Supporting Information

Synthesis of Unnatural Amino Acids Functionalized with Sterically Shielded Pyrroline Nitroxides

*Ying Wang, Joseph T. Paletta, Kathleen Berg, Erin Reinhart, Suchada Rajca, Andrzej Rajca**

Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588-0304.

E-mail address: arajca1@unl.edu

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1. Experimental Section.

1.a General procedures and materials.

Throughout the following paragraphs labels "JTP-4-08f1G" and alike correspond to sample or experiment codes directly traceable to the laboratory notebooks or raw data.

Chemicals and per-deuterated solvents for NMR spectroscopy were obtained from commercial sources and used as received unless otherwise indicated. Amino acids, such as *L*-cysteine Boc-dap-OH, were obtained from a commercial source. Purification and titration of *m*-CPBA were followed as previously described.[S1] Dess-Martin periodinane (DMP) was prepared as previously reported.[S2] Column chromatography was carried out on flash grade silica gel, using 0 – 20 psig pressure. Preparative TLC (PTLC) was carried out using tapered silica plates with a preadsorbent zone. Reverse-phase column chromatography (0–20 psig pressure) was carried out on regular column with reverse-phase silica gel (octadecyl, C_{18}, bonded silica gel, 14% carbon content, 35-75 micron particle size, 150 Å pore size) with the mixture of methanol/water/ammonia (28~30% aq.) as the eluent, monitoring by analytical reverse-phase HP-TLC plates (octadecyl, C18, bonded silica gel, tapered with a preadsorbent zone).

NMR spectra were obtained using commercial spectrometers (^{1}H, 400, 500 and 600 MHz) using methanol-d_4 (CD_3OD) and chloroform-*d* ($CDCl_3$) as solvent. The 500 MHz instrument was equipped with a cryoprobe. The chemical shift references were as follows: (^{1}H) methanol-d_3, 3.31 ppm, (^{1}H) chloroform, 7.26 ppm. Typical 1D FID was subjected to exponential multiplication with an exponent of 0.3 Hz (For selected spectra smaller values of LB were used to resolve closely spaced resonances, as indicated in the spectral data summaries). IR spectra were obtained using a commercial instrument, equipped with an ATR sampling accessory. MS analyses were carried out at the local mass spectrometry facility.

1.b EPR spectroscopy and kinetic studies.

EPR spectroscopy. CW X-band EPR spectra for nitroxides in solution were acquired on an X-band EPR instrument, equipped with a frequency counter and nitrogen flow temperature control (130–300 K). The spectra were obtained using a dual mode cavity; all spectra were recorded using an oscillating magnetic field perpendicular (TE_{102}) to the swept magnetic field. DPPH powder (g = 2.0037) was used as a g-value reference.

Kinetic studies. The results of kinetic measurements are summarized in Table S1 (this Section). The ascorbate solution was made with ascorbic acid, diethylenetriaminepentaacetic acid (DTPA, 0.1 mM), sodium hydroxide, and sodium phosphates (<30 ppm transition metals) at pH 7.4, measured with pH/ion analyzer. Phosphate buffer was made with sodium phosphates and DTPA (0.1 mM) at pH 7.4 and used to make nitroxide solutions. Typically, kinetic runs were carried out with a 20-fold molar excess of ascorbate and 25-fold molar excess of GSH. Prior to a typical kinetic run, solutions of nitroxide and ascorbate/GSH were filtered through a 0.45 μm nylon syringe filter, combined in equal portions, vortexed for 6 seconds, and then the resultant mixture was drawn into an EPR-quality quartz capillary tube (0.6-mm I.D.). The capillary was stoppered with parafilm and placed in a 5-mm O.D. EPR sample tube in the cavity of X-band spectrometer. The peak height (PH, Table S1) and the integrated peak height (IPH, Table S1) of the low-field line of the triplet were measured as a function of time. Microwave power was kept under 6.5 mW and temperature was controlled at 295 K with nitrogen flow system. Typical EPR parameters for kinetics of *gem*-diethyl nitroxides: power 15 dB, modulation amplitude 4 G, sweep width 20 G, 2 scans, receiver gain 4.48E+04, conversion time 20.48 ms, time constant 20.48 ms, and sweep time 10.49 s. Typical EPR parameters for kinetics of spirocyclohexyl nitroxides: power 15 dB, modulation amplitude 4 G, sweep width 30 G, 2 scans, receiver gain 1.00E+05, conversion time 20.48 ms, time constant 20.48 ms, and sweep time 10.49 s.

Table S1. Kinetics of reduction of 0.2 or 1 mM nitroxides with 20-fold molar excess of ascorbate and 25-fold molar excess of GSH.

								Initial Kinetics (<1 h)				Range of fit	
Compd	Sample Label	Run No.	Run Label	Data used	Nitrox. Conc. (mM)	Asc. Conc. (mM)	GSH conc. (mM)	k' $\times 10^4$ (s^{-1})	R^2	$k \times 10^4$ ($M^{-1}s^{-1}$)	Avg $k \times 10^4$ ($M^{-1}s^{-1}$)	Time (s)	Remaining radical (%)
1	YW12-31colA	1	JP870	IPH	0.2	4.0	5	3.324	0.9989	831.0	839.6 ± 16.04	840	71
				PH				3.338	0.9970	834.5	844.6 ± 17.96	720	71
	YW12-31colA	2	JP871	IPH	0.2	4.0	5	3.387	0.9989	846.9		900	68
				PH				3.391	0.9982	847.8		660	71
	YW12-31colA	3	JP872	IPH	0.2	4.0	5	3.363	0.9987	840.8		840	71
				PH				3.406	0.9968	851.6		660	72
3	YW13-11col	1	JP876	IPH	0.2	4.0	5	5.358	0.9940	1340	1335 ± 22.72	840	63
				PH				5.458	0.9929	1365	1370 ± 23.44	660	66
	YW13-11col	2	JP877	IPH	0.2	4.0	5	5.289	0.9937	1322		840	64
				PH				5.446	0.9936	1361		660	67
	YW13-11col	3	JP878	IPH	0.2	4.0	5	5.371	0.9936	1343		902	63
				PH				5.531	0.9953	1383		660	66
5	JTP108f1	1	JP881	IPH	0.2	4.0	5	2.022	0.9993	505.6	502.0 ± 20.11	840	84
CyHx				PH				2.088	0.9935	522.0	509.4 ± 38.62	660	86
Acid	JTP108f1	2	JP882	IPH	0.2	4.0	5	1.962	0.9996	490.6		840	85
				PH				1.949	0.9910	487.2		660	87
	JTP108f1	3	JP883	IPH	0.2	4.0	5	2.039	0.9988	509.7		840	84
				PH				2.076	0.9979	519.1		660	86
6	JTP1021f1	1	JP887	IPH	1	20	25	0.3091	0.9948	15.46	15.59 ± 0.3079	480	97
Et4				PH				0.2414	0.9922	12.07	12.15 ± 0.3124	593	97
Acid	JTP1021f1	2	JP888	IPH	1	20	25	0.3109	0.9931	15.55		480	97
				PH				0.2465	0.9919	12.33		420	97
	JTP1021f1	3	JP889	IPH	1	20	25	0.3152	0.9936	15.76		480	97
				PH				0.2410	0.9950	12.05		480	97
2	YW13-08crp2	1	JP891	IPH	1	20	25	0.3655	0.9832	18.28	17.40 ± 1.902	420	97
				PH				0.2588	0.9927	12.94	13.97 ± 5.431	480	97
	YW13-08crp2	2	JP892	IPH	1	20	25	0.3277	0.9942	16.39		480	97
				PH				0.3409	0.9824	17.05		420	98
	YW13-08crp2	3	JP893	IPH	1	20	25	0.3504	0.9917	17.52		420	97
				PH				0.2383	0.9897	11.92		480	97
4	YW13-17col	1	JP894	IPH	1	20	25	0.5618	0.9991	28.09	27.01 ± 2.288	420	94
				PH				0.5333	0.9954	26.67	25.14 ± 3.209	480	95
	YW13-17col	2	JP895	IPH	1	20	25	0.5424	0.9940	27.12		420	96
				PH				0.5056	0.9928	25.28		360	96
	YW13-17col	3	JP896	IPH	1	20	25	0.5162	0.9920	25.81		480	95
				PH				0.4694	0.9976	23.47		360	96
PROXYL	PROXYL	1	JP899	IPH	0.2	4.0	5	2.435	0.9997	608.8	608.0 ± 4.233	480	89
				PH				2.361	0.9990	590.3	602.6 ± 24.90	480	89
	PROXYL	2	JP8100	IPH	0.2	4.0	5	2.438	0.9997	609.6		480	89
				PH				2.410	0.9996	602.4		540	89
	PROXYL	3	JP1101	IPH	0.2	4.0	5	2.423	0.9998	605.6		600	86
				PH				2.461	0.9996	615.2		480	86

[c] $k = 0.07$ $M^{-1}s^{-1}$ was reported for **5** at rt, however, the temperature was not well controlled in this measurement.[S3]

1.c Analysis of paramagnetic ^{1}H NMR spectra.

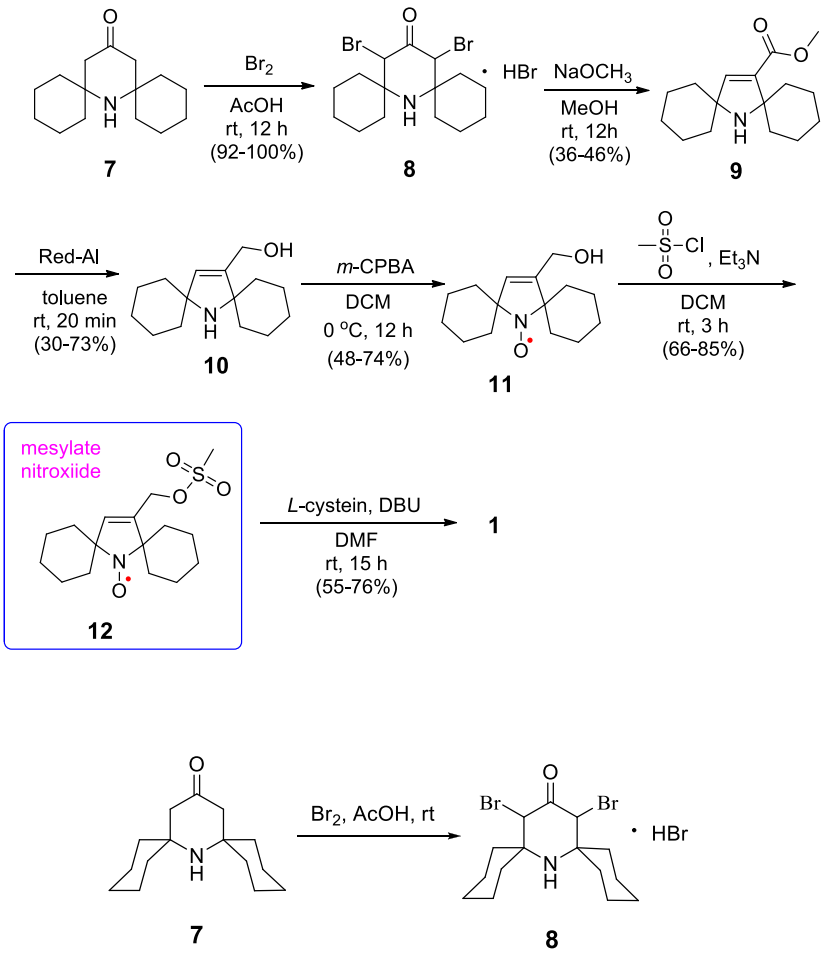
Table S2. Analyses of paramagnetic ^{1}H NMR spectra for pyrroline nitroxides and amino acids **1** – **4**.

Compd.	Sample label	Paramagnetic integrals		Diamagnetic integrals		Int (H_2O)	Concentration of nitroxide (M)	Minimum apparent purity (%)[a]
		Values	∑	Values	∑			
11	YW11-72col	1.000, 4.420, 6.748, 0.758, 2.408, 10.868, 3.707, 0.595	30.504	0.054, 0.108, 0.272	0.434		0.73	99
12	YW11-77col1	1.309, 1.948, 3.000, 0.349, 0.907, 2.054, 1.134	10.842	0.065, 0.047, 0.121	0.233		0.70	98
1	YW11-88colA2	0.748, 1.040, 1.390, 1.439, 1.163, 1.759, 0.423	7.962	0.205, 0.047, 0.031, 0.088, 0.052, 0.636, 0.320	1.379		0.095	85[b]
17	YW12-71col	1.000, 1.859, 0.948, 0.190	3.997	0.001, 0.018, 0.096	0.115		0.30	97
18	YW12-78col	2.919, 3.935, 1.840, 3.000, 0.972, 1.035	13.701	0.003, 0.048, 0.261, 0.062	0.371		0.54	97
2	YW13-08crp2	3.902, 1.627, 2.000, 0.615, 0.578, 0.831, 0.607	10.16	0.038, 0.152, 0.079	0.269		0.14	97
19	CRR-1-55-f2	1.000, 2.086, 3.047, 4.668, 2.813, 1.590, 2.074, 0.692, 1.111, 0.574	19.655	0.069, 0.760	0.829		1.8	96
5	JTP-10-8-f1	1.000, 0.142, 6.541, 3.498, 4.395	15.576	0.010, 0.179, 0.130	0.319		1.4	98
20	JTP-10-14-f1	0.710, 4.000, 1.718, 0.855, 0.932	8.215	0.060, 0.275	0.335	0.109	0.5	96
3	YW13-11col	0.264, 1.335, 2.530, 0.741, 0.853, 12.000, 2.222, 0.491	20.436	0.008, 0.483, 0.121	0.612		0.10	97
21	JTP-10-20-f1	5.654, 5.614, 3.000, 1.771, 0.751, 0.487, 1.043	18.320	0.035, 0.253, 0.309, 0.008	0.605		2.5	97
6	JTP-10-21-f1	10.000, 14.717, 3.408, 3.313	31.438	0.310, 0.268, 0.024, 0.106	0.708	0.045	1.3	98
22	JTP-10-23-f1	1.554, 3.062, 4.554, 4.000, 0.024, 1.670, 0.311, 2.054	17.229	0.079, 0.163, 0.079, 0.153, 0.321, 0.063	0.858	0.178	0.8	95
4	YW13-17col	1.998, 0.380, 1.309, 2.338, 1.120, 1.115, 12.000, 1.080	21.34	0.027, 0.029, 0.085	0.141		0.17	99

[a] Minimum apparent purity (%) is computed under the assumption that some of the paramagnetic peaks are missed (or under-integrated) because they are too broadened to be detected at relatively low concentrations of nitroxides. [b] Very low concentration (<0.095 M, saturated solution) of nitroxide amino acid **1** was used because of its low solubility in common organic solvents (see: footnote "a").

2. Experimental Section: Synthesis of Pyrroline Nitroxides and Amino Acids 1 – 4.

Scheme S1. Synthesis of amino acid **1**.

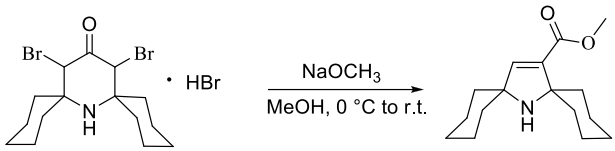


Summary for preparation of **8**:

Run	ID	SM (g/mmol)	Br_2 (mL/equiv)	AcOH (mL)	Yield (g/%)	Label
1	YW1039	1.00/4.3	0.88/4.0	5.0	1.86/92	YW10-39PPT
2	YW1080	0.96/4.1	1.05/5.0	3.5	1.99/100	YW10-80PPT
3	YW1155	2.1/8.9	2.8/6.1	8.2	4.16/98	YW11-55PPT
4	YW1156	2.12/9.0	2.8/6.0	8.2	4.3/100	YW11-56PPT

YW10_80: Starting material, 7-aza-3,11-dithiadispiro[5.1.5.3]hexadecane-15-one (**7**) was prepared according to the previously published procedure.[S4] This reference[S3] was followed in preparation of **8**. To the stirred solution of 7-Azadispiro[5.1.5.3]hexadecan-15-one (**7**) (label: BF2-71; 0.96 g, 4.1 mmol, 1.0 equiv) in acetic acid (2.0 mL), a solution of Br_2 (1.05 mL, 20.4 mmol, 5.0 equiv) in acetic acid (1.5 mL) was added drop by drop. A yellow precipitate was formed at the beginning and finally a red-orange mixture was obtained. The mixture was stirred at ambient temperature for overnight. The precipitate was filtered off, which was washed with acetic acid

(totally 25 mL), then diethyl ether (3 mL × 2) and evacuated under high vacuum at ambient temperature to yield the product **8** (1.99 g, yield: 100%; label: YW1080PPT) as a yellow powder which was directly used for next step without further purification.



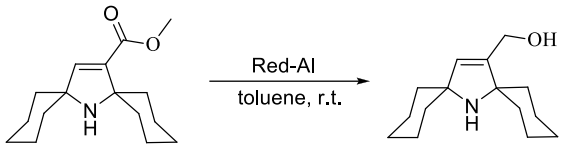
8 **9**

Summary for preparation of compound **9**:

Run	**ID**	**SM** (g/mmol)	**Na** (mL/equiv)	**MeOH** (mL)	**Yield** (g/%)	**Label**
1	YW1040	1.68/3.5	0.41/5.0	14	0.48/43	YW10-40crp1
2	YW1085	1.99/5.1	0.84/7.2	18	0.51/46	YW10-85-fr2&3
3	YW1157	8.36/21.5	4.0/8.1/	120	1.69/36	YW11-57crp2&3

YW10_85: We followed this reference[S5] for preparation of *gem*-dimethyl pyrroline methyl ester. In argon bag, metal sodium (0.84 g, 36.3 mmol, 7.16 equiv) was dissolved into anhydrous methanol (12 mL) in a round bottom flask to give a sodium methoxide solution in methanol. The flask was sealed, then an argon balloon was attached. To this solution in ice-water bath, the mixture of starting material **8** (1.99 g, 5.1 mmol, 1.0 equiv; label: YW10-80cr) in 6.0 mL anhydrous methanol was added dropwise at 0 °C in five portion during 50 min. The ice-water bath was removed, and the reaction mixture was stirred at ambient temperature for 12 h. The mixture was concentrated under reduced pressure until most the methanol was removed. The residue was re-dissolved in 10% aqueous K_2CO_3, then extracted with ethyl ether. The ether layer was washed with brine (× 3), dried over Na_2SO_4, concentrated under reduced pressure. Purification on silica gel flash column chromatography (ether/hexanes, 1/12) to give the product **9**, 0.51 g (label: YW10-85fr2&3; Yield: 46%), as a light yellow pasty. R_f = 0.24 (ether/hexanes, 1/12). ^{1}H NMR ($CDCl_3$, 400 MHz, YW11-57crp2), δ = 6.85 (s, 1H), 3.74 (s, 1H), 2.13-2.05 (m, 2H), 1.66-1.28 (m, 18H). ^{13}C-NMR ($CDCl_3$, 100MHz, YW11-57crp2): δ = 165.0, 147.9, 139.4, 67.8, 66.4, 51.3, 40.0, 37.6, 25.6, 25.4, 23.4, 22.6. IR (ZnSe, cm^{-1}, YW11-57crp2): 2922, 2952, 1716, 1449, 1434, 1327, 1226, 1139, 1031, 1001, 915, 906, 765. LRMS-ESI (0.1% HCOOH in MeOH, YW11-57crp2), m/z (ion type, % RA for m/z, 150–2000) at $[M+H]^+$: 264.4 (100%), 265.3 (17%). TOF-HRMS-EI (1%

CH_3COONa in 3:1 (v/v) MeOH/H_2O; ion type, %RA for m/z, deviation from the formula; sample label: YW11-57crp2): Calcd. for $^{12}C_{16}H_{26}NO_2$ at $[M+H]^+$: 264.1964; found: 264.1969 (2.1 ppm).

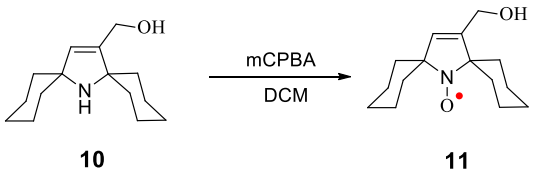


9 **10**

Summary for preparation of compound **10**:

Run	ID	SM (g/mmol)	Red-AL (mL/equiv)	toluene (mL)	Yield (g/%)	Label
1	YW1051	0.17/0.66	0.80/3.9	1.5	0.045/30	YW10-51fr3
2	YW1096	0.25/0.95	0.80/2.7	2.0	0.12/52	YW10-96col
3	YW1124	0.25/0.95	0.59/2.0	2.0	0.14/63	YW11-24col
4	YW1162	1.98/7.52	4.8/2.1	12	0.81/73	YW11-62crp1,2&3

YW11_62: The procedure was slightly modified from the one which was published before for preparation of 3-(hydroxymethyl)-2,2,5,5-tetramethyl-3-pyrroline.[S6] To the solution of ester **9** (label: YW11-57-crp1-3; 1.98 g, 7.52 mmol, 1.0 equiv) in anhydrous toluene, sodium bis(2-methoxyethoxy)aluminumhydride (Red-Al, 65% solution in toluene, 3.215 M; 4.8 mL, 15.4 mmol, 2.05 equiv) was added dropwise in argon atmosphere at –40 °C. The mixture was warmed up to ambient temperature and stirred for 20 min. The mixture was cooled back to 0 °C, quenched with aqueous KOH solution (20%, 10 mL), then extracted with ethyl ether. The organic layer was washed with brine (× 3), dried over Na_2SO_4, then concentrated under reduced pressure. Purification on silica gel flash column chromatography (ether/hexanes, 20/80 to 30/70) to give the product **10**, 0.81 g (label: YW11-62fr2,3&5; Yield: 73%), as almost colorless pasty. R_f = 0.23 (ether/hexanes, 40/60). ^{1}H NMR ($CDCl_3$, 400 MHz, YW11-62crp2), δ = 5.78 (s, 1H), 4.20 (d, 2H, J = 1.6 Hz), 1.66-1.41 (m, 20H). ^{13}C-NMR ($CDCl_3$, 100MHz, YW11-62crp2): δ = 147.9, 129.4, 67.5, 66.2, 59.3, 41.0, 38.4, 25.8, 25.6, 23.9, 25.6. IR (ZnSe, cm^{-1}, YW11-62crp2): 3301, 2091, 2850, 1448, 1414, 1066, 1014, 908, 673. LRMS-ESI (0.1% HCOOH in MeOH, YW11-62crp2), m/z (ion type, % RA for m/z, 150–2000) at $[M+H]^+$: 236.3 (100%), 237.3 (17%). TOF-HRMS-EI (1% CH_3COONa in 3:1 (v/v) MeOH/H_2O; ion type, %RA for m/z, deviation from the formula; sample label: YW11-62crp2): Calcd. for $^{12}C_{15}H_{26}NO$ at $[M+H]^+$: 236.2014; found: 236.2004 (-4.4 ppm).

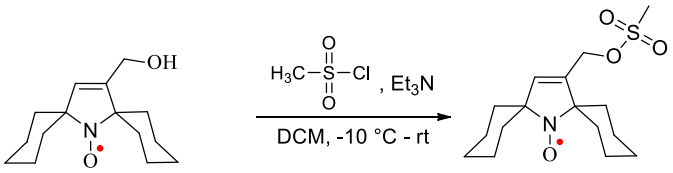


10 11

Summary for preparation of compound **11**:

Run	ID	SM (g/mmol)	mCPBA (g/equiv)	Product			Comment
				Yield (mg/%)	Spin Conc (%)	Label	
1	YW1114	0.010/0.043	0.0089/1.20	0.0051/48	104	YW11-14fr2	
2	YW1123	0.055/0.23	0.066/1.63	0.0344/58	96	YW11-23col	
3	YW1153	0.12/0.52	0.17/1.91	0.047/36	/	YW12-53crp1	
4	YW1170	0.19/0.80	0.23/1.64	0.63/74	99	YW11-72col&col3	Reserved 28 mg pure For characterization
5	YW1172	0.61/2.6	0.72/1.61				

YW11_23: To compound **10** (label: YW10-96col; 55.1 mg, 0.23 mmol, 1.0 equiv) in anhydrous DCM (2.2 mL) at argon atmosphere, *meta*-Chloroperoxybenzoic acid (mCPBA: commercial compound which washed with PBS buffer, YW1165; 66.0 mg, 0.38 mmol, 1.6 equiv) in anhydrous DCM (2.2 mL) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 24 h to give a yellow suspension mixture. The mixture was concentrated under reduced pressure. The residue was redissolved into ether, washed with saturated aqueous $NaHCO_3$ (× 3) then brine (× 2), dried over Na_2SO_4, then concentrated under reduced pressure. Purification on silica gel flash column chromatography (benzene/acetone, 100/3) to give the product **11**, 34.4 mg (label: YW11-23col; Yield: 58%, spin concentration 96%) as yellow pasty which solidify after kept at freezer (–20 °C) for 3 days. R_f = 0.26 (ether/hexanes, 40/60) and 0.48 (acetone/benzene, 10/90). M.p. 92–97 °C (under argon, YW11-72col); lit.:[S3] yellow crystals, mp 106−108 °C. Paramagnetic ^{1}H NMR spectrum for **11**, see: Fig. S9. EPR (sample label: YW11-72col; 1.11 mM in $CHCl_3$): *g*-value = 2.006; a_N = 14.72 G; spin concentration 99% (data label: YW1281r5&6, YW1324r5). IR (ZnSe, cm^{-1}, YW11-72col): 3406, 2924, 2855, 1448, 1413, 1063, 1049, 997, 906, 829, 689; lit.:[S3] 3362, 3059, 2930, 2858, 1454, 1443, 1408, 1356, 1207, 1194, 1173, 1130, 1047, 1018, 1007, 905, 841, 673, 638, 608, 554. LRMS-ESI (0.1% TFA in DCM, YW11-72col), m/z (ion type, % RA for m/z, 120–800) at $[M]^+$: 250.4 (61%, *N*-oxomethanaminium); at $[M+2H]^+$: 252.3 (100%, protonated hydroxylamine). TOF-HRMS-EI (1% CH_3COONa in 3:1 (v/v) MeOH/H_2O; ion type, %RA for m/z, deviation from the formula; sample label: YW11-72col): Calcd. for $^{12}C_{15}H_{24}N^{23}NaO_2$ at $[M+Na]^+$: 273.1705; found: 273.1708 (1.2 ppm).

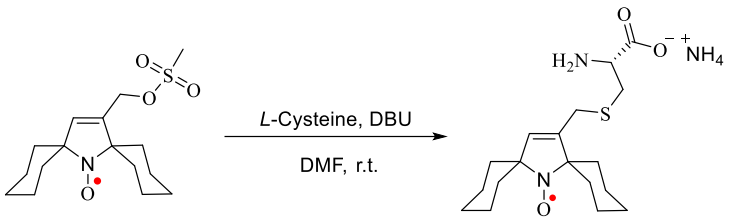


11 **12**

Summary for preparation of **12**:

Run	ID	SM (g/mmol)	Sulfonyl Chloride (mL/equiv)	Et_3N (mL/equiv)	Product		
					Yield (g/%)	Spin Conc (%)	Label
1	YW1130	0.032/0.127	0.012/1.22	0.022/1.25	0.0355/85	99	YW11-30col
2	YW1177	0.60/2.40	0.23/1.20	0.44/1.31	0.54/68	102	YW11-77col1&3

YW11_77: The starting material **11** (label: YW11-72col; 0.60 g, 2.4 mmol, 1.0 equiv) was placed in a Schlenk vessel, and then evacuated under high vacuum for 12 h. The vessel was charged with argon. To the vessel, anhydrous DCM (8 mL) was added, following the addition of triethylamine (redistilled, 0.44 mL, 3.2 mmol, 1.3 equiv). The resultant colorless solution was cooled down to –15 °C with ice/acetone bath, then methanesulfonyl chloride (225 μL, 2.9 mmol, 1.2 equiv) was added drop by drop, providing a yellow suspended mixture. The mixture was warmed up to ambient temperature and stirred for 3 h. The mixture was diluted with DCM, washed with 5% aqueous $NaHCO_3$ (× 3) then brine (× 2). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification on silica gel flash column chromatography (benzene/acetone, 100/1) to give the product **12**, 0.54 g (label: YW11-77col&col3; Yield: 68%, spin concentration 100%, data label: YW1186r5) as yellow solid. R_f = 0.48 (benzene/acetone, 95/5). M.p. 122–125 °C (under argon, YW11-77col1); lit.:[S3] yellow crystals, mp 125−127 °C. Paramagnetic ^{1}H NMR spectrum for **12**, see: Fig. S12. EPR (YW11-77col1, 0.52 mM in $CHCl_3$): *g*-value = 2.006; α_N = 14.69 G; spin concentration 100% (data label: YW1186r5&6, YW1324r8). IR (ZnSe, cm^{-1}, YW11-77col1): 2930, 2857, 1454, 1355, 1174, 966, 924, 907, 859, 833; lit.:[S3] 3024, 3003, 2945, 2926, 2856, 1445, 1414, 1355, 1173, 972, 961, 908, 841, 808, 737, 673, 528, 492. LRMS-ESI (0.1% TFA in DCM, YW11-77col1), *m/z* (ion type, %RA for *m/z*, 120–800) at $[M]^+$: 328.3 (96%, *N*-oxomethanaminium); at $[M+2H]^+$: 330.3 (100%, protonated hydroxylamine). TOF-HRMS-EI (1% CH_3COONa in 3:1 (v/v) MeOH/H_2O; ion type, *m/z*, sample label: YW11-77col1): Calcd. for $^{12}C_{16}H_{26}NO_4{}^{23}NaS$ at $[M+Na]^+$: 351.1480; found: 351.1470 (-2.9 ppm).



12 **1**

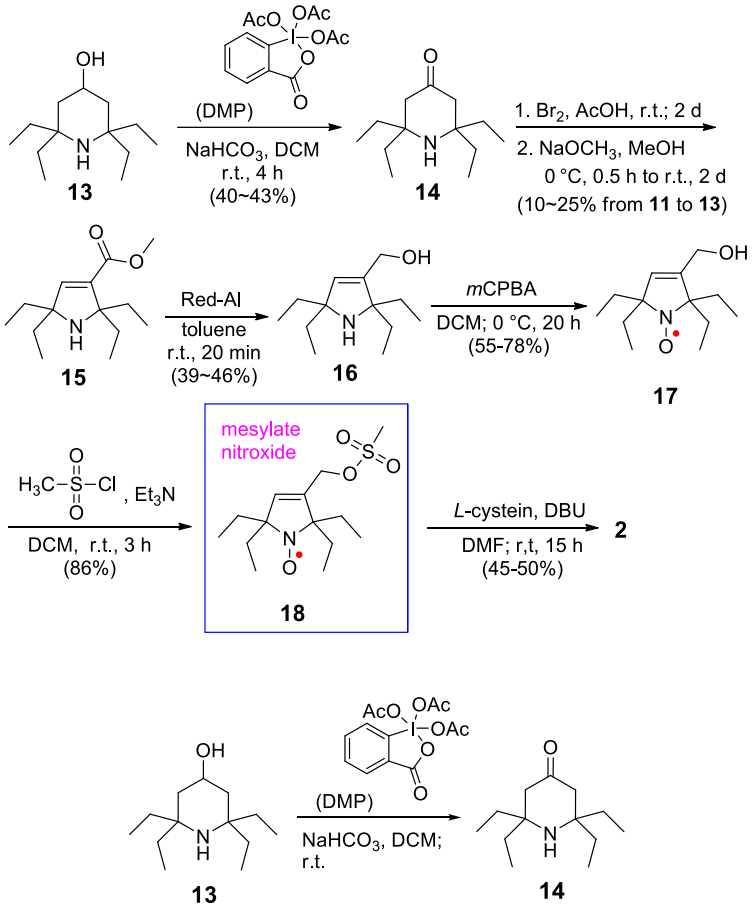
Summary for preparation of amino acid **1**:

Run	ID	SM (g/mmol)	Cysteine (g/mmol)	DBU (mL/equiv)	Product			Comment
					Yield (mg/%)	Spin Conc (%)	Label	
1	YW1114	0.034/0.10	0.020/1.60	0.028/1.82	37.2/102	72	YW11-78PPT2	
2	YW1183	0.105/0.32	0.062/1.61	0.087/1.82	86/76	89	YW11-83col	
3	YW1188	0.165/0.50	0.97/1.60	0.135/1.81	97/55	100 94	YW11-88colA2 YW11-88colB3	
4	YW1231	0.166/0.51	0.97/1.58	0.140/1.85	93.6/52	94 87	YW12-31colA	

YW11_88: To *L*-cysteine (96.5 mg, 0.80 mmol, 1.60 equiv) crystals in a Schlenk vessel prefilled with argon, anhydrous DMF (2.6 mL) was added, following the addition of 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU, redistilled; 135 μL, 0.90 mmol, 1.81 equiv). The mixture was stirred at ambient temperature for 1 h, providing a homogeneous colorless solution with tiny undissolved *L*-cysteine crystal suspended. The mixture was cooled to 0 °C with ice-water bath. Then nitroxide **12** (sample label: YW11-77col&col3; 163.5 mg, 0.50 mmol, 1.0 equiv) in anhydrous DMF (1.2 mL) was added. The mixture was stirred at ambient temperature for 12 h. The resultant pale yellow milk mixture was concentrated under high vacuum, re-dissolved into methanol (3 mL), and centrifuged to remove the white precipitate. The obtained yellow homogenous solution was concentrated under reduced pressure. The residue was purified on silica gel (reverse phase, C18, 40% surface coverage) flash column chromatography (MeOH/H_2O/(28%, aq.)NH_4OH, 10/80/10) twice to give the product **1**, 59.2 mg (label: YW11-88colA2) with spin concentration of 100% and 37.8 mg (label: YW11-88colB3) with spin concentration of 94% as yellow solid. R_f = 0.52 (reverse phase silica gel, C18, 14% C; MeOH/H_2O/(28%)NH_4OH, 60/30/10). M.p. 150–155 °C (under argon, YW11-88colA2). Paramagnetic ^1H NMR spectrum for **1**, see: Fig. S15. EPR (YW11-88colA2, 1.00 mM in MeOH): α_N = 14.95 G; spin concentration 100% (EPR label: YW1189r3). IR (ZnSe, cm^{-1}, YW11-88colA2): 3067, 2926, 2855, 1644, 1620, 1535, 1452, 1412, 1390, 1343, 906. Optical rotation (sample label: YW11-88colA2; 2.39 mg in 5.0 mL methanol): $[\alpha]_{20}^{D}$ = –10.5°. LRMS-ESI (0.1% HCOOH in MeOH, YW11-88colA2), m/z (ion type, % RA for m/z, 150–2000) at

$[M+H]^+$: 354.3 (100%); at $[2M+H]^+$: 707.3 (35%). TOF-HRMS-EI (1% CH_3COONa in 3:1 (v/v) MeOH/H_2O; ion type, %RA for m/z, deviation from the formula; sample label: YW11-88colA2): Calcd. for $^{12}C_{18}H_{29}N_2O_3{}^{23}NaS$ at $[M+Na]^+$: 376.1797; found: 376.1800 (0.9 ppm).

