Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Enzymatic Triggering of C—ON bond Homolysis of Alkoxyamines.

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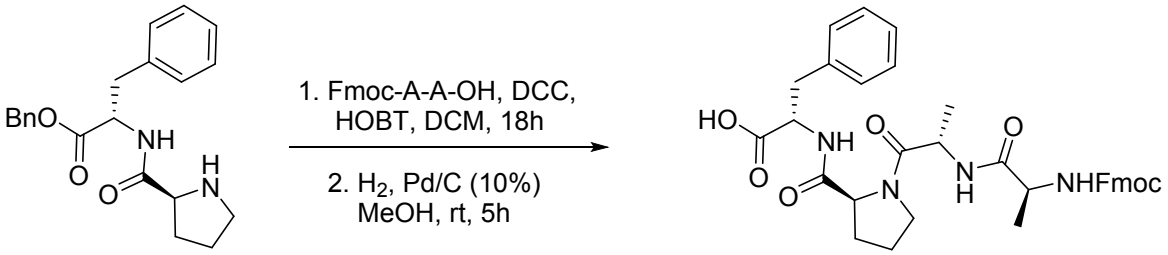
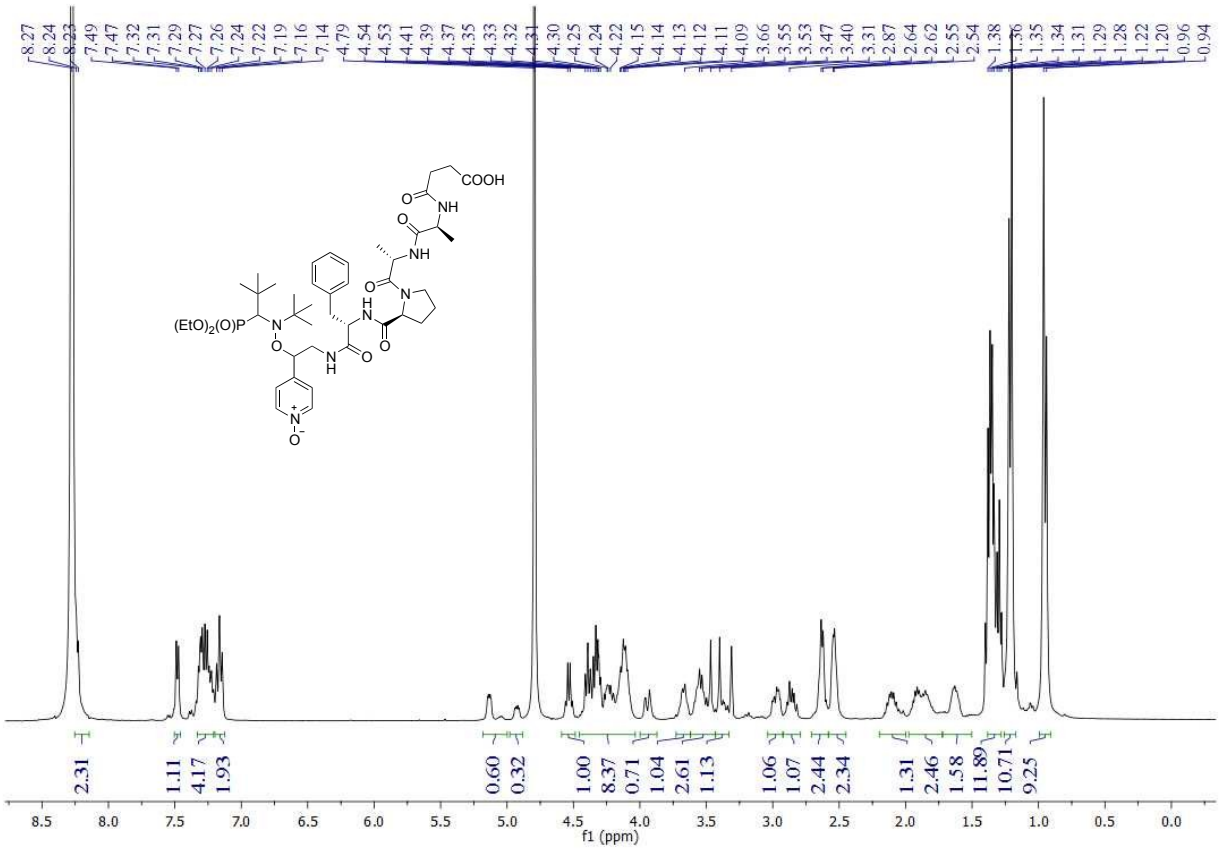
Experimental section



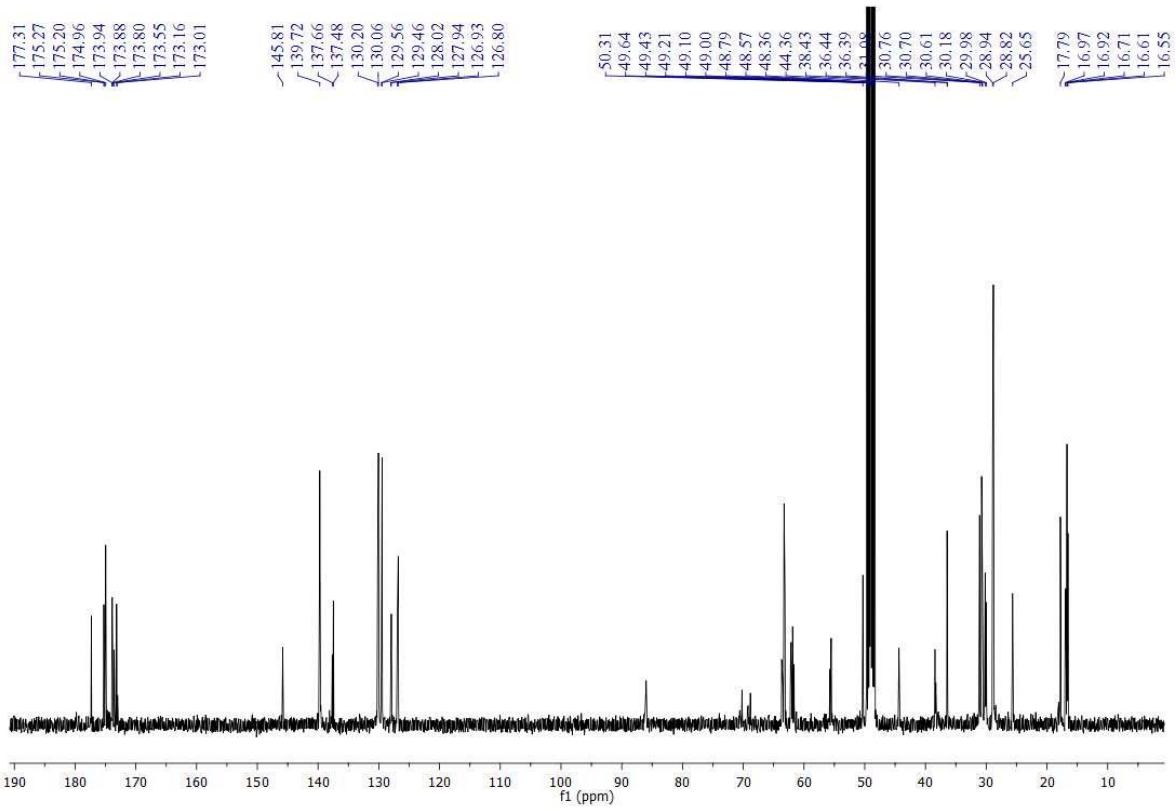
Scheme 1SI. Preparation of peptide **10**

NMR Analysis

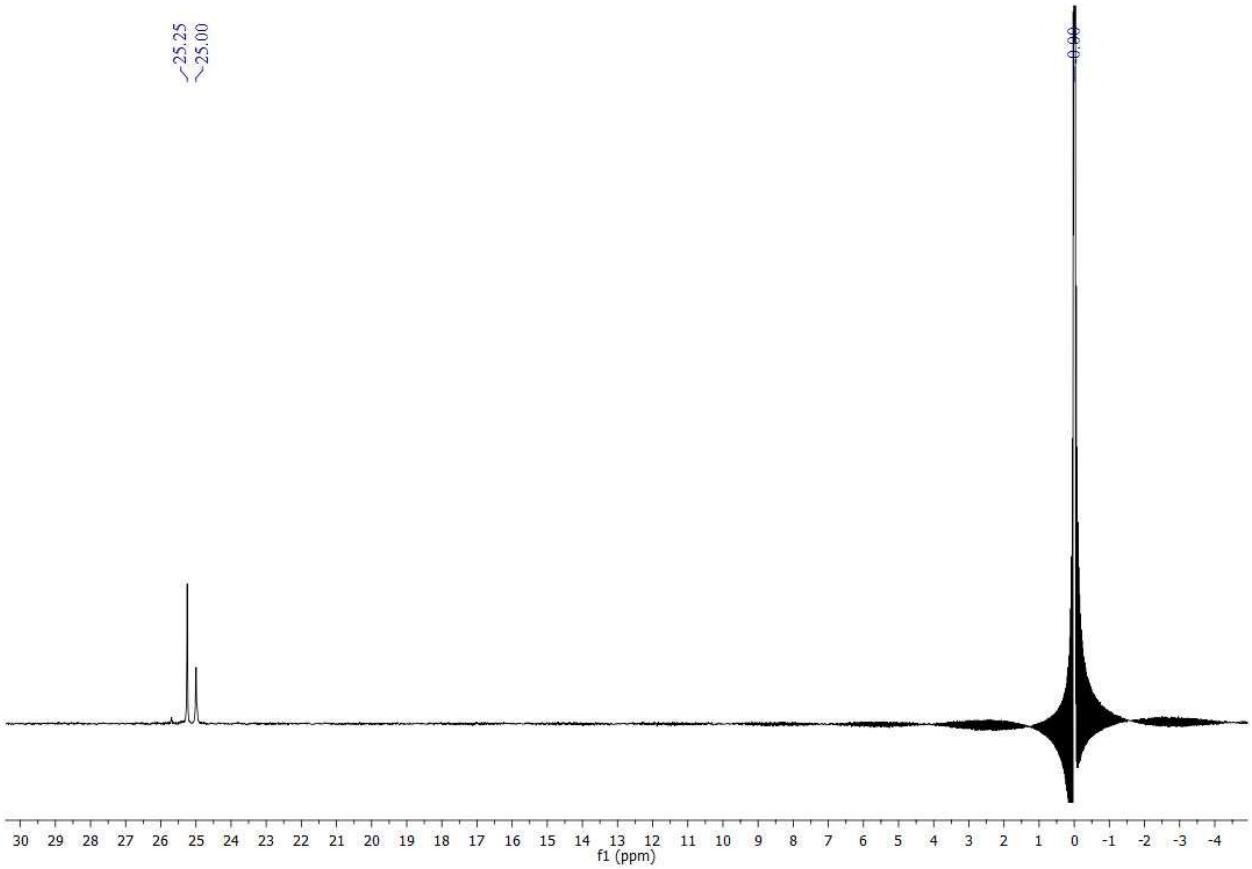
^{1}H NMR of 1



^{13}C NMR of 1.

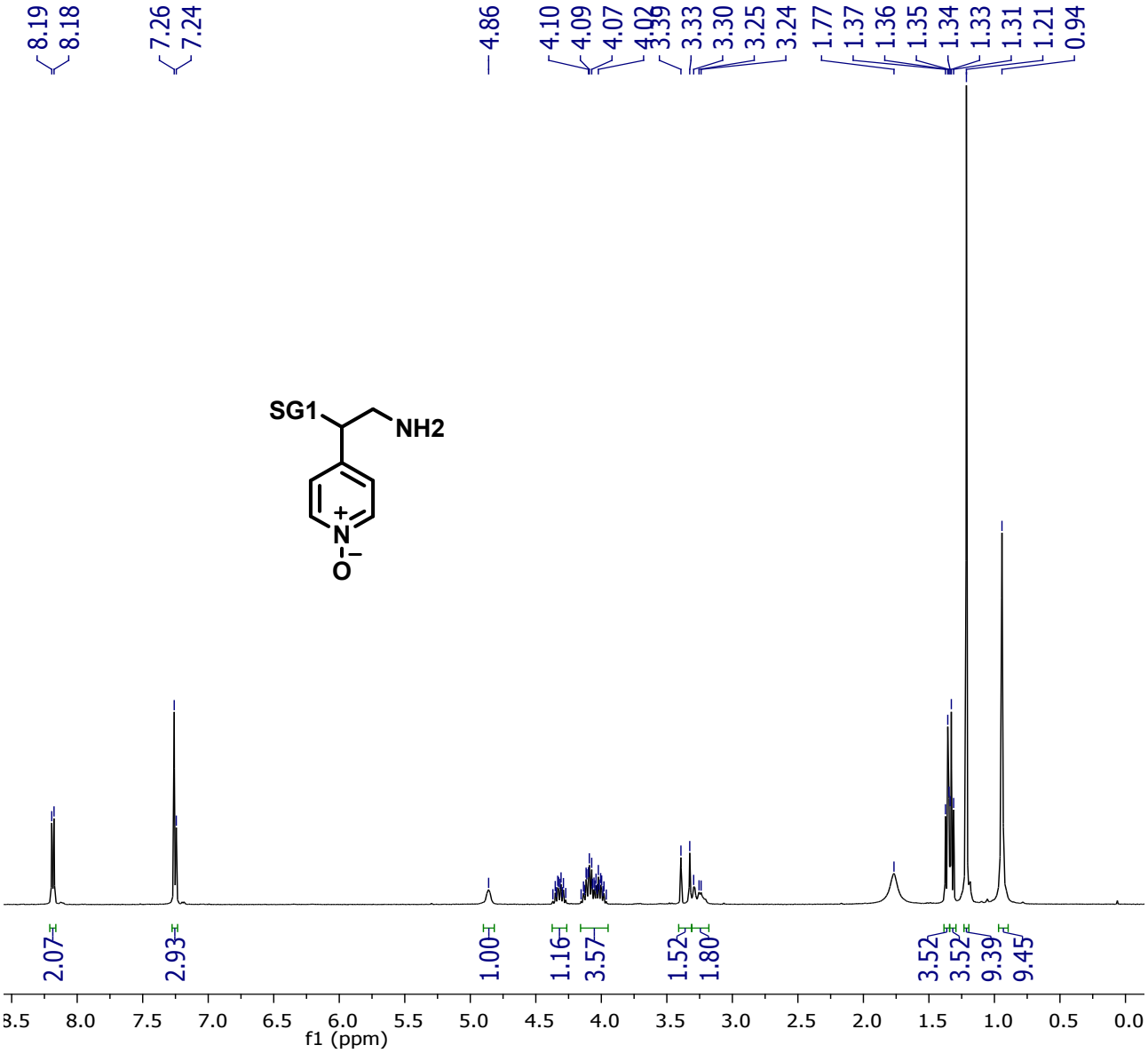


^{31}P NMR of 1

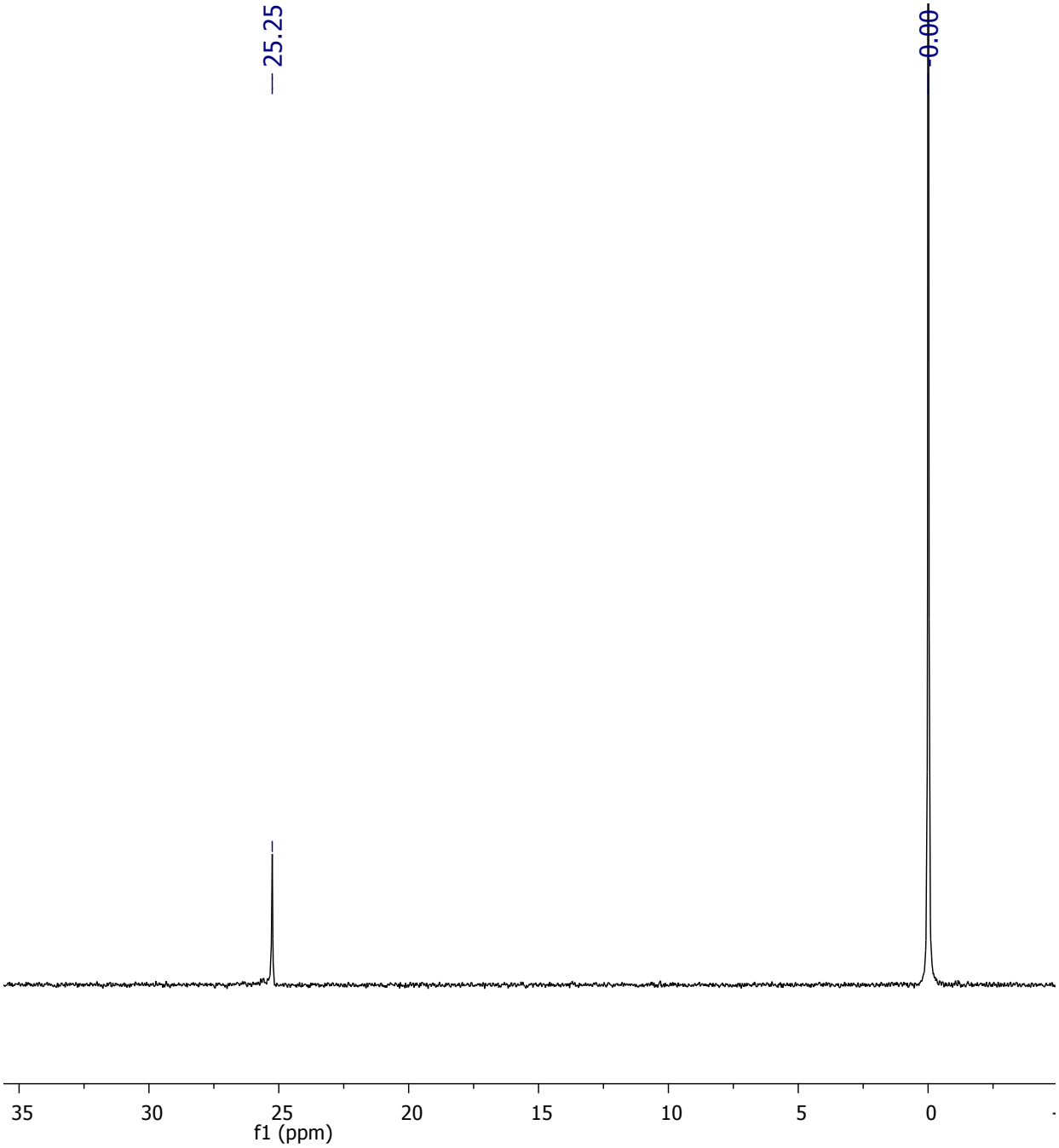

25.25
25.00
0.00
30 29 28 27 26 25 24 23 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)

NMR of *RS/SR*-2

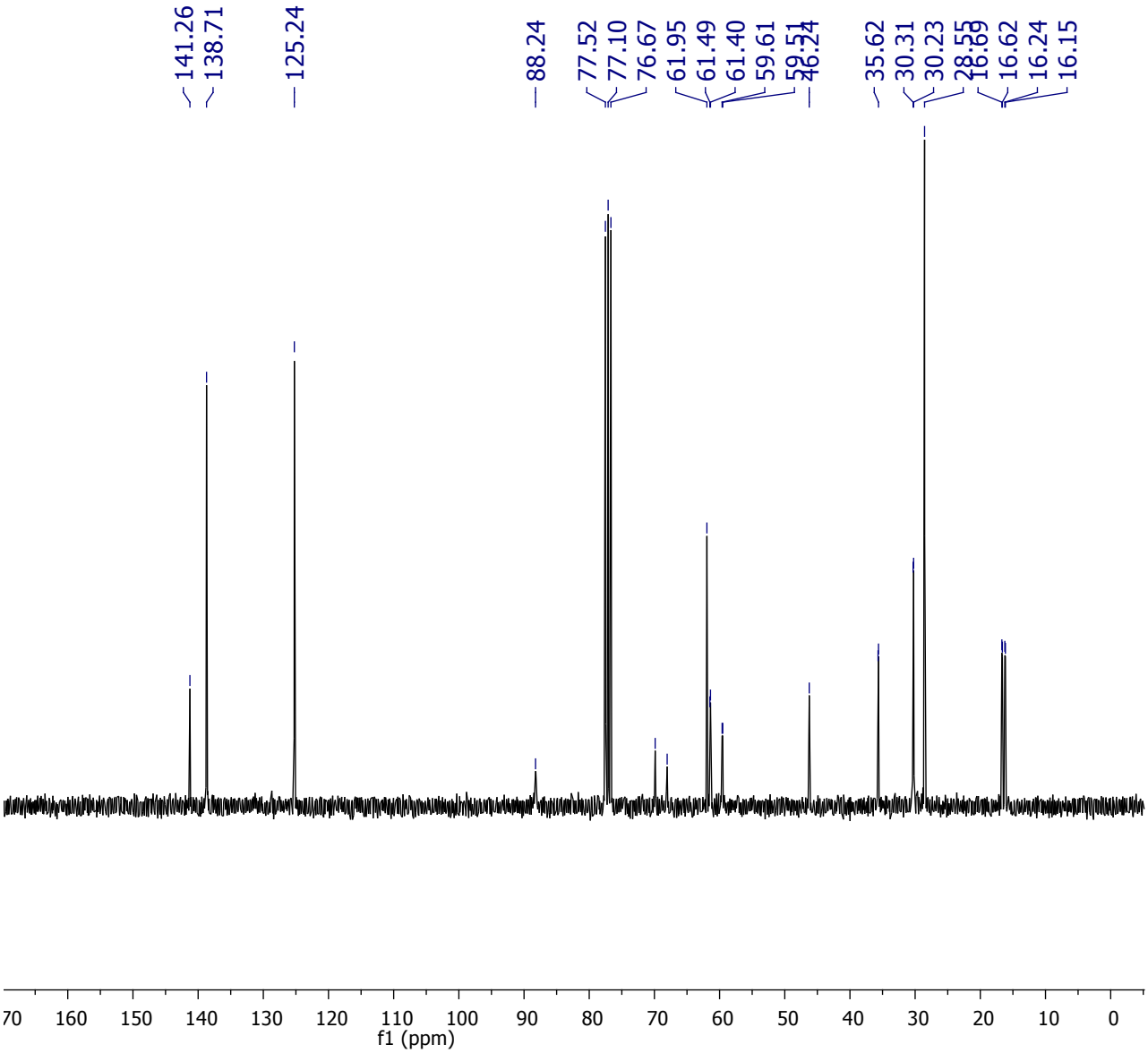
^{1}H NMR Spectrum ($CDCl_3$)



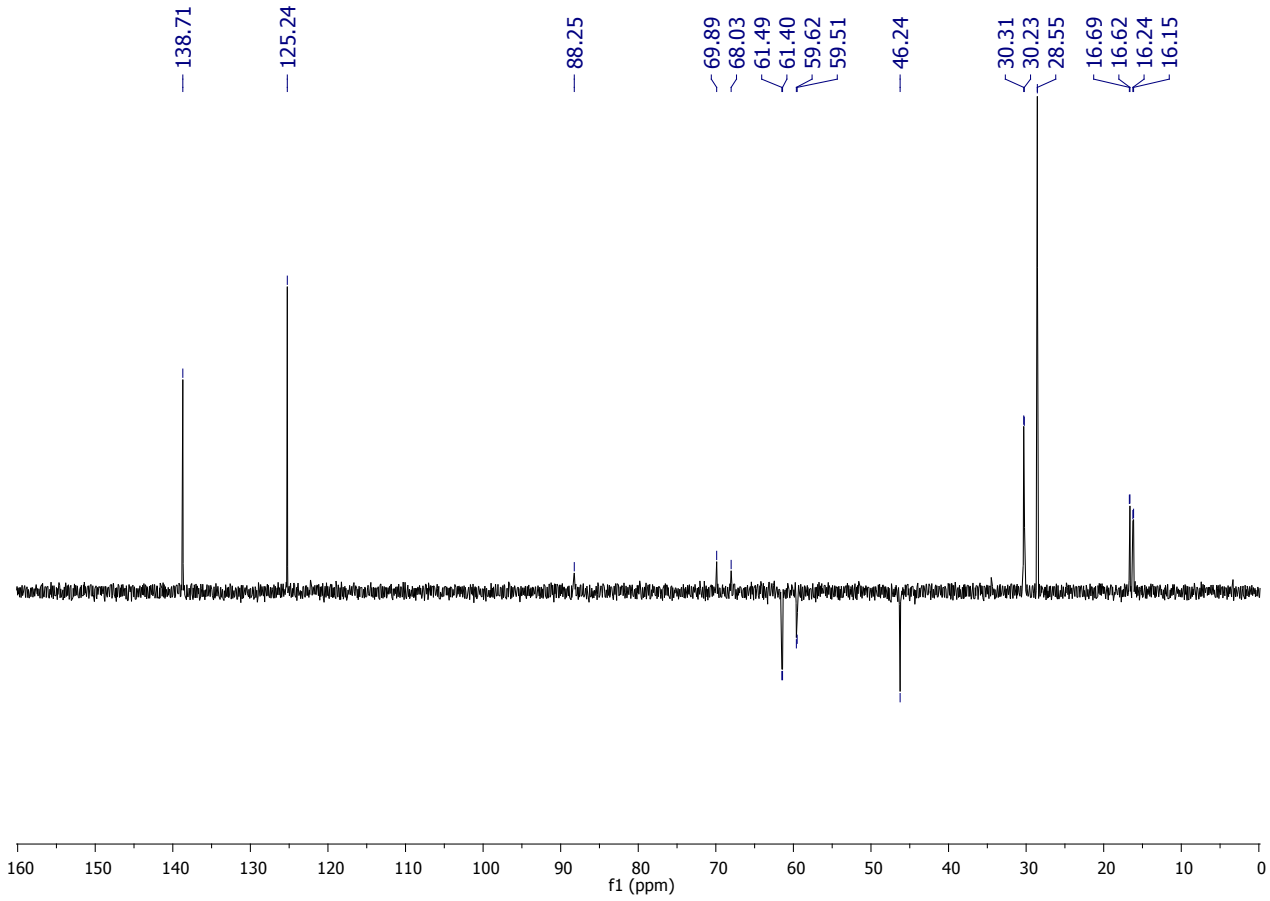
^{31}P NMR Spectrum ($CDCl_3$)



