Expanding the Synthetic Accessibility of Thiocarbamate (TCM) and Dithiocarbamate (DTCM) Donors for Hydrogen Sulfide (H_2S) and Carbon Disulfide (CS_2) Delivery

By

RACHEL E. LUTZ

Presented to the Department of Chemistry and Biochemistry
and the Robert D. Clark Honors College at the University of Oregon
in partial fulfillment of the requirements for the degree of Bachelor of Science

May 2020

ABSTRACT

Title: Expanding the Synthetic Accessibility of Thiocarbamate and Dithiocarbamate Donors for Hydrogen Sulfide and Carbon Disulfide Delivery

Approved: _____*Dr. Michael D. Pluth*_____
Primary Thesis Advisor

The goal of this thesis is to bridge the gap in the literature by developing a suite of thiocarbamate and dithiocarbamate donor synthesis with azide and *tert*-butyl ester triggers. Based on previous work with esterase-triggered TCM H_2S donors, we have developed a library H_2S donors both with and without the nitrogen of the payload methylated, and have installed various EWGs and EDGs on the payload. Furthermore, we have developed a new method for modular TCM synthesis that supports diversification of the alkyl group of the aniline. This method allows us to block an unproductive deprotonation-based side pathway to more clearly study the effect of changing the payload electron density on the self-immolation of this donor motif. Blocking deprotonation of the payload expands the synthetic utility of these donors, which make them less reactive under basic conditions and allowing for more harsh synthetic conditions. Due to the ease of synthetic variation of the TCM donors, it is natural to consider broadening their application beyond H_2S release to another biologically relevant molecule, CS_2. Our TCM donor motif can yield this small molecule by designing the compound to contain a dithiocarbamate. Rather than releasing COS upon self-immolation, the donor should release CS_2. Thus, I synthesized N-methylated CS_2 DTCM donors using the same synthetic scheme as with methylated S-alkyl TCM donors.

ACKNOWLEDGEMENTS

My accomplishments and successes in research are directly related to the wonderful support of my mentors and fellow labmates of the Pluth lab. The members of the Pluth group inspire a positive attitude towards research, problem solving, and asking questions. I've been so lucky to spend my last two years of college surrounded by such driven and kind people. I owe a special thanks to my graduate student mentor, Carrie (soon to be Dr. Levinn), who not only worked on the research in this paper with me but has also been an outstanding role model both in and out of the lab. It has been an honor to work with her over the years and I will always be grateful for the experiences I have had in the Pluth lab.

I also owe deep thanks to Professor Pluth, who has pushed me to perform undergraduate research at a high level and has always encouraged me to seek further research opportunities. Through work as an undergraduate in his lab I have become proficient in performing and presenting research, which are skills that will help me for the rest of my life. I am thankful that he has agreed to be on my thesis committee and mentor me through the long and rewarding process of performing this research.

I'd like to thank both the PURS program and the Kuntz-Swinehart Memorial fund through the Chemistry and Biochemistry department at the University of Oregon for funding and encouraging my research. The PURS program emphasized the

importance of research in our community, as well as the importance of communicating that research to others.

To my Clark Honors College thesis advisor, Dr. Frank, thank you for your unwavering support of my interests both in and out of science. Your passion is contagious, and I am so lucky to have you as an advisor and mentor.

Lastly, I'd like to acknowledge the Partnership for Scientific Inquiry (PSI) program at Oregon Health and Sciences University (OHSU) for encouraging my interest in research right from the start, when I was still a high school student. My mentor at the time, Dr. Messer, taught me that there is a marriage of humanity and innovation in research which has continued to inspire me through college. It was through the diagnosis and treatment of a close family friend at OHSU that my passion for research began, and I will always humbly remember those beginnings.

TABLE OF CONTENTS

INTRODUCTION

Hydrogen Sulfide (H_2S)

H_2S as a Biologically Relevant Molecule

Hydrogen sulfide (H_2S) is notorious as the culprit behind the foul, rotten egg smell of volcanic vents, sewers, and decomposition. Its lethality is also well established, as exposure to H_2S levels above 500 ppm causes death.[1] However, there is much more to H_2S than death and decay. In fact, H_2S was recently identified as a gasotransmitter,[2] joining the ranks of carbon monoxide (CO) and nitric oxide (NO). Gasotransmitters are small, gaseous molecules that are produced endogenously and have important physiological functions.[3] This discovery has prompted the need for synthetically derived H_2S donors to better understand the role of H_2S. Now, H_2S is no longer merely a noxious toxin, but also broadly accepted as a biologically relevant molecule with many roles in the human body.

H_2S in Human Physiology

Since its identification as a gasotransmitter, H_2S has been found to play a role in many organ systems such as the cardiovascular system, the digestive system, the nervous system, the lungs, the liver as well as in pro- and anti- apoptotic pathways.[4] H_2S is supplied to tissues throughout the body by three enzymes, cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (MST).[4] These enzymes are responsible for some of the endogenous production of H_2S.

---

[1] National Research Council (US) Committee on Acute Exposure Guideline Levels. Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 9. Washington (DC): National Academies Press (US); 2010, 4, Hydrogen Sulfide Acute Exposure Guideline Levels
[2] Wang, R. *FASEB* **2002**, *16*, 1792–1798.
[3] Wang, R. *Antioxid. Redox Signal.* **2004**, *5*, 493-501
[4] Levinn, C. M.; Cerda, M. M.; Pluth, M. D. *Antioxid. Redox Signal.* **2020**, *32(2)*, 96-109

A substantial amount of H_2S is also produced by bacteria that live in the human microbiome. In the nervous system, H_2S plays a neuroprotective role and contributes to neurotransmission. In the cardiovascular system, H_2S has been found to be cardioprotective, and helps aid in vasodilation. It also aids the lungs as a bronchodilator and helps with insulin production and blood flow regulation in the liver.[4] Due to its global role in the body, it is natural to suspect mis-regulation of H_2S as a culprit for some diseases. It is thought to play a key role in diseases such as hypertension, diabetes and asthma.[5] Elucidation of the role H_2S plays in disease is essential; however, further study of H_2S in biological systems will require the development of a library of synthetically derived H_2S donors to be utilized for research.

Prior H_2S Small Molecule Donors

Small molecule H_2S donors are useful in studying cellular processes because they provide a toolbox for altering H_2S levels in cells. Some H_2S donor platforms can even be targeted to certain cell types or specific organelles such as the mitochondria[6] and the lysosome. In a lysosome-targeted H_2S donor, Lyso-pHTCM, the donor releases H_2S when it enters a pH-specific window specific to the lysosome.[7] This design affords research of H_2S in acidic microenvironments. By designing donors for organelle-specific delivery, researchers can investigate the role of H_2S in the functions of specific organelles.

Due to the precarious role of H_2S as both a gasotransmitter and a toxin, it is essential to release H_2S in a controlled fashion to both mimic natural production and

[5] Wang, R. *Physiol. Rev.* **2012**, *92*, 791-896.
[6] Szczesny, B.; et. al, *Nitric Oxide* **2014**, *41*, 120-130.
[7] Gilbert, A. K.; Zhao, Y.; Otteson, C. E.; Pluth, M. D. *J. Org. Chem.* **2019**, *84*, 14469–14475.

avoid cell death.[8] Many studies of H_2S in biological systems rely on NaSH or Na_2S as a source for H_2S.[9] Rather than mimicking the slow endogenous release of H_2S, these sources cause a rapid release of H_2S which can cause cell death and deviates significantly from natural H_2S release.[10] These sulfur sources are also not triggerable or tunable, making their release fairly uncontrolled. Furthermore, they do not afford control compounds such as CO_2 releasing or triggerless compounds. The use of NaSH or Na_2S for rapid H_2S release is often incompatible with biological processes.

It is also desirable to create donors that can provide on-demand, controlled release of H_2S when exposed to certain stimuli. Thus, it is important to understand how the structure of donor molecules contributes to their function in terms of tunability of release rates, triggerability, and stability. This provides a synthetic challenge for organic chemists. To meet these needs, the Pluth lab at the University of Oregon has developed various types of H_2S donors which can deliver H_2S through a myriad of mechanisms. Some recent examples include acid-mediated donors that become activated at a specific pH window,[7] cysteine-triggered donors,[11] and esterase-triggered self-immolative thiocarbamates (TCMs),[12]

Carbonyl Sulfide (COS) Based H_2S Delivery

The Pluth lab has developed a TCM donor motif for triggerable H_2S release *via* COS intermediate which allows for the controlled release of H_2S. In the

[8] Steiger, A.K.; Marcatti, M.; Szabo, C.; Szczesny, B.; Pluth, M.D. *ACS Chem. Biol.* **2017**, 12(8), 2117-2123.
[9] Levinn, C. M.; Cerda, M. M.; Pluth, M. D. *Acc. Chem. Res.* **2019**, *52,* 2723-2731.
[10] DeLeon, E. R.; Gilbrian F. S.; Kenneth R. O. *Anal. Biochem.* **2012**, *421.1,* 203-207.
[11] Cerda, M. M.; Newton, T. D.; Zhao, Y.; Collins, B. K; Hendon, C. H.; Pluth, M. D. *Chem. Sci.* **2019**, *10*, 1773-1779.
[12] Levinn, C. M.; Steiger, A. K., Pluth, M. D. *ACS Chemical Biology* **2019**, *14,* 170-175.

proposed mechanism the triggering event activates the donor, resulting in self-immolation in which the donor breaks down to release an amine payload, and COS (Figure 1). The COS is subsequently converted to H_2S by carbonic anhydrase (CA), a ubiquitous enzyme in many organisms.

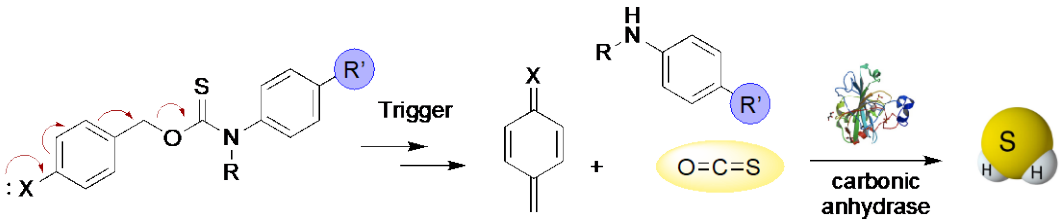


Figure 1. *Self-immolation of TCM donors and subsequent conversion of COS to H_2S*

Unlike other donor types, these TCM donors can be easily designed to produce control compounds that are trigger-less or release CO_2 rather than H_2S. A trigger-less control compound allows for researchers to assess how much H_2S release is initiated by the triggering event compared to 'leaky' release of H_2S as a baseline. A control compound that releases CO_2 rather than H_2S can be used to help confirm that none of the byproducts of self-immolation result in biological activity or a false signal of H_2S release. Ideally, these control compounds allow researchers to study the effects of the byproducts of self-immolation independently of COS production. While these controls afford useful insights into these donor platforms, they do not control for differences in subcellular localization that may occur.

Furthermore, the design of these compounds can be altered in multiple ways to tune the rate of H_2S release. For example, the trigger in the 'X' position can be interchanged, the designation of an *O*-alkyl or *S*-alkyl donor can be selected, and both the R group bound to the amine and the R' group on the amine payload can be

manipulated. This modular design allows for controlled optimization of H_2S release through various design choices.

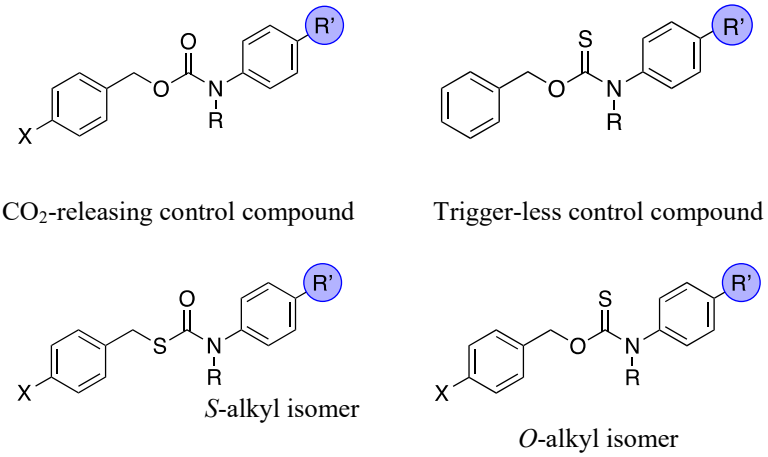


Figure 2. *Control Compounds afforded by Donor Scaffold*

The designation of an *O*-alkyl or *S*-alkyl donor is significant because this provides another modulation of H_2S release rate. Some *S*-alkyl donors have been found to release H_2S very slowly, whereas the corresponding *O*-alkyl donors have been found to have a more rapid release rate, on the order of minutes rather than hours.[13] Furthermore, the *O*-alkyl donors will isomerize to the *S*-alkyl over time, because the *S*-alkyl isomer is the thermodynamic sink.

The mechanism of H_2S release *via* COS intermediate occurs through a self-immolation event through the benzyl group, releasing a para-quinone methide and the TCM anion which then breaks down to release COS. During the self-immolation event, electron density flows across the donor from the trigger into the thiocarbamate group and the amine payload portion. Thus, it is intuitive to think that installing electron withdrawing groups (EWGs) in the para position of the payload (R' in Figure 2.) would assist in the natural flow of the breakdown. Contrastingly, electron donating groups

[13] Zhao, Y.; Henthorn, H. A.; Pluth, M. D., *J. Am. Chem. Soc.* **2017**, *139, 45*, 16365-16376

(EDGs) in the para position would counter the movement of electron density that is required for breakdown, hindering the process. These events are summarized by Figure 3.

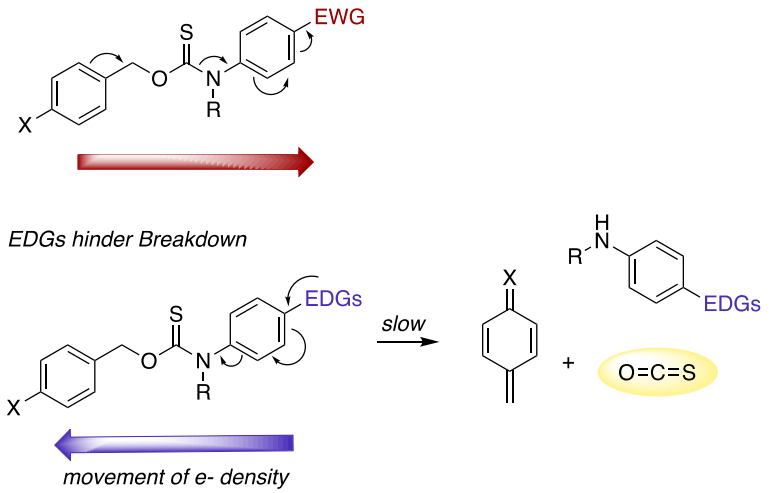


Figure 3. *The Role of Electronics in Triggered Self-Immolation of TCM Donor Scaffold*

Recent research by Levinn *et. al* demonstrated that a *tert*-butyl ester group can function as a trigger for self-immolative TCM donors via esterase hydrolysis and subsequent conversion of COS to H_2S by the CA enzyme. It also showed that the installment of electron withdrawing and electron donating groups on the payload of esterase-triggered TCM donors yielded no clear trend in H_2S release. This raised questions on the mechanism of self-immolation in these donors. With the discovery that installing EWGs did not always necessarily accelerate the release of H_2S when compared to EDGs, the mechanistic hypothesis was not sufficient to explain the lack of trend.

Upon further examination of the TCM donors in the Levinn *et. al*, a competing pathway hypothesis was developed to explain the results. This hypothesis recognizes that in TCM H_2S donors with EWGs in the para position, the hydrogen bound to the

amine portion of the donor is acidified. Rather than aiding in the flow of self-immolation, this acidic amine could result in a non-productive deprotonation-based pathway that competes with the traditional mechanism. These two pathways are shown in Figure 3.

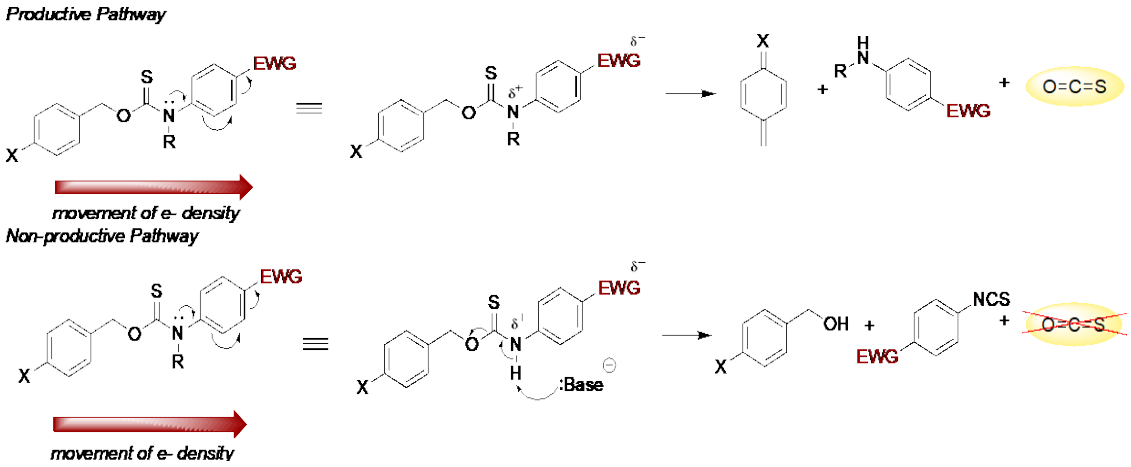


Figure 4. *The Competing Pathway Hypothesis*

Carbon Disulfide (CS_2)

CS_2 as a Biologically Relevant Molecule

Carbon disulfide (CS_2) is another sulfur-containing biologically relevant molecule. In rats, it has been found to be metabolized to both carbon dioxide (CO_2) and carbonyl sulfide (COS).[14] There is debate on the status of COS as a possible gasotransmitter, and it is a known caged source of H_2S in biological systems.[15] Thus, the metabolism of CS_2 to COS could have implications on H_2S research. Furthermore, CS_2 has biological implications in its own right. Research using mouse models have shown that mice have a system developed for detection of CS_2, which has been found to be related to socially transmitted food behaviors in mice. This system includes a specialized olfaction with olfactory neurons that express the receptor guanylyl cyclase GC-D, the cyclic nucleotide-gated channel subunit CNGA3, and an isoform of carbonic anhydrase (CA).[16] It has also long been known there is a correlation between patients with schizophrenia and increased CS_2 levels in the breath, indicating that there are increased levels of CS_2 in the blood as well.[17] Synthetic donors for CS_2 release could provide a method for exogenous CS_2 to be used for further research into this biomolecule.

Prior CS_2 Small Molecule Donors

There are very few CS_2 donors in the literature. Due to its toxicity, CS_2 has not been investigated as a biomolecule until recently, leaving a gap in synthetically available CS_2 donors. Currently, dithiocarbamate (DTCM) anions have been used for

[14] Ramesh, R.; Dalvi, R.; Poore, R. E.; Neal, R. A., *Life Sci.* **1974**, *14(9), 1785-1796*.
[15] Zhao, Y.; Steiger, A. K.; M. D. Pluth, *J. Am. Chem. Soc.* **2019,** *141.34,* 13610-13618.
[16] Munger, S.D., *et. al. Curr. Biol.* **2010**, *20*, 1438-1444.
[17] Phillips, M.; Sabas, M.; Greenberg, J. *J. Clin. Pathol.* **1993**, *46*, 861-864.

thermal release of CS_2. This class of molecules shows a broad range of release rates that don't follow a clear trend, and release is monitored by tracking the disappearance of the DTCM group by *UV-vis* spectroscopy.[18] These donors are summarized in Figure 4. Unlike the self-immolative TCM donor scaffold that the Pluth lab has developed, these donors are not readily tunable, lack a triggerable handle, and do not afford suitable control compounds. Thus, there is a significant need for a diverse toolbox of CS_2 donors.

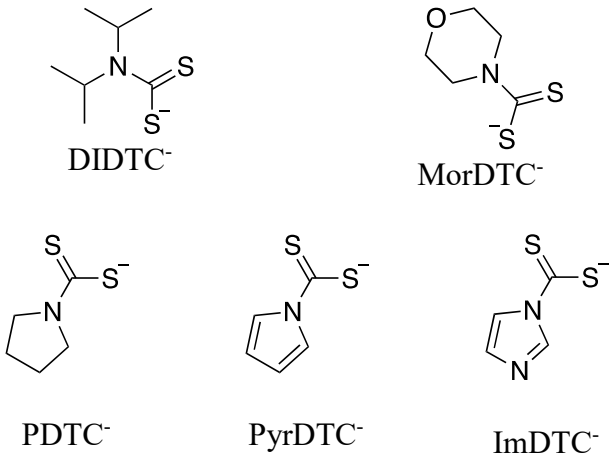


***Figure 5**. Current Dithiocarbamate CS_2 donors in Literature*[17]

[18] DeMartino, A. W.; Souza, M.L.; Ford, P. C., *Chem. Sci.* **2017**, *8*, 7186-7196.