Scheme S2. Synthesis of amino acid **2**



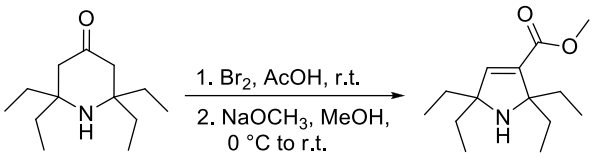
Summary for preparation of **14**:

Run	ID	SM (g/mmol)	DMP (g/equiv)	$NaHCO_3$ (g)	Yield (g/%)	Label	Comments
1	YW1055	0.020/0.093	0.055/1.4	0.118	0.0079/40	YW11-55crp1 [a]	
2	YW1128A	0.50/2.35	1.50/1.5	3.04/15.4	0.42/43	YW11-28col [b]	
3	YW1128B	0.51/2.37	1.51/1.5	3.13/15.7			
4	YW1132A	0.79/3.72	2.37/1.5	4.69/15.0	1.59/99	YW11-32cr [c]	
5	YW1132B	0.78/2.69	2.36/1.5	4.66/15.0			
6	YW1239A	1.16/5.47	3.50/1.5	6.92/15.1	1.77/78	YW12-39cr [c]	
7	YW1239B	1.12/5.26	3.38/1.5	6.66/15.1			
8	YW1295A	0.58/2.71	1.74/1.5	3.57/15.7	1.09/98	YW12-95cr [c]	
9	YW1295B	0.40/1.89	1.30/1.6	2.43/15.3			

[a] Purified by silica gel flash column chromatography at the first step. [b] Passed through a very short gel flash column chromatography. [c] Did not purified. Crude NMR showed it was almost pure.

For reactions on very small scale, typically only tiny amount of water is needed to accelerate the reaction.[S7,S8] In these cases, small amount of degassed water (for example, 10 μL) was added into comparatively large amount of anhydrous DCM (for example, 2.0 mL) in a vial under argon atmosphere, sonicated to give a milk solution, then part of the mixture was transferred to the reaction. Then, sonication and transfers in portion were repeated until enough water was added.

YW12_95A: To starting material 2,2,6,6-tetraethylpiperidin-4-ol (**13**) (0.579 g, 2.71 mmol, 1.0 equiv; sample label: EFR-1-18cr) in 50 mL round bottle flask, $NaHCO_3$ (Aldrich, 3.56 g, 42.5 mmol, 15.7 equiv) was added. The mixture was briefly evacuated under high vacuum for about 3 min, then charged with argon, following the addition of anhydrous DCM (from purification system, 13 mL). After the suspension mixture was cooled down to 0 °C, to this mixture, Dess–Martin periodinane[S8] (1.74 g, 4.1 mmol, 1.52 equiv) in anhydrous DCM (10 mL) was added drop by drop. The mixture was warmed up to ambient temperature, then to this mixture, degassed pure water (57.4 μL, 3.19 mmol, 1.18 equiv) was added dropwise within 45 min (9 drops, 1 drop per 5 min). After stirred at ambient temperature for totally 4 hours, the brown-yellow suspension was concentrated under reduced pressure. By the same approach, another reaction (experiment label: YW1295B) with 0.505 g starting material was also taken parallel. The residues were combined. To the residue, a mixture of 10% $Na_2S_2O_4$/(sat.) $NaHCO_3$ (1:1) was added until the pH was adjusted to 8~9, then taken up with ethyl ether (15 mL×4). The combined organic layers was washed with brine (×3) and water, dried over Na_2SO_4 and concentrated under reduced pressure again to provide the brown crude mixture **14**, 1.09 g (crude yield: 98%), with crude ^{1}H NMR which showed it was almost pure.



14 **15**

Summary for preparation of **15**:

Run	ID	SM (g/mmol [b])	SM Label	Br_2 (1st step) (mL/equiv)	Na (2nd Step) (g/equiv)	Yield (g/%)	Label
1	YW1084 [a] YW1101	0.095/0.45 - /0.45	YL1-71col YW10-84cr [c]	0.19/8.3 /	/ 0.45/39.5	0.0276/26	YW11-01crp2&3
2	YW1141A	0.42/1.97	YW11-28col	0.84/8.3	1.92	*d*	/
3	YW1141B	0.54/2.39	YW11-32cr [c]	1.0/8.1	2.2/40.0	0.184/10 [e]	YW11-54crp1
4	YW1244 YW1246 YW1251	1.77/8.38 - /4.19 - /4.19	YW12-39cr [c] YW12-44cr [c] YW12-44cr [c]	3.5/8.1 / /	/ 3.92/40.7 9.95/41.0	0.50/25 [e]	/ YW12-46col YW12-51col
5	YW1297 YW1307	1.08/4.60 - /4.60	YW12-95cr [c] YW12-97cr [c]	1.9/8.0 /	/ 4.33/0.9	0.163/15 [e]	/ YW13-07crp1&2

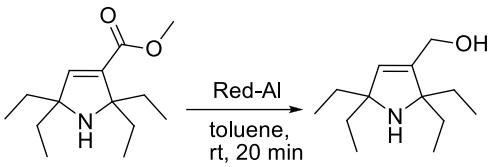
[a] Purified by silica gel flash column chromatography at the first step. [b] If the starting material was a crude compound, the molar amount of starting material was calculated according to the amount of its precursor. [c] Crude starting material was used. [d] Products decomposed after the crude seat in fridge for three weeks. [e] Yield for three steps including the reaction of oxidation by DMP.

YW12_44, YW12_46 & YW12_51:

Bromination: To compound **14** (crude material obtained from the previous step, 1.77 g, 8.38 mmol, 1.0 equiv; sample label: YW12-39cr) in 25 mL round bottle flask, acetic acid (7.0 mL) was added. The brown mixture was cooled down to 0 °C. To the flask, bromine (Br_2, 3.5 mL, 68.0 mmol, 8.1 equiv) was added at 0 °C drop by drop. The obtained dark brown mixture was warmed up to ambient temperature and stirred under the light-free condition for 48 h. The dark solution was evaporated with the flowing of nitrogen, and the exhaust was absorbed with 1:1 (v/v) saturated $Na_2S_2O_3$(aq.)/NaOH (aq.) solution. Then DCM (10 mL) was added and evaporated with nitrogen again. By repeating this for 2~3 times, most of the residual bromine was removed. The obtained brown pasty was evacuated under high vacuum line with two additional liquid nitrogen traps, providing brown foam. To remove the possible trace amounts of acetic acid, the foam was further evacuated under high vacuum for 24 h.

Favorskii rearrangement: In a nitrogen-filled glove bag, the foam from previous step as re-dissolved into 4 mL anhydrous DCM, and half of the brown solution (4.19 mmol, 1.0 equiv) was transferred to a Schlenk vessel, evaporated with the flowing of nitrogen and evacuated under high vacuum for 12 h then re-dissolved into 10 mL anhydrous methanol. In an argon-filled glove bag,

metal sodium (3.92 g, 170.4 mmol, 40.7 equiv) was dissolved into anhydrous methanol (60 mL) in a 250 mL round bottom flask to give a sodium methoxide solution in methanol. The flask was sealed and an argon balloon was attached. The solution was cooled down to 0 °C. Then to this solution, the brown solution of di-bromo compound was added dropwise at 0 °C, providing a light brown suspension. The reaction mixture was stirred at 0 °C for 30 min, then warmed up to ambient temperature and stirred for 48 h. The mixture was concentrated under reduced pressure until most the methanol was removed. The residue was re-dissolved into cool (0 °C) 100 mL 10% aqueous K_2CO_3, then extracted with ethyl ether (× 5). The combined ether layer was washed with brine (× 3), dried over Na_2SO_4, concentrated under reduced pressure. Using this procedure, the other half of dibromo crude compound was also converted to **15**. Purification of the crude mixtures on silica gel flash column chromatography (ether/hexanes, 10/90) gave the product **15** in the total amount of 0.50 g as a light pale pasty (label: YW12-46col and YW12-50col; yield for conversion from **13** to **15**: 25%). R_f = 0.53 (ether/hexanes, 20/80). ^{1}H NMR ($CDCl_3$, 400 MHz, YW12-46col2), δ = 6.72 (s, 1H), 3.72 (s, 3H), 1.73-1.64 (m, 4H), 1.54-1.51(m, 4H), 0.88 (t, 6H, J = 7.2 Hz), 0.83 (t, 6H, J = 7.2 Hz). ^{13}C-NMR ($CDCl_3$, 100MHz, YW12-46col2): δ = 164.9, 148.8, 137.0, 72.2, 66.2, 51.4, 32.2, 31.5, 9.1, 8.9. IR (ZnSe, cm^{-1}, YW12-46col): 2962, 2937, 2869, 1717, 1458, 1436, 1319, 1269, 1236, 1160, 1071, 996, 928, 763. TOF-HRMS-EI (1% CH_3COONa in 3:1 (v/v) MeOH/H_2O; ion type, %RA for m/z, deviation from the formula; sample label: YW12-46col): Calcd. for $^{12}C_{14}H_{26}NO_2$ at $[M+H]^+$: 240.1964; found: 240.1968 (1.9 ppm).



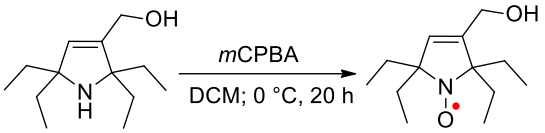
15 **16**

Summary for preparation of **16**:

Run	ID	SM (g/mmol)	Red-Al [a] (mL/equiv)	toluene (mL)	Yield (g/%)	Label
1	YW1237	0.059/0.25	0.30/2.5	0.5	0.020/39	YW12-37col
2	YW1259	0.48/2.0	2.9/3.0	3.5	0.20/46	YW12-59fr1

[a] 65% (w/w, 3.215 M) solution in toluene.

YW12_59: To the solution of ester **15** (label: YW11-01crp2, YW12-46col, YW12-51col; 0.48 g, 2.0 mmol, 1.0 equiv) in anhydrous toluene, sodium bis(2-methoxyethoxy)aluminumhydride (Red-Al, 65% solution in toluene, 3.215 M; 2.9 mL, 6.1 mmol, 3.0 equiv) was added dropwise at –40 °C under the flowing of argon. The mixture was warmed up to ambient temperature and stirred for 20 min. Then the mixture was cooled back to 0 °C, quenched with aqueous KOH solution (20%, 5 mL), and extracted with ethyl ether. The organic layer was washed with brine (× 3), dried over Na_2SO_4 and concentrated under reduced pressure. Purification on silica gel flash column chromatography (acetone/DCM, 10/90 to 25/75) to give the product **16**, 0.20 g (label: YW12-59fr1; Yield: 46%), light-yellow pasty. R_f = 0.35 (ether/hexanes, 40/60). ^{1}H NMR ($CDCl_3$, 400 MHz, YW12-37col), δ = 5.63 (s, 1H), 4.07 (t, 2H, *J* = 1.6 Hz), 1.52-1.41 (m, 8H), 0.84 (t, 12H, *J* = 7.6 Hz). ^{13}C-NMR ($CDCl_3$, 100MHz, YW12-37col): δ = 145.2, 130.1, 77.8, 69.1, 59.6, 32.3, 32.1, 9.1, 8.9. IR (ZnSe, cm^{-1}, YW12-37col): 3321, 2961, 2921, 2876, 1458, 1413, 1378, 1100, 1040, 992, 921, 845. LRMS-ESI (0.1% HCOOH in MeOH, YW12-37col), m/z (ion type, % RA for m/z, 150–1000) at $[M+H]^+$: 212.4 (100%), 213.4 (12%). TOF-HRMS-EI (1% CH_3COONa in 3:1 (v/v) MeOH/H_2O; ion type, %RA for m/z, deviation from the formula; sample label: YW12-37col): Calcd. for $^{12}C_{13}H_{26}NO$ at $[M+H]^+$: 212.2014; found: 212.2018 (1.7 ppm).

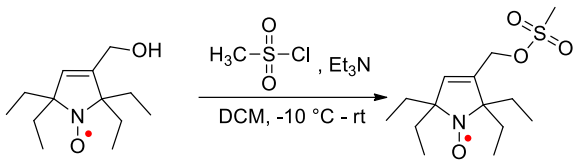


16 **17**

Summary for preparation of **17**:

Run	ID	SM (g/mmol)	mCPBA (g/equiv)	Yield (mg/%)	Spin Conc. (%)	Label
1	YW1243	0.0056/0.026	0.0074/1.61	0.0031/55	97	YW12-43col
2	YW1265	0.0175/0.083	0.0258/1.80	0.0104/56	90	YW12-65col
3	YW1271	0.177/0.84	0.265/1.83	0.148/78	103	YW12-71col

YW12_71: To compound **16** (label: YW12-59fr1; 176.6 mg, 0.84 mmol, 1.0 equiv) in anhydrous DCM (9.0 mL) at argon atmosphere, *meta*-chloroperoxybenzoic acid (*m*-CPBA: commercial compound washed with PBS buffer,[S1] YW1165; 264.8 mg, 1.53 mmol, 1.8 equiv) in anhydrous DCM (6.0 mL) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 20 h to give a yellow suspension mixture. The mixture was concentrated under reduced pressure. The residue was re-dissolved into ether, washed with saturated aqueous $NaHCO_3$ (× 3) then brine (× 2), dried over Na_2SO_4, and then concentrated under reduced pressure. Purification on silica gel flash column chromatography (benzene/acetone, 9/1) to give the product **17**, 148.2 mg (label: YW12-71col; Yield: 78%, spin concentration 103% (original one from calculation)) as yellow pasty. R_f = 0.25 (ether/hexanes, 40/60) and 0.23 (acetone/benzene, 10/90). Paramagnetic ^{1}H NMR spectrum for **19**, see: Fig. S33. EPR (sample label: YW12-71col; 2.3 mM in $CHCl_3$): *g*-value = 2.006; a_N = 14.31 G; spin concentration 103% (data label: YW1274r5&6). IR (ZnSe, cm^{-1}, YW12-71col): 3408, 2967, 2937, 2879, 1458, 1415, 1377, 1111, 1051, 1025, 934, 906, 828, 679. TOF-HRMS-EI (1% CH_3COONa in 3:1 (v/v) MeOH/H_2O; ion type, %RA for *m/z*, deviation from the formula; sample label: YW12-71col): Calcd. for $^{12}C_{13}H_{24}N^{23}NaO_2$ at $[M+Na]^+$: 249.1705; found: 249.1709 (1.7 ppm).

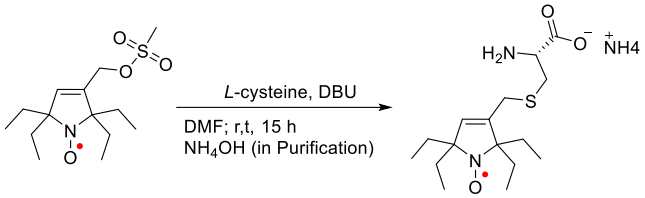


17 **18**

Summary for preparation of **18**:

Run	ID	SM (mg/mmol)	Sulfonyl Chloride (μL/equiv)	Et_3N (μL/equiv)	Product		
					Yield (mg/%)	Spin Conc (%)	Label
1	YW1278	147/0.652	61/1.20	120/1.32	172/86	103	YW12-78col

YW12_78: To starting material **17** (label: YW12-65col & YW12-71col; 147.5 mg, 0.65 mmol, 1.0 equiv) in a schlenk vessel pre-filled with argon, anhydrous DCM (8 mL) was added, following the addition of triethylamine (redistilled, 120 μL, 0.86 mmol, 1.3 equiv). The resultant colorless solution was cooled down to –15 °C with ice/acetone bath, then methanesulfonyl chloride (Aldrich, 61 μL, 0.79 mmol, 1.2 equiv) was added drop by drop, providing a yellow suspended mixture. The mixture was warmed up to ambient temperature and stirred for 3 h, then diluted with DCM, washed with 5% aqueous $NaHCO_3$ (× 3) then brine (× 2). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification on silica gel flash column chromatography (benzene/acetone, 100/2) to give the product **18**, 172 mg (label: YW12-78col; Yield: 87%, spin concentration 103% (original datum from calculation), data label: YW1281r2-3) as yellow solid. R_f = 0.36 (benzene/acetone, 95/5). M.p. 58–61 °C (under argon, YW12-78col). Paramagnetic ^{1}H NMR spectrum for **18**, see: Fig. S27. EPR (YW12-78col, 2.67 mM in $CHCl_3$): *g*-value = 2.006; α_N = 14.25 G; spin concentration 103% (original one from calculation, data label: YW1281r2-3). IR (ZnSe, cm^{-1}, YW12-78col): 2969, 2939, 2880, 1459, 1417, 1354, 1173, 967, 923, 859, 827. TOF-HRMS-EI (1% CH_3COONa in 3:1 (v/v) MeOH/H_2O; ion type, %RA for m/z, deviation from the formula; sample label: YW12-78col): Calcd. for $^{12}C_{14}H_{26}NO_4{}^{23}NaS$ at $[M+Na]^+$: 327.1480; found: 327.1477 (-1.0 ppm).



18 **2**

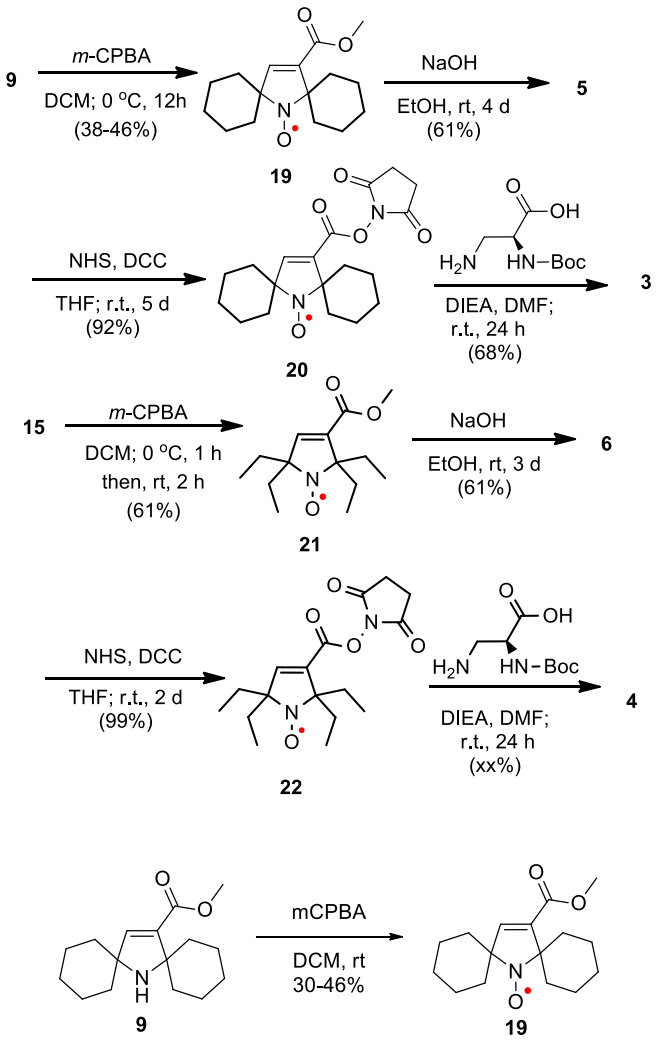
Summary for preparation of spin-labeled amino acid **2**:

Run	ID	SM (mg/μmol)	*L*-cysteine (mg/equiv)	DBU (μL/equiv)	Product			Comment
					Yield (mg/%)	Spin Conc (%)	Label	
1	YW1282	10.2/33.5	6.5/1.6	9/1.8	5.8/50	62	YW12-82col	
2	YW1292	6.2/20.4	3.9/1.6	6/2.0	1.94/28 1.33/19	63 39	YW12-92col-t3 YW12-92col-t4	
3	YW1308	25.2/82.8	16.0/1.6	25/2.0	3.95/14 8.92/31	99 102	YW13-08crp1 YW13-08crp2	

YW13_08: To *L*-cysteine (Aldrich, 16.0 mg, 132 μmol, 1.6 equiv) crystal in a schlenk vessel prefilled with argon anhydrous DMF (180 μL) was added, following the addition of 1,8-Diazabicyclo[5.4.0] undec-7-ene (DBU, Aldrich, redistilled; 25 μL, 167 μmol, 2.0 equiv). The mixture was stirred at ambient temperature for 1 h, providing a homogeneous colorless solution. The mixture was cooled to 0 °C with ice-water bath. Then nitroxide **18** (sample label: YW12-78col; 25.2 mg, 82.8 μmol, 1.0 equiv) in anhydrous DMF (150 μL) was added. The mixture was stirred at ambient temperature for 15 h. The resultant pale yellow milk mixture was centrifuged. The supernatant homogenous yellow solution was concentrated with the flowing of nitrogen, then evacuated under high vacuum. The residue which could dissolved into 0.15 mL methanol to form a homogenous solution was purified on silica gel (reversed phase, C18, 40% surface coverage) flash column chromatography (MeOH/H_2O/(28%)NH_4OH, 10/80/10) to give the product **2** of 3.95 mg (label: YW13-08crp1) with spin concentration of 99% and 8.92 mg (label: YW13-08crp2) with spin concentration of 102% (original datum from calculation) as yellow solid. R_f = 0.80 (reversed phase silica gel, C18, 14% C; MeOH/H_2O/(28%)NH_4OH, 60/30/10). M.p. 163–165 °C (crystalline-like particles after evaporated from methanol and smashed; under argon, YW13-08crp2). Paramagnetic ^{1}H NMR spectrum for **2**, see: Fig. S30. EPR (YW13-08crp1, 0.97 mM in MeOH): *g*-value = 2.006; α_N = 14.67 G; spin concentration 99% (data label: YW1313r5). IR (ZnSe, cm^{-1}, YW13-08crp2): 3124, 2968, 2936, 2869, 1628, 1507, 1460, 1394, 1341, 1095, 935, 832, 668. TOF-HRMS-EI (in 3:1 (v/v) MeOH/H_2O; ion type, %RA for m/z, deviation from the formula; sample label: YW13-08crp2): Calcd. for $^{12}C_{16}H_{29}N_2O_3{}^{23}NaS$ at $[M+Na]^+$: 352.1797;

found: 352.1802 (1.5 ppm). Optical rotation (sample label: YW13-08crp1&2; 10.36 mg in 2.3 mL methanol): $[\alpha]_{20}^{D}$ = –7.7°.

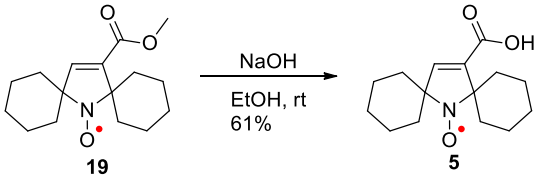
Scheme S3. Synthesis of *N*-Boc-protected amino acids **3** and **4**



Reaction label	SM (mg/mmol)	*m*-CPBA (g/mmol)	DCM (mL)	Time (h)	Yield (mg/%)	Product label	Spin conc. (%)
CRR-1-45	59/0.225	0.158/0.915	3	35	18.9/30	CRR-1-45-f10	97
CRR-1-55	205/0.778	0.530/3.071	11	45	99.0/46	CRR-1-55-f2	102*
JTP-7-78	487/1.849	1.317/7.63	18.2	74	195.9/38	JTP-7-78	
JTP-9-3	1595/6.058	4.314/24.99	60	60	938/56	JTP-9-3-f1	106*
JTP-9-97	497/1.893	1.275/7.39	20	46	205/39	JTP-9-97-f1	

CRR-1-55: A 25 mL three neck round bottom flask was charged with ester-amine **9** (204.8 mg, 0.778 mmol), evacuated, charged with nitrogen and dissolved in DCM (4 mL), the resulting solution was cooled in an ice water bath and a solution of *m*-CPBA in DCM (0.530 g, 3.071 mmol

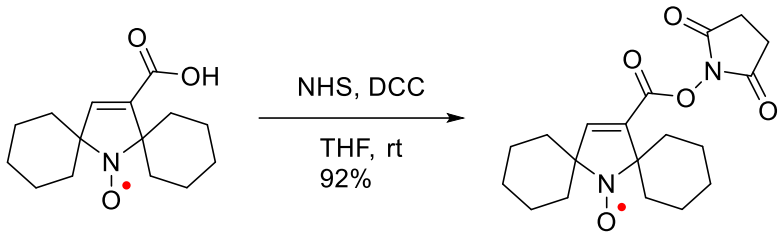
in 7 mL) was added dropwise over 5 minutes. The resulting green solution was stirred at rt for 45 h, over which time the solution became yellow, and finally was evaporated. The resulting solid was dissolved in diethyl ether (25 mL) and washed at 0 °C with saturated aqueous sodium bicarbonate solution (3 × 20 mL), then a 1.5 M aqueous solution of sodium bisulfate (2 × 10 mL), then saturated aqueous sodium bicarbonate solution again (2 × 10 mL). After drying over magnesium sulfate, evaporation in rotary evaporator gave a yellow crude oil which was purified by column chromatography (silica gel, chloroform/ethyl ether/hexanes, 1:1:18) to give nitroxide **19** as yellow crystals (99 mg, 46 %). mp 92–93 °C. EPR; spin concentration ~100%. LR-ESI MS (0.1 % TFA in DCM): *m/z* ion type (%RA for *m/z* = 150–800): 262.4 $[M\text{-}O]^+$ (100%). Paramagnetic ^1H NMR spectrum for **19**, see: Fig. S33. EPR (sample label: CRR-1-55-f2; 1.0 mM in $CHCl_3$): *g*-value = 2.006; a_N = 14.59 G; spin concentration 102% (data label: JP819r7&13). IR (diamond, cm^{-1}, JTP-9-3-f1): 2925, 2856, 1920, 1626, 1436, 1357, 1310, 1237, 1192, 1163, 1118, 1063, 1040, 1024, 257, 908, 840, 775, 760, 731. TOF-HRMS-EI (methanol/sodium acetate, label: JTP-9-3-f1): *m/z* ion type (%RA for *m/z* = 95-820): 301.1641 $[M+Na]^+$ (75%, 4.3 ppm for $C_{16}H_{24}NO_3Na$).



Reaction label	SM	SM (mg/mmol)	NaOH (mg/mmol)	EtOH (mL)	Yield (mg/%)	Product label
JTP-10-2	JTP-9-97-f1	8/0.028	7/0.17	0.3	Yes by HRMS	
JTP-10-8	JTP-9-97-f1	130/0.468	138/3.425	5.8	76/61	JTP-10-8-f1

JTP-10-8: **19** (130 mg, 0.468 mmol), sodium hydroxide (138 mg, 3.43 mmol), and ethanol (5.8 mL) were added to a vial, and then stirred at rt for 4 days. Subsequently, the yellow solution was concentrated. The resultant oil was diluted with water (2 mL) and washed with diethyl ether (2 × 2 mL), acidified with aqueous sodium bisulfate (1.5 M, 3 mL), and extracted chloroform (3 × 2 mL). The organic phase was dried over sodium sulfate and evaporated, yielding a crude yellow solid which was purified by column chromatography (silica gel, diethyl ether/chloroform, 1:24). The yellow band corresponding to the product (the only intensely colored band) was collected, to give nitroxide **5** as a yellow solid (76 mg, 61%). M.p. 203 °C (decomp.); lit.:[S3] light-yellow

crystals, mp 196−198 °C (ethyl acetate). Paramagnetic ^{1}H NMR spectrum for **5**, see: Fig. S35. EPR (sample label: JTP-10-8-f1; 0.4 mM in PBS): *g*-value = 2.005; a_N = 16.03 G; spin concentration 102% (data label: JP864r4&9). IR (diamond, cm^{-1}, JTP-10-8-f1): ~3000 (broad), 2956, 2929, 2863, 2849, 1719, 1626, 1446, 1421, 1293, 1229, 1167, 1116, 995, 932, 907, 859, 780, 759, 684, 668; lit.:[S3] 2955, 2932, 2862, 1722, 1626, 1449, 1421, 1294, 1231, 1171, 1117, 995, 933, 908, 860, 845, 779, 760, 685. TOF-HRMS-EI (methanol/sodium acetate, label: JTP-10-8-f1): *m/z* ion type (%RA for *m/z* = 50-300): 264.1605 $[M]^+$ (11%, 2.0 ppm for $C_{15}H_{22}NO_3$).

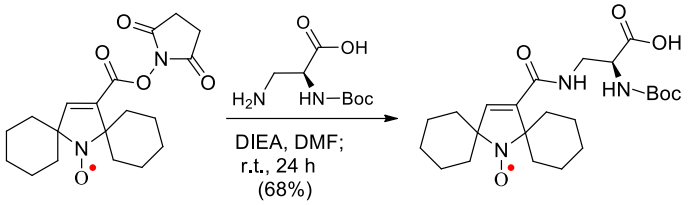


5 **20**

Reaction label	SM	SM (mg/mmol)	NHS (mg/mmol)	DCC (mg/mmol)	THF (mL)	Yield (mg/%)	Product label	Spin conc. (%)
JTP-10-12	JTP-10-8-f1	8/0.031	7/0.062	11/0.055	0.4	Yes TLC		
JTP-10-14	JTP-10-8-f1	58/0.219	34/0.295	66/0.319	3.5	73/92	JTP-10-14-f1	100

JTP-10-14: Nitroxide **5** (58 mg, 0.219 mmol), *N*-Hydroxysuccinimide (34 mg, 0.295 mmol), and *N,N′*-Dicyclohexylcarbodiimide (66 mg, 0.319 mmol) were placed in a 10 mL conical flask, and then briefly evacuated and filled with nitrogen gas. THF (3.5 mL, freshly distilled from sodium and benzophenone) was added and the resulting yellow solution was stirred at rt, protected from light, for 5 days. The reaction mixture was evaporated in rotary evaporator and filtered through cotton with dichloromethane, evaporation of which yielded a crude yellow oil. The crude was dissolved in chloroform and purified by column chromatography (silica gel, chloroform/acetone, 97:3). The product was the only colored (yellow) band. Evaporation of solvents yielded active ester **20** as a yellow solid (73 mg, 92%, label JTP-10-14-f1). A portion (32 mg) was purified by column chromatography (silica gel, chloroform/acetone, 97:3), yielding a yellow solid (31 mg, label JTP-10-14-f1). M.p. 198–201 °C; lit.:[S3] yellow crystals, mp 203−204 °C (methanol). Paramagnetic ^{1}H NMR spectrum for **20**, see: Fig. S37. EPR (sample label: JTP-10-14-f1; 1.0 mM in $CHCl_3$): *g*-value = 2.006; a_N = 14.46 G; spin concentration 100%. IR (diamond, cm^{-1}, JTP-10-14-f1): 2930, 2858, 1768, 1738, 1447, 1368, 1305, 1202, 1080, 1066, 1047, 993, 964, 908, 892, 741; lit.:[S3] 2934, 2856, 1767, 1738, 1620, 1450, 1427, 1371, 1306, 1225, 1203, 1084, 1069,

993, 964, 895, 766, 743, 648, 594. TOF-HRMS-ESI (methanol/sodium acetate, label: JTP-10-14-f1): *m/z* ion type (%RA for *m/z* = 90-900): 416.1921 $[M+CH_3OH+Na]^+$ (98%, 0.6 ppm for $C_{20}H_{29}N_2O_6Na$), 438.1829 $[M+CH_3O+Na_2]^+$ (87%, 20 ppm for $C_{20}H_{28}N_2O_6Na_2$).



