Electronic Supplementary Information (ESI)

Synthesis and preliminary evaluation of the anti-cancer activity on A549 lung cancer cells of a series of unsaturated disulfides

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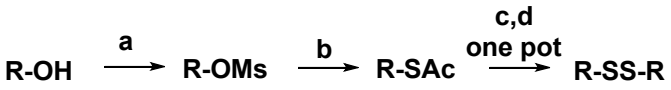
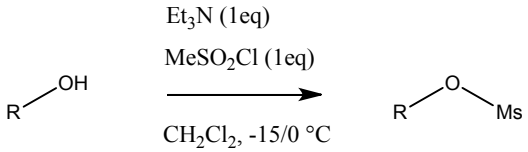
INDEX

1. Total reaction scheme



2. General reaction scheme for the synthesis of organic mesylates.

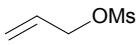


2.1 General procedure for the synthesis of organic mesylates.

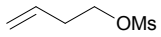
All the mesylates were synthetized starting from alcohol following the same procedures reported in literature. [1,2] The reactions were almost quantitative after work up and the freshly prepared product were used directly for the next step. All GC-MS and NMR spectra were comparable to those reported in literature.[3] The isolate yield was calculated in the usual way as percentage yield from the ratio between the actual yield and the theoretical yield. The theoretical yield was obtained considering the alcohol as the limiting reactant. The actual yield was obtained weighing the product after the mentioned work up. [3]

In a one neck round bottom flask, 4.6 mmol of the corresponding alcohol were dissolved in 20 ml of CH_2Cl_2 and the mixture cooled down at 0 °C. 4.6 mmol of triethylamine were added under stirring. After that, 4.6 mmol of methane sulfonyl chloride were added dropwise under stirring. The mixture was stirred open flask for one hour and half. The reaction was quenched with 2 ml of HCl 1N and the solution washed with $NaHCO_3$ saturated aqueous solution. The organic phase was extracted and washed three times with water. The organic phase was dried over Na_2SO_4. The solvent was removed under vacuum to obtain a pink liquid. Yield (>90%). [3]

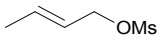
2.2 General, Experimental and Analytical Information



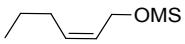
Allyl mesylate (1a) , in accordance to general procedure, was prepared from allyl alcohol (0.5g, 8.6 mmol) , triethylamine (1eq, 8.6 mmol) and methane sulfonyl chloride (1 eq, 8.6 mmol), dissolved in 20 mL of CH_2Cl_2 , under stirring at -15 °C. After 1 hour and half the reaction mixture was quenched with 2ml of HCl 1N and the solution washed with $NaHCO_3$ saturated aqueous solution. The organic phase was washed three times with water and dried over Na_2SO_4. The solvent was removed under vacuum to afford a pink yellow liquid in quantitative yield. ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C) δ = 3.02 (3H, s, CH_3), 4.72 (2H, d, ^3J = 6Hz, CH_2O), 5.38 (1H, dd, ^3J=10.3Hz, ^4J= 0.9Hz, CH_2=), 5.46 (1H, dd, ^3J=17.1Hz, ^4J= 1.3Hz,CH_2=), 5.97(1H, ddt, ^3J=17Hz, ^3J=10.4Hz, ^3J=6Hz, CH=). ^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 38.04 (CH_3-S), 70.38 (CH2-O), 120.79 (CH_2=), 130.48 (CH=CH_2). NMR comparable to those reported in literature. [3]



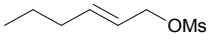
4-methanesulfonyloxy-1-butene (2a) , in accordance to general procedure, was prepared from homoalylic alcohol (0.5g, 5.8 mmol) , triethylamine (1eq, 5.8mmol) and methane sulfonyl chloride (1 eq, 5.8 mmol), dissolved in 20 mL of CH_2Cl_2 , under stirring at -15 °C. After 1 hour and half the reaction mixture was quenched with 2ml of HCl 1N and the solution washed with $NaHCO_3$ saturated aqueous solution. The organic phase was washed three times with water and dried over Na_2SO_4. The solvent was removed under vacuum to afford a pink yellow liquid in quantitative yield. GC-MS(ESI): m/z (%) , 149 (M-1, 0.4), 109 (52), 79 (95), 54 (100), 45 (9), 41 (52) . NMR comparable to those reported in literature.[4]



Trans-2-butenyl mesylate (3a) , in accordance to general procedure, was prepared from crotyl alcohol (0.5g, 6.9 mmol) , triethylamine (1eq, 6.9mmol) and methane sulfonyl chloride (1 eq, 6.9 mmol), dissolved in 20 mL of CH_2Cl_2 , under stirring at -15 °C. After 1 hour and half the reaction mixture was quenched with 2ml of HCl 1N and the solution washed with $NaHCO_3$ saturated aqueous solution. The organic phase was washed three times with water and dried over Na_2SO_4. The solvent was removed under vacuum to afford a pink yellow liquid in quantitative yield. GC-MS(ESI): m/z (%) , 150 (M^+, 0.4), 80 (11), 79 (15), 71 (42), 65 (11), 55 (100), 43 (27). ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C) δ = 1,77 (3H, d, ^3J=6.5Hz, CH_3CH), 3.00 (3H, s, CH_3S), 4.67 (2H, d, ^3J=6.9Hz, CH_2O), 5.58-5.68 (1H, m, CH=), 5.87-5.97(1H, m, CH=). ^{13}C-NMR : δ = 17.73 (CH_3-CH), 38.21 (CH_3S), 70.78 (CH_2S), 123.49 ($CHCH_2$), 134.57($CHCH_3$). NMR data comparable to those reported in literature.[5]

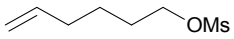


Cis-2-hexenyl mesylate (4a), in accordance to general procedure, was prepared from trans-hexenyl alcohol (0.5g, 4.9 mmol) , triethylamine (1eq, 6.9 mmol) and methane sulfonyl chloride (1 eq, 6.9 mmol), dissolved in 20 mL of CH_2Cl_2 , under stirring at -15 °C. After 1 hour and half the reaction mixture was quenched with 2ml of HCl 1N and the solution washed with $NaHCO_3$ saturated aqueous solution. The organic phase was washed three times with water and dried over Na_2SO_4. The solvent was removed under vacuum to afford a pink yellow liquid in quantitative yield. GC-MS(ESI): m/z (%) , 149 (0.4), 82 (59), 79 (27), 67 (100), 57 (31), 55 (43), 41 (55). [6]



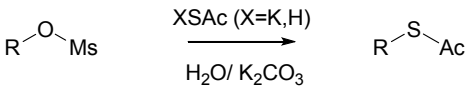
Trans-2-hexenyl mesylate (5a), in accordance to general procedure, was prepared from trans-hexenyl alcohol (0.5g, 4.9 mmol) , triethylamine (1eq, 6.9 mmol) and methane sulfonyl chloride (1 eq, 6.9 mmol), dissolved in 20 mL of CH_2Cl_2 , under stirring at -15 °C. After 1 hour and half the reaction mixture was quenched with 2ml of HCl 1N and the solution washed with $NaHCO_3$ saturated aqueous solution. The organic phase was washed three times with water and dried over Na_2SO_4. The solvent was removed under vacuum to afford a pink yellow liquid in quantitative yield. GC-MS(ESI): m/z (%) , 149 (0.4), 82 (59), 79 (27), 67 (100), 57 (31), 55 (43), 41 (55). ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C): δ = 0.90 (3H, t, ^3J=7.3Hz, CH_3CH_2), 1.42 (2H,

sextuplet, ^{3}J=7.4Hz, CH_2CH_3), 2.06 (2H, q, ^{3}J=7.2Hz, CH_2CH), 2.99 (3H, s, CH_3S),4.67 (2H, d, ^{3}J=6.8Hz, CH_2S), 5.58-5.63(1H, m, CH=), 5.88-5.93(1H, m, CH=). ^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 13.58 (CH_3-CH_2), 21.82 (CH_2CH_3), 34.21, 38.24, 70.93 (CH_2S), 122.27($CHCH_2S$), 139.58($CHCH_2CH_2$). NMR comparable to those reported in literature. [3]



5-hexenyl mesylate (6a) , in accordance to general procedure, was prepared from 5-hexen-1-ol (0.5g, 4.9 mmol) , triethylamine (1eq, 4.9mmol) and methane sulfonyl chloride (1 eq, 4.9 mmol), dissolved in 20 mL of CH_2Cl_2 , under stirring at -15 °C. After 1 hour and half the reaction mixture was quenched with 2ml of HCl 1N and the solution washed with $NaHCO_3$ saturated aqueous solution. The organic phase was washed three times with water and dried over Na_2SO_4. The solvent was removed under vacuum to afford a pink yellow liquid in quantitative yield. GC-MS(ESI): m/z (%) , 150 (0.4), 82 (31), 79 (29), 67 (100), 54 (96), 53 (4), 42 (4), 41 (38) . NMR comparable to those reported in literature.[7]

3. General reaction scheme for the synthesis of organic thioacetates



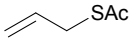
3.1 General procedure for the synthesis of allylic thioacetates

Freshly prepared mesylate was put in a one neck round bottom flask. In the same flask aqueous potassium carbonate 0.4 M solution were added. 1.5 eq of potassium thioacetate or acetic acid were added dropwise to the solution under stirring. The mixture was stirred at 40 °C with a reflux condenser for almost 2 hours. The organic phase was extracted with a small portion of diethyl ether and washed three times with water. The organic phase was dried over Na_2SO_4 and the solvent removed under vacuum. The product was recovered as a yellow liquid. The isolate yield was calculated in the usual way as percentage yield from the ratio between the actual yield and the theoretical yield. The theoretical yield was obtained considering the organic mesylate as the limiting reactant. The actual yield was obtained weighing the product after the mentioned work up.[3]

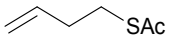
3.2 General procedure for the synthesis of primary thioacetates

Freshly prepared mesylate was put in a one neck round bottom flask. In the same flask aqueous potassium carbonate 0.4 M solution were added. 1.5 eq of potassium thioacetate or acetic acid were added to the solution under stirring. The mixture was stirred at 80 °C under reflux for almost 2 hours. The organic phase was extracted with a small portion of diethyl ether and washed three times with water. The organic phase was dried over Na_2SO_4 and the solvent removed under vacuum. The product was recovered as a yellow liquid. The isolate yield was calculated in the usual way as percentage yield from the ratio between the actual yield and the theoretical yield. The theoretical yield was obtained considering the organic mesylate as the limiting reactant. The actual yield was obtained weighing the product after the mentioned work up.[3]

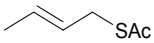
3.3 General, Experimental and Analytical Information



Allyl thioacetate (1b) was prepared in accordance to the general procedure.
Allyl mesylate (2a) (0.37g, 2.7 mmol) was put in a one neck round bottom flask. In the same flask 20 ml of 0.4 M aqueous potassium carbonate solution were added. 1.5 eq of potassium thioacetate or acetic acid were added dropwise to the solution under stirring. The mixture was stirred at 40 °C with a reflux condenser for almost 2 hours. After the usual work up the product was recovered as a yellow liquid obtained in 96 % yield (GC-MS) . Isolated yield after work up: 92%. ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C) δ = 2.33 (3H, s, CH_3), 3.53 (2H, d, ^3J = 7Hz, CH_2S), 5.08-5.26 (2H, m, $CH=CH_2$), 5.73-5.93 (1H, m, $CH=CH_2$). ^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 30.48 (CH_3), 32.01 (CH_2S), 117.88 ($CH=CH_2$), 133.07 ($CH=CH_2$), 195.12 (C=O). NMR comparable to those reported in literature. [3]

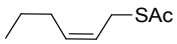


Thioacetic acid S-but-3-enyl ester (2b) was prepared in accordance to the general procedure.
4-butenyl mesylate (3c) (0.42g, 2.8 mmol) was put in a one neck round bottom flask. In the same flask 20 ml of 0.4 M aqueous potassium carbonate solution were added. 1.5 eq of potassium thioacetate or acetic acid were added dropwise to the solution under stirring. The mixture was stirred at 80 °C under reflux for almost 2 hours. After the usual work up the product was recovered as a yellow liquid obtained in 96% yield (GC-MS) . Isolated yield after work up: 93%. GC-MS(ESI): m/z (%) , 130 (M^+, 0.4), 88 (23), 87 (15), 71 (19), 54 (10), 45 (6), 43 (100) . ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C): δ = 2.29-2.35 (2H, m, CH_2-CH), 2.33 (3H,s, CH_3), 2.94 (2H, t, ^3J=7.3Hz, CH_2-CH_2), 5.02-5.11 (2H, m, CH_2=), 5.71-5.85 (1H,m). ^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 28.26(CH_2), 30.50 (CH_3),33.50 (CH_2), 116.34(CH_2), 135.99 (CH), 195.46(C=O). NMR comparable to those reported in literature. [3]

