A simple method of synthesis of 3-carboxy-2,2,5,5-tetraethylpyrrolidine-1-oxyl and preparation of reduction-resistant spin labels and probes of pyrrolidine series

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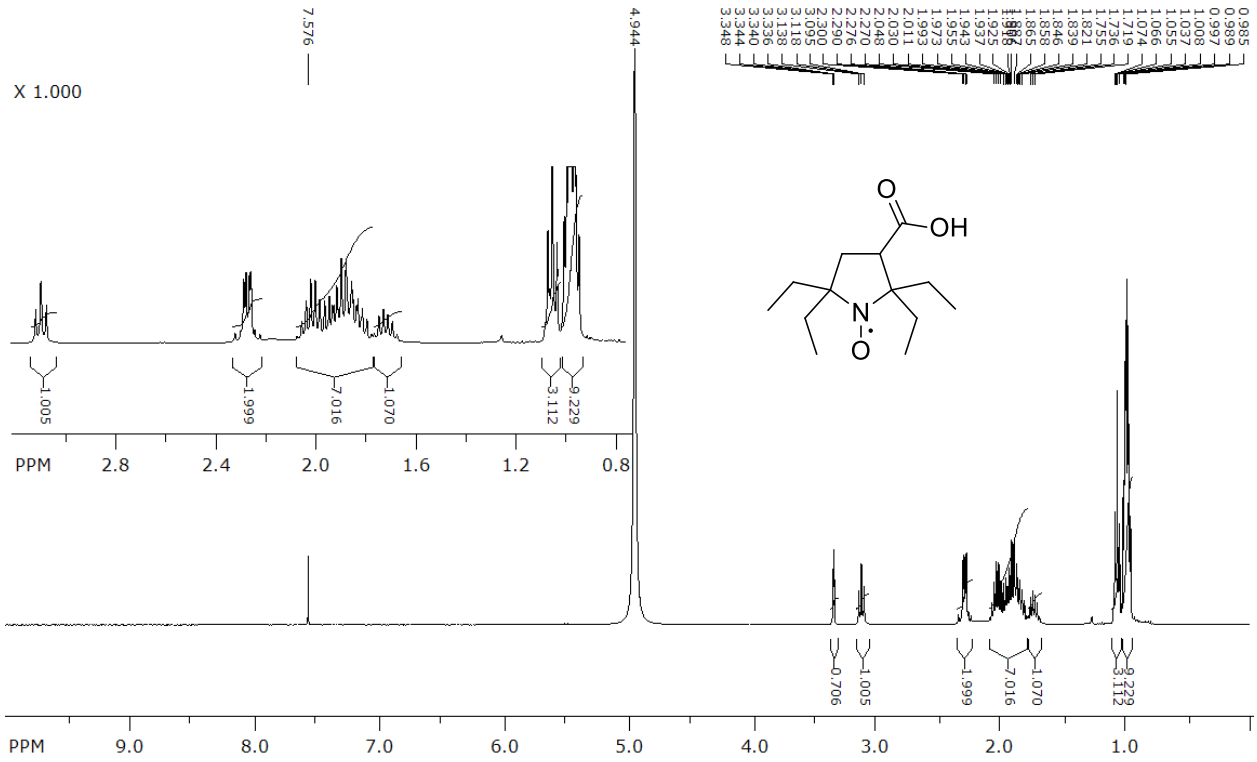
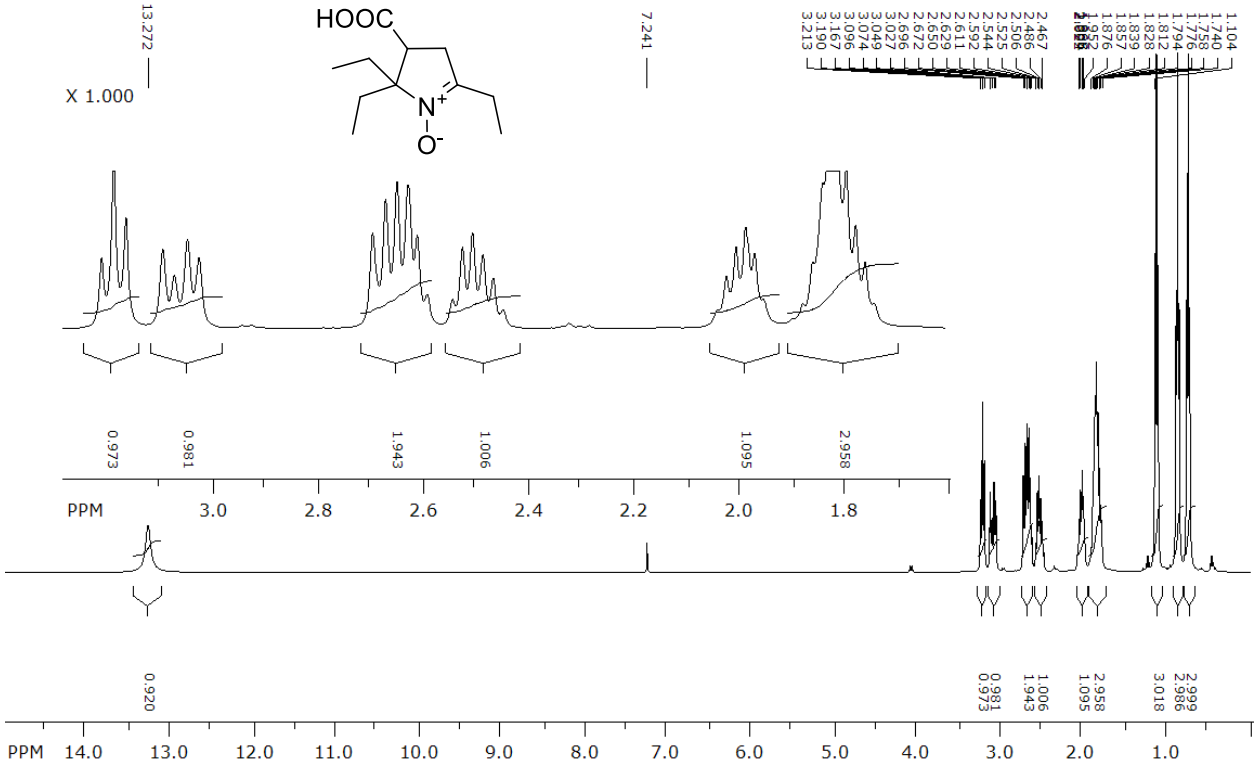
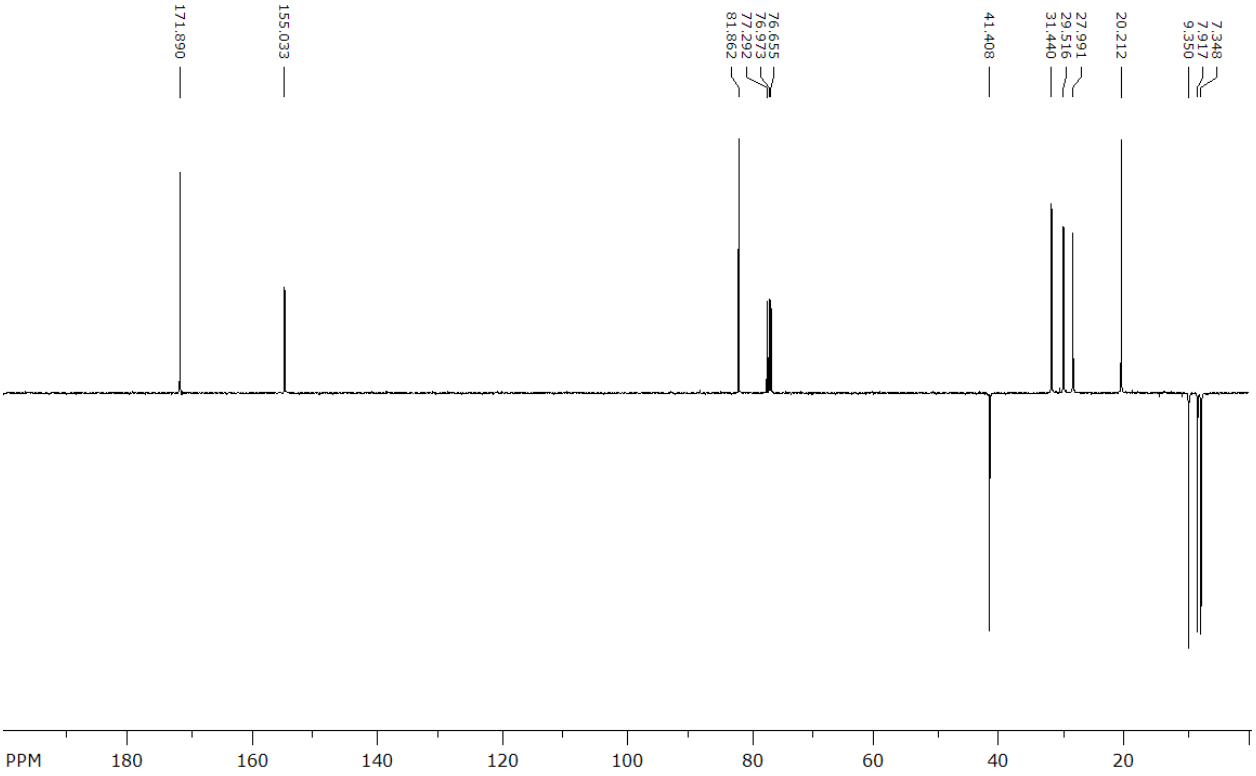


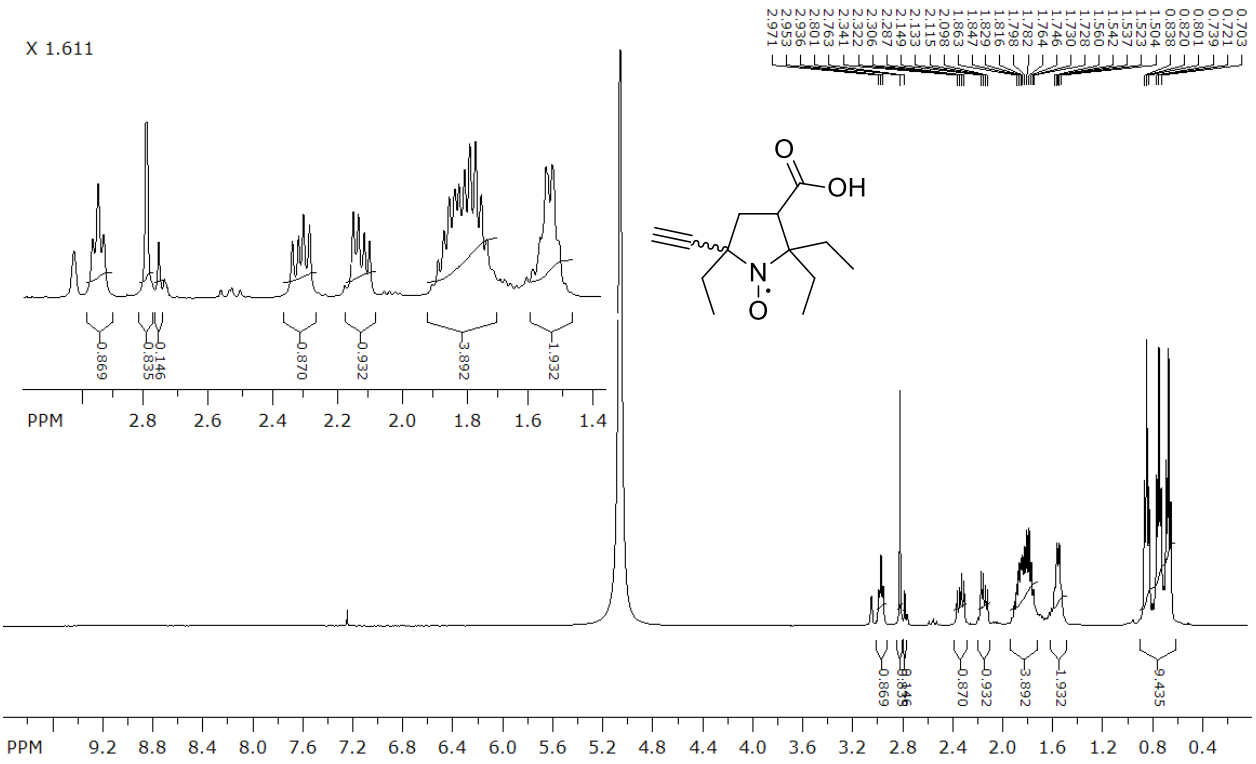
^{1}H NMR (400 MHz; $CDCl_3$) of 3-carboxy-2,2,5-triethyl-3,4-dihydro-2*H*-pyrrole 1-oxide (8).



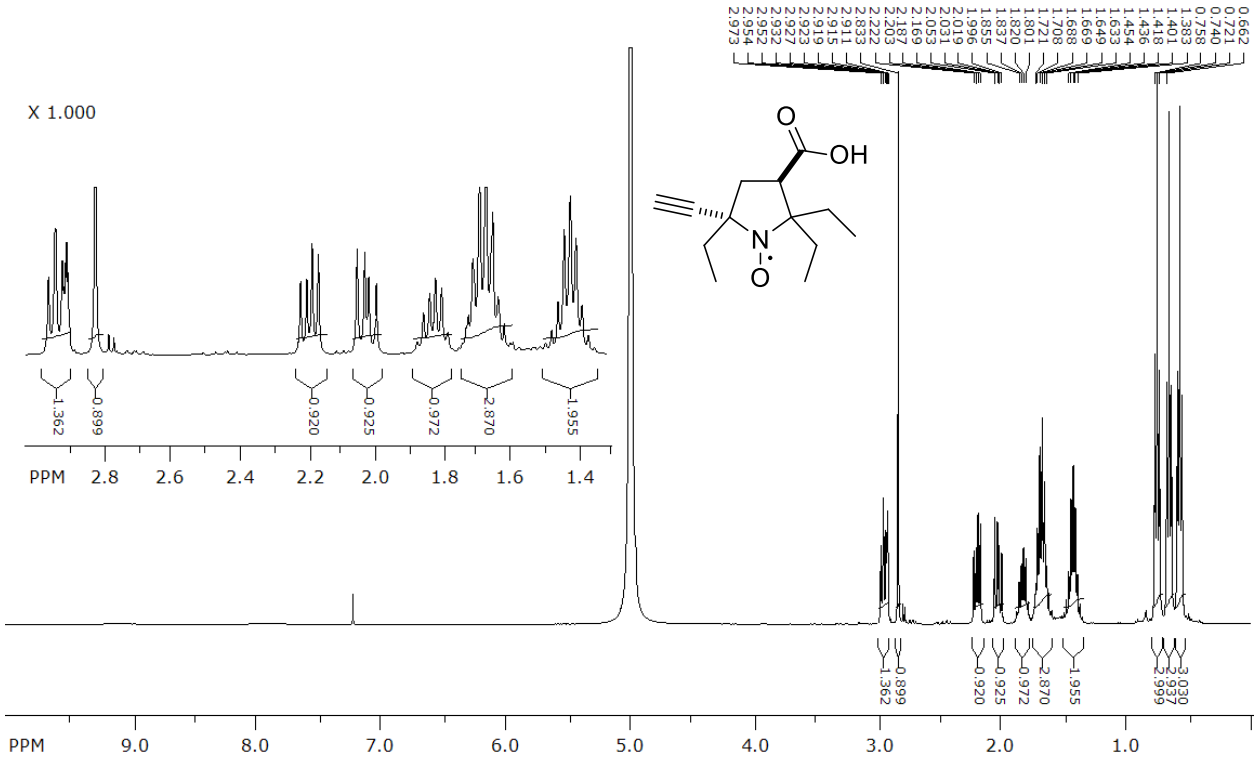
$^{13}C\{^{1}H\}$ NMR (100 MHz; $CDCl_3$) of 3-carboxy-2,2,5-triethyl-3,4-dihydro-2*H*-pyrrole 1-oxide (8).



