Hemisynthesis of deuteriated adenosylhopane and conversion into bacteriohopanetetrol by a cell-free system of *Methylobacterium organophilum*

Supplementary material

Wenjun Liu,[1] Anne Bodlenner[2] and Michel Rohmer[1]

1 Université de Strasbourg/CNRS, Institut Le Bel, 4 rue Blaise Pascal, F 67070 Strasbourg, Cedex, France

2 Université de Strasbourg/CNRS, ECPM, 25 rue Becquerel, F 67087 Strasbourg, Cedex 2, France

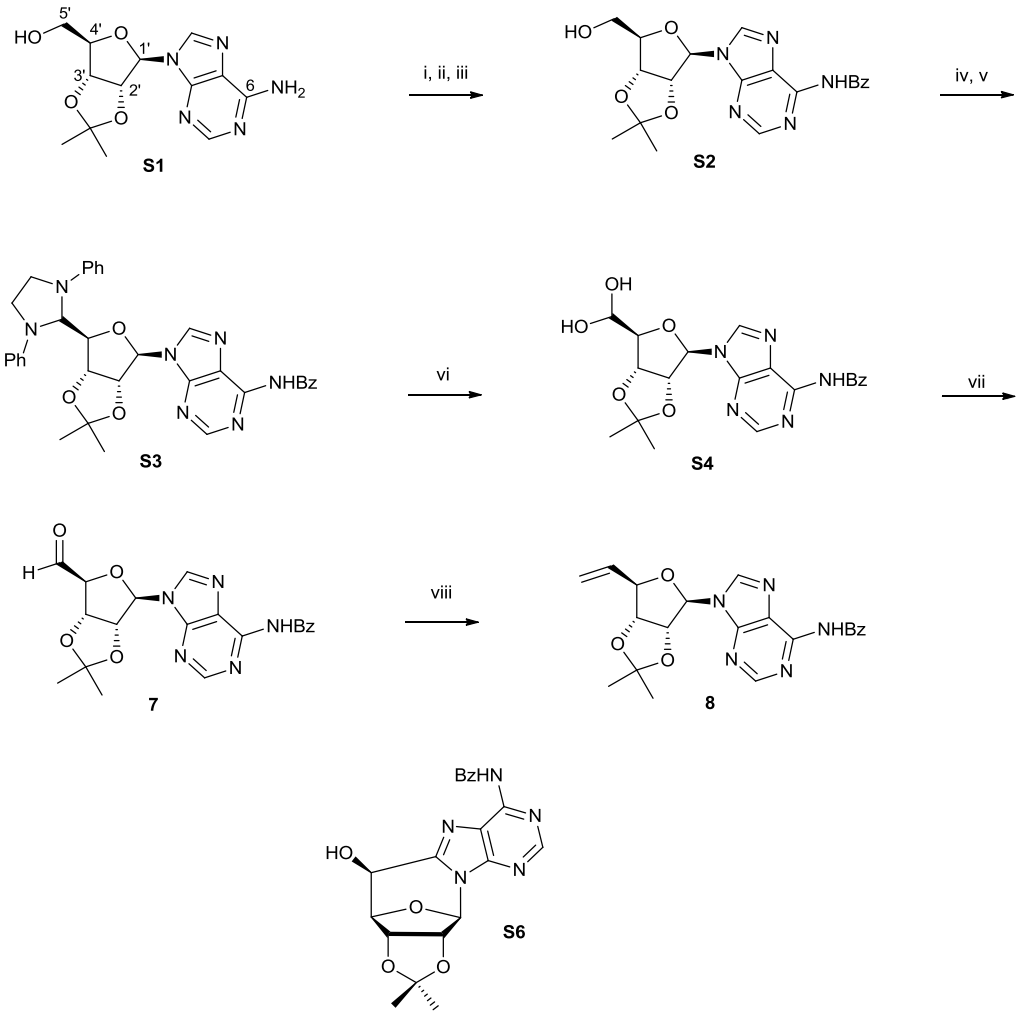
Table of Contents

1. Synthesis of 6-*N*-Benzoyl-5'-methylene-2',3'-*O*-isopropylidene adenosine (8): experimental procedures and characterization of compounds S2, S3, S4, 7 and 8

In the synthesis of the protected 5'-methyleneadenosine **8**, the aldehyde **7** is an important intermediate (Main text, Fig. 2; supplementary material, Scheme S1). It was obtained by an adaptation of the methods of Ranganathan *et al*. and Eppacher *et al*. (Scheme S1).[1a,b] Protection of the 6-amino group is necessary, otherwise the yield of the following metathesis would be largely affected.[2] The parent nucleoside 6-*N*-benzoyl-24,34-*O*-isopropylideneadenosine **S2** was prepared from commercial acetonide **S1** via a one-pot reaction.[3] Oxidation of alcohol **S2** was achieved by treatment with DMSO and *N*,*N*'-dicyclohexylcarbodiimide in the presence of dichloroacetic acid. The resulting crude aldehyde cannot be purified by silica gel chromatography, as it readily epimerises at C-4' or eliminates the acetonide function yielding 3',4'-unsaturated aldehyde upon attempted flash chromatography.[4] The crude aldehyde was therefore protected with *N*,*N*'-diphenylethylenediamine, and the resulting aminal **S3** was recrystallized from ethanol. In order to obtain the quite sensitive aldehyde **7**, the imidazolidine **S3** must be carefully purified. In some cases a quick flash chromatography was required before recrystallisation. It is worth to mention that the aminal **S3** may also epimerize at C-4' after prolonged or repeated flash chromatography on silica gel. Treatment of the aminal **S3** with Dowex 50 (H^+) resin in aqueous THF at room temperature regenerated the aldehyde as a pure and stable hydrate **S4**. Prolonged stirring with the resin can lead to the appearance of the 2',3'-*O*-deprotected

product. After careful washing with water and predrying under vacuum, the aldehyde hydrate **S4** was dehydrated by azeotropic distillation with benzene using a Dean-Stark trap to afford free aldehyde **7**. Care should also be taken since the cyclonucleoside **S5**, a less polar, benzene insoluble product, can be easily obtained upon prolonged distillation.

NMR spectra of adenosine 5'-aldehyde hydrate **S4** and free aldehyde **7** were obtained using ($^2H_6$)DMSO as solvent and with ($^2H_5$)DMSO (δ = 2.50 ppm) as internal standard for ^{1}H-NMR and ($^2H_6$)DMSO (δ = 39.52 ppm) for ^{13}C-NMR.



Scheme S1. Synthesis of 6-*N*-Benzoyl-2',3'-*O*-isopropylideneadenosine-5'-aldehyde **11**.
i) TMSCl, Py; ii) BzCL, Py; iii) NH_4OH, 94% (3 steps); iv) DCC, Cl_2CHCO_2H, DMSO, than (CO_2H), H_2O, MeOH; v) $(PhNHCH_2)_2$, MeOH, 59% (2 steps); vi) Dowex-50 H^+, THF, H_2O, 64%; vii) benzene/reflux, 75 %; viii) Ph_3PCH_3Br, *n*-BuLi, THF, -40°C, 75%.

6-*N*-benzoyl-2',3'-*O*-isopropylideneadenosine (S2). To 2',3'-*O*-isopropylideneadenosine **S1** (1.4 g, 4.7 mmol) in freshly distilled pyridine (35 mL) is added TMSCl (4.7 mL, 37 mmol) and the mixture was stirred at ambient temperature overnight. After adding benzoyl chloride (1.1 mL, 9.3 mmol) at 0 °C, the mixture was allowed to warm up to room temperature and stirred for two additional hours. The reaction was stopped by adding water (10 mL) at 0 °C with stirring. After stirring 5 min at 0 °C and another 5 min at room temperature, 30% aqueous ammonia (20 mL) is added. After stirring an additional 30 min the mixture is partitioned between equal volumes (40 mL) of DCM and phosphate buffer (pH = 7). The organic phase was washed with water (3 ×10 mL), dried with anhydrous Na_2SO_4 and evaporated to dryness. The crude compound was purified by FCC (DCM/ MeOH 100:3, 100:4, 100:4.5) to yield crystalline product **S2** (1.8 g, 94%).

Analytical data match those described in the literature.[3]

^{1}H NMR (300MHz, $CDCl_3$): δ/ppm = 9.37 (1H, br., -NH) 8.74 (1H, s, 2-H), 8.10 (1H, s, 8-H), 8.03-8.00 (2H, m, arom. H), 7.62-7.47 (3H, m, arom. H), 5.96 (1H, d, J = 4.6 Hz, 1'-H), 5.21 (1H, dd, J = 5.9, 4.6 Hz, 2'-H), 5.09 (1H, dd, J = 5.9, 1.3 Hz), 4.52 (1H, m, 4'-H), 3.96 (1H, dd, J = 12.6, 1.7 Hz, 5'$_b$-H), 3.79 (1H, dd, J = 12.6, 2.2 Hz, 5'$_a$-H), 1.63 and 1.37 (3H, s, Me_2C).

^{13}C NMR (75MHz, $CDCl_3$): δ/ppm = 164.7 (C=O), 152.2 (2-C), 150.5 (6-C), 150.3 (4-C), 142.5 (8-C), 133.4, 132.9, 128.8, 128.0, 124.2 (5-C), 114.2 (Me_2C), 94.0 (1'-C), 86.3 (4'-C), 83.2 (2'-C), 81.5 (3'-C), 63.2 (5'-C), 27.5 (Me_2C), 25.2 (Me_2C).

MS (ESI): m/z = 434 [M+Na$^+$].

6-*N*-Benzoyl-5'-deoxy-2',3'-*O*-isopropylidene-5',5'-(*N*,*N'*-diphenylethylenediamino)adenosine (S3). To a solution of 6-*N*-benzoyl-2',3'-*O*-isopropylideneadenosine **S2** (1.8 g, 4.38 mmol) and *N*,*N'*-dicyclohexylcarbodiimide (2.7 g, 13.1 mmol) in dry DMSO (14 mL) was added dropwise dichloroacetic acid (0.18 mL, 2.2 mmol) in DMSO (1.5 mL) at 0 °C. After stirring for 2.5 h at ambient temperature, a solution of oxalic acid dihydrate (1.1 g, 8.8 mmol) in methanol (4.5 mL) was added slowly. The mixture was then stirred for another 30 min and filtered after. The crystalline residue of dicyclohexylurea was washed with ice-cold methanol. *N*,*N'*-Diphenylethylenediamine (975 mg, 4.6 mmol) was added to the combined filtrate and washings and the resulting solution was stirred at room temperature overnight. The mixture was diluted with EtOAc after being concentrated under vacuum, and washed with water three times. After evaporating all the solvent, the crude compound was purified by FCC (DCM/MeOH, 100:1.5). Proper fractions

were collected, concentrated and then recrystallized in EtOH to give *N,N'*-diphenylimidazolidine **S3** as light brown crystals (2.0 g, 76%).

Analytical data match those described in the literature.[1b]

^{1}H NMR (300MHz, $CDCl_3$): δ/ppm = 9.19 (1H, br. s, -NH), 8.73 (1H, s, 2-H), 8.04-8.02 (2H, m, arom. H) 7.81 (1H, s, 8-H), 7.63-7.50 (3H, m, arom. H), 7.23-7.14 (4H, m, arom. H), 6.82-6.70 (6H, m, arom. H), 6.17 (1H, d, *J* = 2.2 Hz, 1'-H), 5.75 (1H, d, *J* = 2.6 Hz, 5'-H), 5.21 (1H, dd, *J* = 6.2, 4.6 Hz, 3'-H), 5.17 (1H, dd, *J*= 6.2, 2.2 Hz, 2'-H), 4.63 (1H, dd, *J* = 4.6, 2.6 Hz, 4'-H), 3.74-3.57 (4H, m, CH_2CH_2), 1.49 AND 1.33 (6H, 2s, Me_2C).

^{13}C NMR (75MHz, $CDCl_3$): δ/ppm = 164.6 (C=O), 152.8 (2-C), 151.2 (6-C), 149.5 (4-C), 146.4, 141.6 (8-C), 133.6, 132.7, 129.3, 129.1, 128.8, 127.9, 122.9 (5-C), 118.3, 118.2, 115.0, 113.5, 113.4, 88.4 (1'-C), 86.9 (4'-C), 83.7 (2'-C), 80.1 (3'-C), 73.3 (5'-C), 58.3, 47.7, 46.8, 27.3, 25.7.

MS (ESI): *m/z* = 604 [M+H$^+$] .

6-*N*-Benzoyl-9-(2,3-*O*-isopropylidene-*β*-*D*-ribo-pentodialdo-1,5-furanosyl)adenine hydrate (**S4).** Dowex 50 (H$^+$) resin (3 g) was added to a solution of **S3** (2.8 g 4.6 mmol) dissolved in THF/H_2O 1:1 (240 mL) and stirred for 4 h at room temperature. The resin was removed by filtration and washed with THF (5×10 mL). The combined filtrates were concentrated to *ca.* ½ of the volume and the resulting white, amorphous solid was removed, washed with water, and dried *in vacuo* to afford **S4** (1.3 g, 67%) as a stable hydrate.

Analytical data match those described in the literature.[1b]

^{1}H NMR (300MHz, DMSO-D6): δ/ppm = 11.21 (1H, br. s, -NH), 8.76 (1H, s, 2-H), 8.64 (1H, s, 8-H), 8.06-8.03 (2H, m, arom. H), 7.68-7.53 (3H, m, arom. H), 6.32 (1H, d, *J* = 5.8 Hz, -OH), 6.27 (1H, d, *J* = 2.6 Hz, 1'-H), 6.20 (1H, d, *J* = 6.1Hz, -OH), 5.37(1H, dd, *J* = 2.7, 6.1 Hz, 2'-H), 5.07 (1H, dd, *J* = 1.7, 6.1 Hz, 3'-H) 4.85 (1H, ddd, *J* = 4.9, 5.7, 6.1 Hz, 5'-H), 4.08 (1H, dd, *J* = 1.7, 4.8 Hz, 4'-H), 1.56 & 1.35 (6H, 2s, Me_2C)

MS (ES): *m/z* = 450 [M+Na$^+$].

6-*N*-Benzoyl-2',3'-*O*-isopropylideneadenosine-5'-aldehyde (7). A suspension of hydrated aldehyde **S4** (1.3 g, 3.1 mmol) in benzene (75 mL) was heated under reflux for 2 h using a Dean-Stark condenser and evaporated. The residue was dried *in vacuo* to afford aldehyde **7** (1.1 g, 85%) and cyclonucleoside **S5** (190 mg, 15%) as an inseparable mixture.

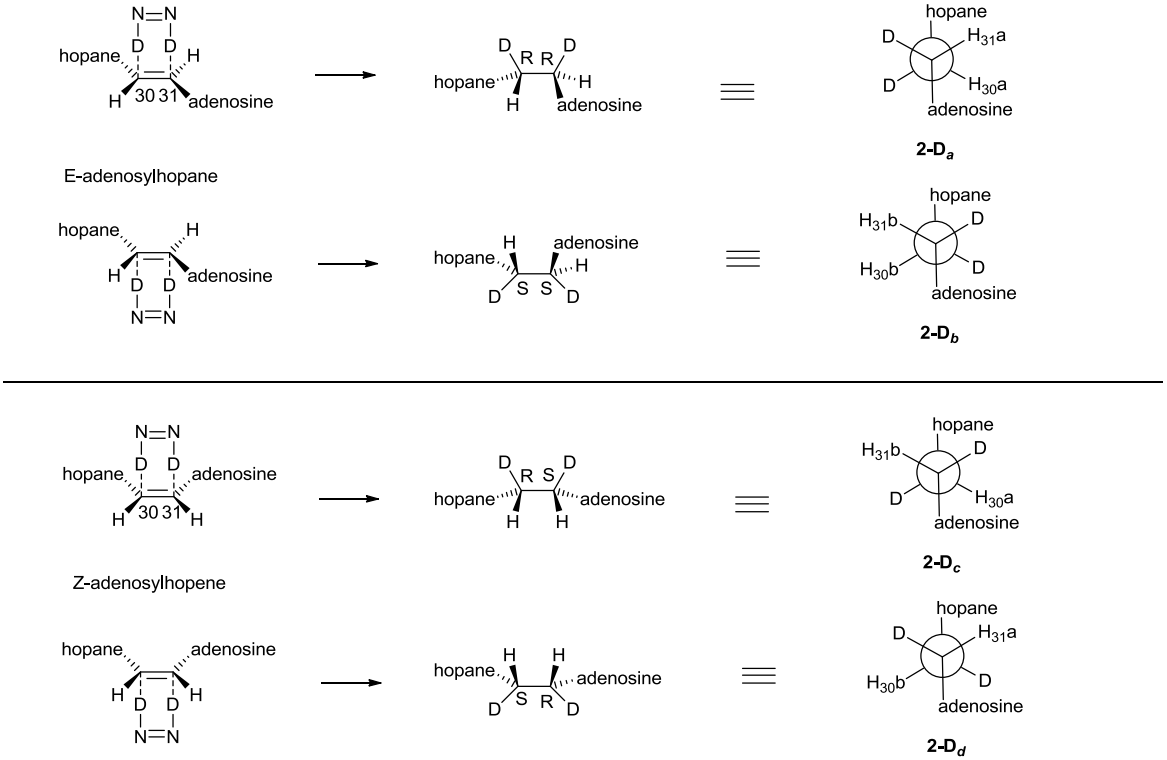
Aldehyde **7** has been described in the literature,[1a,b] as well as the cyclonucleoside **S5**.[1b]

^{1}H NMR (300MHz, DMSO-D6): δ/ppm = 11.24 (1H, br. s, -NH), 9.33 (1H, s, CHO), 8.63 (1H, s, 2-H), 8.60 (1H, s, 8-H), 8.06-8.03 (2H, m, arom. H) 7.68-7.53 (3H, m, arom. H), 6.58 (1H, s, 1'-H), 5.51 (1H, dd, *J* = 1.7, 6.0 Hz, 3'-H), 5.43 (1H, d, *J* = 6.0 Hz, 2'-H), 4.8 (1H, d, *J* = 1.6 Hz, 4'-H), 1.55 & 1.37 (6H, 2s, Me_2C).
MS (ES): *m/z* = 432 [M+Na^+].

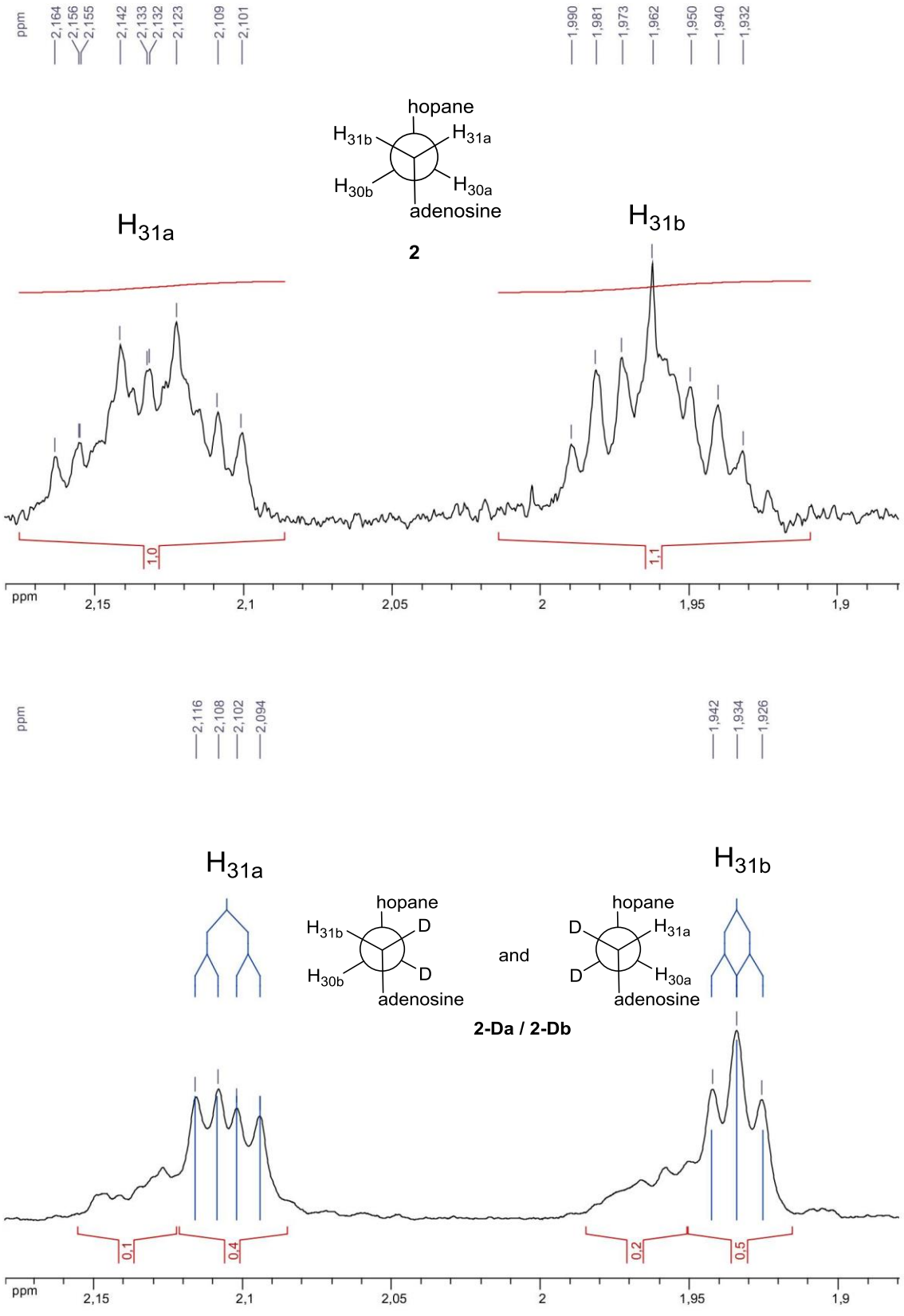
6-*N*-Benzoyl-5'-methylene-2',3'-*O*-isopropylidene adenosine (8). To a stirred suspension of methyltriphenylphosphonium bromide (1.8 g, 5 mmol) in dry THF (80 mL) under argon was added a solution of *t*-BuLi in THF (3.1 mL, 2.1 mmol) at –40 °C. The bright yellow solution was warmed up to 0 °C and stirred for another 1 h before cooled to –40 °C again. The mixture of aldehyde **7** (Scheme 2) and cyclonucleoside **S2** (Supplementary material and Scheme S1) (880 mg, 85/15, mol/mol) in anhydrous THF (40 mL) was added slowly and stirring continued at –40 °C for 2 h and overnight at 0 °C. Saturated NH_4Cl/H_2O (60 mL) was added. The layers were separated. The aqueous layer was extracted with EtOAc. The two organic fractions were combined and washed with $NaHCO_3/H_2O$ and brine. After drying over anhydrous $NaSO_4$, the residue was concentrated *in vacuo* and purified by flash column chromatography (CH_2Cl_2/MeOH, 100:4) to give terminal olefin **8** as a colourless foam (588 mg, 67%).
^{1}H NMR (300MHz, $CDCl_3$): δ/ppm = 8.78 (1H, s, 2-H), 8.08 (1H, s, 8-H), 8.02-8.7.99 (2H, m, arom. H), 7.60-7.46 (3H, m, arom. H), 6.17 (1H, d, *J* = 2.0 Hz, 1'-H), 5.88 (1H, ddd, *J* = 17.2, 10.5, 6.8 Hz, 5'-H), 5.54 (1H, dd, *J* = 6.2, 2.0 Hz, 2'-H), 5.24 (1H, ddd as dt, *J* = 17.2, 1.3 Hz, 6'-H_a), 5.13 (1H, ddd as apparent dt, *J* = 10.5, 1.1 Hz, 6'-H_b), 5.01 (1H, dd, *J* = 6.2, 3.3 Hz, 3'-H), 4.71 (1H, dd, *J* = 6.8, 3.3 Hz, 4'-H), 1.62 & 1.40 (6H, 2s, Me_2C).
^{13}C NMR (75MHz, $CDCl_3$): δ/ppm = 164.7, 152.6, 151.3, 149.7, 142.2, 134.7, 133.5, 132.7, 128.8, 127.8, 123.5, 118.4, 114.5, 90.7, 88.4, 84.4, 84.2, 27.0, 25.3.
MS (ESI): *m/z* = 408 [M+H] $^{+}$.

2. Structure of the bisdeuteriated isotopomer of adenosylhopane (2-D): NMR-data interpretation

The structure of protected bisdeuteriated (31,32-$^{2}H_2$)adenosylhopane **2-D** was confirmed by comparing its ^{1}H- and ^{13}C-NMR spectra recorded in ($^{2}H_5$)pyridine with those of the corresponding natural abundance adenosylhopane derivative **2**. Four diastereoisomers can be potentially obtained from the diimide *syn*-reduction of alkene (*Z*) and (*E*). Given that diimide reacts faster with the protected (*E*)-adenosylhopene **12**, which is the dominant isomer, the two major products resulting from *syn*-addition of deuteriated diimide are acetonide protected (30*R*,31*R*-$^{2}H_2$)- and (30*S*,31*S*-$^{2}H_2$)adenosylhopane **2-D$_a$** and **2-D$_b$**. The (30*R*,31*S*-$^{2}H_2$)- and (30*S*,31*R*-$^{2}H_2$)adenosylhopane diastereomers **2-D$_c$** and **2-D$_d$** are also generated from the reduction of protected (*Z*)-adenosylhopene by diimide (Scheme S2).



Scheme S2. Stereochemistry of (30,31-$^{2}H_2$)adenosylhopane **2-D**.



