Diels-Alder reaction of *N*-phenylmaleimide with *in situ* generated buta-1,3-diene

Supplementary Material

Notes:

This work is planned for two sessions of 3 h each in a laboratory equipped with two rotary evaporators. It has been offered as a laboratory classroom work, for more than four years, to second year undergraduate chemistry students (about 30 students a year, in classes of 16 students, working in groups of two). Students are challenged to synthesize a Diels-Alder cycloadduct. The experimental work illustrates a cycloaddition reaction between a conjugated diene (buta-1,3-diene), generated *in situ* by thermal extrusion of SO_2 from 3-sulfolene, and *N*-phenylmaleimide. The reaction is performed in refluxing toluene (the oil bath must be kept at a temperature above 120 °C) in order to generate the buta-1,3-diene.

Both 3-sulfolene and *N*-phenylmaleimide are commercial compounds but the *N*-phenylmaleimide can be prepared previously by the students following the procedure in the experiment entitled “Synthesis of *N*-arylmaleimides”.

The condenser must be refrigerated to avoid the evaporation of the toluene (Figure SM **10.1.**2).

The reaction may be monitored by TLC. In this case it is necessary to cool down the reactional flask before the removal of the sample to be analysed.

Silica gel F_{254} should be used both in the analytical and preparative TLC; dichloromethane is a good eluent to develop the chromatogram.

In the analysis of the crude reaction mixture by TLC, it is recommended the use of the starting *N*-phenylmaleimide (dissolved in dichloromethane) as reference: the reference and the reaction mixture should be spotted on the TLC plate about 1 cm apart and 1 cm from the bottom. Retention factors (R_f) can be used to confirm the presence of unreacted *N*-phenylmaleimide in the crude oil. To determine the retention factor, the students should mark with a pencil the solvent front immediately after the removal of the TLC plates from the TLC chamber and outline the spots visualized under the UV-lamp. The cycloadduct has a lower retention factor than *N*-phenylmaleimide.

During the visualization of the TLC plates under the UV-lamp the students must wear safety glasses at all times.

The aim of this experiment is to illustrate the formation of a six-membered ring via a cycloaddition reaction. For the characterization of the resulting product by NMR it is just required a small portion of

pure cycloadduct. So, it is not necessary to purify by preparative thin layer chromatography (TLC) all the crude product obtained. The yield of the reaction was always calculated for the crude product (55-65%), since only a small amount was purified by TLC for the NMR analysis.

Figure SM 10.1.1 - Reaction setup apparatus.

Figure SM 10.1.2 - Filtration apparatus.

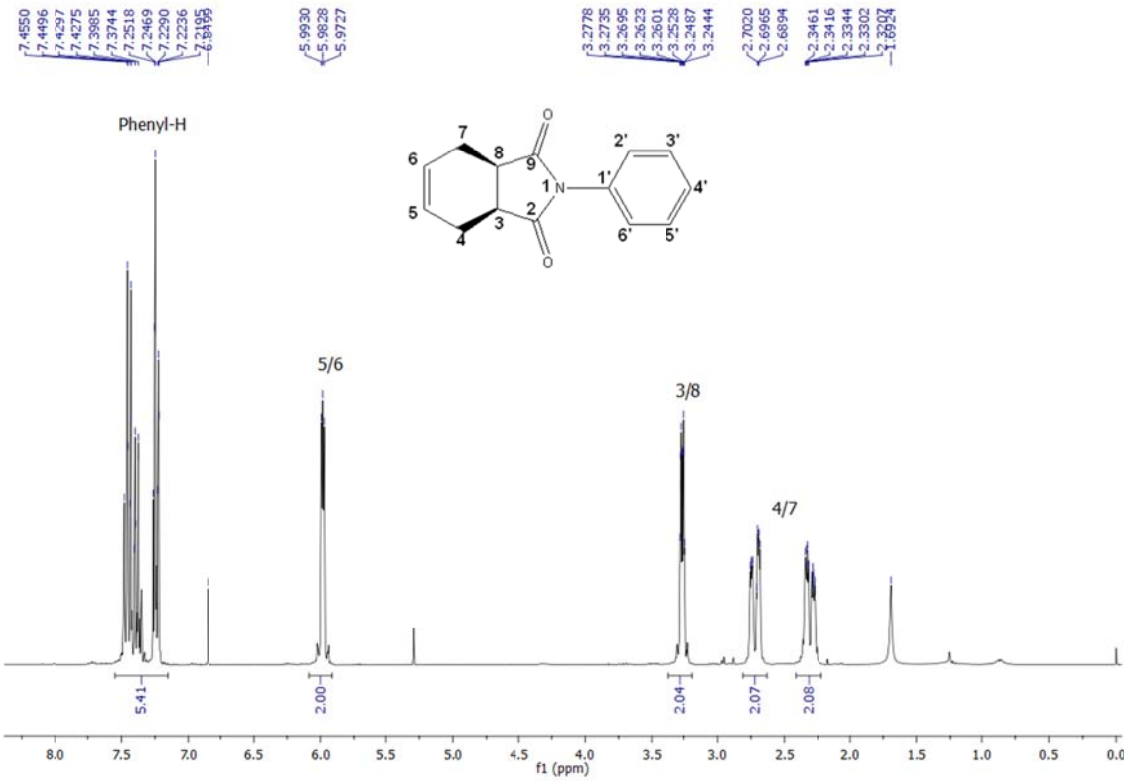


Figure SM 10.1.3 - ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the Diels-Alder cycloadduct.

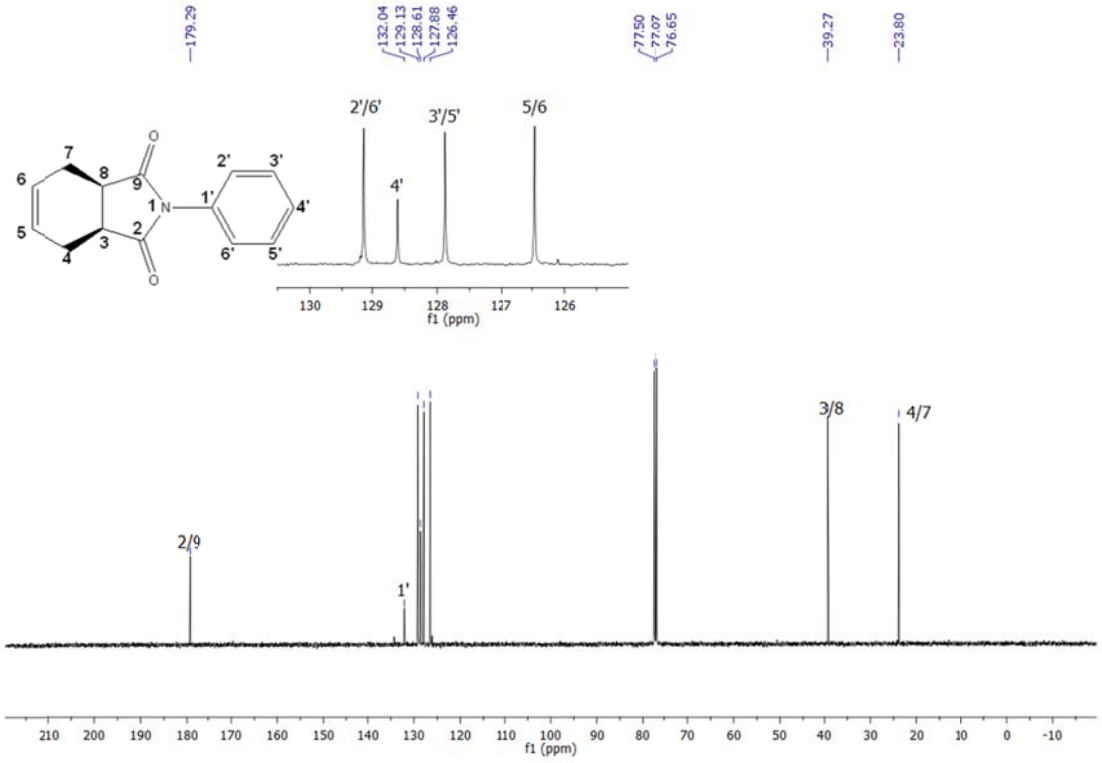


Figure SM 10.1.4 - ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of the Diels-Alder cycloadduct.

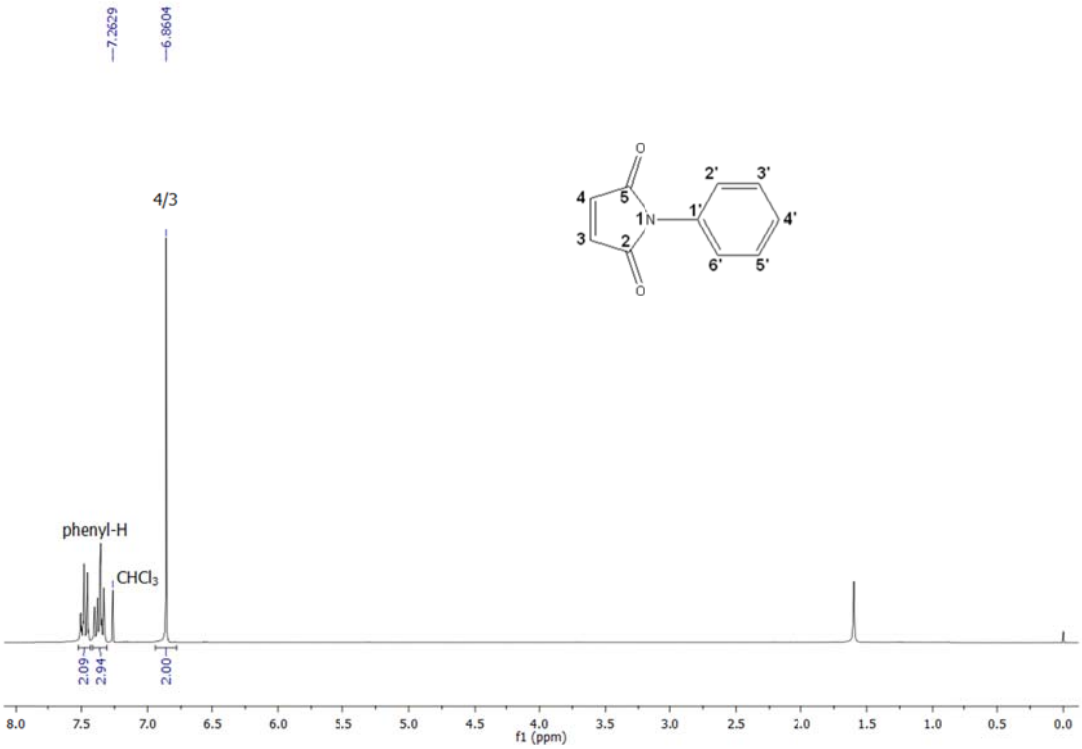


Figure SM 10.1.5 - ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of *N*-phenylmaleimide.

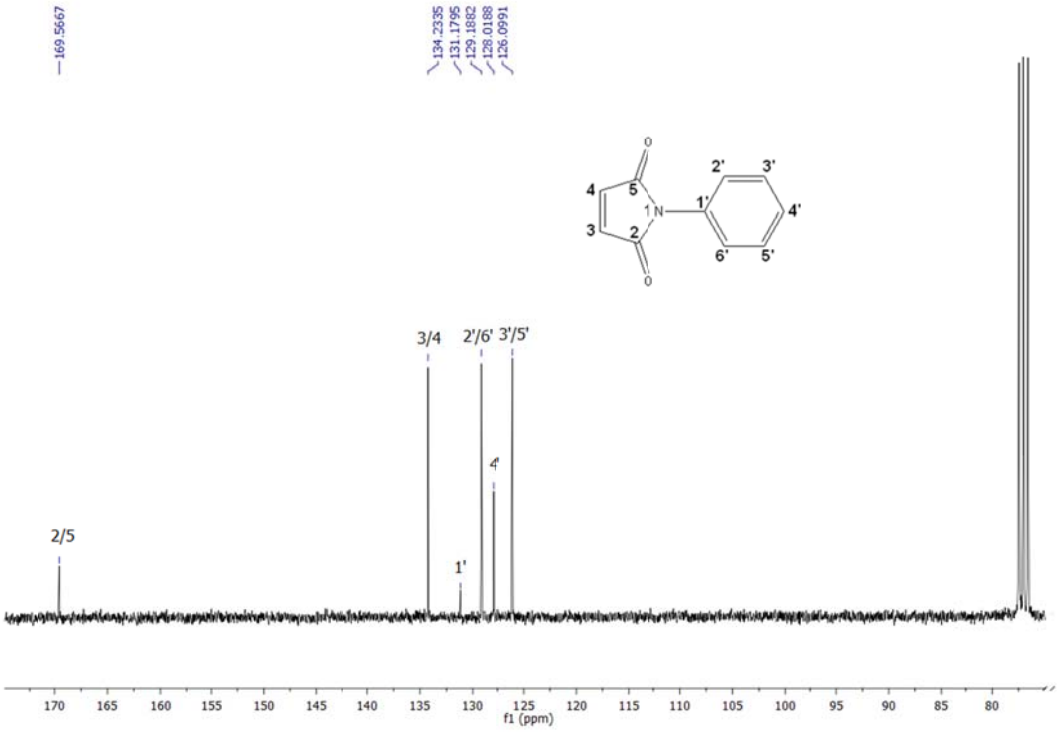


Figure SM 10.1.6 - ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of *N*-phenylmaleimide.

Synthesis of isomeric bicyclopropyls from conjugated dienes

Supplementary Material

Leiv K. Sydnes, Magne O. Sydnes

Recommended study level

This experiment is indeed easy to carry out successfully on the condition that the required equipment is available and the students have learnt the basic laboratory operations and can execute them properly. From an experimental point of view the synthesis may therefore be included in a laboratory course at an intermediate level. However, cyclopropane chemistry is oftentimes not incorporated in curricula at this level, a consequence of which is that the experiment may appear somewhat out of context if the purpose of the laboratory course is to illustrate the material covered in the course lectures and the accompanying textbook(s). On the other hand, if the idea is that the practical work should be regarded as a supplement to the theory presented, the synthesis should be considered as an attractive option.

The synthesis of 2,2,2',2'-tetrachloro-3,3,3',3'-tetramethylbicyclopropyl as presented here has been carried out by students at intermediate and advanced levels a number of times. No serious problems have been observed. Preparations have been carried out on a 15 mmol to 0.10 mol scale, and the isolated yield after recrystallization has ranged from as low as 10% to as high as 93%. (The lowest yields were obtained by students with inferior recrystallization skills, the consequence of which was a significant loss of product.) The colour of the product was not consistent even in successful experiments; in one case it was white, but in most cases it appeared to be yellowish to light brown or somewhat grey (see the picture in the Photo section). The isomeric composition was not always determined accurately, only the melting point was measured consistently, but when analyses were carried out, the ratio was in the 7:3 to 3:1 range in favour of the *meso* isomer after recrystallization.

The isomeric composition can be determined by both ^{1}H-NMR spectroscopy and GLC analysis (see the section Product data). The compound is too volatile to be analysed by TLC.[1]

Theory contextualization

Cyclopropanes have become quite prominent compounds in organic chemistry the last few decades, both as a structural moiety incorporated to introduce strain in a carbon skeleton, and as intermediates in organic synthesis. In spite of this many chemistry students regard three-membered carbocycles as quite unstable due to strain (about 28 kcal/mol) caused by the ring's significant deviation from the ideal tetragonal angle. Synthesis of a stable cyclopropane can therefore serve the students beyond giving them practical experience with phase-transfer catalysis which is applied here.

