Synthesis of paracetamol by acetylation

Supplementary Material

The object of the present experiment is to synthesize an aromatic amide by addition-elimination reaction with acetic anhydride. This organic experiment is done in our laboratory for more than 20 years (approximately 200 students per year) without any difficulty or problem and has the advantage to introduce students to the medicinal chemistry field. As a matter of fact this is normally their first synthesis of a pharmaceutical active ingredient which always generates great motivation and expectation. In addition, it can also be adequate to introduce the topic of paracetamol (acetaminophen) toxicity, as an acute overdosage is relatively common and may cause severe liver damage, although it is an analgesic widely used to treat mild to moderate pain, and often found in familial pharmacies.[1,2,3,4]

Synthesis and crystallization

As a pre-lab assignment, students should make a table showing the physical properties (molecular formula, molecular mass, purity grade of the reagents, m.p., b.p., solubility, and theoretical and used mass in grams and moles) of 4-aminophenol, acetic anhydride (etanoic anhydride) and paracetamol. This table must have a footnote with the corresponding bibliography sources. In addition, the instructor can ask students to draw a chart as the one shown in Figure SM 3.1.1.1**.**

The reaction is done in an Erlenmeyer flask but, if possible, it can take place in a round-bottom flask with a reflux condenser. If not, and for security reasons, students must previously fix the Erlenmeyer with a clamp holder to a universal support or hold it with a wood clamp (Figure SM 3.1.1.2a). In this last situation, the reaction can be done without magnetic stirring. To reduce class time, water baths should be provided already near boiling. It should be noted that extending the time of the reaction may lead to the formation of the diacetylated derivative of 4-aminophenol.

Note that acetic anhydride should be added to the aqueous suspension of paracetamol.

After completion of the synthesis, the crude solution often appears slight yellow or pinkish. A good filtration and washing with cold water is mandatory as it is the quantitative recovery of the product (Figure SM 3.1.1.2b).

During the hot dissolution of paracetamol for crystallization the solution can also be coloured and the use of activated charcoal does not greatly improve the situation. However, slow crystallization and a careful wash of the crystals allow obtaining pearly crystals (Figure SM 3.1.1.2c) and ensure a good melting point. In addition, 4-aminophenol can also be crystallized before synthesis takes place.

Crystallization of paracetamol occurs easily when the solution cools. Nevertheless, if crystallization doesn't occur or occurs with difficulty, it may be induced with a glass rod by gently rubbing the inside surface of the crystallization vessel. With this option, crystallization is almost immediate but very small crystals are formed.

After cooling to room temperature, the crystallization vessel is placed in an ice bath for some minutes.

As the washing of the crystals is carried out with water, they will be placed in an oven with appropriate temperature and/or stored in the desiccator until constant weight. As a rule, and for the objectives pursued, we consider constant weight a difference of less than 5 mg between two weightings with intermediate drying. The yields range from 35% to 70%.

If necessary, class time can be reduced by performing crystallization (and crystals wash) with hexane (or petroleum ether). This will decrease the product drying time to constant weight. In this situation, attention should be drawn to the fact that it is more difficult to perform the crystallization by first year students as the volatile solvent will evaporate during the operation. But, if this is the option, students should be encouraged to think about what to do for the solvent elimination which must be collected in a conveniently labelled non-halogenated organic solvent container for posterior treatment and recovery.

The yields, TLC (Fig. SM 3.1.1.3a and b), m.p., and IR spectra (Fig. SM 3.1.1.4a and b), are registered and the results discussed. If student's background allows it, the purity and structure elucidation of the product may also be evaluated by ^{1}H- and ^{13}C-NMR spectra (Fig. SM 3.1.1.5. a and b).

If paracetamol m.p. is unsatisfactory (169-170.5 ºC),[5] it may be speculated if the reaction was complete or if the diacetylated derivative was formed. To purify a product containing the diacetylated derivative, dissolve the crystals in 10% NaOH, v/v (cold dilute alkali will not hydrolyze the amide bond of acetyl but only the ester) and reprecipitate with 10% HCl (v/v).[6]

The IR spectra (KBr pellet) were collected on an IR Affinity-1 Shimadzu spectrophotometer. The IR spectrum of 4-aminophenol shows the two *N*-H amine bands at 3340 and 3282 cm^{-1}, emerging from the broadband of the phenolic OH (Fig. SM 3.1.1.4a). On the other hand, the IR spectrum of paracetamol (Fig. SM 3.1.1.4b) shows the *N*-H amide band near 3325 cm^{-1} although it is on top of the broad band of phenolic O-H that is at its right. Other important informative bands are the appearance of the amide carbonyl band at 1654 cm^{-1} and *N*-H band at 1564 cm^{-1}.

The ^{1}H-NMR spectrum of paracetamol (Fig. SM 3.1.1.5a) shows signals with chemical shifts in agreement with the proposed structure and with the literature data. In the aromatic region, the four signals are indicative of a 1,4-substituted aromatic ring with two different substituents: two upfielded

singlets of the NH (δ=9.68 ppm) and OH (δ=9.14 ppm), and two downfielded ortho-coupled doublets of the aromatic protons at δ=7.35 and δ=6.68 ppm. The observation of a large singlet, integrating for three protons at δ=1.97 ppm, corresponds to the methyl protons (Fig. SM 3.1.1.5a). DMSO (δ=3.4 ppm) and water contamination (δ=2.5 ppm) can also be observed in the spectra provided.

^{13}C-NMR spectrum of paracetamol reveals four signals in the aromatic region: one C-OH at 153.56 ppm, one C-NH at 131.49 ppm and two pairs of equivalent C-H (121.24 and 115.44 ppm). A deshielded carbonyl carbon at 167.96 ppm, and the methyl carbon at 24.20 ppm (Fig. SM 3.1.1.5b) are also observed.

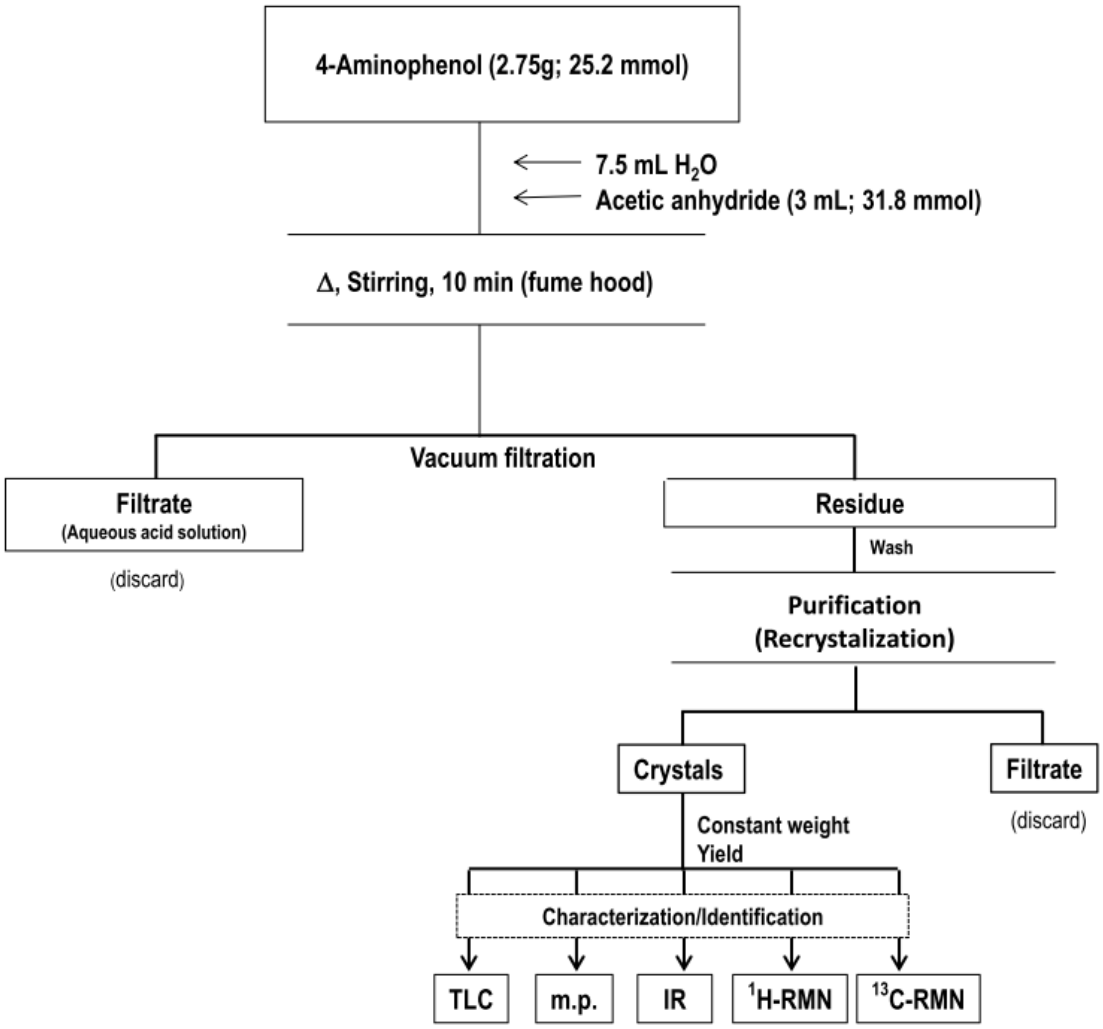


Figure SM 3.1.1.1. Flowchart for the synthesis, purification and characterization of paracetamol.

a)

b)

c)

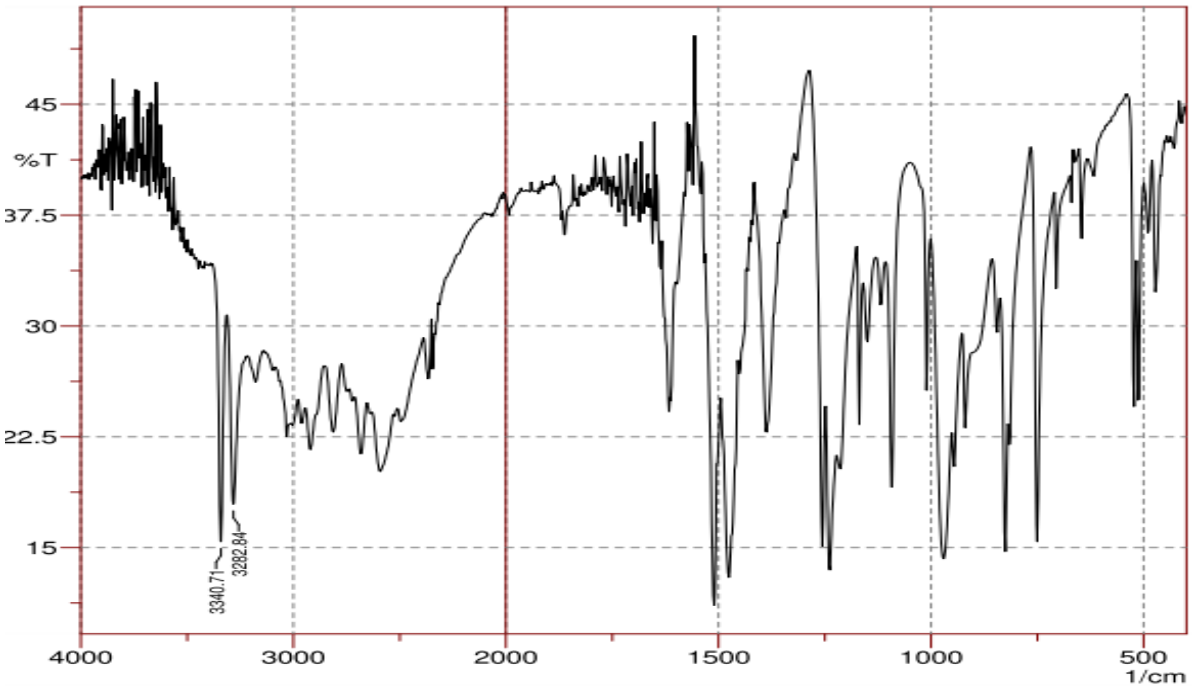
Figure SM 3.1.1.2. a) Holding the Erlenmeyer with a wood clamp. **b)** Washing and quantitative recovery of the crude material. **c)** Product recovery and pearly crystals of paracetamol after crystallization.

a) **b)**

Figure SM 3.1.1.3. a) Preparing 4-aminophenol and paracetamol solutions for TLC. **b)** Visualization of the TLC plate in UV_{254} with (A) Standard 4-aminophenol, (B) Standard paracetamol and (P) Product of the synthesis.

IR spectra

a)



b)

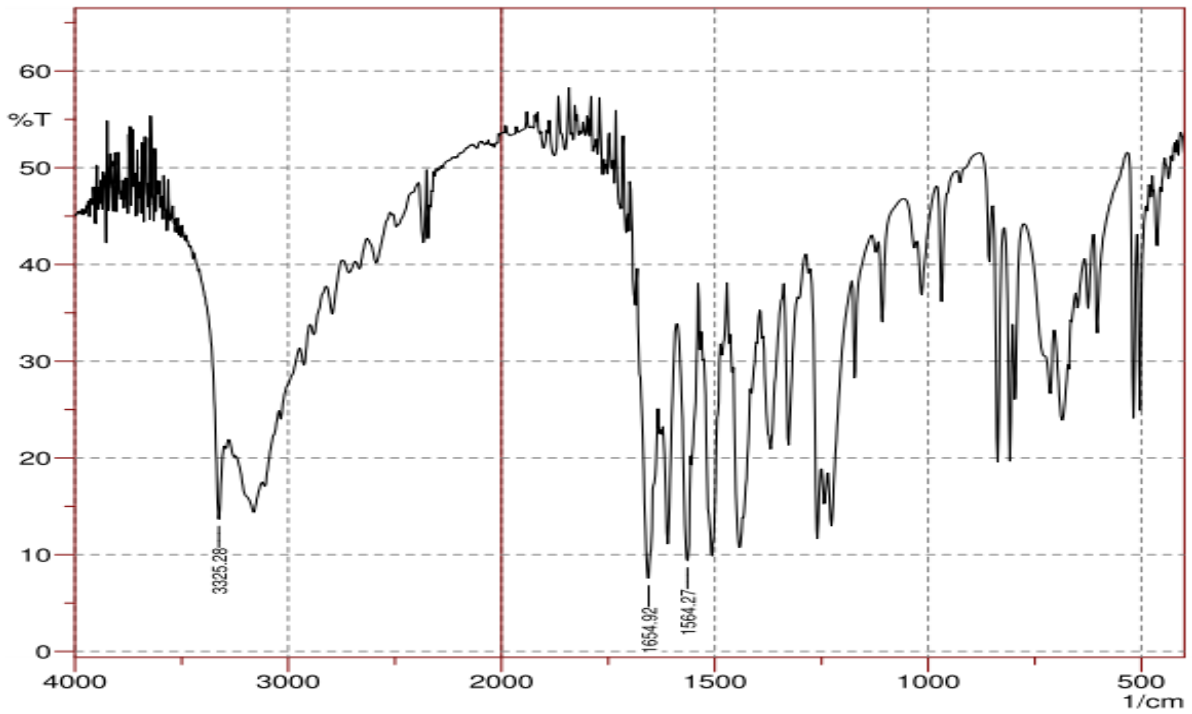
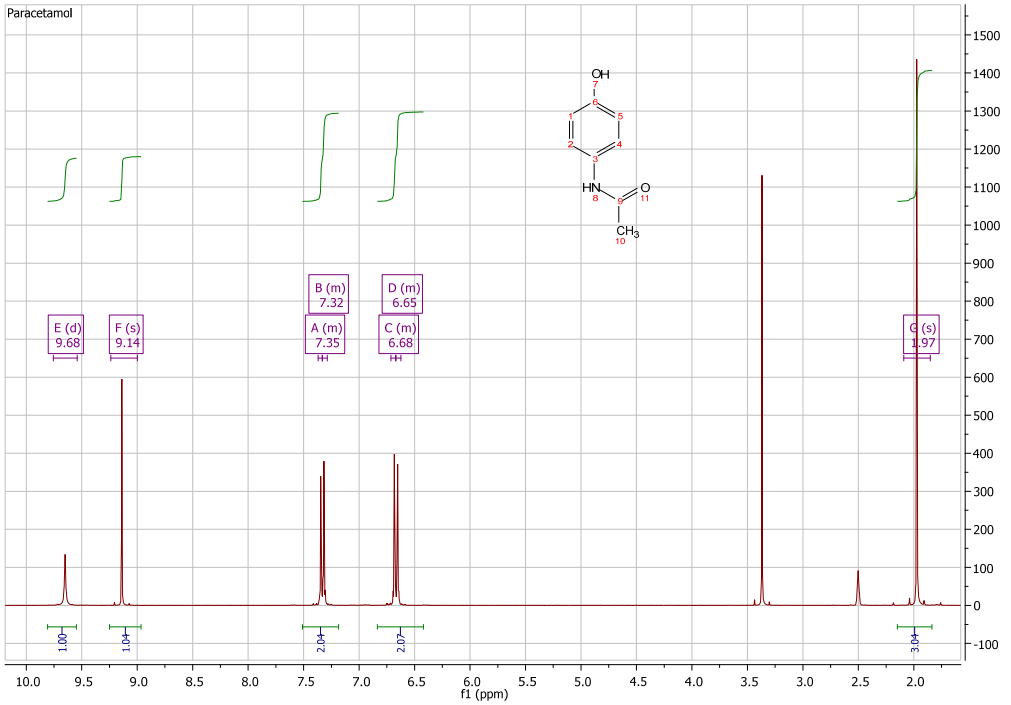


Figure SM 3.1.1.4. Infrared spectra of **a)** 4-aminophenol and **b)** paracetamol (KBr pellet).

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

a)



b)

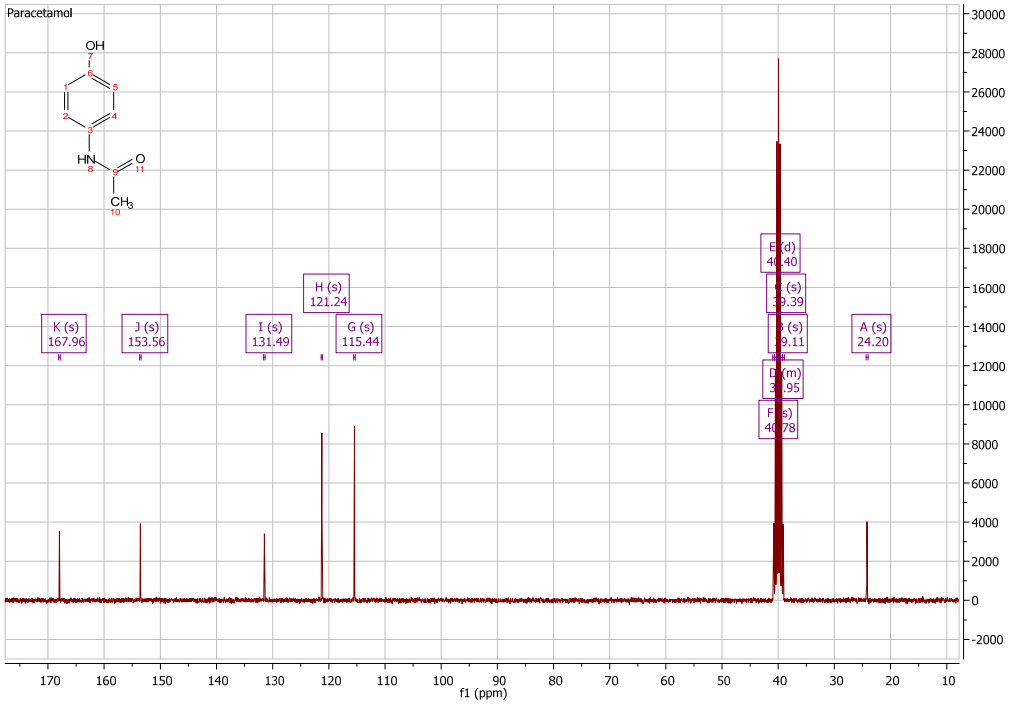


Figure SM 3.1.1.5. a) ^{1}H-NMR spectrum (300 MHz) and **b)** ^{13}C-NMR spectrum (75 MHz) of paracetamol dissolved in DMSO d_6 (≈40.00 ppm).

References

[1] *Martindale, The Complete Drug Reference*, ed. S. C. Sweetman, Pharmaceutical Press, London, 37th ed., 2011, volume A, p. 112.

[2] A. C. Moffat, M. D. Osselton, and B. Widdop (eds), *Clarke's Analysis of Drugs and Poisons*, Pharmaceutical Press, London, 4th ed., 2011, volume 2, pp. 1856-1858.

[3] G. G. Graham, M. J. Davies, R. O. Day, A. Mohamudally, and K. F. Scott, *Inflammopharmacology* 2013, 21, 201-232.

[4] J. A. Hinson, D. W. Roberts, and L. P. James, *Handbook of Experimental Pharmacology*, *Adverse Drug reactions*, Springer, Berlin, 2010, chapter 12, 369-405. doi:10.1007/978-3-642-00663-0_12

[5] *The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals*, Merck and Co., Whitehouse Station NJ, 14th ed., 2006, p. 9 and p. 78.

[6] A. I. Vogel, *Vogel's Textbook of Practical Organic Chemistry*, Longman Scientific & Technical, New York, 5th ed., 1989, Experiment 6.109, p. 985.

Synthesis and characterization of *N,N'*-dicyclohexyl-*N,N'*-dimethyl-propan-1,3-diamide

Supplementary Material

Experiment Notes for Instructors

The synthesis of *N,N'*-dicyclohexyl-*N,N'*-dimethyl-propan-1,3-diamide relying on the addition-elimination reaction of *N*-cyclohexylmethylamine to dimethyl malonate can be considered a classic and simple reaction in synthetic organic chemistry; accordingly, the involved experimental procedures are straightforward, and adequate for students basically acquainted with the main techniques adopted in any organic chemistry lab.

This experiment has been repeated several times in the authors' research lab, but typically involving higher quantities than the ones proposed herein. Students should be warned that if they allow the reflux temperature go above 150ºC, they are likely to achieve lower yields. The yields of crude *N,N'*-dicyclohexyl-*N,N'*-dimethyl-propan-1,3-diamide are usually in the range 70-75%, reducing to 55-60% after recrystallization. Approximate records have been obtained for a total time reaction of 1 hour and 30 minutes, including the distillation step. As suggested in topic 4 of the experimental procedure, the extent of the reaction can be monitored by FTIR, because the stretching vibrations of the carbonyl groups of the diester (reagent, 1750 cm^{-1}) and diamide (product, 1650 cm^{-1}) are easily distinguishable, allowing a possible shorter time for the reflux. If the *N*-cyclohexylmethylamine flask has been opened for some time, revealing a darker and brownish appearance, the final yields of the amide product show tendency to decrease. Cold ethyl acetate readily solubilizes the coloured impurities from the bulk *N,N'*-dicyclohexyl-*N,N'*-dimethyl-propan-1,3-diamide, and it is simultaneously adequate as recrystallization solvent, since it dissolves the product when hot, and promotes its crystallization upon cooling.

This experiment can be completed in a 4h session providing the students are already basically acquainted with the involved techniques and procedures. If that is not the case, an additional session of about 2h may be necessary (for instance, the recrystallization and characterization techniques may be left to the second session). Furthermore, the overall experiment bears an interesting degree of versatility; after a simple synthesis, the instructor can enhance the level of study to intermediate if requiring a detailed analysis of the ^{1}H NMR spectrum.

The melting point ranges observed for this compound are rather variable in supporting literature. Reference 5 of the protocol reports a range of 117-118ºC, whereas 111-112ºC has been found by researchers at the authors' lab (reference 3). Some lower melting point values, varying between 106-109ºC, have recently been determined in our lab, but chromatographic and spectroscopic data point out unequivocally to the existence of the desired compound with a high purity grade. Assuming that all the involved melting-point apparatus are properly calibrated, the observed behaviour may suggest that

the presence of small impurity traces in the synthesized *N,N'*-dicyclohexyl-*N,N'*-dimethyl-propan-1,3-diamide seems to decisively affect the melting point or, alternatively, different crystalline forms of the solid are eventually being produced.

Photos of the Experiment

Figure SM 3.1.2.1 – Simple distillation for methanol removal.

Figure SM 3.1.2.2 – Reflux after distillation.

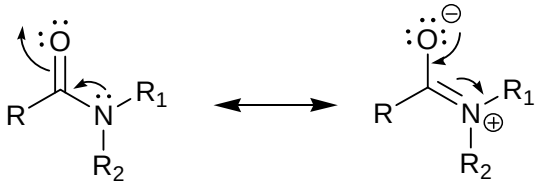
Figure SM 3.1.2.3 – Appearance of the reaction mixture just before crystallization.

Figure SM 3.1.2.4 – Final appearance of the product after recrystallization.

FTIR and ^{1}H NMR Spectroscopic Data

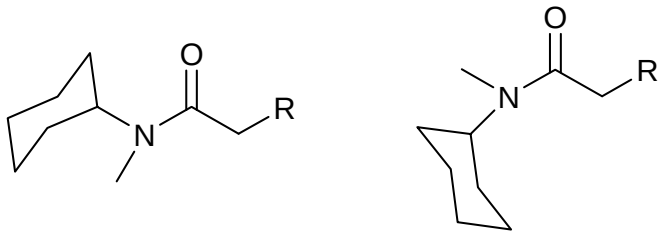
The C=O stretching vibration of the FTIR spectrum of *N,N'*-dicyclohexyl-*N,N'*-dimethyl-propan-1,3-diamide appears in the range 1650-1652 cm^{-1}.

The correspondent ^{1}H NMR spectrum of this tertiary amide puts in evidence a phenomenon, typical for this sort of compound, related with the "partial" character of double bond for the C-N. The two most important resonance forms for tertiary amides are displayed in Scheme SM 3.1.2.1.



Scheme SM 3.1.2.1 – Resonance forms of tertiary amides (R, R_1 and R_2: alkyl groups).

The contribution of the second resonance form leads to a stronger and more rigid C-N bond, and accordingly, with less conformational flexibility. Depending on the effects caused by the substituents R_1 and R_2, the rotation can be more or less rapid; for the former case, the peaks assigned to the protons of those substituents only suffer an enlargement. However, if rotation along the C-N axis is relatively slow for the NMR time scale, the proton signals multiply, reflecting all possible conformations the molecule can adopt. Considering one C-N bond only, the usual classification given to these conformers is *syn* (when the more complex substituent is on the same side as the oxygen atom) and *anti* (when the more complex substituent and the oxygen atom are in opposite sides) – see Scheme SM 3.1.2.2.



Scheme SM 3.1.2.2 – Examples of *syn* and *anti* conformations, respectively.

For *N,N'*-dicyclohexyl-*N,N'*-dimethyl-propan-1,3-diamide, a symmetric tertiary 1,3-diamide, a few possible conformers may exist; that is why all the proton signals suffer the correspondent multiplication.

The ^{1}H NMR spectrum of *N,N'*-dicyclohexyl-*N,N'*-dimethyl-propan-1,3-diamide is depicted in Figure SM 3.1.2.5.

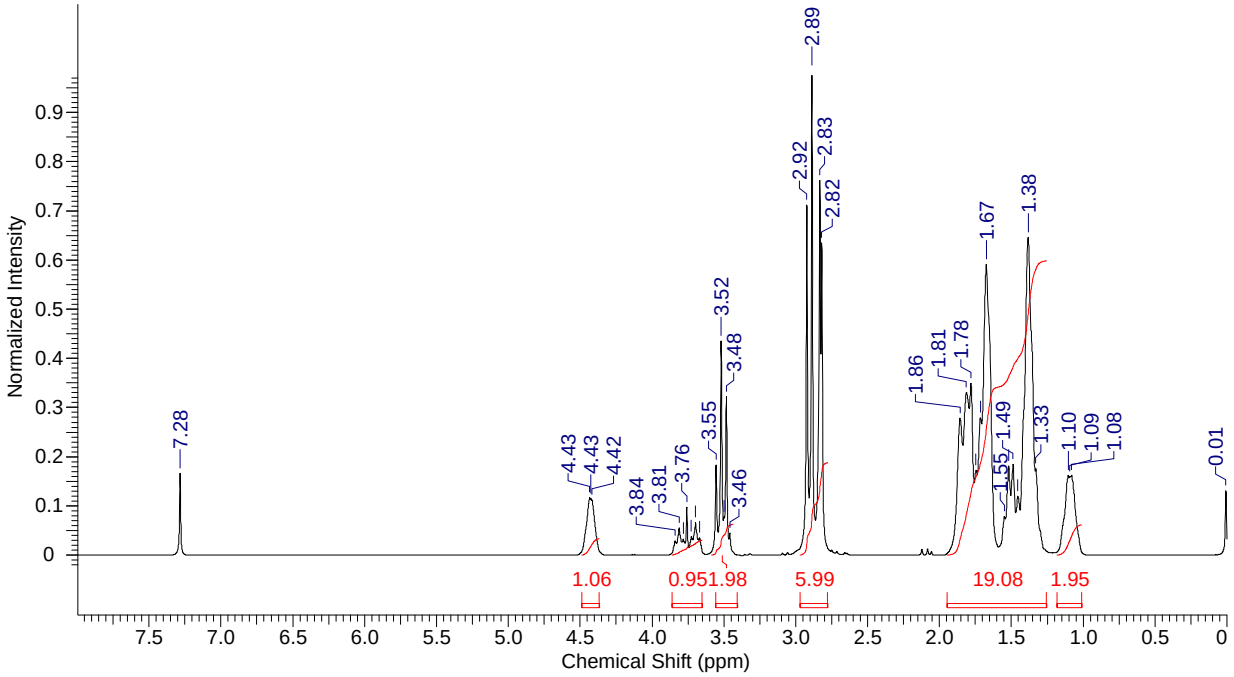


Figure SM 3.1.2.5 – ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of *N,N'*-dicyclohexyl-*N,N'*-dimethyl-propan-1,3-diamide.

Figures SM 3.1.2.6 and SM 3.1.2.7 show amplifications of specific parts of the ^{1}H NMR spectrum of *N,N'*-dicyclohexyl-*N,N'*-dimethyl-propan-1,3-diamide, for a better visualization of the signals multiplication.

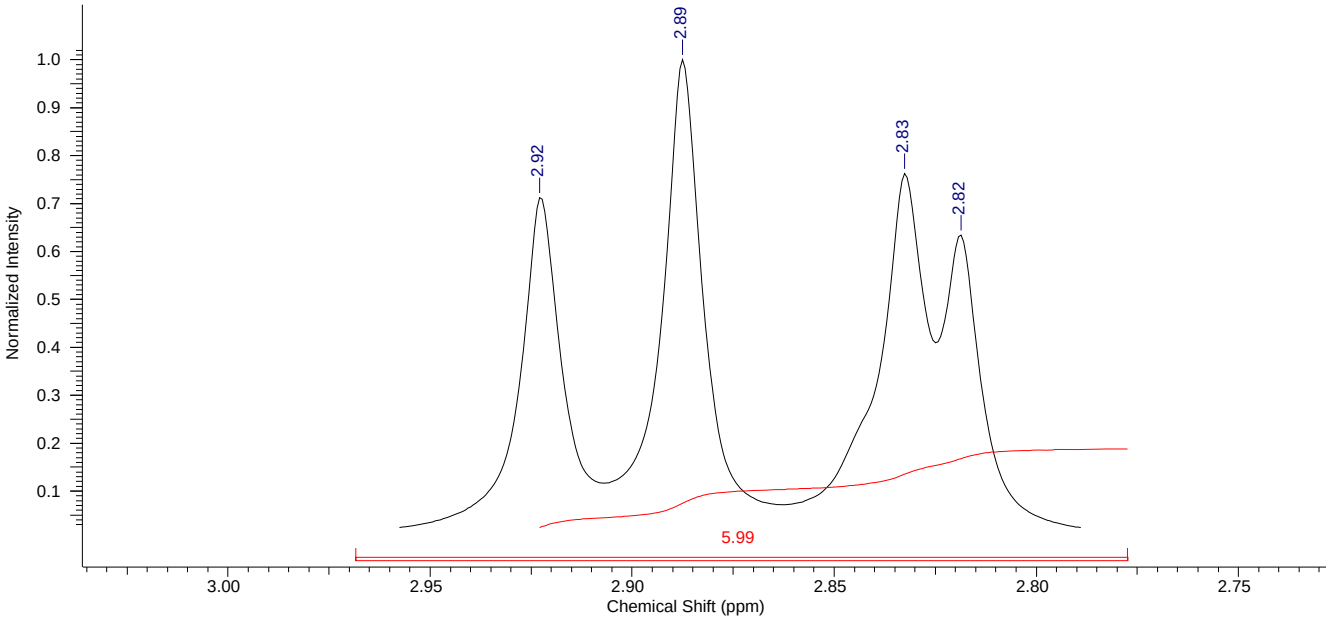


Figure SM 3.1.2.6 – ^{1}H NMR signals of the methyl groups attached to the nitrogen atoms (400 MHz, $CDCl_3$).

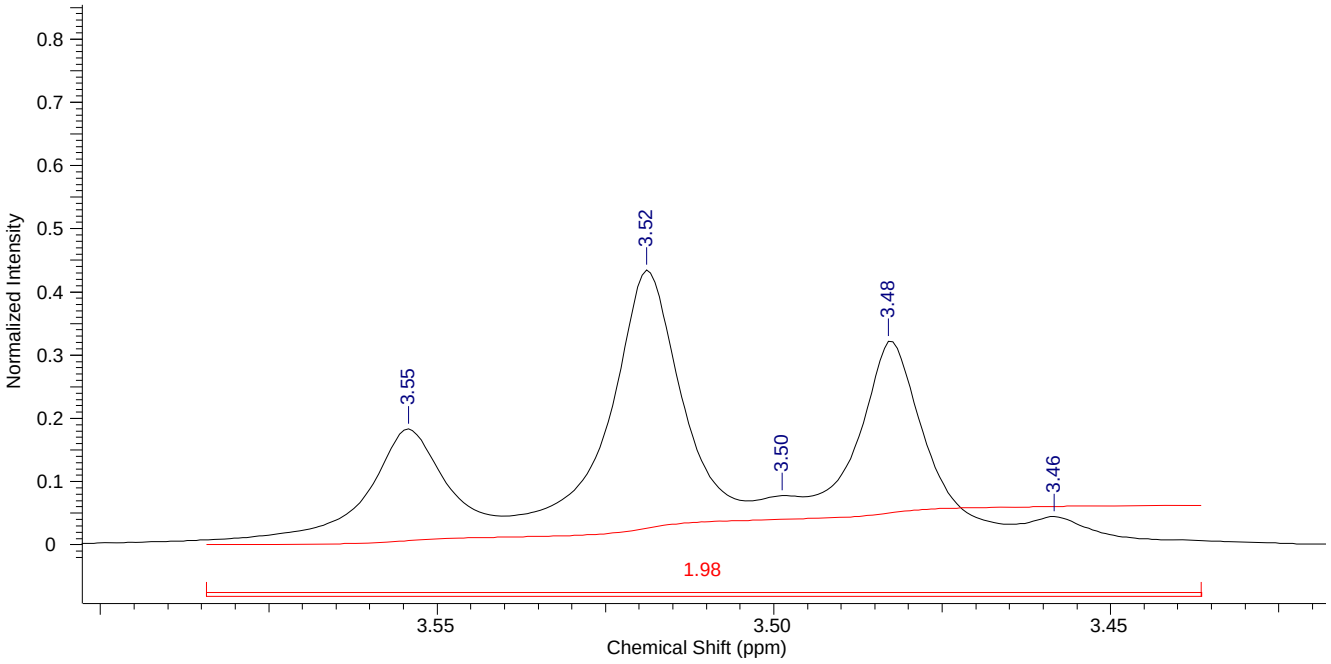


Figure SM 3.1.2.7 – ^{1}H NMR signals of the methylene protons between the two carbonyl groups (400 MHz, $CDCl_3$).

The same phenomenon (multiplication of signals) can obviously be observed in the correspondent ^{13}C NMR spectrum as well. As an example, an amplification of the region of the carbonyl groups is depicted in Figure SM 3.1.2.8.

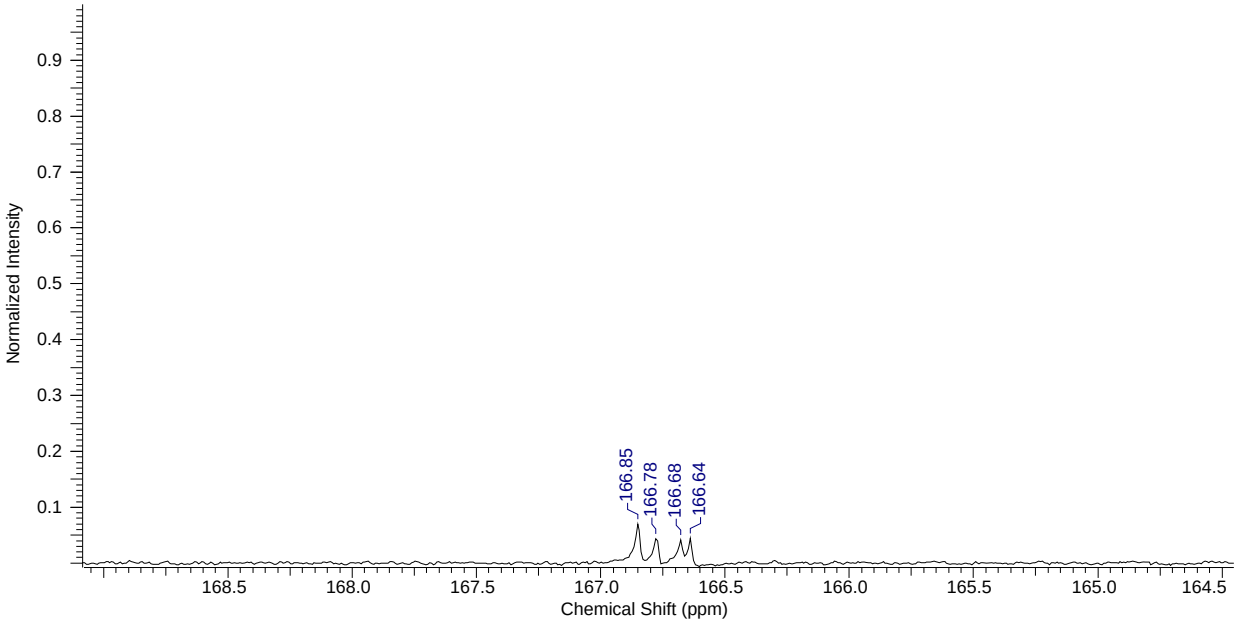
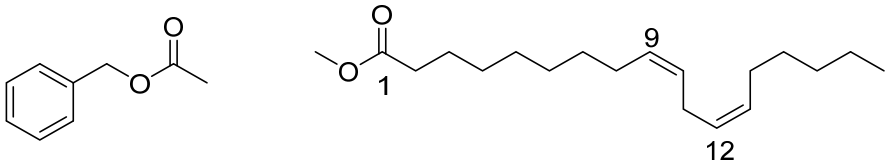


Figure SM 3.1.2.8 – ^{13}C NMR signals of the carbons of the two carbonyl groups (100 MHz, $CDCl_3$).

Synthesis and characterisation of an ester from 4-nitrobenzoyl chloride

Supplementary Material

This experiment is aimed at first year undergraduate students who have had training in basic synthetic organic chemistry work. The corresponding lecture course would be expected to cover core organic chemistry, including carbonyl chemistry. This exercise allows students to practice assembly of appropriate glassware for heating under reflux, liquid-liquid separation and vacuum filtration. The purification step involves recrystallistion of products which are readily crystallised, and analysis by melting point and NMR spectroscopy. This experiment has been used routinely in laboratory sessions for 35-45 first year undergraduate students at a time (total class sizes ranging from 140-185 students). In the laboratory manual introduction, the students are introduced to the best known routes for preparation of esters (Fischer esterification and nucleophilic substitution of acyl halides) and some topical examples (Scheme SM 3.1.3.1).



Benzyl acetate
(isolated from Jasmine)

methyl Z,Z-9,12-octadecadienoate
or "methyl linoleate"
(component of biodiesel)

Scheme SM 3.1.3.1 Example ester containing structures

The students do not necessarily need to know the identity of the ester product they will prepare; the alcohols can be labelled as unknowns A, B and C and the students can be asked to identify the product and hence identify the alcohol they started with. A table of melting points can be provided to aid the students, the examples in table SM 3.1.3.1 allow the students to narrow the candidates down after comparison with their observed melting points but in addition they require their NMR results to unambiguously identify the product they have made.

Ester	Melting Point
sec-butyl 4-nitrobenzoate	25°C
propyl 4-nitrobenzoate	36°C
n-butyl 4-nitrobenzoate	37°C
ethyl 4-nitrobenzoate	57°C
iso-butyl 4-nitrobenzoate	70°C
benzyl 4-nitrobenzoate	85°C
iso-propyl 4-nitrobenzoate	108°C
phenyl 4-nitrobenzoate	127°C

Table SM 3.1.3.1 – Selected melting points of alkyl-4-nitrobenzoates

General notes for preparative steps.

The students can select alternative heating sources if available. The experiment has been tested with steam baths, oil baths, heating mantles and aluminium heating blocks. The main procedure uses anti-bumping granules to control excessive reflux, however a magnetic stirring bar can be used instead if desired. It has been found more convenient to supply the students with a pre-weighed sample of the 4-nitrobenzoyl chloride in a sample vial labelled with the precise mass of the contents. The reaction should be allowed to cool to room temperature before any attempting to transfer the flask contents to the separating funnel and rinsing with diethyl ether. The liquid-liquid extraction step should be supervised carefully since novice students tend to shake the funnel contents too hard, the extraction mixture releases carbon dioxide as the residual HCl gas is neutralised. In all cases it is important that there is access to an efficient rotary evaporator to ensure any residual alcohol is removed from the crude product. Any residual alcohol can interfere with the purification steps and the corresponding signals will be visible in the NMR spectra. The work-up and purification is slightly different depending on the alcohol used in the reaction, specific notes and instructions are provided. Typical yields obtained in all three cases are in the 40-80% range. Lower isolated yields usually result from poor recrystallization technique and transfer losses when insufficient care is taken.

Ethyl 4-nitrobenzoate (from unknown alcohol A) – Precipitation of colourless material can occur when the sodium bicarbonate solution is added to the reaction mixture. Addition of 30-40 mL of water and gentle shaking should be sufficient to dissolve any precipitate formed. Once the ether layer is concentrated and dried, the crude product should be recrystallized from aqueous ethanol (10 -15 mL total volume of 1:1 EtOH: H_2O should typically be enough).

Isopropyl 4-nitrobenzoate (from unknown alcohol B) – Precipitation of colourless material can occur when the reaction mixture is cooled to room temperature, this material is soluble in diethyl ether and remains dissolved when sodium bicarbonate solution is added. Once the ether layer is concentrated and dried, the crude product should be recrystallized from ethanol.

Propyl 4-nitrobenzoate (from unknown alcohol C) – Precipitation is not normally observed when the reaction mixture is cooled or when sodium bicarbonate solution is added. The product in this case is a low-melting solid (~ 36°C), the crude will probably be observed as an oil in the rotary evaporator flask so it should be allowed to stand for several minutes to solidify before transfer to a vial or flask. If the product does not solidify, this is usually due to residual 1-propanol being present, this can be removed by reconnecting the flask to a rotary evaporator.

NMR samples and assignments

All three products are highly soluble in deuterochloroform so there should be no problems obtaining ^{1}H NMR spectra. If ^{13}C NMR spectra are desired, the products are soluble enough to give saturated solutions. Assignments of spectra (and copies all the NMR spectra are provided) in the “Answers to additional questions” section.

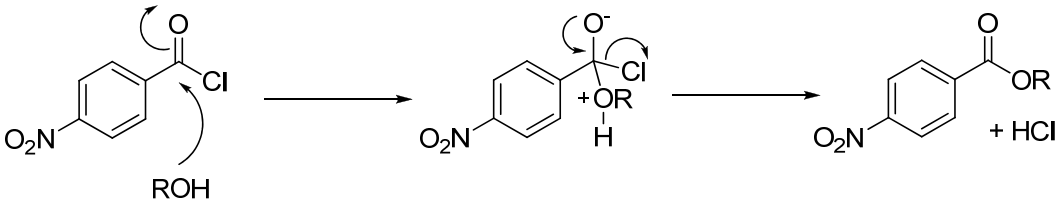
Answers to additional questions

1. Interpret the ^{1}H NMR spectrum you obtained. Use the NMR spectrum and melting point to confirm the structure of your compound unambiguously and explain why the spectrum fits the proposed structure.

The ^{1}H and ^{13}C NMR spectra (see Figures SM 3.1.3.1 – SM 3.1.3.6, in conjunction with melting points) allow unambiguous identification of all three products. The ^{1}H spectrum of the ethyl ester shows a characteristic triplet/quartet pattern which is consistent with the ethyl group. The ^{13}C spectrum of the ethyl ester has diagnostic signals at 61.9 and 14.2 ppm with correspond to the CH_2 and CH_3 group respectively. The ^{1}H spectrum of the isopropyl ester shows a characteristic septet/doublet pattern which is consistent with the isopropyl CH and CH_3 groups. The ^{13}C spectrum of the isopropyl ester has two signals attributed to the alkyl group, these can be distinguished from the ethyl example since the CH and CH_3 groups are observed at higher chemical shift (69.7 and 21.8 ppm respectively). The ^{1}H spectrum of the propyl ester shows three signals as would be expected (2 triplets and a sextet). The ^{13}C spectrum of the propyl ester has three diagnostic signals at 67.5, 22.0 and 10.4 ppm with correspond to the two CH_2 units and the CH_3 group respectively.

The signals for the aromatic regions are virtually identical in each case, both the ^{1}H and ^{13}C NMR spectra are consistent with a symmetrical 4-substituted aromatic system.

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



3. If your reaction is run in tetrahydrofuran as the solvent in the presence of triethylamine, an insoluble white precipitate appears in addition to the soluble product. This precipitate does not

dissolve in most organic solvents, but is soluble in water. Using your mechanistic explanation, identity of this precipitate?

The precipitate is $Et_3NH^+Cl^-$ so is soluble in water and essentially insoluble in most organic solvents.

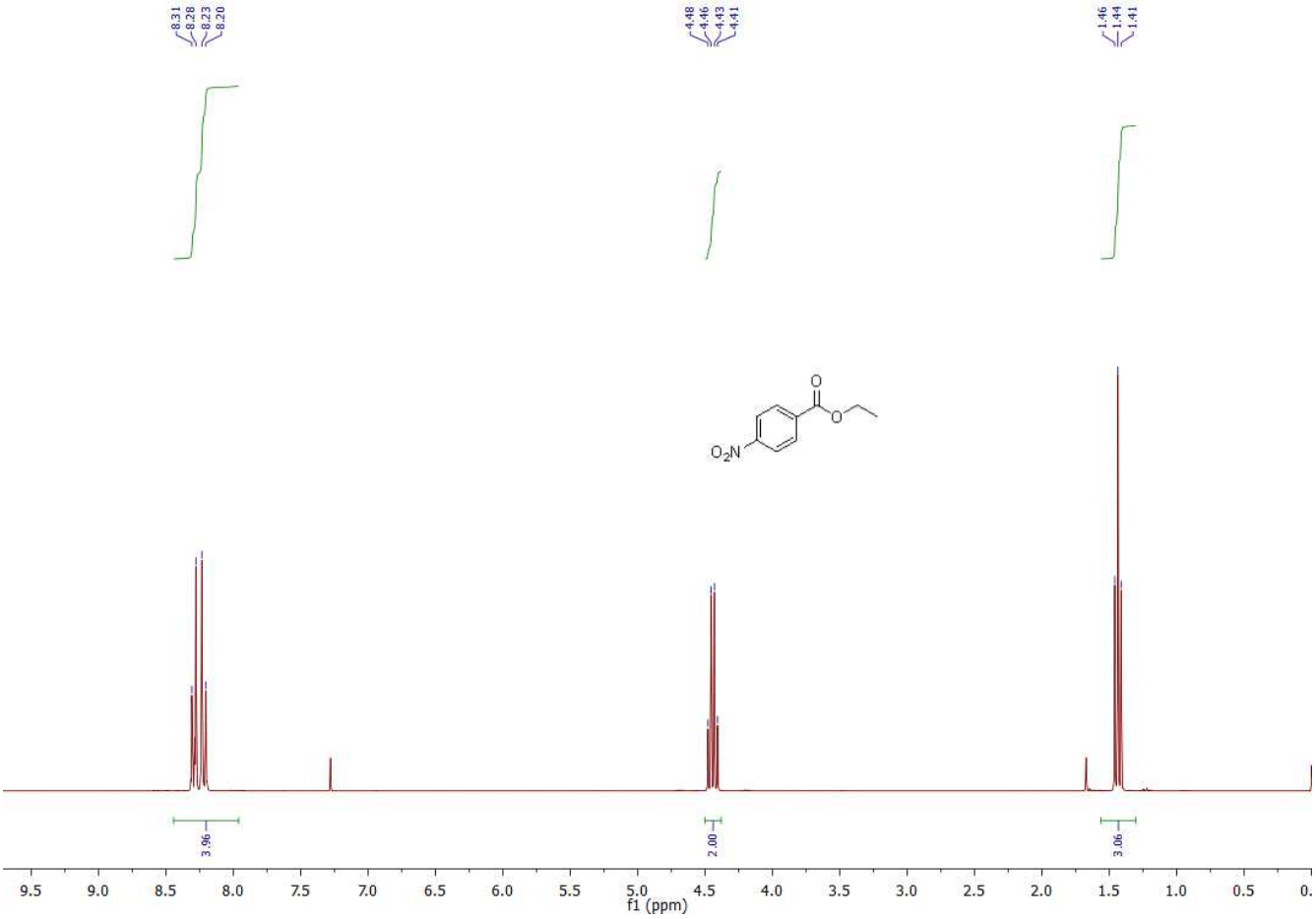
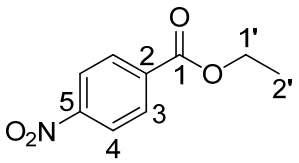


Figure SM 3.1.3.1 – ^{1}H NMR spectrum of ethyl 4-nitrobenzoate (300 MHz, $CDCl_3$)



δ_H (300 MHz, $CDCl_3$, Me_4Si) 8.29 (2H, d, $^4J_{HH}$ = 9 Hz, H-4), 8.22 (2H, d, $^4J_{HH}$ = 9 Hz, H-3), 4.44 (2H, t, $^3J_{HH}$ = 7 Hz, H-1') and 1.44 (3H, q, $^3J_{HH}$ = 7 Hz, H-2').

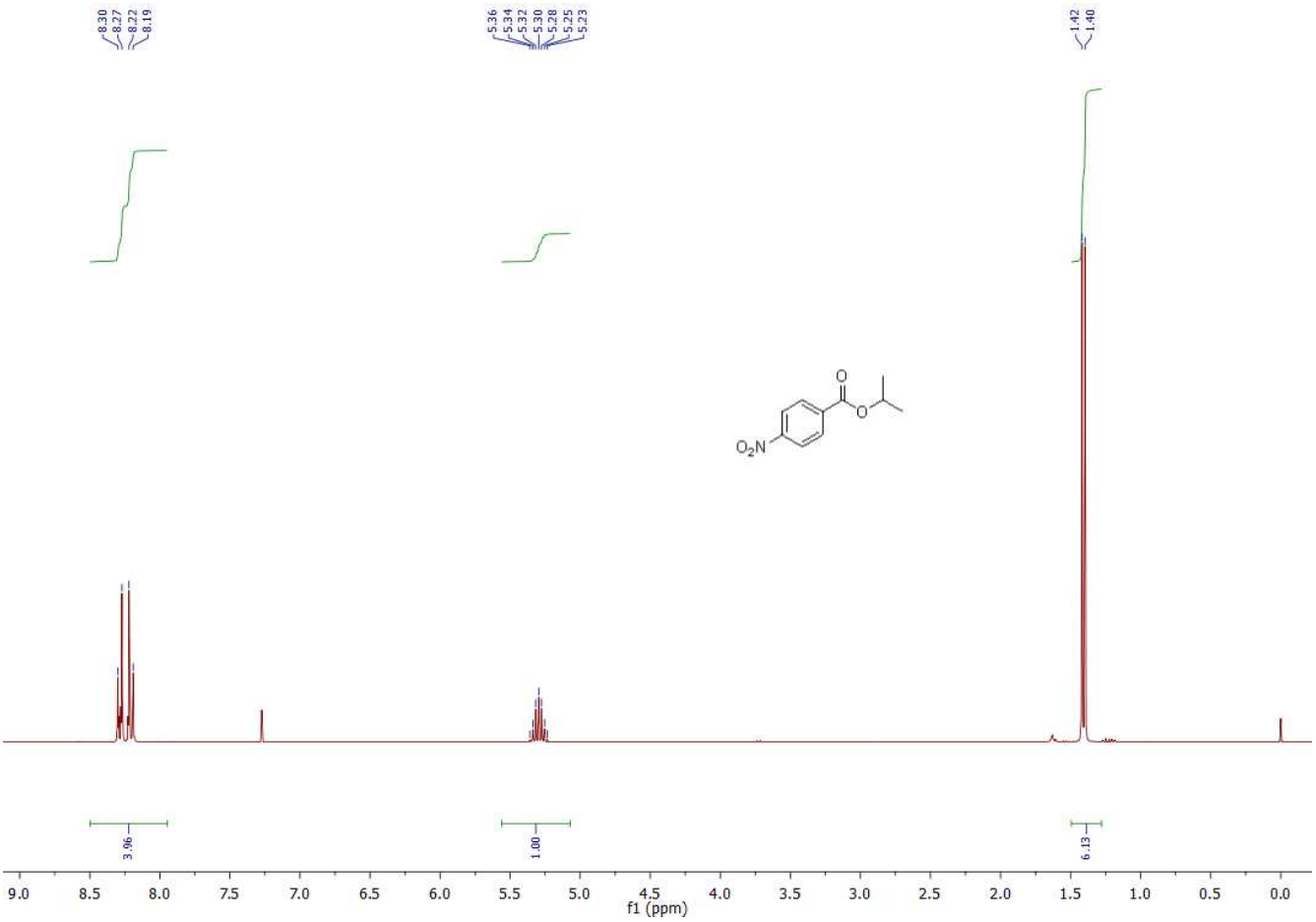
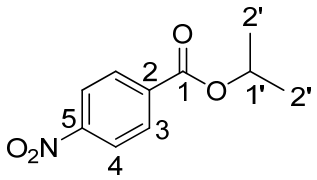


Figure SM 3.1.3.2 – ^{1}H NMR spectrum of isopropyl 4-nitrobenzoate (300 MHz, $CDCl_3$)



δ_H (300 MHz, $CDCl_3$, Me_4Si) 8.28 (2H, d, $^{4}J_{HH}$ = 9 Hz, H-4), 8.21 (2H, d, $^{4}J_{HH}$ = 9 Hz, H-3), 5.30 (1H, sept, $^{3}J_{HH}$ = 6 Hz, H-1') and 1.41 (6H, d, $^{3}J_{HH}$ = 6 Hz, H-2').

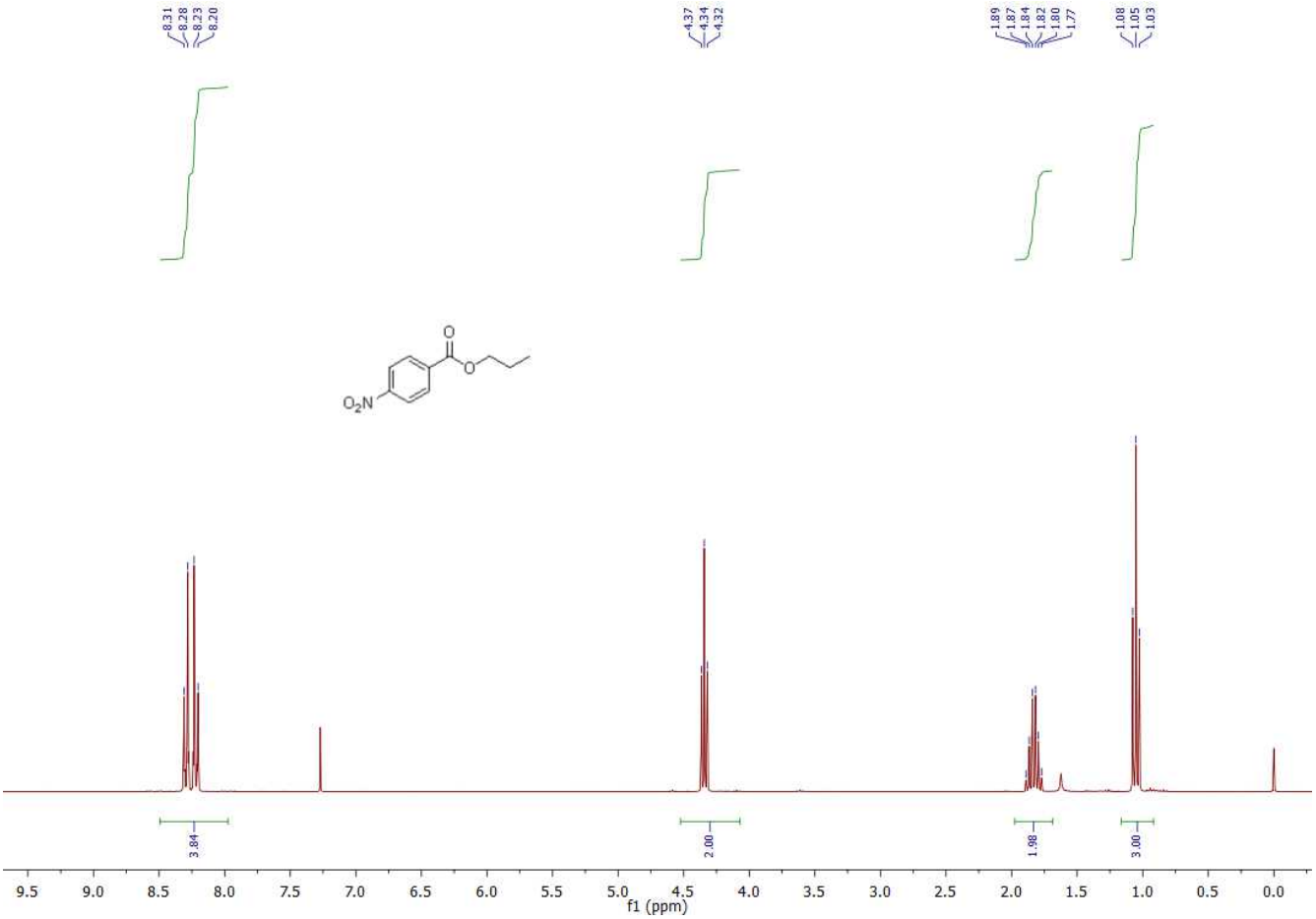
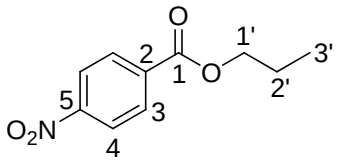


Figure SM 3.1.3.3 – ^{1}H NMR spectrum of propyl 4-nitrobenzoate (300 MHz, $CDCl_3$)



δ_H (300 MHz, $CDCl_3$, Me_4Si) 8.29 (2H, d, $^{4}J_{HH}$ = 9 Hz, H-4), 8.21 (2H, d, $^{4}J_{HH}$ = 9 Hz, H-3), 4.34 (2H, t, $^{3}J_{HH}$ = 7 Hz, H-1'), 1.81 (2H, sext, $^{3}J_{HH}$ = 7 Hz, H-1') and 1.05 (3H, t, $^{3}J_{HH}$ = 7 Hz, H-3').

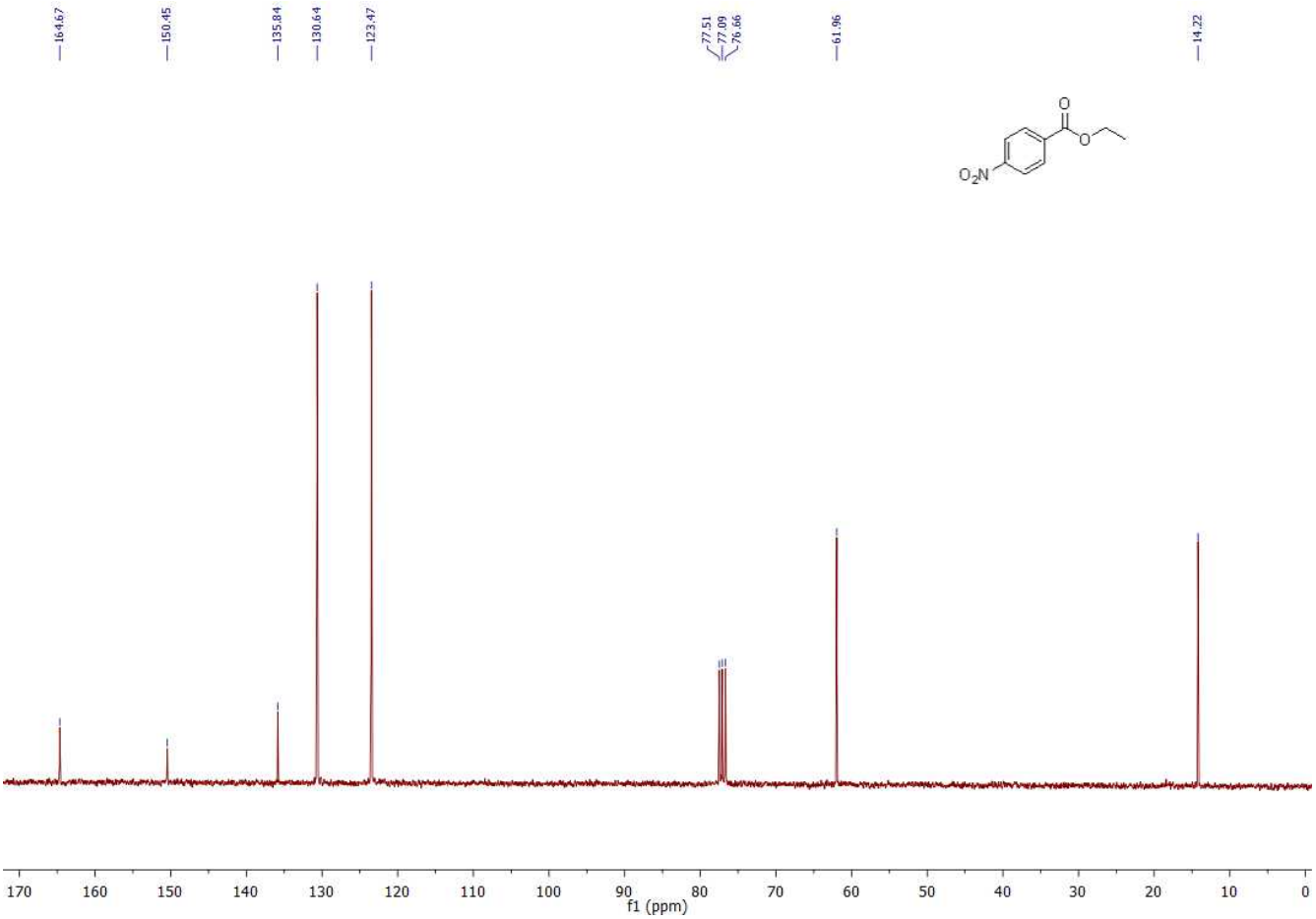
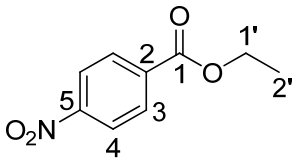


Figure SM 3.1.3.4 – ^{13}C NMR spectrum of ethyl 4-nitrobenzoate (75.45 MHz, $CDCl_3$)



δ_C (75.45 MHz, $CDCl_3$, Me_4Si) 164.7 (C-1, C=O), 150.5 (C-5, 4ry), 135.8 (C-2, CH), 130.6 (C-4, CH), 123.5 (C-3, CH), 61.9 (C-1', CH_2), 14.2 (C-2', CH_3).

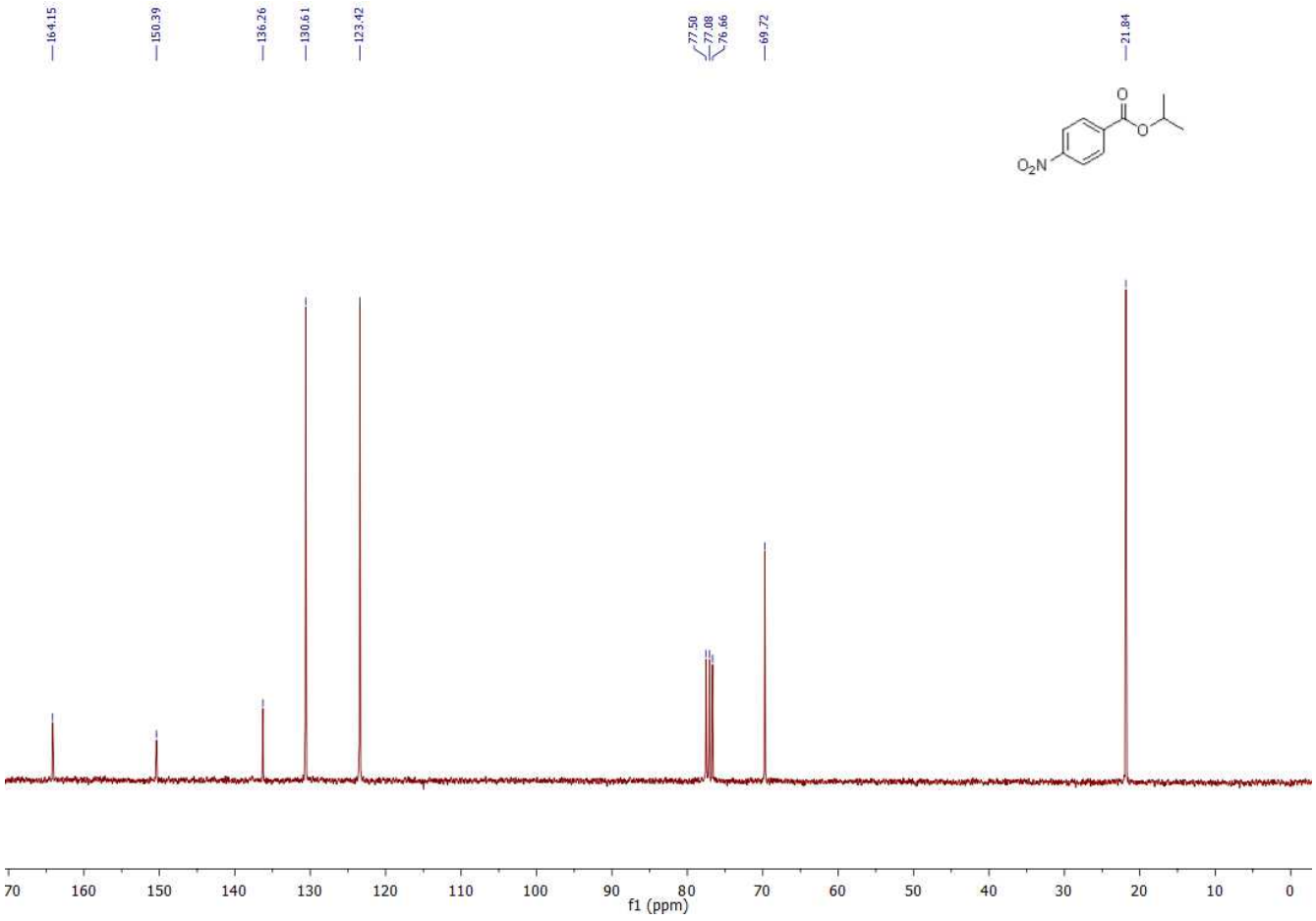
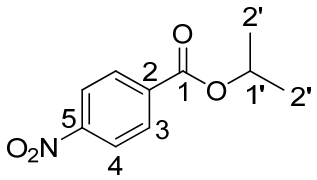


Figure SM 3.1.3.5 – ^{13}C NMR spectrum of isopropyl 4-nitrobenzoate (75.45 MHz, $CDCl_3$)



δ_C (75.45 MHz, $CDCl_3$, Me_4Si) 164.2 (C-1, C=O), 150.4 (C-5, 4ry), 136.3 (C-2, CH), 130.6 (C-4, CH), 123.4 (C-3, CH), 69.7 (C-1', CH_2), 21.8 (C-2', CH_3).

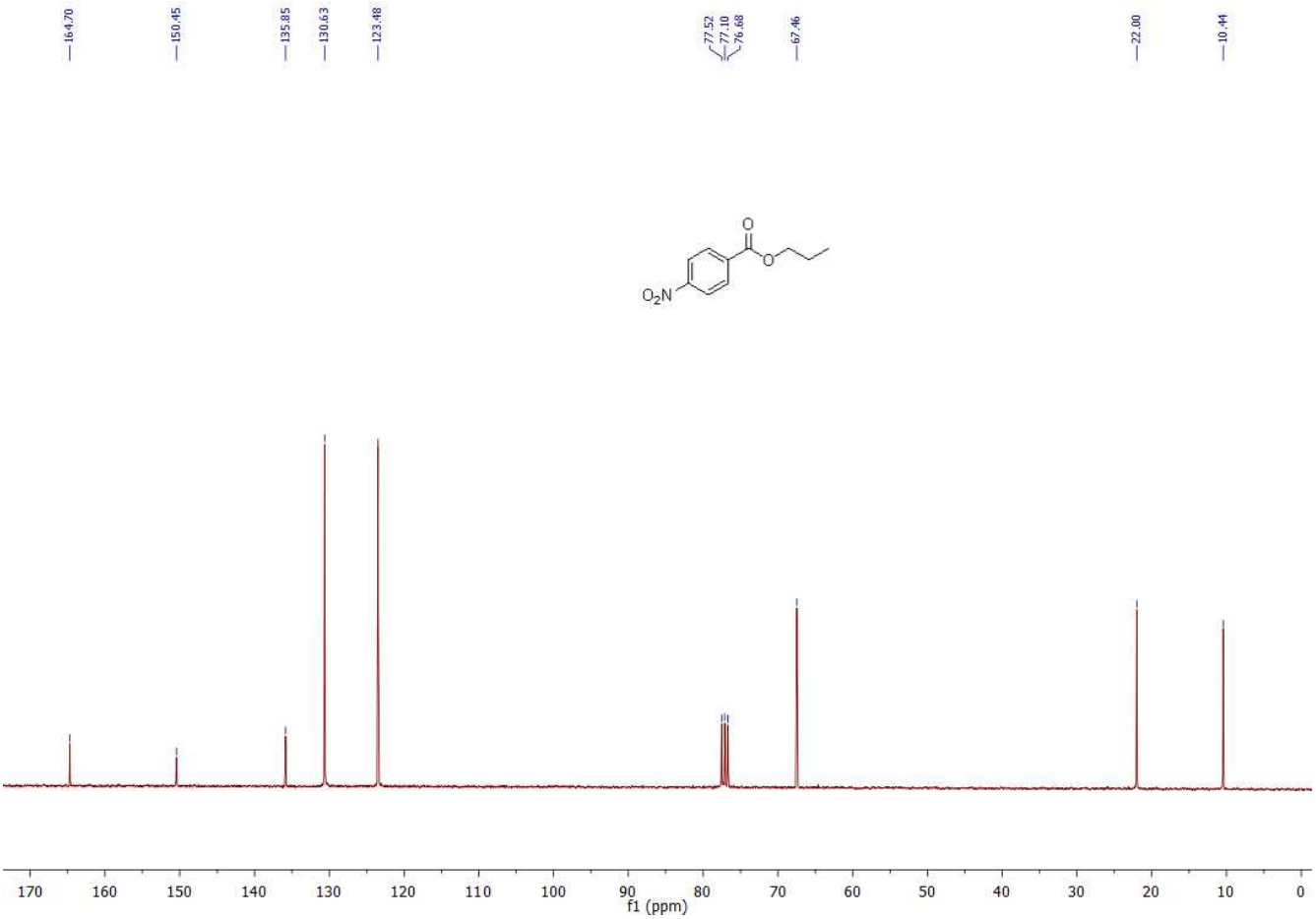
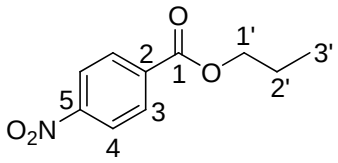


Figure SM 3.1.3.6 – ^{13}C NMR spectrum of propyl 4-nitrobenzoate (75.45 MHz, $CDCl_3$)



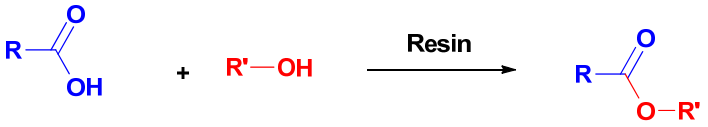
δ_C (75.45 MHz, $CDCl_3$, Me_4Si) 164.7 (C-1, C=O), 150.5 (C-5, 4ry), 135.9 (C-2, CH), 130.6 (C-4, CH), 123.5 (C-3, CH), 67.5 (C-1', CH_2), 22.0 (C-2', CH_2), 10.4 (C-3', CH_3).

Green esterification: the synthesis of aromas in the presence of an acid resin

Supplementary Material

The aim of this experiment is a synthesis of aromas in the presence of heterogeneous catalyst (a commercial resin). This experiment can be done to the preparation of other flavors using different carboxylic acids and alcohol available at the lab (Scheme SM 3.1.4.1).
This experiment is carried out by 12 students (4 groups) in a curricular unit, as an introduction to heterogeneous catalysis.

Scheme SM 3.1.4.1 Esters with different flavors obtained by esterification of a carboxylic acid with an alcohol.



Carboxylic acid	Alcohol	Ester	Flavor
Acetic acid	Isoamylic alcohol	3-methylbutyl ethanoate	pears, bananas
Acetic acid	Octyl alcohol	octyl ethanoate	oranges
Butanoic acid	Methyl alcohol	methyl butanoate	apples
Butanoic acid	Ethyl alcohol	ethyl butanoate	pineapples
Butanoic acid	Isoamylic acohol	3-methylbutyl butanoate	apples

In order to obtain major yield of ester, the esterification reaction can be carry out during 3-4 h. Also, due to the thermal stability of resin, the reaction temperature should not overcome 120 ºC (maximum operating temperature). Usually, the yields of this reaction, in the proposed conditions, are between 40-60%.
The boiling point of 3-methylbutyl ethanoate is 254 ºC.

The GC analysis were carried using a Hewlett Packard GC instrument equipped with 30 m×0.25 mm DB-1 column and He as carrier gas. The injector and detector temperatures were, respectively, 260 and 300 ºC. The oven temperature program was as follows: start at 60 ºC (6 min), ramp at 10 ºC min^{-1} to 300 ºC.

The catalytic activity of resin (Question 3 and Question 4) can be expressed as

$$\text{catalytic activity} = \frac{\text{amount of limiting reagent consumed (mol)}}{\text{amount of catalyst (g)}.\,\text{reaction time (h)}}$$

Usually, the catalytic activity of resin obtained by students is between 0.06-0.08 mol/h.g_{cat}.

The spectra of the compounds obtained by students could be compared with published spectra (see, for instance, the database: http://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi).

Figure SM 3.1.4.1 Reaction setup apparatus needed to perform the experiments.

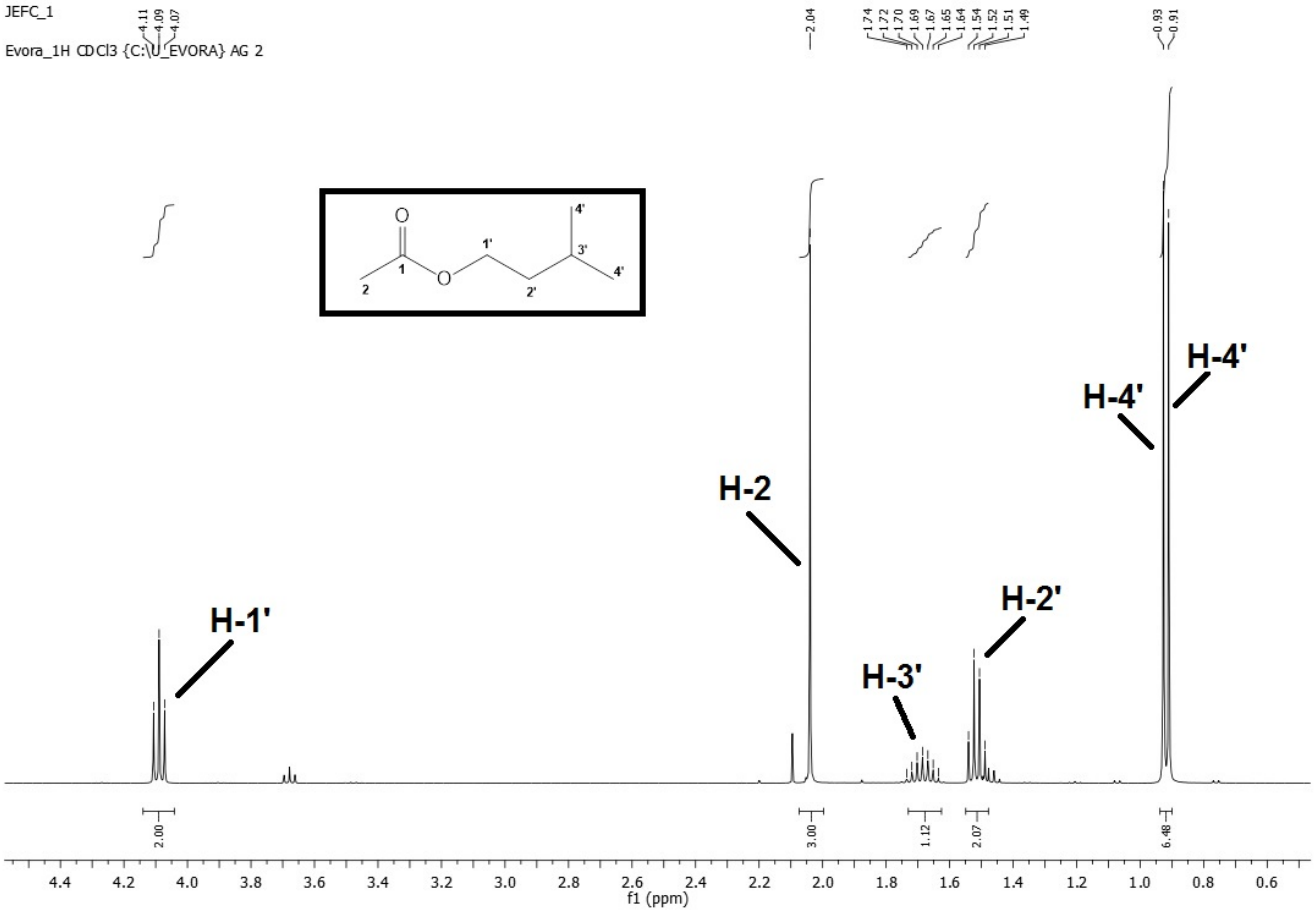


Figure SM 3.1.4.2 ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 3-methylbutyl ethanoate.

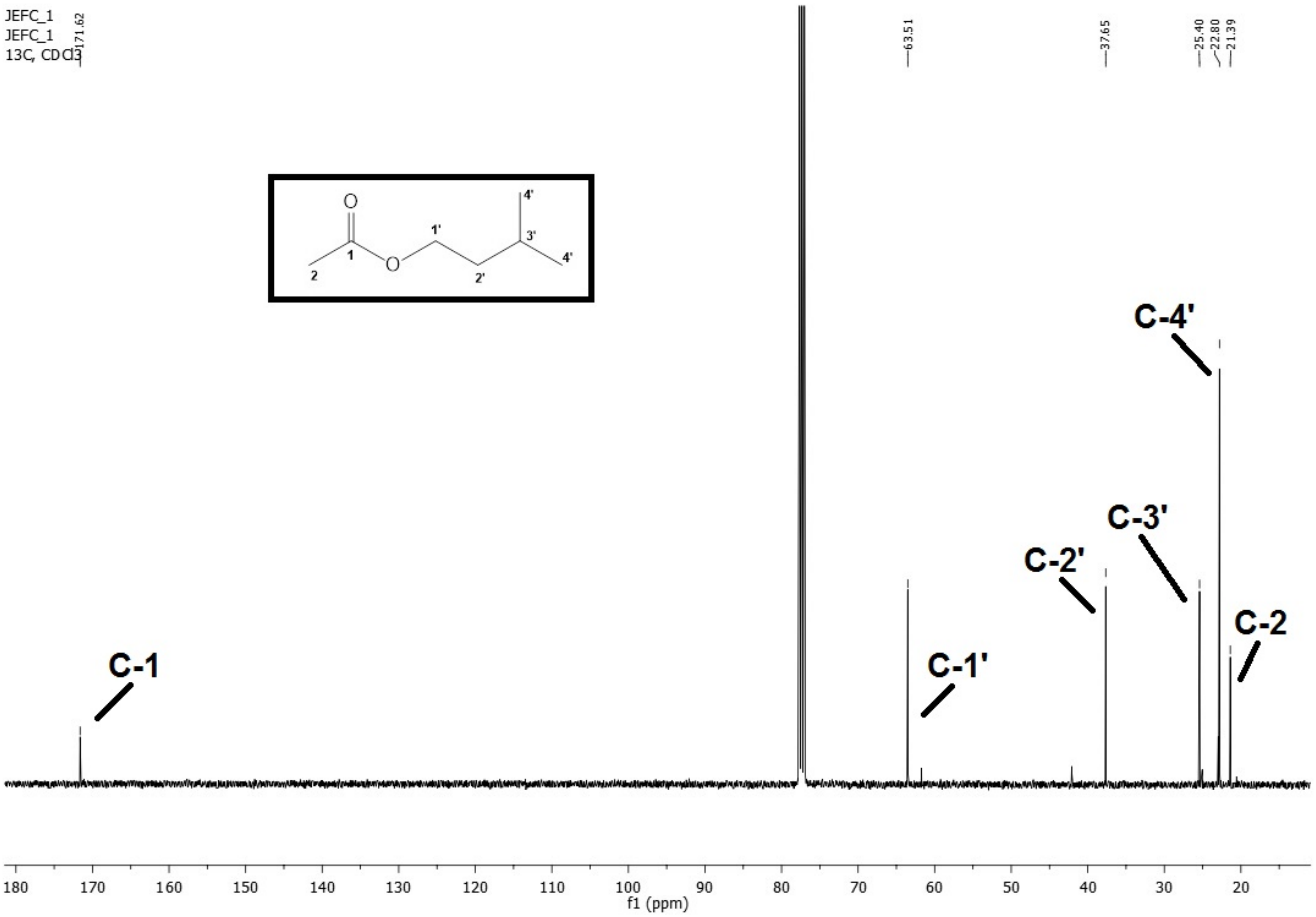


Figure SM 3.1.4.3 ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of 3-methylbutyl ethanoate.

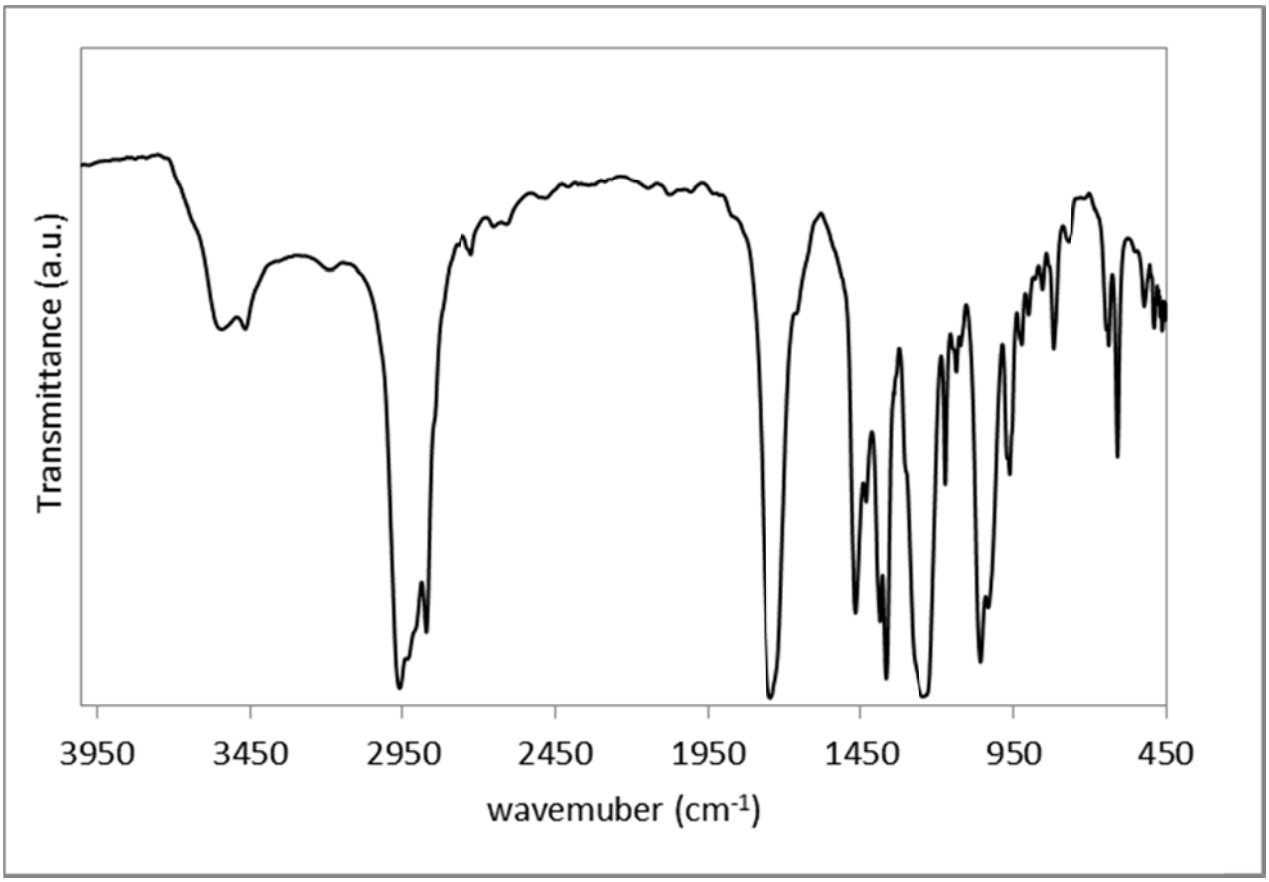


Figure SM 3.1.4.4 IR spectrum of 3-methylbutyl ethanoate (thin film KBr plates).

Acetylation of cholesterol and purification by column chromatography

Supplementary Material

Experiment notes:

The aim of this experiment is to execute an easily performed acetylation of the alcohol function in the cholesterol molecule, and apply chromatographic techniques to the purification of the reaction mixture. The reaction is not complete and as such a mixture of the acetylated and non acetylated cholesterol will be obtained. The students can then practice the chromatographic methods both column (CC) and thin layer chromatography (TLC) of the reaction mixture. The separation is easy because cholesterol and cholesteryl acetate have different polarities. Since both compounds are not colored and do not show significant absorbance to UV light the visualization must be done with a staining agent*. This visualization is another method the students will be in contact with.

The column chromatography (CC) and the TLC should be executed in a fume hood or in a bench with appropriate ventilation. The acetic acid and anhydride addition must be executed in the fume cupboard and the measuring glassware must be put in a container half filled with water in the fume hood in order to avoid the acid and anhydride vapors to spread in the laboratory room. Also TLC visualization by acid spraying must be executed in a fume hood, to avoid acid vapors to spread in the lab.