Challenges in Measuring CS_2 Release

In addition to the very narrow selection of CS_2 delivery molecules, there is also a significant need for improved CS_2 detection techniques. Current methods have a high limit for detection (20 uM), which is incompatible with endogenously relevant concentrations of CS_2 that would be studied in biological settings. Previously, the Pluth lab designed DTCM donors, but were not able to observe any CS_2 release.[13] Surprisingly, some H_2S release was observed in N-H DTCM donors, but this was attributed to the deprotonation of the acidic proton, yielding isothiocyanate which slowly hydrolyzed.

METHODS

Synthesis

Synthesis of all compounds in this paper was performed at the University of Oregon in the Pluth lab in a well-ventilated fume hood by Rachel Lutz or Carolyn Levinn. Characterization of the compounds made was performed primarily by NMR spectroscopy.

Many of the donor compounds were designed to include an azide trigger which was installed in the first step of the synthesis. Working with azides is hazardous, as the azide can participate in an exothermic breakdown if given sufficient activation energy. Azides are known for their toxicity, photosensitivity, and shock sensitivity, so it is essential to treat them with care. Thus, all steps of synthesis after the installment of an azide were performed in low light by covering reaction flasks with aluminum foil and without even gentle heating during workup.

Synthesis of H_2S Donors

Azide-Triggered N-H H_2S donors

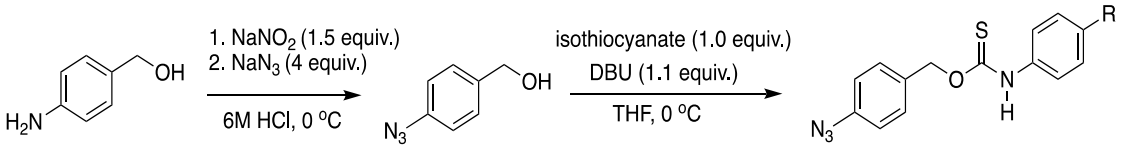


Figure 6. *Synthesis of Azide-Triggered N-H H_2S Donors*

The first reaction is a Sandmeyer reaction to install the azide. First, 4-amino benzyl alcohol (1.0 equiv.) was dissolved in 6 M HCl (0.1 M) in a round bottom flask with a stir bar. The flask was wrapped in aluminum foil and placed in an ice water bath. The reaction flask was cooled to 0 °C. Separately, 1.5 equivalents of $NaNO_2$ and 4 equivalents of NaN_3 were dissolved in two scintillation vials, each containing 10 mL of

water. First, the $NaNO_2$ was added dropwise from the scintillation vial to the reaction flask. After stirring for five minutes, the NaN_3 was added dropwise. The reaction was monitored for 2 hours by TLC and quenched with addition of sodium bicarbonate upon completion. The organic compounds were extracted with dichloromethane (DCM), combined, and dried over anhydrous magnesium sulfate ($MgSO_4$). The resulting crude mixture was concentrated under reduced pressure and the products were purified by silica column chromatography.

In the next step, 4-azido benzyl alcohol was reacted with isothiocyanate and a bulky base to yield the N-H donor of interest. 1.0 equivalent of the starting material was dissolved in dry THF (0.1 M) with a stir bar in a round bottom flask. The reaction flask was subjected to an inert gas (N_2). The flask was wrapped in aluminum foil and placed in an ice bath. Then, 1.0 equivalent of isothiocyanate was added. The isothiocyanate contained the variable *R* group which allowed for modification of the final donor. Next, 1.1 equivalents of DBU were added dropwise. The use of a bulky base here was important to avoid. The reaction was carefully monitored by TLC with variable run times. Upon completion, the reaction was quenched with the addition of brine. The organic components were extracted with ethyl acetate, combined, and dried over anhydrous $MgSO_4$. The resulting crude mixture was concentrated under reduced pressure and purified by silica column chromatography. The purified product was stored in the freezer and wrapped in foil.

Esterase-Triggered N-H H_2S donors

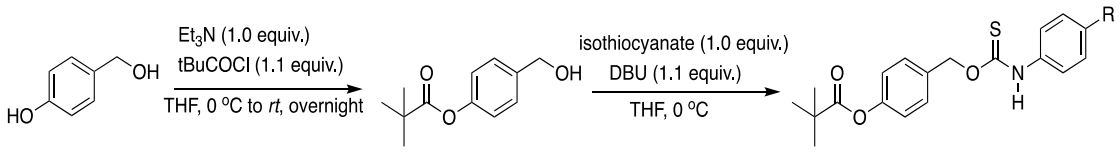


Figure 7. *Synethesis of Eserase-Triggered N-H H_2S Donors*

In the first step, 1.0 equivalent of 4-hydroxy benzyl alcohol was dissolved into a flask with dry THF (0.1 M) and a stirring bar. The reaction flask was put under an inert atmosphere (N_2 gas), and cooled to 0 °C in a ice water bath. Once the solution was cooled to 0 °C, 1.0 equivalent of triethylamine (Et_3N) was added dropwise to deprotonate the benzyl alcohol and let stir for 5 minutes. Subsequently, *tert*-butyl carbonyl chloride (1.1 equiv.) was added dropwise. It was important to perform this step slowly to promote selective reaction with the deprotonated phenol over the more nucleophilic benzyl alcohol. An outlet needle attached to a balloon was used to allow for pressure release and equilibration, while protecting the hood manifold from hydrogen chloride (HCl) acid gas corrosion. The reaction was allowed to warm to room temperature and stirred overnight. The following morning the reaction mixture was quenched by addition of brine, the organic compounds extracted with ethyl acetate, and the combined organic layers dried over anhydrous magnesium sulfate ($MgSO_4$). The resulting crude mixture was concentrated under reduced pressure and the products were purified by silica column chromatography.

Synthesis of the N-H donors was performed following the second step in the above scheme. The *R* indicates that different groups were used depending on the reaction. First, one equivalent of *tert*-butyl ester starting material was dissolved in a round bottom flask with dry THF (0.1 M). The reaction flask was put under an N_2 atmosphere and cooled to 0 °C in an ice water bath. Once the reaction was cooled to 0

°C, 1.0 equivalent of isothiocyanate was added. Lastly, DBU (1.1 equiv.) was added dropwise. The reaction was monitored by TLC and remained on ice for the duration of the reaction. Once the reaction was stopped, the product was purified by column chromatography using a silica column, following the procedure described above. The purified product was stored in the freezer.

Synthesis of Coupling Partners (CP) for N-Me donor synthesis

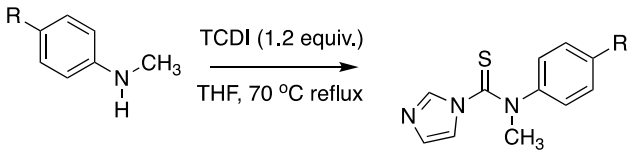


Figure 8. *Synthesis of Coupling Partners*

Synthesis of the coupling partners involves a variable component, *R* which can be interchanged to make a variety of coupling partners and subsequent N-Me donors. Synthesis of these compounds involves a ratio of at least 1.2:1 of thiocarbonyldiimidazole (TCDI) to N-Me aniline. This ratio helps to ensure that the aniline adds in once to the TCDI rather than twice. Thus, it was essential to first dissolve the TCDI by itself, then add aniline so that the TCDI was always in excess. The scale of this reaction varied. First, 1.2 equivalents of TCDI was dissolved into dry THF (0.1 M) with a stir bar and under N_2 atmosphere. Then, 1.0 equivalent of the N-Me aniline was added. The reaction was then heated to 75 °C under reflux overnight. Heating under reflux is essential to obtaining a high yield with this reaction. Once the reaction was stopped, the product was dry-loaded directly onto silica and purified by column chromatography using a silica column.

Esterase-Triggered N-Me H_2S donors

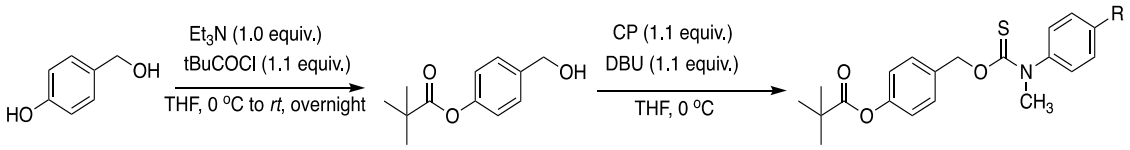


Figure 9. *Synthesis of Esterase-Triggered N-Me H_2S Donors*

Synthesis of the *tert*-butyl ester was performed as described previously. Synthesis of the N-Me donors utilized coupling partners with varied *R* groups, which allows for modification of the para-position of the donor. Synthesis of the N-Me donors was performed following the second step in the above scheme. First, 1.0 equivalent of the *tert*-butyl ester was dissolved in a flask with a stir bar and dry THF (0.1 M) under N_2 atmosphere and in an ice water bath. Once the solution cooled to 0 °C, 1.1 equivalents of the coupling partner were dissolved into the solution. Lastly, 1.1 equivalents of DBU were added dropwise. The reaction was monitored by TLC and remained on ice for the duration of the reaction. Reaction times varied. Upon completion, the reaction was quenched upon addition of brine. The organic compounds were extracted with ethyl acetate, and the organic layers were dried with anhydrous $MgSO_4$. The crude organic materials were concentrated under low pressure and purified by silica column chromatography.

Synthesis of CS_2 donors

The synthesis of CS_2 donors involves more steps than the previous syntheses because it requires the preparation of a reactive thiol nucleophile rather than an alcohol for the final step.

Synthesis of Thiol Containing tert-butyl ester Trigger

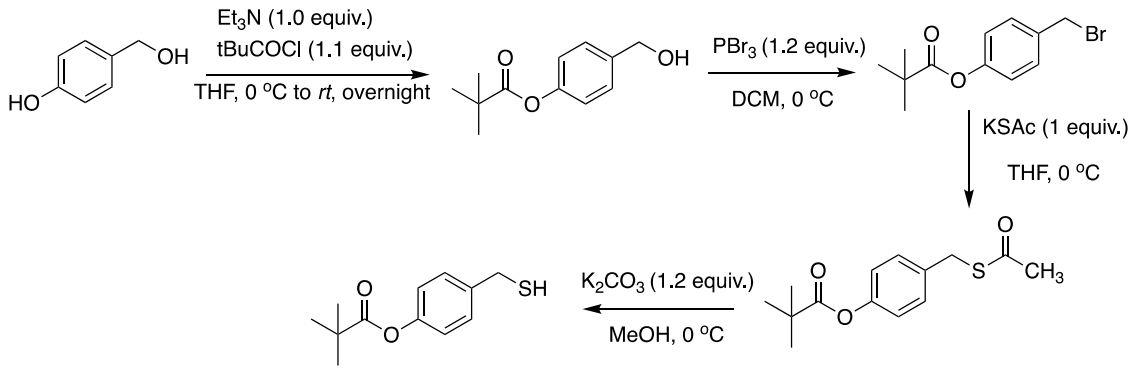


Figure 10. *Synthesis of Thiol Containing tert-butyl ester Trigger*

Synthesis of the benzyl thiol starting materials involves four different steps. The first step installs the *tert*-butyl ester trigger and was performed as explained previously. In the second step, bromine is installed as a leaving group. First, 1.0 equivalent of the para-azido benzyl alcohol was dissolved in dry DCM (0.1 M). The reaction flask was put under an inert atmosphere (N_2 gas), and cooled to 0 °C in an ice water bath. An outlet needle attached to a balloon was used to allow for pressure release and equilibration, while protecting the hood manifold from hydrogen bromide (HBr) acid gas corrosion. Once the solution was cooled to 0 °C, 1.2 equivalents of phosphorous tribromide (PBr_3) was added dropwise and let stir. The reaction was monitored by TLC and ran no more than two hours. The reaction was quenched upon addition of $NaHCO_3$. The organic compounds were extracted with DCM, and the organic layers were dried with anhydrous $MgSO_4$. The crude organic materials were concentrated under low pressure and purified by silica column chromatography.

In the third reaction, 1.0 equivalent of the *tert*-butyl benzyl bromide compound was dissolved in dry THF (0.1 M) in a round bottom flask with a stir bar and put under an N_2 atmosphere. The flask was placed in an ice bath and allowed to cool to 0 °C. Once the starting material was dissolved, 1.0 equivalent of potassium thioacetate was added.

The reaction stirred and continued overnight. The next day, the reaction was quenched with addition of brine. The organic compounds were extracted with ethyl acetate, and the organic layers were combined and dried with anhydrous $MgSO_4$. The crude organic materials were concentrated under low pressure and purified by silica column chromatography.

In the last step, the acetate needed to be removed to yield the thiol. To achieve this, one equivalent of the thioacetate compound was dissolved with a stir bar in methanol (0.1 M) in a round bottom flask and placed in an ice bath until it cooled to 0 ^oC. Then 1.2 equivalents of potassium carbonate were added into the solution. The reaction was carefully monitored by TLC and worked up upon the disappearance of starting material and appearance of product spot. The reaction was quenched with addition of brine. The organic compounds were extracted with ethyl acetate, and the organic layers were combined and dried with anhydrous $MgSO_4$. The crude organic materials were concentrated under low pressure and purified by silica column chromatography.

Synthesis of Thiol Containing Azide Trigger

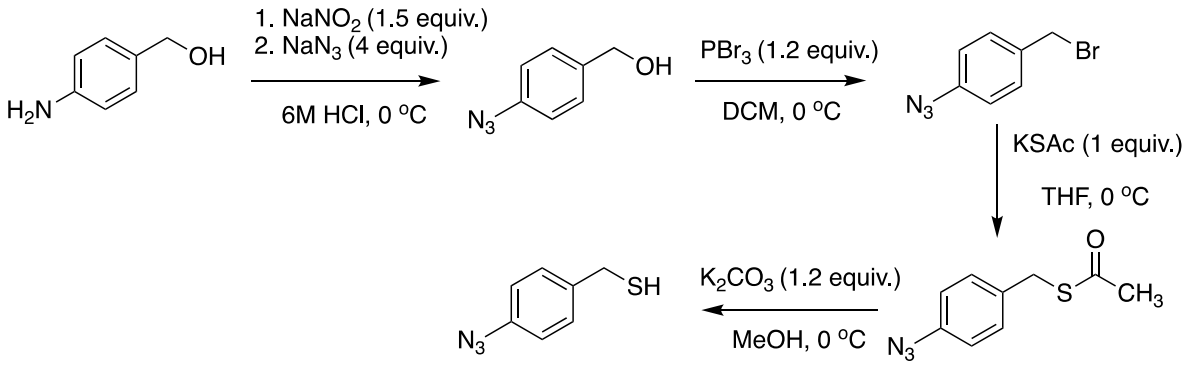


Figure 11. Synthesis of Thiol Containing Azide Trigger

Synthesis of the benzyl thiol starting materials involves four different steps. The first step is a Sandmeyer reaction to install the azide. This step was performed as explained previously. In the second reaction, 1.0 equivalent of 4-azido benzyl alcohol was dissolved in dry DCM (0.1 M) in a round bottom flask with a stir bar under an N_2 atmosphere. The round bottom flask was wrapped in aluminum foil, placed in a water bath, and the solution cooled to 0 ^oC. Next, 1.2 equivalents of PBr_3 were added to the reaction flask dropwise. An outlet needle attached to a balloon was used to allow for pressure release and equilibration, while protecting the hood manifold from hydrogen bromide (HBr) acid gas corrosion. Once the solution was cooled to 0 ^oC, 1.2 equivalents of phosphorous tribromide (PBr_3) was added dropwise and let stir. It was important to perform this step slowly to prevent a runaway reaction from occurring. The reaction was monitored by TLC and ran no more than two hours. The reaction was quenched upon addition of $NaHCO_3$. The organic compounds were extracted with DCM, and the organic layers were dried with anhydrous $MgSO_4$. The crude organic materials were concentrated under low pressure and purified by silica column chromatography.

In the third reaction, 1.0 equivalent of 4-azido benzyl bromide was dissolved in dry THF (0.1 M) in a round bottom flask with a stir bar and put under an N_2 atmosphere. Again, the flask was wrapped in aluminum foil and the solution cooled to 0 ^oC. Once the starting material was dissolved, 1.1 equivalents of potassium thioacetate was added. The reaction continued overnight. The reaction was quenched with addition of brine. The organic compounds were extracted with ethyl acetate, and the organic layers were combined and dried with anhydrous $MgSO_4$. The crude organic materials were concentrated under low pressure and purified by silica column chromatography.

In the final step, the acetate needs to be removed to yield the thiol. To achieve this, the thioacetate compound was dissolved with a stir bar in methanol (0.1 M) in a foiled round bottom flask and placed in an ice bath until it cooled to 0 ºC. Then, 1.2 equivalents of potassium carbonate were added to the solution. The reaction was carefully monitored by TLC and worked up upon the disappearance of starting material and appearance of product spot. The starting material and product are difficult to separate, so it is important that the product runs to completion. The reaction was quenched with addition of brine. The organic compounds were extracted with ethyl acetate, and the organic layers were combined and dried with anhydrous $MgSO_4$. The crude organic materials were concentrated under low pressure and purified by silica column chromatography. It is important not to let this reaction overreact because the product is unstable and can react with itself to form a polysulfide.

Synthesis of Esterase-Triggered N-Me CS_2 donors

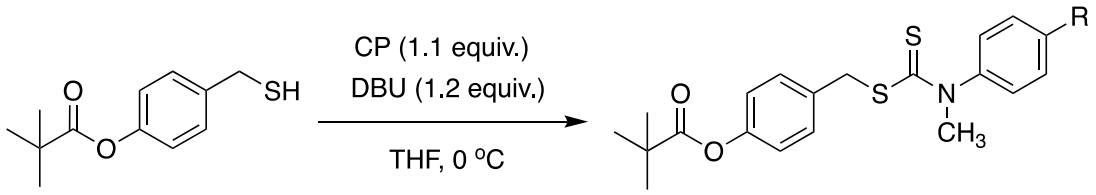


Figure 12. Synthesis of Esterase-Triggered N-Me CS_2 donors

Synthesis of the *tert*-butyl ester N-Me donors was performed following the thiol synthesis and coupling partner synthesis described previously. The *R* indicates that different groups were used depending on the reaction. First, one equivalent of *tert*-butyl ester benzyl thiol was dissolved in a round bottom flask with dry THF (0.1 M). The reaction flask was put under an N_2 atmosphere and cooled to 0 °C in an ice water bath. Once the reaction was cooled to 0 °C, 1.1 equivalent of coupling partner (CP) was added. Lastly, DBU (1.1 equiv.) was added dropwise. The reaction was monitored

carefully by TLC and remained on ice for the duration of the reaction. Upon appearance of product spot and disappearance of starting material spot, the reaction was quenched with brine. The organic components were extracted with ethyl acetate, combined, and dried over anhydrous $MgSO_4$. The resulting crude mixture was concentrated under reduced pressure and purified by silica column chromatography.

Synthesis of Azide-Triggered N-Me CS_2 donors

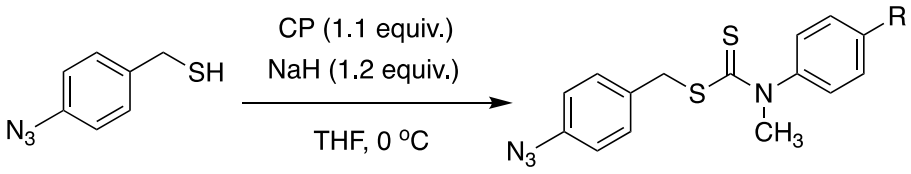


Figure 13. *Synthesis of Azide-Triggered N-Me CS_2 donors*

Synthesis of the azide-triggered N-Me donors was performed following the thiol synthesis and coupling partner synthesis described previously. The *R* indicates that different groups were used depending on the reaction. First, one equivalent of 4-azido benzyl thiol was dissolved in a foiled round bottom flask with dry THF (0.1 M). The reaction flask was put under an N_2 atmosphere and cooled to 0 °C in an ice water bath. Once the reaction was cooled to 0 °C, 1.1 equivalent of coupling partner (CP) was added. Lastly, NaH (1.2 equiv.) was added. The reaction was monitored carefully by TLC and remained on ice for its duration. Upon appearance of product spot and disappearance of starting material spot, the reaction was quenched with brine. The organic components were extracted with ethyl acetate, combined, and dried over anhydrous $MgSO_4$. The resulting crude mixture was concentrated under reduced pressure and purified by silica column chromatography. The purified product was protected from light with foil and stored in the freezer.

