Synthesis of robalzotan, ebalzotan and rotigotine precursors *via* stereoselective multienzymatic cascade reduction of *α,β*-unsaturated aldehydes

Elisabetta Brenna,[†] Francesco G. Gatti,*[,†] Luciana Malpezzi,[†]
Daniela Monti,[‡] Fabio Parmeggiani,[†] and Alessandro Sacchetti*[,†]

[†] Dipartimento di Chimica, Materiali ed Ingegneria Chimica "G. Natta",
Politecnico di Milano, Milano, Italy

[‡] Istituto di Chimica del Riconoscimento Molecolare, C. N. R., Milano, Italy

SUPPORTING INFORMATION

Contents

* Corresponding author.
Address: Dipartimento di Chimica, Materiali ed Ingegneria Chimica "G. Natta", Politecnico di Milano, P.zza Leonardo da Vinci, 32, 20133, Milano, Italy. Telephone: +39 02 23993072. Fax: +39 02 23993080. E-mail: francesco.gatti@polimi.it, alessandro.sacchetti@polimi.it

Overexpression of OYEs and GDH in *E. coli* BL21 (DE3)

LB medium (5 mL) containing the appropriate antibiotic (50 μg mL^{-1} kanamycin for pET30a-OYE2 and pET30a-OYE3, 100 μg mL^{-1} ampicillin for pKTS-GDH) was inoculated with a single colony from a fresh plate and shaken overnight at 37°C and 220 rpm. This starter culture was used to inoculate 200 mL of medium, which was in turn shaken overnight at the same conditions and used to inoculate 1.5 L of medium. The latter was vigorously aerated at 37°C and 220 rpm until OD_{600} reached 0.4-0.5 and then enzyme expression was induced by the addition of 0.1 mM IPTG (50 ng mL^{-1} anhydrotetracycline was also added for the pKTS-GDH plasmid). After 5-6 h the cells were harvested by centrifugation (5000 *g*, 20 min, 4 °C), resuspended in 50 mL of lysis buffer (20 mM phosphate buffer pH 7.0, 300 mM NaCl, 10 mM imidazole) and disrupted by sonication (Omni Ruptor 250 W Ultrasonic Cell Disruptor, five sonication cycles, 15 s each, 50% duty). The cell-free extract, after centrifugation (20000 *g*, 20 min, 4°C), was chromatographed on an IMAC stationary phase (Ni-Sepharose Fast Flow, GE Healthcare) with a mobile phase composed of 20 mM phosphate buffer pH 7.0, 300 mM NaCl and a 10-300 mM imidazole gradient. Protein elution was monitored at 280 nm, the fractions were collected according to the chromatogram and dialyzed twice against 1.0 L of 20 mM phosphate buffer pH 7.0 (4°C) to remove imidazole and salts. Purified protein aliquots were stored frozen at –80°C.

HPLC chromatograms of the products of preparative scale bioreductions

Figure S1. HPLC chromatograms (Chiralcel OD) of *rac*-**5c** and of (*S*)-**5c** obtained from cascade bioreduction with OYE2 and HLADH.

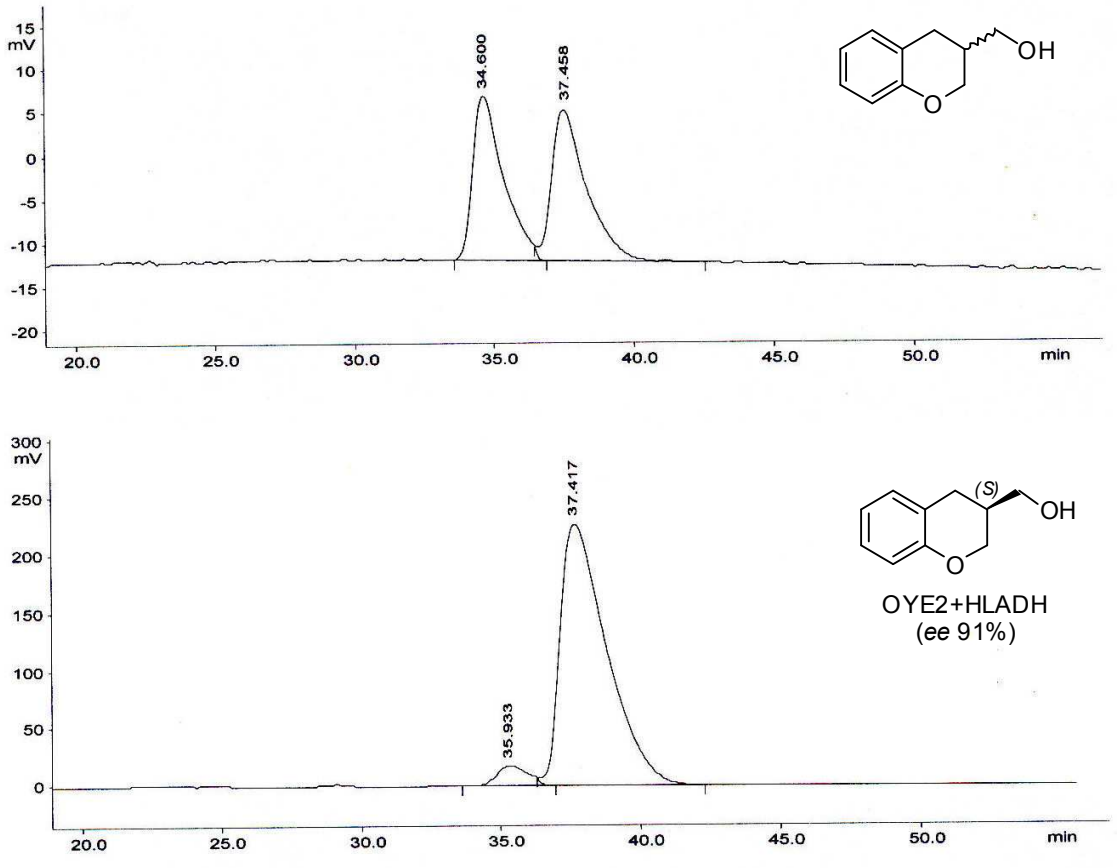
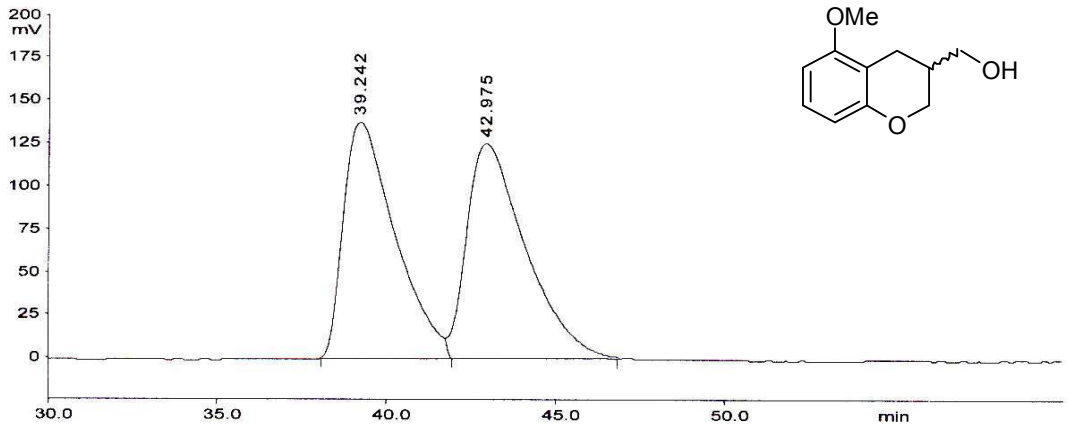
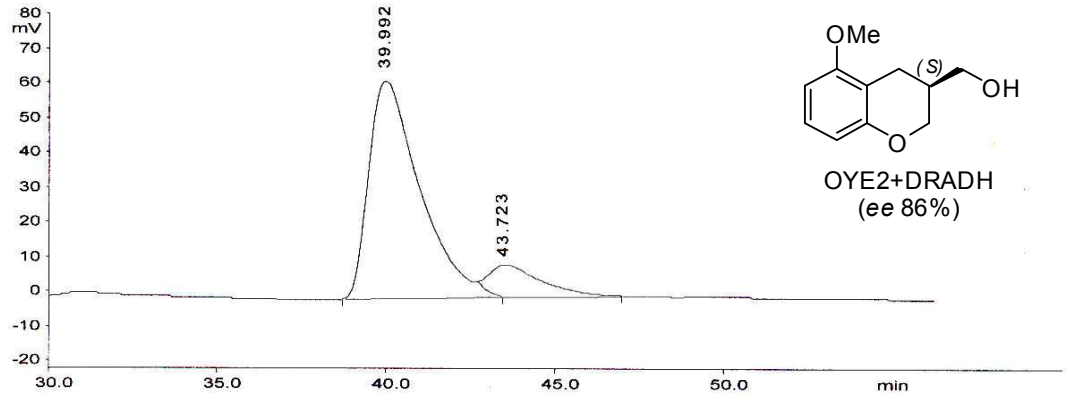


Figure S2. HPLC chromatograms (Chiralcel OD) of *rac*-**6c** and of (*S*)-**6c** obtained from cascade bioreduction with OYE2 and either DRADH or HLADH.





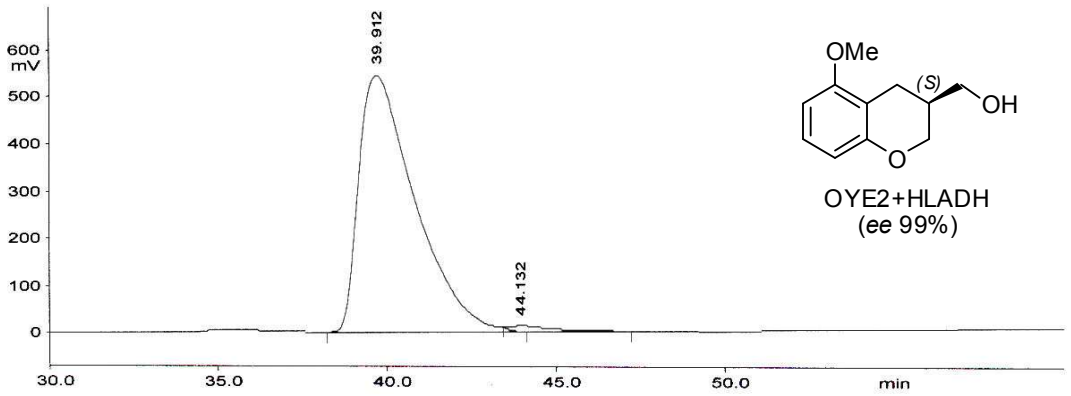


Figure S3. HPLC chromatograms (Chiralcel OD) of *rac*-**8c** and of (*S*)-**8c** obtained from cascade bioreduction with OYE2 and HLADH.

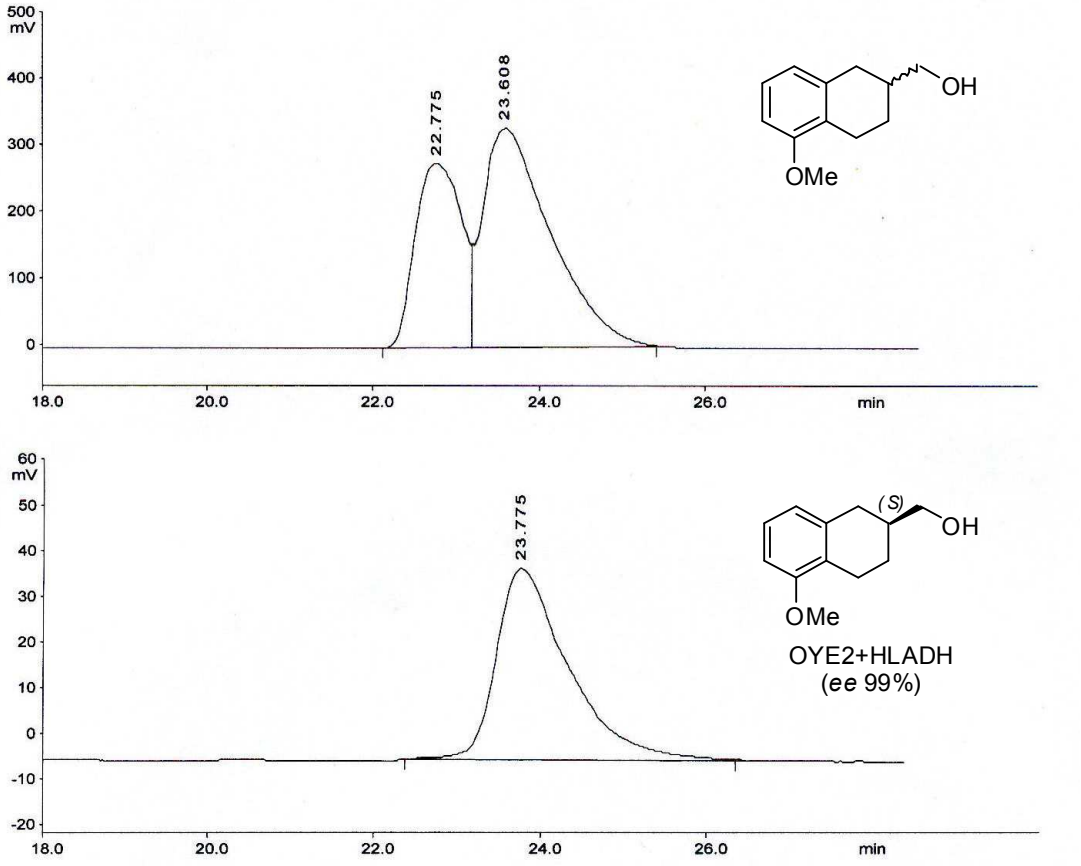


Figure S4. HPLC chromatograms (Lux 5u Cellulose-3) of *rac*-**7c** (*anti/syn* ratio 6:4), of (1*S*,3'*R*)-**7c** obtained from one-pot sequential bioreduction with OYE2 and CPADH (followed by recrystallization), and of (1*R*,3'*R*)-**7c** obtained from one-pot sequential bioreduction with OYE2 and KRED (followed by recrystallization).

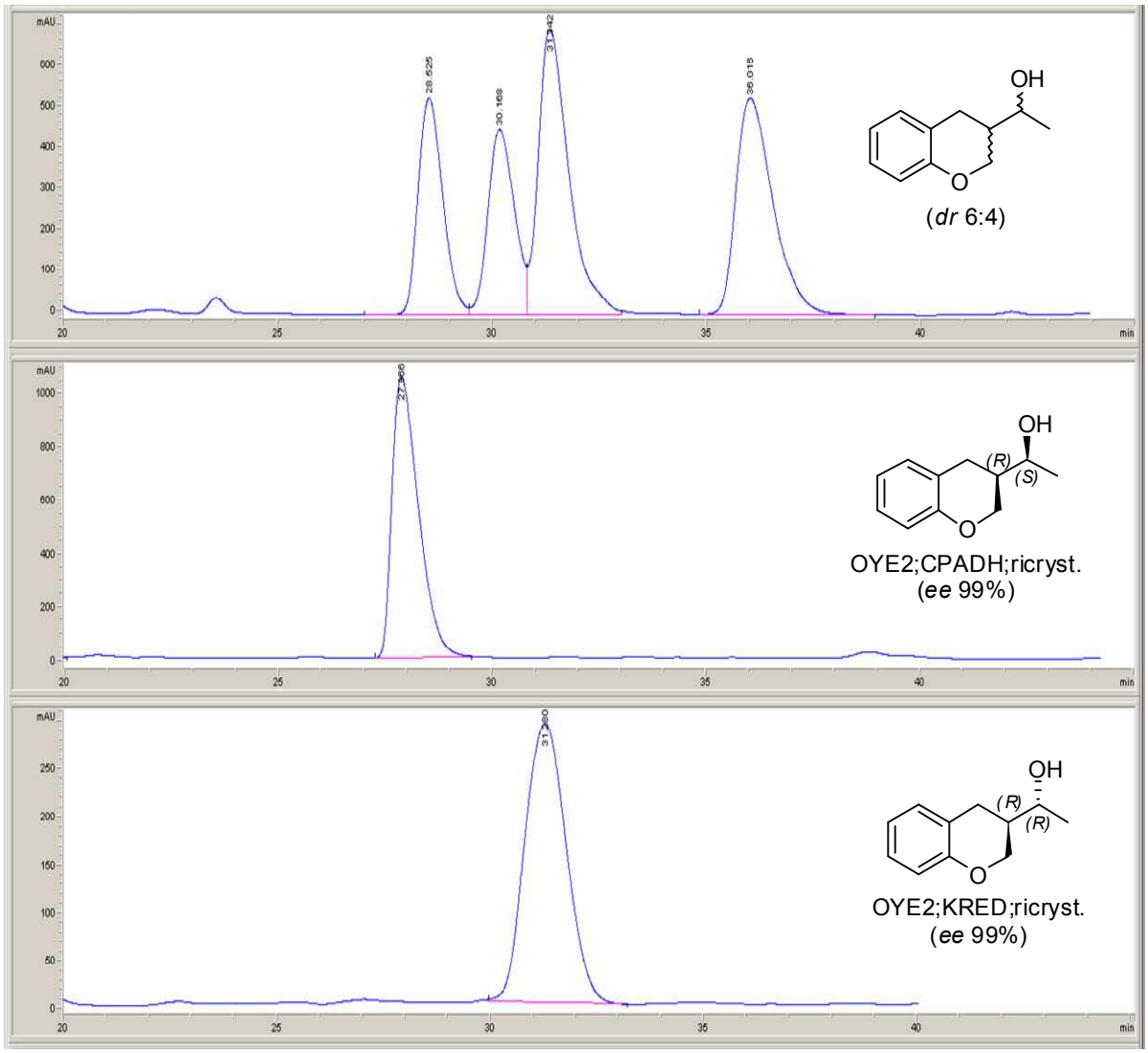
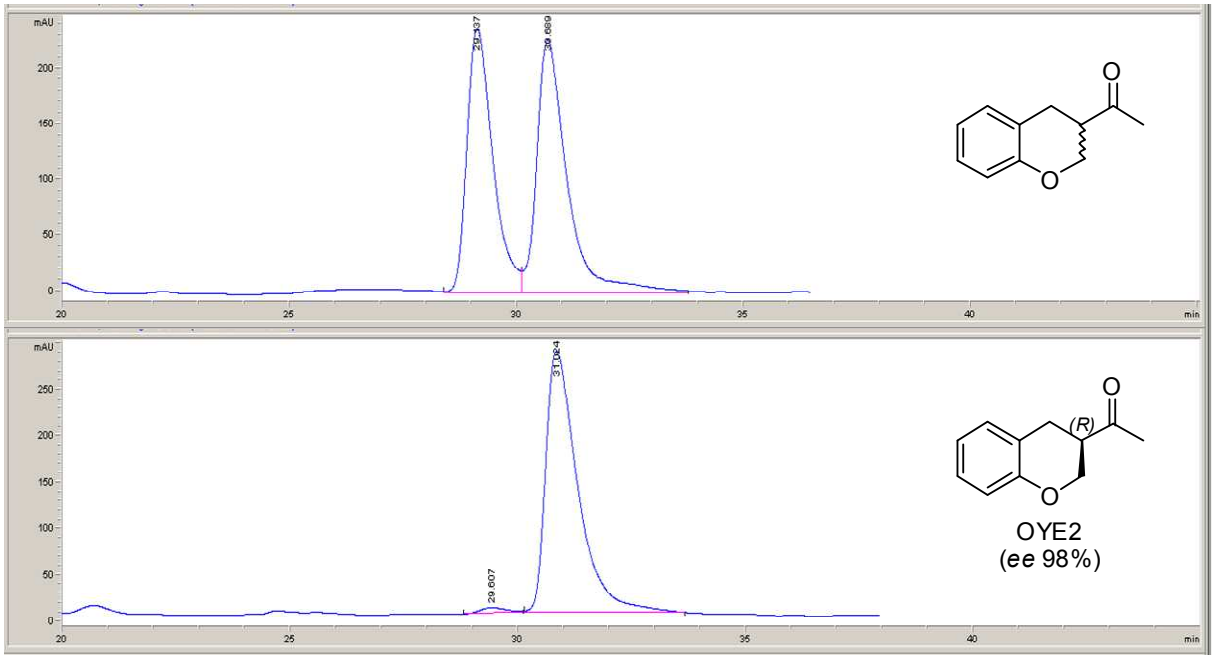
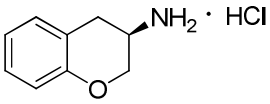


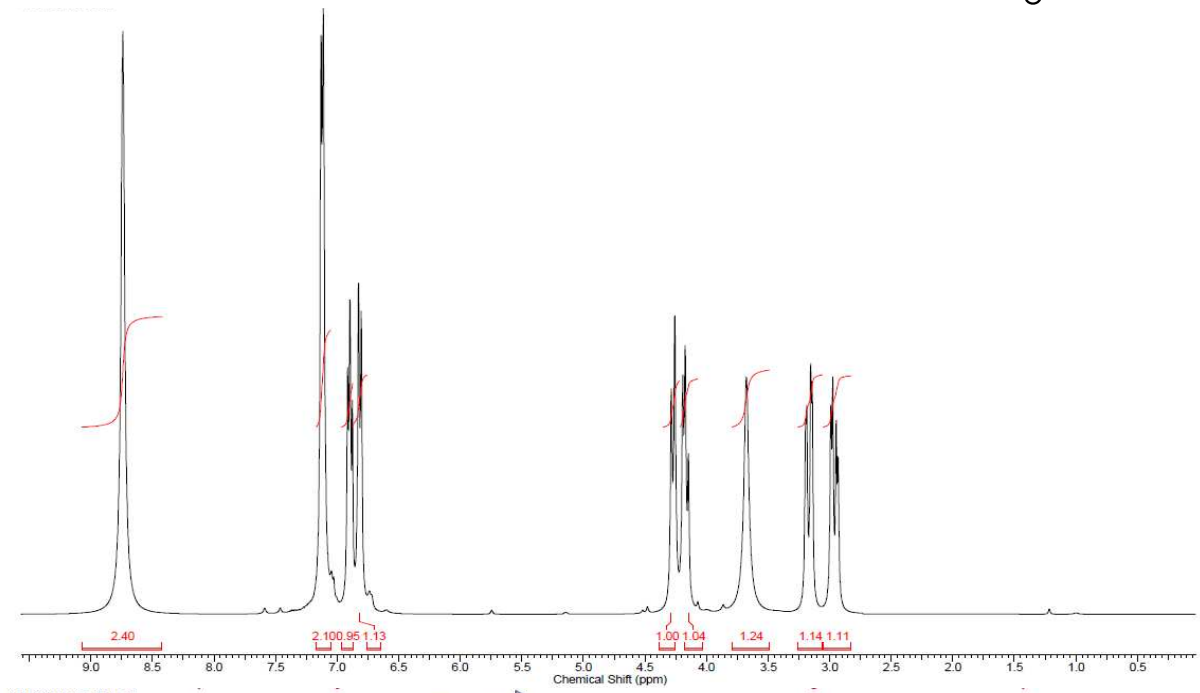
Figure S5. HPLC chromatograms (Lux 5u Cellulose-3) of *rac*-**7b** and of (*R*)-**7b** obtained from bioreduction with OYE2.

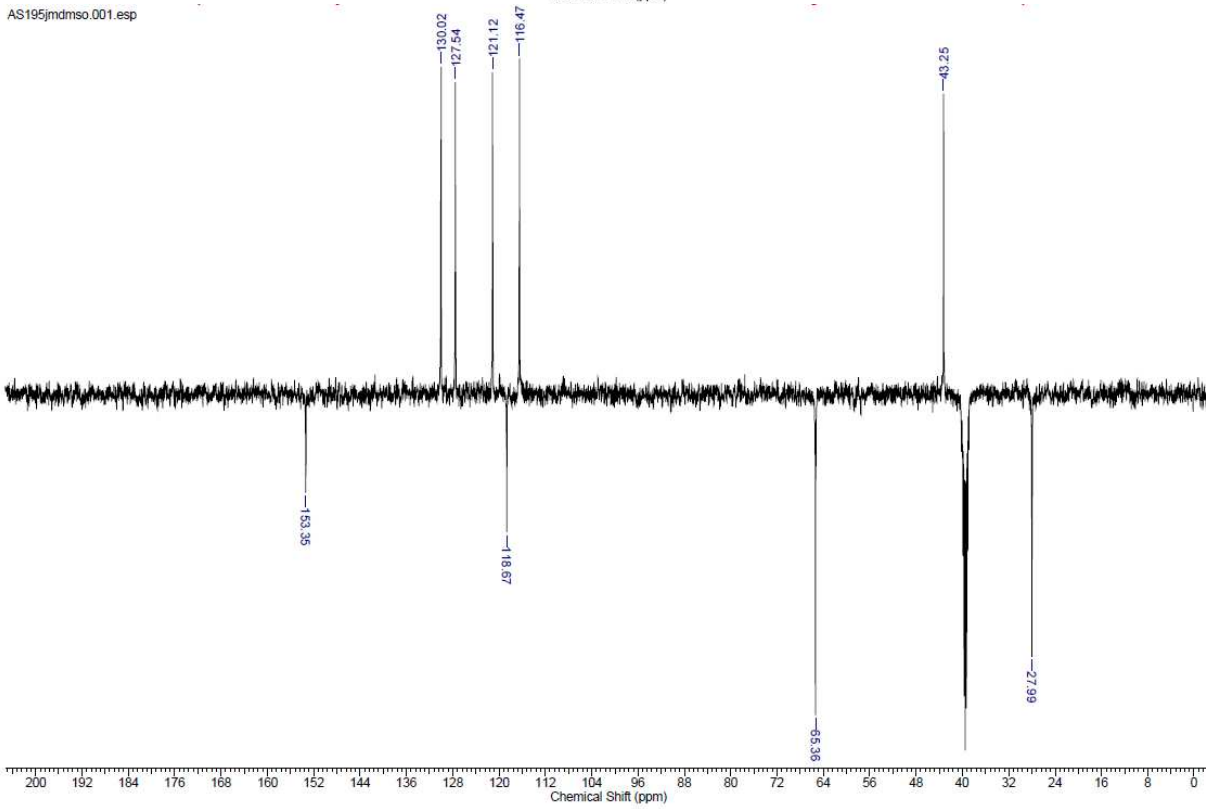


^{1}H and ^{13}C NMR spectra of the characterized compounds

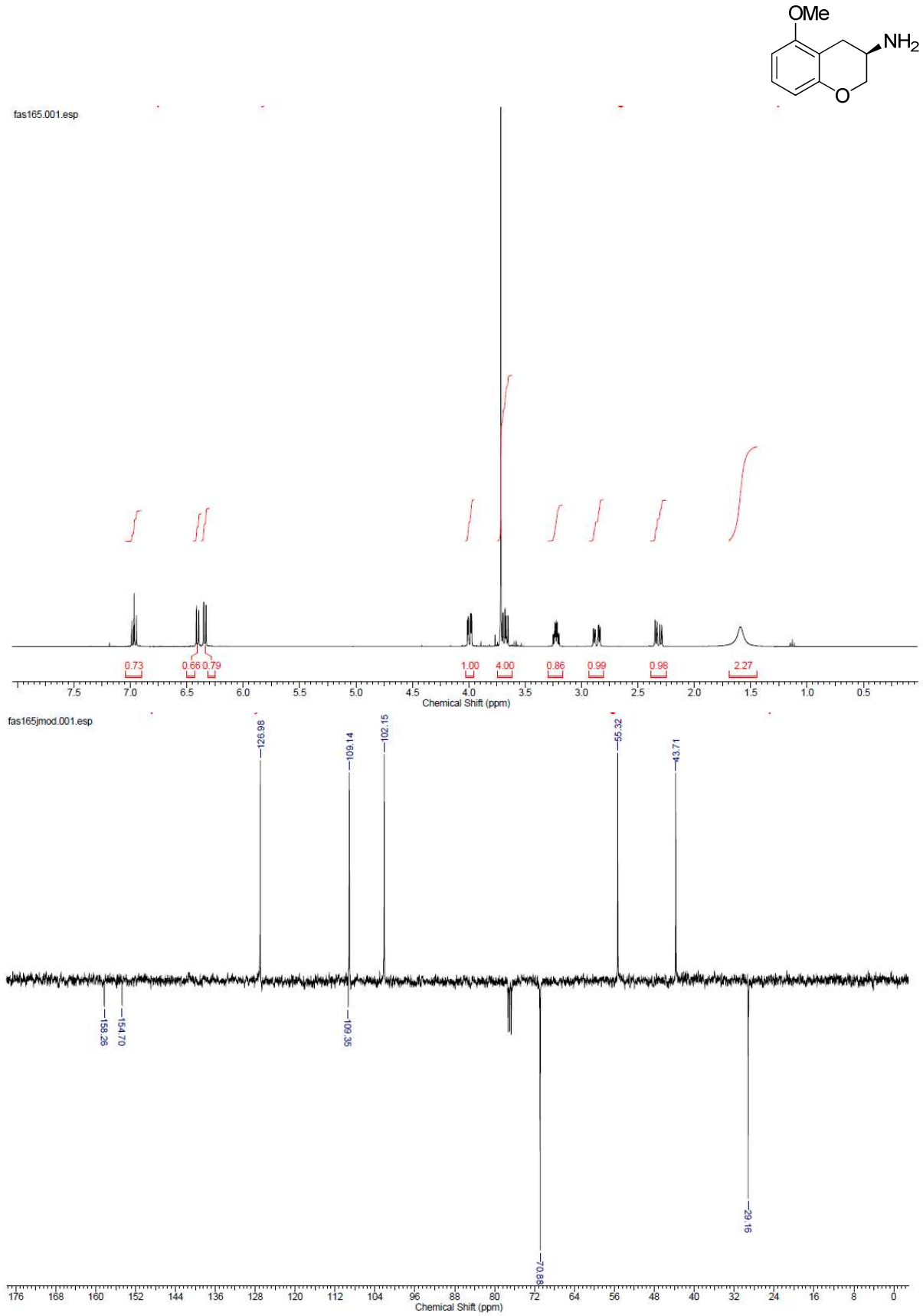
^{1}H and ^{13}C NMR spectra of compound **1**·HCl