^{13}C {^{1}H} NMR Spectrum ($CDCl_3$)

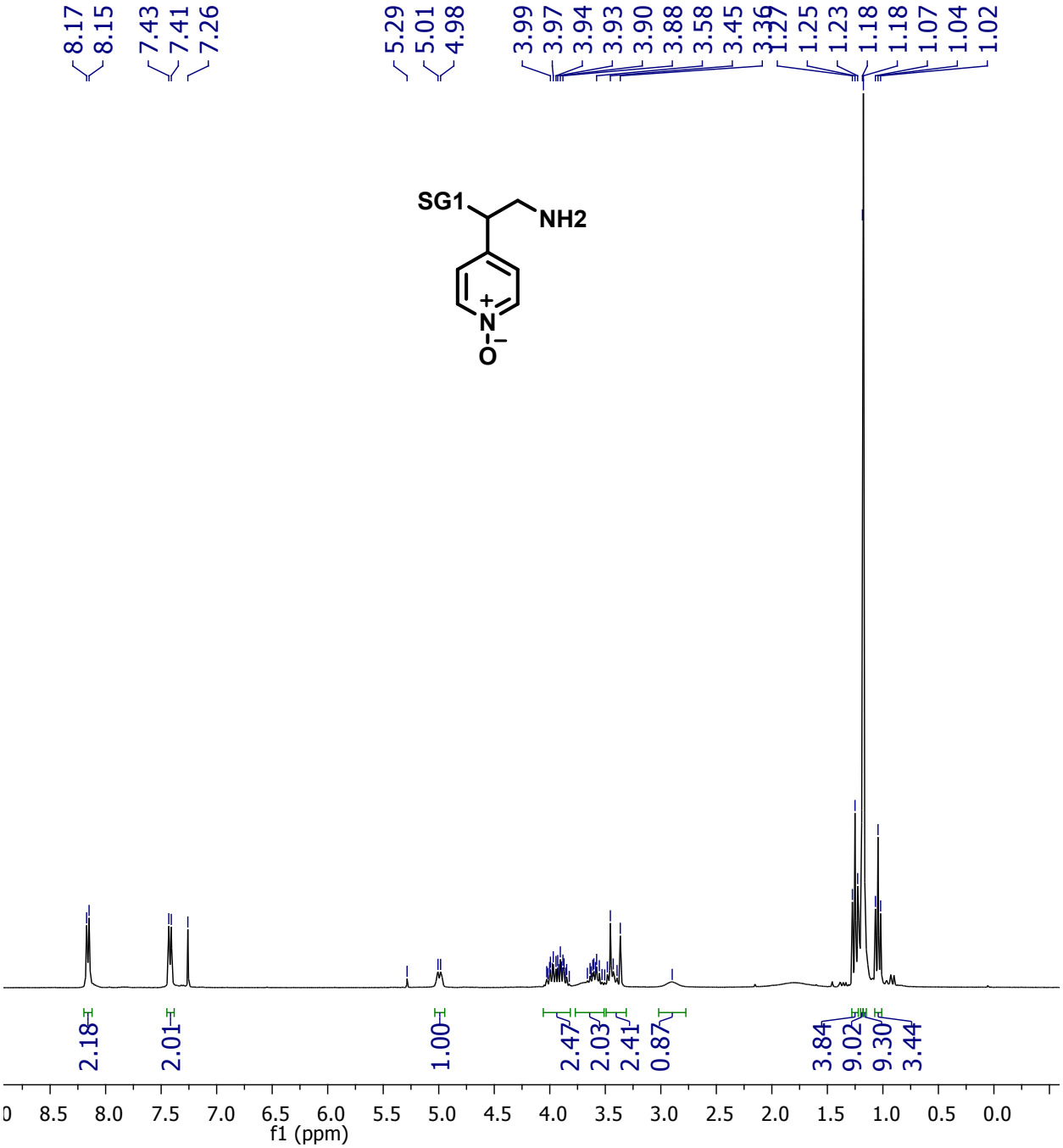


^{13}C DEPT 135 NMR Spectrum ($CDCl_3$)

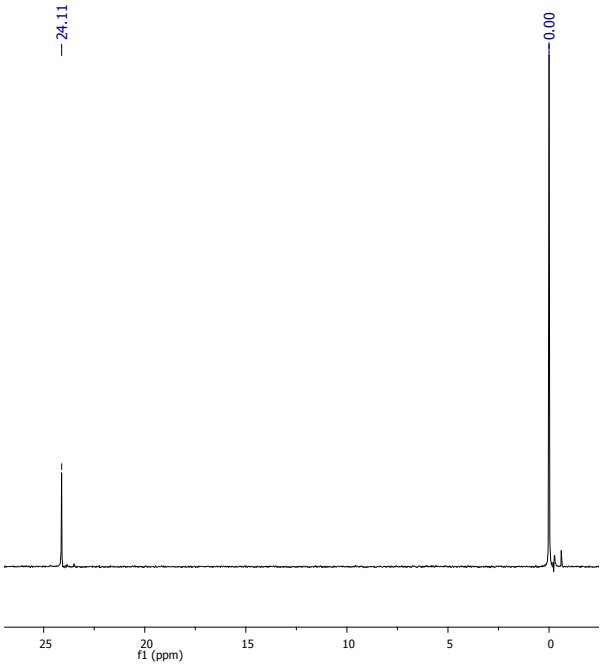


NMR of *RR/SS*-2

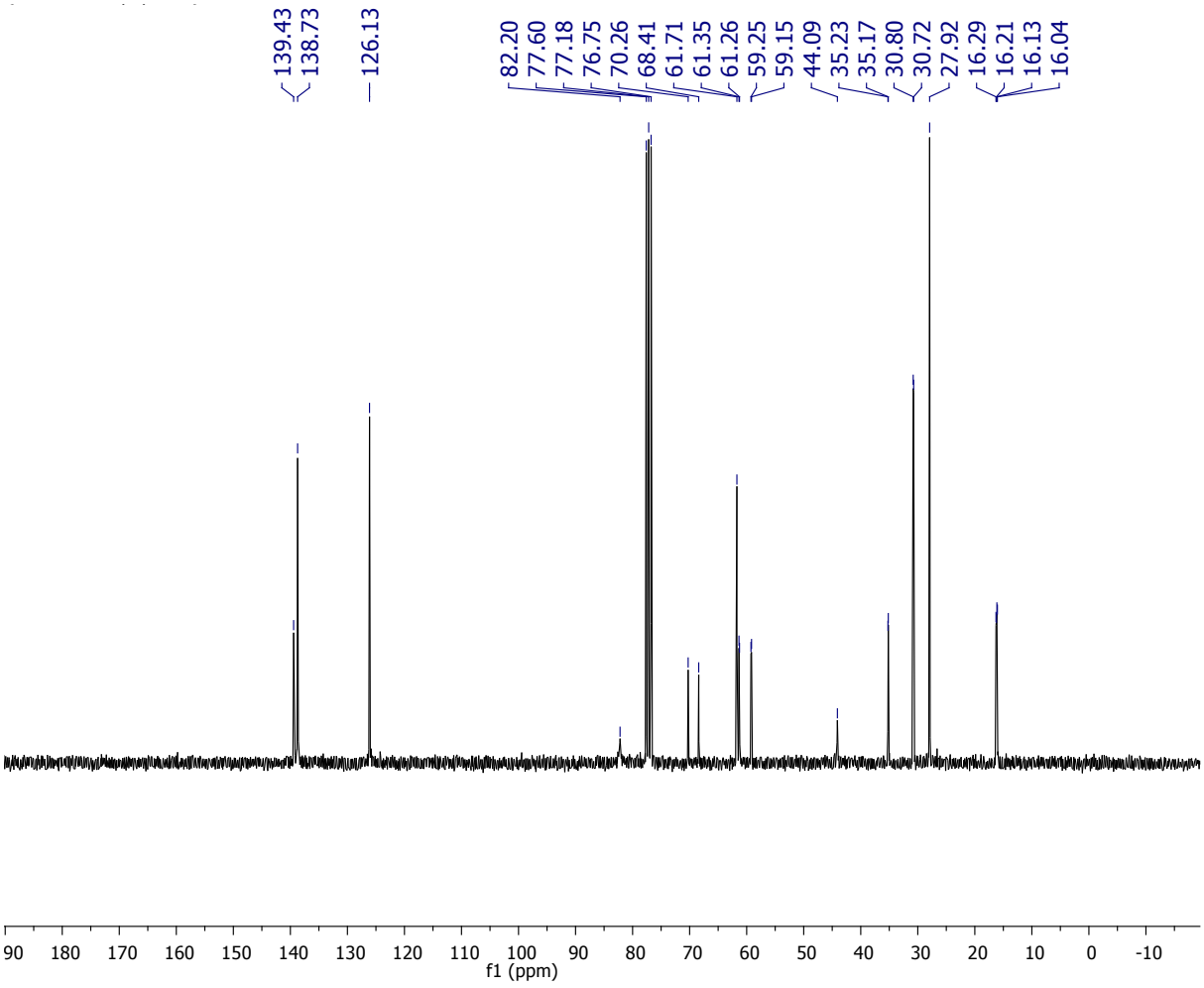
^{1}H NMR Spectrum ($CDCl_3$)



^{31}P NMR Spectrum ($CDCl_3$)

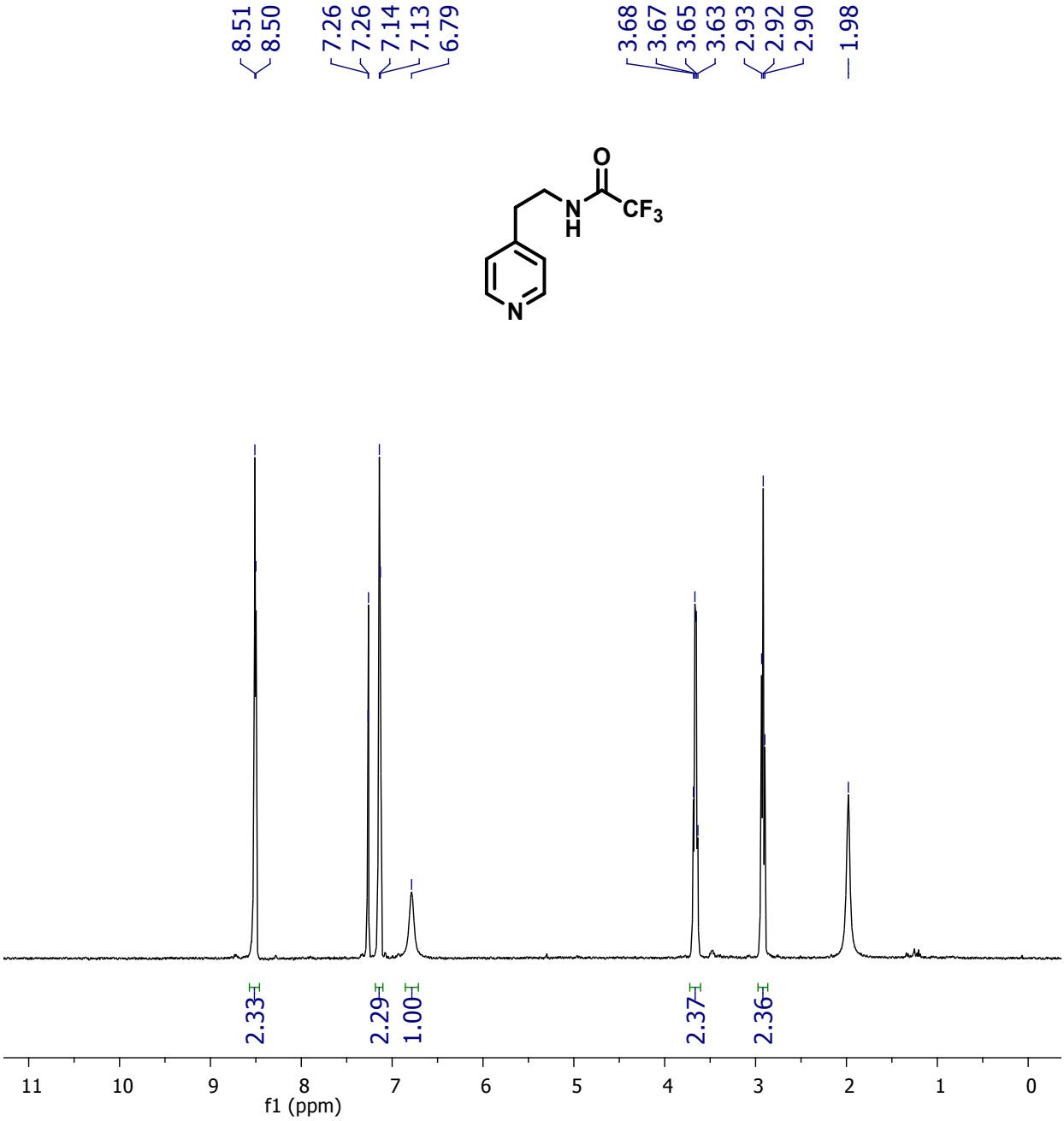


^{13}C {^{1}H} NMR Spectrum ($CDCl_3$)

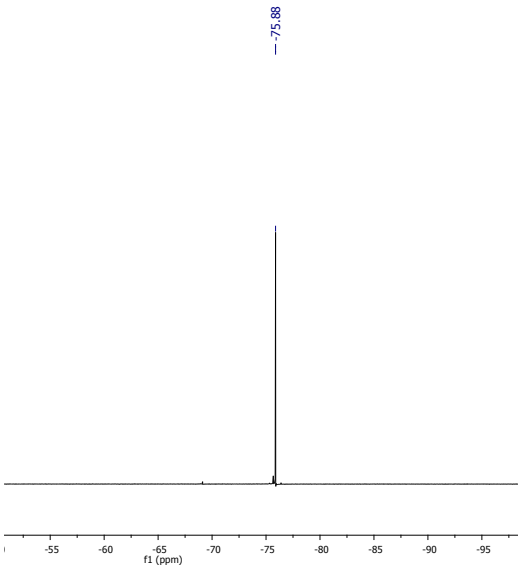


NMR of 5

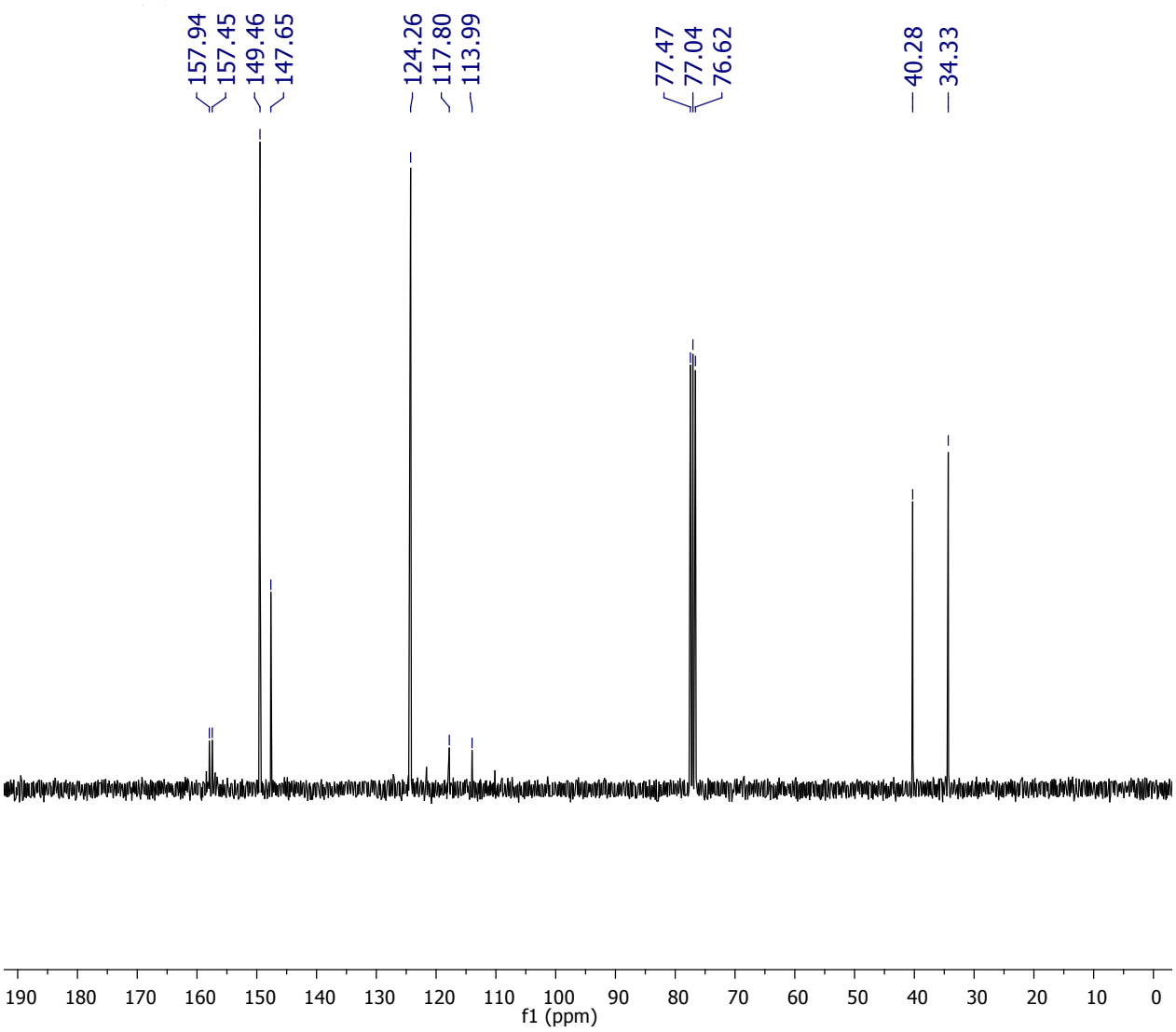
^{1}H NMR Spectrum ($CDCl_3$)



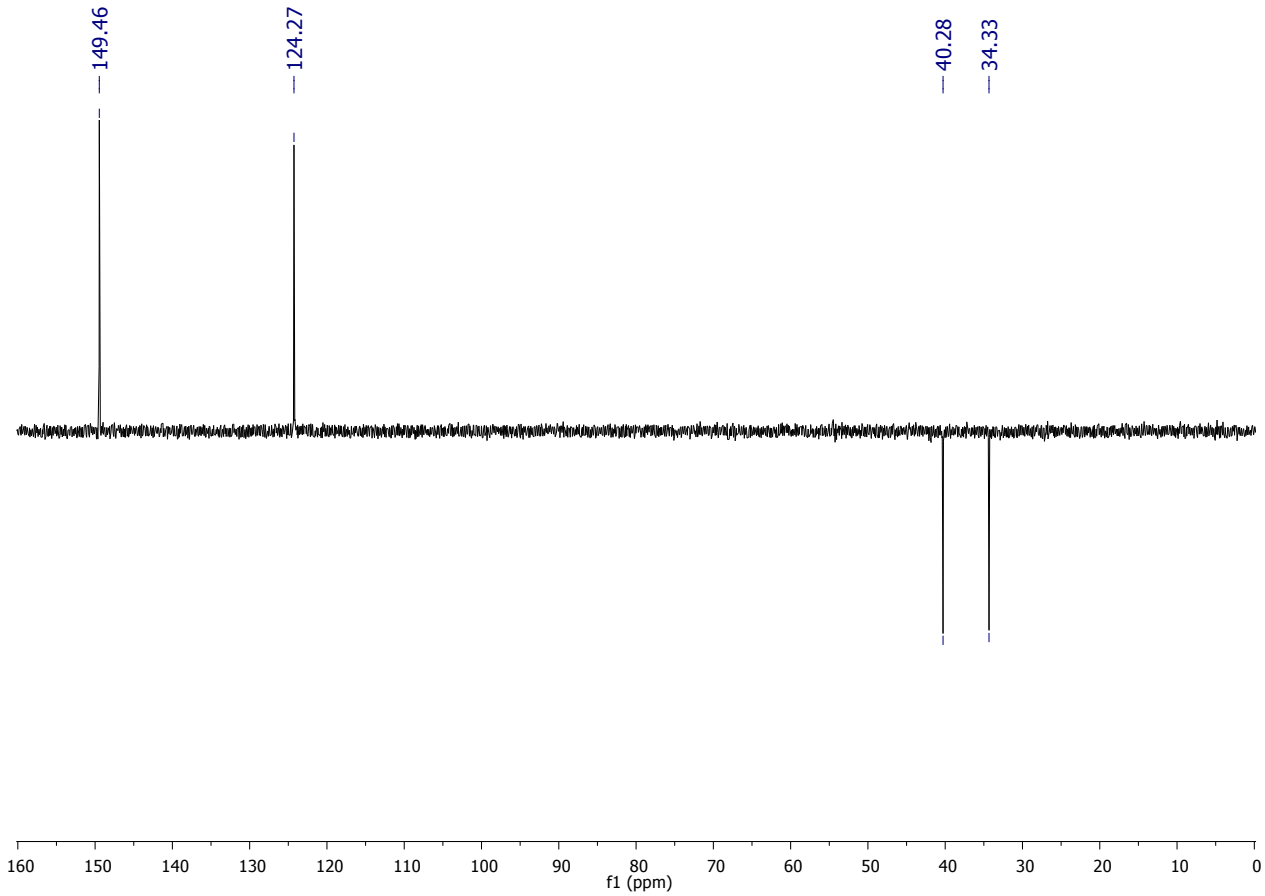
^{19}F NMR Spectrum ($CDCl_3$)



^{13}C {^{1}H} NMR Spectrum ($CDCl_3$)