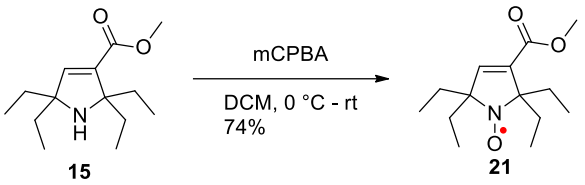
20 **3**

Summary for preparation of *N*-Boc-protected amino acid **3**:

Run	ID	SM (mg/μmol/equiv)	Boc-dap-OH (mg/equiv)	Hünig's base (DIEA) (μL/equiv)	Yield (mg/%)	Spin Conc. (%)	Label
1	YW1310	9.3/25.7/1.1	4.8/1.0	14/3.4	20.3/48	95	YW13-11col
2	YW1311	28.6/79.1/1.1	14.6/1.0	43/3.4	11.6/27	97	YW13-11col2

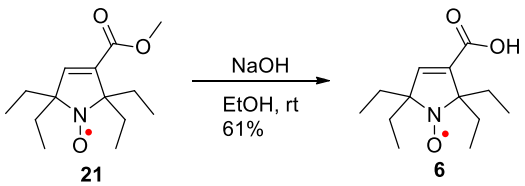
YW13_10 and YW13_11: To aminoacid Boc-dap-OH (Bachem; 4.8 mg, 23.5 μmol, 1.0 equiv) in a schlenk vessel under argon atmosphere, *N*,*N*-Diisopropylethylamine (DIEA; 14 μL, 80.2 μmol, 3.4 equiv) was added. The mixture was cooled to 0 °C. Then to the vessel, nitroxide active ester **20** (9.3 mg, 25.7 μmol, 1.1 equiv) in 0.18 mL anhydrous DMF was added dropwise. The suspension mixture was stirred at ambient temperature with light-free for 24 h. The obtained yellow homogenous solution was concentrated by the flowing of nitrogen, then evacuated under high vacuum for 12 h to give a yellow pasty. With the same procedure, another reaction with 28.6 mg of **20** was also prepared. The two crude pasty were combined and purified on silica gel flash column chromatography (DCM/MeOH/AcOH, 96/4/1) to give the product **3** (20.3 mg, label: YW13-11col) with spin concentration of 95% and 11.6 mg (label: YW13-11col2) with spin concentration of 97% as yellow solid (Yield: 68%). R_f = 0.38 (DCM/MeOH/AcOH, 95/5/1). M.p. 186–188 °C (under argon, YW13-11col2). Paramagnetic ^{1}H NMR spectrum for **3**, see: Fig. S39. EPR (YW13-11col2, 0.99 mM in MeOH): *g*-value = 2.006; α_N = 14.89 G; spin concentration 97% (data label: YW1312r5). IR (ZnSe, cm^{-1}, YW13-11col): 3426, 3348, 2935, 2858, 1748, 1669, 1618, 1538, 1502, 1430, 1366, 1314, 1230, 1149, 848, 782, 666. LRMS-ESI (0.1% HCOOH in MeOH, YW13-11col2), m/z (ion type, % RA for m/z, 150–2000) at $[M+H]^+$: 451.5 (22%); $[M+Na]^+$: 473.7 (17%); at $[2M+Na]^+$: 923.9 (100%). TOF-HRMS-EI (0.1% TFA in 3:1 (v/v) MeOH/H_2O; ion type, %RA for m/z, deviation from the formula; sample label: YW13-11col): Calcd. for

$^{12}C_{23}H_{35}N_3{}^{23}Na_2O_6$ at [M–H+2Na]$^+$: 495.2321; found: 495.2335 (2.8 ppm). Optical rotation (sample label: YW13-11col.CB; 23.93 mg in 2.0 mL methanol): $[\alpha]_{20}^{D}$ = –2.5°.



Reaction label	SM	SM (mg/mmol)	*m*-CPBA (mg/mmol)	DCM (mL)	Time (h)	Yield (mg/%)	Product label
JTP-10-19	YW12-46col1	11/0.046	57/0.330	0.7	6	7/58	JTP-10-19-f1
JTP-10-20	YW13-07crp1 YW13-07crp2	163/0.681	307/1.779	5.3	3	128/74	JTP-10-20-f1

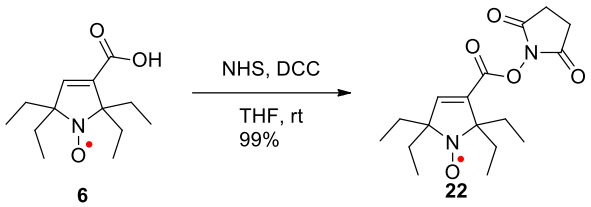
JTP-10-20: A small vial was charged with ester-amine **15** (163 mg, 0.681 mmol) under nitrogen, and dissolved in DCM (1.9 mL), the resulting solution was cooled in an ice water bath and a solution of *m*-CPBA in DCM (307 mg, 1.779 mmol in 3.4 mL) was added dropwise. The resulting green solution was stirred for 1 h, then the ice bath was removed and stirred for 2 h at rt, over which time the solution became yellow, and finally was evaporated. The resulting solid was dissolved in diethyl ether (5 mL) and washed with an aqueous solution of sodium bisulfate (1.5 M, 2 × 3 mL), then saturated aqueous sodium bicarbonate solution (3 × 5 mL). After drying over sodium sulfate, evaporation in rotary evaporator gave a yellow crude oil which was purified by column chromatography (silica gel, diethyl ether/pentane, 1:9) to give nitroxide **21** as a yellow oil (128 mg, 74 %). Paramagnetic ^{1}H NMR spectrum for **21**, see: Fig. S42. EPR (sample label: JTP-10-20-f1; 0.5 mM in $CHCl_3$): *g*-value = 2.006; a_N = 14.22 G. IR (diamond, cm^{-1}, JTP-10-20-f1): 2966, 2938, 2879, 1719, 1627, 1458, 1437, 1375, 1276, 1247, 1207, 1165, 1084, 962, 940, 783, 755. TOF-HRMS-ESI (methanol/sodium acetate, label: JTP-10-20-f1): *m/z* ion type (%RA for *m/z* = 80-920): 277.1664 [M+Na]$^+$ (75%, 3.6 ppm for $C_{14}H_{24}NO_3Na$).



Reaction label	SM	SM (mg/mmol)	NaOH (mg/mmol)	EtOH (mL)	Yield (mg/%)	Product label
JTP-10-21	JTP-10-20-f1	110/0.430	147/3.675	4.3	63/61	JTP-10-21-f1

JTP-10-21: Charge **21**, sodium hydroxide (147 mg, 3.675 mmol), and ethanol (4.3 mL) to a vial and stir at rt for 3 days before concentrating the yellow solution. The resulting oil was diluted

with water (5 mL) and washed with chloroform (3 × 5 mL), acidified with aqueous sodium bisulfate (1.5 M, 3 mL), and extracted chloroform (3 × 5 mL). The organic phase was dried over sodium sulfate and evaporated, yielding a crude yellow solid which was purified by column chromatography (silica gel, diethyl ether/chloroform, 1:24). The yellow band corresponding to the product (the only intensely colored band) was collected, to give nitroxide **6** as a yellow solid (63 mg, 61%). M.p. 126–129 °C. Paramagnetic ^{1}H NMR spectrum for **6**, see: Fig. S44. EPR (sample label: JTP-10-21-f1; 0.4 mM in PBS): *g*-value = 2.006; a_N = 15.76 G; spin concentration 93%. IR (diamond, cm^{-1}, JTP-10-21-f1): ~3000 (broad), 2979, 2944, 2878, 1685, 1624, 1445, 1427, 1288, 1255, 1174, 1089, 943, 749, 680. TOF-HRMS-EI (methanol/sodium acetate, label: JTP-10-21-f1): *m/z* ion type (%RA for *m/z* = 50-300): 240.1600 $[M]^+$ (15%, 0.1 ppm for $C_{13}H_{22}NO_3$).

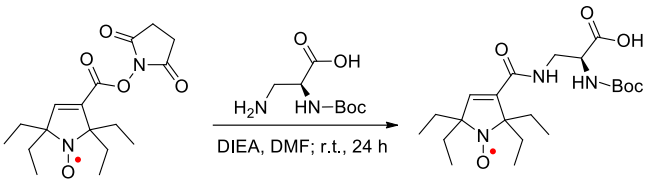


6 22

Reaction label	SM	SM (mg/mmol)	NHS (mg/mmol)	DCC (mg/mmol)	THF (mL)	Yield (mg/%)	Product label
JTP-10-23	JTP-10-21-f1	41/0.171	42/0.362	56/0.270	3	57/99	JTP-10-23-f1

JTP-10-23: Nitroxide **6** (41 mg, 0.171 mmol), *N*-hydroxysuccinimide (42 mg, 0.362 mmol), and *N,N′*-dicyclohexylcarbodiimide (56 mg, 0.270 mmol) were charged to a 10 mL conical flask, briefly evacuated and filled with nitrogen. THF (3 mL, freshly distilled from sodium and benzophenone) was added and the resulting yellow solution was stirred at rt, protected from light, for 2 days. The reaction mixture was evaporated in rotary evaporator and filtered through cotton with dichloromethane, evaporation of which yielded a crude yellow oil. The crude was dissolved in chloroform and purified by column chromatography (silica gel, chloroform/acetone, 97:3). The product was the only colored (yellow) band. Evaporation of solvents yielded active ester **22** as a yellow solid (57 mg, 99%). M.p. 90.5–92.0 °C. Paramagnetic ^{1}H NMR spectrum for **22**, see: Fig. S46. EPR (sample label: JTP-10-23-f1; 0.8 mM in $CHCl_3$): *g*-value = 2.006; a_N = 14.13 G; spin concentration 96% (data label: JP875r2&4). IR (Diamond, cm^{-1}, JTP-10-23-f1): 2972, 2939, 2881, 1769, 1736, 1622, 1458, 1421, 1369, 1235, 1197, 1136, 1065, 992, 978, 942, 910, 881, 841,

813, 779, 739, 644. TOF-HRMS-EI (methanol/sodium acetate, label: JTP-10-23-f1): *m/z* ion type (%RA for *m/z* = 50-400): 337.1752 $[M]^+$ (12%, 3.4 ppm for $C_{17}H_{25}N_2O_5$).



22 **4**

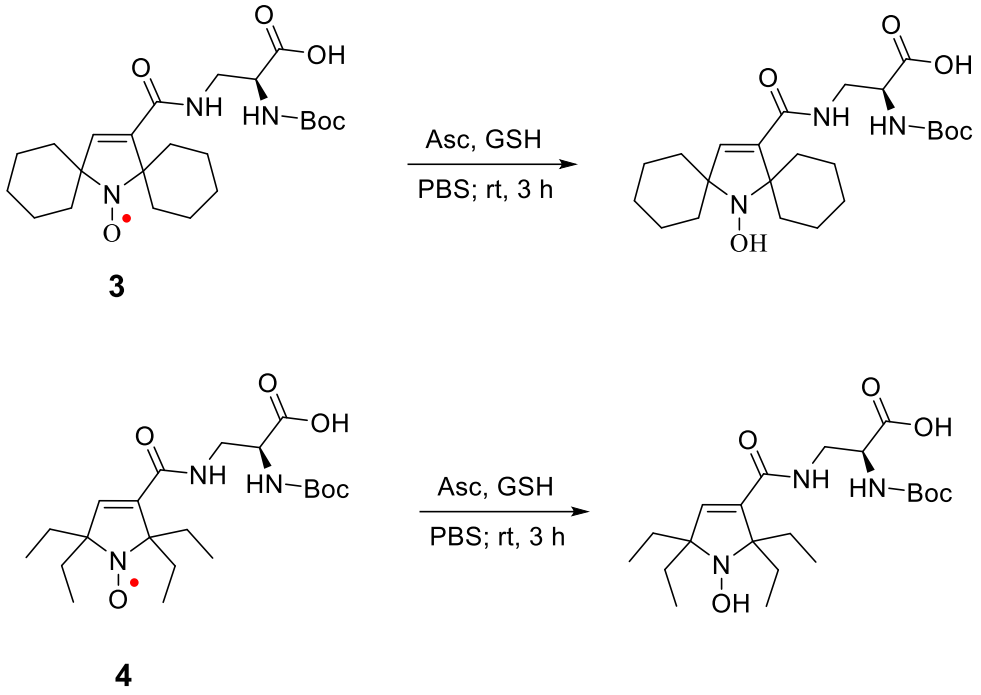
Summary for preparation of *N*-Boc-protected amino acid **4**:

Run	**ID**	**SM** (mg/μmol/equiv)	**Boc-dap-OH** (mg/equiv)	**Hünig's base (DIEA)** (μL/equiv)	**Yield** (mg/%)	**Spin Conc.** (%)	**Label**
1	YW1317	28.2/83.6/1.1	15.5/1.0	45/3.4	26.8/83	100	YW13-17col

YW13_17: To aminoacid Boc-dap-OH (15.5 mg, 75.9 μmol, 1.0 equiv) in a Schlenk vessel under argon atmosphere, *N*,*N*-Diisopropylethylamine (DIEA; 45 μL, 0.26 mmol, 3.4 equiv) was added. The mixture was cooled to 0 °C. Then to the vessel, nitroxide active ester **22** (28.2 mg, 83.6 μmol, 1.1 equiv) in 0.20 mL anhydrous DMF was added dropwise. The suspension mixture was stirred at ambient temperature with light-free for 24 h. The obtained yellow homogenous solution was concentrated by the flowing of nitrogen, then evacuated under high vacuum for 12 h to give a yellow pasty. It was purified on silica gel flash column chromatography (DCM/MeOH/AcOH, 96/3.5/1). The resultant solution was concentrated under reduced pressure. Azeotropic distillation of the concentrated solution (with some residual TFA) with methanol (5 mL × 5) and DCM (5 mL × 3) rapidly, then re-dissolved in 0.2 mL DCM, evaporated with the flowing of nitrogen, and evacuated under high vacuum to give the product **4** of 26.8 mg as yellow foam (label: YW13-17col; Yield: 83%) with spin concentration of 100%. R_f = 0.38 (DCM/MeOH/AcOH, 95/5/1). M.p. 58–67 °C (under argon, YW13-17col). Paramagnetic ^{1}H NMR spectrum for **4**, see: Fig. S48. EPR (YW13-17col, 0.97 mM in MeOH): *g*-value = 2.006; α_N = 14.59 G; spin concentration 97% (data label: YW1318r5). IR (ZnSe, cm^{-1}, YW13-11col): 3336, 2970, 2935, 2877, 1697, 1655, 1611, 1520, 1367, 1300, 1250, 1160, 1048, 1021, 936, 851, 774, 747. LRMS-ESI (0.1% HCOOH in MeOH, YW13-17col), m/z (ion type, % RA for m/z, 150–2000) at $[M+H]^+$: 427.5 (29%); $[M+Na]^+$: 449.7 (11%); at $[2M+Na]^+$: 975.9 (100%). TOF-HRMS-EI (0.1% TFA in 3:1 (v/v) MeOH/H_2O; ion type, %RA for m/z, deviation from the formula; sample label: YW13-11col): Calcd. for

$^{12}C_{21}H_{35}N_3{}^{23}Na_2O_6$ at $[M–H+2Na]^+$: 471.2321; found: 471.2340 (4.0 ppm). Optical rotation (sample label: YW13-11col.CB; 23.93 mg in 2.0 mL methanol): $[\alpha]_{20}^{D}$ = –3.1°.

Scheme S4. Reduction of *N*-Boc-protected amino acids **3** and **4**



3

4

YW13_22: *N*-Boc-protected amino acid **3** (label: YW13-11col.CB; 2.16 mg, 4.79 μmol, 1.0 equiv) was dissolved into 1.5 mL phosphate buffered saline (PBS; 125 mM) to give a homogenous solution with initial spin concentration of 3.27 mM (data label: YW1322r3-4). To this solution, *L*-glutathione reduced (36.86 mg, 120 μmol, 25.0 equiv) was added, following the addition of *L*-ascorbic acid (16.82 mg, 95.5 μmol, 19.9 equiv). The pH value of this solution was adjusted rapidly by NaOH powder to 7.3. The mixture was stirred at ambient temperature with the protection from light for 3 h.

In parallel, to amino acid **4** (label: YW13-17col; 2.25 mg, 5.28 μmol, 1.0 equiv) in 1.0 mL PBS (125 mM) with initial spin concentration of 5.20 mM (data label: YW1322r5-6), *L*-Glutathione reduced (40.62 mg, 132 μmol, 25.0 equiv) and *L*-ascorbic acid (18.36 mg, 104 μmol, 19.7 equiv) was added in sequence. After its pH value was adjusted to 7.3 rapidly, it was stirred at ambient temperature with the protection from light for 3 h.

EPR spin concentration measurement showed that, after 3 hours, the spin concentration of the solution of **3** decreased to 0.0198 mM (conversion: 99.4%), and that of **4** to 3.462 mM (conversion:

33.4%). Then respectively, the solutions were acidified with 0.5 M $NaHSO_4$ to pH 4, extracted with ethyl acetate (2 mL× 6). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure then evacuated under high vacuum to give the residue from the reaction of **3** of 1.23 mg (label: YW13-11col.CBR), and from **4** of 1.91 mg (label: YW13-17colR). The residue were dissolved into CD_3OD and characterized by ^1H NMR (Fig S1 and S2).

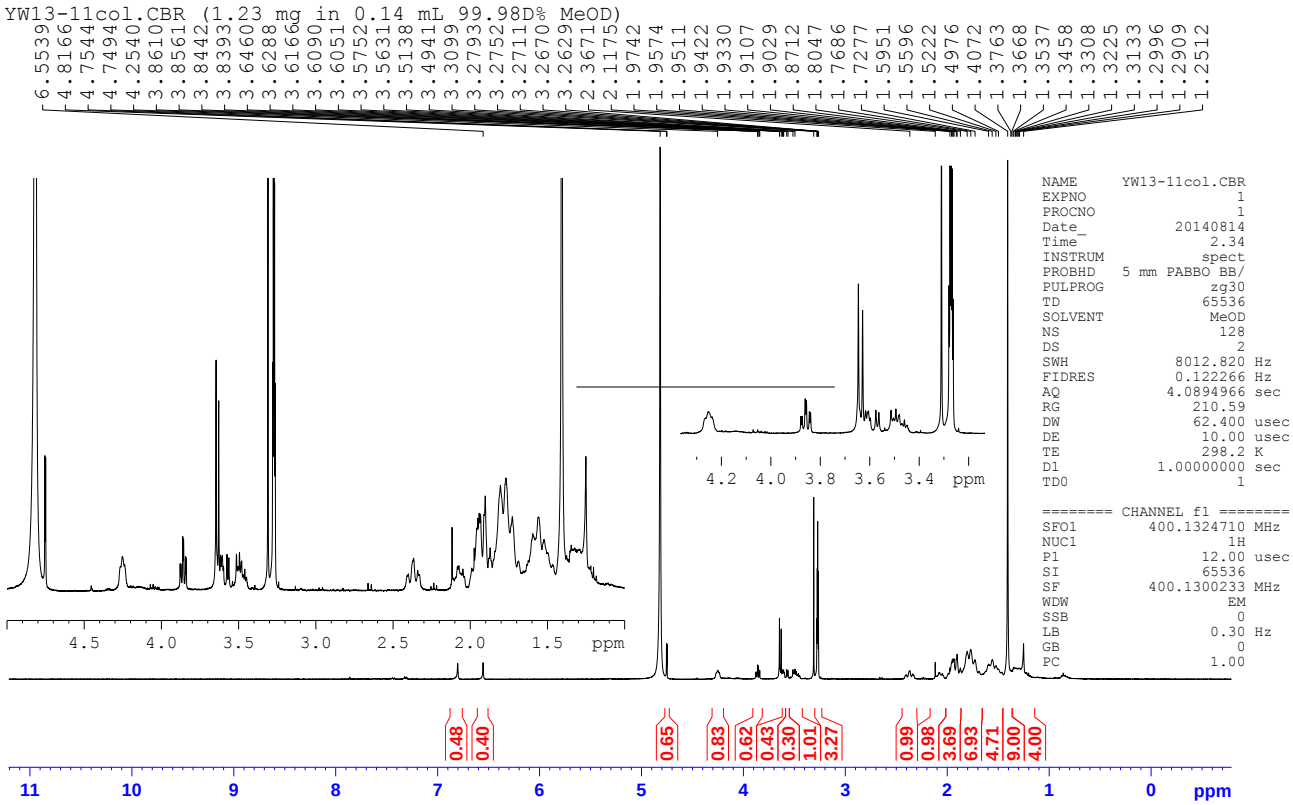


Figure S1. ^1H NMR spectrum (400 MHz, CD_3OD) of the extracted residue from the reduced solution of amino acid **3** (label: YW13-11col.CBR).

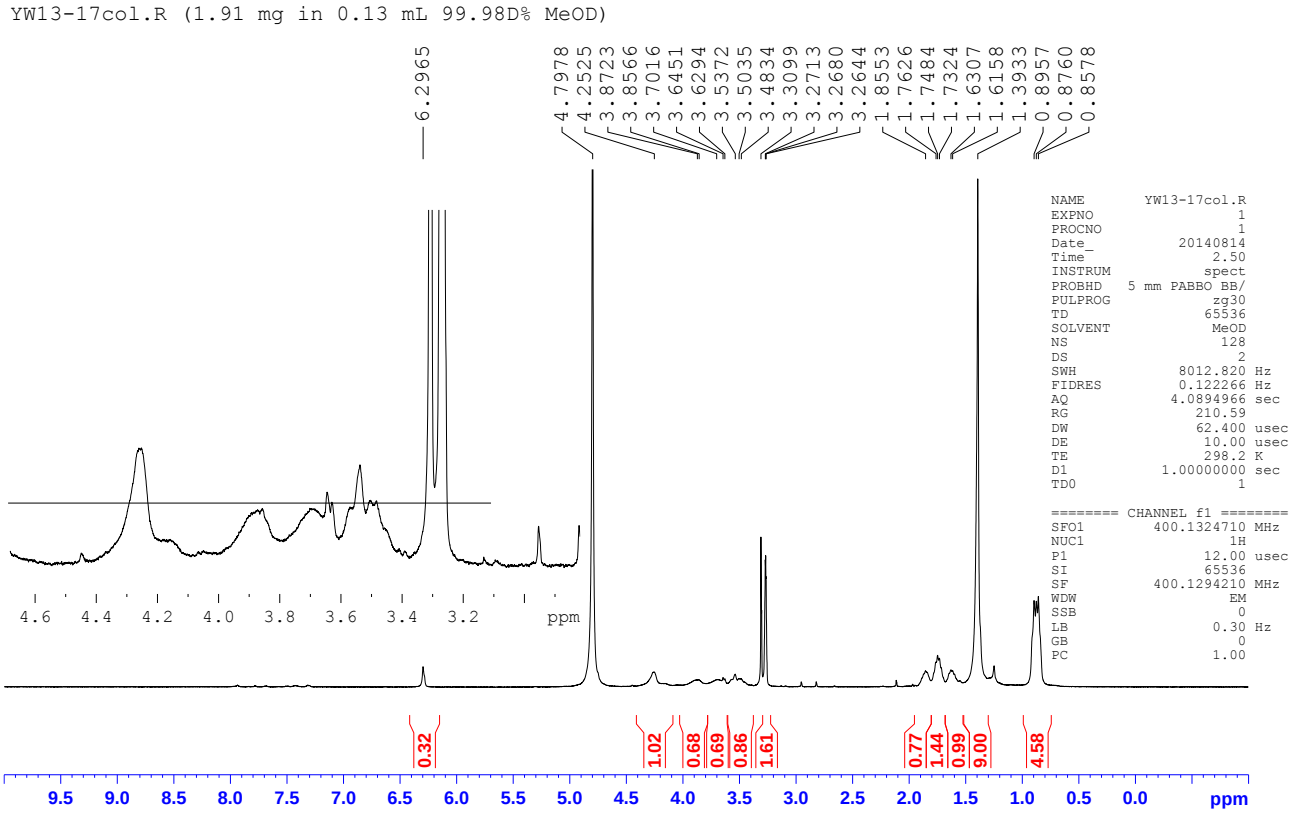


Figure S2. ^{1}H NMR spectrum (400 MHz, CD_3OD) of the extracted residue from the reduced solution of amino acid **4** (label: YW13-17colR).

3. Spectra of Nitroxides (EPR, Paramagnetic ^{1}H NMR, and IR) and Spectra of Diamagnetic Synthetic Intermediates.

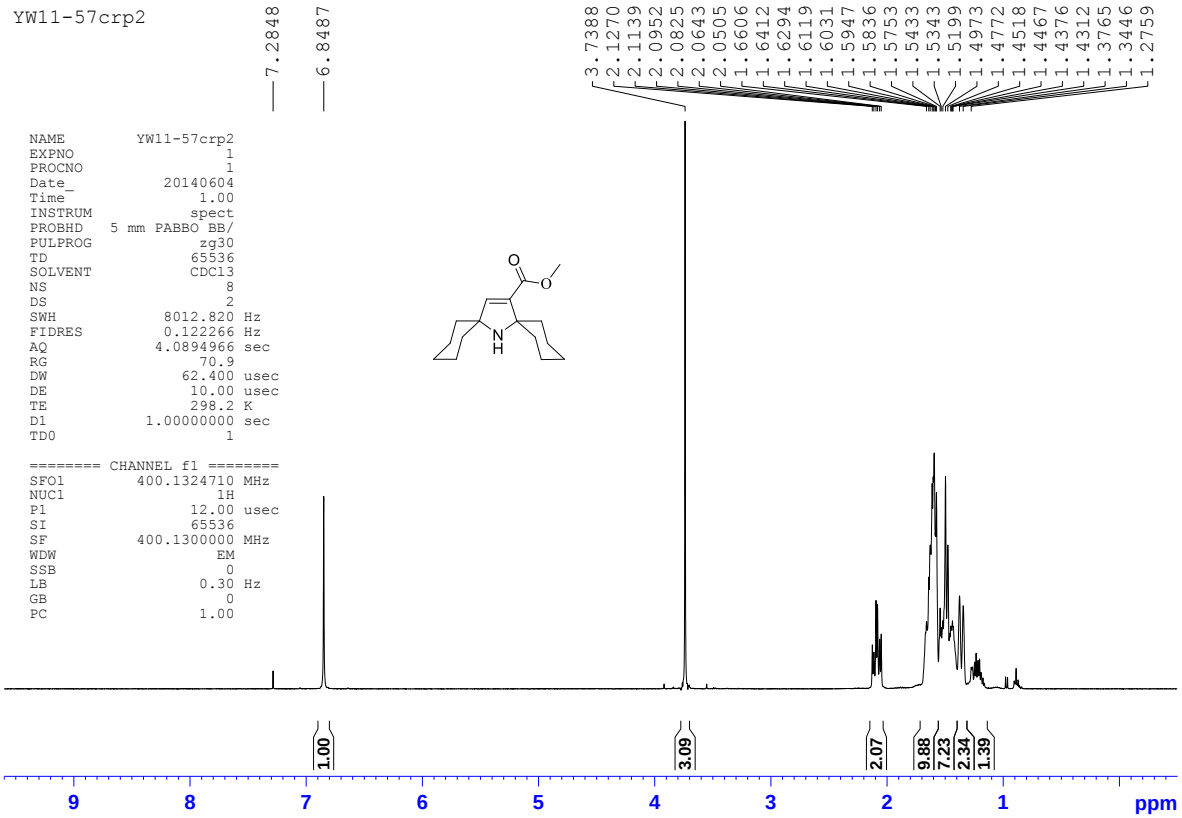


Figure S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **9** (label: YW11-57crp2).

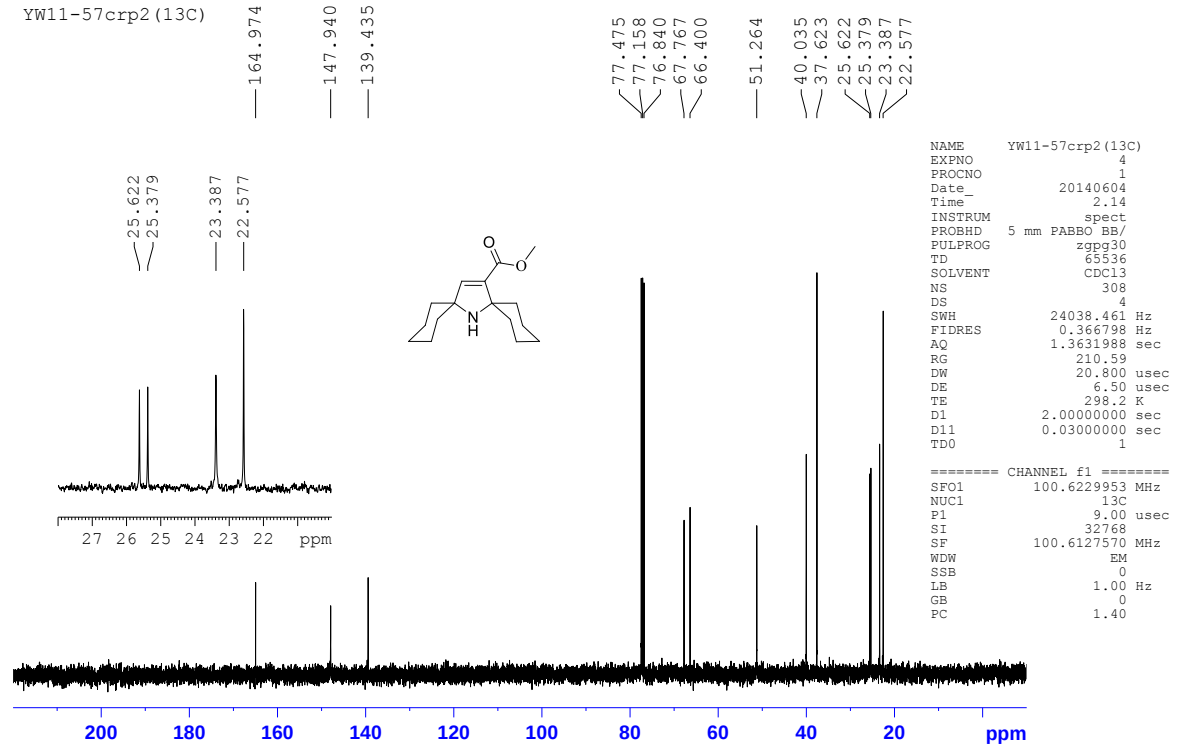


Figure S4. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **9** (label: YW11-57crp2).

