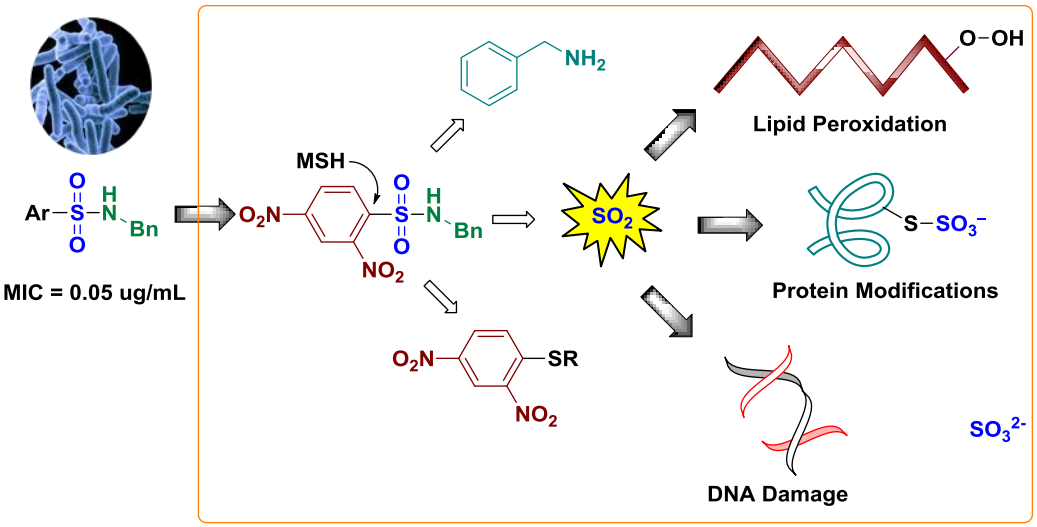


Figure 8. Proposed Mechanism of *Mtb* Inhibition

In addition to thiol depletion, possible mechanisms of antimycobacterial activity of **6** include ability of SO_2 to induce stress by affecting cellular redox equilibrium and causing damage to biomacromolecules such as lipids, proteins and DNA (Figure 8).[13-15]

3A.2.8 Structure-activity relationship: We prepared a library of structural analogues of lead compound **6** in order to further understand the relationship between thiol mediated sulfur dioxide generation and *Mtb* inhibitory activity. Analogues with comparable clogPs to **6** were synthesized starting from various benzyl amines, anilines and DNsCl. (Figure 9). Various benzylamine analogues **18-21** (Figure 9) with electron donating and electron withdrawing groups, aniline derivatives **22-24** were also prepared. In order to study the effect of changing sterics around the nitrogen bearing the DNs group, we prepared **25** and **26** with α-methyl substituent (Figure 9). The 1-phenyl-2-aminoethyl derivative **27** was prepared by treatment of the corresponding amine with DNsCl (Table 4, entry 11). Finally, the alkylated derivatives **28-31** were prepared by treatment of the corresponding alkyl halide with a suitable 2,4-dinitrosulfonamide (Figure 9).

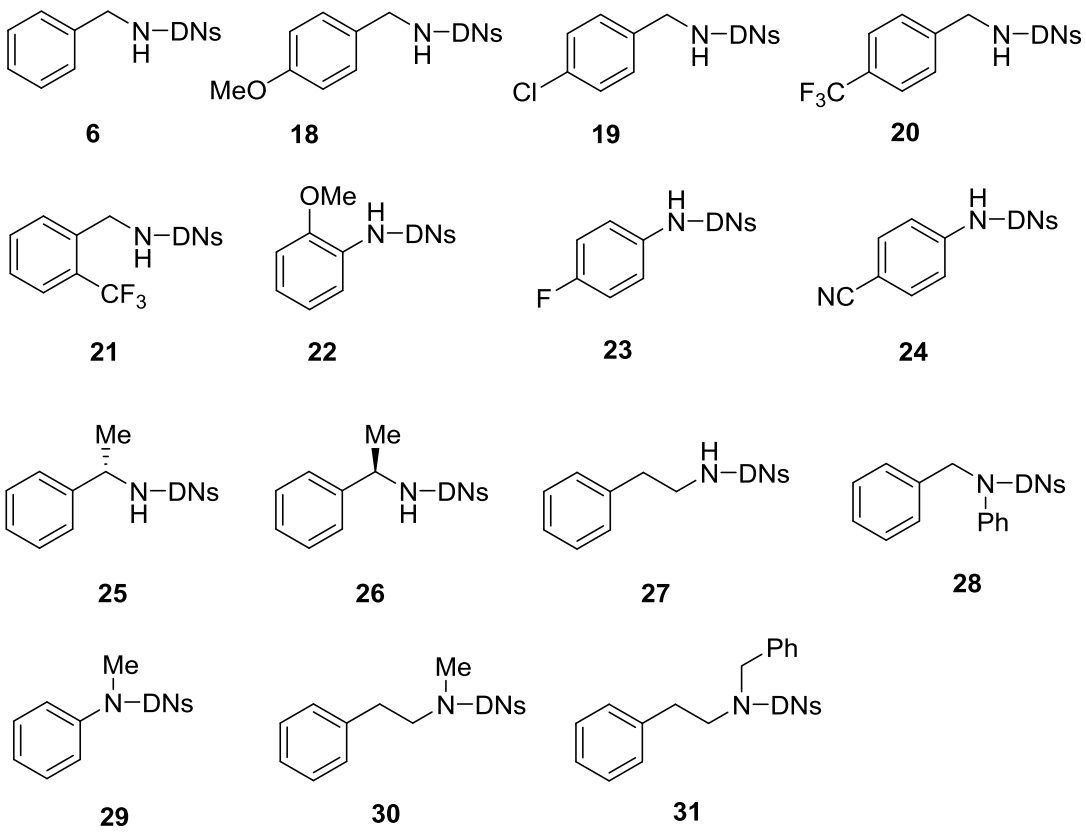


Figure 9. Structure-activity relationship

3A.2.9 SO_2 yields and Anti-mycobacterial activities of sulfonamides prepared for studying SAR: Cysteine mediated decomposition study of this library of compounds in pH 7.4 buffer was conducted and yields of SO_2 after 5 min and 30 min were determined.

Table 4. Synthesis, calculated partition coefficients (c log P), thiol-mediated sulfur dioxide generation and anti-mycobacterial activities of 2,4-dinitrosulfonamides prepared in this study and related compounds

Entry	Compd	- *c*logP[a]	SO_2 yield, 5 min (μM)[b]	SO_2 yield, 30 min (μM)[b]	pK_{aH}[c]	MIC (μg mL^{-1})[d]	MIC (μM)
1	**6**	2.87	83	100	9.51	0.05	0.15
2	**18**	2.87	74	84	9.50	0.25	0.68
3	**19**	3.58	77	96	9.44	0.4	1.07
4	**20**	3.75	75	79	9.45	0.4	0.98
5	**21**	3.75	81	84	9.23	1.56	3.84
6	**22**	2.69	57	86	4.42	3.13	8.85
7	**23**	2.92	18	55	3.80	6.25	18.31
8	**24**	2.24	6	5	1.63	>50	>100
9	**25**	3.17	80	97	9.73	1.56	4.44
10	**26**	3.17	78	94	9.73	1.56	4.44
11	**27**	3.19	76	100	9.79	0.4	1.13
12	**28**	3.87	44	94	3.92	1.56	4.44
13	**29**	2.10	89	94	5.64	0.78	2.31
14	**30**	2.74	79	84	10.13	0.78	2.13
15	**31**	4.50	37	75	9.88	3.13	7.09
16	Isoniazid	-0.66		-	-	0.05	0.37
17	Ethambutol	2.08		-	-	1.56	7.63
18	Pyrizinamide	-0.67		-	-	6.25	50.8

[a] Calculated using Chembiodraw Ultra.[b] Sulfur dioxide as sulfite was quantified using an ion chromatograph equipped with a conductivity detector: yields are 5 min and 30 min after treatment of compound (100 µM) with 10 equiv of cysteine in pH 7.4 phosphate buffer.[c]Values are for the corresponding amine and were calculated using Marvinsketch 5.7.1. [d]Minimum inhibitory concentration (MIC) is the minimum concentration of the compound required to inhibit 99% of bacterial growth and was found against *Mycobacterium tuberculosis* $H_{37}R_v$ strain. Data provided by our collaborator Dr. Sriram, BITS Hyderabad, India.

The benzyl amine derivative **6** gave 100 % yield after 30 min (Table 4, entry 1) and its analogues gave 80-90% yield after 30 min (Table 4, entry 2-5) suggesting that presence of electron donating and withdrawing group does not affect SO_2 yields significantly. The 2-methoxyaniline (Table 4, entry 6) derivative gave 86% of SO_2 yield after 30 min higher than aniline derivative (55%, Table 1, entry 9).

These results suggest the presence of electron donating group on aromatic ring of aniline significantly affects the electronics on aniline ring and increases the rate of reaction, hence SO_2 yield after 30 min. When an electron withdrawing group such as 4-CN- or 4-F- was present on aromatic ring of aniline, SO_2 yields were significantly lowered with 4-F giving 55% and 4-CN- giving 5% after 30 min. When an additional alkyl, aryl, or methylene group was present, either comparable or slightly diminished yield of SO_2 in comparison with **6** was observed (Table 4,entries 9–15).

The ability of compounds in this library to inhibit *Mycobacterium tuberculosis* ($H_{37}R_v$) growth was evaluated using a reported protocol.[8] We found MICs ranging from 0.05 to >100 µg/mL (Table 4). While **6** was still the best *Mtb* inhibitor in this series, some of the derivativess **18**–**20**, **27**, **29** and **30** had potent *Mtb* inhibitory activities with MICs <1 µg/mL (Table 4, entries 2–4, 11, 13 and 14); better than those of ethambutol and pyrizinamide, both clinically used tuberculosis drugs, evaluated under similar assay conditions (Table 4, entries 17 and 18).

3A.2.10 Statistical analysis of % SO_2 yields, MICs and pK_{aH}s of amine: Data analysis revealed that the most potent *Mtb* inhibitors generated elevated levels of SO_2 in 30 min (>75% yield). We did statistical analysis of SO_2 yields, MICs and pK_{aH} of amines. In statistics, Spearman's rank correlation coefficient, ρ (rho) is a measure of statistical

dependence between two variables X and Y. It assesses how well the relationship between two variables can be described using a monotonic function. If there are no repeated data values, a perfect Spearman correlation of +1 or −1 occurs when each of the variables is a perfect monotone function of the other. If Y tends to increase when X increases, the Spearman correlation coefficient is positive. If Y tends to decrease when X increases, the Spearman correlation coefficient is negative.

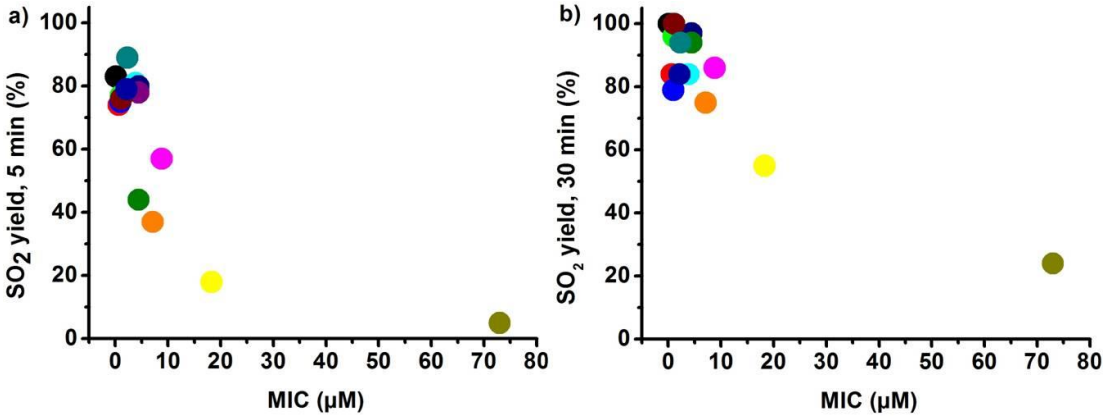


Figure 10. Relationship between sulfur dioxide yield generated during cysteine mediated decomposition of 2,4-dinitrophenylsulfonamides prepared in this study and their *Mtb* inhibitory activity. (a) SO_2 yield was after 5 min; Spearman rank correlation analysis of SO_2 yield and MIC gave a Spearman correlation coefficient ρ = - 0.69 (P-value = 0.001). (b) SO_2 yield was after 30 min; Spearman rank correlation analysis of SO_2 yield and MIC gave a Spearman correlation coefficient ρ = - 0.50 (P-value = 0.01).

Spearman rank correlation analysis of MICs with sulfur dioxide yields after 30 min (Figure 10b) shows a moderate negative correlation value of Spearman rank correlation coefficient ρ = - 0.50 (*P*-value = 0.02) between MICs and SO_2 yields. Similarly, for SO_2 yields after 5 min, ρ = - 0.69 (*P*-value = 0.001), showing good correlation with MICs (Figure 10a) Therefore SO_2 yields can be correlated with the observed *Mtb* inhibitory activity of this series of compounds.

The Pearson correlation coefficient (*r*) is a measure of the linear correlation (dependence) between two variables X and Y, giving a value between +1 and −1 inclusive, where 1 is total positive correlation, 0 is no correlation, and −1 is total negative correlation. It is widely used in science as a measure of the degree of linear dependence between two variables. The correlation coefficient ranges from −1 to 1. A value of 1 implies that a linear equation describes the relationship

between X and Y perfectly, with all data points lying on a line for which Y increases as X increases. A value of −1 implies that all data points lie on a line for which Y decreases as X increases. A value of 0 implies that there is no linear correlation between the variables. We found a good positive correlation r = 0.79 (P-value < 0.0001) between pK_{aH} of the amine and SO_2 yields for 5 min and r = 0.71 (P-value < 0.001) for 30 min (Figure 11b). These results support the dependence of rate of decomposition on protonation of the amine (Scheme 7).

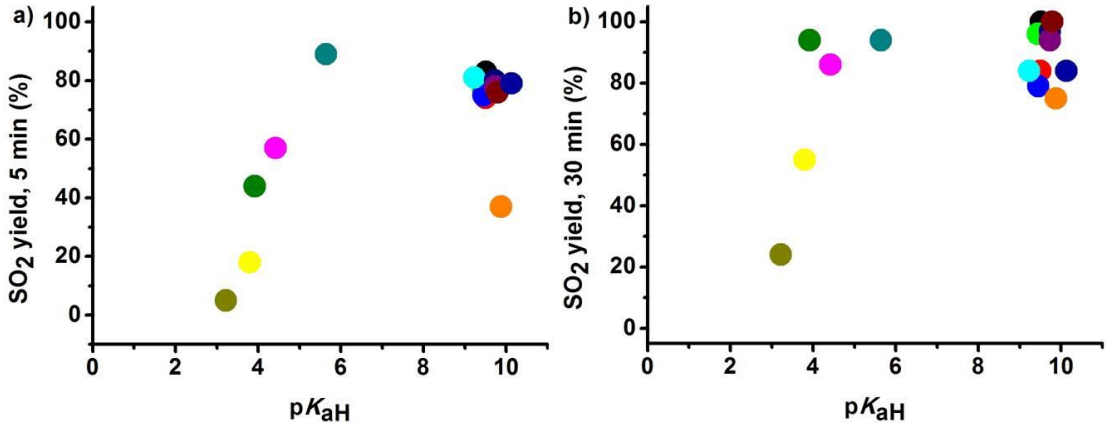


Figure 12. Relationship between sulfur dioxide generated during cysteine-mediated decomposition of 2,4-dinitrophenylsulfonamides prepared in this study and pK_{aH} of the amine from which the corresponding sulfonamide was prepared. (a) SO_2 yield was after 5 min; Pearson correlation analysis of SO_2 yield and pK_{aH} gave a correlation coefficient r = 0.79 (P-value < 0.001) (b) SO_2 yield was after 30 min; Pearson correlation analysis of SO_2 yield and pK_{aH} gave a correlation coefficient r = 0.71 (P-value < 0.0001).

In case of *Mtb* active compounds PA-824, a nitroimidazole,[10,16-19] dinitrobenzamides (DNBs), and benzothiazinones (BTZs)[20-26] the reduction of nitro group present plays an important role (Figure 12). Although all these compounds have one or

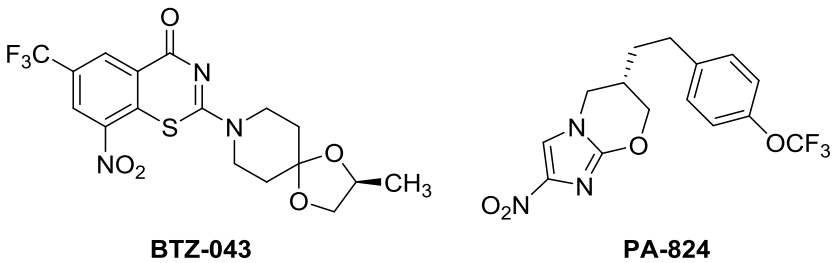


Figure 12. Structure of BTZ-043 and PA-824

more nitro groups, their mechanisms of action and molecular targets in *Mtb* differ suggesting that reduction of an aromatic nitro group may have wide-ranging consequences to *Mtb* growth.

At this time, the involvement of nitro group reduction as a possible mechanism of action of 2,4-dinitrophenylsulfonamides reported in this work is unclear. However, most of the active sulfonamides prepared were completely decomposed in 30 min, probably as they would not stay unreacted, especially in the presence of (estimated) millimolar concentration of mycothiols in *Mtb*.[27]

3A.3 Conclusions:

In conclusion, we prepared various 2,4-dinitrophenylsulfonamides as a thiol activated sources of sulfur dioxide under physiological condition having controlled rate of SO_2 generation, with antimycobacterial activity. The observed *Mtb* inhibitory activity depends upon rate of generation of SO_2, which in turn depends upon pK_{aH} of amine present in sulfonamide.

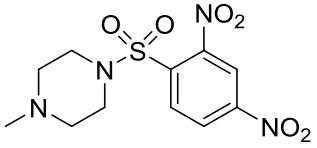
3A.4 Experimental Section

The 2,4-dinitrobenzenesulfonamides **1**, **2**, **3**, **4**, **12**, **13**, **14**, **15 18, 25, 27,** and **29** are previously reported and our data matched with reported data.[29]

A. General procedure for synthesis of 2,4-Dinitrophenylsulfonamides: To a solution of the amine (1 eq.) and DNsCl (1.1 eq.), pyridine/NEt_3 (1.1 eq.) was added dropwise at -78 °C in DCM or THF . This mixture was stirred at -78 °C for 2 h after which 10 mL of water was added. The organic later layer was separated, dried on Na_2SO_4 and concentrated under reduced pressure to get crude product, which was purified by silica gel column chromatography using mixtures of petroleum ether/EtOAc or DCM as the eluant to afford the desired compound as a yellow solid (unless otherwise stated). The 2,4-dinitrobenzenesulfonamides **1-6, 9-15** and **18-27** were prepared by this procedure.

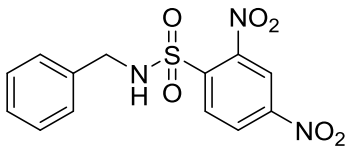
B. General procedure for alkylation of 2,4-Dinitrophenylsulfonamides: To a solution of the 2,4-dinitrophenylsulfonamide in DMF, K_2CO_3 (2 eq.) and alkyl halide (2 eq.) were added at RT and reaction mixture was stirred for 2 h. The reaction was quenched with water and extracted with EtOAc. The organic layer was dried on Na_2SO_4 and concentrated under reduced pressure to get crude product, which was purified by silica gel column chromatography using mixtures of petroleum ether/EtOAc or DCM as the eluant to get desired compound as a yellow solid (unless otherwise stated). The 2,4-dinitrobenzenesulfonamides **7-8,** and **29-31** were prepared by this procedure.

1-(2,4-Dinitrophenylsulfonyl)-4-methylpiperazine (5). Prepared according to general procedure **A**, starting from N-methyl piperazine (0.25 g, 2.49 mmol) to afford **5** (0.51 g, 62%) as a brown solid: mp 164-165 °C; FTIR (ν_{max}, cm^{-1}): 3437, 3108, 2943, 2842, 2803, 1545, 1361, 1169; ^1H NMR (400 MHz, $CDCl_3$): δ 8.50 (dd, *J* = 2.2, 8.6 Hz, 1H), 8.46 (d, *J* = 2.1 Hz, 1H), 8.19 (d, *J* = 8.8 Hz, 1H), 3.37 (t, *J* = 4.8, 5.2 Hz, 4H), 2.49 (t, *J* = 5.2, 4.8 Hz, 4H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.8, 148.4, 137.3, 132.6, 126.0, 119.7, 54.4, 46.1, 45.9; HRMS (ESI-TOF) $C_{11}H_{15}N_4O_6S$ [M + H]+: 331.0712. Found: 331.0714.

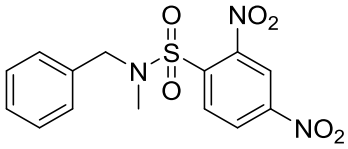


***N*-Benzyl-2,4-dinitrobenzenesulfonamide (6).** Prepared according to general procedure **A**, starting from benzylamine (0.50 g, 4.66 mmol) to afford **6** (0.64 g, 41%) as pale yellow

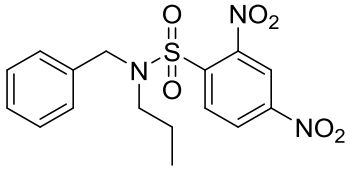
solid: mp 150-151 ^{o}C; FTIR (ν_{max}, cm^{-1}): 3379, 3098, 2345, 1537, 1352, 1161; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05 (t, J = 5.8 Hz, 1H), 8.85 (d, J = 2.4 Hz, 1H), 8.51 (dd, J = 8.8, 2.4 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.26-7.20 (m, 5H), 4.20 (d, J = 6.0, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 149.9, 147.9, 138.5, 137.4, 131.8, 128.8, 128.1, 127.9, 127.5, 120.4, 46.7; HRMS (ESI-TOF) $C_{13}H_{11}N_3O_6S$ [M + Na]+: 360.0266. Found: 360.0275.



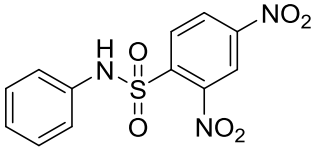
***N*-Benzyl-*N*-methyl-2,4-dinitrobenzenesulfonamide (7).** Prepared according to general procedure, starting from **6** (0.050 g, .148 mmol) to afford **7** (0.045 g, 86%) as **B** a brown solid: mp 132-133 °C; FTIR (ν_{max}, cm^{-1}): 3436, 3105, 2922, 1547, 1357, 1163; ^{1}H NMR (400 MHz, $CDCl_3$):δ 8.50 – 8.47 (m, 2H), 8.23-8.21 (m, 1H), 7.36 – 7.29 (m, 5H), 4.44(s, 2H), 2.83 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.7, 148.2, 138.1, 134.6, 132.8, 129.0, 128.4, 128.3, 126.1, 119.7, 54.2, 34.4; HRMS (ESI-TOF) $C_{14}H_{14}N_3O_6S$ [M + H]+: 352.0605. Found: 352.0603.



***N*-Benzyl-*N*-(1-propyl)-2,4-dinitrobenzenesulfonamide (8).** Prepared according to general procedure **B**, starting from **6** (0.050 g, .148 mmol) to afford **8** (0.041 g, 73%) as brown solid: mp 120-121 °C; FTIR (ν_{max}, cm^{-1}): 3098, 2927, 1545, 1352, 1155, 1020; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (d, J = 2.2 Hz, 1H), 8.38 (dd, J = 8.8, 2.4 Hz, 1H), 8.12 (d, J = 8.8 Hz, 1H), 7.32-7.23 (m, 5H), 4.54 (s, 2H), 3.26 (t, J = 8.0, 7.6 Hz, 2H), 1.52-1.43 (m, 2H), 0.77 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.4, 147.8, 139.6, 135.0, 132.5, 128.8, 128.1, 125.8, 119.6, 51.2, 49.4, 20.9, 10.9; HRMS (ESI-TOF) $C_{16}H_{18}N_3O_6S$ [M + H]+: 380.0920. Found: 380.0916.

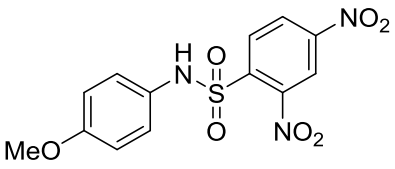


***N*-Phenyl-2,4-Dinitro-benzenesulfonamide (9).** Prepared according to general procedure **A**, starting from aniline (0.40 g, 4.29 mmol) to afford **9** (0.41 g, 30%) as a orange solid: mp 117-8 °C; FTIR (ν_{max}, cm^{-1}): 3316, 3096, 2347, 1539, 1353, 1163; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (d, J = 2.4 Hz, 1H), 8.37 (dd, J = 8.8, 2.4 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.33-7.19 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.0, 148.4, 137.5, 134.4, 133.5, 129.7, 127.3,

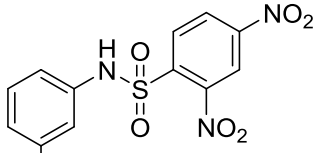


126.7, 123.5, 120.6; HRMS (ESI-TOF) $C_{12}H_9N_3O_6S$ [M + Na]+: 346.0110. Found: 346.0181.

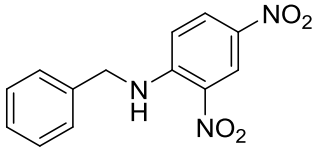
***N*-(4-Methoxyphenyl)-2,4-dinitrobenzenesulfonamide (10).).** Prepared according to general procedure **A**, starting from 4-methoxy aniline (0.10 g, 0.812 mmol) to afford **10** (0.19 g, 66 %) as a orange solid: mp 115°C; FTIR (ν_{max}, cm^{-1}): 3345, 3098, 2343, 1539, 1357, 1246, 1169, 1029 ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.87 (d, *J* = 2.0 Hz, 1H), 8.58 (d, *J* = 8.8 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 1H), 7.04, (d, *J* = 7.2 Hz, 2H), 6.86 (d, *J* = 7.2 Hz, 2H), 3.69 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.9, 150.4, 148.3, 136.8, 132.1, 128.5, 127.6, 125.1, 120.7, 115.1, 56.7; HRMS (ESI-TOF) $C_{13}H_{11}N_3O_7S$ [M + Na]+: 376.0215. Found: 376.0214.



***N*-(3-Fluorophenyl)-2,4-dinitrobenzenesulfonamide (11).** Prepared according to general procedure **A**, starting from 3-fluoro aniline (0.25 g, 2.24 mmol) to afford **11** (0.54 g, 70 %) as a orange solid: mp 172-173 °C; FTIR (ν_{max}, cm^{-1}): 3445, 3267, 3093, 1541, 1354, 1171; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (d, *J* = 2.2 Hz, 1H), 8.43 (dd, *J* = 2.3, 1.8 Hz, 1H), 8.12 (d, *J* = 8.7 Hz, 1H), 7.35 (s, 1H), 7.29-7.24 (td, *J* = 6.4, 10.1 Hz, 1H), 7.06-7.02 (dt, *J* = 2.2, 9.6 Hz, 1H), 6.97-6.90 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.2-161.7 (d, *J* = 248 Hz) 150.1-148.4 (d, *J* = 173 Hz), 137.2, 136.0-135.9 (d, *J* = 9 Hz), 133.5, 131.1-131.0 (d, *J* = 8.58 Hz), 126.9, 120.8, 118.4-118.3 (d, *J* = 11 Hz), 114.2-114.0 (d, *J* = 21 Hz), 110.5-110.3 (d, *J* = 25 Hz); MALDI-TOF MS (m/z): Calculated $C_{12}H_8FN_3O_6S$ [M+K]+: 379.9452. Found [M+K]+: 379.9755.

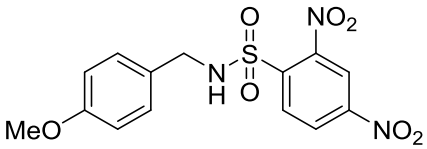


***N*-Benzyl-2,4-dinitroaniline (17).** To a solution of 1-fluoro-2,4-dinitrobenzene (0.25 g, 0.134 mmol) in 5 mL dry THF, benzylamine (0.176 mL,1.608 mmol) in 2 mL dry THF was added at room temperature. The reaction mixture was stirred for two hours. Then THF was evaporated on rotary evaporator, to get yellow solid, purified by silica gel column chromatography (DCM) to afford **17** (0.325 g, 88 %) as a yellow solid: mp 115-116 °C; FTIR (ν_{max}, cm^{-1}): 3375, 1621, 1586, 1523, 1493, 1455, 1376, 1364, 1337, 1152, 1124; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.16 (d, *J* = 2.7 Hz, 1H), 8.92 (s, 1H), 8.22 (dd, *J* = 9.6, 2.8 Hz, 1H),

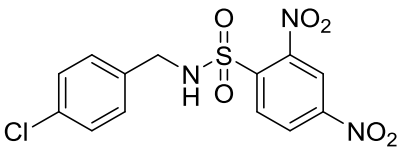


7.39 – 7.33 (m, 5H), 6.91 (d, *J* = 9.2 Hz, 1H), 4.65 (d, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.2, 136.5, 135.6, 130.8, 130.4, 129.3, 128.4, 127.1, 124.3, 114.4, 47.6; HRMS (ESI-TOF) C13H12N3O4 [M + H]+ : 274.0835, Found: 274.0828.

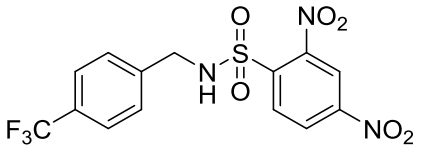
***N*-(4-methoxybenzyl)-2,4-dinitrobenzenesulfonamide (18).** Prepared according to general procedure **A**, starting from 4-methoxy benzylamine (0.25 g, 1.81 mmol) to afford **18** (0.11 g, 33 %) as a pale yellow solid: mp 163-164 °C; FTIR (ν_{max}, cm^{-1}): 3388, 1604, 1553, 1513, 1392, 1348, 1302, 1248, 1163; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (s, 1H), 8.83 (d, *J* = 2.1 Hz, 1H), 8.48 (dd, *J* = 8.4, 2.2 Hz, 1H), 8.05 (d, *J* = 8.7 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 2H), 6.77 (d, *J* = 8.5, 2H), 4.13 (s, 2H), 3.67 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 159.1 149.8, 147.8, 138.7, 131.9, 129.7, 129.2, 127.5, 120.4, 114.1, 55.5, 46.4; HRMS (ESI-TOF): Calculated $C_{14}H_{13}N_3O_7S$ $[M+Na]^+$: 390.0372. Found $[M+Na]^+$: 390.0372.



***N*-(4-chlorobenzyl)-2,4-dinitrobenzenesulfonamide (19).** Prepared according to general procedure **A**, starting from 4-chloro benzylamine (0.20 g, 1.41 mmol) to afford **19** (0.28 g, 54 %) as a pale yellow solid: mp 161-162 °C; FTIR (ν_{max}, cm^{-1}): 3385, 3100, 1540, 1346, 1165; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.06 (s, 1H), 8.86 (d, *J* = 1.8 Hz, 1H), 8.53 (dd, *J* = 6.9, 1.8 Hz, 1H), 8.12 (d, *J* = 6.9 Hz, 1H), 7.32 (d, *J* = 6.7 Hz, 2H), 7.25 (d, *J* = 6.7 Hz, 2H), 4.20 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 149.7, 147.6, 138.4, 136.2, 132.6, 132.0, 130.0, 128.7, 127.5, 120.4, 46.0; HRMS (ESI-TOF): Calculated $C_{13}H_{10}ClN_3O_6S$ $[M+Na]^+$: 393.9877. Found $[M+Na]^+$: 393.9879.

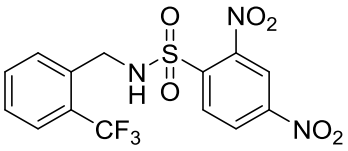


***N*-(4-(trifluoromethyl)benzyl)-2,4-dinitrobenzenesulfonamide (20).** Prepared according to general procedure **A**, starting from 4-trifluoromethyl benzylamine (0.20 g, 1.14 mmol) to afford **20** (0.20 g, 43 %) as a pale yellow solid: mp 176-177 °C; FTIR (ν_{max}, cm^{-1}): 3389, 1854, 1354, 1322, 1170, 1120; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.15 (s, 1H), 8.85 (s, 1H), 8.49 (d, *J* = 6.9 Hz, 1H), 8.10 (d, *J* = 6.8 Hz, 1H), 7.61 (d, *J* = 6.4 Hz, 2H), 7.44 (d, *J* = 6.3 Hz, 2H), 4.32 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 149.7, 147.5, 141.9, 138.4, 132.0, 128.9, 127.4, 125.8,

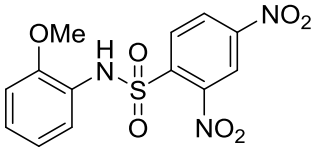


125.5, 120.4, 120.3, 46.2; HRMS (ESI-TOF): Calculated $C_{14}H_{10}F_3N_3O_6S$ $[M+Na]^+$: 428.0140. Found $[M+Na]^+$: 428.0136.

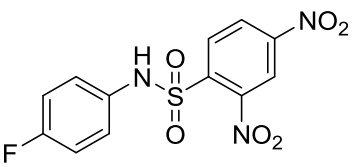
***N*-(2-(trifluoromethyl)benzyl)-2,4-dinitrobenzenesulfonamide (21).** Prepared according to general procedure **A**, starting from 2-trifluoromethyl benzylamine (0.20 g, 1.14 mmol) to afford **21** (0.25 g, 54 %) as a pale yellow solid: mp 157-158 °C; FTIR (ν_{max}, cm^{-1}): 3385, 1556, 1540, 1422, 1350, 1313, 1171, 1121, 1104, 1038; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.23 (s, 1H), 8.90 (d, *J* = 2.2 Hz, 1H), 8.59 (dd, *J* = 8.7, 2.2 Hz, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.65-7.60 (m, 2H), 7.47 (dd, *J* = 7.8, 2.2 Hz, 1H), 4.39 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.2, 147.9, 138.1, 135.8, 133.2, 131.7, 129.9, 128.5, 127.8, 126.3, 120.6, 119.2, 43.1; HRMS (ESI-TOF): Calculated $C_{14}H_{10}F_3N_3O_6S$ $[M+Na]^+$: 428.0140. Found $[M+Na]^+$: 428.0141.



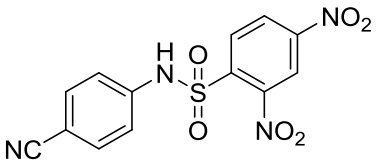
***N*-(2-methoxyphenyl)-2,4-dinitrobenzenesulfonamide (22).** Prepared according to general procedure **A**, starting from 2-methoxy aniline (0.150 g, 1.21 mmol) to afford **22** (0.30 g, 70 %) as a orange solid: mp 172-173 °C; FTIR (ν_{max}, cm^{-1}): 3268, 3101, 1605, 1545, 1498, 1410, 1357, 1172, 1132; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.30 (s, 1H), 8.87 (d, *J* = 2.2 Hz, 1H), 8.60 (dd, *J* = 8.7, 2.3 Hz, 1H), 8.13 (d, *J* = 8.7 Hz, 1H), 7.26-7.16 (m, 2H), 6.97-6.90 (m, 2H) 3.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.1, 150.1, 147.7, 138.6, 132.0, 128.9, 128.4, 127.3, 124.0, 121.1, 120.3, 112.5, 55.7; HRMS (ESI-TOF): Calculated $C_{13}H_{11}N_3O_7S$ $[M+Na]^+$: 376.0215. Found $[M+Na]^+$: 376.0215.



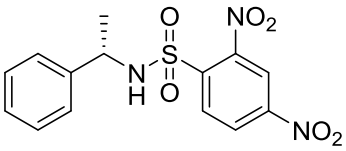
***N*-(4-fluorophenyl)-2,4-dinitrobenzenesulfonamide (23).** Prepared according to general procedure **A**, starting from 4-fluoro aniline (0.175 g, 1.57 mmol) to afford **23** (0.26 g, 48 %) as a orange solid: mp 127-128 °C; FTIR (ν_{max}, cm^{-1}): 3299, 1553, 1536, 1348, 1171; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.02 (s, 1H), 8.89 (s, 1H), 8.59 (d, *J* = 8.6 Hz, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 7.15 (d, *J* = 5.4 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.1, 159.1, 150.5, 148.3, 136.5, 132.4, 132.1, 127.7, 124.7 (d, *J* = 8 Hz), 120.8, 116.7 (d, *J* = 22 Hz); HRMS (ESI-TOF): Calculated $C_{12}H_8FN_3O_6S$ $[M+K]^+$: 379.9755. Found $[M+K]^+$: 379.9755.



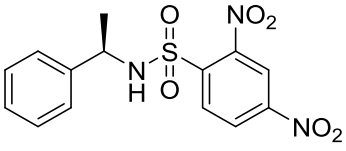
***N*-(4-cyanophenyl)-2,4-dinitrobenzenesulfonamide (24).** Prepared according to general procedure **A**, starting from 4-cyano aniline (0.200 g, 1.69 mmol) to afford **24** (0.28 g, 47 %) as a orange solid: mp 186-187 °C; FTIR (ν_{max}, cm^{-1}): 3252, 2231, 1607, 1555, 1538, 1469, 1346, 1165; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.79 (s, 1H), 8.93 (s, 1H), 8.60 (d, *J* = 8.5 Hz, 1H), 8.30 (d, *J* = 8.6 Hz, 1H), 7.78 (d, *J* = 8.3 Hz, 2H), 7.27 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.8, 148.2, 141.3, 136.3, 134.4, 132.2, 128.0, 121.1, 120.1, 119.0, 107.1; HRMS (ESI-TOF): Calculated $C_{13}H_8N_4O_6S$ $[M+Na]^+$: 371.0062. Found $[M+Na]^+$: 371.0062.