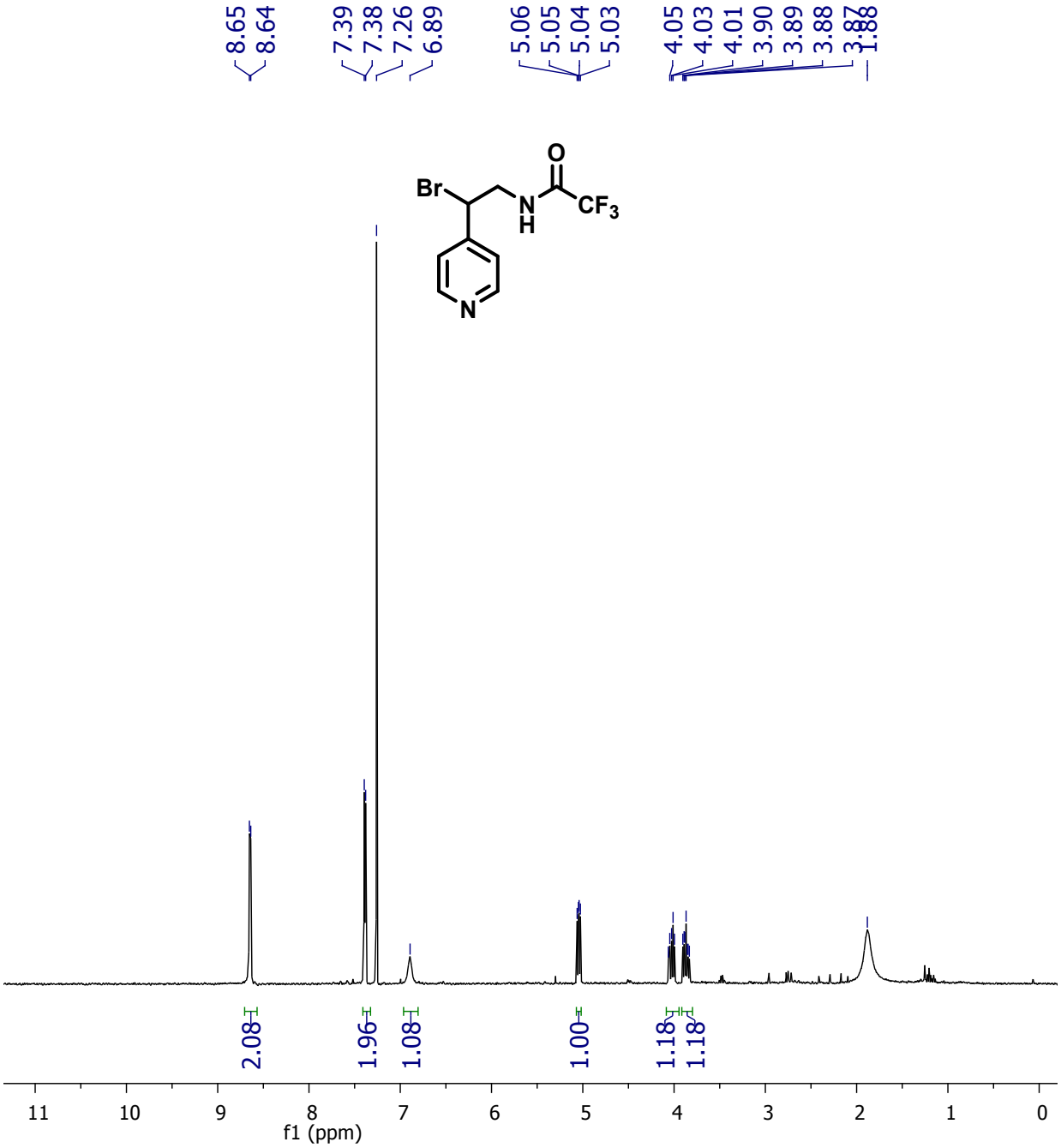


^{13}C DEPT 135 NMR Spectrum ($CDCl_3$)

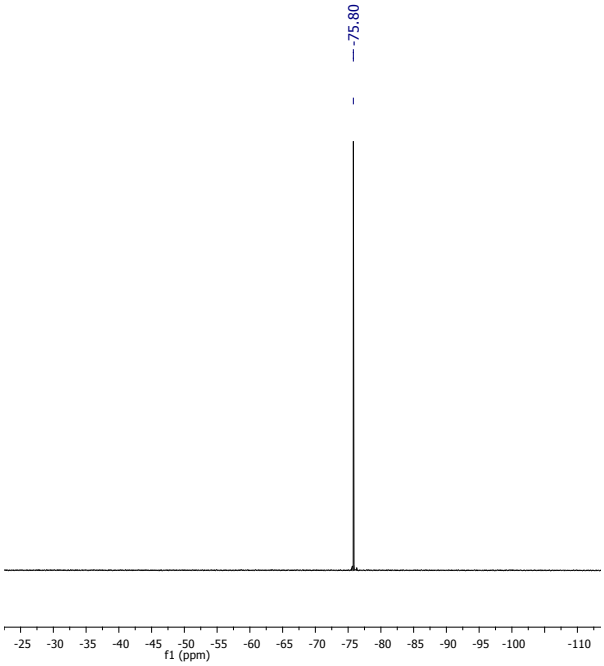


NMR of 6

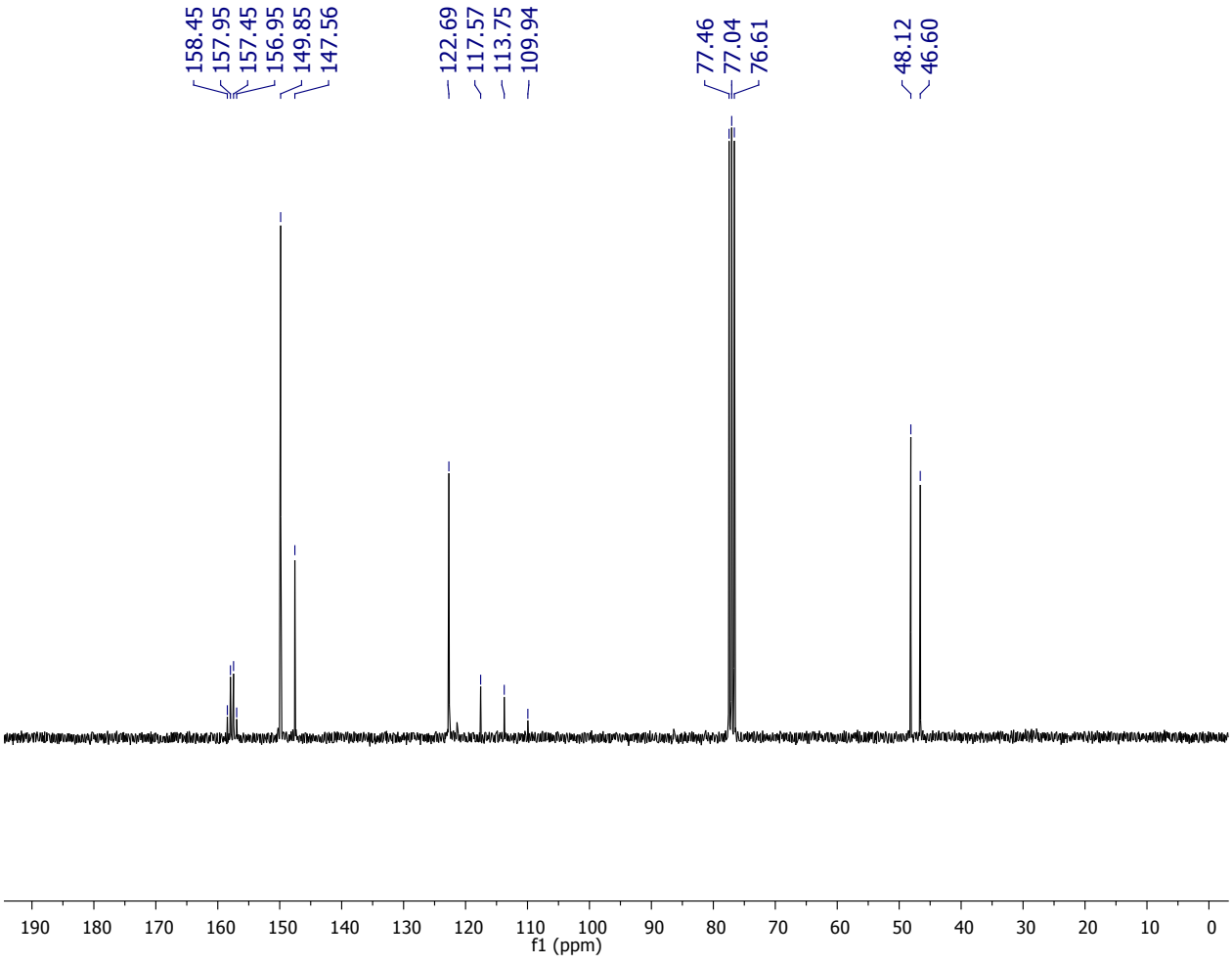
^{1}H NMR Spectrum ($CDCl_3$)



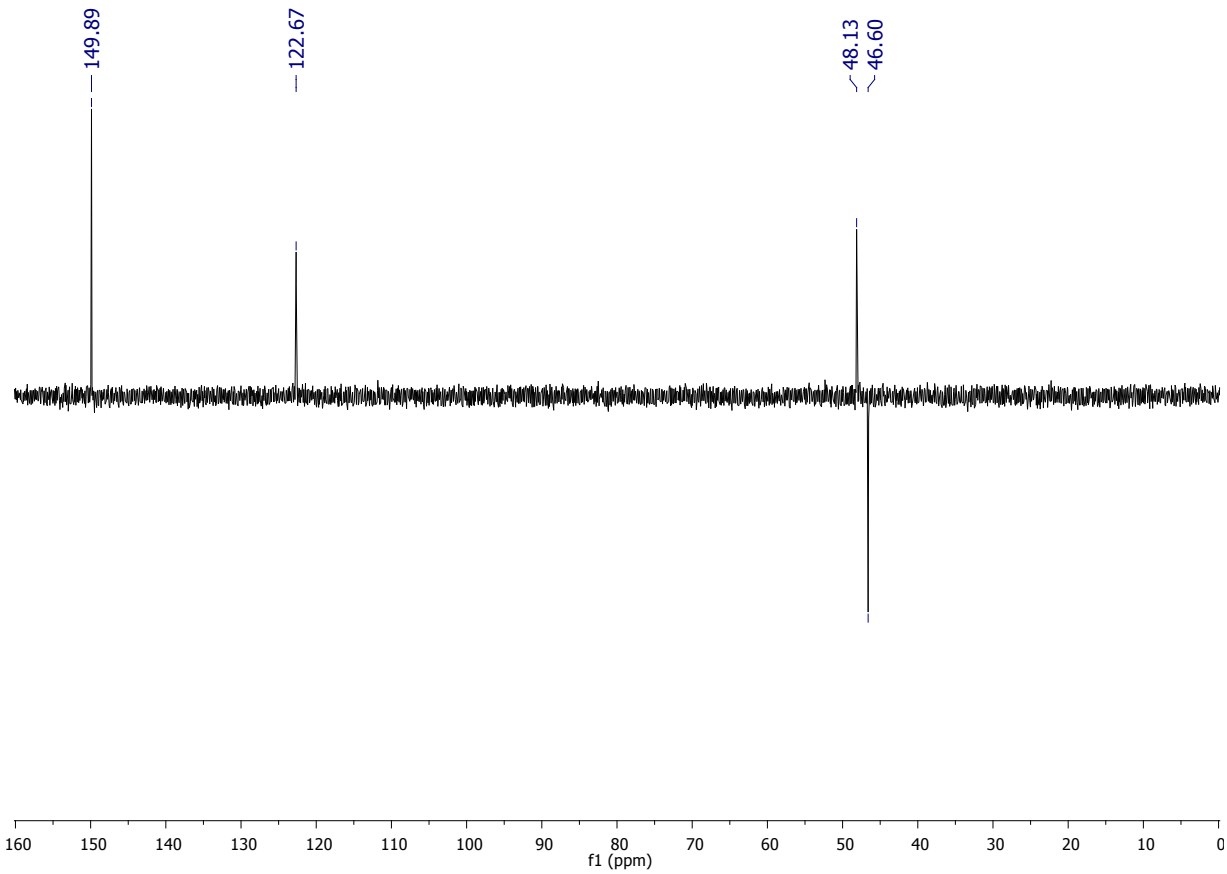
^{19}F NMR Spectrum ($CDCl_3$)



^{13}C {^{1}H} NMR Spectrum ($CDCl_3$)

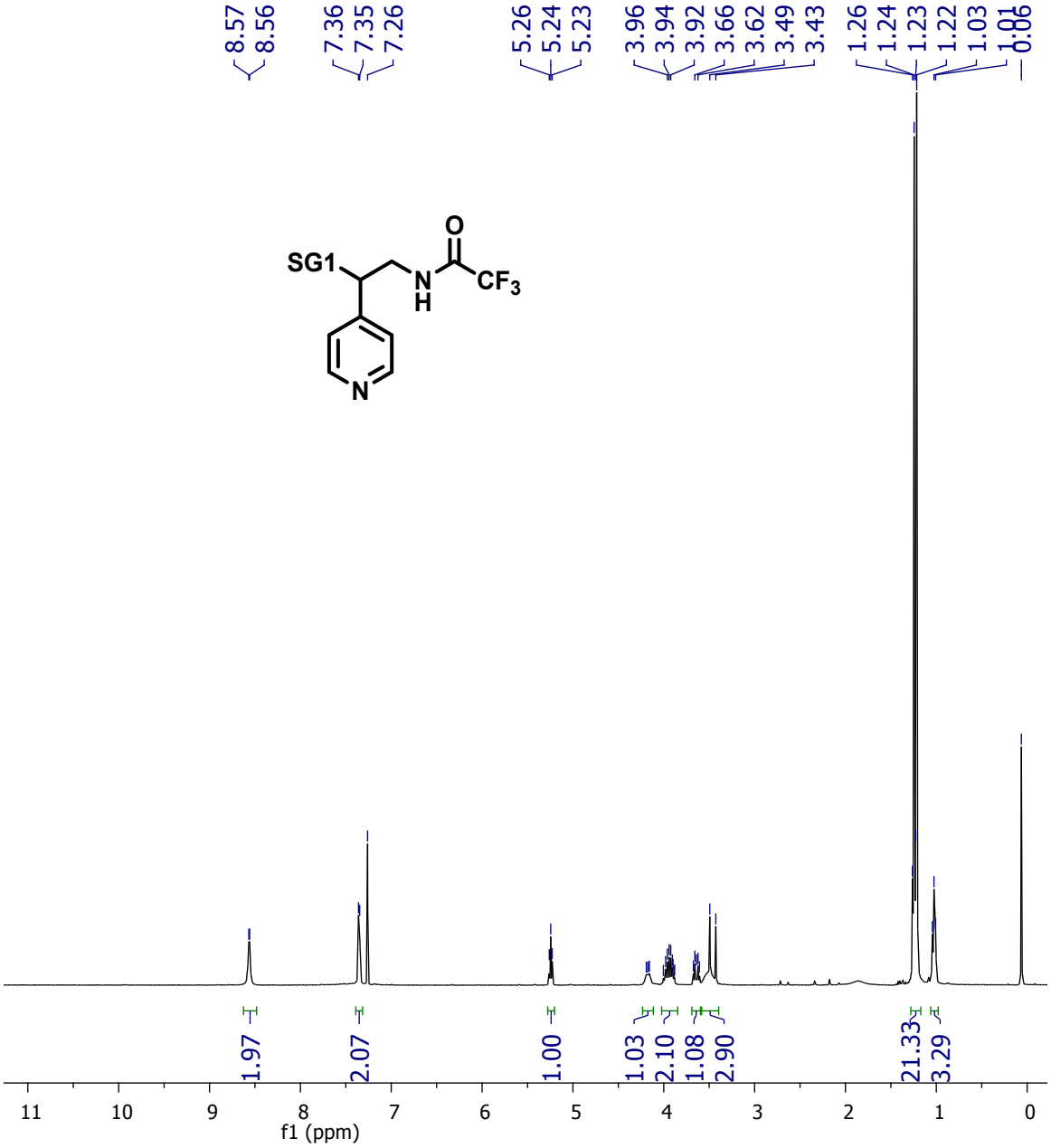


^{13}C DEPT 135 NMR Spectrum ($CDCl_3$)

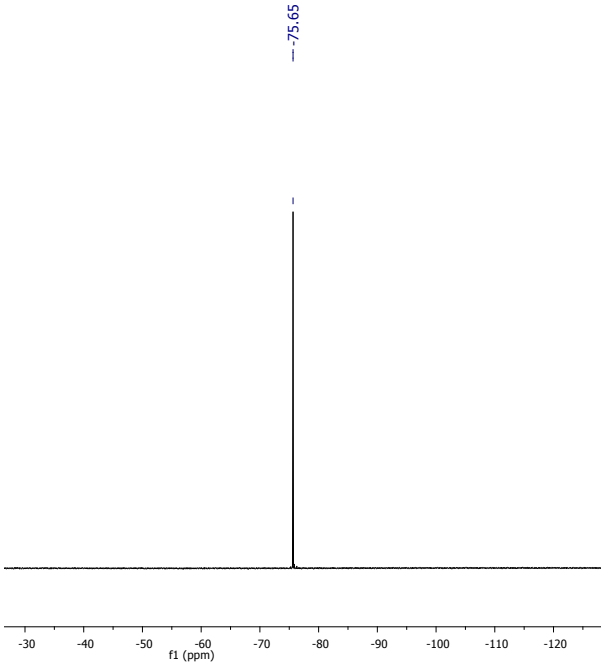


NMR of *RR/SS*-7

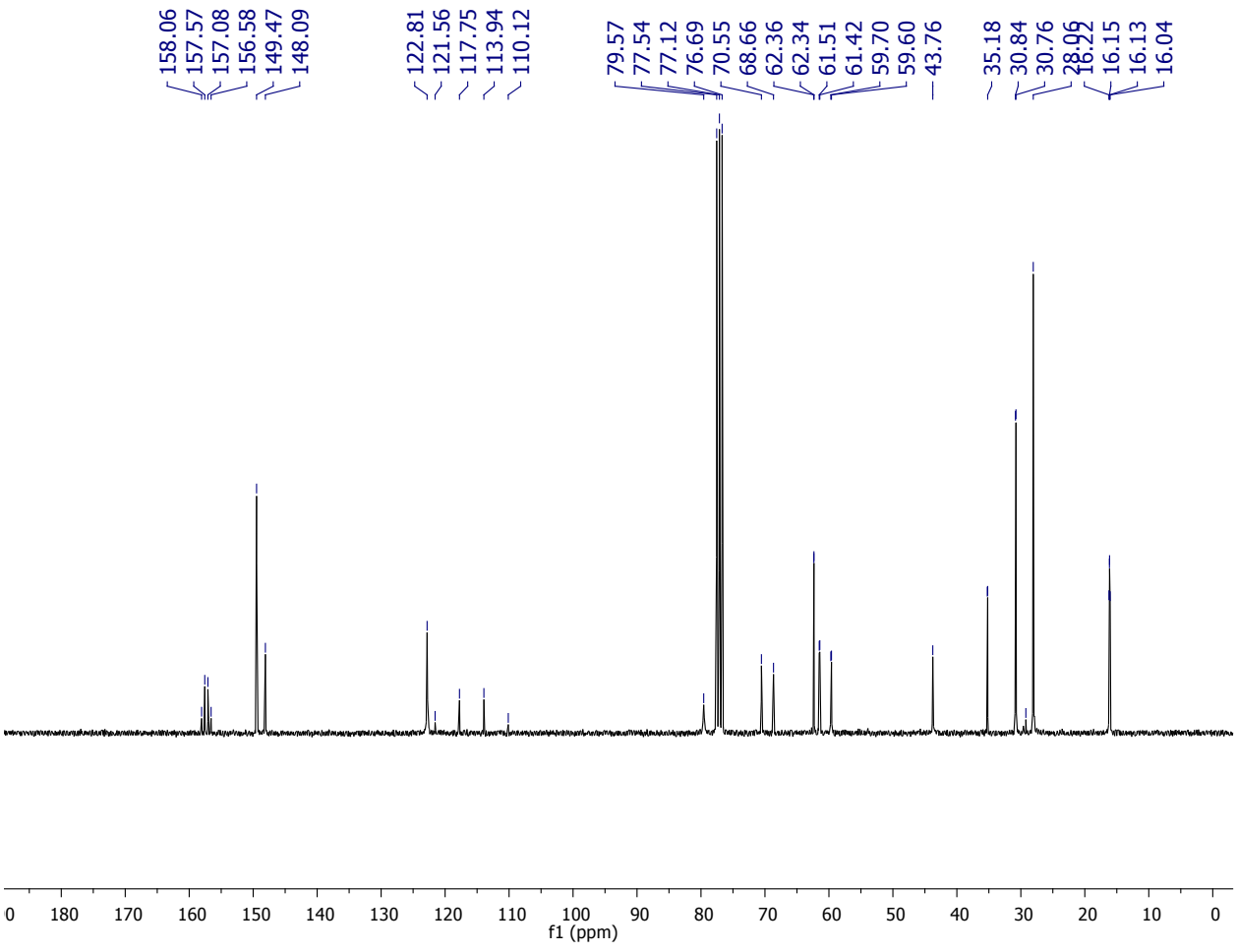
^{1}H NMR Spectrum ($CDCl_3$)



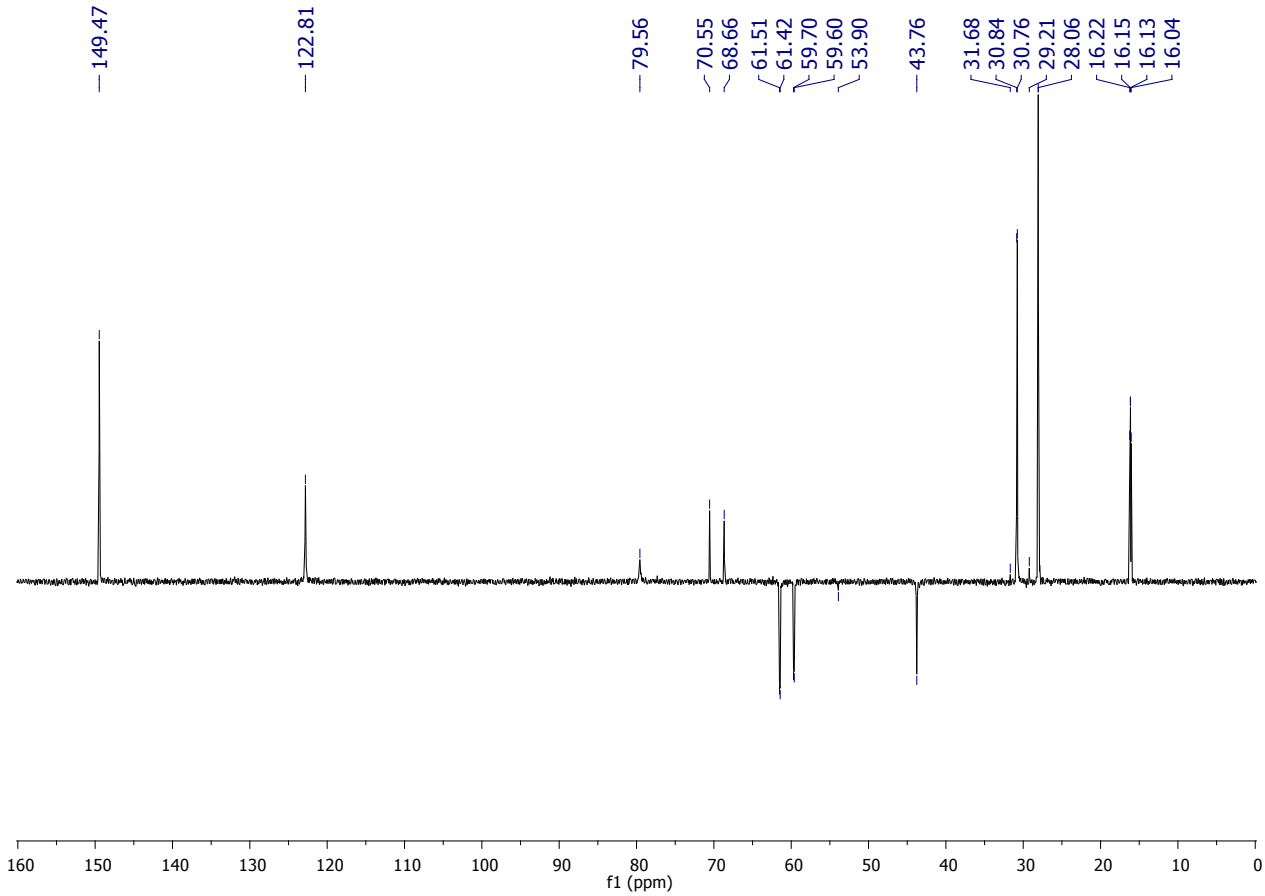
^{19}F NMR Spectrum ($CDCl_3$)



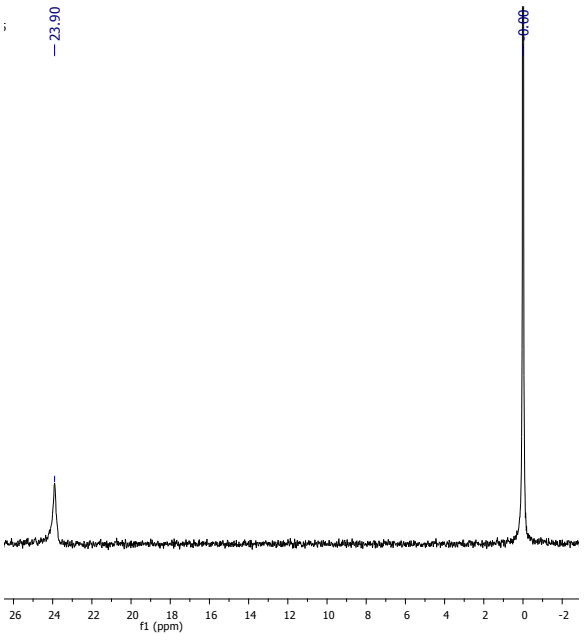
^{13}C {^{1}H} NMR Spectrum ($CDCl_3$)