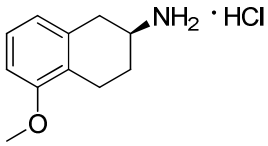


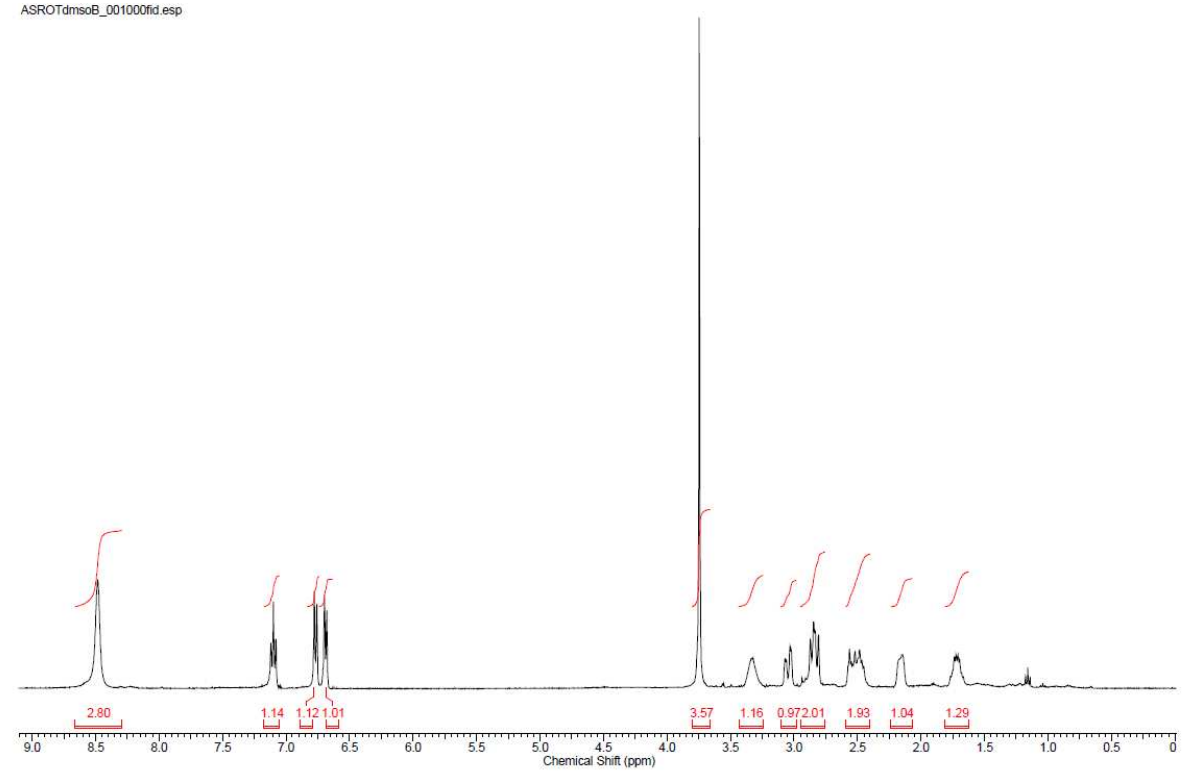


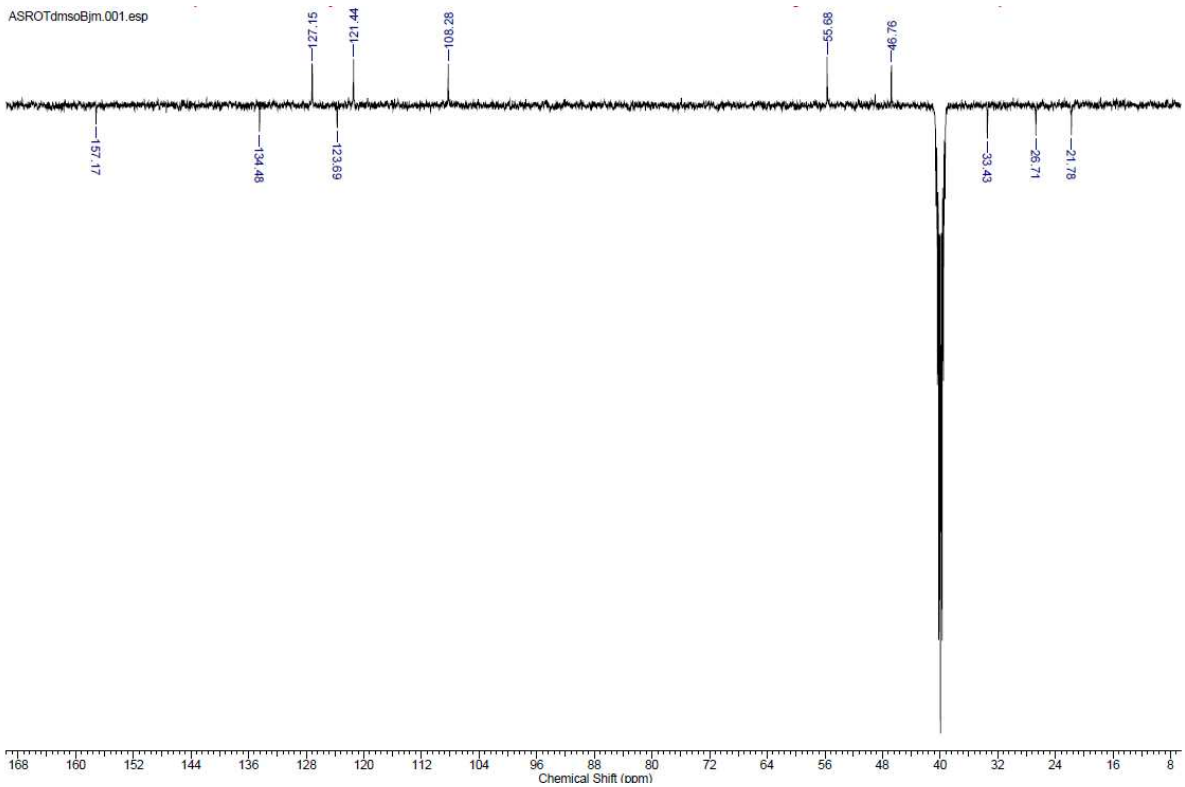
^{1}H and ^{13}C NMR spectra of compound **2**



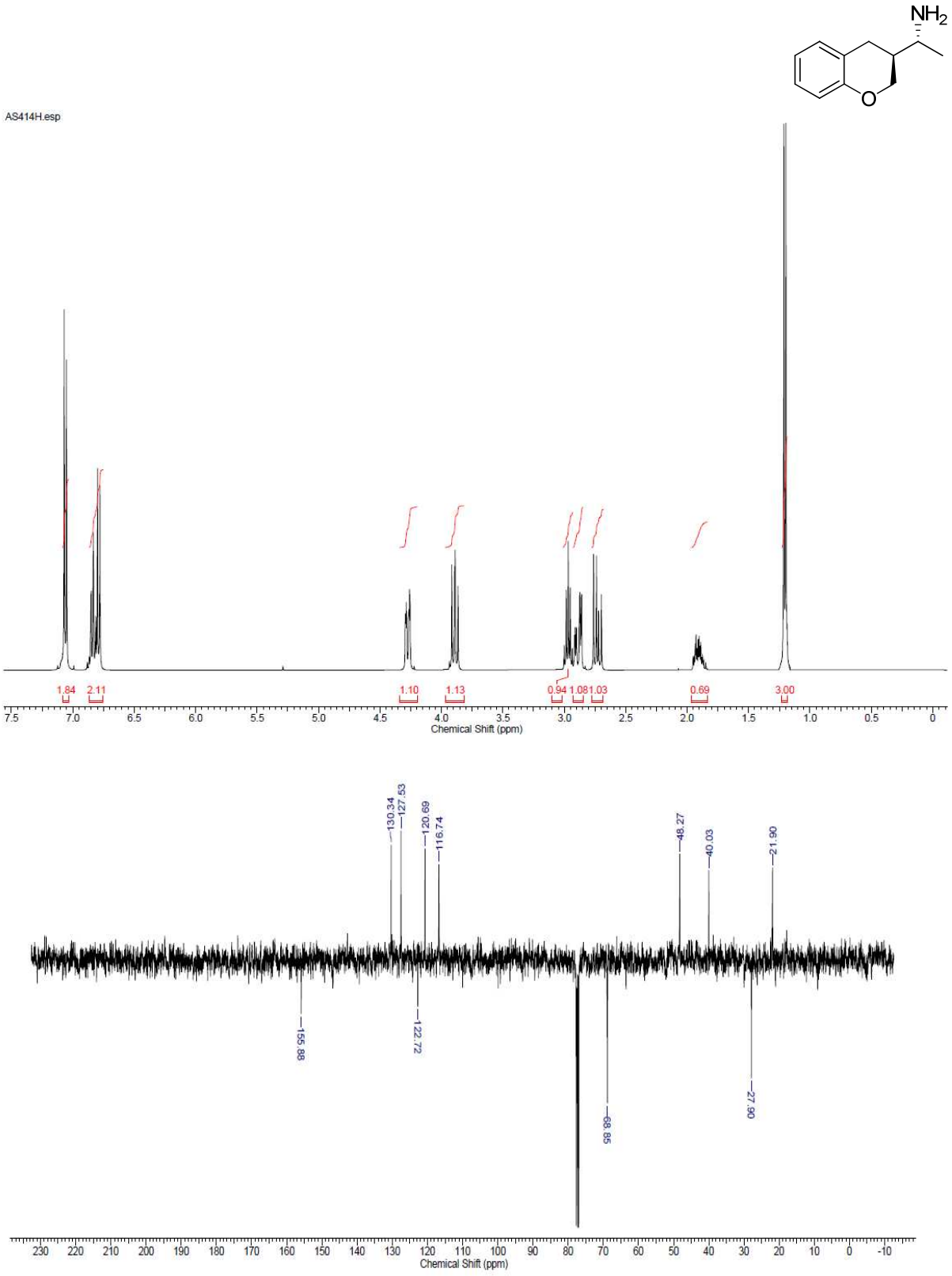
^{1}H and ^{13}C NMR spectra of compound **3**·HCl



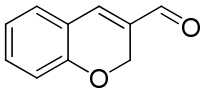


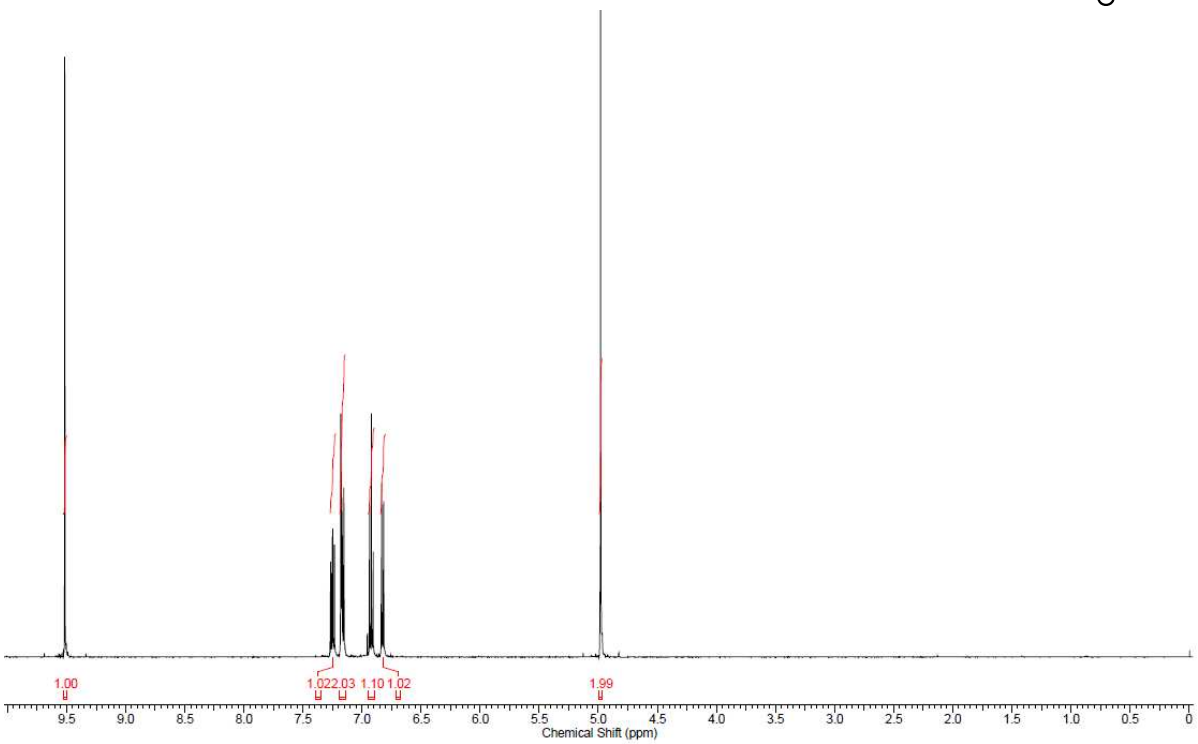


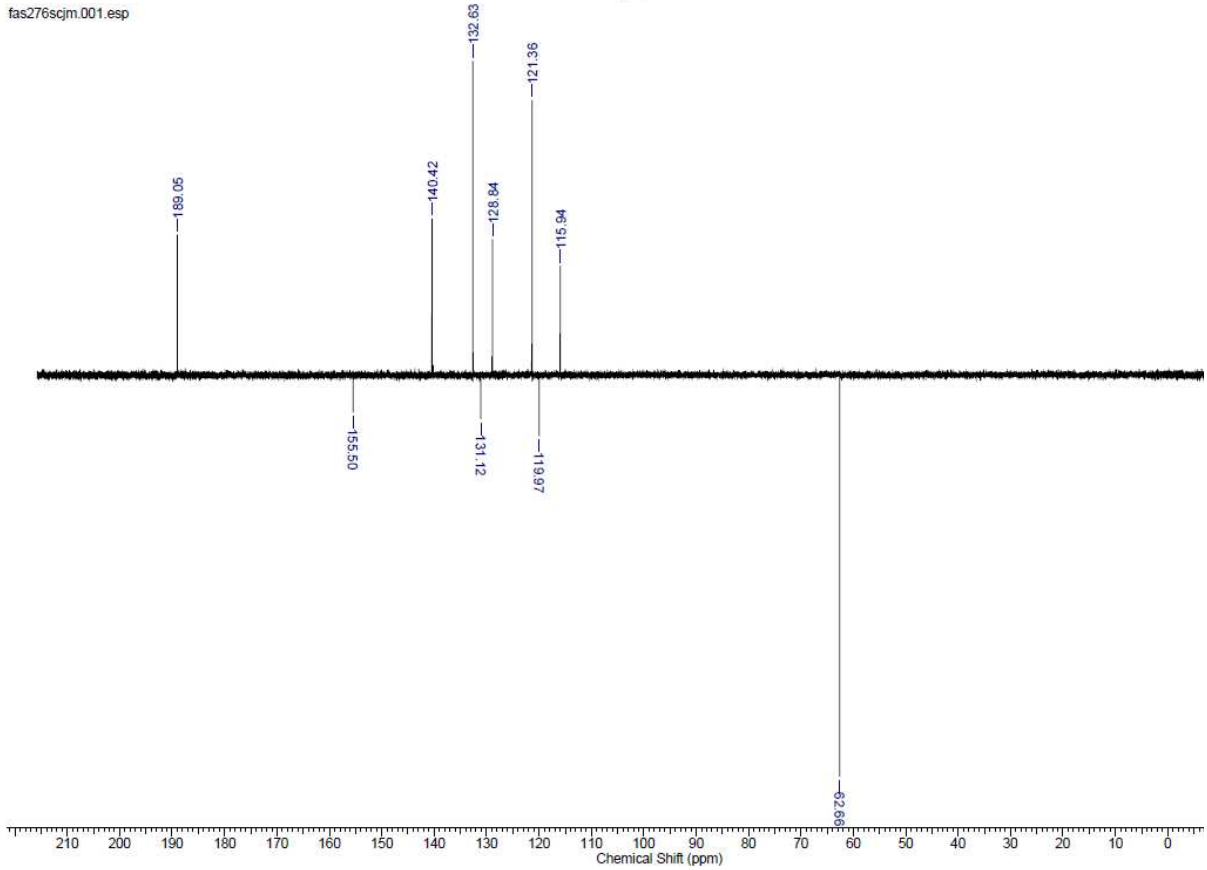
^{1}H and ^{13}C NMR spectra of compound **4**



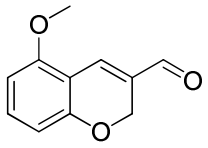
^{1}H and ^{13}C NMR spectra of compound **5a**

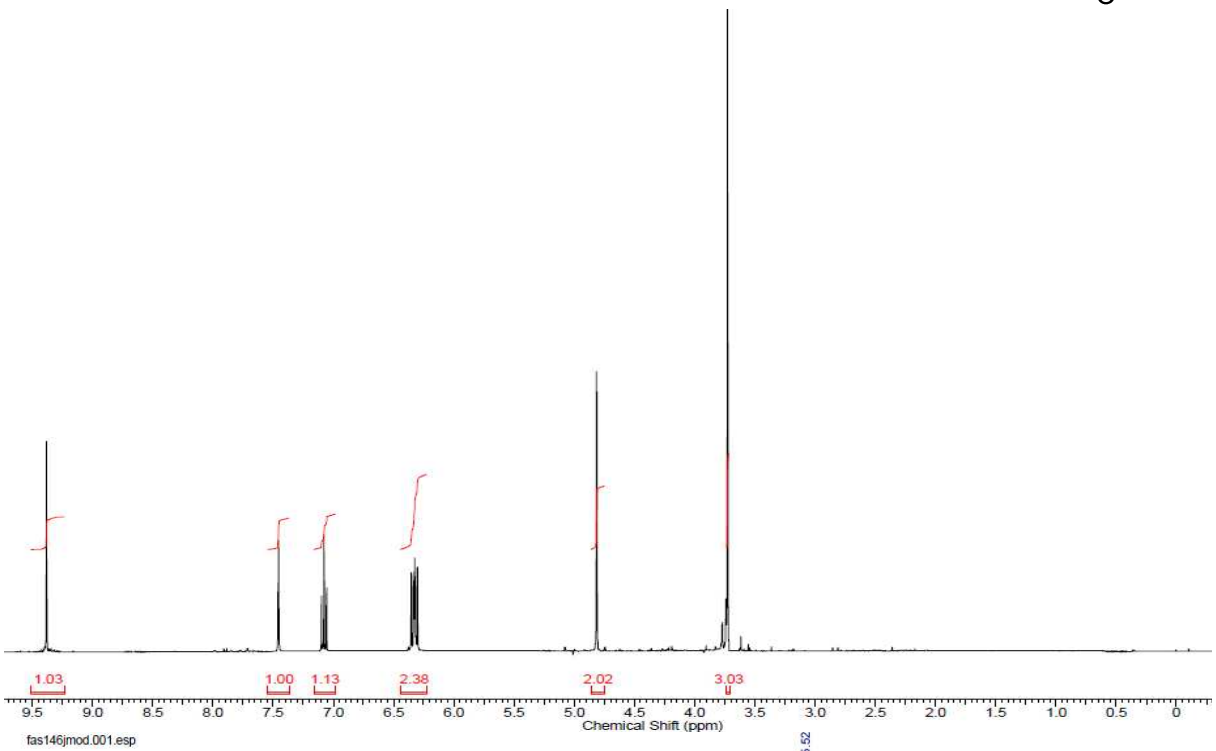


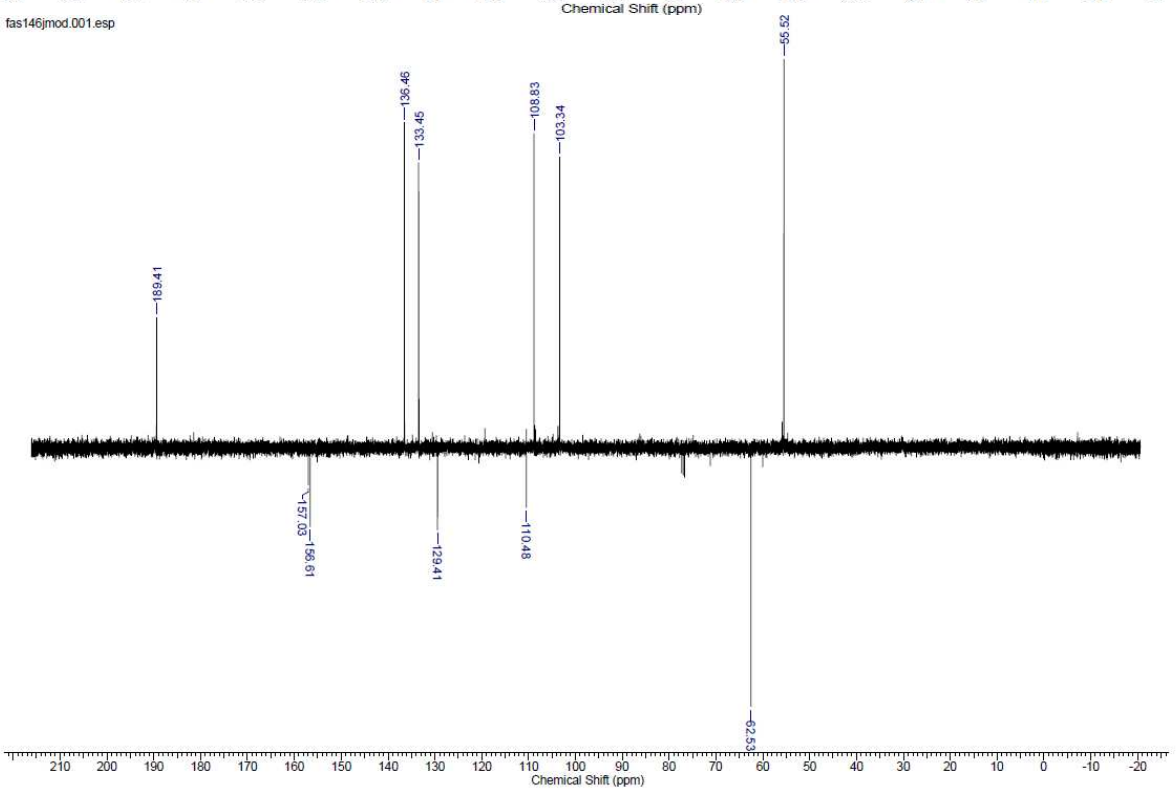




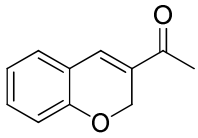
^{1}H and ^{13}C NMR spectra of compound **6a**

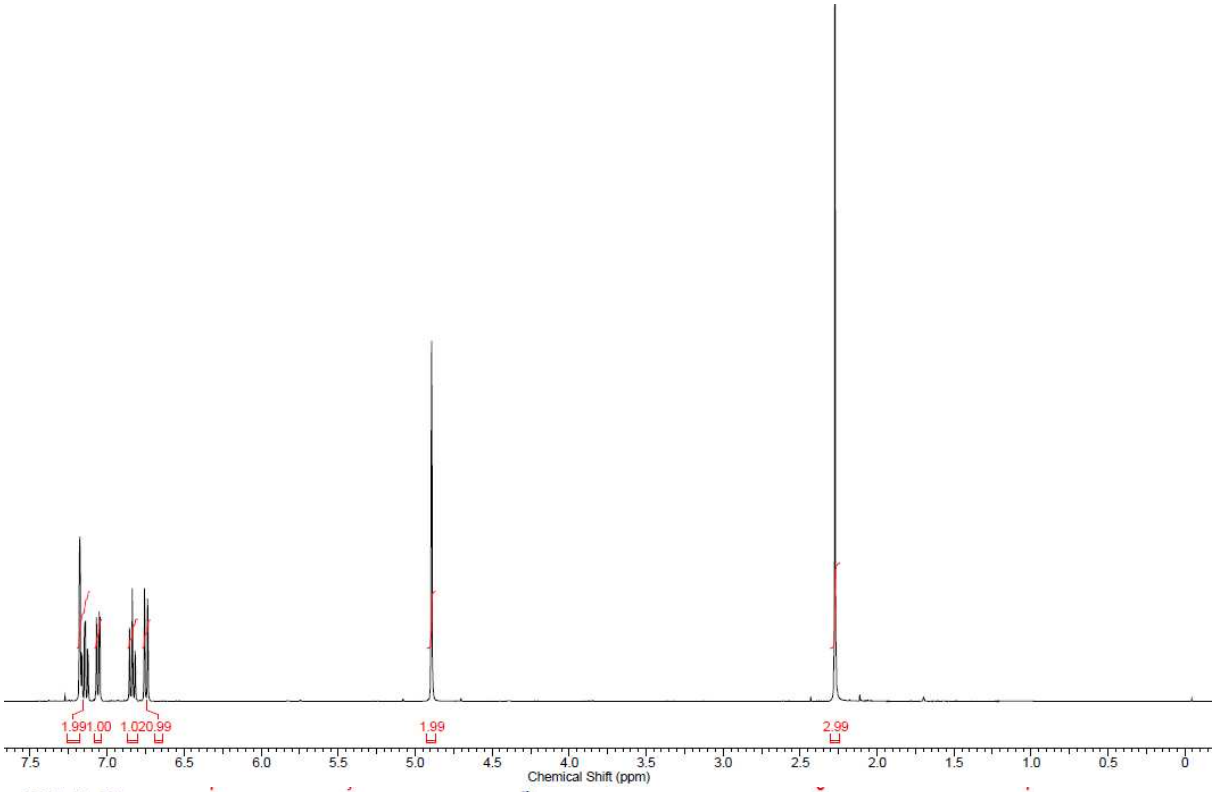


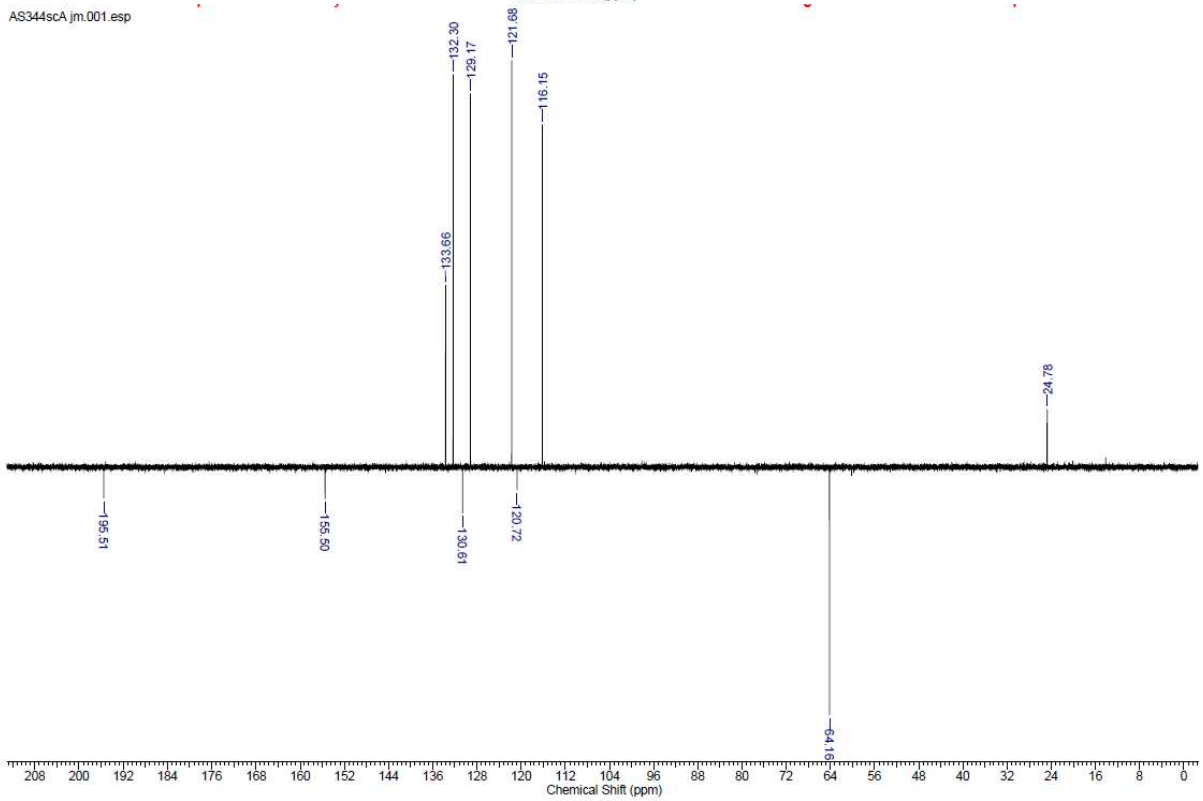




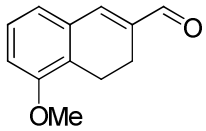
^{1}H and ^{13}C NMR spectra of compound **7a**

