Synthesis of fructone

Supplementary Material

<u>Experiment notes</u>

The aims of this experiment include on the theory side an understanding that in molecules with more than one functional group there could be a need to selectively protect a functional group. For ketones this can be achieved by the formation of acetals. The second concept is the influencing of equilibria by selectively removing one of more products or increasing one of more starting materials. The removal of water as the lowest boiling component in the reaction mixture seems logic to students and this experiment also introduces azeotropic mixtures. Theoretical aspects and mechanisms are extensively discussed by Clayden *et al.*[a]

From a practical side these concepts are involved: Dean and Stark traps, liquid-liquid extraction, rotary evaporation (distillation under reduced pressure). The Dean and Stark trap as well as liquid-liquid extraction using a separating funnel necessitate that the layering of immiscible solvents is governed by their densities.

Depending on prior knowledge and observation skills students could figure out that the final wash with saturated brine clears the organic layer to a certain degree by removing some water. Turbidity can in some cases also be seen in the Dean and Stark trap as the two phases separate. The final step in removing water from an organic phase is drying with anhydrous $MgSO_4$ or Na_2SO_4 – thus allowing for a discussion of different ways of doing so, and when these are most suitable.

The experiment offers additionally an opportunity to raise awareness of solvent purity and ingress of water to the reaction mixture. PTSA is used as monohydrate and toluene and ethylene glycol might not be anhydrous – water would be removed during the reaction and collected in the trap. Often an amount higher than stoichiometrically expected will be observed for these reasons.

The reaction can be conducted for just 30 min or 90-120 min. However, in most cases sufficient amounts of product are produced within 45-60 min. The yield for this experiment is calculated by the students and is often very high, sometimes students' calculations afford more than is theoretically possible. The main reason is residual toluene after evaporation (the endpoint is sometimes difficult to spot for more inexperienced students). Ideally the evaporation takes place at 10 mbar with a bath temperature of 80 °C under careful observation of the distillation. Once it slows down significantly or stops the evaporation should be interrupted/stopped. However, this offers an opportunity to discuss possible explanations for this result. A good pointer for solvent contamination of the product is the IR spectrum: C-H stretches of the aromatic ring of toluene (likely solvent) or O-H stretches from ethylene glycol or water (rare) can be clearly distinguished from the product's absorptions.

The total removal of toluene also depends on the quality of the vacuum. If a high end vacuum (or rotary evaporation) is not possible this experiment can be extended by a second lab session which could be dedicated to a normal / standard distillation as opposed to rotary evaporation. The product is stable at elevated temperatures and a distillation was successfully performed previously.

After removal of all toluene typical yields range between 40 % and 80 %.

Optional features

The actual yield is not important as the product is used after purity is established by ^{1}H NMR in another experiment (see experiment 13.5 *"Synthesis of allylic esters by reduction of fructone followed by Wittig olefination"*). If raising the level of study to medium is desired, the ^{1}H NMR and the ^{13}C NMR of the product can be discussed, possibly even in conjunction with the ^{1}H NMR of the starting material, although ethyl acetoacetate does not exhibit as nice a spectrum with clearly distinguishable keto-enol-tautomers as other analogues (*e.g.* isopropyl acetoacetate or ethyl 4,4,4-trifluoro-3-oxo-butanoate).

Another aspect that could be discussed during lab classes circles around the partition coefficient, depending on physical chemistry background. Calculations for given equilibrium constants (solubility of a substrate in aqueous and organic phase, respectively) would show how often an extraction step should be performed to effectively remove/collect most of the desired by-product/product.

Spectra

Figure SM 4.1.2.1.1: IR (ATR) of ethyl acetoacetate
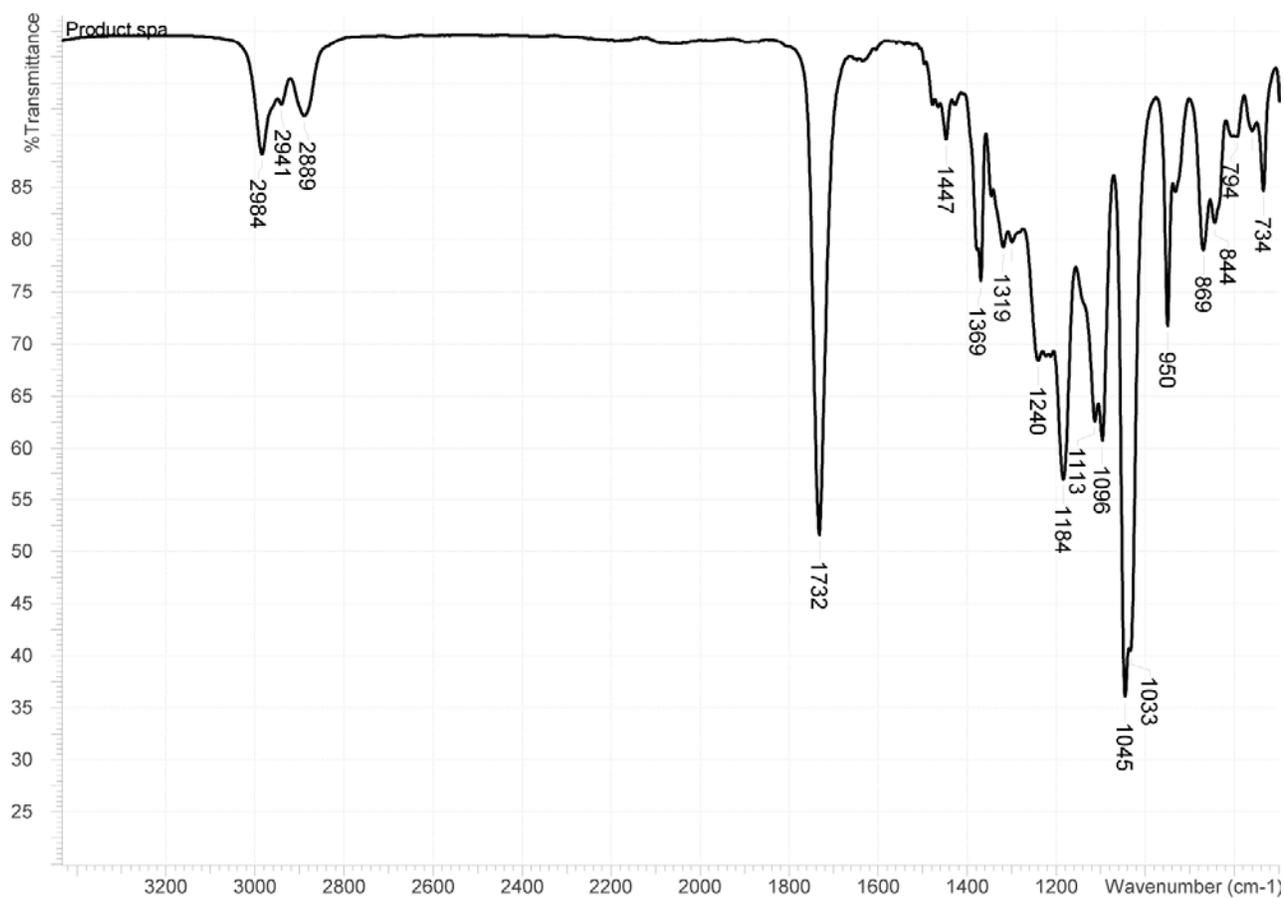
Figure SM 4.1.2.1.2: IR (ATR) of ethyl 2-(2-methyl-1,3-dioxolan-2-yl)acetate
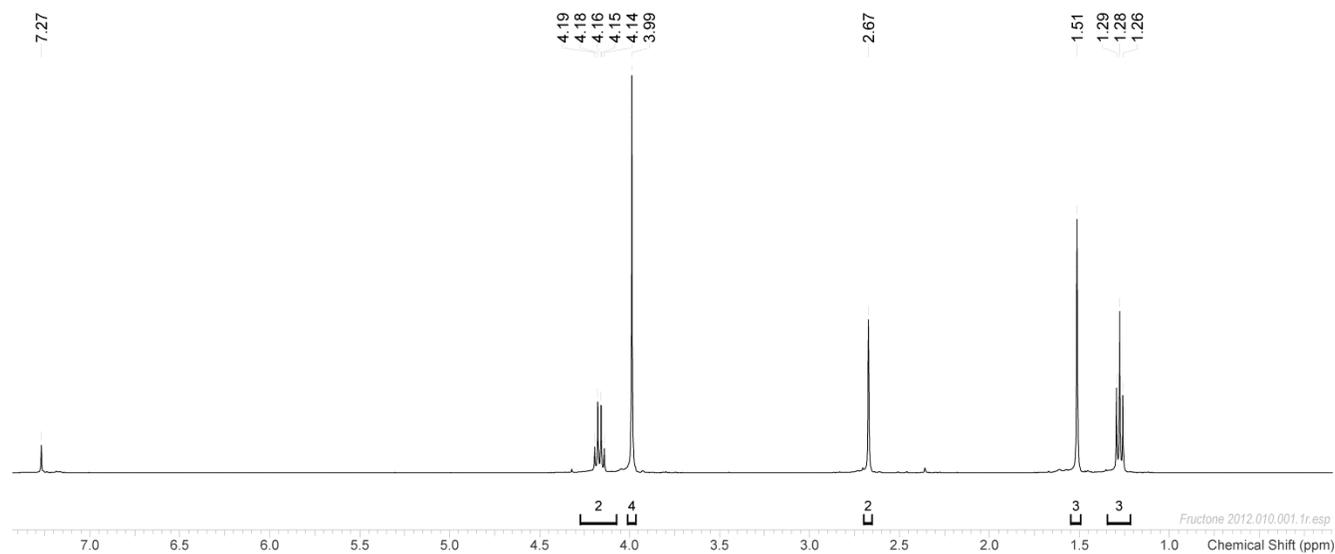
Figure SM 4.1.2.1.3: ^{1}H NMR (400 MHz, CDCl$_3$) of ethyl 2-(2-methyl-1,3-dioxolane-2-yl)acetate

169.46   107.63   77.32 77.00 76.69   64.78 60.53   44.26   24.47   14.18
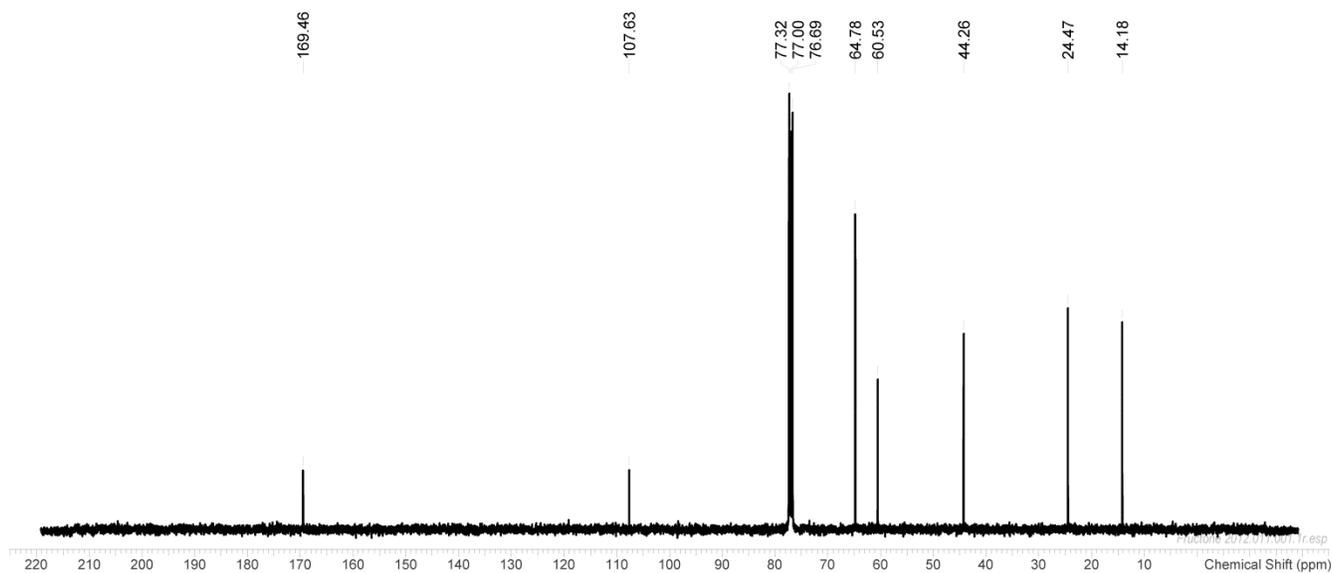
Figure SM 4.1.2.1.4: ^{13}C NMR (100 MHz, CDCl$_3$) of ethyl 2-(2-methyl-1,3-dioxolane-2-yl)acetate

References

[a] J. Clayden, N. Greeves, S. Warren, *Organic Chemistry*, OUP, Oxford, 2nd ed., 2012, 222-228.