***(S) N*-(1-phenylethyl)-2,4-dinitrobenzenesulfonamide (25).** Prepared according to general procedure **A**, starting from (S)-1-phenylethan-1-amine (0.250 g, 2.06 mmol) to afford **25** (0.34 g, 47%) as a pale yellow solid; mp 162-163 °C; FTIR (ν_{max}, cm^{-1}): 3342, 1541, 1442, 1353, 1166; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05 (d, *J* = 7.6 Hz, 1H), 8.78 (d, *J* = 2.2 Hz, 1H), 8.39 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.99 (d, *J* = 8.6 Hz, 1H), 7.23-7.10 (m, 5H), 4.56-4.49 (m, 1H), 1.37 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 149.8, 147.6, 142.8, 138.5, 132.1, 128.7, 127.6, 127.1, 126.6, 120.1, 54.0, 23.7; HRMS (ESI-TOF): Calculated $C_{14}H_{13}N_3O_6S$ $[M+Na]^+$: 374.0423. Found $[M+Na]^+$: 374.0422.

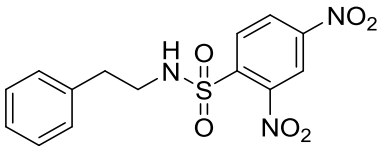


***(R) N*-(1-phenylethyl)-2,4-dinitrobenzenesulfonamide (26).** Prepared according to general procedure **A**, starting from (R)-1-phenylethan-1-amine (0.250 g, 2.06 mmol) to afford **26** (0.27 g, 38%) as a pale yellow solid; mp 160-161 °C; FTIR (ν_{max}, cm^{-1}): 3342, 1541, 1442, 1353, 1166; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05 (d, *J* = 8.2 Hz, 1H), 8.78 (d, *J* = 2.1 Hz, 1H), 8.39 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.99 (d, *J* = 8.6 Hz, 1H), 7.23-7.10 (m, 5H), 4.56-4.49 (m, 1H), 1.37 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 149.8, 147.6, 142.9, 138.6, 132.1, 128.7, 127.6, 127.2, 126.6, 120.2, 54.0, 23.8; HRMS (ESI-TOF): Calculated $C_{14}H_{13}N_3O_6S$ $[M+Na]^+$: 374.0423. Found $[M+Na]^+$: 374.0424.

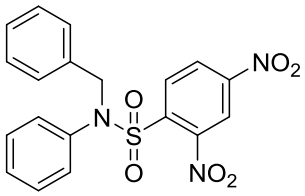


***N*-phenethyl-2,4-dinitrobenzenesulfonamide (27).** Prepared according to general procedure **A**, starting from 2-phenylethan-1-amine (0.20 g, 1.65 mmol) to afford **27** (0.46 g, 38%) as a pale yellow solid: mp 127-128 °C; FTIR (ν_{max}, cm^{-1}): 3348, 1537, 1428, 1342,

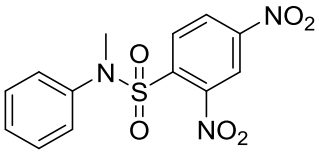
1166, 1101; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.85 (d, J = 2.2 Hz, 1H), 8.59 (s,1H), 8.53 (dd, J = 8.7, 2.2 Hz, 1H), 8.12 (d, J = 8.7 Hz, 1H), 7.31-7.11 (m, 5H), 3.20 (t, J = 7.5, 7.1 Hz, 2H), 2.72 (t, J = 7.4, 7.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.0, 147.9, 138.8, 138.2, 131.7, 129.2, 128.8, 127.7, 126.8, 120.5, 44.8, 35.8; HRMS (ESI-TOF): Calculated $C_{14}H_{13}N_3O_6S$ [M+Na]$^+$: 374.0423. Found [M+Na]$^+$: 374.0423.



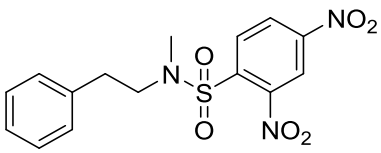
***N*-benzyl-2,4-dinitro-*N*-phenylbenzenesulfonamide (28).** Prepared according to general procedure **B**, starting from **9** (0.25 g, 0.773 mmol) to afford **28** (0.15 g, 47%) as a pale yellow solid: mp 134-135 °C; FTIR (ν_{max}, cm^{-1}): 1604, 1550, 1536, 1351, 1169, 1106; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.01 (d, J = 2.2 Hz, 1H), 8.53 (dd, J = 8.7, 2.3 Hz, 1H), 7.99 (d, J = 8.7 Hz, 1H), 7.31-7.14 (m, 10H), 4.96 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.7, 148.1, 132.9, 129.8, 129.5, 129.1, 129.0, 128.7, 128.3, 127.2, 120.5, 55.4; HRMS (ESI-TOF): Calculated $C_{19}H_{15}N_3O_6S$ [M+Na]$^+$: 436.0579. Found [M+Na]$^+$: 436.0578.



***N*-methyl-2,4-dinitro-*N*-phenylbenzenesulfonamide (29).** Prepared according to general procedure **B**, starting from **9** (0.25 g, 0.773 mmol) to afford **29** (0.21 g, 81%) as a pale yellow solid: mp 156-157 °C; FTIR (ν_{max}, cm^{-1}): 1556, 1541, 1365, 1184, 1061; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.99 (d, J = 2.2 Hz, 1H), 8.52 (d, J = 8.7, 2.2 Hz, 1H), 7.88 (d, J = 8.7 Hz, 1H), 7.42-7.38 (m, 3H), 7.26-7.24 (m, 2H), 3.3 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.7, 148.3, 140.1, 134.5, 132.7, 129.9, 128.8, 127.6, 127.2, 127.1, 120.5; HRMS (ESI-TOF): Calcd. $C_{13}H_{11}N_3O_6S$ [M+Na]$^+$: 360.0266. Found [M+Na]$^+$: 360.0266.

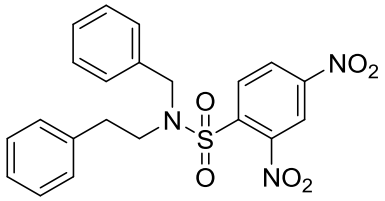


***N*-methyl-2,4-dinitro-*N*-phenethylbenzenesulfonamide (30).** Prepared according to general procedure **B**, starting from **27** (0.20 g, 0.56 mmol) to afford **30** (0.20 g, 95%) as a oil: FTIR (ν_{max}, cm^{-1}): 1552, 1537, 1352, 1163; ^{1}H NMR (400 MHz, $CDCl_3$):δ 8.40 (s, 1H), 8.37 (d, J = 6.9 Hz, 1H), 8.05 (d, J = 6.8 Hz, 1H), 7.24-7.15 (m, 5H), 3.53 (t, J = 6, 5.7 Hz, 2H), 2.99 (s, 3H), 2.92-2.89 (t, J = 5.8, 5.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.5, 147.9, 138.3, 137.7, 132.4, 128.9, 128.7, 126.9, 126.1, 119.7, 52.1, 35.0, 34.5;



HRMS (ESI-TOF): Calculated $C_{15}H_{15}N_3O_6S$ $[M+Na]^+$: 388.0579. Found $[M+Na]^{+:}$ 388.0579.

***N*-benzyl-2,4-dinitro-*N*-phenethylbenzenesulfonamide (31).** Prepared according to general procedure **B**, starting from **27** (0.10 g, 0.28 mmol) to afford **31** (0.10 g, 80%) as a pale yellow solid: mp 111-112 °C; FTIR (ν_{max}, cm^{-1}): 1543, 1370, 1347, 1158; ^1H NMR (400 MHz, DMSO-d_6): δ 8.93 (d, *J* = 2.2 Hz, 1H), 8.45 (dd, *J* = 8.8, 2.3 Hz, 1H), 8.25 (d, *J* = 8.7 Hz, 1H), 7.39-7.33 (m, 5H), 7.19-7.02 (m, 5H), 4.62 (s, 2H), 3.37 (t, *J* = 8.1, 7.6 Hz, 2H), 2.61 (t, *J* = 7.9, 7.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.4, 147.7, 138.31, 137.2, 136.2, 132.2, 129.2, 129.1, 128.9, 128.8, 128.5, 127.5, 126.9, 120.6, 51.4, 49.3, 34.2; HRMS (ESI-TOF): Calculated $C_{21}H_{19}N_3O_6S$ $[M+Na]^+$: 464.0892. Found $[M+Na]^+$: 464.0893.



Cysteine-activated decomposition and sulfur dioxide release.

Sulfite analysis: To 3 mL of 1 mM stock solution of compound in acetonitrile (21 or 24 mL) of phosphate buffer (pH = 7.4, 20 mM) and 3/0 mL of acetonitrlie was added (to make final volume 10/20%, ACN/PB), vortexed for 20 s. To this mixture, 3 mL of 10 mM cysteine solution (pH7.4) was added and the reaction mixture was stirred at RT under inert atmosphere. Aliquots at appropriate time intervals were analyzed by IC. Maximum sulfur dioxide yield was calculated based on completion of the reaction with no further increase in sulfite formation. Ion chromatography analysis: An ion chromatograph attached with a conductivity detector was used for sulfite analysis.1 mM $NaHCO_3$/3.2 mM Na_2CO_3 was the eluant and the flow rate was 0.7 mL/min. Using stock solutions of sulfite, a calibration curve was generated (R^2 = 0.9999).

Calibration curve for Sulfite: A stock solution (100 mM) of sodium sulfite, Na_2SO_3 (Sigma-Aldrich) was prepared in dI water containing 1% paraformaldehyde. This stock solution was diluted to prepare 10, 40, 60, 80, 100, 120 and 160 µM sodium sulfite solutions. Injections of 20 µL were carried out to generate a calibration curve for sulfite, R^2 was found as 0.9999, and slope 0.0044.

Calibration curve for Sulfate: Sulfite is susceptible to aerobic oxidation to form sulfate, which was detected in small amounts in several instances during our study. In order to quantify sulfate, a calibration curve was generated by preparing a 10 mM solution of sulfate, which was diluted to prepare solutions of suitable concentrations for injections into the IC. As the levels of sulfate were below 5 μM, calibration curve was generated with concentrations below 10 μM (Figure S3). R^2 was found as 0.9965 and slope 0.135.

Reaction of 6 with various nucleophiles. A general procedure involved preparation of a stock solution of **6** in acetonitrile and treatment of 10 eq. of the nucleophile in pH 7.4 buffer. HPLC analysis was carried out to estimate amount of **6** remaining after 4 h

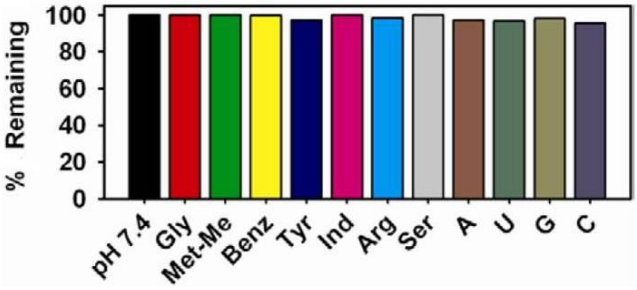


Figure S1. Results of HPLC analysis to estimate amount of **6** remaining after 4 h upon treatment with 10 eq. of Gly: glycine, Met-Me: Methionine, methyl ester; Benz:benzoic acid; Tyr: tyrosine; Ind: indole; Arg: arginine; Ser: serine; A: adenine; U: uracil; G: guanine and C: cytosine.

***In vitro* cell viability activity.**[28] Human Embryonic Kidney 293 cells (HEK) were obtained from National Centre for Cell Science (Pune, India) were cultured in RPMI-1640 medium (HiMedia, India) supplemented with 2 mM glutamine (Himedia, India), antibiotics (100 U/mL penicillin A and 100 U mL−1 streptomycin; Himedia, India) and 10% heat-inactivated fetal bovine serum (HiMedia, India). Cells were cultured in 75 cm2 flasks with loosened caps and incubated in 5% CO2 humidified air at 37°C. The toxicity of compounds was determined by means of a reported 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT) assay. Fresh stock solutions of each compound were prepared in DMSO at a concentration of 100 mM. Dilution in 50:50 RPMI: DMSO mixtures produced stock solutions of compounds ranging from 10^{-8} M to 10^{-4} M. Cells were diluted to a final concentration of 1 x 10-5 cells/mL. 50 μL of the cell suspension were sowed into the wells of a 96-well culture plate (Axygen, USA) and

treated with varying concentrations of compounds in triplicates. After incubation with compounds at 37 °C, 5% CO2 in humidified atmosphere, XTT reagent was freshly prepared with XTT-labelling reagent and electron-coupling reagent in a ratio of 50:1, and 50 µL of this mixture was added to each well of the 96-well plate. The plates were incubated for about 72 h at 37 °C, 5% CO_2 in humidified atmosphere and read out after incubation. Quantification of cell viability was performed in an ELISA plate reader (Bio-Rad, Munchen, Germany) at 450 nm with a reference wavelength of 655 nm.

Table S1. *In vitro* cell viability, antimycobacterial activity and selectivity indices

Entry	Compd	IC50 (µM)[a]	MIC (µM)[b]	SI[c]
1	**6**	7.0	0.15	47
2	**7**	8.8	1.1	8
3	**8**	8.9	8.25	1.1

[a]50% inhibitory concentration determined against human embryonic kidney (HEK293) cells. bMinimum inhibitory concentration (MIC) is the minimum concentration of the compound required to inhibit 99% of bacterial growth and was found against *Mycobacterium tuberculosis* $H_{37}R_v$ strain. [c]SI = IC_{50}/MIC.