Care must be taken during the reaction mixture extractions to avoid over-pressure in the separatory funnel. The organic and aqueous layers must be confirmed in each extraction. All the aqueous and organic layers must be kept by the students until the end of the work to avoid any phase mistakes. Aqueous solutions will then be discharged in identified appropriate containers. Since the acetylation reaction is performed in acidic media, the mixture treatment will involve washing of the organic phase with water and neutralization with alkaline sodium hydroxide diluted solution. This step must be done with care, as the neutralization of the remaining acid may release significant heat.

The preparation of the chromatography column and the selection of the eluents for both chromatographic techniques will be done along with the cholesterol acetylation reaction.

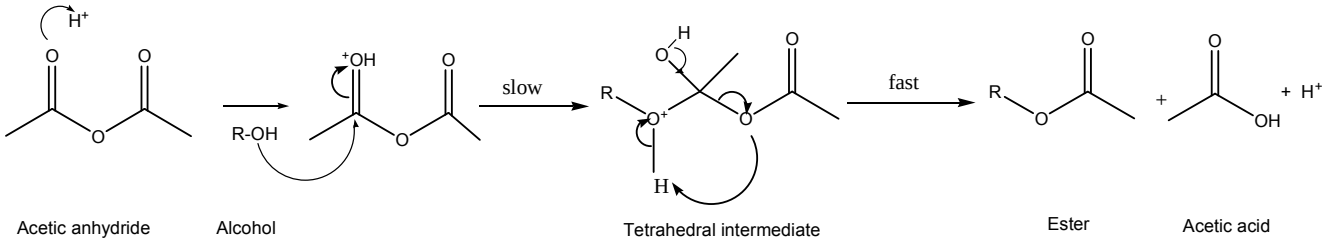
During the column chromatography preparation is important to avoid any air bubbles within the silica solid support. Also either during the column preparation or during its elution it is important to keep always a solvent layer above the column silica solid support. Before the column elution the test tubes support must be placed under the column such that it can be moved from one tube to another without closing the column stopper.

It is possible to separate this work in two 2 hours sessions. If that is the case, in the first session the students will perform the reaction with the reaction mixture workup and the TLC system testing. In the second session the students will perform the chromatography column separation with TLC control. In that case the crude solid product with the reaction mixture can be kept in desiccators with the student's identification.

Alternatively if more time is available in a second session the work can be extended in order to isolate also the unreacted cholesterol.

After vacuum evaporation the combined column fractions must reach constant weight. This can be evaluated if the weight variation is lower than 5 mg after 10 minutes in an oven at an appropriate temperature.

The reaction mechanism can be devised in the following scheme for the general acetylation of an alcohol by acetic anhydride originating an ester and acetic acid as the reaction products.



Scheme SM 3.1.5.1 – General acetylation reaction mechanism of an alcohol with acetic anhydride in acidic media.

The purity of the product may be also confirmed by the melting point[1]

Cholesteryl acetate – 115-116 ºC

Cholesterol - 148.5 ºC

*__TLC Revelation mixture__ – sulphuric acid-acetic acid-ethanol (4:80:16)

Eluent – Hexane - ethyl acetate (9:1)

$R_{f\ \text{Cholesterol}} = 0.05$

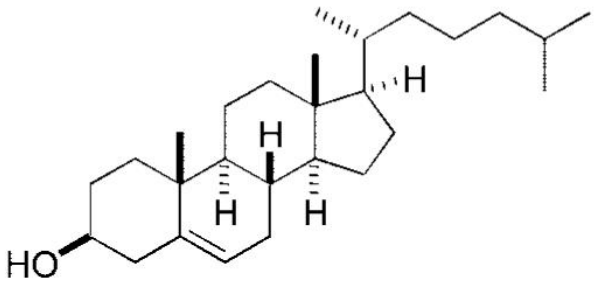
$R_{f\ \text{Cholesteryl Acetate}} = 0.5$

The difference on the R_f of the alcohol cholesterol and the ester cholesteryl acetate is due to the different polarities of both molecules. Cholesterol being an alcohol, forms easily hydrogen bonds with the silica solid support oxygen atoms (SiO_2) whereas in the ester the alcohol function -OH is blocked,

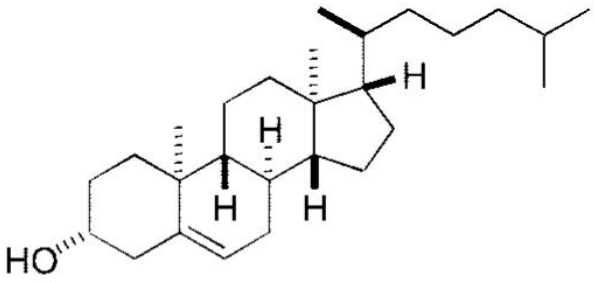
so that hydrogen bonding with the silica solid support do not exist and therefore the molecule presents a higher R_f value.

This organic experiment has been done by Pharmaceutical Sciences students (1st / 2nd year) in the Organic Chemistry laboratory of the Faculty of Pharmacy – Universidade de Lisboa, Portugal - for more than 20 years. Reaction yields depend on the reaction time, and on efficient heating. We typically obtain yields of about 15% for a 15 minutes acetylation reaction and *ca* 35% - 40% with a 30 min reaction performed in a boiling water bath. This same experiment has been tried by another group with the water bath set up at T=50ºC and no reaction has been observed after one hour.[5] That same group obtained a 20% yield with the acetylation for 30 minutes in a boiling water.[5] To improve the reaction yield and maintain the reaction time as short as possible we advise to use a pre-heated water bath and maintain the reaction for 30 minutes in the boiling water bath. We should stress that a high yield is not fundamental as the objective is to separate cholesteryl acetate from unreacted cholesterol, however the students can register the reaction time and compare the acetylation yields in the final of the experiment.

Also if the student's background will allow, infrared spectra of cholesterol and cholesteryl acetate can be obtained and compared. The disappearance of the alcohol –OH band at around 3400 cm^{-1} and the formation of the carbonyl C=O ester band around 1700 cm^{-1} in the acetylated product will be observed, and can be discussed in a tutorial session – Figure SM 3.1.5.1.
These spectra are also available on-line.. [2,]
Yet Nuclear Magnetic Resonance spectra, proton and carbon, have been run in deuterated chloroform (≈ 20mg / 600 μL solvent) for both cholesterol and cholesteryl acetate – Figure SM 3.1.5.2 – with a Bruker apparatus (300 mHz to proton and 75 mHz to carbon). The acetyl signal can be seen at 2.1 ppm in the ^{1}H NMR spectrum (CH_3C=O) and at 171 ppm (CH_3C=O) in the ^{13}C NMR spectrum. The whole interpretation of these spectra is quite complex and not appropriate for first year or even second year chemistry students and can only be achieved with 2D or even 3D correlations. Any way, cholesterol and cholesteryl acetate NMR spectra interpretation have been published elsewhere and can be used by the instructor if necessary. [3,4]



nat-cholesterol



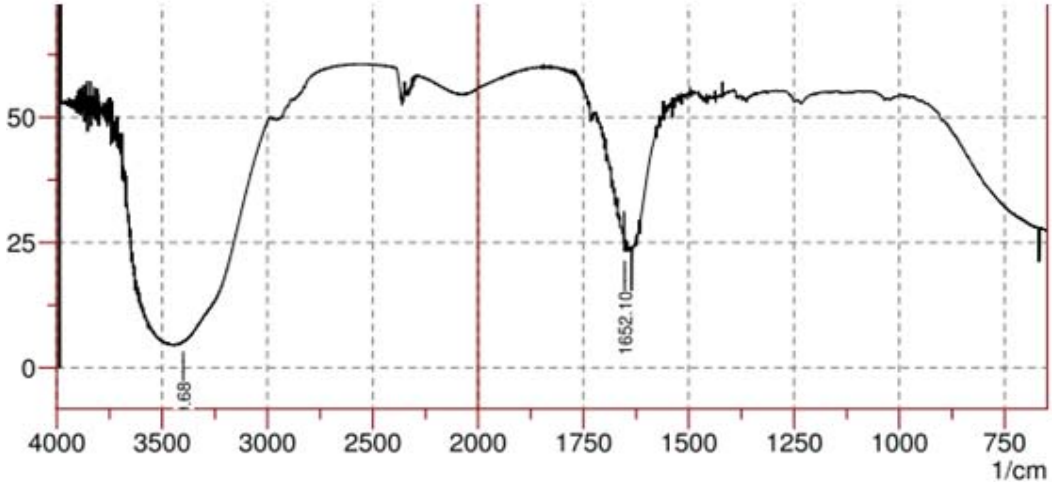
ent-cholesterol

Cholesterol has 8 stereocenters therefore 256 isomers are possible but only the natural cholesterol (*nat*-cholesterol) exists in Nature.

The Systematic IUPAC name of Cholesterol is (10*R*,13*R*)-10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,11,12,14,15,16,17-dodecanohydro-1*H*-cyclopenta[a]phenthren-3-ol.

Cholesterol and Cholesteryl acetate IR spectra:

A - Cholesterol:



B - Cholesteryl acetate:

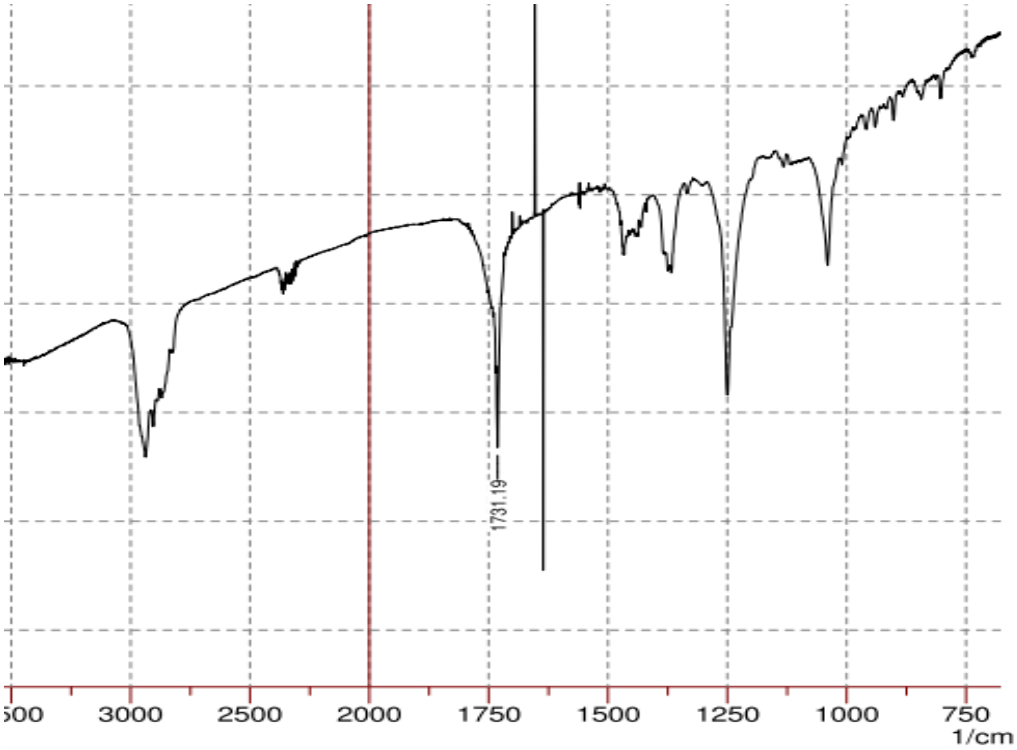
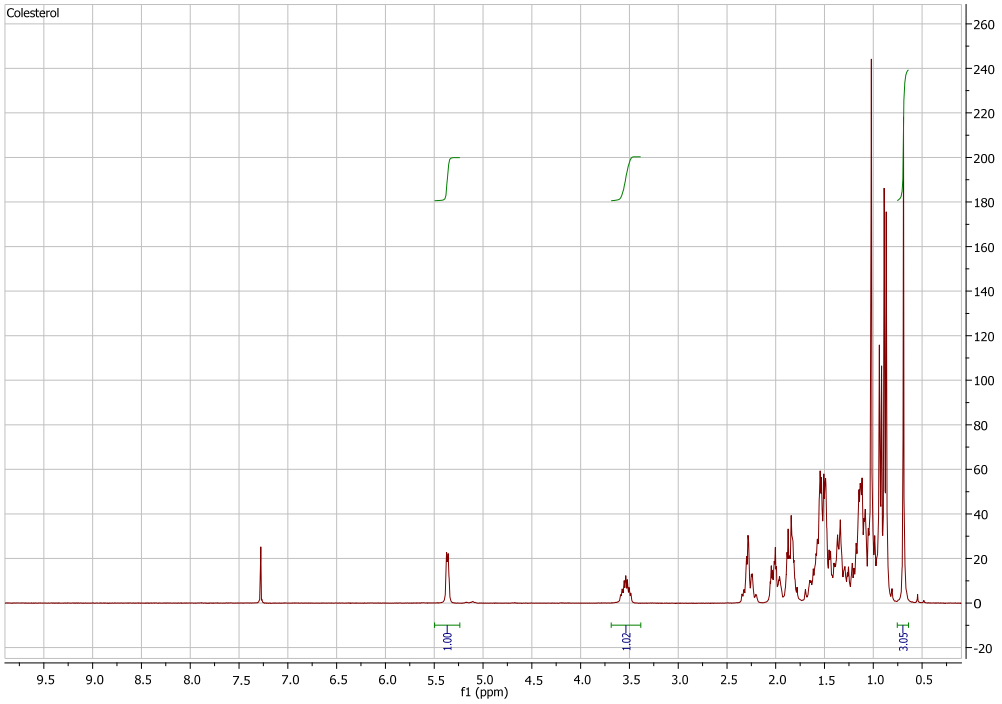
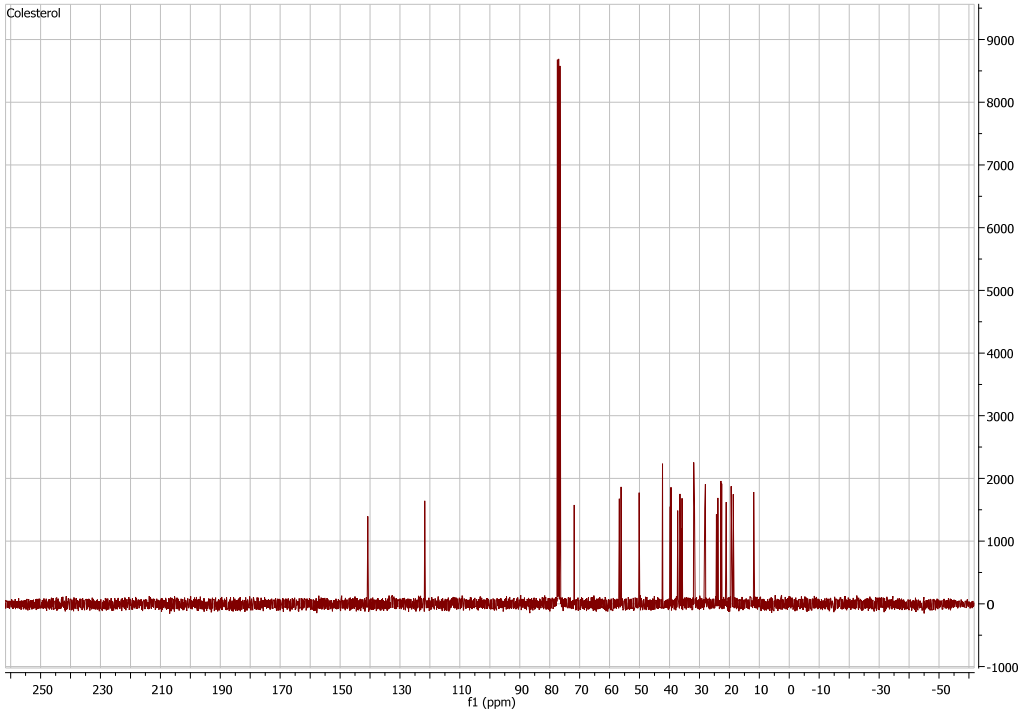


Figure SM 3.1.5.1 – Cholesterol (A) and Cholesteryl Acetate (B) IR spectra obtained with a FTIR IR Affinity - 1 Fourier Transform Infrared Spectrophotometer SHIMADZU from film on a KBr cell. Cholesterol alcohol C-*OH* band about 3400 cm^{-1} (spectrum A) and Cholesteryl acetate ester *C=O* band about 1731 cm^{-1} (spectrum B).

Cholesterol and Cholesteryl acetate NMR spectra:

A - Cholesterol





B – Cholesteryl acetate

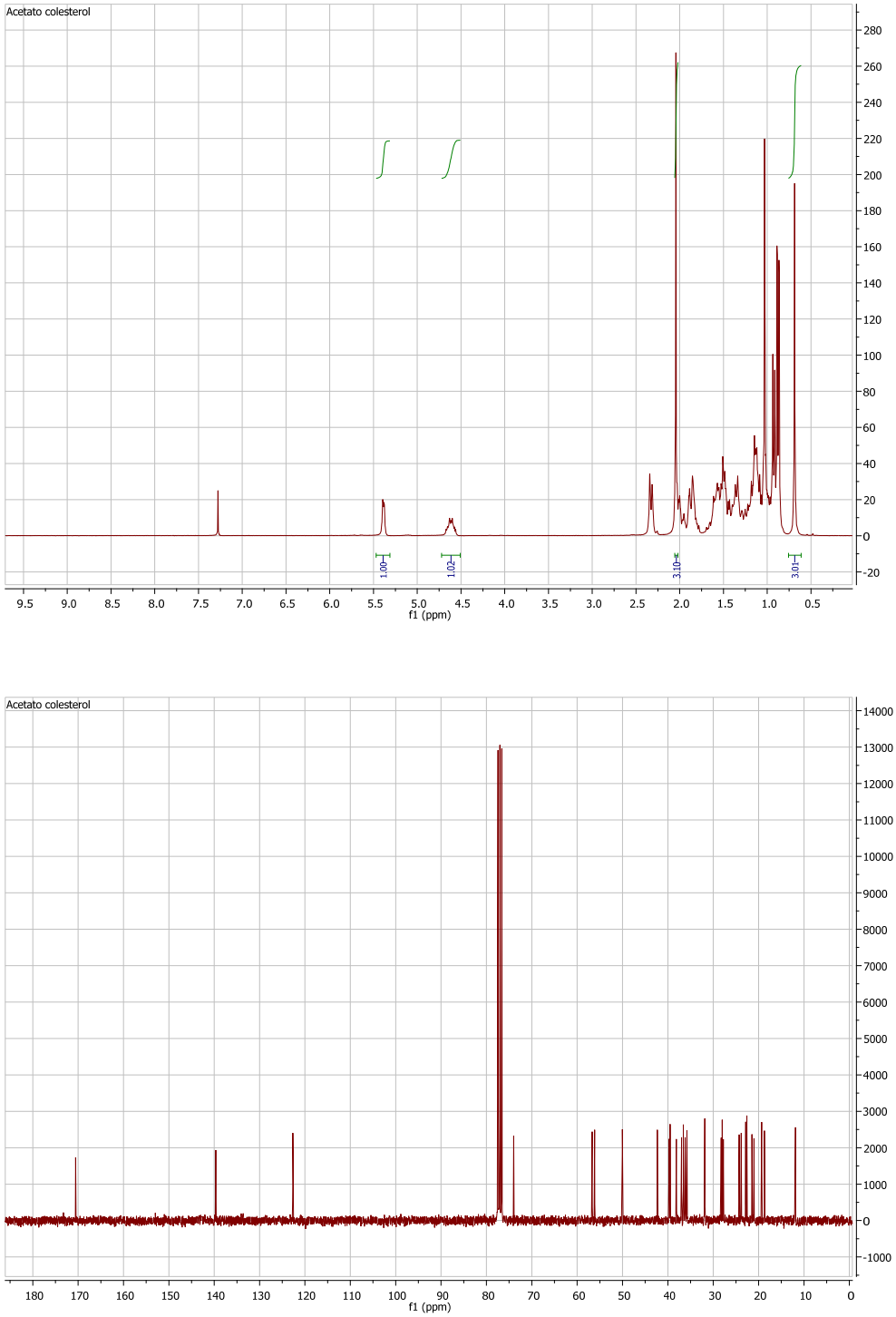


Figure SM 3.1.5.2 – Cholesterol (A) and Cholesteryl acetate (B) ^{1}H and ^{13}C NMR spectra in $CDCl_3$ with a Bruker apparatus (300 mHz ^{1}H and 75 mHz ^{13}C). The acetyl band can be seen at 2.1 ppm ^{1}H (CH_3C=O) and at 171 ppm (CH_3C=O) in the ^{13}C cholesteryl acetate spectra.

References:

[1]-"*The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals*". M. J. O. O'Neil Edn. Merck &Co., Inc, NI, USA 14th Edn 2006.
[2] - Examples of IR spectra websites (accessed 9 September 2015):
[a] Cholesterol - http://webbook.nist.gov/cgi/cbook.cgi?ID=C57885&Type=IR-SPEC&Index=1
[b]-Cholesteryl acetate - http://webbook.nist.gov/cgi/cbook.cgi?ID=C604353&Mask=80
[3] - Muhr, P., Likussar, W., Schubert-Zailavecz, M., *Magnetic Resonance in Chemistry*, (1996) **34**, 137.
[4] - Colesteryl acetate nmr spectra: http://chem.ch.huji.ac.il/nmr/techniques/2d/assigncholac.htm. (accessed at 9 September 2015)
[5] – Information kindly provided by Professor Diogo S. Lüdtke and Dr. Maria Eduarda Contreira from Institute of Chemistry, Universidade Federal do Rio Grande do Sul - UFRGS, Brazil.

Synthesis of Hippuric Acid: an example of amide bond formation

Supplementary Material

1. General Notes:

The main objective of this experiment is to familiarize students with the preparation of amides from acyl chlorides. This will be illustrated using the synthesis of hippuric acid, starting from glycine and benzoyl chloride in a biphasic basic solvent system (Schotten-Baumann conditions). Since no organic solvent is used in the reaction, this synthetic methodology is attractive from a sustainable point of view and emphasis can be given to this aspect, as well as, to the biological role of the synthesised compound.

The experiment was designed to be performed in a 1.05 g scale of glycine and a small excess of benzoyl chloride (1.15 equiv.). It encompasses a relatively short reaction time at room temperature, elementary laboratory techniques such as filtration and recrystallization, and product characterization by melting point and NMR. The procedure is operationally simple and can be performed by any undergraduate student following organic chemistry courses where the concepts of nucleophilic addition to the carbonyl group and NMR spectroscopy are discussed.

At the Faculty of Pharmacy – University of Lisbon (FFUL), this experiment is routinely performed by students (app. 200 per year) attending the laboratory classes of the Pharmaceutical Chemistry II course (4th year of the Integrated Master of Pharmaceutical Sciences Degree). The usual yield of pure product (after recrystallization) is in the range of 40-70%, depending on students skills. The range of melting points obtained by the students normally varies between 185 ºC and 189 ºC.

2. Troubleshooting Information:

- The reaction is complete when it forms a clear yellow solution and when no oil droplets remain at the bottom of the flask (Figure SM 3.1.6.1). If any precipitate is present at this point, students should be instructed to filter the mixture, using vacuum filtration. The filtrate (product) should be kept and the solids discarded.

- In the present methodology, a side reaction of benzoyl chloride with HO^- may occur, leading to the formation of benzoic acid which will co-precipitate with hippuric acid under acidic conditions.

- However, benzoic acid is soluble in diethyl ether while hippuric acid is not and therefore crude product (slightly yellowish solid) should be washed with ether previously to recrystallization (Figure SM 3.1.6.2). To remove benzoic acid more efficiently it is advisable to instruct students to turn off the vacuum source first and cover the precipitate with 5 to 10 mL of ether, which should then be removed by reapplying the suction. This procedure should be repeated at least two more times.

- After the ether washing, crude hippuric acid should recrystallize nicely using 10 mL of ethanol:water solution (1:3, v/v) as solvent (Figure SM 3.1.6.3). In order to achieve complete solubilisation, the solution must be heated at or near its boiling point and particular attention should be paid to avoid solvent evaporation.

3. Photos of the experiment:

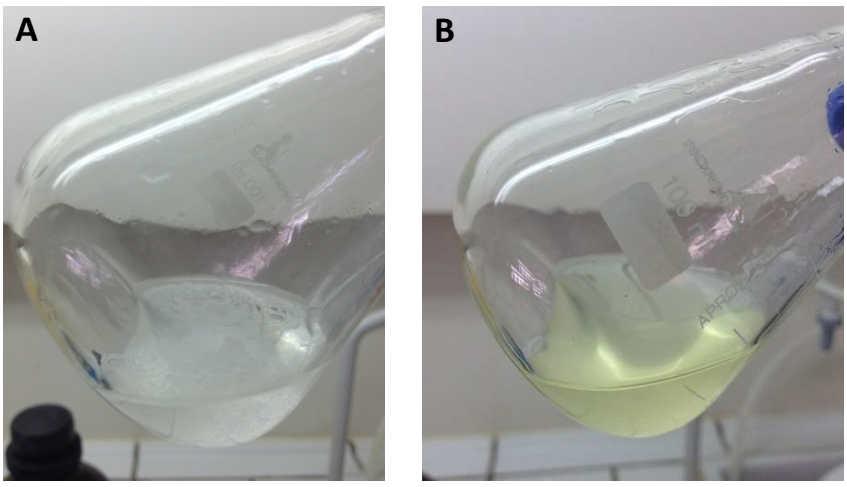


Figure SM 3.1.6.1 – Illustrative photos of the reaction mixture before (A) and after completion at pH 12 (B).

Figure SM 3.1.6.2 – Crude hippuric acid: (A) contaminated with benzoic acid (slightly yellowish solid) and (B) after ether washing (white solid).

Figure SM 3.1.6.3 – Hippuric acid recrystallization using ethanol:water solution (1:3, v/v) as solvent.

Figure SM 3.1.6.4 – Pure hippuric acid (white needles).

4. ^{1}H and ^{13}C NMR spectra:

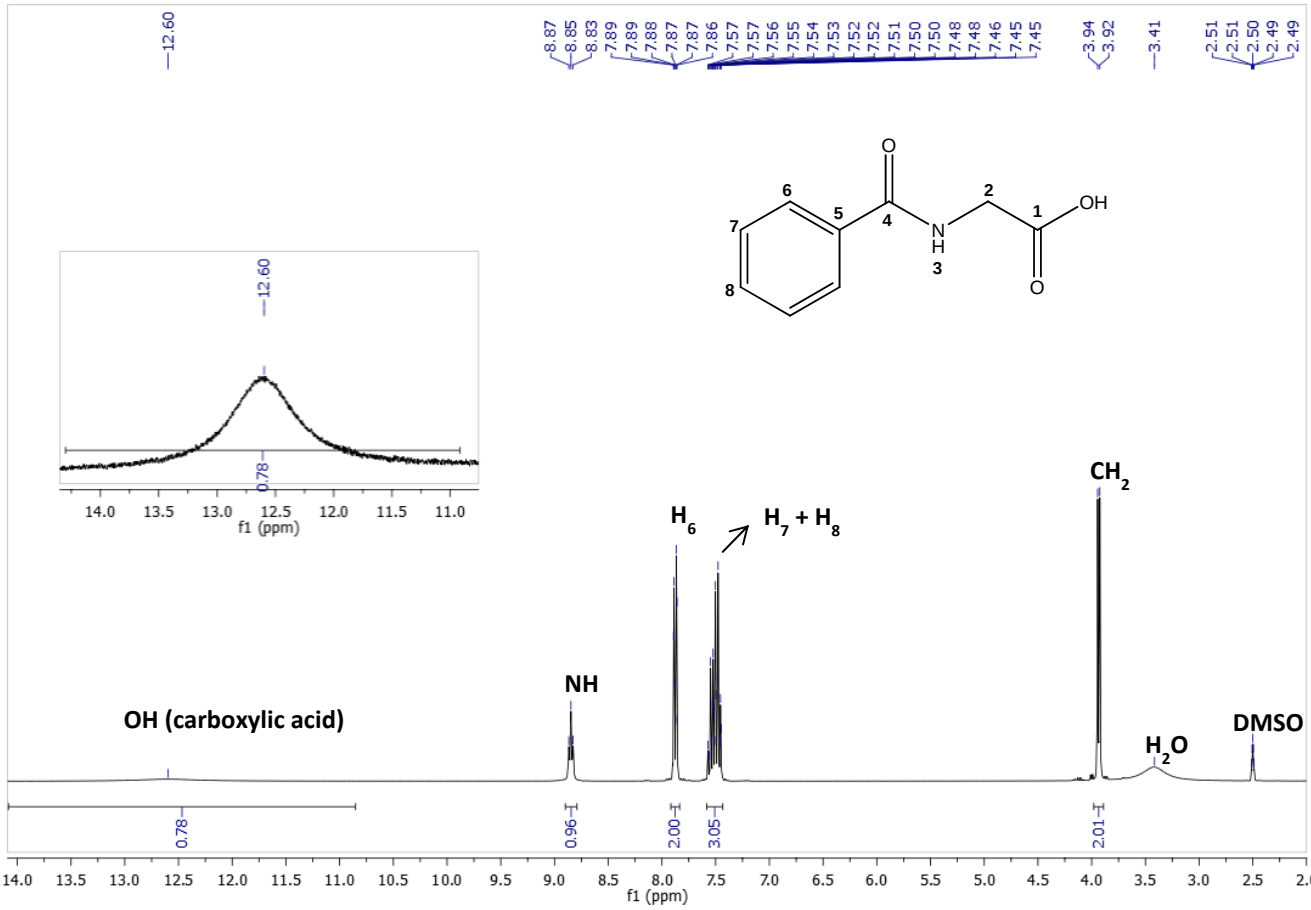


Figure SM 3.1.6.5 – ^{1}H NMR spectrum of Hippuric Acid (300 MHz, DMSO-d_6) and peak assignment.

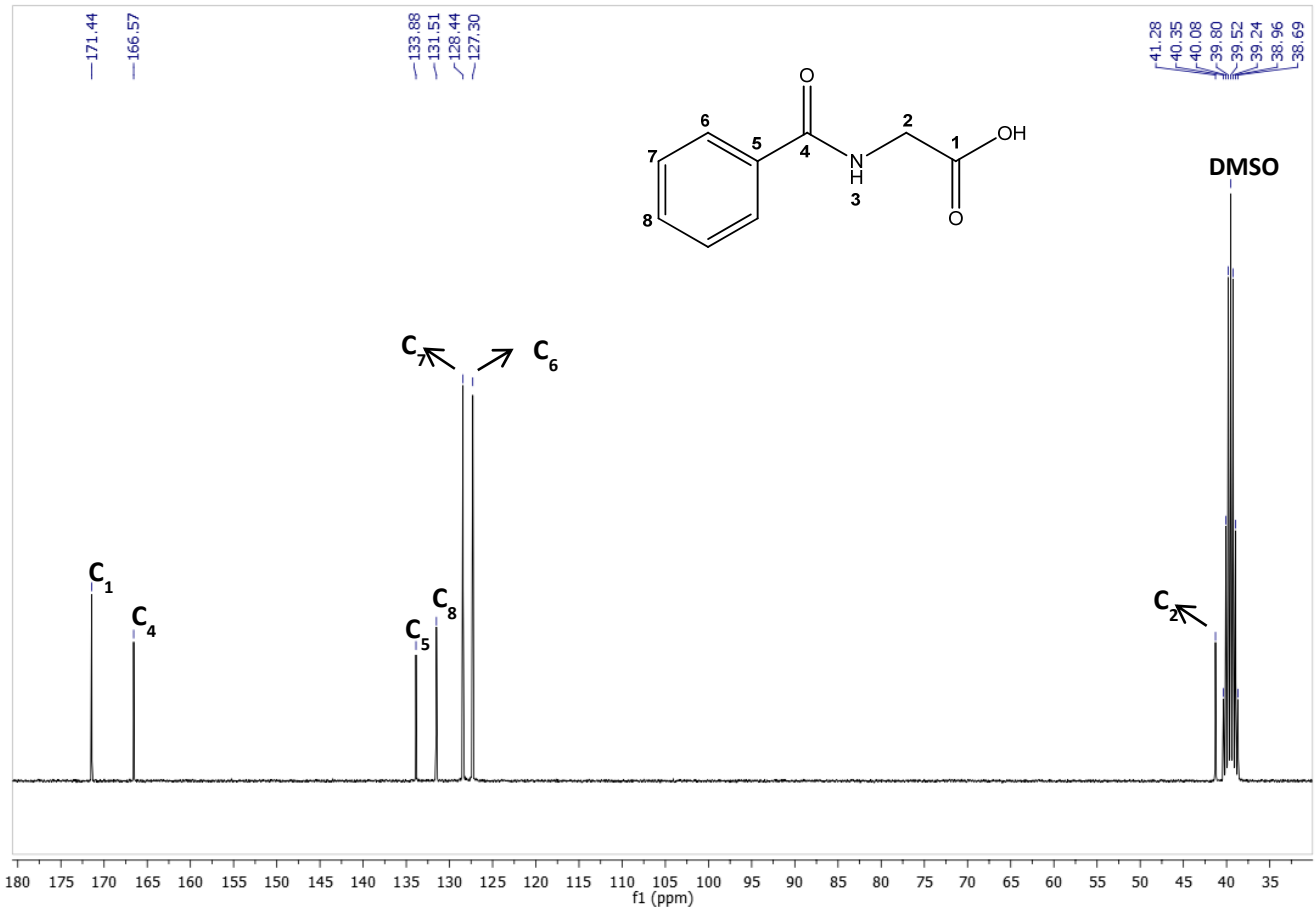


Figure SM 3.1.6.6 – ^{13}C NMR spectrum of Hippuric Acid (300 MHz, DMSO-d_6) and peak assignment.

Synthesis of Hippuric Acid: an example of amide bond formation

Supplementary Material

1. General Notes:

The main objective of this experiment is to familiarize students with the preparation of amides from acyl chlorides. This will be illustrated using the synthesis of hippuric acid, starting from glycine and benzoyl chloride in a biphasic basic solvent system (Schotten-Baumann conditions). Since no organic solvent is used in the reaction, this synthetic methodology is attractive from a sustainable point of view and emphasis can be given to this aspect, as well as, to the biological role of the synthesised compound.

The experiment was designed to be performed in a 1.05 g scale of glycine and a small excess of benzoyl chloride (1.15 equiv.). It encompasses a relatively short reaction time at room temperature, elementary laboratory techniques such as filtration and recrystallization, and product characterization by melting point and NMR. The procedure is operationally simple and can be performed by any undergraduate student following organic chemistry courses where the concepts of nucleophilic addition to the carbonyl group and NMR spectroscopy are discussed.

At the Faculty of Pharmacy – University of Lisbon (FFUL), this experiment is routinely performed by students (app. 200 per year) attending the laboratory classes of the Pharmaceutical Chemistry II course (4th year of the Integrated Master of Pharmaceutical Sciences Degree). The usual yield of pure product (after recrystallization) is in the range of 40-70%, depending on students skills. The range of melting points obtained by the students normally varies between 185 ºC and 189 ºC.

2. Troubleshooting Information:

- The reaction is complete when it forms a clear yellow solution and when no oil droplets remain at the bottom of the flask (Figure SM 3.1.6.1). If any precipitate is present at this point, students should be instructed to filter the mixture, using vacuum filtration. The filtrate (product) should be kept and the solids discarded.

- In the present methodology, a side reaction of benzoyl chloride with HO^- may occur, leading to the formation of benzoic acid which will co-precipitate with hippuric acid under acidic conditions.

- However, benzoic acid is soluble in diethyl ether while hippuric acid is not and therefore crude product (slightly yellowish solid) should be washed with ether previously to recrystallization (Figure SM 3.1.6.2). To remove benzoic acid more efficiently it is advisable to instruct students to turn off the vacuum source first and cover the precipitate with 5 to 10 mL of ether, which should then be removed by reapplying the suction. This procedure should be repeated at least two more times.

- After the ether washing, crude hippuric acid should recrystallize nicely using 10 mL of ethanol:water solution (1:3, v/v) as solvent (Figure SM 3.1.6.3). In order to achieve complete solubilisation, the solution must be heated at or near its boiling point and particular attention should be paid to avoid solvent evaporation.

3. Photos of the experiment:

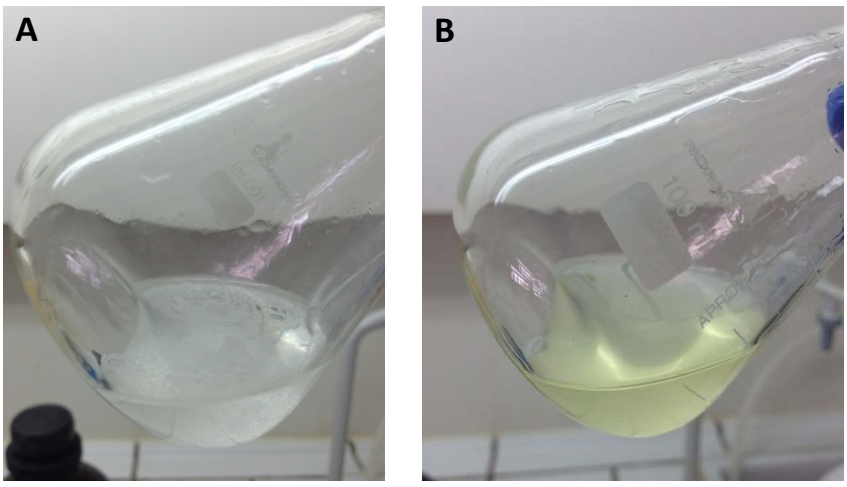


Figure SM 3.1.6.1 – Illustrative photos of the reaction mixture before (A) and after completion at pH 12 (B).

Figure SM 3.1.6.2 – Crude hippuric acid: (A) contaminated with benzoic acid (slightly yellowish solid) and (B) after ether washing (white solid).

Figure SM 3.1.6.3 – Hippuric acid recrystallization using ethanol:water solution (1:3, v/v) as solvent.

Figure SM 3.1.6.4 – Pure hippuric acid (white needles).

4. ^{1}H and ^{13}C NMR spectra:

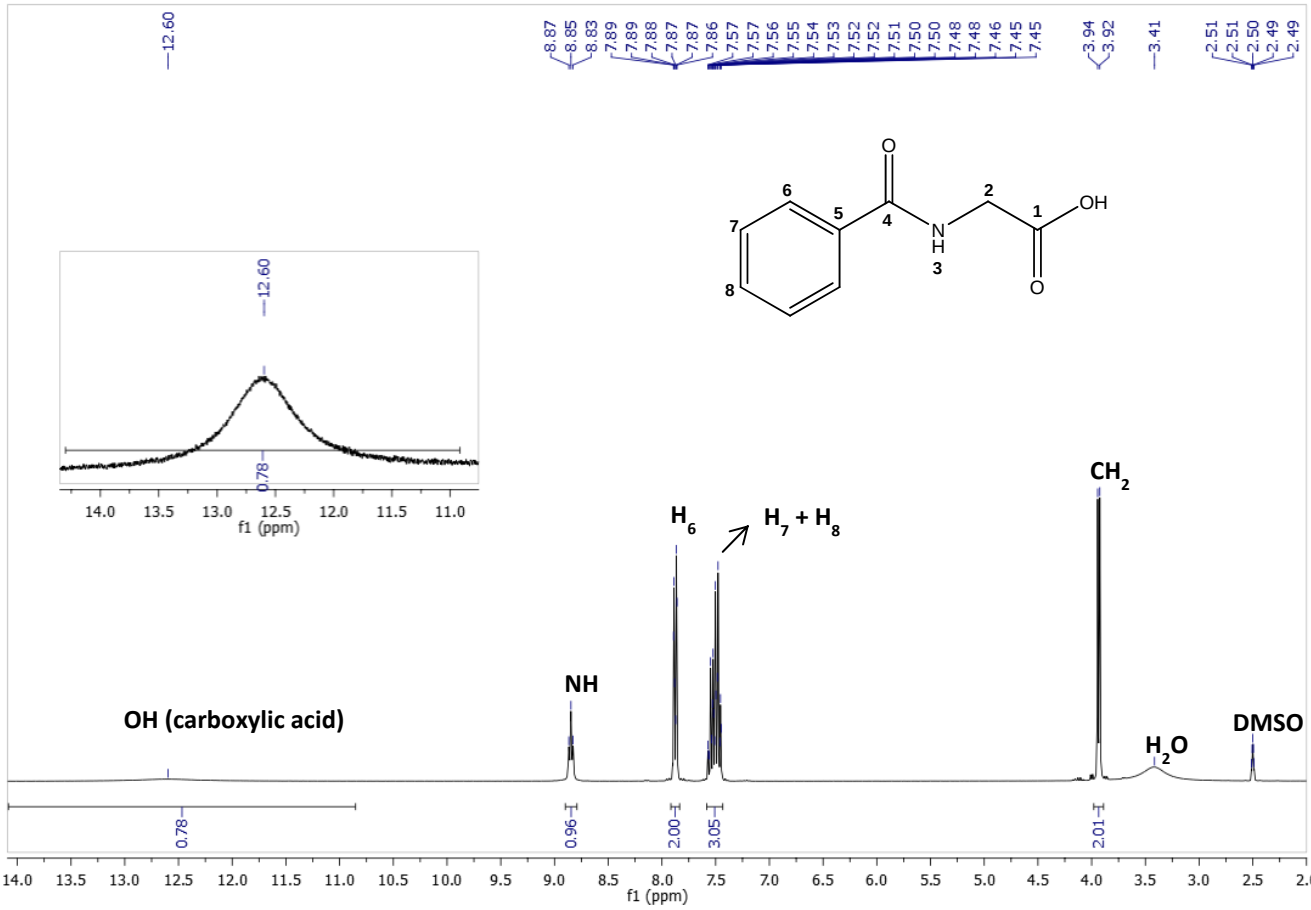


Figure SM 3.1.6.5 – ^{1}H NMR spectrum of Hippuric Acid (300 MHz, DMSO-d_6) and peak assignment.

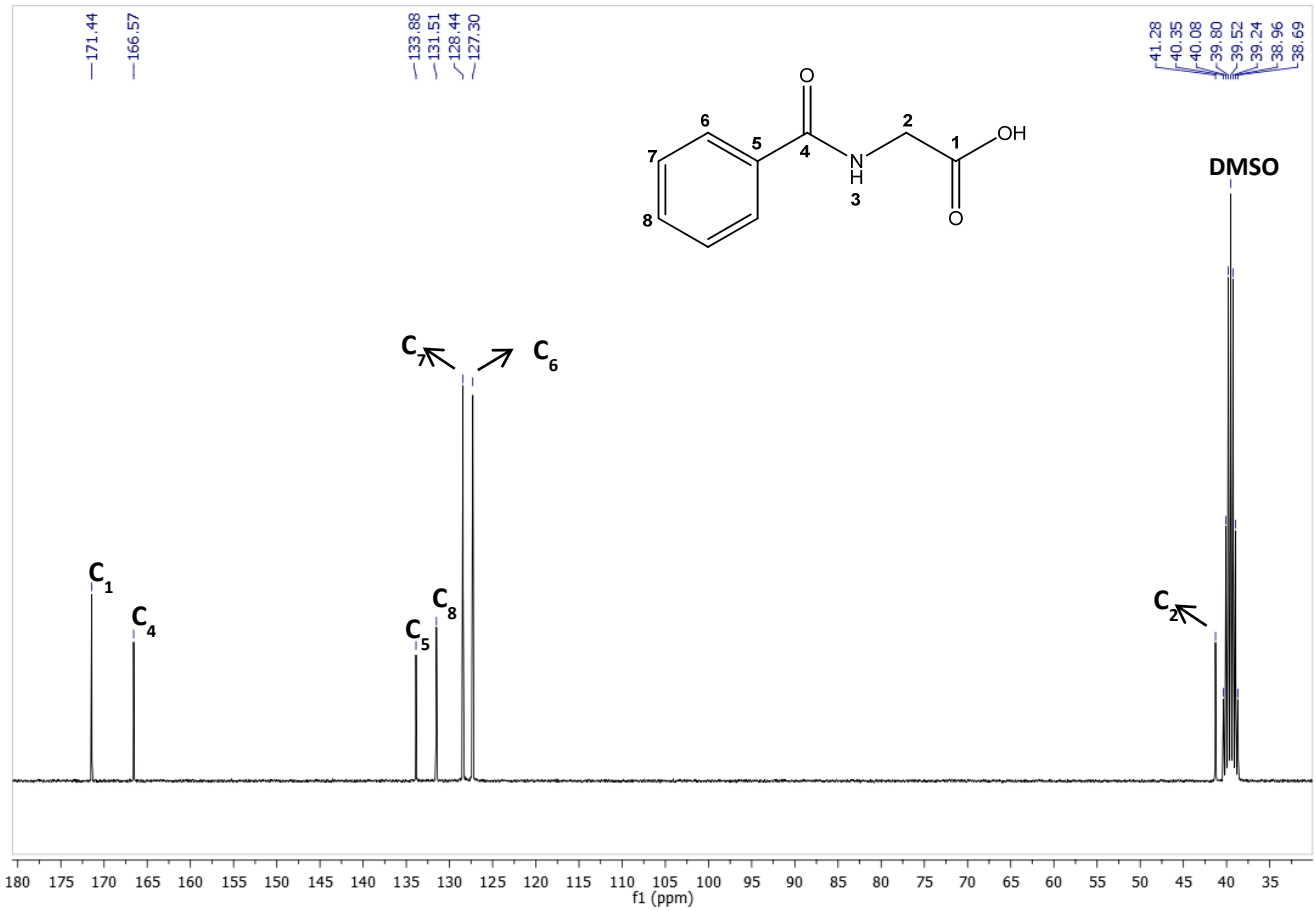


Figure SM 3.1.6.6 – ^{13}C NMR spectrum of Hippuric Acid (300 MHz, DMSO-d_6) and peak assignment.

Preparation of a sulfathiazole prodrug via N-acylation with succinic anhydride

Supplementary Material

General notes

In this experiment a carbon-nitrogen bond forming reaction between anhydride and aromatic amine leading to a new amide bond and a carboxylic acid group is explored. This reaction is a classical organic chemistry transformation that is taught during the chemistry of the carbonyl group, in the first years of organic chemistry teaching. We believe that the synthesis of a prodrug is ideal to increase the student interest in this simple organic reaction. This experiment has been executed successfully on a yearly-basis by hundreds of Bachelor/Master degree Pharmacy students (about 15 students) divided in groups of two. The students execute this reaction without any problems due to the ease of execution. Typically, depending on the student's recrystallization abilities, the yield can vary from 12 to 65 %, however the most frequent range of isolated yields is about 50%.

This experiment is extremely useful to allow students to become more experienced in purification of organic compounds by recrystallization with thermal gradient. This recrystallization is particularly challenging for the students because it uses high boiling point solvents, reinforcing the need to use the minimal amount of boiling solvent mixture to achieve the purification. Using too much solvent will penalize students by delaying their work as these solvents are not easily evaporated. Typically for the scale used herein it will be necessary to use about 8 mL of recrystallization solvents, but as they have high boiling point it is advisable to allow the students to try dissolving in 5 mL, a add gradually 1 mL until complete dissolution.

It is important to use a mild temperature to dissolve the sulfathiazole to minimize the formation of degradation side-products. The isolated product can be difficult to dry within the class time-frame, and for this reason can be kept under vacuum in a desiccator for several hours for later accurate yield determination and melting point measurement. Generally, students obtain melting point ranging from 182-183ºC to 188-189ºC, depending on whether the samples are completely dried.

Succinyl sulfathiazole

- CAS Number: 116-43-8
- Molecular Weight: 355.39 g/mol
- Melting point: 189ºC (with decomposition)

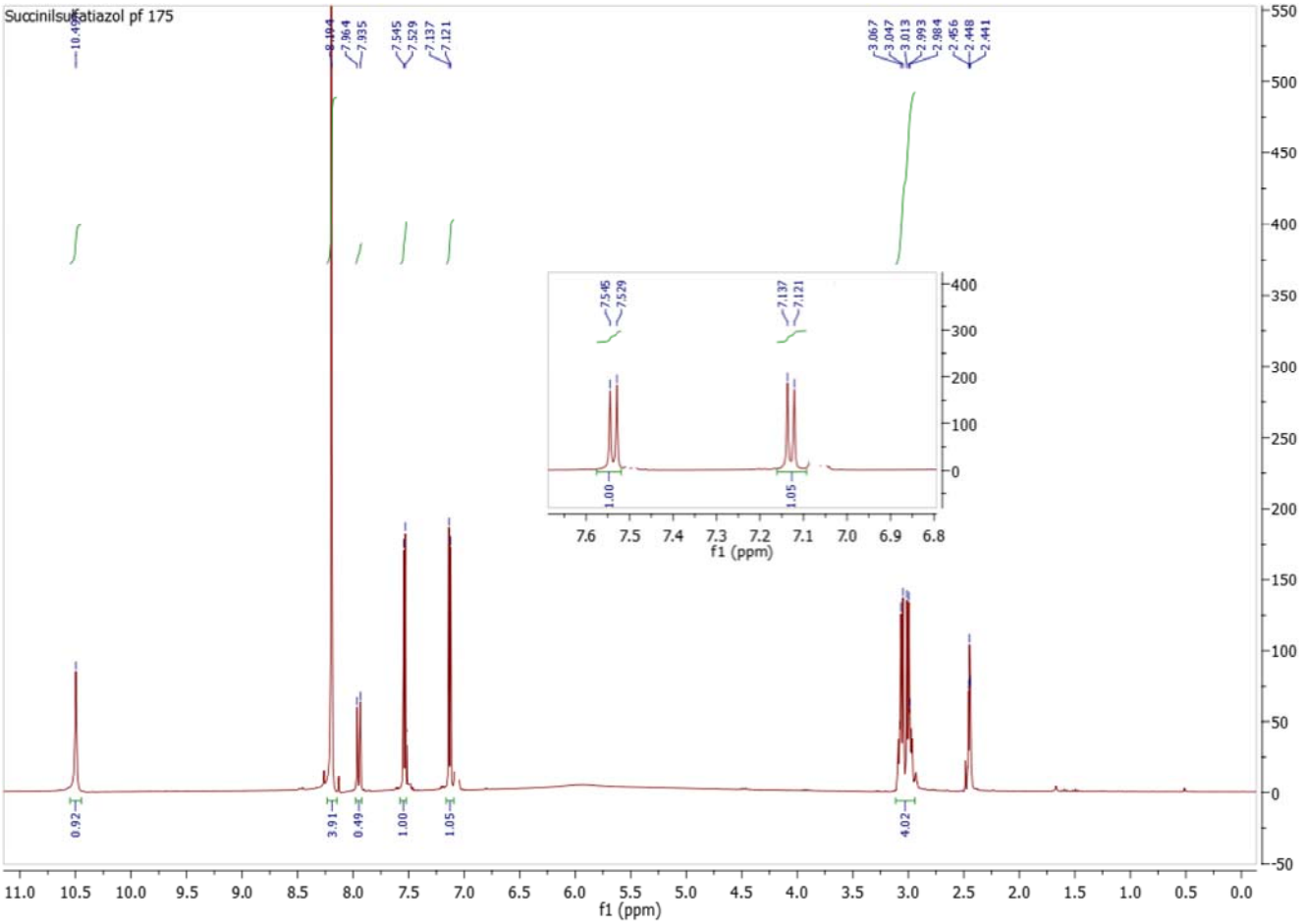


Figure SM 3.1.7.1 – Proton NMR of succinyl sulfathiazole in deuterated DMSO

Hints for the questions:

1 – carboxylic acid: 10.5 ppm 1H

Sulfonamide phenyl ring: 8.2 ppm 4H

Thiazole ring: 7.5 ppm 1H, near nitrogen

Thiazole ring: 7.1 ppm 1H, near Sulphur

Typical chemical shift of succinic anhydride in deuterated DMSO is 2.9 ppm.

2 – The most probable contaminants in the final product are sulfathiazole (unreacted or hydrolyzed during recrystallization), succinic anhydride or succinic acid.

3 – Students are expected to understand that aliphatic amines are more nucleophilic than anilines, due to the resonance with the aromatic ring. For this reason, they should also predict that the nitroaniline is the least reactive.

4 – From the pK_a the students are expected to understand that an aliphatic carboxylic acid is stronger acid than protonated aniline and aniline. For this reason, the conjugated base of the carboxylic acid should be the best leaving group in the tetrahedral intermediate.

Determination of the Absolute Configuration of Enantioenriched Secondary Alcohols via Thin-Layer Chromatography

Alexander J. Wagner; Shawn M. Miller; Scott D. Rychnovsky; Renée D. Link

Supplementary Material

Experiment Background

This experiment aims to establish the structure and the absolute configuration of an unknown enantioenriched alcohol using ^{1}H NMR spectroscopy and the Competing Enantioselective Conversion method. The Competing Enantioselective Conversion method was first reported in 2011[1] and can be applied to a number of reactive functional groups.[1-4] A single enantioenriched substrate is reacted with both enantiomers of a catalytic or stoichiometric chiral reagent in parallel reactions. One enantiomer of chiral reagent is "matched" with the enantiomer of chiral substrate, resulting in a faster reaction leading to higher conversion. The conversion of both reactions is measured using an analytical tool, and the absolute configuration of the substrate is assigned based on which reaction proceeded to higher conversion by comparison to a predictive mnemonic (Figure SM 3.1.8.1).

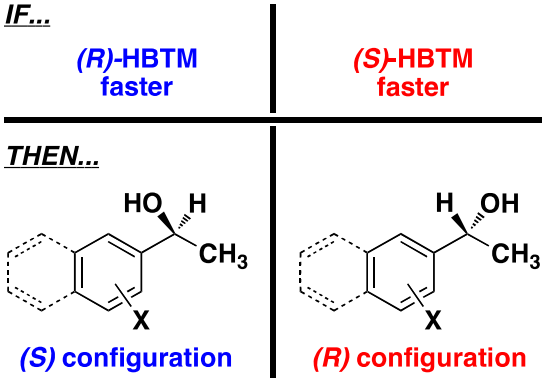


Figure SM 3.1.8.1. Predictive mnemonic for secondary alcohols adjacent to π-system. Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

Enantioenriched compounds are often difficult to use in undergraduate laboratories due to a lack of commercial availability. Enantioenriched alcohols are used in this experiment because of their relative abundance on the commercial market and their ubiquity in organic chemistry. In particular, this experiment utilizes chiral benzylic alcohols. A non-exhaustive list of commercially available benzylic alcohols can be found on page 33. If time and resources allow, it is more cost-effective to synthesize the enantioenriched alcohols from ketones that can be purchased in bulk, and the procedure can be found in the original publication of this experiment.[5] Similarly, while commercially available, the catalyst used in this experiment can also be synthesized cost-effectively according to the procedure found in the original publication of this experiment.[5] There is significant room in this experiment for variation based on class needs and instructor desires, which is expanded upon on page 30.

In this experiment, students identify the structure and establish the absolute configuration of an unknown enantioenriched alcohol. Students are given alcohol **1**, **2**, or **3** (Figure SM 3.1.8.2) and use a provided ^{1}H NMR spectra to determine the structure. Enantiomers provide identical NMR spectroscopic data. Therefore students will need another tool to identify which enantiomer of their alcohol they possess. The students then assign the absolute configuration using the CEC method.

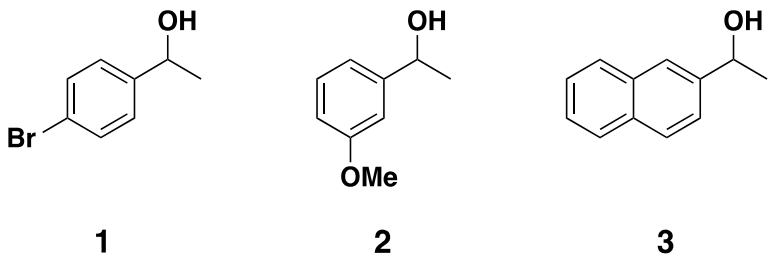


Figure SM 3.1.8.2. Chiral alcohols used in this experiment. Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

Student Learning Outcomes

Lab Skills

1. Students should be able to set up microscale esterification reactions.

2. Students should be able to run, stain, and qualitatively analyze thin-layer chromatography plates.

3. Students should be able to use ImageJ to quantitatively analyze thin-layer chromatography plates.

Critical Thinking and Understanding Concepts

1. Students should be able to explain the Competing Enantioselective Conversion method for determining absolute configuration. In particular, they should be able to explain why both enantiomers of HBTM are used and why two reactions are being run in parallel.

2. Students should be able to understand the reason for the difference in the rate of the parallel reactions and the reaction's selectivity and be able to articulate their understanding using discussions of energy diagrams and kinetics.

3. Students should be able to explain why the mnemonic for assigning absolute configuration using the CEC method works.

4. Students should be able to explain how the secondary alcohol interacts with the chiral catalyst (HBTM) to produce the ester in the matched-case transition state.

5. Students should be able to explain the mechanism for the esterification reaction of their unknown alcohol using HBTM.

6. Students should be able to interpret the ^{1}H NMR spectroscopic data for their unknown to determine which secondary alcohol they were given.

Catalytic Cycle of the Esterification Reaction

Upon the addition of the anhydride to the reaction mixture in the experiment procedure, reactive intermediate **6** is formed from the HBTM catalyst (**4**) and the anhydride (**5**) (Figure SM 3.1.8.3). This process is reversible and the equilibrium favors the starting catalyst and anhydride.[6] Interaction of the unknown alcohol substrate (**7**) with intermediate **6** affords the key carbon-oxygen bond in the ester formation. Proton transfer from the tetrahedral intermediate to the carboxylate anion produces propionic acid, which is sequestered by the amine base present in the reaction. Collapse of the tetrahedral intermediate affords the ester product **8** while regenerating catalyst **4**. Previous research has shown that the rate law for this reaction is first-order in catalyst, first-order in anhydride, and first-order in alcohol.[6] The fact that the rate law contains three chemical species may seem unusual, but the explanation is straightforward. The rate-determining step in this cycle is the carbon-oxygen bond formation between alcohol **7** and reactive intermediate **6**. As a result, the rate is dependent on the concentration of the alcohol and the concentration of the reactive intermediate. The same study of this catalytic system showed that under relevant reaction conditions with a constant concentration of catalyst **4**, the correlation between concentration of anhydride **5** and the concentration of intermediate **6** was linear.[6] The concentration of the reactive intermediate, due to the equilibrium of formation and considering relevant reaction concentrations, is therefore dependent on the concentration of the HBTM catalyst and the anhydride. Therefore, the concentrations of the HBTM catalyst and the anhydride directly influence the rate of the reaction despite not being directly involved in the rate-determining step.

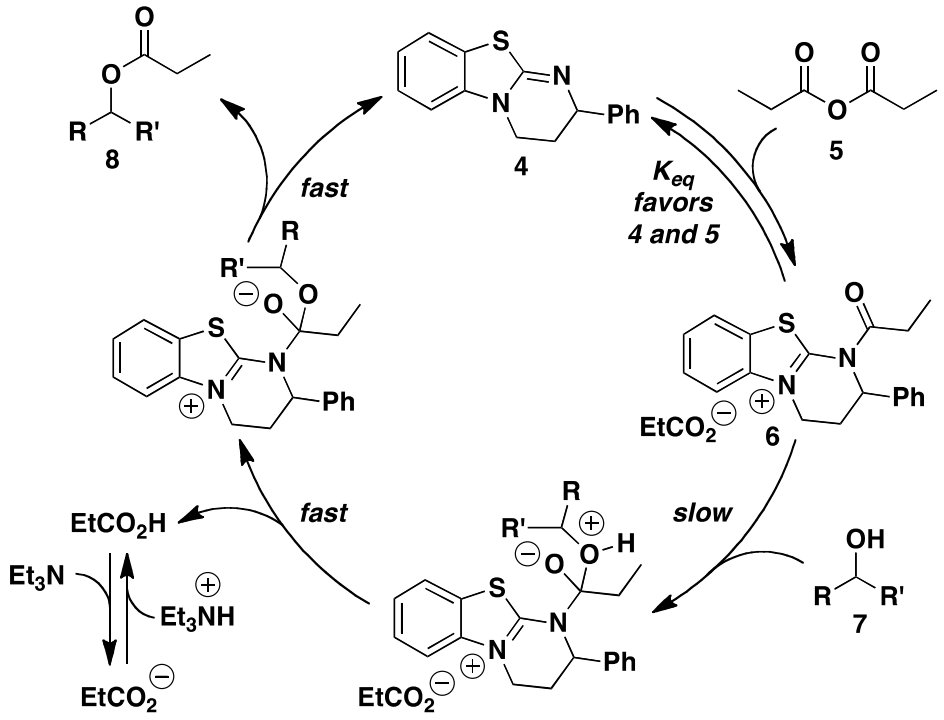


Figure SM 3.1.8.3. Catalytic cycle of the esterification reaction. Adapted with permission from *Org. Lett.*, 2013, **15**, pp 5504–5507. Copyright 2014 American Chemical Society.

Mechanism of the Esterification Reaction

Both the formation of reactive intermediate **6** and the subsequent attack of the alcohol onto **6** proceed through classic carbonyl chemistry. In each case, the nucleophile attacks the carbonyl to form a tetrahedral intermediate, ultimately followed by collapse of that intermediate to form the product (Figure SM 3.1.8.4). In the interaction of catalyst **4** with anhydride **5**, the more nucleophilic nitrogen on catalyst **4** can be rationalized by showing a resonance structure before conducting the bond formation step in the mechanism. Additionally, the proton transfer required after intermediate **6** interacts with alcohol **7** has been generically shown as "B:" to indicate a base is required for the acid-base reaction. A more specific identification of the base and a discussion of the close ion pair of intermediate **6** with the carboxylate anion, as shown in the catalytic cycle on page 4, is not required to give undergraduate students a thorough understanding of the reaction mechanism.

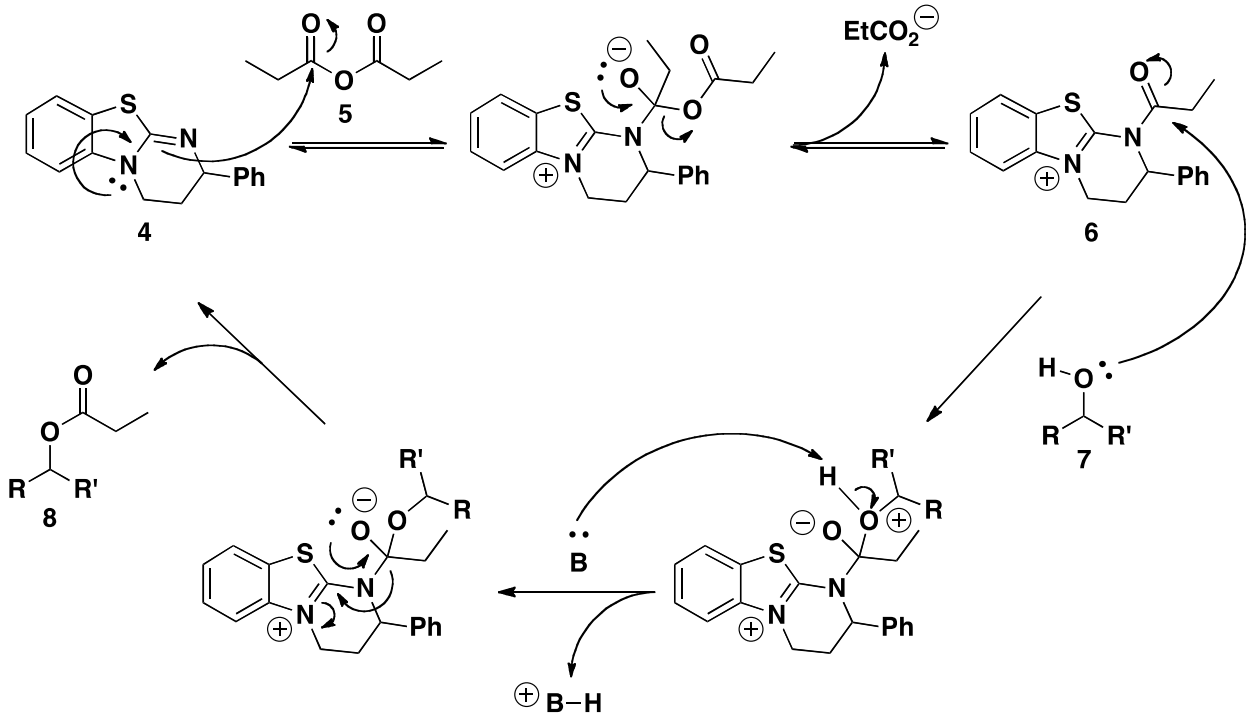


Figure SM 3.1.8.4. Mechanism for the formation of reactive intermediate **6** and the subsequent esterification reaction. Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

Source of Enantioselectivity of HBTM Catalyst

Spartan 3-D Image **Molecular Structure**

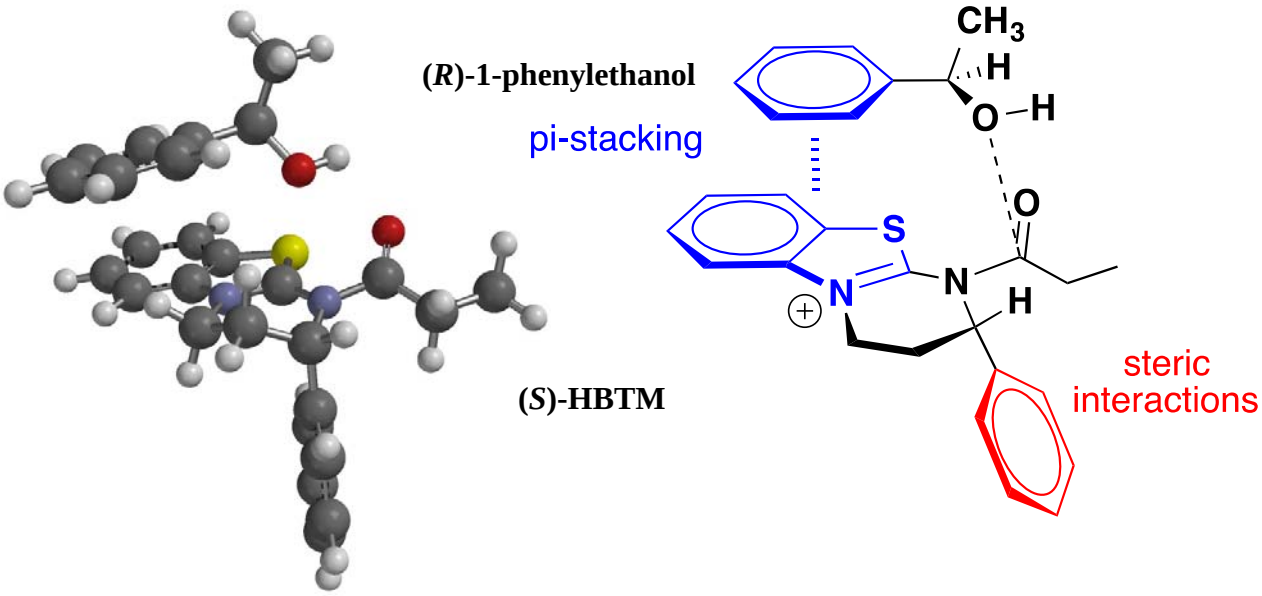


Figure SM 3.1.8.5. Two representations of the approach of the alcohol when attacking the activated HBTM catalyst intermediate in a "matched" case. Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

Figure SM 3.1.8.5 shows the interaction of the *R* enantiomer of a representative secondary alcohol with the (*S*)-HBTM catalyst. A few factors are hypothesized to dictate this interaction.

First, notice the red phenyl ring in the molecular structure on the (*S*)-HBTM catalyst. This ring serves to block the alcohol from interacting with the catalyst from the bottom face, as drawn. The result of blocking one face is called a steric effect. Therefore, the alcohol should interact with the catalyst from the top face.

Second, notice the blue aromatic systems highlighted on both the catalyst and the alcohol in the molecular structure. These aromatic systems are participating in pi-stacking. This effect brings the alcohol and the catalyst together, much like what we see in the base pairs of our DNA!

Finally, notice the position of the alcohol in proximity to the carbonyl of the catalyst intermediate (highlighted with a dashed line in the molecular structure). The *R* enantiomer of the alcohol will have the alcohol facing directly over the carbonyl of the activated catalyst, providing an easy path for the (*R*)-alcohol to attack the carbonyl. If we were to consider the *S* enantiomer of the alcohol attempting to react with (*S*)-HBTM in the same way, the alcohol OH group will be positioned farther away from the carbonyl of the activated catalyst and will therefore not be able to reach the carbonyl as easily. Therefore, the *R* enantiomer of the alcohol will be the "fast" reaction with (*S*)-HBTM and the *S* enantiomer of the alcohol will be the "slow" reaction with (*S*)-HBTM.

Experiment Preparation

Stock solutions should be prepared using volumetric glassware if available and stored in tightly sealed containers. Example solution preparation sheets using volumetric glassware for Versions **α** and **β** of this experiment are shown below. In small classes, individual students/groups can be given personal vials of all solutions. For larger classes, community solutions may be more feasible. In the case of community solutions, care must be taken to avoid cross contamination at all costs. Any mixing of enantiomers will have a detrimental effect on student results with the added problem of enantiomeric separation of contaminated samples being unfeasible in most situations. Solutions, particularly the small volumes of the unknown solutions given to individual groups/students, should not be dispensed into vials more than 12 hours prior to the experiment to minimize solvent evaporation.

Example Solution Preparation Sheet for Version α of the Determination of the Absolute Configuration of Enantioenriched Secondary Alcohols via Thin-Layer Chromatography

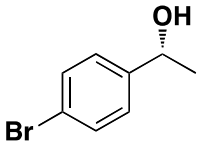
**********PREP is for a class size of 1040 students**********

Alcohols (one 100 mL volumetric flask and one 50 mL volumetric flask per compound)

Notes: This prep makes at least 20% more solution than required to account for waste. After preparation, all alcohol stock solutions should be kept in independent sealed large bottles until just before the experiment. Then, dispense 1.0 mL of solution labeled with an unknown number for each pair of students to use as their unknown.

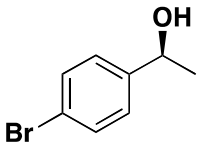
***1.* (*R*)-1-(*p*-bromophenyl)ethanol**
0.30 M in toluene

100 mL volumetric flask: 6.0318 g
50 mL volumetric flask: 3.0159 g



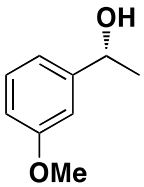
***2.* (*S*)-1-(*p*-bromophenyl) ethanol**
0.30 M in toluene

100 mL volumetric flask: 6.0318 g
50 mL volumetric flask: 3.0159 g



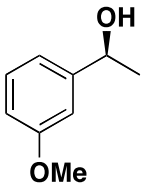
***3.* (*R*)-1-(*m*-methoxyphenyl)ethanol**
0.30 M in toluene

100 mL volumetric flask: 4.5657 g
50 mL volumetric flask: 2.2829 g

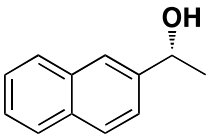


***4.* (*S*)-1-(*m*-methoxyphenyl)ethanol**
0.30 M in toluene

100 mL volumetric flask: 4.5657 g
50 mL volumetric flask: 2.2829 g

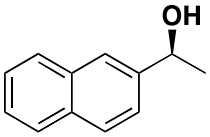


***5.* (*R*)-1-(naphthalen-2-yl)ethanol**
0.30 M in toluene



100 mL volumetric flask: 5.1666 g
50 mL volumetric flask: 2.5833 g

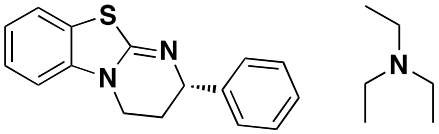
***6.* (*S*)-1-(naphthalen-2-yl)ethanol**
0.30 M in toluene



100 mL volumetric flask: 5.1666 g
50 mL volumetric flask: 2.5833 g

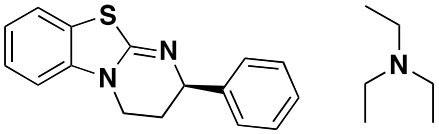
Catalysts and Base (one 500 mL volumetric flask per catalyst/base solution)

***7.* (*S*)-HBTM + NEt_3**
0.0075 M in toluene with respect to (*S*)-HBTM
0.60 M in toluene with respect to NEt_3



500 mL volumetric flask: 0.9989 g *S*-HBTM and 41.814 mL NEt_3

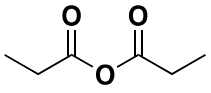
***8.* (*R*)-HBTM + NEt_3**
0.0075 M in toluene with respect to (*R*)-HBTM
0.60 M in toluene with respect to NEt_3



500 mL volumetric flask: 0.9989 g (*R*)-HBTM and 41.814 mL NEt_3

Anhydride (one 1000 mL volumetric flask)

***9.* propionic anhydride**
0.60 M in toluene



1000 mL volumetric flask: 76.930 mL propionic anhydride

Example Solution Preparation Sheet for Version β of the Determination of the Absolute Configuration of Enantioenriched Secondary Alcohols via Thin-Layer Chromatography

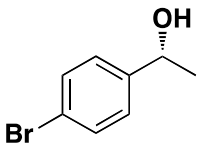
**********PREP is for a class size of 1040 students**********

Alcohols (one 100 mL volumetric flask and one 10 mL volumetric flask per compound)

Note: This prep makes at least 20% more solution than required to account for waste. After preparation, all alcohol stock solutions should be kept in independent sealed large bottles until just before the experiment. Then, dispense 1.0 mL of solution labeled with an unknown number for each pair of students to use as their unknown.

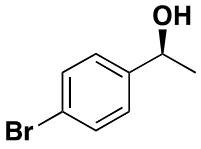
***1.* (*R*)-1-(*p*-bromophenyl)ethanol**
0.075 M in toluene

100 mL volumetric flask: 1.5080 g
10 mL volumetric flask: 0.1508 g



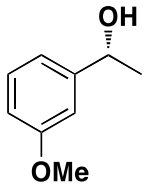
***2.* (*S*)-1-(*p*-bromophenyl) ethanol**
0.075 M in toluene

100 mL volumetric flask: 1.5080 g
10 mL volumetric flask: 0.1508 g



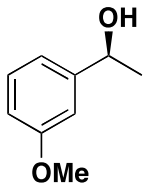
***3.* (*R*)-1-(*m*-methoxyphenyl)ethanol**
0.075 M in toluene

100 mL volumetric flask: 1.1414 g
10 mL volumetric flask: 0.1141



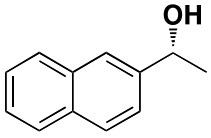
***4.* (*S*)-1-(*m*-methoxyphenyl)ethanol**
0.075 M in toluene

100 mL volumetric flask: 1.1414 g
10 mL volumetric flask: 0.1141



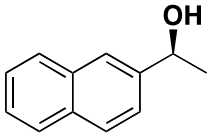
***5.* (*R*)-1-(naphthalen-2-yl)ethanol**
0.075 M in toluene

100 mL volumetric flask: 1.2917 g
10 mL volumetric flask: 0.1292



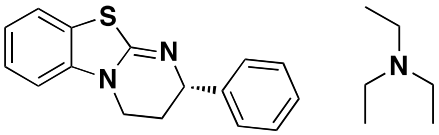
***6.* (*S*)-1-(naphthalen-2-yl)ethanol**
0.075 M in toluene

100 mL volumetric flask: 1.2917 g
10 mL volumetric flask: 0.1292



Catalysts and Base (one 250 mL volumetric flask and one 100 mL volumetric flask per catalyst/base solution)

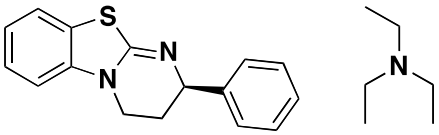
***7.* (*S*)-HBTM + NEt_3**



0.0009375 M in toluene with respect to (*S*)-HBTM
6.0 M in toluene with respect to NEt_3

250 mL volumetric flask: 0.0624 g (*S*)-HBTM and 209.07 mL NEt_3
100 mL volumetric flask: 0.0250 g (*S*)-HBTM and 83.86 mL NEt_3

***8.* (*R*)-HBTM + NEt_3**

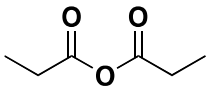


0.0009375 M in toluene with respect to (*R*)-HBTM
6.0 M in toluene with respect to NEt_3

250 mL volumetric flask: 0.0624 g (*R*)-HBTM and 209.07 mL NEt_3
100 mL volumetric flask: 0.0250 g (*R*)-HBTM and 83.86 mL NEt_3

Anhydride (one 500 mL volumetric flask and one 250 mL volumetric flask)

***9.* propionic anhydride**



6.0 M in toluene

500 mL volumetric flask: 384.65 mL propionic anhydride
250 mL volumetric flask: 192.33 mL propionic anhydride

Student Analysis of Data

Both a qualitative and quantitative analysis of the TLC results in this experiment have been developed. The qualitative analysis has students visually observing which reaction produced a larger ester product spot on the TLC plate and/or a smaller starting alcohol spot. The quantitative analysis uses ImageJ, which is a free program developed by the National Institutes of Health and can be downloaded at http://rsbweb.nih.gov/ij/. Detailed instructions on how to use ImageJ can be found on Page 21. Students acquire an image of their TLC plate and manipulate it using ImageJ in order to measure values for the spot densities of the spots on the TLC plate. Detailed instructions on how to use ImageJ can be found later in this document.

Student Demographics and Student Accuracy

Version **α** of this experiment has been performed with class sizes of over 1000 students composed primarily of sophomore undergraduates that have taken 2 quarters of organic chemistry lecture and 1 quarter of organic chemistry laboratory. Version **β** of this experiment has been performed in a class with 500 students comprised primarily of a mixture of students that have taken 1 semester of organic chemistry lecture or were concurrently taking a semester of organic chemistry lecture. Both versions are scheduled towards the end of the course. An analysis of student accuracy of a class performing Version **α** of this experiment using both qualitative and quantitative analyses showed that the vast majority of students were able to correctly assign the structure and absolute configuration of their unknown alcohol (Table SM 3.1.8.1). An analysis of responses where the absolute configuration was not correctly identified can be found in Table SM 3.1.8.2.

Table SM 3.1.8.1. Analysis of Student Laboratory Conclusions

Student Conclusion	Yes (%)[a]	No (%)[a]	Omitted (%)[a]
Correct Qualitative Analysis of TLC	933 (93.6)	64 (6.4)	—
Correct Quantitative Analysis via ImageJ	916 (91.9)	81 (8.1)	—
Correct Structure via ^{1}H NMR	842 (84.5)	63 (6.3)	92 (9.2)

[a] The percentages were based on the number of reports received (997); thirty-nine students did not turn in a report.

Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

Table SM 3.1.8.2. Student Errors for Incorrect Qualitative Analyses

Error	Number of Students
Misinterpreted TLC plate	19
Conclusions match provided TLC plate, but not unknown number (switched HBTM enantiomers for reaction A and B)	10
Claims both reactions went to completion	9
Performed ImageJ analysis incorrectly and matched quantitative result to qualitative result	8
Used mnemonic incorrectly	8
Mislabeled fast reaction	4
Claims reactions did not run	3
Unclear, may have guessed	3
Total	64

Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

Photos of Experiment Setup and TLC Plates

Figure SM 3.1.8.6. Vials A and B after the addition of all solutions

Figure SM 3.1.8.7. Experiment Version **α**: High reagent concentrations, high contrast between lanes Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

Figure SM 3.1.8.8. Experiment Version **β**: Low reagent concentrations, lower contrast between lanes

Analysis of ^{1}H NMR Spectra of 1, 2, and 3

The key differentiating spectroscopic component between the three possible enantioenriched alcohol compounds is the aromatic system. Compound **1** (1-(4-bromophenyl)ethanol) has a *para* substituent, allowing the student to recognize what they believe to be "doublets" for the aromatic protons.* The total number of aromatic protons is four. Compound **2** (1-(3-methoxyphenyl)ethanol) has a *meta* substituent, giving a more complex aromatic region of "multiplets".** The total number of aromatic protons is four. The other key difference here is the appearance of a methyl singlet from the methoxy group. Compound **3** (1-(naphthalene-2-yl)ethanol) has a naphthyl aromatic group, which is clearly differentiated from the others because it possesses seven aromatic protons.

The ^{1}H NMR spectra that the students receive when picking up their unknown is used to rule out two of the three possible compounds in order to provide them logical evidence that the ^{1}H NMR spectra they receive matches the other compound.

A summary of things to look for when ruling out the other two compounds for each possible unknown are listed below.

For compound **1**, things to look for are:
- -"doublets" in the aromatic region
- -a total of 4 protons in the aromatic region
- -no singlet integrating to 3 protons (which would be found in the *m*-methoxy compound)

For compound **2**, things to look for are:
- -a total of 4 protons in the aromatic region
- -a lack of "doublets" in the aromatic region
- -a singlet integrating to 3 protons

For compound **3**, things to look for are:
- -a total of 7 protons in the aromatic region
- -no singlet integrating to 3 protons (which would be found in the *m*-methoxy compound)

---

* Although the signals of compound **1** in the aromatic region look like doublets, they are actually second-order spectrum patterns displaying a AA'BB' pattern. We do not address this in the laboratory experiment to the students because our students are not introduced to second-order spectra in their introductory organic laboratory studies. Therefore, the aromatic protons for compound **1** are referred to as "doublets" for clarity to the student's current understanding of the NMR spectral data. If interested in more on second-order spectra **for the instructor's reference**, see: P. Crews, J. Rodríguez, M. Jaspars, Interpretation and Use of Proton or Carbon Coupling Constants. In *Organic Structure Analysis*, K. N. Houk, G. M. Loudon, Eds.; Oxford University Press, Inc.: New York, NY, 1998; pp 103–128. If interested in more on second-order spectra **for the student's reference**, see: D. L. Pavia, G. M. Lampman, G. S. Kriz, J. A. Vyvyan, Part Three: Spin-Spin Coupling. In *Introduction to Spectroscopy*, 4th ed.; L. Lockwood, B. Kirksey, B. Kauser, K. Brown, Eds.; Cengage Learning: Belmont, CA, 2009; pp 268–277.

** Although the signals of compound **2** in the aromatic region appear to be multiplets, they are actually second-order spectrum patterns displaying a ABCD pattern. We also do not address this in the laboratory experiment to our students for the same reasons as in footnote 1; the same references are useful for additional reading on this subject.

