KJ 2022
Spectroscopic Methods in Organic Chemistry
Laboratory exercise
Report

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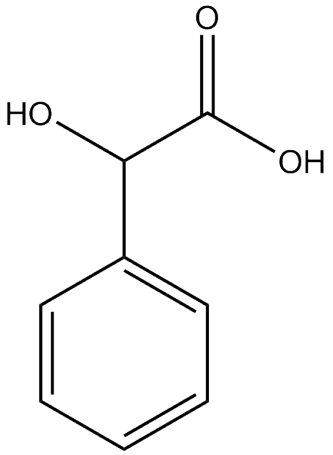

NTNU

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1 Introduction

This report is part of the compulsory laboratory exercise in the subject KJ 2022 Spectroscopic methods in organic chemistry at NTNU in the spring of 2013. The aim of this exercise is to elucidate the structure of an unknown compound. This is to be done using four spectroscopic methods: UV, IR, MS and NMR. The experiments will be carried out on the equipment mentioned, and the structure of the compound puzzled together piece by piece.

From the experimental spectroscopy data, the unknown compound assigned to Group 2 was found to be mandelic acid. UV and IR didn't give any big clues, but from MS it was clear that mandelic acid was the only possible compound. Both ^{1}H NMR and ^{13}C NMR later confirmed that mandelic acid was the right decision.

2 Theory

2.1 UV/VIS spctroscopy

UV-VIS spectroscopy is based on a compound's absorption of certain electromagnetic wavelengths in the visible spectrum as well as in the UV region of the electromagnetic spectrum. The reason for this absorption of energy is that electrons in π systems as well as non-bonding electrons may be exited to orbitals of higher energy. The normal excitation pattern is from a bonding π orbital (or non-bonding p orbital) to the anti-bonding π^* og σ^*.

The bigger the gap between the HOMO and LUMO orbital, the shorter the wavelengths have to be for the light to excite the electrons. This means that if short wavelengths are required, the chemical bonds need a lot of energy to get excited. The energy of electromagnetic radiation ΔE depends on the wavelength λ.

$$\Delta E = h \cdot \nu = h \cdot \frac{1}{\lambda} \tag{2.1}$$

where ν is the frequency and h is the Planck constant. The energy can be absorbed as electronic, vibrational or rotational energy, which are the energies making up the total energy of a molecule.

UV-VIS spectroscopy is particularly useful for compounds containing conjugated chromophores, such as dienes, aromatic rings and enones. However, for molecules not containing such systems, UV-VIS spectroscopy is useless and gives no information at all.

The UV-VIS instrument works by sending electromagnetic radiation of different wavelengths through a cuvette containing the sample solution. The intensities of the radiation that makes it through is measured, and compared to a beam that has been sent through a reference cuvette. The way to do this in the instrument is usually solved in either of two ways. If the instrument is a double beam instrument, it has one lamp producing one beam, which is then split in two by a half mirror. The two beams are then sent through the sample and reference respectively. A

single beam instrument can not split the beam, and have to analyze the sample and the reference separately.

The signal from the reference cuvette with the pure solvent is used as a measurement for the background signal which is assumed to be the same as for the incident beam for the sample cuvette. The transmittance is then calculated as the ratio of the intensity if the beam hitting the sample I equal to the one emerging from the reference and the intensity of the beam emerging from the reference cuvette I_0.

$$T = \frac{I}{I_0} \tag{2.2}$$

Beer-Lamberts law can be used to predict the absorption for a certain compound. It was proposed in 1729 by Lambert and then improved by Beer in 1852. It also gives the relation between the inverse transmittance and the absorbance.

$$A = \varepsilon l c = \log_{10}(I/I_0) \tag{2.3}$$

where ε is the molar absorptivity of the solute, c is the conscentration of the solute, l is the lights pathlength through the cuvette.

The Woodward-Fieser rules are a set of empirical rules for predicting the wavelength of maximum absorption λ_{max}. In order to calculate this, one needs to know which *chromophores* are present in the molecule. Chromophores are the parts of the compound which are responsible for the colour, and in the Woodward-Fieser rules chromophores are generally functional groups. *Auxochromes* are substituents to the chromophore and change its absorption maxima.

If the auxochrome moves the absorption maxima to a longer wavelength, it's called a *bathochromic shift*, in contrast to a *hypsochromic shift* where the maxima is moved to a shorter wavelength.

2.2 IR spectroscopy

Infrared (IR) radiation refers to that part of the electromagnetic spectrum between the visible light and the microwave regions. Organic compounds will absorbe and convert infrared radiation between 10 000-100 cm^{-1} into energy of molecular vibration, but the most interesting region is between 4000-400 cm^{-1}. The whole molecule can vibrate or just some of the individual bonds or functional groups within the molecule. There are two types of molecular vibration: stretching and bending. It is only the vibrations that result in a change in the dipole moment that can be observed in the IR spectra. It is possible with both asymmetrical and symmetrical vibration. Since symmetrical vibrations doesn't result in a change of dipole moment, they are inactive in IR spectroscopy.

2.2.1 Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a technique used to obtain an infrared spectrum. A beam of light containing the whole range of IR frequencies is passed through the sample, and measure how much of the beam is absorbed by the sample. A second beam, which has travelled a longer path length, is combined with the first beam and an interferogram is produced. A computer uses Fourier transformation to convert this interferogram into a plot of absorption against frequency.

2.2.2 Sample preparation

Infrared spectra may be obtained for liquids, solids and gases. Liquids can either be examined neat or in solutions. If it is examined neat, a few drops of the liquid is placed between two sodium chloride plates and the spectrum can be recorded. Both liquids and solids can be dissolved in a solvent. The solution is placed in an IR solution cell which consists of two rectangular sodium chloride plates. IR spectra for solids can also be recorded in the form of a KBr disk. The sample is mixed with potassium bromide and is exposed for a very high pressure with the use of a hydraulic press. This high pressure causes potassium bromide to become fluid and the result is a KBr disk containing the solid sample. Potassium bromide

is transparent throughout the IR range and therefore doesn't interfere with the spectra.

2.3 Mass Spectroscopy

The concept of mass spectrometry is that a compound is ionized and the ions are separated based on their mass/charge ratio, and the number of ions representing each mass/charge unit is plotted as a spectrum. The spectrum will consist of x-axis with mass/ratio and intensity of the mass/ratio units at the y-axis.

The mass spectrometry instrument consist of sample introduction, ionization method, mass analyzer/ion separation method and detector. Some instruments have direct insertion of the sample into the ionization chamber.

There are different types of ion sources, one example is electron impact ionization. In the electron impact ionization method the mass spectrometer bombards molecules in vapour phase with a high-energy electron beam. This result in the ejection of an electron from the sample molecule to produce a radical cation, molecular ion. Molecular ion, M^+, then represents the intact molecule with one less electron which is removed by the electron beam. The smaller ions are fragments of the molecular ion. The molecular ion is fragmented because of excess energy recieved from the bombarding electron. Covalent bonds in the molecular ion breaks in "predictable" patterns and is useful in the identification of an unknown compound.