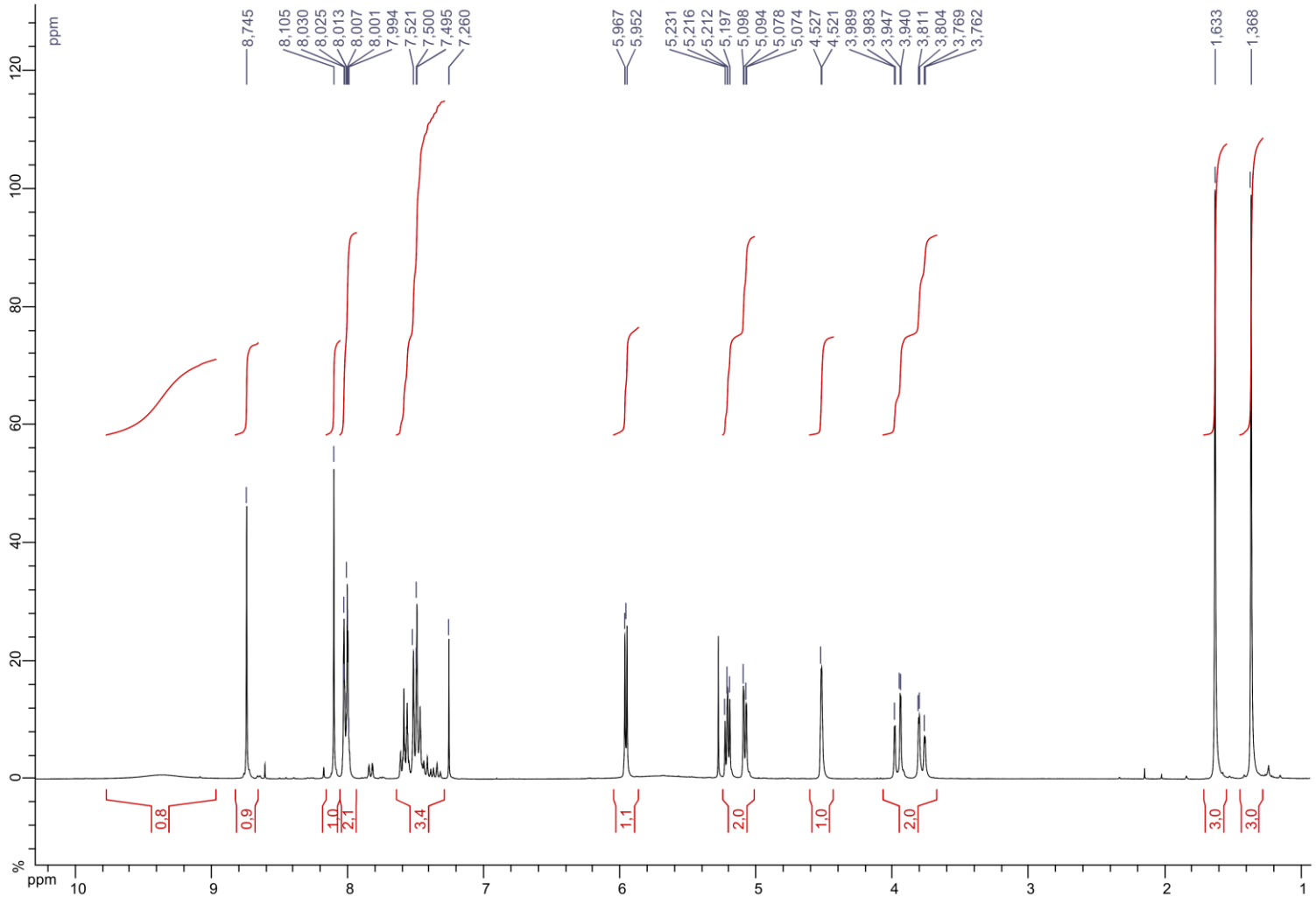
Scheme S3. Multiplicities of the signals of protons 31_a and 31_b of (30,31-$^2H_2$)adenosylhopane **2-D**.

For natural abundance adenosylhopane **2**, the two protons at C-31 (31-H_a and 31-H_b) are diastereotopic and magnetically non-equivalent. They are respectively observed as multiplets at 2.13 and 1.96 ppm. After diimide reduction, the observed multiplicities are simplified, showing two major compounds, arising from the *syn*-reduction of the (*E*) alkene on both sides, and minor diasteroisomers as smaller multiplets. Compounds **2-D_a** and **2-D_b** have the following ^{1}H NMR pattern: one compound has a 31-H_a proton at 2.11 ppm as a doublet of doublet ($J_{31a,32}$ = 8.3 Hz and $J_{30,31a}$ = 4.6 Hz) and no signal for 31-H_b due to the presence of a deuterium, and the other compound has no signal for 31-H_a but a pseudo triplet at 1.93 ppm ($J_{30,31b}$ = $J_{31b,32}$ = 5.0 Hz) for its 31-H_b (Scheme S3). On the one hand, absence of a J_{gem} (~11-12 Hz) indicates that there is only one deuterium at C-31. On the other hand, the presence of the J_{gauche} (4.6 and 5.0 Hz) coupling constant result from the *gauche* relative positions of 30-H and 31-H. Signals of remaining protons at C-30 were hidden under a large multiplet and not attributed. The signals of the minor isotopomers (30*R*,31*R*-$^2H_2$)- and (30*S*,31*S*-$^2H_2$)adenosylhopane derived from the reduction of the (*Z*)-isomer are too weak and multiplicities were not clearly visible.

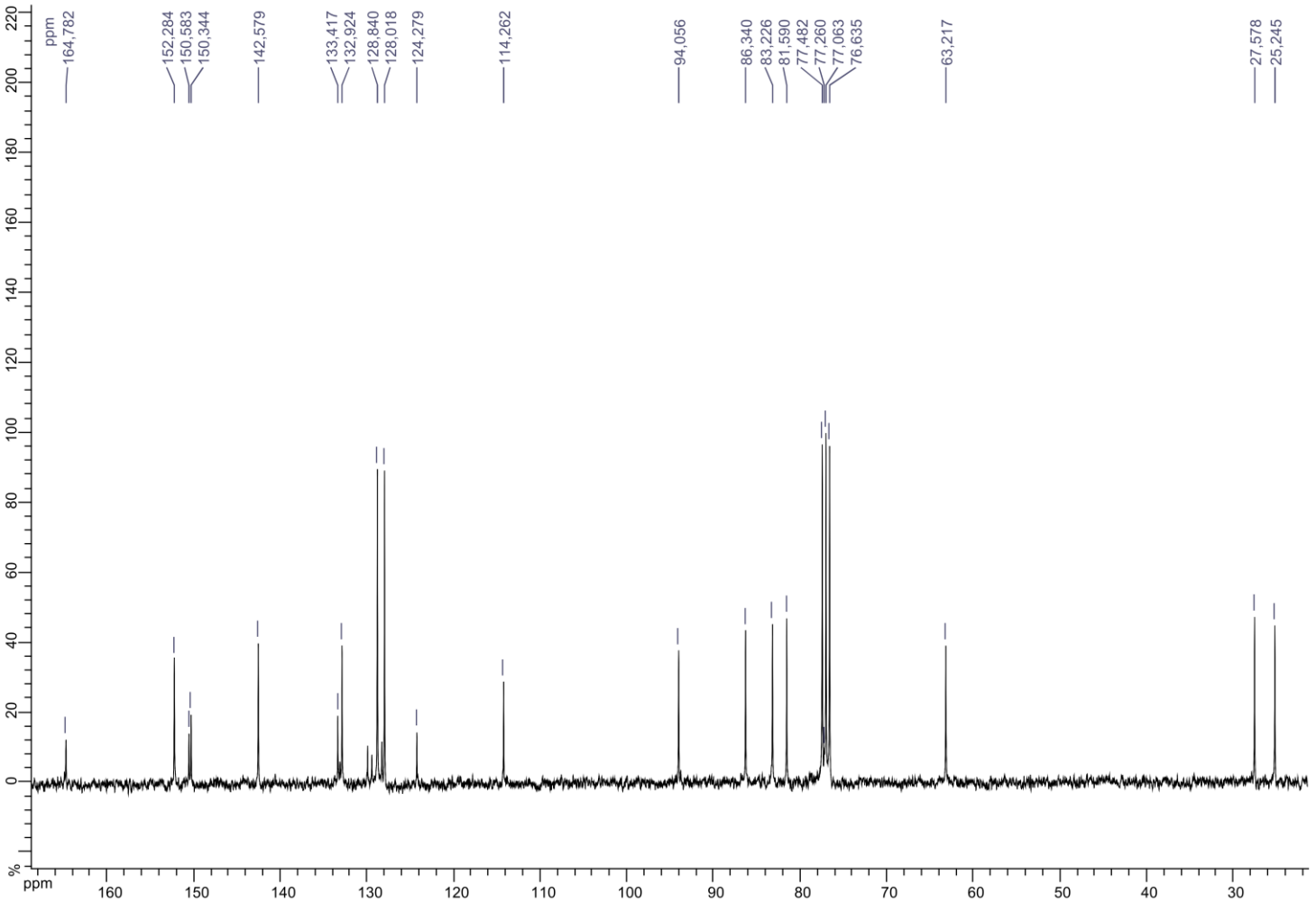
The only partial deuteriation and the occurrence of four bisdeuteriated diastereomers prevented a detailed interpretation of the ^{13}C-NMR spectrum. The disappearance of the two singlets for carbon atoms C-30 and C-31 pointed out the presence of a deuterium on each of these positions. Rough α+β shifts were, however, found from edited HSQC spectrum, –430 ppb and –410 ppb for the C-30 and C-31 signals respectively. C-31, C-22 and C-32 were characterized by a complex signal pattern due to the presence of deuterium at C-30 and/or C-31, and β and γ shift values could not be determined.

3. NMR, MS and HRMS spectra

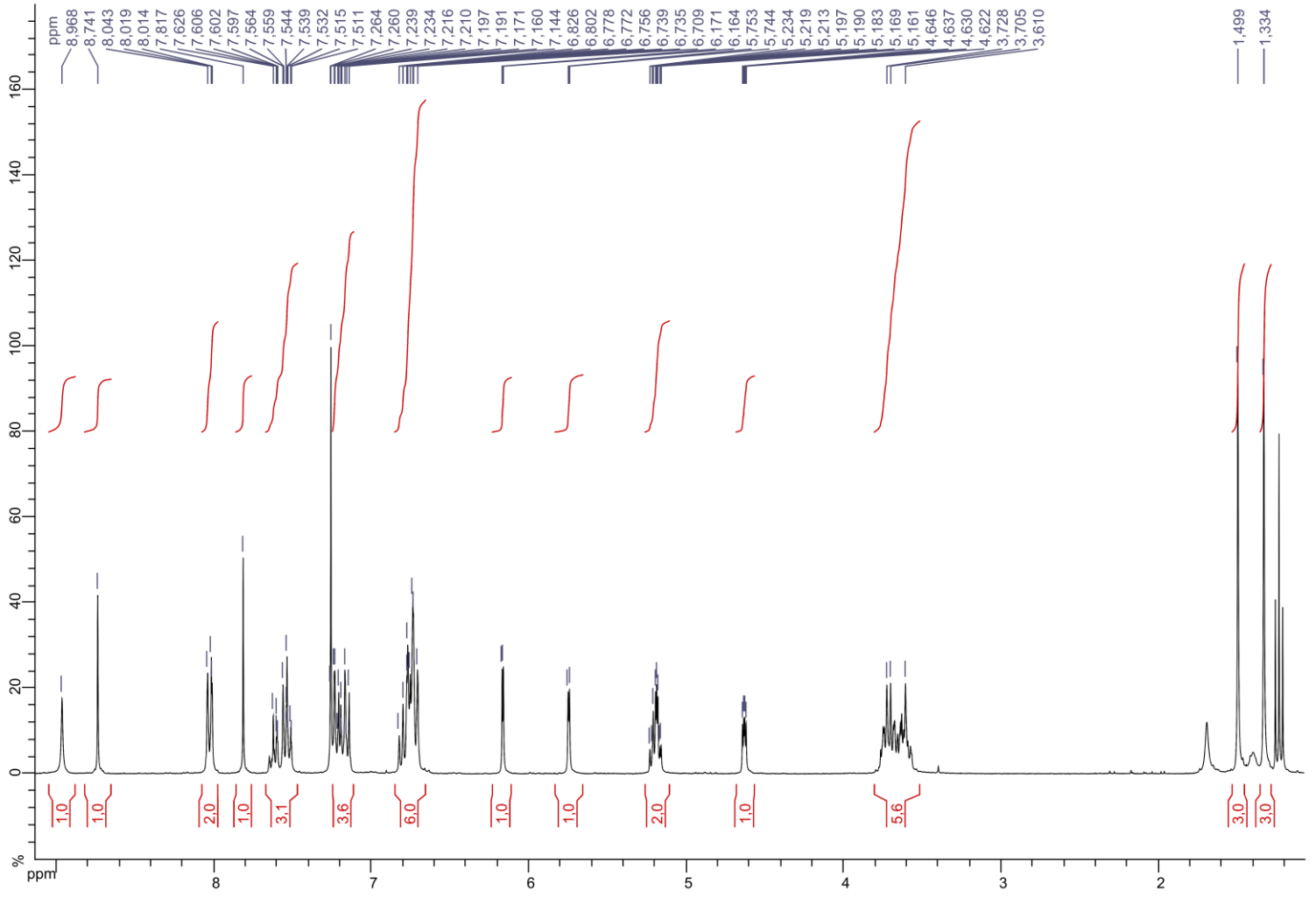
^{1}H-NMR spectrum ($C^{2}HCl_3$, 300 MHz) of compound S2



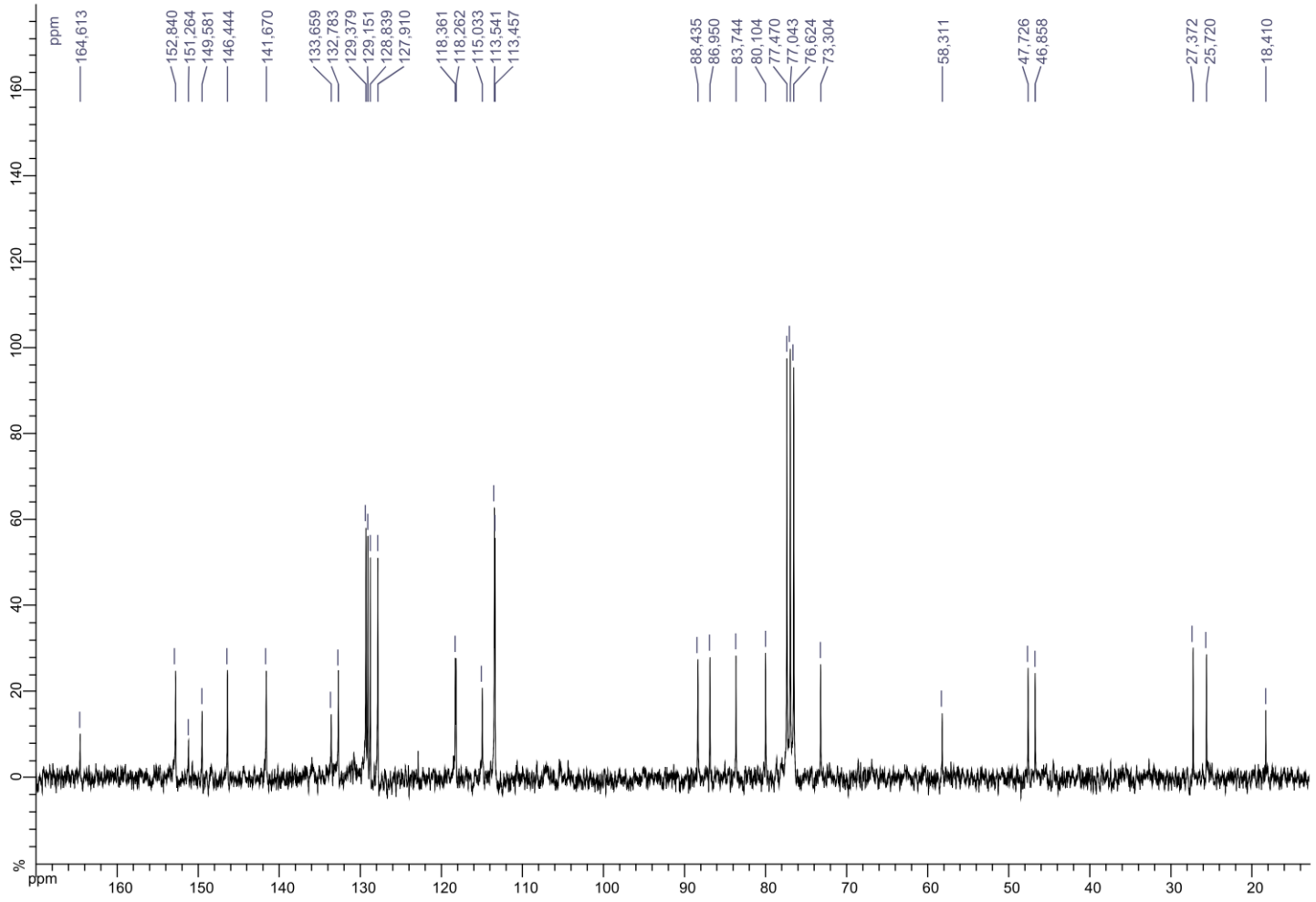
^{13}C-NMR spectrum (C^2HCl_3, 75 MHz) of compound S2



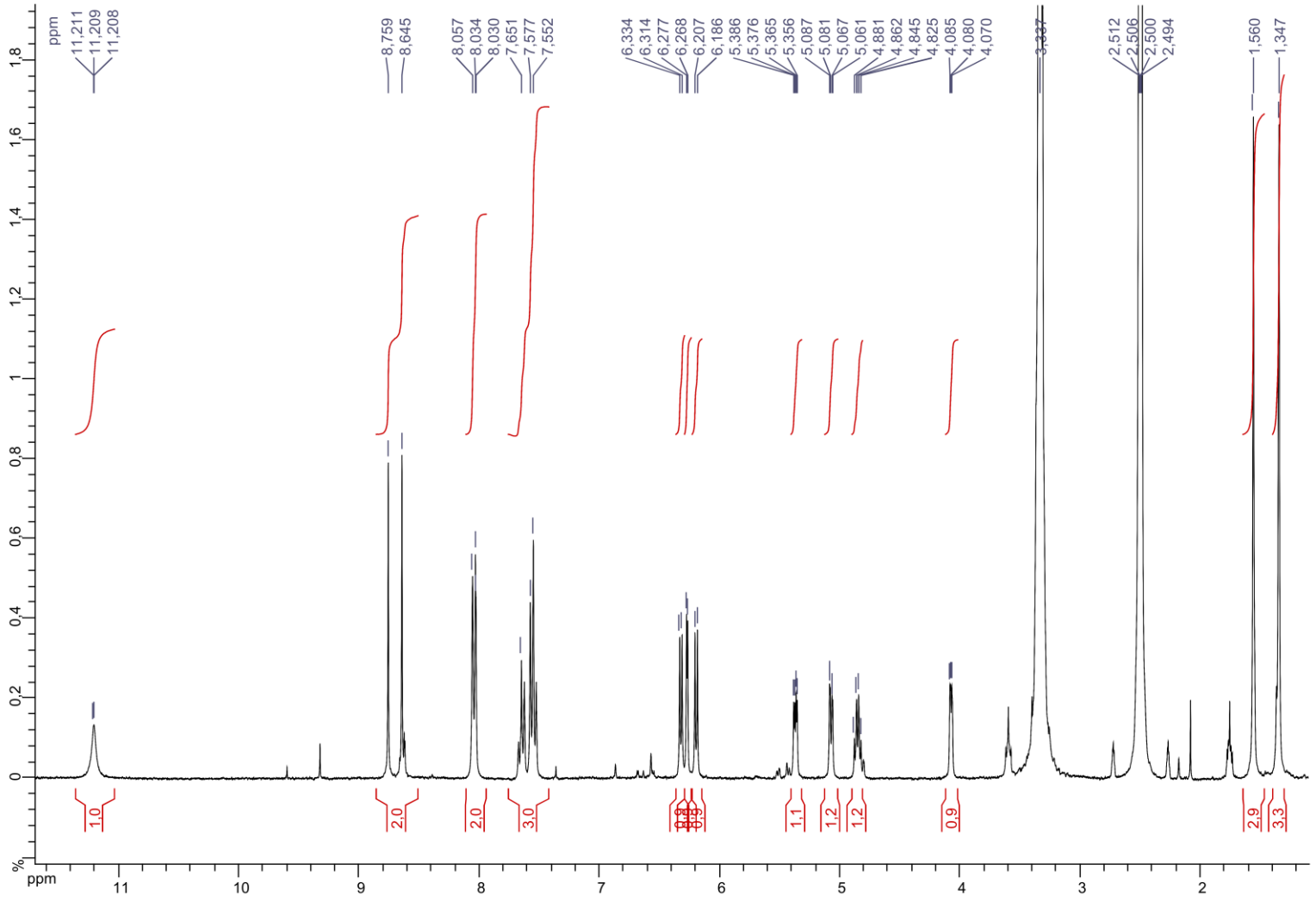
^{1}H-NMR spectrum ($C^{2}H\ Cl_3$, 300 MHz) of compound S3



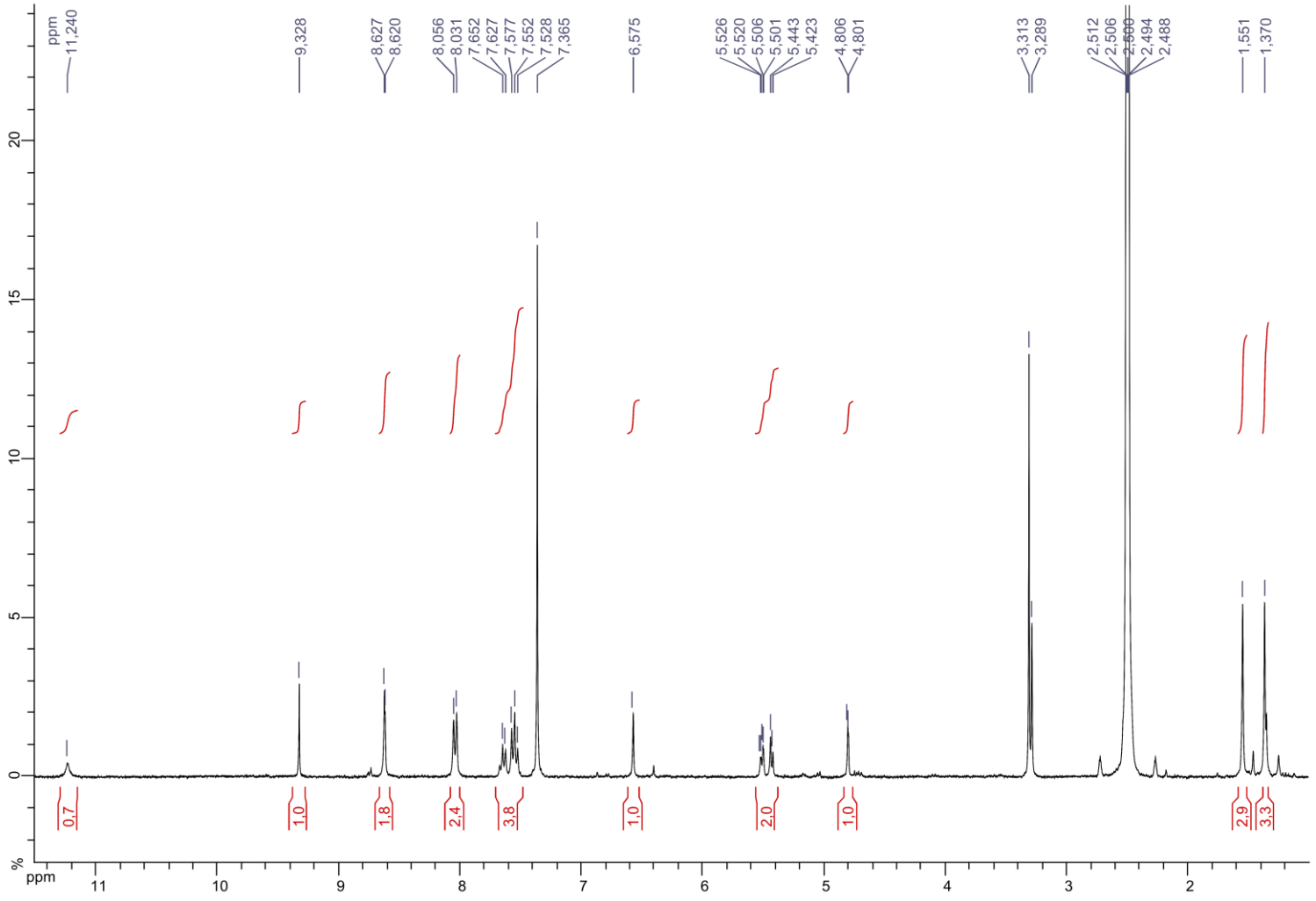
^{13}C-NMR spectrum (C^2HCl_3, 75 MHz) of compound S3



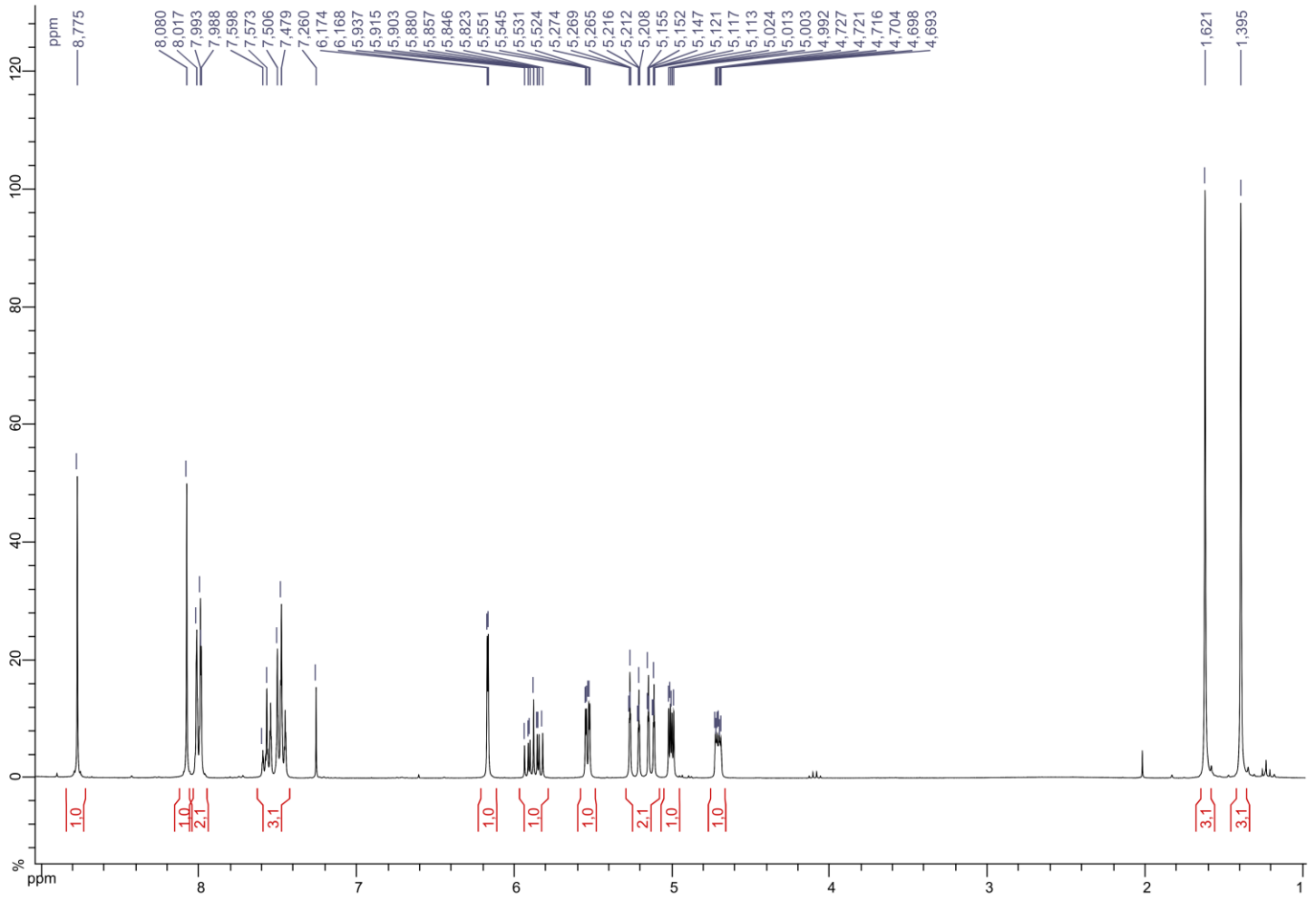
^{1}H-NMR spectrum ($^{2}H_6$)DMSO, 300 MHz) of compound S4