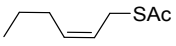


***trans*-2-butenyl thioacetate (3b)** was prepared in accordance to the general procedure.

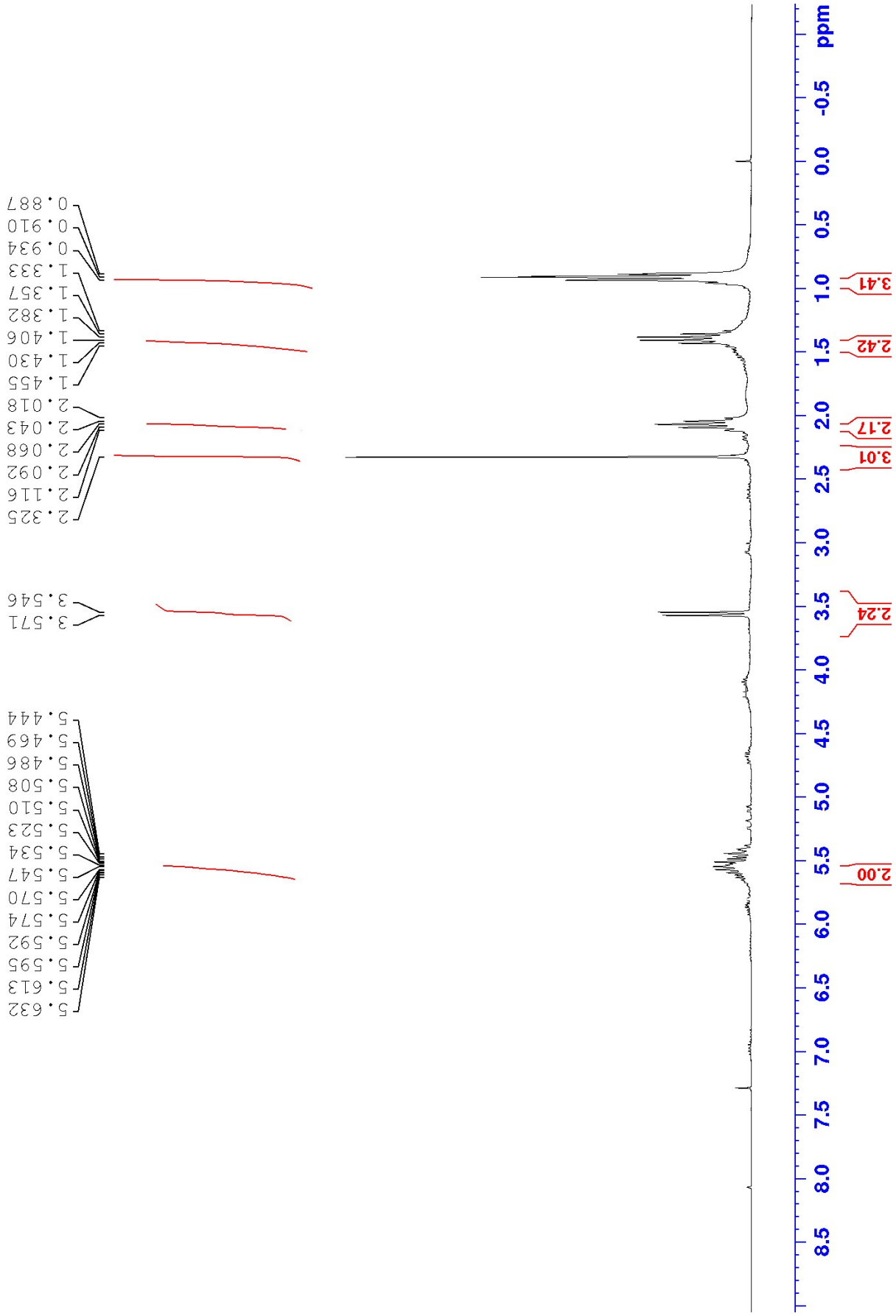
trans-2-butenyl mesylate (3a) (0.40g, 2.7 mmol) was put in a one neck round bottom flask. In the same flask 20 ml of 0.4 M aqueous potassium carbonate solution were added. 1.5 eq of potassium thioacetate or acetic acid were added dropwise to the solution under stirring. The mixture was stirred at 40 °C with a reflux condenser for almost 2 hours. After the usual work up the product was recovered as a yellow liquid obtained in 97% yield (GC-MS) . Isolated yield after work up: 91%. GC-MS(ESI): m/z (%) , 149 (M^+, 31), 88 (35), 55 (73), 53 (15), 43 (100). ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C) δ = 1.65-1.68 (3H, m, CH_3), 2.32 (3H, s, CH_3), 3.48 (2H, bd, ^3J =7Hz, CH_2), 5.37-5.48 (1H, m, CH=CH), 5.57-5.72 (1H, m, CH=CH) . ^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 17.53 (CH_3-CH), 30.29(CH_3-C=0), 31.25 (CH_2-S), 125.63 (CH=CH), 129.03 (CH=CH), 195.04 (C=0). NMR comparable to those reported in literature. [3]



cis-2-hexenyl thioacetate (4b) was prepared in accordance to the general procedure.
cis-2-hexenyl mesylate (4a) (0.48g, 2.7 mmol) was put in a one neck round bottom flask. In the same flask 20 ml of 0.4 M aqueous potassium carbonate solution were added. 1.5 eq of potassium thioacetate or acetic acid were added dropwise to the solution under stirring. The mixture was stirred at 40 °C with a reflux condenser for almost 2 hours. After the usual work up the product was recovered as a yellow liquid obtained in 96% yield (GC-MS) . Isolated yield after work up: 89%. GC-MS(ESI): m/z (%) , 158 (M^+, 0.4), 116 (19), 82 (81), 73 (9), 67 (30), 55 (67), 43 (100), 41 (38) . ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C) δ = 0.91 (3H, t, ^3J=7Hz, CH_3), 1.39 (2H,sextuplet, ^3J=7Hz, CH_2-CH_3), 2.02-2.12 (2H, m , CH_2-CH=), 2.32 (3H, s, CH3), 3.56 (2H, bd, ^3J=7.44Hz, CH_2-S), 5.44-5.63 (2H, m, CH=CH). ^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 13.67 (CH_3-CH_2), 22.53(CH2-CH_3), 26.26, 29.17, 30.32, 123.76(CH=CH), 133.76(CH=CH), 195.61(C=0).

cis-2-hexenyl thioacetate 

^{1}H-NMR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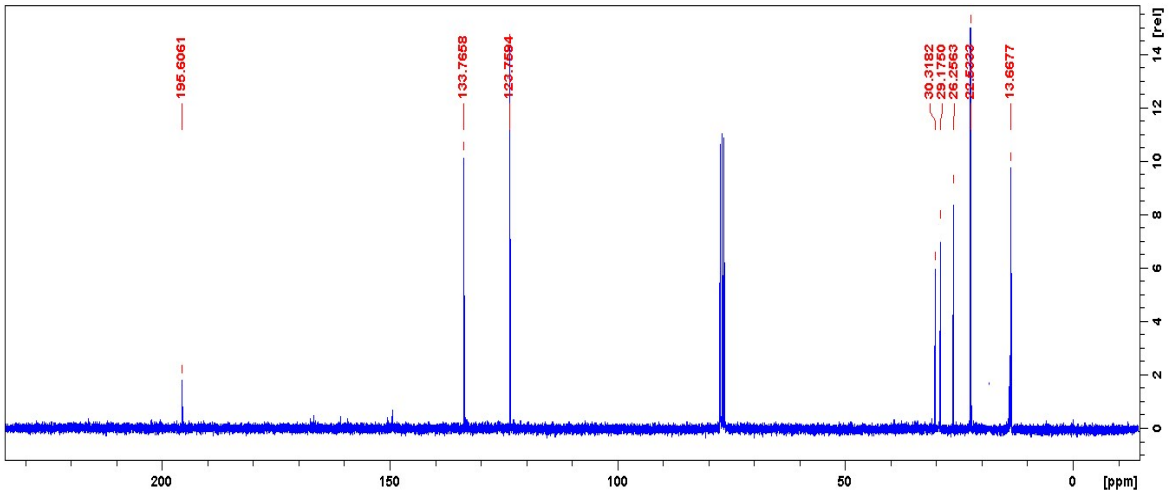
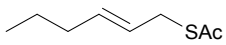
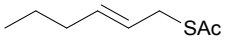
^{13}C-NMR



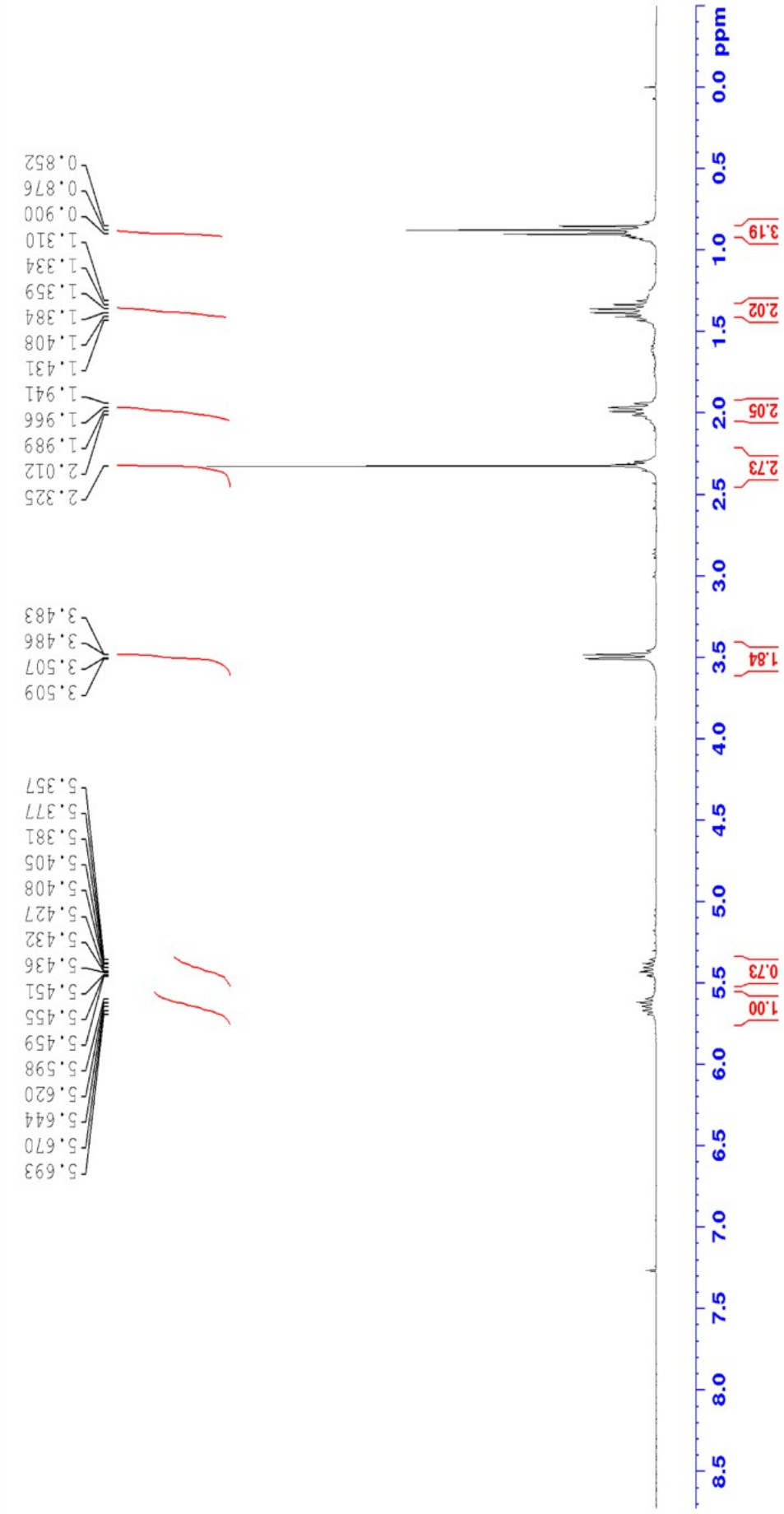


***trans*-2-hexenyl thioacetate (5b)** was prepared in accordance to the general procedure.

t*rans*-2-hexenyl mesylate (4a) (0.48g, 2.7 mmol) was put in a one neck round bottom flask. In the same flask 20 ml of 0.4 M aqueous potassium carbonate solution were added. 1.5 eq of potassium thioacetate or acetic acid were added dropwise to the solution under stirring. The mixture was stirred at 40 °C with a reflux condenser for almost 2 hours. After the usual work up the product was recovered as a yellow liquid obtained in 93% yield (GC-MS) . Isolated yield after work up: 89%. GC-MS(ESI): m/z (%) , 158 (M^+, 0.4), 116 (19), 82 (81), 73 (9), 67 (30), 55 (67), 43 (100), 41 (38) . ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C) δ = 0.87 (3H, t, ^3J=7.2Hz, CH_3), 1.37 (2H,sextuplet, ^3J=7.3Hz, CH_2-CH_3), 1.98 (2H,dt , ^3J =7Hz, ^{3}J=7Hz, CH_2-CH=), 2.32 (3H, s, CH3), 3.49 (2H, bd, ^3J=7Hz, CH_2-S), 5.36-5.46 (1H, m, CH=CH), 5.60-5.69 (1H, m, CH=CH). ^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 13.55 (CH_3-CH_2), 22.21(CH2-CH_3), 30.45, 31.48, 34.28, 124.55(CH=CH), 134.54(CH=CH), 195.41(C=0).