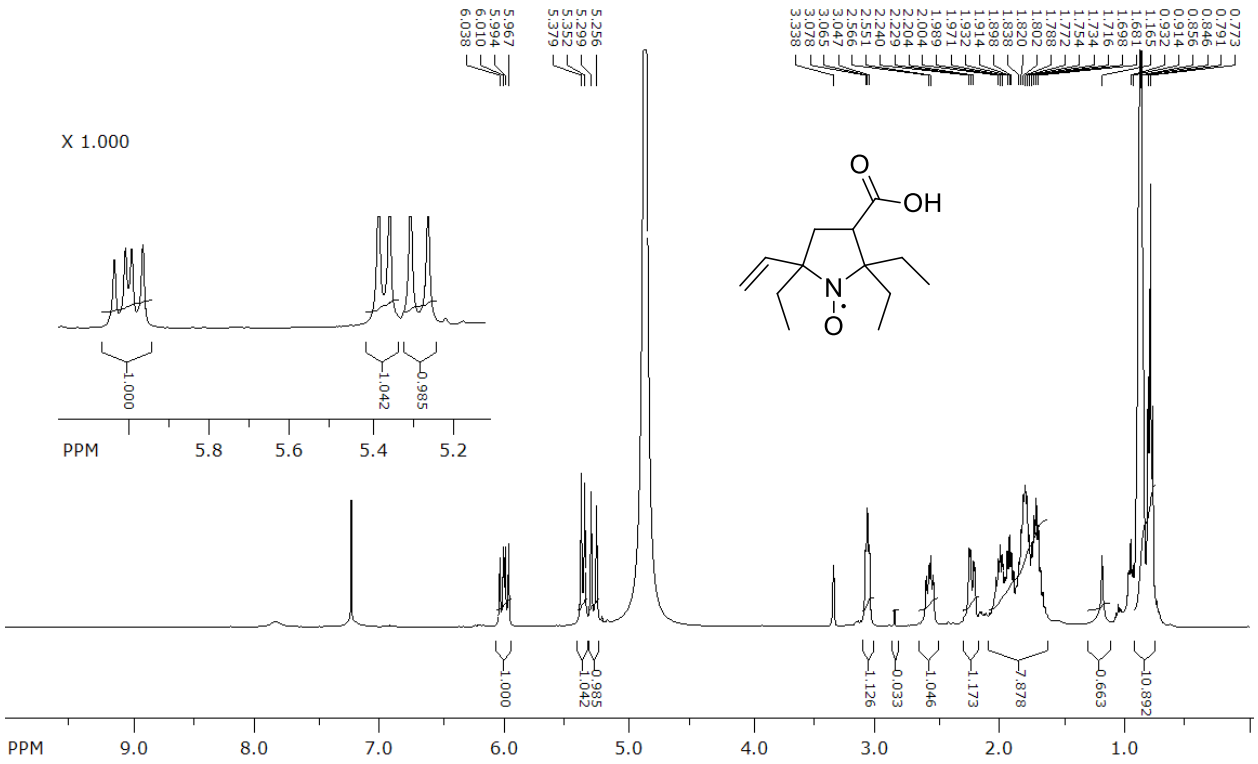
^{1}H NMR (400 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH) of 3-carboxy-5-ethynyl-2,2,5-triethylpyrrolidine-1-oxyl – mixture of isomers (10a,b).



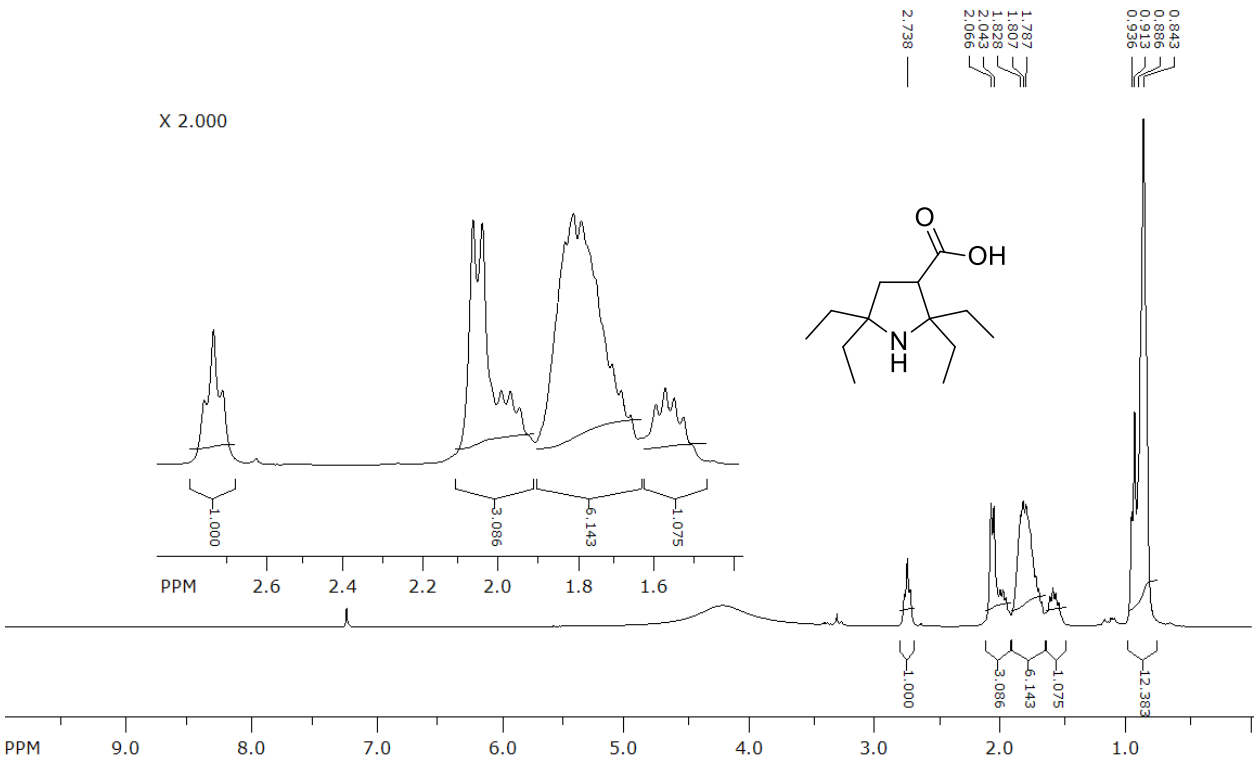
^{1}H NMR(400 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH) of 3-carboxy-5-ethynyl-2,2,5-triethylpyrrolidine-1-oxyl –major isomer (10a).



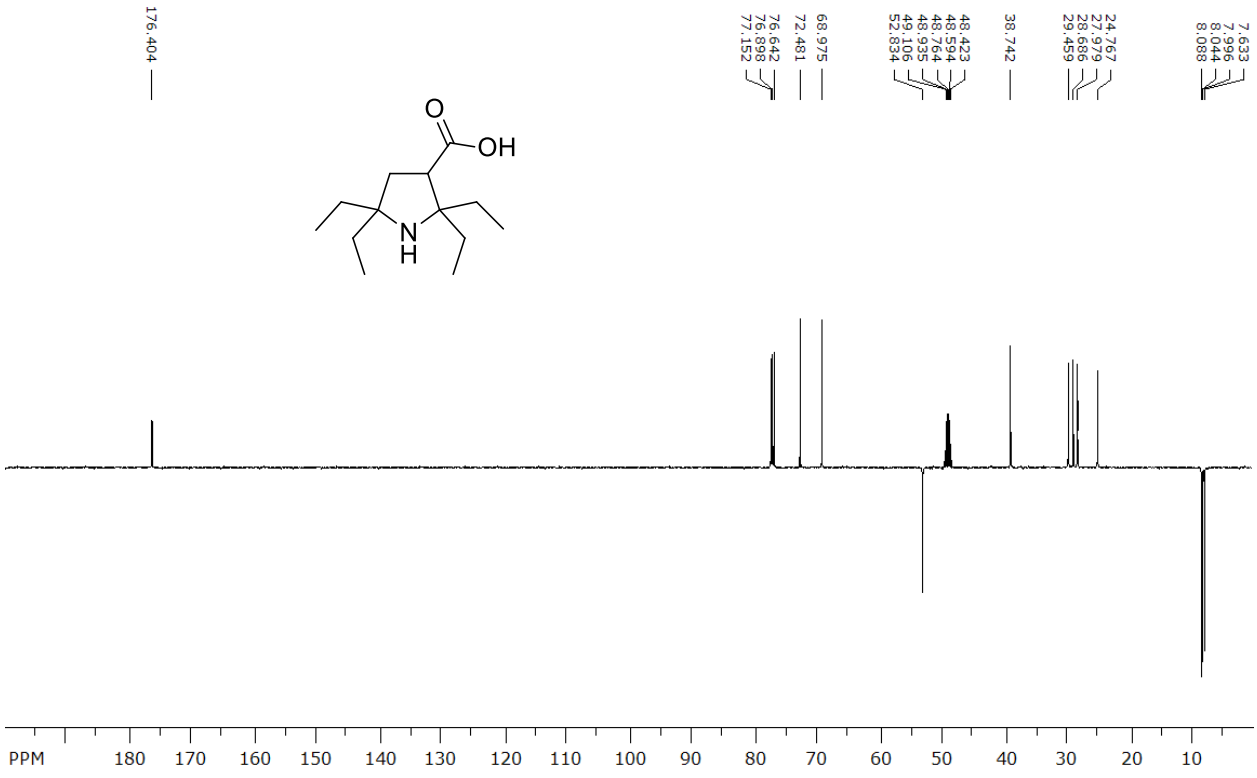
^{1}H NMR(400 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH) of 3-carboxy-5-vinyl-2,2,5-triethylpyrrolidine-1-oxyl (14).



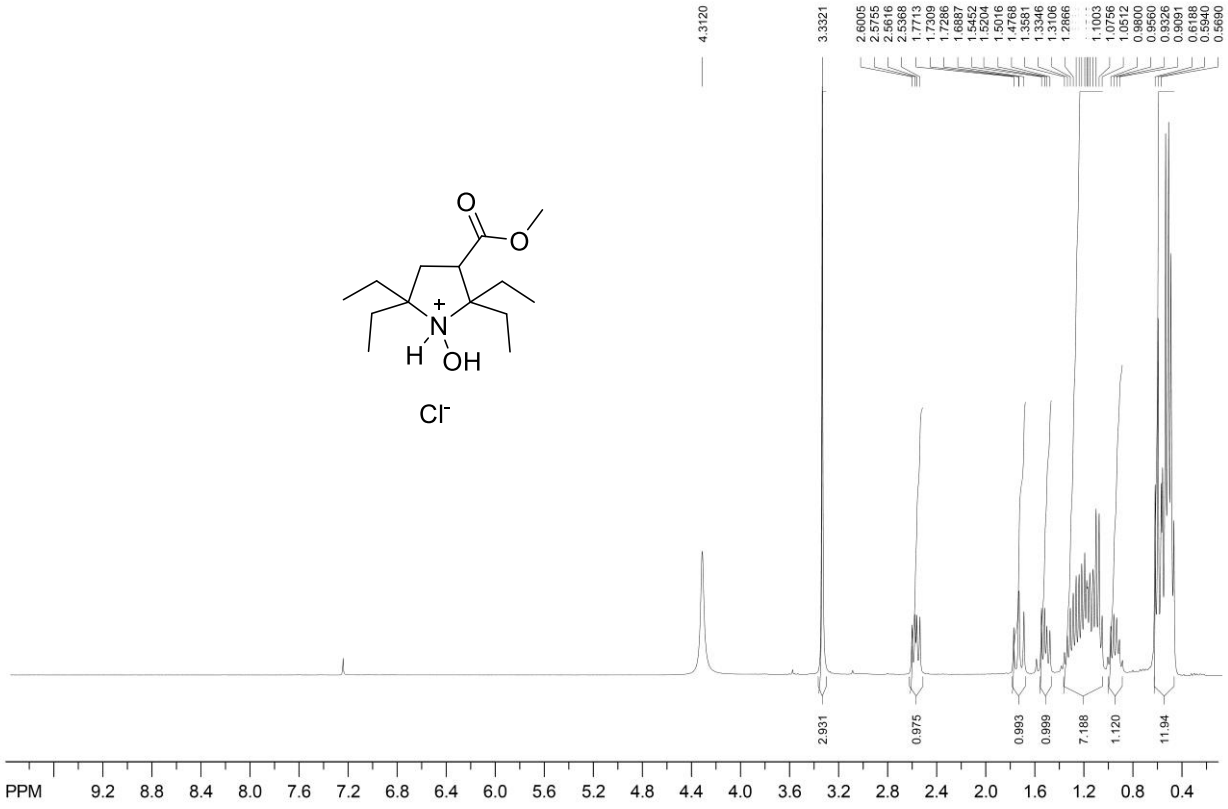
^{1}H NMR (400 MHz; $CD_3OD/CDCl_3$) of 3-carboxy-2,2,5,5-tetraethylpyrrolidine (15).