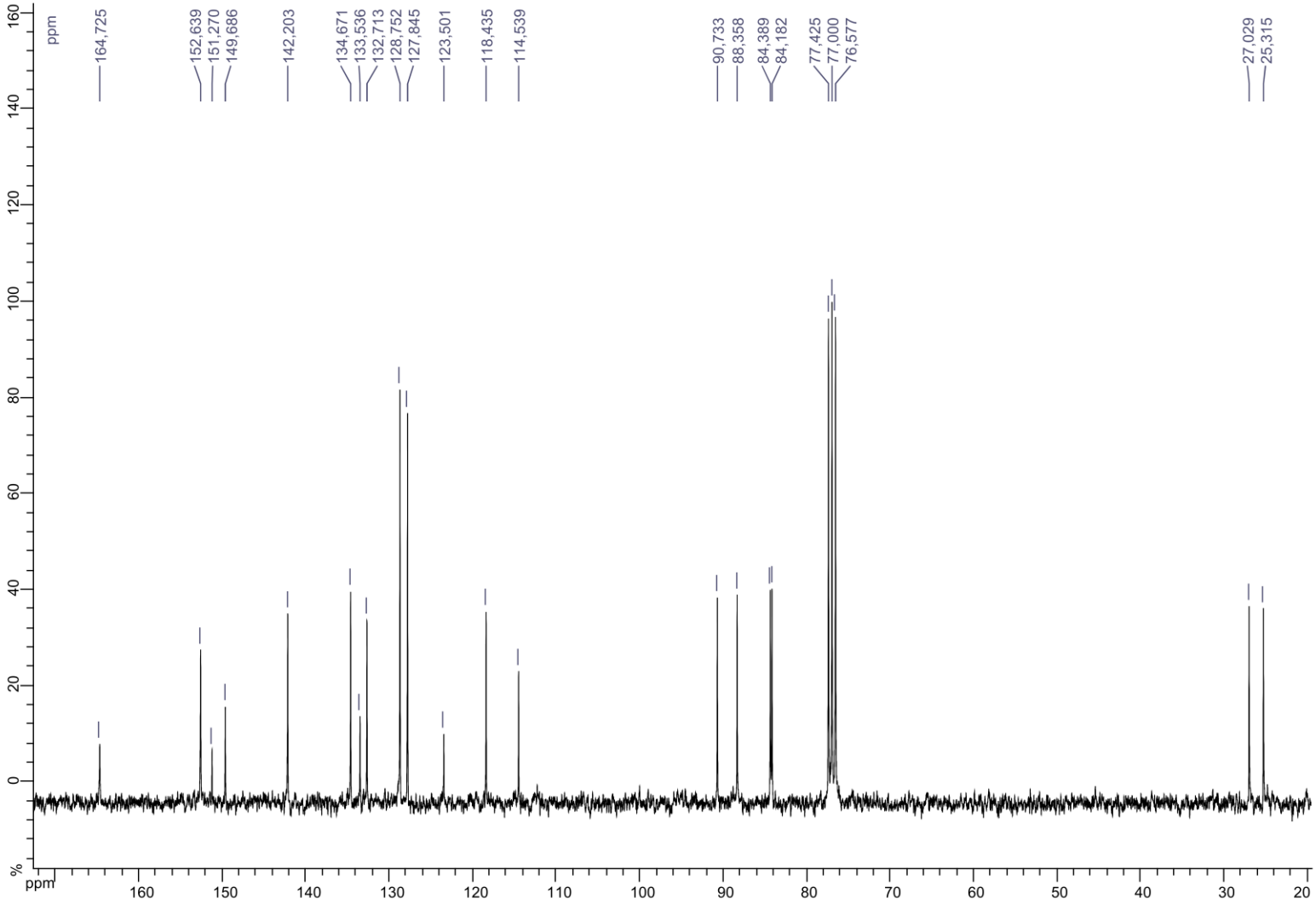
^{1}H-NMR spectrum ($C^{2}HCl_3$, 300 MHz) of compound 7



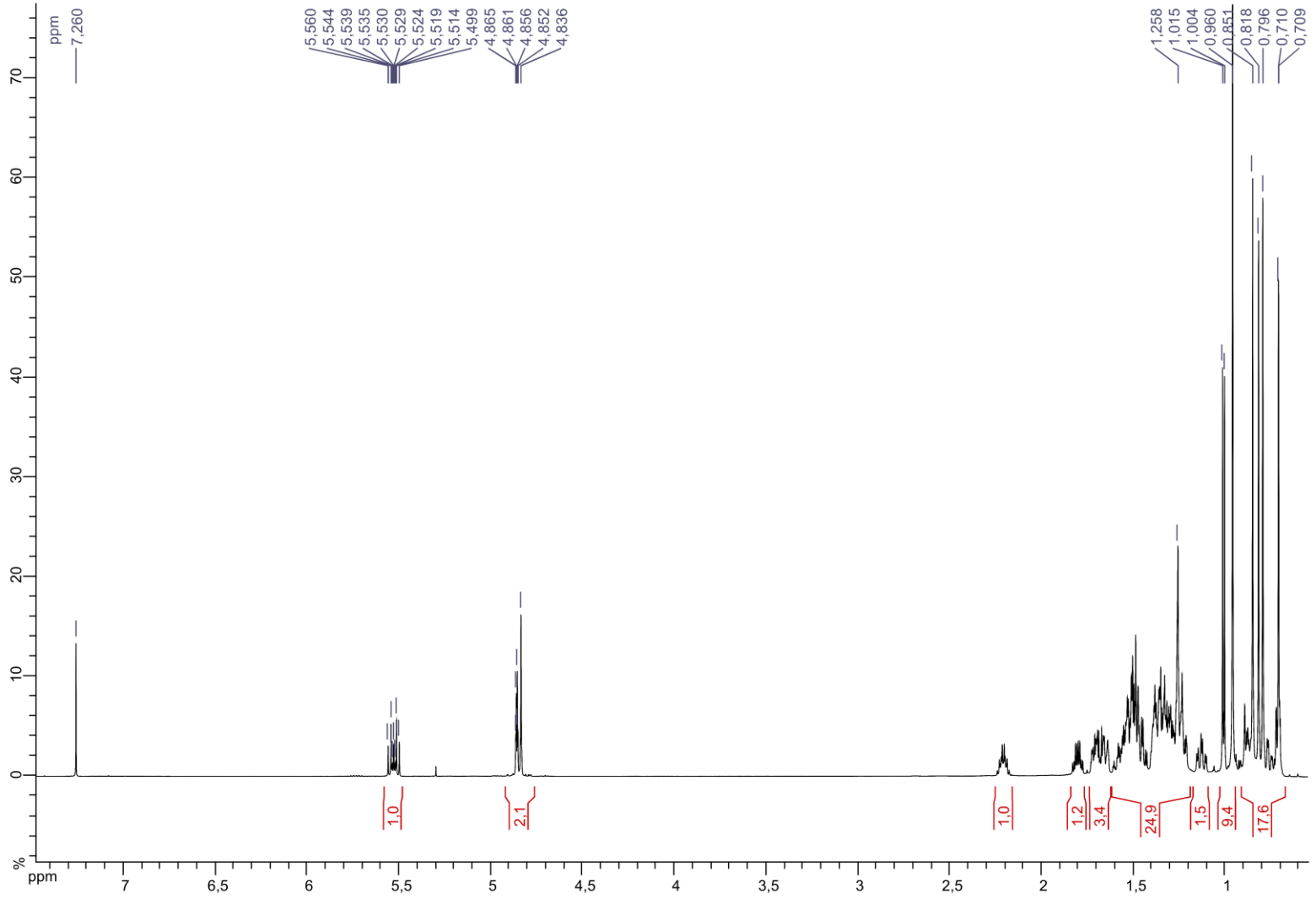
^{1}H-NMR spectrum ($C^{2}HCl_3$, 300 MHz) of compound 8



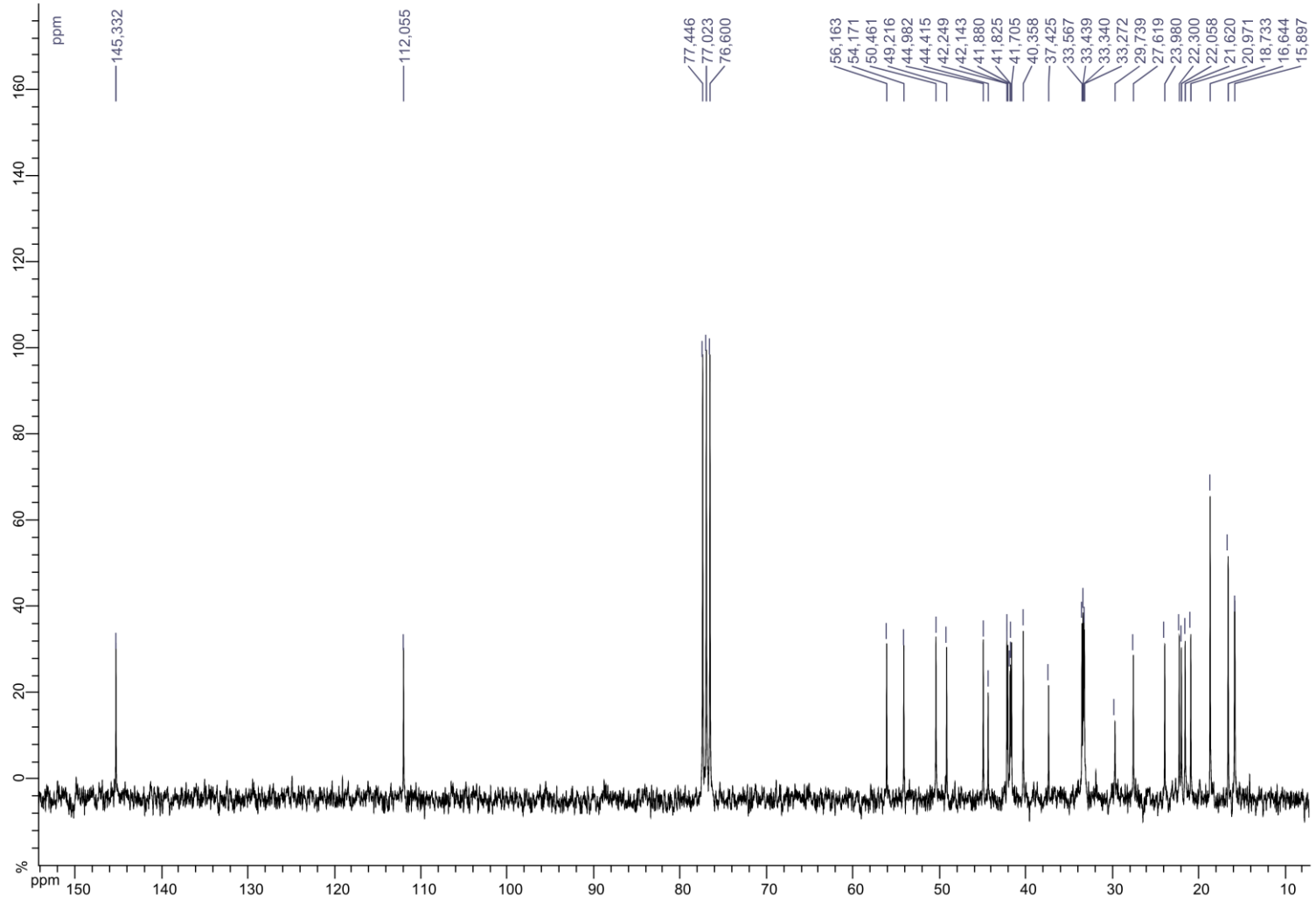
^{13}C-NMR spectrum (C^2HCl_3, 75 MHz) of compound 8



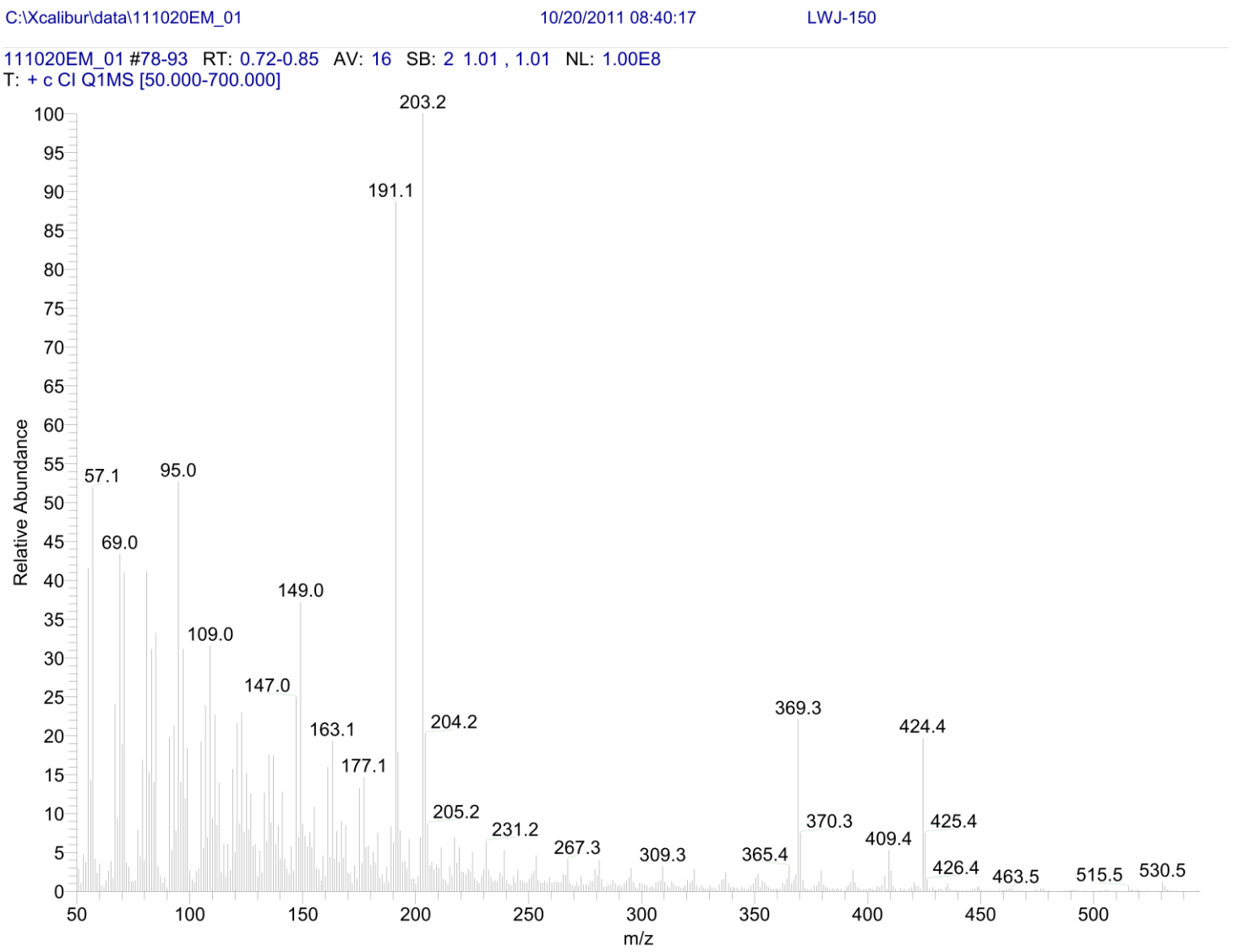
^{1}H-NMR spectrum ($C^{2}HCl_3$, 300 MHz) of compound 10



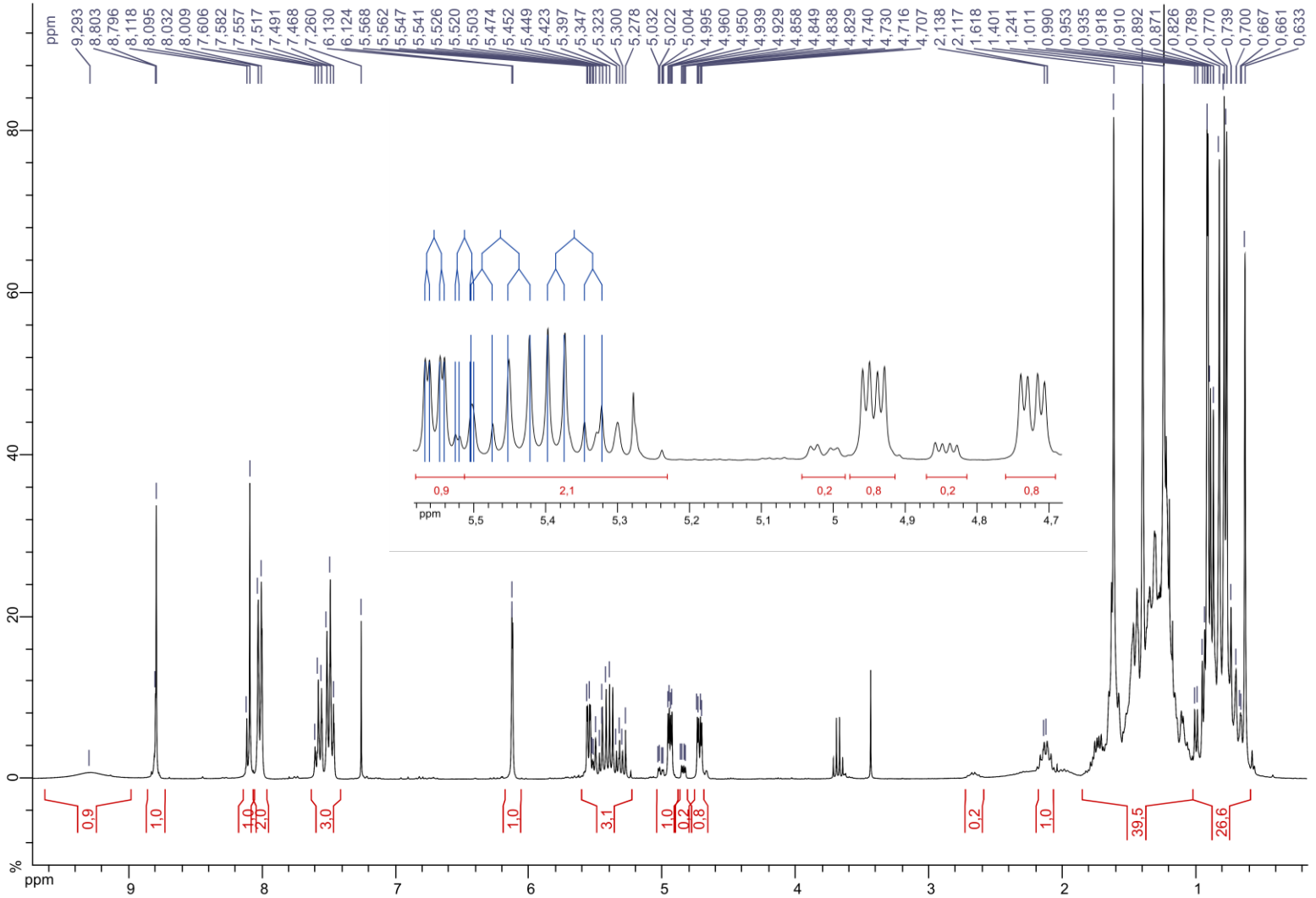
^{13}C-NMR spectrum (C^2HCl_3, 75 MHz) of compound 10



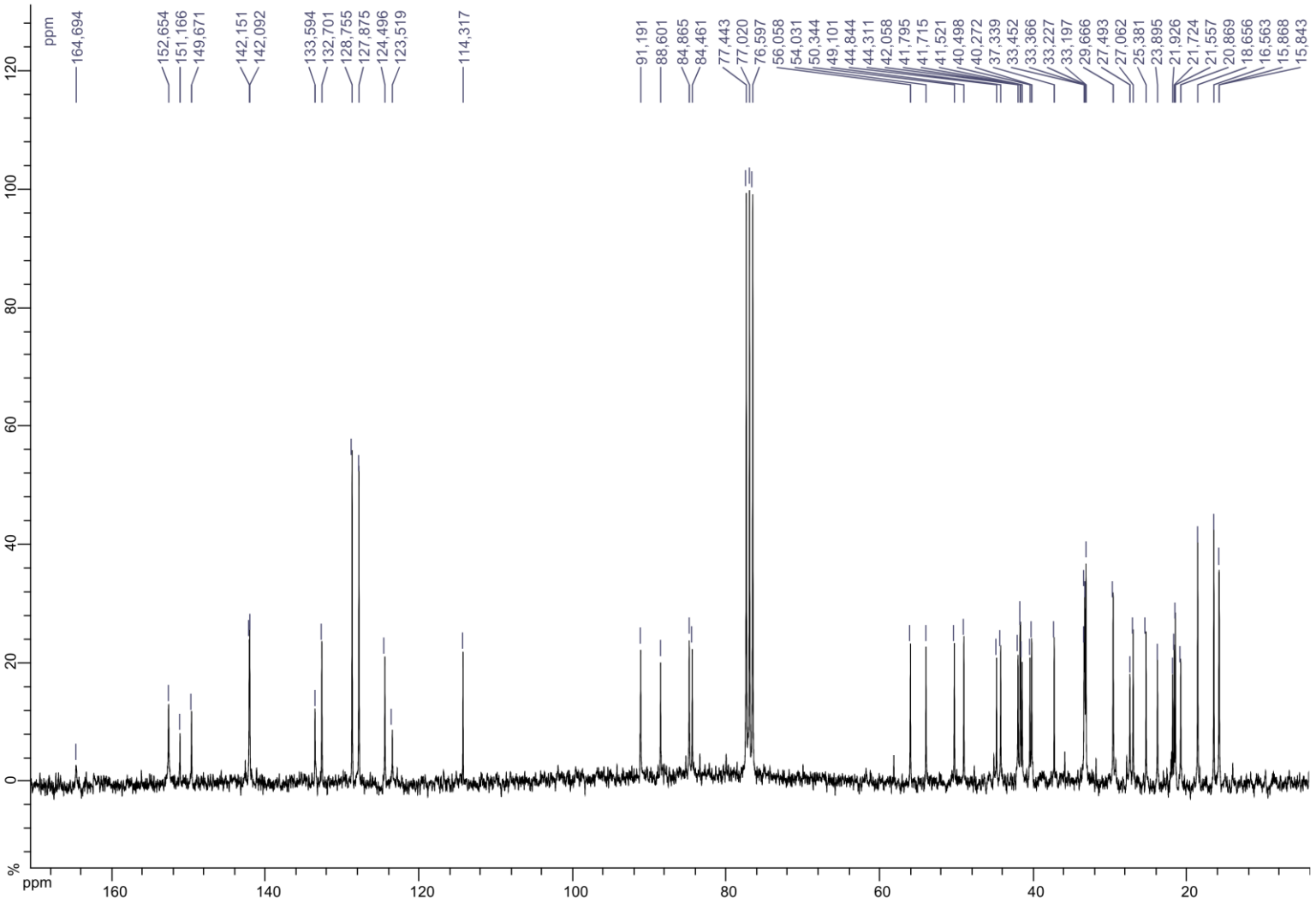
EI-MS (direct inlet, positive mode 70 eV) of compound 10



^{1}H-NMR spectrum ($C^{2}HCl_3$, 300 MHz) of compound 11



^{13}C-NMR spectrum (C^2HCl_3, 75 MHz) of compound 11



HRMS of compound 11

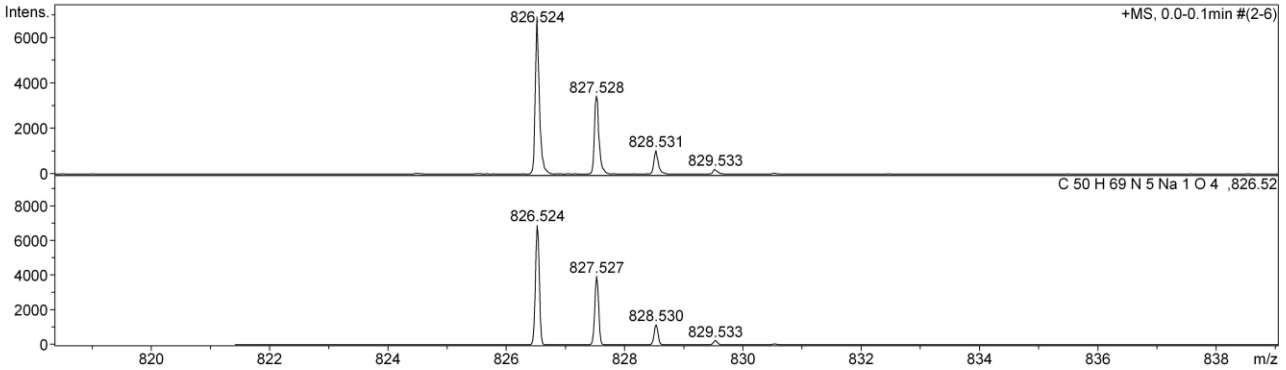
Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Service masse\O15643ML.d	Acquisition Date	10/17/2011 4:19:23 PM
Method	esi wide pos.m	Operator	Administrator
Sample Name	LWJ-232	Instrument	micrOTOF 66
Comment			

