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Towards the Total Synthesis of Thioviridamide: Thiyl Radical Approach to the Beta-Thioenamide Linkage Formation

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TOWARDS THE TOTAL SYNTHESIS OF THIOVIRIDAMIDE: A THIYL RADICAL APPROACH TO β-THIOENAMIDE LINKAGE FORMATION

by

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GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

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ABSTRACT

TOWARDS THE TOTAL SYNTHESIS OF THIOVIRIDAMIDE: A THIYL RADICAL APPROACH TO THE *β*-THIOENAMIDE LINKAGE FORMATION

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Department of Chemistry and Biochemistry

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We developed an approach to the *β*-thioenamide linkage contained in the *S*-(2-aminovinyl)cysteine (avCys) residue of thioviridamide.[1,2] Kinetic and thermodynamic control of radical additions of thiols to ynamides were studied for the formation of *β*-thioenamide linkage. Thiyl radicals are electrophilic and ynamides are electron-rich alkynes. This complementary polarity of the radical and acceptor increases the likelihood of a successful radical addition reaction. Because little is known about these types of compounds (*β*-thioenamides), we were unsure what kinds of yields and stereoselectivities (cis vs. trans) to expect. The adduct stability is another issue to consider. Fortunately, under typical radical addition conditions, the two separable isomers (cis and trans) are formed in good yield. Selective formation of kinetic (cis) and thermodynamic (trans) isomers are controlled by reaction time and equivalents of thiol. We converted the kinetic isomer to the thermodynamic isomer to confirm that isomerization can occur under the reaction conditions. Alkyl and aryl thiols including cysteine-derived thiols with different ynamides were used in this process.

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CHAPTER 1. INTRODUCTION

1.1 Thioviridamide

Thioviridamide (**1**) was isolated from *Streptomyces olivoviridis* by Hayakawa and co-workers.[1,2] Using 3Y1 rat fibroblasts transformed with adenovirus oncogenes, Hayakawa and co-workers also found antitumor antibiotic activity from extracts of *Streptomyces olivoviridis* which contained active substance **1**. Thioviridamide induces apoptosis selectively in Ad12-3Y1 cells (IC_{50}=3.9 ng/mL) and E1A-3Y1 cells (IC_{50}=32 ng/mL).[1] Additionally, it was tested in 3Y1 rat normal fibroblasts and SV40 (SV-3Y1), v-*src* (SR-3Y1) or v-H-*ras* (HR-3Y1) cancer cells. Although thioviridamide inhibited the growth of these cell-lines including normal fibroblasts at higher concentrations, it did not show toxicity towards these cell lines. Ad12-3Y1 and E1A-3Y1 cell lines both contain the adenovirus E1A oncogene, which can inactivate the retinoblastoma tumor suppressor protein (pRB). E1A is reported as an apoptosis inducer in pRB-deficient cells. These facts make **1** a possible anticancer agent. Its structure was analyzed by a variety of two-dimensional NMR techniques including COSY, HMBC, CT-HMBC, and HMBC-HOHAHA.

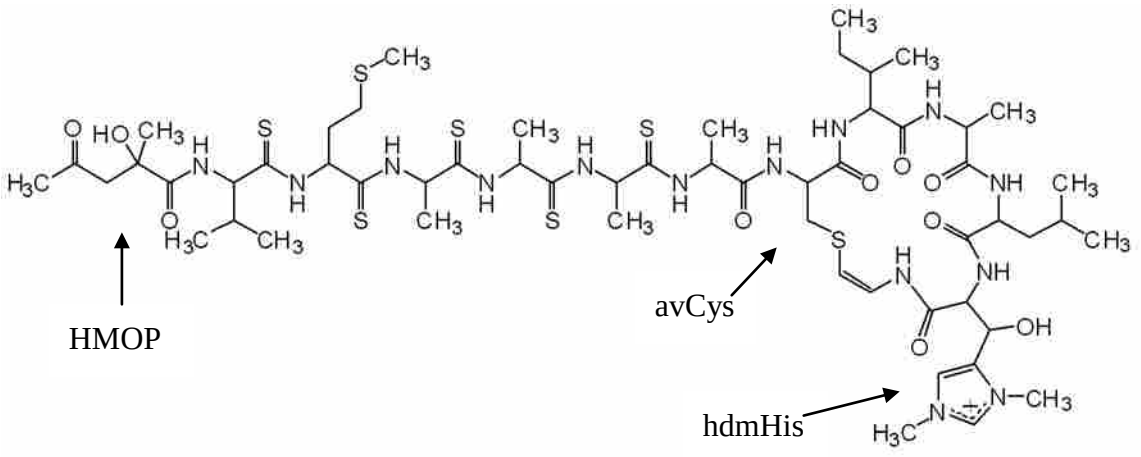


Figure 1. Thioviridamide (**1**)

As depicted in Figure 1, thioviridamide has an interesting structure, which includes seven amide linkages and five thioamide linkages. Especially interesting is the cyclic peptide on the right side. In that substructure are contained two novel amino acids: *β*-hydroxy-N^1,N^3-dimethylhistidinium (hdmHis) and *S*-(2-aminovinyl)cysteine (avCys).[2] The linkage of these residues forms an unusual *β*-thioenamide system (Figure 2). On the left side of the molecule, the peptide chain contains all five thioamides, which are separated from the cyclic peptide structure by a solitary alanine. The chain also contains a terminal 2-hydroxy-2-methyl-4-oxopentanoyl (HMOP) group. A few single-thioamide-containing natural products have been isolated from natural sources,[3] but **1** is an unprecedented example of a multi-thioamide-containing natural product. Despite the combination of challenging structural motifs with useful biological activity, to date no synthetic studies toward **1** have been reported. Except for the cis configuration of the *β*-thioenamide system, all stereochemical details of **1** are unknown. Thus, our goals in this project are a total synthesis and a structural determination of **1**.

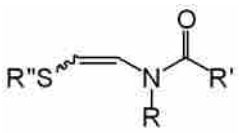


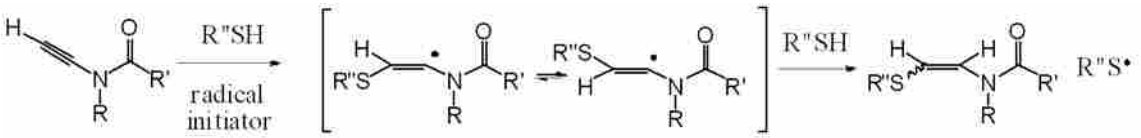
Figure 2. *β*-Thioenamide

1.2 *S*-(2-aminovinyl)cysteine (avCys)

Due to the numerous complex structural features of **1**, the initial studies focused on the preparation of the *β*-thioenamide contained in the avCys residue. For the formation of *β*-thioenamide linkage, we wanted to explore a radical addition of thiols to ynamides.

Thiyl radicals are electrophilic, and ynamides are electron-rich alkynes. These complementary polarities of the radical and the radical acceptor increase the likelihood of a successful radical addition reaction.[4] Electron-deficient radicals will be formed from thiols, and these thiyl radicals will be added to electron-rich alkynes to form electron-rich radicals. The chain reaction will propagate via hydrogen atom transfer from the thiol to the intermediate vinyl radical. Scheme 1 shows the proposed reaction.

Scheme 1. Synthesis of β-Thioenamide by Radical Reaction



Ynamides have been synthesized by several groups,[5] including compounds 3-ethynyloxazolidin-2-one (**2)** and *N*-benzyl-*N*-ethynylbenzamide (**3)** (Figure 3). We decided to begin this study by testing additions of simple thiols to **2** and **3**, and then exploring the use of a protected cysteine derivative (Figure 4).

2 **3**

Figure 3. Basic Forms of Ynamide Acceptors

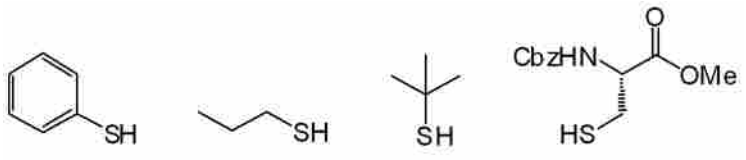
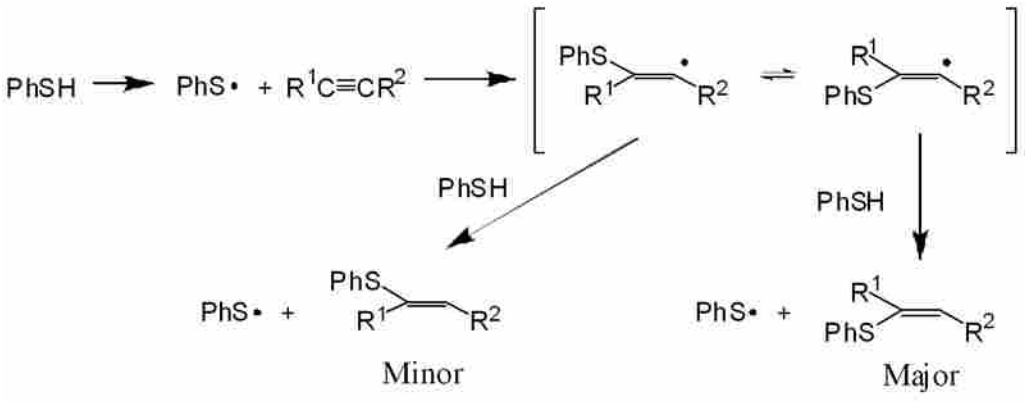


Figure 4. Simple Thiols and Protected Cysteine Derivative

Because little is known about these types of compounds (*β*-thioenamides), we were unsure about what kinds of yields and stereoselectivities (cis vs. trans) to expect. An early free-radical reaction study[6] of benzenethiol and diphenyl disulfide with simple alkynes shows that (*Z*)-vinyl sulfide adducts are the kinetic products due to the fast hydrogen extraction by alkene radical intermediate from a thiol on the less hindered side (Scheme 2). The study also suggested the possibility of isomerization between (*Z*) and (*E*) adducts. After the rapid formation of (*Z*) adducts in the reaction conditions, excess thiyl radicals might add to the resulting alkene. *β*-elimination of thiyl radical could then afford the less crowded (*E*) adducts.

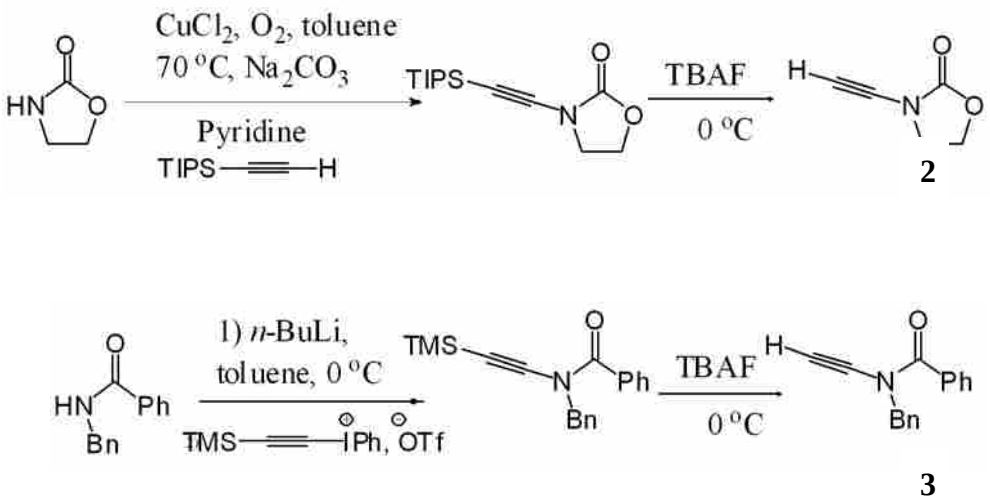
Scheme 2. Free-Radical Reaction Products



CHAPTER 2. SYNTHESIS OF THE YNAMIDES AND RADICAL ADDITION REACTIONS WITH SIMPLE THIOLS

In order to study the proposed thiyl radical additions, we began by preparing ynamides **2** and **3**. Ynamide **3** was prepared from *N*-benzylbenzamide, and **2** was prepared from 2-oxazolidone. The syntheses of **2** and **3** are given in Scheme 3. Both ynamides **2**[5e] and **3**[5d] are known compounds. Copper-catalyzed aerobic oxidative amidation of terminal alkynes was used to prepare compound **2**. By using trimethylsilylethynyliodonium triflate, compound **3** was obtained through N-alkynylation. Both TIPS and TMS groups were removed by TBAF. Simple alkyl thiols,

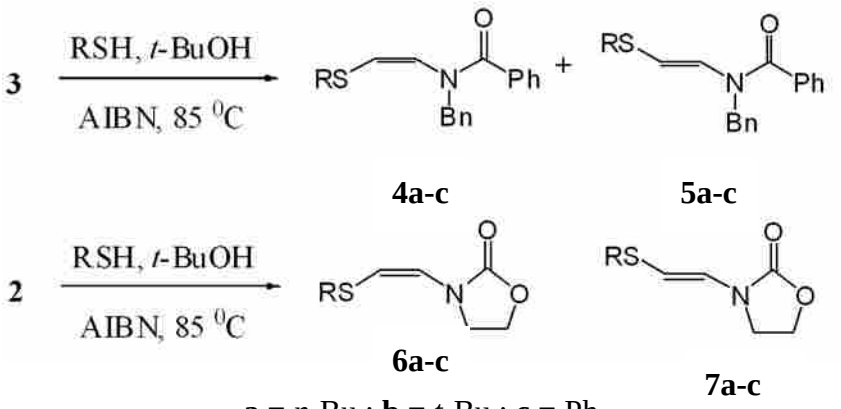
Scheme 3. Preparation of Ynamides



n-butyl thiol and *t*-butyl thiol were used to probe the effect of different sized alkyl groups on the radical addition. Phenyl thiol was also tested in the radical addition reactions. A

reaction vial with all reagents including ynamide, thiol, and AIBN was flushed with argon and stirred in an oil bath at 85 °C for a variable period of time to obtain *β*-thioenamide products (see Table 1).[7] In general, shorter reaction times with fewer equivalents of thiols and AIBN favored cis products. By contrast, longer reaction times with more equivalents of thiols and AIBN favored trans products. The results are given in Table 1.

