

Dissecting AI-2-mediated quorum sensing through C5-analogue synthesis and biochemical analysis

Karen C. Collins, Kyoji Tsuchikama, Colin A. Lowery, Jie Zhu, Kim D. Janda*

Departments of Chemistry and Immunology, The Skaggs Institute for Chemical Biology, Worm Institute of Research and Medicine (WIRM), The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States.

Calculation of Equilibrium Constants

Equilibrium constants of hydration were calculated using the method of Casado (Figure S1).¹ Under standard conditions (298.15 K, 1 atm), the equilibrium constant for **(2S,4S)-SH-DPD** was calculated to be 8.31, comparable to those of (2R,4S)-DHMF and (2S,4S)-DHMF, which were calculated to be 12.34 and 4.60, respectively. These values suggest that under aqueous conditions, the cyclised form of **C5-SH-DPD** has comparable hydration to that of DPD. Furthermore, the validity of these calculations are supported by the 4.3:1 hydrate/ketone ratio observed for DPD by ¹H-NMR spectroscopy.²

	G (ketone)/a.u.	G (hydrate)/a.u.	$\Delta G_{exchange}/a.u.$	$\log K_{hyd}$
acetone	-193.1310337	-269.5474811	-	-
(2S, 4S)-SH-DPD	-819.1470804	-895.5717334	-0.008205719	0.92
(2R, 4S)-DHMF	-496.1726171	-572.5976328	-0.008563396	1.09
(2S, 4S)-DHMF	-496.1718362	-572.5959201	-0.007636544	0.66

$$\log(K_{hyd}) = \log[K(\text{acetone})_{hyd,exp}] - \frac{\Delta G_{exchange}}{\ln(10RT)}$$

Figure S1. Calculations of the Equilibrium Constants of Hydration

DFT calculations were performed using the B3LYP/6-31++(d,p) level of theory and the PCM solvation model (solvent: water) in the Gaussian 09 package. The obtained values were corrected by applying a scaling factor of 0.95. $\log[K(\text{acetone})_{hyd,exp}]$ = experimentally determined equilibrium constant of hydration of acetone $(-2.85)^3$, R = gas constant $(8.3145 \text{ J K}^{-1} \text{ mol}^{-1})$, T = temperature (298.15 K)

Numerical Bacterial Assay Data

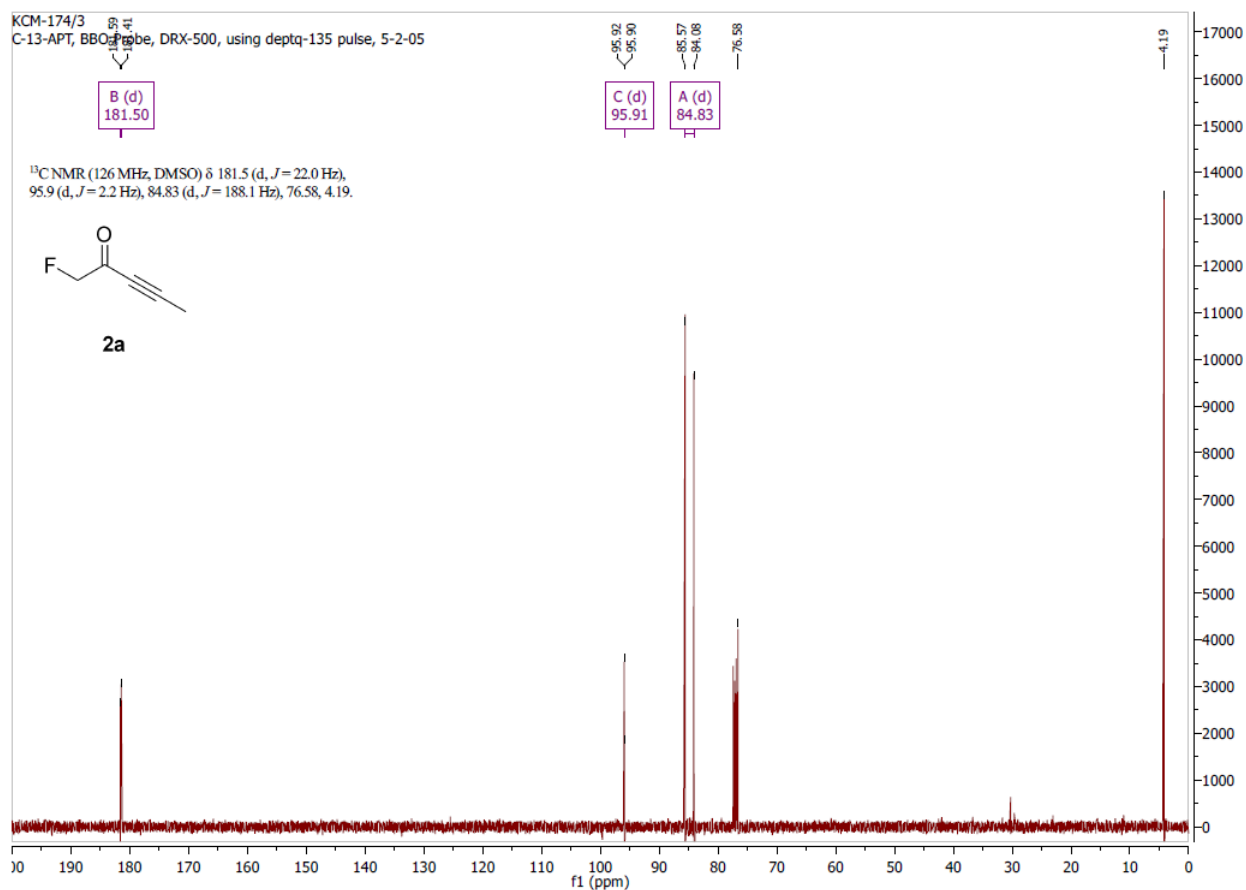
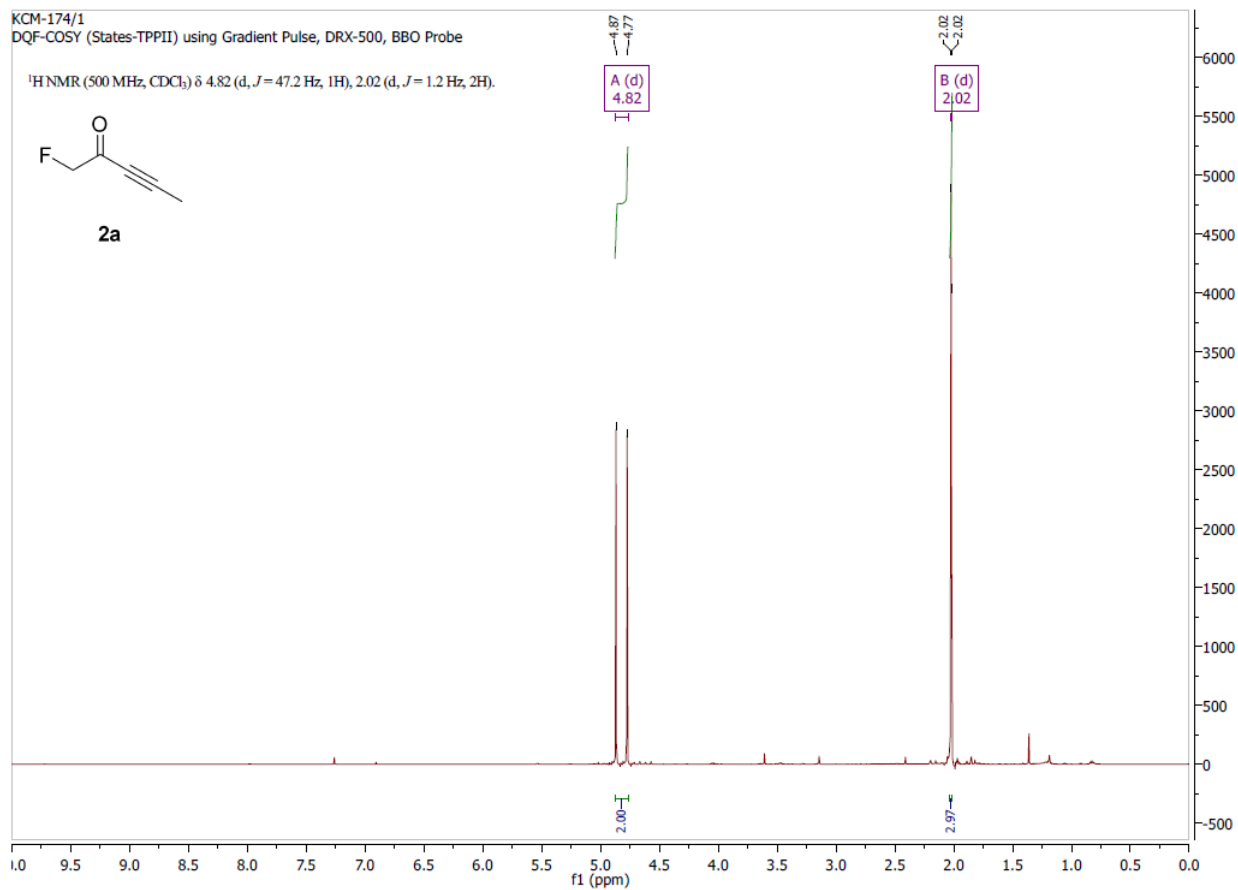
	C5-SH-DPD	C5-Cl-DPD	C5-F-DPD	C5-OMe-DPD	DPD	DMSO
50 μM	-1.50 ± 1.93	0.573 ± 1.41	-2.05 ± 1.05	-2.49 ± 7.91	100 ± 4.0	0.00 ± 1.07
200 μM	-0.688 ± 2.73	4.22 ± 2.01	-3.19 ± 1.06	-5.60 ± 4.09		

	C5-SH-DPD	C5-Cl-DPD	C5-F-DPD	C5-OMe-DPD	DPD	Pr-DPD (25 μM)	DMSO
50 μM	106 ± 1.1	98.8 ± 5.18	124 ± 2.7	89.3 ± 5.97	100 ± 1.9	2.03 ± 0.30	0.00 ± 0.93
200 μM	94.3 ± 3.00	103 ± 11.6	128 ± 4.3	92.2 ± 3.79			

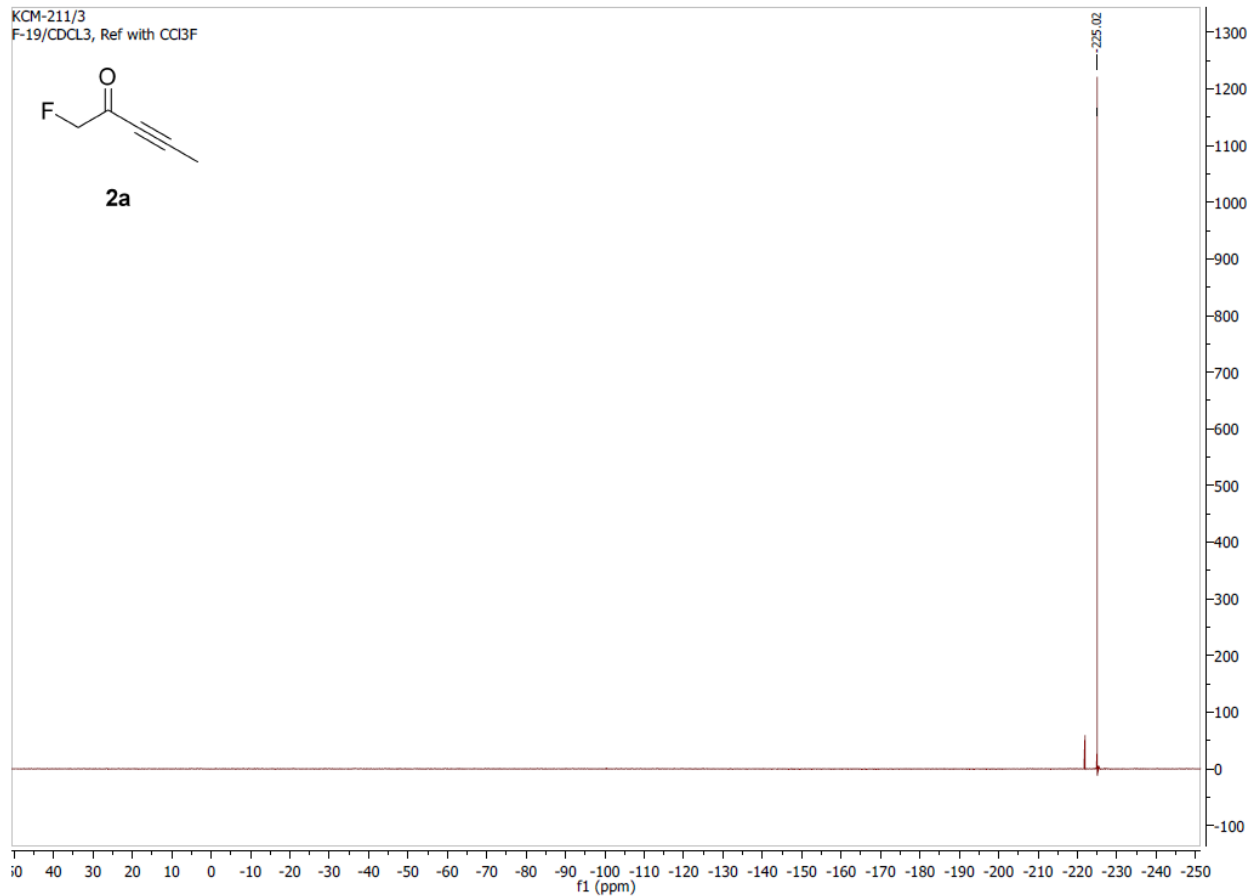
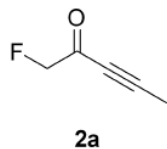
Table S1 and S2. Agonism and antagonism of C5-DPD analogues in *V. harveyi* and *S. Typhimurium*.

In the case of agonism, all contain 50 μM DPD; Pr-DPD used as a positive antagonism control. β -Galactosidase activity in was normalised to cell density. All data was performed in triplicate, errors represent SEM.

NMR Spectra

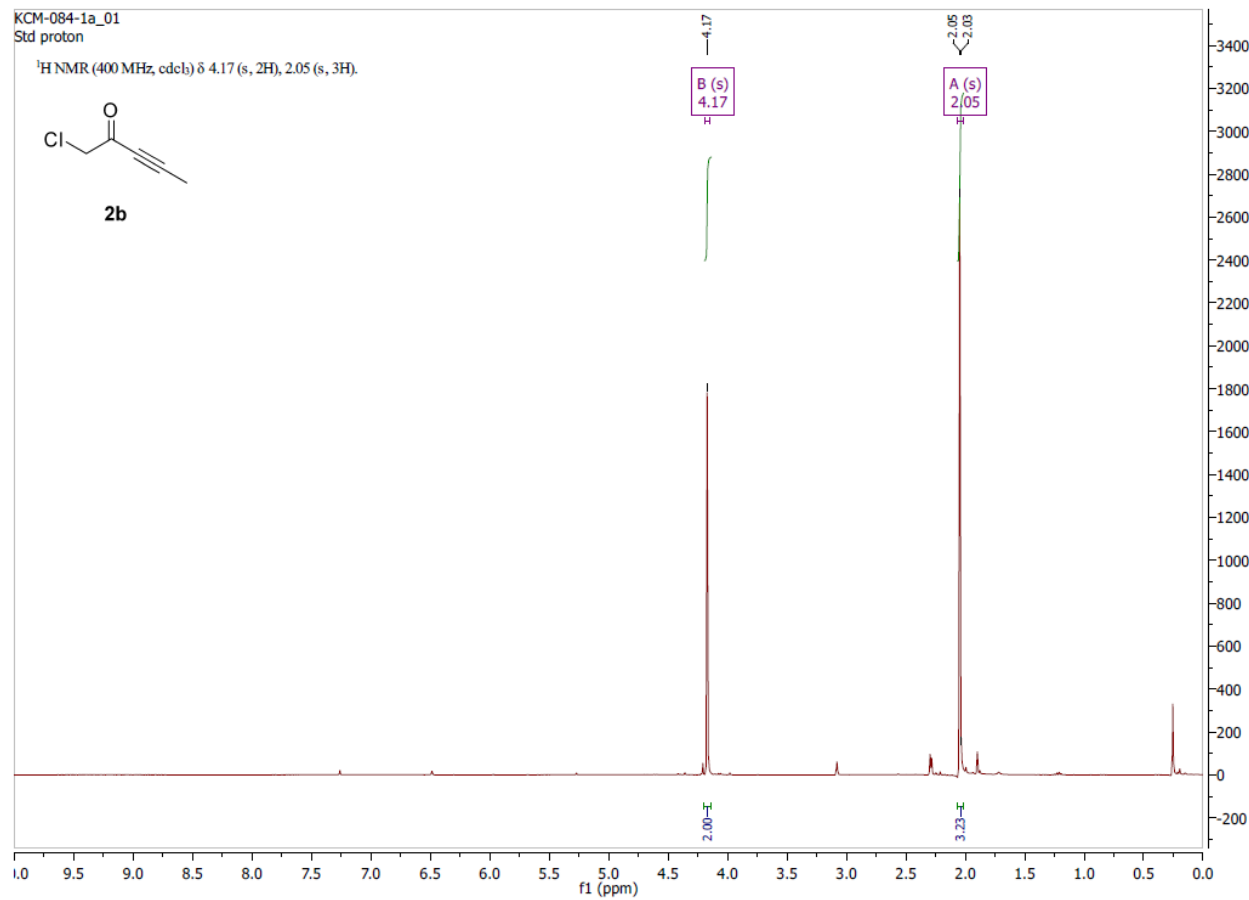
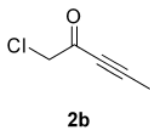


