Knorr Pyrazole Synthesis of Edaravone

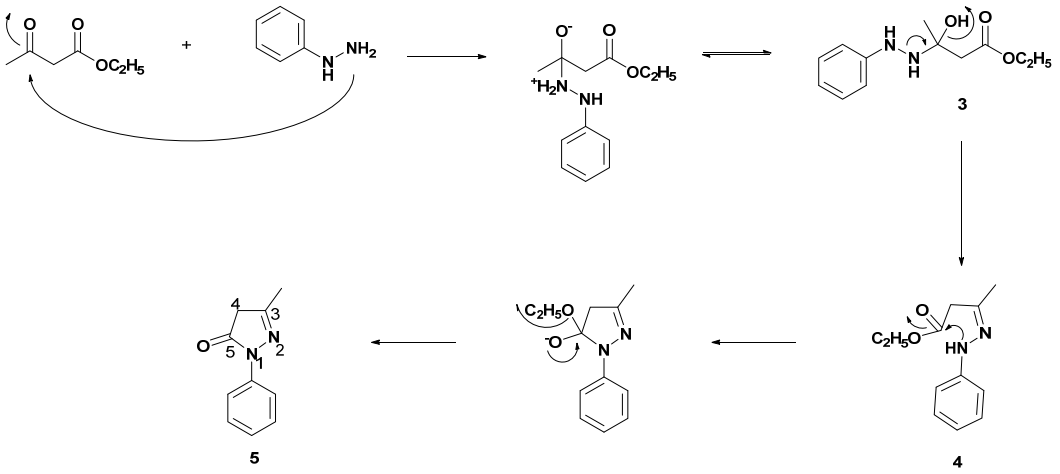
Supplementary Material

Experiment notes

The objective of this experimental work is to introduce the students to the synthesis of heterocycles using the Knorr pyrazole reaction. The experimental work is easy to perform and at the same time is an excellent example for teaching students about the concepts of regioselectivity (**Scheme SM 4.2.3.1.1**) and tautomerism. Solubility of edaravone in acidic and basic solutions could be discussed with reference to its tautomeric forms (**Scheme SM 4.2.3.1.3**).

Mechanism of the reaction

Mechanistically, reaction proceeds through the carbinolamine **3**, and hydrazone **4,** which cyclizes intramolecularly, to give edaravone **5** (**Scheme SM 4.2.3.1.1**). The regioselectivity of the reaction is governed by the higher reactivity towards nucleophiles of the ketone moiety over ester, and the more nucleophilic and least hindered nitrogen atom of hydrazine.



Scheme SM 4.2.3.1.1. Mechanism for the formation of edaravone

Acidity of edaravone

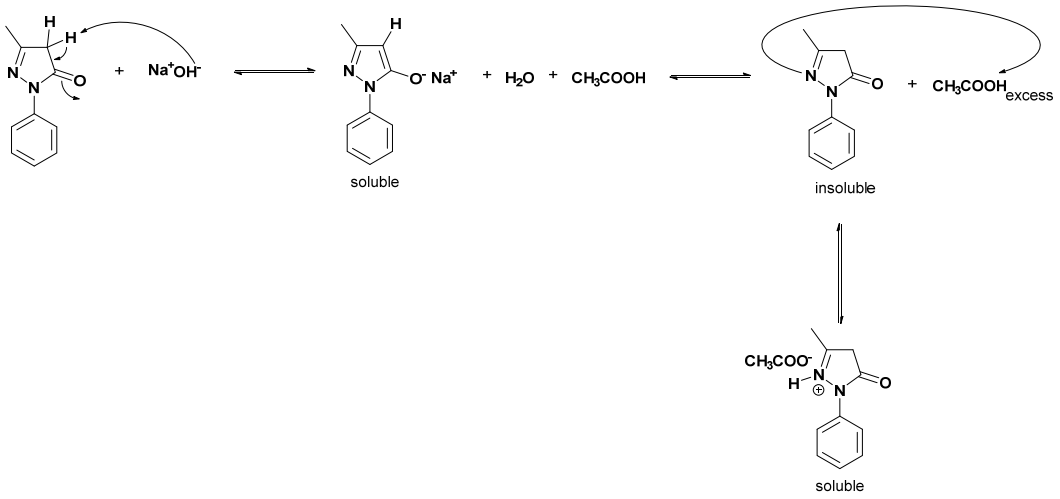
The methylene hydrogen atoms of edaravone are slightly acidic due to the resonance of the corresponding conjugate base, which exists in the enolate form. (**Scheme SM 4.2.3.1.2**).

Basicity of edaravone

The imine like N-2 nitrogen atom is basic and can be protonated in acid aqueous solutions. In contrast, the amide like N-1 nitrogen atom is non-basic.

Solubility tests

Solubility tests confirm the amphoteric character of edaravone and the chemical reactions (**Scheme SM 4.2.3.1.2**) illustrate the acidic and basic properties of the compound.



Scheme SM 4.2.3.1.2. Acidic and basic reactions of edaravone

The reproducibility of the experiment was assessed by its repetitive execution by the fourth year of the Integrated M.Sc. students from Faculty of Pharmacy - University of Lisbon (about 200 students/year)

Experimental concerns

Addition of both reagents into the round bottom flask is slightly exothermic and the students will see the formation of some drops of water (derived from imine formation) in the walls of the reaction flask (**Figure SM 4.2.3.1.1**).

Figure SM 4.2.3.1.1. Reaction mixture. The mixing of both reagents is slightly exothermic

Figure SM 4.2.3.1.2. Heating the reaction in a mantle with a reflux assembly

The heating must be done carefully; the round bottom flask must stay away from the bottom and the walls of the mantle, to avoid overheating (**Figure SM 4.2.3.1.2**). Otherwise it is possible to see projections of the reaction mixture into the walls of the flask during the heating process.

The principal concern in the experiment is related with precipitation of the product with diethyl ether. The addition of the ether must be done after cooling the syrup (**Figure SM 4.2.3.1.3**), with caution and the mixture must be stirred vigorously, while maintained in the ice bath (**Figure SM 4.2.3.1.4**). Addition of a small volume of the solvent (2 mL) and in small portions is also important for good practice. If the total volume (8 mL) of diethyl ether is added at once it will be very difficult to induce the precipitation of the product, as it will separate as oil.

Figure SM 4.2.3.1.3. Mixing the first portion of diethyl ether

Figure SM 4.2.3.1.4. Recrystallized edaravone

Care should be taken during recrystallization. Addition of an excess of the solvent results in low yields as the product solubilizes quite well in ethanol 95%.

Solubility tests should be first exemplified by the instructor, in order to show students how to estimate the correct amount of compound necessary to perform the tests and to determine the amount of basic and acidic solutions for neutralization and precipitation of the product.

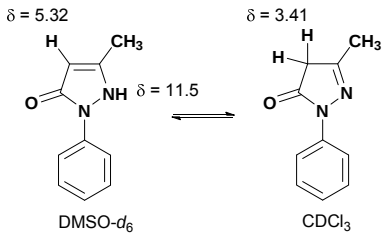
Product analysis, spectra and additional information

The reproducibility of the experiment was assessed by its repetitive execution by the students of the fourth year of the Integrated M.Sc. from Faculty of Pharmacy - University of Lisbon (14 classes of 16 students in groups of two), average results (**Table SM 4.2.3.1.1**).

Table SM 4.2.3.1.1. Average class results for the synthesis of edaravone as an example in the first five classes

	Uield	**Melting point**
Class 1	η = 15-60%	Mp (ºC) = 121-128
Class 2	η = 29-40%	Mp (ºC) = 129-134
Class 3	η = 23-70%	Mp (ºC) = 126-130
Class 4	η = 20-57%	Mp (ºC) = 126-131
Class 5	η = 23-85%	Mp (ºC) = 126-130
Similar results were obtained in the other classes		

Edaravone was analysed by ^{1}H NMR in $CDCl_3$ and DMSO-d_6. Spectra were acquired on a 300 MHz Bruker spectrometer and reported in parts per million (δ) referenced against a residual solvent peak. In general the students obtained pure products (**Figure SM 4.2.3.1.4**), but in some spectra is possible to identify residual peaks from reagents. Performing the analysis in $CDCl_3$ and DMSO permits the identification of the signals corresponding to the enol form of edaravone (**Scheme SM 4.2.3.1.3**).



Scheme SM 4.2.3.1.3. Two major tautomeric forms of edaravone in DMSO-d_6 and $CDCl_3$

Edaravone: IR (KBr) ν_{max}/cm^{-1} : 3431, 3129, 1602, 1599, 1580; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (d, J = 7.5 Hz, 2H, Ar*H*), 7.40 (m, 2H, Ar*H*), 7.18 (m, 1H, Ar*H*), 3.41 (d, *J* =0.6 Hz, 2H, CH_2), 2.19 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): 170.6, 156.4, 130.1, 128.8, 125.0, 118.9, 43.1, 17.0; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.5 (bs, 1H, N*H*), 7.71 (m, 2H, Ar*H),* 7.40 (m, 2H, Ar*H*), 7.22 (m, 1H, Ar*H*), 5.36 (s, 1H, C*H*), 2.12 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6):171.7, 158.9, 148.7, 139.2, 138.6, 129.3,125.4, 124.8, 118.4, 43.5, 17.1, 14.2.

These values are in accordance with the previous published in literature[1]. In the carbon spectrum in DMSO presented in Figure SM 4.2.3.1.8 is evident the presence of the two major tautomeric structures of edaravone, signals are identified by different colours in both structures in the figure. Also in the IR analysis of the solid material (Figure SM 4.2.3.1.9) is possible to see either the NH form (ν_{max}/cm^{-1}, 3129), the OH form (ν_{max}/cm^{-1}, 3431) and the C=O (ν_{max}/cm^{-1}, 1599) of the enol and keto tautomeric forms of edaravone.

Spectra

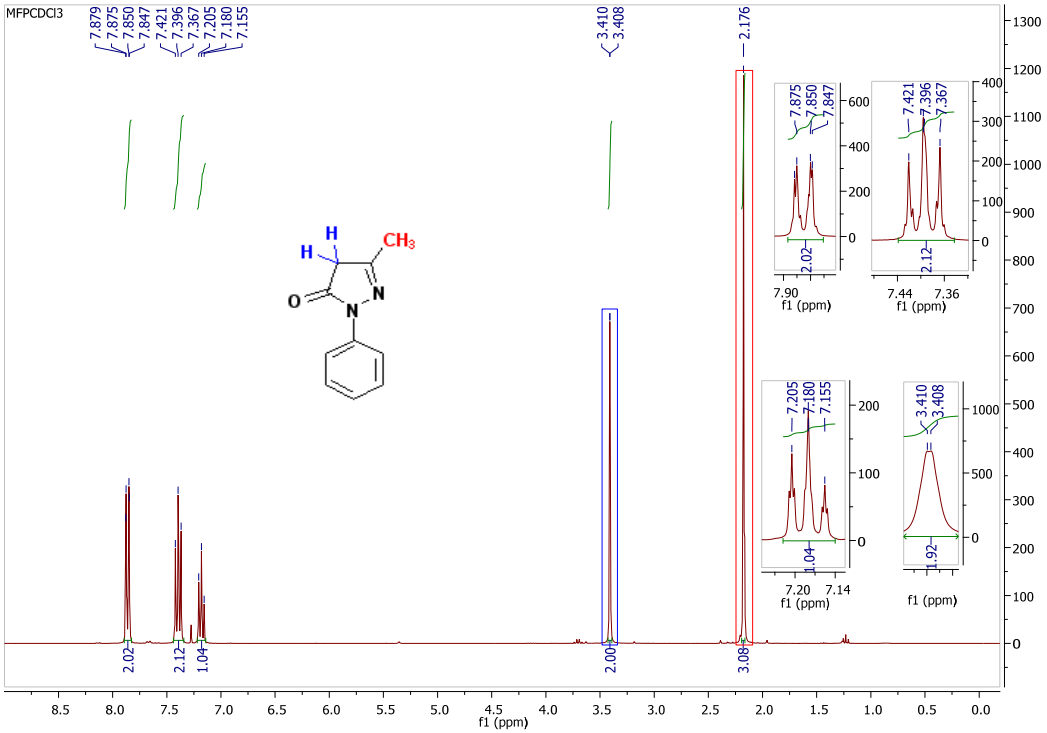


Figure. SM 4.2.3.1.5. ^{1}H NMR of 3-methyl-1-phenyl-5-pyrazolone in $CDCl_3$

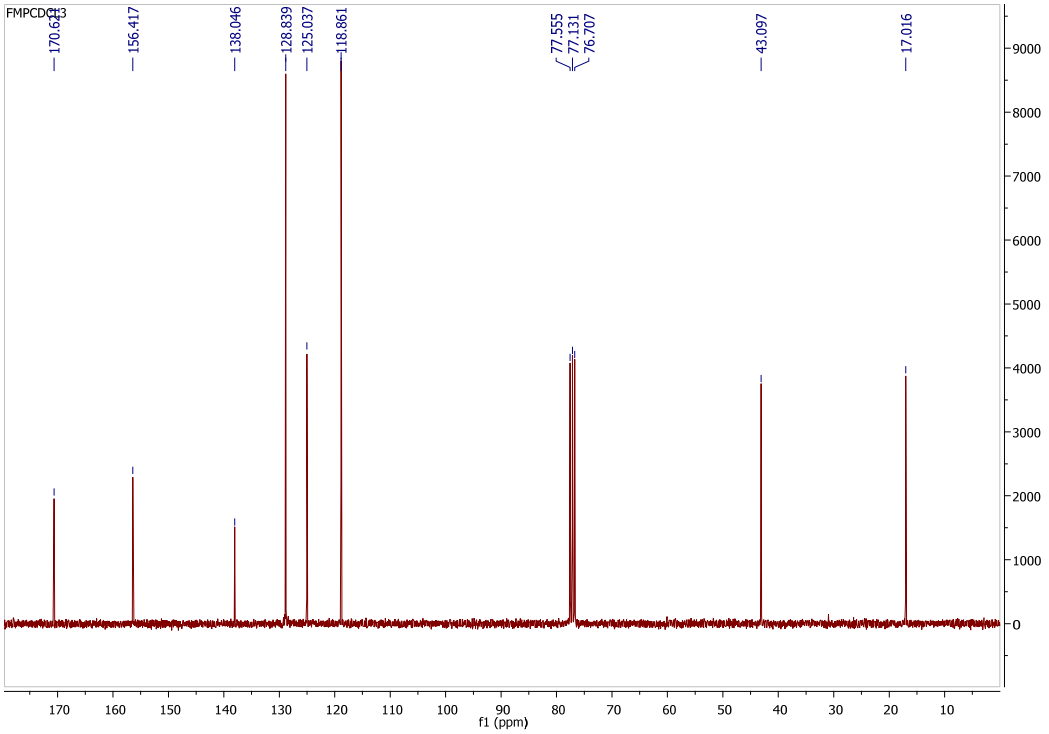


Figure. SM 4.2.3.1.6. ^{13}C NMR of 3-methyl-1-phenyl-5-pyrazolone in $CDCl_3$

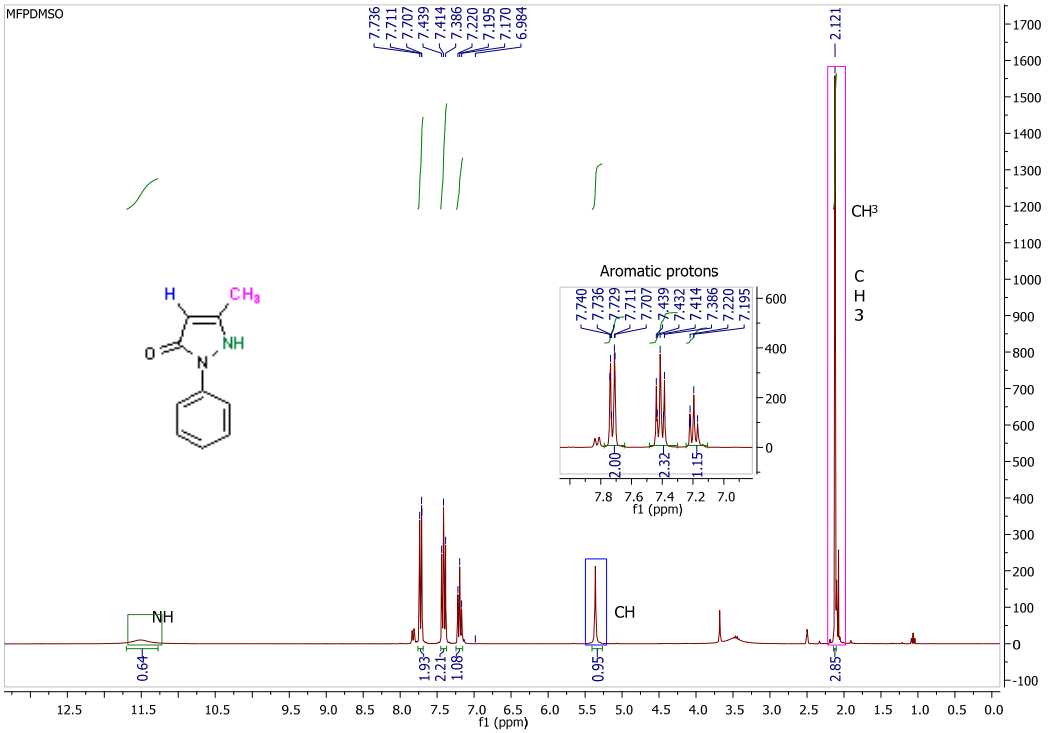


Figure SM 4.2.3.1.7. ^{1}H NMR of 3-methyl-1-phenyl-5-pyrazolone in DMSO

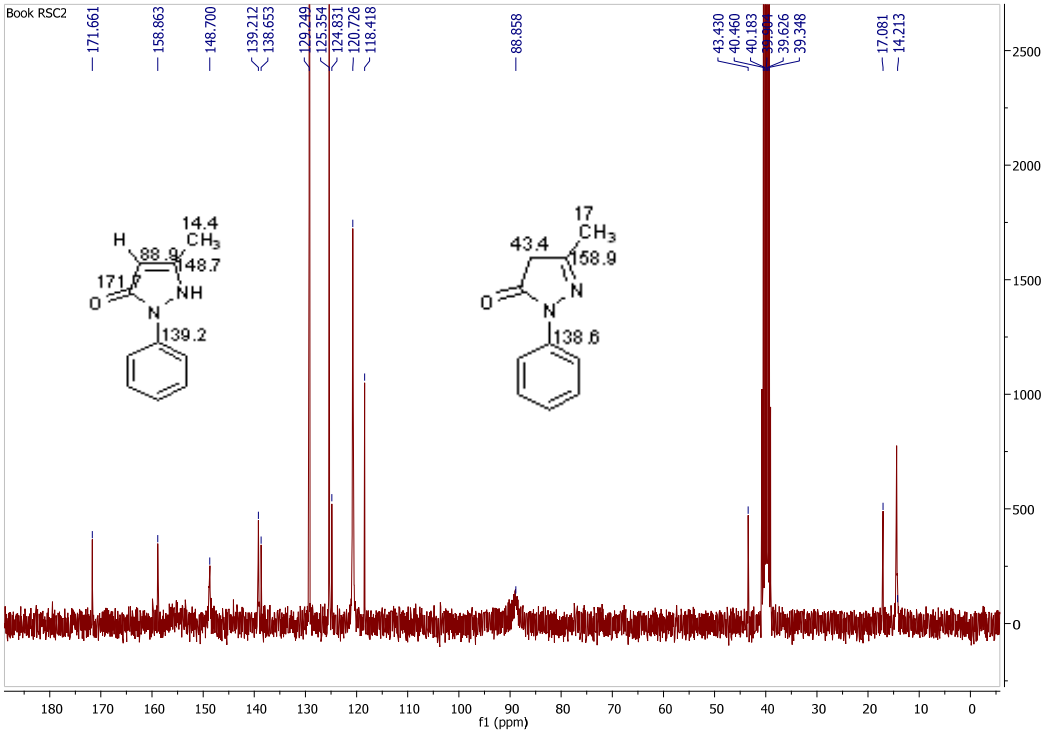


Figure SM 4.2.3.1.8. ^{13}C NMR of 3-methyl-1-phenyl-5-pyrazolone in DMSO

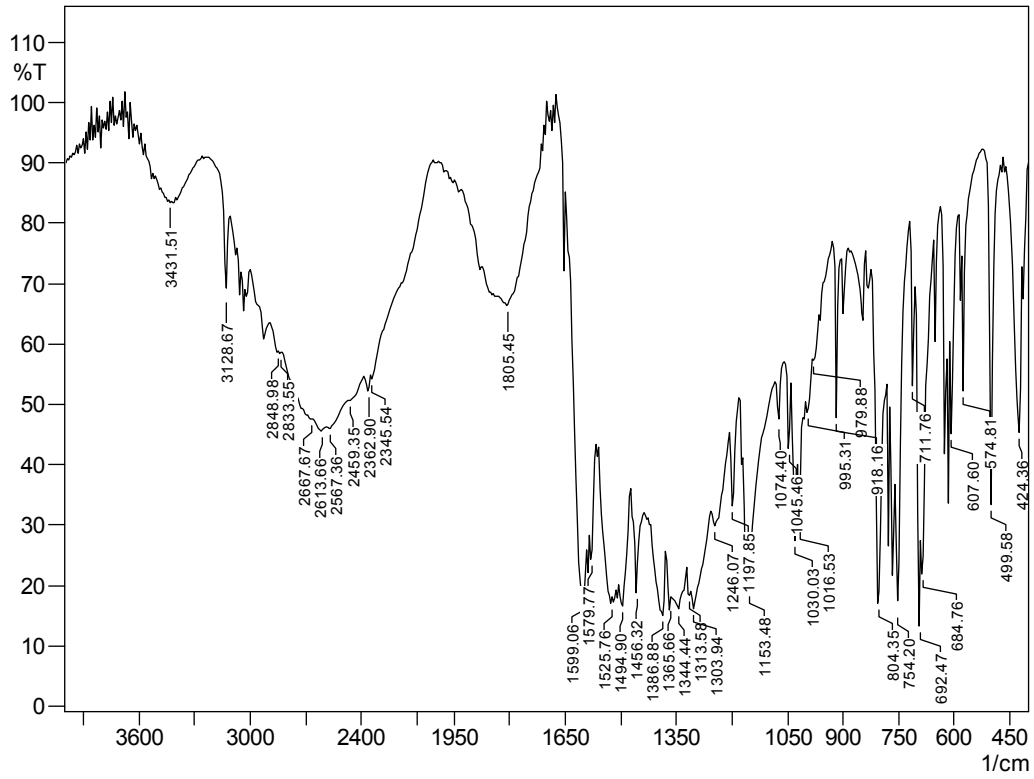


Figure SM 4.2.3.1.9. IR spectra of 3-methyl-1-phenyl-5-pyrazolone (KBr)

1. S. Pal, J. Mareddy and N. S. Devi, *J. Braz. Chem. Soc.*, 2008, **19**, 1207.

Greener Solvent Substitution in a Verley-Doebner Condensation

Supplementary Material

The Verley-Doebner condensation of malonic acid with 4-methoxybenzaldehyde is a straightforward reaction incorporating a range of laboratory techniques. The experiment is intended for introductory-level to intermediate-level undergraduate organic chemistry students. The techniques utilized during this experiment include solution reflux, precipitation by acidification, vacuum filtration, and solid purification via recrystallization. The reaction also showcases a variety of concepts including the formation of a nucleophilic enolate, use of an organocatalyst (β-alanine), decarboxylation as a driving force for the reaction, and application of greener solvents in organic reactions.

The reaction must be undertaken in a fumehood or other suitably-ventilated space, and requires a high-temperature reflux: a temperature of approximately 160°C is suitable, although it need not necessarily be that high. However, room temperature is not sufficient to drive the reaction over the course of the laboratory session (see Table SM 4.2.3.2.1). Carbon dioxide gas will vigorously be evolved during the reaction, which is visibly different from the actual refluxing of the solvent mixture. As such, under no circumstances should the condenser and round-bottomed flask be capped. It is also important to note that when adding concentrated HCl to the reaction mixture, fumes will form and the reaction will be highly exothermic. Consequently, this addition must be carried out very slowly.

The yield of the reaction was found to be quite consistent between students in the third-year undergraduate organic chemistry course (annual enrollment of 35-45 students)[1] where this experiment is incorporated (typically 25 - 85%, average of 50% after product recrystallization, mp ~173-174°C).[2] Before the solvent mixture was optimized to 70:30 v/v triethylamine:PEG-400, a number of solvent mixtures were analyzed. Table SM 4.2.3.2.1 lists the yields from these different tests. While pyridine resulted in the highest yield of the cinnamic acid product, its toxicity makes it less than ideal for this reaction. Not only is pyridine highly flammable and toxic to aquatic environments, it is also extremely toxic to the liver, kidney and central nervous system.[3] From the Pfizer solvent selection guide, triethylamine is a suggested replacement in reactions where pyridine is used as a base.[4] While triethylamine is still toxic, this property stems primarily from its caustic nature.[5] A number of recent solvent selection guides have highlighted the many hazards associated with different solvents including pyridine and triethylamine.[6] Students are encouraged to research and explore the different guides as part of the results and discussion aspect of the experiment, and to consider the various health and environmental factors that determine which solvents should be used in different chemical reactions. Their research should lead them to conclude that PEG-400 is an ideal solvent in terms of health, environmental impact and safety for organic reactions, provided that the nature of the reaction is not strongly altered (i.e. the effect of the solvent on the reaction is taken into account, solubility is not an issue, boiling point of the solvent does not strongly affect the reflux conditions or reaction, etc.).

Table SM 4.2.3.2.2 summarizes the published results from the GlaxoSmithKline (GSK) solvent selection guide for the solvents studied herein, and highlights some examples of factors to consider when comparing the different solvents. Some or all of these factors may be included in the answers to the discussion questions in the experiment. Particular emphasis should be given to the life cycle scores for pyridine and triethylamine in the GSK guide. The life cycle score encompasses all of the factors that are important to the lifetime of a solvent and its applicability to a particular reaction or process. This includes its manufacture (considering the reagents needed for its synthesis, the waste generated, any greenhouse gas emissions produced during synthesis, etc.), its use in a process or reaction (toxicity, flammability, etc.), and its recyclability or disposal (boiling point and distillation issues, greenhouse gas emissions from incineration, etc.).[7] This "cradle-to-grave" approach helps to identify important metrics beyond just use of the solvent during the reaction, and is an important point in industrial processes and pharmaceutical reactions. This is particularly relevant here, as pyridine is very difficult to recycle due to its high boiling point and its ability to form azeotropes with other solvents. More details can be found in the report by GSK on the life cycle assessment of a variety of organic solvents.[7] A similar assessment is given by Capello et al. for other common organic solvents.[8]

Solvent[a]	**PEG-400**	**PEG-400**	**TEA:PEG (40:60)**	**TEA:PEG (70:30)**	**TEA**	**Pyridine**
Temperature[b] (°C)	25	160	160	160	160	160
Yield (%)	---	28	51	64	76	93

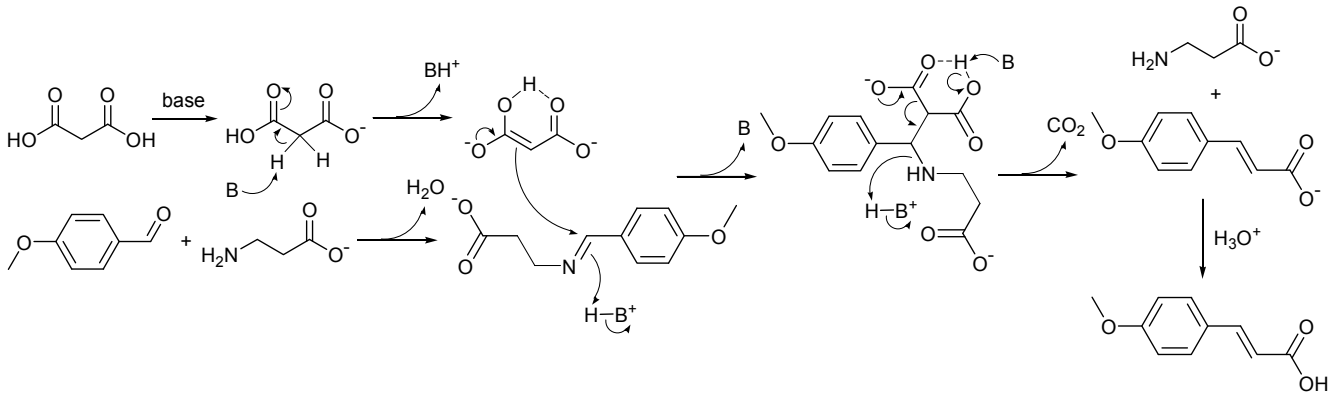
Table SM 4.2.3.2.1. Solvent systems tested for reaction of 4-methoxybenzaldehyde with malonic acid in the presence of 17 mol % β-alanine. Yields are quoted after product recrystallization in ethanol. [a]TEA = triethylamine, the ratios in brackets are the volume ratios of the two solvents. [b]The temperature of 160°C is the reflux temperature, as measured using a thermocouple in the solution during reaction.

	LC_{50} (4h, mg/m^3)	**LD_{50} (ppm)**	**boiling point (°C)**	**Waste**	**Environ-mental Impact**	**Health**	**Flammability**	**Reactivity /Stability**	**Life Cycle Score**	**Cost$^+$ ($/L)**
pyridine	7125	891	115	3	4	4	7	9	2	86.60
TEA	7100	730	88	4	5	3	4	8	7	89.10
PEG-400*	------	28,000	>200	(6)	(8)	(6)	(10)	(9)	(7)	74.10

Table SM 4.2.3.2.2. Toxicity data,[3,5,9] pricing,[9-11] and solvent guide scores from the GlaxoSmithKline (GSK) Solvent Selection Guide.[12] Scoring is out of 10, with 10 being the best score and 1 being the worst score. (*) The GSK scores listed for PEG-400 are those for tri-(ethylene glycol) as this is the closest compound in nature to PEG-400 listed in the GSK Solvent Guide, and hence these are shown in brackets. ($^+$) Costs were compared for 1 L of solvent at ≥99.0% purity from several different companies, with the cheapest cost available listed in the table.

While PEG-400 may seem like the ideal choice of solvent, the yield of the reaction is low in pure polyethylene glycol. This is due to the mechanism of the reaction (Scheme SM 4.2.3.2.3), where a

basic solvent is required to deprotonate malonic acid and catalyze the overall reaction.[13,14] As expected, PEG-400 is a weak base and the presence of triethylamine is necessary to promote the condensation reaction. The reason for the difference in yield between triethylamine and pyridine is less obvious, and a look into the pKa of their conjugate acids may yield a clue. The pK_a of the conjugate acid of pyridine is 5.5,[14] while the pK_a of the conjugate acid of triethylamine is 10.7.[15] This suggests that triethylamine should be a far better base for the deprotonation of the methylene group of the 1,3-dicarbonyl compound (pK_a of about 13).[14] However, the imine intermediate in the reaction (Scheme SM 4.2.3.2.3) is likely to be protonated in the presence of pyridine (and a sufficient concentration of protons in solution from the reaction), as opposed to deprotonated in the presence of triethylamine due to their difference in pK_a. This may have a strong effect on the nucleophilic attack of the enolate species. In addition, the steric bulk of the ethyl groups of triethylamine may have an effect on the overall rate of the condensation reaction as well.



Scheme SM 4.2.3.2.3. Reaction mechanism for the condensation of malonic acid and 4-methoxybenzaldehyde. The β-alanine organocatalyst is regenerated in the final step, and acidic workup of the carboxylate affords the α,β-unsaturated product.

The *trans*-4-methoxycinnamic acid product formed in this reaction is easily isolated in moderate to high yields after purification by recrystallization from ethanol. The crude product is generally a beige or off-white powder, while the recrystallized product exhibits needle-like crystals. A ^{1}H NMR spectrum of the product is given in Figure SM 4.2.3.2.4, with the resonances and coupling constants labeled. The ^{1}H NMR spectrum is acquired in either $CDCl_3$ or DMSO-d_6 (the latter is favourable for solubility reasons). Students can be asked to assign the resonances of the product (and of any impurities), and should be able to explain the multiplet splitting in the spectrum. A representative IR spectrum is shown in Figure SM 4.2.3.2.5, and the stretching frequencies should likewise be assigned to the appropriate functional groups in the *trans*-4-methoxy-cinnamic acid product. The IR spectrum is acquired using Nujol and NaCl plates with a standard IR spectrometer under transmission mode, or using an attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectrometer and directly examining the recrystallized product.

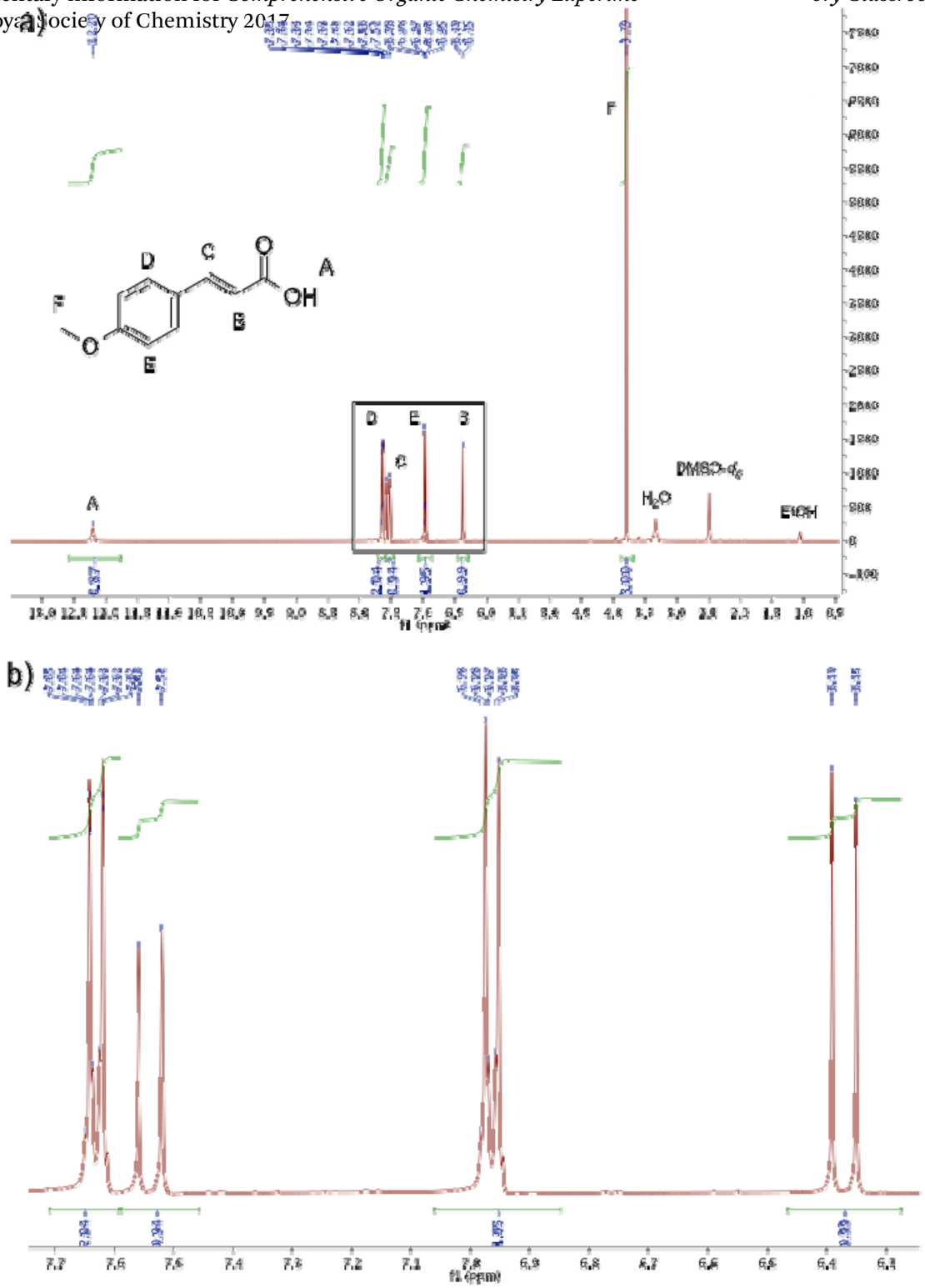


Figure SM 4.2.3.2.4. ^{1}H NMR spectrum of the recrystallized *trans*-4-methoxycinnamic acid product. a) Full spectrum, with different proton resonances indicated. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.79 (s, 3H), 6.37 (d, 1H, J = 16.0 Hz), 7.06-6.85 (m, 2H), 7.54 (d, 1H, J = 16.0 Hz), 7.71-7.59 (m, 2H), 12.20 (s, 1H). b) Expansion of the squared region in Fig. SM 4.2.3.2.4.a), highlighting the aromatic and vinylic resonances.

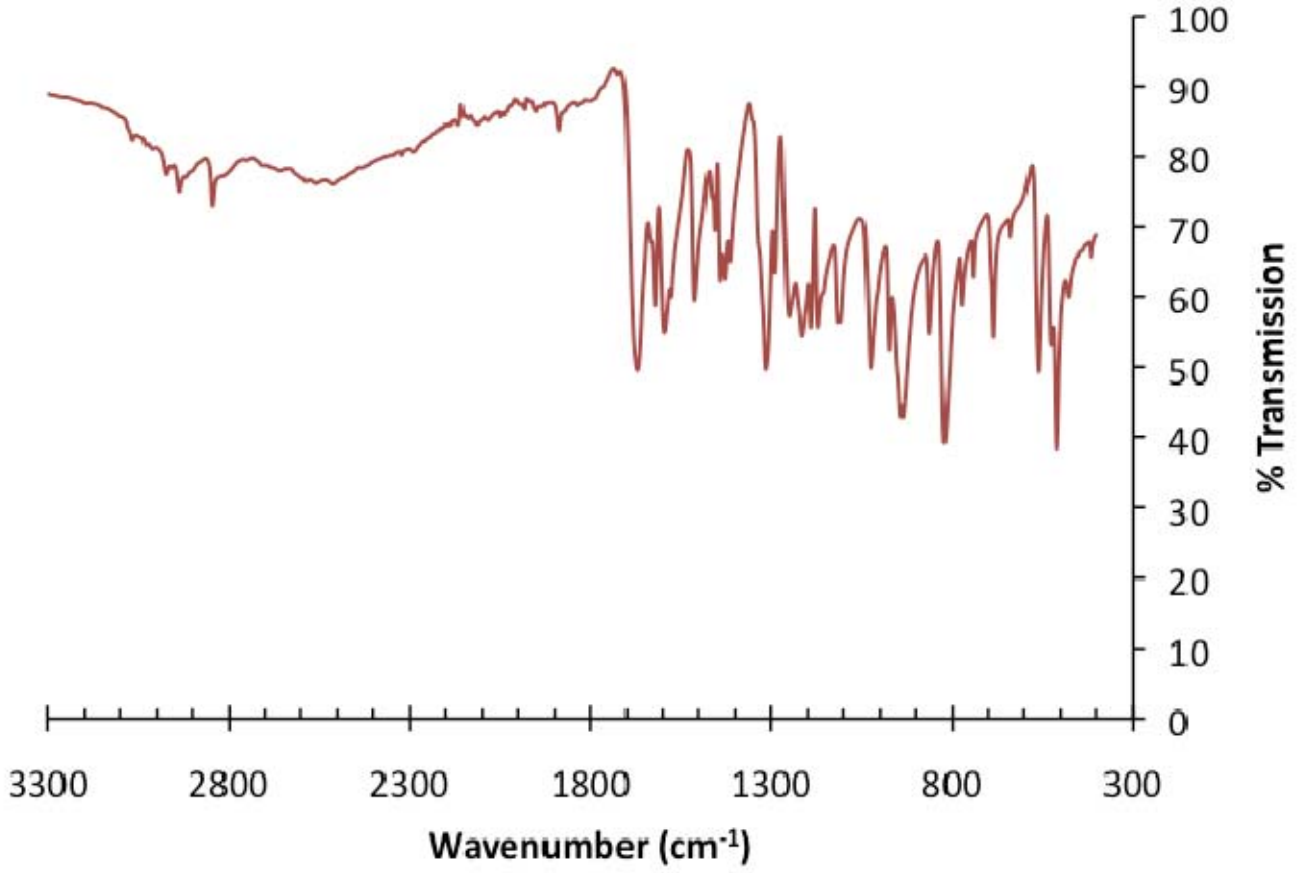
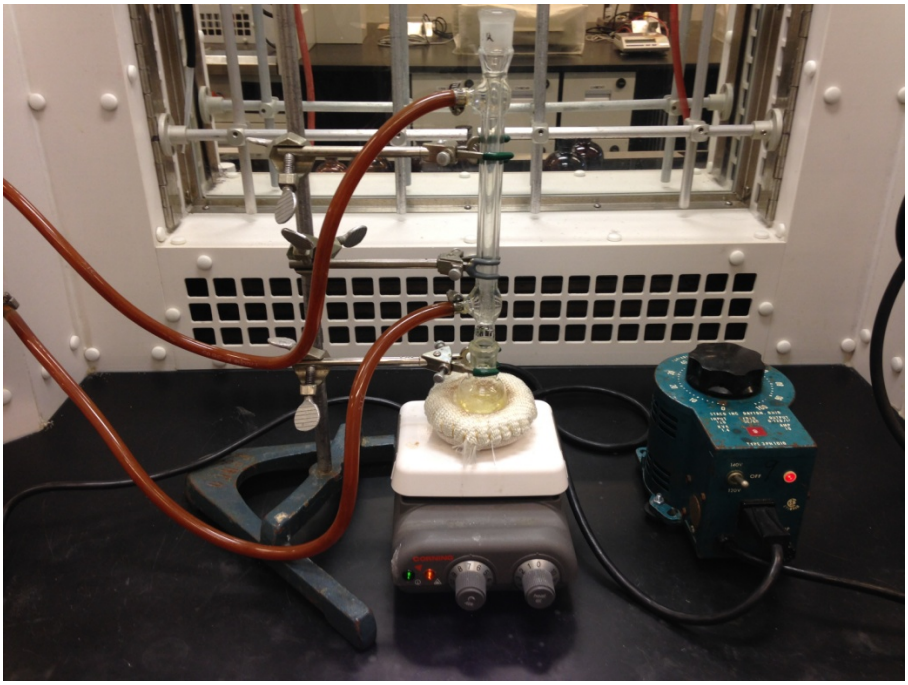
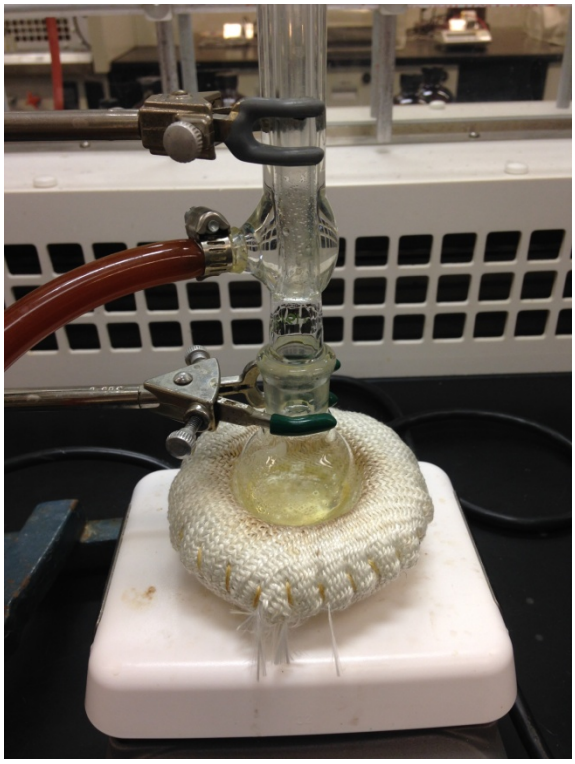


Figure SM 4.2.3.2.5. IR spectrum of the recrystallized *trans*-4-methoxycinnamic acid product. IR (ATR-FTIR) v_{max}/cm^{-1} 2970w (C=C-H stretch), 2930w (OCH_3 asym. stretch), 2840m (OCH_3 sym. stretch), 1670s (C=O stretch), 1590m (trans C=C stretch), 1510-1430 (aryl C=C stretch, OCH_3 asym. bend), 1310s (OCH_3 sym. stretch), 1220-1110 (OCH_3), 954-932s (C=C-H bending), 819s (OH bending), 561-510s (aryl C-H bending).

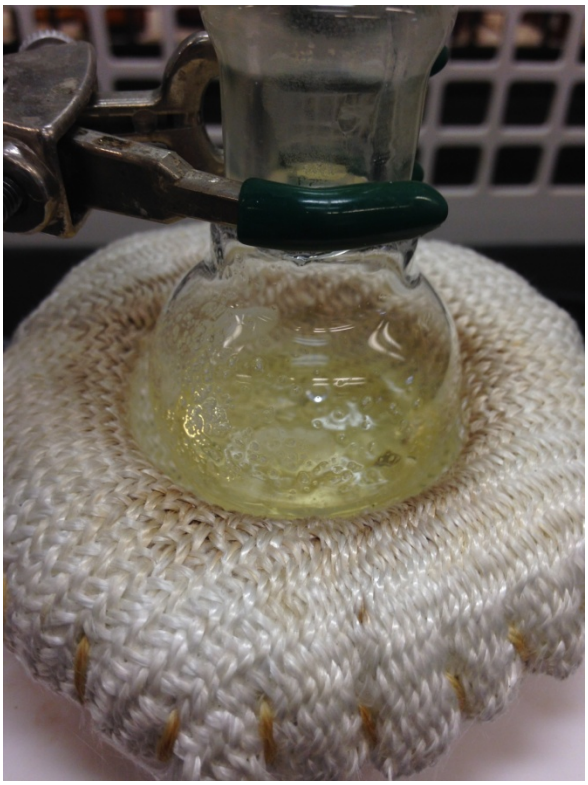
Experiment photographs



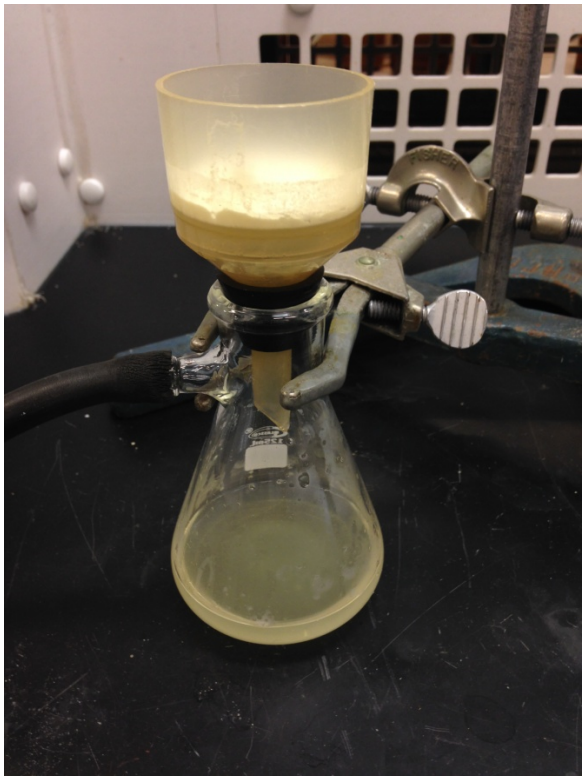
Reflux apparatus set-up (1)



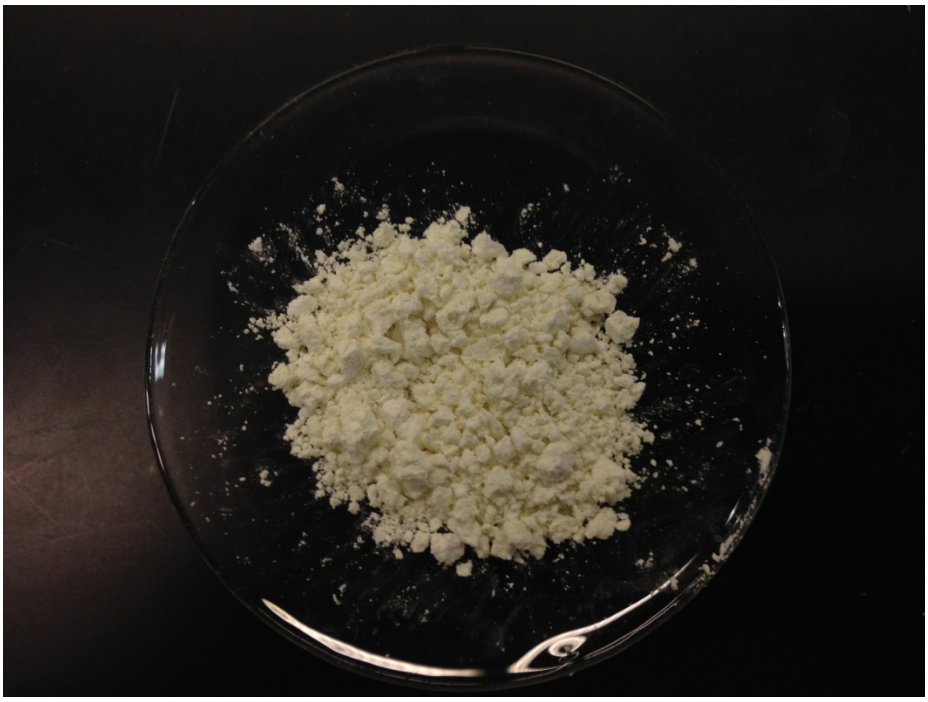
Reflux apparatus set-up (2)



Close-up of reaction mixture



Vacuum filtration apparatus



trans-4-Methoxycinnamic acid product (crude)

trans-4-Methoxycinnamic acid product (recrystallized)

References

1. A. P. Dicks and R. A. Batey, *J. Chem. Educ.*, 2013, **90**, 519.
2. L. R. Moore, E. C. Western, R. Craciun, J. M. Spruell, D. A. Dixon, K. P. O. Halloran, and K. H. Shaughnessy, *Organometallics*, 2008, **27**, 576.
3. Sigma-Aldrich. Pyridine, ACS Reagent, ≥99.0%. www.sigmaaldrich.com (accessed March 30, 2015): MSDS information for pyridine.
4. K. Alfonsi, J. Colberg, P. J. Dunn, T. Fevig, S. Jennings, T. A. Johnson, H. P. Kleine, C. Knight, M. A. Nagy, D. A. Perry, and M. Stefaniak, *Green Chem*., 2008, **10**, 31.
5. Sigma-Aldrich Co. Triethylamine, ≥99%. www.sigmaaldrich.com (accessed March 30, 2015): MSDS information for triethylamine.
6. D. Prat, J. Hayler, and A. Wells, *Green Chem.*, 2014, **16**, 4546.
7. J.-G. Concepcion, A. D. Curzons, D. J. C. Constable, and V. L. Cunningham, *Clean Technol. Environ. Policy*, 2005, **7**, 42.
8. C. Capello, U. Fischer, and K. Hungerbühler, *Green Chem.*, 2007, **9**, 927.
9. Acros Organics. Poly(ethylene glycol), average M.W. 400. www.acros.com (accessed March 30, 2015): MSDS and pricing information for PEG-400.
10. Acros Organics. Pyridine, 99+%, extra pure. www.acros.com (accessed March 30, 2015): pricing information for pyridine.
11. Acros Organics. Triethylamine, 99%, pure. www.acros.com (accessed March 30, 2015): pricing information for triethylamine.
12. R. K. Henderson, C. Jiménez-González, D. J. C. Constable, S. R. Alston, G. G. A. Inglis, G. Fisher, J. Sherwood, S. P. Binks, and A. D. Curzons, *Green Chem.*, 2011, **13**, 854.
13. J. M. Stacey, A. P. Dicks, A. A. Goodwin, B. M. Rush, and M. Nigam, *J. Chem. Educ.*, 2013, **90**, 1067.
14. J. Clayden, N. Greeves, S. Warren, and P. Wothers, *Organic Chemistry*, Oxford University Press, New York, NY, USA, 2001.
15. National Center for Biotechnology Information (National Institutes for Health (NIH)), Open Chemistry Database. Triethylamine. http://pubchem.ncbi.nlm.nih.gov//compound/8471?from=summary#section=Top (accessed March 30, 2015): physical properties for triethylamine.

Knorr synthesis of diethyl 3,5-dimethyl-1H-pyrrole-2,4-dicarboxylate

Supplementary Material

Experiment Notes

This classic Knorr pyrrole synthesis is a good opportunity to study the formation of an aromatic compound from open chain starting material.

Although commercially available ethyl acetoacetate is employed, it may be also synthesized by the student (if the instructor considers it appropriate). The synthesis of ethyl acetoacetate by Claisen reaction from ethyl acetate is a well-known reaction and its procedure is well documented (Org. Syn. Coll. Vol. I, **1941**, 235).

The experiment is divided into three sessions, but they can be reduced to two ones (since sessions 1 and 2 can be considered as one larger session). For a better nitrosation, the addition of aq. sodium nitrite should be done slowly at low temperature but with vigorous stirring.

If the temperature rises over 10 ºC an increasing in the color is observed in the obtained crystals. When the mixture reaches rt the formed oxime can be left overnight instead of being left only for 3 h. (An increasing of the yield is observed when is left overnight). Oxime reduction with zinc dust in acetic acid media produces a temperature spike because the process is exothermic, but with the small amounts of this experiment this fact is hardly perceptible. If the reaction were scaled up to 20-50 g of starting material, the control of temperature should be much more efficient, maintaining the flask in cold water. When addition is finished, Zn dust over the walls of the flask can be drawn into with some acetic acid. When the reaction is under reflux, the stirring must be efficient. After reflux, the dilution of the still warmed mixture with water can be done in a beaker instead of an Erlenmeyer flask, but with these amounts it is easier collecting crystals in the latter.

Recrystallization- The student has to add the minor amount of solvent in which the precipitate is solved when heated. It is better add a minimum of the solvent and later add more if it is necessary. When the heated solution is filtered, sometimes the student can watch the leftover Zn dust. The first crop of crystals is more suitable for NMR experiments. If a second crop of crystal were obtained, students should obtain and compare both melting points as criteria of purity. The elucidation of RMN data might be done by students before of comparing with literature data.

This experiment was carried out by students in the third year of Chemistry.