Table 1. Thiyl Radical Addition of Alkyl and Aryl Thiols to Ynamides **2** and **3**



a = *n*-Bu ; **b** = *t*-Bu ; **c** = Ph

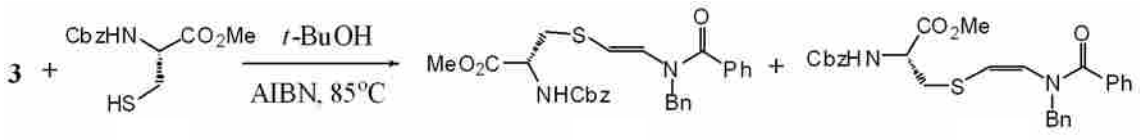
Acceptor	R (eq)	AIBN(eq)	Time	Cis : Trans	Yield
3	*n*-Bu (1)	0.5	10 min	**4a** (11) : **5a** (1)	82 %
3	*n*-Bu (4)	2	3 hrs	**4a** (1) : **5a** (15)	79 %
3	*t*-Bu (4)	2	6 hrs	**4b**	41 %
3	Ph (1)	0.5	10 min	**4c** (4.3) : **5c** (1)	41 %
3	Ph (4)	2	5 hrs	**4c** (1) : **5c** (33)	quant.
2	*n*-Bu (2)	1	30 min	**6a** (1.3) : **7a** (1)	75 %
2	Ph (2)	1	30 min	**6c** (0) : **7c** (1)	71 %

Reactions of ynamide **3** with *n*-butyl thiol and phenyl thiol resulted primarily in compounds **4a** and **4c** respectively under conditions of shorter reaction time with 1 equivalent of thiol and 0.5 equivalents of AIBN. Compound **5a** and **5c** were predominantly formed in longer time reactions with 4 equivalents of thiol and 2 equivalents of AIBN. Attempts to obtain the *t*-butyl trans isomer (**5b)** were not successful. Longer reaction times with increased equivalents of *t*-butyl thiol and AIBN resulted in decomposition of cis isomer (**4b)** and starting materials. Extensive reaction time in a hot oil bath with increased equivalents of thiols and AIBN resulted in side reactions. Thiyl radical addition with *n*-butyl thiol and phenyl thiol to ynamide **2** yielded interesting results. Under moderate reaction conditions (see Table 1) with ynamide **2,** a reaction with *n*-butyl thiol formed cis isomer (**6a)** more than trans isomer (**7a)**. Despite the same reaction conditions, reaction with phenyl thiol to ynamide **2** favored trans isomer (**7c)** over cis isomer (**6c**). Based upon our observation of thiyl radical addition with ynamide **2**, we concluded that isomerization of thiyl radical adducts occurs more rapidly with phenyl thiols than with *n*-butyl thiols.

CHAPTER 3. RADICAL ADDITION REACTIONS OF CYSTEINE-DERIVED THIOLS TO YNAMIDES **3** AND ISOMERIZATION OF RADICAL ADDITION PRODUCTS

After the study of radical addition reactions of simple thiols to compounds **2** and **3**, we focused on the thiyl radical addition of a cysteine-derived thiol (**8**)[8] to compound **3**. The results are given in Table 2.

Table 2. Thiyl Radical Addition of Cysteine-derived Thiols to Ynamides **3**



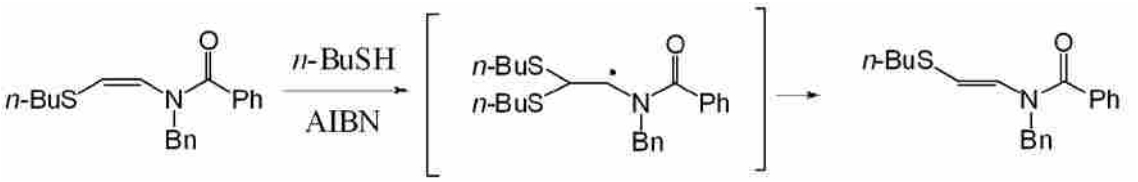
8 **9** **10**

8 (eq)	**AIBN(eq)**	**Time**	**Cis : Trans**	**Yield**
1	0.5	10 min.	**9** (6.1) : **10** (1)	79 %
2	2	3 hrs	**9** (1) : **10** (8.5)	77 %

As seen in Table 1, Table 2 illustrates that one isomer was predominantly synthesized over the other under different reaction conditions. Compound **9** was dominant in a reaction with shorter reaction time and fewer equivalents of compound **8** and AIBN. In contrast, compound **10** was dominant in a reaction with longer reaction time and more equivalents of compound **8** and AIBN.

According to the results in Table 1 and Table 2, we hypothesized that the cis isomers are kinetic products and the trans isomers are thermodynamic products. After the rapid formation of cis isomers in the reaction conditions, excess thiyl radicals might add to the resulting alkene. β-elimination of thiyl radical could then afford the less crowded trans isomer. Based on this hypothesis, we performed a reaction to confirm the isomerization. Scheme 4 shows the cis dominant isomeric mixture reacted under the thiyl radical addition reaction conditions, and it resulted in a trans dominant isomeric mixture.

Scheme 4. Isomerization

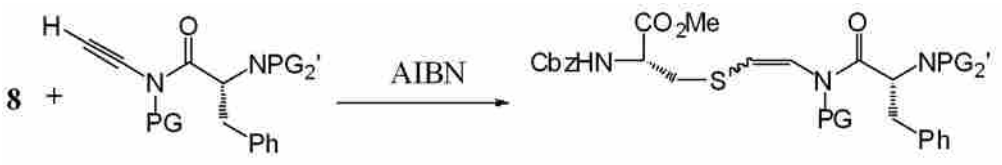


Cis vs. trans ratio converted from 3.6 : 1 to 1 : 5.3

CHAPTER 4. FUTURE WORK AND CONCLUSIONS

Due to the successful results of thiyl radical addition to ynamides, the research can now focus on thiyl radical addition of cysteine-derived thiols to phenylalanine-derived ynamides that have removable protecting groups on the amide- and terminal-nitrogen atoms (See Scheme 5). Because of the structural similarity between Phe and hdmHis, Phe-derived ynamides will be used for the model study. Possible protecting

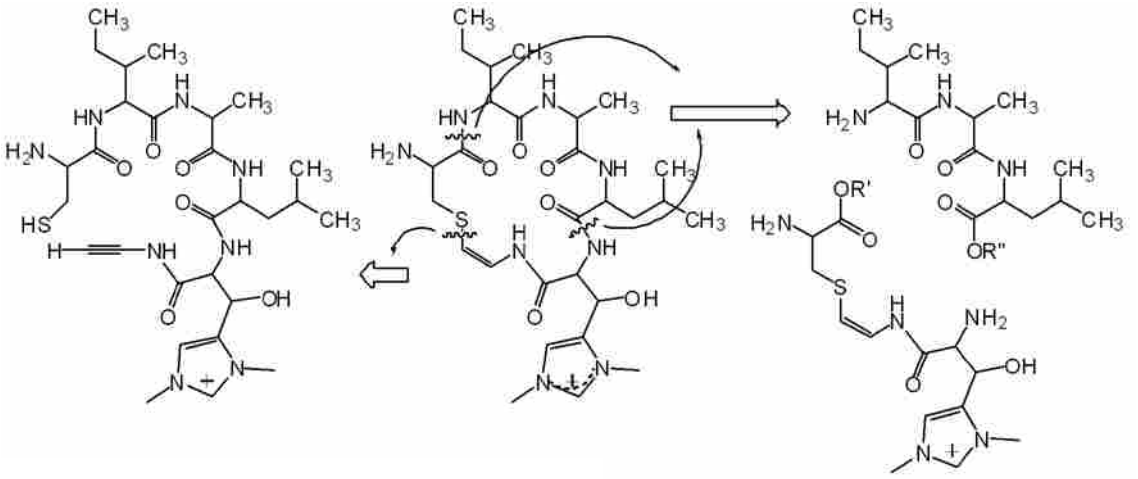
Scheme 5. Radical Reaction with Phenylalanine-derived Ynamides



groups for the amide-nitrogen are *p*-methoxybenzyl (PMB), dimethoxybenzyl (DMB), and [2-(trimethylsilyl)ethoxy]methyl (SEM). For the terminal-nitrogen, carbamates, benzyl-type protecting groups, or a combination thereof are possibilities. Removal of terminal nitrogen protecting groups is essential for the subsequent peptide coupling to

apply this method in the total synthesis of thioviridamide.

Macrolactamization of the thioviridamide ring (Figure 5) will be the next phase that we can feasibly undertake. Due to challenges in macrocyclization, formation of the uncommon β-thioenamide linkage, and synthesis of a novel amino acid (hdmHis), the thioviridamide ring preparation will be a difficult task. Macrolactamization at either the avCys/Ile or the Leu/hdmHis peptide bonds will be tested for the thioviridamide ring formation because those peptide linkages are the peptide bonds between the novel amino acids and common amino acids. This approach will lead us to a convergent synthesis of the thioviridamide ring. Macrocyclization via an intramolecular thiyl radical-ynamide addition is another approach we can possibly take to prepare the thioviridamide ring because with this approach, we can likely avoid racemization, which might be a problem if we perform macrolactamization for the ring preparation.



Intramolecular thiyl radical-ynamide addition

Peptide coupling, macrolactamization

Figure 5. Thioviridamide Peptide Ring

Although the stereochemistry of the thioviridamide ring is unknown, a computational study will be helpful to determine the most probable stereochemistry of the thioviridamide ring. After we construct the ring in Gaussian 03 to predict its NMR data, we will compare this calculated data with the published experimental NMR data. By varying the stereochemistry in our computations, we hope to find a calculate set of data that matches the experimental data well.

In conclusion, we have developed a new methodology for the synthesis of cis and trans *β*-thioenamide products via thiyl radical addition that is controlled by the reaction time and equivalents of reagents. To the best of our knowledge, this is the first use of ynamides as radical addition acceptors.[9] *β*-Thioenamides have been synthesized previously.[10] For example, *β*-thioenamides are found in carbapenem antibiotics PS-5 and thienamycin, 9-(2-aminoethenylthio)-9-deoxy derivatives of clavulanic acid, and 3-vinylthio- and 3-vinylthiomethylcephem derivatives. In synthetic study of carbapenem antibiotics PS-5 and thienamycin, the Ishibashi group[10d] used a *β*-thioenamide as a radical cyclization precursor to construct an azetidin-2-one derivative. The *β*-thioenamide present in the target structure was then annulated onto the azetidin-2-one via a lengthy multistep process. Brooks and coworkers[10c] also prepared *β*-thioenamides from bromoacetaldehyde diethyl acetal. Reaction with ethanethiol and sodium ethoxide in ethanol afforded 1,1-diethoxy-2-ethylthioethane, which on acid-catalyzed (*p*-toluene sulfonic acid) reaction with an amide gave the *β*-thioenamide system. These studies employ time-consuming methods with poor yields to construct the *β*-thioenamide system. Also, the reactions were not stereoselective. Often, they used harsh conditions that we cannot use in peptide synthesis. By contrast, we have developed a useful new pathway to construct a *β*-thioenamide system via thiyl radical addition. Our approach is

highly stereoselective, and the selectivity can be switched. The thiyl radical reaction is clean, and the conditions used are mild. Accordingly, we can expect high yields and stereoselectivities in peptide synthesis. Based on these promising preliminary results, we will move toward macrocyclization which ultimately will facilitate the total synthesis of thioviridamide.

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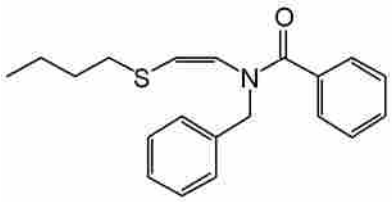
CHAPTER 5. EXPERIMENTAL AND SPECTROSCOPIC DATA

5.1 General Methods

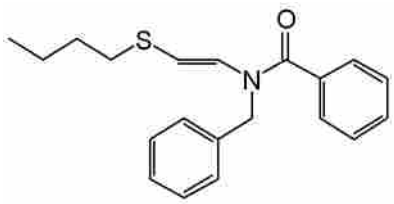
Flash chromatography was carried out using 60-230 mesh silica gel. ^{1}H NMR spectra were obtained on either a Varian 300 MHz or a Varian 500 MHz spectrometer with chloroform (7.27 ppm) or trimethylsilane (0.00 ppm) as internal reference. Signals are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), dd (doublet of doublets), dt (doublet of triplets), bs (broad singlet), m (multiplet). Coupling constants are reported in hertz (Hz). ^{13}C NMR spectra were obtained on Varian spectrometers operating at 75 or 125 MHz with chloroform (77.23 ppm) or trimethylsilane (0.00 ppm) as internal reference. Infrared spectra were obtained on a Nicolet Avatar 360 FT-IR Spectrometer. Mass spectral data were obtained using FAB techniques by the Brigham Young University mass spectrometry facility.

5.2 Experimental Details



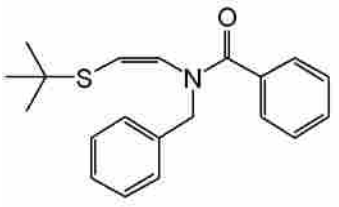
(*Z*)-*N*-Benzyl-*N*-(2-(butylthio)vinyl)benzamide (4a) A reaction vial with ynamide **3** (10 mg, 0.043 mmol) and *n*-butyl thiol (4.6 μL, 0.043 mmol) in dry *t*-butanol (0.3 mL) was flushed with argon for 5 minutes. AIBN (3.5 mg, 0.022 mmol) was added to the reaction mixture at room temperature, and the reaction mixture was flushed with argon for another 2 minutes. The reaction mixture was heated at 85 ˚C for 10 minutes. The completion of the reaction was determined by

consumption of starting material (TLC). The solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (1 mL) and stirred with saturated $NaHCO_3$ solution (1 mL) for 2 h. The aqueous layer was extracted with CH_2Cl_2 (3 x 1 mL) and the combined CH_2Cl_2 extracts were washed with water (2 x 1 mL), brine solution (1 x 1 mL), and dried (Na_2SO_4). The solution was evaporated under reduced pressure. The crude product (11:1 **4a**:**5a**) was purified by silica gel column chromatography using gradient elution with ethyl acetate:hexanes (1.5:8.5) to afford **4a** (10.5 mg, 0.032 mmol, 75%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.58 (d, *J* = 7.0 Hz, 2H), 7.43–7.30 (m, 6H), 7.28–7.25 (m, 2H), 6.07 (bs, 1H), 5.59 (bs, 1H), 5.00 (bs, 2H), 2.58 (t, *J* = 7.5 2H), 1.62–1.45 (m, 2H), 1.38–1.28 (m, 2H), 0.89 (t, *J* = 7.5 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.1, 137.7, 136.1, 130.6 (2C), 128.6 (4C) 128.1 (2C), 127.5 (2C), 126.9, 122.2, 49.0, 34.5, 32.6, 21.8, 13.9; IR (film) ν_{max} 3030, 2956, 2928, 1651, 1602, 1495, 1446, 1376, 1274, 1138, 1074, 982, 790, 723, 701 cm^{-1}; HRMS (ESI) m/z 326.1573 (MH^+, $C_{20}H_{23}NOS$ requires 326.1573).