^{13}C DEPT 135 NMR Spectrum ($CDCl_3$)

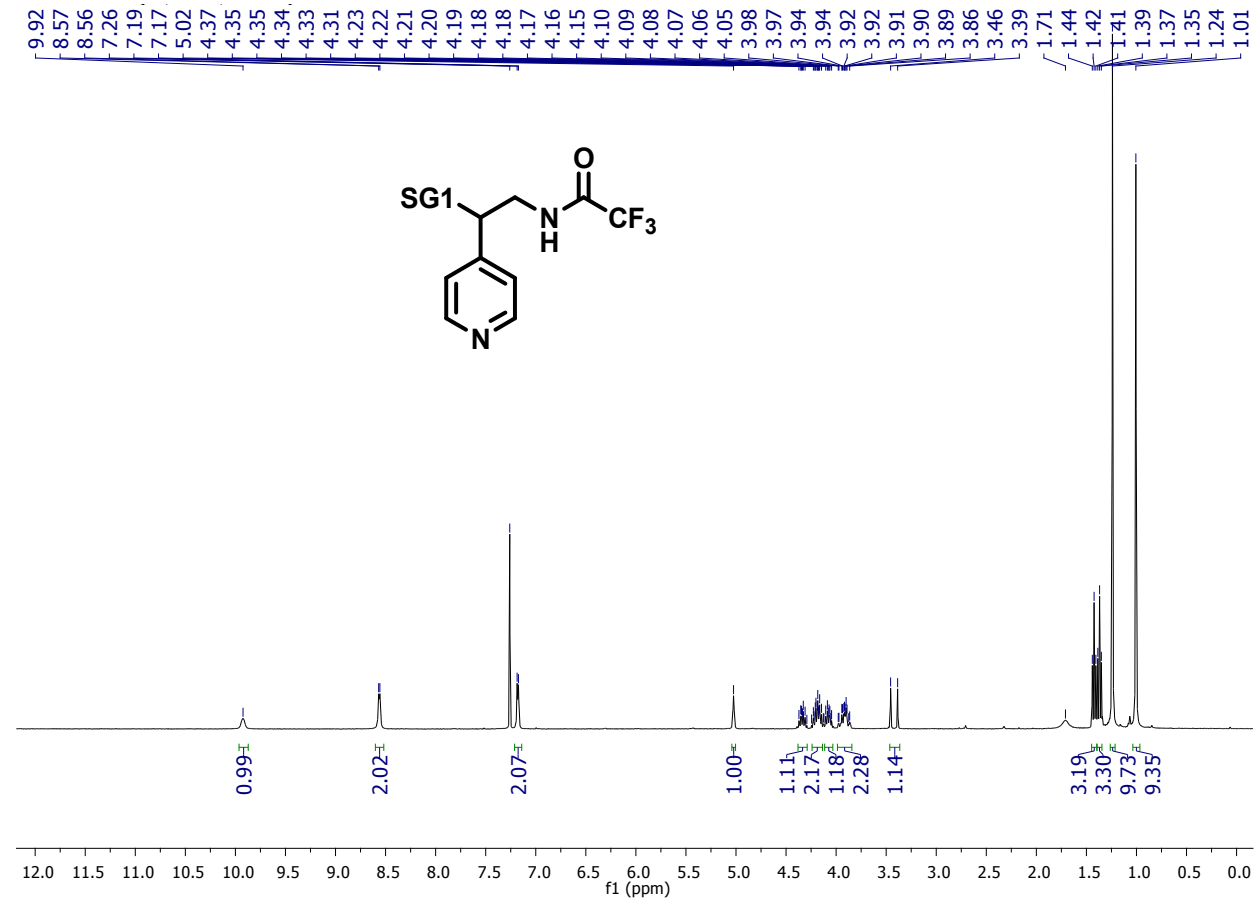


^{31}P NMR Spectrum ($CDCl_3$)

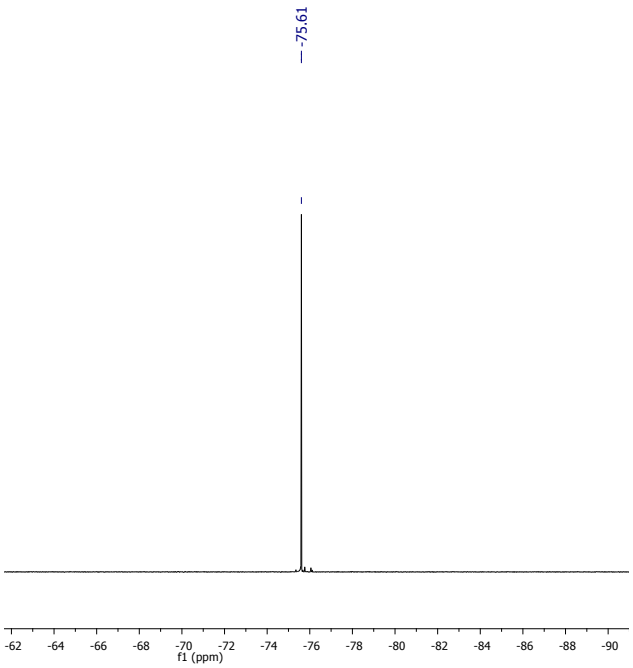


NMR of *RS/SR*-7

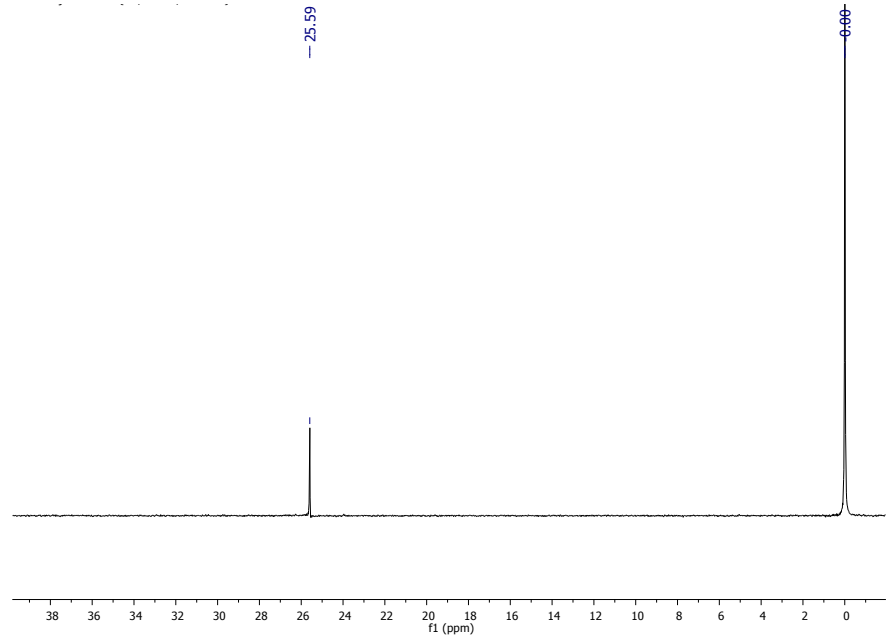
^{1}H NMR Spectrum ($CDCl_3$)



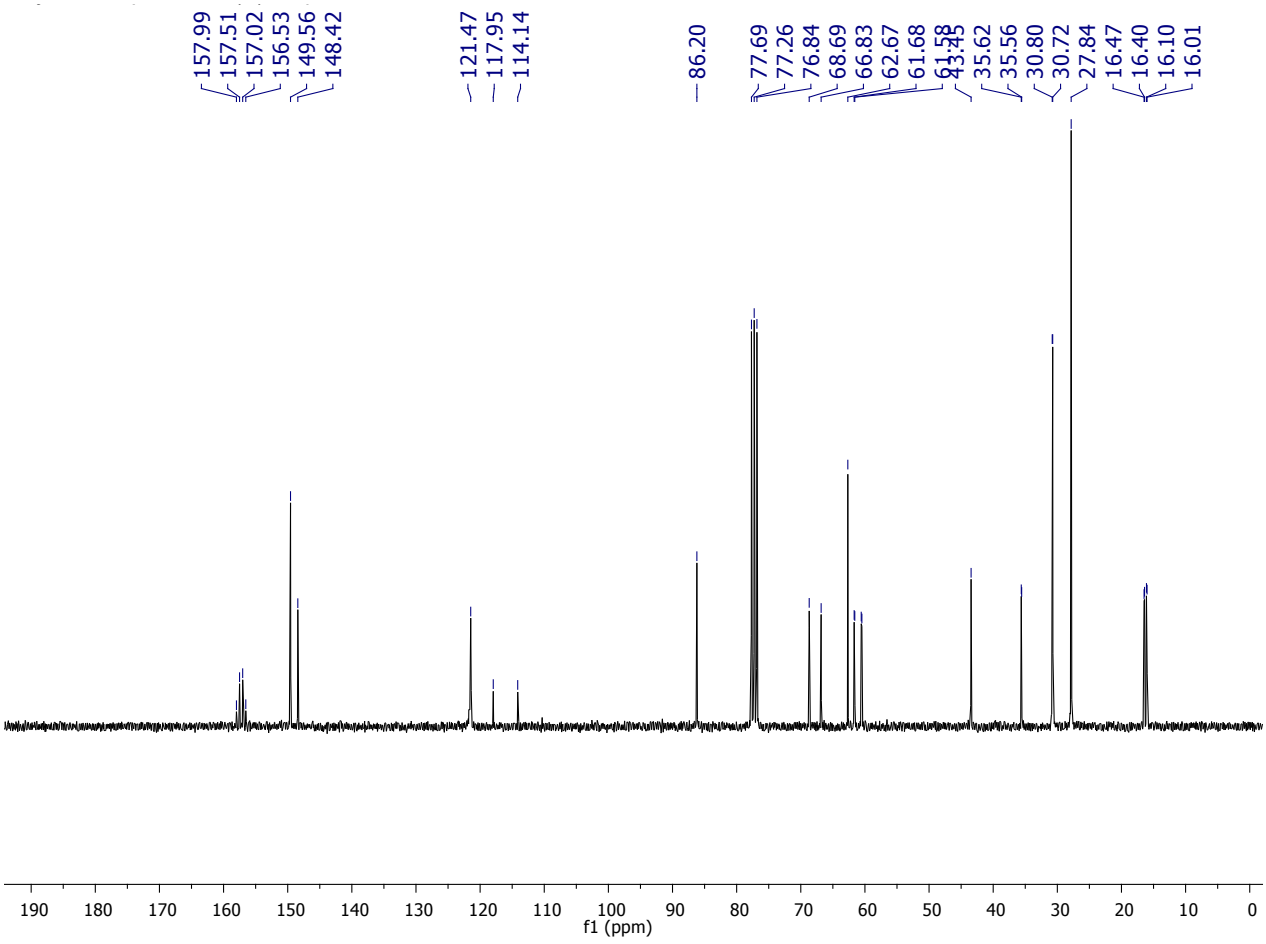
^{19}F NMR Spectrum ($CDCl_3$)



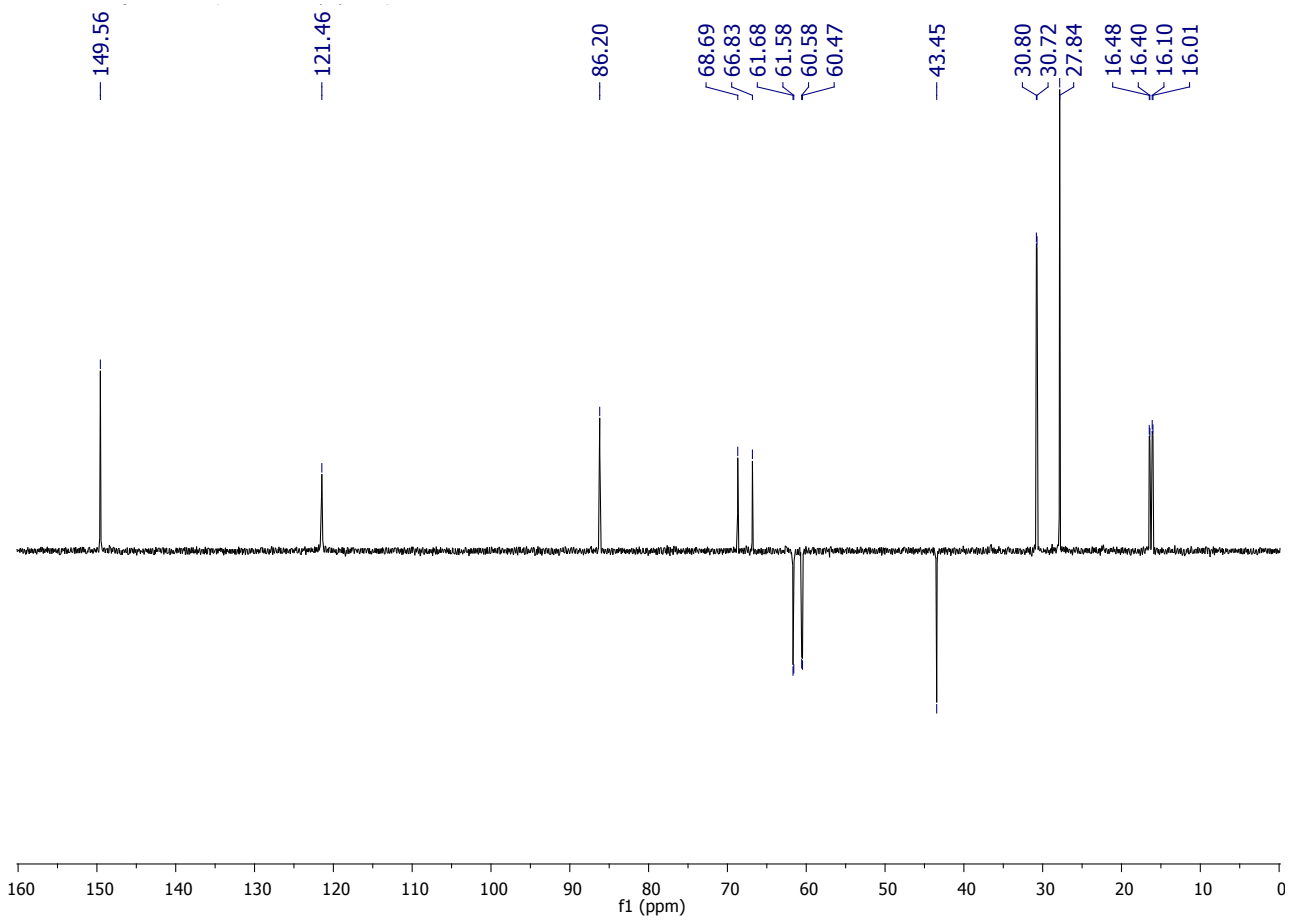
^{31}P NMR Spectrum ($CDCl_3$)



^{13}C {^{1}H} NMR Spectrum ($CDCl_3$)

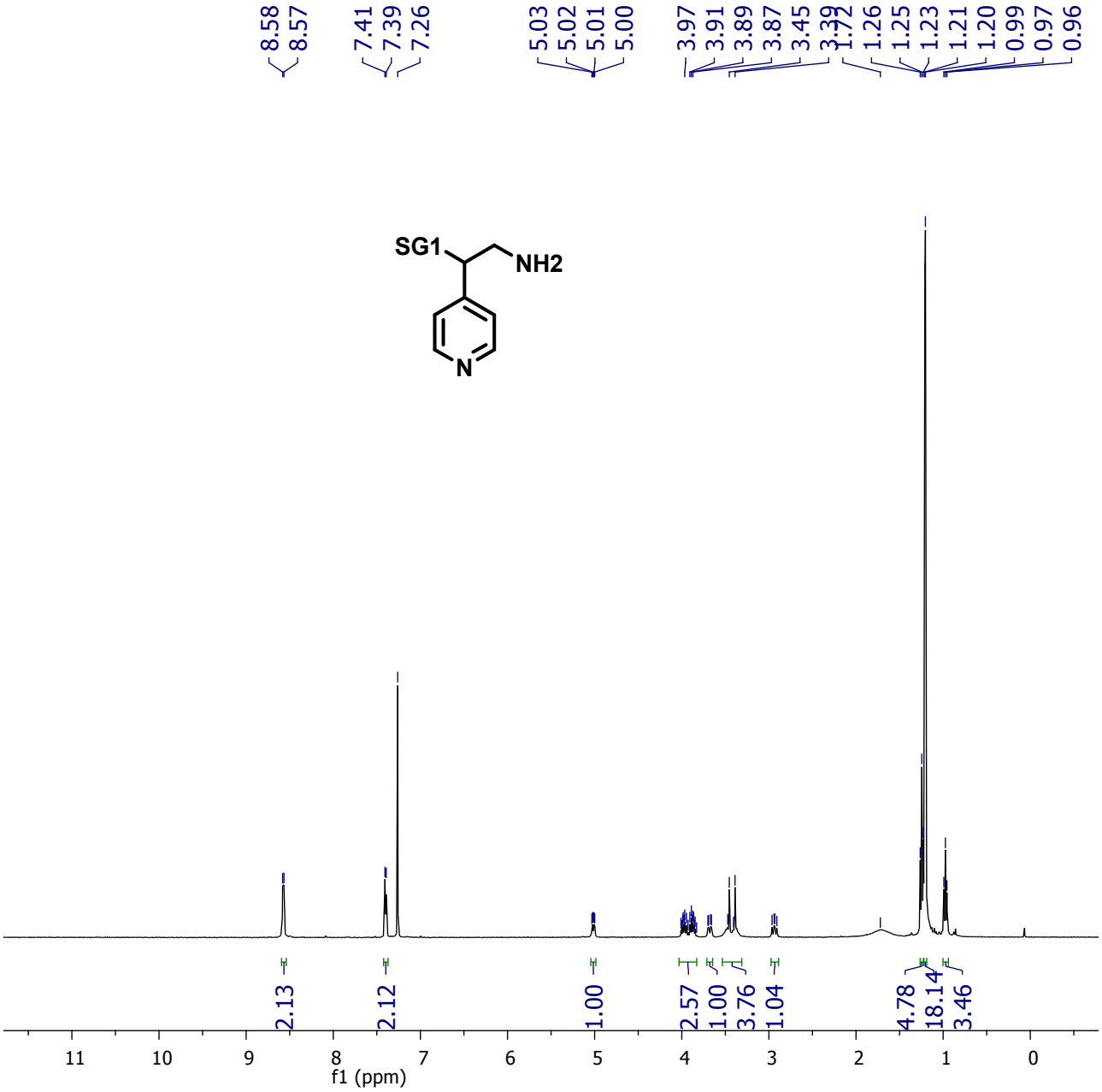


^{13}C DEPT 135 NMR Spectrum ($CDCl_3$)

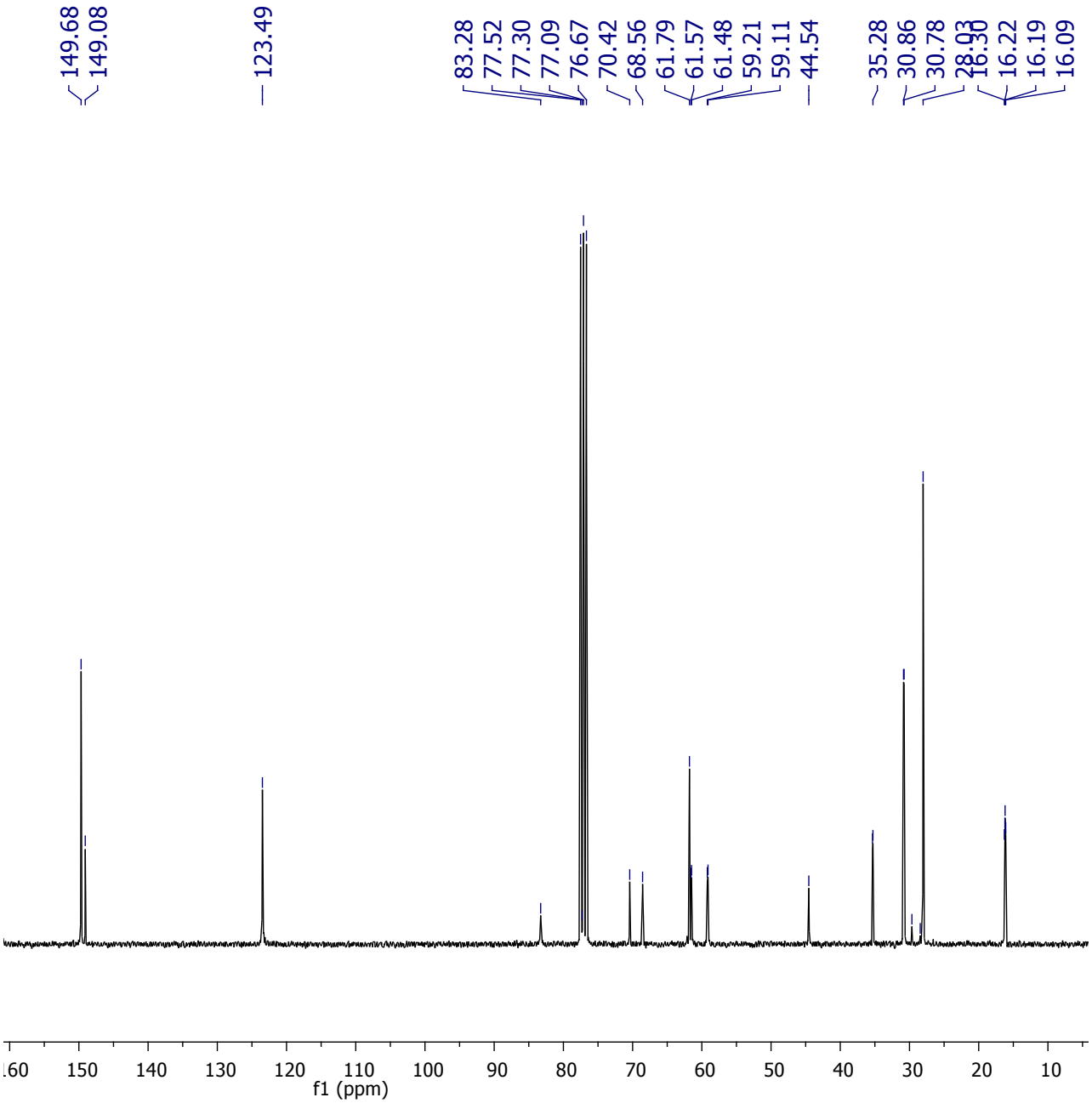


NMR of *RR/SS*-8

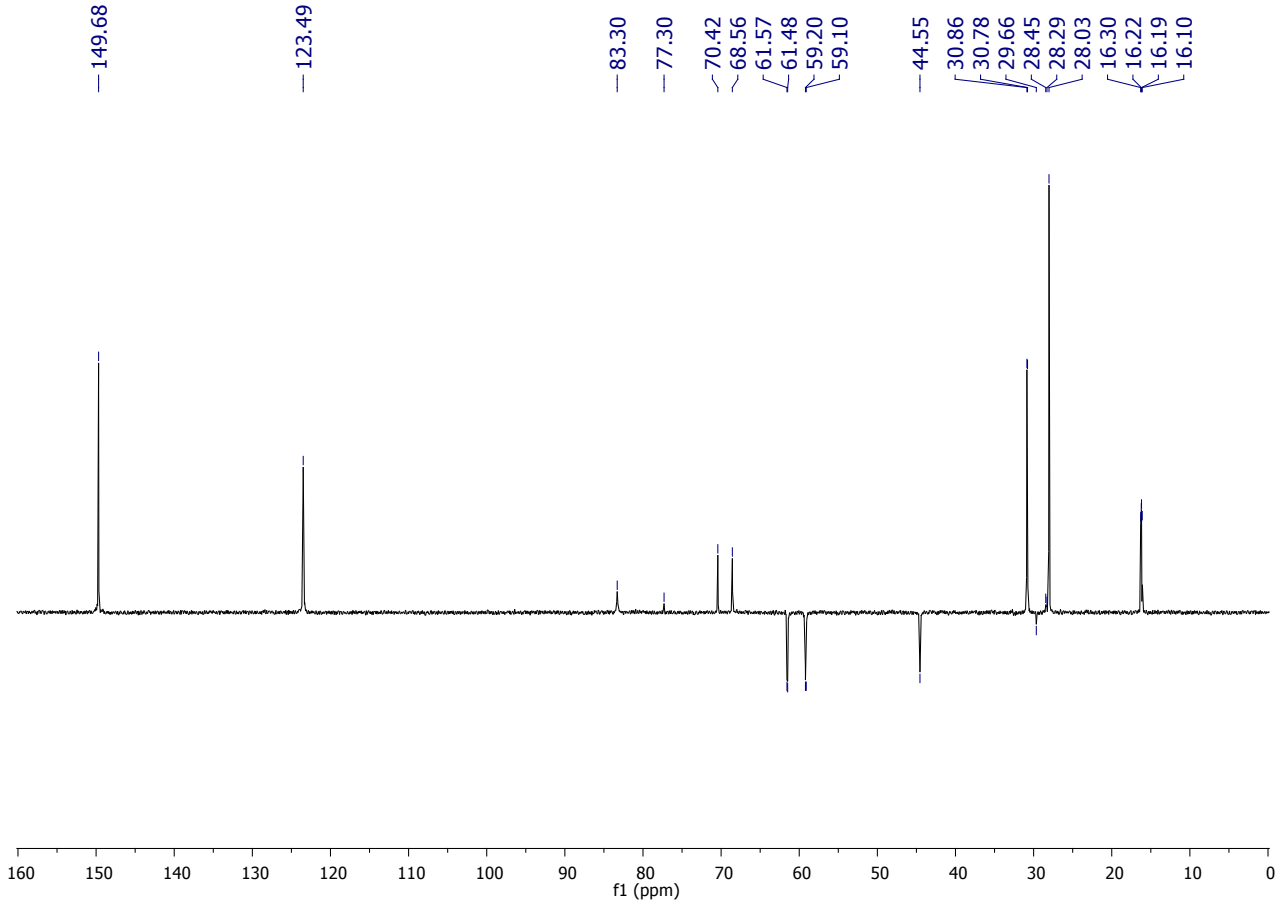
^{1}H NMR Spectrum ($CDCl_3$)