The obtained total yield of the recrystallized product in different experiments runned out is in the range 26 up to 65 %. The lower yield was due to an increasing of the temperature in $NaNO_2$ addition.

m.p.: 133-134 ºC up to 136-137 ºC. Literat. (CAS Registry N. 2436-79-5) 126-130 °C up to 136-137 °C

Photos of the experiment

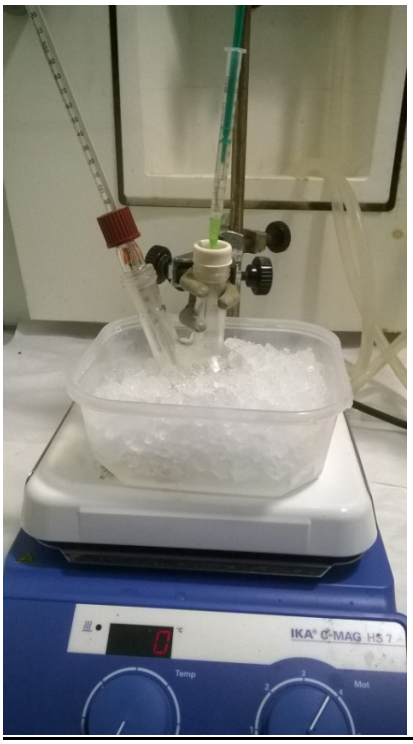


Figure SM 4.2.3.3.1 – Oxime formation

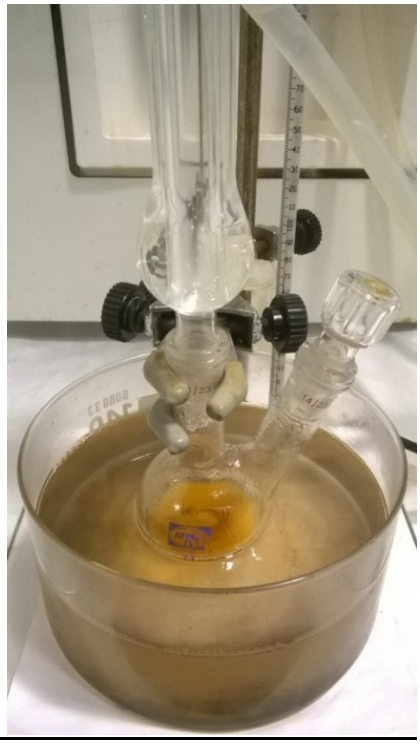


Figure SM 4.2.3.3.2– Oxime reduction

Figure SM 4.2.3.3.3– Filtration of the crude product

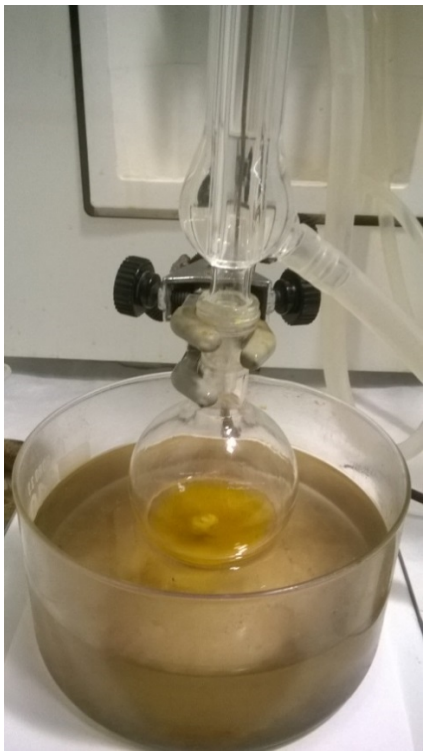


Figure SM 4.2.3.3.4– Reflux for recrystallization

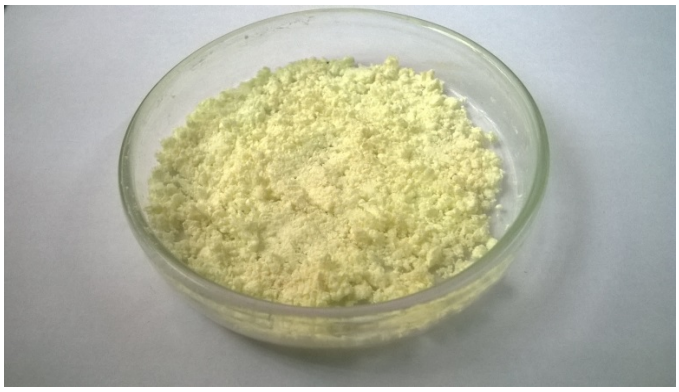


Figure SM 4.2.3.3.5– Recrystallized product

^{1}H NMR and ^{13}C NMR spectra

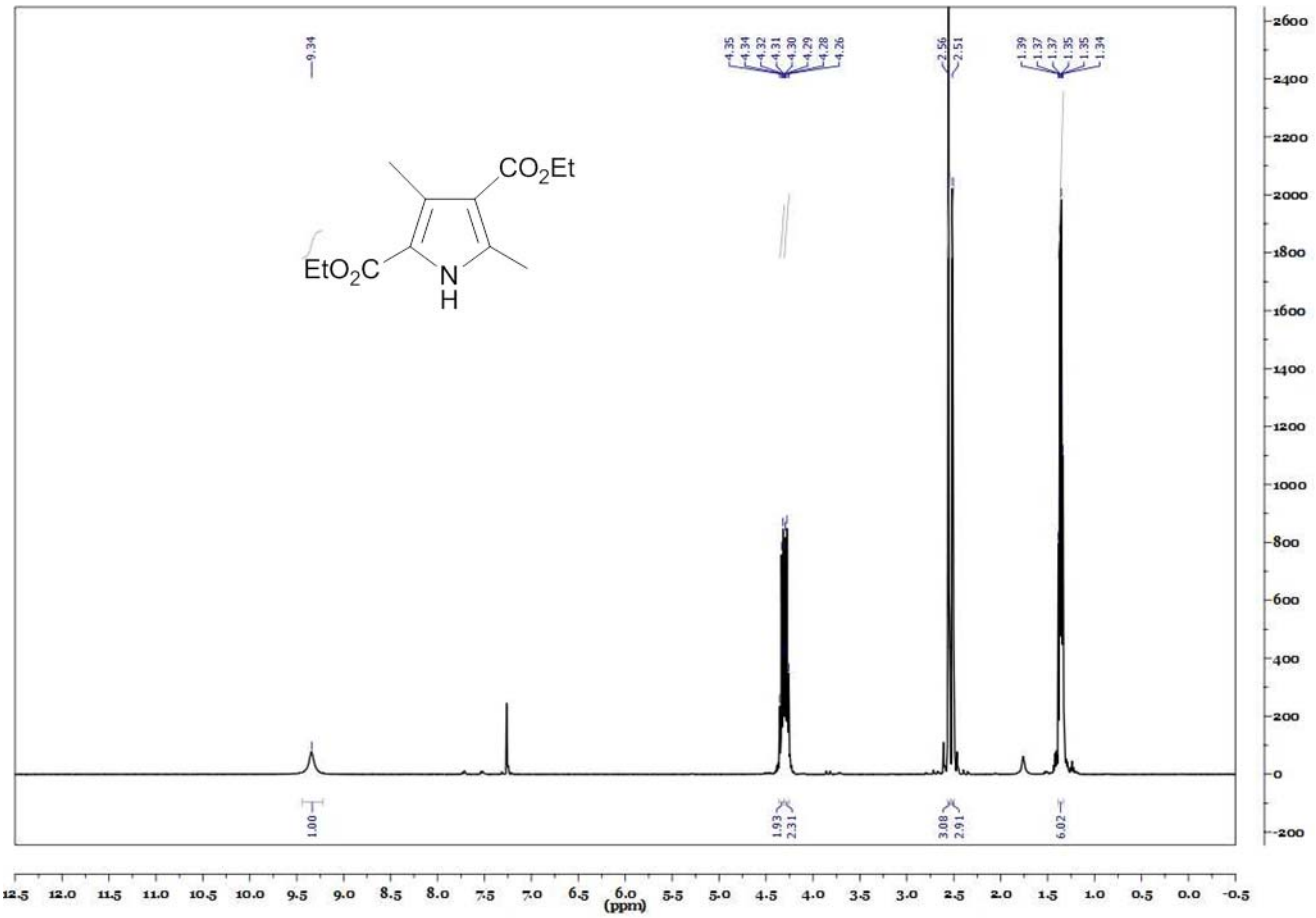


Figure SM 4.2.3.3.6–^{1}H NMR spectrum (400MHz, $CDCl_3$) of diethyl 3,5-dimethyl-1H-pyrrole-2,4-dicarboxylate.

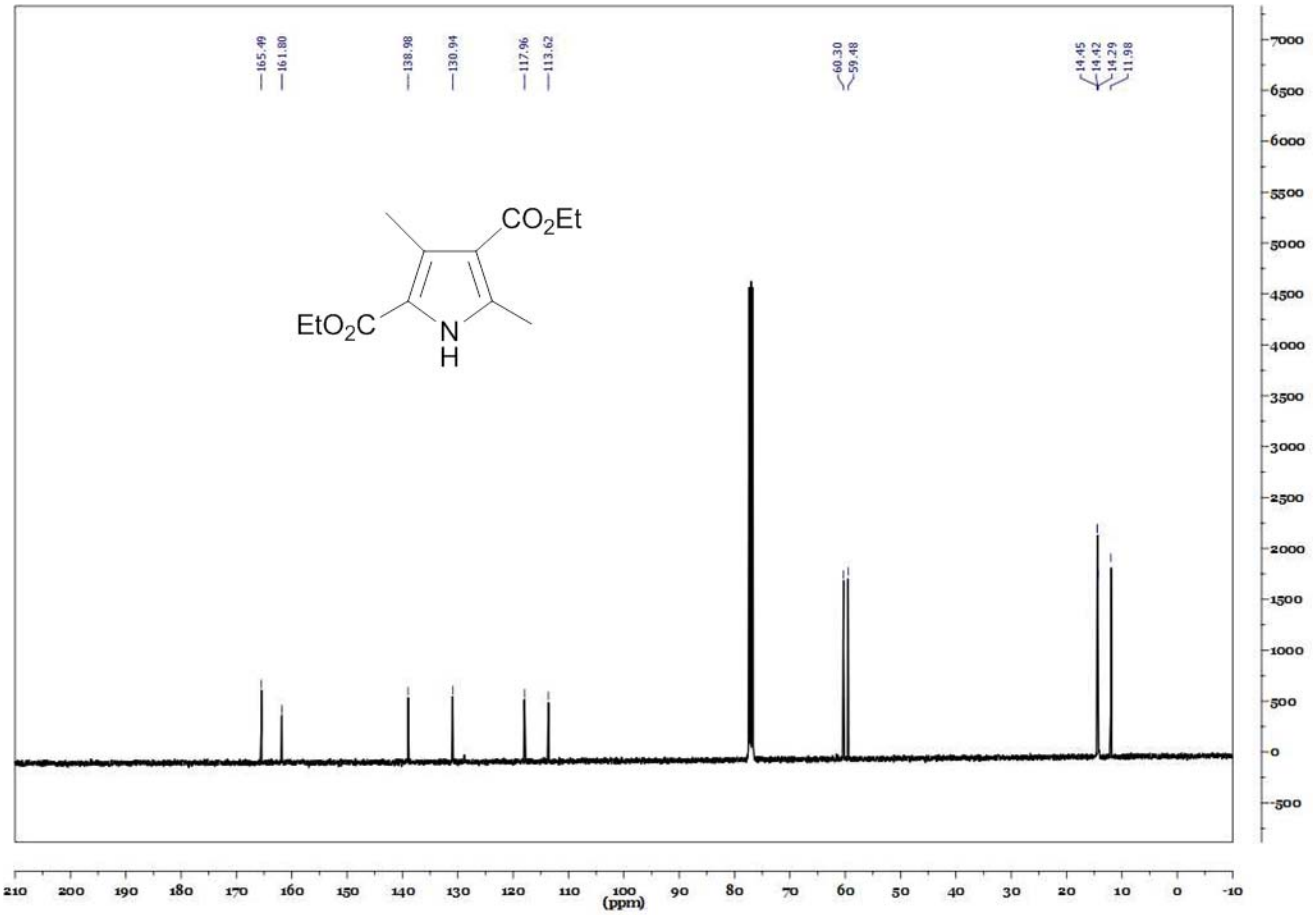


Figure SM 4.2.3.3.7–^{13}C NMR spectrum (100MHz, $CDCl_3$) of diethyl 3,5-dimethyl-1H-pyrrole-2,4-dicarboxylate.

Synthesis of coumarin-3-carboxylic acid

Supplementary Material

Experimental notes

This experiment aims at the preparation of coumarin-3-carboxylic acid, in two steps, through the Knoevenagel condensation of salicylaldehyde with diethyl malonate followed by basic hydrolysis. The experiment requires two 2h sessions but the products are easily isolated by filtration after precipitation. This experiment is very reproducible and easy to perform but due to the concepts involved, the experiment is more adequate to 2nd year chemistry students.

The initial solution is yellow and after one hour at reflux there is still a small amount of the salicylaldehyde, which can be easily detected by TLC. Increasing the reaction time does not lead to higher yields. The residual salicylaldehyde does not interfere with the isolation of the coumarin ester as the addition of cold water to the reaction mixture leads to the immediately formation of white crystals of the coumarin ester while the salicylaldehyde rests in solution. It is thus important to wash the crystals to assure its purity. The yields of this first step vary between 82-83% and the melting points, 88-92ºC, are similar to those reported in the literature (92-95ºC, Bhat, M. A., Siddiqui, N., Khan, S. A. (2008) Synthesis of novel 3-(4-acetyl-5H/methyl-5-substituted phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)-2H-chromen-2-ones as potential anticonvulsant agents. *Acta Poloniae Pharmaceutica Drug Research*, Vol. 65 No. 2 page. 235-239)

The second step is a classic basic hydrolysis. The initial reaction mixture is opaque, due to low solubility of the coumarin ester in the water/ethanol mixture, but as the mixture is heated under reflux it progressively becomes transparent. After 30 min the reaction is complete and upon acid addition there is the instantly formation of white crystals of the coumarin acid. It is not necessary to recrystallize the product as it appears pure by TLC and NMR. The measurement of the weight and melting point of the product can only be made after drying the product overnight in a oven The yields of the second step vary between 80-88% and the melting points, 190-192ºC , are similar to those reported in the literature (189-192ºC, Karami, B., Farahi, M. and Khodabakhshi, S. (2012), Rapid Synthesis of Novel and Known Coumarin-3-carboxylic Acids Using Stannous Chloride Dihydrate under Solvent-Free Conditions. *Helvetica Chimica Acta*, Vol. 95 pp 455–460). The global yield is thus between 70-81%.

Photos of experiment

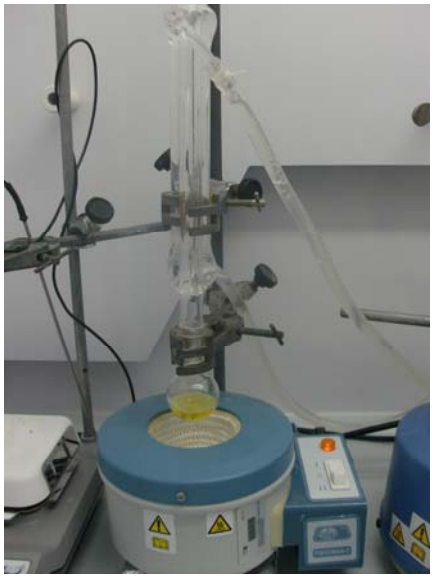


Figure SM 4.2.3.4.1. Reaction apparatus with the initial yellowish solution

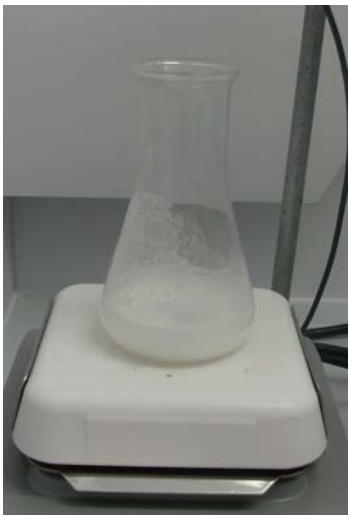


Figure SM 4.2.3.4.2. Addition of HCl to the basic aqueous solution and formation of the final product

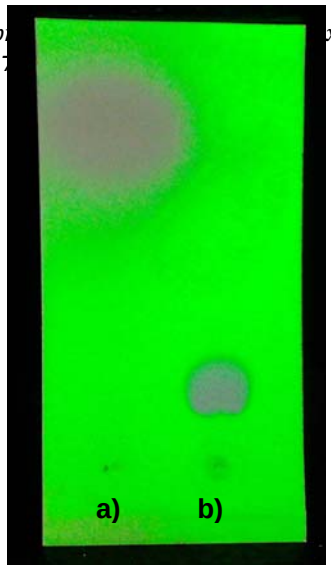


Figure SM 4.2.3.4.3. 1st step TLC plate. 60% diethyl ether/petroleum ether. a) Salicylaldehyde b) Product (coumarin ester)

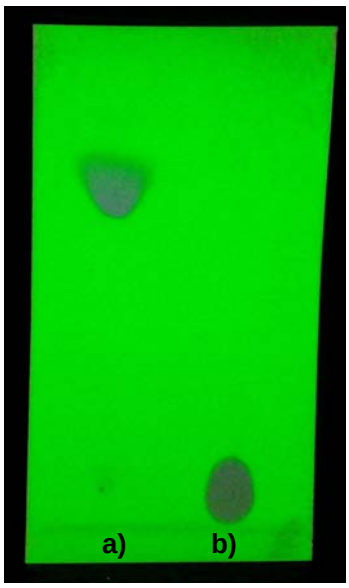


Figure SM 4.2.3.4.4. 2nd step TLC plate. 80% diethyl ether/petroleum ether. a) Coumarin ester b) Product (coumarin-3-carboxylic acid)

^{1}H NMR and IR Spectra

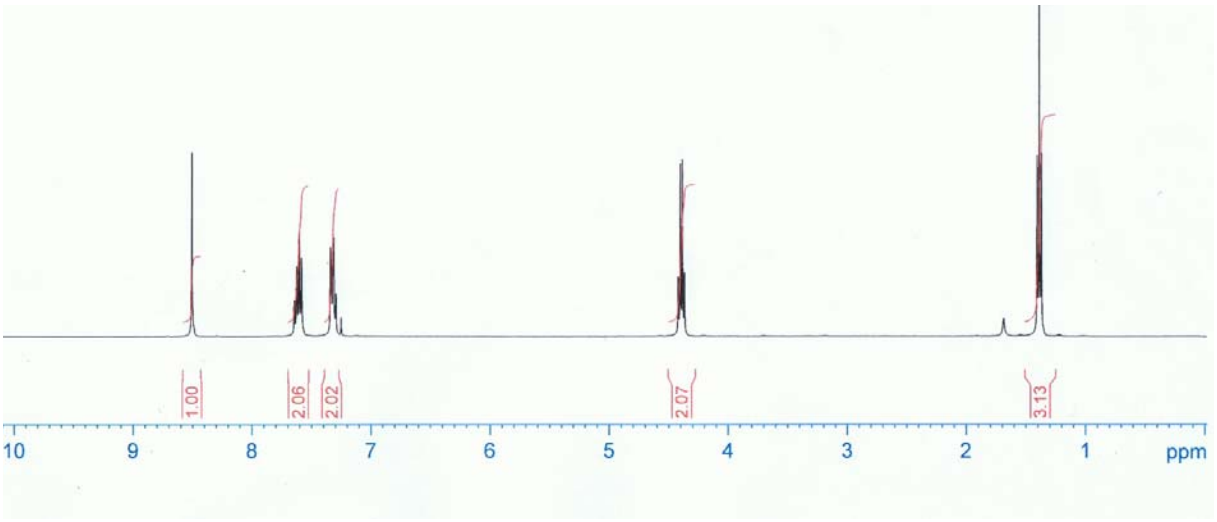


Figure SM 4.2.3.4.5. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of the coumarin ester

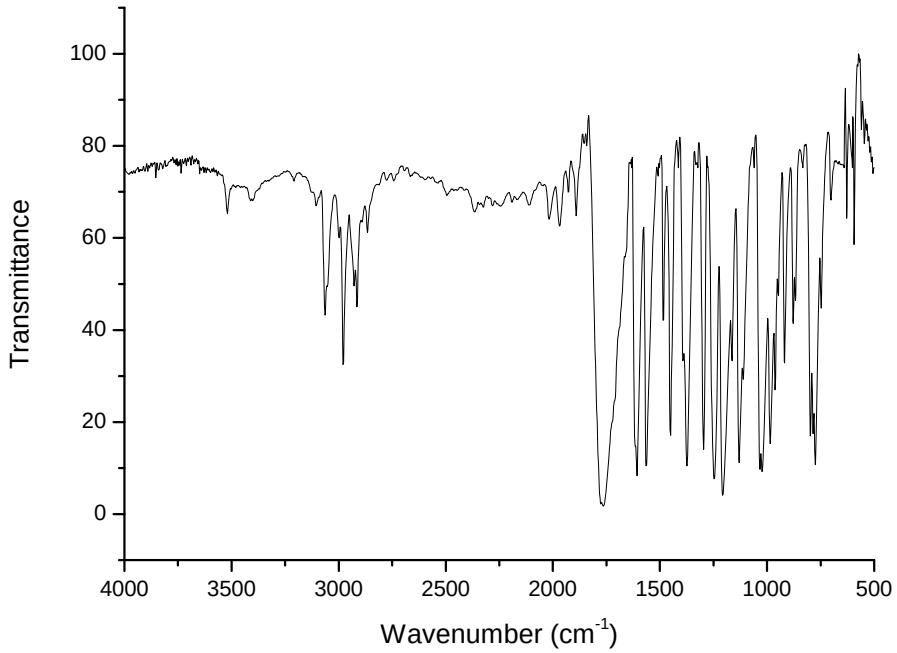


Figure SM 4.2.3.4.6. IR spectra of the coumarin ester

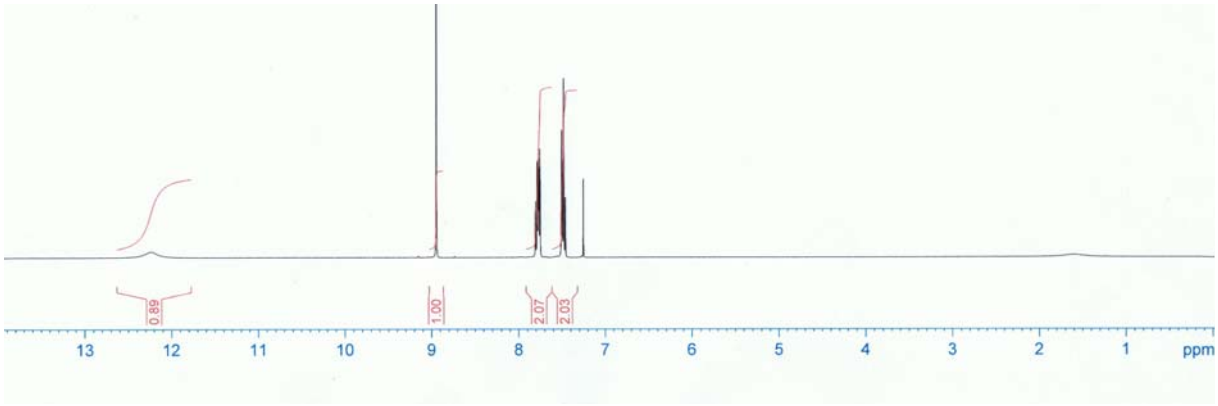


Figure SM 4.2.3.4.7- ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of the coumarin-3-carboxylic acid

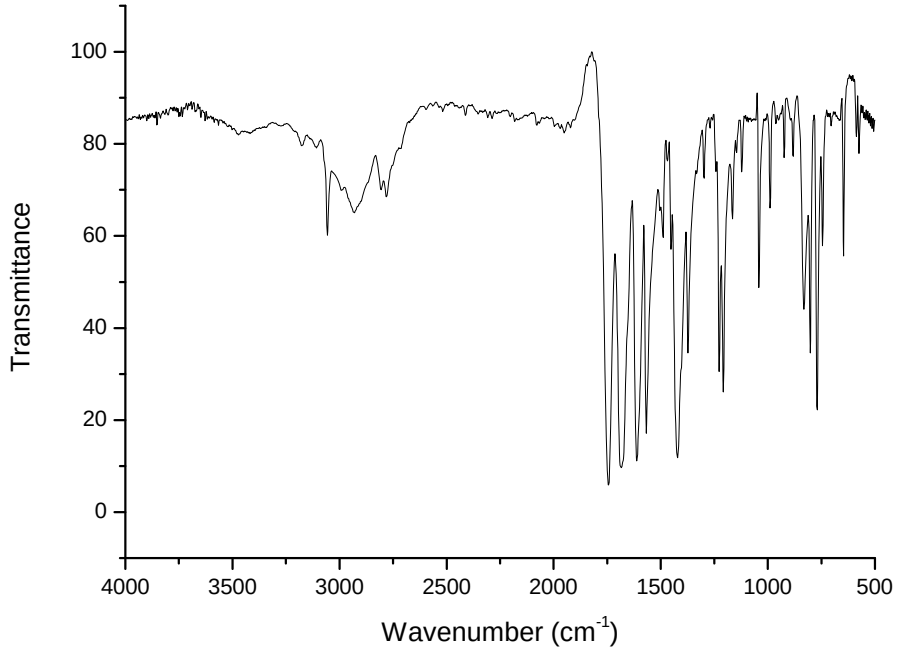


Figure SM 4.2.3.4.8. IR spectra of the coumarin-3-carboxylic acid

Synthesis of lipophilic antioxidants based on natural models

Supplementary material

Antioxidants play an important role in the protection of cells from the damage caused by reactive species (RS). Hydroxycinnamic acids (HCAs) have particular interest due to their plethora of biological activities such as antioxidant, anti-inflammatory and/or antimicrobial activity. However, their hydrophilicity hinders their application in lipophilic media. Within this experimental framework, the students will become familiarized with the classic synthetic approaches used for enhancing a drug's lipophilicity and an *in vitro* method often used for the evaluation of antioxidant activity. To validate the effect of the structural modifications performed on the natural antioxidant, the last step of the current protocol envisages the theoretical evaluation of lipophilicity using an online freeware program. The experiments proposed were designed for chemistry-based curricula, namely chemistry or medicinal chemistry courses, and are best suited for students that have organic chemistry (theoretical and practical) foundations. The experiments were tested in a first year master chemistry course including 30 students. They were easy to perform and produced clear results. The average success of students in the integrated experience was of 95%.

Additional notes on the synthesis of 3-(hexyloxy)-3-oxopropanoic acid

This experience has been implemented to synthesize the monomalonate needed for the subsequent reaction in a simple and effective fashion. In normal conditions, the reaction is complete after 4h. The progress of the reaction can be monitored by TLC using ethyl acetate or petroleum ether/ethyl acetate (6:4)) as eluent. Any emulsions formed in the extraction step can be easily solved by adding a small amount of brine. The highest yield obtained was around 75-85 %. The synthesized monomalonate is used in the following reaction without further purification.

Additional notes on the preparation of hexyl (E)-3-(3,4-dihydroxyphenyl)acrylate

This procedure was a modification of Knoevenagel condensation reaction, and was obtained yields around 65-70 %. Due to the use of pyridine the reaction work-up should be performed inside the fume hood. The mixture is heated at 90-100 °C, cooled in an ice bath and neutralized with HCl 1 mol/L. In this step the mixture should be left with

stirring for approximately 30 minutes. The formation of the long chain alkyl product is easily visualized by TLC (petroleum ether/ethyl acetate (6:4) with UV revelation, since the compound display a higher R_f value than the starting material 3.4-dihydroxybenzaldehyde. The spots can be visualized under UV detection (254 and 366 nm) and iodine vapour. In addition, the chromatogram can be also sprayed with a $FeCl_3$ solution (10% in MeOH) as phenolic compounds gave a positive reaction (from blue, violet, purple, green, to red-brown color). The crude product is purified by flash chromatography using silica gel as stationary phase (petroleum ether/ethyl acetate (6:4)). The identity of hexylcaffeate is confirmed by ^{1}H NMR experiments performed in MeOD (Figure **SM 4.2.3.5.1**). As *cis* and *trans* coupling appear differently on NMR spectrum the isomerism of the synthesized compound can be determined. In the present case a coupling constant $J_{\alpha,\beta}$ = 16.0 Hz is observed indicating the formation of a *trans* (*E*) isomer. The aromatic protons H(2) and H(5) appear as doublets (J_{H2} = 2 Hz, due to *meta* coupling with H(5) and J_{H5} = 8 Hz due to *ortho* coupling with H(6)), and the proton H(6) appears as a doublet of doublets (J_1 = 8 Hz and J_2 = 2 Hz).

^{1}H NMR data was acquired, at room temperature, on a Brüker Avance III spectrometer operating at 400 MHz.

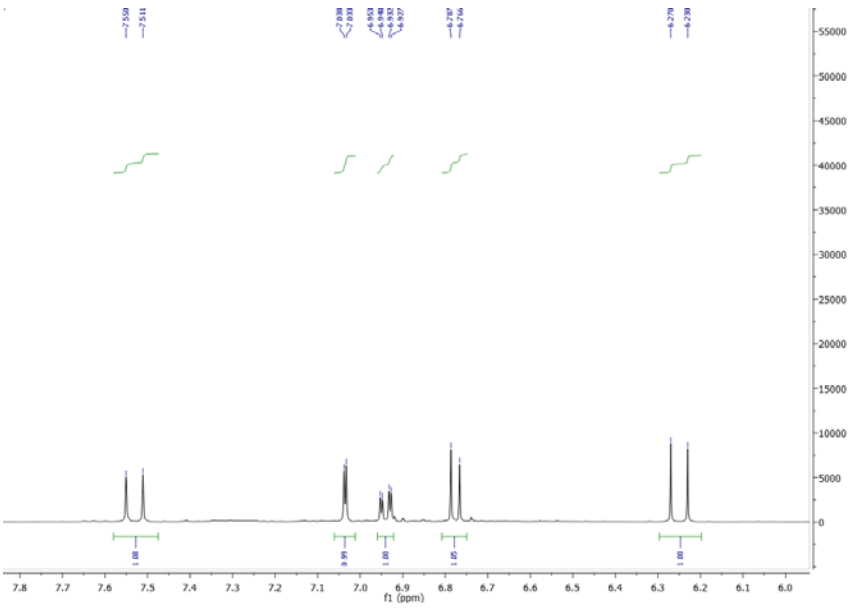


Figure SM 4.2.3.5.1. ^{1}H NMR spectrum of hexyl caffeate (400 MHz, MeOD)

Additional notes on the synthesis of (*E*)-3-(3,4-dihydroxyphenyl)-N-hexylacrylamide

The synthesis of amides is a classic chemical modification to obtain drugs enhanced not only in lipophilicity but also in plasmatic half-life, since this bond is physiologically more stable than the ester bond. In order to synthesize amides from the corresponding carboxylic acids several strategies can be used, namely the use of coupling agents. From the array of coupling reagents described so far, PyBOP was selected due to its advantages over other coupling agents, namely easier purification, mild reaction conditions and reduced toxicity. Reaction progress can be monitored by TLC (dichloromethane/methanol (95:5)). The crude product is purified by flash chromatography using silica gel as stationary phase and dichloromethane/methanol (95:5)) as eluent. The reaction yield is about 60%. ^{1}H NMR experiments can be performed in DMSO-*d6* (Figure **SM 4.2.3.5.2**). A coupling constant $J_{\alpha,\beta}$ = 16.0 Hz is observed indicating the formation of a *trans* (*E*) isomer. The aromatic protons H(2) and H(5) appear as doublets (J_{H2} = 2 Hz, due to *meta* coupling with H(5) and J_{H5} = 8 Hz, due to *ortho* coupling with H(6)) and proton H(6) appears as a doublet of doublets (J_1 = 8 Hz and J_2 = 2 Hz).

^{1}H NMR data was acquired, at room temperature, on a Brüker Avance III spectrometer operating at 400 MHz.

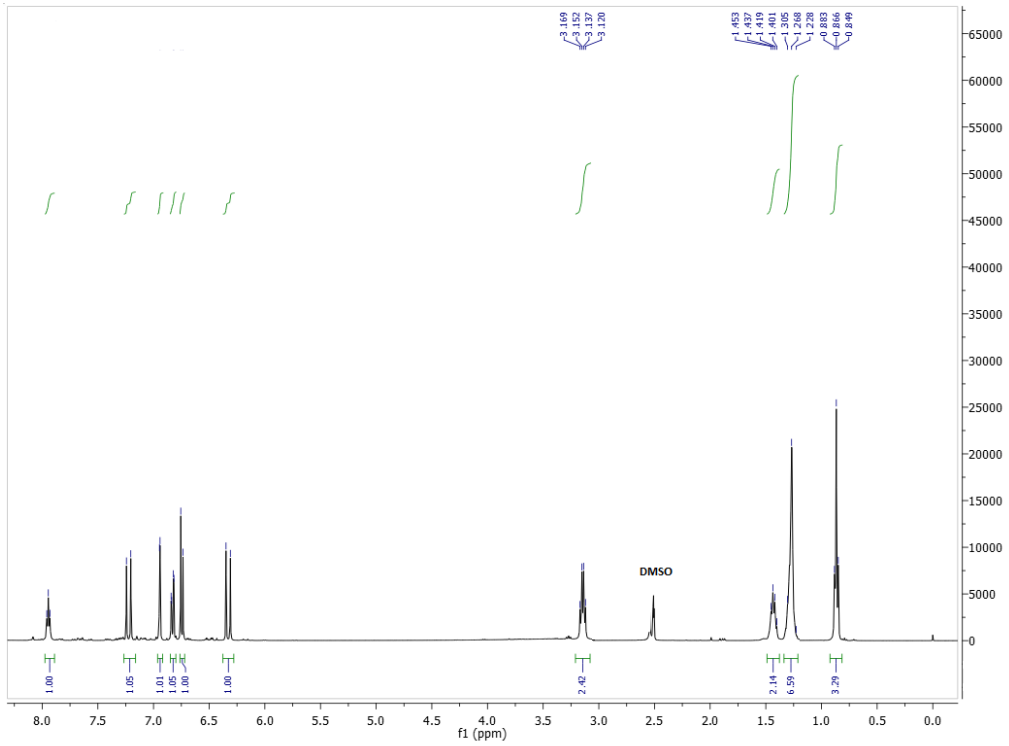


Figure SM 4.2.3.5.2. ^{1}H NMR spectrum of *N*-hexylcaffeoyl amide (400 MHz, DMSO)

Additional notes on the DPPH assay

This spectrophotometric assay is based on the decrease on the absorbance at 515 nm when the radical is neutralized by an antioxidant. To measure the radical scavenging activity of the compounds under study, the radical solution is incubated with increasing concentrations of the compound and for each treatment the percentage of inhibition (% inhibition) is calculated according to the following formula:

$$\% \text{ Inhibition}_{sample} = (A_{control} - A_{sample})/(A_{control})$$

where $A_{control}$ is the absorbance of the DPPH solution without antioxidant and A_{sample} is the absorbance of the tested antioxidant. The initial solution of the radical should be sonicated for at least 15 min to ensure that DPPH is fully dissolved. The solutions should always be capped and stored in a cool place to avoid the evaporation of EtOH and consequent concentration variation. When the assay is complete, the % inhibition for each concentration is calculated using the formula described above. The IC_{50} can be determined by plotting % inhibition vs concentration and adjusting the data to a linear fit using the minimum squares method.

Under these experimental conditions, the mean values obtained for the reference antioxidant and parent compounds are shown on Table **SM 4.2.3.5.1**.

Table SM 4.2.3.5.1. Partition coefficients and results for the DPPH assay.

Compounds	Log P (Theor.)[a]	DPPH IC_{50} ± S.D. (μM)
Caffeic acid	0.94	21.7 ± 0.2
Hexyl caffeate	4.01	30.1 ± 0.3
***N*-hexylcaffeoyl amide**	3.25	29.9 ± 0.3
Trolox	3.07	30.8 ± 0.1
Vitamin E	9.04	30.9 ± 0.9

[a] Determined with Molinspiration Cheminformatics Software ®

These values will function as positive controls to validate the experiments. Generally, antioxidant activity is enhanced by the number of OH functions in the aromatic ring, which is in accordance with the results shown on Table **1** (IC_{50} for caffeic acid is lower than the reference compounds). The same outcome is expected for the corresponding ester and amide derivatives. Hexyl caffeate is more lipophilic than *N*-hexylcaffeoyl amide but exhibit similar antioxidant activity. Vitamin E is as expected the more

lipophilic compound. However, it must be stressed that the correlation between logP and membrane permeation is non-linear; the permeation decreases at both low and high ends of logP values, and often a logP value of 5 is considered as the upper limit for lipophilicity.

Additional notes

- Stock solutions of DPPH and antioxidants could be provided by the instructor, but students should be asked to bring in the calculations needed to prepare them.
- Instructors interested in more reliable results may consider the use of several replicates. However, the use of replicates will result in a longer lab session.
- A limitation of the DPPH radical scavenging assay is related with the evaluation of the antioxidant activity of coloured antioxidants. In fact some antioxidants can have absorption at the wavelength under study (517 nm). In order to overcome this constraint, instructors must include in the lab session, a spectrophotometric scan of each of the antioxidants in the wavelength interval between 490-560 nm.

Additional notes on the theoretical evaluation of partition coefficient

The theoretical determination of the partition coefficient is straightforward and enables an accurate estimation of the lipophilicity scale within a set of derivatives. In this case, and in accordance with the rational for the performed chemical modifications, the amidation and esterification of the parent HCAs results in a significant increase in lipophilicity, as seen by the LogP values (Table **1**).

Additional Reading

A. Ault, Techniques and Experiments for Organic Chemistry, University Science Books, Sausalito, 6th edition, 1998.

D.L. Pavia, G.M. Lampman, G.S. Kriz, J.A. Vyvyan, Introduction to Spectroscopy, Cengage Learning, Stamford, 5th edition, 2014.

F. Shahidi, M. Naczk, Phenolics in Food and Nutraceuticals, CRC Press, Boca Raton, 2004.

A simple and ecological preparation of a chromene-3-carboxamide derivative

Supplementary Material

The use of safe, green laboratory experiments for reactions studied within basic organic chemistry courses are increasingly relevant in the educational context.

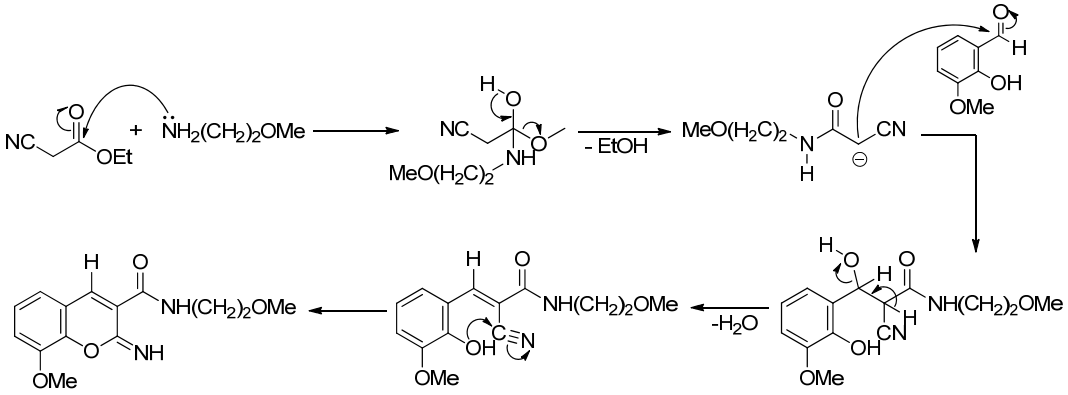
This experiment demonstrates the viability to synthesize a simple organic molecule, present in diverse bioactive compounds (a chromene-3-carboxamide) under simple and eco-friendly conditions. The work is initiated with the solventless synthesis of a cyanoacetamide by combining equimolar amounts of ethylcyanoacetate and amine. The reaction is fast and quantitative and the product can be directly used for the reaction with a salicylaldehyde derivative, in mild aqueous base, at room temperature.

The difficulty level of the experiment is basic as it illustrates simple operations performed in organic chemistry laboratories: stirring at room temperature and isolation of the solid product by vacuum filtration. The product can be characterized by NMR spectroscopy and the atom economy of the process can also be determined.

The theoretical concepts associated with the experiment are focused on the chemistry of carbonyl compounds, also at a basic level. The reactions involved are:

a) Amide formation from the combination of an ester with an amine;
b) Aldol-type condensation (Knoevenagel reaction) of an active methylene compound and an aldehyde;
c) Intramolecular nucleophilic addition of a phenolic oxygen to a cyano group, eventually followed by hydrolysis of the imine function, if aqueous acid is used in a subsequent step.

The mechanism of reaction can be summarized as follows:



The concept of atom economy as a measure of conversion efficiency in a chemical process is important in the design of eco-friendly experimental procedures. Besides

high yields, friendly solvents and non-toxic reagents and products, it is also important to minimize waste formation. The atom economy quantifies the amount of waste produced in a chemical transformation and can be calculated using the equation:

$$\text{Atom economy} = \frac{\text{Molecular mass of desired product}}{\text{Molecular mass of all reagents}} \times 100$$

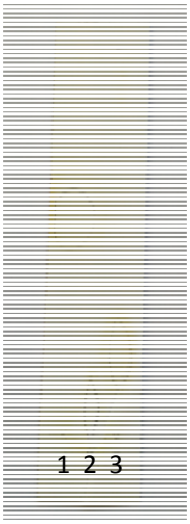
Additional notes on the preparation of chromene-3-carboxamide derivative (Session 2): This procedure was successfully implemented at the described scale and temperature, with a product yield of around 60 %, when performed by experienced researchers (six final year undergraduate students performed the experiment and isolated the product in 52-60% yield). In the original procedure[1] the aqueous base solution combining the aldehyde and 2-cyanoacetamide was stirred at room temperature for approximately 20 h, leading to a quantitative yield of the product. This may be a better approach if the work is carried out in two separate sessions, in consecutive days.

The monitoring of the reaction progress by TLC can be performed using silica plates eluted with Petroleum Ether (40-60)/EtOAc 1:2 after sample dilution with ethanol. For session 1, the TLC should be visualized using an iodine chamber. For session 2, the TLC spots can be visualized either by UV light or by staining with iodine (to identify the product from session 1).

The NMR spectrum should be performed in DMSO-d_6, since the product is not soluble enough in $CDCl_3$ and only spectra of poor quality will be obtained in this solvent.

Photo Gallery

Figure SM 4.2.3.6.1: Reaction set-up for laboratory session 1 and solid product after completing reaction 1



1: Ethyl-cyanoacetate

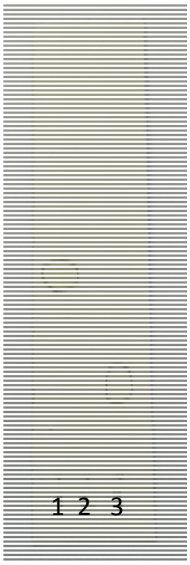
2: 3-methoxyethylamine

3: Product

Figure SM 4.2.3.6.2: TLC at the end of session 1 (stained with iodine)

Figure SM 4.2.3.6.3: Reaction set-up for laboratory session 2 and solid product after completing reaction 2

Figure SM 4.2.3.6.4: Filtration set-up



1: Aldehyde

2: 2-Cyanoacetamide

3: Product

Figure SM 4.2.3.6.5: TLC of the final isolated product visualized by UV light

^{1}H NMR spectra

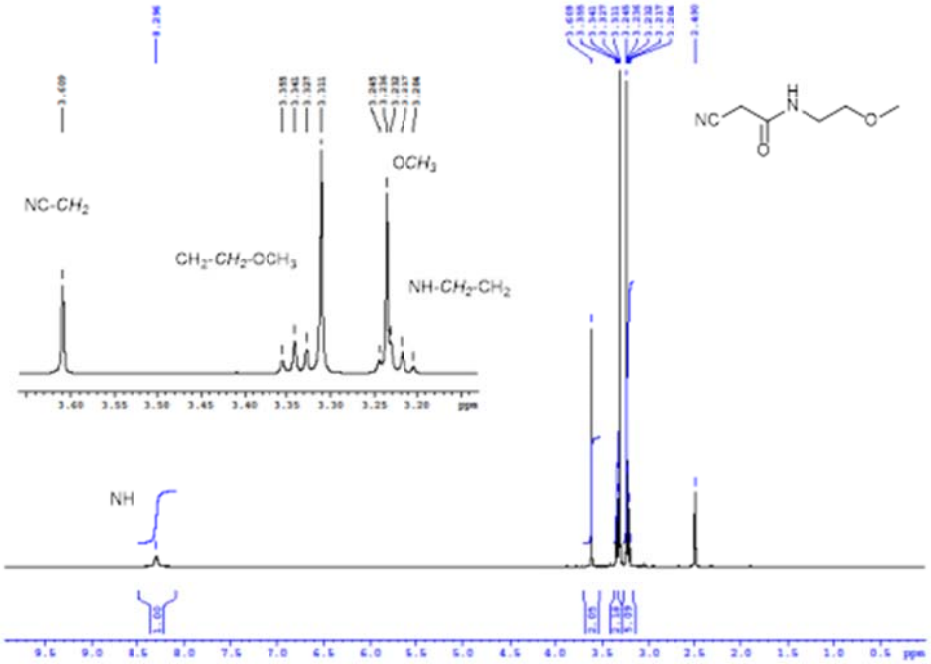


Figure SM 4.2.3.6.6 ^{1}H NMR spectrum for 2-cyano-*N*-(2-methoxyethyl)acetamide (400 MHz, dmso-d_6).

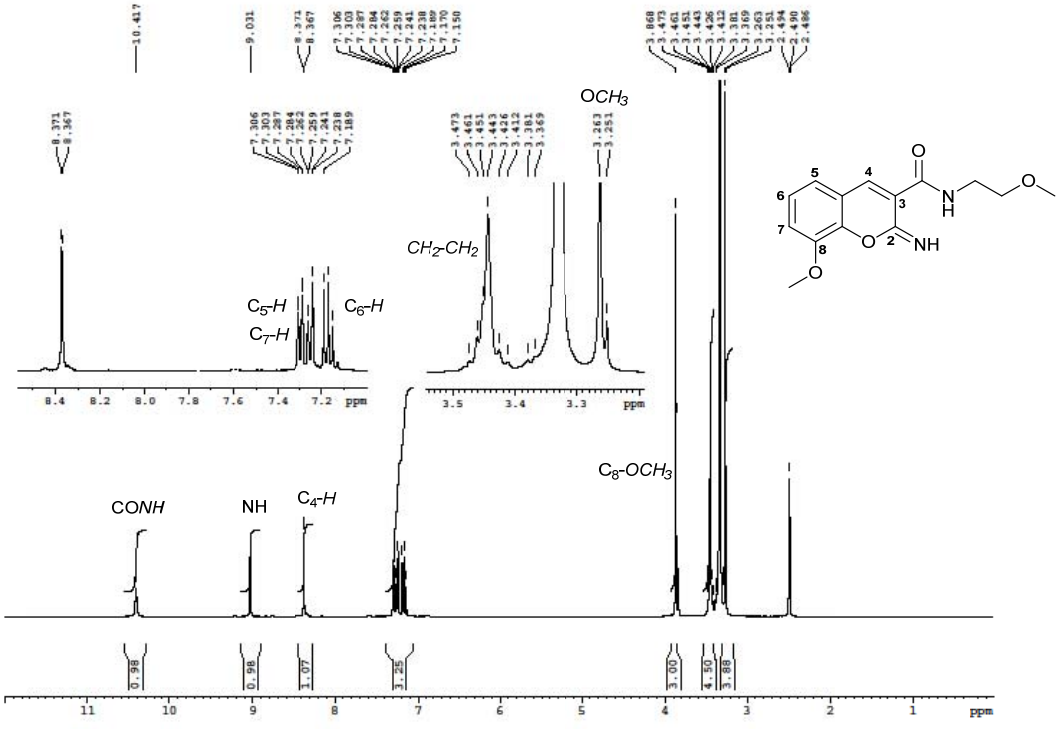


Figure SM 4.2.3.6.7 ^{1}H NMR spectrum for 2-imino-2*H*-chromene-3-carboxamide (400 MHz, dmso-d_6).

Acknowledgement

Reprinted (adapted) from *Green Chem.*, 2008, **10**, 995.

1 M. Costa and F. Proença, *Green Chem.*, 2008, **10**, 995

Synthesis of a biologically active oxazol-5(4*H*)-one *via* Erlenmeyer-Plöchl reaction

Catarina A. B. Rodrigues*[a], José M. G. Martinho[b] and Carlos A. M. Afonso*[a]

[a] Instituto de Investigação do Medicamento (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003, Lisboa, Portugal,

[b] Centro de Química-Física Molecular and IN-Institute of Nanoscience and Nanothecnology, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

catarinarodrigues@ff.ulisboa.pt, carlosafonso@ff.ulisboa.pt

Supplementary Material

1. Results

This experiment describes the synthesis of (*Z*)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4*H*)-one, a potent immunomodulator and tyrosinase inhibitor. This well-known reaction was applied by us for the synthesis of new two-photon probes[1] and further optimized in order to achieve conditions that can be used in a teaching environment. The temperature was reduced from 120ºC to 100ºC which allow to use a water bath, the reaction time was reduced from 2 hours to only 15 minutes and the purification process involved only the washing of the crude with ethanol. This optimization improved the previous result of 66% yield[1] to 81-86%.

The experiment was reproduced in the teaching laboratory environment by the students attending the organic chemistry laboratory from the first years of pharmaceutical sciences course (5 years course). The results obtained by the students are presented in Table SM 4.2.3.7.1.

Table SM 4.2.3.7.1: Results from the students experiments using authors optimized conditions.

Group[(a)]	Product Amount[(b)](g)	Yield (%)	Melting Point (ºC)
1	0.804	97	230-232
2	0.694	84	236-240
3	0.689	84	220-223
4	0.694	83	241-242
5	0.681	83	233-235
6	0.752	91	240-246
7	0.652	79	233-236
8	0.689	81	237-241
9	0.687	84	245-247
10	0.716	87	245-249

One or two students per group, (b) obtained product 1 starting from hippuric acid (~0.5 g) *each shading in the table corresponds to one laboratory session with several groups.

Products from groups 1-3 were the less pure. In order to increase the purity, some changes were done to the procedure namely: (a) the amount of acetic anhydride was increased from 0.8 mL to 1mL (3 eq. to 4 eq.), to get a more homogeneous and fluid reaction mixture and (b) the students were instructed to smash better the crude reaction mixture with ethanol in order to improve the washing purification.

By addressing those changes, groups 4 to 8 also have got some impurities but some achieved better results, so a final change was made: the amount of aldehyde was decreased from 1.1 eq to 1 eq. Finally groups 9 and 10 got very good results of yield (84 and 87%) and purity.

The purity was accessed by TLC (eluent Hexane 8:2 AcOEt) and in all cases no visible impurities were observed (usually only one spot with Rf=0.57 is observed at 254 nm). The obtained products were also compared to a pure sample of (*Z*)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4*H*)-one for identification of the compound.

The melting point temperatures were determined by the students and a difference of more than 15 degrees between them and when compared to the literature value was observed. (Literature melting point is 246-247ºC.[2]) The students concluded that these differences are justified by contaminations with solvent (resulting from insufficient product drying time).

In Figure SM 4.2.3.7.1 is provided a comparison of the observed ^{1}H NMR spectra between the purified product sample of (*Z*)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4*H*)-one obtained by us (i) and from the groups students (ii) to (iv) randomly selected, which shows the same spectra for all samples.

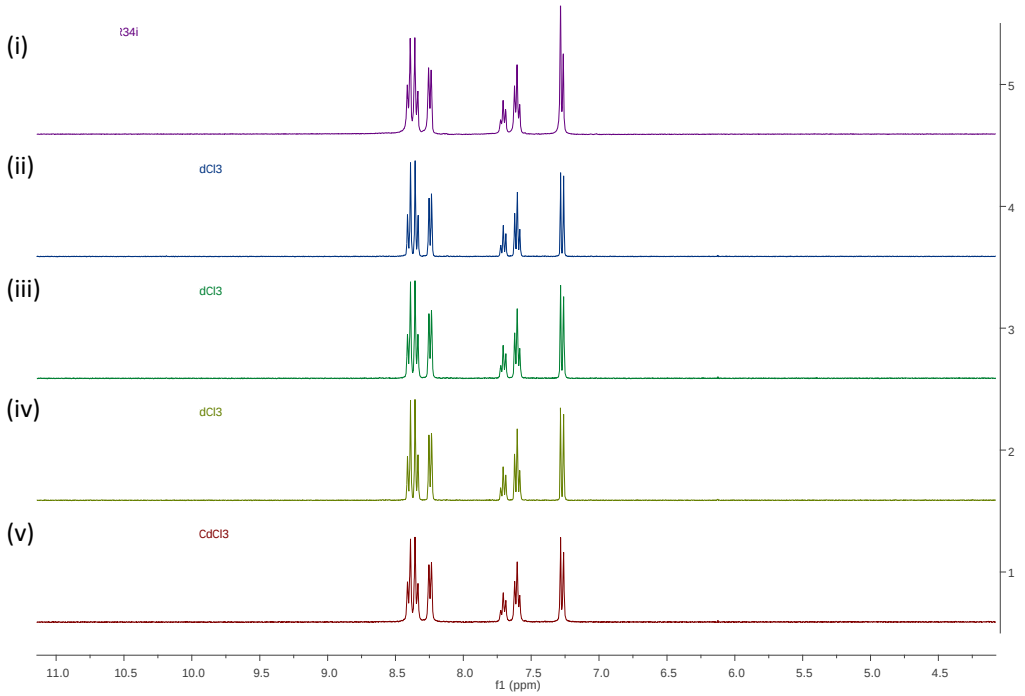


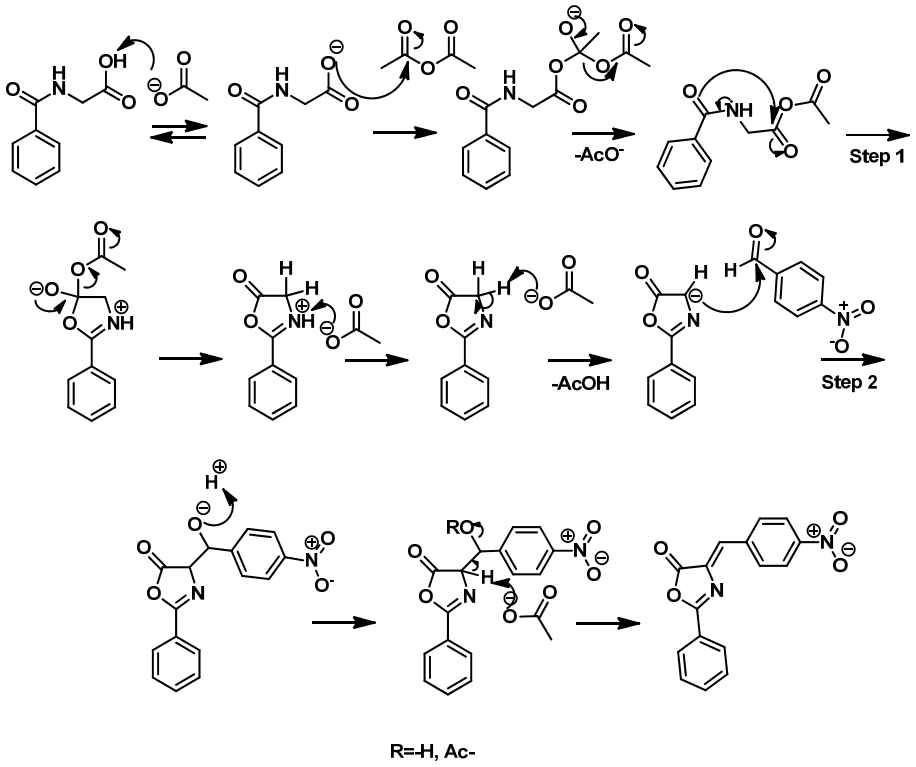
Figure SM 4.2.3.7.1: Comparison between the ^{1}H NMR spectra of (*Z*)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4*H*)-one 1 prepared by us (i) and some of the products obtained by the students randomly selected group 2 (ii), group 4 (iii), group 6 (iv) and group 10 (v).

The ^{1}H NMR spectra of (Z)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4H)-one shows second order aromatic protons despite it seems first order spectra. At this stage the students are not able to identify second order spectra so the NMR spectra are reported in the 'Characterization section' as a first order spectra, but the correct report is as follow:

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38, 8.32 (ABq, J_{AB} = 8.8 Hz, 4H, 2x C$\underline{H}_{Ar}$), 8.22 (d, *J* = 7.6 Hz, 2H, 2x C$\underline{H}_{Ar}$), 7.68 (t, *J* = 7.2 Hz, 1H, 1x C$\underline{H}_{Ar}$), 7.58 (t, *J* = 7.7 Hz, 2H, 2x C$\underline{H}_{Ar}$), 7.24 (s, 1H, -C=C$\underline{H}$-).

2. Notes for the Instructor

2.1. General mechanism of Erlenmeyer-Plöchl Reaction



Scheme SM 4.2.3.7.1: Proposed mechanism of Erlenmeyer Plöchl synthesis of (*Z*)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4*H*)-one[3, 4]

2.2. The Hammett Equation

The students attempted to apply these conditions to other aldehydes: *p*-(dimethylamine)benzaldehyde, and benzaldehyde, but these trials were not successful. No reaction was observed after two series of 15 minutes heating for the three additional aldehydes. To understand this result the instructors carried out the study of the influence of aldehyde substituent on this reaction applying the Hammett equation.

Hammett equation

The Hammett equation correlates the structure and reactivity trough the quantification of reaction rates changes when varying the aromatic substituents. The reaction constant (ρ) is experimentally determined as the slope of a Hammett plot given by equation SM 4.2.3.7.1:[5-8]

$$\rho\sigma_X = \log\frac{k_X}{k_0}$$

Equation SM 4.2.3.7.1: Hammett Equation

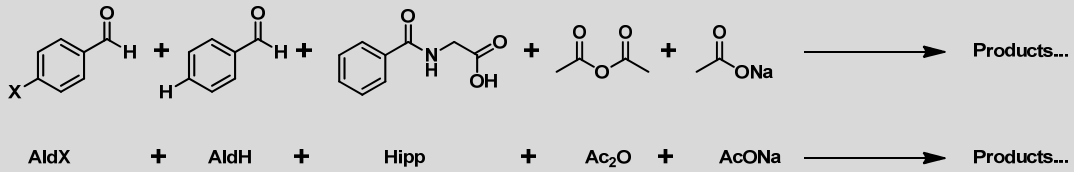
Where σ_x is the substituent constant that quantifies the electronic contribution of

substituent X; ρ is a reaction constant that gives information about the mechanistic rate-determining step; k_x and k_0 are the constant rates of the reaction with the ring substituted with X and the unsubstituted ring respectively.

Hammett σ_x values were established, in 1937 by Louis P. Hammett, for *meta*- and *para*-aromatic substituents, resulting from the acidity study of substituted benzoic acid derivatives.[9] Electron-withdrawing groups stabilize an increase in negative charge in the transition state of the rate-determining step and present positive σ_x, whilst, electron-donating substituents stabilize the increment of positive charge in the rate-determining transition state and have negative σ_x.

The value of ρ gives information about the mechanistic rate-determining step: a positive value of ρ indicates rate acceleration by electron-withdrawing groups and a negative ρ value means rate acceleration by electron donating groups. If ρ value is much greater than +1.00 or much less than –1.00, the reaction in study is very sensitive to substituent effects, if ρ value is near zero the rate determining step of the mechanism is independent of the substituents.[8]

The reaction rates applied to the system in study (Scheme SM 4.2.3.7.3) are expressed by equation SM 4.2.3.7.2 and equation SM 4.2.3.7.3:



Scheme SM 4.2.3.7.2: Competitive Erlenmeyer-Plöchl Reaction

$$\frac{d[AldX]}{dt} = k_1[AldH][Hipp][Ac_2O][AcONa][AldX] = k_X[AldX] \Rightarrow k_X = k_1[AldH][Hipp][Ac_2O][AcONa]$$

Equation SM 4.2.3.7.2: *p*-substituted aldehyde reaction rate

$$\frac{d[AldH]}{dt} = k_2[AldX][Hipp][Ac_2O][AcONa][AldH] = k_H[AldH] \Rightarrow k_H = k_2[AldX][Hipp][Ac_2O][AcONa]$$

Equation SM 4.2.3.7.3: Benzaldehyde reaction rate

Applying these equations to Hammett expression we obtain the simplified equation SM 4.2.3.7.4:

$$\rho\sigma_X = \log\frac{k_X}{k_H} = \log\frac{k_1[AldH][Hipp][Ac_2O][AcONa]}{k_2[AldX][Hipp][Ac_2O][AcONa]} = \log\frac{k_1[AldH]}{k_2[AldX]} \Leftrightarrow \rho\sigma_X = \log\frac{k_1}{k_2} + \log\frac{[AldH]}{[AldX]}$$

$$\Leftrightarrow \rho\sigma_X - \log\frac{k_1}{k_2} = \log\frac{[AldH]}{[AldX]}$$

Equation SM 4.2.3.7.4: Hammett Equation

To calculate the Hammett equation of this system four competitive reactions were performed; in each reaction were used 2 equivalents of aldehyde mixture: one equivalent of benzaldehyde and another equivalent of a representative *para*- substituted benzaldehyde. The original Erlenmeyer-Plöchl reaction conditions were used: heating at 120ºC during 1 hour, 1 eq. of sodium acetate and 3 eq. of acetic anhydride. The crude reaction was analyzed by ^{1}H NMR in order to determine the unreacted amount of each aldehyde. The ratio of ^{1}H NMR aldehyde peak integration gives the ratio of aldehydes concentrations, a value needed for the construction of Hammett equation. These ratios were also confirmed by the integration of other characteristic ^{1}H NMR peaks of oxazolone and respective aldehyde. Using these results we considered that there are no secondary reactions in this system. In Table 2 are presented the results obtained from these experiments:

Table SM 4.2.3.7.2: Hammett constants and results obtained for each *para* substituted aldehyde

R^1	σ_X[10]	AldH[(a)]	AldX[(b)]	$\frac{[AldH]}{[AldX]}$	$\log\frac{[AldH]}{[AldX]}$
NMe_2	-0,82	1	5.62	0,18	-0,74
Me	-0,17	1	1.36	0,73	-0,14
H	0	-	-	1,00	0,00
Cl	0,23	1	0.94	1,06	0,03
NO_2	0,78	1	0.17	5,88	0,77

[(a)]^{1}H NMR aldehyde peak integration of benzaldehyde, [(b)]^{1}H NMR aldehyde peak integration of representative benzaldehyde,

The Hammett plot for the substituted aldehydes is displayed in Figure SM 4.2.3.7.2:

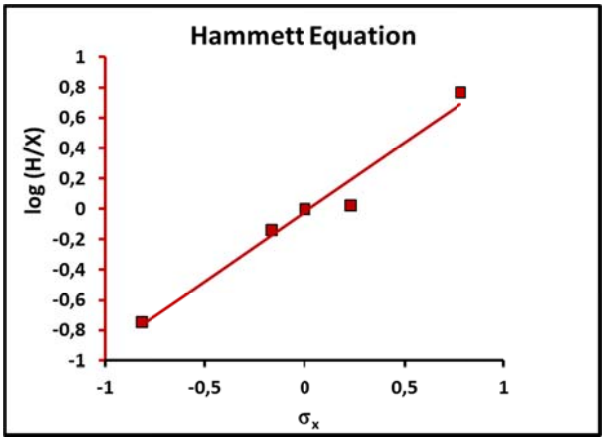


Figure SM 4.2.3.7.2: Hammett Plot for *para* substituted aldehydes

$$\log\frac{[AldH]}{[AldX]} = 0.91\,\sigma_X - 0.02$$

Equation SM 4.2.3.7.5

The experimental points are well fitted by a linear regression given by Equation SM 4.2.3.7.5. In this system the reaction constant ρ is 0.91, indicating that this transformation is sensitive to the substituent electronic nature and the reaction rate is increased by electron-withdrawing groups that enhances the electrophilicity of aldehyde carbon.