^{1}H NMR Spectra of 1, 2, and 3

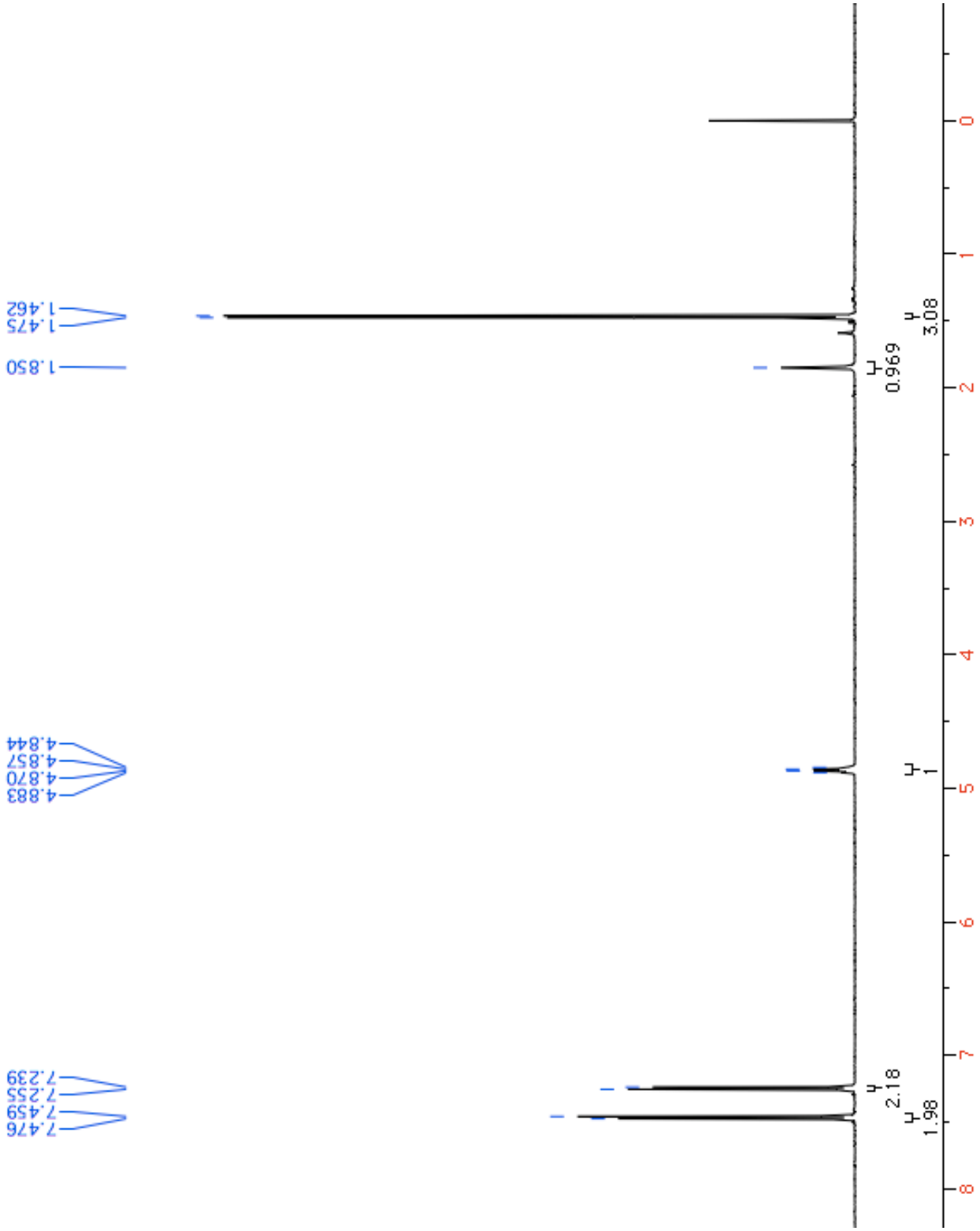


Figure SM 3.1.8.9. ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of **1.** Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

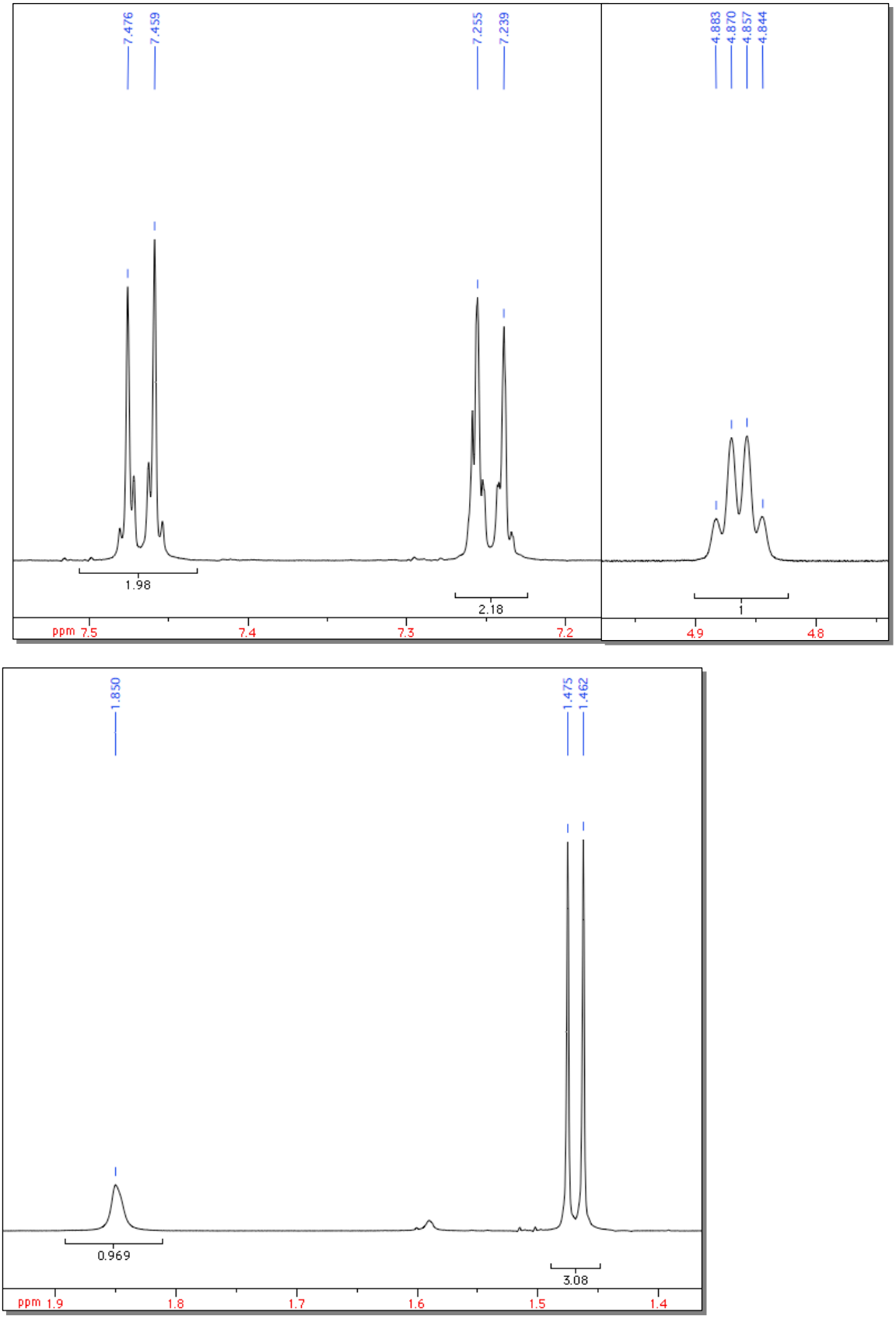


Figure SM 3.1.8.10. Enlarged ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of **1.** Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

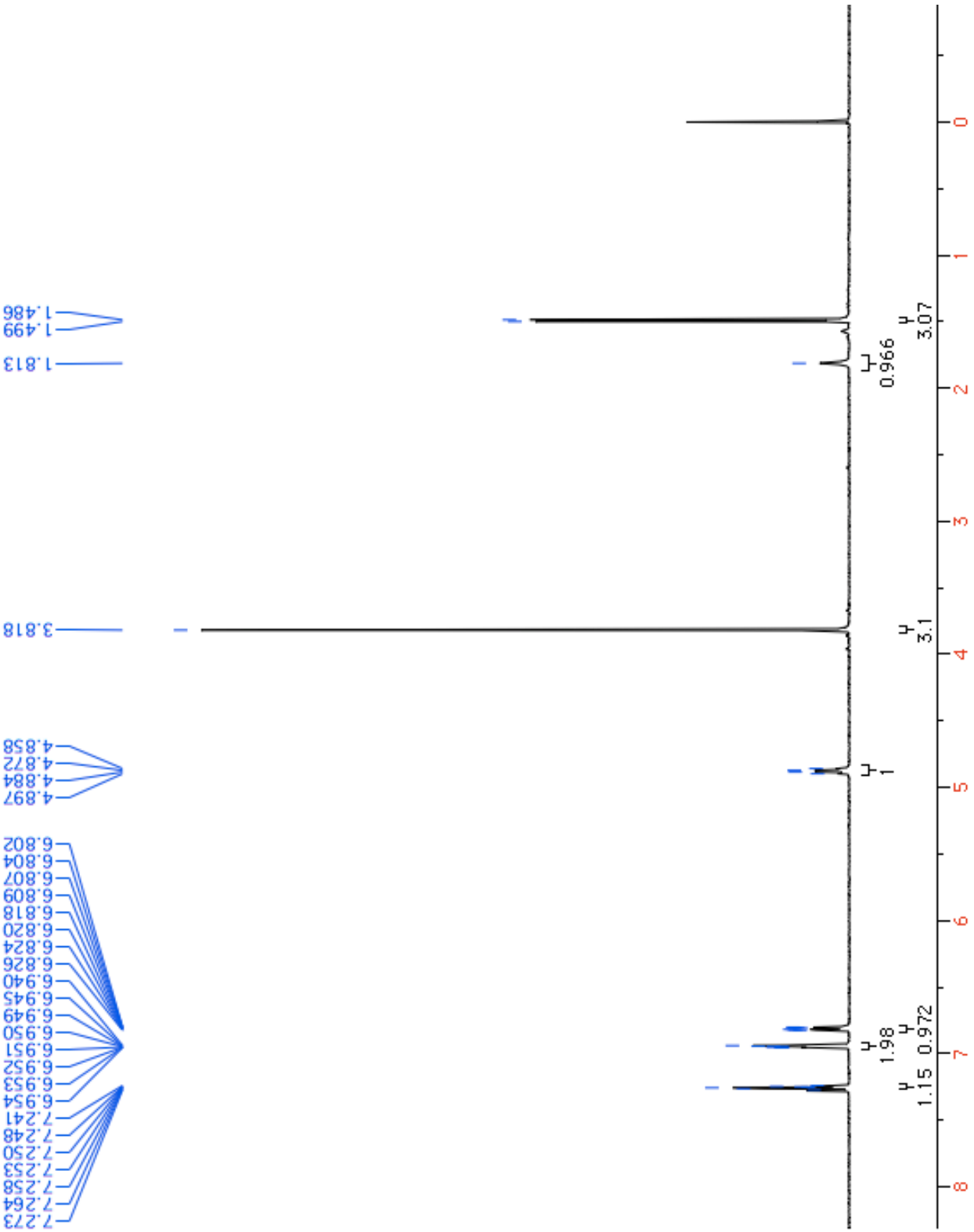


Figure SM 3.1.8.11. ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of **2.** Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

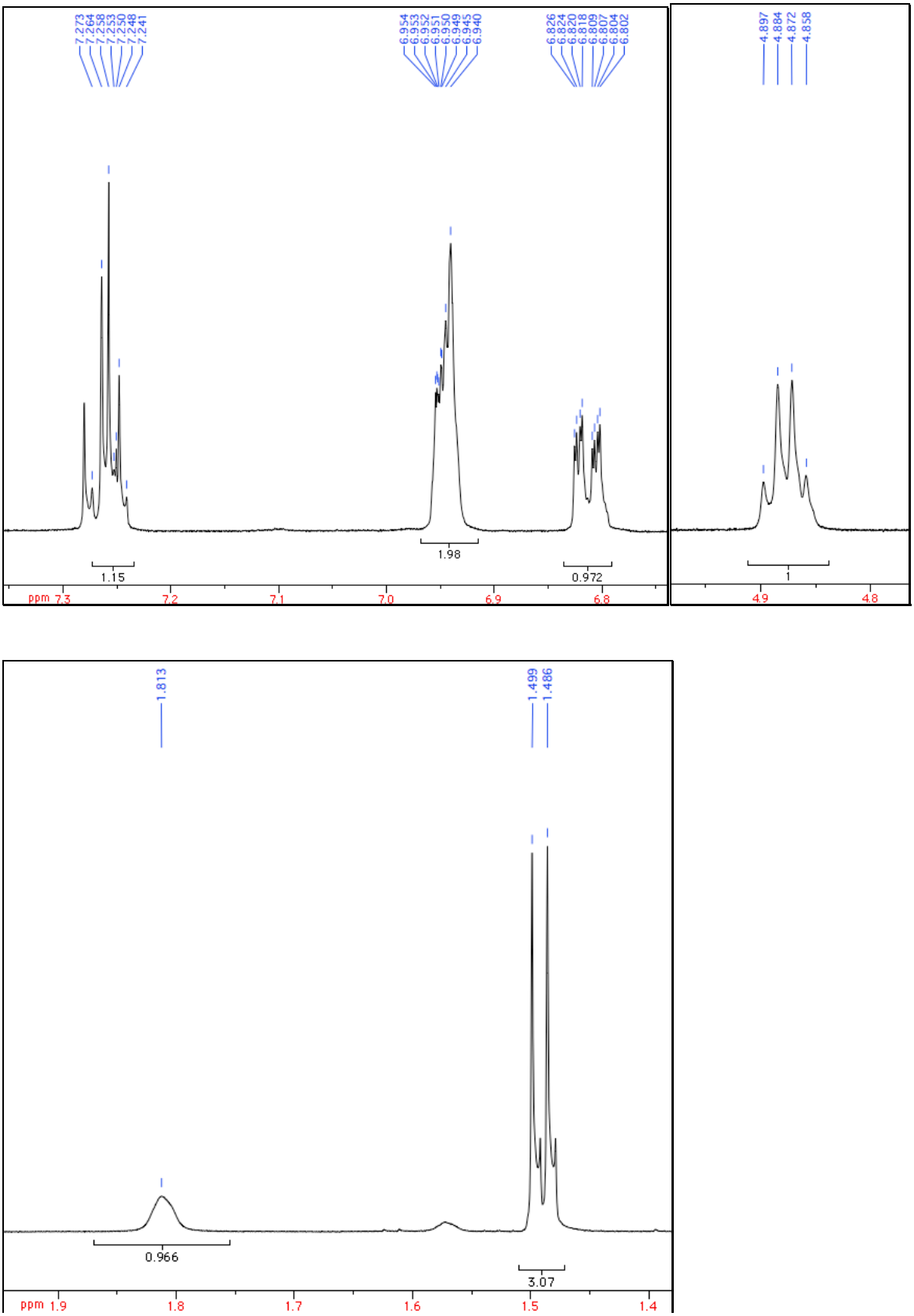


Figure SM 3.1.8.12. Enlarged ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of **2.** Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

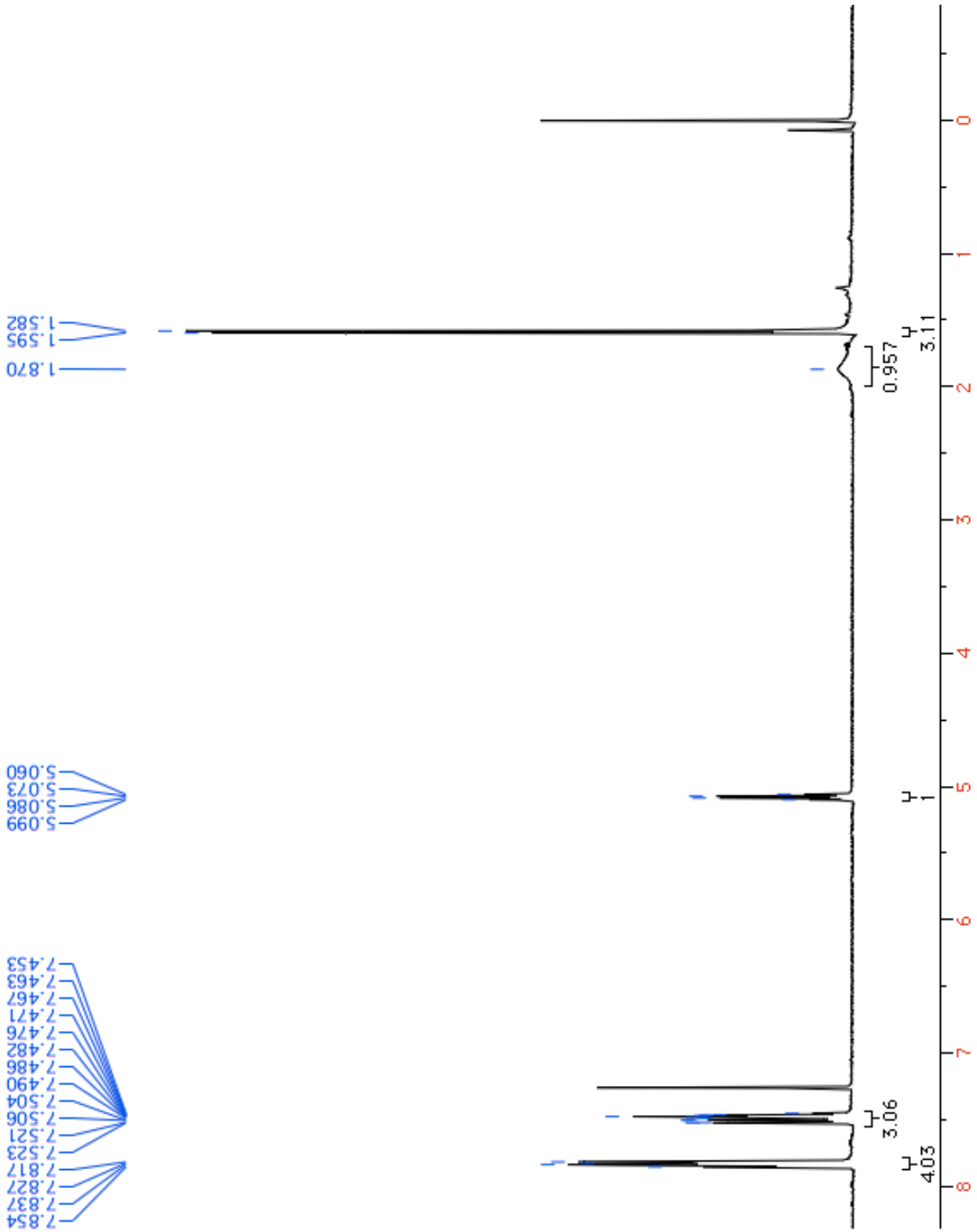


Figure SM 3.1.8.13. ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of **3.** Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

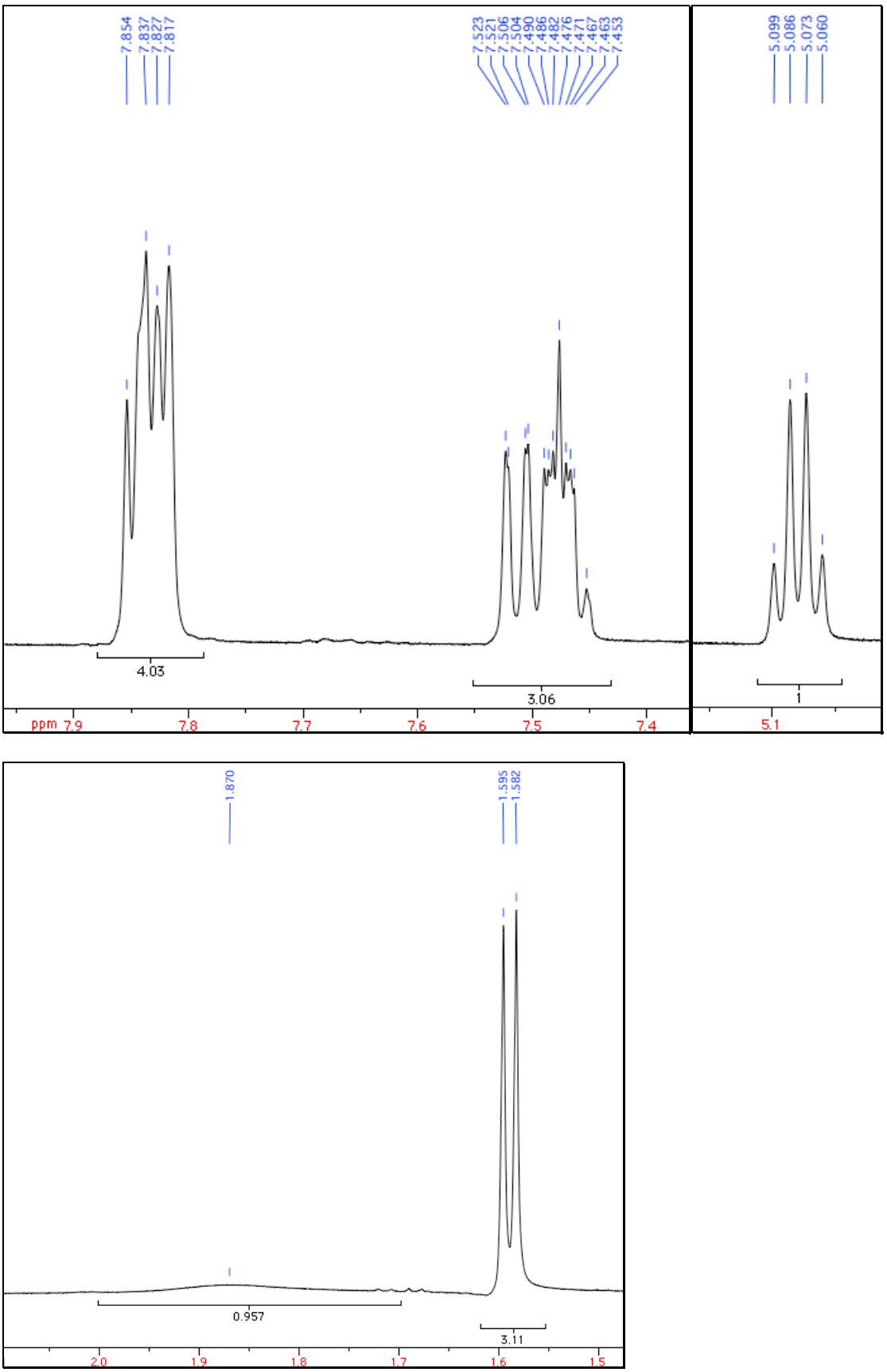


Figure SM 3.1.8.14. Enlarged ^{1}H NMR Spectrum (500 MHz, $CDCl_3$) of **3.** Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

Quantitative Analysis of TLC Plates using ImageJ

Pages 22-25 contain instructions on the use of ImageJ in the quantitative analysis of this experiment using data obtained from Version **α**. Pages 26-29 contain instructions on the use of ImageJ in the quantitative analysis of this experiment using data obtained from Version **β**. The instructions have been separated from the rest of this document to allow instructors to print them to use as a handout to give to students. A video version of these instructions can be provided upon contacting Dr. Renée Link at rlink@uci.edu.

Instructions for Quantitative Analysis of TLC Plates using ImageJ

ImageJ is a program that can be used to compare the density of bands or spots and can be downloaded for free at http://rsbweb.nih.gov/ij/. It is most commonly used for biochemistry techniques. These instructions assume that your picture from your TLC stain is a .tif, .jpg, or .png file.

1. Open the ImageJ program.

2. Open the image file using *File* > *Open* in ImageJ. Your TLC plate should be vertical in your image. Rotate it if necessary using *Image* > *Transform*

3. Make sure the *Rectangle Selections* tool is clicked (Figure 1).

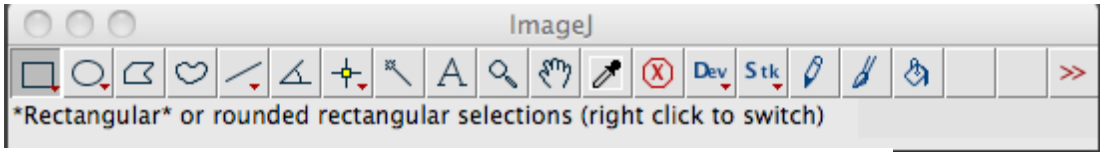


Figure 1. Selection of the *Rectangle Selections* tool in ImageJ**.**

4. Draw a rectangle around the first lane.

5. After drawing the rectangle around your first lane, press **1** on your computer keyboard. This will leave lane 1 in place, while creating a second rectangle directly on top of it (Figure 2).

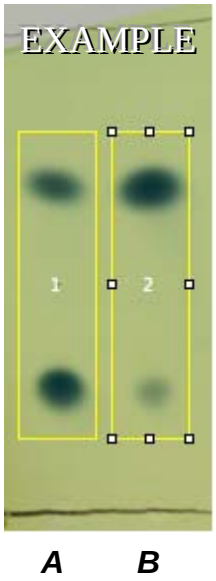


Figure 2. TLC plate with rectangles drawn to separate lanes using ImageJ.

6. Use your arrow keys to move the second rectangle over the second lane.

7. Press **3** on your computer keyboard. This will automatically bring up a window that has integrated the lanes for density. This window represents the relative density of the contents of the rectangle over each lane. The rectangles are arranged top to bottom on the profile plot (Figure 3)

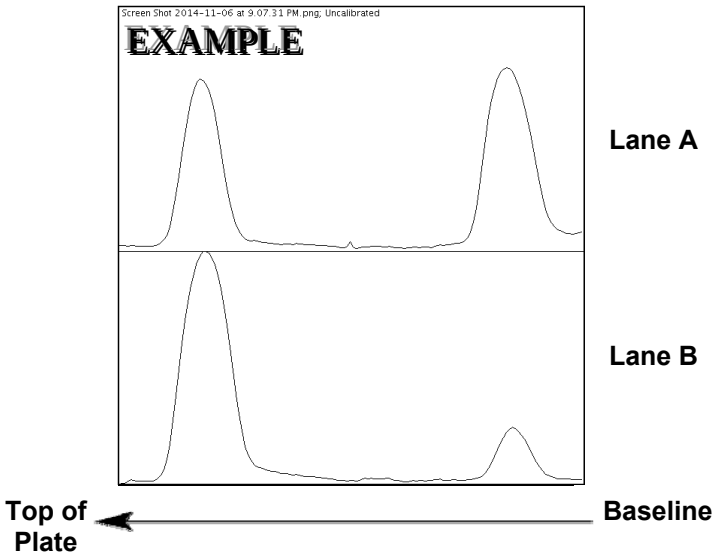


Figure 3. Readout window of an analysis of a TLC plate using ImageJ.

8. Now and at any point, you may save the image using File > Save As. The image format should be Jpeg or PNG)

9. Select the *Line* tool (Figure 4).

Figure 4. Selection of the *Line* tool in ImageJ.

10. Use the *Line* tool to draw a line across the baseline for each peak in the TLC lanes. Images will have some background signal, so the peaks likely won't reach down to the baseline of the profile plot. This may make the peak appear to float above the baseline. It is necessary to make sure to close off the peak in order to measure its size.

11. Click the *Wand* tool (Figure 5).

Figure 5. Selection of the *Wand* tool in ImageJ.

12. Use the *Wand* tool to click inside of each of the peaks in the TLC lanes. As you click on a peak, it will be highlighted in yellow. There will also be a window that pops up to give area values for each of the peaks. Keep track of the order that you click on the peaks, as that is the order they will show up on the Results window (Figure 6).

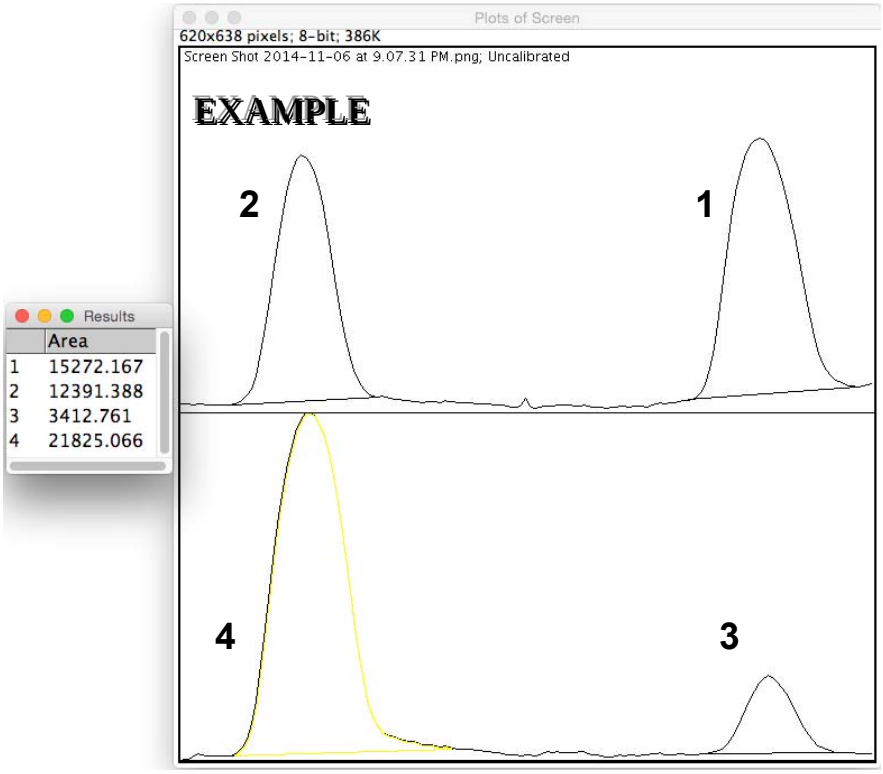


Figure 6. Spot density integrations of a TLC plate using ImageJ. The labeling numbers in the figure have been added using an image program to aid in visualization. **IMAGEJ WILL NOT PROVIDE YOU WITH LABELING NUMBERS.** Students must remember the order in which they select peaks and match those peaks to the numbers in the pop-up window.

Use the integrated value of the unknown alcohol (starting material) spot and of the ester (product) spot for both reaction **A** and reaction **B** to calculate reaction conversion using the below equation.

$$\text{Reaction Conversion (\%)} = \frac{\text{Integrated value of ester spot}}{\text{Integrated value of alcohol spot} + \text{Integrated value of ester spot}} \times 100\%$$

13. Determine which reaction, and therefore which enantiomer of the HBTM catalyst, reacted faster.

14. Determine the absolute configuration of the unknown alcohol based on the reaction conversions via the predictive mnemonic provided.

Instructions for Quantitative Analysis of TLC Plates using ImageJ

ImageJ is a program that can be used to compare the density of bands or spots and can be downloaded for free at http://rsbweb.nih.gov/ij/. It is most commonly used for biochemistry techniques. These instructions assume that your picture from your TLC stain is a .tif, .jpg, or .png file.

1. Open the ImageJ program.

2. Open the image file using *File* > *Open* in ImageJ. Your TLC plate should be vertical in your image. Rotate it if necessary using *Image* > *Transform*

3. Make sure the *Rectangle Selections* tool is clicked (Figure 1).

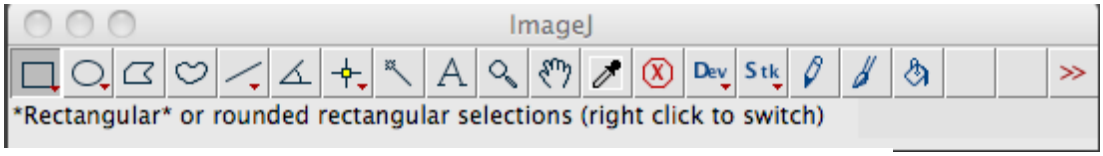


Figure 1. Selection of the *Rectangle Selections* tool in ImageJ**.**

4. Draw a rectangle around the first lane.

5. After drawing the rectangle around your first lane, press **1** on your computer keyboard. This will leave lane 1 in place, while creating a second rectangle directly on top of it (Figure 2).

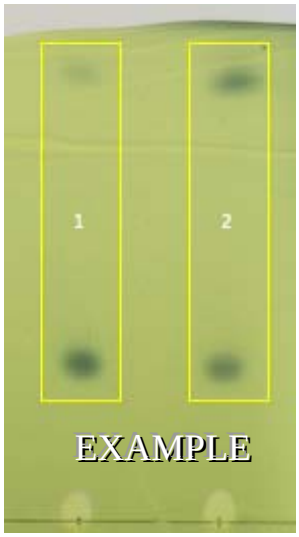


Figure 2. TLC plate with rectangles drawn to separate lanes using ImageJ.

6. Use your arrow keys to move the second rectangle over the second lane.

7. Press **3** on your computer keyboard. This will automatically bring up a window that has integrated the lanes for density. This window represents the relative density of the contents of the rectangle over each lane. The rectangles are arranged top to bottom on the profile plot (Figure 3)

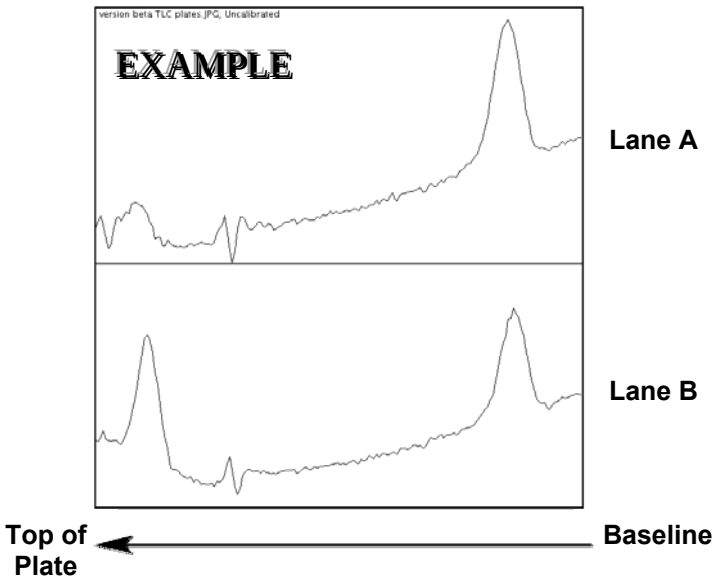


Figure 3. Readout window of an analysis of a TLC plate using ImageJ.

8. Now and at any point, you may save the image using File > Save As. The image format should be Jpeg or PNG)

9. Select the *Line* tool (Figure 4).

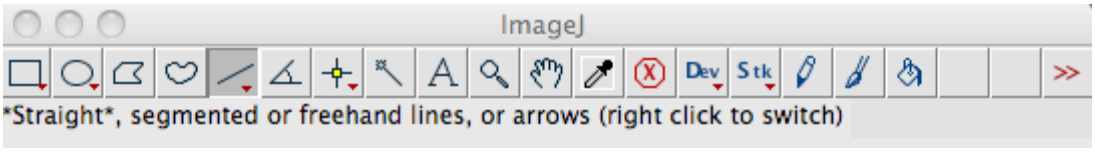


Figure 4. Selection of the *Line* tool in ImageJ.

10. Use the *Line* tool to draw a line across the baseline for each peak in the TLC lanes. Images will have some background signal, so the peaks likely won't reach down to the baseline of the profile plot. This may make the peak appear to float above the baseline. It is necessary to make sure to close off the peak in order to measure its size.

11. Click the *Wand* tool (Figure 5).

Figure 5. Selection of the *Wand* tool in ImageJ.

12. Use the *Wand* tool to click inside of each of the peaks in the TLC lanes. As you click on a peak, it will be highlighted in yellow. There will also be a window that pops up to give area values for each of the peaks. Keep track of the order that you click on the peaks, as that is the order they will show up on the Results window (Figure 6).

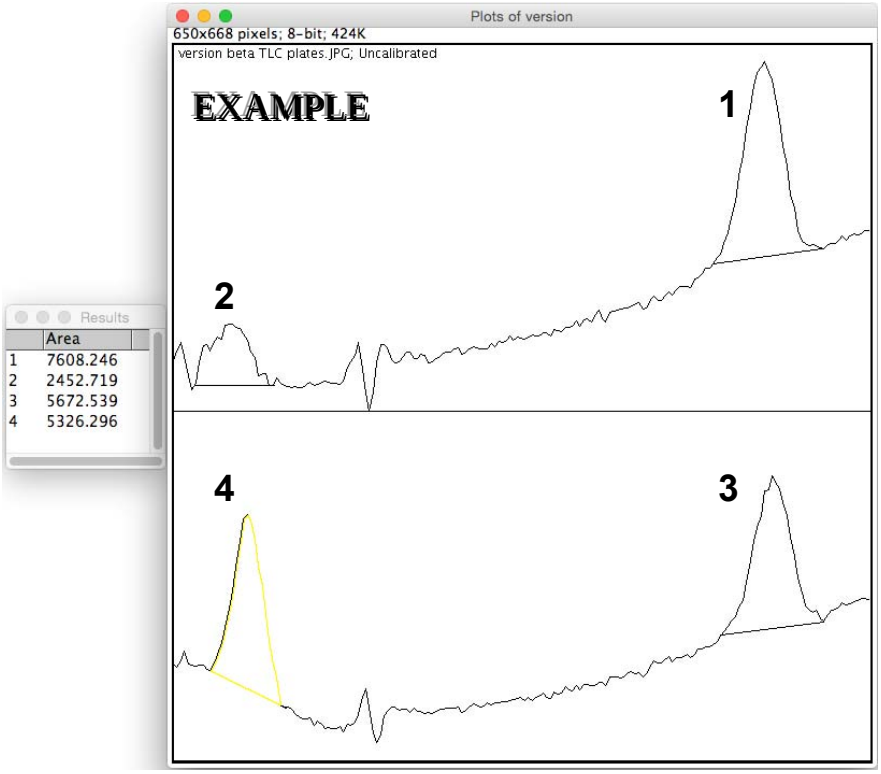


Figure 6. Spot density integrations of a TLC plate using ImageJ. The labeling numbers in the figure have been added using an image program to aid in visualization. **IMAGEJ WILL NOT PROVIDE YOU WITH LABELING NUMBERS.** Students must remember the order in which they select peaks and match those peaks to the numbers in the pop-up window.

Use the integrated value of the unknown alcohol (starting material) spot and of the ester (product) spot for both reaction **A** and reaction **B** to calculate reaction conversion using the below equation.

$$\text{Reaction Conversion (\%)} = \frac{\text{Integrated value of ester spot}}{\text{Integrated value of alcohol spot} + \text{Integrated value of ester spot}} \times 100\%$$

13. Determine which reaction, and therefore which enantiomer of the HBTM catalyst, reacted faster.

14. Determine the absolute configuration of the unknown alcohol based on the reaction conversions via the predictive mnemonic provided.

Optional Resources for Discussion

The following topics can be used as a jumping off points for class discussions on related to the experiment that may be covered in other courses or other aspects of the course.

Brief History of Chirality

The concept of molecular dissymmetry was developed by the French chemist Louis Pasteur in 1848. Pasteur's experiment with the sodium ammonium salt of tartaric acid showed the first example of two isolated enantiomers of the same compound. Lord Kelvin later proposed the term chirality in 1894, which has become the predominant term utilized for the concept of molecular dissymmetry.[7]

The asymmetric carbon atom was proposed independently by Jacobus Henricus van 't Hoff and Joseph Achille Label in 1874, where they suggested that four different substituents on the same carbon atom would result in the opportunity for two different geometric assemblies of the substituents that were non-superimposible mirror images. Emil Fischer built upon this concept starting in 1884 with his experiments detailing the asymmetric synthesis of sugars. Fischer reported chemical stereoselectivity in enzymatic reactions of sugars, finding separate cases that displayed enantioselectivity and diastereoselectivity. His work led him to propose the lock-and-key model for enzymatic transformations. Van't Hoff and Fischer were awarded the first and second Nobel prizes in chemistry in 1901 and 1902, respectively, for their work.[7]

Emil Fischer's pioneering work with asymmetric synthesis in the enzymatic reactions of sugars and his development of the understanding of enzymatic recognition with the lock-and-key model ignited interest in the field of asymmetric organic synthesis and methodology, which has only strengthened over the past 130 years.[7] With the development of asymmetric transformations and improvements in the isolation of single enantiomers of natural products came a need to be able to identify and determine the absolute configuration of the enantioenriched stereocenter(s) of interest.[8-10]

The Cahn-Ingold-Prelog (CIP) system was developed in 1966 to aid in this endeavor by assigning either the *R* or *S* configuration to a stereocenter depending on the priorities of the substituents attached to that stereocenter.[11] The CIP system serves as an effective descriptor of a stereocenter, as opposed to optical rotation, which is only a characteristic of the entire molecule regardless of the number of stereocenters.

The Mismatched Case Transition State

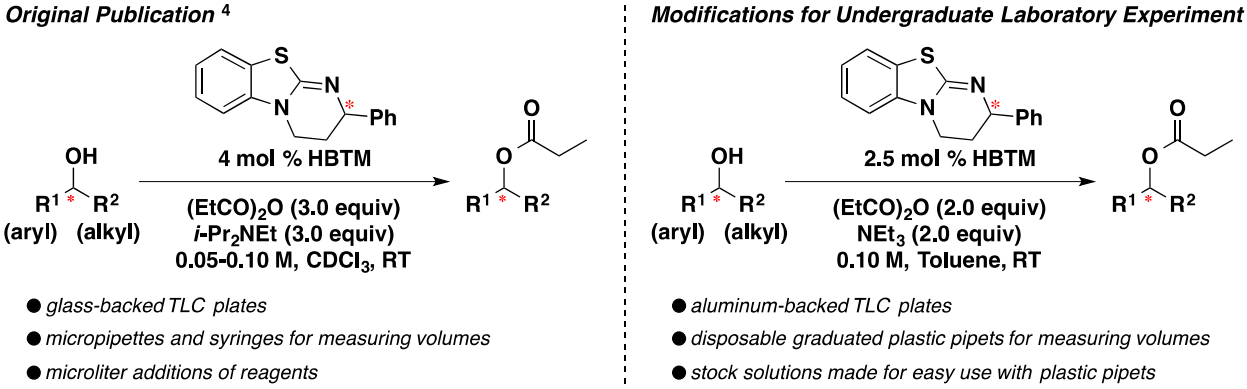
The type of mismatched interaction of the unknown alcohol and the HBTM catalyst has been examined with a similar catalytic system and is discussed in detail.[12] The lowest energy conformation provides a more complicated view of the transition state for the mismatched case. We did not discuss this topic with our students unless they inquired out of curiosity.

Experimental Modifications

Historical Perspective: Modifications from Published Work to Version α

A number of key modifications to a previously reported system[4] were made in order to apply it to an undergraduate experiment (Figure SM 3.1.8.15). First, the solvent was changed from $CDCl_3$ to toluene. Chloroform is a known carcinogen and a chlorinated solvent. Changing the

solvent for the reaction to toluene resulted in a less hazardous solvent for the students and a non-chlorinated more environmentally friendly solvent for waste disposal. Toluene is also less volatile, which was important for maintaining more accurate stock solutions over a week's time. Second, the base was changed from Hünig's base to triethylamine. We found these bases behaved similarly, but triethylamine was considerably less expensive. Third, the catalyst loading was reduced to 2.5 mol % in the reaction. This decreases the overall cost of the experiment, as the catalyst is an expensive component. With this modification, a group of two students uses only 1.0 mg of each catalyst. The anhydride and base were also reduced from 3.0 to 2.0 equivalents to reduce waste. Fourth, the TLC plates were changed from glass-backed to aluminum-backed to reduce cost and increase ease of cutting the plates. Fifth, plastic graduated pipets were used for measuring volumes that students added to the reaction. The result of all of these changes is a more cost-effective, less hazardous, and simpler procedure that can be



easily implemented for a large number of students.

Figure SM 3.1.8.15. Modifications made to adapt protocol to undergraduate laboratory experiment. Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright 2014 American Chemical Society.

Historical Perspective: Modifications from Version α to Version β

In recognition of the cost of commercially available enantiopure alcohols and HBTM catalyst, Version **β** was designed to minimize the amounts of those reagents used in the experiment. As discussed on page 4, previous research has shown that the rate law for this reaction is first-order in catalyst, first-order in anhydride, and first-order in alcohol.[6] For example, doubling the concentration of anhydride would double the rate of reaction. Compared to Version **α**, the concentrations of alcohol and catalyst were reduced to a quarter and an eighth of their original concentrations, respectively. To compensate, the concentration of anhydride was increased ten-fold. The final change in rate of the reactions as a result of these new concentrations was a net reduction of ca. 60%. The reactions were able to proceed to an acceptable extent, while simultaneously capable of being visualized on a TLC plate.

Instructor Options for Experimental Modifications

Versions **α** and **β** of this experiment mark the extremes of what concentrations of reagents can be used in this experiment. Version **α** of this experiment is designed to maximize the visual quality of the final TLC plate used for analysis, but requires higher concentrations of reagents. Version **β** of this experiment is designed to drastically lower concentrations of chiral reagents to minimize cost, with the tradeoff being a reduction in the intensities of the spot densities on the

TLC plate. Example images of TLC plates from Versions **α** and **β** can be found on Page 13. It is possible to change reagent concentrations between the two extremes of Versions **α** and **β** as well alter the length of the reactions to suit the desires of the course instructor.

This experiment requires a quantitative analysis using ImageJ. If access to photographic or computer equipment is limited, or if the quantitative analysis is not desired, the quantitative analysis can be removed without damaging the core learning experience.

The ^{1}H NMR spectra given later in this document are genuine ^{1}H NMR spectra of the substrate alcohols. Computer generated ^{1}H NMR spectra can be used for other alcohols if acquiring genuine spectra is not feasible.

The alcohols students use in the reaction portion of this experiment do not need to correspond to the ^{1}H NMR spectra provided to the students. For example, students could be given the ^{1}H NMR spectrum for **1** while their unknown solution could contain an enantiomer of a less expensive commercially available alcohol such as 1-phenylethanol. In this scenario, the experimental learning outcomes are still achieved even though students do not know that their ^{1}H NMR spectrum does not match their unknown alcohol in solution, but the cost of the experiment is significantly reduced.

As of this publication, propionic anhydride is classified as a List I chemical by the United States Drug Enforcement Administration, which may limit its availability in some locations. Butyric anhydride (CAS: 106-31-0) can be substituted for propionic anhydride.

This document details the use of alcohols **1**, **2**, and **3**, but the CEC method is not limited to these three substrates. Any enantioenriched secondary alcohol with an adjacent aromatic system is a viable candidate for use in this experiment. This can be useful when looking to limit costs or adding variety to the experiment. A non-exhaustive listing of commercially available alcohols is shown on Page 33.

Listing of Commercially Available Enantioenriched Alcohols

* (*R*)-(+)-1-phenylethanol (CAS: 1517-69-7)

* (*S*)-(−)-1-phenylethanol (CAS: 1445-91-6)

***** (*S*)-(−)-α-methyl-2-naphthalenemethanol (CAS: 27544-18-9)

***** (*R*)-(+)-α-methyl-2-naphthalenemethanol (CAS: 52193-85-8)

***** (*R*)-4-bromo-α-methylbenzyl alcohol (CAS: 76155-78-7)

***** (*S*)-4-bromo-α-methylbenzyl alcohol (CAS: 100760-04-1)

***** (*S*)-(−)-2-bromo-α-methylbenzyl alcohol (CAS: 114446-55-8)

* (*R*)-(+)-2-bromo-α-methylbenzyl alcohol (CAS: 76116-20-6)

***** (*R*)-4-chloro-α-methylbenzyl alcohol (CAS: 75968-40-0)

***** (*S*)-4-chloro-α-methylbenzyl alcohol (CAS: 99528-42-4)

***** (*S*)-(−)-2-chloro-α-methylbenzyl alcohol (CAS: 131864-71-6)

* (*R*)-(+)-2-chloro-α-methylbenzyl alcohol (CAS: 120466-66-2)

(*1R,2S*)-(−)-*N*-methylephedrine (CAS: 552-79-4)

(*1S,2R*)-(+)-*N*-methylephedrine (CAS: 42151-56-4)

(*S*)-(−)-α-methyl-1-naphthalenemethanol (CAS: 15914-84-8)

(*R*)-(+)-α-methyl-1-naphthalenemethanol (CAS: 42177-25-3)

*****These commercially available enantioenriched alcohols have been used successfully with this laboratory experiment.

Acknowledgement

Adapted with permission from *J. Chem. Educ.*, 2014, **91**, pp 716–721. Copyright (2014) American Chemical Society.

References

[1] A. J. Wagner, J. G. David, S. D. Rychnovsky, *Org. Lett.* 2011, **13**, 4470−4473.
[2] M. A. Perry, J. V. Trinidad, S. D. Rychnovsky, *Org. Lett.* 2013, **15**, 472−475.
[3] S. M. Miller, R. A. Samame, S. D. Rychnovsky, *J. Am. Chem. Soc.* 2012,**134**, 20318−20321.
[4] A. J. Wagner, S. D. Rychnovsky, *J. Org. Chem.* 2013, **78**, 4594−4598.
[5] A. J. Wagner, S. M. Miller, S. Nguyen, G. Y. Lee, S. D. Rychnovsky, R. D. Link, *J. Chem. Educ.*, 2014, **91**, 716–721.
[6] A. J. Wagner, S. D. Rychnovsky, *Org. Lett.* 2013, **15**, 5504–5507; 2014, **16**, 4348.
[7] J. Gal, P. Cintas, P. *Top. Curr. Chem.* 2013, **333**, 1–40.
[8] E. L. Eliel, S. H. Wilen, *Stereochemistry of Organic Compounds*; Wiley-Interscience: Hoboken, NJ, 1994; pp 101−147 and 991−1105.
[9] J. M. Seco, E. Quiñoá, R. Riguera, *Chem. Rev.* 2004, **104**, 17−118.
[10] T. J. Wenzel, C. D. Chisholm, *Chirality* 2011, **23**, 190−214.
[11] R. S. Cahn, C. Ingold, V. Prelog, *Angew. Chem., Int. Ed.* 1966, **5**, 385–415.
[12] X. Li, P. Liu, K. N. Houk, V. B. Birman, *J. Am. Chem. Soc.* 2008, **130**, 13836−13837.

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

Anhydride aminolysis: synthesis of *N*-arylmaleamic acids

Supplementary Material

Experimental notes

Figures

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

Notes:

This work was planned for a 4 h session. Students may work individually or in groups of two.

The reaction between maleic anhydride and the aniline (aniline, *p*-toluidine or *p*-anisidine) is carried out in diethyl ether but other solvents can be used, namely THF or dichloromethane, for instance. A solvent-free synthesis of *N*-arylmaleamic acids has been reported but that methodology was not tested in our labs (J. Trujillo-Ferrara, J. Correa-Basurto, J. Espinosa, J. García, F. Martínez and R. Miranda, *Synthetic Commun*. 2005, **35**, 2017–2023).

The reaction of maleic anhydride with anilines is exothermic. Because of that, it is advisable (but not mandatory) to keep the reaction flask in a water bath. The condenser must be water-refrigerated to avoid the evaporation of the diethyl ether (Figure SM 3.1.9.2).

p-Toluidine and *p*-anisidine are solids and must be dissolved in diethyl ether. Aniline can be used neat or diluted with diethyl ether.

The colour of the reaction mixture is very different depending on the substituent present on the benzene ring: white for R = H, yellow for R = CH_3, and greenish for R = OCH_3.

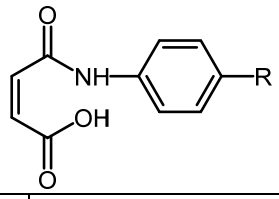
The *N*-arylmaleamic acids are obtained directly by filtration from the reaction mixture. Good quality material for structural characterization (and further transformation, see experiment “Synthesis of *N*-arylmaleimides”) is obtained just by filtration and washing the solid with diethyl ether.

The use of a Buchner funnel to filtrate the reaction product may be problematic (due to the high volatility of the diethyl ether, the filter paper has tendency to roll up). If possible, a filtration unit (figure SM 3.1.9.3) should be used.

This work has been carried out since several years by undergraduate students at their first Organic Chemistry laboratorial course unit. Classes have typically seven groups of two students. The yields of the *N*-arylmaleamic acids obtained by the students are similar, irrespectively of the aniline derivative used, and range from 76% to 100%. The average yields are reported in the Table of Results.

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

Table of Results: Average yields and melting points of the *N*-arylmaleamic acids synthesized by the students.



Entry	R	Average yield (%)	Melting point (ºC)
1	H	96	199-200
2	CH_3	97	188-189
3	OCH_3	97	187-188

Figure SM 3.1.9.1 – Reaction setup apparatus.

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

Figure SM 3.1.9.2 – Synthesis of *N*-phenylmaleamic acid – a suspension is formed immediately after the addition of some drops of aniline (the water bath was removed for clarity).

Figure SM 3.1.9.3 – Filtration unit.

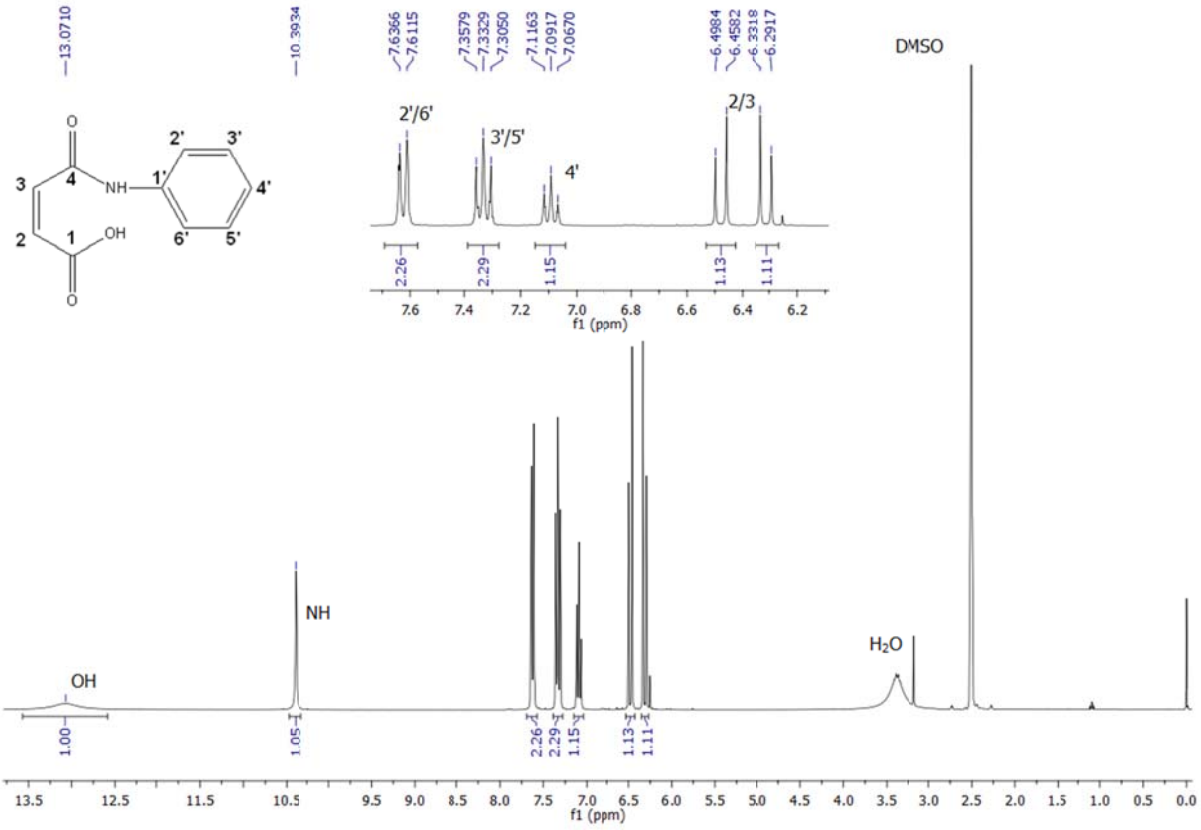


Figure SM 3.1.9.4 – ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of *N*-phenylmaleamic acid

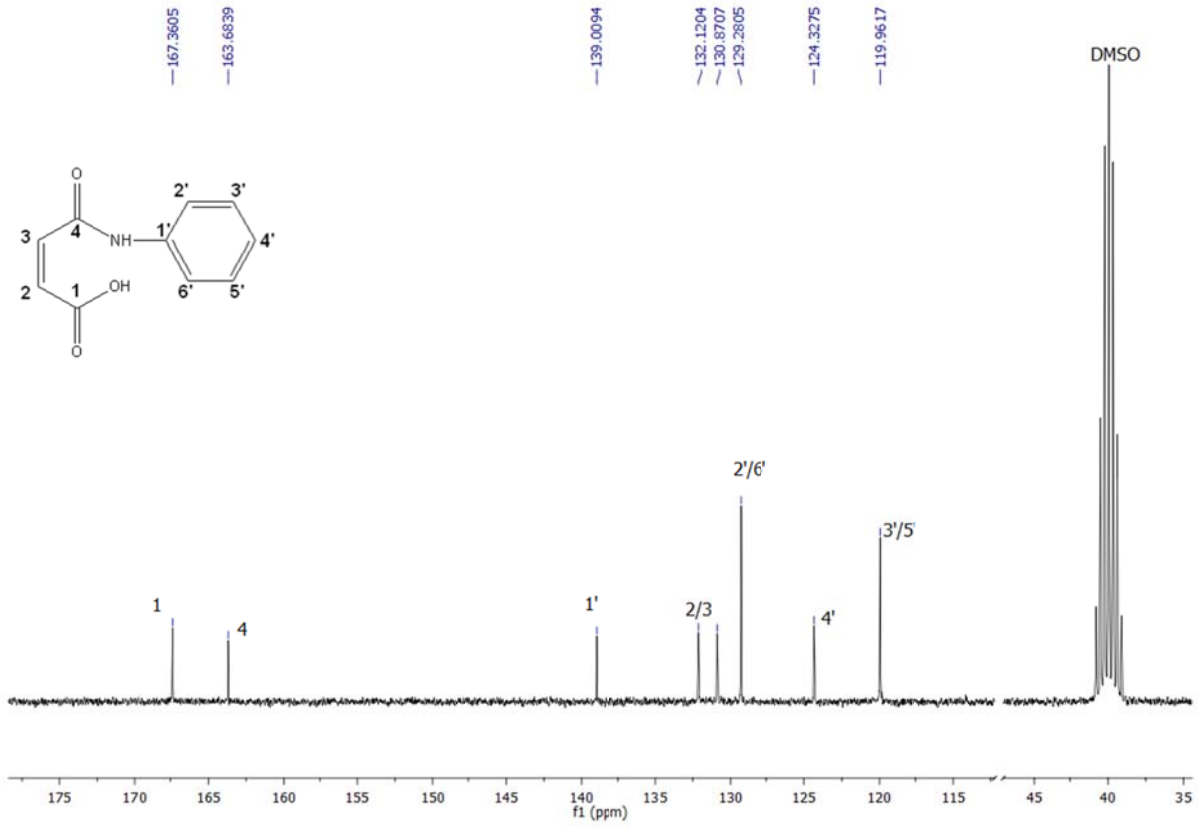


Figure SM 3.1.9.5 – ^{13}C NMR spectrum (75 MHz, DMSO-d_6) of *N*-phenylmaleamic acid.

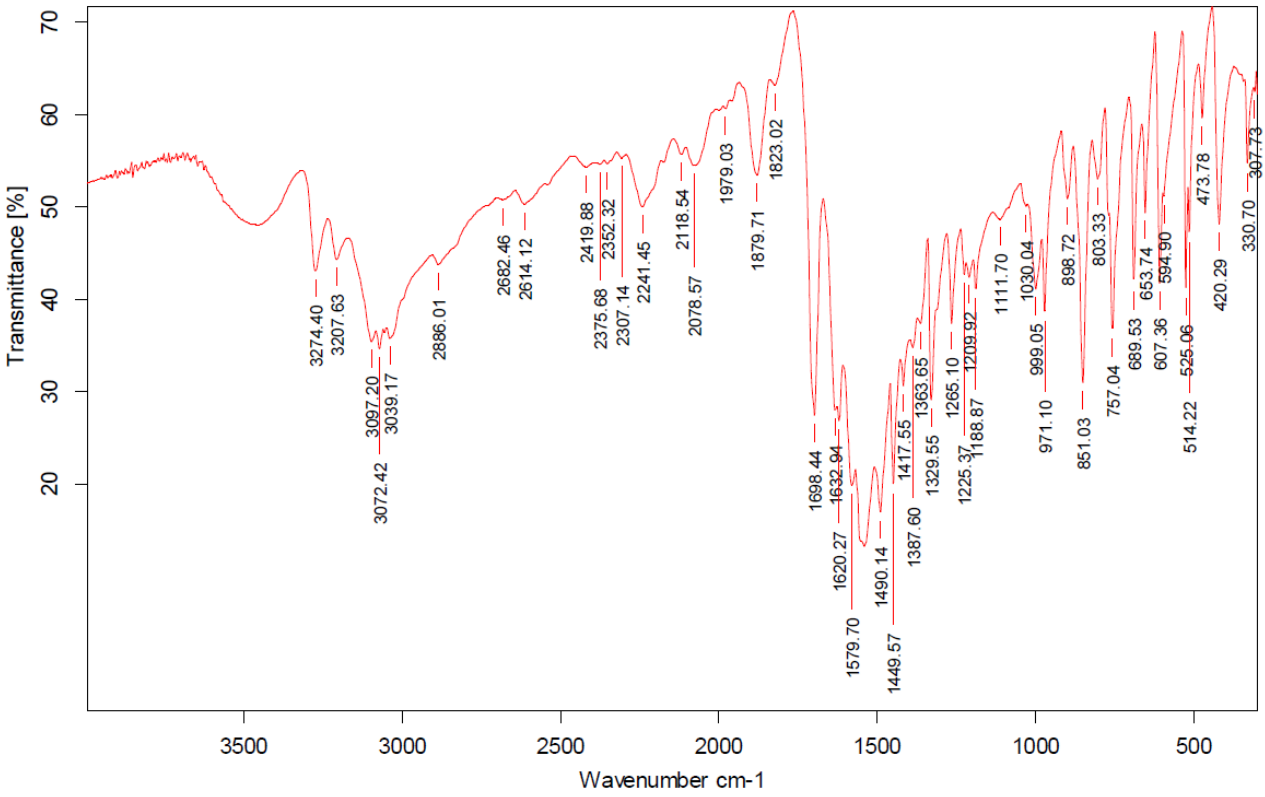


Figure SM 3.1.9.6 – IR spectrum of *N*-phenylmaleamic acid (KBr pellet).

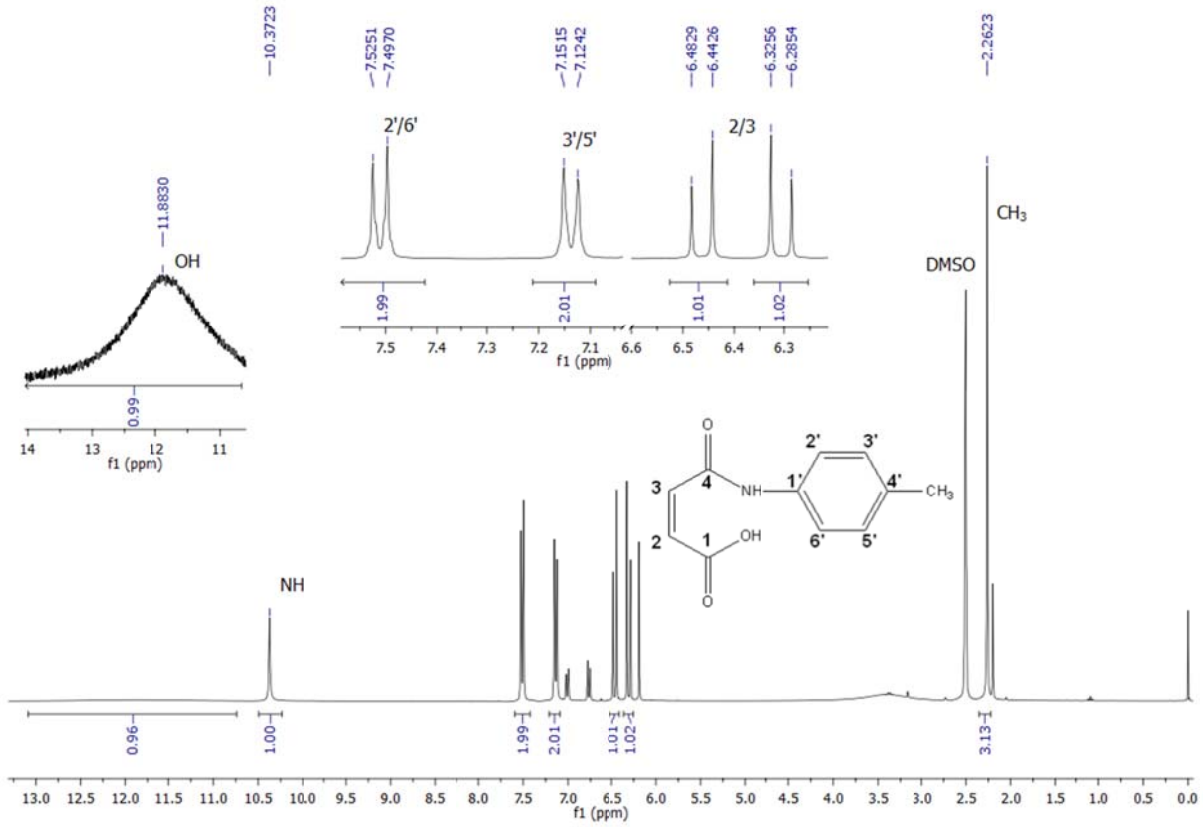


Figure SM 3.1.9.7 – ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of *N*-(4-methylphenyl)maleamic acid.

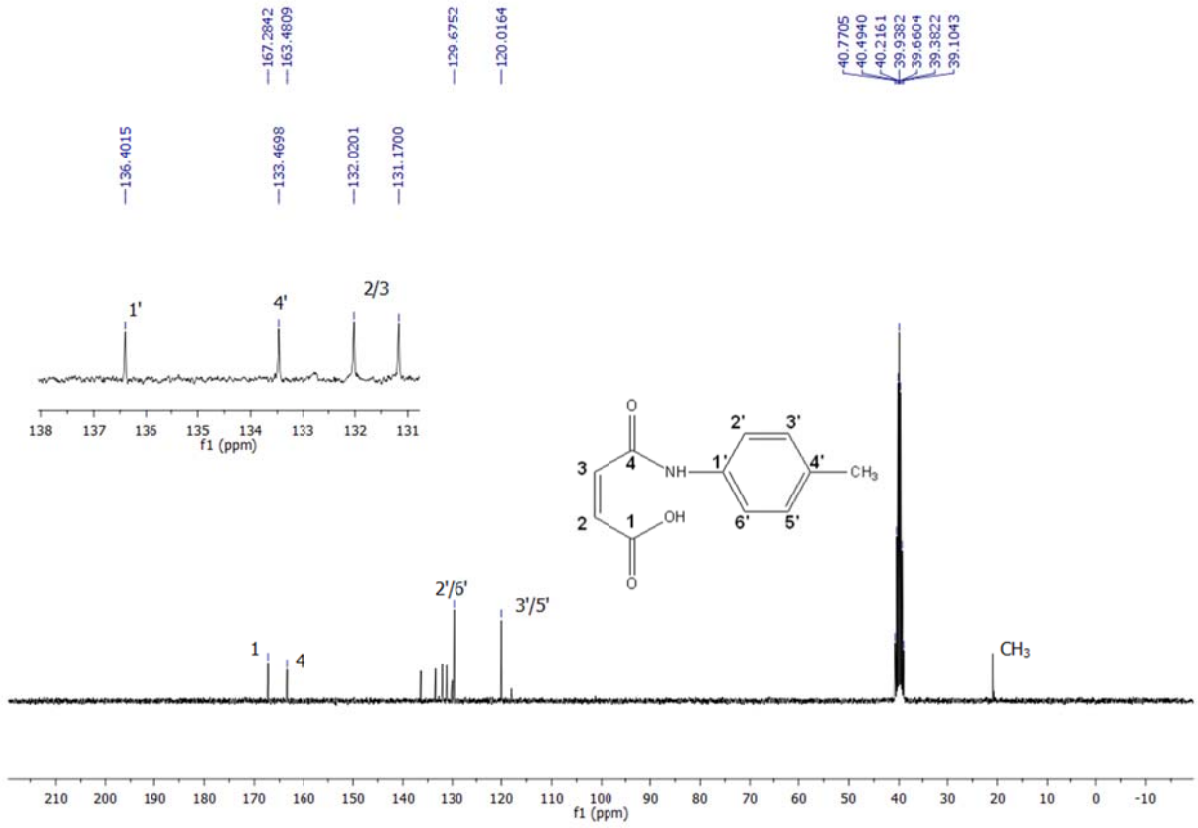


Figure SM 3.1.9.8 – ^{13}C NMR spectrum (75 MHz, DMSO-d_6) of *N*-(4-methylphenyl)maleamic acid.

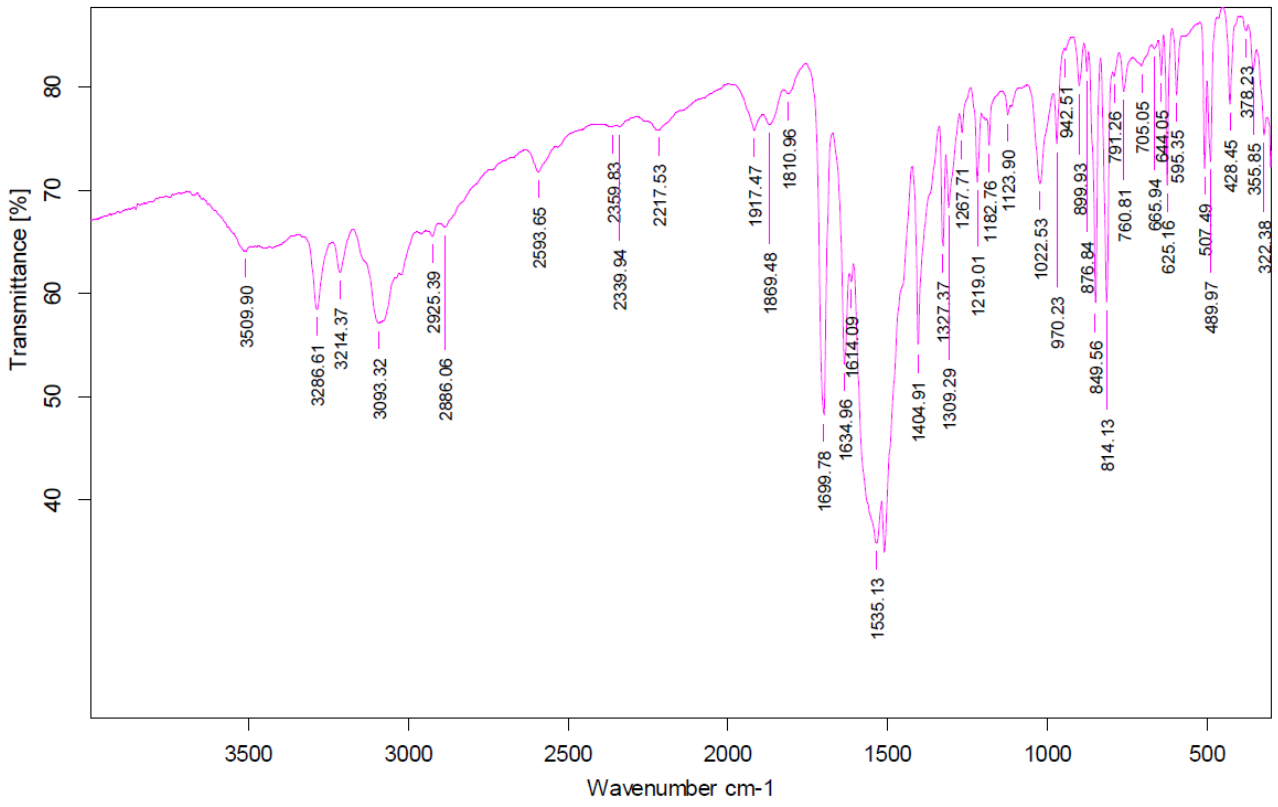


Figure SM 3.1.9.9 – IR spectrum of *N*-(4-methylphenyl)maleamic acid (KBr pellet).

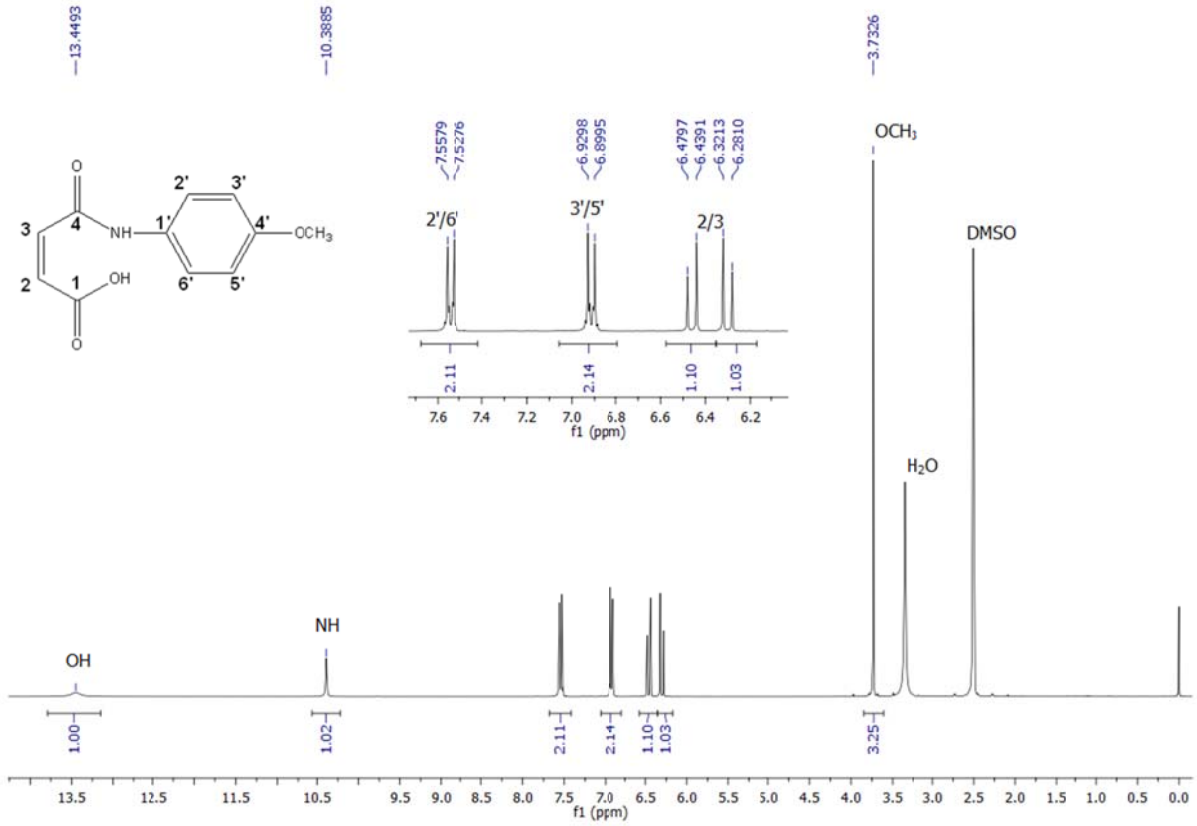


Figure SM 3.1.9.10 – ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of *N*-(4-methoxyphenyl)maleamic acid

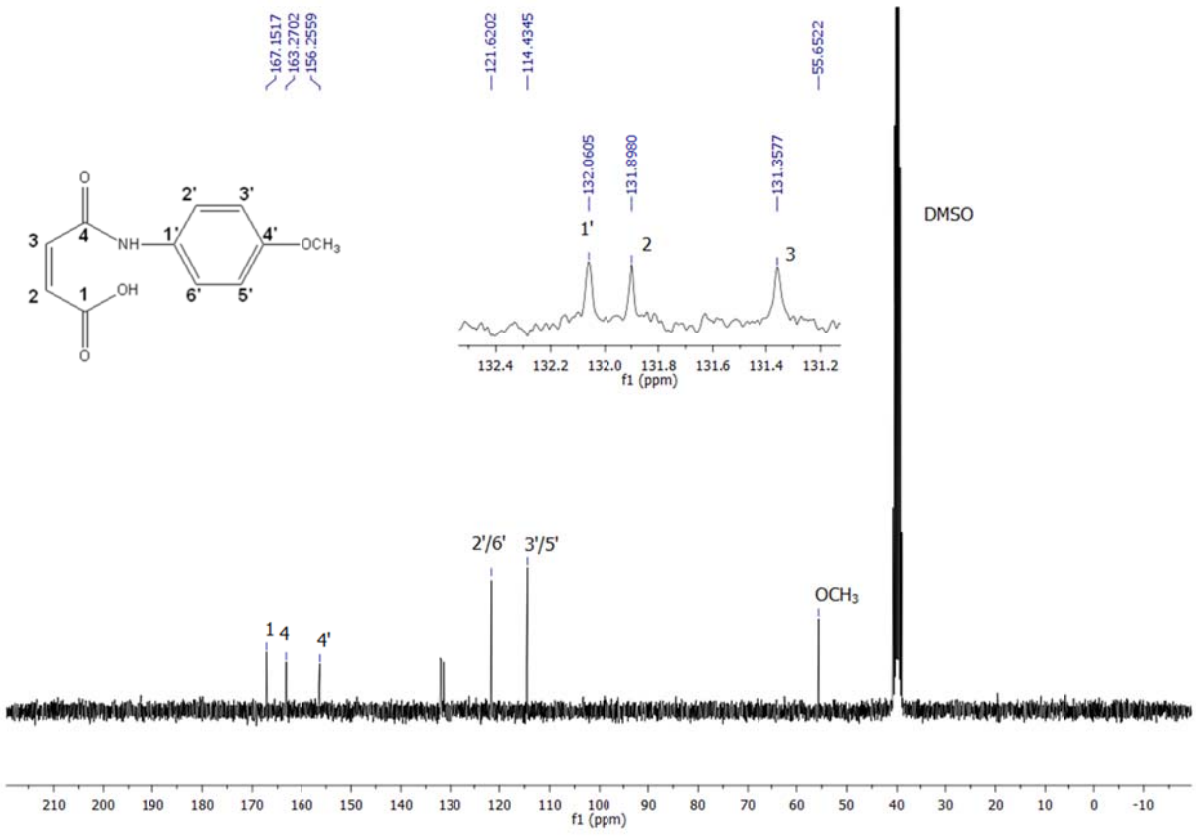


Figure SM 3.1.9.11 – ^{13}C NMR spectrum (75 MHz, DMSO-d_6) of *N*-(4-methoxyphenyl)maleamic acid.

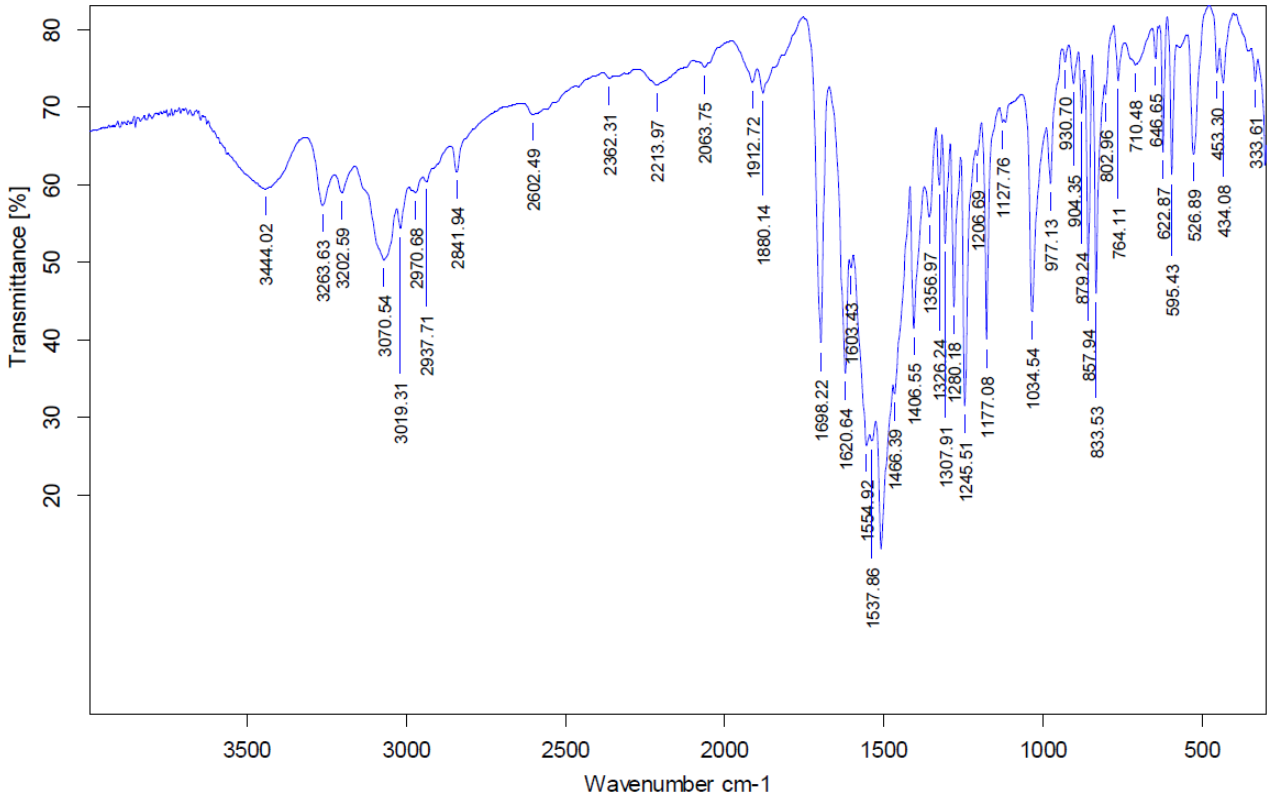


Figure SM 3.1.9.12 – IR spectrum of *N*-(4-methoxyphenyl)maleamic acid (KBr pellet).

Synthesis of *N*-arylmaleimides

Supplementary Material

Experimental notes

Figures

Notes:

This work is planned for a 4 hours session.

It has been offered as a laboratory classroom work, for more than five years, to second year undergraduate chemistry students (about 60 students a year, in classes of 16, working in groups of two). Yields obtained by the students for the different maleimides are usually very high and melting points normally close to the values reported in the literature.

N-arylmaleimides are synthesized from *N*-arylmaleamic acids, usually prepared in a previous laboratory session (*see Experiment 3.1.9 - Anhydride aminolysis: synthesis of N-arylmaleamic acids*), through an intramolecular reaction.

The reaction should be performed in an oil bath, and not in a water bath, in order to keep a dry environment. A drying tube filled with dried silica gel should be fitted to the top of the condenser. (Figure SM 3.1.10.1).

After the addition of the reaction mixture to an ice/water mixture a solid is formed and the crude product can be isolated by filtration. However, if a large excess of acetic anhydride is present, an oil is formed instead. In this case, carefully decant the water and repeatedly wash the oil with cold water until a solid is obtained.

After filtration in the Buchner funnel, the solid should be washed several times with cold water and then with a small amount of cold ethanol to facilitate the drying process.

The *N*-arylmaleimides thus obtained are yellowish (Figure SM 3.1.10.2) and all yield calculations are based on the crude products.

Small portions of the crude *N*-arylmaleimides can be further purified by flash column chromatography for NMR and melting point determination. The *N*-arylmaleimides elute first as yellowish fractions.

A 20 mm diameter flash chromatography column, about 30 cm long, should be used.

Pack the column with flash silica (0.035-0.070 mm) by the slurry method [silica is mixed with the eluent in a beaker and this slurry is transferred to the column] up to a height close to 15 cm. During the packing process the silica should not dry. Carefully add 1 cm of sand to the top of the of silica gel column.

Securely clamp the column adapter to the column and connect it to an airline. Apply a little pressure until the solvent is just above the sand layer.

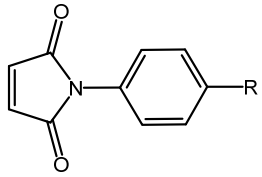
Remove the column adapter and, with a Pasteur pipet, add the crude product, dissolved in 2-3 mL of the eluent, to the top of the flash column. Then briefly apply some pressure to the column and push

this mixture onto the top of the silica gel, taking care not to let the eluent level dip below the level of the silica gel. Rinse the container that contained the crude product with another 2-3 mL of eluent and load this onto the column as you did above. Repeat this procedure several times until there is little or no colour left in the solution. Then, carefully fill the column with eluent and use the air to force the eluent through the column at a slow rate. When the eluent level is 1 cm from the top of the sand, remove the air and add more eluent. Continue this process until about 200 mL of eluent has been collected.

At no time should the eluent level be allowed to drop below the level of the silica gel. Do not discard the column until confirmation that the product has been isolated.

If 0.06-0.20 mm silica is used, it may be necessary to adjust the polarity of the eluent (typically a 1:1 mixture of dichloromethane/hexane is adequate).

Table of results: Average yields and melting points of the *N*-arylmaleimides synthesized by the students.



Entry	R	Average yield (%)	Melting point (ºC)
1	H	90	89-90
2	CH_3	92	150-151
3	OCH_3	92	149-150

Figure SM 3.1.10.1 - Reaction setup apparatus.

Figure SM 3.1.10.2 - Aspect of the dried products without purification. Left: *N*-phenylmaleimide; middle: *N*-(4-methylphenyl)maleimide; right: *N*-(4-methoxyphenyl)maleimide.

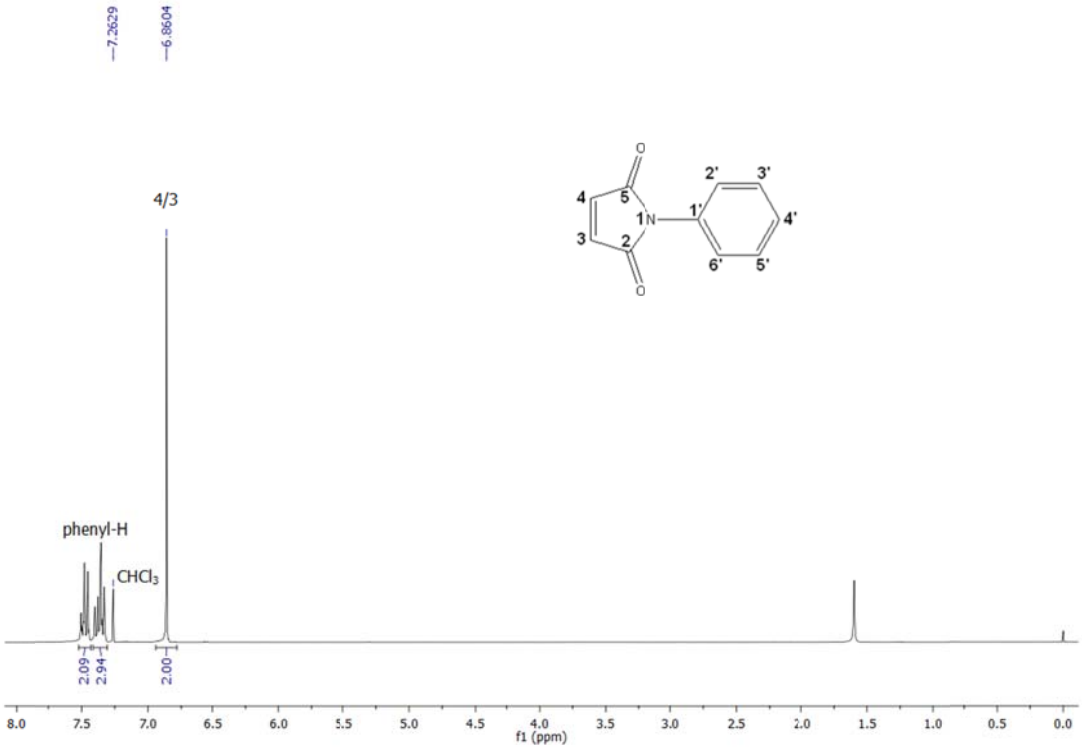


Figure SM 3.1.10.3 - ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of *N*-phenylmaleimide.

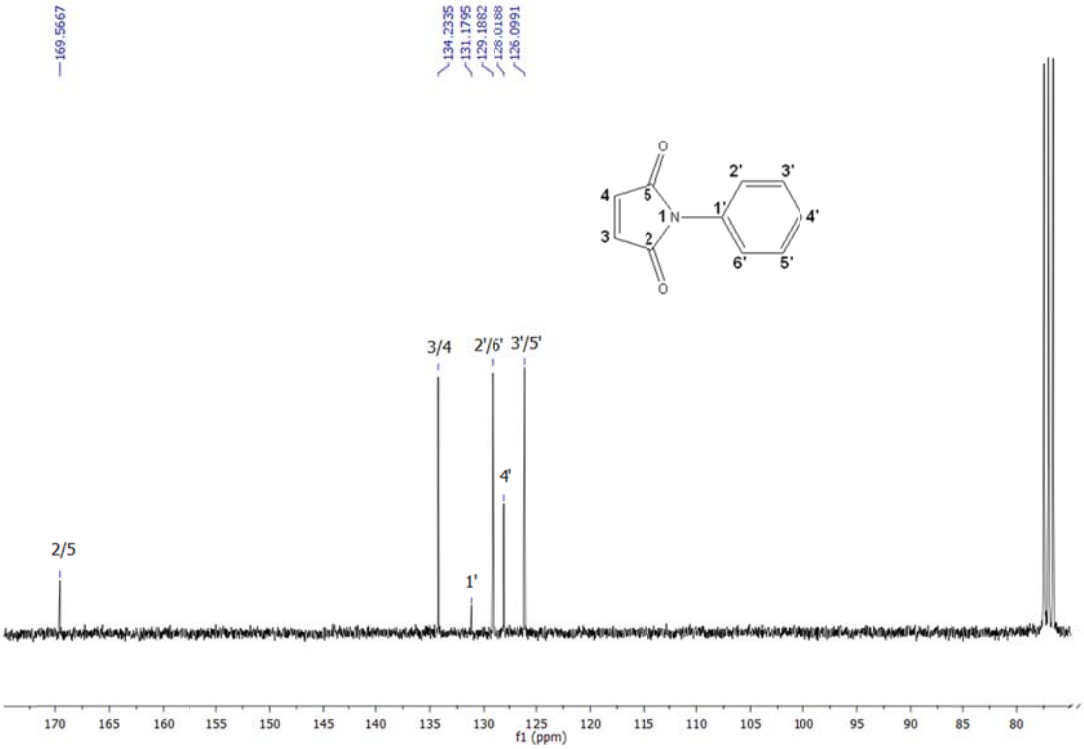


Figure SM 3.1.10.4 - ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of *N*-phenylmaleimide.

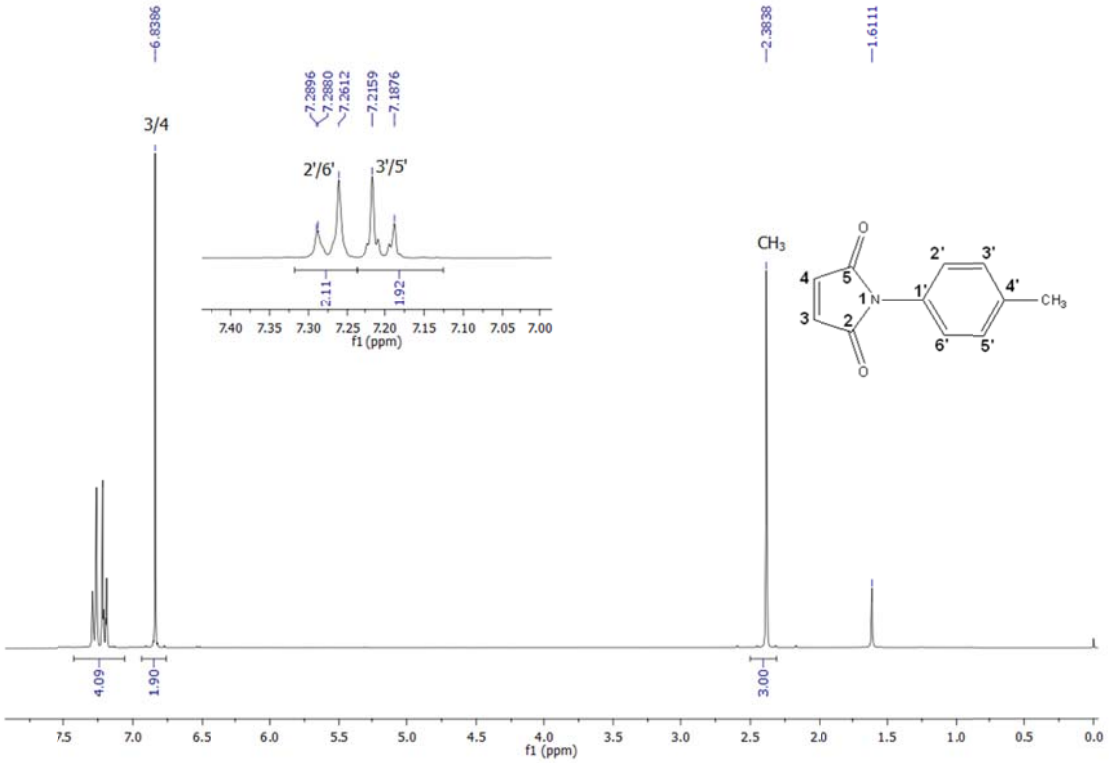


Figure SM 3.1.10.5 - ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of *N*-(4-methylphenyl)maleimide.