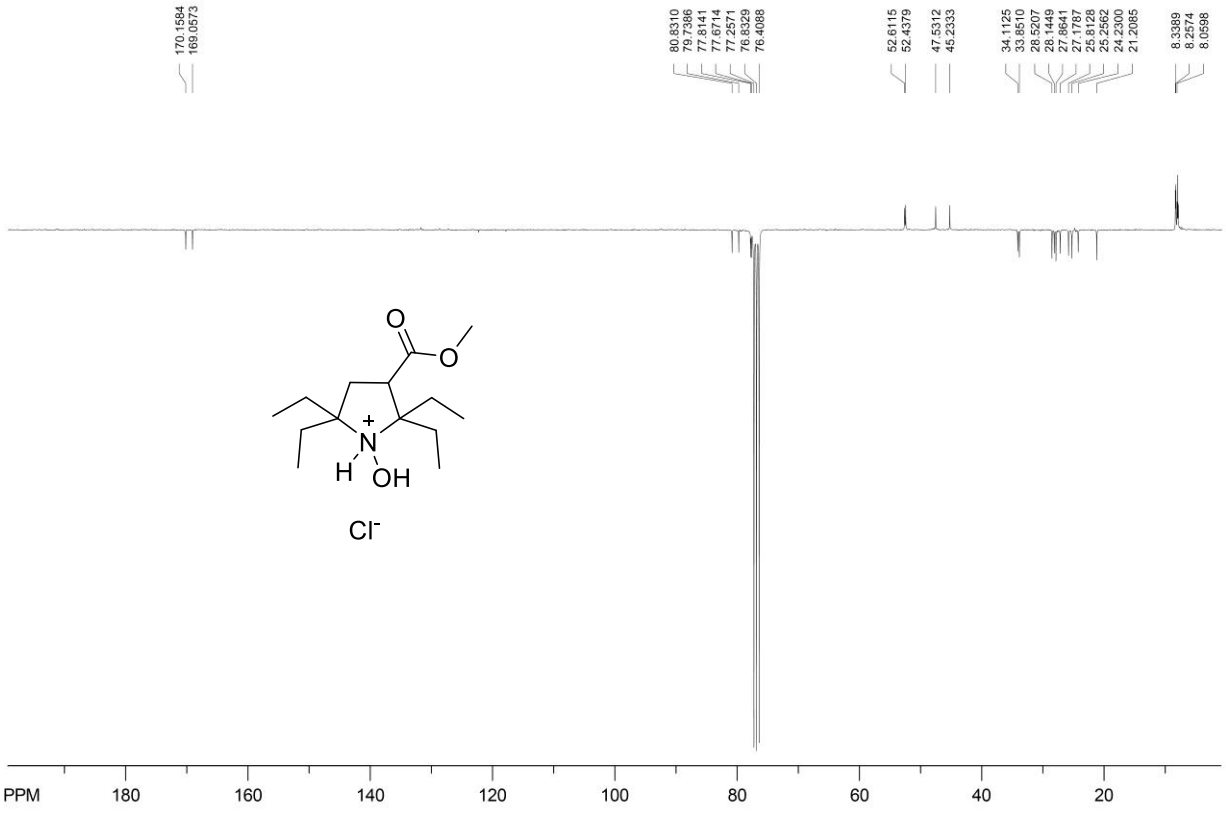
^{13}C{^{1}H} NMR (100 MHz; $CD_3OD/CDCl_3$) of 3-carboxy-2,2,5,5-tetraethylpyrrolidine (15).



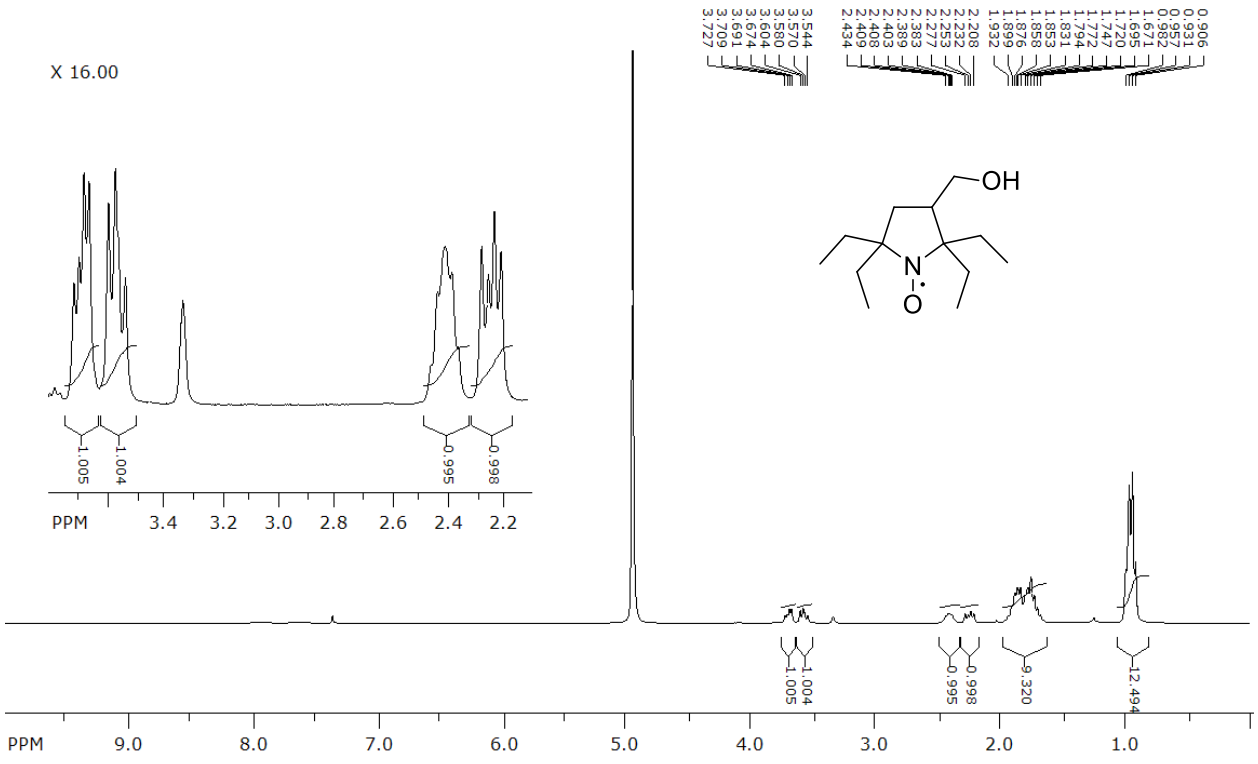
^{1}H NMR (100 MHz;$CDCl_3$) of 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetraethylpyrrolidine hydrochloride (17a,b)



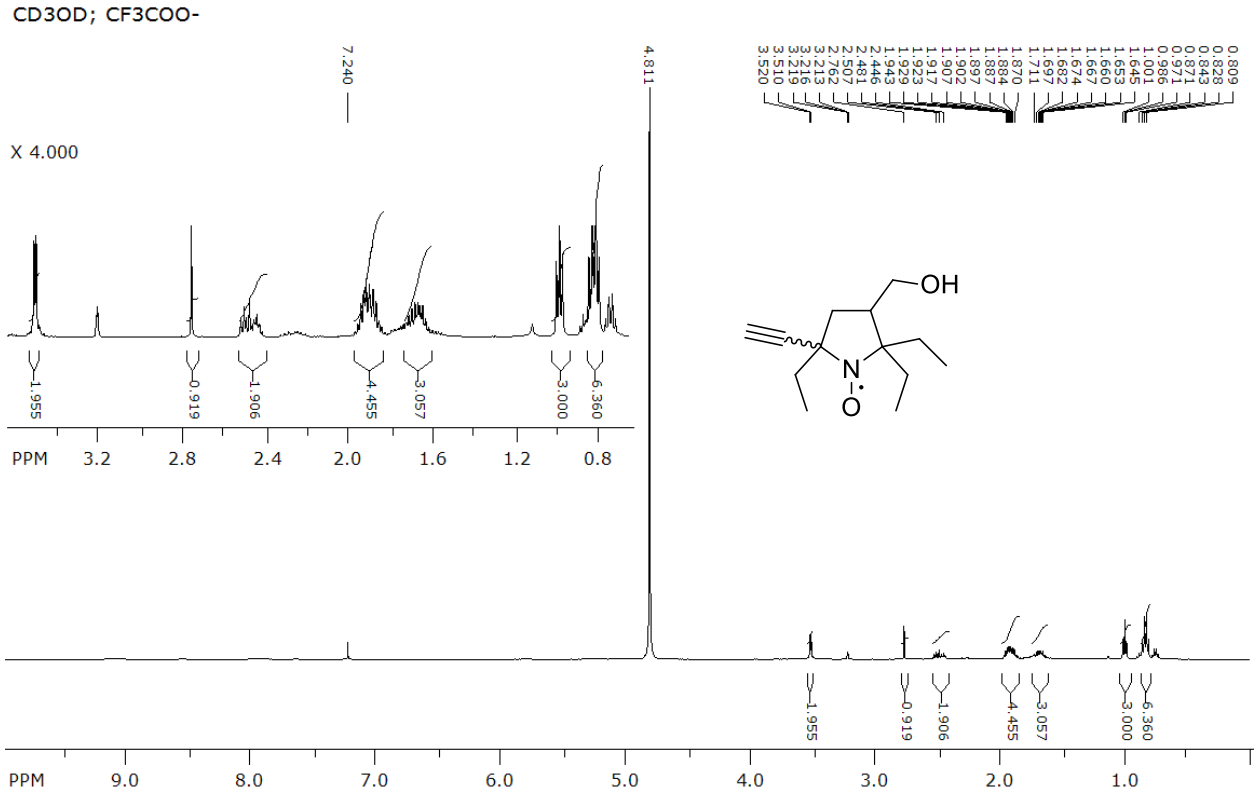
$^{13}C\{^{1}H\}$ NMR (75 MHz; $CDCl_3$) of 1-Hydroxy-3-methoxycarbonyl-2,2,5,5-tetraethylpyrrolidine hydrochloride (17a,b)



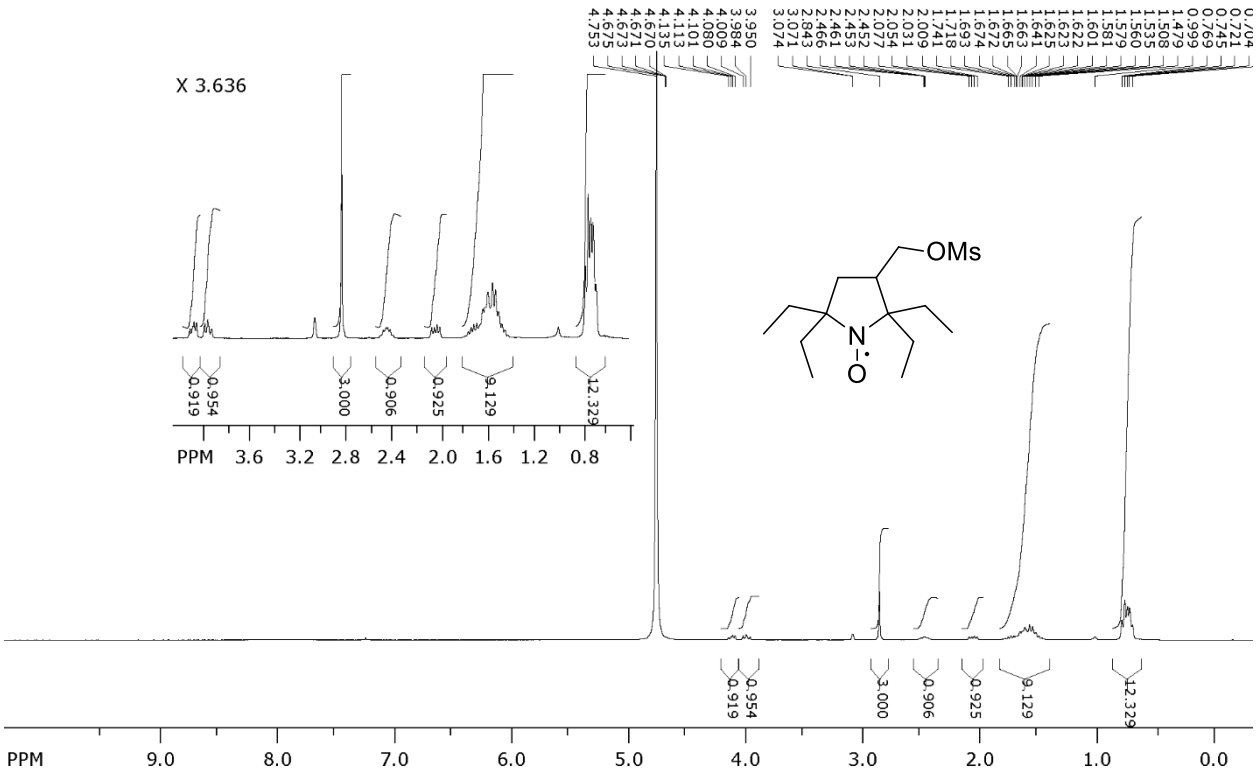
^{1}H NMR (300 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH) of 3-hydroxymethyl -2,2,5,5-tetraethylpyrrolidine-1-oxyl (18).



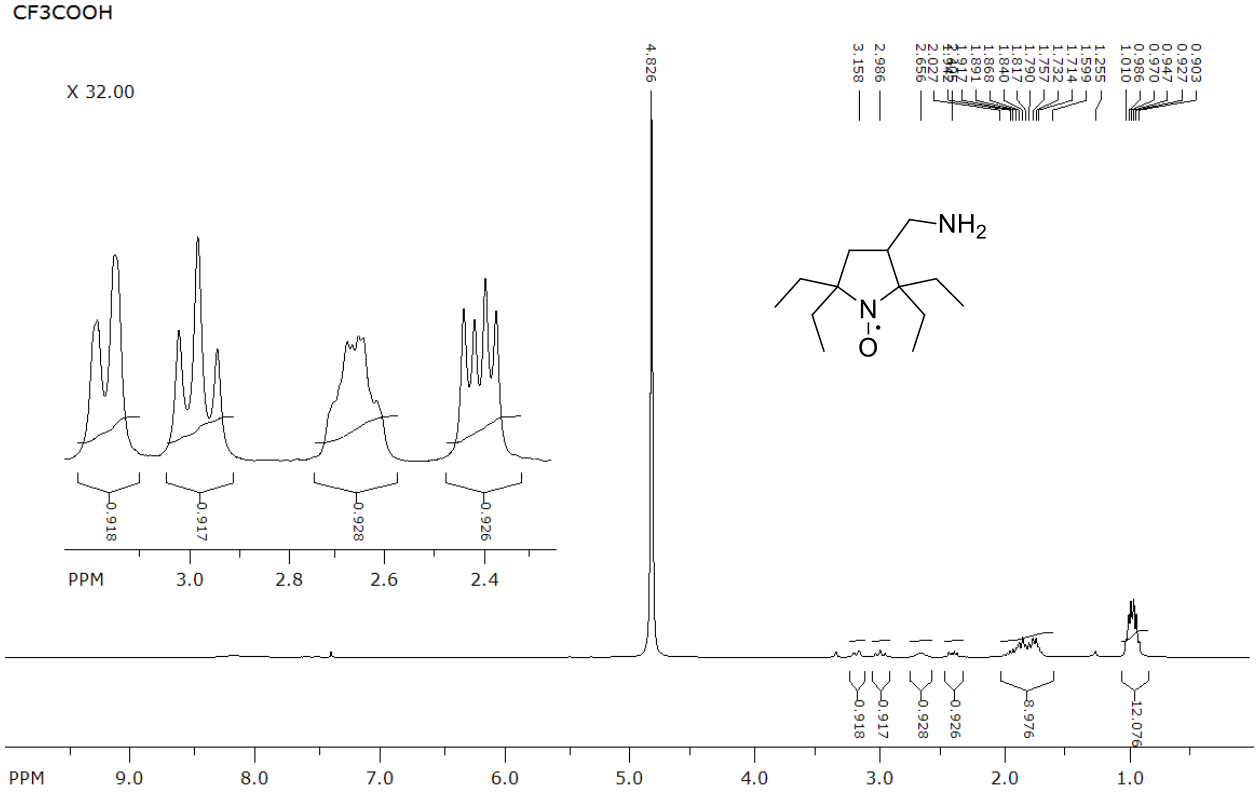
^{1}H NMR (500 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH) of 3-hydroxymethyl-5-ethynyl-2,2,5-triethylpyrrolidine-1-oxyl mixture of isomers (19a,b).