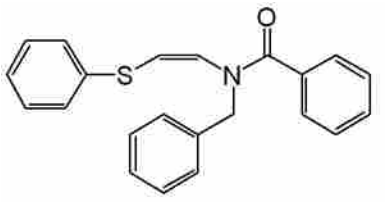


(*E*)-*N*-Benzyl-*N*-(2-(butylthio)vinyl)benzamide (5a) Prepared from **3** (10 mg, 0.043 mmol) and *n*-butyl thiol (18 μL, 0.17 mmol) according to the procedure given in the Experimental Section for the synthesis of **4a**, with AIBN (14 mg, 0.085 mmol), and the reaction was stirred at 85 ˚C for 3 hrs. The crude product (15:1 **5a**:**4a**) was purified by silica gel column chromatography using gradient elution with ethyl acetate:hexanes (1.5:8.5) to afford **5a** (10 mg, 0.031 mmol, 74%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 (d, *J* = 7.0 Hz, 2H), 7.50–7.42 (m, 3H), 7.29–7.26 (m, 2H), 7.28 (d, *J* = 8.5 Hz, 4H), 5.48 (d, *J* = 13.50 Hz, 1H), 5.02 (bs, 2H), 2.42 (t, *J* =

7.5 2H), 1.40–1.34 (m, 2H), 1.31–1.24 (m, 2H), 0.85 (t, *J* = 7.5 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.4, 136.8, 134.4, 130.9 (3C), 129.0 (4C) 128.8, 127.5 (2C), 126.9, 105.5, 48.0, 34.3, 31.6, 21.9, 13.8; IR (film) ν_{max} 3054, 3045, 2926, 1659, 1604, 1495, 1446, 1375, 1315, 1245, 1137, 1076, 982, 935, 788, 727, 696 cm^{-1}; HRMS (ESI) m/z 326.1581 (MH^+, $C_{20}H_{23}NOS$ requires 326.1573).

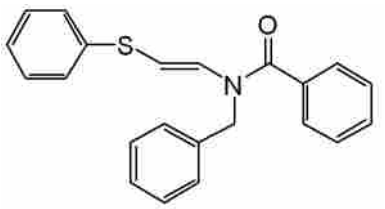


(*Z*)-*N*-Benzyl-*N*-(2-(*tert*-butylthio)vinyl)benzamide (4b) Prepared from **3** (10 mg, 0.043 mmol) and *t*-butyl thiol (19 μL, 0.17 mmol) according to the procedure given in the Experimental Section for the synthesis of **4a**, with AIBN (14 mg, 0.085 mmol), and the reaction was stirred at 85 ˚C for 6 hrs. The crude product was purified by silica gel column chromatography using gradient elution with ethyl acetate:hexanes (1.5:8.5) to afford **4b** (5.7 mg, 0.017 mmol, 41%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.57 (d, *J* = 7.0 Hz, 2H), 7.48–7.29 (m, 7H), 7.28–7.18 (m, 1H), 6.12 (bs, 1H), 5.79 (bs, 1H), 5.01 (bs, 2H), 1.29 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.0, 137.6, 136.0, 130.3, 130.0, 128.3 (5C), 127.8 (2C), 127.2 (2C), 48.8, 44.5, 30.8 (3C); IR (film) ν_{max} 3030, 2960, 1741, 1652, 1600, 1494, 1455, 1367, 1260, 1141, 1075, 1027, 983, 929, 791, 704, 667 cm^{-1}; HRMS (ESI) m/z 326.1573 (MH^+, $C_{20}H_{23}NOS$ requires 326.1573)



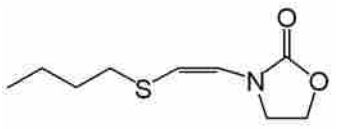
(*Z*)-*N*-Benzyl-*N*-(2-(phenylthio)vinyl)benzamide (4c) Prepared from **3** (10 mg, 0.043

mmol) and phenyl thiol (4.3 μL, 0.043 mmol) according to the procedure given in the Experimental Section for the synthesis of **4a**, with AIBN (3.5 mg, 0.021 mmol), and the reaction was stirred at 85 ˚C for 10 minutes. The crude product (4.3:1 **4c**:**5c**) was purified by silica gel column chromatography using gradient elution with ethyl acetate:hexanes (1.5:8.5) to afford **4c** (4.9 mg, 0.014 mmol, 33%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.62 (d, *J* = 7.0 Hz, 2H), 7.45–7.36 (m, 5H), 7.34–7.32 (m, 2H), 7.30–7.19 (m, 4H), 7.09 (d, *J* = 7.5 Hz, 2H), 6.31 (bs, 1H), 5.80 (d, *J* = 6.5 Hz, 1H), 5.07 (bs, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.1, 137.4, 135.9, 135.2, 130.7, 129.8 (2C), 129. 3 (2C), 128.7 (4C), 128.4 (2C), 128.2 (2C), 127.6, 127.3, 119.5, 119.4, 49.9; IR (film) ν_{max} 3068, 3058, 2991, 1644, 1601, 1494, 1477, 1439, 1368, 1340, 1274, 1178, 1138, 1074, 1025, 981, 790, 741, 703 cm^{-1}; HRMS (ESI) m/z 346.1259 (MH^+, $C_{22}H_{19}NOS$ requires 346.1260).

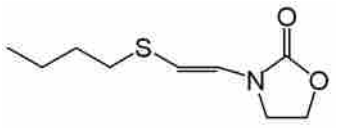


(*E*)-*N*-Benzyl-*N*-(2-(phenylthio)vinyl)benzamide (5c) Prepared from **3** (10 mg, 0.043 mmol) and phenyl thiol (17 μL, 0.17 mmol) according to the procedure given in the Experimental Section for the synthesis of **4a**, with AIBN (14 mg, 0.085 mmol), and the reaction was stirred at 85 ˚C for 3 hrs. The crude product (33:1 **5c**:**4c**) was purified by silica gel column chromatography using gradient elution with ethyl acetate:hexanes (1.5:8.5) to afford **5c** (14 mg, 0.041 mmol, 97%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 (d, *J* = 7.0 Hz, 2H), 7.50–7.42 (m, 3H), 7.29–7.26 (m, 2H), 7.28 (d, *J* = 8.5 Hz, 4H), 5.48 (d, *J* = 13.50 Hz, 1H), 5.02 (bs, 2H), 2.42 (t, *J* = 7.5 2H), 1.40-1.34 (m, 2H), 1.31-1.24 (m, 2H), 0.85 (t, *J* = 7.5 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.4, 136.8, 134.4, 130.9 (3C), 129.0 (4C) 128.8, 127.5 (2C), 126.9, 105.5, 48.0, 34.3, 31.6, 21.9,

13.8; IR (film) ν_{max} 3060, 2899, 1662, 1604, 1495, 1478, 1439, 1371, 1315, 1243, 1207, 1135, 1076, 1024, 980, 935, 788, 736, 696, 650 cm^{-1}; HRMS (ESI) m/z 346.1267 (MH^+, $C_{22}H_{19}NOS$ requires 346.1260).

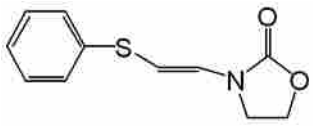


(*Z*)-3-(2-(butylthio)vinyl)oxazolidin-2-one (6a) Prepared from **2** (10 mg, 0.090 mmol) and *n*-butyl thiol (9.7 μL, 0.09 mmol) according to the procedure given in the Experimental Section for the synthesis of **4a**, with AIBN (7.4 mg, 0.045 mmol), and the reaction was stirred at 85 ˚C for 30 minutes. The crude product (1.3:1 **6a**:**7a**) was purified by silica gel column chromatography using gradient elution with ethyl acetate:hexanes (3:7) to afford **6a** (7.7 mg, 0.038 mmol, 42%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.59 (d, *J* = 8.5 Hz, 1H), 5.27 (d, *J* = 9.0 Hz, 1H), 4.41 (dd, *J* = 8.7, 6.7 Hz, 2H), 4.23 (dd, *J* = 8.7, 7.2 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 1.63-1.59 (m, 2H), 1.44-1.40 (m, 2H), 0.93 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.9, 127.8, 103.8, 62.4, 42.8, 34.4, 31.5, 22.0, 13.9; IR (film) ν_{max} 3027, 2956, 2925, 2871, 1760, 1653, 1617, 1558, 1540, 1506, 1479, 1407, 1326, 1267, 1205, 1078, 1031, 976, 929, 782, 751, 697, 670 cm^{-1}; HRMS (ESI) m/z 202.0901 (MH^+, $C_9H_{15}NO_2S$ requires 202.0896).

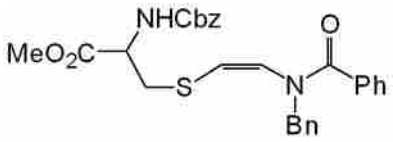


(*E*)-3-(2-(butylthio)vinyl)oxazolidin-2-one (7a) Prepared from **2** (10 mg, 0.090 mmol) and *n*-butyl thiol (19 μL, 0.18 mmol) according to the procedure given in the Experimental Section for the synthesis of **4a**, with AIBN (14 mg, 0.090 mmol), and the reaction was stirred at 85 ˚C for 30 minutes. The crude product (1:1.3 **7a**:**6a**) was purified by silica gel column chromatography

using gradient elution with ethyl acetate:hexanes (3:7) to afford **7a** (5.9 mg, 0.029 mmol, 33%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.97 (d, *J* = 13.5 Hz, 1H), 5.35 (d, *J* = 14.0 Hz, 1H), 4.46 (dd, *J* = 8.2, 8.0 Hz, 2H), 3.75 (dd, *J* = 9.0, 7.0 Hz, 2H), 2.62 (t, *J* = 7.5 Hz, 2H), 1.61-1.56 (m, 2H), 1.44-1.40 (m, 2H), 0.93 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.9, 123.3, 106.5, 62. 6, 45.0, 35.1, 31.9, 21. 7, 13.6; IR (film) ν_{max} 7058, 2956, 2923, 2871, 1996, 1755, 1628, 1558, 1506, 1479, 1399, 1371, 1332, 1274, 1231, 1070, 1035, 977, 847, 755, 667 cm^{-1}; HRMS (ESI) m/z 202.0899 (MH^+, $C_9H_{15}NO_2S$ requires 202.0896).

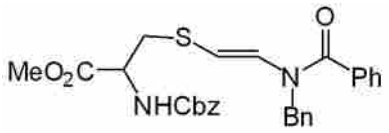


(*E*)-3-(2-(phenylthio)vinyl)oxazolidin-2-one (7c) Prepared from **2** (10 mg, 0.090 mmol) and phenyl thiol (18 μL, 0.18 mmol) according to the procedure given in the Experimental Section for the synthesis of **4a**, with AIBN (14 mg, 0.090 mmol), and the reaction was stirred at 85 ˚C for 30 minutes. The crude product was purified by silica gel column chromatography using gradient elution with ethyl acetate:hexanes (3:7) to afford **7c** (5.9 mg, 0.029 mmol, 71%) as a white powder: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36–7.28 (m, 4H), 7.28 (d, *J* = 14.5 Hz, 1H), 7.25–7.18 (m, 1H), 5.55 (d, *J* = 23 Hz, 1H), 4.52 (dd, *J* = 13.5, 13.5 Hz, 2H), 3.84 (dd, *J* = 15, 13.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.7, 137.0, 132.0, 129.0 (2C), 127. 8 (2C), 126. 1, 100.6, 62. 3, 42. 5; IR (film) ν_{max} 3068, 2922, 1748, 1623, 1582, 1510, 1479, 1439, 1418, 1329, 1279, 1210, 1085, 1031, 976, 926, 897, 877, 812, 779, 755, 741, 689, 669, 578, cm^{-1}; HRMS (ESI) m/z 222.0592 (MH^+, $C_{11}H_{11}NO_2S$ requires 222.0583).



(*E*)-Methyl 3-(2-(*N*-benzylbenzamido)vinylthio)-2-(benzyloxycarbonylamino)

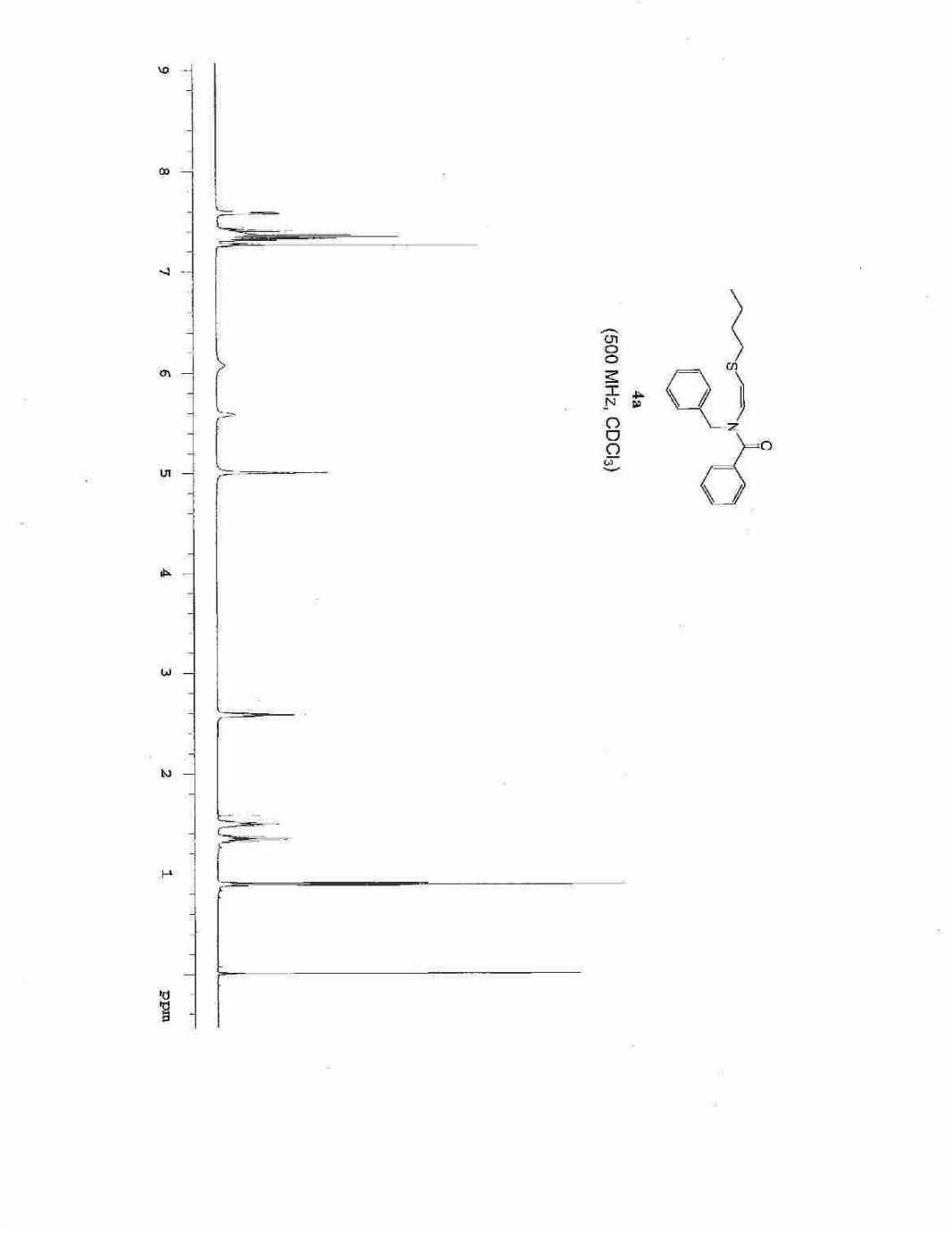
propanoate (9) Prepared from **3** (10 mg, 0.043 mmol) and **8** (13 mg, 0.043 mmol) according to the procedure given in the Experimental Section for the synthesis of **4a**, with AIBN (3.5 mg, 0.021 mmol), and the reaction was stirred at 85 ˚C for 10 minutes. The crude product (6.1:1 **9**:**10**) was purified by silica gel column chromatography (2.5% MeOH in CH_2Cl_2 gradient elution) to afford **9** (15 mg, 0.029 mmol, 68%) as a colorless oil: $[\alpha]^{25}_D$ –30 (c 0.5, EtOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.55 (d, *J* = 7 Hz, 2H), 7.41 (d, *J* = 7 Hz, 1H), 7.39–7.30 (m, 12H), 7.27 (d, *J* = 7 Hz, 1H), 6.03 (bs, 1H), 5.43 (d, *J* = 6 Hz, 1H), 5,34 (bs, 1H), 5.10 (bs, 2H), 4.94 (dd, *J* = 37, 15 Hz, 2H), 4.52–4.51 (m, 1H), 3.73 (s, 3H), 3.29–3.12 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.1, 170.6, 155.8, 136. 5, 136.3, 135.9, 134. 7, 131.1, 129.1 (2C), 128.9 (2C), 128.8 (2C), 128.6 (2C), 128.4 (2C), 128.2, 127.7 (2C), 126.9, 102.4, 67.4, 53.7, 52.7, 48.1, 37.6; IR (film) ν_{max} 3352, 3022, 2992, 2981, 1717, 1653, 1507, 1423, 1207, 1110, 1008, 950, 708, 655, 650 cm^{-1}; HRMS (ESI) m/z 505.1795 (MH^+, $C_{28}H_{28}N_2O_5S$ requires 505.1792).