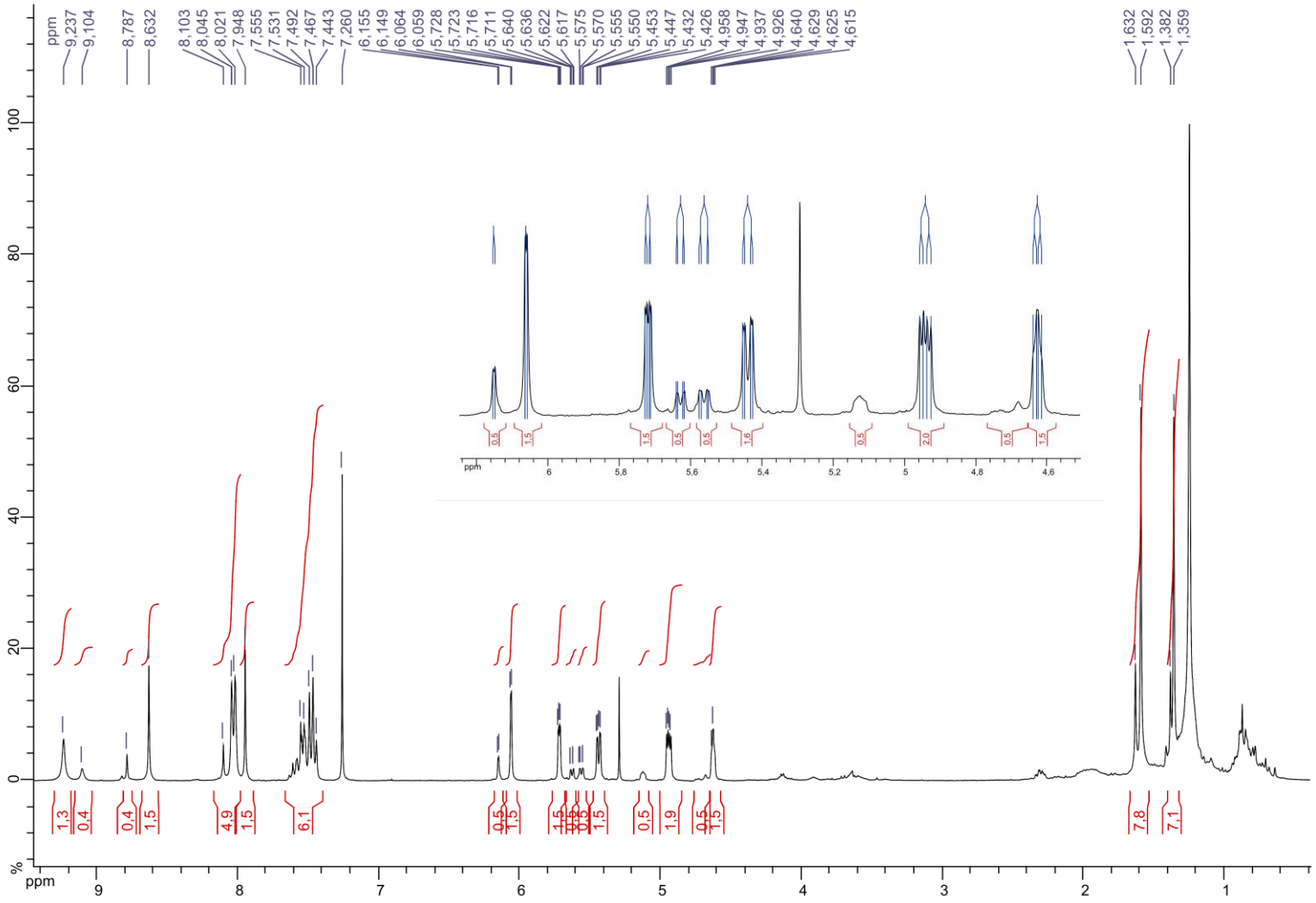
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	79 V
Scan Range	n/a	Capillary Exit	150.0 V	Set Pulsar Pull	799 V
Scan Begin	50 m/z	Hexapole RF	300.0 V	Set Pulsar Push	799 V
Scan End	3000 m/z	Skimmer 1	50.0 V	Set Reflector	1700 V
		Hexapole 1	24.3 V	Set Flight Tube	8600 V
				Set Detector TOF	2050 V

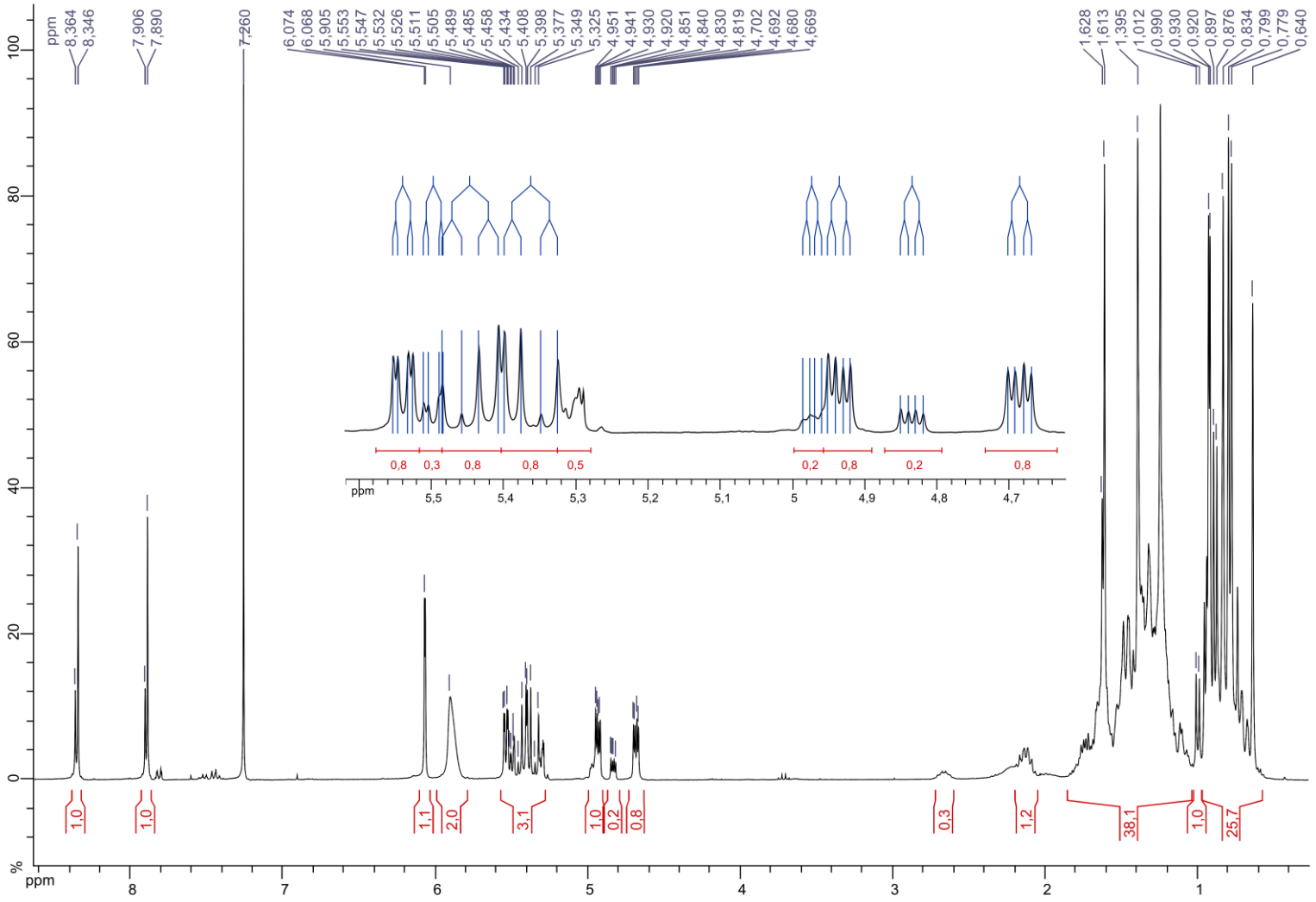


Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
C 50 H 69 N 5 Na 1 O 4	0.04	826.524	-0.02	-0.10	18.50	ok	even
C 50 H 68 N 5 Na 1 O 4	0.57	825.516	-5.62	-6.00	19.00	-	odd
C 50 H 67 N 5 Na 1 O 4	0.75	824.509	-11.36	-12.82	19.50	ok	even

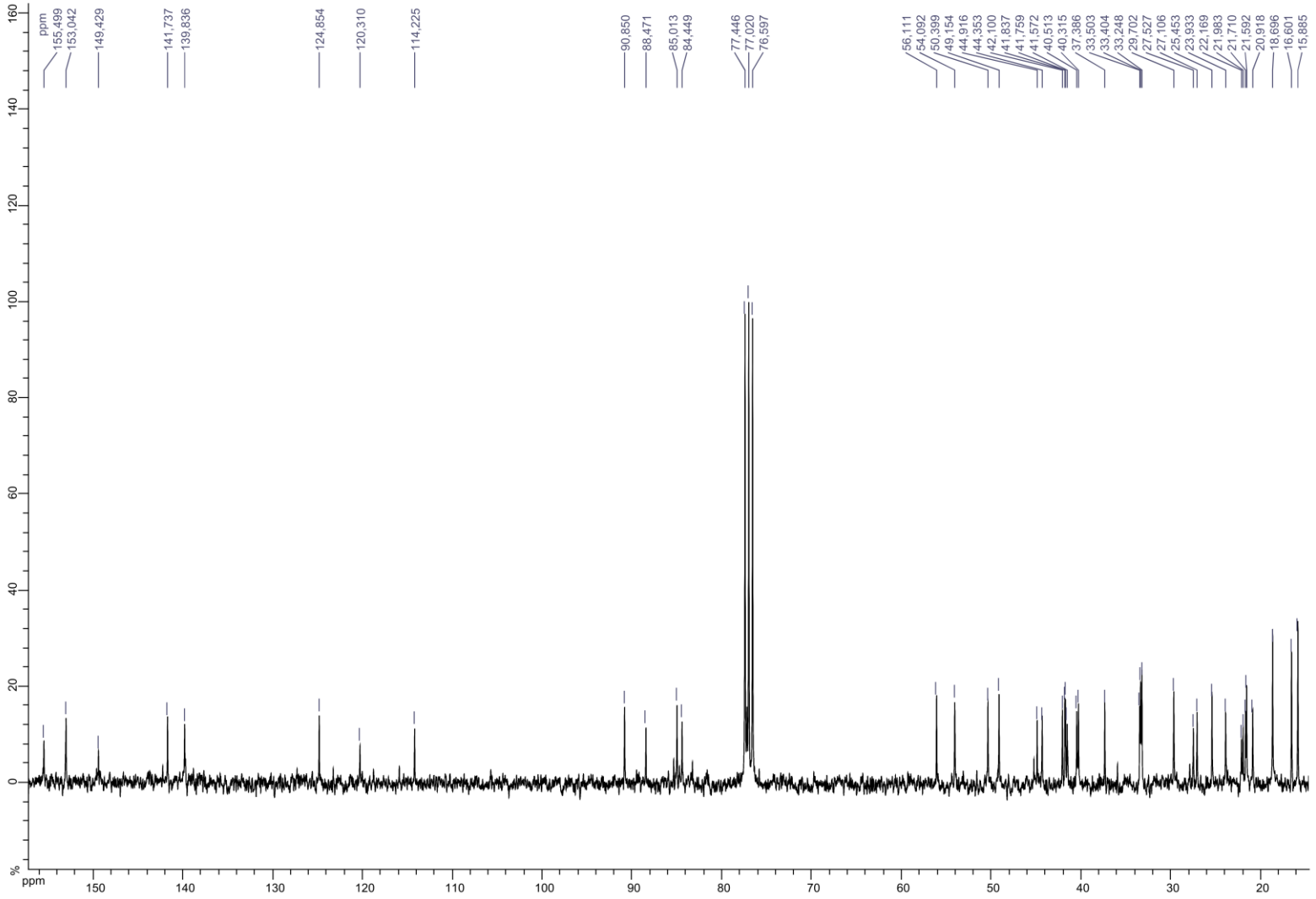
^{1}H-NMR spectrum ($C^{2}HCl_3$, 300 MHz) of compound 15



^{1}H-NMR spectrum ($C^{2}HCl_3$, 300 MHz) of compound 12



^{13}C-NMR spectrum (C^2HCl_3, 75 MHz) of compound 12



HRMS of compound 12

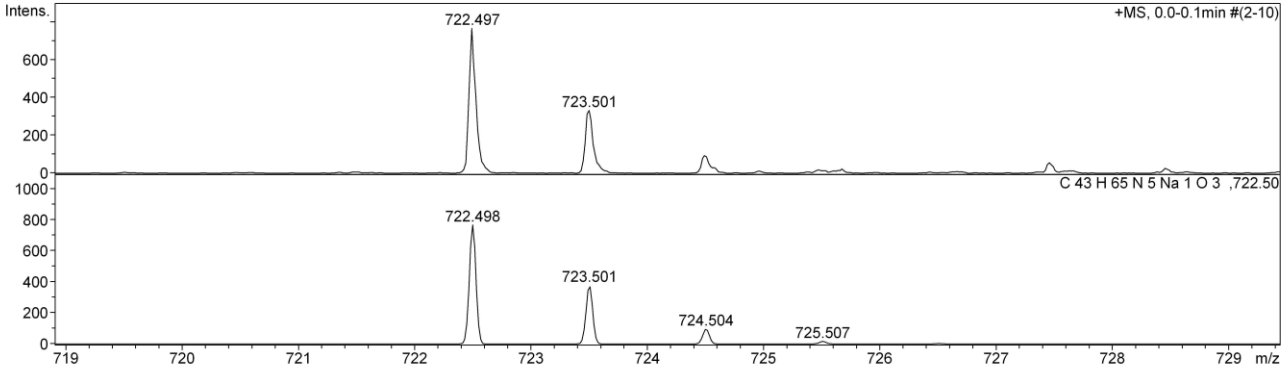
Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Service masse\O15638ML.d	Acquisition Date	10/17/2011 3:45:54 PM
Method	esi wide pos.m	Operator	Administrator
Sample Name	LWJ-235	Instrument	micrOTOF 66
Comment			

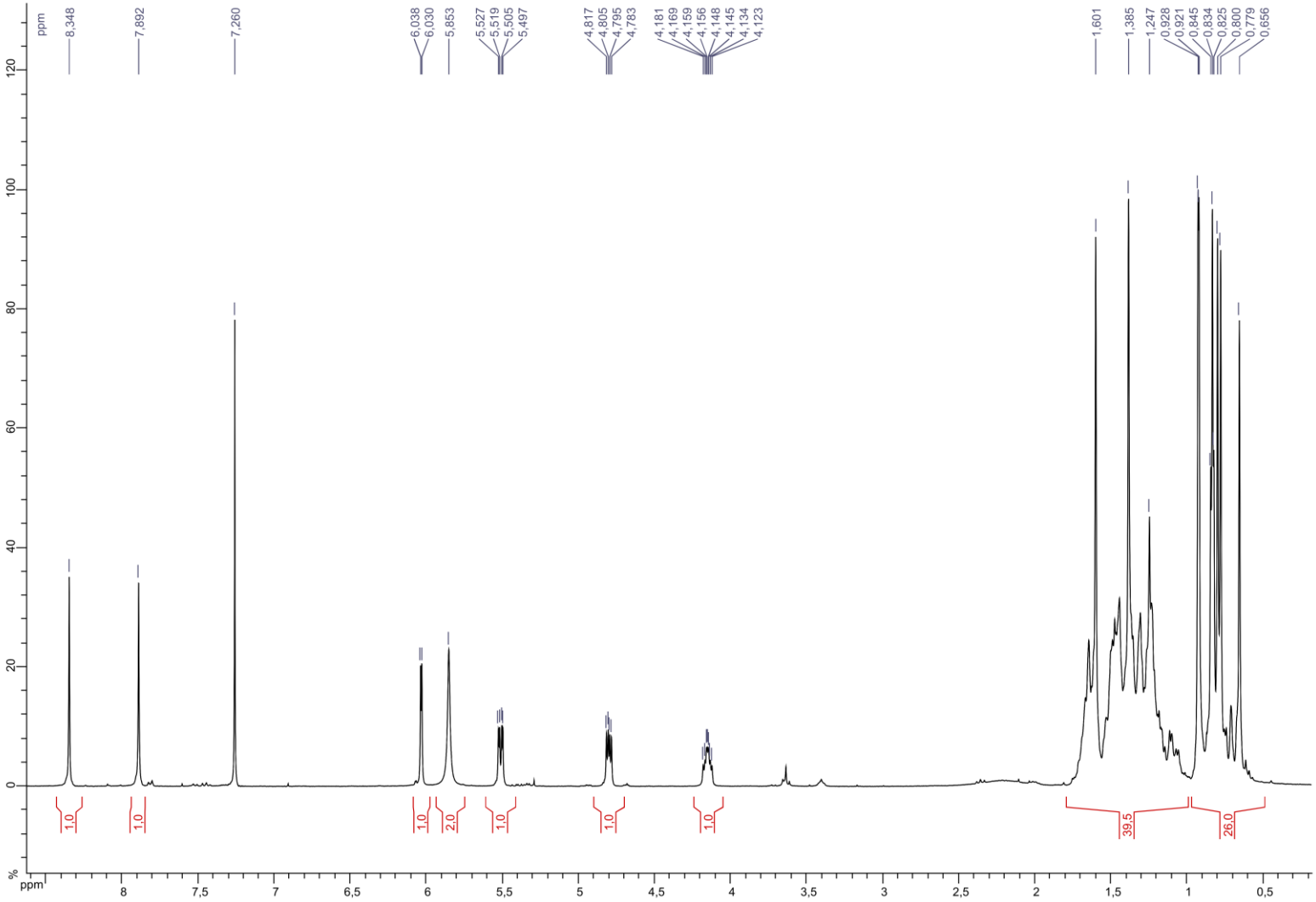
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	79 V
Scan Range	n/a	Capillary Exit	150.0 V	Set Pulsar Pull	799 V
Scan Begin	50 m/z	Hexapole RF	300.0 V	Set Pulsar Push	799 V
Scan End	3000 m/z	Skimmer 1	50.0 V	Set Reflector	1700 V
		Hexapole 1	24.3 V	Set Flight Tube	8600 V
				Set Detector TOF	2050 V

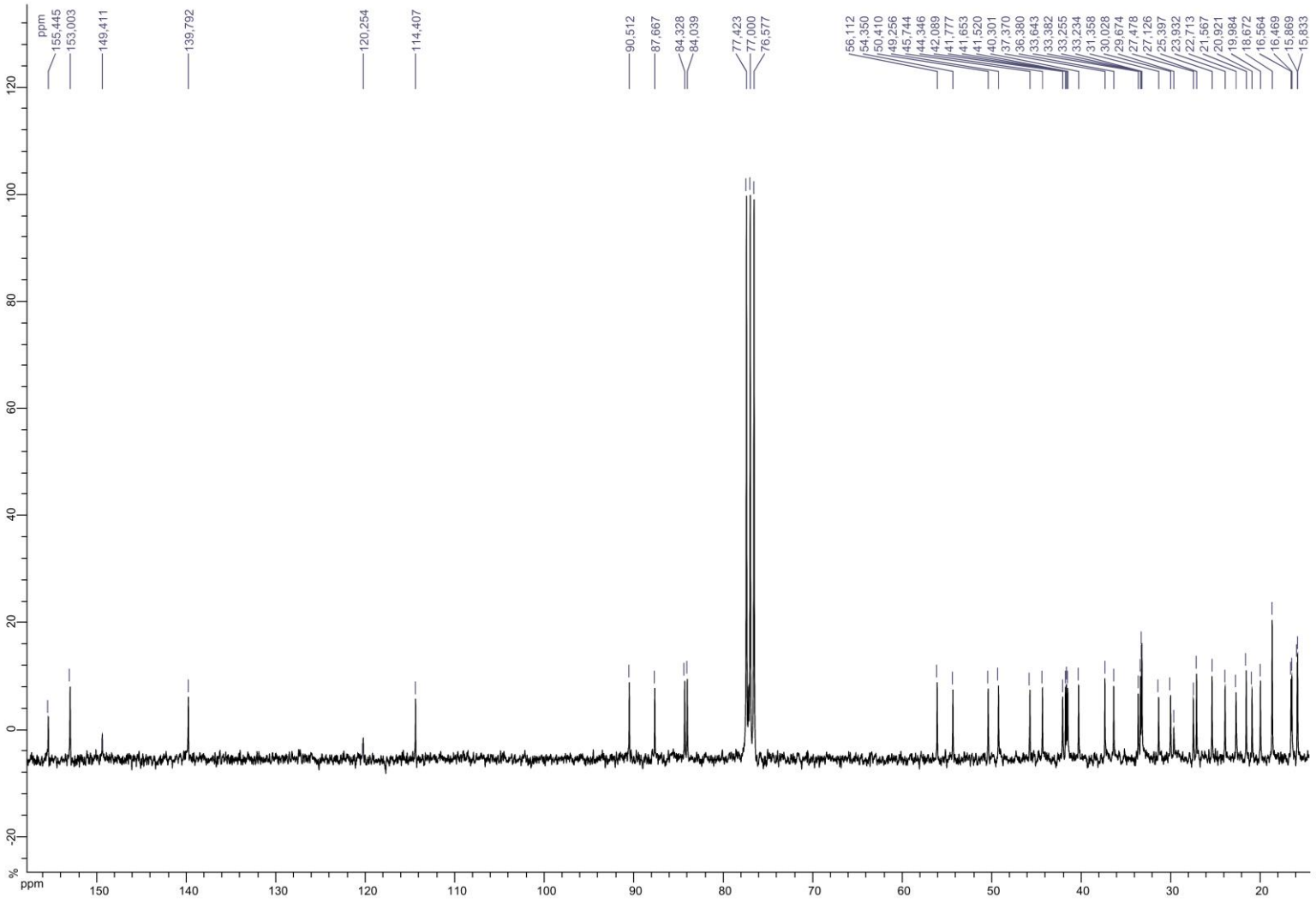


Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
C 43 H 65 N 5 Na 1 O 3	0.07	722.498	1.55	1.10	13.50	ok	even
C 43 H 64 N 5 Na 1 O 3	0.58	721.490	-4.90	-5.68	14.00	-	odd
C 43 H 63 N 5 Na 1 O 3	0.74	720.482	-11.50	-13.11	14.50	ok	even

^{1}H-NMR spectrum ($C^{2}HCl_3$, 300 MHz) of compound 13



^{13}C-NMR spectrum (C^2HCl_3, 75 MHz) of compound 12



HRMS of compound 13

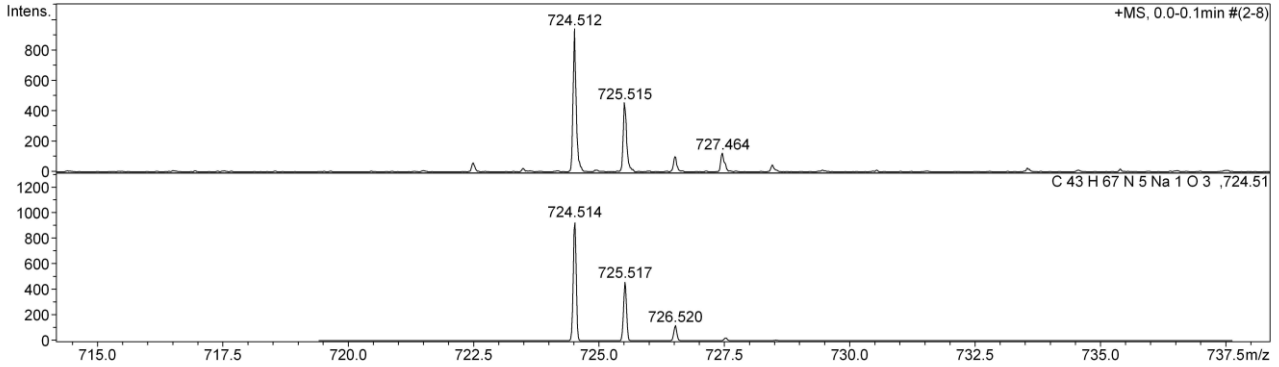
Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Service masse\O15639ML.d	Acquisition Date	10/17/2011 3:48:54 PM
Method	esi wide pos.m	Operator	Administrator
Sample Name	LWJ-236	Instrument	micrOTOF 66
Comment			

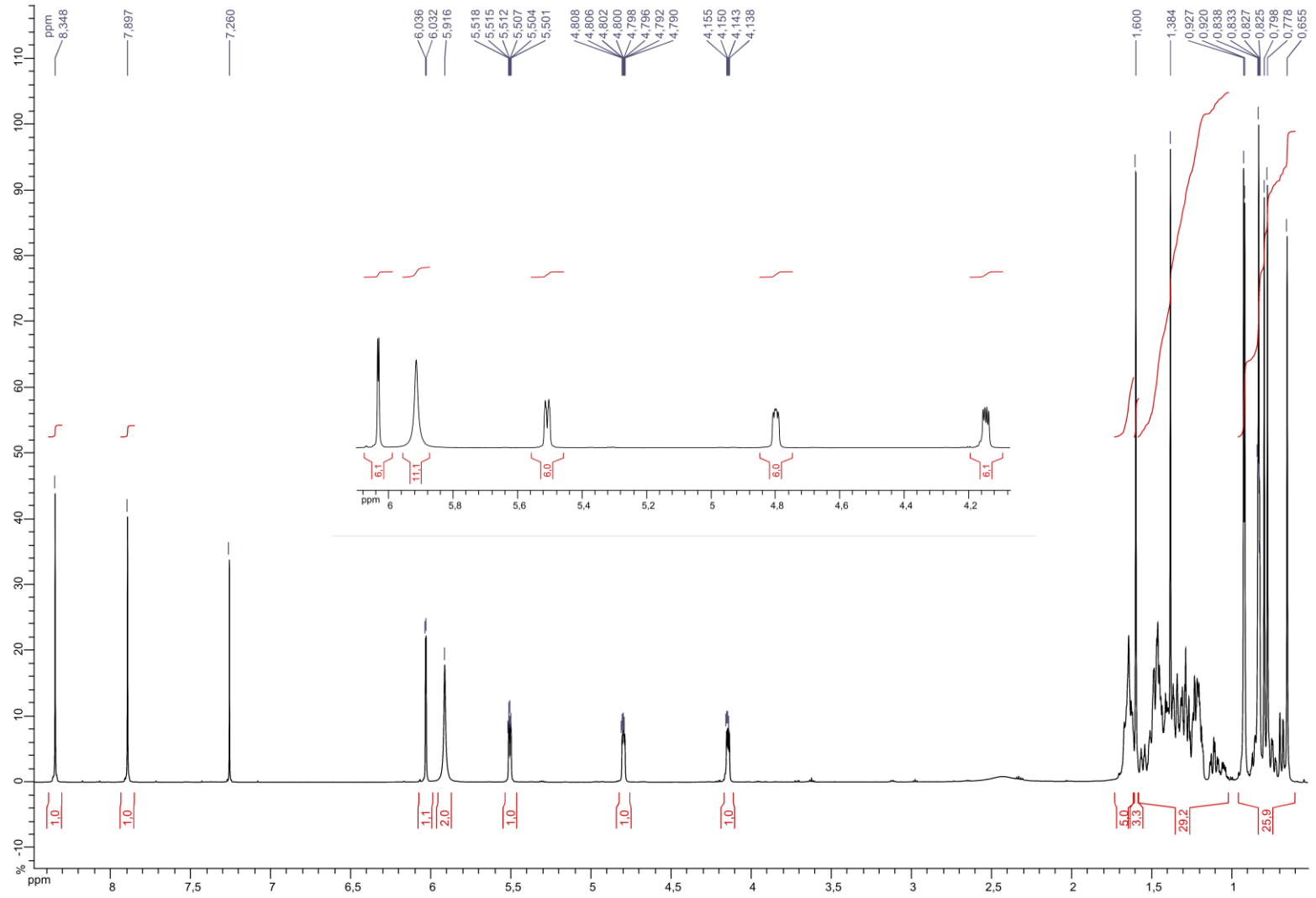
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	79 V
Scan Range	n/a	Capillary Exit	200.0 V	Set Pulsar Pull	799 V
Scan Begin	50 m/z	Hexapole RF	300.0 V	Set Pulsar Push	799 V
Scan End	3000 m/z	Skimmer 1	50.0 V	Set Reflector	1700 V
		Hexapole 1	24.3 V	Set Flight Tube	8600 V
				Set Detector TOF	2050 V