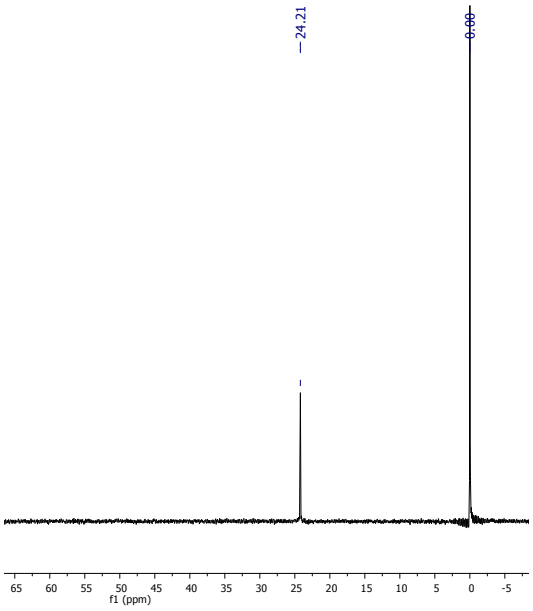
^{13}C {^{1}H} NMR Spectrum ($CDCl_3$)



^{13}C DEPT 135 NMR Spectrum ($CDCl_3$)

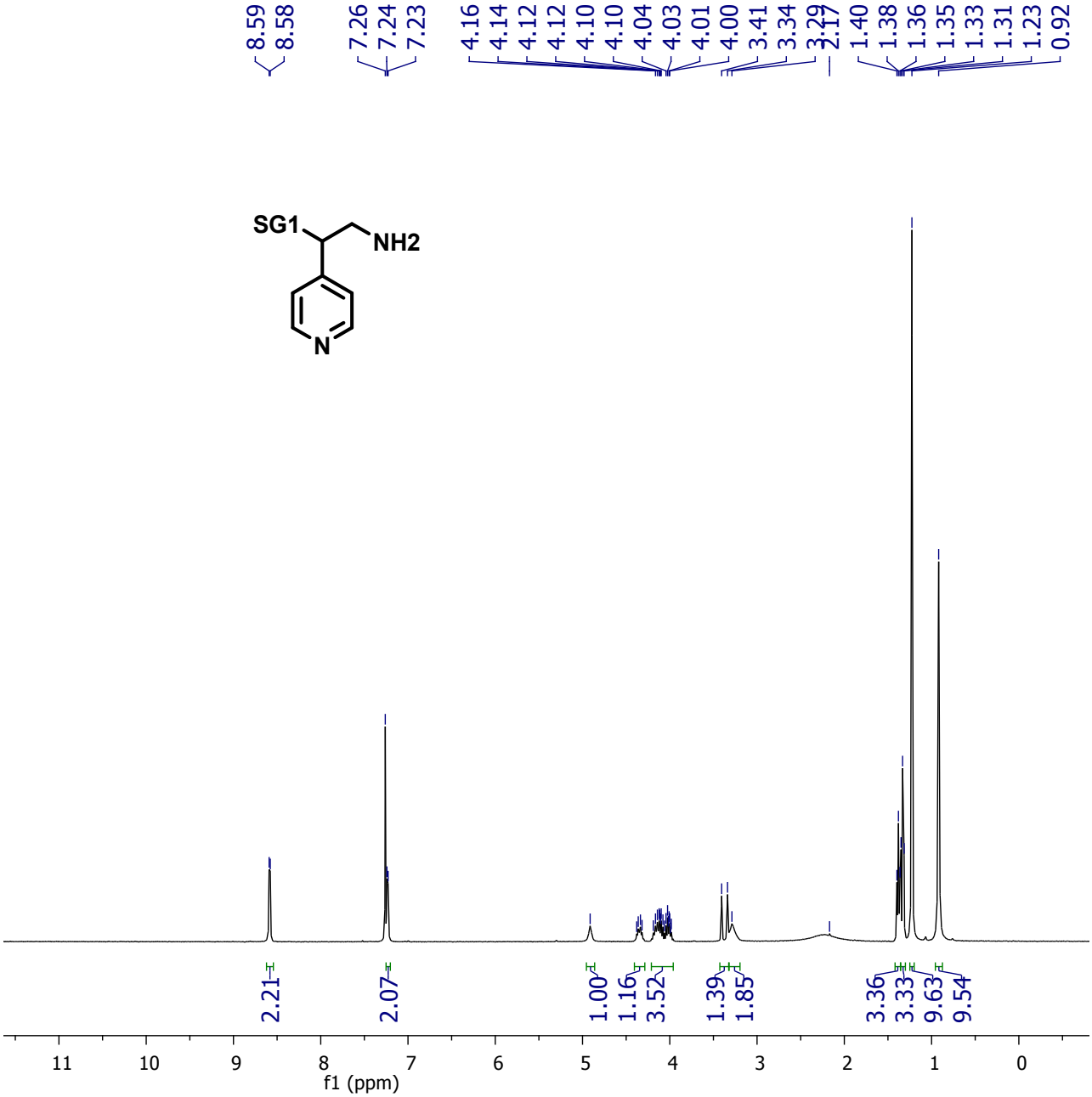


^{31}P NMR Spectrum ($CDCl_3$)

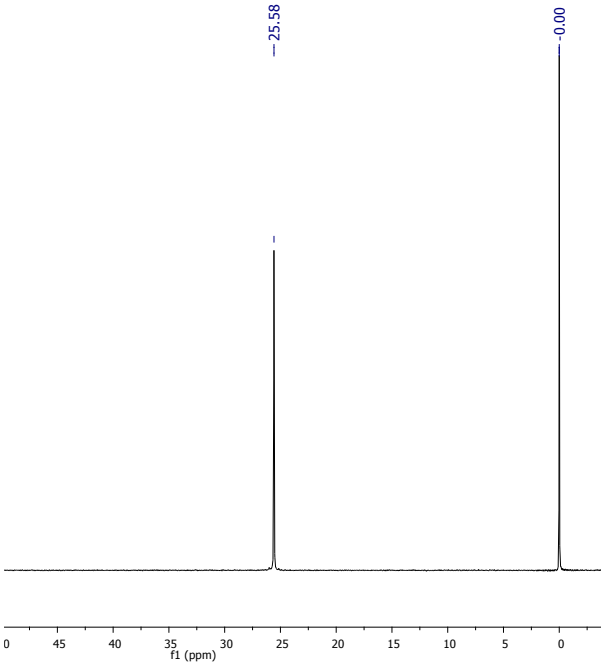


NMR of *RS/SR*-8

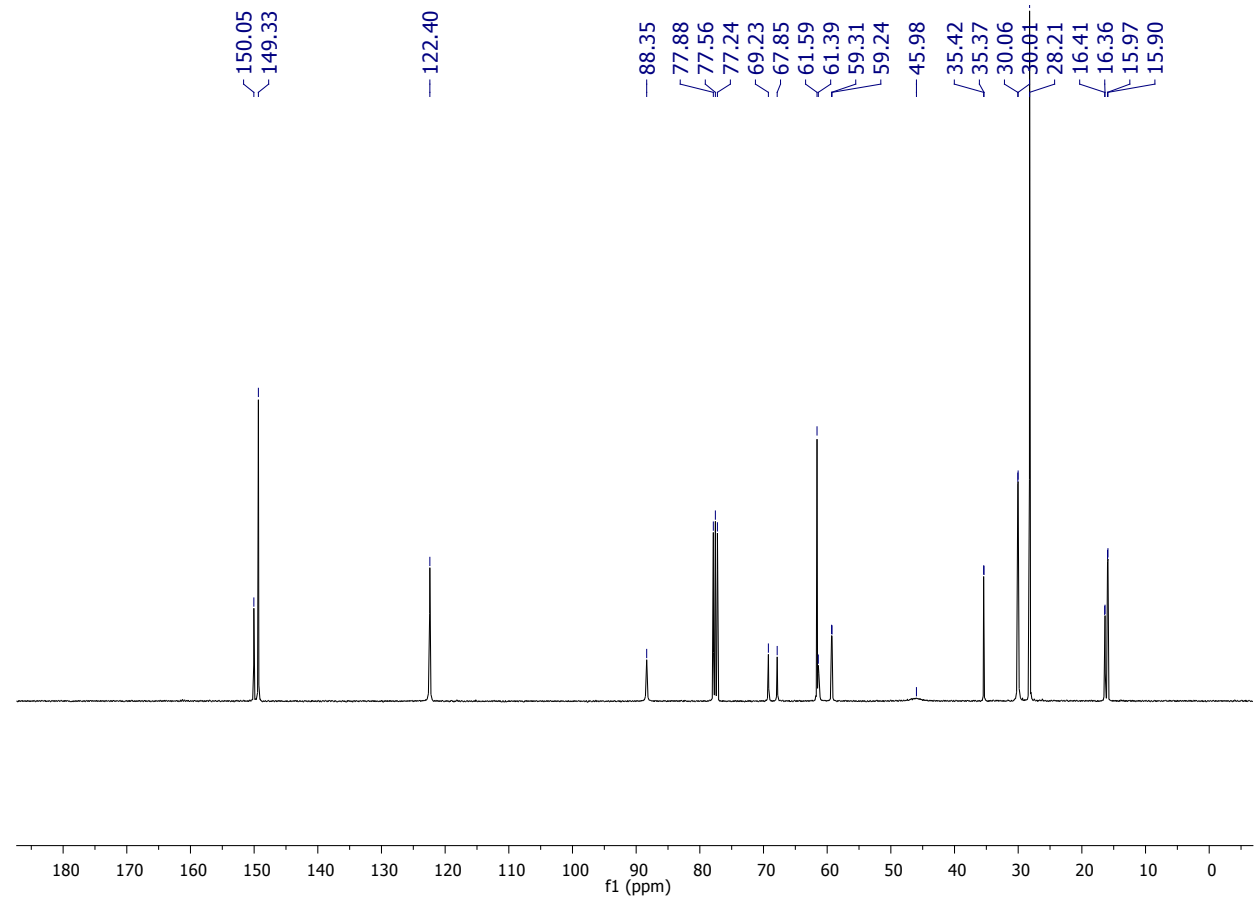
^{1}H NMR Spectrum ($CDCl_3$)



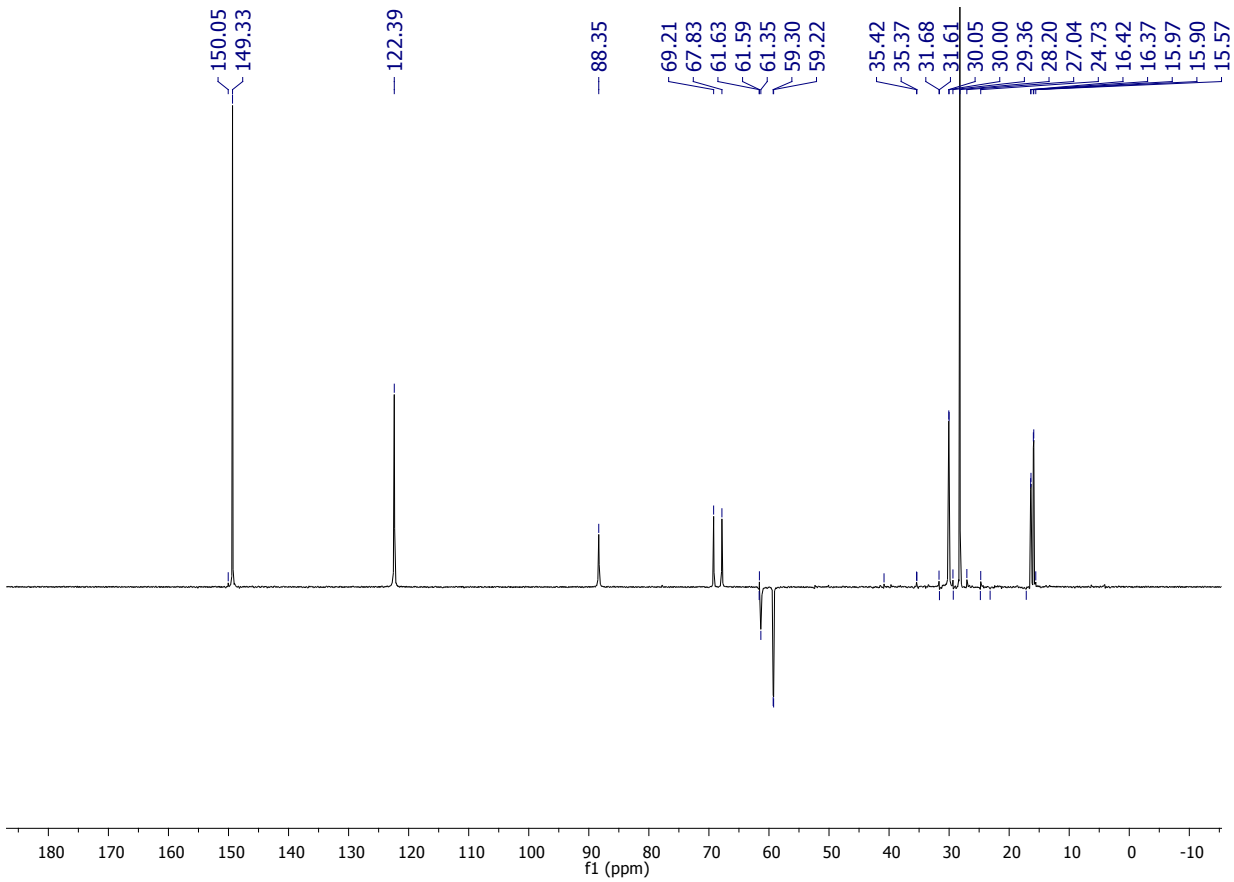
^{31}P NMR Spectrum ($CDCl_3$)



^{13}C {^{1}H} NMR Spectrum ($CDCl_3$)

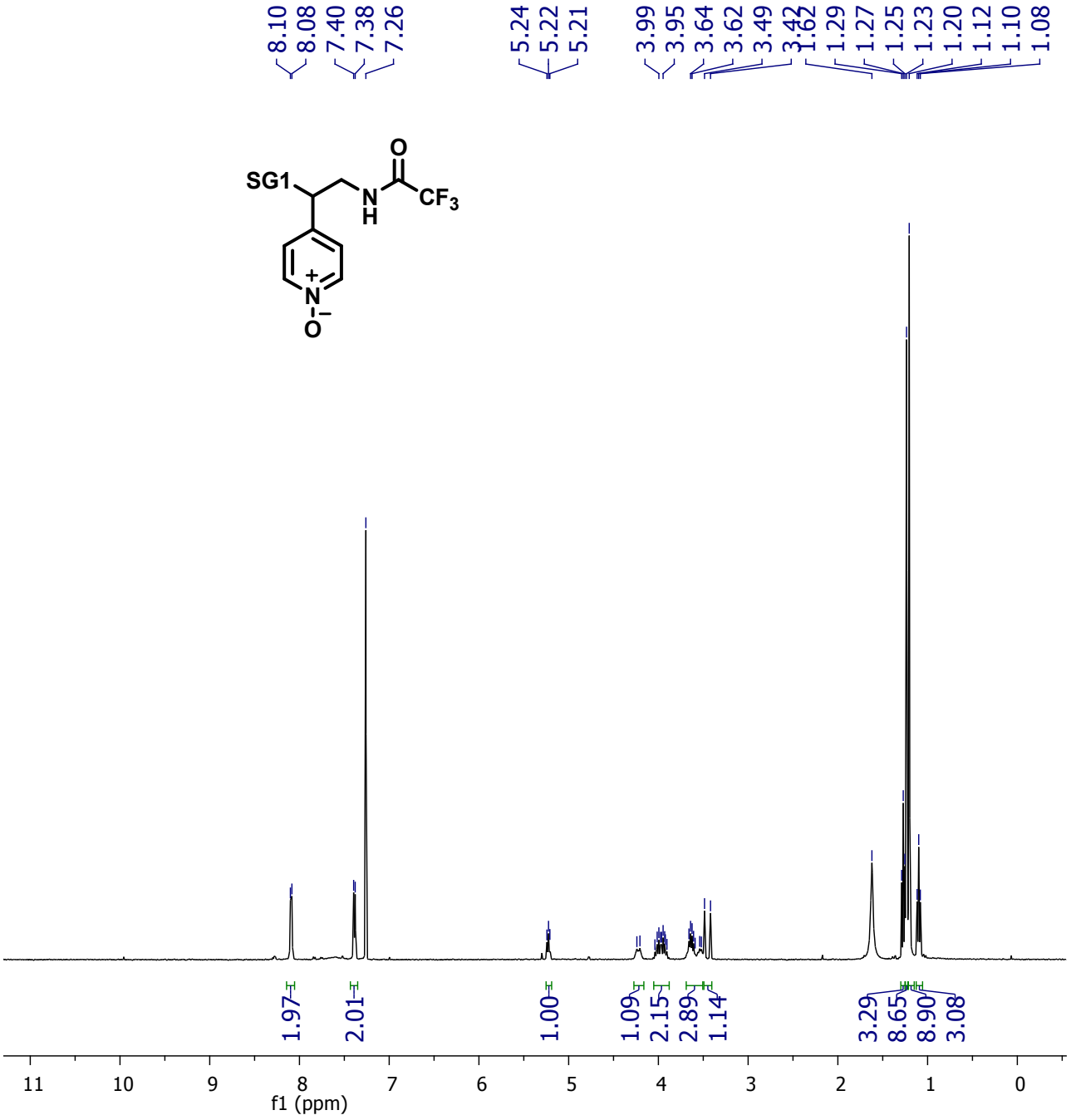


^{13}C DEPT 135 NMR Spectrum ($CDCl_3$)

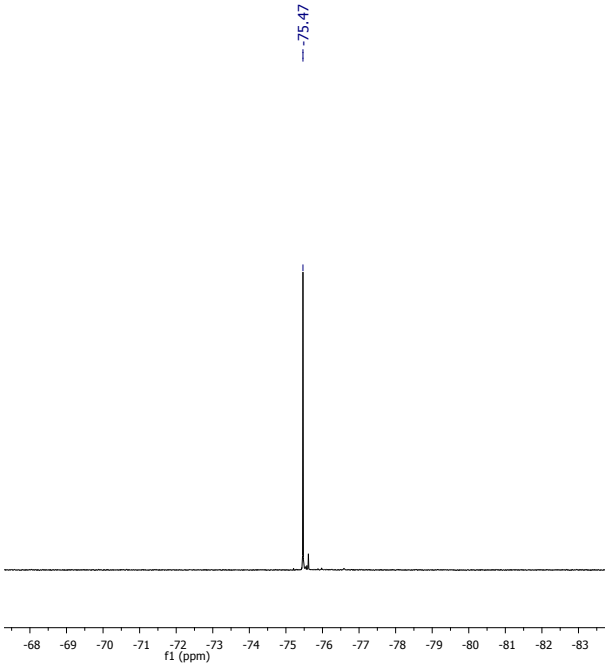


NMR of *RR/SS*-9

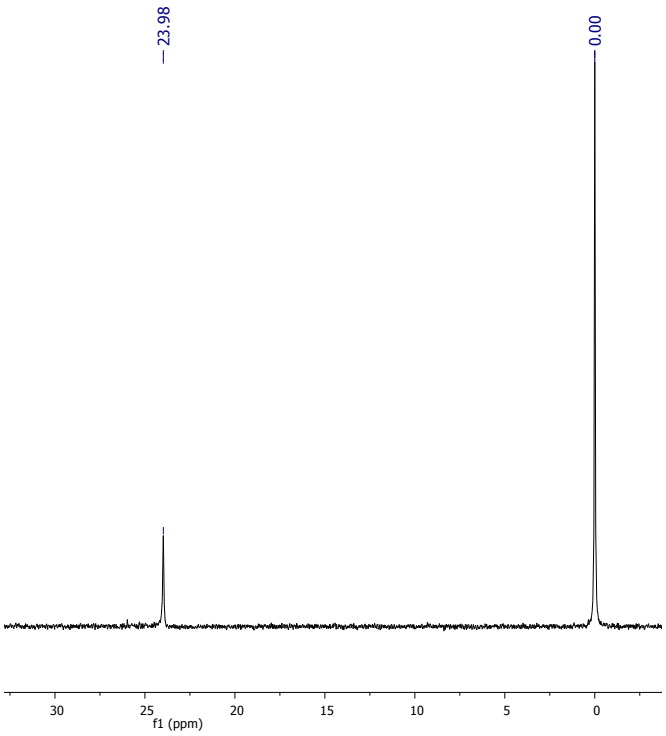
^{1}H NMR Spectrum ($CDCl_3$)



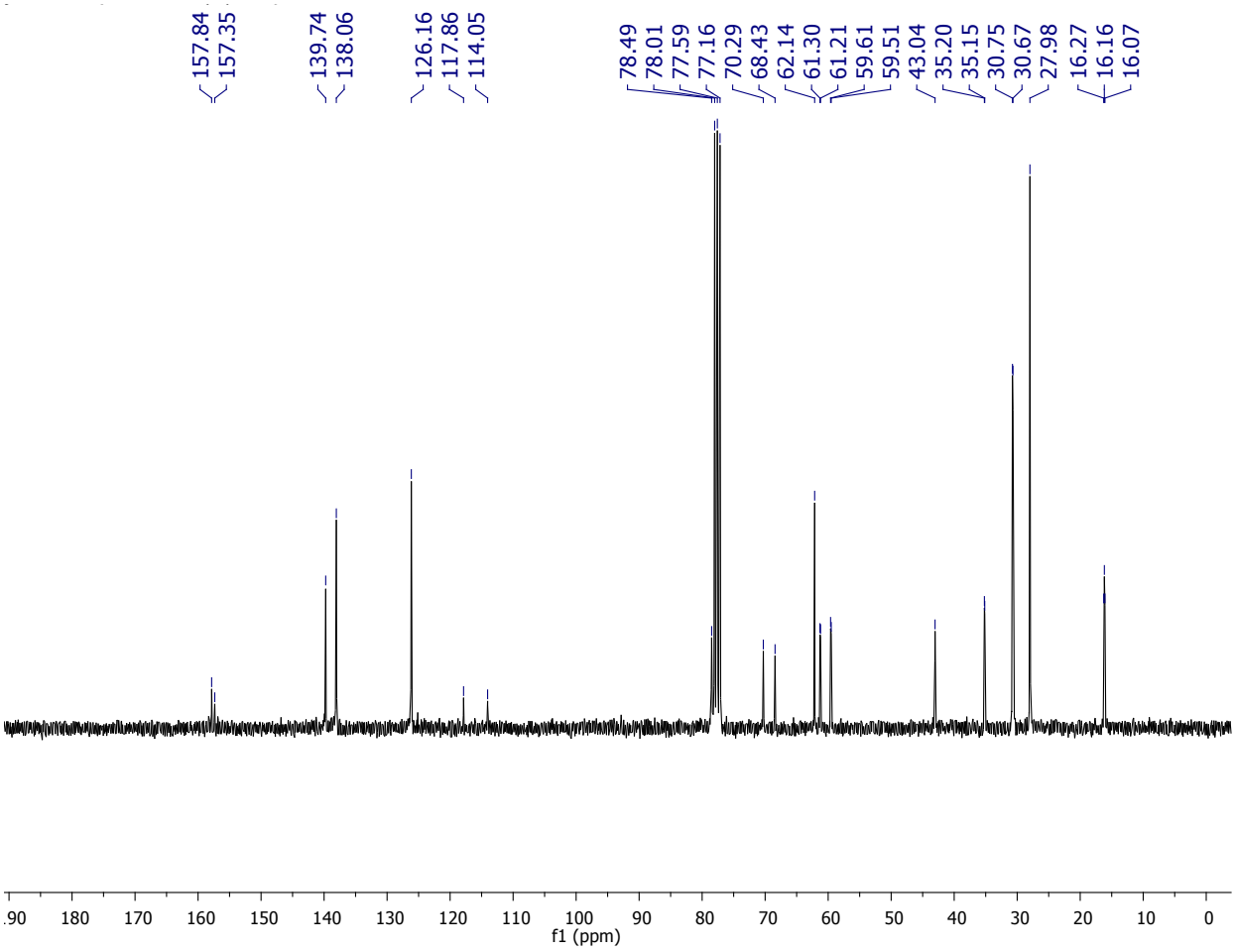
^{19}F NMR Spectrum ($CDCl_3$)