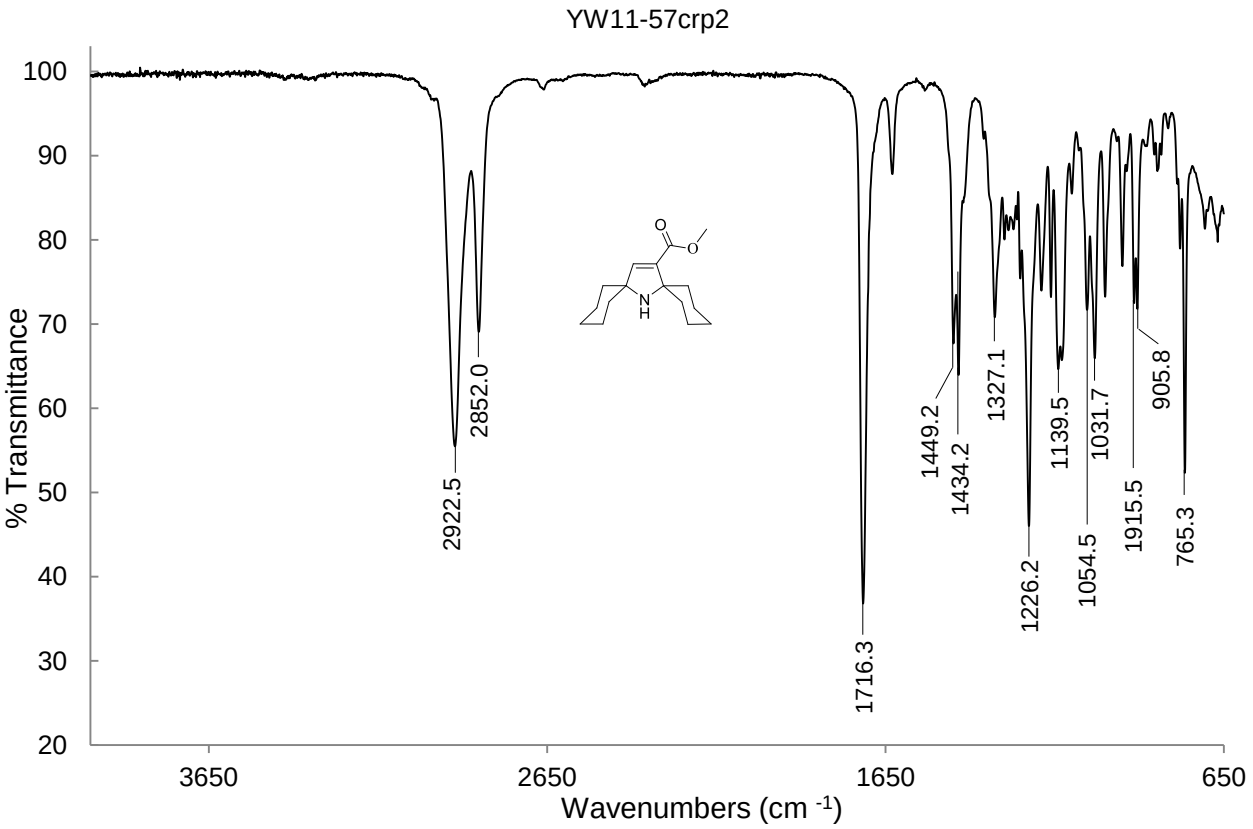


Figure S5. IR spectrum (ATR, ZnSe) of compound **9** (label: YW11-57crp2)

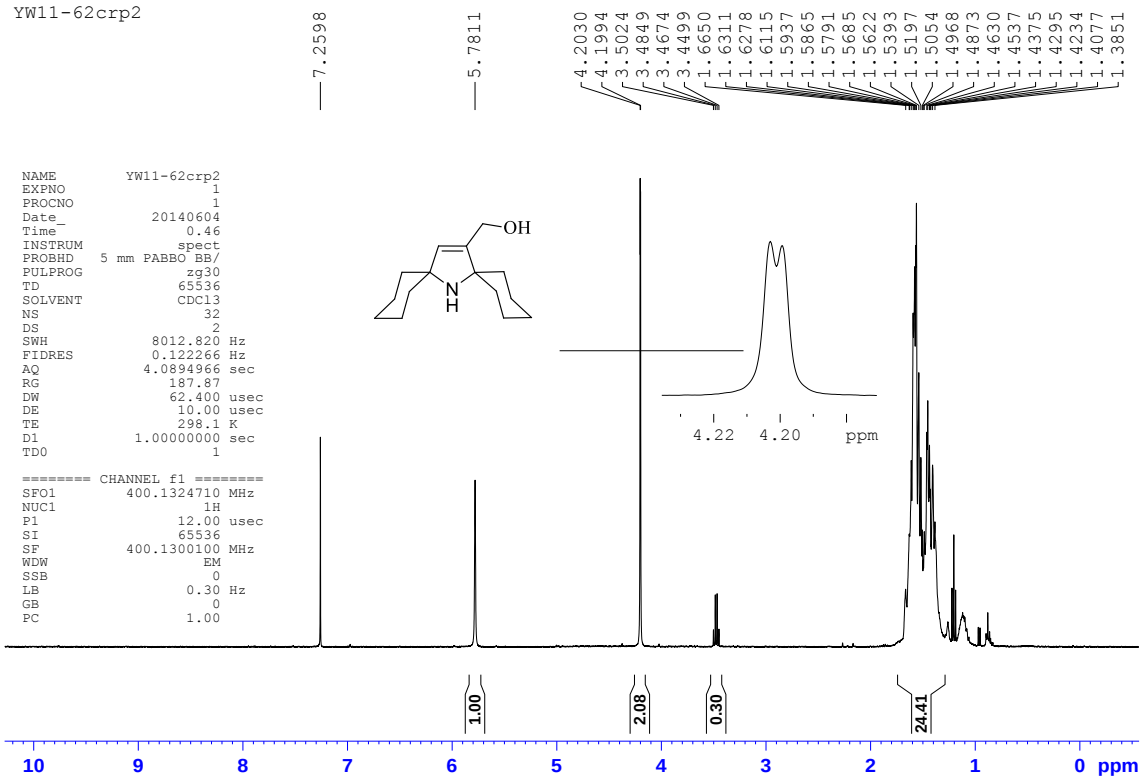


Figure S6. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **10** (label: YW11-62crp2).

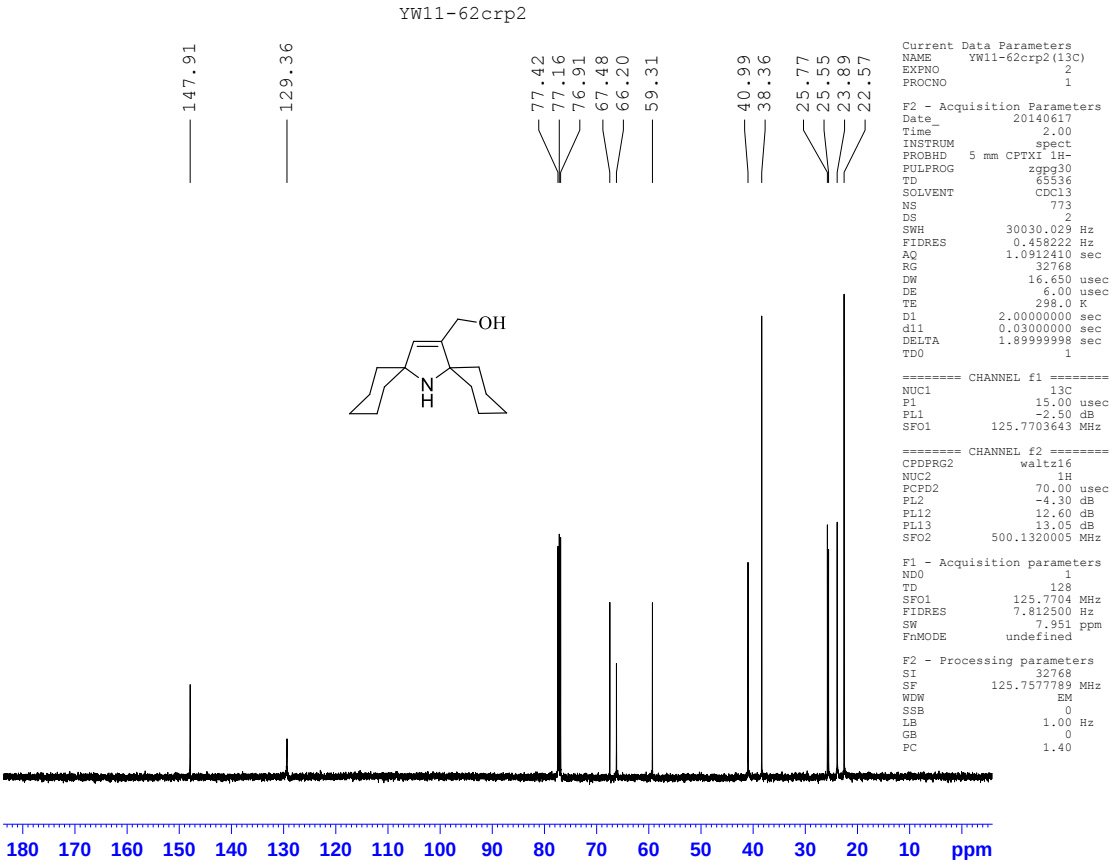


Figure S7. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of **10** (label: YW11-62crp2).

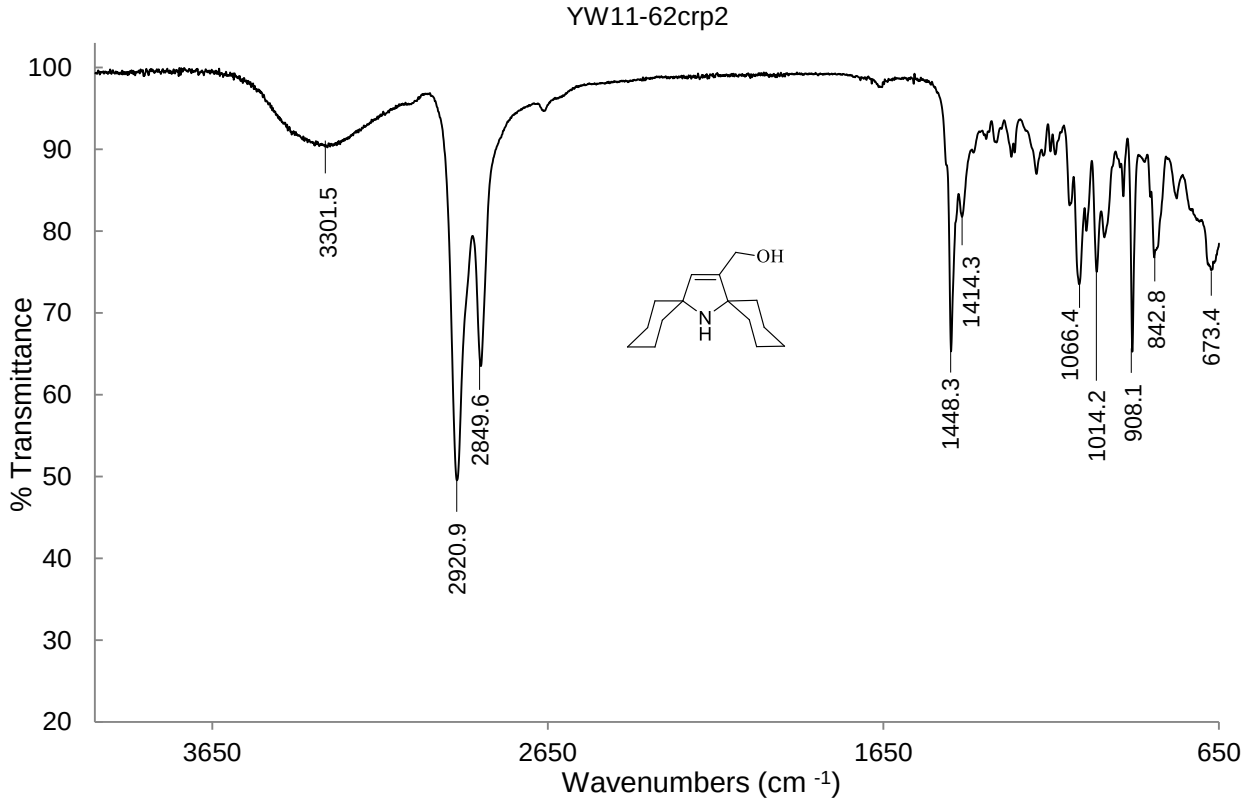


Figure S8. IR spectrum (ATR, ZnSe) of compound **10** (label: YW11-62crp2)

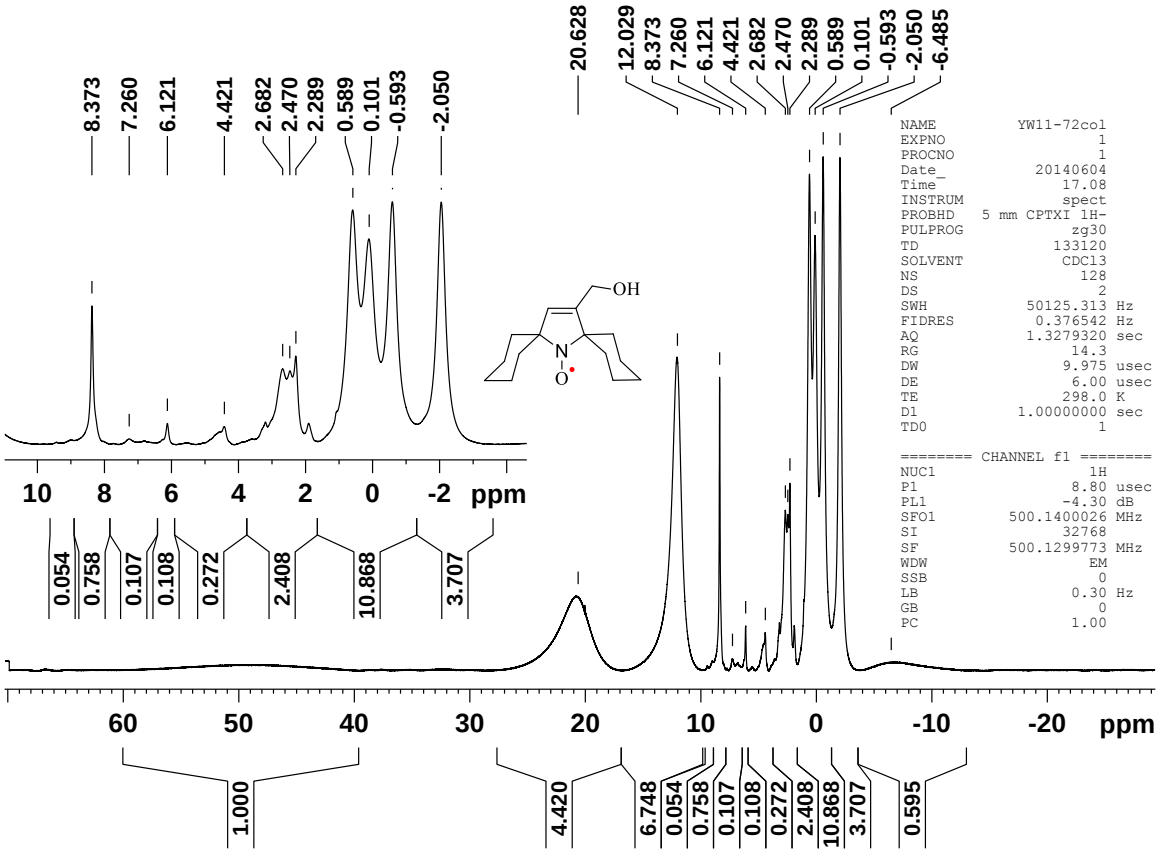


Figure S9. ^{1}H NMR spectrum (500 MHz, 0.73 M in $CDCl_3$) of **11** (label: YW11-72col).

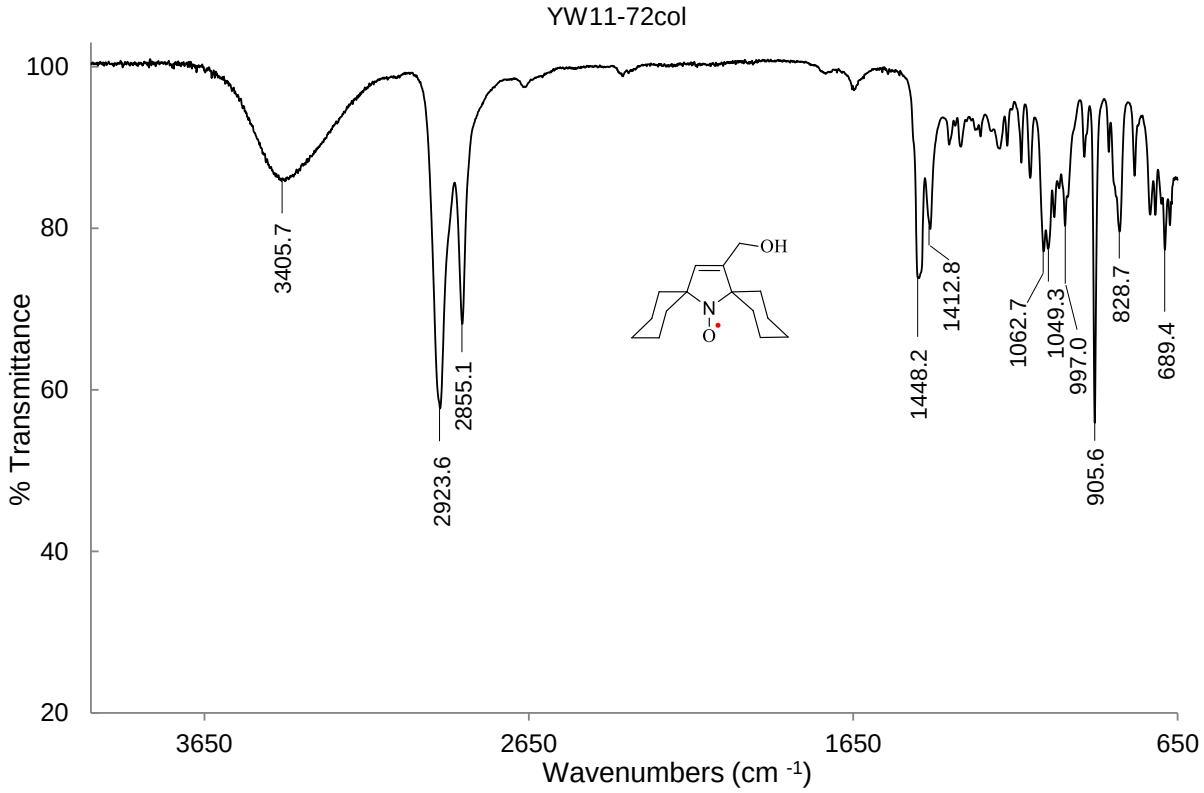


Figure S10. IR spectrum (ATR, ZnSe) of compound **11** (label: YW11-72col)

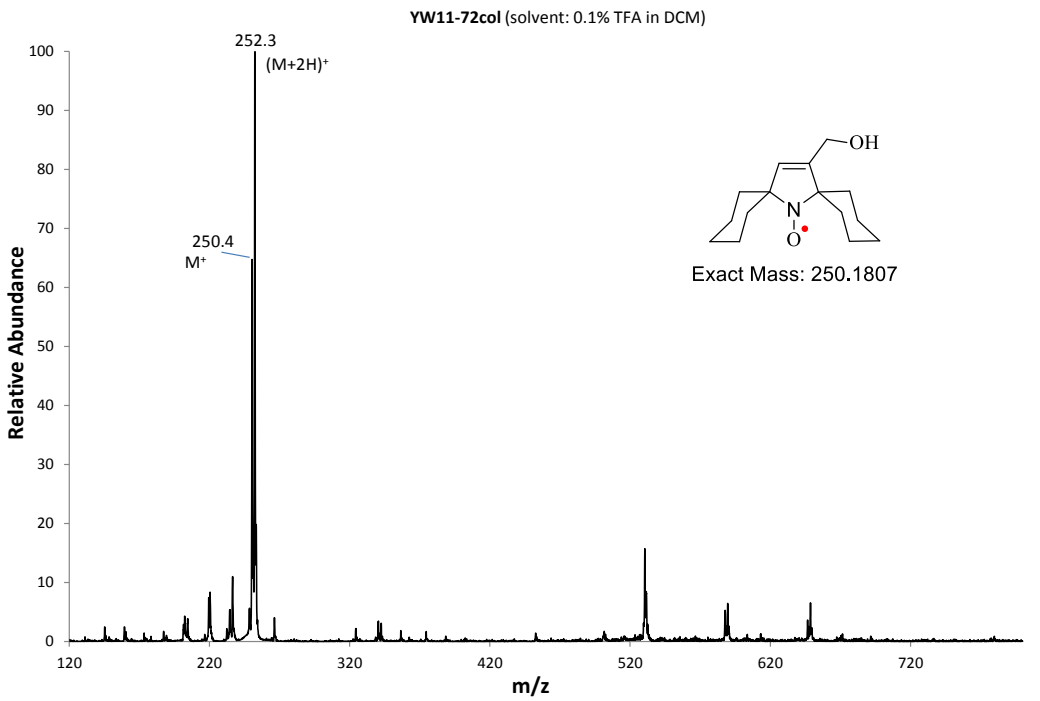


Figure S11. LRMS-ESI spectrum (solvent: 0.1% TFA in DCM) of compound **11** (label: YW11-72col)

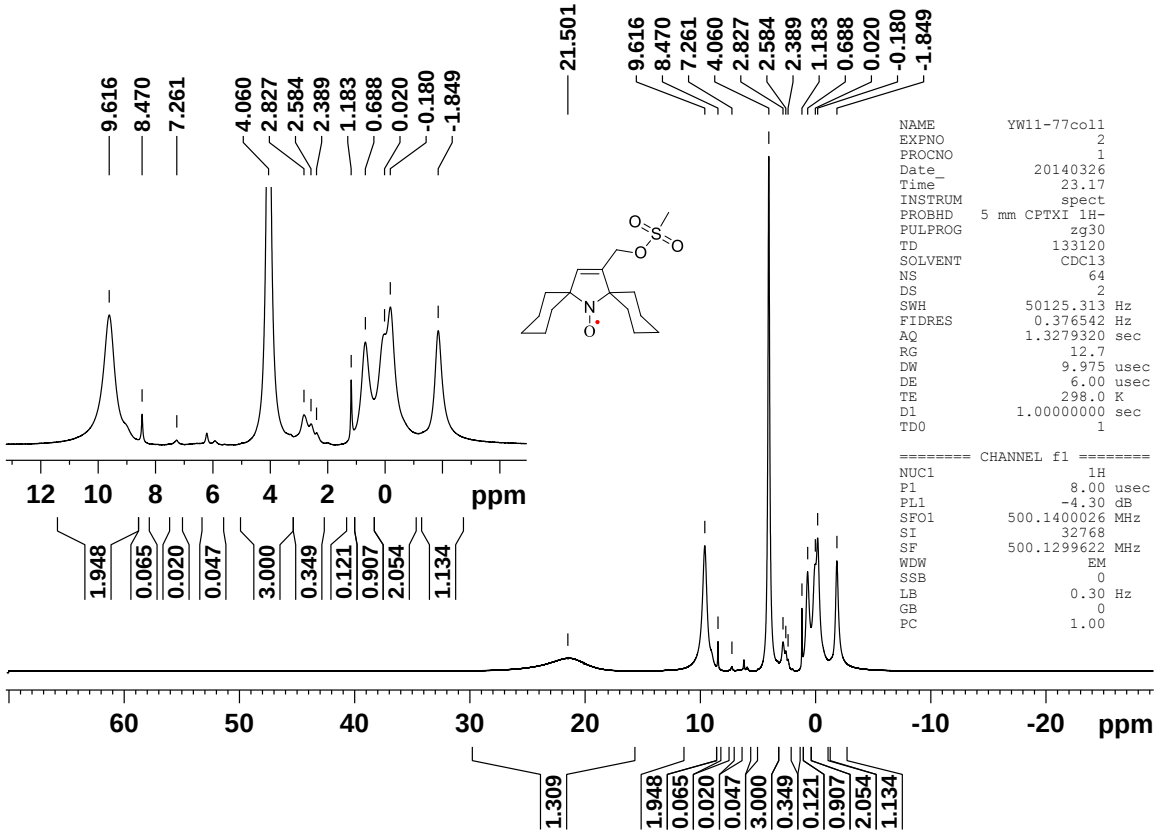


Figure S12. ^{1}H NMR spectrum (500 MHz, 0.70 M in $CDCl_3$) of **12** (label: YW11-77col1).

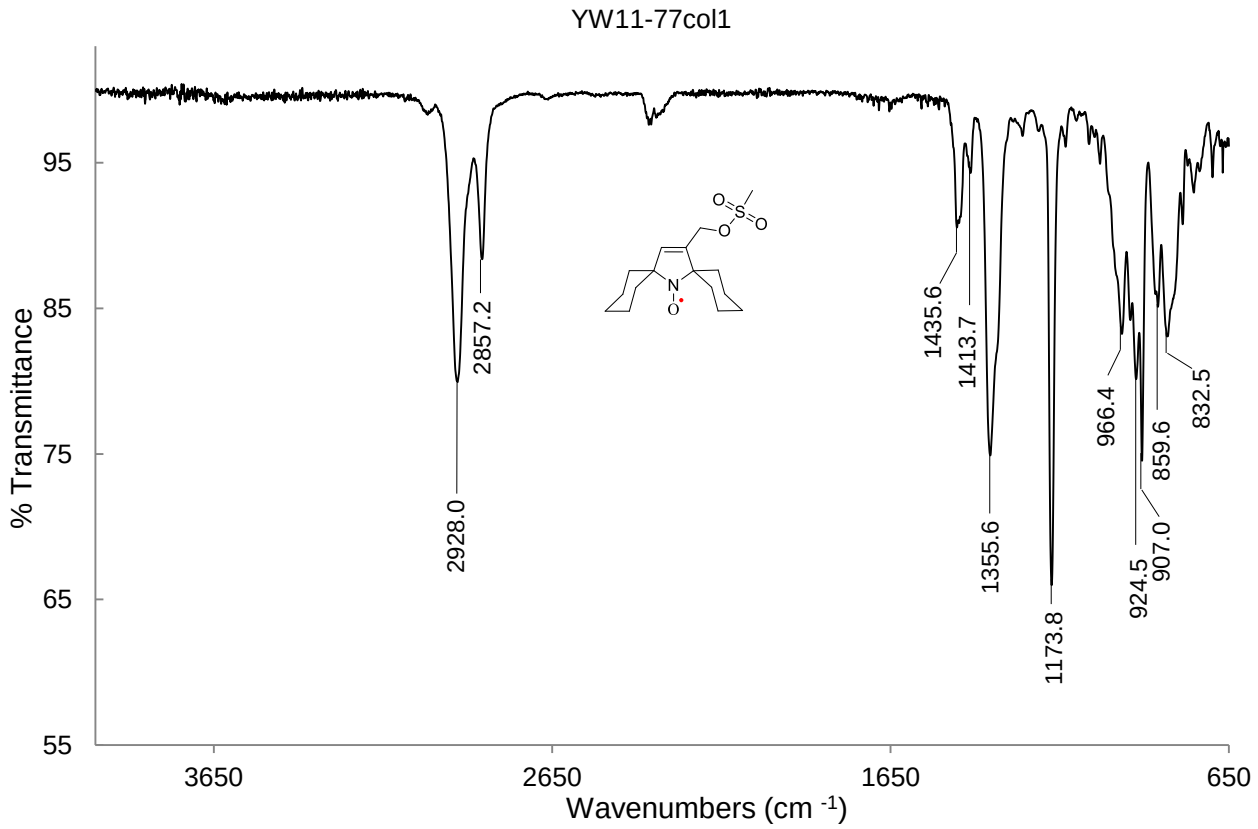


Figure S13. IR spectrum (ATR, ZnSe) of compound **12** (label: YW11-77col1)

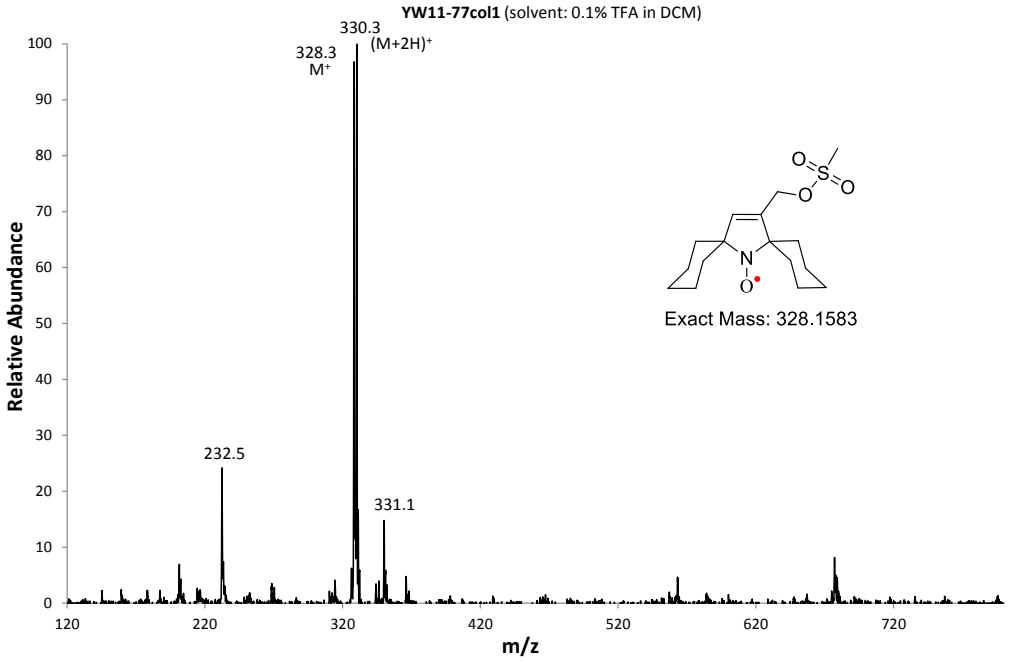


Figure S14. LRMS-ESI spectrum (solvent: 0.1% TFA in DCM) of **12** (label: YW11-77col1)

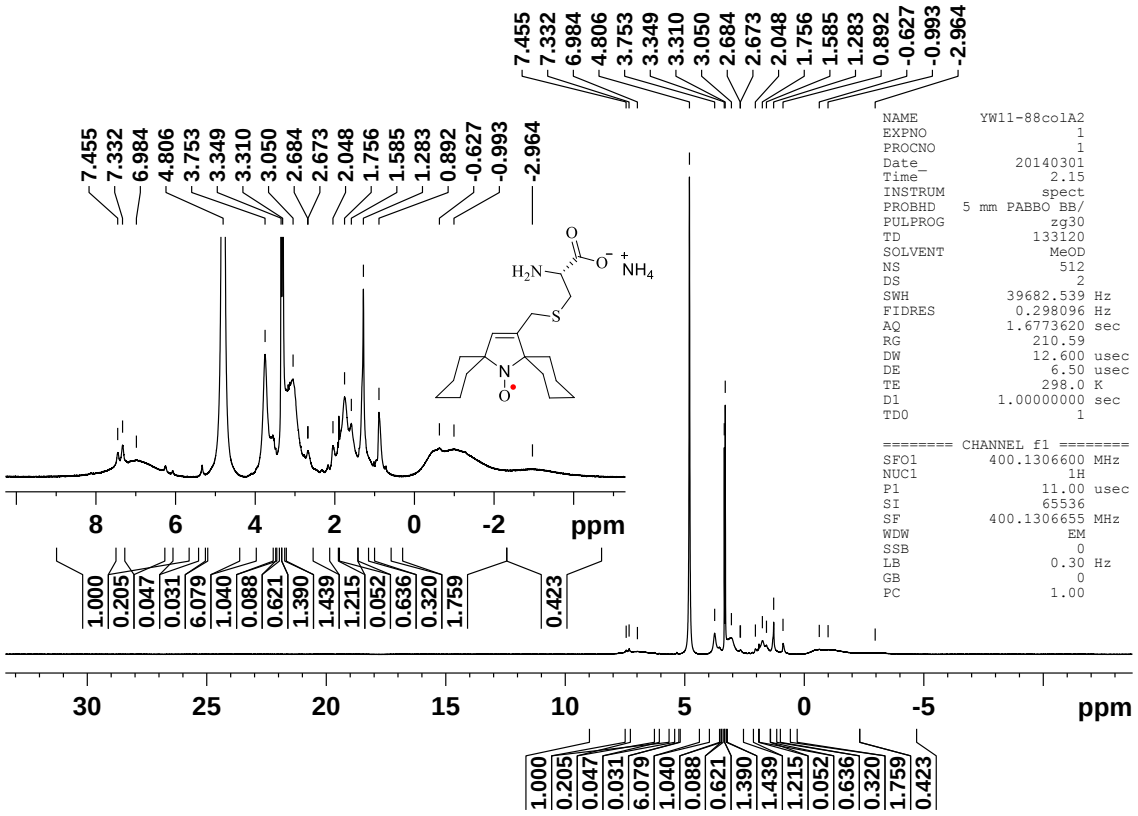


Figure S15. ^{1}H NMR spectrum (400 MHz, 0.095 M) of saturated **1** (label: YW11-88colA2) in CD_3OD.