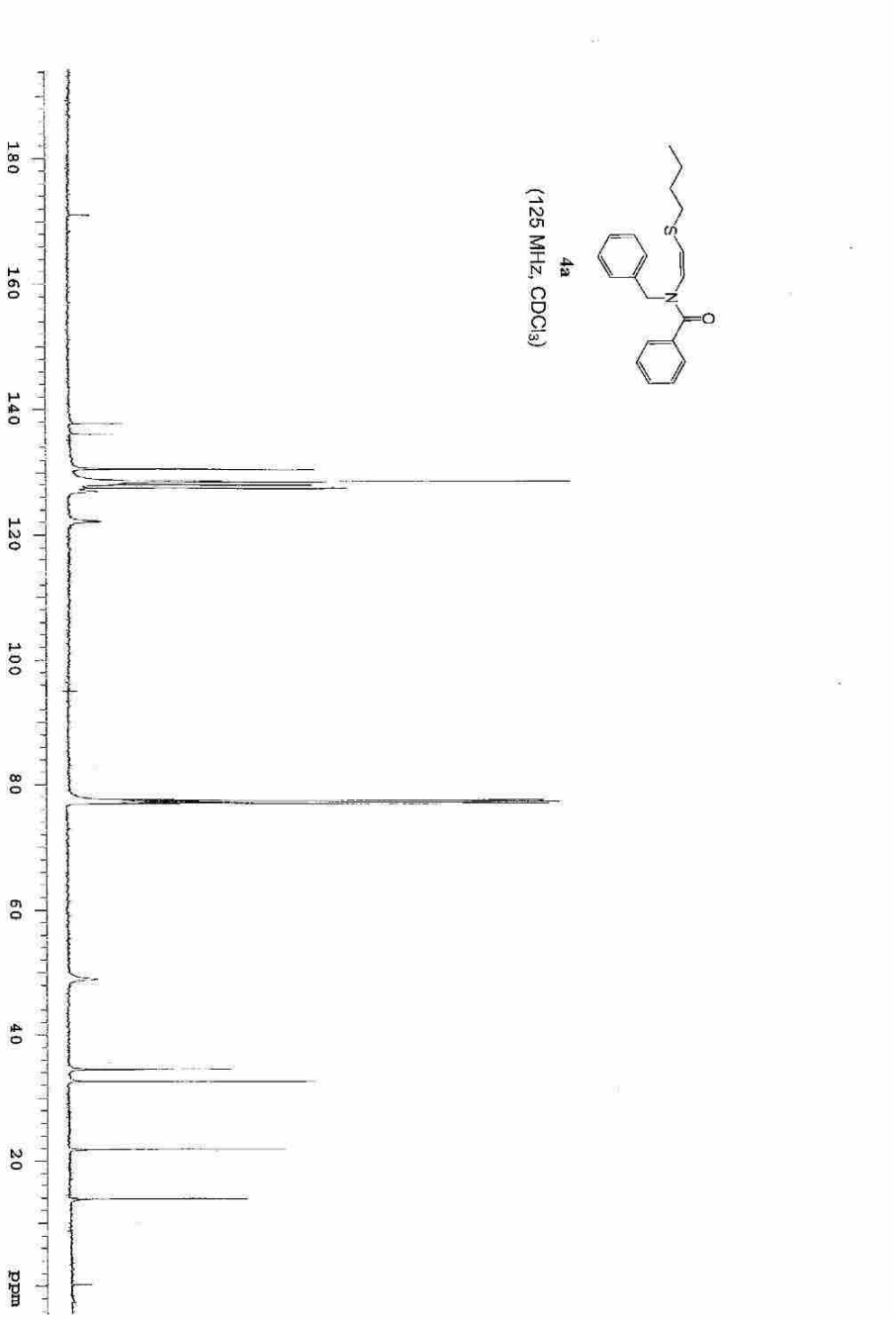


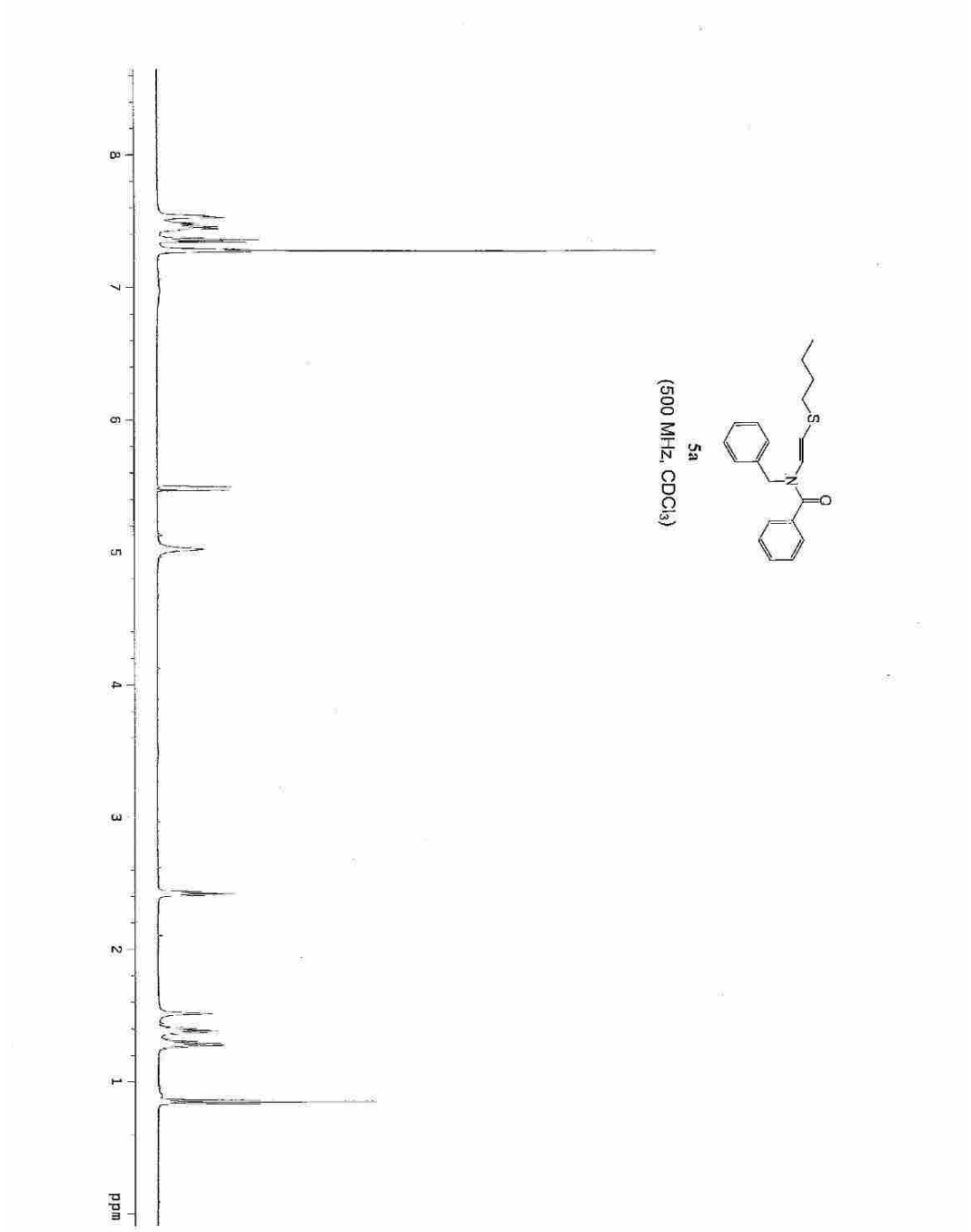
(*Z*)-Methyl 3-(2-(*N*-benzylbenzamido)vinylthio)-2-(benzyloxycarbonylamino) propanoate (10) Prepared from **3** (10 mg, 0.043 mmol) and **8** (16 mg, 0.086 mmol) according to the procedure given in the Experimental Section for the synthesis of **4a**, with AIBN (14 mg, 0.086 mmol), and the reaction was stirred at 85 ˚C for 3 hrs. The crude product (8.5:1 **10**:**9**) was purified by silica gel column chromatography (2.5% MeOH in CH_2Cl_2 gradient elution) to afford **10** (16 mg, 0.030 mmol, 69%) as a colorless oil: $[\alpha]^{25}_D$ –4.7 (c 0.15, EtOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ7.58–7.51 (m, 2H), 7.50–7.41 (m, 2H), 7.40–7.31 (m, 7H), 7.30–7.22 (m, 4H), 5.45 (bs, 1H), 5.42 (d, *J* = 13.5 Hz, 1H), 5.10 (dd, 7.58–7.51 (m, 2H), 7.50–7.41 (m, 2H), 7.40–7.31 (m, 7H), 7.30–

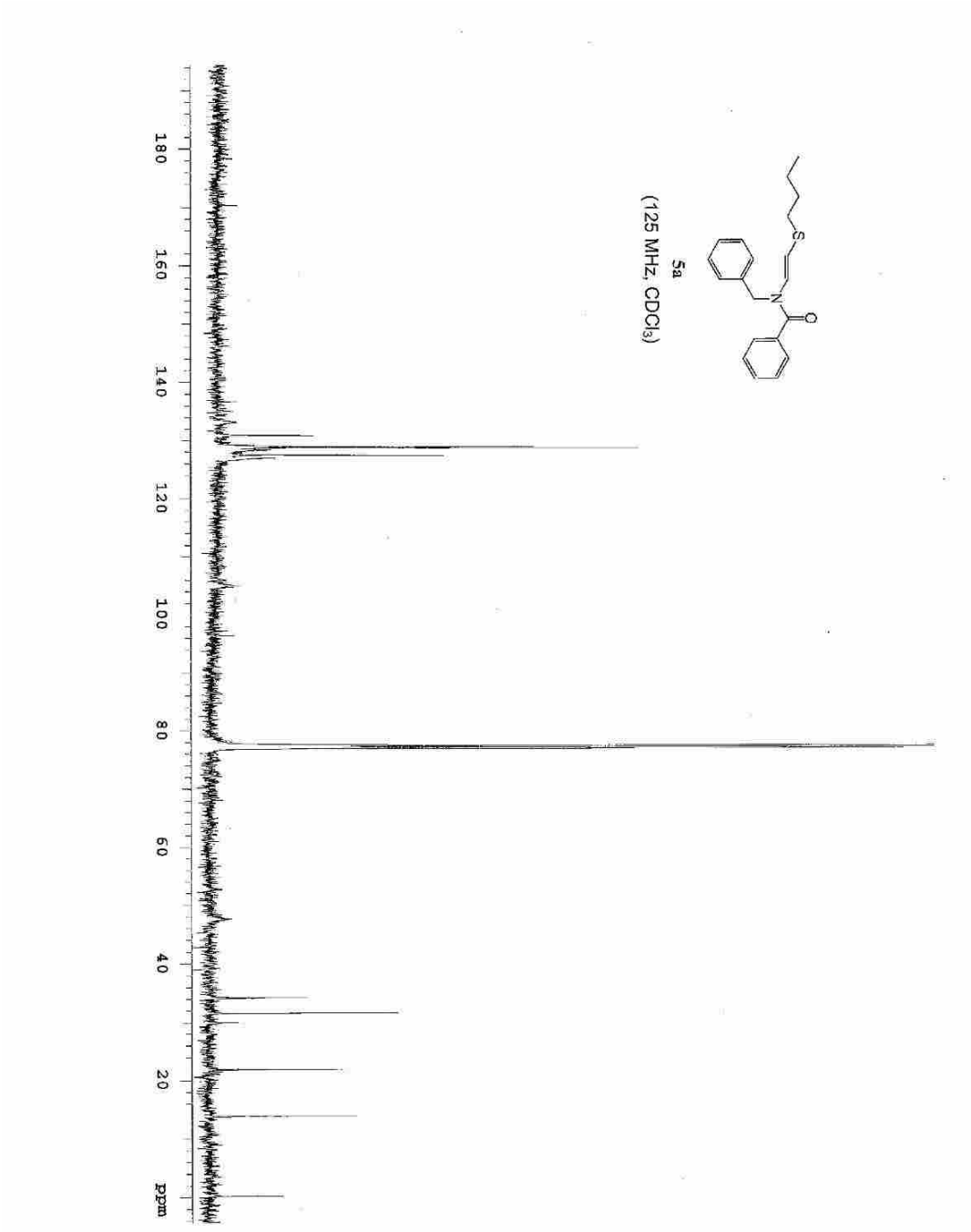
7.22 (m, 4H), 5.45 (bs, 1H), 5.42 (d, *J* = 37, 15 Hz, 2H), 4.97 (bs, 2H), 4.52–4.46 (m, 1H), 3,61 (s, 3H), 2.93–2.80 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.0, 170.4, 155.9, 137.3, 136.1, 130.5, 128.9, 128.8 (3C), 128.7, 128.6, 128.5 (2C), 128.5 (2C), 128.4 (2C), 128.2 (2C), 127.7, 120.3, 67. 5, 54.4, 53.5, 53.0, 41.4; IR (film) ν_{max} 3334, 3032, 2953, 1718, 1652, 1517, 1437, 1343, 1214, 1055, 912, 734, 698 cm^{-1}; HRMS (ESI) m/z 505.1798 (MH^+, $C_{28}H_{28}N_2O_5S$ requires 505.1792).