Following a similar reasoning and using the reported data[4] presented in Table SM 4.2.3.7.2 and Equation SM 4.2.3.7.6 the Hammett plot and equation for *para* substituted hippuric acids was determined.

$$\rho\sigma_X - \log\frac{k_1}{k_2} = \log\frac{[OxaX]}{[OxaH]}$$

Equation SM 4.2.3.7.6

Table SM 4.2.3.7.3: Hammett constants and results obtained for each *para* substituted hippuric acid

R^1	σ_X[10]	%conversion Oxa[4]	$\frac{[OxaX]}{[OxaH]}$	$\log\frac{[OxaX]}{[OxaH]}$
H	0	86	1,00	0,00
F	0,06	59	0,69	-0,16
NO_2	0,78	20	0,23	-0,63

The Hammett plot and equation for the substituted hippuric acids are displayed in Figure SM 4.2.3.7.2 and in Equation SM 4.2.3.7.7:

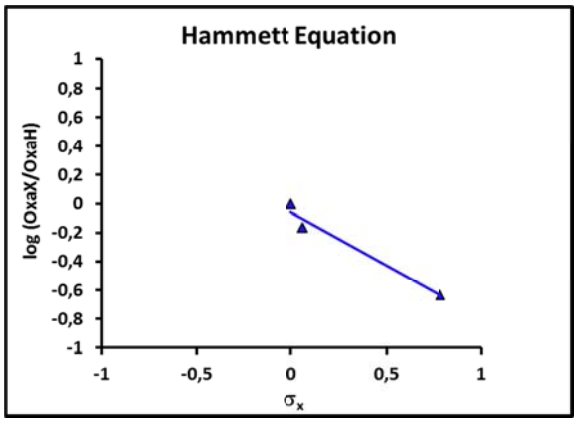


Figure SM 4.2.3.7.3: Hammett Plot for *para* substituted hippuric acids

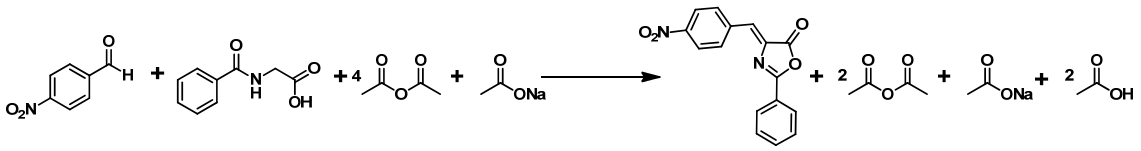
$$\log\frac{[OxaX]}{[OxaH]} = -0.74\,\sigma_X - 0.06$$

Equation SM 4.2.3.7.7

The experimental points are well fitted by a linear regression given by Equation 6. As calculated from reported results[4], the reaction constant ρ is -0.74, indicating that this reaction is sensitive to the substituent electronic nature and the reaction rate is increased by electron-donating groups that enhances the amide carbonyl group nucleophilicity.

2.3. E-factor

The chemical equation for the synthesis of (*Z*)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4*H*)-one is presented in Scheme SM 4.2.3.7.3.



Scheme SM 4.2.3.7.3: Chemical equation for synthesis of (*Z*)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4*H*)-one

Considering this chemical equation we can calculate the theoretical amounts of waste and desired product obtained (see Table SM 4.2.3.7.4).

Table SM 4.2.3.7.4: Calculated amounts of waste and product obtained

	Substance	Mass (g) used/produced
Waste	ethanol	23,4
	acetic acid	0,64
	sodium acetate	0,23
	acetic anhydride	0,54
Product	(*Z*)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4*H*)-one	0,821

Based in these values we are able to determine the theoretical E factor of our system using the following Equation SM 4.2.3.7.8:

$$\boldsymbol{E\ factor} = \frac{\mathbf{0.64}\boldsymbol{g}\ (\boldsymbol{AcOH}) + \mathbf{23.4}\boldsymbol{g}\ (\boldsymbol{EtOH}) + \mathbf{0.54}\boldsymbol{g}\ (\boldsymbol{Ac_2O}) + \mathbf{0.23}\boldsymbol{g}\ (\boldsymbol{AcONa})}{\mathbf{0.821}\ \boldsymbol{g}\ \left((\boldsymbol{Z}) - \mathbf{4} - (\mathbf{4} - \mathbf{nitrobenzylidene}) - \mathbf{2} - \mathbf{phenyloxazol} - \mathbf{5}(\mathbf{4}\boldsymbol{H}) - \mathbf{one}\right)} = \mathbf{30.2}$$

Equation SM 4.2.3.7.8

Taking into account the reaction yield obtained by each group and using the same calculation method the experimental *E*-factor can be determined (Table SM 4.2.3.7.5):

Table SM 4.2.3.7.5: Determined E factors for each student group

Group	Product amount (g)	E factor
1	0,804	30,9
2	0,694	35,7
3	0,689	36,0
4	0,694	35,7
5	0,681	36,4
6	0,752	33,0
7	0,652	38,0
8	0,689	36,0
9	0,687	36,1
10	0,716	34,6

The *E*-factors obtained range from 30 to 38, which places the synthesized oxazol-5-(4H)-one in fine chemicals class (E factor range from 5 to 50).[11] The E factors are high due to the purification process that requires several milliliters of ethanol to achieve a proper purity.

3. Experiment's Photos

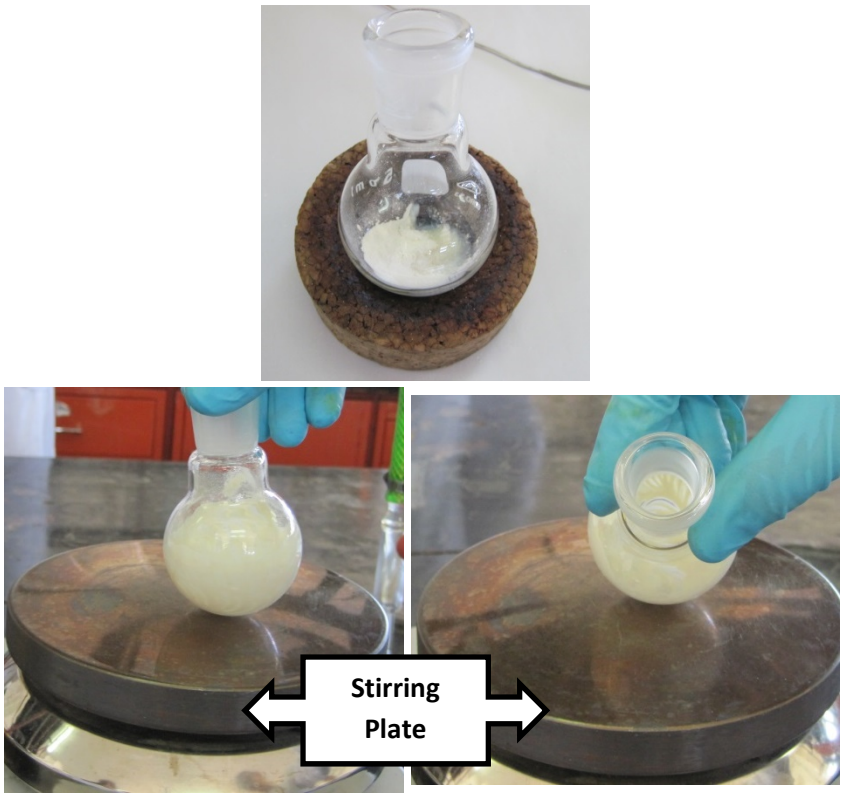


Figure SM 4.2.3.7.1: Homogenization and spreading of the reaction mixture in the flask walls

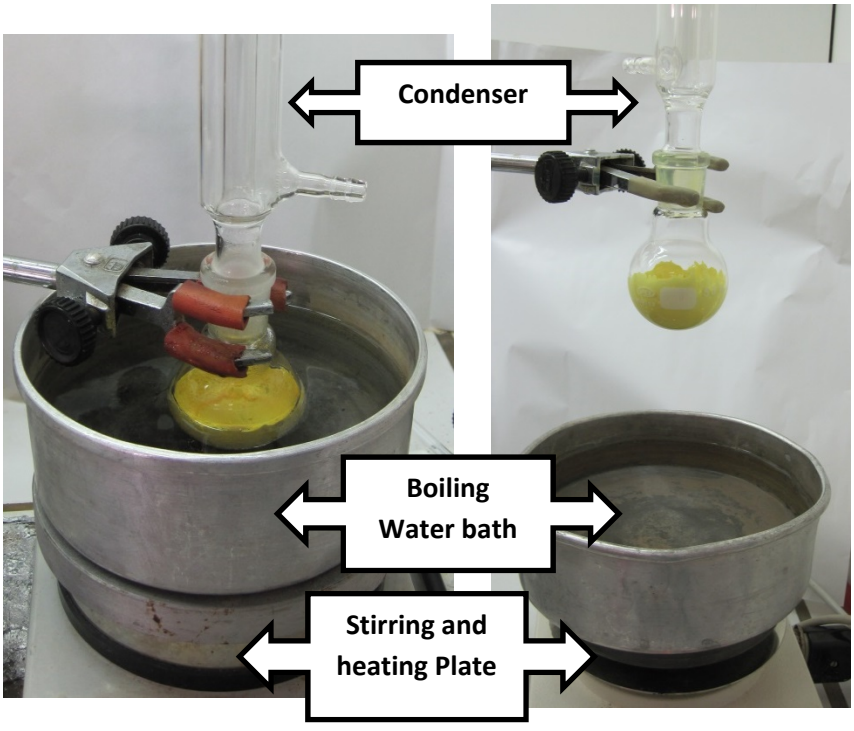


Figure SM 4.2.3.7.2: Heating the reaction flask in a boiling water bath.

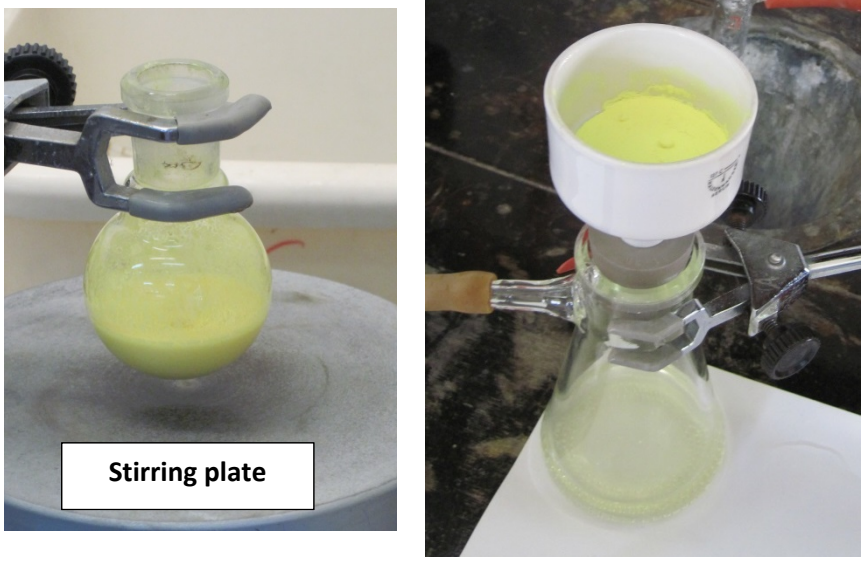
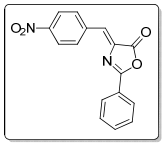
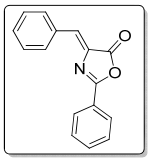


Figure SM 4.2.3.7.3: Work Up – washing with ethanol and filtration

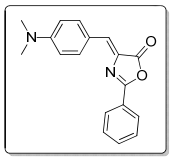
4. Compounds Characterization



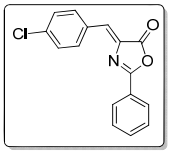
(*Z*)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(*4H*)-one (1): Yellow solid, yield: 66% (washed with methanol). **M.p.:** 242-244 ºC (decomposition) (Lit. 246-247ºC)[2]. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.38 (d, *J* = 8.7 Hz, 2H, 2x C$\underline{H}_{Ar}$), 8.32 (d, *J* = 8.8 Hz, 2H, 2x C$\underline{H}_{Ar}$), 8.22 (d, *J* = 7.6 Hz, 2H, 2x C$\underline{H}_{Ar}$), 7.68 (t, *J* = 7.2 Hz, 1H, 1x C$\underline{H}_{Ar}$), 7.58 (t, *J* = 7.7 Hz, 2H, 2x C$\underline{H}_{Ar}$), 7.24 (s, 1H, -C=C$\underline{H}$-). **^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm)** 166.75, 165.66, 148.31, 139.39, 136.31, 134.26, 132.75, 129.17, 128.83, 127.56, 124.95, 123.96. **IR (KBr) ν:** 3103.46, 3066.82, 3043.67, 1797.66, 1761.01, 1654.92, 1641.42, 1598.99, 1579.7, 1556.55, 1519.91, 1489.05, 1452.4, 1413.82, 1361.74, 1344.38, 1325.1, 1296.16, 1240.23, 1224.8, 1163.08, 1109.07, 1093.64, 1070.49, 1001.06, 979.84, 894.97, 883.4, 867.97, 862.18, 848.68, 823.6, 777.31, 763.81, 746.45, 700.16, 684.73, 669.3 cm^{-1}**. MS (ESI+) m/z calc for [$C_{16}H_{10}N_2O_4$+1]:** 295.26**, found:** 349.2 $[M+MeOH+Na]^+$, 295.1 $[M+H]^+$. **Elemental analysis: (Found:** %N, 9.23; %C, 65.28; %H, 3.64. **Calc. for $C_{16}H_{10}N_2O_4$:** % N, 9.52, % C, 65.31, % H, 3.43.)



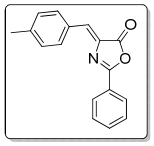
(Z)-4-benzylidene-2-phenyloxazol-5(4H)-one: Light yellow solid, yield: 64% (washed with methanol**). M.p.:** 166-167ºC (Lit. 165-166.5ºC)[2]. **^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm):** 8.21 (td, *J* = 8.4, 1.7 Hz, 4H, 4x C$\underline{H}_{Ar}$), 7.66 – 7.58 (m, 1H, 1x C$\underline{H}_{Ar}$), 7.58 – 7.40 (m, 5H, 5x C$\underline{H}_{Ar}$), 7.26 (s, *J* = 1.4 Hz, 1H). **^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm)** 167.64, 163.54, 133.52, 133.37, 133.27, 132.48, 131.79, 131.22, 128.95, 128.92, 128.39, 125.58. **IR (KBr) ν:** 1793.8, 1768.72, 1653, 1595.13, 1552.7, 1489.05, 1448.54, 1327.03, 1317.38, 1296.16 , 1163.08, 983.7, 866.04, 767.67, 698.23 , 686.66, 551.64 **cm^{-1}. HRMS calc for $C_{16}H_{11}NO_2$:** 249.078430, **found:** 249.077825. **MS (ESI+) m/z calc for [$C_{16}H_{11}NO_2$ +H]$^+$:** 250.07, **found:** 282.1 $[M+MeOH+Na]^+$, 250.1 $[M+H]^+$. **Elemental analysis:** (Found: %N, 5.55; %C, 76.92; %H, 4.73. Calc. for $C_{16}H_{11}NO_2$: % N, 5.62, % C, 77.10, % H, 4.45.).



(Z)-4-[4-(dimethylamino)benzylidene]-2-phenyloxazol-5(4H)-one: Bright orange needles. Yield: 45% (recristalized from ethyl acetate). **M.p.:** 218-219 ºC (Lit. 220-221ºC)[2]. **^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm)** 8.14 (d, *J* = 6.9 Hz, 4H, 4x$C\underline{H}_{Ar}$), 7.65 – 7.42 (m, 3H, 3x$C\underline{H}_{Ar}$), 7.20 (s, 1H,-C$\underline{H}$-C=), 6.75 (d, *J* = 8.8 Hz, 2H, 2x$C\underline{H}_{Ar}$), 3.09 (s, 6H,- N($C\underline{H}_3$)$_2$). **^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm)** 168.55, 160.58, 152.16, 134.86, 133.35, 132.30, 128.79, 128.33, 127.77, 126.34, 121.82, 111.82, 40.11 (N($\underline{C}H_3$)$_2$). **IR (KBr) ν:** 3080.32, 3035.96, 3022.45, 3005.1, 1784.15, 1762.94, 1647.21, 1606.7, 1595.13, 1581.63, 1568.13, 1529.55, 1490.97, 1448.54, 1375.25, 1323.17, 1296.16, 1195.87, 1161.15, 1126.43, 856.39, 813.96 **cm^{-1}. HRMS calc for $C_{18}H_{16}N_2O_2$:** 292.120629, **found:** 292.120841. **MS (ESI+) m/z calc for [$C_{18}H_{16}N_2O_2$+1]:** 293.12, **found:** 315.1 [M+Na]$^+$, 293.1 [M+H]$^+$. **Elemental analysis:** (Found: %N, 9.32; %C, 73.93; %H, 5.30. Calc. for $C_{18}H_{16}N_2O_2$: % N, 9.88, % C, 73.95, % H, 5.52.)

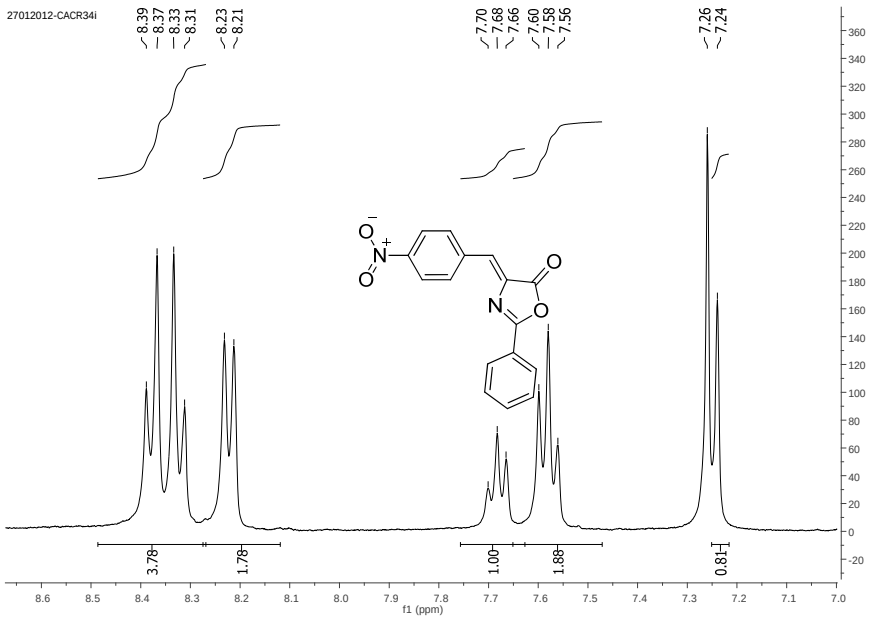


***(Z)-4-(4-chlorobenzylidene)-2-phenyloxazol-5(4H)-one*:** Yellow solid, yield: 79% (washed with methanol), **M.p.:** 196-198 ºC (Lit. 196.5-197.5ºC)[2]. ^{1}H **NMR (300 MHz, $CDCl_3$) δ** 8.25 – 8.07 (m, 4H,4x $C\underline{H}_{Ar}$), 7.68 – 7.59 (m, 1H, $C\underline{H}_{Ar}$), 7.59 – 7.49 (m, 2H, 2X$C\underline{H}_{Ar}$), 7.48 – 7.41 (m, 2H,2X$C\underline{H}_{Ar}$), 7.18 (s, 1H, -C$\underline{H}$=C). **^{13}C NMR (75 MHz, $CDCl_3$) δ** 167.39, 163.90, 137.29, 133.63, 133.57, 133.53, 132.01, 130.04, 129.25, 129.01, 128.46, 125.43. **IR (KBr) ν:** 3047.53, 2937.59, 2850.79, 1982.82, 1923.03, 1878.87, 1830.45, 1795.73, 1766.8, 1637.56, 1629.85, 1589.34, 1543.05, 1487.12, 1450.47, 1409.96, 1325.1, 1300.02, 1282.66, 1161.15, 1093.64, 1070.49, 1001.06, 983.7, 900.76, 887.26, 864.11, 825.53, 777.31, 694.37, 555.5, 487.99 **cm^{-1}, MS (ESI+) m/z calc for [$C_{16}H_{10}ClNO_2$+1]:** 284.04, found: 284 [M+H]$^+$ **Elemental analysis:** (Found: %N, 5.06; %C, 67.39; %H, 3.56. Calc. for **$C_{16}H_{10}ClNO_2$**: % N, 4.94, % C, 67.74, % H, 3.55)

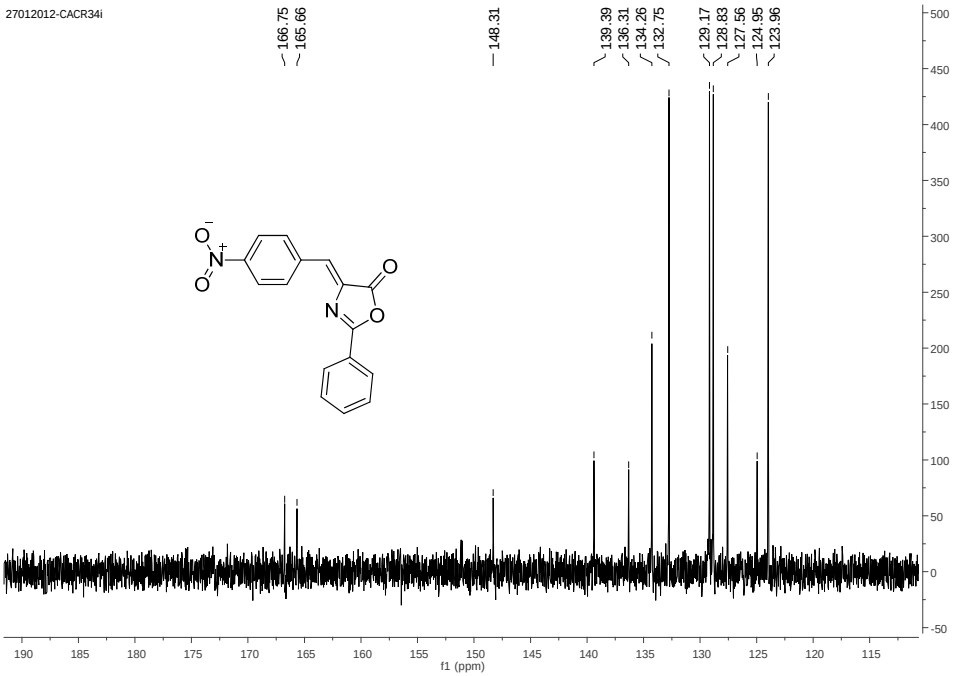


***(Z)-4-(4-methylbenzylidene)-2-phenyloxazol-5(4H)-one*:** Yellow solid, yield: 60% (washed with methanol), **M.p.:** 142-143 ºC (Lit. 141.5-143ºC)[2]. **^{1}H NMR (300 MHz, $CDCl_3$) δ** 8.22 – 8.14 (m, 2H, 2X$C\underline{H}_{Ar}$), 8.10 (d, *J* = 8.2 Hz, 2H, 2x$C\underline{H}_{Ar}$), 7.59 (dd, *J* = 5.0, 3.6 Hz, 1H, 1x$C\underline{H}_{Ar}$), 7.57 – 7.49 (m, 2H, 2x$C\underline{H}_{Ar}$), 7.29 (d, *J* = 8.1 Hz, 2H, 2x$C\underline{H}_{Ar}$), 7.24 (s, 1H, C$\underline{H}$=C), 2.43 (s, 3H, $C\underline{H}_3$). **^{13}C NMR (75 MHz, $CDCl_3$) δ** 167.99, 163.18, 142.27, 133.33, 132.68, 132.58, 132.23, 131.04, 129.89, 129.06, 128.43, 125.87, 21.96 ($\underline{C}H_3$). **IR (KBr) ν:** 3047.53, 2914.44, 2852.72, 1924.96, 1890.24, 1870.95, 1793.8, 1770.65, 1718.58, 1649.14, 1629.85, 1606.7, 1544.98, 1490.97, 1448.54, 1363.67, 1327.03, 1315.45, 1294.24, 1161.15, 1107.14, 1095.57, 1068.56, 983.7, 904.61, 889.18, 860.25, 815.89, 773.46, 694.37, 684.73, 557.43, 514.99, 487.99 **cm^{-1},MS (ESI+) m/z calc for [$C_{17}H_{13}NO_2$+1]:** 263.09, found: 264 [M+H]$^+$, **Elemental analysis:** (Found: %N, 5.13; %C, 75.75; %H, 5.03. Calc. for **$C_{17}H_{13}NO_2.0.5CH_3OH$**: % N, 5.01, % C, 75.25, % H, 5.41)

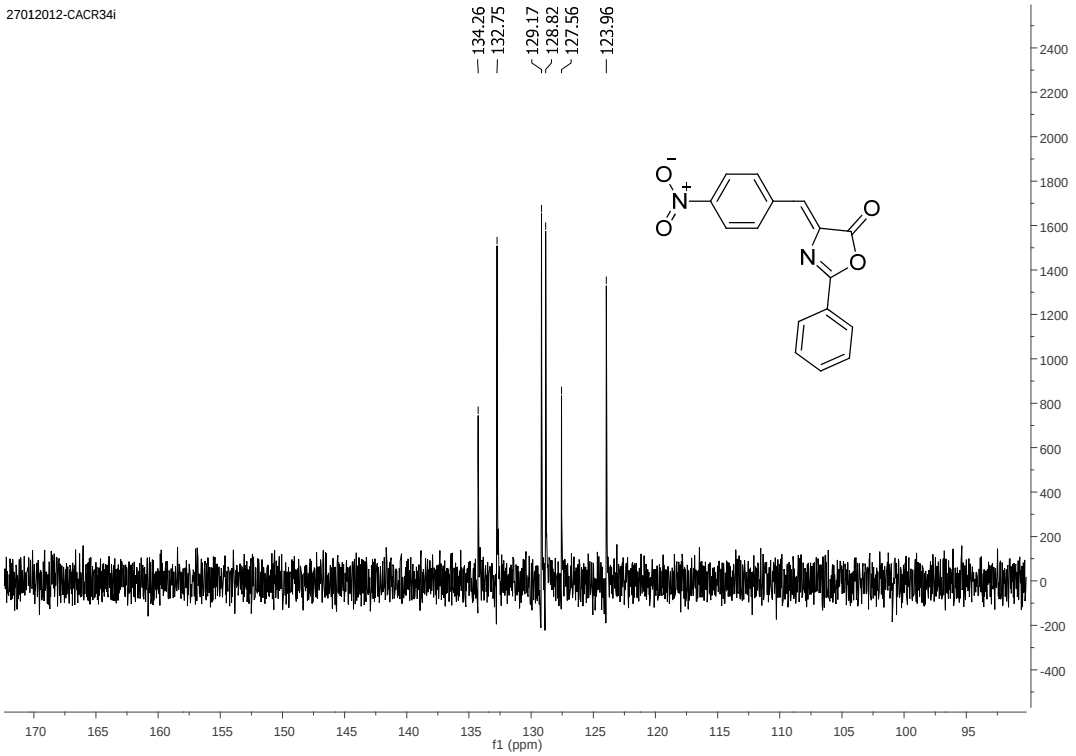
4.1. (Z)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4H)-one



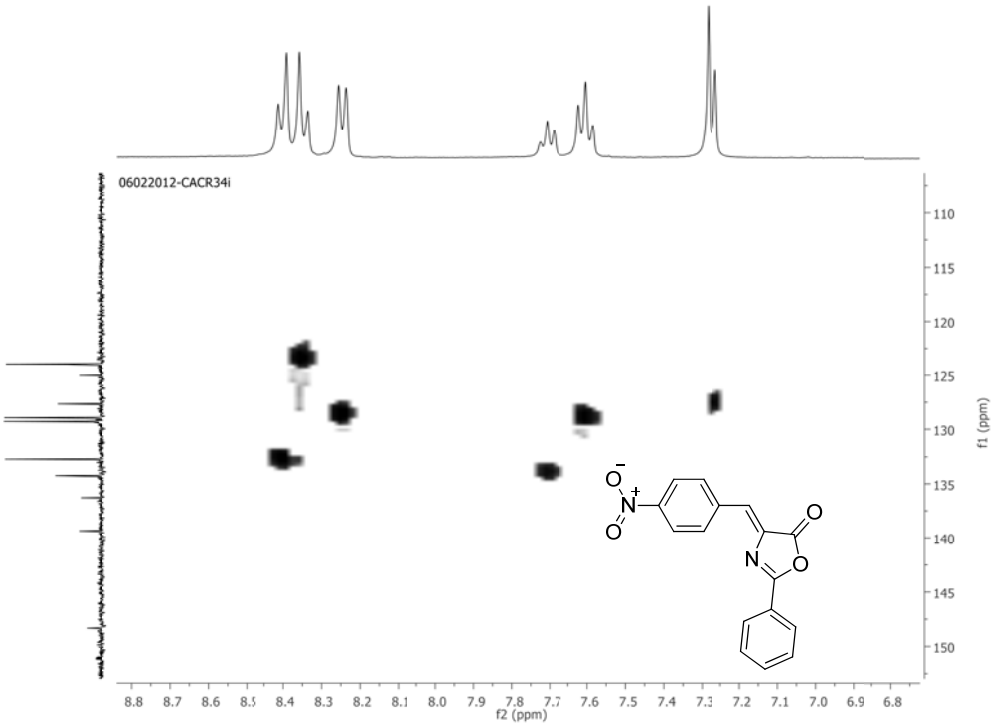
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 8.7 Hz, 2H, 2x C<u>H</u>$_{Ar}$), 8.32 (d, *J* = 8.8 Hz, 2H, 2x C<u>H</u>$_{Ar}$), 8.22 (d, *J* = 7.6 Hz, 2H, 2x C<u>H</u>$_{Ar}$), 7.68 (t, *J* = 7.2 Hz, 1H, 1x C<u>H</u>$_{Ar}$), 7.58 (t, *J* = 7.7 Hz, 2H, 2x C<u>H</u>$_{Ar}$), 7.24 (s, 1H, -C=C<u>H</u>-).

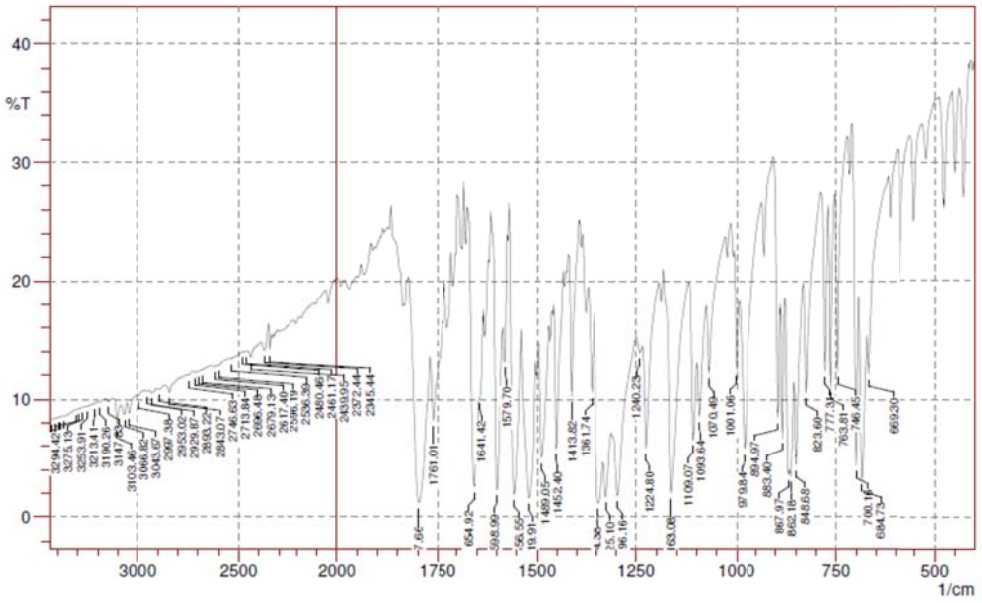


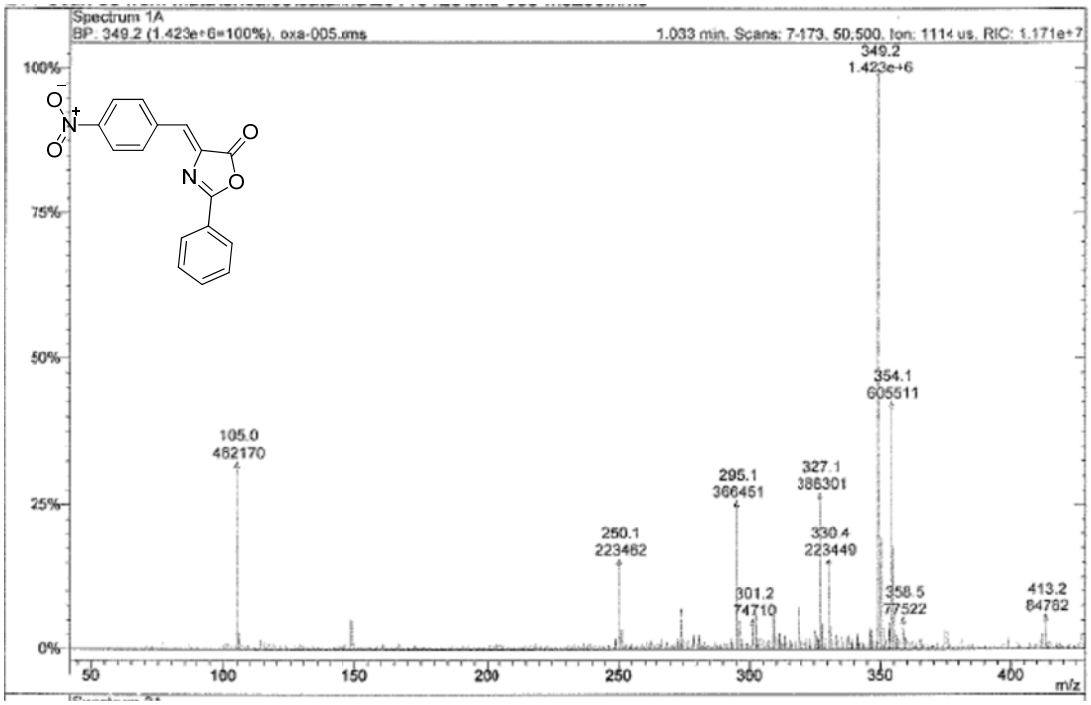
^{13}C NMR (101 MHz, $CDCl_3$) δ 166.75, 165.66, 148.31, 139.39, 136.31, 134.26, 132.75, 129.17, 128.83, 127.56, 124.95, 123.96.



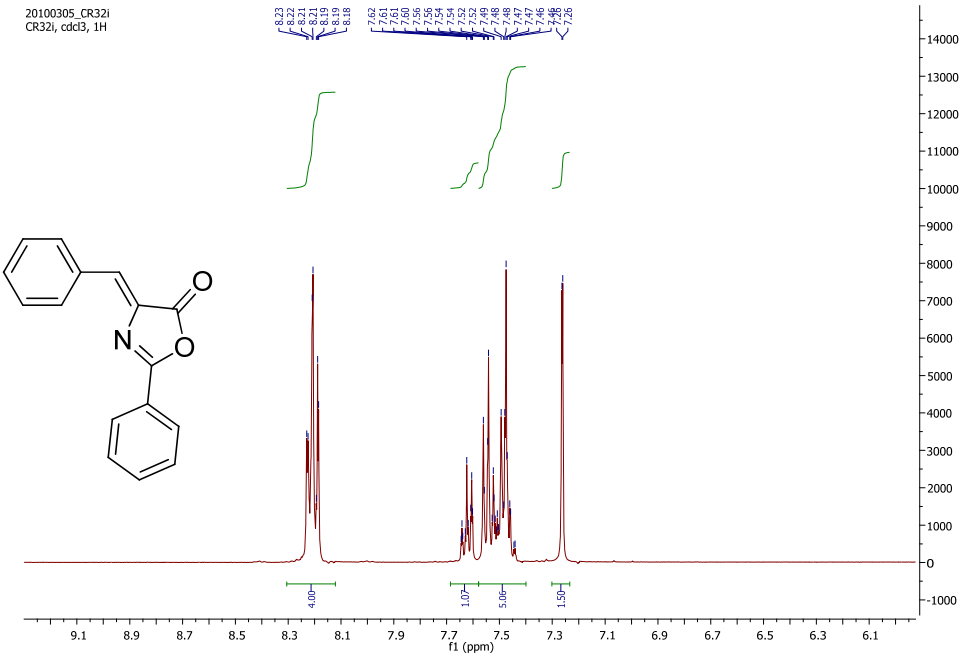
^{13}C NMR (101 MHz, $CDCl_3$) δ 134.26, 132.75, 129.17, 128.82, 127.56, 123.96.



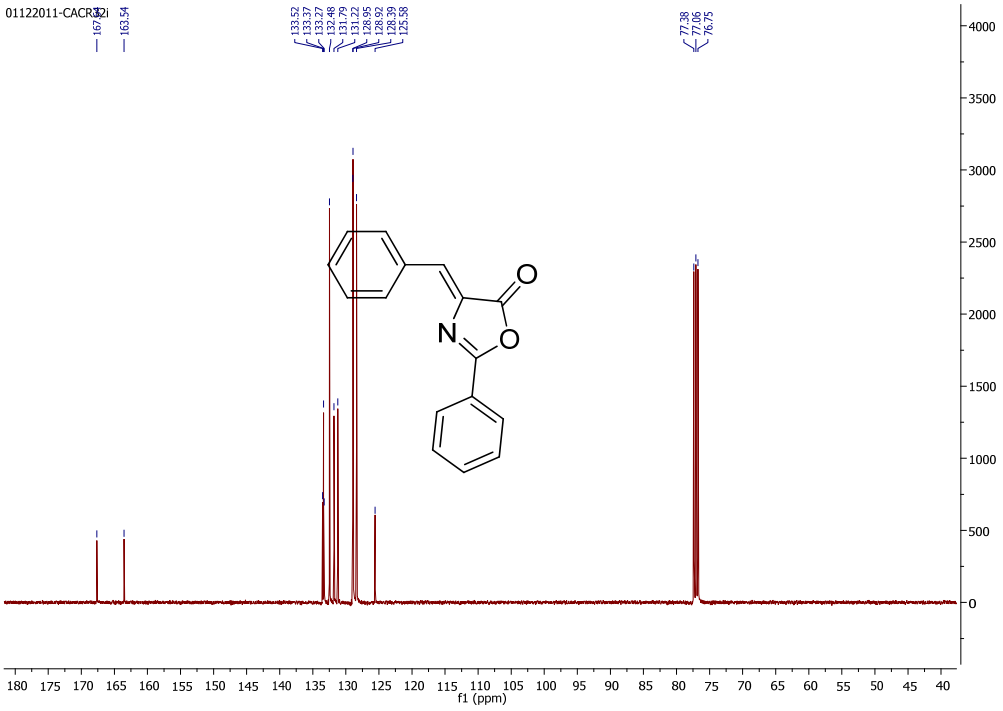




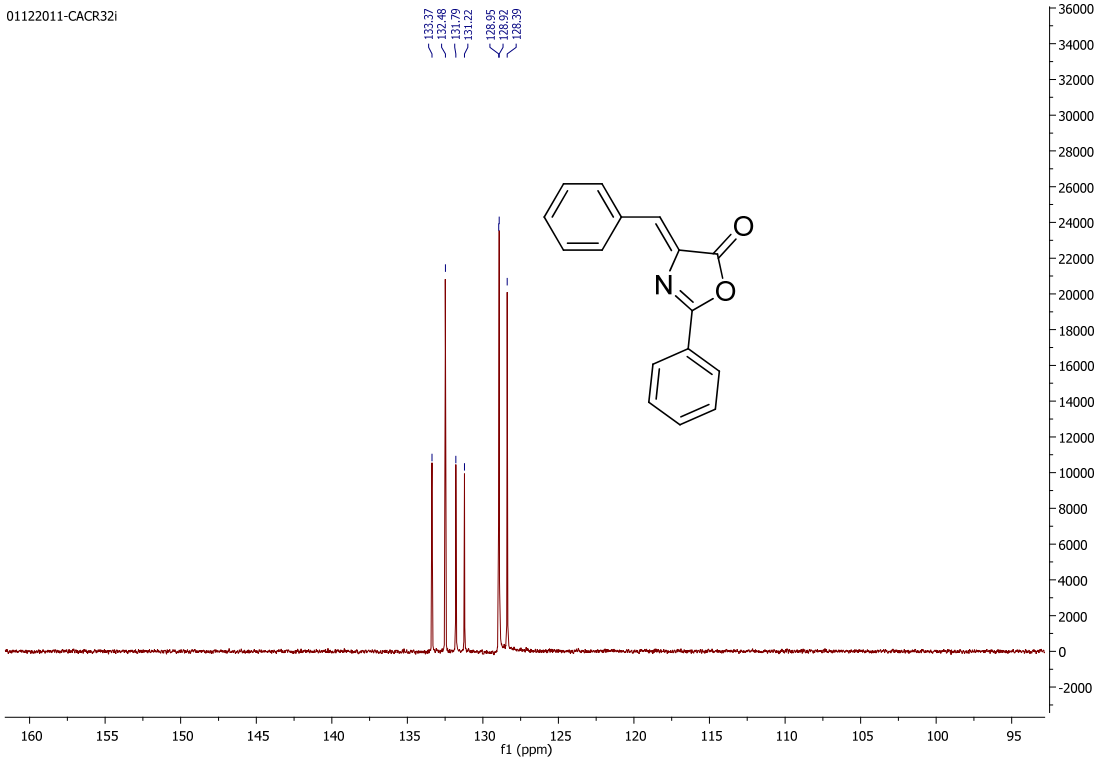
4.2. (Z)-4-benzylidene-2-(phenyl)oxazol-5(4H)-one



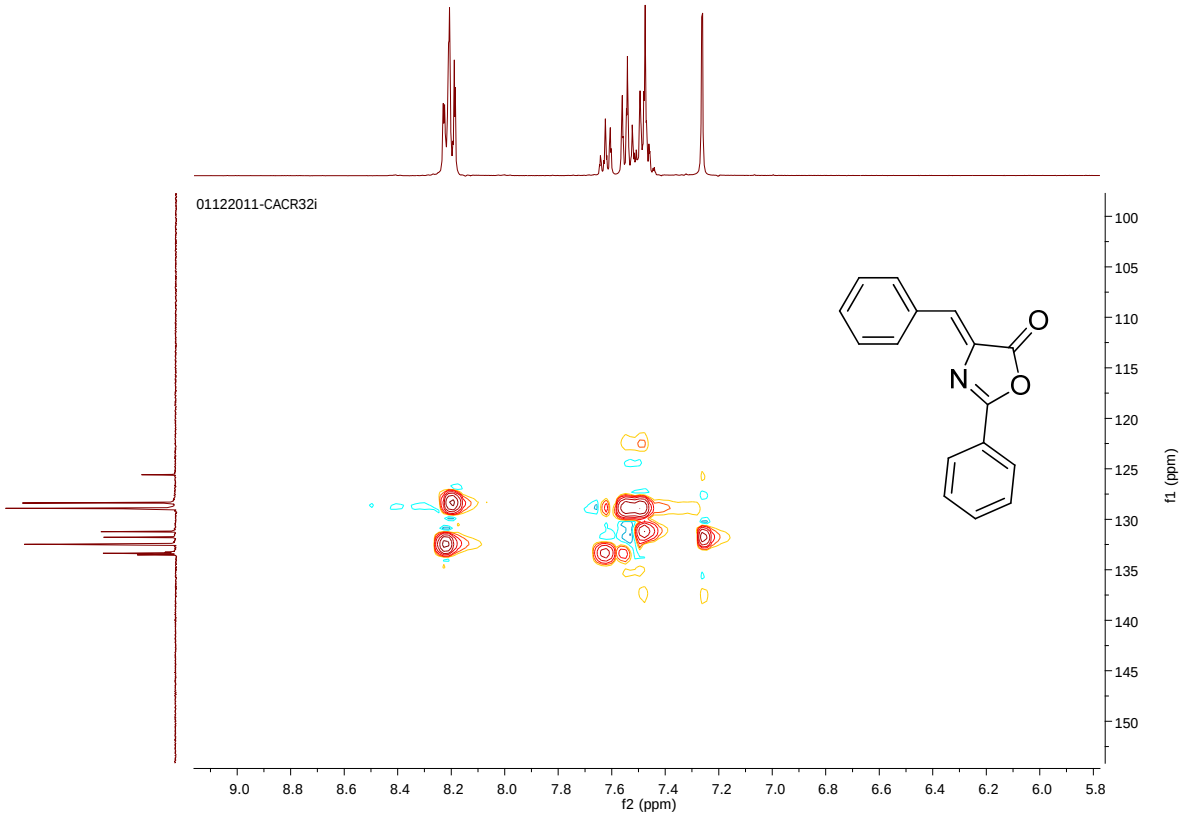
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (td, *J* = 8.4, 1.7 Hz, 4H, 4x C$\underline{H}_{Ar}$), 7.66 – 7.58 (m, 1H, 1x C$\underline{H}_{Ar}$), 7.58 – 7.40 (m, 5H, 5x C$\underline{H}_{Ar}$), 7.26 (s, *J* = 1.4 Hz, 1H).

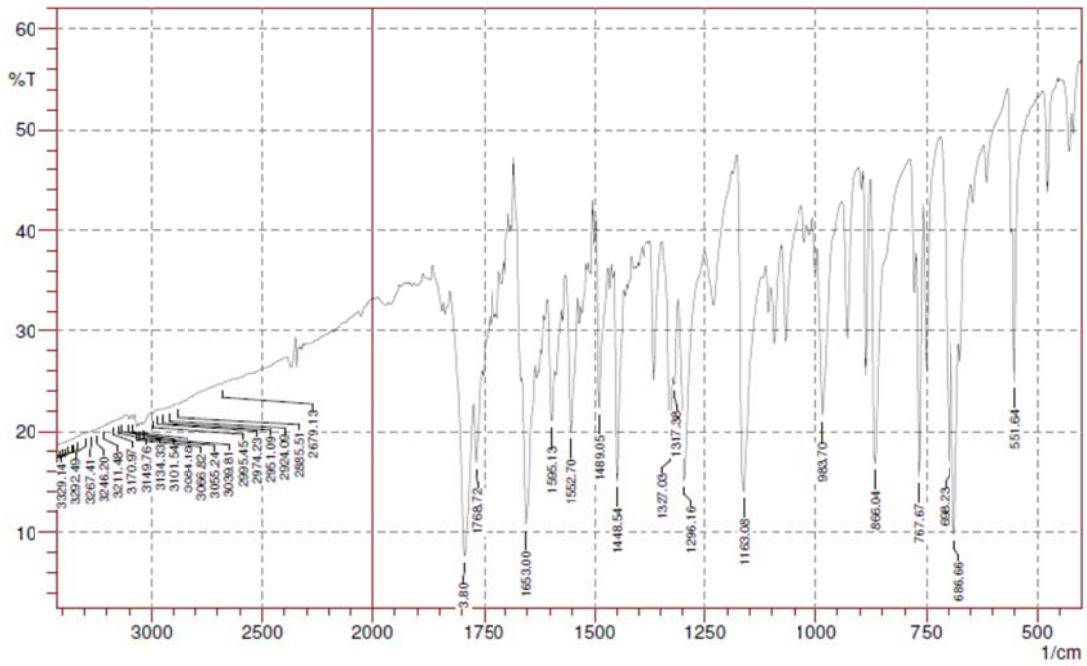


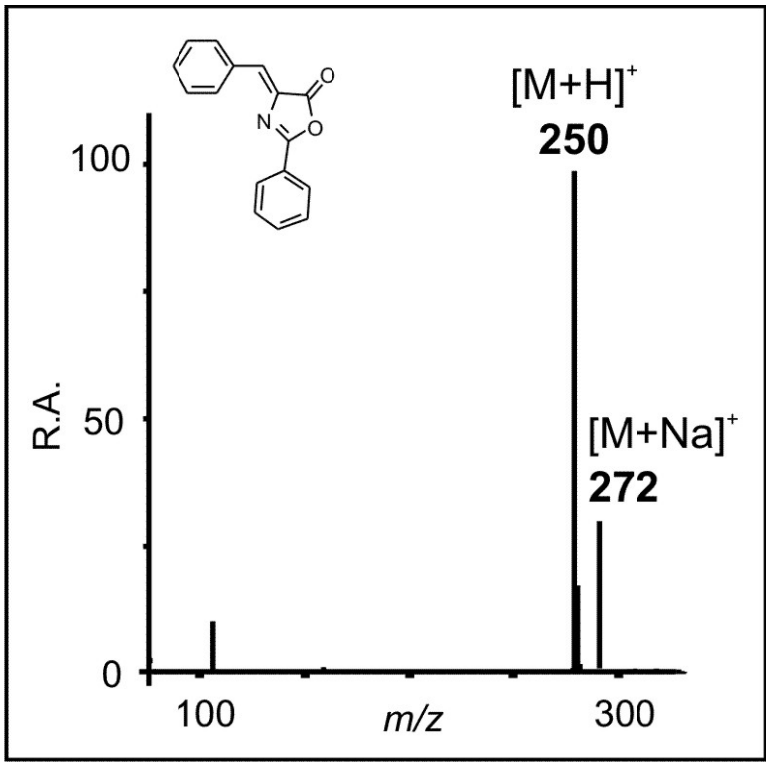
^{13}C NMR (101 MHz, $CDCl_3$) δ 167.64, 163.54, 133.52, 133.37, 133.27, 132.48, 131.79, 131.22, 128.95, 128.92, 128.39, 125.58.



^{13}C NMR (101 MHz, $CDCl_3$) δ 133.37, 132.48, 131.79, 131.22, 128.95, 128.92, 128.39.