***trans*-2-hexenyl thioacetate** 

^{1}H-NMR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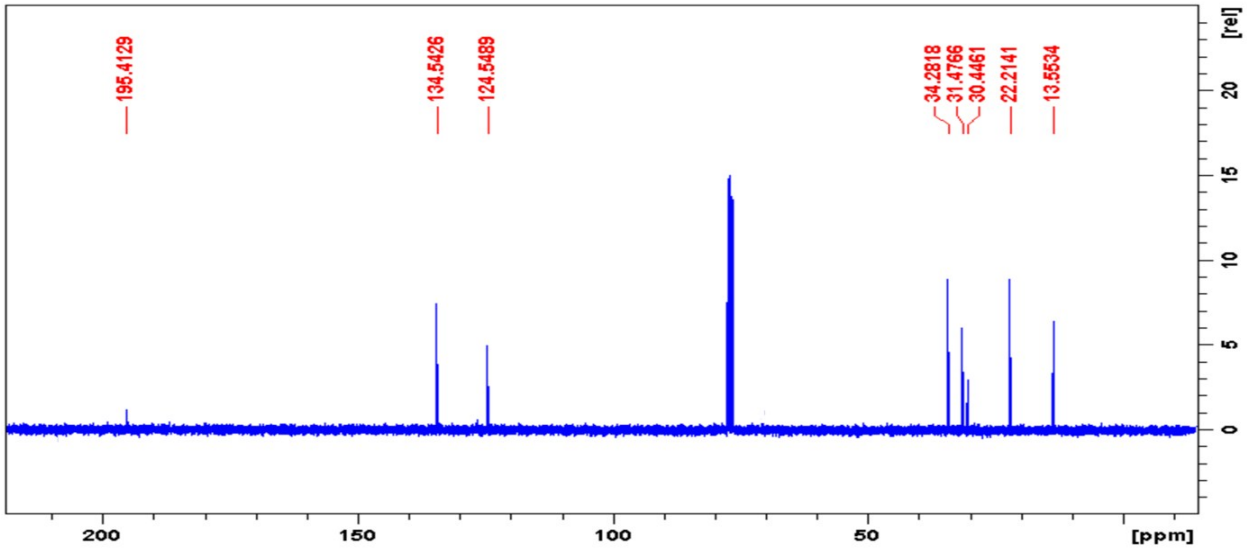
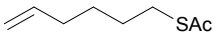
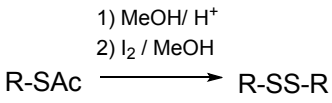
^{13}C-NMR





6-Acetylsulfanyl-hex-1-ene (6b) was prepared in accordance to the general procedure.
5-hexenyl mesylate (1c) (0.50g, 2.8 mmol) was put in a one neck round bottom flask. In the same flask 20 ml of 0.4 M aqueous potassium carbonate solution were added. 1.5 eq of potassium thioacetate or acetic acid were added dropwise to the solution under stirring. The mixture was stirred at 80 °C under reflux for almost 2 hours. After the usual work up the product was recovered as a yellow liquid obtained in 96% yield (GC-MS) . Isolated yield after work up: 92%. GC-MS(ESI): m/z (%) , 158 (M^+, 0.4), 115 (69), 100 (11), 87 (6), 81 (11), 67 (8), 54 (4), 43 (100), 41 (11) . ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C): δ = 1.43-1.50 (2H, m, CH_2), 1.54-1.61 (2H,m, CH_2), 2.06 (2H, m, CH_2-CH_2-CH_2), 2.33 (3H, s, CH_3), 2.87 (2H,t, ^3J=7Hz,CH_2S), 4.94-5.04 (2H, m, CH_2=C), 5.74-5.83 (1H, m, CH=C). ^{13}C-NMR : δ = 27.99, 28.9, 28.9 30.60, 33.18, 114.75 (CH_2=), 138.33(CH=), 195.88 (C=O). NMR comparable to those reported in literature. [3]

4. General reaction scheme for the synthesis of symmetric unsaturated disulfides

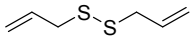


4.1 General procedure for the synthesis of symmetric unsaturated disulfides

Step 1. Organic thioacetate was dissolved in methanol and some drops of HCl 37% were added. The mixture was stirred under reflux overnight.
Step 2. The mixture was cooled down at 0°C, and a solution of iodine 5% in methanol was added until the solution becomes colored. The mixture was stirred open air for 45 minutes. Iodine in excess was quenched

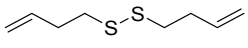
with sodium thiosulfate. The solvent was removed under vacuum and the crude product was dissolved in water and the organic phase extracted in diethyl ether. The organic phase was washed again with water. The solvent was removed under high vacuum and the crude product was purified with column chromatography using alumina as stationary phase and hexane as eluting solvent. [8]

4.2 General, Experimental and Analytical Information



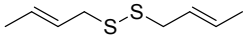
X

Diallyl disulfide (**X).** 250 mg of **Allyl thioacetate** (2.1 mmol) were dissolved in 5 ml of MeOH and HCl 37% (drops) was added. The mixture was stirred at 50 °C overnight. The mixture was cooled down at 0°C and Iodine solution (5% in methanol) was added dropwise until the colour becomes dark. After 45 minutes , $Na_2S_2O_3$ was added to quench the iodine in excess , and the solution turns colorless. The solvent was removed under high vacuum and after that the crude product was dissolved in diethyl ether and washed three times with water. The crude product was subjected to flash column chromatography with alumina and hexane as elution solvent to give 82% of product as a pale yellow oil. The isolated yield refers to percentage yield calculated from the ratio between the theoretical yield and the actual yield after weighing the product.
GC-MS(ESI): m/z (%) , 146 (M^+, 8.3), 113 (11), 85 (8), 81 (27), 45 (16), 41 (100) . Data in accordance to literature.[9] ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C) δ = 3.49 (4H, dd, ^{3}J=7.3Hz, CH_2S), 5.11-5.28 (4H, m, CH_2=CH), 5.78-5.94 (2H, m, CH=CH_2). ^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 41.71 (CH_2=S), 119.09 (CH_2=CH), 132.74 (CH=CH_2). Data in accordance to literature.[10]



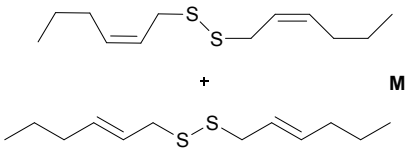
A

1,2-di(but-3-en-1-yl)disulfide(**A).** 250 mg of **Thioacetic acid S-but-3-enyl ester** (1.9 mmol) were dissolved in 5 ml of MeOH and HCl 37% (drops) was added. The mixture was stirred at 50 °C overnight. The mixture was cooled down at 0°C and Iodine solution (5% in methanol) was added dropwise until the colour becomes dark. After 45 minutes , $Na_2S_2O_3$ was added to quench the iodine in excess , and the solution turns colorless. The solvent was removed under high vacuum and after that the crude product was dissolved in diethyl ether and washed three times with water. The crude product was subjected to flash column chromatography with alumina and hexane as elution solvent to give 84% of product as a pale yellow oil. The isolated yield refers to percentage yield calculated from the ratio between the theoretical yield and the actual yield after weighing the product. GC-MS(ESI): m/z (%) , 174 (M^+, 23), 120 (23), 87 (21), 55 (100), 53 (13), 45 (23), 41 (1f0) . Data in accordance to literature.[11] ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C): δ = 2.42-2.49 (4H, m, CH_2-CH), 2.76 (4H, t, ^{3}J=7.2Hz, CH_2S), 5.05-5.13 (4H, m, CH_2=CH), 5.77-5.90 (2H, m, CH=CH_2).^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 17.87(CH_2=CH), 41.86 (CH_2S),126.17 (CH_2=CH), 129.76(CH=CH_2). Data in accordance to literature.[11]

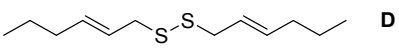


B

Trans-di(but-2-enyl)disulfide (**B**). 250 mg of ***Trans*-2-butenyl thioacetate** (1.9 mmol) were dissolved in 5 ml of MeOH and HCl 37% (drops) was added. The mixture was stirred at 50 °C overnight. The mixture was cooled down at 0°C and Iodine solution (5% in methanol) was added dropwise until the colour becomes dark. After 45 minutes , $Na_2S_2O_3$ was added to quench the iodine in excess , and the solution turns colorless. The solvent was removed under high vacuum and after that the crude product was dissolved in diethyl ether and washed three times with water. The crude product was subjected to flash column chromatography with alumina and hexane as elution solvent to give 80% of product as a pale yellow oil. The isolated yield refers to percentage yield calculated from the ratio between the theoretical yield and the actual yield after weighing the product. GC-MS(ESI): m/z (%) , 174 (M^+, 6), 120 (6), 55 (100), 53 (6), 45 (6). Data in accordance to literature.[12] ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C) δ = 1.73 (6H, dd, ^{3}J=6.3Hz , CH_3), 3.28 (4H, dd, ^{3}J=7.3Hz ,CH_2CH), 5.47-5.67 (4H, m, CH=CH), 5.37-5.48 . ^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 17.87 (CH_3-CH), 41.86 (CH_2-S), 126.17, 129.76. Data in accordance to literature.[13]

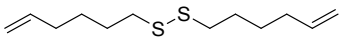


(Z)-1-[[(Z)-hex-2-henyl]disulfanyl]hex-2-ene + (E)-1-[[(E)-hex-2-henyl]disulfanyl]hex-2-ene, mixture 65/35 by ^{13}C-NMR (**M**)**.** 250 mg of ***Cis*-2-hexenyl thioacetate** (1.6 mmol) were dissolved in 5 ml of MeOH and HCl 37% (drops) was added. The mixture was stirred at 50 °C overnight. The mixture was cooled down at 0°C and Iodine solution (5% in methanol) was added dropwise until the colour becomes dark. After 45 minutes , $Na_2S_2O_3$ was added to quench the iodine in excess , and the solution turns colorless. The solvent was removed under high vacuum and after that the crude product was dissolved in diethyl ether and washed three times with water. The crude product was subjected to flash column chromatography with alumina and hexane as elution solvent to give 81% of product as a pale yellow oil. The isolated yield refers to percentage yield calculated from the ratio between the theoretical yield and the actual yield after weighing the product. GC-MS(ESI): m/z (%) , 230 (M^+, 5), 148 (5), 83 (66), 55 (100), 41 (42) . ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C) δ = 0.90-0.95 (12H, m, CH_3CH_2), 1.35-1.45 (8H,m, CH_2-CH_3), 2.02-2.13 (8H, m , CH_2CH), 3.32-3.45 (8H, m, CH_2S), 5.41-5.67 (8H, m, CH-CH). ^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 13.67, 13.81, 22.35, 22.82, 29.40, 34.53, 36.35, 41.83, 124.37, 125.02, 134.34, 135.17.



(E)-1-[[(E)-hex-2-henyl]disulfanyl]hex-2-ene (**D**). 250 mg of ***Trans*-2-hexenyl thioacetate** (1.6 mmol) were dissolved in 5 ml of MeOH and HCl 37% (drops) was added. The mixture was stirred at 50 °C overnight. The mixture was cooled down at 0°C and Iodine solution (5% in methanol) was added dropwise until the colour becomes dark. After 45 minutes , $Na_2S_2O_3$ was added to quench the iodine in excess , and the solution turns colorless. The solvent was removed under high vacuum and after that the crude product was dissolved in diethyl ether and washed three times with water. The crude product was subjected to flash column chromatography with alumina and hexane as elution solvent to give 82% of product as a pale yellow oil. The isolated yield refers to percentage yield calculated from the ratio between the theoretical yield and the actual yield after weighing the product. GC-MS(ESI): m/z (%) , 230 (M^+, 5), 148 (5), 83 (66), 55 (100), 41 (42) .

^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C) δ = 0.91 (6H, t,^{3}J= 7.3Hz, CH_3CH_2), 1.41 (4H,sextet, ^{3}J= 7.5Hz, CH2-CH_3), 1.99-2.06 (4H, m , CH_2CH), 3.29 (4H, dd, ^{3}J= 7.6Hz, ^{4}J= 0.7Hz, CH_2S), 5.39-5.65 (4H, m, CH=CH). ^{13}C-NMR : (75 MHz, $CDCl_3$, 25°C) δ = 13.69 (CH_3CH_2), 22.37 (CH_2CH_3), 34.52 (CH_2CH_2), 41.88 (CH_2S), 125.00 (CH=CH), 135.15 (CH=CH).