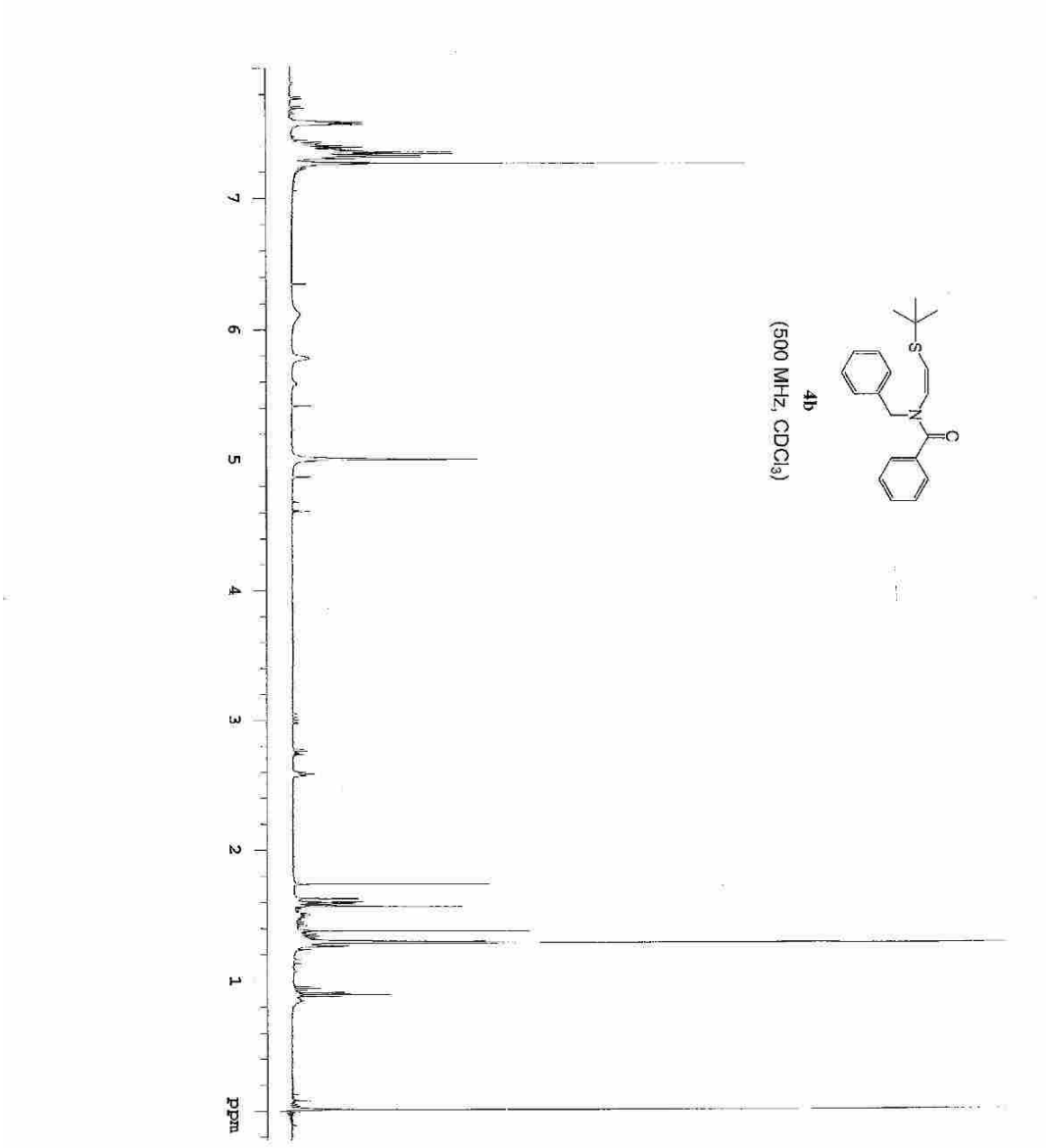
5.3 Selected NMR Spectra

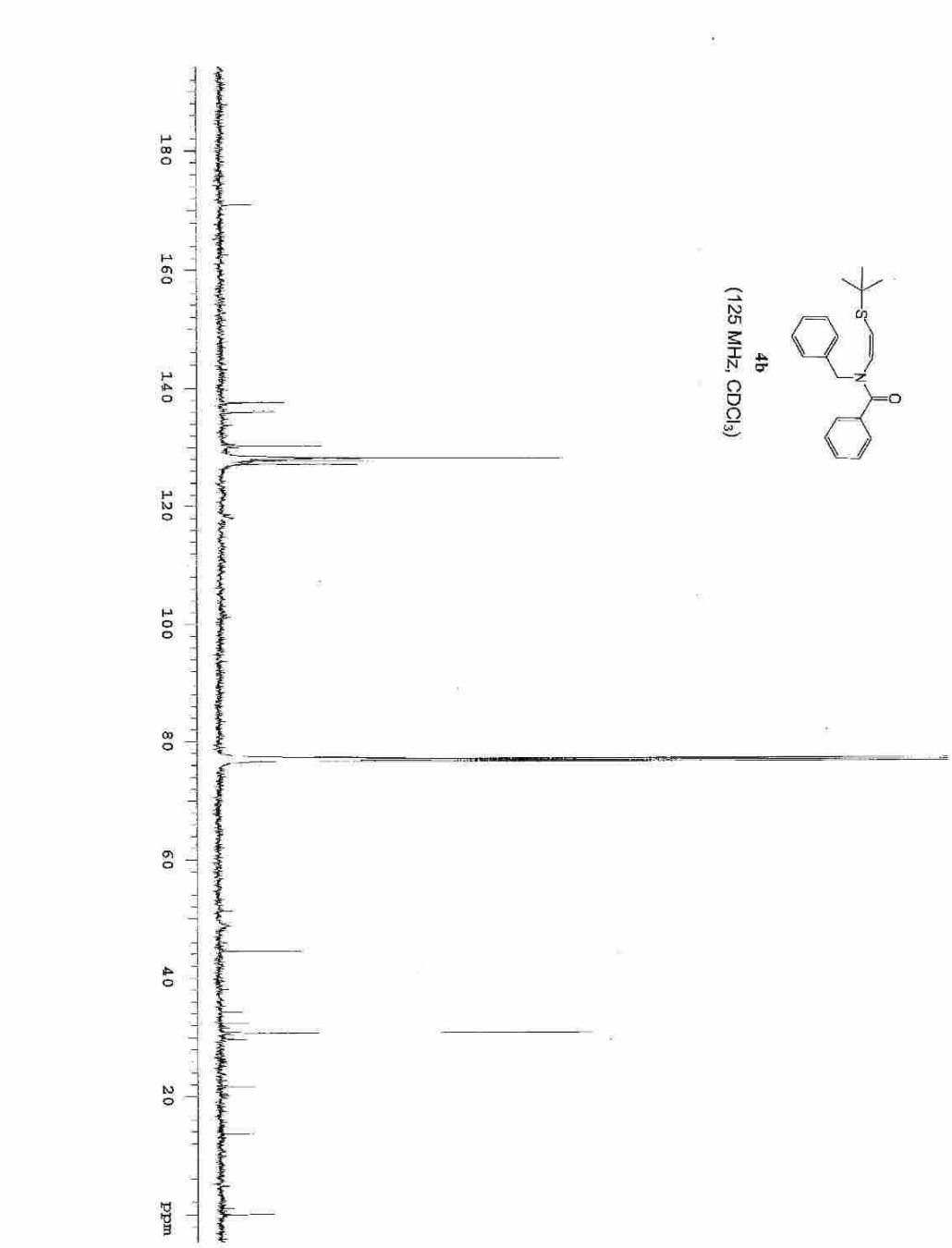


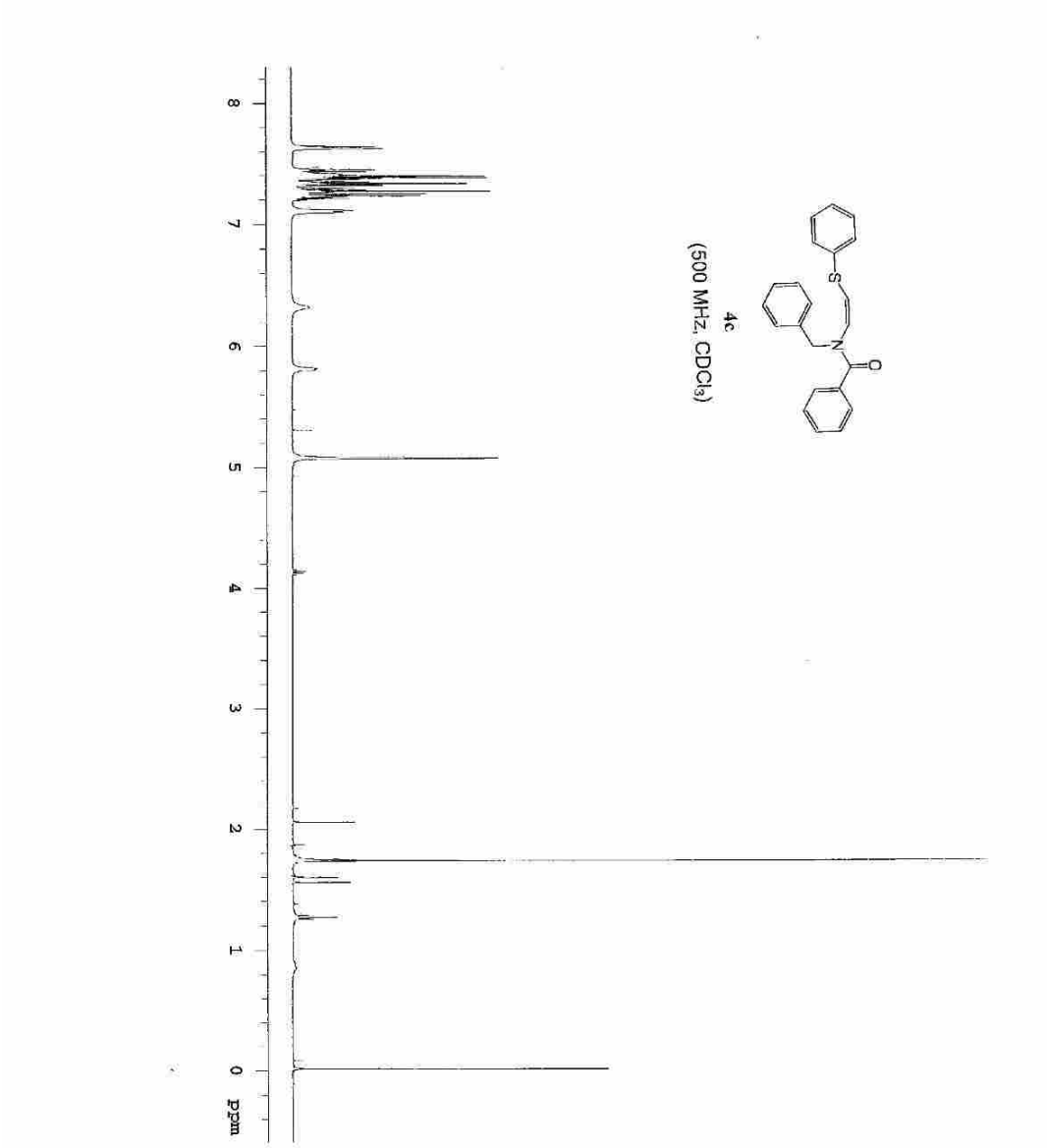

4a
(125 MHz, CDCl3)
S
N
O
180
160
140
120
100
80
60
40
20
ppm

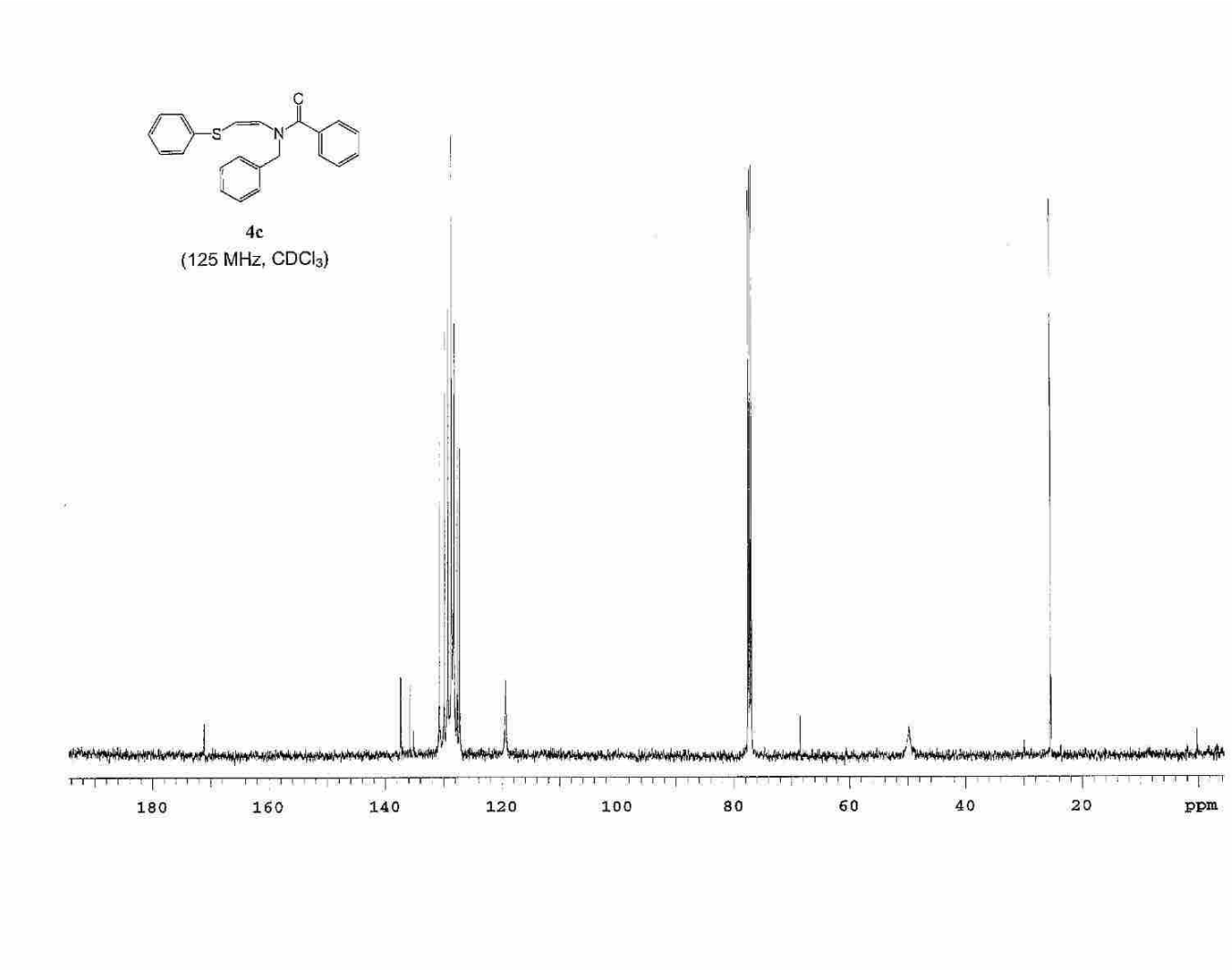

8
7
6
5
4
3
2
1
ppm
5a
(500 MHz, CDCl3)
S
N
O

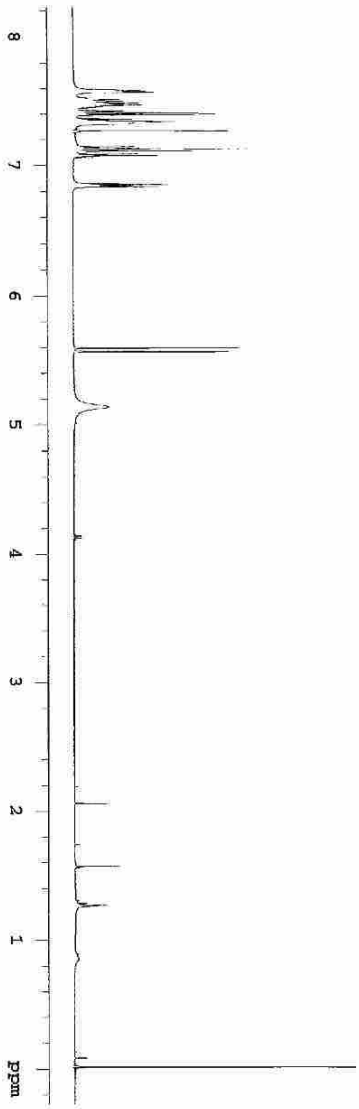

5a
(125 MHz, CDCl3)
180
160
140
120
100
80
60
40
20
ppm