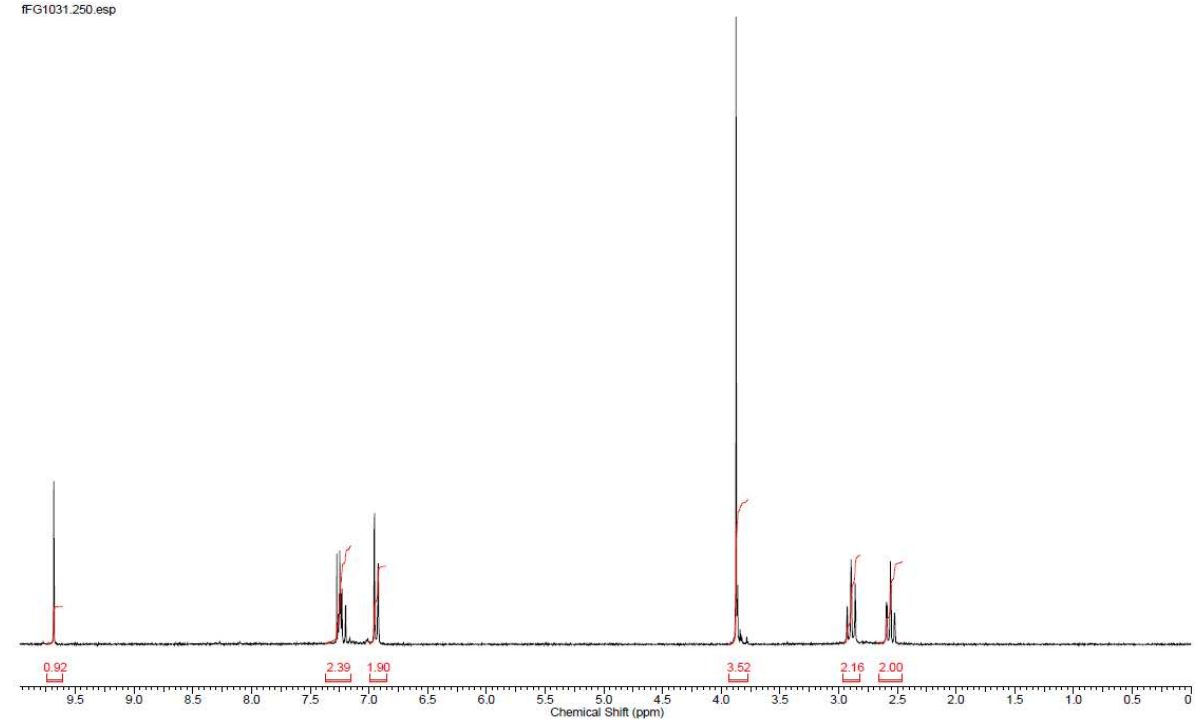


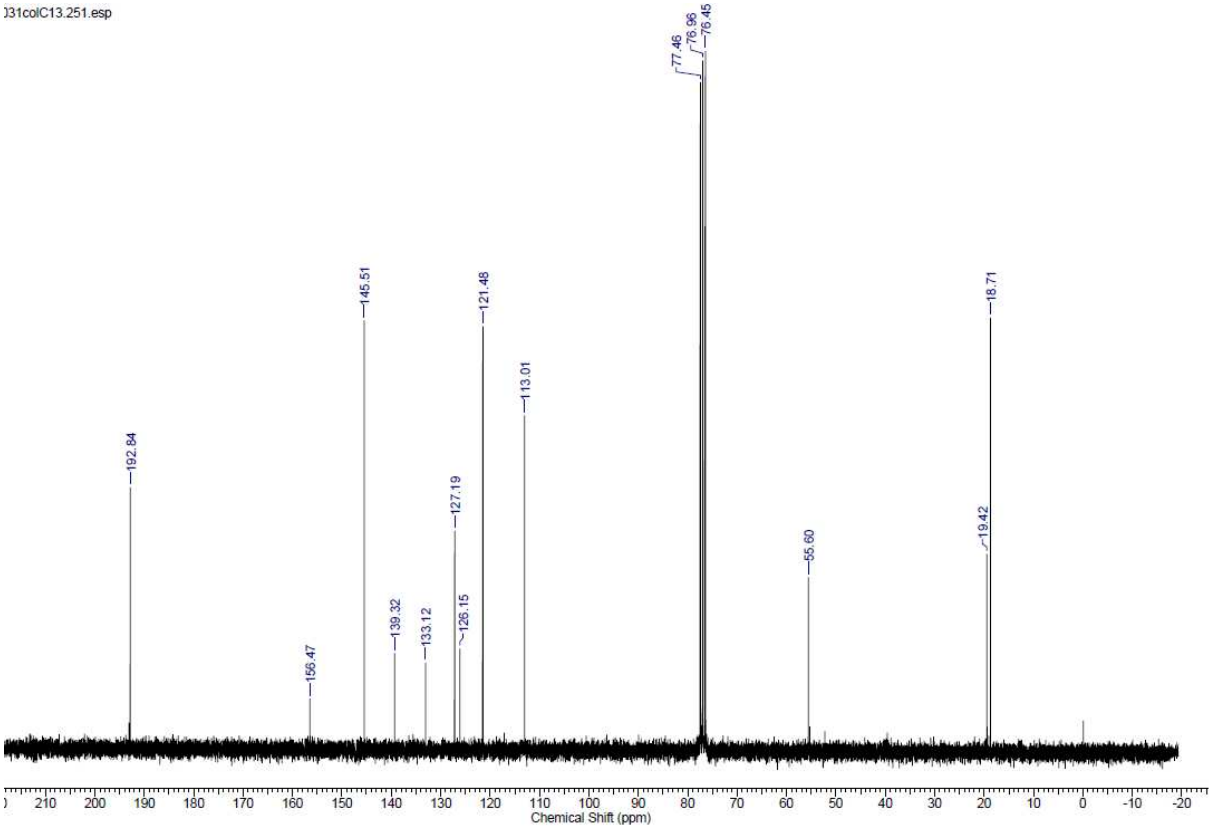




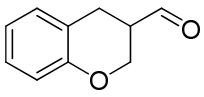
^{1}H and ^{13}C NMR spectra of compound **8a**

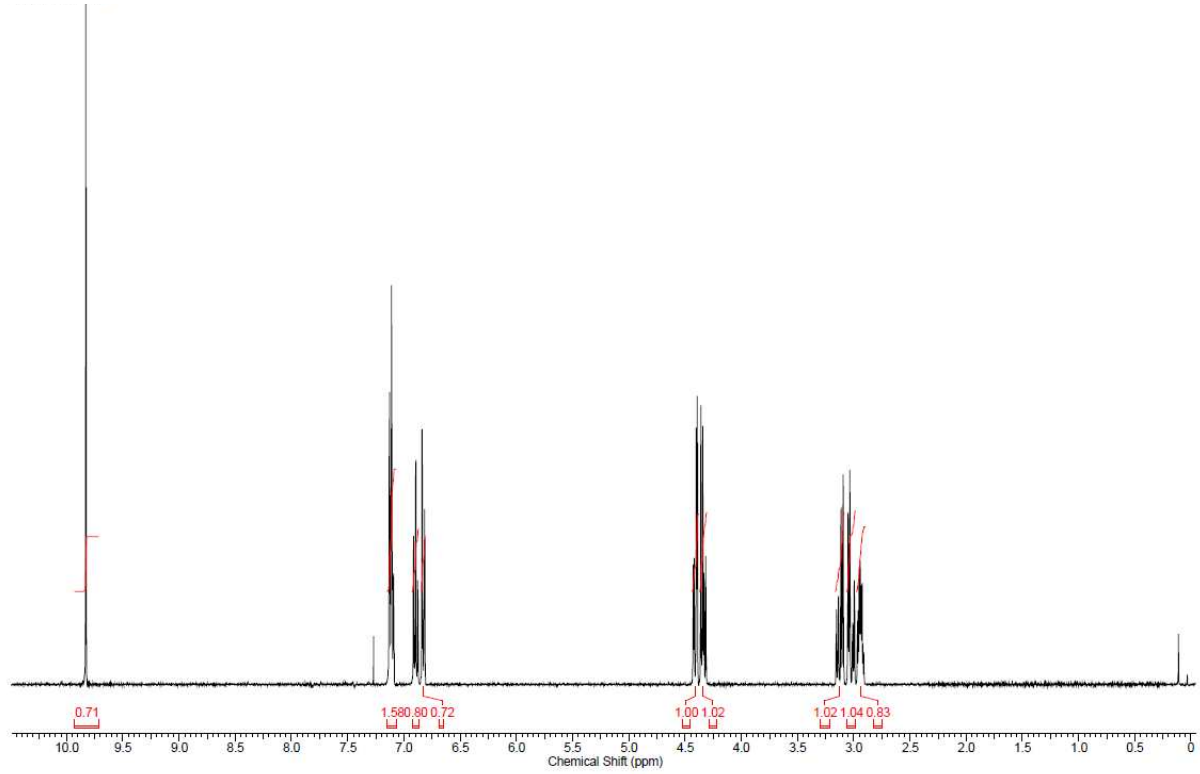


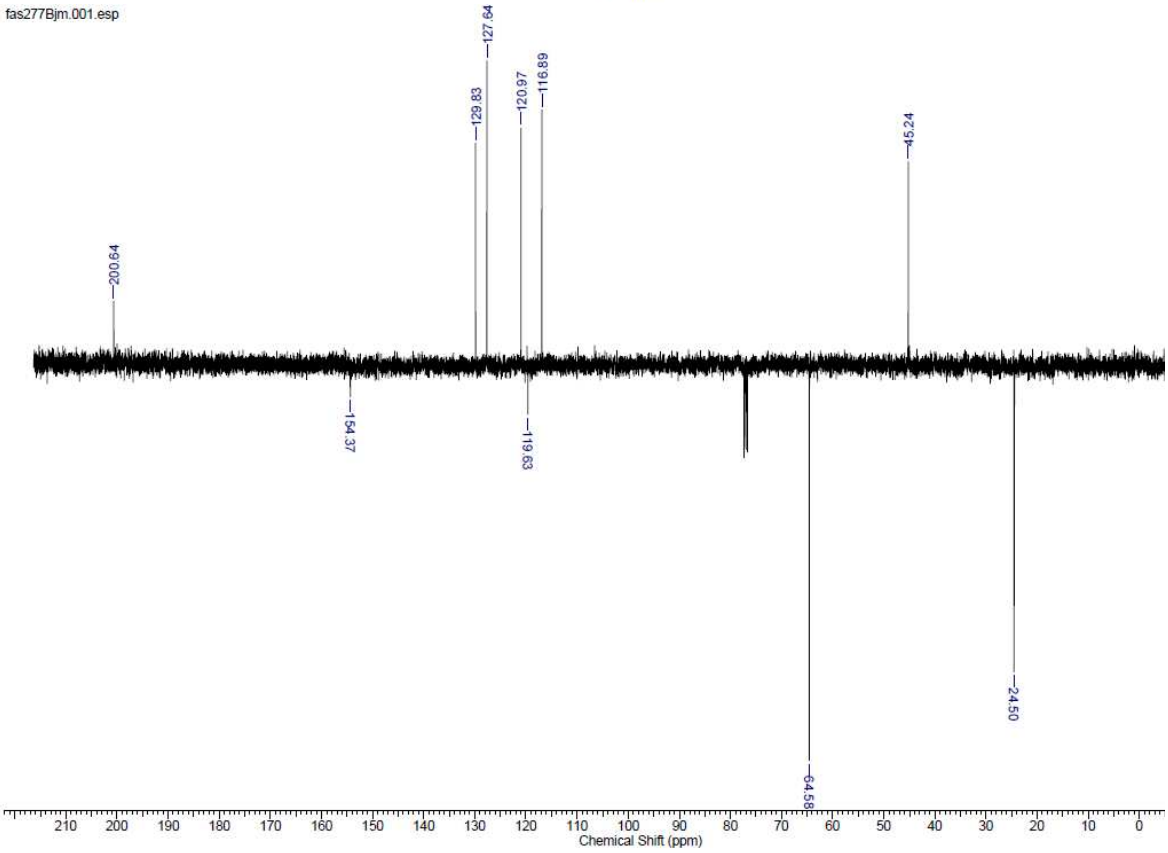




^{1}H and ^{13}C NMR spectra of compound **5b**







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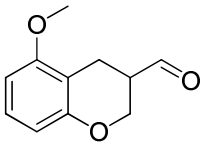

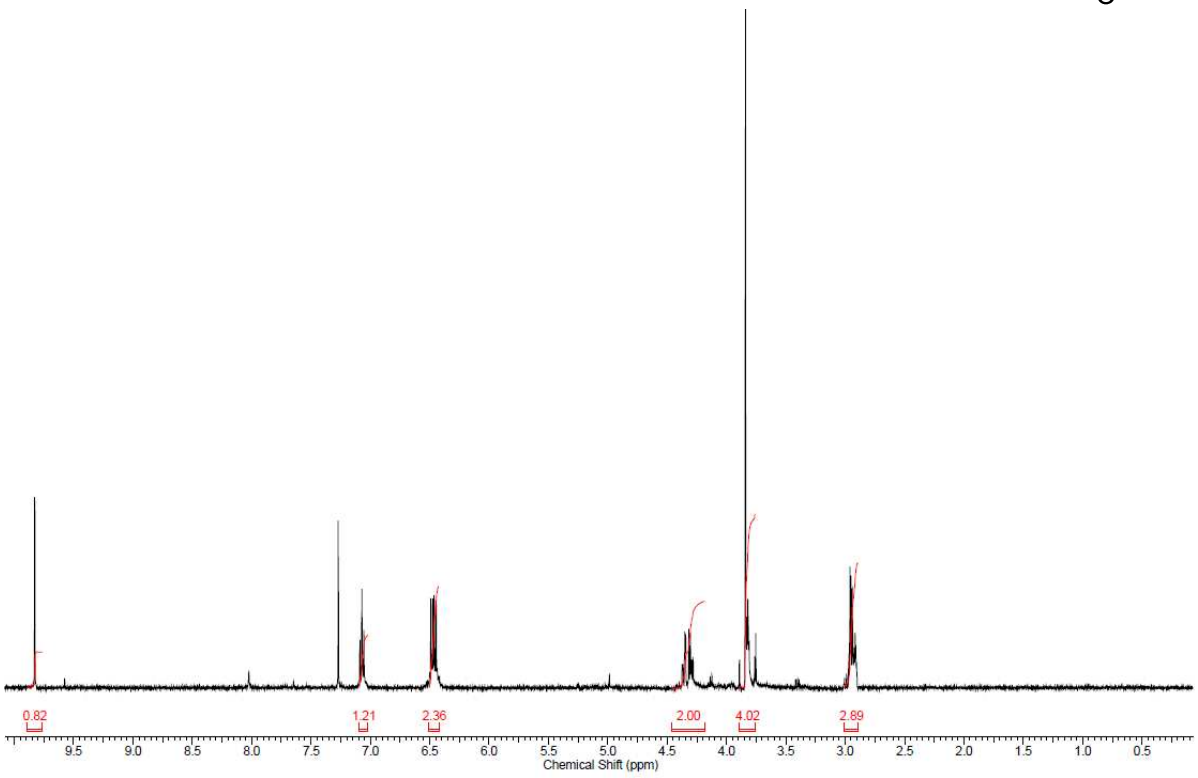

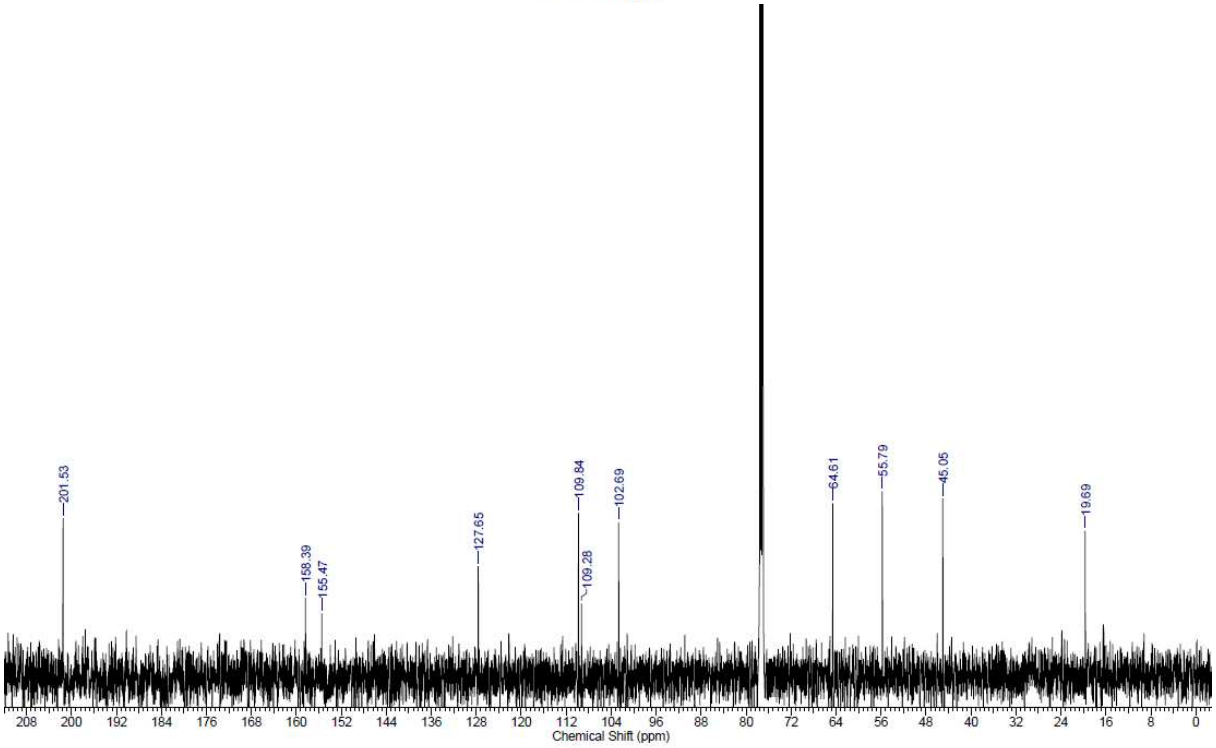

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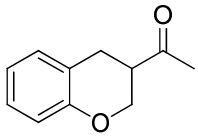
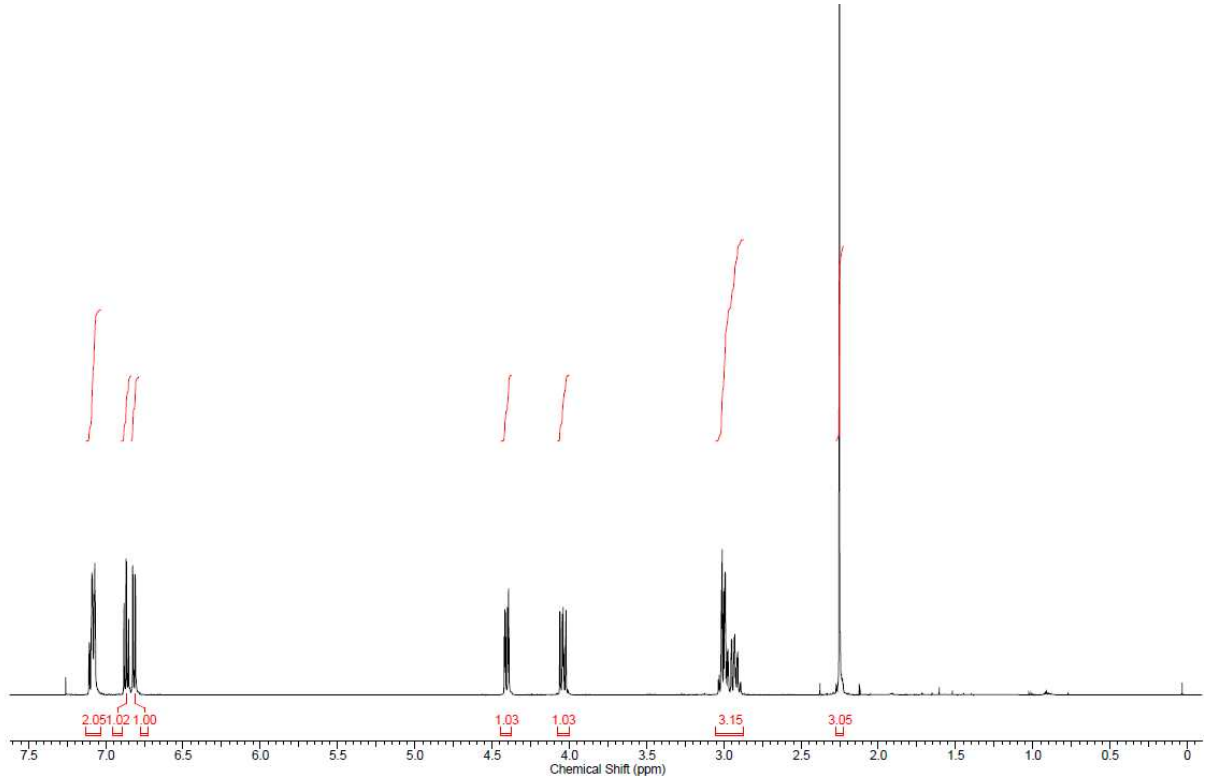
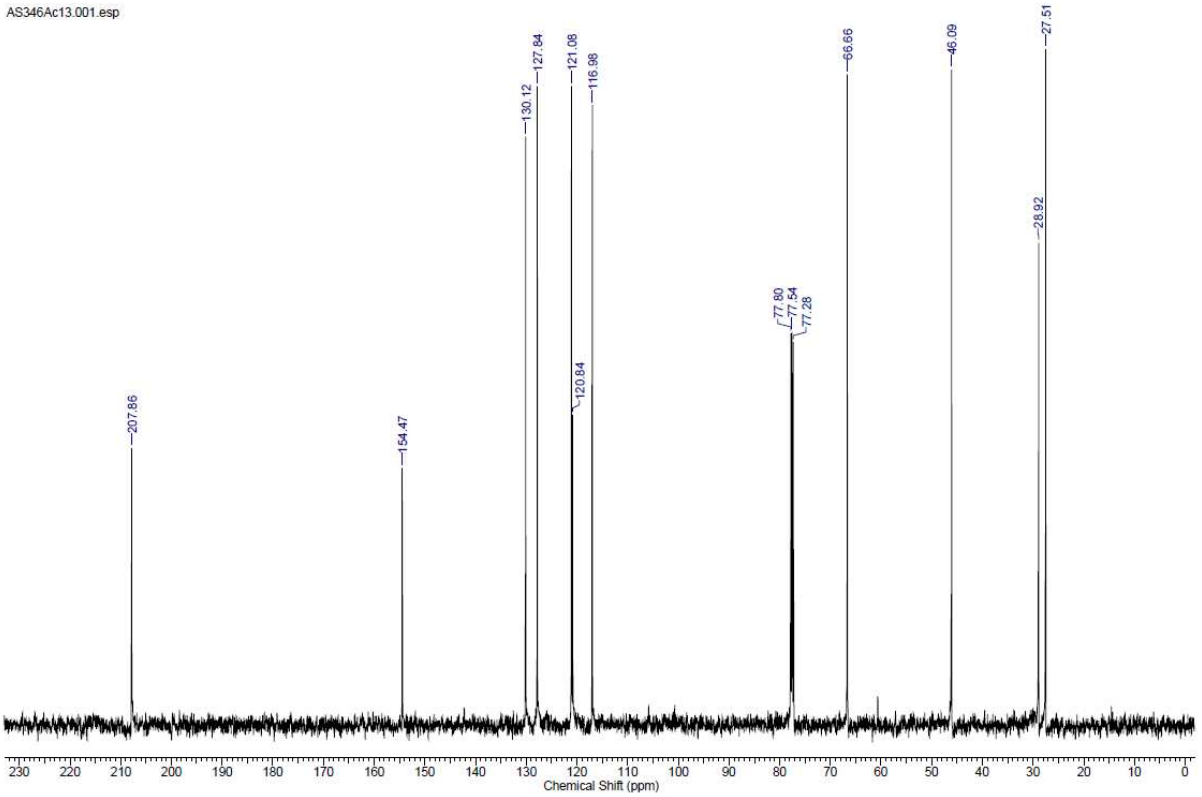
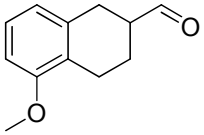
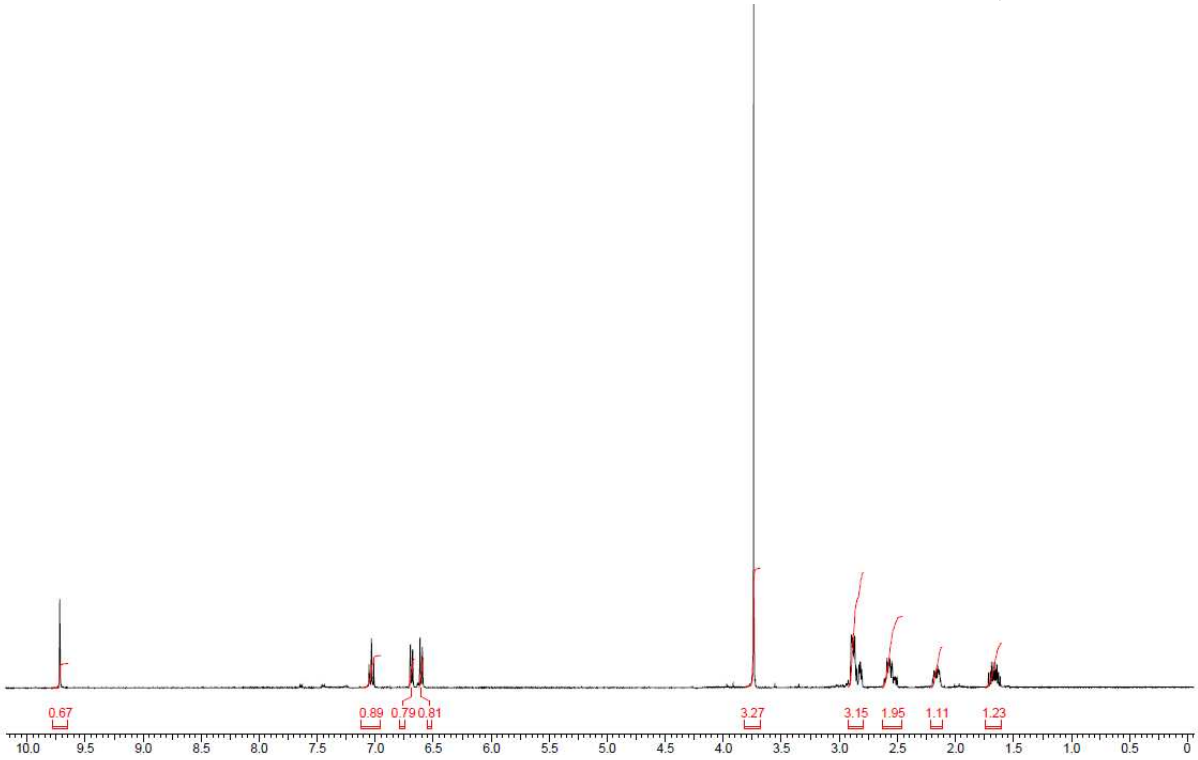