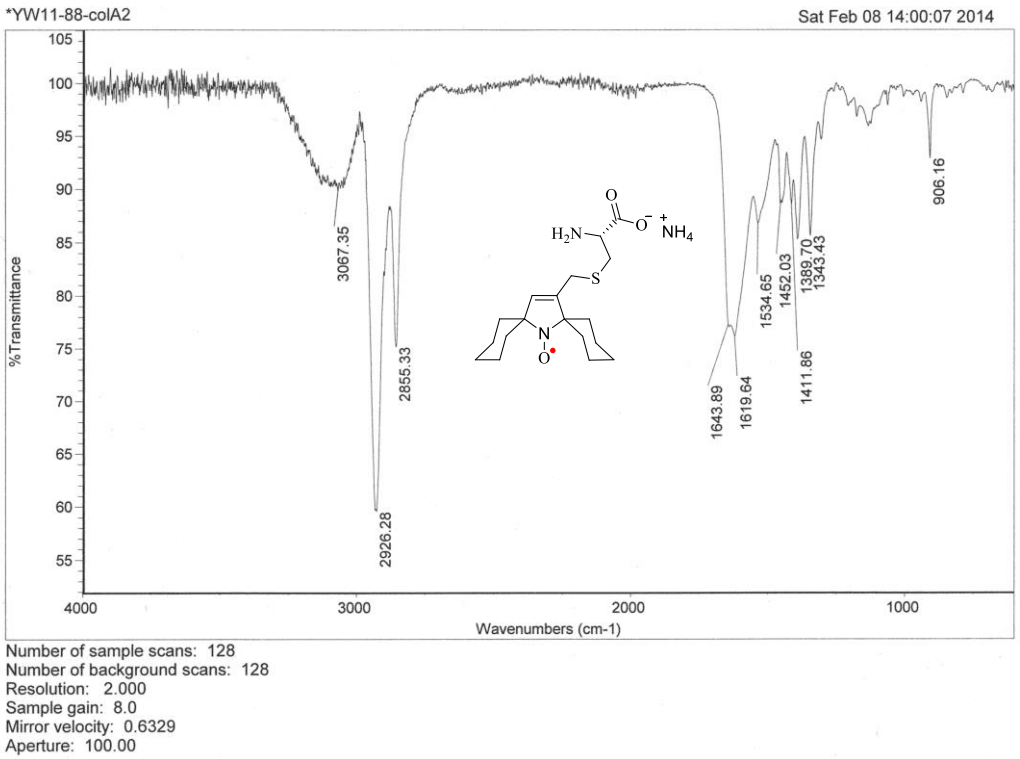


Figure S16. IR spectrum (ATR, ZnSe) of compound **1** (label: YW11-88colA2)

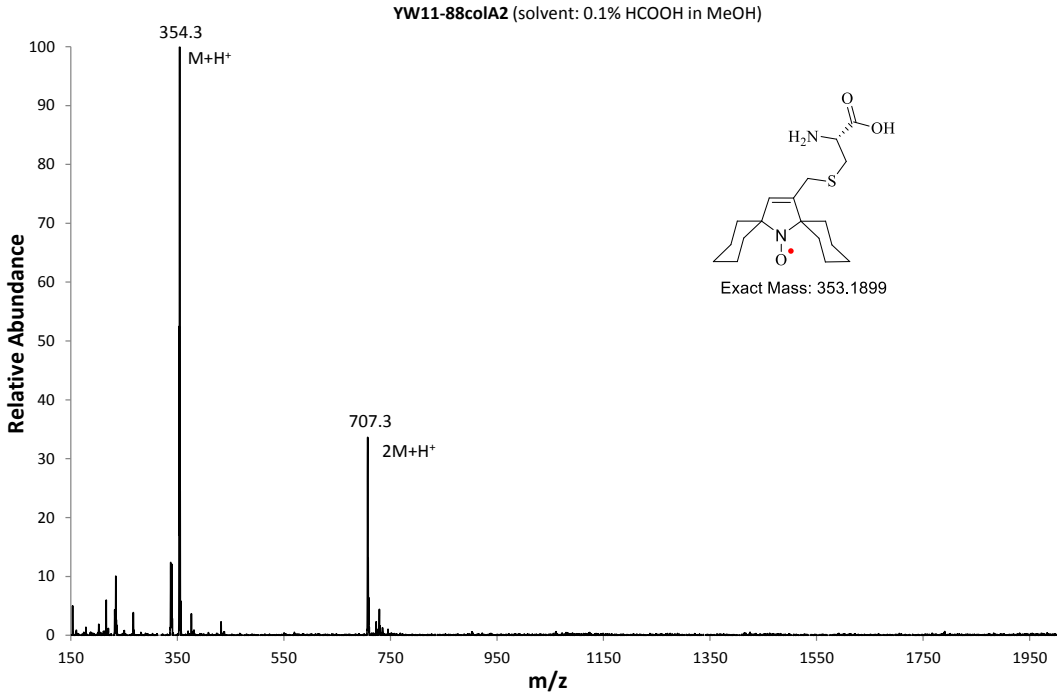


Figure S17. LRMS-ESI spectrum (solvent: 0.1% HCOOH in MeOH) of compound **1** (label: YW11-88colA2)

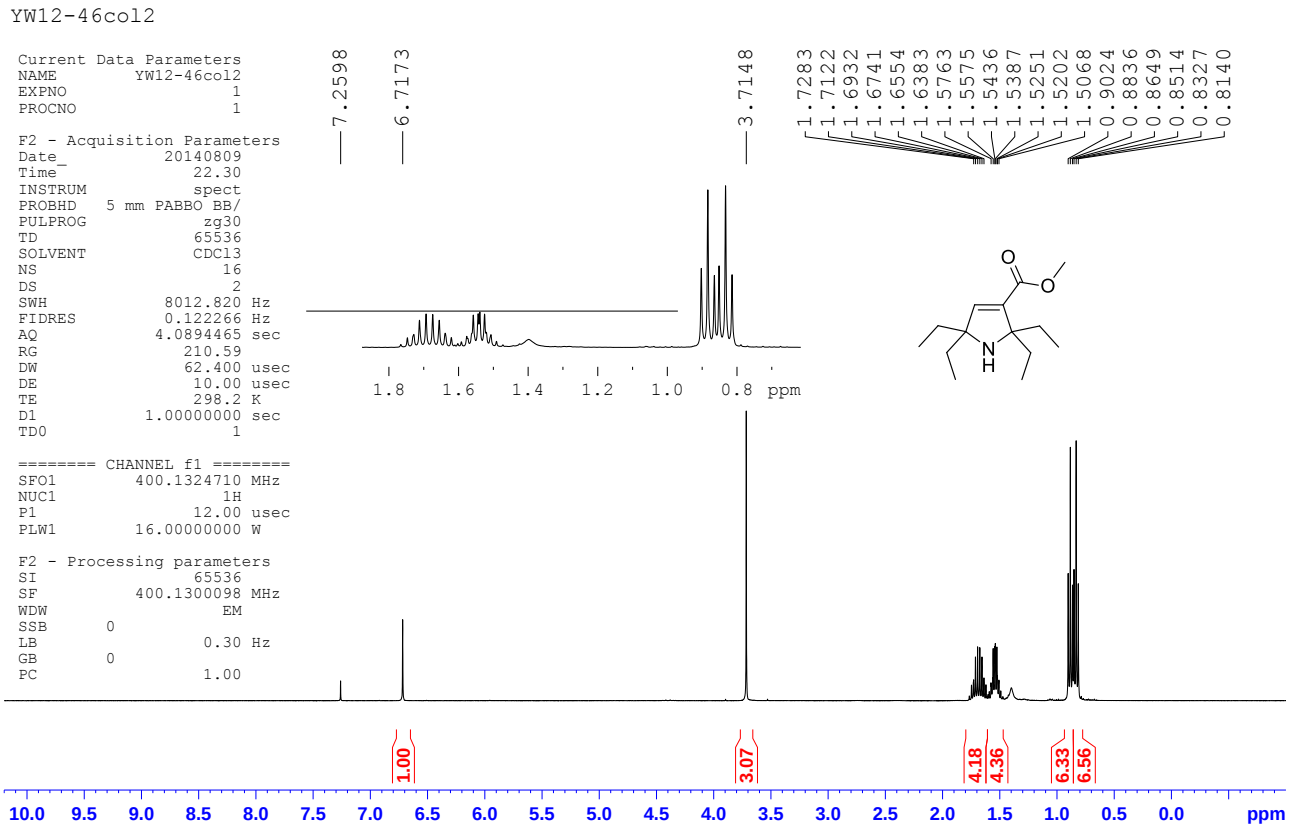


Figure S18. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **15** (label: YW12-46col2).

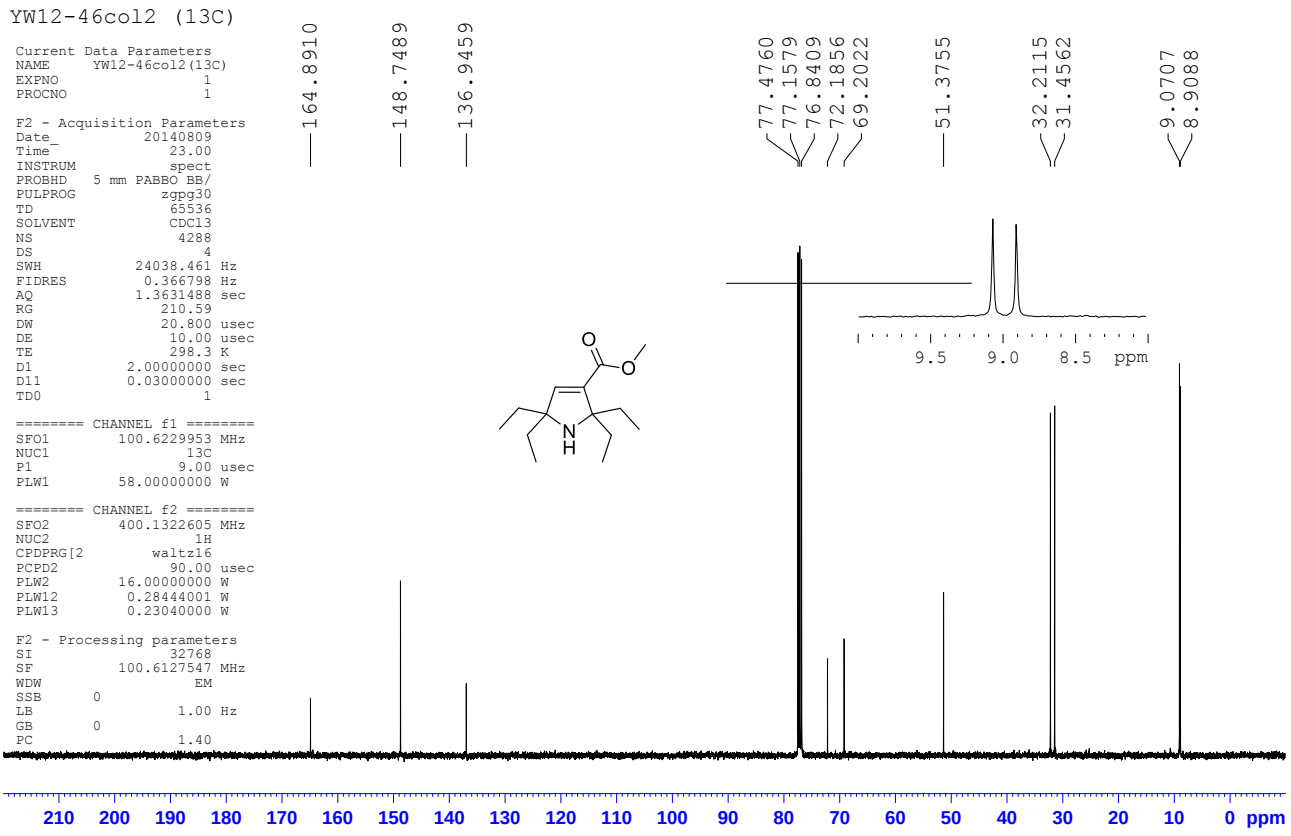


Figure S19. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **15** (label: YW12-46col2).

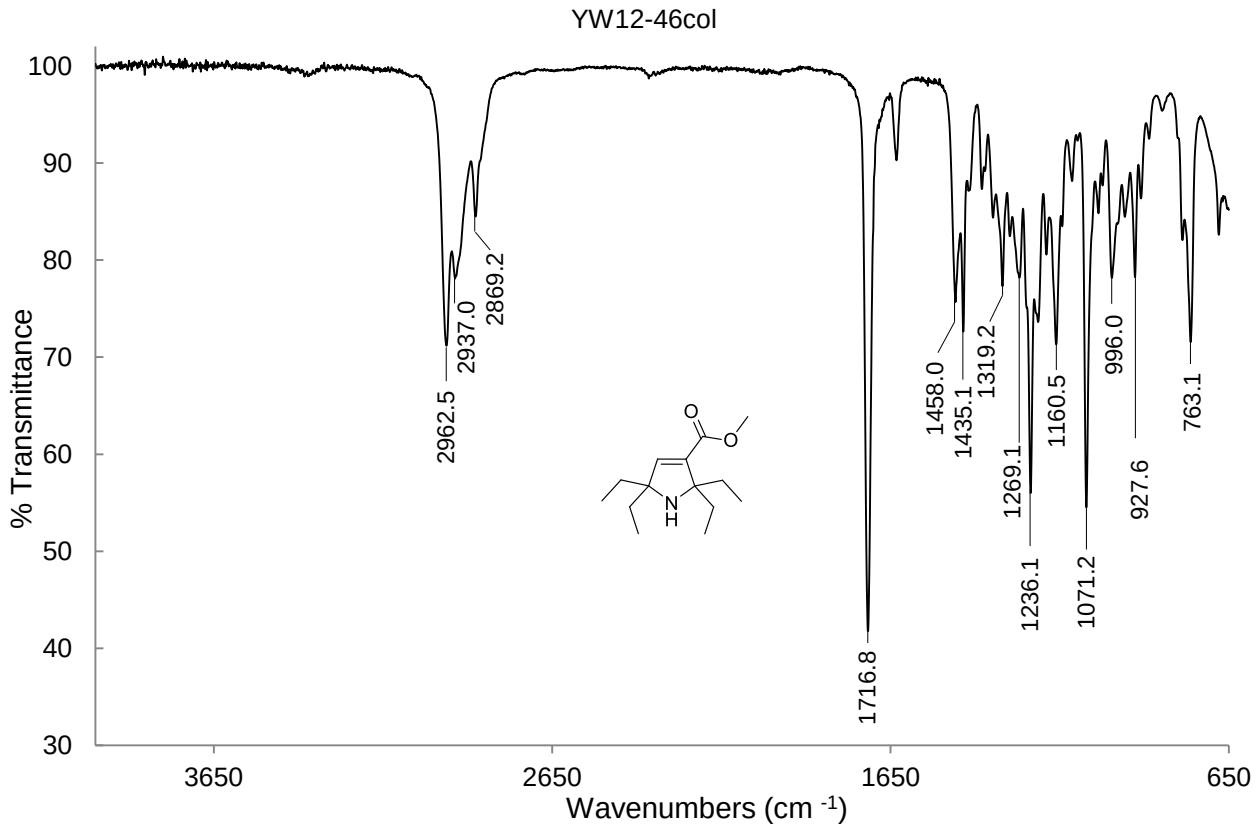


Figure S20. IR spectrum (ATR, ZnSe) of compound **15** (label: YW12-46col)

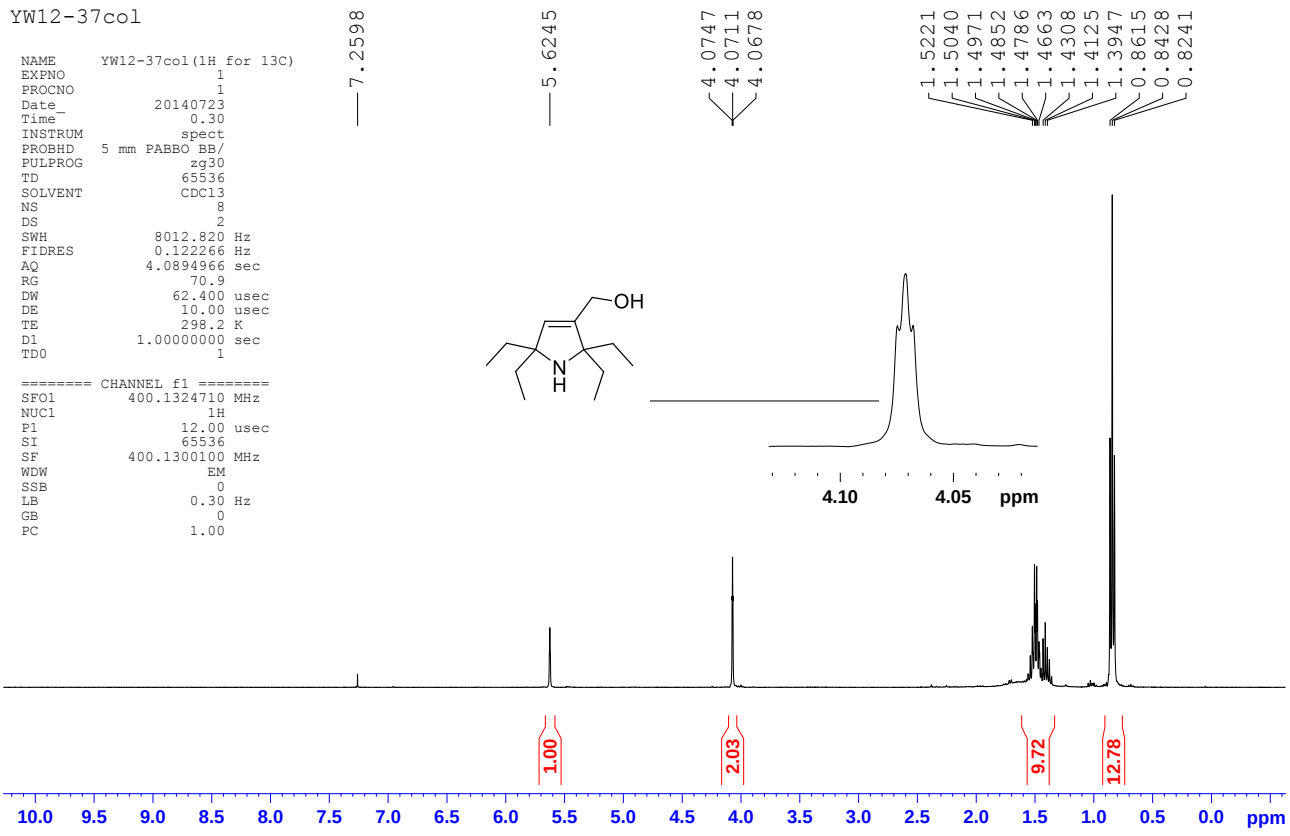


Figure S21. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **16** (label: YW12-37col).

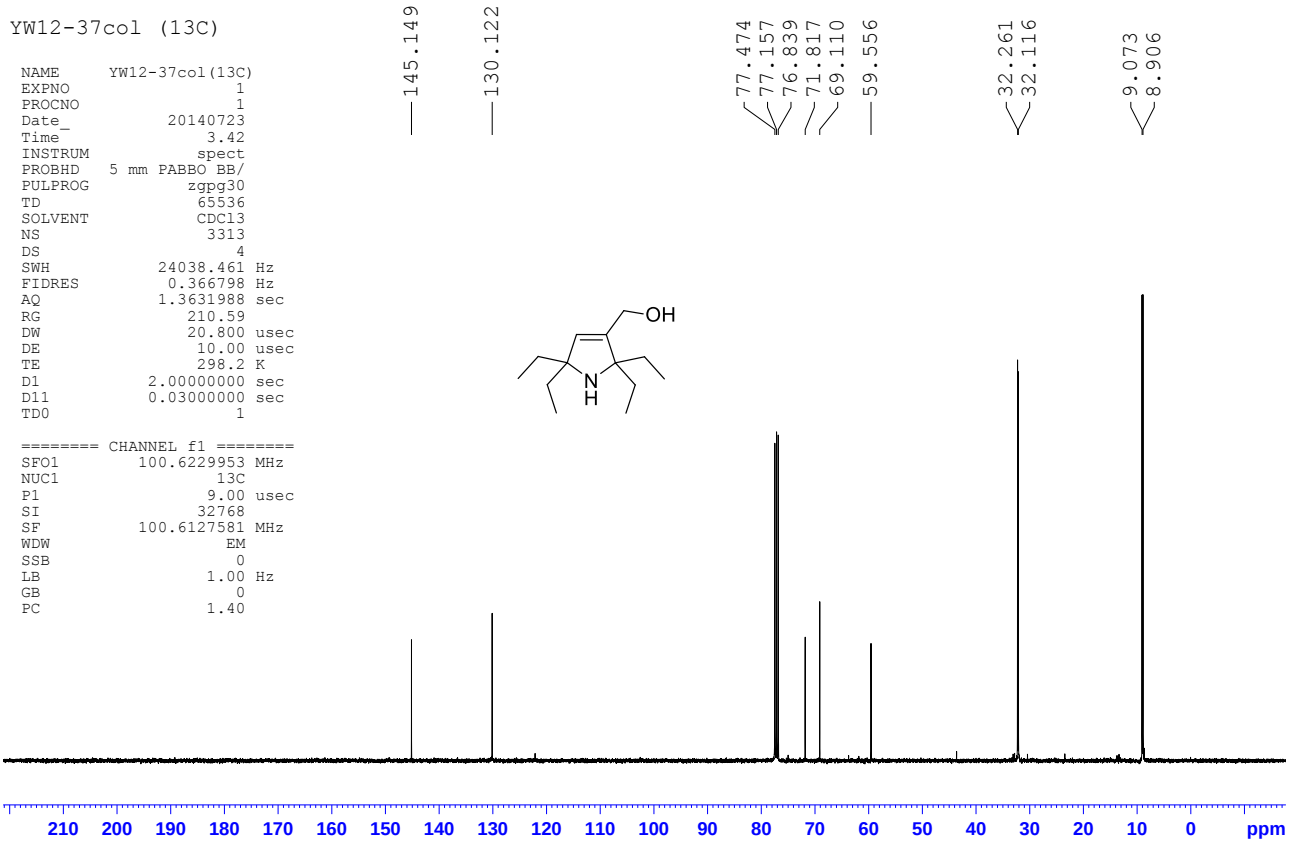


Figure S22. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **16** (label: YW12-37col).

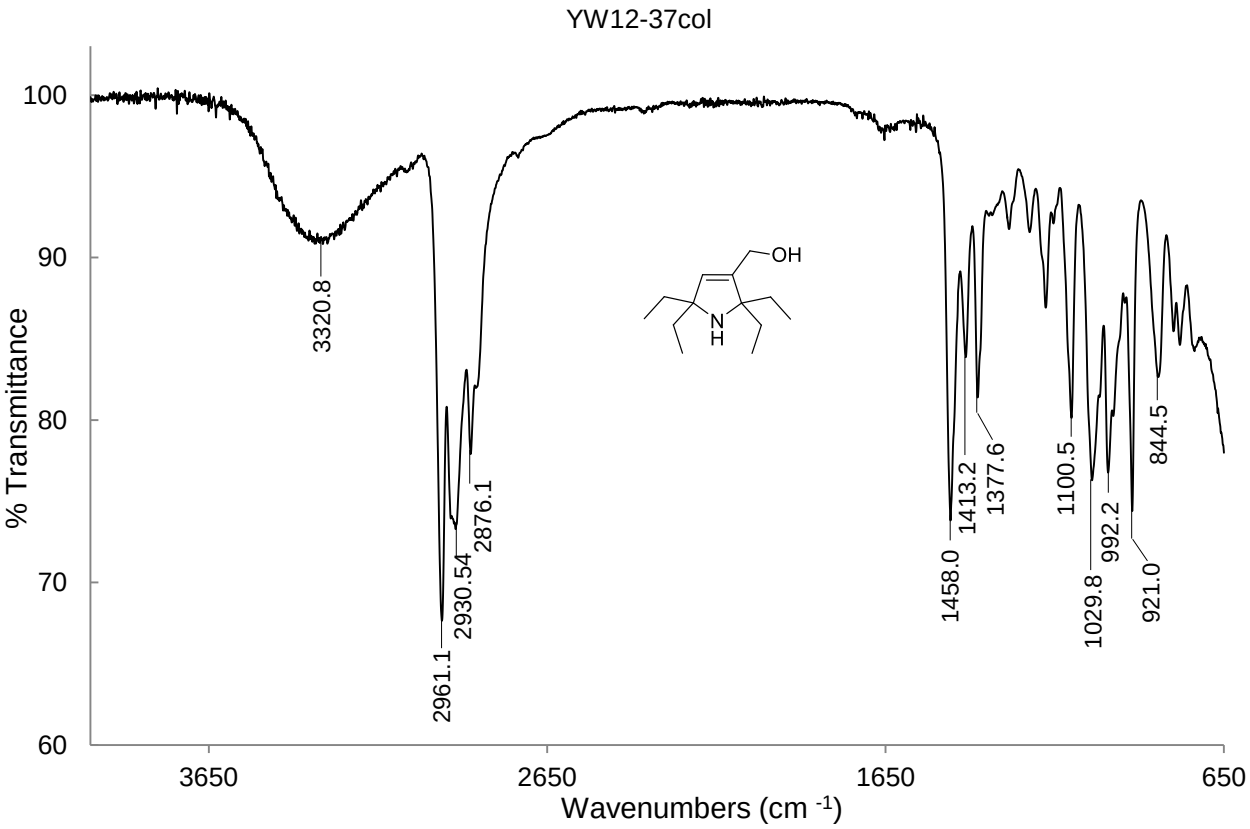


Figure S23. IR spectrum (ATR, ZnSe) of compound **16** (label: YW12-37col)

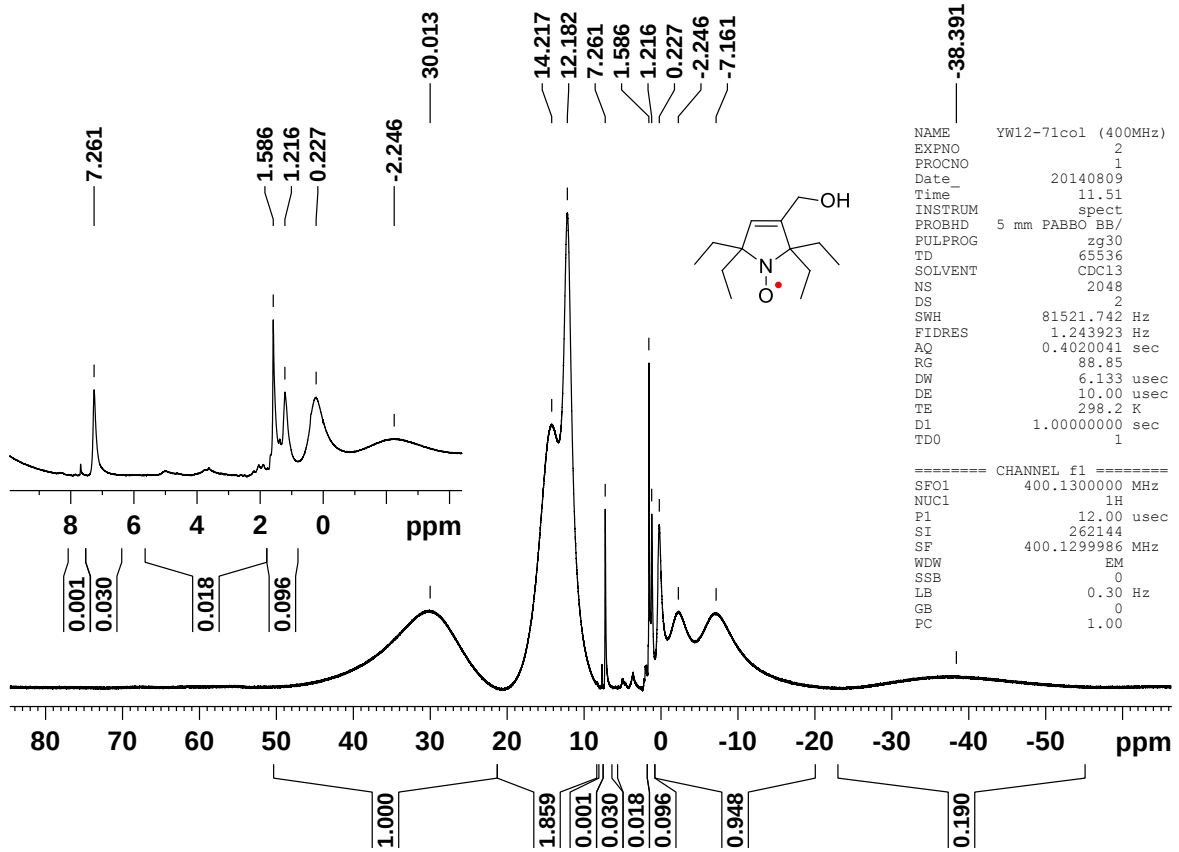


Figure S24. ^{1}H NMR spectrum (400 MHz, 0.30 M in $CDCl_3$) of **17** (label: YW12-71col).

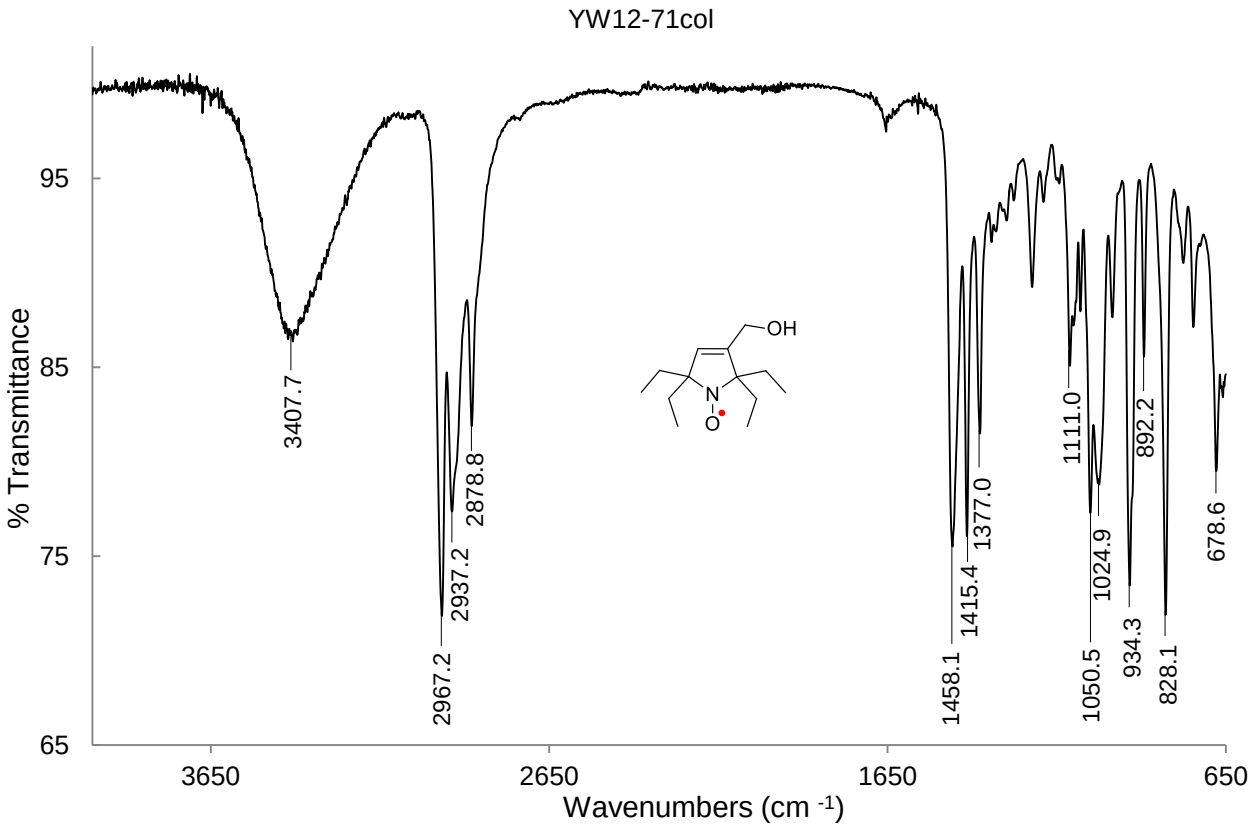


Figure S25. IR spectrum (ATR, ZnSe) of compound **17** (label: YW12-71col).