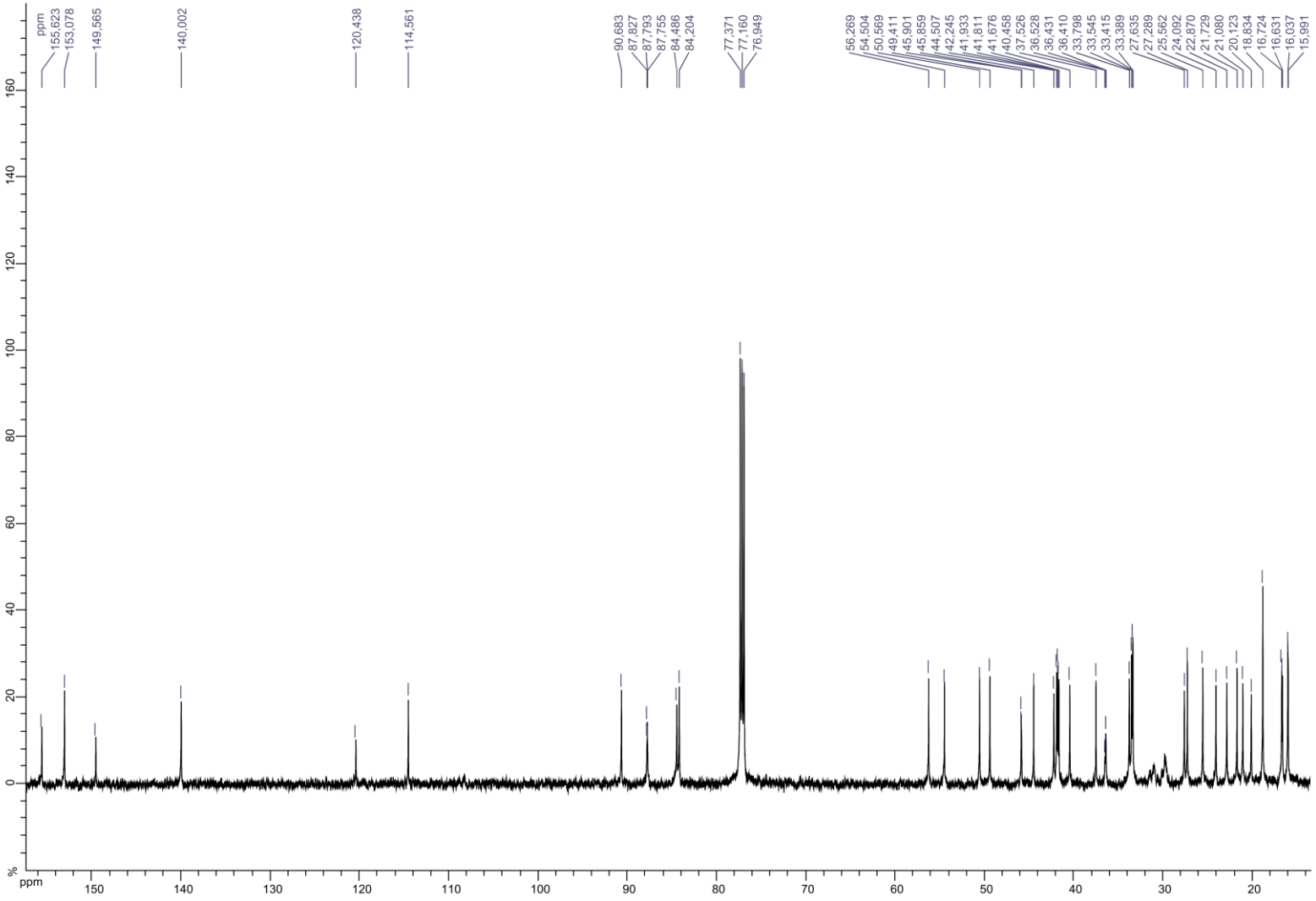


Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
C 43 H 67 N 5 Na 1 O 3	0.06	724.514	2.27	7.92	12.50	ok	even
C 43 H 66 N 5 Na 1 O 3	0.59	723.506	-4.16	-0.58	13.00	-	odd
C 43 H 65 N 5 Na 1 O 3	0.75	722.498	-10.74	-11.79	13.50	ok	even

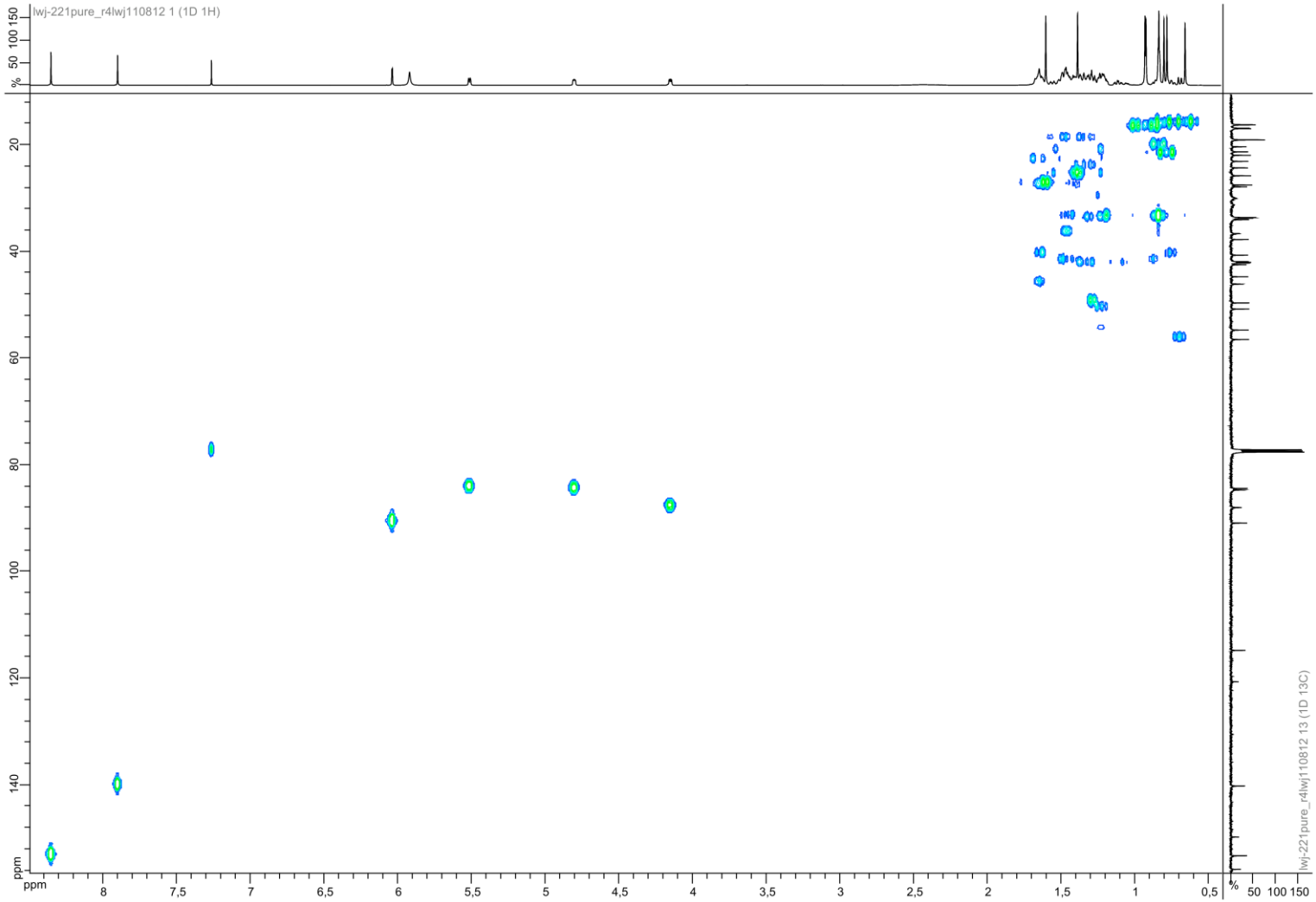
^{1}H-NMR spectrum ($C^{2}HCl_3$, 600 MHz) of compound 13D

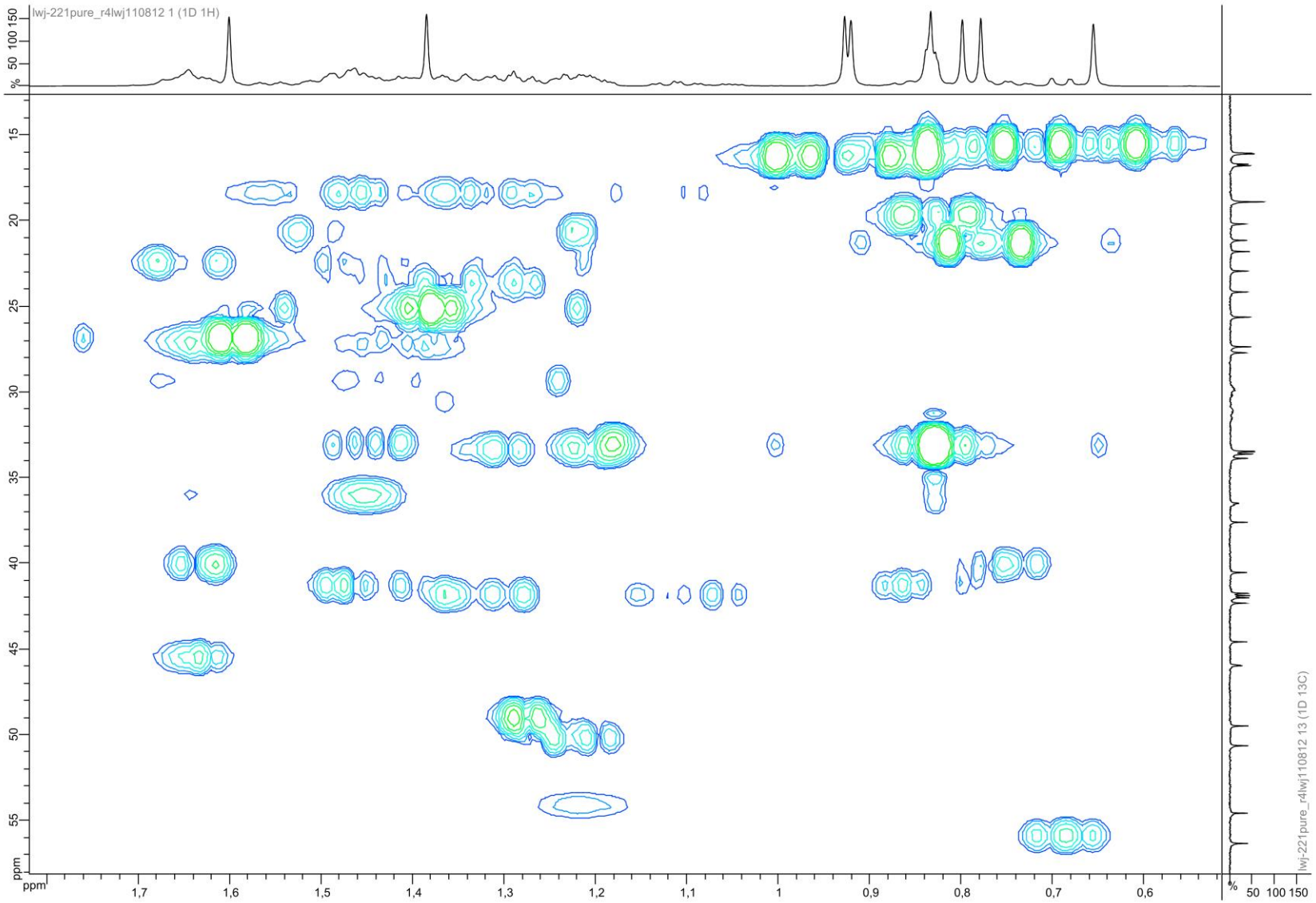


^{13}C-NMR spectrum (C^2HCl_3, 150 MHz) of compound 13D

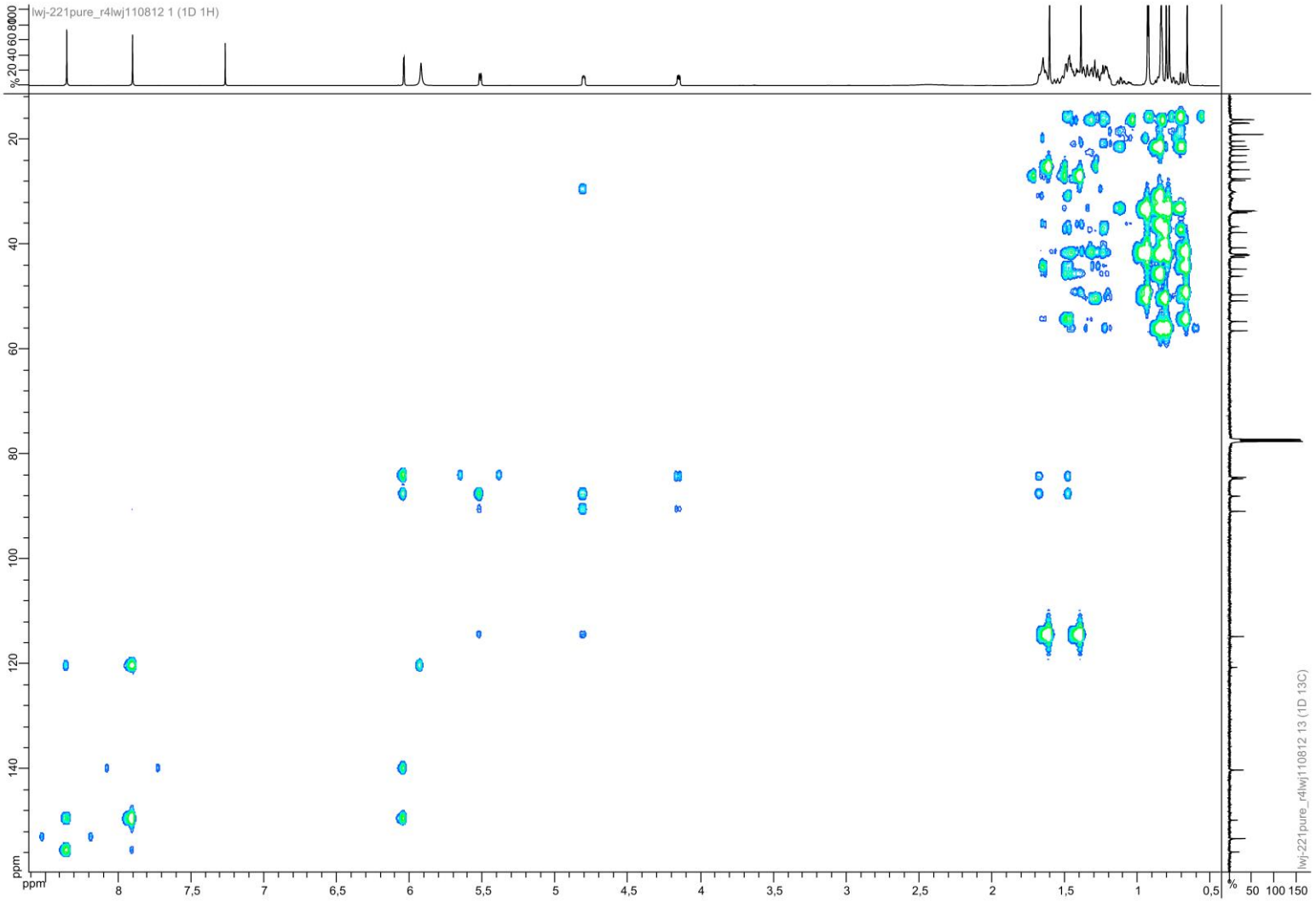


2D-NMR spectrum HSQC $^{1}H/^{13}C$ ($C^{2}HCl_3$, 600 MHz) of compound 13D




lwj-221pure_r4lwj110812 1 (1D 1H)
% 50 100 150
15
20
25
30
35
40
45
50
55
ppm
1,7
1,6
1,5
1,4
1,3
1,2
1,1
1
0,9
0,8
0,7
0,6
% 50 100 150
lwj-221pure_r4lwj110812 13 (1D 13C)

2D-NMR spectrum HMBC $^{1}H/^{13}C$ ($C^{2}HCl_3$, 600 MHz) of compound 13D



HRMS of compound 13-D

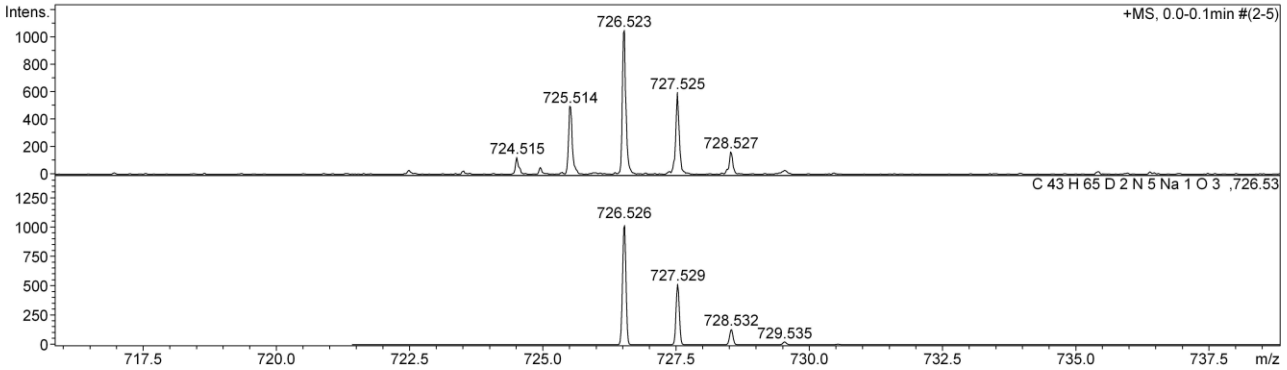
Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Service masse\O15640ML.d	Acquisition Date	10/17/2011 3:52:13 PM
Method	esi wide pos.m	Operator	Administrator
Sample Name	LWJ-221	Instrument	micrOTOF 66
Comment			

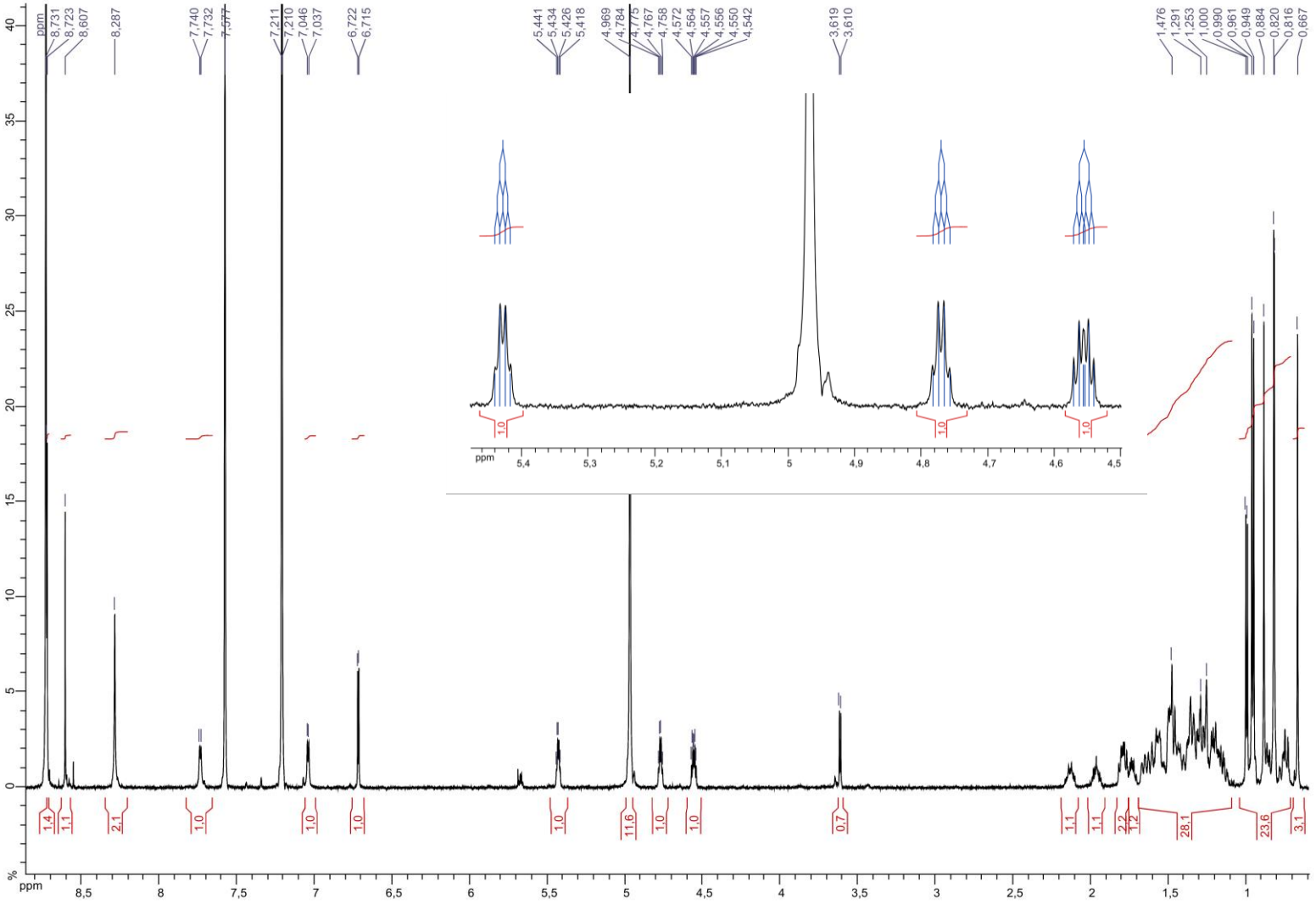
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	79 V
Scan Range	n/a	Capillary Exit	200.0 V	Set Pulsar Pull	799 V
Scan Begin	50 m/z	Hexapole RF	300.0 V	Set Pulsar Push	799 V
Scan End	3000 m/z	Skimmer 1	50.0 V	Set Reflector	1700 V
		Hexapole 1	24.3 V	Set Flight Tube	8600 V
				Set Detector TOF	2050 V

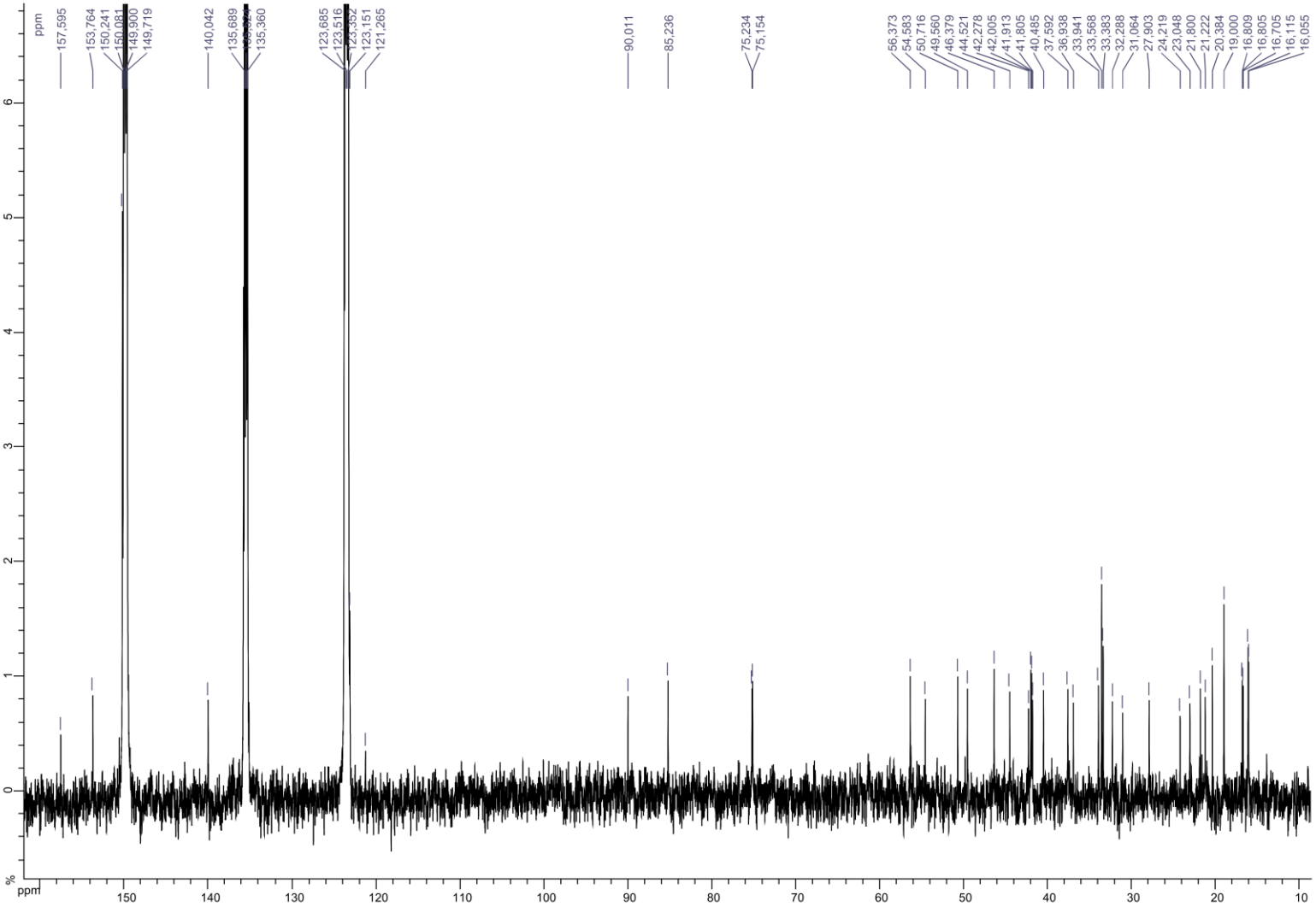


Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
C 43 H 65 D 2 N 5 Na 1 O 3	0.04	726.526	4.93	5.48	12.50	ok	even
C 43 H 64 D 2 N 5 Na 1 O 3	0.43	725.518	-1.49	0.37	13.00	-	odd
C 43 H 63 D 2 N 5 Na 1 O 3	0.68	724.511	-8.06	-6.23	13.50	ok	even
C 43 H 62 D 2 N 5 Na 1 O 3	0.74	723.503	-14.75	-12.93	14.00	-	odd