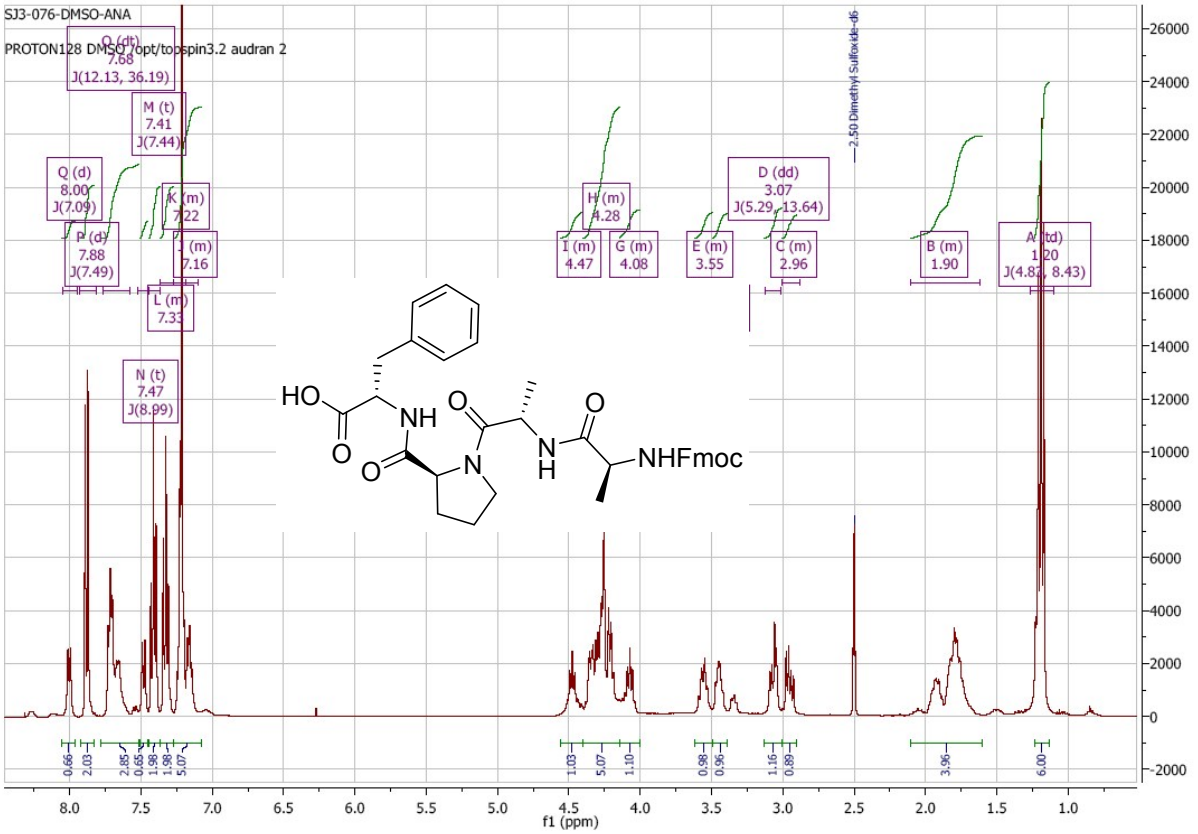
^{31}P NMR Spectrum ($CDCl_3$)



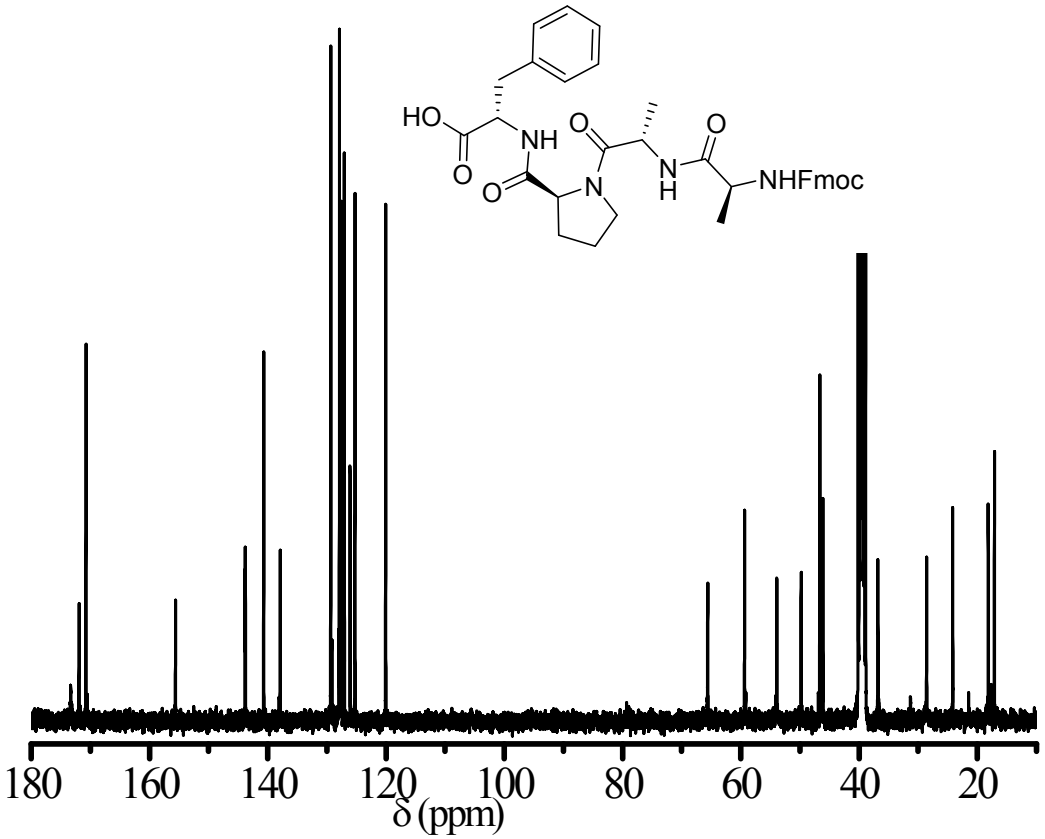
^{13}C {^{1}H} NMR Spectrum ($CDCl_3$)



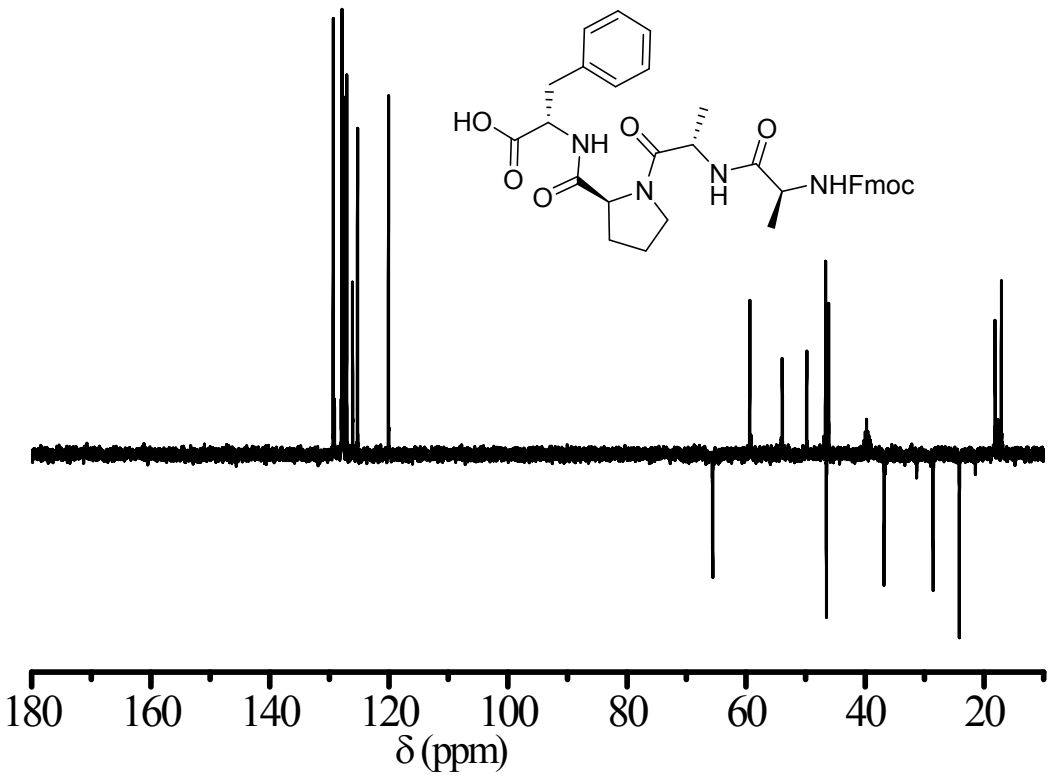
^{1}H NMR of 10.



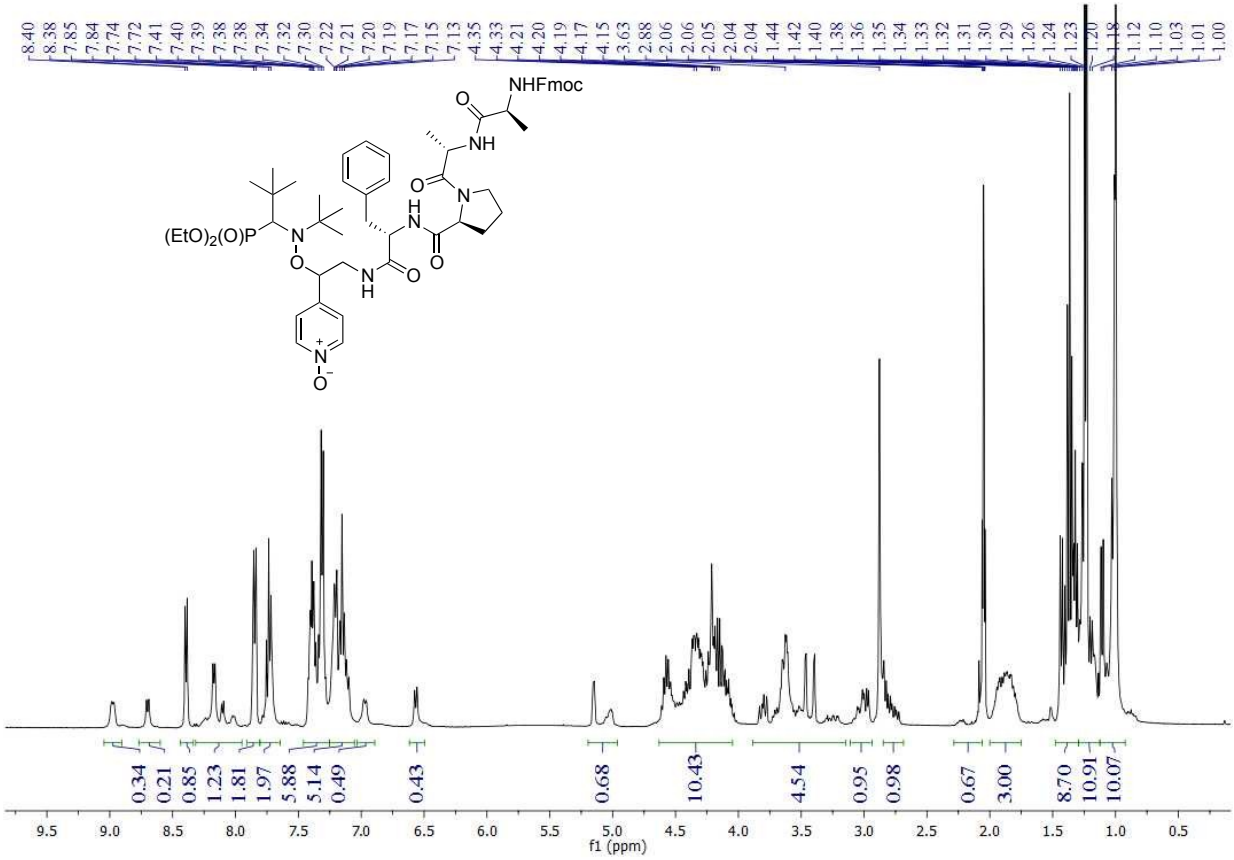
^{13}C NMR of 10.



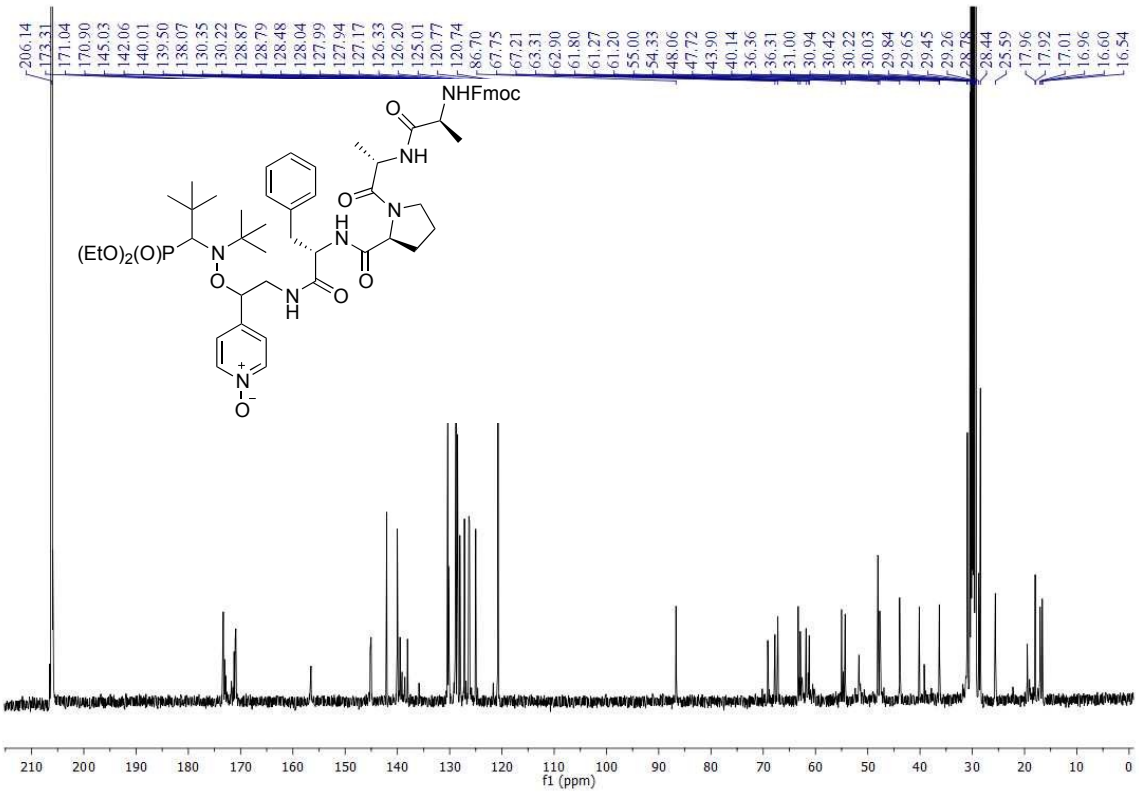
DEPT of 10.



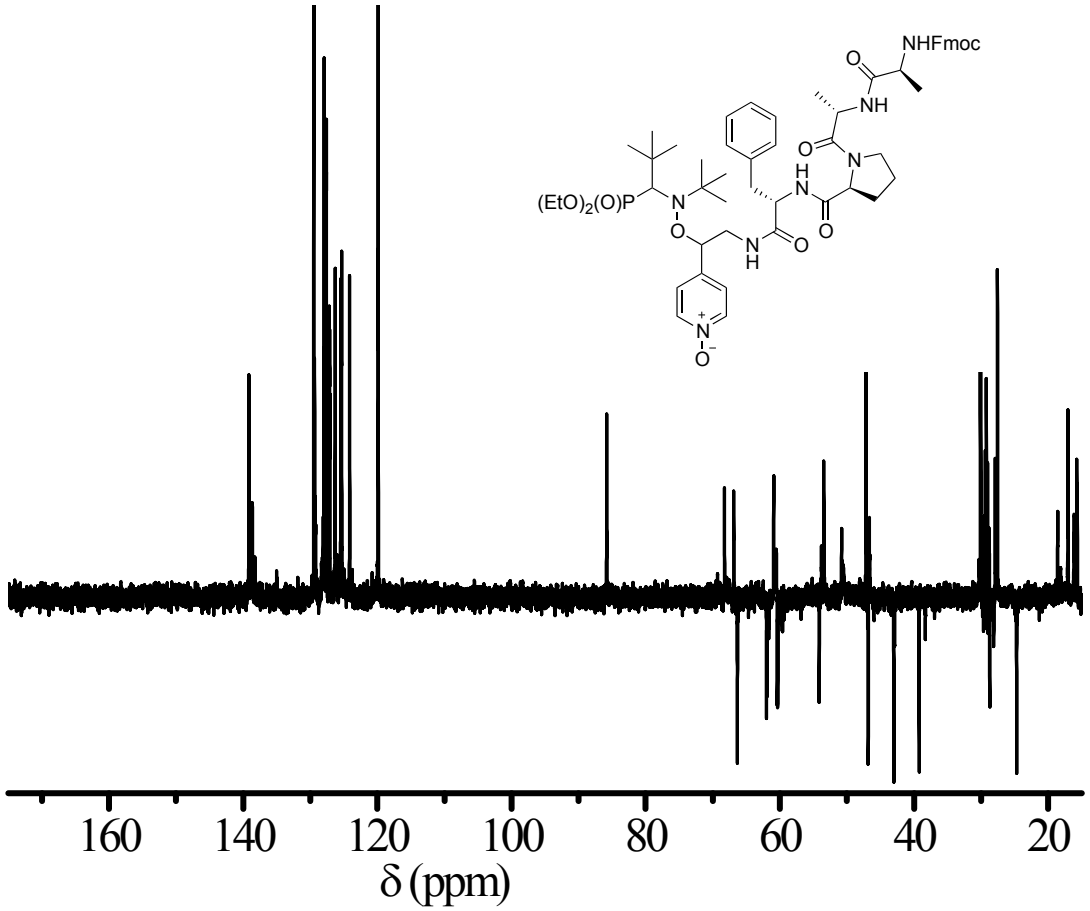
^{1}H NMR of 11.



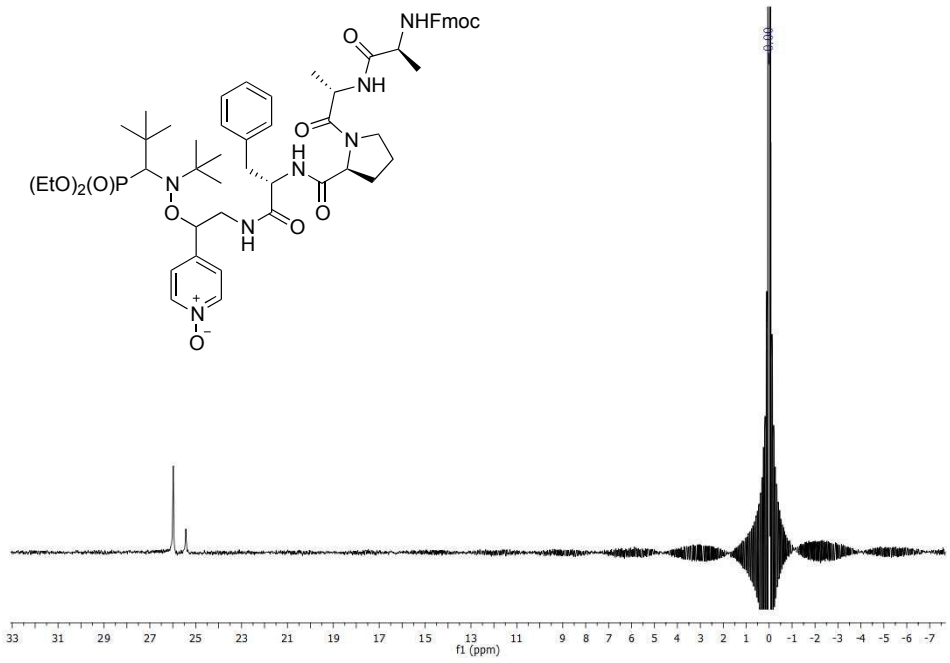
^{13}C NMR of 11.



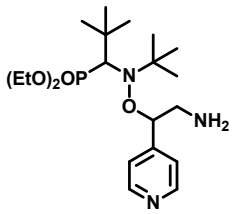
DEPT of 11



^{31}P NMR of 11



Intramolecular Hydrogen bond investigation in 8:



Diastereoisomers ratio ***RS/SR*:*RR/SS*** 1:2

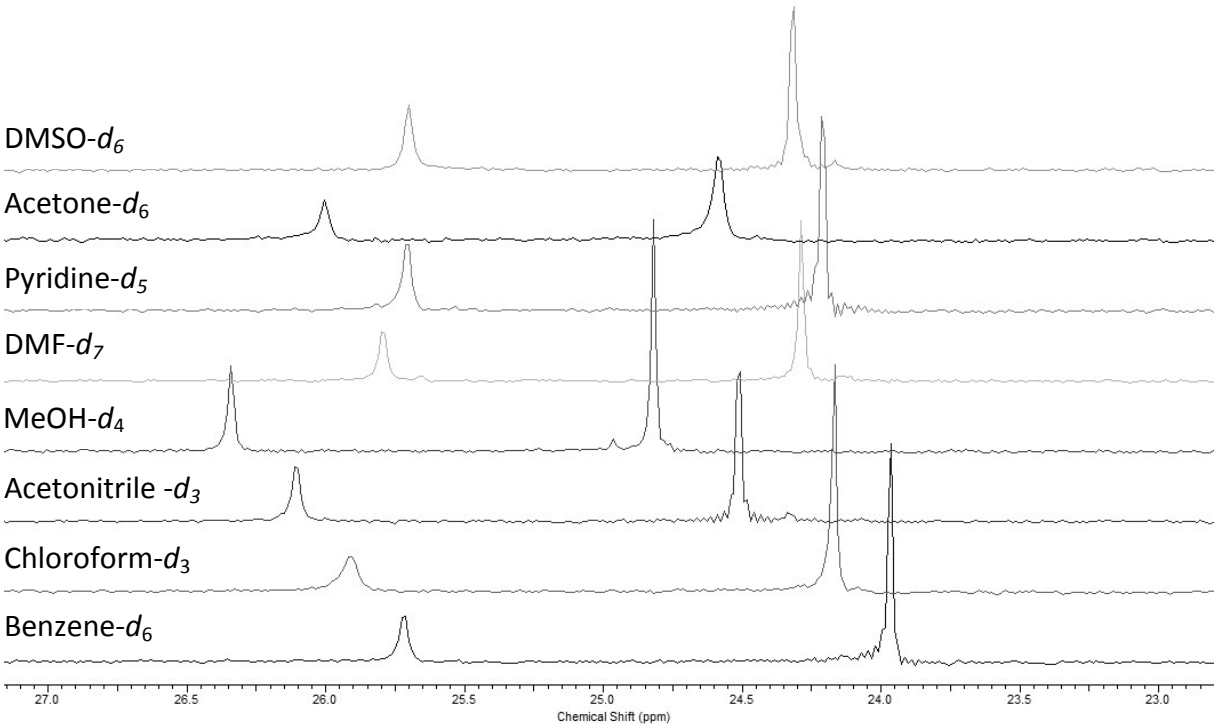


Figure 1SI. ^{31}P NMR of 1:2 mixture of *RS*/*SR*-8 and *SS*/*RR*-8 in various deuterated solvents

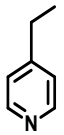
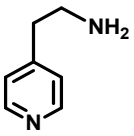
Table 1SI. ^{31}P NMR chemical shift (δ_P in ppm) and difference in δ_P ($\Delta\delta_P$ in ppm) for *RR*/*SS*-**8** and *RS*/*SR*-**8** in various deuterated solvents

	δ_p		$\Delta\delta_p$
Solvant RMN	***RS/SR***	***RR/SS***	
DMSO-d_6	25.7	24.3	1.39
Acetone-d_6	26.0	24.6	1.41
Pyridine-d_5	25.7	24.2	1.48
DMF-d_7	25.8	24.3	1.51
MeOH-d_4	26.3	24.8	1.52
Acetonitrile -d_3	261	24.5	1.60
Chloroforme-d_3	25.9	24.2	1.74
Benzene-d_6	25.7	24.0	1.75

pKa measurements:

The dependence of the chemical shifts of alkoxyamine **2 - 8** upon protonation was investigated by means of ^{1}H NMR spectroscopy (Table 2SI). 0.02 M samples in a D_2O/CD_3OD (v/v 1:1) mixture of alkoxyamine were used. The values of pH* were adjusted with DCl or NaOD (0.2 M) and controlled by a Hanna Instruments HI-2211 pH-meter with SI Analyticsmicro-pH electrode N 6003 (o.d. 3 mm glass, 180 mm stem length) and converted into pH-values *via* the correlation pH = 0.929·pH* + 0.42.[1] ^{1}H NMR spectra were recorded on a Bruker 400 MHz Advance spectrometer.