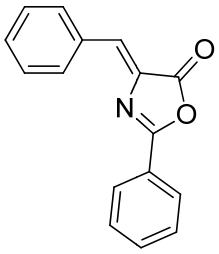


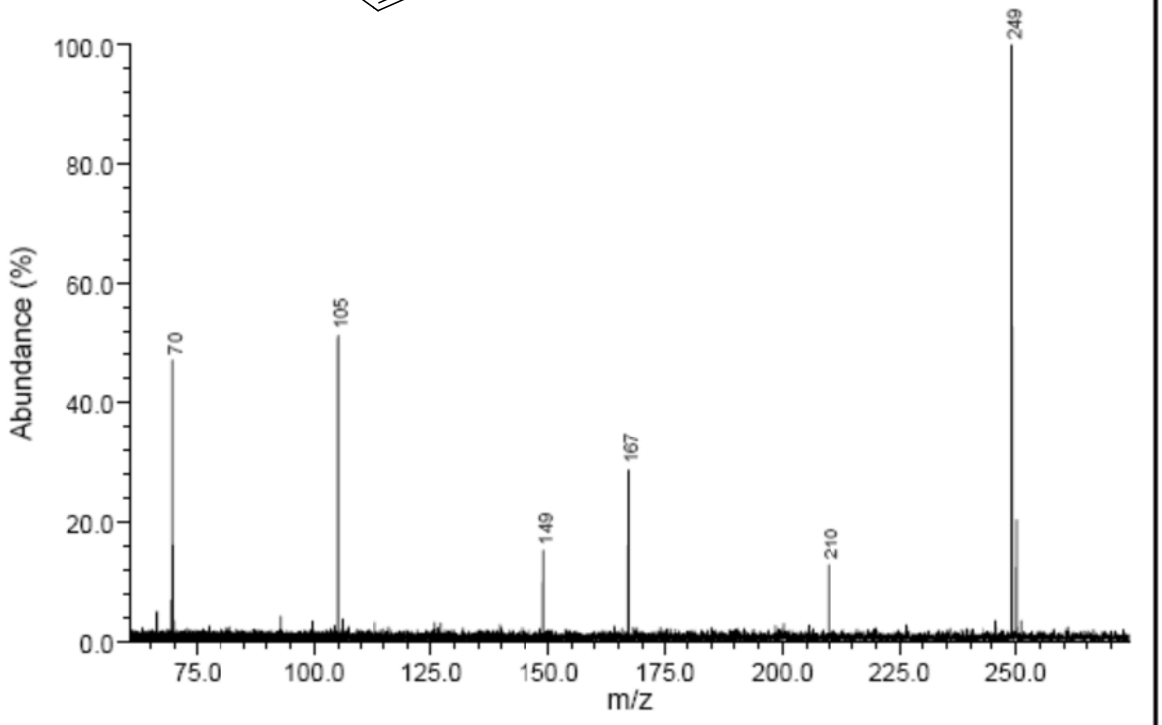




No. of peaks:	9
Height and Width:	From Spectrum Points
Peak Fit Type:	3-Point Quadratic
Peak Threshold Set:	Automatically
Min. Peak Width:	3
Search Start:	60.43584 m/z
Search Start:	308.40565 m/z
Peak Thresh:	5.09 Relative (%)
Noise Thresh:	0.63 Relative (%)
Peak S/N:	5.00 (S/N)

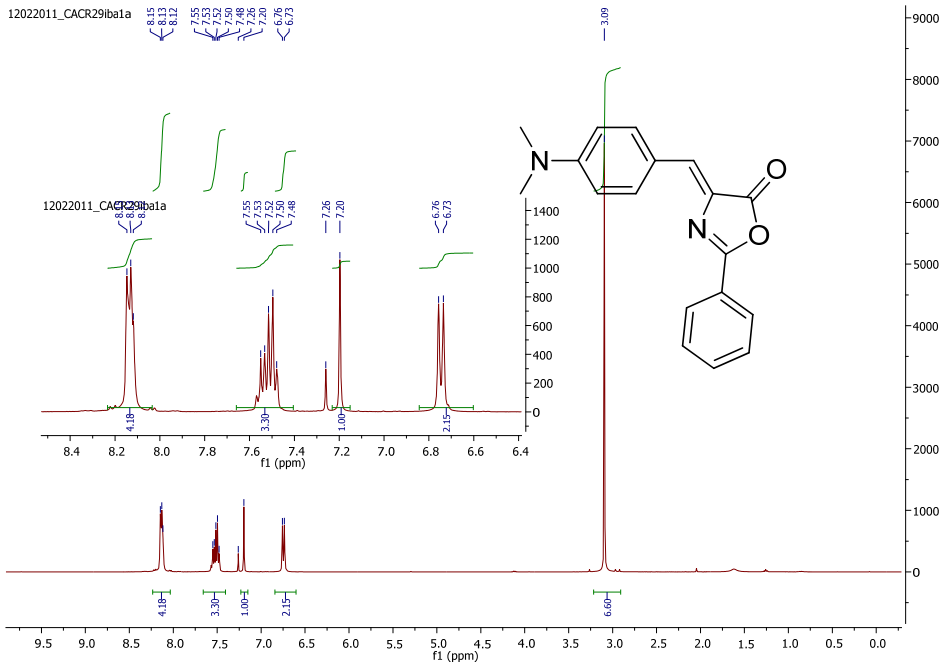
Peak Number	Mass (m/z)	Abundance (%)	FWHH (m/z)	Resolving Power
1	250.08239	20.335	0.06681	3743
2	249.07783	100.000	0.06593	3778
3	209.68836	12.756	0.04521	4638
4	167.03358	28.720	0.02925	5711
5	149.02336	15.249	0.02298	6484
6	105.03349	51.121	0.01214	8650
7	69.73811	16.090	0.00505	13816
8	69.68737	47.101	0.00480	14506
9	69.50167	6.835	0.00479	14503



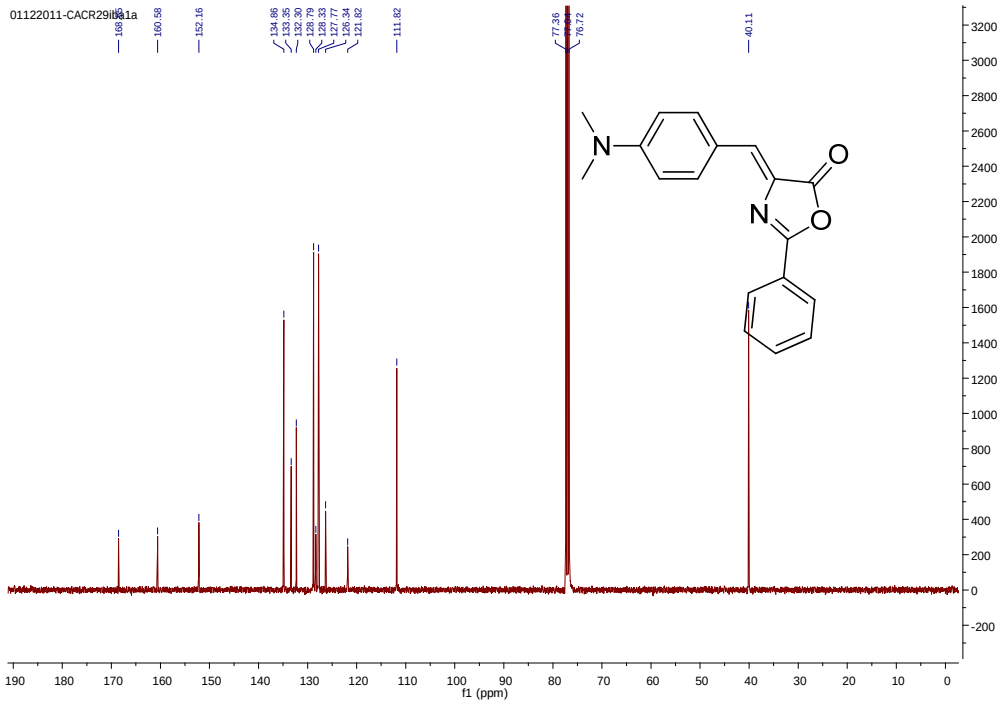


Slice_6 *OXA004_EI-4*

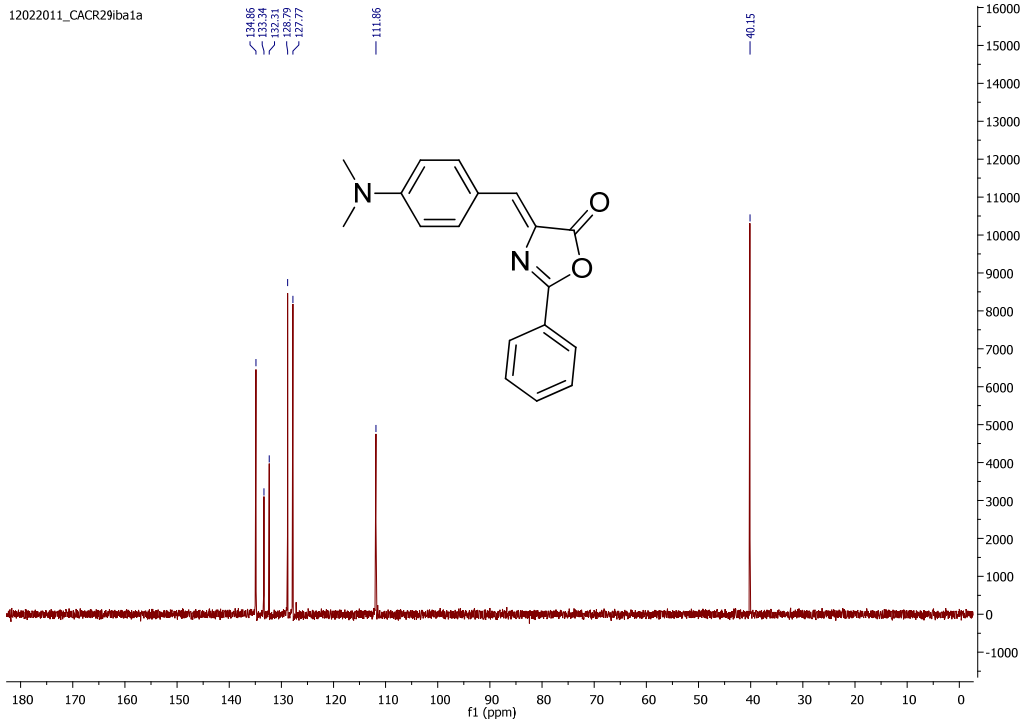
4.3. (Z)-4-[4-(dimethylamino)benzylidene]-2-phenyloxazol-5(4H)-one



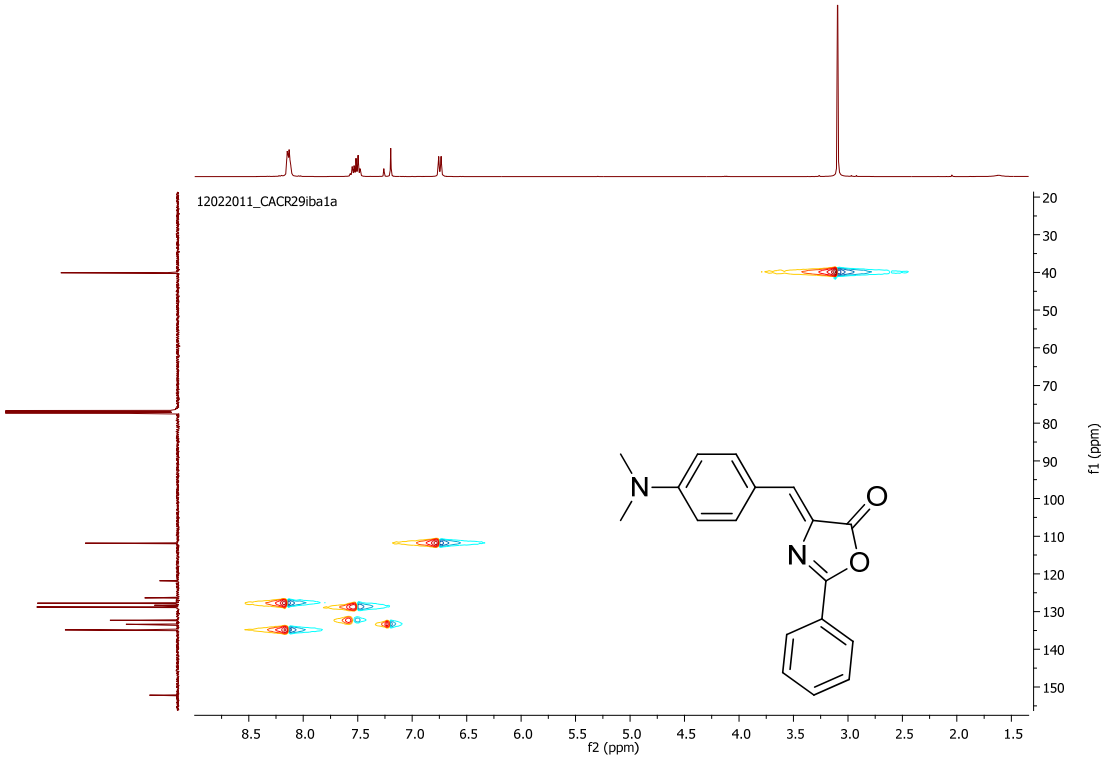
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 6.9 Hz, 4H, 4xC<u>H</u>$_{Ar}$), 7.65 – 7.42 (m, 3H, 3xC<u>H</u>$_{Ar}$), 7.20 (s, 1H,-C<u>H</u>-C=), 6.75 (d, *J* = 8.8 Hz, 2H, 2xC<u>H</u>$_{Ar}$), 3.09 (s, 6H,- N(C<u>H</u>$_3$)$_2$).

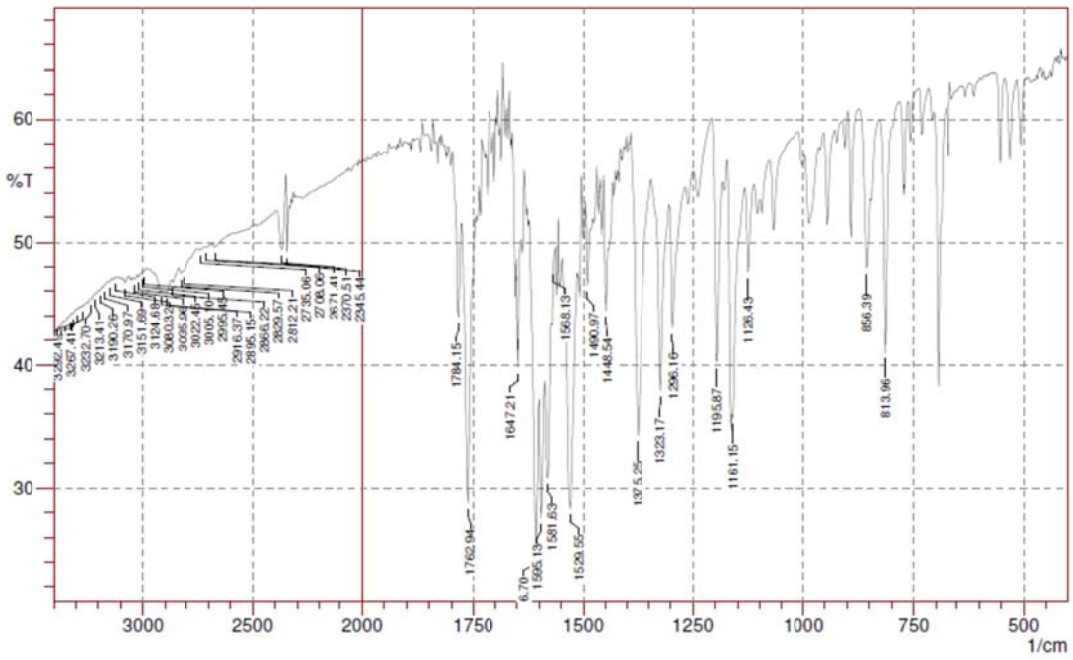


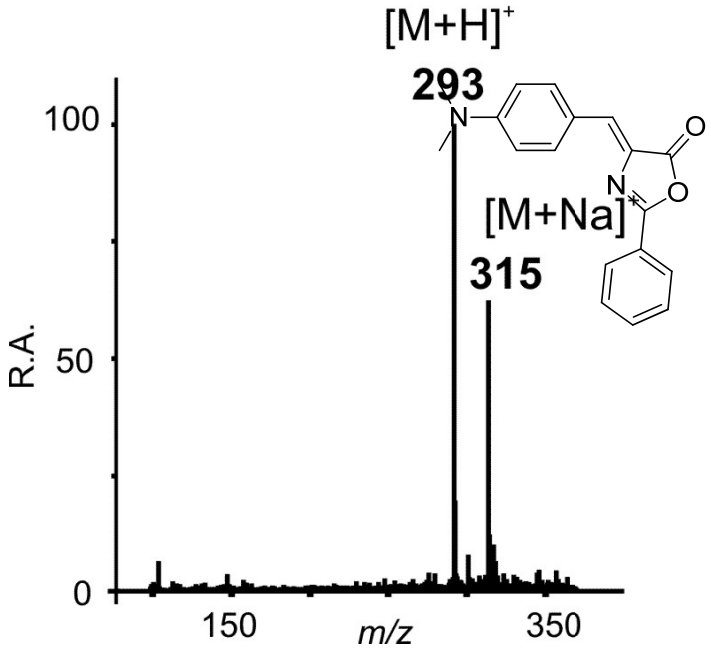
^{13}C NMR (101 MHz, $CDCl_3$) δ 168.55, 160.58, 152.16, 134.86, 133.35, 132.30, 128.79, 128.33, 127.77, 126.34, 121.82, 111.82, 40.11 (N(<u>C</u>H$_3$)$_2$).



^{13}C NMR (101 MHz, $CDCl_3$) δ 134.86, 133.34, 132.31, 128.79, 127.77, 111.86, 40.15.





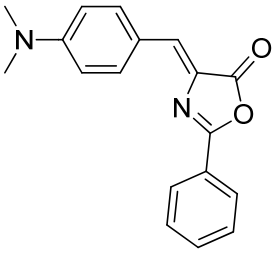


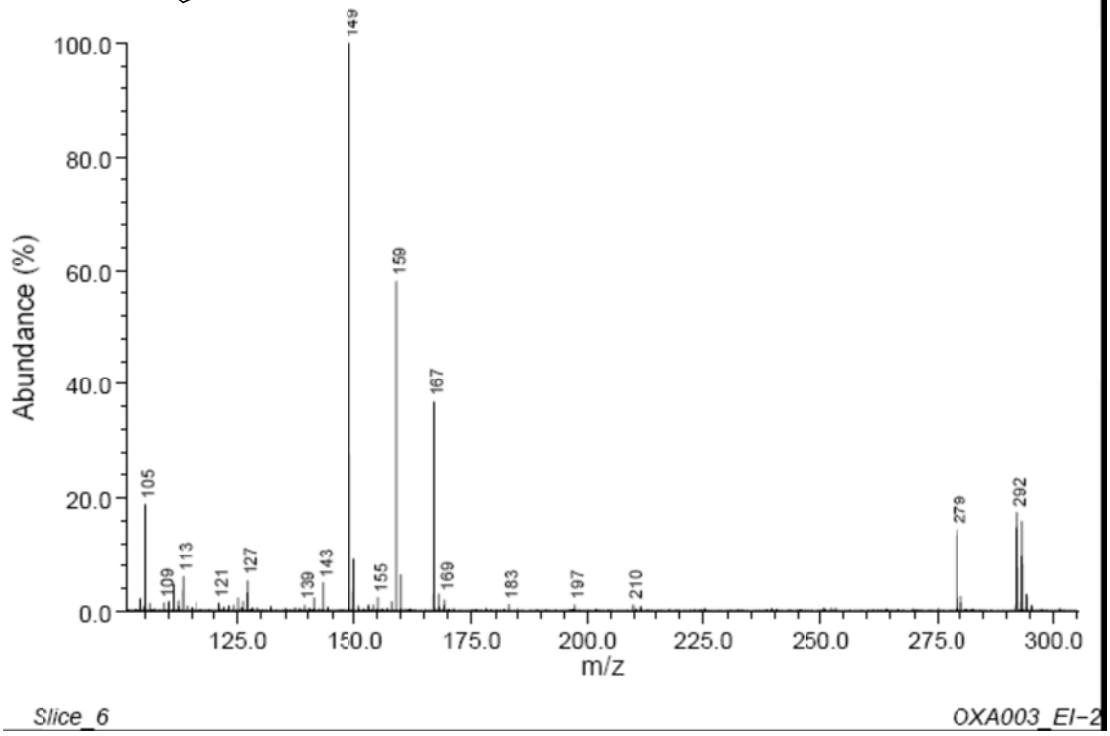
No. of peaks: 43

Height and Width: From Spectrum Points
Peak Fit Type: 3-Point Quadratic
Peak Threshold Set: Automatically
Min. Peak Width: 3
Search Start: 97.94063 m/z
Search Start: 306.29475 m/z
Peak Thresh: 0.96 Relative (%)
Noise Thresh: 0.09 Relative (%)
Peak S/N: 5.00 (S/N)

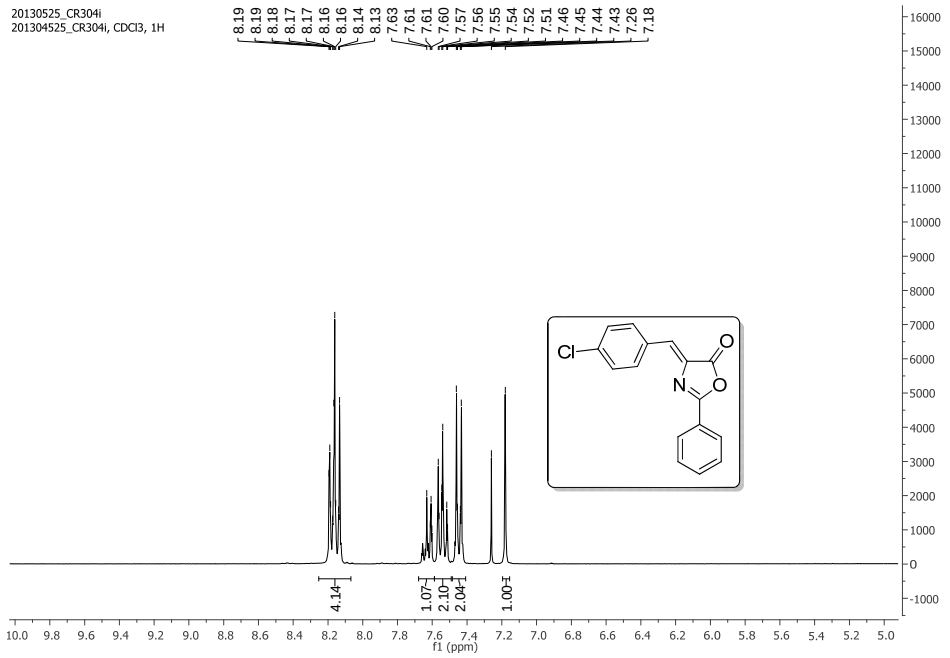
Peak Number	Mass (m/z)	Abundance (%)	FWHH (m/z)	Resolving Power
1	295.26240	1.031	0.12883	2292
2	294.19150	2.963	0.08762	3358
3	293.18654	15.934	0.08660	3385
4	292.12084	17.467	0.09973	2929
5	291.97685	1.409	0.07831	3729
6	280.17140	2.595	0.08189	3421
7	279.16923	14.412	0.07948	3512
8	209.67661	1.132	0.04194	4999
9	197.23008	1.106	0.03987	4946
10	183.21539	1.174	0.03409	5375
11	169.19661	1.997	0.03024	5595
12	168.03955	3.026	0.02993	5614
13	167.03563	36.742	0.02921	5718
14	160.09642	6.391	0.02771	5778
15	159.09156	58.022	0.02822	5638
16	159.04808	2.611	0.02242	7095
17	158.08329	1.736	0.02787	5672
18	155.18102	2.394	0.02566	6047
19	154.17340	1.109	0.02389	6452
20	153.16462	1.120	0.02404	6371
21	150.02805	9.250	0.02332	6432
*22	*149.02371	*100.000	*0.02412	*6179

* additional peaks not listed

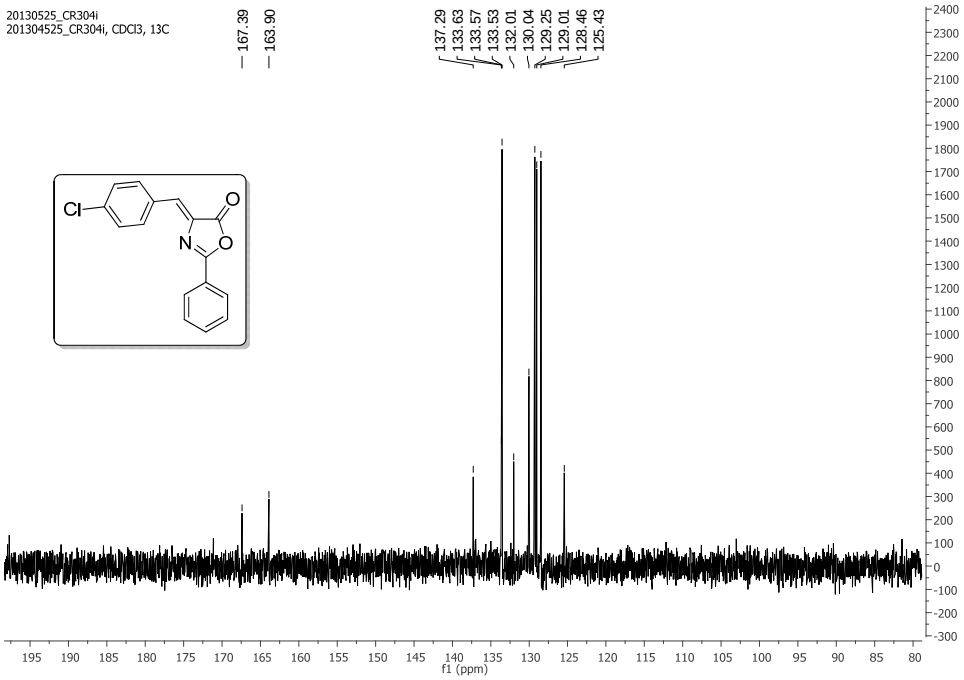




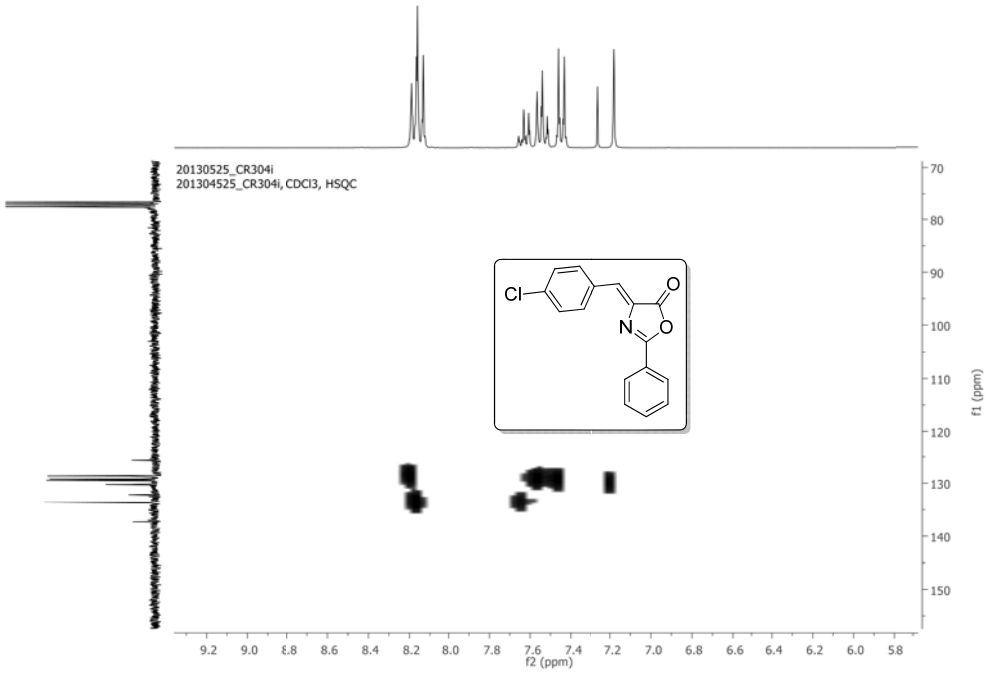
4.4. (Z)-4-(4-chlorobenzylidene)-2-phenyloxazol-5(4H)-one

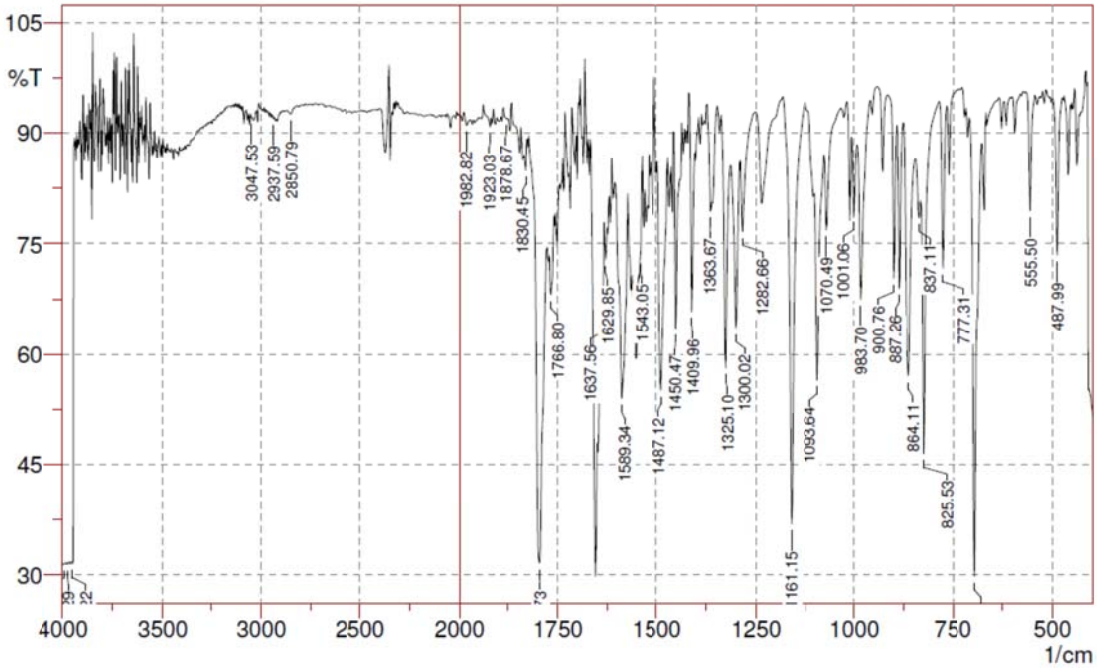


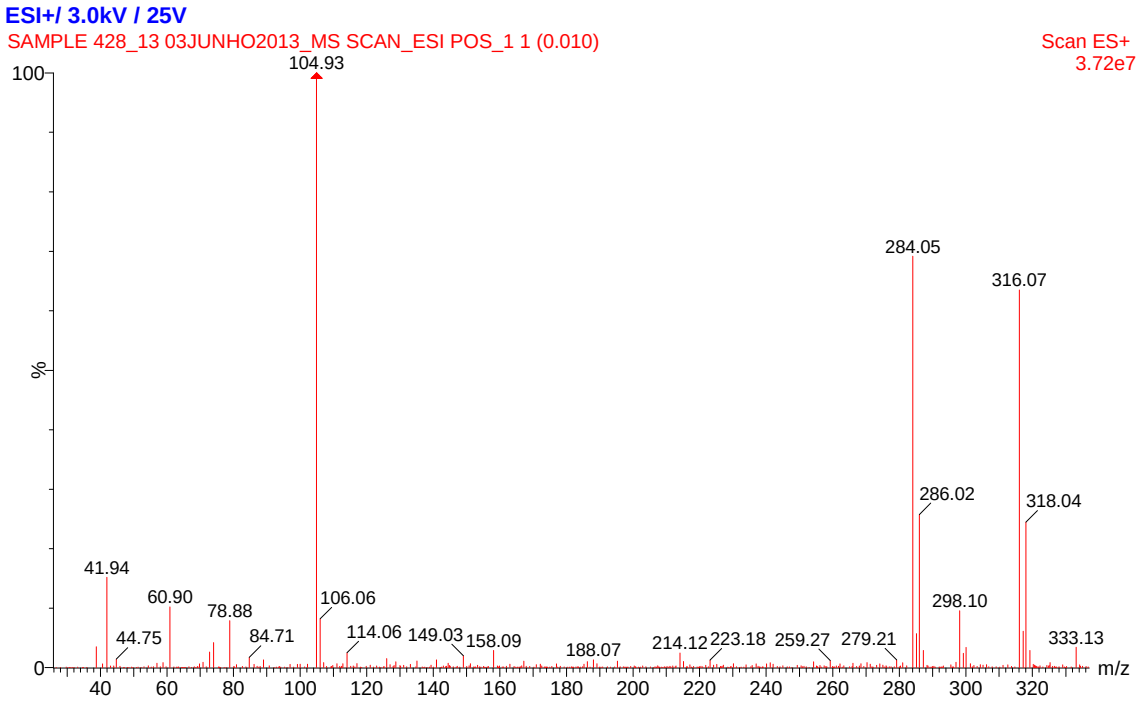
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 – 8.07 (m, 4H,4x C<u>H</u>$_{Ar}$), 7.68 – 7.59 (m, 1H, C<u>H</u>$_{Ar}$), 7.59 – 7.49 (m, 2H, 2XC<u>H</u>$_{Ar}$), 7.48 – 7.41 (m, 2H,2XC<u>H</u>$_{Ar}$), 7.18 (s, 1H, -C<u>H</u>=C).



^{13}C NMR (75 MHz, $CDCl_3$) δ 167.39, 163.90, 137.29, 133.63, 133.57, 133.53, 132.01, 130.04, 129.25, 129.01, 128.46, 125.43.

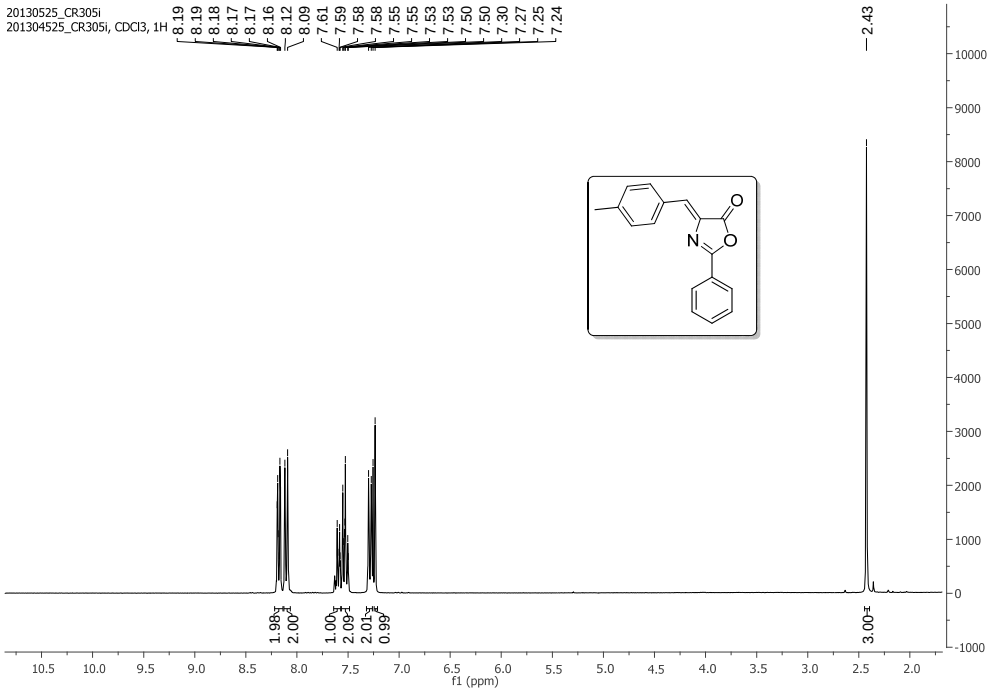




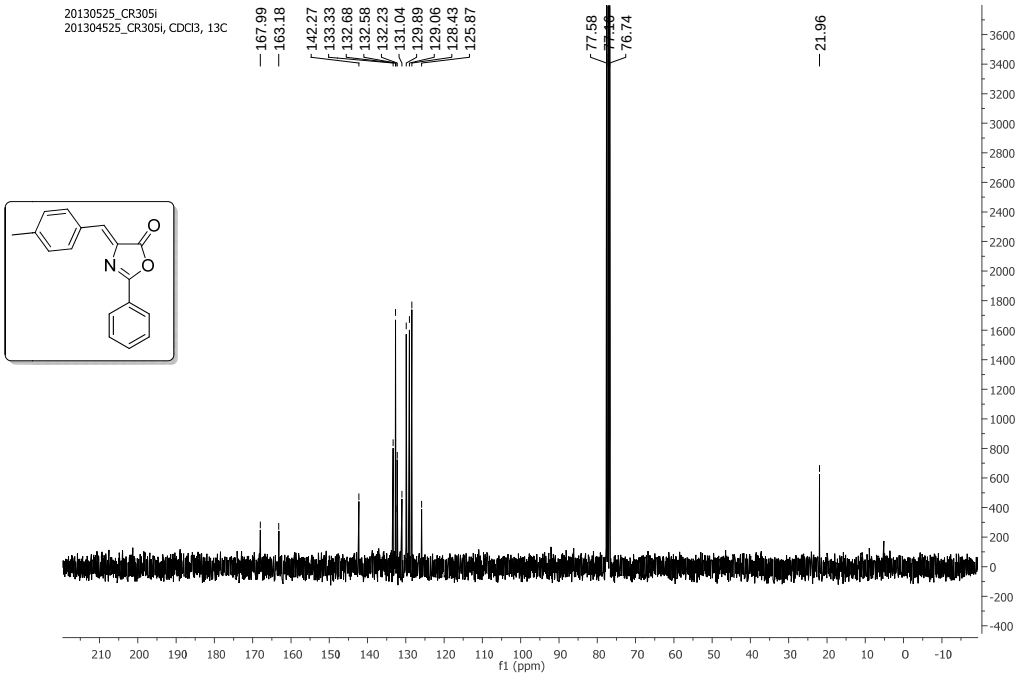


MS (ESI+) m/z calc for [$C_{16}H_{10}ClNO_2$+1]: 284.04, found: 284 $[M+H]^+$

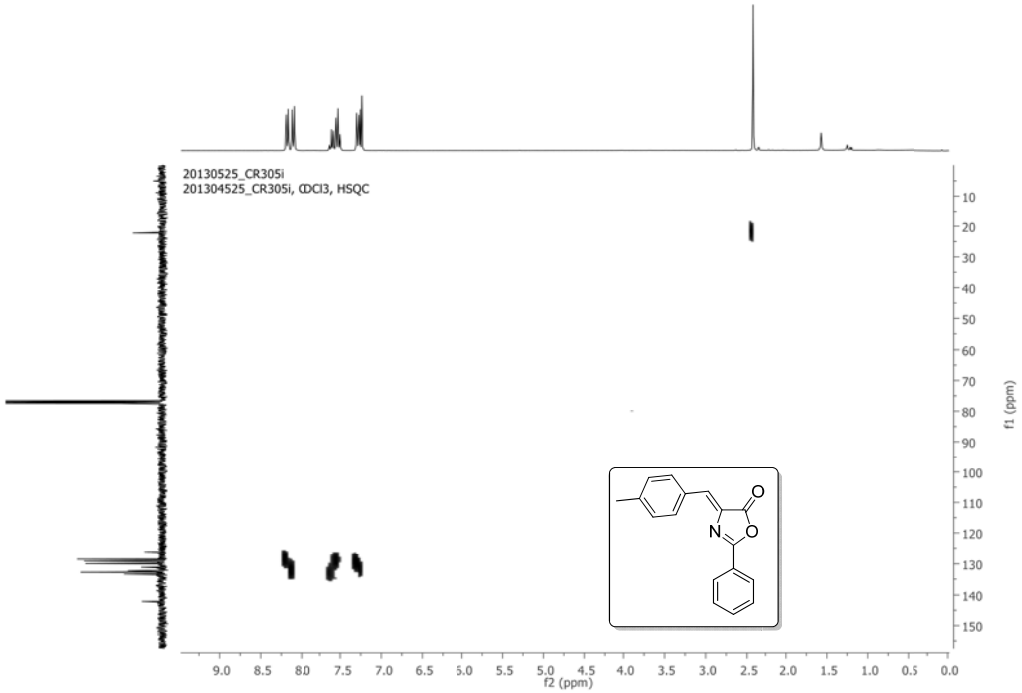
4.5. (Z)-4-(4-methylbenzylidene)-2-phenyloxazol-5(4H)-one

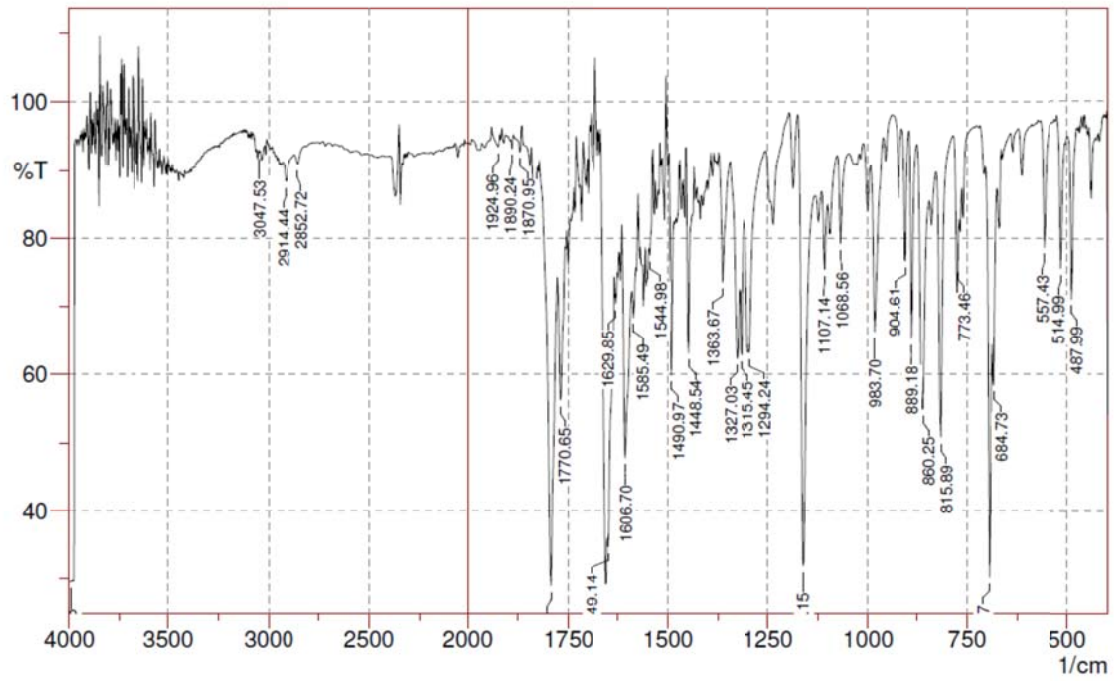


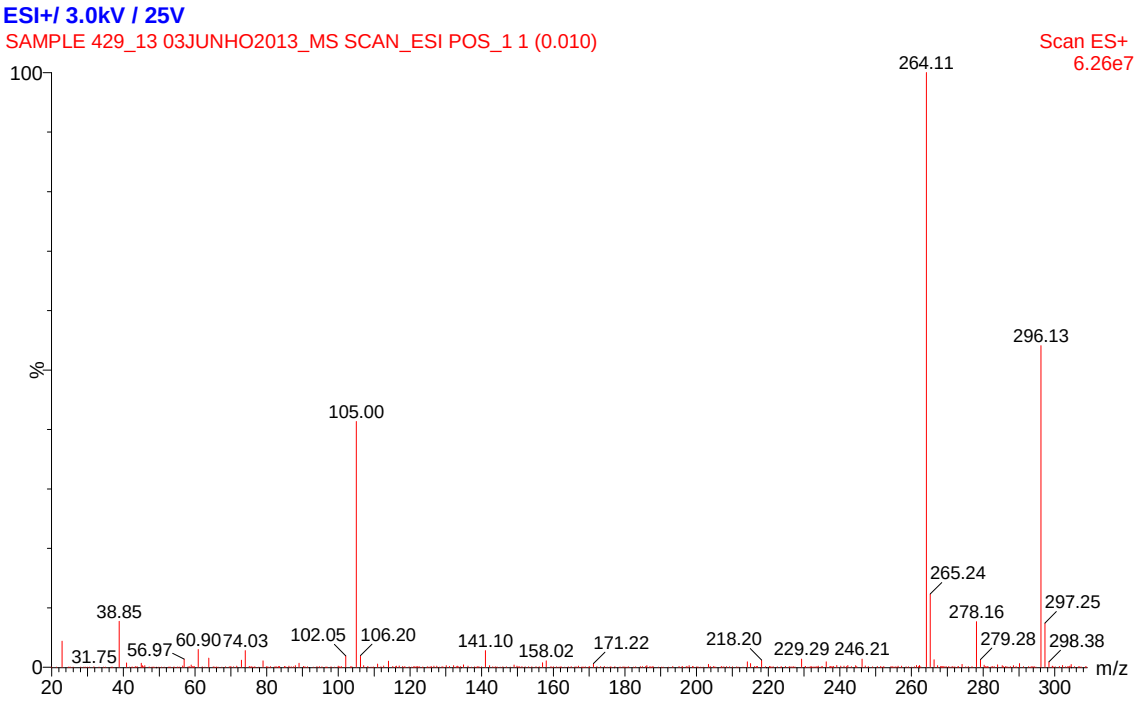
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 – 8.14 (m, 2H, 2XC<u>H</u>$_{Ar}$), 8.10 (d, *J* = 8.2 Hz, 2H, 2xC<u>H</u>$_{Ar}$), 7.59 (dd, *J* = 5.0, 3.6 Hz, 1H, 1xC<u>H</u>$_{Ar}$), 7.57 – 7.49 (m, 2H, 2xC<u>H</u>$_{Ar}$), 7.29 (d, *J* = 8.1 Hz, 2H, 2xC<u>H</u>$_{Ar}$), 7.24 (s, 1H, C<u>H</u>=C), 2.43 (s, 3H, C<u>H</u>$_3$).



^{13}C NMR (75 MHz, $CDCl_3$) δ 167.99, 163.18, 142.27, 133.33, 132.68, 132.58, 132.23, 131.04, 129.89, 129.06, 128.43, 125.87, 21.96 (<u>C</u>H$_3$).







MS (ESI+) m/z calc for [$C_{17}H_{13}NO_2$+1]: 263.09, found: 264 $[M+H]^+$

5. References

1. C. A. B. Rodrigues, I. F. A. Mariz, E. M. S. Macoas, C. A. M. Afonso and J. M. G. Martinho, *Dyes Pigments*, 2012, **95**, 713-722.
2. M. Kitazawa, R. Higuchi, M. Takasashi, T. Wada and H. Sasabe, *J. Phys. Chem.*, 1995, **99**, 14784-14792.
3. F. Chavez, N. Kennedy, T. Rawalpally, R. T. Williamson and T. Cleary, *Organic Process Research & Development*, 2010, **14**, 579-584.
4. T. Cleary, T. Rawalpally, N. Kennedy and F. Chavez, *Tetrahedron Letters*, 2010, **51**, 1533-1536.
5. A. Jacobs, *Understanding Organic Reaction Mechanisms*, Cambridge University Press, 1997.
6. P. S. Marrs, *J. Chem. Educ.*, 2001, **78**, 527.
7. S. L. Keenan, K. P. Peterson, K. Peterson and K. Jacobson, *J. Chem. Educ.*, 2008, **85**, 558.
8. G. K. Ikeda, K. Jang, S. O. C. Mundle and A. P. Dicks, *J. Chem. Educ.*, 2006, **83**, 1341.
9. L. P. Hammett, *J. Am. Chem. Soc.*, 1937, **59**, 96-103.
10. L. R. Domingo, P. Perez and R. Contreras, *J. Org. Chem.*, 2003, **68**, 6060-6062.
11. R. A. Sheldon, *Green Chemistry*, 2007, **9**, 1273-1283.

Synthesis of a long-wavelength absorbing squaraine dye

Supplementary Material

Notes

This experiment aims at the preparation of a symmetrical squaraine dye from 3-butyl-2-methylbenzothiazolium iodide and squaric acid. Since the starting reagents are scarcely soluble in the reaction medium at room temperature, the reaction is carried out under reflux. Being a heterogeneous reaction, vigorous stirring is of pivotal importance. During the process it is possible to observe a change of the reaction mixture's color, which starts as yellowish, then turns to green and finally to deep blue.

The progress of the reaction is followed by TLC. After elution, the TLC plates are observed under UV light at a wavelengths of 254 nm. The disappearance of the starting benzothiazolium salt can also be followed by spraying the TLC plates after elution with a Dragendorff's reagent solution (the spot of the salt acquires an orange color).

Once the reaction is complete, diethyl ether is added and the reaction mixture is stored in a refrigerator, to allow the formation of crystals, until the next session. Alternatively, the reaction mixture can be placed on ice to promote crystallization. However, in this way the crystals precipitate rapidly and often show a lower degree of purity.

After being collected by filtration under reduced pressure, the crystals must be washed firstly with water, to remove residues of pyridine and 1-butanol, and then with cold diethyl ether to remove traces of water (careful must be taken since diethyl ether may dissolve the crystals slightly). If necessary methyl *tert*-butyl ether (MTBE) can be used instead of the diethyl ether.

After being transferred to a weighed flask, the crystals are dried in a vacuum desiccator and weighed to determine the reaction yield.

Squaraine dye **2** was obtained in 87% yield, as green crystals. Melting point: 265 °C (dec.). UV/Vis (MeOH/CH_2Cl_2 (99/1, v/v)) λ_{max}: 652 nm.

Photos of the experiment

Figure SM 4.2.3.8.1 - Squaraine dye **2** obtained in the form of green crystals.

Figure SM 4.2.3.8.2 - Solution of squaraine dye **2** in MeOH/CH_2Cl_2 (99/1, v/v)

UV/Vis absorption spectrum

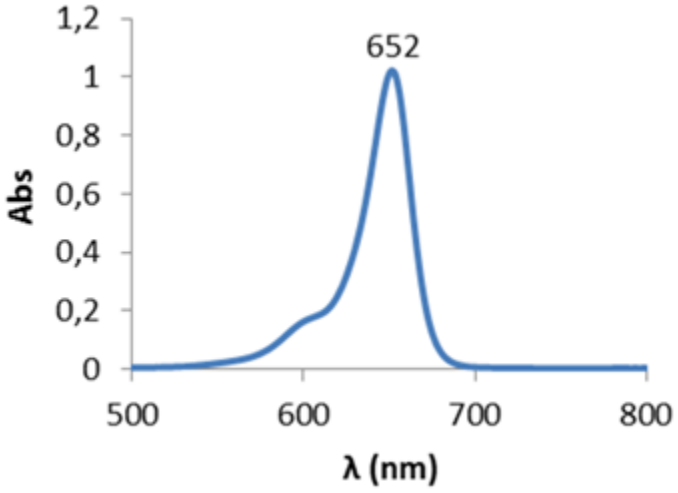


Figure SM 4.2.3.8.3 - UV/Vis absorption spectrum of squaraine dye **2** in MeOH/CH_2Cl_2 (99/1, v/v).

IR spectrum

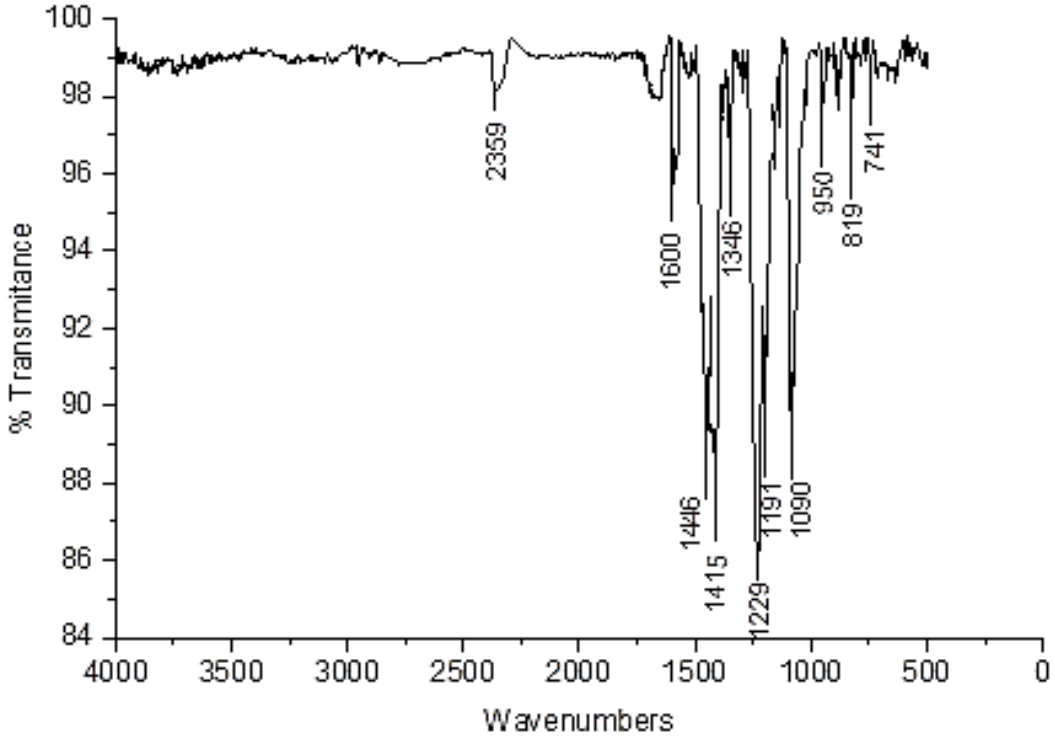


Figure SM 4.2.3.8.4 - IR spectrum (KBr) of squaraine dye **2**.

^{1}H-NMR spectrum

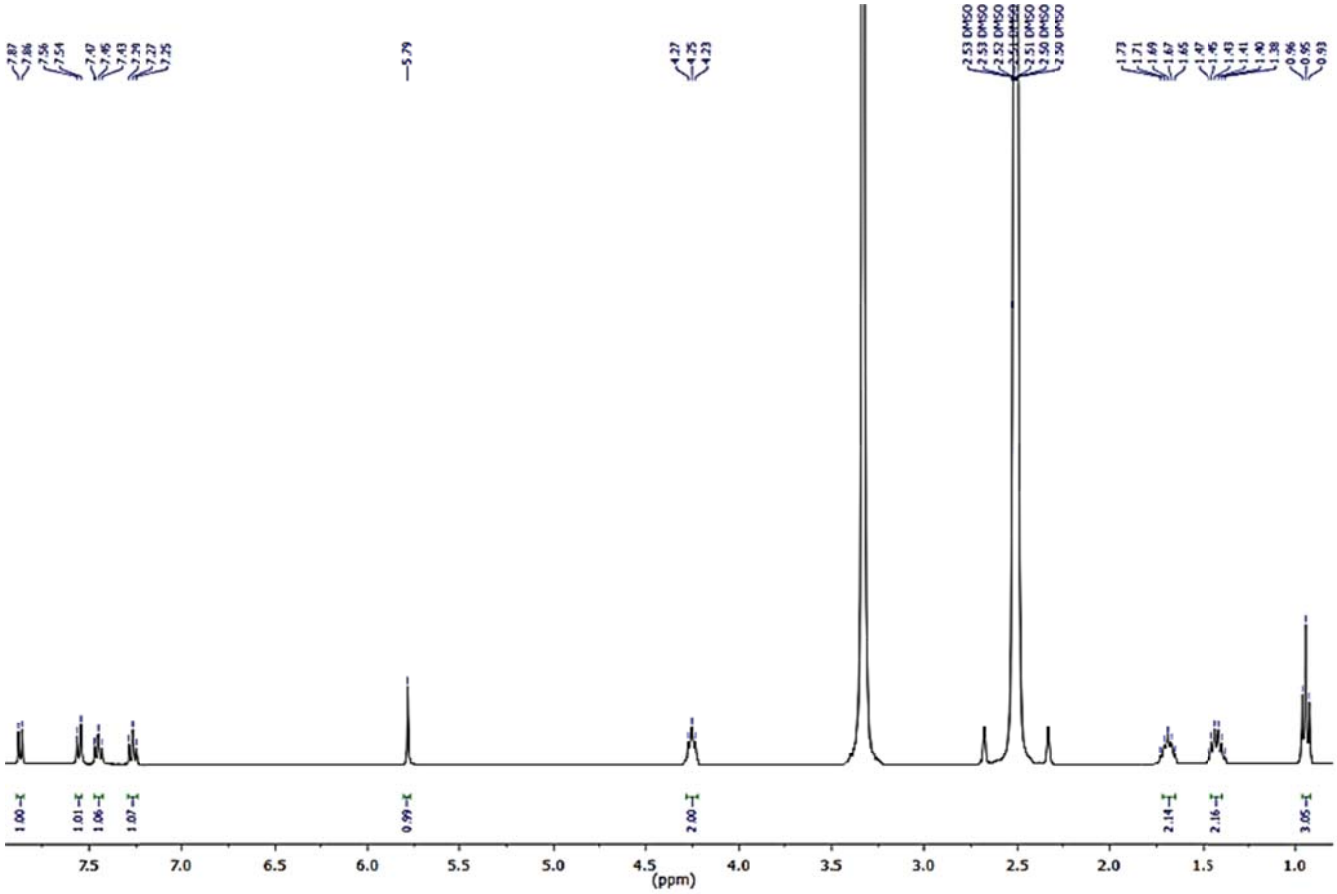


Figure SM 4.2.3.8.5 - ^{1}H-NMR spectrum (400.13 MHz, DMSO-d_6) of squaraine dye **2**.

Synthesis of π-conjugated systems using formylchromone as building block

Supplementary Material

The proposed lab experiences can be integrated into organic chemistry and medicinal chemistry courses as they are linked to the design and synthesis of heterocyclic compounds.

To reach the objectives different organic reactions have been employed:

a) Vilsmeir-Haack reaction that encircles an addition reaction followed by an intramolecular cyclisation to attain the chromone;

b) Knoevenagel-Doebner condensation that allow the formation of a C-C bond and the introduction of a vynilic exocyclic bond.

Furthermore, the experiments include the use of purification techniques and the identification of the synthesized compounds by spectrophotometric methods. The IR technique was introduced as they are useful tools to follow the course of the reactions and to characterize the final compounds. The data of UV-Vis, IR and NMR (^{1}H and ^{13}C) spectra of the synthesized compounds are given as auxiliary material.

The experiments were designed for chemistry-based curricula, namely chemistry or medicinal chemistry courses, and are best suited for students that have organic chemistry (theoretical and practical) foundations. The experiments were tested in a first year master chemistry course including 30 students. They were easy to perform and produced clear results which directed the students in a new practical and real organic chemistry lesson. The average success of students in the integrated experience was of 95%. The yields of the reactions were around 50% for the first reaction and 90% for the second one. In general, students obtained adequate amount of compounds to complete the hands-on experience of structural characterization. No difficulties in the purification step and acquisition of NMR spectra have been detected. However, in the interpretation of NMR data, some drawbacks have been noticed; students were advised to use molecular model kits to assist in the visualization process. A lab report was written according standard guidelines.[1]

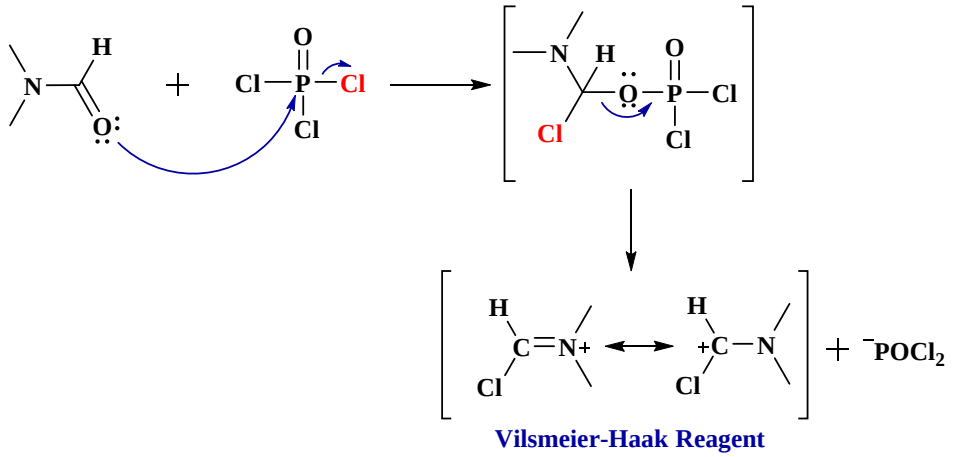
Notes

- Students should prepare a procedure flow chart of the experience to be followed in the class;

- Students must be aware that the reaction of HCl and pyridine results in a pyridinium salt and so pyridine and its unpleasant odour, is eliminated;
- Students must review UV-Vis and IR techniques and concepts.

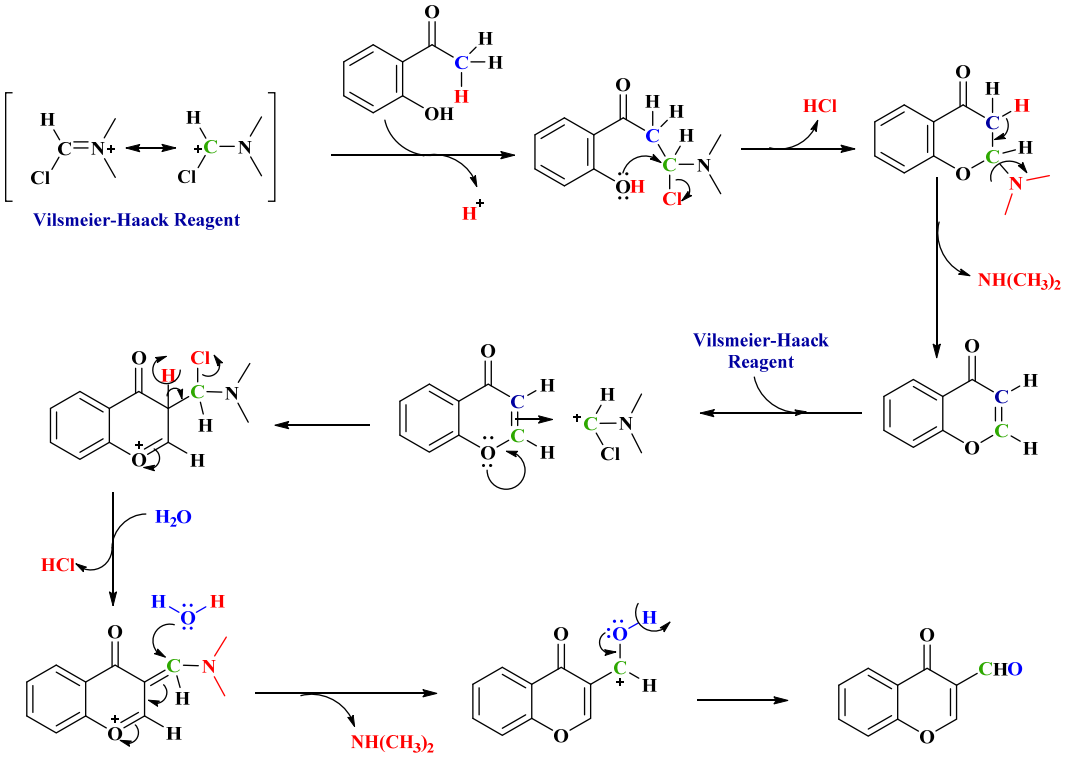
Synthesis of 4-oxo-4*H*-chromone-3-carbaldehyde

The Vilsmeir-Haack reaction starts with the *in situ* formation of the so-called Vilsmeier-Haack reagent, a halomethylenium salt, generated by the reaction of a *N,N*-disubstituted formamide, such as dimethylformamide (DMF), with phosphorus oxychloride ($POCl_3$) (Scheme SM 4.2.3.9.1) [8]. This reaction can be applied for the formylation of both aromatic and heteroaromatic substrates.



Scheme SM 4.2.3.9.1 Proposed mechanism for the formation theVilsmeier-Haack reagent

The Vilsmeier-Haack reaction can also be used for chromone synthesis. The proposed reaction mechanism encompasses the formation of an enolate intermediate, generated from the *in situ* reaction of the Vilsmeier-Haack reagent and 2-hydroxyacetophenone. The obtained intermediate undergoes an intramolecular annulation, with subsequent formation of a benzopyran-4-one ring. Subsequently, the double bond of the benzopyran ring reacts with the Vilsmeier-Haack reagent and reactive intermediate is obtained. Finally, a rapid hydrolysis of this intermediate occurs, giving rise to 4-oxo-4*H*-benzopyran-3-carbaldehyde [8].