Methylene Blue Assay

The methylene blue assay was utilized in this study to quantity the release of H_2S from donors. This experiment involves a cocktail of chemicals that react with hydrogen sulfide to form a colored complex called methylene blue, which has a peak absorbance at 670 nm.[19] Figure 14 shows the reaction with H_2S that forms the colored methylene blue.

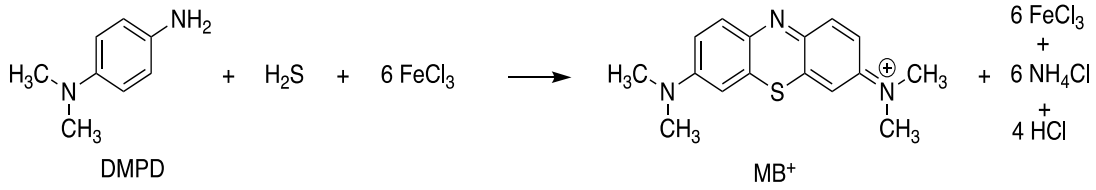


Figure 14. *Methylene Blue Reaction Scheme*

The reaction is initiated by adding a solution of donor molecule, buffer, trigger, and carbonic anhydrase (CA) to cuvettes with a pre-prepared mixture of the methylene blue cocktail, a highly acidic solution of N,N-dimethyl-p-phenylenediamine, ferric chloride, and zinc acetate. The donor solution is added to time trials spanning from 1 minute to 90 minutes. During the reaction, the donor is triggered, undergoes self-immolation to produce COS, and the COS is converted to H_2S *via* CA. This happens in an air-free scintillation vial with degassed buffer and reagents, to ensure that no oxygen is present which can oxidize the H_2S and confound the results. Aliquots from this reaction mixture are taken at different time points and quenched into the cuvettes of the prepared methylene blue cocktail, at which point any H_2S present is then captured and produces methylene blue, which absorbs light upon interacting with ferric iron. The absorbance of the solutions in each cuvette is measured at 670 nm *via UV-vis* to give a range of absorbances over time, indicating the release rate of H_2S by donor. The absorbance values are then compared to a calibration curve prepared using solutions of

[19] Lawrence, N. S.; Davis, J.; Jiang, L.; Jones, T. G. J.; Davies, S. N.; Compton, R. G. *Electroanalysis* **2000**, *12*, 1453-1460.

known H_2S concentration made with NaHS, to give the actual concentrations of H_2S released. This process is summarized by Figure 15.

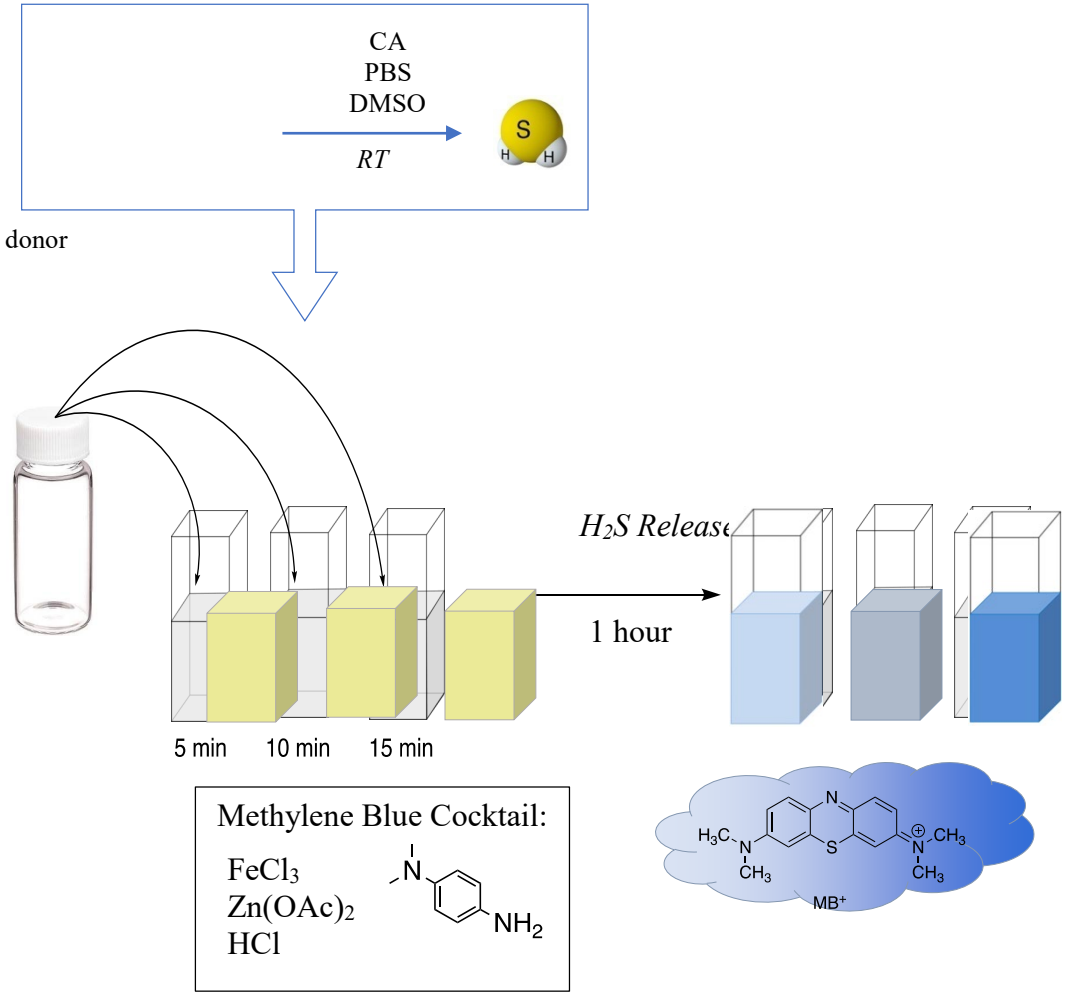


Figure 15. *Methylene Blue Assay Design*

NMR Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is a widely used tool for characterization of chemical compounds. This technique has been essential for characterization since as early as the 1960's, and the technology and instruments involved have vastly improved for modern research. Modern spectrometers are highly sensitive and can be used to collect coupling constants, chemical shifts, multiplicity,

and NMR spectra. These data are specific to certain functional groups and can be used to definitively identify many compounds.

The values of peaks in a given spectrum are a result of the chemical makeup of a compound, and for some types of NMR spectroscopy, integration of those peaks is related to the ratio of atoms present in a molecule with the given nucleus of interest. For this research, ^{1}H NMR, proton decoupled ^{13}C NMR, and ^{19}F NMR spectroscopy were performed to characterize compounds.

MATERIALS

UV-vis

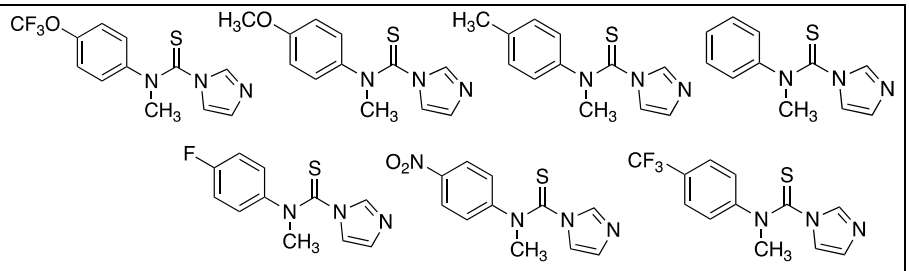
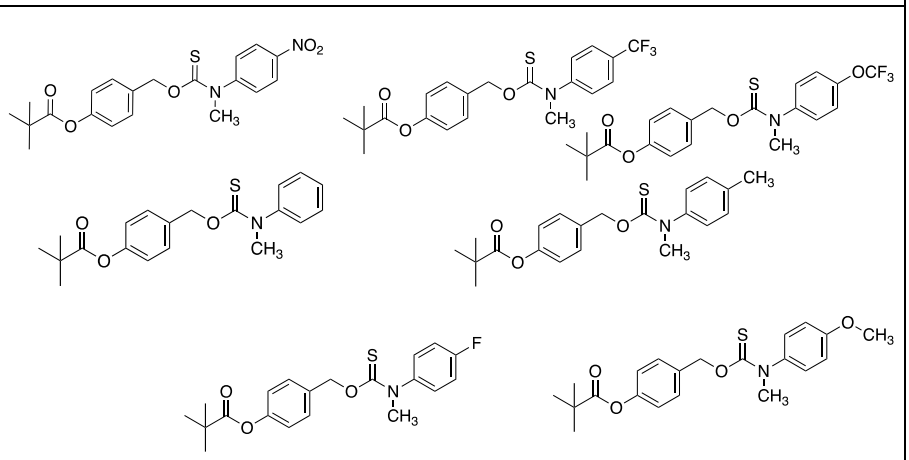
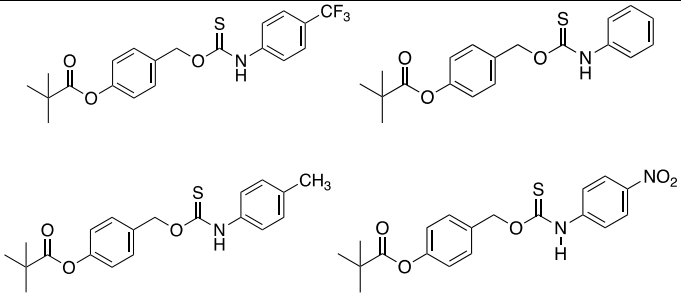
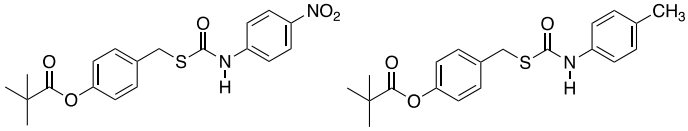
Methylene blue data was collected by measuring absorbances *via* UV-vis. Absorbance data was measured using an Agilent Cary 60 UV-Vis spectrometer.

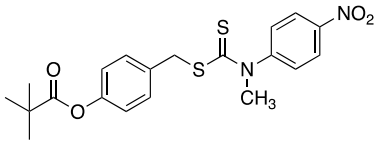
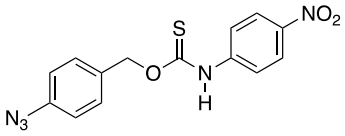
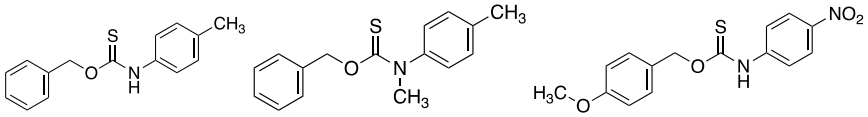
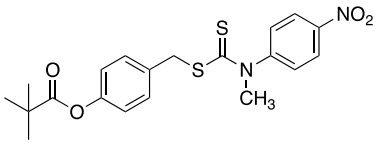
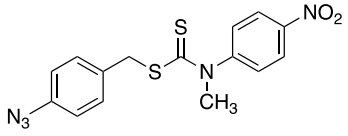
Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectra were recorded using a Bruker 500 MHz or a Bruker 600 MHz NMR instrument.

RESULTS & DISCUSSION

Table I. Synthetic Overview

Coupling Partners (CPs)	
Esterase-Triggered N-Me H_2S Donors	
Esterase-Triggered N-H *O*-alkyl H_2S donors	
Esterase-Triggered N-H *S*-alkyl H_2S donors	

Esterase-Triggered CS_2 Donors	
Azide-Triggered N-H H_2S Donors	
Triggerless H_2S donors	
Esterase-Triggered N-Me CS_2 Donors	
Azide-Triggered N-Me CS_2 Donors	

Synthesis of the full library of compounds in Table I was performed in collaboration with my graduate student mentor, Carolyn Levinn.

NMR Analysis

This synthetic work utilized NMR analysis as a key tool for monitoring the extent of reactions, identifying products, and assessing their purity. In ^{1}H NMR analysis, each peak in the spectra represents one or more protons occupying a particular chemical environment, certain types of protons tend to appear within a specific range of chemical shifts, and the integrals under the peaks can be used to compare the ratio of protons in each peak to one another. The following sample ^{1}H NMR spectra are representative of their respective compounds.

Coupling Partner ^{1}H NMR Spectra

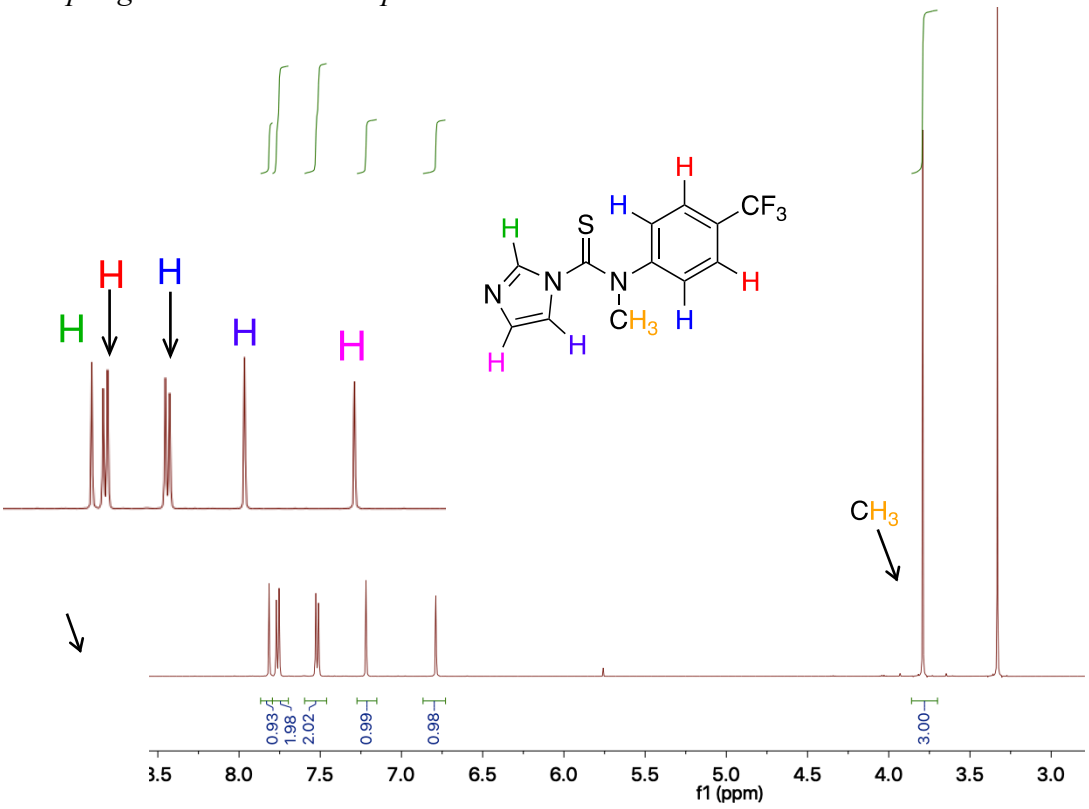


Figure 16. ^{1}H (500 MHz, DMSO) NMR Spectrum

In identifying coupling partners, the peaks in the aromatic region (typically from 6.5 – 8.5 ppm) and the methyl peak were used as representative peaks. For coupling partners that did not contain additional protons on the *R* group, there were six distinct groups of protons. In the imidazole portion, there are three distinct protons, each integrating to one. Bound to the nitrogen is a methyl group (CH_3), which contained three protons that were chemically identical. In the aromatic region of the benzyl group, there were two types of chemically distinct protons, each containing two protons. The methyl group in this compound is a distinctive peak and easily identifiable, so was set to integrate to 3.0 protons, and all other peaks in the spectrum were integrated in reference to this. Next, the aromatic protons of the imidazole and the benzene ring were integrated downfield. Integrating these peaks showed two doublets that integrated to two, which represent the two sets of protons in the benzene ring, and three distinct singlets that integrated to one, which represent the protons in the imidazole group. With each proton accounted for, the only significant remaining peak at 3.33 ppm was attributed to DMSO, which is the solvent. Overall, this spectrum indicates that this compound was successfully synthesized and is pure.

Coupling Partner ^{19}F NMR Spectra

This particular coupling partner, shown in Figure 17, also contains fluorine atoms, so a ^{19}F NMR spectrum was used to confirm that the CF_3 group was installed. The ^{19}F isotope has similar abundance to the ^{1}H isotope, so this spectrum was collected fairly easily. For this compound, the three fluorine atoms bound to the carbon

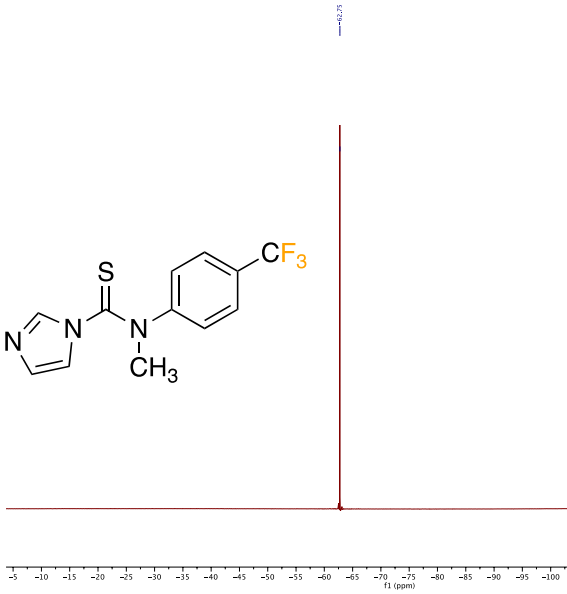


Figure 17. ^{19}F (564 MHz, $CDCl_3$) NMR Spectrum

were expected to be chemically identical, like the methyl group from the previous spectrum. Thus, one singlet peak is anticipated. This is present at 62.75 ppm in this spectrum, as expected.