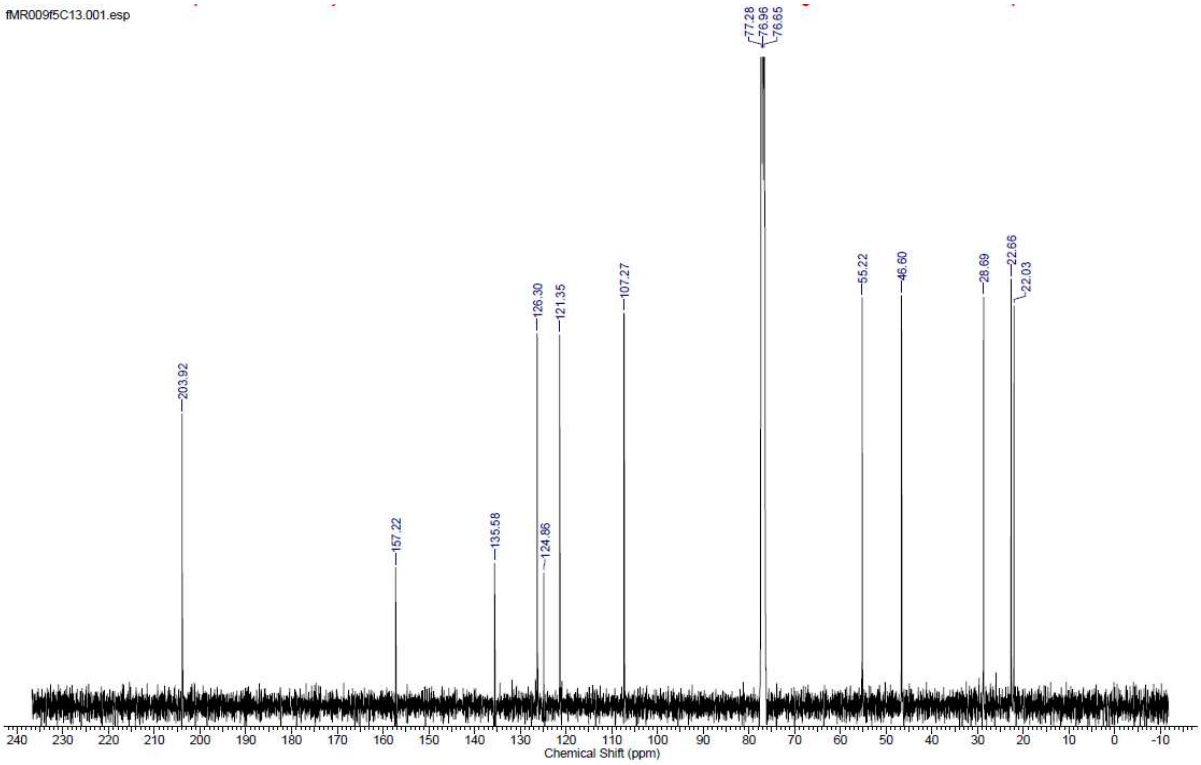




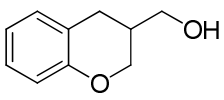
^{1}H and ^{13}C NMR spectra of compound **8b**

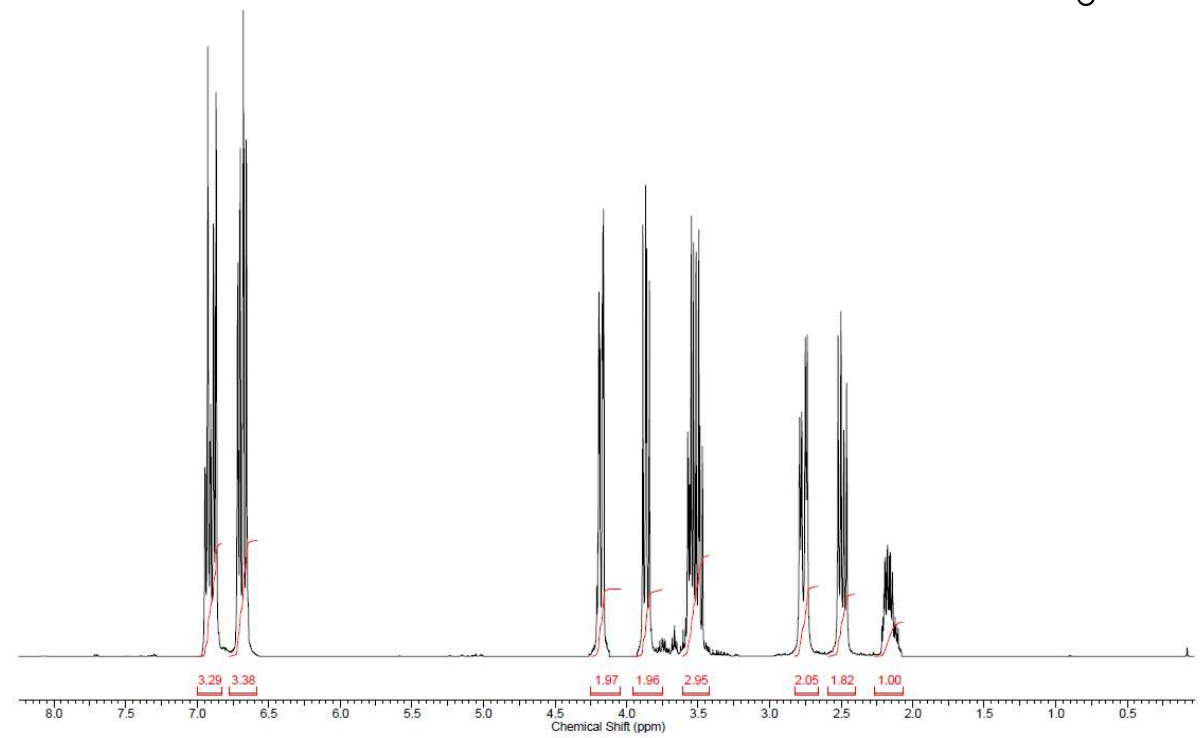


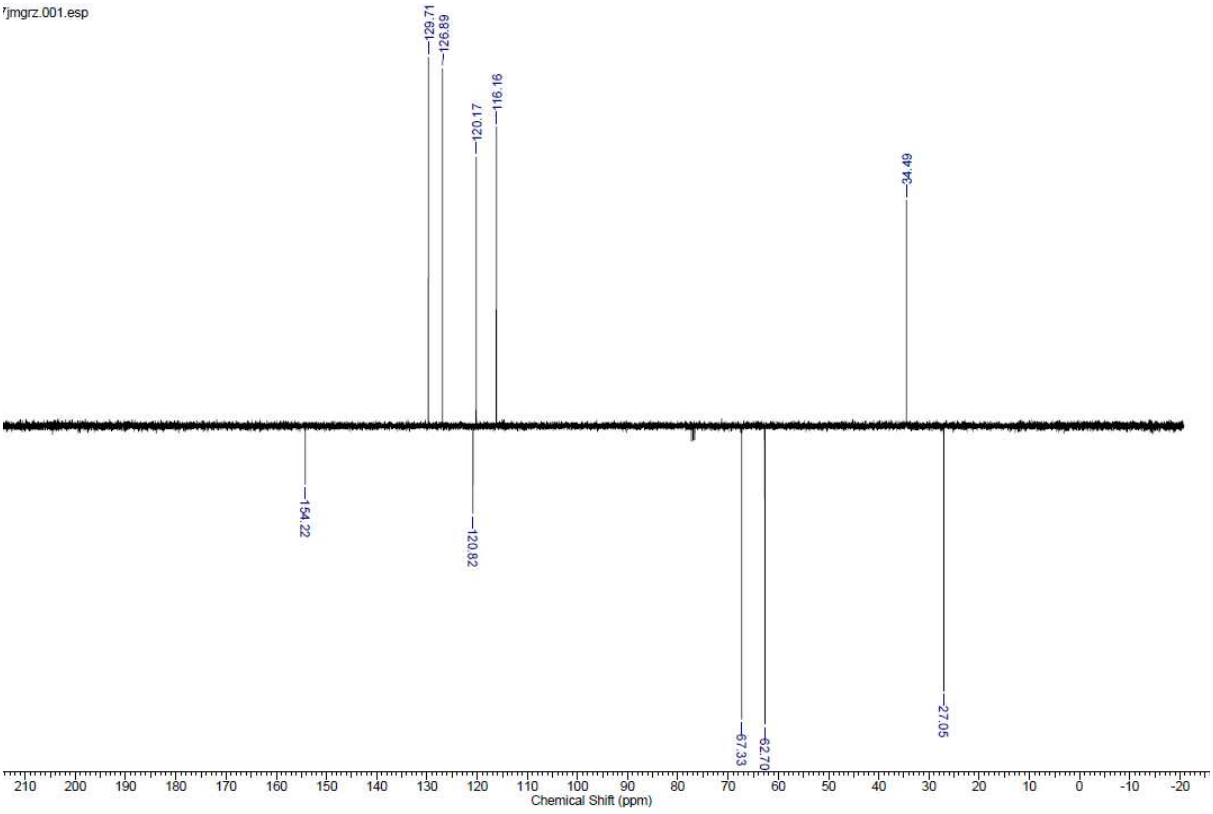




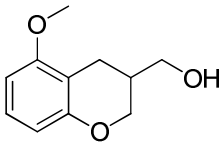
^{1}H and ^{13}C NMR spectra of compound **5c**

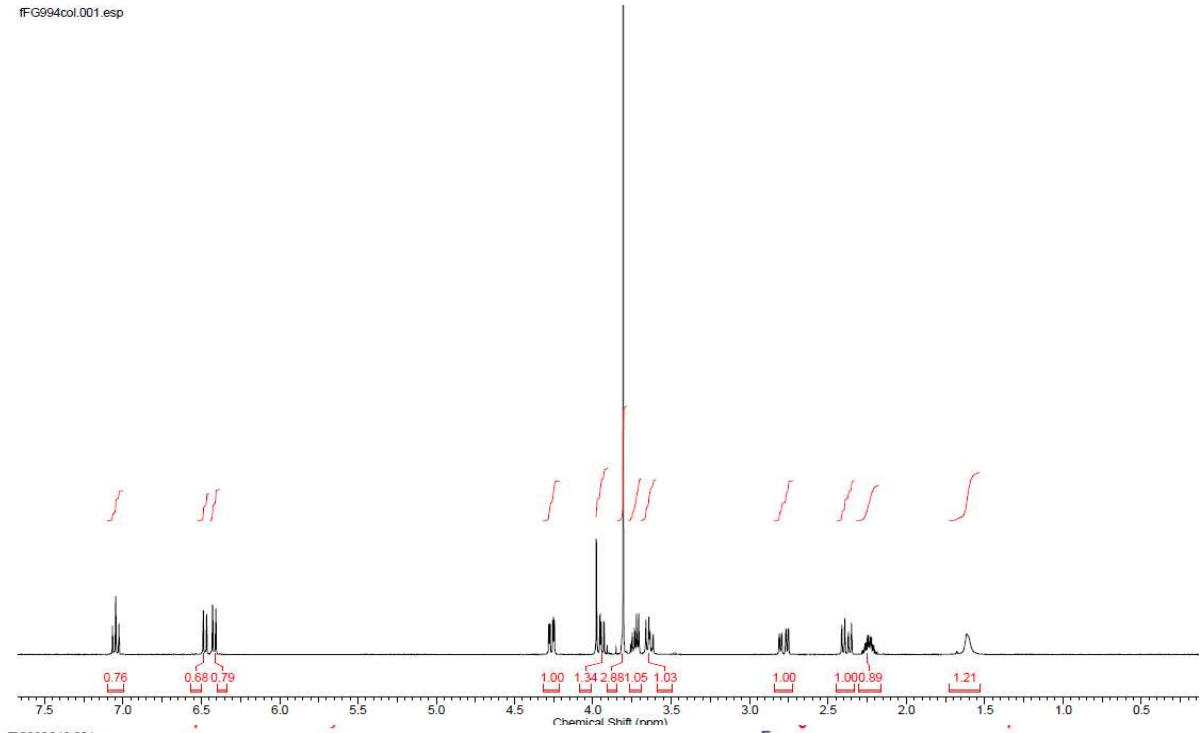


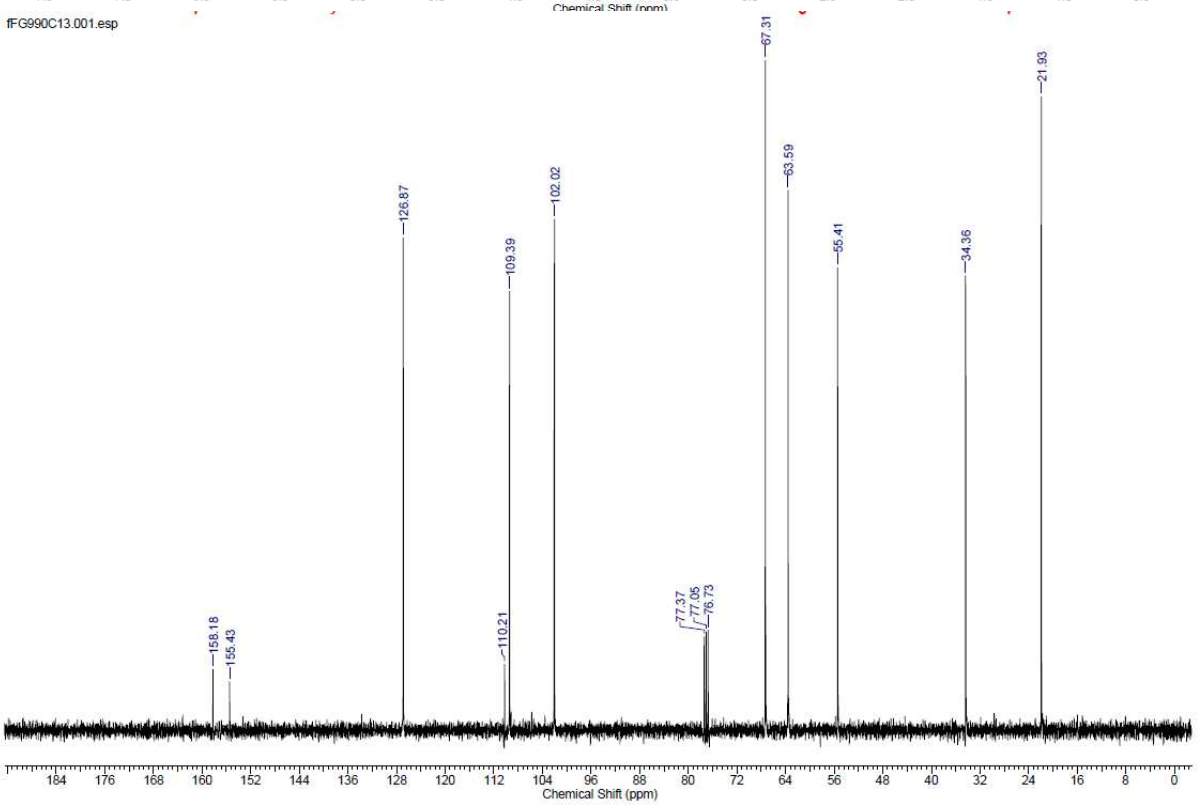




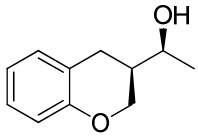
^{1}H and ^{13}C NMR spectra of compound **6c**

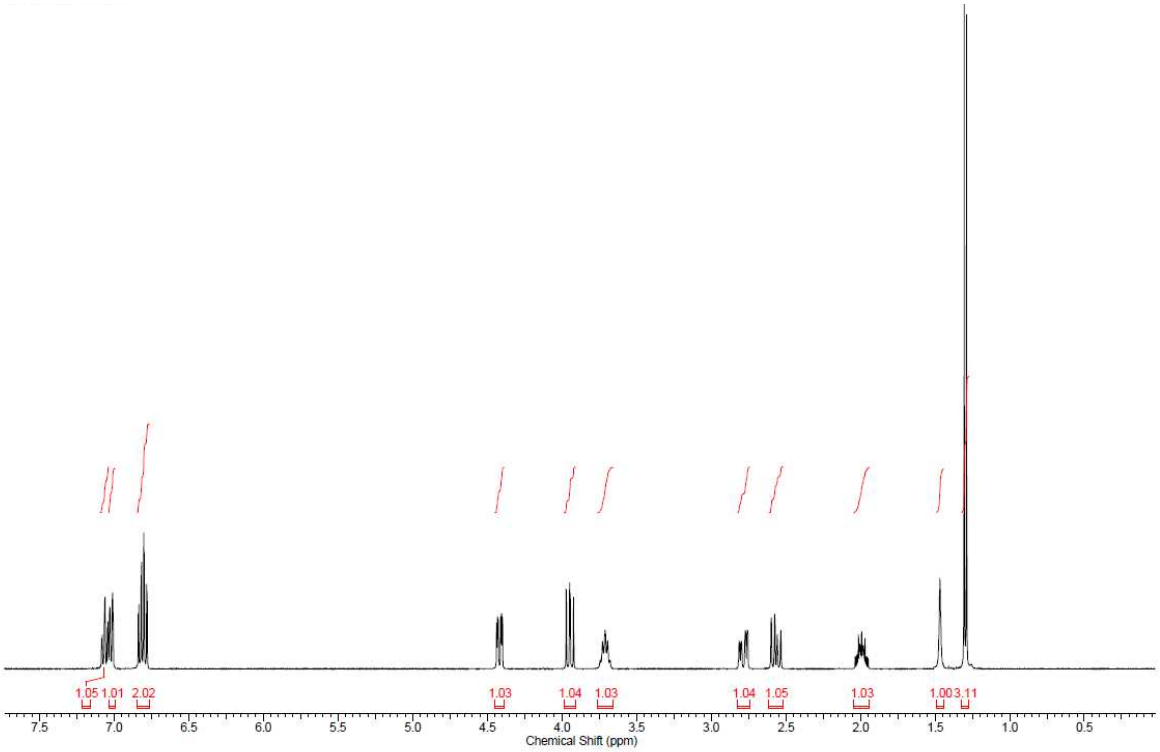


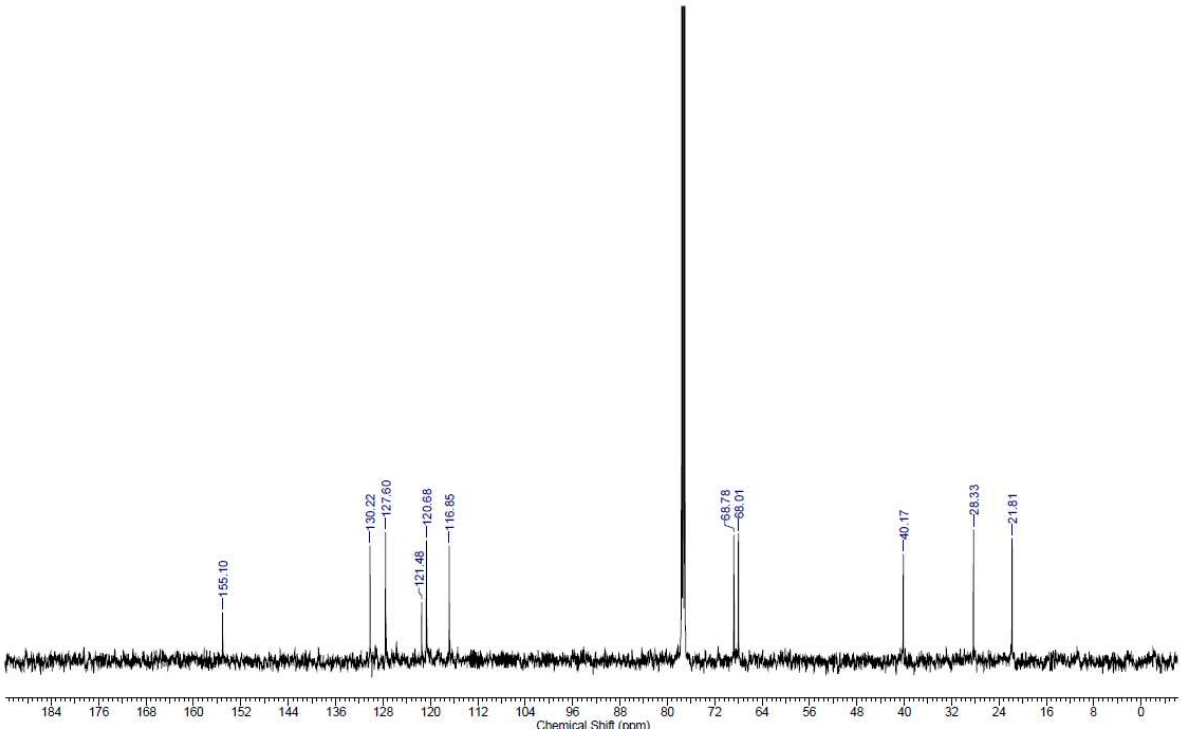




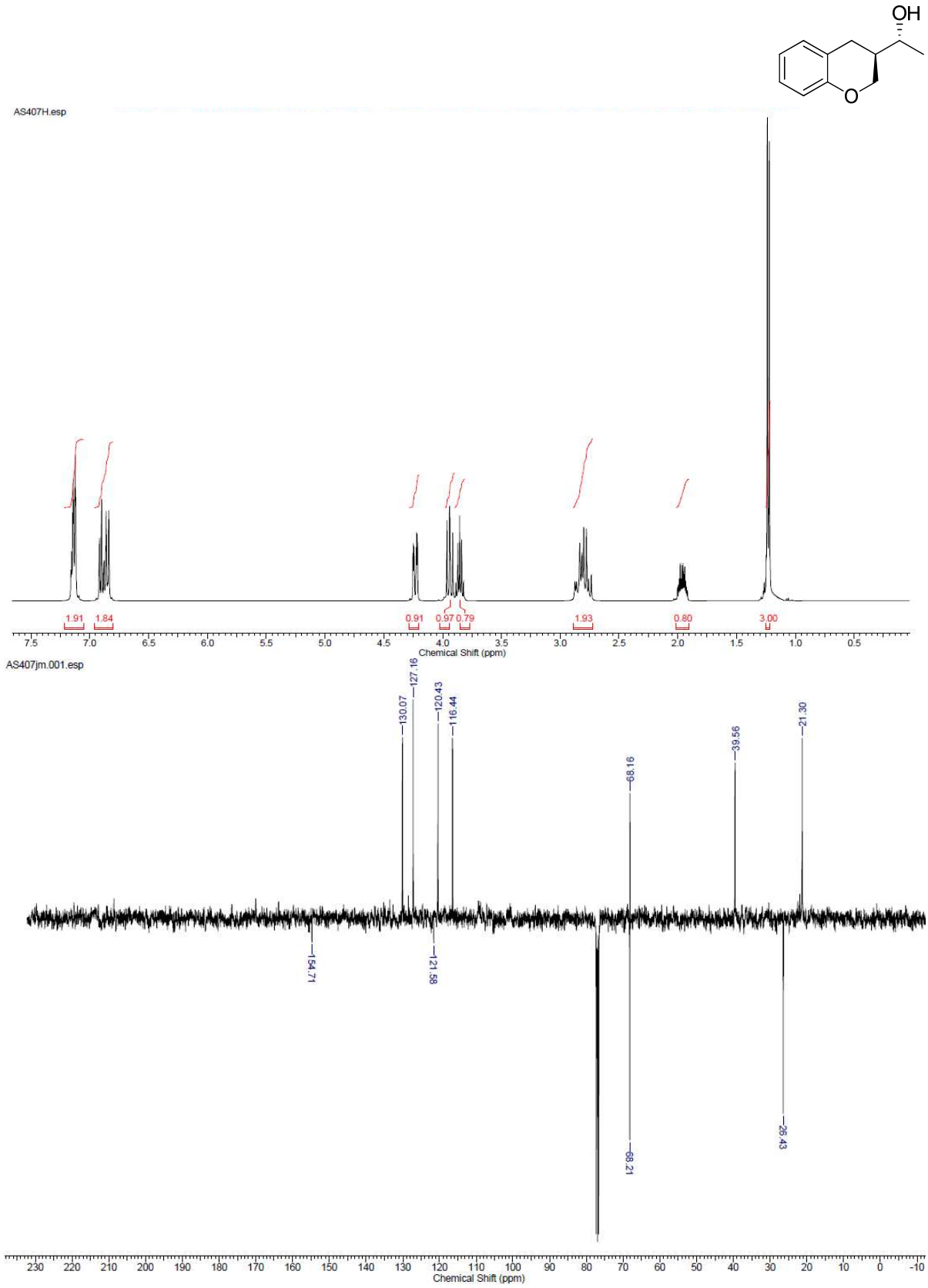
^{1}H and ^{13}C NMR spectra of compound (1*S*,3'*R*)-**7c**