The cyclopropane synthesized here is a so-called *gem*-dichlorocyclopropane. As pointed out in the introduction to the experimental section, a large number of methods are available for the preparation of such compounds.[2] The method of choice in a given case depends on several factors, but when the substrate is an alkene without other reactive moieties the Makosza method[3,4] appears very attractive. This is the method applied here because it has a number of advantages over most of the alternatives: a) The reaction is carried out under aqueous conditions; b) Only moderate cooling is required (ice/water); c) No heating is necessary; d) The dichlorocarbene precursor, chloroform, is stable, commercially available, and inexpensive; f) No problematic waste is generated.

An attractive and educational feature with Makosza's method is the phase-transfer mechanism by which the dichlorocarbene is formed. As illustrated in Figure **SM 10.2.1** (Cl = X) the first reaction (Eq.1) is the equilibrium which was studied by Hine,[5] but which under aqueous conditions, in the absence of an organic phase and an ammonium ion, furnishes dichlorocarbene that reacts rapidly with water and/or hydroxide and forms formate and carbon monoxide. In the presence the triethylbenzylammonium ion, however, the ammonium ion replaces the sodium ion and forms a salt (Eq. 2) which dissolves in the organic phase (Eq. 3). Here the salt decomposes and affords

dichlorocarbene (Eq. 4) which reacts with a C=C moiety before any moisture dissolved in the organic phase intervenes; this gives the desired product, the corresponding 1,1-dichlorocyclopropane (Eq. 5).

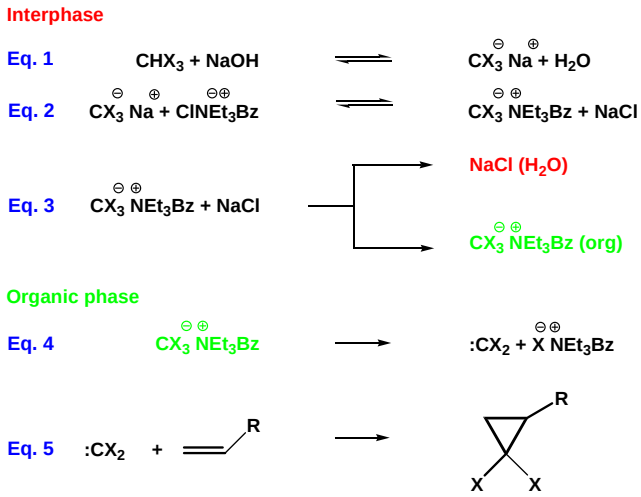


Figure SM 10.2.1. The reactions taking place at the interface under phase-transfer cyclopropanation.

The cyclopropane formation involves a cheletropic reaction which is one of the fundamental reactions exhibited by singlet carbenes. The reaction can be analyzed by the tools developed by Woodward and Hoffmann,[6] but the thorough studies published by Moss and co-workers give deep insight into the nature of the process.[7,8] What is important is the interaction between the HOMO and the LUMO orbitals of the reacting species. As depicted in Figure **SM 10.2.**2 the HOMO-LUMO interactions bring the carbene and the alkene together and lead to cyclopropane formation.[9,10]

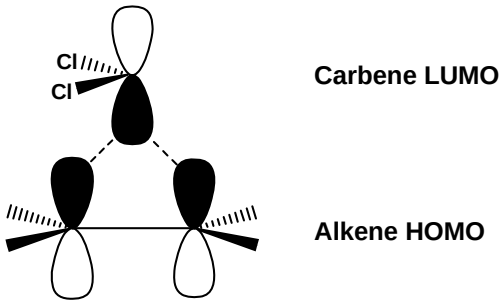


Figure SM 10.2.2. One possible HOMO-LUMO interaction between the carbene and the alkene.

There has been no indication whatsoever of formation of 2,2-dichloro-3,3-dimethy-1-(2-methylprop-1-enyl)cyclopropane, the expected mono-adduct from 2,5-dimethyl-2,4-hexadiene, in the preparation of 2,2,2',2'-tetrachloro-3,3,3',3'-tetramethylbicyclopropyl. In the synthesis described here that is perhaps that surprising since a significant excess of carbene is applied, but even when an excess of the diene is used only the bis-adduct is obtained. This clearly indicates that 2,2-dichloro-3,3-dimethy-1-(2-methylprop-1-enyl)cyclopropane is more reactive toward dichlorocarbene than the diene.

Experimental aspects

As long as the procedure is followed and the basic standard laboratory operations are carried out properly, it is not necessary to apply any special tricks to succeed in making 2,2,2',2'-tetrachloro-3,3,3',3'-tetramethylbicyclopropyl. However, the following points should be observed:

1. Make sure that the stirring is fast to secure good mixing and a large interfacial surface; that will facilitate the first two reactions in the multistep transformation that is involved in the conversion of C=C bond to a cyclopropane moiety (Figure **SM 10.2.**1).
2. The amount of NaOH added is not crucial, which is an advantage because whether the base is measured in a graduated cylinder or prepared in an Erlenmeyer flask, some of the base will remain in the glassware and not be transferred to the dropping funnel (approximately 0.5 g). About the same amount will be attached to the inside wall of the dropping funnel and not be added to the reaction mixture. Therefore, to make sure that about 16 g of NaOH solution reaches the organic phase, about 17 g of 50% aqueous NaOH have either to be prepared in an Erlenmeyer flask (from 8.5 g of NaOH and 8.5 mL of water) or added from a stock solution (11.0 mL).
3. The NaOH solution required can either be prepared by each student or be obtained from a stock solution. The preparation is carried out by adding about 8.5 g of NaOH to a small Erlenmeyer flask followed by the same amount of water in one portion, and swirling the flask vigorously until all the NaOH pellets have been dissolved. The temperature will increase

quickly and this facilitates the dissolution. Add the base warm to the dropping funnel; that will prevent precipitation of NaOH.

4. The 50% NaOH solution reacts gradually with glass, so if a stock solution is made, it should be stored in a plastic bottle. Make sure that the screwcap is tight so that carbonate formation is prevented as much as possible. If the stock solution has suffered extensive conversion of hydroxide to carbonate, the yield of *gem*-dihalocyclopropanes may drop significantly when Makosza's method is applied.
5. Hydrochloric acid is added to the reaction mixture in the work-up to neutralize any unreacted NaOH and make the aqueous phase somewhat acidic. That facilitates phase separation during the extraction.

A few words about the colour of the product are warranted. The synthesis described here has been performed according to the given procedure a number of times, but in spite of essentially identical reaction conditions the colour of the product has varied. In a couple of cases the product has been fairly brownish and grey. If that happens it might help to purify the crude product with active charcoal before recrystallization is performed.

As the number of sessions and their duration indicate, this synthesis can be carried out in a very flexible way and be performed in parallel with another synthesis. Thus, in step 4 in the experimental procedure the reaction time can be extended quite a bit without any consequences (reaction times up to 72 hours have been tested). The same is the case with step 7. Drying for 1.5 hours is really given to be able to carry out the experiment in three sessions; better results are usually obtained if the extract is dried until the next day, but drying for several days is acceptable as well. And finally, the recrystallization can without problems be extended to several days (step 8). (It goes without saying that melting-point determination and recording of NMR spectra can be postponed as desired/needed).

Photos

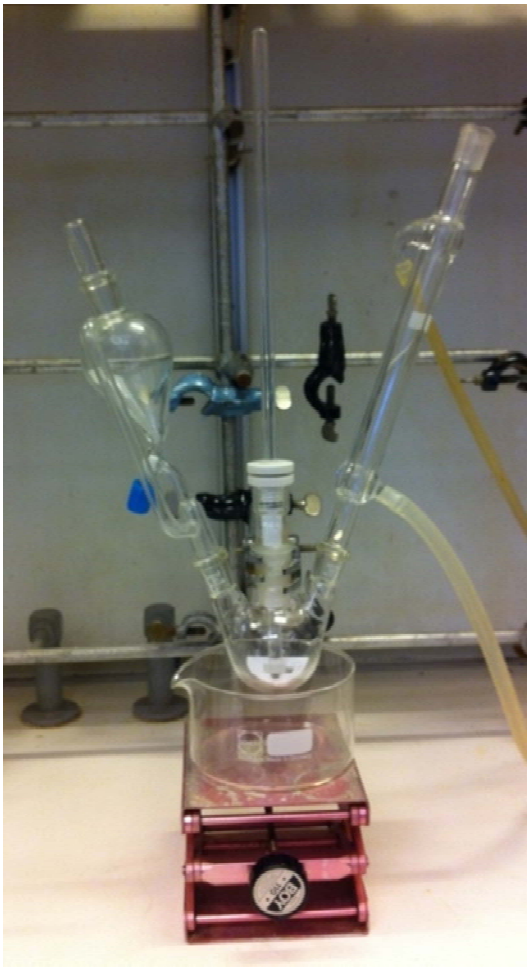


Figure SM 10.2.3. The glassware used to carry out the synthesis. The condenser is not really necessary, but is attached as a precaution. Furthermore, it is not necessary to use a dropping funnel with a pressure equalizer and a stopcock either; a simple dropping funnel will do.

Figure SM 10.2.4. In order to secure fast and steady stirring a stirring rod with a bearing and a Teflon blade is used. Adequate equipment can be purchased for instance from Kontes, Vineland, NJ, USA.

Figure SM 10.2.5. The product may get a brownish look or almost grey like here. When dried under vacuum at around 50 ^{o}C for some hours, the crystalline material may become a powder.

Product data

2,2,2',2'-Tetrachloro-3,3,3',3'-tetramethylbicyclopropyl is formed as a diastereoisomeric mixture. After recrystallization the melting point (uncalibrated) has been measured to 79 - 80 ^{o}C and 80 - 81 ^{o}C (lit.[11] 78 ^{o}C). NMR spectra have been recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C, using TMS as internal reference (0.00 ppm) for the former nucleus and $CDCl_3$ as internal reference (77.03 ppm) for the latter. The following data were obtained:^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 and 1.38 (2 s in a 3:1 ratio, 6H), 1.34 and 1.21 (2 s in a 3:1 ratio, 6H), 1.17 and 1.10 (2 s in a 1:3 ratio, 2H) [see Figure **SM 10.2.**6]. ^{13}C NMR (100 MHz, $CDCl_3$) δ 71.3, 36.0, 36.0, 5.9, 35,9, 35.8, 35.8, 30.2, 29.4, 24.4, 24.3, 18.4 [see Figure **SM 10.2.**7]. The isomers have previously been separated by preparative GLC, and the ^{1}H NMR data have been reported in the literature.[1]

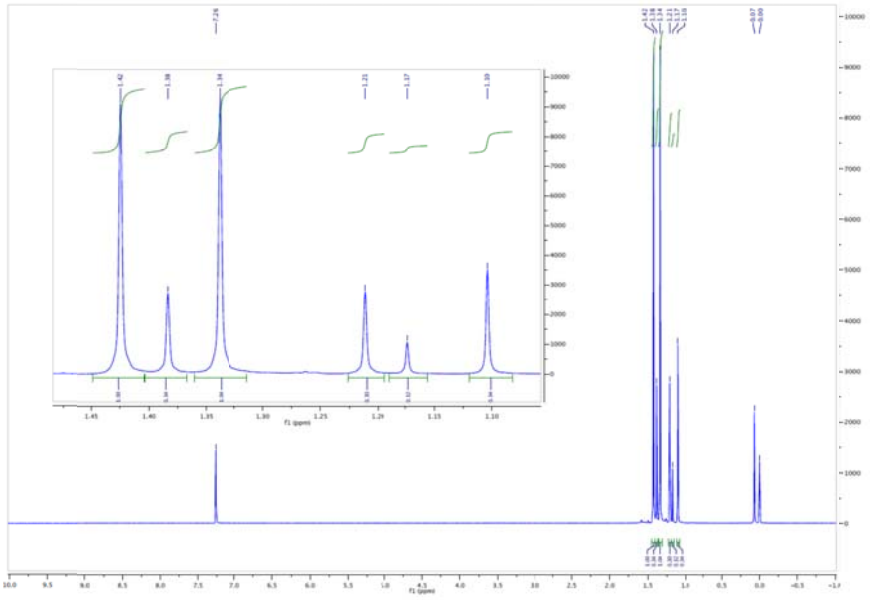


Figure SM 10.2.6. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of a recrystallized sample of a mixture of *meso* and *racemic* 2,2,2',2'-tetrachloro-3,3,3',3'-tetramethylbicyclopropyl.

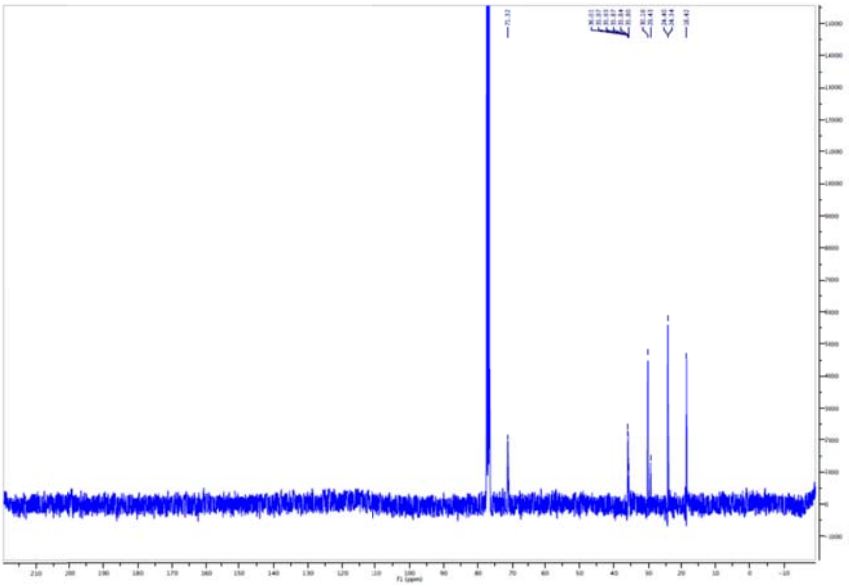


Figure SM 10.2.7. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of a recrystallized sample of a mixture of *meso* and *racemic* 2,2,2',2'-tetrachloro-3,3,3',3'-tetramethylbicyclopropyl.

As pointed out in the literature 2,2,2',2'-tetrachloro-3,3,3',3'-tetramethylbicyclopropyl is so volatile that it cannot be analyzed by TLC. However, GC analysis works very well, and when analyzed using the

equipment and the conditions compiled in Table **SM 10.2.**1, the chromatogram in Figure **SM 10.2.**8 is obtained.

Table SM 10.2.1. The equipment and conditions used to perform GLC analysis of an ethyl-acetate solution of 2,2,2',2'-tetrachloro-3,3,3',3'-tetramethylbicyclopropyl.