CS_2 N-Me Donor ^1H NMR Spectra

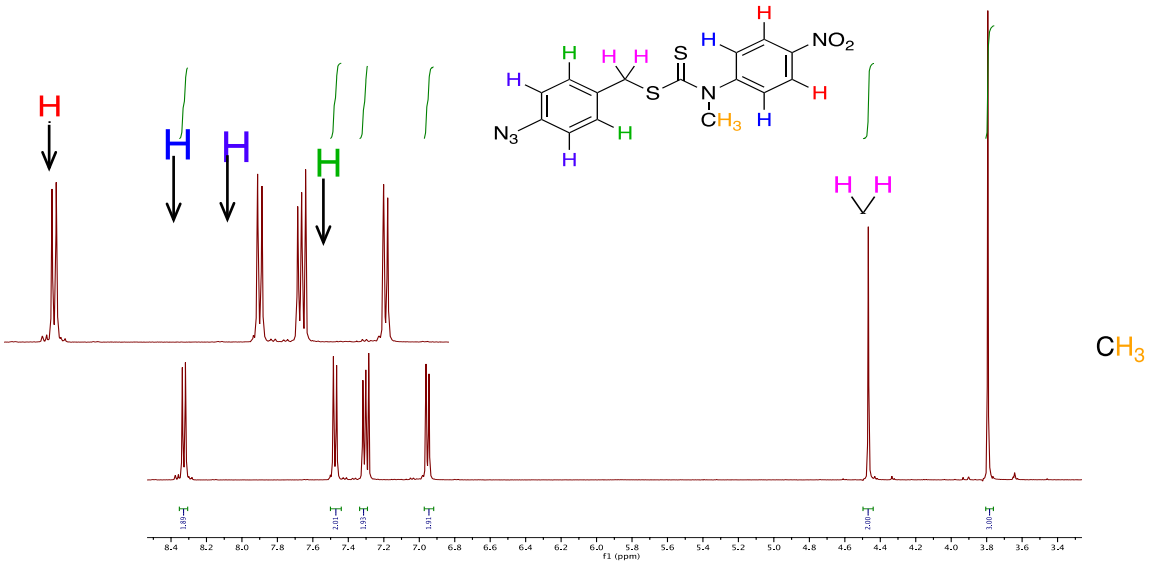
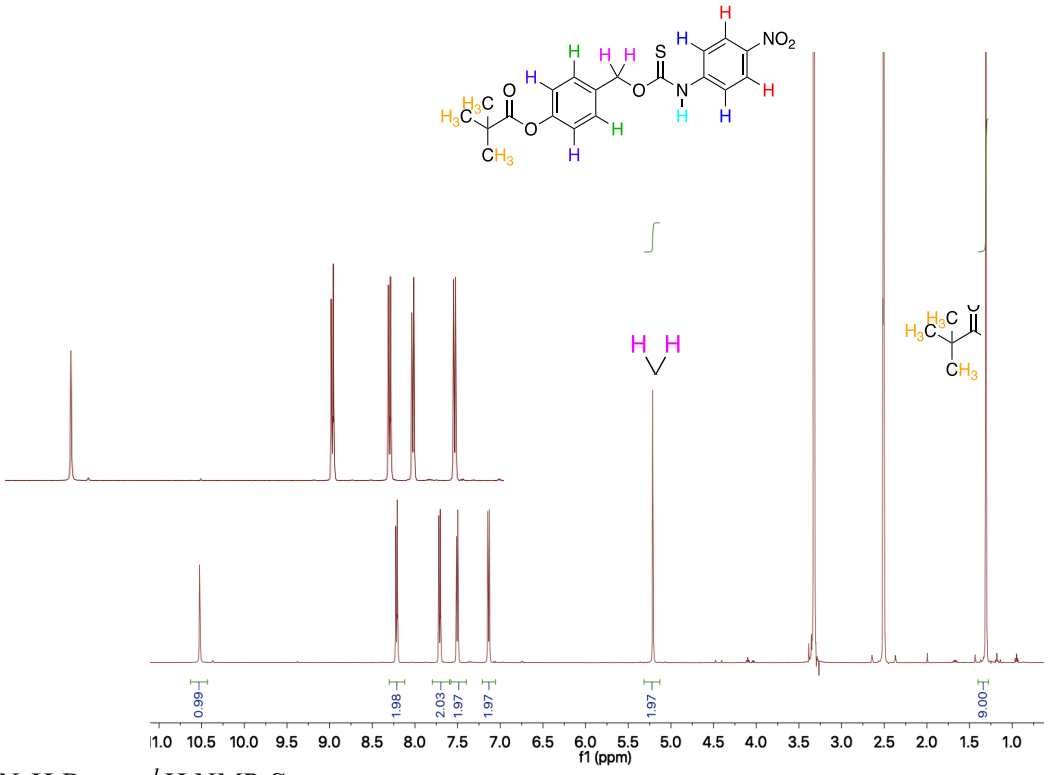


Figure 18. ^1H (500 MHz, $CDCl_3$) NMR spectrum

The same principles of structural analysis were used to analyze CS_2 donor compounds. With these compounds, the methylene peak, methyl handle, and aromatic peaks were used to confirm donor identity. When the trigger on the donor is an azide, it does not contribute more protons to the spectrum. With these donors, the methyl handle was again used to establish a baseline for integration. The methylene peak is also a characteristic doublet that integrated to two, as expected. The four sets of aromatic doublet peaks that each integrated to two are also consistent with the two substituted benzene rings in the donor. The chloroform peak can be seen at 7.28 ppm, and the

spectrum shows a few small impurities. Overall, this analysis shows that the compound was successfully synthesized and pure.



H_2S N-H Donor ^1H NMR Spectra

Figure 19. ^1H (500 MHz, DMSO) at 60 °C NMR Spectrum

For COS/H_2S N-H donors, there was an additional intricacy that required attention in NMR analysis. Here, it is important to determine if the compound was *O*-alkyl donor or if it had isomerized to *S*-alkyl. In this case, the chemical shift of the methylene peak was used to determine the identity of the isomer. Similar to the CS_2 donors, the methylene peak, aromatic peaks were used as representative peaks. With this particular donor, the *tert*-butyl group also produced a representative peak used in the analysis, and the proton bound to the nitrogen (N-H) was also present.

As was described previously, the *tert*-butyl group was used as a handle in analysis and set to be integrated to a value of 9.0. The next representative peak of an *O*-alkyl donor is the methylene peak, which was found to be at about 5.25 ppm in this spectrum. This resonance was notably downfield relative to the methylene peak of the *S*-alkyl CS_2 donor that was described previously, which was at about 4.50 ppm. The downfield methylene peak indicated that this compound is an *O*-alkyl isomer that has not isomerized. Again, the four sets of aromatic doublet peaks that each integrated to two are also consistent with the two para-substituted benzene rings in the donor. The DMSO peak can be seen at 3.33 ppm, and the spectrum shows a few small impurities. Overall, this analysis shows that the compound was successfully synthesized and pure.

Development of Library of Azide-triggered donors

One of the benefits of using an azide trigger over the *tert*-butyl ester handle shown in this work is that the azide does not require an enzyme to initiate the self-immolation event. Rather, it can be readily triggered by a phosphine. Comparatively, the *tert*-butyl ester handle becomes activated by an esterase protein, which cleaves the ester group and activates the donor. Thus, the azide-triggered donor compounds do not require this step. Proteins are very large molecules that can cause solubility issues and significant noise in NMR studies. Developing these azide donors that do not require activation by enzymes is particularly useful in the study of CS_2 based donor motifs, as this allows for the exploration of NMR based techniques for CS_2 detection. However, this donor type has a downside, in that it is incompatible with the methylene blue assay, as both the phosphine trigger and the azide consume the H_2S that is produced.

Impacts of Methylation of the Amine in TCM Donor Scaffold

In this work we synthesized *tert*-butyl ester triggered *O*-alkyl H_2S donors both with and without methylated amine. It is well known that the *O*-alkyl H_2S donors readily isomerize to the *S*-alkyl isomer over time, and methylation of the amine in the donor may have a stabilizing effect. For esterase-triggered N-Me TCM donors, H_2S release was assessed *via* methylene blue assay as summarized in Figure 20.

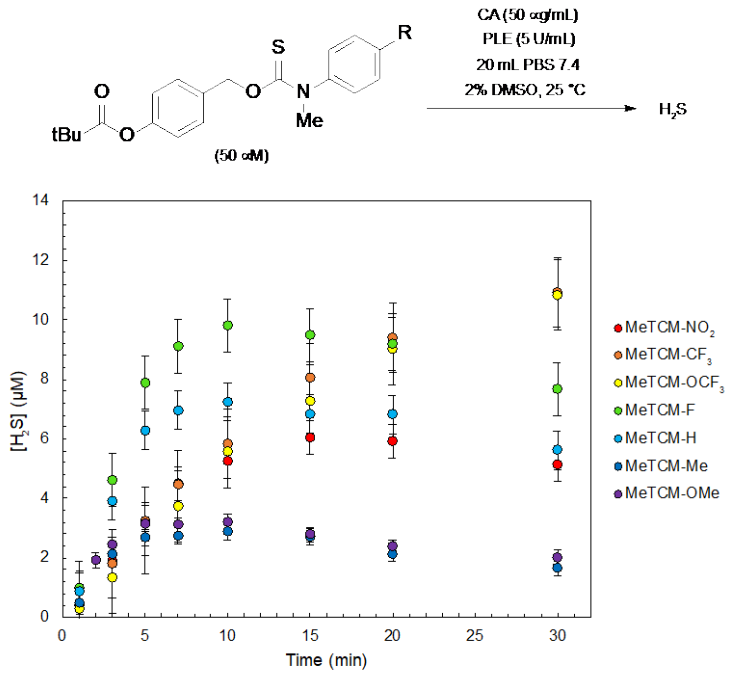


Figure 20. Methylene Blue assay for N-Me H_2S donors

Donors in this experiment were synthesized in collaboration with Carolyn Levinn of the Pluth lab at the University of Oregon, and this research is pending publication. This experiment was designed to assess the effects of N-Me of donors with various ED or EW groups in the para position of the amine payload. The alternative pathway hypothesis suggested that upon self-immolation, N-H donors with EWGs on the payload faced a competitive non-productive pathway as a result of acidification of

the N-H proton. If this were true, then methylation of the amine would effectively block this pathway, which would result in EWGs dominating in a faster release of H_2S compared to EDG donors. The data illustrated in Figure 20 somewhat contradicts this hypothesis. The fastest releasing donor contains an EWG (Me TCM-F); however, other highly electron-withdrawing donors do not release as rapidly (Me TCM-NO_2). The most electron donating groups perform the slowest release of H_2S (Me TCM-Me and Me TCM-OMe); however, there are clear breaks in the trend with the Me TCM-NO_2 donor releasing more slowly than the Me TCM-H donor. This data demonstrates that there may be alternative mechanisms at play in the rate of H_2S release from this COS based donor scaffold, and further research is necessitated to elucidate them.

Development of CS_2 donors using Pluth lab's donor scaffold

This work demonstrates the expansion of the synthetic utility of the Pluth lab's DTCM donor motif. The current CS_2 donors in the literature lack the tunability and trigger ability afforded by this donor scaffold, so this is a significant development in CS_2 donor chemistry. We synthesized two different kinds of N-Me CS_2 donors, an azide triggered donor and a *tert*-butyl ester triggered donor. Unlike the previous donors made in this motif, these donors do not move through a carbonyl sulfide intermediate; Rather, they should directly release CS_2 upon self-immolation. Since previous work showed 'leaky' release of H_2S from N-H CS_2 donors due to deprotonation of the amine, our newly synthesized N-Me donors should prevent this effect.

Despite this improvement in synthesis of CS_2 donors, there still remains significant challenges in CS_2 detection. Current methods involve observation of the disappearance of the dithiocarbonyl peak by UV-*vis*; however, this method only

measures the breakdown of the donor, not the formation of CS_2.We performed ^{1}H and ^{13}C NMR experiments in multiple solvents in an attempt to observe CS_2 resonances over time. Unfortunately, we encountered solubility issues and struggled to obtain clear results. This could be addressed in the future by preparing N-Me DTCM CS_2 donors with a solubilizing group on the payload.

FUTURE DIRECTIONS

H_2S Delivery

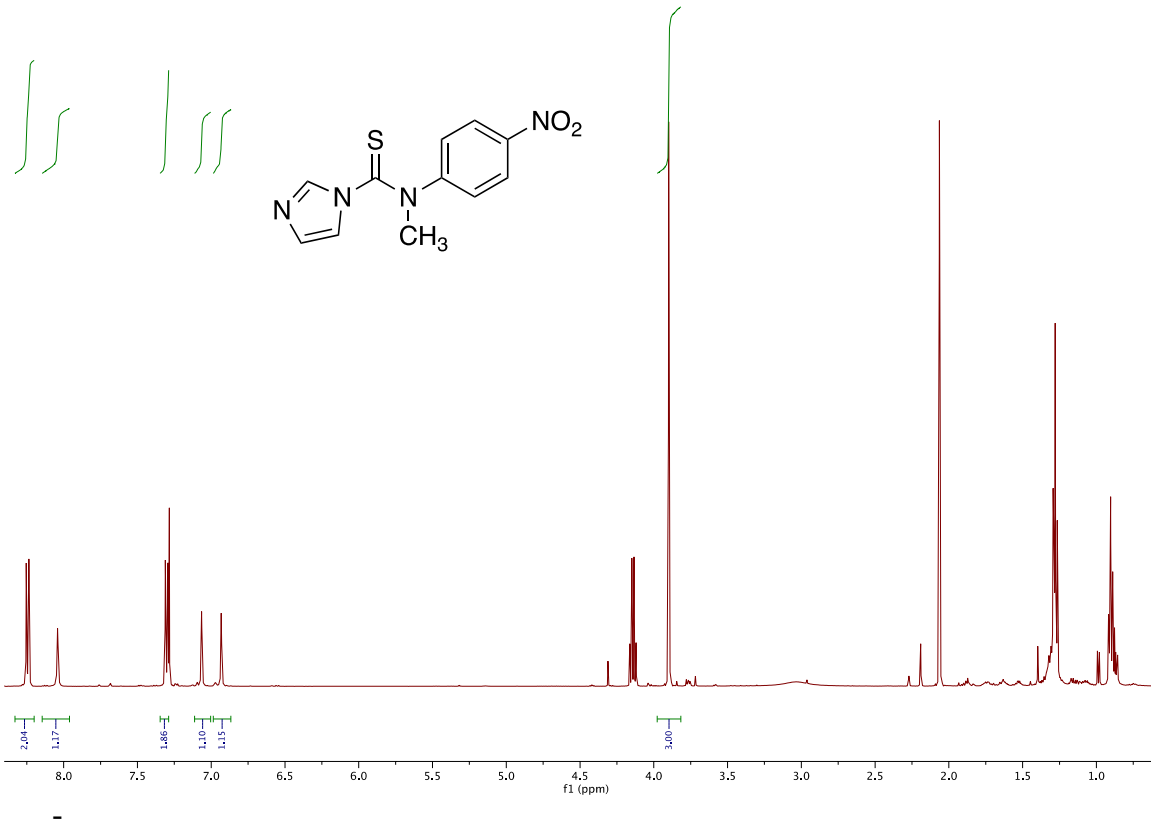
The TCM donor motif for triggerable H_2S release *via* COS intermediate developed by the Pluth lab is a useful donor scaffold for H_2S release that allows for synthetically accessible donor compounds and controlled release of COS. The controlled release of COS is essential for maintaining a slow release of H_2S and avoiding H_2S toxicity in cells. While these donors have been widely used, the TCMs themselves are sensitive compounds and very unstable, which limits their synthetic accessibility and utility. To continue to expand the synthetic utility of these donors it is important to further investigate synthetic choices that will optimize stability and tunability. Further studies may be performed to provide mechanistic insights on *O*-alkyl isomerization, and the role of interchanging *R* groups bound to the amine payload, as well as the roles of EWGs and EDGs in the para position in the release rate of COS. The more that is known about these processes, the more that these donors can be tuned for specific purposes in research.

CS_2 Delivery

The biological relevance CS_2 and lack of synthetically diverse CS_2 donors available makes this a great candidate for expanding the Pluth lab's TCM donor scaffold to include DTCMs capable of CS_2 release. However, there is not currently a method of CS_2 detection with a significantly low limit of detection, requiring further investigation of CS_2 detection techniques. The azide-triggered DTCM donor platform could be utilized to perform NMR studies of CS_2 release by developing new donors with solubilizing groups in the para position of the payload. Improved solubility would yield clearer results and provide a novel method of CS_2 quantification.

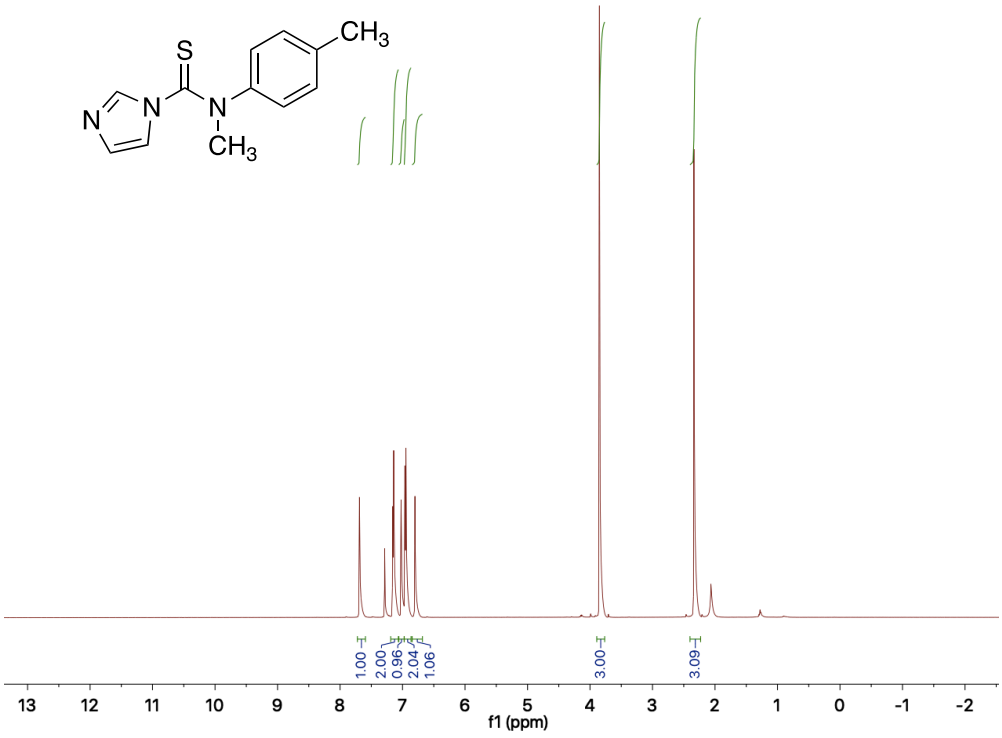
Coupling Partners (CPs)

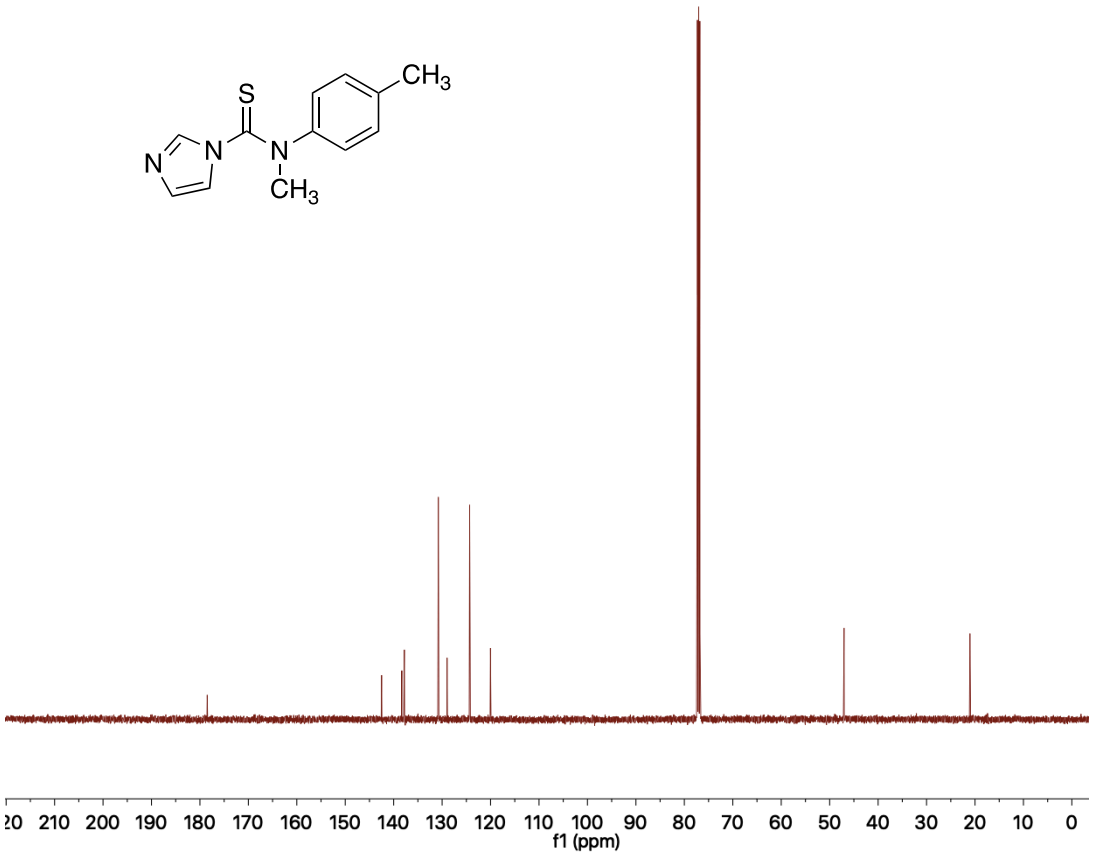
^{1}H (500 MHz, $CDCl_3$) NMR Spectrum (20.3% yield)