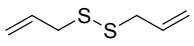


E

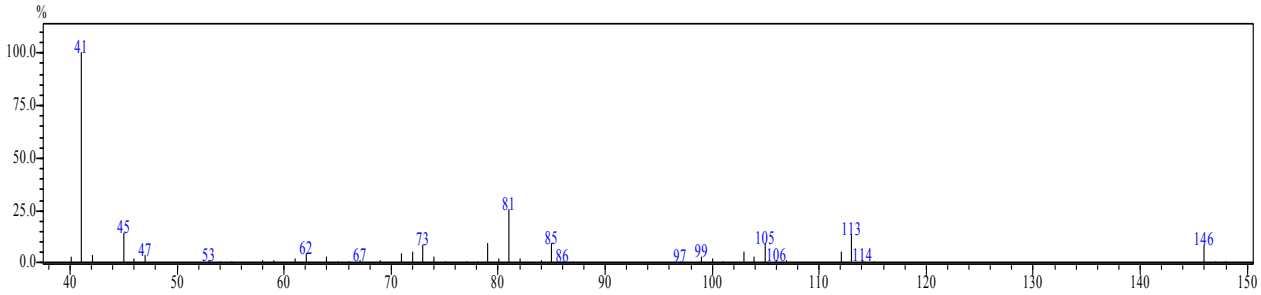
1,2-di(hex-5-en-1-yl)disulfide (**E**)**.** 250 mg of **6-Acetylsulfanyl-hex-1-ene** (2.5 mmol) were dissolved in 5 ml of MeOH and HCl 37% (drops) was added. The mixture was stirred at 50 °C overnight. The mixture was cooled down at 0°C and Iodine solution (5% in methanol) was added dropwise until the colour becomes dark. After 45 minutes , $Na_2S_2O_3$ was added to quench the iodine in excess , and the solution turns colorless. The solvent was removed under high vacuum and after that the crude product was dissolved in diethyl ether and washed three times with water. The crude product was subjected to flash column chromatography with alumina and hexane as elution solvent to give 83% of product as a pale yellow oil. The isolated yield refers to percentage yield calculated from the ratio between the theoretical yield and the actual yield after weighing the product. GC-MS(ESI): m/z (%) , 230 (M^+, 7), 115 (100), 83 (21), 79 (7), 67 (5), 67 (8), 55 (96), 41 (53) . ^{1}H-NMR: (300 MHz, $CDCl_3$, 25°C): δ = 1.49 (4H, quintet, ^{3}J= 7.1Hz), 1.70 (4H, quintet, ^{3}J= 7.3Hz), 2.04-2.11 (4H, m, CH_2CH), 2.69 (4H, t, ^{3}J= 7.2Hz, CH_2S), 4.93-4.95 (4H, m, CH_2=CH), 4.97-4.99 (4H, m, CH=CH_2). ^{13}C-NMR : δ = 27.73, 28.66, 33.32, 39.98 (CH_2S), 114.74 (CH_2=CH), 138.43 (CH_2=CH).

4.3 GC-MS (ESI) spectra

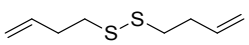
Diallyl disulfide (X)



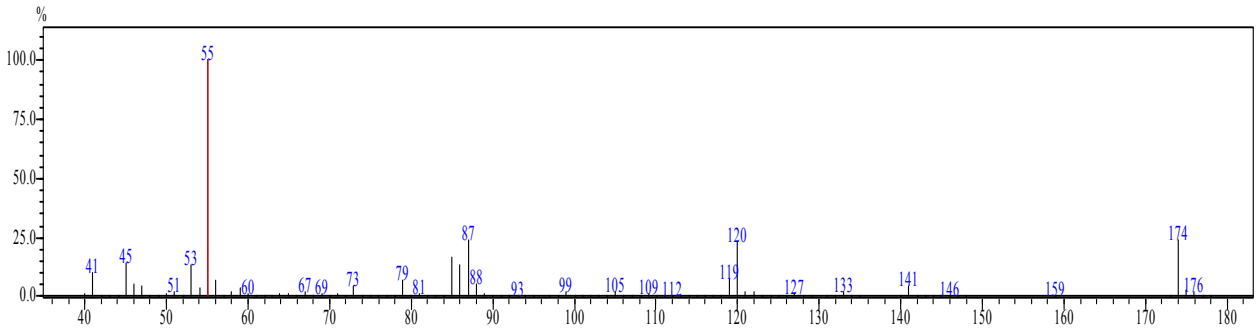
X



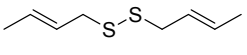
1,2-di(but-3-en-1-yl)disulfide (A)



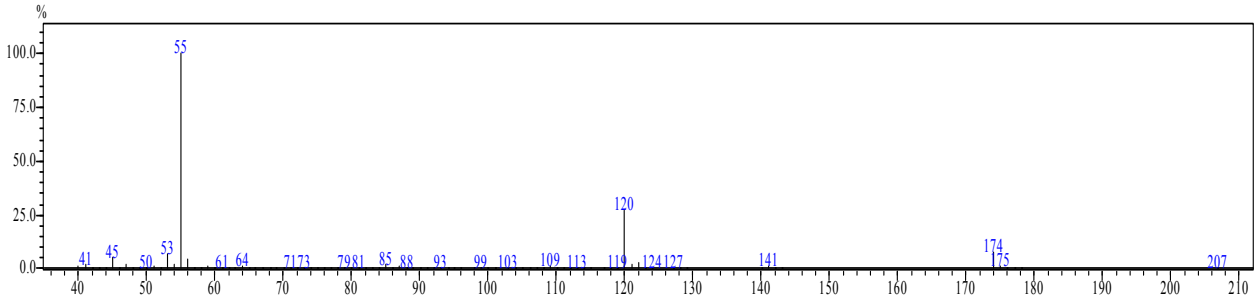
A

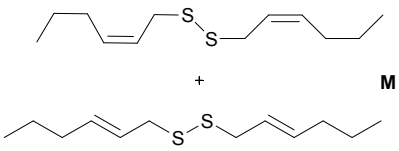


Trans-di(but-2-enyl)disulfide (B)



B

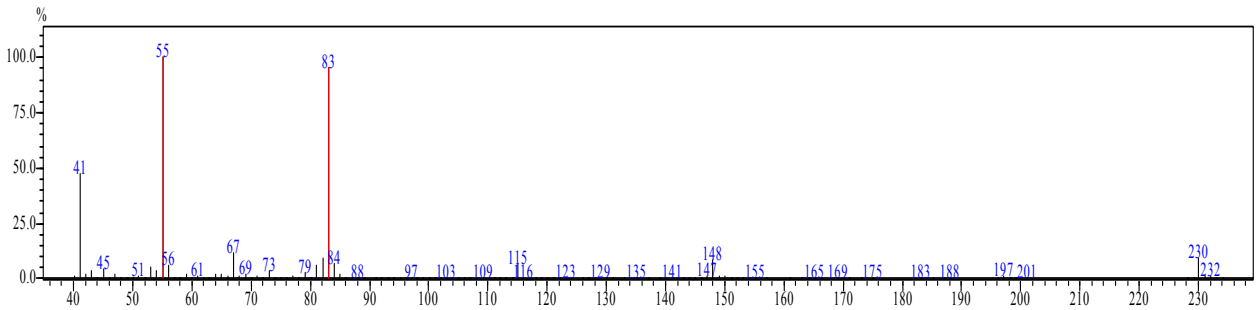


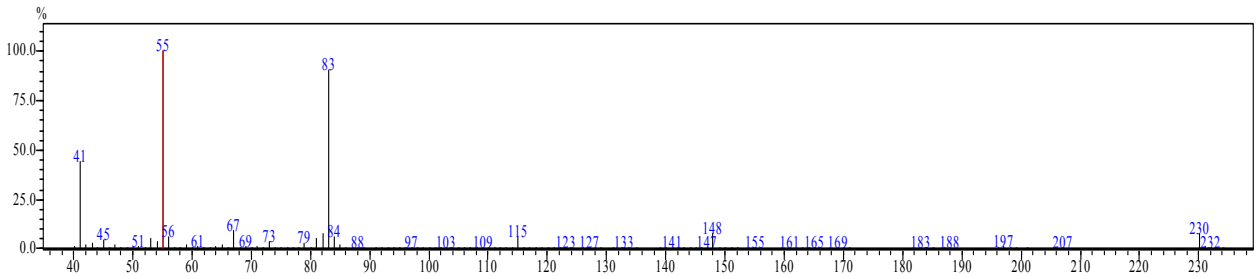


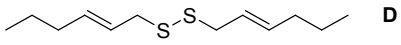
+

M

(Z)-1-[[(Z)-hex-2-henyl]disulfanyl]hex-2-ene + (E)-1-[[(E)-hex-2-henyl]disulfanyl]hex-2-ene, mixture 65/35 by ^{13}C-NMR (C)

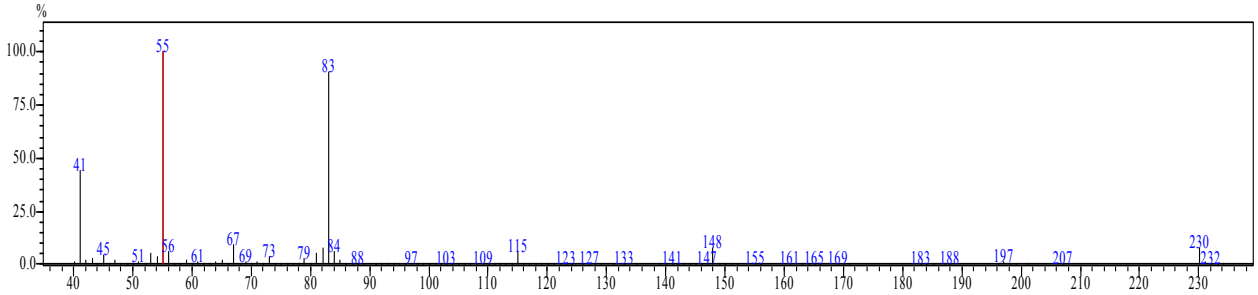


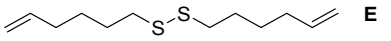




D

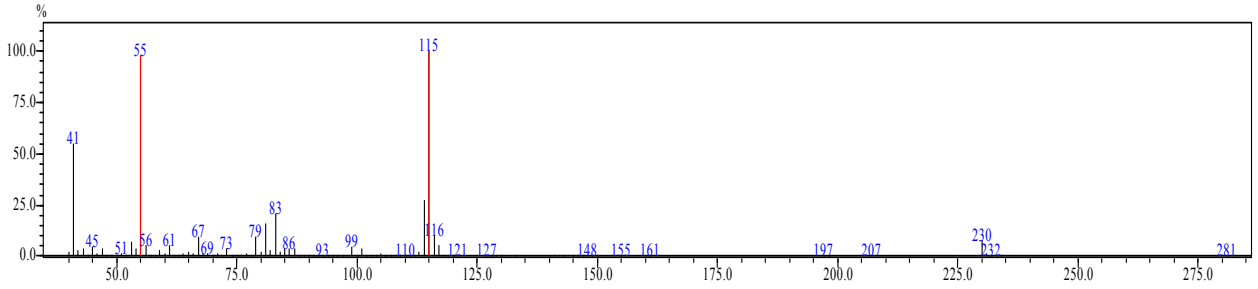
(E)-1-[[(E)-hex-2-henyl]disulfanyl]hex-2-ene (D)



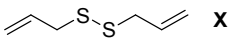


E

1,2-di(hex-5-en-1-yl)disulfide (E)