The detector consist of collimating slits that direct only one set of ions at a time into a collector where they are detected and amplified by an electron multiplier. The signals are saved and plotted.

The fragmentation pattern and the molecular ions are helpful in the identification of an unknown compound. From the molecular ion you find the molecular mass of the compound. From the different fragments it is possible to find the different units the compound consist of and how the compound is constructed. The most

intense peak in the spectra is called the base peak (100%) and the other fragments are measured in percentage to this base peak. Often the base peak is the molecular ion, this is however only when there are no fragments that are more stable than the molecular ion. The molecular ion is usually the peak with the highest m/z value. The probability that a given bond will be cleaved is related to the strength of the bond, to the possibilty of low energy transition, and to the stability of the formed fragments. There are some general signal and fragment patterns listed below:

- For straight-chained compounds the relative height of M^+ is greatest, and the height decreases with the degree of branching in the molecule.
- With an increasing molecular weight in a homologous series, the relative height of M^+ decreases accordingly.
- Cleavage is favored at the alkyl-substituted carbon. The more substituted, the more likely is a cleavage. Generally the largest substituent is removed first, then the subsituent leaves as a radical.
- The double bonds, cyclic structures and aromatic systems stabilize the molecular ion.
- Allylic cleavage is favored for double bonds, because the allylic carbocation is resonance-stabilized.
- Saturated rings generally lose their alkyl side chains at the α position.
- In alkyl substituted aromatic compounds there is often a cleavage at the bond β to the ring, since this gives a resonance-stabilized ion.
- The carbon-carbon bond next to a heteroatom is easily cleaved. The charge is then left on the fragment containing the heteroatom.
- Elimination of small, neutral molecules often result in cleavage. Example of such small neutral molecules are carbon monoxide or water.

A computer calculate the molecular formula for the analyzed compound. From the calculated molecular formula the index of hydrogen deficiency (IHD) can be found using Equation (2.4) relating it the the chemical formula $C_nH_mX_XN_yO_z$. The hydrogen deficency index is the number of hydrogen molecules missing from the linear saturated version of the compound. From the hydrogen deficency one therefore find if and how many multiple bonds and rings the compound contain.

$$IHD = n - \frac{m}{2} - \frac{x}{2} + \frac{y}{2} + 1 \tag{2.4}$$

2.4 NMR spectroscopy

Nuclear magnetic resonance (NMR) is an absorption spectrometry. A sample can absorb electromagnetic radiation in the radio frequency region when it is in a magnetic field. A plot of the frequencies of the absorption peaks against peak intensities is a NMR spectra, and it provides more information about the molecular structure than any other spectroscopic method.

All nuclei carry a charge, and if this charge spins on the nuclear axis a magnetic dipole will be generated. Nuclear angular momentum is related to quantum spin number I. These numbers can have values of 0, 1/2, 1, 3/2 and so on. All isotopes that contain an odd number of protons and/or neutrons, such as hydrogen, nitrogen and carbon-13, will have half-integer spin quantum number and will be NMR active. It is the atomic nuclei that absorbs the electromagnetic radiation. This results in change in the angular momentum. After some time the atomic nuclei will relax back to ground state of angular momentum while emitting electromagnetic radiation. This radiation is measured and plotted in the NMR spectra.

2.4.1 Chemical shift

Protons in a molecule is shielded by its electron cloud to a very small extent. The intensities of the shielding varies with the chemical environment that leads to differences in chemical shift positions. The degree of shielding depends on the density of the circulating electrons and the inductive effect of other groups attached to the carbon molecule. The difference in the absorption position of a proton from

the absorption position of a reference proton is called the chemical shift of the proton.

2.4.2 Spin coupling

Spin coupling is a phenomenon caused by coupling of proton spins through the intervening bonding electrons. This spin coupling results in multiplets in the NMR spectra. The number of peaks in a multiplet is n+1, where n is the number of hydrogen atoms at the neighbouring carbon atoms. Spin coupling is typical for ^{1}H NMR.

2.4.3 NMR spectra

There are different types of NMR spectra, for example ^{1}H NMR, ^{13}C NMR, HSQC, HMBC and COSY. They can be further divided in to one dimensional and two dimensional spectra. The one dimensional ^{1}H NMR spectra illustrate what type of chemical environment the different hydrogen atoms have in the compound. ^{13}C NMR is similar to ^{1}H NMR except that in ^{13}C NMR the spin coupling is not expressed. The two dimensional spectra is otained by preforming sevral one dimensional analysis and plotting them together. COSY stands for Correlation spectroscopy. In COSY spectra coupling between hydrogen atoms in the compound is displayed. HSQC stands for Heteronuclear single quantum coherence. In HSQC NMR the coupling between carbon atoms and hydrogen atoms displayed. HMBC stands for Heteronuclear multiple bond correlation. In HMBC spectra coupling between carbon atoms and hydrogen atoms two to four carbons away from the given carbon. COSY, HSQC and HMBC are all two dimensional methods/analysis.

3 Results

3.1 UV/VIS

The UV/VIS-spectroscopy analysis revealed a very weak absorption for the unknown compound, and the λ_{max} was observed at 354 nm. The absorption spectrum is attached as Appendix A.

3.2 IR

The diamond tip FTIR spectroscopy experiment gave an absorption spectrum attached as Appendix C. The spectrum gave the following absorption for the unknown compound. IR (diamond tip, cm^{-1}): 3437.14 (O-H stretch), 2894.48 (ν_{as} (CH) stretch), 1710.02 (C=0 stretch), 1490.55 (C-C stretch), 1450.73 (C-C stretch), 1241.33 (C-O stretch, COOH), 1190.45 (C-C stretch), 1095.47 (C-O stretch, sec. alc.), 1060.45 (C-C aromatic stretch).

3.3 MS

The MS spectrum is attached as Appendix B. The spectrum clearly shows the molecular ion peak at 152.1 m/z. And from high resolution MS the molecular ion peak was determined to have a mass over charge ratio of exactly 152.0470. Which leaves two potential formulas: $C_8H_8O_3$ (152.0468) and CH_8ON_6S (152.0475).

The most important peaks in the MS spectrum are explained in Table 3.1, and the important fragments are shown in Figure 3.1 and Figure 3.2.

Table 3.1: List of peaks and assosiated fragments from the MS analysis.