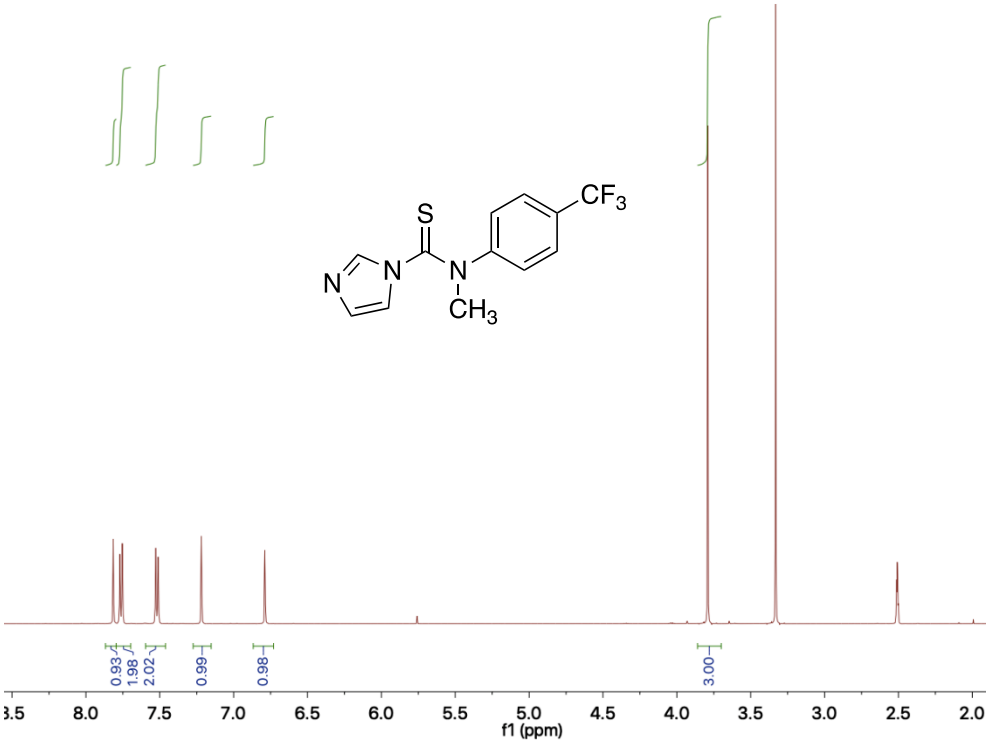
-

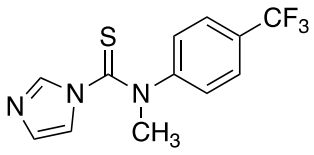
^{1}H (500 MHz, $CDCl_3$) and ^{13}C (150 MHz, CDCl3) NMR Spectra (72.0% yield)

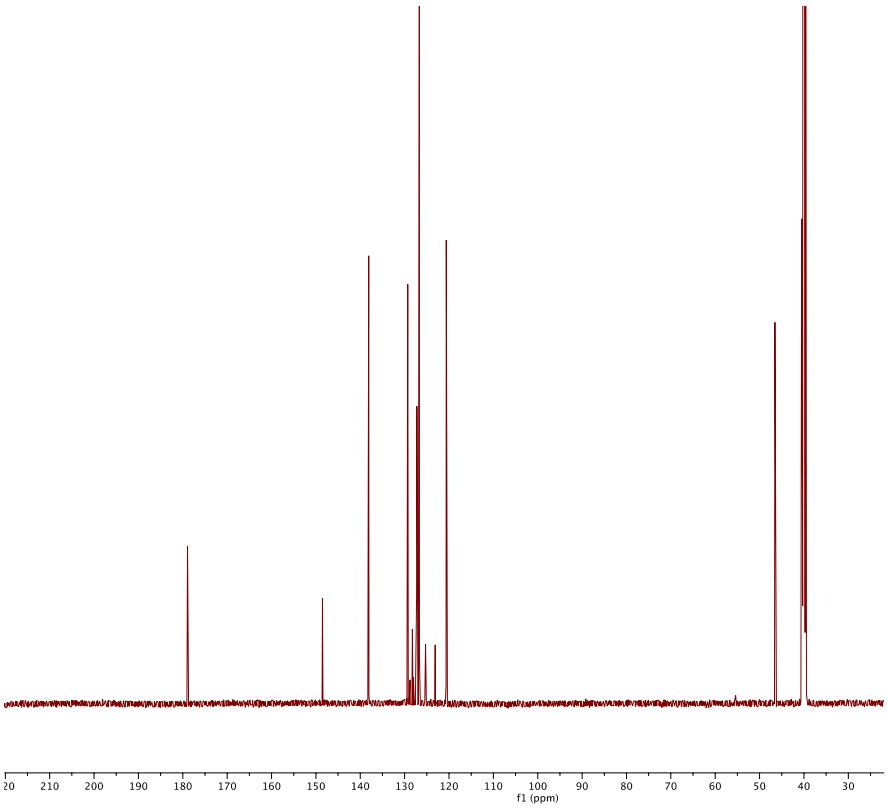


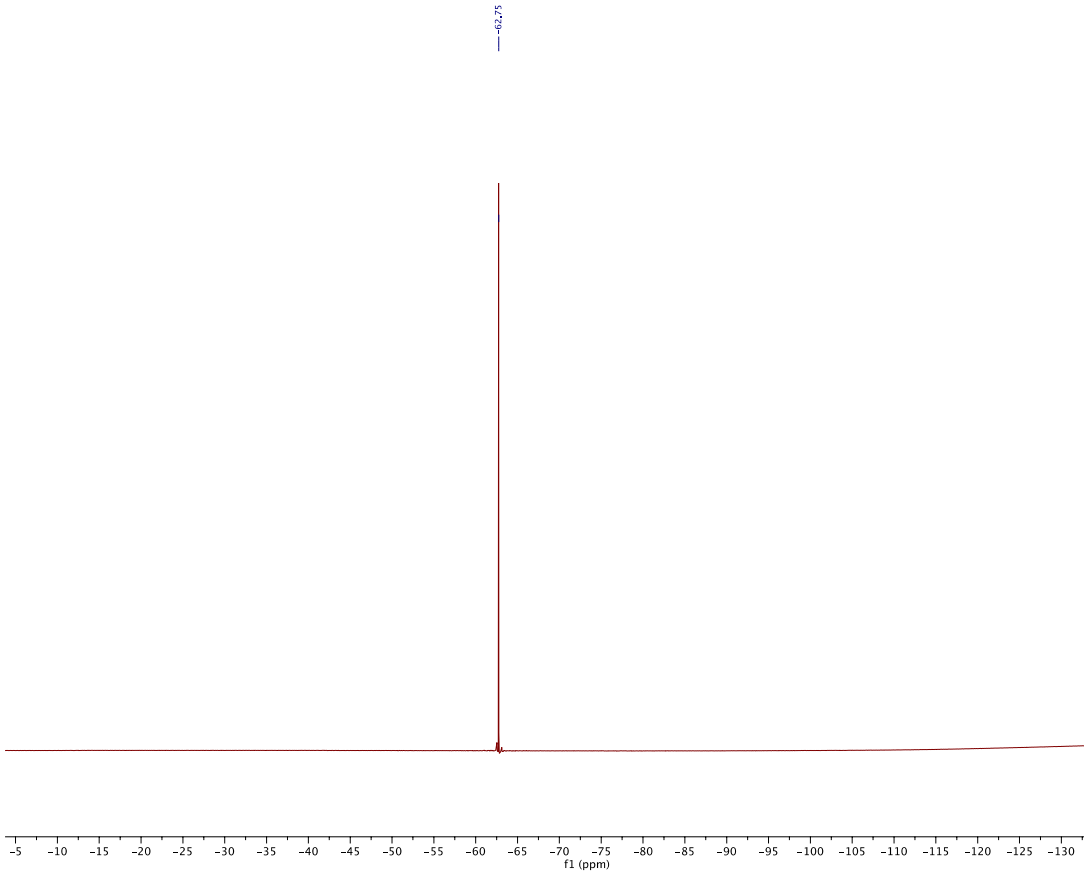


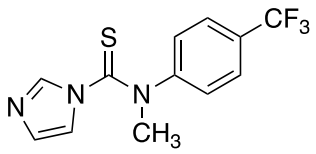
^{1}H (500 MHz, DMSO) at 60 ºC, ^{13}C (150 MHz,DMSO) at 60 ºC and ^{19}F (564 MHz, $CDCl_3$) NMR Spectra (57.8% yield)



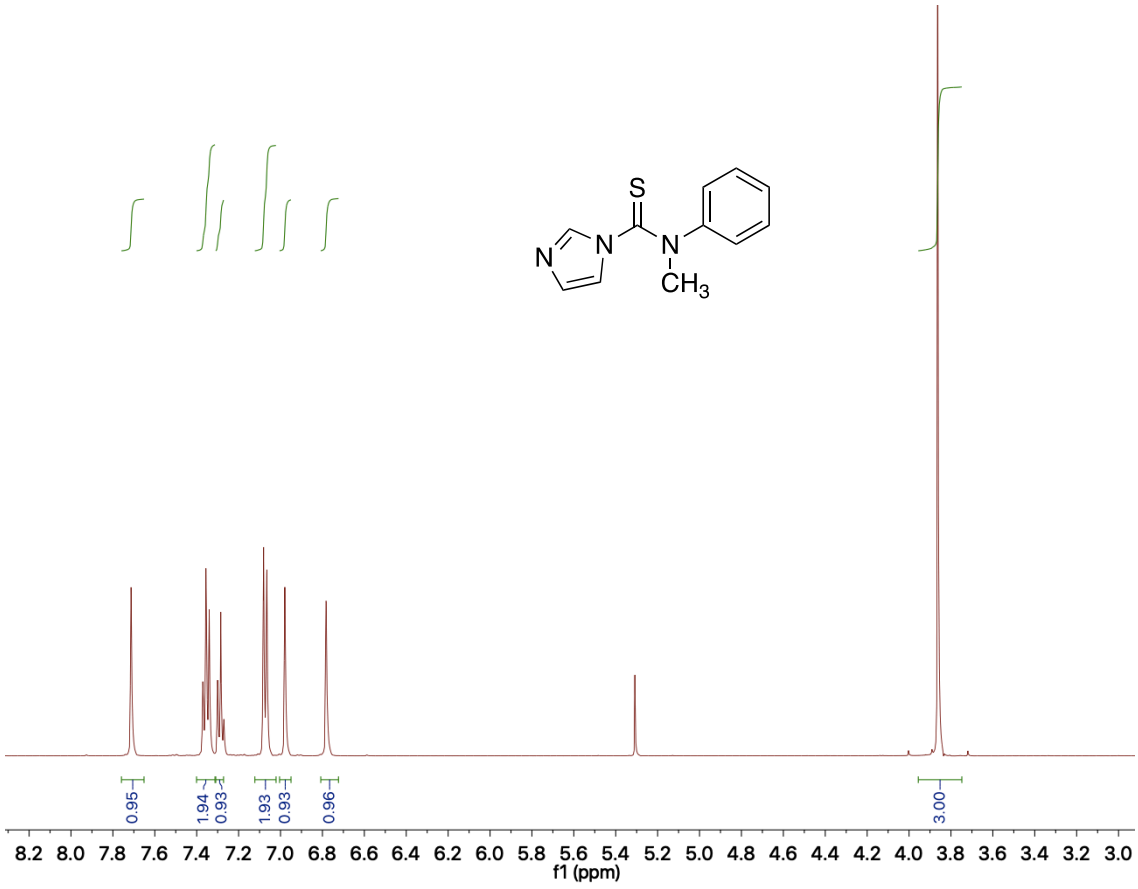



220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30
f1 (ppm)

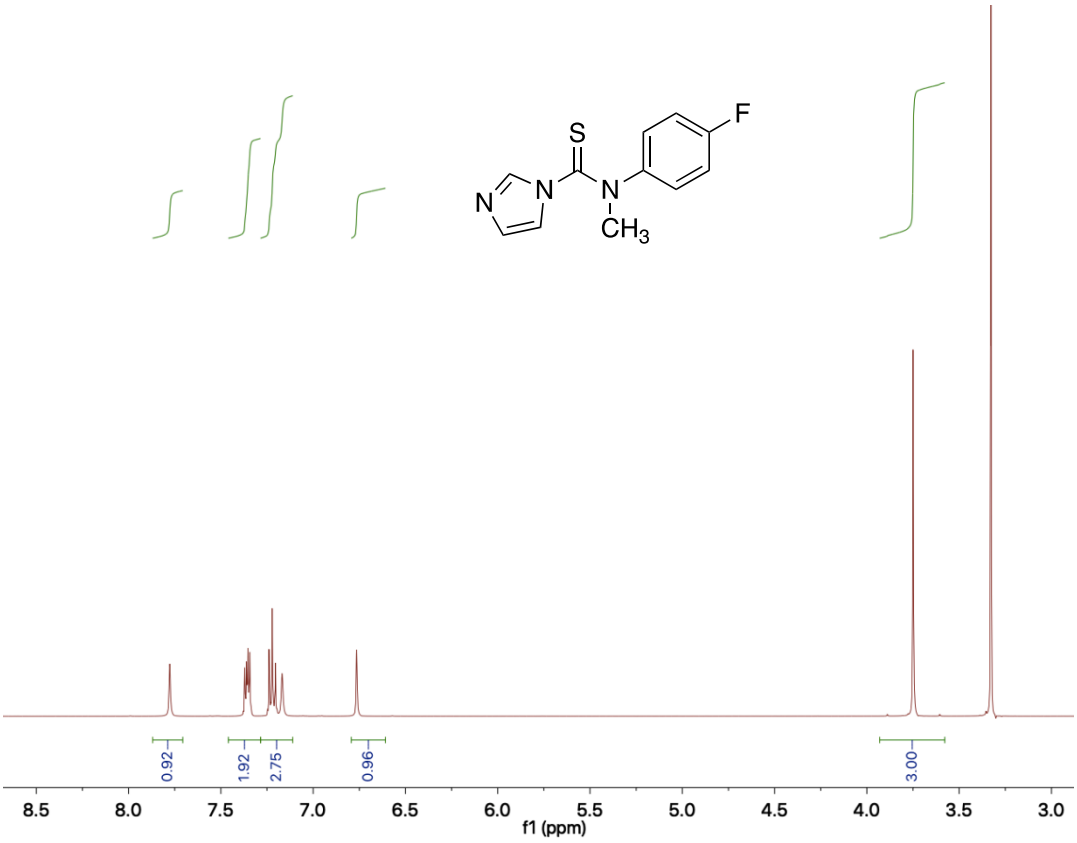

-62.75
-5 -10 -15 -20 -25 -30 -35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115 -120 -125 -130
f1 (ppm)

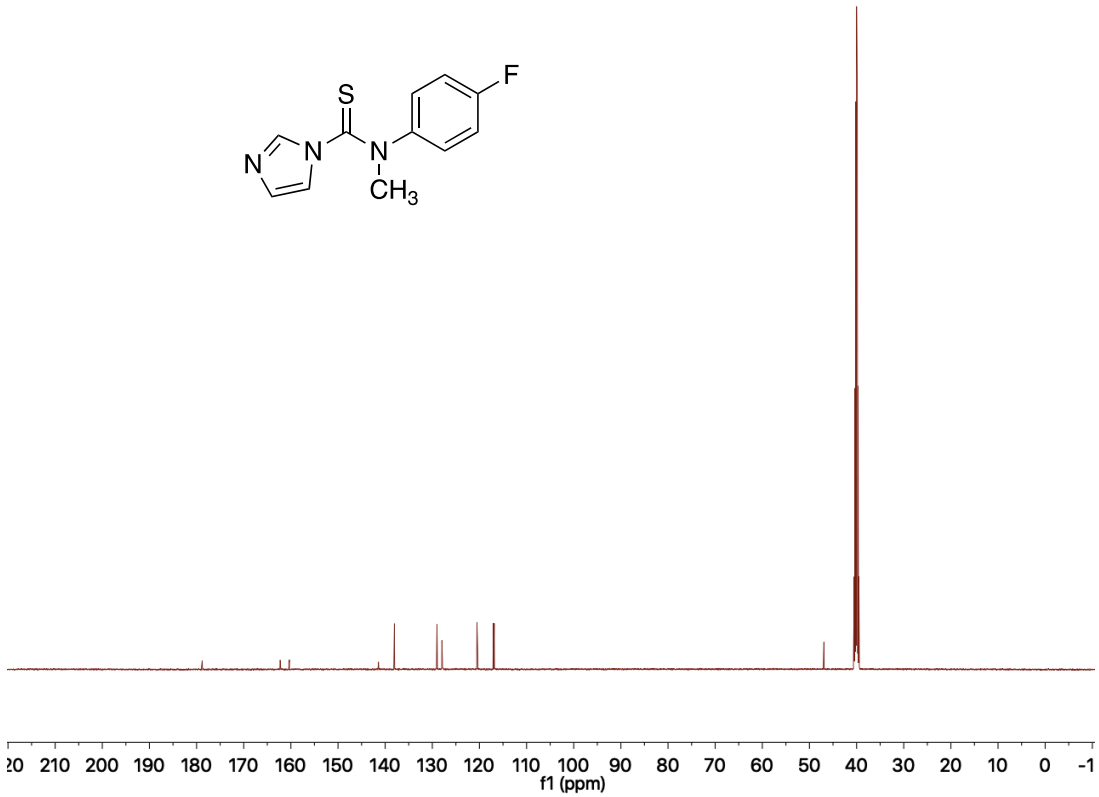

S
N
N
N
CH3
CF3

^{1}H (500 MHz, $CDCl_3$) NMR Spectrum (72.0% yield)



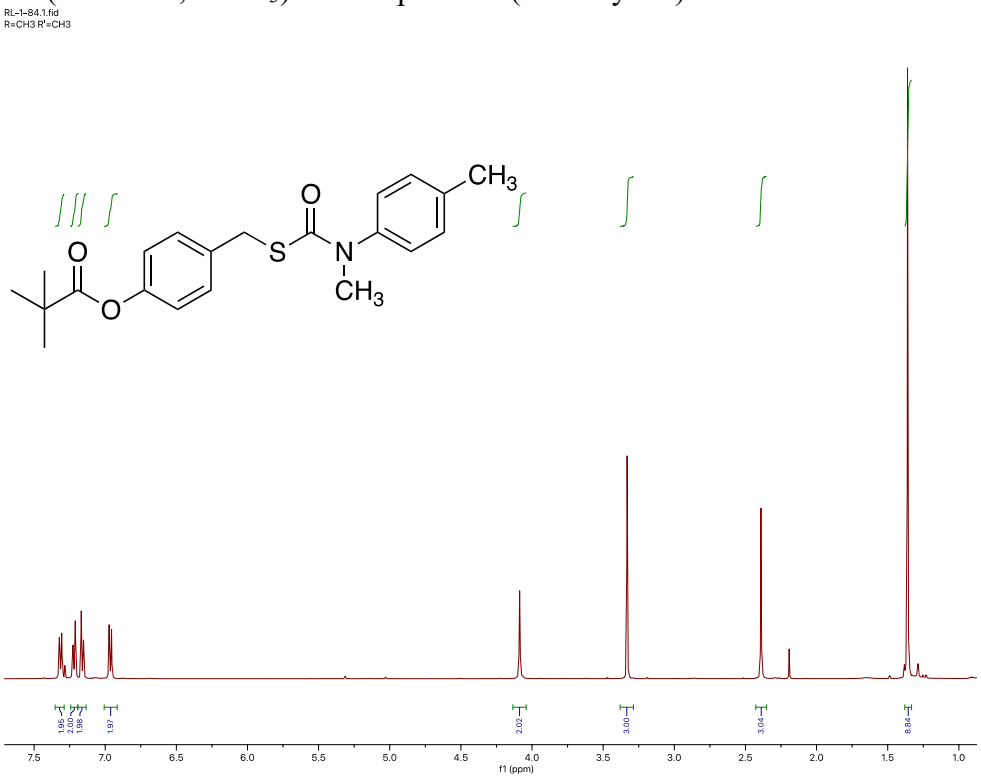
^{1}H (500 MHz, DMSO) at 60 ºC, ^{13}C (150 MHz,DMSO) at 60 ºC NMR Spectra (55.5% yield)