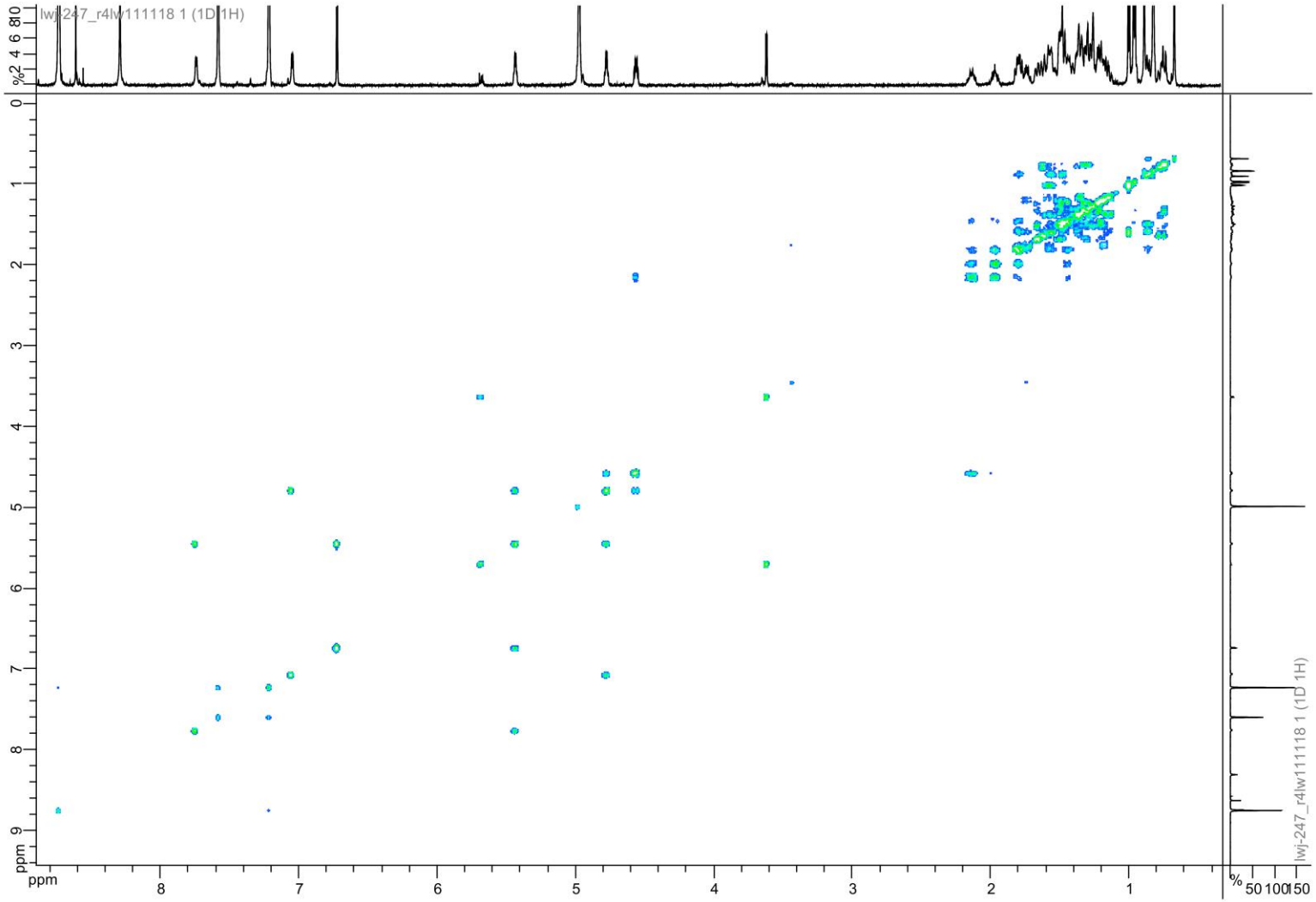
^{1}H-NMR spectrum (($^{2}H_5$)pyridine, 600 MHz) of compound 2



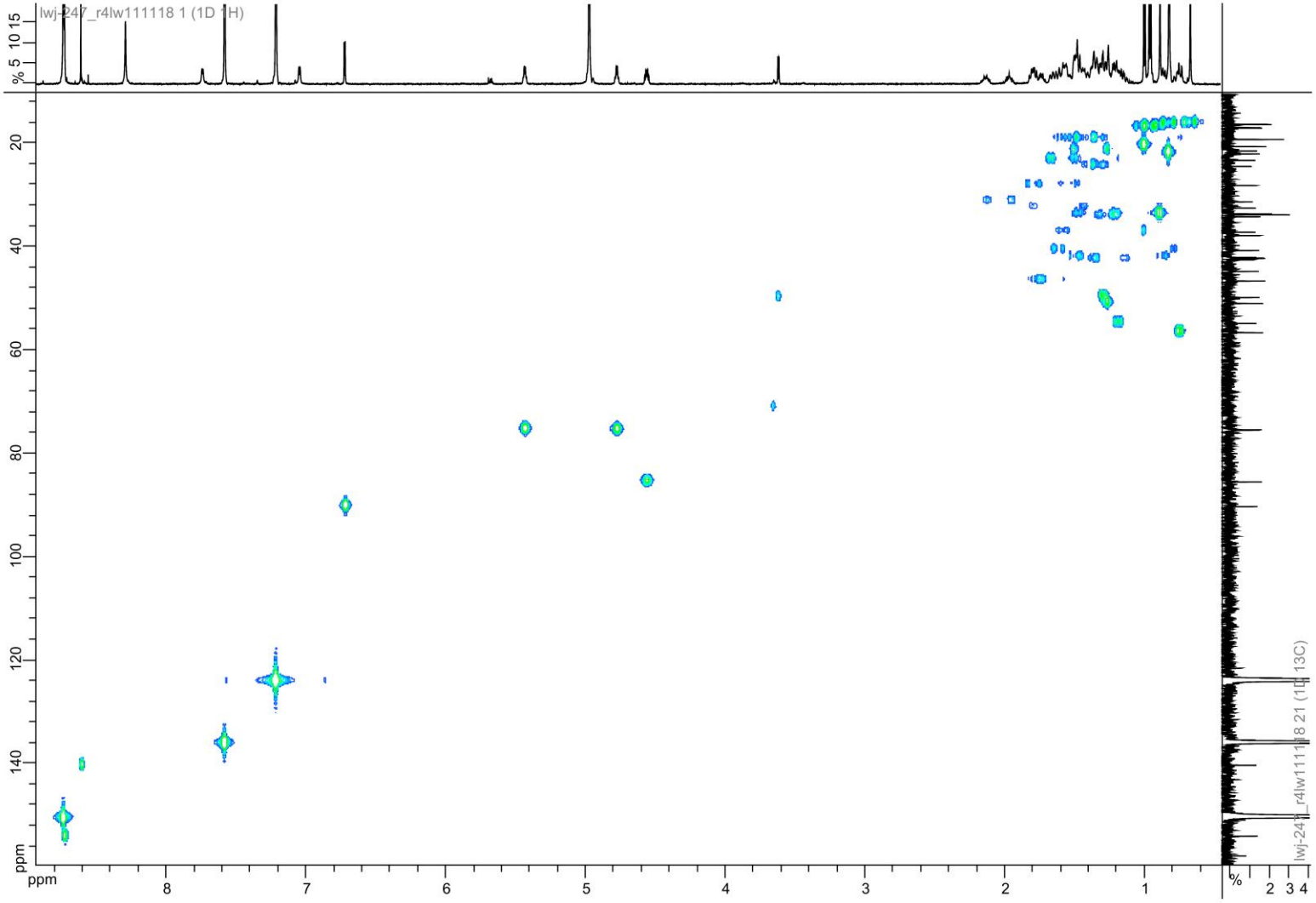
^{13}C-NMR spectrum (($^{2}H_5$)pyridine, 150 MHz) of compound 2



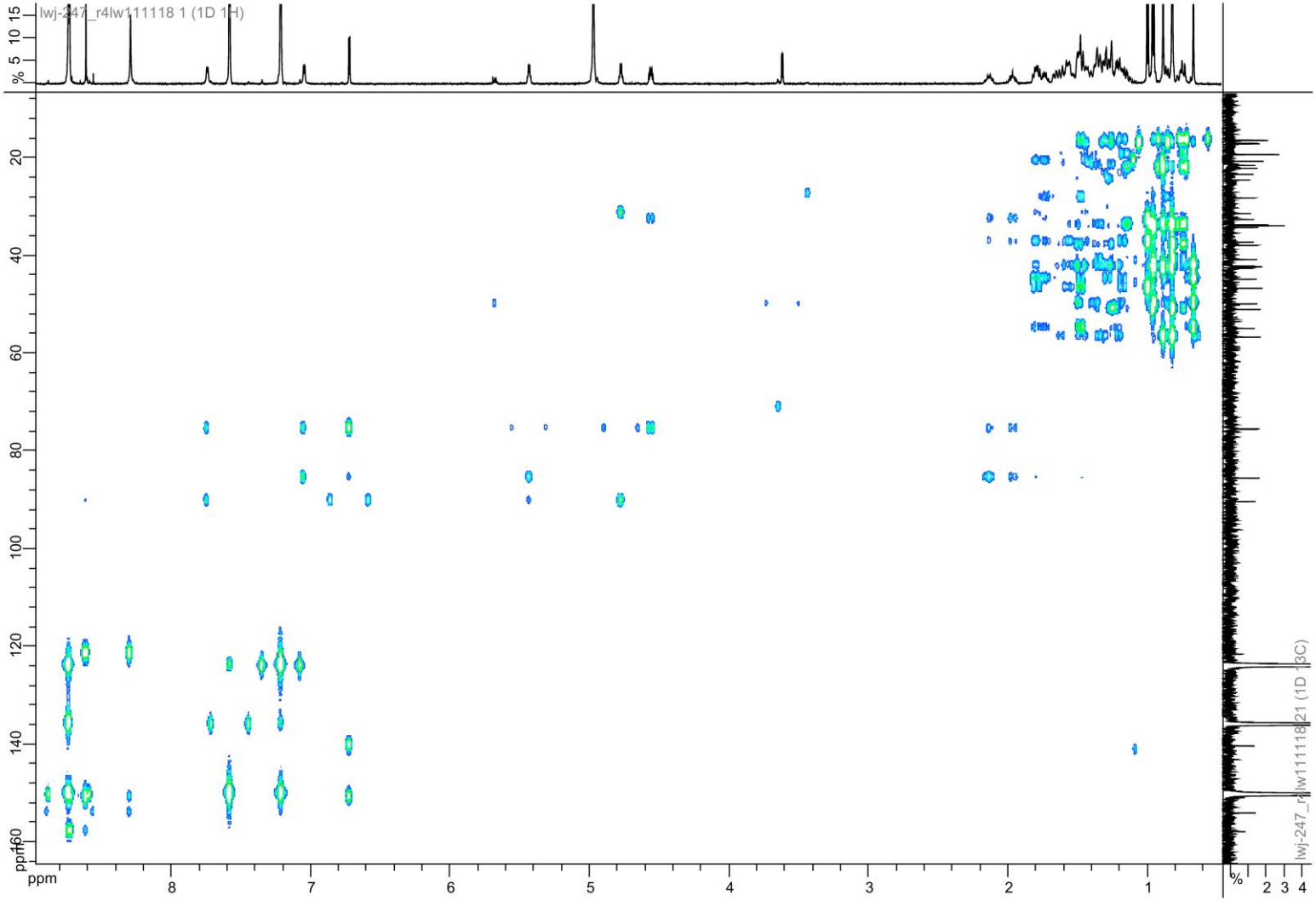
2D-NMR spectrum COSY $^{1}H/^{1}H$ ($(^{2}H_5)$pyridine, 600 MHz) of compound 2



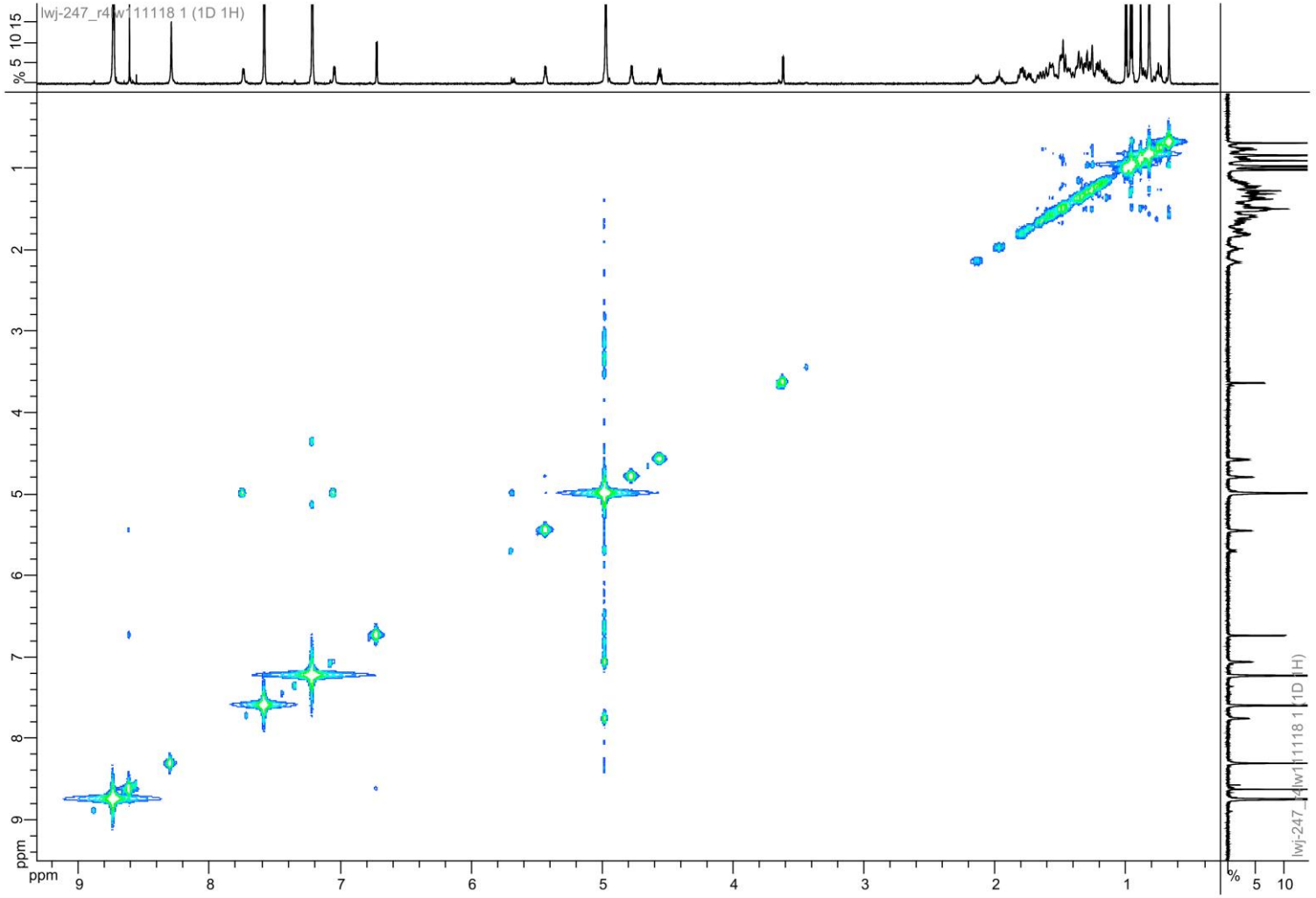
2D-NMR spectrum HSQC $^{1}H/^{13}C$ ($(^{2}H_5)$pyridine, 600 MHz) of compound 2



2D-NMR spectrum HMBC $^{1}H/^{13}C$ ($(^{2}H_5)$pyridine, 600 MHz) of compound 2



2D-NMR spectrum ROESY $^{1}H/^{1}H$ ($(^{2}H_5)$pyridine, 600 MHz) of compound 2



HRMS of compound 2

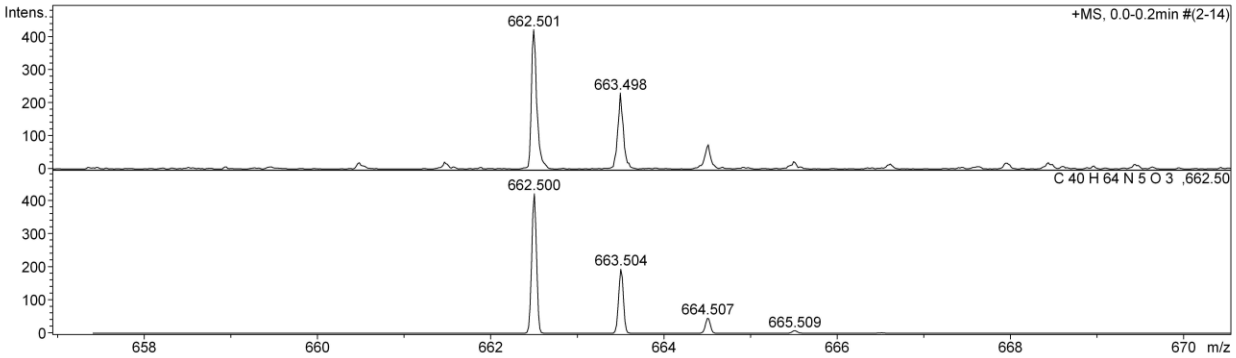
Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Service masse\O15637ML.d	Acquisition Date	10/17/2011 3:42:01 PM
Method	esi wide pos.m	Operator	Administrator
Sample Name	LWJ-247	Instrument	micrOTOF 66
Comment			

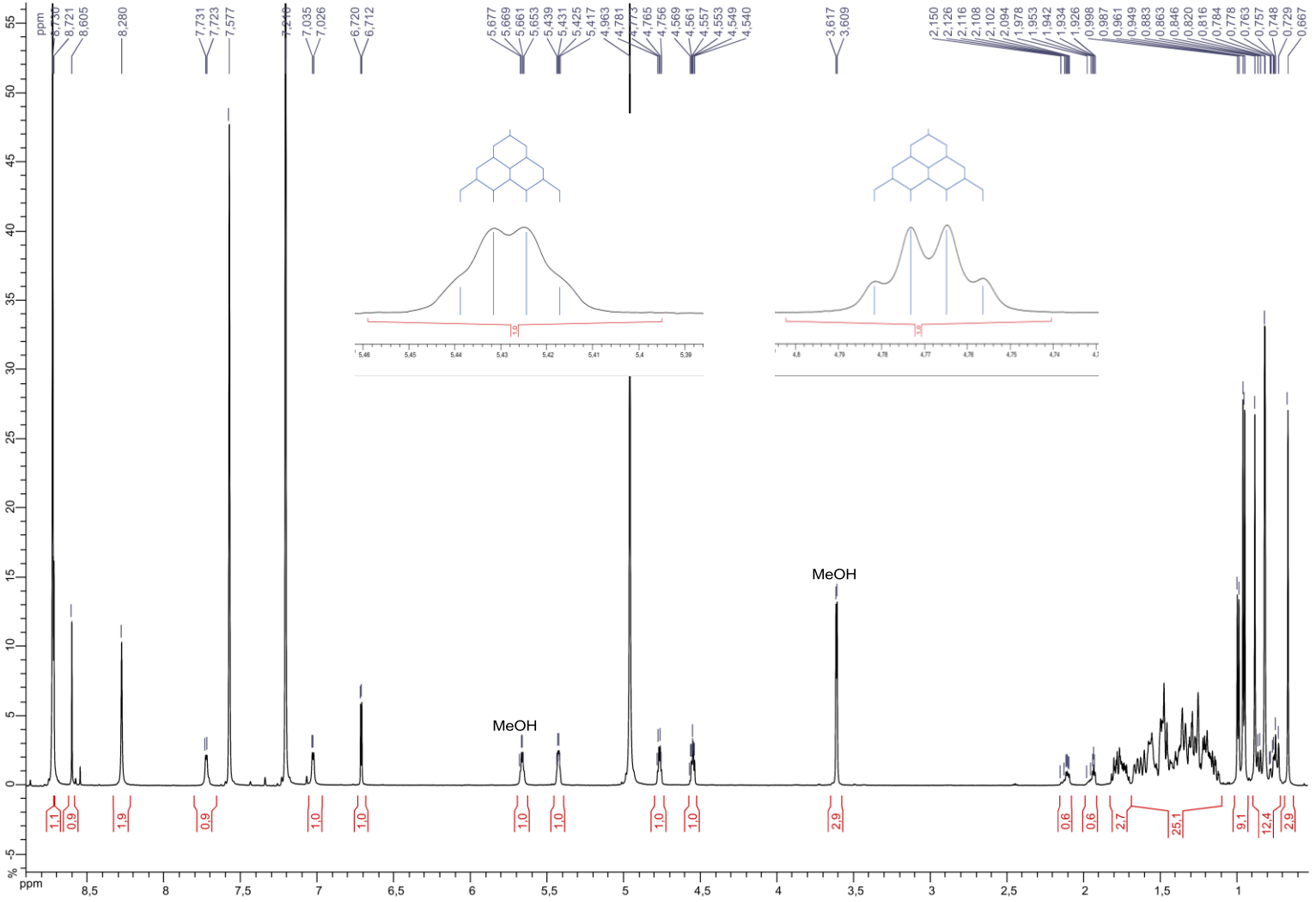
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	79 V
Scan Range	n/a	Capillary Exit	200.0 V	Set Pulsar Pull	799 V
Scan Begin	50 m/z	Hexapole RF	300.0 V	Set Pulsar Push	799 V
Scan End	3000 m/z	Skimmer 1	50.0 V	Set Reflector	1700 V
		Hexapole 1	24.3 V	Set Flight Tube	8600 V
				Set Detector TOF	2050 V

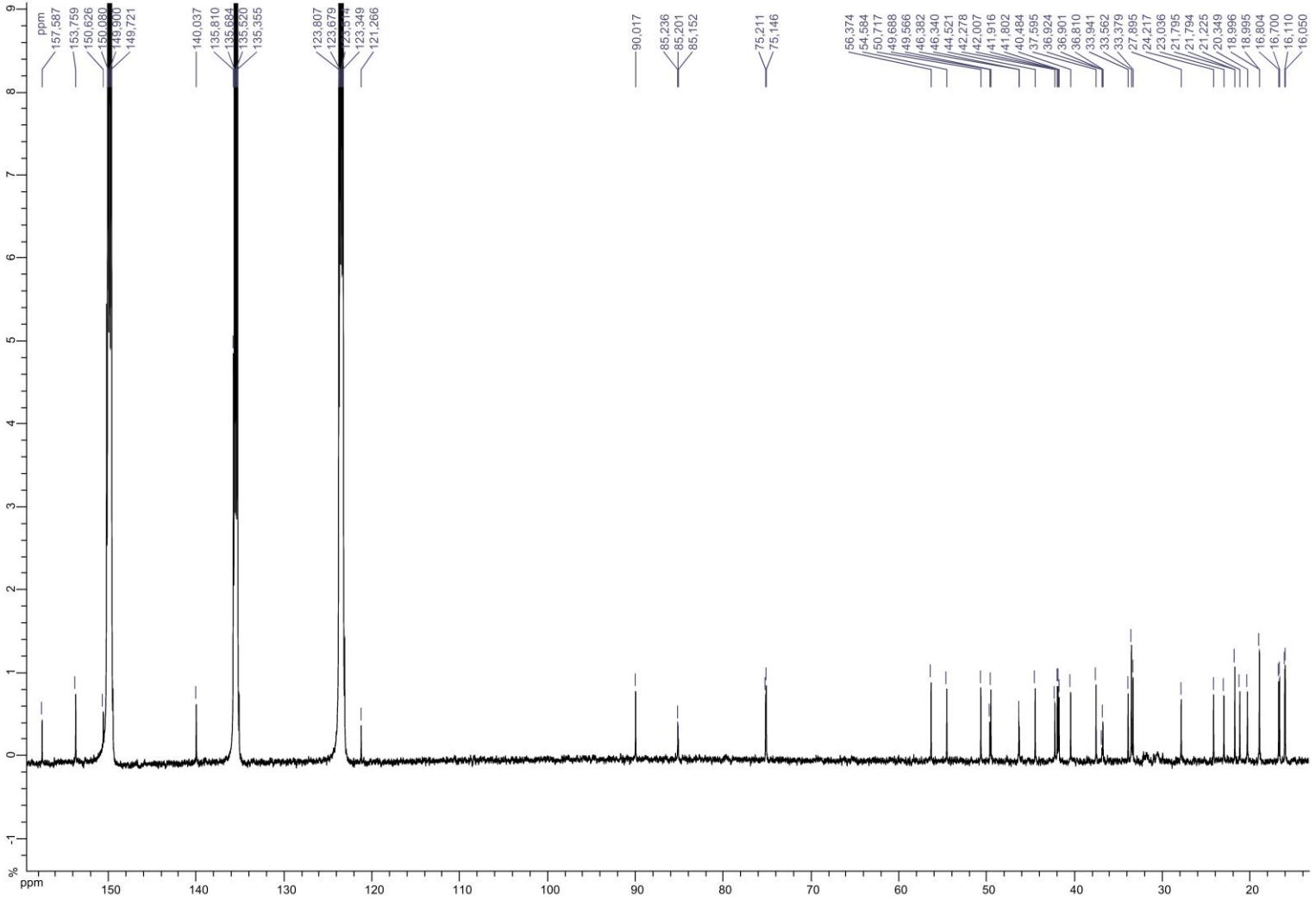


Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
C 40 H 64 N 5 O 3	0.07	662.500	-1.51	1.76	11.50	ok	even
C 40 H 63 N 5 O 3	0.61	661.493	-8.57	-5.85	12.00	-	odd