Column	**Oven**
Model: Agilent 19091J-413 HP-5 Column: 5% phenyl methyl siloxane Nominal length: 30.0 m Nominal diameter: 320.00 μm Nominal film thickness: 0.25 μm Mode: Constant flow Inifial flow: 1.5 mL/min Nominal init pressure: 56.5 Kpa Average velocity: 28 cm/sec	Initial temp: 70 °C Initial time: 1.50 min Ramps: # Rate Final temp Final time 1 30.0 240 °C 2.00 min Run time: 9.17 min
Inlet	**Detector (FID)**
Mode: Split Initial temp: 240 °C Pressure: 56.4 KPa Split ratio: 100:1 Split flow: 149.7 mL/min Total flow: 154.3 mL/min Gas type: Helium	Temperature: 280 °C Hydrogen flow: 40.0 mL/min Air flow: 450.0 mL/min Mode: Constant makeup flow Makeup flow: 30.0 mL/min Makeup gas type: Nitrogen

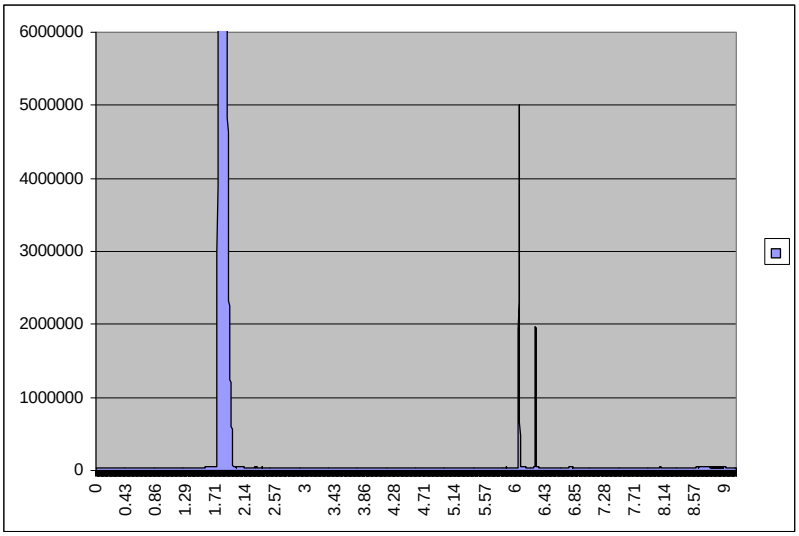
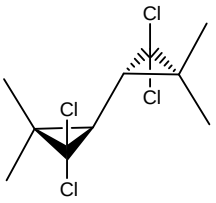


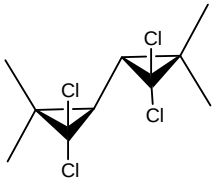
Figure SM 10.2.8. The GC chromatogram of an ethyl-acetate solution of a mixture of *meso* and *racemic* 2,2,2',2'-tetrachloro-3,3,3',3'-tetramethylbicyclopropyl. The equipment and conditions listed in Table X.1 were applied. The peak with retention time around 2 minutes is due to the solvent.

Topics for additional discussion

1. As stated in question 2 in "Results interpretation and additional questions" in the procedure, the isomeric composition can conveniently be determined by ^{1}H NMR spectroscopy. When this NMR spectrum is treated as outlined in the question, the percentage of the more abundant isomer (X) becomes for example X = 100% (A_1 + A_3 + A_6)/ $\sum_{i=1}^{6} Ai$.
2. The two isomers are quite volatile and the isomer composition can also be determined by GC analysis assuming identical response factors. On a nonpolar column the most abundant isomer has the shorter retention time.
3. It is important to appreciate the principle of the phase-transfer reaction which was published by Professor Makosza and co-workers in 1969.[28] An important point in this context is the phase-transfer step, which prevents hydrolytic decomposition of chloroform under basic conditions from occurring.[2-4]
4. The product consists of a *meso* form and a pair of enantiomers that exhibit rather different polarity due to different dipole moments (μ which is measured in Debye [D]). Each ring has a dipole directed towards the dichloro-substituted carbon. Thus, if the conformations of the isomers are approximately as depicted below, the dipoles in the two rings are more or less antiparallel in the *meso* form, giving a low total dipole moment, and more or less parallel in the pair of enantiomers, giving a high total dipole moment. For the compounds below μ = 1.08 D for the *meso* form whereas μ = 3.74 D for the pair of enantiomers.[25]



meso form



pair of enantiomers

Equations describing the variation of the total dipole moments for *meso* forms and pairs of enantiomers for bicyclopropyl compounds are given in reference 25. The same paper does also give dipole moments for a number of other cyclopropanes.

Synthesis of other bicyclopropyls

The procedure used here to prepare 2,2,2',2'-tetrachloro-3,3,3',3'-tetramethylbicyclopropyl has also been applied to make two 2,2,2',2'-tetrabromobicyclopropyl derivatives, *viz*. 2,2,2',2'-tetrabromo-1,1'-dimethylbicyclopropyl and 2,2,2',2'-tetrabromo-3,3,3',3'-tetramethylbicyclopropyl. Both compounds are reported in the literature as products from dibromocarbene addition to the corresponding dienes using essentially the Doering-Hoffmann method.[1,12,13]

Preparation of 2,2,2',2'-tetrabromo-1,1'-dimethylbicyclopropyl was carried out once on a large scale (0.50 mol) from 2,3-dimethyl-1,3-butadiene. The equipment was adjusted to the larger quantities, and the procedure was stepwise modified as follows: Bromoform (8.0 eq), TEBA 2.0 g [step 2]; NaOH (8.0 eq) [step 3]; dropwise addition 1 hour, stirring 66 hours at ambient temperature [step 4]; quenching 1.3 L water [step 5]; extraction 4 x 200 mL with diethyl ether [step 6]; recrystallization from EtOH [step 8]. The yield was 95.5 g (45%), melting point 94-96 ^{o}C (lit.[1,12] 96-99 ^{o}C and 99 ^{o}C). The ^{1}H-NMR spectrum is in accordance with the literature.[12]

Preparation of 2,2,2',2'-tetrabromo-3,3,3',3'-tetramethylbicyclopropyl has been carried out twice on approximately 0.25 mol scale with 2,5-dimethyl-1,3-hexadiene. The equipment was adjusted to the larger quantities, and the procedure was stepwise modified as follows: Bromoform (8.0 eq), TEBA 1.0 g [step 2]; NaOH (8.0 eq) [step 3]; dropwise addition 40 – 45 minutes, stirring 48 - 96 hours at ambient temperature [step 4]; hydrolysis 1.3 L water [step 5]; extraction 4 x 200 mL with diethyl ether [step 6]; recrystallization from EtOH [step 8]. The yields were 18% and 26% and the melting point 107 ^{o}C (lit.[1,12] 109 ^{o}C). The ^{1}H-NMR spectrum is in accordance with the literature.[1]

Unlike 2,2,2',2'-tetrachloro-3,3,3',3'-tetramethylbicyclopropyl both the tetrabromobicyclopropyls can be analyzed by TLC. The conditions and the results are in accordance with the literature.[1]

References and literature

[1] C. Rømming and Leiv K. Sydnes, *Acta Chem. Scand*. 1976, **B 30**, 963.
[2] L. K. Sydnes, *Preparation of* gem-*dihalocyclopropanes; a short summary of some classical work*, attachment to the supplement to this contribution, 2014, 9 pages.
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[4] M. Makosza and M. Fedorynski, *Synthetic Commun*. 1973, **3**, 305.
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[7] R. A. Moss, *Acc. Chem. Res.* 1980, **13**, 58.
[8] R. A. Moss, L. A. Perez, Wlostowska, W. Guo, and K. Krogh.Jespersen, *J. Org. Chem*. 1982, **47**, 4177.
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[10] J. March, *Advanced Organic Chemistry; Reactions, Mechanisms, and Structure*, John Wiley & Sons, New York, NY USA, 4th edition, 1992, chapters 5 and 10,5-50, 195-202 and 866-873.
[11] H. Komrsova and J. Farkas, *Collect. Czech. Chem. Commun*. 1960, **25**, 1977.
[12] L. Skattebøl, *J. Org. Chem.* 1964, **29**, 2951.
[13] K. Kleveland and L. Skattebøl, *Acta Chem. Scand.* 1975, **B 29**, 191.

In addition to the references given above and quoted in the experimental section, the following literature contains a lot of valuable material about carbenes and cyclopropane chemistry:

Chem. Rev. 2003, **103**, issue number 4.

The chemistry of the cyclopropyl group, ed. S. Patai and Z. Rappoport, John Wiley & Sons, Chichester, UK, 1987.

G. Boche and H. M. Walborsky, *Cyclopropane derived reactive intermediates*, ed. S. Patai and Z. Rappoport, John Wiley & Sons, Chichester, UK, 1990.

Advances in Strain in Organic Chemistry, ed. B. Halton, JAI Press, London, UK; monograph series that started in 1991.

Carbenes I, ed. R. A. Moss and M. Jones, Wiley & Sons, New York, NY USA, 1973.

Carbenes II, ed. R. A. Moss and M. Jones, Wiley & Sons, New York, NY USA, 1975.

M. O. Sydnes, *Applications of* gem-*Dihalocyclopropanes in Synthesis*, PhD thesis, Australian National University, Canberra Australia, 2004, chapter 1, pp. 1-23; attachment 2.

Diels-Alder reaction between *p*-benzoquinone and cyclopentadiene and subsequent photochemical [2π+2π] cycloaddition

Supplementary Material

Experiment Notes

Background information

Yield, melting point, IR, TLC

Table 1 – ^{1}H NMR data for Diels-Alder product **A**

Table 2 – Inferences from the COSY spectrum of Diels-Alder product **A**

Table 3 – Complete assignment of all ^{1}H and ^{13}C NMR signals for Diels-Alder product **A**

Figures

Photos of the experiment

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

HSQC, COSY and NOESY spectra of Diels-Alder product **A**

IR spectrum of **B**

The procedures for this experiment have been developed for a Year 3 Organic Chemistry lab class to support a lecture course on cycloadditions and MO theory. Since 2013, about 40 students, working individually, have successfully carried out the reaction sequence.

The Diels-Alder reaction has been adapted and scaled down from known literature procedures.[1,2] The photochemical cycloaddition to yield pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione (**B**) has been reported in the literature before, but is usually carried out using a high-power (300 W) mercury lamp and more expensive quartz or pyrex reactors.[3,4,5] However, the photochemical product **B** will form even when **A** is left exposed to sunlight long enough.[6] Irradiation of a solution of **A** in $CDCl_3$ with a simple blacklight takes about 1 hour and works well in an undergraduate laboratory setting. It allows the reaction product to be identified by ^{1}H NMR spectroscopy without the need for any work up.

Costs: The starting materials for this experiment are cheap. The blacklight (20 W low energy, B22 bayonet fitting, high UV light intensity) was purchased from Amazon.co.uk for under £10.

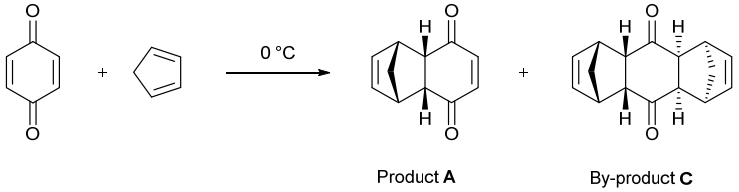
Step 1: Diels-Alder Reaction

Yield: Cyclopentadiene (2.18 g, 2.7 mL, 27.0 mmol) is the limiting the reagent. Student yields tend to vary a lot: 25 – 86%, with an average around 51%.

Practical notes: Cyclopentadiene was prepared by a lab technician ahead of the lab session by thermal cracking of dicyclopentadiene, then stored in small sample vials in the freezer.

The cycloaddition should be carried out at 0 °C or below. Higher temperature (even room temperature) causes **A** to undergo a second cycloaddition with another molecule of

cyclopentadiene;[7] the mixture of the two cycloadducts **A** and **C** is not easy to separate (Scheme **SM 10.3.**1).[2,6] For this reason, cyclopentadiene was chosen as the limiting reagent.



Scheme SM 10.3.1 – Diels-Alder reaction between *p*-benzoquinone and cyclopentadiene.

A is photosensitive and will isomerise slowly to **B** when exposed to daylight or room light. The compound should be stored in the dark, for example inside a bench cupboard.
M.p.: 76-78 °C, dirty yellow or yellow-brown crystals. Most student samples tend to be contaminated by varying amounts of *p*-benzoquinone which darkens the colour but does not affect the subsequent photochemical cycloaddition.

IR: 2990, 2945 cm^{-1} (alkyl C–H stretch), 1665 cm^{-1} (C=O stretch) [8]

TLC (petroleum ether/ethyl acetate, 5:1): R_f = 0.44

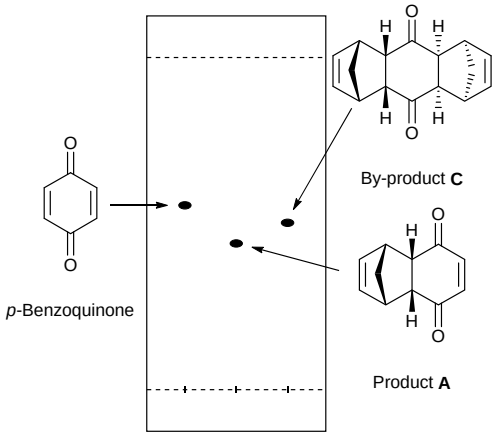


Figure SM 10.3.1 – Typical TLC of the Diels-Alder reaction product and common impurities (step 1).

Step 2: Photochemical Cycloaddition

Yield: **A** has an absorption maximum at 360 nm which coincides with the UV band emitted by the blacklight.[5] Irradiation time should be about 1 hour. Less time, or not placing the NMR tube directly under the blacklight, results in mixtures of **B** and unreacted **A**. After 15 min, conversion is about 50%, after 30 min about 85%. The most likely by-products that can be detected by NMR are:

unreacted **8A** (if irradiation time was too short); and left-over *p*-benzoquinone (recognisable by a singlet at δ_H 6.8).

Photos of the experiment

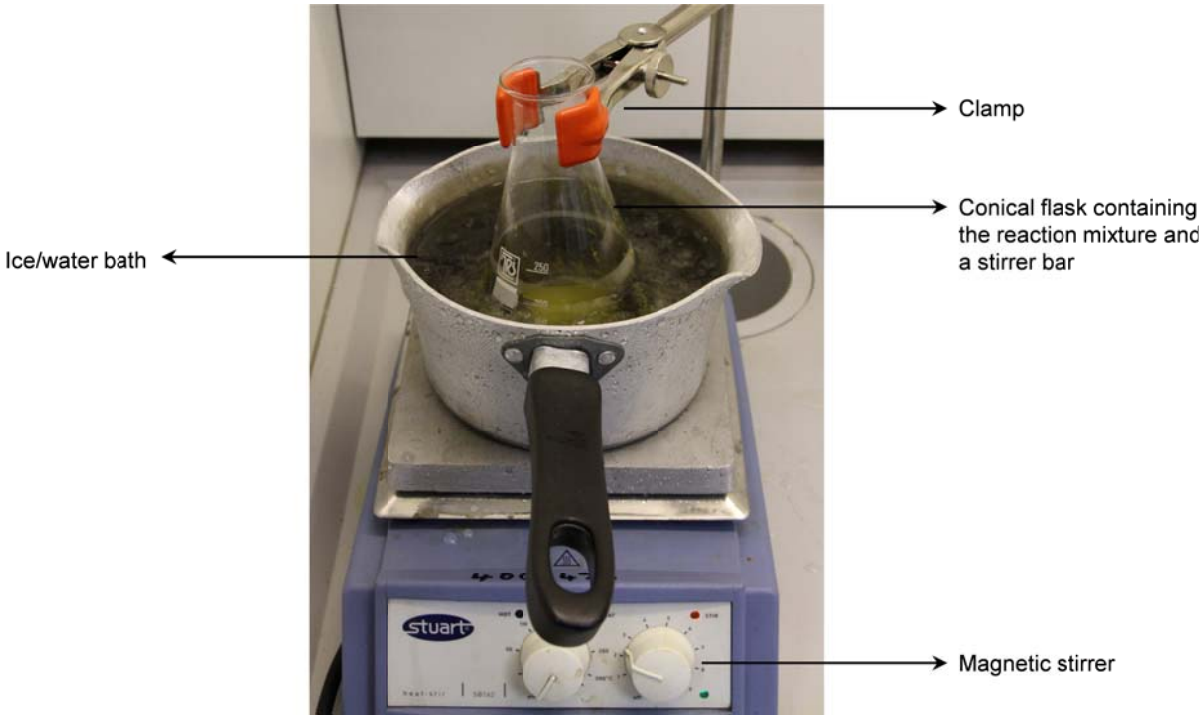


Figure SM 10.3.2 – Set-up for carrying out the Diels-Alder reaction (step 1).