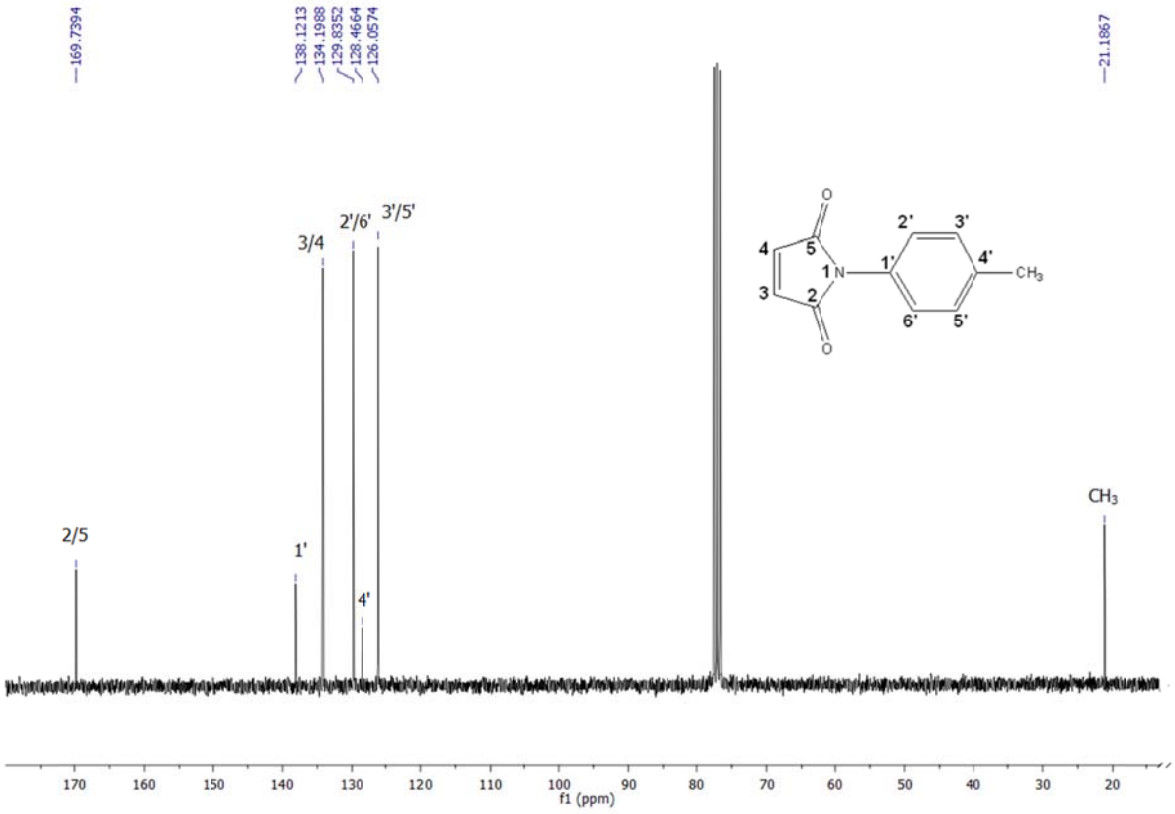


Figure SM 3.1.10.6 - ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of *N*-(4-methylphenyl)maleimide.

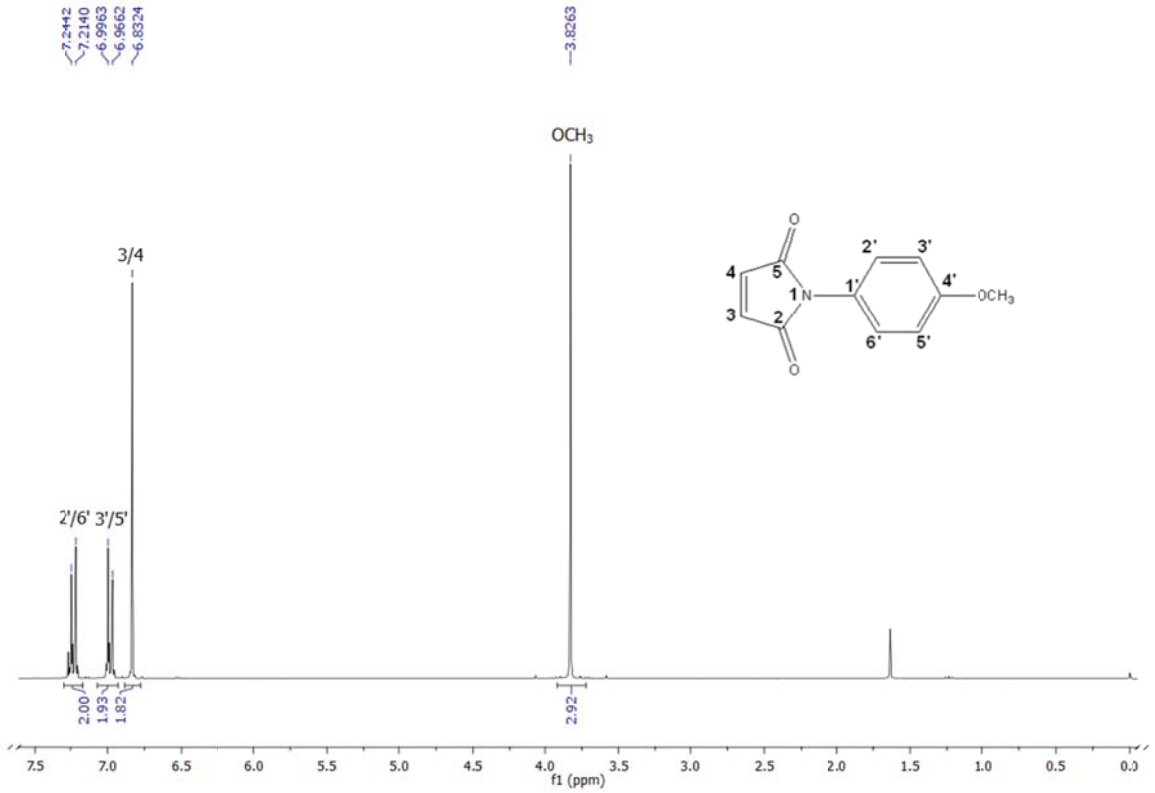


Figure SM 3.1.10.7 - ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of *N*-(4-methoxyphenyl)maleimide.

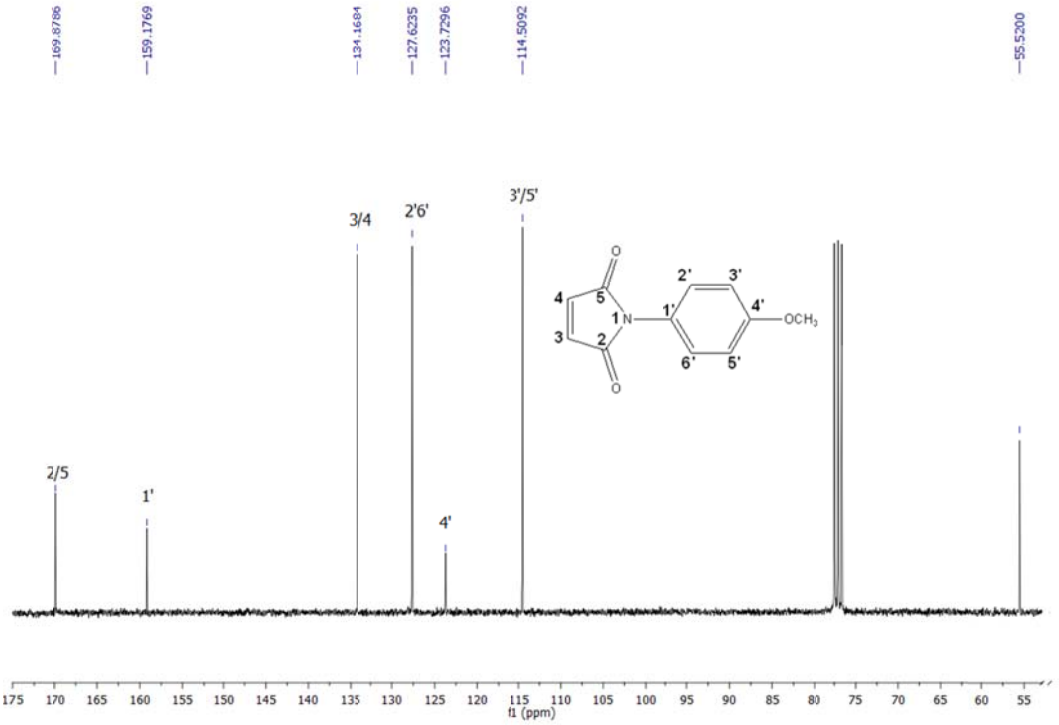


Figure SM 3.1.10.8 - ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of *N*-(4-methoxyphenyl)maleimide.

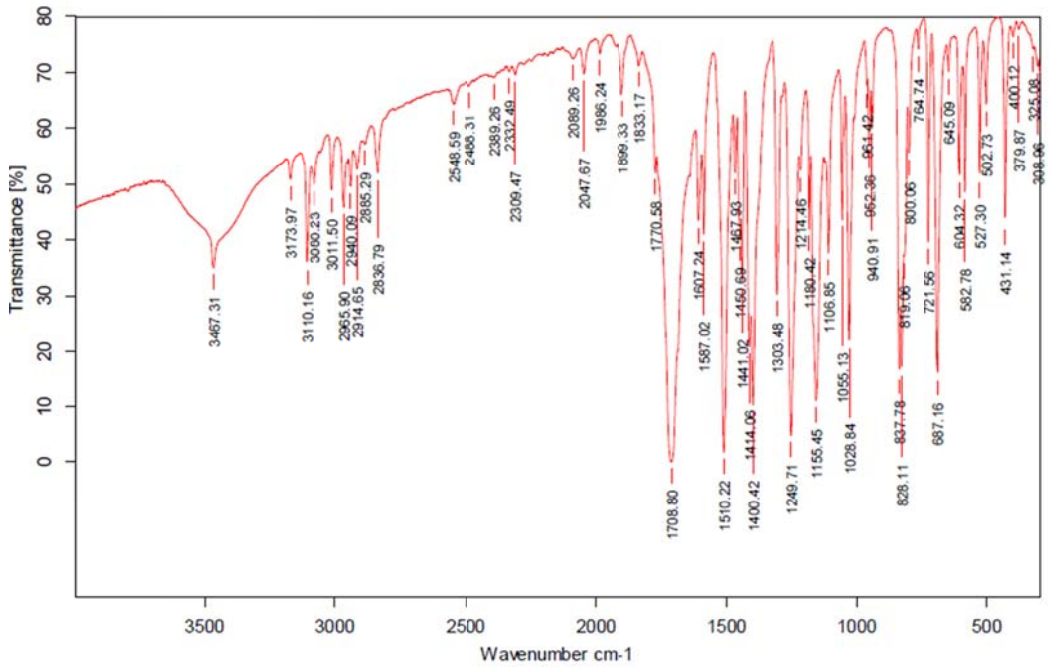


Figure SM 3.1.10.9 - IR spectrum of *N*-phenylmaleimide

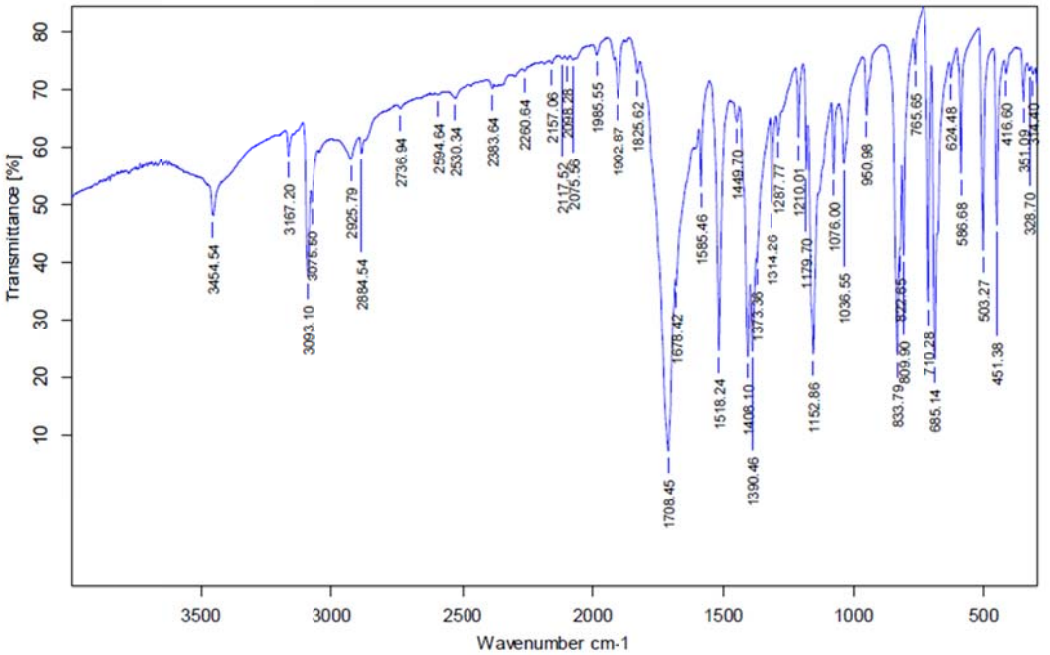


Figure SM 3.1.10.10 - IR spectrum of *N*-(4-methylphenyl)maleimide

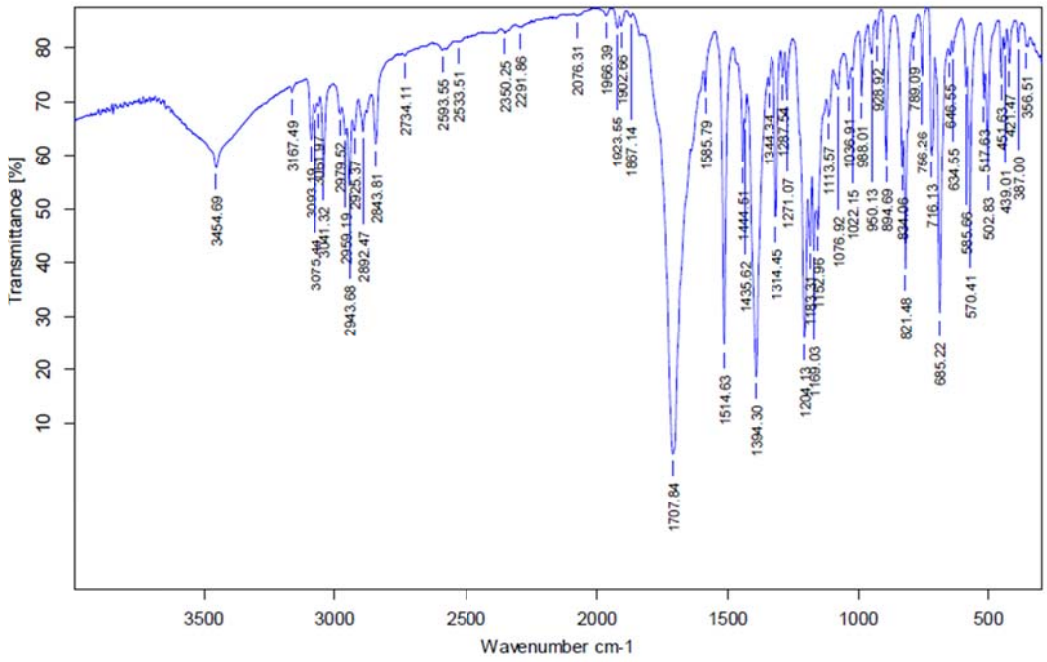


Figure SM 3.1.10.11 - IR spectrum of *N*-(4-methoxyphenyl)maleimide

Synthesis and characterization of *N*-cyclohexyl-*N*-methyloctanamide

Supplementary Material

The synthesis of *N*-cyclohexyl-*N*-methyloctanamide, based on the conversion of octanoic acid into octanoyl chloride and subsequent reaction of the latter with *N*-cyclohexylmethylamine, consists on a series of addition-elimination reactions. The transformation of a carboxylic acid into an amide, *via* acyl chloride, can be considered a classic reaction in synthetic organic chemistry; the involved experimental procedures are accessible, but chemicals such as thionyl chloride, and octanoyl chloride, require that students need to be warned that their handling should be carried out with additional care (*e.g.*, these reagents should not be removed from the fume hood at any time, at least without a proper stopper). Apart to the utilization of the rotary evaporator, all the remaining procedures are rather suitable for students basically acquainted with the principal techniques performed in any organic chemistry lab.

Additional Notes on the Preparation of *N*-Cyclohexyl-*N*-Methyloctanamide

The 2-neck round-bottomed flask for the reflux, if not available in the undergraduate laboratory, can be replaced by a round-bottomed flask with only one entry, providing that a thermostatic heating block to accurately maintain the temperature at 76ºC be used instead of the oil bath. Accordingly, the addition of thionyl chloride can therefore be made through the top of the condenser.

In the first 15-30 minutes of the reflux, the release of persistent and abundant white fuming at the top of the condenser (thionyl chloride, hydrochloric acid and sulphur dioxide) is normal. The inclusion of the rotary evaporator in the fume hood is strongly advised, and may be ensured prior to the experiment.

The cease of the experiment after the production of octanoyl chloride is not convenient, since the compound is reactive and may decompose until the next session. The reaction of octanoyl chloride with the mixture of amines is quite exothermic; therefore, a convenient cooling of the amines solution, prior to the careful and slow addition of the octanoyl chloride, is advisable. Due to the abundant formation of solid ammonium salts, this latter reaction is better accomplished in a beaker, since major losses of the solid and viscous products meanwhile formed are better prevented. However, if preferred, the reaction can also be carried out in a proper round-bottomed flask. Concerning the period of time for stirring, reference 4 of the manuscript suggests 3 hours, based on the monitoring of the amide formation by FTIR. Relying on our experience, we found 2 hours enough. However, 1 hour agitation may also be adopted, as the only probable consequence should be the attainment of lower yields. The placement of the two laboratory sessions in the same or in consecutive days is also recommended.

This experiment has been carried out several times in the authors' research lab, but typically involving higher quantities than the ones proposed herein. Students should be alerted that if they allow the reflux temperature go above 80ºC, they are likely to achieve lower yields. The determination of the mass of crude octanoyl chloride allows them to calculate the yield of *N*-cyclohexyl-*N*-

methyloctanamide taking this data into account. Regarding the liquid-liquid extractions, the first washing with water removes the major part of the abundant and viscous solids, the 6 M HCl solution eliminates the amine traces, water is added in sequence to reduce the acid amount in the organic phase prior to its washing with the sodium bicarbonate solution, and finally sodium chloride solution is used, to sequester as much water as possible out of the organic layer.

The yields obtained for *N*-cyclohexyl-*N*-methyloctanamide are usually in the range 80-88%. If the *N*-cyclohexylmethylamine flask has been opened for some time, revealing a darker and brownish appearance, the final yields of the amide product show tendency to decrease (75-77%).

If the instructor thinks the application of NMR spectroscopy can be suppressed, the duration of the second session of this experiment may be shortened. Nevertheless, due to the caution required for the handling of the chemicals referred to previously, the adjustment of this experiment to beginner organic chemistry students may not be prudent.

Photos of the Experiment

Figure SM 3.1.11.1 – Reflux apparatus for octanoyl chloride production.

Figure SM 3.1.11.2 – Appearance of *N*-cyclohexyl-*N*-methyloctanamide just before the washings.

Figure SM 3.1.11.3 – Appearance of *N*-cyclohexyl-*N*-methyloctanamide at the end of the experiment.

FTIR and ^{1}H NMR Spectroscopic Data

The C=O stretching vibration on the FTIR spectrum of *N*-cyclohexyl-*N*-methyloctanamide appears in the range 1644-1650 cm^{-1}. The complete FTIR spectrum can be observed in Figure SM 3.1.11.4.

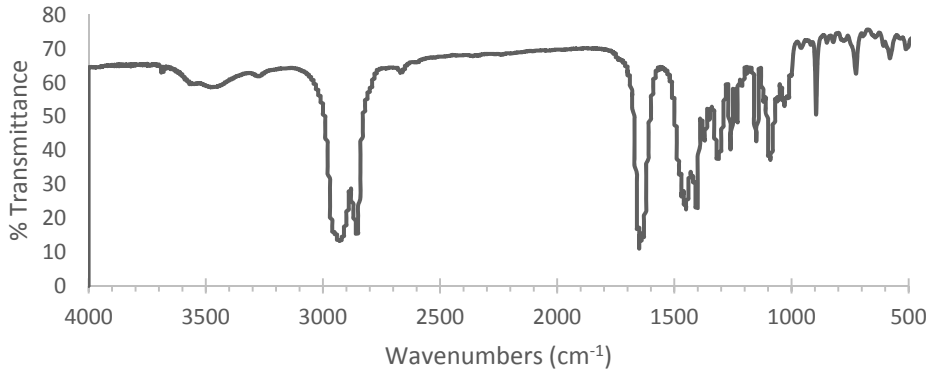
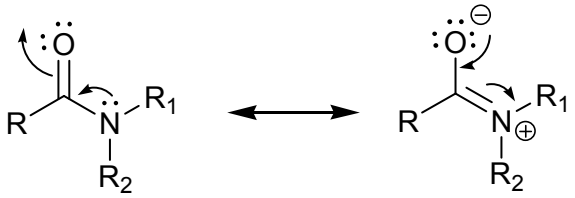


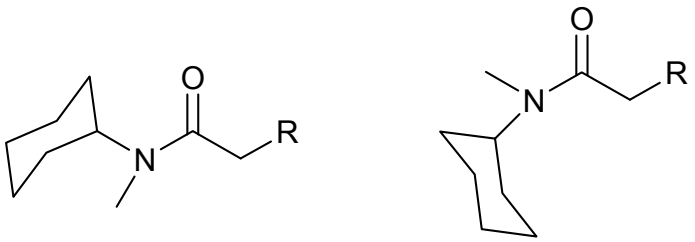
Figure SM 3.1.11.4 – FTIR spectrum of *N*-cyclohexyl-*N*-methyloctanamide (in NaCl cells).

The correspondent ^{1}H NMR spectrum of this tertiary amide evidences a phenomenon, representative of this type of compound, associated with the "partial" double character of the C-N bond. The two most important resonance forms for tertiary amides are displayed in Scheme SM 3.1.11.1.



Scheme SM 3.1.11.1 – Resonance forms of tertiary amides (R, R_1 and R_2: alkyl or aryl groups).

The contribution of the second resonance structure produces a stronger C-N bond, more rigid, hence with less conformational flexibility. Depending on the nature of the groups R_1 and R_2, the rotation can be more or less rapid; if fast, the peaks assigned to the protons of those substituents only enlarge, but if the rotation along the C-N axis is relatively slow for the NMR time scale, the proton signals duplicate, revealing the two conformations the molecule can exhibit. The usual classification given to these conformers is *syn* (when the more complex substituent is on the same side of the oxygen atom) and *anti* (when the more complex substituent and the oxygen atom are at opposite sides) – see Scheme SM 3.1.11.2.



Scheme SM 3.1.11.2 – Examples of *syn* and *anti* conformations, respectively.

The ^{1}H NMR spectrum of *N*-cyclohexyl-*N*-methyloctanamide is depicted in Figure SM 3.1.11.5.

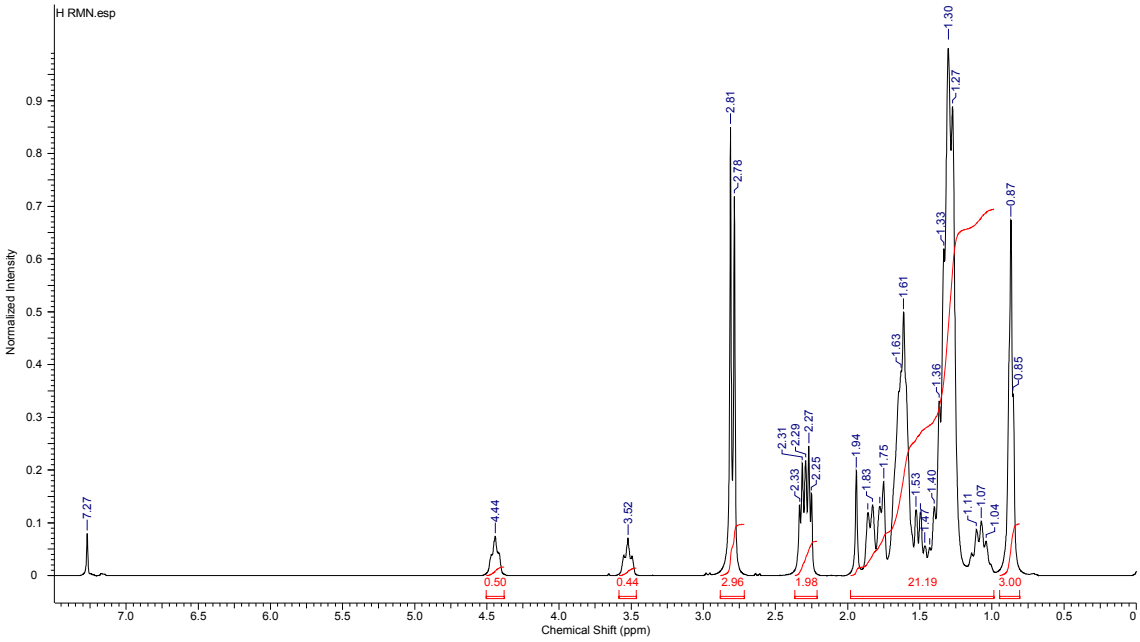


Figure SM 3.1.11.5 – ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of *N*-cyclohexyl-*N*-methyloctanamide.

Figures SM 3.1.11.6 and SM 3.1.11.7 show amplifications of some specific parts of the ^{1}H NMR spectrum of *N*-cyclohexyl-*N*-methyloctanamide, for a clearer visualization of the duplication of signals.

Taking into account the relative proportion of the CH proton of the cyclohexyl group, at δ = 3.52 and 4.44 ppm, and the ratio of the three protons corresponding to the N-CH_3 substituent (at δ = 2.78 and 2.81), the ratio of the *syn-anti* conformers for this compound is of about 55%-45%, respectively. The instructor may encourage students to use molecular models to better understand the detection of the two conformers by NMR, if found appropriate.

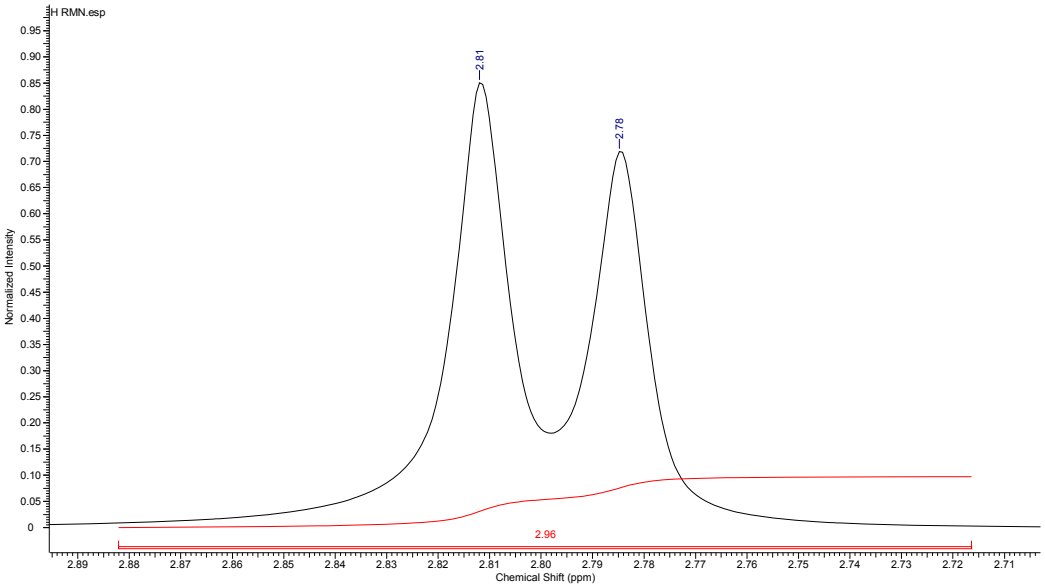


Figure SM 3.1.11.6 – ^{1}H NMR signals of the methyl group attached to the nitrogen atom (400 MHz, $CDCl_3$).

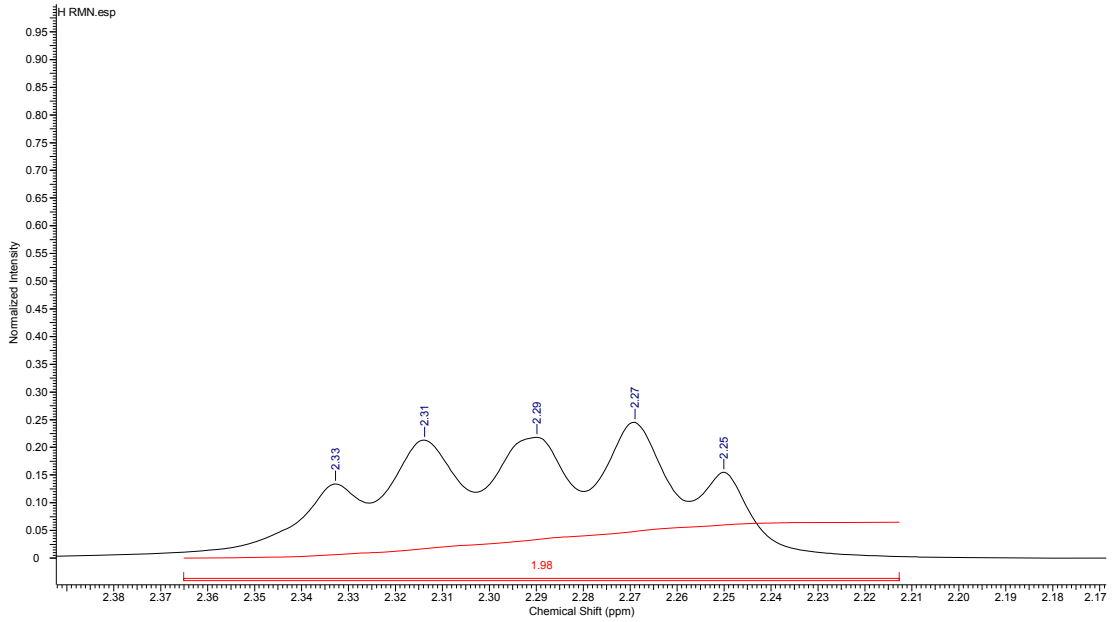


Figure SM 3.1.11.7 – ^{1}H NMR signals of the methylene substituent adjacent to the carbonyl group (400 MHz, $CDCl_3$).

The same phenomenon (duplication of signals) can obviously be observed in the correspondent ^{13}C NMR spectrum as well. As an example, an amplification of the region of the carbonyl group is depicted in Figure SM 3.1.11.8.

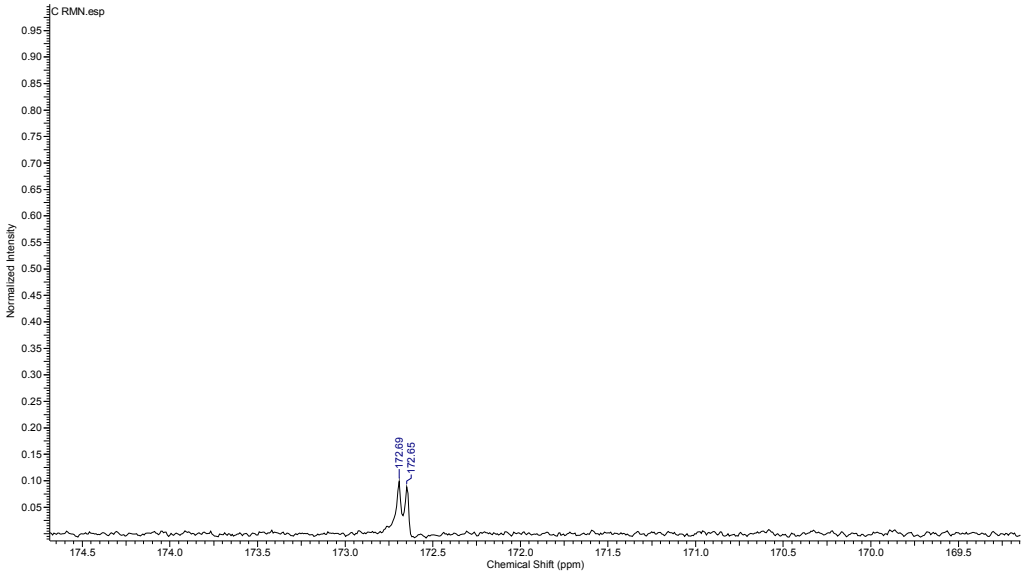
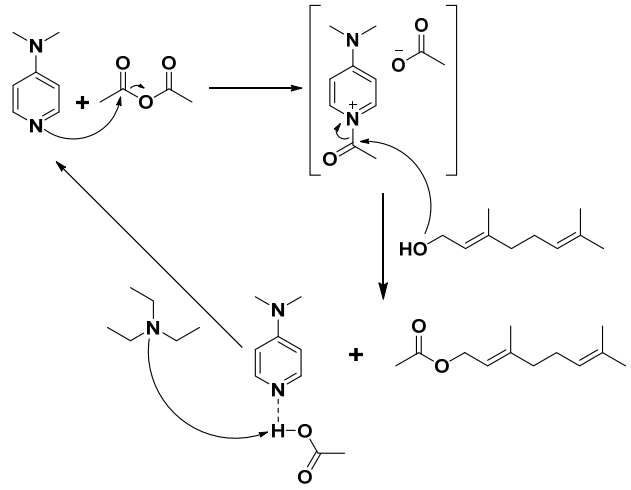


Figure SM 3.1.11.8 – ^{13}C NMR signals corresponding to the carbonyl group (100 MHz, $CDCl_3$).

Effect of a catalyst in the acylation of alcohols with acetic anhydride: Manipulation of a natural aroma

Supplementary Material

This experiment was developed in order to demonstrate to students the effect of a catalyst, by simple TLC observation (demonstration of the potentialities of this technique). It is shown that in the absence of the catalyst DMAP the reaction does not occur or is too slow, however in its presence the reaction is almost immediate (Figure SM 3.1.12.1). The reaction mechanism first goes through a pre-equilibrium between DMAP and acetic anhydride, forming the acetylpyridinium cation and the acetate anion. Then the alcohol attacks the acetyl group of the acetylpyridinium cation, in the rate-determining step, to form the ester product and the protonated DMAP. In the last step, triethylamine deprotonates the DMAP, regenerating the catalyst (Scheme SM 3.1.12.1).



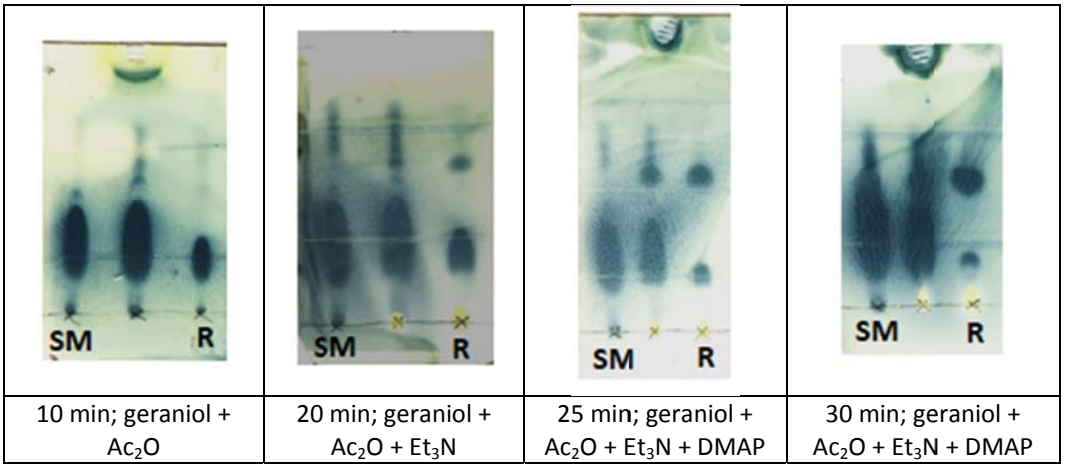
Scheme SM 3.1.12.1 – Mechanism of the acetylation of geraniol, catalyzed by DMAP.

Initially, this procedure was performed at room temperature, however under those conditions it was possible to observe the acylation product formation without the catalyst, which made the effect from the addition of the catalyst less noticeable. On the other hand, performing the reaction at low temperature, just using ice or preferentially ice-cooking salt (which shows the students an easy way to obtain a temperature slightly lower than 0 ºC) it is possible to clearly see the effect of the catalyst.

After demonstration of the catalytic effect, the reaction mixture was allowed to warm up to room temperature to reduce the reaction time. Although it is more appropriate to carry out the reaction under anhydrous conditions it was found that the experiment can be performed using commercial dichloromethane and triethylamine. Students obtained yields in the range of 70 to 90%. The results allow the instructors to stimulate a discussion about the reaction mechanism involved, effect of the catalyst and the reactivity of several carboxylic acid derivatives (*eg.* ketenes, acyl halides, carboxylic acids, esters and amides). In Figures SM 3.1.12.4 to SM 3.1.12.7 are depicted the spectral data of the starting material as well as the final product.

Students also tested other amines as potential catalysts, such as dimethylaniline and pyridine, by the same procedure but using dimethylaniline (0.2 mL, 0.2 equiv.) or pyridine (0.1 mL, 0.2 equiv.) instead of using DMAP. However it was observed their lower efficiency by TLC (Figure SM 3.1.12.2 and SM 3.1.12.3). Students performed this study during the same class where different groups tested different catalysts. Using dimethylamine as base, this can also act as nucleophile and react with acetic anhydride forming a stable product, *N,N*-diethylacetamide. However, even if the trietilamine acts as a nucleophile and react with the anhydride, the product formed is unstable and may undergo attack by another nucleophile.

In another procedure, other group tested a competitive reaction between acetic anhydride and benzoic anhydride (1:1) in order to evaluate the reactivity between these two anhydrides. In Figure SM 3.1.12.8 is depicted the resulting ^{1}H NMR spectrum where is showed exclusively the acetylation product, demonstrating the low reactivity of benzoic anhydride when compared to acetic anhydride.



10 min; geraniol + Ac_2O	20 min; geraniol + Ac_2O + Et_3N	25 min; geraniol + Ac_2O + Et_3N + DMAP	30 min; geraniol + Ac_2O + Et_3N + DMAP

Figure SM 3.1.12.1 – Example of TLCs (eluent: dichloromethane; detection with a 10% ethanolic solution of phosphomolybdic acid) performed over time, by the students (t = 0 min is the addition of acetic anhydride; application of the geraniol starting material (SM) on the left; application of the reaction mixture (R) on the right; and in the middle the co-application of the starting material and reaction mixture). Reaction performed at T < 0 ºC (ice-cooking salt bath).

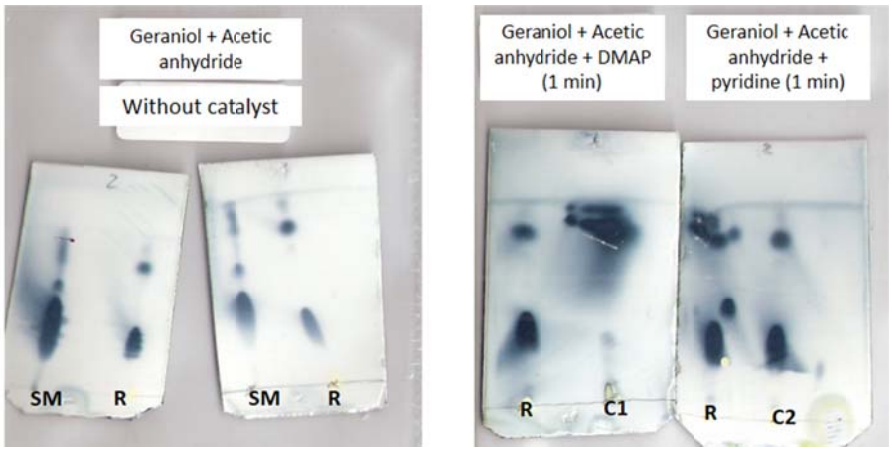


Figure SM 3.1.12.2 – TLCs obtained by the groups that studied the catalytic efficiency of pyridine over DMAP (SM – geraniol starting material, R – reaction of the acetic anhydride with geraniol without catalyst, C1 – reaction catalyzed by DMAP after 1 min, C2 – reaction catalyzed by pyridine after 1 min.

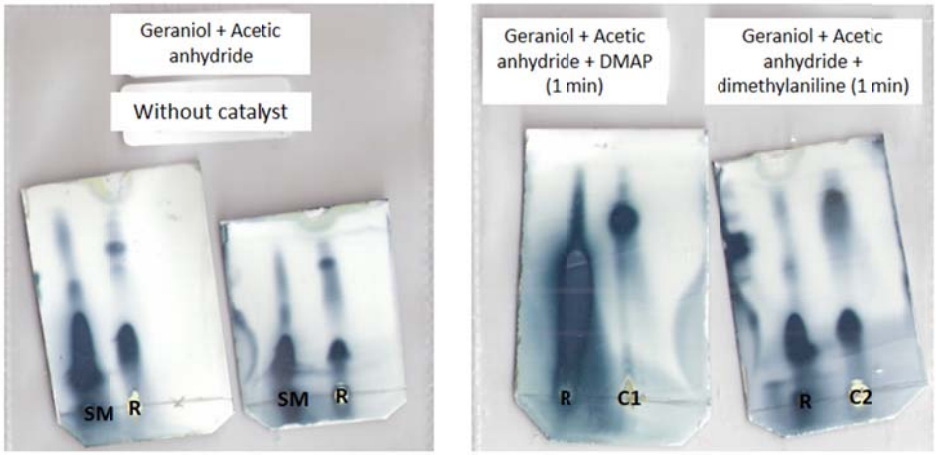


Figure SM 3.1.12.3 - TLCs obtained by the groups that studied the catalytic efficiency of dimethylaniline over DMAP (SM – geraniol starting material, R – reaction of the acetic anhydride with geraniol without catalyst, C1 – reaction catalyzed by DMAP after 1 min, C2 – reaction catalyzed by dimethylaniline after 1 min.

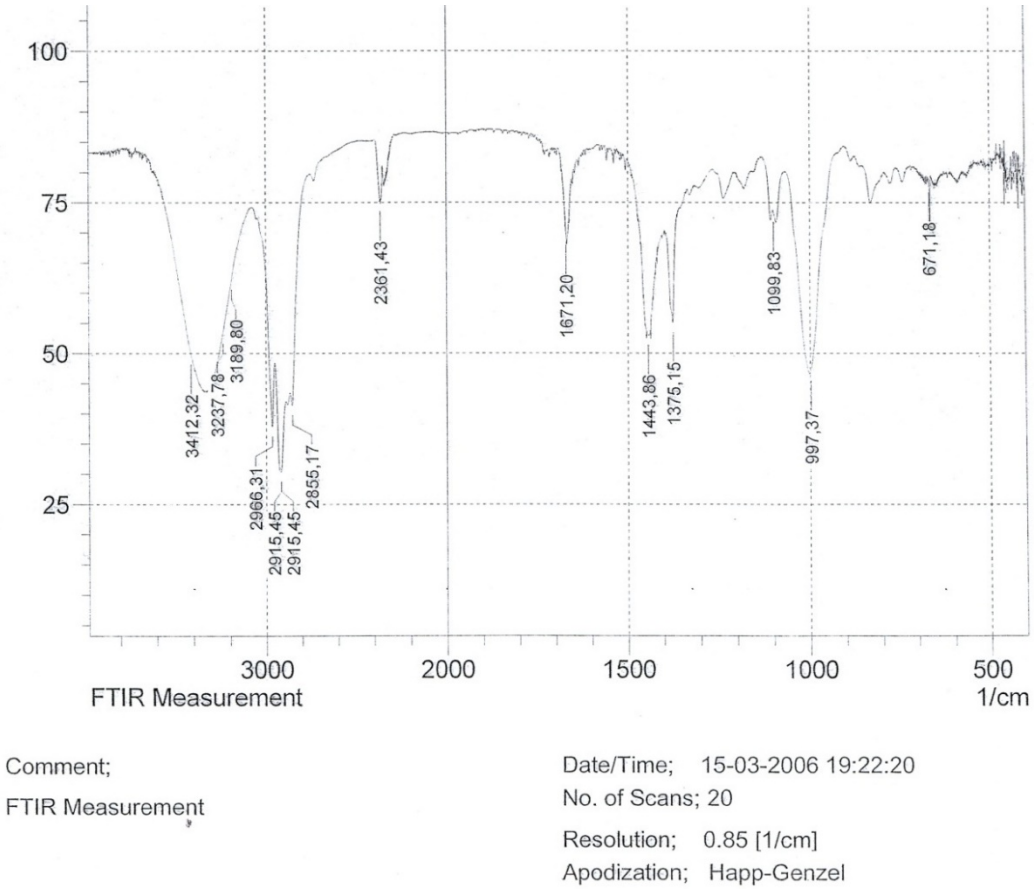


Figure SM 3.1.12.4 – Obtained IR (film) spectrum of starting material geraniol.

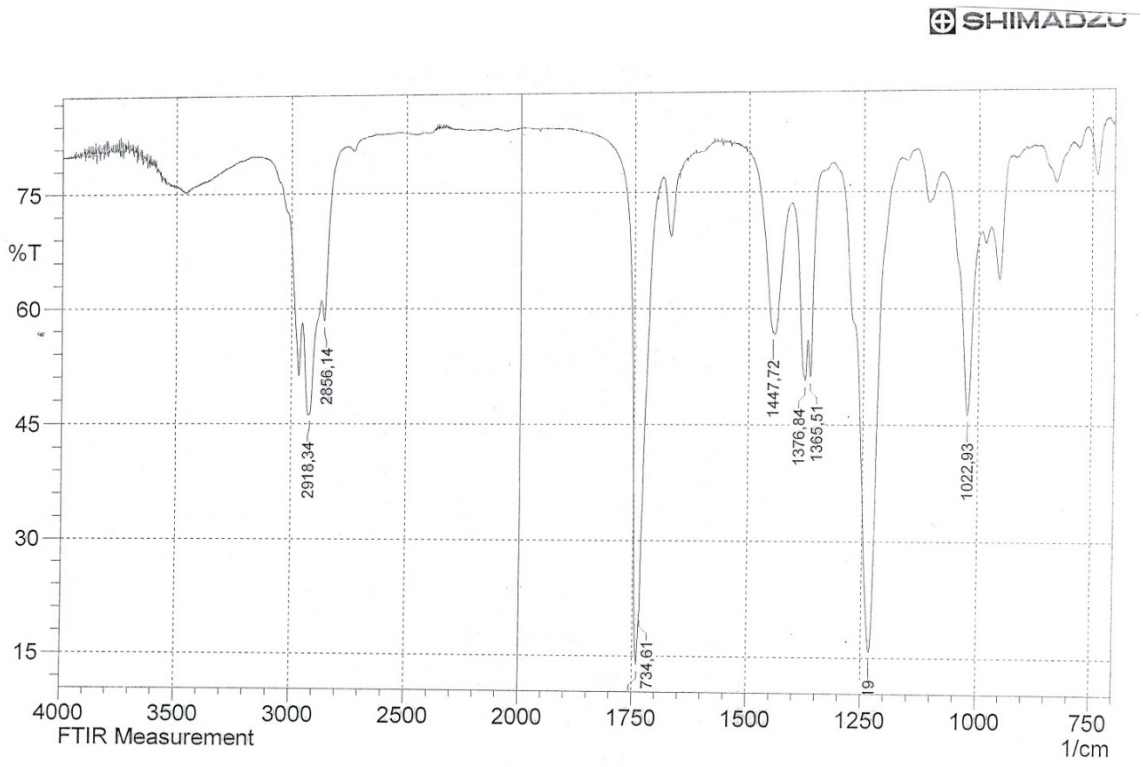


Figure SM 3.1.12.5 – Obtained IR (film) spectrum for the isolated product.

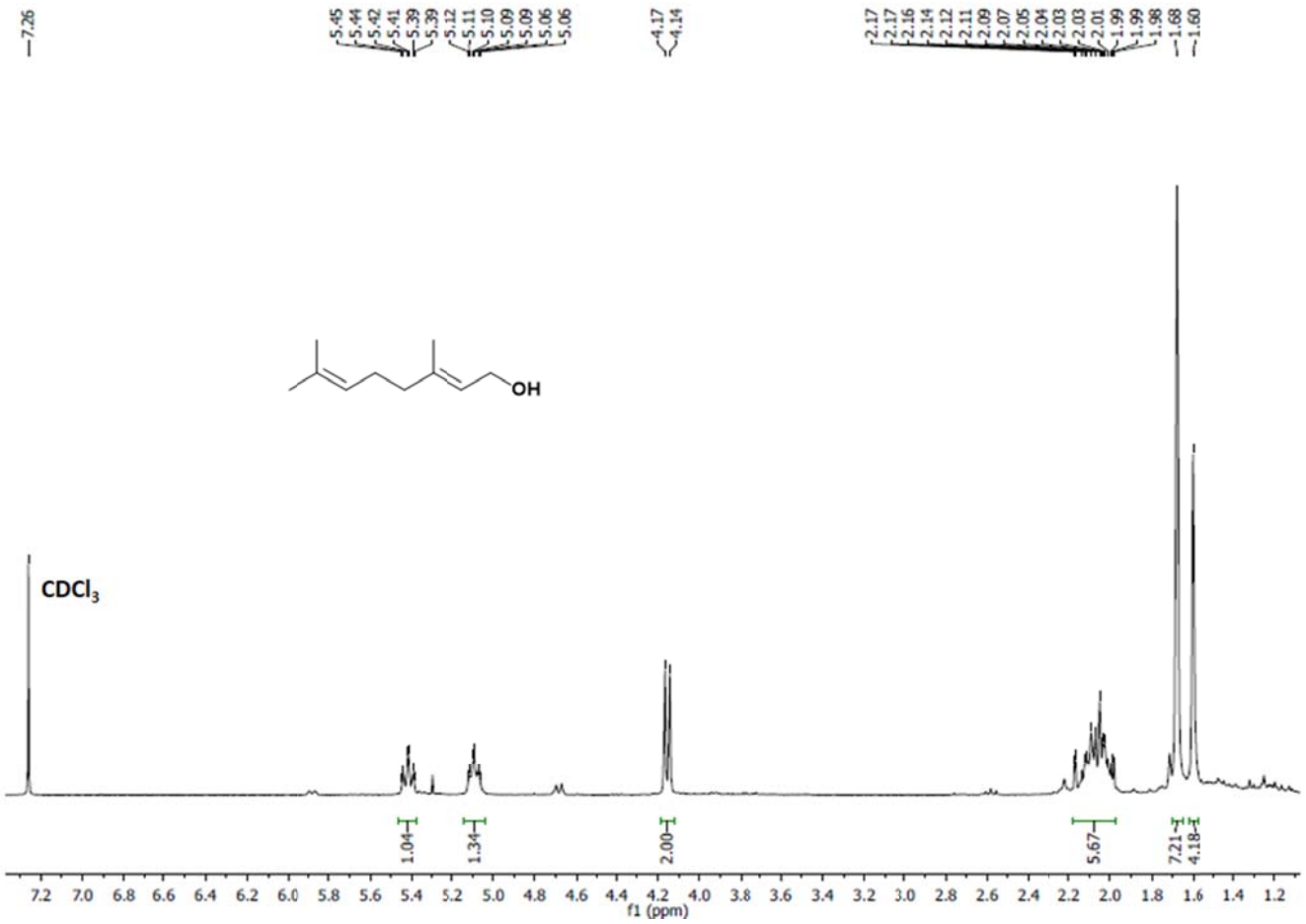


Figure SM 3.1.12.6 – ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of starting material geraniol.

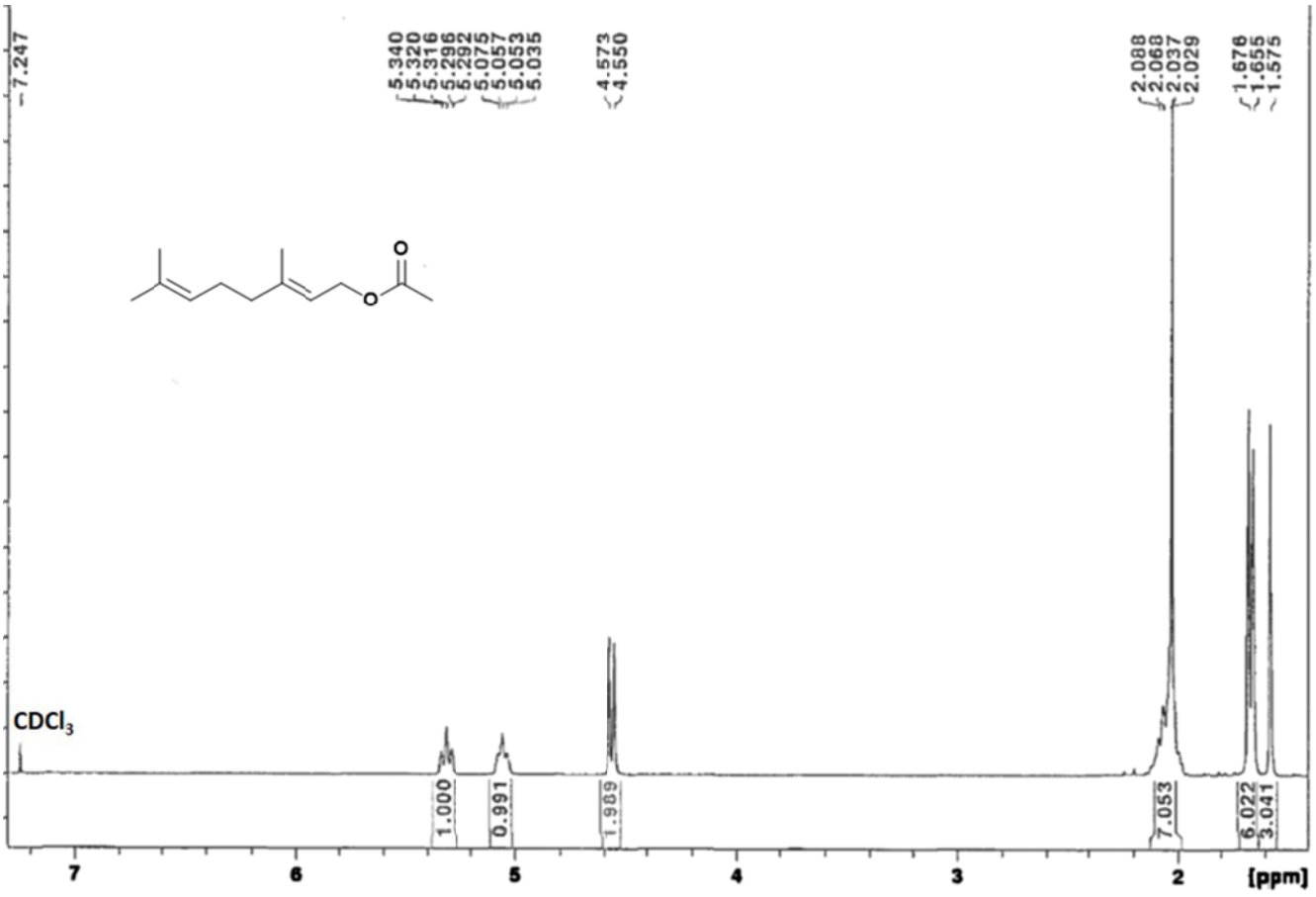


Figure SM 3.1.12.7 – ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of geraniol acetate.

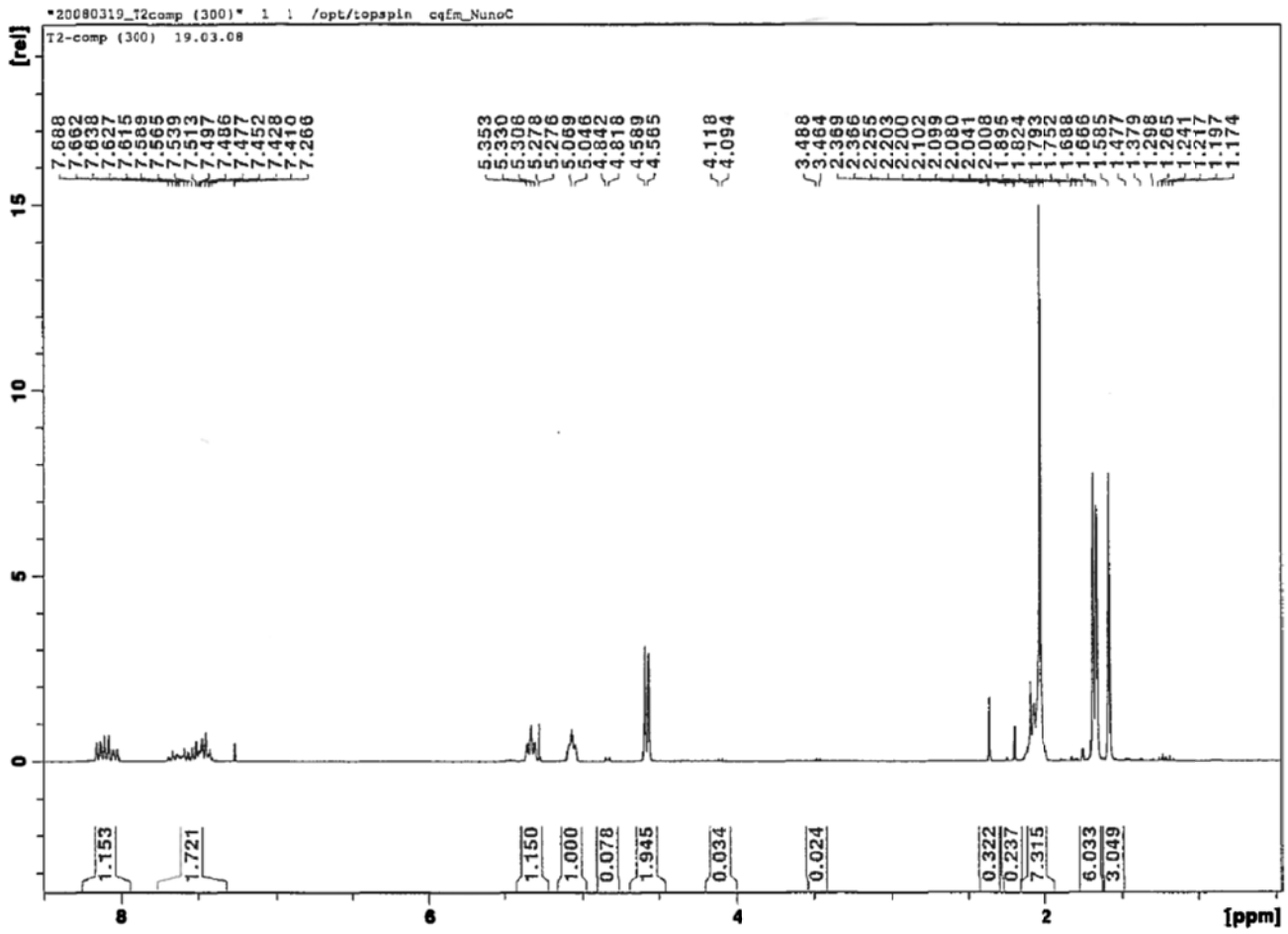


Figure SM 3.1.12.8 – Obtained ^{1}H NMR (400 MHz, $CDCl_3$) spectrum for the product of the competitive reaction between anhydrides.

Synthesis of 4,5-dichloro-1,2-dicyanobenzene

Supplementary Material

This experiment proposal was prepared by the principal author during her research, and used as precursor in the synthesis of aromatic dithiols (also included in this book) once it was not available on the market. The first synthetic way followed was described in the literature[1] and employed $(NH_4)_2CO_3$ with formation of imide and amide mixture however both products could be transformed in the diamide. In 1993 another work emerged with an improved procedure[2] and was adapted to classroom for 2nd year undergraduate students of intermediate organic chemistry as a short project involving bibliographic research and experimental work (three groups of two students)[3]. They could study several chemical transformations concerning carbonyl group, and they had the opportunity to learn how to work in anhydrous conditions and under inert atmosphere. This experiment can be made in only 3 steps, starting from the inexpensive 4,5-dichlorophthalic anhydride, in mild conditions and easy work-up. The main disadvantages are the long-time reactions for a 4-5h classroom and big stirring periods but without need of surveillance. According to schedules students they can follow the reactions. Vacuum filtrations may be left for the next session. The principal problem is avoiding contact with water in each step to prevent hydrolysis.

Additional notes on the preparation of 4,5-dichlorophthalic anhydride:

This product is easily obtained by precipitation without need of slow distillation of acetic acid formed during reaction as described in literature[3]. Yield 70-85%; mp 183-184ºC (187.9ºC[4]).

Additional notes on the preparation of 4,5-dichlorophthalimide:

4,5-dichlorophthalimide is obtained in 85-95% yield and mp 192-193ºC (193-195ºC[2]).

Additional notes on the preparation of 4,5-dichlorophthalamide:

4,5-dichlorophthalamide obtained in 60-70% yield and mp 243-245ºC (245-247ºC[2]).

Additional notes on the preparation of 4,5-dichloro-1,2-dicyanobenzene:

Thionyl chloride was distilled before use. N,N-dimethylformamide was dried over magnesium sulphate and distilled under reduced pressure. An ice/water bath is sufficient to cool the reaction until 0ºC. At the end of lab session, students may change the inert gas inlet by a balloon filled with argon or nitrogen. The reaction set apparatus can be seen in **Figure SM 3.1.13.1**. All reagents must be dry to prevent hydrolysis. Thionyl chloride reacts vigorously with water. Yield 40-50%; mp 181-182ºC (182-184ºC[2]). The overall yield is 40-45%.

Figure SM 3.1.13.1 – Reaction set apparatus for 4,5-dichloro-1,2-dicyanobenzene

IR spectra:

Students easily identify the strong absorption of C=O stretching bands in the intermediates **Figures SM 3.1.13.2 – SM 3.1.13.4**, N-H stretching bands in **Figures SM 3.1.13.3** and **SM 3.1.13.4** and the characteristic absorption band due to CN triple bond stretching at 2240 cm^{-1} in **SM 3.1.13.5**.

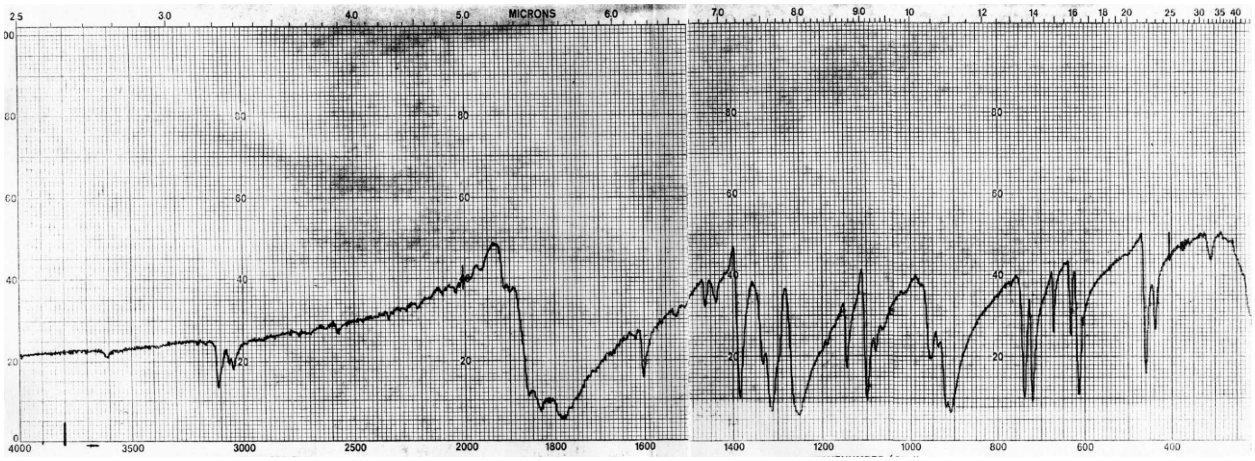


Figure SM 3.1.13.2 - IR (KBr) of 4,5-dichlorophthalic anhydride

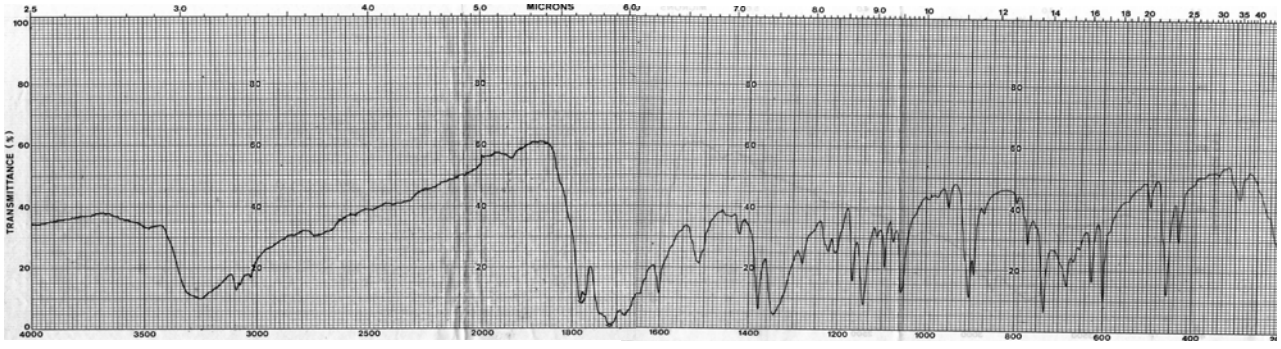


Figure SM 3.1.13.3 - IR- (KBr) of 4,5-dichlorophthalimide

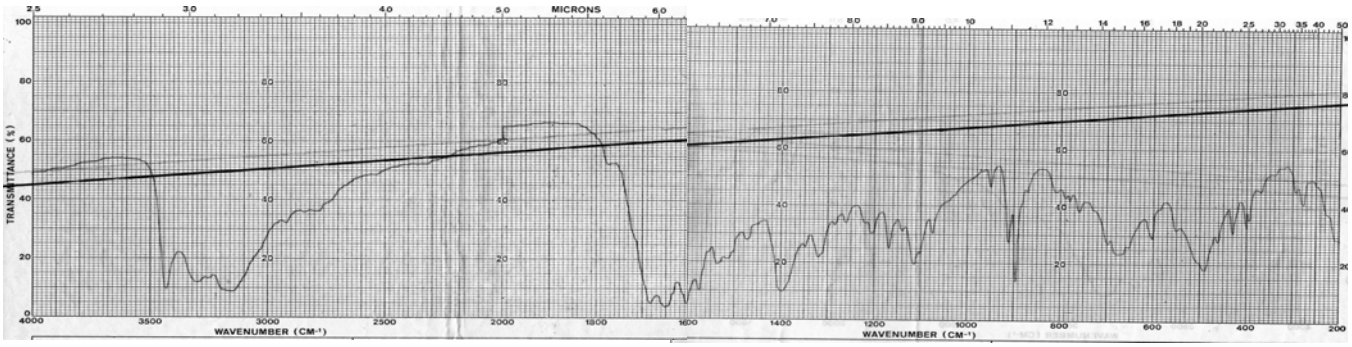


Figure SM 3.1.13.4 - IR (KBr) of 4,5-dichlorophthalamide

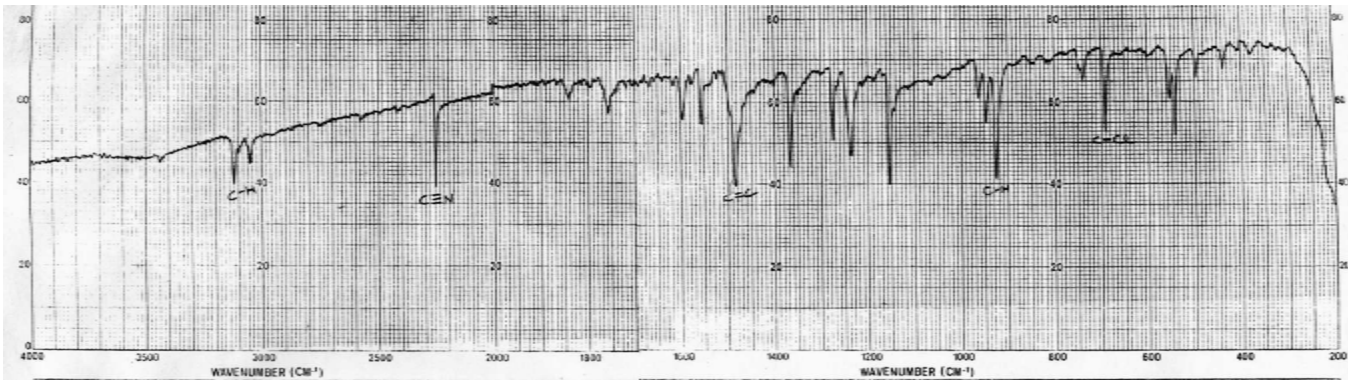


Figure SM 3.1.13.5 - IR (KBr) of 4,5-dichloro-1,2-dicyanobenzene

NMR spectra:

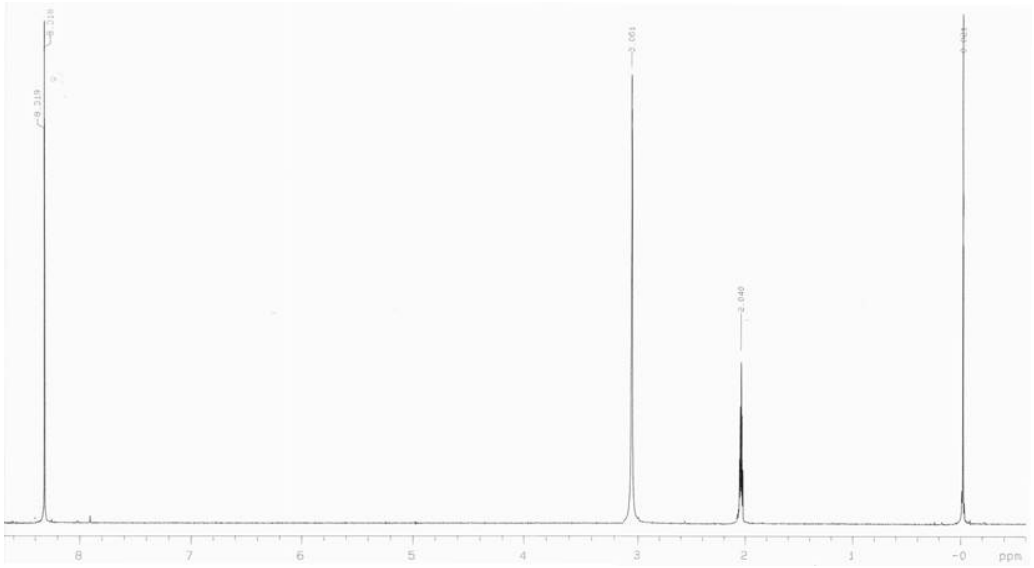


Figure SM 3.1.13.6 - ^{1}H RMN of 4,5-dichlorophthalic anhydride (300 MHz, DMSO-d_6)

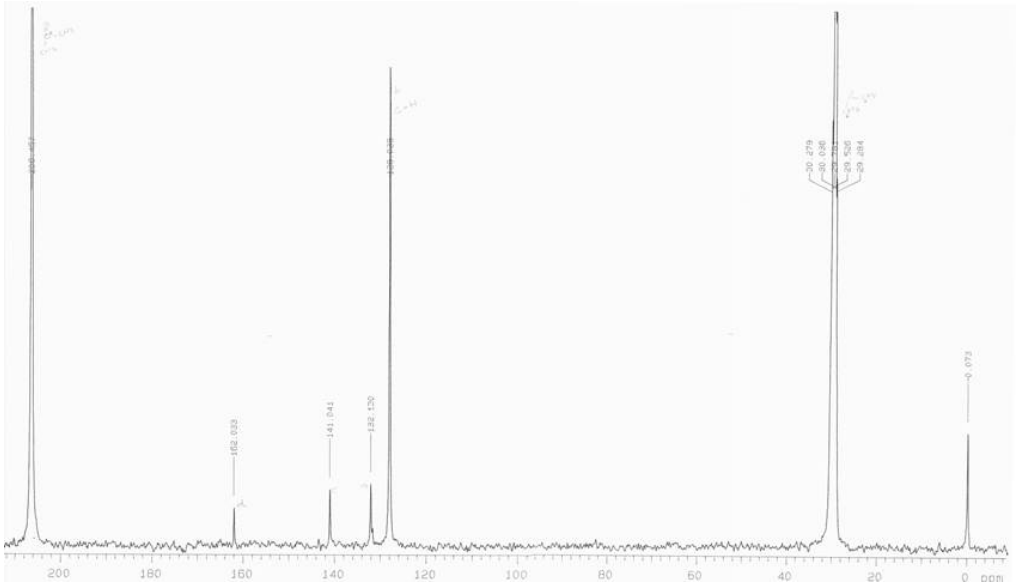


Figure SM 3.1.13.7 – ^{13}C RMN of 4,5-dichlorophthalic anhydride (300 MHz, DMSO-d_6)

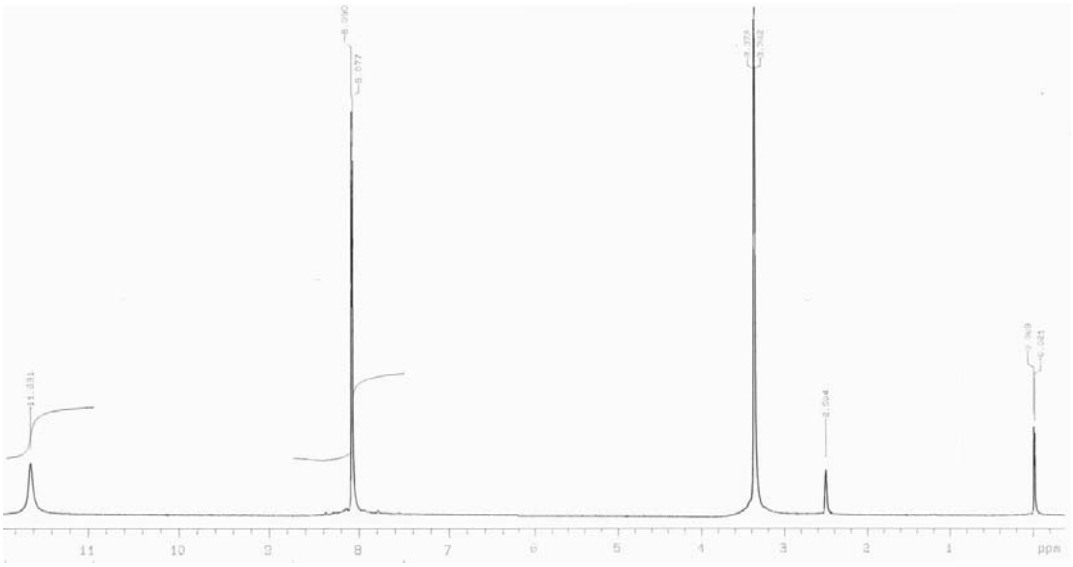


Figure SM 3.1.13.8 - ^{1}H RMN of 4,5-dichlorophthalimide (300 MHz, DMSO-d_6)

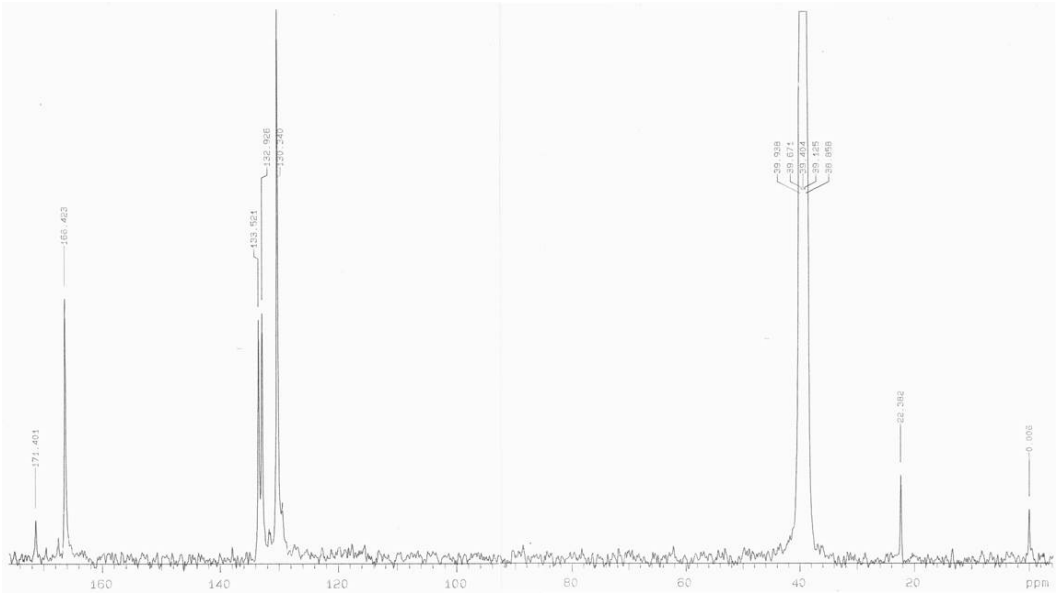


Figure SM 3.1.13.9 – ^{13}C RMN of 4,5-dichlorophthalimide (300 MHz, DMSO-d_6)

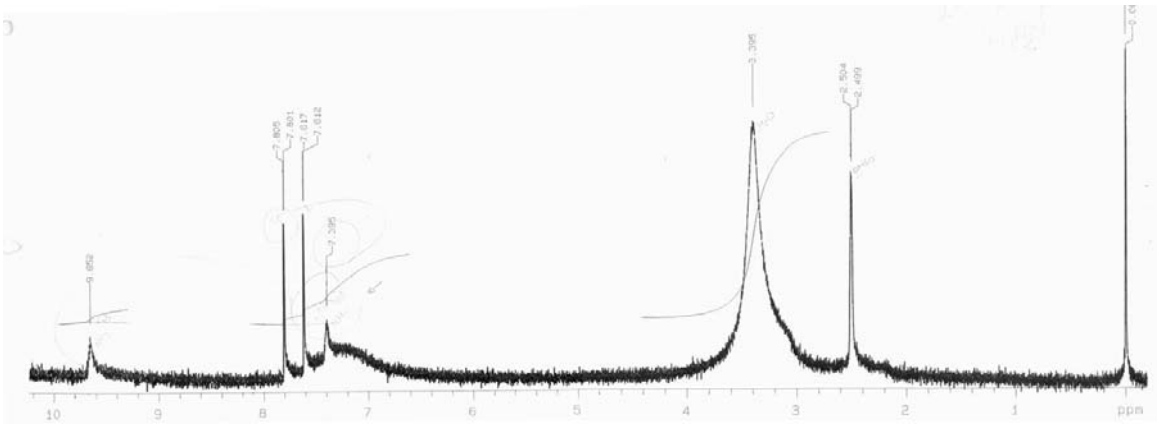


Figure SM 3.1.13.10 - ^{1}H RMN of of 4,5-dichlorophthalamide (300 MHz, DMSO-d_6)

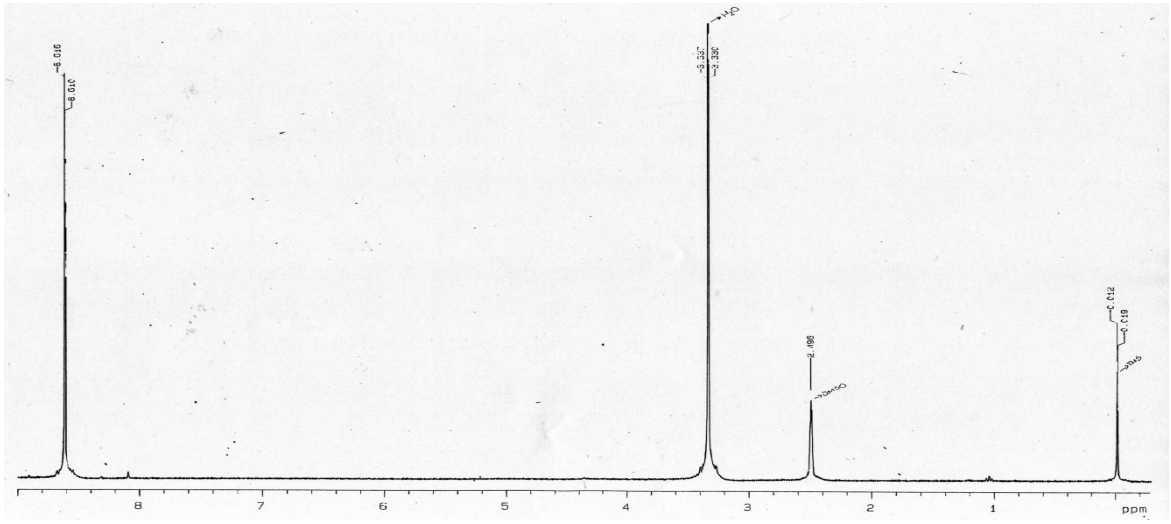


Figure SM 3.1.13.11 - ^{1}H RMN of of 4,5-dichloro-1,2-dicyanobenzene (300 MHz, DMSO-d_6)

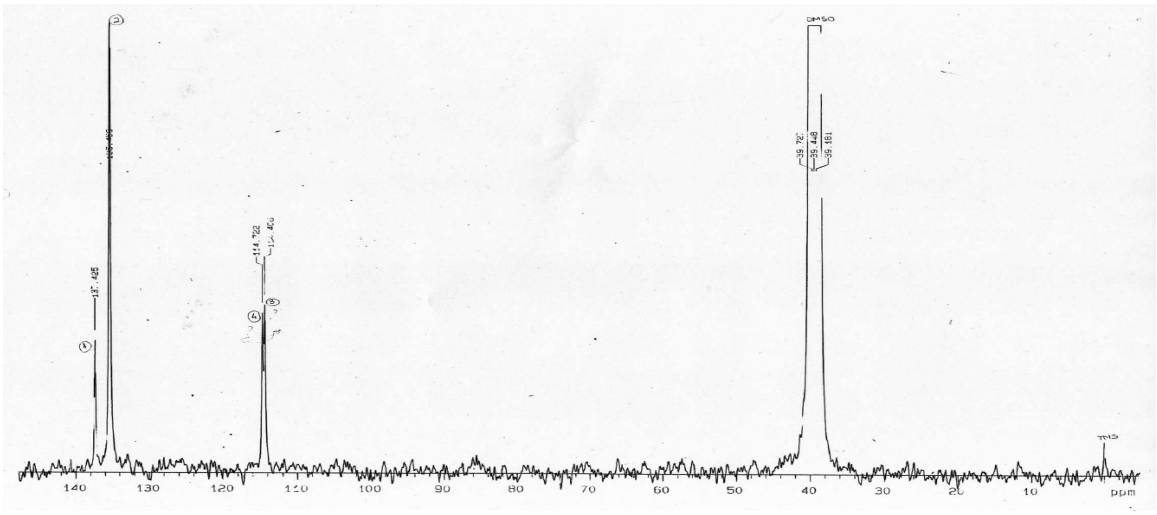


Figure SM 3.1.13.12 – ^{13}C RMN of 4,5-dichloro-1,2-dicyanobenzene (300 MHz, DMSO-d_6)

In the same reference[2] more information can be found concerning mass spectra and elemental analysis.

[1] a) B. Nicolet *et al*., *Org. Synth. Coll.I,* 1941, 410; b) W. Noyes *et al*., *Org. Synth. Coll.I,*, 1941, 457; c) Hargreaves, *Chem. Rev*., 1970, 439; d) Yamato *et al*., *Tetrahedron Lett.,* 1970, 4383.
[2] D. Wöhrle *et al*., *Synthesis,* 1993, 194.
[3] C. A. M. Afonso, D. P. Simão, L. P. Ferreira, M. S. Serra, M. M. M. Raposo, *100 Experiências de Química Orgânica*, Copyright © IST Press 2011, 313.
[4] Handbook of Chemistry and Physics, CRC Press,1st Student Ed., C-431.

Synthesis of *N-tert*-butyloxycarbonyl-3-nitro-L-tyrosine methyl ester

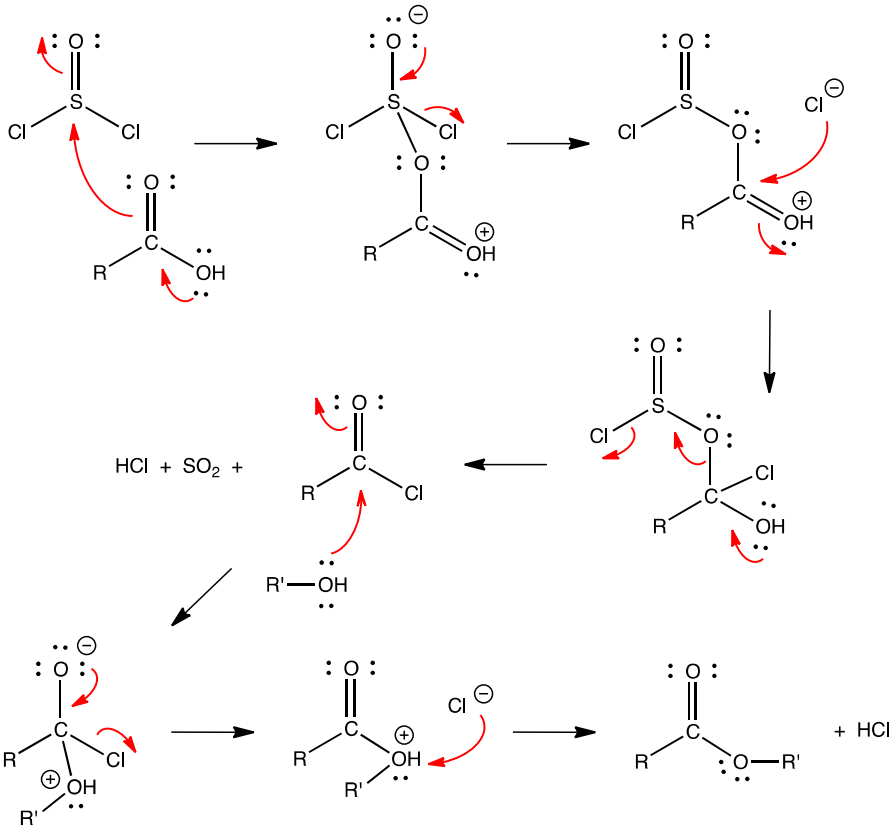
Supplementary Material

1. Experiment notes

This experiment involves simple experimental techniques and commercially available reagents, and it is expected that the students possess previously acquired practical skills (in terms of isolation and purification techniques) and theoretical background (synthesis, reactivity and spectroscopic data interpretation). Therefore, this experiment may be appropriate for 3rd year Chemistry students or 1st Chemistry Master students.

The aim of this work is the synthesis of a protected amino acid using simple experimental techniques and commercially available reagents, in two sessions. All the procedures are classical functional group transformations.
The protection at the amino acid *N*- and C-terminals is achieved with carbamate and ester groups, respectively. The protecting groups used in the protection of the amino and carboxylic acid groups are the classical *tert*-butyloxycarbonyl (Boc) and methyl ester (OMe) groups. This procedure for the introduction of the protecting groups may be applied to all natural α-amino acids.