KCM-211/3
F-19/CDCl₃, Ref with CCl₃F

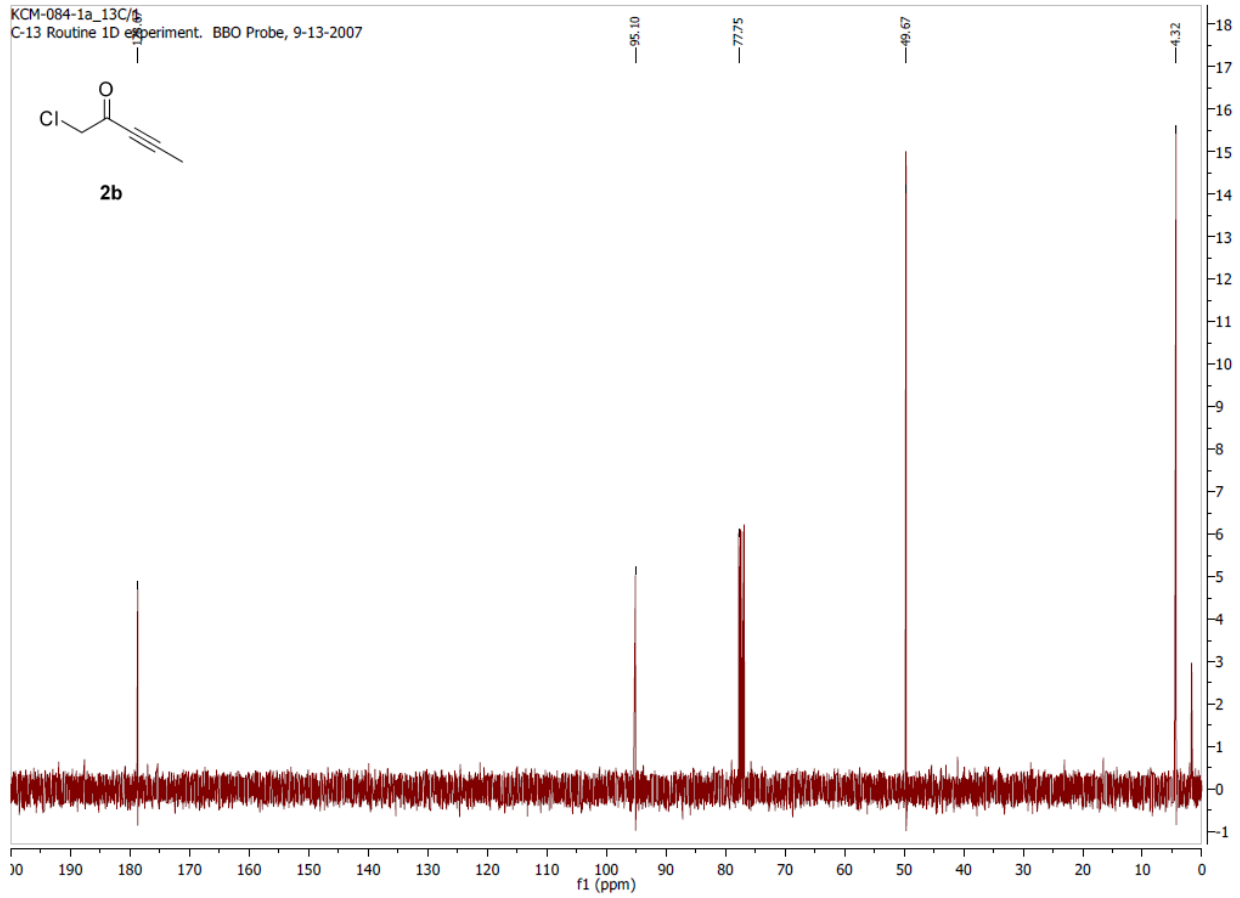


KCM-084-1a_01
Std proton

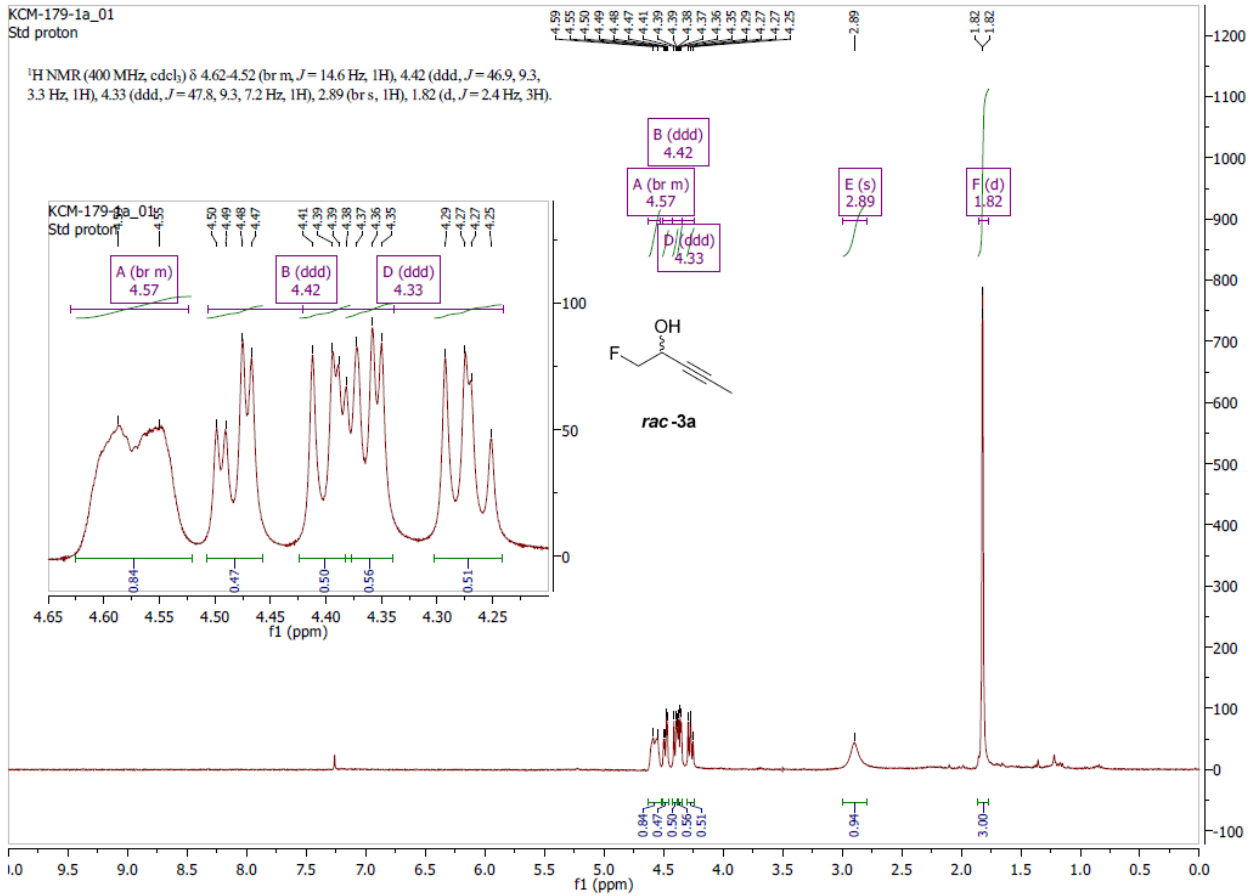
¹H NMR (400 MHz, cdcl₃) δ 4.17 (s, 2H), 2.05 (s, 3H).

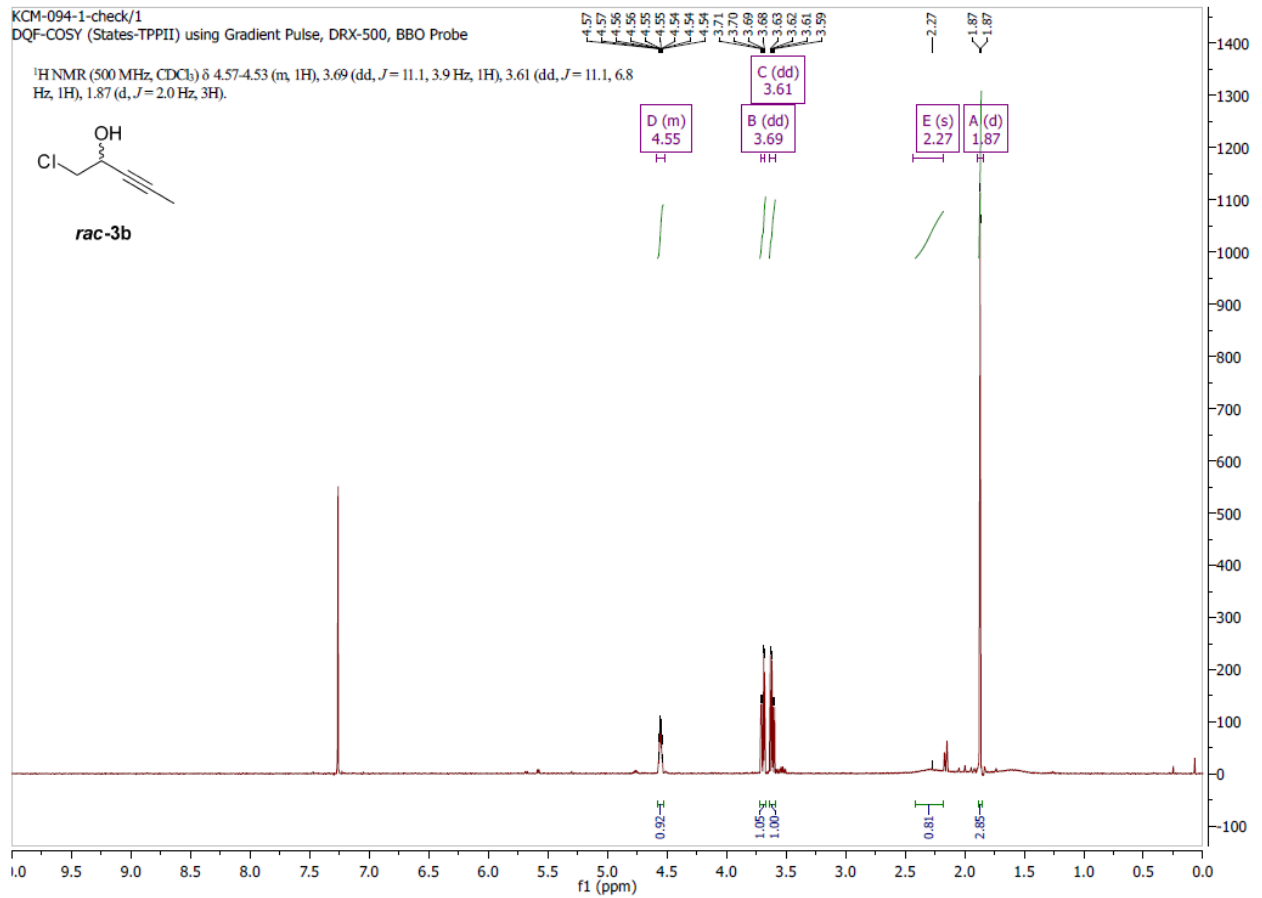
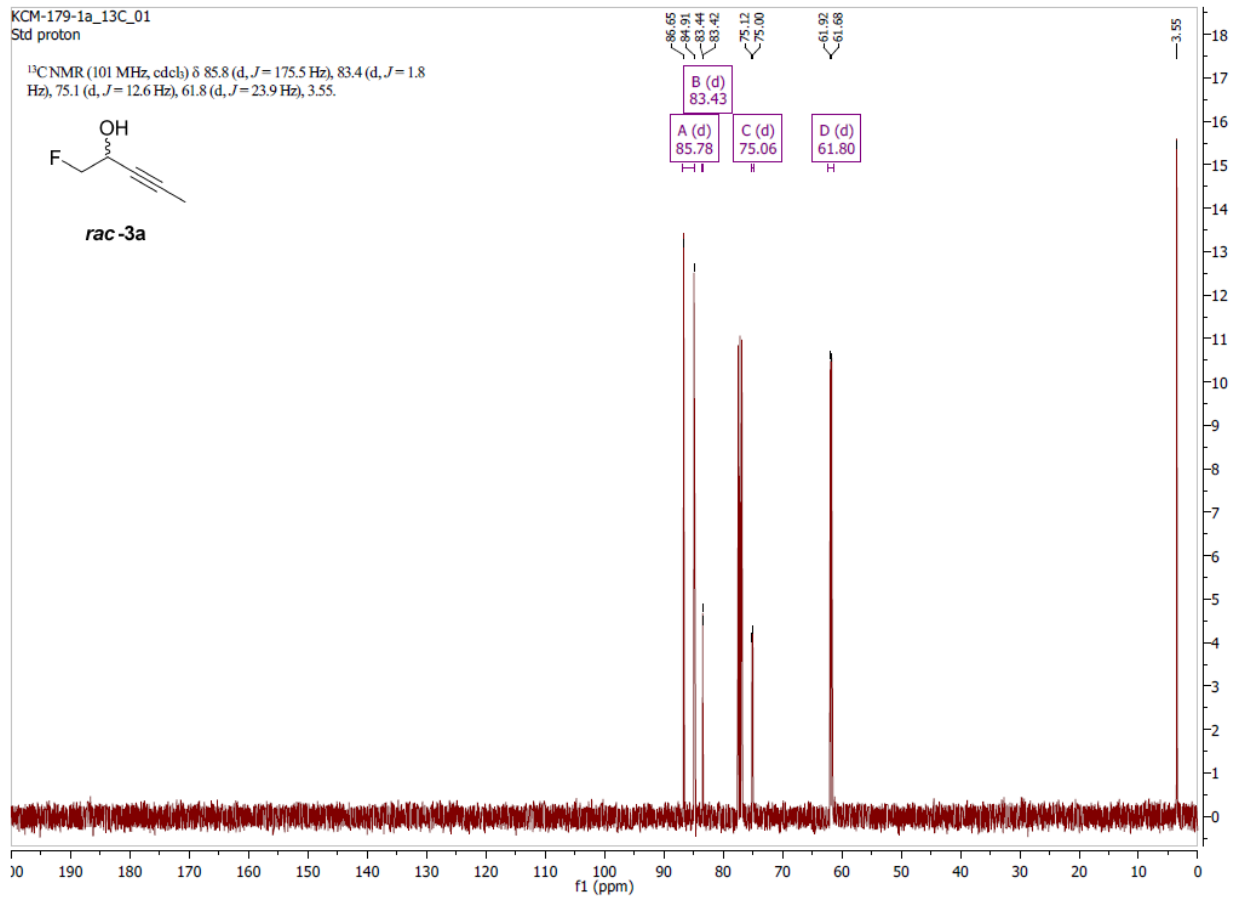


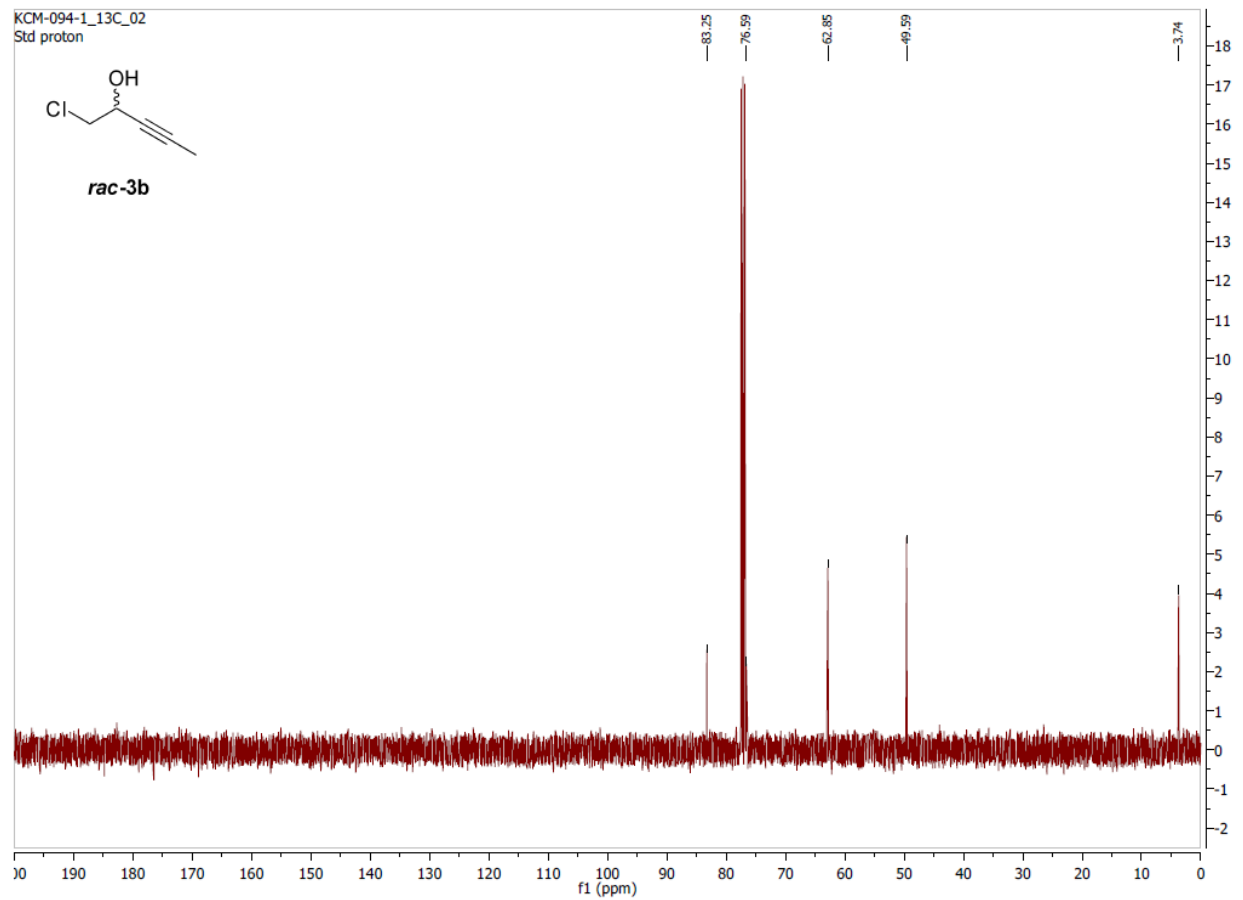
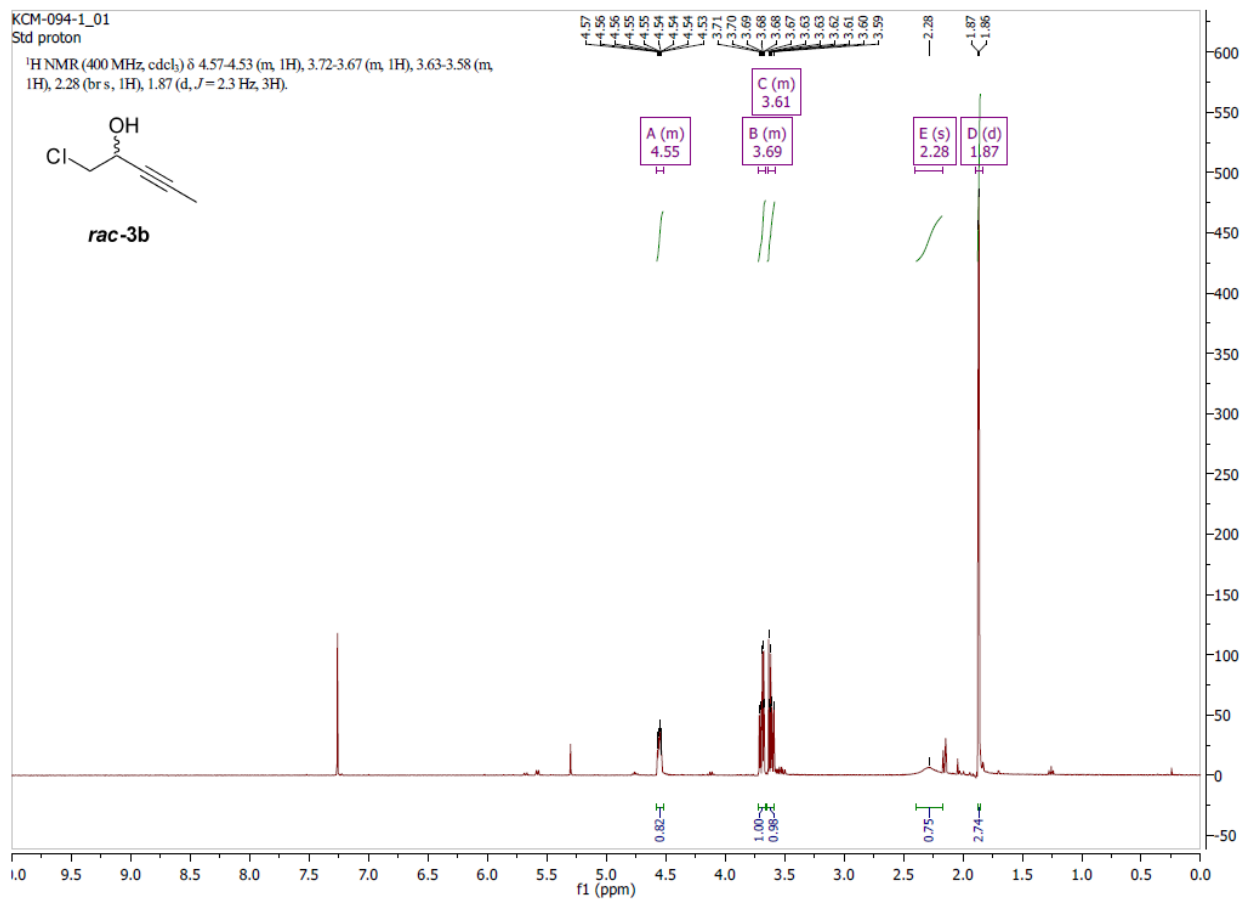
KCM-084-1a_13C/8
C-13 Routine 1D experiment. BBO Probe, 9-13-2007