Peak	**m/z**	**Fragment**	**Comment**
Molecular ion	152.1	-	Molecular ion M^+
Base peak	107.1	COOH	Loss of COOH group (m/z 45), usual clevage at α-carbon for aromatic carboxylic acids
Strong peak	79.1	$C_6H_7^+$	Characteristic cluster from 77 to 79 derived from monoalkylbenzene, from benzyl alcohol
Weaker peaks	51.1	$C_4H_4^+$	Loss of acetyl group from fenyl ion
	90.1	$C_7H_7^+$	Tropylium ion usual fragment from alkyl substituted benzene
	105.1	$C_7H_5O(Ph{-}C^{\cdot}{=}O)$	Benzaldehyd cation

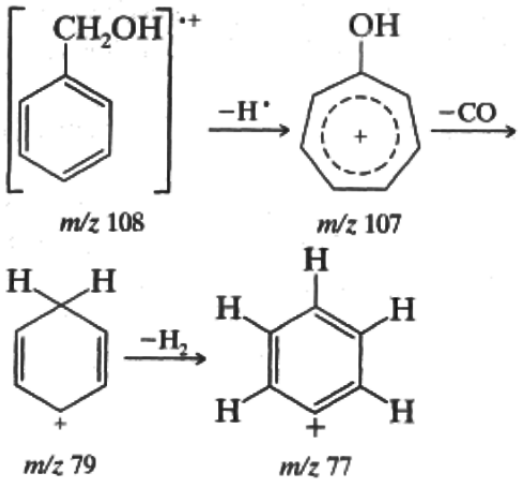

Figure 3.1: Fragmentation patterns common for aromatic alcohol fragments.

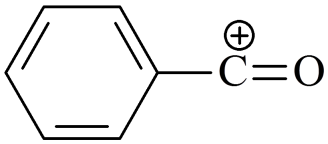

Figure 3.2: Fragment responsible for the 105.1 m/z signal in the MS spectrum.

From the molecular formula, one can find the hydrogren defficiency index of the compound with Equation (2.4). For the unknown compound the calculation will be:

$$IHD = 8 - \frac{8}{2} - \frac{0}{2} + \frac{0}{2} + 1$$

This means that in order to fully saturate the molecule, one would have to add 5 molecules of H_2.

3.4 NMR

The various NMR spectrums are attached as Appendix D. It is clear from the ^{13}C NMR spectrum that the compound has eight carbon atoms, the *ortho* and *meta* carbons being equivalent and showing higher intensities.

The integrals from the ^{1}H NMR spectrum indicated that there are a ratio of 5 to 1 of aromatic hydrogens to aliphatic hydrogens.

The following shifts were found from the ^{1}H NMR and the ^{13}C NMR spectrums.
^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 7.44 - 7.39 (m, 2H, CH), 7.38 - 7.31 (m, 2H, CH), 7.31 - 7.25 (m, 1H, CH), 5.02 (s, 1H, C-H).
^{13}C NMR (400 MHz, DMSO-d$_6$) δ: 174.08 (1H, COOH), 140.25 (1H, C), 128.11 (2H, CH), 127.62 (1H, CH) , 126.62 (2H, CH), 72.37 (1H, CH).

Table 3.2 shows the correlations found using HSQC, while Table 3.3 shows the correlations found using HMBC. The COSY spectrum gave little information because of extensive coupling between almost all the protons.

Table 3.2: Correlations found using HSQC found from ^{1}H NMR and ^{13}C NMR spectrums run at 400 MHz in DMSO-d$_6$

$\delta_{^1H}$ [ppm]	$\delta_{^{13}C}$ [ppm]
5.02	72.37
7.44 - 7.39	126.62
7.31 - 7.25	127.62
7.38 - 7.31	128.11
-	140.25
-	174.08

Table 3.3: Correlations found using HMBC found from ^{1}H NMR and ^{13}C NMR spectrums run at 400 MHz in DMSO-d$_6$

$\delta_{^1H}$ [ppm]	$\delta_{^{13}C}$ [ppm]
7.44 - 7.39	72.37
	127.62
7.38 - 7.31	128.11
	140.25
7.31 - 7.25	126.62

4 Experimental

4.1 UV/VIS

2.5 mg of the unknown compound was dissolved in methanol and transferred to a volumetric flask. The solution was then diluted to 10 ml. 1 ml of the first solution was transferred to another 10 ml volumetric flask and diluted to 10 ml. This was done once again, so that three different dilutions of the compound had been prepared. After analysis, another two samples were prepared, with even stronger concentrations. The strongest of these concentrations (9.7 mg / 10 ml) gave barely visible UV absorption.

4.2 IR

A spatula tip of compound was transferred to the diamond tip FTIR instrument. The diamond tip was lowered until it was touching the compound. The IR spectrum was obtained using a 32 smart scan setting.

4.3 NMR

The compound was dissolved in deuturated DMSO, transferred to an NMR tube, placed in a spinner and then loaded into the NMR instrument. The full characterization of the compound was performed, giving ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, COSY, DEPT 95, DEPT 135 spectrums. During a meeting in the NMR lab the NMR spectras were handed over from the supervisor for the NMR lab.

4.4 MS

The MS spectrum was handed over from the superviser for the MS lab. The MS-spectrum was obtained with Thermo Quest MAT95X double focusing high resolution instrument using electron impact ionization (EI) at 70eV.

5 Discussion

The low absorbance of the unknown compound was a bit unexpected, seeing that it has an aromatic ring. The π-system of the conjugated ring should be quite visible in UV/VIS spectroscopy, and the recorded λ_{max} is higher than expected from our compound. The low absorbance might come from the UV/VIS machine possibly being broken on the day of the experiment. The most important information gathered from the UV/VIS spectrum is that the compound shows signs of absorbance. This indicates that there are some conjugated electrons present.

From the IR spectrum there are a few more pieces of information to notice. The primary being that there is a sharp absorbance peak around 1710 cm^{-1}. This is a very clear indication of a carbonyl group with the C=O stretching vibration. Also the absorbance at 2894 cm^{-1} might indicate a methine C-H stretching vibration, as well as the 3437 which might be an indication of an O-H stretching vibration. In the fingerprint region between 1500 and 500 cm^{-1} the IR spectrum shows a lot of absorption which is in correspondence with aromatic C-H stretching vibrations.

From the high resolution MS spectrum the molecular ion peak gave the molecular mass of the unknown compound, 152.0470, which corresponds well with $C_8H_8O_3$ (152.0468) and CH_8ON_6S (152.0475), where only the first is a real molecule. The base peak of [M-45] is characteristic for aromatic carboxylic acids, by cleavage at the α-carbon, and loss of a COOH group. The 107.1 m/z peak comes from benzyl alcohol, which follows the fragmentation pattern shown in Figure 3.1. This explains the strong peaks at 79.1 m/z and 77 m/z as well.