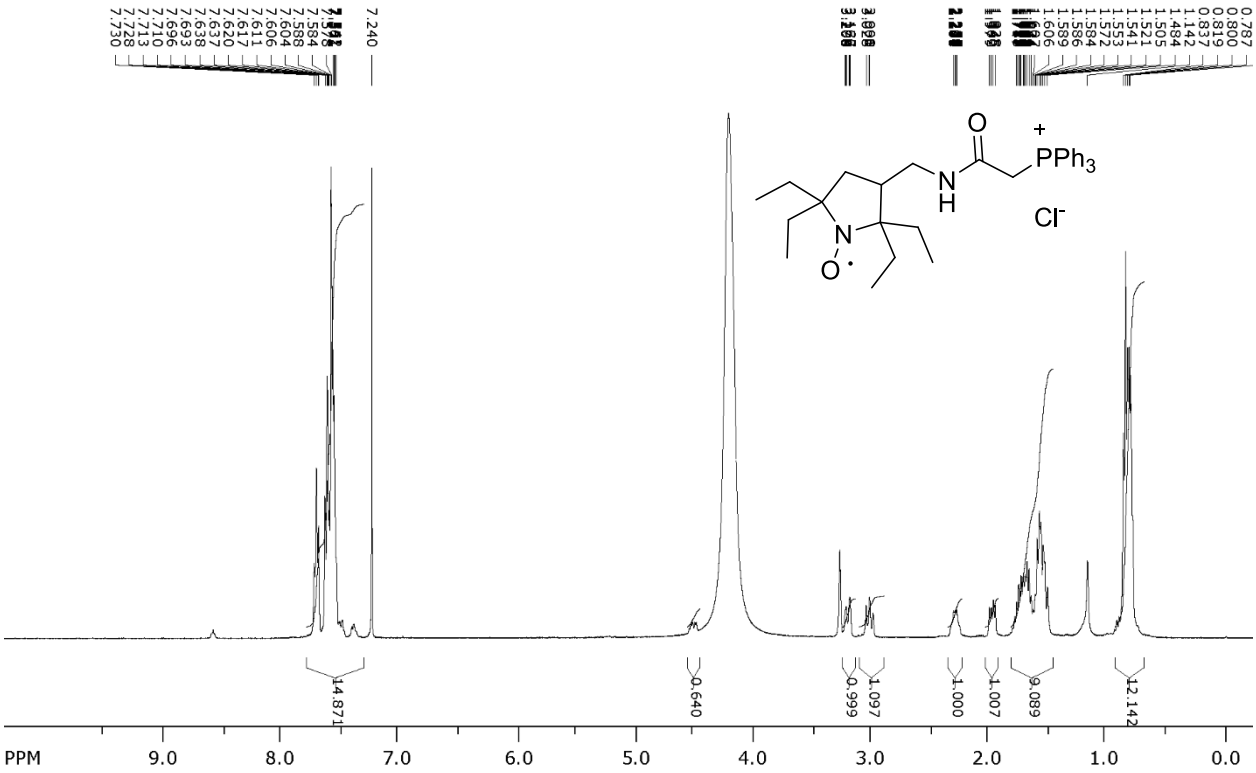
^{1}H NMR (300 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH) of 3-methansulfonyloxy-methyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl (20).



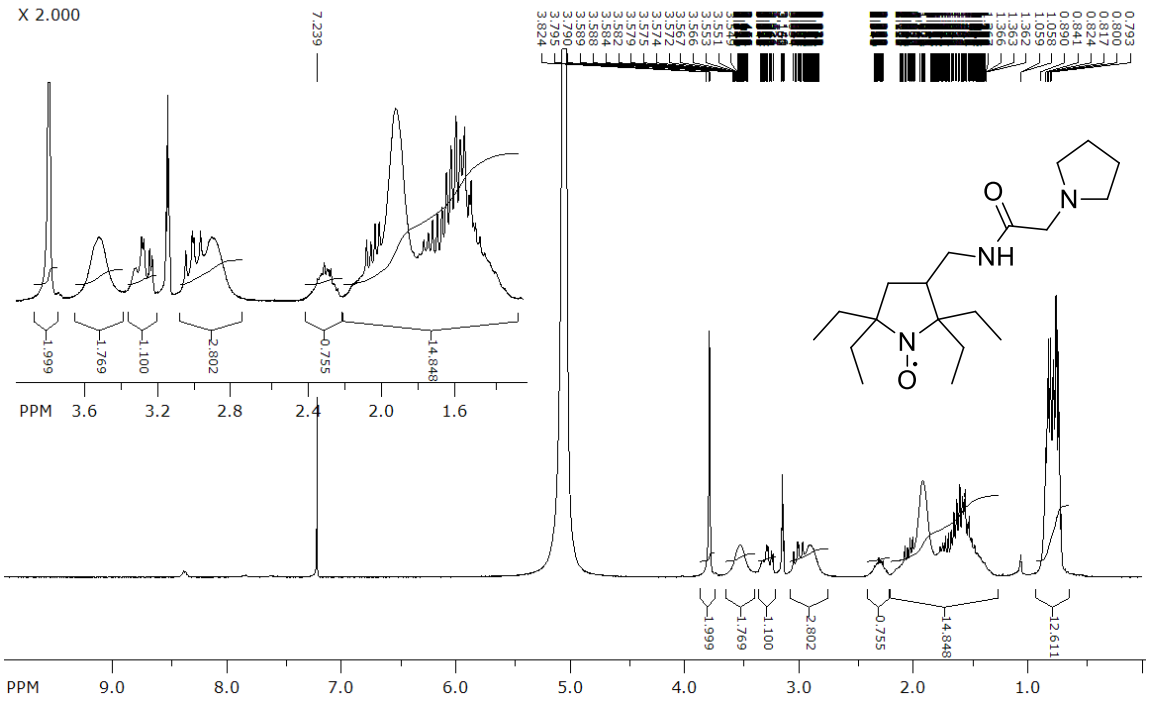
^{1}H NMR (300 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH) of 3-aminomethyl -2,2,5,5-tetraethylpyrrolidine-1-oxyl (22).



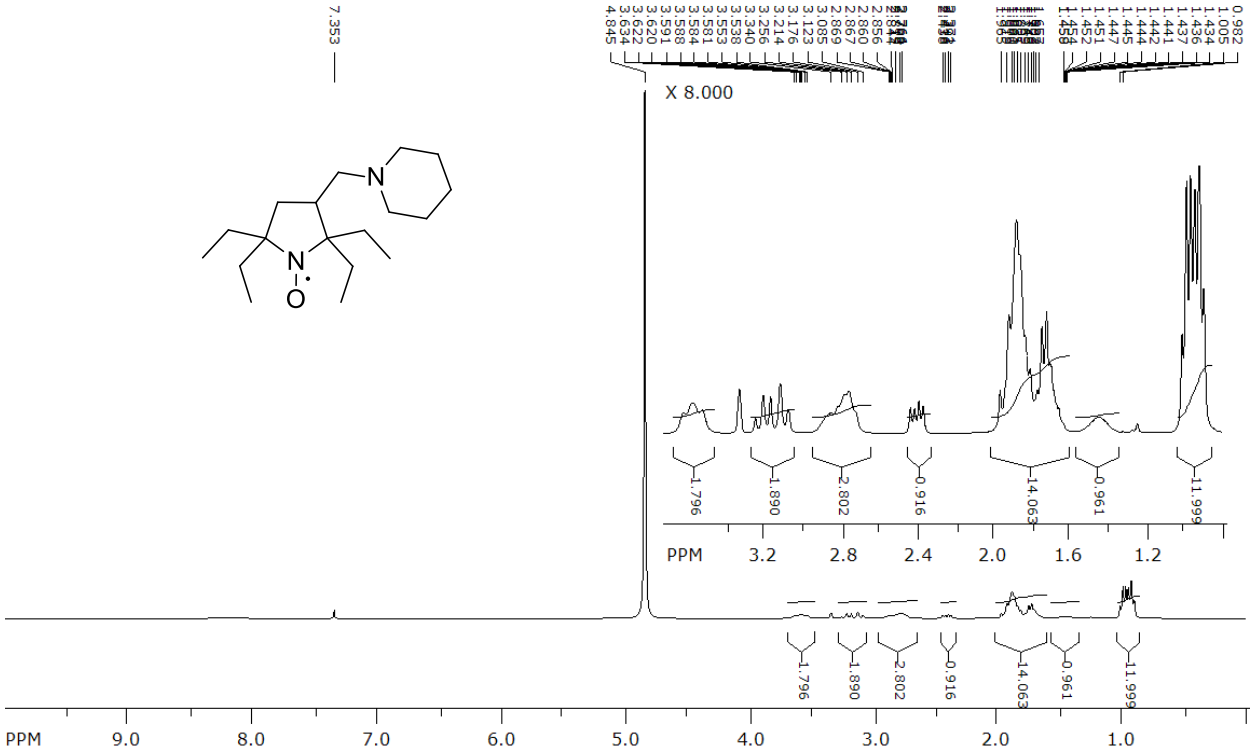
^{1}H NMR (300 MHz; CD3OD/CDCl3, Zn/CF3COOH) of 3-(2-triphenylphosphonio-acetamido)methyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl chloride (28).



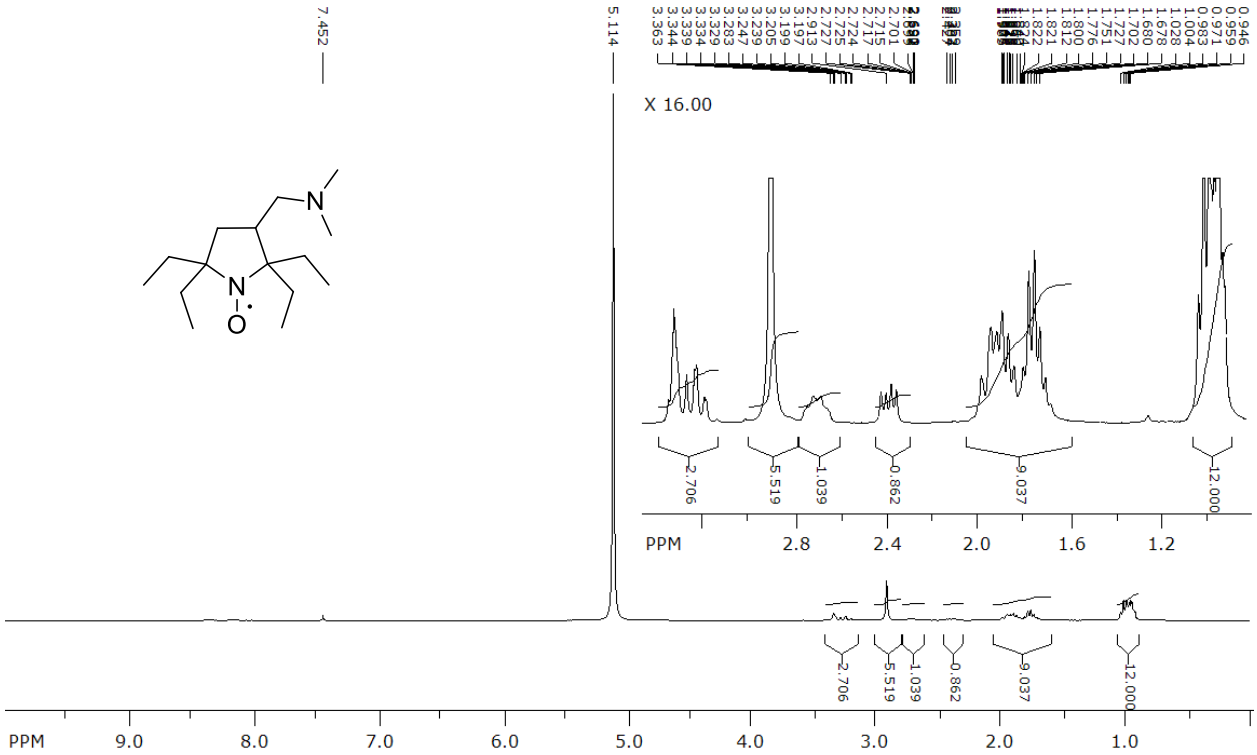
^{1}HNMR (300 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH) of 3-(2-(pyrrolidin-1yl)acetamidomethyl)-2,2,5,5-tetraethylpyrrolidine-1-oxyl (29).



^{1}HNMR (300 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH) of 3-((piperidin-1-yl)methyl)-2,2,5,5-tetraethylpyrrolidine-1-oxyl (30).



^{1}H NMR(300 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH) of 3-(dimethylaminomethyl)-2,2,5,5-tetraethylpyrrolidine-1-oxyl (31).



Line shape analysis of multiplets for mixture of isomers 10a,b.

The analysis was performed using the gNMR 5.0 software is shown in Fig. S1. Parameters of spin system are shown in Table S1 [Budzelaar, P. H. M. "gNMR, version 5.0. 6.0." *Ivorysoft, Nijmegen, Netherlands* (2006).]