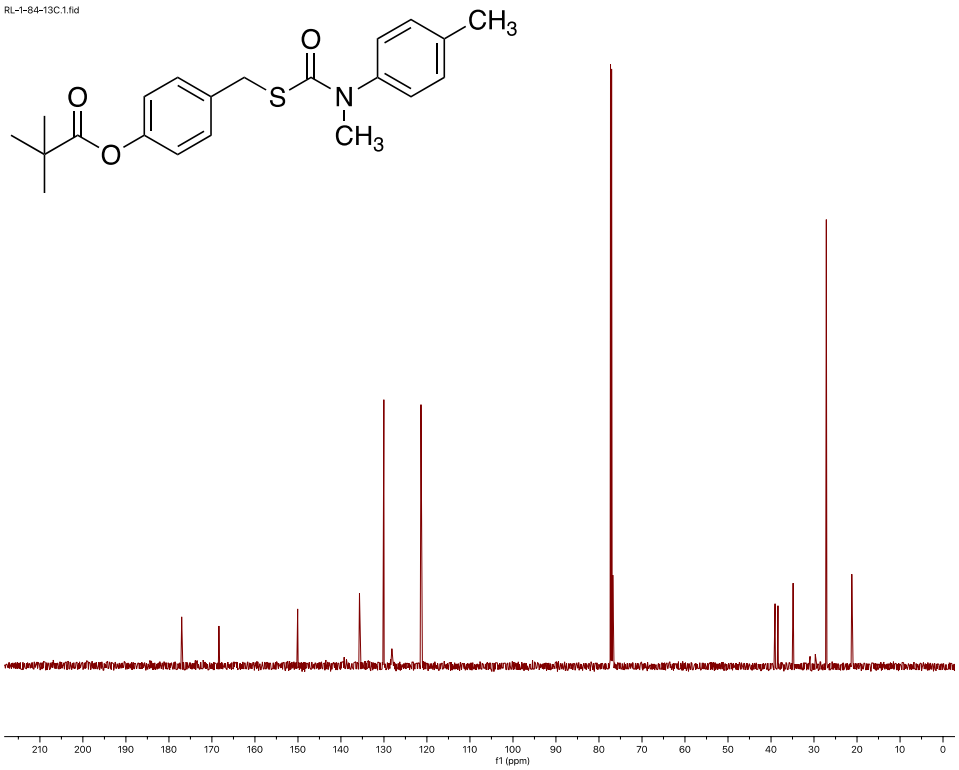



S
F
N
N
N
CH3
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

Esterase-Triggered N-Me H_2S donors

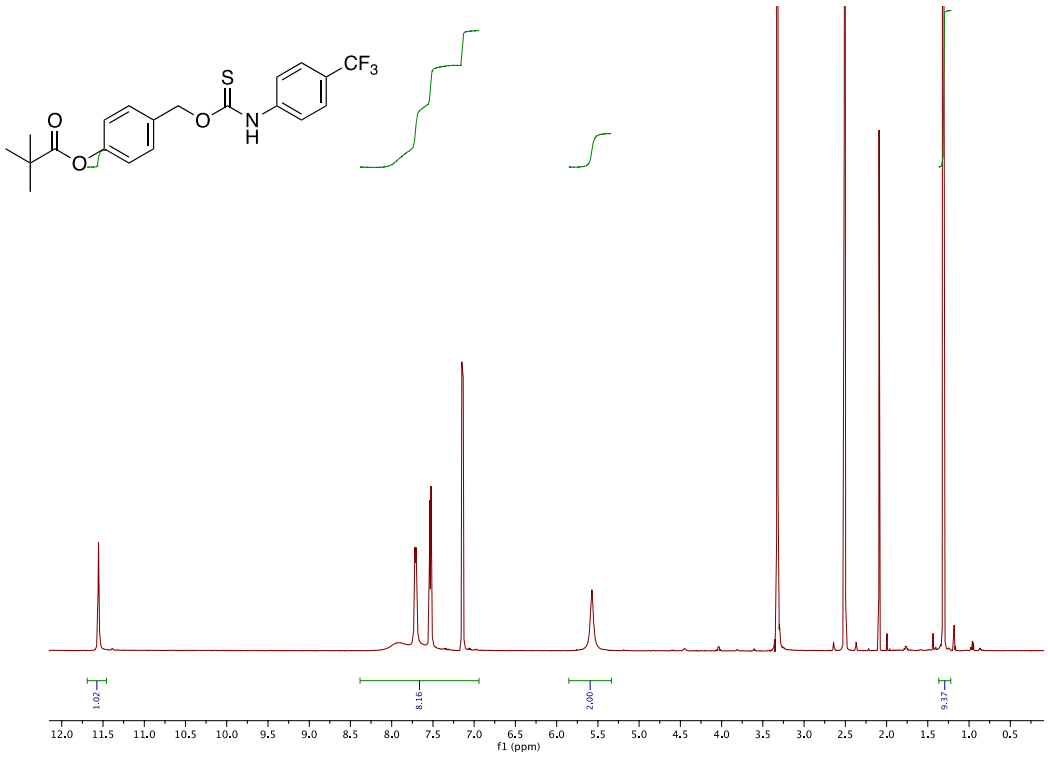
^{1}H (500 MHz, $CDCl_3$) NMR Spectrum (17.7% yield)

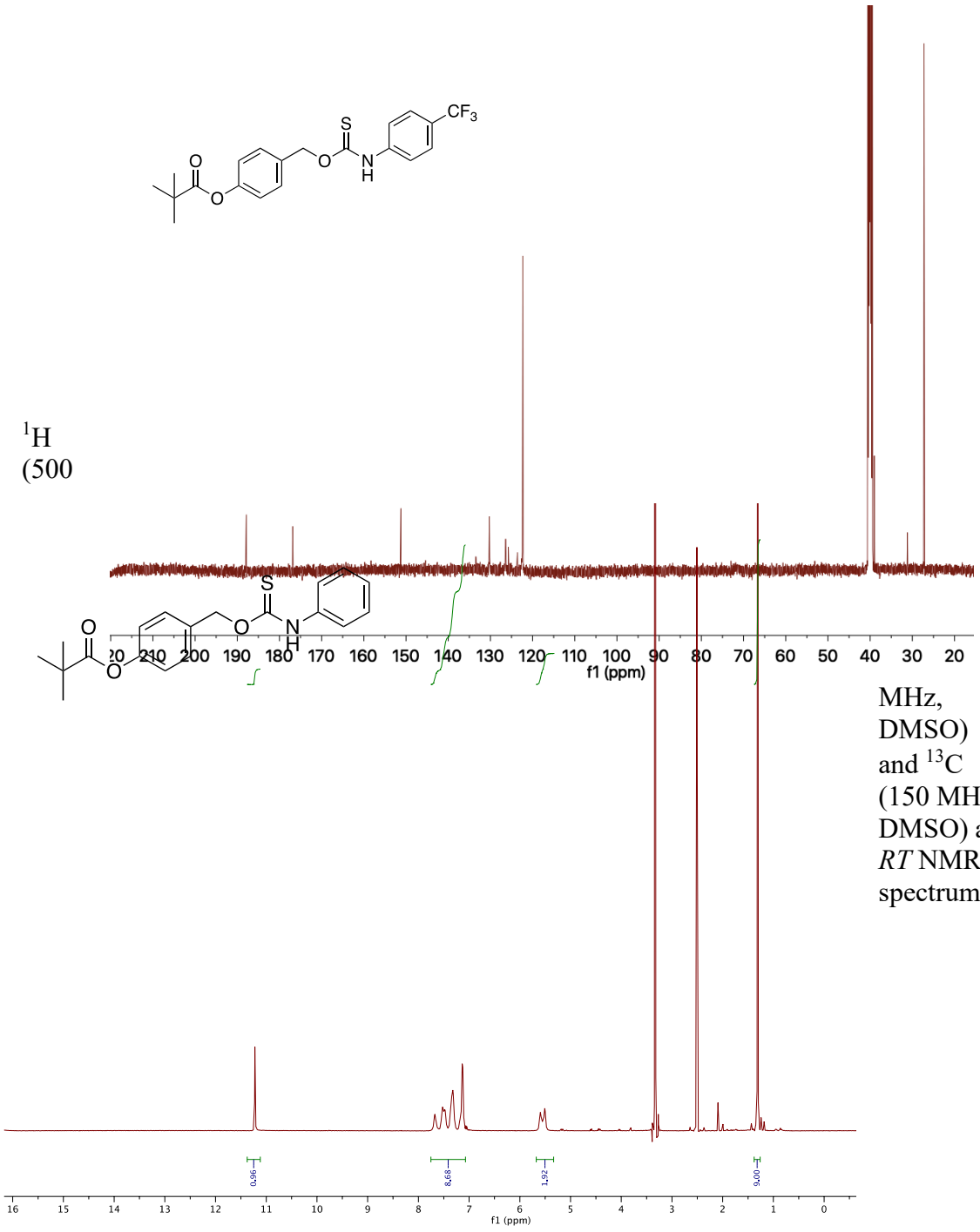




Esterase-Triggered N-H O-alkyl H_2S donors

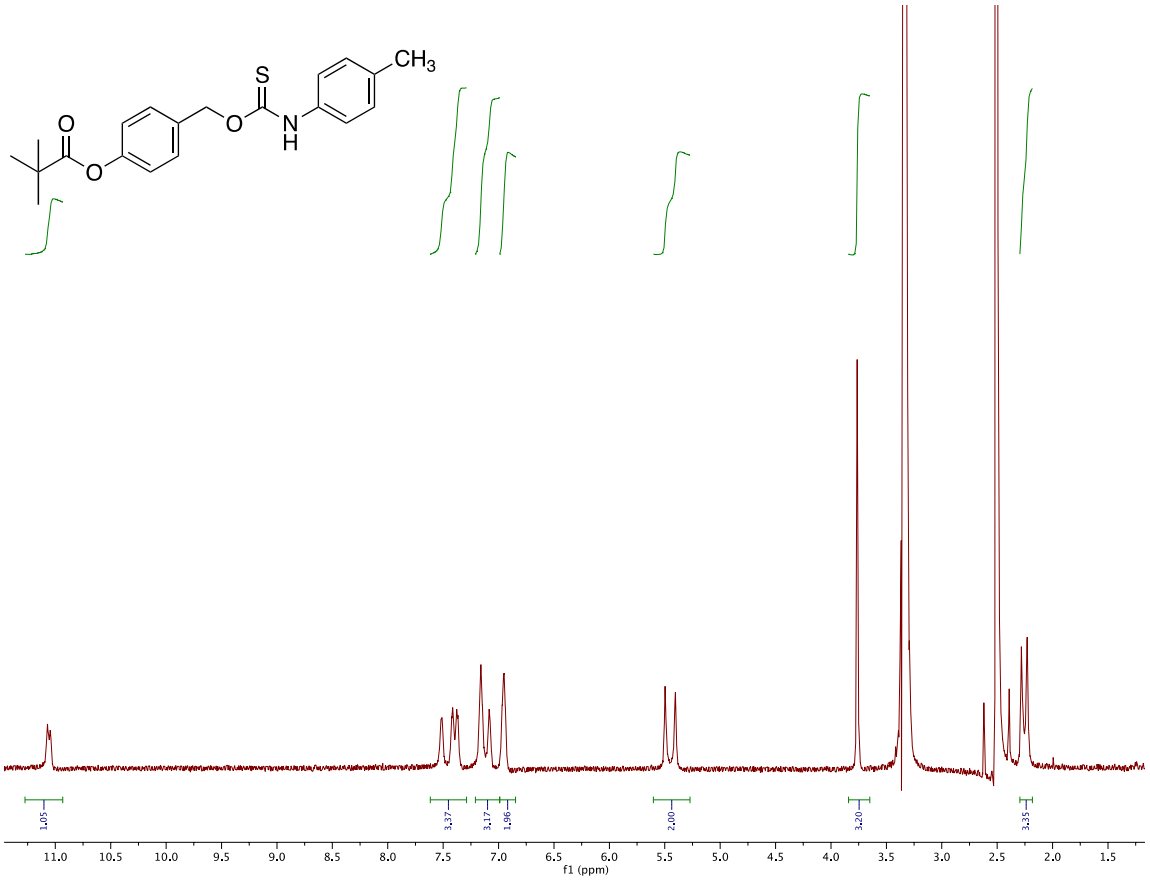
^{1}H (500 MHz, DMSO) and ^{13}C (150 MHz, DMSO) at 60 ºC NMR spectra (32.9% yield)



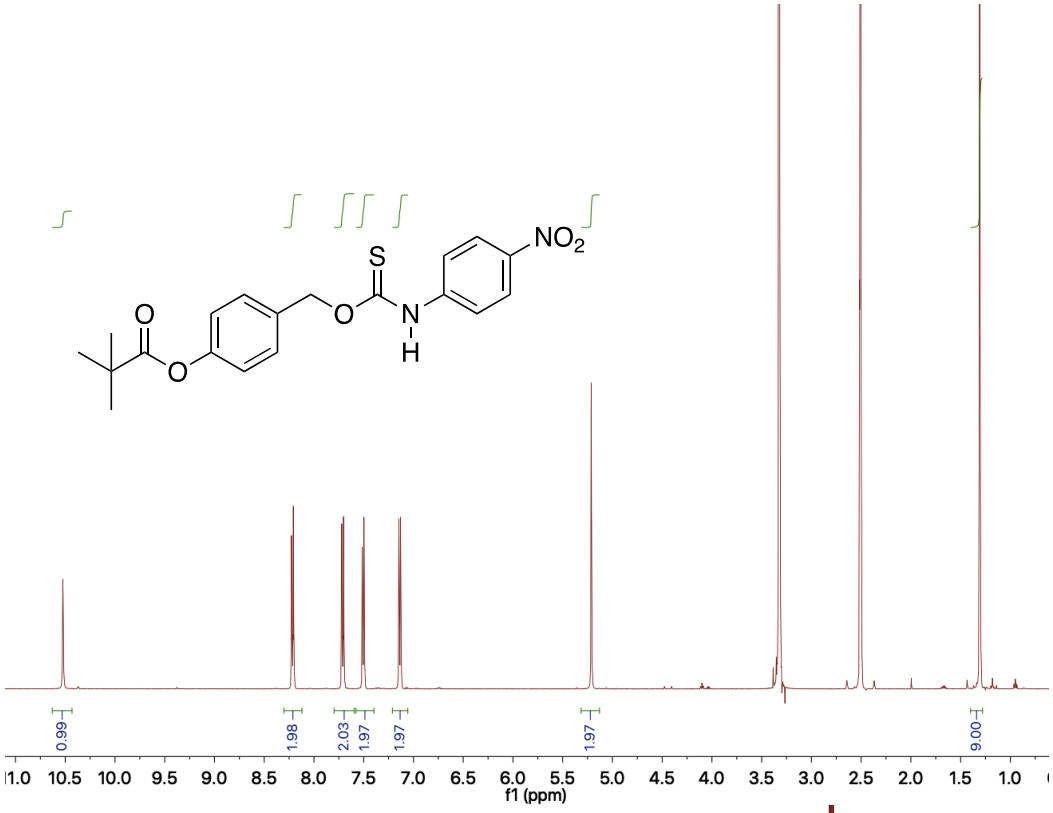


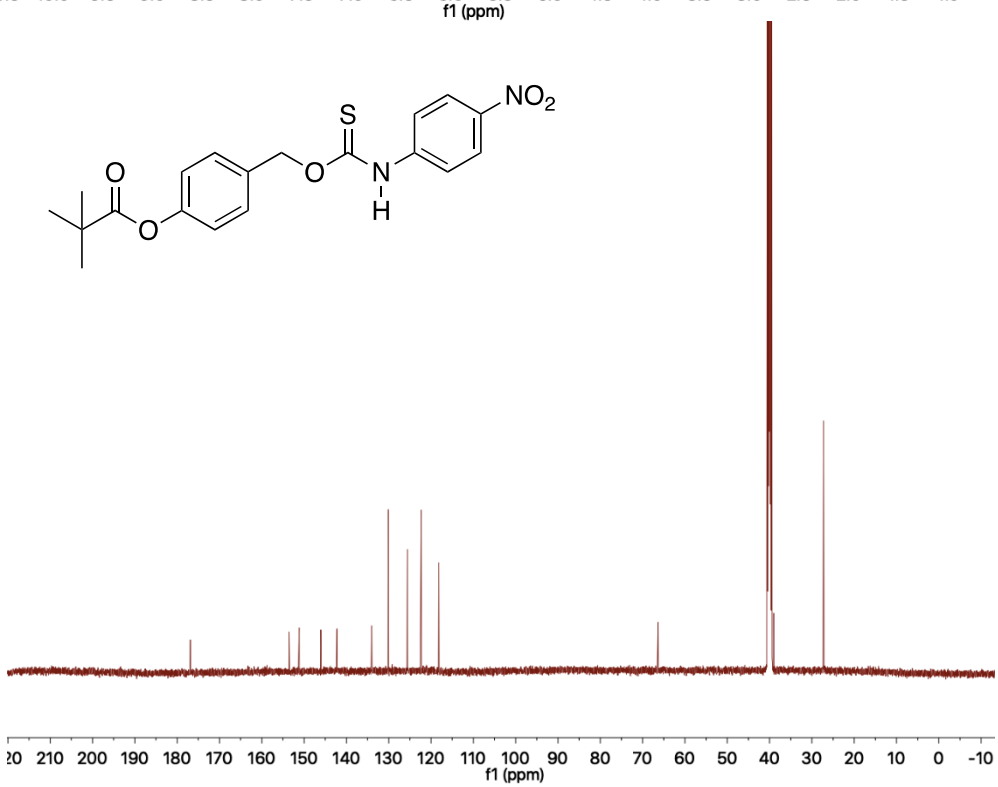
^{1}H (500 MHz, DMSO) and ^{13}C (150 MHz, DMSO) at *RT* NMR spectrum

^{1}H (600 MHz, DMSO) at *rt* NMR spectrum


CH3
S
O
O
N
H
O
1.05
3.37
3.17
1.96
2.00
3.20
3.35
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

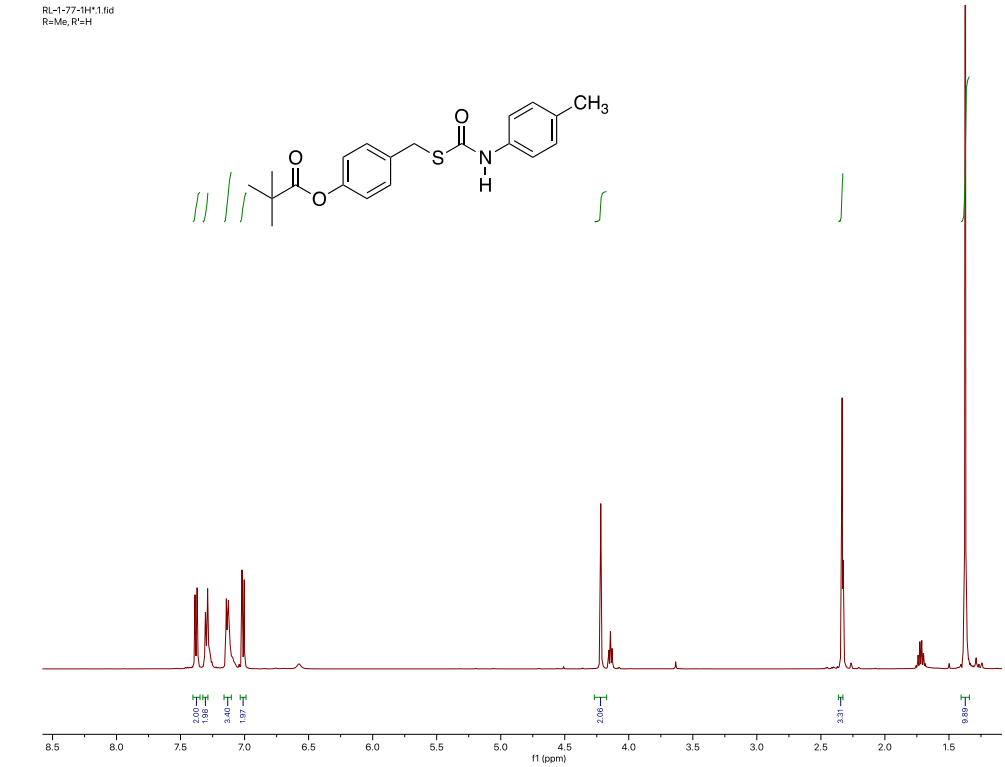
^{1}H (600 MHz, DMSO) and ^{13}C (150 MHz, DMSO) at 60 ^{o}C NMR Spectra ^{1}H (25.1% yield)