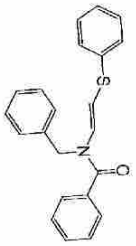

4b
(500 MHz, CDCl3)
S
N
O
7
6
5
4
3
2
1
ppm


4b
(125 MHz, CDCl3)
S
N
O
180
160
140
120
100
80
60
40
20
ppm

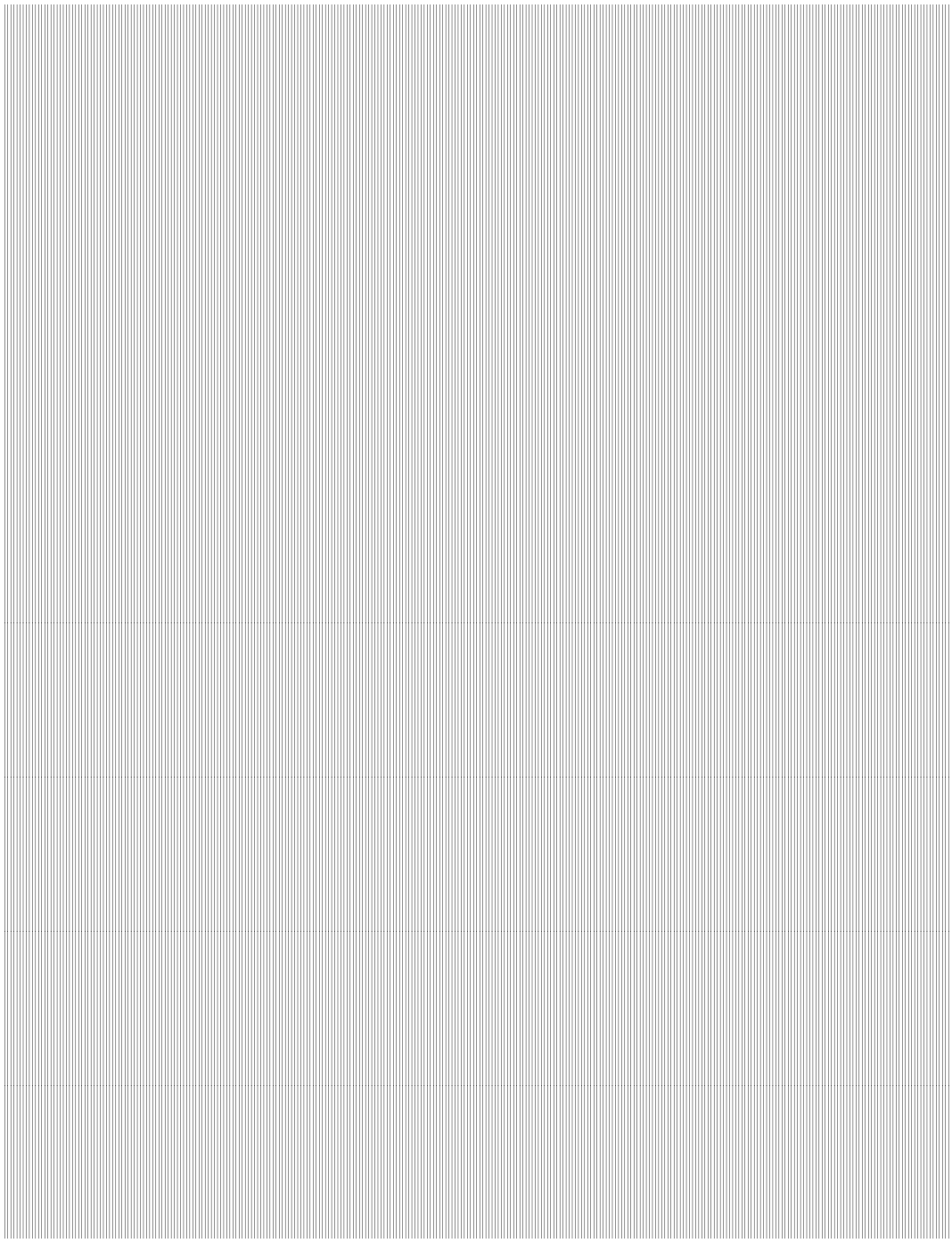

4c
(500 MHz, CDCl3)
S
N
O
8
7
6
5
4
3
2
1
0
ppm

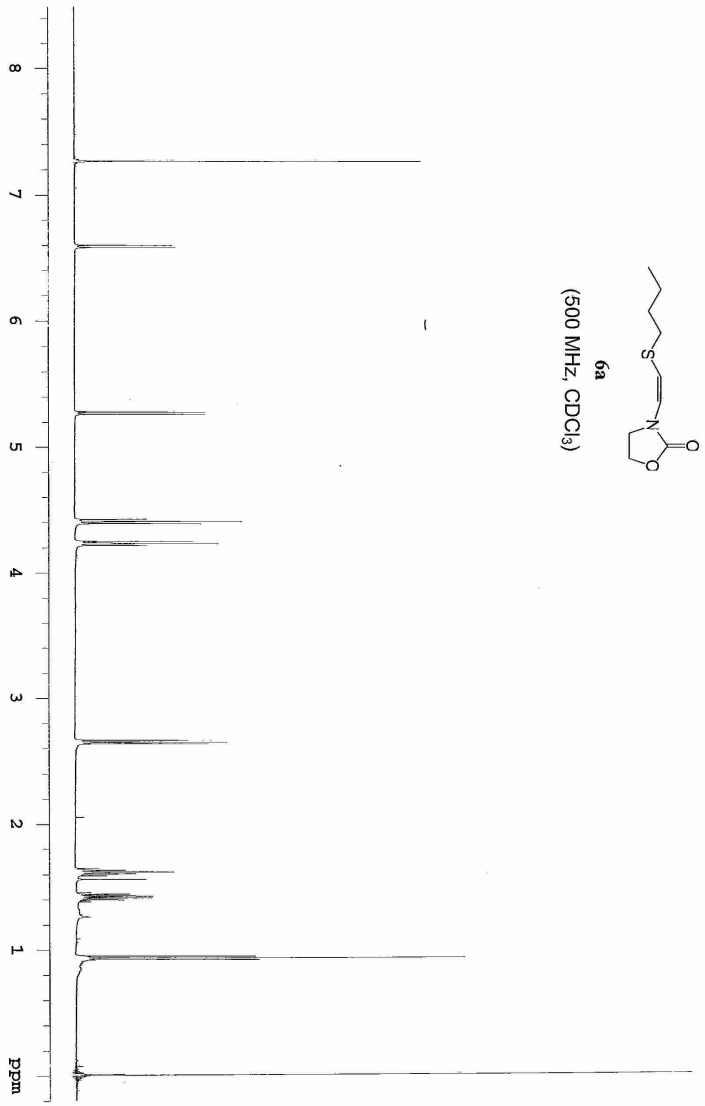

S
N
O
4c
(125 MHz, CDCl3)
180
160
140
120
100
80
60
40
20
ppm





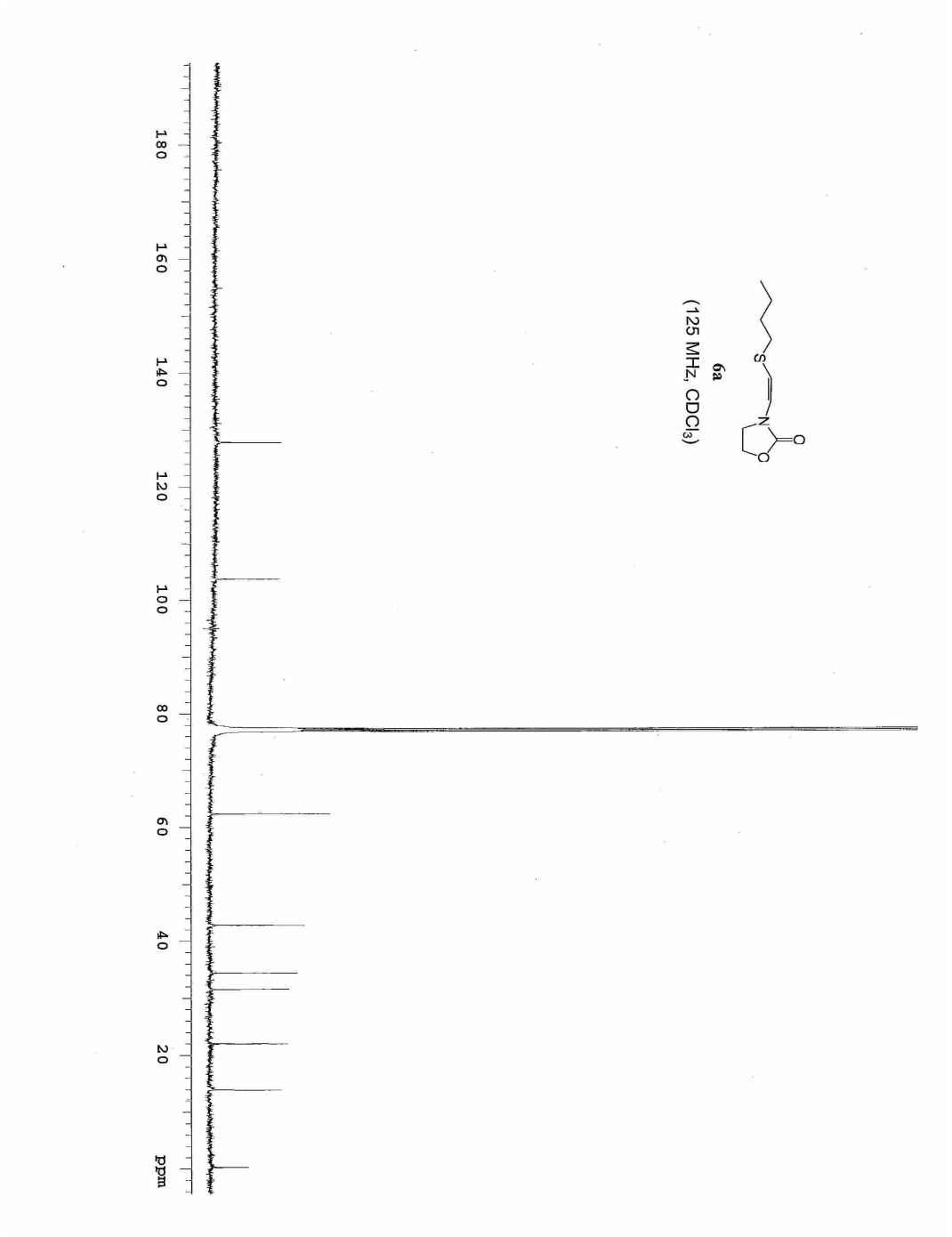
5c
(500 MHz, $CDCl_3$)