Synthesis of flavone
Supplementary Material

This synthesis was first introduced in the 1990's to intermediate organic chemistry students as a short project involving bibliographic research and experimental work, and it was later adapted to classroom for 2nd year undergraduate students of intermediate organic chemistry. No more than 8 students working in pairs have done this work in the same classroom. They could study several chemical transformations concerning carbonyl group. It was found that base-catalyzed rearrangement known as Baker-Venkataraman rearrangement can also be named as Fries rearrangement[1]. The mechanisms for flavone synthesis can be found in literature[2]. Experimental procedure can be easily performed by them in only 3 steps of at least 3 hours each. They have the opportunity to learn how to work in anhydrous conditions.

Additional notes on the preparation of *o*-benzoyloxyacetophenone:

In order to prevent hydrolysis of benzoyl chloride, some precautions should be made: pyridine used must be previously dried and redistilled and lab material needed for this synthesis should remain in the oven and only removed prior to its use (**Figure SM 4.1.2.2.1**).

Figure SM 4.1.2.2.1 – Reaction set-up apparatus for *o*-benzoyloxyacetophenone

The average yield of *o*-benzoyloxyacetophenone is 75-80% and the experimental melting point varies between 86 and 90ºC (87-88ºC[1], 88ºC[3]).

Additional notes on the preparation of *o*-hydroxydibenzoylmethane:

In this step it is also necessary to protect reaction mixture from moisture, and thus the recommendations for the preparation of *o*-benzoyloxyacetophenone must be followed. The mortar is pre-heated in the oven to 100ºC, but it is removed some time before use. When the students quickly grind KOH, it is warm and they avoid absorb moisture. When acetic acid is added with stirring, the reaction mixture changes its color from yellow to green before it returns to yellow again.

Figure SM 4.1.2.2.2 – Reaction set-up apparatus for *o*-hydroxydibenzoylmethane

The final product can be recrystallized from ethanol, although it is pure enough to be used on the next step. Yield (average): 85-89%. Experimental melting point varies between 114 and 120°C (120-121°C[1], 121°C[2]).

Additional notes on the preparation of flavone:

The crude flavone obtained from filtration should be washed with water to remove any residual acetic acid. If not done properly it leads to a higher final weight and consequently to a false high yield. The recrystallization solvent (petroleum ether 60-80°C) can be replaced by acetone/water dissolving the product in the least amount possible of hot acetone and add cold water to precipitate.

Figure SM 4.1.2.2.3 – Copper funnel (previously heated by flame) for hot filtration during flavone recrystallization from petroleum ether and vacuum filtration set-up

The average yield is 85-90% and overall yield for the 3-step flavone synthesis is 55%. Experimental melting points were determined for the crude (between 92 and 97°C) and for the recrystallized product (between 98 and 100°C). For the latter, the melting point range was never higher than 2°C (98-99°C[1], 98°C[2] or 100°C[4]).

IR spectra:
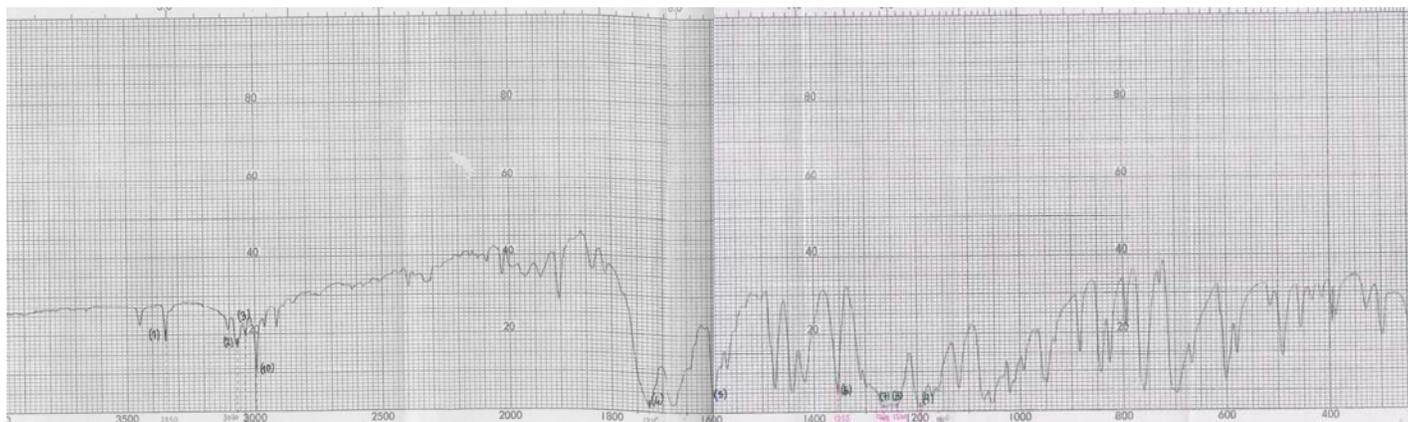
Figure SM 4.1.2.2.4 - IR (KBr) of *o*-benzoyloxyacetophenone

IR spectrum of *o*-benzoyloxyacetophenone can be found online at the National Institute of Standards and Technology database[5].
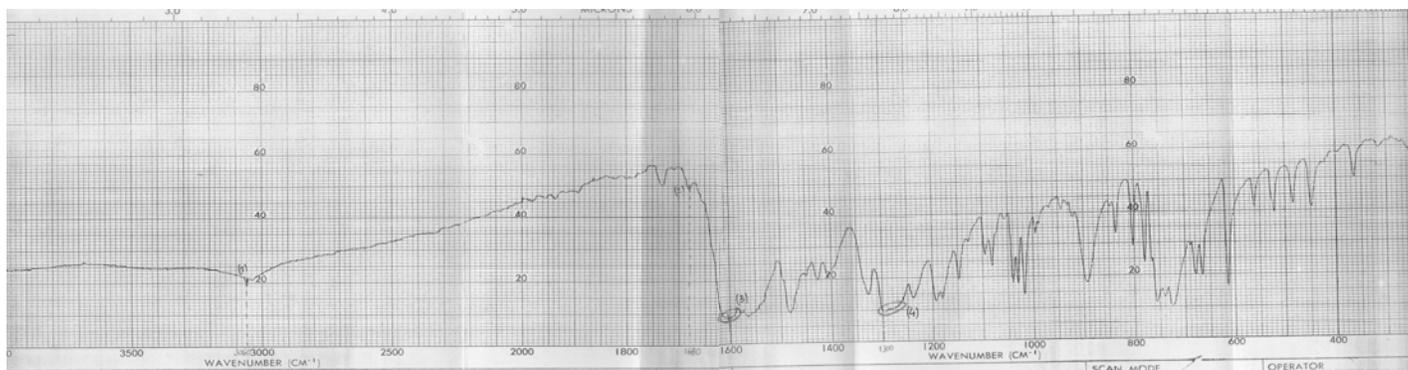
Figure SM 4.1.2.2.5 - IR (KBr) of *o*-hydroxydibenzoylmethane
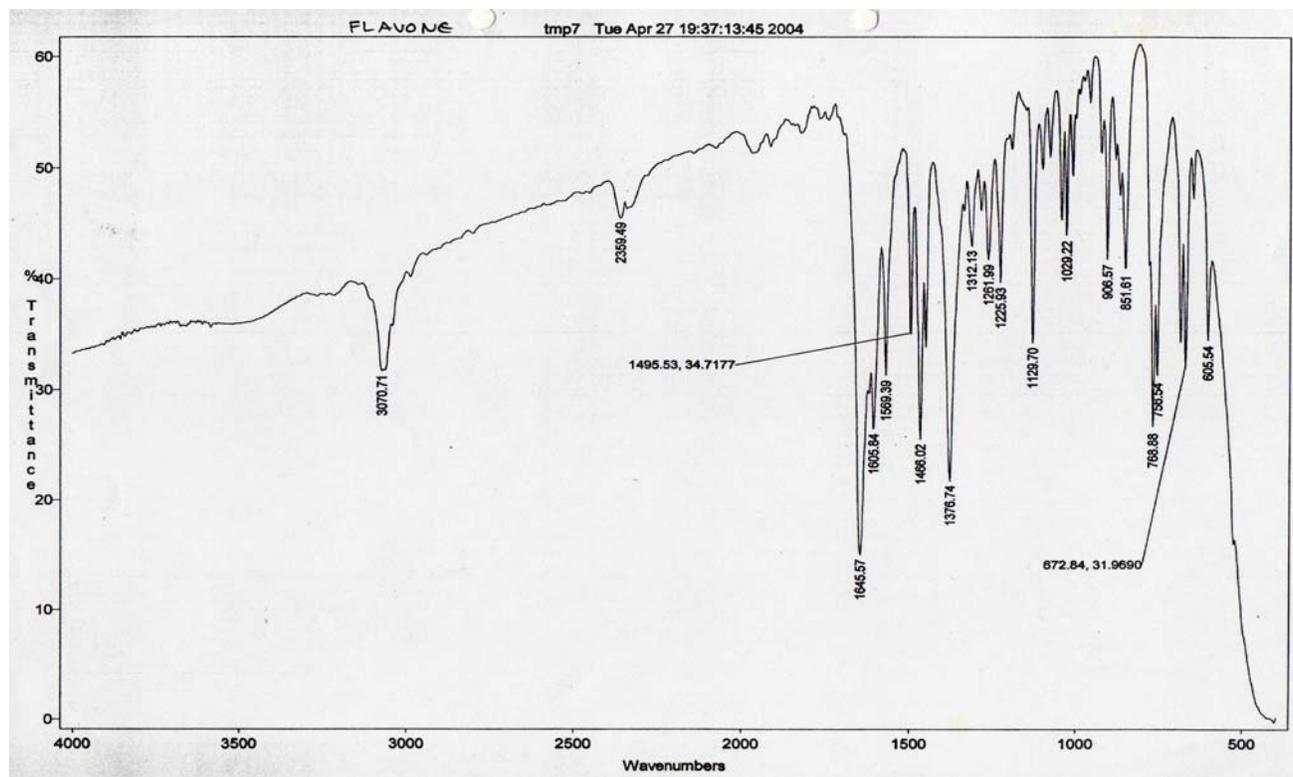
Figure SM 4.1.2.2.6 - IR (KBr) of flavone

IR spectrum of flavone can be found in literature[6] and in Spectral Database for Organic Compounds, SDBS[7], under number 2359. Students easily identify strong absorption bands near 1700-1650 cm^{-1} due to the C=O stretching vibrations.

NMR spectra:

^{1}H NMR data of flavone[6] and intermediates can be found in literature as well other spectroscopic information[1]. Flavone ^{1}H NMR spectrum was obtained with student's samples where a signal with a chemical shift of 2.05 ppm is a impurity (acetone). Students easily identify in **Figure SM 4.1.2.2.7** the vinylic proton at 6.95 ppm. In **Figure SM 4.1.2.2.8**, is visible the peak solvent (chloroform) at 7.26 ppm.
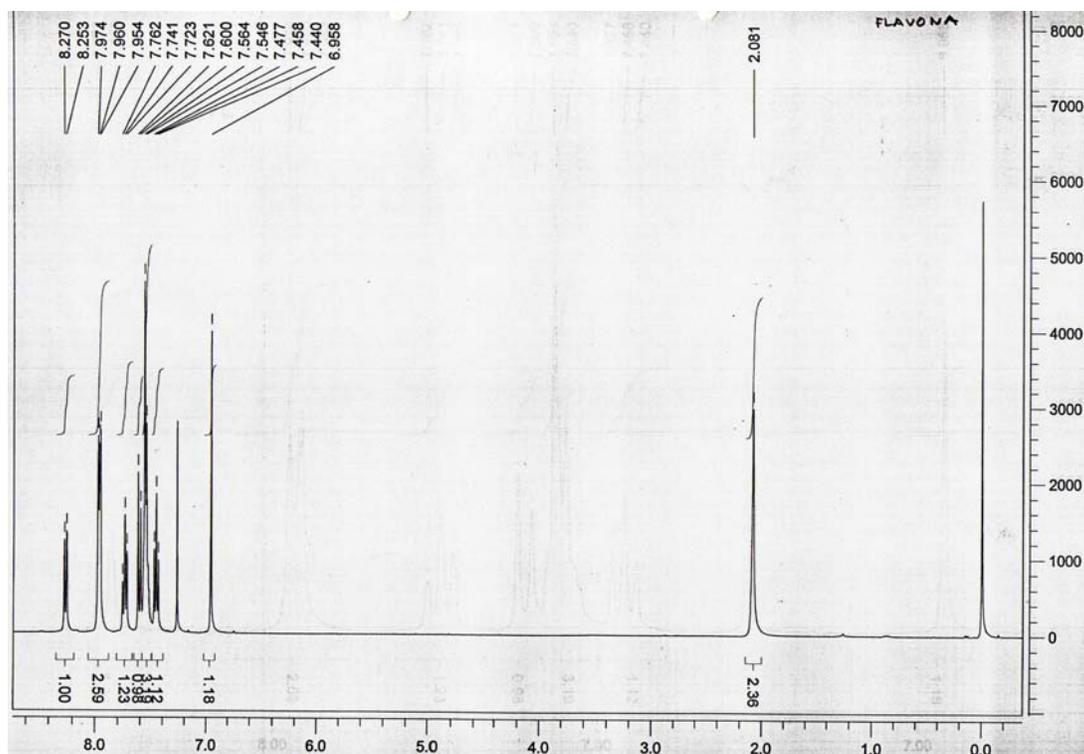
Figure SM 4.1.2.2.7 - ¹H NMR (CDCl₃) of flavone
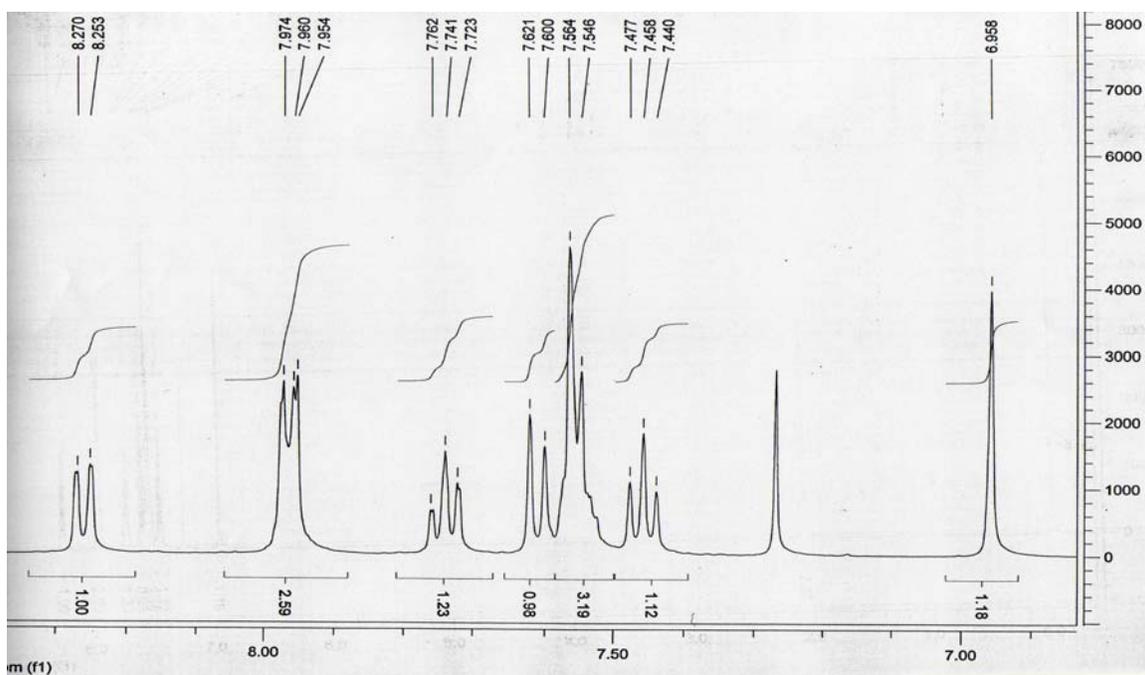
Figure SM 4.1.2.2.8 - ¹H NMR (CDCl₃) of flavone (range 6.8-8.3 ppm)

