Synthesis of 2-(5'-phenylthien-2'-yl)benzothiazole

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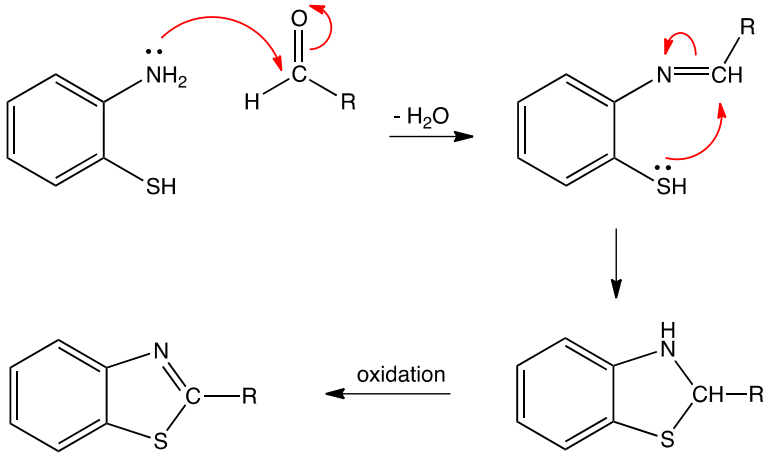
1. Experiment notes

This experiment involves simple experimental techniques and commercially available reagents, and it is expected that the students possess previously acquired practical skills (in terms of isolation and purification techniques) and theoretical background (synthesis, reactivity and spectroscopic data interpretation). Therefore, this experiment may be appropriate for last year project in Chemistry degree or as the practical component of chemistry subjects at Master level.

The purpose of this experiment is the synthesis of 2-(5'-phenylthien-2'-yl) benzothiazole from the condensation of an aldehyde with 2-aminobenzenethiol. The reaction is initiated by the formation of the corresponding imine, by nucleophilic attack of the nitrogen to the carbon of the carbonyl group, that cyclises spontaneously, yielding the intermediate benzothiazoline, which is oxidised to the benzothiazole (Scheme SM 12.2.1.1).



Scheme SM 12.2.1.1. Mechanism of the intramolecular oxidative cyclization.

The use of DMSO as reaction solvent avoids the use of an oxidizing agent as DMSO promotes the conversion of the intermediate benzothiazoline to benzothiazole due to its oxidant character. Also, DMSO has a high boiling point and conducting the reaction at high temperature favours the formation of the desired product in shorter reaction time and elimination of water as the reaction by-product.

Samples dissolved in DMSO cannot be as easily recovered compared to other solvents, as it is very difficult to remove all traces of DMSO by conventional rotary evaporation. In this experiment, liquid-liquid extraction is used to fully recover the product: the reaction mixture is diluted with water (some precipitation of the product may occur) and extracted with ethyl acetate (the precipitate dissolves in ethyl acetate). The column chromatography on silica gel can be carried out with petroleum ether 40-60, as described, or with hexanes.

Using the present reaction conditions, 2-(5'-phenylthien-2'-yl) benzothiazole was prepared in 90% yield with m.p. 149.5-150.8 ºC.[1] In these conditions, in various repetitions carried out by third year Chemistry students, the product was obtained in 88-93% yield as a off-white solid.

This synthetic method has been applied in the preparation of other functionalized thienylaryl, (oligo)thienyl, and thienylpyrrolyl benzothiazoles, from the corresponding aldehydes and 2-aminobenzenethiol, in good yields by third year Chemistry students.[2]
The yield for the various derivatives was influenced by the electronic character of the substituents at the heterocyclic ring bearing the aldehyde and was higher for the derivatives with electron-withdrawing groups, as expected from the mechanism.
As for the influence of substituent groups on the 2-aminobenzenethiol, it was confirmed that electron-donating groups at suitable positions resulted in higher yields, due to an increase of electron density at the nitrogen atom of the amino group, thus increasing its nucleophilicity.

The title benzothiazole could be synthesised by using other reagents mentioned in the background section of this experiment, for example from 2-aminobenzenethiol and an adequate carboxylic acid, acyl chloride or nitrile, instead of the aldehyde.

2. Figures

2.1. ^{1}H NMR spectra

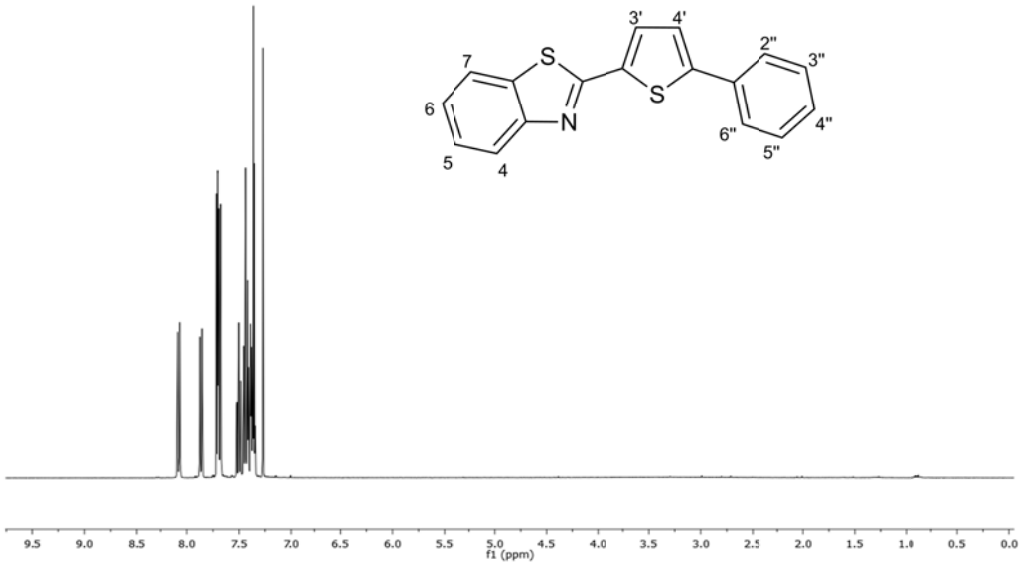


Figure SM 12.2.1.**1** – ^{1}H NMR spectrum of 2-(5'-phenylthien-2'-yl) benzothiazole in $CDCl_3$, obtained using a Bruker Avance III spectrometer operating at 400 MHz at 25ºC.

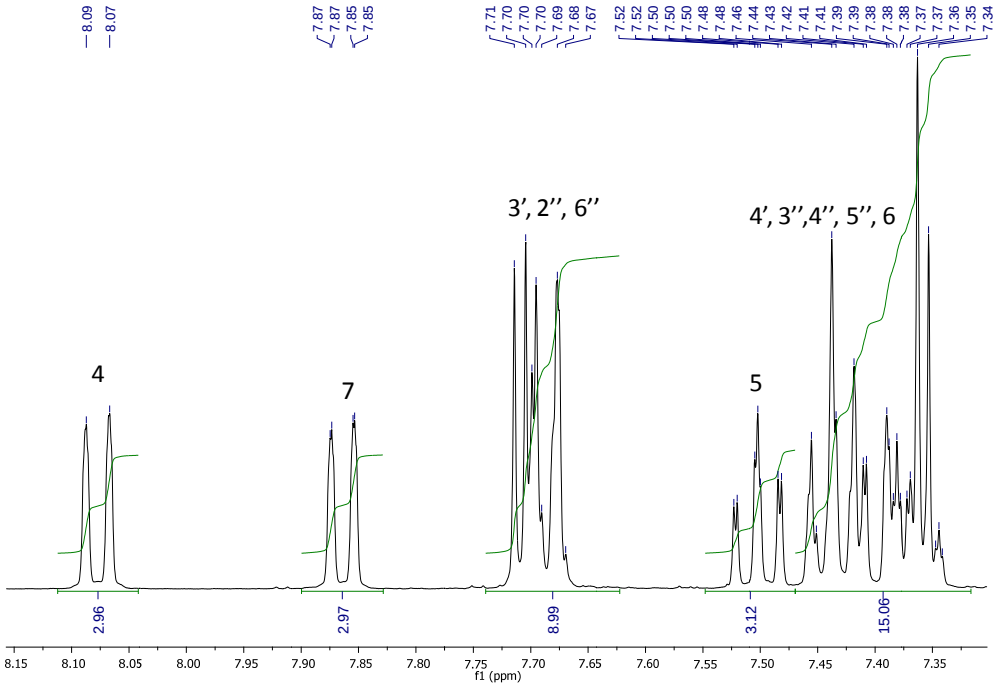


Figure SM 12.2.1.**2** – Expansion of the aromatic zone of the ^{1}H NMR spectrum of 2-(5'-phenylthien-2'-yl)benzothiazole in $CDCl_3$.

2.2 ^{13}C NMR spectra

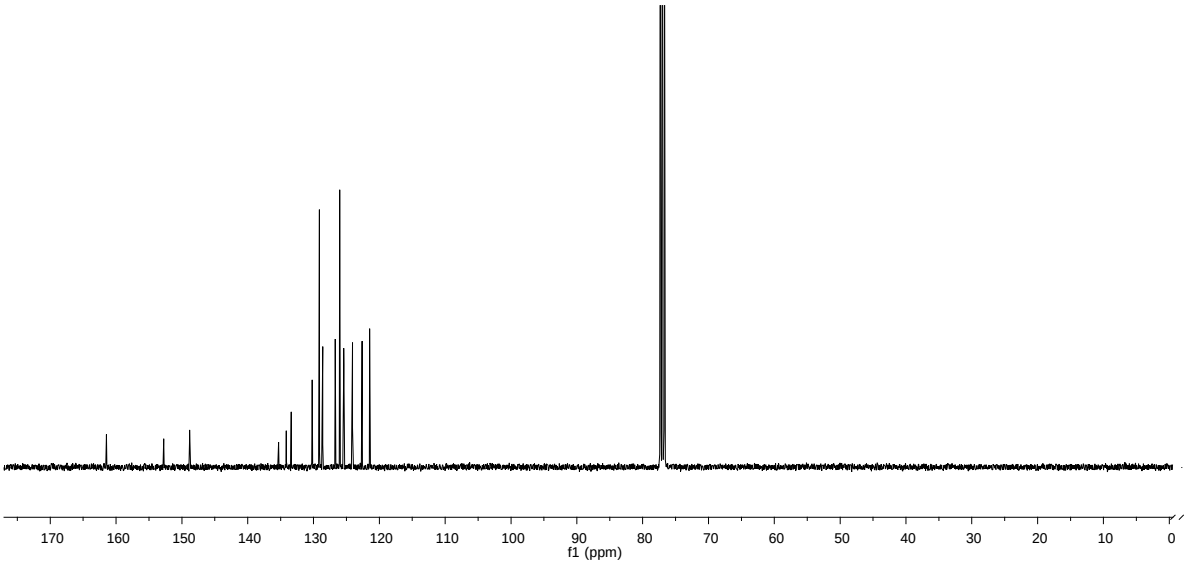


Figure SM 12.2.1.**3** – ^{13}C NMR spectrum of 2-(5'-phenylthien-2'-yl)benzothiazole in $CDCl_3$, obtained using a Bruker Avance III spectrometer operating at 100.6 MHz at 25ºC.

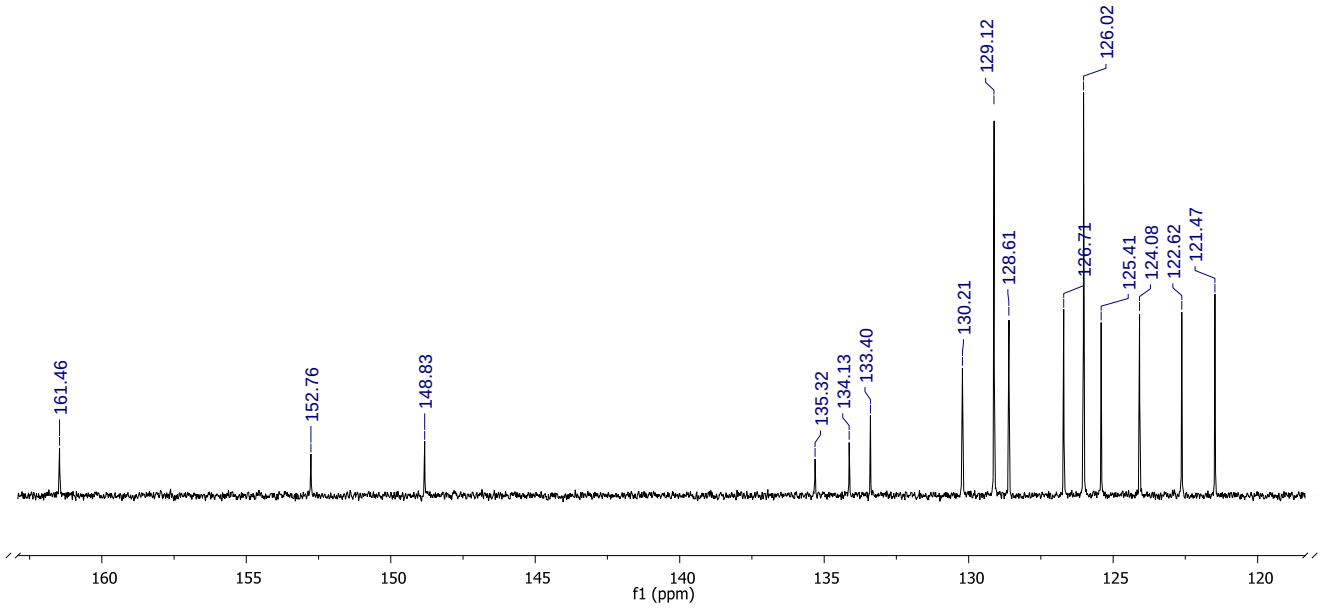


Figure SM 12.2.1.**4** – Expansion of the ^{13}C NMR spectrum of 2-(5'-phenylthien-2'-yl)benzothiazole $CDCl_3$.

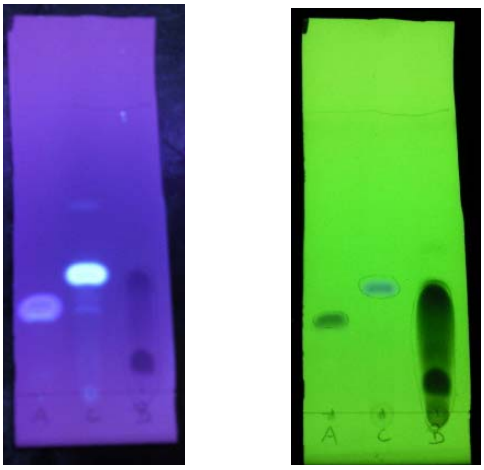
2.3. Photos of reaction and purification setups

Figure SM 12.2.1.**5** – Reaction setup for the intramolecular oxidative cyclisation reaction (laboratory session 1).

Figure SM 12.2.1.**6** – Liquid-liquid extraction setup for the isolation of the product (laboratory session 1).

2.4. Photo of the TLC plate

The course of the reaction and the contents of the column chromatography fractions were checked by TLC. The spots on the TLC plate were visualized under a UV lamp and the formation of the product was easily detected by its high fluorescence, when compared to the reagents. Although the aminothiol has a similar retention factor, its appearance under the UV lamp is completely different (it leaves a non-fluorescent spot with a tail upon elution).



	Retention factors, R_f
A – reagent: 5-phenyl-2-thiophenecarboxaldehyde	$R_f = 0.30$
B – reagent: 2-aminobenzenethiol	$R_f = 0.38$
C – product: 2-(5'-phenylthien-2'-yl)benzothiazole	$R_f = 0.36$

Figure SM 12.2.1.**6** – Photo of the TLC plate with the reagents and product (eluent: dichloromethane/petroleum ether 1:1): visualization under a 365 nm lamp (left) or a 254 nm lamp (right).

References

[1] S. P. G. Costa, J. A. Ferreira, G. Kirsch, A. M. F. Oliveira-Campos, *J. Chem. Res.*, 1997, (*S*) 314-315; (*M*) 2001-2013.

[2] a) R. M. F. Batista, S. P. G. Costa, M. M. M. Raposo, *Tetrahedron Lett*., 2004, **45**, 2825. b) S. P. G. Costa, R. M. F. Batista, P. Cardoso, M. Belsley, M. M. M. Raposo, *Eur. J. Org. Chem*., 2006, 3938. c) R. M. F. Batista, S. P. G. Costa, E. L. Malheiro, M. Belsley, M. M. M. Raposo, *Tetrahedron*, 2007, **63**, 4258.