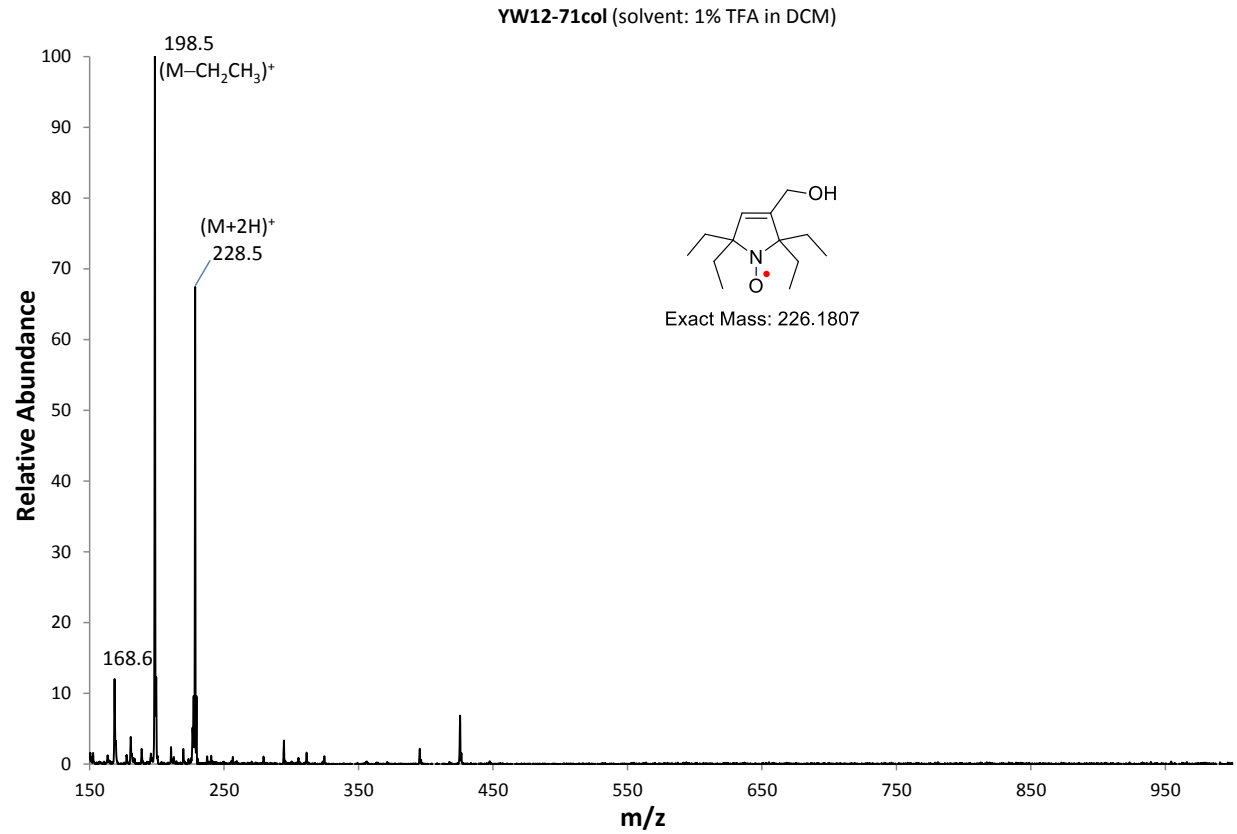


Figure S26. LRMS-ESI spectrum (solvent: 1% TFA in DCM) of **17** (label: YW12-71col)

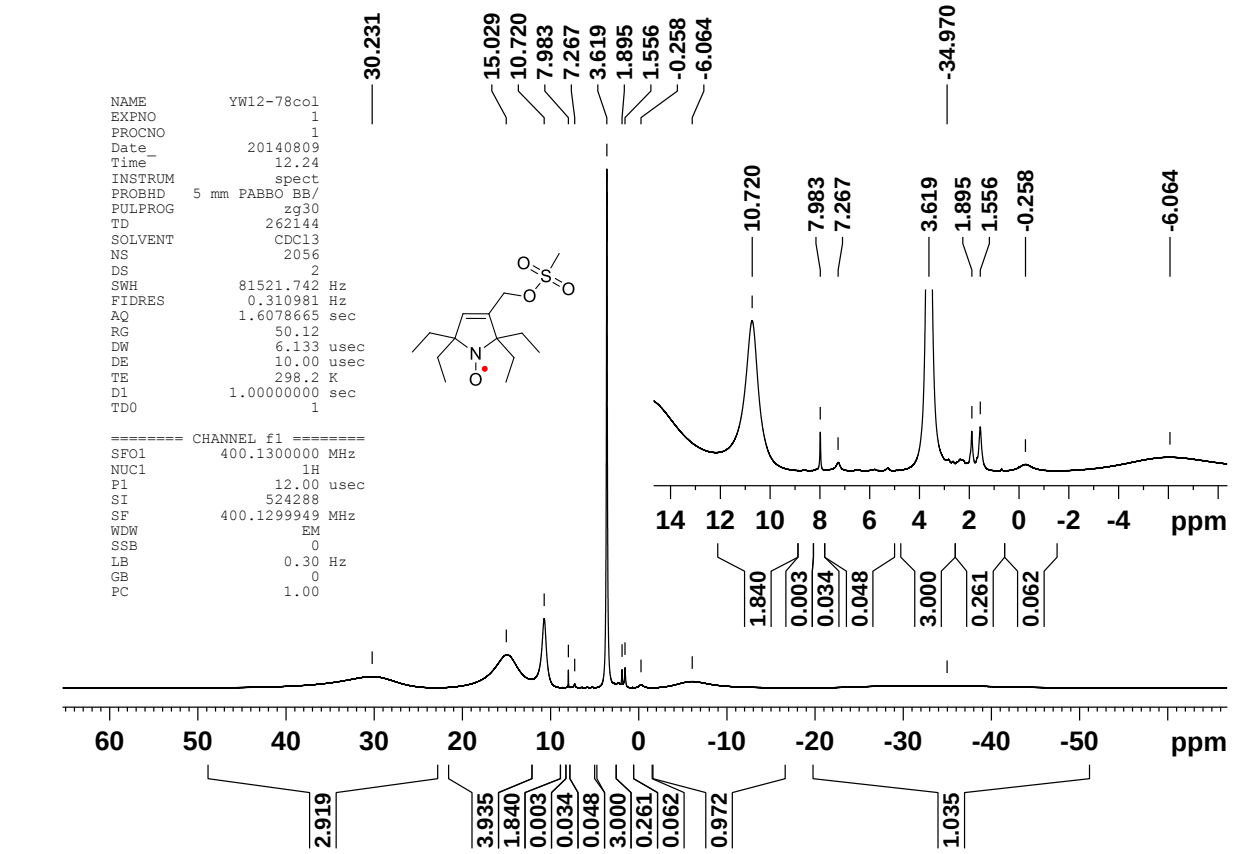


Figure S27. ^{1}H NMR spectrum (400 MHz, 0.54 M in $CDCl_3$) of **18** (label: YW12-78col).

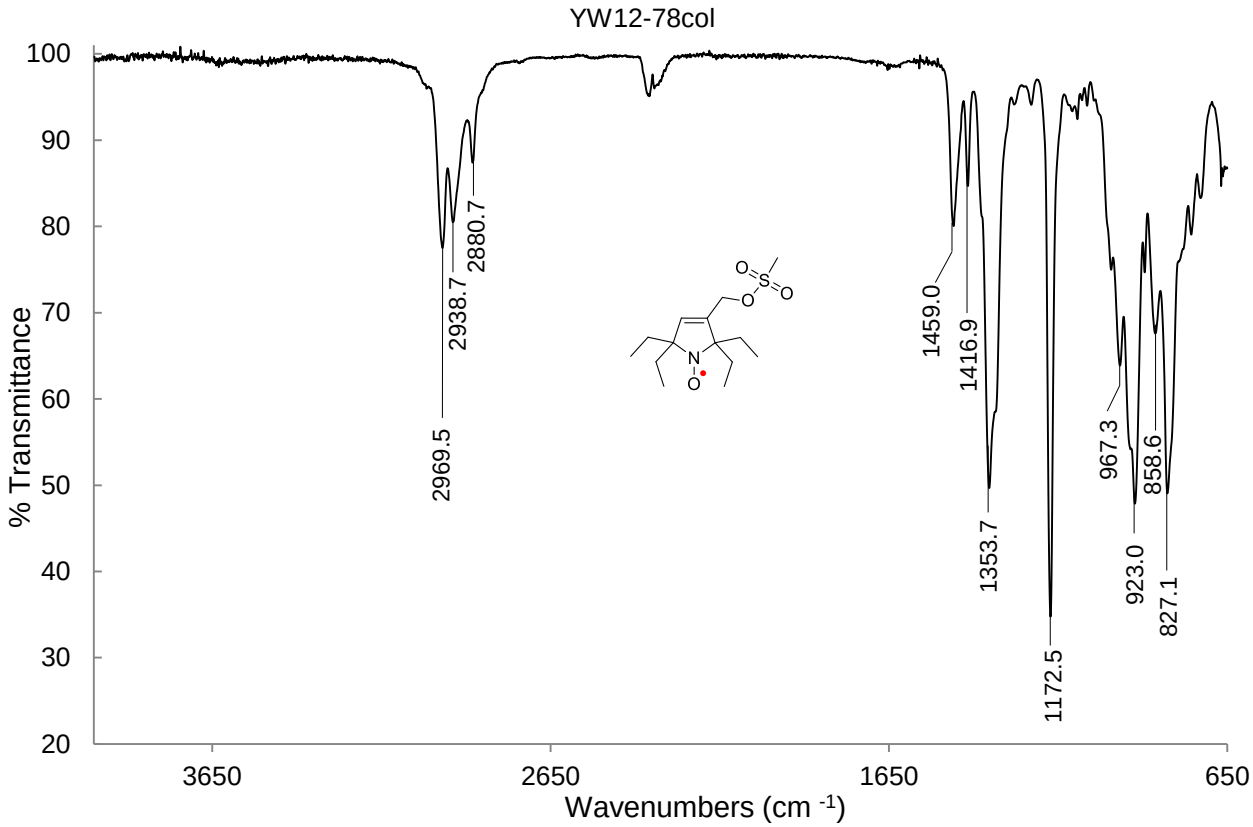


Figure S28. IR spectrum (ATR, ZnSe) of compound **18** (label: YW12-78col).

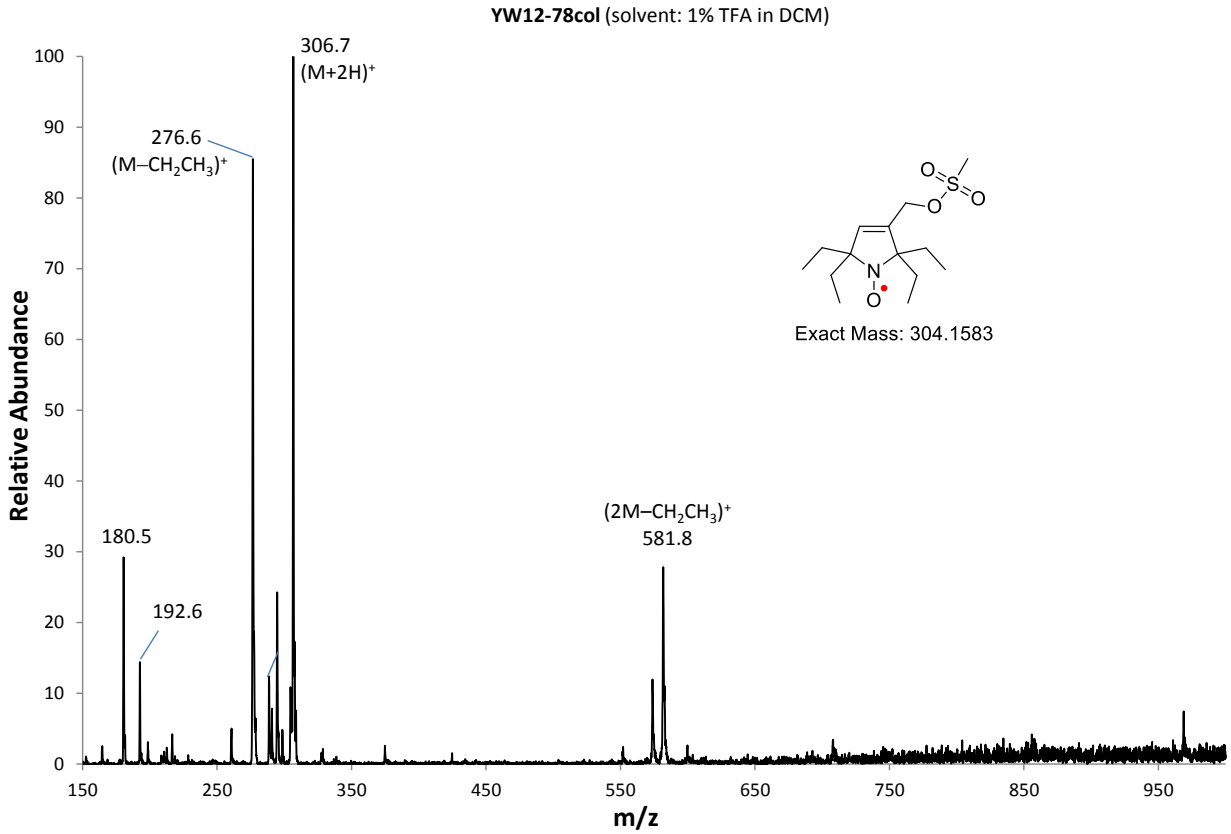


Figure S29. LRMS-ESI spectrum (solvent: 1% TFA in DCM) of **18** (label: YW12-78col)

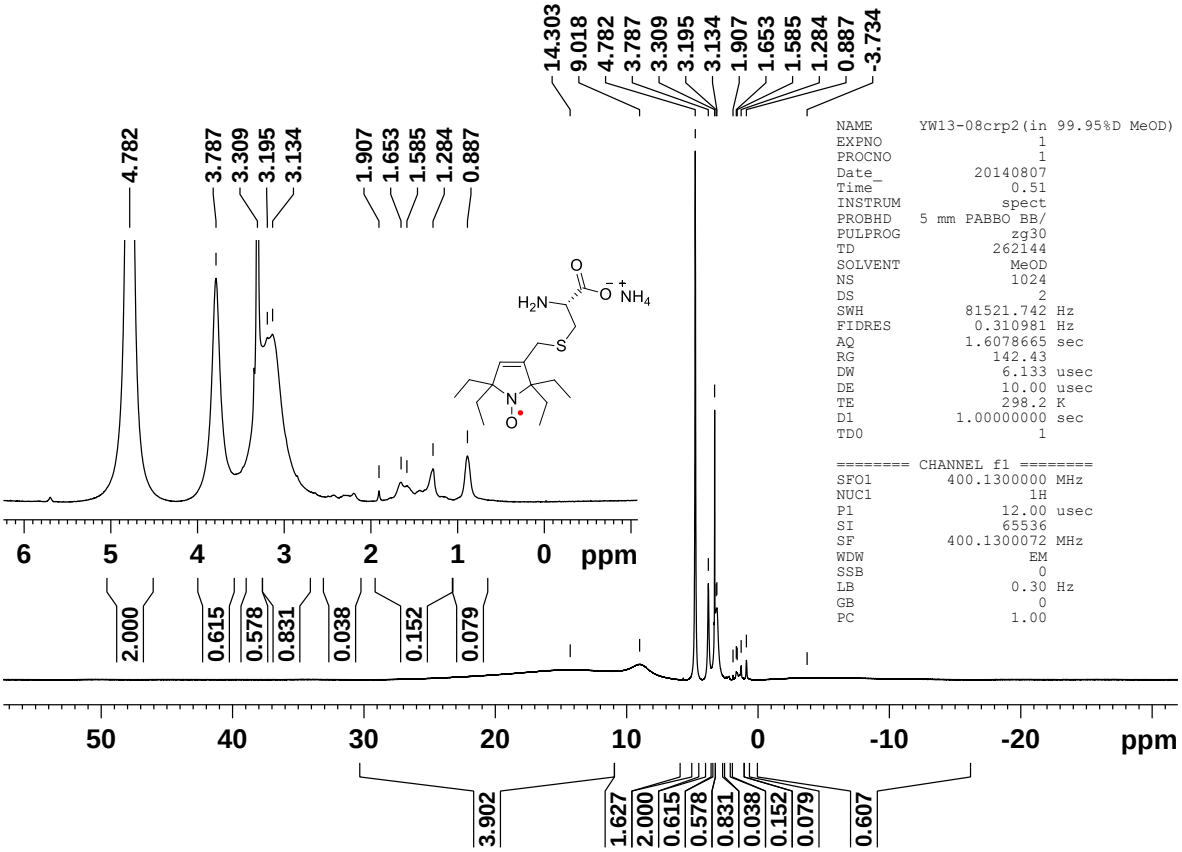


Figure S30. ^{1}H NMR spectrum (400 MHz, 0.14 M in CD_3OD) of **2** (label: YW13-08crp2).

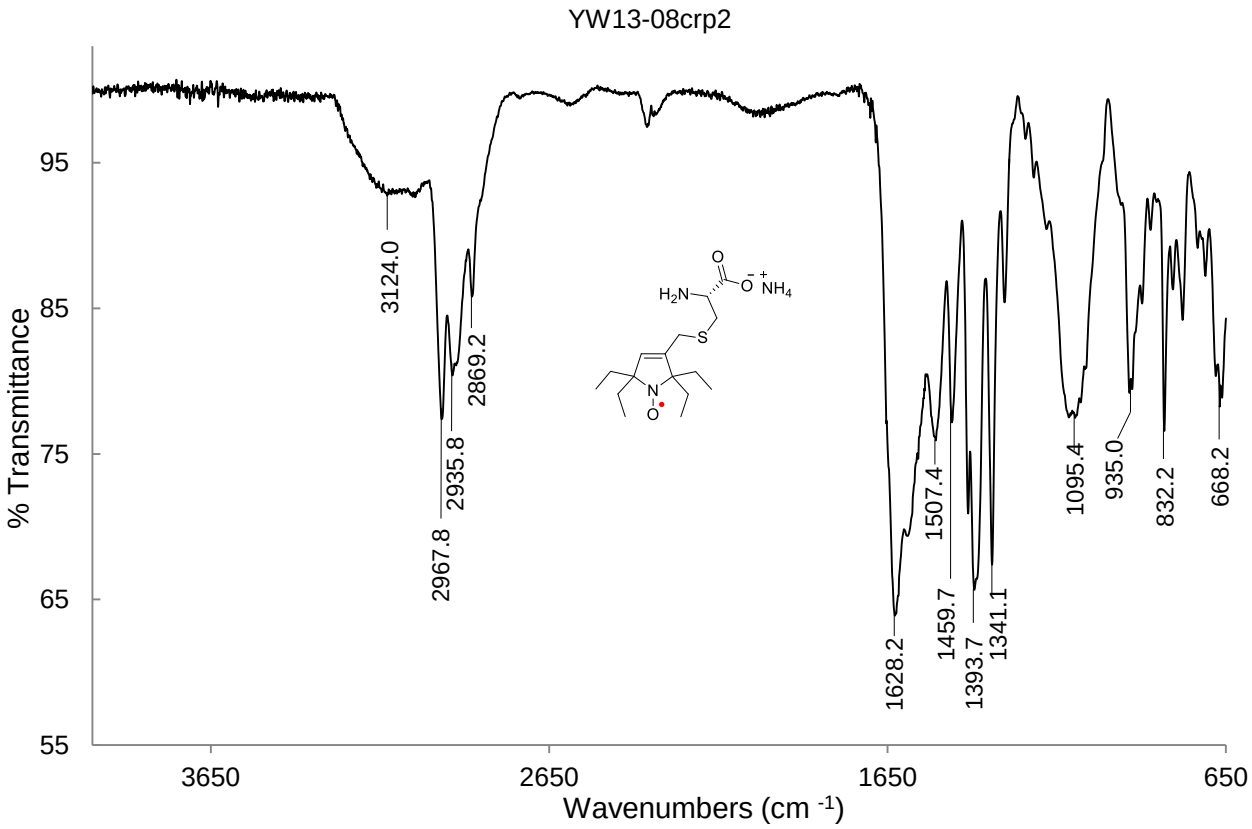


Figure S31. IR spectrum (ATR, ZnSe) of compound **2** (label: YW13-08crp2).

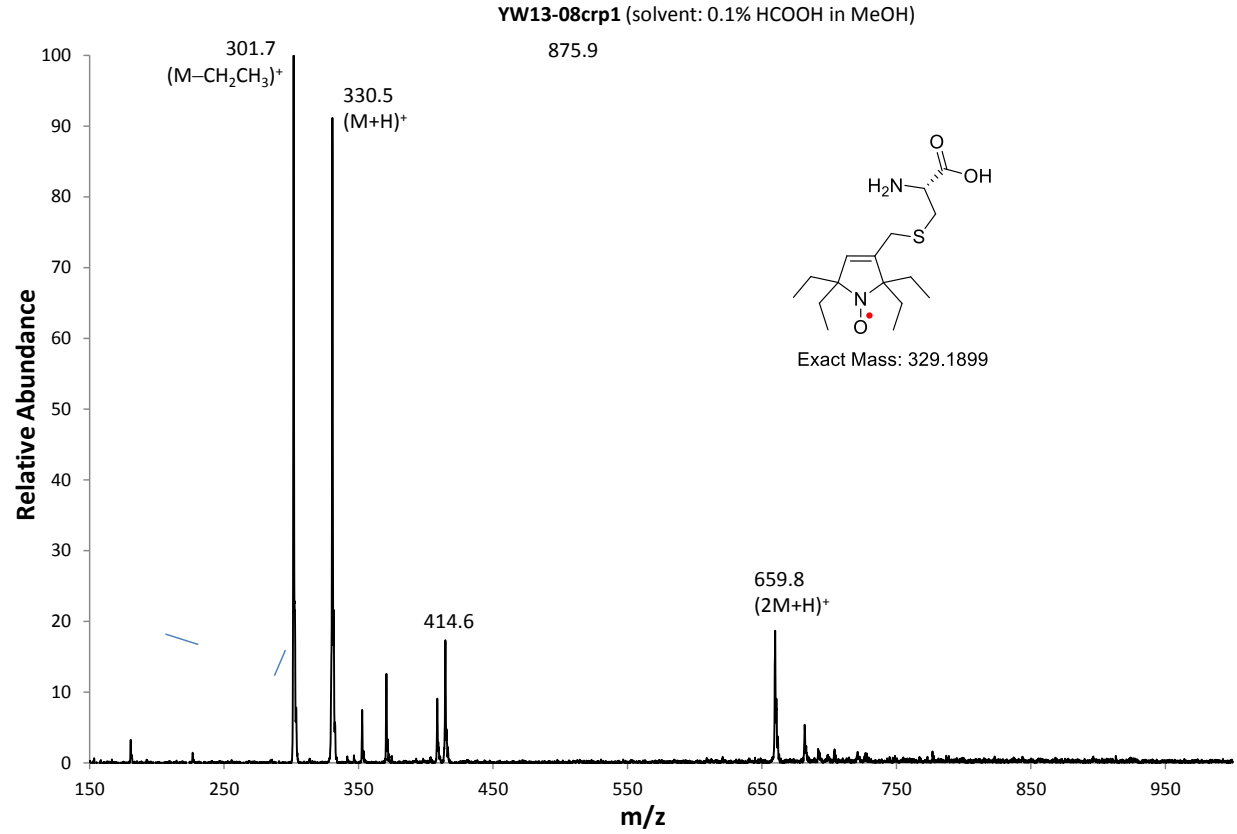


Figure S32. LRMS-ESI spectrum (solvent: 0.1% HCOOH in MeOD) of **2** (label: YW13-08crp1)

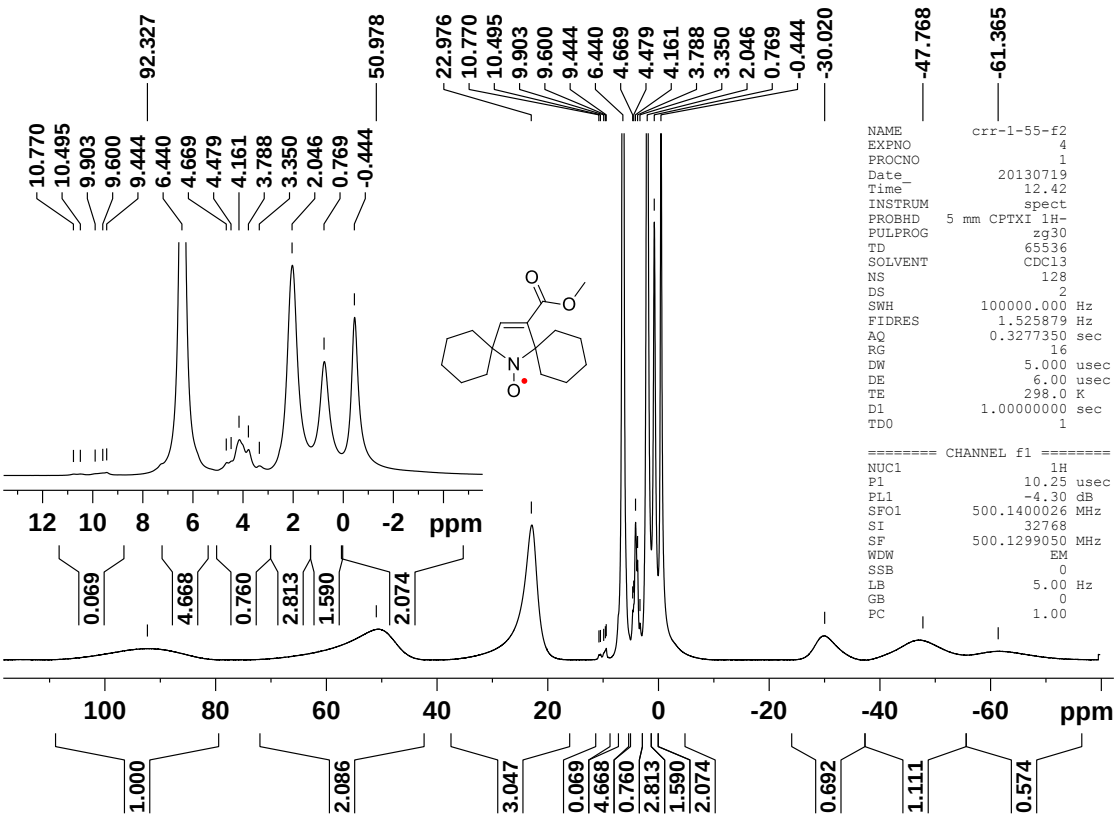


Figure S33. ^{1}H NMR spectrum (500 MHz, 1.8 M in $CDCl_3$) of **19** (label: CRR-1-55-f2).

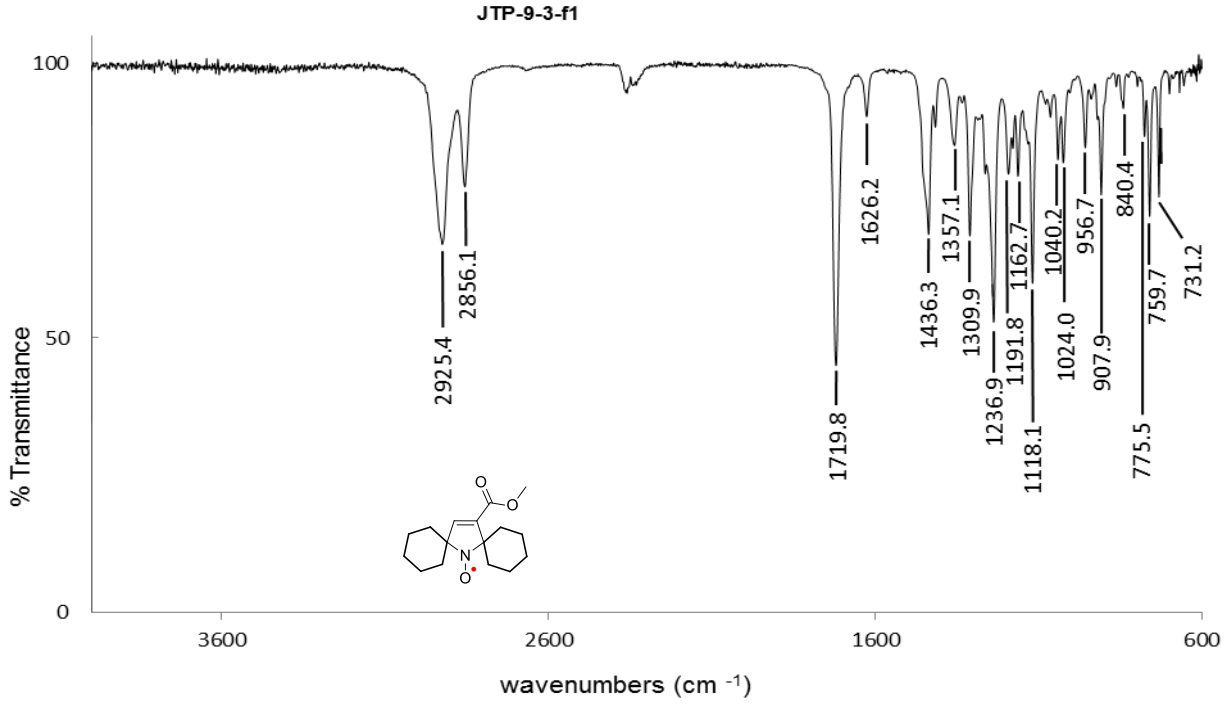


Figure S34. IR spectrum (ATR, diamond) of compound **19** (label: JTP-9-3 -f1).

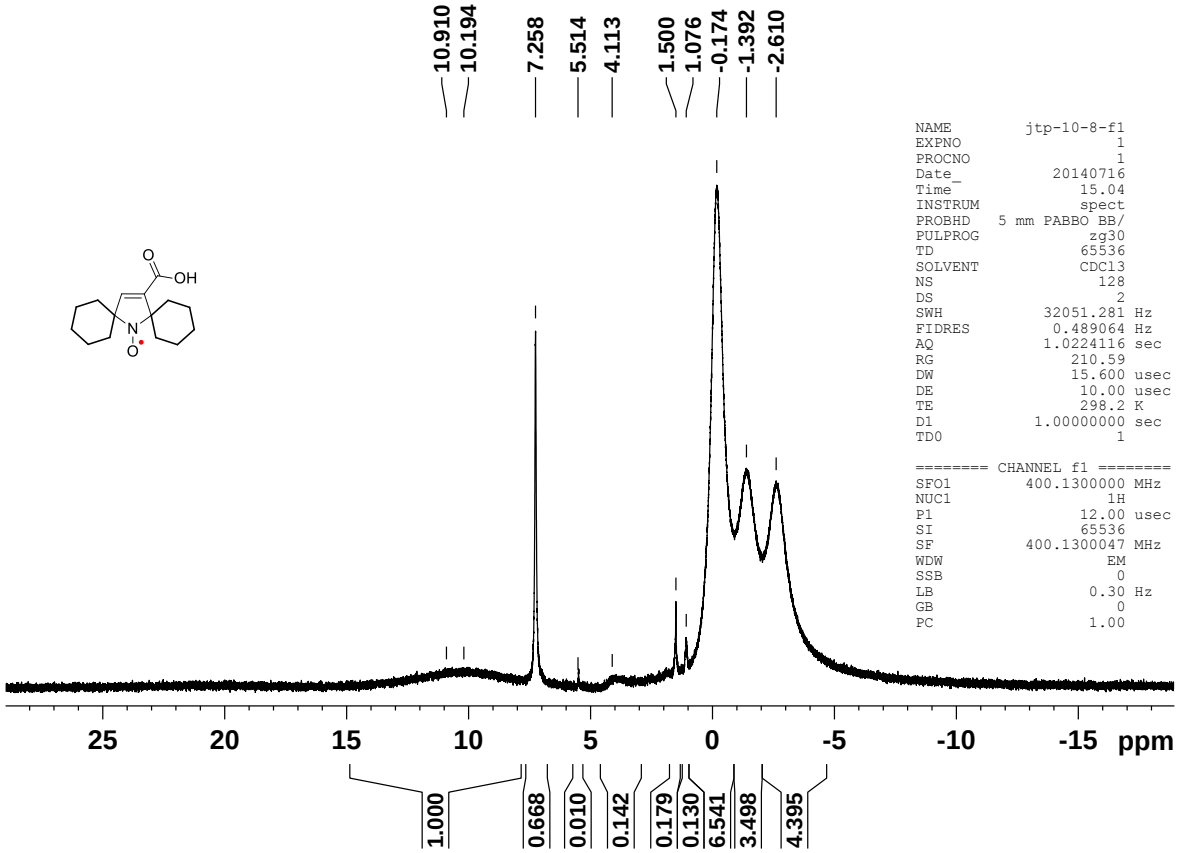


Figure S35. ^{1}H NMR spectrum (500 MHz, 1.4 M in $CDCl_3$) of **5** (label: JTP-10-8-f1).

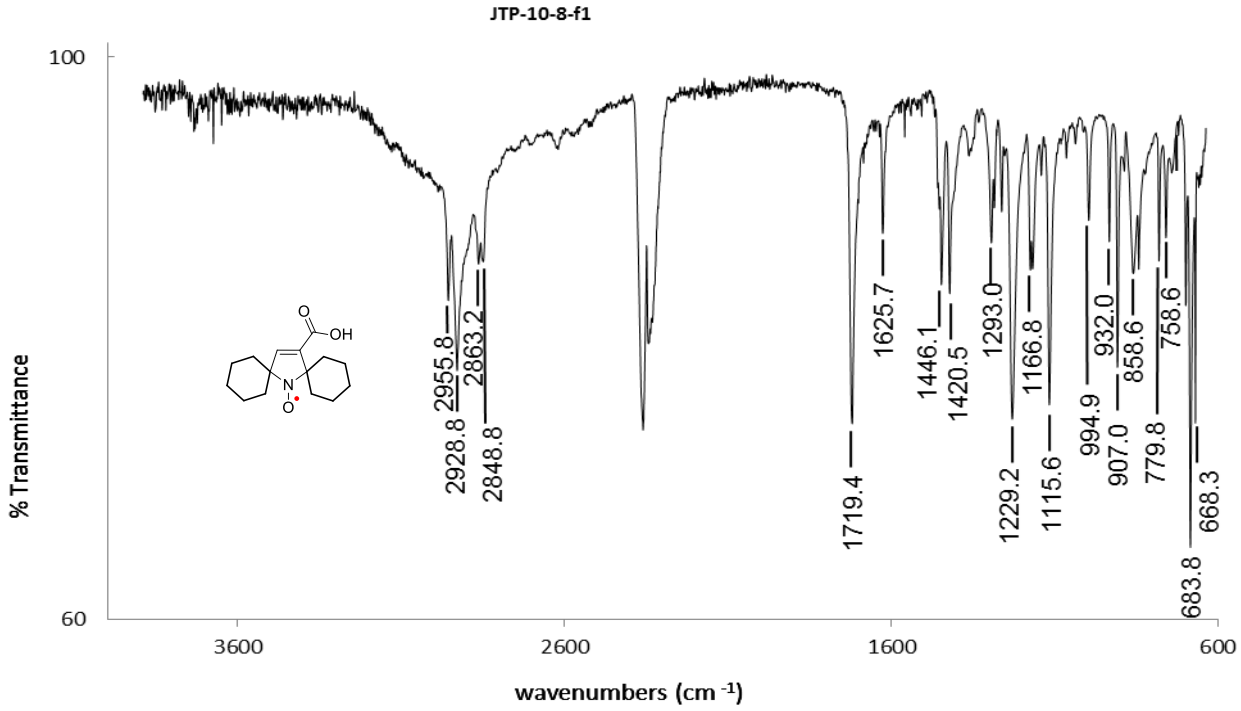


Figure S36. IR spectrum (ATR, diamond) of compound **5** (label: JTP-10-8-f1).