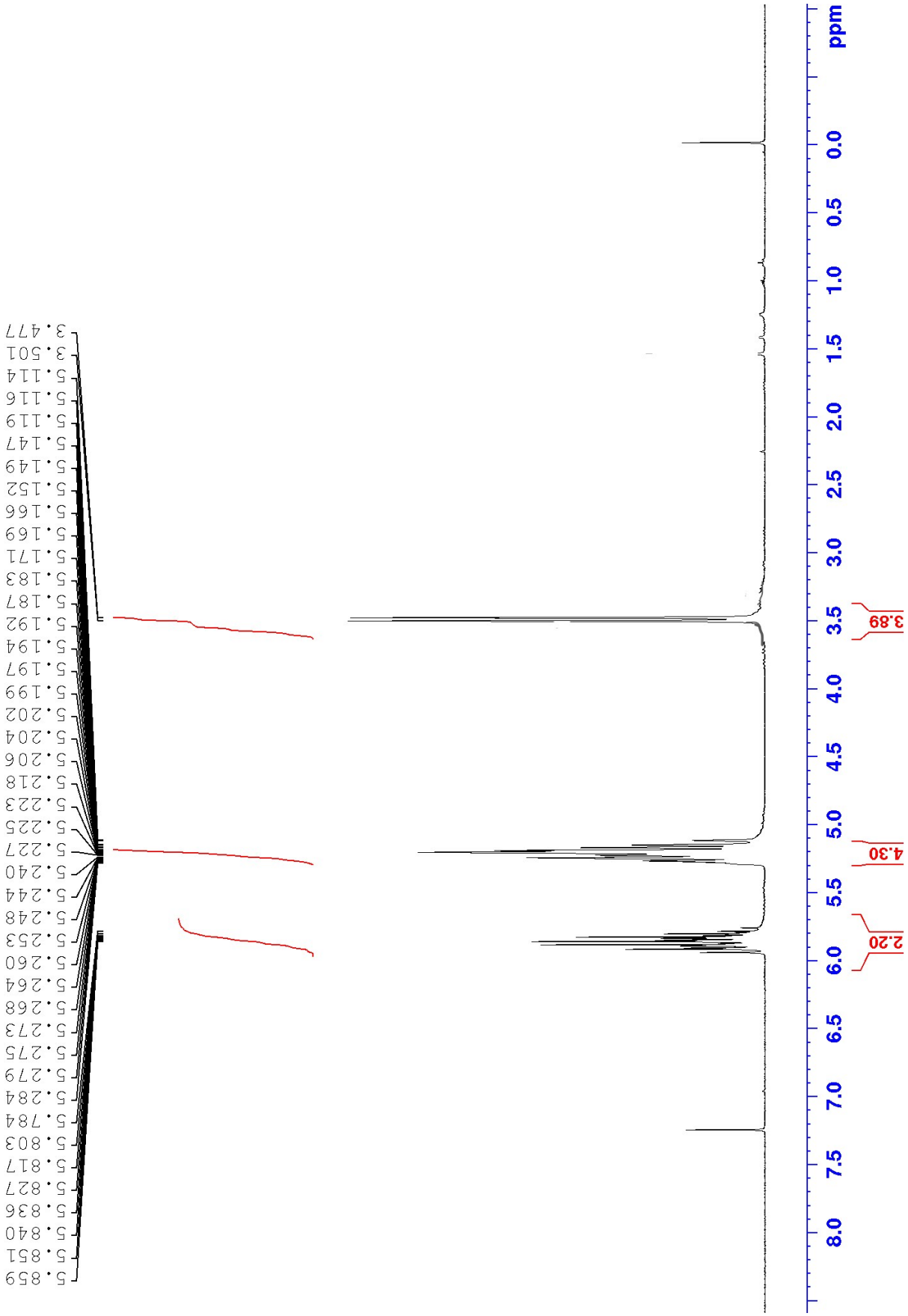


4.4 NMR spectra



X

^{1}H-NMR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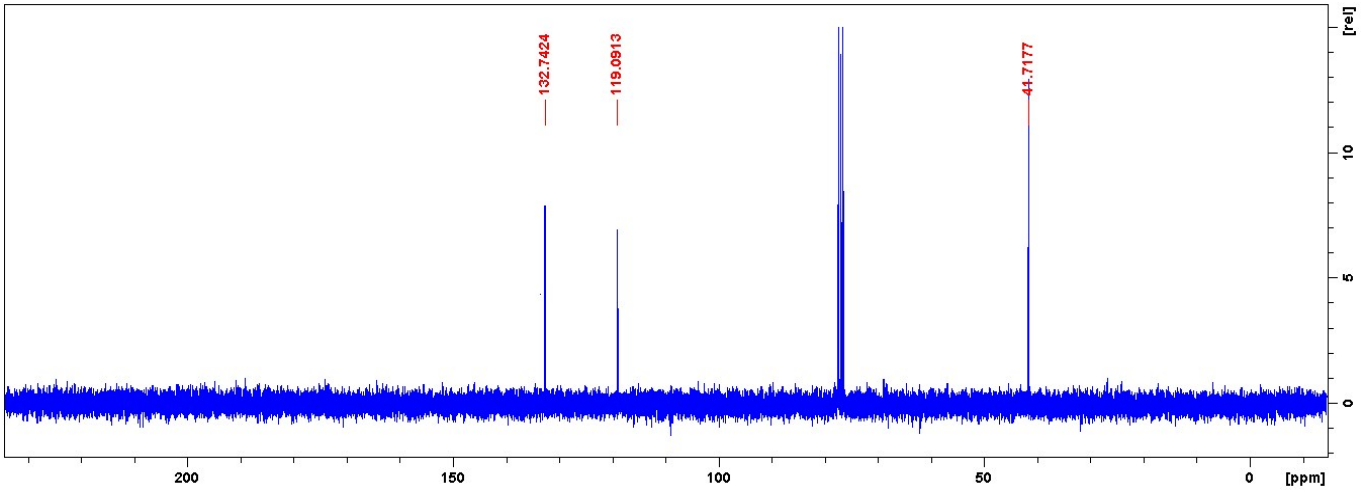
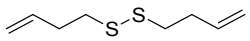
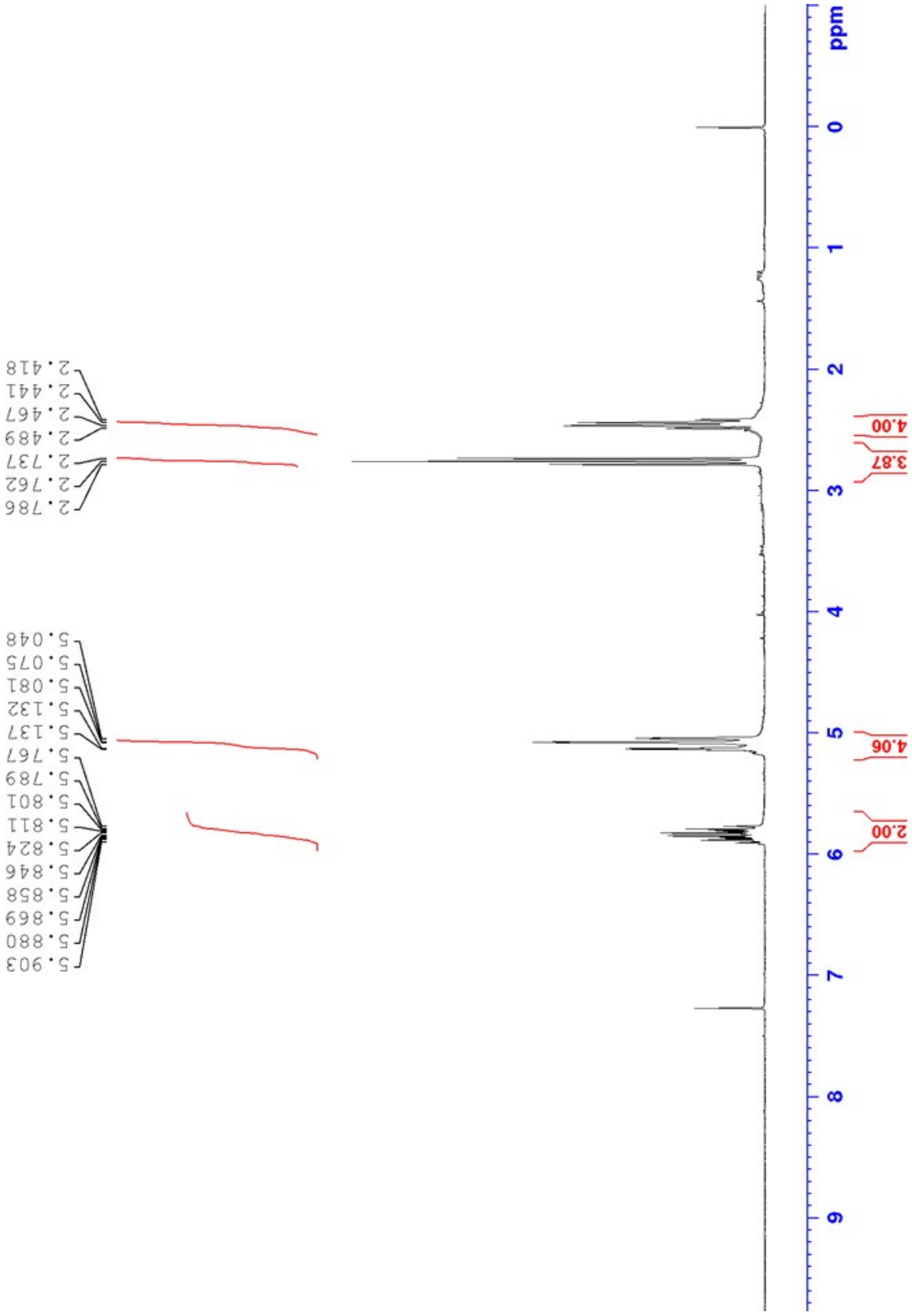
^{13}C-NMR





A

^{1}H-NMR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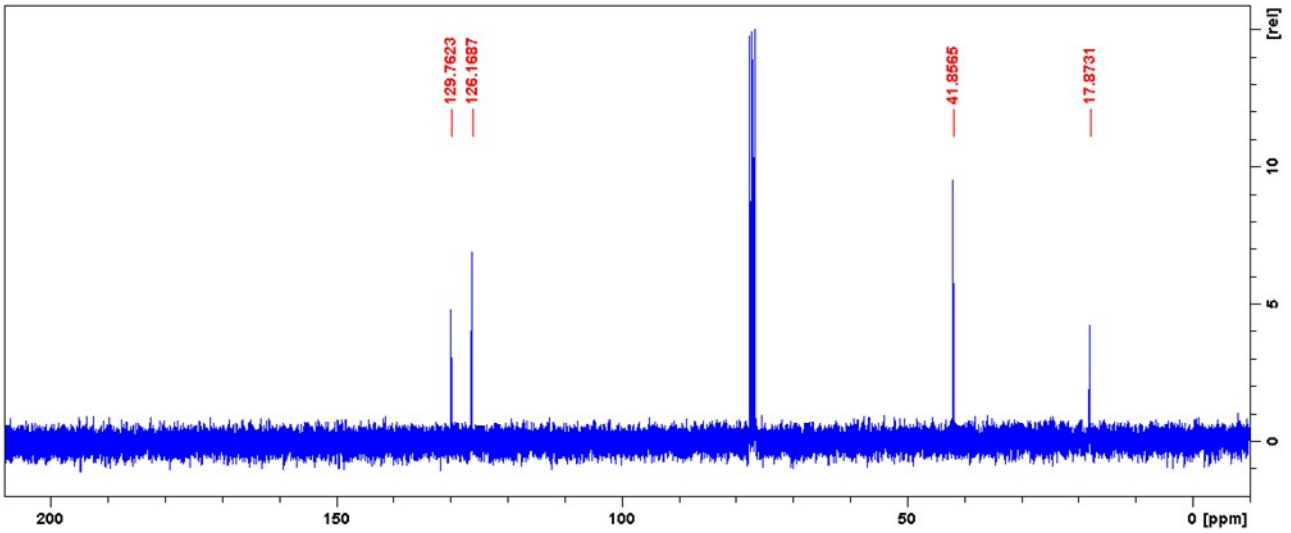
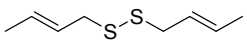
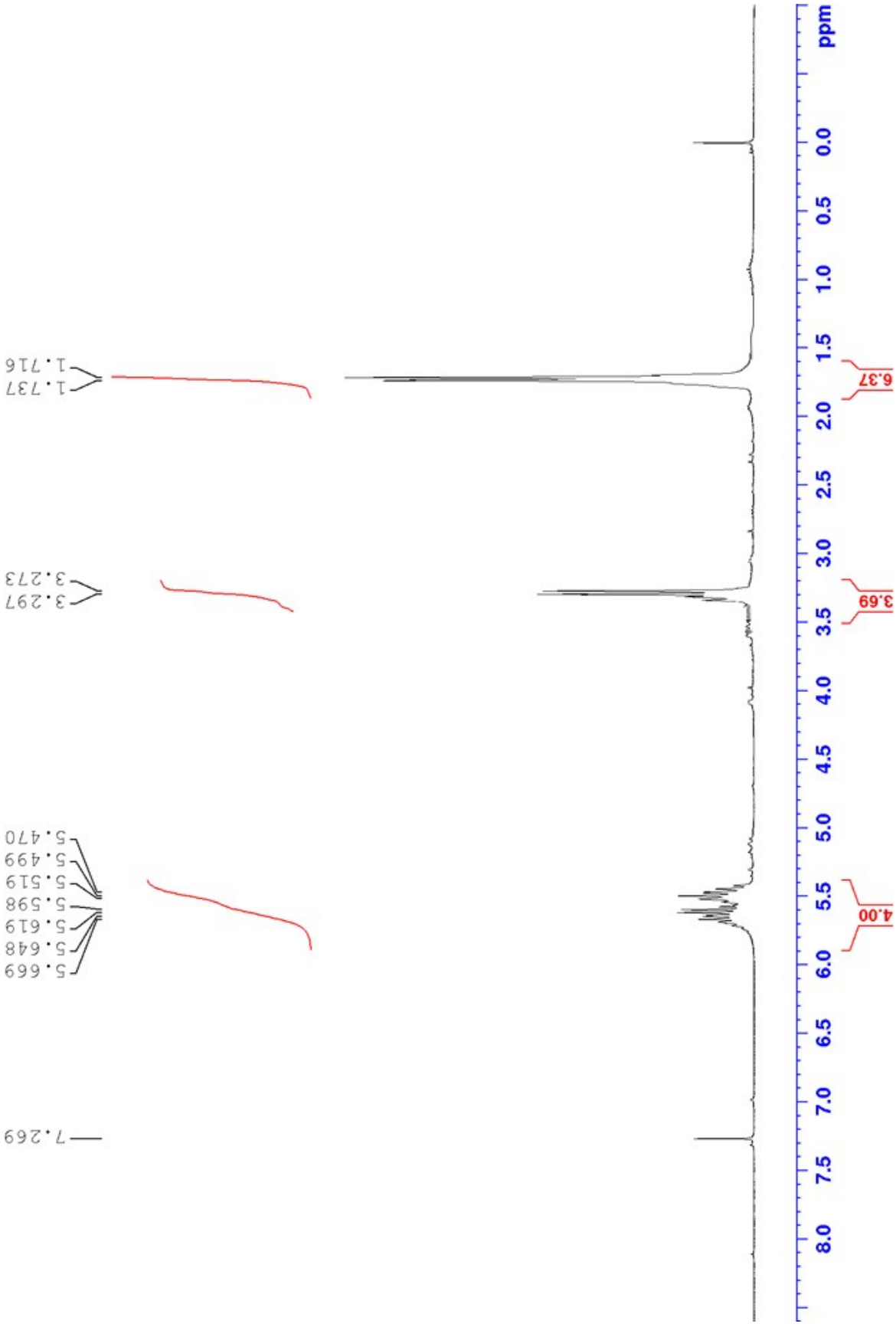
^{13}C-NMR