The 105.1 m/z peak likely comes from loss of H_2 from the benzyl alcohol. The 90.1 m/z peak is usually strong in all alkyl substituted benzyl compounds, and is also clearly visible in our spectrum. The peaks in the MS spectrum is in accordance with already reported peaks for mandelic acid, as well as the spectrum [2]. None of the other possible structures with the same molecular formula coincide with the MS-spectrum

The unknown compound consist of eight carbon atoms, this can be seen in the ^{13}C NMR spectrum. In the spectrum there are six peaks, were two of them have very strong intensities, indicating that there are two equivalent carbons for each peak, so eight carbon atoms in total.

A ratio of 5 to 1 of aromatic hydrogen to aliphatic hydrogen is found from the ^{1}H NMR spectrum. In the ^{1}H NMR the total integral of hydrogen atoms is 5 in the aromatic part of the spectrum and 1 in the aliphatic part of the spectrum. This indicates a ring with substitution in the ratio 5:1 hydrogen atoms. From the MS it is clear that there are 8 hydrogen atoms in the compound, the proton NMR only shows 6. Therefore one can conclude that there are two hydrogens bound through heteroatons, in this case oxygen atoms.

The correlations between hydrogen and hydrogen atoms, and hydrogen and carbon atoms in the HSQC and HMBC spectra fits with the obtained structure.

6 Conclusion

The structure of the unknown compound was initially found using the data for the molecular mass from the MS experiment as well as MS spectrums. The molecular weight was compared to a database, and mandelic acid was the only compound with a match. IR and NMR was used to confirm the results obtained from MS. The proposed structure with the assigned carbon and proton NMR shifts is shown in Figure 6.1.

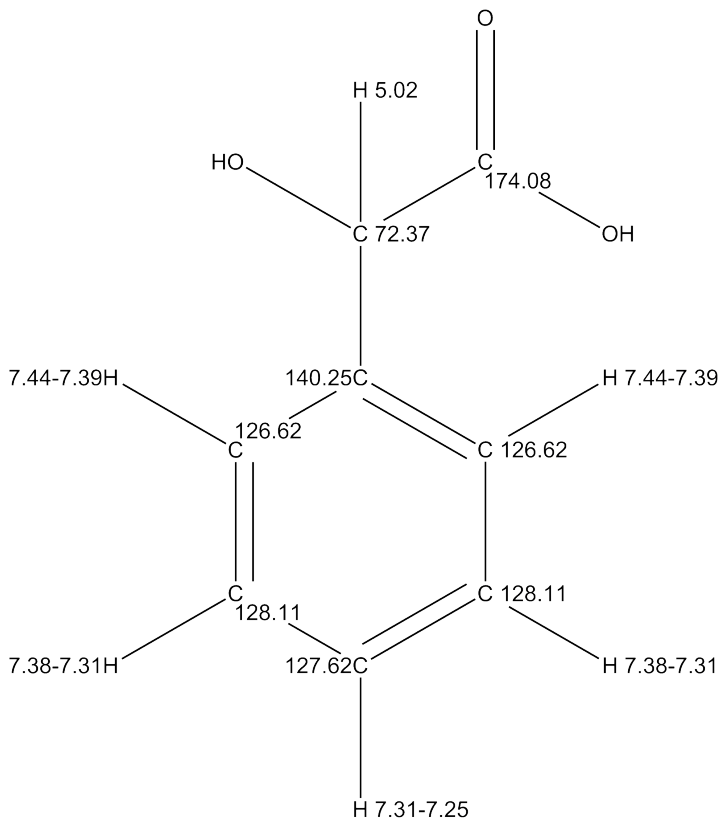

Figure 6.1: Structure of the unknown compound with the assigned carbon and proton NMR shifts

Trondheim, May 2, 2013

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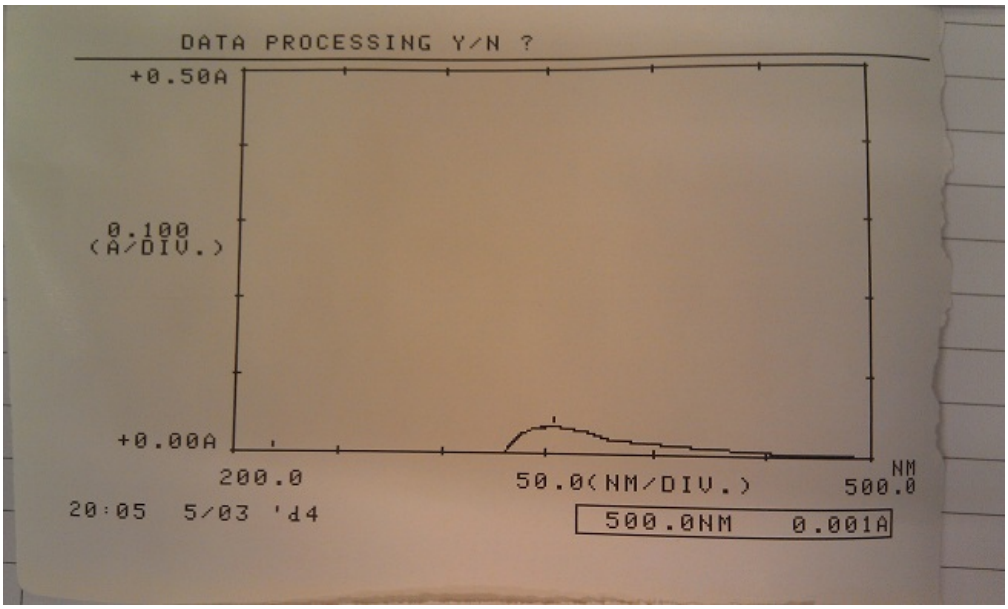
Silje Henriksen	Alexander Asplin

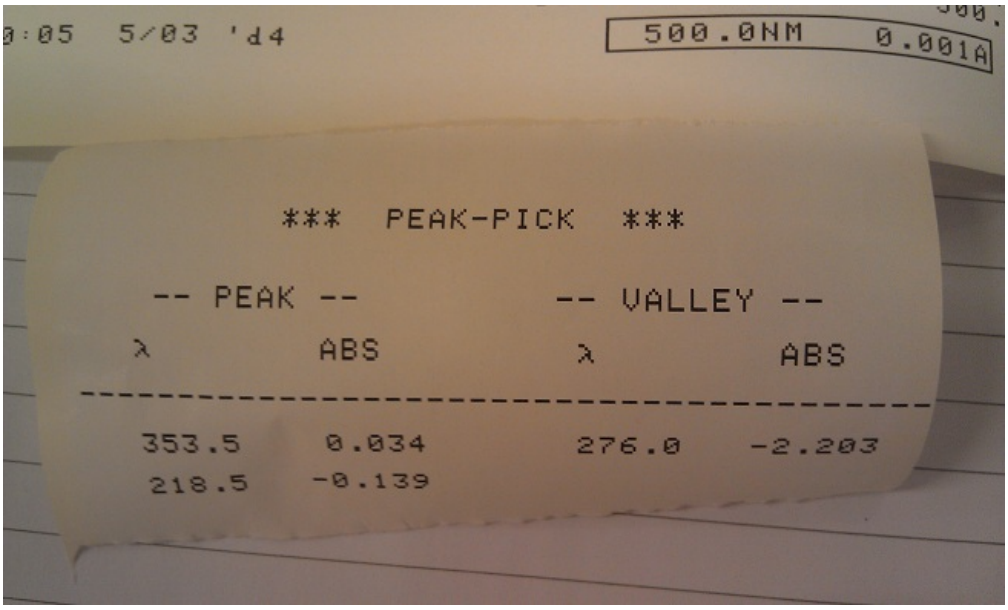
References

[1] Silverstein, R., Webster, F. & Kiemle, D. Spectrometric Identification of Organic Compounds, 7th ed., John Wiley & Sons Inc., 2005, Hoboken, USA.