Table 2SI. pK_a values of **3** and **4**

	Structures	pK_a *RR/SS*		pK_a *RS/SR*
4-éthylpyridine **3**		-	6.03	-
4		-	4.41/8.53	-

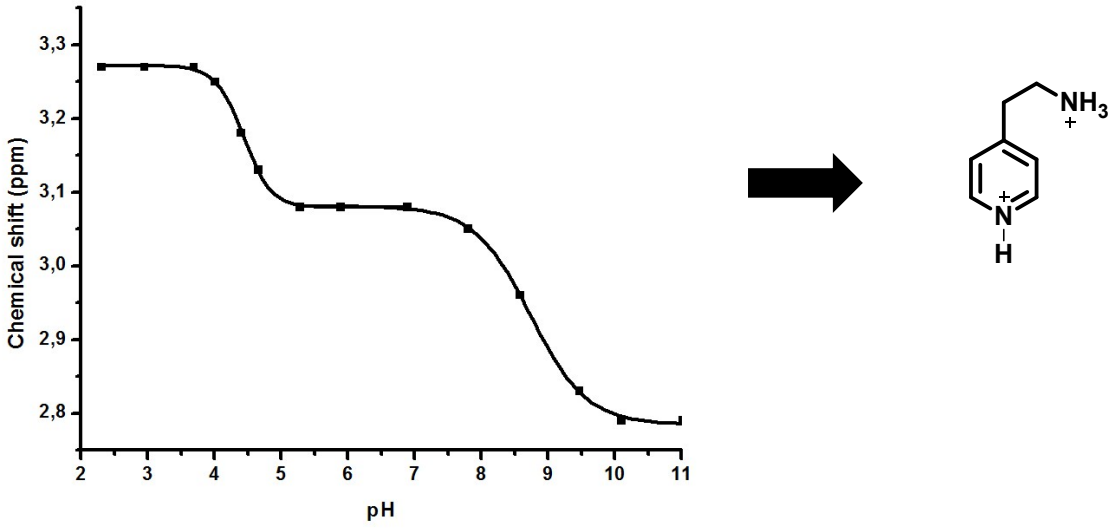


Figure 2SI. pH versus δ_H for **4**

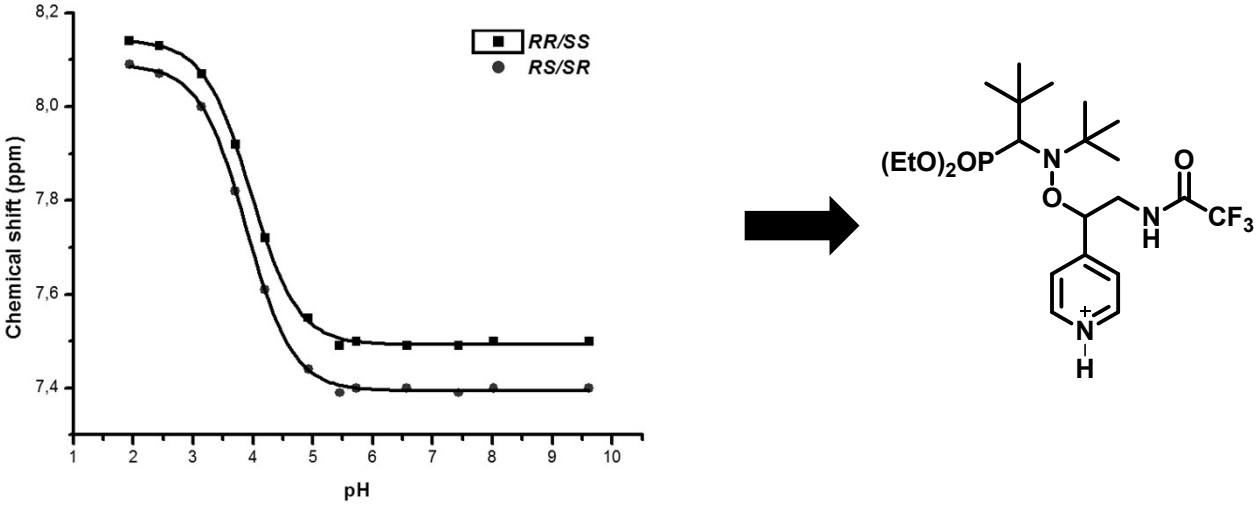


Figure 3SI. pH versus δ_H for 7

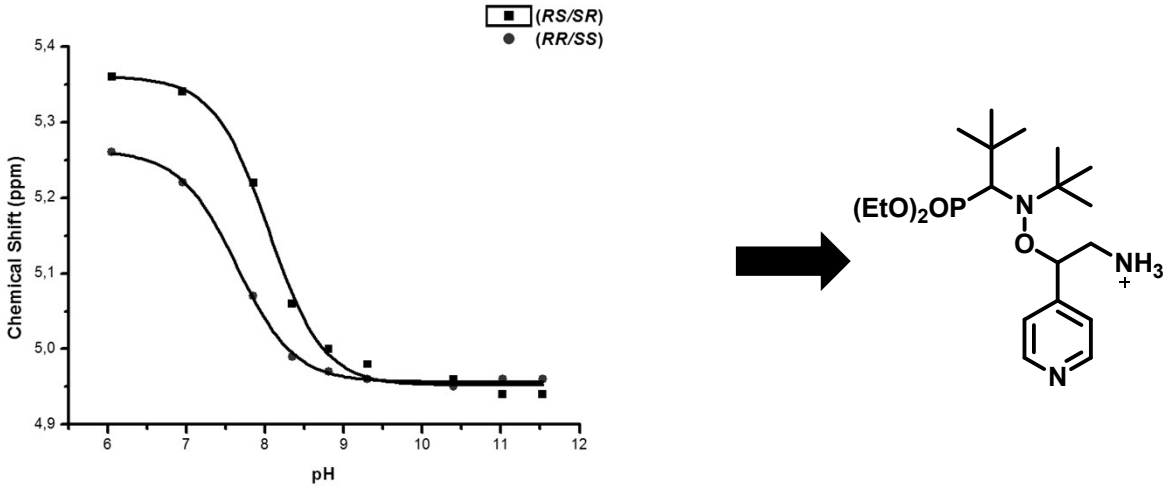


Figure 4SI. pH versus δ_H for the protonation of amino group in **8**

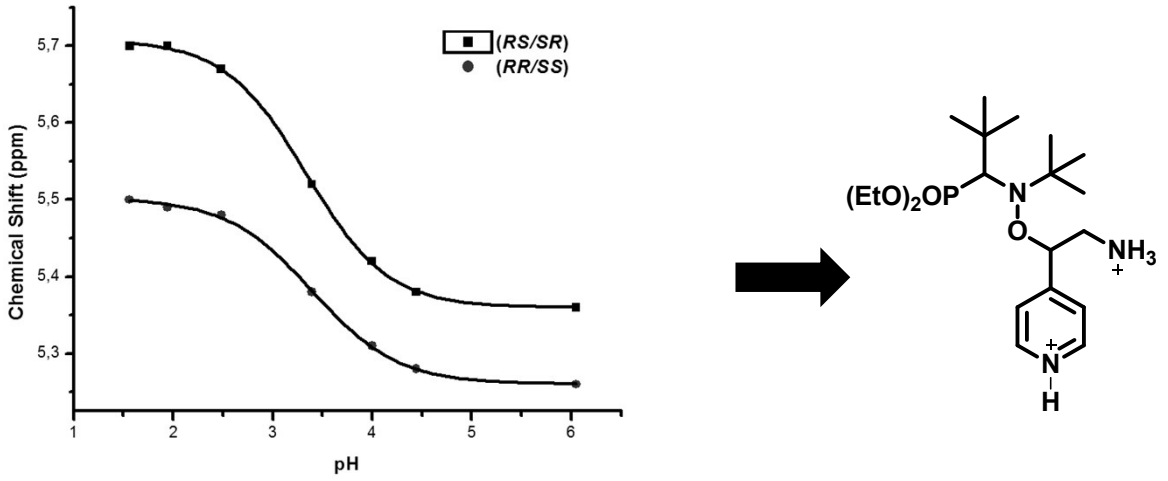


Figure 5SI. pH versus δ_H for the protonation of both the amino group and pyridyl moiety in 8

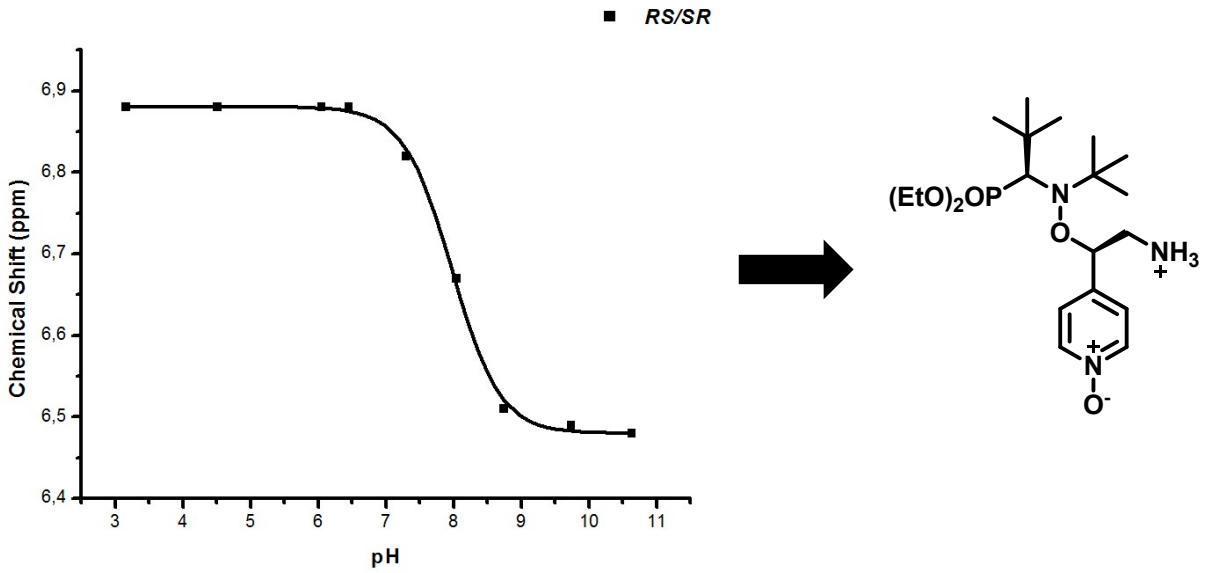


Figure 6SI. pH versus δ_H for the protonation of amino group in *RS*/*SR*-**9**

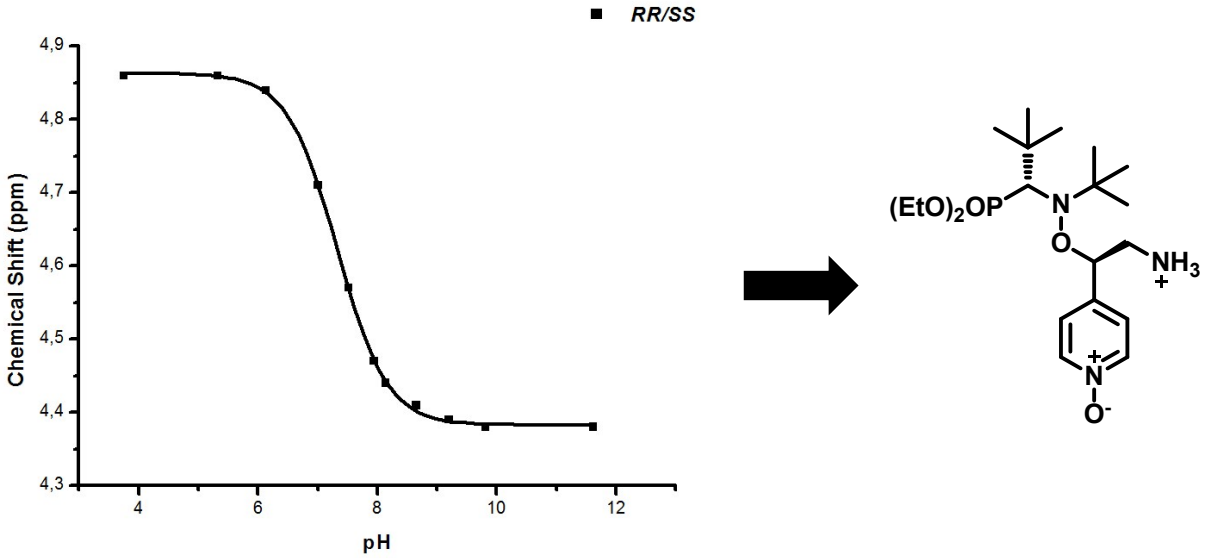


Figure 7SI. pH versus δ_H for the protonation of amino group in *RR/SS*-9

Kinetic measurements

EPR experiments were performed on X-band EMX machine (Bruker) equipped with temperature control unit. The values of k_d were measured by recording ESR spectra upon heating of 10^{-4} M solutions of alkoxyamines in the presence of oxygen as alkyl radical scavenger. Profiles of the relative concentration are obtained by integration of the EPR signal of **SG1** and the data are fitted linear in semi logarithmic coordinates with eq. 1

$$ln\frac{[C]_0-[C]}{[C]_0} = -k_d t \quad \text{eq. 1}$$

The values of k_d are gathered in Table 3SI. Activation energies E_a are given by the Arrhenius equation (eq. 2) using the value of 2.4 10^{14} s^{-1} as frequency factor A_0[2] and half-life time $t_{1/2}$ by eq. 3.

$$k_d = A_0 \times e^{-\frac{E_a}{RT}} \quad \text{eq. 2}$$

$$t_{1/2} = \frac{\ln 2}{k_d} \quad \text{eq. 3}$$

In all cases, error of les than 3% is reported when eq. 1 is used to determine k_d values.

Table 3SI. Experimental conditions (temperature *T*, solvents, pH) and experimental rate constants k_d for **1, 2,** and **7-9**. Errors on values are less than 1%.

Structures	Conditions	*T* (°C)	k_d (× 10^{-4} s^{-1})	
			RR/SS	***RS/SR***
1	***t*-BuPh**	81		4.5
7	***t*-BuPh**	130		243.0
		110	31.9	
7H+	***t*-BuPh + 2eq. TFA**	70	3.2	0.9
8	***t*-BuPh**	130		227.0
		120	110.0	
8H+	***t*-BuPh + 1eq. TFA**	89		7.7
		81	5.0	

8H2+	***t*-BuPh + 2eq. TFA**	90		13.7
		79	4.3	
	***t*-BuPh + 3eq. TFA**	90		14.6
		81	5.1	
9	***t*-BuPh**	70		1.0
		50	16.8	
2	***t*-BuPh**	50		0.4
		80	26.6	
2H+	***t*-BuPh + 2eq. TFA**	50		3.1
		80	154.0	
7	**H2O/MeOH (1:1)**	90		8.6
		71	3.0	
7H+	**H_2O/MeOH (1:1) pH = 1.9**	80	176.0	116.0
8	**H2O/MeOH (1:1) pH = 10**	90		14.0
		80	6.1	
8H+	**H_2O/MeOH (1:1) pH = 6**	90		55.9
		80	56.1	
8H2+	**H2O/MeOH (1:1) pH = 1**	60		48.5
		50	39.4	

9	**H_2O/MeOH (1:1)**	70		6.1
		50	3.2	
2	**H2O/MeOH (1:1) pH = 10**	50		1.0
		70	15.7	
2H+	**H_2O/MeOH (1:1) pH = 6**	50		7.6
		60	60.1	
2H+	**H_2O HEPES pH 7.4**	37		1.1
1	**H_2O HEPES pH 7.4**	37		0.2
1 + Chymotrypsin	**H_2O HEPES pH 7.4**	37		0.83
1 + Subtilisin A	**H_2O HEPES pH 7.4**	37		1.04
1 + trypsin	**H_2O HEPES pH 7.4**	37		0.15
1 + pancreatic elastase	**H_2O HEPES pH 7.4**	37		0.13

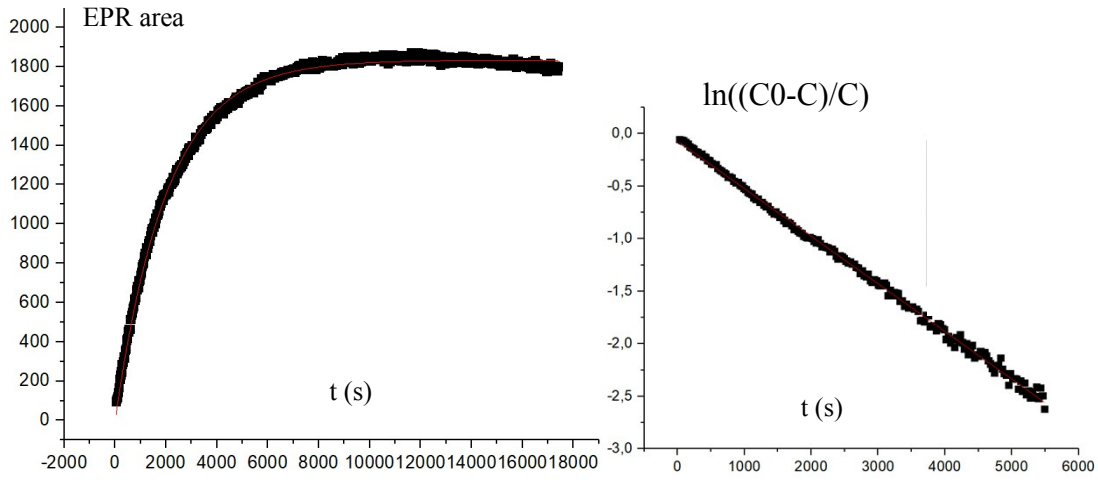


Figure 8SI. Alkoxyamine *RS/SR*-1 in tBuPh. (left) plot EPR area vs *t* and (right) plot ln((C_0-C)/C) vs *t*

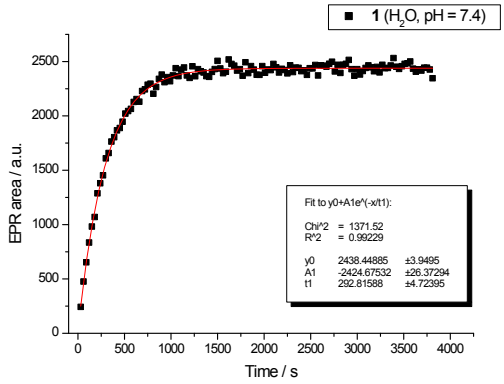


Figure 9SI. Alkoxyamine *RS/SR*-1 in HEPES

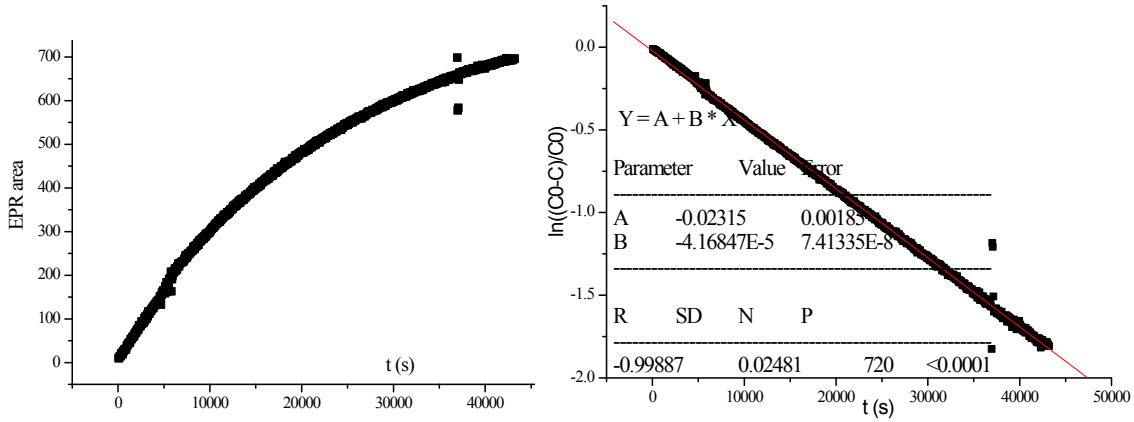


Figure 10SI. Alkoxyamine *RS/SR*-2 in tBuPh. (left) plot EPR area vs *t* and (right) plot ln((C_0-C)/C) vs *t*

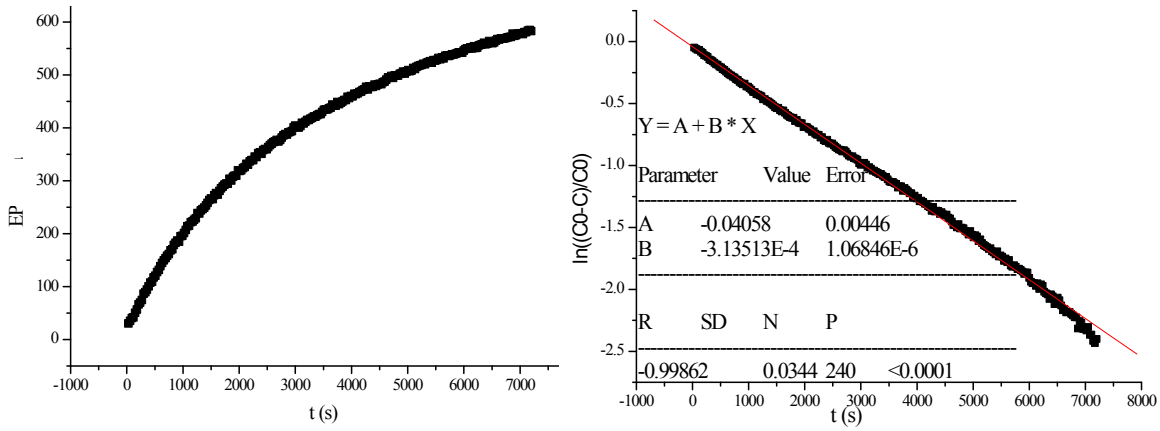


Figure 11SI. Alkoxyamine *RS/SR*-2 in tBuPh + 2 TFA. (left) plot EPR area vs *t* and (right) plot ln((C_0-C)/C) vs *t*

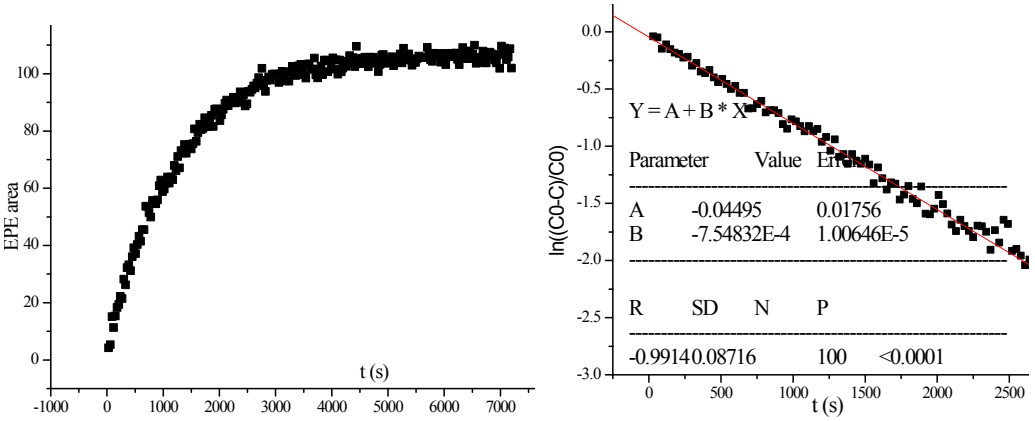


Figure 12SI. Alkoxyamine *RS/SR*-2 in tBuPh pH = 6. (left) plot EPR area vs *t* and (right) plot ln((C_0-C)/C) vs *t*