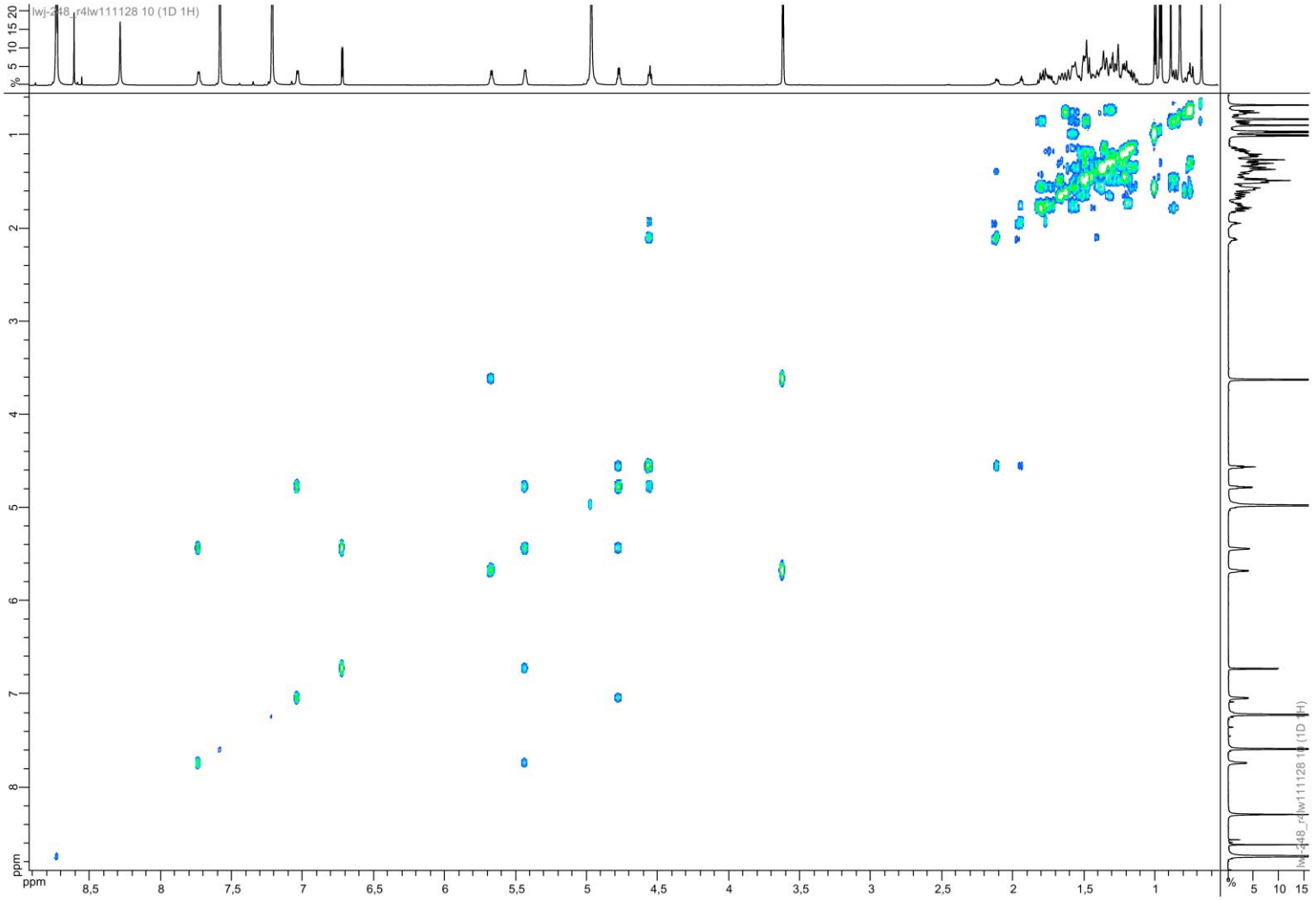
^{1}H-NMR spectrum (($^{2}H_5$)pyridine, 600 MHz) of compound 2-D



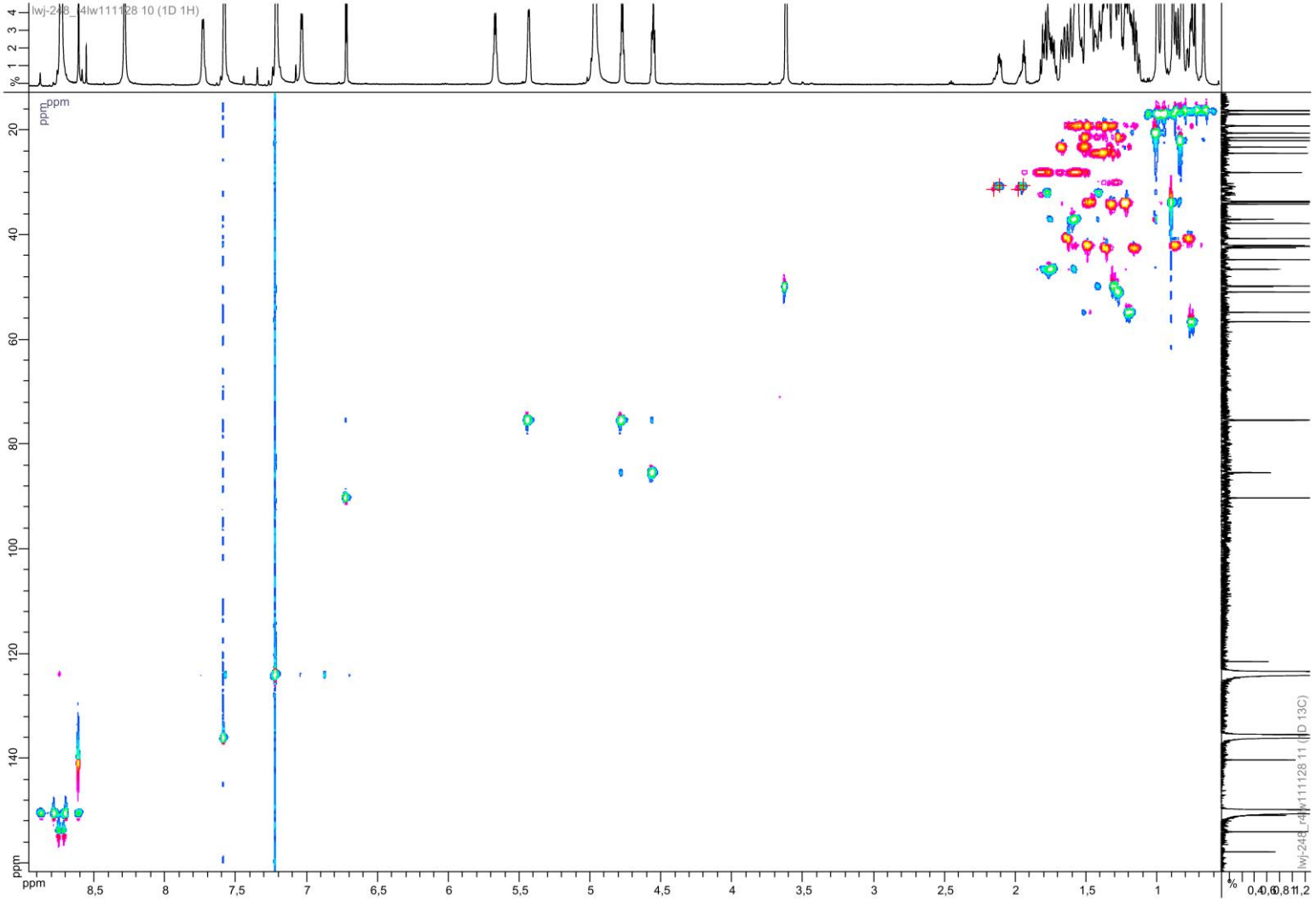
^{13}C-NMR spectrum (($^{2}H_5$)pyridine, 150 MHz) of compound 2D



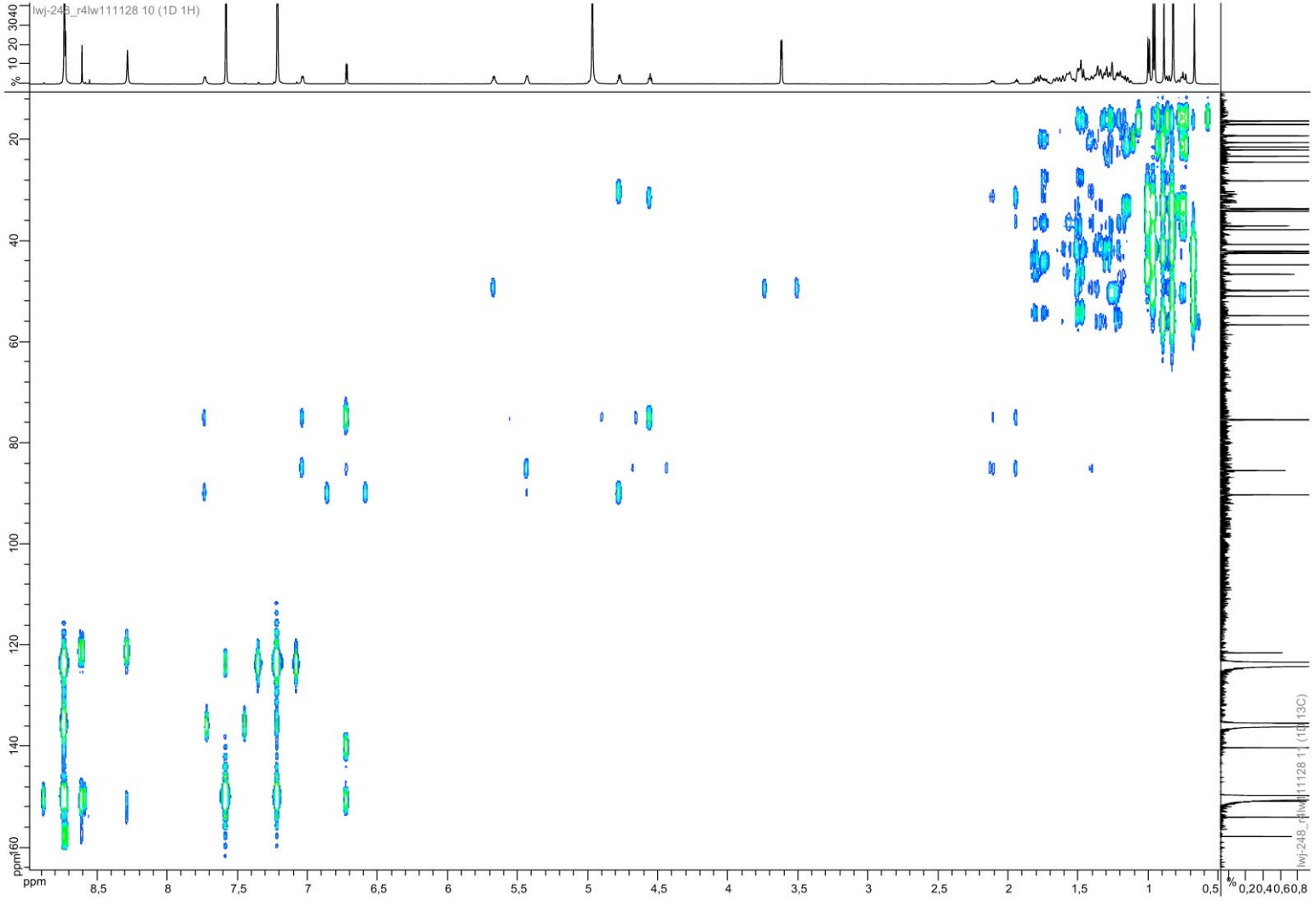
2D-NMR spectrum COSY $^{1}H/^{1}H$ ($(^{2}H_5)$pyridine, 600 MHz) of compound 2D



2D-NMR spectrum HSQC $^{1}H/^{13}C$ ($(^{2}H_5)$pyridine, 600 MHz) of compound 2D



2D-NMR spectrum HMBC $^{1}H/^{13}C$ ($(^{2}H_5)$pyridine, 600 MHz) of compound 2D



HRMS of **compound 2-D**

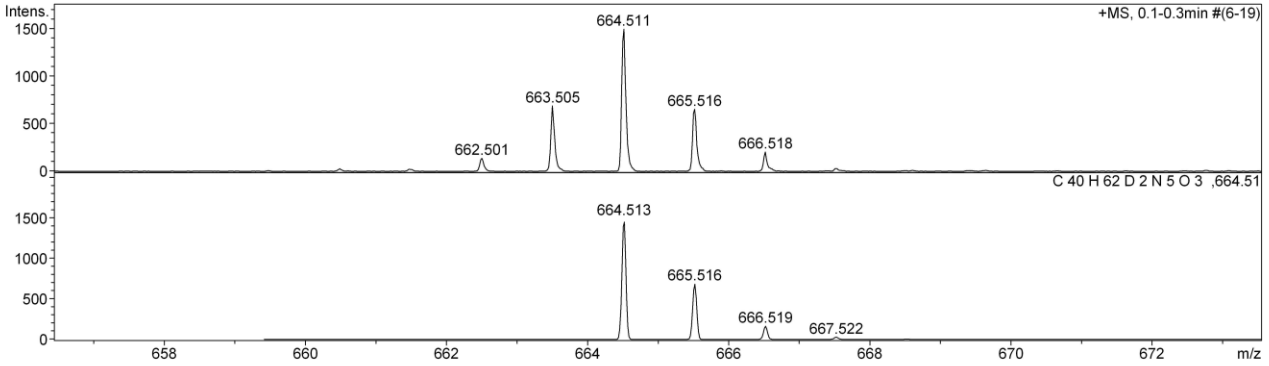
Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Service masse\O15636ML.d	Acquisition Date	10/17/2011 3:31:45 PM
Method	esi wide pos.m	Operator	Administrator
Sample Name	LWJ-248	Instrument	micrOTOF 66
Comment			

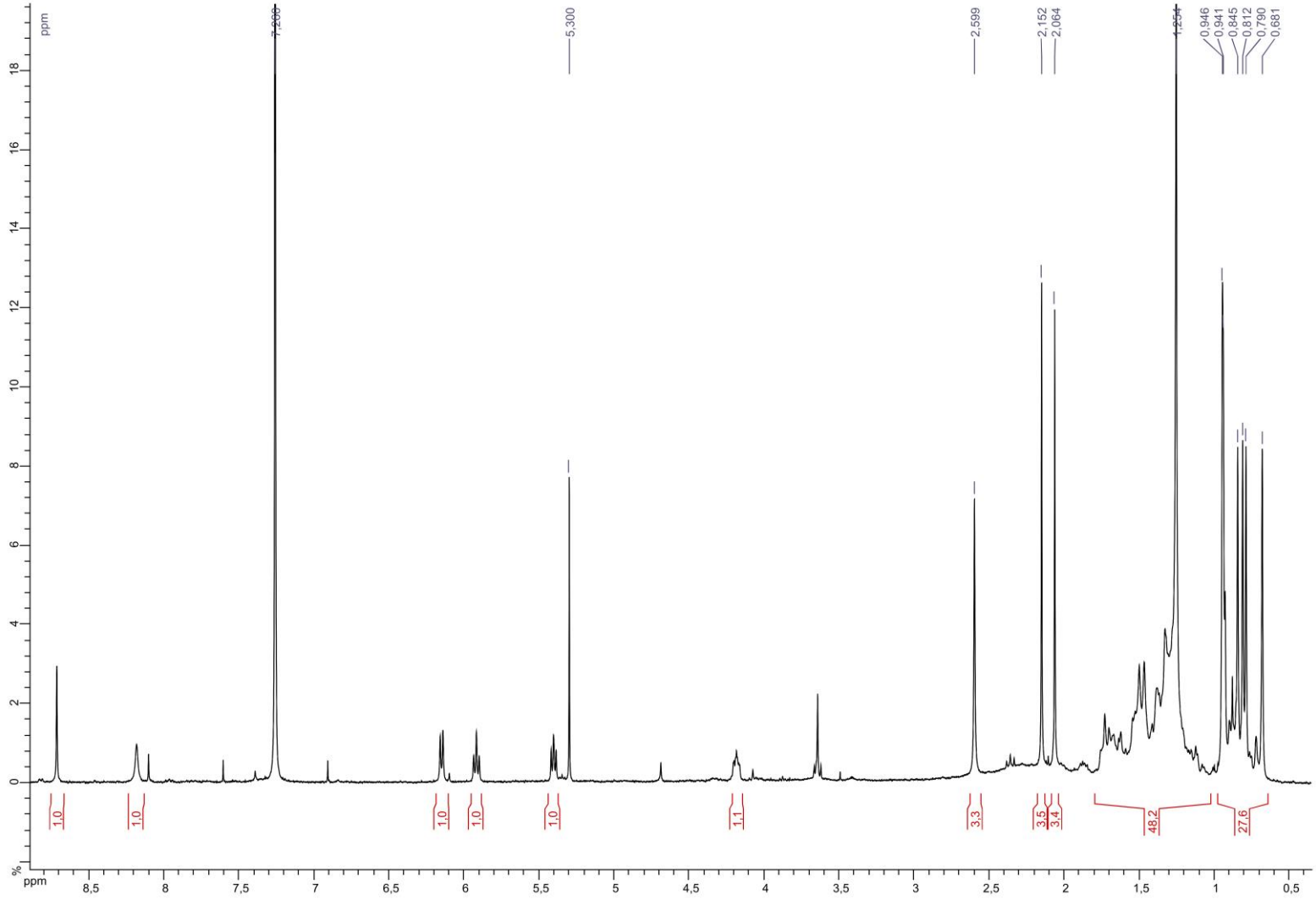
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Corrector Fill	79 V
Scan Range	n/a	Capillary Exit	80.0 V	Set Pulsar Pull	799 V
Scan Begin	50 m/z	Hexapole RF	300.0 V	Set Pulsar Push	799 V
Scan End	3000 m/z	Skimmer 1	50.0 V	Set Reflector	1700 V
		Hexapole 1	24.3 V	Set Flight Tube	8600 V
				Set Detector TOF	2050 V

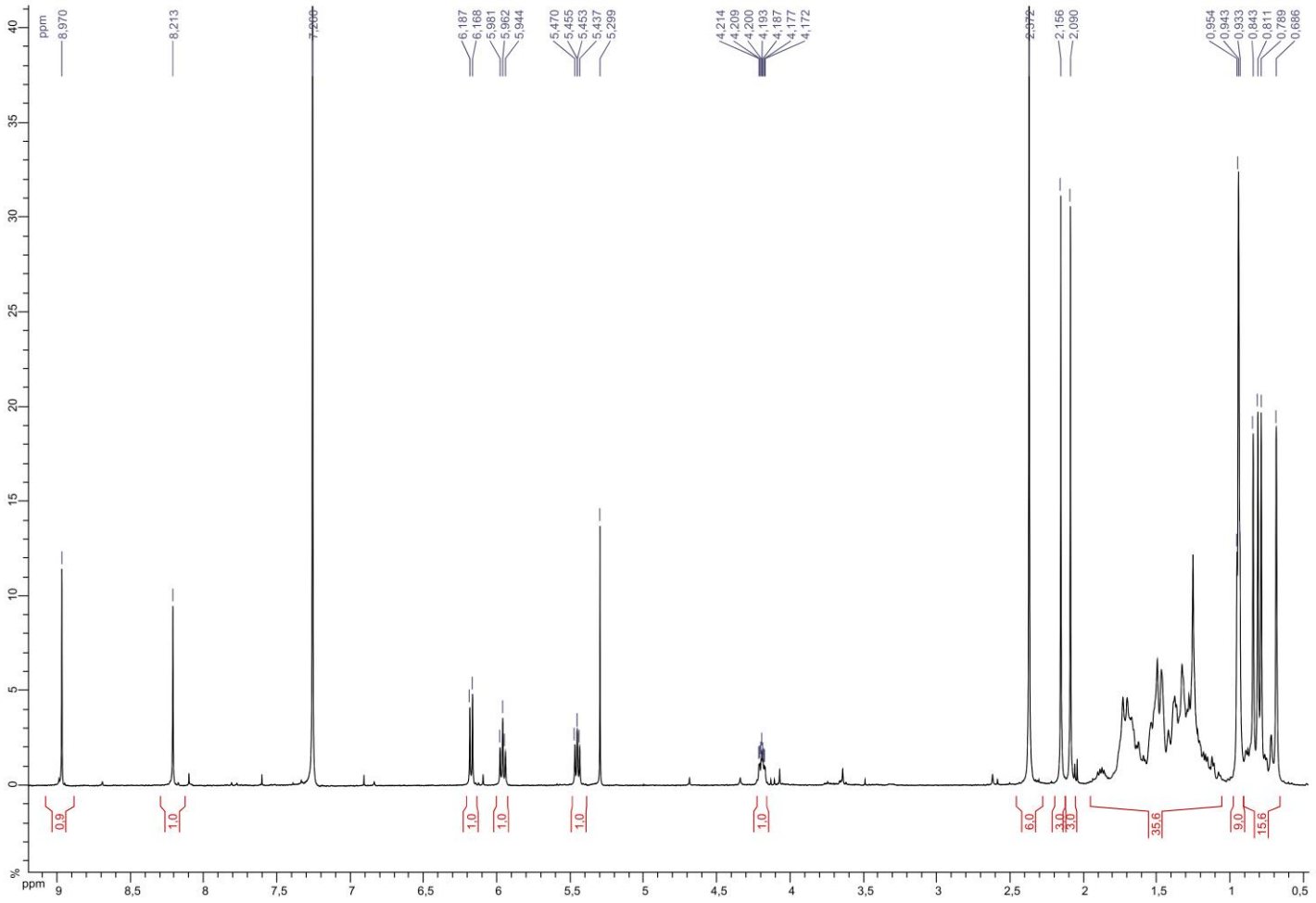


Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	rdb	N Rule	e⁻
C 40 H 62 D 2 N 5 O 3	0.02	664.513	2.15	1.46	11.50	ok	even
C 40 H 61 D 2 N 5 O 3	0.42	663.505	-4.89	-4.45	12.00	-	odd
C 40 H 60 D 2 N 5 O 3	0.67	662.497	-12.12	-11.40	12.50	ok	even