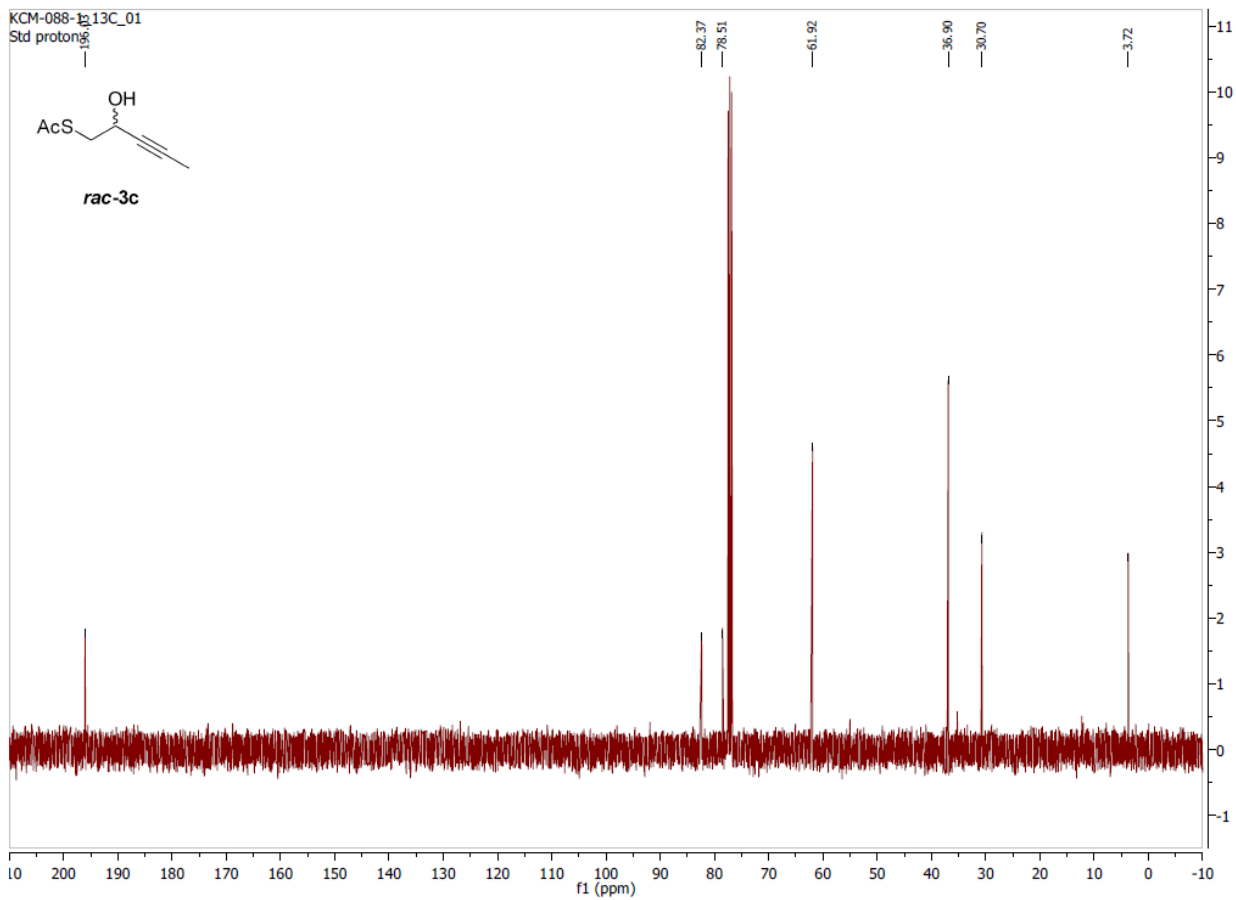
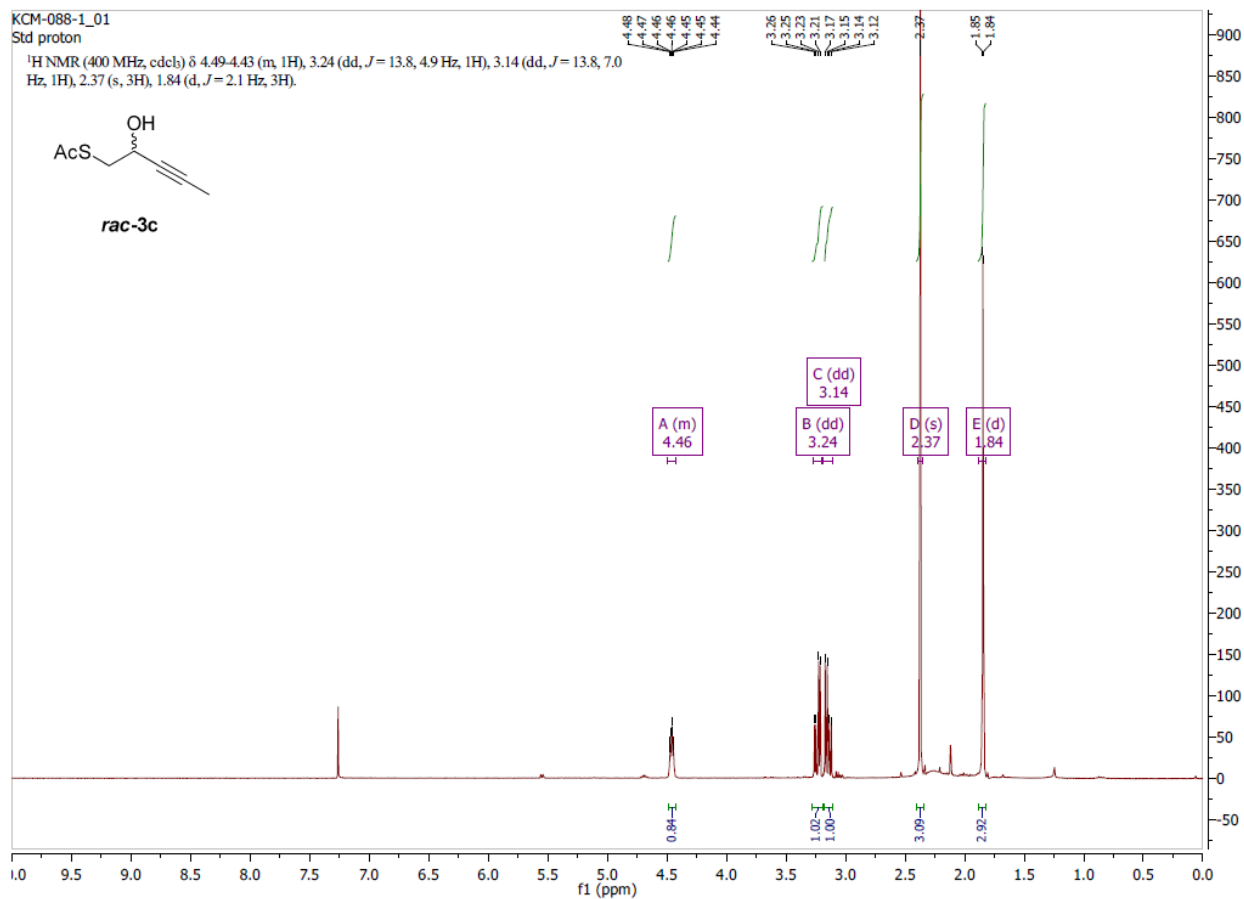


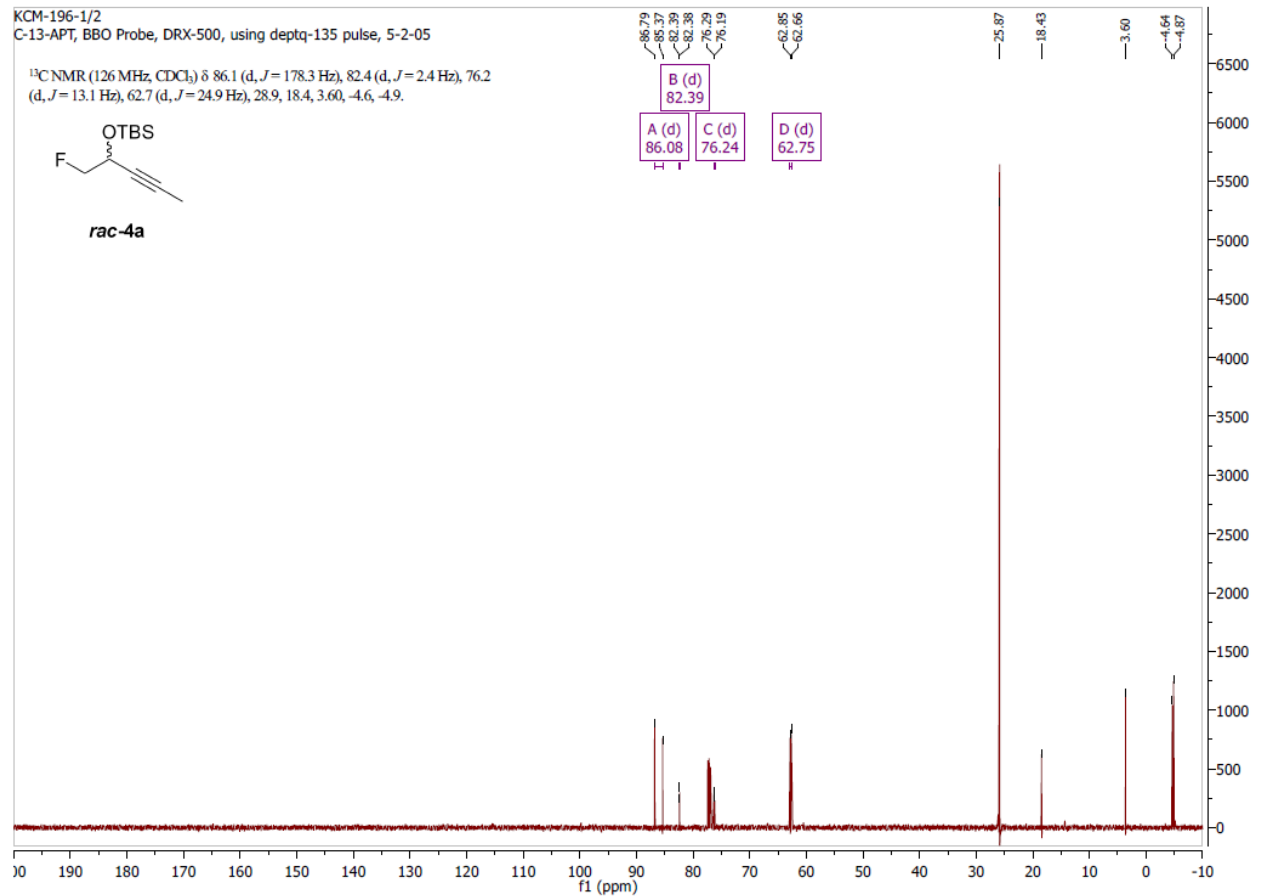
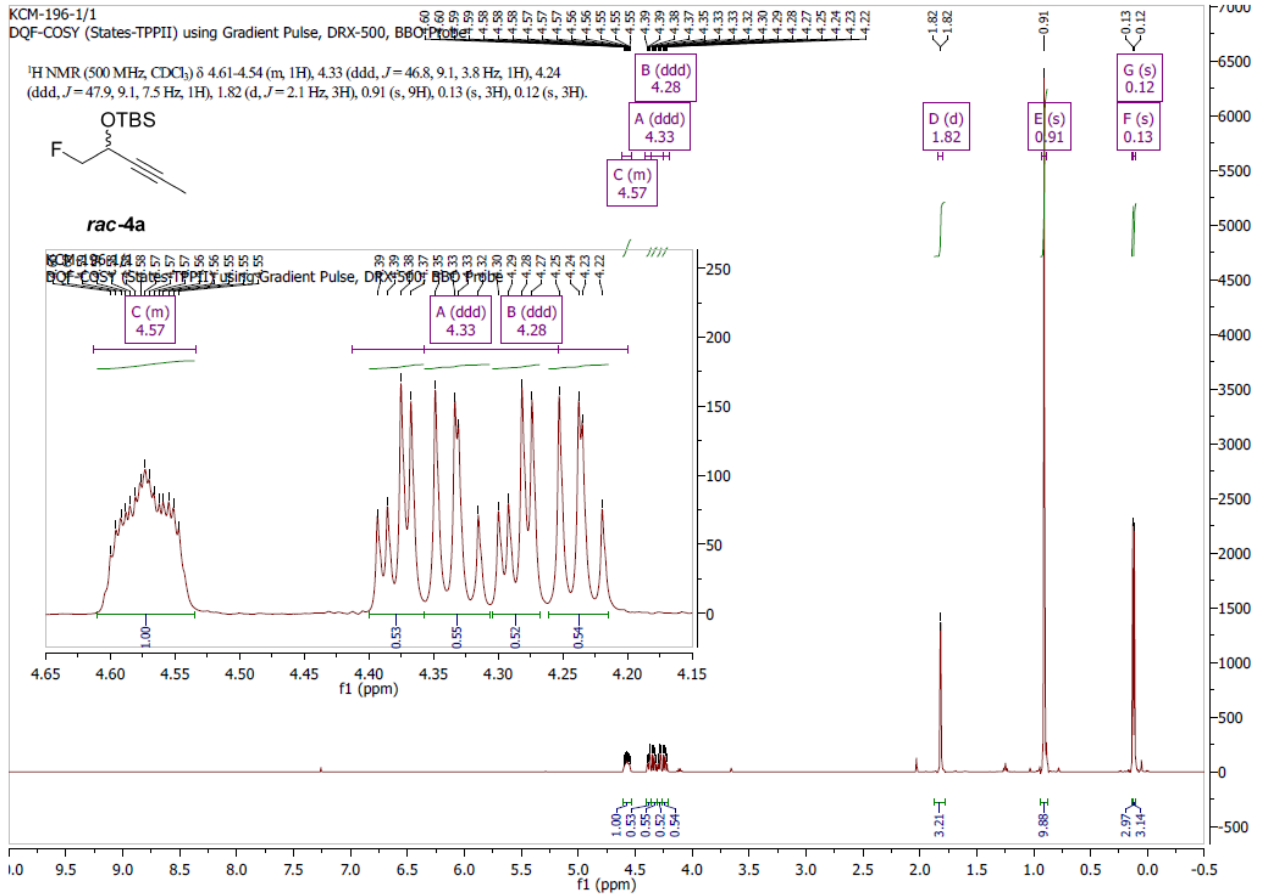
KCM-179-1a_01
Std proton