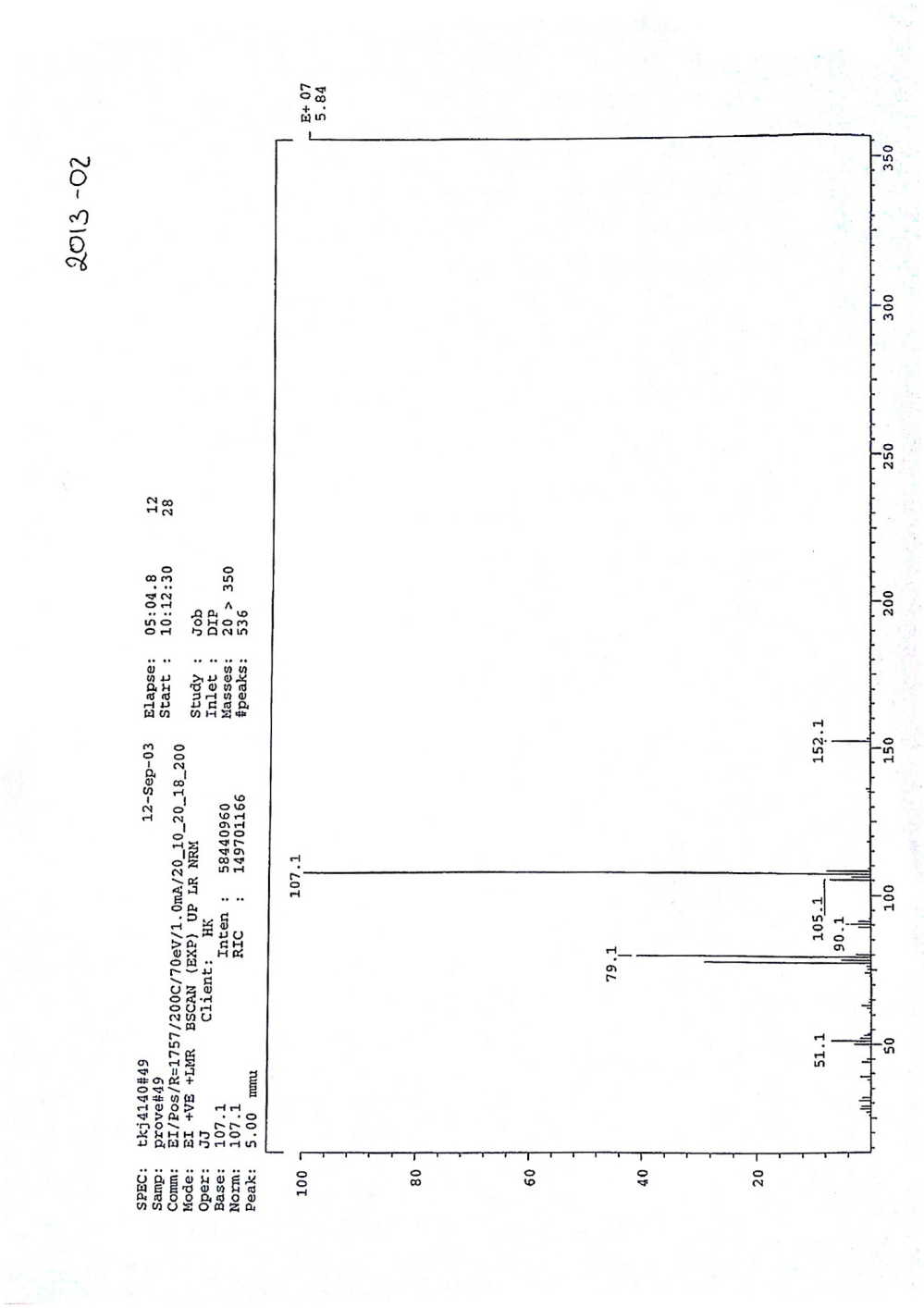
[2] SDBSWeb : *http://sdbs.riodb.aist.go.jp*, National Institute of Advanced Industrial Science and Technology, 02.05.2013, CAS nr: 90-64-2.