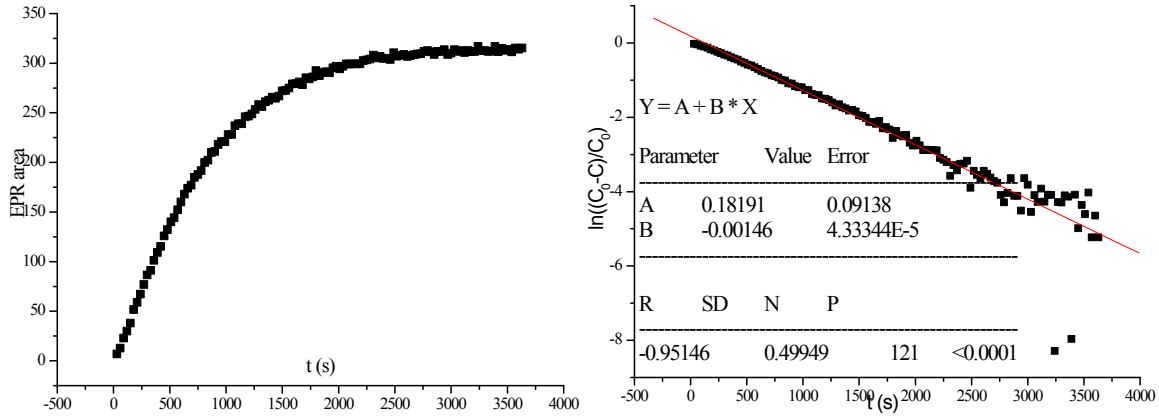


Figure 13SI. Alkoxyamine RS/SR-8 in tBuPh + 3TFA. (left) plot EPR area vs *t* and (right) plot ln((C_0-C)/C) vs *t*

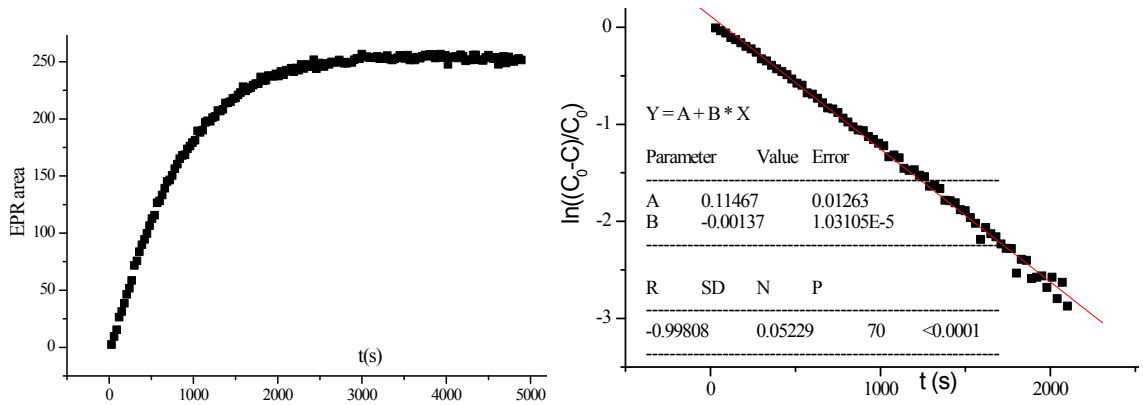


Figure 14SI. Alkoxyamine *RS/SR*-8 in tBuPh + 2TFA. (left) plot EPR area vs *t* and (right) plot ln((C_0-C)/C) vs *t*

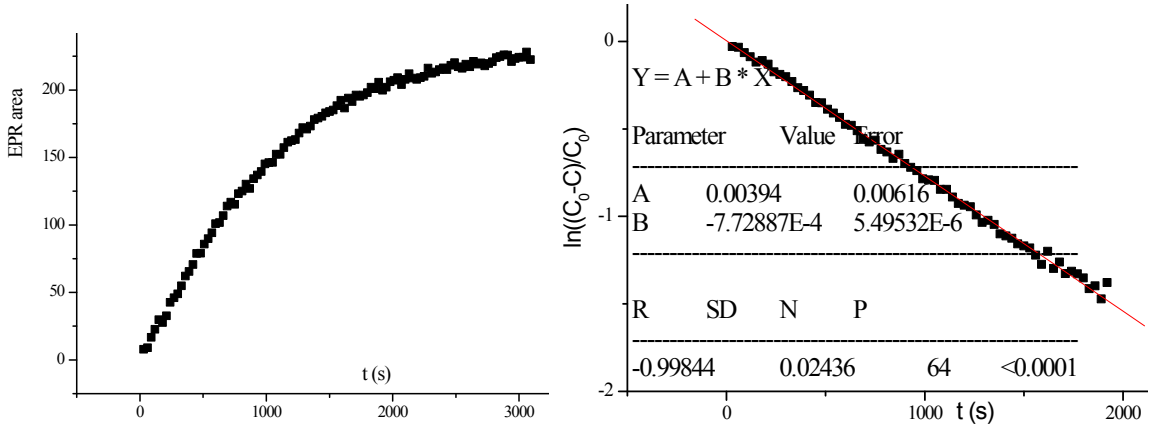


Figure 15SI. Alkoxyamine *RS/SR*-8 in tBuPh + 1TFA. (left) plot EPR area vs *t* and (right) plot ln((C$_0$-C)/C) vs *t*

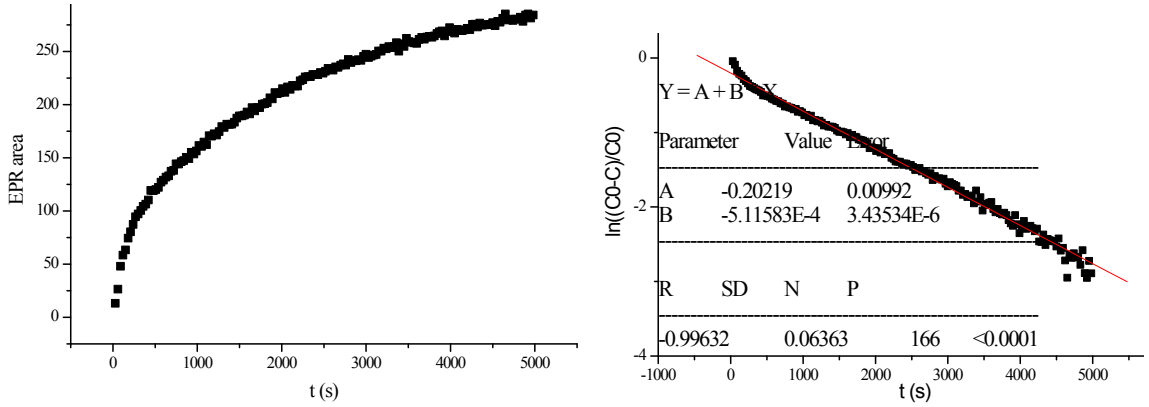


Figure 16SI. Alkoxyamine *RR/SS*-8 in tBuPh + 3eqTFA. (left) plot EPR area vs *t* and (right) plot ln((C$_0$-C)/C) vs *t*

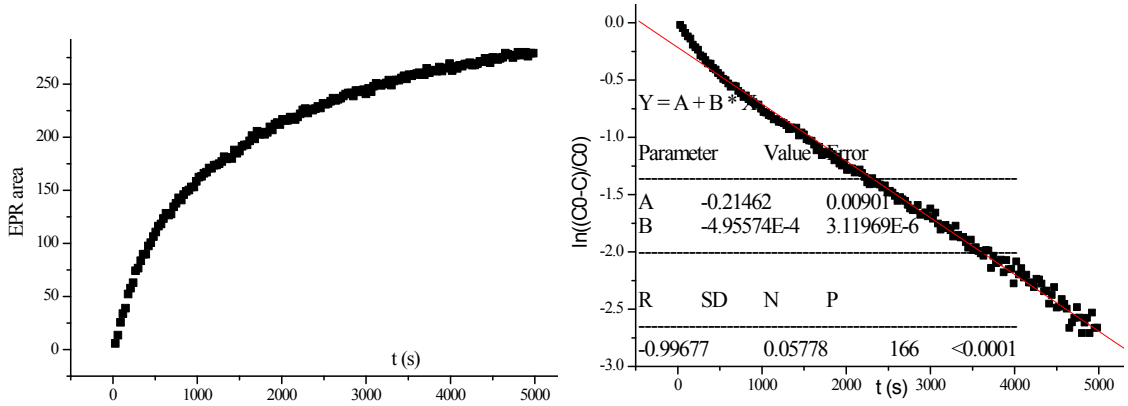


Figure 17SI. Alkoxyamine *RR/SS*-8 in tBuPh + 1eqTFA. (left) plot EPR area vs *t* and (right) plot ln((C$_0$-C)/C) vs *t*

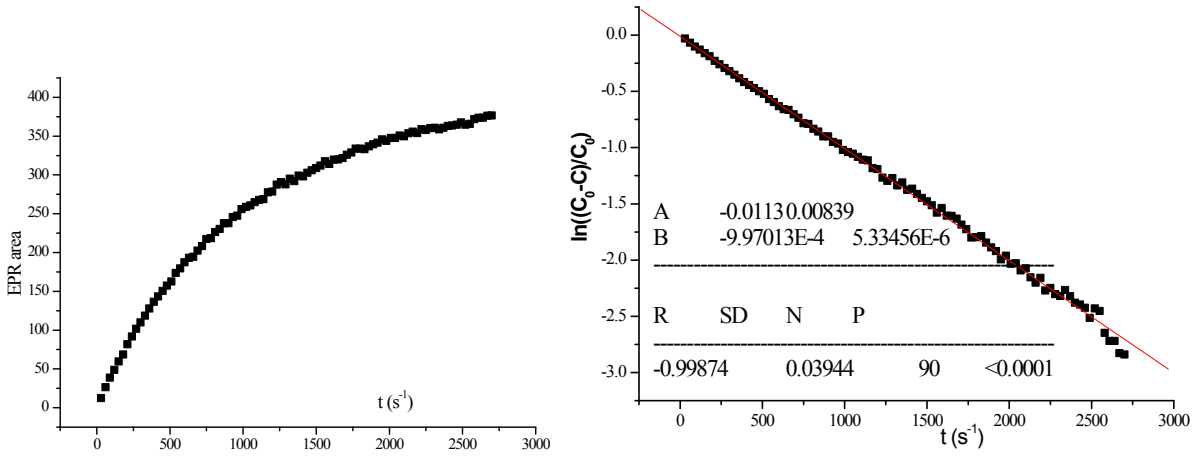


Figure 18SI. Alkoxyamine *RS/SR*-9 in tBuPh. (left) plot EPR area vs *t* and (right) plot ln((C_0-C)/C) vs *t*

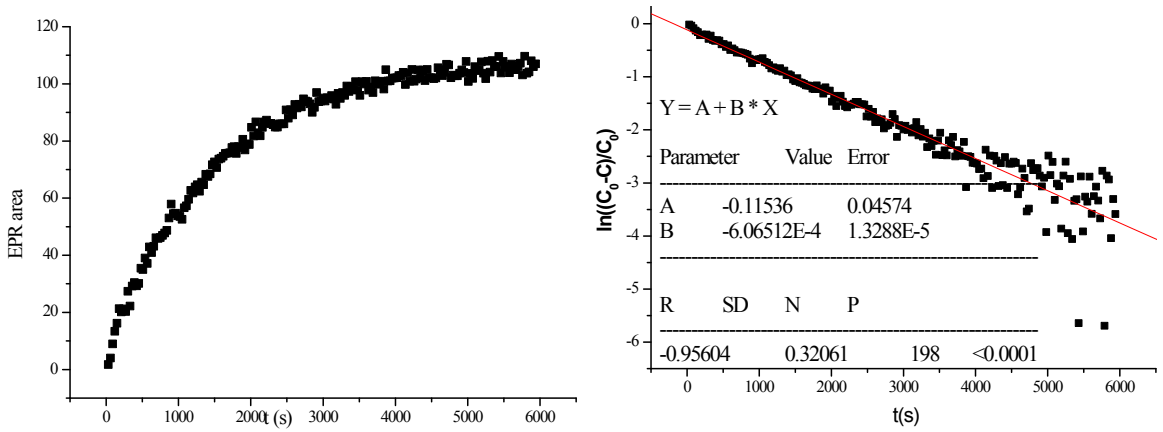


Figure 19SI. Alkoxyamine *RS/SR*-9 in MeOH/water. (left) plot EPR area vs *t* and (right) plot ln((C_0-C)/C) vs *t*

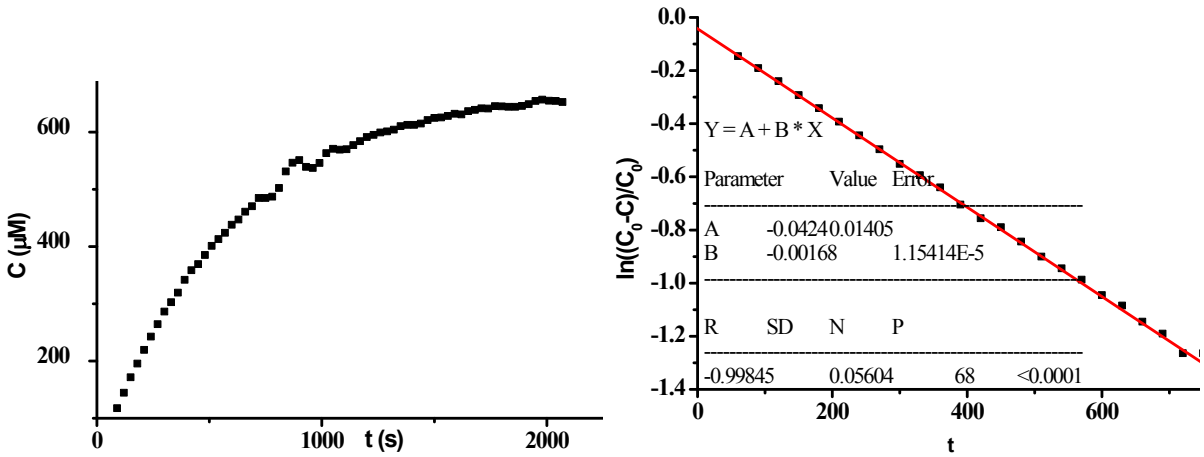


Figure 20SI. Alkoxyamine *RR/SS*-9 in tBuPh. (left) plot EPR area vs *t* and (right) plot ln((C_0-C)/C) vs *t*

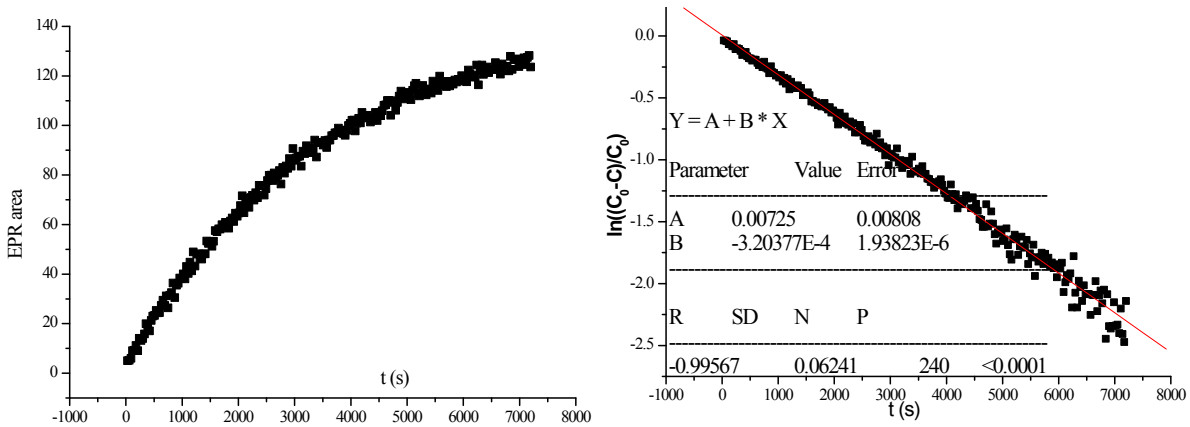


Figure 21SI. Alkoxyamine *RR/SS*-9 in MeOH/water. (left) plot EPR area vs *t* and (right) plot ln((C_0-C)/C) vs *t*

Table 4SI. Statistical report and kinetic model for the activation of **1** in the presence of different enzymes displayed in Figure 2.

Model	kineticsfirstorder (User)				
Equation	y = A0 - A0*exp(-k1*x)+y0				
		Value	Standard Error	R^2	χ^2
1	A0	35.5	0.09	0.99	0.71
1	k1	2.0E-5	1.1E-7		
1	y0	2.4	0.1		
2H+	A0	52.1	0.21	0.99	0.945
2H+	k1	1.1E-4	8.2E-7		
2H+	y0	3.6	0.21		
1+Chymotripsin	A0	41.2	0.18	0.98	0.96
1+ Chymotripsin	k1	8.3E-5	6.6E-7		
1+ Chymotripsin	y0	-1.4	0.18		
1+PPE	A0	38.2	0.08	0.99	0.71
1+PPE	k1	1.3E-5	8.2E-8		
1+PPE	y0	3.5	0.09		
1+Subtilisin	A0	47.2	0.27	0.97	1.12
1+Subtilisin	k1	1.0E-4	9.2E-7		
1+Subtilisin	y0	1.0	0.28		
1+Trypsin	A0	28.6	0.07	0.98	0.52
1+Trypsin	k1	1.4E-5	8.2E-8		
1+Trypsin	y0	3.0	0.07		

XRD Analysis

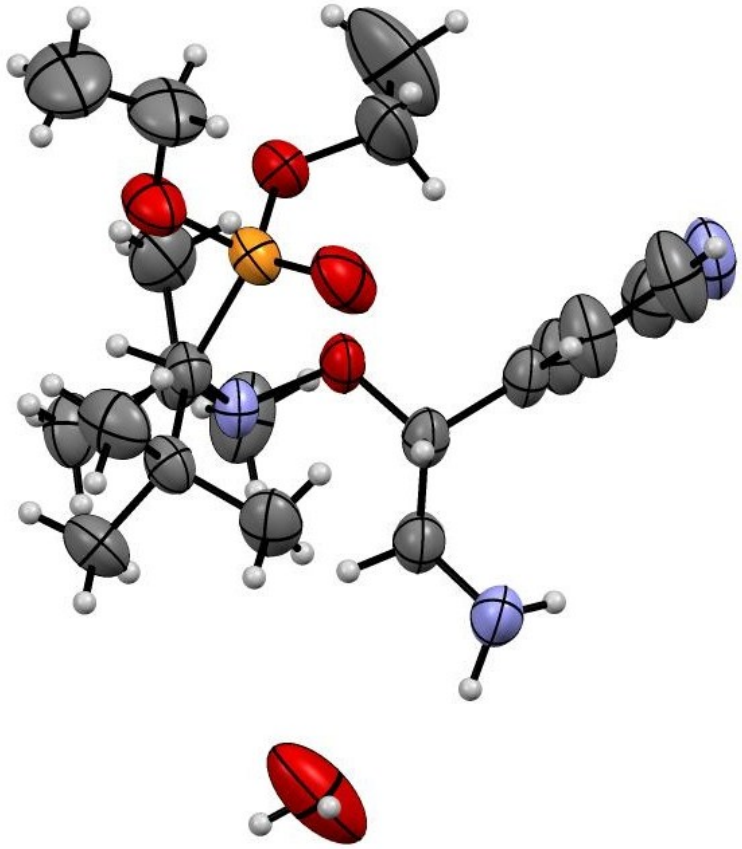


Figure 22SI. ORTEP of *RR/SS*-8. Ellipsoïd at 50%. Crystallized with diethyl ether in fridge.

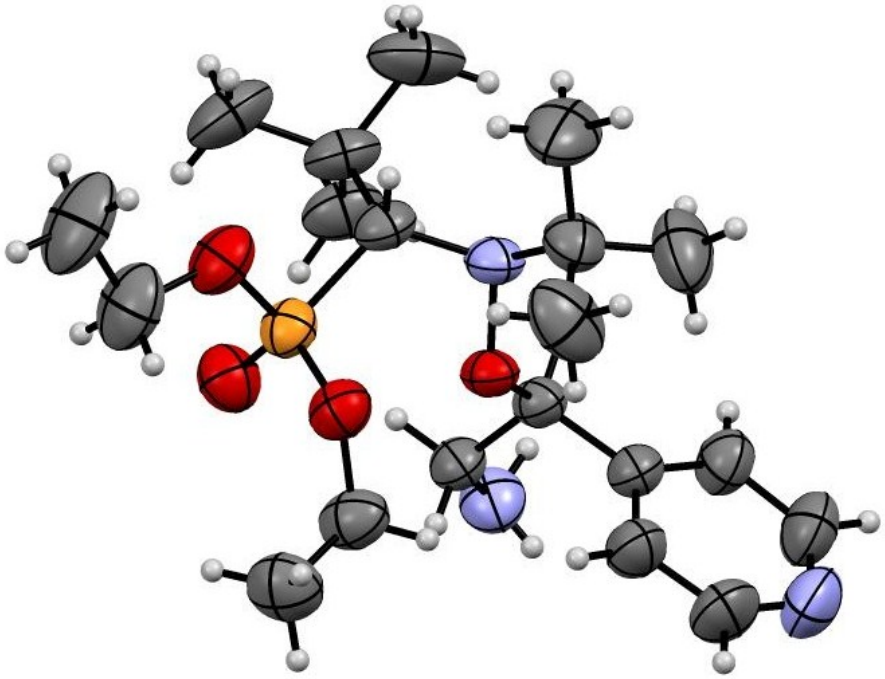


Figure 23SI. ORTEP of *RS/SR*-8. Ellipsoïd at 50%. Crystallized with diethyl ether in fridge.

Table 5SI. XRD data for *RR*/*SS*-**8** and *RS*/*SR*-**8.**

Compound	*RR/SS*-**8**	*RS/SR*-**8**
Empirical formula	$C_{20}H_{38}N_3O_4P$	$C_{20}H_{38}N_3O_4P$
Formula weight	415.50	415.50
Temperature K	293(2)	293(2)
Wavelength Å	1.54184	1.54184
Crystal system	triclinic	monoclinic
Space group	P -1	P 2_1/c
Unit cell dimensions *a* Å	3.2786(6)	8.8282(6)
b Å	9.5213(6)	22.0666(14)
c Å	15.4265(6)	12.5309(6)
α °	75.673(4)	90.0
β °	76.438(4)	96.936(5)
γ °	71.300(5)	90.0
Volume Å^3	1232.76 (11)	2423.(2)
Z	2	4
Density (calcd.) Mg.m^{-3}	1.168	1.139
Abs. coefficient mm^{-1}	1.256	1.228
F(000)	472	904
Crystal size mm^3	0.22	0.22
Θ range for data collection °	73.728	73.81
Index ranges	-10/11; -10/18; -17/18	-10/10; -26/26; -15/15
Reflections collected	7179	8051
Independent reflections	4718	4694
Completeness to θ %	66.97	98.99
Data / restraints / parameters	4038/0/273	3780/0/267
Goodness-of-fit on F^2	1.053	1.04
Final R indices $I > 2\sigma(I)$	0.0522	0.0477
Final R indices (all data)	0.0601	0.0584
Largest diff. peak / hole e.Å^{-3}	0.405/-0.315	0.182/-0.415
CCDC	1880849	1880850

References

[1] Krężel, A.; Bal, W. Formula for Correlating P K a Values Determined in D_2O and H_2O. *J. Inorg. Biochem.* **2004**, *98*, 161-166.

[2] Bagryanskaya, E. G.; Marque, S. R. A. RSC Polymer Chemistry Series, n°=19, Nitroxide Mediated Polymerization: From Fundamentals to Applications in Materials Sciences Gigmes, D., Ed., Royal Society of Chemistry, **2016**, chapter 2, 45-113