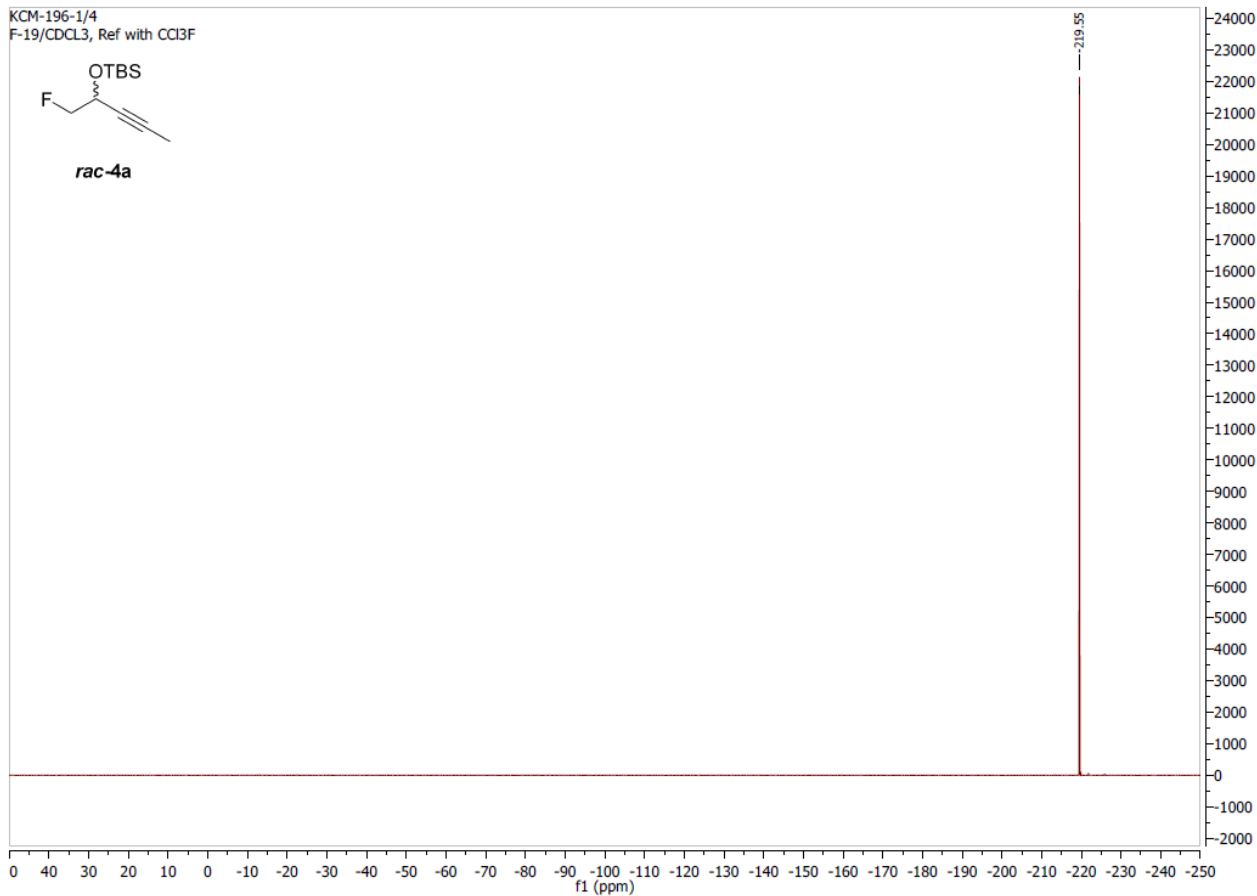
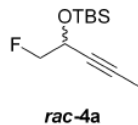








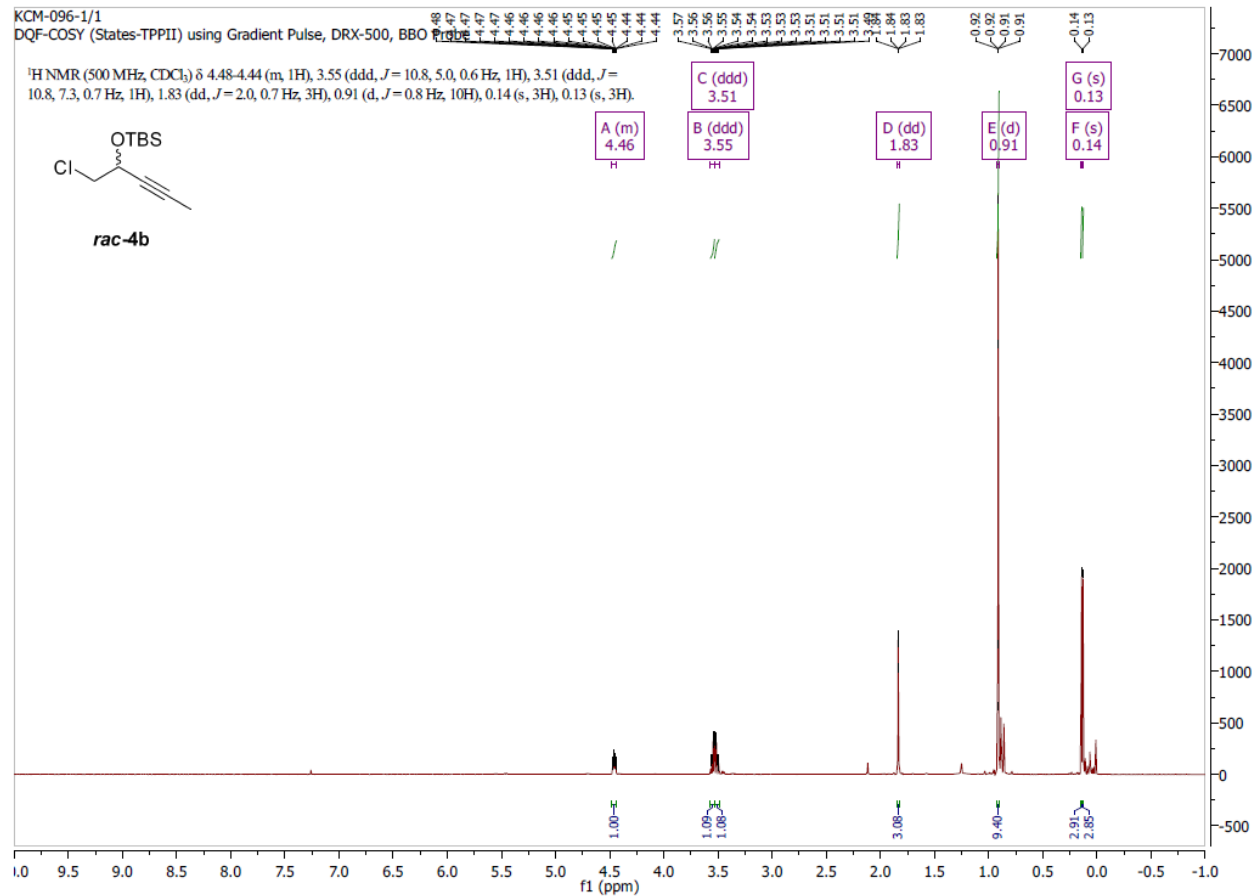
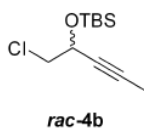
KCM-196-1/4
F-19/CDCL3, Ref with CCl3F



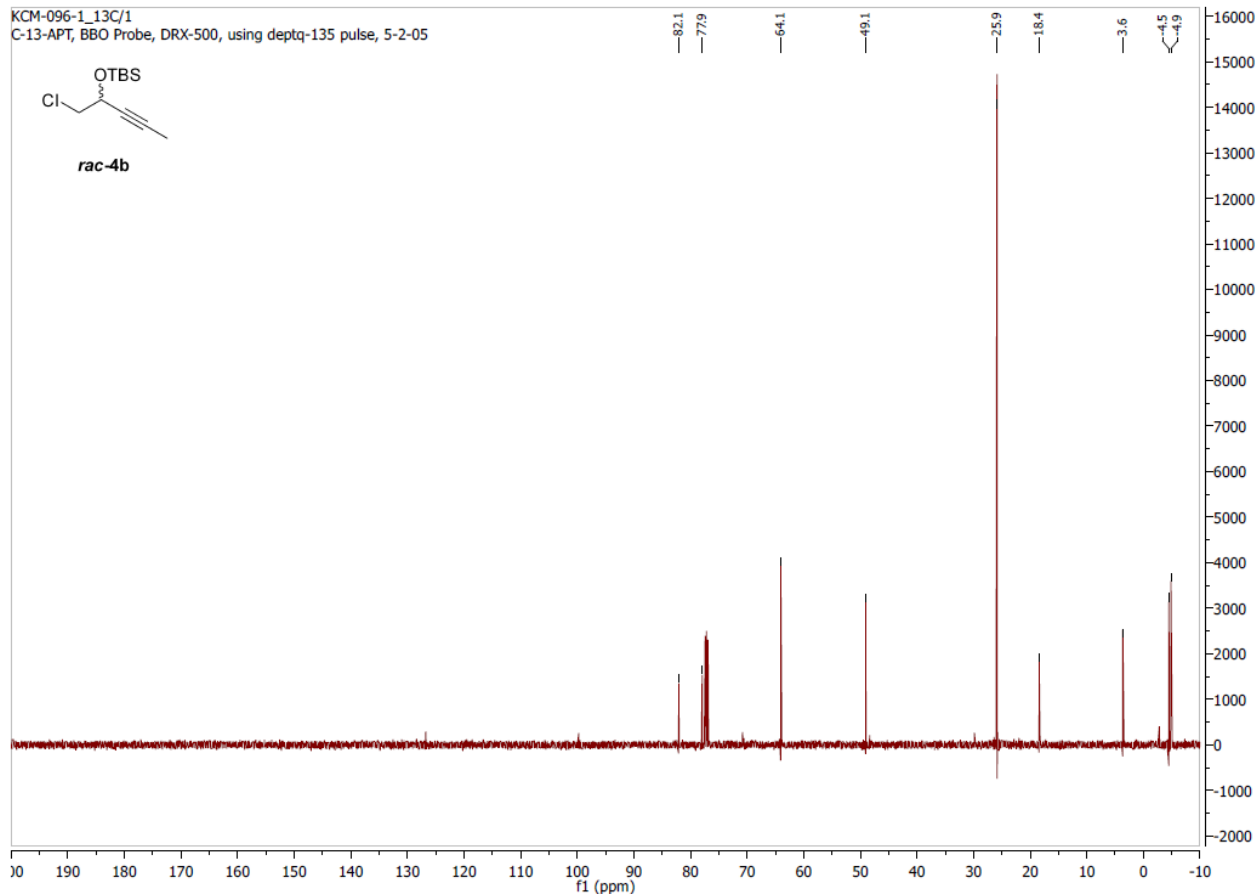
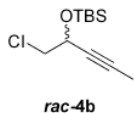
KCM-096-1/1

DQF-COSY (States-TPPII) using Gradient Pulse, DRX-500, BBO Probe

¹H NMR (500 MHz, CDCl₃) δ 4.48-4.44 (m, 1H), 3.55 (ddd, *J* = 10.8, 5.0, 0.6 Hz, 1H), 3.51 (ddd, *J* = 10.8, 7.3, 0.7 Hz, 1H), 1.83 (dd, *J* = 2.0, 0.7 Hz, 3H), 0.91 (d, *J* = 0.8 Hz, 10H), 0.14 (s, 3H), 0.13 (s, 3H).

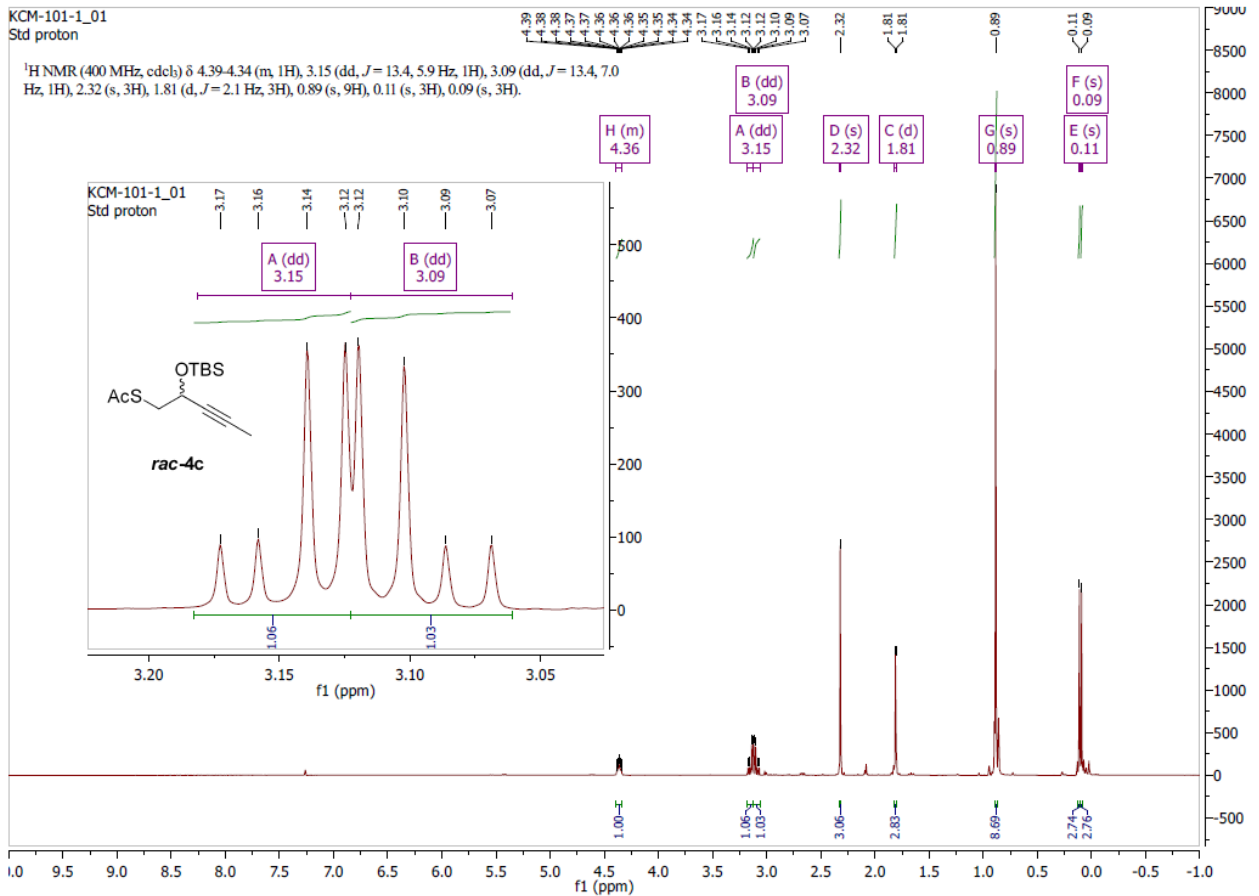
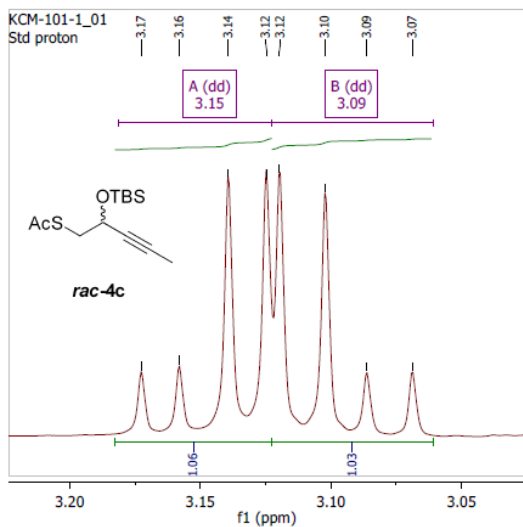


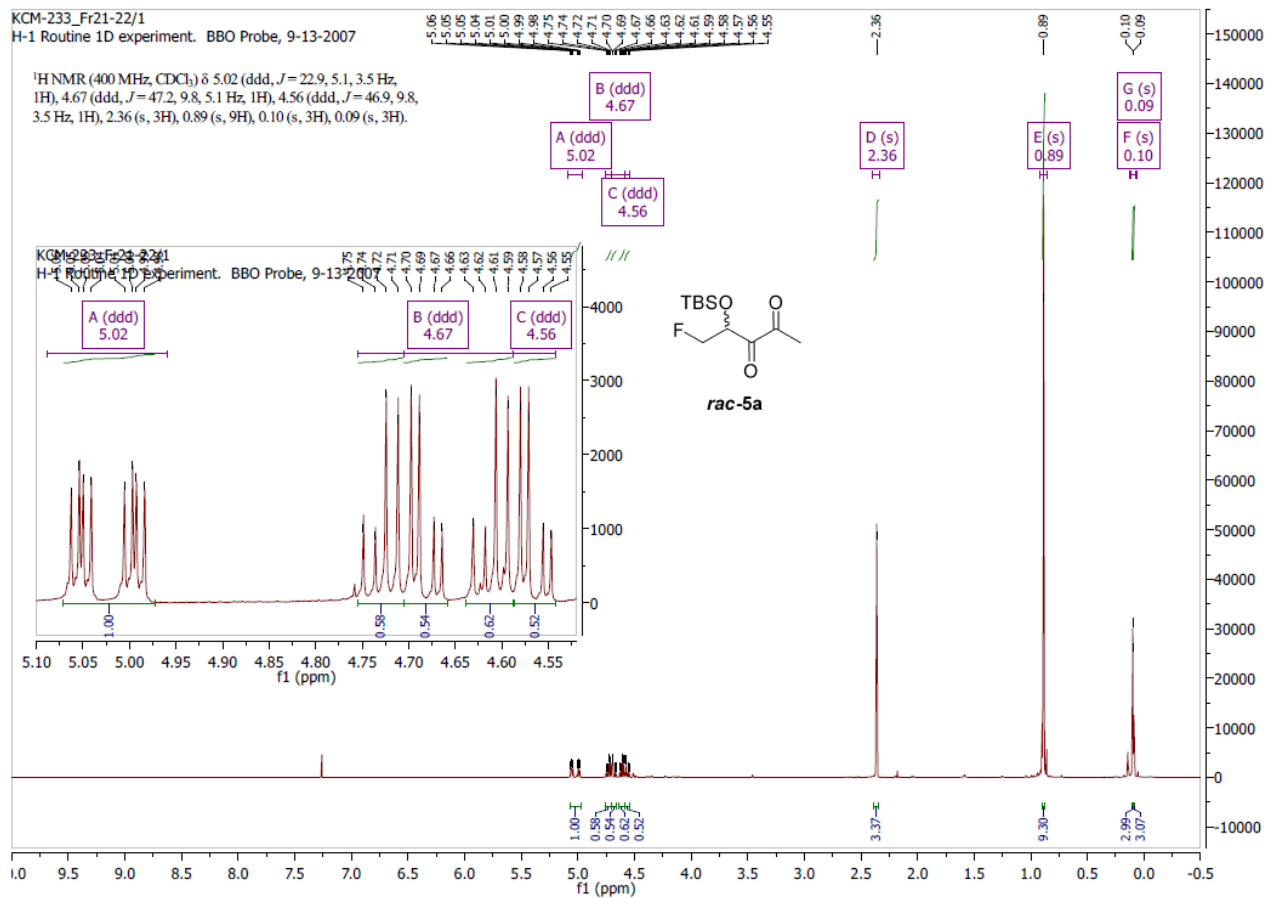
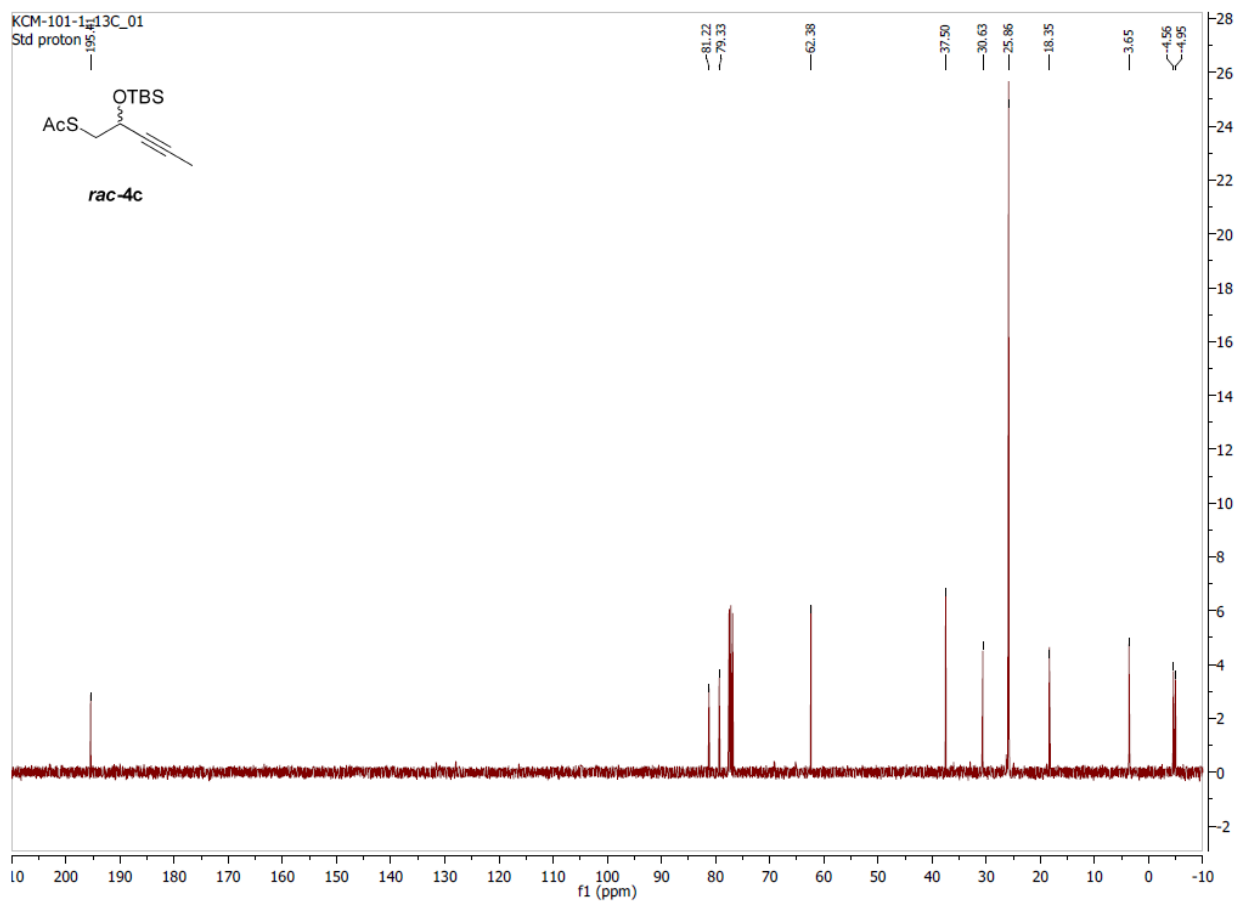
KCM-096-1_13C/1
 C-13-APT, BBO Probe, DRX-500, using deptq-135 pulse, 5-2-05