Synthesis of *N-tert*-butyloxycarbonyl-[2-(thien-2'-yl)benzoxazol-5-yl]-L-alanine methyl ester

Supplementary Material

1. Experiment notes 1

2. Figures 3

^{1}H NMR spectra of the prepared compounds 3

^{13}C NMR spectra of the prepared compounds 4

IR spectra of the prepared compounds 5

1. Experiment notes

This experiment involves simple experimental techniques and commercially available reagents, and it is expected that the students possess previously acquired practical skills (in terms of isolation and purification techniques) and theoretical background (synthesis, reactivity and spectroscopic data interpretation). This experiment has been carried out in various occasions by third year Chemistry degree students and first year Chemistry MSc students, with final product yield ranging between 90-95%. This experiment has also been replicated with different aldehydes and the procedure and results are consistent (very high final product yield).

The aim of this work is the synthesis of a heterocyclic amino acid in two sessions, starting from a previously synthesised 3-aminotyrosine derivative (experiment 3.1.14) that will be reacted with an aldehyde, resulting an intermediate imine. The benzoxazolyl moiety will be obtained through an oxidative intramolecular cyclization of a phenol derivative bearing an imine, in the presence of $Pb(OAc)_4$ as the oxidant. The protecting groups at the amino and carboxylic acid groups (*tert*-butyloxycarbonyl and methyl ester) are compatible with the reaction conditions and thus unaffected.

<u>Session 1: Preparation of *N-tert*-butyloxycarbonyl-3-[(thien-2'-ylmethylene)amino]-L-tyrosine methyl ester</u>

Condensation of *N-tert*-butyloxycarbonyl-3-amino-L-tyrosine methyl ester with the aldehyde yields the intermediate imine by nucleophilic substitution with loss of a water molecule.

It is important to perform the reaction in anhydrous conditions to prevent hydrolysis of the imine and it is also required to store it in dry conditions. This imine is also sensitive to silica

gel and to minimize degradation the imine crude does not require purification. Conversion to the imine is quantitative as confirmed by ^{1}H NMR (see spectra).

This experiment has been scaled up to 10 mmol of the starting aminotyrosine with reproducible results by repetitive execution.

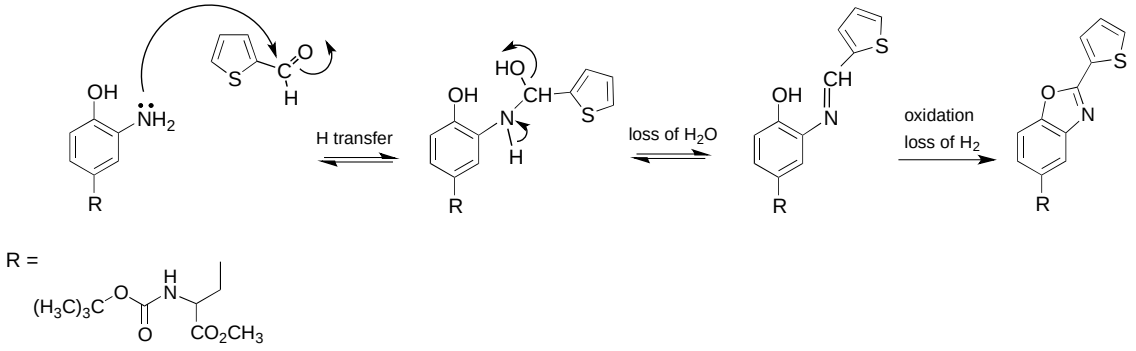
Session 2: Synthesis of *N-tert*-butyloxycarbonyl [2-(thien-2'-yl)benzoxazol-5-yl]-L-alanine methyl ester

The previously prepared imine suffers intramolecular cyclization by nucleophilic attack of the hydroxyl oxygen on the imine carbon. The action of $Pb(OAc)_4$, aided by the oxidant character of DMSO as solvent, results in aromatization and a new oxazole ring fused to the phenyl is formed.

When performing the chromatography, the final product (benzoxazolyl-alanine) will appear on the TLC plate as a highly fluorescent spot, due to the presence of the benzoxazole unit in its structure.

In the present conditions the final compound was isolated as off-white solid in 90% yield, with melting point 106.6-107.8 ºC. This experiment has been scaled up to 10 mmol of the reagents with reproducible results by repetitive execution.

The mechanism for both steps is given in Scheme SM 12.2.2.1.



Scheme SM 12.2.2.1. Mechanism for the formation of imine (session 1) and oxidative cyclization (session 2).

Lead tetraacetate should be kept in the refrigerator, and taken out only for rapid weighing, as acetic acid fumes may evolve if left too long out of the refrigerator. Handling of lead tetraacetate requires caution as it a toxic hazard and special care should be taken in discarding it in appropriate containers.

^{1}H NMR spectroscopy

In the NMR spectra, the students can see the influence of:

- the electronic effects on the ^{1}H chemical shift by comparing the spectra of the starting aminotyrosine (obtained previously in experiment 3.1.14), the intermediate imine and the final benzoxazole;
- the electronic effects on the ^{13}C chemical shift by comparing the spectra of the starting aminotyrosine (obtained previously in experiment 3.1.14), the intermediate imine and the final benzoxazole.

IR spectroscopy

In the IR spectra, the students can see the influence of the electronic effects on wavenumbers of:

- the NH stretching band by comparing the spectra of the reagent (amine), intermediate imine and final benzoxazole.

2. Figures

2.1 ^{1}H NMR spectrum of the crude imine intermediate

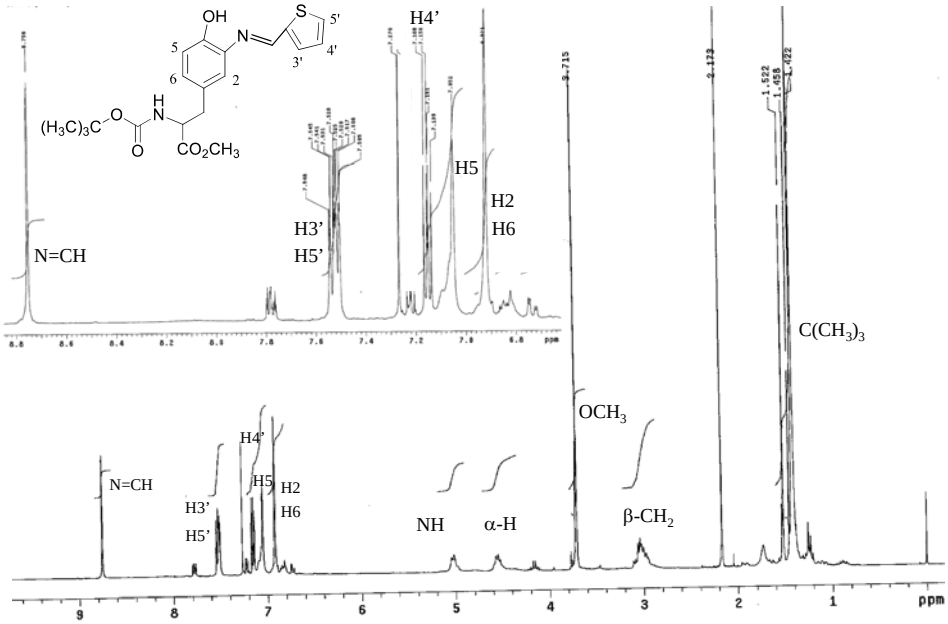


Figure SM 12.2.2.1. ^{1}H NMR of the crude imine in $CDCl_3$ obtained in a Varian Unity Plus spectrometer operating at 300 MHz at 25ºC.

2.2 ^{1}H NMR spectrum of final benzoxazolyl-alanine

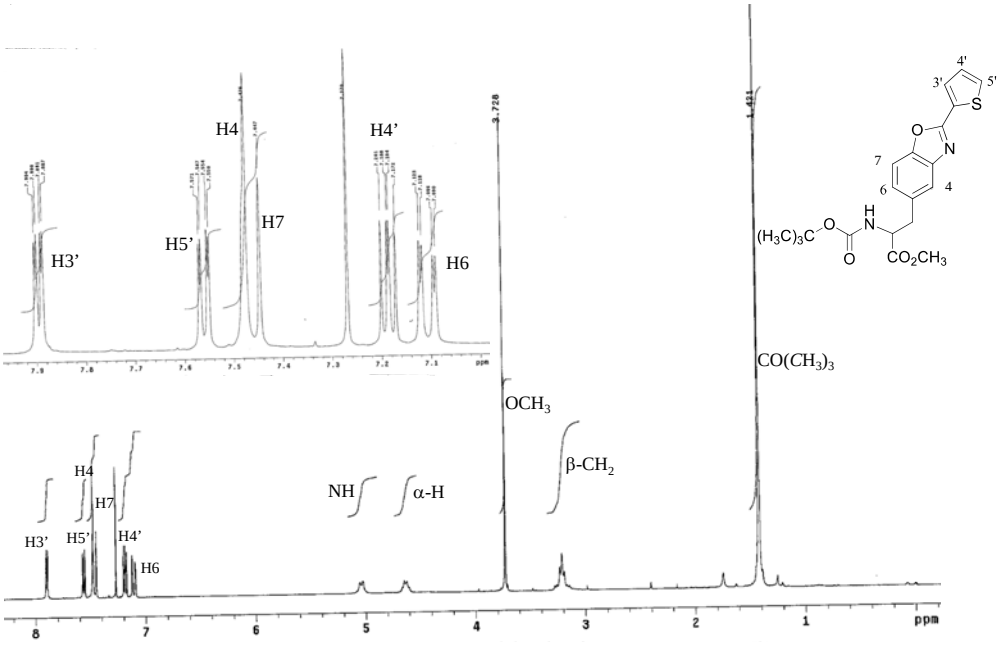


Figure SM 12.2.2.2 ^{1}H NMR of benzoxazolyl-alanine in $CDCl_3$ obtained in a Varian Unity Plus spectrometer operating at 25ºC.

2.3 ^{13}C NMR spectrum of the crude imine intermediate

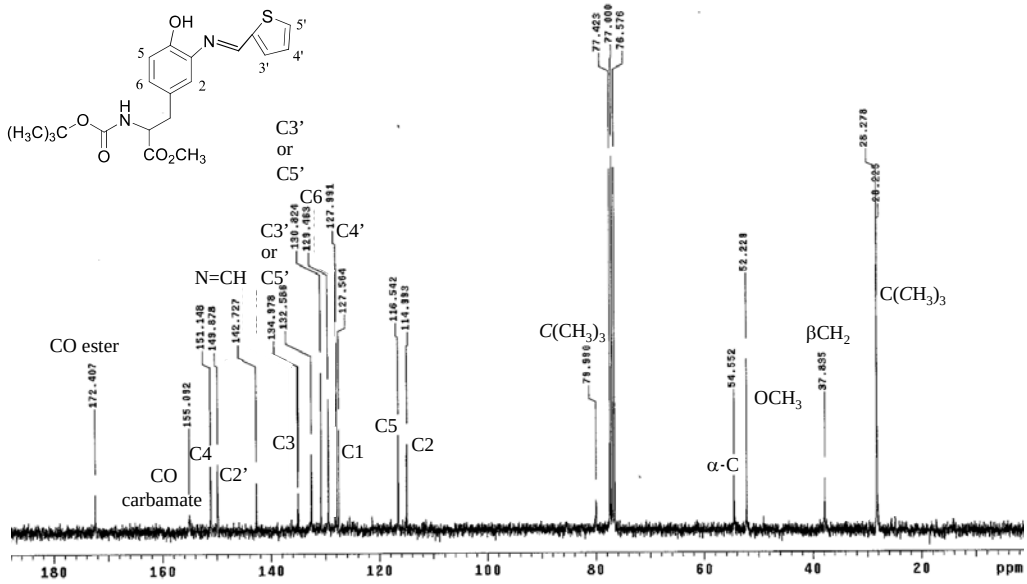


Figure SM 12.2.2.3. ^{13}C NMR of the crude imine in $CDCl_3$ obtained in a Varian Unity Plus spectrometer operating at 75.4 MHz at 25ºC.

2.4 ^{13}C NMR spectrum of final benzoxazolyl-alanine

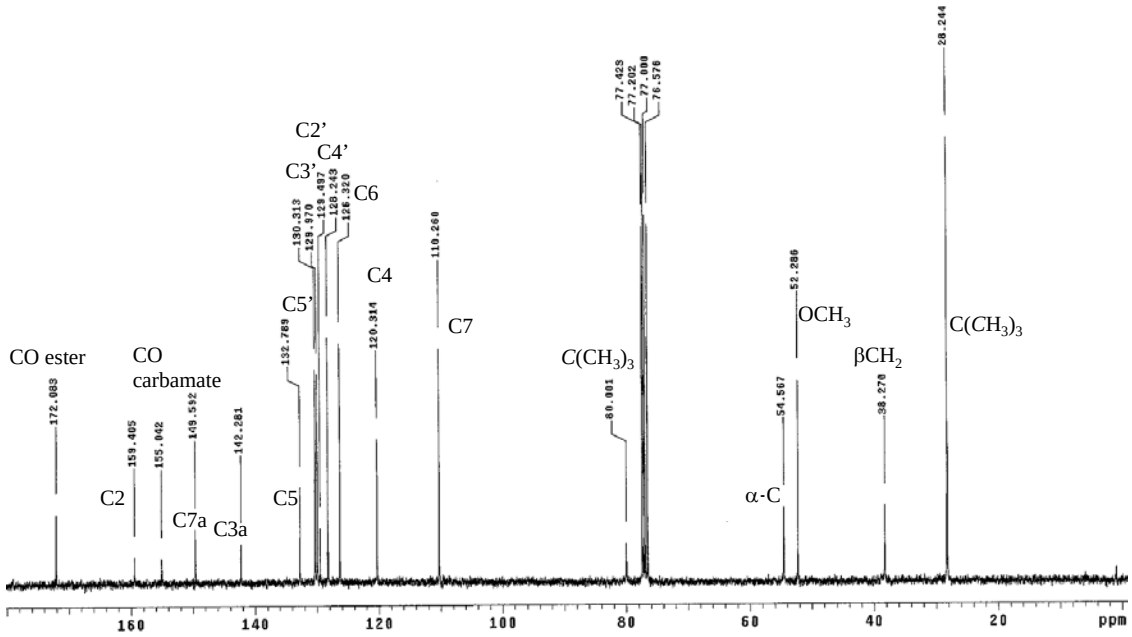


Figure SM 12.2.2.4. ^{13}C NMR of benzoxazolyl-alanine in $CDCl_3$ obtained in a Varian Unity Plus spectrometer operating at 75.4 MHz at 25ºC.

2.5 IR spectrum of the crude imine intermediate

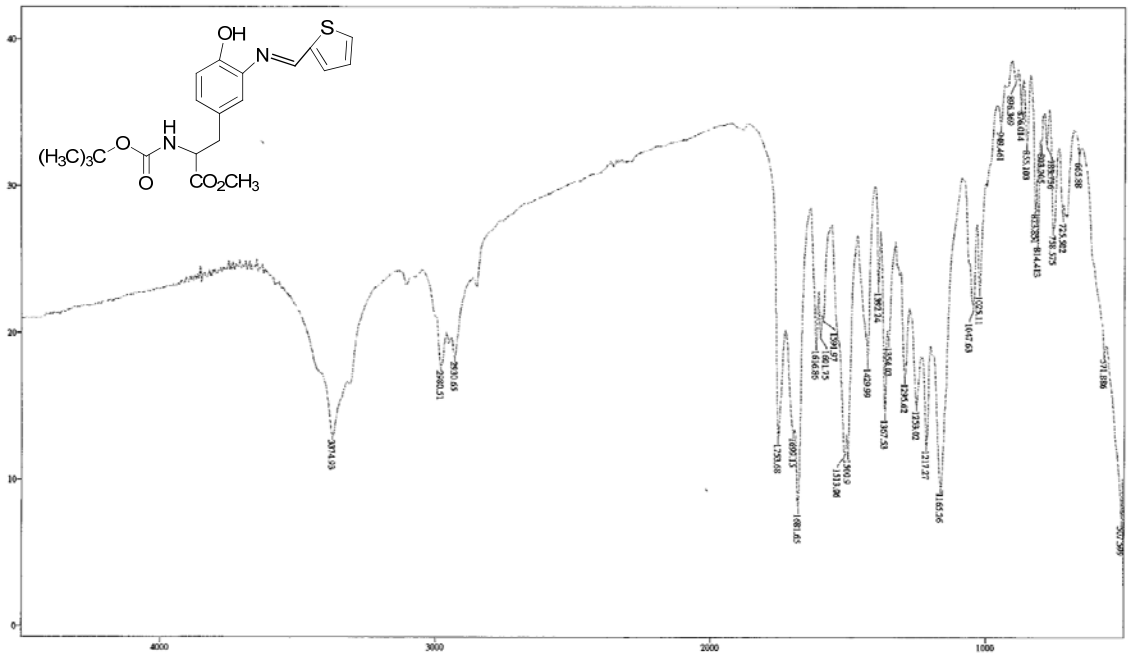


Figure SM 12.2.2.5. IR spectrum of crude imine in KBr disc obtained in a Perkin Elmer FTIR-1600 spectrophotometer.