[1] Arh-Hwang Chen, Wei-Bao Kuo and Chia-Wen Chen, *Journal of the Chinese Chemical Society*, 2003, **50**, 123.

[2] A.I. Vogel, *Vogel's Textbook of Practical Organic Chemistry*, Longman Scientific and Technical, 5th ed. 1989, 1190-1195.

[3] *CRC Handbook of Chemistry and Physics*, R. Weast, CRC Press, 69th ed. (1st Student ed.), 1988, C-127.

[4] *CRC Handbook of Chemistry and Physics*, R. Weast, CRC Press, 69th ed. (1st Student ed.), 1988, C-275.

[5] http://webbook.nist.gov/cgi/cbook.cgi?ID=C4010337&Type=IR-SPEC&Index=1#IR-SPEC, access in July 2015.

[6] L. M. Harwood, C. J. Moody, J. M. Percy, *Experimental Organic Chemistry*, Blackwell Science, 2nd ed. 1999, 626.

[7] http://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi, access in July 2015.

Synthesis and characterisation of H$_2$salen: an introduction to 1D and 2D

NMR spectroscopy

Supplementary Material

The aim of this experiment is to integrate NMR spectroscopy with synthesis procedures, providing students with an opportunity to have a real-life experience in characterising the structure of an expected product after a synthesis experiment, using 1D and 2D NMR techniques.

This experiment is part of an extended coordination chemistry lab mini-project which has been given to 2nd or 3rd year Chemistry students from DQB-FCUL and included the preparation and study of a cobalt complex, based on published procedures.[1,2,3]

The preparative chemistry involved is quite simple, while the NMR analysis requires students to be familiar with ^{1}H and ^{13}C NMR spectroscopy. The set of NMR spectra can be programmed to run overnight so that the spectra can be analysed on the following session. During the *prelab* lecture, students review 1D proton and carbon-13 spectroscopy and solve NMR problems involving the analysis of chemical shifts, spin-spin couplings and intensities in order to get structure-related information for the protons and carbons of the samples. Following the review, students can be given a short tutorial on 2D data interpretation, even if they have not been previously introduced to NMR correlation spectroscopy.

Owing to time constraints on some undergraduate laboratories, it can be a challenge to integrate NMR with synthesis procedures and frequently only the ^{1}H NMR spectrum is recorded. Although the synthesis of H$_2$salen can be confirmed by ^{1}H NMR, students should be trained on how to use different NMR techniques to make proton and carbon assignments. As high field FT-NMR has been included in the undergraduate Chemistry and Biochemistry curricula in a traditional Spectroscopy course, some selected compounds synthesised in organic or inorganic lab courses can be studied there in more

detail. The structure of H_2salen has suitable features for teaching 1D and 2D NMR in a short introductory course, providing students with practice on the acquisition, processing and interpretation of several 1D and 2D NMR spectra. Alternatively, copies of the spectra can be provided to the students to be analysed in the classroom or as a homework exercise. This has been an option when acquisition and processing are not available for a large number of students. As students become more familiar with the use of different techniques, they will develop the skills to analyse more complicated structures and to identify unknowns.

Although published methods for the synthesis of H_2salen involve reflux or heating,[1,3,4] most of the students who performed this experiment in our lab courses observed precipitation of the product immediately after addition of all the reagents at room temperature (Yields: 60-100 %). However, if this is not the case, heating the reaction mixture (in a sand or water bath, for safety reasons) may be necessary.

This experiment is flexible, allowing the instructor to determine the level of coverage and depth, depending on the student body, resources and time. Infrared and electronic spectra can be recorded and the melting point measured. Furthermore, the synthesis and characterization can be extended to other salen-type ligands.[4]

It should be noted that the NMR spectra of the compound could change significantly (chemical shifts and proton splitting) if different deuterated solvents and magnetic fields are used. Concentration of the sample can also influence the resolution, especially in proton spectra.

Some of the samples collected from students showed a rather broad resonance for the OH proton in the ^{1}H NMR spectrum, probably due to slight contamination with ethanol. This may occur as a result of deficient rinsing with diethyl ether and incomplete drying of the product. However it did not interfere with the NMR analysis (and was sometimes used to illustrate the effect of chemical exchange in ^{1}H NMR resonances).

The NMR spectra presented in this experiment were collected in a Bruker Avance 400 spectrometer equipped with an automatic sample changer and a QNP probe, working at 400 MHz for ^{1}H and at 100 MHz for ^{13}C, using standard pulse programs from Bruker library. TOPSPIN 2.6 software, also from Bruker was used for acquisition and TOPSPIN 3.0 for processing.

The sample was prepared using approximately 15 mg of compound in 0.5 mL $CDCl_3$ in a 5 mm NMR tube. ^{1}H and ^{13}C NMR spectra were referenced to $CDCl_3$ residual signals (7.26 and 77.16 ppm, respectively).

The total acquisition time for the set of NMR spectra presented in this work was approximately 3 h, each experiment time being specified in Table SM 4.1.2.3.2.

The infrared spectrum was recorded as KBr pellet in a Shimadzu IR Affinity-1 FT IR.
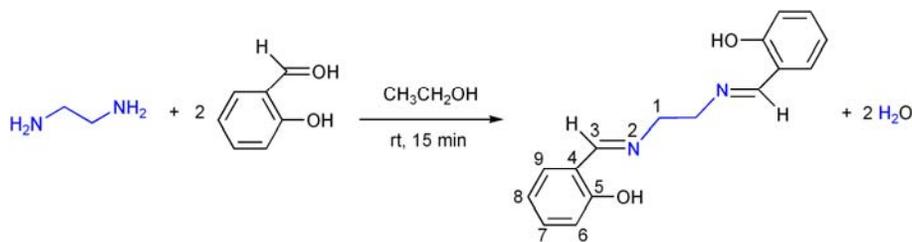
Figure SM 4.1.2.3.1 – Reaction scheme with the atom labeling used for NMR assignment

Analysis of the 1D and 2D NMR spectra

The molecule possesses a two-fold symmetry axis (C_{2h}) bisecting the ethylene carbon-carbon bond (C1-C1'), which is in accordance with the number of signals observed in the ^{1}H and ^{13}C NMR spectra (Figures SM 4.1.2.3.2 and SM 4.1.2.3.5, respectively). For simplicity, only one part of the molecule will be considered on the NMR analysis, using the atom labeling depicted in the reaction scheme (Figure SM 4.1.2.3.1). The splitting pattern abbreviations used are as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets); br (broad).

^{1}H NMR spectrum (Figure SM 4.1.2.3.2)

In the proton spectrum, the singlet at 3.94 ppm can immediately be assigned to the methylene protons H1 based on integration. Furthermore, it is the only signal to be expected in the lower frequency region of the spectrum, although under the deshielding influence of the electronegative nitrogen neighbour. No splitting is observed for this resonance because, even though the protons on C1 and on its symmetrical counterpart are close enough for vicinal coupling (^3J), they are chemically equivalent.

The four aromatic protons (all in different environments) show the ABCD pattern typically observed in *ortho*-disubstituted benzene rings, with two doublets corresponding to protons H6 and H9 and two triplet-like signals belonging to protons H7 and H8 (the more deshielded one showing partial overlap with the solvent residual peak at 7.26 ppm). Their individual assignment, however, needs information from the 2D spectra. First order coupling can be used to analyse the splitting patterns, although second order effects are apparent (note a slight "roof effect" on the aromatic region). Partial overlapping of the expected doublet of doublets (dd) for H7 and H8 yielded the triplet-like signals with different coupling constants (7 and 8 Hz), as often occurs in related systems. (Note: some samples gave more resolved ^{1}H NMR spectra where *meta* interactions ($^4J_{HH}$) were also observed).

The singlet at 8.36 ppm corresponds to the imine proton H3, which lies in the magnetic anisotropy deshielded zone of the aromatic ring. The downfield shift of this proton is a clear example of the additivity of deshielding effects (sp^2 hybridisation on C3, electronegativity of the C3-attached nitrogen atom and magnetic anisotropy of π-bonds).

The OH protons correspond to the most deshielded broad singlet at 13.22 ppm, consistent with the existence of intramolecular hydrogen-bonding between the OH groups and the nearby nitrogen atoms, as has been detected in the solid-state structure of the compound.[5]

Addition of D_2O to the sample causes replacement of the O**H** hydrogen by deuterium (according to the general equation: RO**H** + $D_2O \rightarrow$ ROD + **H**DO), causing it to disappear from the spectrum or to have its intensity reduced. This has been suggested as an optional experiment and is illustrated in figures SM X.3 and SM X.4.

^{13}C-APT NMR spectrum (Figure SM 4.1.2.3.5)

In the ^{13}C-APT NMR spectrum, eight signals were expected, due to the intrinsic symmetry of the molecule. Four of these can safely be assigned from their chemical shift values and multiplicities: the aliphatic carbon C1 at 59.86 ppm, the imine carbon C3 at 166.62 ppm and the two quaternary carbons. The resonance at 161.10 ppm corresponds to the oxygen-substituted C5 and the signal at 118.74 ppm is assigned to C4. The four aromatic CH resonances will only be assigned with the help of the 2D spectra. (Note the small peak intensity of the quaternary carbons and the solvent resonance at 77 ppm which consists of a 1:1:1 triplet due to ^{13}C coupling to deuterium (I=1), with $^1J_{CD}$= 32 Hz.)

COSY (Figure SM 4.1.2.3.6)

The COSY spectrum shows that the doublet at 6.94 ppm is coupled to the most deshielded triplet at 7.29 ppm, which in turn is connected to the most shielded triplet at 6.86 ppm and this in turn is coupled to the doublet at 7.23 ppm. However, the assignment of the signals requires analysis of the HMBC spectrum. Note an additional spot of much lower intensity correlating protons H1 and H3, four bonds apart, suggesting an allyl-type interaction (with a coupling constant too small to be detected in the proton spectrum with the actual resolution). The COSY method can sometimes detect interactions between nuclei that extend beyond three bonds.

HSQC (Figure SM 4.1.2.3.7)

The HSQC spectrum confirms the assignments for C1 and C3 due to their correlation signals with H1 and H3, respectively. Note that OH protons can also be identified by this method due to their lack of connectivity to any carbon, as well as the quaternary carbons, which lack correlations to protons. The connectivity between each proton and its directly bound carbon-13 ($^1J_{CH}$ couplings) can be identified but an individual assignment is not yet possible.