Scheme SM 4.2.3.9.2. Proposed mechanism for the synthesis of 3-formyl chromone

Additional notes

- Compound synonyms: 3-formylchromone, chromone-3-carboxaldehyde, chromone-3-aldehyde, 4-oxochromene-3-carbaldehyde (IUPAC), 3-formyl-4*H*-1-benzopyran-4-one, 4-oxo-4*H*-chromene-3-carbaldehyde, 4-oxo-1-benzopyran-3-carboxaldehyde, 4-oxo-4*H*-1-benzopyran-3-carboxaldehyde, 4-oxo-4*H*-benzopyran-3-carboxaldehyde.
- The implementation of the reaction should be easy for students in advanced level of studies. Nevertheless, some extra care should be taken with $POCl_3$ as it is water-reactive and have a pungent, irritating odour releasing vapours at room temperature.
- The reaction should be performed in a fume hood and with reflux apparatus. Visually, the solution turns yellow with the addition of $POCl_3$ to the DMF. No colour alteration is detected when 2-hydroxyacetophenone is added.
- In TLC chromatogram, the starting material and product have slight different retention factors (Rf of 2-hydroxyacetophenone ≈ 0.77 and Rf of 3-formylchromone ≈ 0.71).

- In the work-up attention should be paid to the order of purification techniques. Students must have special care when the content of the reaction is poured into ice as the reaction is strongly exothermic.
- The average yield is 85 %.
- The purified solid must be dried, for instance in a vacuum oven, before UV-VIS and IR spectra acquisition.
- Additionally, the compound can also be elucidated by NMR spectroscopic techniques.

Compound structural elucidation:

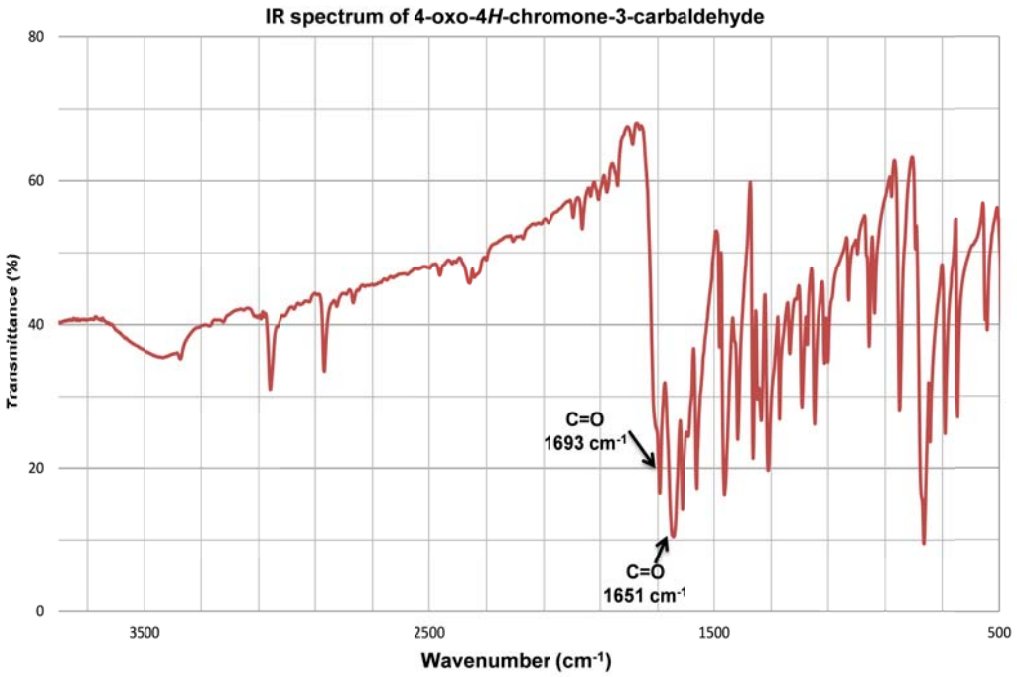
1. **UV-VIS data**

The spectrum was acquired in a UV-VIS spectrophotometer (Shimadzu UV-1700 PharmaSpec, Japan) and a scan between 200-800 was performed. The λ_{max} within the wavelength interval is reported in the following table:

λ_{max}	Abs
341.5	0.2611
293.0	1.0424
225.5	2.0396
208.0	1.1914

2. **FTIR data**

The IR spectrum was acquired in a Nicolet 6700 FT-IR spectrometer using potassium bromide disk.

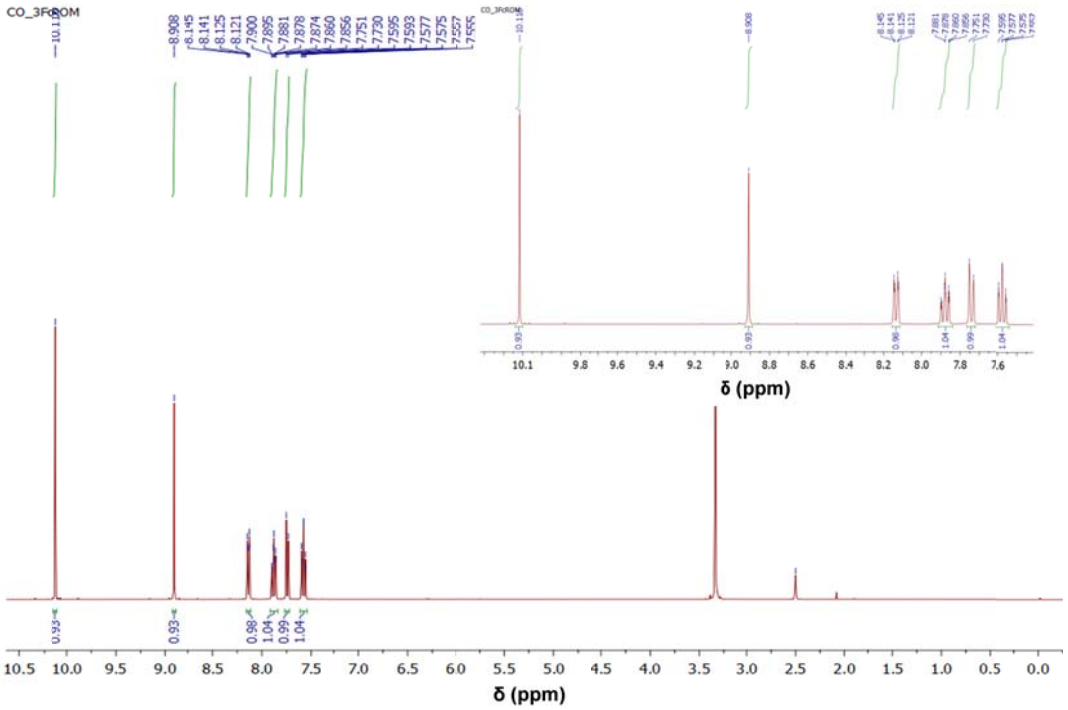


3. NMR Data

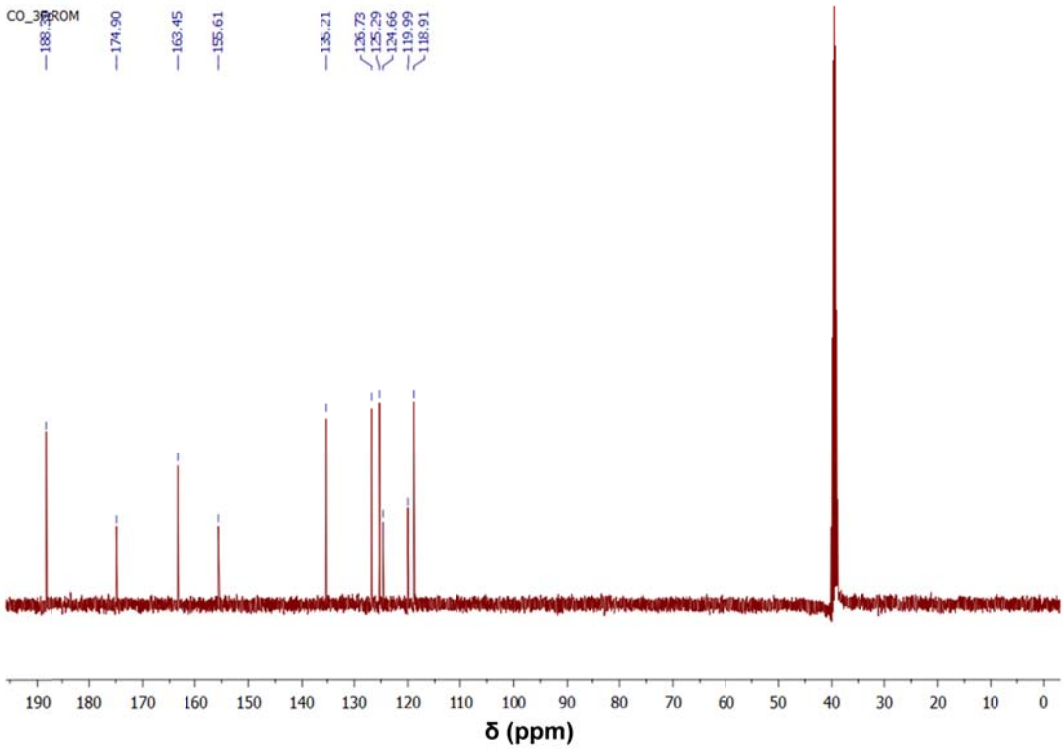
^{1}H and ^{13}C NMR spectra were obtained on a BRUKER AVANCE III 400, operating at room temperature at 400.15 MHz and 100.62 MHz respectively. The solvent used was d_6-dimethylsulfoxide, and Chemical shifts are expressed as ppm values relative to tetramethylsilane (TMS) as an internal reference; coupling constants (J) are given in Hz.

^{1}H NMR (400 MHz, DMSO) δ: 7.58 (1H, *ddd*, *J* = 8.0, 8.0, 0.8 Hz, H6), 7.74 (1H, *d*, *J* = 8.4 Hz, H8), 7.89 (1H, *ddd*, *J* = 8.6, 7.2, 1.6 Hz, H7), 8.13 (1H, *dd*, *J* = 8.0, 1.6 Hz, H5), 8.91 (1H, *s*, H2), 10.1 (1H, CHO)

^{13}C NMR (101 MHz, DMSO) δ: 118.9 (C8), 120.0 (C3), 124.7 (C4a), 125.3 (C6), 126.7 (C5), 135.2 (C7), 155.6 (C8a), 163.4 (C2), 174.9 (CO), 188.4 (CHO)



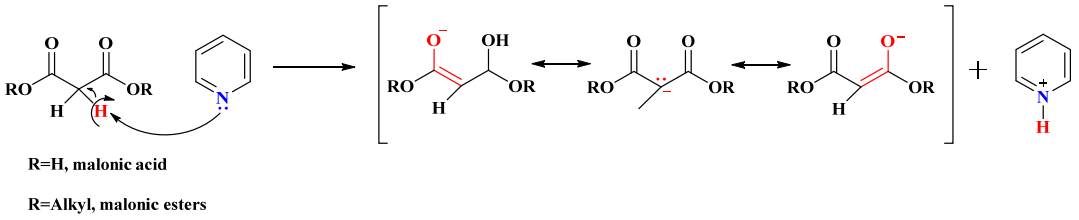
^{1}H NMR of 4-oxo-4*H*-chromone-3-carbaldehyde with an expansion from 11 ppm to 7.5 ppm



^{13}C NMR of 4-oxo-4H-chromone-3-carbaldehyde

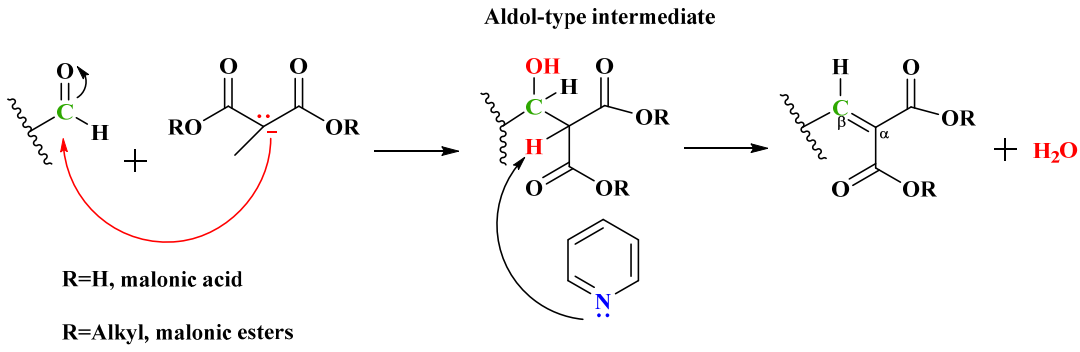
Synthesis of (*trans*)-3-(4-oxo-4*H*-chromen-3-yl)acrylic acid

The synthesis of 3-(4-oxo-4*H*-chromen-3-yl)acrylic acid herein described was performed *via* Knoevenagel–Doebner condensation. This reaction is considered to be a modification of an aldol condensation, involving a nucleophilic addition of a CH-acidic methylene compound (*e.g.* malonic acid and its ester derivatives) to aldehydes or ketones. Typically, this reaction occurs in the presence of a weak amine base such as piperidine. The subsequent step is a dehydration reaction in which a molecule of water is eliminated. Mechanistically, the Knoevenagel-Doebner reaction starts with the formation of a resonance stabilized enolate intermediate, formed from the reaction of malonic acid or its esters derivatives, with a weak amine (e.g. pyridine). In fact, the employment of pyridine as a catalyst is known as the Doebner modification[11] (Scheme SM 4.2.3.9.3).



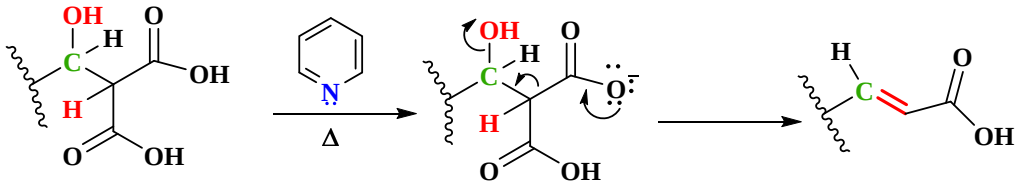
Scheme SM 4.2.3.9.3. Proposed mechanism for the formation of a resonance stabilized enolate intermediate

The Knoevenagel–Doebner proposed mechanism to attain the *α, β*-unsaturated compound is highlighted in scheme SM 4.2.3.9.4[11]. It involves a nucleophile attack of the enolate intermediate to the carbonyl group of the aldehyde function. The resulting aldol-type intermediate undergoes a rapid base-induced dehydration process to give the *α, β*-unsaturated compound (Scheme SM 4.2.3.9.4) [11].



Scheme SM 4.2.3.9.4. Proposed mechanism of Knoevenagel–Doebner condensation

When the CH-acidic methylene compound corresponds to malonic acid and the reaction is conducted in refluxing pyridine, the aldol-type intermediate may undergo decarboxylation, giving rise to the correspondent *α,β*-unsaturated carboxylic acid (Scheme SM 4.2.3.9.5) [12].



Scheme SM 4.2.3.9.5. Decarboxylation step

Additional notes

- Compound synonyms: (*E*)-3-(4-oxochromen-3-yl)prop-2-enoic acid;
- The assembly of the reaction mixture is very simple, being perhaps the major struggle the use of a water-jacketed reflux condenser.
- Due to safety issues, and the unpleasant odour of pyridine, the reaction must be performed in a fume hood.
- The condensation of 3-formylchromone with malonic acid in the presence of pyridine is carried out at 120 ºC. The reaction mixture will immediately turn a reddish coloration.
- The work-up of the reaction encompasses pyridine neutralization by addition of concentrated hydrochloride acid (HCl 37%) dropwise until the solution tests weakly acidic to pH paper (use universal indicator paper). The yellow solid Collect is collected by vacuum filtration using a Buckner funnel and washed thoroughly with diluted HCl (1M) and water. To partially dry the material, continue the suction for an additional 15 min.
- The average yield is 95 %.
- The purified solid must be dried, for instance in a vacuum oven, before UV-VIS and IR spectra acquisition.
- Additionally the compound can also be elucidated by NMR spectroscopic techniques.
-

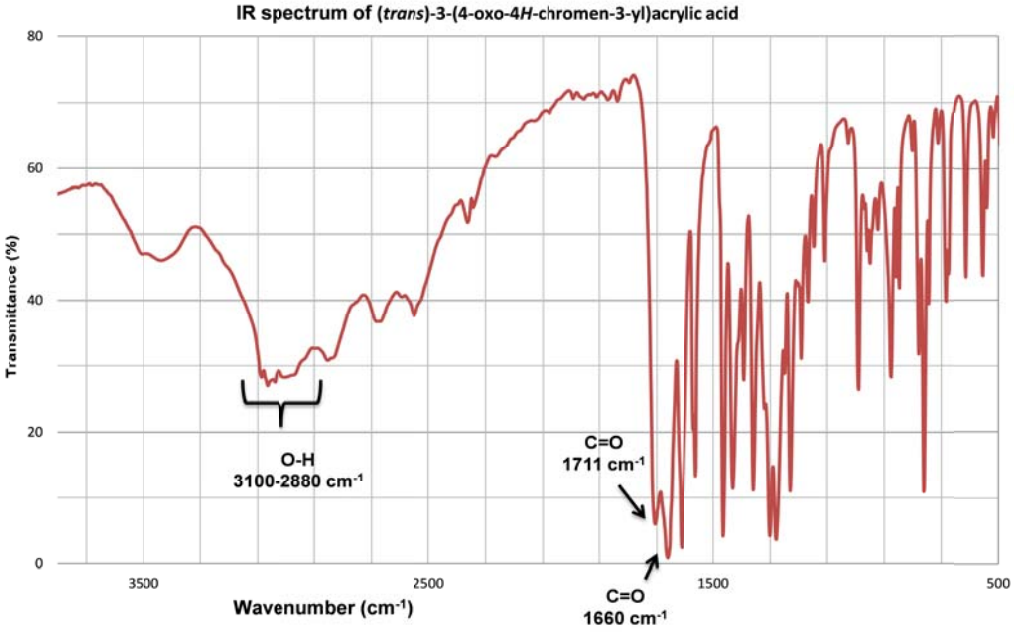
Compound structural elucidation:

1. **UV-VIS data**

The spectrum was acquired in a UV-VIS spectrophotometer (Shimadzu UV-1700 PharmaSpec, Japan) and a scan between 200-800 was performed. The λ_{max} within the wavelength interval is reported in the following table:

λmax	Abs
289.0	1.4747
265.5	2.9082
218.5	1.0414

2. FTIR data

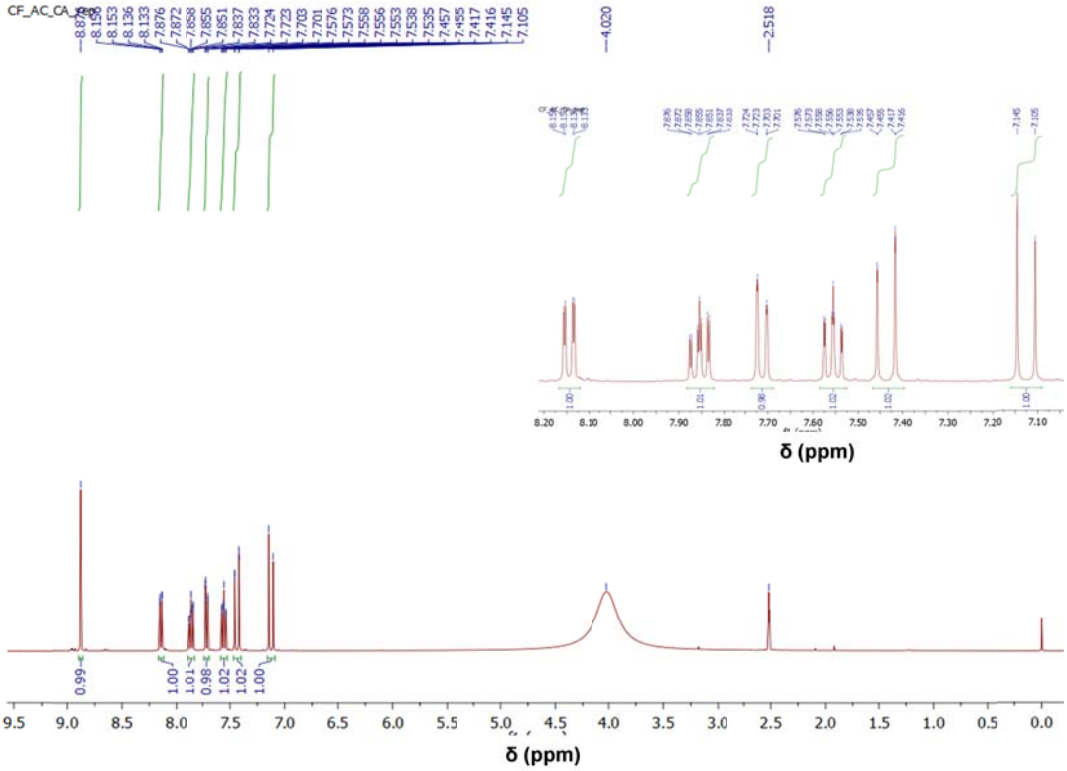


3. NMR Data

^{1}H and ^{13}C NMR spectra were obtained on a BRUKER AVANCE III 400, operating at room temperature at 400.15 MHz and 100.62 MHz respectively. The solvent used was d_6-dimethylsulfoxide and Chemical shifts are expressed as ppm values relative to tetramethylsilane (TMS) as an internal reference; coupling constants (J) are given in Hz.

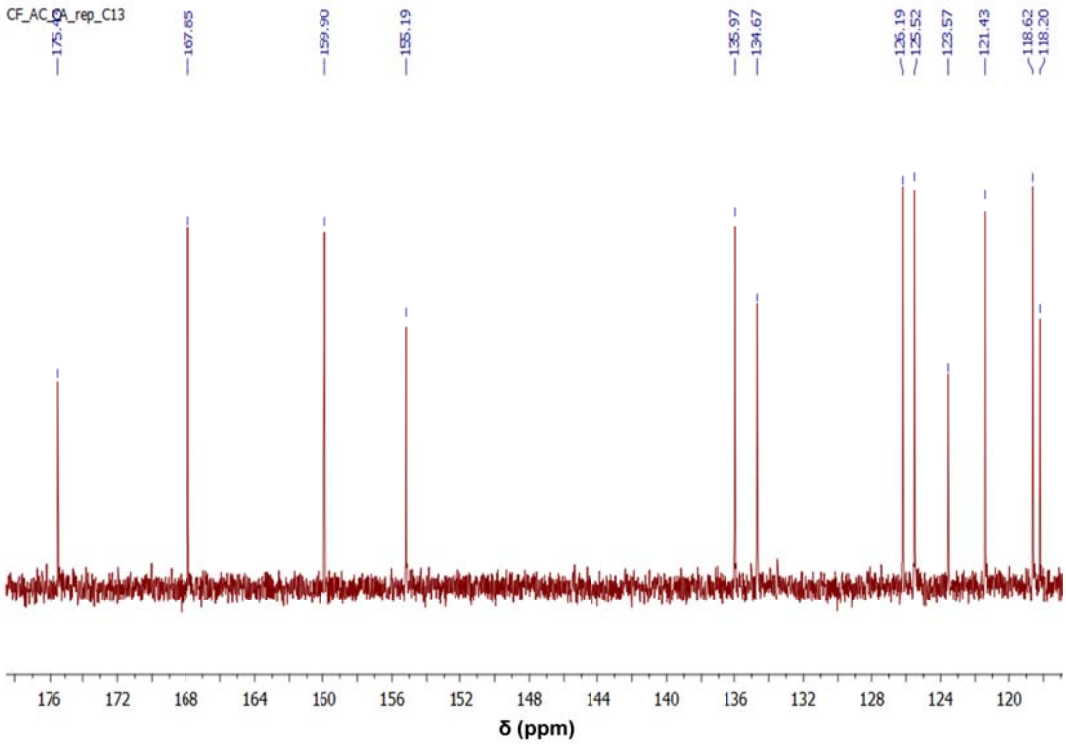
^{1}H NMR (400 MHz, DMSO) δ: 7.13 (1H, *d*, *J* = 16 Hz, H_α), 7.44 (1H, *d*, *J* = 16 Hz, H_β), 7.56 (1H, *ddd*, *J* = 7.4, 7.2, 0.8 Hz, H6), 7.71 (1H, *dd*, *J* = 8.8, 0.4 Hz, H8), 7.85 (1H, *ddd*, *J* = 7.4, 7.2, 0.8 Hz, H7), 8.14 (1H, *dd*, *J* = 8.8, 0.4 Hz, H5), 8.88 (1H, *s*, H2).

^{13}C NMR (101 MHz, DMSO) δ: 118.2 (C8), 118.6 (C3), 121.4 (C4a), 123.6 (C_α), 125.5 (C6), 126.2 (C5), 134.7 (C7), 136.0 (C_β), 155.2 (C8a), 159.9 (C2), 167.8 (COOH), 175.3 (CO)



^{1}H NMR of 3-(4-oxo-4H-chromen-3-yl)acrylic acid with an expansion from 8.2 ppm to 7.0 ppm

Note: From the ^{1}H NMR it is possible to infer what type of diastereoisomers (cis/trans) is obtained. The coupling constants (*J*) for *cis* hydrogen atoms have values near 10 Hz, while the *J* for *trans* is around 16 Hz. The NMR spectrum shows the presence of two signals with the multiplicity of doublet possessing a *J*=16 Hz and assigned at δ=7.13 and 7.44 respectively. So, it is possible to conclude that the synthesized compound is the *trans* stereoisomer.



^{13}C NMR of 3-(4-oxo-4H-chromen-3-yl)acrylic acid

1. W. R. Dolphin, Writing Lab Reports and Scientific Papers; McGraw-Hill: New York, 1997. http://www.mhhe.com/biosci/ genbio/maderinquiry/writing.html

Additional Reading

John Gilbert, Stephen Martin, *Experimental Organic Chemistry: A Miniscale and Microscale Approach* (5 th ed.), Cengage Learning.

Robert M. Silverstein, Francis X. Webster, David Kiemle, *Spectrometric Identification of Organic Compounds* (7 th ed.), John Wiley & Sons Inc.

Ernö Pretsch, Philippe Bühlmann, Martin Badertscher *Structure Determination of Organic Compounds* (4th ed.), Springer-Verlag Berlin Heidelberg.

A multistep synthesis of imidazolin-5-ones

Supplementary Material

The purpose of this experiment is to train a beginner in the handling of multistep organic synthesis and also briefly introduce NMR and column chromatography. The final product (imidazolin-5-one) is an analogue of the *green fluorescent protein* chromophore. Imidazolin-5-one heterocycle is a fluorescent molecule and is also known to show pharmacological properties. In the first step, the reactant become soluble in water but the product (acetyl glycine) precipitate from the water reaction medium, so there is no need of monitoring the reaction progress by TLC analysis. The product yield in the first step is around 60-70%. The second step, oxazolone synthesis was reported previously.[1-6] The oxazolone can be synthesized by cyclodehydration condensation between acetyl glycine and aromatic aldehyde by *Erlenmeyer-Polchl* reaction. After refluxing the reaction mixture, the ice pieces are added to initiate precipitation by vigorous stirring. This precipitate contains the product (oxazolone) and unreacted aromatic aldehyde as an impurity. In column chromatography, unreacted aldehyde elutes first followed by the pure product. In the oxazolone purification, make sure that aromatic aldehyde impurity has been removed properly before proceedings the next step, because the excess aromatic aldehyde further react with imidazolin-5-one (product of next step) in the presence of Lewis acid $ZnCl_2$ and form the product with extended conjugation (Figure SM 4.2.3.10.4),[5-7] known as *Red Fluorescent Protein* chromophore analogues. The presence of electron withdrawing group in aromatic aldehyde ring increases the yield of oxazolone synthesis in step 2 (See mechanism in Figure SM 4.2.3.10.2). The pure oxazolone is very stable and can be stored under any condition. The third and last step is a moisture and air sensitive reaction, performed under solvent free condition. This step requires an inert moisture free atmosphere. Cold-water circulation in the reflux condenser mainly ensures that the primary amine condenses back into the reaction mixture and consequently becomes an important factor for getting good yields. After the reaction work-up, the colored crude product will have to be purified by column chromatography. The silica binds to the imidazolin-5-one due to its acidic nature

and consequently product sticks to the column and separation is very poor. So to avoid this problem, the column should be packed with neutral alumina. The pure product yield varies from 45-85 %, depending upon the substituent present in the benzene as well as imidazolinone ring.

Here, the synthesis of oxazolone and imidazolin-5-one were performed by master and doctoral level students respectively. Some of the synthesized and characterized oxazolone and imidazolon-5-one are given in figure SM 4.2.3.10.1.The reaction condition, M.P., R_f values and ^{1}H-NMR spectra are collected from literature[3-6] and are given below, which will be helpful in the synthesis.

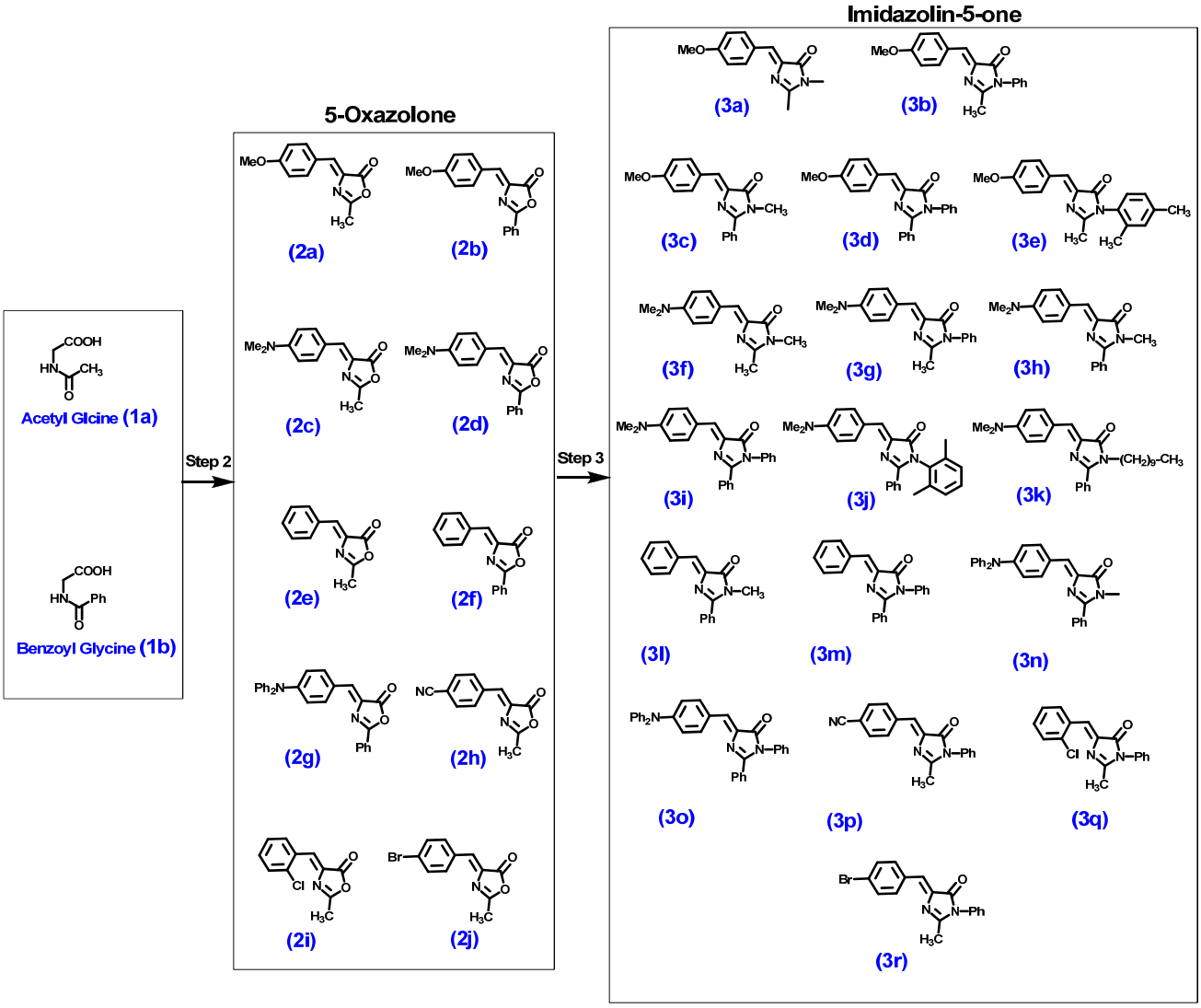


Figure SM 4.2.3.10.1: Some examples of synthesized oxazolone and imidazolin-5-one

Compound No.	Reaction Temperature(°C)	Reaction Time(hours)	Yield (%)	R_f values (In EtOAc : Pet ether)	M.P. (°C)
Oxazolone compounds					
2a	90-110	2	55-65	0.5 (20% EtoAc)	85-87
2b	130-150	2	55-65	0.5 (20% EtoAc)	150-152
2c	90-110	2	65-75	0.5 (25% EtoAc)	159-160
2d	150-170	2	65-75	0.5 (25% EtoAc)	198-200
2e	90-110	2	55-65	0.5 (20% EtoAc)	150-151
2f	130-150	2	60-65	0.5 (20% EtoAc)	165-167
2g	150-160	2	68-75	0.5 (20% EtoAc)	168-170
2h	120-140	2	50-60	0.5 (30% EtoAc)	170-172
2i	90-110	2	50-60	0.5 (25% EtoAc)	159-160
2j	90-110	2	52-60	0.5 (20% EtoAc)	184-186
Imidazolin-5-one compounds					
3a	110-120	1.5	60-70	0.5 (30% EtoAc)	128-129
3b	135-145	1.5	55-60	0.5 (20% EtoAc)	131-132
3c	155-165	2	70-78	0.5 (20% EtoAc)	133-135
3d	140-160	1.5	65-70	0.5 (20% EtoAc)	154-155
3e	90-110	1.5	80-85	0.5 (20% EtoAc)	96-98
3f	140-160	2	55-60	0.5 (30% EtoAc)	198-200
3g	140-160	1.5	80-88	0.5 (20% EtoAc)	159-161
3h	165-170	2	65-70	0.5 (20% EtoAc)	162-163
3i	165-170	2	65-70	0.5 (30% EtoAc)	198-200
3j	165-170	2	60-65	0.5 (30% EtoAc)	169-170
3k	165-170	2	65-75	0.5 (20% EtoAc)	70-72
3l	140-160	1.5	70-75	0.5 (20% EtoAc)	124-126
3m	140-160	1.5	58-65	0.5 (20% EtoAc)	162-163
3n	165-170	2	57-65	0.5 (30% EtoAc)	187-189
3o	165-170	2	55-62	0.5 (20% EtoAc)	216-218
3p	80-110	1.5	80-88	0.5 (20% EtoAc)	197-198
3q	80-110	1.5	75-80	0.5 (20% EtoAc)	181-182
3r	90	2	45-55	0.5 (20% EtoAc)	121-123

Mechanism of Oxazolone Synthesis

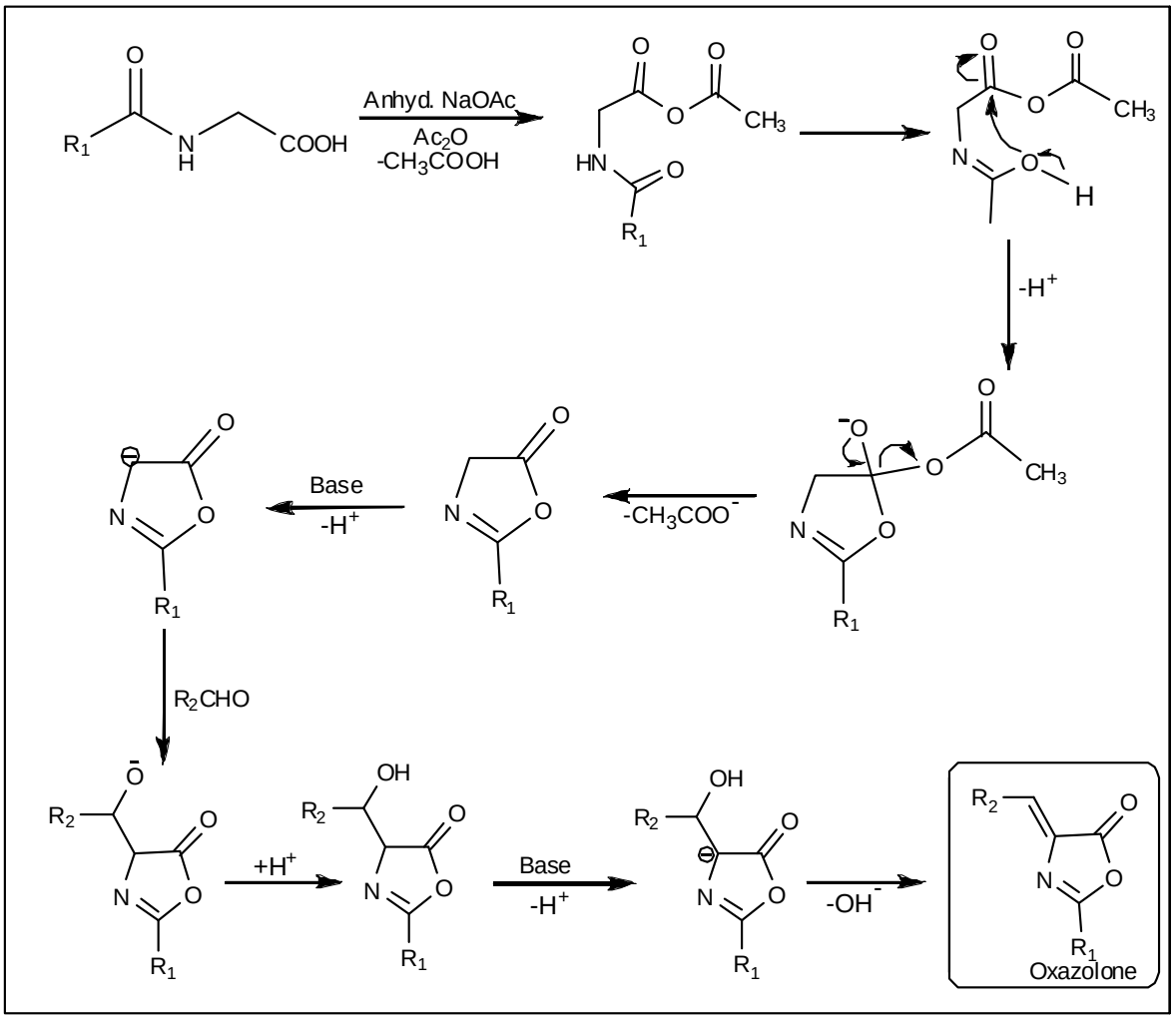


Figure SM 4.2.3.10.2: Proposed mechanism of oxazolone synthesis

Mechanism of Imidazolin-5-one Synthesis

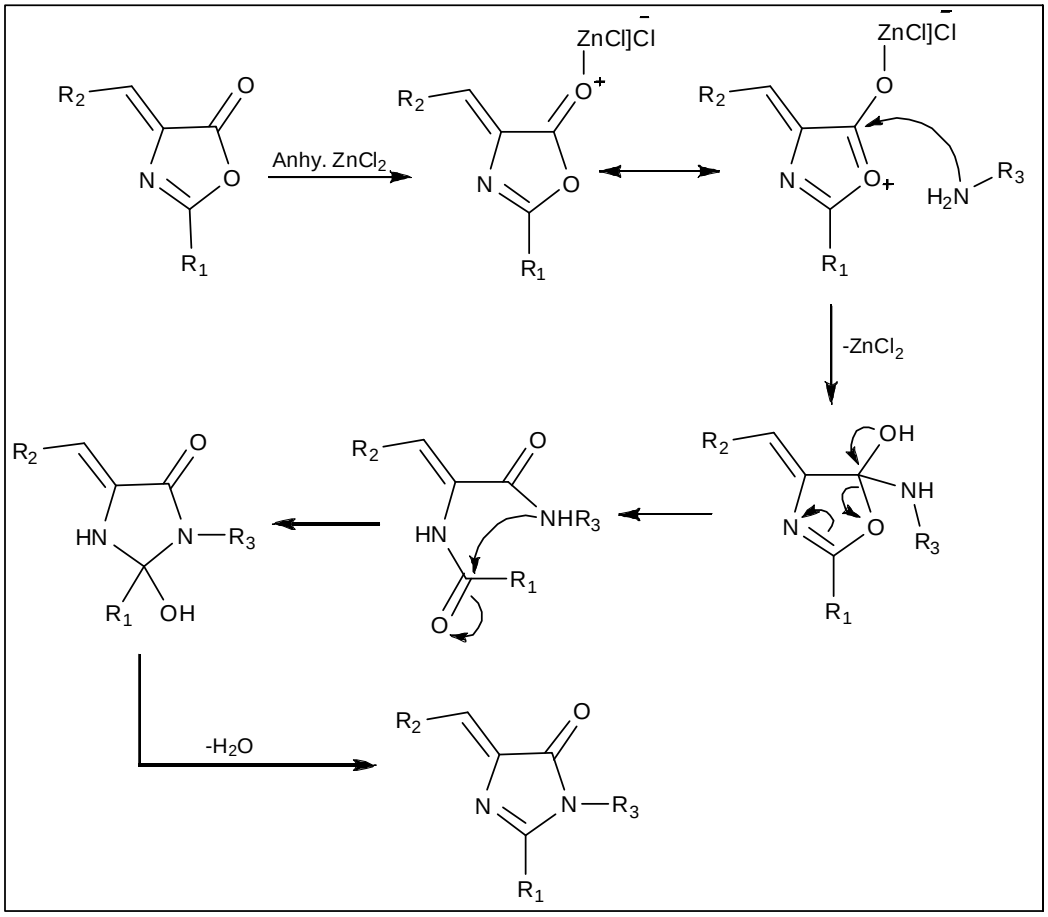


Figure SM 4.2.3.10.3: Proposed mechanism of imidazolin-5-one synthesis

Side reaction during imidazolinone synthesis in presence of unreacted aldehyde

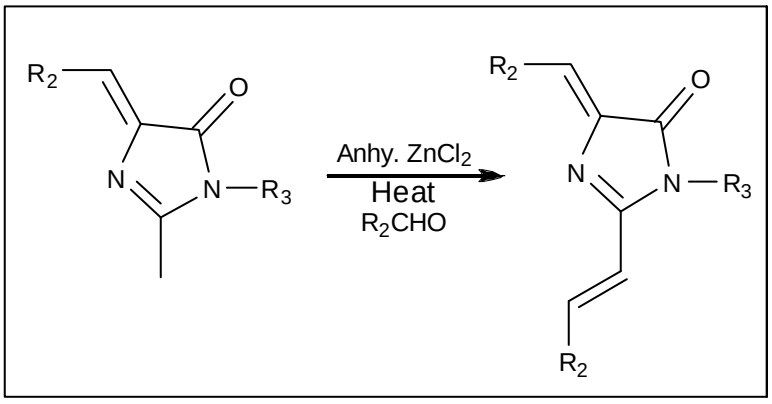


Figure SM 4.2.3.10.4: Side reaction in step 3

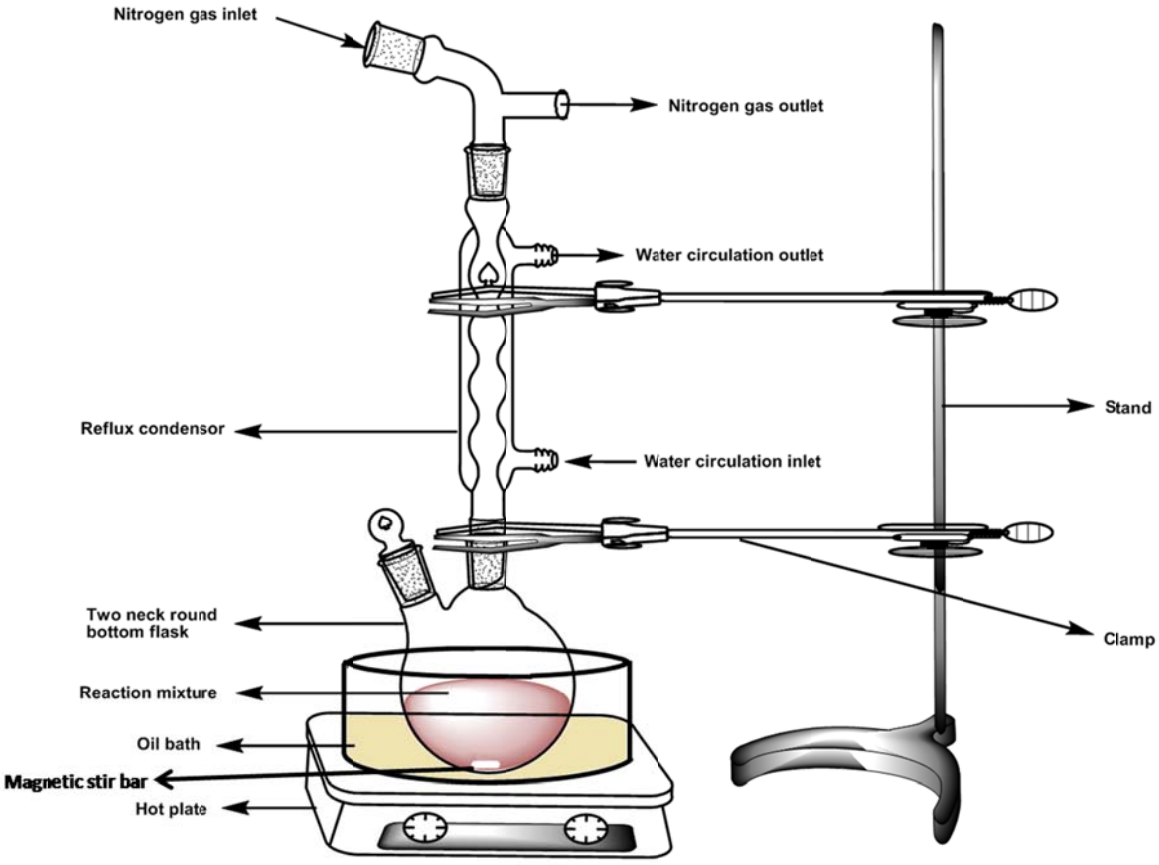


Figure SM 4.2.3.10.5: Reaction setup for synthesis of imidazolin-5-one

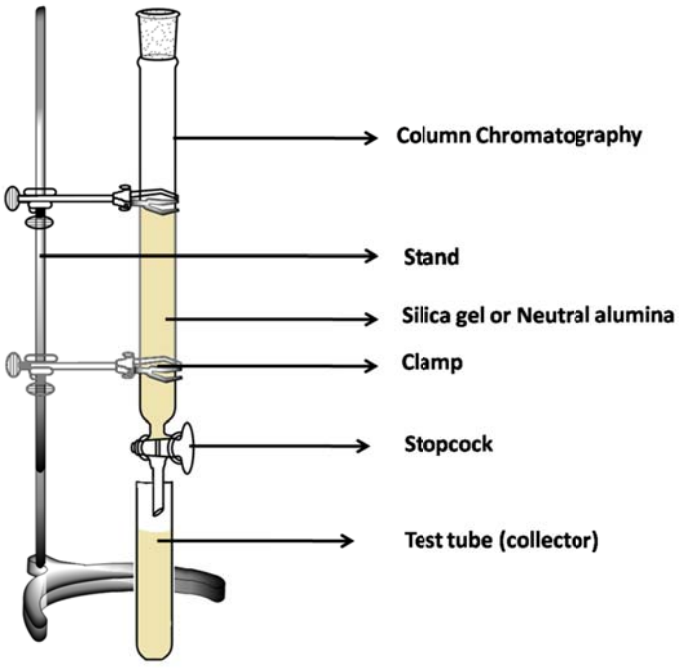
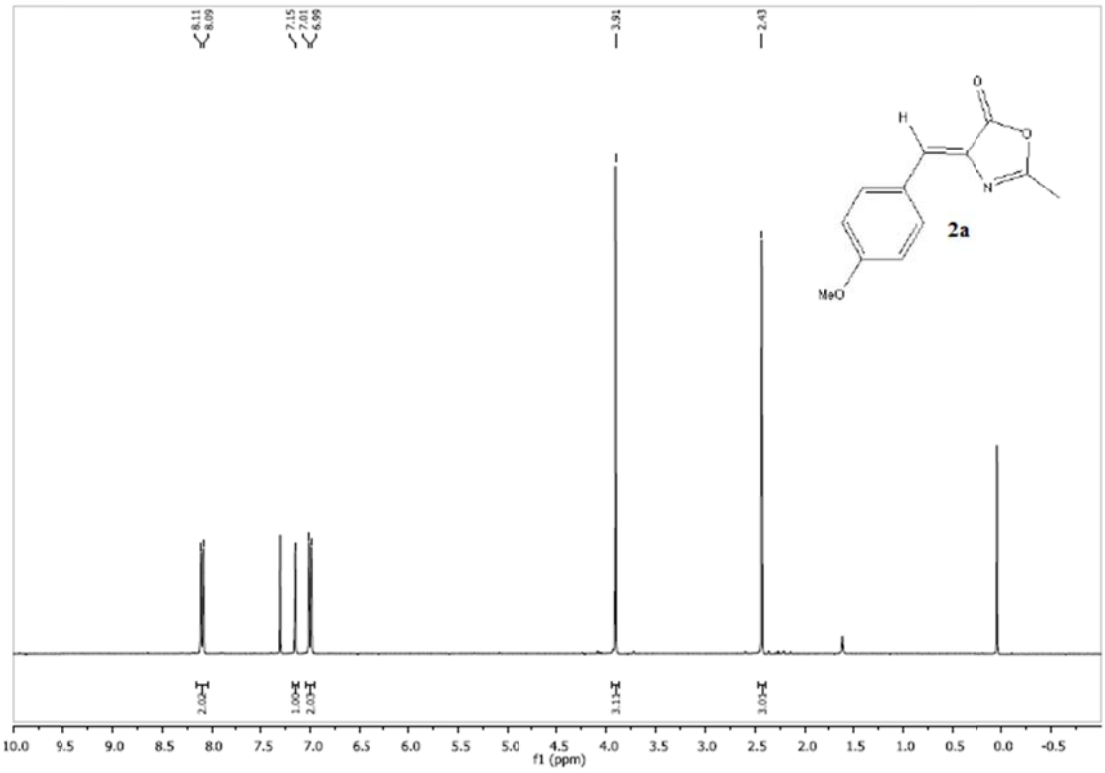
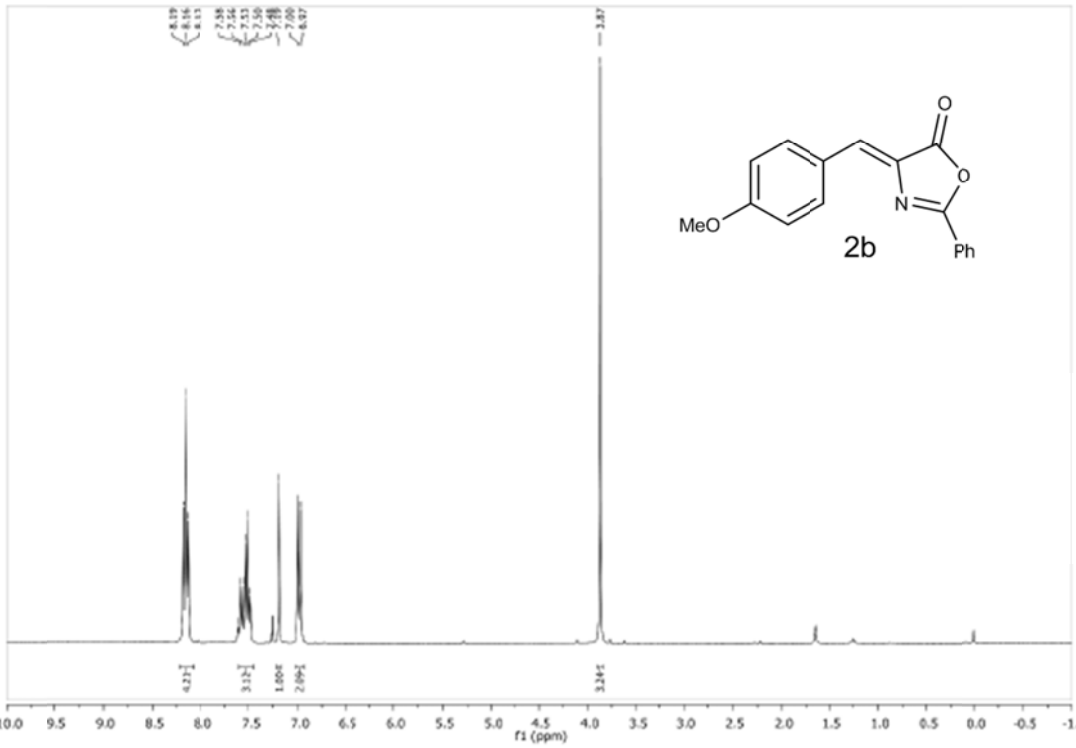
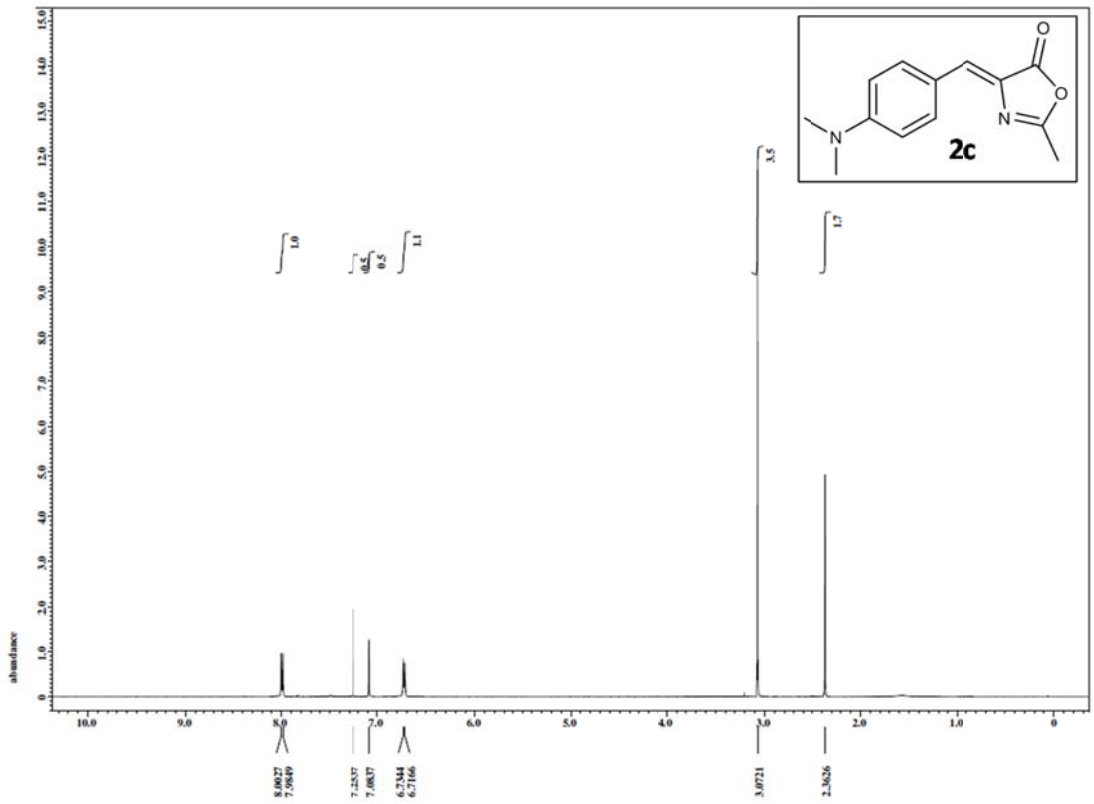


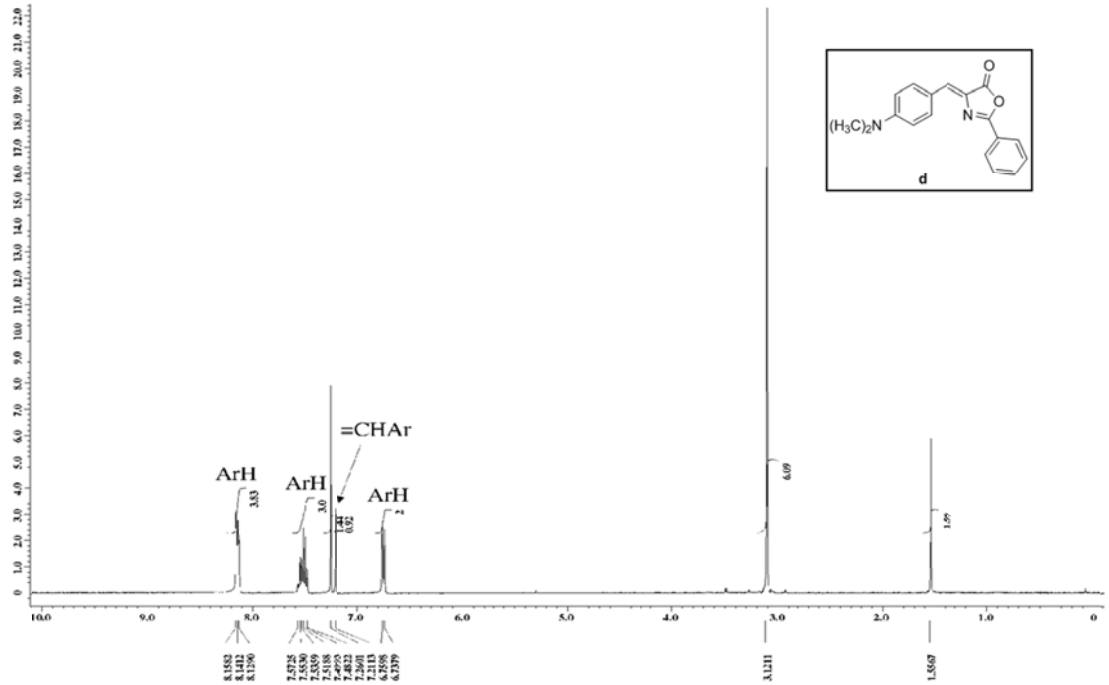
Figure SM 4.2.3.10.6: A picture of compound purification by Column Chromatography