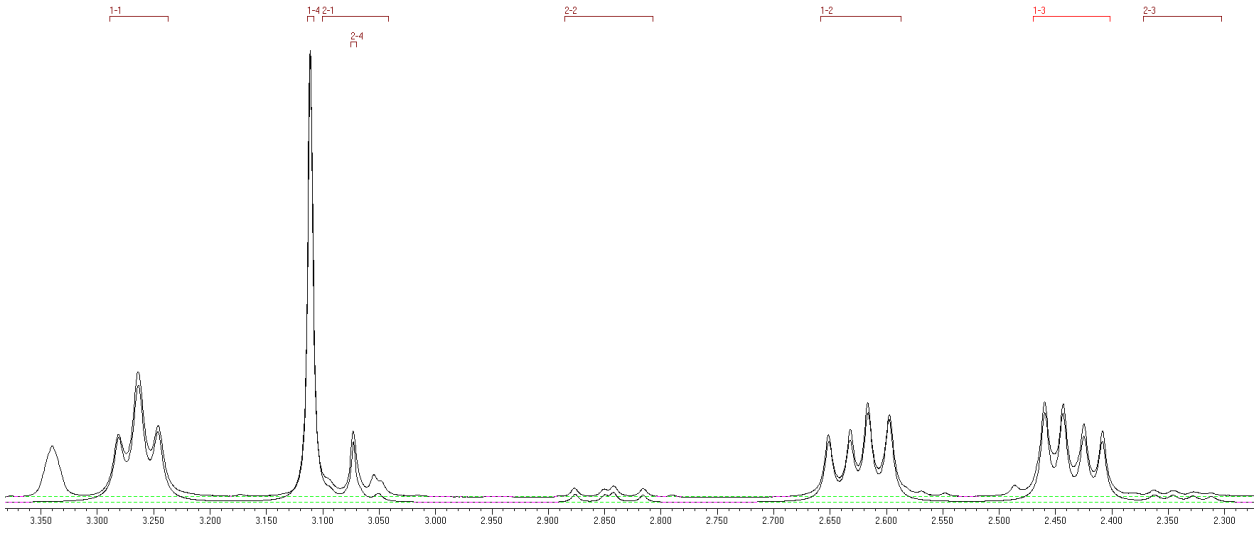


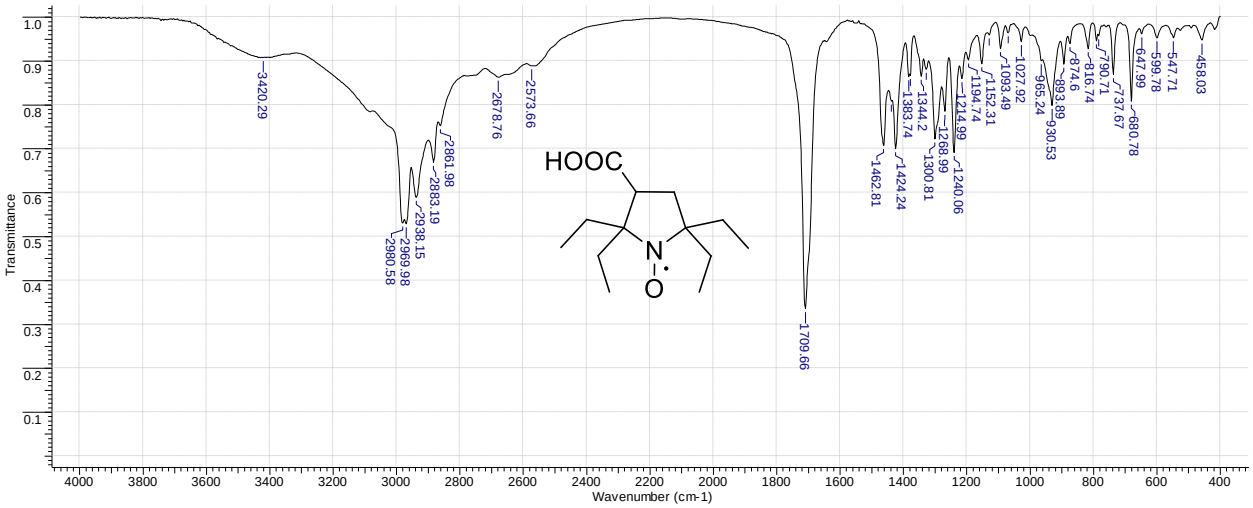
Fig. S1 Simulation of spin system for ^{1}H NMR (400 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH system) of 3-carboxy-5-ethinyl-2,2,5-triethylpyrrolidine-1-oxyl – mixture of isomers (10a,b).

Table S1.Simulation of spin system parameters for ^{1}H NMR (400 MHz; $CD_3OD/CDCl_3$, Zn/CF_3COOH system) of 3-carboxy-5-ethinyl-2,2,5-triethylpyrrolidine-1-oxyl – mixture of isomers (10a,b).

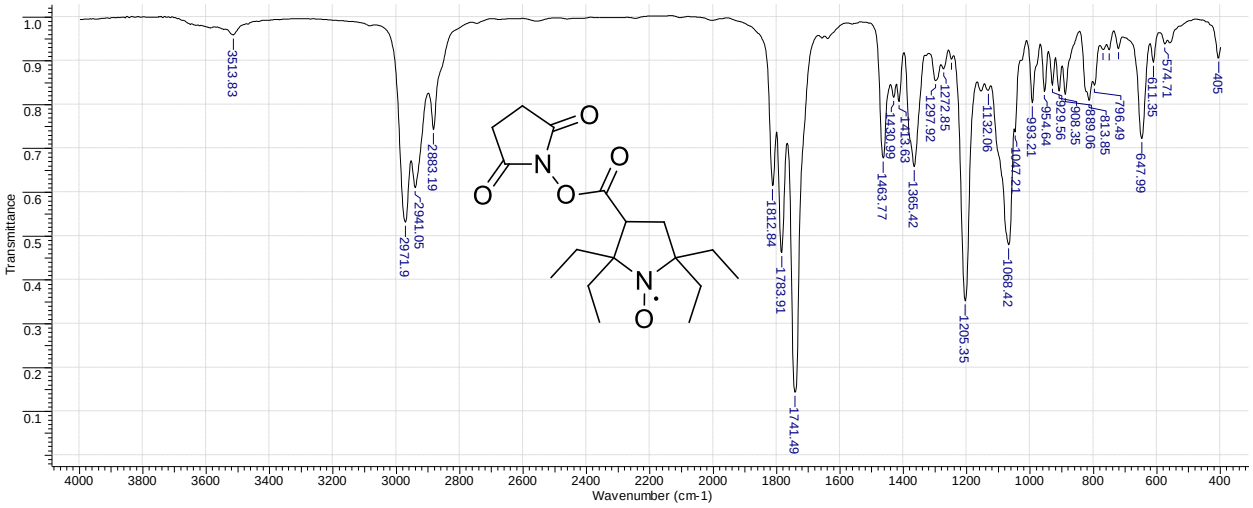
Major isomer – 10a							
#	Nucleus	n	Shift	J[1]	J[2]	J[3]	J[4]
1	^{1}H	1	3.263	-			
2	^{1}H	1	2.623	7.55			
3	^{1}H	1	2.436	6.49	-13.94		
4	^{1}H	1	3.111	-	-	-	
Concentration: 86.87975							
Minor isomer -10b							
#	Nucleus	n	Shift	J[1]	J[2]	J[3]	J[4]
1	^{1}H	1	3.071				
2	^{1}H	1	2.846	10.51			
3	^{1}H	1	2.337	6.65	-13.72		
4	^{1}H	1	3.073	-	-	-	-
Concentration: 8.25143							

IR spectra

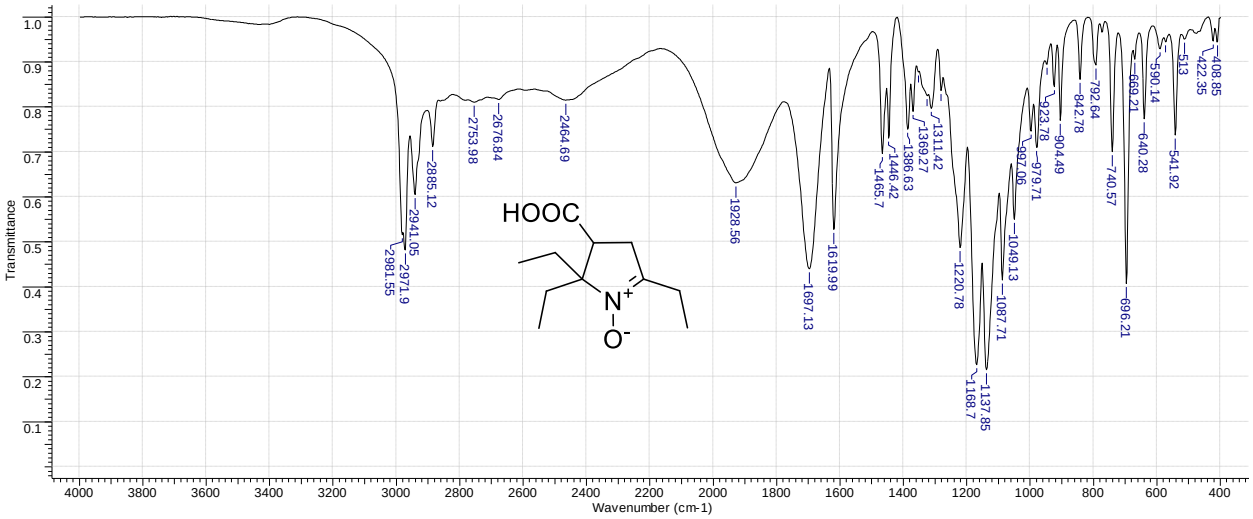
IR (KBr) of 3-carboxy-2,2,5,5-tetraethylpyrrolidine-1-oxyl (1).



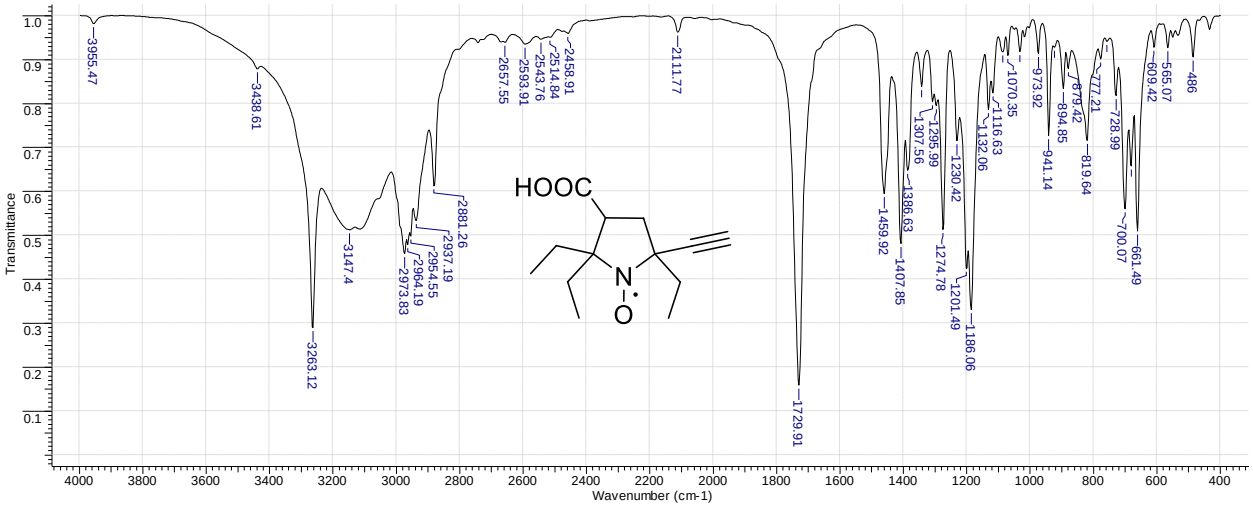
IR (neat) of 3-[[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl]-2,2,5,5-tetraethylpyrrolidine-1-oxyl (3).



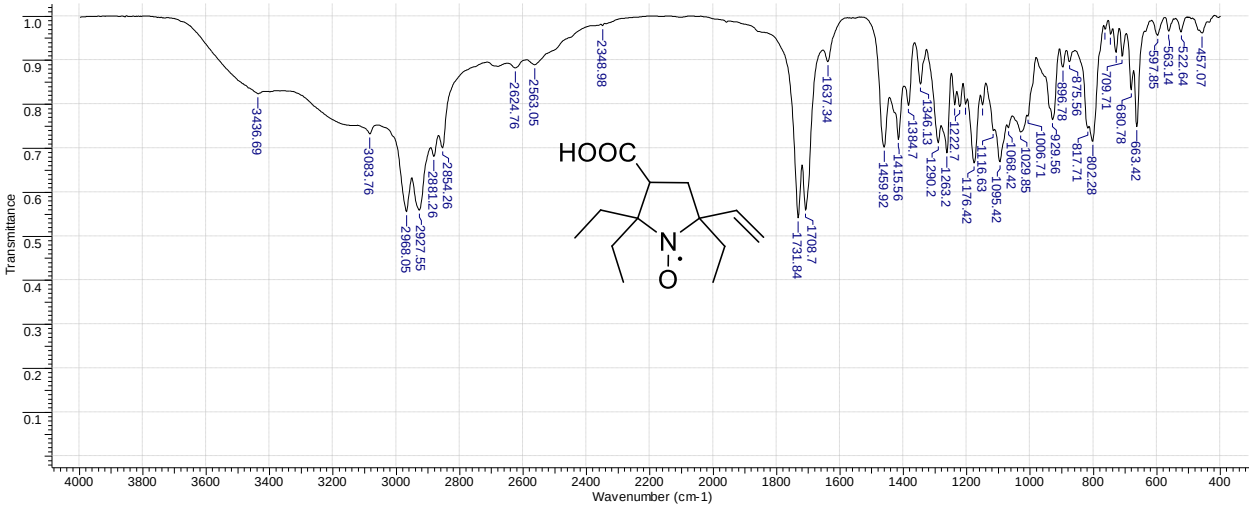
IR (KBr) of 3-carboxy-2,2,5-triethyl-3,4-dihydro-2H-pyrrole 1-oxide (8).



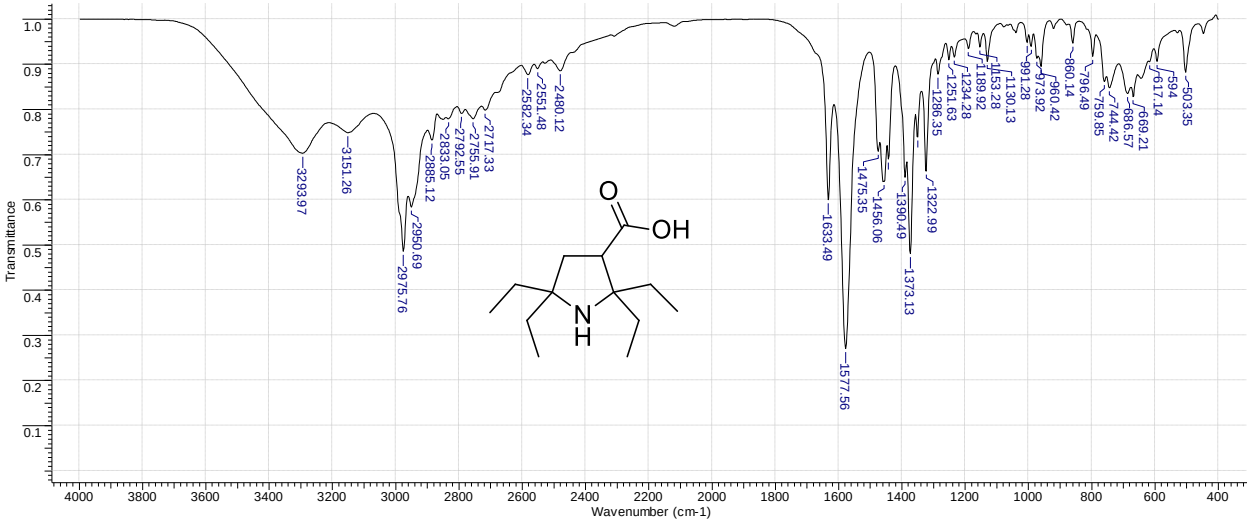
IR (KBr) of 3-carboxy-5-ethynyl-2,2,5-triethylpyrrolidine-1-oxyl (10a,b).