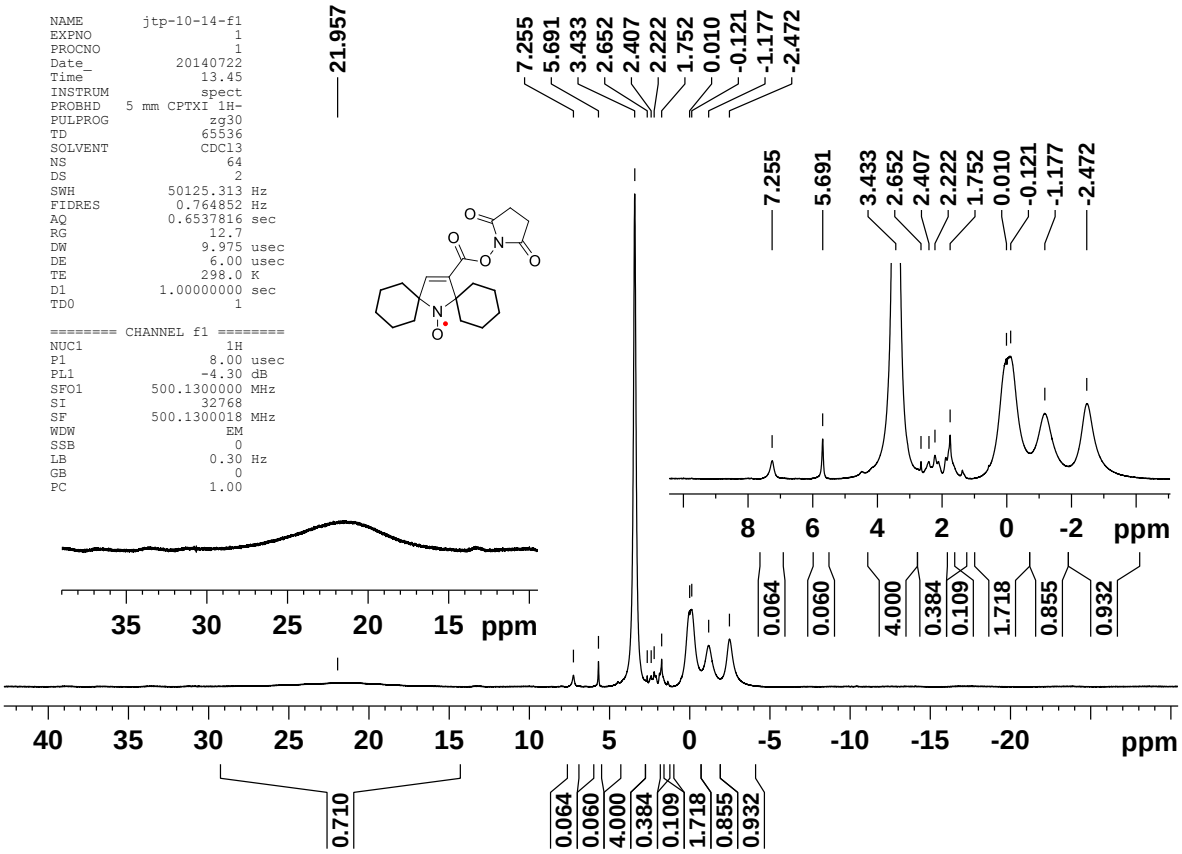


Figure S37. ^{1}H NMR spectrum (500 MHz, 0.5 M in $CDCl_3$) of **20** (label: JTP-10-14-f1).

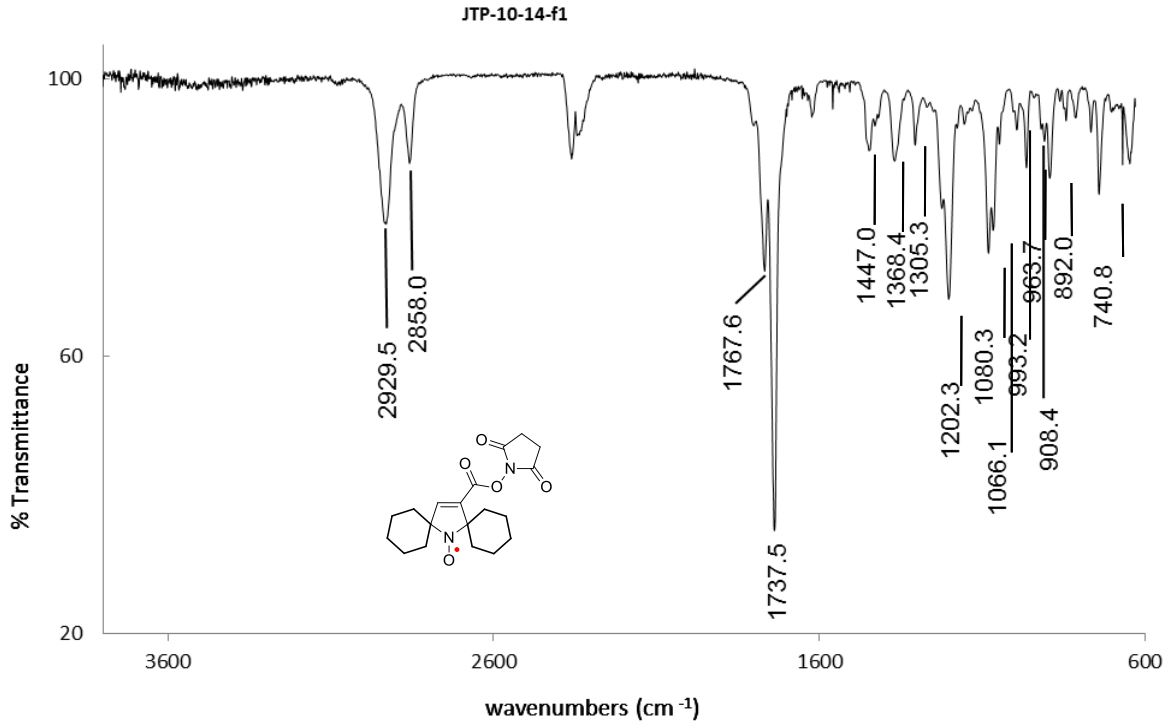


Figure S38. IR spectrum (ATR, diamond) of compound **20** (label: JTP-10-14-f1).

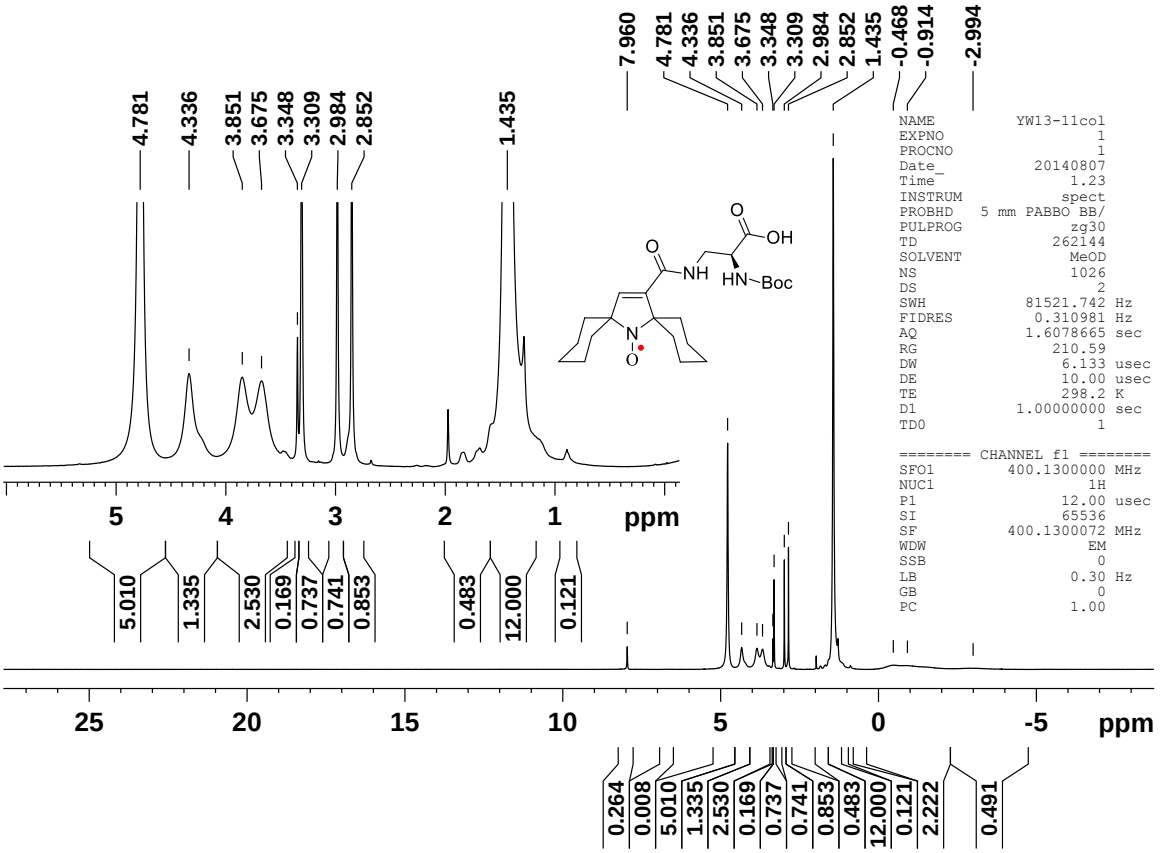


Figure S39. ^{1}H NMR spectrum (400 MHz, 0.10 M in CD_3OD) of **3** (label: YW13-11col).

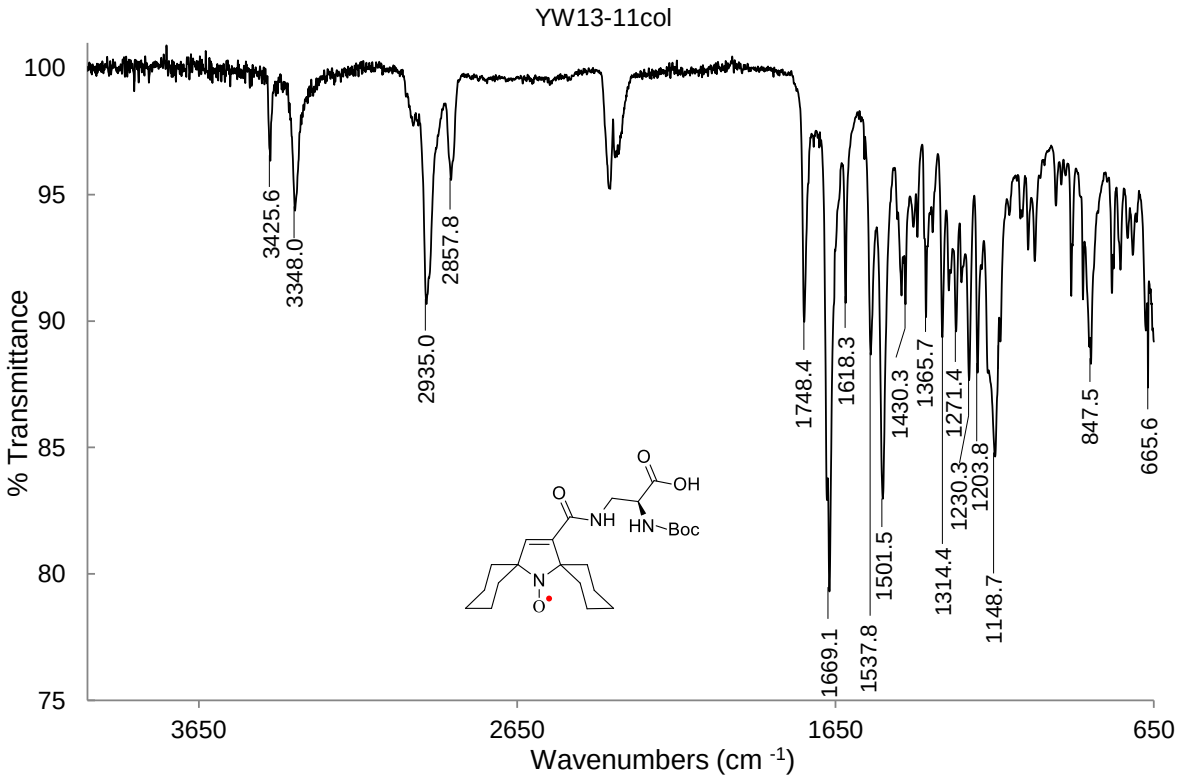


Figure S40. IR spectrum (ATR, ZnSe) of compound **3** (label: YW13-11col)

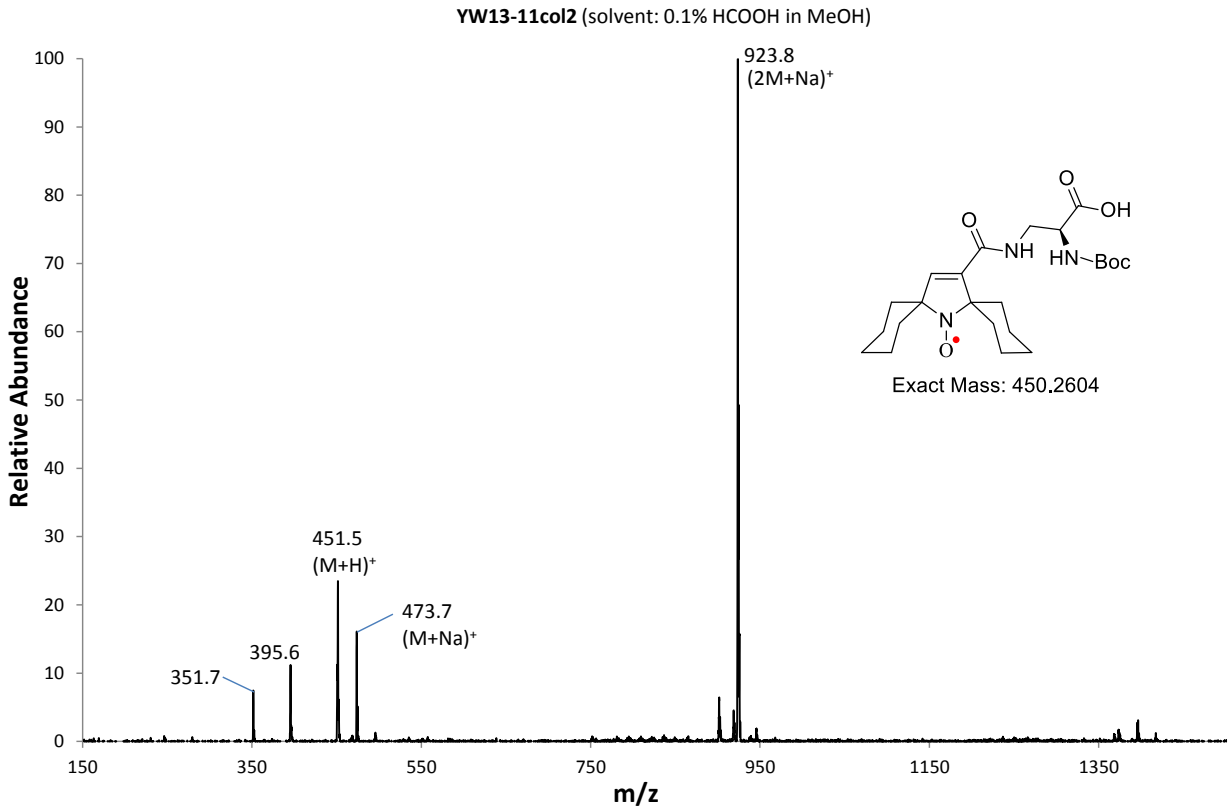


Figure S41. LRMS-ESI spectrum (solvent: 0.1% HCOOH in MeOH) of **3** (label: YW13-11col2).

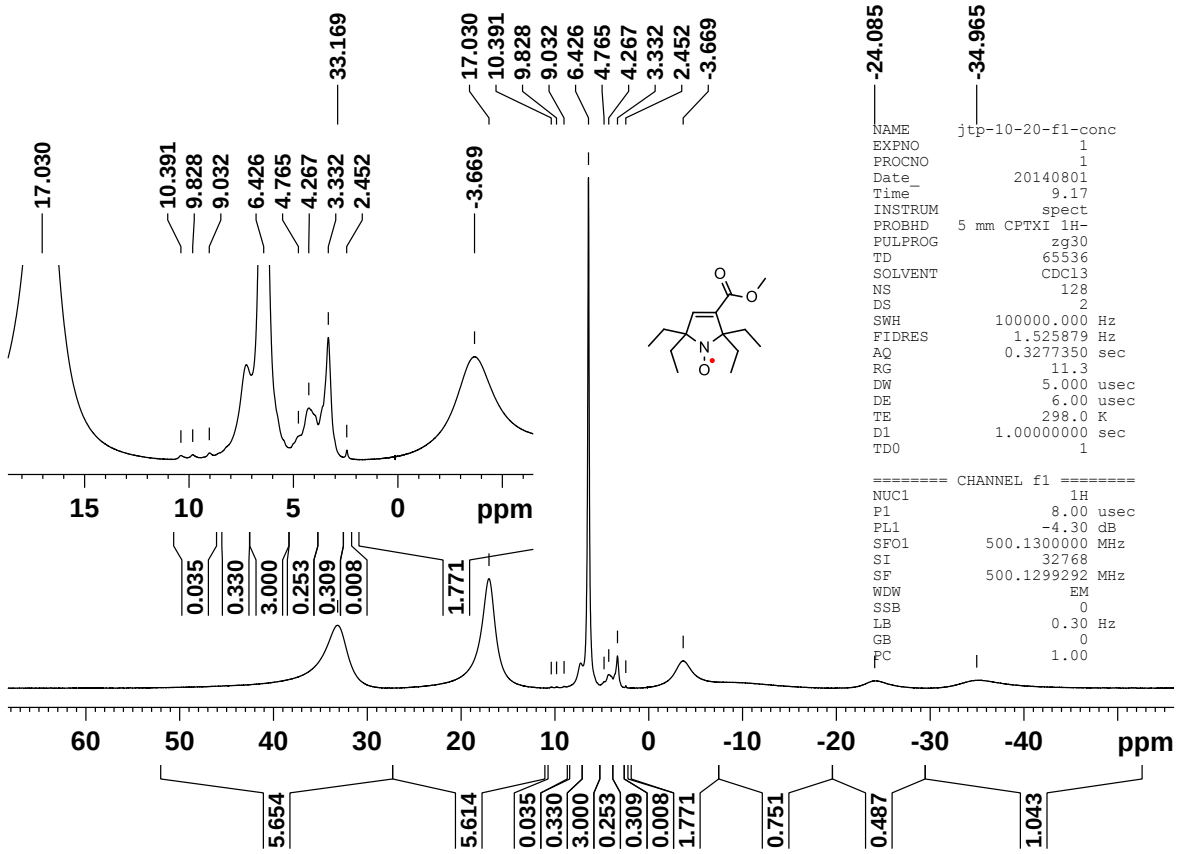


Figure S42. ^{1}H NMR spectrum (500 MHz, 2.5 M in $CDCl_3$) of **21** (label: JTP-10-20-f1).

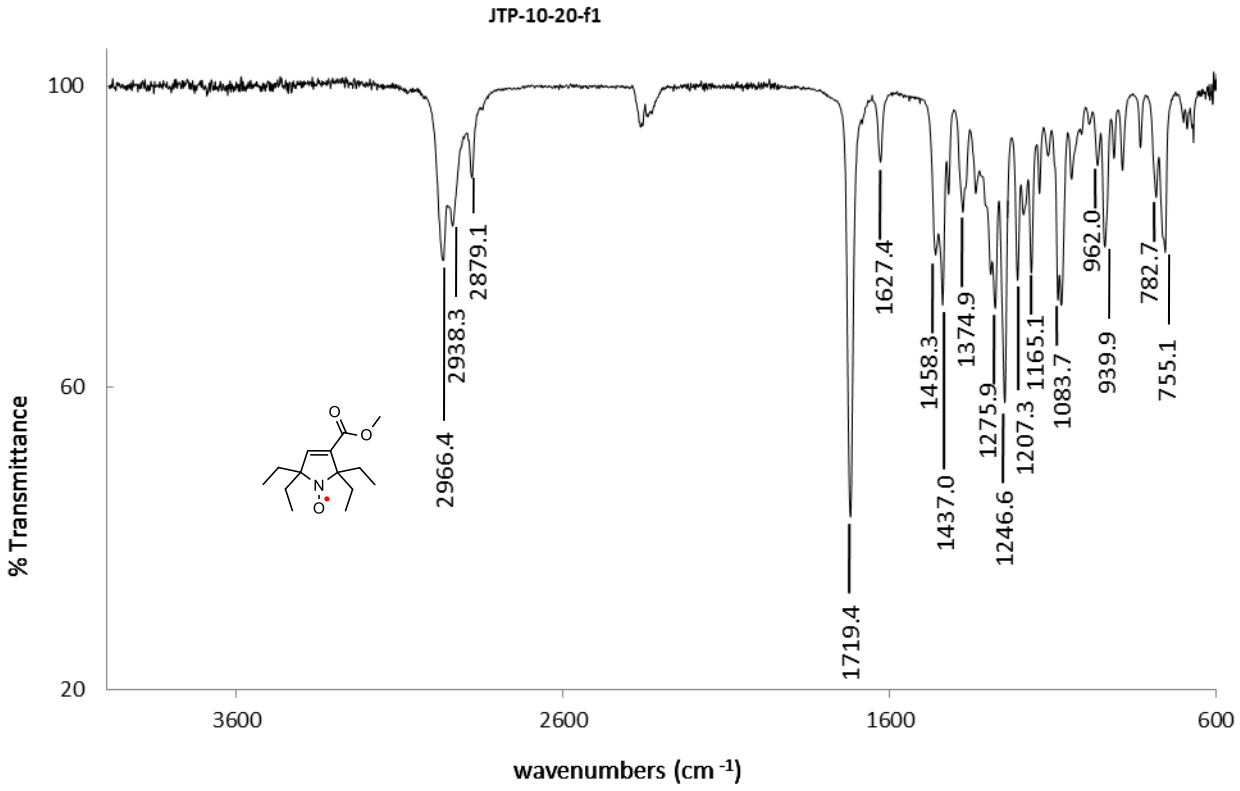


Figure S43. IR spectrum (ATR, diamond) of compound **21** (label: JTP-10-20-f1).

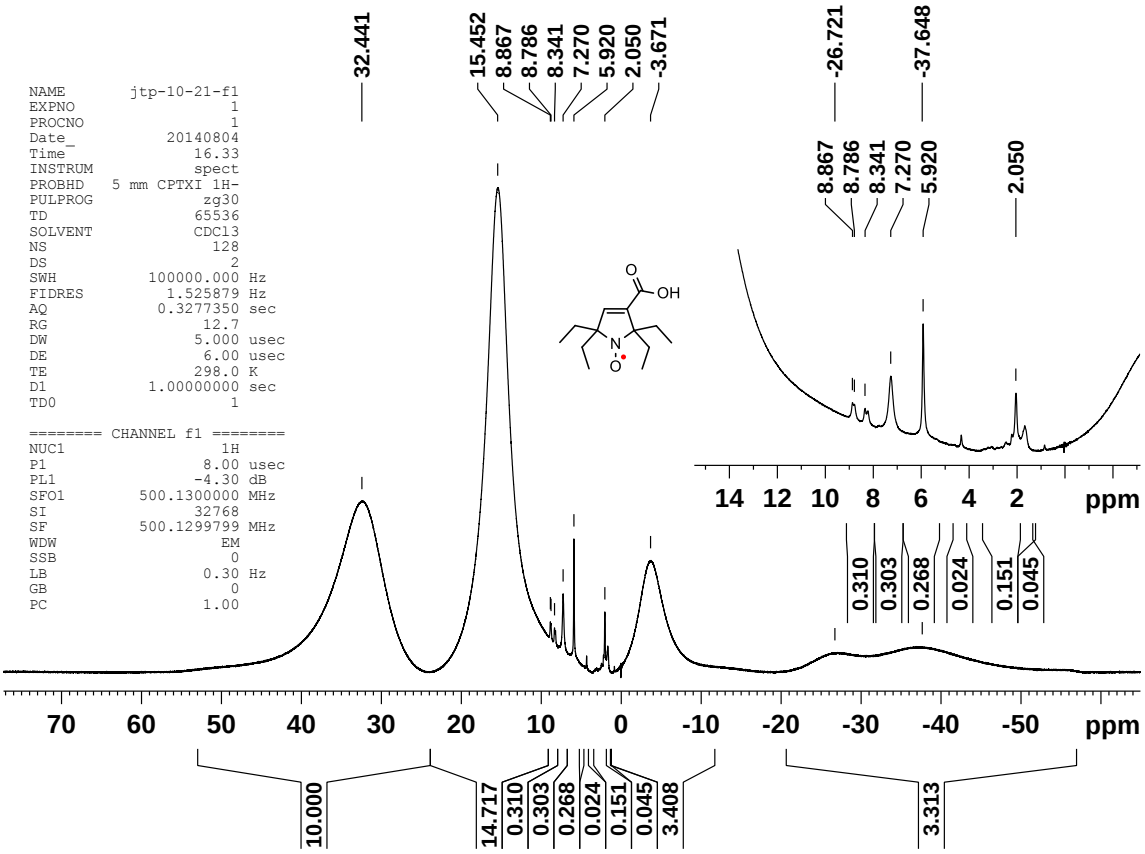


Figure S44. ^{1}H NMR spectrum (500 MHz, 1.3 M in $CDCl_3$) of **6** (label: JTP-10-21-f1).

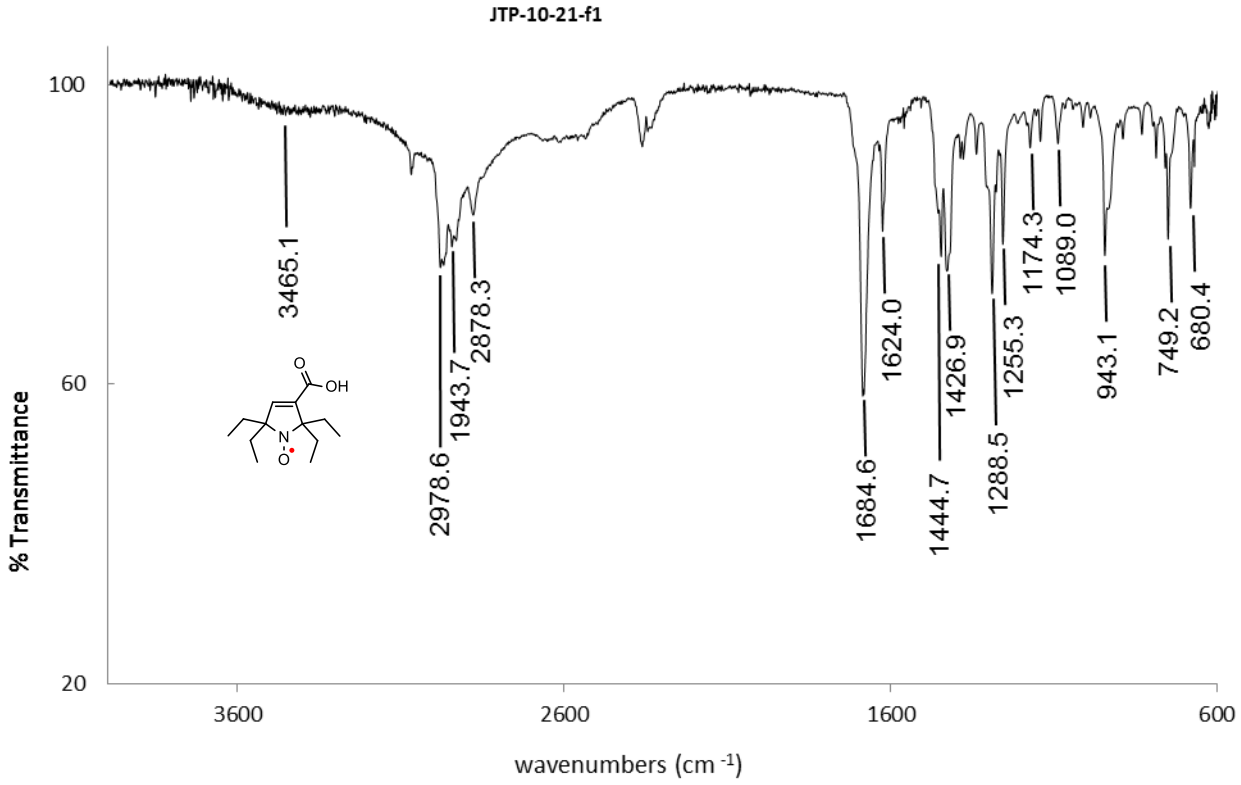


Figure S45. IR spectrum (ATR, diamond) of compound **6** (label: JTP-10-21-f1).

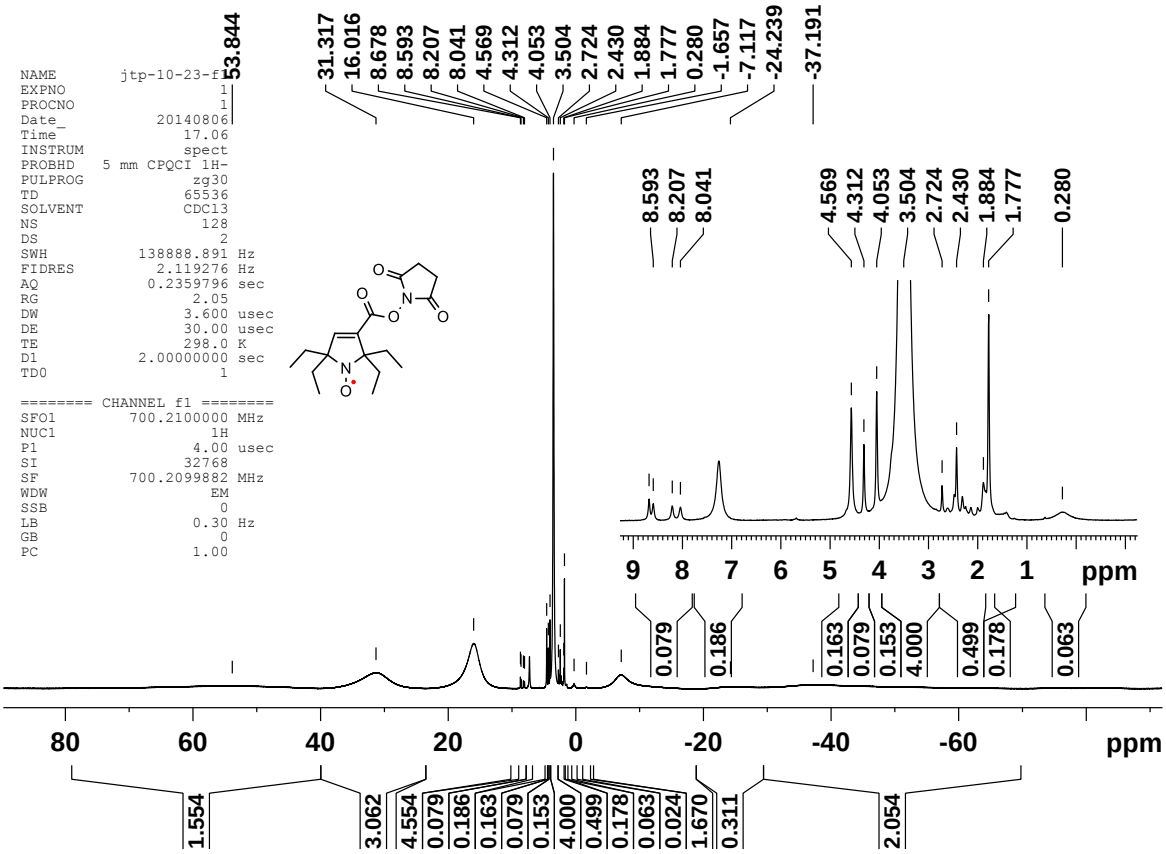


Figure S46. ^{1}H NMR spectrum (500 MHz, 0.8 M in $CDCl_3$) of **22** (label: JTP-10-23-f1).