HMBC (Figure SM 4.1.2.3.8)

The HMBC spectrum is the key to solve the open question, namely the assignment of one of the aromatic protons or carbons. Inspection of the correlation signals for C3, for instance, reveals

connectivity to H1 and to the more deshielded doublet at 7.23 ppm, which identifies the proton signal H9. The subsequent assignment of the aromatic protons H6, H7 and H8 follows by consideration of the COSY results and the corresponding carbon signals will be easily assigned via the HSQC spectrum.

The results of the NMR analysis are displayed in table SM 4.1.2.3.1.

Table SM 4.1.2.3.1 - NMR data for H_2salen in CDC_3 (QC=quaternary carbon)

^{13}C Signals δ / ppm	Type of C	Assignment	^{1}H Signals δ / ppm (multiplicity, J / Hz)
166.62	CH	3	8.36 (s)
161.10	QC	5	-
132.51	CH	7	7.29 (t, J = 7 and 8)
131.60	CH	9	7.23 (d, $J_{9,8}$ = 7)
118.80	CH	8	6.86 (t, J = 7 and 8)
118.74	QC	4	-
117.06	CH	6	6.94 (d, $J_{6,7}$ = 8)
59.86	CH_2	1	3.94 (s)
-	-	OH	13.22 (br s)
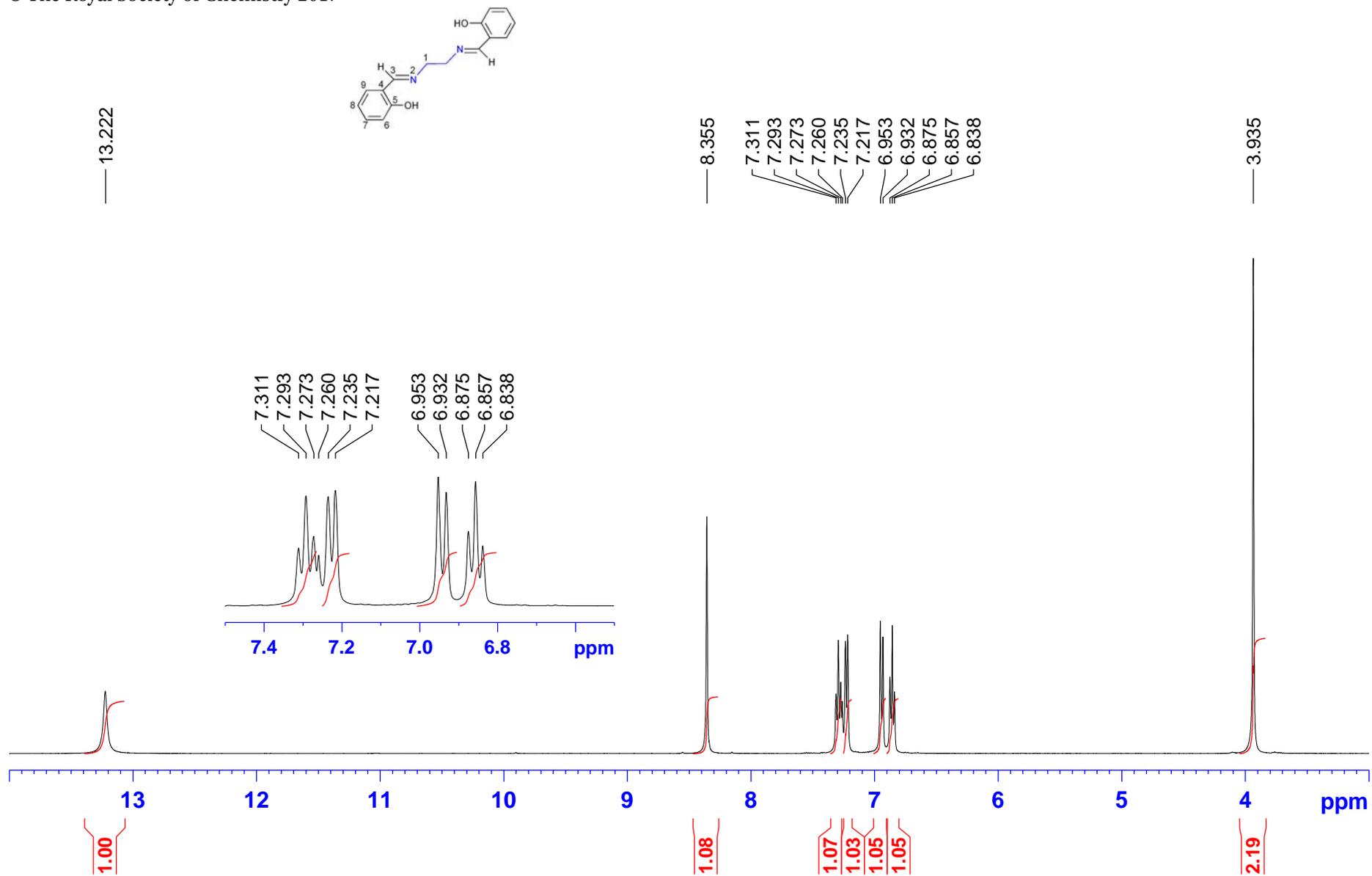
Figure SM 4.1.2.3.2 - ^{1}H NMR spectrum at 400 MHz in CDCl$_3$ with expansion of the aromatic region
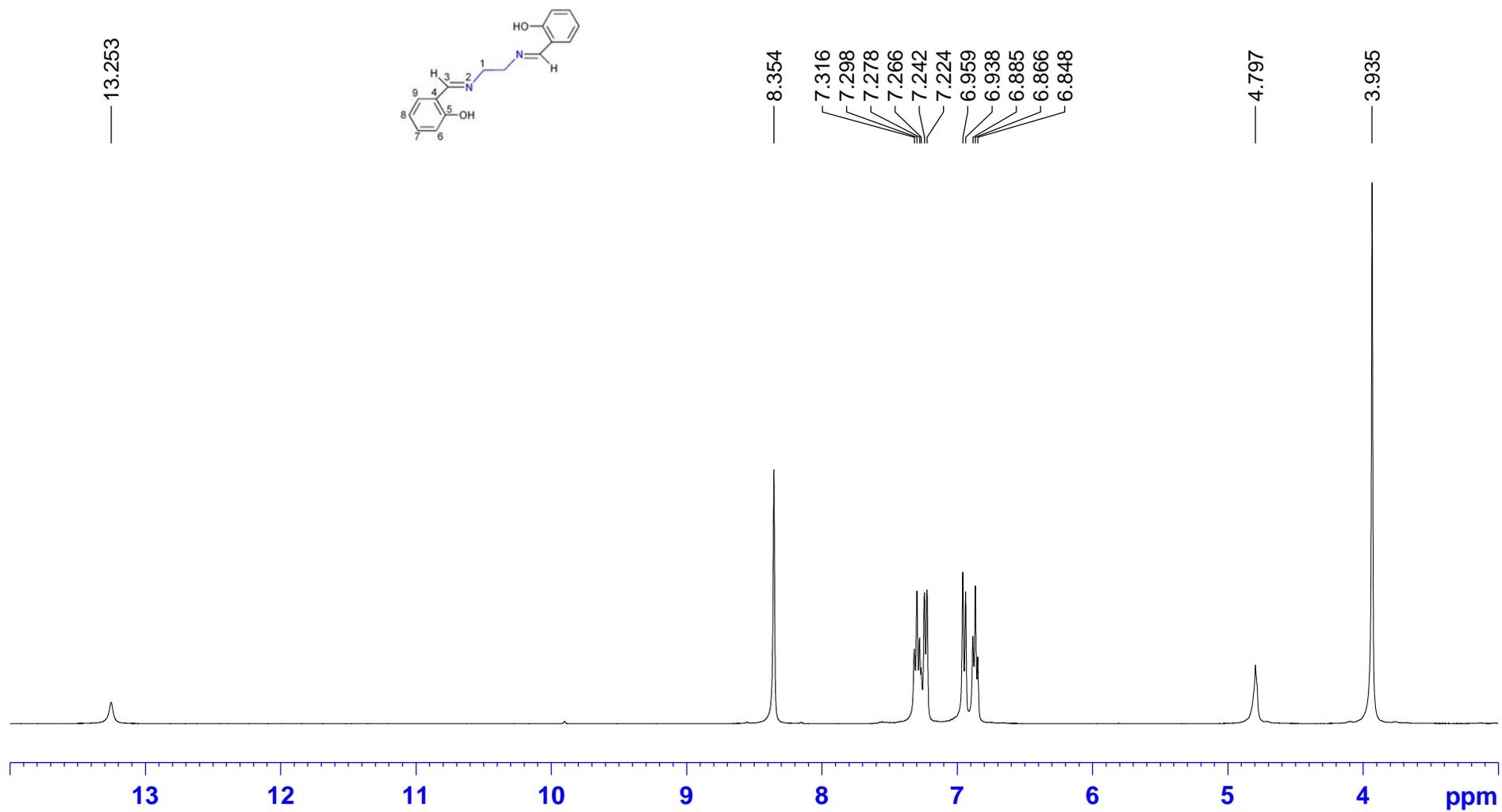