3A.5 Spectral Data

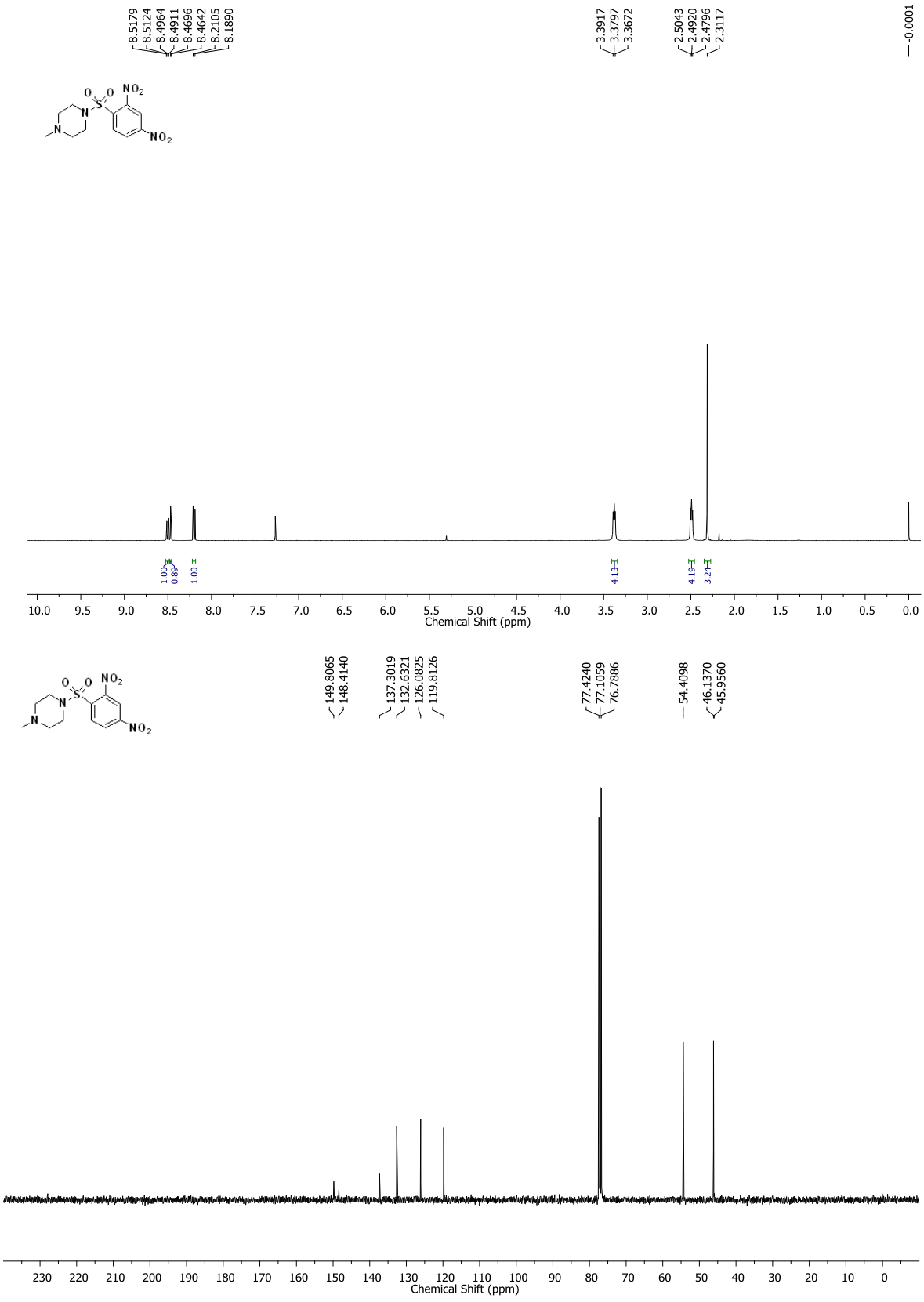


Figure S2. NMR spectra of **5**

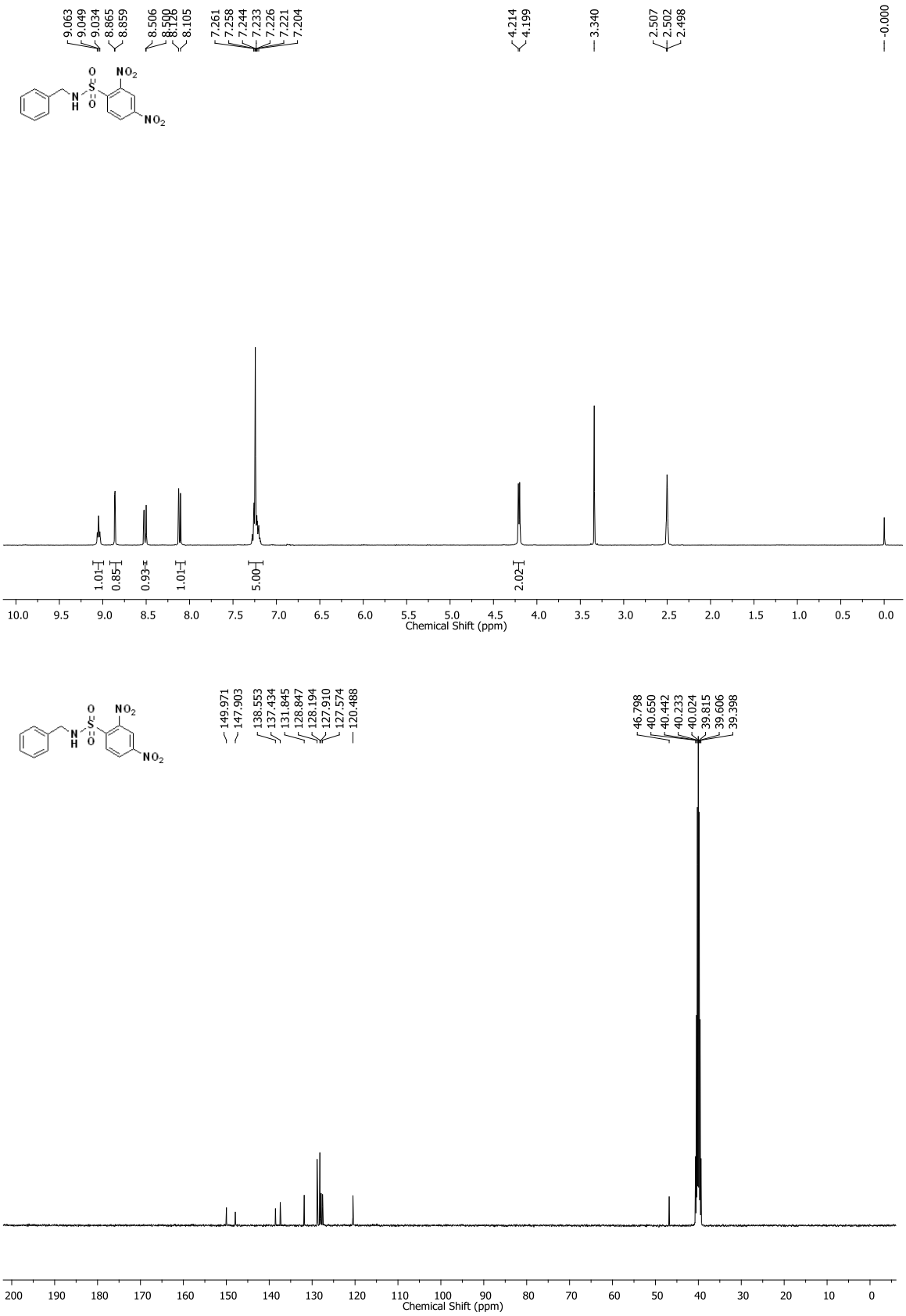


Figure S3. NMR spectra of **6**

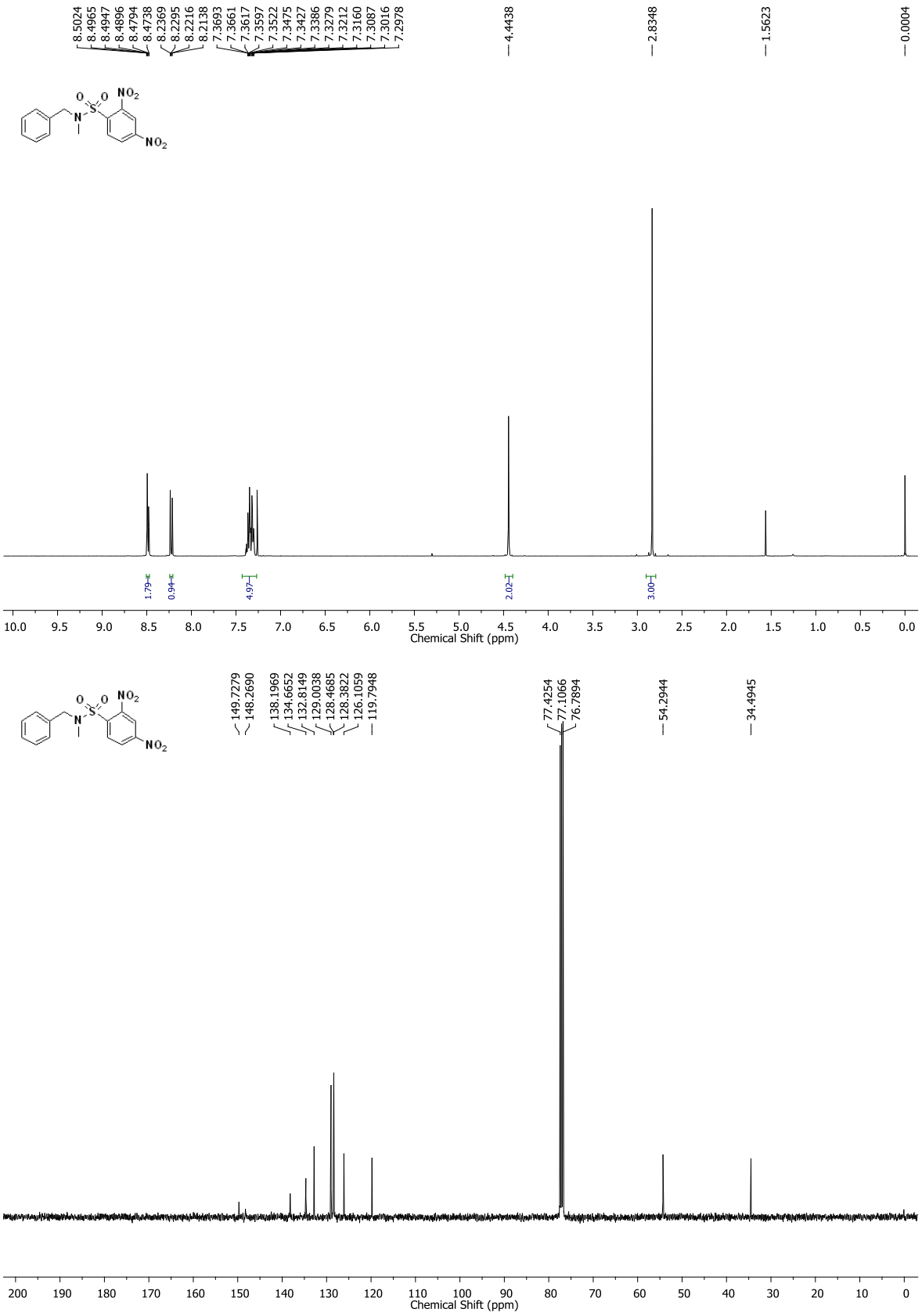


Figure S4. NMR spectra of **7**

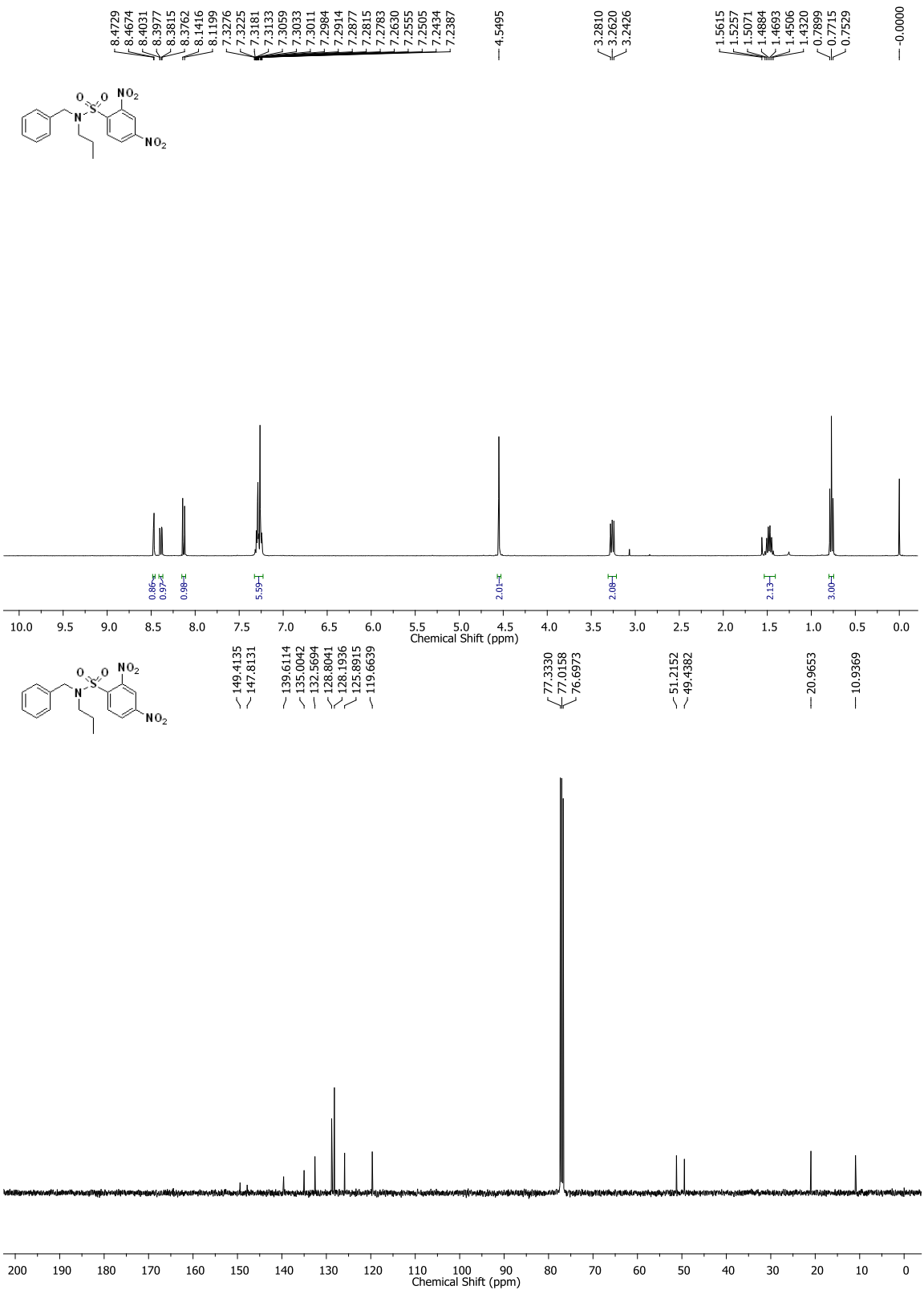


Figure S5. NMR spectra of **8**

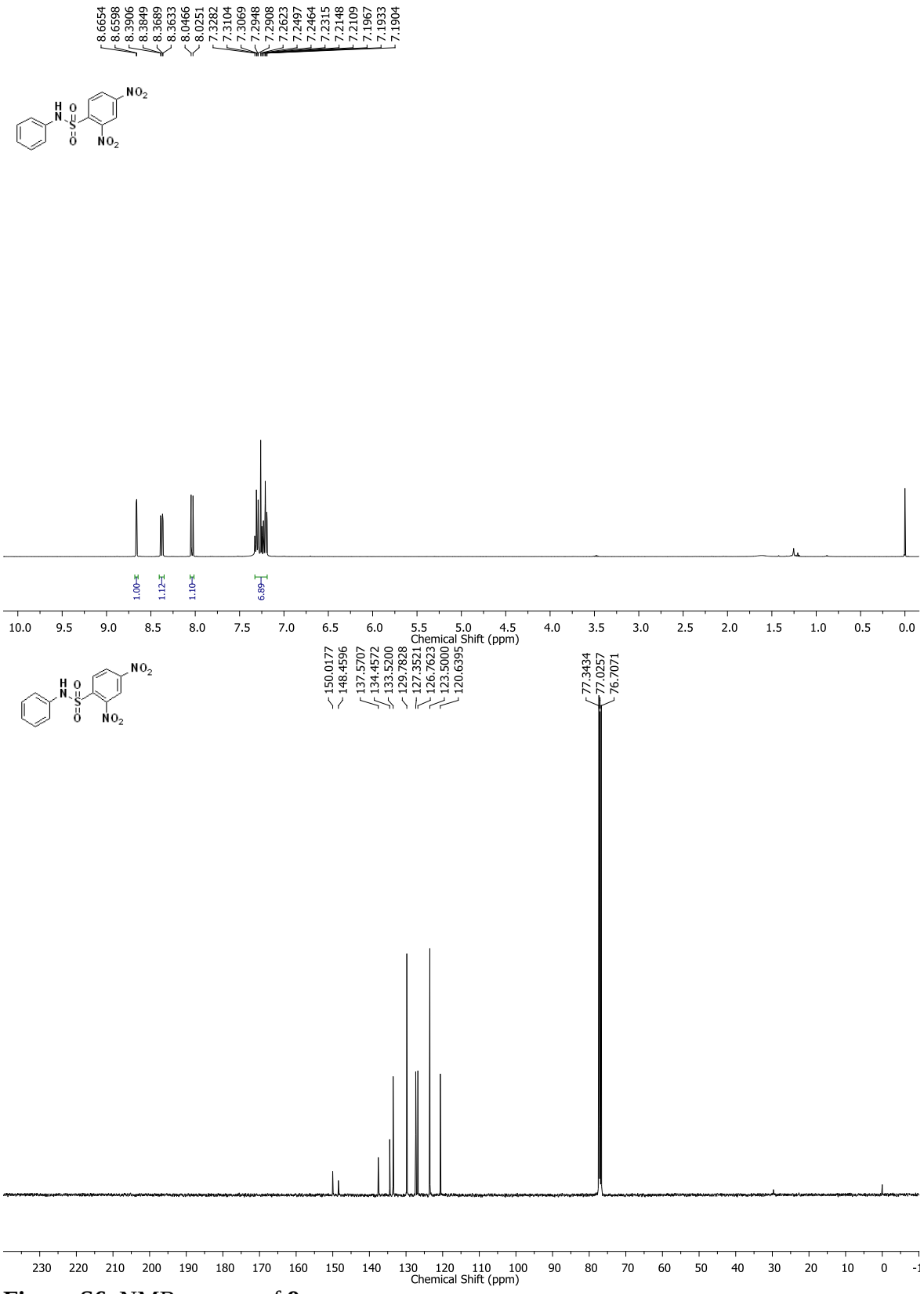


Figure S6. NMR spectra of **9**

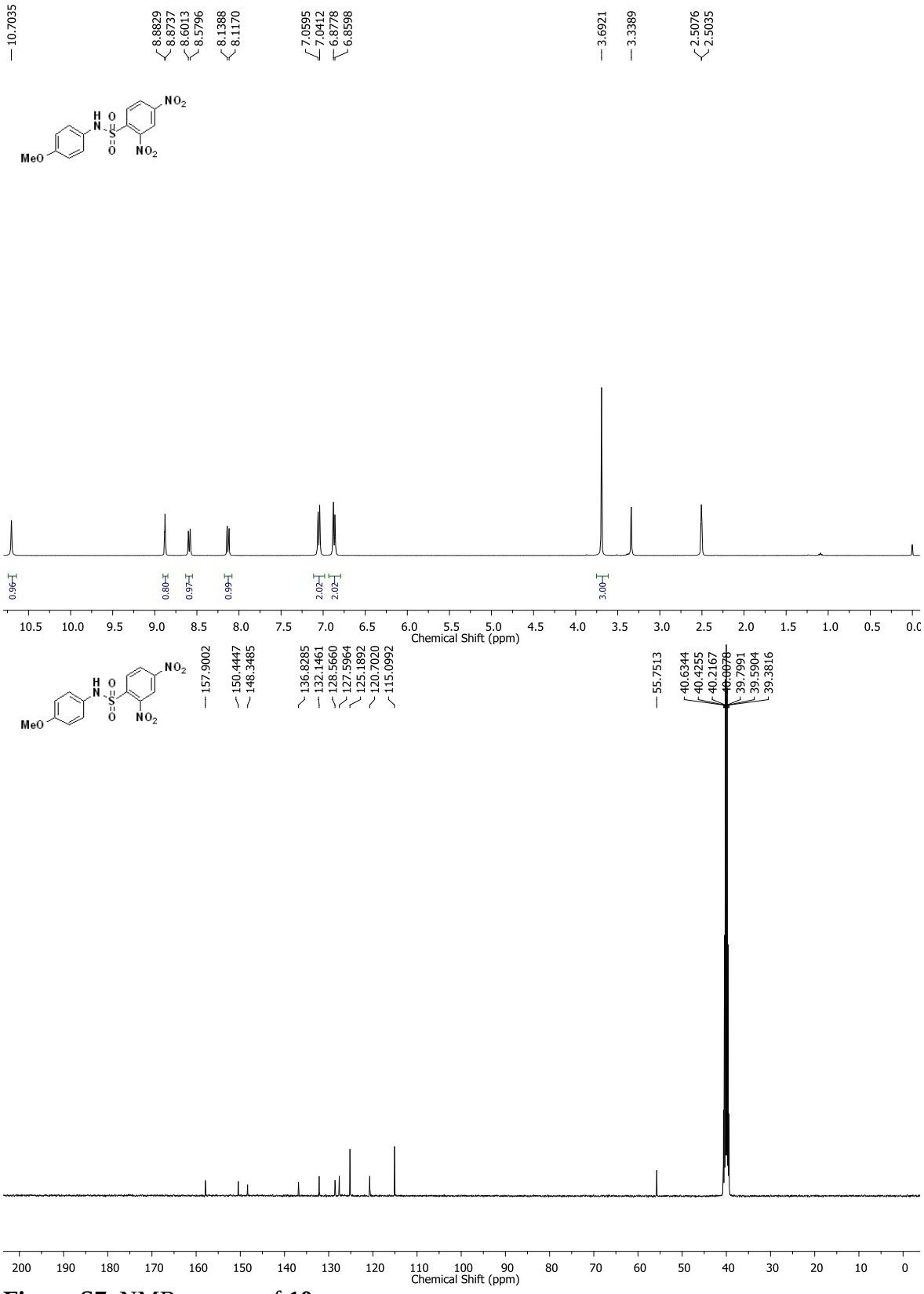


Figure S7. NMR spectra of **10**

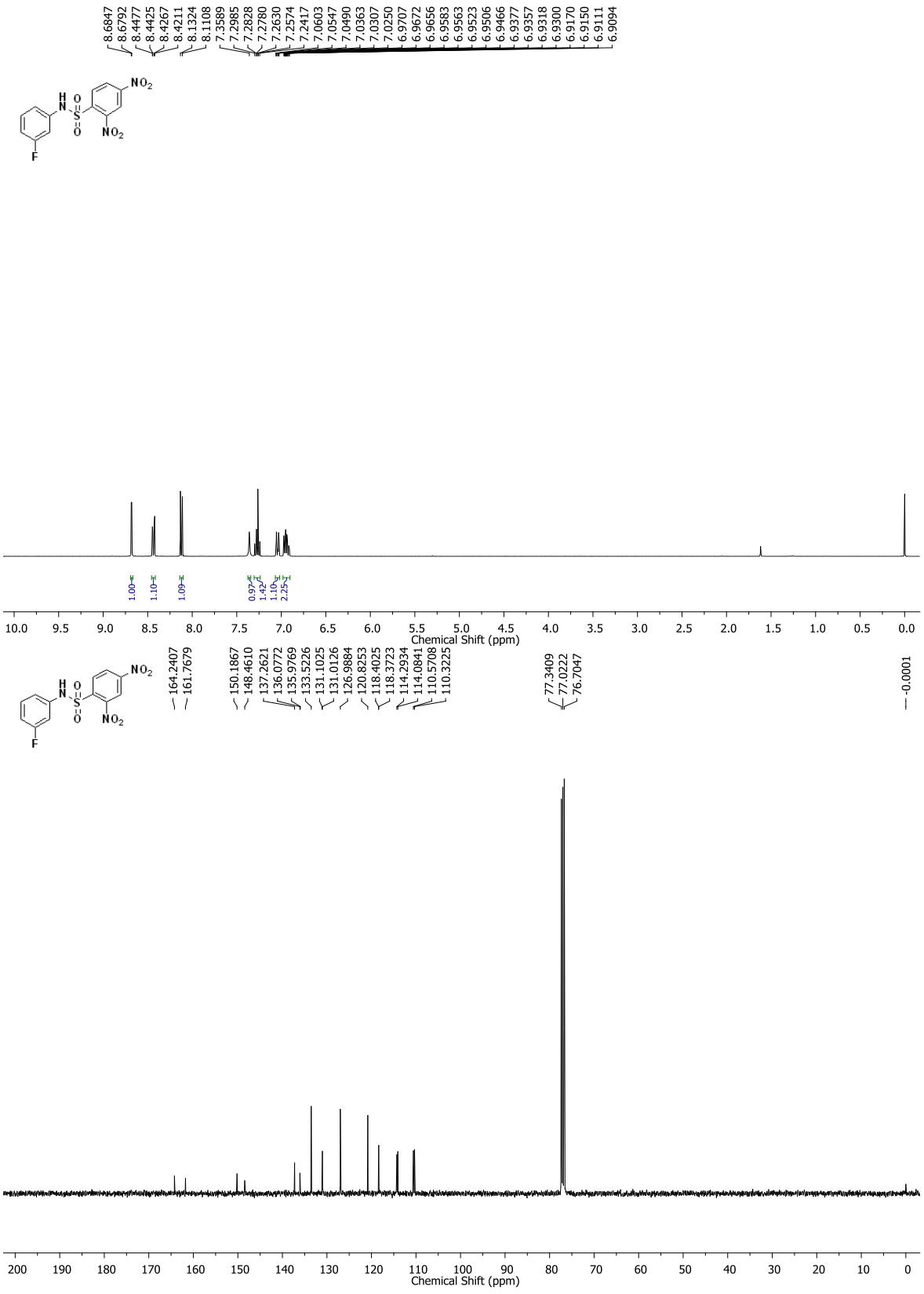


Figure S8. NMR spectra of **11**

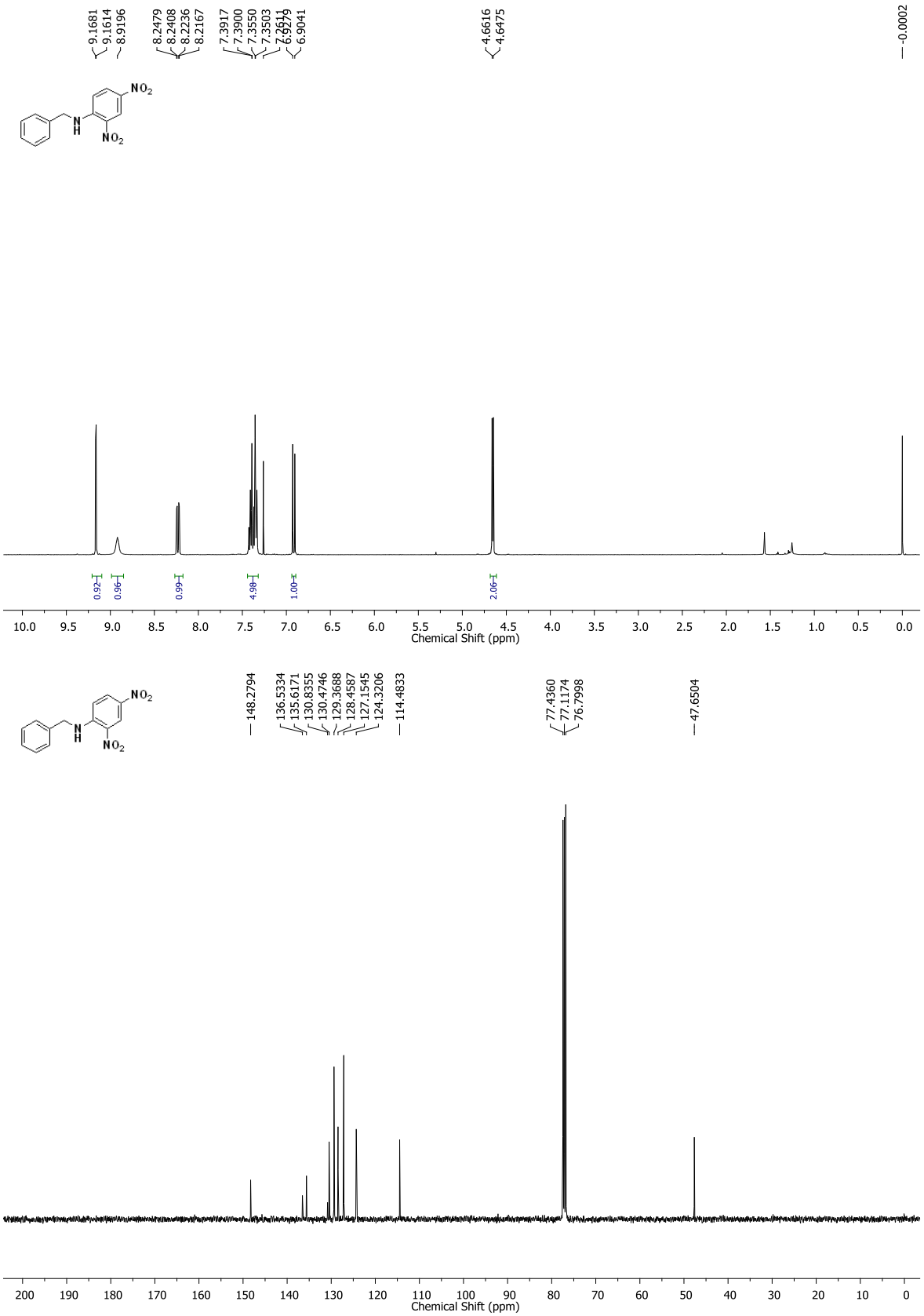


Figure S9. NMR spectra of **17**

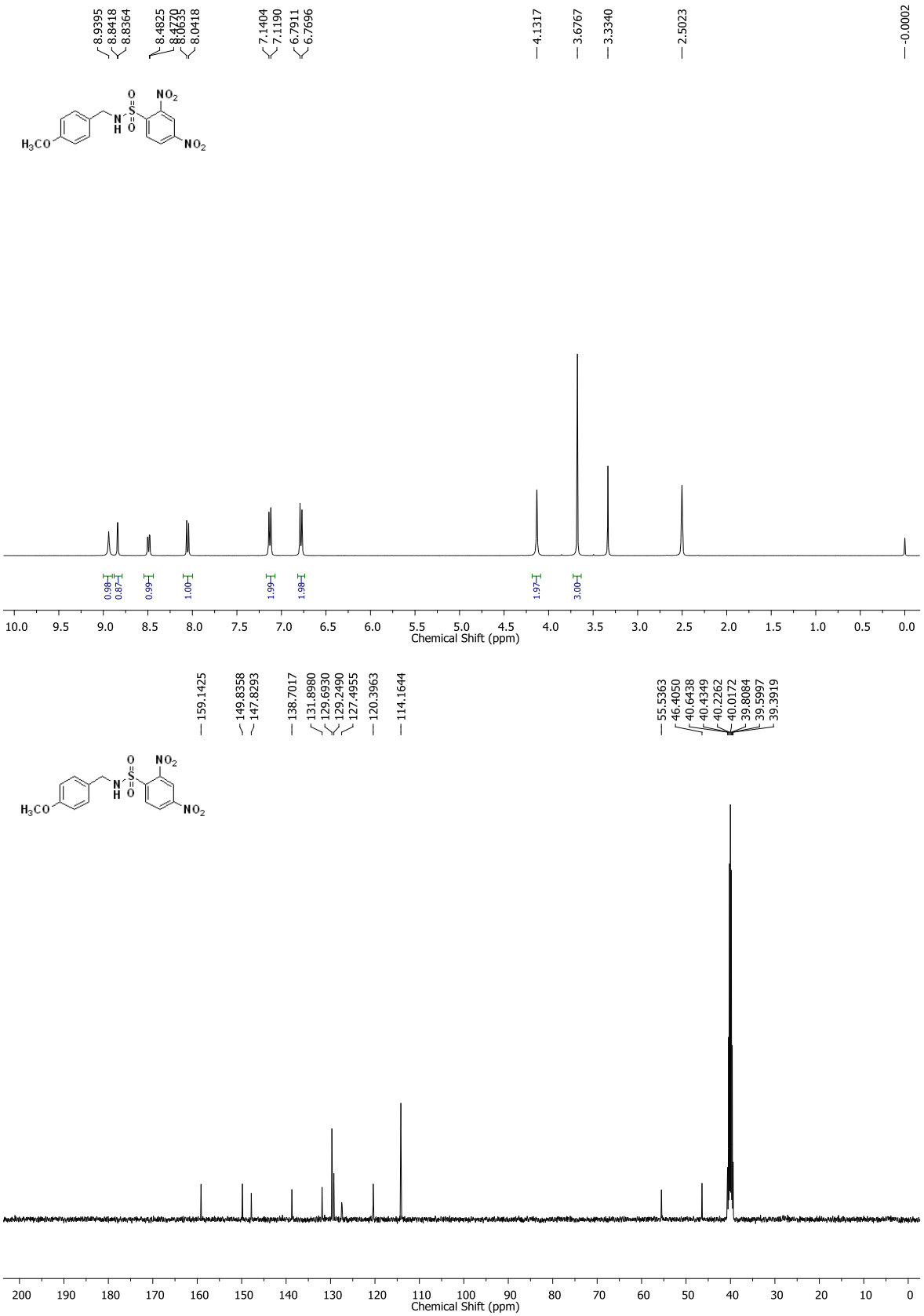


Figure S10. NMR spectra of **18**

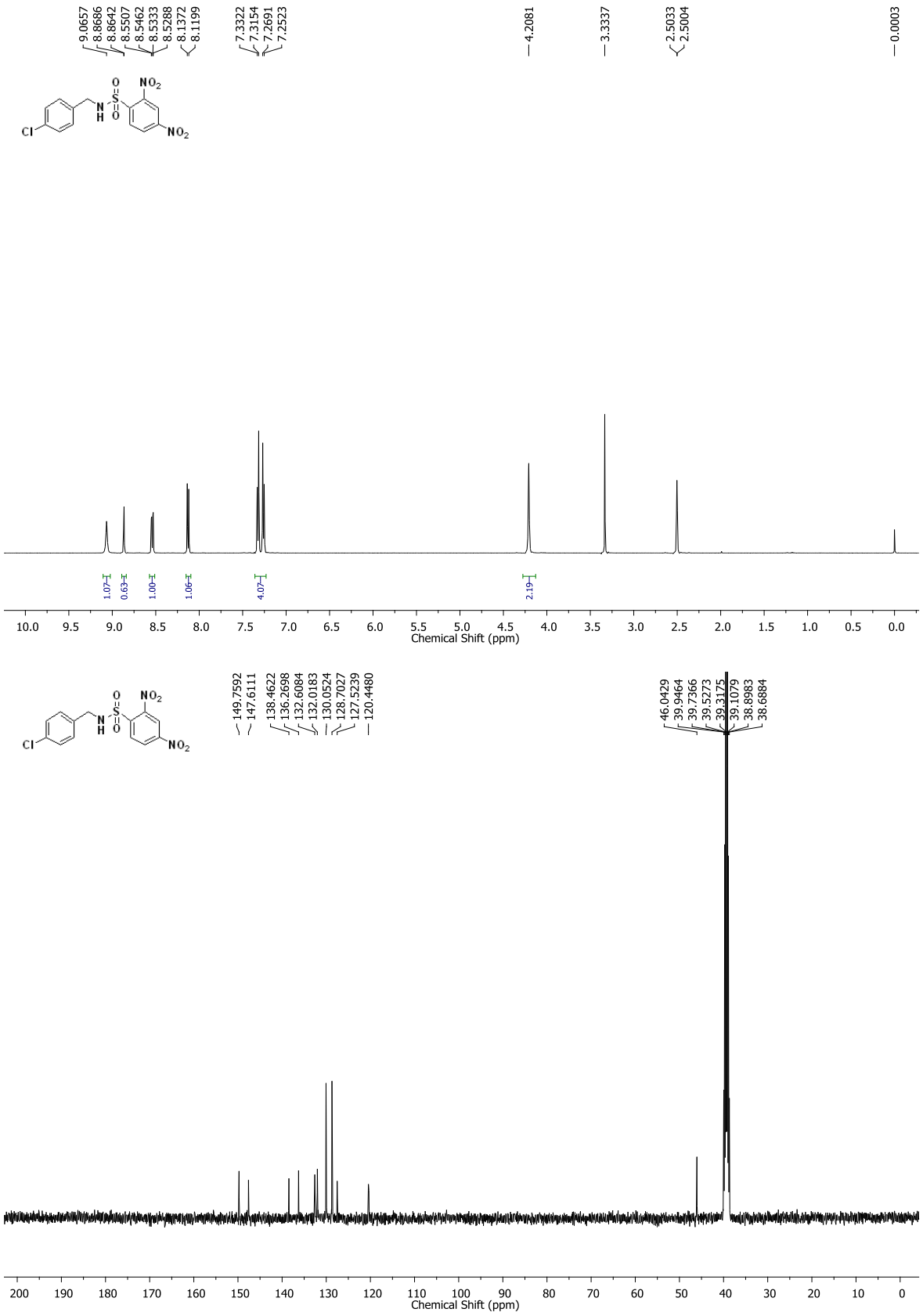


Figure S11. NMR spectra of **19**

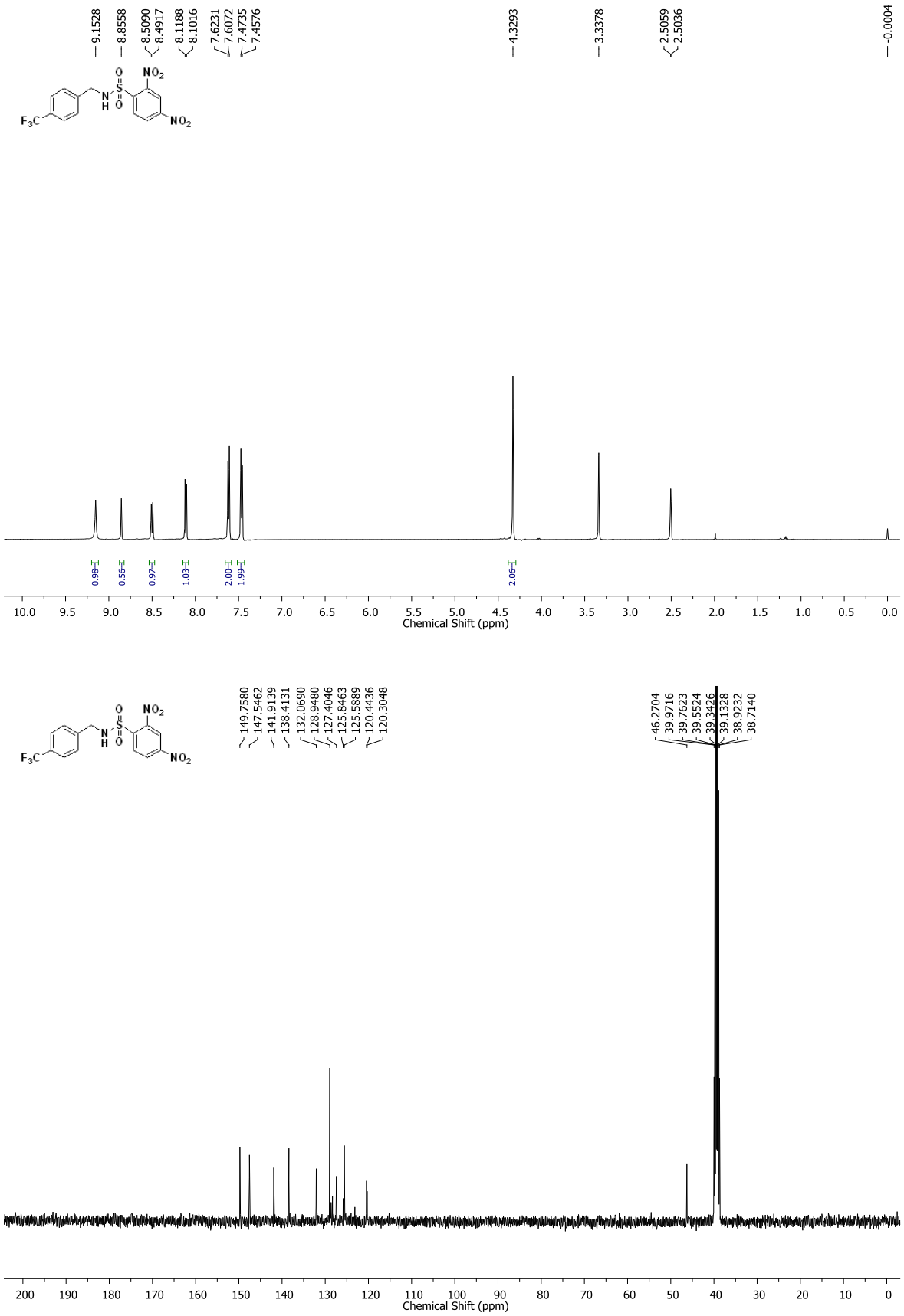


Figure S12. NMR spectra of **20**

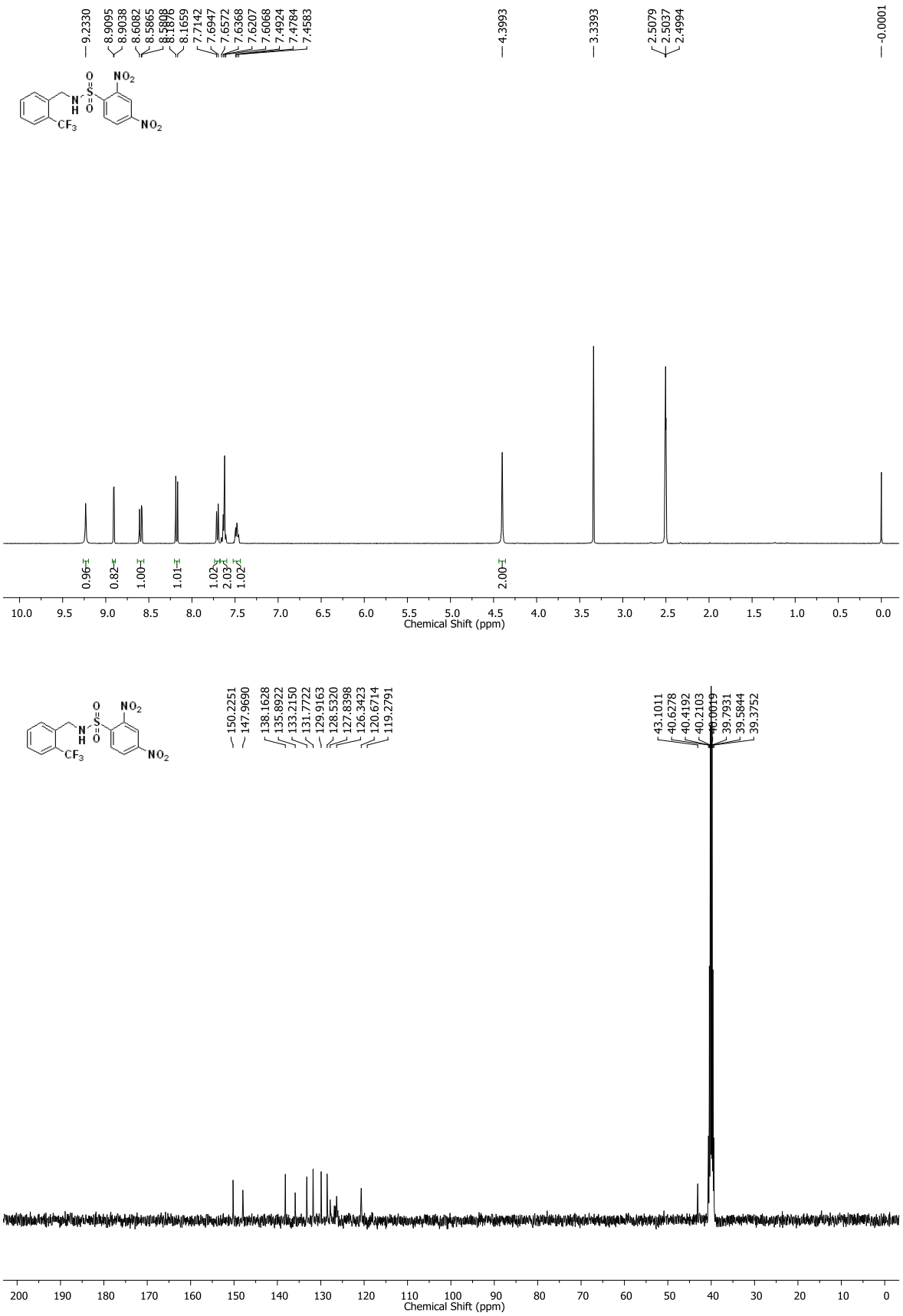


Figure S13. NMR spectra of **21**

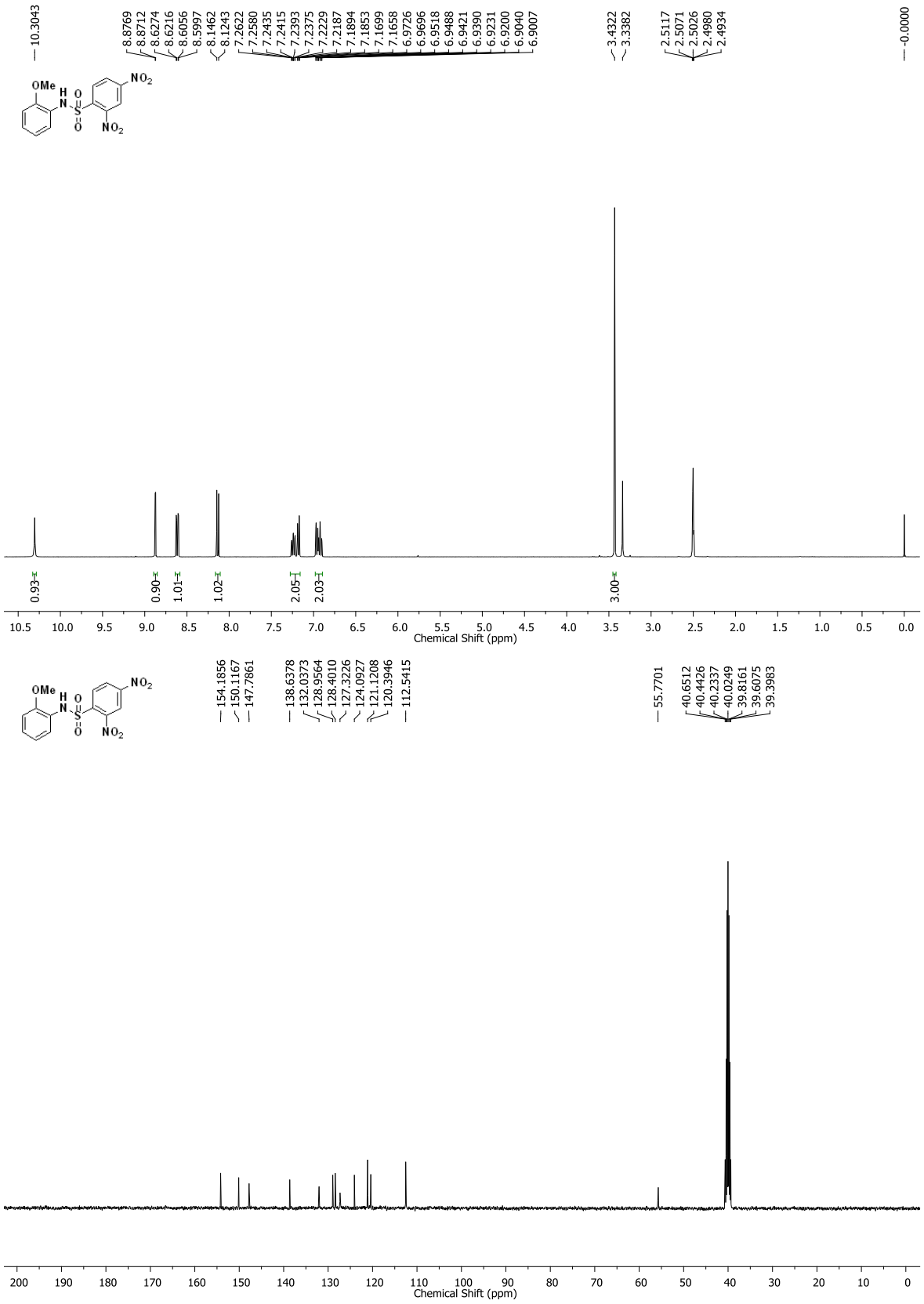


Figure S14. NMR spectra of **22**

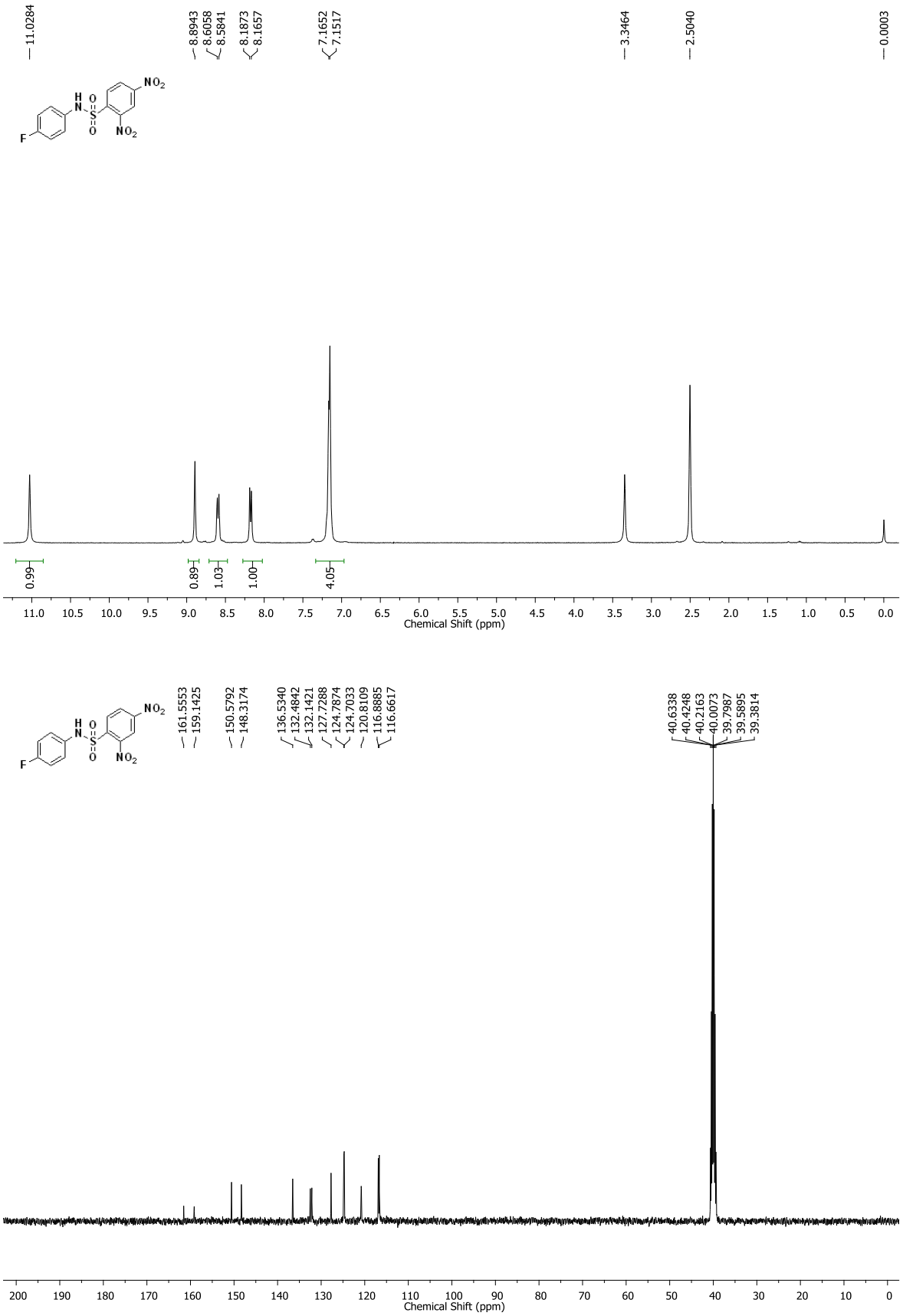


Figure S15. NMR spectra of **23**

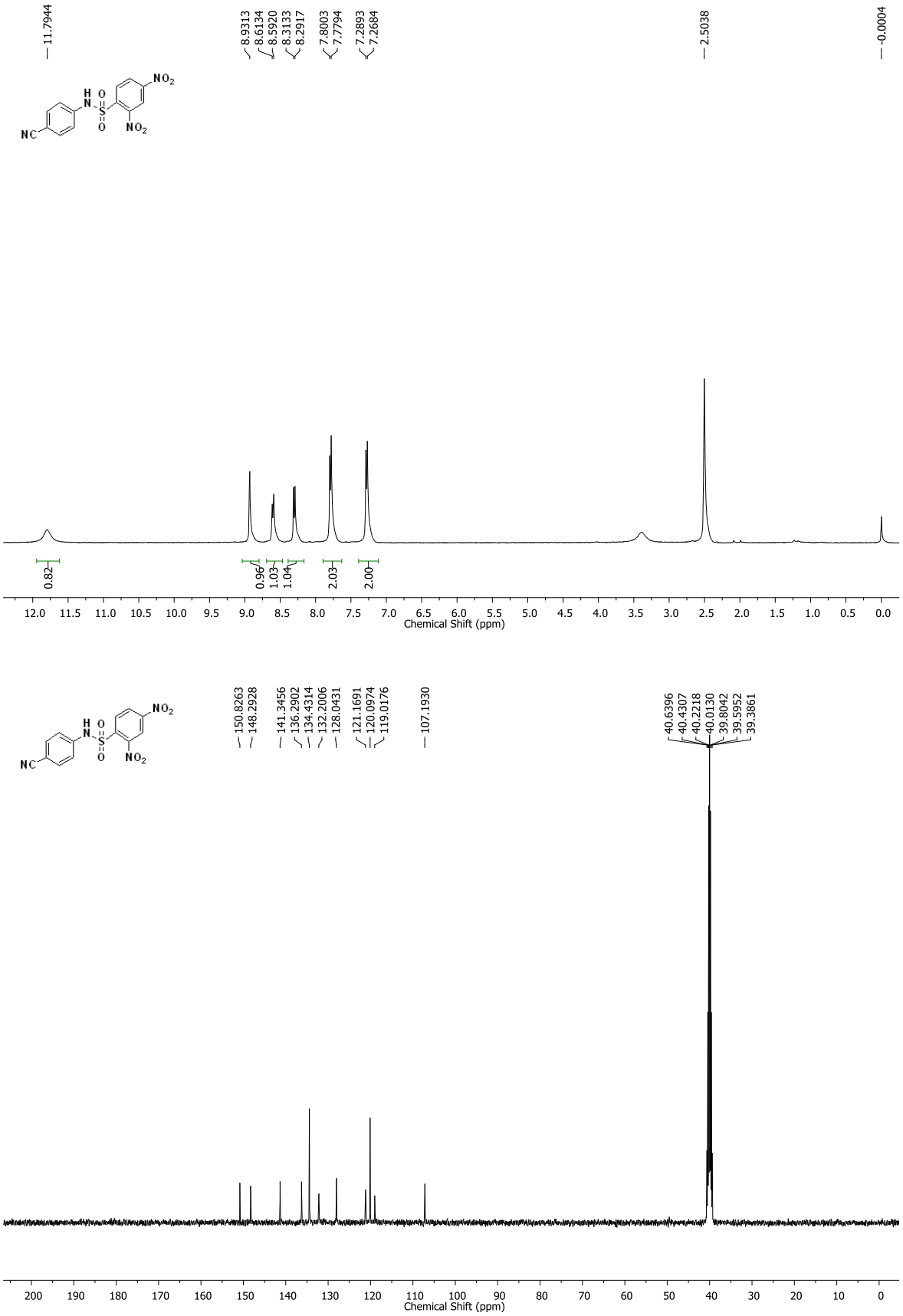


Figure S16. NMR spectra of **24**

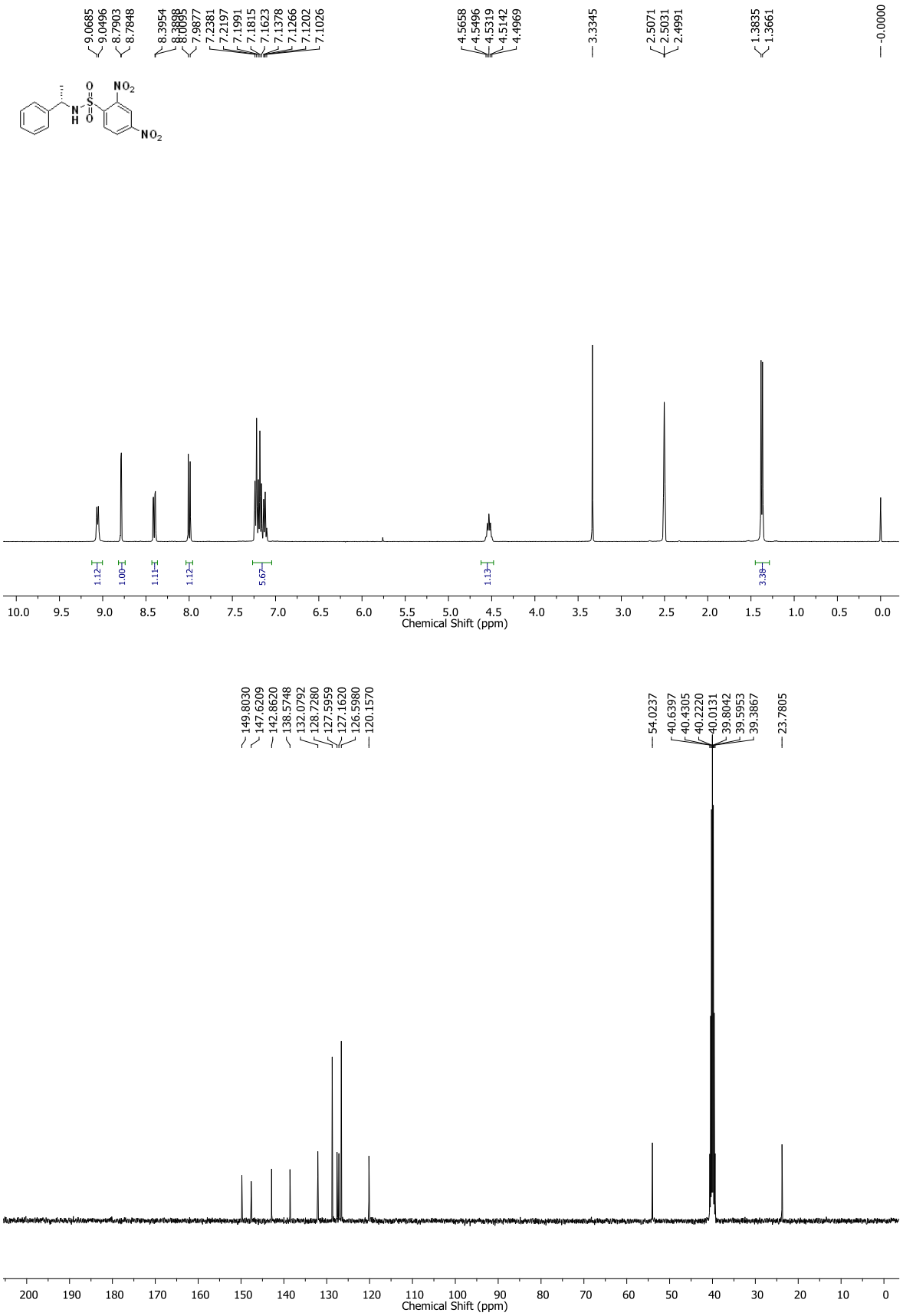


Figure S17. NMR spectra of **25**

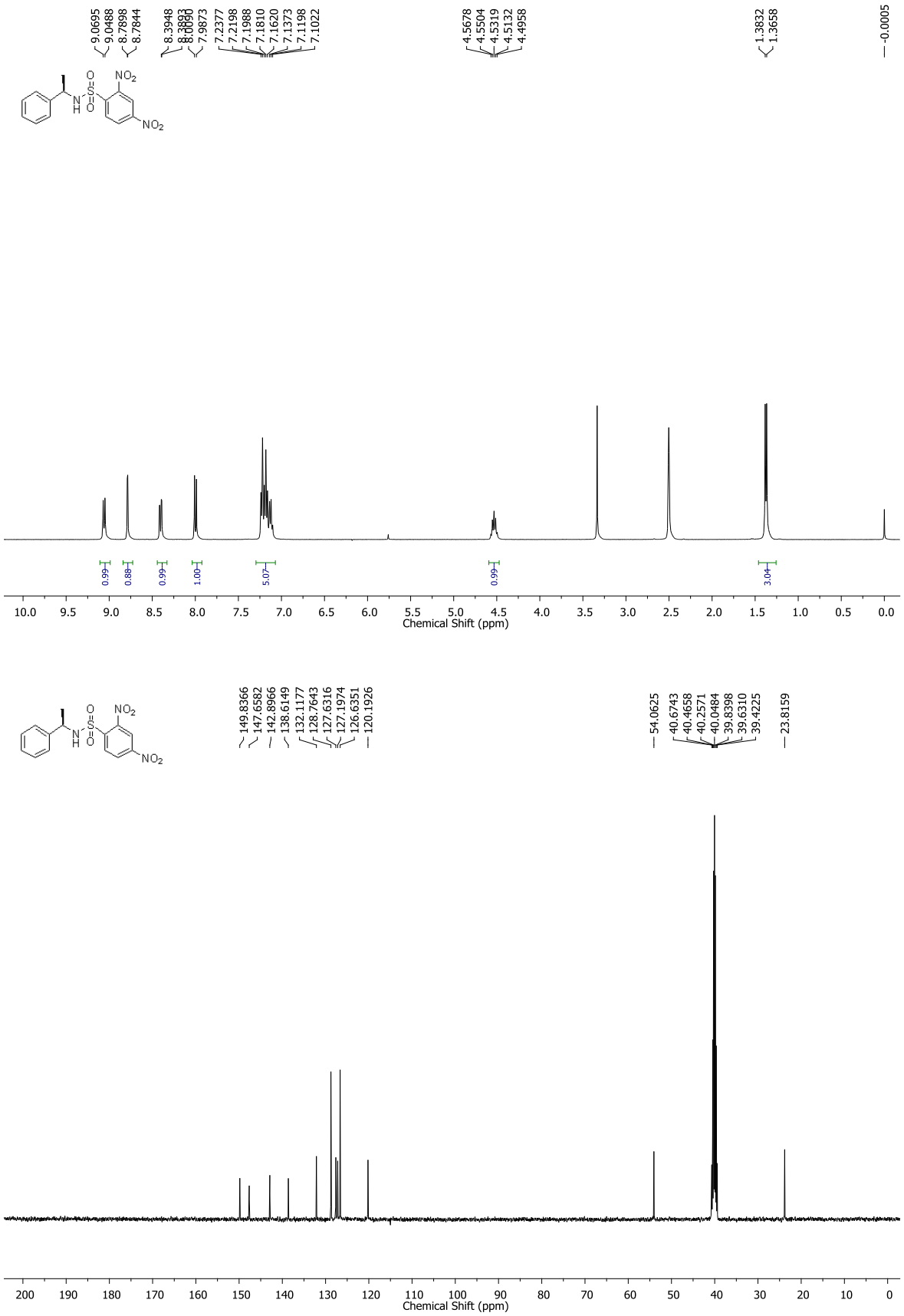


Figure S18. NMR spectra of **26**

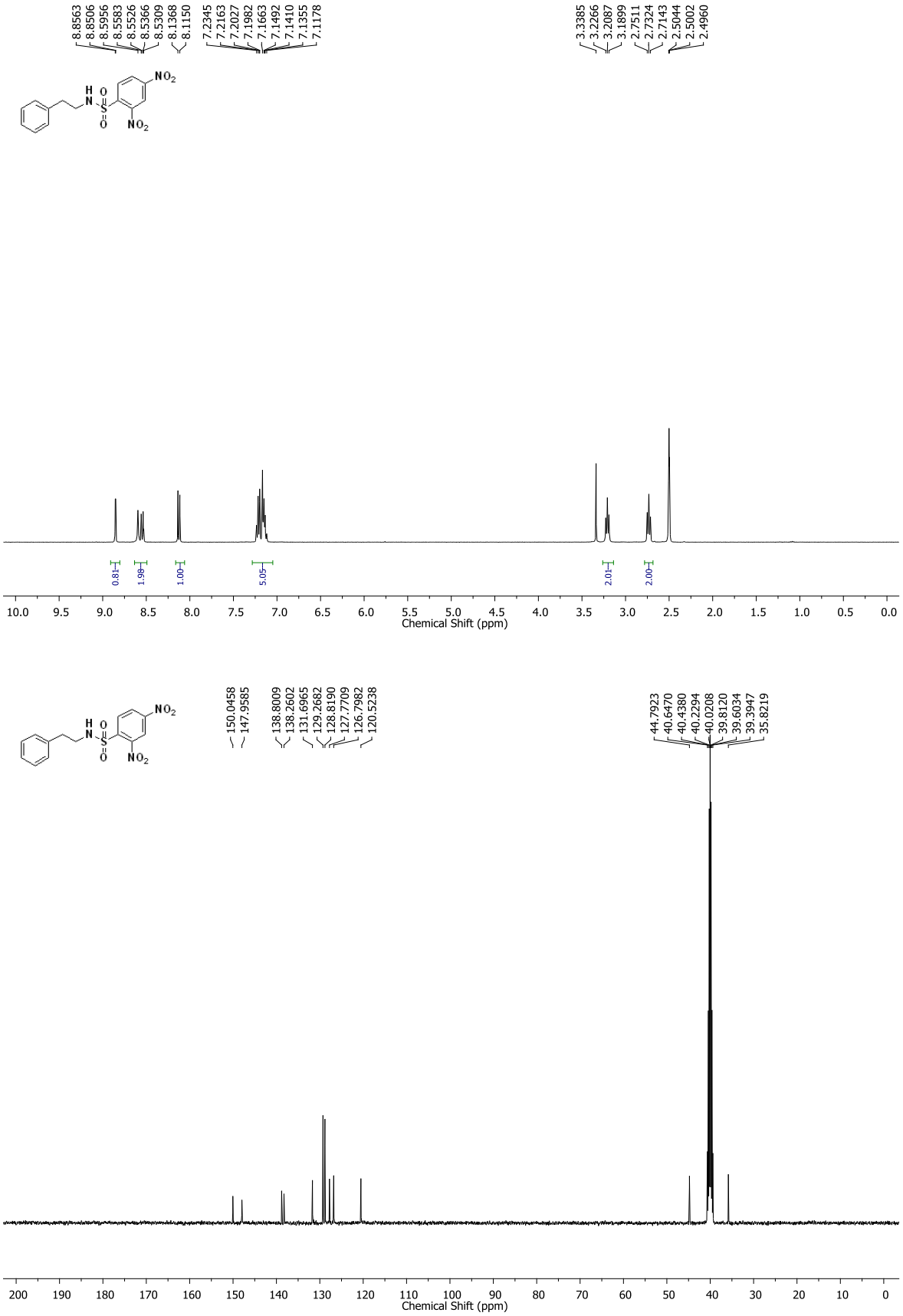


Figure S19. NMR spectra of **27**

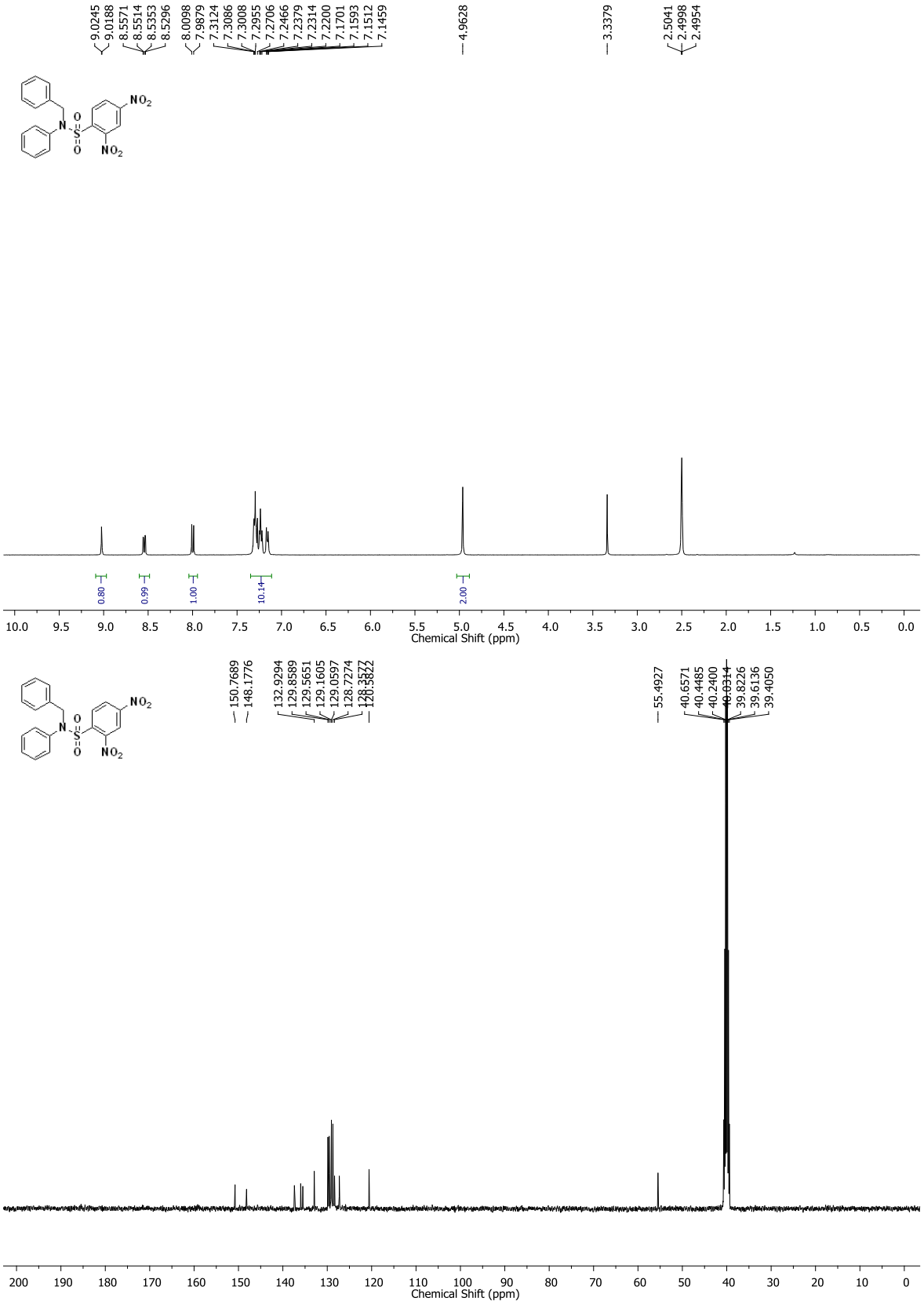


Figure S20. NMR spectra of **28**

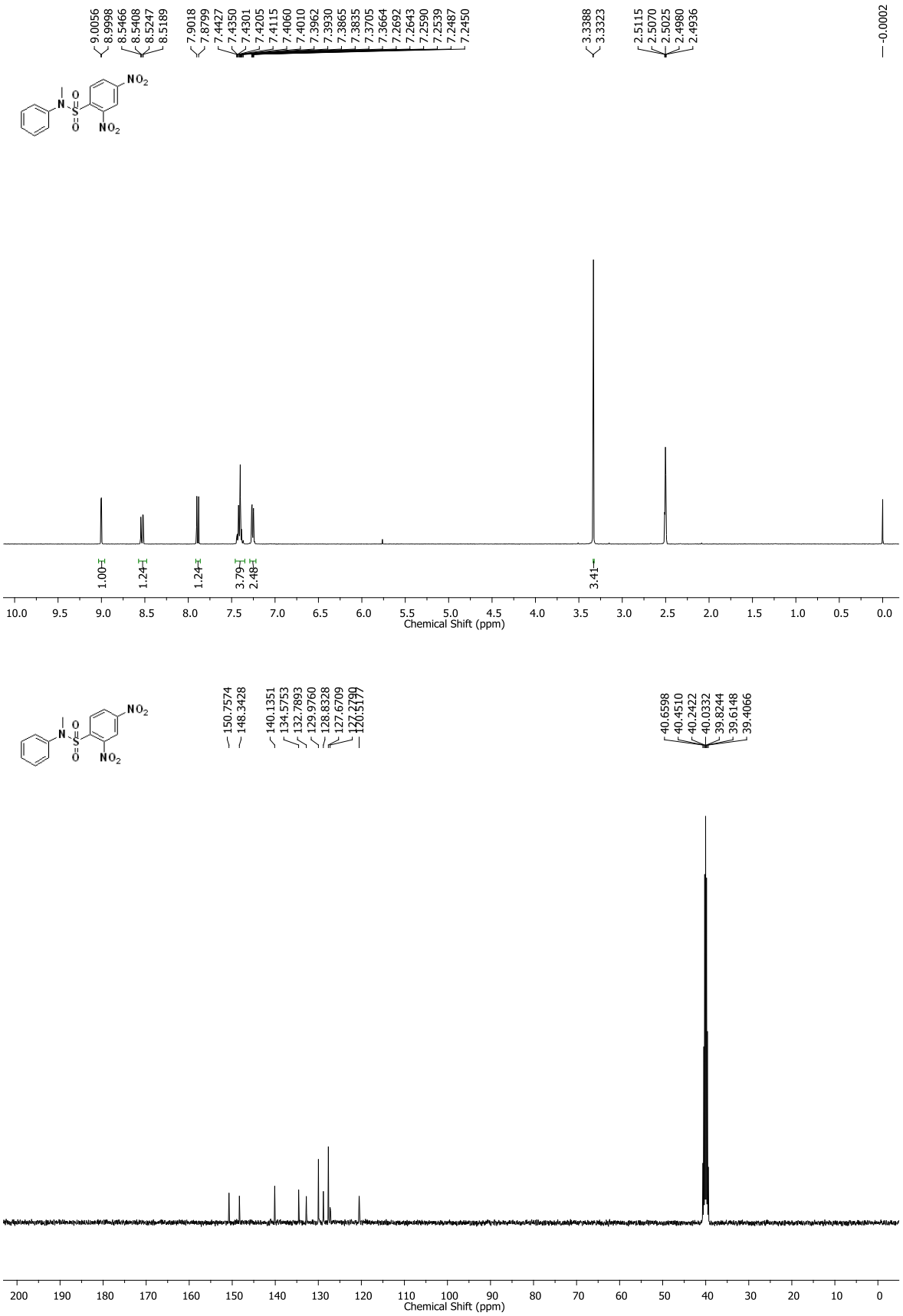


Figure S21. NMR spectra of **29**

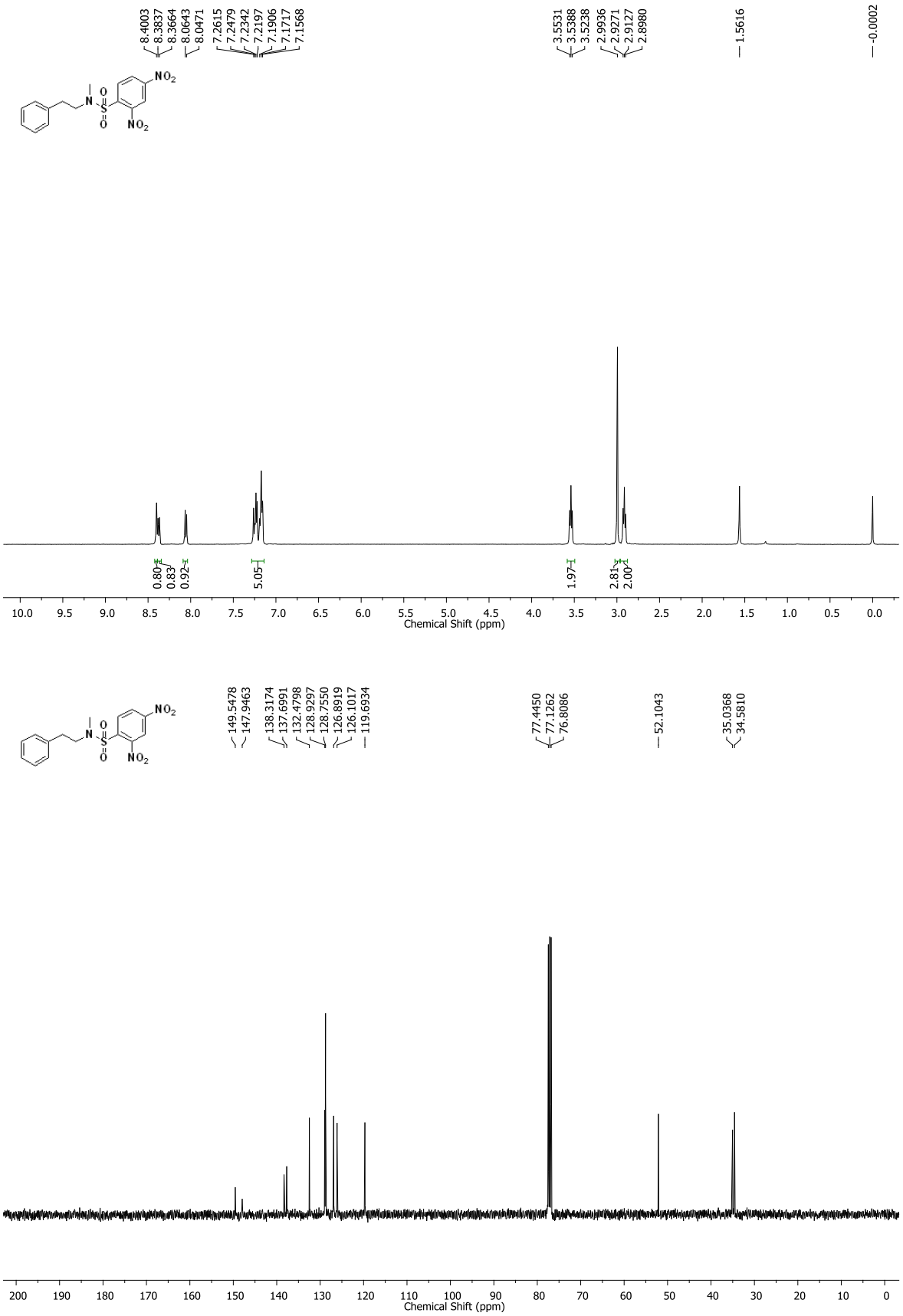


Figure S22. NMR spectra of **30**

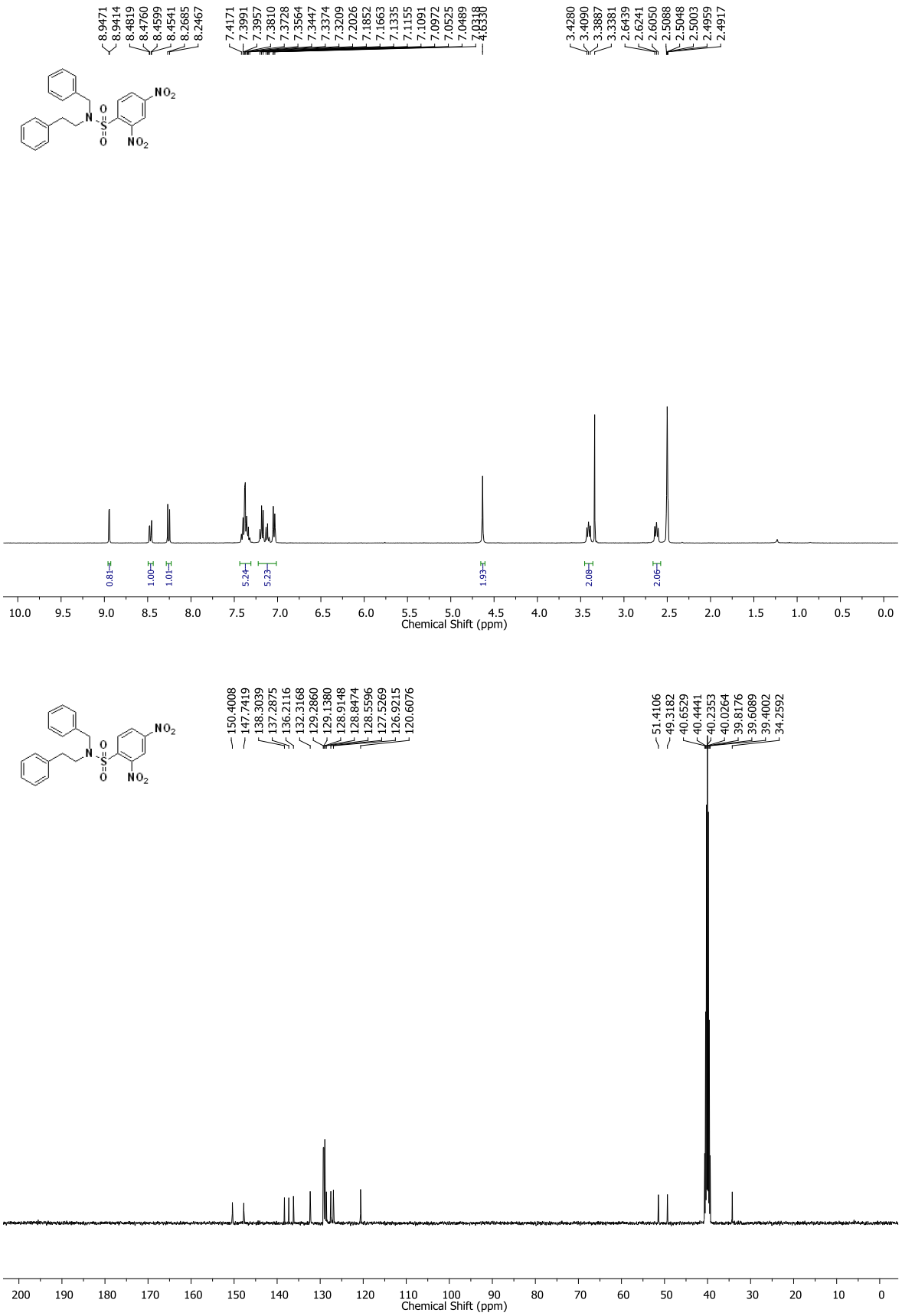


Figure S23. NMR spectra of **31**

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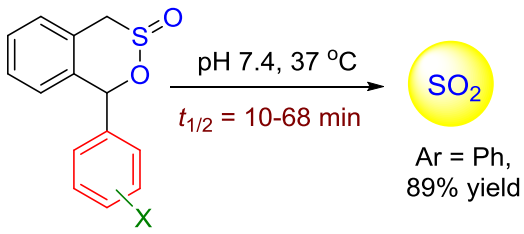
Chapter 3
Section B: Synthesis and Evaluation of 1,2-Cyclic Sulfite Diesters as SO_2 Donors

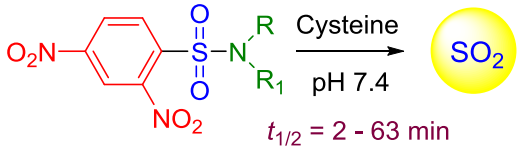
Chapter 3: Section B: Synthesis and Evaluation of 1,2-Cyclic Sulfite Diesters as SO_2 Donors

3B.1 Introduction: In Chapter 2, we have shown benzosultines as SO_2 donors with controlled rate of generation of SO_2 (Scheme 1). Benzosultines act as temperature dependent SO_2 donors under physiological condition.[1]



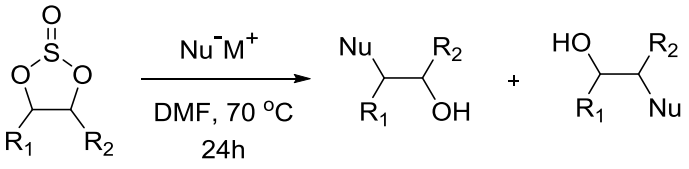
Scheme 1. Benzosultines as temperature dependent sulfur dioxide (SO_2) donors.

In section A of Chapter 3, 2,4-dinitrophenylsulfonamides as thiol activated SO_2 donors with potent *Mtb* inhibitory activity were prepared.[2,3] The rate of generation of SO_2 is important for observed inhibitory activity, where intracellular thiols act as a trigger for SO_2 generation (Scheme 2).



Scheme 2. 2,4-Dinitrophenylsulfonamides as thiol-activated sulfur dioxide (SO_2) donors.

Benzosultine synthesis is a multistep process and purification is challenging. In the case of 2,4-dinitrophenylsulfonamides thiol is used as a trigger, which may complicate the studies because targets of SO_2 include biologically relevant disulfides and thiols.[4,5] Svendsen *et al.* reported that 1,2-cyclic sulfite diesters undergoes nucleophilic substitution with various nucleophiles such as LiCl, NaCl, CsCl, triethylbenzylammonium chloride (TEBACl), triethylbenzylammoniumcyanide (TEBACN), NaN_3 and $(CH_3OOC)_2CH^-Na^+$ at one of the activated carbon atoms (Scheme 3).

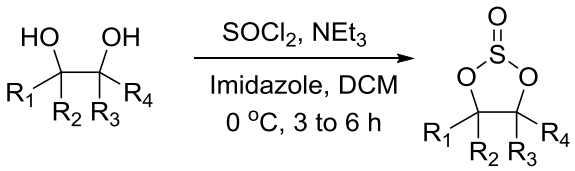


Scheme 3. Nucleophilic ring opening of 1,2-cyclic sulfite diesters

This reaction would produce sulfite monoester as the intermediate. Decomposition of this intermediate gives SO_2 and an alcohol. When reacted with a hard nucleophiles such as Grignard reagents, the reaction has a different outcome.[6,7] Here the nucleophile adds to the sulfite sulfur atom. We hypothesized that modulating substituents R_1 and R_2 would enable us to tune the rate of substitution by a nucleophile. We prepared various 1,2-cyclic sulfite diesters (Figure 1) with different substituents present on activated carbon atoms, for modulating the rate of generation of SO_2. The ability of cyclic diesters to act as SO_2 donors in pH 7.4 was evaluated.

3B.2 Results and discussion:

3B.2.1 Synthesis of 1,2-cyclic sulfite diester: Various 1,2-cyclic sulfite diesters were prepared starting from 1,2-diols by treating with thionyl chloride, triethylamine and imidazole in DCM at 0 °C (Scheme 4). [8,9]



Scheme 4. Synthesis of 1,2-cyclic sulfite diesters.

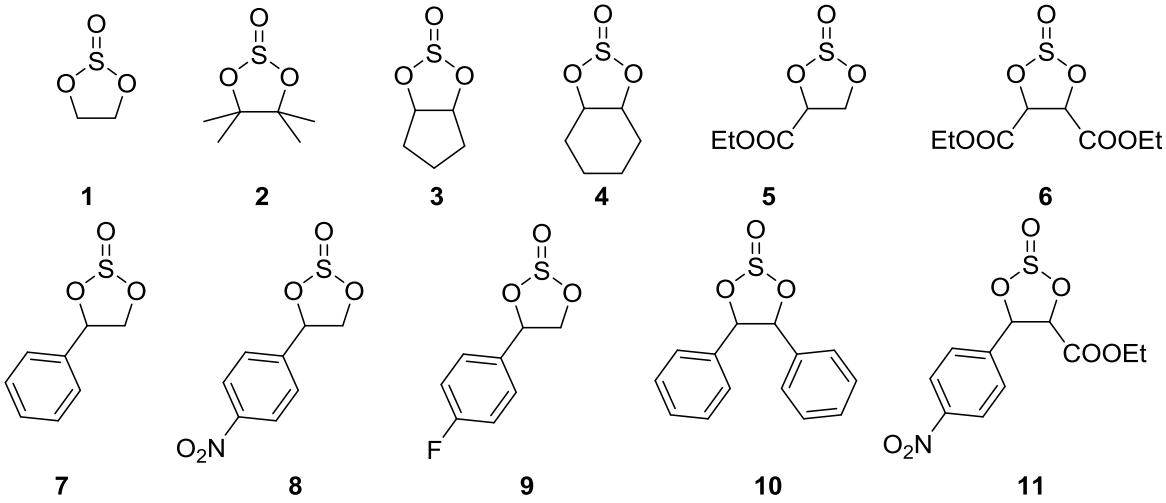
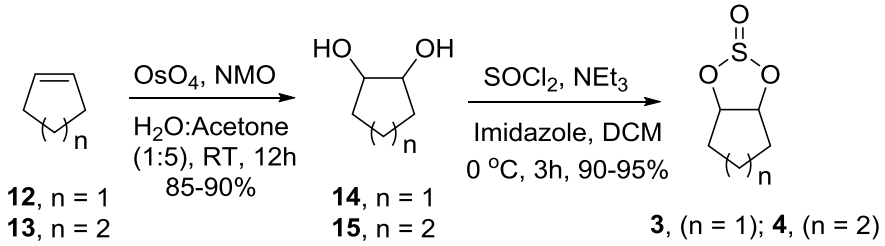


Figure 1. 1,2-Cyclic sulfite diesters prepared in this study.

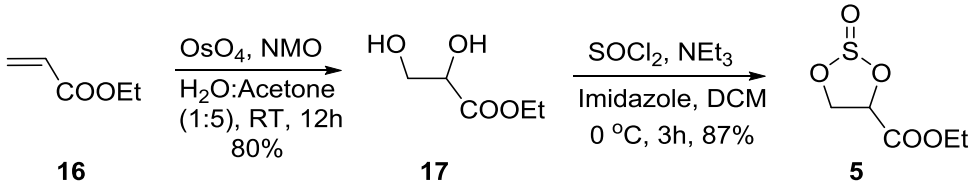
In order to study the effects of sterics on nucleophilic ring opening of 1,2-cyclic sulfite diesters ethylene glycol and pinacol sulfite diesters (**1** and **2**) were prepared by treating 1,2-diol with thionyl chloride, triethylamine and imidazole (Scheme 4). Similarly cyclic sulfite diester (**3** and **4**) of 1,2-cyclopentane diol and 1,2-cyclohexane diol were prepared. The respective diols **14** and **15** were prepared by OsO_4, NMO mediated