2.6 IR spectrum of the final benzoxazolyl-alanine

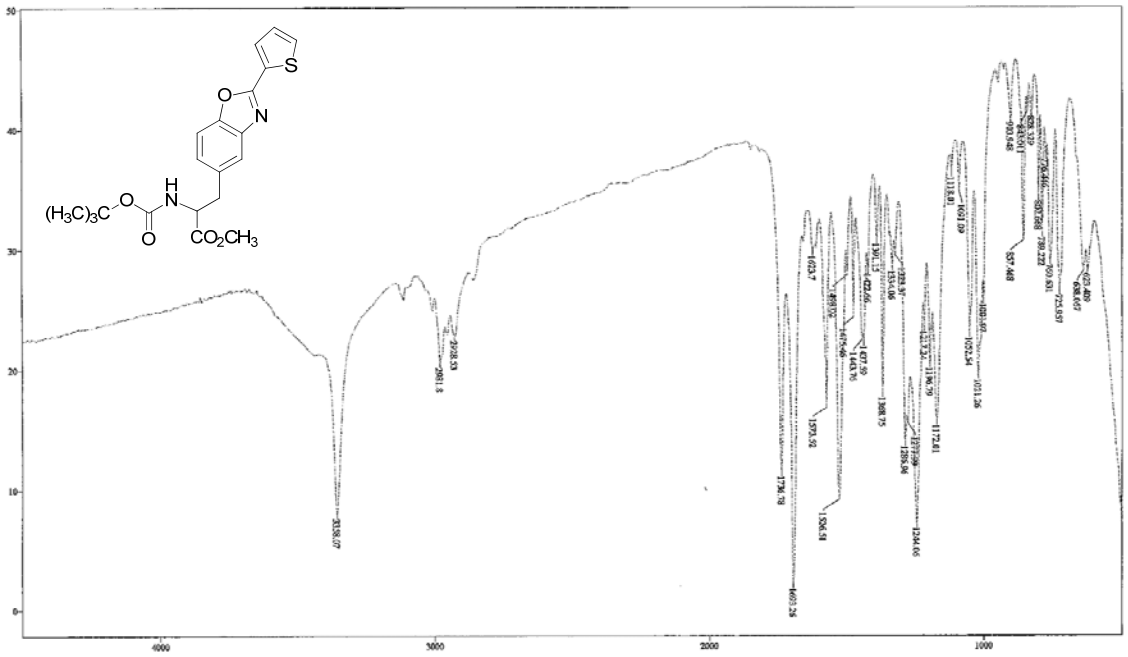


Figure SM 12.2.2.6. IR spectrum of benzoxazolyl-alanine in KBr disc obtained in a Perkin Elmer FTIR-1600 spectrophotometer.

Synthesis of Flavones (2-aryl-4*H*-chromen-4-ones)

Supplementary Material

In this work, which is planned for 1 session or 2 sessions 3 hours each, students (individually or in groups of two) will synthesize flavone derivatives by the cyclodehydrogenation reaction of 2'-hydroxychalcones.

The desired product is obtained directly by filtration or its purification can be used to illustrate chromatographic techniques (in a second session).

The flavone derivatives show different colours depending of the substituent present in the aromatic ring (from R= H, white to R= NO_2 more yellow).

Nearly 500 students performed this experiment and in the following table the average yields and melting points are indicated (Table).

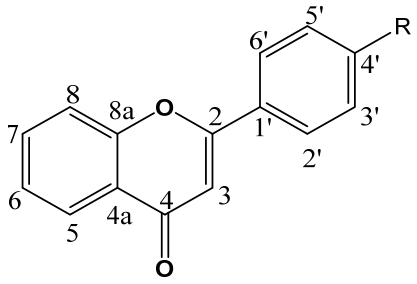


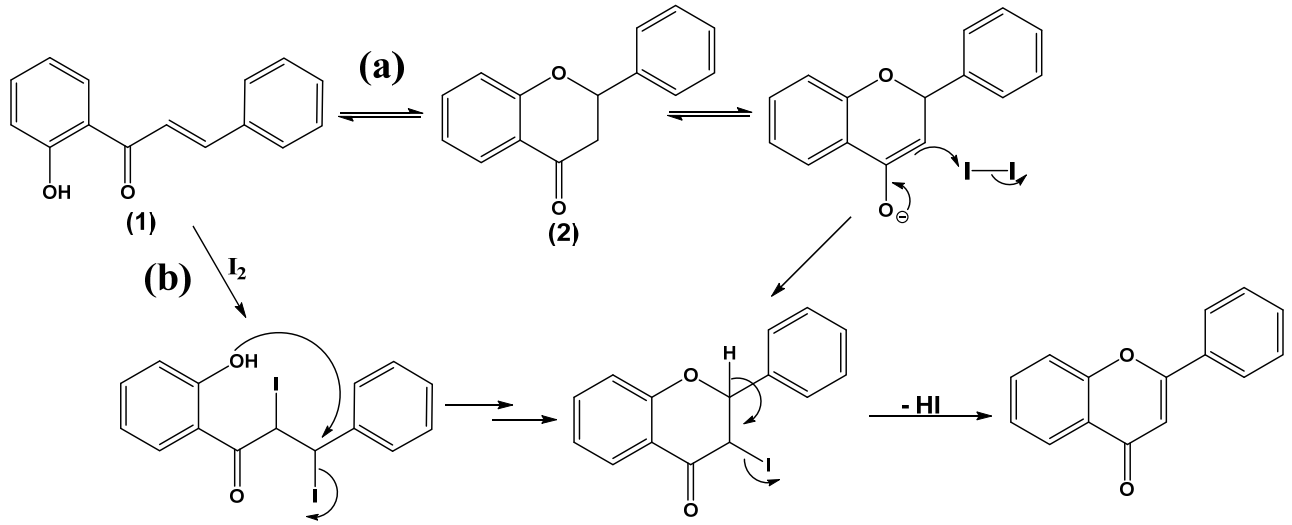
Figure SM 12.2.3.1- Flavones structure

Figure SM 12.2.3.2 - Reaction setup apparatus.

Table of Results: Reaction Yield and Melting Point of some flavones

Entry	Substituent R	Yield of the crude (%)	Melting point (°C)
1	H	90-97	93-95
2	OCH_3	95-97	156-158
3	NO_2	65-75	242-244

This experimental work illustrates the intramolecular cyclisation of organic compounds and simultaneously an *in situ* oxidation. 2'-Hydroxychalcone (**1**) can in slightly acidic conditions isomerize to flavanone (**2**) (Scheme SM **12.2.3.1**). Due to the acidity of α-carbonyl protons a α-iodination of flavanone can occur and a subsequent HI elimination giving the desired flavone (**3**) (Scheme SM **12.2.3.1** - step a). Another possibility is an electrophilic addition of iodine to the 2'-hydroxychalcone double bond followed by intramolecular cyclisation into the 3-iodoflavanone (Scheme SM **12.2.3.1** - step b).



Scheme SM 12.2.3.1- Reaction setup apparatus.

The ^{1}H and ^{13}C NMR spectra of the obtained products are also given herein as examples. The most important aspects in the NMR analysis are the presence of a singlet due to proton H-3 (^{1}H NMR) and the signal due to the carbonyl group carbon C-4 (^{13}C NMR). The deshielding mesomeric and anisotropic effect of the carbonyl group in the resonance of H-5 of the flavone structure is an important aspect to be explored by the students. The deshielding or shielding effects of the proposed substituents on *ortho*-protons can also be explored. In the following figures are given, as examples, the ^{1}H and ^{13}C NMR spectra of flavone (2-phenyl-4*H*-chromen-4-one) and 4'-methoxyflavone [2-(4-methoxyphenyl)-4*H*-chromen-4-one].

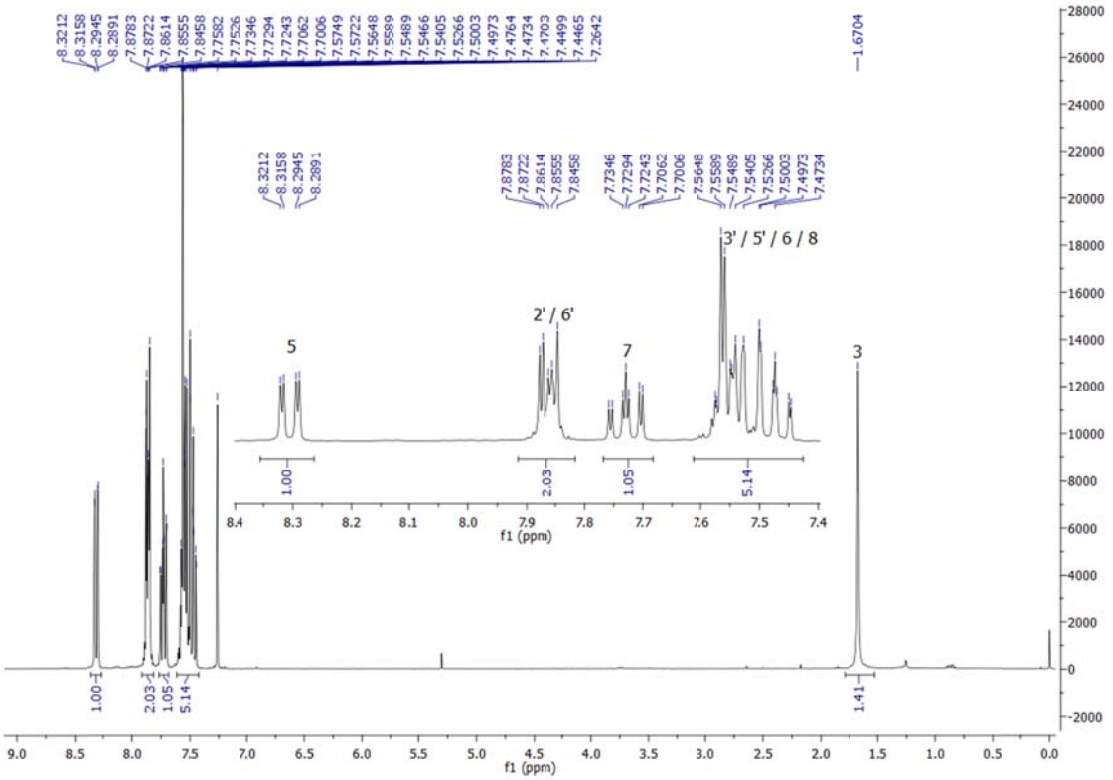


Figure SM 12.2.3.3 - ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the flavone (R = H).

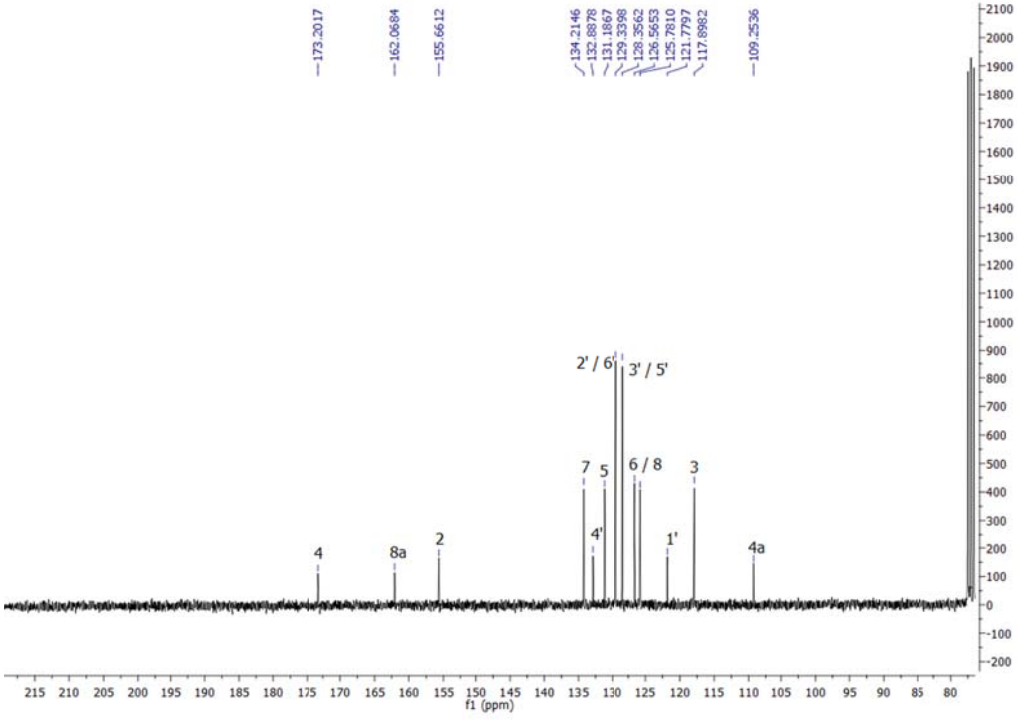


Figure SM 12.2.3.4 - ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of the flavone (R = H).

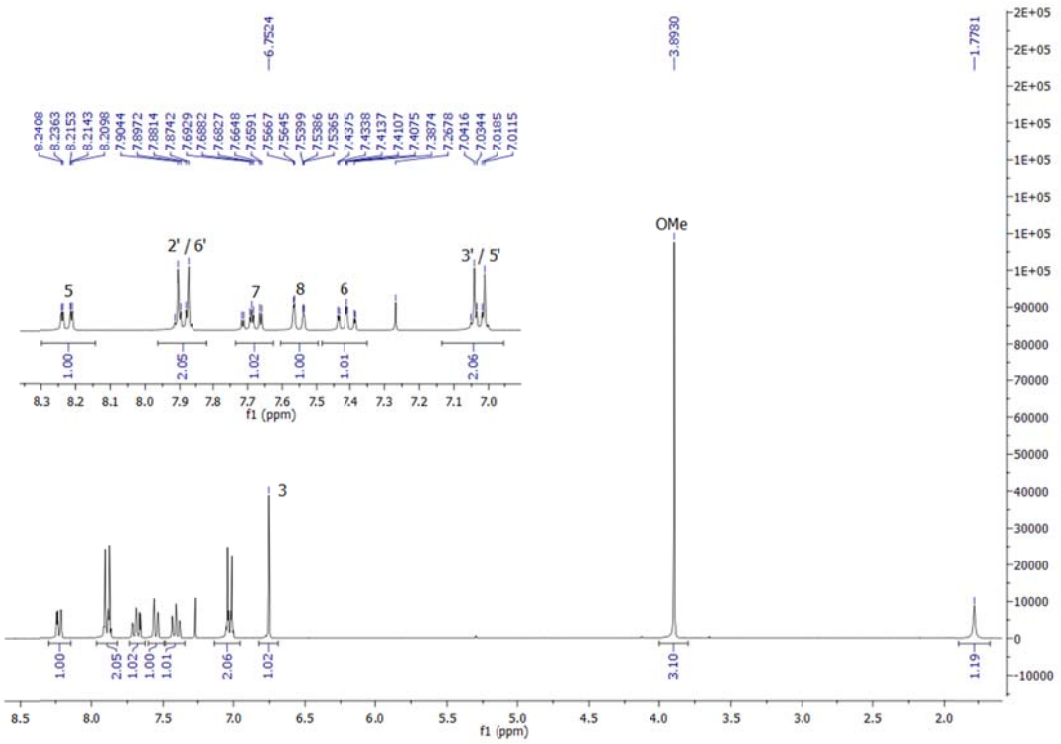


Figure SM 12.2.3.5 - ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the 4'-methoxyflavone (R = OCH_3).