Figure SM 4.1.2.3.3 - ^{1}H NMR spectrum at 400 MHz in CDCl$_3$ after addition of D$_2$O
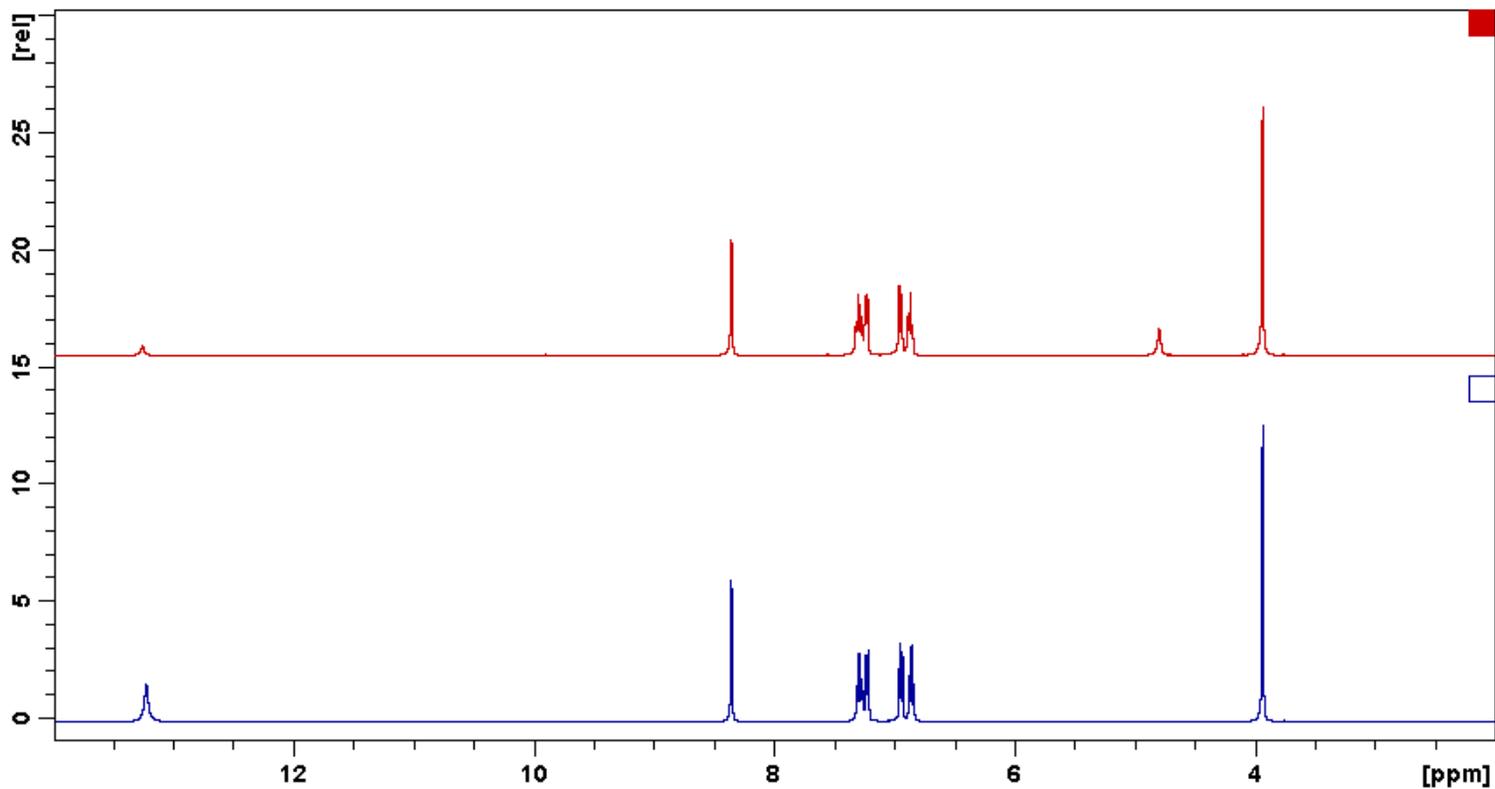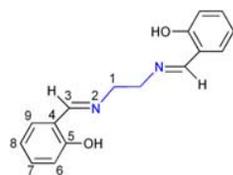
Figure SM 4.1.2.3.4 - ^{1}H NMR spectra of H$_2$salen in CDCl$_3$ (below) and after addition of D$_2$O (above). Note that the peaks due to the exchanged hydrogen atoms (at 13 ppm) "disappear" and a new peak (at 4.8 ppm) is generated due to the HDO formation (above spectrum).
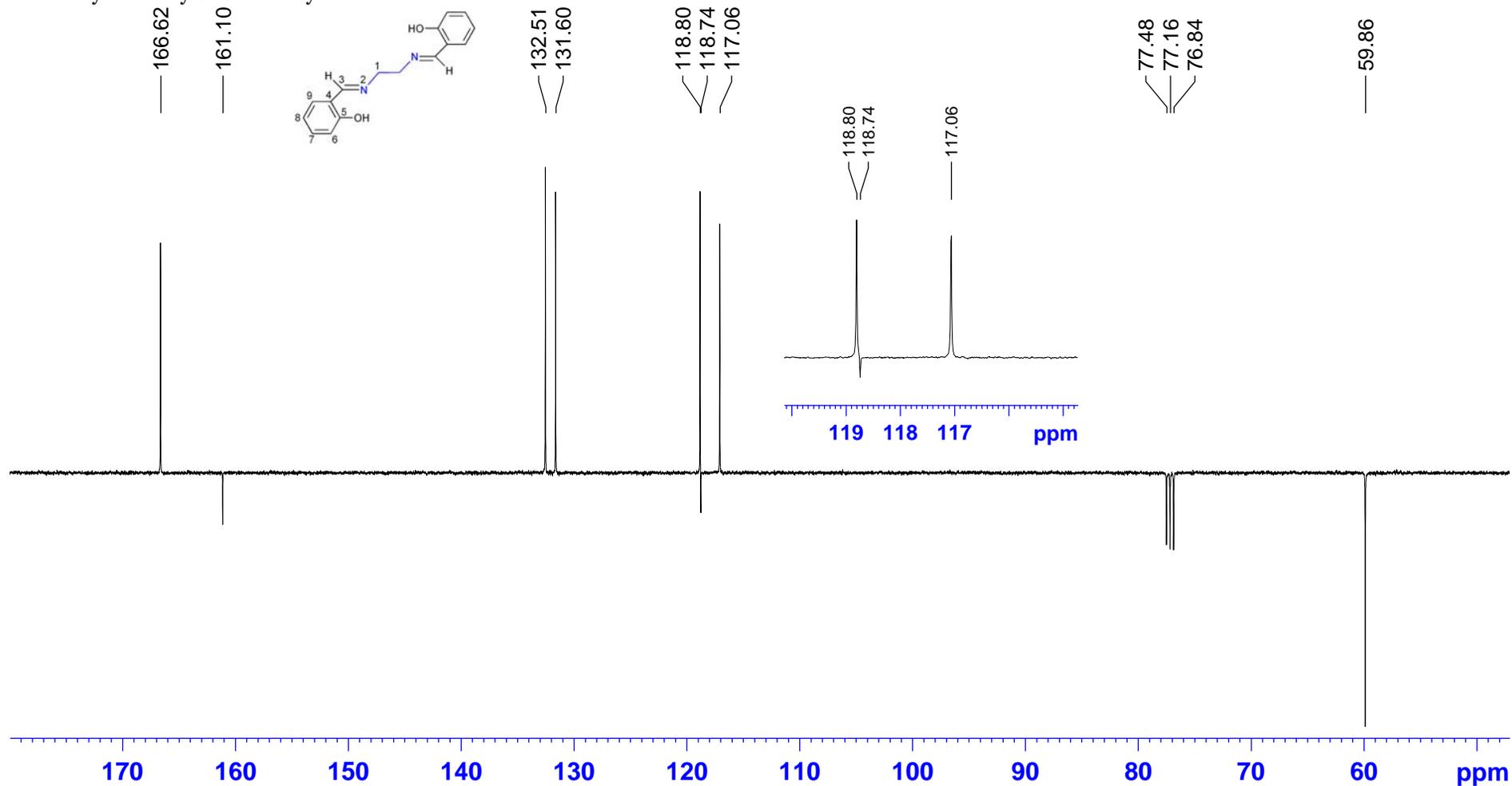
Figure SM 4.1.2.3.5 – APT ^{13}C NMR spectrum at 100 MHz in CDCl$_3$. The signal of the solvent was adjusted to be negative, like the other signals of carbon atoms carrying no protons. Signals of CH and CH$_3$ groups are positive and signals of CH$_2$ groups are negative.
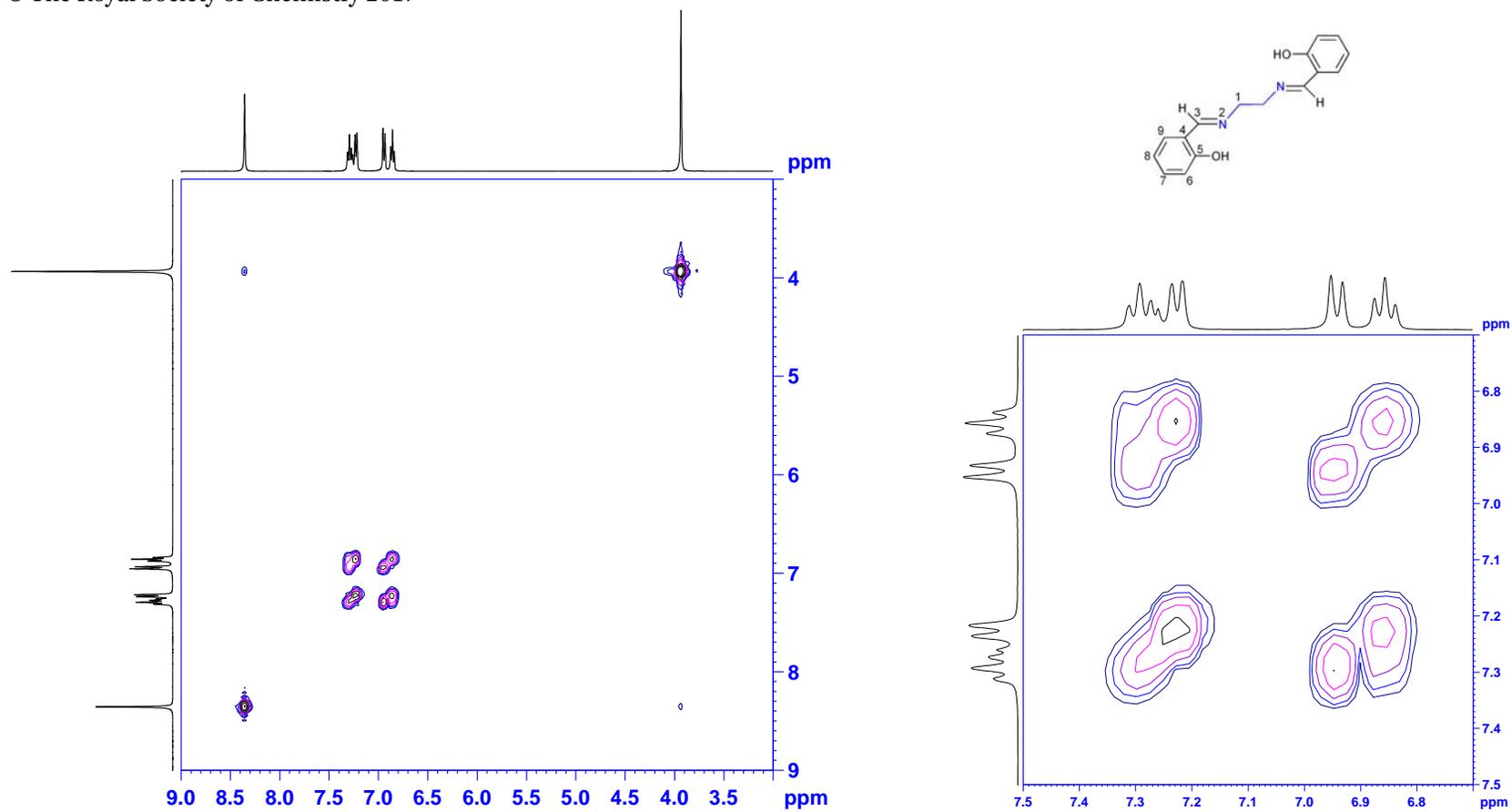
Figure SM 4.1.2.3.6 – COSY spectrum with expansion of the aromatic region
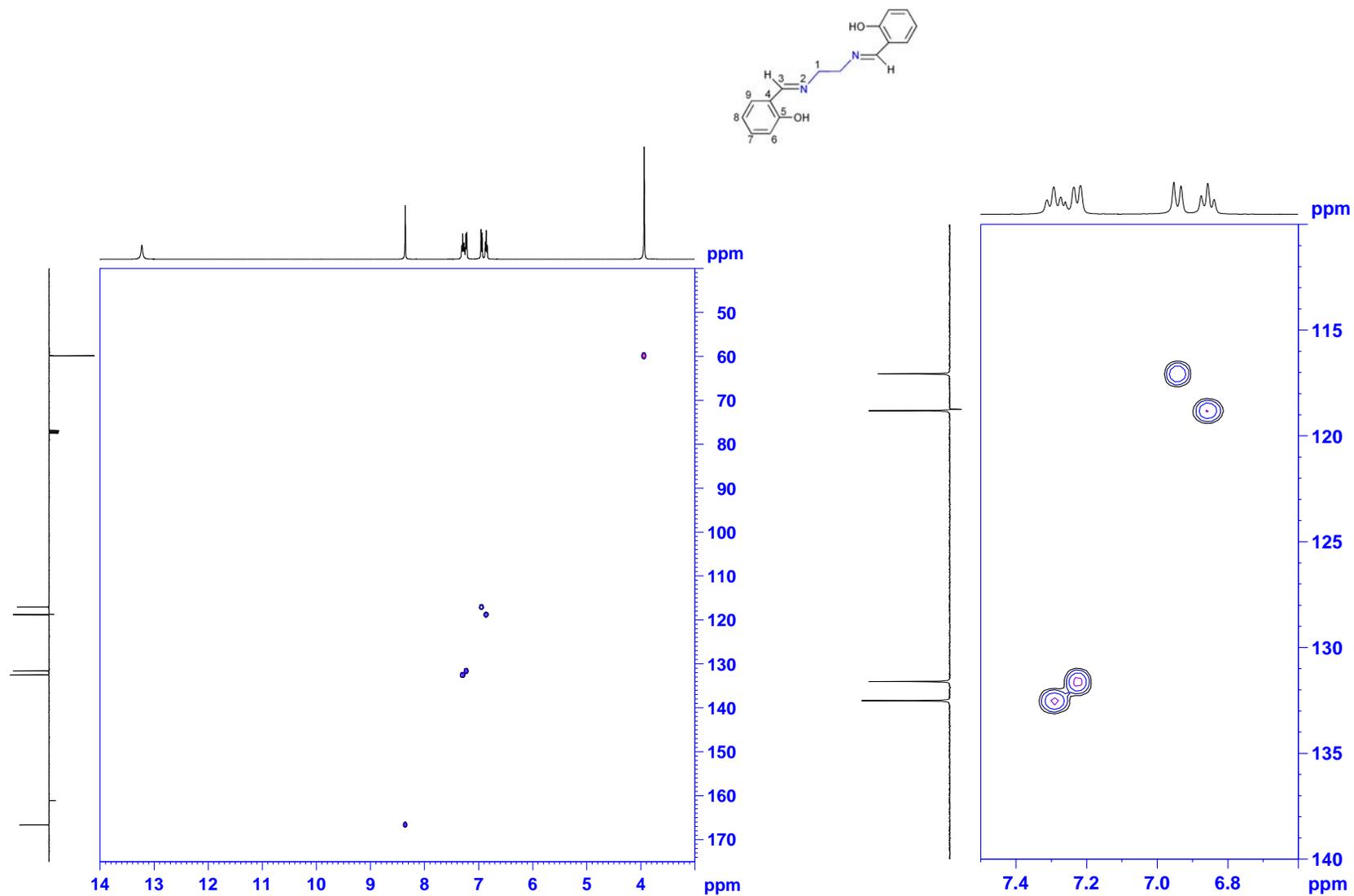
Figure SM 4.1.2.3.7 – HSQC spectrum with expansion of the aromatic region
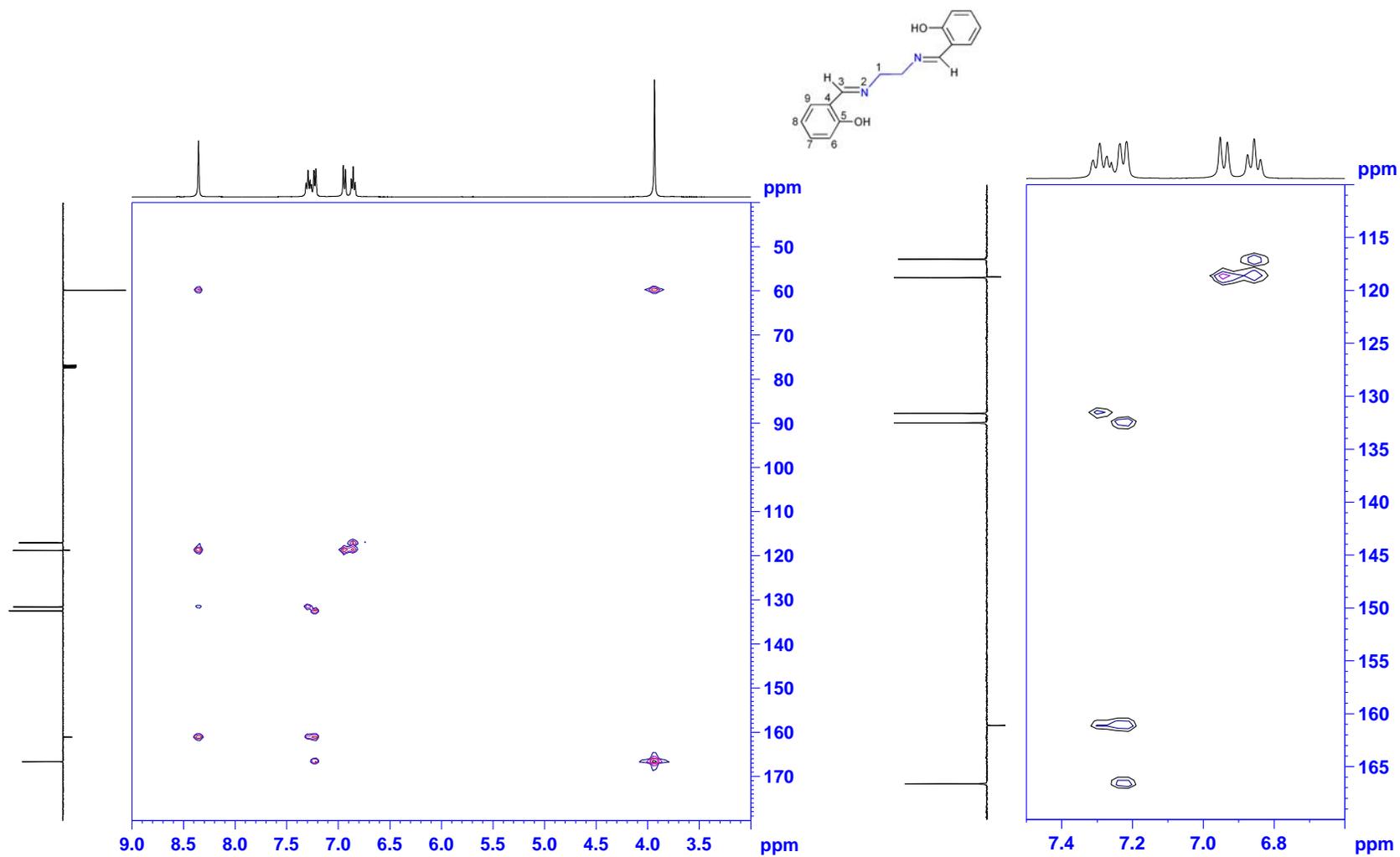
Figure SM 4.1.2.3.8 – HMBC spectrum with expansion of the aromatic region
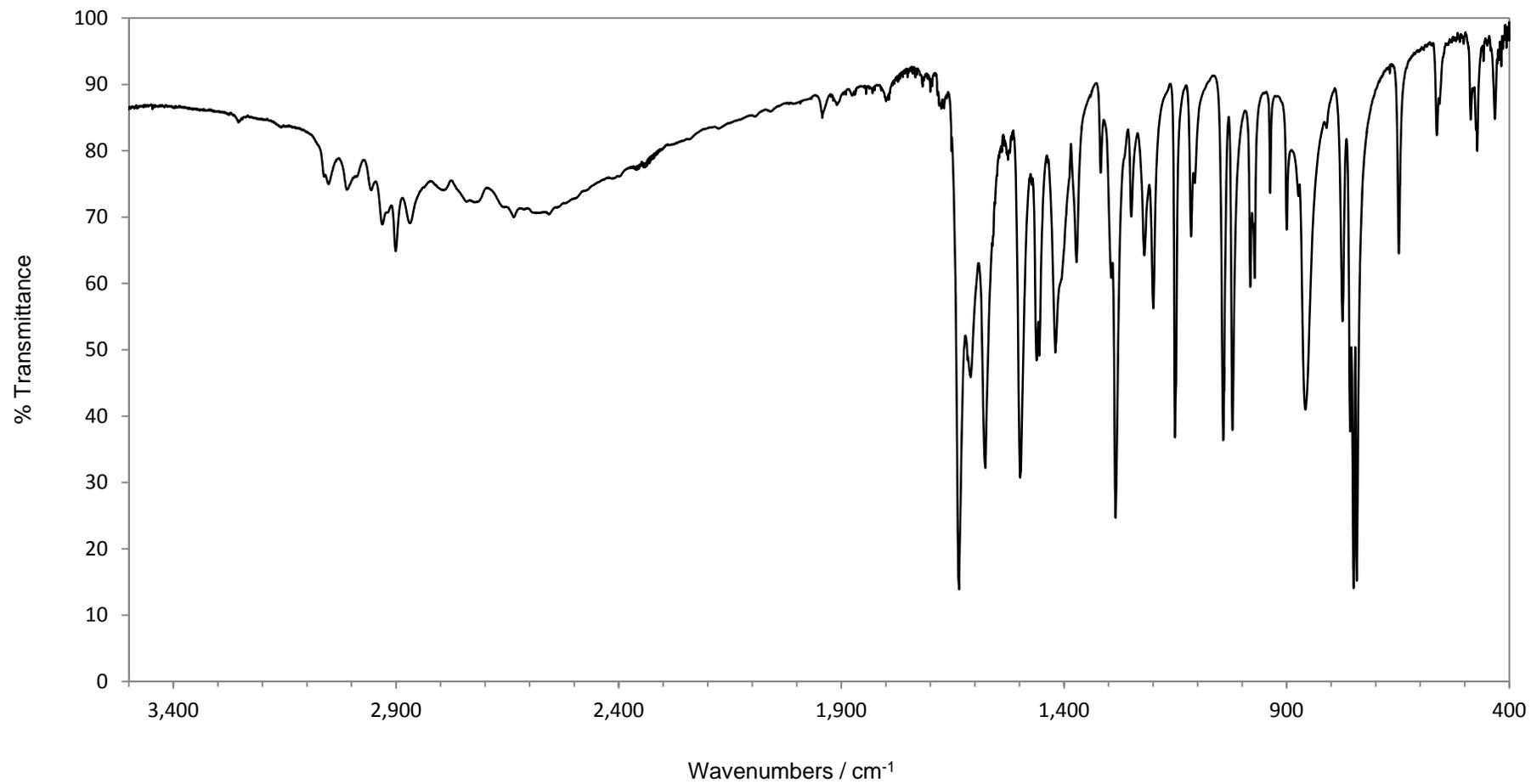
Figure SM 4.1.2.3.9 – Infrared spectrum of H$_2$salen in KBr.