dihydroxylation of respective olefin (Scheme 5).[10] In order to study the effect of electron withdrawing group on nucleophilic ring opening of diester, we prepared carboethoxy diester **5**, from 1,2-diol of ethyl acrylate (Scheme 6) and diethyl tartarate diester **6** starting from L(+)-tartaric acid.



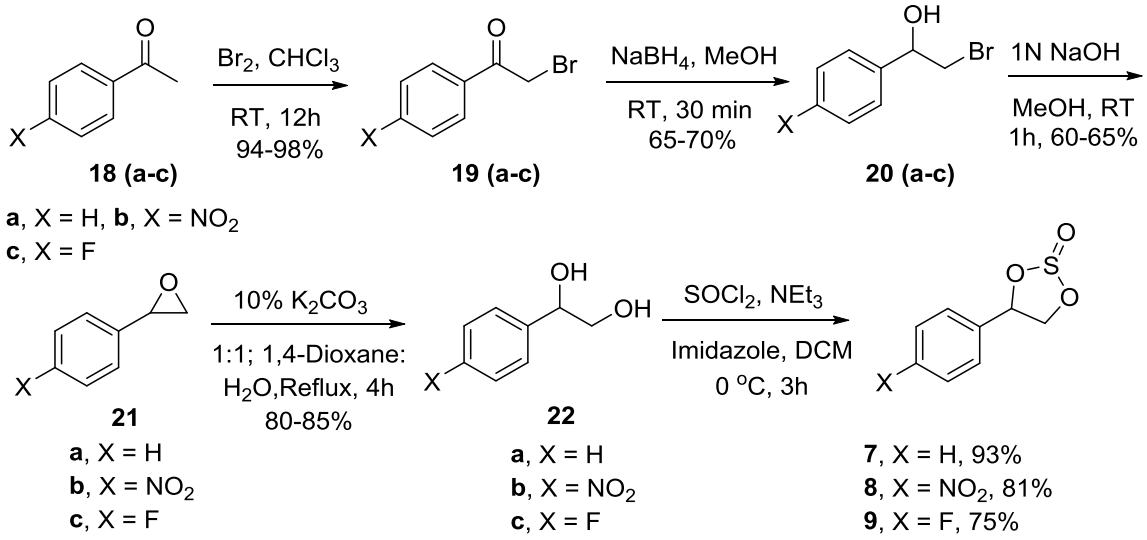
Scheme 5. Synthesis of sulfite diesters **3** and **4**.

In order to study the effect of phenyl ring on nucleophilic ring opening of cyclic sulfite diester we prepared various derivatives **7-11** (Figure 1).



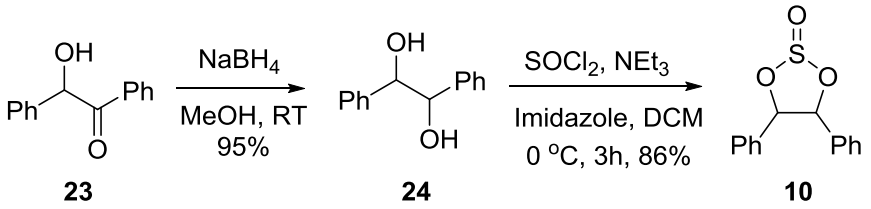
Scheme 6. Synthesis of sulfite diester **5**.

The mono phenyl diester **7** was prepared in two steps. First, styrene oxide opening was effected in basic condition (10% K_2CO_3)[11] to give 1,2-diol **22a**, followed by $SOCl_2$ mediated cyclization to give **7** (Scheme 7).



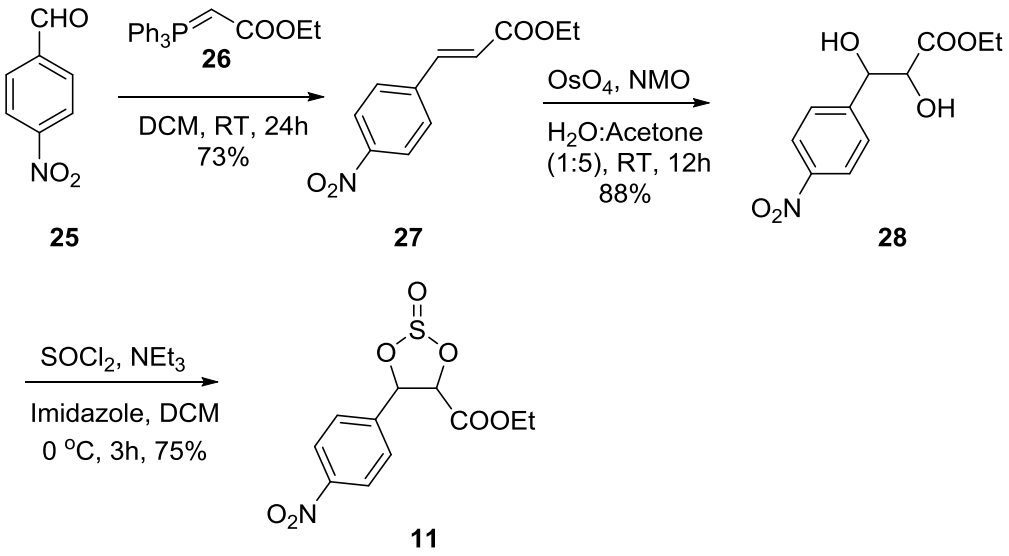
Scheme 7. Synthesis of 1,2-cyclic sulfite diesters **7**, **8** and **9**.

To study the effect of presence of electron withdrawing group on phenyl substituent on nucleophilic ring opening of 1,2-cyclic sulfite diesters, we prepared 4-NO_2- phenyl derivative **8** starting from 4-nitroacetophenone (Scheme 7). The 4-NO_2-phenacyl bromide was prepared by bromination of 4-nitroacetophenone.[12] Sodium borohydride ($NaBH_4$) mediated reduction of **19b**, followed by displacement of bromide under basic conditions (1N NaOH) gave 4-nitro styrene oxide **21b**. $SOCl_2$ mediated cyclization of the resulting 1,2-diol **22b** obtained by epoxide opening gave sulfite diester **8**. Similarly 4-fluoro-phenyl derivative **9** was prepared starting from 4-fluoroacetophenone (Scheme 7). The diphenyl derivative **10** was prepared by reduction of benzoin to give 1,2-diol **24**, followed by $SOCl_2$ mediated cyclization to give **10** (Scheme 8).



Scheme 8. Synthesis of 1,2-cyclic sulfite diester **10**.

Compound **11** was prepared starting from 4-nitrobenzaldehyde (Scheme 9). The 1,2-diol **28** was prepared from olefin **27**, obtained by the Wittig reaction of 4-nitrobenzaldehyde and phosphorane **26**. The cyclic sulfite diester **11** obtained by $SOCl_2$ mediated cyclization.



Scheme 9. Synthesis of compound **11**.

All 1,2-cyclic sulfite diesters were obtained in yields between 75-98% (Table 1).

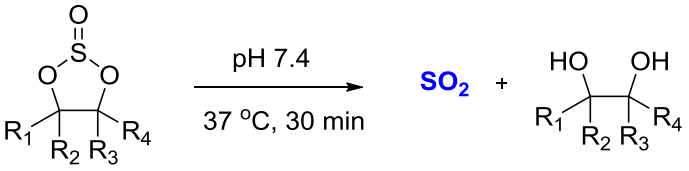
Table 1. 1,2-Cyclic sulfite diesters yields.

Entry	R_1	R_2	R_3	R_4	Compound	Yield (%)
1	-H	-H	-H	-H	**1**	78
2	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	**2**	96
3	$-(CH_2)_3-$	-H	$-(CH_2)_3-$	-H	**3**	90
4	$-(CH_2)_4-$	-H	$-(CH_2)_4-$	-H	**4**	69
5	-COOEt	-H	-H	-H	**5**	87
6	-COOEt	-H	-COOEt	-H	**6**	80
7	-Ph	-H	-H	-H	**7**	93
8	$4\text{-}NO_2\text{-}C_6H_4-$	-H	-H	-H	**8**	81
9	$4\text{-}F\text{-}C_6H_4-$	-H	-H	-H	**9**	75
10	-Ph	-H	-Ph	-H	**10**	86
11	$4\text{-}NO_2\text{-}C_6H_4-$	-H	-COOEt	-H	**11**	75

3B.2.2 Evaluation of 1,2-cyclic sulfite diesters as SO_2 donors in physiological conditions: The aforementioned derivatives **1-11** were evaluated for SO_2 release in pH 7.4 phosphate buffer. First, ethylene glycol derivative **1** was incubated at 37 °C in pH 7.4 buffer for 30 min. The reaction was monitored for SO_2 generation by ion chromatography equipped with an ion conductivity detector;[2] SO_2 was quantified as sulfite, SO_3^{2-}. After 30 min, **1** gave 45% of SO_2 (Table 2, entry 1). The pinacol derivative **2** on the other hand produced negligible amounts of SO_2 and a 2% yield was recorded (Table 2, entry 2). These results suggest that increasing sterics on the carbon bearing the sulfite functional group reduced the propensity for decomposition of the compound. Thus, it appears that SO_2 generation involves a displacement at the carbon bearing the sulfite functional group. Next, decomposition studies of cyclopentane and cyclohexane derivatives **3** and **4** were conducted and 25% and 21 % SO_2 yields, respectively, were recorded. These yields were lower in comparison with **1** but higher than the SO_2 yields from **2**. Again, this result is consistent with sterics being an important determinant of SO_2 yield.

Decomposition of diesters **5** and **6** gave 93% and 98% SO_2, respectively, after 30 min. These compounds contain an electron withdrawing substituent as compared to ethylene glycol diester **1**. The electron withdrawing nature of the ester would increase electrophilicity of carbon bearing the sulfite functional group thus resulting in an increased rate of displacement. Next, decomposition of derivatives with phenyl substituent (**7-11**) was carried out. After 30 min, the phenyl derivative **7** gave 73% of SO_2 (Table 2, entry 7).

Table 2. Sulfur dioxide yields and p*K*a of 1,2-diols.



Entry	Compd	% SO_2 yield after 30 min	p*K*a[a]
1	**1**	45	14.40
2	**2**	2	14.23
3	**3**	25	14.25
4	**4**	21	14.27
5	**5**	93	11.91
6	**6**	98	10.64
7	**7**	73	13.83
8	**8**	96	13.48
9	**9**	68	13.73
10	**10**	83	13.70
11	**11**	95	11.53

[a]Values are for the corresponding 1,2-diol (most acidic proton) calculated using ChemBioDraw Ultra 14.0.