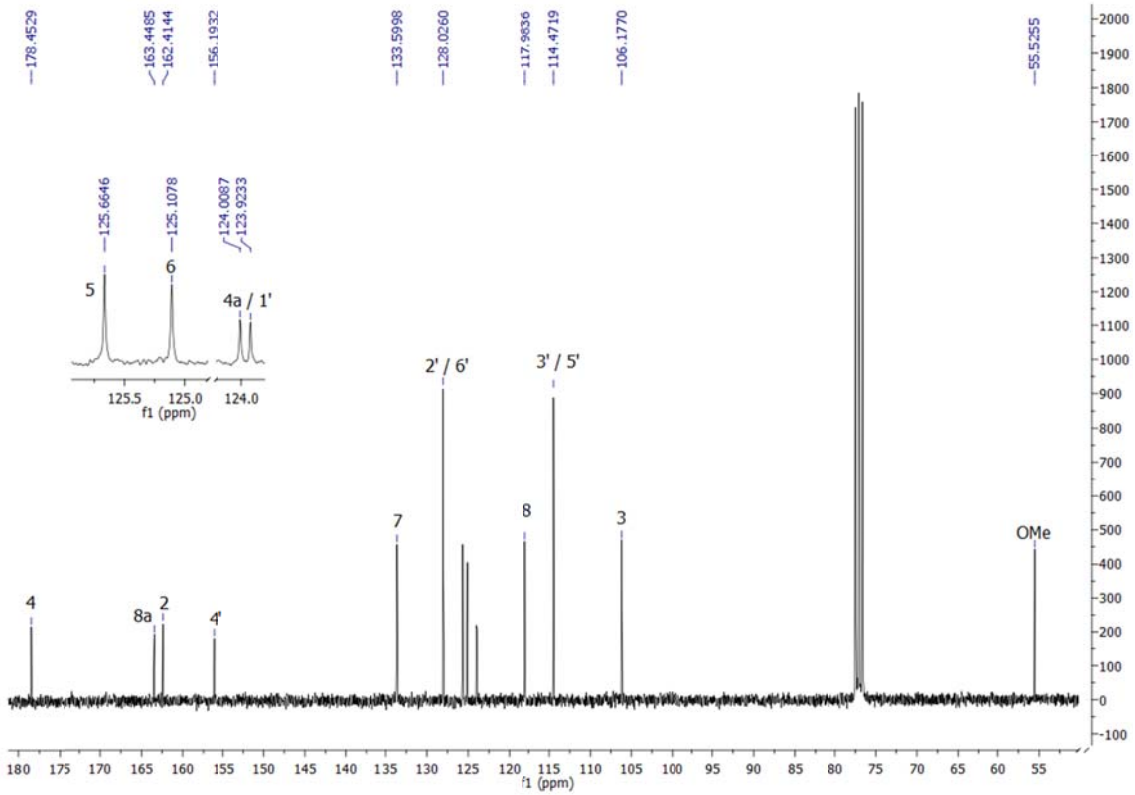


Figure SM 12.2.3.6 - ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of the 4'-methoxyflavone (R = OCH_3).

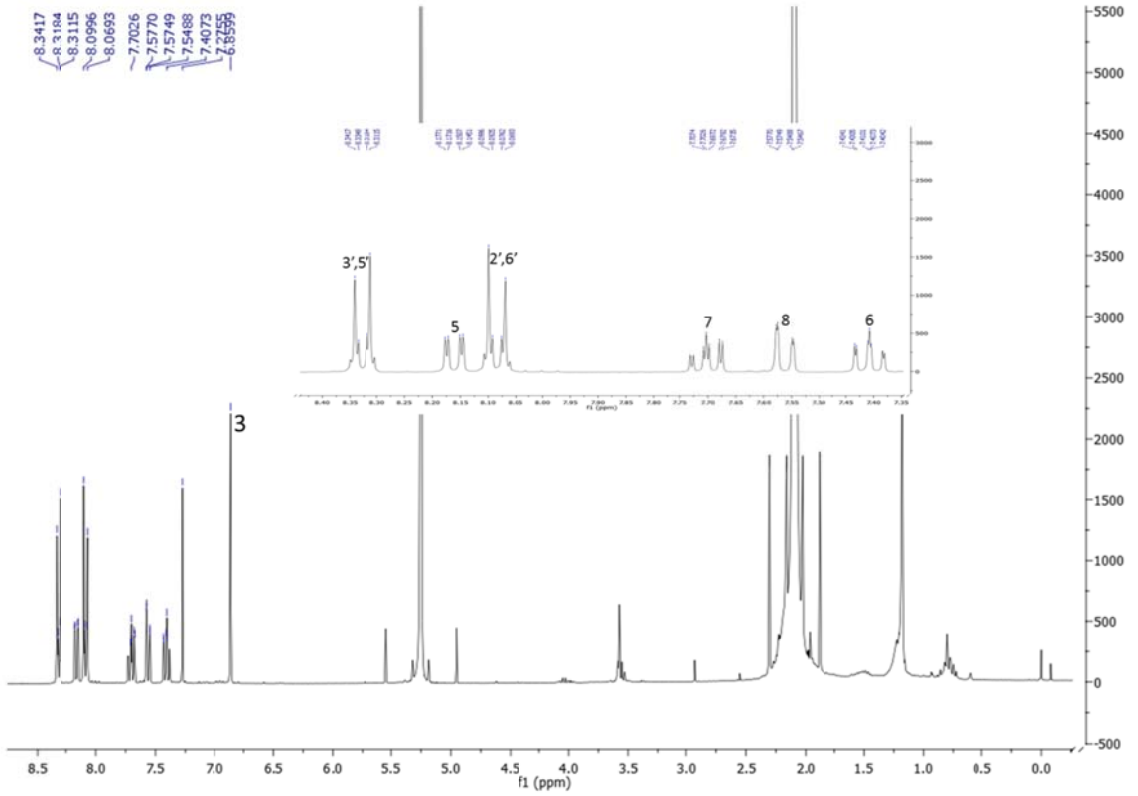


Figure SM 12.2.3.7 - ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the 4'-nitroflavone (R = NO_2).

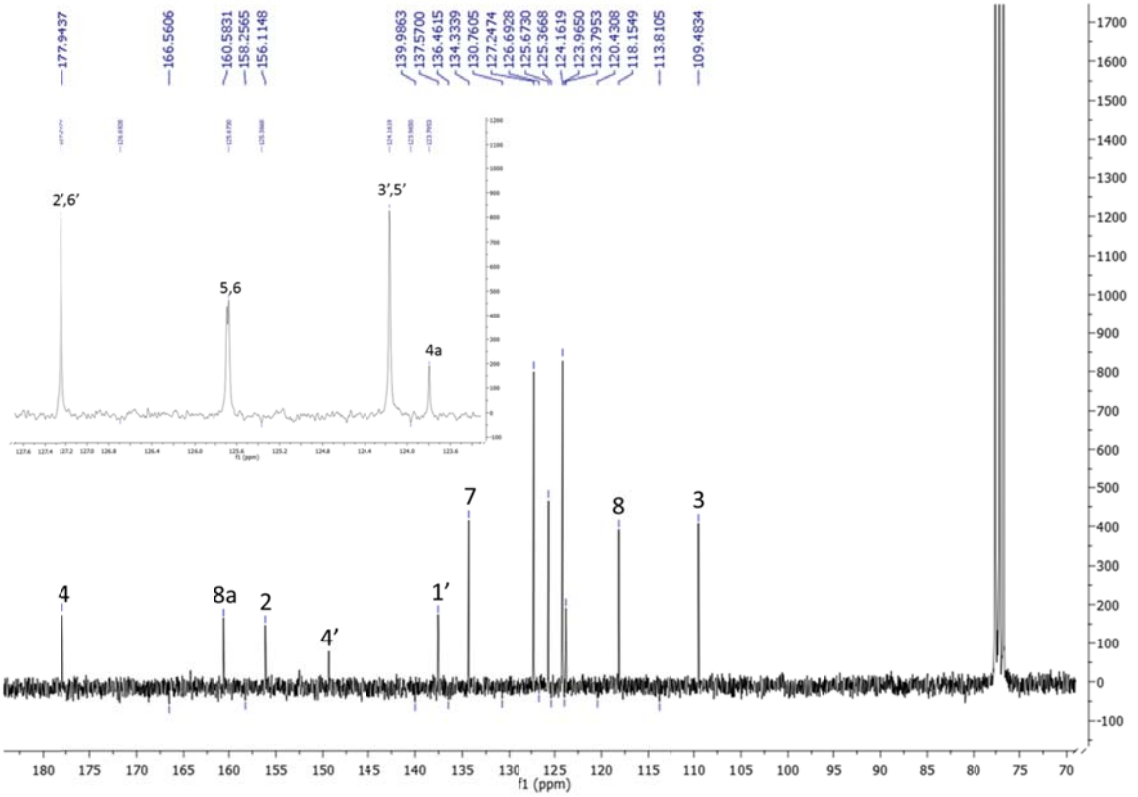


Figure SM 12.2.3.8 - ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of the 4'-nitroflavone (R = NO_2).

Hantzsch Synthesis of 3,5-Diethoxycarbonyl-2,6-dimethylpyridine

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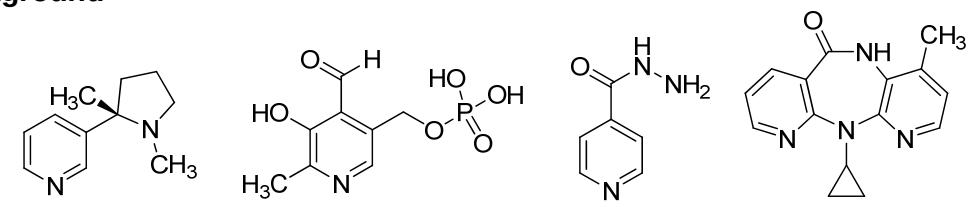
Supplementary Material

Background

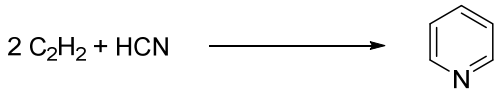


Scheme **12.2.4.**1 Selected examples of compounds containing the pyridine moiety

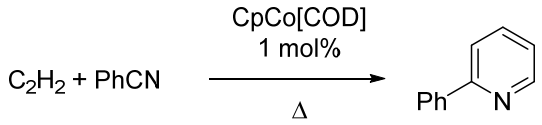
The pyridine ring is a recurring motif in naturally occurring compounds and pharmaceuticals (a selection of some of the more familiar examples is provided in scheme 12.2.4.1).[1,2] Pyridines can be prepared by a variety of routes, a selection of representative examples is shown in scheme 12.2.4.2.[2] Pyridine has been isolated from various sources, coal tar perhaps being the most significant. In 1876, Ramsay reported the first synthesis of pyridine from acetylene and hydrogen cyanide at high temperature,[3] this trimerisation approach has been adapted for modern use by applying transition metal catalysts.[4] The Kröhnke[5] and pyrilium[6] routes are good examples of how varying functionalities and substitution patterns can be installed. One of the oldest and best known methods for making pyridines is the Hantzsch synthesis,[2,7] this is a two-step cyclisation/oxidation process. The first step is a multi-component reaction between a β-keto ester, an aldehyde and an amine (or

ammonia). The product from step one is a 1,4-dihydropyridine, such compounds can be oxidised under relatively mild conditions to afford the corresponding pyridine.

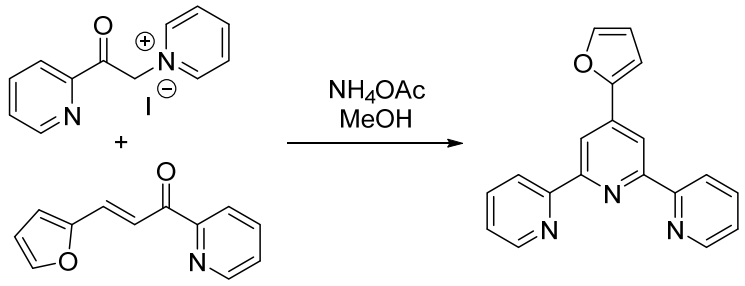
Ramsay Synthesis[3]



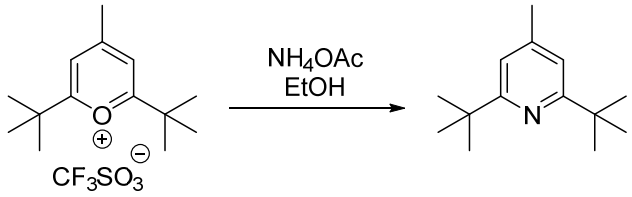
Cobalt Catalysed cyclisation[4]



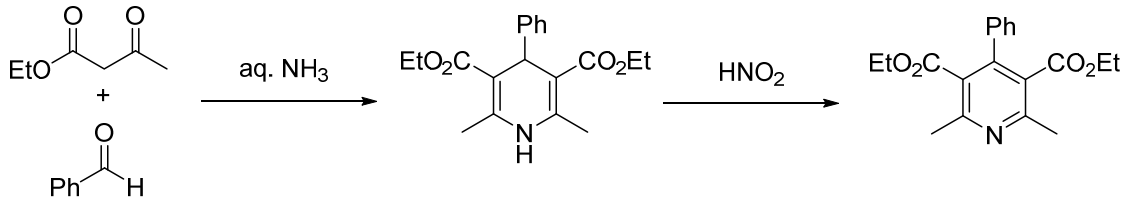
Krohnke Pyridine Synthesis[5]



Trisubstituted Pyridines from Pyrilium Salts[6]



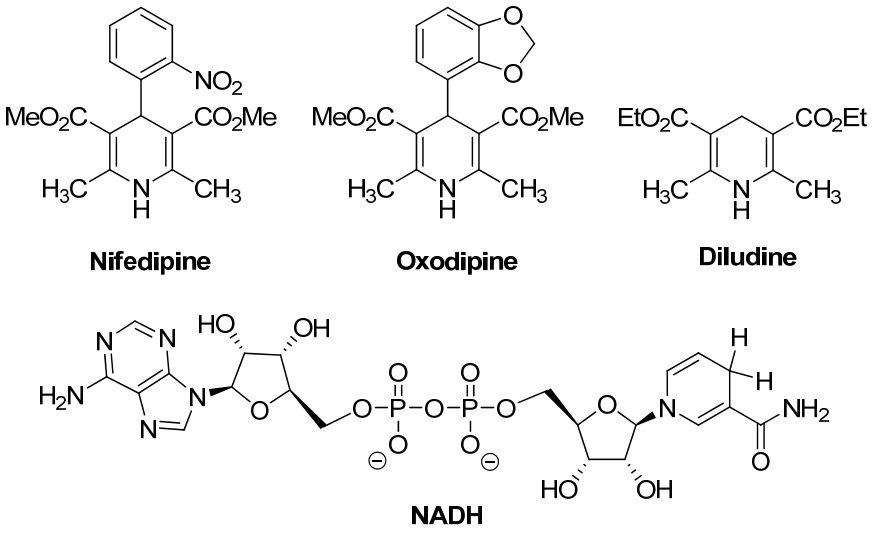
Hantzsch Pyridine Synthesis[7]



Scheme **12.2.4.**2 Selected examples of preparative routes to pyridines

1,4-dihydropyridines are also found in Nature (in the co-factor NADH) and comprise an important family of calcium channel blocking drugs that are used to treat

cardiovascular disease (a selection of some of the more familiar examples is provided in scheme 12.2.4.3).



Scheme **12.2.4.**3 Selected examples of compounds containing the 1,4-dihydropyridine moiety

The aim of this experiment is to prepare Diludine *via* a Hantzsch sequence (originally modified and reported by Dicks *et al*[8]) and then oxidise the product to afford the corresponding pyridine. This experiment is aimed at second or third year undergraduate students who have had training in intermediate to advanced level synthetic organic chemistry work. This experiment has been used routinely in laboratory sessions as part of a synthetic organic chemistry course for 60-70 advanced undergraduate students (third year). The chemistry is directly relevant to lecture material on synthetic and medicinal chemistry; associated lecture courses would be expected to cover aromatic and heterocyclic chemistry. This exercise allows students to practice assembly of appropriate glassware for heating under reflux and vacuum filtration. The students can also be introduced to appropriate manipulation of toxic and corrosive materials (formaldehyde, iodine and urea

hydrogen peroxide). The purification steps involve recrystallistion of products although flash column chromatography can be incorporated if desired. The second stage of the experiment can be monitored by thin layer chromatography and the identity of the products from each section can be confirmed by melting point, IR spectroscopy and ^{1}H NMR spectroscopy.

General notes for preparative steps.

Both steps of the reaction are usually quite straightforward. In the first step it is important to break up the solid crude product well with a glass rod prior to the recrystallization step (in order to aid removal of occluded impurities). Key stages of the reaction are illustrated in figure **12.2.4.**1.