Table SM 4.1.2.3.2 - Data parameters and duration of the NMR experiments

^{1}H NMR (1min 47s)	^{13}C-APT NMR spectrum (18min 28s)
Current Data Parameters NAME Schiff-20140912-mjb EXPNO 10 PROCNO 1 F2 - Acquisition Parameters Date_ 20140912 Time 19.02 INSTRUM spect PROBHD 5 mm QNP 1H/13 PULPROG zg30 TD 65536 SOLVENT CDCl3 NS 16 DS 2 SWH 8278.146 Hz FIDRES 0.126314 Hz AQ 3.9584243 sec RG 161.3 DW 60.400 usec DE 8.00 usec TE 294.9 K D1 2.00000000 sec TD0 1 ======= CHANNEL f1 ======== NUC1 1H P1 8.87 usec PL1 -3.30 dB PL1W 21.48127174 W SFO1 400.1324710 MHz F2 - Processing parameters SI 32768 SF 400.1300095 MHz WDW EM SSB 0 LB 0.30 Hz GB 0 PC 1.00	Current Data Parameters NAME Schiff-20140912-mjb EXPNO 11 PROCNO 1 F2 - Acquisition Parameters Date_ 20140912 Time 20.01 INSTRUM spect PROBHD 5 mm QNP 1H/13 PULPROG jmod TD 65536 SOLVENT CDCl3 NS 1024 DS 4 SWH 23980.814 Hz FIDRES 0.365918 Hz AQ 1.3664756 sec RG 16384 DW 20.850 usec DE 6.50 usec TE 295.4 K CNST2 145.0000000 CNST11 1.0000000 D1 2.00000000 sec D20 0.00689655 sec TD0 1 ======= CHANNEL f1 ======== NUC1 13C P1 8.50 usec P2 17.00 usec PL1 -3.60 dB PL1W 80.56159210 W SFO1 100.6228298 MHz ======= CHANNEL f2 ======== CPDPRG2 waltz16 NUC2 1H PCPD2 100.00 usec PL2 -3.30 dB PL12 17.62 dB PL2W 21.48127174 W PL12W 0.17380406 W SFO2 400.1316005 MHz F2 - Processing parameters SI 32768 SF 100.6127585 MHz WDW EM SSB 0 LB 1.00 Hz GB 0 PC 1.40

Table SM 4.1.2.3.2 - Data parameters and duration of the NMR experiments (continued)

COSY (18min 26s)	HSQC (28min 2s)	HMBC (1h 15min)
| ```
Current Data Parameters
NAME      Schiff-20140912-mjb
EXPNO               12
PROCNO               1

F2 - Acquisition Parameters
Date_         20140912
Time             20.02
INSTRUM           spect
PROBHD  5 mm QNP 1H/13
PULPROG        cosygpqf
TD                1024
SOLVENT           CDCl3
NS                   2
DS                   4
SWH         4789.272 Hz
FIDRES      4.677023 Hz
AQ         0.1069556 sec
RG                 362
DW          104.400 usec
DE            8.00 usec
TE              295.3 K
D0        0.00000300 sec
D1        2.00000000 sec
D13       0.00000400 sec
D16       0.00020000 sec
IN0       0.00020825 sec


======== CHANNEL f1 ========
NUC1                1H
P0            8.87 usec
P1            8.87 usec
PL1            -3.30 dB
PL1W      21.48127174 W
SFO1     400.1321711 MHz

====== GRADIENT CHANNEL =====
GPNAM1        SINE.100
GPZ1            10.00 %
P16          1000.00 usec

F1 - Acquisition parameters
TD                 256
SFO1       400.1327 MHz
FIDRES     18.756222 Hz
SW            12.000 ppm
FnMODE              QF

F2 - Processing parameters
SI                 512
SF       400.1300039 MHz
WDW               SINE
SSB       0
LB        0 Hz
GB        0
PC               1.00

F1 - Processing parameters
SI                 512
MC2                 QF
SF       400.1305737 MHz
WDW       echo-antiecho
SSB       0
LB        0 Hz
GB        0
``` | ```
Current Data Parameters
NAME      Schiff-20140912-mjb
EXPNO               16
PROCNO               1

F2 - Acquisition Parameters
Date_         20140912
Time             23.45
INSTRUM           spect
PROBHD  5 mm QNP 1H/13
PULPROG      hsqcetgpsi2
TD                1024
SOLVENT           CDCl3
NS                   4
DS                  16
SWH         6082.725 Hz
FIDRES      5.940161 Hz
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TE              294.3 K
CNST2       145.0000000
D0        0.00000300 sec
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D4        0.00172414 sec
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D13       0.00000400 sec
D16       0.00020000 sec
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ZGOPTNS

======== CHANNEL f1 ========
NUC1                1H
P1            8.87 usec
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P28           0.50 usec
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PL1W      21.48127174 W
SFO1     400.1327241 MHz

======== CHANNEL f2 ========
CPDPRG2            garp
NUC2               13C
P3            8.50 usec
P4           17.00 usec
PCPD2        80.00 usec
PL2            -3.60 dB
PL12           15.87 dB
PL2W      80.56159210 W
PL12W      0.91018158 W
SFO2     100.6218241 MHz

====== GRADIENT CHANNEL =====
GPNAM1        SINE.100
GPNAM2        SINE.100
GPNAM3        SINE.100
GPNAM4        SINE.100
GPZ1            80.00 %
GPZ2            20.10 %
GPZ3            11.00 %
GPZ4            -5.00 %
P16          1000.00 usec
P19           600.00 usec

F1 - Acquisition parameters
TD                 256
SFO1       100.6218 MHz
FIDRES     78.610802 Hz
SW           200.000 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                1024
SF       400.1300062 MHz
WDW              QSINE
SSB                  2
LB        0 Hz
GB        0
PC               1.40

F1 - Processing parameters
SI                1024
MC2        echo-antiecho
SF       100.6127485 MHz
WDW                 GM
SSB                  2
LB        0 Hz
GB        0
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Current Data Parameters
NAME      Schiff-20140912-mjb
EXPNO               13
PROCNO               1

F2 - Acquisition Parameters
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TD                1024
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NS                   8
DS                  16
SWH         6082.725 Hz
FIDRES      5.940161 Hz
AQ         0.0842228 sec
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DE            8.00 usec
TE              294.9 K
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CNST13        7.0000000
D0        0.00000300 sec
D1        2.02293801 sec
D2        0.00344828 sec
D6        0.07142857 sec
D16       0.00020000 sec
IN0       0.00002260 sec


======== CHANNEL f1 ========
NUC1                1H
P1            8.87 usec
P2           17.74 usec
PL1            -3.30 dB
PL1W      21.48127174 W
SFO1     400.1327178 MHz

======== CHANNEL f2 ========
NUC2               13C
P3            8.50 usec
PL2            -3.60 dB
PL2W      80.56159210 W
SFO2     100.6226690 MHz

====== GRADIENT CHANNEL =====
GPNAM1        SINE.100
GPNAM2        SINE.100
GPNAM3        SINE.100
GPZ1            50.00 %
GPZ2            30.00 %
GPZ3            40.10 %
P16          1000.00 usec

F1 - Acquisition parameters
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SW           219.994 ppm
FnMODE              QF

F2 - Processing parameters
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WDW                 EM
SSB       0
LB               1.00 Hz
GB        0
PC               1.40

F1 - Processing parameters
SI                 512
MC2                 QF
SF       100.6127470 MHz
WDW                °f1
SSB                  2
LB        0 Hz
GB        0
``` |