<u>Session 1: Preparation of 3-nitro-L-tyrosine methyl ester hydrochloride **1**</u>
The preparation of the methyl ester from the carboxylic acid occurs by nucleophilic substitution: firstly the carboxylic acid is converted to the more reactive acid chloride, by reaction with thionyl chloride. Then alcoholysis of the acid chloride with methanol yields the corresponding methyl ester. By-products are sulphur dioxide and hydrogen chloride (Scheme SM 3.1.14.1).



Scheme SM 3.1.14.1. Mechanism for the esterification of a carboxylic acid through formation of an intermediate acid chloride.

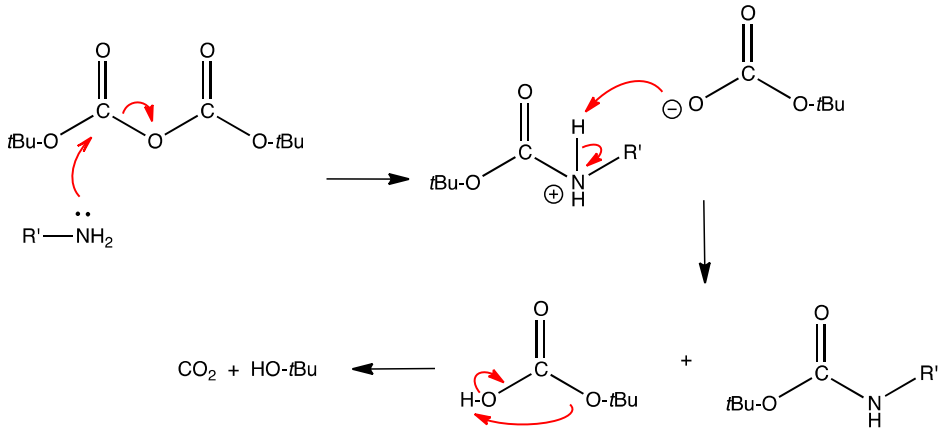
The free amino group in tyrosine is protonated by the released hydrogen chloride and the corresponding chloride salt is isolated as a crystalline solid. Compound **1** was isolated in quantitative yield as yellowish green solid in the present conditions, and in various attempts by 3rd year Chemistry students yields between 95% and quantitative were achieved. This experiment has been scaled up to 25 mmol of the starting tyrosine with reproducible results by repetitive execution, in slightly lower yields (90-95%).

Session 2: Preparation of *N*-*tert*-butyloxycarbonyl-3-nitro-L-tyrosine methyl ester **2**

The preparation of the carbamate from the amine group is achieved by acylation with di-*tert*-butyl dicarbonate. Basic conditions are required to neutralize the starting hydrochloride salt and restore the amine nucleophilic character. The amine nitrogen attacks a carbonyl site on di-*tert*-butyl dicarbonate resulting in *tert*-butyl carbonate leaving as a leaving group. This *tert*-butyl carbonate picks up a proton from the protonated amine and dissociates into gaseous CO_2 and *tert*-butanol, while the carbamate is formed (Scheme SM 3.1.14.2).

Di-*tert*-butyl dicarbonate should be kept in the fridge, and taken out only for the weighing. It is a low melting point solid (22-24°C) and it may become sluggish and difficult to weigh if kept too long out of the fridge.

Compound **2** was isolated in 90% yield as yellow solid with melting point 97.3-98.4°C.[1] This experiment has been scaled up to 25 mmol of the reagent with reproducible results by repetitive execution, in yields between 90 and 95% in this scale, by 3rd year Chemistry students, or in slightly lower yields in 25 mmol scale (80-85%).



Scheme SM 3.1.14.2. Mechanism for the acylation of an amino by reaction with di-*tert*-butyl dicarbonate.

<u>^{1}H NMR and IR spectroscopy rationale</u>

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

- the carbonyl stretching band by comparing the spectra of the reagent (carboxylic acid), compound **1** (ester) and compound **2** (carbamate): accordingly to the electronic effect of the atoms adjacent to the carbonyl group, the order for the bands will be $\nu_{C=O\ ester} \approx \nu_{C=O\ acid} > \nu_{C=O\ carbamate}$;
- the NH stretching band by comparing the spectra of the reagent (amine), compound **1** (ammonium salt) and compound **2** (carbamate): the shape of the band will differ with two bands for the amine, a broad band for the ammonium salt and a single band for the carbamate.

In the ^{1}H NMR spectra, the students can see the influence of:

- the solvent on the chemical shift of the hydroxyl proton by comparing the spectra of compounds **1** and **2**: OH proton appears at larger δ in the H-bonding solvent DMSO-d_6;

2. Figures

2.1 ^{1}H NMR spectrum of compound **1**

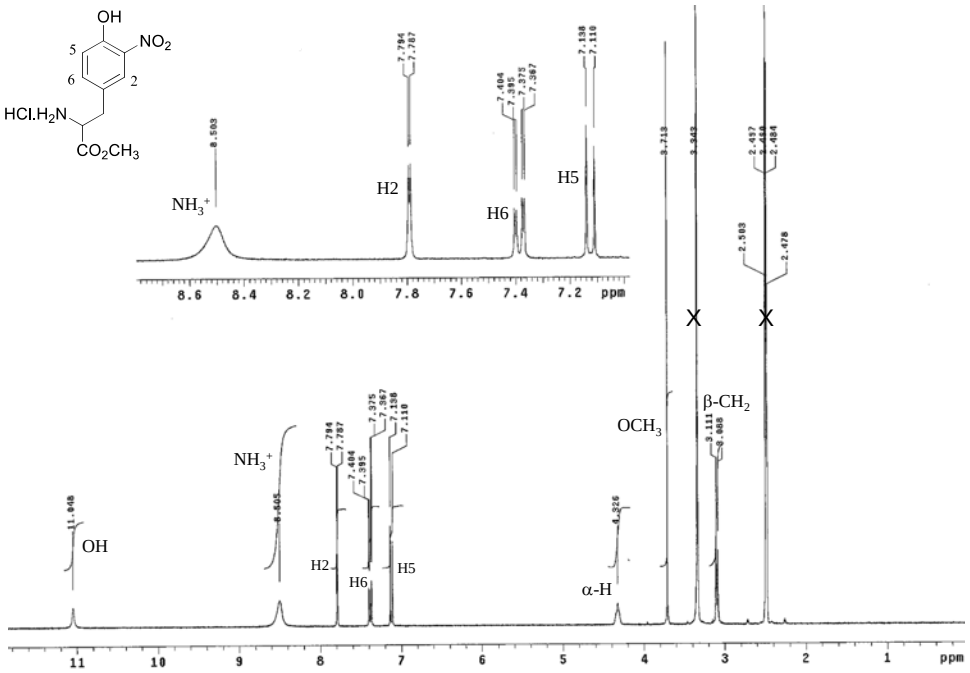


Figure SM 3.1.14.1. ^{1}H NMR of compound **1** in DMSO-d_6 obtained in a Varian Unity Plus spectrometer operating at 300 MHz at 25ºC (spectrum of the crude compound as obtained).

2.2 ^{1}H NMR spectrum of compound **2**

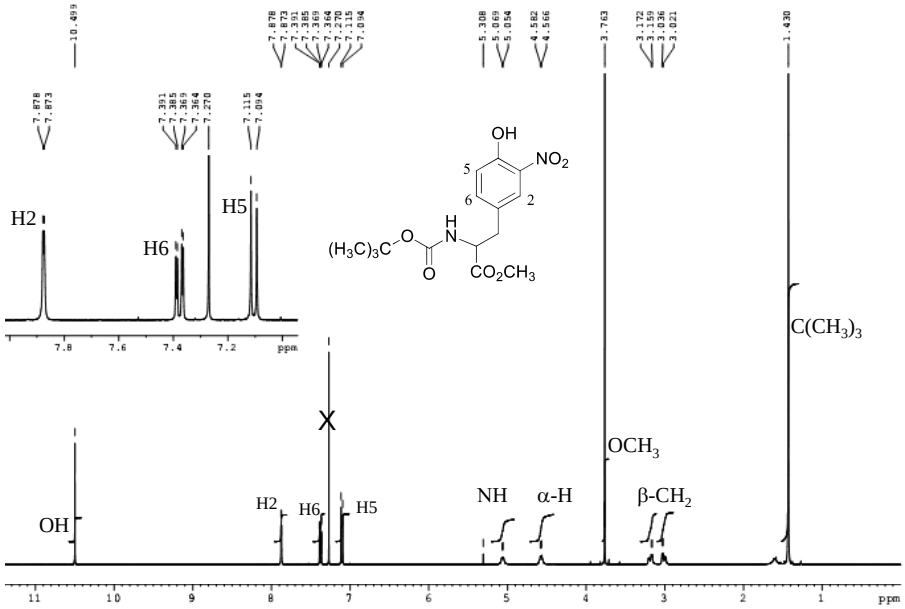


Figure SM 3.1.14.2. ^{1}H NMR of compound **2** in $CDCl_3$ obtained in a Bruker Avance III spectrometer operating at 400 MHz at 25ºC (spectrum of the crude compound as obtained).

2.3 IR spectrum of compound **1**

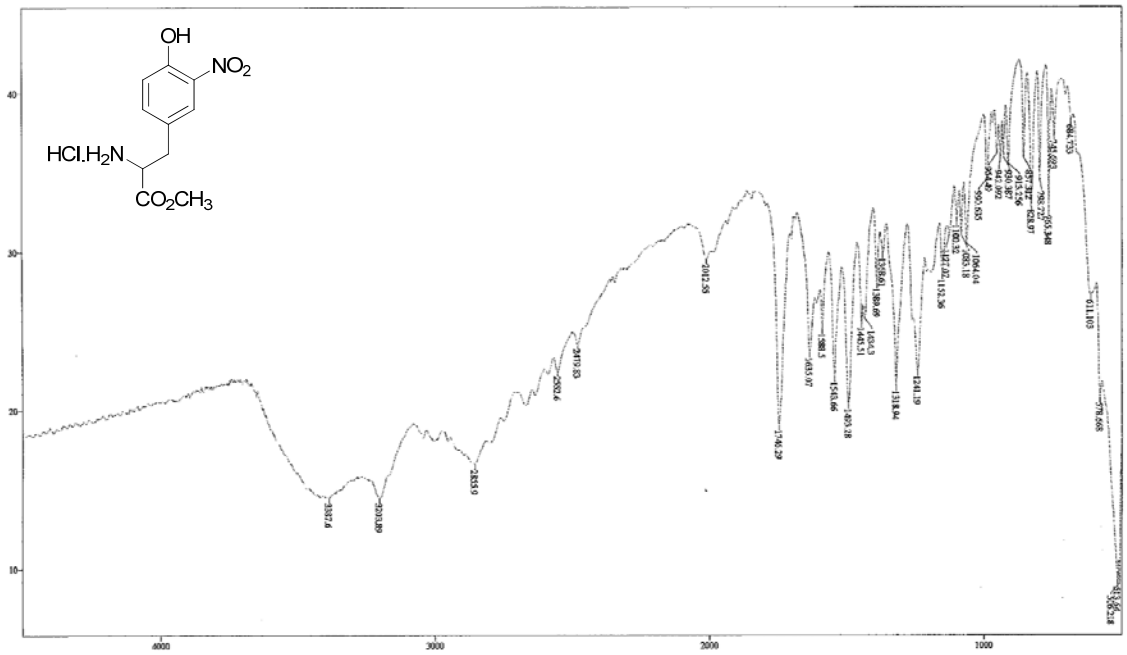


Figure SM 3.1.14.3. IR spectrum of compound **1** in KBr disc obtained in a Perkin Elmer FTIR-1600 spectrophotometer (spectrum of the crude compound as obtained).

2.4 IR spectrum of compound **2**

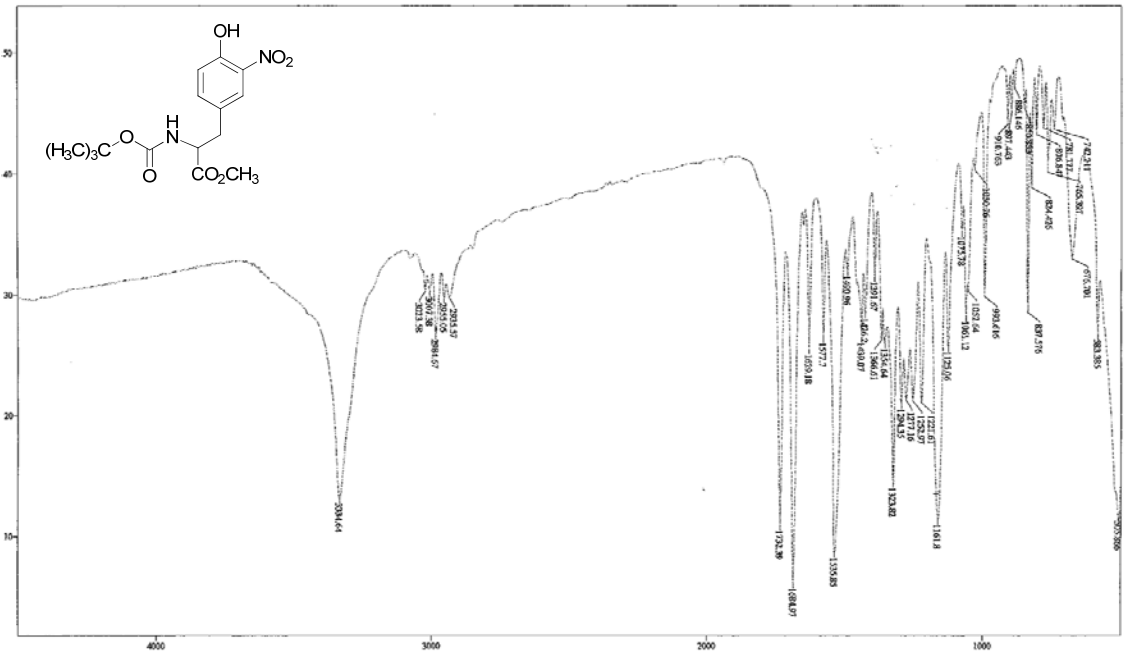


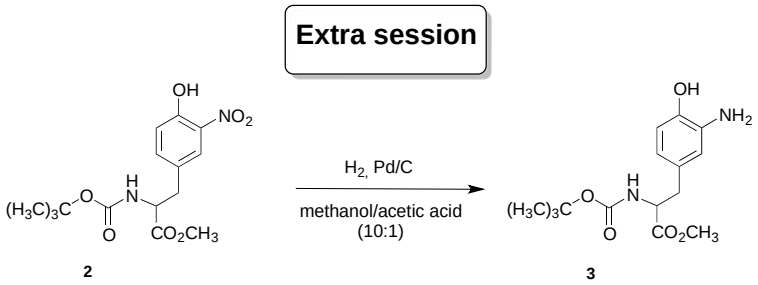
Figure SM 3.1.14.4. IR spectrum of compound **2** in KBr disc obtained in a Perkin Elmer FTIR-1600 spectrophotometer (spectrum of the crude compound as obtained).

References

1. S. P. G. Costa, E. Oliveira, C. Lodeiro, M. M. M. Raposo*, Sensors,* 2007*,* **7***,* 2096-2114*.*

3. Additional procedure: reduction of nitro group

In case there is interest in conducting experiment 12.2.2 in this book and if considered adequate by the teacher, compound **2** can undergo an additional reaction. This additional procedure involves catalytic reduction of the nitro group with hydrogen in the presence of palladium on carbon (Pd/C) catalyst.



Compound **3** was isolated in 98% yield as a light brown solid in these conditions. This experiment has been scaled up to 25 mmol of the reagent with reproducible results by repetitive execution. This procedure has been carried out in various occasions by 3rd year Chemistry students or 1st year Master students and the yields obtained range from 94-98%.

Additional safety

The work should be done in a well-ventilated fume hood, with proper safety equipment, such as protective clothing, gloves and safety goggles. Hydrogen is a highly flammable gas (handle safely, risk of explosion), which may be harmful if inhaled and cause skin, eye and respiratory irritation. Acetic acid is a corrosive acid and may cause severe skin and eye burns, and flammable in liquid and vapor form. Palladium on activated carbon is a flammable solid, sensitive to water and moist, may cause skin and respiratory irritation. Celite may cause eye and respiratory irritation, do not breathe dust.

IMPORTANT: in the scale of the present experiment the risk of fire is kept low as only a small sized balloon filled with hydrogen is used and the catalyst is not weighed to minimize its spread and contact with air.

Experimental procedure

Preparation of *N-tert*-butyloxycarbonyl-3-amino-L-tyrosine methyl ester **3**

1. Fill a 25 mL two-necked round-bottomed flask with a magnetic stirrer with an inert gas, such as argon or nitrogen (use rubber septa in both necks). Add a tip of a small spatula (2-3 mg) of 10% palladium on carbon and methanol/acetic acid (10:1) (5 mL).
2. Add 340 mg (1 mmol, 1 equiv) of *N-tert*-butyloxycarbonyl-3-nitro-L-tyrosine methyl ester **2** dissolved in methanol/acetic acid (10:1) (5 mL), and start stirring the solution, while passing inert gas (argon or nitrogen) through the flask.
3. To one of the flask necks, connect a stopcock adapter with a balloon filled with H_2 and fill the round-bottomed flask with hydrogen (in the other neck, insert a needle in the septa to allow flushing). Maintain the stopcock open and close the flask by removing the needle from the septa.
4. Stir the reaction mixture at room temperature in H_2 atmosphere for 12h. Check the reaction progress by thin layer chromatography (TLC) by comparing with the reagent (eluent: dichloromethane/methanol, 100:1).
5. When the reaction is finished, flush the reaction flask with inert gas (argon or nitrogen) to replace all the hydrogen gas.
6. Filter the reaction mixture through a bed of Celite to remove Pd/C and wash the filter cake with methanol (5 mL) (caution: do not allow the filter cake to dry completely). After filtration, add some water to the filter cake and discard in the appropriate palladium waste container.
7. Evaporate the solvent to dryness in the rotary evaporator.
8. Weigh the solid, measure the melting point, acquire ^{1}H and ^{13}C NMR spectra in $CDCl_3$ and a IR spectra in KBr disc. Compare with literature data (see spectra).[9]

Handling of the effluents

Handling of hydrogen requires caution as it is highly flammable and an explosion hazard. Hydrogen is colourless and odourless and not perceived by the handler's senses.

Handling of Pd/C also requires caution, as it is a flammable solid. Special care should be taken in discarding the Pd/C slurry waste. The Pd/C slurry in Celite should be disposed in a palladium waste container and the filtered magnesium sulphate in the appropriate solid waste container.

Results, interpretation and additional questions

In the IR spectra, the students can see the influence of the electronic effects on wavenumbers of the OH stretching band and the appearance of new NH stretching bands by comparing the spectra of compounds **2** and **3**.

In the ^{1}H NMR spectra, the students can see the influence of:

- the electronic effects on the proton chemical shift by comparing the spectra of compound **2** (nitro group) and compound **3** (amino group);
- the electronic effects on the chemical shift of the hydroxyl proton by comparing the spectra of compound **2** (nitro group) and compound **3** (amino group);

1. What is the function of Pd/C in the preparation of compound **3**?
2. Interpret the ^{1}H NMR spectral data obtained for compound **3**, with the corresponding assignment of signals. Compare with the spectra provided as supplementary material.
3. Interpret the IR spectral data obtained for compound **3**, with the corresponding assignment of bands. Compare with the spectra provided as supplementary material.
4. Compare the ^{1}H NMR chemical shift of the aromatic protons to the structure of compounds **2** and **3**.

3.1 Photo of reduction experimental setup

Figure SM 3.1.14.5. Catalytic reduction experimental setup.

3.2 ^{1}H NMR spectrum of compound **3**

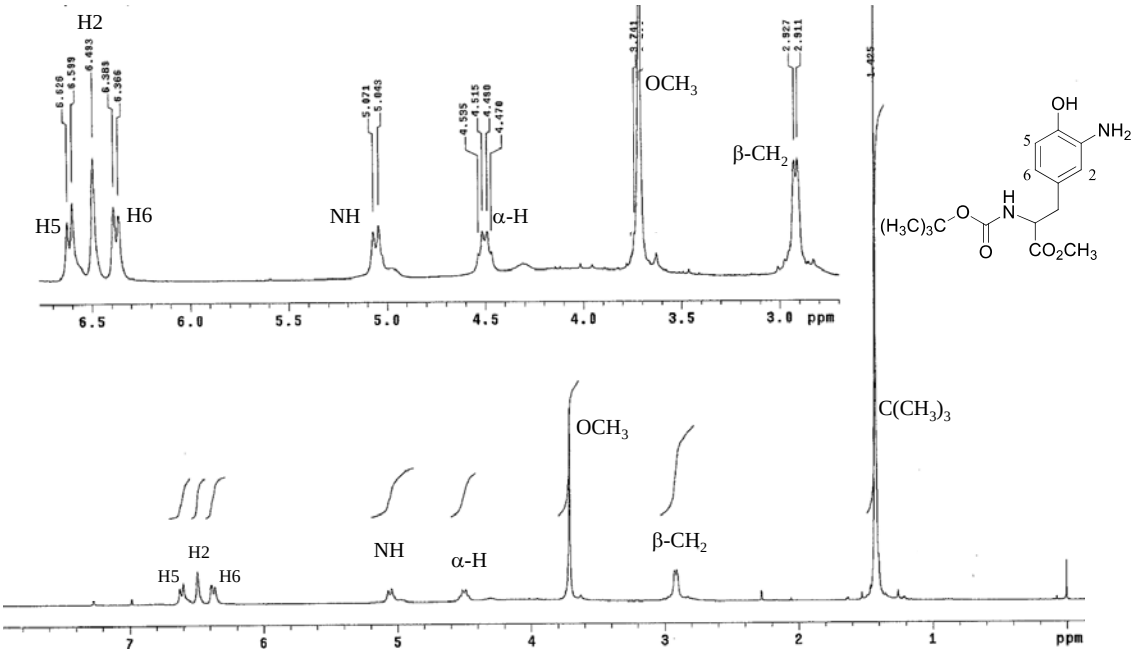


Figure SM 3.1.14.6. ^{1}H NMR of compound **3** in $CDCl_3$ obtained in a Varian Unity Plus spectrometer operating at 300 MHz at 25ºC (spectrum of the crude compound as obtained).

3.3 IR spectrum of compound **3**

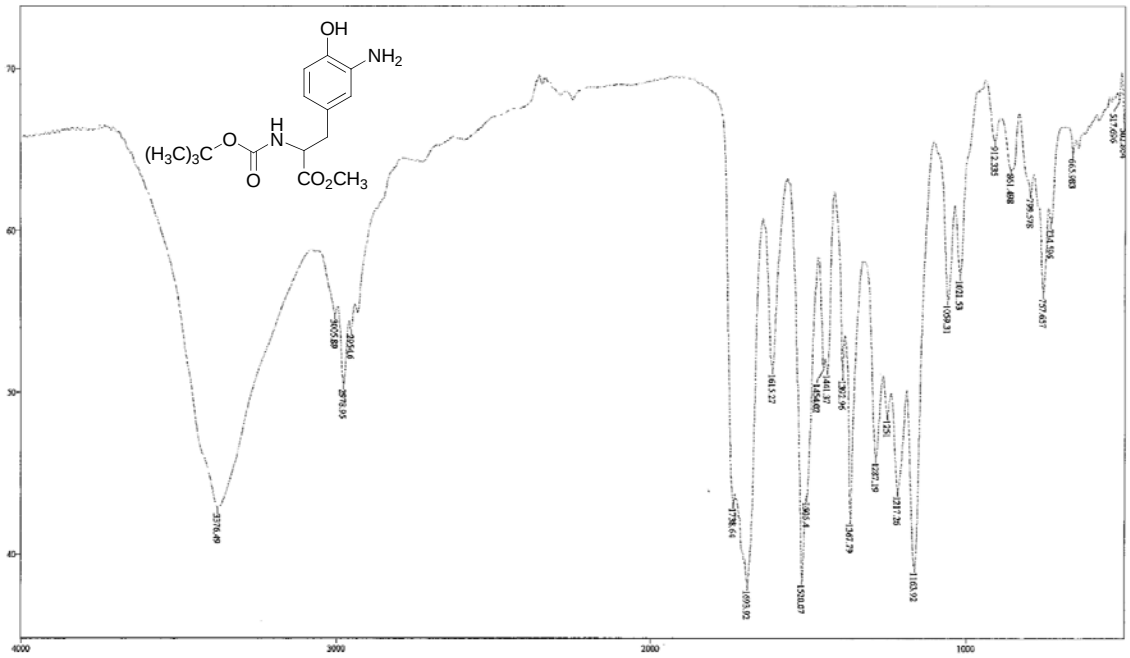


Figure SM 3.1.14.7. IR spectrum of compound **3** in KBr disc obtained in a Perkin Elmer FTIR-1600 spectrophotometer (spectrum of the crude compound as obtained).

Michael Addition Reaction Followed by Elimination Under Solvent-Free Conditions

Supplementary Material

- The objective of this experiment is the preparation of β-enaminones from amination of 1,1,1-trihalo-4-alkoxy-3-alken-2-ones,either using ethanol as the solvent or not using a solvent. The system's needs for this reaction are presented in **Figure SM 3.1.15.1** and **SM 3.1.15.2**.
- In method A, the appearance of the reactants before and after the reaction is the same. In method B, the enone is colorless and liquid before the addition of the appropriated amine (**Figure SM 3.1.15.2**). After the addition of benzylamine, it is possible to follow the reaction by direct observation of the formation of the viscous yellow liquid as the product is being formed (**Figure SM 3.1.15.3a**). In the case of *N*,*N*-Diisobutylamine, it is possible to follow the reaction by direct observation of the formation of the reddish liquid as the product is being formed (**Figure SM 3.1.15.3b**).

- The final product with N,N-Diisobutylamine is washed with distilled water (**Figure SM 3.1.15.4**). **Figure SM 3.1.15.5** shows the final product, after being exposed to reduced pressure for 1 h. **Table SM 3.1.15.1** describes the results obtained in molecular solvent (ethanol) for entries 1 and 3, and in the absence of solvent for entries 2 and 4.
- This experiment allows students to rationalize the reaction mechanism of the synthesis of β-enaminones. Additionally, the importance of solvent-free reactions for Green Chemistry is discussed.

Table SM 3.1.15.1. Experiments conducted in a round-bottomed flask, using 1,1,1-trifluoro-4-methoxypent-4-en-2-one as starting material (5 mmol) and the appropriated amine (5 mmol).

Entry	Solvent	Amine	Temp. (°C)	Reaction Time (min.)	Yield (%)	Mp[c] (°C)
1	Ethanol	Benzylamine	25	5	90–99[a]	69
2	Solvent-free	Benzylamine	25	5	90–99[a]	69
3	Ethanol	N,N-Diisobutylamine	25	5	80–90[b]	35
4	Solvent-free	N,N-Diisobutylamine	25	5	80–90[b]	35

[a]After being exposed to reduced pressure for 1 h, tested by three students. [b]After liquid-liquid extraction and being exposed to reduced pressure for 1 h. [c]Melting point.

Table SM 3.1.15.2. Chemical and physical properties of reagents and product.

Compounds	Mp (°C)	Refractive index	Bp (°C)
1	-	-	42[a,3]
2a	10[4]	1.540[5]	185[b,6]
2b	-73.5[4]	1.409[4]	139[b,4]
3a	59-61[7]	-	-
3b	35[c]	-	-

[a]Determined at 11 Torr. [b]Determined at 760 Torr. [c]Determined in this work.

General Data

(Z)-4-(Benzylamine)-1,1,1-trifluoro-3-pent-2-one ($C_{12}H_{12}F_3NO$).

Decomposition temperature (T_d) 205 °C (determined by derivative TGA); melting point (Mp) 59 °C–61 °C (determined by melting point apparatus); Mp 69 °C (determined by

DSC); ^{1}H NMR ($CDCl_3$, 600MHz): δ, 2.09 (s, CH_3),4.55 (d, CH_2), 5.39 (s, CH) 7.25–7.38 (m, 5H, arom), 11.43 (s, NH) ppm; ^{13}C NMR ($CDCl_3$, 150.9 MHz): δ, 19.44 (CH_3), 47.64 (CH_2), 89.86 (CH), 117.71 (q, CF_3, ^{1}J=288 Hz), 127.00 (CH), 128.0 (2CH), 128.15 (2CH), 129.36 (CH), 136.04 (CH), 169.59 (C), 176.06 (q, C=O, ^{2}J=32 Hz); MS: m/z (%) = 243 (M^+, 45), 91 (100), 174 (81), 65 (52), 146 (13).

(*Z/E*)-4-(N,N-Diisobutylamine)-1,1,1-trifluoro-3-pent-2-one ($C_{13}H_{22}F_3NO$).

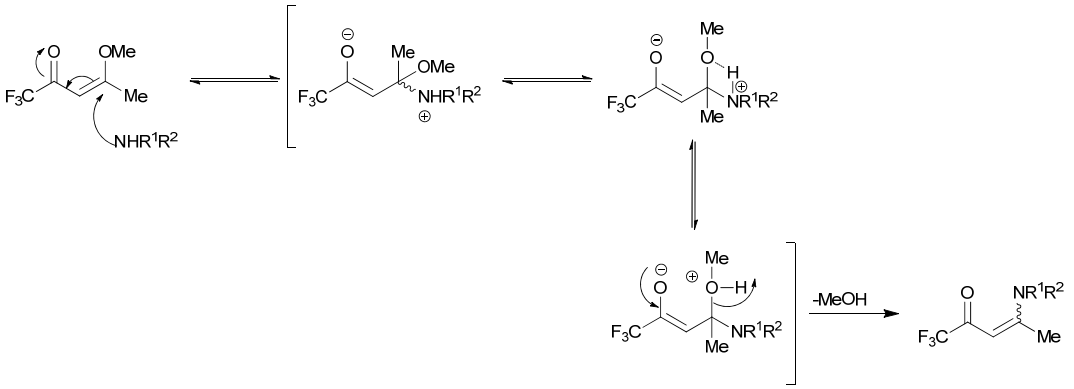
Decomposition temperature (T_d) 198 °C (determined by derivative TGA); melting point (Mp) 35 °C (determined by DSC); ^{1}H NMR ($CDCl_3$, 600MHz): δ 0.95 (d, CH_3), 1.10 (d, CH_3), 1.99 (m, CH) 2.24 (m, CH), 2.63 (s, CH_3), 3.21 (d, CH_2), 3.27 (d, CH_2), 5.29 (s, CH) ppm; ^{13}C NMR ($CDCl_3$, 150.9 MHz): δ, 17.71 (CH_3), 19.94 (CH_3), 20.04 (CH_3), 25.87 (CH), 28.54 (CH), 59.54 (CH_2), 59.69 (CH_2), 87.94 (CH), 118.21 (q, CF_3, ^{1}J=288 Hz), 167.96 (C), 175.11 (q, C=O, ^{2}J = 32 Hz).

How to prepare a NMR sample for analysis

1. Place 0.020 g (20 mg) of the product (M3) into an NMR tube.
2. Add 600 µL of $CDCl_3$ (containing TMS as an internal reference) to the NMR tube.
3. Acquire the ^{1}H NMR and ^{13}C NMR spectra.

- Reaction Mechanism

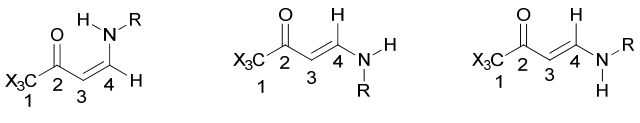
According to the variable transition state model developed for nucleophilic vinylic substitution by Rappoport,[1] when the nucleophile is neutral (mostly an amine), the first intermediate formed is the zwitterion. This reaction involves the initial attack of the amine on the β-carbon of the enone, followed by a charge delocalization to the carbonyl group and the subsequent prototropism, thus making the O-Alk grouping a good leaving group. Finally, the last step is the elimination of the alcohol molecule (**Scheme SM 3.1.15.1**).



Scheme SM 3.1.15.1. Reaction Mechanism.

- Conformational properties of β-enaminones.

Regarding the conformational properties, there are three centers that hinder rotation in the 4-amino-1,1,1-trihalo-3-alken-2-ones: (i) the carbon-carbon double bond; (ii) the carbon-carbon single bond; and (iii) the carbon-nitrogen single bond. Two configurations (Z or E) may exist with respect to the C3-C4 double bond, and two conformations (syn or anti) may exist for the C1-C2 and the C4-N bond (**Scheme SM 3.1.15.2**). The stereochemistry of the C3-C4 double bond of 4-(benzylamine)-1,1,1-trifluoropent-4-en-2-one can be determined by ^{1}H NMR spectroscopy. The downfield peak of the amino protons (10–12 ppm) in compounds suggests the existence of an intramolecular hydrogen bond and, therefore, a cis relationship between the NH and C=O groups, a reason for which a Z-configuration can be deduced. On the other hand, the stereochemistry of the C3-C4 double bond of 4-(*N*,*N*-diisobutylamine)-1,1,1-trifluoropent-4-en-2-one cannot be observed by NMR ^{1}H NMR spectroscopy because there are no amino protons.



ZZE-isomer *ZEZ-isomer* *ZEE-isomer*

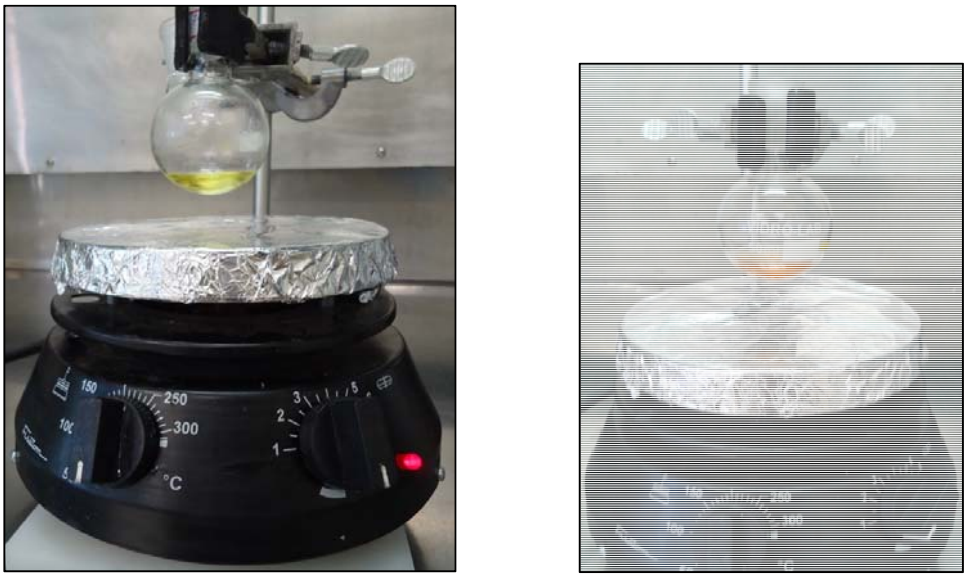
(i) (ii) (iii)

Scheme SM 3.1.15.2. Conformational properties of 4-amino-1,1,1-trihalo-3-alken-2-ones.

Figures

Photos of the experiment

Method A: Ethanol



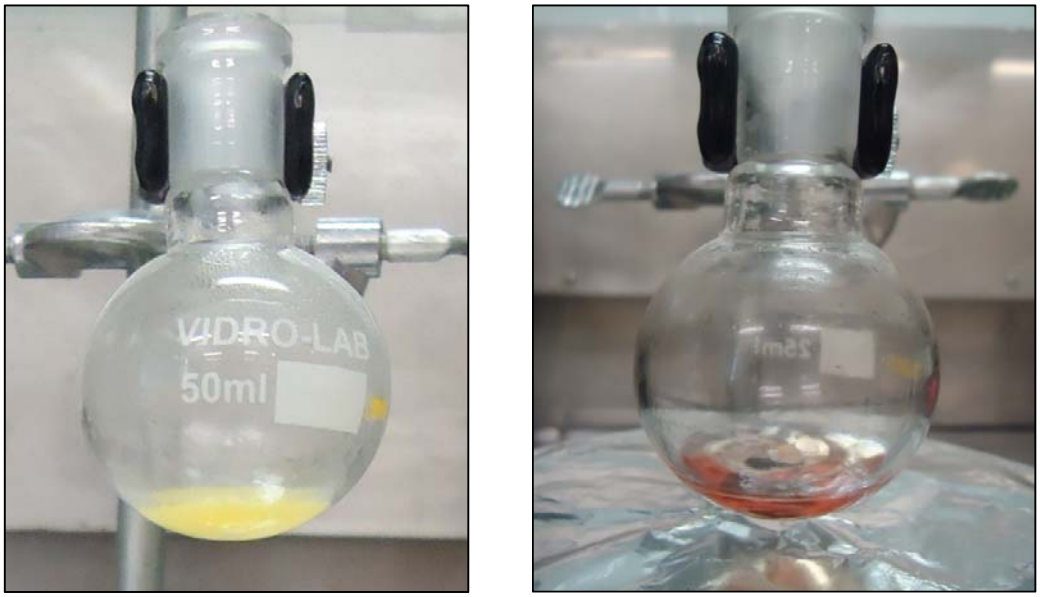
(a) (b)

Figure SM 3.1.15.1. Reagents and solvent: (a) with benzylamine; and (b) with N,N-Diisobutylamine.

Method B: Solvent-Free

(a) **(b)**

Figure SM 3.1.15.2. Reagents and solvent before reaction: (a) reaction with benzylamine; and (b) reaction with N,N-Diisobutylamine.



(a) (b)

Figure SM 3.1.15.3. Products after reaction: (a) reaction with benzylamine; and (b) reaction with N,N-Diisobutylamine.

Figure SM 3.1.15.4. Liquid-liquid extraction of the reaction with N,N-Diisobutylamine.

(a) (b)

Figure SM 3.1.15.5. Products after 1 h in the vacuum pump: (a) reaction with benzylamine; and (b) reaction with N,N-Diisobutylamine.

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

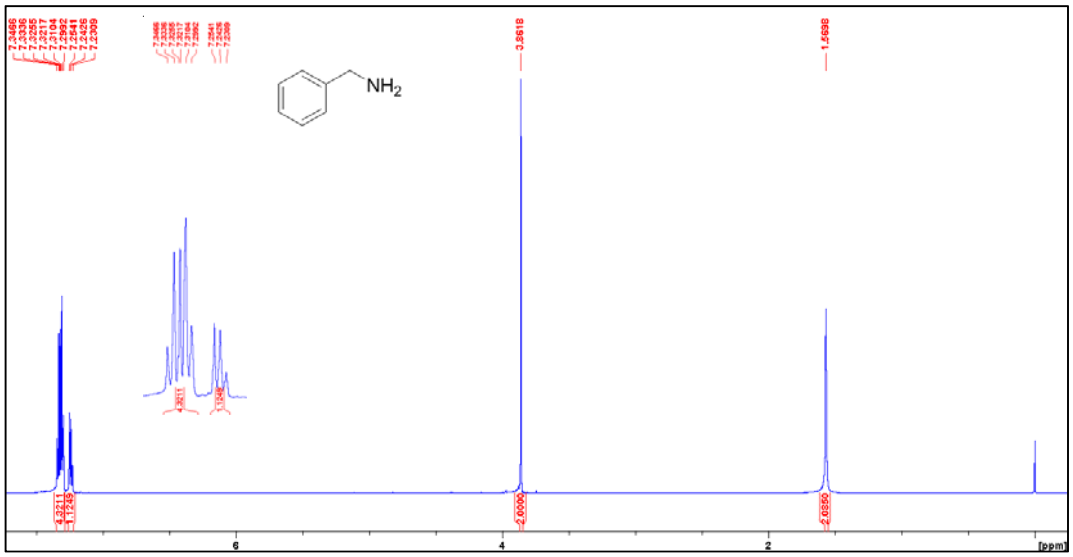


Figure SM 3.1.15.6. ^{1}H NMR spectrum (600MHz, $CDCl_3$, 25°C) of benzylamine.

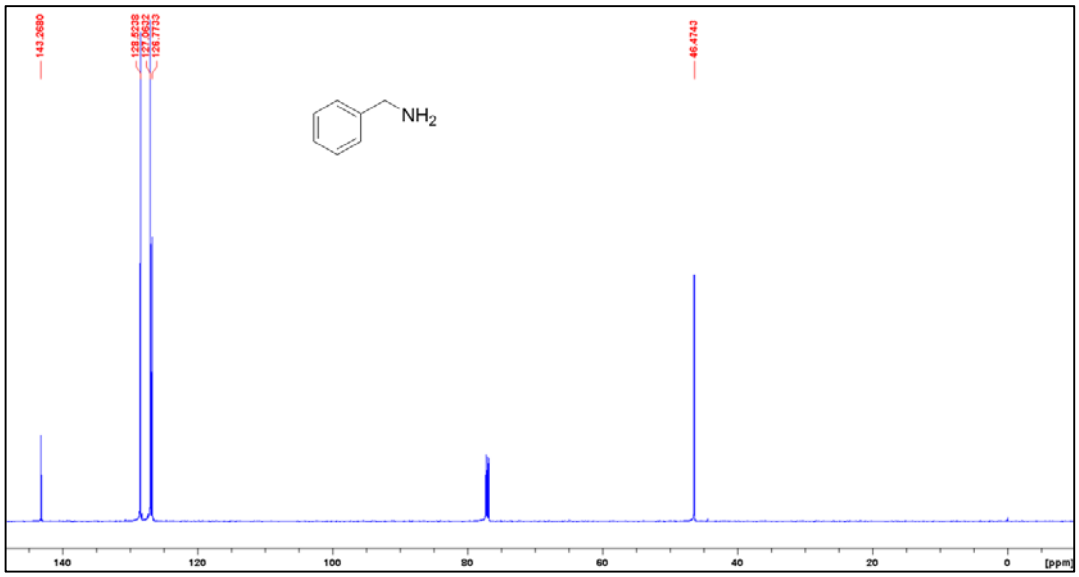


Figure SM 3.1.15.7. ^{13}C NMR spectrum (150.9 MHz, $CDCl_3$, 25°C)of benzylamine.

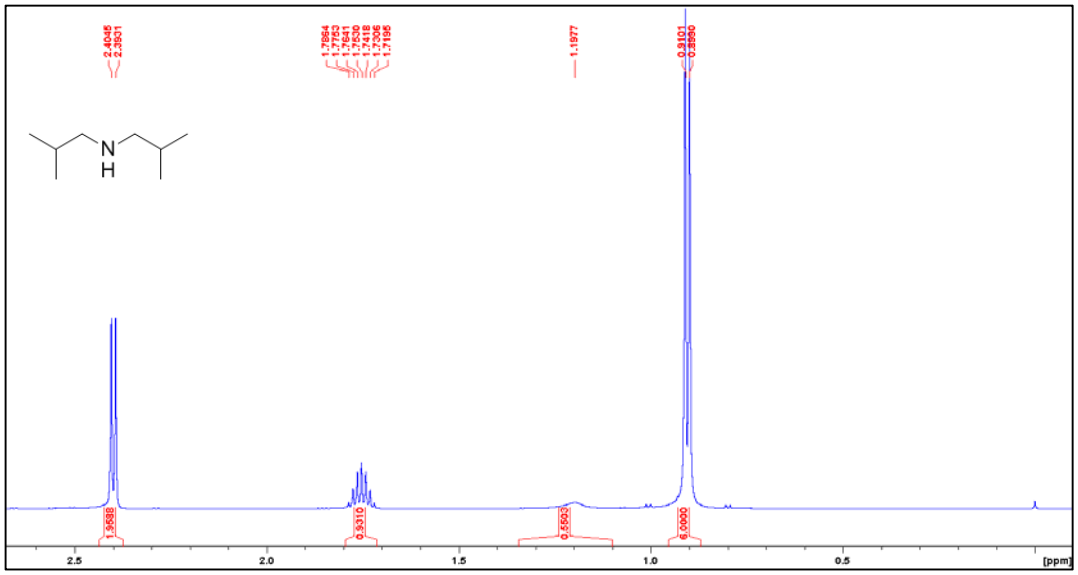


Figure SM 3.1.15.8. ^{1}H NMR spectrum (600MHz, $CDCl_3$, 25°C) of N,N-Diisobutylamine.

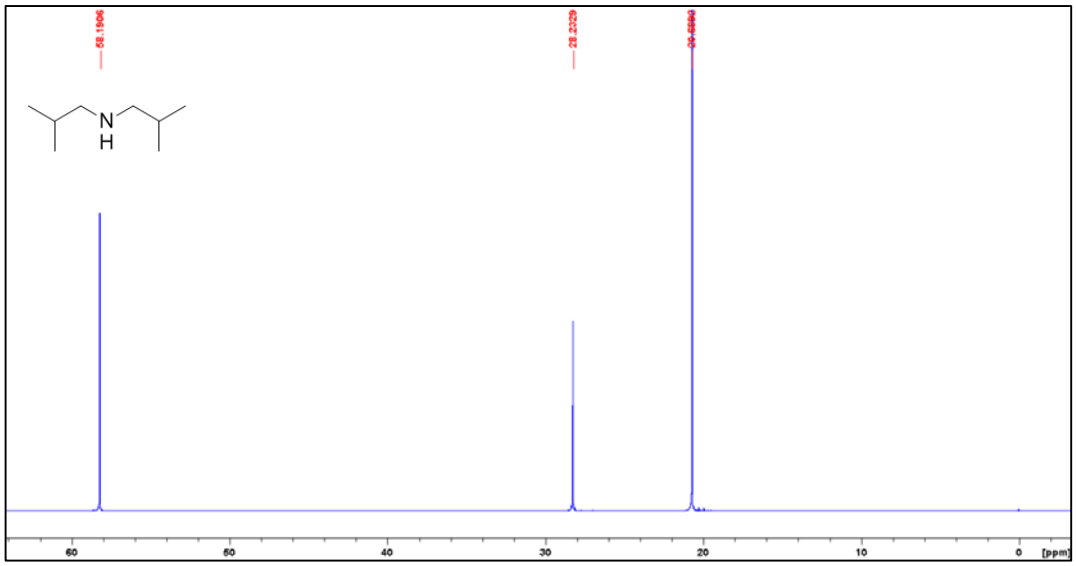


Figure SM 3.1.15.9. ^{13}CNMR spectrum (150.9 MHz, $CDCl_3$, 25°C) of N,N-Diisobutylamine.

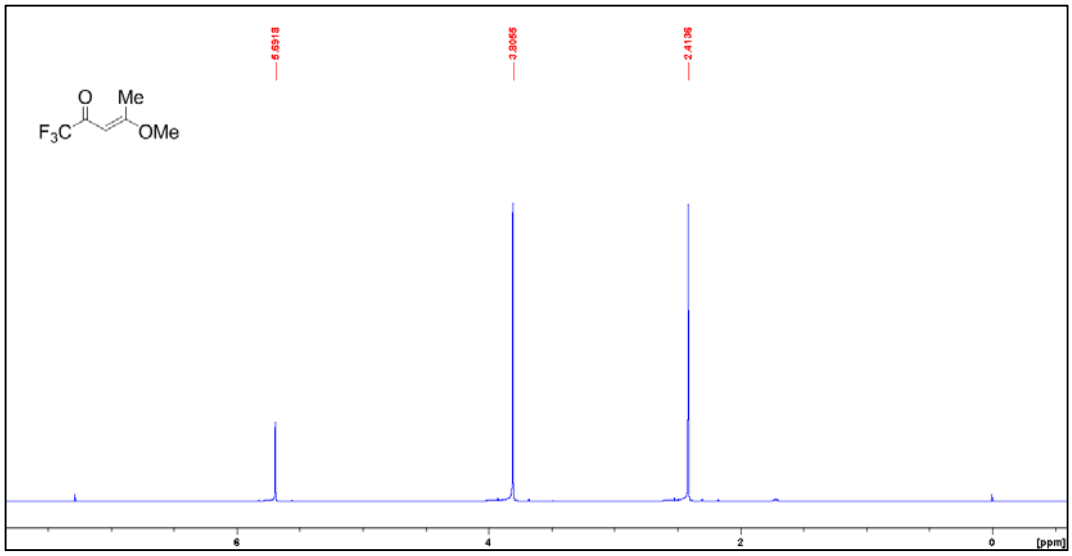


Figure SM 3.1.15.10. ^{1}H NMR spectrum (600MHz, $CDCl_3$,25°C) of 1,1,1-trifluoro-4-methoxypent-4-en-2-one.

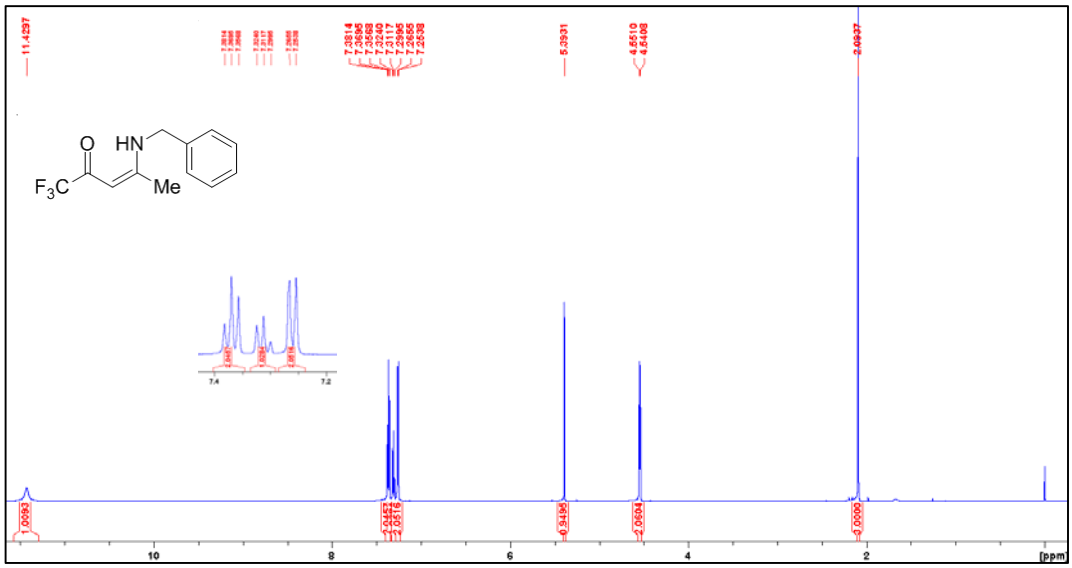


Figure SM 3.1.15.11. ^{1}H NMR spectrum (600MHz, $CDCl_3$,25°C) of the (*Z*)-4-(benzylamine)-1,1,1-trifluoropent-4-en-2-one.

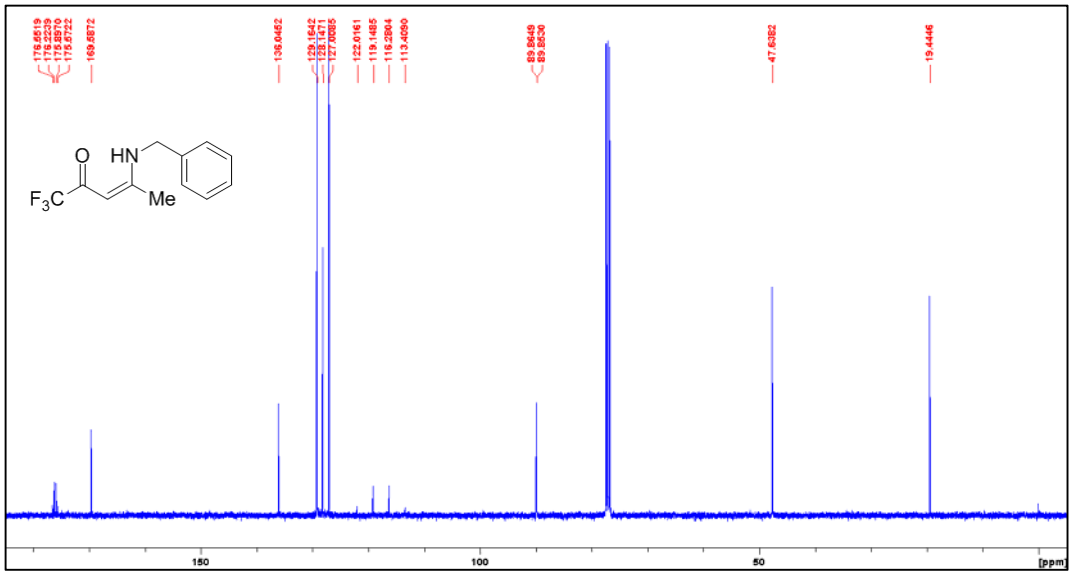


Figure SM 3.1.15.12. ^{13}C NMR spectrum (150.9 MHz, $CDCl_3$,25°C) of (*Z*)-4-(benzylamine)-1,1,1-trifluoropent-4-en-2-one.

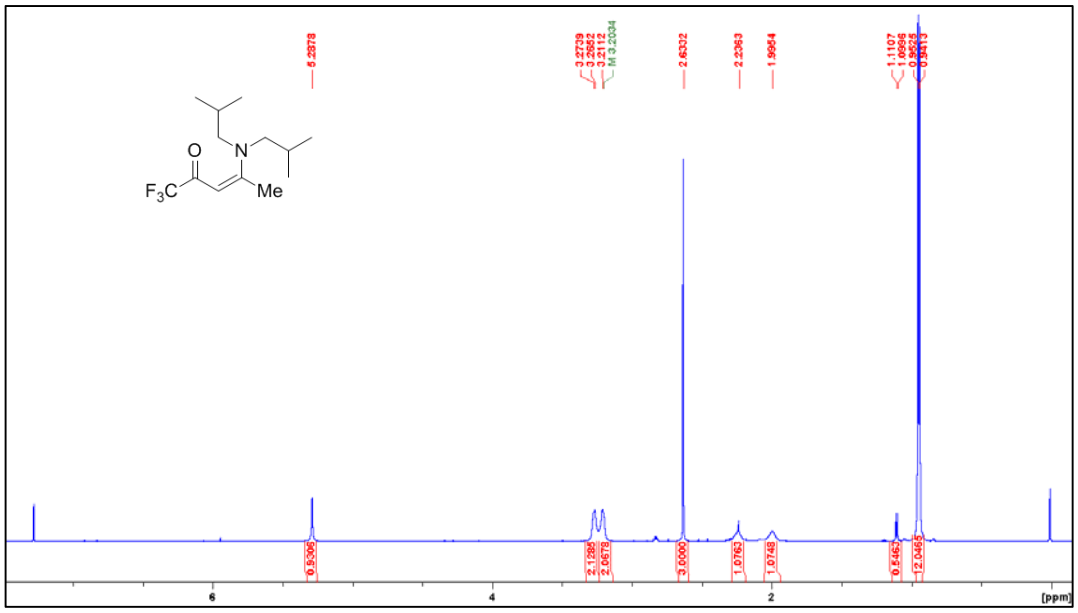


Figure SM 3.1.15.13. ^{1}H NMR spectrum (600MHz, $CDCl_3$, 25 °C) of the (*Z*/*E*)-4-(N,N-Diisobutylamine)-1,1,1-trifluoropent-4-en-2-one.

Figure SM 3.1.15.14. ^{13}C NMR spectrum (150.9 MHz, $CDCl_3$, 25 °C) of the (*Z*/*E*)-4-(N,N-Diisobutylamine)-1,1,1-trifluoropent-4-en-2-one.

Thermal Analysis

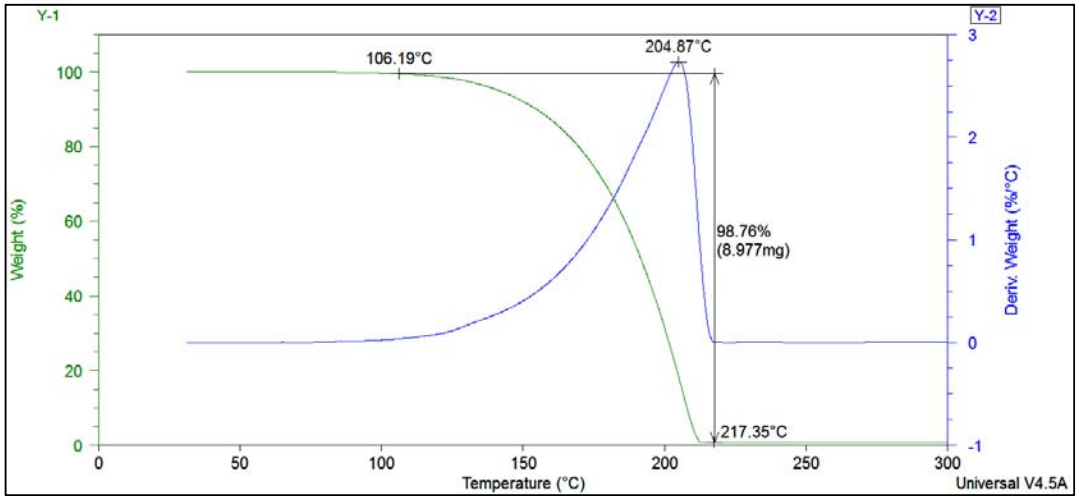


Figure SM 3.1.15.15. Thermogram of the thermogravimetric analysis (TGA) of the compound (*Z*)-4-(benzylamine)-1,1,1-trifluoropent-4-en-2-one (heating rate of 10 °C·min^{-1}).

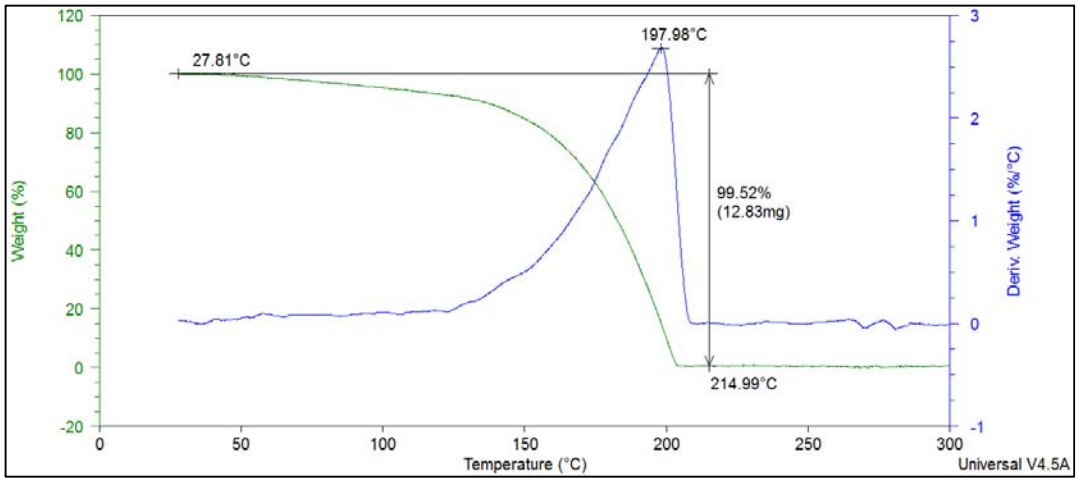


Figure SM 3.1.15.16. Thermogram of thermogravimetric analysis (TGA) of the compound (*Z/E*)-4-(N,N-Diisobutylamine)-1,1,1-trifluoropent-4-en-2-one (heating rate of 10 °C·min^{-1}).

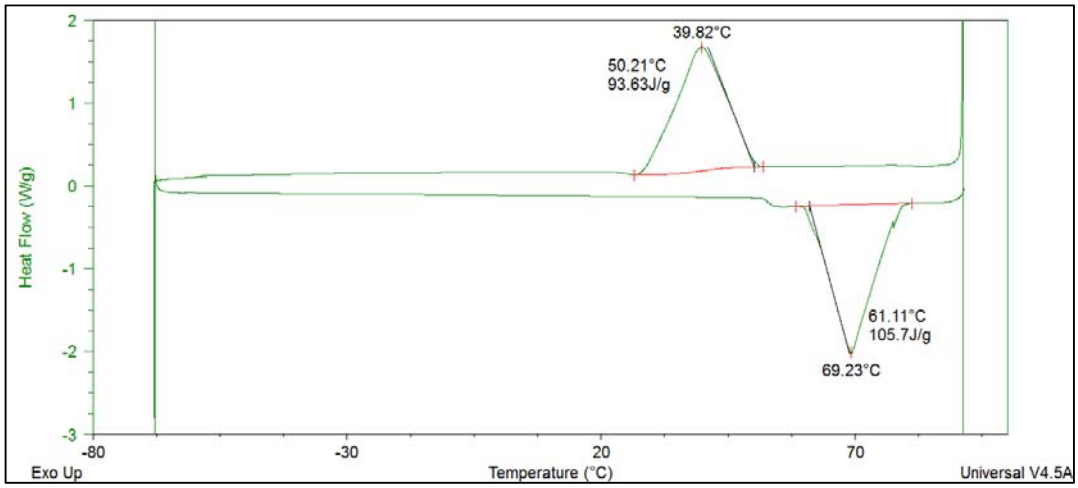


Figure SM 3.1.15.17. Thermogram of the differential scanning calorimetry (DSC) of the compound (*Z*)-4-(benzylamine)-1,1,1-trifluoropent-4-en-2-one(heating rate of 10 °C·min^{-1}).

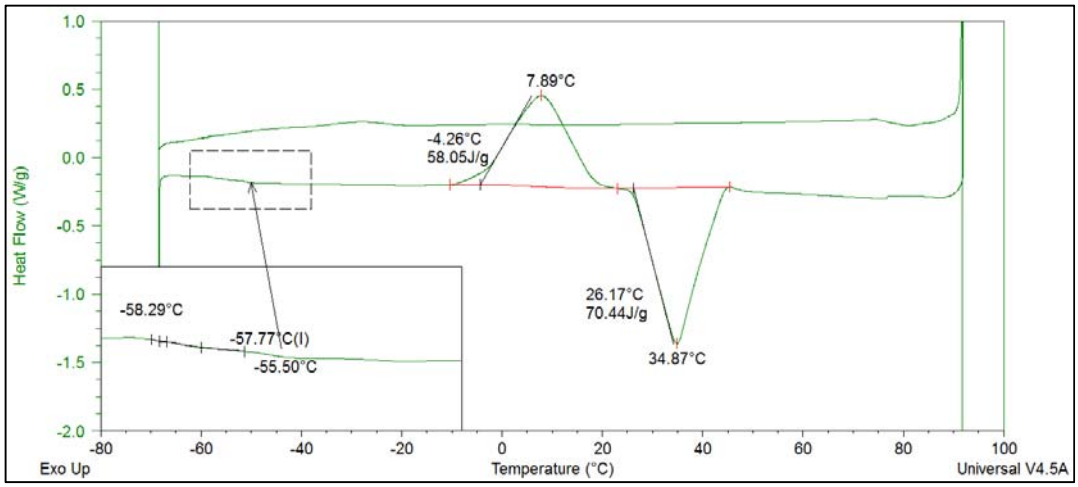


Figure SM 3.1.15.18. Thermogram of the differential scanning calorimetry (DSC) of the compound (*Z/E*)-4-(N,N-Diisobutylamine)-1,1,1-trifluoropent-4-en-2-one (heating rate of 10 °C·min^{-1}).

[1] (a) M. Beit-Yannai, X. Chen, Z. Rappoport, J. Chem. Soc., Perkin Trans. 2 2001, 1534–1545. (b) Z. Rappoport, *Acc. Chem. Res.* 1992, **25**, 474–479. (c) Z. Rappoport, *Rec. Trav. Chim. Pays-Bas* 1985, **104**, 309–349. (d) Z. Rappoport, *Acc. Chem. Res.* 1981, **14**, 7–15. (e) Z. Rappoport, B. Avramovitch, *J. Org. Chem.* 1982, **47**, 1397–1408.
[2] Z. Rappoport, P. Peled, *J. Am. Chem. Soc.* 1979, **101**, 2682–2693.
[3] M. A. P. Martins, W. Cunico, C. M. P. Pereira, A. F. C. Flores, G. Bonacorso, N. Zanatta, *Curr. Org. Synth.* 2004, 391.
[4] "Hazardous Substances Data Bank" data were obtained from the National Library of Medicine (US).
[5] L. M. Soffer, J. Am. Chem. Soc.1956, 78, 1705.
[6] L. J. Exner, M. J. Hurwitz,; P. L. De Benneville, J. Am. Chem. Soc.1955, 77, 1103.
[7] M. A. P. Martins, E. A. Guarda, C. P. Frizzo, M.R. B. Marzari, D. N. Moreira, N. Zanatta, H. G. Bonacorso, *Monatsh Chem.* 2008, **139**, 1321.

Synthesis of a γ-keto amide derived from thiophene using a carboxyl ester as precursor

Supplementary Material

1. Experiment notes

The aim of this experiment is the synthesis of a γ-keto amide derived from thiophene, using several simple experimental techniques and cheap commercially available reagents. The theoretical concepts associated with the work are vast and have an intermediate/advanced degree of difficulty. The students will be introduced to several classical reactions in organic chemistry such as alkaline hydrolysis of a γ-keto ester (saponification), nucleophilic substitution (conversion of a carboxylic acid to amide) using two different synthetic methodologies: *i)* mixed anhydride or *ii)* direct amidation of a carboxylic acid in the presence of DCC/OHBt coupling agents.[1-4]

The preparation of 1-(piperidin-1-yl)-4-(thiophen-2-yl)butane-1,4-dione through two different methods will allow the students to compare the efficiencies of the two experimental procedures as well as study two different reaction mechanisms. Other synthesis methods of amides could also be discussed in order to give a broad introduction to this subject.[1]

The characterization of the compounds through the usual spectroscopic methods (especially ^{1}H NMR and IR) is extremely interesting and could be used in the comparative analysis of the spectra of the desired product (amide) and the by-product obtained (ester) *via* the mixed anhydride method.

Several experimental techniques will be used such as heating at reflux, liquid-liquid extraction, recrystallization, evaporation of solvent with a rotary evaporator, thin layer chromatography (TLC), gravity and vacuum filtration, column chromatography on silica gel and melting point.[5]

The students should interpret the ^{1}H NMR and IR spectroscopic data of all the synthesized products in order to identify the obtained compounds as well to check their purity.[6]

The use of the synthetic methodology described in this experiment will allow the preparation of several thienyl-4-oxo-butanamides.[7]

This experiment was previously developed in the research group of the author and was performed later by students of the 4th year of the undergraduate Chemistry degree course at the University of Minho as well as by Erasmus students from the undergraduate Chemistry degree course at the University of Metz, France. This experiment could be performed by undergraduate Chemistry who have previously acquired some skills with the experimental techniques involved used as well some knowledge regarding the theoretical concepts presented (synthesis, reactivity and spectroscopic data interpretation).

Session 1: Synthesis of 4-oxo-4-(thiophen-2-yl)butanoic acid

In session 1 the methyl 4-oxo-4-(thiophen-2-yl)butanoate (synthesised in experiment 131) is used as precursor for the synthesis of 4-oxo-4-(thiophen-2-yl)butanoic acid. However, this compound is also commercially available in Acrös [4-oxo-4-(2-thienyl)butanoic acid, 97%, code: 124500250].

4-Oxo-4-(2-thienyl)butanoic acid: beige solid (78%). Mp: 119-120.5 ^{o}C[7] (lit.[8] 119-120 ^{o}C). ^{1}H NMR ($CDCl_3$) δ 2.83 (2H, t, J = 6.7 Hz, CH_3), 3,28 (2H, t, J = 6.7 Hz, CH_2), 7.13-7.18 (1H, m, 4´-H), 7.66 (1H, dd, J = 5.0 and 1.0 Hz, 5´-H), 7,78 (1H, dd, J = 4.0 and 1.0 Hz, 3´-H), 10.8 (1H, large s, OH). IR (Nujol): ν 3250-2682 (OH), 1694 (C=O), 1644 (C=O) cm^{-1}.

The range of yields obtained earlier by students of the 4th year of the degree course in Chemistry of University of Minho as well as by Erasmus students was 70-85%.

Session 2: Synthesis of 1-(piperidin-1-yl)-4-(thiophen-2-yl)butane-1,4-dione

In session 2 (mixed anhydride method): the ethyl chloroformate used must be previously distilled or obtained from a freshly opened bottle, in order to avoid the presence of carboxylic acid which would lower the yields obtainable. The DMF used must be previously dried[5] or purchased as an anhydrous grade reagent. In order to

dry the solvent it should be putted on calcium hydride overnight, filtered and distilled from molecular sieves 3 Å.

The synthesis of the amide using the mixed anhydride method gives two products: the desired amide and the ethyl 4-oxo-4-(thiophen-2-yl)butanoate as a by-product. When the reaction mixture is submitted to column chromatography with a silica gel the first eluted compound is the γ-keto ester, obtained as a pale yellow oil, while the second eluted compound is the amide which emerges as a beige oil.

Ethyl 4-oxo-4-(thiophen-2-yl)butanoate:[7,9] beige oil (43%). ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, *J* = 6.7 Hz, OCH_2CH_3), 2.76 (2H, t, *J* = 6.7 Hz, CH_2), 3.26 (2H, t, *J* = 6.7 Hz, CH_2), 4.18 (2H, q, *J* = 6.7 Hz, OCH_2 CH_3, 7.12-7.16 (1H, m, 4´-H), 7.65 (1H, dd, *J* = 5.1 and 1.2 Hz, 5´-H), 7.78 (1H, dd, *J* = 4.0 and 1.2 Hz, 3´-H). IR (liquid film): 1730 (C=O), 1666 (C=O), cm^{-1}.

1-(Piperidin-1-yl)-4-(thiophen-2-yl)butane-1,4-dione:[7] beige oil (50%). ^{1}H NMR ($CDCl_3$) δ 1.64 (6H, m, 3xCH_2) 2.78 (2H, large t, *J* = 6.4 Hz, CH_2), 3.30 (2H, large t, *J* = 6.4 Hz, CH_2), 3,47 (2H, t, *J* = 5.9 Hz, NCH_2), 3.56 (2H, t, *J* = 5.9 Hz, NCH_2), 7.12-7.16 (1H, m, 4´-H), 7,63 (1H, dd, *J* = 5.3 and 1.2 Hz, 5´-H), 7.82 (1H, dd, *J* = 4.1 and 1.2 Hz, 3´-H). IR (liquid film): ν 1638 (C=O) cm^{-1}.

The range of yields obtained earlier by students of the 4th year of the degree course in Chemistry of University of Minho as well as by Erasmus students was 39-43% for ethyl 4-oxo-4-(thiophen-2-yl)butanoate and 45-50% for 1-(piperidin-1-yl)-4-(thiophen-2-yl)butane-1,4-dione.

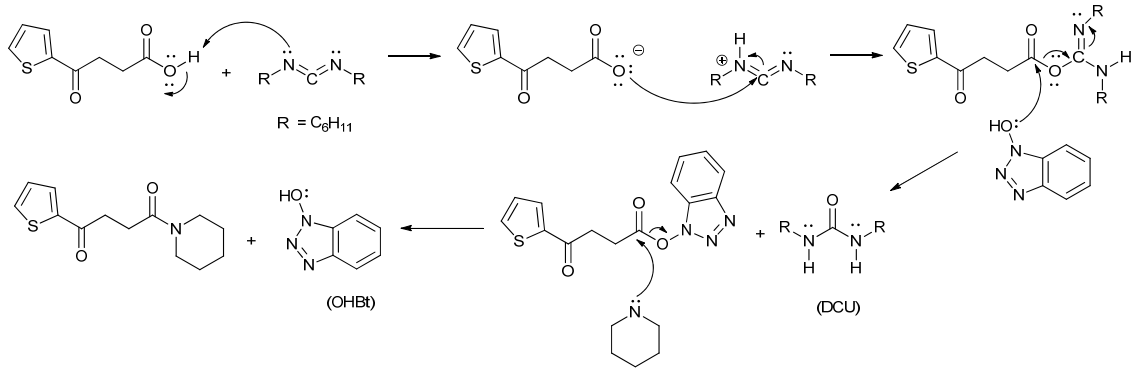
The synthesis of amide through the direct amidation method using DCC/OHBt coupling agents (session 4) gives a less complex reaction mixture compared to the mixed anhydride method. The reaction mixture consists of the title compound and some residual dicyclohexylurea (DCU). The presence of DCU in the mixture is confirmed by ^{1}H NMR and the purification of the amide will be carried out by inducing the precipitation of DCU. This is accomplished by dissolving the reaction mixture in acetone using a water bath, followed by cooling of the solution at 0-4 ^{o}C (refrigerator).

The synthesis of the amide through direct amidation of the acid with DCC and HOBt coupling agents proves to be more a efficient method due to several factors such as mild reaction conditions, better yield (84%), less complex reaction mixture (without the formation of by-products) and easy purification.

The range of yields obtained earlier by students of the 4th year of the degree course in Chemistry of University of Minho as well as by Erasmus students was 77-84%.

2. Mechanism of direct amidation in presence of coupling reagents

The mechanism of amidation in the presence of DCC is well known.The reaction is initiated by protonation of the DCC, yielding the carboxylate. Nucleophilic attack of the carboxylate anion, at the protonated carbon of the DCC yields the *o*-acylurea derivative. Subsequent attack of the OHBt by the *o*-acylurea carbonyl gives the activated ester of the carboxylic acid. This rapidly acylates the amine yielding the corresponding amide and dicyclohexylurea (DCU) with recovery of the catalyst (OHBt) (Scheme SM 3.1.16.1.).[1,10]



Scheme SM 3.1.16.1. Mechanism of direct amidation of 4-oxo-4-(thiophen-2-yl)butanoic acid with piperidine in the presence of DCC/HOBt coupling agents.

References

[1] C. A. G. N. Montalbetti and V. Falque, *Tetrahedron* 2005, **61**, 10852.

[2] F. Albericio, *Curr. Opin. Chem. Biol.*, 2004, **8**, 211–221.

[3] (a) M. Bodansky, “Peptide Chemistry: A Pratical Textbook”; Springer: New York, 1988. (b) *Handbook of Reagents for Organic Synthesis: Activating Agents and Protecting Groups*”; A. J. Pearson, W. R. Roush Eds., John Wiley and Sons: Chichester, 2000, p. 133.

[4] (a) M. Bodansky, *J. Protein Chem.*, 1989, **8**, 461. (b) *Handbook of Reagents for Organic Synthesis: Activating Agents and Protecting Groups*"; A. J. Pearson, W. R. Roush Eds., John Wiley and Sons: Chichester, 2000, p. 220.

[5] W.-L.F. Armarego and D. D. Perrin, "Purification of laboratory chemicals", 4th Ed.; Butterworth Heinemann, Oxford, 2000.

[6] (a) R. M. Silverstein, F. X. Webster and D. J. Kiemle, "Spectrometric Identification of Organic Compounds", 7th Edition, John Wiley and Sons: New York, 2005. (b) E. Pretsch, P. Buhlmann and C. Affolter, "Structure Determination of Organic Compounds" 3rd Edition, Springer-Verlag: Berlin, 2000.

[7] M. M. M. Raposo and G. Kirsch, *Heterocycles*, 2001, **55**, 1487.

[8] L. Fieser and R. G. Kennelly, *J. Am. Chem. Soc.,* 1935, **57**, 1616.

[9] H. Stetter, M. Schreckenberg and K. Wiemann, *Chem. Ber.,* 1976, **109**, 541.

[10] J. G. Moffatt and V. Falque *J. Org. Chem.*, 1971, **36**, 1909.

3. Figures

3.1 Photos for the apparatus used in laboratory session 1.

(A)

(B)

(C)

(D)

Figure SM 3.1.16.1 (A) - Photo of the apparatus for heating at reflux; (B) Photo of the apparatus for evaporation of the solvent with a rotary evaporator; (C) Photo of the apparatus for the liquid-liquid extraction; (C) Photo of the apparatus for filtration with vacuum. (Laboratory session 1).

3.2 Photo of the TLC plate with the reagent and product for laboratory session 4.

Figure SM 3.1.16.2. Photo of the TLC plate with the reagent and product (eluent: dichloromethane:MeOH; 9.9:0.1), laboratory session 4. A- acid; B – amide

3.3 ^{1}H NMR and IR spectra of the products

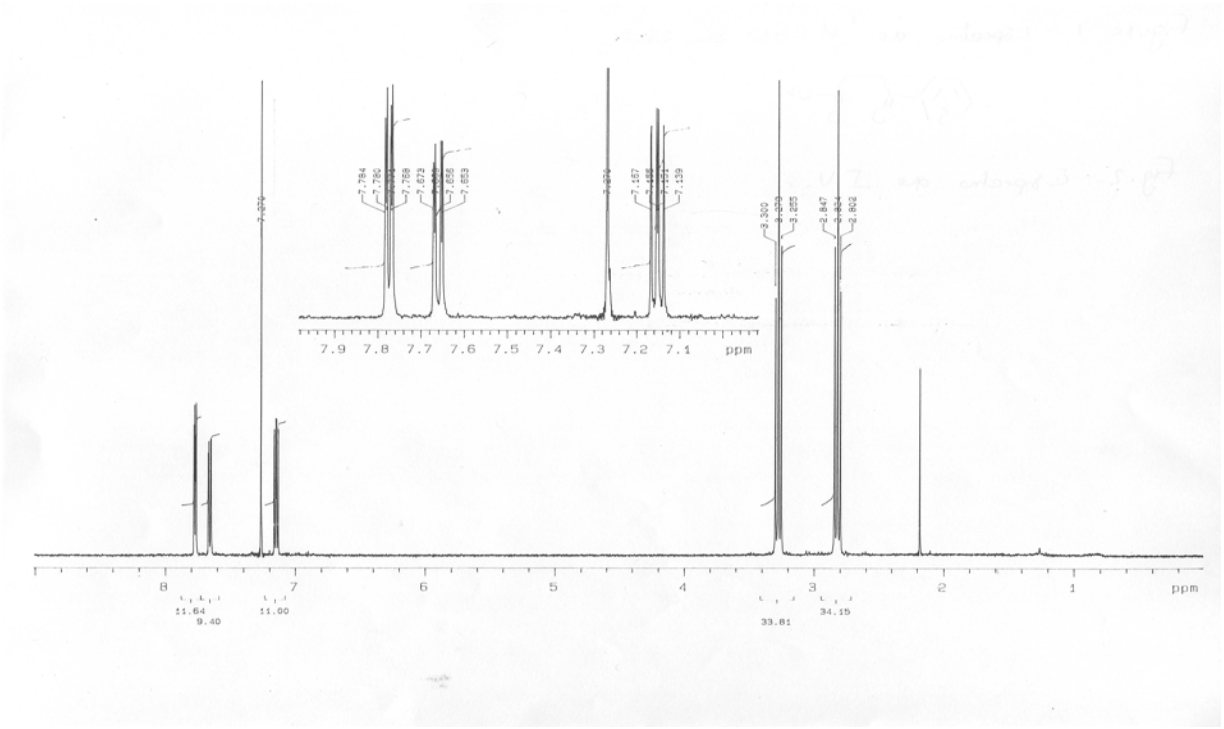


Figure SM 3.1.16.3. ^{1}H NMR Spectrum of 4-oxo-4-(2-thienyl)butanoic acid in $CDCl_3$ obtained using a Varian Unity Plus spectrometer operating at 300 MHz and 25 ^{o}C.

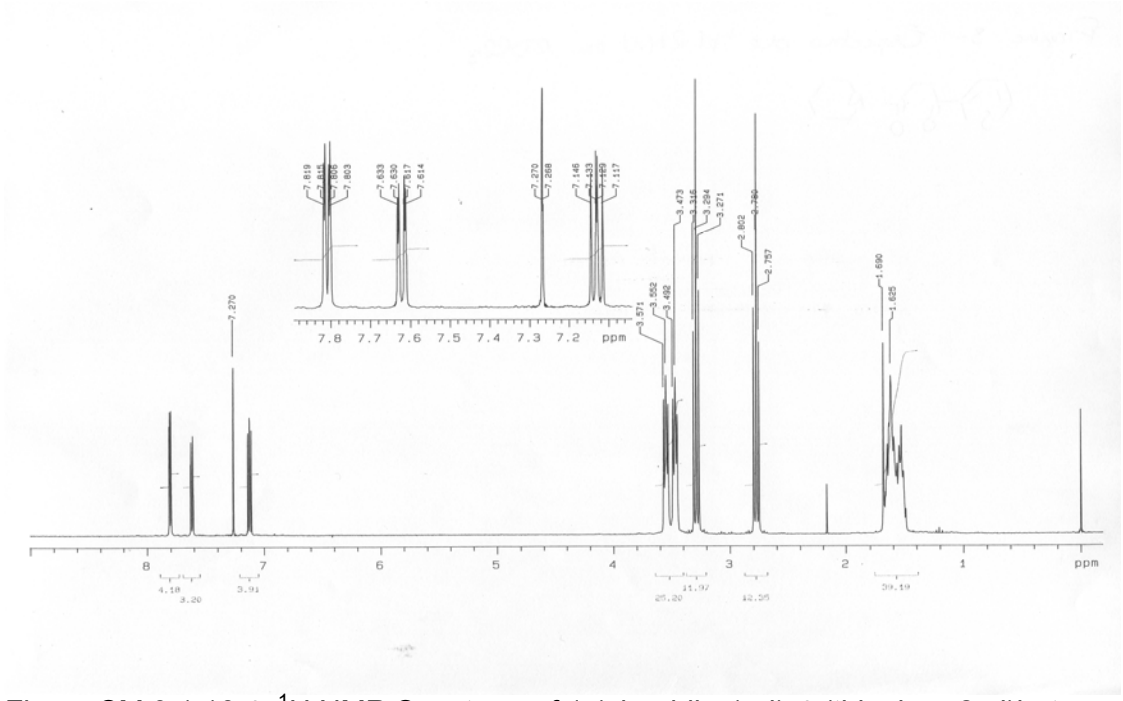


Figure SM 3.1.16.4. ^{1}H NMR Spectrum of 1-(piperidin-1-yl)-4-(thiophen-2-yl)butane-1,4-dione in $CDCl_3$ obtained using a Varian Unity Plus spectrometer operating at 300 MHz and 25 ^{o}C.

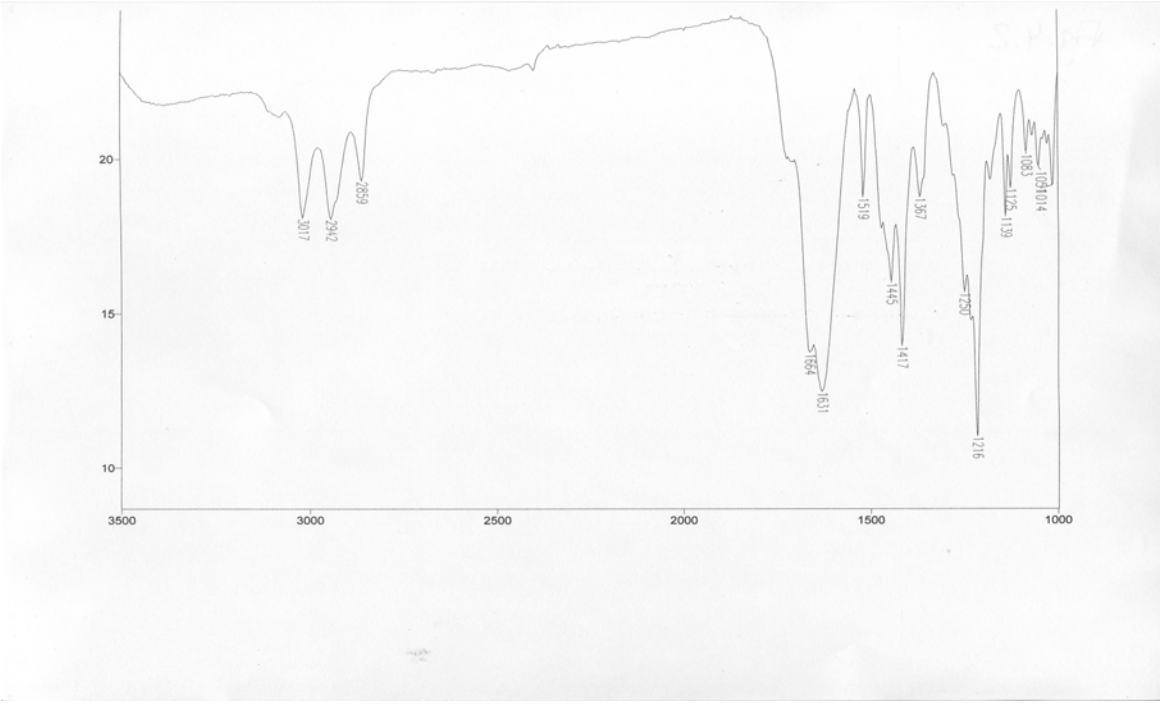


Figure SM 3.1.16.5. IR spectrum of 1-(piperidin-1-yl)-4-(thiophen-2-yl)butane-1,4-dione in liquid film obtained using a Perkin Elmer FTIR-1600 spectrophotometer.

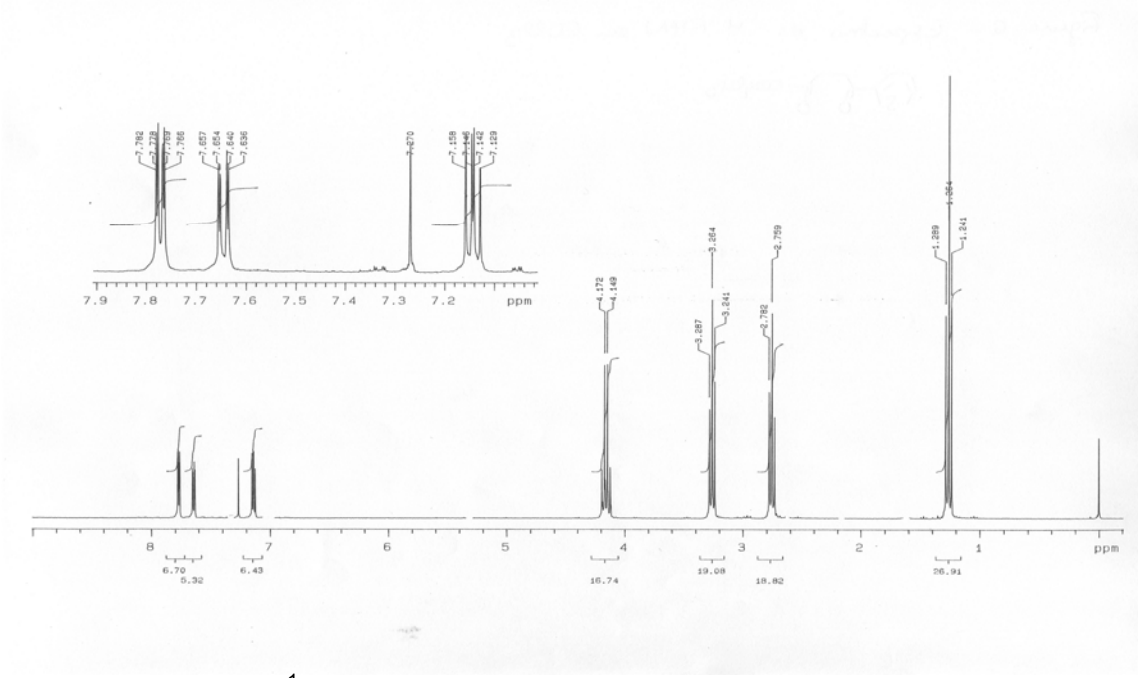


Figure SM 3.1.16.6. ^{1}H NMR Spectrum of ethyl 4-oxo-4-(thiophen-2-yl)butanoate in $CDCl_3$ obtained using a Varian Unity Plus spectrometer operating at 300 MHz and 25 ^{o}C.