B

^{1}H-NMR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5.519
5.499
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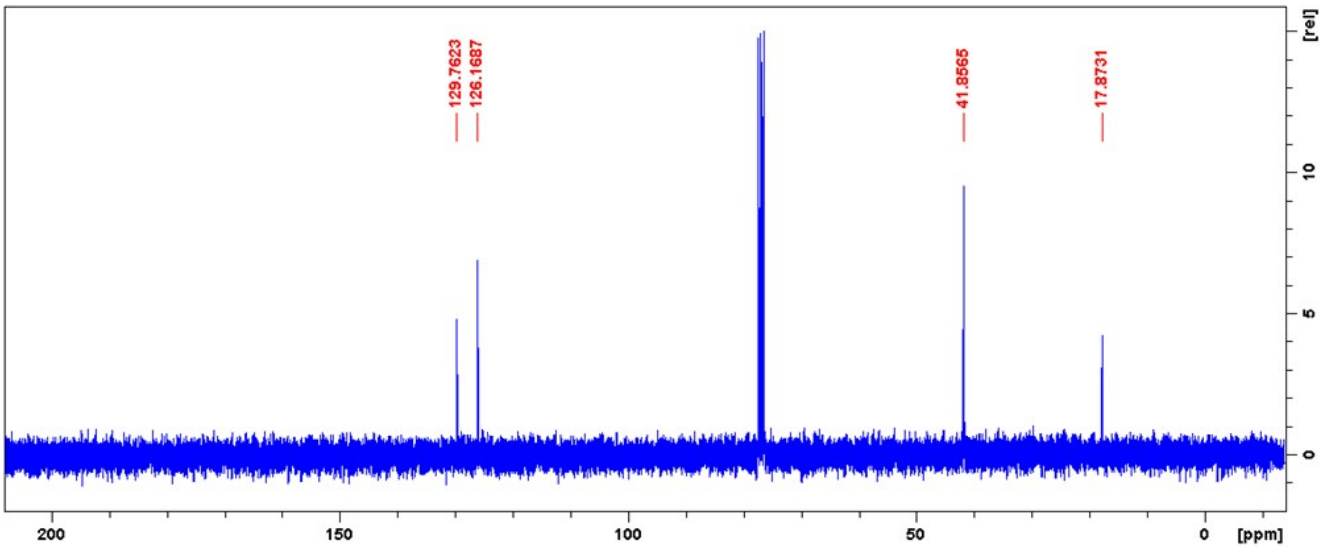
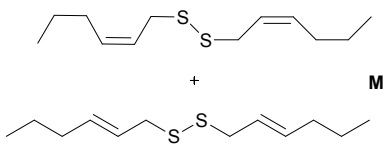
7.269

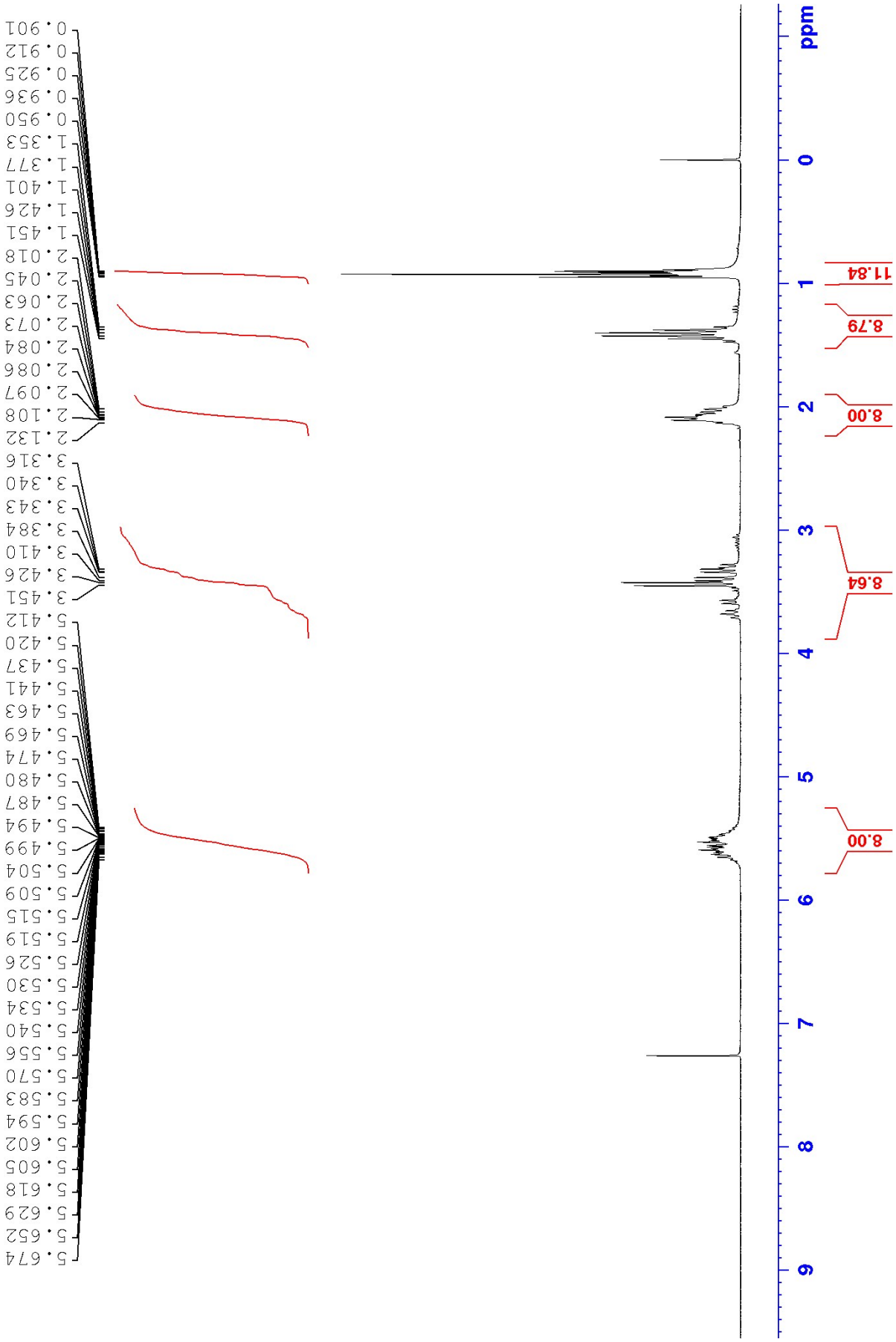
^{13}C-NMR



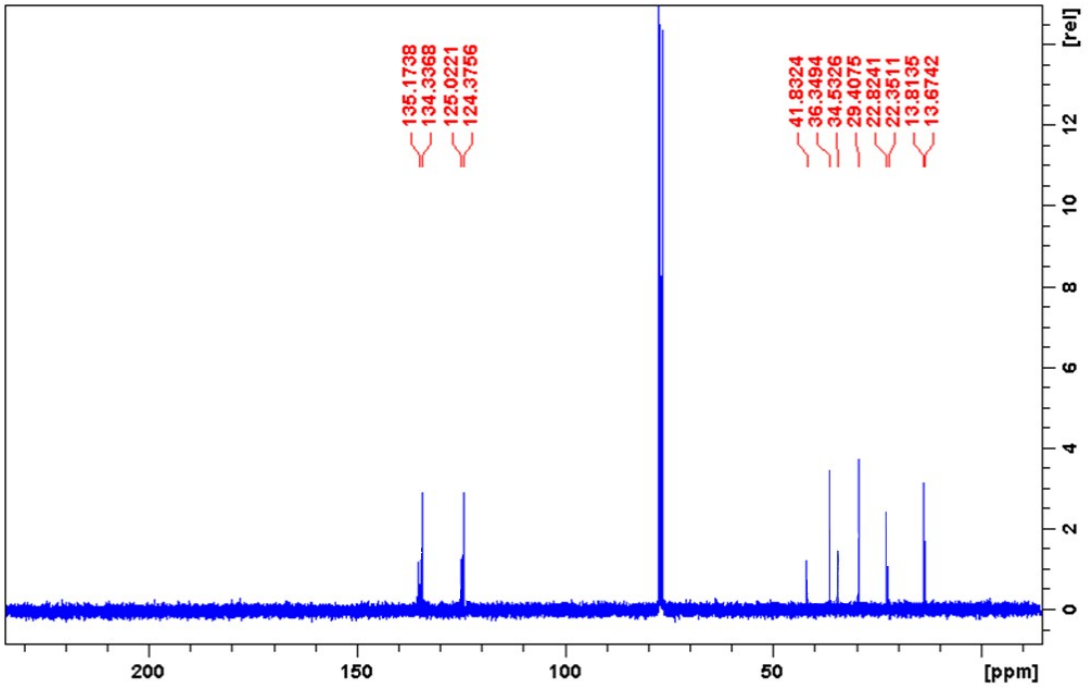


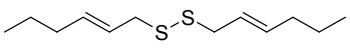
M

^{1}H-NMR


ppm
0
1
2
3
4
5
6
7
8
9
11.84
8.79
8.00
8.64
8.00
0.901
0.912
0.925
0.936
0.950
1.353
1.377
1.401
1.426
1.451
2.018
2.045
2.063
2.073
2.084
2.086
2.097
2.108
2.132
3.316
3.340
3.343
3.384
3.410
3.426
3.451
5.412
5.420
5.437
5.441
5.463
5.469
5.474
5.480
5.487
5.494
5.499
5.504
5.509
5.515
5.519
5.526
5.530
5.534
5.540
5.556
5.570
5.583
5.594
5.602
5.605
5.618
5.629
5.652
5.674