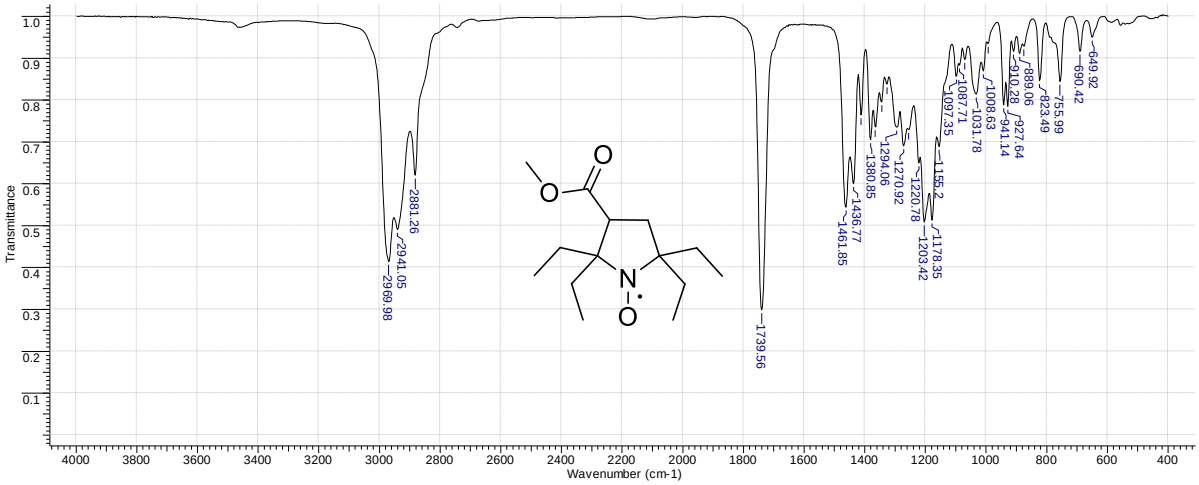
IR (KBr) of 3-carboxy-5-vinyl-2,2,5-triethylpyrrolidine-1-oxyl (14).



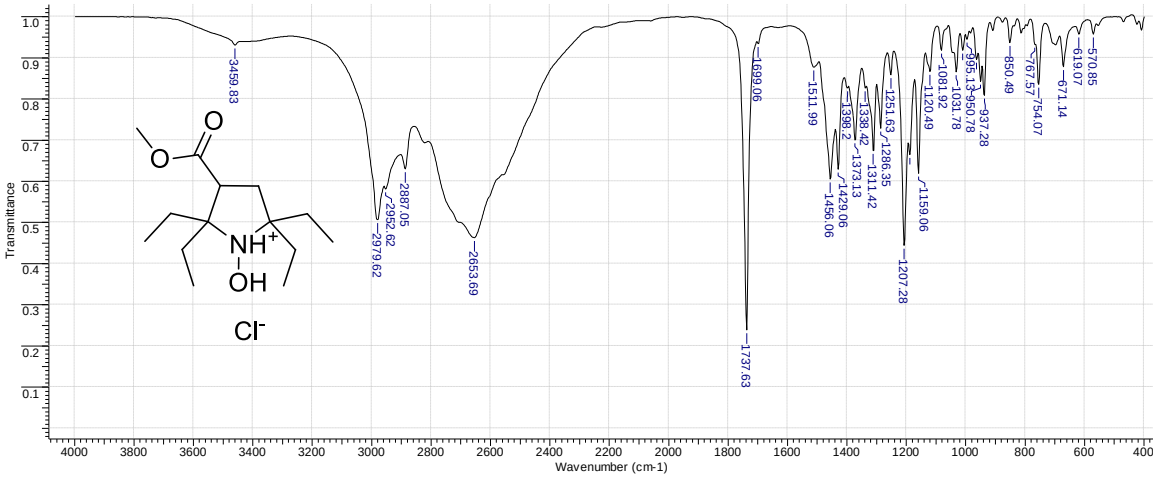
IR (KBr) of 3-carboxy-2,2,5,5-tetraethylpyrrolidine (15).



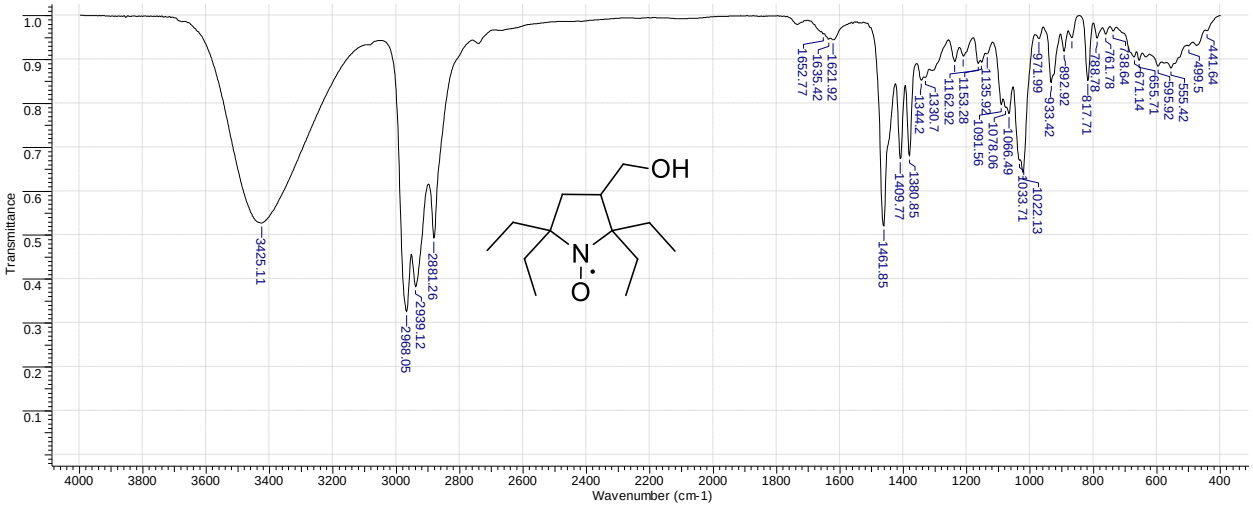
IR (neat) of 3-methoxycarbonyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl (16).



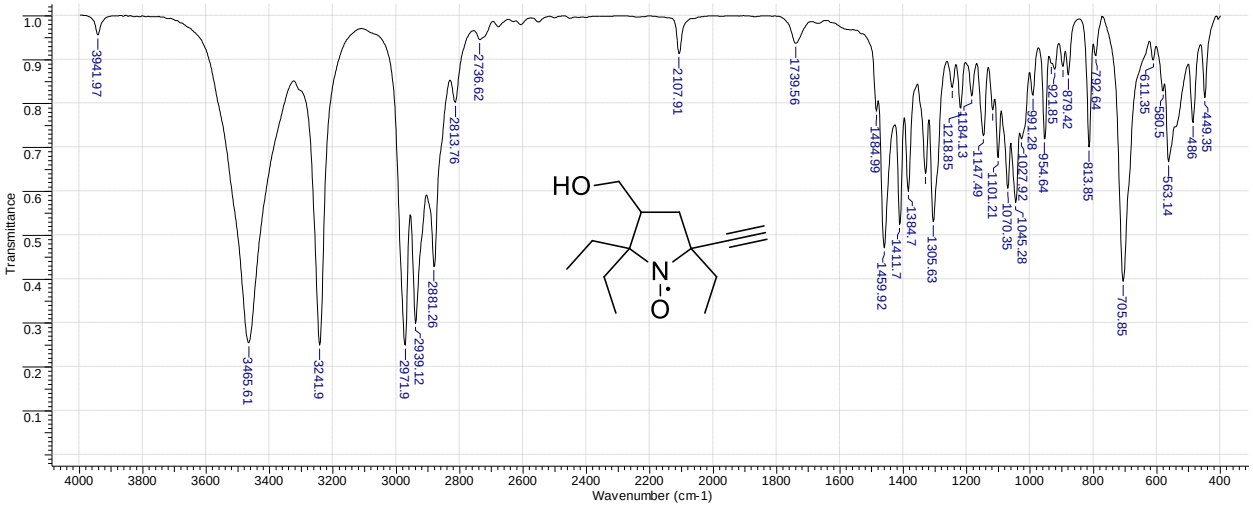
IR (KBr) of 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetraethylpyrrolidine hydrochloride (17)



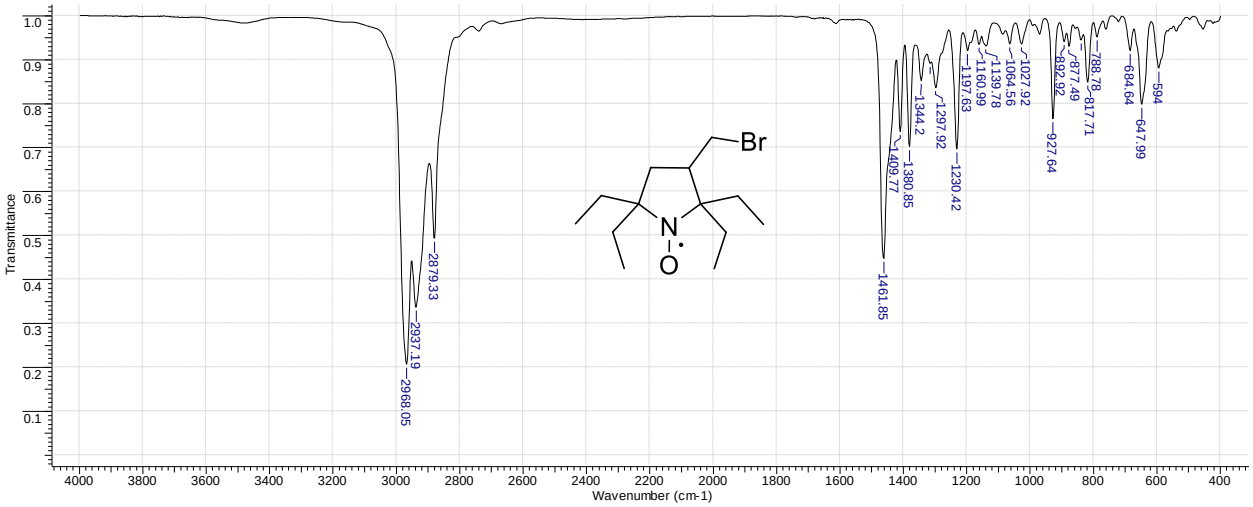
IR (neat) of 3-hydroxymethyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl (18).



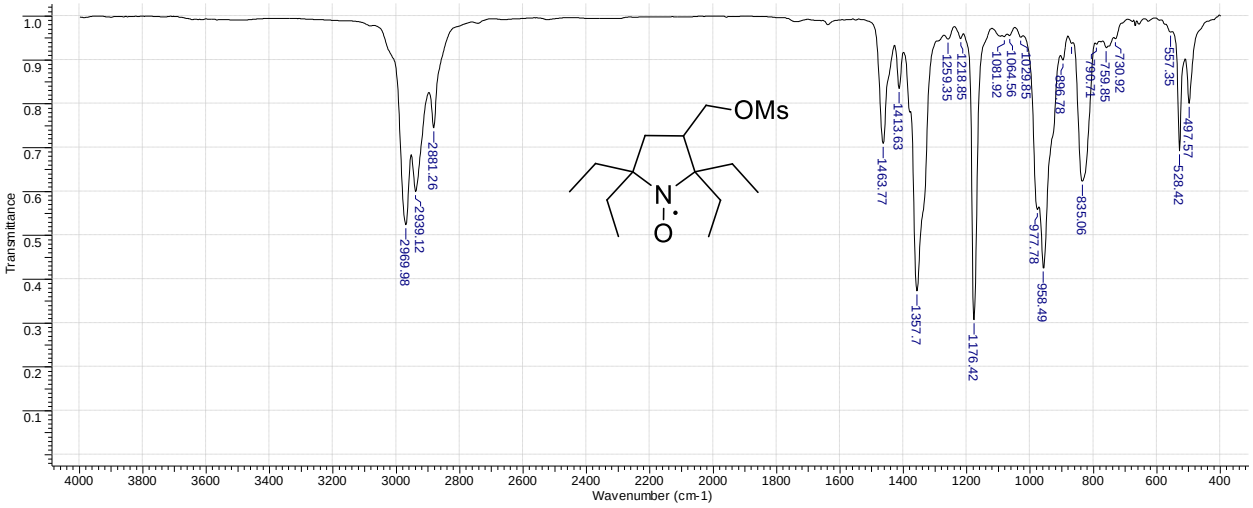
IR (neat) of 3-hydroxymethyl-5-ethinyl-2,2,5-triethylpyrrolidine-1-oxyl (19a,b).



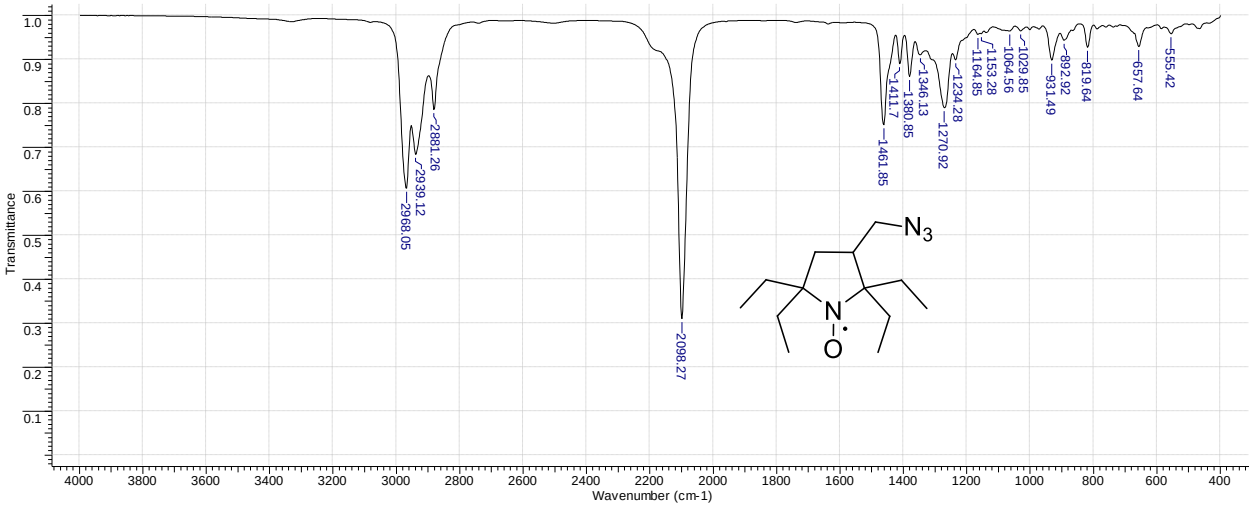
IR (neat) of 3-brommethyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl (20a).