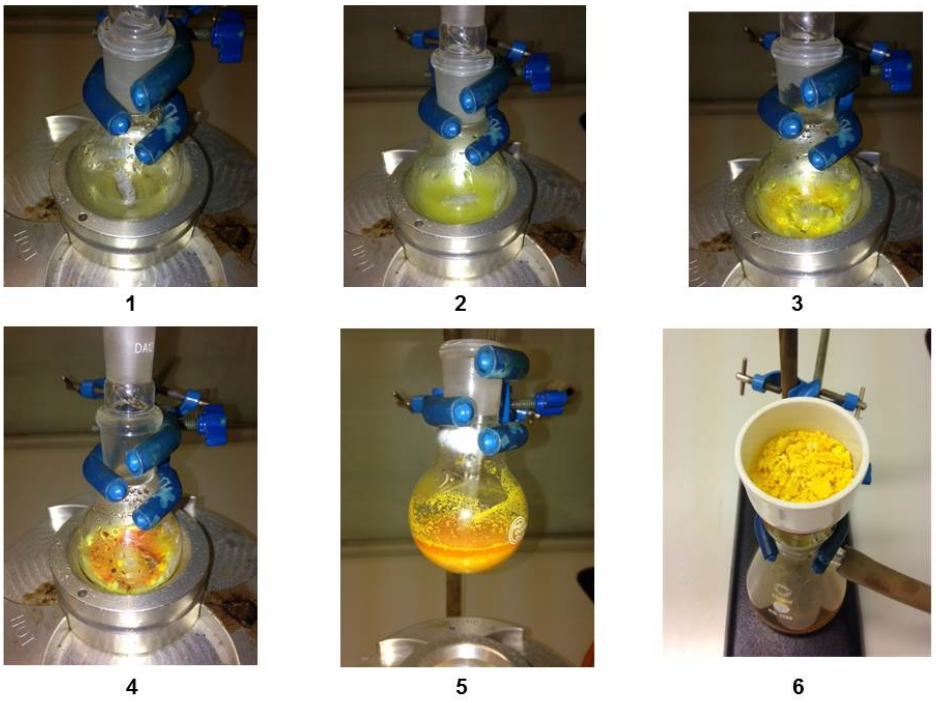


Figure **12.2.4.**1 – Illustrations for part one (DHP formation): pictures 1 – 4 show the reaction mixture transforming from a liquid to a solid during the heating stage. Picture 5 shows the result of addition of water (10 mL) and breaking up the solid with a glass rod. The resulting material is filtered prior to recrystallization (picture 6).

The product is amenable to recrystallization from ethanol (ca. 25 mL), if this has not been performed well there is usually residual ethyl acetoacetate present in the ^{1}H NMR spectrum. The 1,4-dihydropyridine can begin to oxidise if stored in solution for prolonged periods of time, this is most frequently encountered if students leave their NMR samples in $CDCl_3$. If oxidation has occurred it is obvious from ^{1}H NMR evidence (see figure **12.2.4.**6 for an example spectrum containing both compounds and see "Answers to additional questions" section for further discussion of both NMR spectra). If partial oxidation occurs it is actually a useful discussion point for the students (how has this occurred? Is this a problem?). Not all students will notice the diagnostic signals and assume that these are due to a solvent or some other unidentified impurity. The 1,4-dihydropyridine and pyridine can be separated by further recrystallization if desired. Typical yields range between 40 to 90%. Melting point of Diludine is 182-184 °C (lit.)[10]

The second step proceeds smoothly, the 1.5 hour reaction time is probably optimal use of teaching laboratory time, however the results from the original study suggest that stirring for longer periods of time (up to 24 hours) should afford the product in yields >60%. The reaction mixture remains brown until diluted with water and treated with sodium metabisulfite (see figure **12.2.4.**2 for key illustrations).

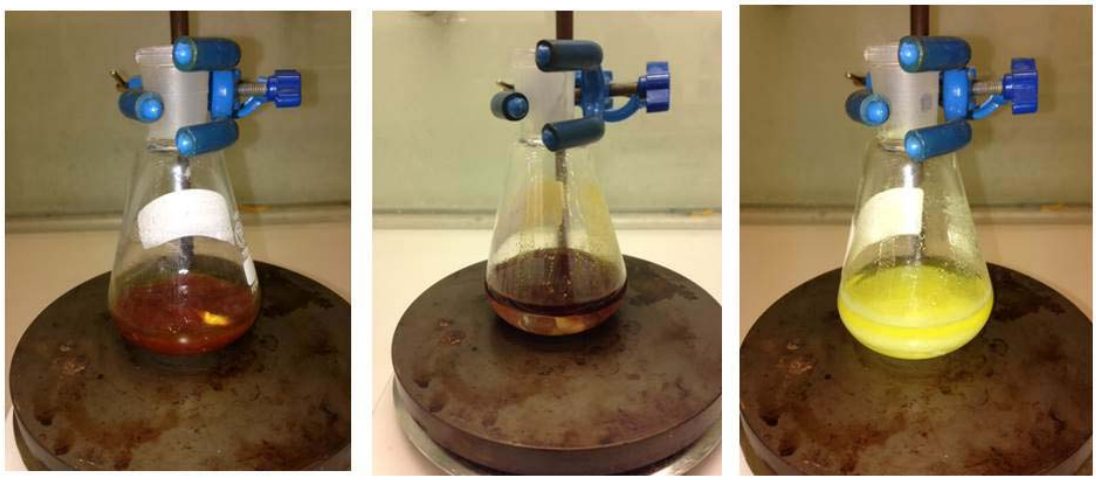


Figure **12.2.4.**2 – Illustrations for part two (DHP oxidation): Picture 1 shows the initial reaction mixture. Picture 2 shows the biphasic solution obtained on addition of water. Picture 3 shows the mixture obtained after excess iodine is removed by addition of sodium metabisulfite.

The reaction can be monitored by thin-layer chromatography [4:1 (petrol:EtOAc): Diludine Rf = 0.24 and 3,5-Diethoxycarbonyl-2,6-dimethylpyridine Rf = 0.59 **or** 9:1 (petrol:EtOAc): Diludine Rf = 0.15 and 3,5-Diethoxycarbonyl-2,6-dimethylpyridine Rf = 0.41] An example set of TLC samples run in 9:1 petrol:EtOAc is shown in scheme **12.2.4.**3.

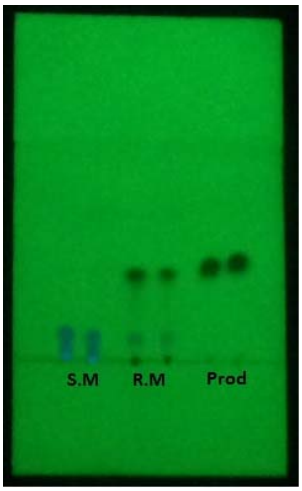


Figure **12.2.4.**3 – Oxidation step TLC (visualised under UV-light). S.M = Diludine, R.M = Reaction Mixture and Prod = 3,5-Diethoxycarbonyl-2,6-dimethylpyridine.

If desired, IR spectroscopy can be included as additional evidence that aromatisation has occurred in the second step. Diludine displays a signal attributed to the NH group at 3348 cm^{-1} (figure *12.2.4.*7), this signal is not observed in the IR spectrum of 3,5-Diethoxycarbonyl-2,6-dimethylpyridine (figure *12.2.4.*8).

Although the main procedure suggests purification of the product by recrystallization, more advanced students can be directed to use flash chromatography instead (9:1 40-60 petroleum spirit: EtOAc, silica gel as stationary phase). In our experience, if this purification option is selected, it is best to allow the students an extra 2-3 hour session. Typical yields range between 20 to 60%. Melting point of 3,5-Diethoxycarbonyl-2,6-dimethylpyridine is 72-74 °C (lit.)[10]

NMR samples and assignments

All the products are soluble in deuterochloroform so there should be no problems obtaining ^{1}H NMR spectra. Assignments of spectra (and copies all the NMR spectra are provided) in the "Answers to additional questions" section.

Answers to additional questions

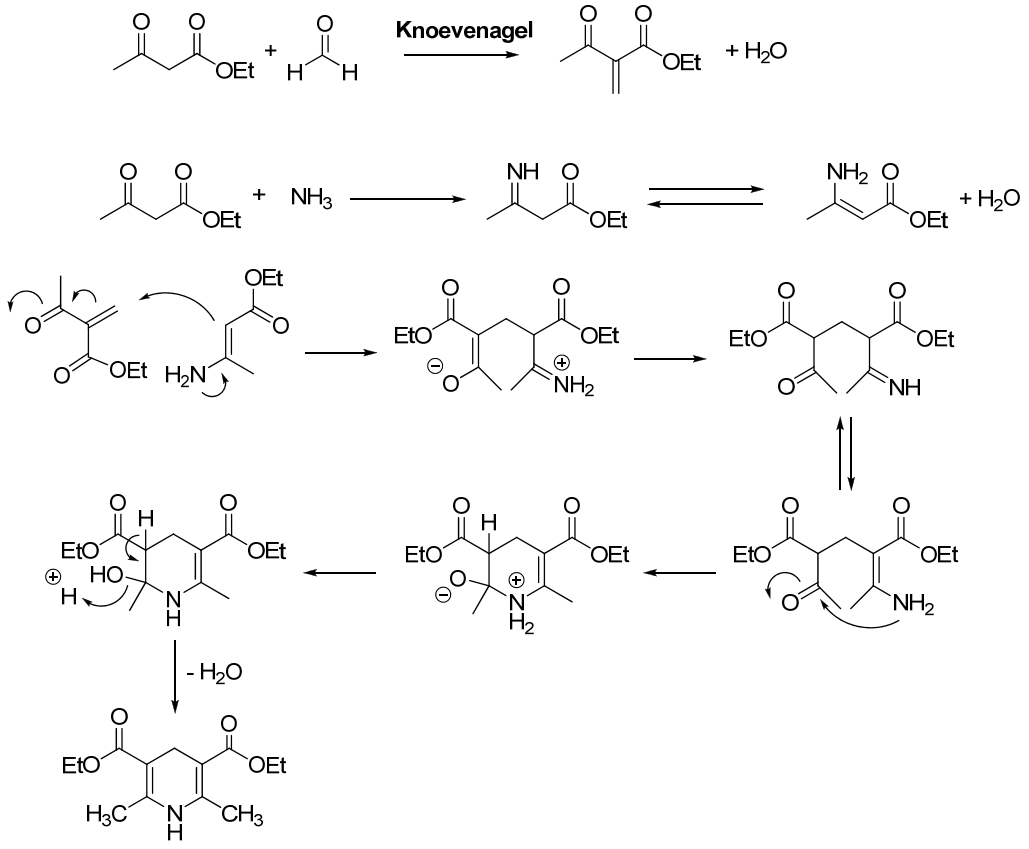
1. Interpret the ^{1}H NMR spectra you obtained from both reaction steps. Use the NMR spectra and melting points to confirm the structure of your compounds unambiguously and identify diagnostic peaks that demonstrate oxidation of the 1,4-dihydropyridine intermediate was successfully achieved.

The ^{1}H NMR spectra (see Figures **12.2.4.**1 and **12.2.4.**2), in conjunction with melting points allow unambiguous identification of both products.

Key evidence that confirms successful aromatization has taken place comes from the chemical shift difference between the protons attached to the ring in each compound. In the ^{1}H NMR spectrum of Diludine, the signal at 3.30 ppm can be attributed to the CH_2 unit within the 1,4-dihydropyridine ring. After step two, the ring proton signal becomes significantly more deshielded (8.71 ppm) and the integral of the signal is reduced by half, these observations are consistent with the removal of a proton and the system becoming aromatic. Additional evidence is that a broad signal consistent with the ring NH is observed at 5.23 ppm in diludine and there is no NH signal observed in the pyridine product. The signals for the methyl and ester substituents are similar in both products, however the methyl substituent signals move to higher frequency in the spectrum of the pyridine (shift from 2.23 ppm to 2.88 ppm).

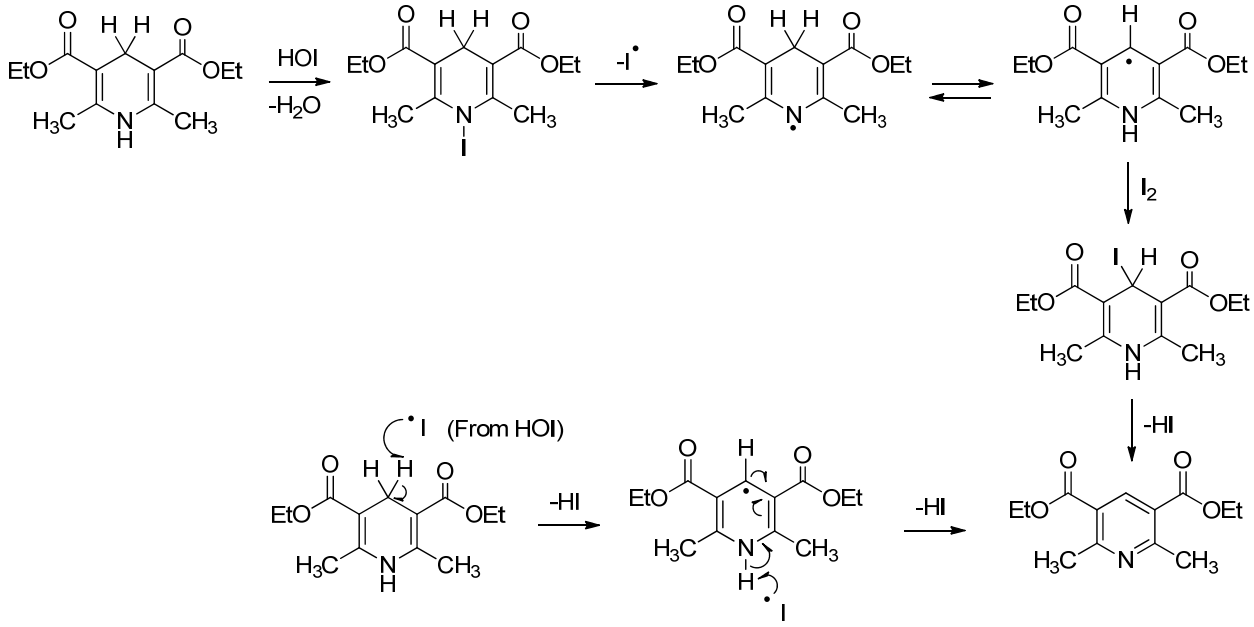
2. Provide a curly arrow mechanism for the reaction you carried out in step 1.

Plausible mechanism of DHP formation



3. In this experiment oxidation of the dihydropyridine ring was achieved using UHP in the presence of iodine. You should suggest a plausible arrow-pushing mechanism for this process. Hint- hydrogen peroxide and iodine can react to form hypoiodous acid *in-situ*.

Plausible mechanisms for oxidation of using 1,4-DHPs using UHP/I_2.



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[10] B. E. Norcross, G. Clement and M. Weinstein, *J. Chem. Educ*., 1969, **46**, 694.

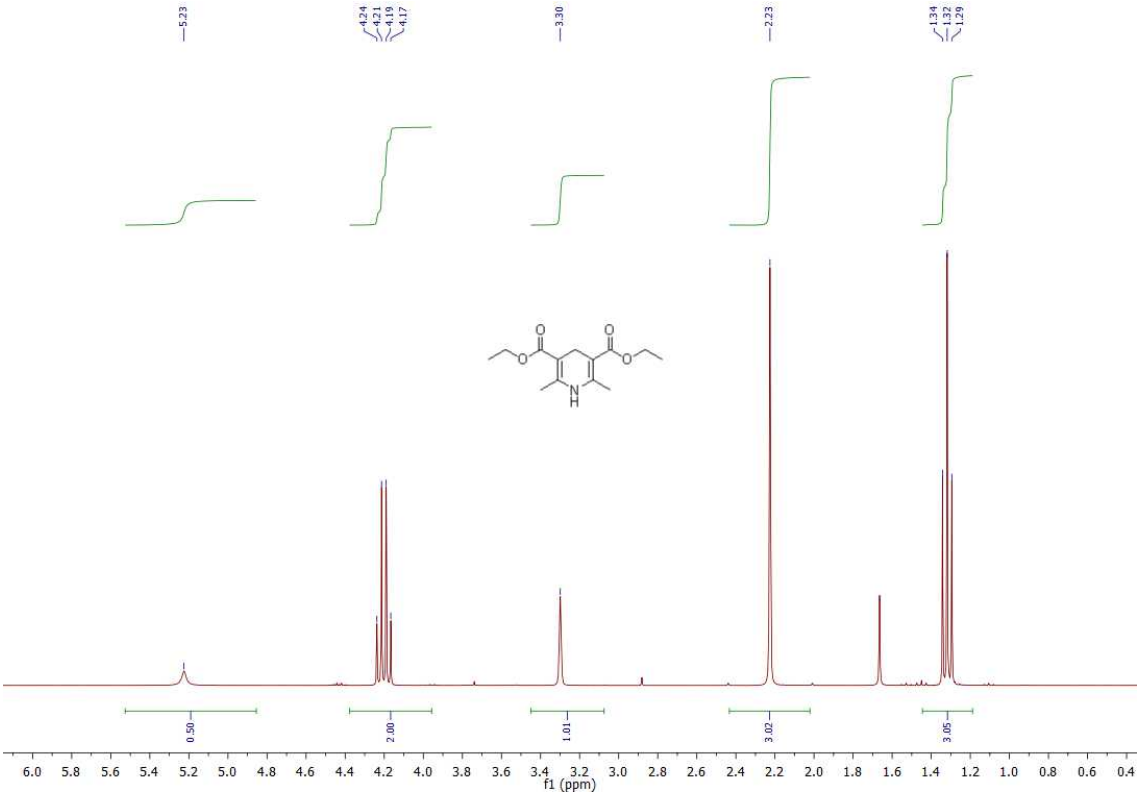
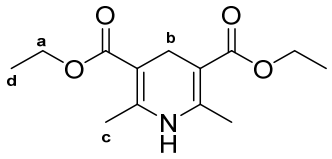


Figure **12.2.4.**4 – ^{1}H NMR spectrum of Diludine (300 MHz, $CDCl_3$)



δ_H (300 MHz, $CDCl_3$, Me_4Si) 5.23 (1H, bs, NH), 4.20 (4H, q, $^{3}J_{HH}$ = 7 Hz, H-a), 3.30 (2H, s, H-b), 2.23 (6H, s, H-c) and 1.32 (6H, t, $^{3}J_{HH}$ = 7 Hz, H-d).