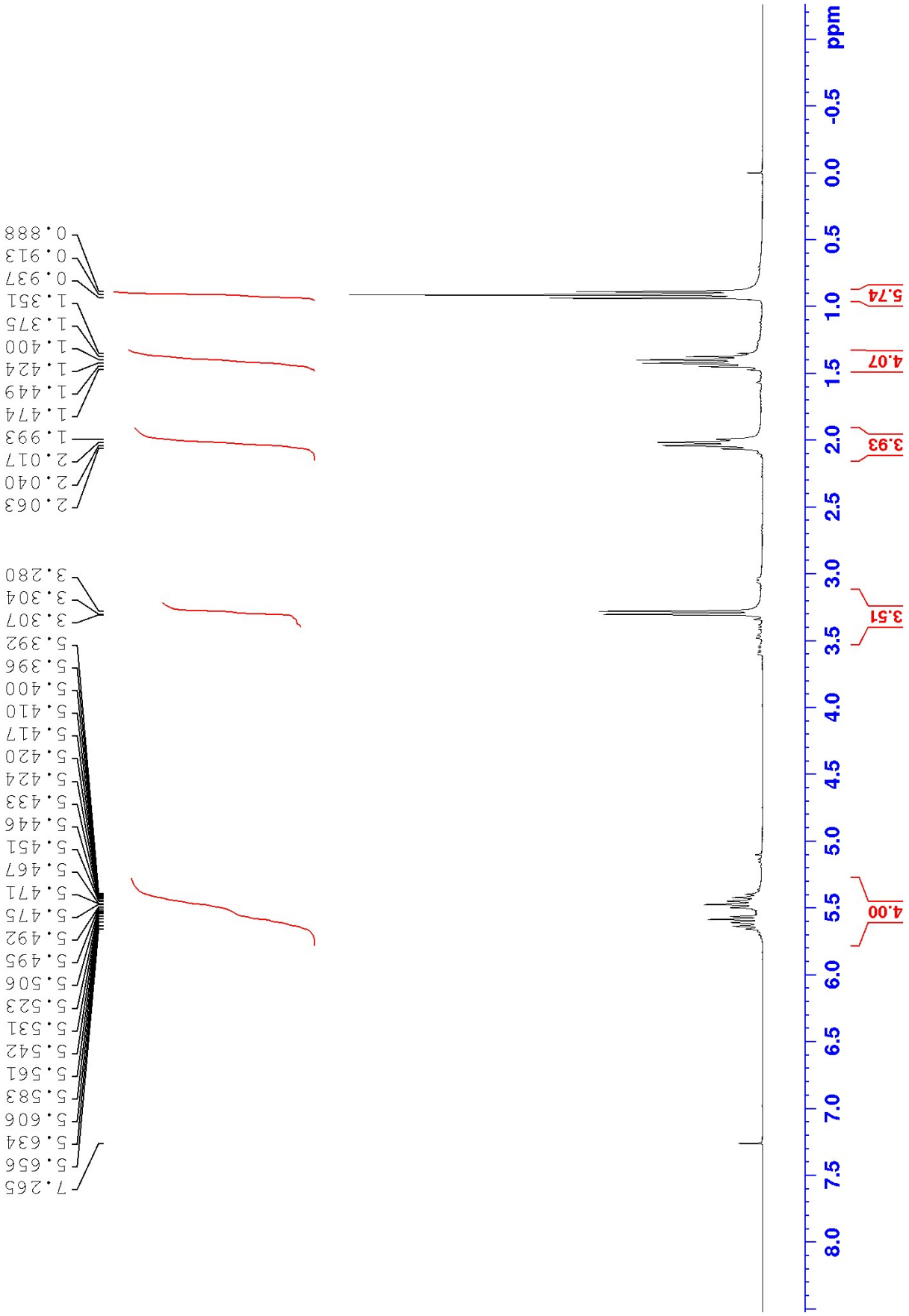
^{13}C-NMR



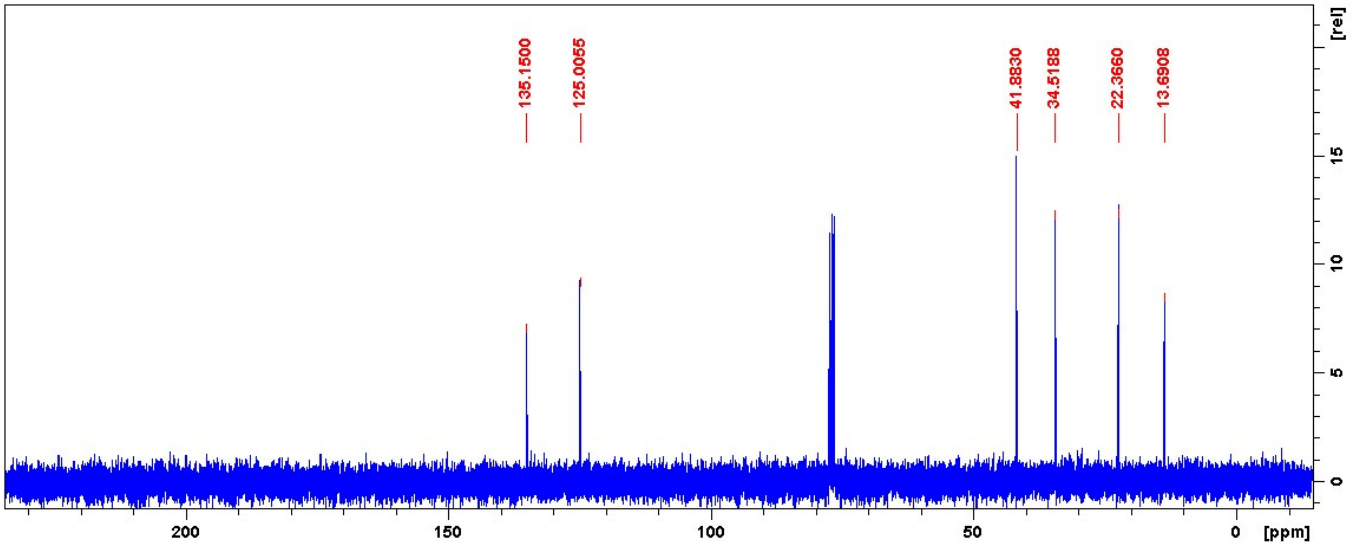


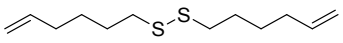
D

^{1}H-NMR


0.888
0.913
0.937
1.351
1.375
1.400
1.424
1.449
1.474
1.993
2.017
2.040
2.063
3.280
3.304
3.307
5.392
5.396
5.400
5.410
5.417
5.420
5.424
5.433
5.446
5.451
5.467
5.471
5.475
5.492
5.495
5.506
5.523
5.531
5.542
5.561
5.583
5.606
5.634
5.656
7.265
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
5.74
4.07
3.93
3.51
4.00

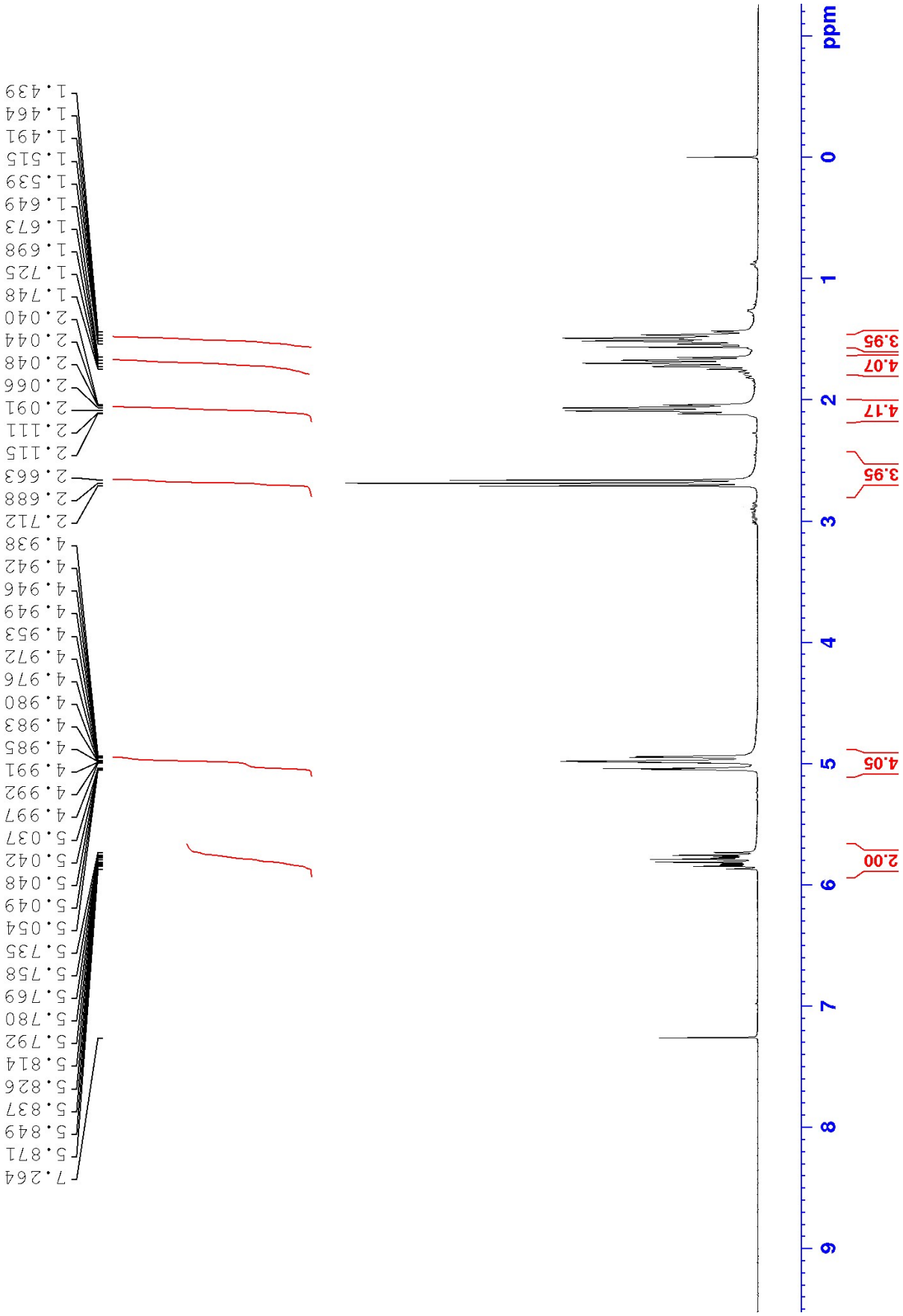
^{13}C-NMR



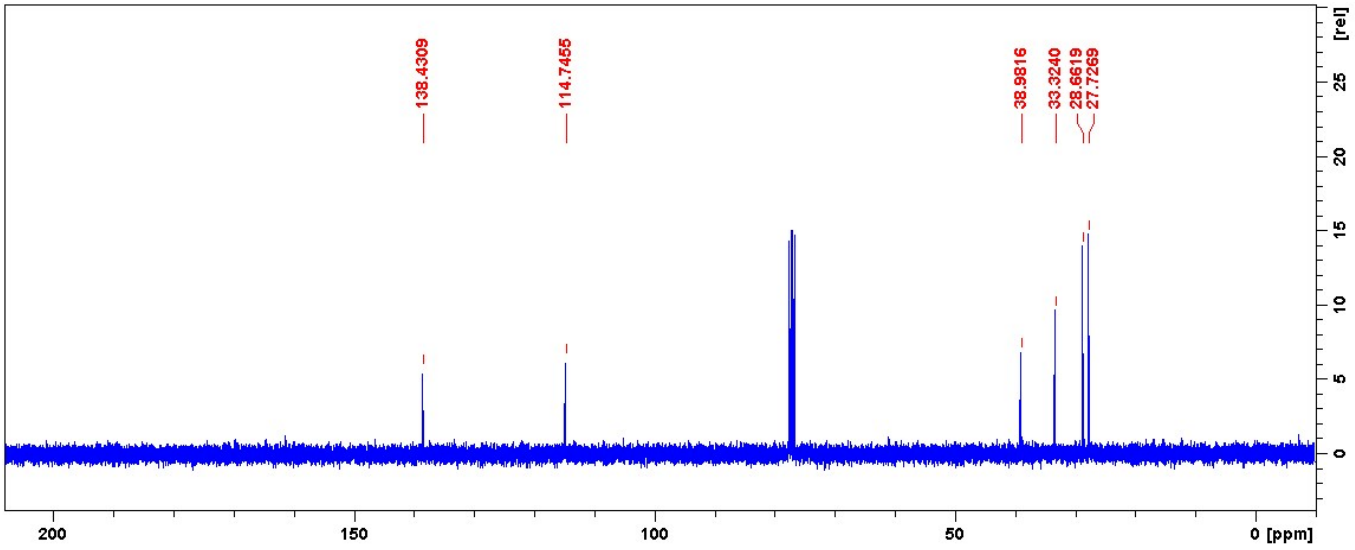


E

^{1}H-NMR


ppm
0
1
2
3
4
5
6
7
8
9
3.95
4.07
4.17
3.95
4.05
2.00
1.439
1.464
1.491
1.515
1.539
1.649
1.673
1.698
1.725
1.748
2.040
2.044
2.048
2.066
2.091
2.111
2.115
2.663
2.688
2.712
4.938
4.942
4.946
4.949
4.953
4.972
4.976
4.980
4.983
4.985
4.991
4.992
4.997
5.037
5.042
5.048
5.049
5.054
5.735
5.758
5.769
5.780
5.792
5.814
5.826
5.837
5.849
5.871
7.264

^{13}C-NMR

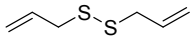

138.4309
114.7455
38.9816
33.3240
28.6619
27.7269
[rel]
25
20
15
10
5
0
200
150
100
50
0 [ppm]