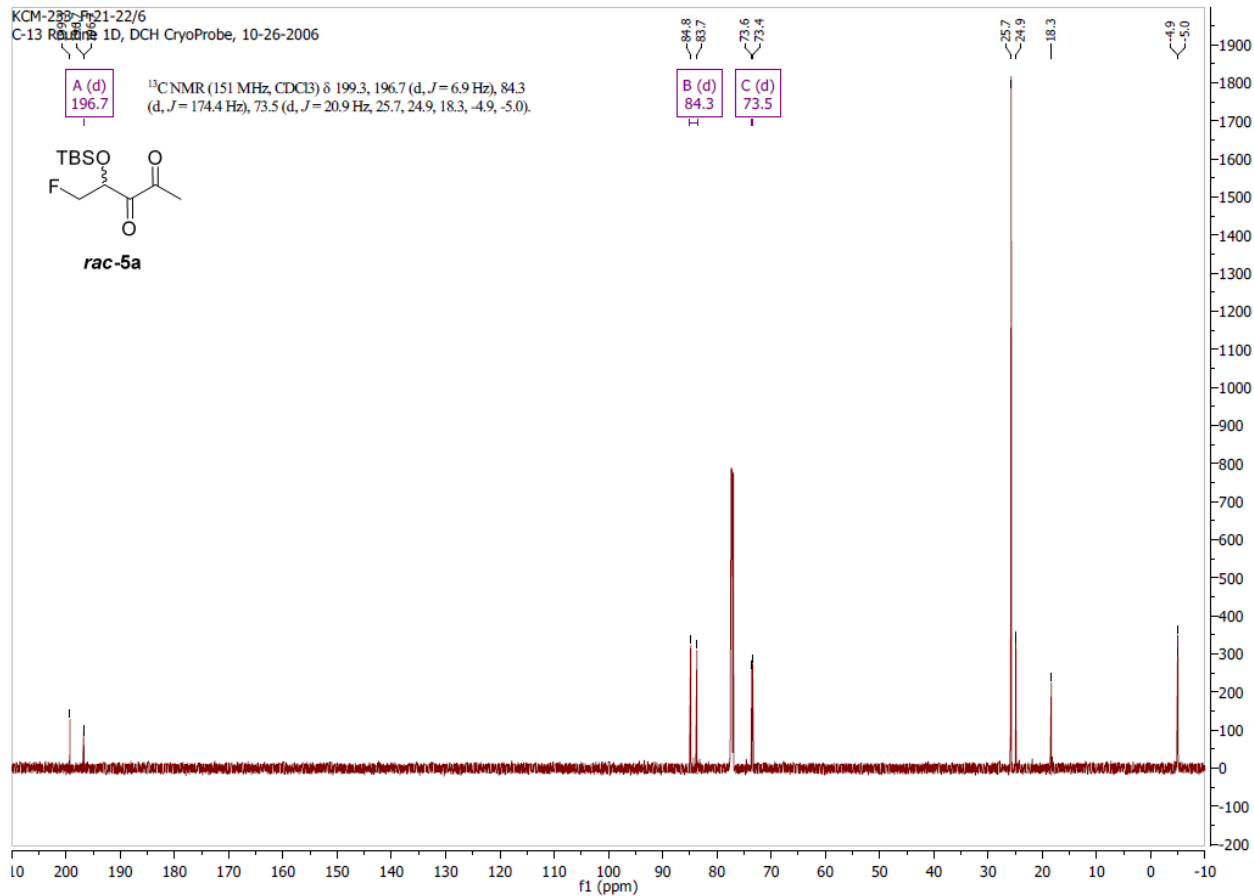
KCM-101-1_01
 Std proton

¹H NMR (400 MHz, cdcl₃) δ 4.39-4.34 (m, 1H), 3.15 (dd, *J* = 13.4, 5.9 Hz, 1H), 3.09 (dd, *J* = 13.4, 7.0 Hz, 1H), 2.32 (s, 3H), 1.81 (d, *J* = 2.1 Hz, 3H), 0.89 (s, 9H), 0.11 (s, 3H), 0.09 (s, 3H).

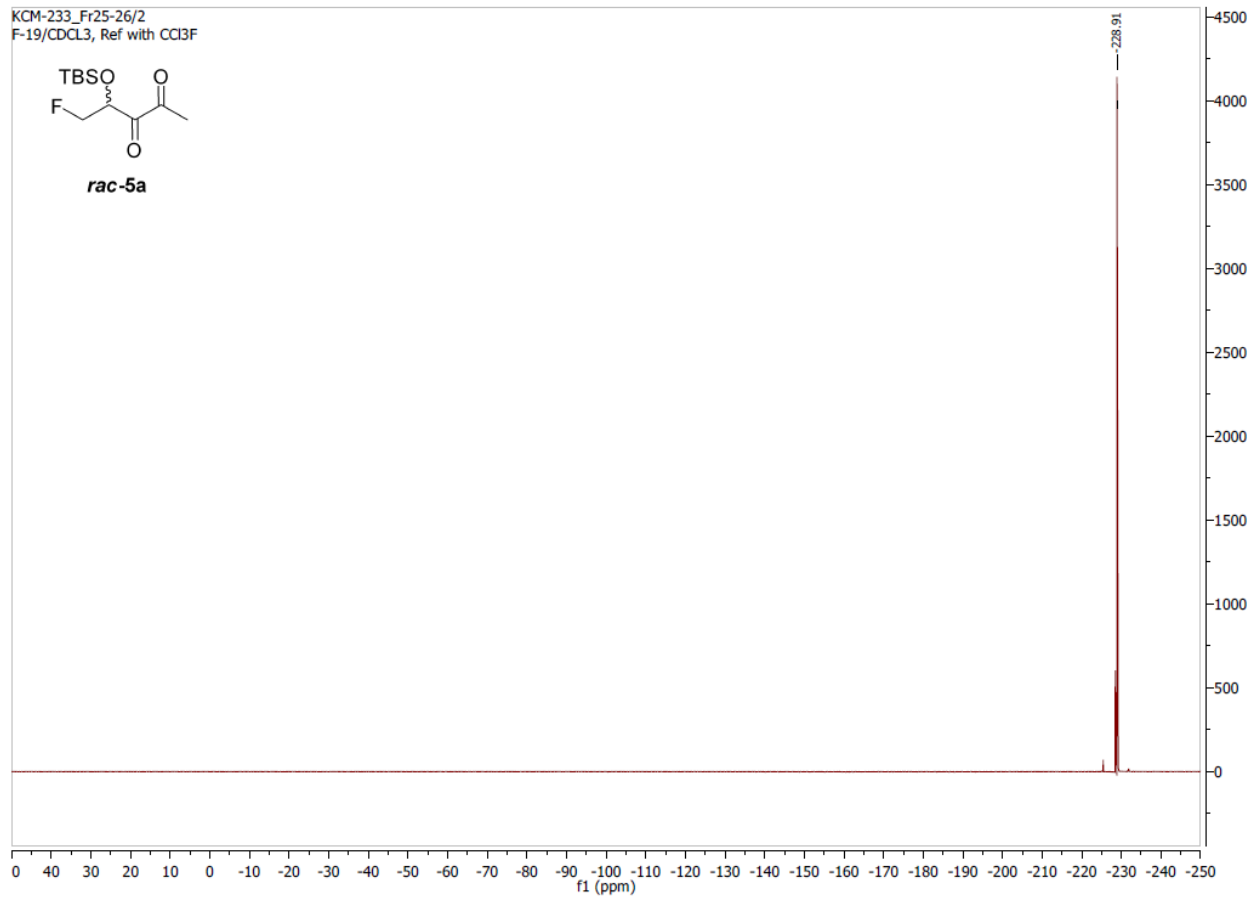




KCM-233_Fr21-22/6
C-13 Routine 1D, DCH CryoProbe, 10-26-2006



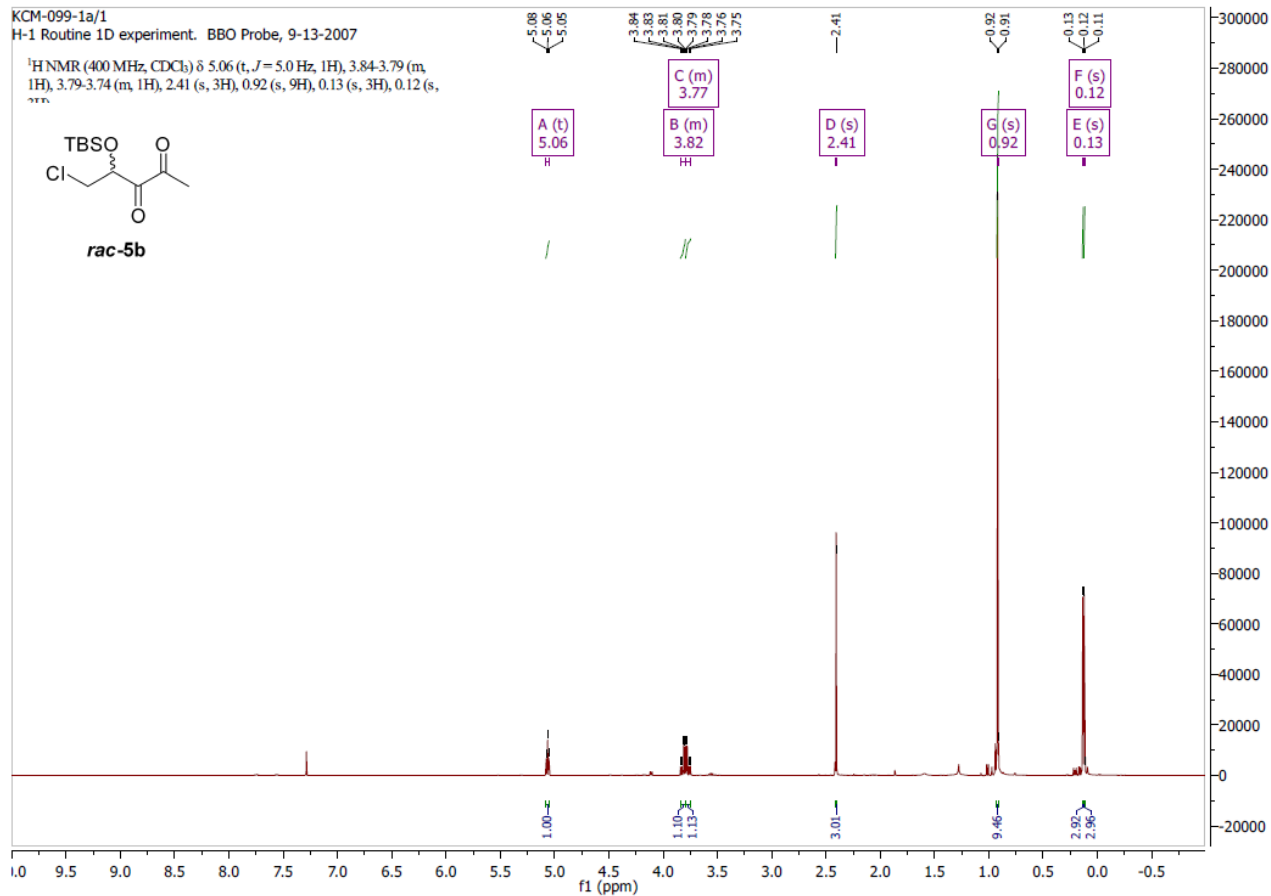
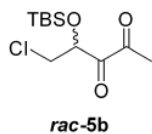
KCM-233_Fr25-26/2
F-19/ CDCl_3 , Ref with CCl_3F



KCM-099-1a/1

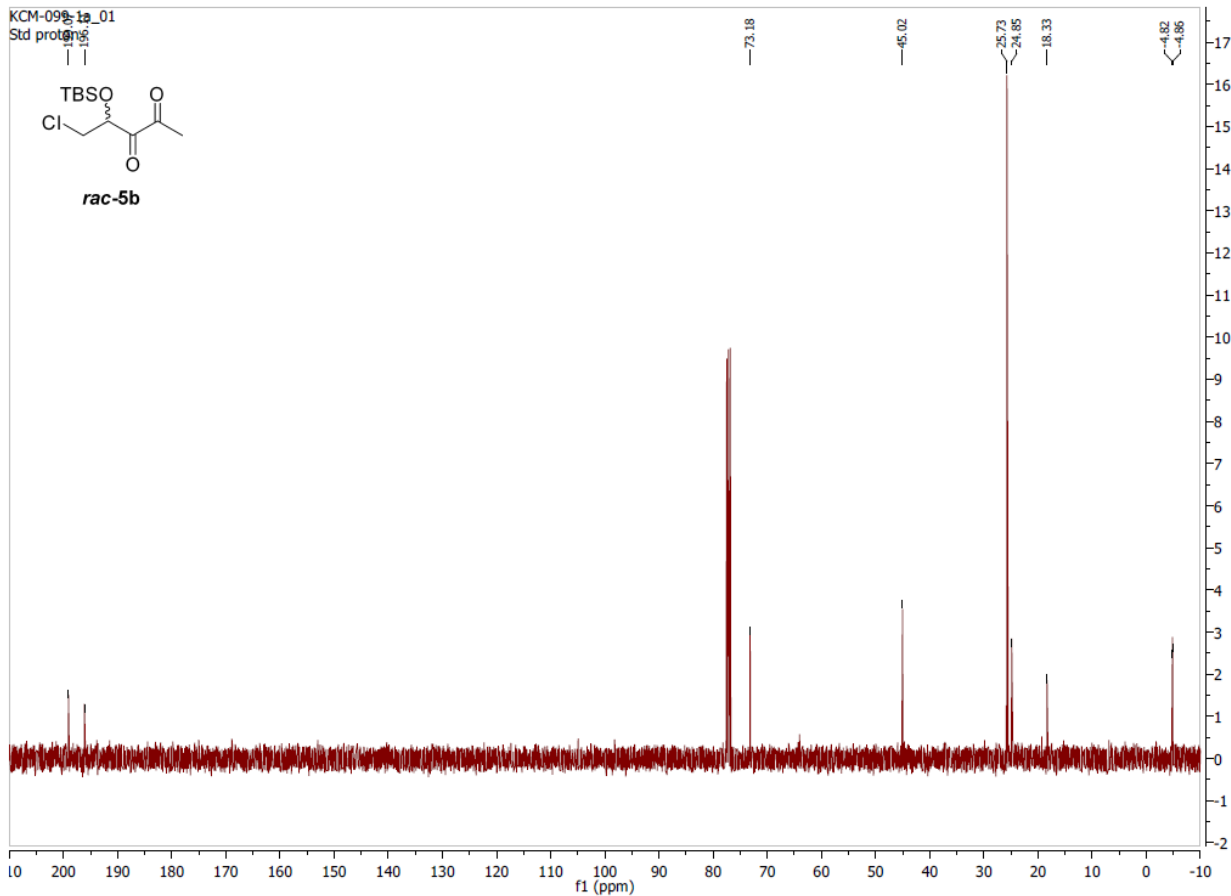
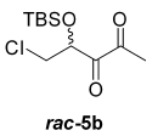
H-1 Routine 1D experiment. BBO Probe, 9-13-2007

^1H NMR (400 MHz, CDCl_3) δ 5.06 (t, $J = 5.0$ Hz, 1H), 3.84-3.79 (m, 1H), 3.79-3.74 (m, 1H), 2.41 (s, 3H), 0.92 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H).