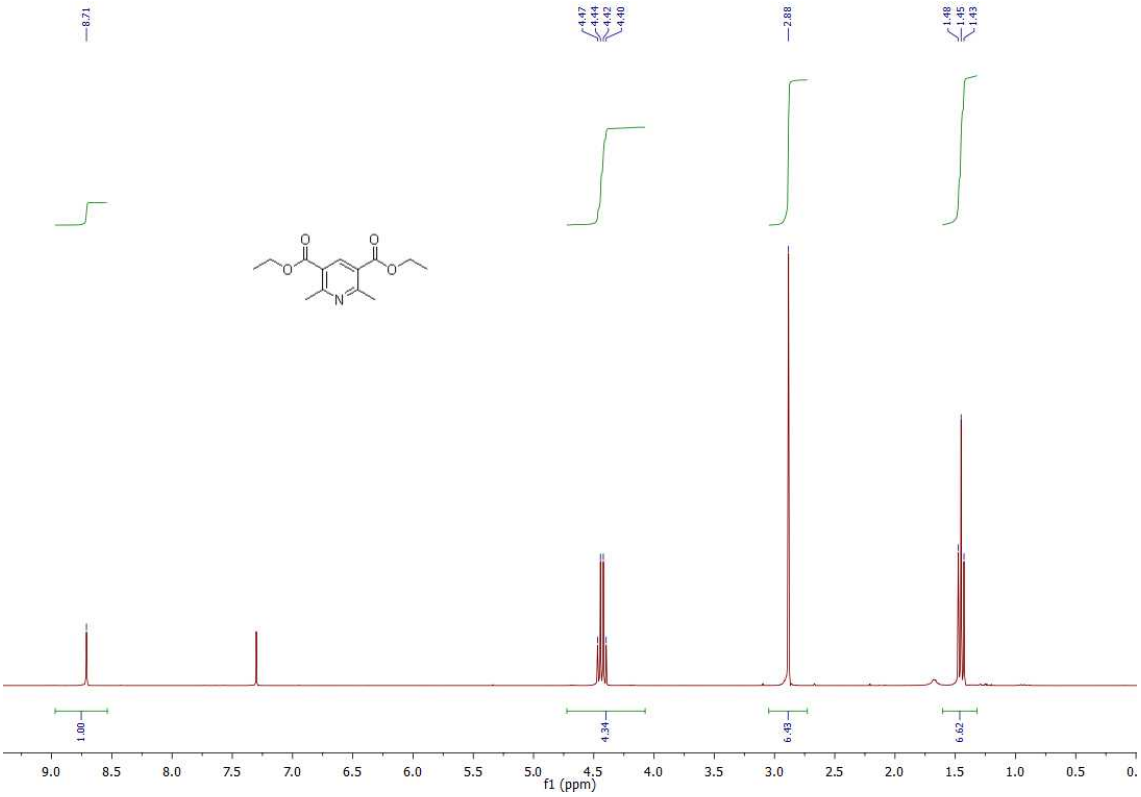
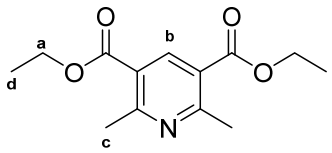


Figure **12.2.4.**5 – ^{1}H NMR spectrum of 3,5-Diethoxycarbonyl-2,6-dimethylpyridine (300 MHz, $CDCl_3$)



δ_H (300 MHz, $CDCl_3$, Me_4Si) 8.71 (1H, s, H-b), 4.43 (4H, q, $^{3}J_{HH}$ = 7 Hz, H-a), 2.88 (6H, s, H-c) and 1.45 (6H, t, $^{3}J_{HH}$ = 7 Hz, H-d).

Note that the ^{1}H NMR spectra illustrated were acquired by students, as noted above, oxidation of the 1,4-dihydropyridine to the corresponding pyridine can be observed on prolonged storage in $CDCl_3$. Figure **12.2.4.**6 shows a typical example of a ^{1}H NMR spectrum of a sample that has undergone partial oxidation

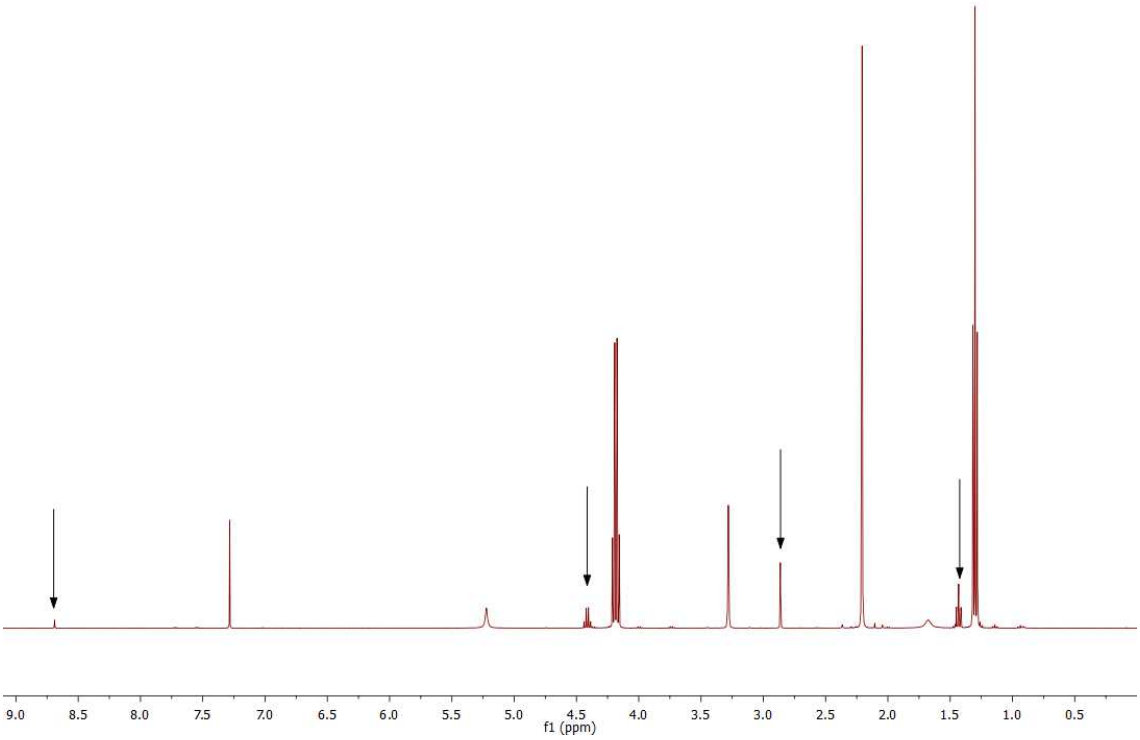


Figure **12.2.4.**6 – ^{1}H NMR spectrum showing Diludine and traces 3,5-Diethoxycarbonyl-2,6-dimethylpyridine (300 MHz, $CDCl_3$). Arrows indicate the positions of the pyridine signals.

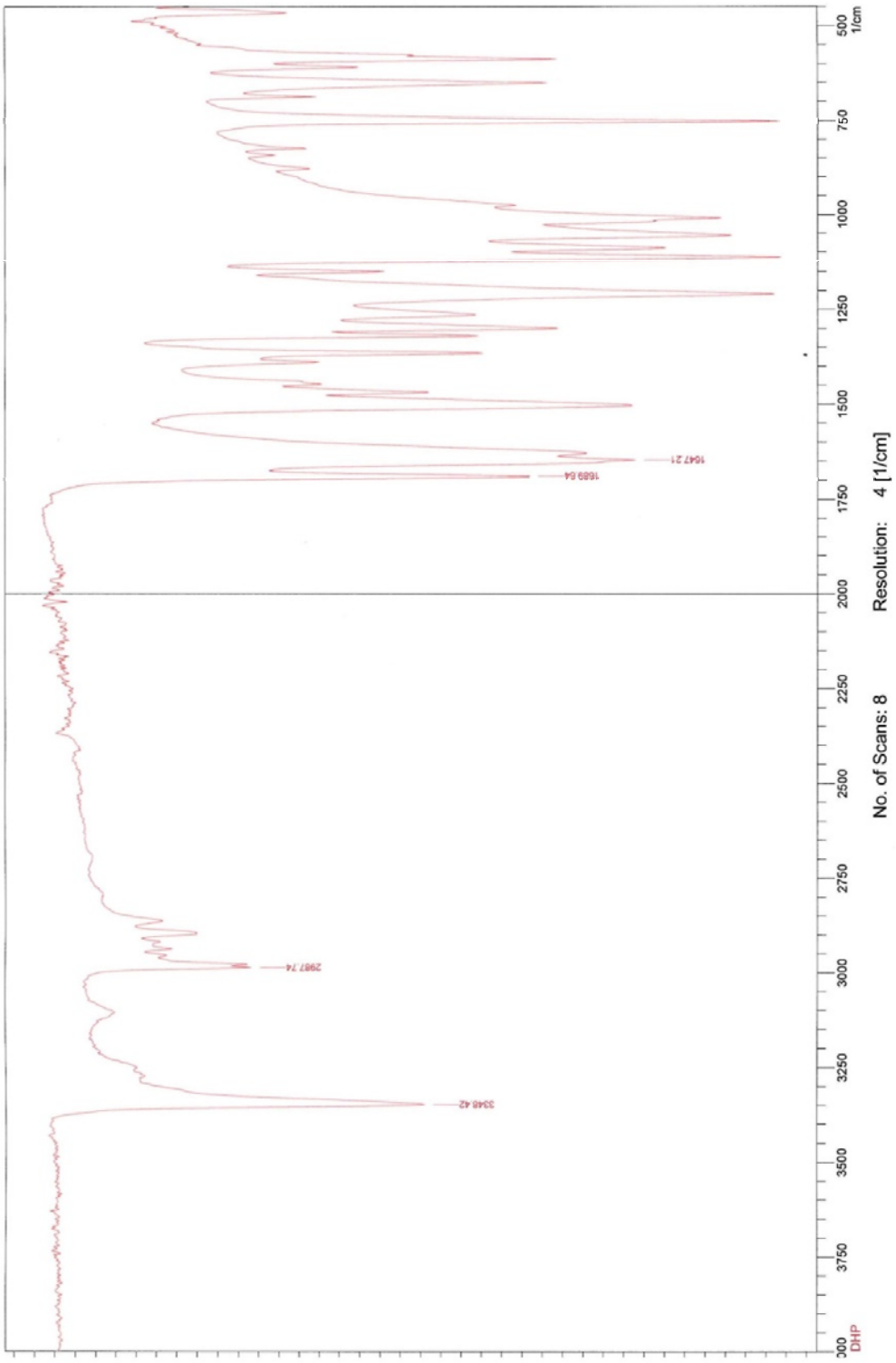


Figure **12.2.4.**7 – ATR-IR spectrum of Diludine

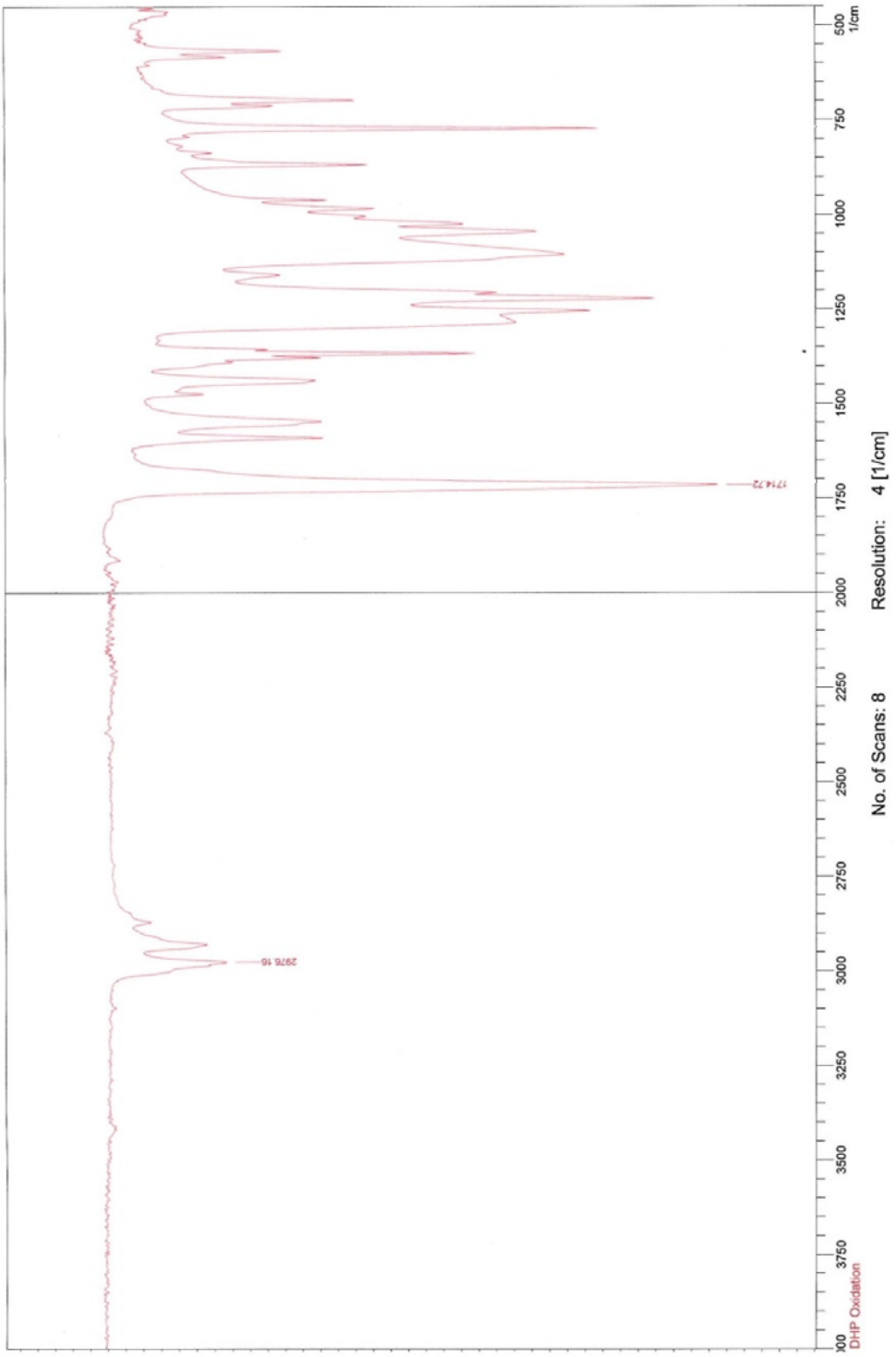


Figure **12.2.4.**8 – ATR-IR spectrum of 3,5-Diethoxycarbonyl-2,6-dimethylpyridine

An "out-of-the-box" example in heterocyclic chemistry: synthesis of 3,6-bis-(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine

Supplementary Material

Experiment Notes **1**

Synthesis of triaminoguanidine mono hydrochloride 2

Synthesis of 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine 3

Synthesis of 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine 5

Spectral characterization

FTIR spectra for triaminoguanidine mono hydrochloride 6

^{1}H NMR for 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine 7

^{13}C NMR for 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine 8

^{1}H NMR for 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine 9

^{13}C NMR for 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine 10

Experiment Notes

This experiment describes a modified process for the synthesis of *s*-tetrazines that does not follow the usual Pinner method. The procedure involves C-N heteroatom bond formation through nucleophilic addition of an amine to a carbonyl group followed by intermolecular cyclization. The final step is the oxidation of the 1,2-dihydro-tetrazine with a mild agent – isoamyl nitrite. The goal of this experiment is to provide a good challenge in the sense that students face an unfamiliar heterocyclic compound with a total of eight N-heteroatoms for which both the procedure and the work-up of the reactions are simple. Furthermore, the

pyrazolyl moieties can act as soft leaving groups and a range of substituents can easily be introduced in the *s*-tetrazine ring.[3,9] This experiment can thus be combined with Exp. 6.5 , which focuses on how the *s*-tetrazine prepared can be further modified to yield a large panel of symmetric or unsymmetrical tetrazine derivatives, providing the students with a valuable synthetic tool .