The 4-NO_2- phenyl derivative **8** on the other hand produced higher amount of SO_2 and a 96% yield was obtained (Table 2, entry 8). The 4-F-phenyl derivative **9** gave nearly similar SO_2 yield as that of phenyl derivative **7** (Table 2, entry 9). These results suggest that electron withdrawing group on phenyl substituent increases the rate of decomposition, again consistent with a direct displacement mechanism. The diphenyl derivative **10** gave 83% SO_2 after 30 min (Table 2, entry 10). Thus, presence of diphenyl substituent gave slightly increased yields of SO_2. The derivative having 4-NO_2-phenyl and ester group, **11** produced higher amount of SO_2 and a 95% yield was obtained (Table 2, entry 11).

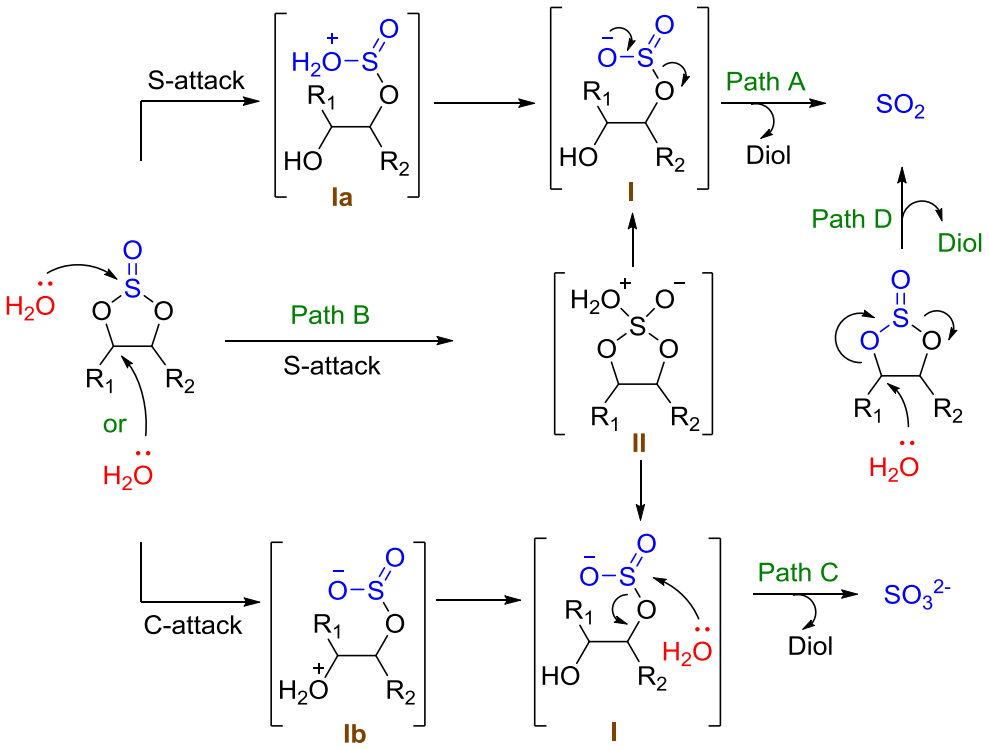
The 1,2-diols used for synthesis of sulfite diesters **1-4**, have nearly similar pK_a values for the most acidic proton (Table 2, entries 1-4). The diesters **1**, **3** and **4** gave SO_2 yields > 20% after 30 min, whereas pinacol derivative **2** gave 2% of SO_2. These results suggest that when leaving group was similar the important determinant of observed reaction rates was sterics. Sulfite diesters **5** and **6** having electron withdrawing ester group present on activated carbon has pK_a values of 11.91 and 10.64, lesser than ethylene glycol (14.40), gave nearly quantitative SO_2 yields after 30 min (Table 2, entries 5 and 6). Thus, when electron withdrawing ester group was present on activated carbon atoms, would

increase electrophilicity of activated carbon thus resulting in decreased pK_a values of 1,2-diols and increased rate of displacement. These results support pK_a values being as an important factor affecting observed SO_2 yields. The sulfite diester derivatives with phenyl substituent **7**, **9** and **10** having comparable pK_a values (13.83, 13.73 and 13.70) gave nearly similar SO_2 yields (Table 2, entries 7, 9 and 10). The diester **8** with 4-NO_2-phenyl substituent and **11** having 4-NO_2-phenyl as well as ester substituent, has pK_a values (13.48 and 11.53) lesser than ethylene glycol (14.40) gave almost quantitative SO_2 yields (Table 2, entries 1, 8 and 11). These results support sterics and pK_a being as important determinants of SO_2 yields.

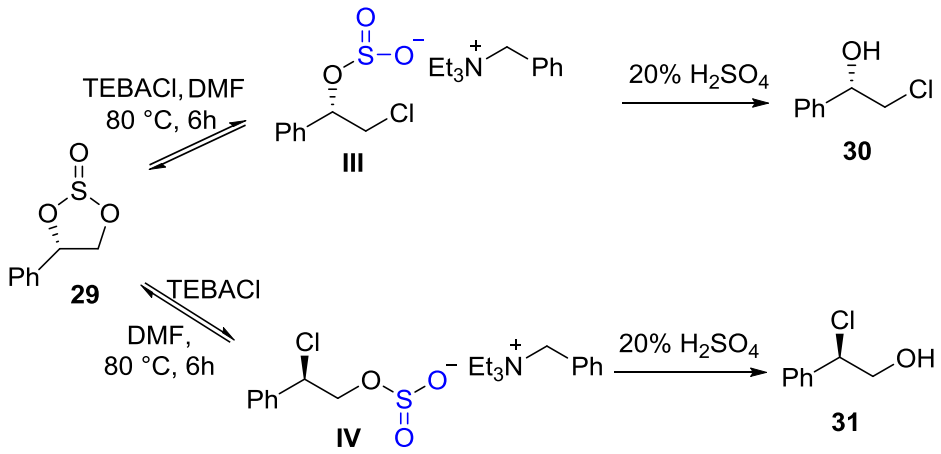
3B.2.3 Mechanisms for nucleophilic ring opening of 1,2-cyclic sulfite diesters: Four possible modes for decomposition of cyclic sulfite diester under aqueous conditions were considered (Scheme 10). The cyclic sulfite is a candidate for nucleophilic attack.[13,14] Sulfite diester undergoes ring opening by nucleophilic attack of H_2O either on activated carbon atoms or sulfite sulfur atom to give identical sulfite monoester intermediate **I** or intermediate **II** (Scheme 10). With cyclic sulfites, the presence of an unshared pair of electrons on sulfur partially suppresses the double bond character of the sulfur atom and the ring oxygen atoms. Thus, in the nucleophilic substitution of cyclic sulfites, attack at the sulfur atom competes with substitution at carbon.[14]

The intermediate **I** can decompose by path A or C, to give sulfur dioxide (SO_2) or its hydrated form sulfite (SO_3^{2-}) and 1,2-diol as decomposition products. In path A, an intramolecular attack of the O^- of sulfite monoester **I** on the sulfur atom take place to give SO_2 and respective diol (Scheme 10). In the path C, sulfur atom of sulfite monoester **I** was attacked by water to give sulfite (SO_3^{2-}), which is the hydrated form of sulfur dioxide in pH 7.4 buffer (Scheme 10).

Svendsen *et al.* have shown that, when the sulfite diester **29** was treated with triethylbenzylammonium chloride (TEBACl) the formation of the chlorohydrin was the dominant outcome. Despite being a harder nucleophile, Cl^- reacts at C-4 as well as C-3 instead of sulfite sulfur atom to give mixture of chlorohydrin **30** and **31** via sulfite monoester intermediates, with overall yield of 90% (Scheme 11).[6] Thus, nucleophilic attack of harder nucleophile on activated carbon atoms of sulfite diester could be possible.



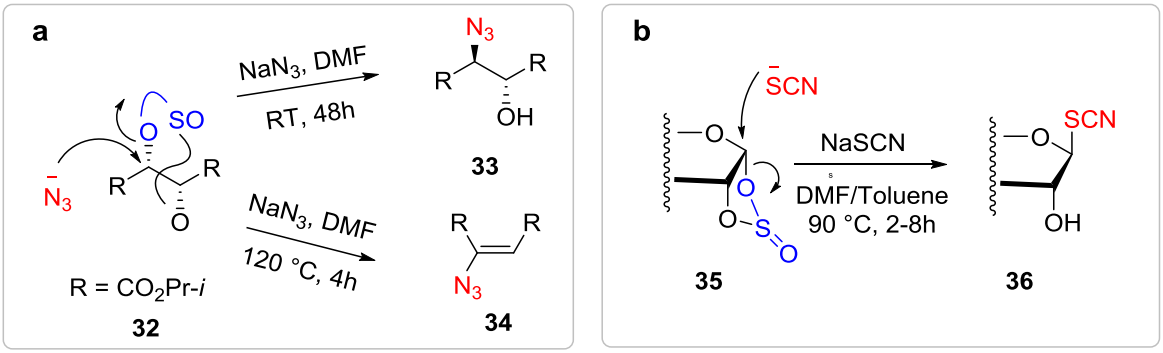
Scheme 10. Possible mechanisms for decomposition of cyclic sulfite diester to give SO_2/SO_3^{2-}.



Scheme 11. Reaction of cyclic sulfite **25** with TEBACl.

The sulfite monoester intermediated **III** formed (Scheme 11), in 20% H_2SO_4 gave chlorohydrin **30** with the retention of configuration, indicating that water doesn't attack on the benzylic carbon atom. The intermediate **III**, can rearrange intramolecularly to give SO_2 or nucleophilic attack of water on sulfite sulfur atom of **III** could generate SO_3^{2-} instead of SO_2.

Various cyclic sulfites react with sodium or lithium azide at elevated temperature, where nucleophilic attack of azide takes place on activated carbon to give azido alcohols in high yields (Scheme 12a).[15] When 1,2-O-sulfinyl-α-D-glycopyranose or furanose derivatives were treated with sodium thiocyanate, stereospecific reaction at the anomeric carbon with inversion of configuration was observed, supporting nucleophilic attack on activated carbon to give exclusively 1,2-trans glycoside **36** (Scheme 12b).[16]



Scheme 12. Reaction of cyclic sulfite diesters with NaN_3 and NaSCN.

These results suggest that in case of pinacol derivative **2**, sterics may be decreasing the rate of nucleophilic displacement by H_2O on activated carbon atoms and thus, negligible amount of SO_2 was generated. Whereas in case of ethylene glycol diester **1**, due to absence of steric effect the nucleophilic attack of water on activated carbon atoms favoured, thus giving higher SO_2 yields as compare to pinacol diester **2**.

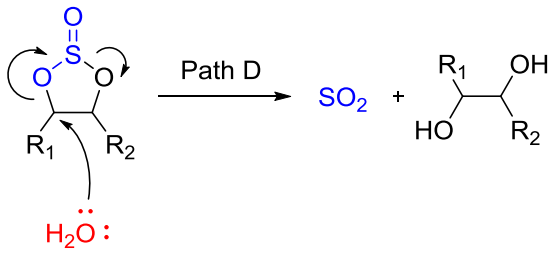
When decomposition study of pinacol derivative **2** was carried out, after 30 min incubation at 37 °C in pH 7.4 buffer, 2 % SO_2 was obtained, whereas ethylene glycol derivative **1** gave 45% of SO_2 after 30 min (Table 2, entries 1 and 2). Due to similar p*K*a values of pinacol and ethylene glycol, it is unlikely that their leaving group ability is very different.

If nucleophilic attack of H_2O takes place on the sulfite sulfur atom, common sulfite monoester intermediate **I** will be generated, same is also formed by nucleophilic attack of H_2O on activated carbons. The sulfite monoester will presumably decompose to give a SO_2 yield higher than actual 2% yield of SO_2. These results suggest that nucleophilic attack of water on activated carbon is more favoured over attack on the sulfur atom of sulfite functional group.

Once formed the sulfite monoester intermediate **I** can decompose by two different paths A or B to give SO_2 or SO_3^{2-}(Scheme 10). At moment whether path A or B is favoured is not clear, but as SO_3^{2-} is hydrated form of SO_2 in physiological pH, even if

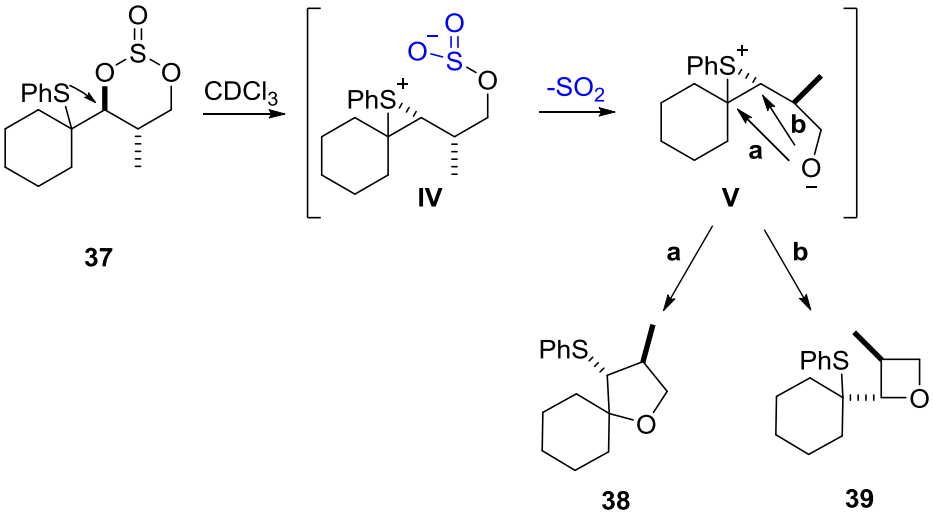
path B is favoured over path A, sulfite diester would be helpful for generating the hydrated form of SO_2.

Alternatively a concerted mechanism for generation of SO_2 is also possible (Scheme 13).



Scheme 13. Concerted SO_2 release by nucleophilic ring opening.

Warren *et al.* have shown that, during attempted synthesis of cyclic sulfite diester **37**, oxetanes **38** and **39** in a 97:3 ratio were formed instead of **37**. The sulfite diester **37** presumably decomposes in concerted manner to give SO_2 and oxetanes.[17] When decomposition was followed by NMR, the reaction mixture gave 80:20 ratio of **38**:**39** and sulfite monoester intermediate was not observed. These results indicate that concerted pathway could be possible during nucleophilic displacement reaction of sulfite diester.



Scheme 14. Rearrangement of cyclic sulfite **37** to give oxetanes with loss of SO_2.

When HPLC study of decomposition of cyclic sulfite diester **8** (Figure 1) was carried out in pH 7.4, showed % compound decomposed giving nearly similar % of SO_3^{2-} (followed by ion chromatography). These results suggest that under these reaction conditions, no intermediate was formed during decomposition and SO_2 is likely to be

generated in a concerted manner via path D (Scheme 14). Thus, path B could be disfavoured as no intermediate was observed. Further investigations are underway in order to understand which mechanistic pathway is more favourable.

3B.3 Conclusions:

In conclusion, we prepared various 1,2-cyclic sulfite diesters as SO_2/SO_3^{2-} donors under physiological conditions, with controlled rate of generation. The rate of reaction can be modulated by varying substituents present on the activated carbon atoms.

3B.4 Experimental Section

General: The 1,2-cyclic sulfite diesters **1**,[20] **4**,[21] **6**,[22] **7**,[23] and **10**[23] were previously reported and our data matched with reported spectral data.

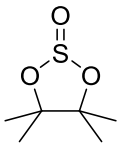
General procedure A: To a solution of 1,2-diol (1 eq.) in dry DCM, imidazole (4.2 eq.) was added portionwise at room temperature. Then solution of $SOCl_2$ in DCM (2 mL, 2.0 eq.) and triethylamine in DCM (2 mL, 2.4 eq.) were added dropwise at 0 °C. The RM was stirred at 0 °C until complete consumption of starting material. Then to reaction mixture 5 mL of water was added and extracted in 2×10 mL of DCM. The combined organic layer dried on Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure on rotory evaporator to give 1,2-cyclic sulfite diester, can be used without further purification. The 1,2-cyclic sulfite diesters **1-11** were prepared by this procedure.

General procedure B: To a solution of olefin (1 eq.) in acetone: H_2O (4:1), OsO_4 (0.01 eq.) and NMO (50% aqueous solution, 1.5 eq.) were added at room temperature. The reaction mixture was stirred at room temperature until complete consumption of SM. To reaction mixture 5 mL of brine was added and extracted in ethyl acetate (4×10 mL).The combined organic layer was dried on Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure on rotory evaporator to give crude product. The crude was purified by silica gel column chromatography in EtOAc/PE (1:1) system to give corresponding 1,2-diol. The 1,2-diols required for synthesis of sulfite diesters **3-5**, **11** were prepared by this procedure.

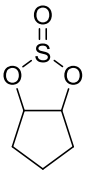
General procedure C: To a solution of epoxide (0.500 g) in 10 mL 1,4-dioxane, 15 mL 10% aq. K_2CO_3 solution was added and RM was refluxed until complete consumption of SM. The RM was cooled gradually to RT and extracted in 4×10 mL of ethyl acetate. The combined organic layer dried on Na_2SO_4 and filtered. The resulting filtrate was concentrated under reduced pressure on rotory evaporator to give crude product. The crude was purified by silica gel column chromatography in EtOAc/PE (1:1) system to give corresponding 1,2-diol. The 1,2-diols **7-9** were prepared by this procedure.

Decomposition study: The 10 mM stock solution of compound was prepared in DMSO and 10 µL was added to 990 µL of phosphate buffer (pH 7.4) to give 100 µM of compound in 1% DMSO/PB. This solution was incubated at 37°C and after appropriate time interval RM was injected in an ion chromatograph equipped with ion conductivity detector to quantify SO_2 as SO_3^{2-}.

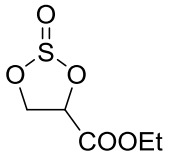
4,4,5,5-tetramethyl-1,3,2-dioxathiolane 2-oxide (2). Prepared according to general procedure **A**. Starting from pinacol (0.050 g, 0.423 mmol) to give **2** (0.060 g, 86%) as a colorless oil; FTIR (ν_{max}, cm^{-1}): 1453, 1376, 1199, 1128;^{1}H NMR (400 MHz, $CDCl_3$):δ 1.56 (s, 6H), 1.34 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 91.1, 24.3, 23.9; HRMS (ESI-TOF): $C_6H_{12}O_3S$ $[M+K]^+$: 203.0144. Found $[M+K]^+$: 203.0512.



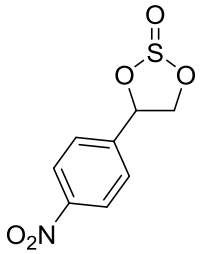
Tetrahydro-4H-cyclopenta[d][1,3,2]dioxathiole 2-oxide (3). Prepared according to general procedure **A**. Starting from cyclopentane-1,2-diol (0.050 g, 0.489 mmol) to give **3** (0.065 g, 90%) as a colorless oil; FTIR (ν_{max}, cm^{-1}): 1448, 1351, 1209, 1142 ; ^{1}H NMR (400 MHz, $CDCl_3$):Isomers in the ratio 1:0.65 were obtained, for major isomer δ 5.41-5.38 (m, 2H), 2.26-2.16 (m, 2H), 1.79-1.65 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): Mixture of isomers, δ 89.6, 85.4, 33.3, 32.5, 21.9, 21.4; HRMS (ESI-TOF): $C_5H_8O_3S$ $[M+H]^+$: 149.0272. Found $[M+Na]^+$: 149.0245.



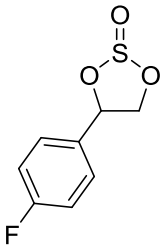
Ethyl 1,3,2-dioxathiolane-4-carboxylate 2-oxide (5). Prepared according to general procedure **A**. Starting from ethyl 2,3-dihydroxypropanoate (0.050 g, 0.373 mmol) to give **5** (0.058 g, 87%) as a pale yellow oil; FTIR (ν_{max}, cm^{-1}): 1740, 1468, 1293, 1200, 1017; ^{1}H NMR (400 MHz, $CDCl_3$): Mixure of isomers (~ 1:1), δ 5.31-5.28 (m, 1H), 4.99-4.86 (m, 3H), 4.74-4.66 (m, 2H), 4.35-4.26 (m, 4H), 1.35-1.31 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): Mixure of isomers, δ 167.4, 167.2, 77.3, 76.8, 69.5, 68.9, 62.7, 14.1; HRMS (ESI-TOF): $C_5H_8O_5S$ $[M+Na]^+$: 202.9990. Found $[M+Na]^+$: 202.9987.



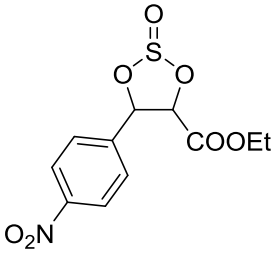
4-(4-nitrophenyl)-1,3,2-dioxathiolane 2-oxide (8). Prepared according to general procedure A.Starting from **22b** (0.150 g, 0.819mmol) to give **8** (0.152 g, 81%) as a pale yellow oil; FTIR (ν_{max}, cm^{-1}): 1644, 1603, 1514, 1339, 1188; ^{1}H NMR (400 MHz, $CDCl_3$): Isomers in the ratio 1:0.87 were obtained, for major isomer δ 8.27 (d, *J* = 8.6 Hz, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 6.00 (t, *J* = 6.5 Hz, 1H), 5.04 (dd, *J* = 6.7, 8.6 Hz, 1H), 4.22 (dd, *J* = 6.3, 8.6 Hz, 1H), ^{13}C NMR (100 MHz, $CDCl_3$): For major isomer δ 142.11, 128.2, 127.2, 124.3, 83.6, 79.5, 73.2, 71.6; HRMS (ESI-TOF): $C_8H_7NO_5S$ $[M+Na]^+$: 251.9943. Found $[M+Na]^+$: 251.9939.



4-(4-fluorophenyl)-1,3,2-dioxathiolane 2-oxide (9). Prepared according to general procedure A.Starting from **22c** (0.050 g, 0.320mmol) to give **9** (0.048 g, 75%) as a colorless oil; FTIR (ν_{max}, cm^{-1}): 1460, 1323, 1216, 1081; ^{1}H NMR (400 MHz, $CDCl_3$): Isomers in the ratio 1:0.65 were obtained, for major isomer δ 7.37-7.32 (m, 2H), 7.14-7.08 (m, 2H), 5.91 (t, *J* = 6.8 Hz, 1H), 4.94 (dd, *J* = 6.4, 8.6 Hz, 1H), 4.18 (dd, *J* = 7.3, 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): Mixture of isomers δ 164.6, 162.1, 130.5, 130.4, 129.8, 129.7, 129.6, 129.5, 128.7, 128.6, 116.4, 116.3, 116.2, 116.1, 84.9, 80.3, 73.4, 71.4; HRMS (ESI-TOF): $C_8H_7FO_3S$ $[M+H]^+$: 203.0178 Found $[M+H]^+$: 203.0509



Ethyl 5-(4-nitrophenyl)-1,3,2-dioxathiolane-4-carboxylate 2-oxide (11). Prepared according to general procedure A.Starting from **28** (0.075 g, 0.294 mmol) to give **11** (0.048 g, 75%) as a pale yellow oil; FTIR (ν_{max}, cm^{-1}): 1758, 1606, 1525, 1347, 1208; ^{1}H NMR (400 MHz, $CDCl_3$): Mixture of isomers (~ 1:1) δ 8.31-8.28 (m, 4H), 7.73 (d, *J* = 8.9 Hz, 2H), 7.65 (d, *J* = 8.9 Hz, 2H), 6.26 (d, *J* = 7.1 Hz, 1H), 5.70 (d, *J* = 8.1 Hz, 1H), 5.18 (d, *J* = 8.2 Hz, 1H), 4.79 (d, *J* = 7.0 Hz, 1H), 4.37-4.33 (m, 4H), 1.36-1.32 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): Mixture of isomers δ 166.7, 165.7, 148.7, 148.6, 141.7, 141.2, 128.5, 127.6, 124.3, 124.2, 85.9, 83.3, 81.8, 81.7, 63.3, 63.2, 14.1; HRMS (ESI-TOF): $C_{11}H_{11}NO_7S[M+Na]^+$: 324.0153. Found $[M+Na]^+$:324.0151.