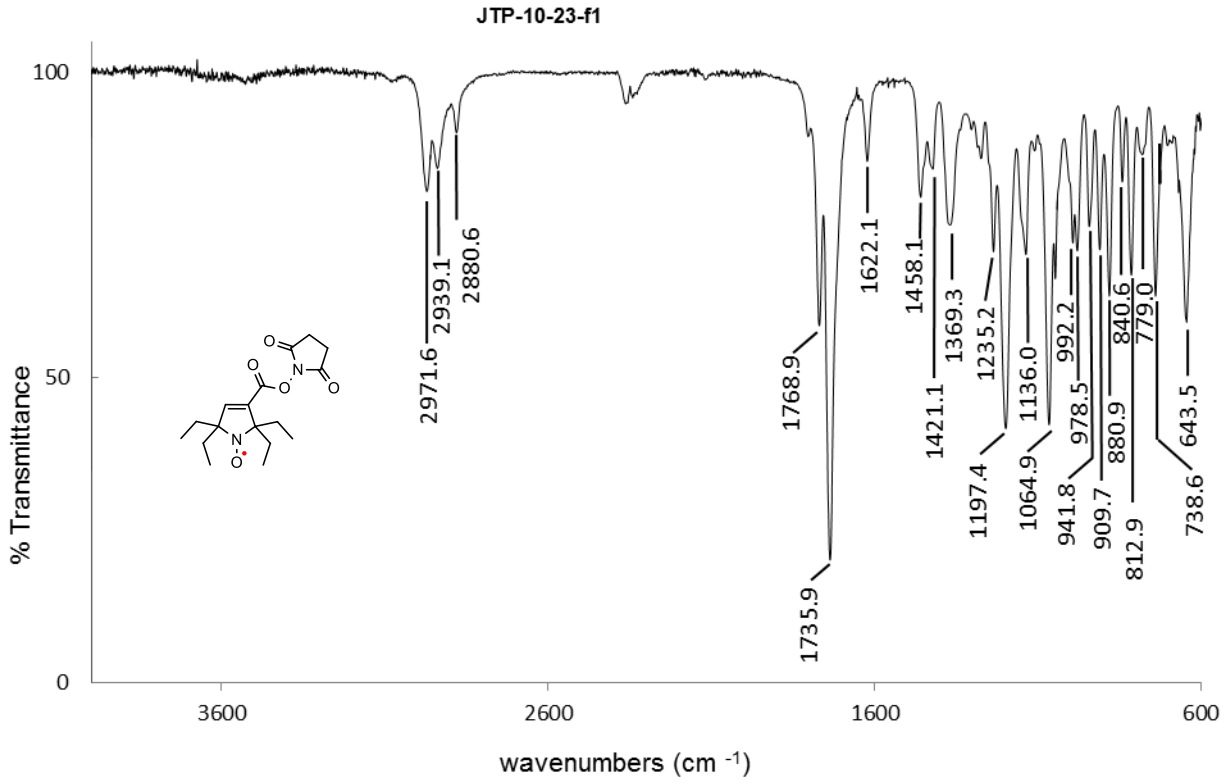


Figure S47. IR spectrum (ATR, diamond) of compound **22** (label: JTP-10-23-f1).

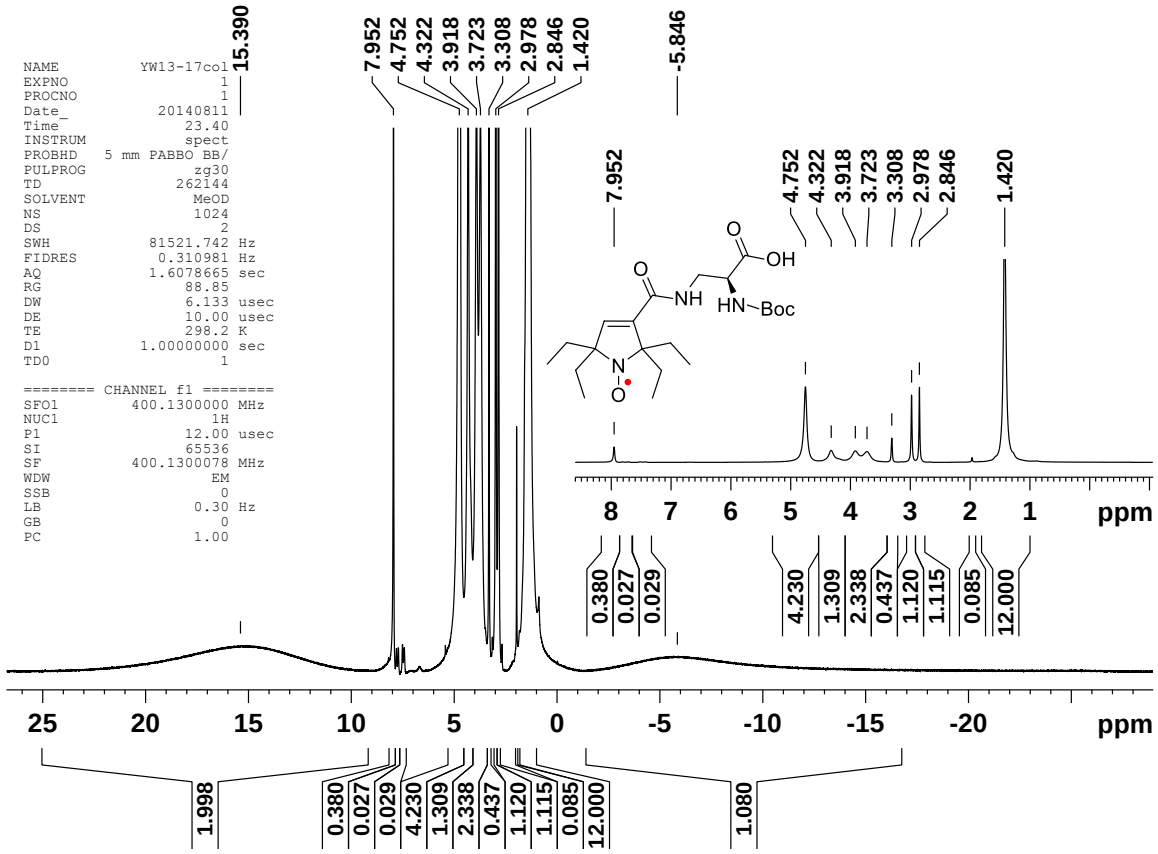


Figure S48. ^{1}H NMR spectrum (400 MHz, 0.17 M in CD_3OD) of **4** (label: YW13-17col).

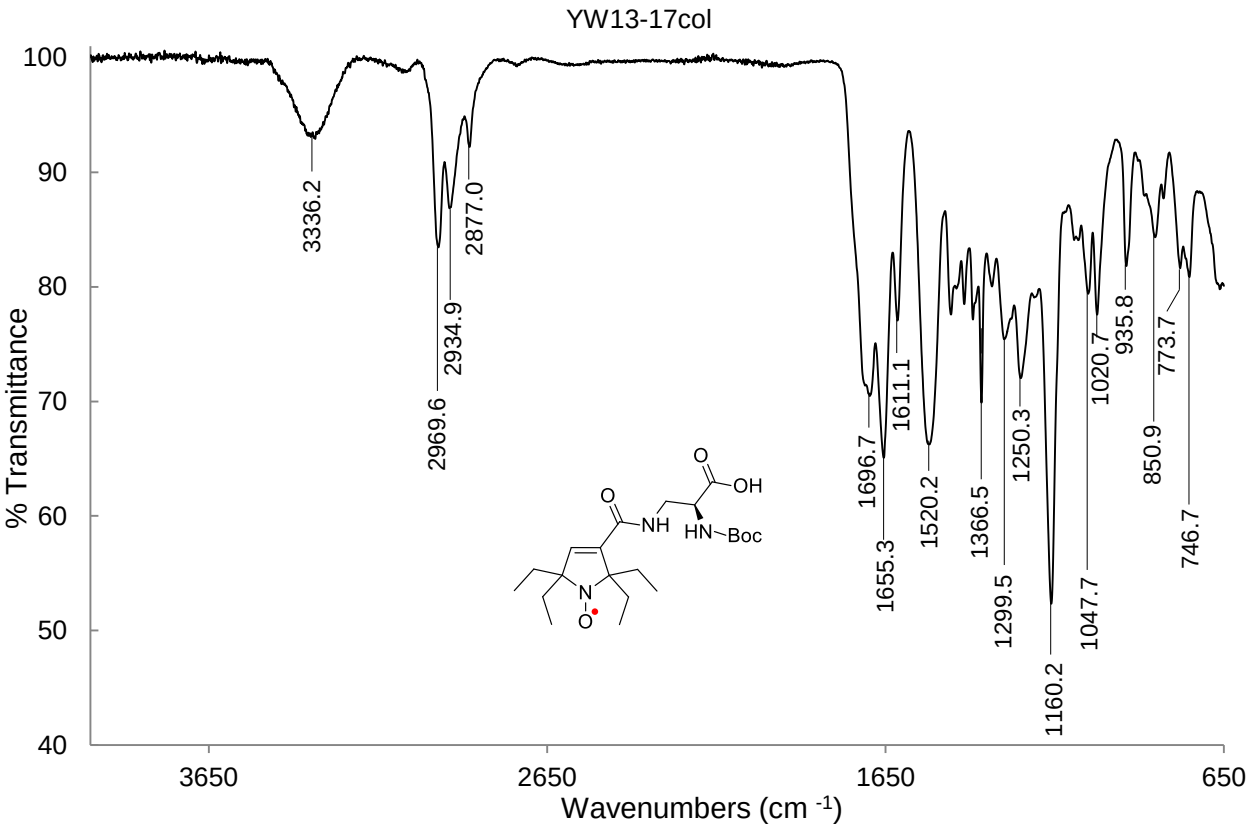


Figure S49. IR spectrum (ATR, ZnSe) of compound **4** (label: YW13-17col)

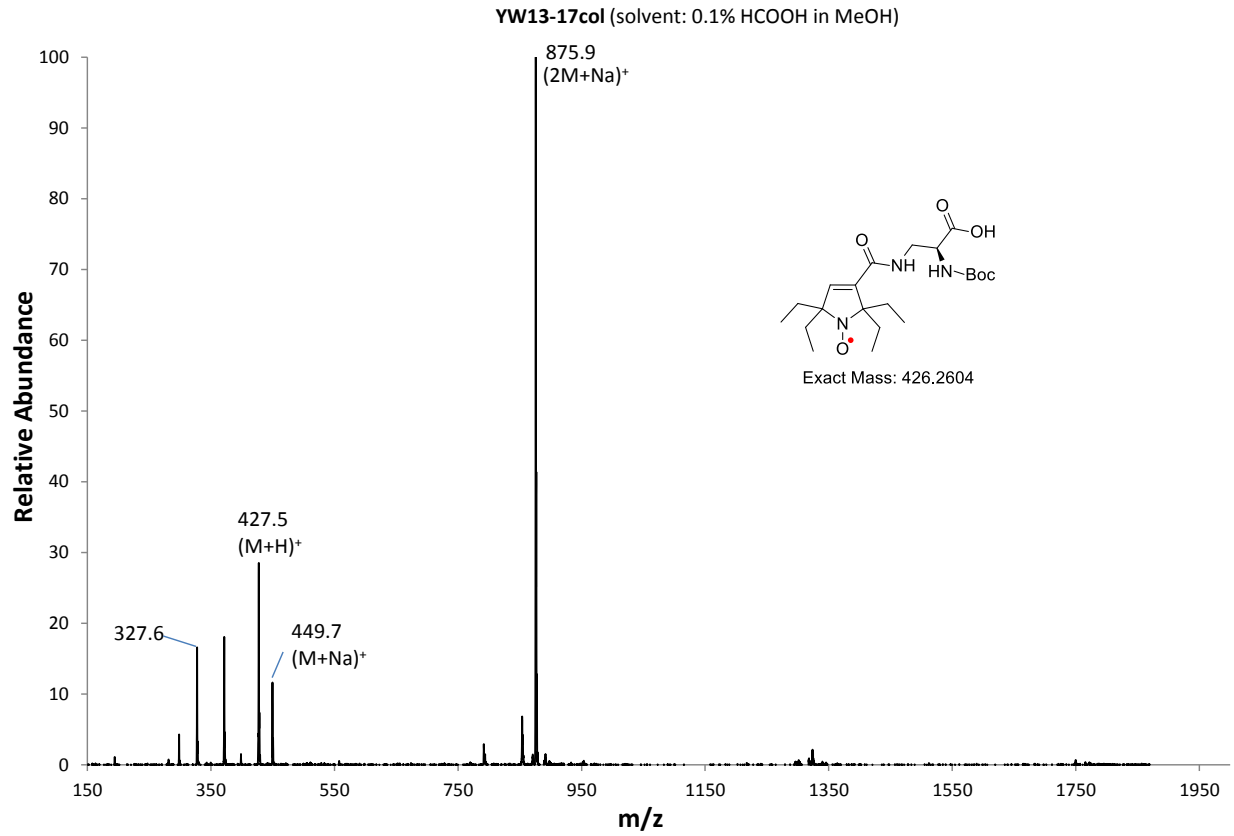


Figure S50. LRMS-ESI spectrum (solvent: 0.1% HCOOH in MeOH) of **4** (label: YW13-17col)

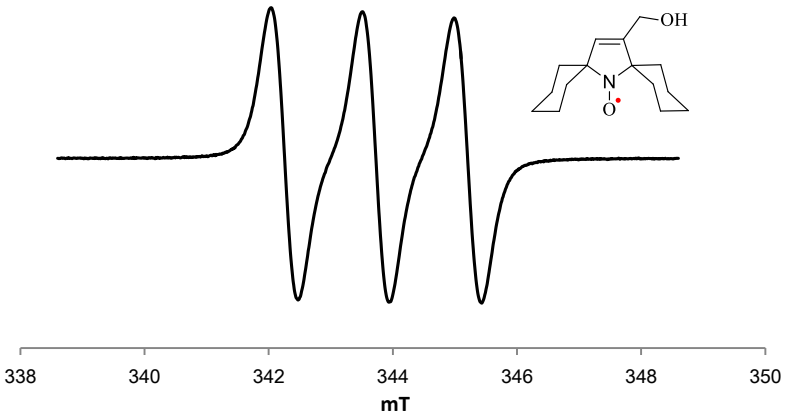


Figure S51. EPR (X-band) spectrum of **11** (sample label: YW11-72col; 1.11 mM in $CHCl_3$; EPR label: YW1324r5, parameters: power, 30 dB, 12.59 μW; modulation amplitude 0.8 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 5.02×10^4).

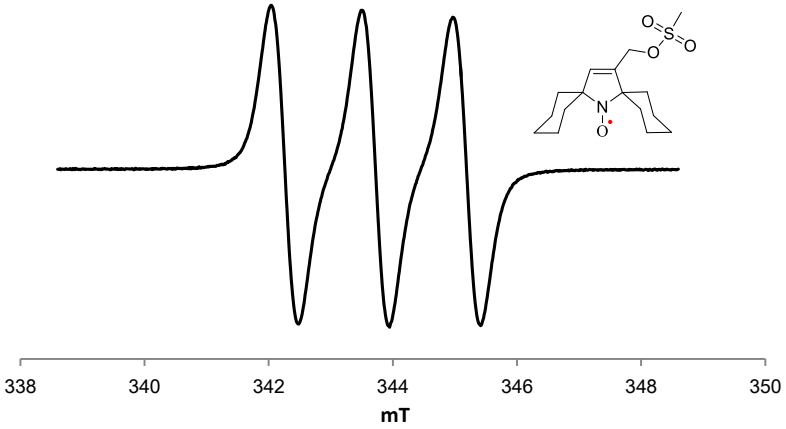


Figure S52. EPR (X-band) spectrum of **12** (sample label: YW11-77col1; 0.99 mM in $CHCl_3$; EPR label: YW1324r8, parameters: power, 30 dB, 12.59 μW; modulation amplitude 0.8 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 5.02×10^4).

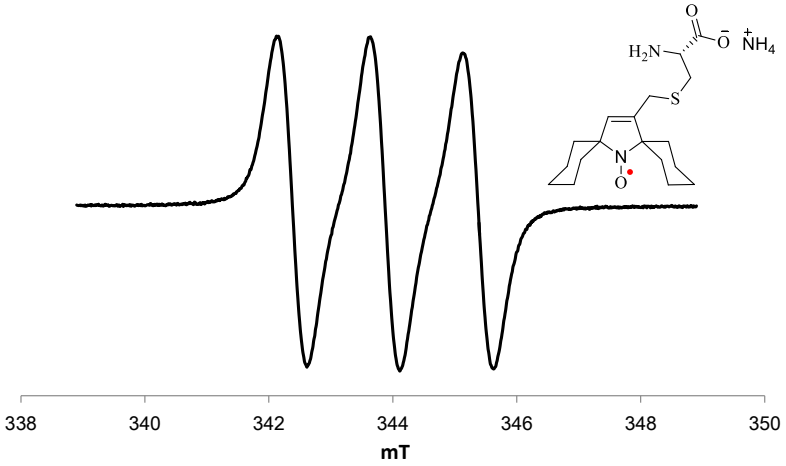


Figure S53. EPR (X-band) spectrum of **1** (sample label: YW11-88colA2; 1.0 mM in MeOH), used for spin concentration determination (EPR label: YW1189r3, parameters: power, 20 dB, 2.046 mW; modulation amplitude 1.0 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 7.96×10^4).

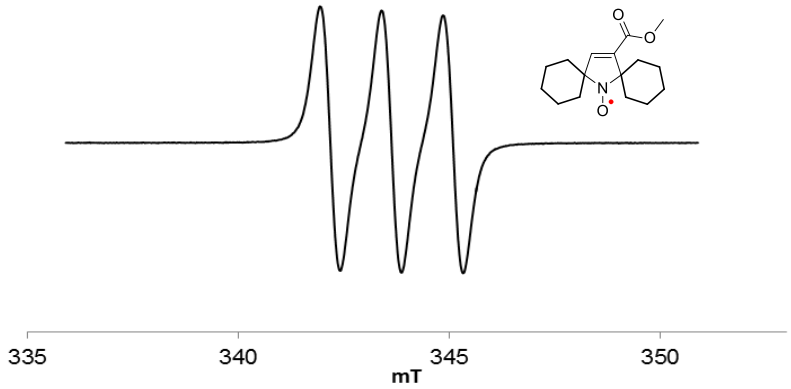


Figure S54. EPR (X-band) spectrum of **19** (sample label: CRR-1-11-f1; 1.0 mM in $CHCl_3$; EPR label: JP819r7, parameters: power, 30 dB, 12.59 μW; modulation amplitude 2.0 G; conversion time 40.96 ms; time constant 1.28 ms; resolution in X, 1024 points; receiver gain, 2.00×10^4).

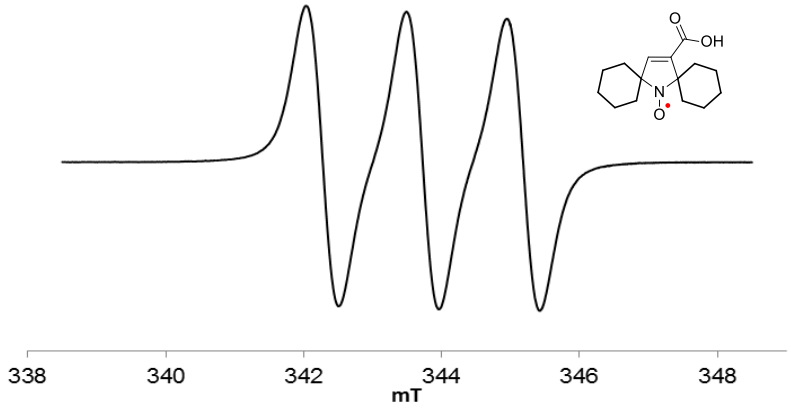


Figure S55. EPR (X-band) spectrum of **5** (sample label: JTP-10-8-f1; 1.2 mM in $CHCl_3$; EPR label: JP864r4, parameters: power, 30 dB, 12.59 μW; modulation amplitude 2.0 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 1.12×10^4).

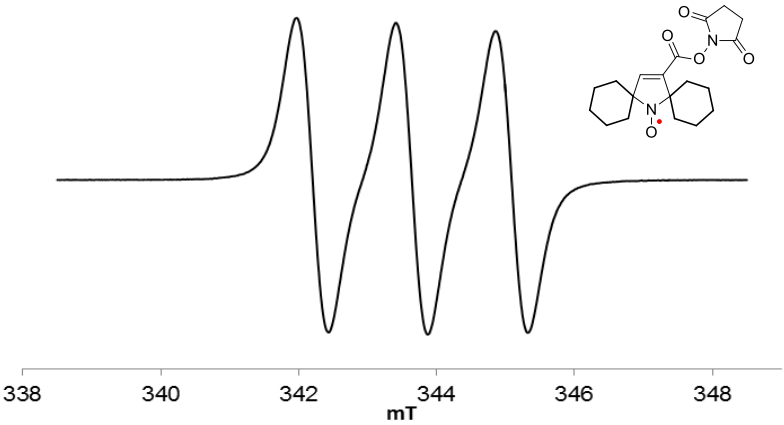


Figure S56. EPR (X-band) spectrum of **20** (sample label: JTP-1 0-14-f1; 1.1 mM in $CHCl_3$; EPR data label: JP864r5, parameters: power, 30 dB, 12.59 μW; modulation amplitude 2.0 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 1.42×10^4).

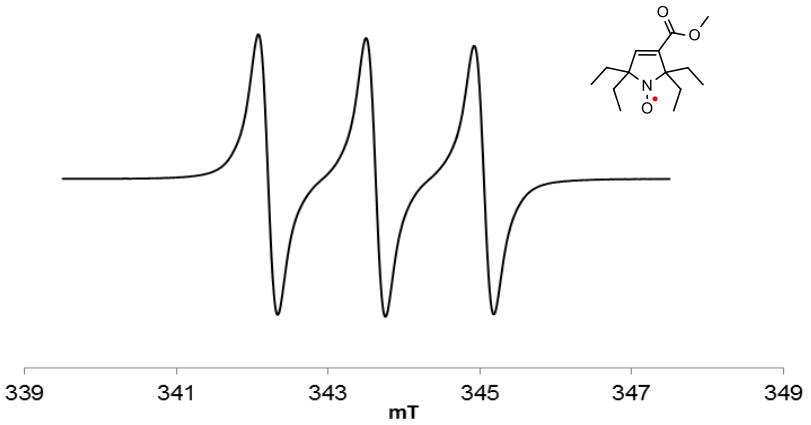


Figure S57. EPR (X-band) spectrum of **21** (sample label: JTP-10-20-f1; 0.5 mM in $CHCl_3$; EPR label: JP1102r4, parameters: power, 20 dB, 2.046 mW; modulation amplitude 0.5 G; conversion time 40.96 ms; time constant 1.28 ms; resolution in X, 1024 points; receiver gain, 5.64×10^3).

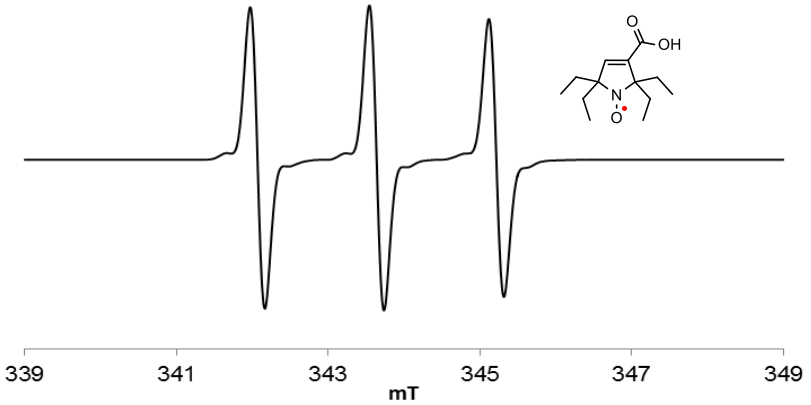


Figure S58. EPR (X-band) spectrum of **6** (sample label: JTP-10-21-f1; 0.9 mM in PBS; EPR label: JP869r2, parameters: power, 15 dB, 6.469 mW; modulation amplitude 2.0 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 5.64×10^3).

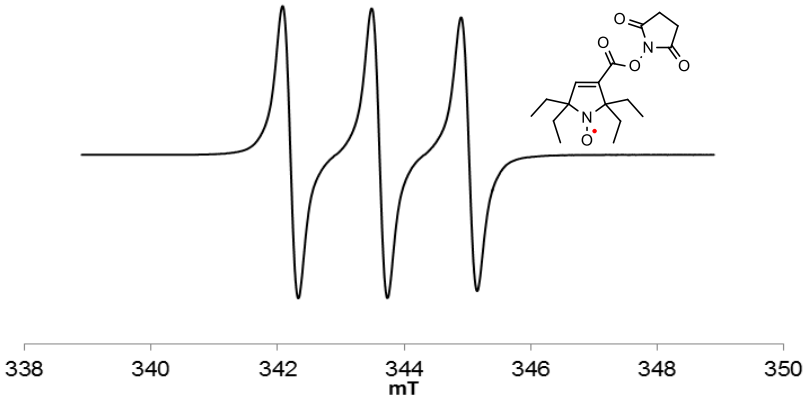


Figure S59. EPR (X-band) spectrum of **22** (sample label: JTP-10-23-f1; 0.8 mM in $CHCl_3$; EPR label: JP875r2, parameters: power, 30 dB, 12.59 μW; modulation amplitude 2.0 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 8.93×10^3).

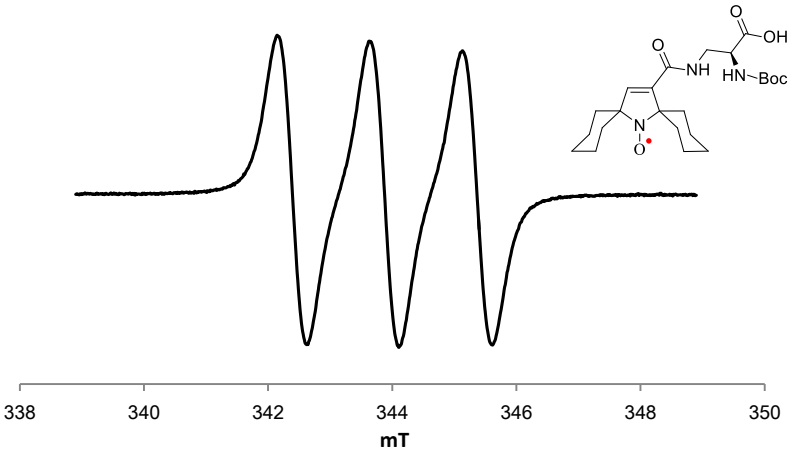


Figure S60. EPR (X-band) spectrum of **3** (sample label: YW13-11col2; 0.99 mM in CH_3OH; EPR label: YW1312r5, parameters: power, 20 dB, 2.046 mW; modulation amplitude 1.0 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 7.96×10^4).

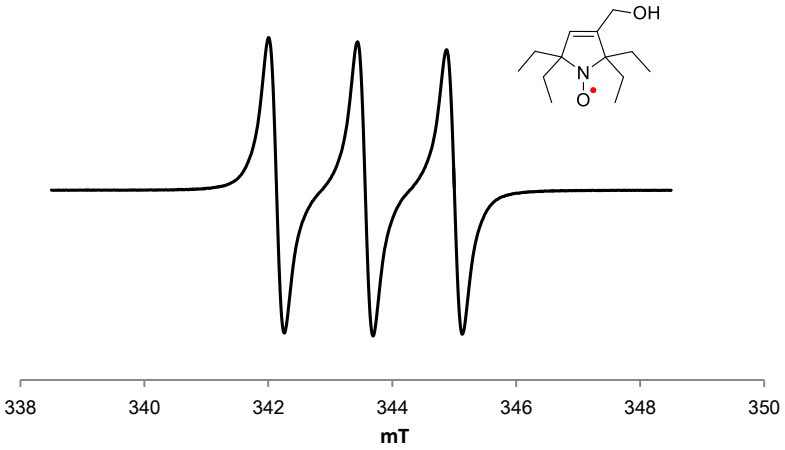


Figure S61. EPR (X-band) spectrum of **19** (sample label: YW12-71col; 2.25 mM in $CHCl_3$; EPR label: YW1274r10, parameters: power, 30 dB, 12.59 μW; modulation amplitude 0.5 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 2.24×10^4).

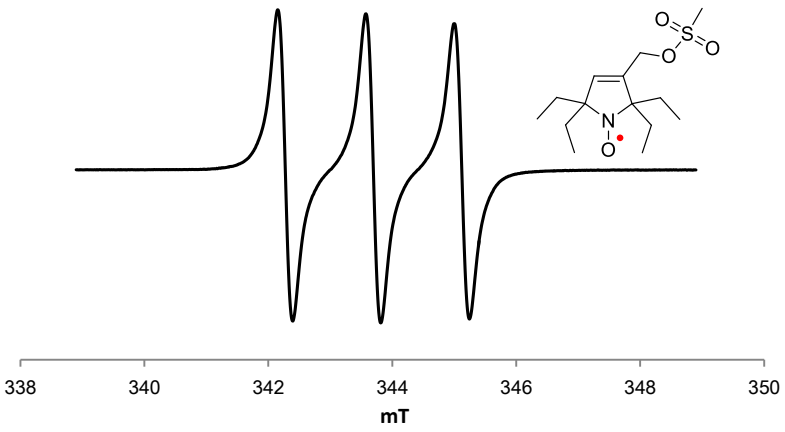


Figure S62. EPR (X-band) spectrum of **20** (sample label: YW12-78col; 2.66 mM in $CHCl_3$; EPR label: YW1281r3, parameters: power, 30 dB, 12.59 μW; modulation amplitude 0.5 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 1.59×10^4).

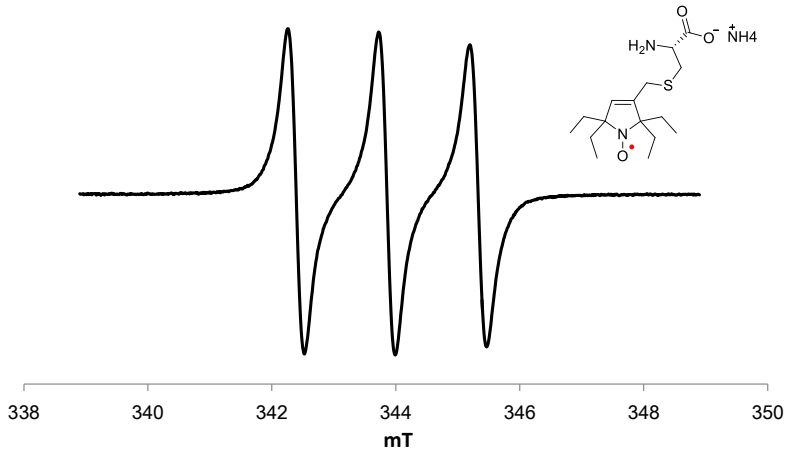


Figure S63. EPR (X-band) spectrum of **2** (sample label: YW13-08crp2; 0.97 mM in CH_3OH; EPR label: YW1313r5, parameters: power, 20 dB, 2.046 mW; modulation amplitude 0.5 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 7.96×10^4).

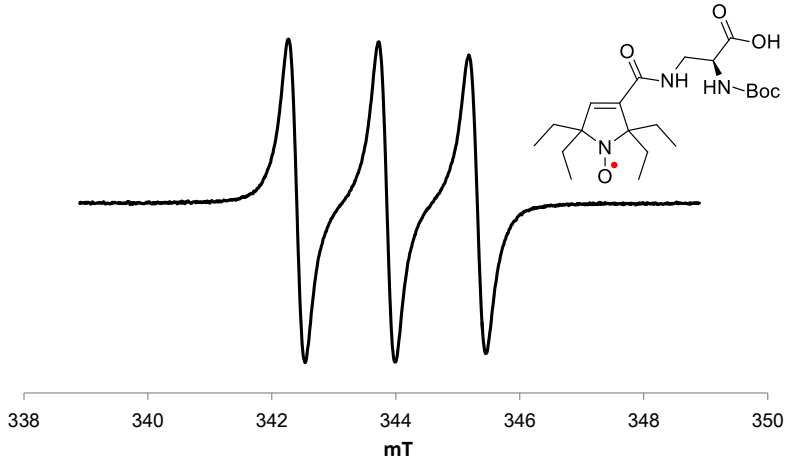


Figure S64. EPR (X-band) spectrum of **4** (sample label: YW13-17col; 0.97 mM in CH_3OH; EPR0 label: YW1318r5, parameters: power, 20 dB, 2.064 mW; modulation amplitude 0.5 G; conversion time 40.96 ms; time constant 10.24 ms; resolution in X, 1024 points; receiver gain, 7.96×10^4)

4. Supporting References.

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