A UV/VIS spectrum



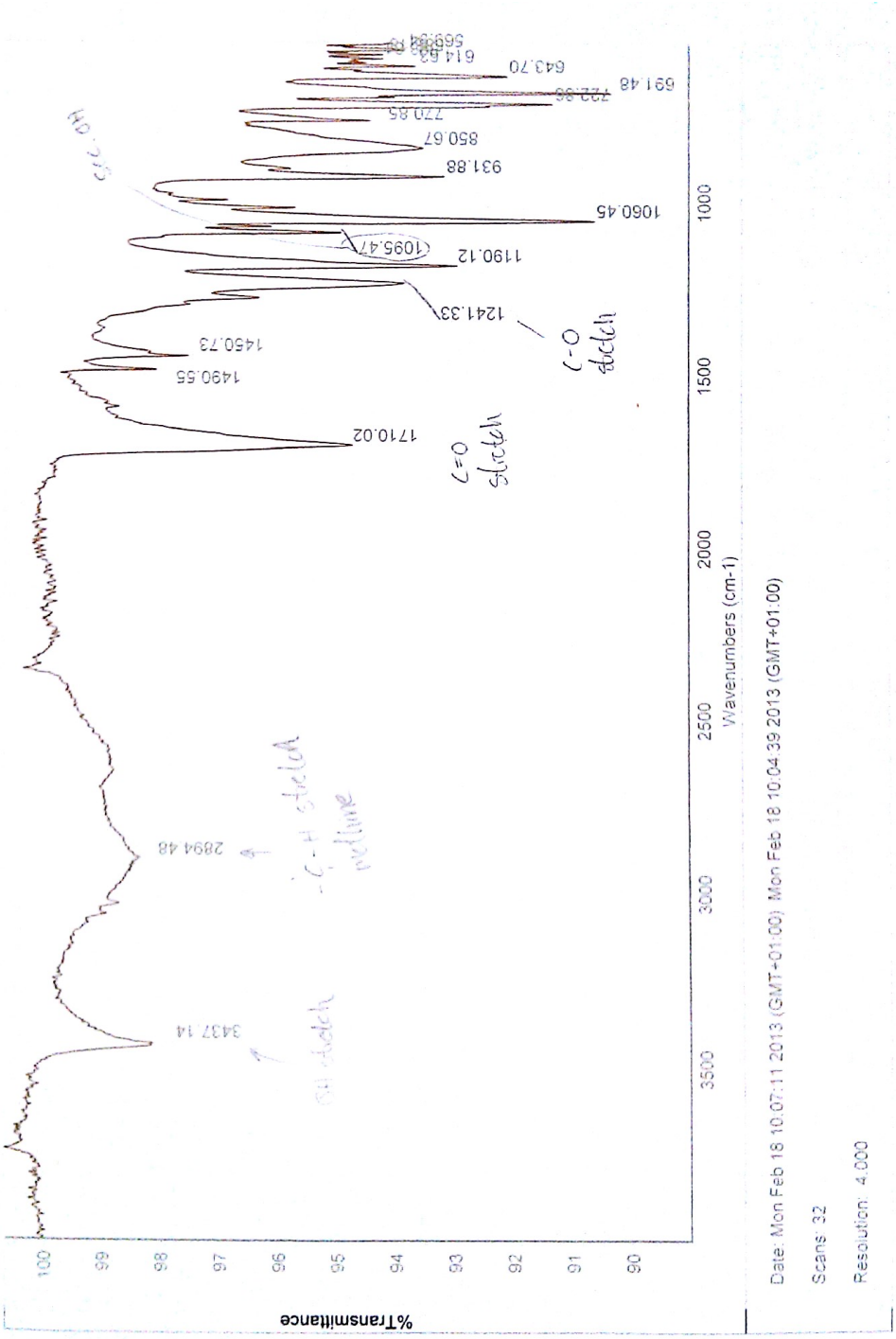


B MS spectrum



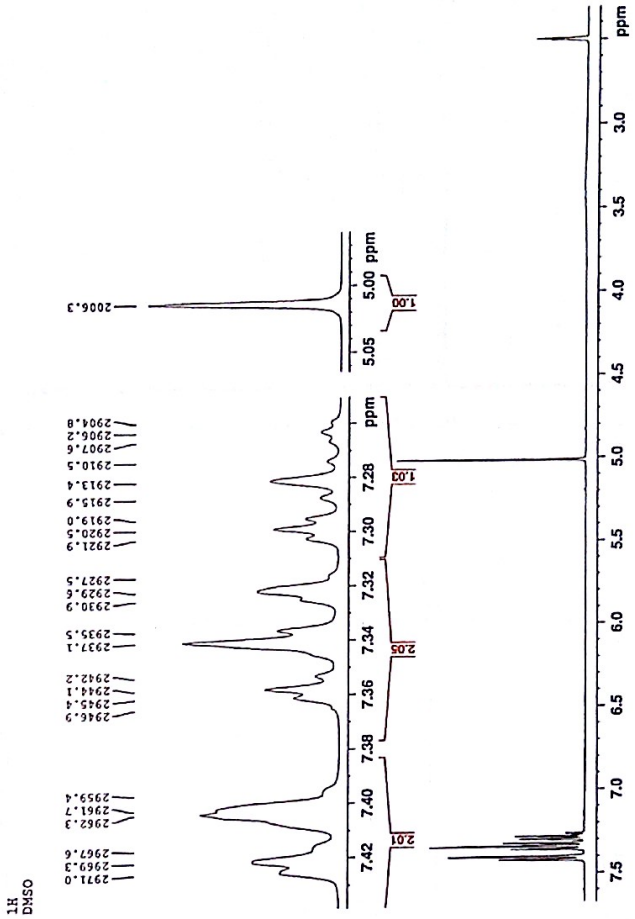
HRMS EI+	Theo. Mass m/z	Delta (ppm)	RDB equiv.	Composition
2013-01 220.1825	220.1825 220.1822	0.1 1.51	-0.5 4	C11 H27 O N P C15 H24 O
2013-02 152.0470	152.0468 152.0475	1.34 -3.16	5.0 1.0	C8 H8 O3 C H8 O N6 S
2013-03 154.1349	154.1352 154.1339	-2.05 6.66	2.0 2.5	C10 H18 O C8 H16 N3
2013-04 222.0247	222.0244 222.0244 222.0250 222.0237 222.0258 222.0231	1.16 1.18 1.32 4.72 4.86 7.21	3. 5 9.0 1.0 1.5 8.5 4	C6 H8 O8 N C5 H2 O3 N8 C8 H15 O2 Br C6 H13 O N3 Br C7 H4 O4 N5 C4 H6 O7 N4
2013-05 114.0677	114.0675	1.48	2.0	C6H10O2
2013-06 154.0989	154.0988 154.0975	0.45 9.16	3.0 3.5	C9 H14 O2 C7 H12 O N3
2013-07 134.0726	134.0726 134.0713	-0.05 9.97	5.0 5.5	C9 H10 O C7 H8 N3
2013-08 152.1196	152.1196 152.1182	0.22 9.0	3.0 3.5	C10 H16 O C8 H14 N3
2013-09 164.0834	164.0832 164.0839	1.33 -2.84	5.0 1.0	C10 H12 O2 C3 H12 N6 S
2013-10 230.0300	230.0301 230.0302 230.0304 230.0295 230.0295 230.0309	0.34 -0.93 -1.72 2.05 2.07 -3.76	3.0 1.5 4.5 5.5 11.0 10.5	C10 H15 O Br C H8 O5 N7 S C9 H12 O2 N S2 C8 H8 O7 N C7 H2 O2 N8 C9 H4 O3 N5
2013-11 154.1349	154.1352 154.1339	-2.05 6.66	2 2.5	C10 H18 O C8 H16 N3
2013-12 136.1248	136.1247	1.09	3	C10 H16