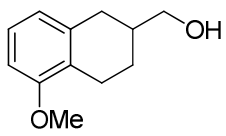


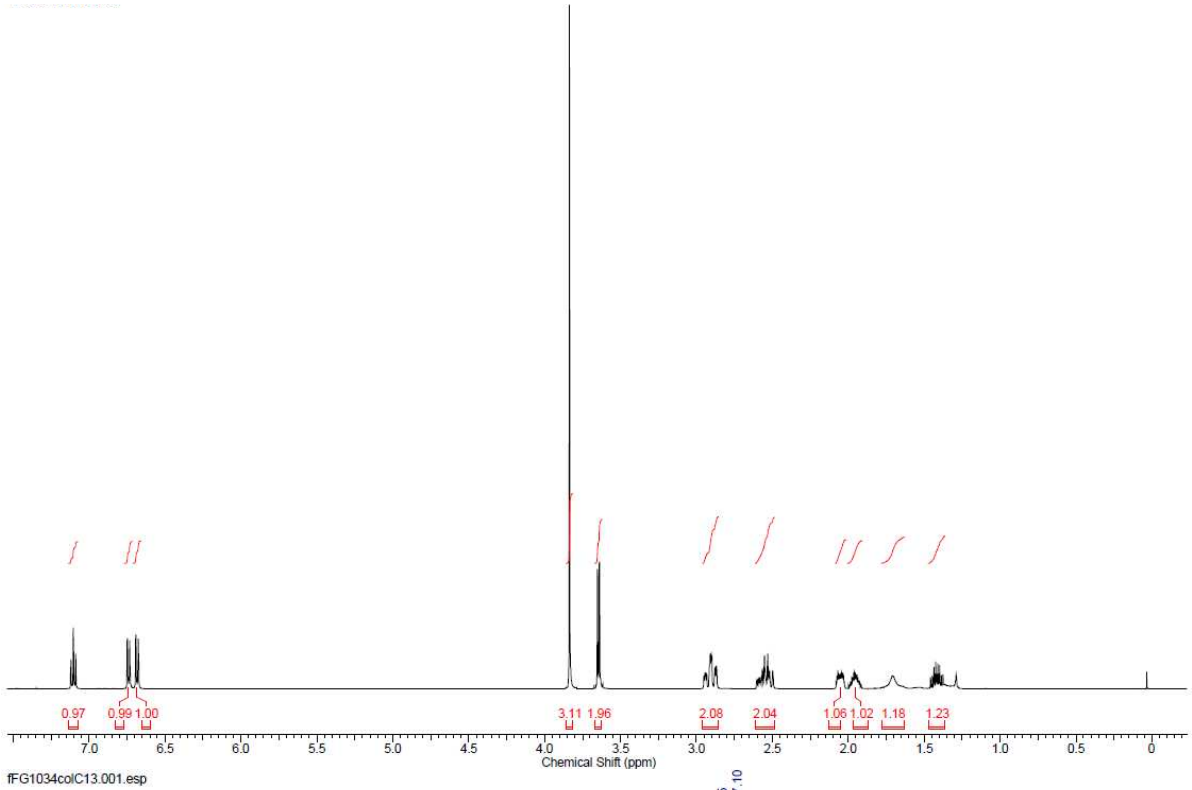


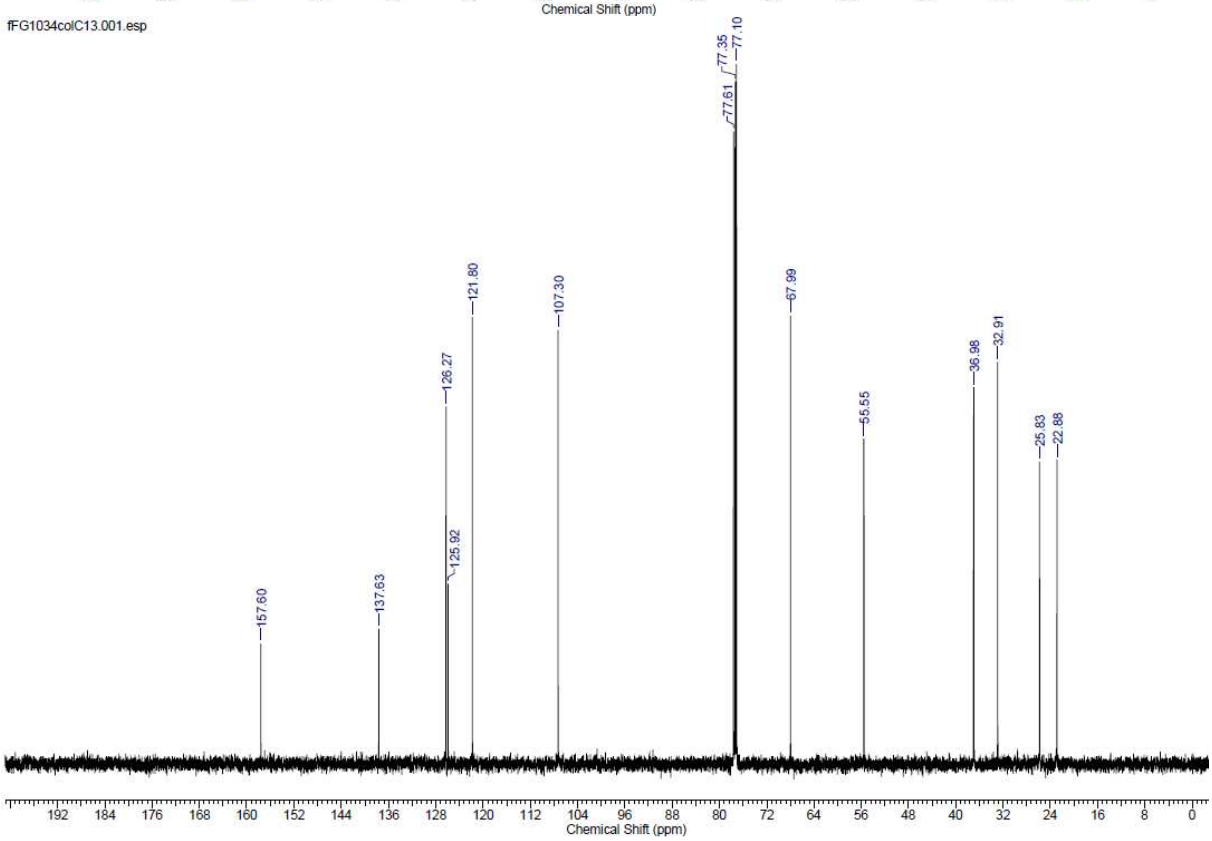
^{1}H and ^{13}C NMR spectra of compound (1*R*,3'*R*)-**7c**



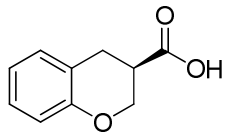
^{1}H and ^{13}C NMR spectra of compound **8c**

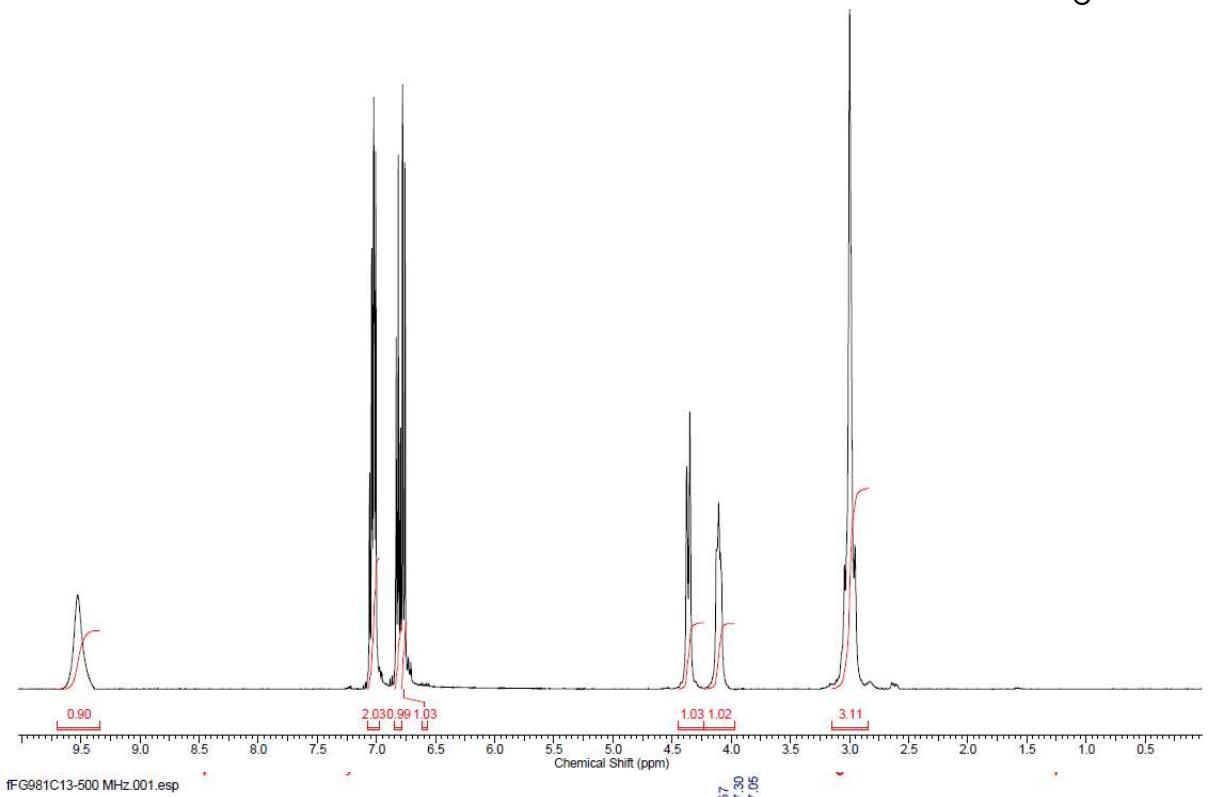




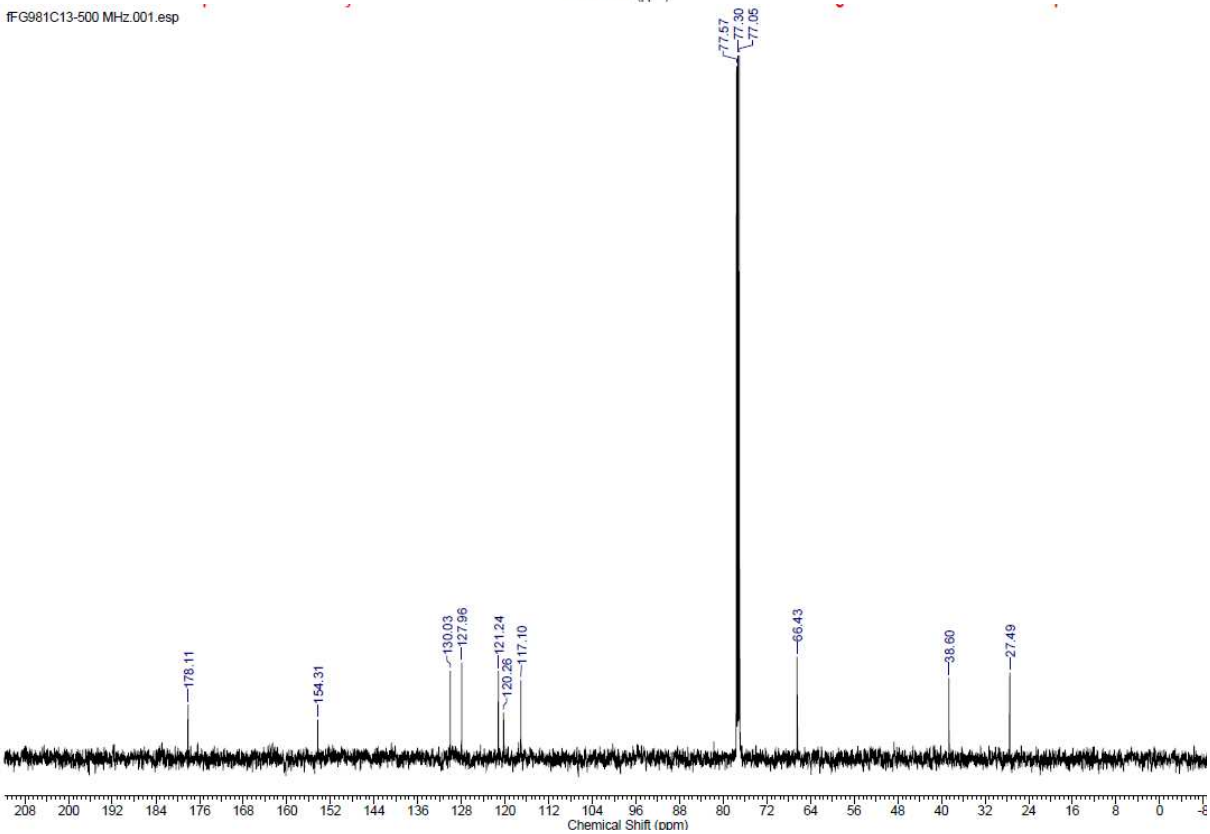


^{1}H and ^{13}C NMR spectra of compound **5e**

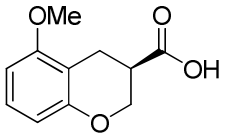


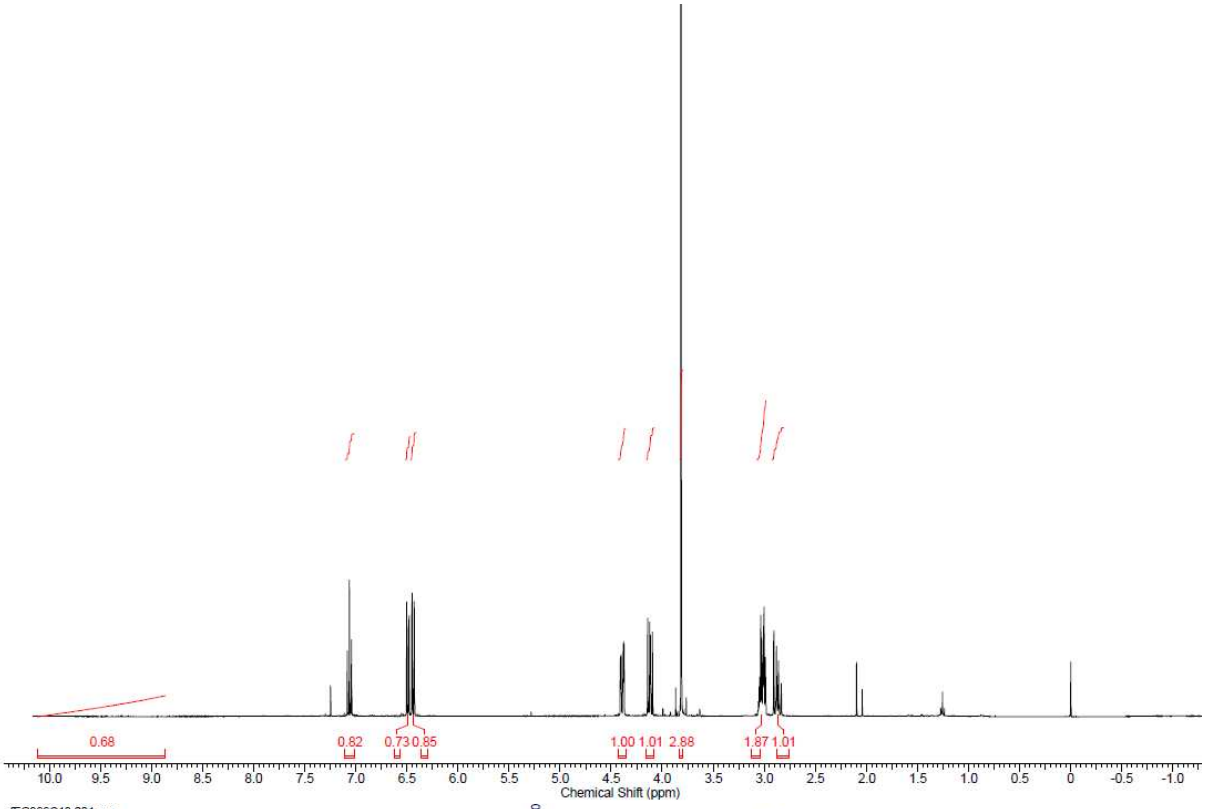


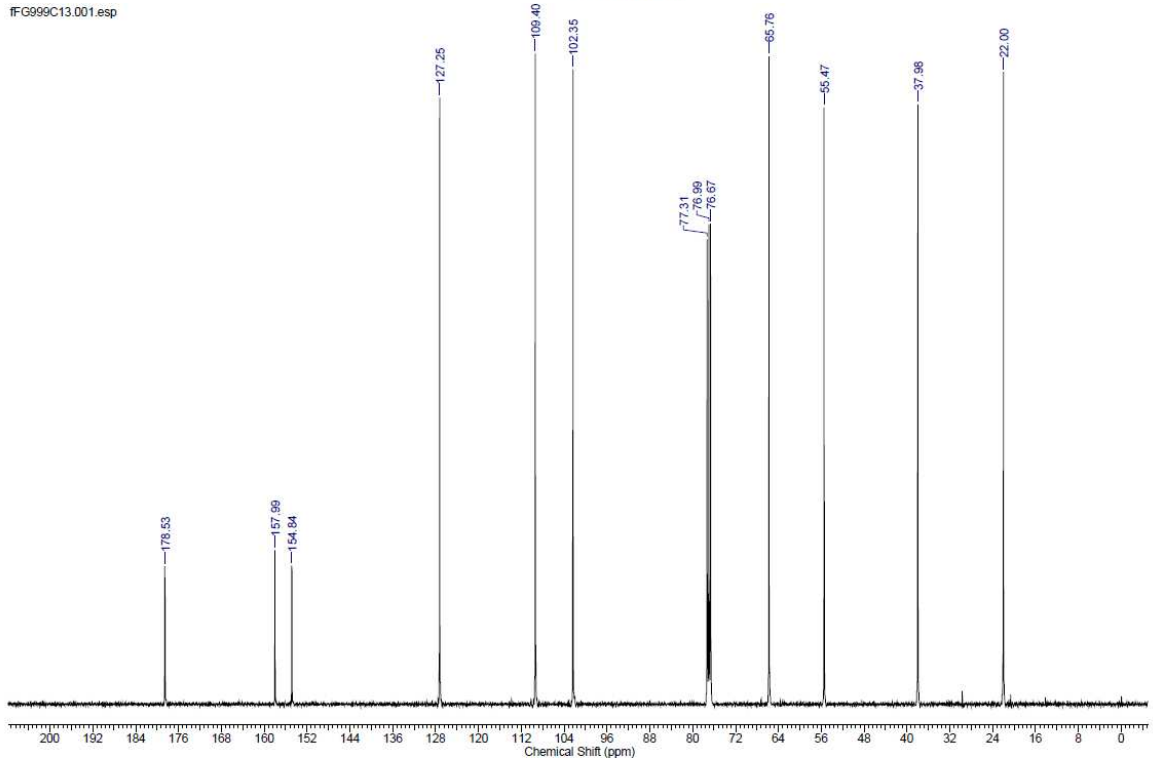
fFG981C13-500 MHz.001.esp



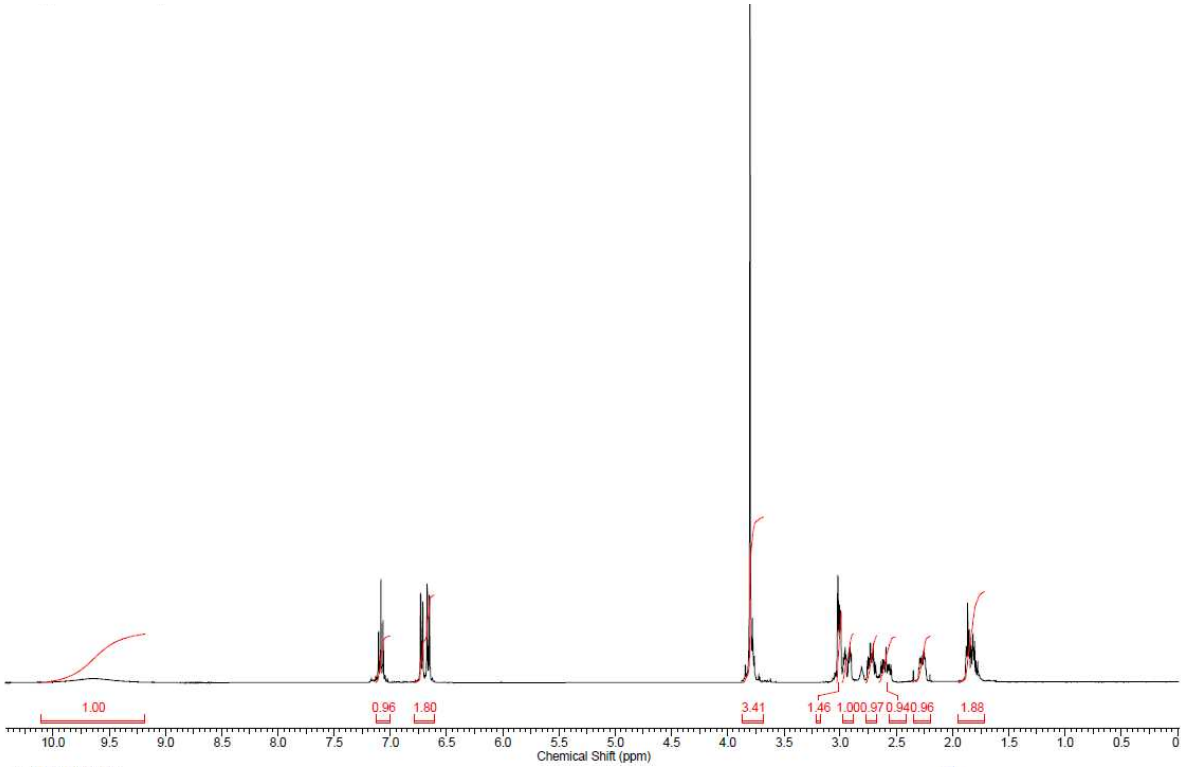
^{1}H and ^{13}C NMR spectra of compound **6e**

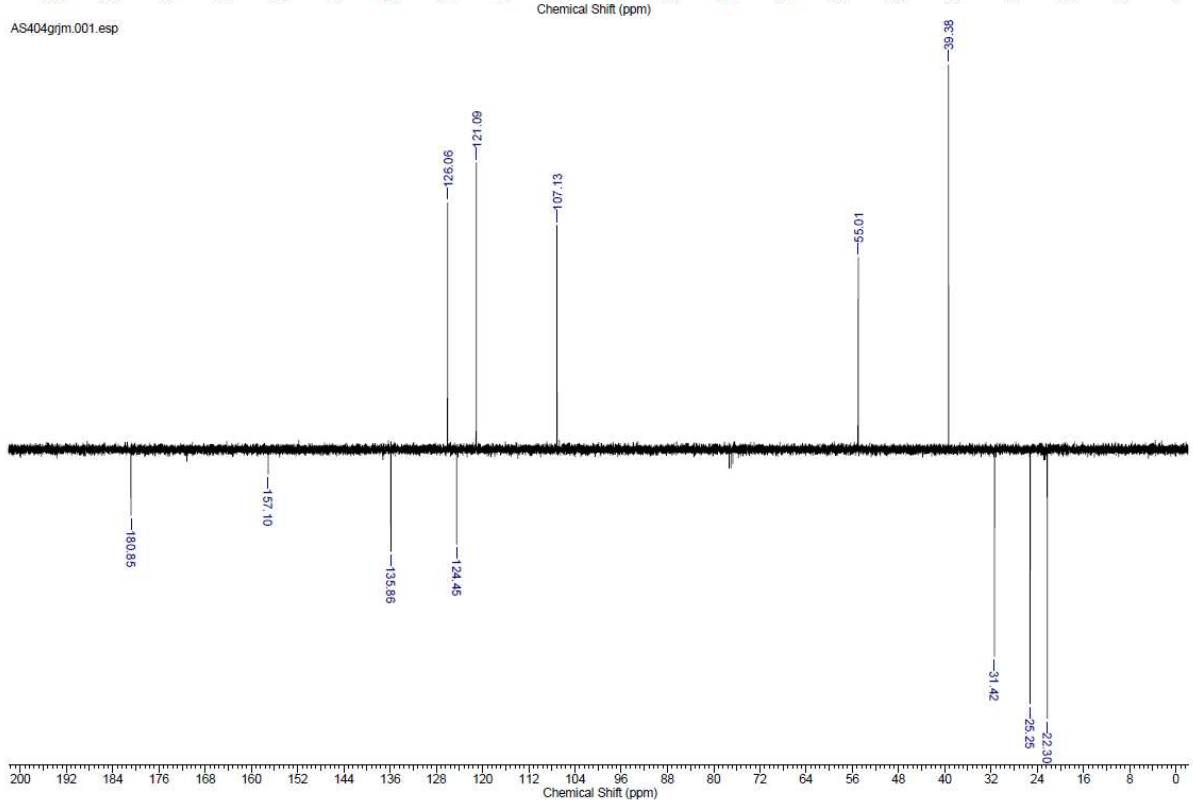




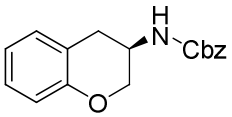


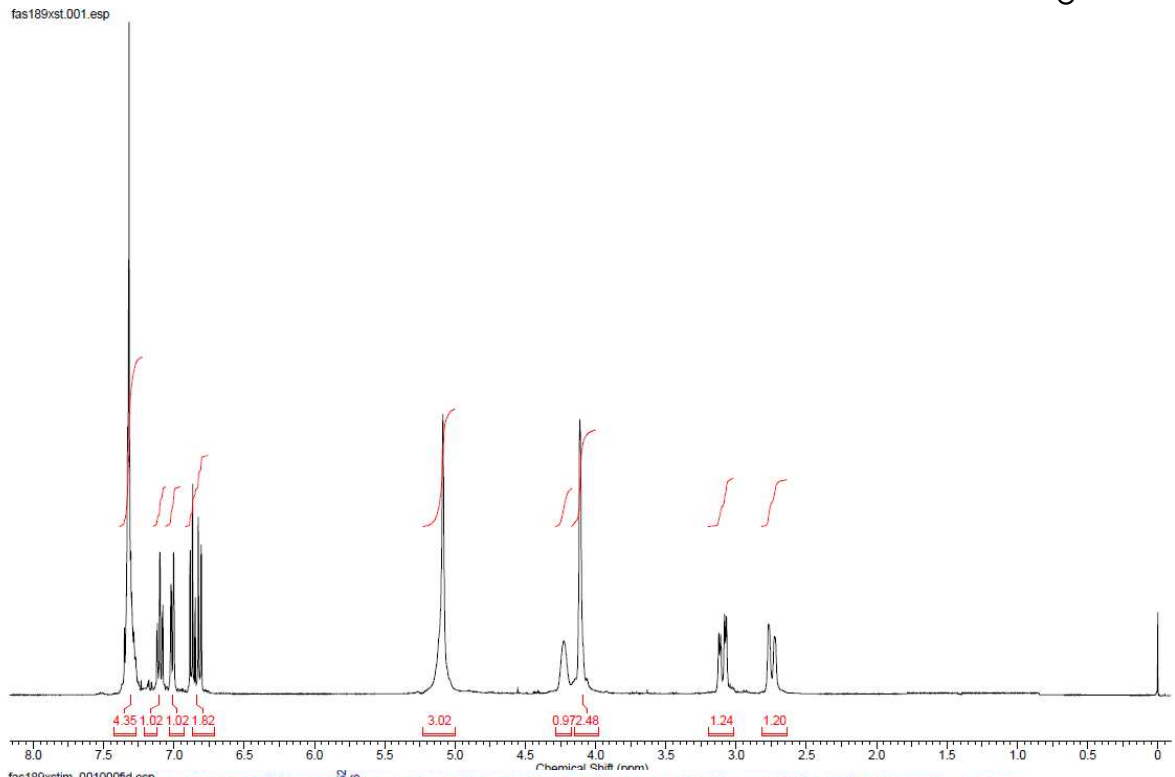
^{1}H and ^{13}C NMR spectra of compound **8e**

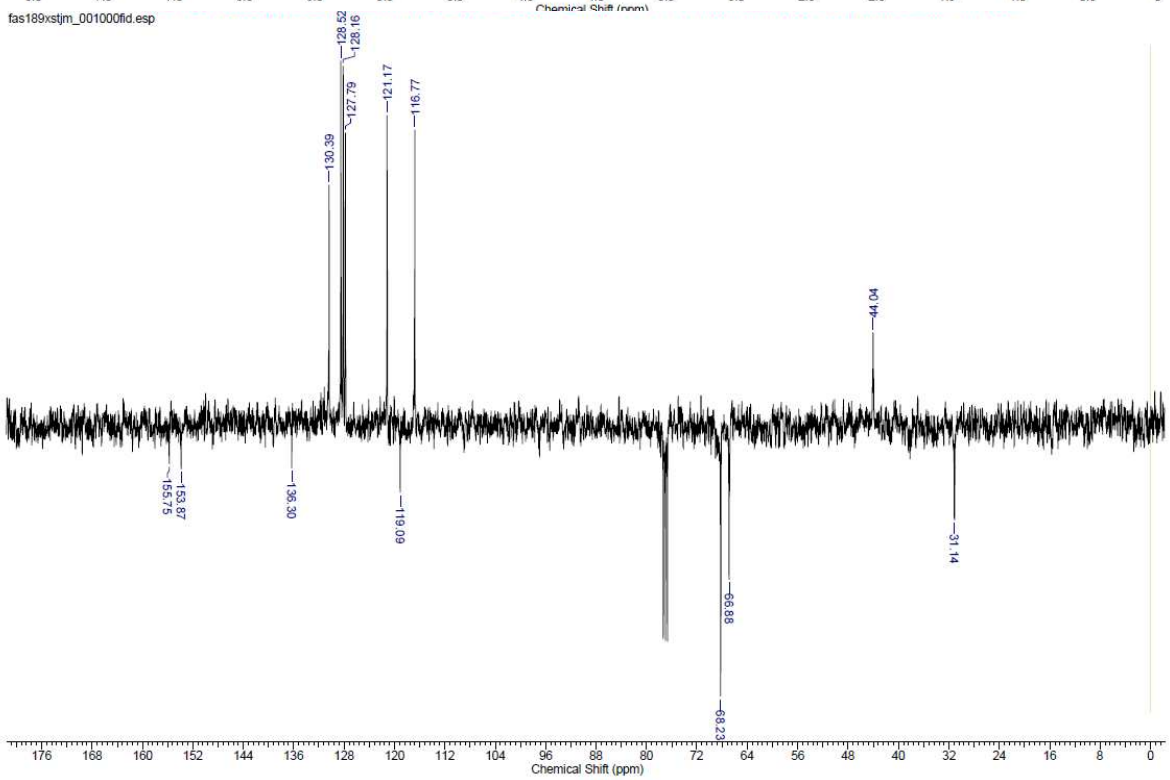




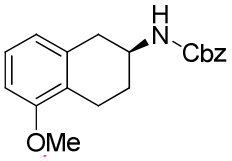
^{1}H and ^{13}C NMR spectra of compound **5f**