3B.5 Spectral Data

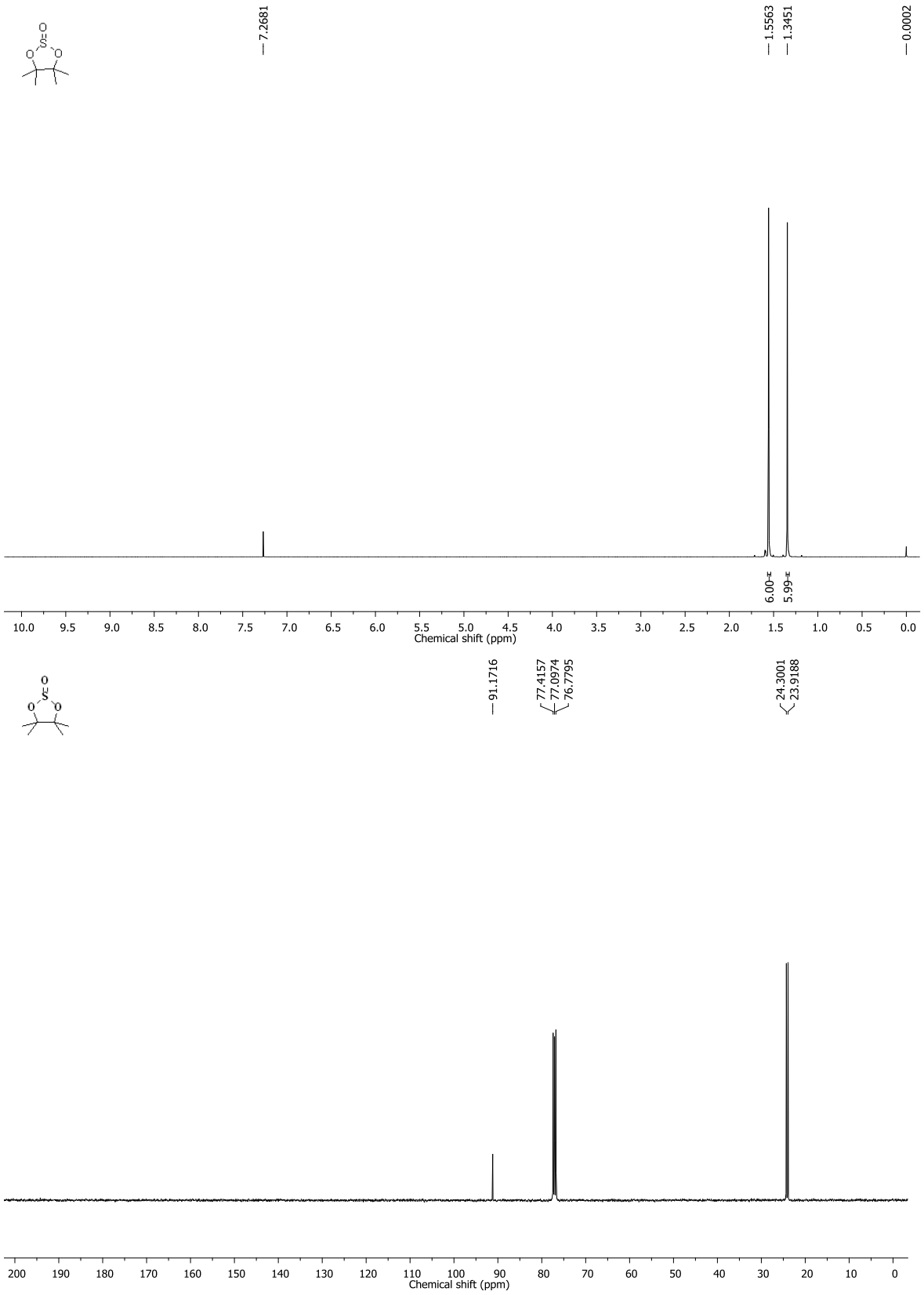


Figure S1. NMR spectra of **2**

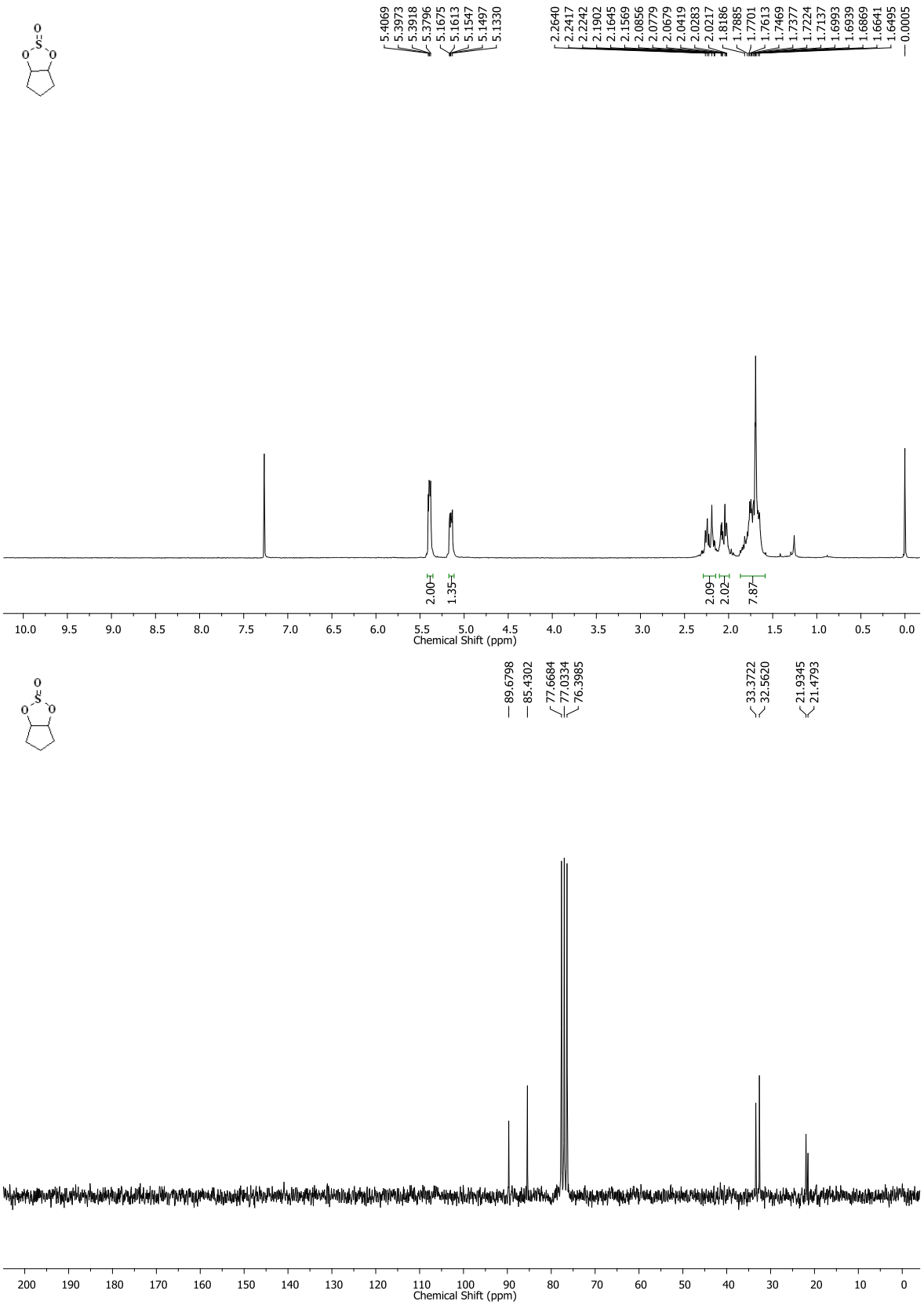


Figure S2. NMR spectra of **3**

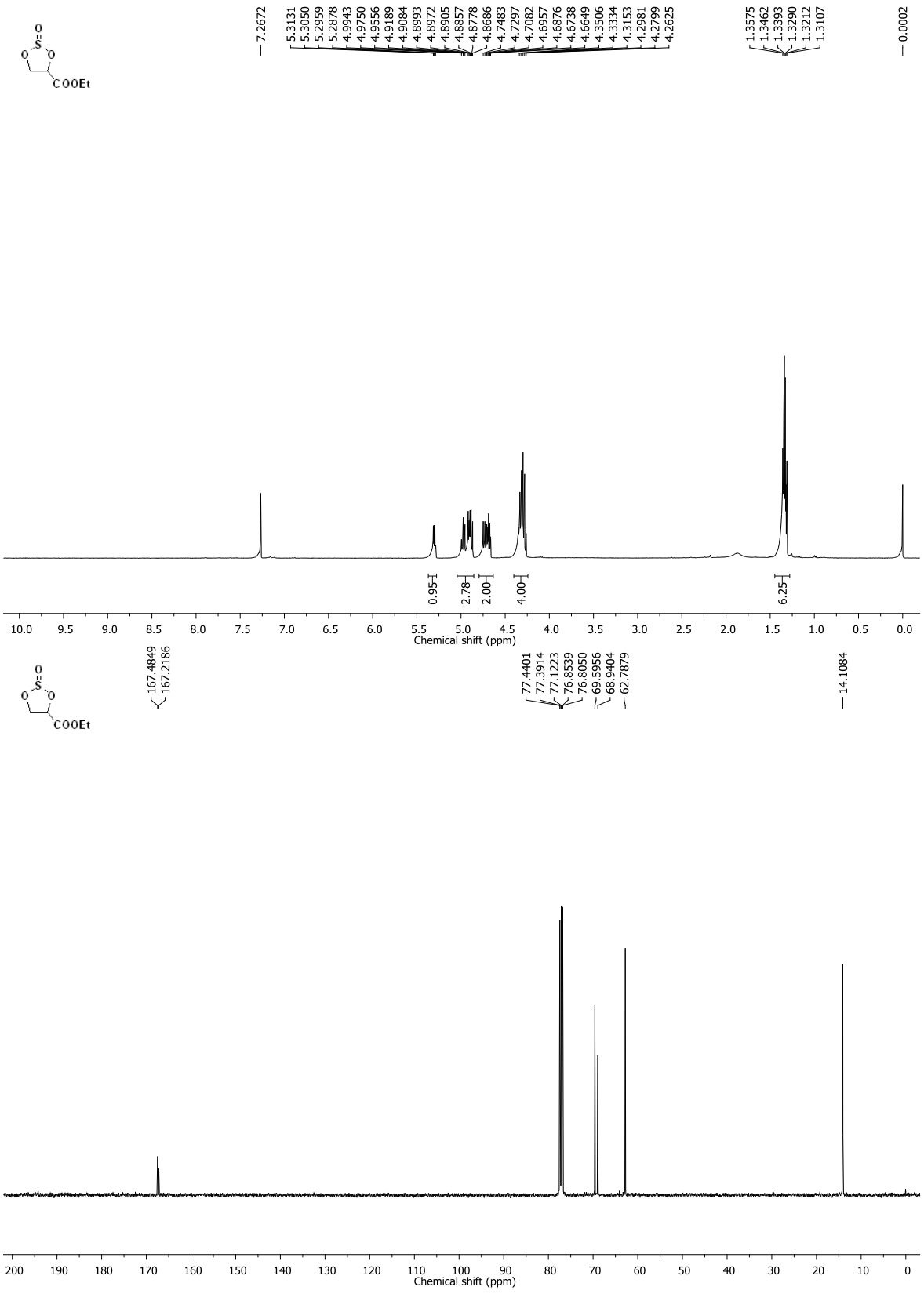


Figure S3. NMR spectra of **5**

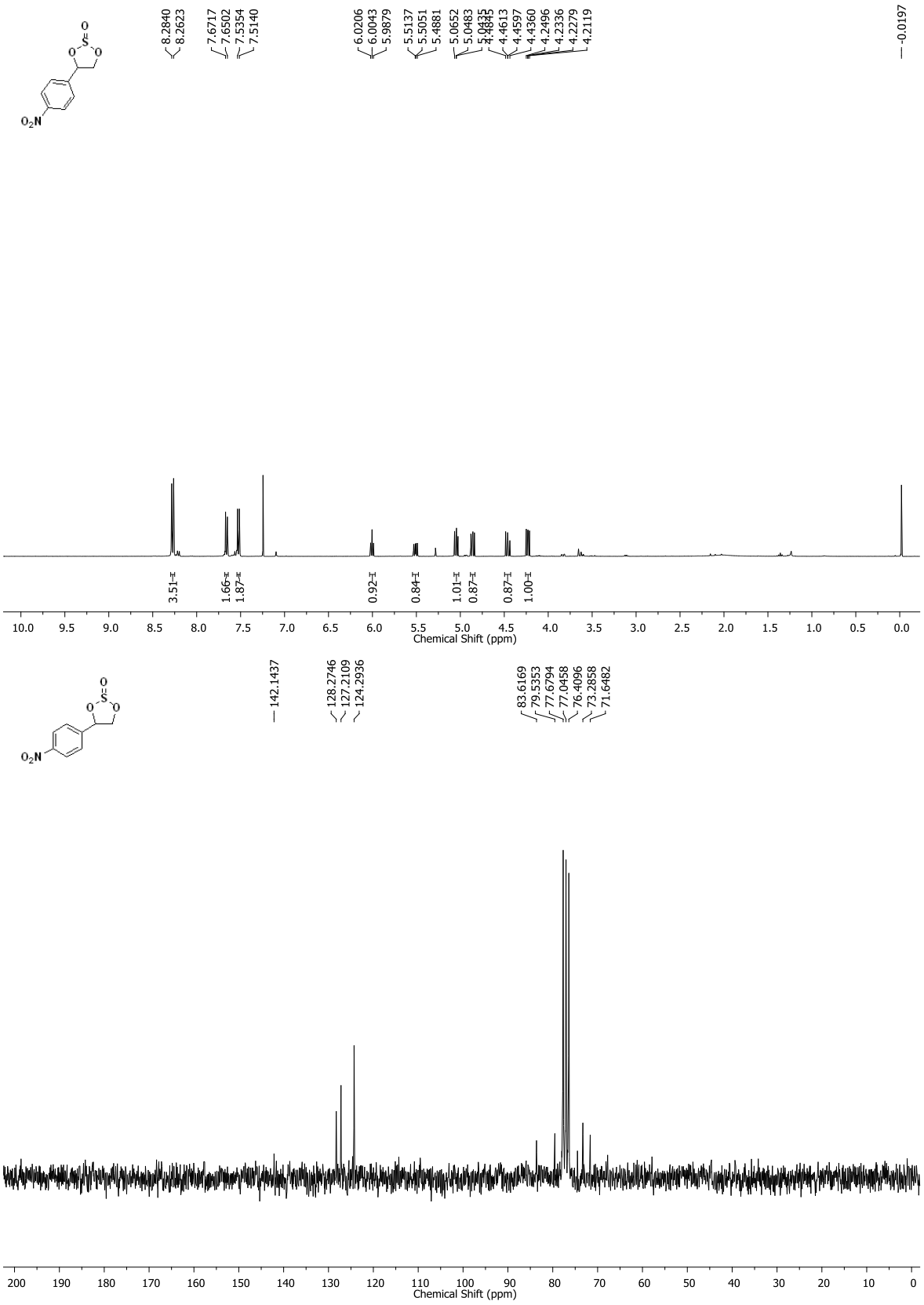


Figure S4. NMR spectra of **8**

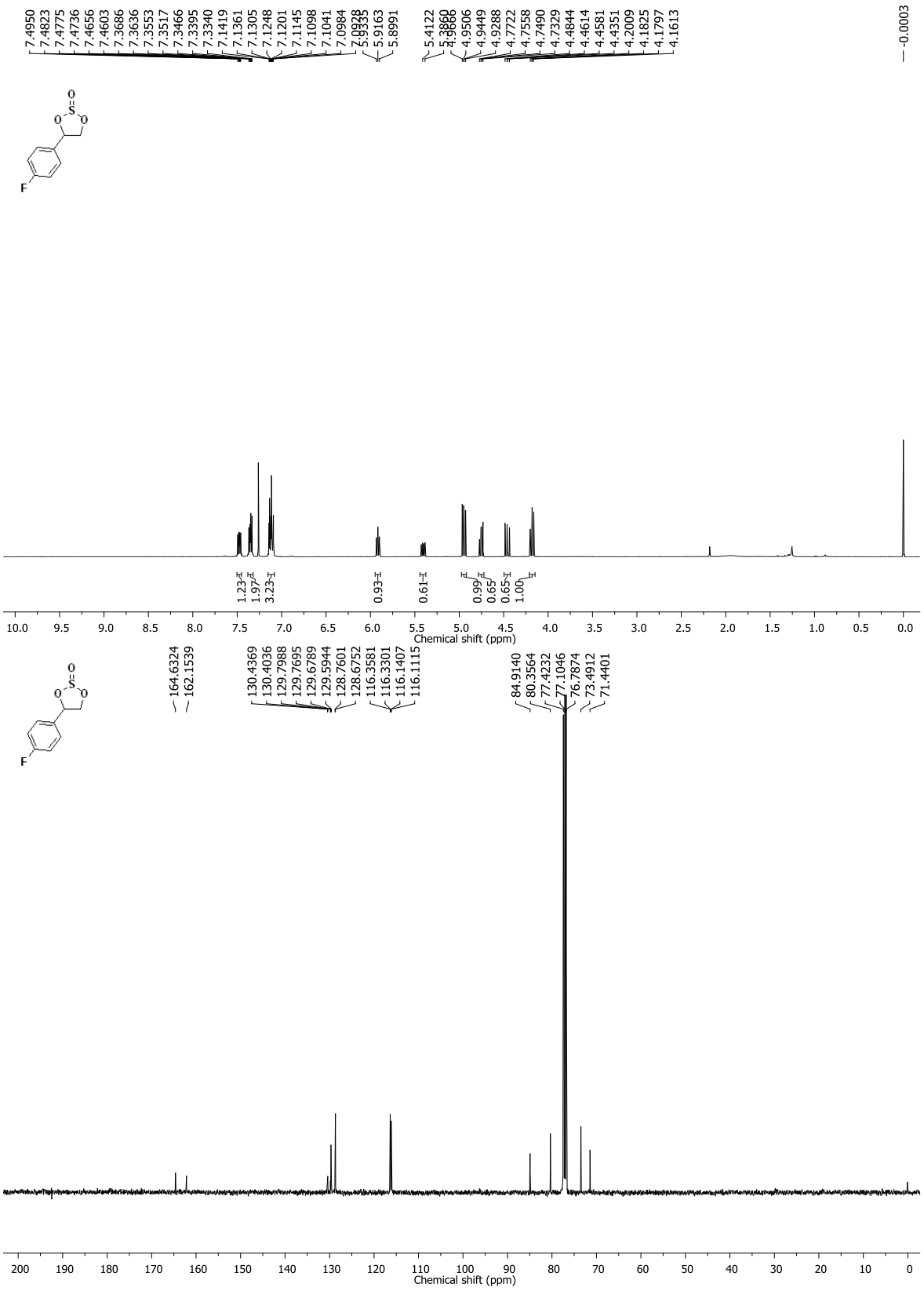


Figure S5. NMR spectra of **9**

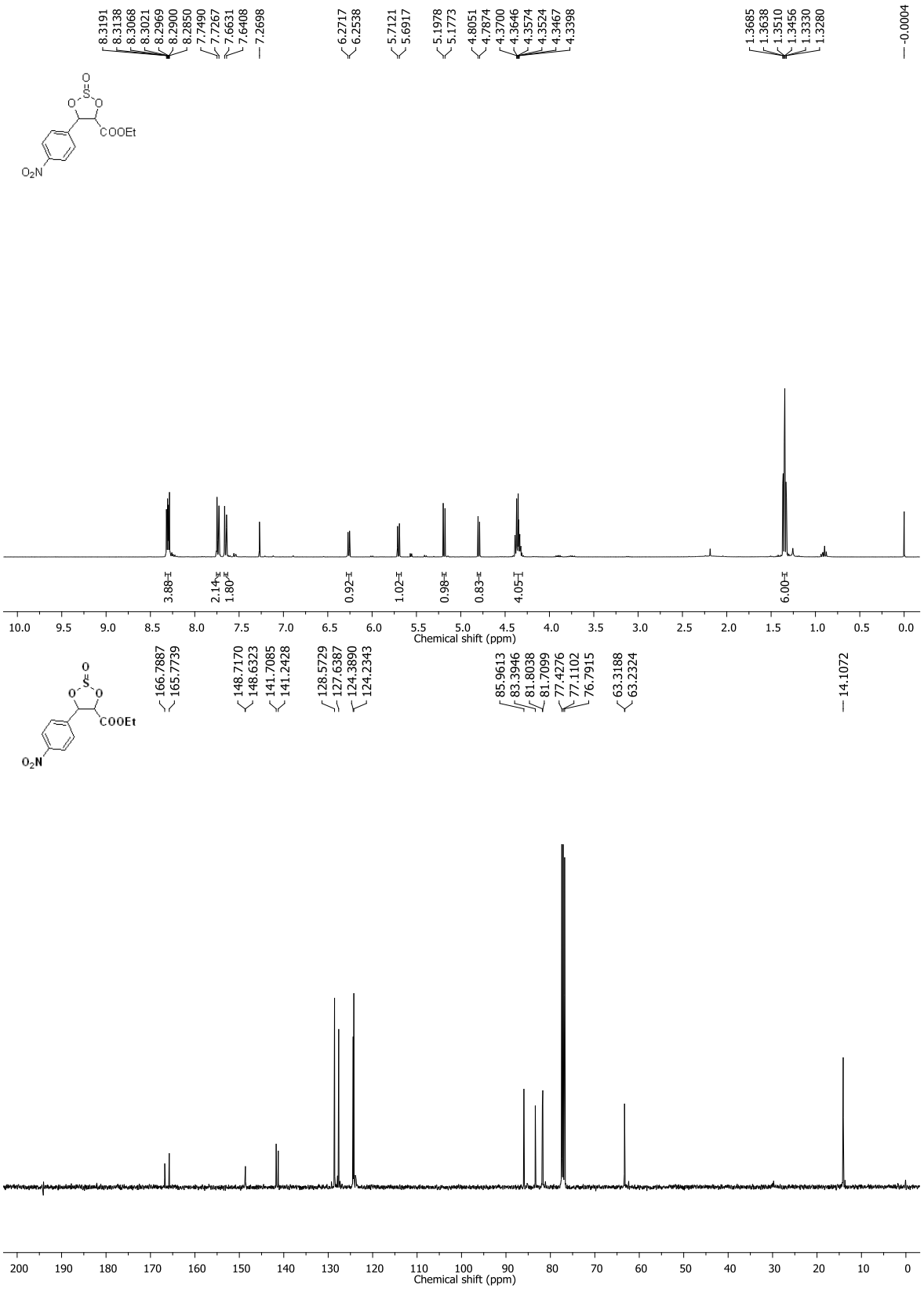


Figure S6. NMR spectra of

3B.6 References:

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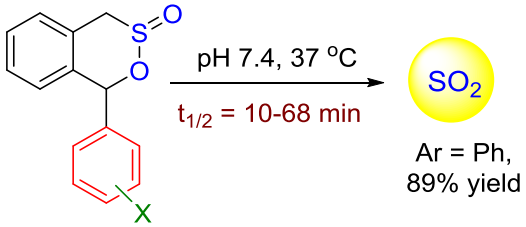
Chapter 4

Benzosulfones as Photochemically Activated Sulfur Dioxide (SO_2) Donors

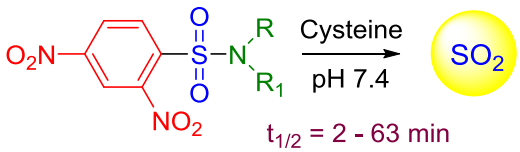
Chapter 4: Benzosulfones as Photochemically Activated Sulfur Dioxide (SO_2) Donors.

4.1 Introduction: In Chapter 2, we described benzosultines as SO_2 donors that would enable us to understand the precise biological role of SO_2.[1] These compounds undergo a retro Diels-Alder reaction to produce SO_2 with half-lives ranging from a few minutes to an hour (Scheme 1). Benzosultines showed SO_2 like activity in a DNA cleavage assay suggesting potential applications for molecular biological assays.[1] However, using only heat as a trigger for SO_2 generation might hinder site-directed delivery applications for this series of compounds.



Scheme 1. Benzosultines as temperature dependent sulfur dioxide (SO_2) donors.

In Chapter 3: Section A, we showed that 2,4-dinitrophenylsulfonamides were activated by biological thiols in physiological pH to generate SO_2.[2] A number of these derivatives were also found to have *Mycobacterium tuberculosis* (*Mtb*) inhibitory activity.[3] Compounds that were better at generating SO_2 in a short period were superior inhibitors of *Mtb* suggesting an important role for SO_2 (Scheme 2).[3]



Scheme 2. 2,4-Dinitrophenylsulfonamides as thiol-activated sulfur dioxide (SO_2) donors.

However, as thiols themselves are targets for modification by SO_2, other methods for activation to produce SO_2 are necessary. Hence SO_2 donors which are stable at room temperature but require a trigger that is not a biological thiol are desirable but yet unavailable. Furthermore, recently the use of SO_2 as a reagent in synthesis has assumed importance with methodologies being developed using small molecule sources of SO_2. Thus, developing new small molecule-based methodologies for generating SO_2 for chemical as well as biological applications would be useful. Targeted drug delivery plays

an essential role in the detection, diagnosis and treatment of life-threatening diseases, including cancers. UV and short visible (<400 nm) light can be used as a trigger for drug release from various delivery vehicles.[4,5] This can be achieved by specifically delivering enough of a drug in its inactive form at specific area and use of a light as a stimulus to release the active drug locally in/around the region. The ability to control the timing, location and dosage of delivery are key advantages to using photochemical methods for drug delivery.

Cava and co-workers have reported that 1,3-diphenyl-1,3-dihydrobenzo[c]thiophene 2,2-dioxide undergoes photolysis when irradiated with UV light to give SO_2 and cyclized product with 13% yield in benzene.[6] We considered benzosulfones as possible candidates for light activated sulfur dioxide donors (Figure 1). These scaffolds provide structural handles to modulate SO_2 generation and can be classified in three series. First, substituents on the aryl ring of the benzosulfone (A) can be varied to modulate electronically the SO_2 release profile. Second, in series B an aryl ring on the α-position of the sulfonyl functional group would offer opportunities to perturb SO_2 generation, presumably by forming benzyl radical with electron donating or withdrawing group present on benzene ring. Finally, steric effects on photolysis could be studied by studying series C. Photolysis of these sulfones in phosphate buffer (pH 7.4) will be carried out by irradiating UV light of appropriate intensity followed by quantification of SO_2 by ion chromatography.[7]

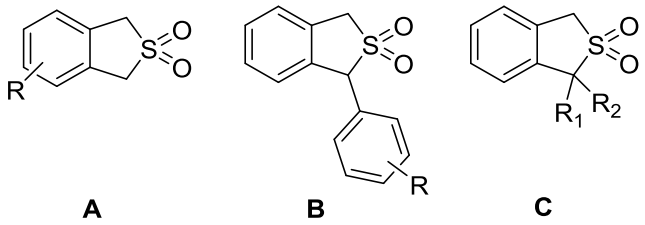
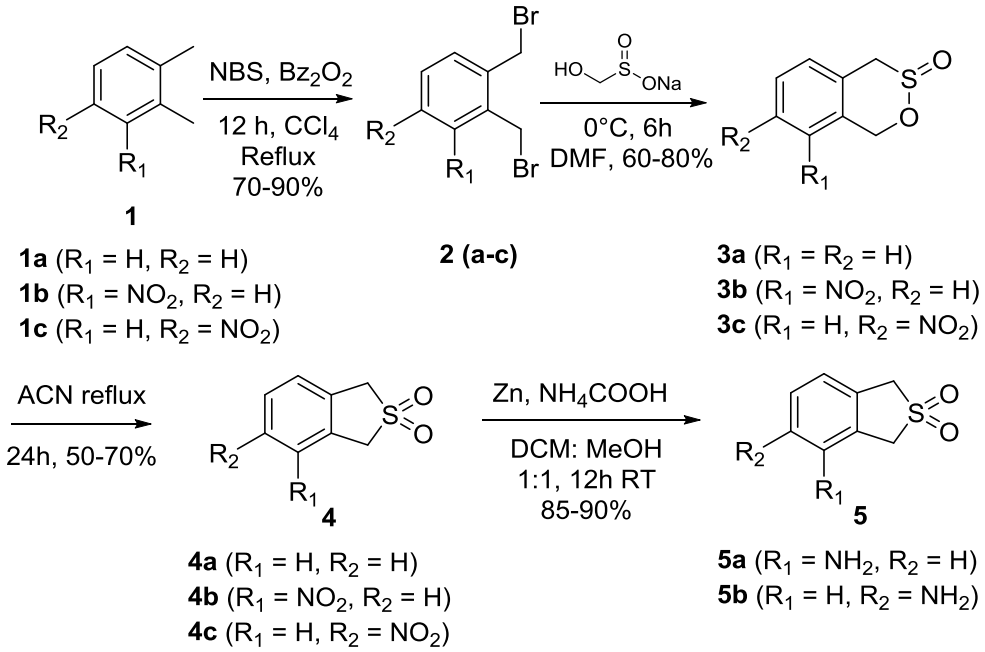


Figure 1. Benzosulfone to be studied for photolysis.

4.2 Results and discussion:

4.2.1 Synthesis of benzosulfones having electron donating or withdrawing group on the aromatic ring: Benzosulfones having electron donating or withdrawing group on the aromatic ring (series A) were synthesized as described in Scheme 3. Ortho xylene **1a** was dibrominated by 2.0 equivalents of NBS,[8] to give **2a**. Treatment of **2a** with 2-hydroxymethane sulfinate (rongalite) gave cyclic sulfinate ester **3a**.[9] This sultine was refluxed in acetonitrile wherein a retro Diels-Alder reaction followed by in situ chelotropic

addition of SO_2 gave the sulfone **4a** (Scheme 3).[10] Similarly, nitro sulfones **4b** and **4c** were prepared starting from **1b** and **1c**. Sulfones with an electron donating amino group **5a** and **5b**, were obtained by Zn/NH_4COOH mediated reduction of **4b** and **4c**.[11]

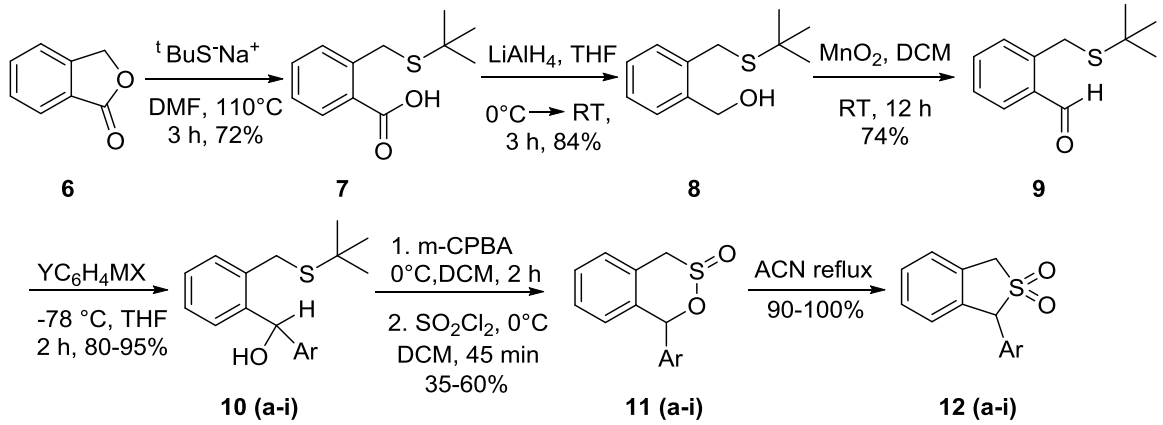


Scheme 3. Synthesis of sulfone with substituent present on aryl part.

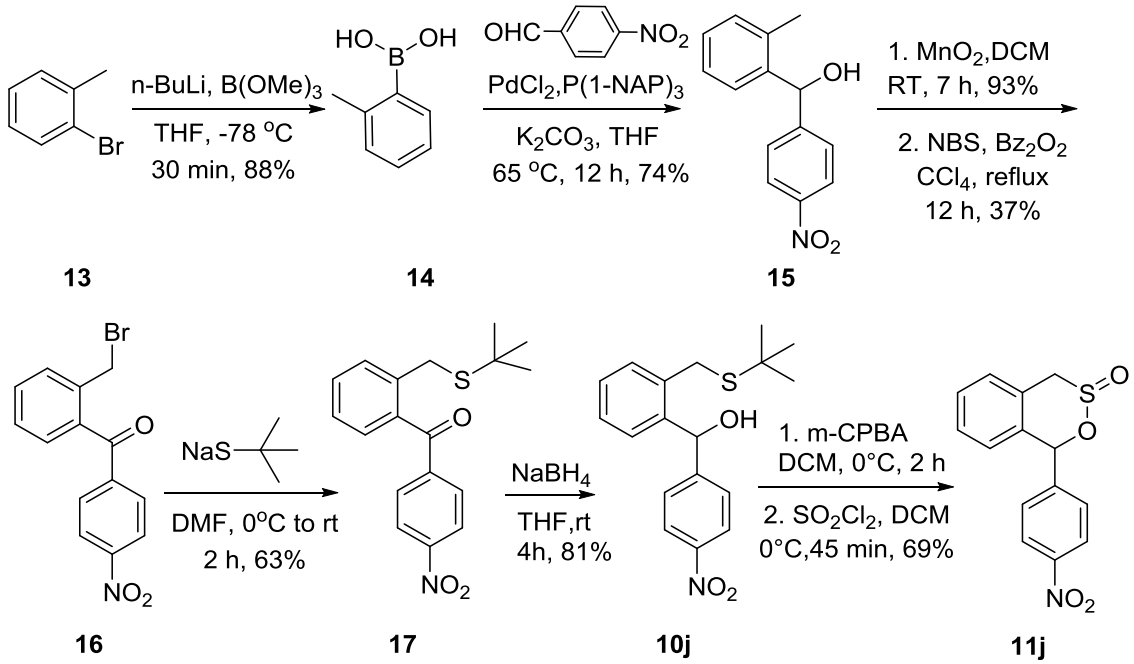
4.2.2 Synthesis of benzosulfones having α-aryl substituent with electron donating or withdrawing group: In order to study the effect of aryl substituent with electron donating or withdrawing group, starting from phthalide, using a reported procedure we synthesized aldehyde **9** in three steps (Scheme 4).[1] This aldehyde was treated with various Grignard reagents to produce **10a**-**10i** using reported methods (Table 1). Attempts to synthesize the 4-nitrophenyl derivative using this methodology failed. Hence, starting from 2-bromotoluene, **10j** was prepared in eight steps as described in Scheme 5. Using a reported two-step sequence involving *m*-CPBA oxidation followed by sulfuryl chloride mediated cyclization, the thioether **10a** was converted to corresponding benzosultine **11a**.[1] Refluxing benzosultine **11a** in acetonitrile gave **12a** presumably through a retro Diels-Alder reaction followed by an in situ chelotropic addition of SO_2 (Table 1).

When this two step sequence was attempted to prepare the 2-methoxy derivative **11b**, we instead isolated the benzosulfone **12b** presumably due to the poor stability of the sultine under the reaction conditions (Table 1). A similar observation was recorded for the 4-methoxy derivative and we isolated **12f** as the major product (Table 1, entries 2 and 6). Previously, rates of retro Diels-Alder reactions of 1-aryl-benzosultines were recorded and

Hammett analysis yielded a ρ-value of –0.621 suggesting that electron donating groups accelerated retro Diels-Alder reaction.[1] Our observation is consistent with this result. Thus, it appears that the sultine, once formed, rearranges to the sulfone without the need for heating above room temperature. Similarly, benzosulfones **12c**-**12e** and **12g**-**12j** were synthesized from the corresponding benzosultines (Table 1).

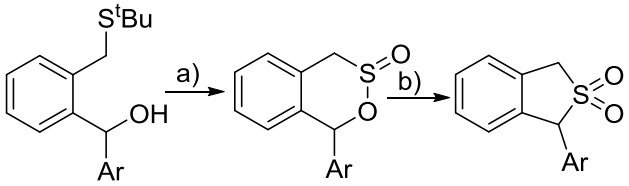


Scheme 4. General scheme for synthesis of various benzosultines.



Scheme 5. Synthesis of 1-(4-nitrophenyl)-1,4-dihydrobenzo[d][1,2]oxathiine 3-oxide (**11j**).

Table 1. Synthesis of 1-aryl sulfones

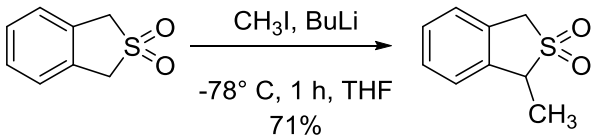


10 (a-i) **11a-11i** **12a-12j**

Entry	Ar	Starting compd	Starting compd	Product
1	Ph	**10a**	**11a**	**12a**
2	2-OMePh	**10b**	**11b**	**12b**
3	2-NO_2Ph	**10c**	**11c**	**12c**
4	3-OMePh	**10d**	**11d**	**12d**
5	3-CNPh	**10e**	**11e**	**12e**
6	4-OMePh	**10f**	**11f**	**12f**
7	4-MePh	**10g**	**11g**	**12g**
8	4-FPh	**10h**	**11h**	**12h**
9	4-CNPh	**10i**	**11i**	**12i**
10	4-NO_2Ph	**10j**	**11j**	**12j**

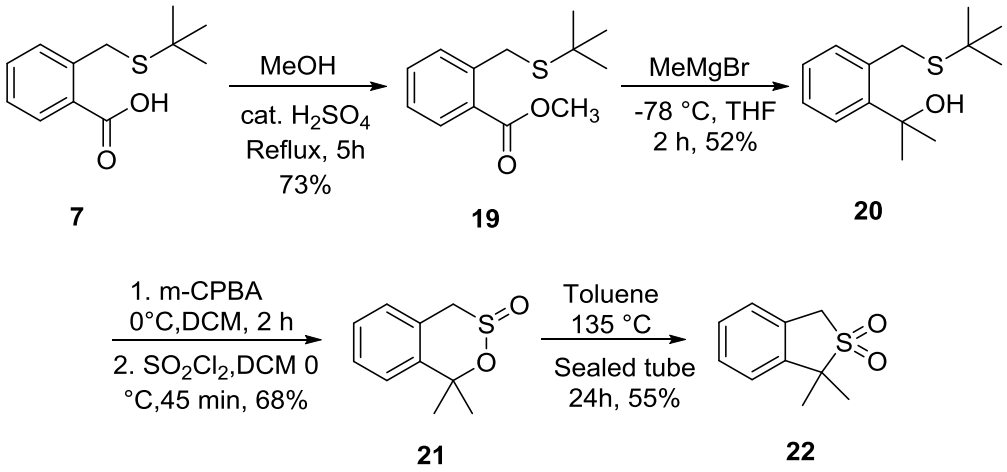
a) 1. *m*-CPBA, 0 °C, DCM, 2h, 2. SO_2Cl_2, 0°C DCM, 45 min b) ACN, Reflux

4.2.3 Synthesis of benzosulfones having α-alkyl substituent: To study the effect of alkyl substituent on α-position of benzosulfone we synthesized methyl derivative (Scheme 6). Sulfone **4a** was alkylated at α-position using butyl lithium and methyl iodide[12] to give α-methyl derivative **18** (Scheme 6). Next 1,1-dimethyl-1,4-dihydrobenzo[d][1,2] oxathiine 3-oxide, **21** was prepared starting from phthalide (Scheme 7). First, nucleophilic ring opening by *tert*-butyl thiolate takes place to give acid **7**.[1] The acid was converted in to ester **19** followed by reaction with MeMgBr to give tertiary alcohol **20**. Thioether **20** was oxidized to sulfoxide by *m*-CPBA, followed by sulfuryl chloride mediated cyclization to give sultine **21**, which was converted to benzosulfone **22**.



4a **18**

Scheme 6. Synthesis of sulfone with methyl substituent on α-position.



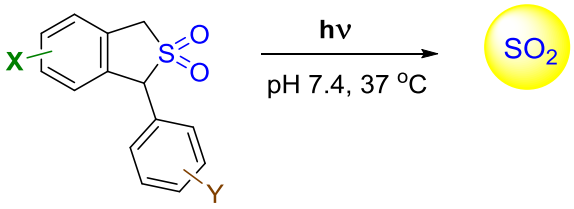
Scheme 7. Synthesis of 1,1-dimethyl sulfone.

The above benzosulfones were subjected to photolysis in phosphate buffer and % SO_2 generated was quantified as sulfate by ion chromatography.[2]

4.2.4 Photolysis of benzosulfones in pH 7.4 buffer to generate SO_2: When sulfone **4a** was irradiated for 10 min, 49% of SO_2 was generated (Table 2, entry 1). In the presence of an electron withdrawing nitro group as in the cases of **4b** and **4c**, the yields of SO_2 were significantly lower giving 30% and 12% SO_2, respectively, after 10 min (Table 2, entries 2-3). In the cases of **5a** and **5b**, where an electron donating amino group was present, the yield of SO_2 was significantly higher than what we obtained for **4a** (Table 2, entry 4 and 5). In 1 h, nearly quantitative SO_2 yield was obtained for **4a**, **5a** and **5b** (Table 2, entries 1, 4 and 5) whereas the yields were diminished in the cases of **4b** and **4c**. A SO_2 yield of 80% was recorded during photolysis of **4c** after 4 h (Table 2, entry 3). Hence it appears that an electron donating group on the fused aromatic ring increases efficiency of photolysis.

Next, in order to understand the effect of aryl substituent on α-position, photolysis of compounds **12a-12j** was carried out (Table 2, entries 6-15). Introduction of a phenyl ring at the α –position resulted in increased yield of SO_2 with **12a** giving nearly 84% of SO_2 after 10 min (Table 2, entry 6). When strong and mild electron donating group was present on the α-aryl ring, similar SO_2 yields was obtained after 10 and 60 min suggesting no major electronic effect on photolysis of these 1-aryl benzosulfones (Table 2).

Table 2. Results of photolysis of benzosulfones prepared in this study



Entry	Compound	% SO_2 after 10 min[a]	% SO_2 after 60 min[a]
1	**4a**	49	98
2	**4b**	30	75
3	**4c**	12	43 (80)
4	**5a**	72	100
5	**5b**	81	100
6	**12a**	84	97
7	**12b**	92	100
8	**12c**	58	96
9	**12d**	79	100
10	**12e**	81	97
11	**12f**	79	96
12	**12g**	80	97
13	**12h**	77	93
14	**12i**	77	99
15	**12j**	51	100
16	**18**	66	95
17	**22**	62	94

[a]% SO_2 was determined as sulfate using an ion chromatograph equipped with a conductivity detector. [b]The yield of SO_2 produced after 4 h is reported in parenthesis.

Previously, benzosulfones have been reported to undergo photolysis when irradiated with UV light through a radical mechanism.[6] Thus, the stability of the radical should dictate the propensity of the benzosulfone to undergo photolysis. Our results indicate that photolysis is sensitive to substituent effects. Among the benzosulfones **4a-4c** and **5a-5b**, electron withdrawing groups appear to hinder photolysis while the electron donating amino groups promoted photolysis.

4.2.5 Substituent effect on stability of benzyl radicals: The stability order of free radicals is tertiary > secondary > primary, suggesting field effects and hyperconjugation contributes to stability analogous to that in carbocation.[13] Benzyl radical is more stable than simple alkyl radical due to extended conjugation.[14] Singh and co-workers reported that the radical constants for para substituted toluenes were in the order of NH_2>NO_2 supporting that the formation of the benzylic radical containing an amino substituent was favoured.[15] Ozaki and co-worker reported that the rate constants (k_{O2}) for oxygenation of para-substituted benzyl radicals decrease in the order of *p*- OCH_3 (3.0×10^9 $M^{-1}s^{-1}$) > *p*-NO_2 (~ 0.9×10^9 $M^{-1}s^{-1}$). These results suggest that strong electron withdrawing para-substituents extensively slows down the oxygenation possibly due to decreased stability of the incipient radical.[16]

When phenyl substituent is present on α-position of benzosulfone **4a**, as in the case of **12a**, the yield of SO_2 during photolysis is significantly higher when compared with **4a** (Table 2). This observation could be rationalized by stabilization afforded by extended conjugation of the additional aryl ring at the α-position to stabilize the radical.[13] However, changing substituents on this aryl ring did not significantly alter photolysis yields except in the cases of a NO_2 substituent being present on the 2- or 4- position of 1-phenyl ring i.e. **12c** and **12j**, wherein a marginally decreased yield of SO_2 after 10 min was observed (Table 2, entries 8 and 15). However, in all the aforementioned cases, we obtained excellent yields of SO_2 after 1 h. This result indicates a diminished electronic substituent effect on photolysis possibly due to a transition state devoid of any partial charges.

Finally, in order to understand the role of alkyl substituent on 1-position we conducted the irradiation experiment with **18** and **22** (Table 2, entries 16-17). In both cases, the presence of an alkyl group marginally enhanced yields of SO_2 as compared to **4a**.

4.3 Conclusions:

In conclusion, we report a series of benzosulfones that undergo photolysis to generate SO_2 in physiological conditions. The yields of SO_2 can be modulated by altering substituents pattern. The possibility of using these compounds for localized delivery of SO_2 is being explored.

4.4 Experimental Section

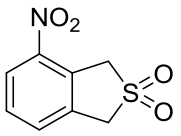
General: The Compounds **4a**,[17] **4c**,[18] **5b**,[18] **7-9**, **10**(**a-i**),[1] **11**(**a-i**)[1] **12a**,[1] **12**(**c-e**),[1] **12**(**g-i**),[1] **15**[19] and **18**,[12] were previously reported and our data matched with reported spectral data.

General procedure A: NBS bromination.[8] To a solution of of o-xylene or substituted o-xylene (0.050 mol) in dry CCl_4 (25 mL), N-bromosuccinamide (0.100 mol) and benzoyl peroxide (0.010 mol) were added portionwise at room temperature. The reaction mixture was refluxed until the starting material was completely consumed (TLC analysis). The reaction mixture was cooled gradually to room temperature and filtered. The resulting filtrate was concentrated under reduced pressure to give crude product. The crude was used for next step without further purification. This procedure was used for synthesis of dibromides **2a-2c**.

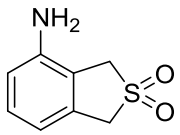
General procedure B: Formation of cyclic sulfinate ester (sultine) by reaction with rongalite.[9] To a solution of 1,2-bis(bromomethyl)-3-nitrobenzene or 1,2-bis(bromomethyl)-4-nitrobenzene (0.032 mol) in dry DMF (10 mL), sodium hydroxymethanesulfinate hydrate (0.130 mol) and tetrabutylammonium bromide (0.0064) were added portionwise at 0 °C. The reaction mixture was stirred at room temperature for 6 h. To the reaction mixture, water was added and extracted in ethyl acetate. The combined organic layer was dried on Na_2SO_4 and filtered. The resulting filtrate was concentrated under reduced pressure to give crude product. The crude was used for next step without further purification. This procedure was used for synthesis of benzosultines **3a-3c**, **11**(**a-j**) and **21**.

General procedure C: Conversion of sultine to sulfone.[10] The solution of sultine in acetonitrile (conc. varied from 0.05M to 0.5M) was refluxed for appropriate time until TLC analysis showed complete conversion to sulfone. The reaction mixture was cooled gradually to room temperature and concentrated under reduced pressure to give crude product. The crude was purified by silica gel flash column chromatography using EtOAc/PE (1:4) as the eluant to produce sulfone. The sulfones **4a**, **4b**, **4c**, **12a**, **12c**, **12d**, **12e**, **12g**, **12h**, **12i** and **12j** were prepared according to this procedure.

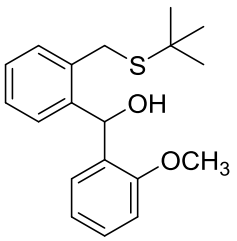
4-nitro-1,3-dihydrobenzo[c]thiophene 2,2-dioxidemp (4b). Prepared according general procedure C. Starting from **3b** (1.5 g, 7.04 mmol) in ACN (15 mL, 24h reflux) **4b** (0.80 g, 53%) was isolated as a yellow solid: mp 177-178 °C; FTIR (ν_{max}, cm^{-1}): 3000, 1525, 1453, 1389, 1355, 1310, 1137 ; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (d, *J* = 8.2 Hz, 1H), 7.83 (d, *J* = 7.6 Hz, 1H), 7.70-7.66 (m, 1H), 4.87 (s, 2H), 4.67 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 145.9, 135.9, 132.6, 129.8, 128.5,124.5, 56.4, 55.2; HRMS (ESI-TOF): $C_8H_7NO_4S$ $[M+Na]^+$: 235.9993. Found $[M+Na]^+$: 235.9993.



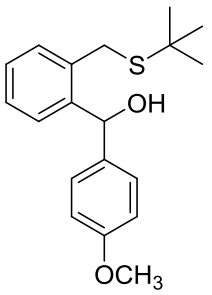
4-amino-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (5a).[11] To a solution of 4-nitro-1,3-dihydrobenzo[c]thiophene 2,2-dioxide **4b** (0.230 g, 1.07 mmol) in DCM:MeOH (25 mL, 1:1), activated zinc powder (0.700 g, 10.7 mmol), and ammonium formate (0.270 g, 10.7 mmol), were added portionwise at room temperature. The reaction mixture was stirred at room temperature for 3 h. To reaction mixture 10 mL of water was added and extracted in ethyl acetate (3×10 mL). The combined organic layer was dried on Na_2SO_4 and filtered. The resulting filtrate was concentrated under reduced pressure to produce **5a** (0.180 g, 91%) as a yellow solid: mp 187-188 °C; FTIR (ν_{max}, cm^{-1}): 3452, 3362, 2922, 1636, 1589, 1480, 1307, 1201;^{1}H NMR (400 MHz, DMSO-d_6): δ 7.05(m, 1H), 6.59 (d, *J* = 8.0 Hz, 1H), 6.51 (d, *J* = 7.4 Hz, 1H), 5.29 (s, 2H), 4.37 (s, 2H), 4.18 (s, 2H) ; ^{13}C NMR (100 MHz, DMSO-d_6):): δ 145.6, 133.0, 129.2, 115.9, 113.8, 113,36, 56.9, 54.2 ; HRMS (ESI-TOF): $C_8H_9NO_2S$ $[M+H]^+$: 184.0432. Found $[M+H]^+$: 184.0431



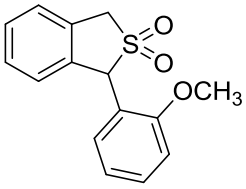
(2-(tert-butylthiomethyl)phenyl)(2-methoxyphenyl)methanol (10b).[1] Prepared according to general procedure in reference 1. Starting from **9** (1.0 g, 4.80 mmol), **10b** (1.4 g, 93% yield) was isolated as a colorless oil: FTIR (ν_{max}, cm^{-1}): 3584, 2959, 1598, 1487, 1460, 1440, 1291, 1245, 1165, 101 6; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.25 (m, 4H), 7.21-7.19 (m, 2H), 6.98-6.94, (m, 1H), 6.87 (dd, *J* = 0.6, 8.2 Hz, 1H), 6.50 (s, 1H), 3.92 (d, *J* = 11.2 Hz, 1H), 3.77 (s, 3H), 3.75 (d, *J* = 12.0 Hz, 1H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.6, 141.7, 135.1, 131.2, 130.3, 128.6, 127.7, 127.6, 127.5, 120.7, 110.4, 67.3, 55.4, 43.3, 30.7, 30.6; HRMS (ESI-TOF): $C_{19}H_{24}O_2S$ $[M+Na]^+$: 339.1395. Found $[M+Na]^+$: 339.1384.



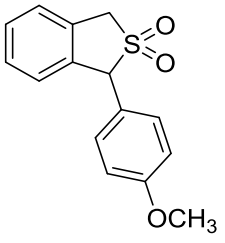
(2-((tert-butylthio)methyl)phenyl)(4-methoxyphenyl)methanol (10f).[1] Prepared according to general procedure in reference 1. Starting from **9** (1.0 g, 4.80 mmol), **10f** (0.87 g, 87%) was isolated as a colorless oil: FTIR (ν_{max}, cm^{-1}): 3421, 2959, 2361, 1610, 1510, 1458, 1364, 1249, 1171, 1034; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34, (d, *J* = 5.6 Hz, 1H), 7.29-7.20 (m, 5H), 6.87 (d, *J* = 6.8 Hz, 2H), 6.19 (s, 1H), 3.80 (s, 3H), 3.77 (d, *J* = 8.9 Hz, 1H), 3.72 (d, *J* = 8.9 Hz, 1H), 2.90 (s, 1H), 1.36 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.9, 142.5, 130.9, 128.2, 128.1, 127.9, 127.7, 116.1, 114.8, 113.8, 72.4, 55.3, 43.5, 33.5, 33.7; HRMS (ESI-TOF): $C_{19}H_{24}O_2S$ $[M+Na]^+$: 339.1395. Found $[M+Na]^+$: 339.1392.