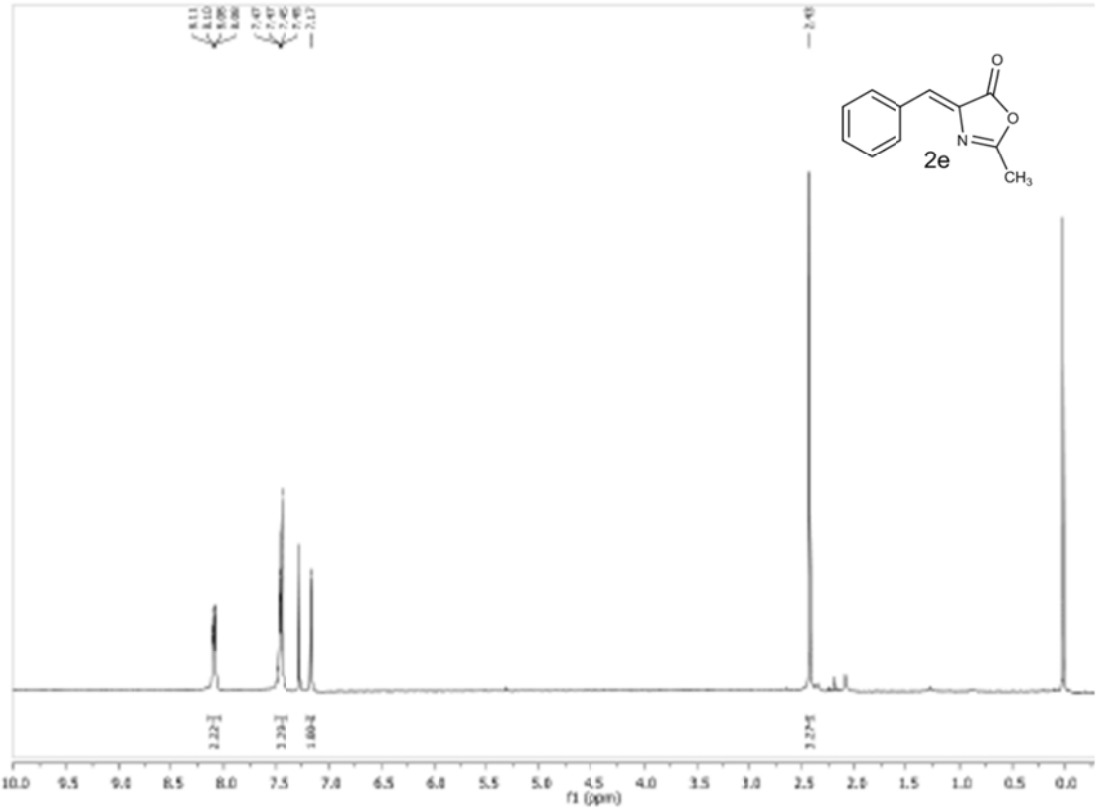
^{1}HNMR- spectra of 5-Oxazolone compounds (2a-2j)

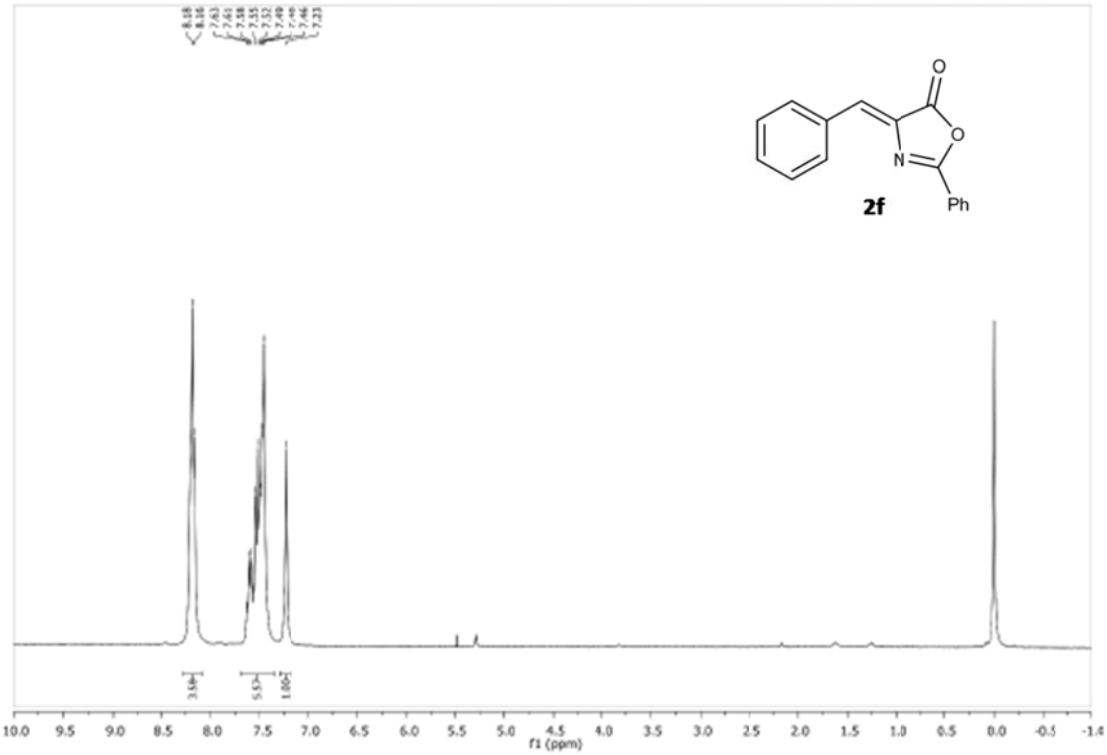


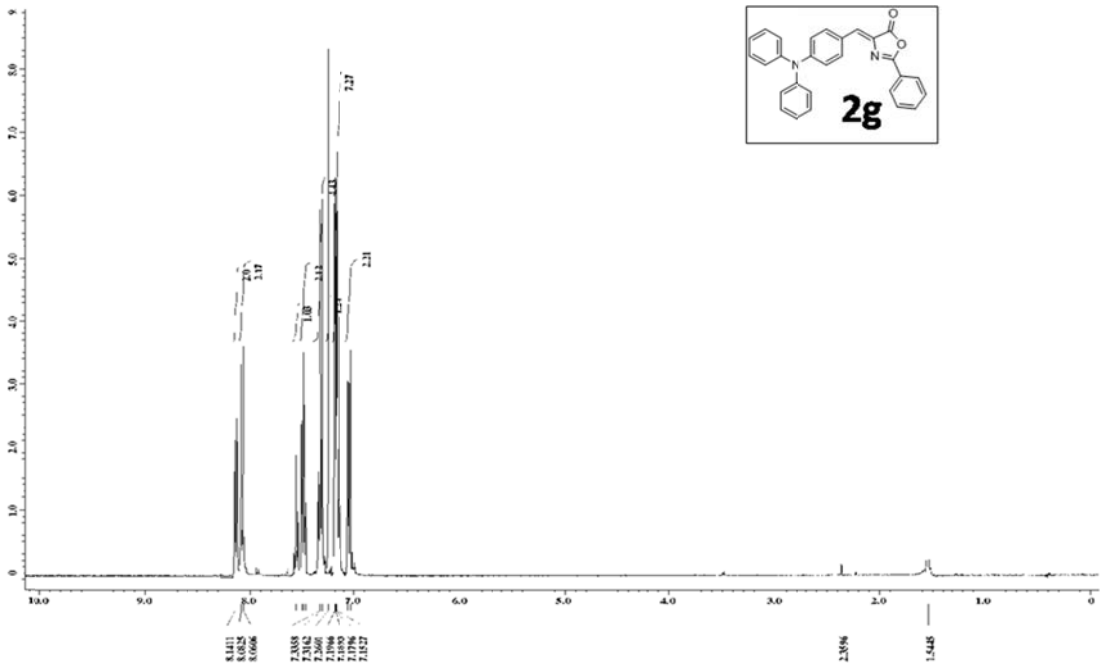


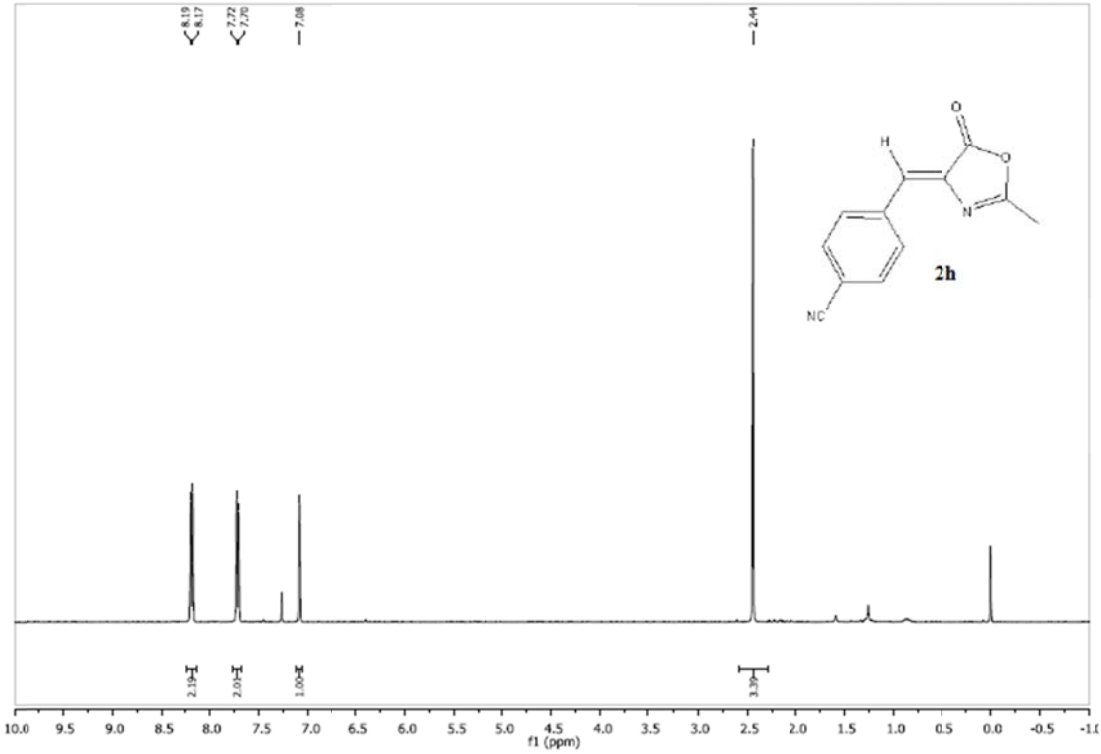


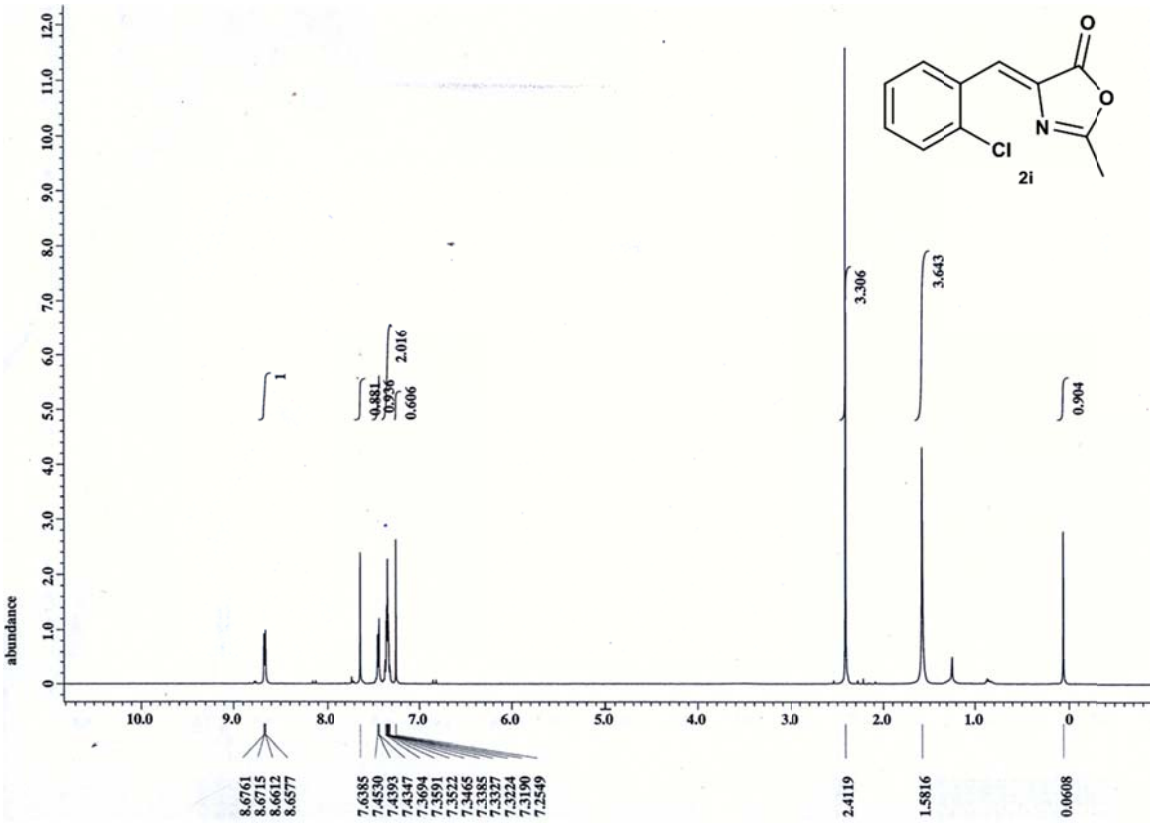


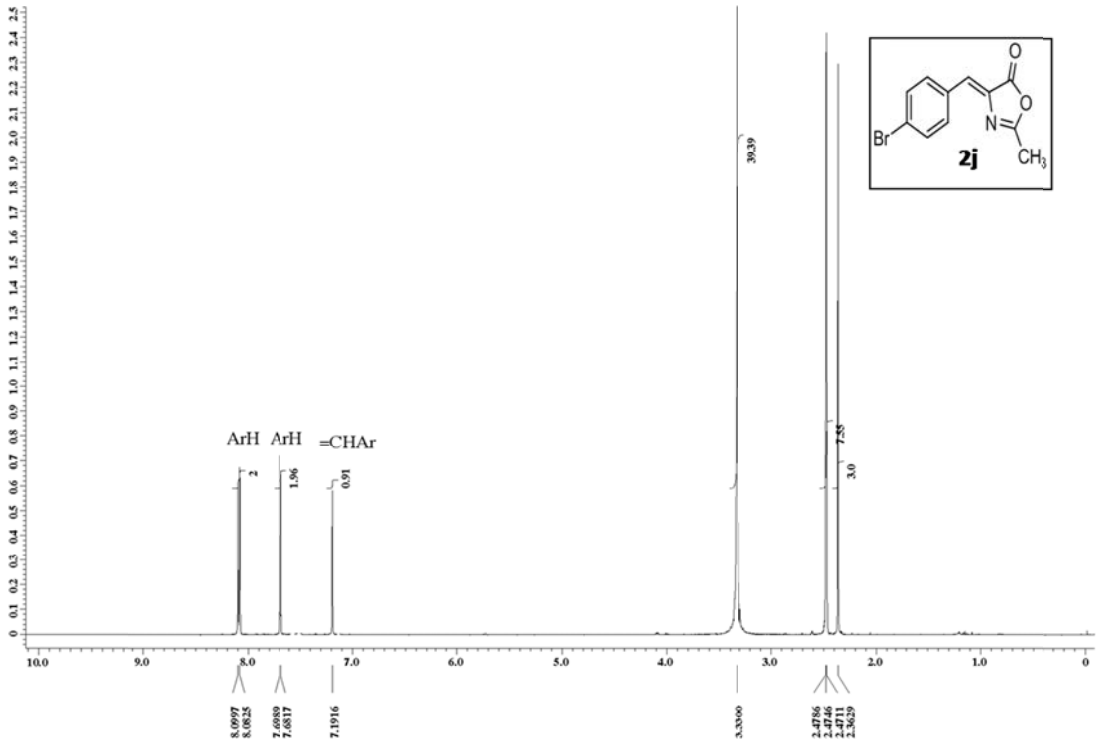




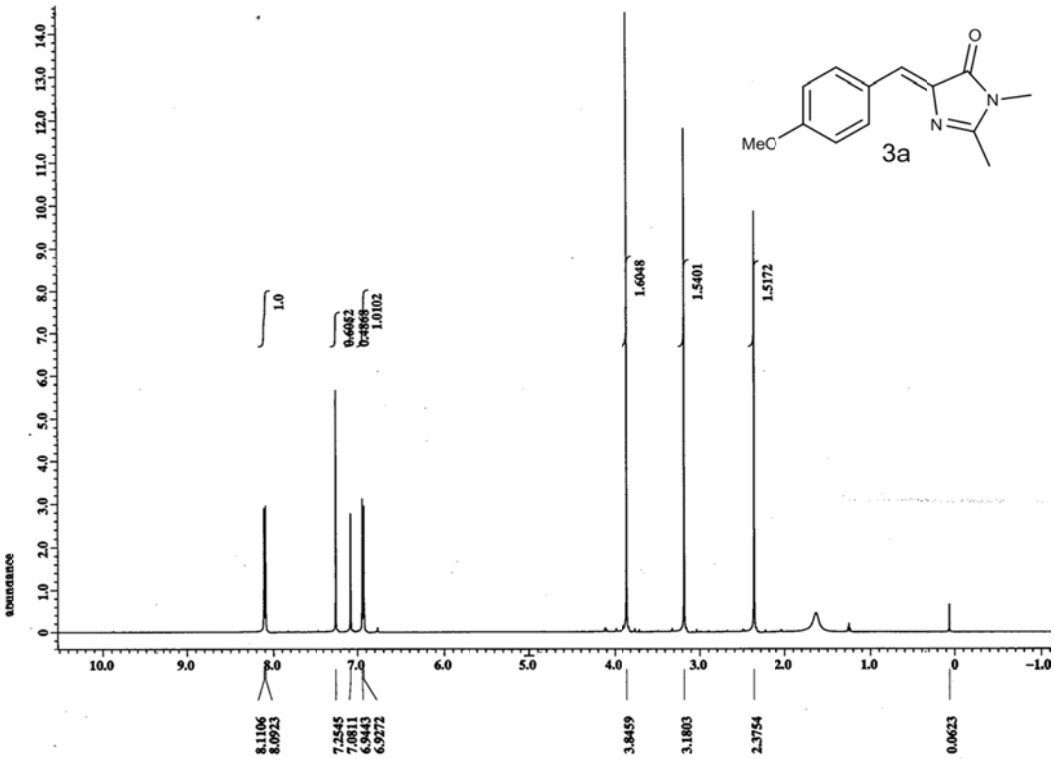


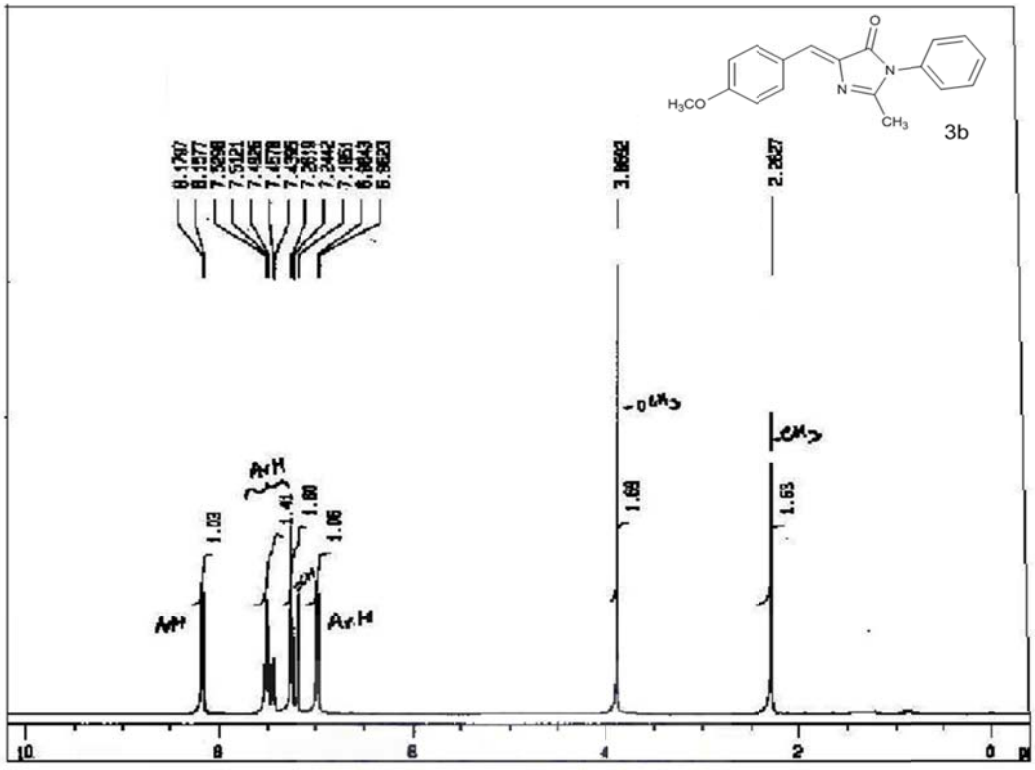


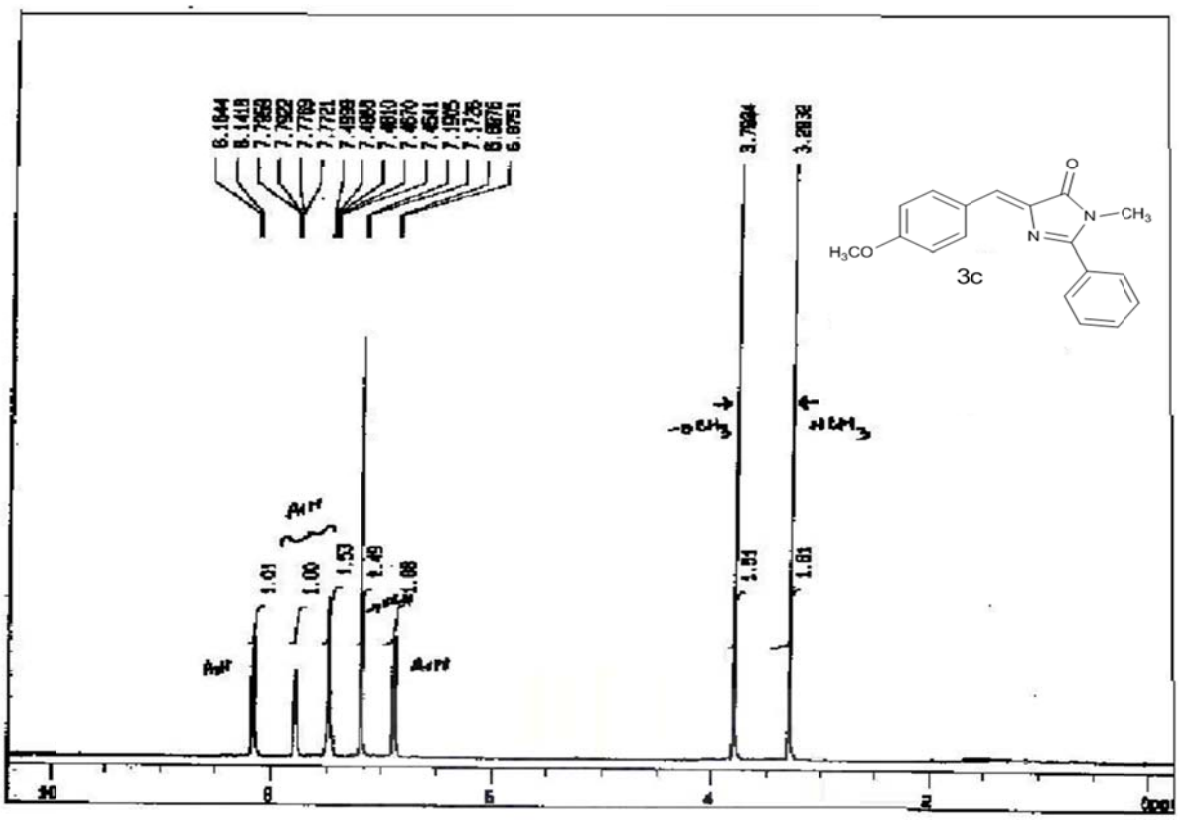


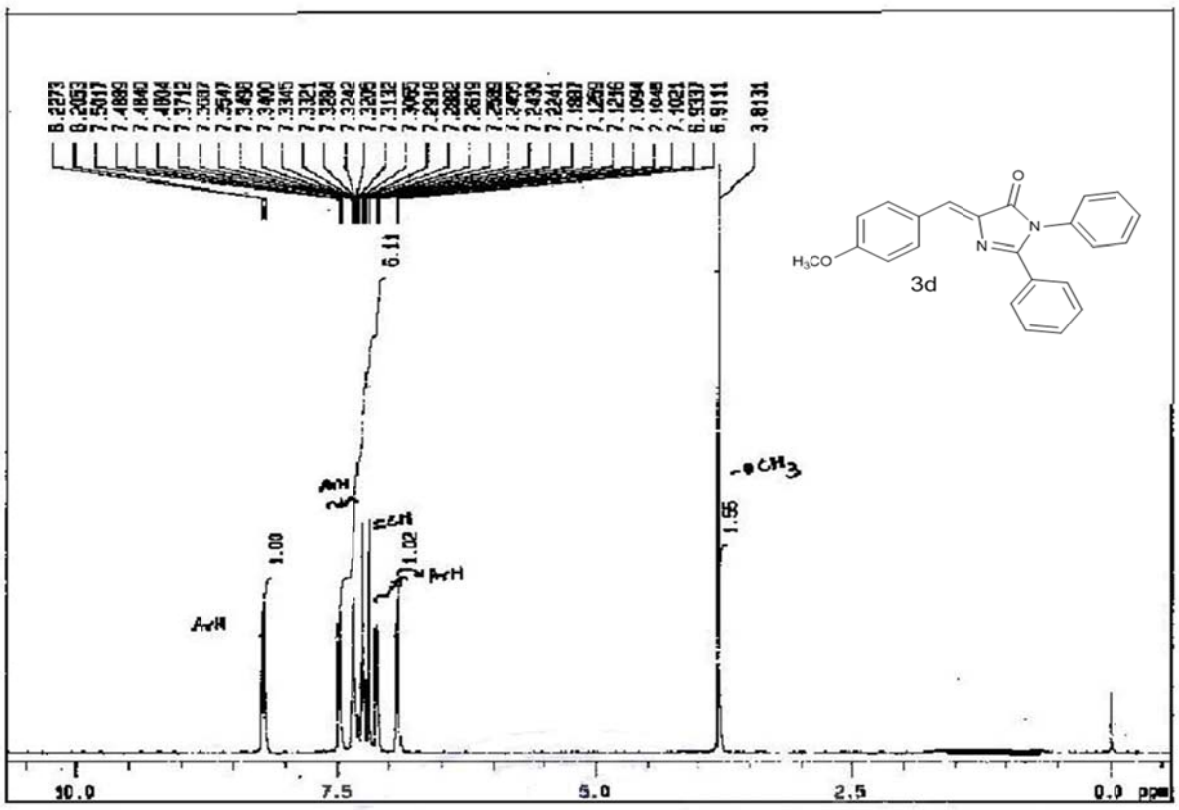


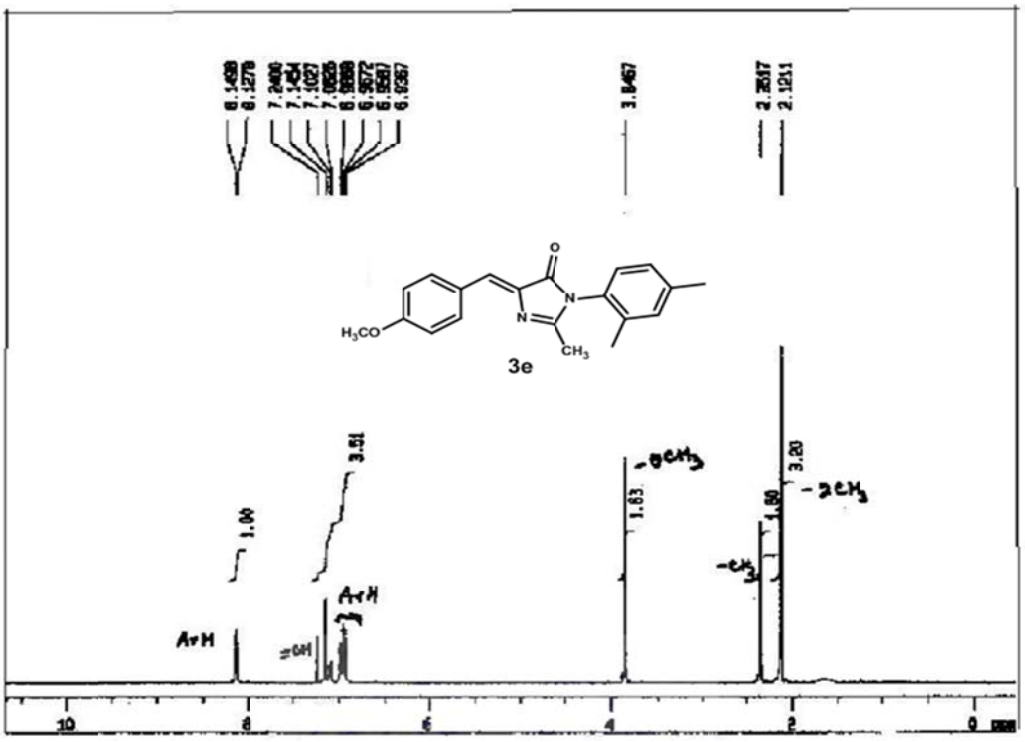
^{1}HNMR- spectra of Imidazolin-5-one compounds (3a-3r)

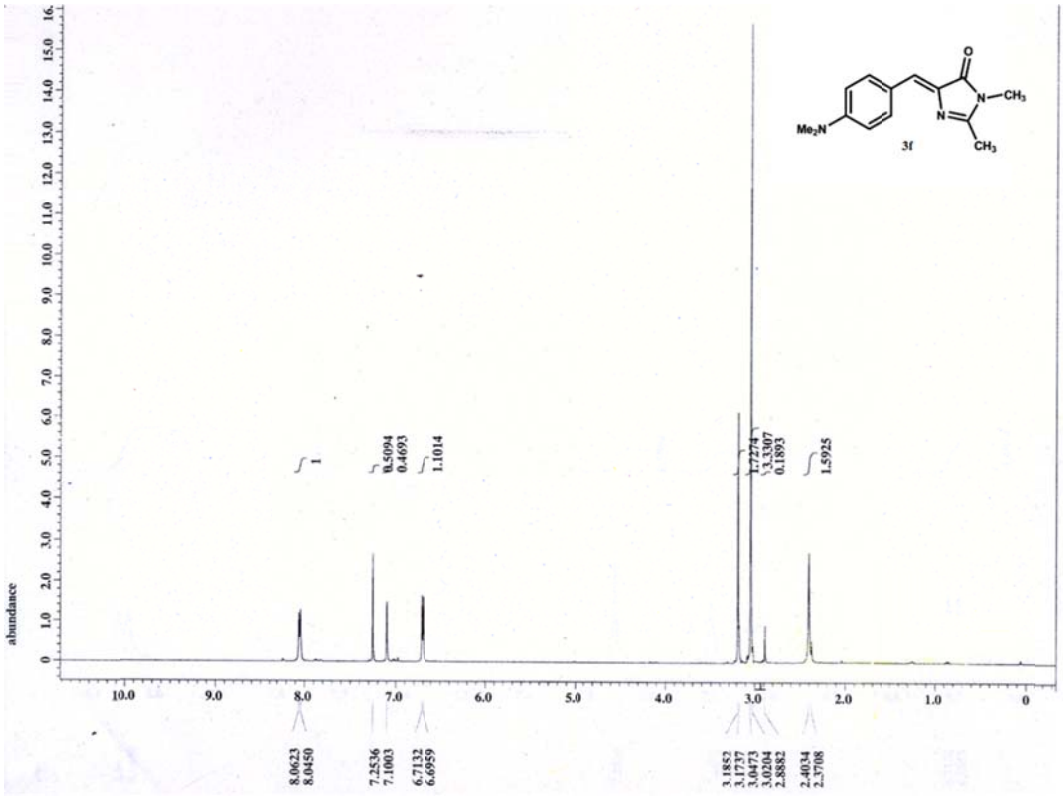


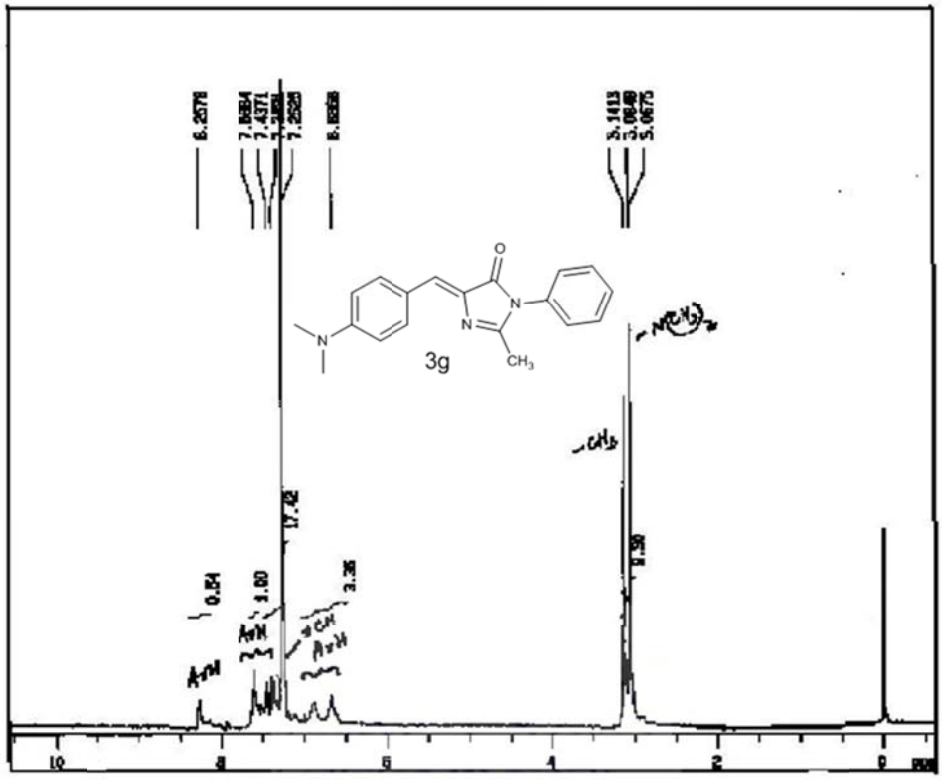


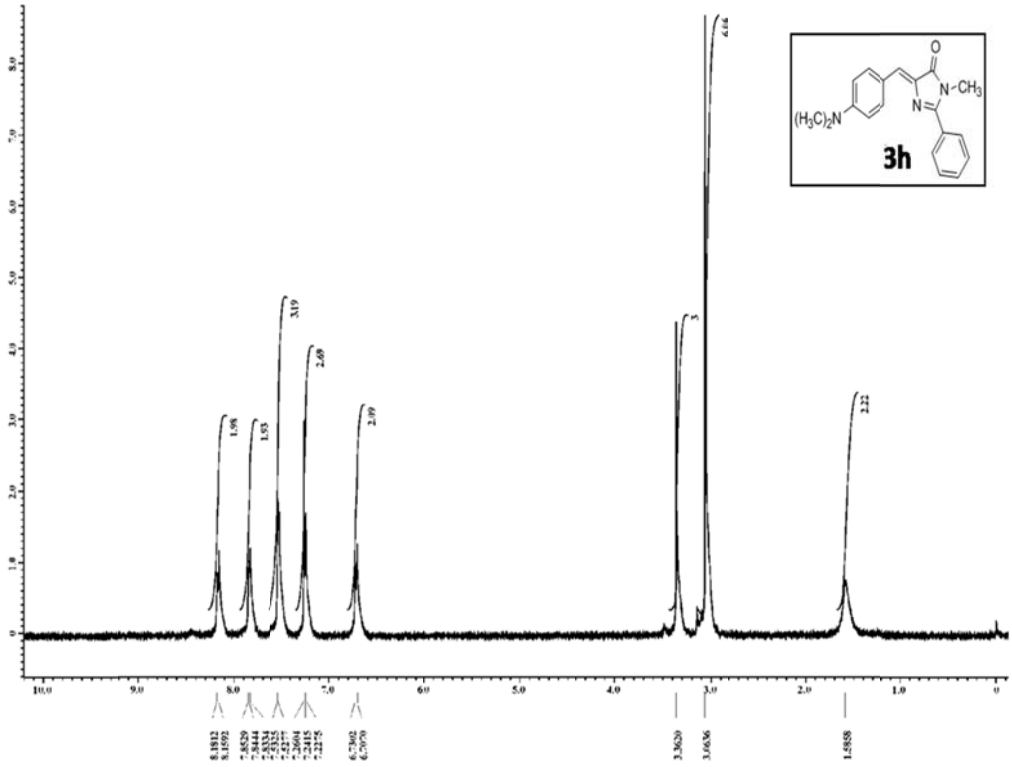


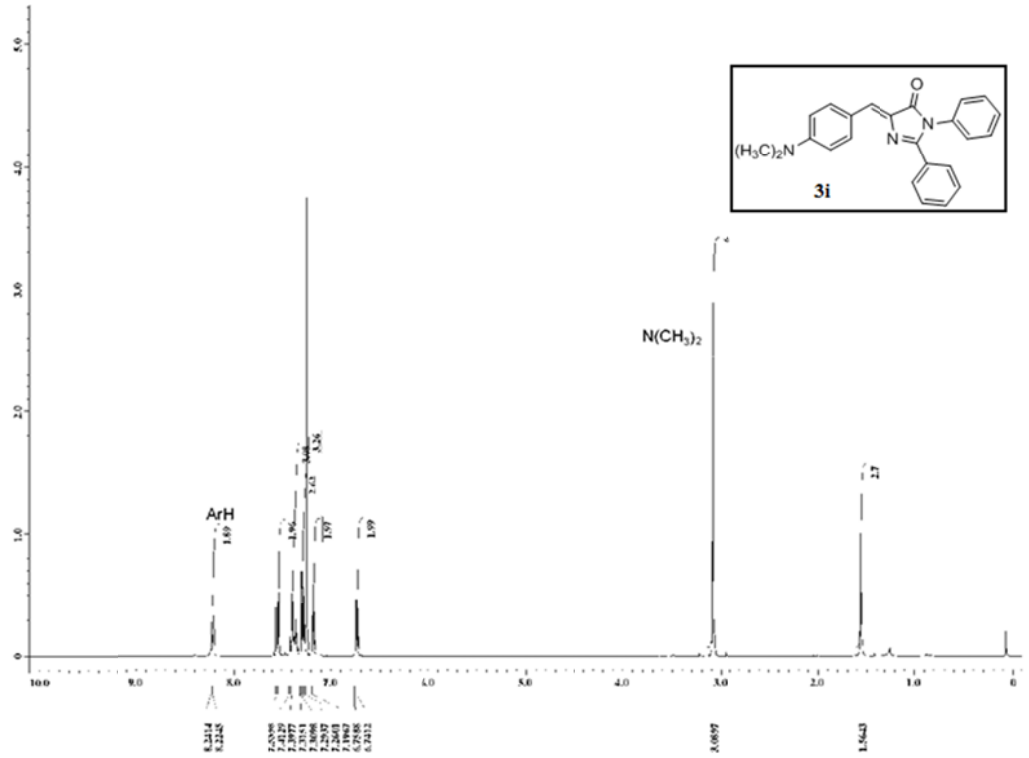


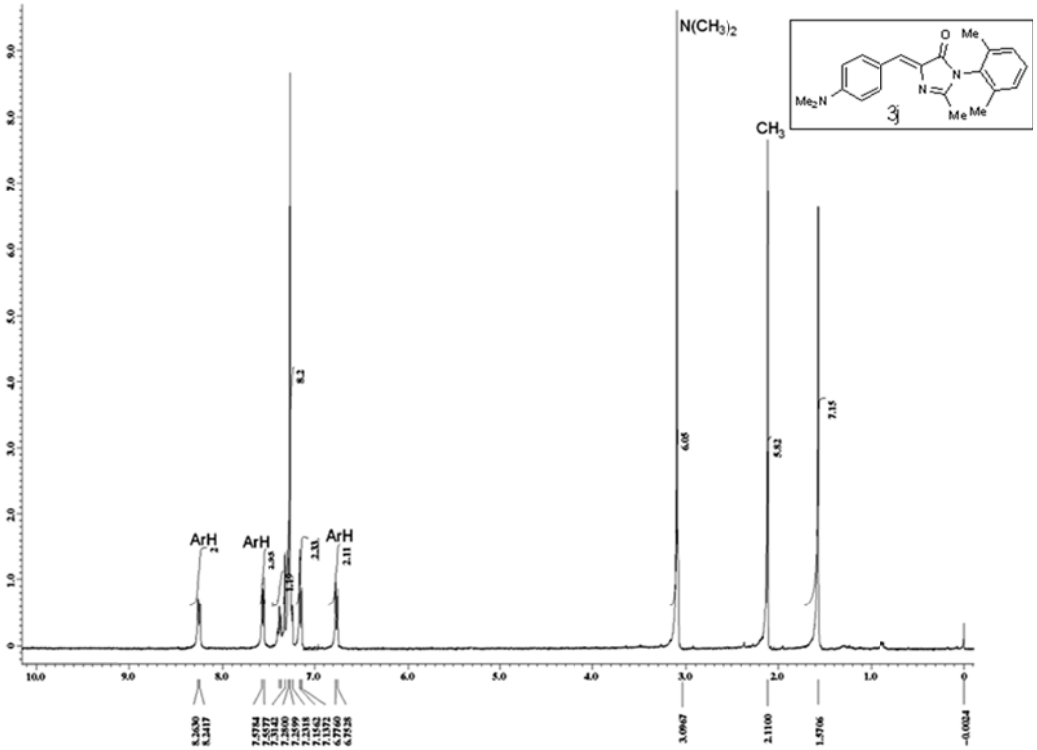


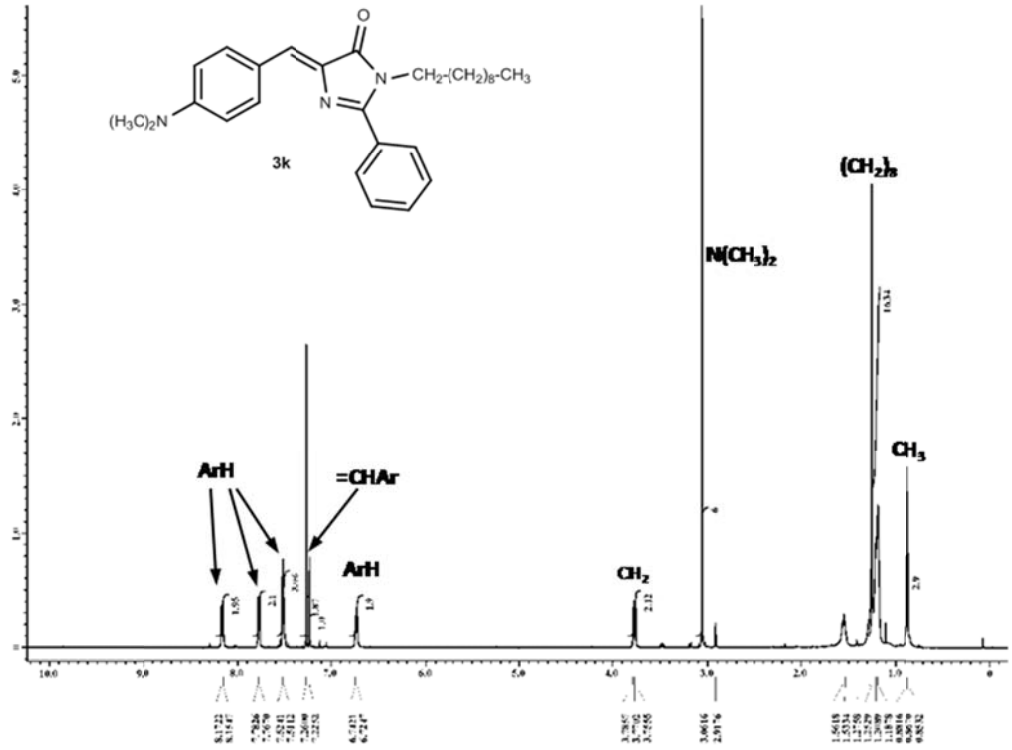


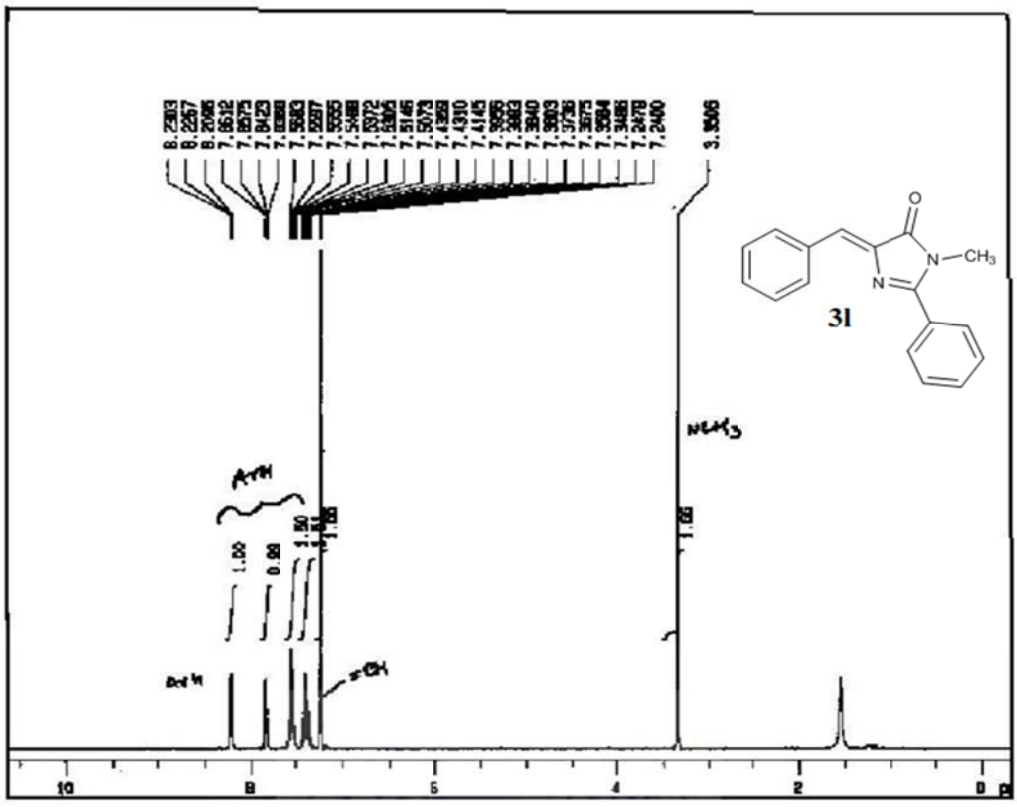


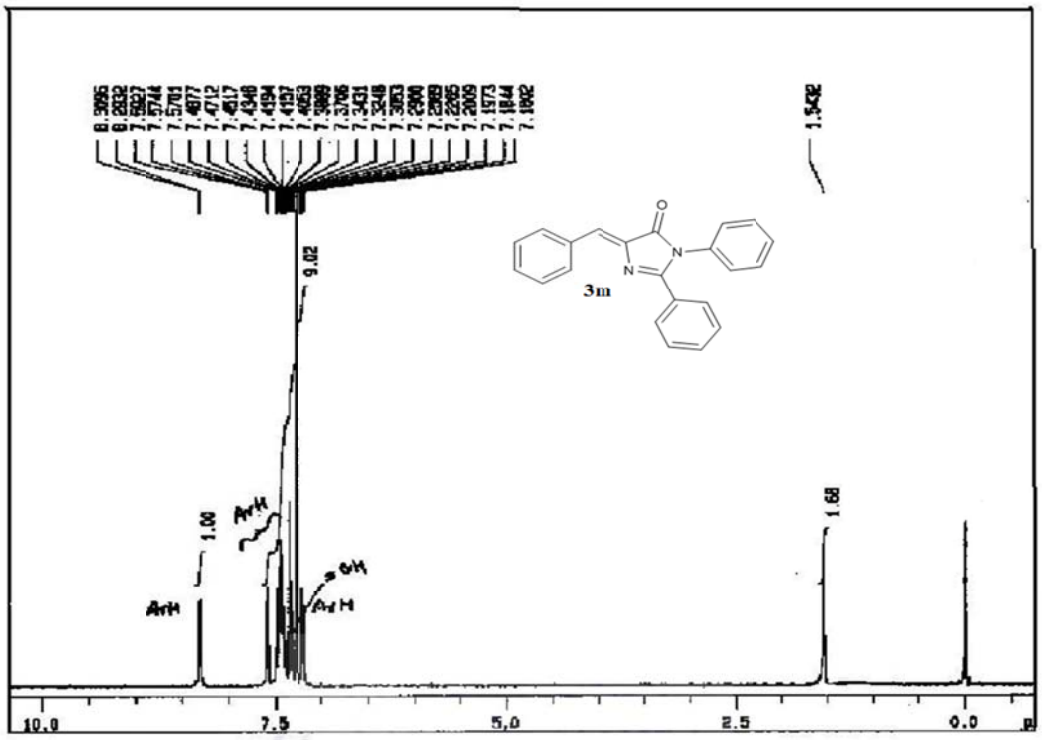


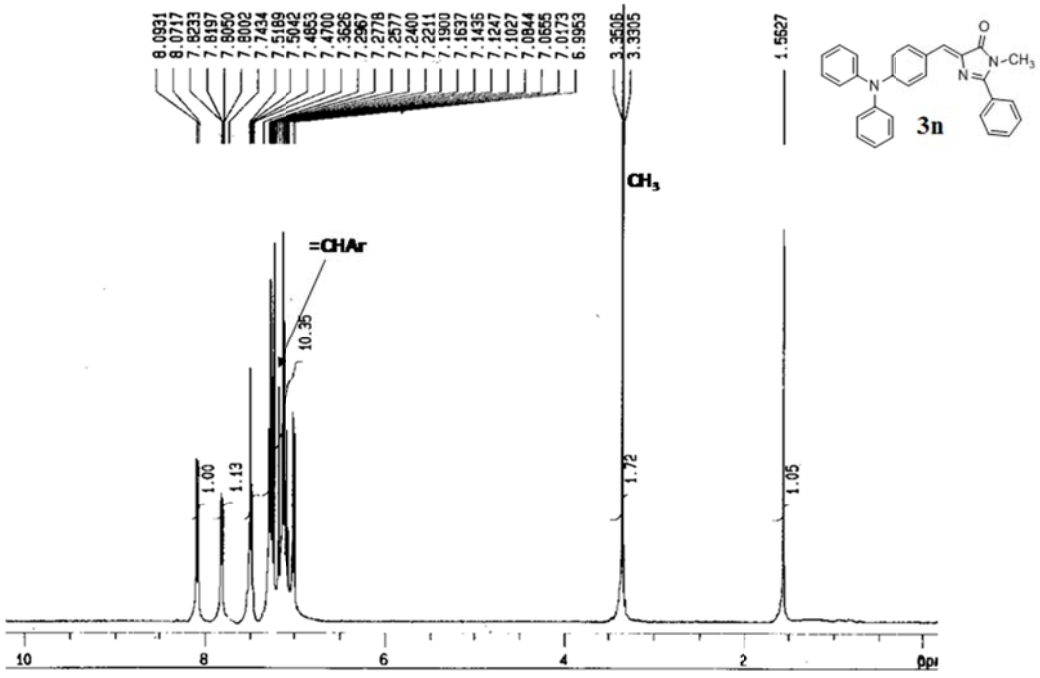


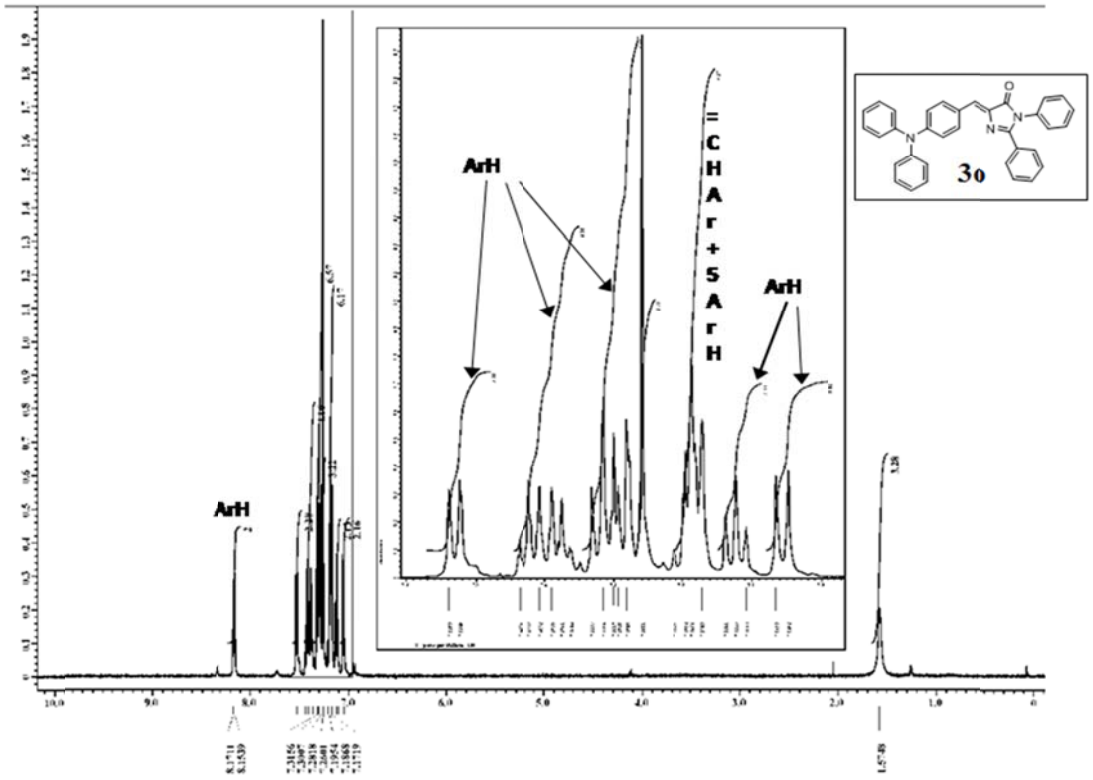


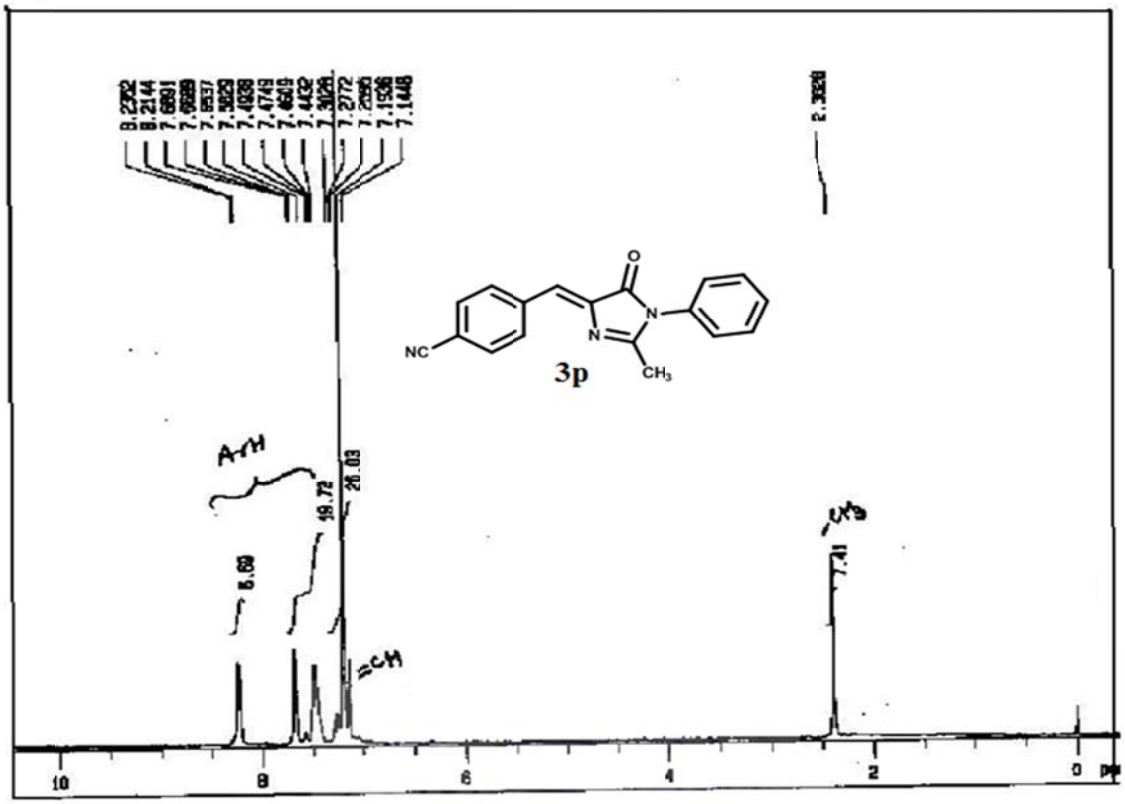


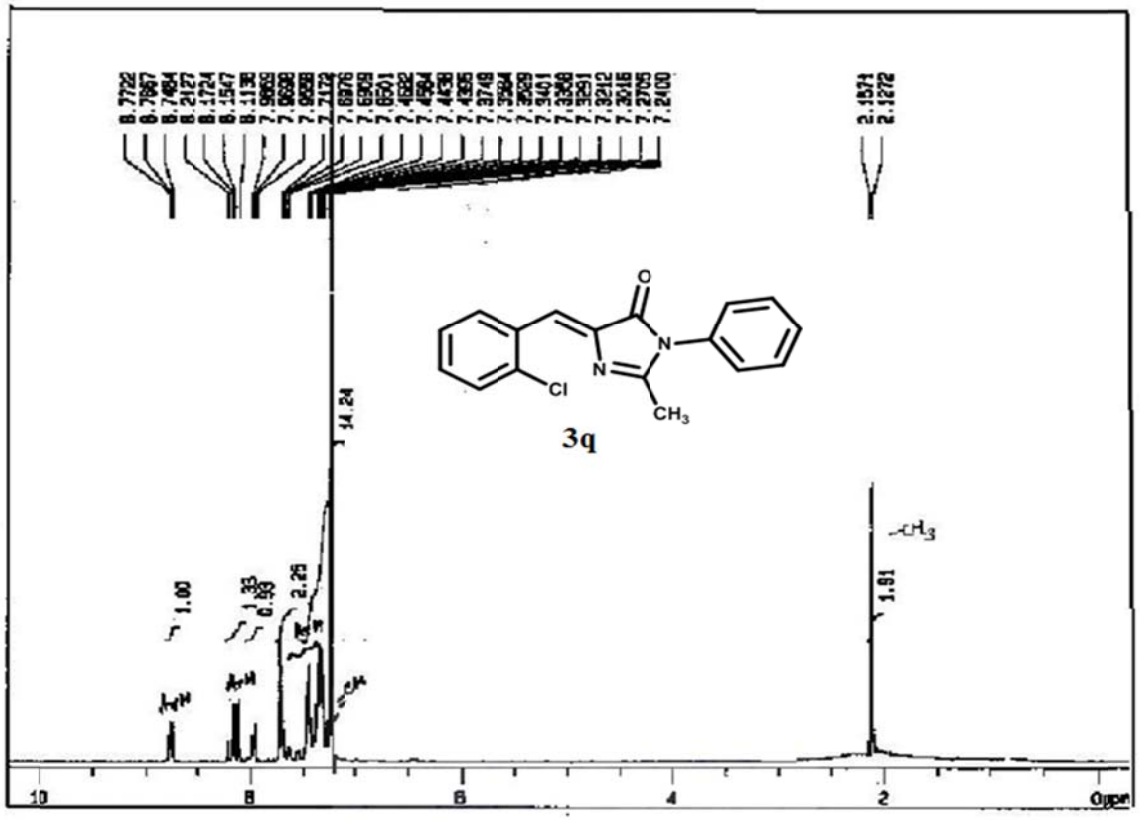


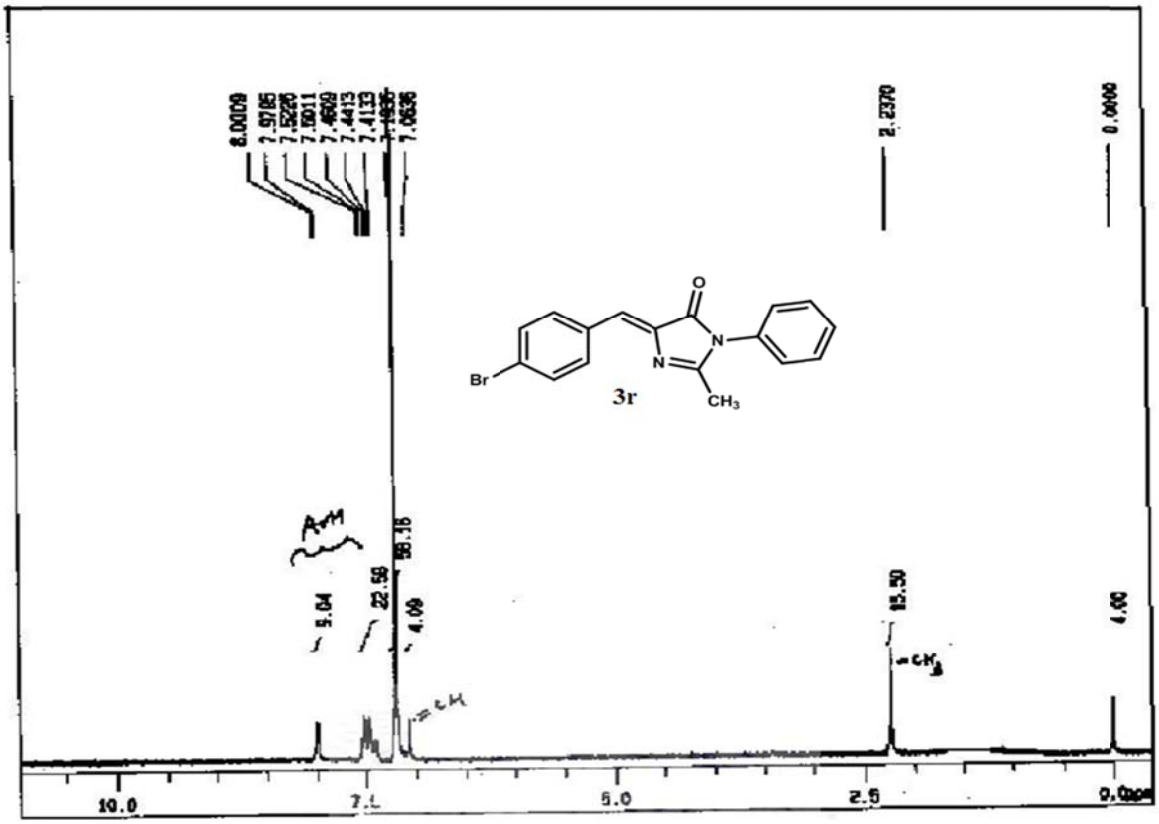












Supplementary information for *Comprehensive Organic Chemistry Experiments for the Laboratory Classroom*

References:

1. V. Taile, K. Hatzade, P. Gaidhane, and V. Ingle, *Turkish Journal of Chemistry*, 2009, **33**, 295.
2. S. Bala, M. Saini and S. Kamboj, *Int.J. ChemTech Res*., 2011, **3**(3), 1102.
3. M. Blanco-Lomas, I. Funes-Ardoiz, P. J. Campos, and D.Sampedro, *Eur. J. Org. Chem.* 2013, 6611.
4. M. Blanco-Lomas, P. J. Campos, and D.Sampedro**,** *Org. Lett*., 2012, **14**, No. 17, 4334.
5. Gitalee Bhattacharya, Ph.D Thesis "From Molecular to Material: Design, Synthesis, Characterization and Possible Application of Imidazolin-5-ones", 2006, Indian Institute of Technology, Kanpur INDIA-208016.
6. Basanta Kumar Rajbongshi, Ph.D Thesis "Photophysical, Crystallographic and Photovoltaic Studies on Imidazolin-5-ones", 2011, Indian Institute of Technology, Kanpur INDIA-208016.
7. G. Bhattacharjya, S. S. Agasti and G. Ramanathan 2006 *ARKIVOC* **x** 152.