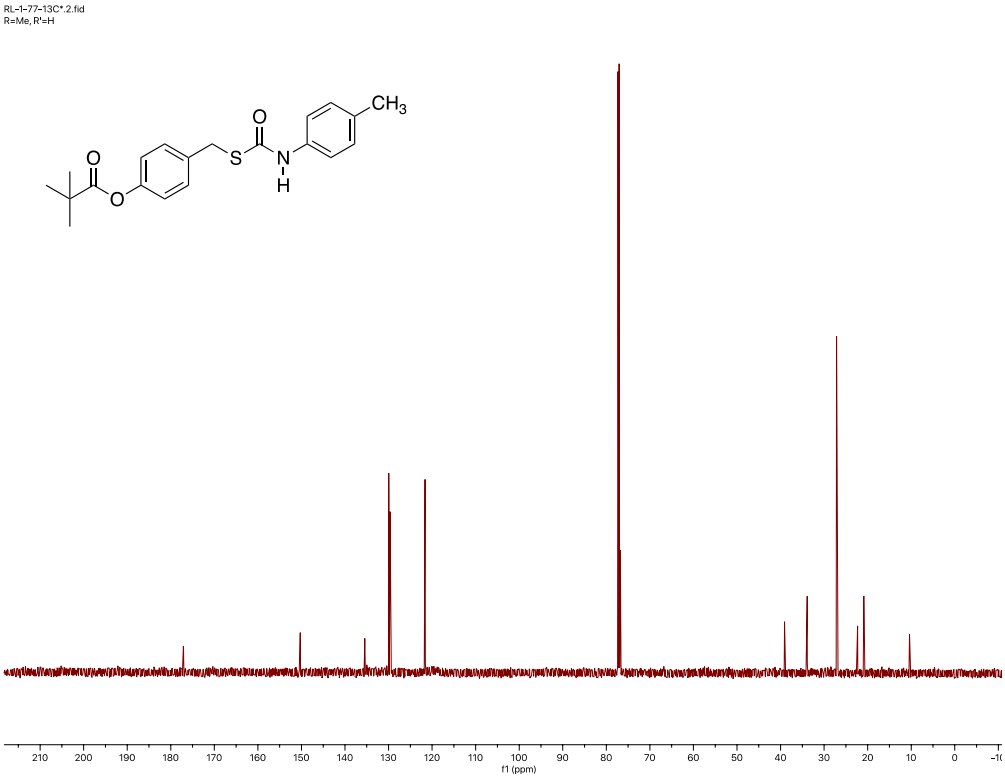




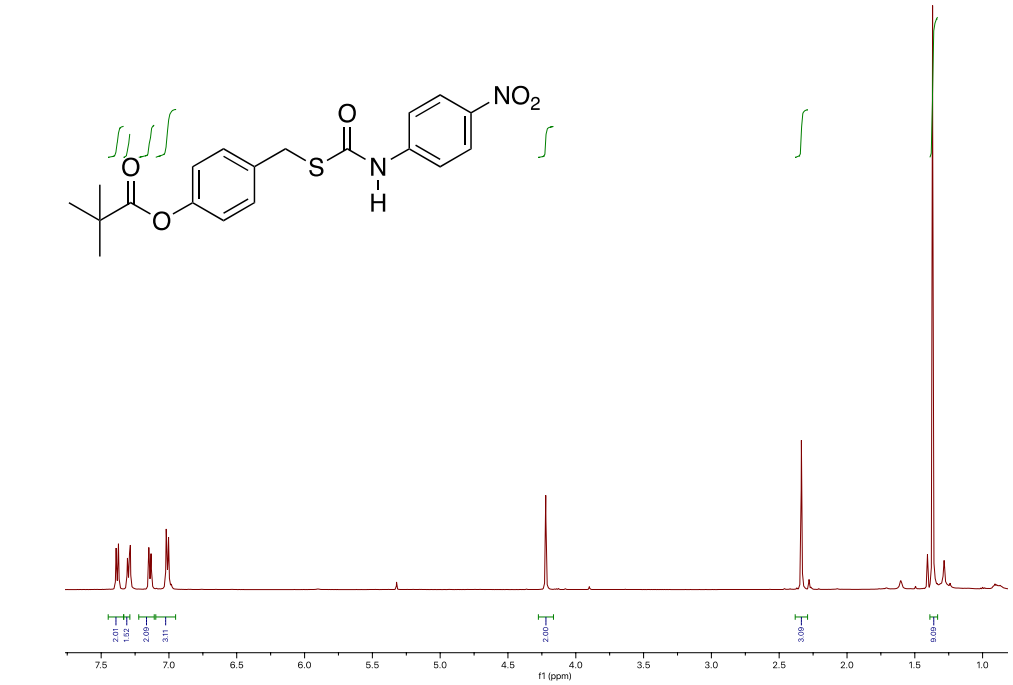
Esterase-Triggered N-H S-alkyl H_2S donors

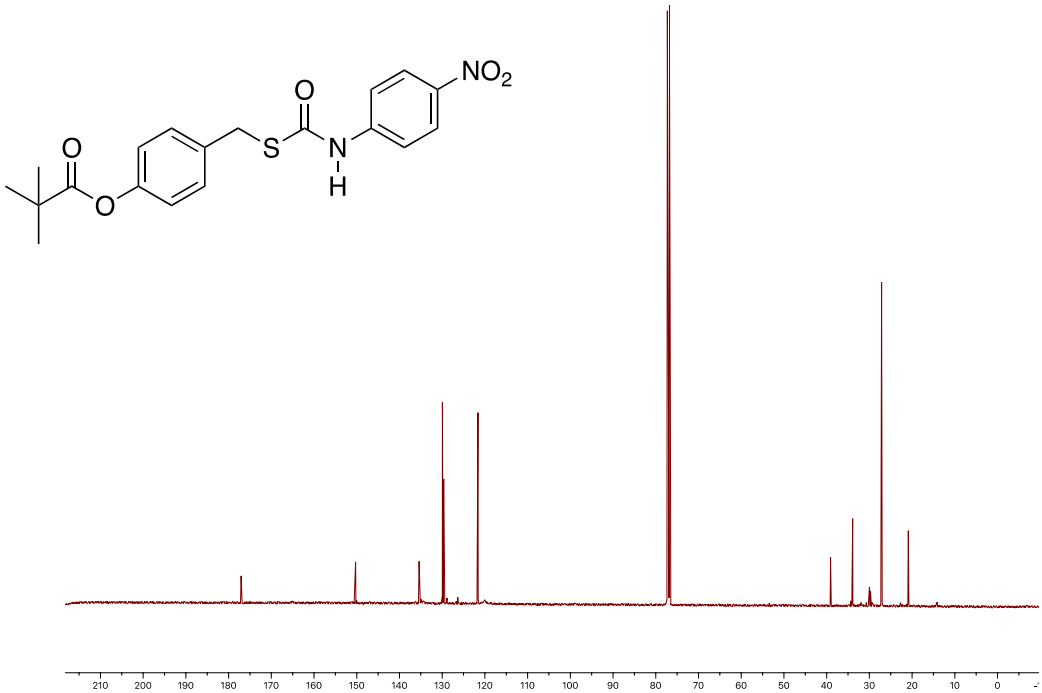
^{1}H (500 MHz, $CDCl_3$) NMR Spectrum (17.7% yield)



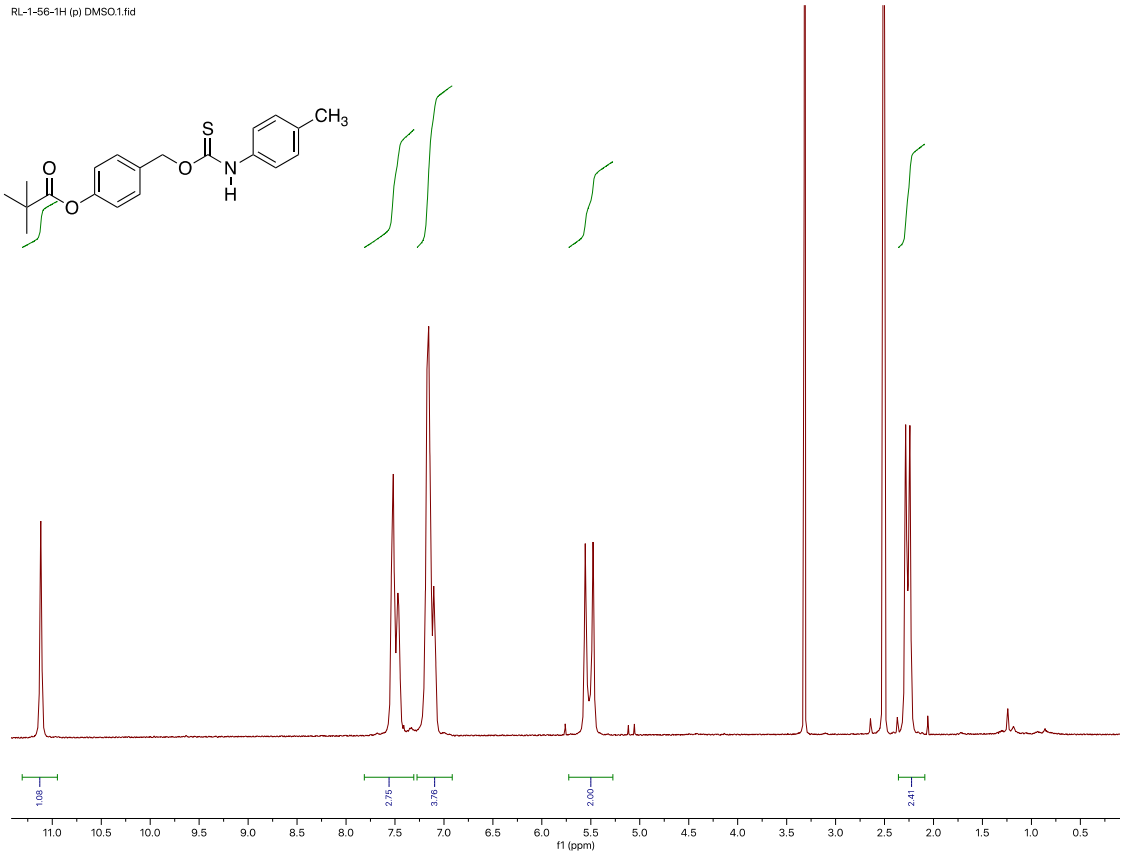


^{1}H (500 MHz, $CDCl_3$) NMR Spectrum (5.6% yield)



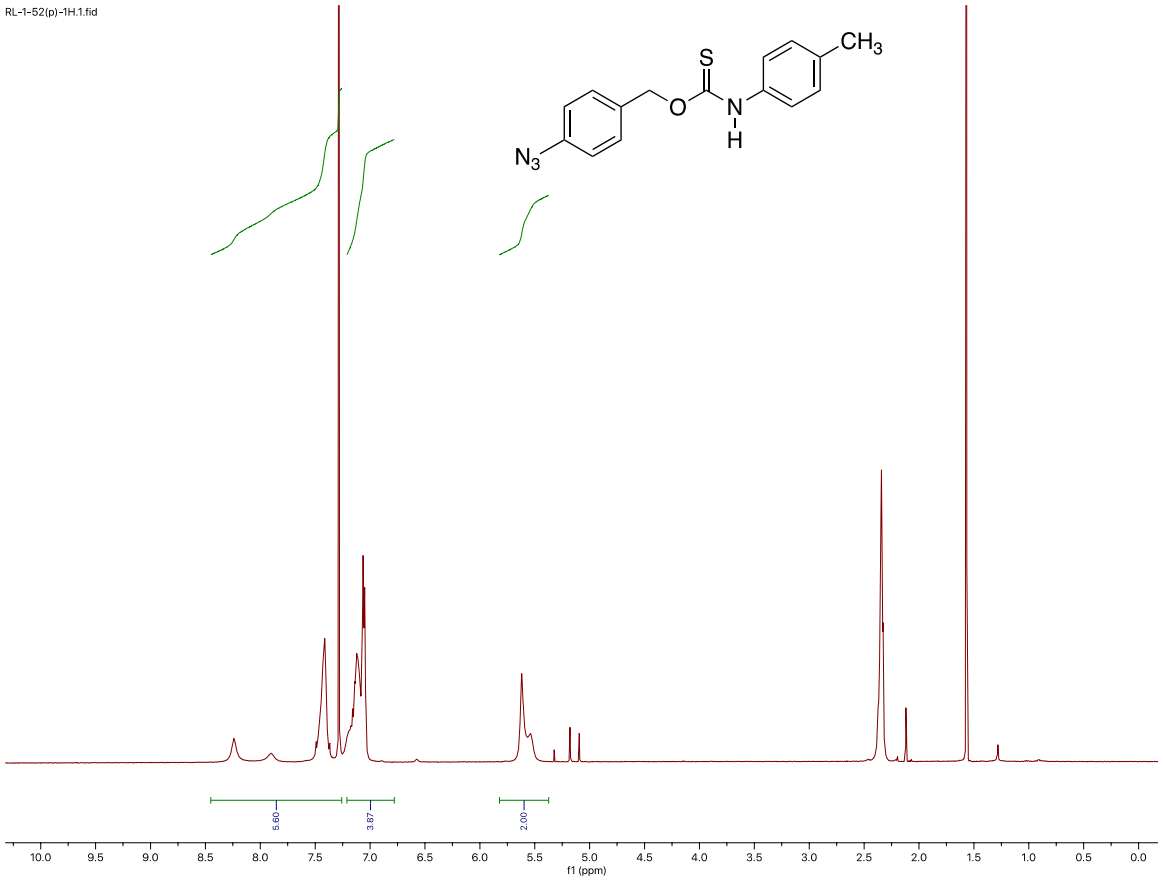


^{1}H (600 MHz, DMSO) NMR Spectrum



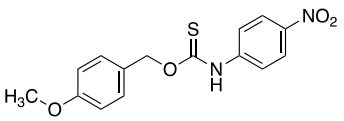
Azide Triggered N-H H_2S Donors

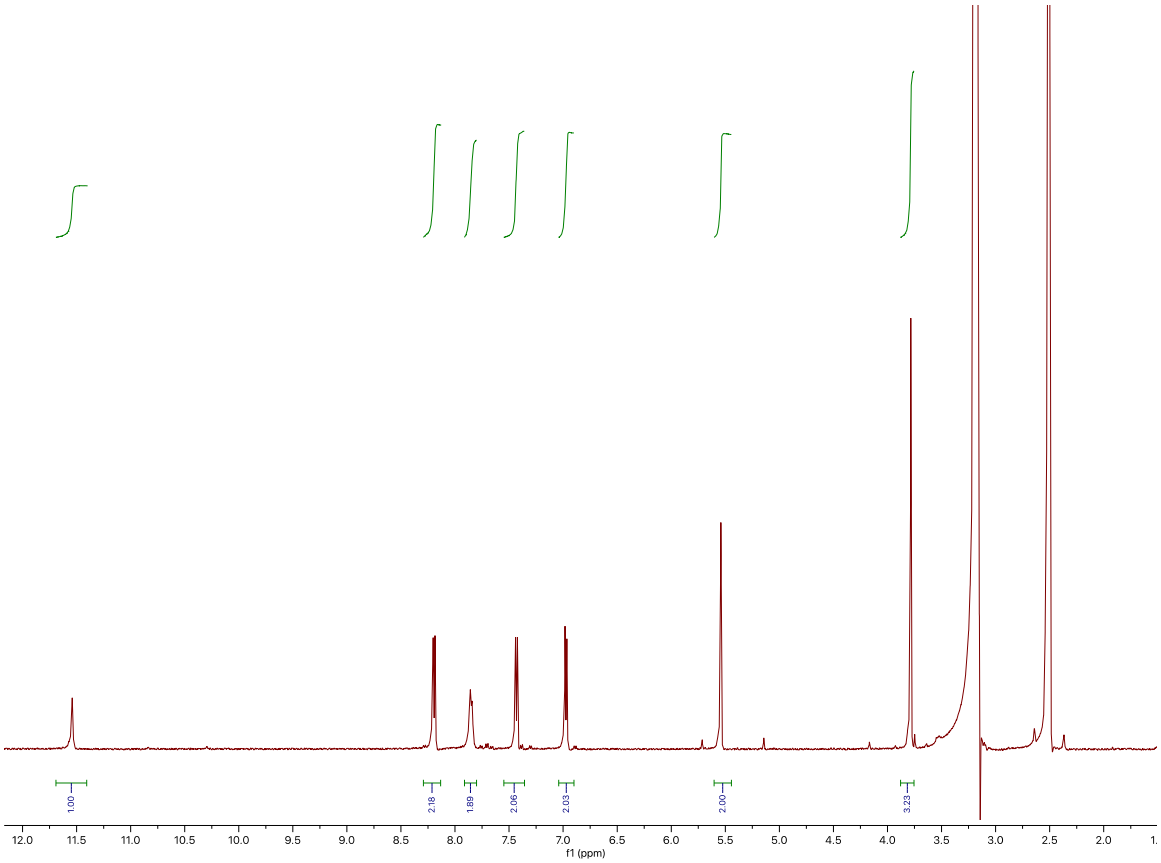
^1H (600 MHz, $CDCl_3$) NMR Spectrum (41.9% yield)



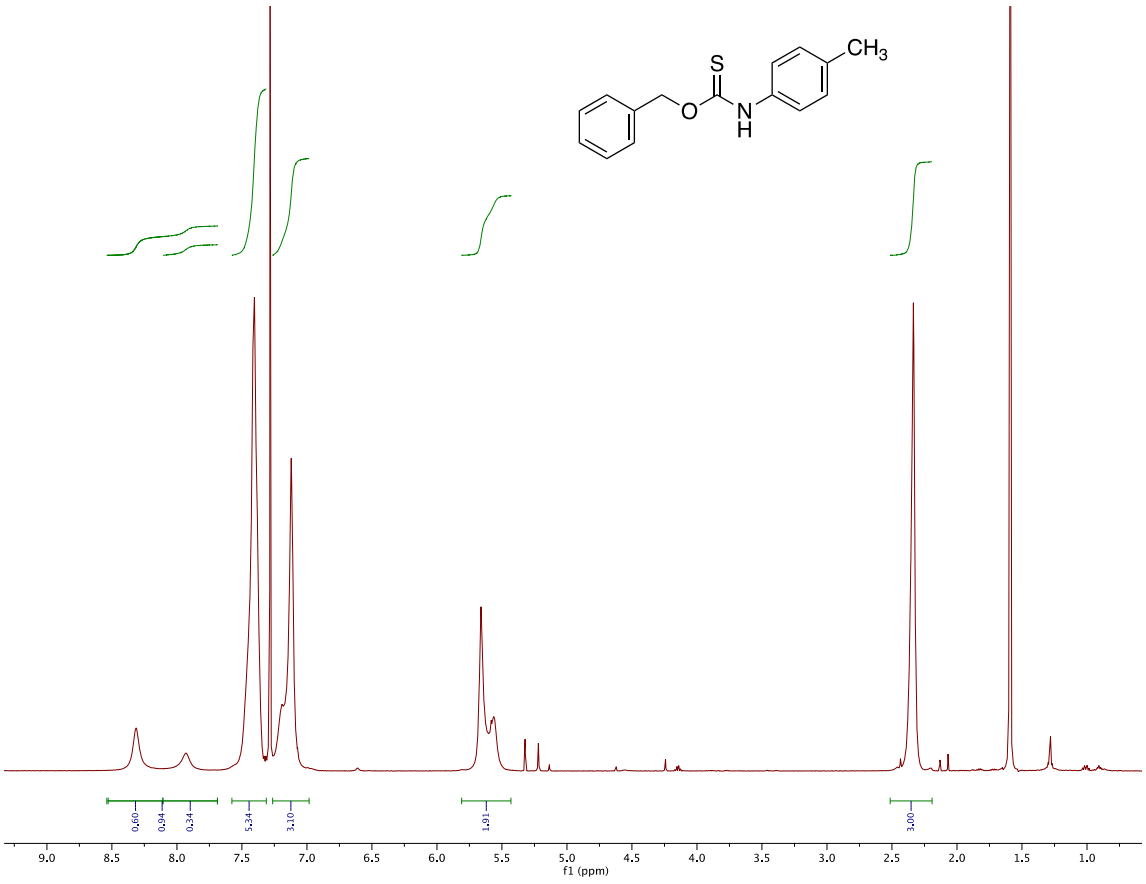
Triggerless H_2S donors

^{1}H (600 MHz, DMSO) at 60 ºC NMR spectrum (19.1% yield)