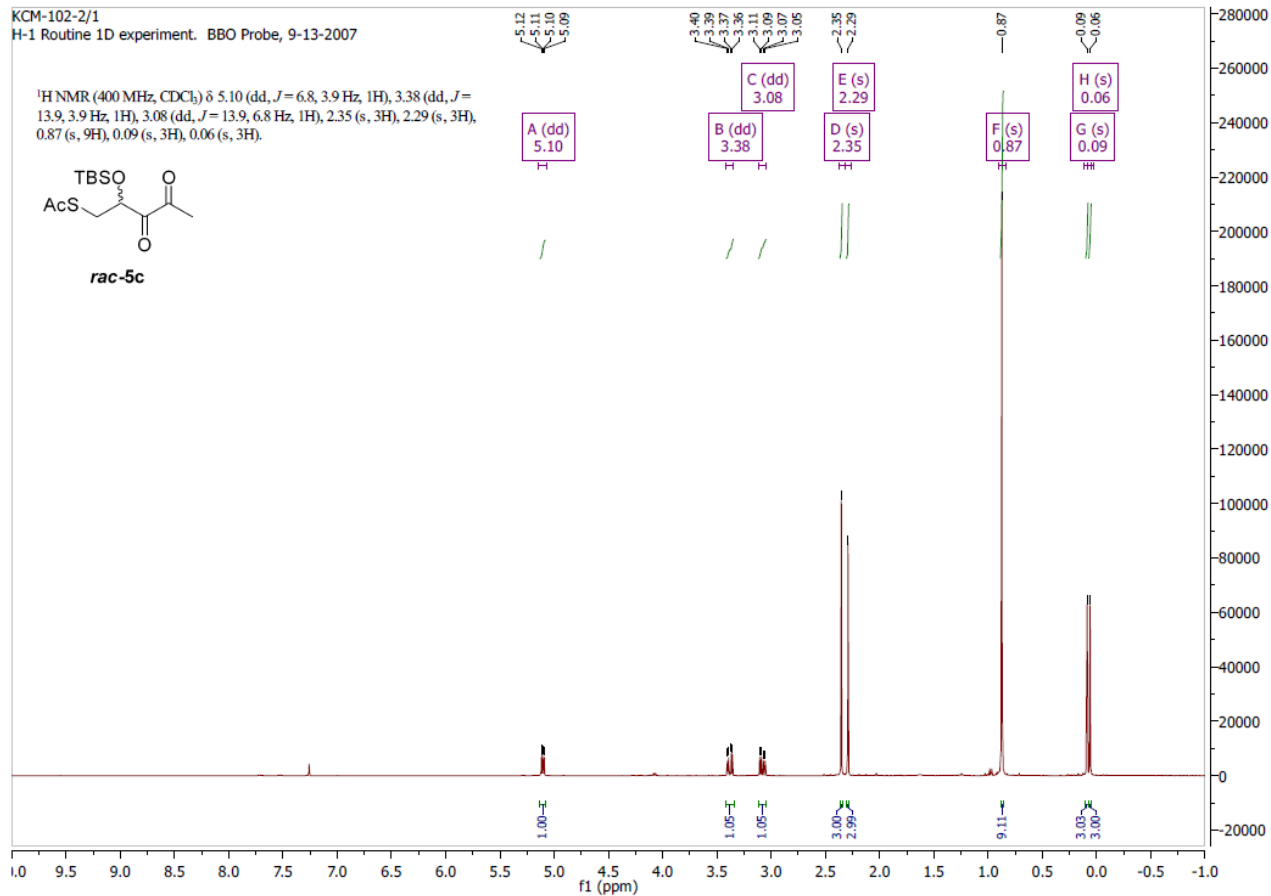
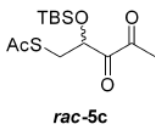
KCM-099-1a_01

Std protngs

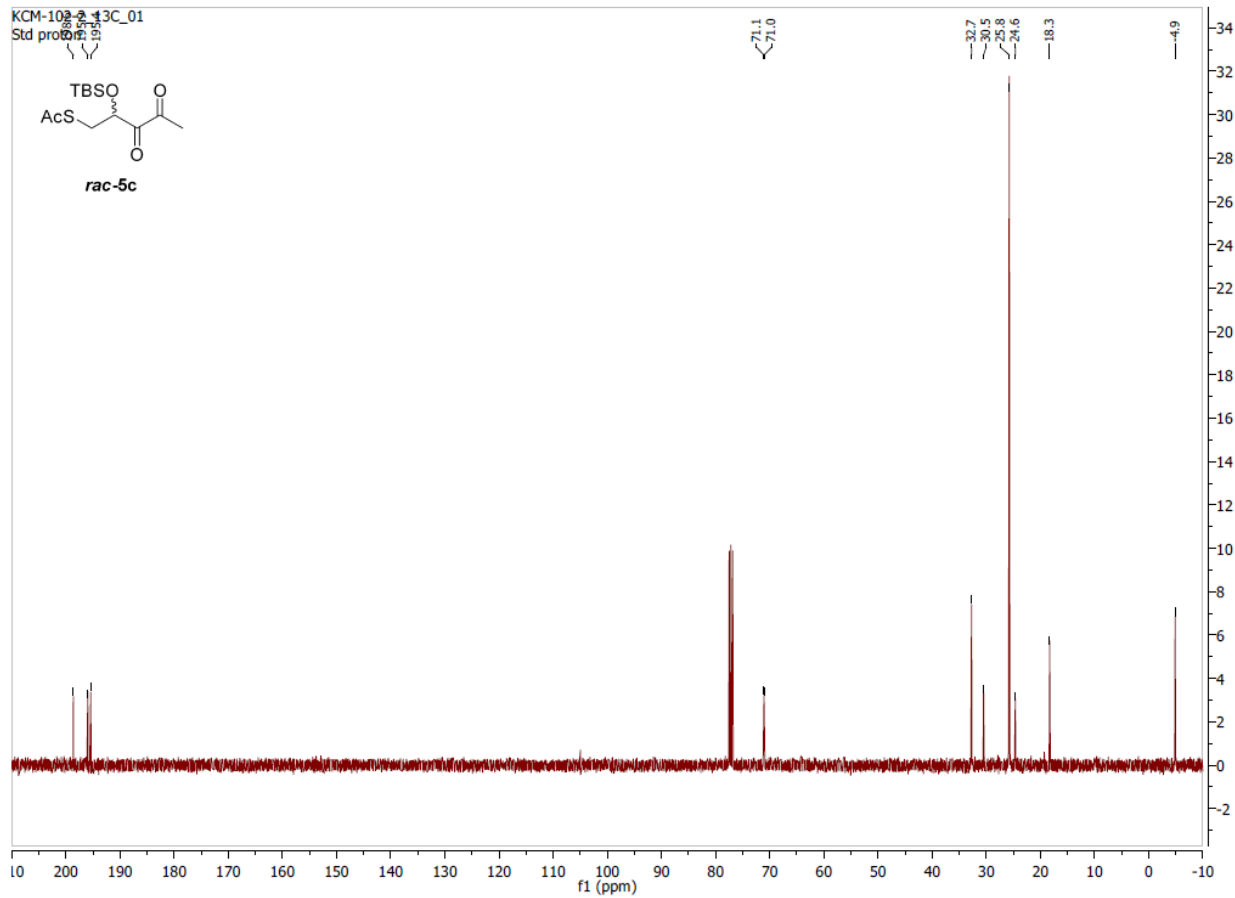
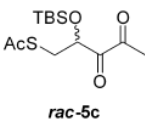


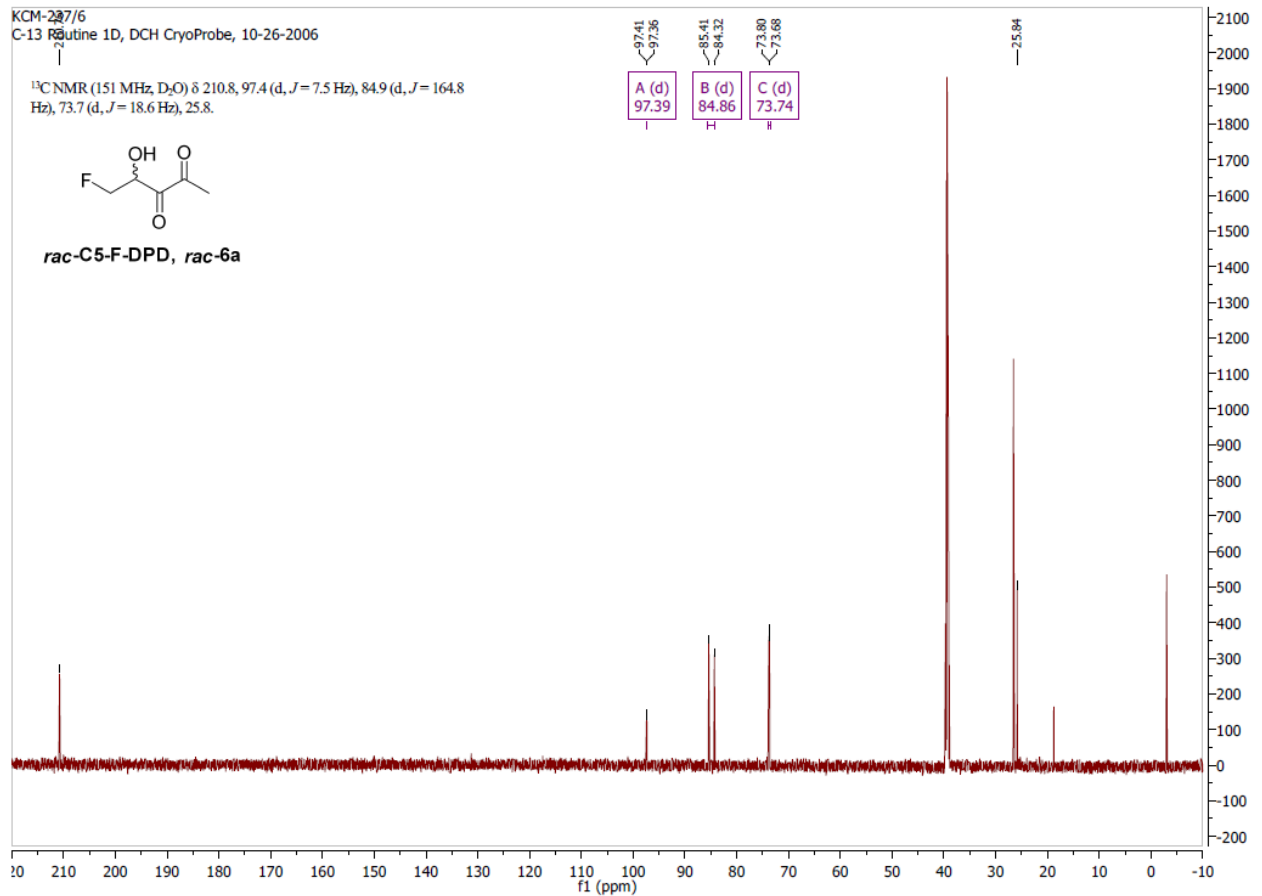
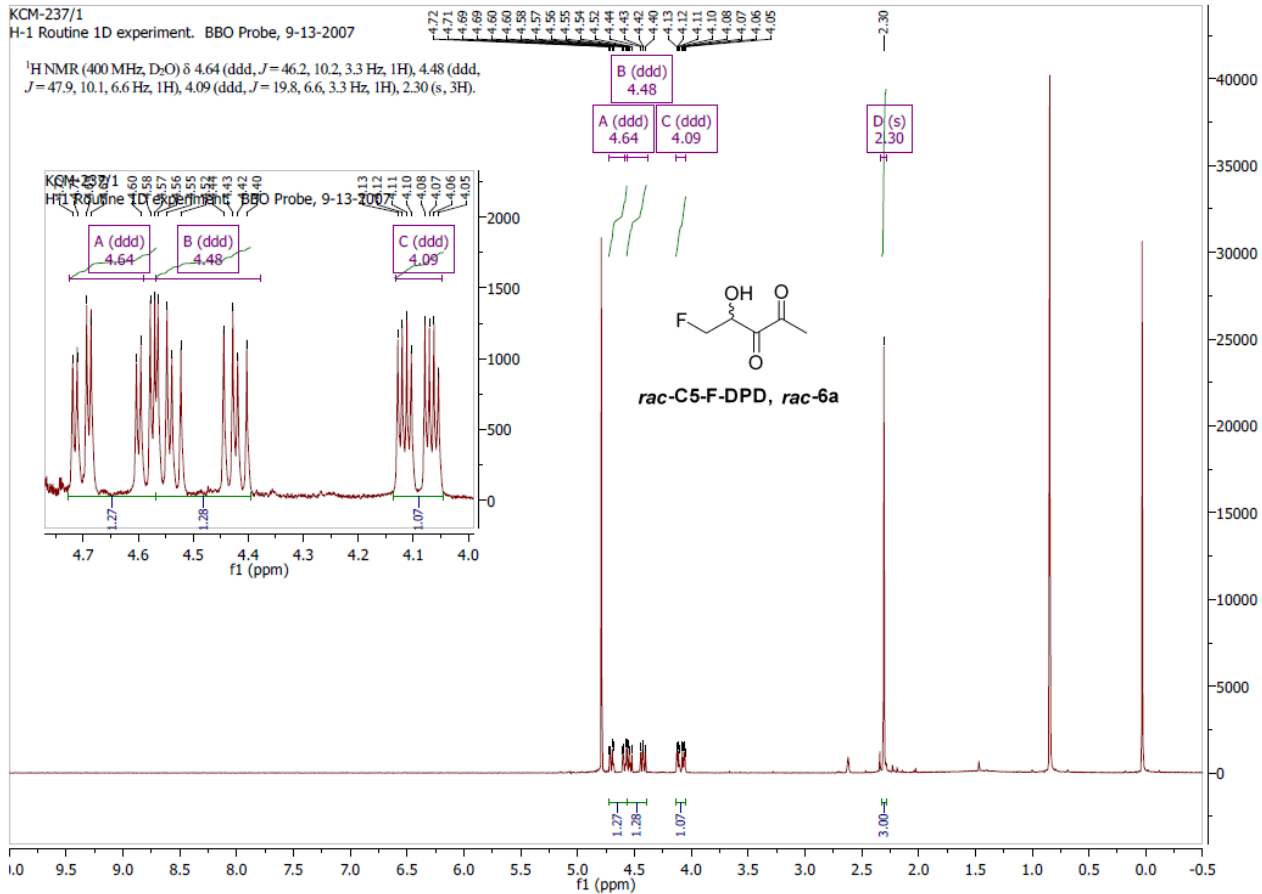
KCM-102-2/1
H-1 Routine 1D experiment. BBO Probe, 9-13-2007

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.10 (dd, $J=6.8, 3.9$ Hz, 1H), 3.38 (dd, $J=13.9, 3.9$ Hz, 1H), 3.08 (dd, $J=13.9, 6.8$ Hz, 1H), 2.35 (s, 3H), 2.29 (s, 3H), 0.87 (s, 9H), 0.09 (s, 3H), 0.06 (s, 3H).