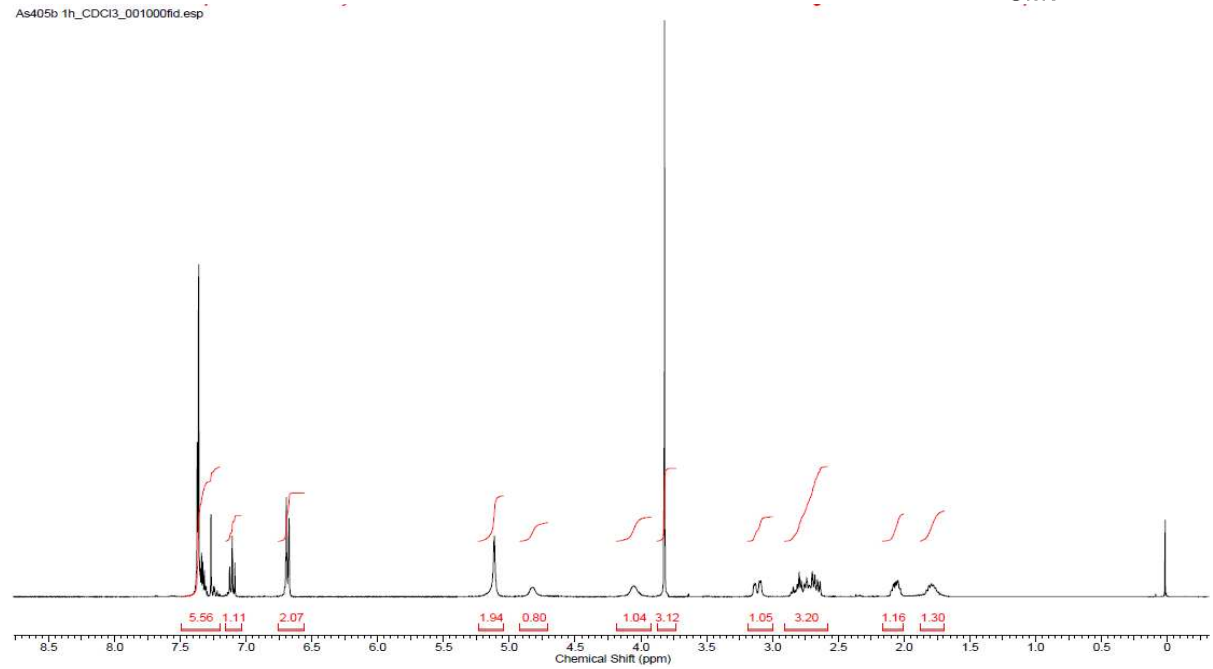


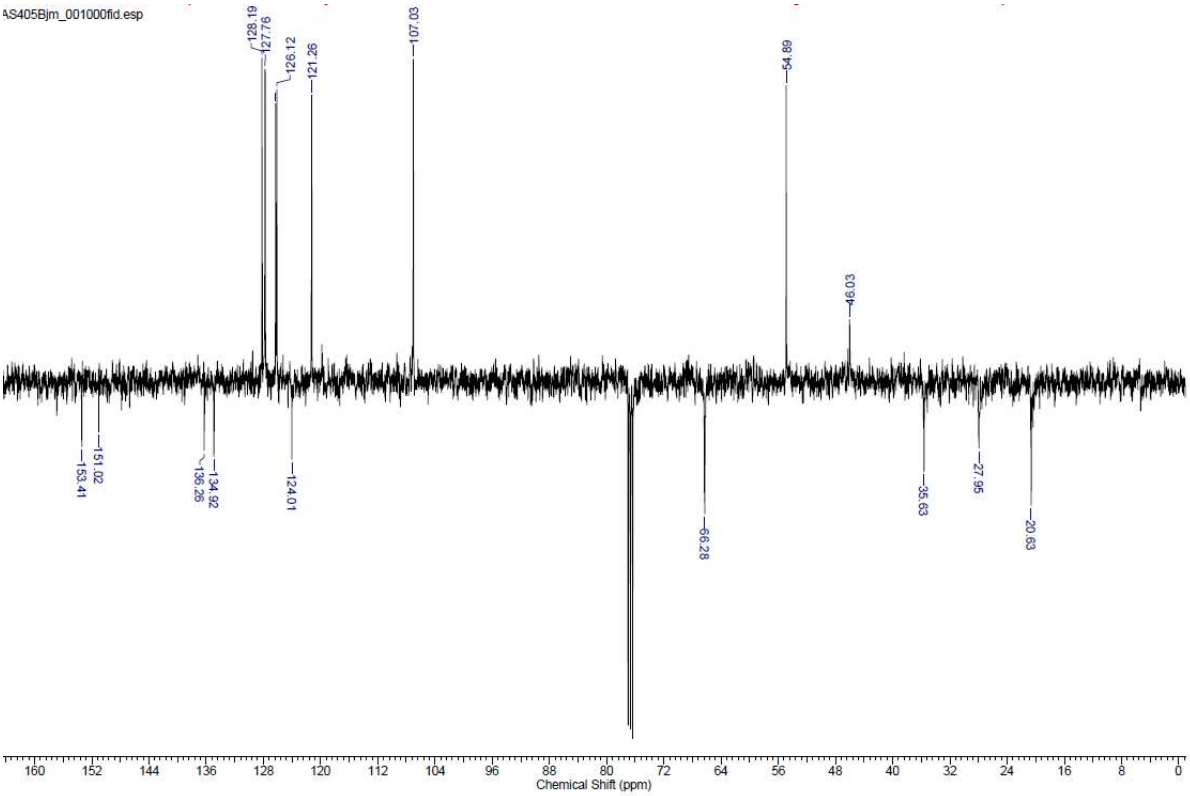




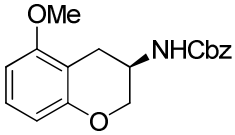
^{1}H and ^{13}C NMR spectra of compound **6f**

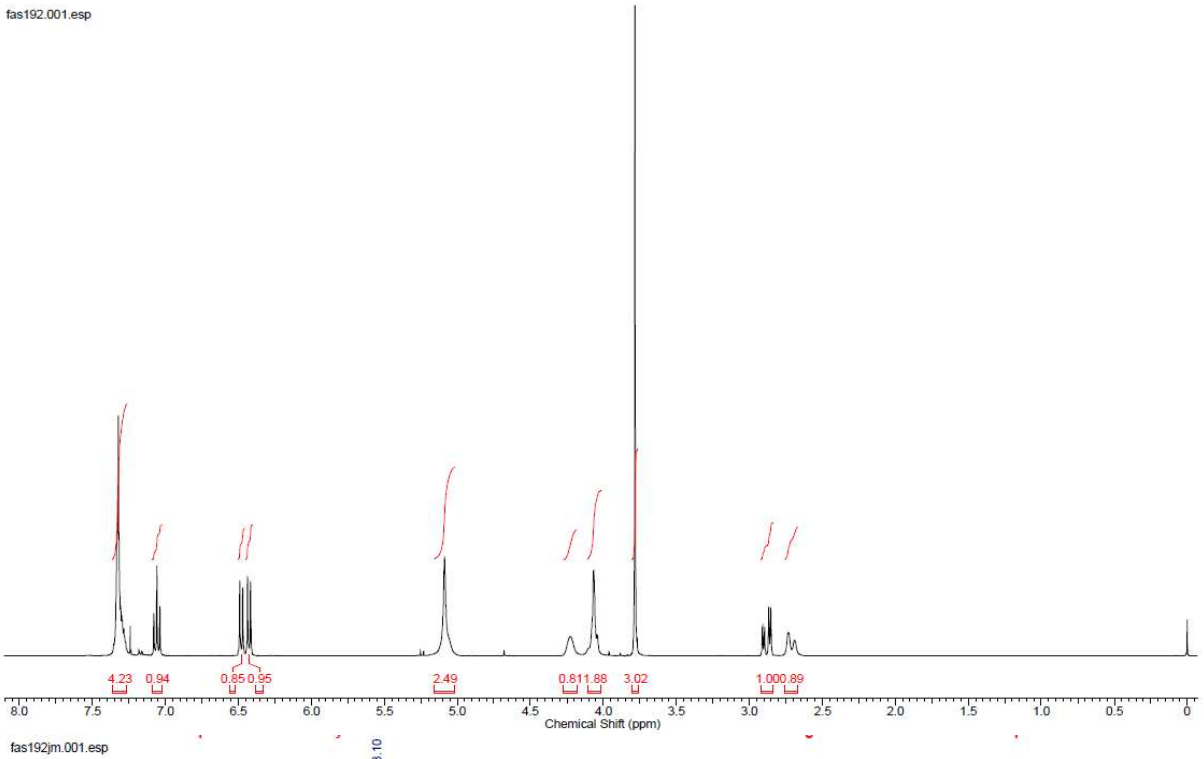


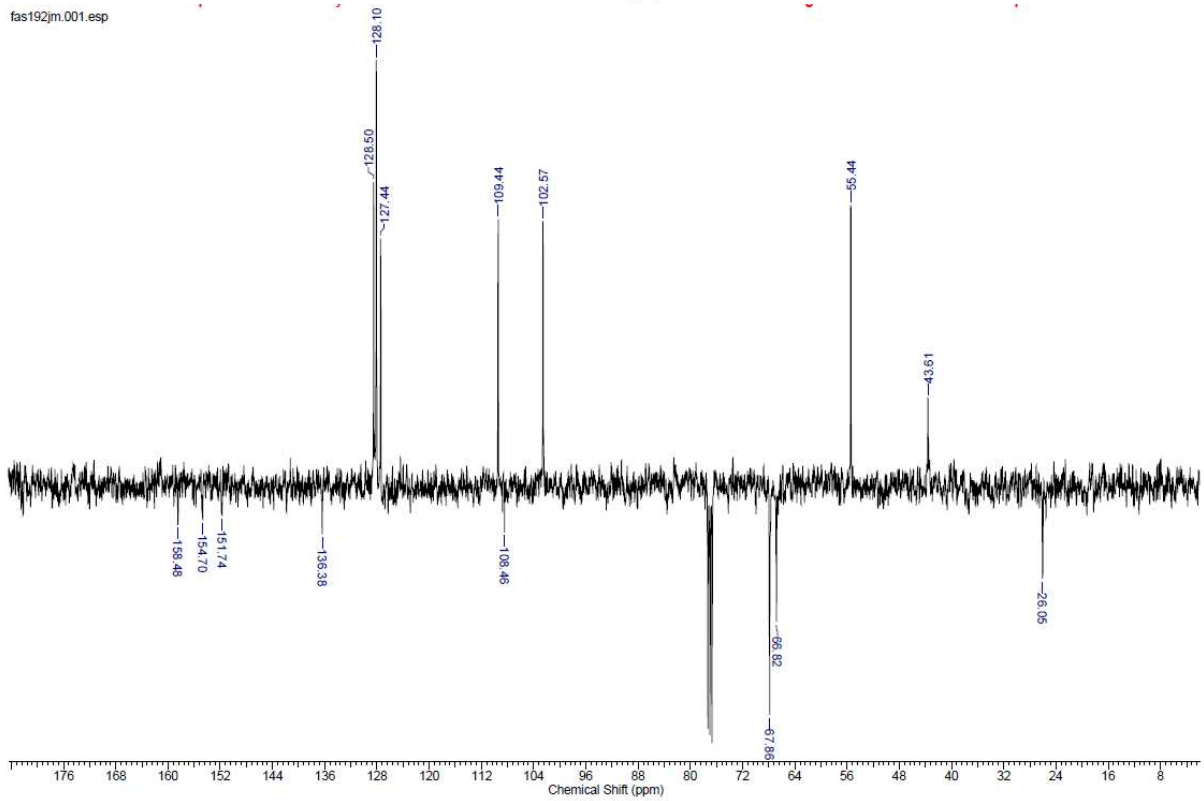




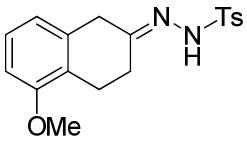
^{1}H and ^{13}C NMR spectra of compound **8f**

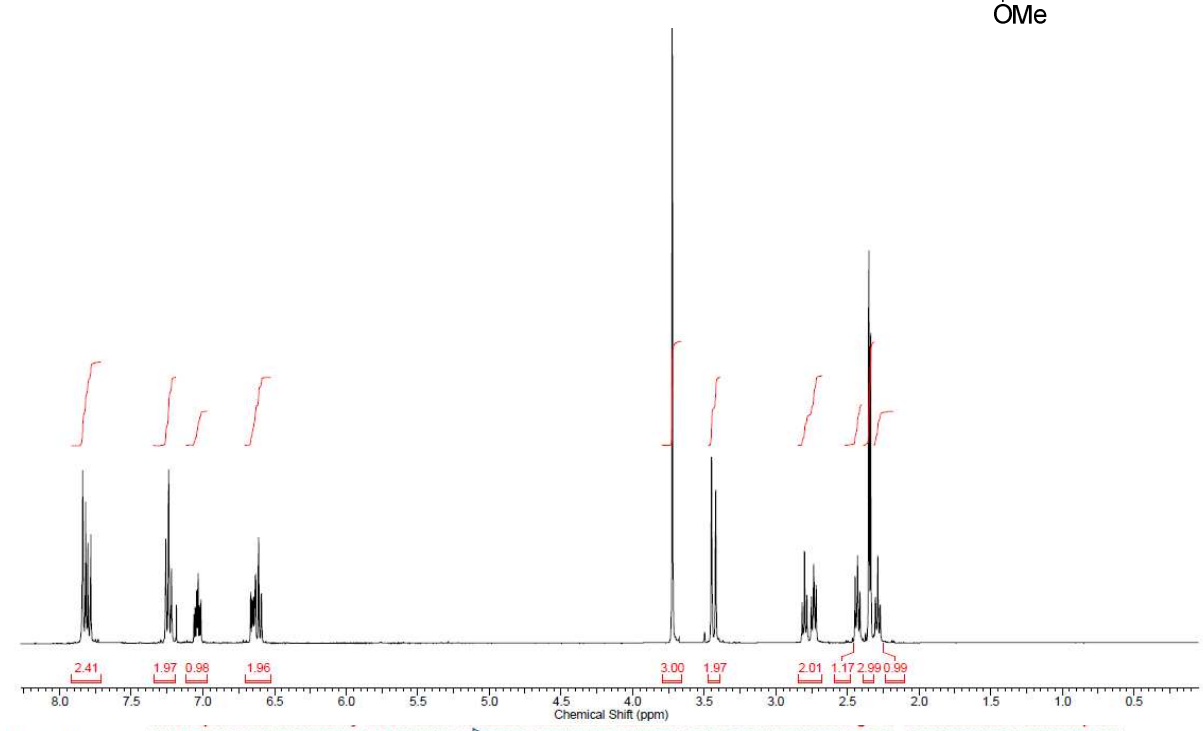


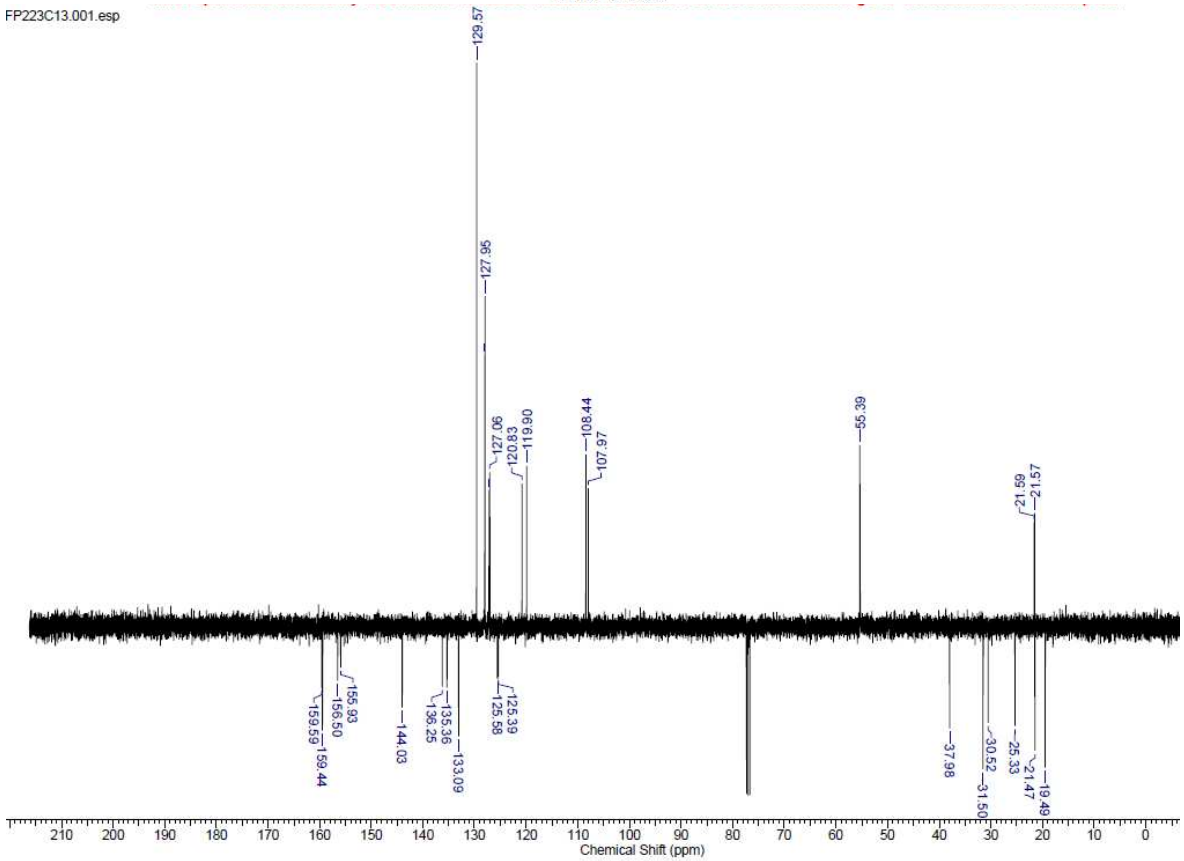




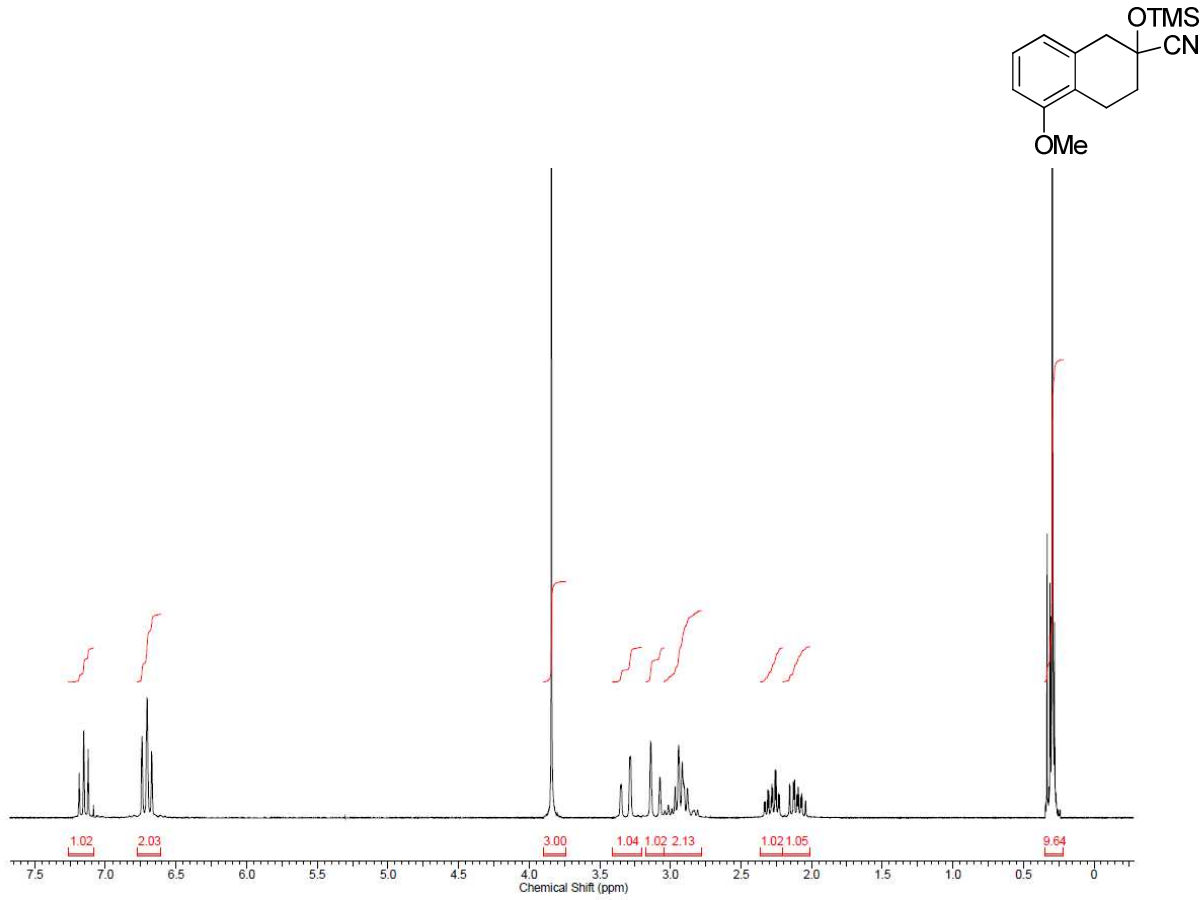
^{1}H and ^{13}C NMR spectra of compound **9**

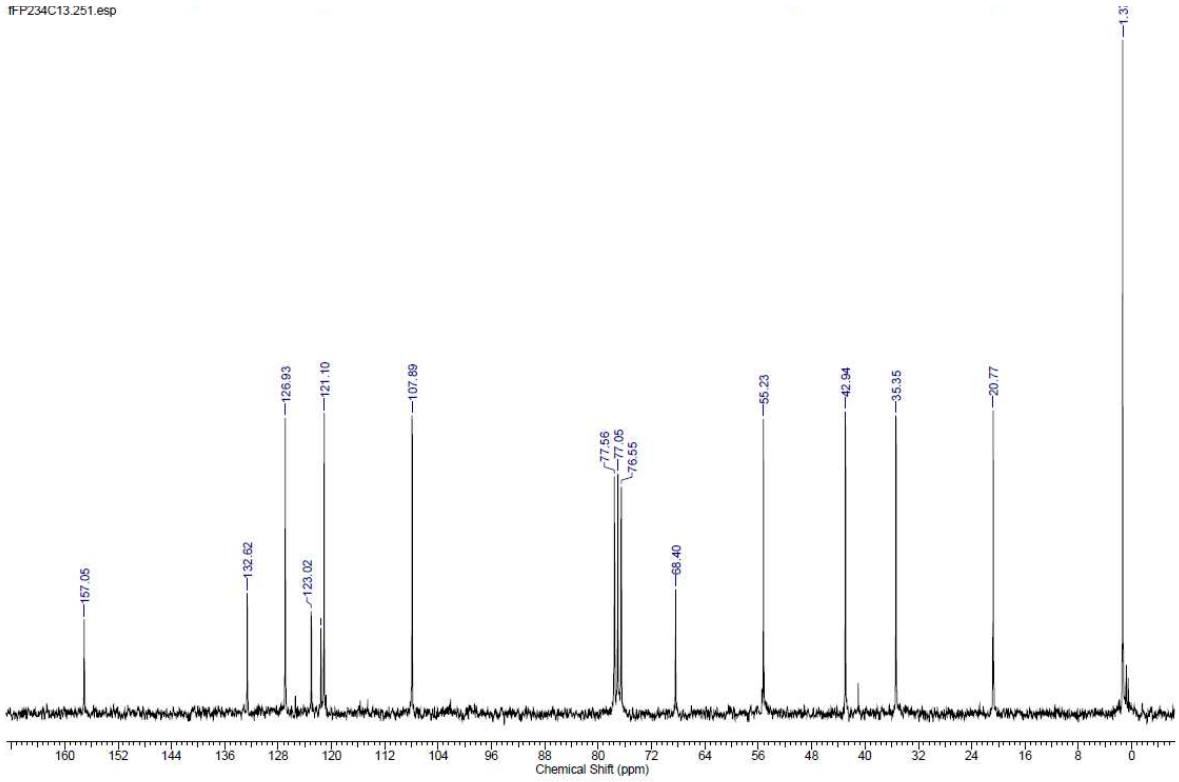




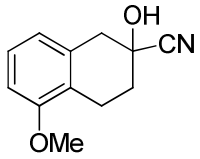


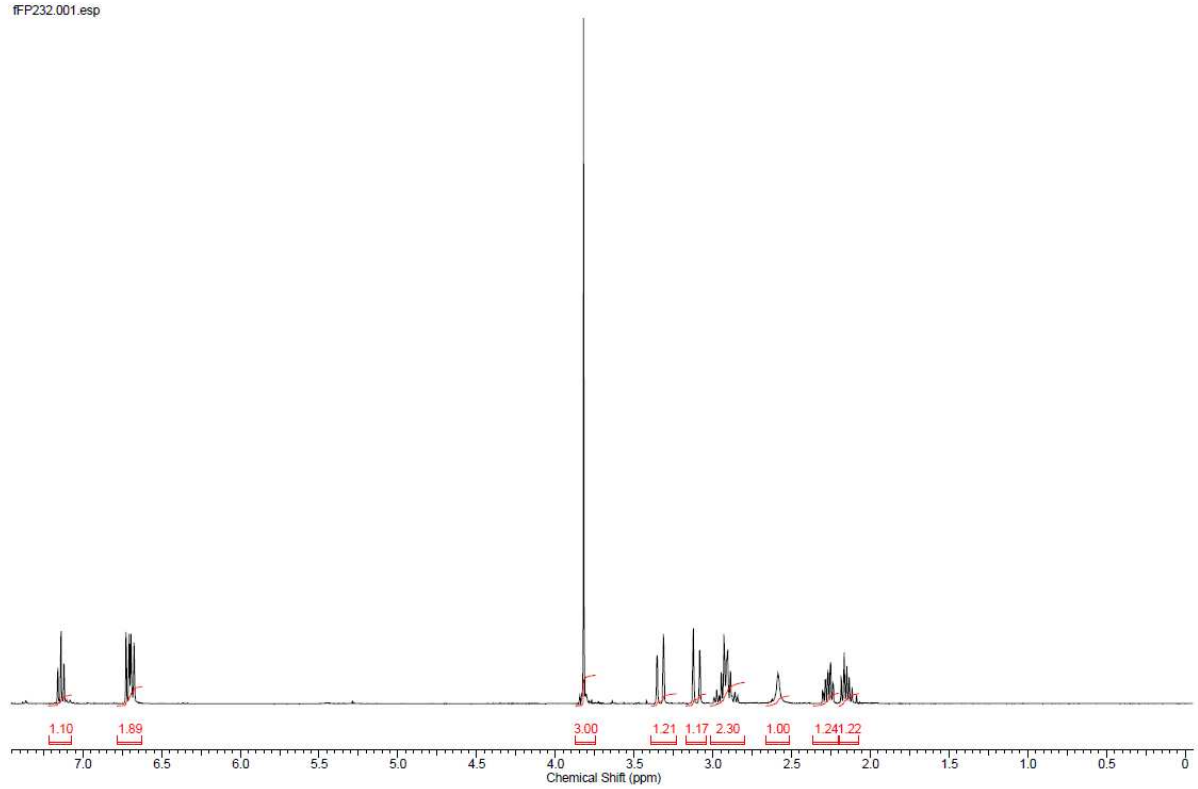
^{1}H and ^{13}C NMR spectra of compound **11**