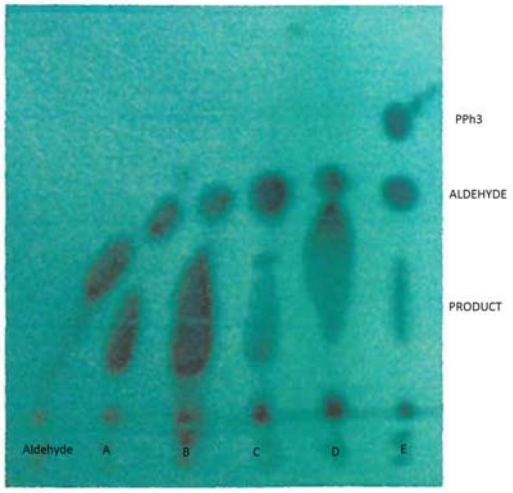
Baylis-Hillman reaction between 4-nitrobenzaldehyde and ethyl acrylate

Supplementary Material

In this experiment is explored a carbon-carbon bond forming reaction between acrylates and aromatic aldehydes, named Baylis-Hillman reaction. This reaction complements well the traditional aldol reactions via enolate chemistry, as in the Baylis-Hillman reaction the same type of nucleophile is formed in situ using a Lewis Base - DABCO. This experiment has been carried on by Bachelor/Master degree students (about 15 students) divided in groups of two. However, due to the simple execution this experiment can also be done by a solo student.

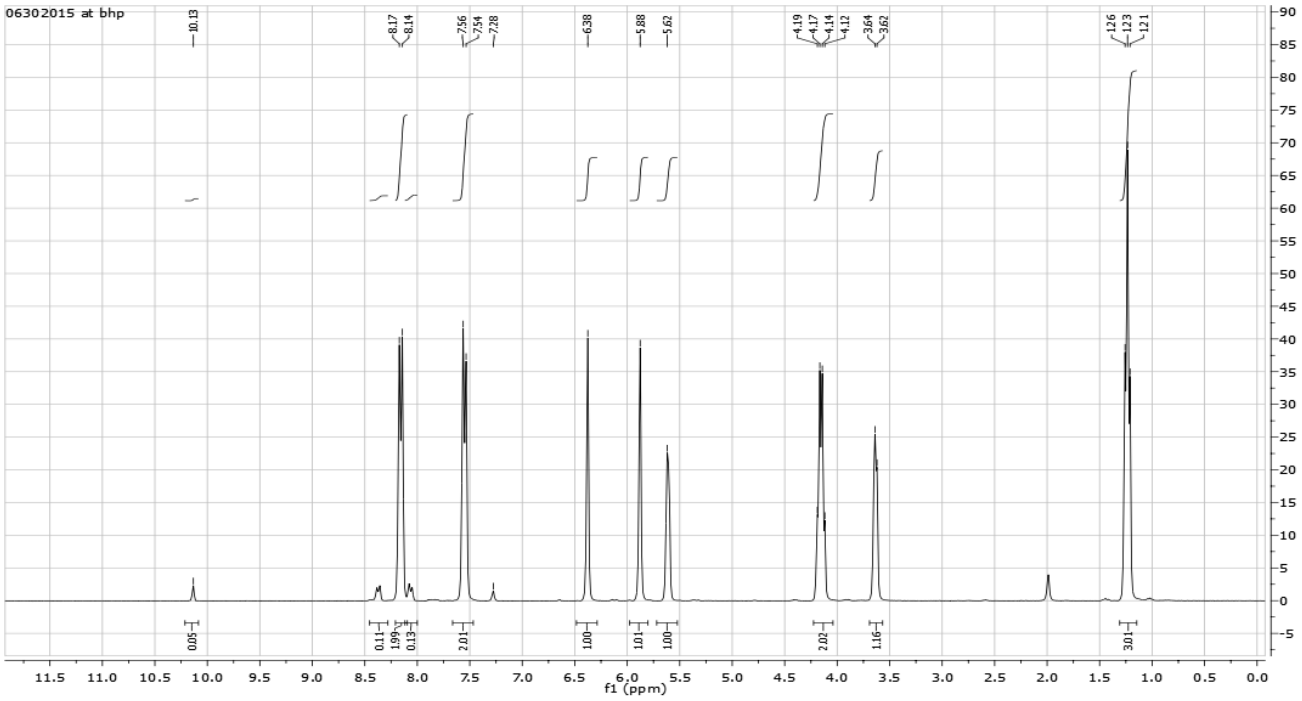
The experimental conditions employed herein using one equivalent of DABCO and mixture of 1,4-dioxane/water were chosen to fit this experiment in typical laboratorial sessions of 4 hours, since the reaction present faster kinetics and is concluded in up to 3 hours. If the reaction is undergone in absence of solvent it takes about one week to reach completion. Another possibility that was tested successfully with students was the evaluation of potential Lewis Base promoters (DABCO 1 eq., DBU 1 eq., DMAP 1eq. and triphenylphosphine 1 eq.). The students evaluated the promoter efficiency by TLC in dichloromethane after 2 hours. The students can comprehend the effect of increasing DABCO, and verify that DBU, DMAP and triphenyl phosphine are poorer promoters of Baylis-Hillman reaction with acrylates.

Mass/Volumn	A	B	C	D	E
Aldehyde	0.754 g				
Ethyl acrylate	1.6 mL				
Promoter (1 mol eq.)	0.55 g (DABCO)	1.12 g (DABCO)	0.76 g (DBU)	0.6 g (DMAP)	1.3 g (PPh_3)
Dioxan (solv)	10 ml				
Water (solv)	10 ml				
Ether wash	20 ml		40 mL		
HCl 1M wash	20 ml		40 mL		
H_2O wash	20 ml		40 mL		

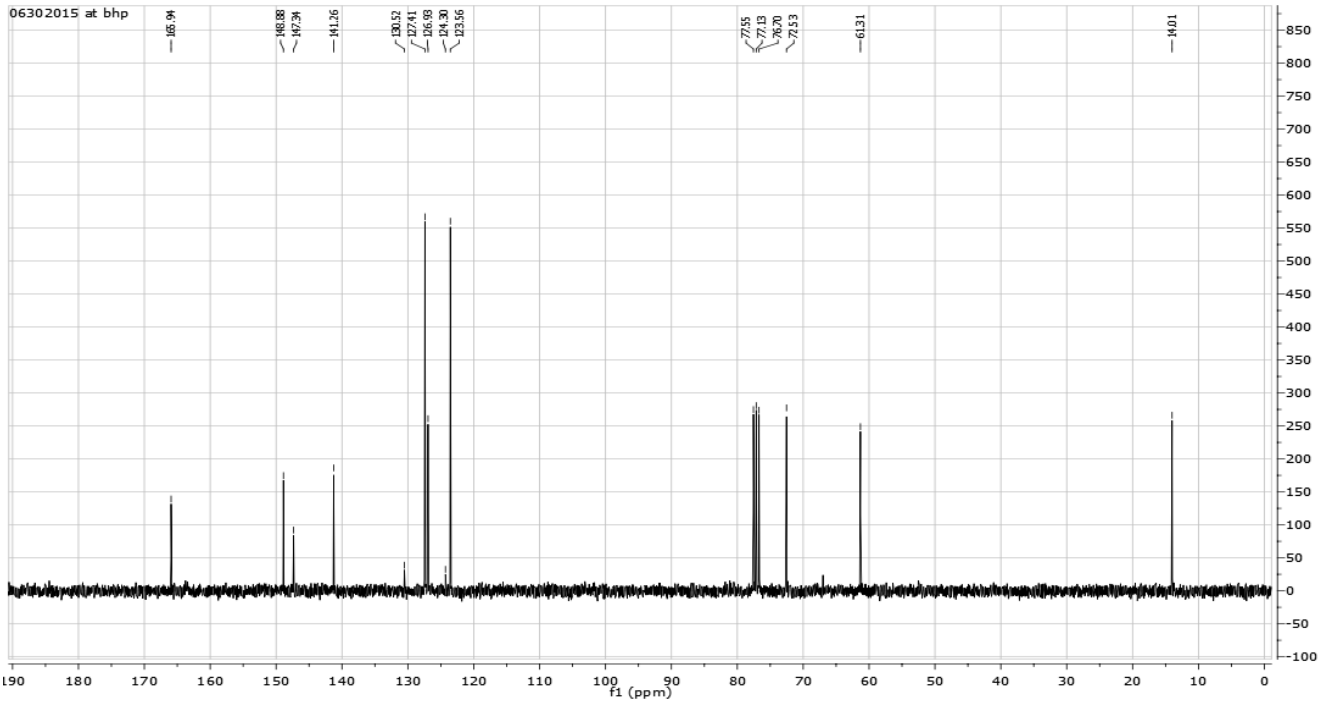


The students carry on this reaction without any problems and the lack of pictures of the experimental setup is due to the low level of difficulty regarding the techniques employed, as well as, due to the absence of alterations in the reaction visual aspect. Depending on how fast can the students prepare the reaction and do the work-up, the celite/silica gel filtration and/or solvent evaporated, plan for the second session can be anticipated. During the reaction period the students are encouraged to answer most of the questions from the short list of question

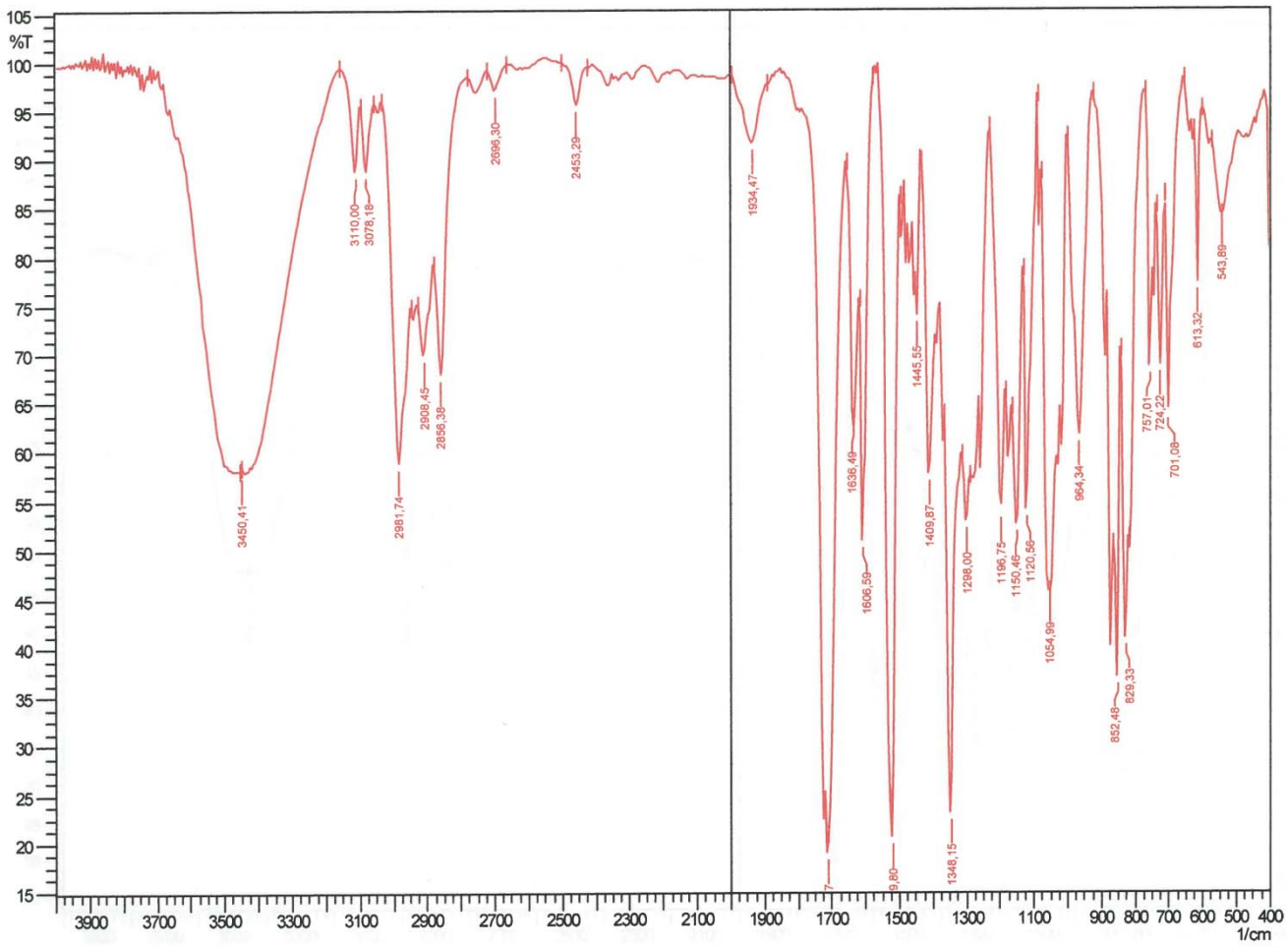
planned herein. Typically, the students can obtain yields around 72 % (up to over 100%). This is mainly due to the incomplete evaporation of ethyl acrylate from the reaction mixture in the rotatory evaporator. From our experience, at 40ºC and 30 mmHg should be possible to evaporate the solvent and ethyl acrylate within 15 mins. In the reaction with 4-nitrobenzaldehyde, the Baylis-Hillman product can be isolated with excellent purity, presenting only about 5% of unreacted aldehyde.



SM 4.2.3.11.1 – Proton NMR of the Baylis-Hillman product ethyl 2-(hydroxy(4-nitrophenyl)methyl)acrylate in deuterated chloroform



SM 4.2.3.11.2 – Carbon NMR of the Baylis-Hillman product ethyl 2-(hydroxy(4-nitrophenyl)methyl)acrylate in deuterated chloroform

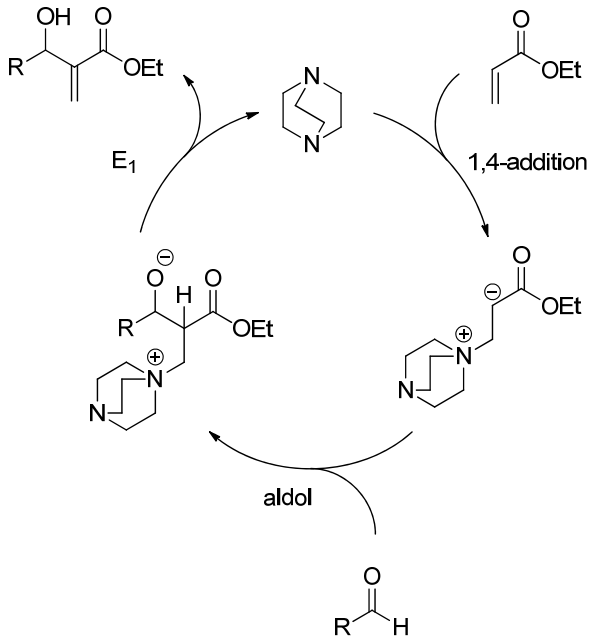


SM 4.2.3.11.3 – IR spectrum of ethyl the Baylis-Hillman product 2-(hydroxy(4-nitrophenyl)methyl)acrylate from reaction A

Hints for the questions:

1 – The students should be able to analyze the proton and carbon NMR to confirm the structure of the product. IR spectrum can also be used to identify the formation of the hydroxyl group. Proton NMR should be used to compare the purity of the desired products, relatively to possible known impurities (ethyl acrylate, dioxane, ether, H_2O, and aldehyde). With this information they should provide a corrected yield, if applicable.

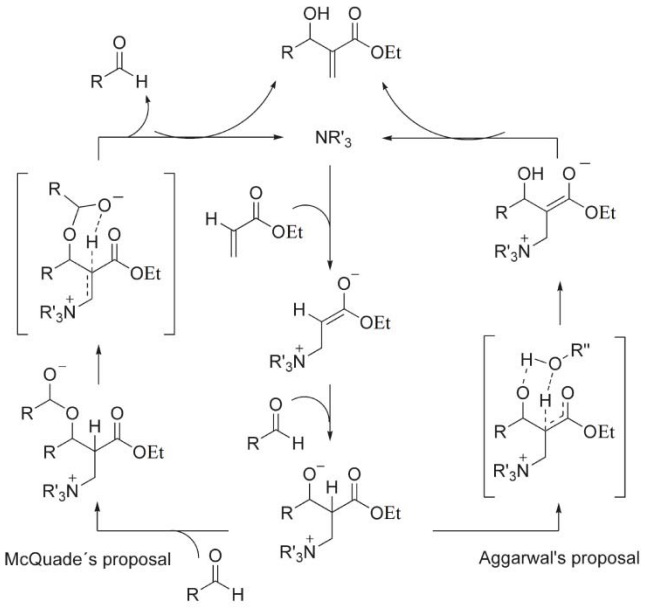
2 – The mechanism of Baylis-Hillman reaction is still under debated, namely regarding which is the rate determining step.[1] However, a general accepted mechanism goes as present in SM 4.2.3.11.4.



SM 4.2.3.11.4 – General reaction mechanism

3 – There is no correct answer for this question. The overall conclusion that one can attain from reading the literature is that the rate limiting step varies according to the reaction conditions (solvent, temperature, catalyst, substrates).

Initial studies from Hill and Isaac groups suggests that the rate determining step is the carbon-carbon bond formation in the aldolic addition reaction of the zwitterionic amine-acrylate adduct and an aldehyde molecule, due to a low kinetic isotopic effect at the alpha proton.[2] Independent studies from McQuade[3] and Aggarwals[4] groups reinvestigated the kinetics of the Morita-Baylis-Hillman reaction by means of the kinetic isotope effect (KIE) employing an α-^{2}H acrylate precursor. In the above-mentioned experiments, Aggarwal also observed that the reaction shows autocatalysis after approximately 20% conversion, while McQuade observed a second-order character for the aldehyde in the reaction. On the basis of these experimental findings two new mechanistic hypotheses were proposed as shown in SM 4.2.3.11.5.



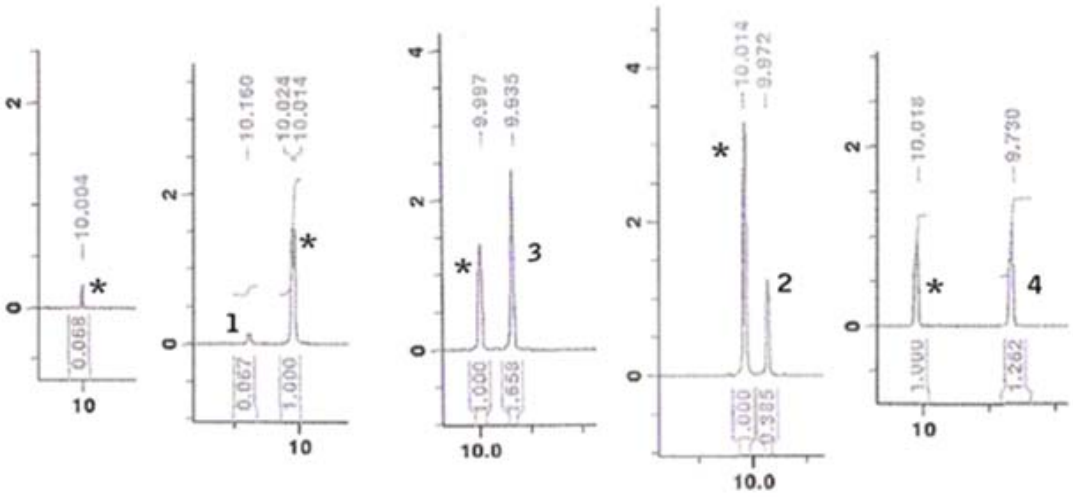
SM 4.2.3.11.5 – Proposed reaction mechanism

More recently, Singleton group suggested that the nature of the proton transfer step do not occur via a proton-shuttle process as advanced by the latter two groups, but rather by a simple acid-base, protonation/deprotonation mechanism.[5]

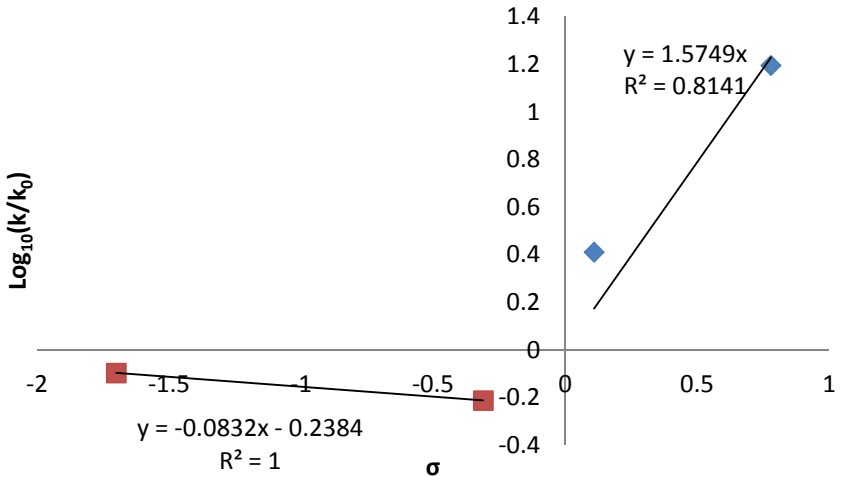
Hence, the objective of this question is to stimulate the students to search in the literature using common Science directed search engines (Web of science, Scopus, Scifinder, etc) for the information needed. They should be able to find the contributions of these four groups to guide them in a critical discussion. This also serves to illustrate to students that it can be difficult to prove with high certainty the mechanism of organic reactions using both experimental data and theoretical calculations.

4 – As the reaction take place by a nucleophilic attack into the aromatic aldehyde, the presence of electron-withdrawing groups should improve the reactivity. In order to access this information, students are encouraged to propose an experiment to determine the reactivity between both aldehydes. The best way to do it is to undergo a competitive reaction using equimolar amounts of benzaldehyde and 4-nitrobenzaldehyde as stoichiometric limiting reagents.

We encouraged students to draw a Hammet correlation graph by doing several competitive reactions between 4-substituted benzaldehydes and benzaldehyde. From which we observed a complex behaviour, which agrees with the mechanistic complexity of this reaction.



SM 4.2.3.11.6 – Compilation of the 10 ppm range of crude NMR samples from competitive reactions. *indicates the proton of benzaldehyde, 1 of *p*-nitrobenzaldehyde, 2 of *p*-methylbenzaldehyde, 3 of *p*-chlorobenzaldehyde e 4 of *p*-dimethylaminobenzaldehyde.



Substituinte	k/k_0	$Log_{10}(k/k_0)$	σ
NO_2	15.67	1.194	0.78
Cl	2.57	0.4102	0.11
CH_3	0.61	-0.2126	-0.31
NMe_2	0.82	-0.0969	-1.7

SM 4.2.3.11.7 – Hammet representation

With this information we can surely say that benzaldehyde is less reactive than 4-nitrobenzaldehyde.

5 – Again this question serves to motivate the students to propose an experiment to evaluate the equilibrium possibility of the Baylis-Hillman reaction. Since they know that 4-nitrobenzaldehyde is more reactive than benzaldehyde, one expected experiment proposal relies in the incubation of the Baylis-Hillman adduct of benzaldehyde, in water/1,4-dioxane in presence of one equivalent of DABCO and 4-nitrobenzaldehyde. If the reaction is indeed in equilibrium, the students should detect the formation by proton NMR the appearance of benzaldehyde and the gradual disappearance of 4-nitrobenzaldehyde.

H and P statements

Compounds	**H statements**	**P statements**
4-nitrobenzaldehyde	H317-H319	P280-P305 + P351 + P338
ethyl acrylate	H225-H302 + H312-H315-H317-H319-H331-H335-H412	P210-P261-P273-P302 + P352 + P312-P304 + P340 + P312-P403 + P233
DABCO	H315-H319-H335-H412	P261-P273-P305 + P351 + P338
1,4-Dioxane	H225-H319-H335-H351	P210-P261-P281-P305 + P351 + P338
Dichloromethane	H315-H319-H335-H336-H351-H373	P261-P281-P305 + P351 + P338
Diethyl ether	H224-H302-H336	P210-P261
Deuterated chloroform	H302-H315-H319-H331-H351-H361-H372	P261-P281-P305 + P351 + P338-P311

References

1 –Wei, Y., Shi, M., *Chem. Rev.* 2013, **113**, 6659
2 – Hill, J. S.; Isaacs, N. S. *J. Phys. Org. Chem.* 1990, **3**, 285
3 – (a) Price, K. E.; Broadwater, S. J.; Jung, H. M.; McQuade, D. T. *Org. Lett.* 2005, **7**, 147. (b) Price, K. E.; Broadwater, S. J.; Walker, B. J.; McQuade, D. T. *J. Org. Chem.* 2005, **70**, 3980
4 – (a) Aggarwal, V. K.; Fulford, S. Y.; Lloyd-Jones, G. C. *Angew. Chem., Int. Ed.* 2005, **44**, 1706. (b) Robiette, R.; Aggarwal, V. K.; Harvey, J. N. *J. Am. Chem. Soc.* 2007, **129**, 15513
5 – Plata, R.E., Singleton, D.A., *J. Am. Chem. Soc.* 2015, **137**, 3811

Synthesis of Ethyl Mandelate through a Rhodium-Catalyzed Arylation Reaction with Ethyl Glyoxylate and Phenylboronic Acid

Supplementary Material

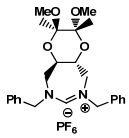
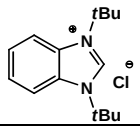
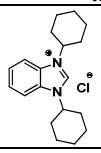
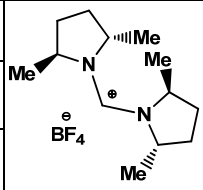
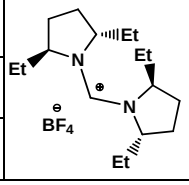
This experiment aims at synthesizing ethyl mandelate (colorless oil) from the corresponding commercially available ethyl glyoxylate (in 50% in toluene) and phenylboronic acid, using an organometallic catalyst (see Figure SM 4.2.3.12.1). The importance of the experiment is based on the application of a catalytic system comprising a transition-metal and an N-Heterocyclic Carbene (NHC) ligand to synthesize a β-hydroxy ester compound under mild reaction conditions. This experiment should be easily conducted by MSc students and others with basic synthetic laboratory experience. There is no need to prepare the pre-formed active complex (Rh-NHC), and all the reagents are added sequentially to the reaction flask. *tert*-Amyl alcohol is the solvent of choice since it easily dissolves the reagents at room temperature. Potassium *tert*-butoxide is the base that is used, and can be used as received (Sigma-Aldrich, reagent grade, ≥98% purity). The reaction yield can be improved by performing the reaction at 60 º C (Table SM 4.2.3.12.1). Various types of NHC ligand can be used (Table SM 4.2.3.12.1), but the most suitable is (2S,3S,4aR,9aR)-6,8-dibenzyl-2,3-dimethoxy-2,3-dimethyl-3,4a,5,8,9,9a-hexahydro-2H-[1,4]dioxino
[2,3-e][1,3] diazepin-6-ium hexafluorophosphate. Since this experiment is divided into two laboratory sessions, the reaction time can be extended by 30 min. In the case of the product isolation, filtration of the solids was achieved with the system shown in Figure SM 4.2.3.12.2. This procedure should be conducted under vacuum since the reaction mixture must be filtered over a layer of *celite* and silica gel (Figure SM 4.2.3.12.2). This step is crucial for the elimination of decomposed organometallic catalyst and other inorganic residues. In order to isolate the desired ethyl mandelate product, simple silica-gel liquid column chromatography and Hexane/AcOEt (5/1) eluent is used. To save time, the columns can be prepared by a laboratory technician under the instructor's guidelines before the class begins. However, it is better that the students prepare them to gain experience in the technique, as it only takes 5 minutes to fill the column with the stationary phase (Figure SM 4.2.3.12.3). TLC (Thin-Layer-Chromatography) should be used for product detection as it elutes from the column (Figure SM

4.2.3.12.4). Although the ethyl mandelate product can be visualized by UV light, it is not so easy for the ethyl glyoxylate substrate and thus a solution of phosphomolybdic acid (5 g) in ethanol (100 ml) should be used (to reveal the spots on the TLC plate, it should be immersed in this staining solution and heated with a hot-air gun).

Table SM 4.2.3.12.1 describes some results obtained using the same quantities of the reagents described in this experiment where several NHC type ligands and will give the students an idea of the type of yields to be expected with this reaction. It is possible to recover unreacted ethyl glyoxylate by liquid chromatography (1st spot on the TLC plates, the ethyl mandelate product is the 2nd spot on the TLC plate, revealed with UV light). This experiment was repeated, under the conditions described, by Silvia Fernandes, an MSc student who obtained yields in the range of 50 to 85% yield (using the (2S,3S,4aR,9aR)-6,8-dibenzyl-2,3-dimethoxy-2,3-dimethyl-3,4a,5,8,9,9a-hexahydro-2H-[1,4]dioxino [2,3-e][1,3] diazepin-6-ium hexafluorophosphate NHC ligand, at room temperature during 1 hour, 85% yield of ethyl mandelate product was obtained – see pictures and ^{1}H and ^{13}C NMR spectra in Figure SM 4.2.3.12.7 and SM 4.2.3.12.8).

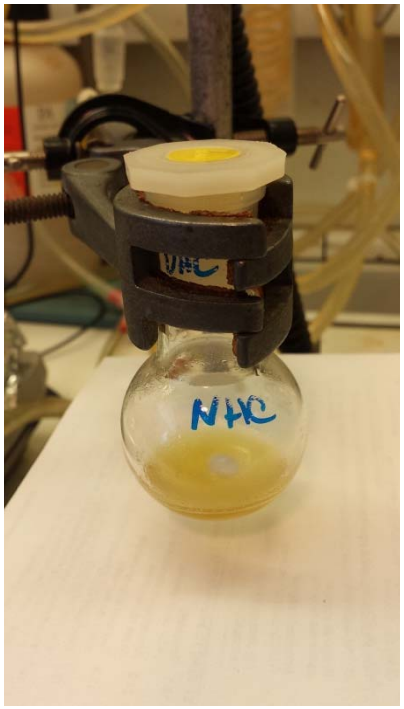
Additional Note: Ethyl glyoxylate could also be stained on the TLC plate using a solution of dinitrophenylhydrazine (DNP) (dissolve 12 g of 2,4-dinitrophenylhydrazine, 60 mL of conc. sulfuric acid, and 80 mL of water in 200mL of 95% ethanol) which is a more specific carbonyl group stain – which forms the corresponding hydrazones with carbonyl groups, giving usually yellow to orange spots.

Table SM 4.2.3.12.1. Experiments conducted in a 25 mL round-bottomed flask with a magnetic stirrer, using ethyl glyoxylate as substrate, phenylboronic acid as phenyl source, Rh-NHC as catalyst, KO*t*Bu as base and *tert*-amyl alcohol as solvent.

Entry	NHC-type ligand		Reaction temperature/ºC	Reaction Time/h	Yield/%
1[a]		Name: (2*S*,3*S*,4a*R*,9a*R*)-6,8-dibenzyl-2,3-dimethoxy-2,3-dimethyl-3,4a,5,8,9,9a hexahydro-2*H*-[1,4]dioxino[2,3-e][1,3] diazepin-6-ium hexafluorophosphate Supplier: ChiraTecnics CAS: 1393381-31-1 Quantity used: 8.6 mg	rt	0.5	>99
2		Name: 1,3-Di-tert-butylbenzimidazolium chloride Supplier: Sigma-Aldrich CAS: 946607-10-9 Quantity used: 4.0 mg	rt	4	46
3			60	4	59
4		Name: 1,3-Dicyclohexylbenzimidazolium chloride Supplier: Sigma-Aldrich CAS: 1034449-15-4 Quantity used: 4.8 mg	rt	4	12
5			60	4	14
6[a]		Name: (2R,5R)-1-{[(2R,5R)-2,5-Dimethylpyrrolidin-1-yl]methylene}-2,5-dimethylpyrrolidinium tetrafluoroborate Supplier: Strem Chemicals CAS: 1204324-14-0 Quantity used: 4.4 mg	60	4	80
7[a]			rt	4	46
8[a]			0	4	88
9[a]		Name: (2R,5R)-1-{[(2R,5R)-2,5-Diethylpyrrolidin-1-yl]methylene}-2,5-diethylpyrrolidinium tetrafluoroborate Supplier: Strem Chemicals CAS: 1204324-20-8 Quantity used: 5.3 mg	60	4	84
10[a]			rt	4	94
11[a]			0	4	70

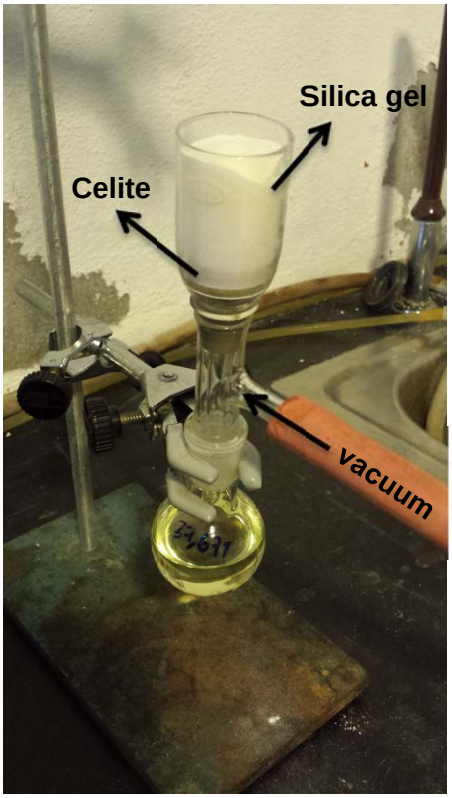
[a]The NHC type ligands used in these experiments are chiral - but since the enantiomeric purity of the ethyl mandelate product were low (ee<20%) we decided not to include any measurements in this experiment. ee= enantiomeric excess.

Photos of the experiment



Round-bottomed flask containing the reaction mixture.

1.



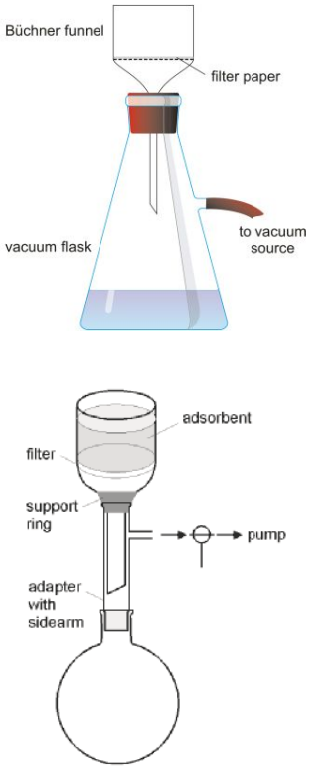


Figure SM 4.2.3.12.2. Filtration apparatus under vacuum.

Liquid Chromatography:

Figure SM 4.2.3.12.3. Glass chromatography column used for the purification and isolation of ethyl mandelate from the reaction mixture.

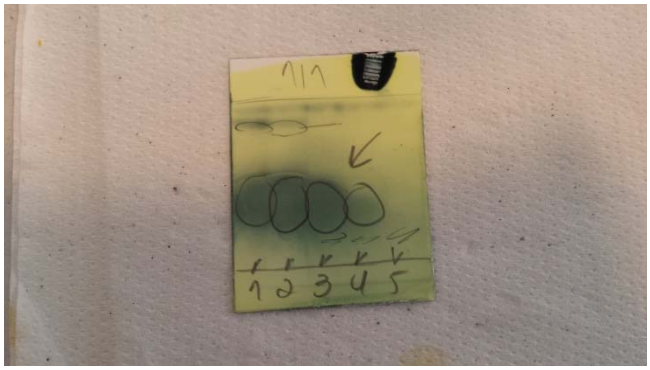


Figure SM 4.2.3.12.4. TLC plate showing the fractions collected from the column.

^{1}H and ^{13}C NMR spectra

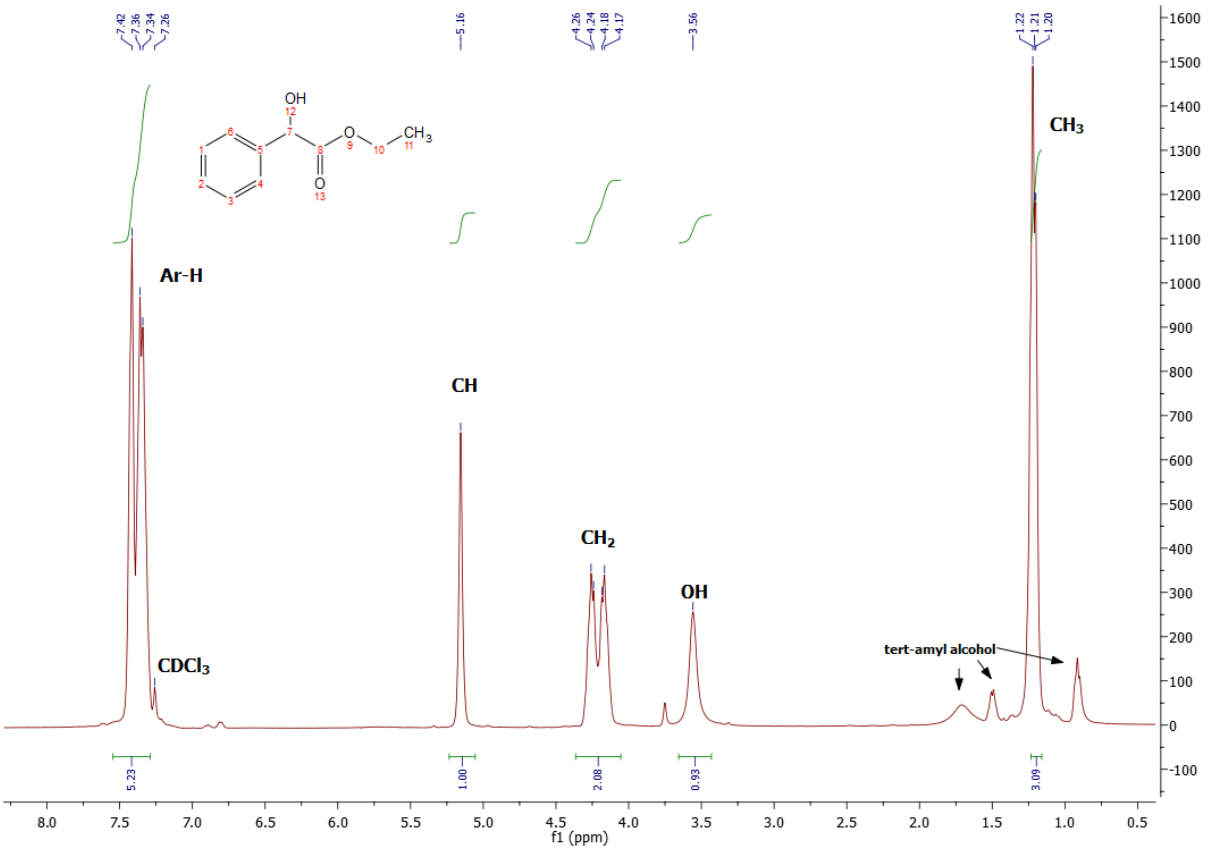


Figure SM 4.2.3.12.5. ^{1}H NMR spectra (400 MHz, $CDCl_3$) of ethyl mandelate.

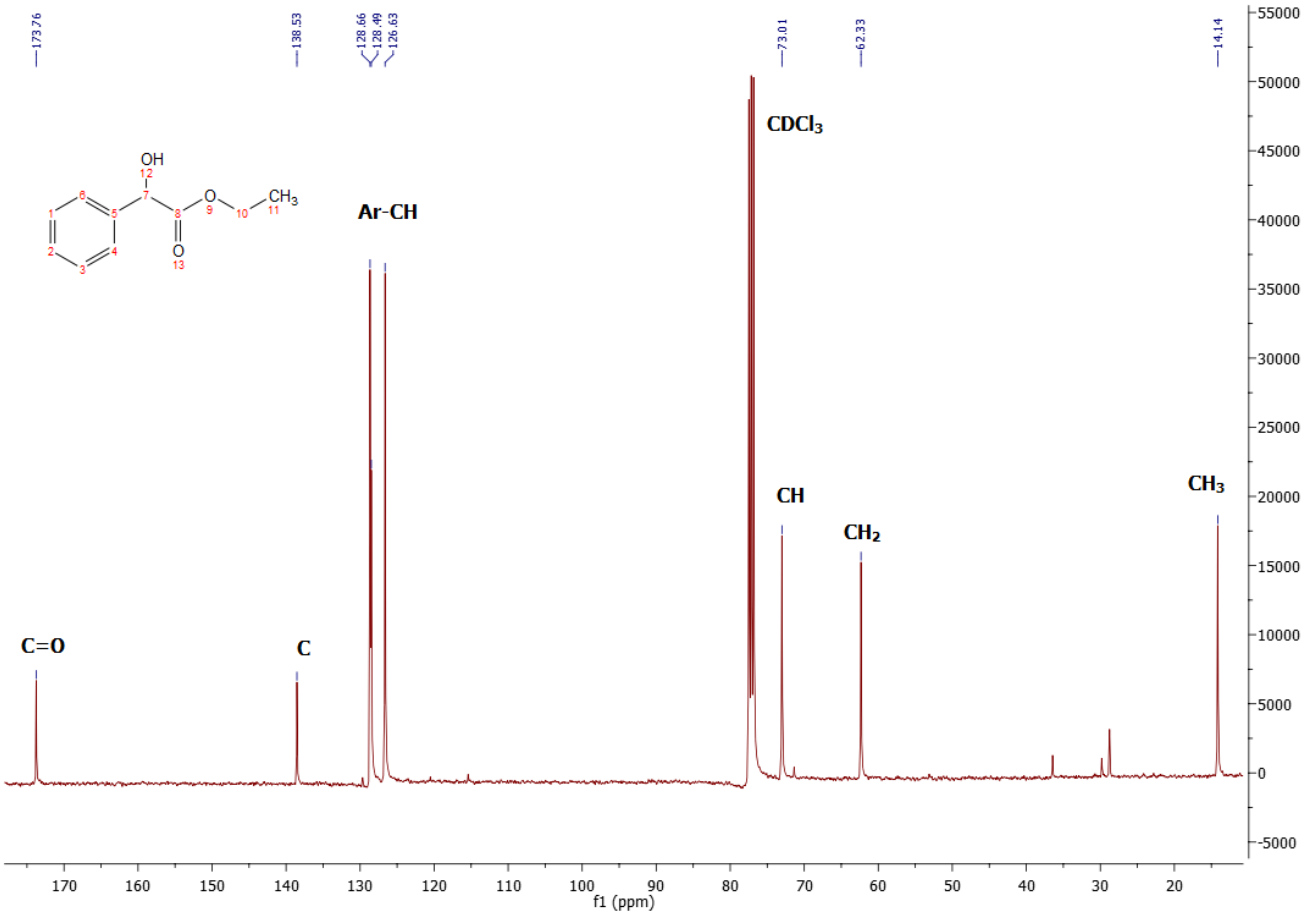


Figure SM 4.2.3.12.6. ^{13}C NMR spectra (100 MHz, $CDCl_3$) of ethyl mandelate.

Results obtained by the MSc Student;
Pictures:

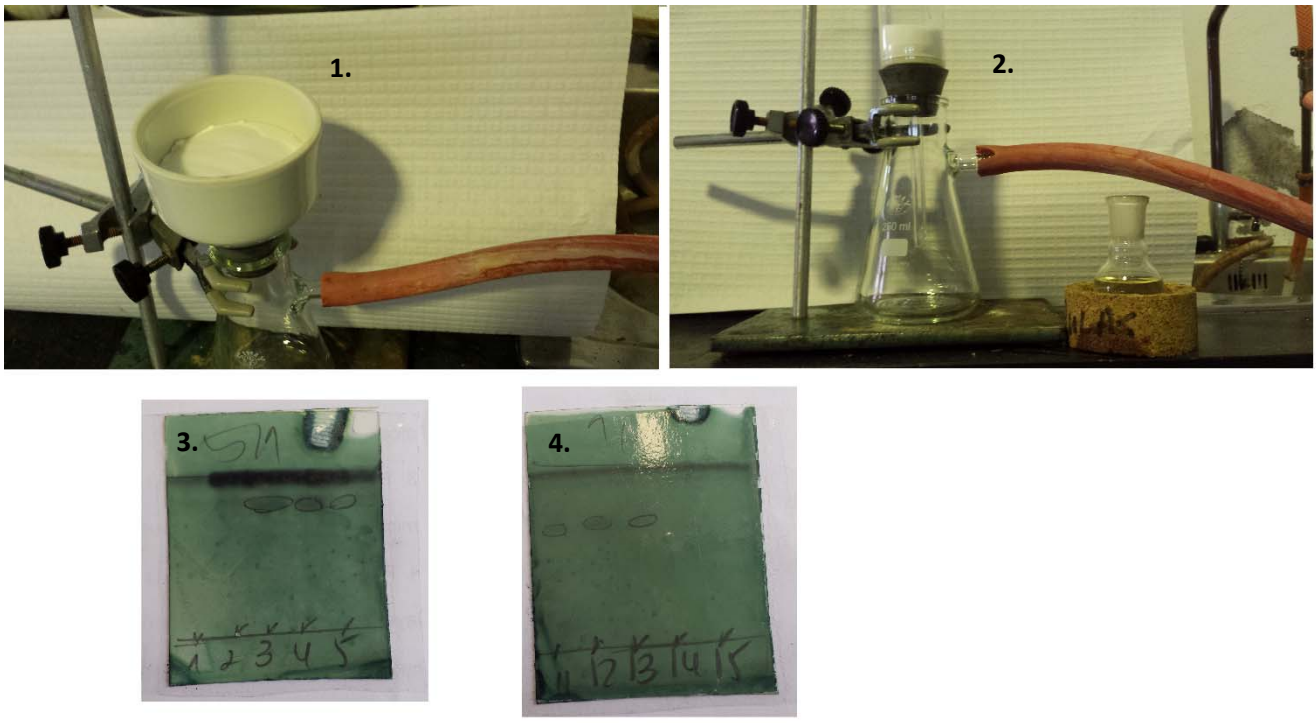
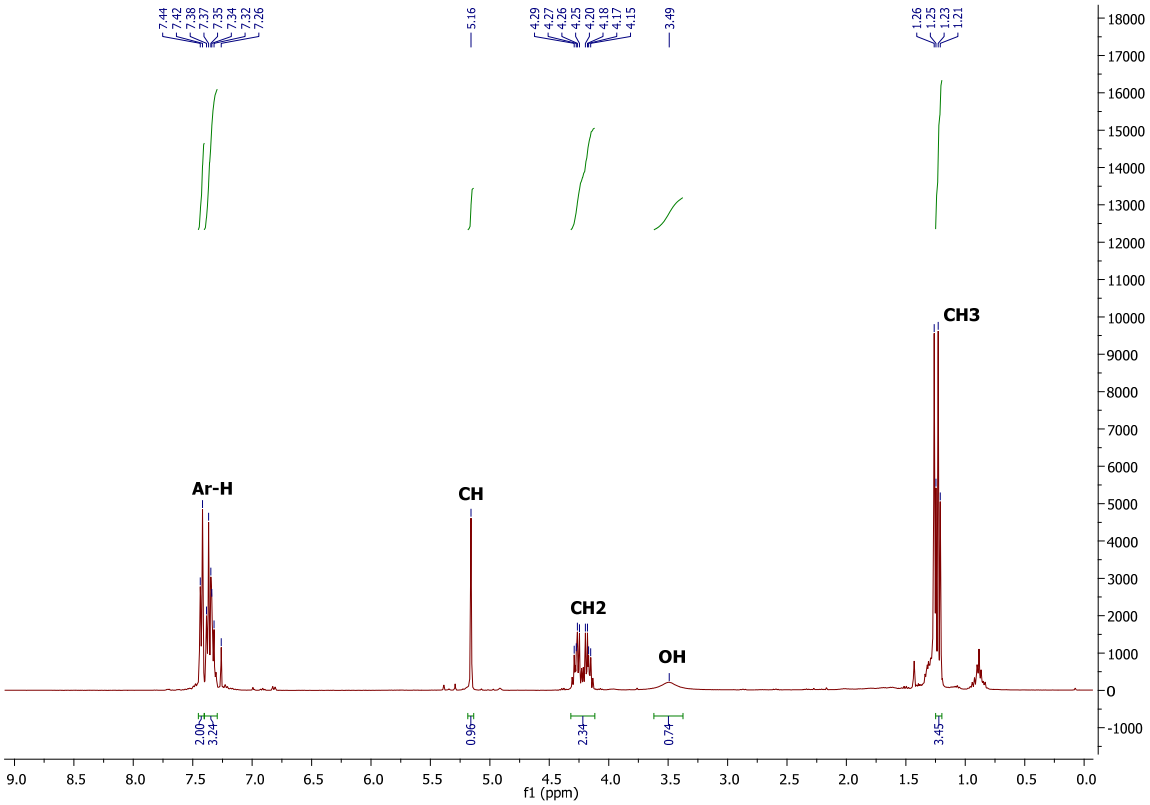
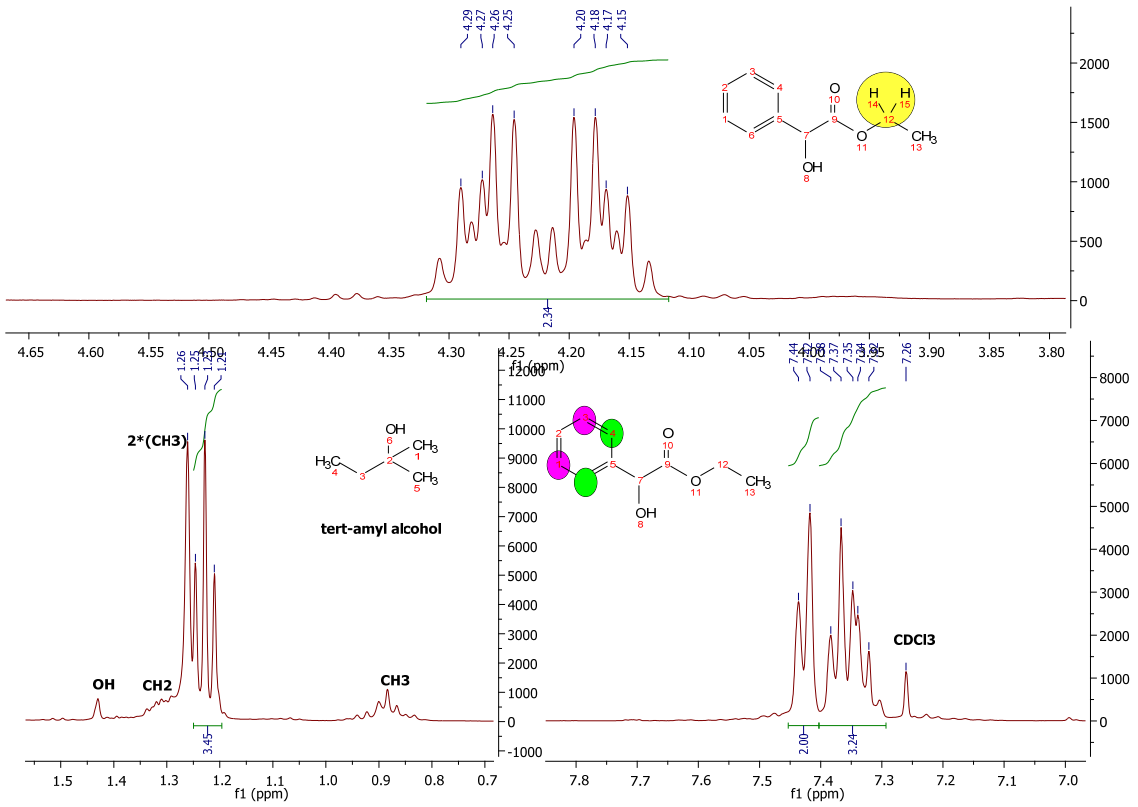


Figure SM 4.2.3.12.7. (1) Filtration apparatus using a Bückner funnel (Method B); **(2)** Filtration apparatus using a sintered glass funnel (Method A); **(3)** TLC plates of the fractions collected from column chromatography eluted in Hexane/AcOEt (5/1) (revealed with phosphomolybdic acid solution); **(4)** TLC plates of the fractions collected from column chromatography eluted in Hexane/AcOEt (1/1) (revealed with phosphomolybdic acid solution).