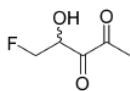


KCM-102-2-13C_01
Std protob

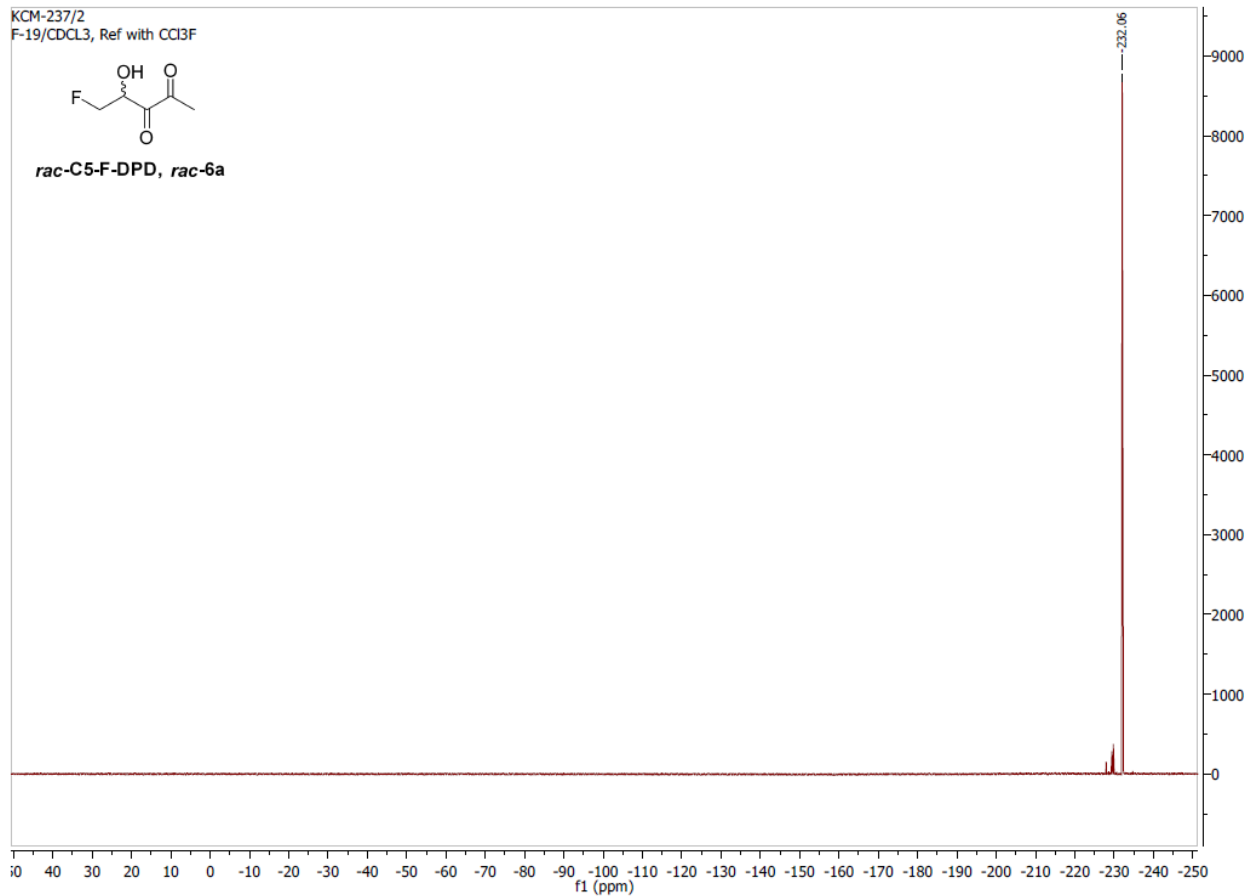




KCM-237/2
F-19/CDCl₃, Ref with CCl₃F

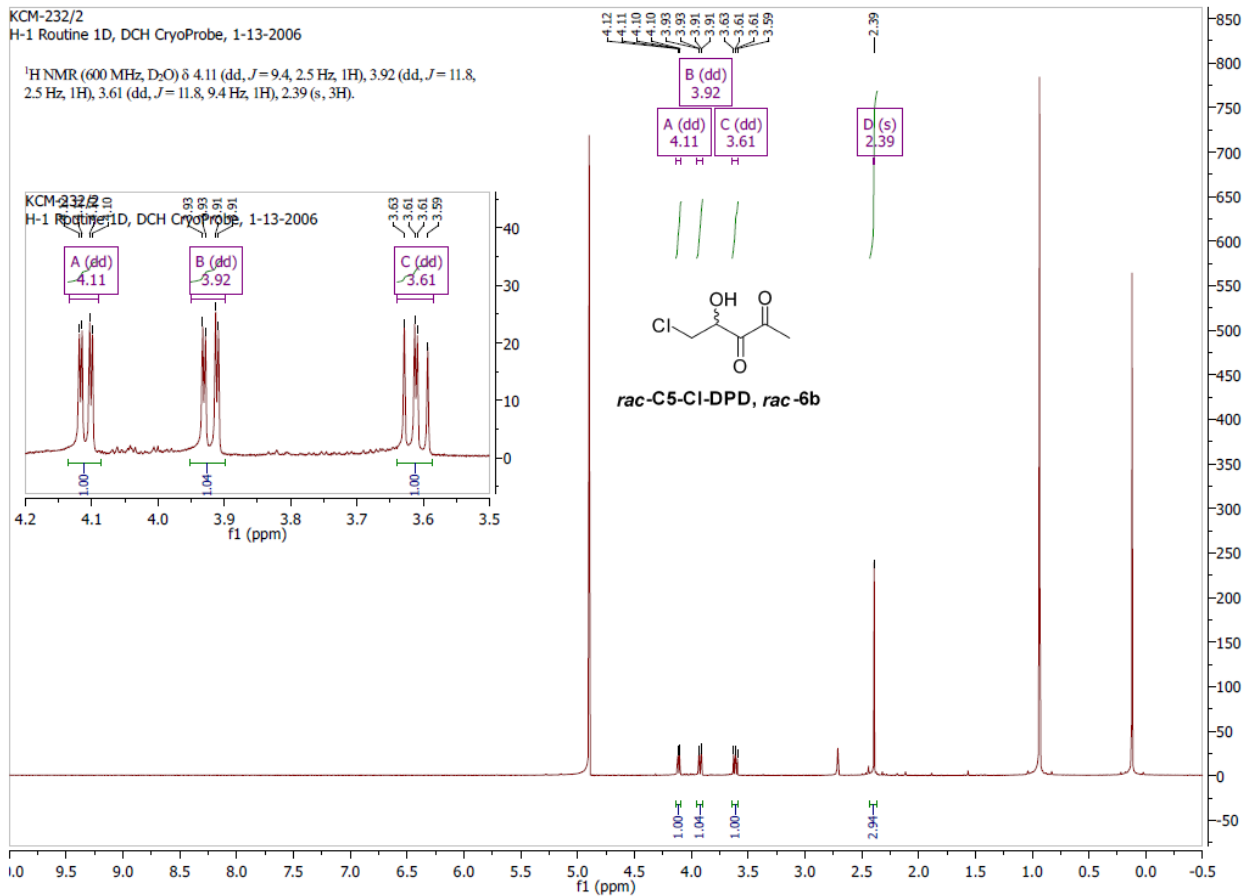


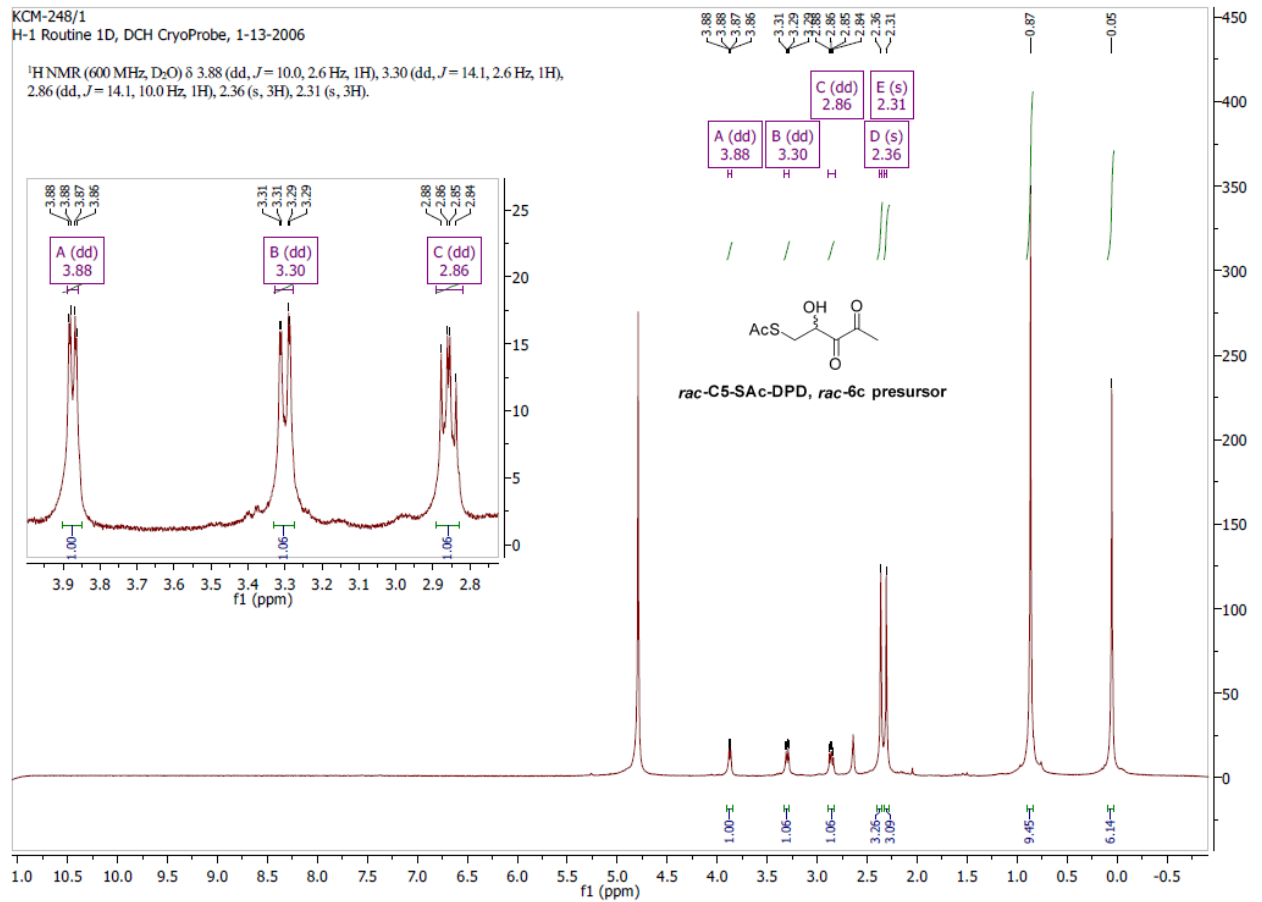
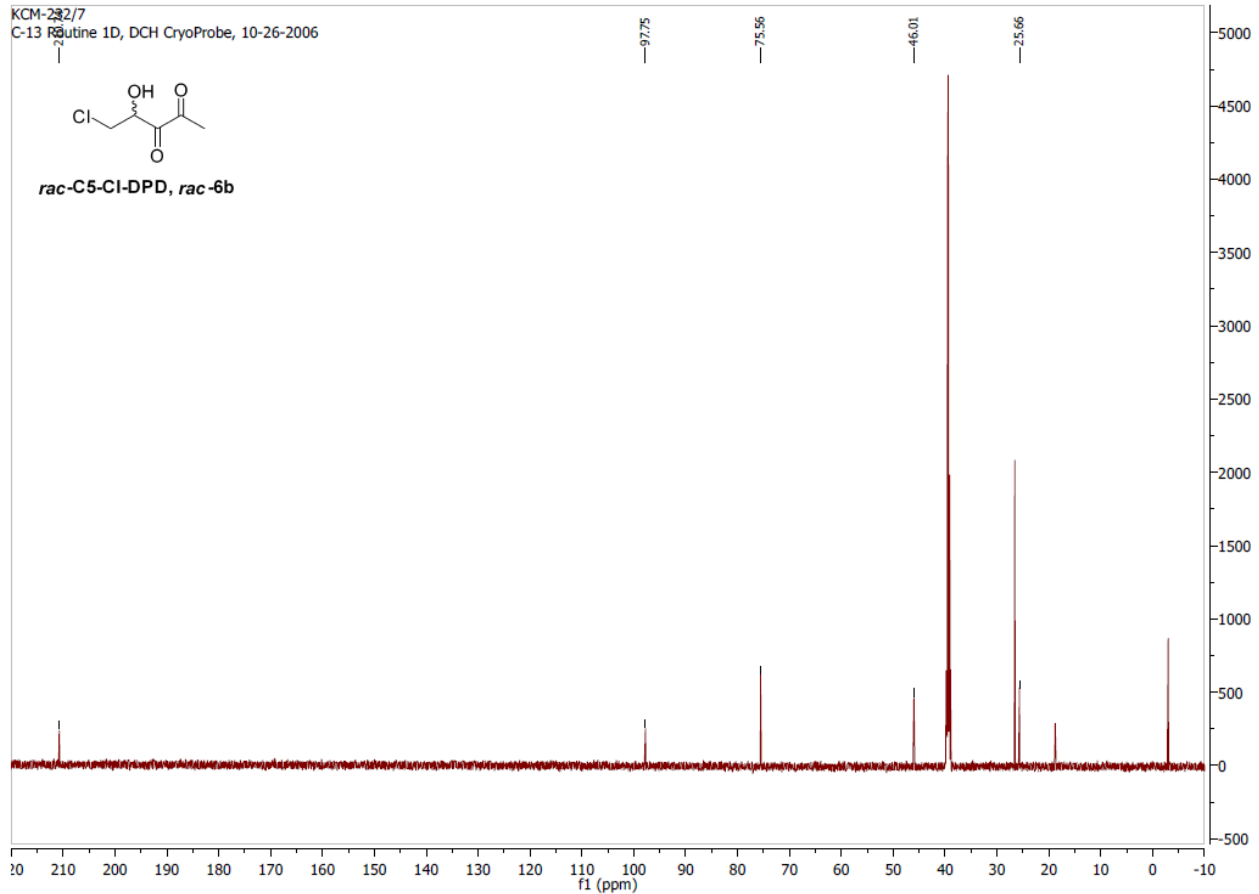
rac-C5-F-DPD, *rac*-6a

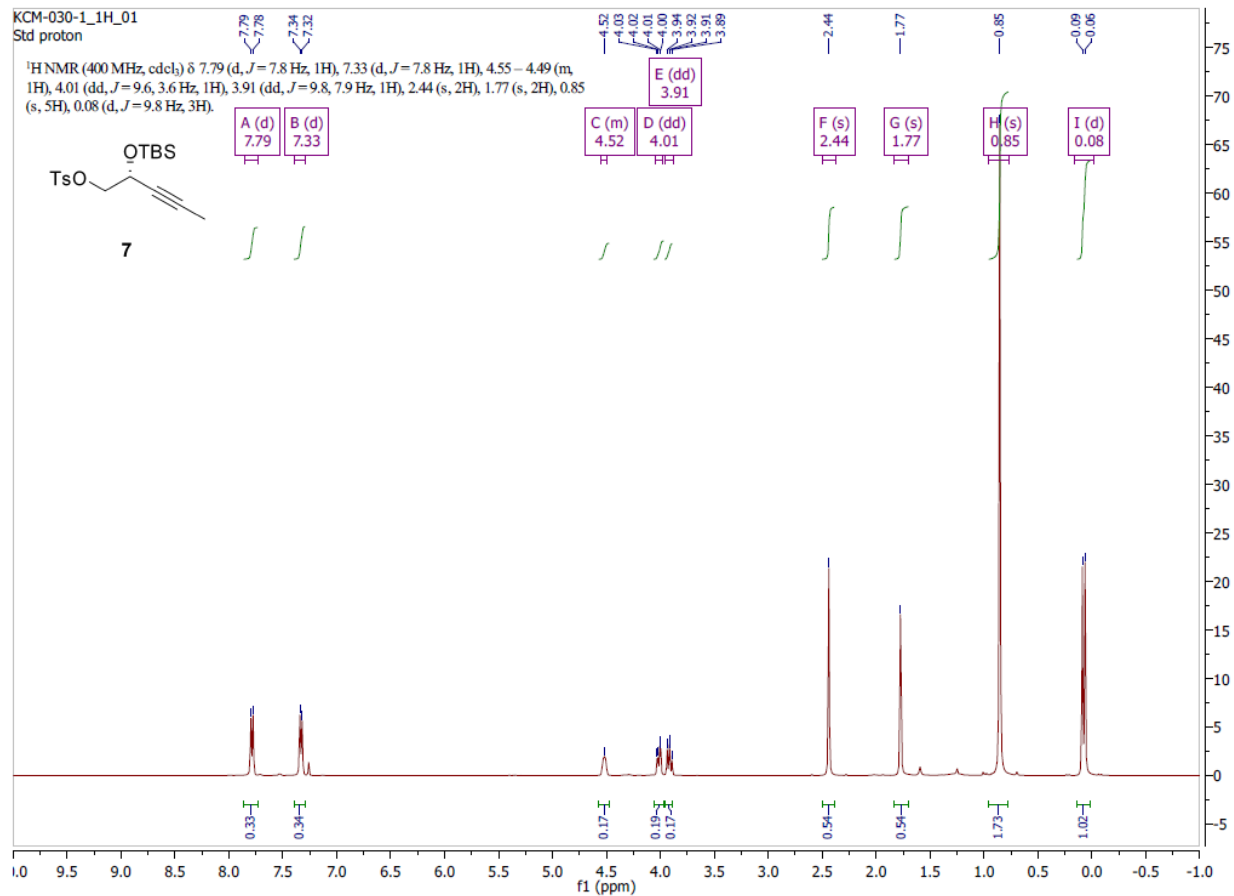
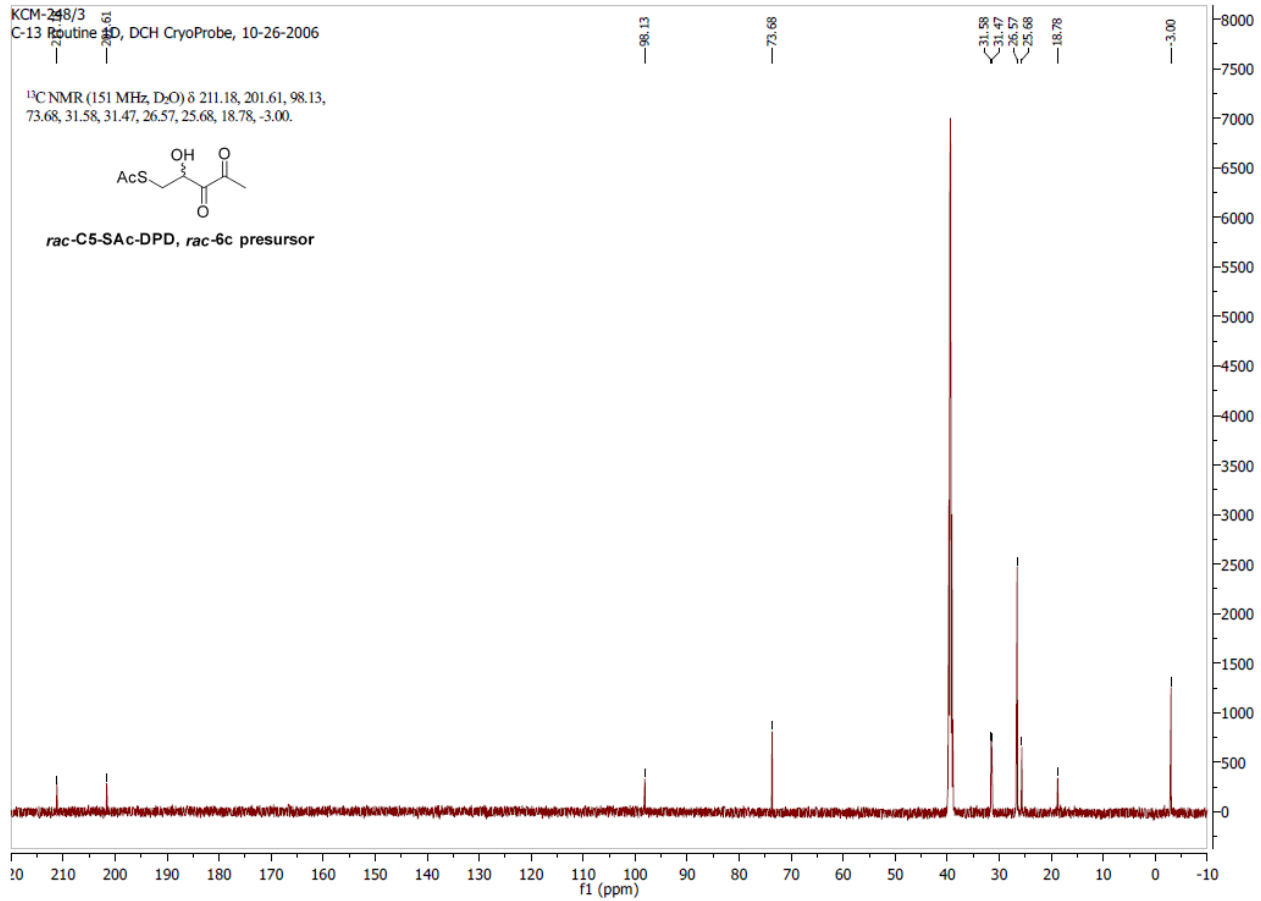