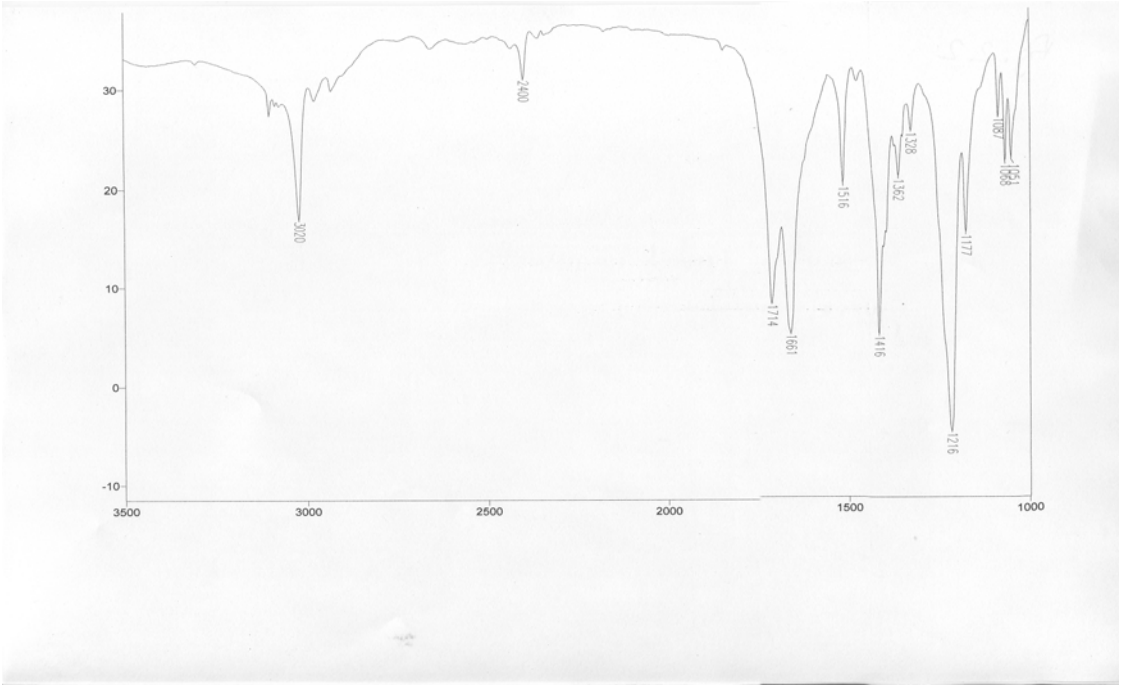


Figure SM 3.1.16.7. IR spectrum of ethyl 4-oxo-4-(thiophen-2-yl)butanoate liquid film obtained using a Perkin Elmer FTIR-1600 spectrophotometer.

Cyclic acetals for regioselective protection in carbohydrate synthesis: A comparative experiment

Supplementary Material

The goal of this set of experiments is to demonstrate the usefulness of cyclic acetal protecting groups to accomplish regioselective manipulation of sugar hydroxy groups, hereby illustrated by acetylation reactions. The full protocol was reproduced by 1st year Chemistry MSc students from the Faculty of Sciences, University of Lisbon.

It is highly recommended that instructors perform the experiments in advance and save some amount of each intermediate compound. This way, students can continue the work even if any step should inadvertently fail. Moreover, it is suggested that final weighting of solid products is performed in the beginning of the following session, along with melting point measurements, to ensure maximum purity.

Experiment Notes

Preparation of methyl 4,6-O-benzylidene-α-D-glucopyranoside

Clean white crystals (**Figure SM 3.1.17.1**, left picture) are obtained in yields up to 69%. The reaction does not reach total completion in 2 hours (or longer) at 80 ºC. Nevertheless, the remaining starting material is not significant and is easily removed during the liquid-liquid extraction due to its high water solubility.

In the experimental procedure, acetonitrile is used as solvent in alternative to the method performed in *N,N*-dimethylformamide at 60 ºC, as suggested by Demchenko *et al*.[1] Solvent evaporation with a rotary evaporator is facilitated when using acetonitrile, which has a boiling point of 81-82 ºC, lower than that of *N,N*-dimethylformamide (153 ºC). Yet, reaction with the latter solvent was more efficient, with yields up to 86%. The melting point of the product determined immediately after recrystallization was 158-160 ºC, lower than the tabled range of 164-167 ºC.[2] Hence, the obtained crystals should be kept in a vacuum desiccator containing also shredded paraffin wax to absorb the remaining solvent until the following session, prior to the final yield calculation and melting point determination. Illustrative NMR spectra are given in **Figures SM 3.1.17.3** and **SM 3.1.17.4**.

Preparation of methyl 2,3-di-O-acetyl-4,6-O-benzylidene-α-D-glucopyranoside

This experimental procedure afforded a white solid (**Figure SM 3.1.17.1**, right picture) in yields usually above 95% after 30 min. reaction time, and high purity. It is important to note that the compound is first obtained as a colourless oil, which slowly crystallizes when sufficiently pure. An efficient aqueous work-up is crucial to remove the pyridine, which reacts with hydrochloric acid to afford the water-soluble pyridinium chloride. The neutralization process should be repeated until the odour of pyridine can no longer be detected. Alternatively, pyridine can be co-evaporated with toluene (twice the volume of pyridine) in a rotary evaporator.

The determined melting point for this compound was 94-96 ºC and no reference value was found in the literature. NMR spectra (as should be obtained by the students) are given in **Figures SM 5** and **SM 6**.

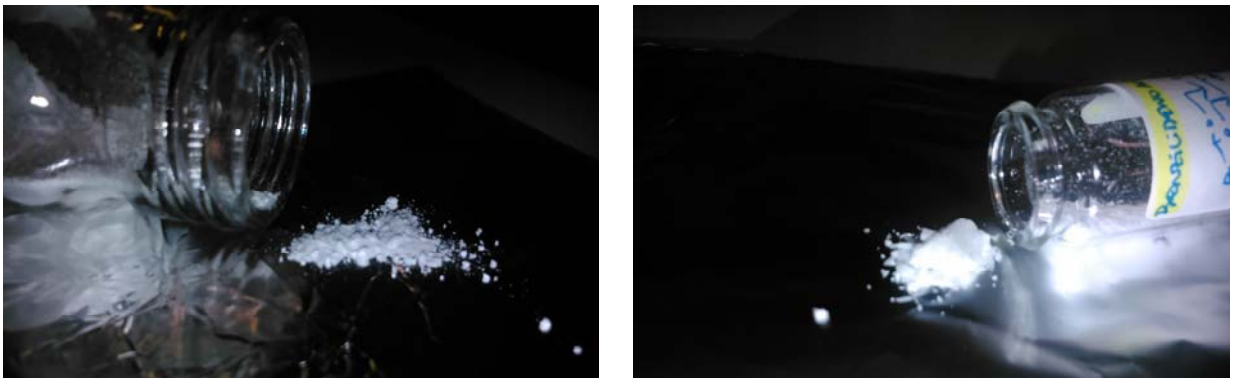


Figure SM 3.1.17.1 Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (white solid, left) and methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (white solid, right).

Preparation of methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside

The product was obtained as a colourless oil in yields usually above 95%. Efficient neutralization is very important in this procedure. Otherwise, trace amounts of solvent may be detected in the ^{1}H NMR spectrum (**Figure SM 3.1.17.7**). Since the experimental protocol is identical to that of the acetylation described in part A of this session, students may be advised to carry out both reactions in parallel. Illustrative NMR spectra are given in **Figures SM 3.1.17.7** and **SM 3.1.17.8**.

Preparation of methyl 2,3-di-O-acetyl-α-D-glucopyranoside

This reaction reaches completion after 2 hours at 50 ºC, as described by Marcelo *et al*.[3] and the final product is obtained as a colourless oil (although it is commercially available as a solid[4]) in yields higher than 95%. Although aqueous work-up featuring extensive extractions with hot ethyl acetate can afford the product in an average 73% yield, purification by silica gel column chromatography is much more efficient (**Figure SM 3.1.17.2, A-C**). Since both the benzaldehyde and reaction end-product have very distinct retention factors when eluted with the solvent mixture petroleum ether/ethyl acetate (1:1) (**Figure SM 3.1.17.2, D**), their separation by column chromatography is straightforward and

accomplished within 1 hour, when eluting the end-product with a much more polar eluent (ethyl acetate/methanol 10:1) after complete removal of the benzaldehyde. It should be noted that the given eluent volumes might vary depending on the specific equipment available in each laboratory, namely column dimension and pressure used. Therefore, it is strongly suggested that those conditions are tested prior to the laboratory sessions. NMR spectra (as should be obtained by the students) are given in **Figures SM 3.1.17.9** and **SM 3.1.17.10**.

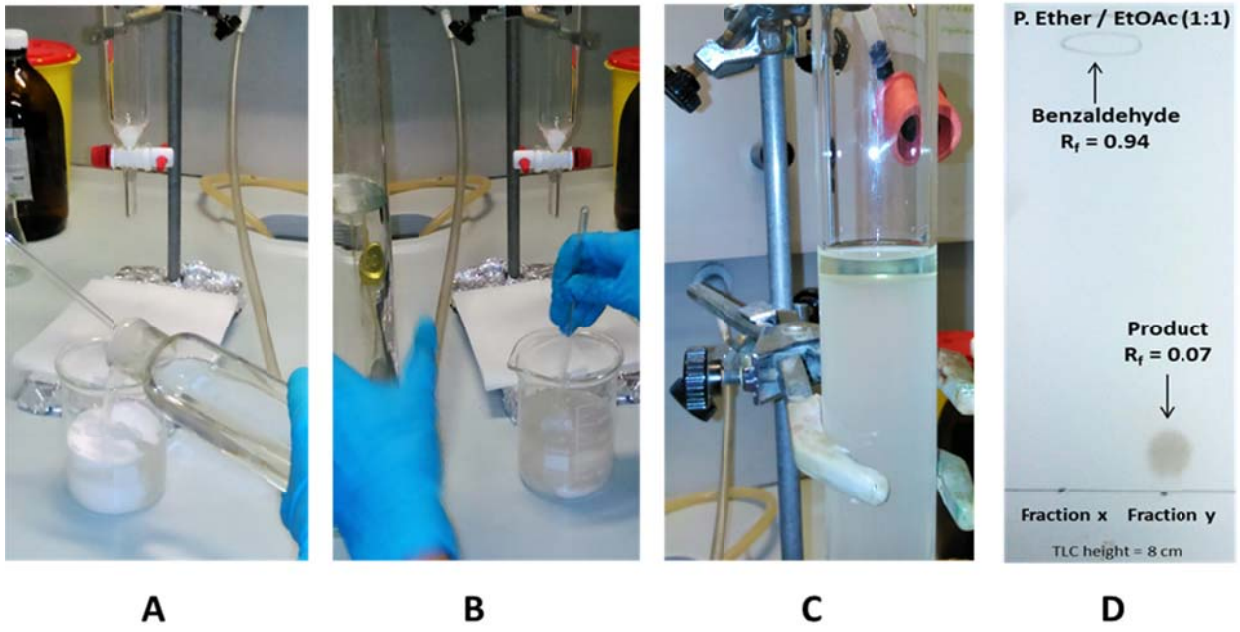


Figure SM 3.1.17.2 Illustration of column chromatography: slurry preparation – **A** and **B**; column chromatography with sample prior to elution – **C**; demonstrative TLC of the achieved separation – **D**.

Results Interpretation and Additional Questions

The proposed discussion was conceived for a group of students with intermediate background on carbohydrate chemistry and NMR analysis. Regarding the 4th question, in particular, it is suggested that chemical shifts of H-2, H-3, H-4 and H-6 are given to the students. The acquisition of ^{13}C and bidimensional (COSY, HMQC and HMBC) NMR spectra of the final products for complete signal attribution and multiplicity analysis are also encouraged.

NMR Spectra

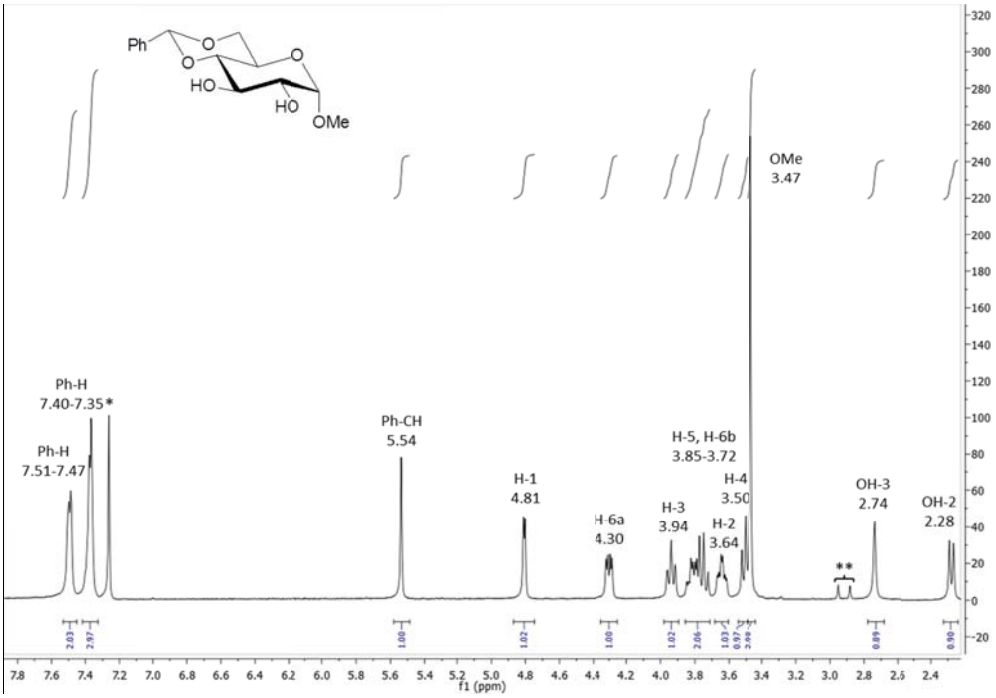


Figure SM 3.1.17.3 ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside. Solvent residual peaks: * chloroform; ** *N,N*-dimethylformamide.

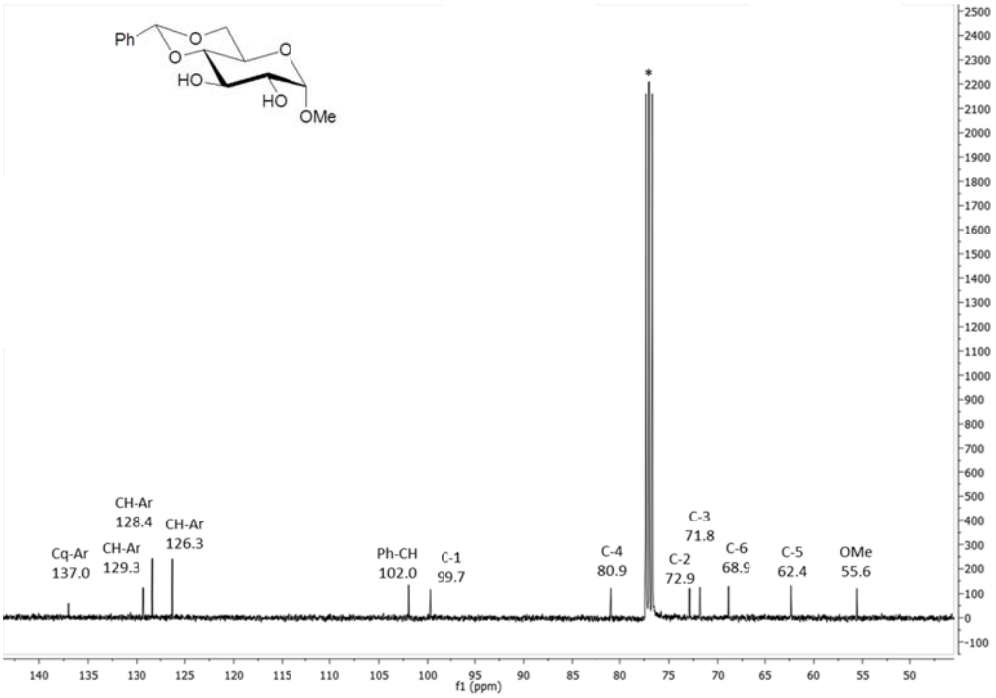


Figure SM 3.1.17.4 ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside. Solvent residual peaks: * chloroform.

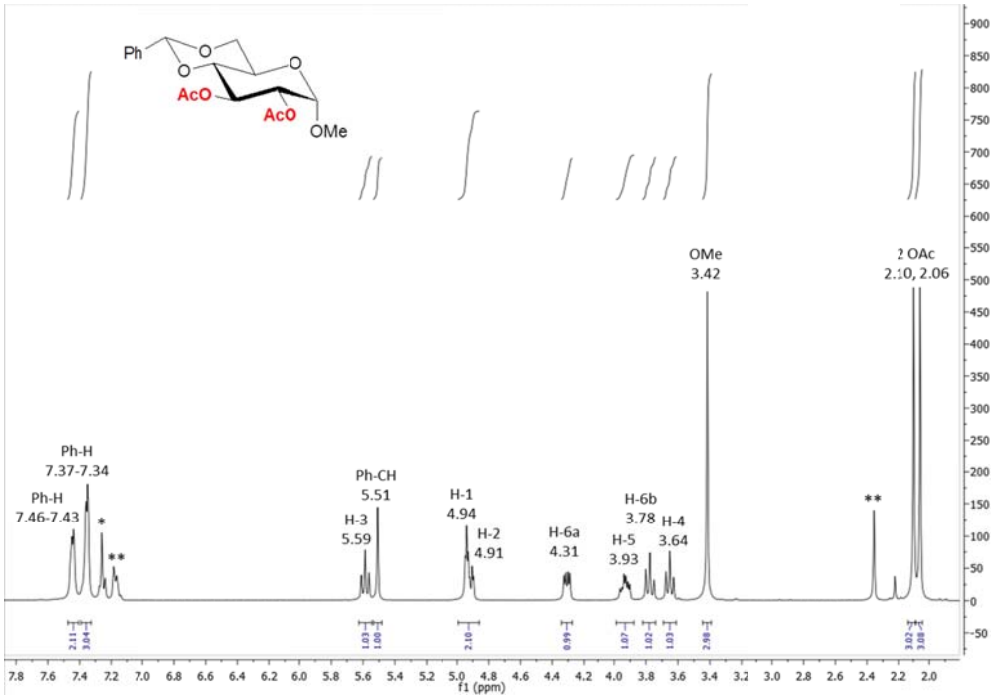


Figure SM 3.1.17.5 ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside. Solvent residual peaks: * chloroform; ** toluene.

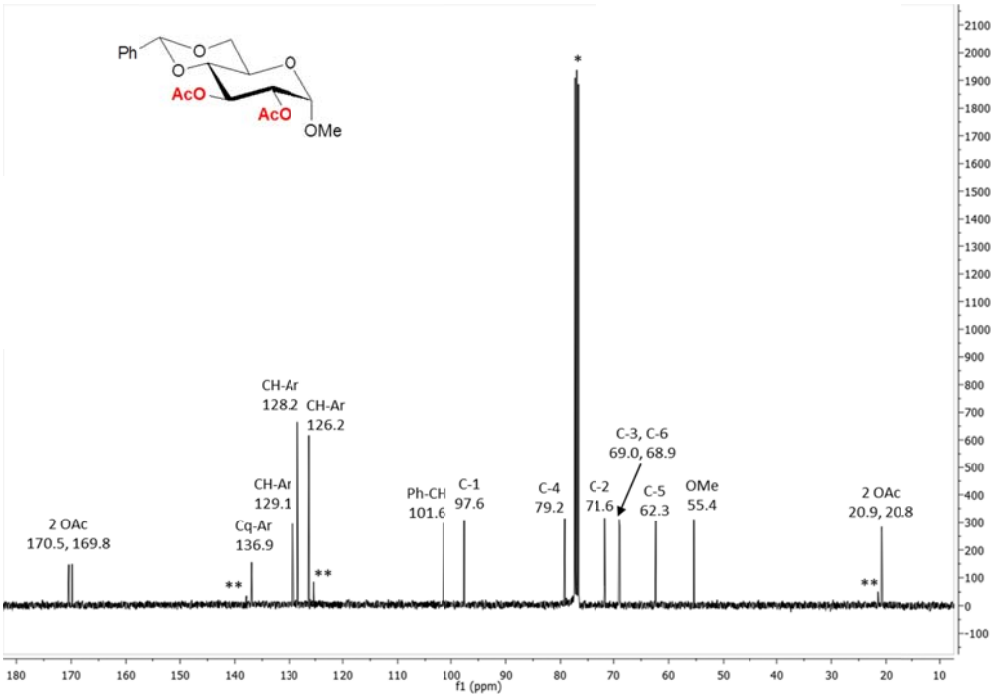


Figure SM 3.1.17.6 ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside. Solvent residual peaks: * chloroform; ** toluene.

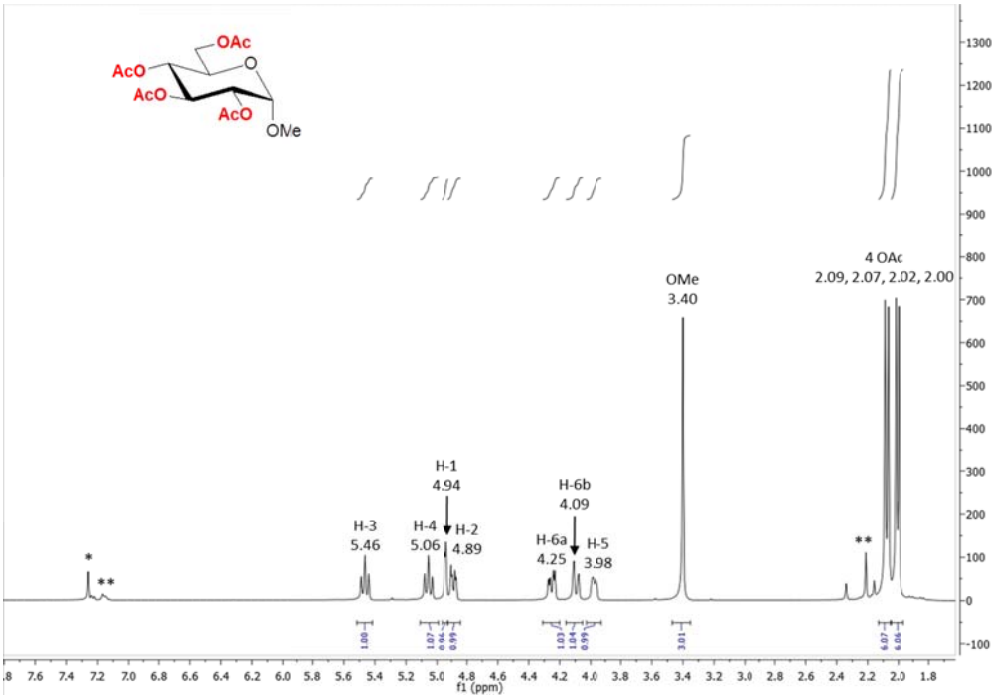


Figure SM 3.1.17.7 ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside. Solvent residual peaks: * chloroform; ** toluene.

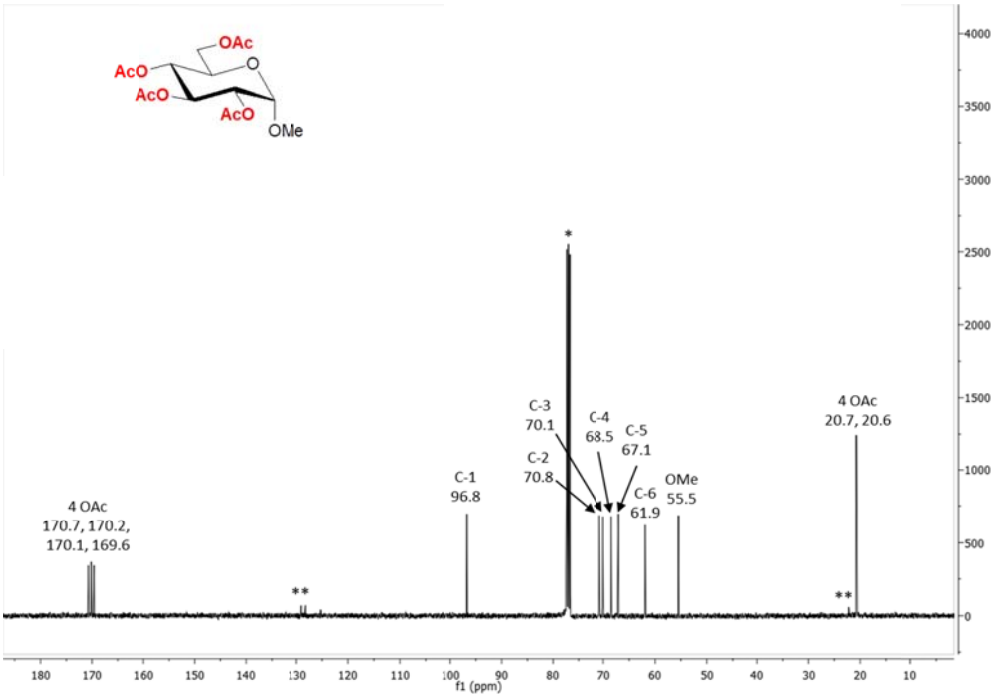


Figure SM 3.1.17.8 ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside. Solvent residual peaks: * chloroform; ** toluene.

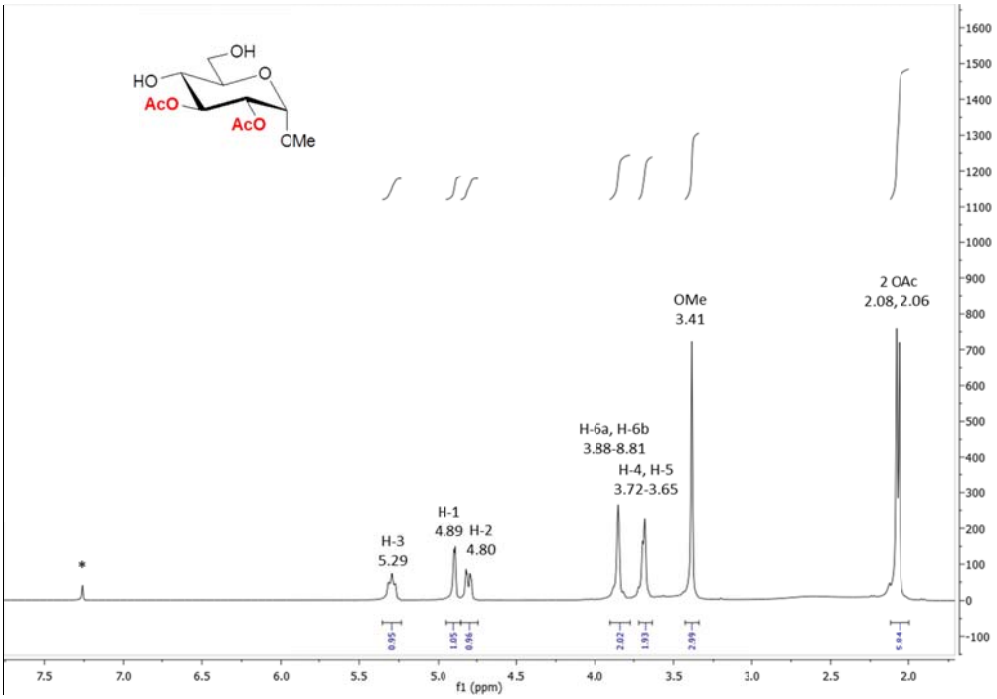


Figure SM 3.1.17.9 ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of methyl 2,3-di-*O*-acetyl-α-D-glucopyranoside. Solvent residual peaks: * chloroform.

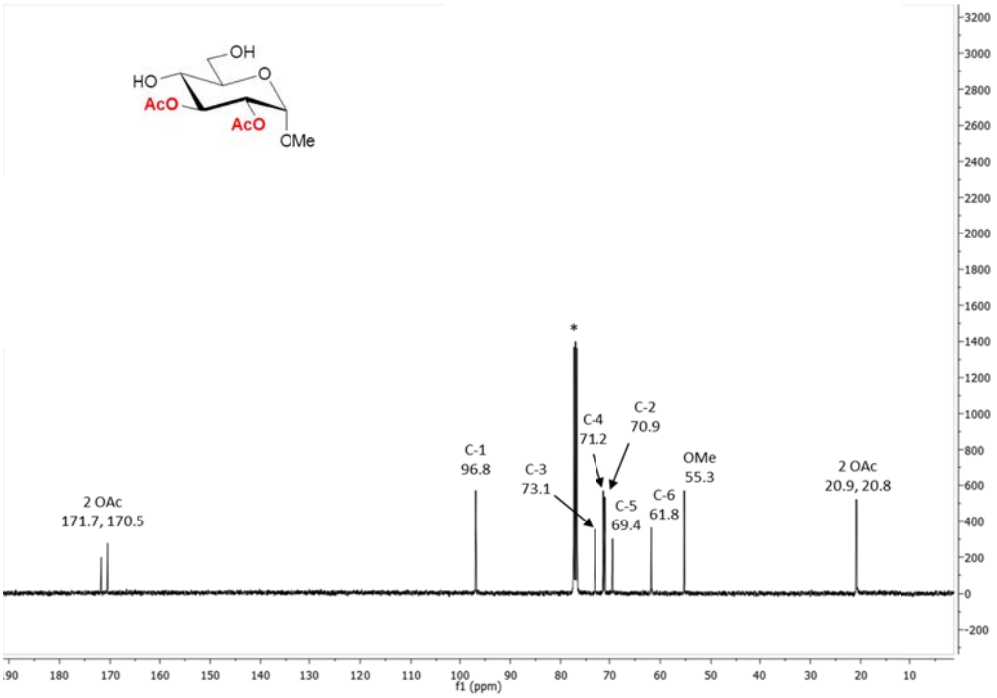


Figure SM 3.1.17.10 ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of methyl 2,3-di-*O*-acetyl-α-D-glucopyranoside. Solvent residual peaks: * chloroform.

[1] A. V. Demchenko, P. Pornsuriyasak, C. De Meo. *J. Chem. Educ*. 2006, **83**, 782-784.

[2] http://www.sigmaaldrich.com/catalog/product/fluka/65895

[3] F. Marcelo, F. V. M. Silva, M. Goulart, J. Justino, P. Sinäy, Y. Blériot, A. P. Rauter, *Bioorg. Med. Chem*. 2009, **17**, 5103-5116.

[4] http://www.sigmaaldrich.com/catalog/product/aldrich/747963?lang=pt&region=PT

Direct diastereoselective synthesis of the tetrahydro-thiazolo[2,3-*b*]isoindole tricyclic ring system

Supplementary Material

Additional notes for the preparation of compound 3: During the reaction precipitation of the product in the reaction medium may occur. In this case dichloromethane must be used in order to ensure complete dissolution of the precipitate. After evaporation of the solvent recrystallization of the product should be carried out with hot diethyl ether, however the process can be time consuming, so it is advisable to simply do trituration of the solid residue with cold diethyl ether. The purity of the product can be assessed by thin layer chromatography (TLC) using silica gel 60 F_{254} plates and a mixture of ethyl acetate/*n*-hexane (1:1) as the solvent system (Figure SM X.1).

The synthetic procedure carried out by two 3rd year B.Sc. students allowed the preparation of compound **3** in yields ranging from 62% to 71%.

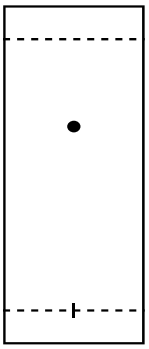


Figure SM 3.1.18.1: TLC of the isolated product in EtOAc/*n*-hexane (1:1), R_f = 0.68.

Additional notes for the measurement optical activity of compound 3: Chiral substances have the property of rotating the plane of plane-polarized light as it passes through them. For this reason, these molecules are referred as optically active. The measurement of the angle of rotation of plane-polarized light (α, optical rotation) can be carried out in a polarimeter (Figure SM 3.1.18.2). The polarimeter is equipped with a single-wavelength light source with a plane-polarizing filter, a sample holder (to put the cell containing the solution of the sample that will be analyzed) and a detector that gives the value of the optical rotation as a digital read-out (for automatic polarimeters). Rotation of the plane-polarized plane to the right leads to a positive value for optical rotation, while the rotation to the left gives a negative one.

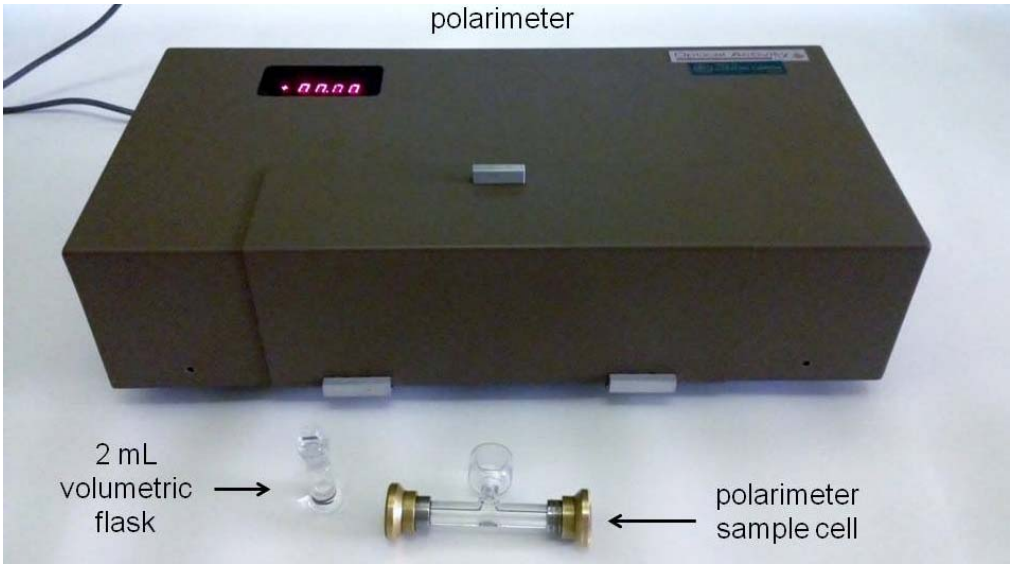
The optical rotation of a sample depends on the following factors: the length of the sample cell, the concentration of the solution, the solvent, the temperature and the light wavelength (normally the measurements were made using the 589.3 nm, the "D line" of a sodium lamp). The specific rotation of [α] is independent of these factors and is quoted as follows, $[\alpha]_{\mathrm{D}}^{t}$, where D refers to the "D line" of sodium and *t* the temperature. The specific rotation of a sample can be calculated by the following formula:

$$[\alpha]_{\mathrm{D}}^{t} = \frac{\alpha}{c.l} \times 100$$

where α is the observed angle, *c* is the concentration of the solution (g/100 mL) and *l* is the length of the polarimeter sample cell (dm).

For the measurement of the optical activity an automatic polarimeter was used. Before carrying out the measurement the polarimeter must be calibrated to zero with the solvent-filled cell in place. It is mandatory to use the same cell for the calibration and the measurement of the optical activity of the sample. When filling the sample cell it is important to avoid any air bubbles.

For research work purposes a solution of 46 mg in 2 mL of dichloromethane (*c* 2.3) can be used, however for the classroom experiment it is advisable to use a 10 mL volumetric flask (*ca*. 230 mg in 10 ml of dichloromethane). The solution must be perfectly clear and free of suspended particles otherwise the dispersion of the polarimeter beam will prevent an accurate reading.



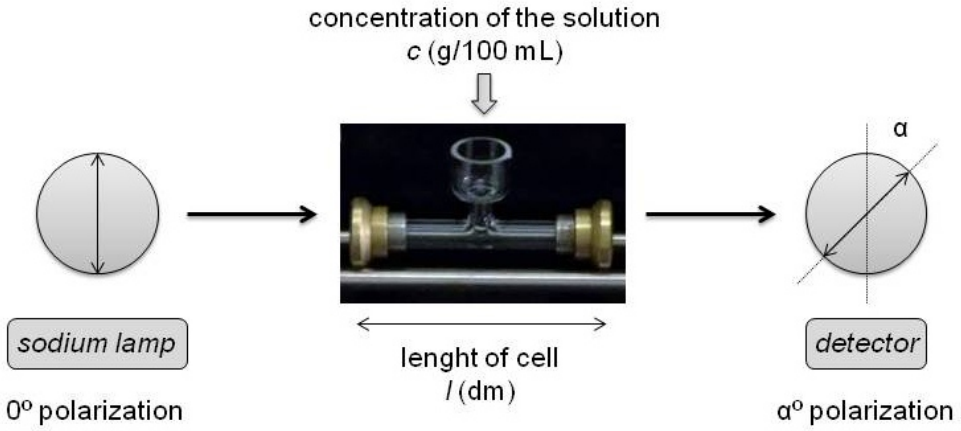
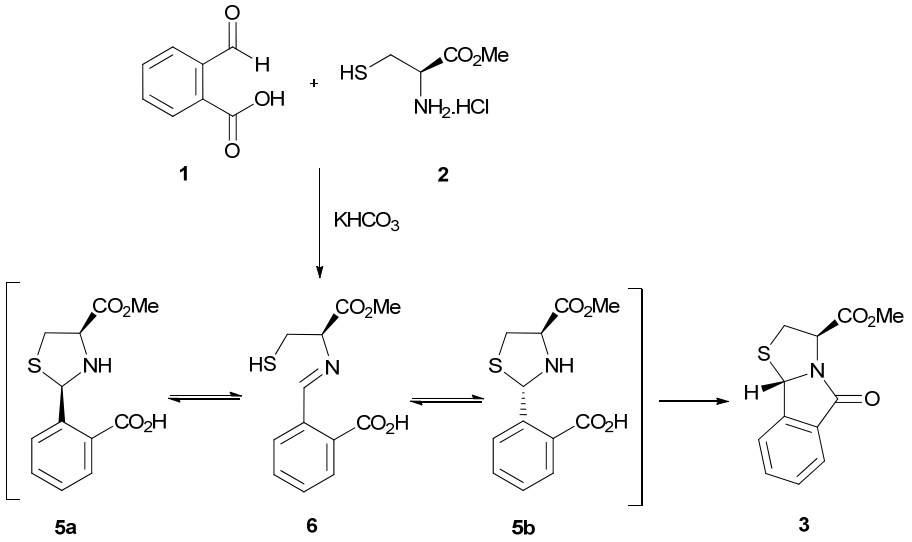


Figure SM 3.1.18.2: Measurement of optical activity using a polarimeter.

Reaction mechanism: The diastereoselective synthesis of the tricyclic product **3** involves the initial condensation of the aldehyde **1** and cysteine methyl ester **2** to give a mixture of (2*S*,4*R*)- and (2*R*,4*R*)-thiazolidines **5**. Thiazolidines **5** generated *in situ* can be interconverted via imine **6**. Under the studied reaction conditions, diastereoselective cyclization occur leading to the corresponding lactam as single enantiomer. This experiment illustrates a straightforward strategy to obtain chiral compounds by using a chiral amino ester as starting material.



Scheme SM 3.1.18.1 – Synthesis of thiazolo[2,3-*b*]isoindole **3**.

Characterization Data of Methyl (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylate (3).

Compound **3** was obtained as a white solid in 71% yield (1.03 g). mp 87-88 ºC; IR (KBr) ν_{max} = 1745, 1710 cm $^{-1}$; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.56-3.65 (2H, m, H2), 3.78 (3H, s, H11), 5.19-5.22 (1H, m, H3), 6.04 (1H, s, H9b), 7.44-7.48 (2H, m, ArH), 7.54-7.57 (1H, m, ArH), 7.76-7.78 (1H, m, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 39.7 (C2), 52.9 (C11), 57.6 (C3), 66.3 (C9b), 123.4, 124.7, 129.5, 130.5, 133.1, 144.8, 170.3 (C5), 170.5 (C11); MS *m/z*: 249 (M^+, 100%), 190 (93), 162 (44), 132 (45); $[\alpha]_D^{25}$ = -400.5 (*c* 2.3, dichloromethane).

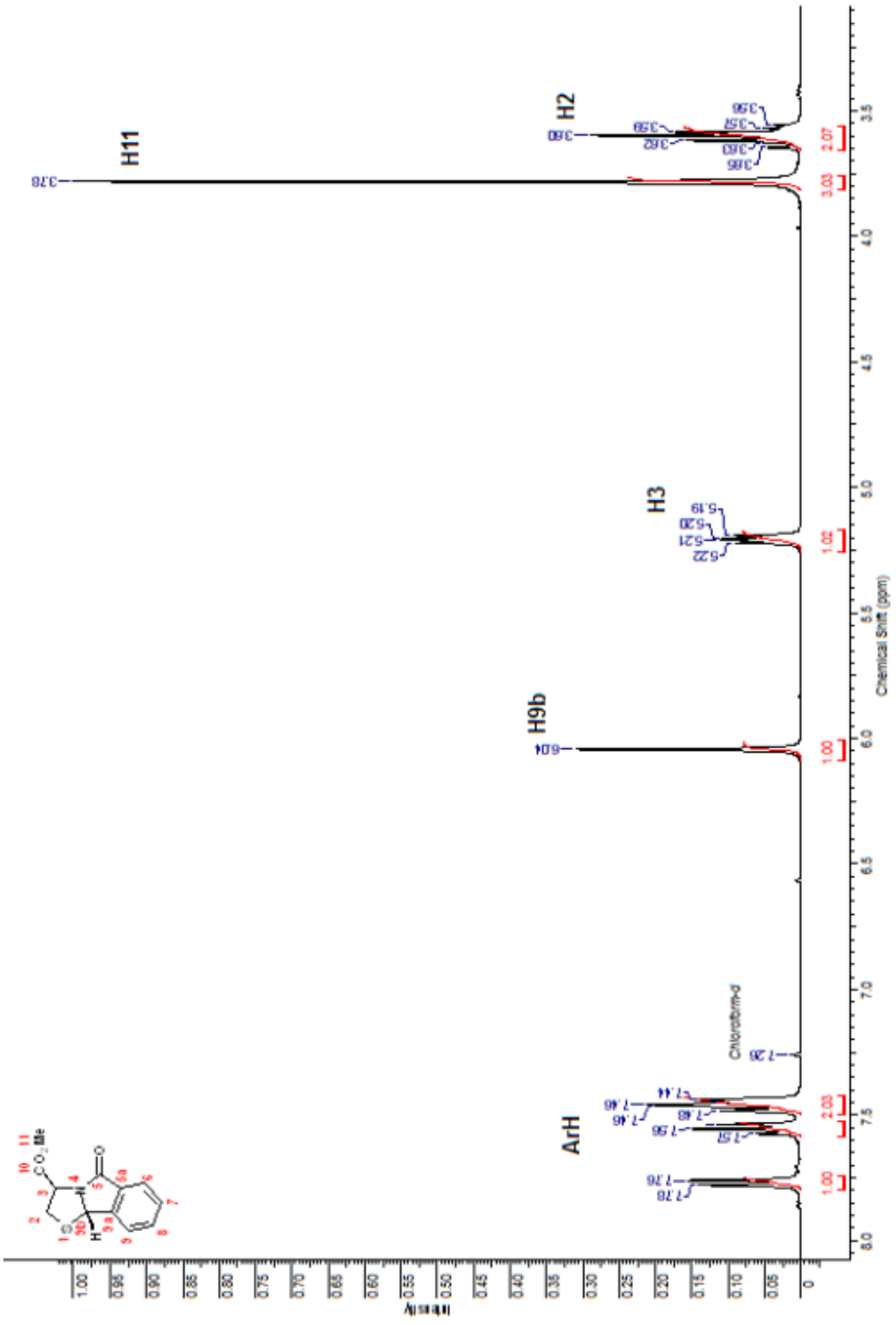


Figure SM 3.1.18.3: ^{1}H NMR spectrum of compound **3** ($CDCl_3$, 400 MHz).

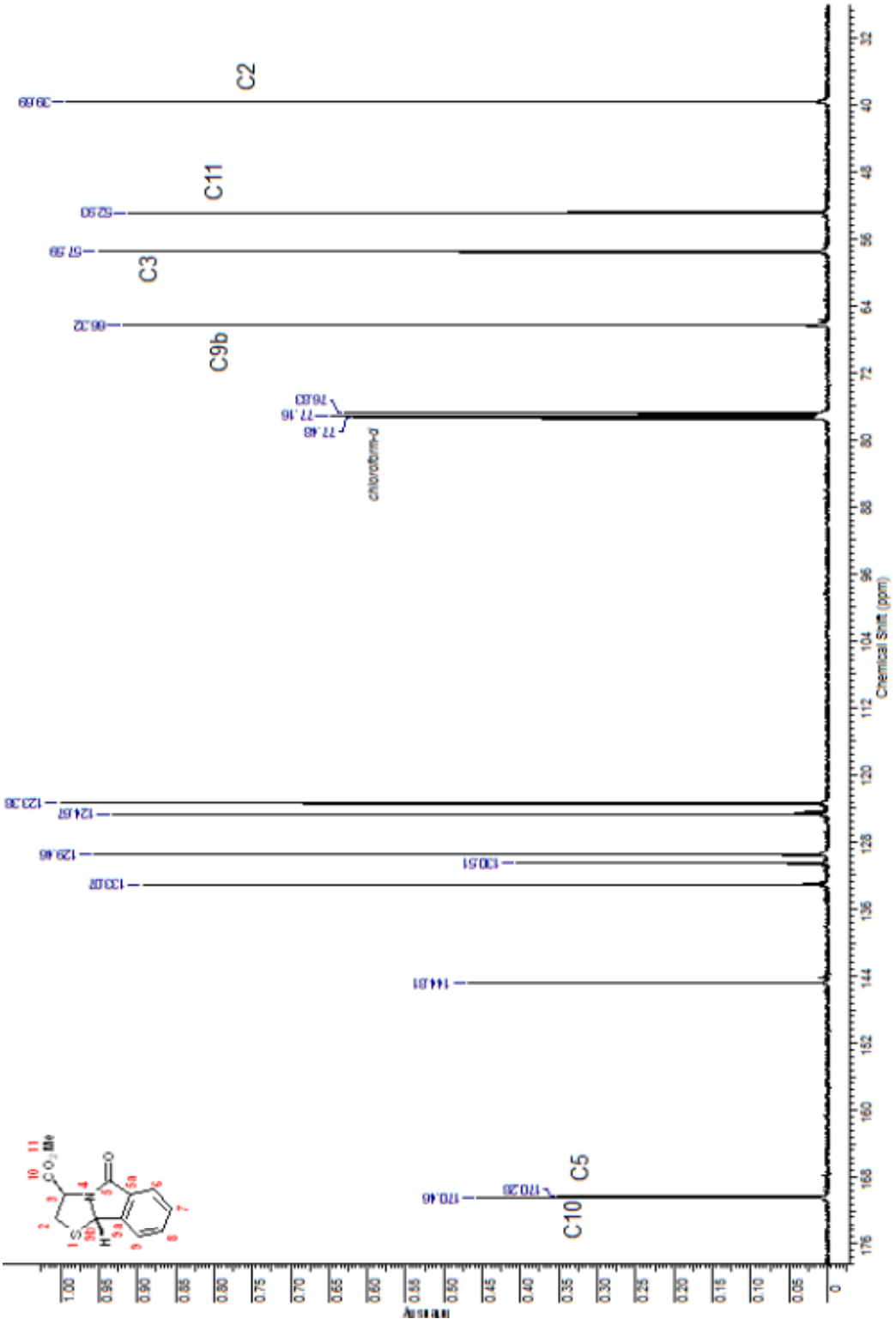


Figure SM 3.1.18.4: ^{13}C NMR spectrum of compound **3** ($CDCl_3$, 100 MHz).

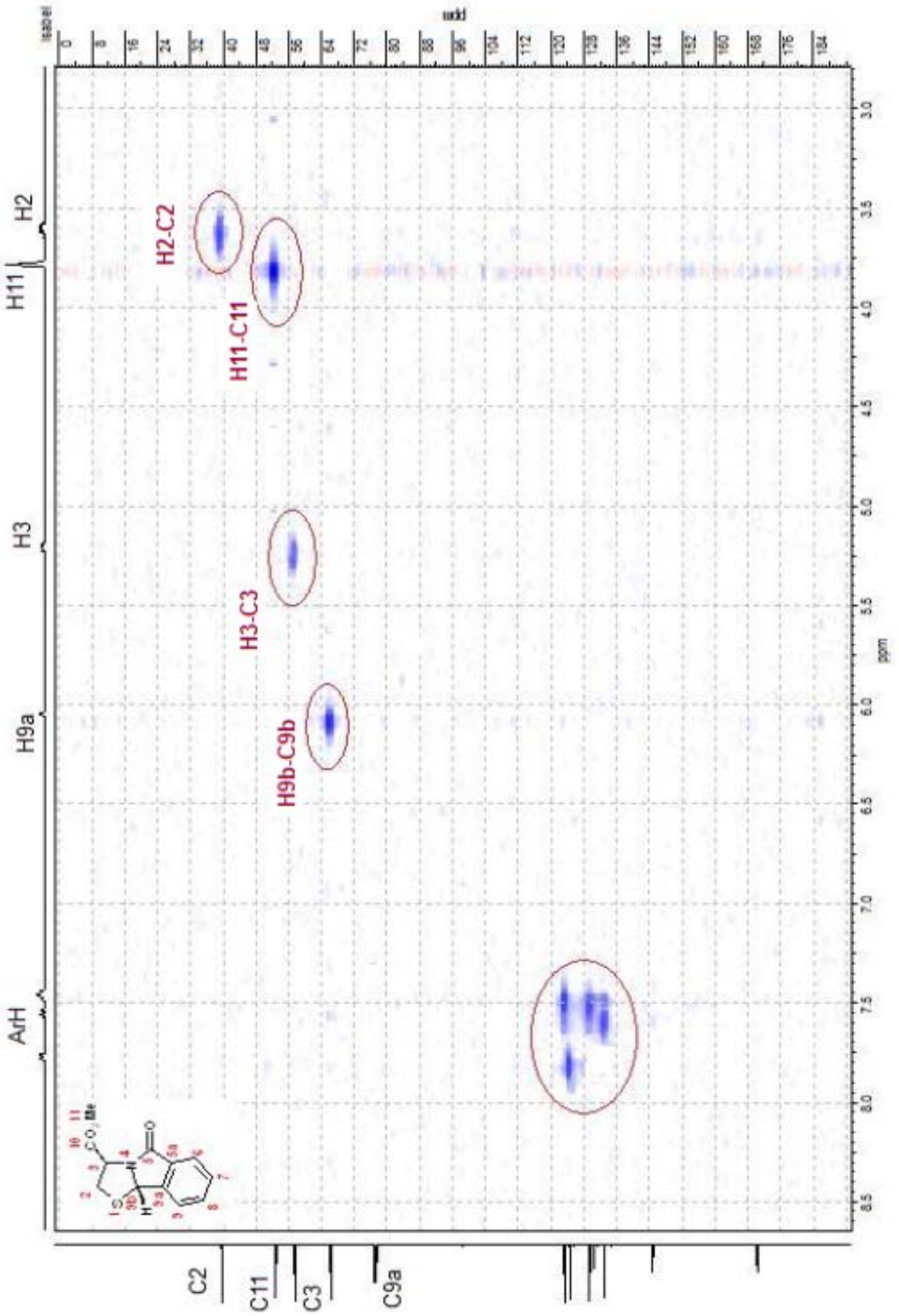


Figure SM 3.1.18.5: HMQC spectrum of compound **3** ($CDCl_3$, 400 MHz).

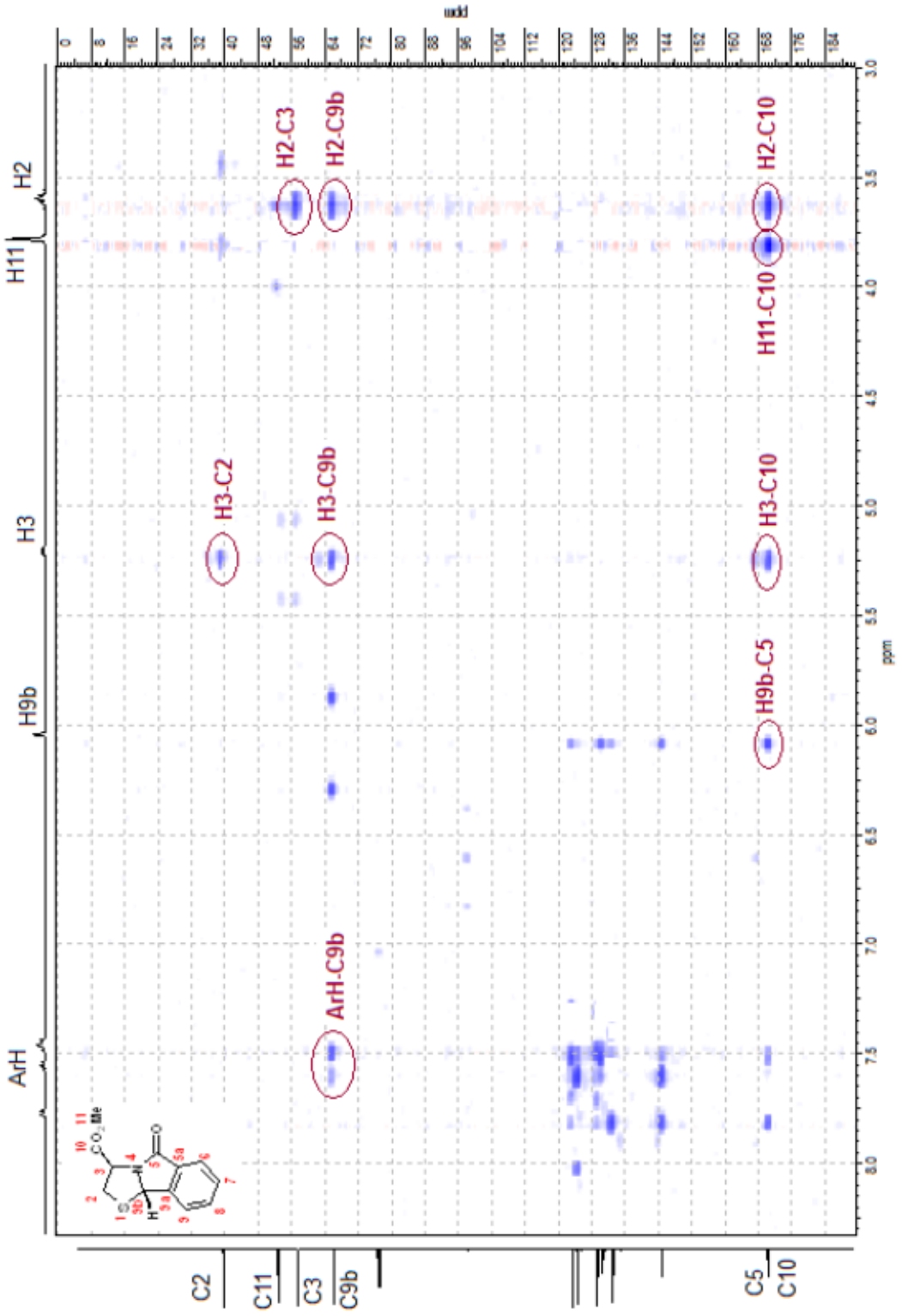


Figure SM 3.1.18.6: HMBC spectrum of compound **3** ($CDCl_3$, 400 MHz).

Asymmetric Cyclocondensation Reaction Induced by Chiral Aminoalcohol

Supplementary Material

This experiment aims at the preparation of (3*S*,9b*R*)-3-benzyl-5-oxo-9b-phenyl-2,3,5,9b-tetrahydrooxazolo[2,3-*a*]isoindole by cyclocondensation reaction of (*S*)-phenylalaninol and 2-benzoylbenzoic acid using a Dean-Stark apparatus. The reproducibility of the experiment was assessed by its repetitive execution, namely by 2nd year Medicinal Chemistry M.Sc. students from Faculty of Pharmacy (Lisbon University, Portugal) and by 4th year undergraduate students from Faculty of Pharmacy (Barcelona University, Spain). Although the reaction does not reach completion in 4h at reflux (one session), the reaction time can be extended to 16-24h (overnight) and yields higher than 80 %, before crystallization, can be achieved.

The student body is students of advanced organic chemistry, in which the concepts of chiral auxiliary/inductor and asymmetric synthesis were taught. The students should be skilled enough in order to perform the reaction at a micro-scale as here planned. This experiment will allow the students to rationalize the reaction mechanism via the formation of an *N*-acyliminium ion, and the release of water.

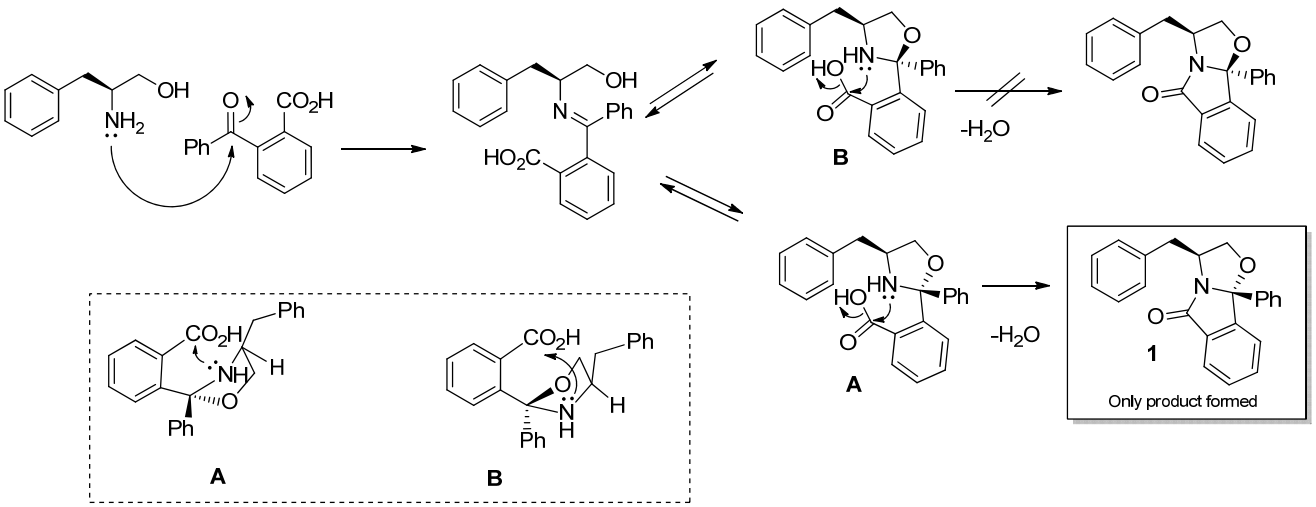
General Information/ Troubleshooting:

- The Dean-Stark apparatus is a piece of laboratory glassware usually used in azeotropic distillations to separate water from a mixture with less dense solvents than it. A common example is the removal of water generated during a reaction in boiling toluene. The apparatus typically consists of a vertical cylindrical piece of glass (which fits with the bottom of the reflux condenser) and a side-arm at the top of the cylinder that connects with the reaction flask. In this experiment, an azeotropic mixture of toluene and water distills out of the reaction, but only the toluene (density = 0.865 g/ml) returns, since it floats on top of the water (density = 0.998 g/ml), which collects in the trap. The Dean-Stark side arm should be covered with cotton and tinfoil to ensure the azeotropic mixture distills out in the condenser. When filling the Dean-Stark cylinder with toluene make sure to fill it until 1-2 drops of solvent falls into the reaction flask.
- The mixture of (*S*)-(-)-2-amino-3-phenyl-1-propanol and toluene is a white suspension which becomes a clear solution after the addition of 2-benzoylbenzoic acid (≈5 min).

- Before heating the reaction mixture be sure that all components of the apparatus are properly connected and that there are no leaks especially between the reaction flask and the Dean-Stark.
- At the end of the reaction, in order to save time, it is advisable that one student prepares the chromatographic column, while the other performs the extractions (in case groups of 2 students perform each experiment). For the column, 300-345 mL of solvent should be enough if the reaction is performed in a 0.1 g scale (advisable to use a column with 2 cm of diameter).
- For the recrystallization, make sure that all the solvent was removed after the column and use a very small amount of Et_2O (<1 pipette) to dissolve the oil (use a water bath at ≈40 ºC to help dissolving it). After the oil is dissolved in the ether add *n*-hexane (2-3 pipettes) and scratch the flask with a spatula. Crystal formation should be immediate. If the product was not completely dry the crystal formation should start to take place within 5-10 min. If necessary, after the flask has cooled to room temperature, place it in an ice bath to increase the yield of product.
- It is also possible to perform directly the recrystallization without previous purification by chromatographic column. In that case, the purity of the final compound will not be so high.
- Yields obtained by the students: after flash chromatography 79-86% (colorless thick oil), after recrystallization 62-78% (white crystals). Melting points obtained by the students: 90 -94ºC (in a range no wider than 3 degrees for each measure).

Mechanism of the reaction

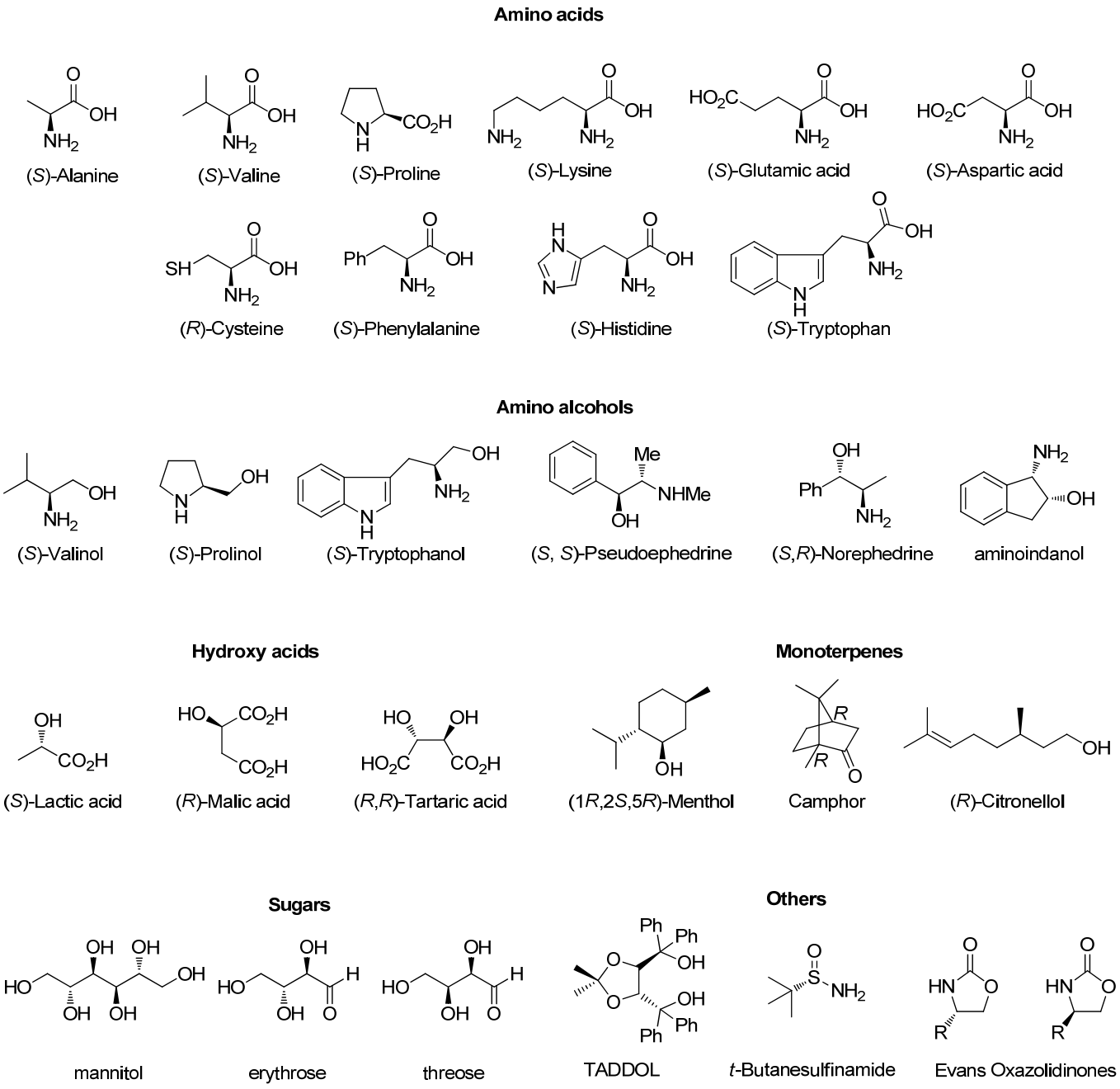
The stereochemical outcome of this reaction can be accounted for by considering that the mixture of two diastereomeric oxazolidines formed initially is in equilibrium through the corresponding imine. The subsequent irreversible lactamization occurs faster for the diastereoisomer that allows the shortest approach of the acid carbonyl group to the nitrogen atom (Scheme SM 3.1.19.1).



Scheme SM 3.1.19.1 – Proposal of reaction mechanism.

Examples of other chiral auxiliaries

There are several families of compounds that can be used as chiral auxiliaries such as amino acids, amino alcohols, hydroxy-acids, terpenes, sugars, and oxazolidinones:



Scheme SM 3.1.19.2 – Some examples of chiral auxiliaries.

Photos of the experiment

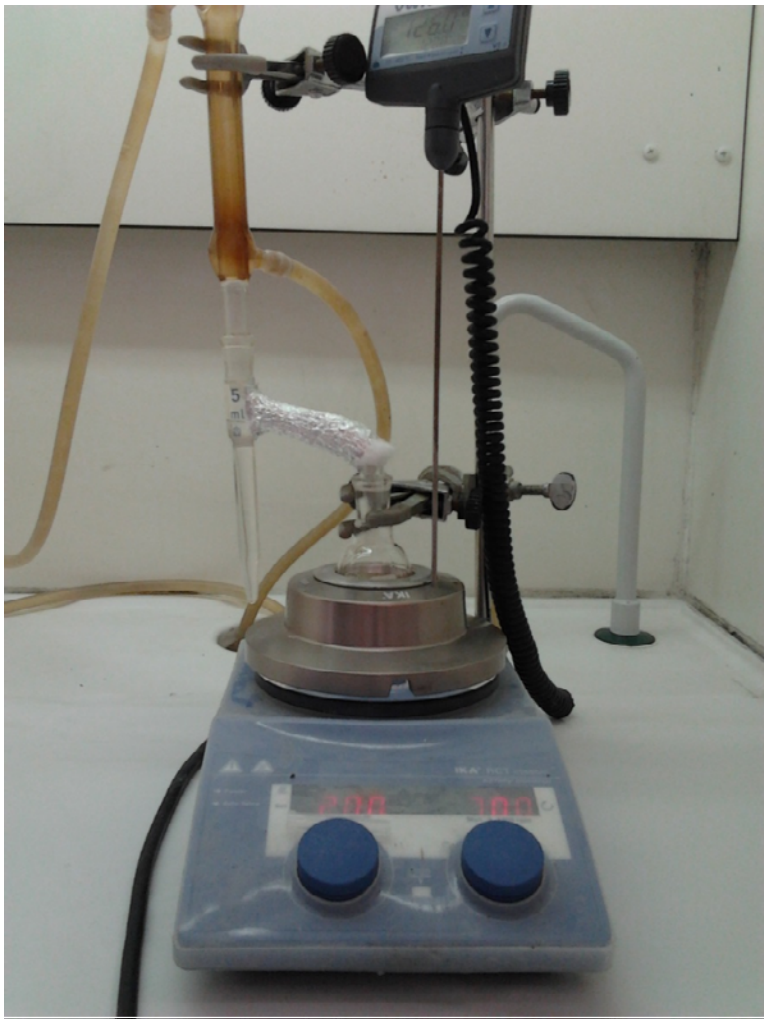


Figure SM 3.1.19.1 - Reaction apparatus.

……..

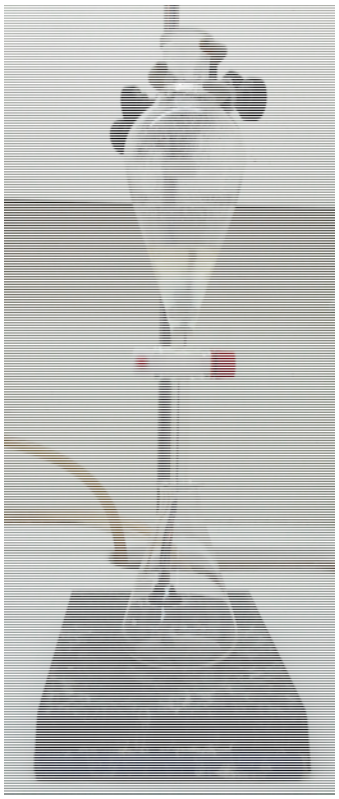


Figure SM 3.1.19.2 – Extraction step.

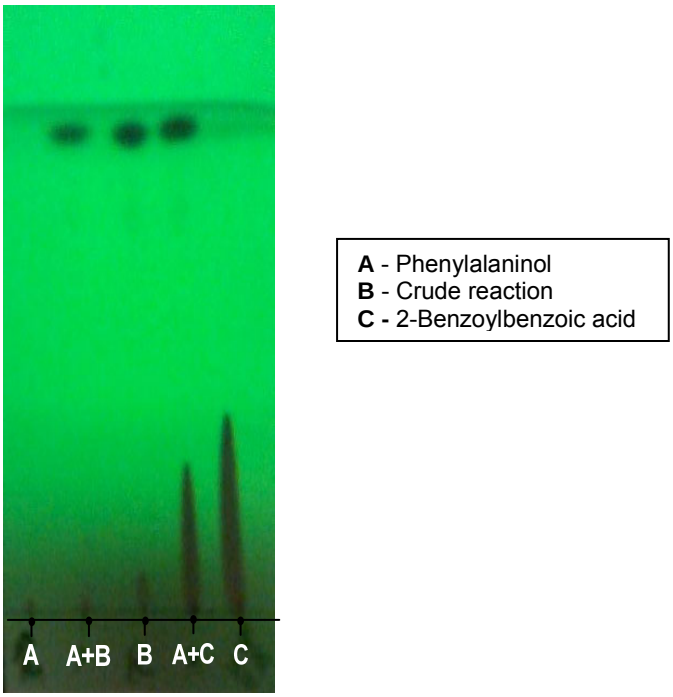


Figure SM 3.1.19.3 – TLC of the reaction (eluent: EtOAc/*n*-hexane 9:1).

Figure SM 3.1.19.4 – Product after crystallization.

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

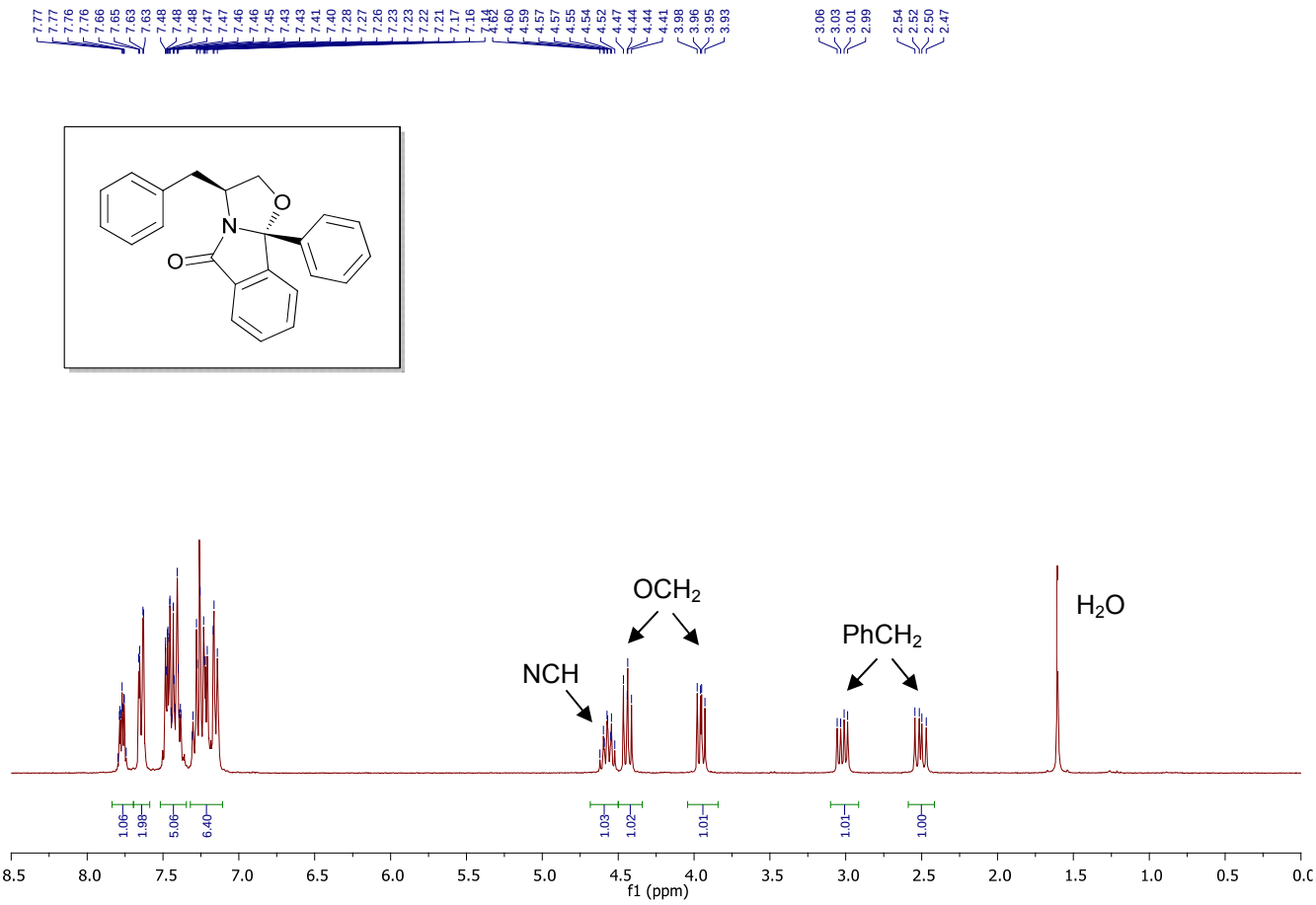


Figure SM 3.1.19.5 – ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the product.

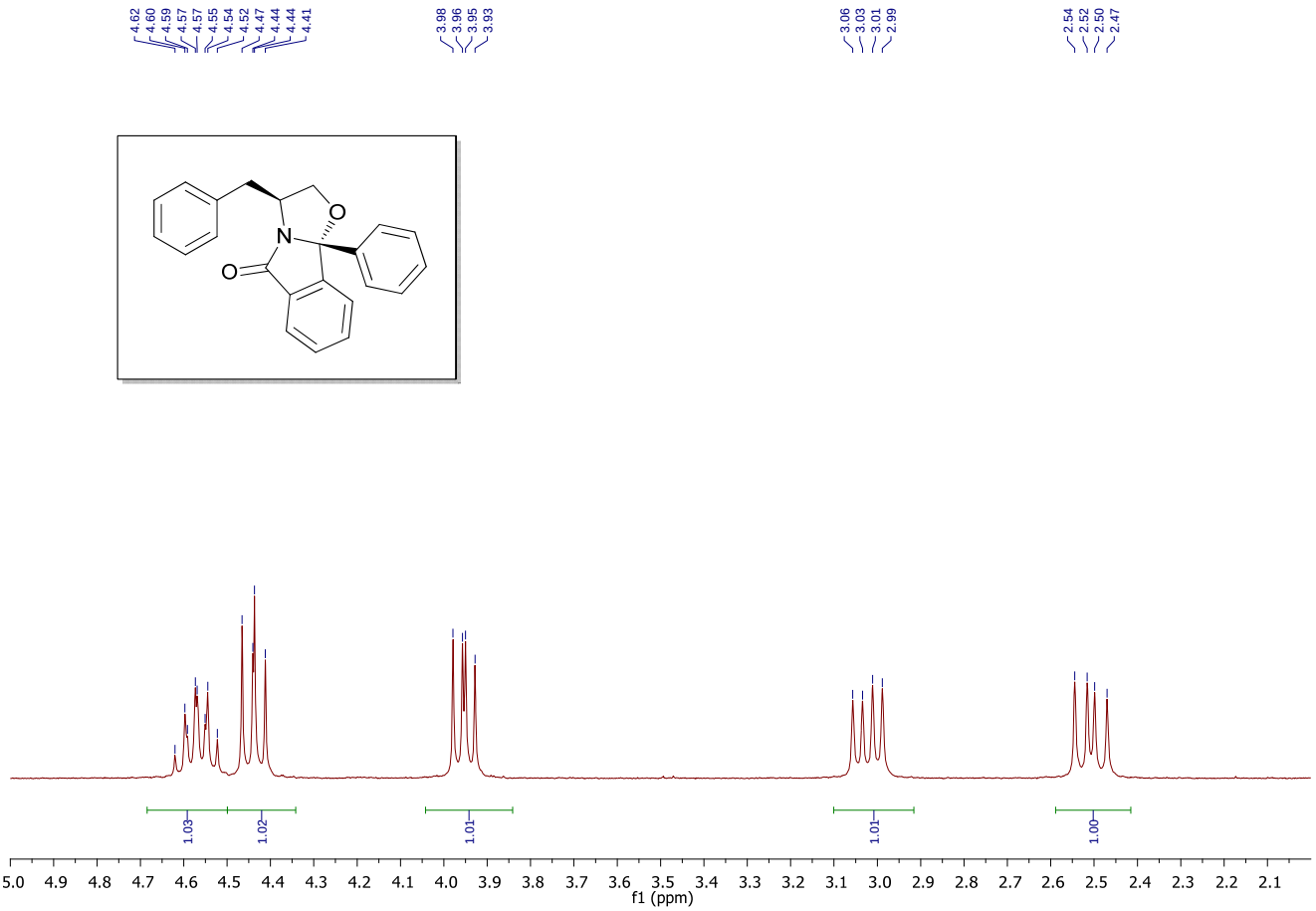


Figure SM 3.1.19.6 – ^{1}H NMR spectrum expansion.

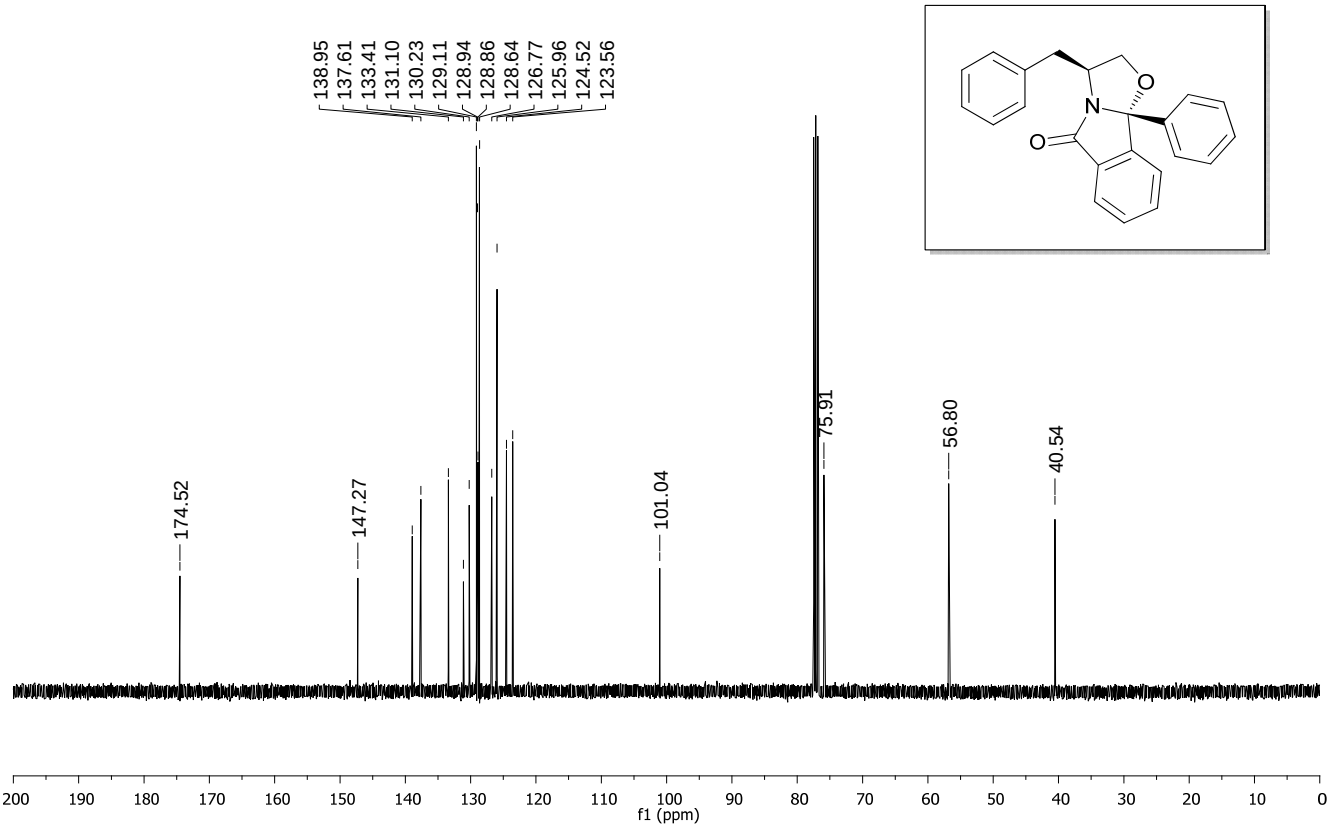


Figure SM 3.1.19.7 – ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of the product.

Synthesis and characterization of biodiesel propyl esters to determine the fatty acid content of unknown plant oils

Supplementary Material

Experimental Notes

We ran this experiment with Stanford University undergraduate students, who had already completed 2–3 quarters (~1 academic year) of organic chemistry lecture training and 1–2 quarters of organic chemistry laboratory training. To confirm reproducibility of results, 10 laboratory sections of ~12 students performed this experiment. Each student in a laboratory section used a different unknown triglyceride-based plant oil (Table SM 3.1.20.1, Figure SM 3.1.20.1). Because of the inclusion of nut-derived oils, we issued an allergy alert to students prior to the distribution of oils.

The plant oil, 1-propanol, and sulfuric acid are refluxed for 1 hour at 97 °C (Figure SM 3.1.20.2). We also investigated the use of methanol, ethanol, 2-propanol, and 1-butanol. However, methanol, ethanol, and 2-propanol have lower boiling points than 1-propanol and 1-butanol. The reactions with the lower-boiling alcohols refluxed at lower temperatures and thus, produced lower yields than the reactions with 1-propanol and 1-butanol. Although similar yields were produced with both 1-propanol and 1-butanol, we chose 1-propanol because the resulting esters were less viscous, which is desirable for biodiesel.

After the reaction has gone to completion, the less dense biodiesel propyl esters comprise the top layer of the reaction, and 1-propanol, glycerol, and sulfuric acid comprise the bottom layer (Figure SM 3.1.20.3). The product mixture is centrifuged, and the bottom layer is removed. The biodiesel is washed with 1 M sodium chloride to remove any remaining 1-propanol, glycerol, sulfuric acid, and polar byproducts (Figure SM 3.1.20.4). Sodium chloride increases the ionic

character of the water and reduces the extent of emulsion formation. The aqueous layer is removed, and the product is dried with magnesium sulfate (Figure SM 3.1.20.5). Magnesium sulfate complexes with water in the emulsion and forms white clumps at the bottom of the test tube. The biodiesel is poured through a pipette filter to remove any particulate matter (Figures SM 3.1.20.6 and SM 3.1.20.7). 0.5 g of biodiesel propyl esters are routinely isolated (Table SM 3.1.20.2).

Table SM 3.1.20.1 – Experiments were conducted with the following oils. Not all oils from a given plant produced the same results, so brands and processing methods are provided. We chose oils derived from single plants[a] that contained more than one type of fatty acid ester.

Oil	Brand	Processing[b]
almond	Spectrum	expeller pressed[c]
avocado	Spectrum	cold pressed[c]
camelina[d]	Omega Maiden	
coconut[d]	365	expeller pressed
Corn	Safeway	
grapeseed	Spectrum	expeller pressed[e]
macadamia nut	Australian MacNut	
Olive	365	cold processed[f]
peanut	La Tourangelle[g]	roasted
pumpkin seed	La Tourangelle	
red palm	Aunt Patty's	expeller pressed[h]
rice bran	Tophe	
safflower[d]	Spectrum	expeller pressed[e]
sesame	Dynasty[i]	
walnut	Spectrum	expeller pressed[e]

[a] Crisco and vegetable oils work well, too, but contain oils from mixtures of plants; canola oil results were inconclusive. [b] Processing completed at factory. [c] Refined for high heat. [d] Organic oil. [e] Refined for medium to high heat. [f] Extra virgin. [g] Spectrum unrefined Hi-Oleic Peanut Oil results were inconclusive. [h] Filter before distributing to students because unrefined. [i] Contains 4 fatty acids; Spectrum and La Tourangelle brands only contain oleic acid.

The isolated biodiesel is characterized with a viscosity test, gas chromatography-mass spectrometry (GC-MS), and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The viscosity test (Figure SM 3.1.20.8) helps students understand why it is necessary to convert triglycerides to alkyl esters before using them as fuel. The composition of the product mixture is

determined by GC-MS (Table SM 3.1.20.3, Figures SM 3.1.20.9 – SM 3.1.20.23). ^{1}H-NMR spectroscopy is used to illustrate whether the reaction goes to completion and forms byproducts, as well as to confirm the composition. For example, the doublet of doublets, labeled $H_ß$ (Figure SM 3.1.20.24), indicates that there are still triglycerides in the almond oil product mixture and that the reaction did not go to completion. Also, the ^{1}H- NMR spectrum (Figure SM 3.1.20.28) of coconut oil shows low abundances of H_c and H_e, which are the protons that indicate the presence of a double bond; this confirms that coconut oil is composed mostly of saturated fatty acid derivatives. Conversely, corn oil (Figure SM 3.1.20.29) is largely comprised of monounsaturated oleic acid chains and has much higher abundances of H_c and H_e.

Table SM 3.1.20.2 – The masses of biodiesel isolated by the students are listed by plant oil. Students were not asked to calculate their percent yields because the exact fatty acid chain compositions of the oils were unknown. The number of samples indicates the number of students who prepared biodiesel from the oil. Q-tests were used to eliminate outlying data.

Plant oil	95% confidence interval	Number of samples
almond oil	[j]	1
avocado oil	0.519 ± 0.253 g	5
camelina oil	0.976 ± 0.286 g	5
coconut oil	0.618 ± 0.344 g	5
corn oil	0.650 ± 0.384 g	3
grapeseed oil	0.767 ± 0.238 g	3
macadamia nut oil	0.784 ± 0.287 g	2
olive oil	0.652 ± 0.301 g	5
peanut oil	0.806 ± 0.212 g	4
pumpkin seed	0.081 ± 0.045 g	4
red palm oil	0.822 ± 0.217 g	5
rice bran oil	0.802 ± 0.320 g	4
safflower	0.357 ± 0.185 g	6
sesame oil	0.734 ± 0.244 g	3
walnut oil	0.462 ± 0.243 g	5

[j] A 95 % confidence interval could not be determined for almond oil because only 1 sample was prepared from almond oil. The yield was 0.051 g.)

Because only a few drops of biodiesel are required for analysis, this reaction can be carried out using microscale quantities. However, we have also done a similar biodiesel laboratory experiment on a larger scale, which worked well. This experiment was completed toward the end of the quarter; therefore, students' laboratory techniques and spectroscopic characterization abilities were advanced enough to focus more on research than basic laboratory skills. In addition to carrying out the reactions, the students researched the fatty acid chain content of the 15 oils and proposed identities for their and their lab partners' unknown oils.

Table SM 3.1.20.3 – Student-derived GC-MS results show the propyl ester content of each oil. MS data is only provided for the 5 most abundant species. The number of samples indicates the number of times each ester was found in student transesterification reactions. Q-tests were used to eliminate outlying data.