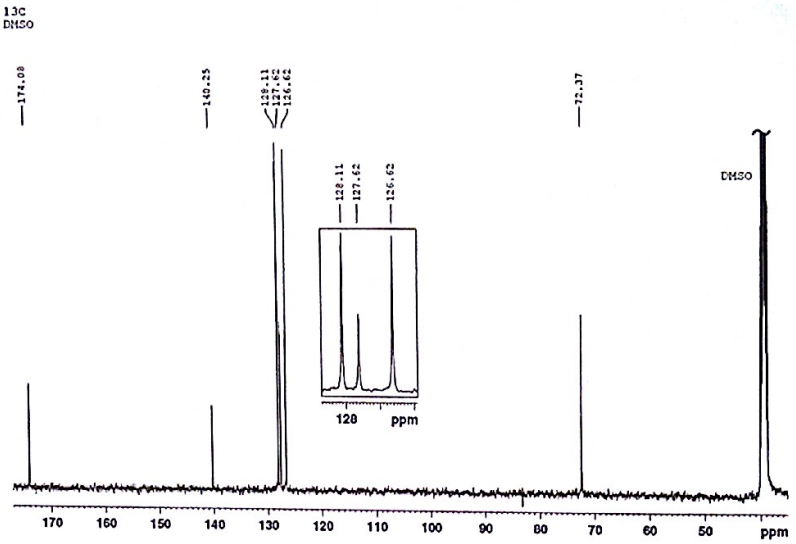
C IR spectrum



D Collection of NMR spectrums

Compound 2013-02

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72.37
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128
ppm
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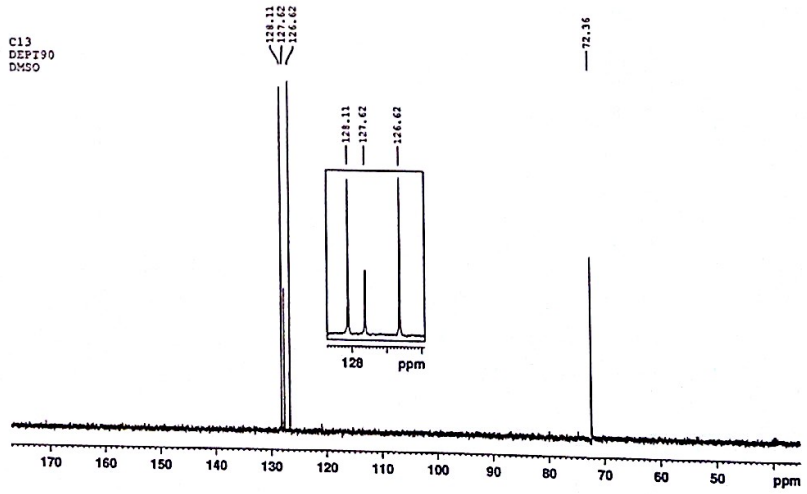
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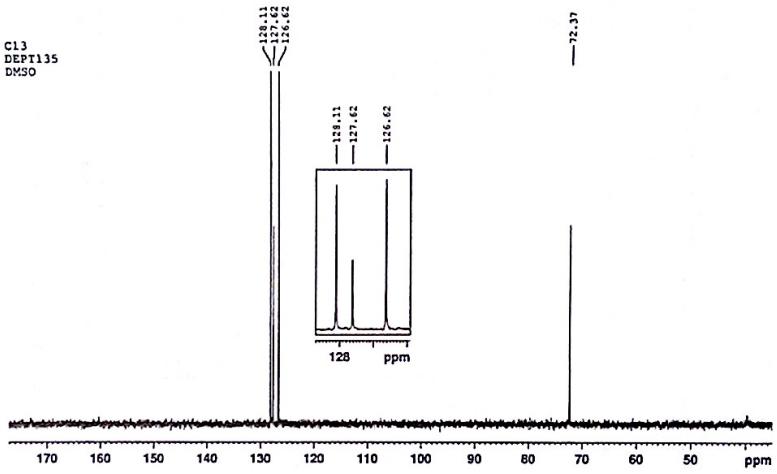
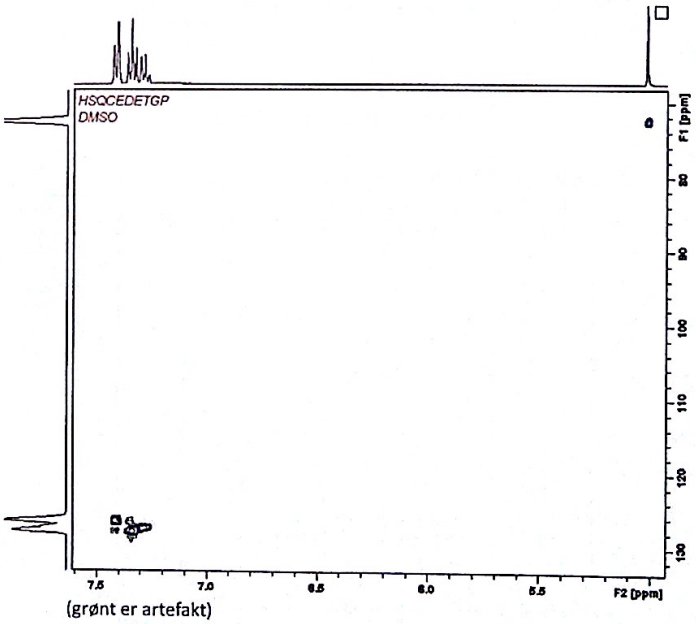
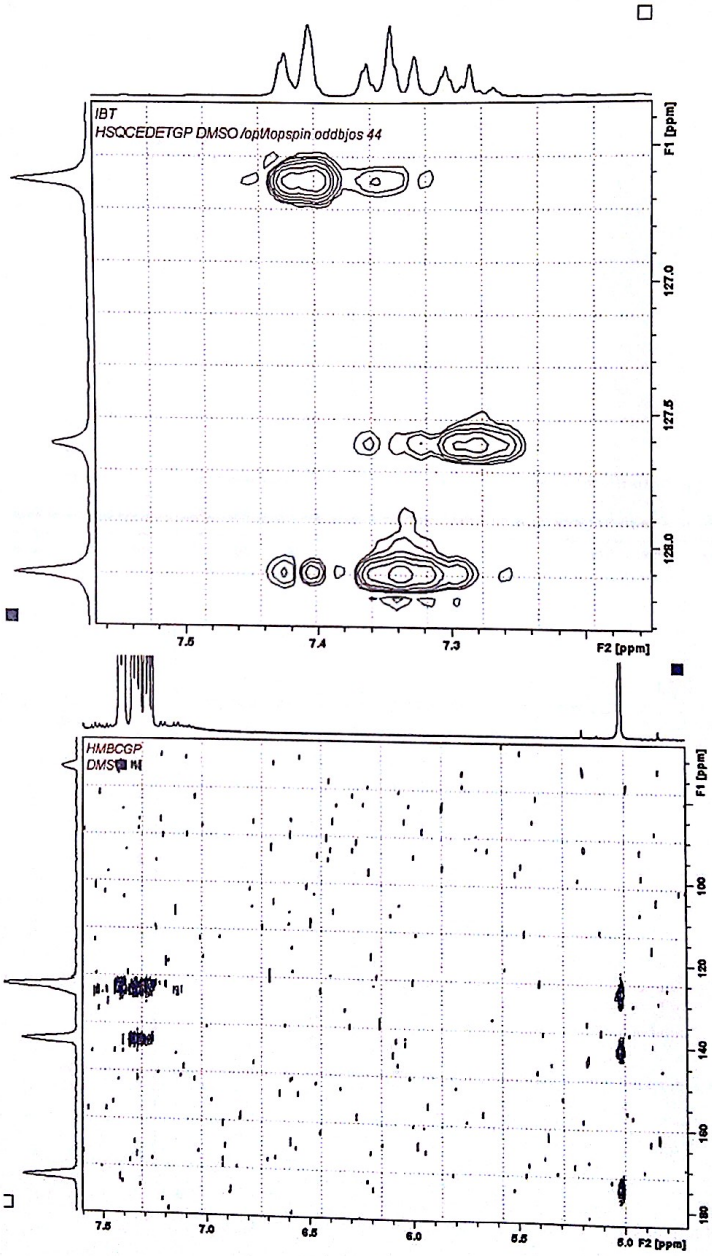
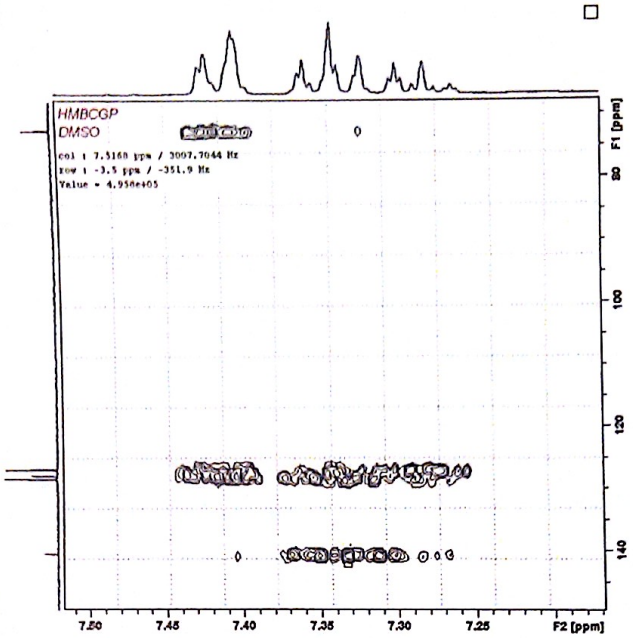
170 160 150 140 130 120 110 100 90 80 70 60 50 ppm