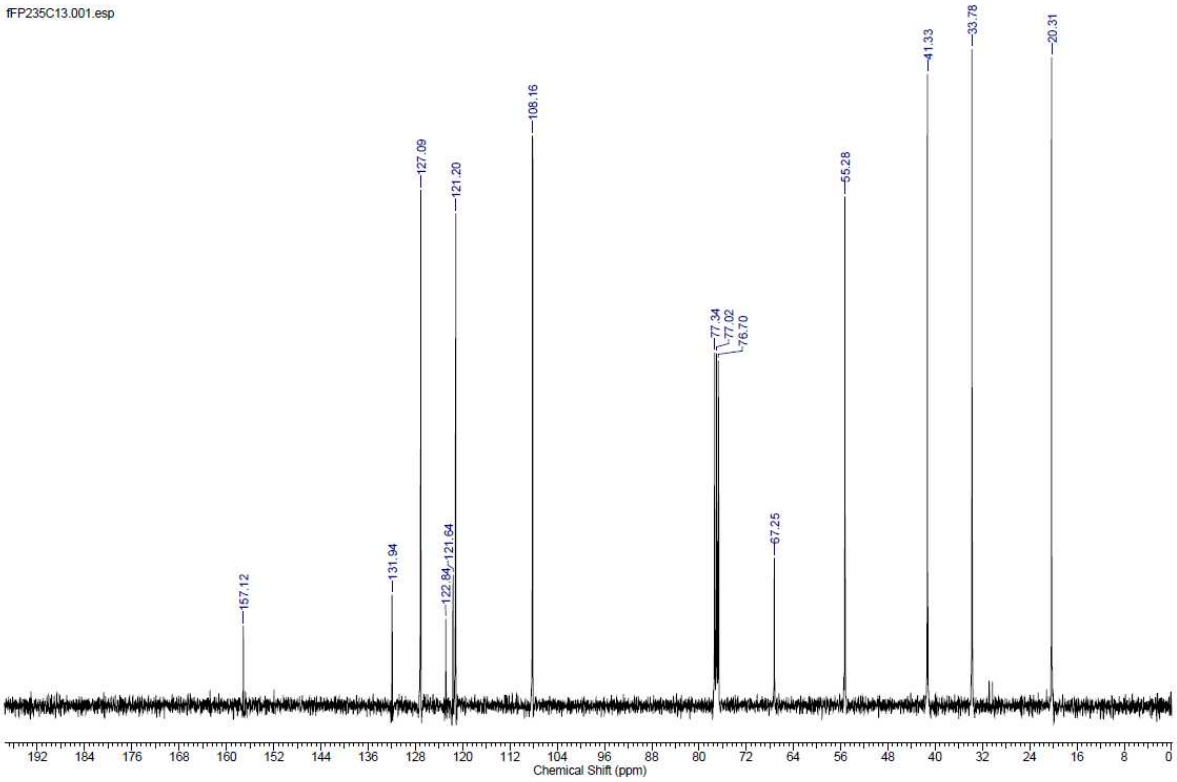




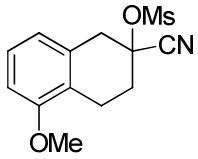
^{1}H and ^{13}C NMR spectra of the cyanohydrin intermediate to compound **12**

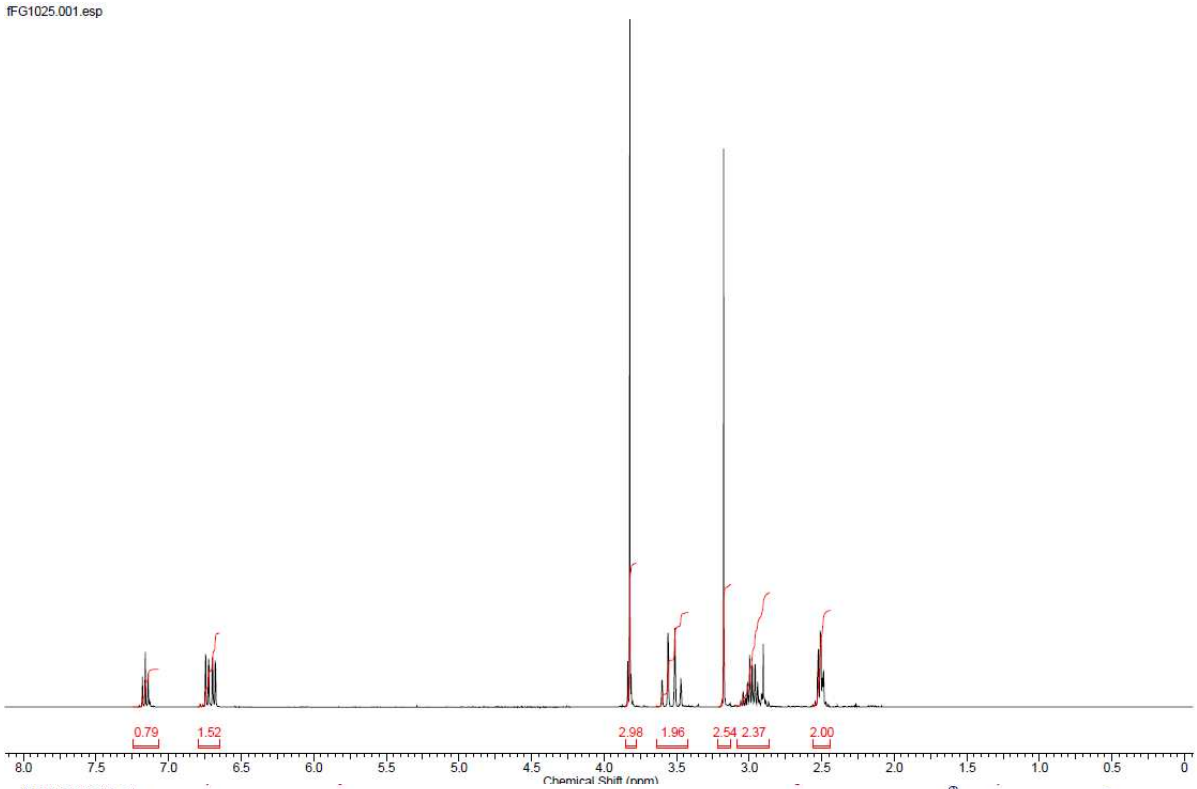


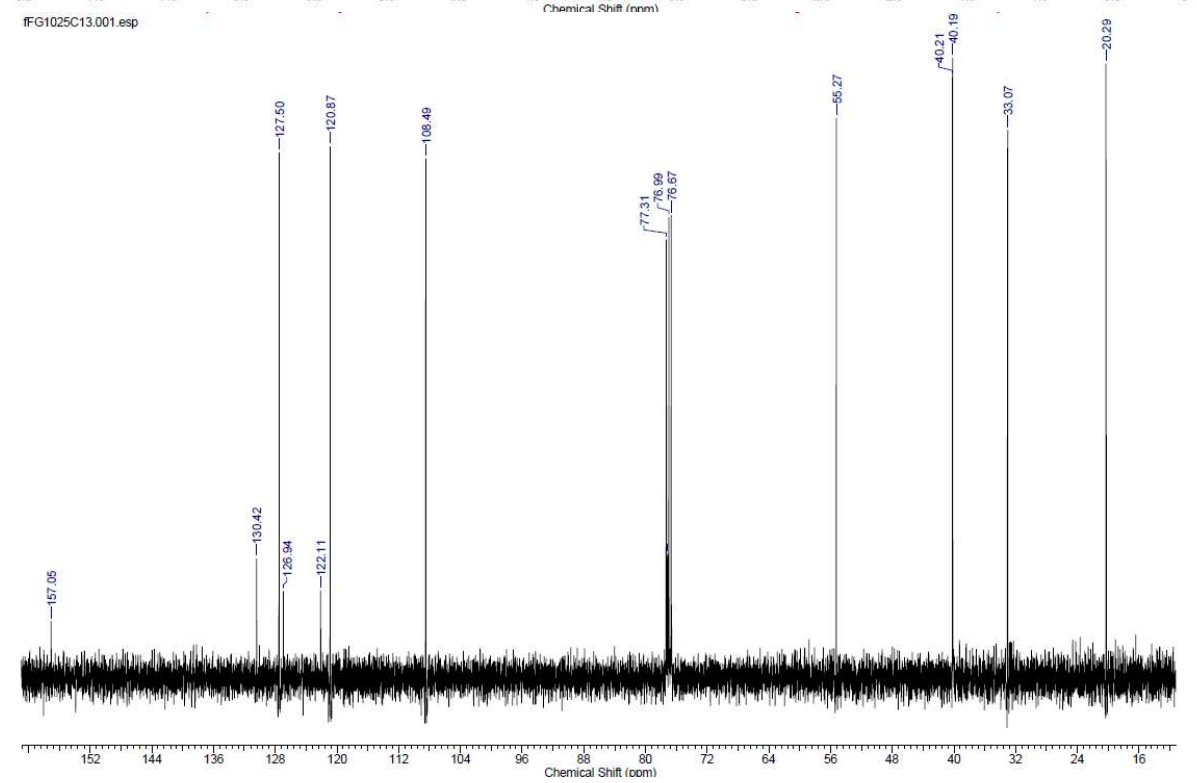




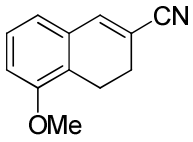
^{1}H and ^{13}C NMR spectra of compound **12**

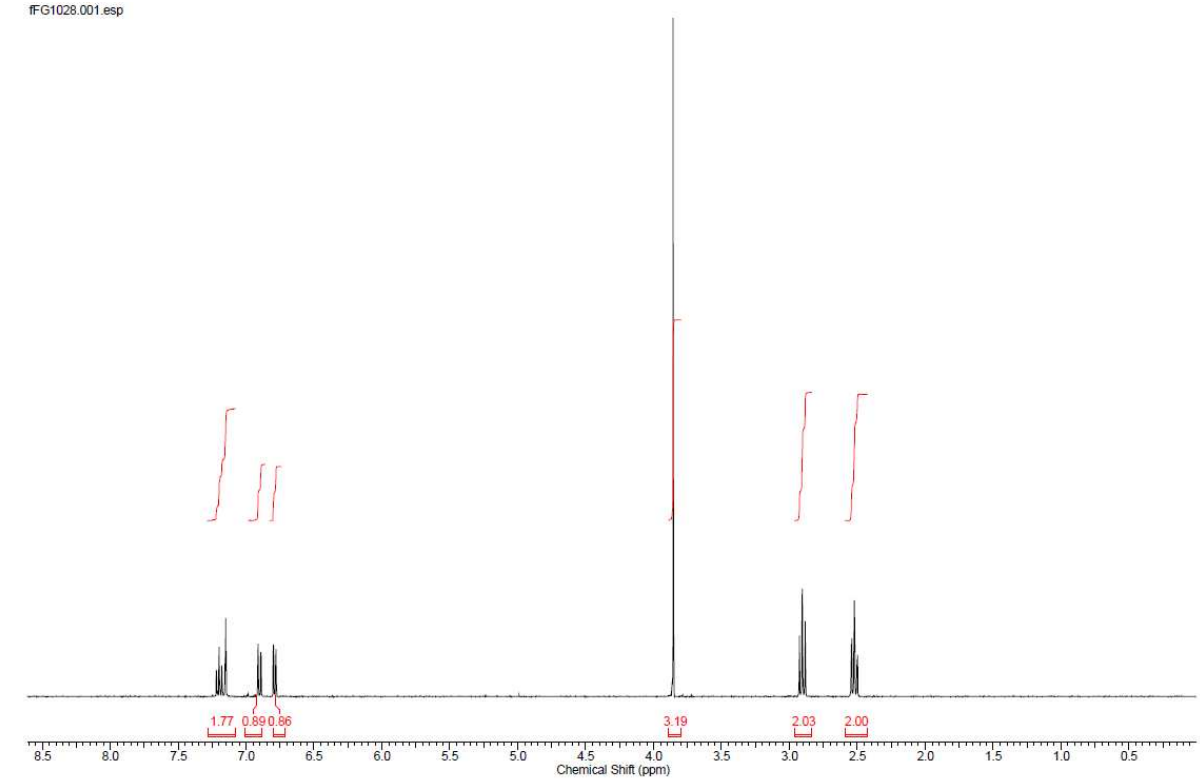


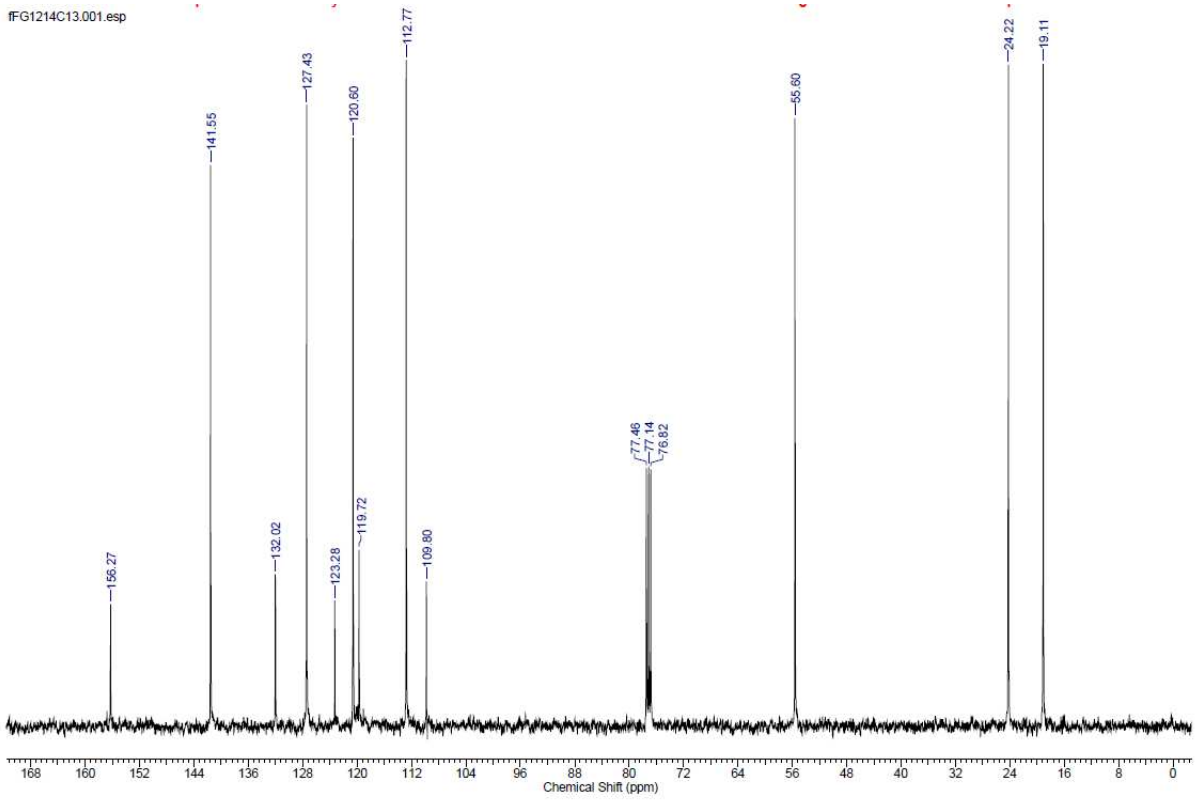




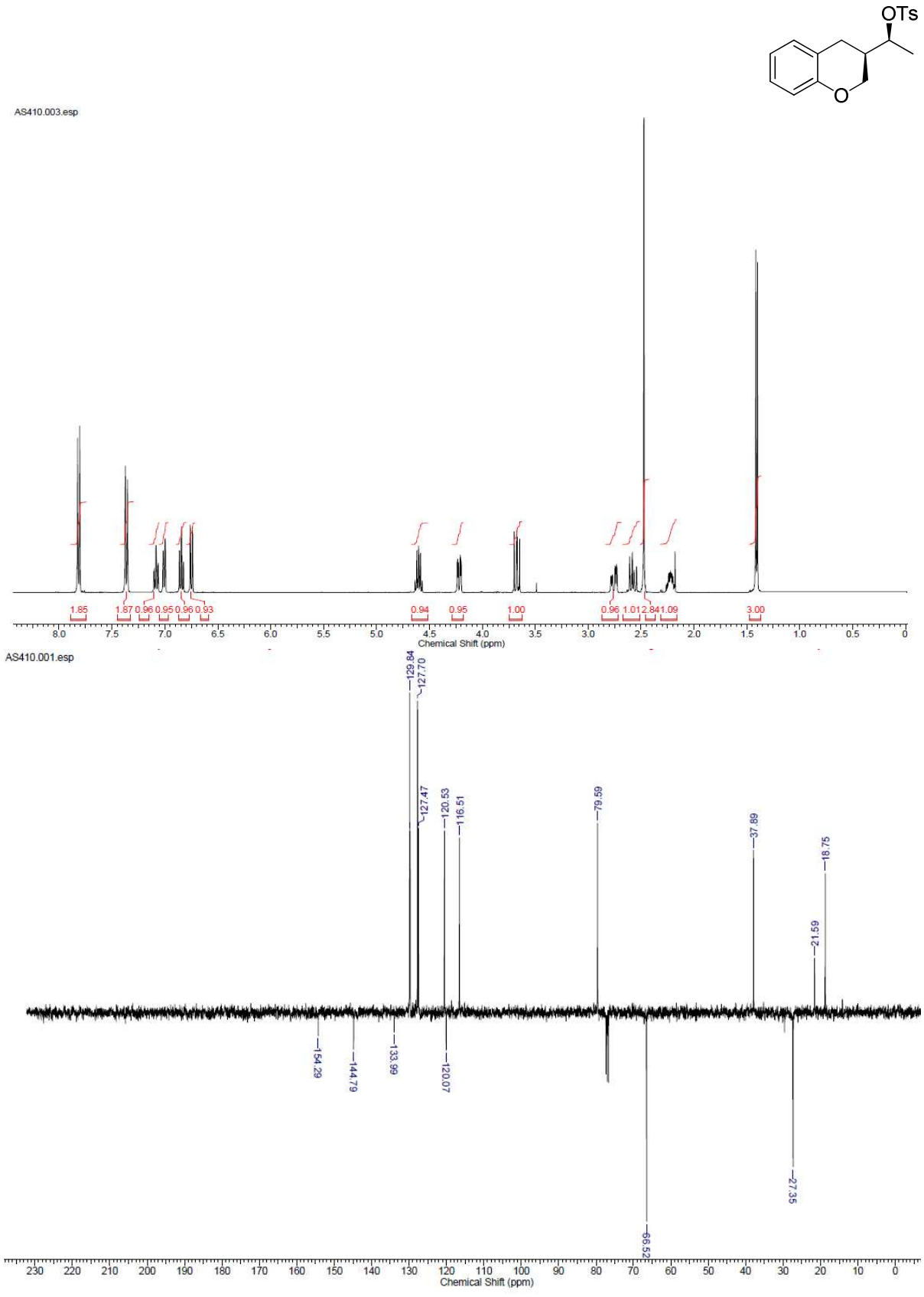
^{1}H and ^{13}C NMR spectra of compound **13**



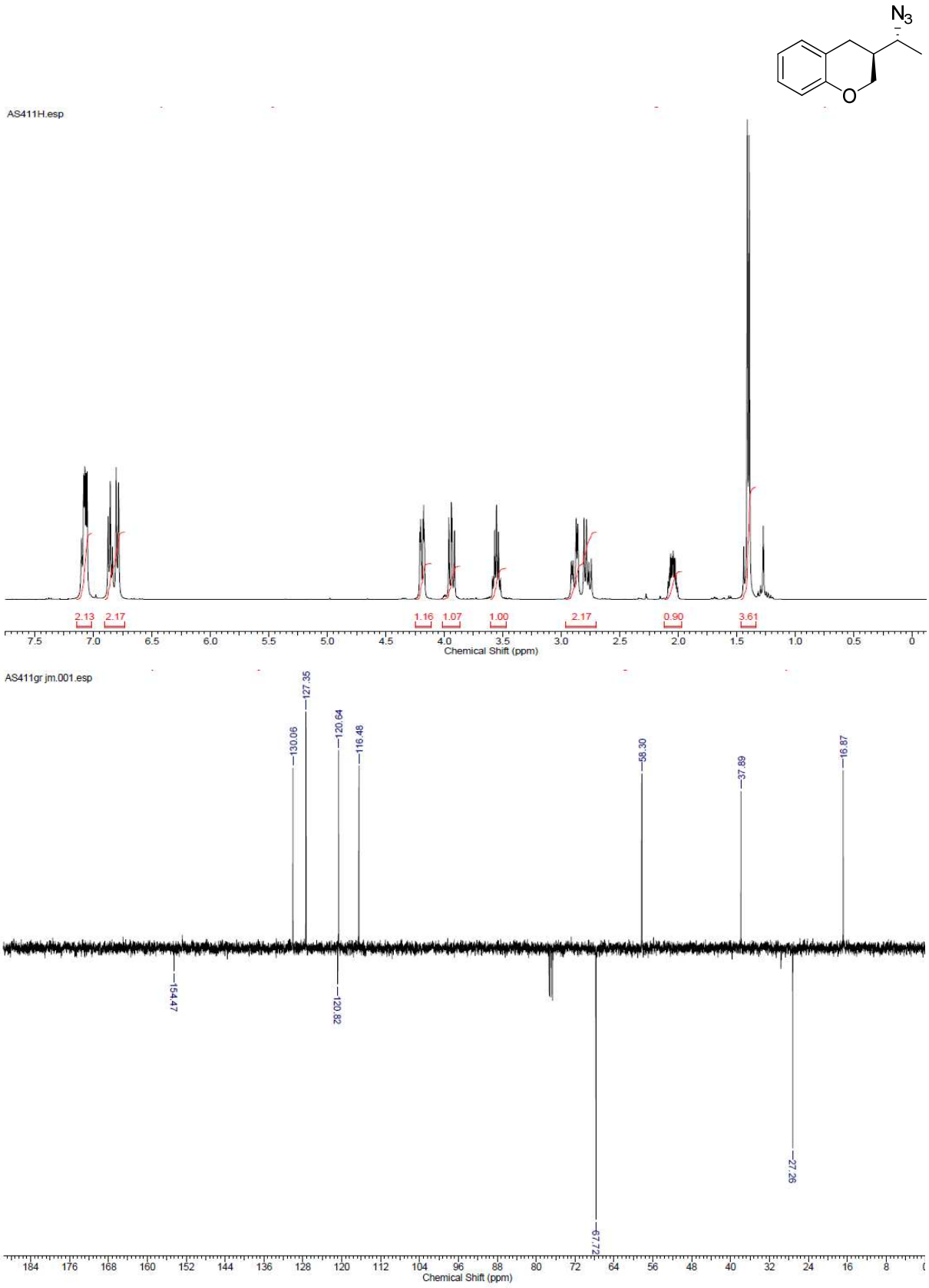




^{1}H and ^{13}C NMR spectra of the tosylate intermediate to compound **14**



^{1}H and ^{13}C NMR spectra of compound **14**



Tables of crystallographic data for *syn*-7c

Table S1. Crystal data and structure refinement for *syn*-**7c**.

Empirical formula	C11 H14 O2
Formula weight	178.22
Temperature	103(2) K
Wavelength	0.71073 A
Crystal system, space group	orthorombic, P $2_12_12_1$
Unit cell dimensions	a = 4.9986(7) A alpha = 90 deg. b = 16.723(3) A beta = 90 deg. c = 23.074(4) A gamma = 90 deg.
Volume	1928.8(6) A^3
Z, Calculated density	8, 1.227 Mg/m^3
Absorption coefficient	0.083 mm^-1
F(000)	768
Crystal size	0.60 x 0.05 x 0.03 mm
Theta range for data collection	2.92 to 24.99 deg.
Limiting indices	-5<=h<=5, -19<=k<=19, -27<=l<=23
Reflections collected / unique	13445 / 3291 [R(int) = 0.1038]
Completeness to theta = 24.99	98.6 %
Max. and min. transmission	0.9975 and 0.9519
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3291 / 0 / 244
Goodness-of-fit on F^2	1.028
Final R indices [I>2sigma(I)]	R1 = 0.0505, wR2 = 0.0995
R indices (all data)	R1 = 0.0741, wR2 = 0.1110
Absolute structure parameter	0.7(14)
Extinction coefficient	0.024(2)
Largest diff. peak and hole	0.282 and -0.255 e.A^-3

Table S2. Atomic coordinates ($\cdot 10^4$) and equivalent isotropic displacement parameters ($Å^2 \cdot 10^3$) for *syn*-**7c**. $U_{(eq)}$ is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
O(1A)	6486(3)	10156(1)	8293(1)	24(1)
O(2A)	1819(3)	8139(1)	8559(1)	24(1)
C(1A)	4126(5)	9671(2)	8348(1)	22(1)
C(2A)	3781(5)	9322(2)	8951(1)	18(1)
C(3A)	3578(5)	10008(1)	9384(1)	20(1)
C(4A)	5548(5)	10667(2)	9256(1)	18(1)
C(5A)	6166(5)	11248(2)	9667(1)	23(1)
C(6A)	8024(5)	11843(2)	9564(1)	27(1)
C(7A)	9308(5)	11873(2)	9031(1)	28(1)
C(8A)	8721(5)	11313(2)	8614(1)	25(1)
C(9A)	6865(5)	10710(2)	8726(1)	19(1)
C(10A)	1360(5)	8777(2)	8967(1)	19(1)
C(11A)	763(6)	8432(2)	9561(1)	28(1)
O(1B)	11756(3)	5608(1)	9213(1)	24(1)
O(2B)	6862(4)	7398(1)	8542(1)	29(1)
C(1B)	9361(5)	6033(2)	9052(1)	22(1)
C(2B)	9071(5)	6152(2)	8404(1)	17(1)
C(3B)	9104(5)	5339(2)	8104(1)	21(1)
C(4B)	11185(5)	4785(2)	8356(1)	21(1)
C(5B)	12016(5)	4103(2)	8064(1)	28(1)
C(6B)	13928(6)	3593(2)	8292(1)	36(1)
C(7B)	15033(5)	3755(2)	8828(1)	33(1)
C(8B)	14231(5)	4425(2)	9129(1)	27(1)
C(9B)	12337(5)	4933(2)	8893(1)	21(1)
C(10B)	6528(5)	6618(1)	8285(1)	19(1)
C(11B)	5861(6)	6702(2)	7648(1)	31(1)

Table S3. Bond lengths (Å) and angles (deg) for *syn*-**7c**.

O(1A)-C(9A)	1.376(3)
O(1A)-C(1A)	1.438(3)
O(2A)-C(10A)	1.443(3)
C(1A)-C(2A)	1.518(3)
C(2A)-C(10A)	1.515(3)
C(2A)-C(3A)	1.523(3)
C(3A)-C(4A)	1.507(3)
C(4A)-C(9A)	1.390(3)
C(4A)-C(5A)	1.393(3)
C(5A)-C(6A)	1.382(4)
C(6A)-C(7A)	1.388(4)
C(7A)-C(8A)	1.373(4)
C(8A)-C(9A)	1.395(3)
C(10A)-C(11A)	1.516(3)
O(1B)-C(9B)	1.380(3)
O(1B)-C(1B)	1.441(3)
O(2B)-C(10B)	1.441(3)
C(1B)-C(2B)	1.517(3)
C(2B)-C(10B)	1.516(3)
C(2B)-C(3B)	1.525(3)
C(3B)-C(4B)	1.509(4)
C(4B)-C(5B)	1.388(4)
C(4B)-C(9B)	1.390(4)
C(5B)-C(6B)	1.385(4)
C(6B)-C(7B)	1.381(4)
C(7B)-C(8B)	1.378(4)
C(8B)-C(9B)	1.384(4)
C(10B)-C(11B)	1.515(3)
C(9A)-O(1A)-C(1A)	115.37(18)
O(1A)-C(1A)-C(2A)	113.0(2)
C(10A)-C(2A)-C(1A)	110.1(2)
C(10A)-C(2A)-C(3A)	112.57(19)
C(1A)-C(2A)-C(3A)	108.5(2)
C(4A)-C(3A)-C(2A)	112.3(2)
C(9A)-C(4A)-C(5A)	117.2(2)
C(9A)-C(4A)-C(3A)	121.3(2)
C(5A)-C(4A)-C(3A)	121.4(2)
C(6A)-C(5A)-C(4A)	122.3(2)
C(5A)-C(6A)-C(7A)	119.3(2)
C(8A)-C(7A)-C(6A)	119.8(3)
C(7A)-C(8A)-C(9A)	120.3(2)
O(1A)-C(9A)-C(4A)	122.5(2)
O(1A)-C(9A)-C(8A)	116.3(2)
C(4A)-C(9A)-C(8A)	121.1(2)
O(2A)-C(10A)-C(2A)	107.60(18)
O(2A)-C(10A)-C(11A)	109.8(2)
C(2A)-C(10A)-C(11A)	114.1(2)
C(9B)-O(1B)-C(1B)	116.14(18)
O(1B)-C(1B)-C(2B)	113.47(19)
C(10B)-C(2B)-C(1B)	108.95(19)
C(10B)-C(2B)-C(3B)	112.7(2)
C(1B)-C(2B)-C(3B)	109.2(2)
C(4B)-C(3B)-C(2B)	112.4(2)
C(5B)-C(4B)-C(9B)	117.1(2)
C(5B)-C(4B)-C(3B)	121.6(2)
C(9B)-C(4B)-C(3B)	121.3(2)
C(6B)-C(5B)-C(4B)	121.8(3)
C(7B)-C(6B)-C(5B)	119.7(3)
C(8B)-C(7B)-C(6B)	119.7(3)
C(7B)-C(8B)-C(9B)	120.0(3)
O(1B)-C(9B)-C(8B)	115.8(2)
O(1B)-C(9B)-C(4B)	122.4(2)
C(8B)-C(9B)-C(4B)	121.7(2)
O(2B)-C(10B)-C(11B)	109.9(2)
O(2B)-C(10B)-C(2B)	107.11(19)
C(11B)-C(10B)-C(2B)	114.0(2)

Table S4. Hydrogen bonds (Å and deg) for *syn*-**7c**.