This experiment is suitable for students at an intermediate level and some familiarity with work in the bench is important. Previous experience on the preparation of a chromatography column is highly desirable, although no other specific skills are required. Given the duration of the reactions involved, this protocol is suitable for laboratory projects taken at an advanced level that do not have to comply with the common 3h lab time restrictions.

The reproducibility of all experiments was assessed by a PhD. student, a MSc. student and undergraduate students (namely 3rd year Chemistry Graduation students) from *Faculdade de Ciências,* ULisboa (Portugal). The intermediate compounds and the final product were obtained in the yields of reaction that are summarized in **Table SM 12.2.5.1**.

Table SM 12.2.5.1. Reaction yields (%) obtained in each step of the synthetic procedure.

Product	Yield range (%)
triaminoguanidine mono hydrochloride (**1**)	80 – 90
3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine (**2**)	50 – 60
3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine (**3**)	60 – 70

Although tetrazine derivatives containing nitrate and perchlorate anions are known for their explosive properties, none of the reactants or products used in this procedure is reported as being explosive. In fact, triaminoguanidine shows good thermal stability in the form of its hydrochloride salt (see e.g. US3813439 patent). Nevertheless it is strongly recommended to handle these chemicals with caution and that all laboratory sessions are executed in a fume hood.

Triaminoguanidine mono hydrochloride is insoluble in most deuterated organic solvents and only modestly soluble in DMSO-*d6* containing a low percentage of D_2O. For that reason this

product was only characterized by infrared spectroscopy, and the FTIR spectrum is provided (see **Figure SM 12.2.5.5**). Students can use this result to confirm the identity of the product obtained. The FTIR spectrum was obtained with a Shimadzu spectrophotometer in KBr pellets. NMR spectra of all the remaining compounds were obtained in a 400 MHz Brucker Avance spectrometer using $CDCl_3$ as solvent. Unless more than 6 hours are available for the laboratory session, NMR spectra must be recorded after the lab sessions, either by the students or by the NMR technician.

Notes for the synthesis of triaminoguanidine mono hydrochloride

The starting material triaminoguanidine mono hydrochloride (CAS No.: 5329-29-3) is commercially available upon request from BOC SCIENCES - Creative Dynamics Inc. (Shirley, New York, USA) within a week. Nevertheless, its synthesis is quite straightforward and it can be prepared in excellent yield either by the students in the first lab session (3 h) or by the laboratory technician if that option is available.

This reaction can be conducted at higher scale by doubling or tripling the amount of reactants and solvents, and increasing the reflux time to 2 hours. The product can then be divided in portions according to the number of students/groups in the class.

Hydrazine is highly toxic, and dangerously unstable in the anhydrous form. For that fact, the use of 80% hydrazine hydrate solution is strongly recommended. Using solutions of lower percentage of hydrazine will reduce the reaction yield due to the product solubility in water.

It is of crucial importance to use a big magnetic stirrer in the reaction since during the reflux time the white precipitate tends to glue to the flask walls. Hence, good stirring power is mandatory for a well-executed experiment.

Step 5 can be done by gravity filtration, but the authors strongly recommend the use of vacuum filtration with a sintered glass filter since is less time consuming. Alternatively, the product can be washed twice with 10 mL of diethyl ether in order to reduce the drying time.

Triaminoguanidine mono hydrochloride is obtained as a white crystalline solid which can turn

pinkish after exposure to air for some time due to its partial decomposition to diaminourea. It is strongly recommended to store it under nitrogen until it is used (if prepared in advance).

Figure SM 12.2.5.1 - Experimental Setup for Reaction 1.

Notes for the synthesis of 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine

Like in the case of Reaction 1, it is mandatory the use of a big magnetic stir bar due to the amount of precipitate that will be formed. The authors found that the ideal temperature to perform the addition of the acetylacetone is 15°C. Temperatures below 15°C will cause the reactant to precipitate and working above 15°C will make the temperature control more difficult for students. A rapid increase of the reaction medium to 20°C within about 3 minutes was typically observed when 1/3 of the acetylacetone was added. If such an increase is observed, the addition ***has to be stopped*** for a few minutes in order to allow the cooling of the pale yellow solution back to 15°C. Nearly at the end of the addition another temperature rise was observed, and again care should be taken. The reaction should be maintained at

15°C for more 15 minutes after complete addition, and then allowed to warm up to room temperature for the remaining 15 minutes. The 4 hours' time can include the heating time in order to make the experiment less time-consuming since no significant changes in yield were observed. Also, during the heating time no condenser is needed since the temperature will not reach reflux temperature.

Filtration of the product can be performed both in a sintered glass filter or a Büchner filter. To save drying time, students can dissolve the solid obtained in 100 mL of dichloromethane and dry the solution with anhydrous sodium sulphate. Evaporation of the solvent in a rotatory evaporator will afford a more dried solid. Although the reaction yield is only in the range of 50-60%, it is worth to note that the final mass of product **2** obtained is around 8 g for an initial mass of 12 g of triaminoguanidine mono hydrochloride. The mechanism for the formation of this 1,2-dihydro-s-tetrazine is included in **Scheme SM 12.2.5.1**.

The 1,2-dihydro-1,2,4,5-tetrazine is rather unstable and tends to oxidize in air. Hence, this product should be characterized straight away and carefully stored under nitrogen until the next session.

Figure SM 12.2.5.2 - Experimental setup for Reaction 2.

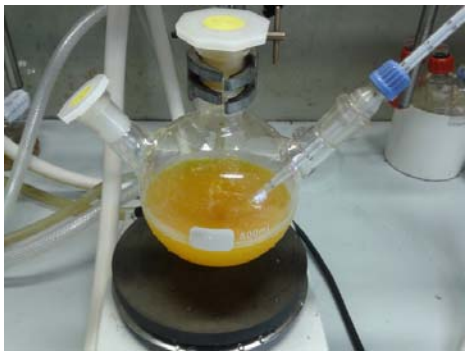


Figure SM 12.2.5.3 – Visual aspect of Reaction 2 after complete addition of acetylacetone.

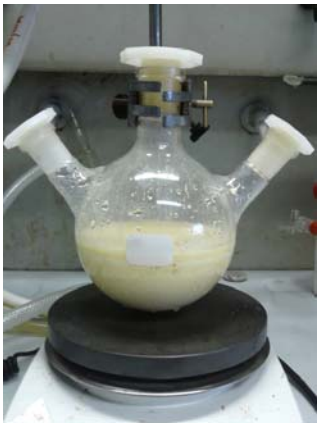


Figure SM 12.2.5.4 – Visual aspect of Reaction 2 after 4 hours at 70°C (the thermometer was removed).

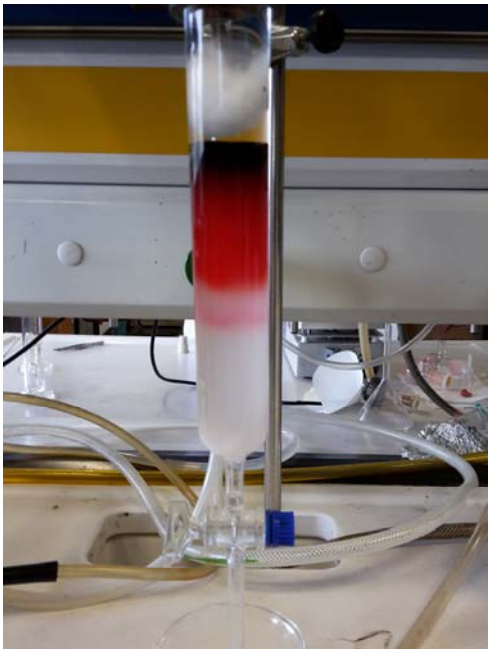
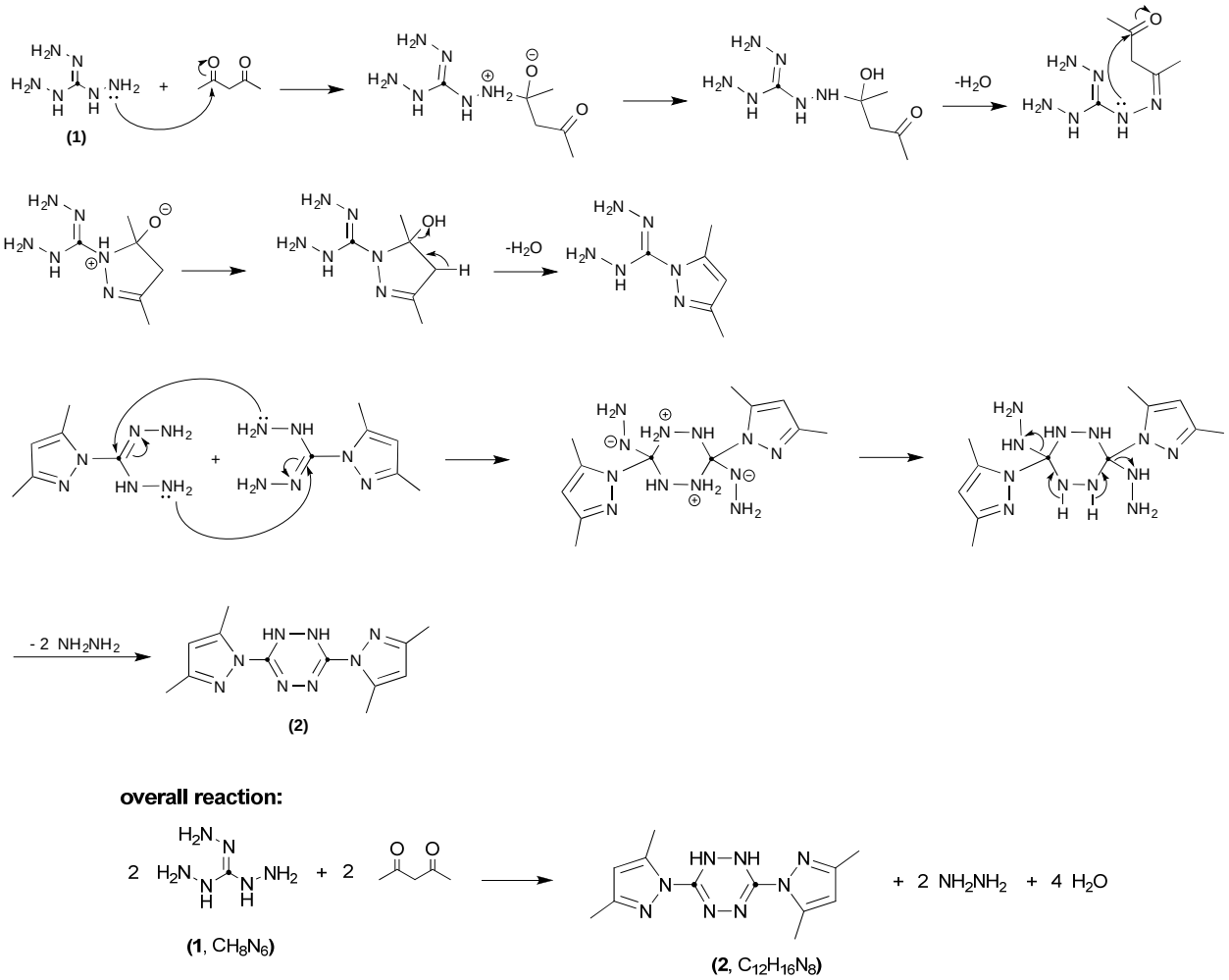


Figure SM 12.2.5.5 – Flash chromatography step with dichloromethane (100%) for reaction mixture 2: the first pinkish band is discarded and the most intense coloured red band is collected until the eluent becomes colourless. (Note that some product keeps absorbed to the silica).

Figure SM 12.2.5.6 – Visual aspect of the reaction products obtained in this experiment: left, white solid triaminoguanidine mono hydrochloride (**1**); middle, orange solid 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine (**2**); right, red solid 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine (**3**).



Scheme SM 12.2.5.1 – Proposed mechanism and overall reaction for the formation of the 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine from the triaminoguanidine precursor and acetylacetone.

Notes for the synthesis of synthesis of 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine

This reaction must be performed **in a well-ventilated fume hood**, since it will involve the formation of nitric oxides. For that matter, the reaction flask should not be covered in order to allow the toxic gases to escape.

The oxidation of 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine can alternatively be performed by sodium nitrite. If this reagent is used, students must add 10 mL of glacial acetic acid to the starting dichloromethane solution, followed by the addition of 2 eq. of sodium nitrite in small portions (aprox. 0.2 eqs each). After washing the organic phase with 5% sodium hydroxide solution, saturated sodium bicarbonate solution and finally water, the work up of the reaction should be continued as described by steps 7-9 of the experimental procedure.

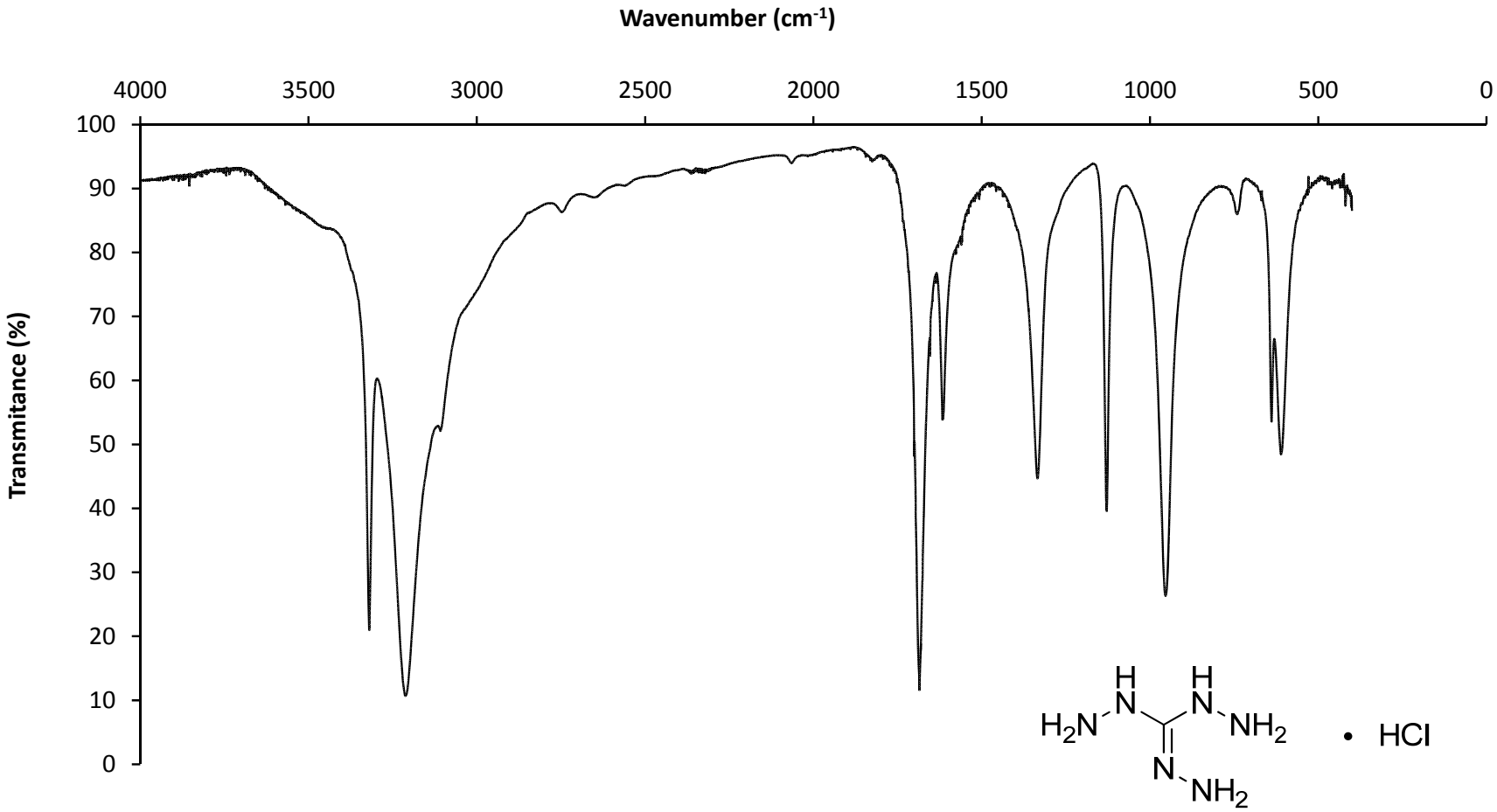


Figure SM 12.2.5.5 – FTIR spectra (in KBr pellets) for triaminoguanidine mono hydrochloride.