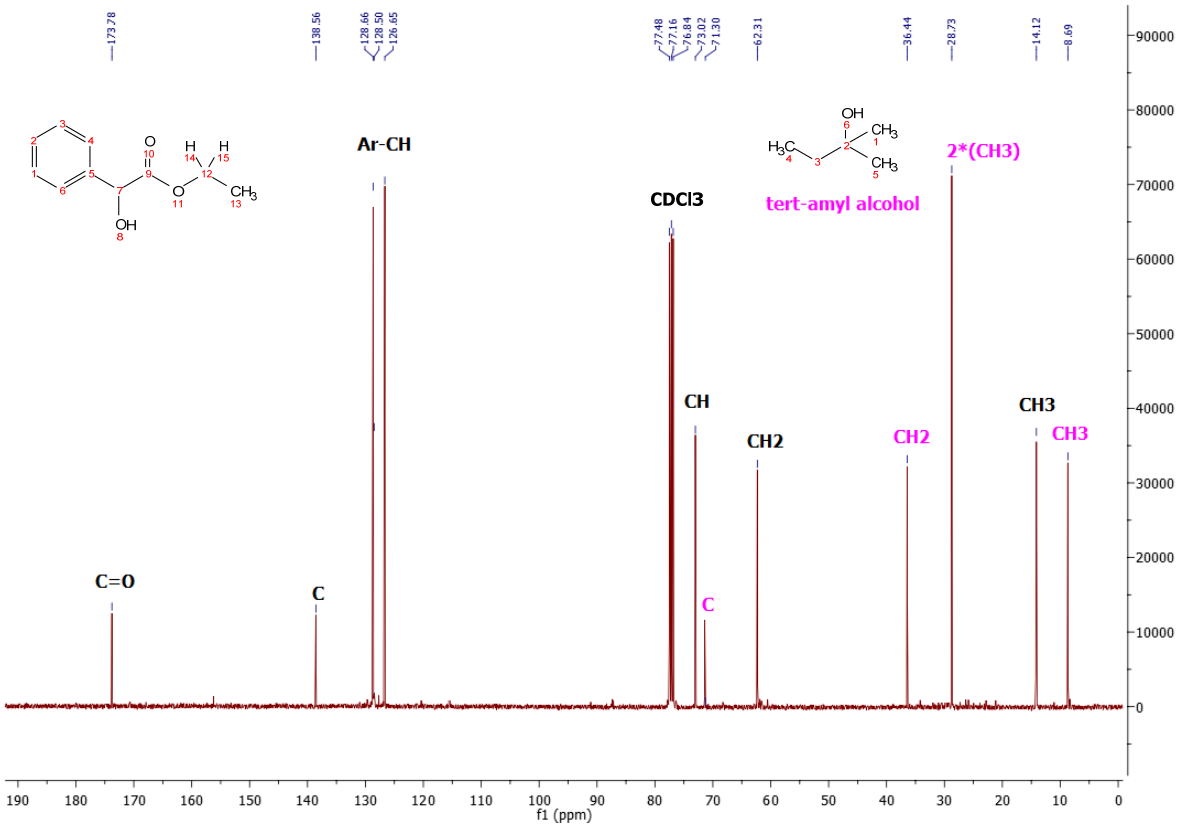
^{1}H and ^{13}C NMR spectra:

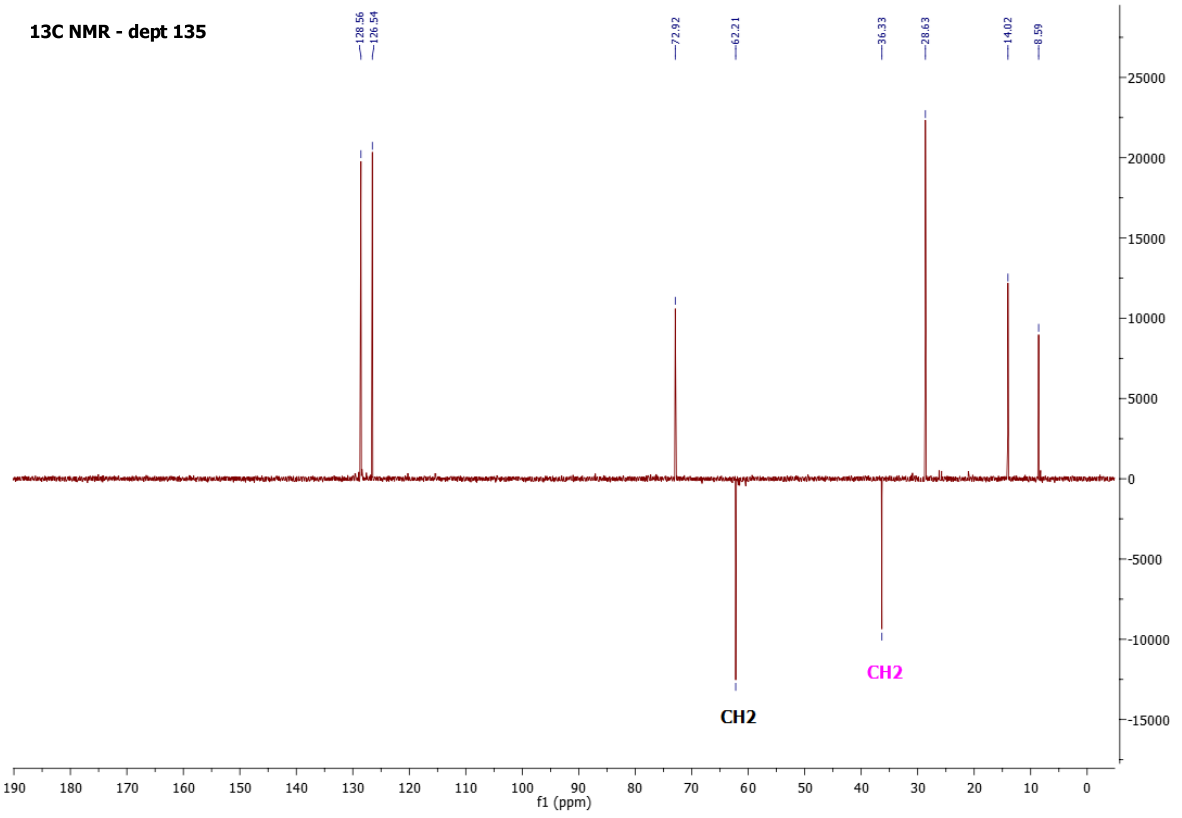
1.





2.





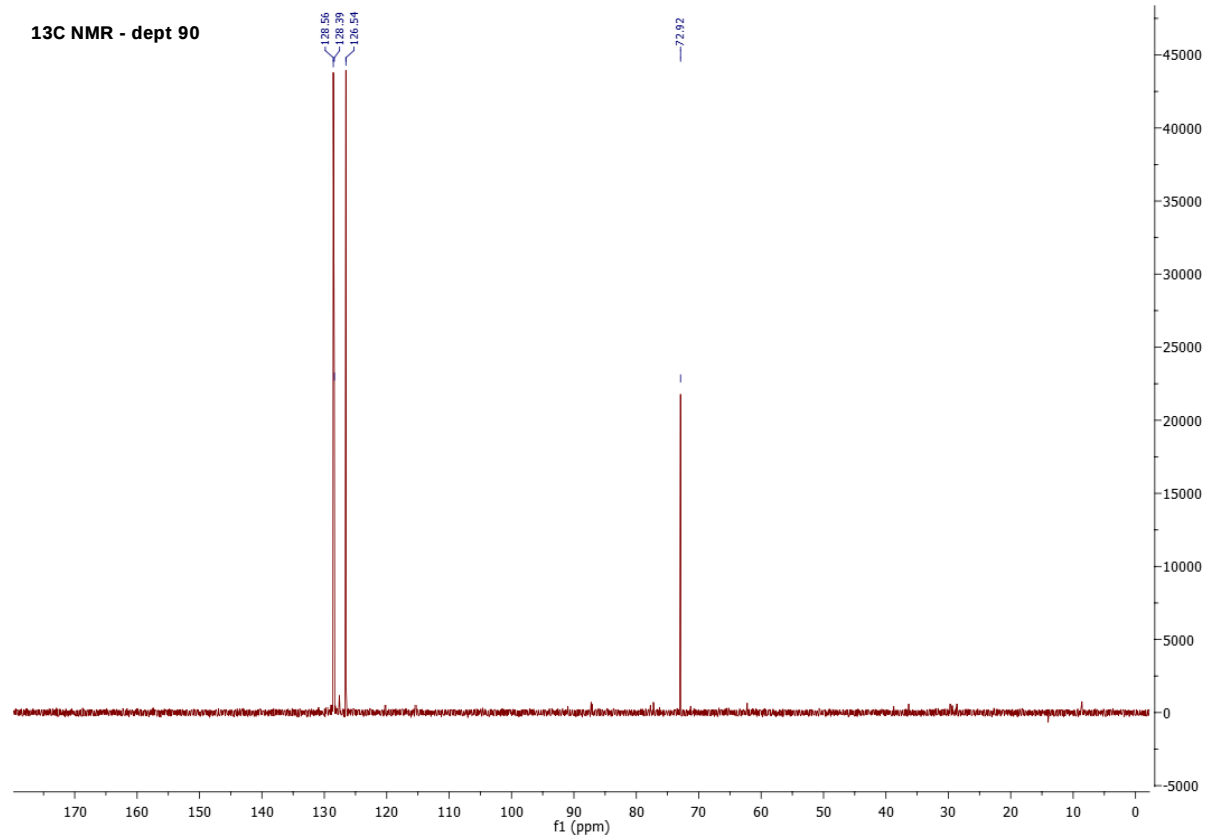


Figure SM 4.2.3.12.8. (1) ^{1}H NMR spectra (400 MHz, $CDCl_3$); **(2)** ^{13}C NMR spectra (100 MHz, $CDCl_3$) along with dept 135 (where the signals from the CH_2 groups are negative, and those from the CH and CH_3 groups are positive) and dept 90 (where only the signals from the CH groups appear) acquisitions.

Additional Notes: The signal at 4.15-4.29 ppm in ^{1}H NMR spectra was of special interest. The concept of diastereotopic protons (CH_2) – two "equivalent" protons attached to the same atom, with different chemical shifts, due to the presence of a chiral center nearby – should be explained to the students. What is observed is a quartet of quadruplets signal due to this diastereotopicity. Dept (Distortionless Enhancement by Polarization Transfer) experiments, in ^{13}C NMR, should also be mentioned as an effective way to determine the multiplicity of carbon atoms substituted with hydrogens.

Enantioselective synthesis and derivatisation of 2-Hydroxy-1,2-diphenylethan-1-one

Supporting Information

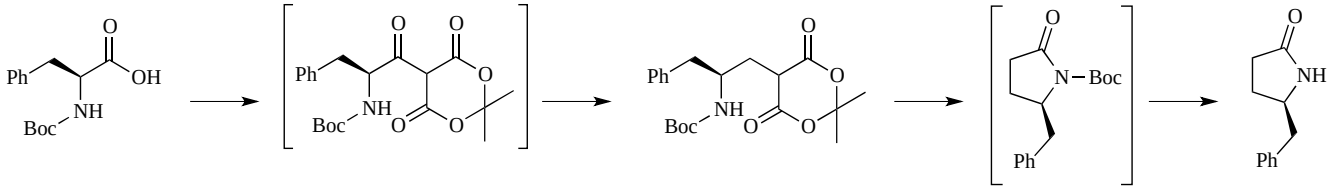
This experiment is designed as a series of 4 sequential steps to introduce third year undergraduate students to multi-step organic synthesis. In addition, the experiment exposes the student cohort to a plethora of practical (handling pyrophoric reagents, vacuum distillation, recrystallisation, utilising dry solvents, column chromatography) and theoretical (planning experimental quantities, time management) skills, which they will utilise going forward to their 4th year studies or take away into industry. Below are highlighted additional comments for each synthetic step along with the associated spectrum of the product.

Additional information for Step 1

To begin this experiment the students had to be provided with (5*R*)-5-benzylpyrrolidinone **1** which **IS NOT** commercially available. Synthesis of this precursor was achieved in 4 steps from commercially available (*R*)-N-Boc-phenylalanine (~100g of amino acid was processed in 25g batches to provide enough material for 116 students) following the procedure outlined below.[1] N.B. The scale of the reactions can be doubled and also ran initially at -5 °C and then warmed to room temperature overnight with no deleterious effect on yield or purity of product.

Synthesis of the catalyst **2** is straightforward. One aspect to be aware of is the recrystallization at the end with methanol. Students tend to use too much methanol and dissolve a lot of their product. This recrystallization should be treated as a trituration. Yields are typically in the range 50 – 75 % as a white crystalline solid, mp (from MeOH) 200 - 204 °C (lit. 195 °C).

Synthesis of (R)-5-Benzyl-pyrrolidin-2-one 1[1]



1

(S)-[1-Benzyl-2-(2,2-dimethyl-4,6-dioxo-[1,3]dioxan-5-yl)-2-oxo-ethyl]-carbamic acid *tert*-butyl ester: A 500 mL round bottom flask was charged with amino acid (12.7 g, 48 mmol), Meldrum's acid (7.6 g, 52.6 mmol), DMAP (8.8 g, 71.7 mmol), and DCM (200 ml) and cooled to -5 °C. To the reaction mixture was added a solution of DCC (10.9 g, 52.6 mmol) in DCM (100 ml) via addition funnel over one hour. The reaction was stirred at at -5 °C overnight. Upon warming to room temperature, the dicyclohexylurea was filtered off and the reaction mixture was washed with 5% $KHSO_4$ (4 x 100 ml) and brine (100 ml) and dried in the refrigerator with $MgSO_4$ for 5 hours. This was filtered and concentrated down to approximately 200 mL and used without further purification.

This step can be started at -5 °C and warmed up overnight to room temperature with no deleterious effects.

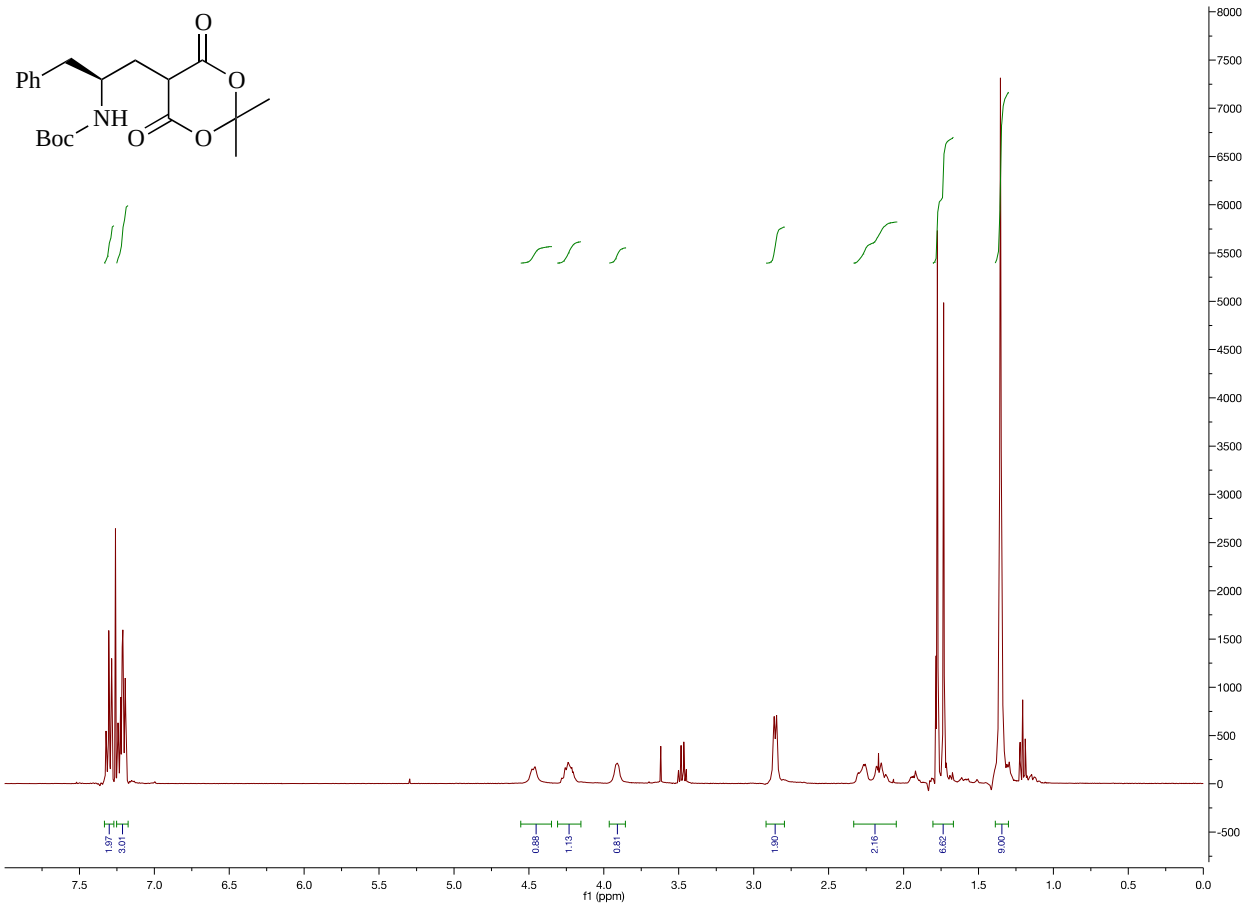
(R)-[1-Benzyl-2-(2,2-dimethyl-4,6-dioxo-[1,3]dioxan-5-yl)-ethyl]-carbamic acid *tert*-butyl ester: A 500 mL round bottom flask was charged with (S)- [1-Benzyl-2-(2,2-dimethyl-4,6-dioxo-[1,3]dioxan-5-yl)-2-oxo-ethyl]- carbamic acid *tert*-butyl ester (approx. 47 mmol, all of the material from the previous step) in DCM (200 ml) and 98% AcOH (30 ml, 531 mmol). The solution was cooled to –5 °C and $NaBH_4$ (4.5 g, 119.5 mmol total) was added via solid addition funnel over 3 hours. The reaction was stirred at –5 °C for 18 hours. The organic layer was washed with water (3 x 100 ml) and brine (2 x 100 ml) and the combined organic extracts were dried with $MgSO_4$ and concentrated down. The desired product was crystallized from diethyl ether (75 ml) to yield (11.7 g, 65 % over 2 steps) as a white solid.

This step can be started at -5 °C and warmed up overnight to room temperature with no deleterious effects.

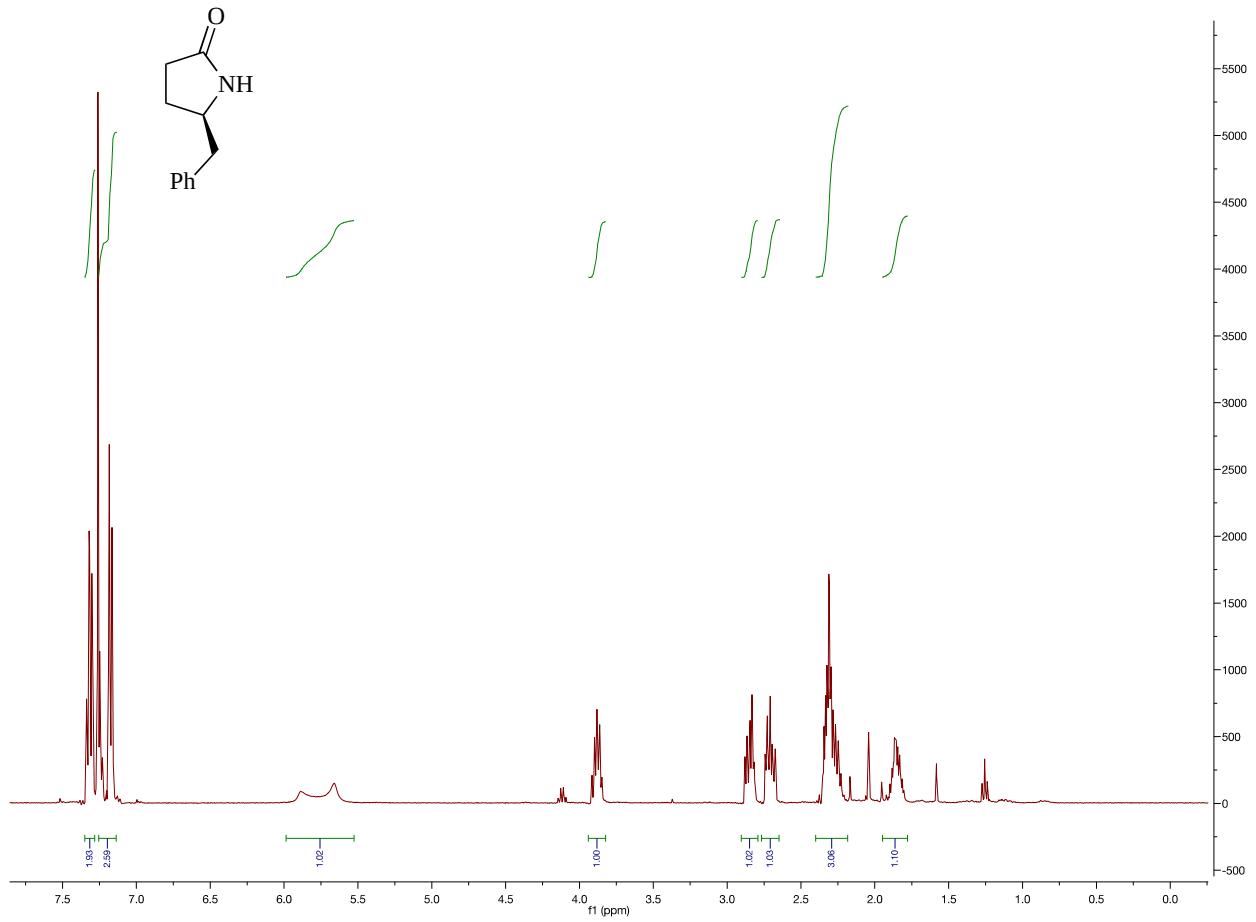
(R)-2-Benzyl-5-oxo-pyrrolidine-1-carboxylic acid *tert*-butyl ester: A 250 mL round bottom flask equipped with a reflux condenser was charged with (R)-[1-Benzyl-2-(2,2-dimethyl-4,6-dioxo-[1,3]dioxan-5-yl)-ethyl]-carbamic acid *tert*-butyl ester (8.7 g, 23 mmol) and toluene (100 mL) and heated to 110 °C for 5 hours. Upon cooling, the organic layer was concentrated under vacuum and used without further purification.

(R)-5-Benzyl-pyrrolidin-2-one: A flame-dried 500 mL round bottom flask was charged with (R)-2-Benzyl-5-oxo-pyrrolidine-1-carboxylic acid *tert*-butyl ester (6.3 g, 23 mmol) and dry DCM (200 mL) and cooled to 0 °C. To this solution was added trifluoroacetic acid (3.9 mL, 51 mmol) dropwise. The reaction was allowed to warm to room temperature and stirred for 4 hours. The reaction was quenched slowly with $NaHCO_3$ and extracted with DCM. The organic layer was washed with water, brine and dried with $MgSO_4$ and concentrated down to yield (3.2 g, 80 % over 2 steps) as cream solid.

^{1}H NMR: (R)-[1-Benzyl-2-(2,2-dimethyl-4,6-dioxo-[1,3]dioxan-5-yl)-ethyl]-carbamic acid *tert*-butyl ester



^{1}H NMR: (R)-5-Benzyl-pyrrolidin-2-one



Additional information for Step 2

Heading the risks associated with the use of n-BuLi is the only real concern with this synthetic step. Students cope admirably, producing colourless oils with yields in the range 75 – 80 %, b.p. 170 - 175 °C (lit. 178 - 179 °C);

Additional information for Step 3

The benzoin condensation can be problematic if students fail to add sufficient base. As potassium tert-butoxide is hygroscopic, newly purchased material should be used in all cases, to alleviate the problem. If still problematic, we found that addition of more potassium tert-butoxide helps the reaction proceed. Students produce a white solid with yields, which range from 40 – 65 %, m.p. 119 - 122 °C (lit. 134 - 138 °C) as a mixture of enantiomers (as shown by the chiral LC) in a ratio of 7:3

Additional information for Step 4

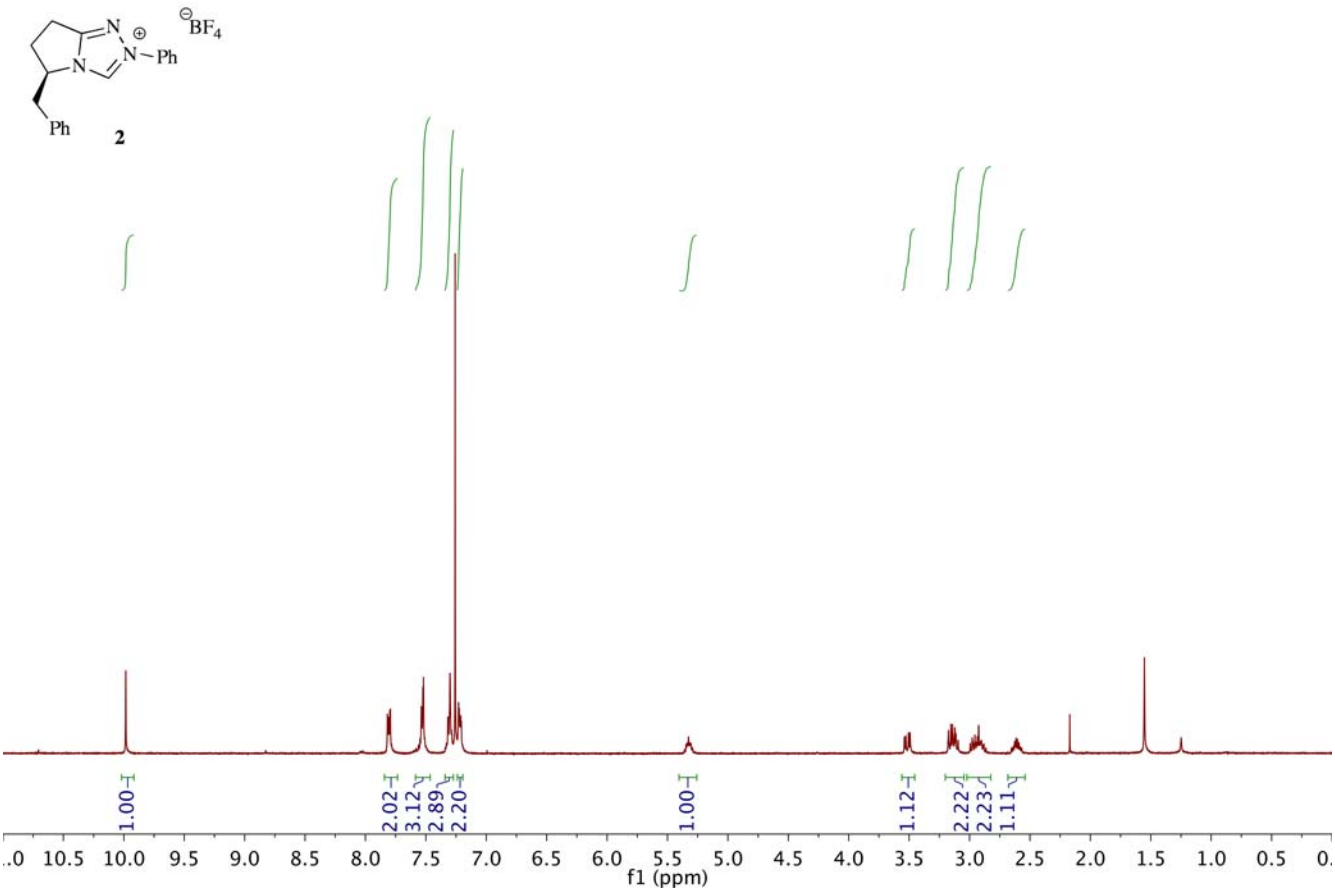
Again, students do not struggle with this synthesis. The reaction takes less than 4 h to complete and is monitored by TLC [Product (R_f = 0.1), s.m. (R_f = 0.3) and another product (R_f = 0.7) in *n*-hexane/EtOAc 9:1]. They achieve yields between 40 – 65 % of the single diastereoisomer. Isolating

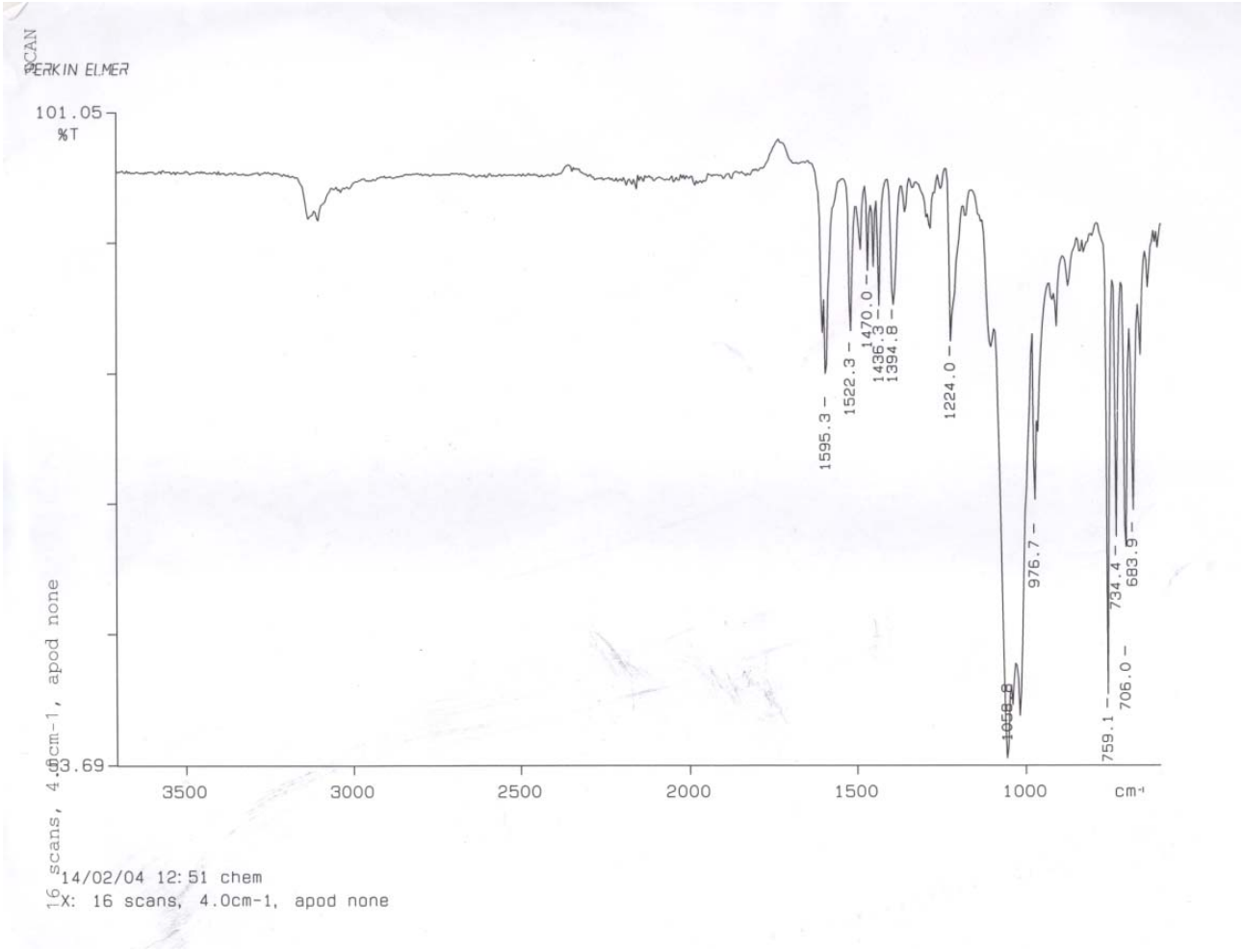
and analysing the other product (R_f = 0.7) reveals that this is not the other diastereoisomer, but a degradation product of derivatisation step. Further manipulation of the column or conditions fails to account for the other diastereoisomer and as such this is lost.

Spectra

Step 1

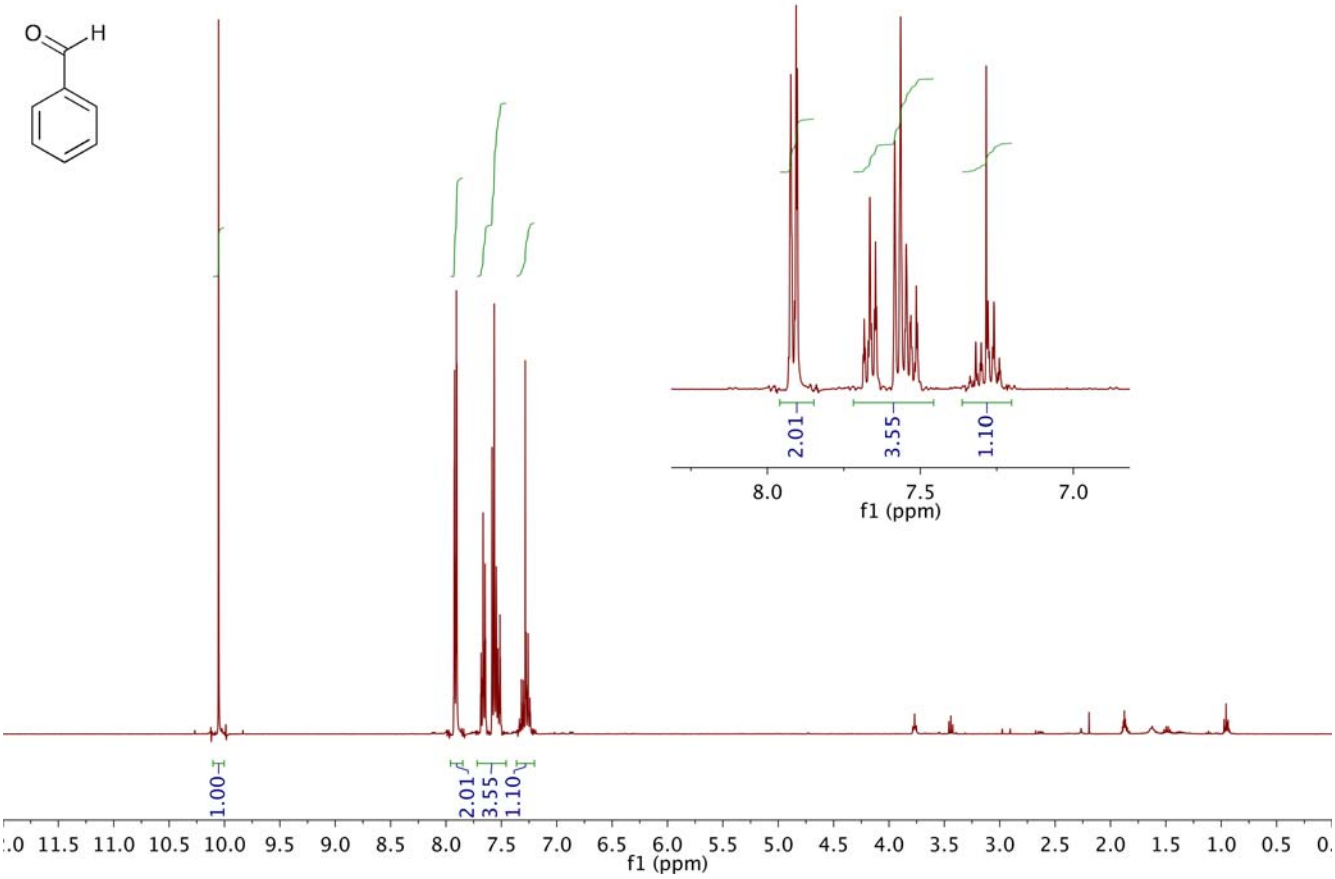
(5R)-5-Benzyl-2-phenyl-6,7-dihydro-5*H*-pyrrolo[2,1-*c*][1,2,4]triazol-2-ium tetrafluoroborate (2)

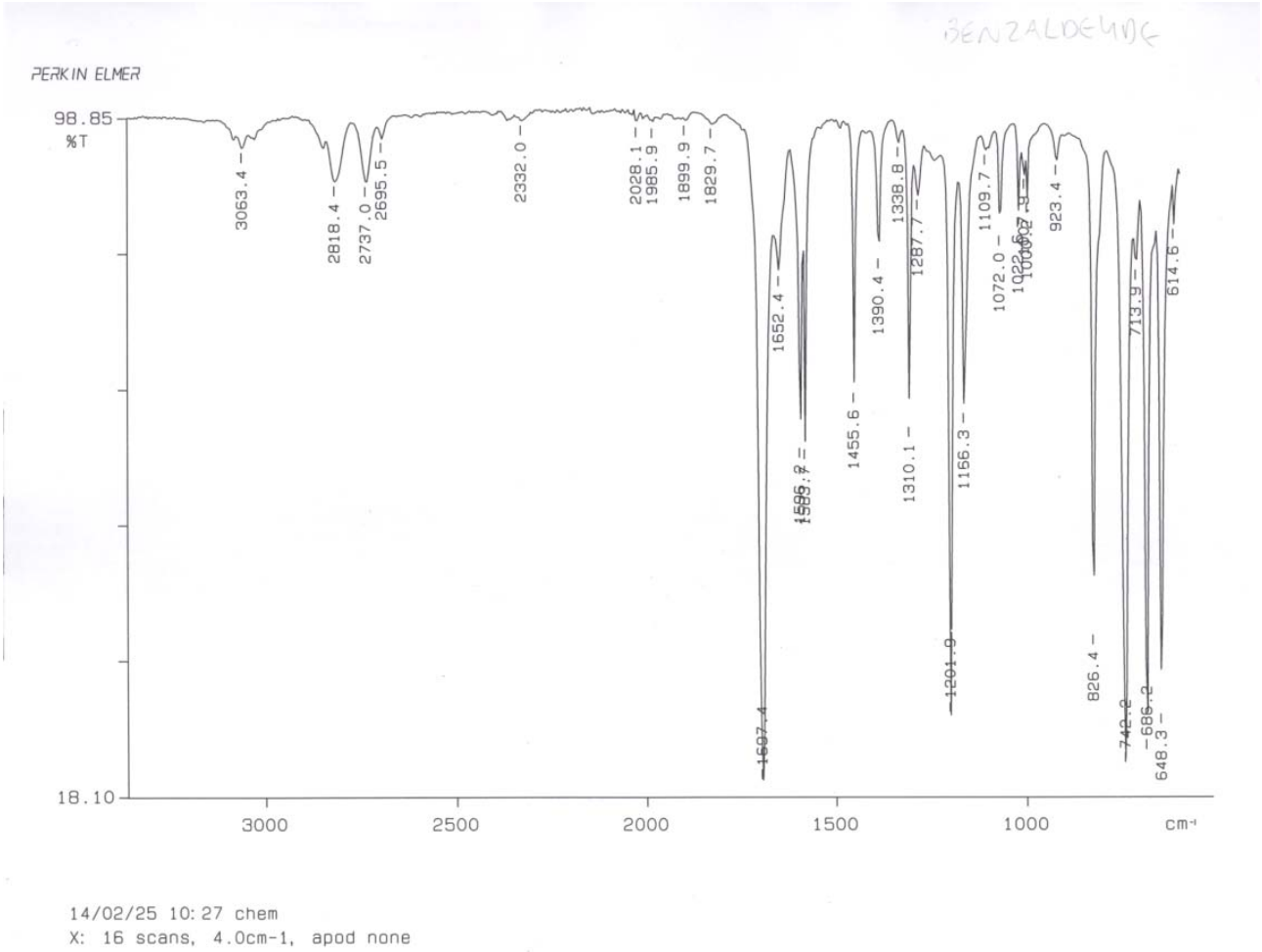




Step 2

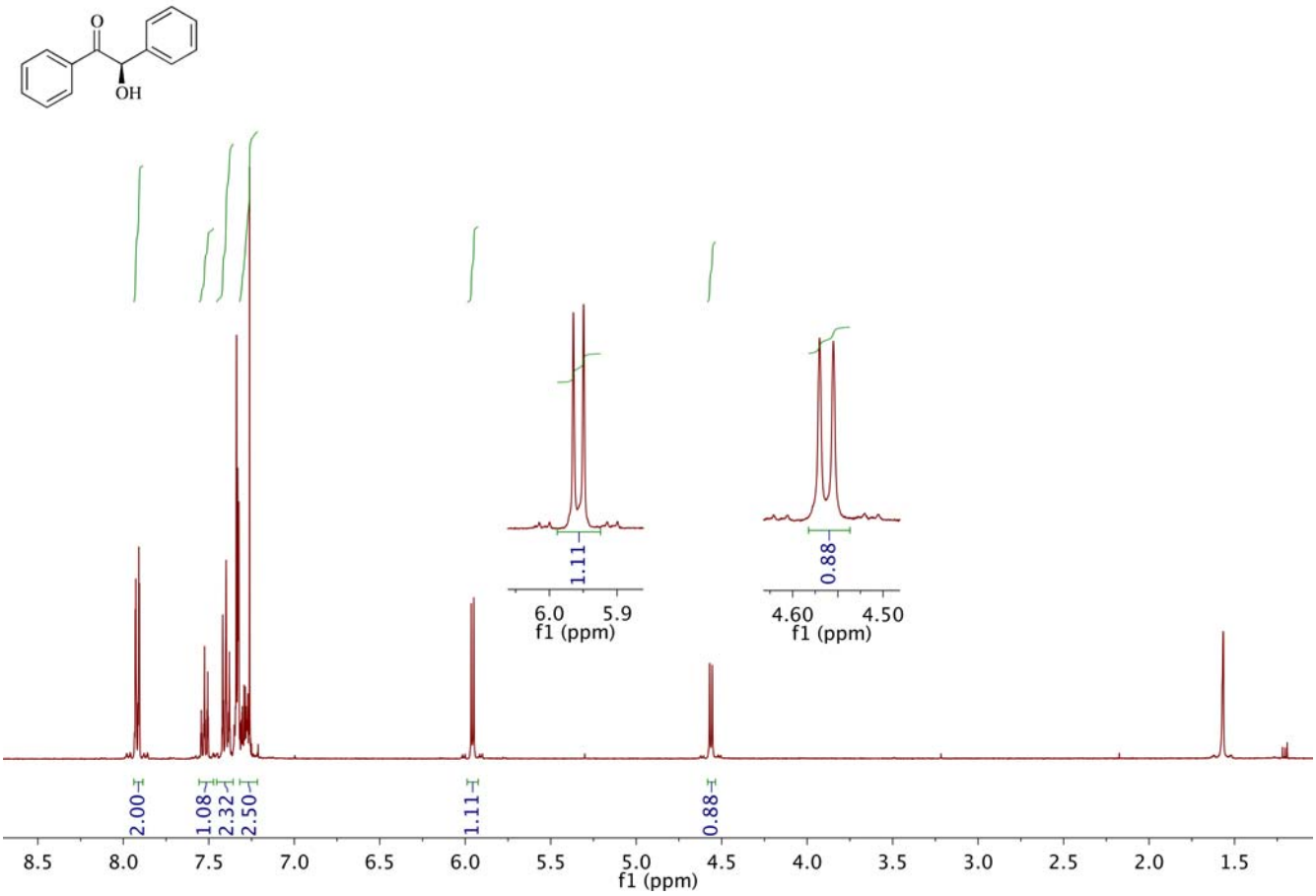
Benzaldehyde (3)

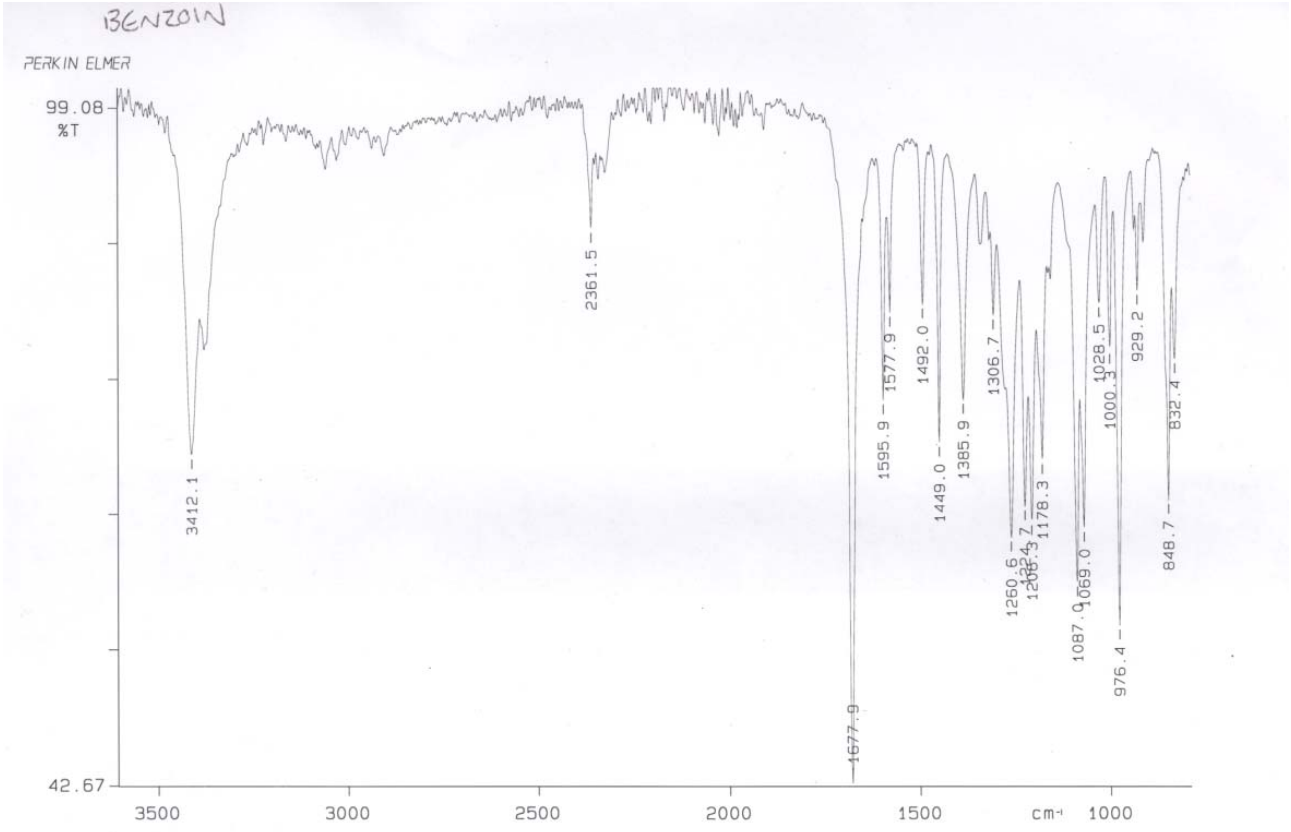




Step 3

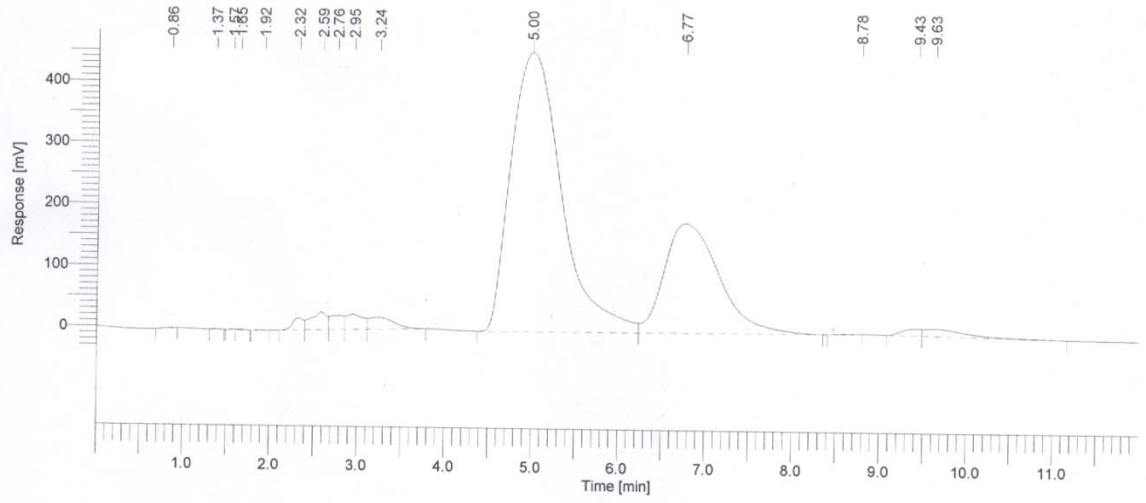
2-Hydroxy-1,2-diphenylethan-1-one (4)





Software Version	: 6.3.1.0504	Date	: 17/02/2014 16:06:35
Reprocess Number	: bioactivehplc2: 8591		
Sample Name	: JHW benzoin		
Instrument Name	: HPLC2	Data Acquisition Time	: 17/02/2014 15:54:25
Rack/Vial	: 0/3	Channel	: A
Sample Amount	: 1.000000	Operator	: dch5lwl
Cycle	: 3	Dilution Factor	: 1.000000

Result File : E:\data\Teaching L3 Org Chiral HPLC\17_02_2014_JHW benzoin_003.rst
Sequence File : E:\data\Teaching L3 Org Chiral HPLC\17th Feb 2014.seq

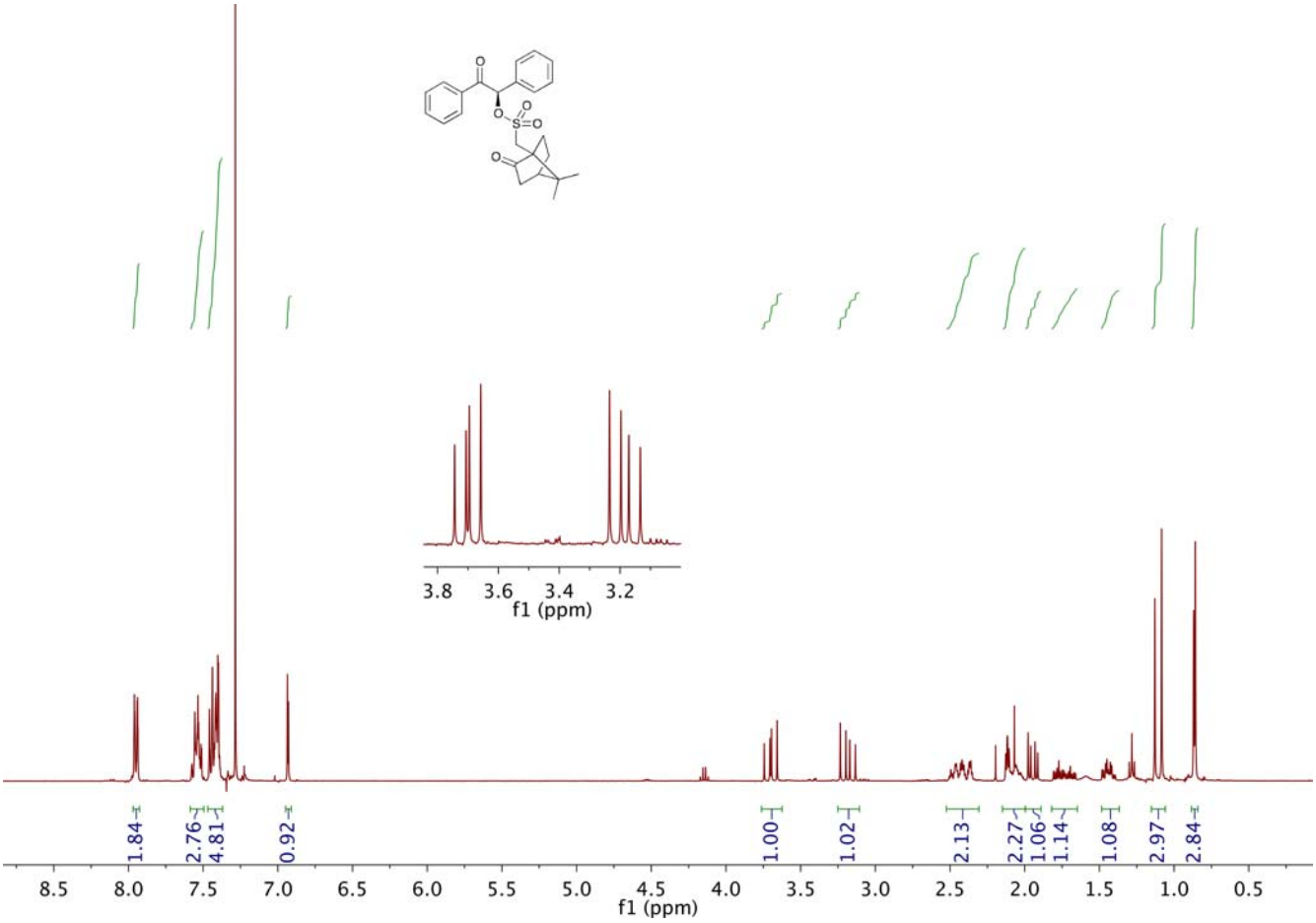


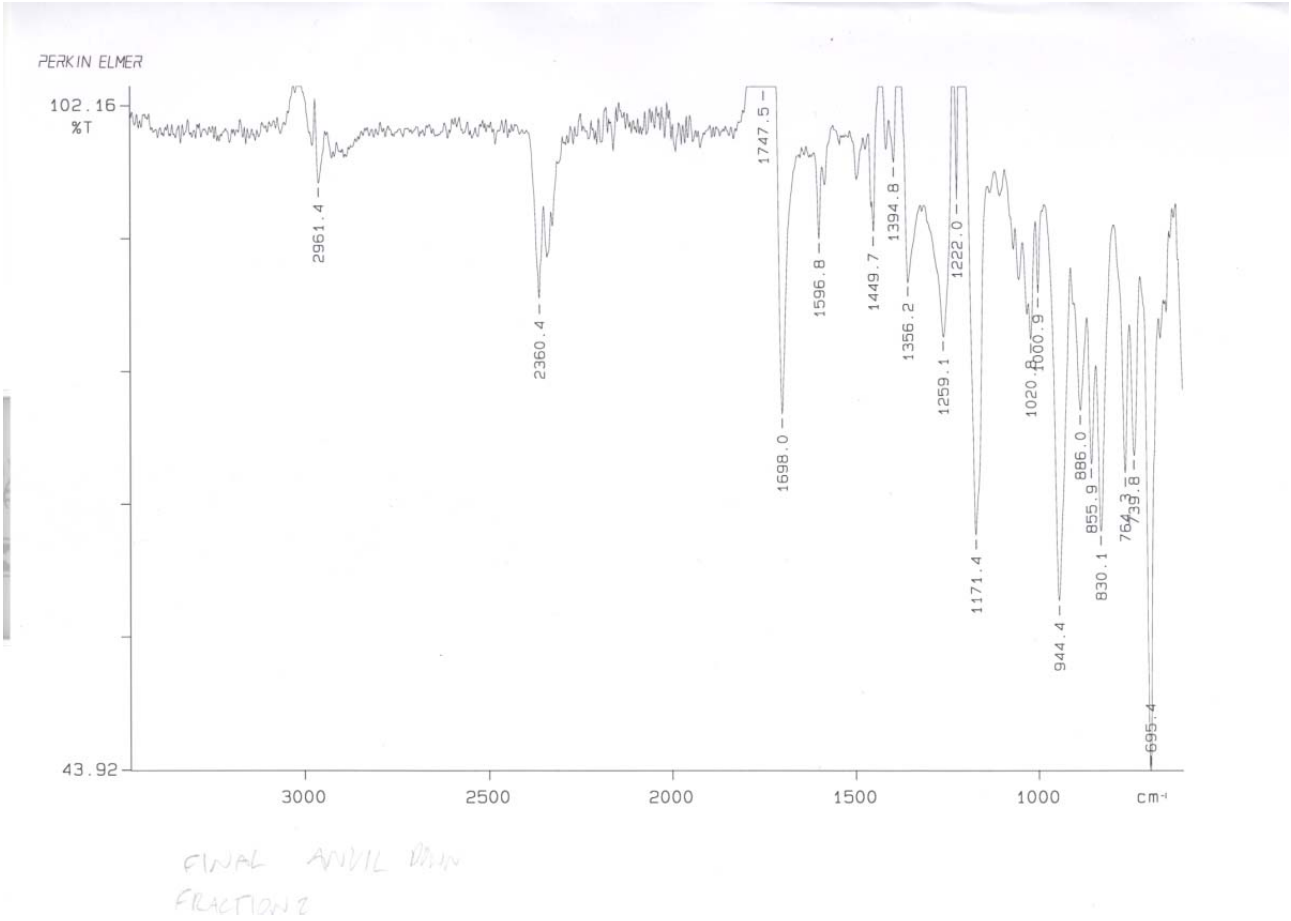
HPLC 2

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]
6	2.318	168173.08	19998.31	0.59	0.59	BV	8.4094
7	2.588	367135.91	29009.39	1.29	1.29	VV	12.6558
8	2.758	248359.75	23016.16	0.87	0.87	VV	10.7907
9	2.945	335246.88	24899.60	1.18	1.18	VV	13.4639
10	3.238	379379.91	19233.27	1.33	1.33	VB	19.7252
11	4.996	18547145.59	454700.48	65.13	65.13	BV	40.7898
12	6.770	7853041.36	178456.95	27.58	27.58	VB	44.0052
14	9.434	166936.20	11211.21	0.59	0.59	BV	14.8901
15	9.630	412791.35	12225.85	1.45	1.45	VB	33.7638
		28478210.03	772751.22	100.00	100.00		

Step 4

2-Oxo-1,2-diphenylethyl[7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl]methanesulfonate (5)





1. M. S. Kerr, J. Read de Alaniz and T. Rovis, *J. Org. Chem.*, 2005, **70**, 5725-5728.