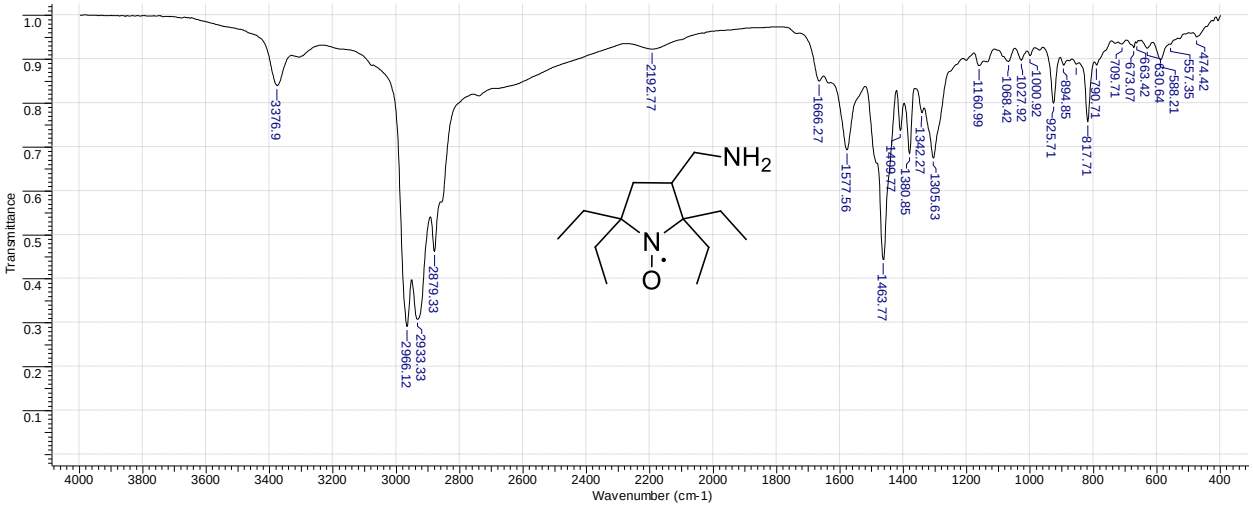
IR (neat) of 3-mesyloxymethyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl (20b).



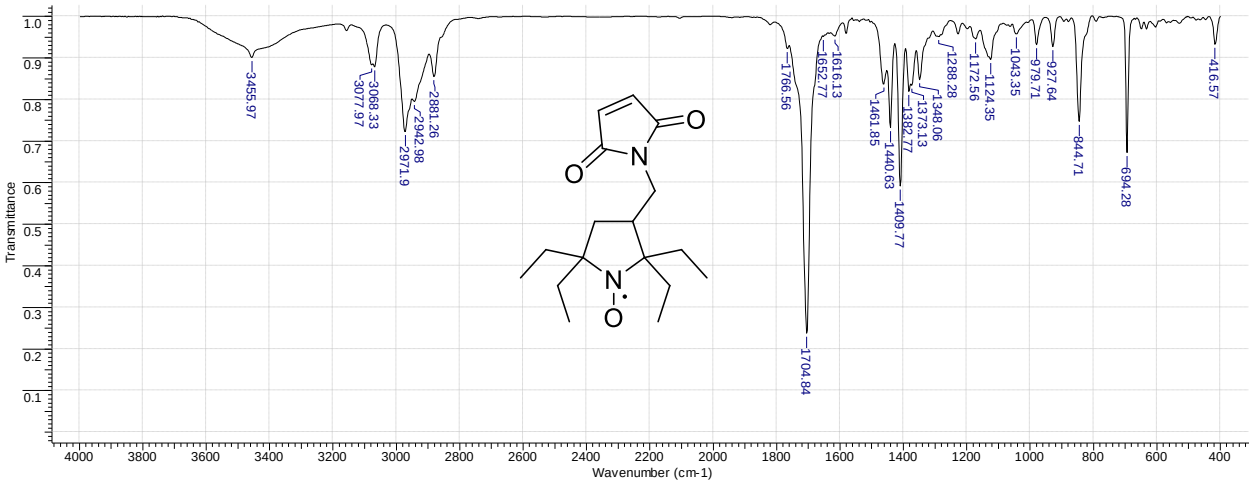
IR (neat) of 3-azidomethyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl (21).



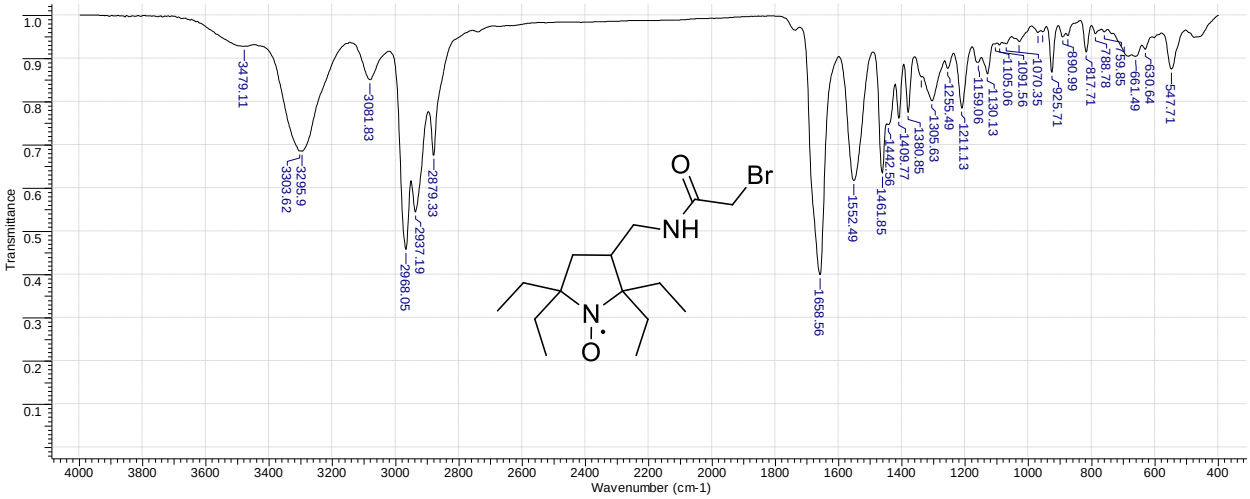
IR (neat) of 3-aminomethyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl (22).



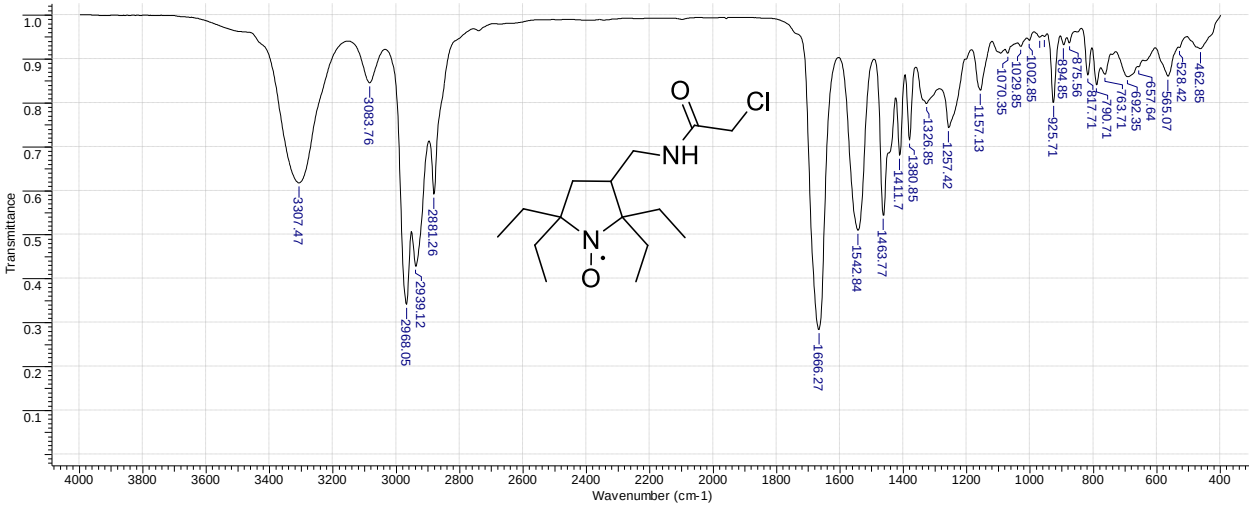
IR (KBr) of 3-maleimidomethyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl (24).



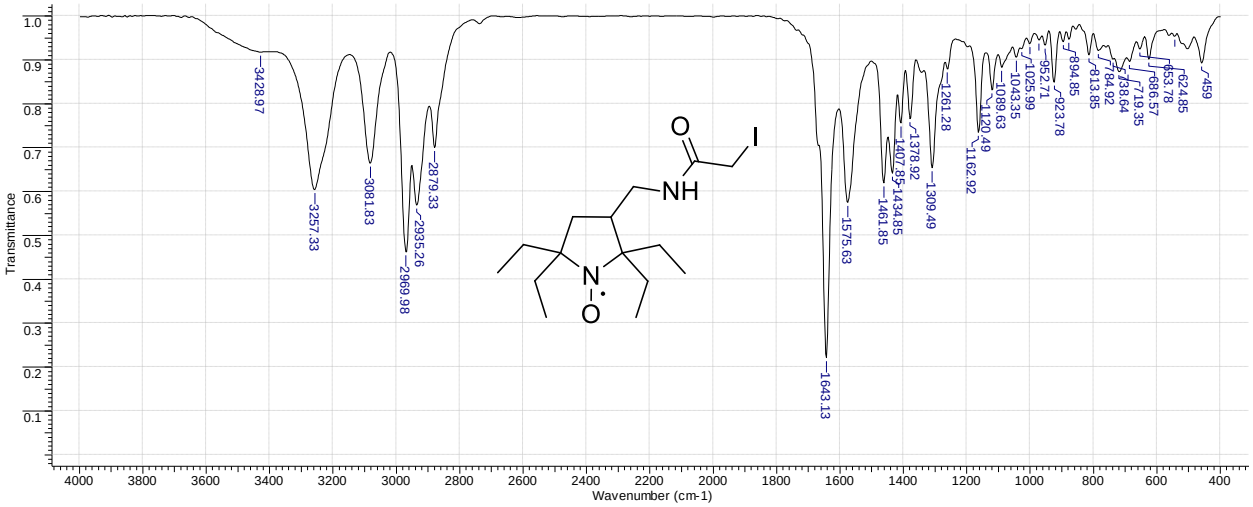
IR (neat) of 3-bromoacetamidomethyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl (25).



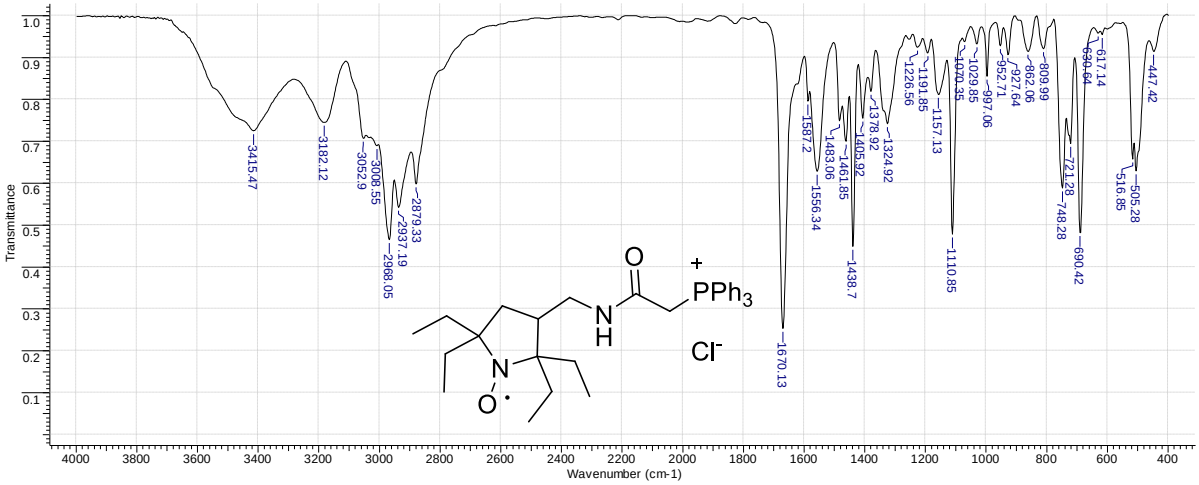
IR (neat) of 3-Chloroacetamidomethyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl (26).



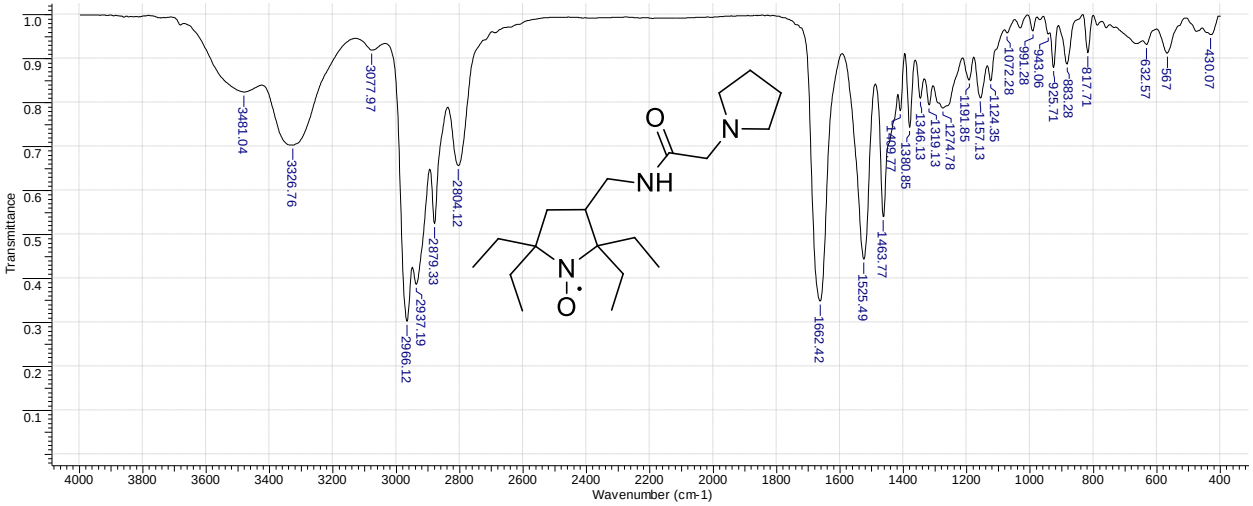
IR (KBr) of 3-Iodoacetamidomethyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl (27).



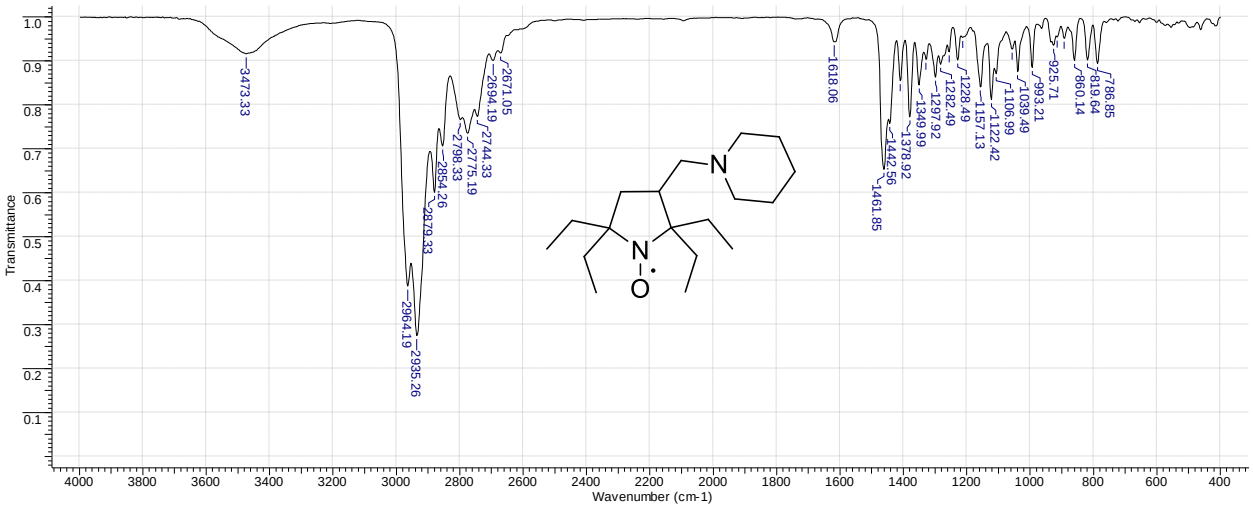
IR (KBr) of 3-(2-triphenylphosphonio-acetamido)methyl-2,2,5,5-tetraethylpyrrolidine-1-oxyl chloride (28).