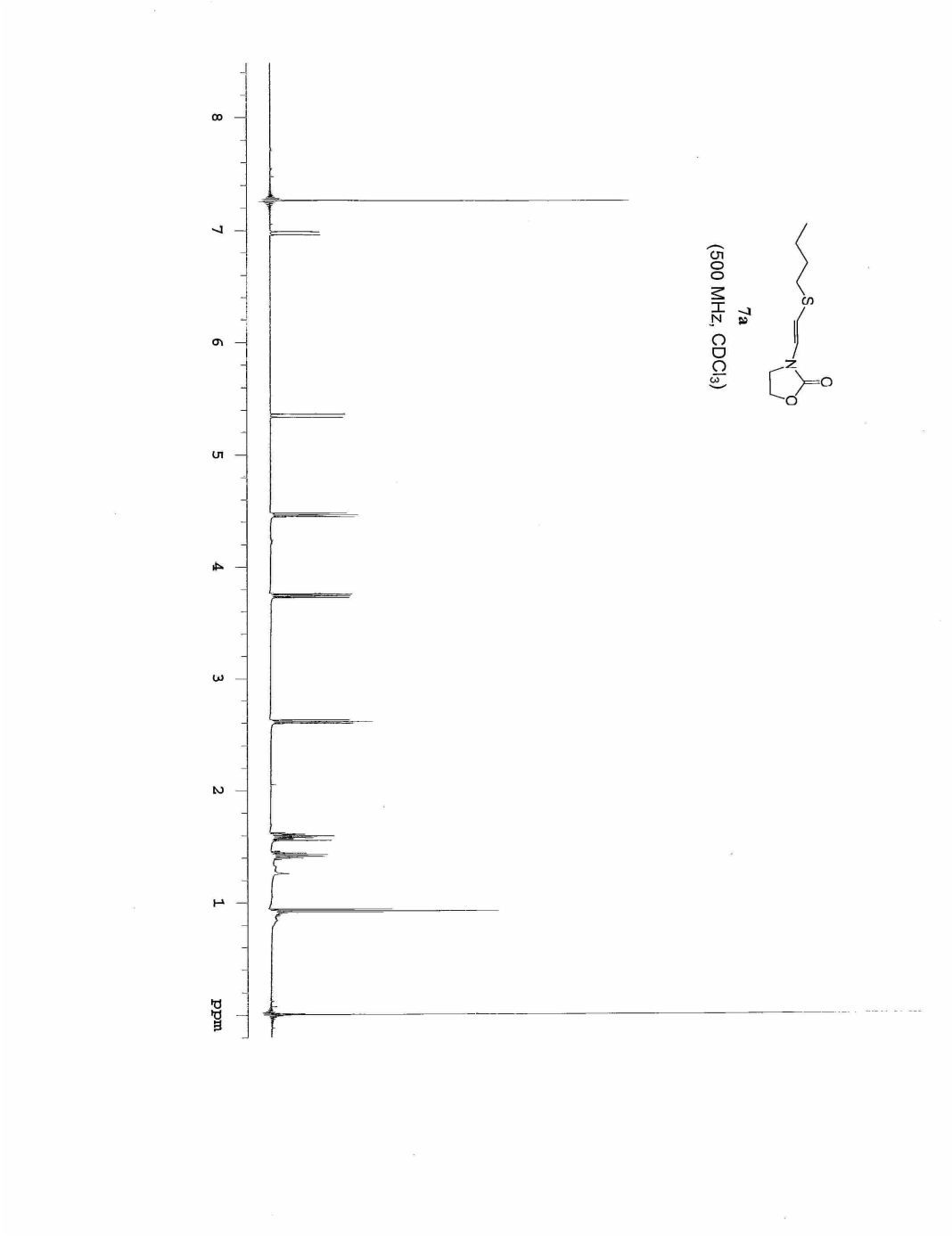


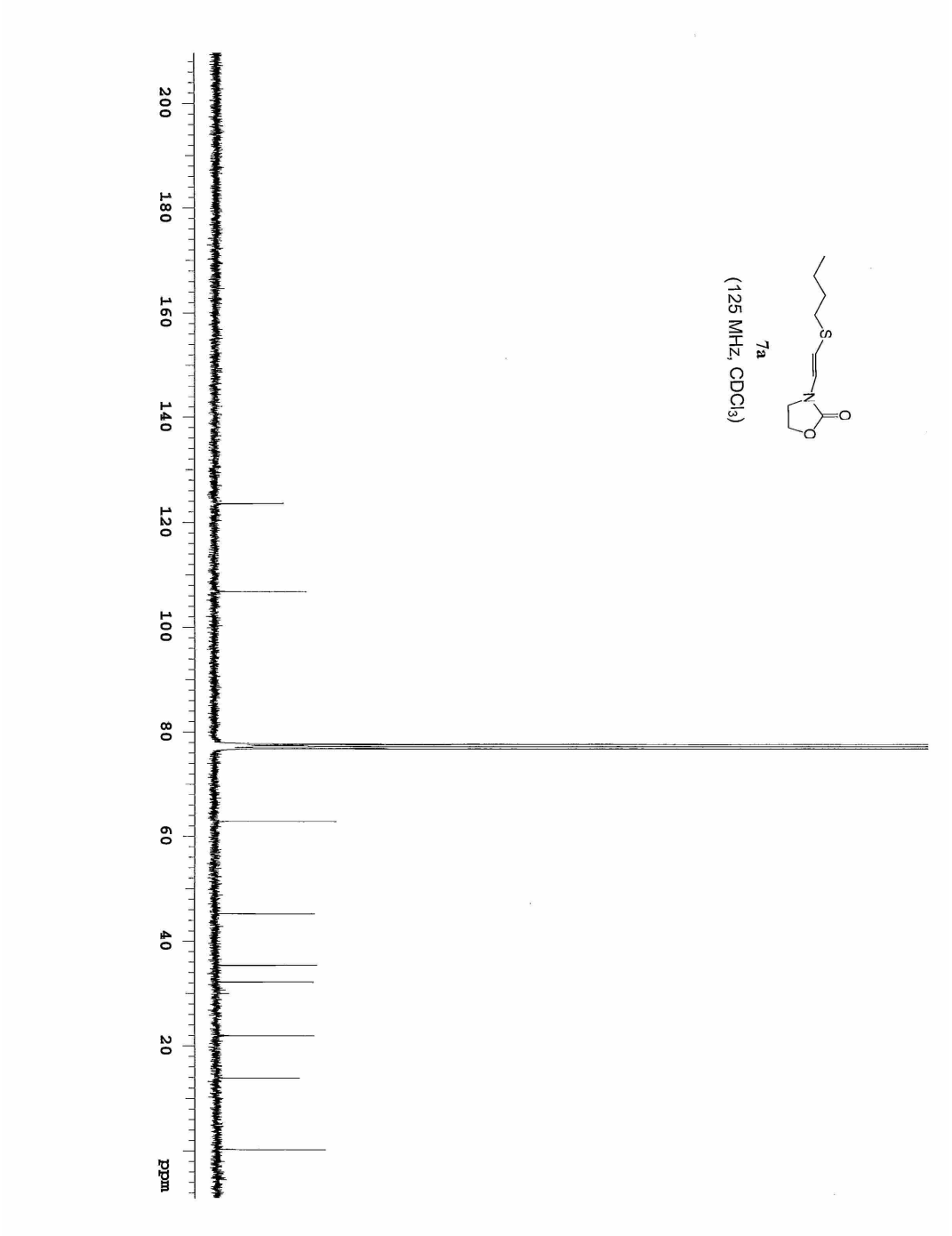


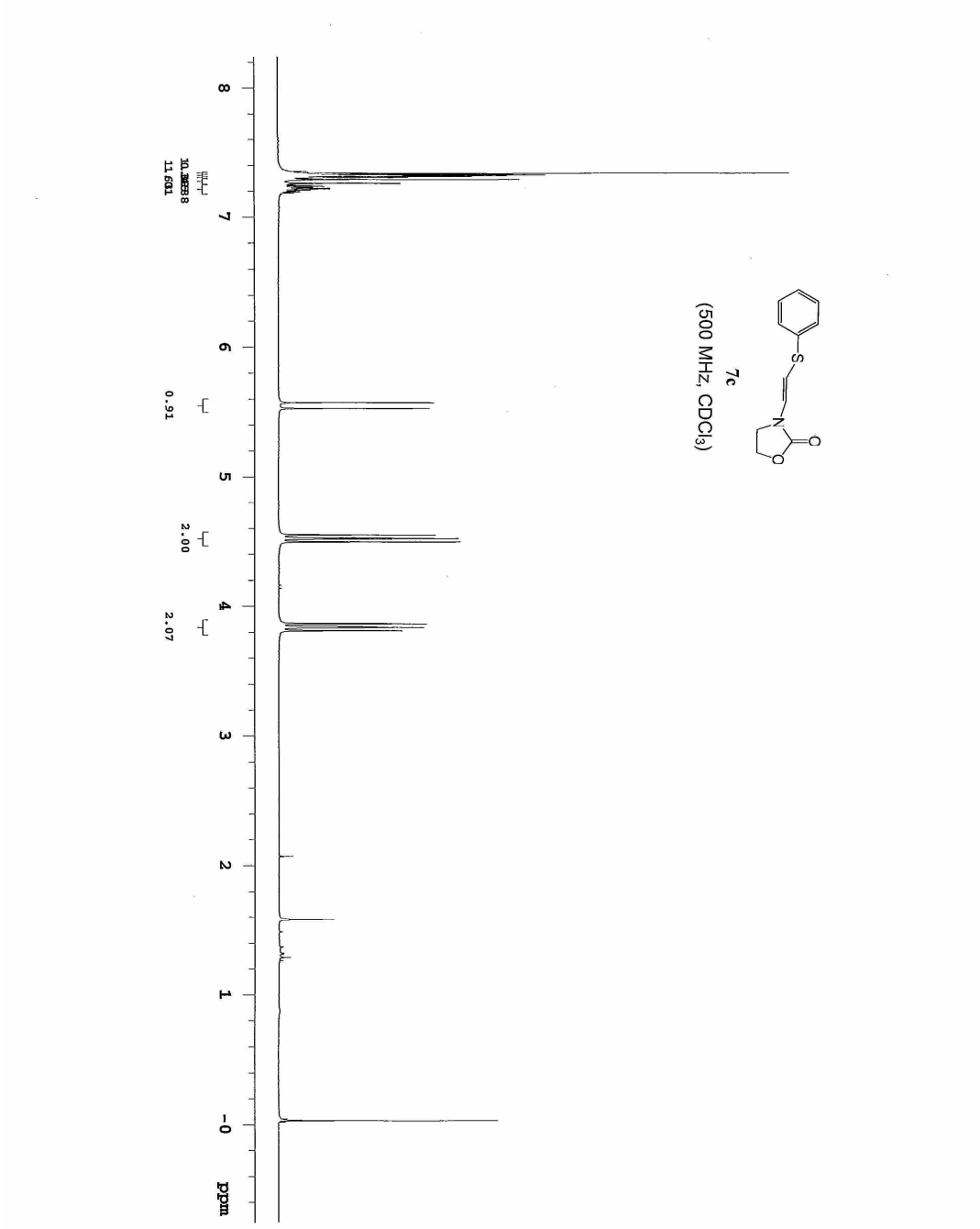
6a

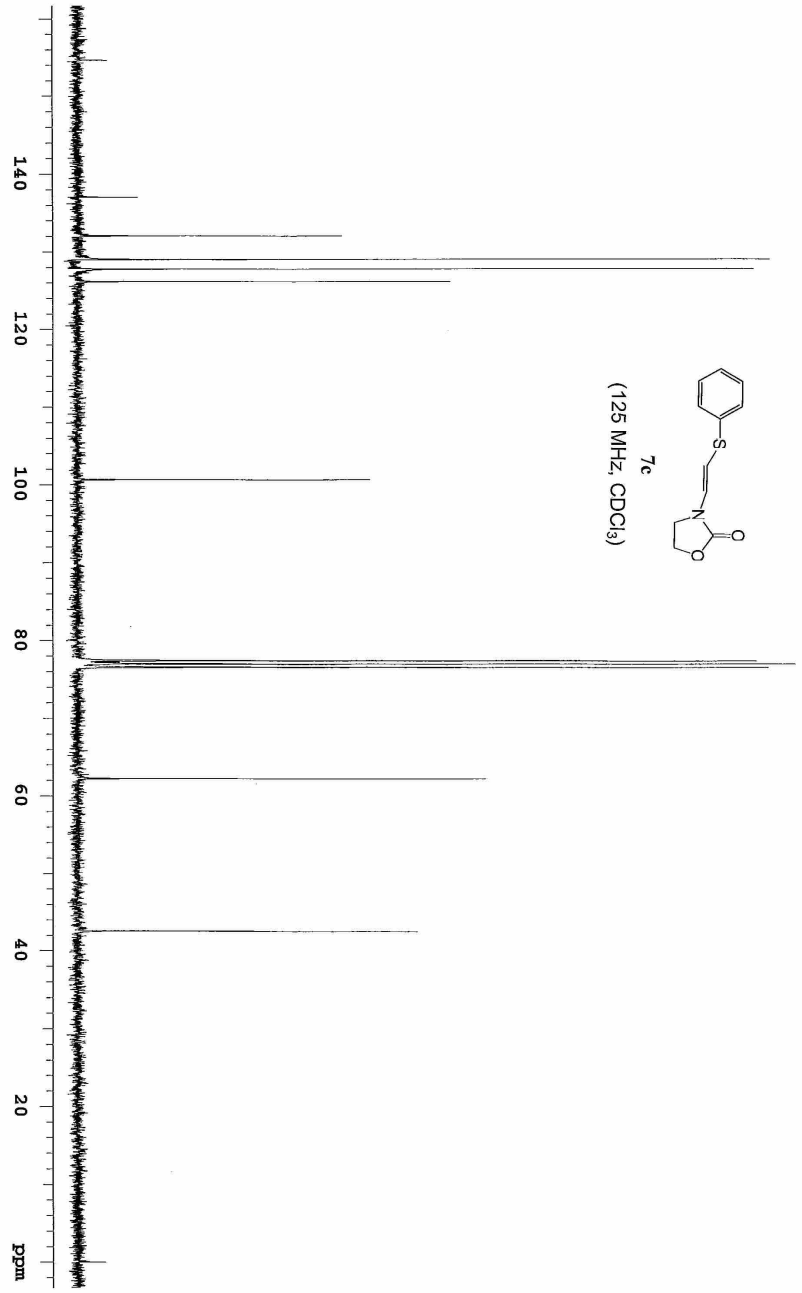
(500 MHz, $CDCl_3$)