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Synthesis of Iophine and conversion into dimers
Supplementary Material

This experiment proposal was tested by students of intermediate organic chemistry in which the concepts of carbonyl group transformations, tautomerism and oxidations reactions were taught. The synthesis of a compound showing phenomena like photochromism, piezochromism increase the interest of all students for this kind of experiments. Experimental procedure can be easily performed by them, but the handling of irritant chemicals requires the use of fume hoods. This work is a great example to illustrate free radical formation. The measurement of kinetics, energetic profiles, the extinction coefficient by radical trapping and ESR signal can be found in literature[1,2]. Additionally, this work allows understanding photochromism, piezochromism, thermochromism phenomena. Iophine can be used in chemiluminescent reactions with hydrogen peroxide, potassium hydroxide and household bleaching[3] although this point is not addressed in the present experiment.

Additional notes on the preparation of Iophine

Yields obtained for Iophine are usually in the range 30-40% and m.p 273-274ºC (273-275ºC[4]). Neutralization with ammonium hydroxide solution leads to low quantity of precipitate. It should be verified that this solution contains 30% of NH_3.

Additional notes on the preparation of Iophine dimers

After ethanolic sodium hydroxide addition to Iophine, the solution becomes orange. In the initial stages of addition of ferricyanide solution, a violet coloration is observed and is replaced by a light grey precipitate. In order to keep the temperature below 10º C, the addition took 1h and 40 minutes instead 1h as refereed. Higher temperatures lead to several products. The yield for crude dimer I is in the range 70-80 % and m. p. 154-155ºC (177-184ºC[2]). Melting points for purified dimers were found in literature: 187.5-188.5ºC for dimer I and 202-202.5ºC for dimer II[2]. The low melting point for crude dimer I is due to the presence of dimer II.

Piezochromism is observed when a small portion of dimer I is ground in a mortar. The light gray solid turns violet (**Figure SM 4.1.2.4.1**).

Figure SM 4.1.2.4.1 – Dimer I before (light gray) and after be grinded (purple) in a mortar (piezochromism)

Photochromism is observed when some crystals are exposed to strong sunlight (**Figure SM 4.1.2.4.2**). The same effect is observed with a toluene solution. In the absence of sun 60 Watt tungsten light can be used although the color change is slower.

Figure SM 4.1.2.4.2 – Dimer I toluene solution before (light gray) and after be exposed to sunlight (purple) (photochromism)

Termochromism is observed when the colorless toluene solution is poured in a warm water bath. The same purple color quickly develops with the temperature.

When the purple solid and solution are stored in the dark, they change to light gray in several hours.

IV spectra:

Students easily identify a sharp band at 1450 cm^{-1} indicative of imidazole ring, a strong absorption bands due to aromatic rings (650 - 800 cm^{-1}) and a broad absorption in the 3200-2300 cm^{-1} (**Figure SM 4.1.2.4.3**).

Figure SM 4.1.2.4.3– IR (KBr) of lophine

IR spectrum of lophine is also available in SDBS (nº 8501)[5].

Figure SM 4.1.2.4.4– IR (KBr) of lophine Dimer

The KBr disk became purple due to the applied pressing. IR spectrum was recorded after KBr became light gray, so this IR spectrum should correspond to dimer II or probably to dimer I and II mixture (**Figure SM 4.1.2.4.4**).

IR spectra of lophine dimers could be found in literature[2].

NMR spectra:

Students easily identify the broad peak of NH proton at 11.7 ppm (**Figure SM 4.1.2.4.5**).

Figure SM 4.1.2.4.5– ^{1}H NMR (300 MHz, DMSO-d$_6$) of lophine

^{1}H and ^{13}C NMR spectra of lophine are available in SDBS (n° 8501)[5].

[1] M. Pickering, *J. Chem. Educ.*, 1980, **57**, 833-834.

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Synthesis of dibenzalacetone 2,4-dinitrophenylhydrazone
Supplementary Material

Experiment notes   1

Session 1 - Synthesis of dibenzalacetone via aldol condensation   1

Session 2 - Synthesis of dibenzalacetone 2,4-dinitrophenylhydrazone   2

Figures

Experiment notes:

Session 1 - Synthesis of dibenzalacetone via aldol condensation

The aim of this experiment is to execute an easy double mixed-aldol condensation reaction between an aldehyde and a ketone – benzaldehyde and acetone, respectively, employing sodium hydroxide as the base. This reaction is appropriate for students of an intermediate organic chemistry level but it can be easily performed by less graduated students. Additionally, this synthesis can be the first part of two sequential experiments aiming a better understanding of the mechanisms involving the carbonyl group and its characterization.

As can be devised from the reaction mechanism (Figure SM 4.1.2.5.1), it is important the order of the reactants addition, to avoid the acetone self-condensation. The sodium hydroxide solution must be the last reactant added and this addition should be slowly performed. After a few minutes (under stirring) the precipitation of a yellow solid should be observed. In some cases the solid mass is too compact and the string velocity must be increased.

When the students are washing the product with the acetic acid solution ensure that the vacuum is turned off to allow the correct elimination of the base and avoid product decomposition. It is advisable to squeeze the crude precipitate between filter paper sheets to eliminate the excess of water.

The dibenzalacetone is quite soluble in ethanol so very little amount of solvent is needed to perform the crystallization.

After crystallized, the dibenzalacetone should be placed in a pre-weighted watch glass and dry until constant weight. This can be evaluated if the weight variation is lower than 5 mg after 10 minutes in an oven at an appropriate temperature (45°C).

Figure SM 4.1.2.5.1. Mechanism of dibenzalacetone synthesis under basic conditions.

This first experiment was assayed by all the students of the 2nd year of the Integrated Masters Degree in Pharmaceutical Sciences, Faculty of Pharmacy, University of Lisbon, (14 classes of 16 students/class, 8 groups of two) who successfully performed the synthesis of dibenzalacetone with average yields of 65-75% and m.p. around 110 °C. The students enjoyed performing this synthesis mainly as result of the color and bright crystals obtained besides the good yields.

Session 2 - Dibenzalacetone 2,4-dinitrophenylhydrazone

The aim of this experiment is to perform an easy condensation reaction between dibenzalacetone, a ketone and 2,4-dinitrophenylhydrazine, with the formation of a new functional group, an imine named 2,4-dinitrophenylhydrazone. This hydrazone is a high melting point solid easily separated from the reaction mixture by precipitation. This reaction can be realized sequentially after the dibenzalacetone synthesis prepared in a previous session by the same students group. This reaction mechanism is presented in Figure SM 4.1.2.5.2.

This laboratorial experiment provides an important application of the carbonyl group reactions and concepts achieved by the students and represent a very good example of the reactivity of this functional group. Also, the synthesis of hydrazones was, for quite a long time, an identification reaction used on the chemical elucidation of aldehydes and ketones structure. This experiment is appropriate to students of intermediate organic chemistry level.

Figure SM 4.1.2.5.2 – Mechanism of the synthesis of dibenzalacetone 2,4-dinitrophenylhydrazone

This synthesis is easily performed by the students, but several precautions must be taken into account:

- The 2,4-dinitrophenylhydrazine reagent acid solution must be previously prepared and kept in fume hood. Care must be taken since this reagent contains hydrazine, which stains the skin, is irritant, toxic and possible carcinogenic, and concentrated sulphuric acid. Therefore, the use of gloves, lab security goggles, and long sleeves lab coat must be mandatory for all the students and staff in the lab. This solution should be placed in a burette, in the fume cupboard, from where the students measure the volume to add to the Erlenmeyer with their reaction mixture. Make sure a beaker is placed under the burette, when not in use, to avoid acid solution drops on the fume cupboard.

- An Erlenmeyer flask with ground-glass jointware should be used with the cap on, when waiting for the reaction to proceed.

- The solid product collected by filtration must be repeatedly washed with cold ethanol to remove all the sulphuric acid residues. Any remaining acid will catalyze the decomposition of the compound when the m.p. is realized, broadening and lowering the value obtained by almost 40ºC. This procedure is extremely important when recrystallization is not performed.

- If necessary crude product may be crystallized. An attempt was tried with crystallization from ethanol, but the product solubility, even near the solvent boiling point, was not good. The appropriate solvent volume was quite high with the consequent decrease in the product final yield. Then the conventional solvent pair technique was tried and several combinations of solvents were assayed. The best results were achieved with acetone/water with an adaptation of the conventional solvent pair technique:

- 250 mg of crystals were dissolved in 10 mL of hot acetone and then 1 mL of hot water (60-70 ºC) was added. The mixture was allowed to cool. The crystals were collected and some bright and shining red needles were obtained. The recrystallization yield was around 70% and the melting point completely fits with the described in the literature (180 ºC).

- The 2,4-dinitrophenylhydrazine reagent acid solution must be previously prepared according to the technique: Dissolve 8 g of 2,4-dinitrophenylhydrazine in 40 mL of concentrated sulphuric acid. Add this solution, carefully and with stirring, to a mixture of 60 mL water and 200 mL ethanol 95 %. Filtrate the final solution if precipitation occurs.

This experiment was successfully carried out by all the students of the 2nd year of the Integrated Master Degree in Pharmaceutical Sciences, Faculty of Pharmacy, University of Lisbon, (14 classes of 16 students/class, 8 groups of two). From those, 32 groups have performed the synthesis and recrystallization (from ethanol) of the hydrazone with average yields of 40% and melting point 177-180ºC. The remaining students just synthesized the compound and in this case the yield was higher (50-55 ºC) with similar melting point. The obtained NMR of the product before and after recrystallization shows almost similar purity.

Photos of the experiment

Session 1 - Synthesis of Dibenzalacetone via Aldol Condensation

Figure SM 4.1.2.5.3. Materials and reagents.

Figure SM 4.1.2.5.4. Synthesis of the

dibenzalacetone.

Figure SM 4.1.2.5.5. Product solid mass.

Figure SM 4.1.2.5.6.Crystallization.

Figure SM 4.1.2.5.7. Dibenzalacetone crystals.

Session 2 - Dibenzalacetone 2,4-dinitrophenylhydrazone

Figure SM 4.1.2.5.8. Material and reagents.

Figure SM 4.1.2.5.9. Synthesis of the hydrazone.

Figure SM 4.1.2.5.10. Crude product.

Figure SM 4.1.2.5.11. Crude hydrazone.

Figure SM 4.1.2.5.12. Hydrazone crystals

(acetone/methanol).

Figure SM 4.1.2.5.13. Hydrazone TLC: **a**. dibenzalacetone;
b. hydrazone before crystallization; **c**. hydrazone crystals.

Spectra:

Session 1 - Synthesis of dibenzalacetone via aldol condensation:

Figure SM 4.1.2.5.14. IR spectrum of dibenzalacetone.

Figure SM 4.1.2.5.15 ^{1}H NMR spectrum of pure dibenzalacetone (300 MHz, CDCl$_3$).

Figure SM 4.1.2.5.16. ^{1}H NMR spectrum of pure dibenzalacetone- expansion (300 MHz, CDCl$_3$).

Figure SM 4.1.2.5.17. ^{13}C NMR spectrum dibenzalacetone (75 MHz, CDCl$_3$).

Figure SM 4.1.2.5.18. ^{13}C NMR spectrum dibenzalacetone- expansion (75 MHz, CDCl$_3$).

Session 2 - Dibenzalacetone 2,4-dinitrophenylhydrazone:

Figure SM 4.1.2.5.19. IR spectrum of dibenzalacetone 2,4-dinitrophenylhydrazone.

Figure SM 4.1.2.5.20. ^{1}H NMR spectrum of crude (before recrystallization) dibenzalacetone 2,4-dinitrophenylhydrazone (300 MHz, CDCl$_3$).

Figure SM 4.1.2.5.21. ^{1}H NMR spectrum of crude (before recrystallization) dibenzalacetone 2,4-dinitrophenylhydrazone - expansion (300 MHz, CDCl$_3$).