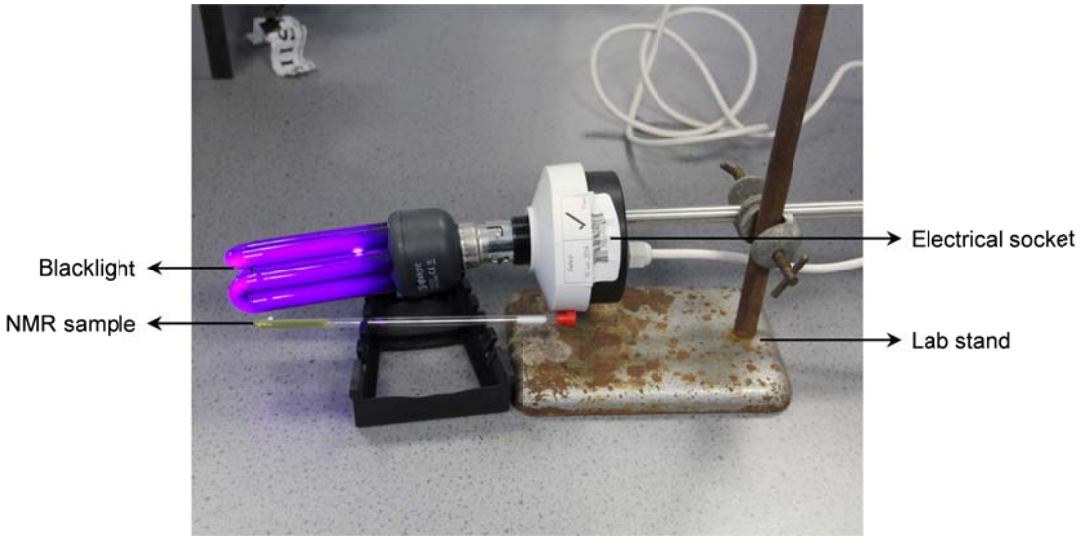


Figure SM 10.3.3 – Set-up for the photochemical cycloaddition using a blacklight and an NMR sample (step 2). During operation the blacklight is covered with a cardboard box. The NMR tube(s) should be placed directly underneath the blacklight bulb. Normally, photochemical reactions are carried out in glassware made from pyrex or quartz. Although the soda lime glass of the NMR

tubes absorbs a some UV light, the photochemical cycloaddition still proceeds at a reasonable rate.

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

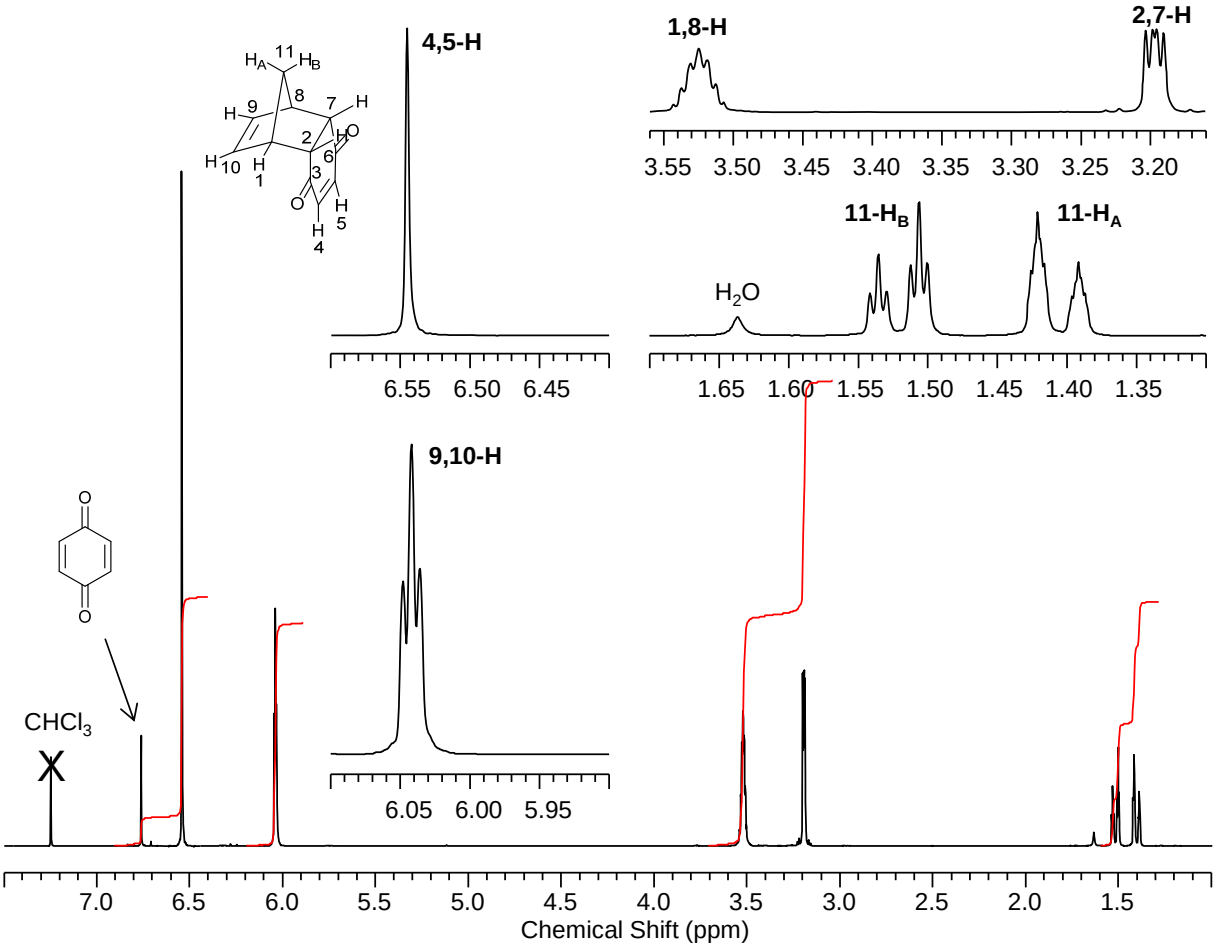


Figure SM 10.3.4 – ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of the Diels-Alder product **A**. Common impurity signals are labelled. Assignment of ^{1}H NMR signals were confirmed by HSQC, COSY and NOESY spectra.

Table SM 10.3.1 – ^{1}H NMR data for Diels-Alder product **A**

δ_H	Integral	Multiplicity	Inferences
1.4	1H	Broad doublet	11-H_A (one of the CH_2 bridge protons with a geminal ^{2}J = 8.7 Hz coupling) [a]
1.5	1H	~Doublet of triplets	11-H_B (one of the CH_2 bridge protons with a geminal ^{2}J = 8.7 Hz and small ^{3}J = 1.7 Hz coupling) [a]

3.2	2H	Multiplet	2,7-H [c]
3.5	2H	Multiplet	1,8-H (bridgehead) [b]
6.0	2H	~Triplet	9,10-H (the broadening of the signal indicates coupling to neighbouring bridgehead H ⇒ norbornene ethylene bridge) [c]
6.5	2H	Singlet	4,5-H (the absence of any coupling indicates that this signal must belong to the 6-ring enone)

a) The two CH_2 protons are diastereotopic; their chemical shifts are slightly different and the two protons couple with each other AB-like (with additional smaller long-range couplings). **b)** Here the COSY provides the key clue: this is the only signal that shows crosspeaks to all other norbornene protons. **c)** The signals are complicated by higher order effects and the observed "simple" multiplicities (which resemble a doublet of doublets, triplet, septet) are deceptive. The norbornene protons are part of a much more complicated AA'MM'XX' spin system; like the AA'XX' of a *p*-disubstituted benzene it looks simple, but the distance between two neighbouring lines no longer equals a coupling constant. Closer inspection reveals small additional lines that are very characteristic of this type of higher-order spectra.

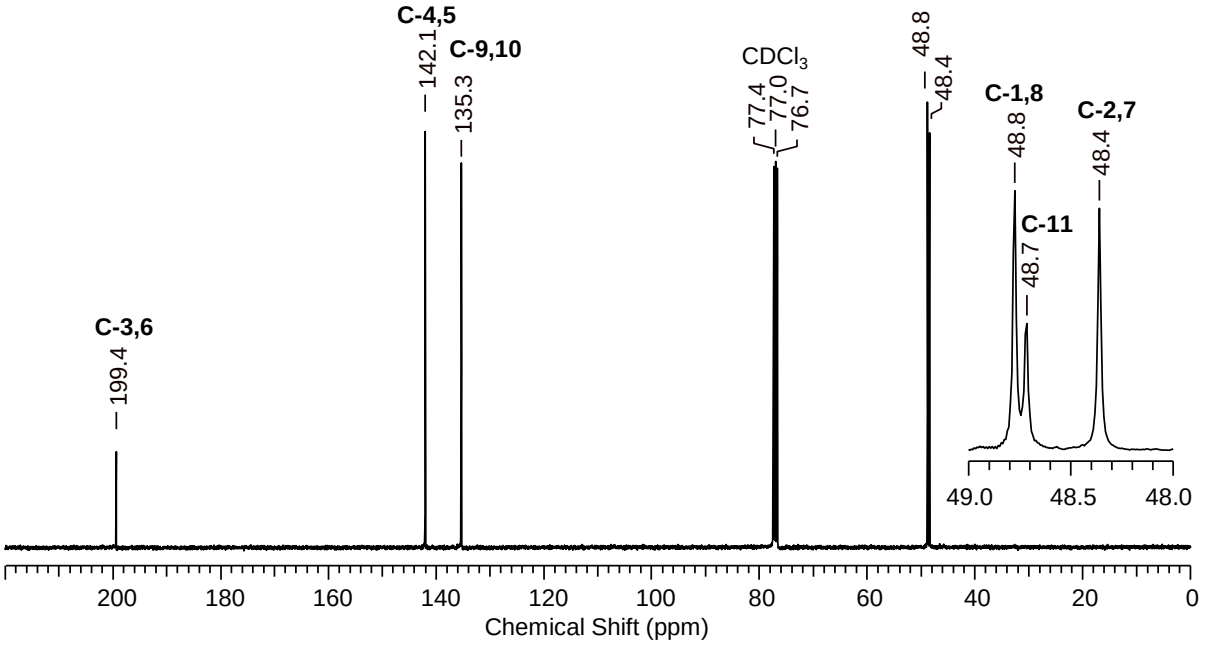


Figure SM 10.3.5 – ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of the Diels-Alder product **A**. Students should note six ^{13}C NMR signals in total. Assignment of ^{13}C NMR signals were confirmed by 2D NMR spectra.

A set of 2D NMR spectra can be used to fully assign all ^{1}H and ^{13}C NMR signals of **A**, as well as confirm the *endo* stereochemistry of the Diels-Alder cycloaddition product (Figures **SM 10.3.**6-8).

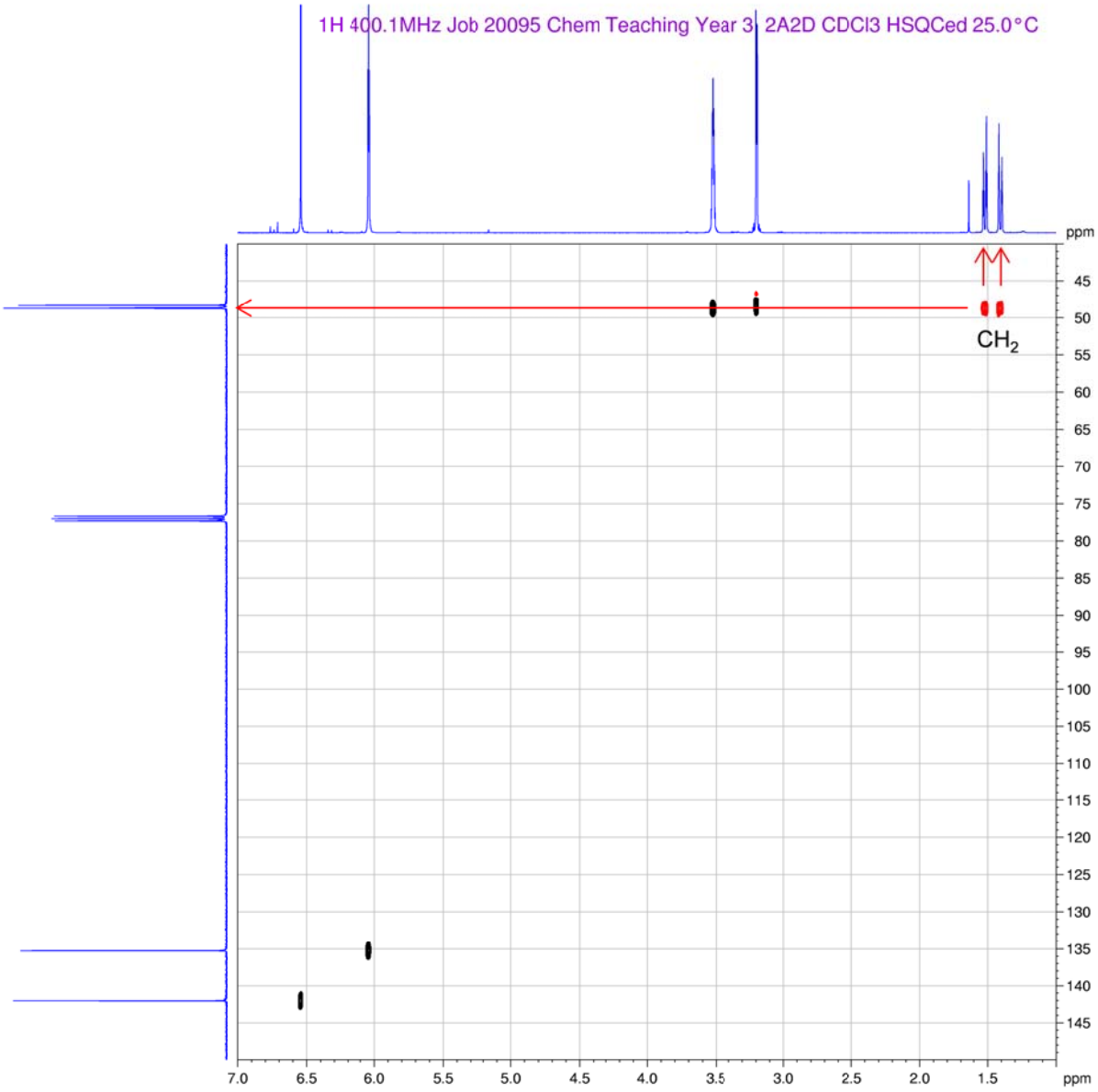


Figure SM 10.3.6 – HSQC C,H correlation of Diels-Alder product **A**. Students should note the different phase (shown in red) for the crosspeak correlating the ^{13}C NMR signal at δ_C 48.7 and the ^{1}H NMR signals at δ_H 1.4 and 1.5; these signals can be assigned to the carbon and two diastereotopic protons of the only CH_2 group in the compound.