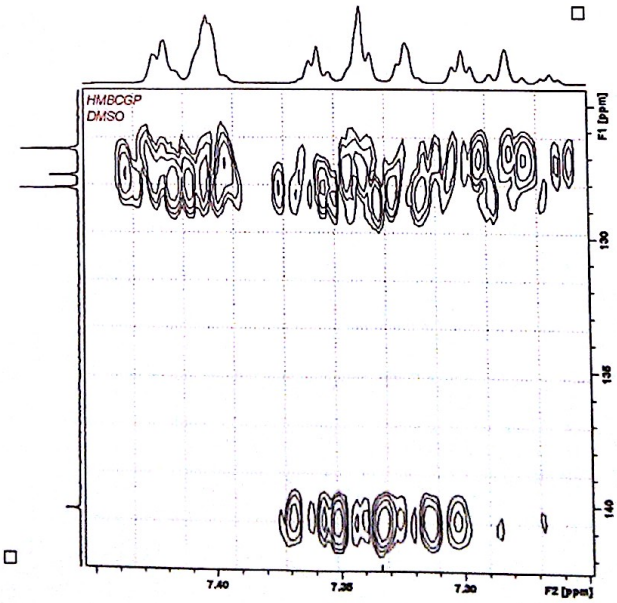


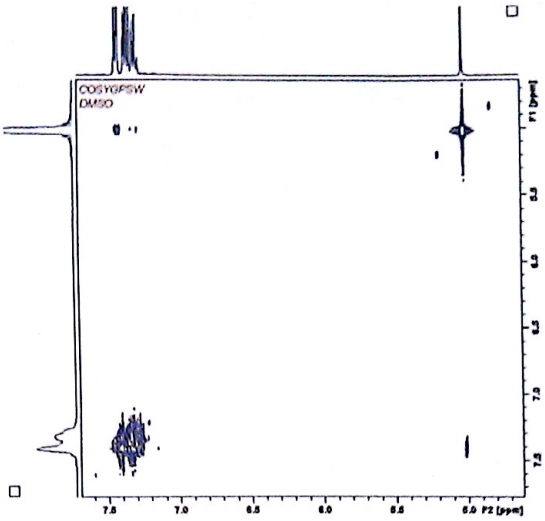


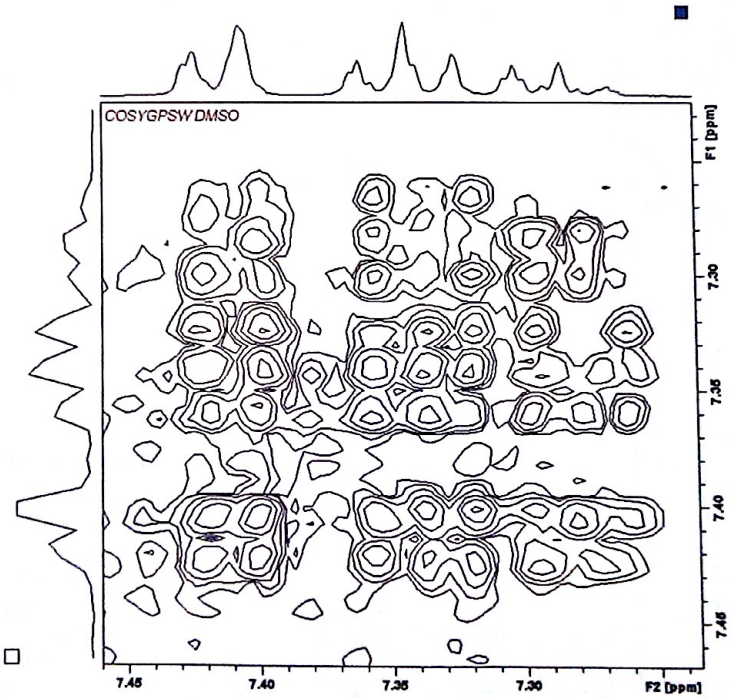
(grønt er artefakt)


IBT
HSQCEDETGP DMSO /opt/topspin oddbjos 44
F1 [ppm]
127.0
127.5
128.0
7.5
7.4
7.3
F2 [ppm]
HMBCGP
DMSO
F1 [ppm]
100
120
140
160
180
7.5
7.0
6.5
6.0
5.5
5.0 F2 [ppm]


HMBCGP
DMSO
col : 7.5168 ppm / 3007.7044 Hz
row : -3.5 ppm / -351.9 Hz
Value = 4.956e+05
F1 [ppm]
80
100
120
140
7.50
7.45
7.40
7.35
7.30
7.25
F2 [ppm]


HMBCGP
DMSO
F1 [ppm]
130
135
140
7.40
7.35
7.30
F2 [ppm]


COSYGPSW
DMSO
F1 [ppm]
F2 [ppm]


COSYGPSW DMSO
F1 [ppm]
F2 [ppm]