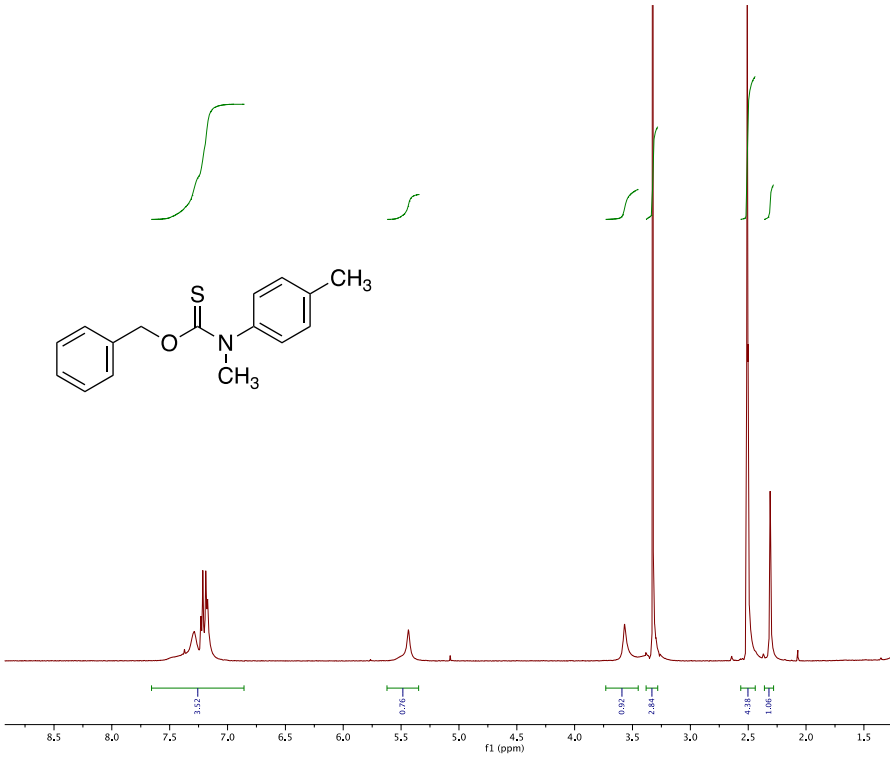


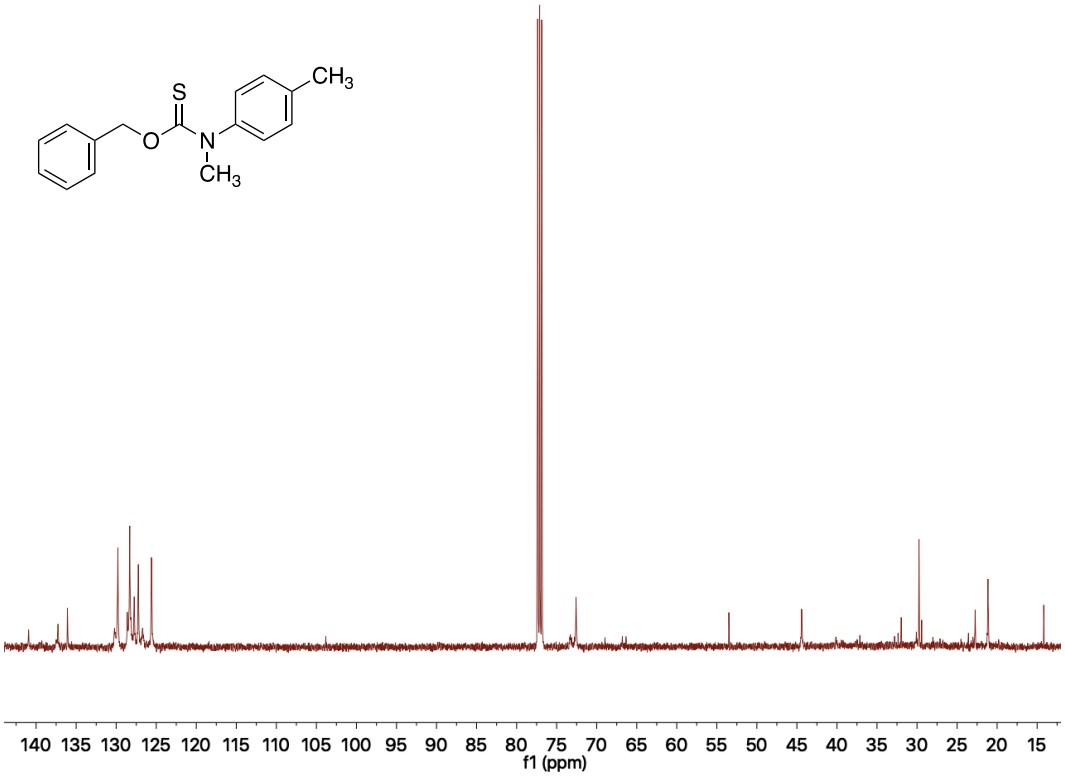

1.00
2.18
1.89
2.06
2.03
2.00
3.23
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

^{1}H (500 MHz, $CDCl_3$) NMR spectrum (38.7% yield)



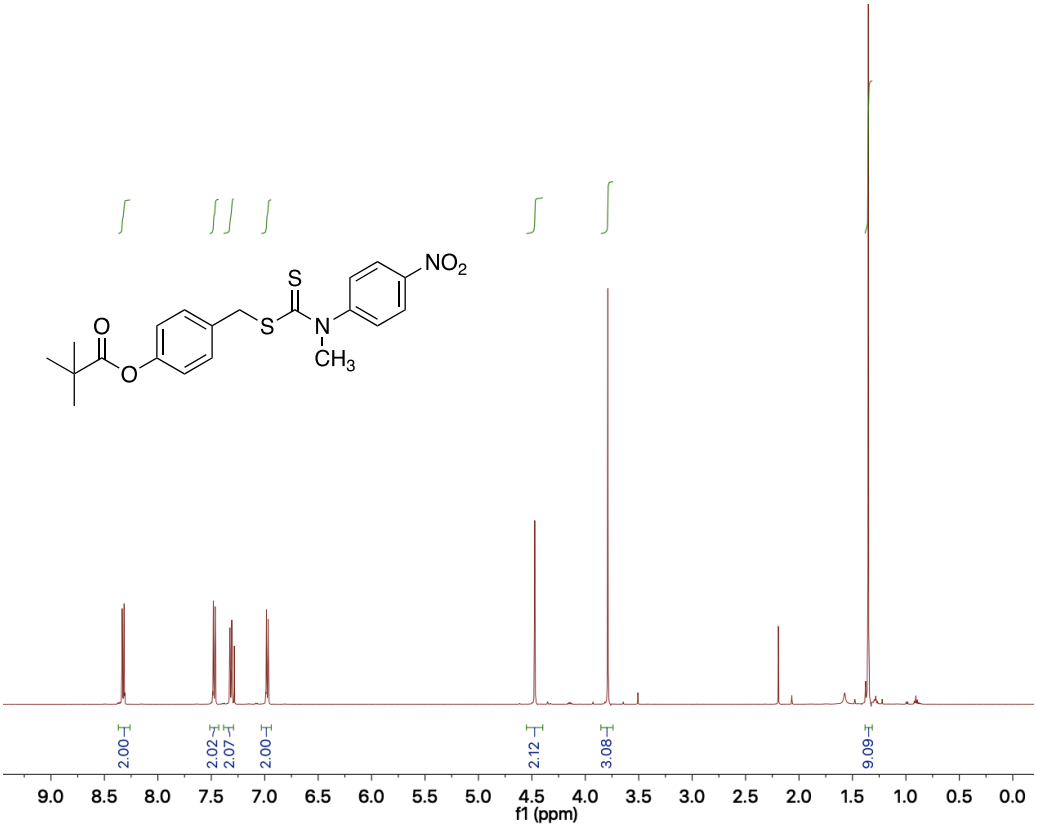
^{1}H (500 MHz, DMSO) and ^{13}C (125 MHz, $CDCl_3$) NMR spectra (33.8% yield)

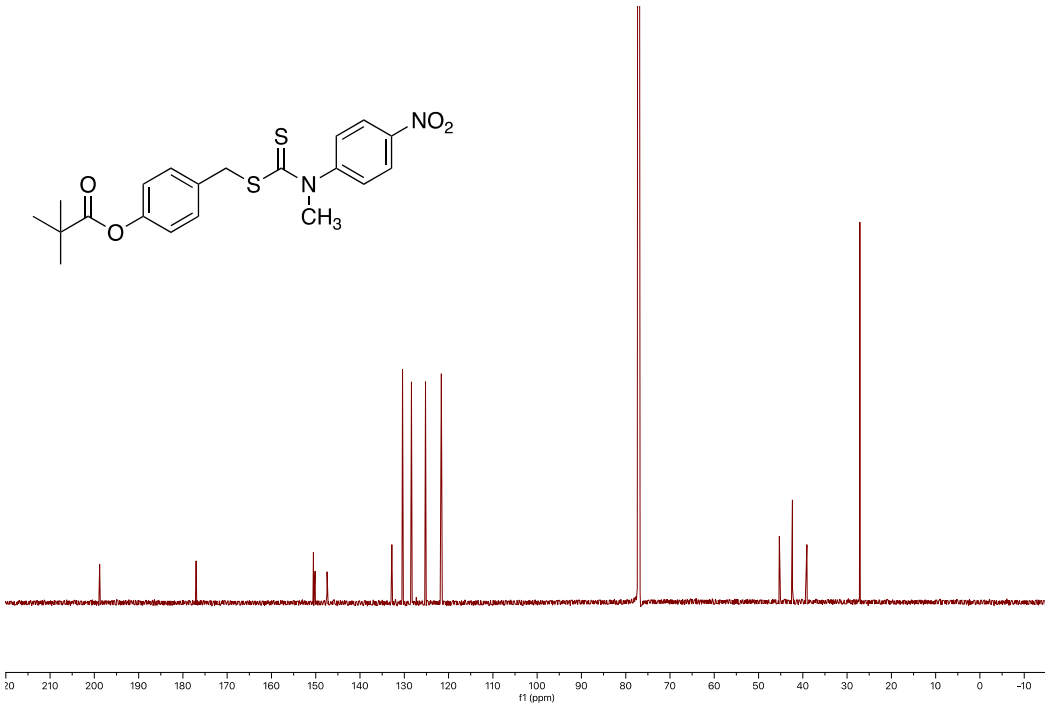




Esterase-Triggered N-Me CS_2 donors

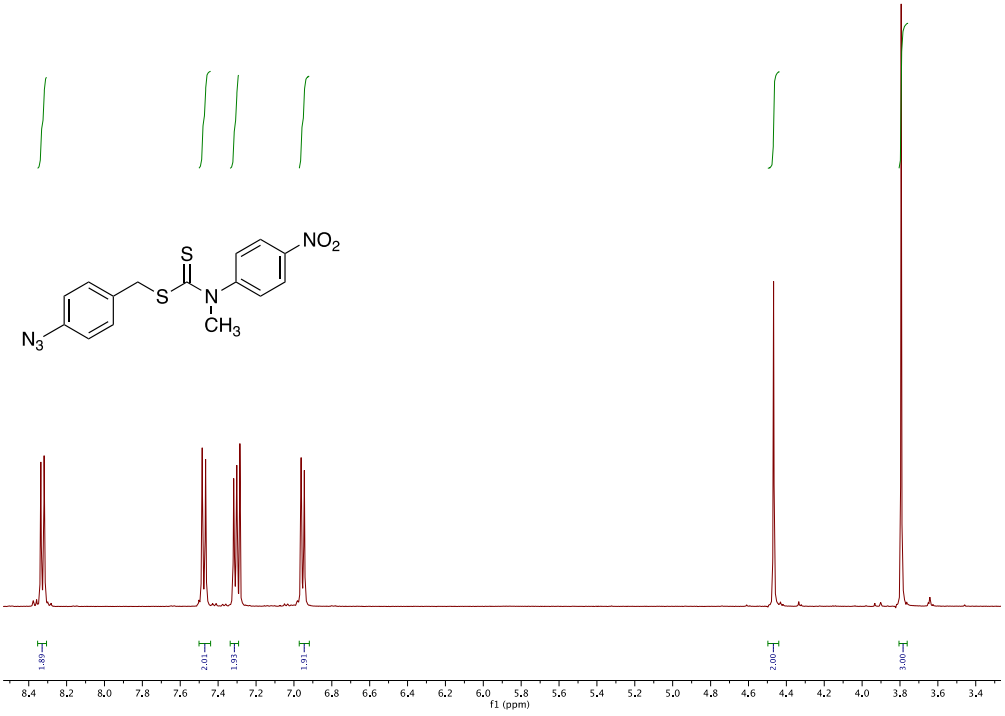
^{1}H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) NMR spectra (64.0% yield)

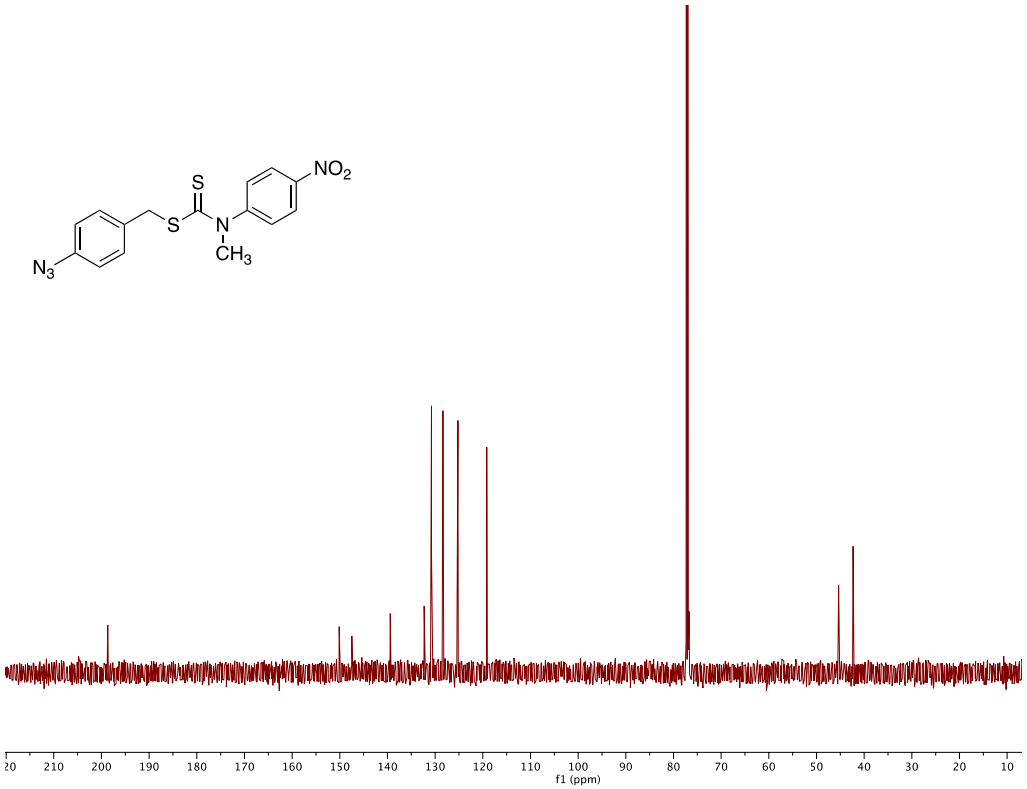




Azide-Triggered N-Me CS_2 donors

^{1}H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) NMR spectra (38.0% yield)





GLOSSARY

Hydrogen Sulfide (H_2S)	A small gaseous molecule that plays an important role in cell signaling events in the human body. H_2S is toxic in small amounts and is known as the source of the rotten-egg smell. It was recently identified as a gasotransmitter, making it a relevant target for synthetic donor design.
Carbon Disulfide (CS_2)	A small, sulfur-containing molecule, CS_2 is a known neurotoxin. It is also known to play a role in the metabolism of COS, a key intermediate of H_2S in the body. CS_2 has physiological implications as it has been found to be related to schizophrenia in humans, and social behaviors in mice.
Carbonyl Sulfide (COS)	A small, sulfur containing molecule, considered to be a caged form of H_2S or possibly a gasotransmitter in its own right. COS can be converted to H_2S by the enzyme carbonic anhydrase.
Carbonic Anhydrase (CA)	A ubiquitous enzyme that aids in the conversion of carbon dioxide to carbonic acid and bicarbonate. It is also known to convert COS to H_2S, which has been utilized for the thiocarbamate donor release motif.
Gasotransmitter	A gaseous signaling molecule that is produced endogenously and contributes to human physiology globally.
Endogenous	Produced within the body.
Thiocarbamate (TCM) Donors	Thiocarbamates in this context refer to a donor motif is a sulfur analog of carbamate. They exist as *O*-alkyl or *S*-alkyl depending on which atom is in the linker position.
Electron Withdrawing Groups (EWGs)	EWGs are functional groups that draw electrons towards themselves.
Electron Donating Groups (EDGs)	EDGs are functional groups that push electrons away from themselves.

Nuclear Magnetic Resonance Spectroscopy (NMR)	A method in analytical chemistry used to determine quality control for content and purity of a sample as well as its atomic structure
Methylene Blue Assay	A method for direct quantification of H_2S release via UV-vis measurements and using Beer's law.
Silica Column Chromatography	A common method for purification in organic synthesis that utilizes a compound's affinity for a given solvent system to separate it from impurities.
Thin Layer Chromatography (TLC)	A useful technique that separates compounds by affinity for a given solvent system using glass or aluminum foil coated in silica.

REFERENCES

1. National Research Council (US) Committee on Acute Exposure Guideline Levels. Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 9. Washington (DC): National Academies Press (US); 2010, 4, Hydrogen Sulfide Acute Exposure Guideline Levels
2. Wang, R. "Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter?" *FASEB J.* **2002**, *16*, 1792–1798.
3. Wang, R. "The gasotransmitter role of hydrogen sulfide." *Antioxid. Redox Signal.* **2004**, *5*, 493-501.
4. Levinn, C. M.; Cerda, M. M.; Pluth, M. D. "Activatable Small-Molecule Hydrogen Sulfide Donors." *Antioxid. Redox Signal.* **2020**, *32(2)*, 96-109
5. Wang, R. "Physiological implications of hydrogen sulfide: a whiff exploration that blossomed." *Physiol. Rev.* **2012**, 92, 791– 896.
6. Szczesny, B.; et. al, "AP39, a novel mitochondria-targeted hydrogen sulfide donor, stimulates cellular bioenergetics, exerts cytoprotective effects and protects against the loss of mitochondrial DNA integrity in oxidatively stressed endothelial cells *in vitro." Nitric Oxide* **2014**, *41*, 120-130.
7. Gilbert, A. K.; Zhao, Y.; Otteson, C. E.; Pluth, M. D. "Development of Acid-Mediated H_2S/COS Donors That Respond to a Specific pH Window." *J. Org. Chem.* **2019**, 84, 22, 14469–14475.
8. Steiger, A.K.; Marcatti, M.; Szabo, C.; Szczesny, B.; Pluth, M.D. "Inhibition of Mitochondrial Bioenergetics by Esterase-Triggered COS/H2S Donors" *ACS Chem. Biol.* **2017**, 12(8), 2117-2123.
9. Levinn, C. M.; Cerda, M. M.; Pluth, M. D. "Development and Application of Carbonyl Sulfide-Based Donors for H_2S Delivery." *Acc. Chem. Res.* **2019**, *52,* 2723-2731.
10. DeLeon, E. R.; Gilbrian F. S.; Kenneth R. O. "Passive loss of hydrogen sulfide in biological experiments." *Anal. Biochem.* **2012**, *421.1,* 203-207.
11. Cerda, M. M.; Newton, T. D.; Zhao, Y.; Collins, B. K; Hendon, C. H.; Pluth, M. D. "Dithioesters: simple, tunable, cysteine-selective H_2S donors." *Chem. Sci.* **2019**, *10*, 1773-1779.
12. Levinn, C. M.; Steiger, A. K., Pluth, M. D. "Esterase-Triggered Self-Immolative Thiocarbamates Provide Insights Into COS Cytotoxicity." *ACS Chemical Biology* **2019**, *14,* 170-175.
13. Zhao, Y.; Henthorn, H. A.; Pluth, M. D., "Kinetic Insights into Hydrogen Sulfide Delivery from Caged-Carbonyl Sulfide Isomeric Donor Platforms." *J. Am. Chem. Soc.* **2017**, *139, 45*, 16365-1637.
14. Ramesh, R.; Dalvi, R.; Poore, R. E.; Neal, R. A., "Studies of the metabolism of carbon disulfide by rat liver microsomes." *Life Sci.* **1974**, *14(9), 1785-1796*.

15. Zhao, Y.; Steiger, A. K.; Pluth M. D. “Cyclic Sulfenyl Thiocarbamates Release Carbonyl Sulfide (COS) and Hydrogen Sulfide (H_2S) Independently in Thiol-Promoted Pathways.” *J. Am. Chem. Soc.* ***2019****, 41(34), 13610-13618.*
16. Munger, S.D., *et. al. Curr. Biol.* “An olfactory subsystem that detects carbon disulfide and mediates food-related social learning.” **2010**, *20*, 1438-1444.
17. Phillips, M.; Sabas, M.; Greenberg, “Increased pentane and carbon disulfide in the breath of patients with schizophrenia.” *J. Clin. Pathol.* **1993**, *46*, 861-864.
18. DeMartino, A. W.; Souza, M.L.; Ford, P. C., “Uncaging Carbon Disulfide. Delivery Platforms for Potential Pharmacological Applications: A Mechanistic Approach.”*Chem. Sci.* **2017**, *8*, 7186-7196.
19. Lawrence, N. S.; Davis, J.; Jiang, L.; Jones, T. G. J.; Davies, S. N.; Compton, R. G. “The Electrochemical Analog of the Methylene Blue Reaction: A Novel Amperometric Approach to the Detection of Hydrogen Sulfide”*Electroanalysis* **2000**, *12*, 1453-1460.