^{1}H-NMR spectrum ($C^{2}HCl_3$, 300 MHz) of compound 14b

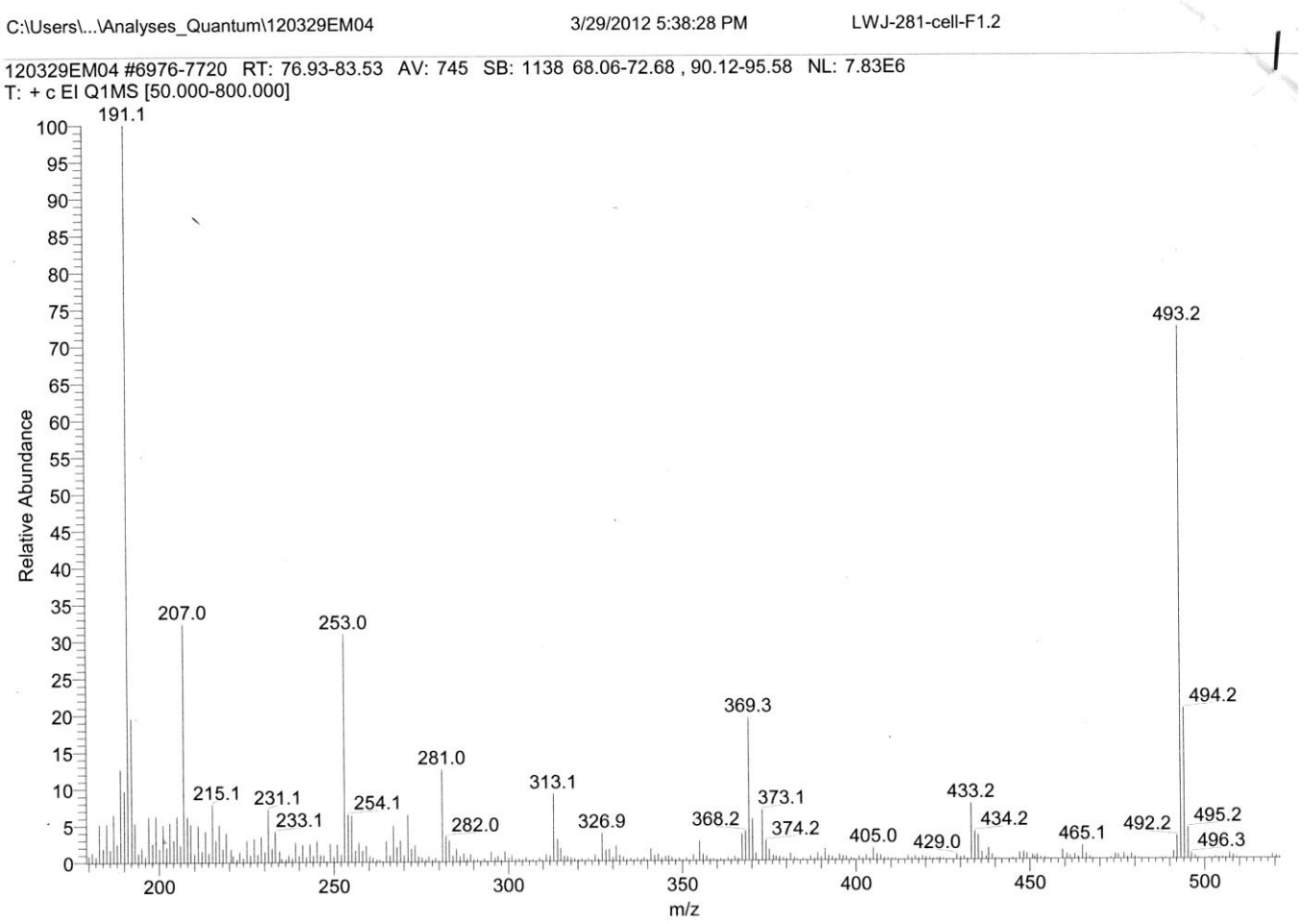


^{1}H-NMR spectrum ($C^{2}HCl_3$, 300 MHz) of compound 14c



4. EI-MS (direct inlet, positive mode 70 eV) of bacteriohopanetetrol tetraacetate

Natural abundance reference



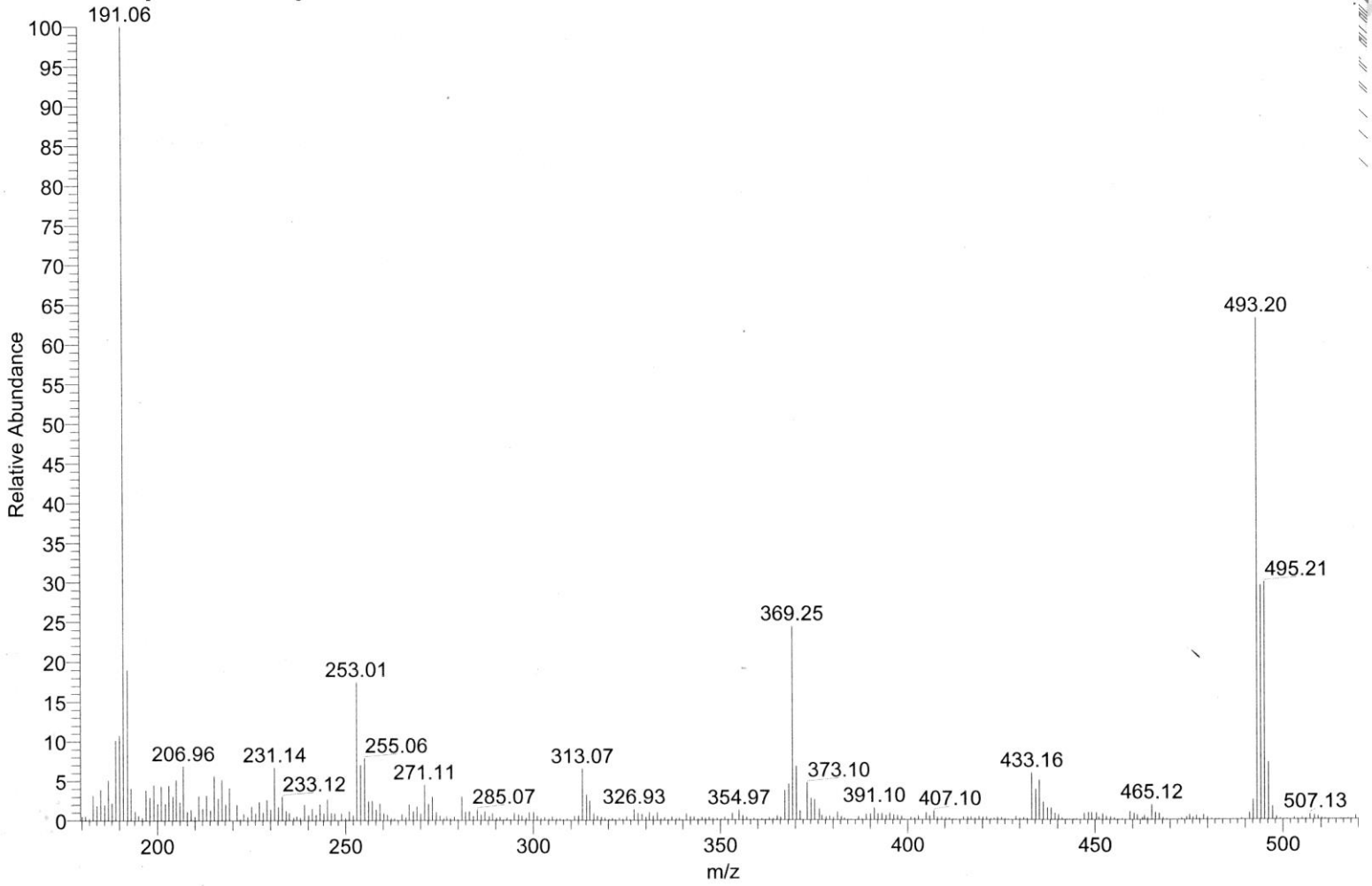
Analyses_Quantum\120329EM04 3/29/2012 5:38:28 PM LWJ-281-cell-F1.2
29EM04#6976-7720 RT: 76.93-83.53 AV: 745
1138 68.06-72.68 , 90.12-95.58
+ c EI Q1MS [50.000-800.000]
m/z= 179.3-521.4

m/z	Intensity	Relative	
183.0	405096.4	5.17	
185.0	409974.7	5.24	
187.0	509300.8	6.51	
189.0	990198.3	12.65	
190.1	759355.8	9.70	
191.1	7828777.6	100.00	
192.1	1528311.0	19.52	
193.0	417712.1	5.34	
197.0	484180.6	6.18	
199.1	492066.4	6.29	
201.1	396438.2	5.06	
203.1	423864.0	5.41	
204.1	234016.4	2.99	
205.0	489084.4	6.25	
207.0	2531971.8	32.34	
208.0	482210.0	6.16	
209.0	402050.0	5.14	
211.1	395592.0	5.05	
213.1	330651.5	4.22	
215.1	615895.6	7.87	
216.1	232660.2	2.97	
217.1	395629.8	5.05	
219.1	311172.2	3.97	
225.0	232829.3	2.97	
227.1	247872.4	3.17	
229.1	267762.8	3.42	
231.1	564890.5	7.22	
233.1	325222.9	4.15	
253.0	2422166.3	30.94	
254.1	507913.1	6.49	
255.1	492661.7	6.29	
267.0	385941.5	4.93	
271.1	501890.7	6.41	
281.0	982460.1	12.55	
282.0	266218.7	3.40	
313.1	719147.5	9.19	
314.2	235499.3	3.01	
326.9	297502.8	3.80	
367.2	283567.2	3.62	
368.2	315526.0	4.03	
369.3	1522436.0	19.45	
370.3	443654.2	5.67	321!
373.1	541324.0	6.91	
433.2	598920.9	7.65	
434.2	301269.1	3.85	
435.2	261683.7	3.34	
492.2	244548.0	3.12	
493.2	5655634.2	72.24	98.4%
494.2	1602273.0	20.47	
495.2	329238.2	4.21	5.83%

Incubation of ($^2H_2$)adenosylhopane: conditions A

C:\Users\...\Analyses_Quantum\120404EM05 4/4/2012 10:29:57 PM LWJ-281A-F1.2

120404EM05 #6868-7087 RT: 75.97-77.91 AV: 220 SB: 418 70.91-72.88 , 83.32-85.04 NL: 3.86E6
T: + c EI Q1MS [50.000-800.000]



...\Analyses_Quantum\120404EM05 4/4/2012 10:29:57 PM

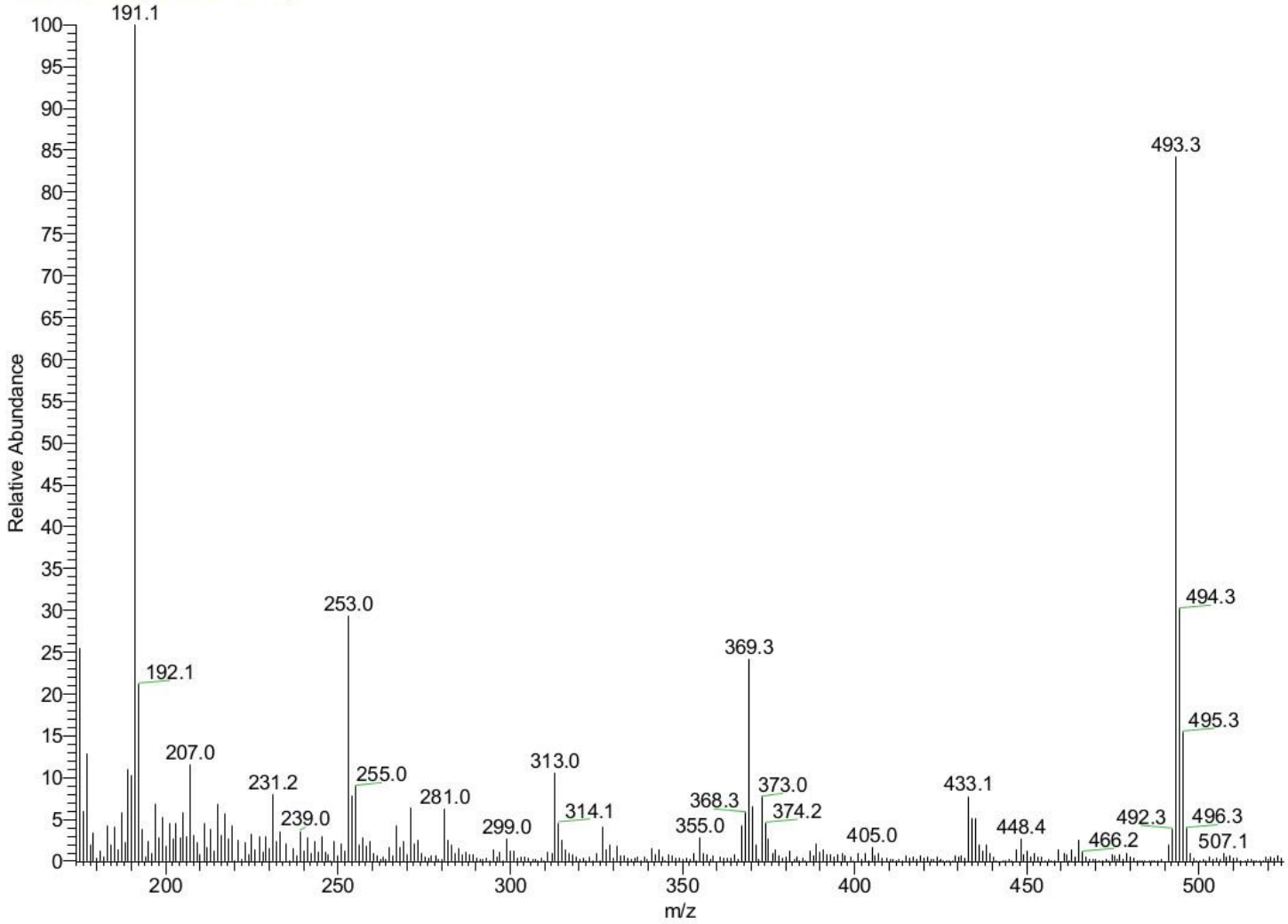
120404EM05#6868-7087 RT: 75.97-77.91 AV: 220
SB: 418 70.91-72.88 , 83.32-85.04
T: + c EI Q1MS [50.000-800.000]
m/z= 179.64-521.64

m/z	Intensity	Relative
182.98	121844.7	3.15
185.02	150598.2	3.90
187.03	197329.3	5.11
189.03	391691.2	10.14
190.05	415437.4	10.76
191.06	3862304.7	100.00
192.12	733186.0	18.98
193.01	156381.5	4.05
197.00	145102.6	3.76
198.04	111702.1	2.89
199.06	172943.4	4.48
201.07	166906.7	4.32
203.06	170900.7	4.42
204.14	115887.5	3.00
205.04	198659.5	5.14
206.96	264446.0	6.85
211.07	119526.3	3.09
213.08	122294.6	3.17
215.11	217053.2	5.62
216.14	106863.6	2.77
217.12	199392.4	5.16
219.13	158244.8	4.10
229.10	101752.0	2.63
231.14	258733.7	6.70
233.12	116797.3	3.02
245.12	103609.9	2.68
253.01	672766.6	17.42
254.05	270430.4	7.00
255.06	303467.7	7.86
257.11	95341.9	2.47
271.11	173836.5	4.50
273.16	115711.5	3.00
280.97	115480.8	2.99
313.07	251421.5	6.51
314.17	124677.7	3.23
367.21	145515.1	3.77
368.23	176537.3	4.57
369.25	942725.7	24.41
370.29	264448.9	6.85
373.10	182665.8	4.73
374.15	106861.6	2.77
375.10	100978.7	2.61
433.16	229570.4	5.94
434.24	149427.6	3.87
435.19	195357.1	5.06
492.13	98721.0	2.56
493.20	2442413.3	63.24
494.21	1142682.3	29.59
495.21	1158490.4	29.99
496.26	281878.6	7.30

Incubation of ($^2H_2$)adenosylhopane: conditions B

C:\Users\...\Desktop\TSQ FTop\120127EM02 1/27/2012 11:32:46 AM LWJ-270A-F1.2

120127EM02 #6579-6673 RT: 74.07-75.06 AV: 95 SB: 164 71.02-72.73 NL: 9.56E6
T: + c EI Q1MS [50.000-800.000]

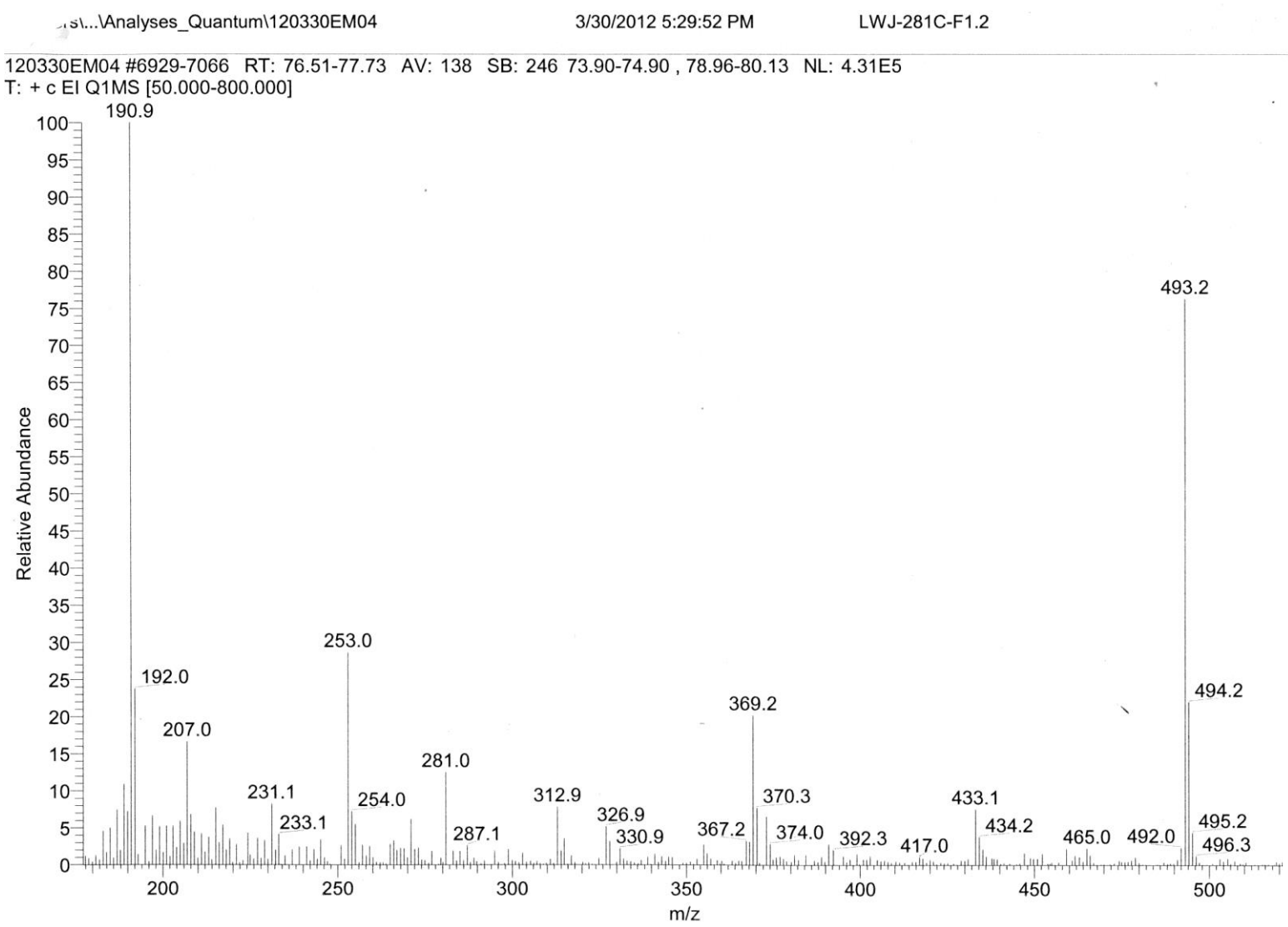


C:\Users\...\Desktop\TSQ FTop\120127EM02 1/27/2012 11

120127EM02#6579-6673 RT: 74.07-75.06 AV: 95
SB: 164 71.02-72.73
T: + c EI Q1MS [50.000-800.000]
m/z= 174.0-524.7

m/z	Intensity	Relative
175.1	2435736.4	25.47
176.1	570225.8	5.96
177.0	1221122.3	12.77
178.9	321294.4	3.36
183.0	406207.2	4.25
185.1	393434.2	4.11
187.1	558780.2	5.84
189.1	1048256.7	10.96
190.1	980016.2	10.25
191.1	9564992.6	100.00
192.1	2020285.3	21.12
193.0	366283.6	3.83
197.0	644375.7	6.74
199.1	498538.8	5.21
201.1	437492.0	4.57
203.1	433497.3	4.53
205.0	553070.7	5.78
207.0	1104844.0	11.55
211.1	430856.6	4.50
213.1	357413.2	3.74
215.1	644765.1	6.74
217.1	539946.9	5.65
219.2	399781.4	4.18
225.0	306272.6	3.20
231.2	761618.2	7.96
233.2	336389.6	3.52
239.0	339631.0	3.55
253.0	2799117.4	29.26
254.0	748096.0	7.82
255.0	851153.0	8.90
267.0	406672.0	4.25
271.1	606440.1	6.34
281.0	590815.7	6.18
313.0	1004672.8	10.50
314.1	434774.9	4.55
327.0	388982.9	4.07
367.3	402937.1	4.21
368.3	549525.2	5.75
369.3	2303511.1	24.08
370.5	624907.6	6.53
373.0	727558.2	7.61
374.2	430142.5	4.50
433.1	737625.8	7.71
434.2	483659.7	5.06
435.2	487578.0	5.10
492.3	362644.3	3.79
493.3	8056882.6	84.23
494.3	2880059.4	30.11
495.3	1471609.6	15.39
496.3	369888.7	3.87

Incubation of ($^2H_2$)adenosylhopane: conditions C



C:\Users\...\Analyses_Quantum\120330EM04 3/30/2012 5:29:52 PM

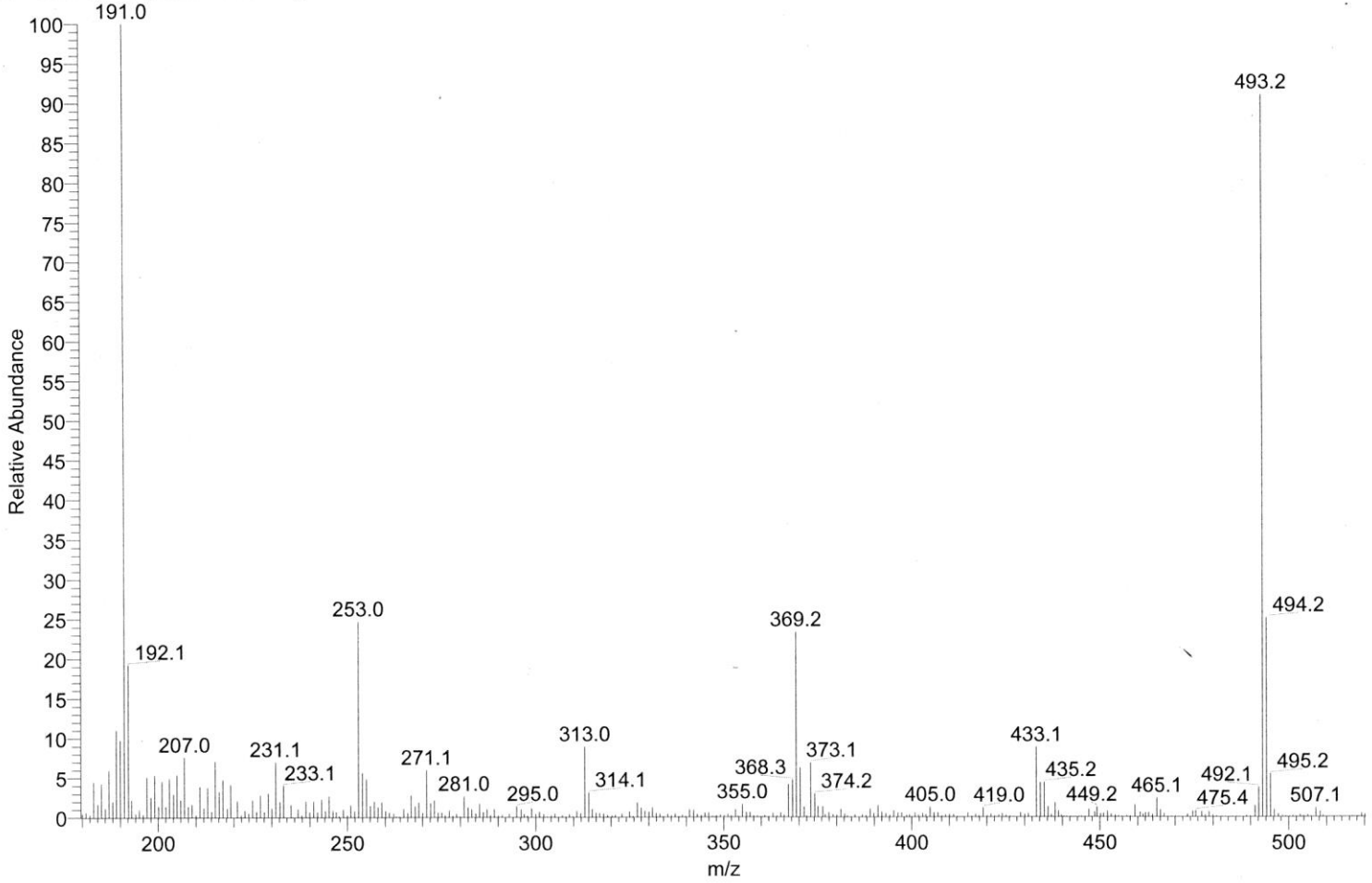
120330EM04#6929-7066 RT: 76.51-77.73 AV: 138
SB: 246 73.90-74.90 , 78.96-80.13
T: + c EI Q1MS [50.000-800.000]
m/z= 177.3-521.5

m/z	Intensity	Relative
183.0	19469.6	4.52
185.0	21511.3	4.99
187.0	32137.9	7.46
188.9	46977.0	10.91
189.9	31224.0	7.25
190.9	430741.0	100.00
192.0	102520.1	23.80
194.9	22753.1	5.28
197.0	28698.1	6.66
199.0	22465.1	5.22
201.0	22654.1	5.26
202.9	22652.2	5.26
204.9	25550.2	5.93
205.9	12686.2	2.95
207.0	71899.3	16.69
208.0	29347.6	6.81
209.0	19157.0	4.45
211.0	18245.8	4.24
213.0	16075.0	3.73
215.1	33280.4	7.73
216.0	12885.6	2.99
217.0	23484.5	5.45
219.0	15181.0	3.52
224.3	18553.0	4.31
227.0	15593.8	3.62
229.1	14374.3	3.34
231.1	35568.3	8.26
233.1	17731.4	4.12
245.0	14719.1	3.42
253.0	123507.1	28.67
254.0	31184.0	7.24
255.0	23755.3	5.51
264.9	12106.3	2.81
266.0	14188.2	3.29
271.0	26826.4	6.23
281.0	53989.1	12.53
312.9	33733.2	7.83
314.9	15349.4	3.56
326.9	22281.6	5.17
328.0	13628.4	3.16
367.2	13851.0	3.22
368.2	13482.5	3.13
369.2	87044.4	20.21
370.3	33233.2	7.72
373.0	27938.0	6.49
433.1	32252.9	7.49
434.2	16166.4	3.75
493.2	328645.8	76.30
494.2	94554.9	21.95
495.2	18689.9	4.34

Incubation of ($^2H_2$)adenosylhopane: conditions D

...\Analyses_Quantum\120402EM03 4/3/2012 12:24:45 PM LWJ-281D-F1.2

120402EM03 #6904-7125 RT: 76.29-78.25 AV: 222 SB: 276 73.07-74.23 , 80.70-81.97 NL: 1.76E6
T: + c EI Q1MS [50.000-800.000]



C:\Users\...\Analyses_Quantum\120402EM03 4/3/2012 12:24:45 PM

120402EM03#6904-7125 RT: 76.29-78.25 AV: 222
SB: 276 73.07-74.23 , 80.70-81.97
T: + c EI Q1MS [50.000-800.000]
m/z= 179.6-520.3

m/z	Intensity	Relative
183.0	78469.7	4.45
185.0	74143.9	4.21
187.0	104338.0	5.92
189.0	194768.6	11.06
190.0	171815.3	9.75
191.0	1761580.4	100.00
192.1	339319.5	19.26
197.0	89784.5	5.10
198.0	44274.7	2.51
199.0	93751.3	5.32
201.0	79805.1	4.53
203.0	86773.3	4.93
204.1	51802.7	2.94
205.0	94405.0	5.36
207.0	133403.4	7.57
211.0	68466.0	3.89
213.1	65312.5	3.71
215.1	124764.0	7.08
216.1	56640.0	3.22
217.1	83534.6	4.74
219.1	72297.0	4.10
225.0	38435.1	2.18
227.1	48734.3	2.77
229.1	53504.3	3.04
231.1	123151.5	6.99
233.1	70444.8	4.00
243.1	39976.0	2.27
245.1	46669.6	2.65
253.0	435221.6	24.71
254.0	98948.8	5.62
255.0	84798.9	4.81
266.9	48858.8	2.77
271.1	105099.5	5.97
281.0	46629.8	2.65
313.0	157111.8	8.92
314.1	55119.6	3.13
367.2	72412.1	4.11
368.3	82561.8	4.69
369.2	411669.8	23.37
370.3	109552.5	6.22
373.1	121174.6	6.88
374.2	51734.7	2.94
433.1	154945.8	8.80
434.2	76577.8	4.35
435.2	77054.1	4.37
465.1	41420.1	2.35
492.1	66126.6	3.75
493.2	1602354.5	90.96
494.2	442805.5	25.14
495.2	94941.6	5.39

5. References

1 (a) R.S. Ranganathan, G.H. Jones, J.G. Moffatt, *J. Org. Chem*., 1974, **39**, 290; (b) S. Eppacher, P.K. Bhardwaj, B. Bernet, J.L.B. Gala, T. Knopfel and A. Vasella, *Helv. Chim. Acta*, 2004, **87**, 2969.

2 Andrei, D. and Wnuk, S. F., *Org. Lett*., 2006, **8**, 5093.

3 L.W. McLaughlin, N. Piel and T. Hellmann, *Synthesis-Stuttgart*, 1985, 322.

4 A. Kjer, D. Kjer and T. Skrydstrup, *Tetrahedron*, 1986, **42**, 1439.