1-(2-methoxyphenyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (12b). Formed as a product while preparing **11b** according to general procedure for synthesis of sultine in reference 1. Starting from **10b** (1.0 g, 3.16 mmol), **12b** (0.58 g, 66%) was isolated as a white solid: mp 179-180 °C; FTIR (ν_{max}, cm^{-1}): 1601, 1587, 1493, 1471, 1306, 1297, 1252, 1203, 1135, 1104; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42-7.34 (m, 4H), 7.14 (d, *J* = 7.0 Hz, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 6.93-6.89 (m, 1H), 6.79 (d, *J* = 7.1 Hz, 1H), 6.05 (s, 1H), 4.38 (s, 2H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.7, 135.9, 131.4, 130.6, 129.8, 129.0, 128.9, 126.8, 125.8, 120.8, 120.7, 111.2, 65.7, 56.0, 55.5; HRMS (ESI-TOF): $C_{15}H_{14}O_3S$ $[M+Na]^+$: 297.0561. Found $[M+Na]^+$: 297.0565; Anal. Calcd. for $C_{15}H_{14}O_3S$: C, 65.67; H, 5.14; S, 11.69. Found: C, 65.01; H, 4.81; S, 11.50.

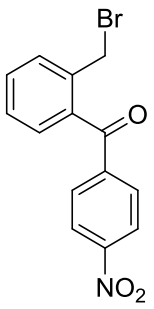


1-(4-methoxyphenyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (12f). Formed as a product while preparing **11f** according to general procedure for synthesis of sultine in reference 1. Starting from **10f** (1.0 g, 3.16 mmol), **12f** (0.266 g, 30%) was isolated as a white solid: mp 121-122 °C; FTIR (ν_{max}, cm^{-1}): 2932, 1610, 1512, 1456, 1317, 1272, 1252, 1194, 1178, 1134; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43-7.37 (m, 3H), 7.19 (d, *J* = 8.7 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 1H), 6.95 (d, *J* = 11.6 Hz, 2H), 5.43 (s, 1H), 4.41 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5, 136.1, 131.7, 131.0, 129.1, 129.0, 126.7, 125.9, 121.9, 114.5, 71.2, 55.4, 55.0; HRMS (ESI-TOF):

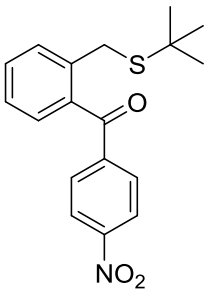


$C_{15}H_{14}O_3S$ $[M+Na]^+$: 297.0561. Found $[M+Na]^+$: 297.0566; Anal. Calcd. for $C_{15}H_{14}O_3S$: C, 65.67; H, 5.14; S, 11.69. Found: C, 65.18; H, 5.08; S, 11.12.

(2-(bromomethyl)phenyl)(4-nitrophenyl)methanone (16). To a solution of (4-nitrophenyl)(o-tolyl)methanol[19] **15** (3.25 g, 13.36 mmol) in dry DCM (25 mL), activated MnO_2 (5.80 g, 66.8 mmol) was added portionwise at 0 °C. The reaction mixture was stirred at room temperature for 6 h. To reaction mixture 20 mL of DCM was added and filtered through a celite bed. The resulting filtrate was dried on Na_2SO_4 and concentrated under reduced pressure to give (4-nitrophenyl)(o-tolyl)methanone (2.95 g, 93%) as a brown solid. The crude was used for next step without further purification. To a solution of (4-nitrophenyl)(o-tolyl)methanone (1.77 g, 7.3 mmol) in dry CCl_4 (40 mL), NBS (1.78 g, 7.3 mmol) and benzoyl peroxide (0.26 g, 1.5 mmol) were added portionwise at room temperature. The reaction mixture was refluxed until starting material was completely consumed (TLC analysis). The reaction mixture was cooled gradually to room temperature and filtered. The resulting filtrate was concentrated under reduced pressure to give crude product. The crude was purified by silica gel column chromatography using EA/PE (1:9) as the eluant to produce **16** (0.86 g, 37%) as a pale yellow solid: mp 124-125 °C; FTIR (ν_{max}, cm^{-1}): 1665, 1600, 1517, 1345, 1294, 1269; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (d, *J* = 8.9 Hz, 2H), 7.98 (d, J = 8.4 Hz, 2H), 7.57-7.51 (m, 2H), 7.41-7.31 (m, 1H), 7.33-7.31 (m, 1H), 4.73 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.7, 150.4, 142.3, 138.3, 136.9, 131.8, 131.5, 131.3, 129.8, 128.1, 123.7, 30.2. HRMS (ESI-TOF): $C_{14}H_{10}BrNO_3$ $[M+K]^+$: 341.9742. Found $[M+K]^+$: 341.9740.

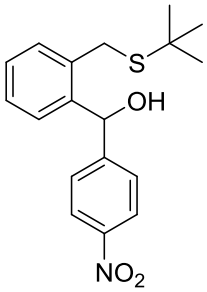


(2-((tert-butylthio)methyl)phenyl)(4-nitrophenyl)methanone (17). To a solution of (2(bromomethyl) phenyl)(4-nitrophenyl)methanone **16** (1.3 g, 4.0 mmol) in dry DMF (25 mL), sodium 2-methylpropane-2-thiolate (0.5 g, 4.6 mmol, solution in 5 mL dry DMF) was added dropwise in 5 min. The reaction mixture was stirred at room temperature for 5 h. To the reaction mixture 300 mL of water was added and extracted in ethyl acetate (3×25 mL). The combined organic layer was dried on Na_2SO_4 and filtered. The resulting filtrate was concentrated under reduced pressure to give crude product. The crude was purified by silica gel column chromatography using EA/PE (1:9) as

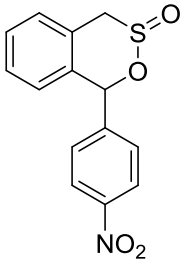


the eluant to produce **17** (0.84 g, 63 %) as a yellow solid: mp 120-121 °C; FTIR (ν_{max}, cm^{-1}): 1663, 1600, 1518, 1346, 1268; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (d, *J* = 8.7 Hz, 2H), 7.98 (d, *J* = 8.7, 2H), 7.50-7.44 (m, 2H), 7.32-7.23 (m, 2H), 3.93 (s, 2H), 1.21 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.0, 150.2, 142.7, 138.7, 137.5, 131.3, 131.2, 129.2, 126.5, 123.8, 123.5, 43.2, 30.5, 30.4. HRMS (ESI-TOF): $C_{18}H_{19}NO3S$ $[M+Na]^+$: 352.0983. Found $[M+Na]^+$: 352.0980.

(2-((tert-butylthio)methyl)phenyl)(4-nitrophenyl)methanol (10j). To a solution of (2-((tert-butylthio)methyl) phenyl)(4-nitrophenyl)methanone **22** (0.27 g, 0.8 mmol) in dry THF (25 mL), sodium borohydride (0.03 g, 1.0 mmol) was added portionwise at room temperature. The reaction mixture was stirred at room temperature for 8 h. To the reaction mixture, 5 mL of water was added and extracted in ethyl acetate (2×15 mL). The combined organic layer was dried on Na_2SO_4 and filtered. The resulting filtrate was concentrated under reduced pressure to give crude product. The crude was purified by silica gel column chromatography using EA/PE (1:4) as the eluant to produce **10j** (0.22 g, 81 %) as a yellow oil; FTIR (ν_{max}, cm^{-1}): 3404, 2960, 1600, 1521, 1346, 1162; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21 (d, *J* = 8.9 Hz, 2H), 7.60 (d, *J* = 8.6 Hz, 2H), 7.33 (dd, *J* = 1.6, 7.4 Hz, 1H), 7.29-7.20 (m, 2H), 7.07 (dd, *J* = 1.5, 7.4 Hz, 1H), 6.26 (d, *J* = 3.0 Hz, 1H), 3.87 (d, *J* = 11.0 Hz, 1H), 3.79 (d, *J* = 11.0 Hz, 1H), 3.45 (d, *J* = 3.9 Hz, 1H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.6, 147.1, 141.5, 135.3, 131.3, 129.2, 128.7, 128.2, 127.4, 123.5, 72.1, 44.0, 31.0, 30.7. HRMS (ESI-TOF): $C_{18}H_{21}NO_3S$ $[M+K]^+$: 370.0879. Found $[M+K]^+$: 370.0876.

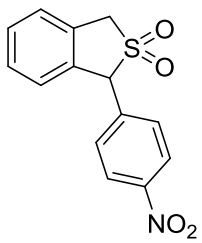


1-(4-nitrophenyl)-1,4-dihydrobenzo[d][1,2]oxathiine 3-oxide (11j).[1] To a solution of (2-((tert-butylthio)methyl)phenyl)(4-nitrophenyl)methanol **10j** (0.85 g, 2.5 mmol) in dry THF (25 mL), *m*-CPBA (0.95 eq. solution in 5 mL THF) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was gradually warmed to room temperature and concentrated under reduced pressure on rotory evaporator to give a white sticky mass. To this crude material satd. aq. $NaHCO_3$ solution (15 mL) was added and resulting mixture was washed with ethyl acetate (3×25 mL). The combined ethyl acetate layer was dried on Na_2SO_4 and filtered. The resulting filtrate was



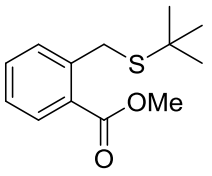
concentrated under reduced pressure to give crude product. The crude material was used for next step without further purification. To a solution of the sulfoxide (0.89 g, 2.56 mmol) in DCM (25 mL), sulfuryl chloride (0.34 mL, 3.8 mmol, in 5 mL DCM) was added dropwise at 0 °C during 5 min. The reaction mixture was stirred at 0 °C for 45 min. After completion of reaction, 10 mL of water was added and the resulting mixture was extracted with DCM (2×15 mL). The combined DCM layer was dried on Na_2SO_4 and filtered. The resulting filtrate was concentrated on a rotary evaporator (bath temperature maintained at 0-5 °C) under reduced pressure to give crude oil. The crude was purified by silica gel flash column chromatography using EA/PE (1:4) as the eluant (Note: the eluant was cooled to 5-10 °C before chromatography) to produce **11j** (0.51 g, 69%) as a white solid: mp 129-130 °C; FTIR (ν_{max}, cm^{-1}):1615, 1573, 1540, 1524, 1488, 1456, 1347; ^{1}H NMR (400 MHz, $CDCl_3$): major isomer, δ 8.31 (d, *J* = 8.6 Hz, 2H), 7.60 (d, *J* = 8.6 Hz, 2H), 7.47-7.38 (m, 1H), 7.32-7.25 (m, 2H), 6.70 (d, *J* = 7.7 Hz, 1H), 6.36 (s, 1H), 4.63 (d, *J* = 15.5 Hz, 1H), 3.70 (d, *J* = 15.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): for mixture of isomers δ 148.3, 145.3, 144.1, 136.5, 134.5, 131.5, 130.2, 130.0, 129.4, 129.1, 128.6, 128.1, 126.6, 126.5, 126.2, 124.2, 124.0, 80.2, 73.3, 57.7, 55.8; HRMS (ESI-TOF): $C_{14}H_{11}NO_4S$ $[M+K]^+$: 328.0046. Found $[M+K]^+$: 328.0043.

1-(4-nitrophenyl)-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (12j).



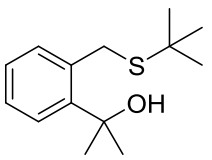
Prepared according to general procedure C. Starting from 1-(4-nitrophenyl)-1,4-dihydrobenzo[d][1,2]oxathiine 3-oxide **11j** (0.10 g, 0.345 mmol) in ACN (5 mL, 5 h reflux), **12j** (0.85 g, 85%) was isolated as a white solid: mp 191-192 °C; FTIR (ν_{max}, cm^{-1}): 1519, 1333, 1323, 1180, 1147; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (d, *J* = 8.7 Hz, 2H), 7.47-7.40 (m, 5H), 7.07 (d, *J* = 7.3 Hz, 1H), 5.58 (s, 1H), 4.51 (d, *J* = 15.6 Hz, 1H), 4.46 (d, *J* = 15.6 Hz, 1H), ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.6, 137.6, 134.7, 131.5, 131.0, 129.8, 129.4, 126.5, 126.2, 124.1, 71.0, 55.6; HRMS (ESI-TOF): $C_{14}H_{11}NO_4S$ $[M+K]^+$: 328.0046. Found $[M+K]^+$: 328.0040.

Methyl 2-(tert-butylthiomethyl)benzoate (19).[20] To a solution of 2-((tert-butylthio)methyl)benzoic acid **7** (0.8 gm, 3.5 mmol) in 5 mL methanol, few drops of conc. H_2SO_4 were added at 0 °C. The reaction mixture was refluxed for 5 h. The reaction mixture was cooled gradually to room temperature and concentrated under reduced pressure on

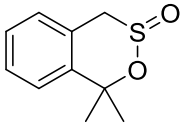


rotory evaporator to give a crude material. To this crude 5 mL of water was added and extracted in ethyl acetate (3×15 mL). The combined organic layer was dried on Na_2SO_4 and filtered. The resulting filtrate was concentrated under reduced pressure to give crude product. The crude was purified by silica gel column chromatography using EA/PE (1:9) as the eluant to give **19** (0.620 g, 73%) as a sticky oil : FTIR (ν_{max}, cm^{-1}): 3441, 2959, 1723, 1434, 1364, 1266, 1163, 1116, 1076; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, *J* = 7.4 Hz, 1H), 7.44.-7.42 (m, 2H), 7.30-7.25 (m, 1H), 4.18 (s, 2H), 3.91 (s, 3H), 1.35 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.9, 140.3, 131.8, 131.3, 130.8, 129.9, 126.9, 52.2, 43.1, 31.3, 30.9. HRMS (ESI-TOF): Calculated $C_{13}H_{18}O_2S$ $[M+Na]^+$: 261.0925. Found $[M+Na]^{+:}$ 261.0927.

2-(2-(tert-butylthiomethyl)phenyl)propan-2-ol (20). [21] To a solution of Methyl 2-(tert-butylthiomethyl)benzoate **19** (0.9 gm, 3.78 mmol) in diethyl ether (10 mL), methyl magnesium bromide (10 mL, 1.0 M in butyl ether, 9.45 mmol) was added dropwise at 0 °C. The reaction mixture was gradually warmed to room temperature and refluxed for 2 h. The reaction mixture was cooled gradually to room temperature. The reaction mixture was quenched with satd. aq. NH_4Cl solution (5 mL). The aqueous phase was extracted with ethyl acetate (3 × 25 mL). The combined organic layer was dried on Na_2SO_4 and concentrated under reduced pressure to get a crude oil. The crude was purified by silica gel column chromatography using EA/PE (1:4) as the eluant to give **20** (0.47 g, 52%) as a sticky oil: FTIR (ν_{max}, cm^{-1}): 3447, 2970, 1723, 1488, 1458, 1364, 1163, 953; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31-7.28 (m, 2H), 7.20-7.17 (m, 2H), 4.17 (s, 2H), 1.66 (s, 6H), 1.41 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 146.4, 134.5, 133.3, 127.2, 127.1, 126.6, 74.6, 43.4, 32.6, 32.4, 30.8. HRMS (ESI-TOF): Calculated $C_{14}H_{22}OS$ $[M+Na]^+$: 261.1289. Found $[M+Na]^{+:}$ 261.1289.

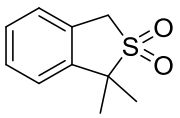


1,1-dimethyl-1,4-dihydrobenzo[d][1,2]oxathiine 3-oxide (21). To a solution of 2-(2-((tert-butylthio)methyl)phenyl)propan-2-ol **20** (0.84 g, 3.52 mmol) in dry THF (25 mL), *m*-CPBA (0.95 eq. in 5 mL THF) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was gradually warmed to room temperature and concentrated under reduced pressure on rotory evaporator to give a white sticky mass. To this crude material, satd. aq. $NaHCO_3$ solution (15 mL) was added and resulting mixture was washed with ethyl acetate (3×25



mL). The combined organic layer was dried on Na_2SO_4 and filtered. The resulting filtrate was concentrated under reduced pressure to give crude product. The crude was used for next step without further purification. To a solution of the sulfoxide (0.89 g, 3.49 mmol) in dry DCM (25 mL), sulfuryl chloride (0.565 mL, 7.0 mmol, in 15 mL DCM) was added dropwise at 0 °C during 5 min. The reaction mixture was stirred at 0 °C for 45 minutes. To reaction mixture, 10 mL of water was added and the resulting mixture was extracted with DCM (2×15 mL). The combined DCM layer was dried on Na_2SO_4 and filtered. The resulting filtrate was concentrated on a rotary evaporator under reduced pressure to give crude oil. The crude was purified by silica gel flash column chromatography using EA/PE (1:4) as the eluant to produce **21** (0.475 g, 68 %) as a white solid: mp 63-64 °C; FTIR (ν_{max}, cm^{-1}): 3440, 2978, 2360, 1493, 1449, 1382, 1363, 1238, 1184, 1104, 869; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40-7.27 (m, 3H), 7.18 (d, *J* = 7.3 Hz, 1H), 4.04 (d, *J* = 14.6 Hz, 1H), 3.85 (d, *J* = 14.6 Hz, 1H), 1.86 (s, 3H), 1.62 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$): δ 140.1, 131.5, 128.6, 127.8, 124.6, 122.2, 82.8, 54.6, 32.1, 31.5; HRMS (ESI-TOF): Calculated $C_{10}H_{12}O_2S$ $[M+H]^+$: 197.0636. Found $[M+H]^{+:}$ 197.0636.

1,1-dimethyl-1,3-dihydrobenzo[c]thiophene 2,2-dioxide (22) The solution of 1,1-dimethyl-1,4-dihydrobenzo[d][1,2]oxathiine 3-oxide **21** (0.300 g, 1.52 mmol) in 5 mL dry toluene was heated at 135 °C oil bath temperature for 24 h in sealed tube. The reaction mixture was cooled gradually to room temperature and concentrated under reduced pressure on rotory evaporator to give crude product. The crude was purified by silica gel flash column chromatography using EA/PE (1:4) as the eluant to give **22** (0.165 g, 55%) as a brown solid: mp 108-109 °C; FTIR (ν_{max}, cm^{-1}): 2925, 1463, 1304, 1209, 1189, 1127, 1105 ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41-7.26 (m, 4H), 4.30 (s, 2H), 1.64 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 142.5, 129.1, 128.8, 128.4, 126.0, 123.5, 63.5, 52.8, 22.7; HRMS (ESI-TOF): $C_{10}H_{12}O_2S$ $[M+H]^+$: 197.0636. Found $[M+H]^+$: 197.0645.



General procedure for photolysis of benzosulfones : A 1mM stock solution of compound was prepared in acetonitrile or ethanol. Using this stock solution, 250 mL of 100 µM solution was prepared in ACN or Ethanol /PB, pH 7.4 (% of ACN or Ethanol varies from 30 to 40%). The above solution was irradiated at 37 °C using 450 W UV light source having broad-spectrum from 200-400 nm, in an immersion

assembly. (Principle wavelengths: 365nm, 313nm and 253nm) After intervals of 10 and 60 min, the lamp was switched off and after 30 min an aliquot of the reaction mixture was injected in an ion chromatograph equipped with conductivity detector. SO_2 was quantified as SO_3^{2-}/SO_4^{2-}.