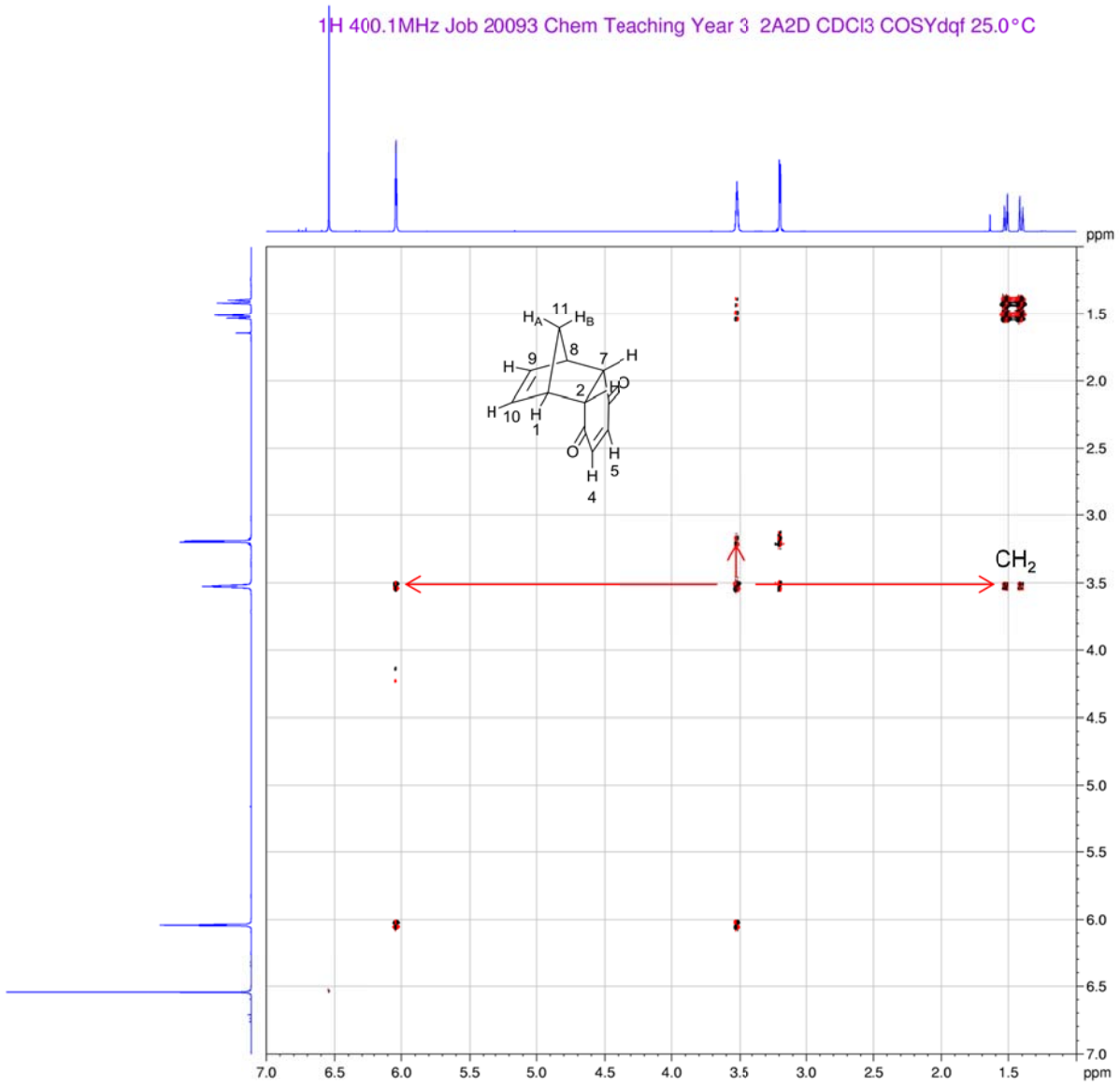


Figure SM 10.3.7 – COSY spectrum of Diels-Alder product **A**. The signal at δ_H 3.5 can be assigned unequivocally to bridgehead protons 1,8-H due to coupling to all proton signals but one (indicated by the arrows).

Table SM 10.3.2 – Inferences from the COSY spectrum of Diels-Alder product **A**

δ_H	Crosspeak(s)	Inferences
1.4, 1.5	3.5	Crosspeak indicates coupling between CH_2 and bridgehead 1,8-H $\Rightarrow$ signal at δ_H 3.5 belongs to 1,8-H
3.5	1.4, 1.5, 3.2, 6.0	Bridgehead 1,8-H show coupling to all but alkene protons 4,5-H $\Rightarrow$ Signal at δ_H 3.2 belongs to 2,7-H; signal at δ_H 6.0 belongs to 9,10-H

3.2	3.5	
6.0	3.5	Signal belongs to alkene protons 9,10-H
6.5	No crosspeaks	Singlet at δ_H 6.5 belongs to alkene protons 4,5-H

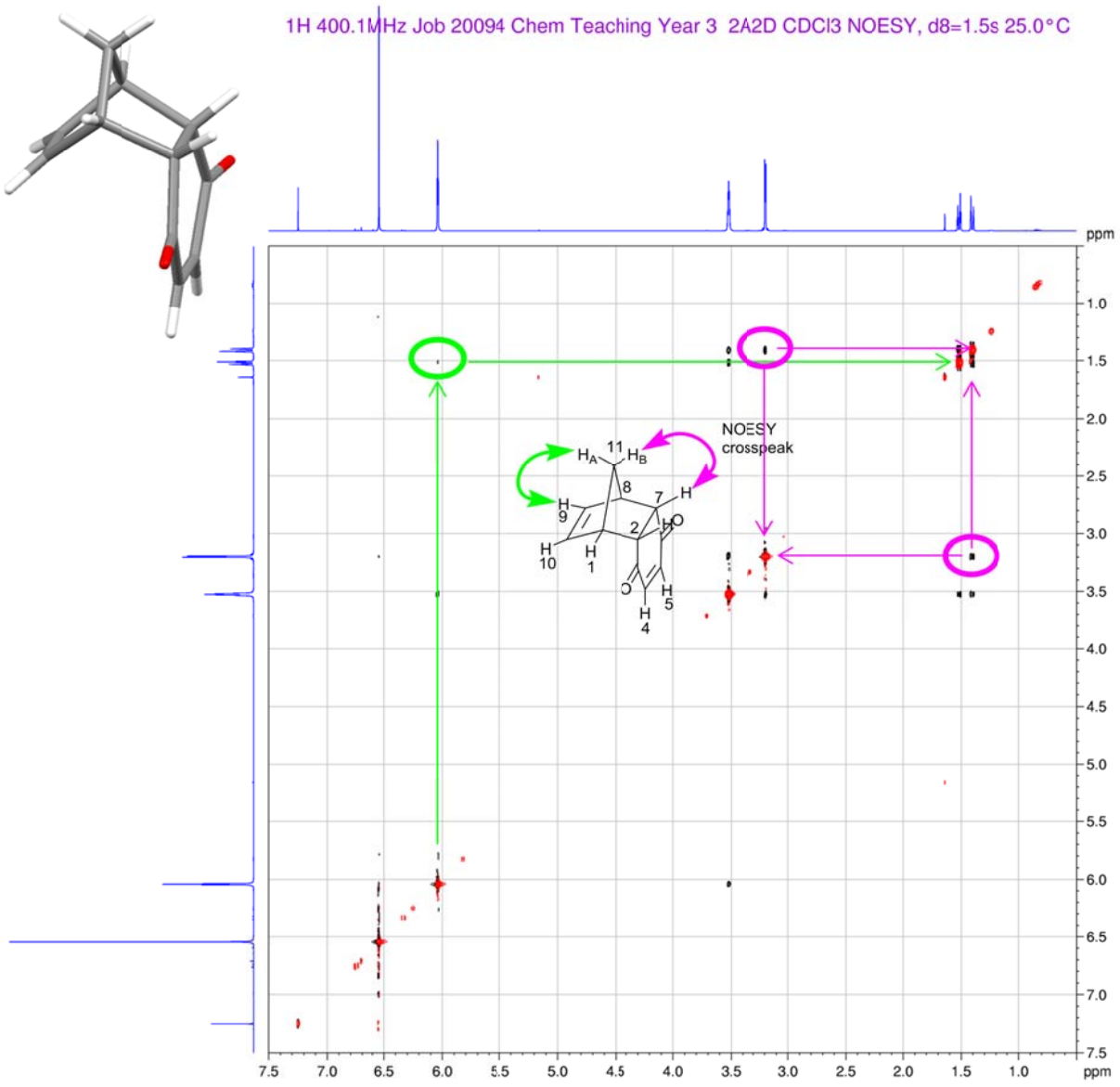


Figure SM 10.3.8 – NOESY spectrum of Diels-Alder product **A** where off-diagonal peaks indicate pairs of nuclei in a molecule that are close together in space — no matter whether they couple with each other or not. One of the CH_2 protons (at δ_H 1.4) shows a clear NOESY crosspeak (highlighted in magenda) to the signal at δ_H 3.2 belonging to the 2,7-H protons. This is only possible if the 2,7-H protons are in *exo* position. It provides spectroscopic proof that the *endo* product has formed in the Diels-Alder reaction. The alkene H signal at δ_H 6.0 has a very weak crosspeak to the bridgehead H (no surprises there) AND to the other CH_2 proton signal at δ_H 1.5 (highlighted in green), indicating that these protons are close in space.

All ^{1}H and ^{13}C NMR signals can be assigned using the 2D NMR evidence (Table 3).

Table SM 10.3.3 – Complete assignment of all ^{1}H and ^{13}C NMR signals for Diels-Alder product **A**

δ_H	Assignment	δ_C
1.4	11-H_A	48.7
1.5	11-H_B	48.7
3.2	2,7-H	48.4
3.5	1,8-H	48.8
6.0	9,10-H	135.3
6.5	4,5-H	142.1
		199.4 (C=O)

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

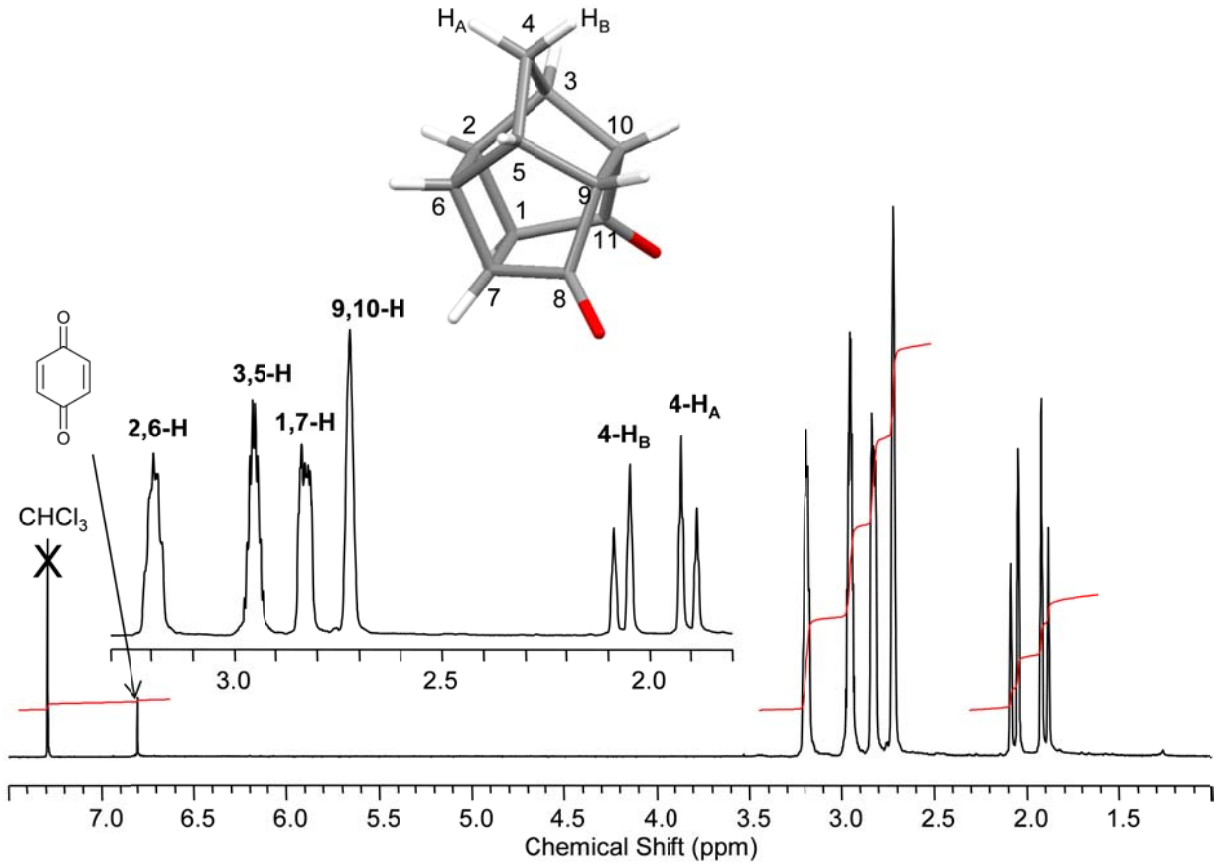


Figure SM 10.3.9 – ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of the photocycloaddition product **B**. Both alkene signals should have vanished unless the irradiation time was too short or the sample has not been properly exposed to UV light source. Assignment of ^{1}H NMR signals were confirmed by HSQC, COSY and NOESY spectra.

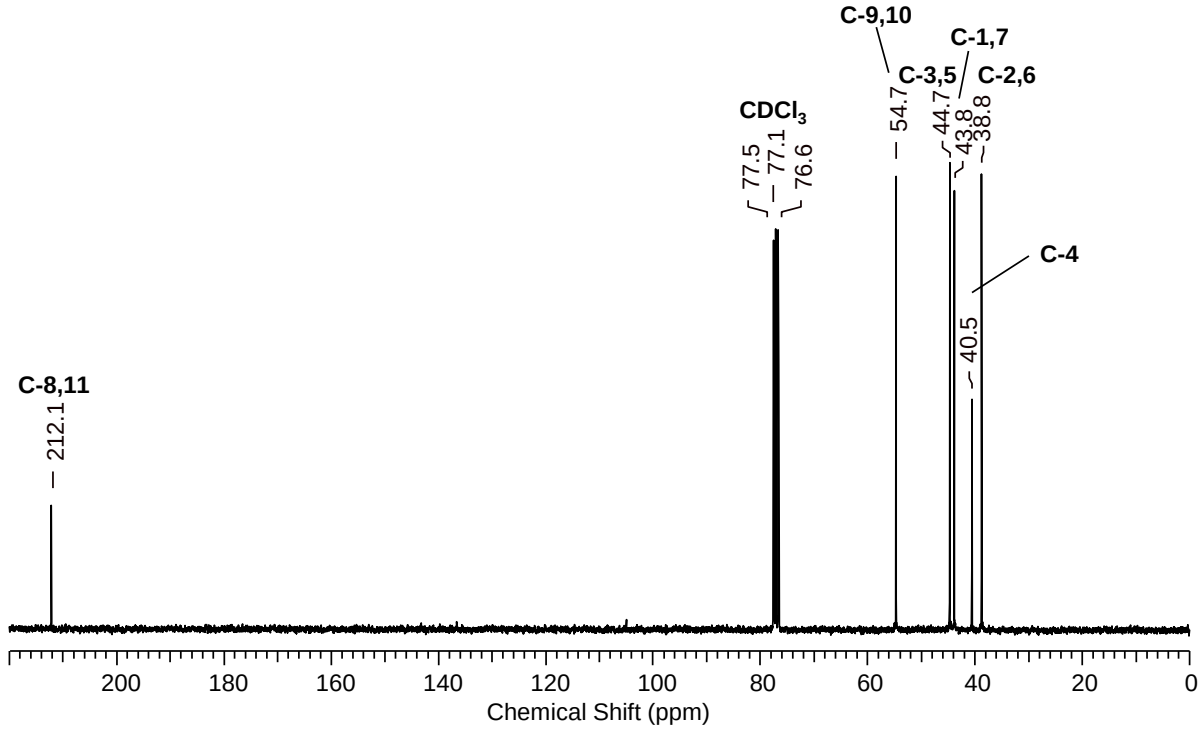


Figure SM 10.3.10 – ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of the photochemical cycloaddition product **B**. Students should note that there are a total of six ^{13}C NMR signals only. The photochemical cycloaddition product is isomeric to **A** and will also have 11 carbons; the small number of ^{13}C NMR signals indicates symmetry. The alkene NMR signals are gone indicating that they have reacted. The assignment of the NMR signals was confirmed by 2D NMR.