Propyl esters	95% Confidence interval	Number of samples
almond oil		
propyl palmitate	3.4 ± 1 %	5
propyl linoleate	21 ± 5 %	3
propyl oleate	87 ± 13 %	6
avocado oil[a]		
propyl palmitate	8.4 ± 2 %	11
propyl oleate	90 ± 3 %	11
camelina oil		
propyl palmitate	3.8 ± 0.3 %	8
propyl α-linolenate	64 ± 0.8 %	8
propyl linoleate	18 ± 2 %	9
propyl stearate	1.6 ± 0.1 %	6
propyl gadoleate	14 ± 0.2 %	7
coconut oil[b]		
propyl caprylate	7.9 ± 0.7 %	9
propyl caprate	4.9 ± 0.7 %	8
propyl laurate	66 ± 3 %	9
propyl myristate	16 ± 1 %	9
propyl palmitate	5.6 ± 0.3 %	8

corn oil[c]		
propyl palmitate	11 ± 4 %	6
propyl linoleate	78 ± 20 %	6
grapeseed oil[d]		
propyl palmitate	3.9 ± 0.9 %	5
propyl linoleate	96 ± 4 %	7
macadamia nut oil[e]		
propyl palmitoleate	16 ± 5 %	5
propyl palmitate	7.8 ± 4 %	5
propyl oleate	75 ± 4 %	4
propyl stearate	2.3 ± 1 %	4
olive oil[f]		
propyl palmitate	7.9 ± 2 %	11
propyl oleate	92 ± 2 %	10
peanut oil[g]		
propyl palmitate	4.2 ± 0.6 %	6
propyl oleate	96 ± 1 %	6
pumpkin seed[h]		
propyl palmitate	8.8 ± 2 %	8
propyl linoleate	34 ± 9 %	8
propyl oleate	48 ± 3 %	7
propyl stearate	3.6 ± 0.1 %	6
red palm oil[i]		
propyl palmitate	51 ± 7 %	10
propyl oleate	41 ± 11 %	10
propyl stearate	2.7 ± 0.4 %	7

rice bran oil		
propyl palmitate	18 ± 4 %	10
propyl oleate	54 ± 9 %	10
propyl linoleate	31 ± 2 %	9
safflower[j]		
propyl palmitate	2.7± 0.5 %	11
propyl oleate	88 ± 14 %	11
sesame oil		
propyl palmitate	6.6 ± 0.6 %	7
propyl linoleate	45 ± 2 %	7
propyl oleate	46 ± 2 %	6
propyl stearate	3.4 ± 0.4 %	6
walnut oil		
propyl palmitate	5.4 ± 2 %	5
propyl linoleate	95 ± 2 %	5

[a] Propyl palmitoleate (1.8, 1.9 %) was found in 2/11 samples and propyl linoleate (8.7 %) in 1/11 samples. [b] Propyl oleate (4.6 %) was found in 1/9 samples. [c] Propyl oleate (27.6, 39.7 %) was found in 2/6 samples. [d] Propyl stearate (2.0, 3.6%) was found in 2/7 samples. [e] Propyl α-linolenate (28.4 %) and propyl gadoleate (1.7 %) were found in 1/5 samples. [f] Propyl stearate (3.8%) and propyl linoleate (43.7 %) were found in 1/11 samples. [g] Propyl stearate (1.1%) was found in 1/6 samples. [h] Propyl α-linolenate (18.4%) was found in 1/8 samples. [i] Propyl linoleate (6.9, 8.1, 14.5 %) was found in 3/10 samples; propyl myristate (3.1 %) and propyl α-linolenate (29.8 %) were found in 1/10 samples. [j] Propyl palmitoleate (2.4, 3.1 %) and propyl α-linolenate (38.9, 55.5 %) were found in 2/11 samples; propyl stearate (1.2 %) was found in 1/11 samples.

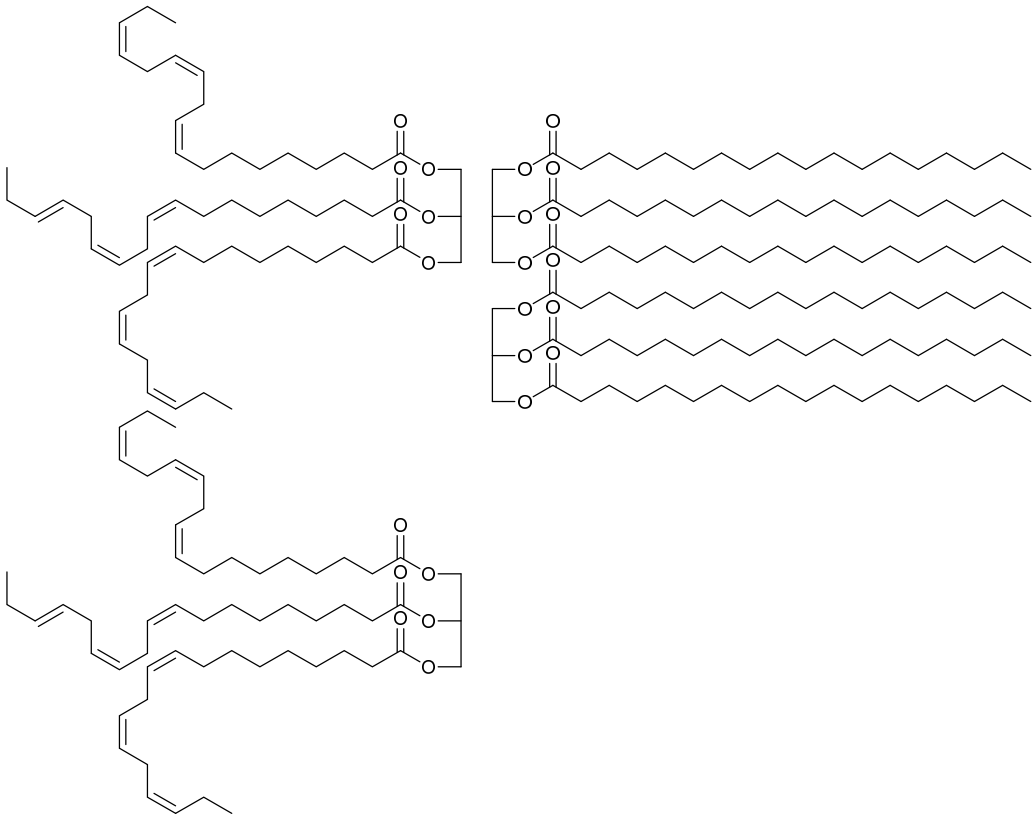
GC-MS protocol – 1 µL of each sample is injected into an Agilent 7890A-5975C GC-MS equipped with a split/splitless inlet operating in split mode (1:100). The instrument is controlled via ChemStation software and uses ultra-high-purity He as the carrier gas, in constant flow mode at 1mL/min. An HP-5ms Ultra Inert ((5%-phenyl)-methylpolysiloxane, equivalent to USP Phase G27) capillary column (30 m x 250 µm i.d. x 25 µm film thickness) is used for the separation. The injector temperature was held at 280 ºC. The oven was initially at 35 ºC and held for 3.75 minutes with subsequent increase at 20 ºC min^{-1} until 320 ºC with a final hold of 7 min. The total runtime was 25 minutes. The transfer line between the GC and MS was held at 280 ºC, and the electron ionization (EI) MS source at 150 ºC. The quadrupole detector operated in full scan mode, with scan range m/z 50–550.

Answers to results interpretation and additional questions

1. Which propyl esters did your biodiesel contain? Draw the propyl esters associated with each of the GC peaks and mass spectra, label the molecular ion peaks on each of the mass spectra, and report the relative ratio of propyl esters from the GC. *See Figures SM 3.1.20.9–SM 3.1.20.23.*

2. Draw a generic saturated or unsaturated fatty acid ester on your ^{1}H-NMR spectrum, and label the relevant shifts. Has your reaction gone to completion? Do you see any peaks associated with the starting material or byproducts? *See Figure SM 3.1.20.24.*

3. Although both plant oils and animal fats are composed primarily of triglycerides, they have different physical properties. Use structures to provide an explanation for this.

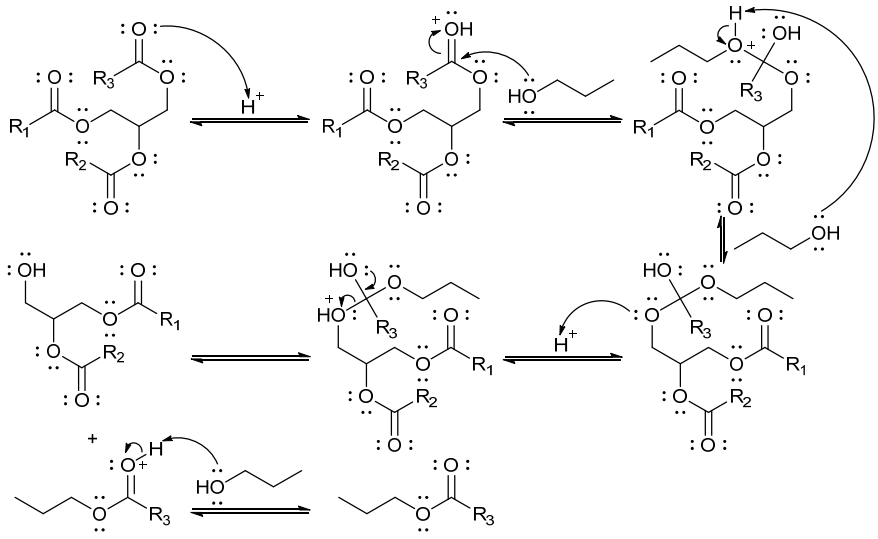


Scheme SM 3.1.20.1 – The triglycerides composed of unsaturated α-linolenic acid chains do not pack as efficiently as the triglycerides composed of saturated stearic acid chains.

The π bonds on the triglyceride with unsaturated fatty acid chains do not allow the sp^2-hybridized carbons to rotate. Thus, the sp^2-hybridized carbons retain their cis orientations and do not allow the chains to pack as close as the triglyceride composed of saturated fatty acid chains. The packing arrangements of the different fatty acid chains explain why saturated fats, such as animal fat, tend to be solids and unsaturated fats, like plant oils, tend to be liquids at room temperature.

4. It would be cheaper and easier to burn plant oil without transesterifying it first. Why is this rarely done? *Plant oil is a less viscous fuel, and the transesterification and extraction process removes impurities that inhibit the performance of the engine (engine sludge).*

5. Draw a detailed mechanism for the first transesterification reaction (triglyceride → diglyceride) in this experiment. Abbreviate the long alkyl chains as R-groups.



Scheme SM 3.1.20.2 — A triglyceride reacts with 1-propanol in acidic conditions to give a diglyceride and a propyl ester.

Photos of the experiment

Figure SM 3.1.20.1 — The unknown oils are numbered, poured into bottles, and presented to students in bottles labeled only with the oil number.

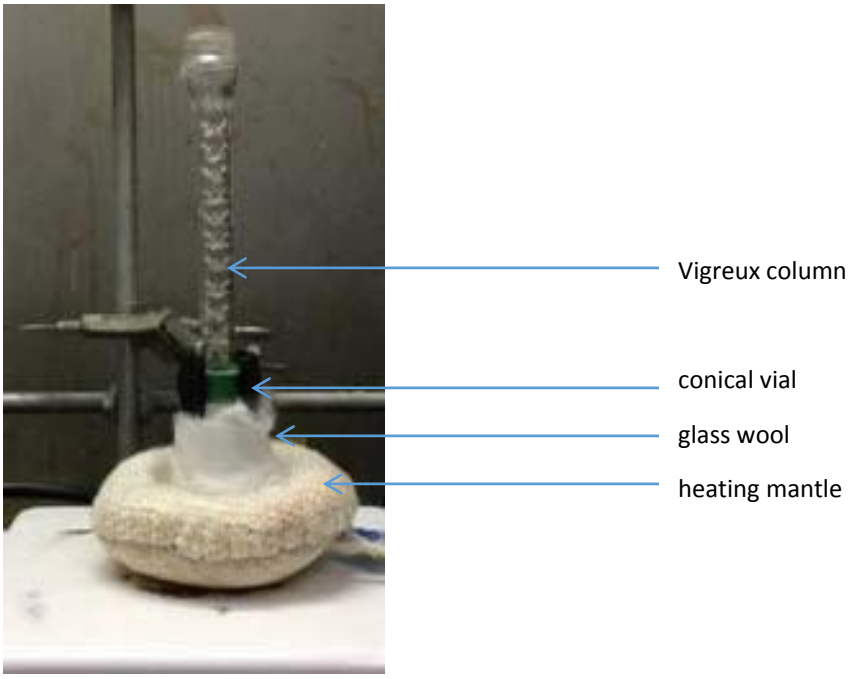


Figure SM 3.1.20.2 — To eliminate the need for a reflux condenser, a Vigreux column is attached to a 5 mL conical vial, which is insulated with glass wool and placed in a heating mantle, on a magnetic stirrer. The vial contains oil, 1-propanol, H_2SO_4, and a spin vane.

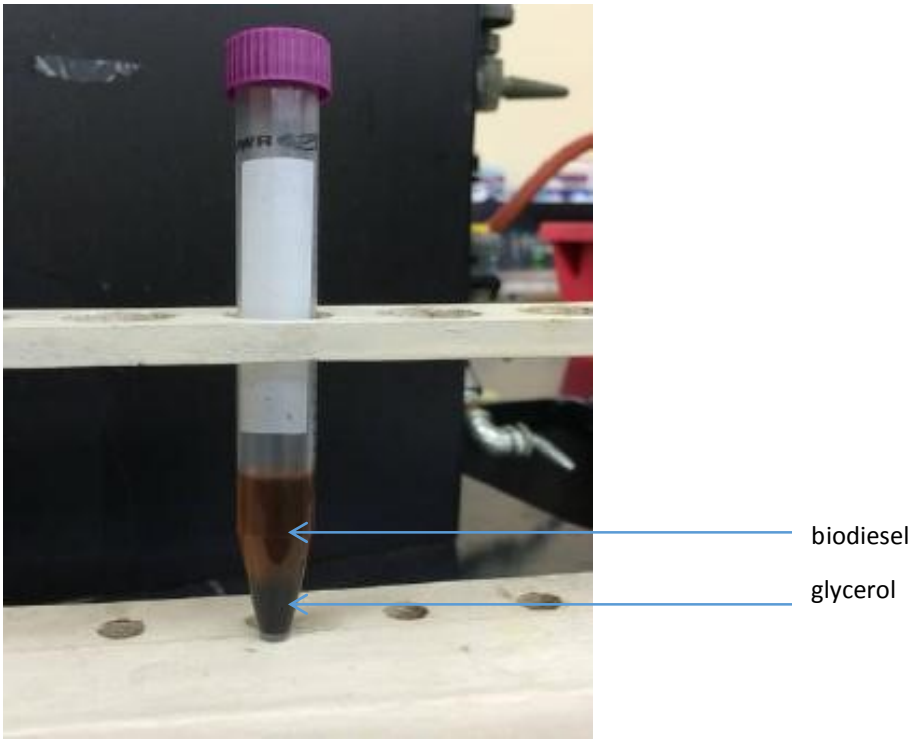


Figure SM 3.1.20.3 — The product mixture is centrifuged to separate the emulsion. The darker glycerol-containing bottom layer is removed, and the biodiesel is then washed with 1 M NaCl.

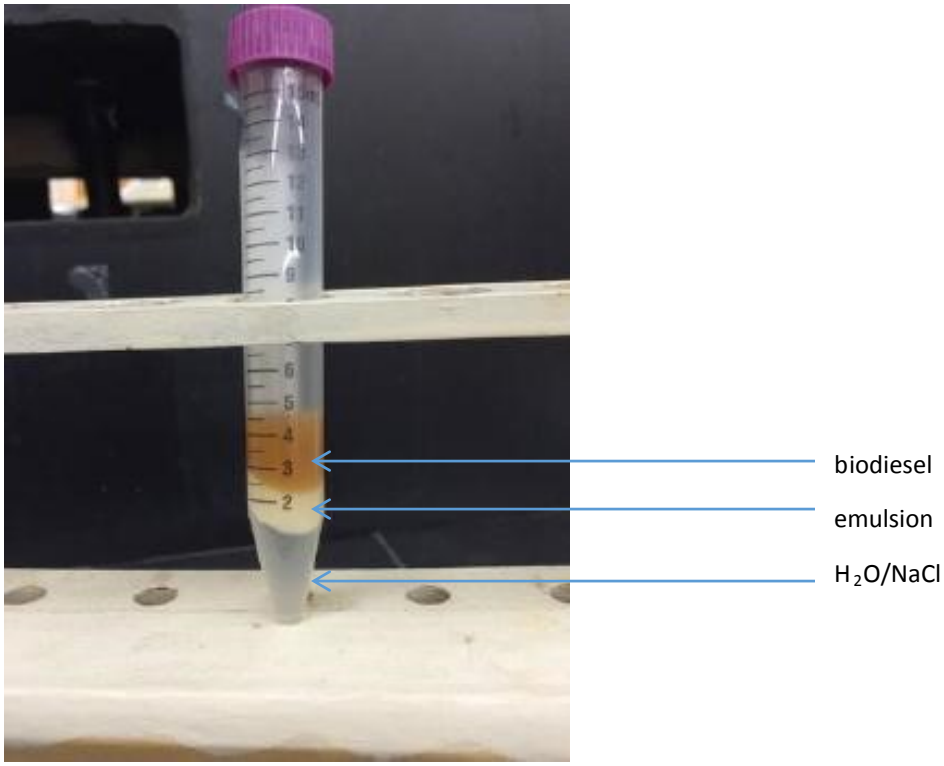


Figure SM 3.1.20.4 — After washing the biodiesel with 1 M NaCl three times, an emulsion has formed. The clear aqueous layer will be removed before $MgSO_4$ is added.

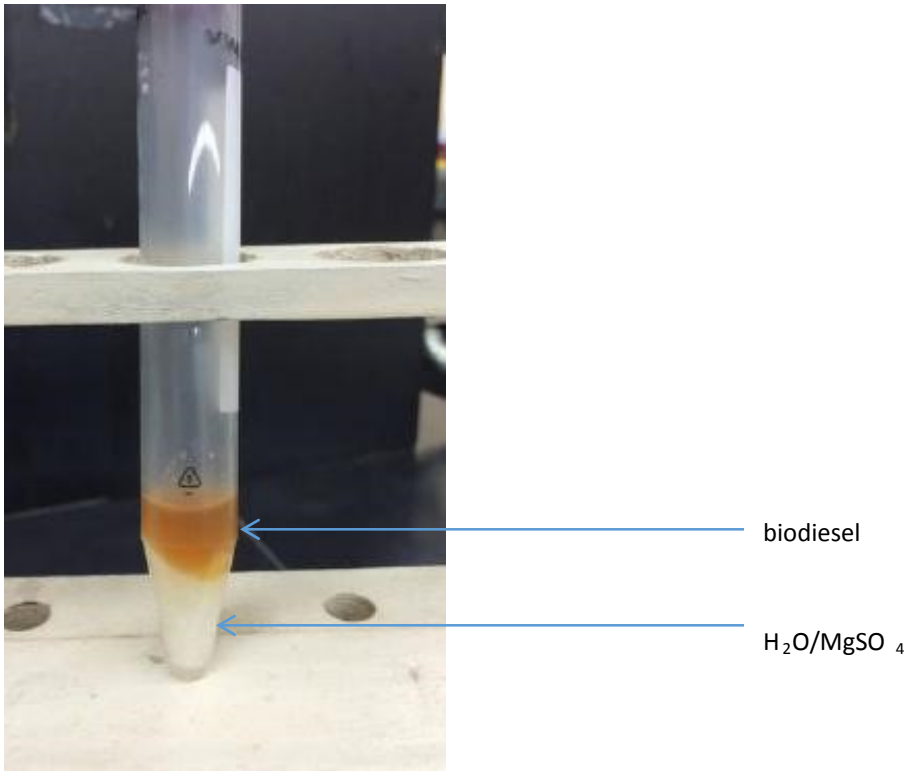


Figure SM 3.1.20.5 — The biodiesel is dried with $MgSO_4$ and centrifuged a fifth time.

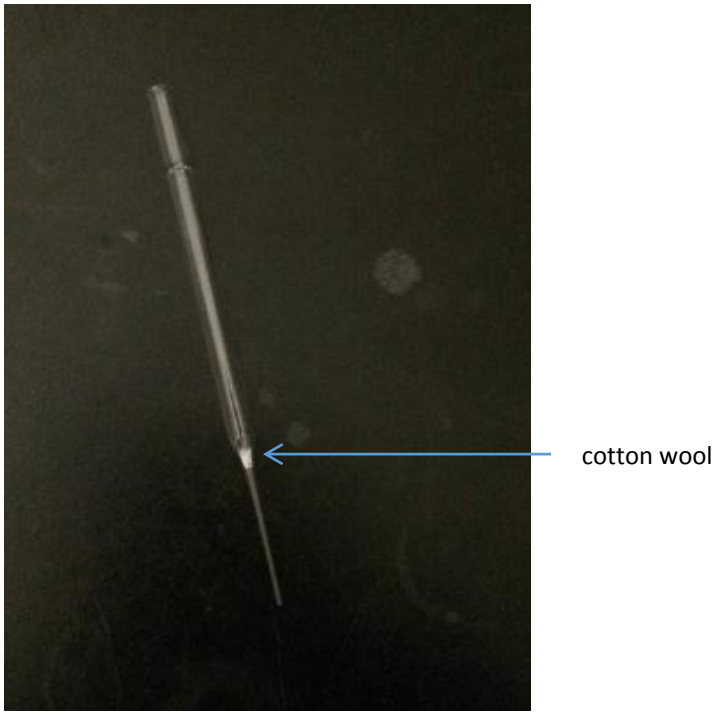


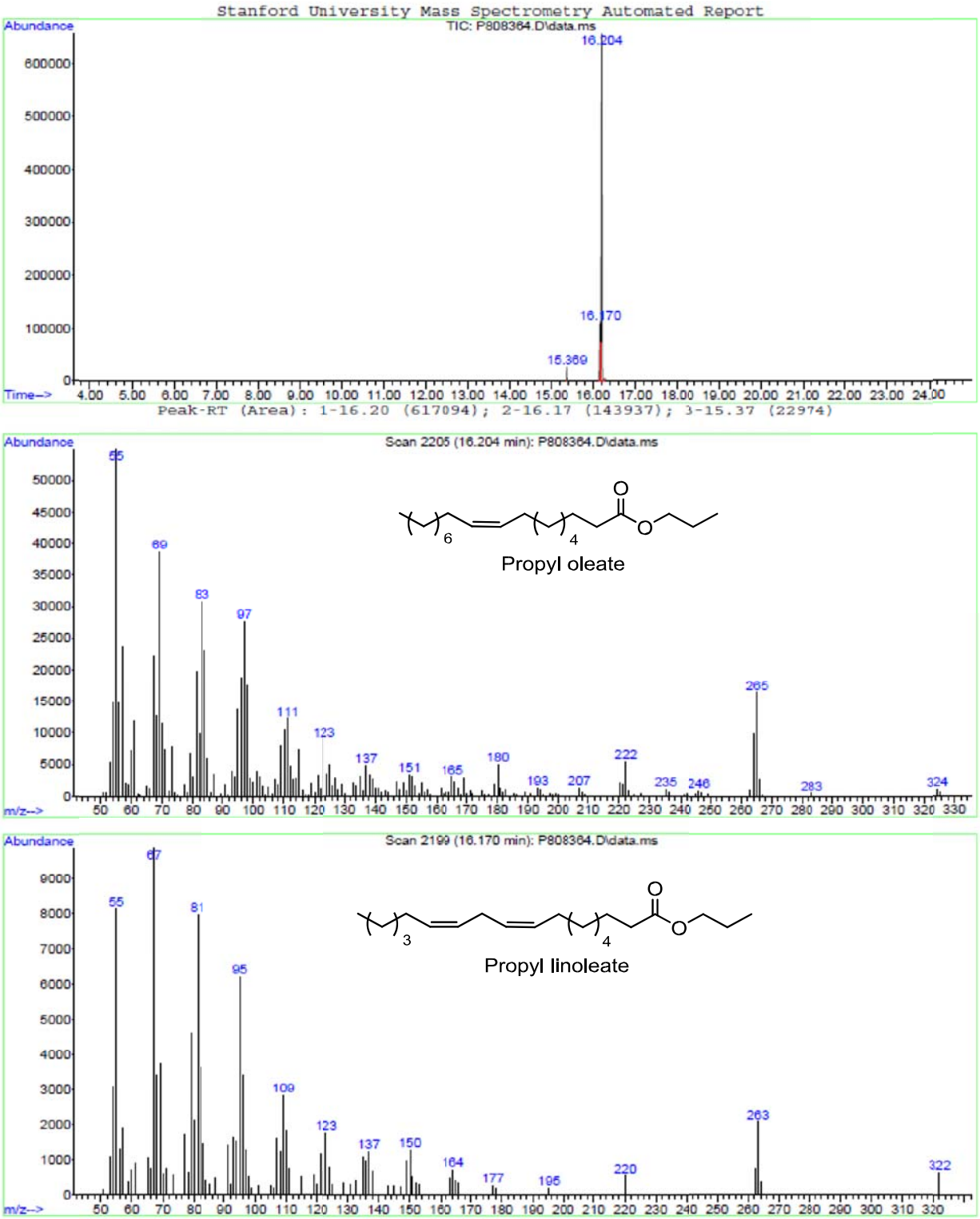
Figure SM 3.1.20.6 — A pipette filter is made by gently packing a Pasteur pipette with cotton wool.

Figure SM 3.1.20.7 — The biodiesel is decanted through the pipette filter into a vial. This removes any particulate matter from the biodiesel.

Figure SM 3.1.20.8 — The viscosities of biodiesel and sesame oil are compared.

Student gas chromatograms and mass spectra

Figure SM 3.1.20.9 — GC-MS of almond oil propyl esters.



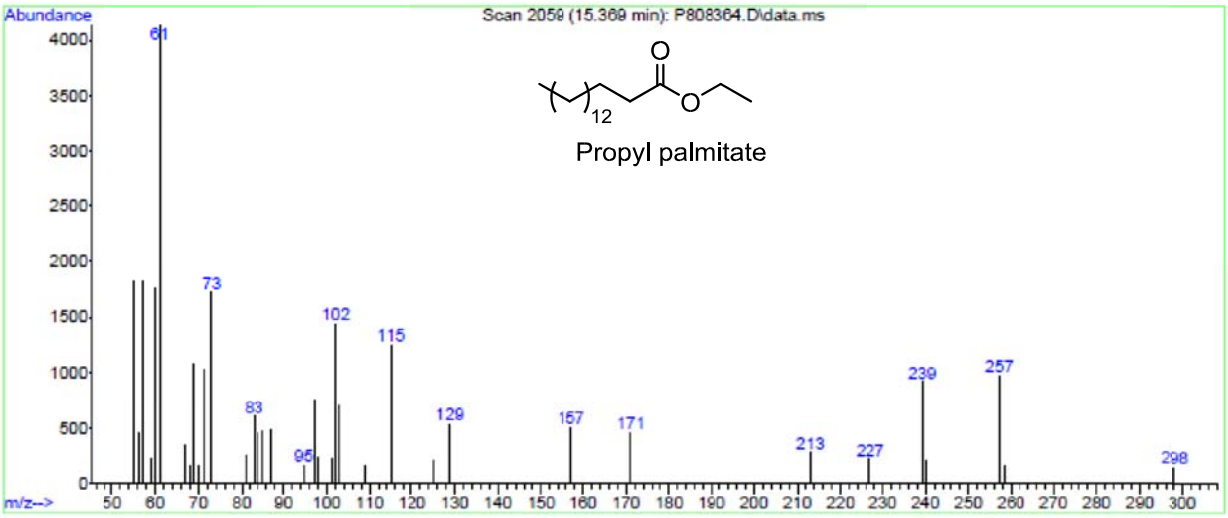


Figure SM 3.1.20.10 — GC-MS of avocado oil propyl esters.

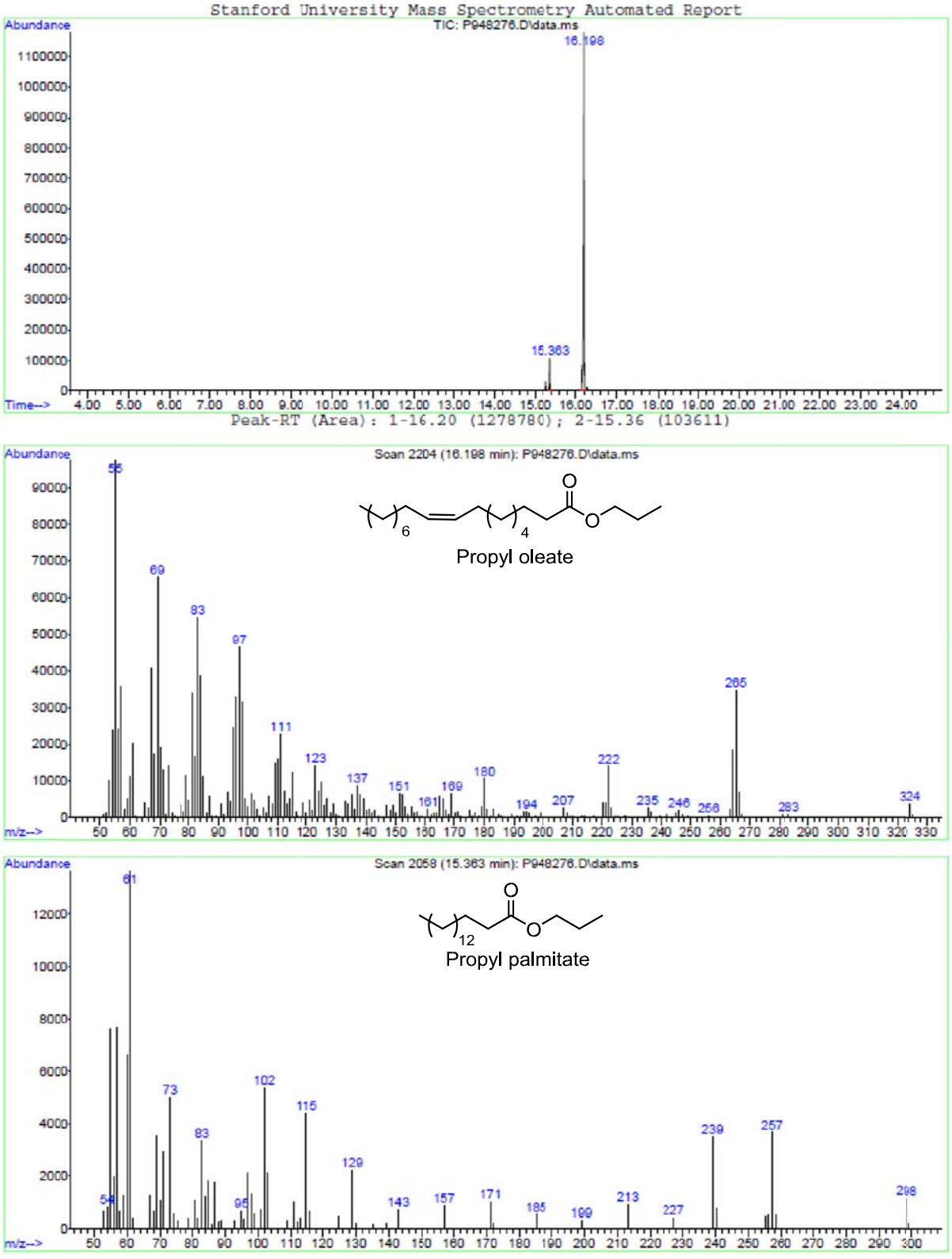
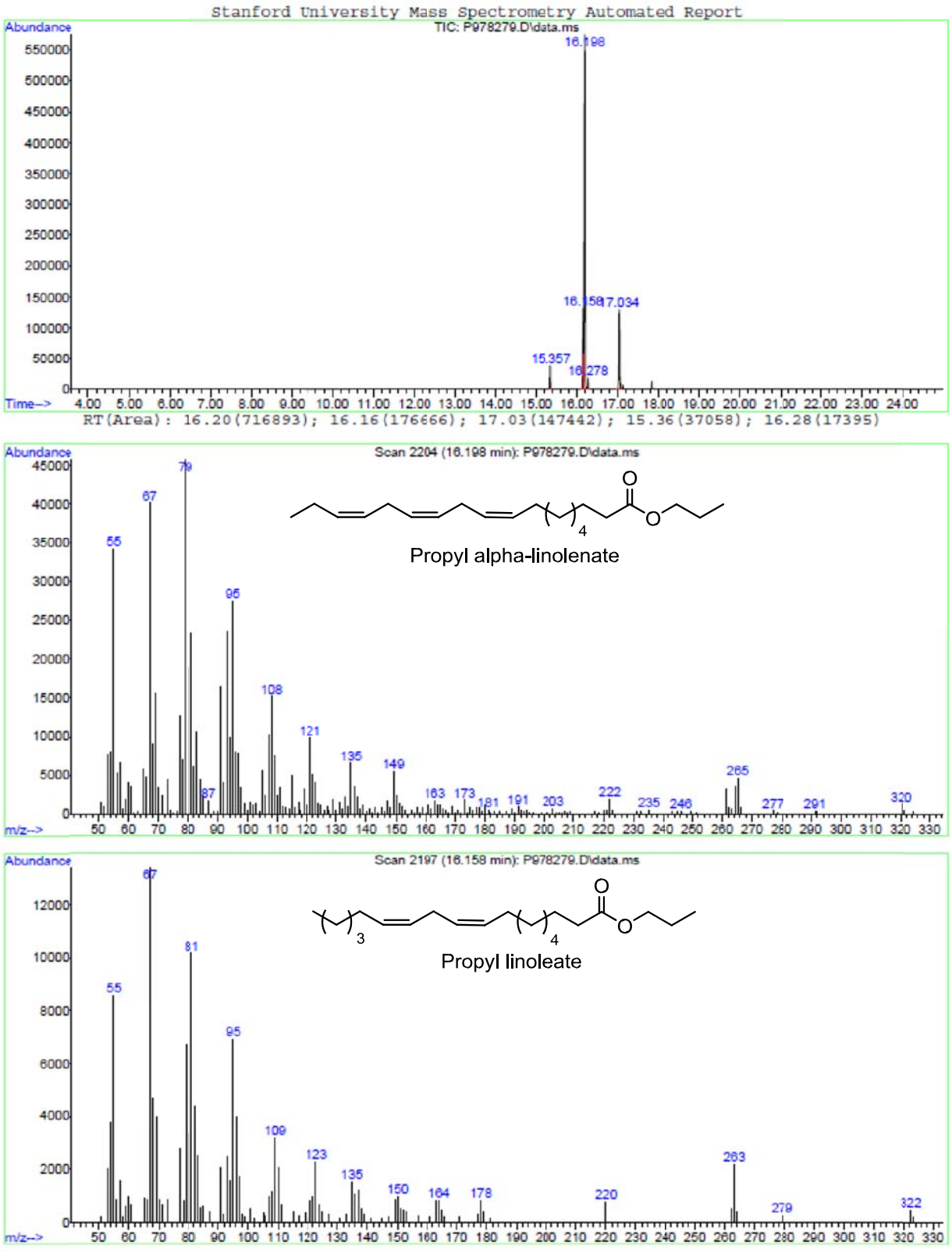
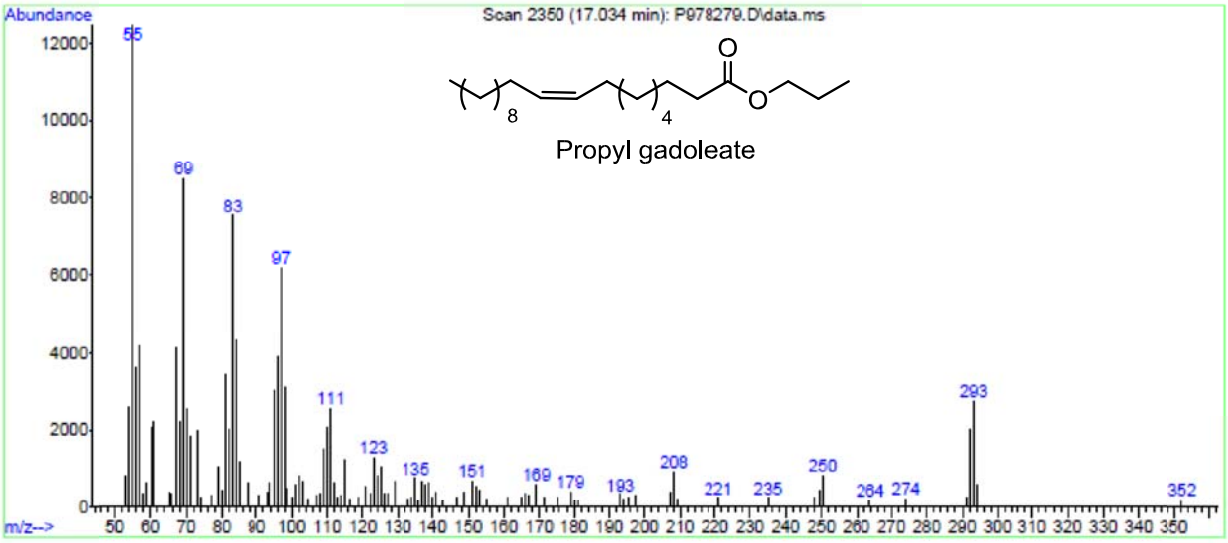
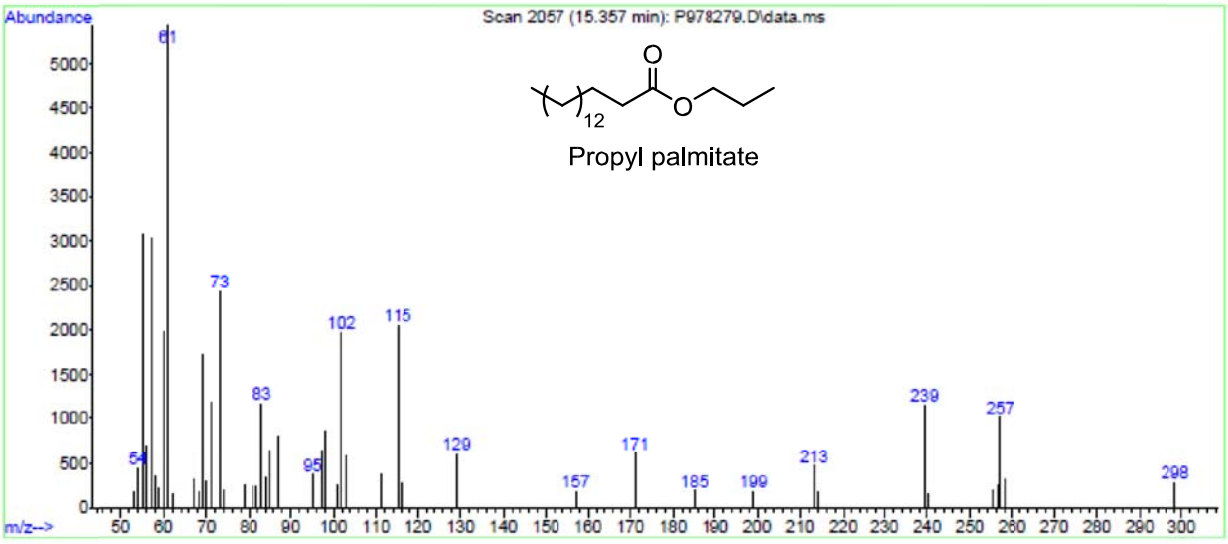


Figure SM 3.1.20.11 — GC-MS of camelina oil propyl esters.




Abundance
Scan 2350 (17.034 min): P978279.D\data.ms
Propyl gadoleate
m/z-->


Abundance
Scan 2057 (15.357 min): P978279.D\data.ms
Propyl palmitate
m/z-->

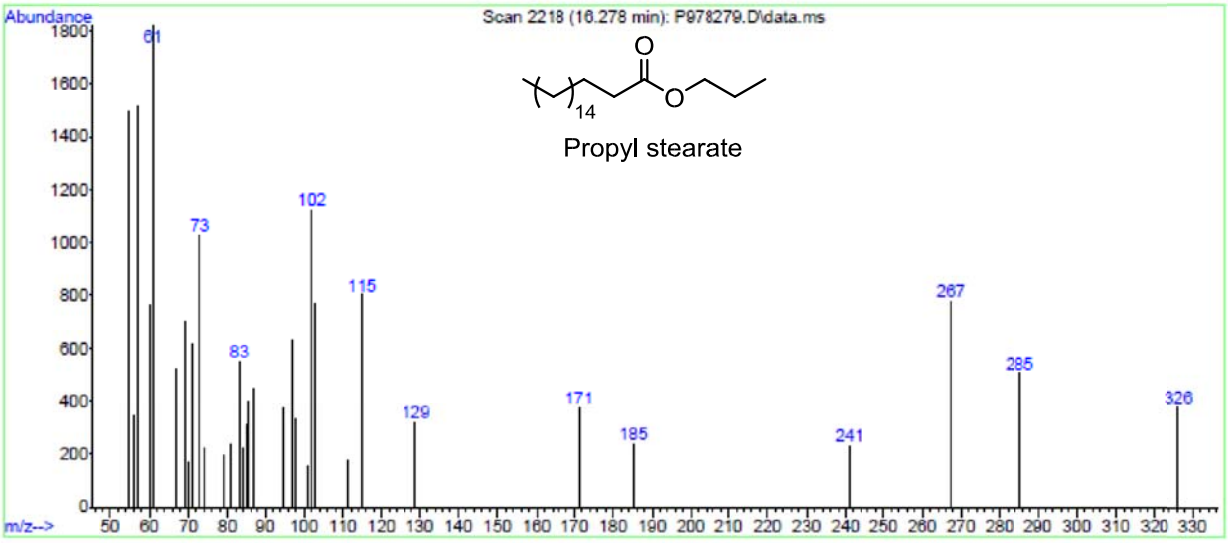
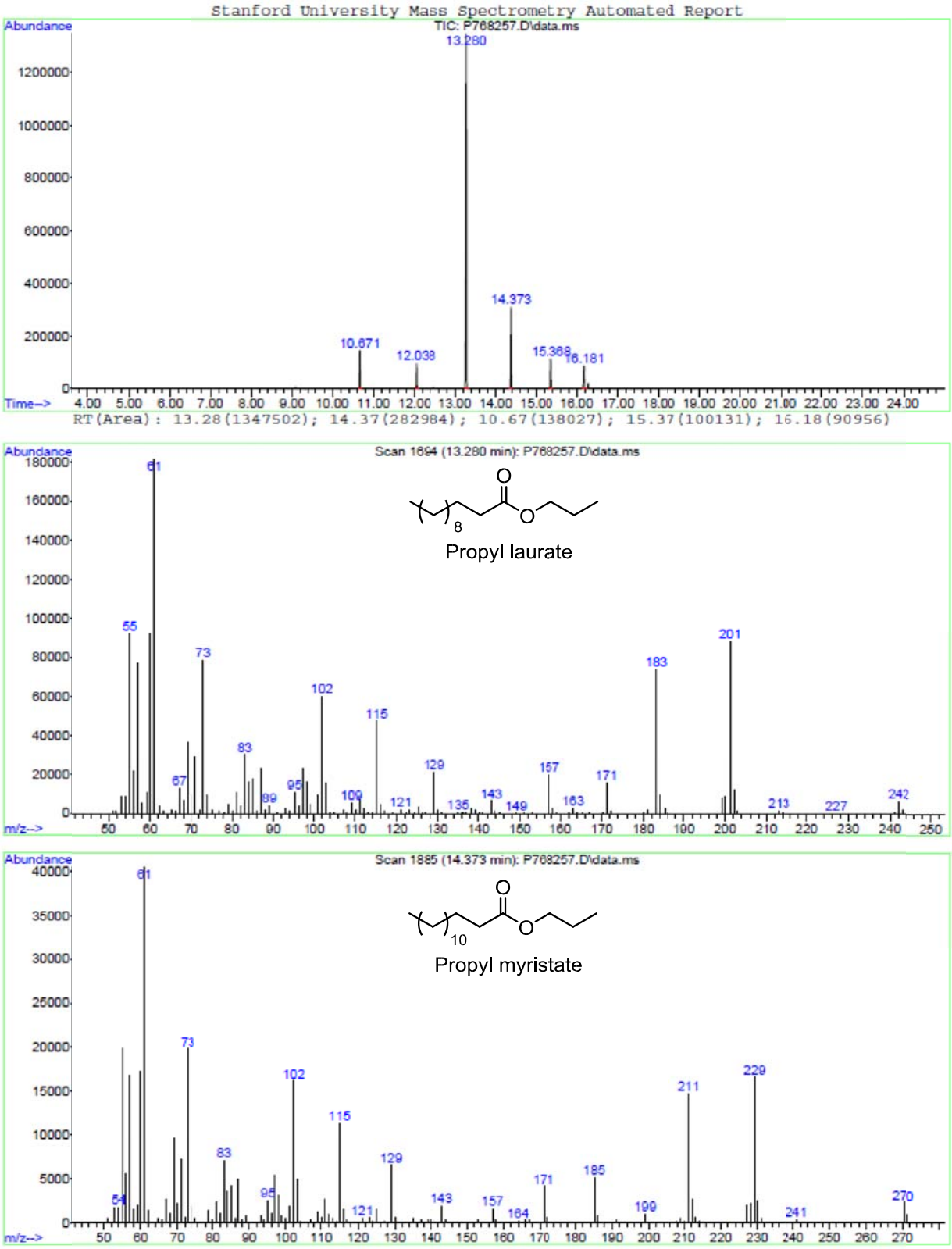
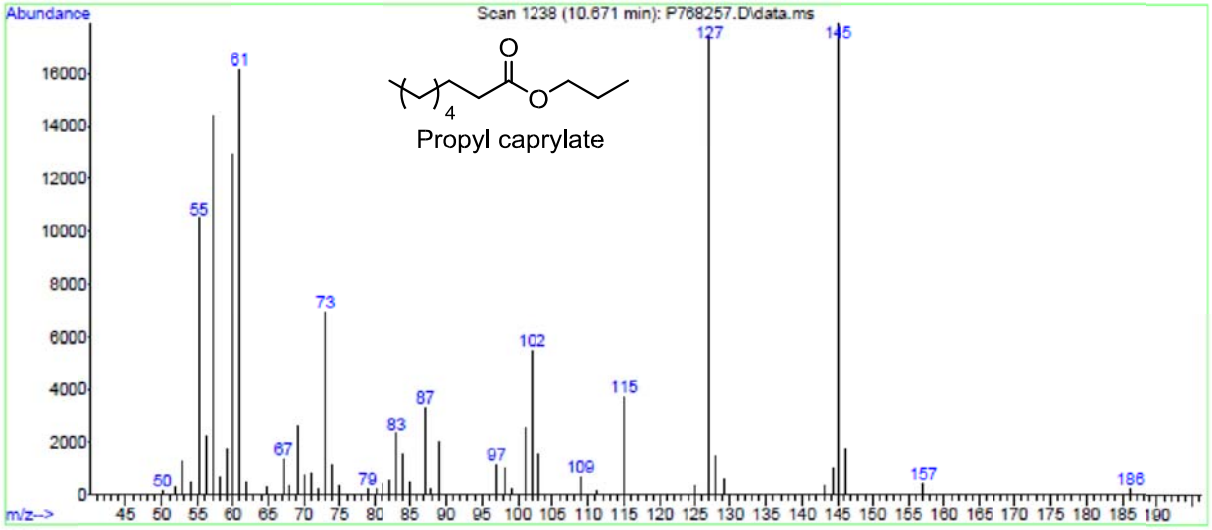
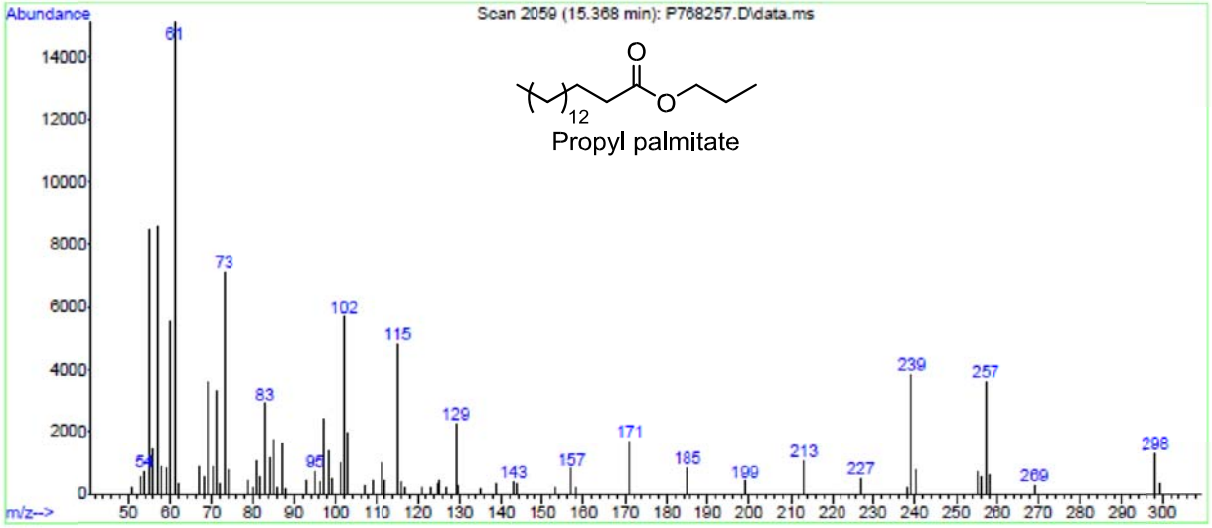

Abundance
Scan 2218 (16.278 min): P978279.D\data.ms
Propyl stearate
m/z-->

Figure SM 3.1.20.12 — GC-MS of coconut oil propyl esters.




Abundance
Scan 1238 (10.671 min): P768257.D\data.ms
Propyl caprylate
m/z-->


Abundance
Scan 2059 (15.368 min): P768257.D\data.ms
Propyl palmitate
m/z-->

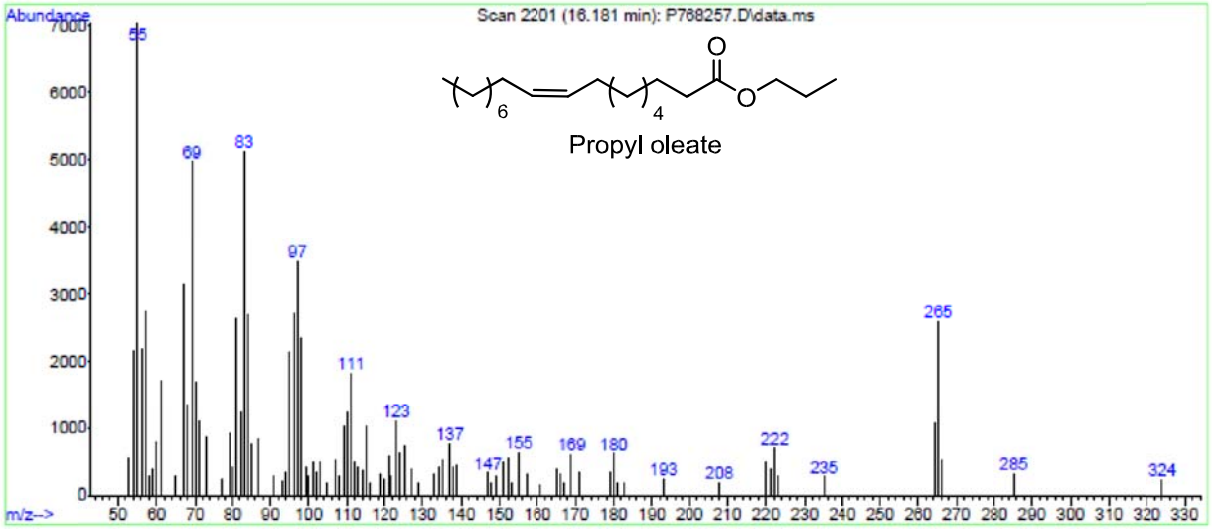
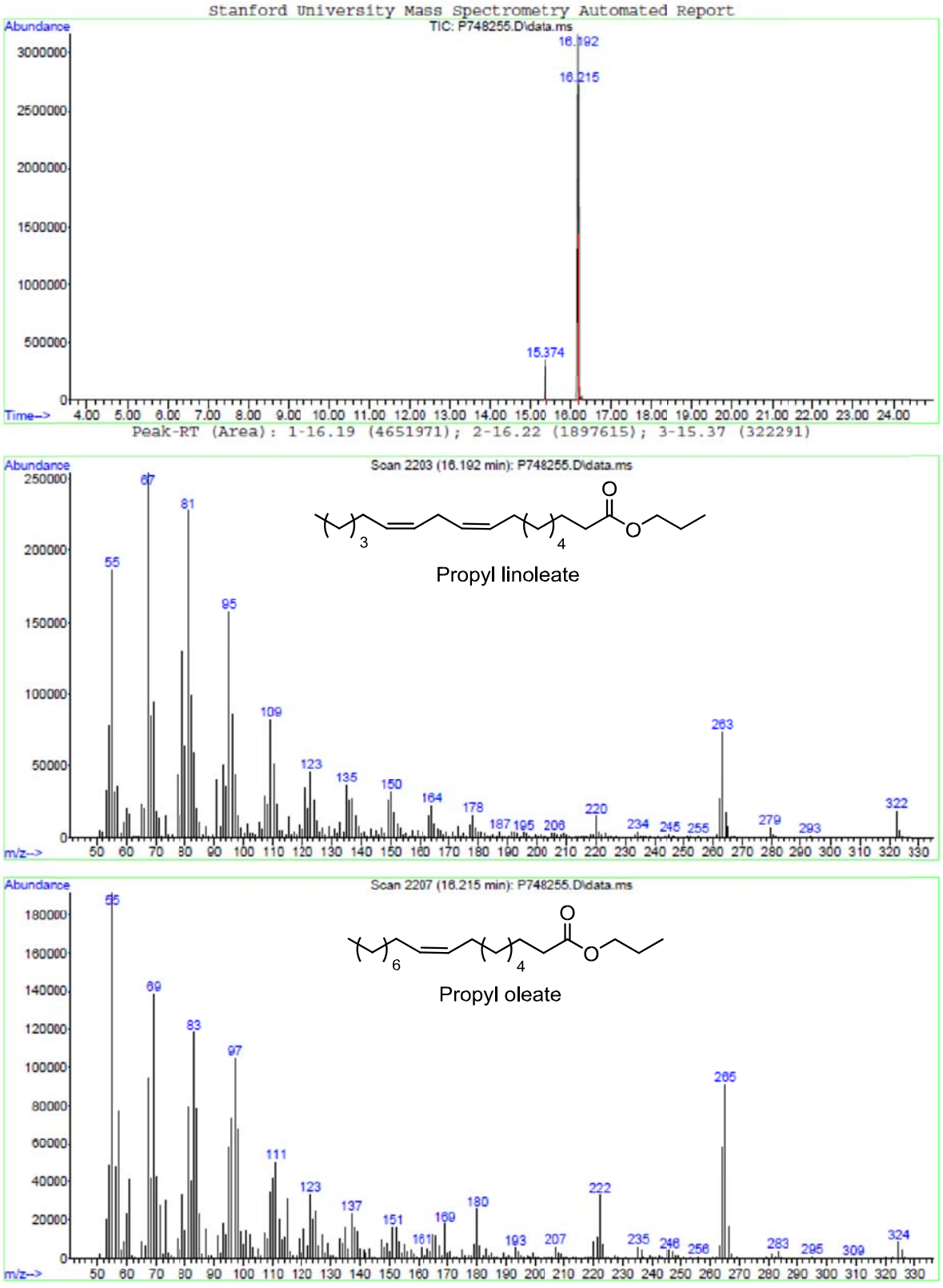

Abundance
Scan 2201 (16.181 min): P768257.D\data.ms
Propyl oleate
m/z-->

Figure SM 3.1.20.13 — GC-MS of corn oil propyl esters



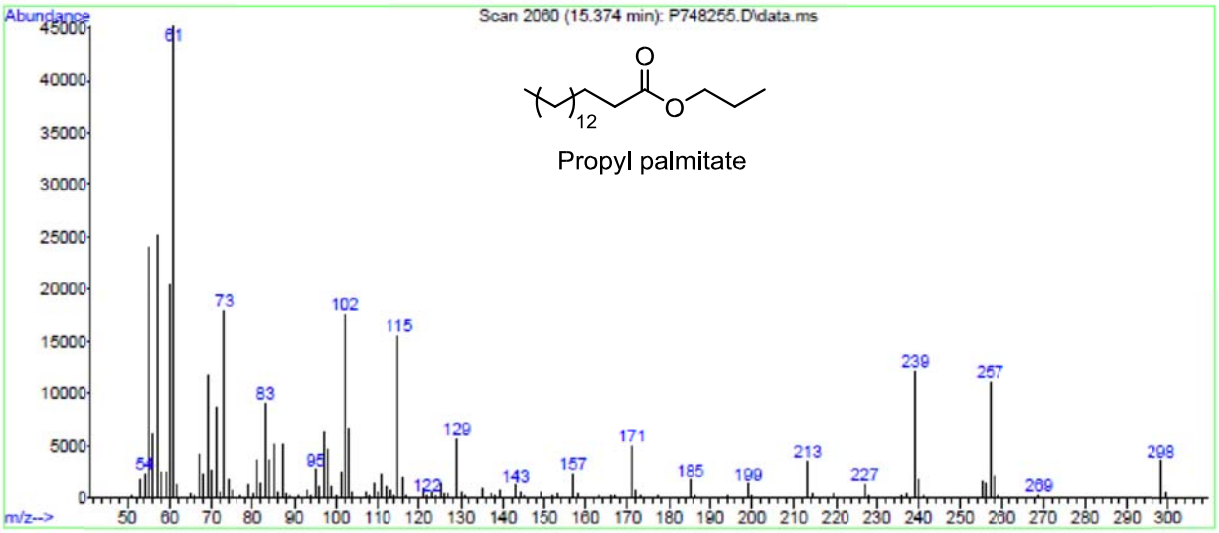

Abundance
Scan 2080 (15.374 min): P748255.D\data.ms
m/z-->
Propyl palmitate

Figure SM 3.1.20.14 — GC-MS of grapeseed oil propyl esters.

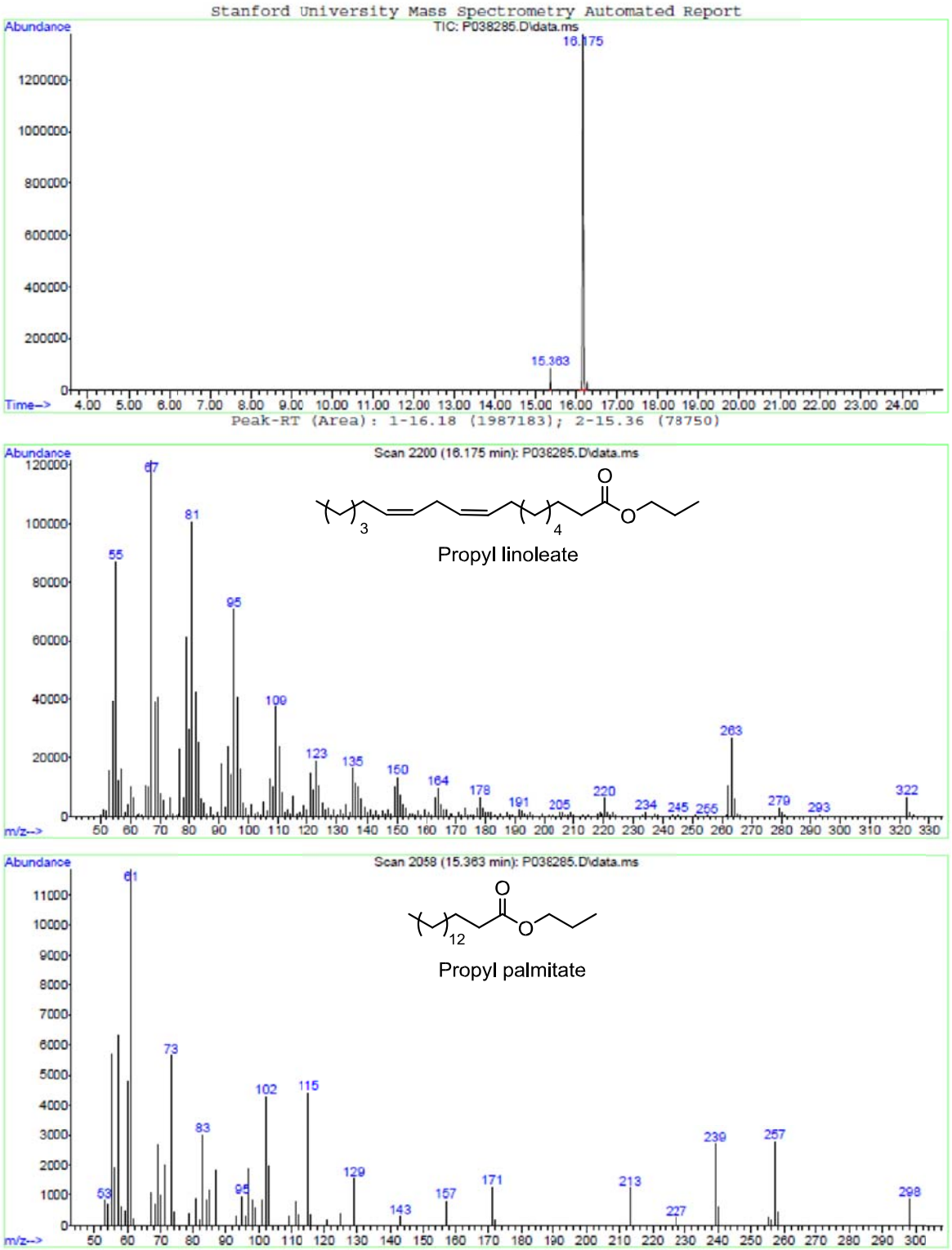
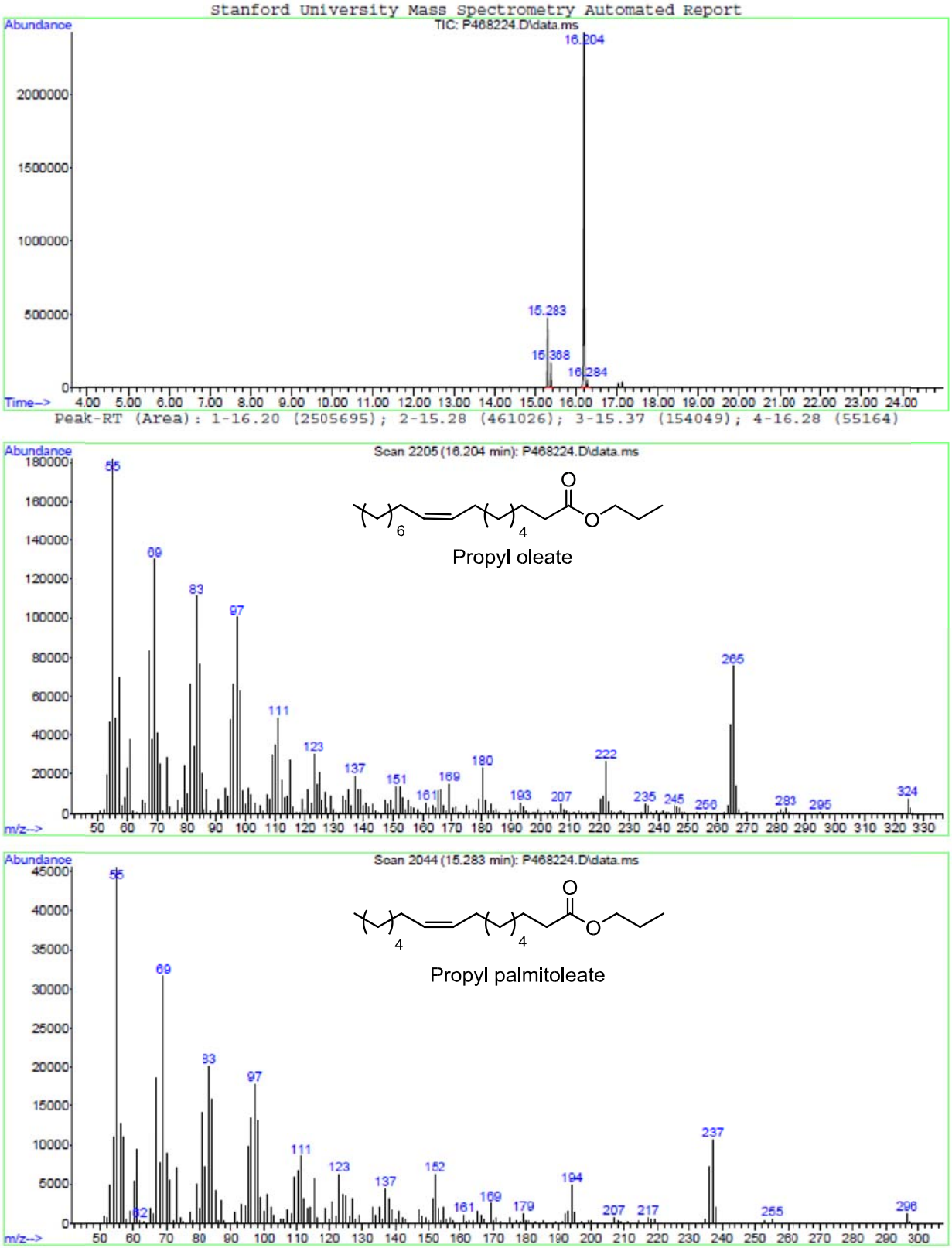
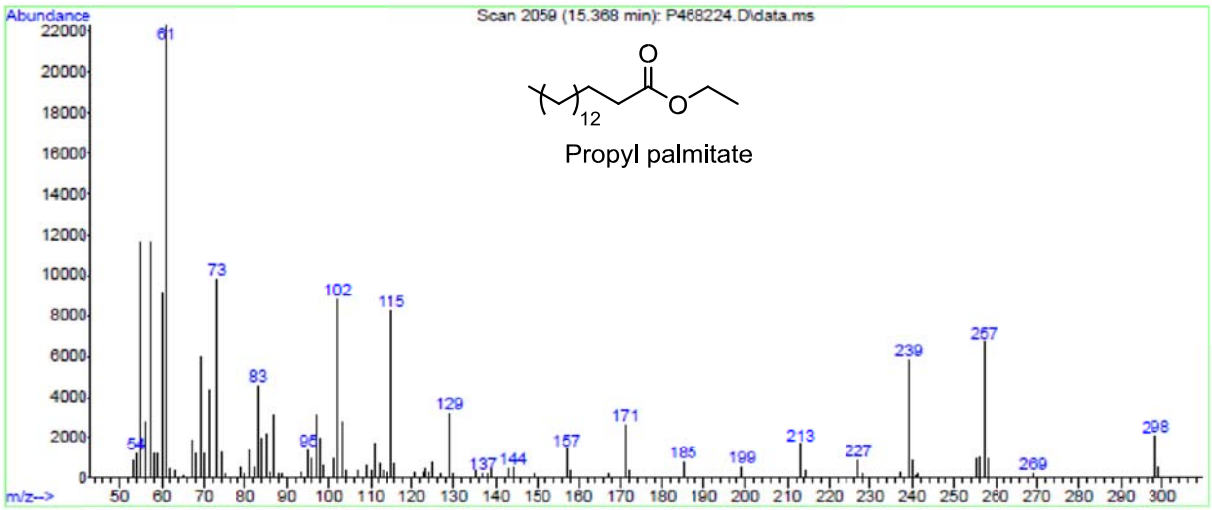


Figure SM 3.1.20.15 — GC-MS of macadamia nut oil propyl esters.




Abundance
Scan 2059 (15.368 min): P468224.D\data.ms
Propyl palmitate
m/z-->

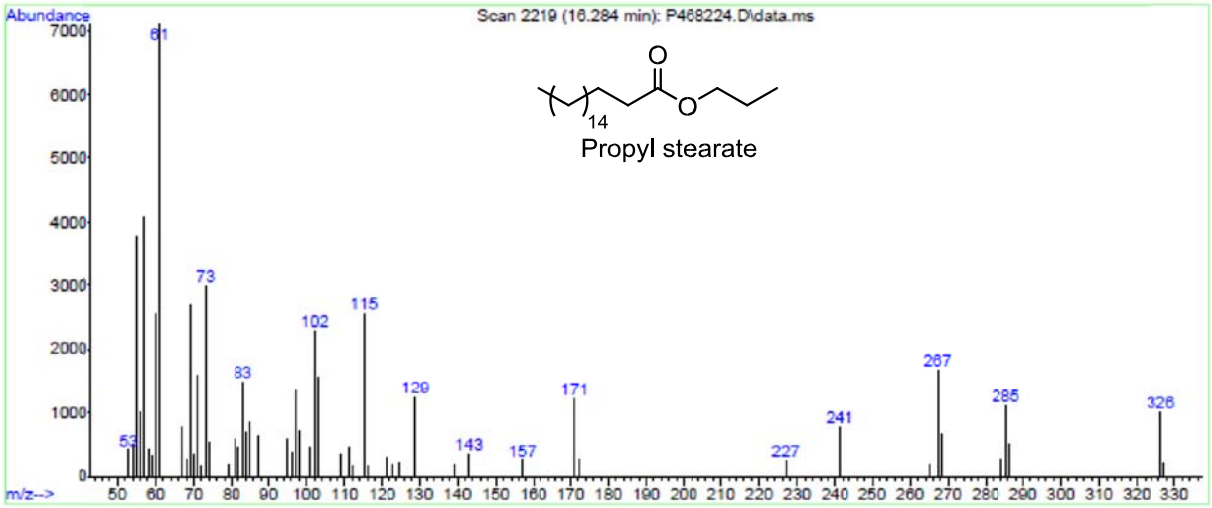

Abundance
Scan 2219 (16.284 min): P468224.D\data.ms
Propyl stearate
m/z-->

Figure SM 3.1.20.16 — GC-MS of olive oil propyl esters.

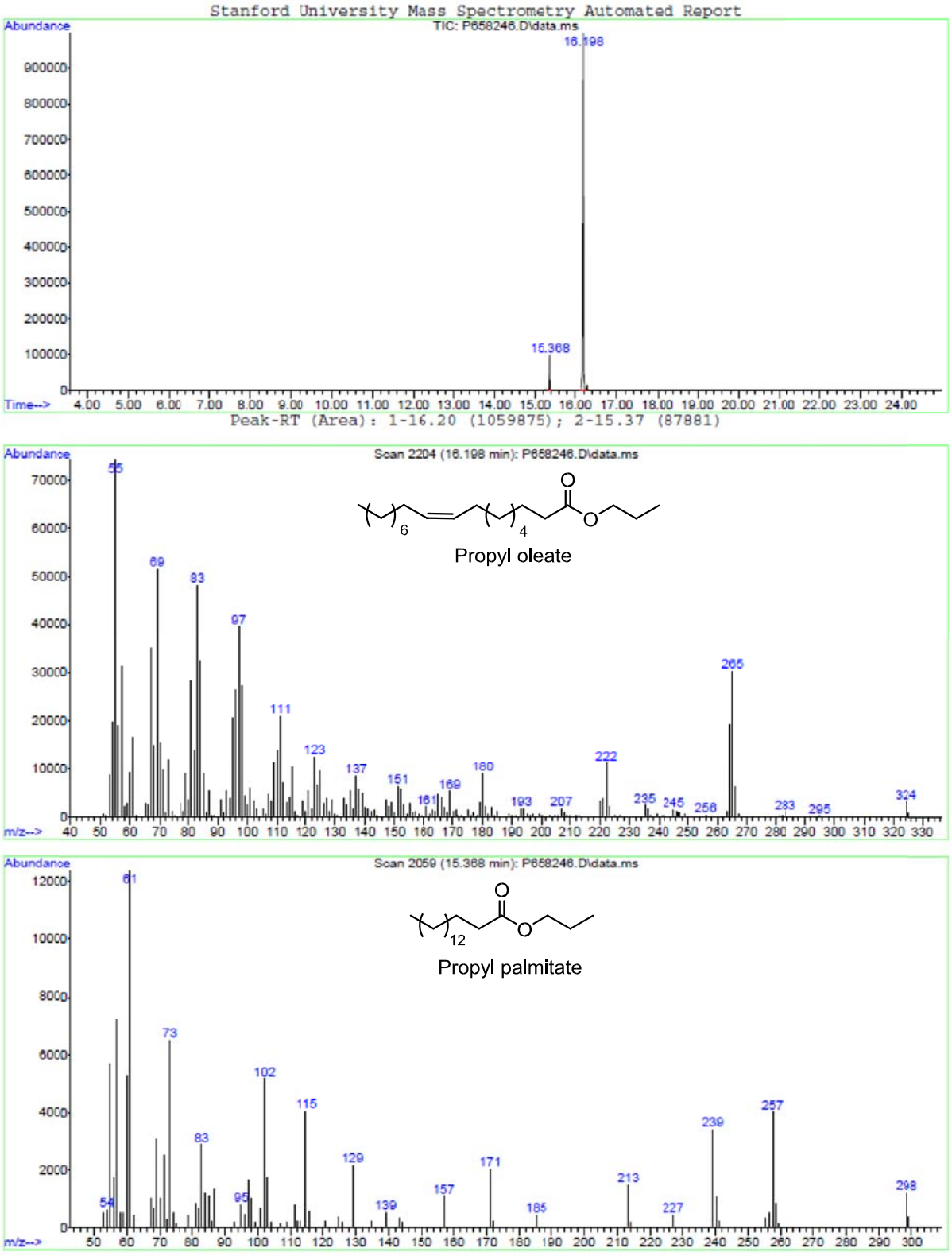


Figure SM 3.1.20.17 — GC-MS of peanut oil propyl esters.

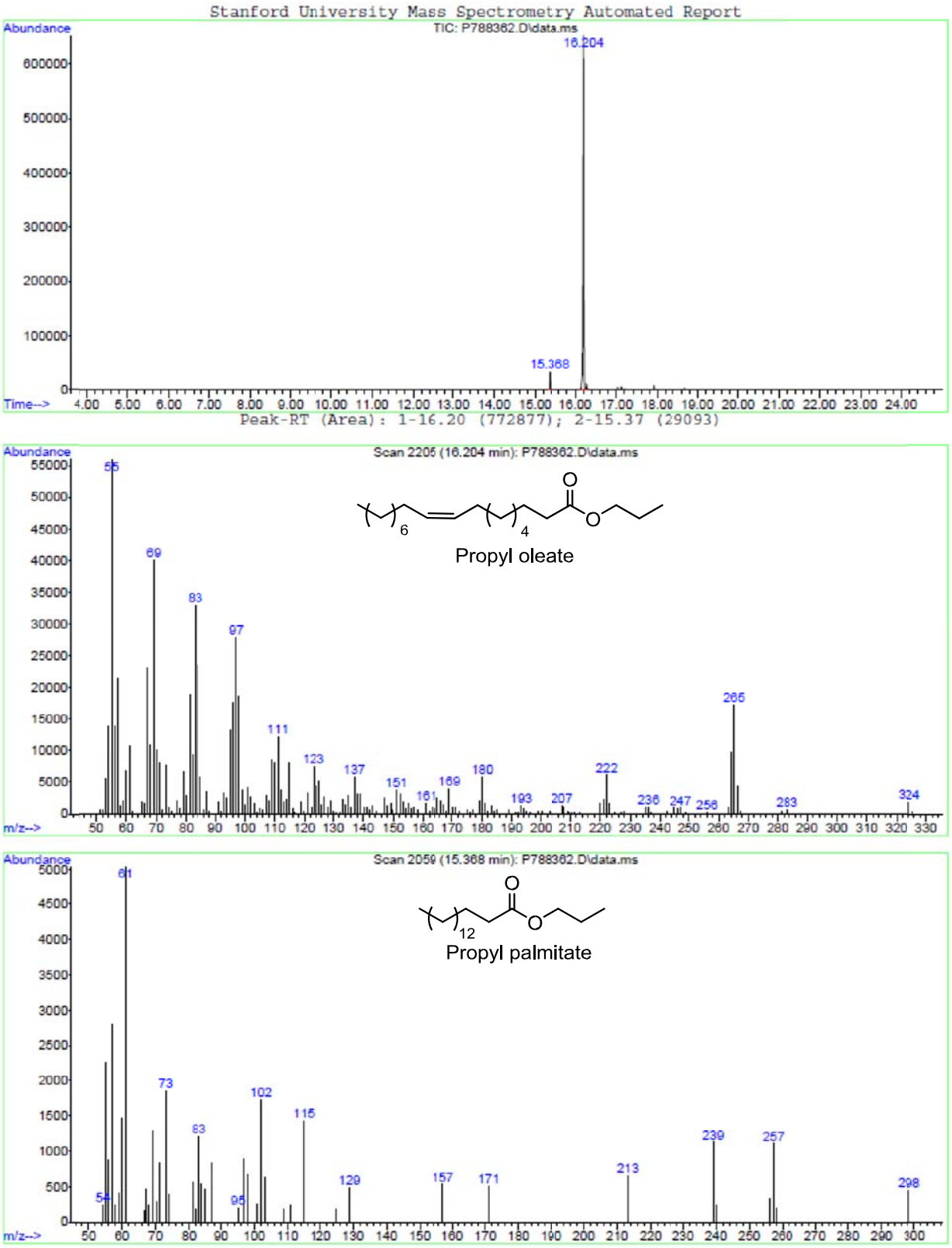
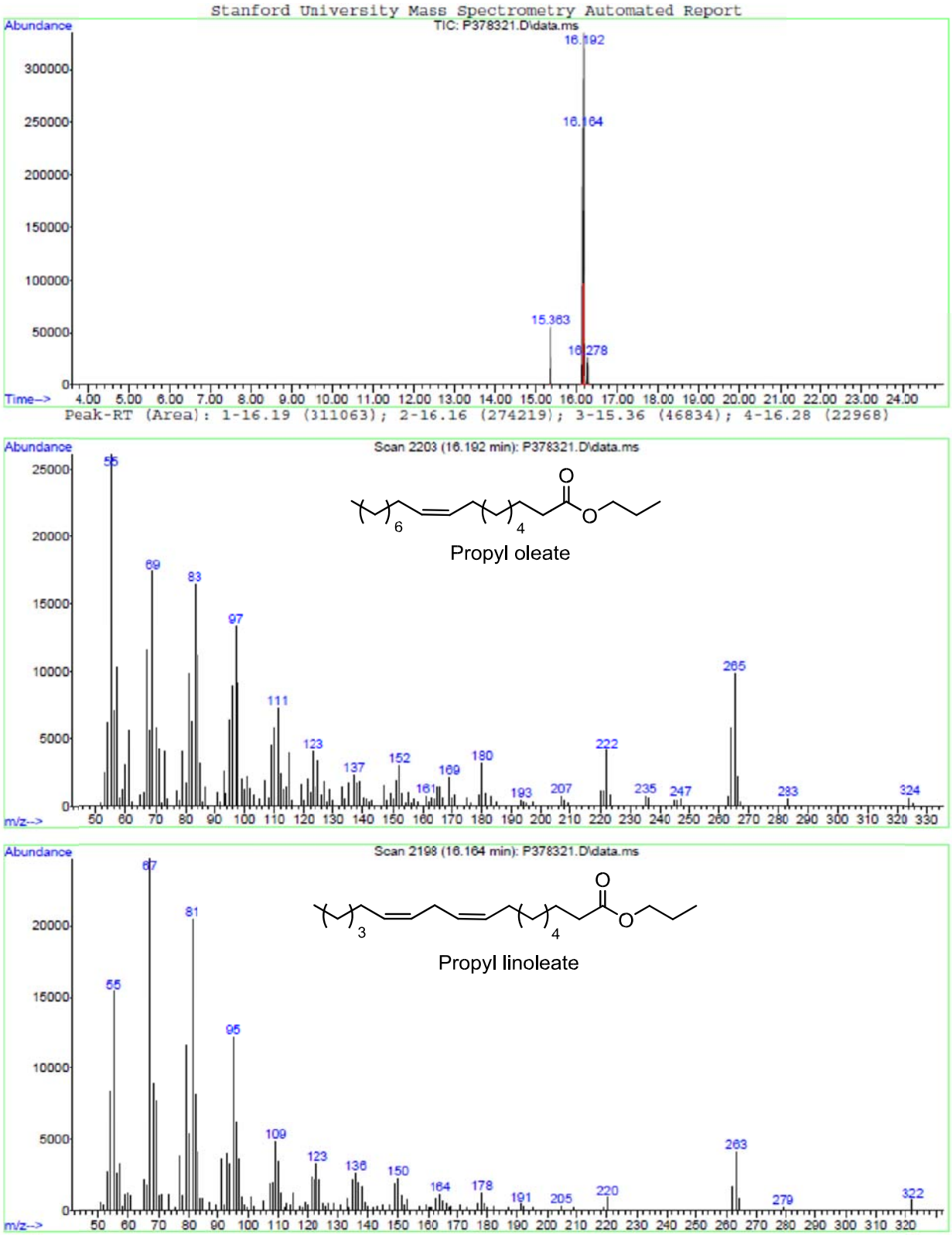
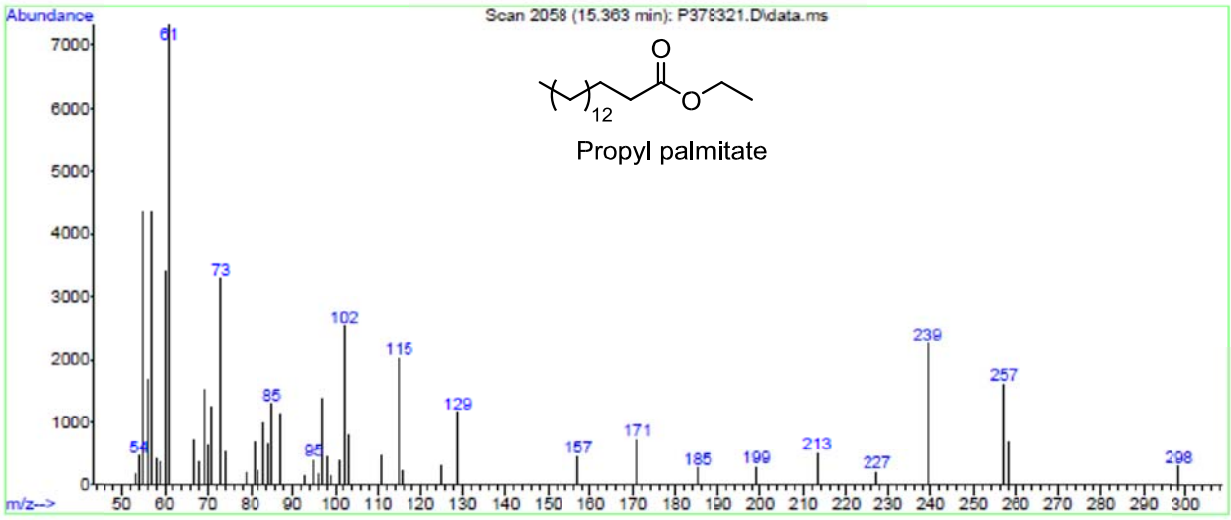


Figure SM 3.1.20.18 — GC-MS of pumpkin oil propyl esters.




Abundance
Scan 2058 (15.363 min): P378321.D\data.ms
61
73
102
115
129
85
54
95
157
171
185
199
213
227
239
257
298
m/z-->
12
Propyl palmitate

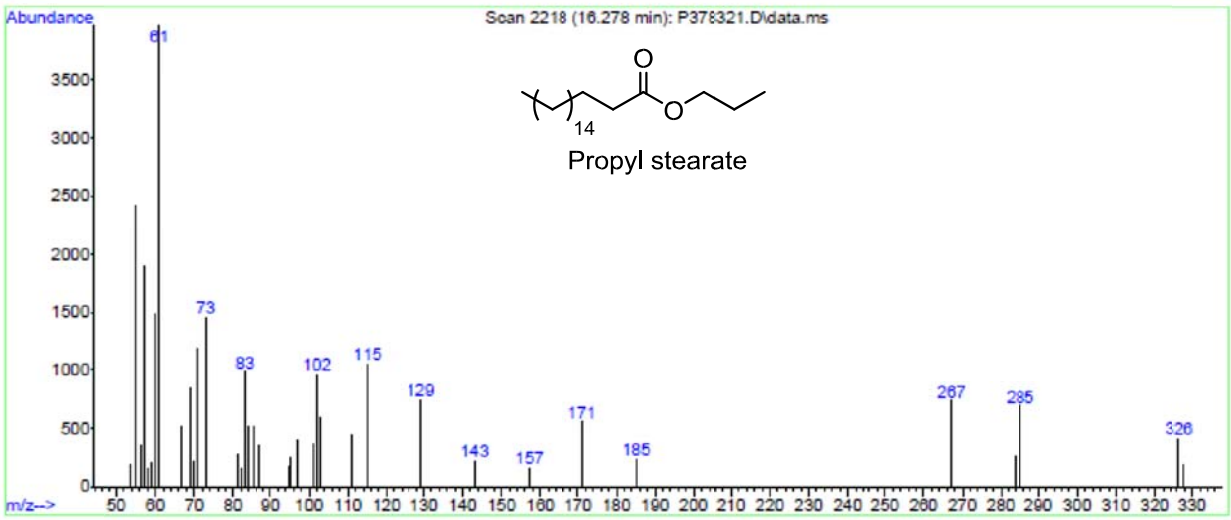
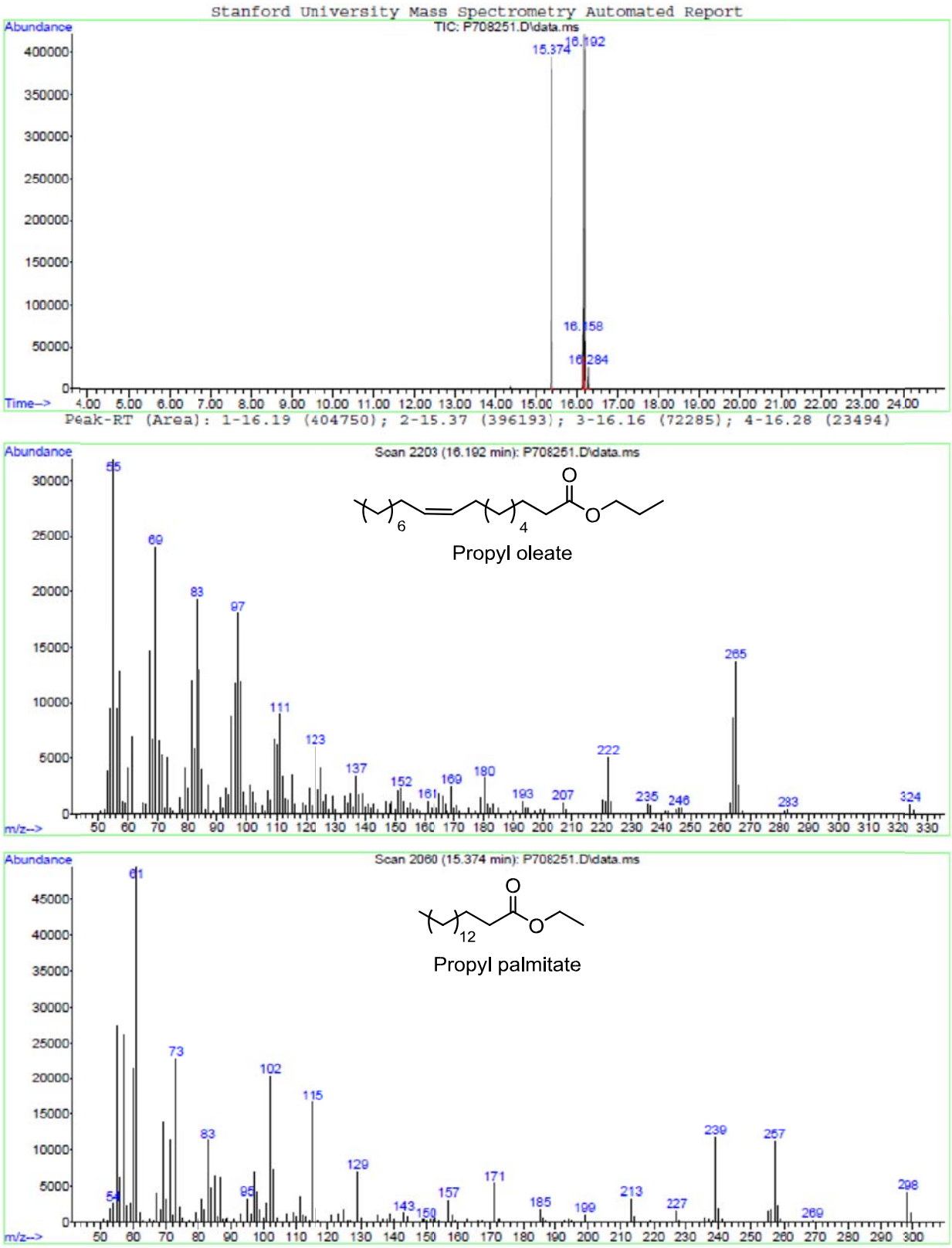
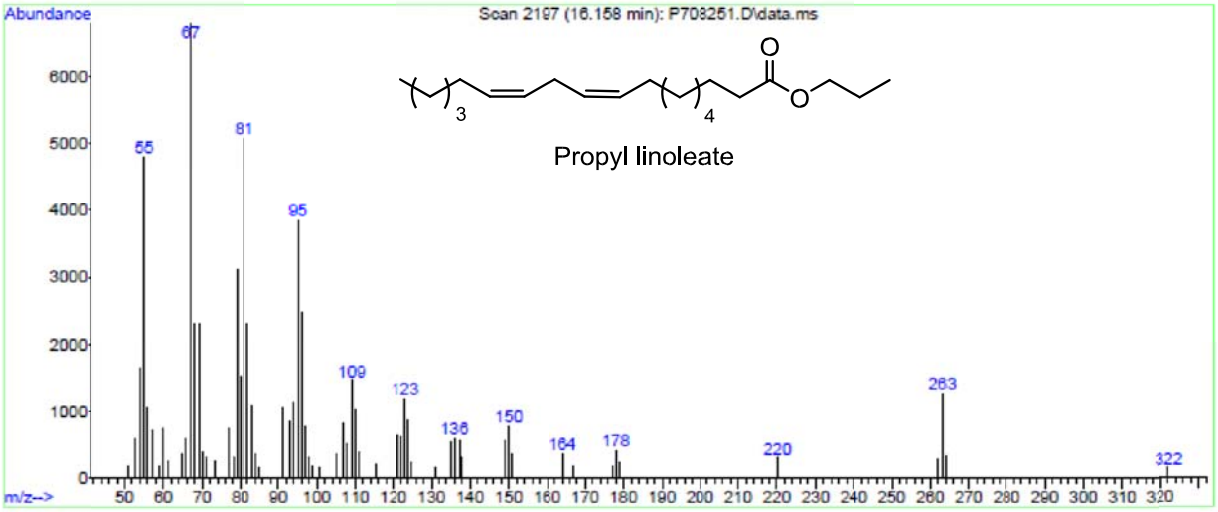

Abundance
Scan 2218 (16.278 min): P378321.D\data.ms
61
73
83
102
115
129
143
157
171
185
267
285
326
m/z-->
14
Propyl stearate

Figure SM 3.1.20.19 — GC-MS of red palm oil propyl esters.