4.5 Spectral Data

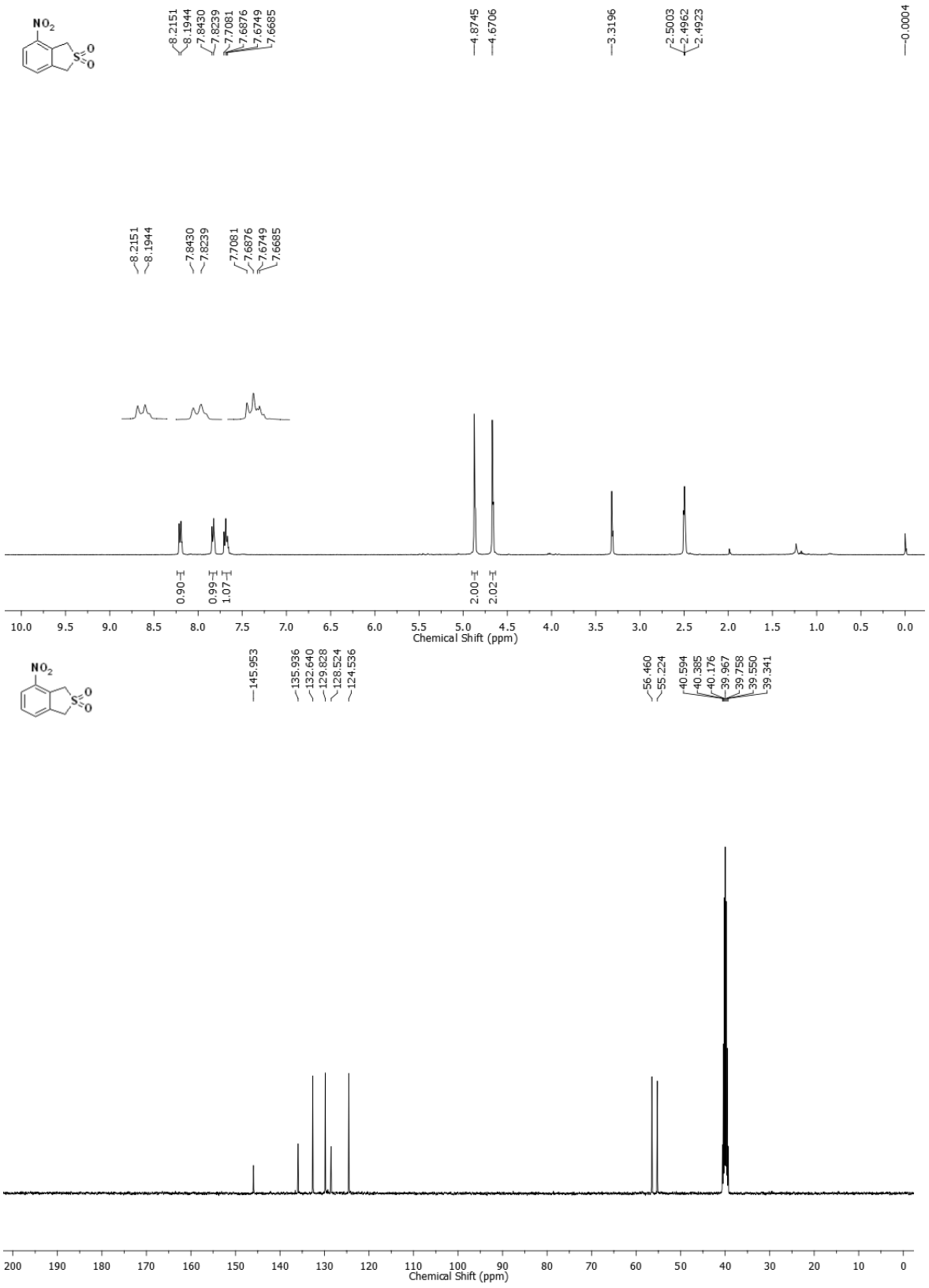


Figure S1. NMR spectra of **4b**

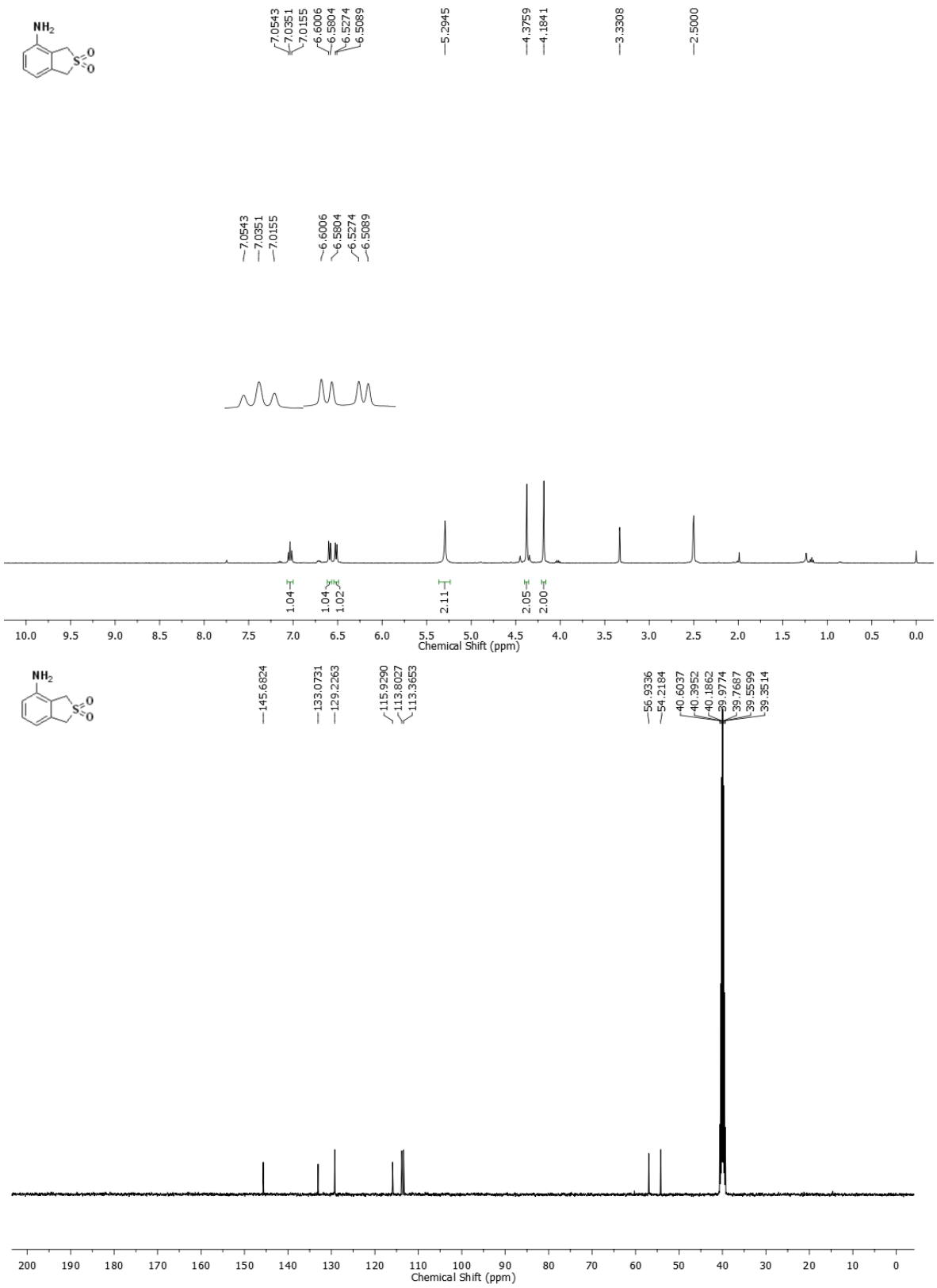


Figure S2. NMR spectra of **5a**

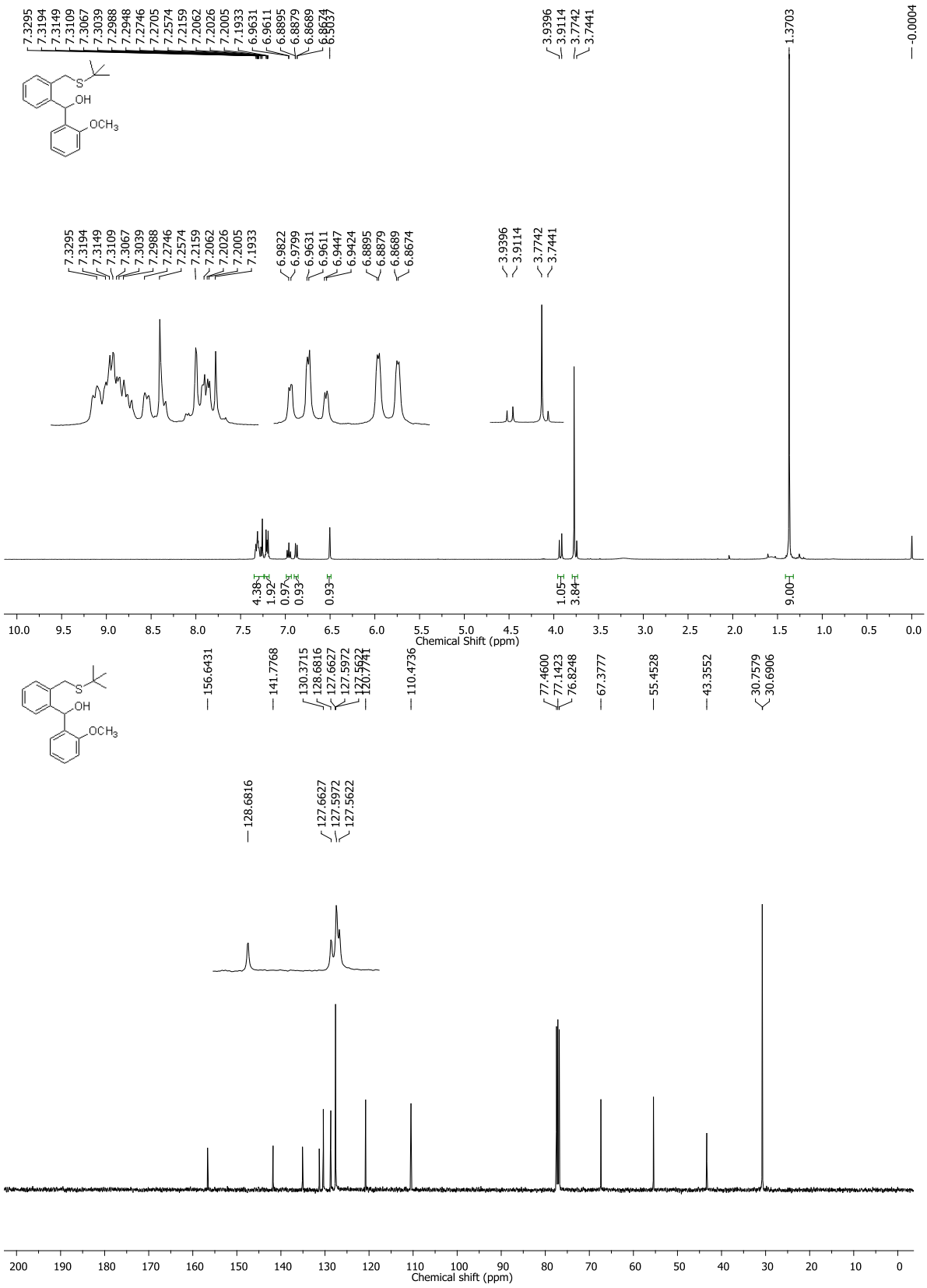


Figure S3. NMR spectra of **10b**

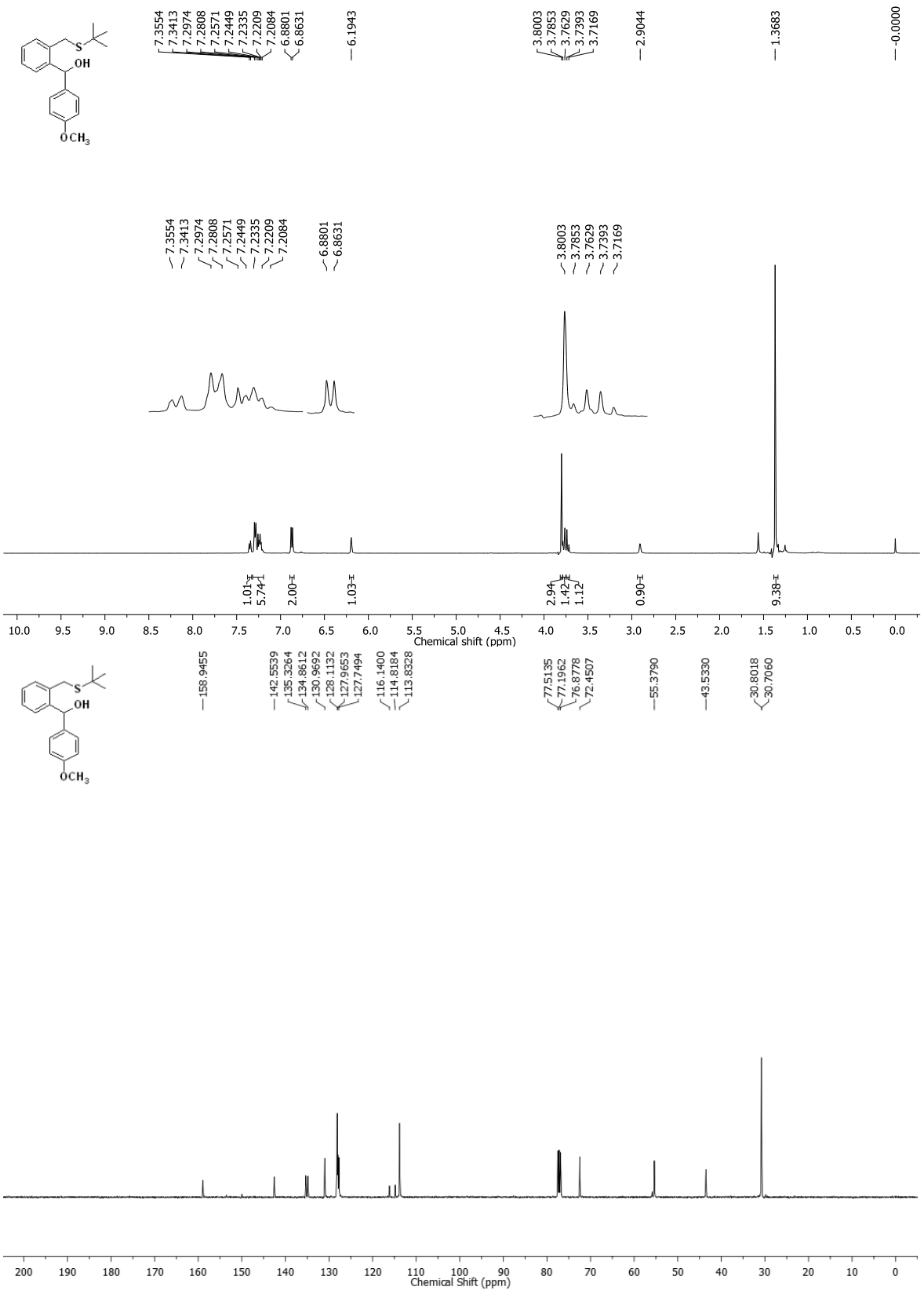


Figure S4. NMR spectra of **10f**

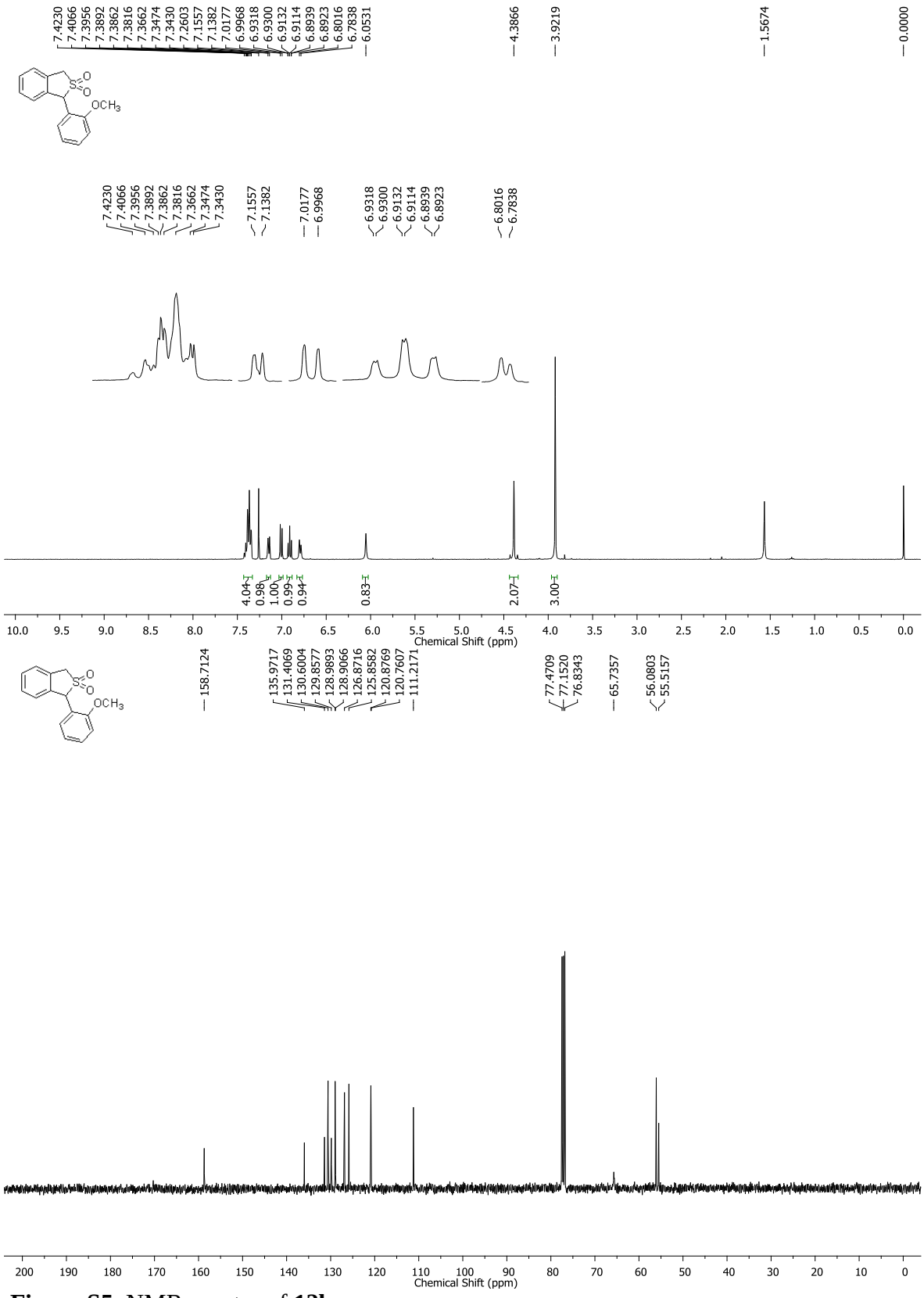


Figure S5. NMR spectra of **12b**

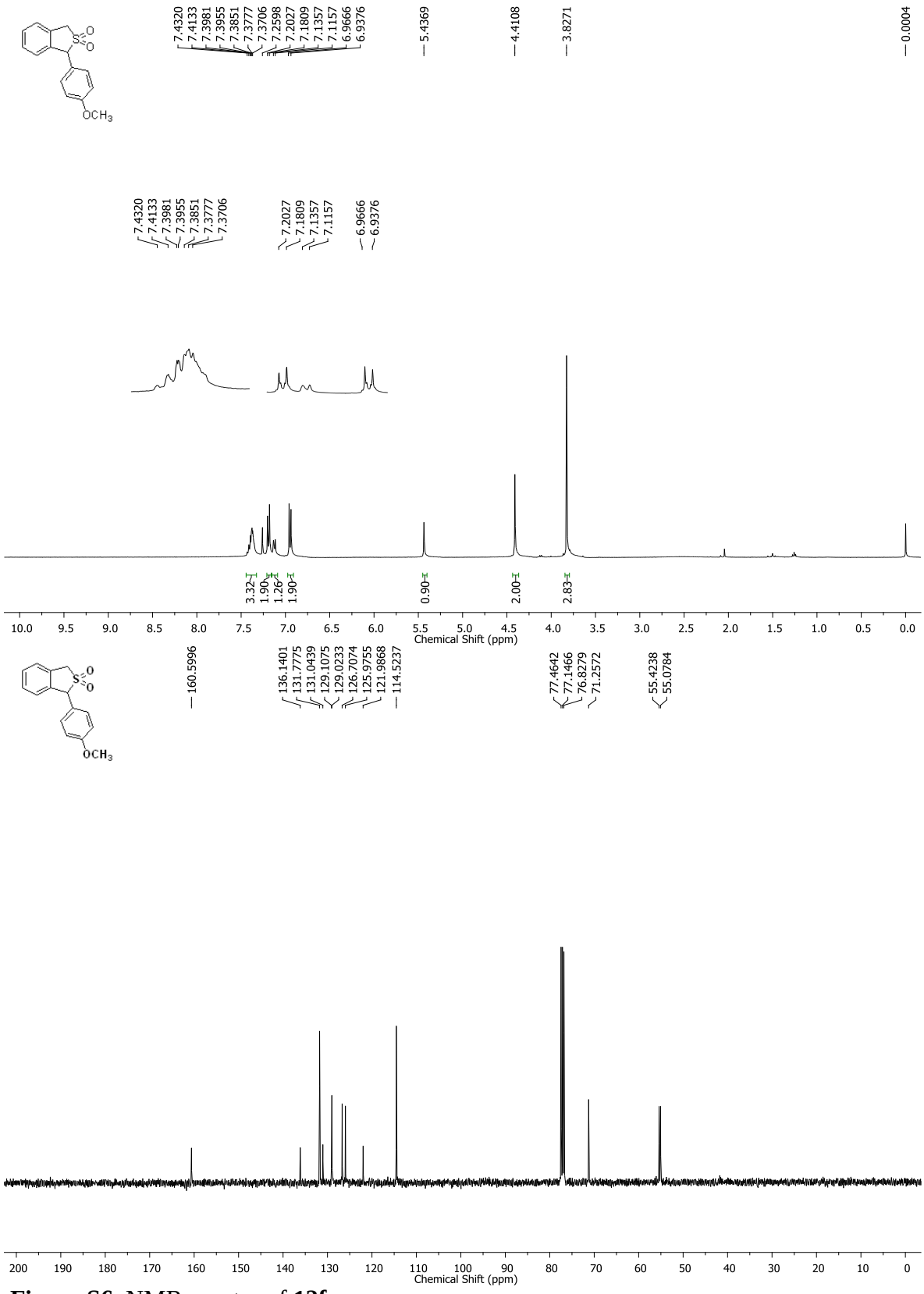


Figure S6. NMR spectra of **12f**

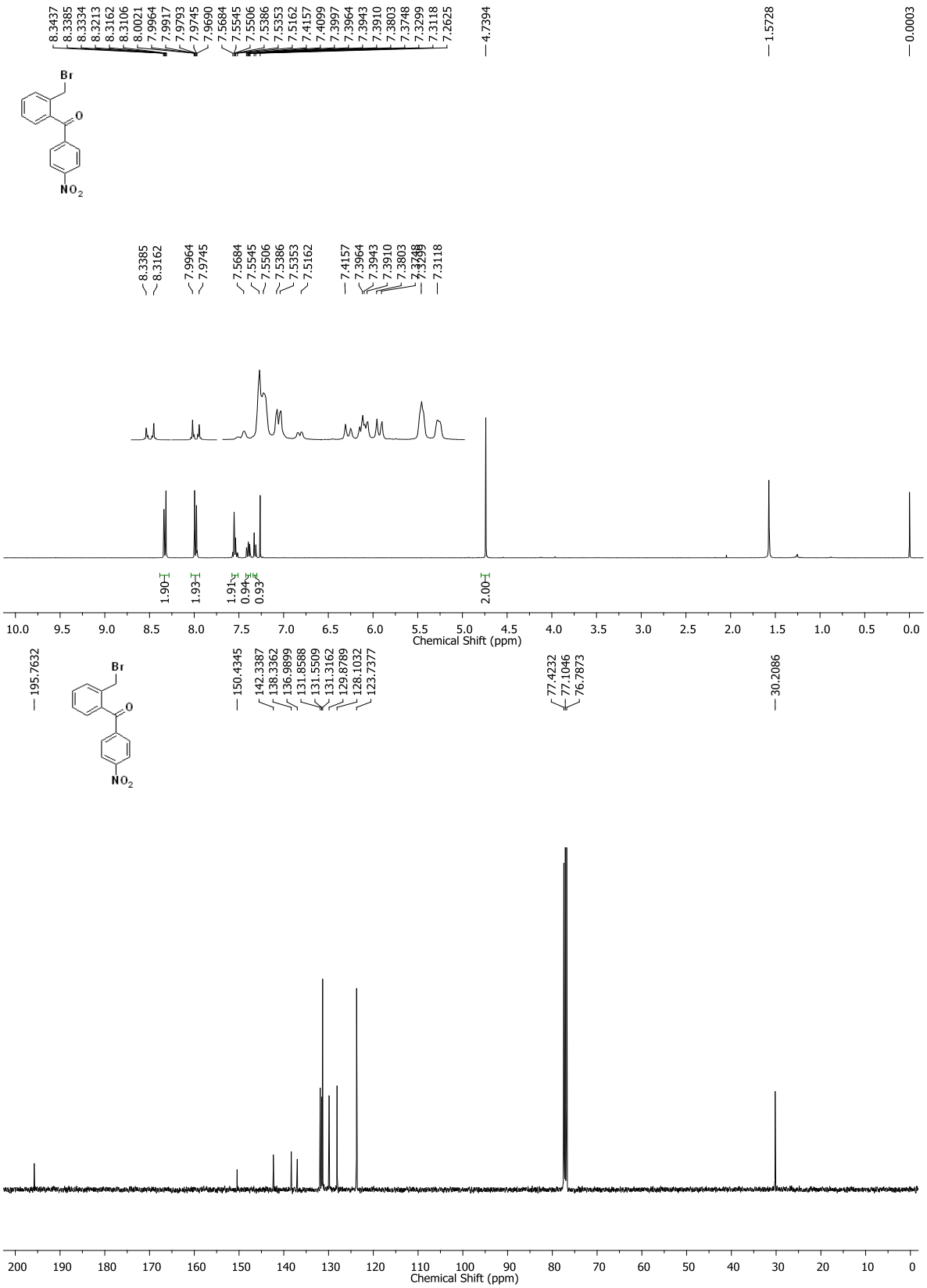


Figure S7. NMR spectra of **16**

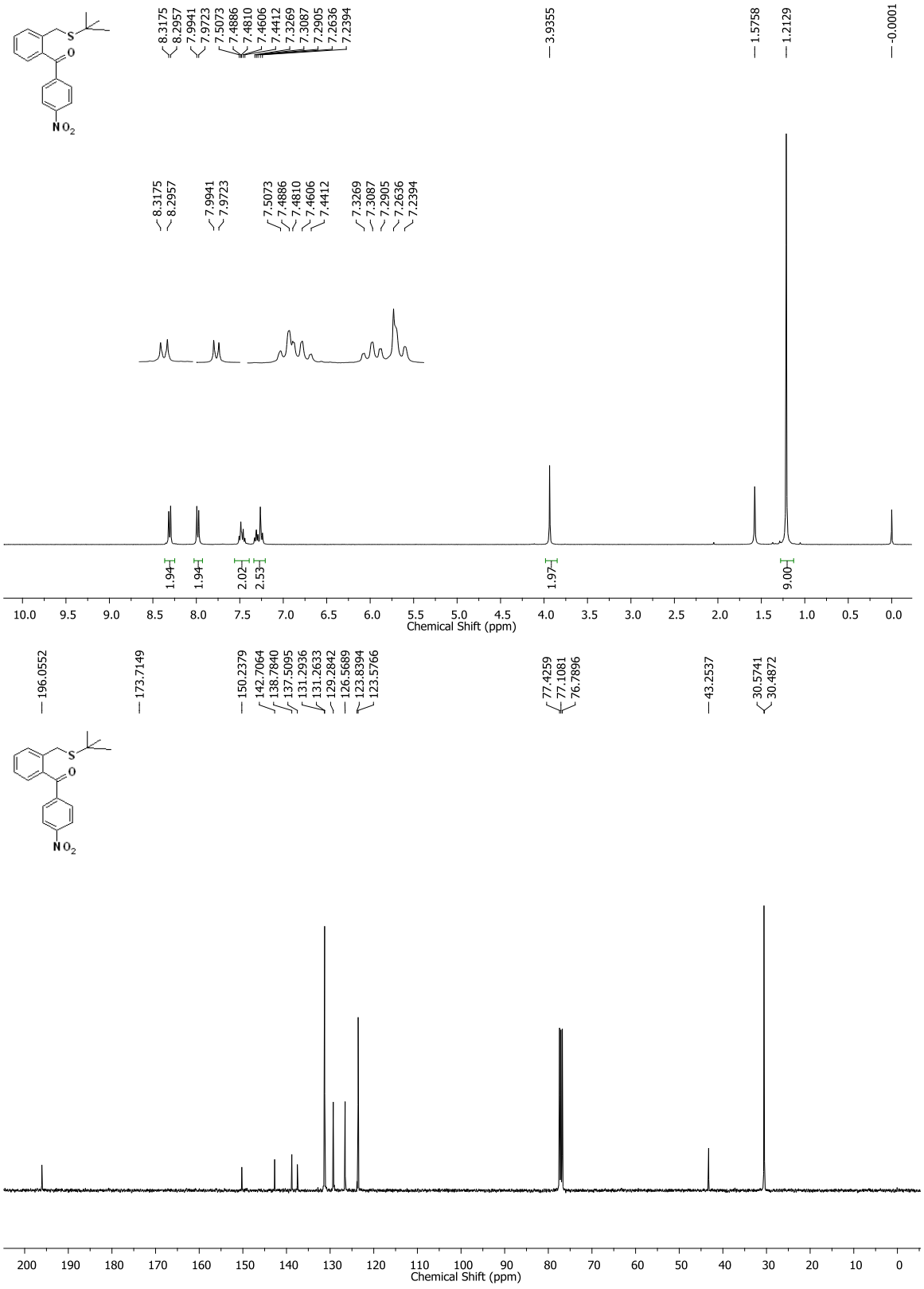


Figure S8. NMR spectra of **17**

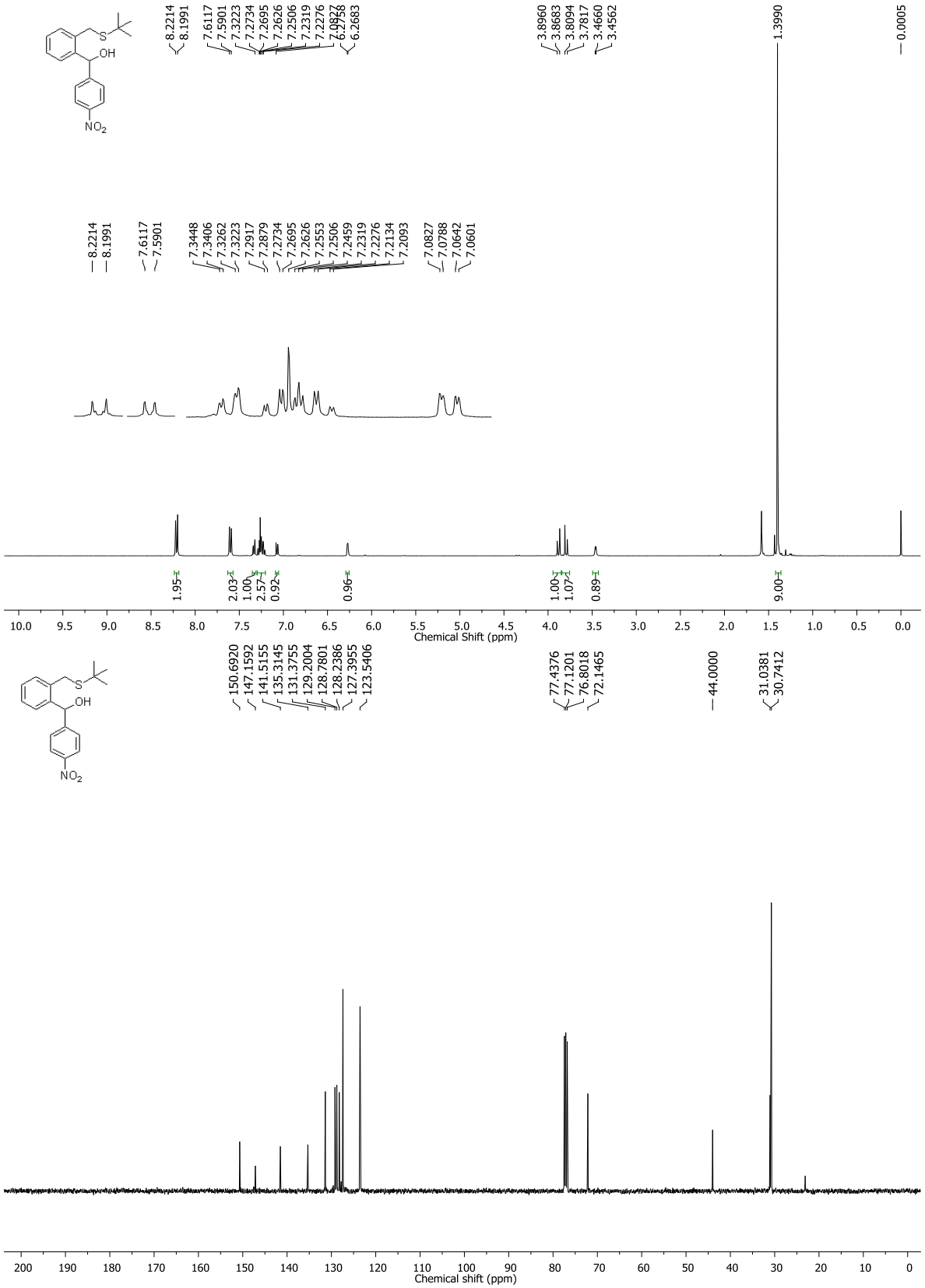


Figure S9. NMR spectra of **10j**

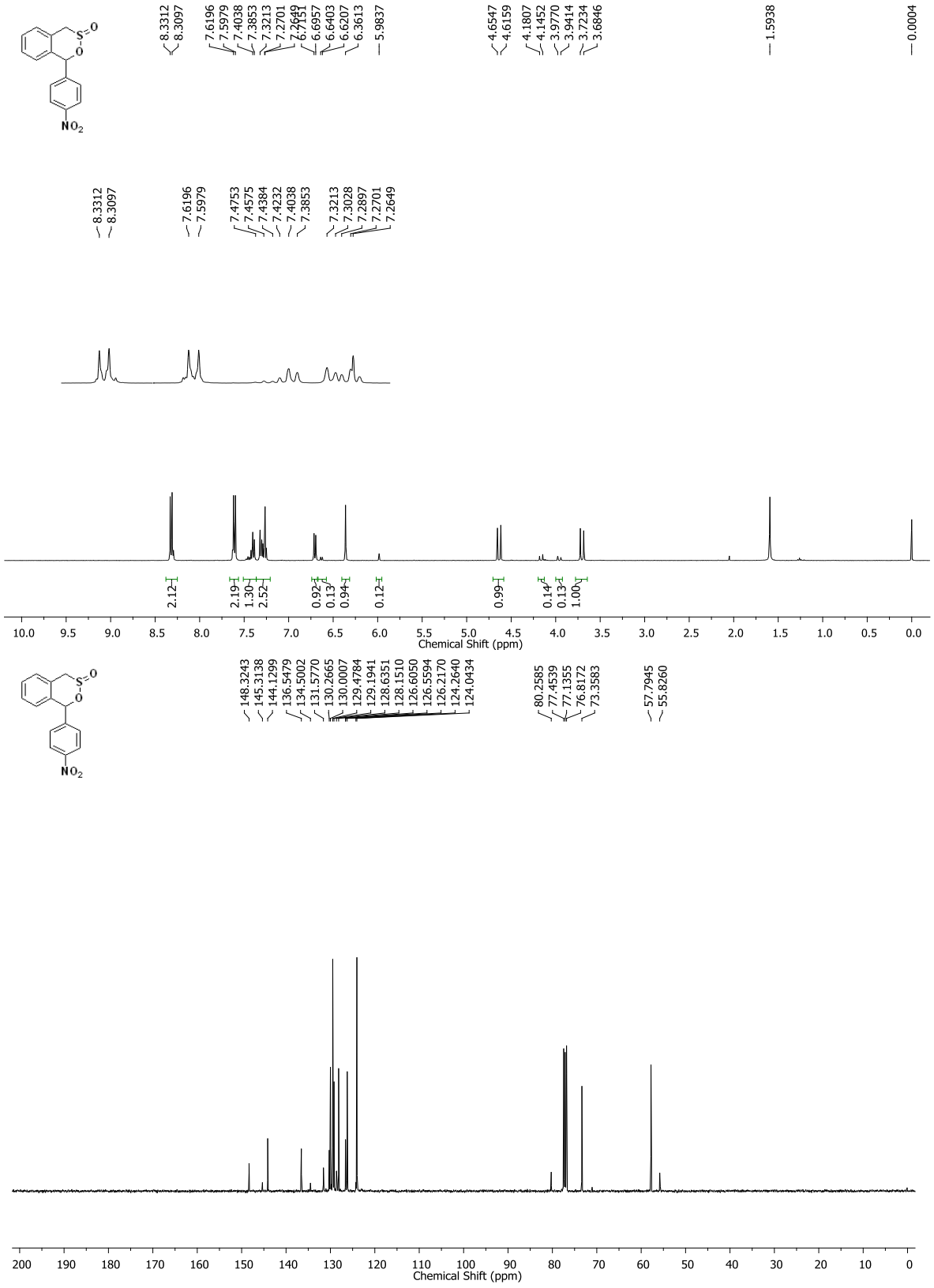


Figure S10. NMR spectra of **11j**

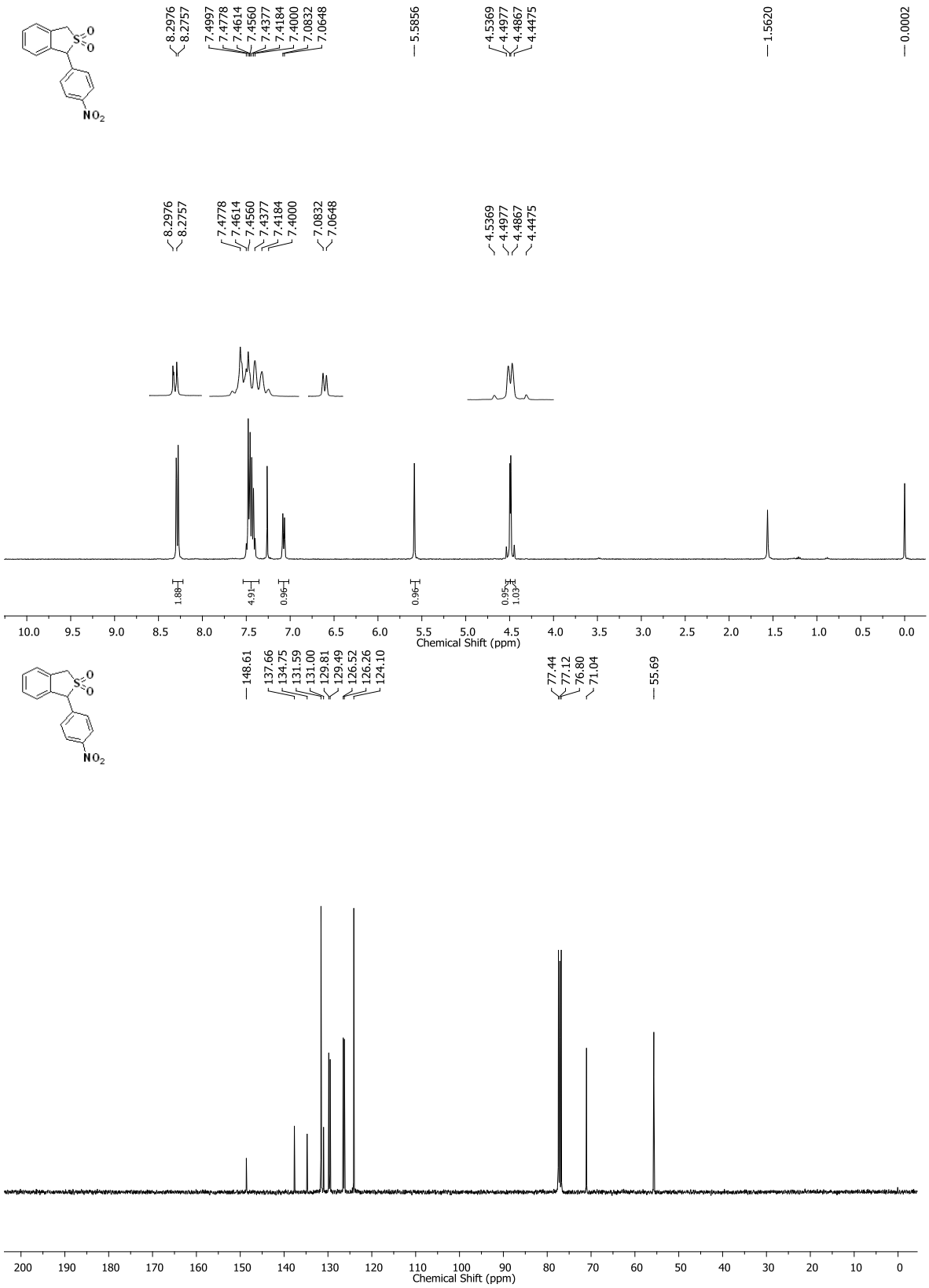


Figure S11. NMR spectra of **12j**

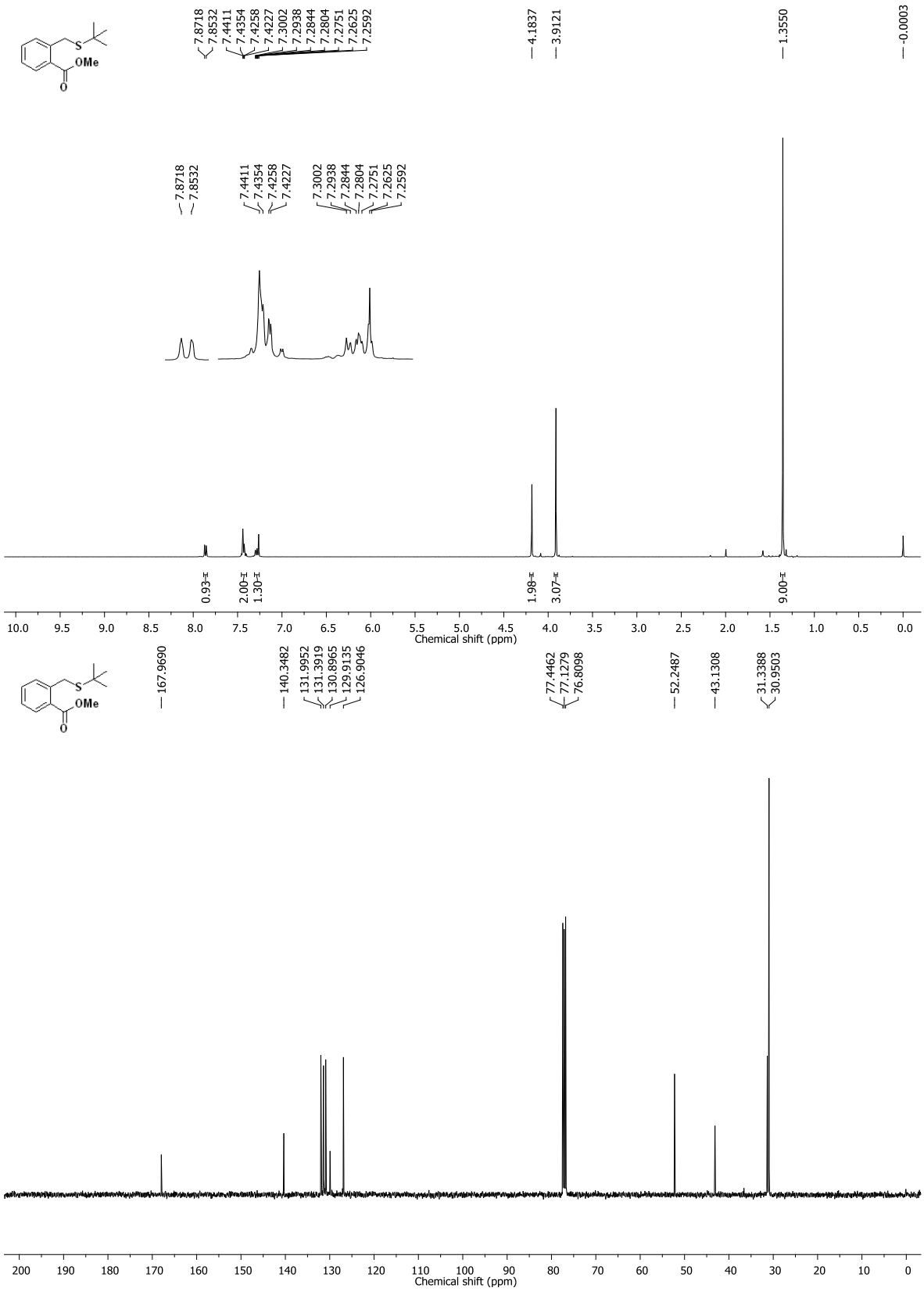


Figure S12. NMR spectra of **19**

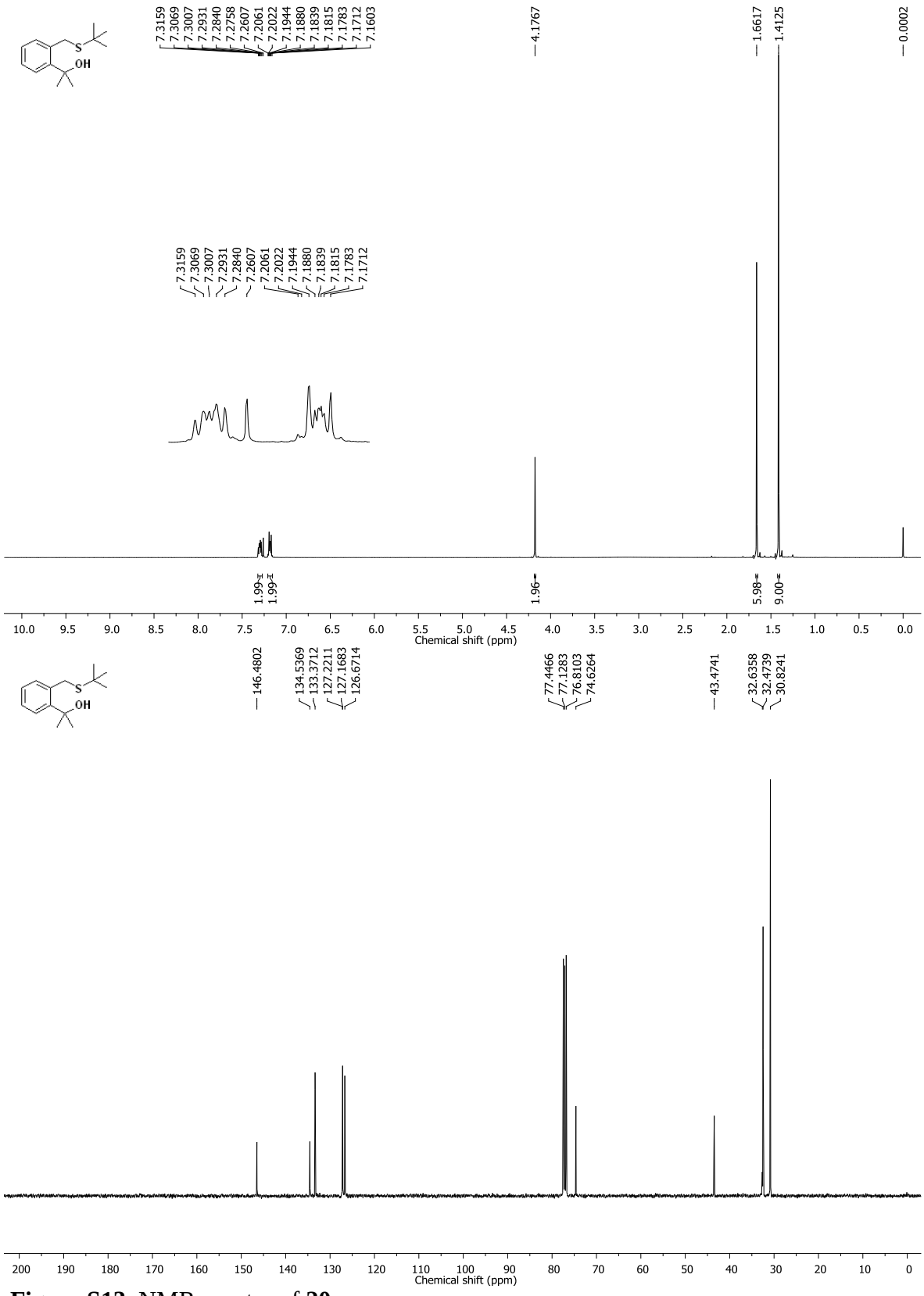


Figure S13. NMR spectra of **20**

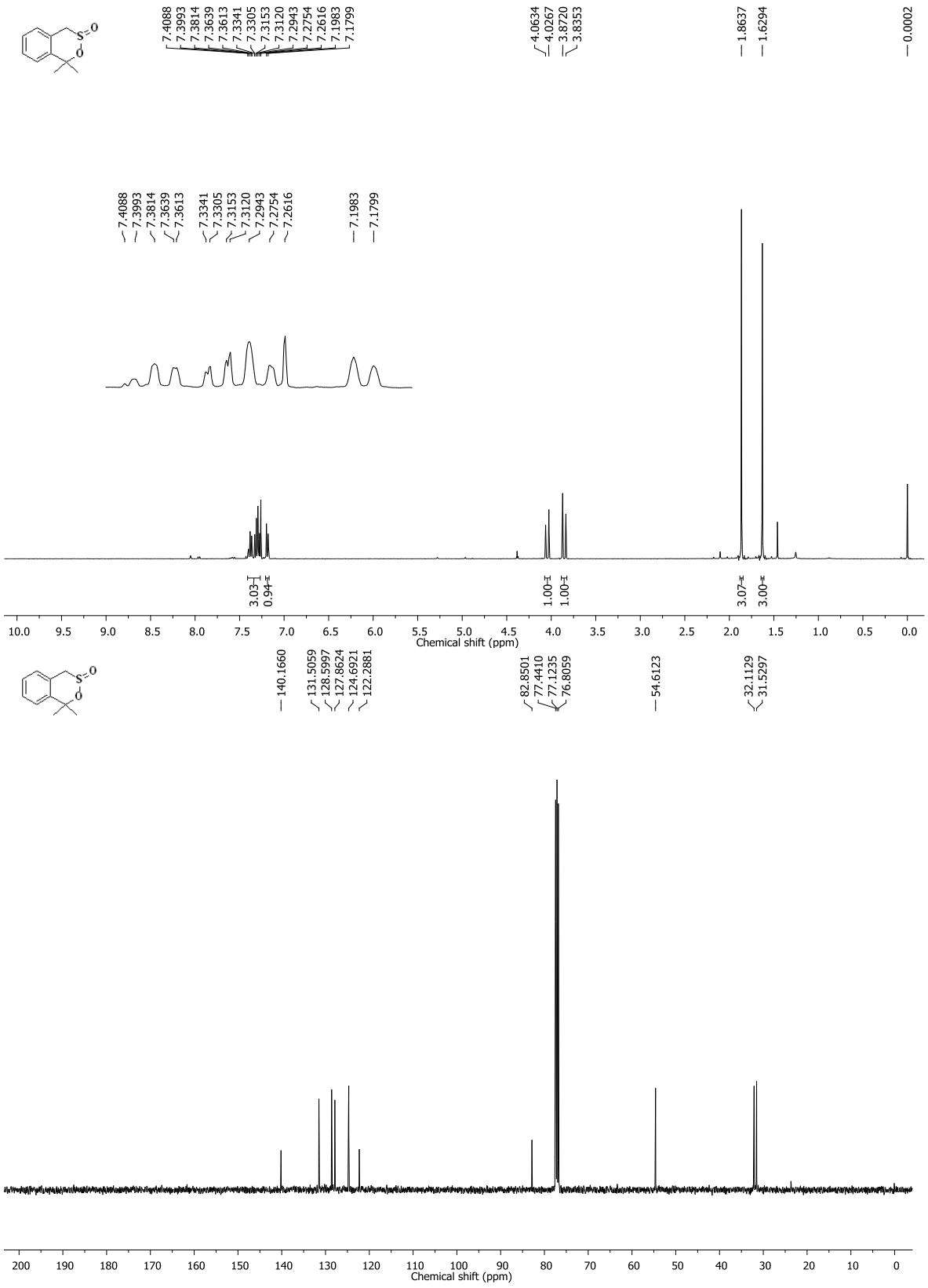


Figure S14. NMR spectra of **21**

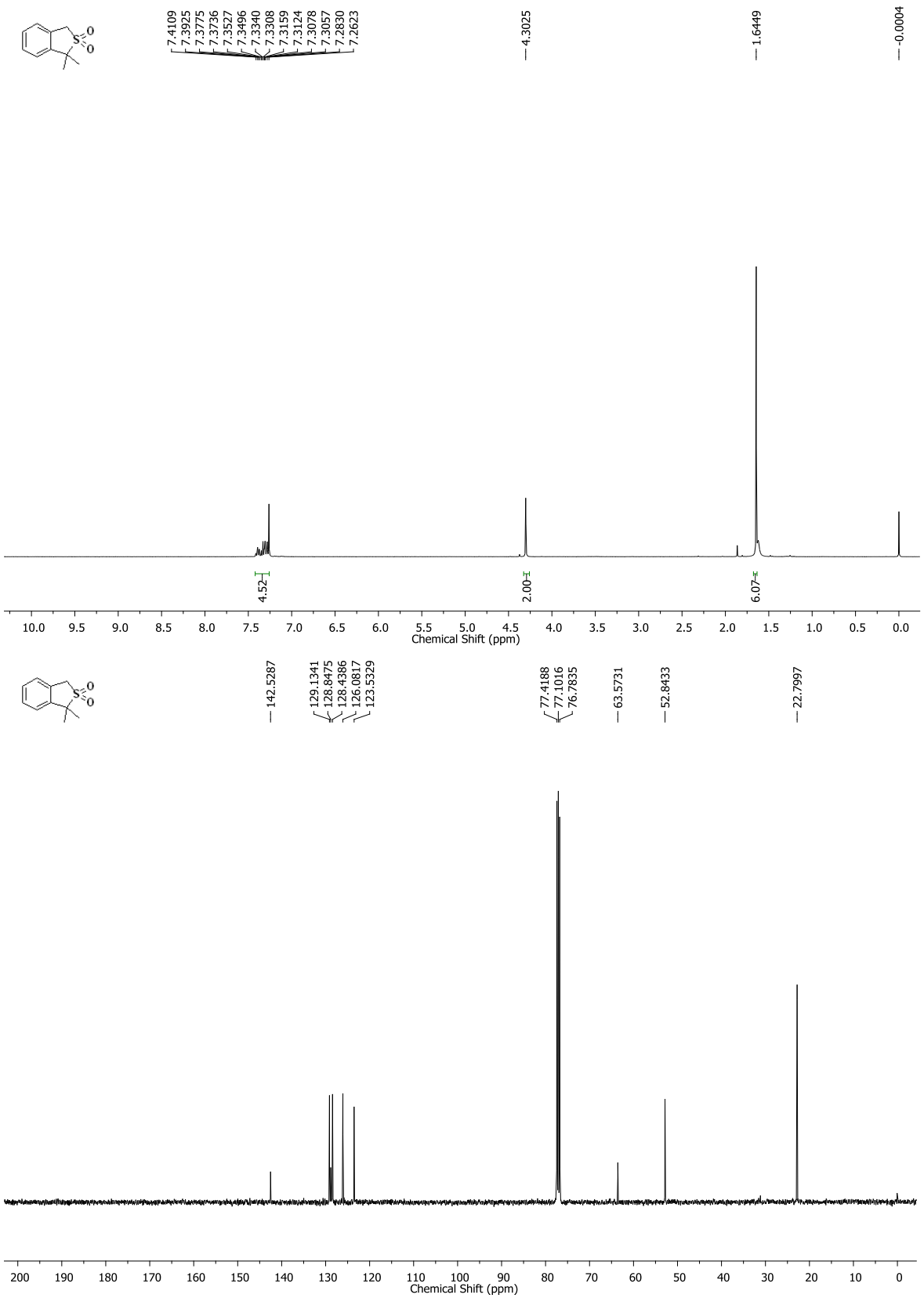


Figure S15. NMR spectra of **22**

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