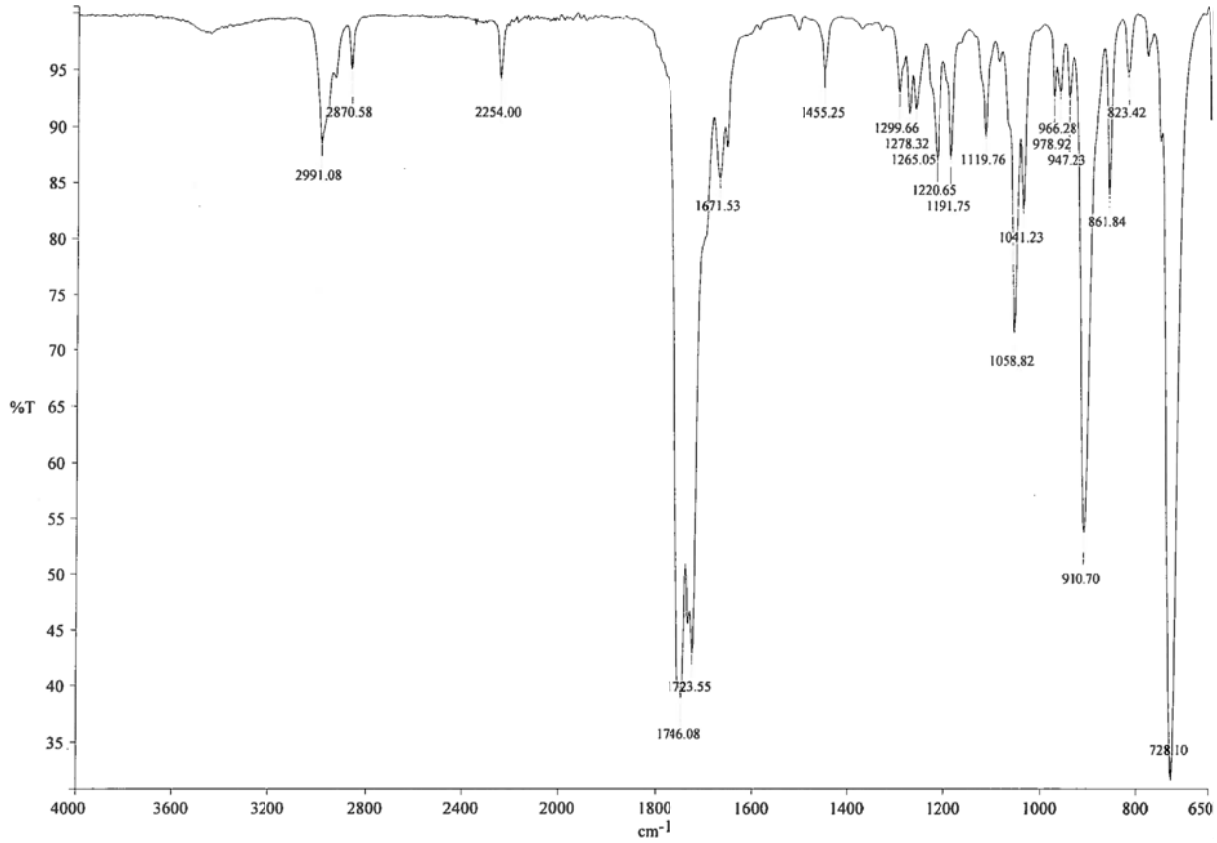


Figure SM 10.3.11 – IR spectrum of the photocycloaddition product **B** showing two C=O stretches at 1746 and 1724 cm^{-1}. Students should realise that the carbonyl stretch has shifted because the carbonyl group is no longer conjugated to an alkene double bond.

IR: 2991, 2871 cm^{-1} (C–H stretches), 1746, 1724 cm^{-1} (C=O stretches)

[1] A. Wassermann, *J. Chem. Soc*., 1935, 1511.
[2] M. Oda, T. Kawase, T. Okada and T. Enomoto, *Org. Synth. Coll. Vol*. 1998, **9**, 186.
[3] R. C. Cookson, E. Crundwell, R. R. Hill and J. Hudec, *J. Chem. Soc*.**,** 1964, 3062.
[4] A. P. Marchand and R. W. Allen, *J. Org. Chem*., 1974, **39**, 1596.
[5] T. Aillet, K. Loubiere, O. Dechy-Cabaret and L. Prat, *Chem. Eng. Proc.*, 2013, **64**, 38.
[6] E. G. Nash, *J. Chem. Educ.*, 1974, 619.
[7] R. Rathore, C. L. Burns and M. I. Deselnicu, *Org. Synth*., 2005, **82**, 1.
[8] The low frequency of the carbonyl stretch indicates that the carbonyl group is in conjugation with an alkene double bond. The C=C stretch is weak (if not absent) due to the symmetry of the alkene (IR selection rules).

Click Chemistry

Supplementary Information

1. Experimental notes 1
2. Reaction Mechanisms 2
3. Photos from the experiment 3
4. Thin Layer Chromatography (TLC) 3
5. ^{1}H-NMR and ^{13}C-NMR Spectra 4

1. Experimental notes

The proposed experimental procedure leads to the synthesis of a 1,4-disubstituted triazole (1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol). The aim of this work is to illustrate the use of a peryciclic (cycloaddition) reaction scheme with 3 steps using high yielded chemical transformations, simple to perform with no need of chromatographic steps for purification, based on the "click chemistry" concept. The experiments were assayed by 3 groups of 2 MSc students.

The students will explore the simple access to 1,2,3-triazoles and understand the variety of scaffolds that can be easily introduced around the triazole central core, not only for a library synthetic purpose, but also that its application may be expanded in the context of bioconjugation and molecular probing.

The synthesis of the starting azide illustrate the stability of aryl azides that are safe enough to be handled with no special safety measures, however appropriate conditions should be taken if storage is necessary. Moreover it points up that a straightforward procedure may be applied for the synthesis of a variety of aryl azides from the corresponding amines via diazonium salt.

Notes on the synthesis of 2-nitrophenyl azide:

The reaction was performed in an open round bottom flask and nitrogen evolution was not closely followed, in addition the apparatus may be settled with a mineral oil bubbler so that the students observe the nitrogen evolution. The instructor should check the precipitation of the desired azide. If further solid is observed in the mother liquids after the first filtration, a second filtration step should be encouraged. The reaction was assayed for 1 g and 0.5 g scale of starting *o*-nitroaniline, and the students obtained the desired azide in good yields (66-70%). After drying the obtained azide, melting point of 49-50 ºC was obtained in a Kofler camera Bock Monoscope M (uncorrected).

Notes on the synthesis of 1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol:

The reaction was assayed in 0.1 g scale and the students obtained the desired product in good yields (81-89%). After evaporation of the organics to dryness the desired product should crystallize in the round bottom flask. Melting point of 96-98 ºC was obtained in a Kofler camera Bock Monoscope M (uncorrected).

2. Reaction Mechanisms

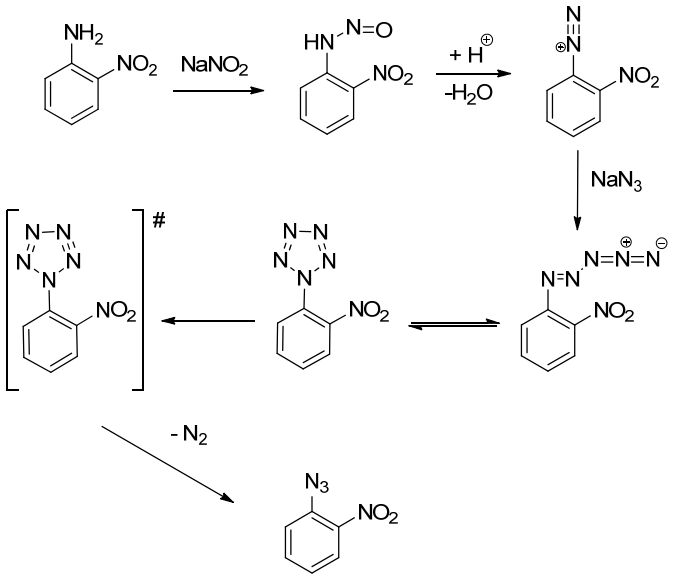


Figure SM 10.4.1. Reaction mechanism for the synthesis of 2-nitrophenyl azide from 2-nitroaniline.

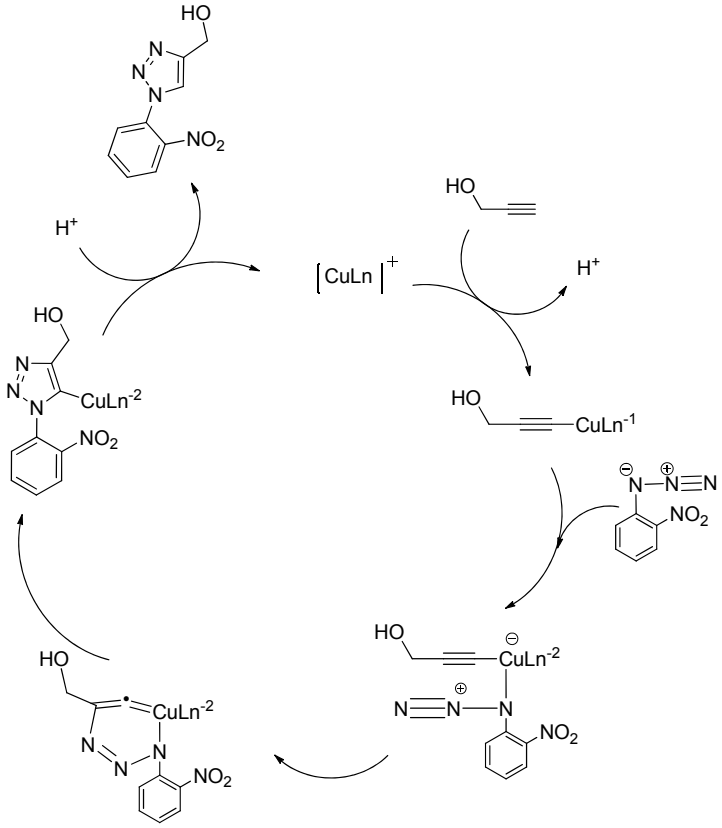


Figure SM 10.4.2. Reaction mechanism for the synthesis of 1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol.

3. Photos from the experiment

Figure SM 10.4.3. a) Step 1 at t=0, 2-nitroaniline orange color; b) Step 1 at t=1h, colorless diazonium salt.

4. Thin Layer Chromatography (TLC)

TLCs were carried out using Merck aluminum backed sheets coated with 60 F254 silica gel. Visualization of the silica plates was achieved using a UV lamp (λmax = 254 nm).

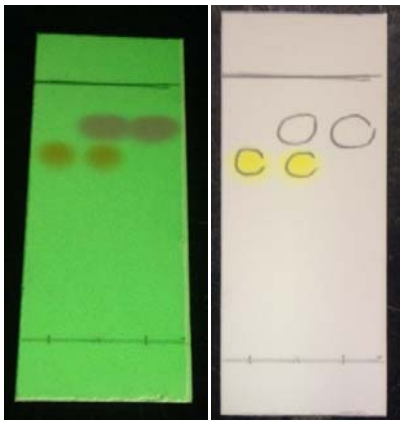


Figure SM 10.4.4. TLC for Step 1. Synthesis of 2-nitrophenyl azide.

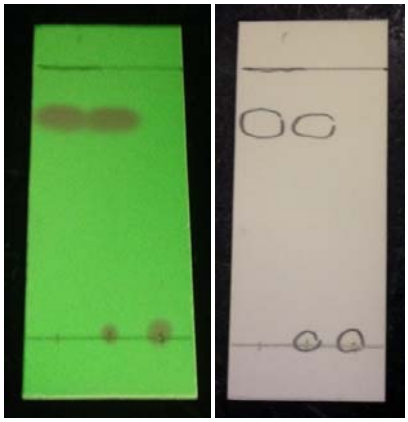


Figure SM 10.4.5. TLC for Step 2. Synthesis of 1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol.

5. ^{1}H-NMR and ^{13}C-NMR Spectra

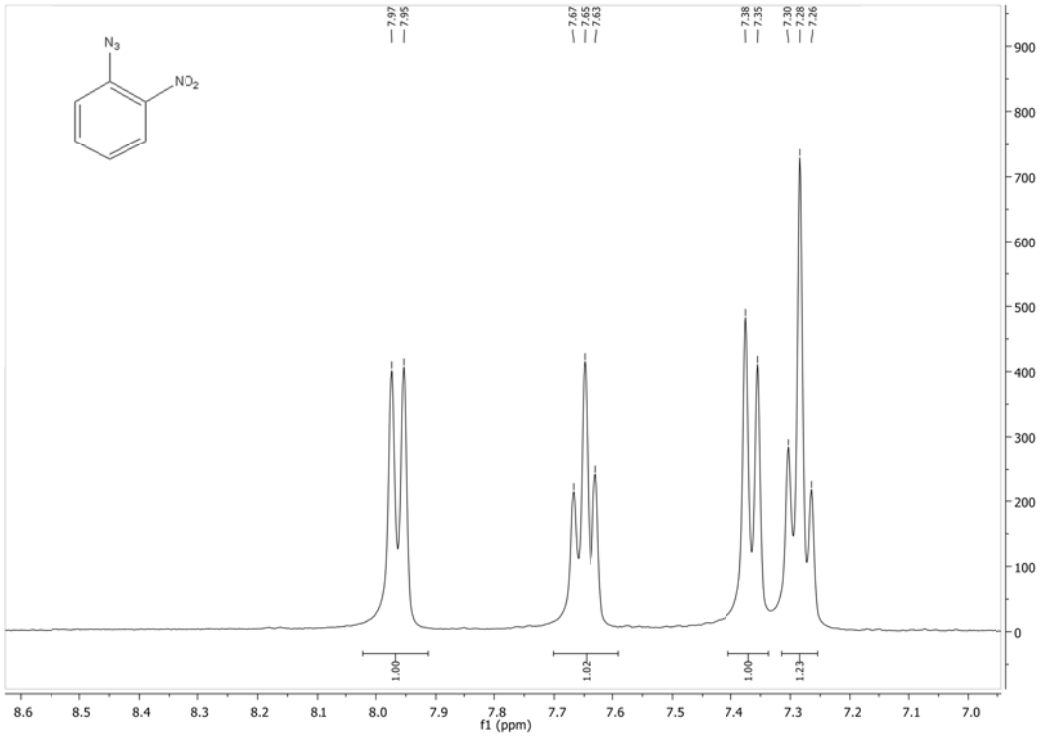


Figure SM 10.4.6. ^{1}H-NMR spectrum for 2-nitrophenyl azide (300 MHz, $CDCl_3$).