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(2B)-H(2OB)...O(2A)	0.86(3)	1.95(3)	2.809(2)	176(3)
O(2A)-H(2OA)...O(2B)#1	0.95(3)	1.84(3)	2.771(3)	165(3)

Symmetry transformations used to generate equivalent atoms:
#1 x-1,y,z

Table S5. Anisotropic displacement parameters ($Å^2 \cdot 10^3$) for *syn*-**7c**.

The anisotropic displacement factor exponent takes the form:
-2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
O(1A)	23(1)	24(1)	24(1)	-2(1)	5(1)	-5(1)
O(2A)	17(1)	21(1)	33(1)	-10(1)	1(1)	-2(1)
C(1A)	19(1)	23(2)	25(1)	0(1)	1(1)	-3(1)
C(2A)	12(1)	20(1)	23(1)	-1(1)	-2(1)	3(1)
C(3A)	19(1)	20(2)	20(1)	3(1)	0(1)	0(1)
C(4A)	15(1)	17(2)	22(2)	3(1)	-2(1)	3(1)
C(5A)	22(1)	22(2)	25(1)	-1(1)	-1(1)	3(1)
C(6A)	26(2)	18(2)	39(2)	-4(1)	-6(1)	0(1)
C(7A)	22(1)	15(2)	46(2)	6(1)	-2(1)	-2(1)
C(8A)	21(1)	21(2)	34(2)	6(1)	4(1)	1(1)
C(9A)	17(1)	18(1)	22(2)	2(1)	-2(1)	5(1)
C(10A)	17(1)	18(1)	22(1)	-4(1)	-1(1)	5(1)
C(11A)	27(2)	29(2)	29(2)	2(1)	-1(1)	-9(1)
O(1B)	22(1)	28(1)	23(1)	-1(1)	-4(1)	3(1)
O(2B)	17(1)	21(1)	50(1)	-12(1)	-3(1)	4(1)
C(1B)	18(1)	22(2)	26(2)	-4(1)	3(1)	1(1)
C(2B)	13(1)	20(1)	20(1)	1(1)	1(1)	-2(1)
C(3B)	21(1)	21(2)	21(1)	-2(1)	0(1)	0(1)
C(4B)	18(1)	19(1)	25(1)	2(1)	6(1)	-1(1)
C(5B)	28(2)	24(2)	33(2)	-4(1)	3(1)	-2(1)
C(6B)	34(2)	19(2)	55(2)	-3(2)	5(2)	4(1)
C(7B)	24(2)	26(2)	49(2)	12(2)	0(2)	4(1)
C(8B)	22(1)	30(2)	30(2)	11(1)	-3(1)	-4(1)
C(9B)	19(1)	20(2)	23(1)	5(1)	7(1)	-3(1)
C(10B)	14(1)	18(1)	25(1)	-3(1)	0(1)	-1(1)
C(11B)	30(2)	34(2)	28(2)	1(1)	-1(1)	9(1)

Table S6. Hydrogen coordinates ($\cdot 10^4$) and isotropic displacement parameters ($Å^2 \cdot 10^3$) for *syn*-**7c**.

	x	y	z	U(eq)
H(2OA)	260(60)	7819(18)	8508(11)	35
H(1AA)	4218	9228	8064	26
H(2AB)	2538	9999	8253	26
H(2A)	5405	8998	9047	22
H(3AA)	1742	10230	9373	24
H(3AB)	3906	9800	9779	24
H(5A)	5280	11234	10031	28
H(6A)	8417	12228	9854	33
H(7A)	10589	12279	8955	33
H(8A)	9583	11337	8247	30
H(10A)	-235	9090	8836	23
H(11A)	-748	8060	9532	42
H(11B)	309	8866	9829	42
H(11C)	2340	8146	9705	42
H(2OB)	5350(60)	7645(19)	8546(13)	44
H(1BA)	9368	6563	9243	26
H(1BB)	7788	5734	9197	26
H(2B)	10629	6471	8261	21
H(3BA)	7319	5088	8142	25
H(3BB)	9468	5415	7686	25
H(5B)	11252	3982	7697	34
H(6B)	14478	3133	8081	43
H(7B)	16338	3406	8989	40
H(8B)	14978	4538	9499	33
H(10B)	5006	6342	8483	23
H(11D)	4243	7028	7604	46
H(11E)	5555	6171	7481	46
H(11F)	7354	6962	7447	46

Table S7. Torsion angles (deg) for *syn*-**7c**.

C(9A)-O(1A)-C(1A)-C(2A)	-47.8(3)
O(1A)-C(1A)-C(2A)-C(10A)	-176.42(19)
O(1A)-C(1A)-C(2A)-C(3A)	60.0(3)
C(10A)-C(2A)-C(3A)-C(4A)	-163.9(2)
C(1A)-C(2A)-C(3A)-C(4A)	-41.7(3)
C(2A)-C(3A)-C(4A)-C(9A)	14.8(3)
C(2A)-C(3A)-C(4A)-C(5A)	-164.0(2)
C(9A)-C(4A)-C(5A)-C(6A)	-0.6(4)
C(3A)-C(4A)-C(5A)-C(6A)	178.1(2)
C(4A)-C(5A)-C(6A)-C(7A)	0.7(4)
C(5A)-C(6A)-C(7A)-C(8A)	0.0(4)
C(6A)-C(7A)-C(8A)-C(9A)	-0.8(4)
C(1A)-O(1A)-C(9A)-C(4A)	17.3(3)
C(1A)-O(1A)-C(9A)-C(8A)	-164.6(2)
C(5A)-C(4A)-C(9A)-O(1A)	177.9(2)
C(3A)-C(4A)-C(9A)-O(1A)	-0.9(3)
C(5A)-C(4A)-C(9A)-C(8A)	-0.1(3)
C(3A)-C(4A)-C(9A)-C(8A)	-178.9(2)
C(7A)-C(8A)-C(9A)-O(1A)	-177.3(2)
C(7A)-C(8A)-C(9A)-C(4A)	0.8(4)
C(1A)-C(2A)-C(10A)-O(2A)	60.8(2)
C(3A)-C(2A)-C(10A)-O(2A)	-177.91(19)
C(1A)-C(2A)-C(10A)-C(11A)	-177.0(2)
C(3A)-C(2A)-C(10A)-C(11A)	-55.8(3)
C(9B)-O(1B)-C(1B)-C(2B)	-45.6(3)
O(1B)-C(1B)-C(2B)-C(10B)	-178.80(19)
O(1B)-C(1B)-C(2B)-C(3B)	57.7(3)
C(10B)-C(2B)-C(3B)-C(4B)	-163.1(2)
C(1B)-C(2B)-C(3B)-C(4B)	-41.8(3)
C(2B)-C(3B)-C(4B)-C(5B)	-163.7(2)
C(2B)-C(3B)-C(4B)-C(9B)	16.8(3)
C(9B)-C(4B)-C(5B)-C(6B)	-0.6(4)
C(3B)-C(4B)-C(5B)-C(6B)	179.9(2)
C(4B)-C(5B)-C(6B)-C(7B)	0.8(4)
C(5B)-C(6B)-C(7B)-C(8B)	-0.3(4)
C(6B)-C(7B)-C(8B)-C(9B)	-0.3(4)
C(1B)-O(1B)-C(9B)-C(8B)	-165.1(2)
C(1B)-O(1B)-C(9B)-C(4B)	17.5(3)
C(7B)-C(8B)-C(9B)-O(1B)	-177.0(2)
C(7B)-C(8B)-C(9B)-C(4B)	0.5(4)
C(5B)-C(4B)-C(9B)-O(1B)	177.2(2)
C(3B)-C(4B)-C(9B)-O(1B)	-3.2(4)
C(5B)-C(4B)-C(9B)-C(8B)	-0.1(4)
C(3B)-C(4B)-C(9B)-C(8B)	179.5(2)
C(1B)-C(2B)-C(10B)-O(2B)	64.1(2)
C(3B)-C(2B)-C(10B)-O(2B)	-174.44(19)
C(1B)-C(2B)-C(10B)-C(11B)	-174.0(2)
C(3B)-C(2B)-C(10B)-C(11B)	-52.6(3)

Crystal structure of *syn*-7c

Figure S6. View of the X-ray molecular structure of (1*S*,3'*R*)-**7c** showing the atom numbering scheme. Displacement ellipsoids are drawn at the 40% probability level.

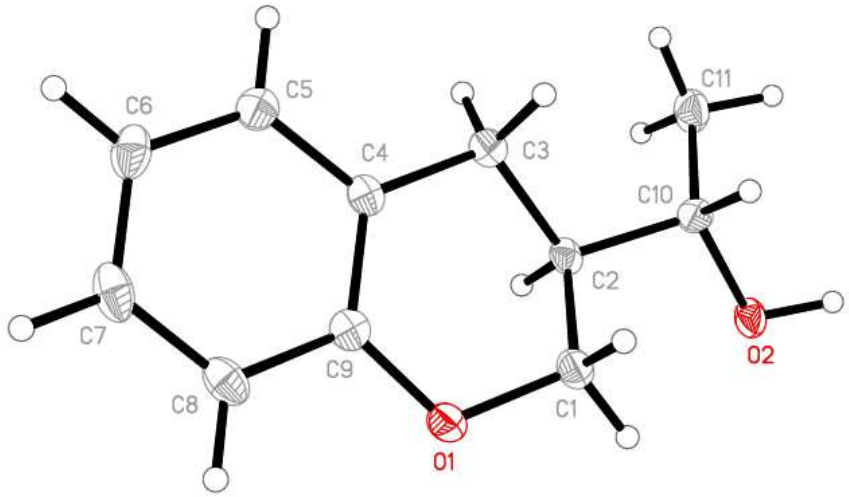
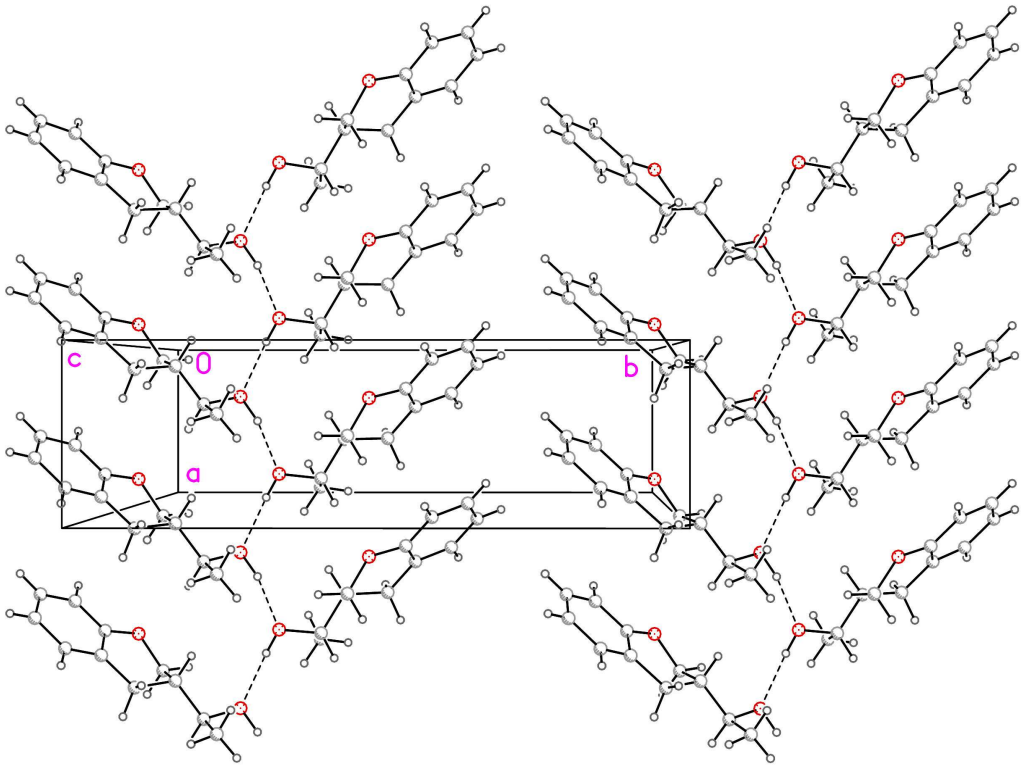


Figure S7. Crystal packing of the molecules of *syn*-**7c**, viewed along the *c* axis. Dashed lines indicate hydrogen bonds.



Expanded NMR spectra of alcohol 7c and of its (*S*)-MTPA derivative

Figure S8. Expanded ^{1}H NMR spectra of optically pure single diastereoisomers of saturated alcohol **7c** and *rac*-**7c** (*anti/syn* ratio 6:4).

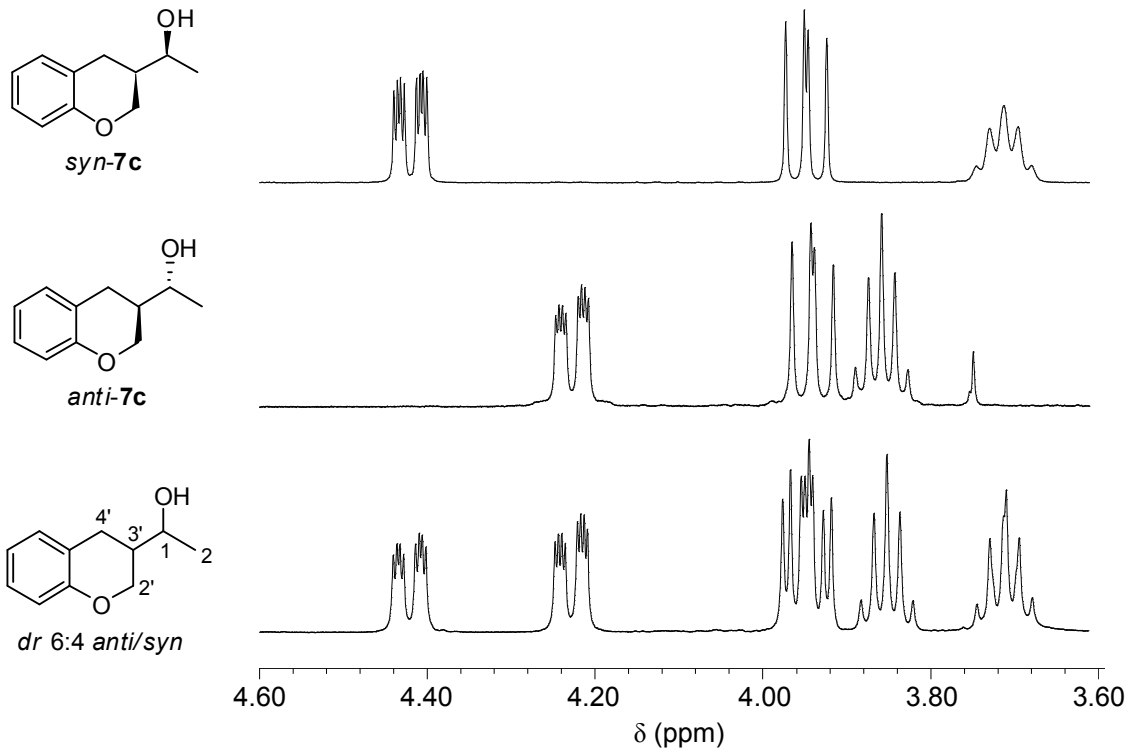


Figure S9. Expanded ^{1}H and ^{13}C NMR spectra of the CH_3 signals of the (*S*)-MTPA derivatives of (1*S*,3'*R*)-**7c** and of *rac*-**7c** (*anti/syn* ratio 6:4).

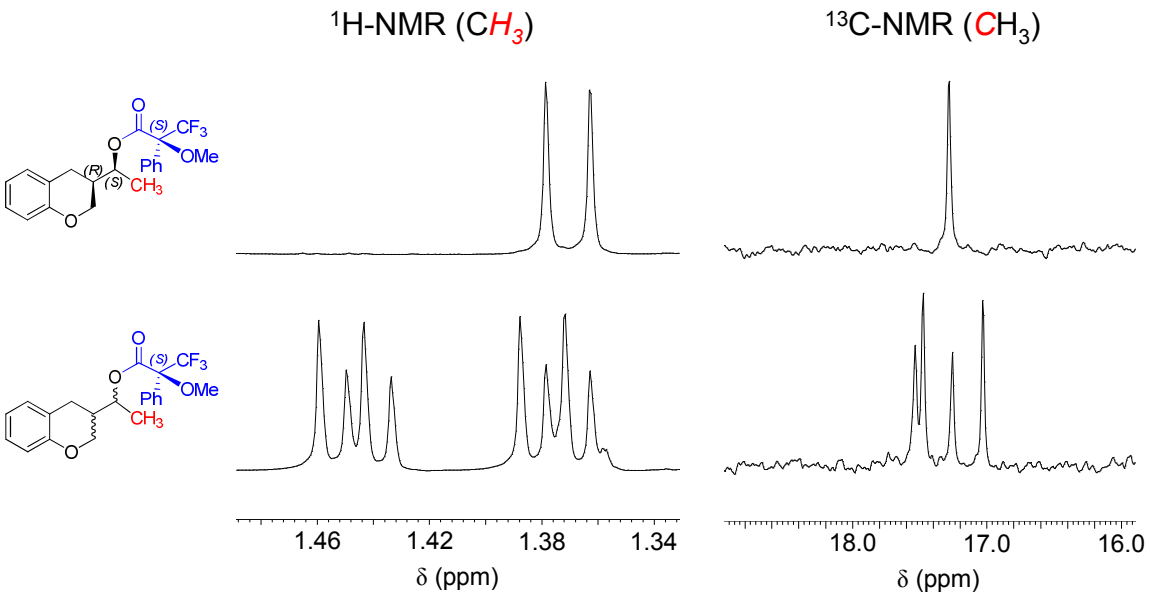


Figure S10. Expanded ^{1}H and ^{13}C NMR spectra of the OCH_2 signals of the (*S*)-MTPA derivatives of (1*S*,3'*R*)-**7c** and of *rac*-**7c** (*anti/syn* ratio 6:4).

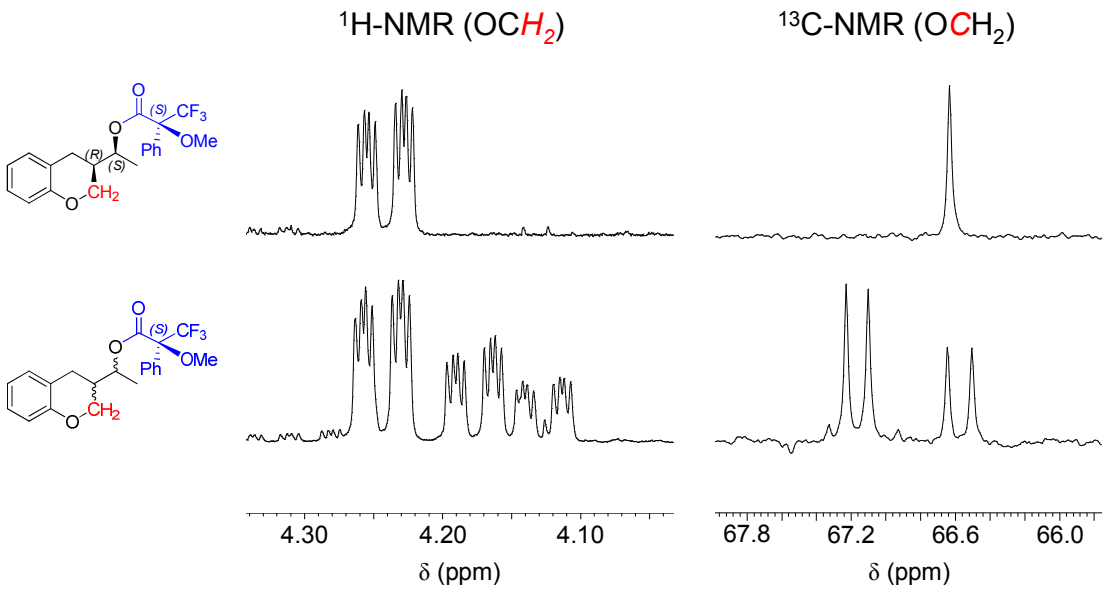


Figure S11. Expanded ^{19}F NMR spectra of the CF_3 signals of the (*S*)-MTPA derivatives of (1*S*,3'*R*)-**7c** and of *rac*-**7c** (*anti/syn* ratio 6:4).

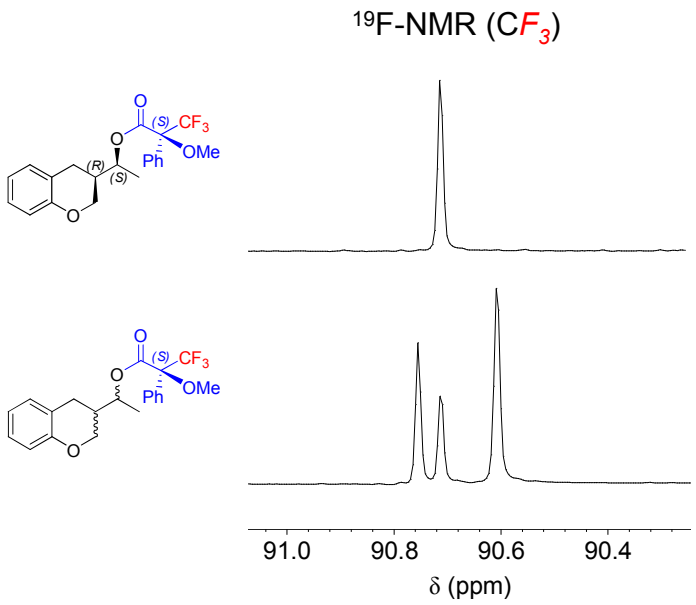
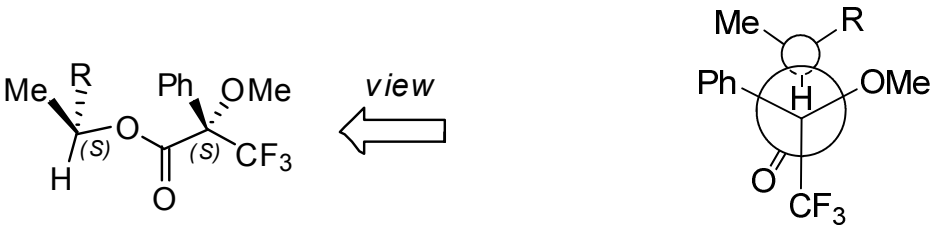


Figure S12. Preferred conformation of the (*S*)-MTPA-(1*S*,3'*R*)-**7c** according to the model proposed by Mosher. The methyl group lies closer to the shielding region of the phenyl ring of (*S*)-MTPA (upfield with respect to the antipode alcohol (1*R*,3'*S*)-**7c**) The CH_2O group, included in the R substituent, lies further away from the phenyl ring of (*S*)-MTPA (downfield with respect to the antipode alcohol (1*R*,3'*S*)-**7c**).



Computational details for (*S*)-MTPA-(1*S*,3'*R*)-7c

Computational studies have been carried out in order to determinate the lowest energy conformer for the Mosher ester derivative of **7c** using the software Spartan '08.[1] First a conformational analysis was performed at the PM3 level to find the lowest energy conformers. The first six conformers (representing the 95% of population according to Boltzmann distribution) were further optimized with DFT at the B3LYP-6-31G* level in chloroform. The lowest energy conformer was assumed for the NMR analysis. It has to be noticed that this conformation is consistent with the standard conformation assumed for a general MTPA ester derivative of chiral secondary alcohols (Figure S10). Images were generated with the software PyMOL.[2]

(1) Spartan'08, Wavefunction, Inc. Irvine, CA. Y. Shao, L.F. Molnar, Y. Jung, J. Kussmann, C. Ochsenfeld, S.T. Brown, A.T.B. Gilbert, L.V. Slipchenko, S.V. Levchenko, D.P. O'Neill, R.A. DiStasio Jr., R.C. Lochan, T. Wang, G.J.O. Beran, N.A. Besley, J.M. Herbert, C.Y. Lin, T. Van Voorhis, S.H. Chien, A. Sodt, R.P. Steele, V.A. Rassolov, P.E. Maslen, P.P. Korambath, R.D. Adamson, B. Austin, J. Baker, E.F.C. Byrd, H. Dachsel, R.J. Doerksen, A. Dreuw, B.D. Dunietz, A.D. Dutoi, T.R. Furlani, S.R. Gwaltney, A. Heyden, S. Hirata, C-P. Hsu, G. Kedziora, R.Z. Khalliulin, P. Klunzinger, A.M. Lee, M.S. Lee, W.Z. Liang, I. Lotan, N. Nair, B. Peters, E.I. Proynov, P.A. Pieniazek, Y.M. Rhee, J. Ritchie, E. Rosta, C.D. Sherrill, A.C. Simmonett, J.E. Subotnik, H.L. Woodcock III, W. Zhang, A.T. Bell, A.K. Chakraborty, D.M. Chipman, F.J. Keil, A.Warshel, W.J. Hehre, H.F. Schaefer, J. Kong, A.I. Krylov, P.M.W. Gill and M. Head-Gordon, *Phys. Chem. Chem. Phys.*, **2006**, *8*, 3172-3191.

(2) The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.

Table S8. Cartesian coordinates (Å) of the lowest energy conformer of (*S*)-MTPA-(1*S*,3'*R*)-**7c**.

Atom	X	Y	Z
1 H H1	4.2731618	-2.7072522	0.1125827
2 C C1	4.6349613	-1.7121661	0.3646891
3 C C4	5.5631563	0.8348398	0.9851932
4 C C2	3.8746382	-0.6040806	-0.0296194
5 C C6	5.8341247	-1.5659719	1.0580849
6 C C5	6.2995105	-0.2830420	1.3629282
7 C C3	4.3546487	0.6739435	0.2974340
8 H H6	6.4050290	-2.4422186	1.3518599
9 H H5	7.2364202	-0.1533019	1.8983217
10 H H4	5.8995935	1.8421590	1.2108463
11 C C7	2.5787783	-0.7663649	-0.7981244
12 H H2	1.7756914	-1.0884971	-0.1211699
13 H H7	2.6887242	-1.5576202	-1.5510348
14 C C8	2.1573913	0.5514779	-1.4701895
15 H H10	2.8376524	0.7595040	-2.3080271
16 C C9	2.3314944	1.6827330	-0.4510870
17 H H8	1.7111362	1.4892745	0.4346625
18 H H11	2.0562787	2.6570022	-0.8611474
19 O O1	3.6972743	1.8253175	-0.0558474
20 C C10	0.7477064	0.4654049	-2.0686468
21 H H9	0.6858786	-0.4203795	-2.7081783
22 C C11	0.2912237	1.6871343	-2.8607841
23 H H12	0.2037422	2.5717838	-2.2226065
24 H H13	-0.6843313	1.4923615	3.3149609
25 H H14	1.0050108	1.9048725	-3.6629250
26 O O2	-0.1728369	0.2560425	-0.9532117
27 C C12	-1.2358030	-0.5299019	-1.1688106
28 O O3	-1.5268523	-1.0310692	-2.2292866
29 C C13	-2.0481346	-0.6970762	0.1498373
30 C C14	-2.6691341	0.6560967	0.5393529
31 C C15	-3.6528405	3.1916847	1.2481601
32 C C16	-1.8910664	1.5369524	1.3037019
33 C C17	-3.9412998	1.0671383	0.1194850
34 C C18	-4.4270185	2.3263731	0.4761129
35 C C19	-2.3804757	2.7918605	1.6602929
36 H H15	-0.8992664	1.2285654	1.6140080
37 H H17	-4.5621417	0.4146308	-0.4815620
38 H H18	-5.4180680	2.6267472	0.1470179
39 H H19	-1.7649026	3.4576258	2.2592872
40 H H20	-4.0364756	4.1698044	1.5252303
41 O O4	-1.0752256	-1.1404055	1.0829948
42 C C20	-1.4968707	-1.4895817	2.4061360
43 H H16	-2.1751668	-0.7416796	2.8296889
44 H H21	-0.5792013	-1.5247512	2.9973141
45 H H22	-1.9783753	-2.4722819	2.4257309
46 C C21	-3.1078903	-1.8179762	-0.0581034
47 F F1	-3.9217855	-1.9007754	1.0228491
48 F F2	-2.5052582	-3.0089498	-0.2000965
49 F F3	-3.9020527	-1.6216411	-1.1259072

Figure S13. Two views of the calculated lowest energy conformer of (*S*)-MTPA-(1*S*,3'*R*)-**7c**.