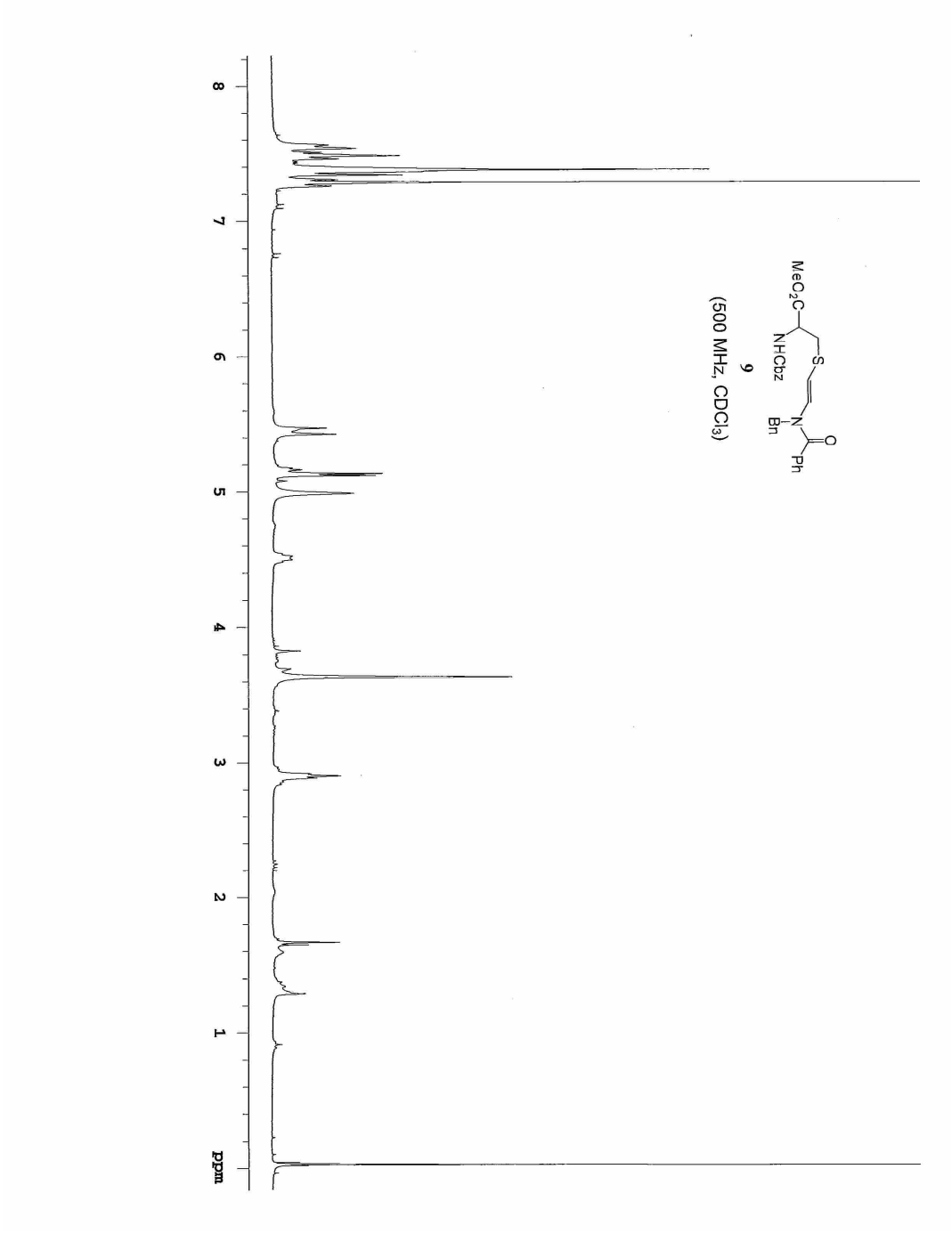

6a
(125 MHz, CDCl3)
S
N
O
O
180
160
140
120
100
80
60
40
20
ppm

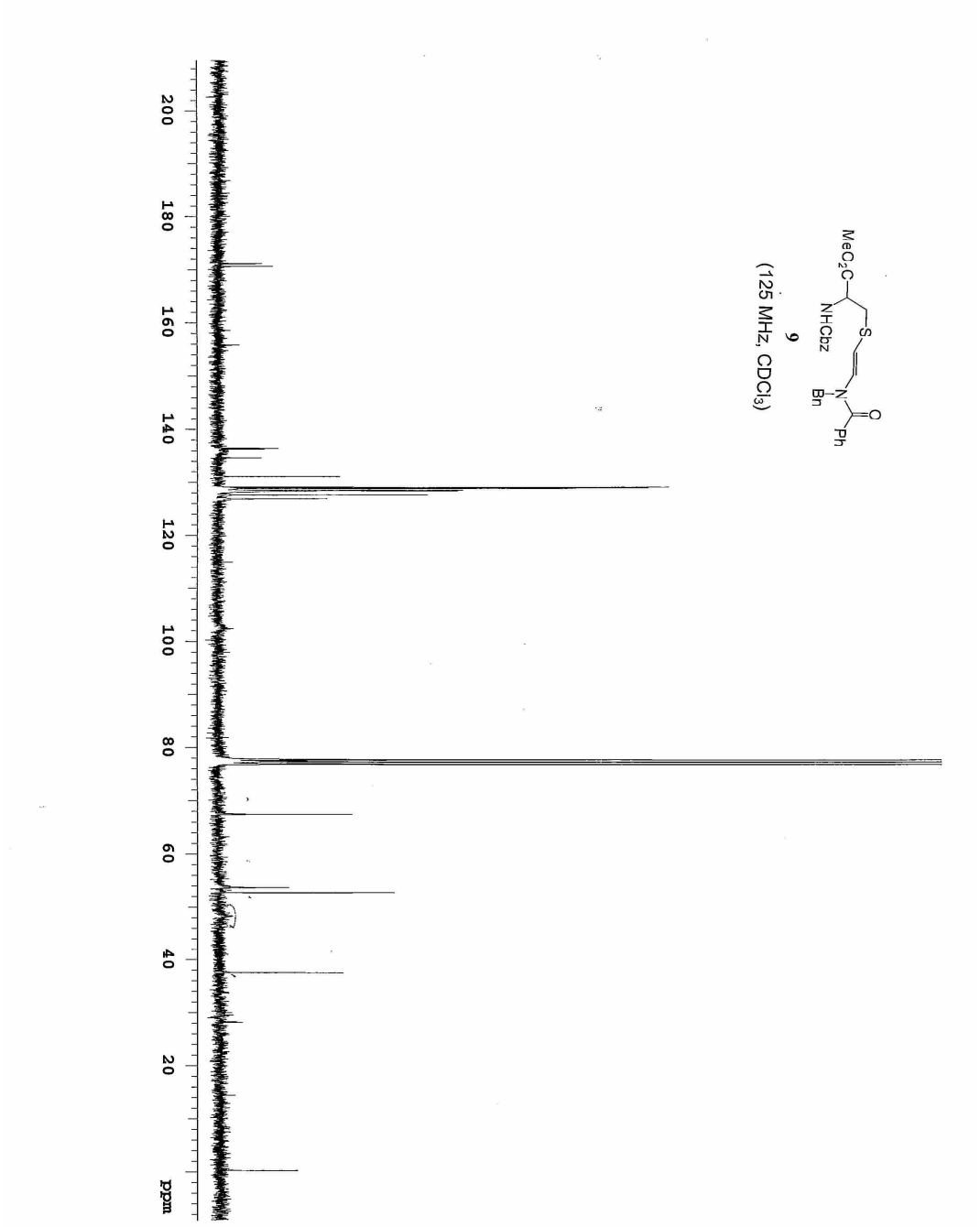

7a
(500 MHz, CDCl3)
S
N
O
O
8
7
6
5
4
3
2
1
ppm

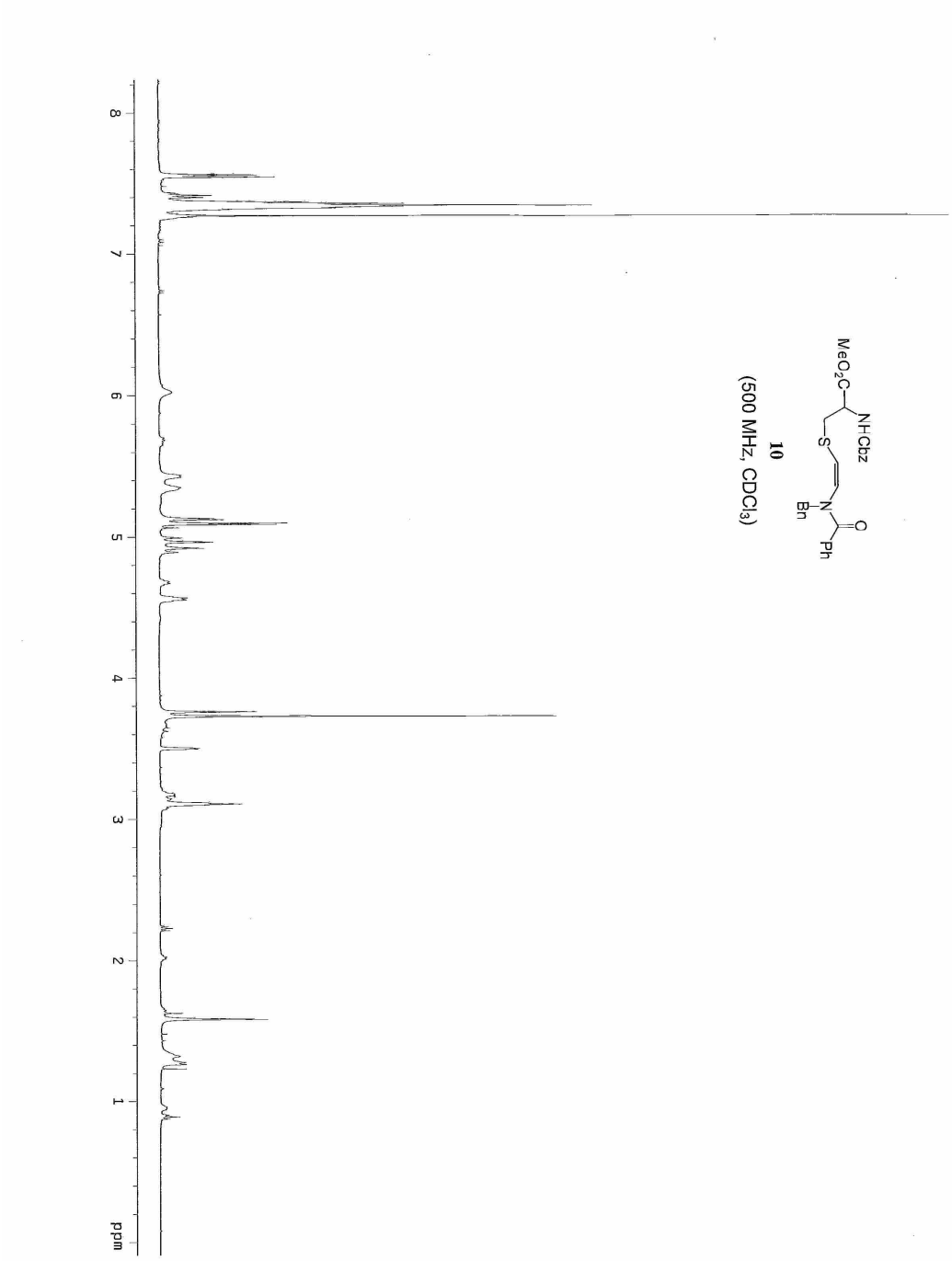

7a
(125 MHz, GDCl3)
S
N
O
O
200
180
160
140
120
100
80
60
40
20
ppm

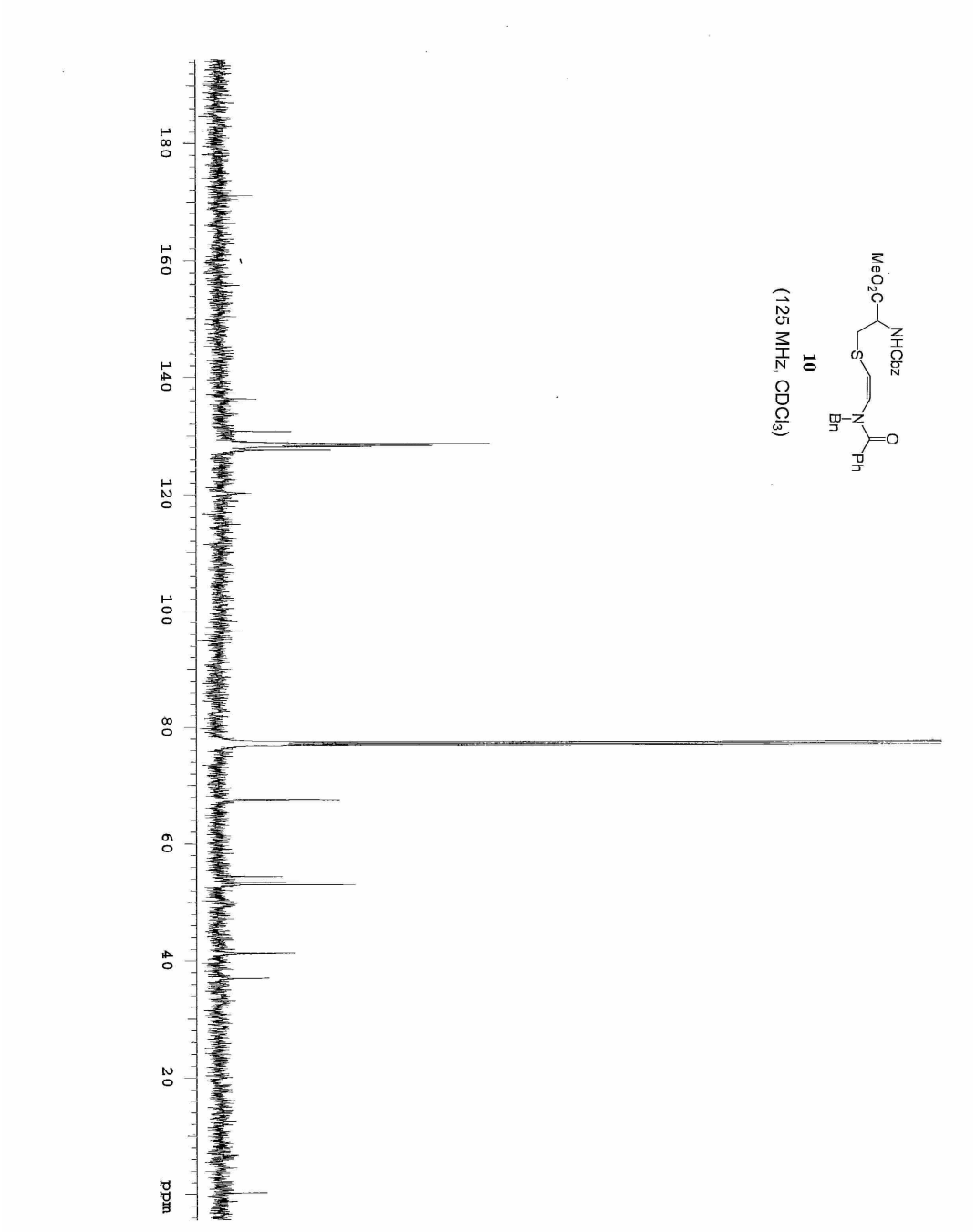

7c
(500 MHz, CDCl3)
S
N
O
O
8
7
6
5
4
3
2
1
-0
ppm
11.601
0.91
2.00
2.07


140
120
100
80
60
40
20
ppm
S
N
O
O
7c
(125 MHz, CDCl3)


MeC2C
NHCbz
S
N
Bn
O
Ph
9
(500 MHz, CDCl3)
8
7
6
5
4
3
2
1
ppm


MeO2C
NHCbz
S
N
Bn
O
Ph
9
(125 MHz, CDCl3)
200
180
160
140
120
100
80
60
40
20
ppm


MeO2C
NHCbz
S
N
Bn
O
Ph
10
(500 MHz, CDCl3)
8
7
6
5
4
3
2
1
ppm


MeO2C
NHCbz
S
N
Bn
O
Ph
10
(125 MHz, CDCl3)
180
160
140
120
100
80
60
40
20
ppm