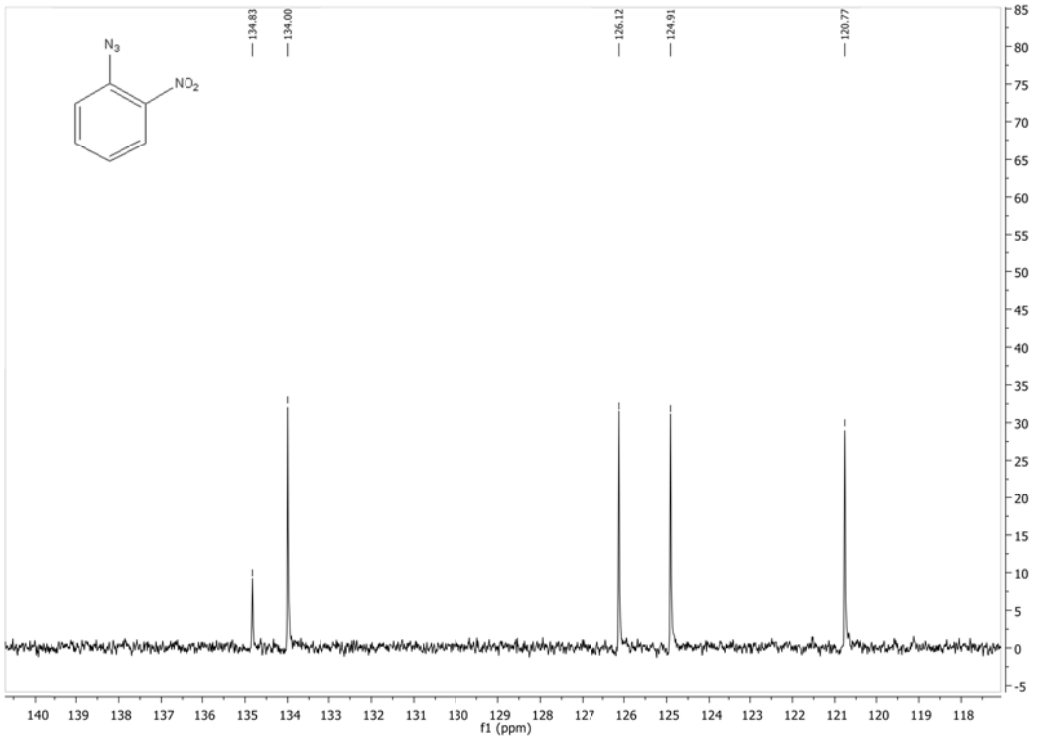


Figure SM 10.4.7. ^{13}C-NMR spectrum for 2-nitrophenyl azide (75 MHz, $CDCl_3$).

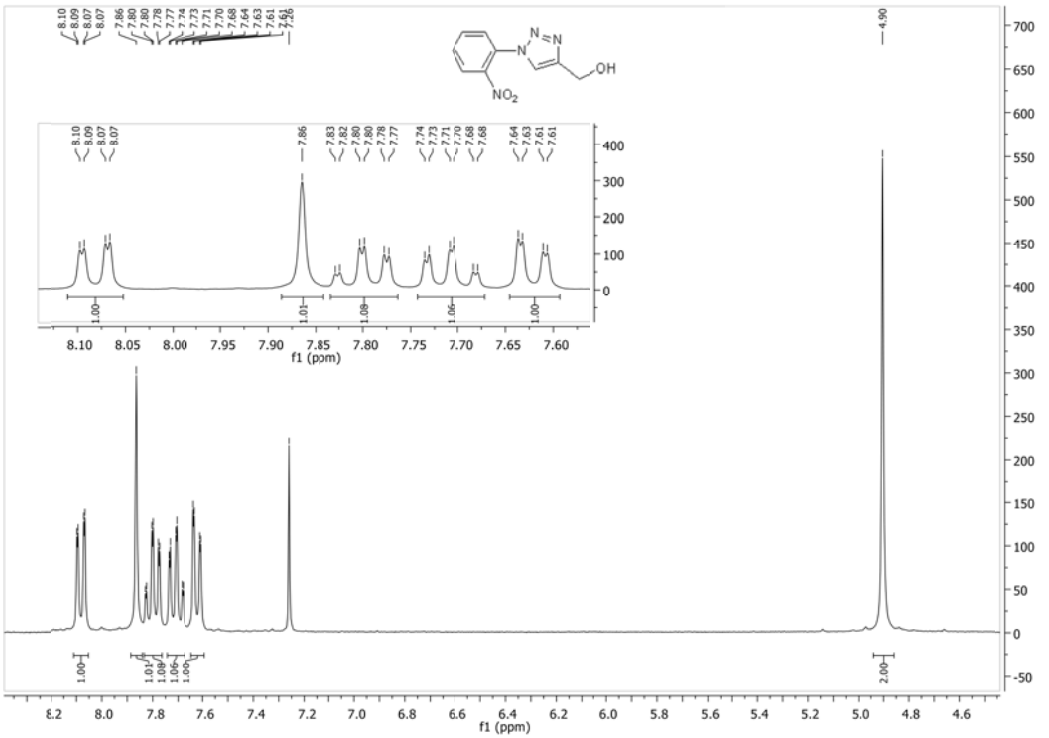


Figure SM 10.4.8. ^{1}H-NMR spectrum for 1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol (300 MHz, $CDCl_3$).

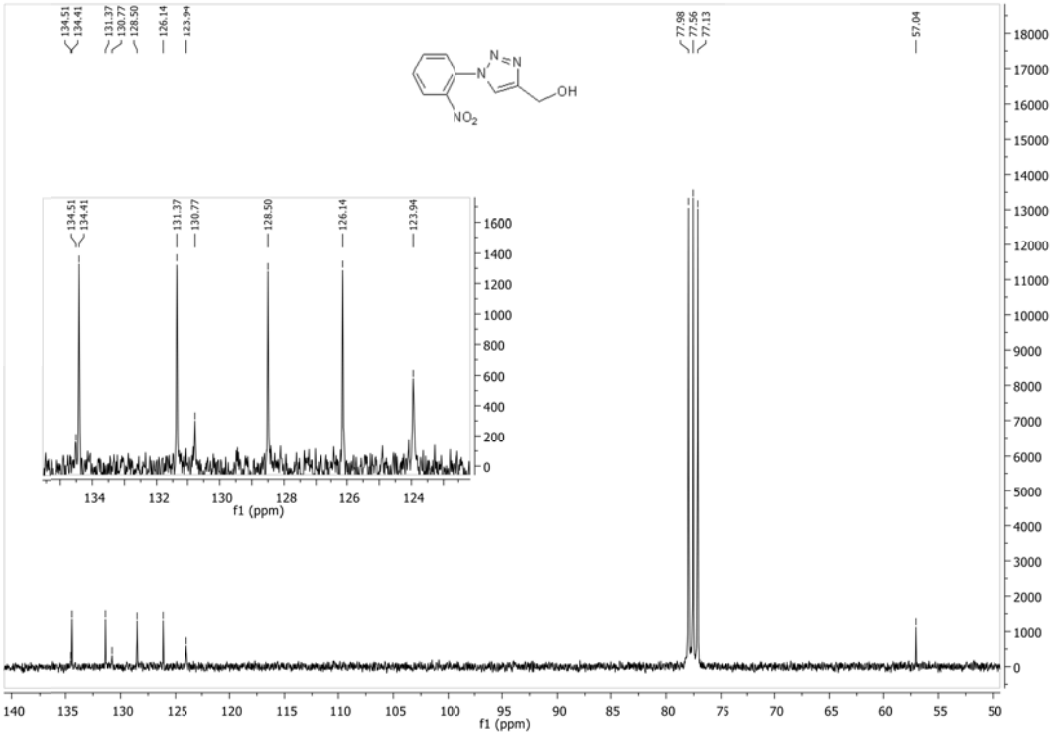


Figure SM 10.4.9. ^{13}C-NMR spectrum for 1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methanol (75 MHz, $CDCl_3$).

Application of 2,4,6-trioxo-pyrimidin-5-ylidene alditol in the synthesis of pyrano[2,3-*d*]pyrimidine containing a sugar moiety by hetero-Diels-Alder reaction

Supplementary Material

Experiment Notes 1

An extensive background on the experiment topic…… … 1

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Photos of experiment…….6

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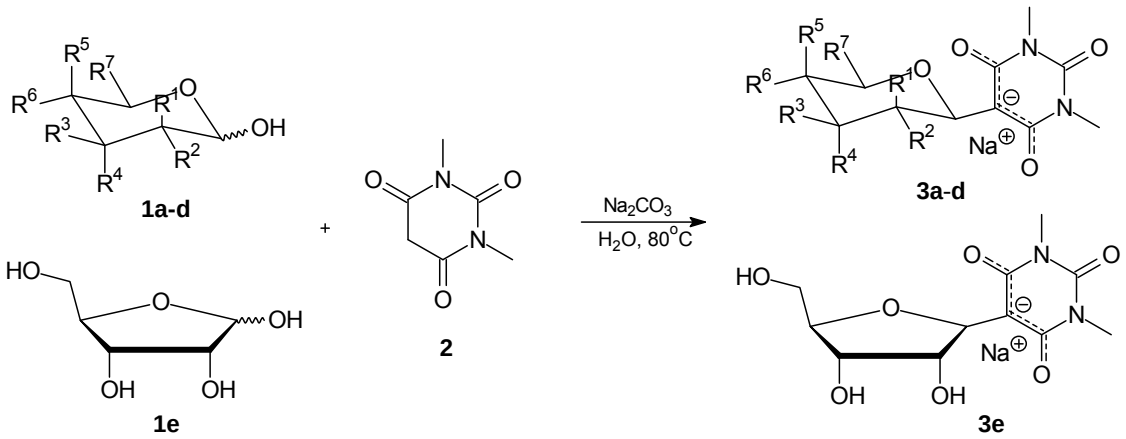
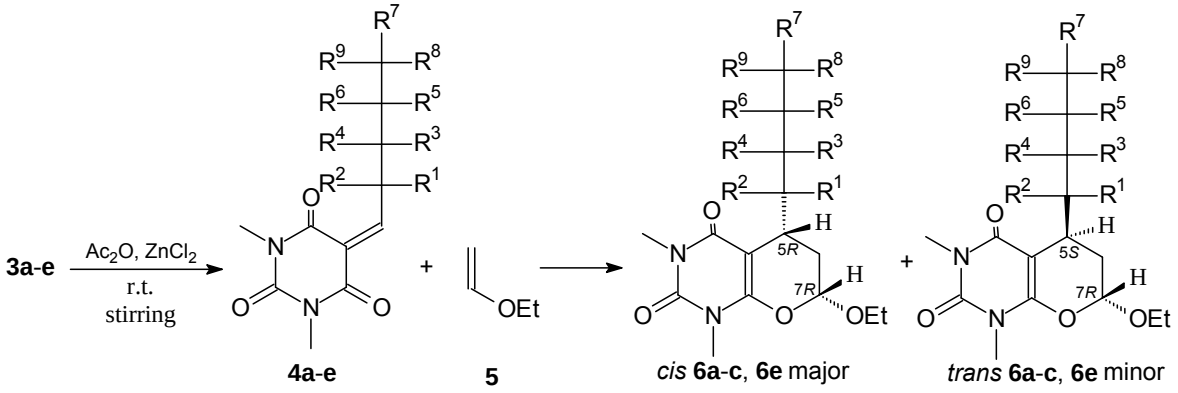
An extensive background on the experiment topic

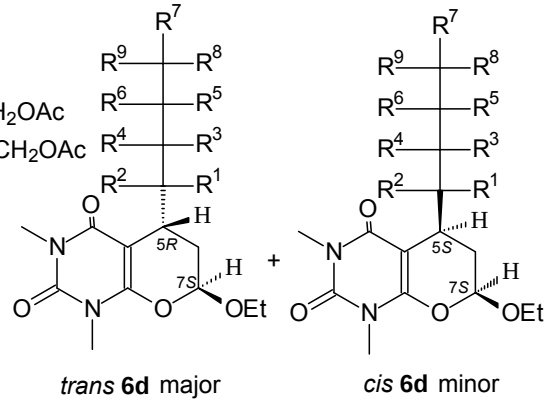
The aim of the experiment is to synthesize fused uracils such as pyrano[2,3-*d*]pyrimidines **6** (Scheme **SM 10.6.**1) containing a sugar moiety at carbon C(5) in the ring system as compounds with potential pharmacological activity. Students can investigate if 5-ylidene barbituric acids **4** with the carbohydrate substituent can act as active heterodienes in hetero-Diels-Alder reactions in synthesis of fused uracils **6**. First, potential heterodienes - 5-ylidene barbituric acids **4** bearing the sugar moiety are synthesized and in the second step their hetero-Diels-Alder reactions with enol ether **5** are conducted (Scheme **SM 10.6.**1).



1a: L-(-)-Xylose, $R^1=R^4=R^5=OH$, $R^2=R^3=R^6=R^7=H$
1b: L-(+)-Arabinose, $R^1=R^4=R^6=R^7=H$, $R^2=R^3=R^5=OH$
1c: D-(+)-Glucose, $R^1=R^4=R^5=H$, $R^2=R^3=R^6=OH$, $R^7=CH_2OH$
1d: D-(+)-Galactose, $R^1=R^4=R^6=H$, $R^2=R^3=R^5=OH$, $R^7=CH_2OH$
1e: D-(-)-Ribose

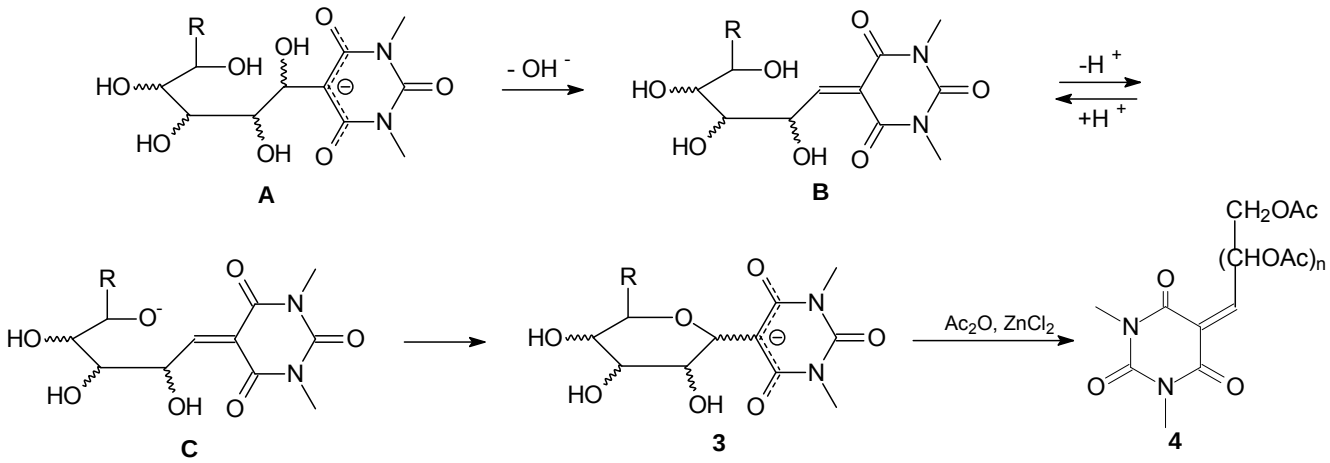


4a: L-Xylo, $R^1=R^4=R^5=R^8=OAc$, $R^2=R^3=R^6=R^7=R^9=H$
4b: L-Arabino, $R^1=R^4=R^6=R^7=R^9=H$, $R^2=R^3=R^5=R^8=OAc$
4c: D-Gluco, $R^1=R^4=R^5=R^8=H$, $R^2=R^3=R^6=R^9=OAc$, $R^7=CH_2OAc$
4d: D-Galacto, $R^1=R^4=R^6=R^8=H$, $R^2=R^3=R^5=R^9=OAc$, $R^7=CH_2OAc$
4e: D-Ribo, $R^1=R^3=R^5=R^7=R^9=H$, $R^2=R^4=R^6=R^8=OAc$



Scheme SM 10.6.1. Knoevenagel condensations of sugars **1a-e** with *N,N'*-dimethylbarbituric acid **2**. Acetylation of **3a**-**e** and hetero-Diels-Alder reactions of 5-ylidene derivatives **4a**-**e** with enol ether **5**.