Figure SM 4.1.2.5.22. ^{13}C NMR spectrum of crude (before recrystallization) dibenzalacetone 2,4-dinitrophenylhydrazone (75 MHz, CDCl$_3$).

Figure SM 4.1.2.5.23. ^{13}C NMR spectrum of crude (before recrystallization) dibenzalacetone 2,4-dinitrophenylhydrazone - expansion (75 MHz, CDCl$_3$).

Figure SM 4.1.2.5.24. ^1H NMR spectrum of purified (after recrystallization) dibenzalacetone 2,4-dinitrophenylhydrazone (300 MHz, CDCl$_3$).

Figure SM 4.1.2.5.25. ^{1}H NMR spectrum of purified (after recrystallization) dibenzalacetone 2,4-dinitrophenylhydrazone - expansion (300 MHz, CDCl$_3$).

Figure SM 4.1.2.5.26. ^{13}C NMR spectrum of purified (after recrystallization) dibenzalacetone 2,4-dinitrophenylhydrazone (75 MHz, CDCl$_3$).

Figure SM 4.1.2.5.27. ^{13}C NMR spectrum of purified (after recrystallization) dibenzalacetone 2,4-dinitrophenylhydrazone - expansion (75 MHz, CDCl$_3$).

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Synthesis and structural characterization of an antitubercular isoniazid hydrazone

Supplementary Material

1. Experiment contextualization

Imine chemistry is a topic usually taught in the first/second Organic Chemistry assignments, included in the carbonyl and amine reactions. The acid catalysed reversible addition-elimination mechanism of its genesis is well documented in textbooks, including the possibility of formation of stereoisomers when the R-groups of the carbonyl reactant are different. Some emphasis is given on the fact that imine formation occurs under equilibrium control and, therefore, to drive the reaction towards the product side, water generated in the process must be removed from the reaction. However, it is important to introduce students to imines that escape from the instability pattern and make them analyse the steric and electronic factors associated with the extra stability of these compounds. Hydrazones (as well as oximes) possess an intrinsic hydrolytic stability higher than common imines, which may be explained by the participation of the lone pair of the amide <u>N</u>H in electron delocalization. One of the main objectives of this experience is precisely to reinforce the students' knowledge about the key role that molecular structure plays in dictating physical and chemical properties. Students should be able to depict the resonance structures of the synthesized hydrazone and to understand the influence of the mesomeric effect on its stability. Moreover, they should be challenged to make a

connection between the stability of the synthesized hydrazone and the reaction experimental conditions.

The incorporation of NMR techniques to assign the structure of the synthesized hydrazone, with a special attention to stereochemistry, is intended to strengthen the students' knowledge on rotation about the amide bond, a classical concept taught to undergraduate Organic Chemistry students. Rotational isomers based on an amide bond play an important role in the regulation of the activity of biologically active peptides and other molecules that have amide skeletons. For instance, although the *trans* conformer is dominant in proteins, information from Protein Structure Data Bank shows that around 0.03% of the secondary amide bonds are assigned as *cis*, and play a key role in protein function.[1] Most of the times, in simple molecules, amide conformers interconvert rapidly at room temperature, but in a few cases, as in the proposed hydrazone, bond rotation is so hindered that *cis* and *trans* isomers can be easily detected by means of NMR. In the NMR spectra analysis of the synthesized hydrazone, students will not only explore the use of this technique to identify the hydrazone structure, including its stereochemistry, but will also be led to reason about the structural features that dictate the stereochemical outcome of the reaction.

All modern Organic Chemistry textbooks have a chapter which introduces the basics of identification of organic compounds by NMR spectroscopy, and many of them even include 2D experiments, and so this work is aligned with basic Organic Chemistry undergraduate *curriculum*. As the present experiment was designed to be performed in 2x3h laboratory periods, running NMR spectra must be done as a spin-off work, and results must be explored in the lab report.

This experiment was already executed by third year undergraduate students, doing their final scientific project, and can be easily carried out in a second year laboratory classroom. The time of implementation is suitable for the time frame of a common laboratory session and the techniques are adequate to an intermediate level Organic Chemistry assignment.

2. Experiment notes

Hydrazone formation was monitored by TLC. This is not a very easy task, since the polarity of the two reagents is very different and the product tends to form an elliptical spot with some tailing. As the stoichiometry of the reaction is 1:1, any of the reagents can be used as control. However, isoniazid is very polar and a good TLC spot is only obtained by using as eluent a mixture of ethyl acetate/methanol/ammonia 4.25:0.75:0.3, which is not adequate for the product. The best results, with good separation from the control and a less tailed spot for the product, were obtained using ethyl acetate as eluent: in this case the aldehyde was used as control and isoniazid remained in the application point. Although the R_f= 0.25 of the product is below what is recommended for flash chromatography separations, the purification was successfully achieved with good yields. The reaction does not reach completion even with longer reaction times but yields are approximately 75%. Hexane/acetone 1:1 can be used as an alternative eluent.

One of the key features of this synthesis is the need to dry thoroughly the crude product before column chromatography. Due to the difficulty of drying properly the product within the timeframe of the lab class, it is recommended to connect a vacuum pump to the desiccator overnight.

As regards the NMR experiments, the ideal situation would be to run ^{1}H, ^{13}C, COSY, DEPT, HSQC, HMBC and NOESY experiments, for a full identification of the compound. Spectra should be run in DMSO-d_6 in order to move the N$\underline{H}$ signal downfield in a zone free of any other signals. However, for the purpose of stereochemistry assignment, ^{1}H, COSY and NOESY experiments will be enough, providing students have access to the literature spectra (see reference 8 of the Experiment). In the ^{1}H NMR spectrum it should be stressed that the observed double signs, with very small intensity, are due to the minor *cis* conformer and not to the *Z* geometric isomer. In this type of compounds, iminic and N$\underline{H}$ protons have quite characteristic and distinct signals, enabling a suitable stereochemistry identification. NOESY experiments reveal a cross peak between the CON$\underline{H}$ proton and the iminic

proton, only possible in the *E* geometry of the double bond. A further cross-peak between the CON<u>H</u>

proton and the pyridine H-3/H-5 protons discloses a major *trans* CO–NH conformation.

3. Physical characteristics of *N'*-(E)-(4-phenoxybenzylidene)isonicotinohydrazide

White crystals; m.p. 175.8-176.8 ºC; soluble in dichloromethane, acetone, diethyl ether and ethanol;

partially soluble in methanol; insoluble in water and hexane.

4. NMR spectra of *N'*-(E)-(4-phenoxybenzylidene)isonicotinohydrazide

Figure SM 4.1.2.6.1: ^{1}H NMR (400 MHz, DMSO-d_6) of *N'*-(*E*)-(4-phenoxybenzylidene)isonicotinohydrazide

Figure SM 4.1.2.6.2: ^{13}C NMR (100.4 MHz, DMSO-d_6) of *N'*-(E)-(4-phenoxybenzylidene)isonicotinohydrazide

N'-(E)-(4-phenoxybenzylidene)isonicotinohydrazide

150.80
150.00
148.88
130.72
130.66
129.69
129.24
124.74
124.59
123.61
122.00
119.97
119.74
118.82
118.67

BRUKER

Current Data Parameters
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EXPNO   12
PROCNO   1

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DE   8.00 usec
TE   300.0 K
CNST2   145.00000000
D1   2.00000000 sec
d2   0.00344828 sec
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MCREST   0 sec
MCWRK   0.01500000 sec

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PL1   -2.00 dB
SFO1   100.6228298 MHz

======== CHANNEL f2 ========
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NUC2   1H
P3   9.60 usec
p4   19.20 usec
PCPD2   100.00 usec
PL2   0 dB
PL12   20.00 dB
SFO2   400.1316005 MHz

F2 - Processing parameters
SI   32768
SF   100.6127690 MHz
WDW   no
SSB   0
LB   0 Hz
GB   0
PC   1.40

160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

Figure SM 4.1.2.6.3: DEPT 135º (DMSO-d_6) of *N'*-(*E*)-(4 phenoxybenzylidene)isonicotinohydrazide

Figure SM 4.1.2.6.4: COSY expansion (DMSO-d_6) of *N'*-(*E*)-(4-phenoxybenzylidene)isonicotinohydrazide

Figure SM 4.1.2.6.5: HMQC expansion (DMSO-d_6) of *N'*-(*E*)-(4-phenoxybenzylidene)isonicotinohydrazide

Figure SM 4.1.2.6.6: HMBC expansion (DMSO-d_6) of *N'*-(*E*)-(4-phenoxybenzylidene)isonicotinohydrazide

Figure SM 4.1.2.6.7: NOESY expansion (DMSO-d_6) of *N'*-(*E*)-(4-phenoxybenzylidene)isonicotinohydrazide

[1] M. J. Deetz, J. E. Fahey and B. D. Smith, *J. Phys. Org. Chem.,* 2001, **14**, 463.

Preparation of a tosylhydrazidyl *N*-glycosyl derivative of D-glucuronic acid via tosylhydrazone formation and intramolecular ring closure
Supplementary Material

The purpose of this work is the preparation of a *N*-glucuronyl sulfonohydrazide by reaction of D-glucuronic acid with tosylhydrazide. The experiment illustrates the possibility of an unprotected reducing sugar to be selectively derivatized at C-1 by undergoing a typical reaction of an aldehyde, which displaces the equilibrium between the cyclic hemiacetal and the open-chain aldehyde to the acyclic form. Glucuronic acid is reacted with a sulfonohydrazide in the presence of acetic acid to form the corresponding hydrazone, which evolves preferentially to the *N*-glucuropyranosyl hydrazide derivative by ring closure. The reaction conditions are mild and the reagents are readily-accessed. The experimental procedure is simple and does not require chromatographic purification to afford the desired product in high purity. The students will carry out the NMR analysis of a carbohydrate derivative, making use of different and complementary NMR experiments (one- and two-dimensional methods) to do a complete structural assignment. The experiment is therefore suitable for students of organic chemistry at the intermediate level with basic background on carbohydrate chemistry and good knowledge and practical experience with NMR spectroscopy.

Three experiments were performed with reproducible findings by the author of this protocol, which was then given to an undergraduate chemistry student who had already completed two semesters of organic chemistry lecture and laboratory training. The student could carry out the experiment with no particular difficulties and confirmed the reproducibility of results.

The reactions were conducted starting from 300 mg and 500 mg of glucuronic acid. No additional tricks are needed to perform the experiment, due to the simplicity of the protocol. It is, however, noteworthy to mention that solvent removal under vacuum (under ca. 10 mBar) takes ca. 30 min and the resulting sticky residue should be vigorously stirred in diethyl ether/dichloromethane (and triturated, if needed) so that a suspension containing powder is obtained.

The product, a novel compound whose synthesis is here reported for the first time, was obtained in yields ranging from 67 to 74% as a pale yellow solid, which is stable under ambient conditions. The student obtained a yield of 70%.

NMR data for 1-(2-tosylhydrazin-1-yl)-β-D-glucopyranuronic acid:

^{1}H NMR (400 MHz, MeOD) δ 7.78 (d, 2 H, H-a, J = 8.0, Ts), 7.39 (d, 2 H, H-b, Ts), 3.85 (d, 1 H, H-1, $J_{1,2}$ = 8.8), 3.70 (d, 1 H, H-5, $J_{4,5}$ = 9.7), 3.55-3.43 (m, 2 H, H-2, H-4), 3.39 (t, 1 H, H-3, $J_{2,3}$ = $J_{3,4}$ = 8.9), 2.43 (s, 3 H, Me, Ts).

^{13}C NMR (100 MHz, MeOD) $δ$: 173.4 (CO), 145.2 (Cq, Ts), 137.2 (Cq, Ts), 130.6, 129.0 (CH, Ts), 91.9 (C-1), 77.6, 77.5 (C-3, C-5), 73.2, 71.0 (C-2, C-4), 21.5 (CH_3, Ts).

Melting point measurements: 95.8-97.6 °C; 96.2-98.5 °C; 97.2-99.0 °C.

Figure SM 4.1.2.7.1. Sample of 1-(2-tosylhydrazin-1-yl)-β-D-glucopyranuronic acid.

NMR spectra: ^{1}H NMR, ^{13}C NMR and Two Dimensional Spectra (COSY and HMQC)

Figure SM 4.1.2.7.2. ^{1}H NMR Spectrum of 1-(2-tosylhydrazin-1-yl)-β-D-glucopyranuronic acid (MeOD, 400 MHz).

Figure SM 4.1.2.7.3. Key correlations observed between the protons of the sugar moiety in the COSY spectrum of 1-(2-tosylhydrazin-1-yl)-β-D-glucopyranuronic acid (MeOD, 400 MHz).

NMX_76

173.35

145.16

137.15
130.63
129.03

91.88

77.58
77.54
73.18
71.04

21.50

f1 (ppm)

Figure SM 4.1.2.7.4. ^{13}C NMR Spectrum of 1-(2-tosylhydrazin-1-yl)-β-D-glucopyranuronic acid (MeOD, 100 MHz).

Figure SM 4.1.2.7.5. HMQC Spectrum of 1-(2-tosylhydrazin-1-yl)-β-D-glucopyranuronic acid (MeOD, 400 MHz).