5. General Bioassey procedure

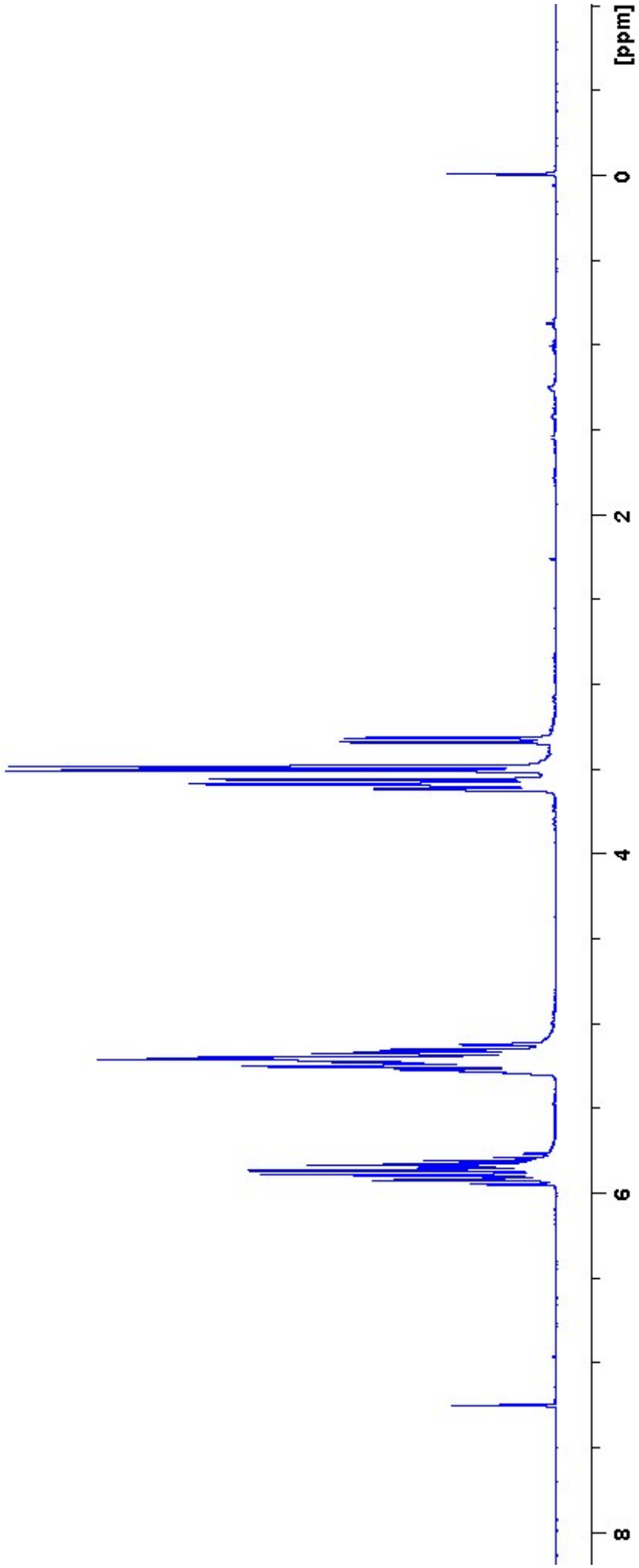
A549 cells were cultured in RPMI1640 medium (Gibco), supplemented with 10% fetal bovine serum (Gibco), and 1% penicillin/streptomycin solution (Gibco). Cells were seeded in 96 well plates (3000 cells/well), and 3 hours later treated with increasing concentrations of disulfide compounds or DMSO, for 24 or 48 hours. DMSO percentage in vehicle-treated cells (controls) was 0.5%, corresponding to the DMSO used for the highest concentration of the compounds (500microM). Then, cell viability was assessed by using CellTiter-Glo® Luminescent Cell Viability Assay (Promega), a homogeneous method for determining the viability of cell cultures based on quantitation of the ATP present, an indicator of metabolically active cells. Procedures were performed according manufacturer's instructions. Briefly, after addition of 100 microliters of Cell Titer Glo reagent, cells were incubated at room temperature for 1 hours under gentle shaking, and then luminescence was measured by the GloMax System (Promega). Results are expressed as percentage of the viability of DMSO-treated cells.

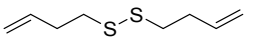
6. Stability test (compounds X, A and B)

Compounds **X**, **A** and **B** were allowed to stay in their pure form at room temperature (an average of 298,15 K) for one week. After one week the purity was confirmed by ^{1}H-NMR. Compounds **X**, **A** and **B** were dissolved in Deuterated Chloroform and were allowed to stay at room temperature (an average of 298,15 K) for one week. Here we report the ^{1}H-NMR spectra of these compounds. No significant changes in the ^{1}H-NMR spectra there are for compounds **A** and **B** respect to the previous reported spectra. Relevant structural changes of the compound **X** are evident from the ^{1}H-NMR spectrum after this test.

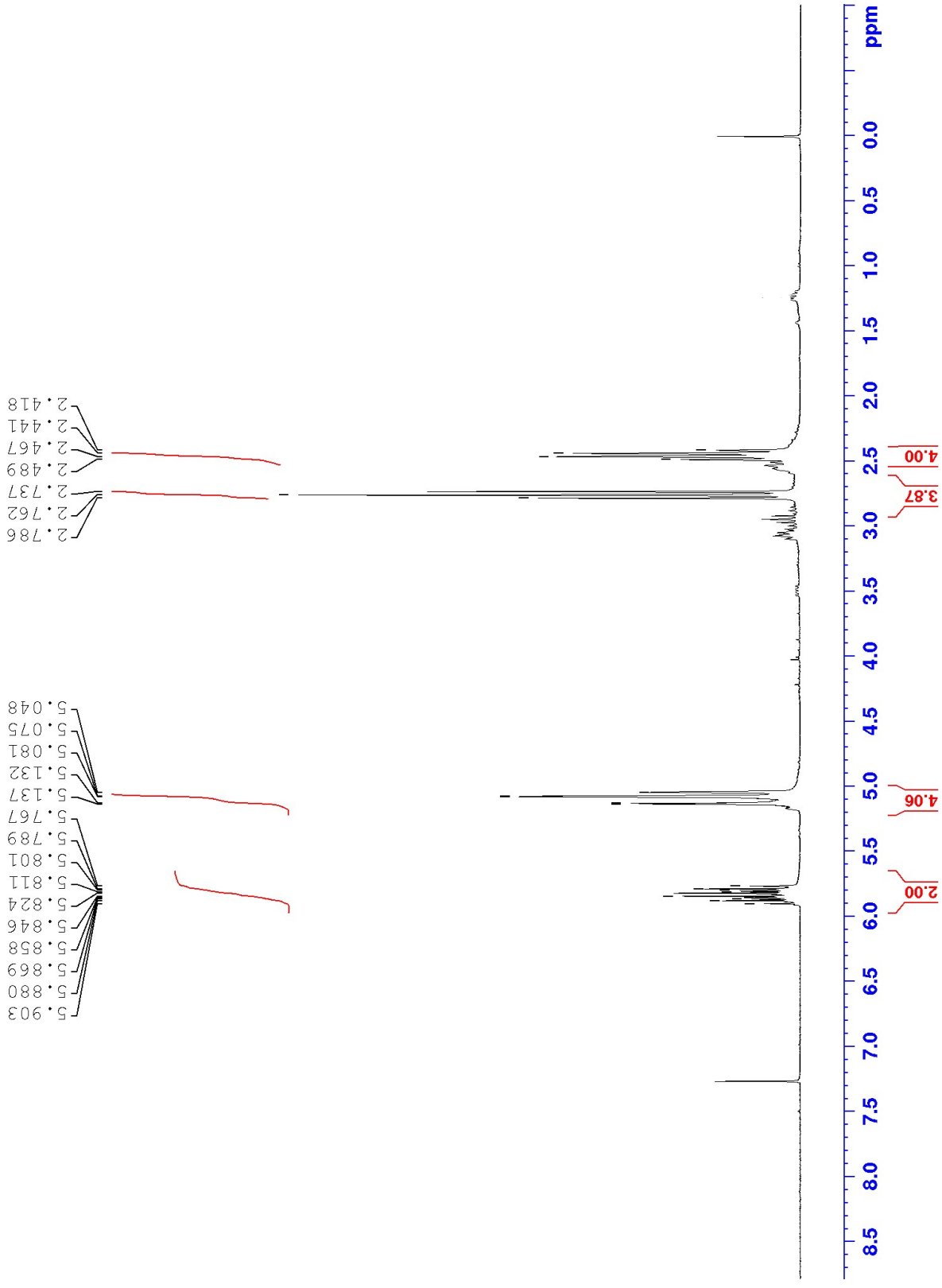
Diallyl disulfide –  **X**

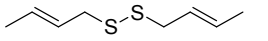
^{1}H-NMR after dissolving the molecule in deuterated chloroform and allowing the sample to stay at an average temperature of 298,15 Kelvin for one week. After purification by chromatography the molecule was estimated 40% pure after one week.


[ppm]
0
2
4
6
8

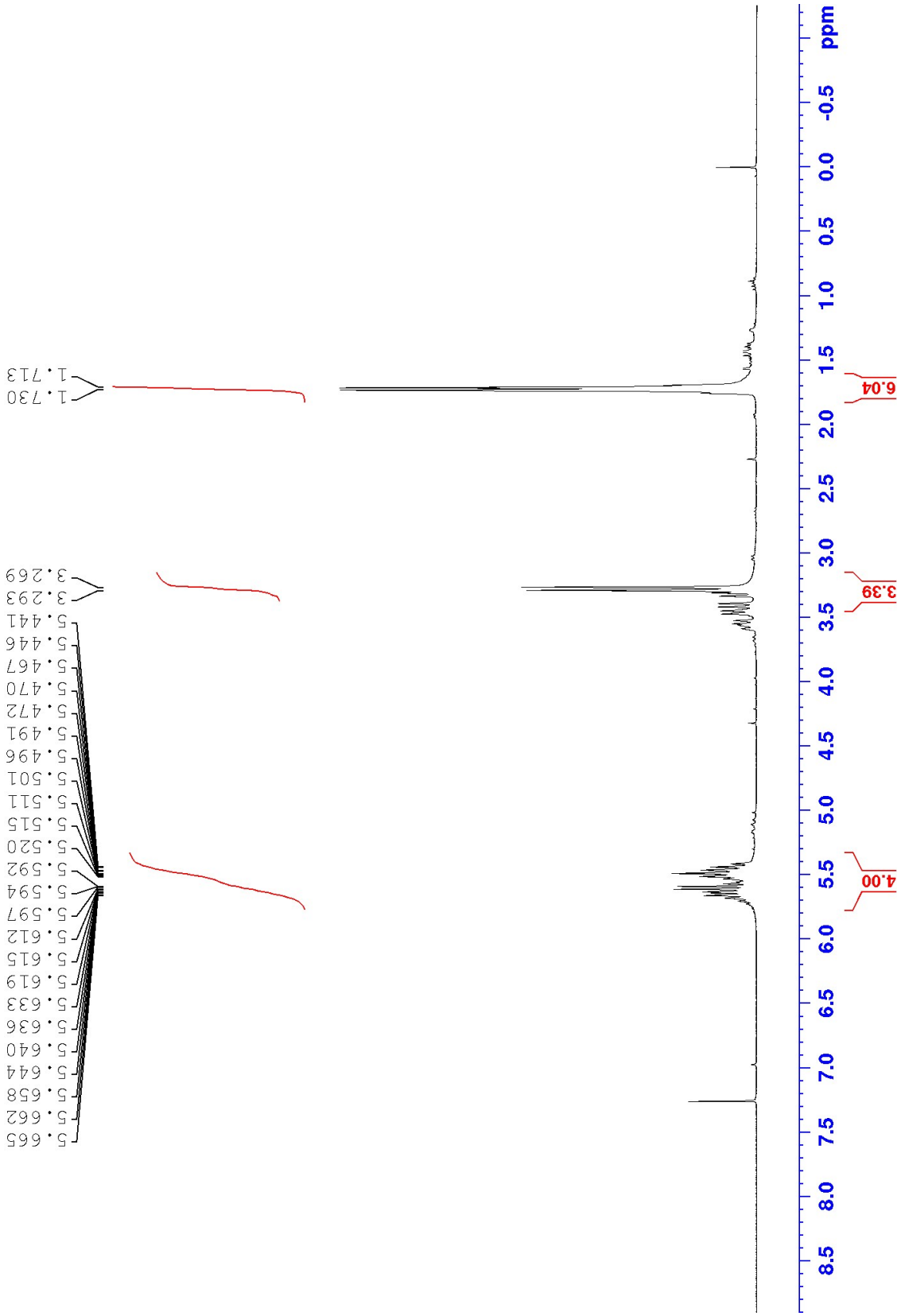
1,2-di(but-3-en-1-yl)disulfide –  **A**

^{1}H-NMR after dissolving the molecule in deuterated chloroform and allowing the sample to stay at an average temperature of 298,15 Kelvin for one week.


ppm
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
4.00
3.87
4.06
2.00
2.786
2.762
2.737
2.489
2.467
2.441
2.418
5.903
5.880
5.869
5.858
5.846
5.824
5.811
5.801
5.789
5.767
5.137
5.132
5.081
5.075
5.048

Trans-di(but-2-enyl)disulfide –  **B**

^{1}H-NMR after dissolving the molecule in deuterated chloroform and allowing the sample to stay at an average temperature of 298,15 Kelvin for one week.


1.713
1.730
3.269
3.293
5.441
5.446
5.467
5.470
5.472
5.491
5.496
5.501
5.511
5.515
5.520
5.592
5.594
5.597
5.612
5.615
5.619
5.633
5.636
5.640
5.644
5.658
5.662
5.665
6.04
3.39
4.00
ppm

7. Notes and references

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