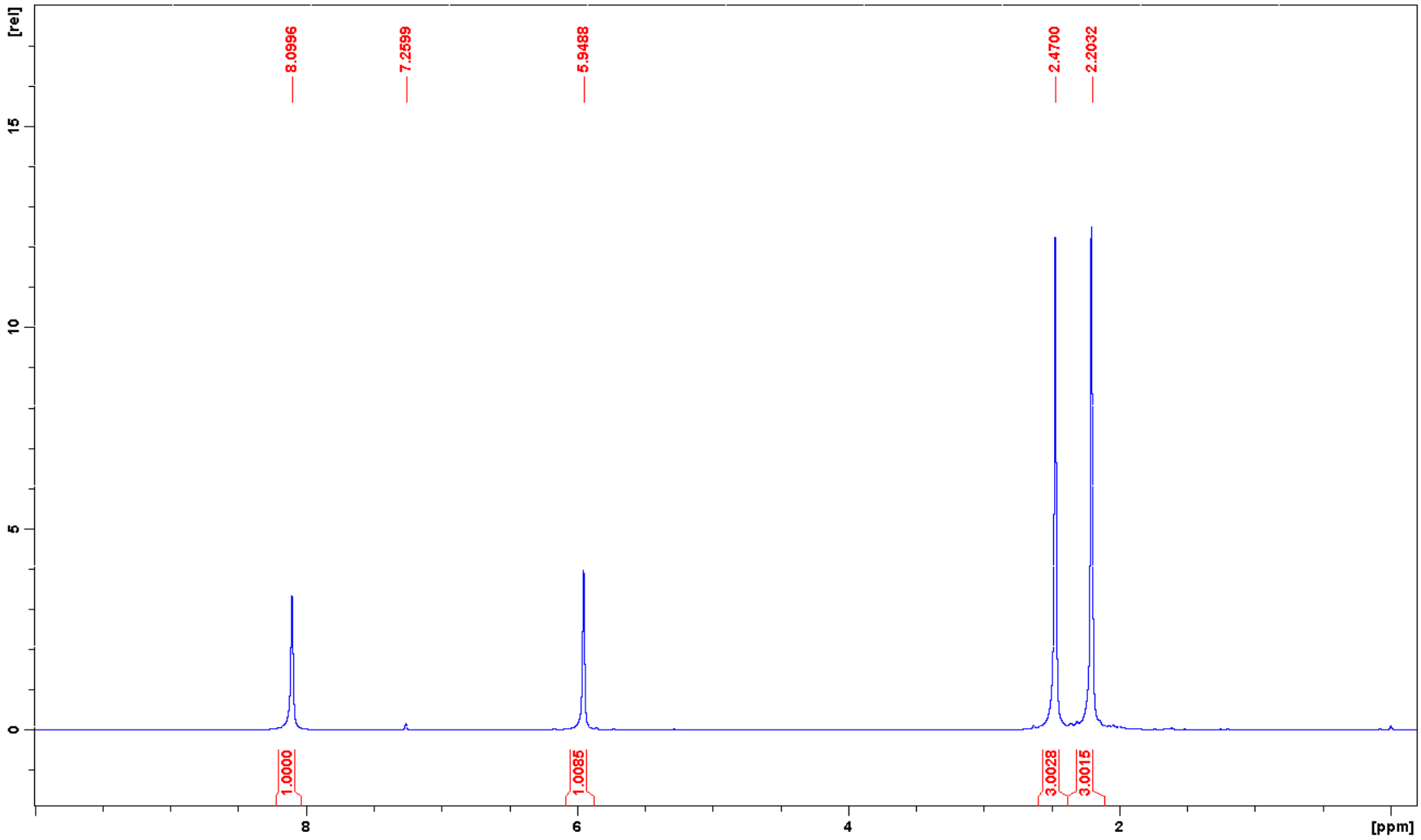


Figure SM 12.2.5.6 – ^{1}H NMR spectra in $CDCl_3$ for 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine.

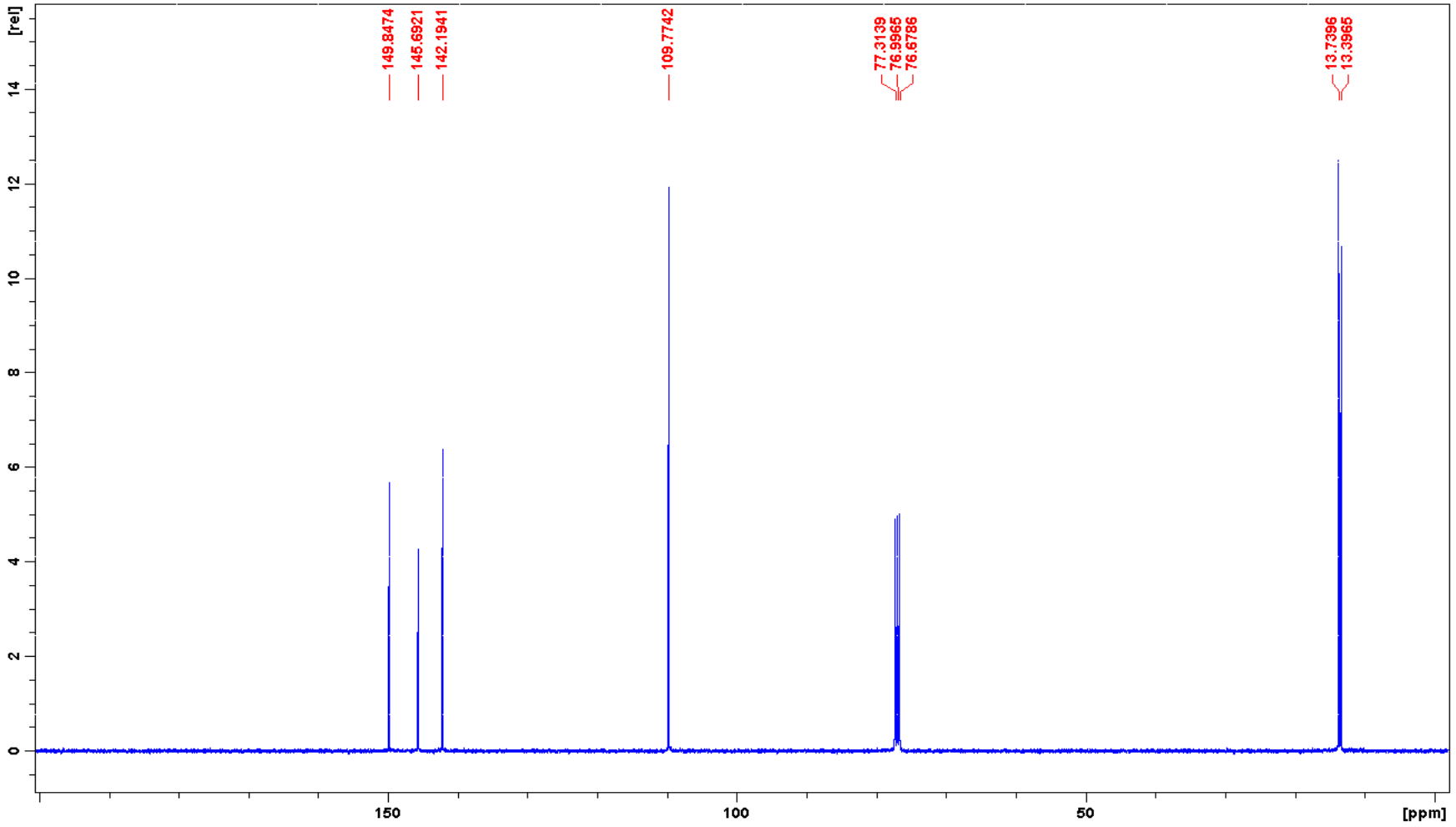


Figure SM 12.2.5.7 – ^{13}C NMR spectra in $CDCl_3$ for 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine.

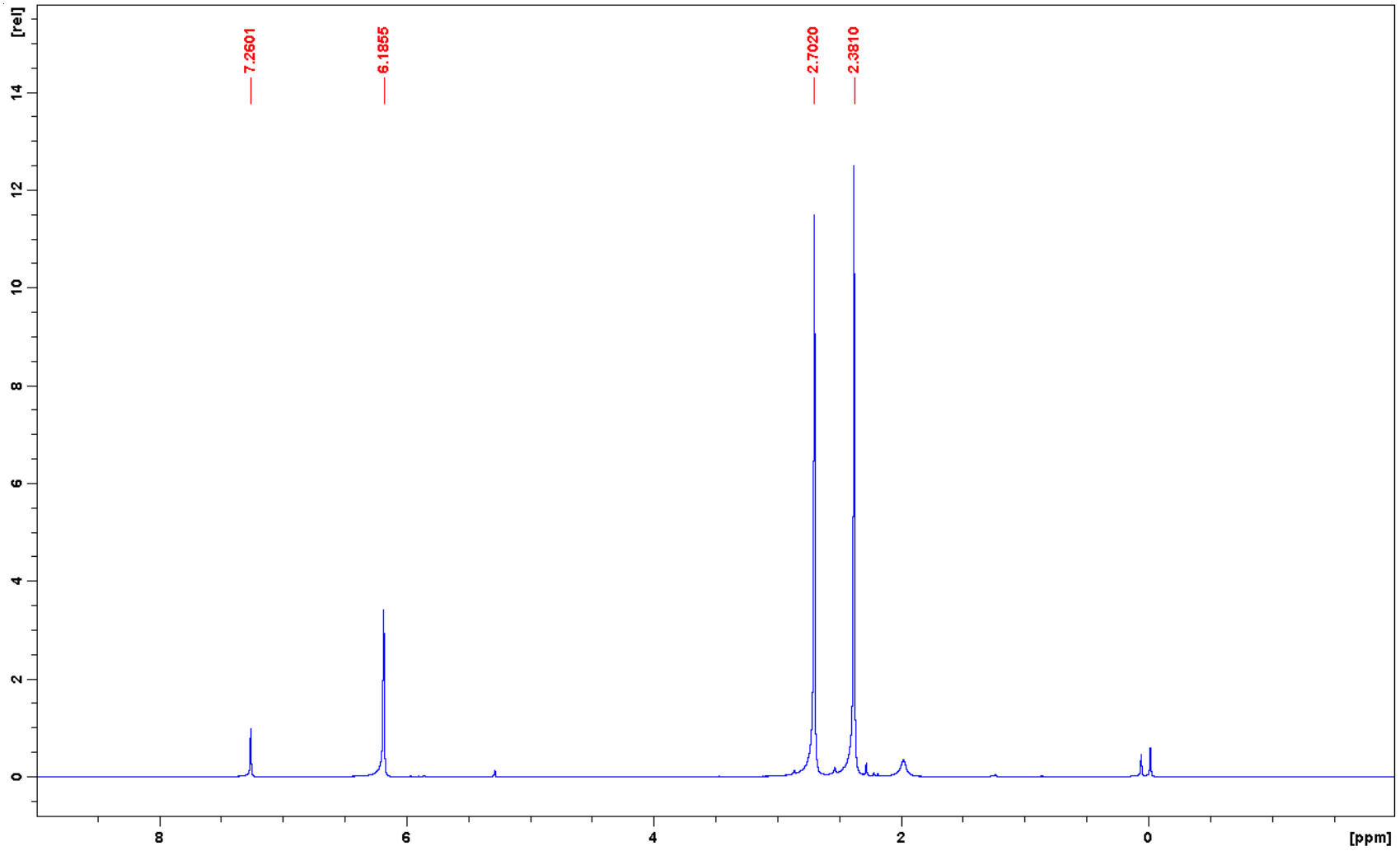


Figure SM 12.2.5.8 – ^{1}H NMR spectra in $CDCl_3$ for 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine.

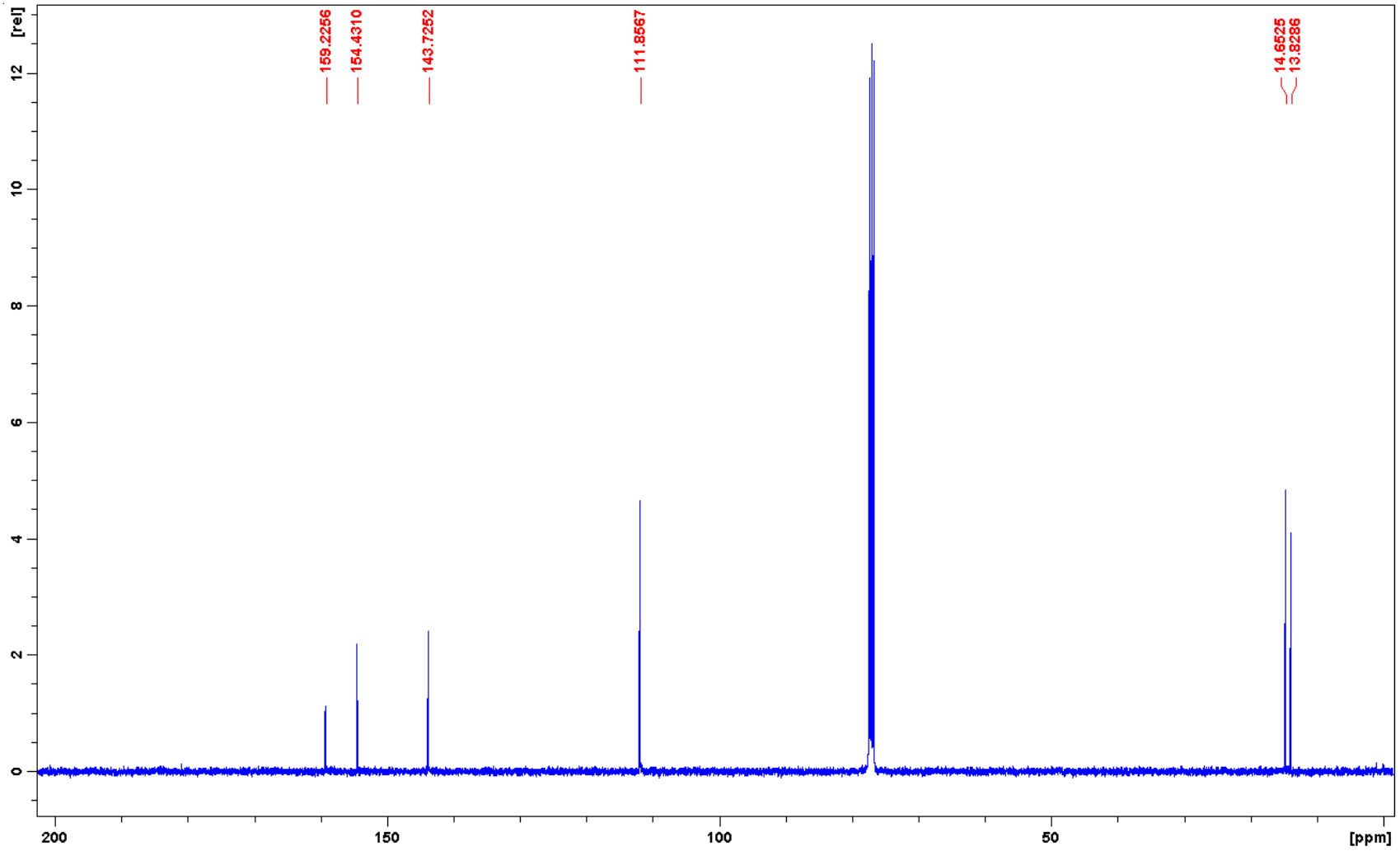


Figure SM 12.2.5.9 – ^{13}C NMR spectra in $CDCl_3$ for 3,6-bis(3,5-dimethyl-1H-pyrazol-1-yl)-1,2,4,5-tetrazine.