Abundance
Scan 2197 (16.158 min): P708251.D\data.ms
67
81
55
95
109
123
136
150
164
178
220
263
322
m/z-->
Propyl linoleate

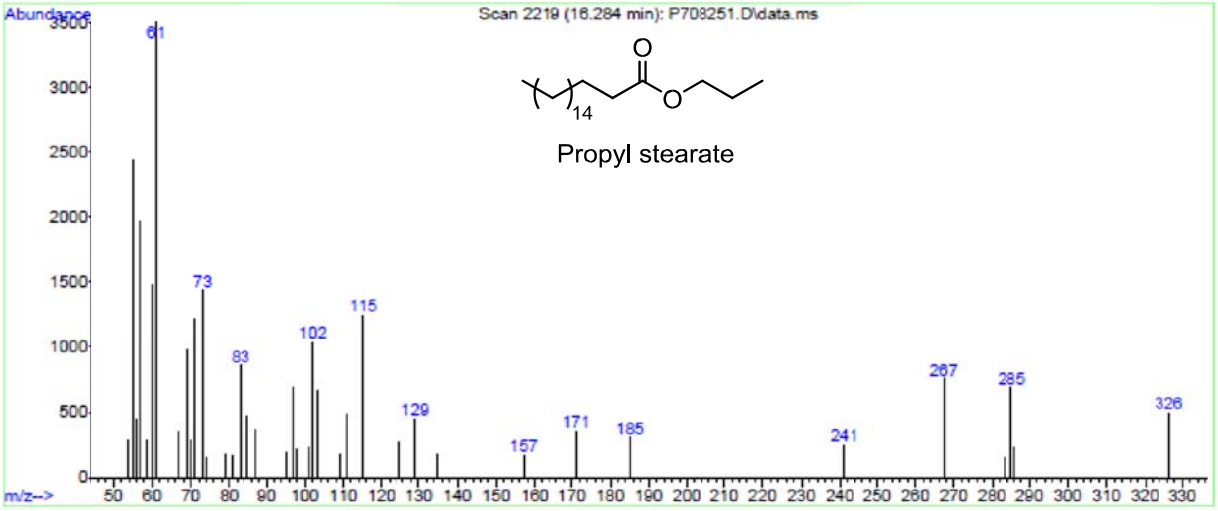
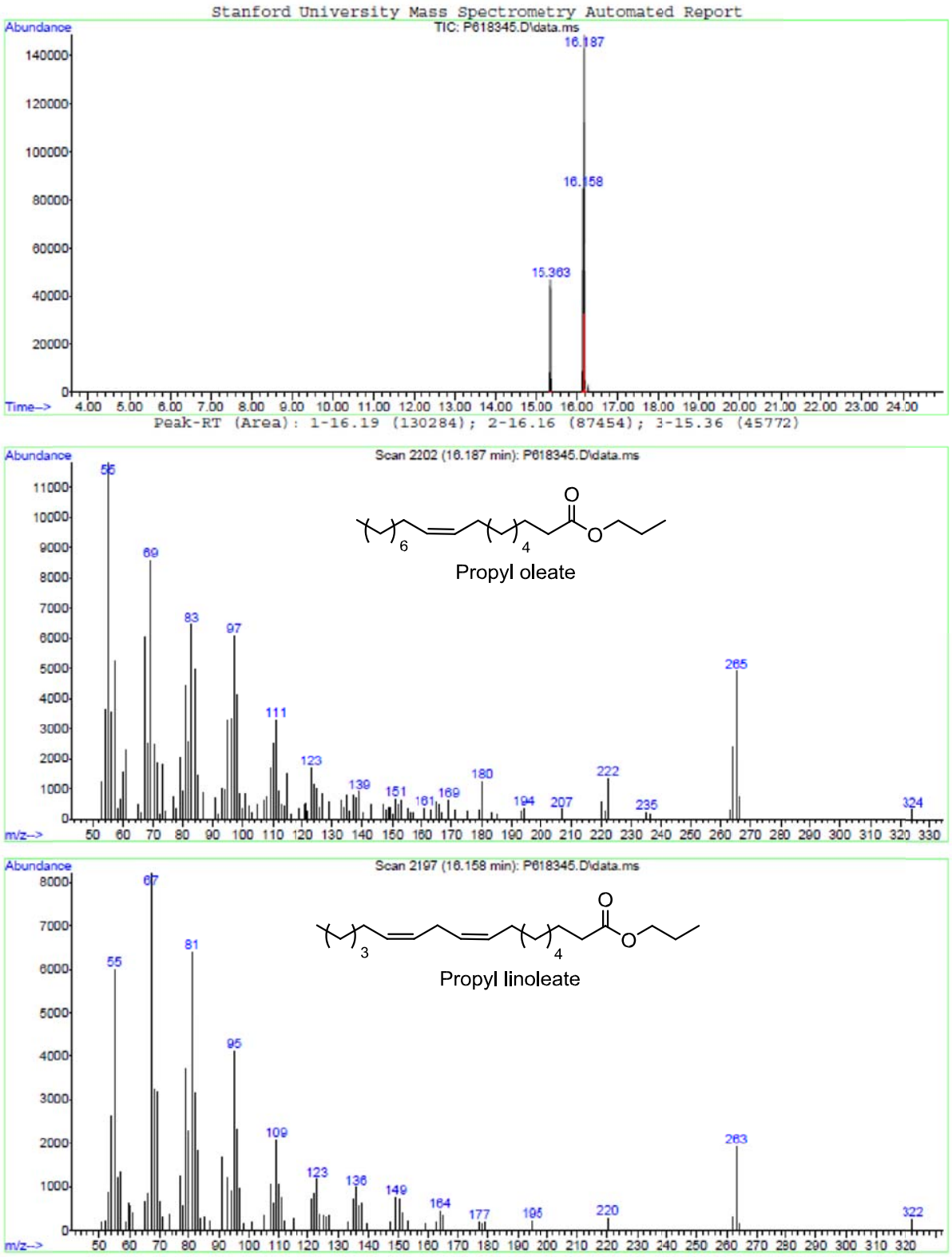

Abundance
Scan 2219 (16.284 min): P708251.D\data.ms
61
73
83
102
115
129
157
171
185
241
267
285
326
m/z-->
Propyl stearate

Figure SM 3.1.20.20 — GC-MS of rice bran oil propyl esters.



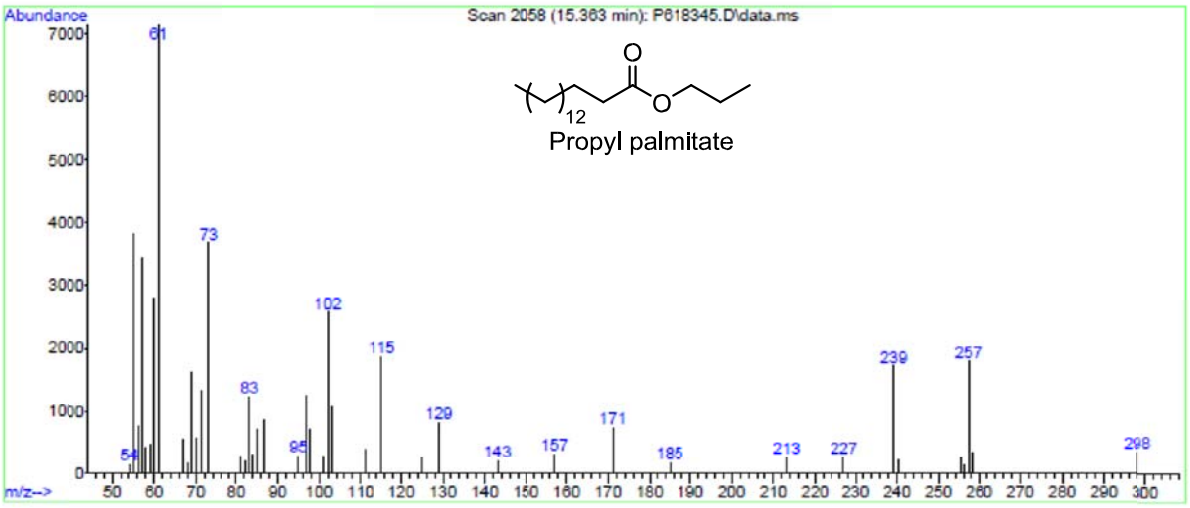

Abundance
Scan 2058 (15.363 min): P618345.D\data.ms
7000
6000
5000
4000
3000
2000
1000
0
m/z-->
61
73
83
95
102
115
129
143
157
171
185
213
227
239
257
298
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300
O
O
12
Propyl palmitate

Figure SM 3.1.20.21 — GC-MS of safflower oil propyl esters.

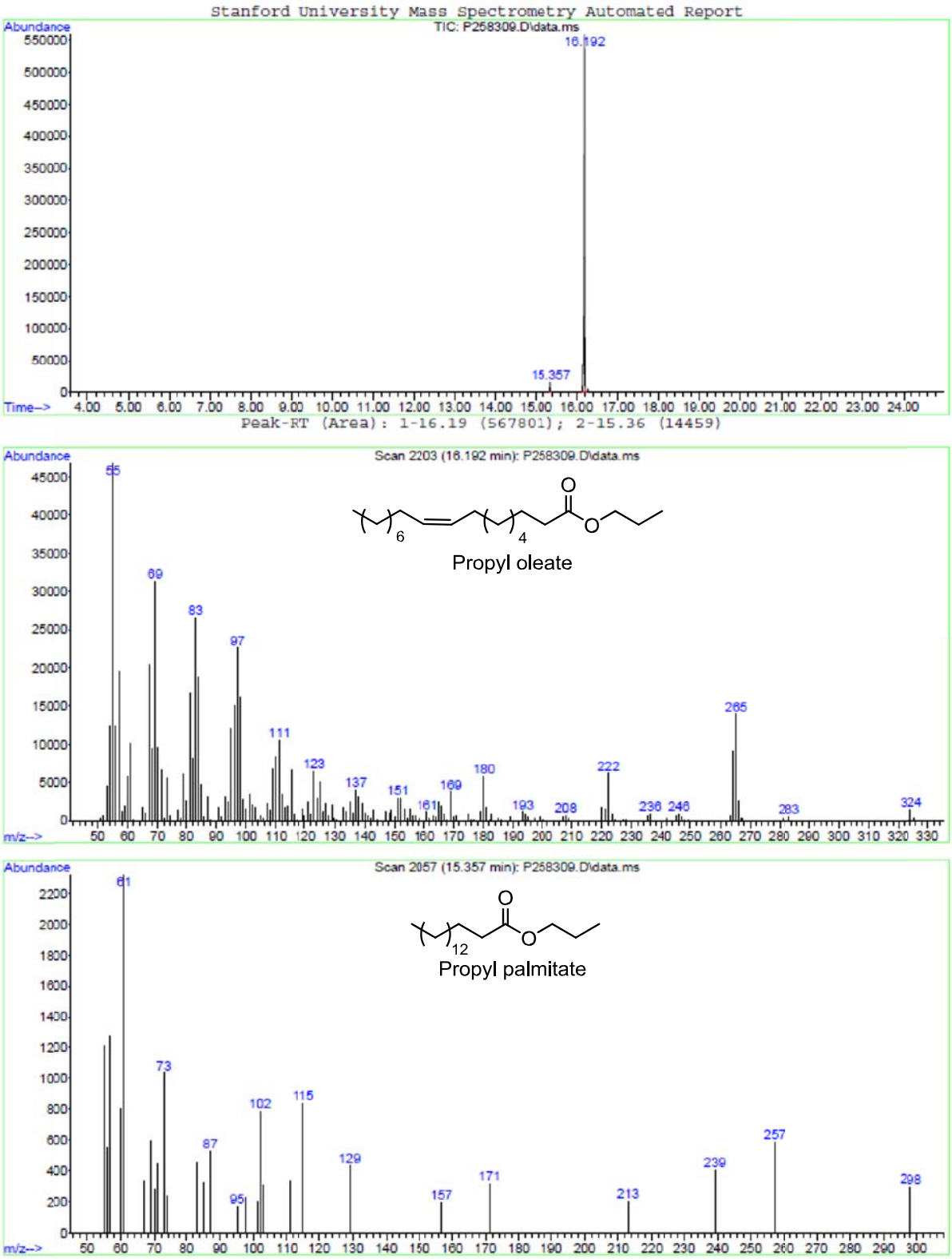
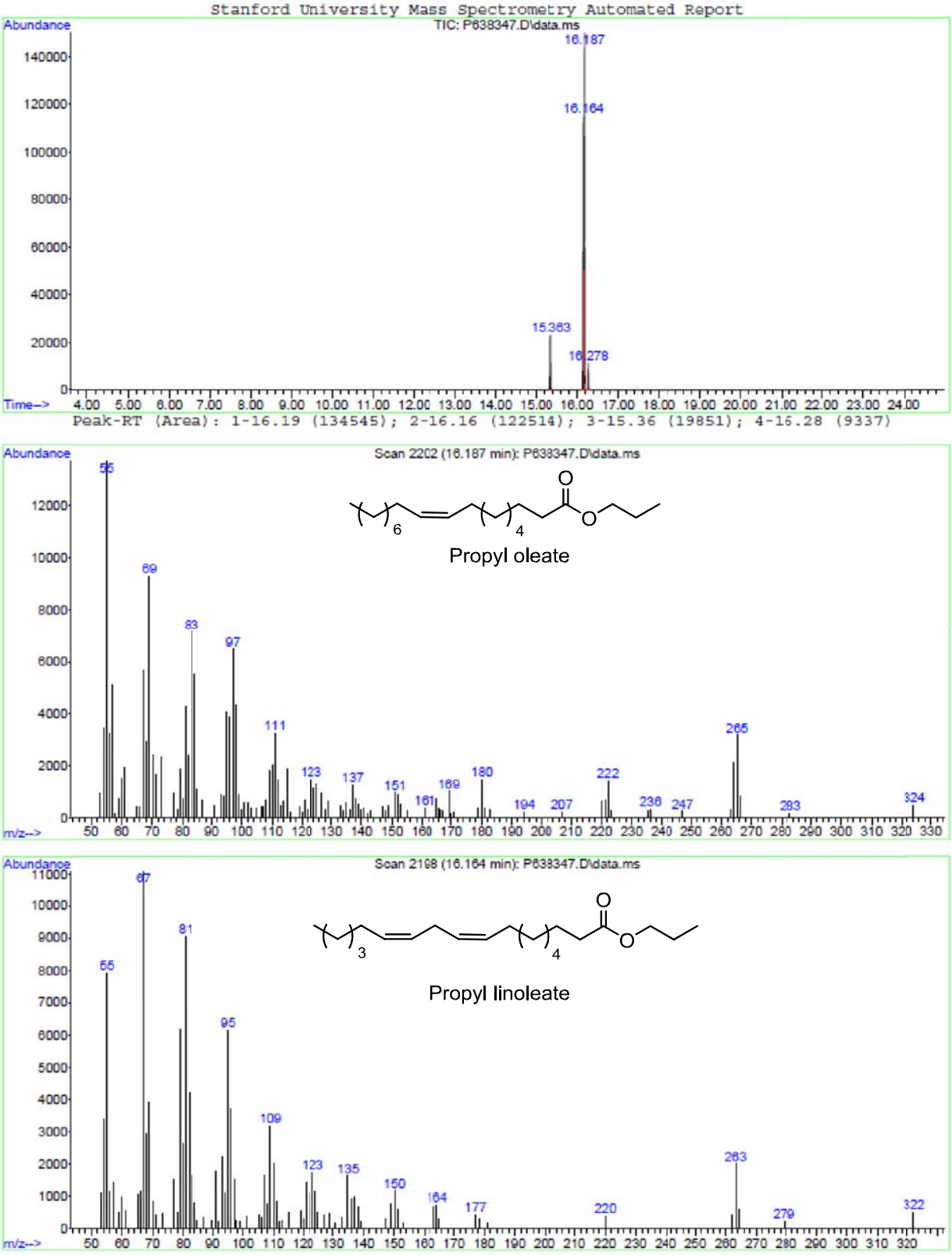
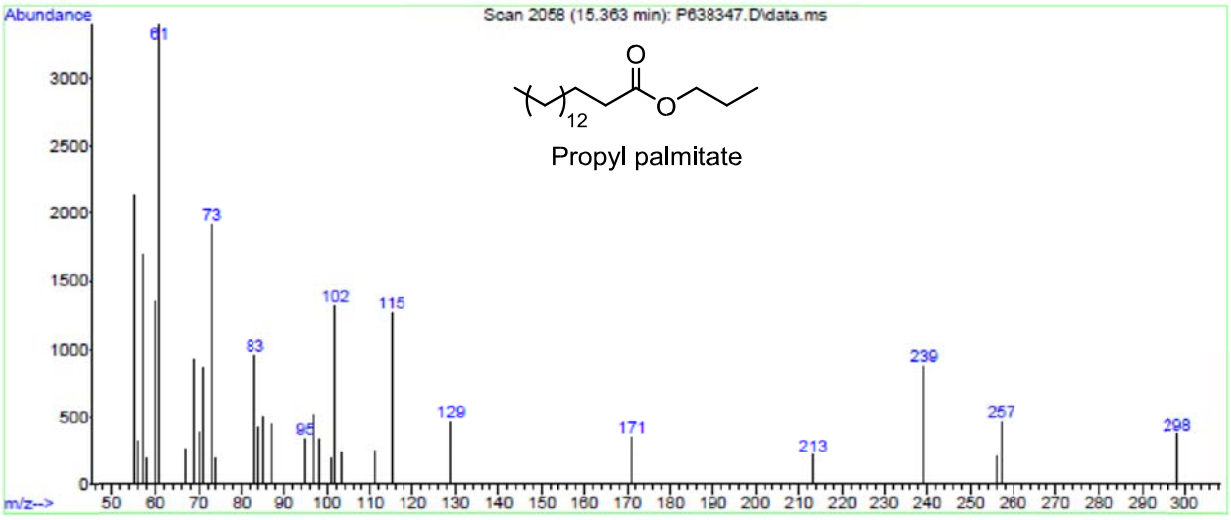


Figure SM 3.1.20.22 — GC-MS of sesame oil propyl esters.




Abundance
Scan 2058 (15.363 min): P638347.D\data.ms
61
73
83
95
102
115
129
171
213
239
257
298
m/z-->
Propyl palmitate

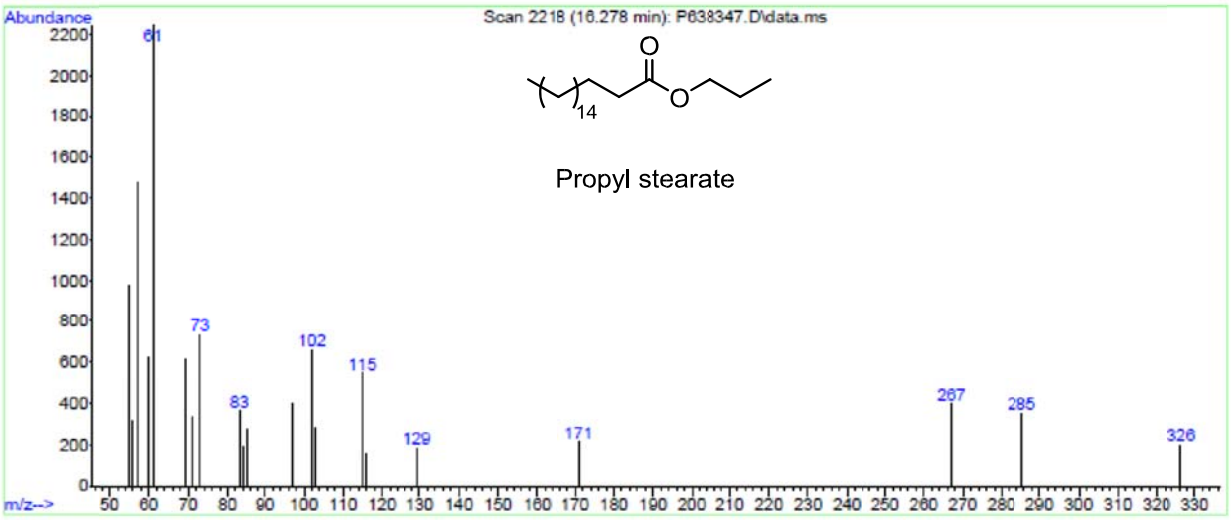
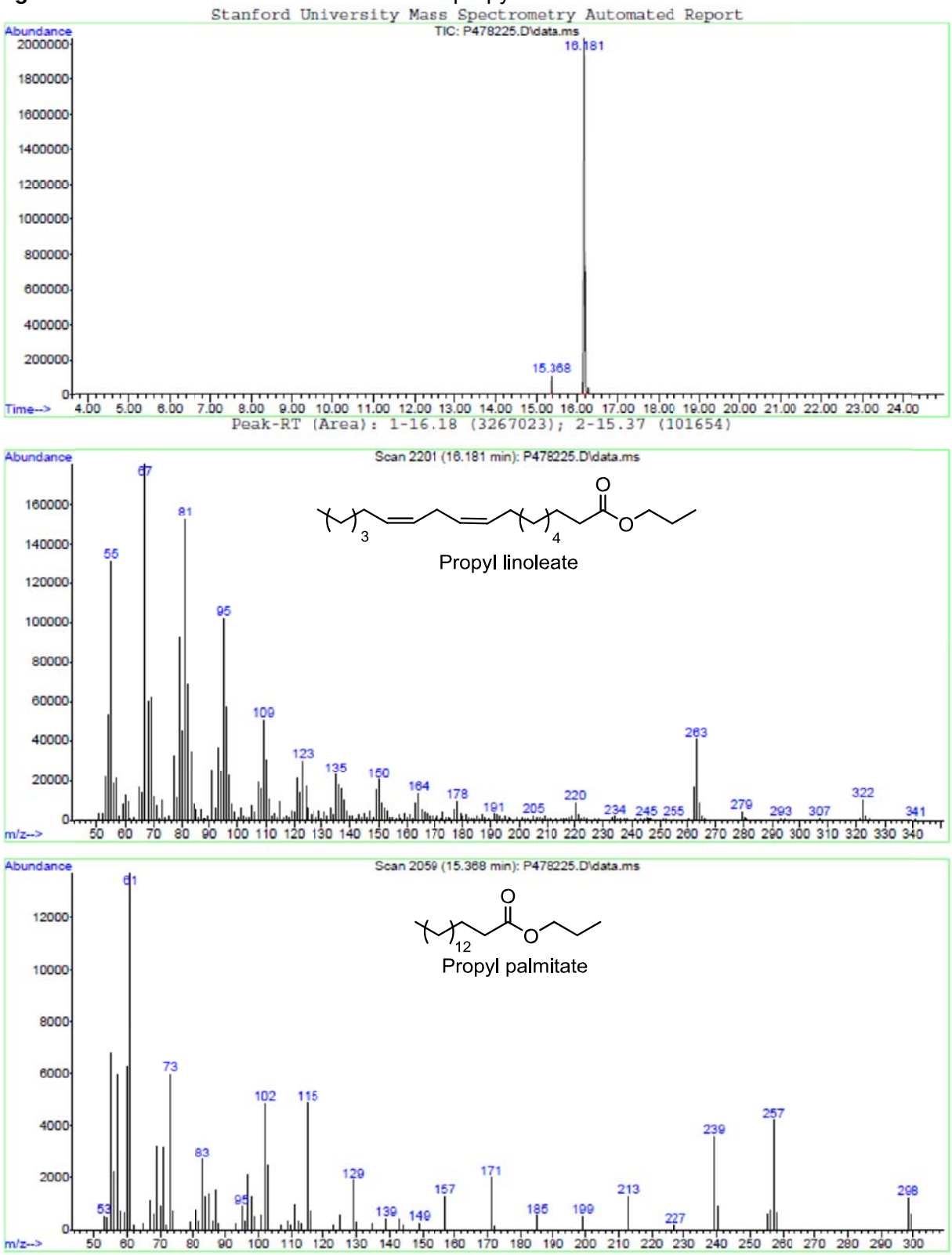

Abundance
Scan 2218 (16.278 min): P638347.D\data.ms
61
73
83
102
115
129
171
267
285
326
m/z-->
Propyl stearate

Figure SM 3.1.20.23 — GC-MS of walnut oil propyl esters.



Student ^{1}H-NMR spectra

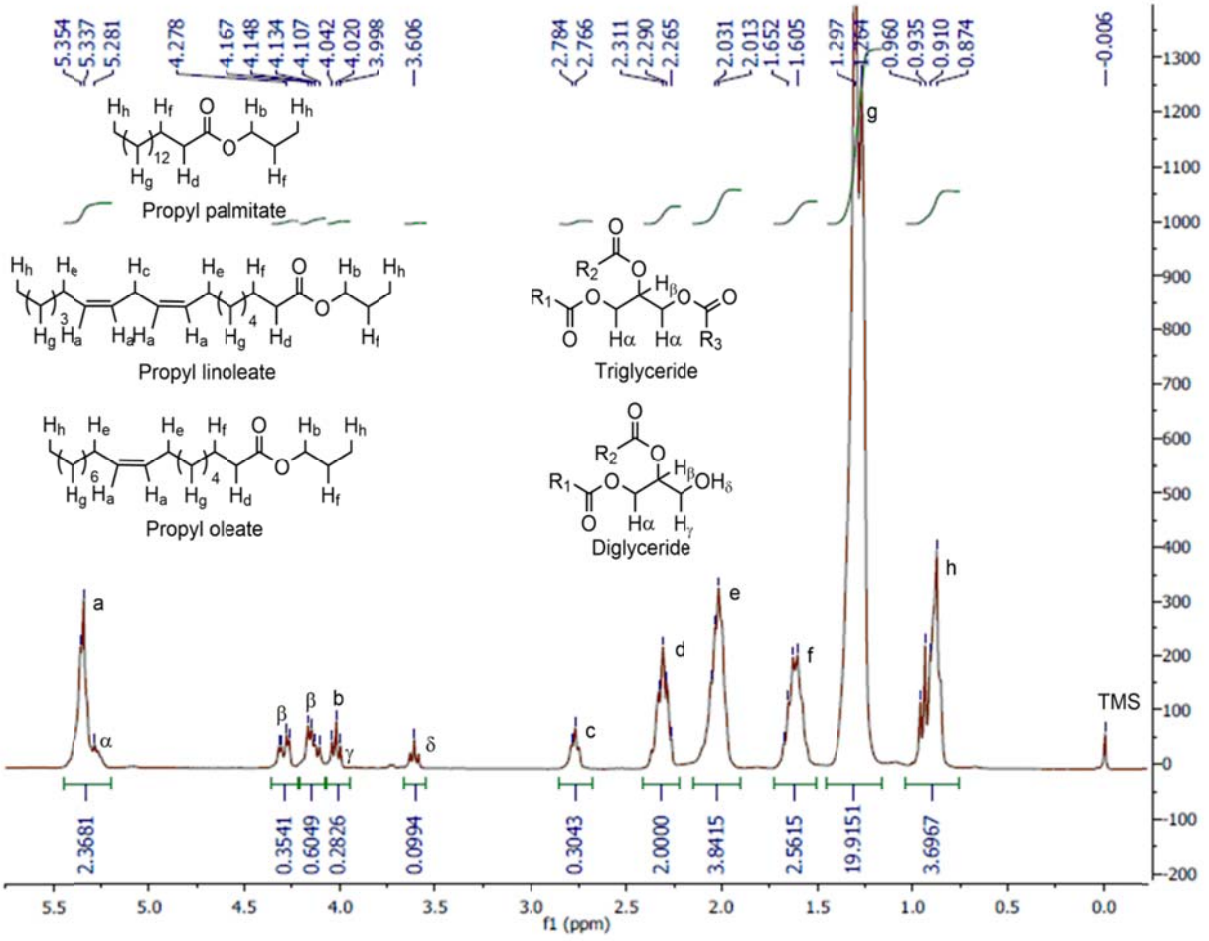


Figure SM 3.1.20.24 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of almond oil propyl esters. This reaction was run for one hour at 70 °C, instead of 100 °C, as stated in the protocol. As a result, the peaks labeled α, ß, γ, and δ indicate that the starting material did not fully react.

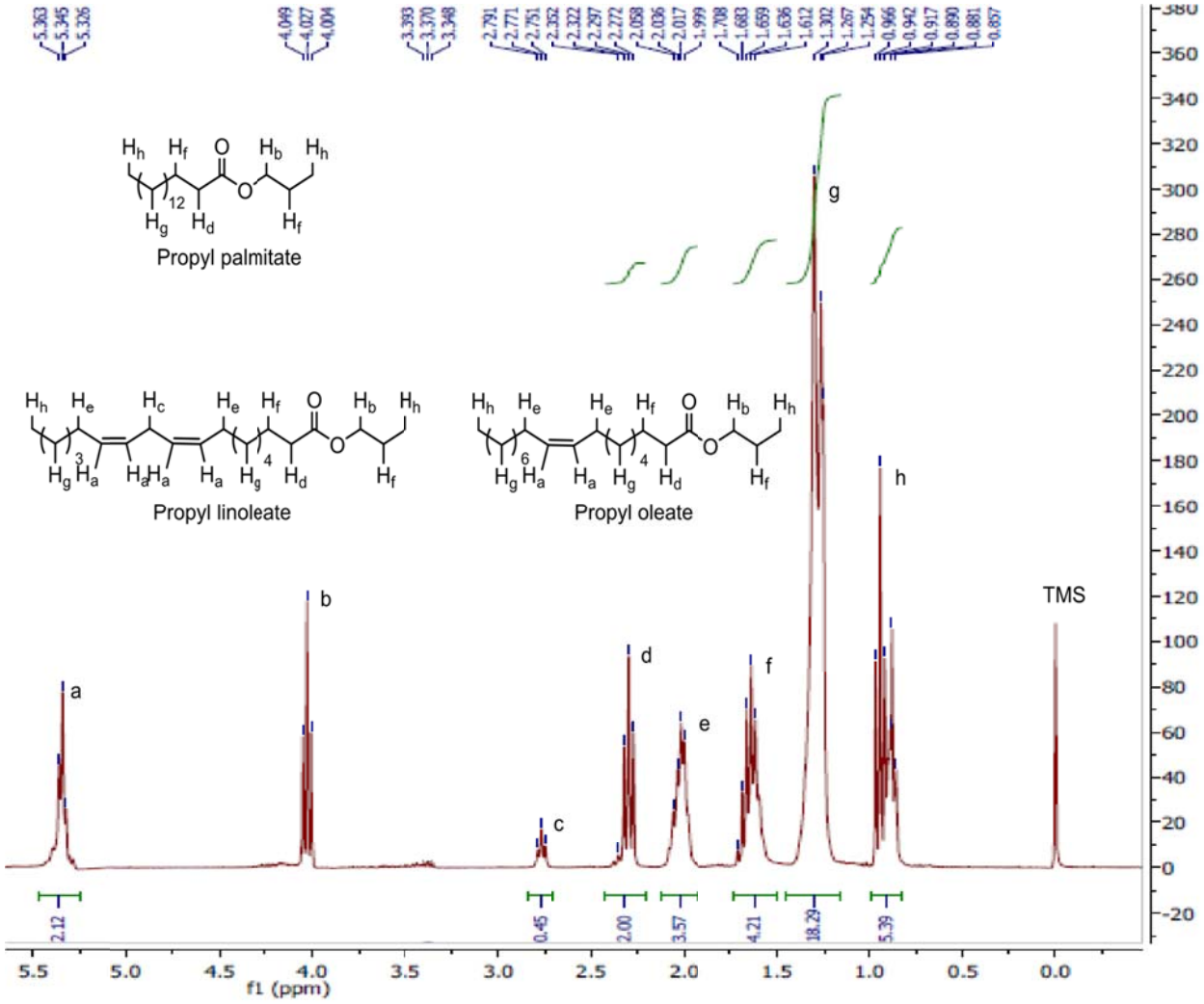


Figure SM 3.1.20.25 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of almond oil propyl esters. This reaction was refluxed for one hour at 100 °C.

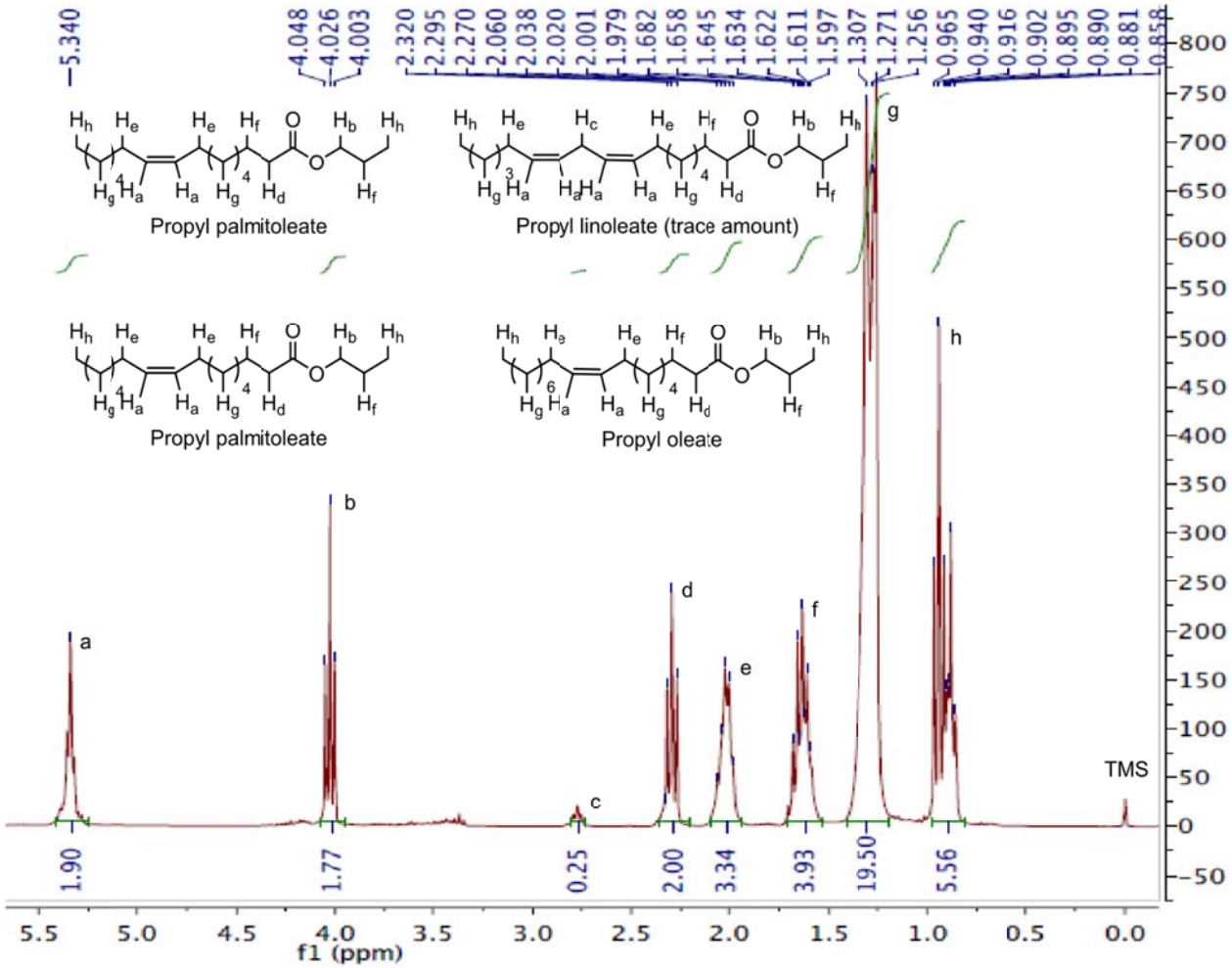


Figure SM 3.1.20.26 —^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of avocado oil propyl esters.

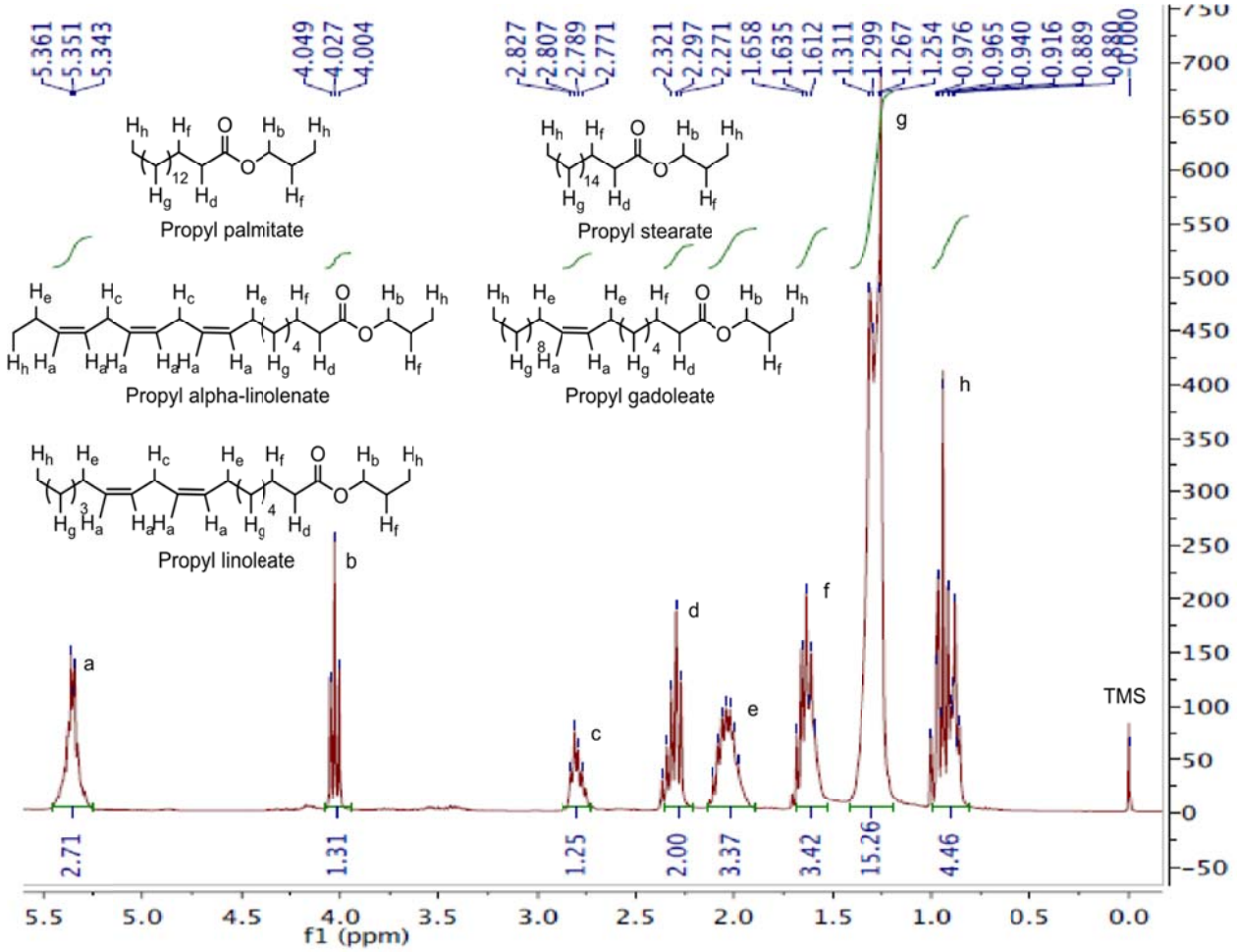


Figure SM 3.1.20.27 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of camelina oil propyl esters.

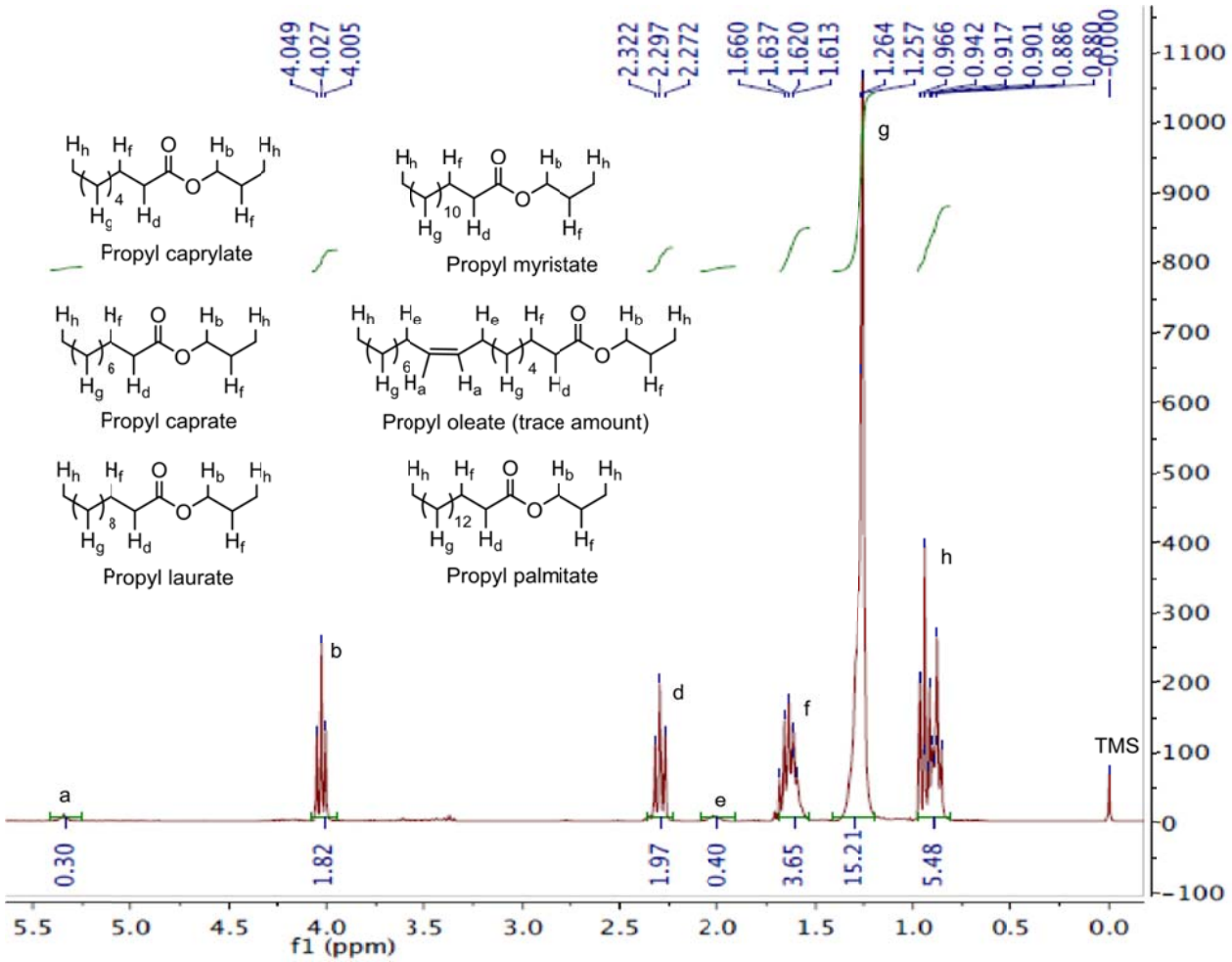


Figure SM 3.1.20.28 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of coconut oil propyl esters.

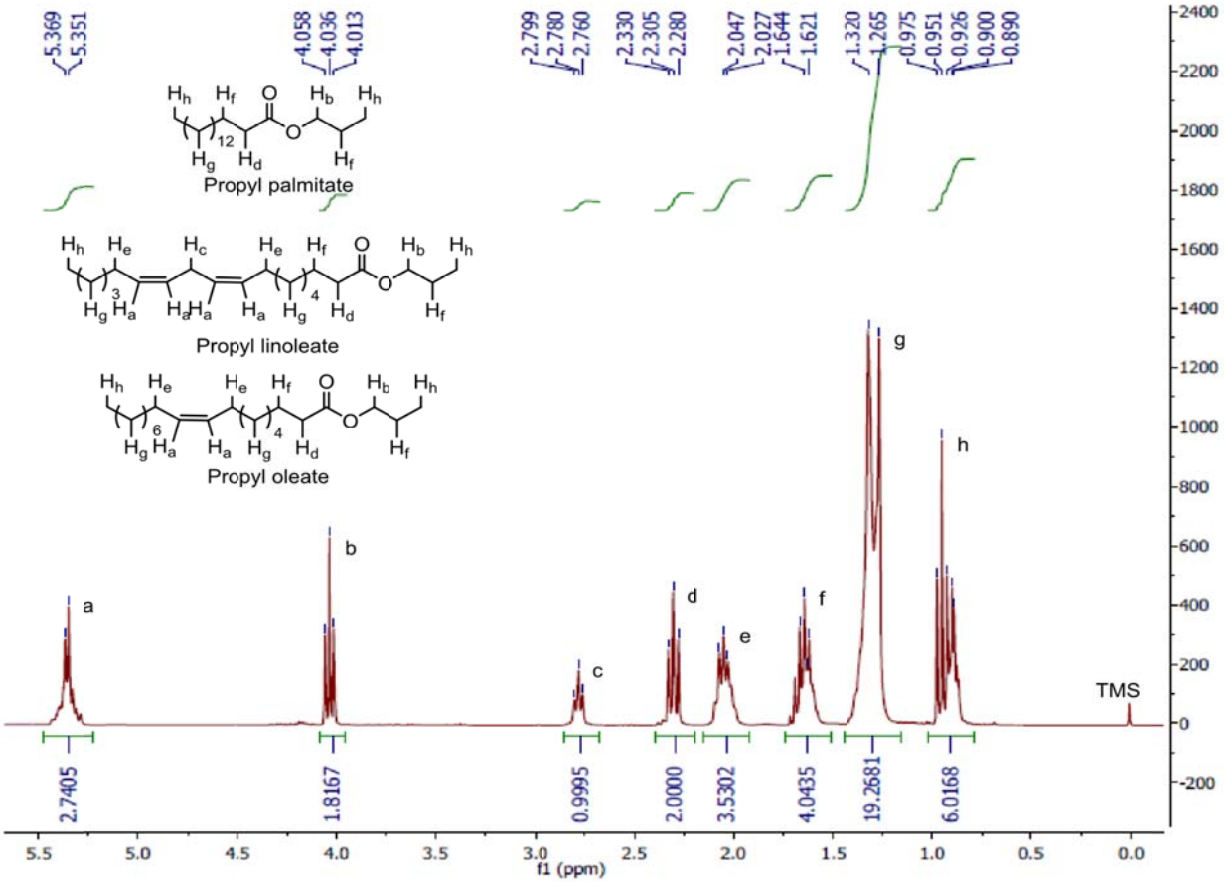


Figure SM 3.1.20.29 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of corn oil propyl esters.

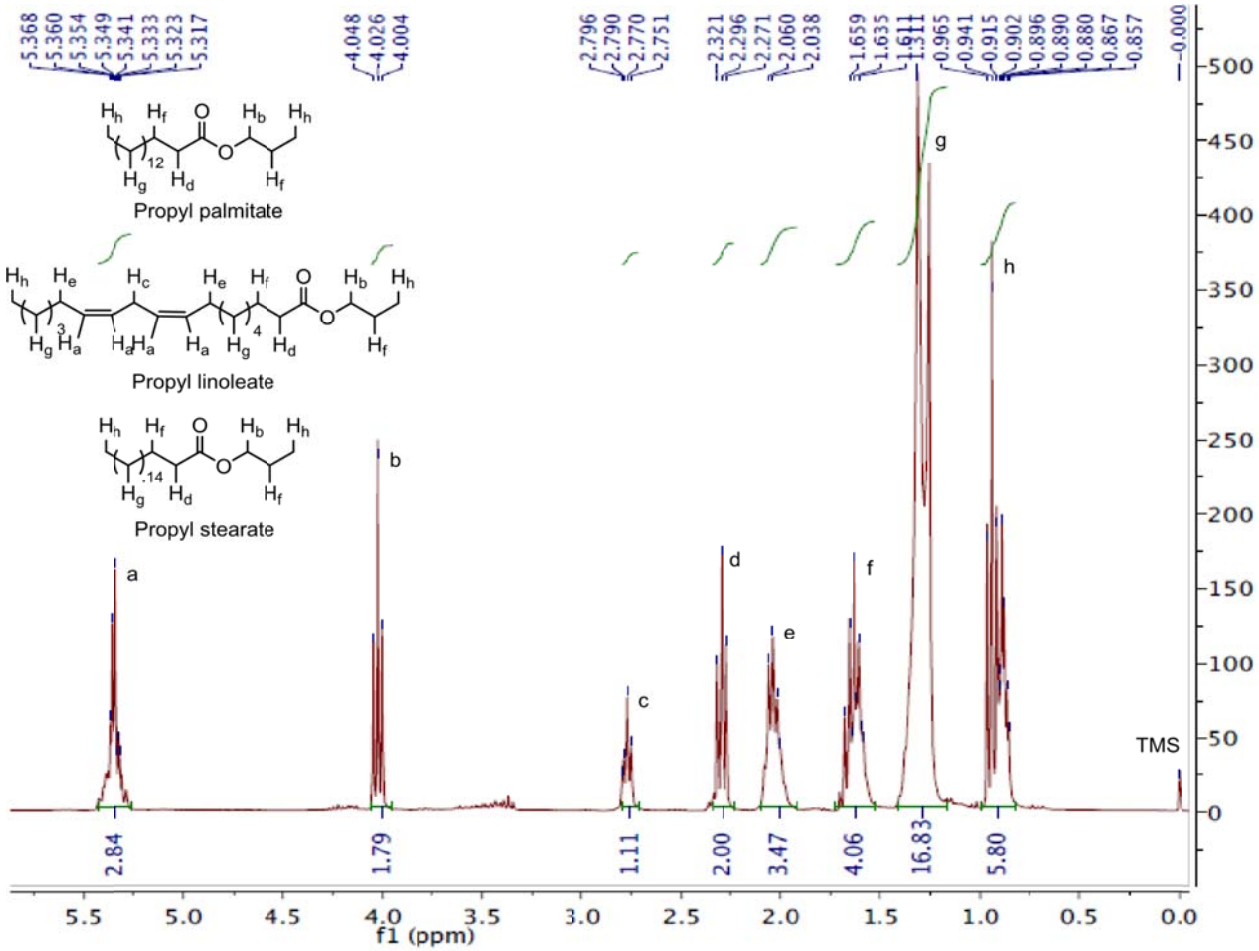


Figure SM 3.1.20.30 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of grapeseed oil propyl esters.

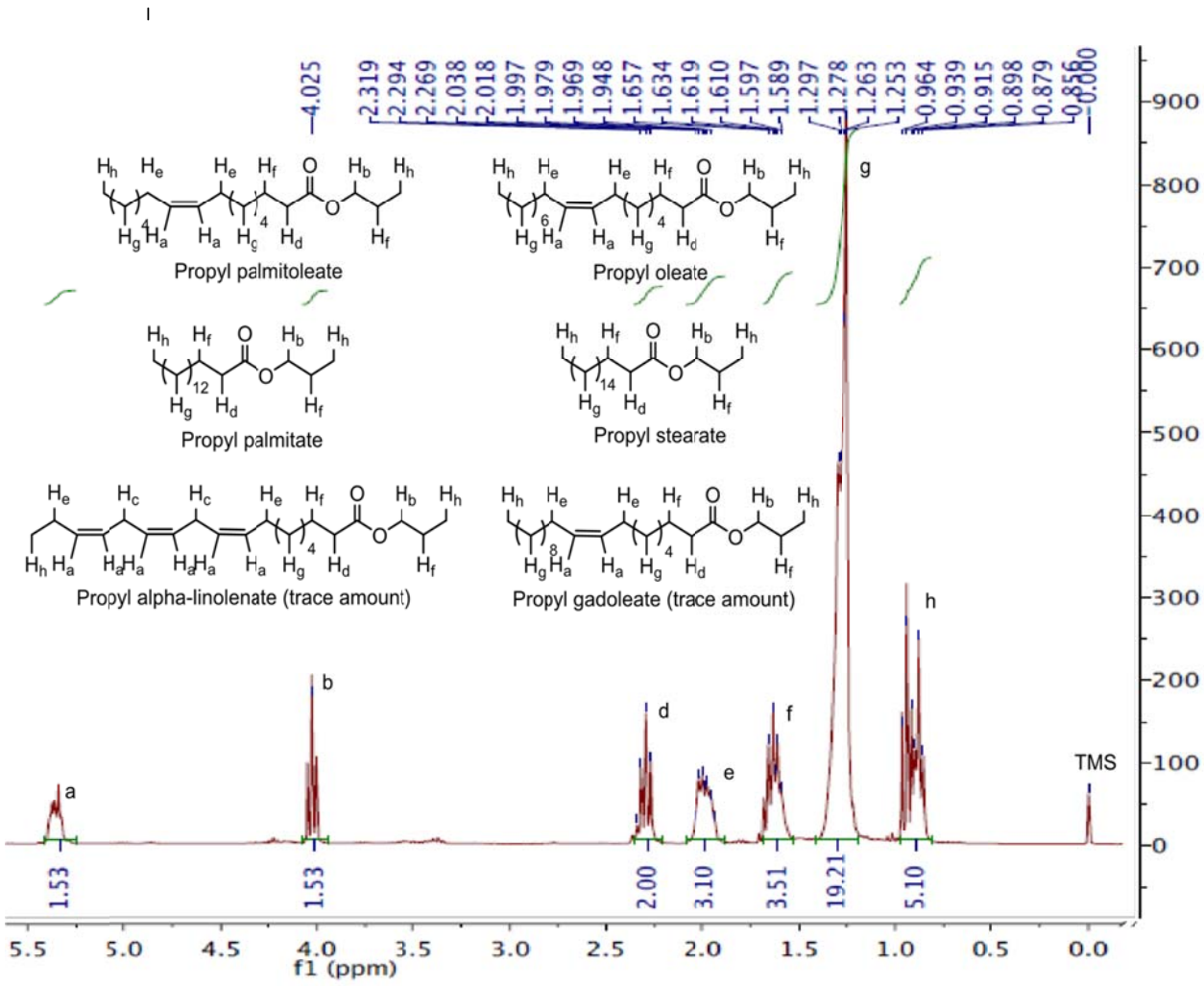


Figure SM 3.1.20.31 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of macadamia nut oil propyl esters.

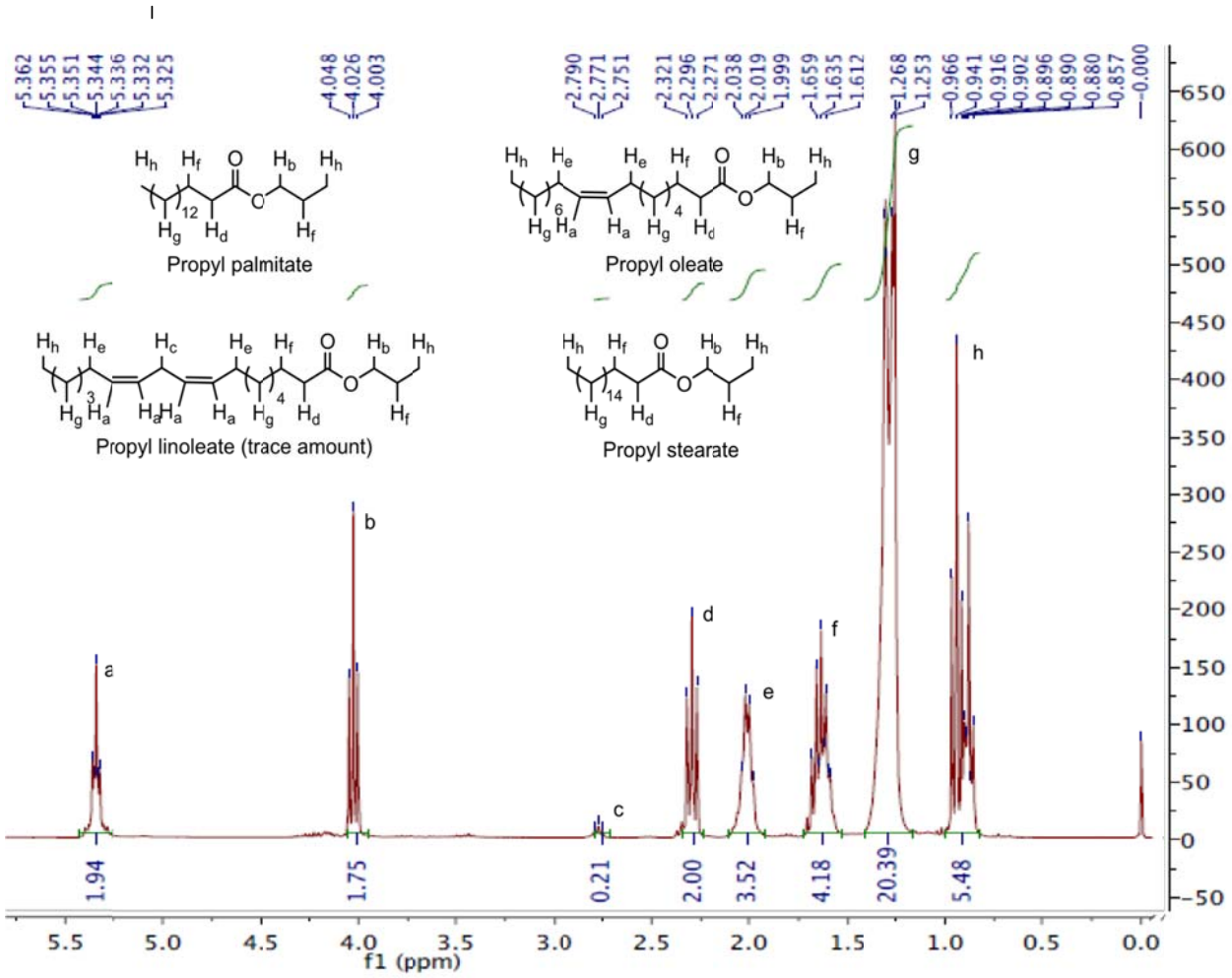


Figure SM 3.1.20.32 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of olive oil propyl esters.

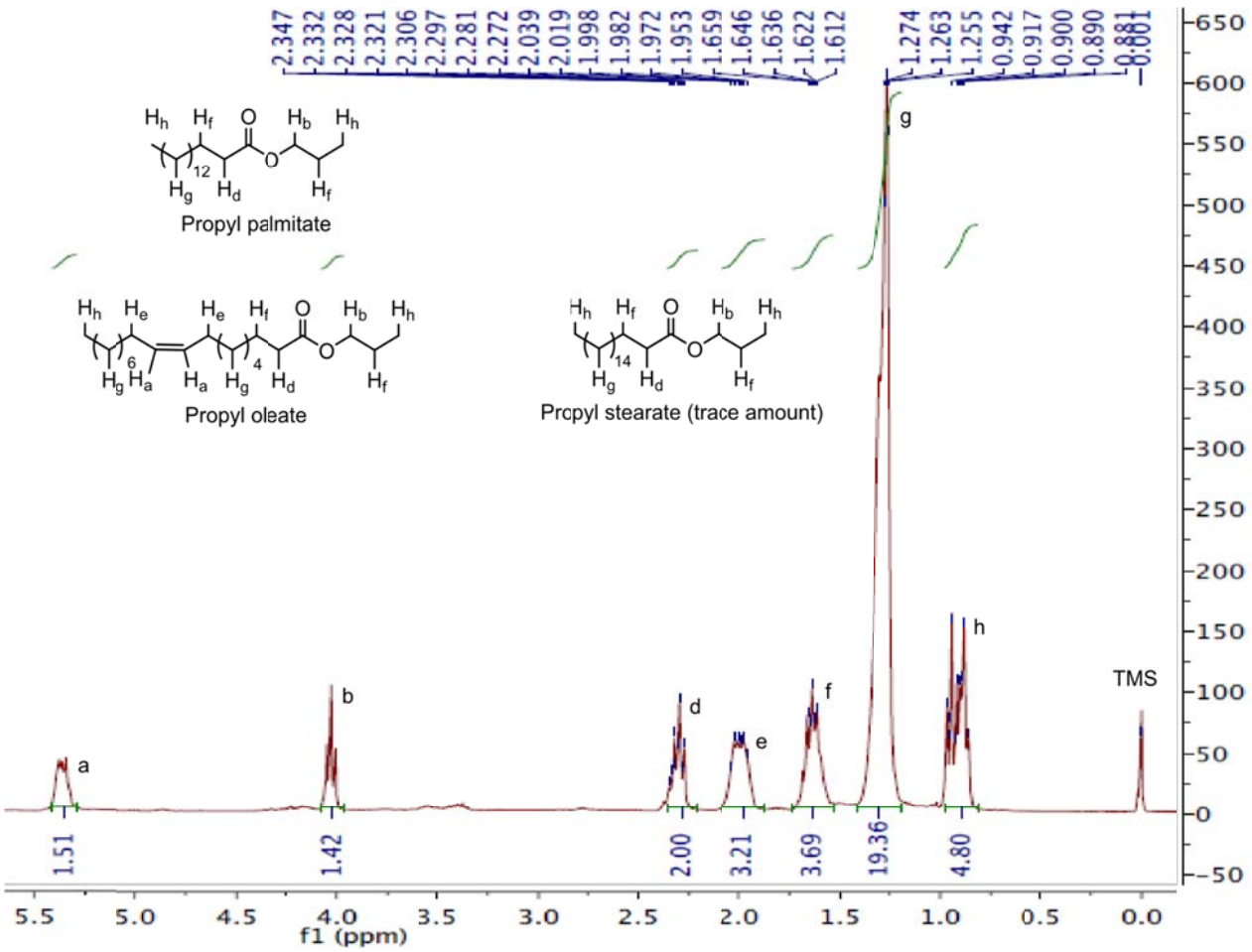


Figure SM 3.1.20.33 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of peanut oil propyl esters.

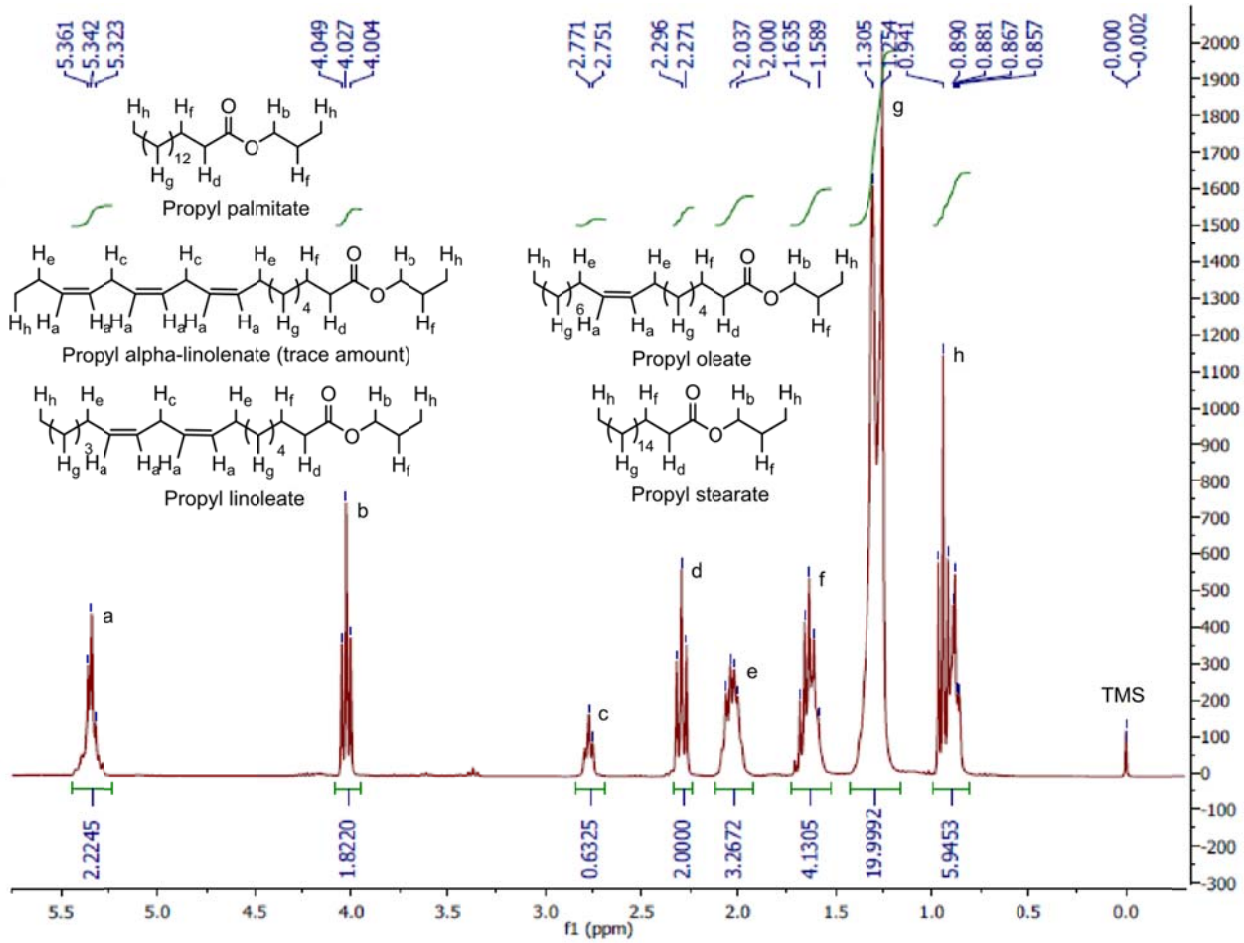


Figure SM 3.1.20.34 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of pumpkin oil propyl esters.

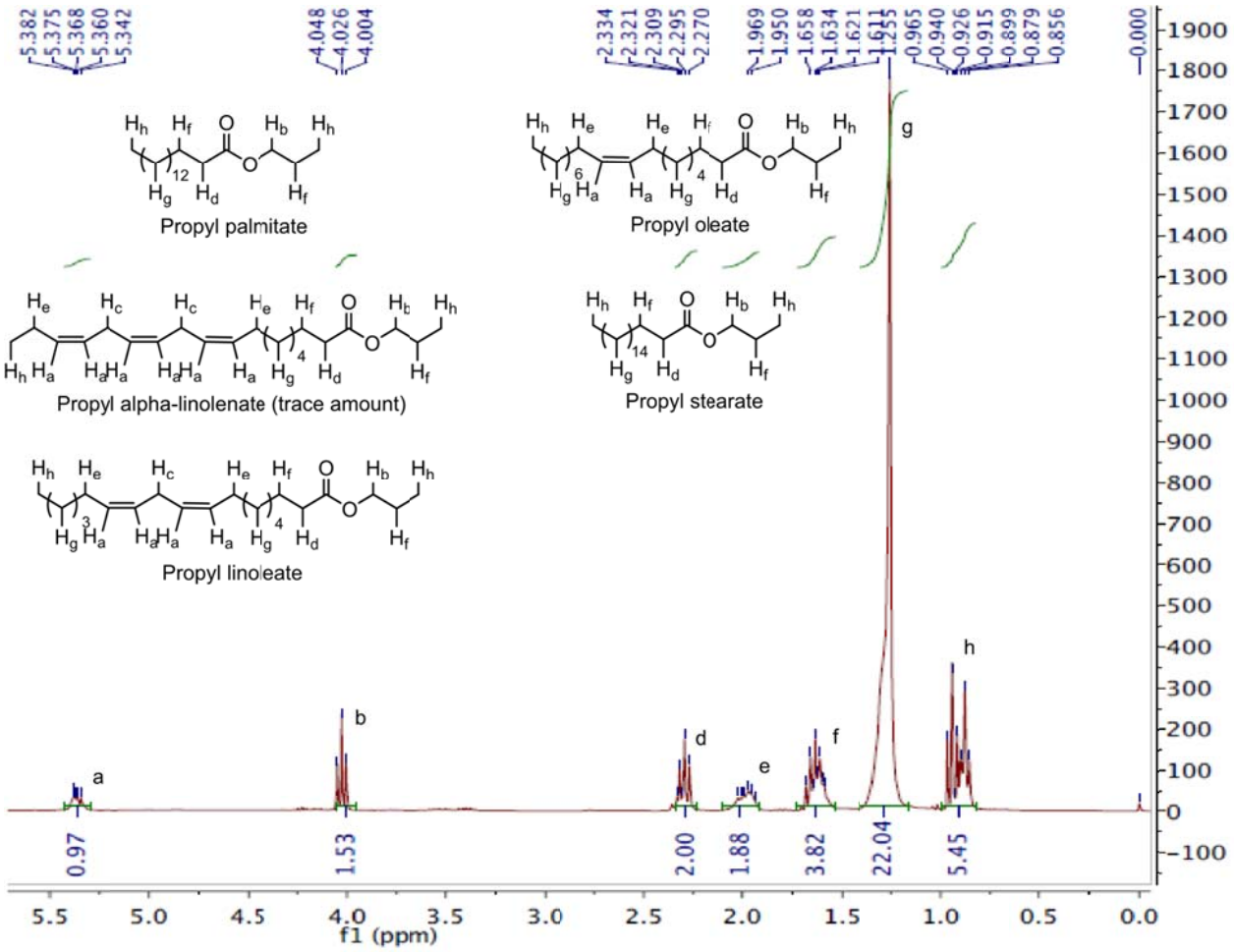


Figure SM 3.1.20.35 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of red palm oil propyl esters.

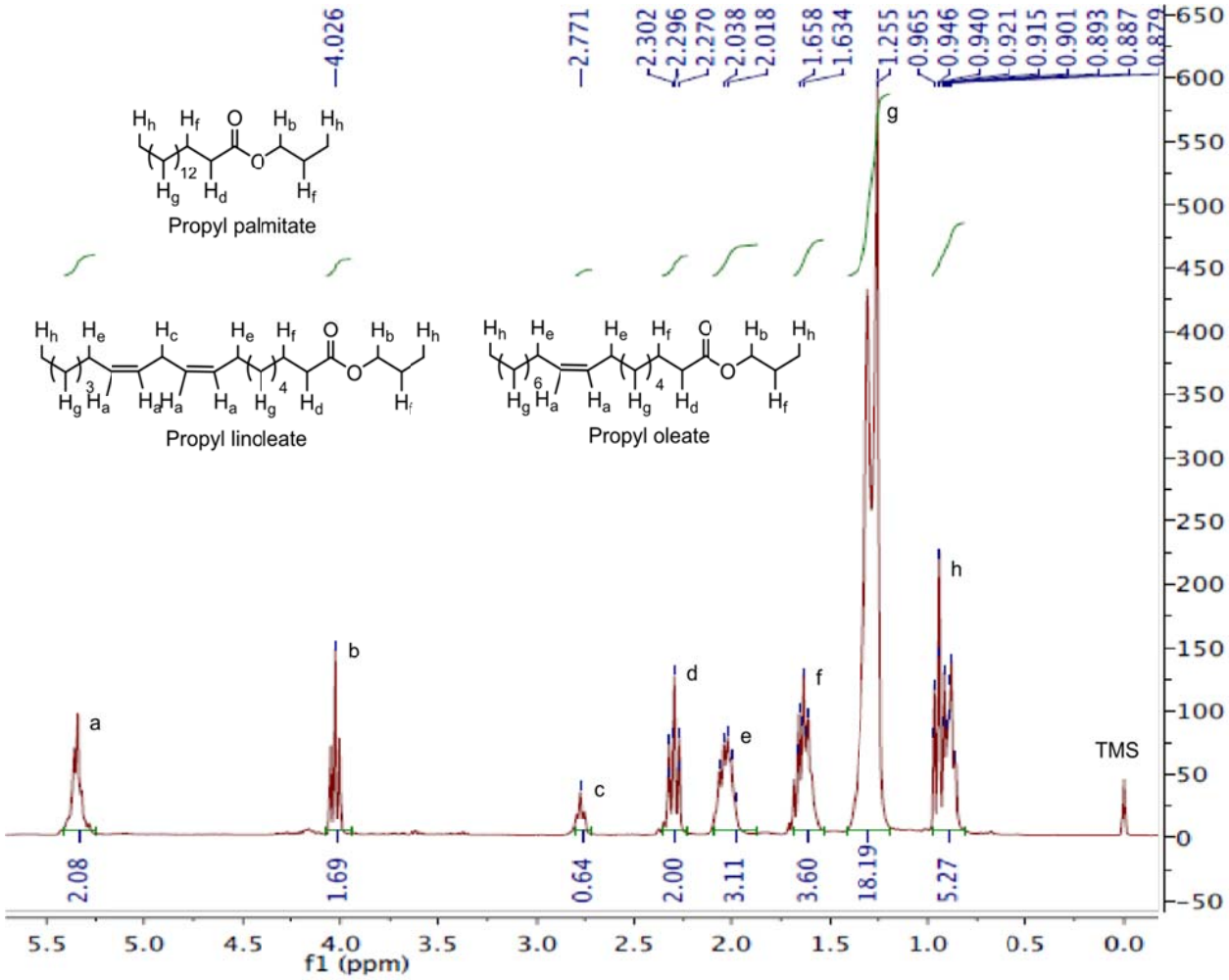


Figure SM 3.1.20.36 —^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of rice bran oil propyl esters.

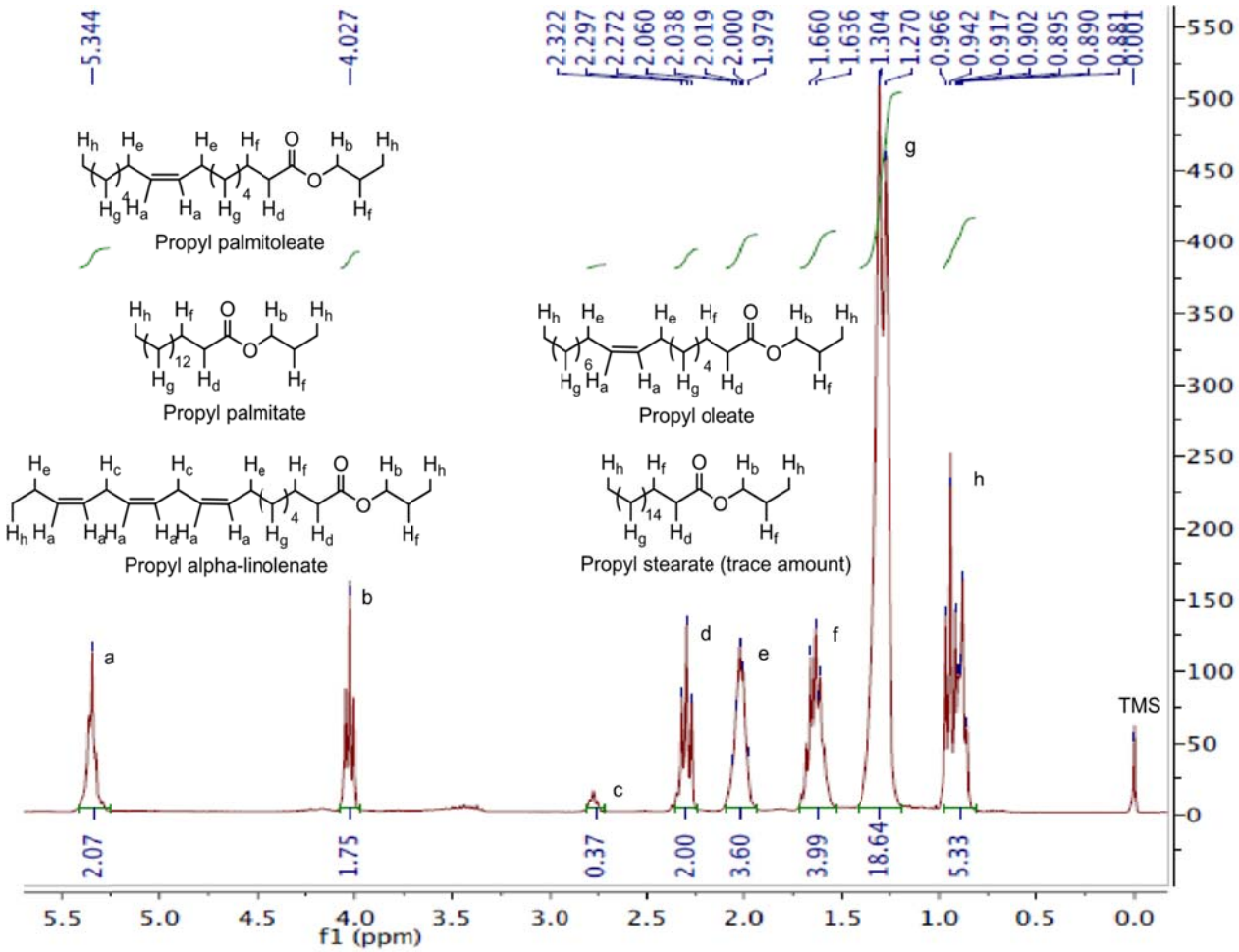


Figure SM 3.1.20.37 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of safflower oil propyl esters.

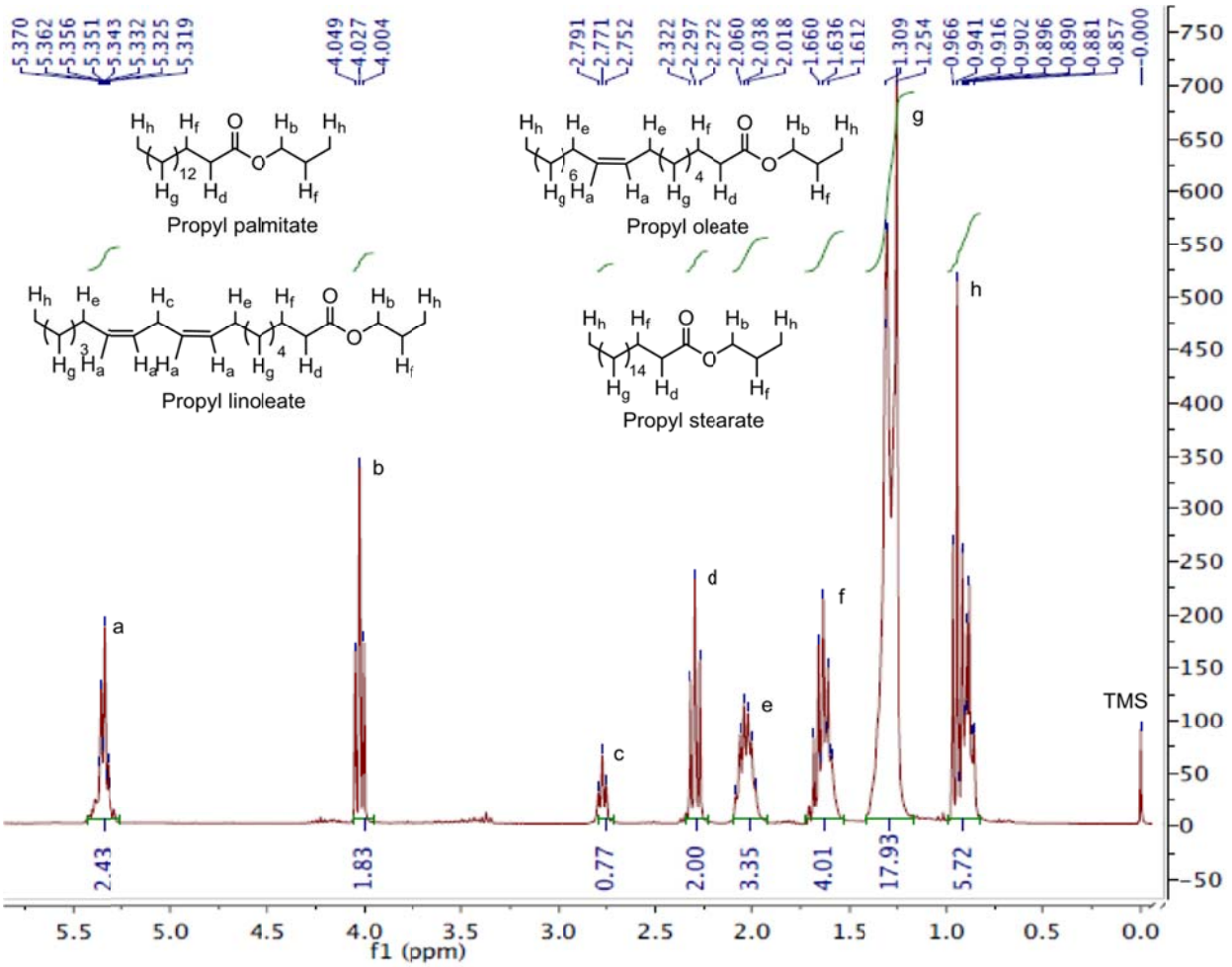


Figure SM 3.1.20.38 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of sesame oil propyl esters.

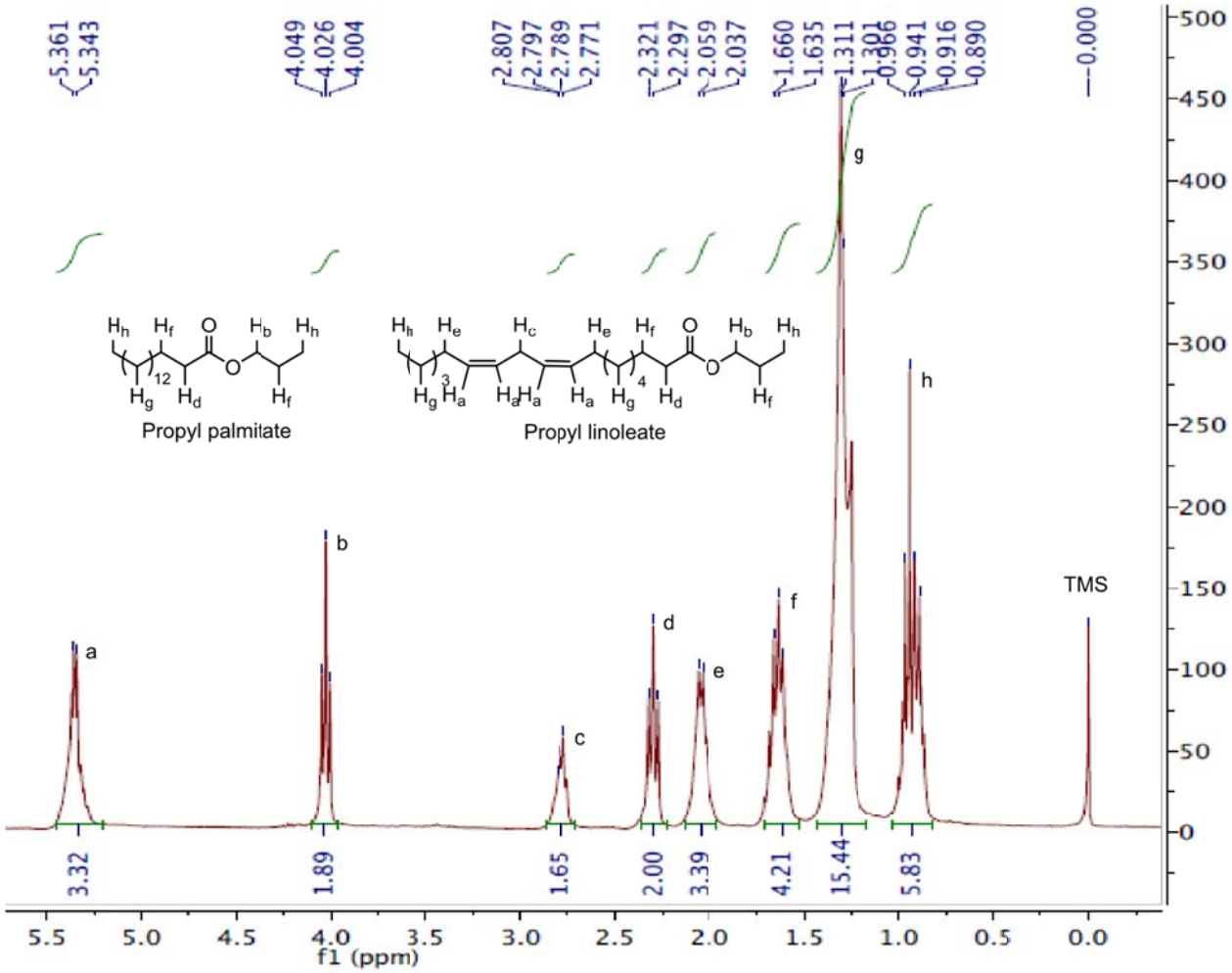


Figure SM 3.1.20.39 — ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of walnut oil propyl esters.