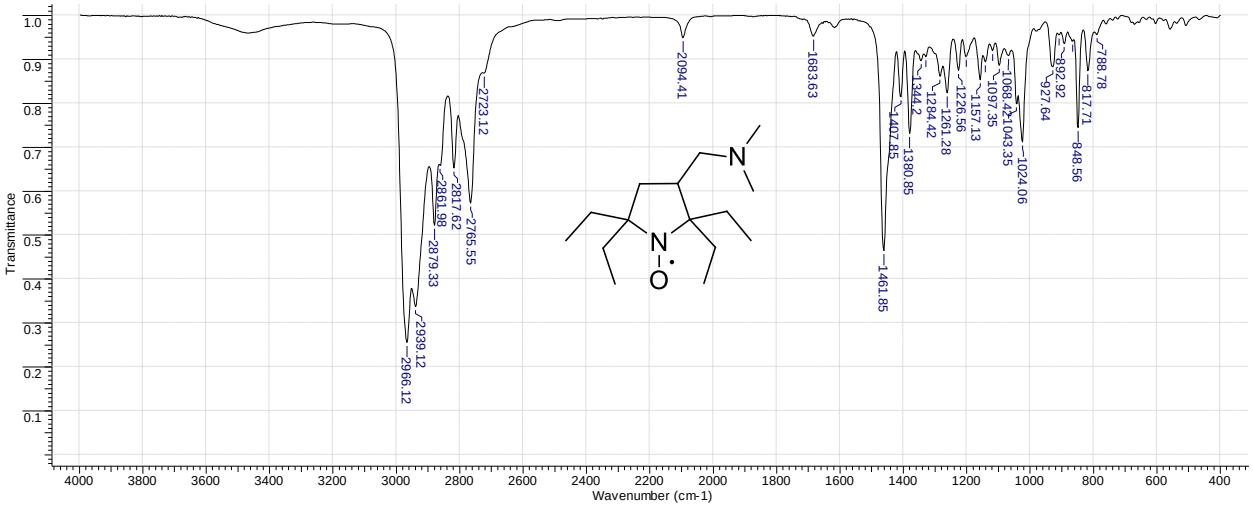
IR (neat) of 3-(2-(pyrrolidin-1yl)acetamidomethyl)-2,2,5,5-tetraethylpyrrolidine-1-oxyl (29).



IR (neat) of 3-((piperidin-1-yl)methyl)-2,2,5,5-tetraethylpyrrolidine-1-oxyl (30).

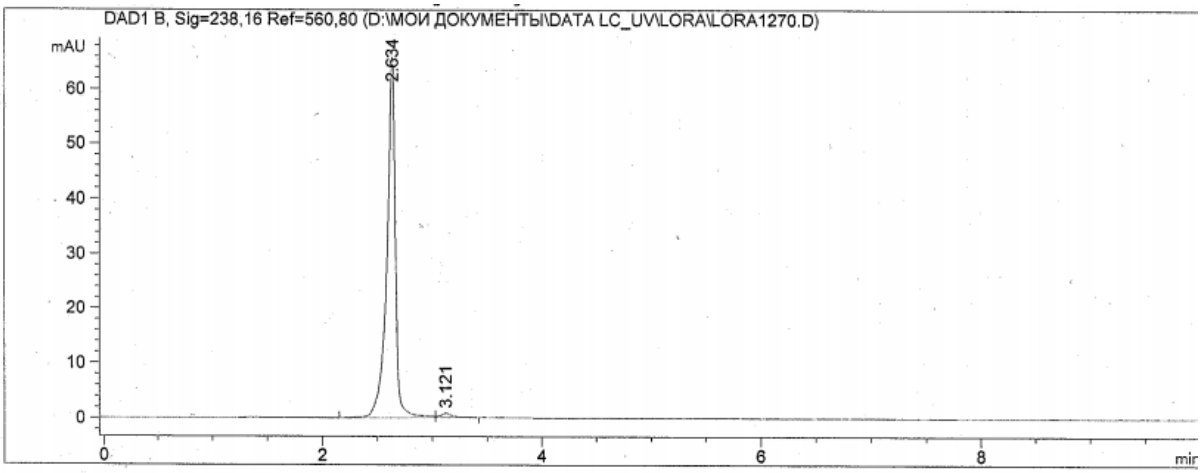


IR (neat) of 3-(dimethylaminomethyl)-2,2,5,5-tetraethylpyrrolidine-1-oxyl (31).



HPLC analyses

HPLC of 3-carboxy-5-ethinyl-2,2,5-triethylpyrrolidine-1-oxyl major isomer(19a).




```
================================================================================
                              Area Percent Report
================================================================================

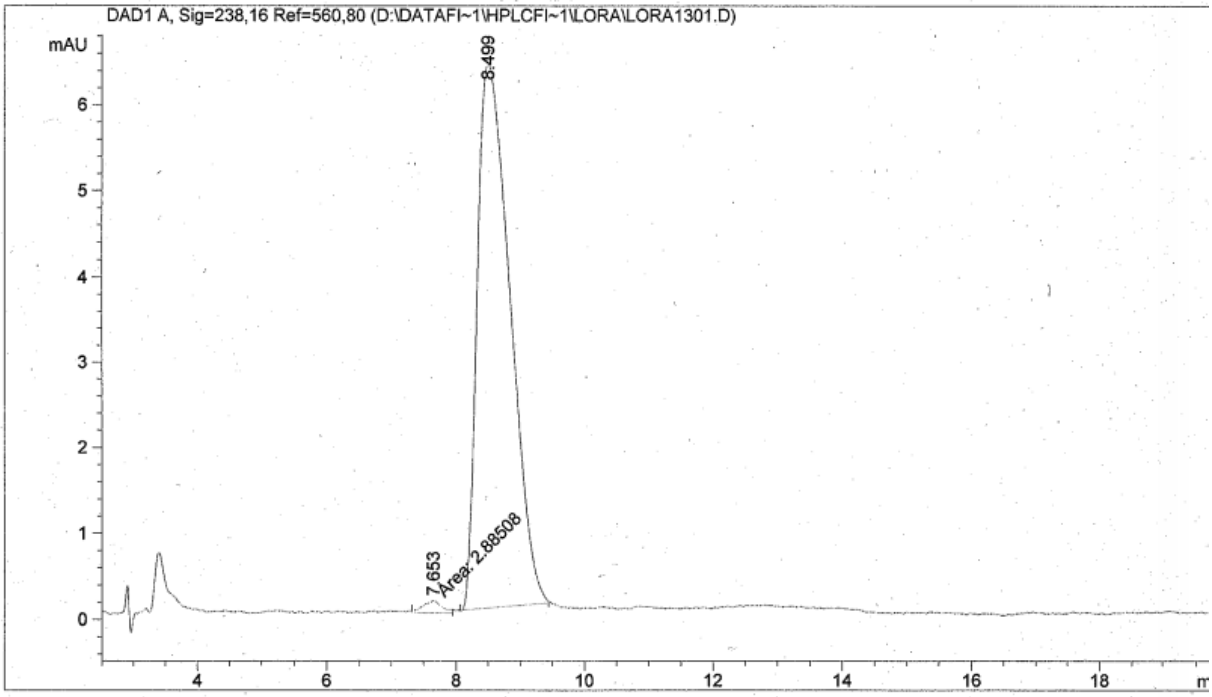
Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=238,16 Ref=560,80

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]    [mAU*s]    [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.634 BV    0.0767  354.74719   66.02786  98.3314
   2   3.121 VB    0.1055    6.01980 8.02957e-1   1.6686

Totals :                   360.76699   66.83082
```

HPLC of 3-((piperidin-1-yl)methyl)-2,2,5,5-tetraethylpyrrolidine-1-oxyl (30).




```
================================================================================
                              Area Percent Report
================================================================================

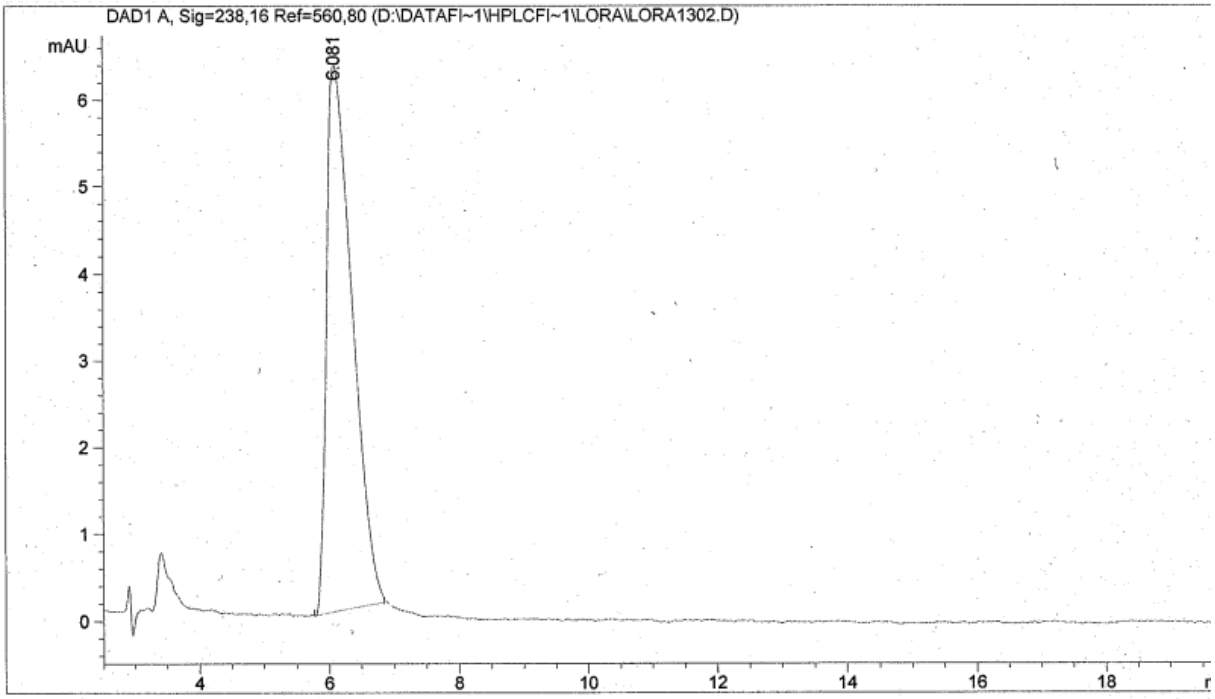
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=238,16 Ref=560,80

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   7.653 MM    0.3434    2.88508 1.40031e-1   1.2723
   2   8.499 BB    0.5095  223.88176    6.34769  98.7277

Totals :                    226.76684    6.48772
```

HPLC of 3-(2-(pyrrolidin-1yl)acetamidomethyl)-2,2,5,5-tetraethylpyrrolidine-1-oxyl (29).

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================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=238,16 Ref=560,80

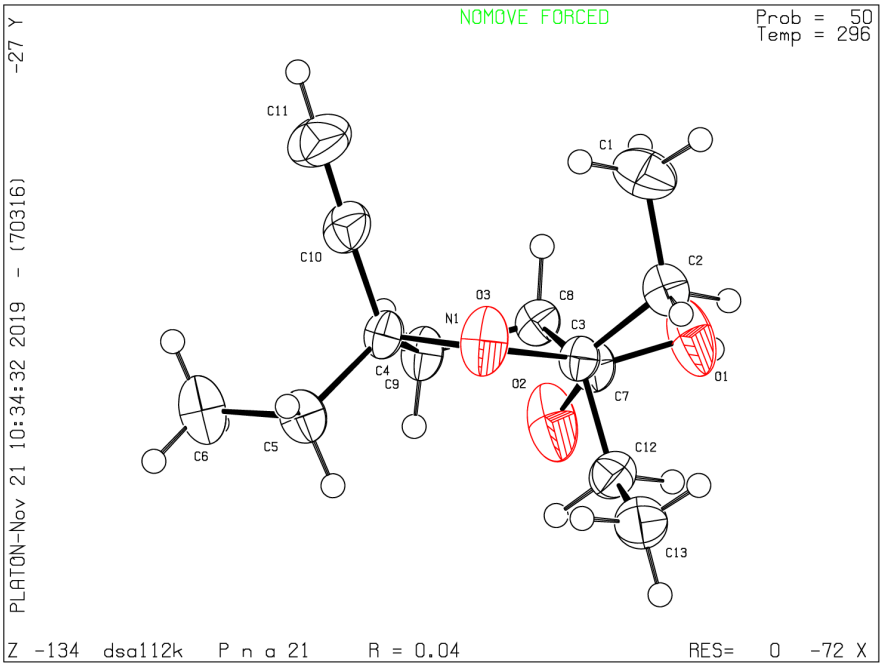
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.081 PB    0.3941  172.00441    6.30903 100.0000

Totals :                   172.00441    6.30903
```

Table S2. X-ray experimental details

Crystal data	Compound **10a** (dsa112k)
Chemical formula	$C_{13}H_{20}NO_3$
M_r	238.30
Crystal system, space group	Orthorhombic, *Pna*2$_1$
Temperature (K)	296
a, *b*, *c* (Å)	19.2686 (9), 8.1112 (3), 8.6742 (3)
V (Å^3)	1355.70 (9)
Z	4
Radiation type	Mo *K*α
μ (mm^{-1})	0.08
Crystal size (mm)	0.61 × 0.32 × 0.12
Data collection	
Diffractometer	Bruker *APEX*-II CCD
Absorption correction	Multi-scan *SADABS2008*/1
T_{min}, T_{max}	0.911, 0.956
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	16081, 3131, 2834
R_{int}	0.044
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.659
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.040, 0.113, 1.03
No. of reflections	3131
No. of parameters	160
No. of restraints	2
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\sigma\rangle_{max}$, $\sigma\rangle_{min}$ (e Å^{-3})	0.34, -0.17
Absolute structure parameter	0.2 (3)
CCDC number	2111214

Fig.S2. X-Ray of 3-carboxy-5-ethynyl-2,2,5-triethylpyrrolidine-1-oxyl (10a).



Computer programs: Bruker *APEX2*, Bruker *SAINT*, *SHELXS2018* (Sheldrick, 2018), *SHELXL2018*/3 (Sheldrick, 2018), Bruker *SHELXTL*.

Fig. S3. The dependences of the first order rates of radical reductions versus ascorbic acid concentrations.

Data were fitted by linear dependences starting at zero. Second order reaction rate constant is a slope of such dependence.