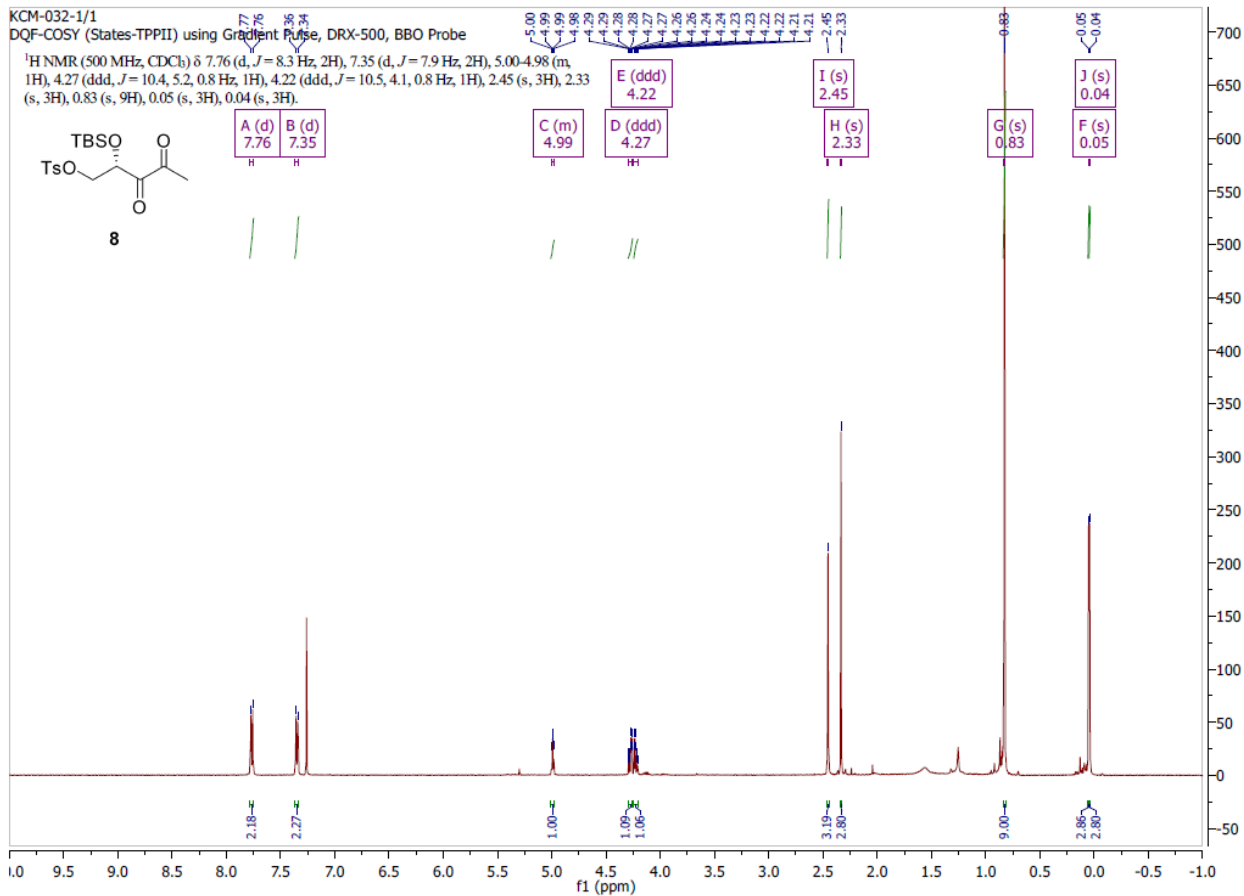
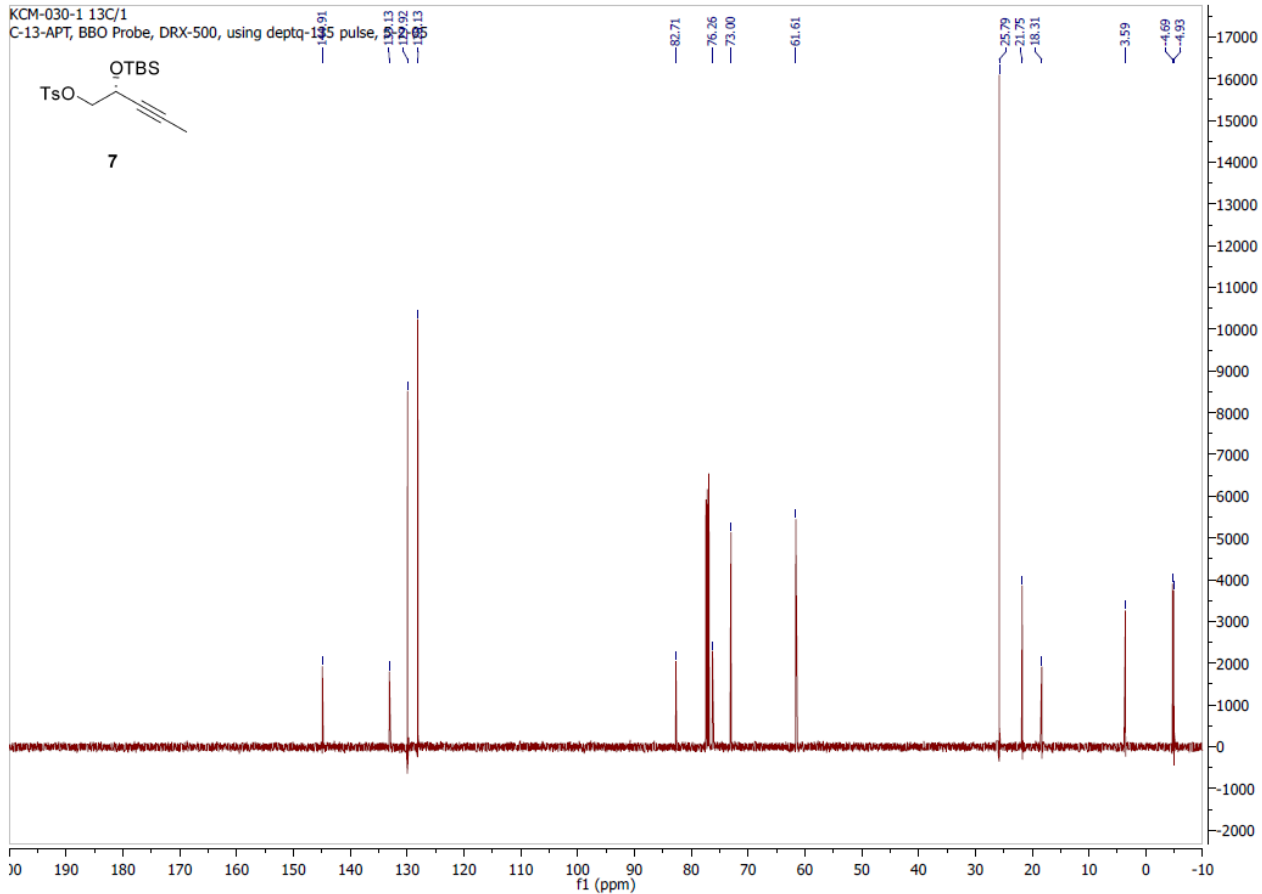
KCM-232/2
H-1 Routine 1D, DCH CryoProbe, 1-13-2006

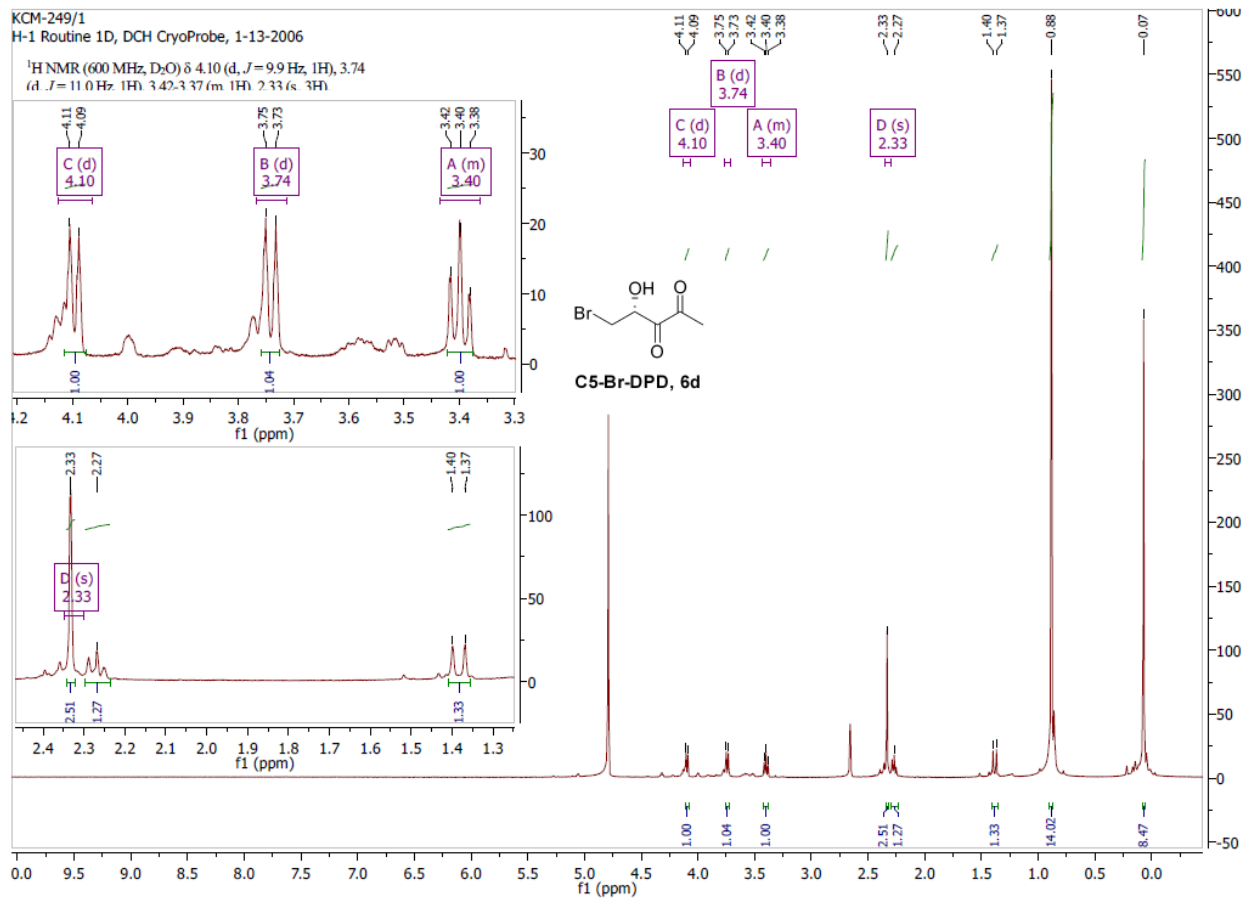
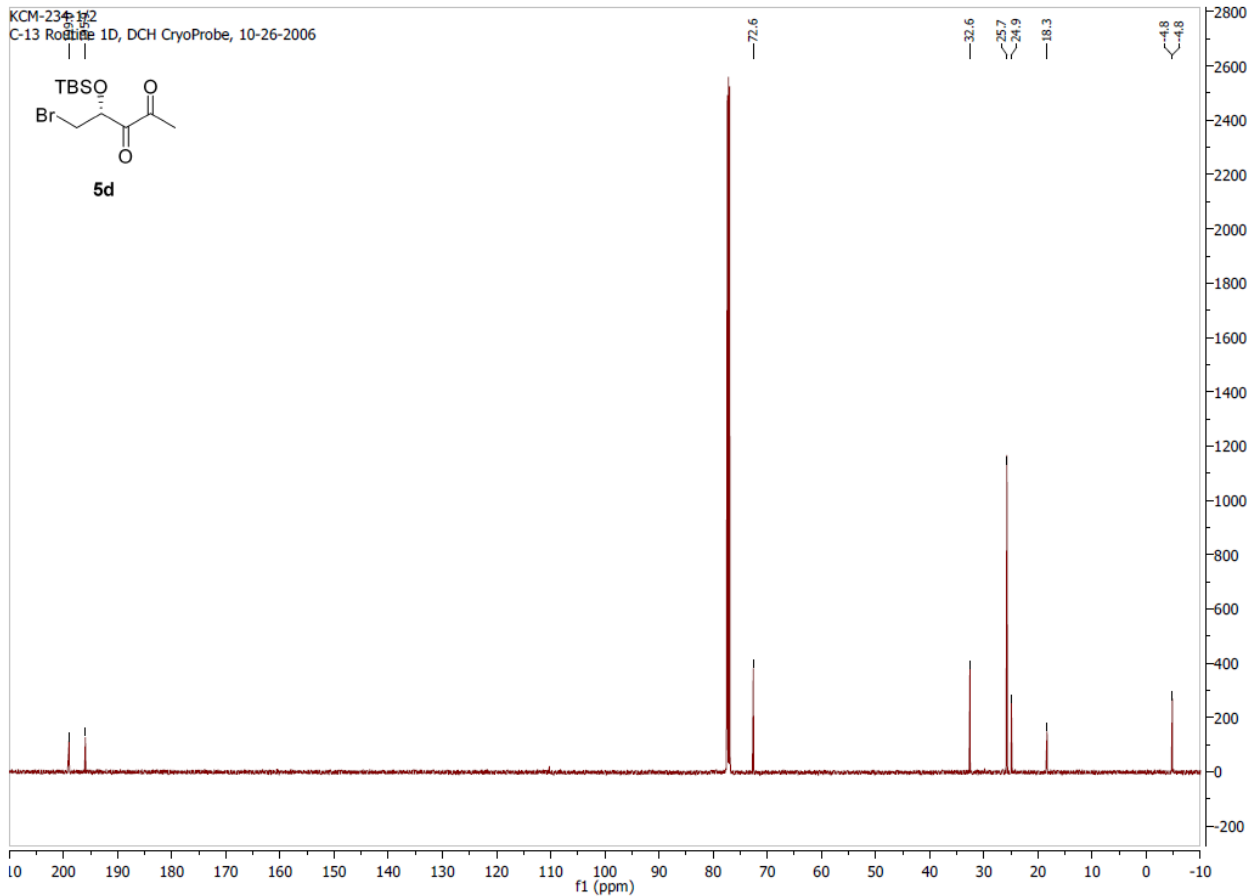
¹H NMR (600 MHz, D₂O) δ 4.11 (dd, *J* = 9.4, 2.5 Hz, 1H), 3.92 (dd, *J* = 11.8, 2.5 Hz, 1H), 3.61 (dd, *J* = 11.8, 9.4 Hz, 1H), 2.39 (s, 3H).

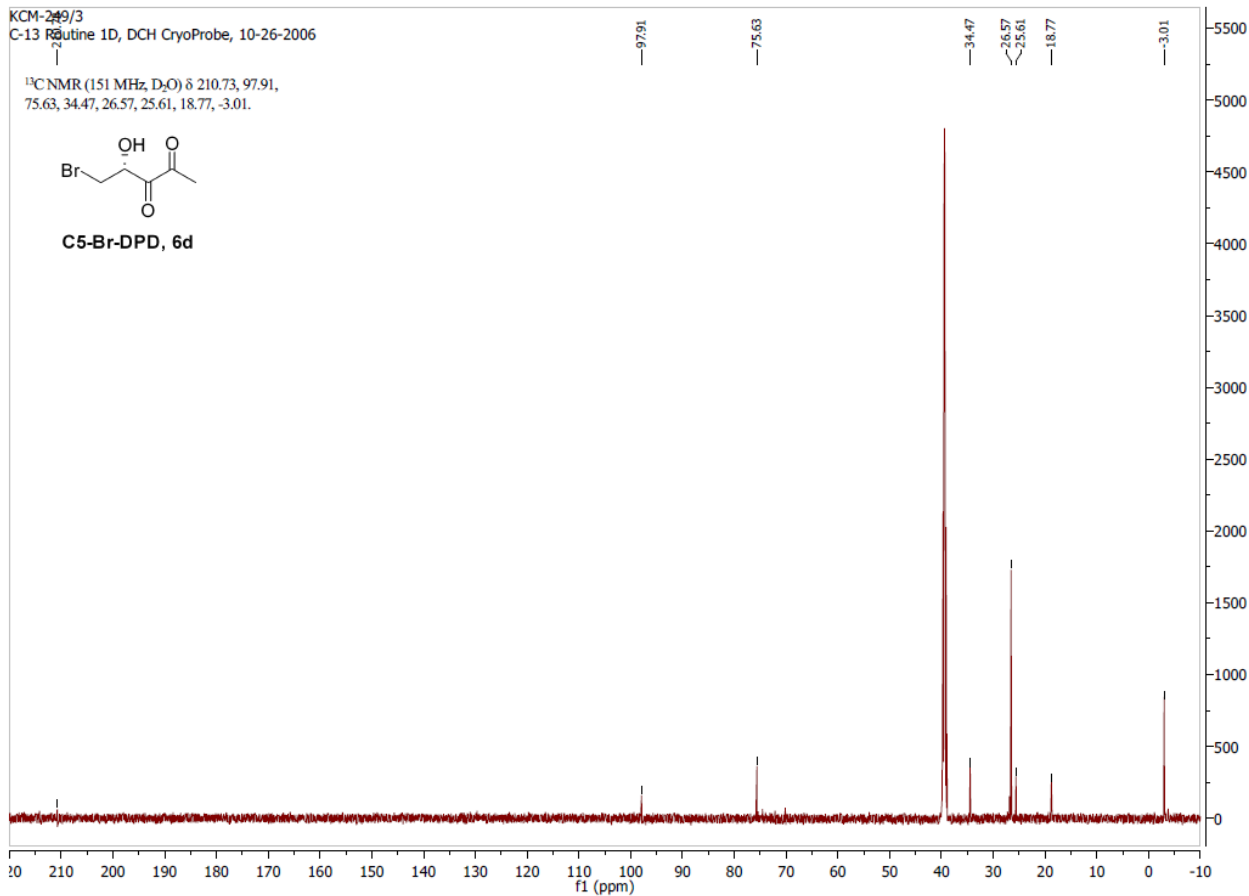












References

1. Gómez-Bombarelli, R.; González-Pérez, M.; Pérez-Prior, M. T.; Calle, E.; Casado, J. J. *Phys. Chem. A* **2009**, *113*, 11423-11428.
2. Globisch, D.; Lowery, C. A.; McCague, K. C.; Janda, K. D. *Angew. Chem. Int. Ed.* **2012**, *51*, 4204-4208.
3. Guthrie, J. P. *J. Am. Chem. Soc.* **2000**, *122*, 5529-5538.