Formation of the 5-arylidene derivatives of barbituric acids **4** can be explained as shown in Scheme **SM 10.6.**2 and have been proposed by Gonzalez *et al*.[12] In the first step, base-catalyzed addition of *N,N*-dimethyl barbituric acid **2** to appropriate sugar **1** provides initial intermediate adduct **A**, which undergoes β-elimination of water to produce the intermediate unsaturated compound **B**. Products **3** are formed by the intramolecular nucleophilic addition of the alkoxide **C** formed from intermediate **B** by loss of the HO-C(5') proton. Although the intermediate **B** has never been isolated, analogous structures **4** have been obtained by acetylation of the *C*-glycosylbarbiturates **3**. This result supports the participation of such olefinic structures in the formation of the *C*-glycosylbarbiturates **3**.[12]



Scheme SM 10.6.2 The mechanism of formation of *C*-glycosylbarbiturates **3**.

According to convenient procedure for Knoevenagel condensation of barbituric acids and unprotected sugars in water described by Gonzalez *et al*.[12] , the condensations of L-(-)-xylose **1a**, L-(+)-arabinose **1b**, D-(+)-glucose **1c**, D-(+)-galactose **1d** and D-(-)-ribose **1e** with 1,3-dimethylbarbituric acid **2** in water solution in the presence of sodium carbonate can be performed at 80 ^{o}C for 5 hours (Scheme **SM 10.6.**1, Figure **SM 10.6.**1). Sodium 5-glycopyranosyl-1,3-dimethylbarbiturates **3** as condensation products, do not represent a 1-oxa-1,3-diene system. Therefore, they do not react as active heterodienes in hetero-Diels-Alder reaction. Treatment of condensation products **3** with acetic anhydride and a large excess of zinc chloride give the *O*-acetylated 1,3-dimethyl-2,4,6-trioxo-

pyrimidin-5-ylidene derivatives **4**.[12] A large excess of zinc chloride is necessary. Treatment of Knoevenagel condensation products with equimolar amounts of acetic anhydride-zinc chloride gave compounds having cyclic pyranoid structures.[12] Acetylation products **4** possess a 1-oxa-1,3-diene system and additionally the introduction of the electron withdrawing acetyl groups, as protection of each of hydroxy groups, can enhance their reactivity in hetero-Diels-Alder reactions. The mixture of anhydrous zinc chloride in acetic anhydride and condensation product **3** should be stirred for 1h and next keep for 24 h at room temperature (Scheme **SM 10.6.**1, Figure **SM 10.6.**2). Acetylation products **4** can be obtained in good yields 78-89 %.[23] The cycloaddition reactions of *O*-acetylated 1,3-dimethyl-2,4,6-trioxo-pyrimidin-5-ylidene derivatives **4** with a ten-fold excess of ethyl-vinyl ether **5** are performed in the absence of solvent at room temperature for 3-5 min and pyrano[2,3-*d*]pyrimidines **6** can be obtained in good 82-88 % yields (Scheme **SM 10.6.**1, Table **SM 10.6.**1, Figure **SM 10.6.**3).[23] The progress of the reactions was monitored by TLC (20:1 CH_2Cl_2/MeOH). The excess of ether was evaporated and the mixture of diastereoisomers **6** was separated and purified by column chromatography on silica gel using dichloromethane/methanol 20:1 as an eluent, column length 60 cm, elution order: (1) minor *trans*-**6**, (2) major *cis*-**6**. (Figure **SM 10.6.**4). The students results reported were first obtained with chloroform instead dichloromethane but due to the known high toxicity of chloroform and considering that its use have been banned from most of the teaching institutions its use cannot be considered.

The reproducibility of the experiment was assessed by its repetitive execution, namely by 2nd year Chemistry M.Sc. students from Faculty of Chemistry of Jagiellonian University (Kraków, Poland).

Table of results of experiments for different sugars

Table SM 10.6.1. Synthesis of pyrano[2,3-*d*]pyrimidines **6** by solvent-free hetero-Diels-Alder reaction of alditols **4** with ethyl vinyl ether **5**.

Sugar derivative alditol **4**	Cycloaddition time (minutes)	**6**	Total yield % of cycloadducts **6**	Ratio of major/minor diastereoisomers[a] **6**	Melting Point (°C) of major diastereoisomer **6**
L-(-)-xylose derivative **4a**	3	**6a**	85-88	4:1	65-66
L-(+)-arabinose derivative **4b**	3	**6b**	82-86	4.8:1	oil
D-(+)-glucose derivative **4c**	5	**6c**	84-86	5.9:1	74-75
D-(+)-galactose derivative **4d**	3	**6d**	83-87	4.8:1	151-152
D-(-)-ribose derivative **4e**	3	**6e**	82-86	2.2:1	oil

[a]Ratio based on ^{1}H NMR (300 MHz) spectra of crude products **6**, analyzing the signal of proton 7-H.[23]

Isolation of *cis* and *trans* diastereoisomers of pyrano[2,3-*d*]pyrimidines by column chromatography

Isolation and purification of *cis* and *trans* diastereoisomers of pyrano[2,3-*d*]pyrimidines **6** by column chromatography can be realized using the setup presented on Figure **SM 10.6.**4. It is a column chromatography on silica gel using dichloromethane/methanol (20:1 v/v) as eluent. Students should collect fractions into test-tubes and control the composition of each fraction by TLC (CH_2Cl_2/CH_3OH 20:1). Next, they should place together the fractions with the same composition in round-bottomed flask and evaporate the solvent under reduced pressure using a rotary evaporator. Pure solid *cis* or *trans* diastereoisomers of pyrano[2,3-*d*]pyrimidines **6** can be isolated by crystallization from the appropriate mixture of *tert*-butyl methyl ether and petroleum ether. Products **6** are soluble in *tert*-butyl methyl ether and insoluble in petroleum ether.

Photos of the experiment

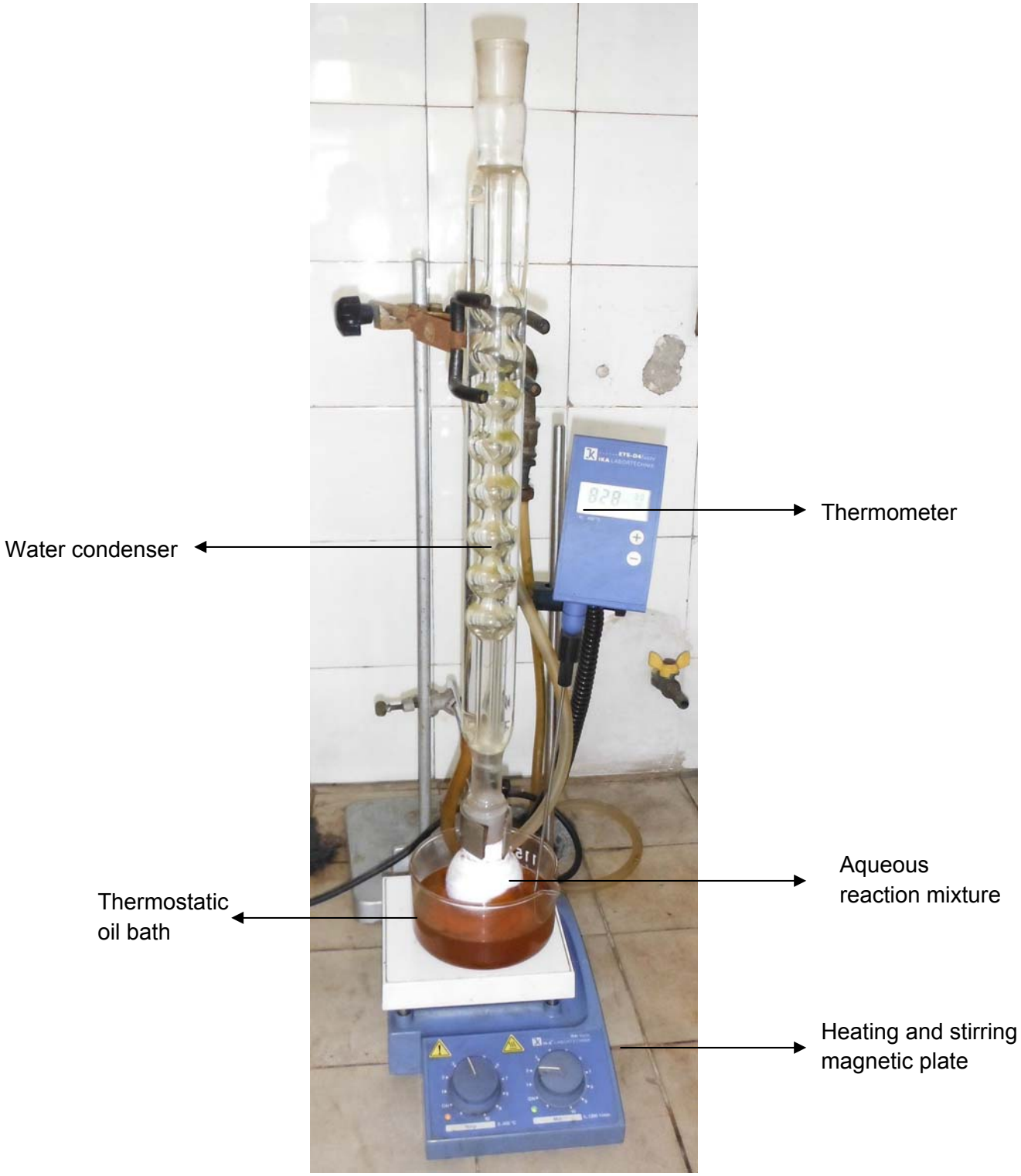


Figure SM 10.6.1 – Knoevenagel condensation setup apparatus with maintaining a temperature of 80 ^{o}C on a magnetic hot plate.

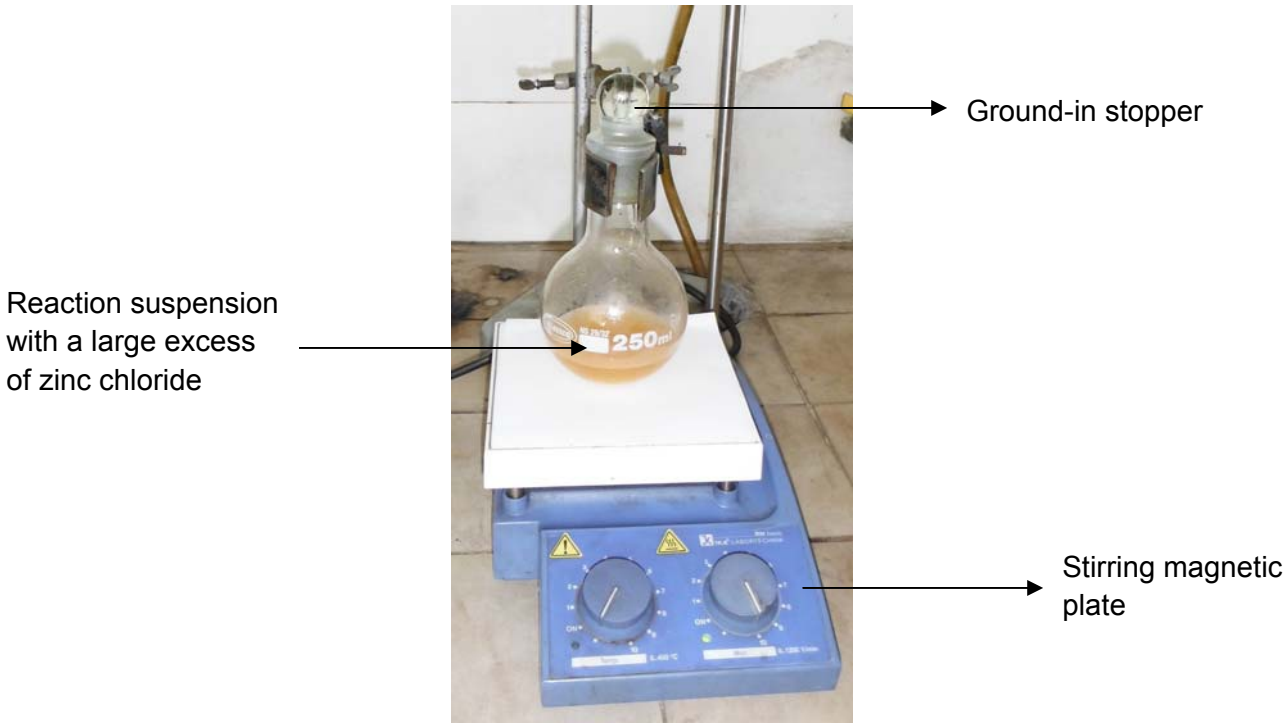


Figure SM 10.6.2 – Acetylation setup apparatus.

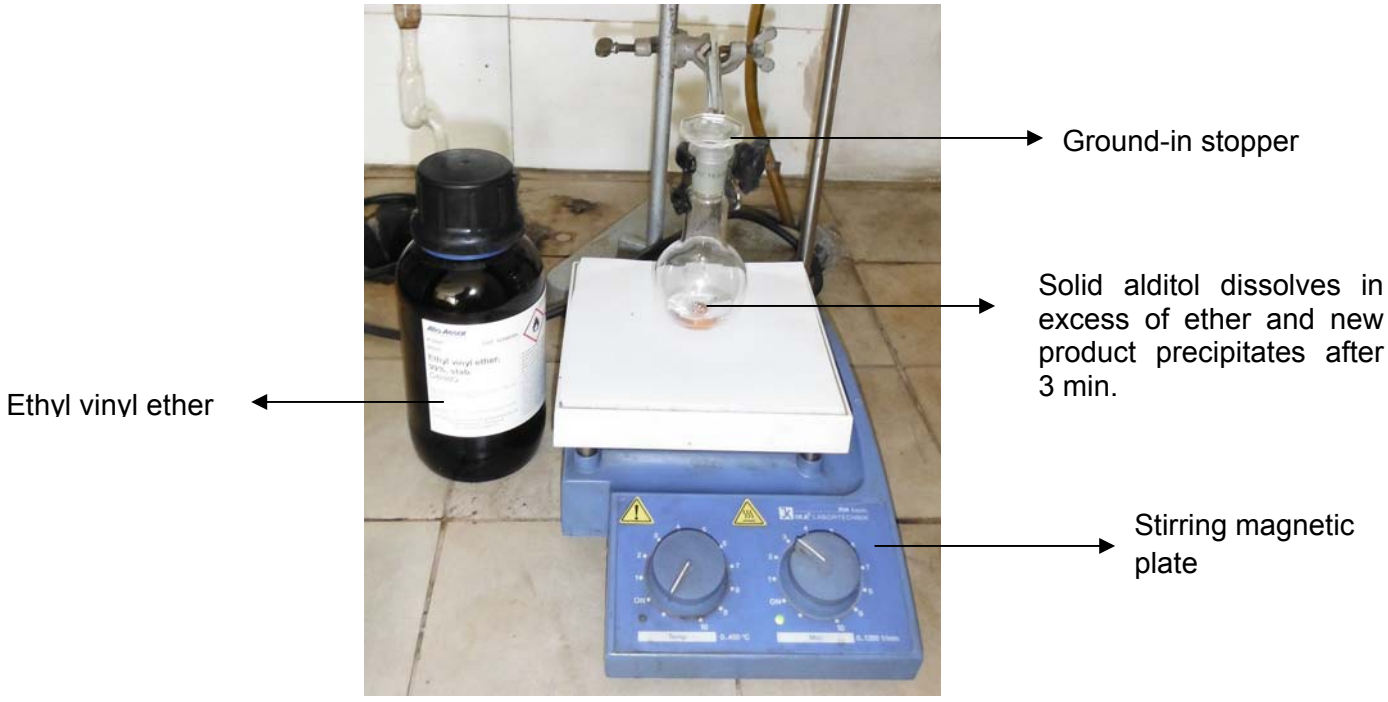


Figure SM 10.6.3 – Solvent-free hetero-Diels-Alder reaction setup apparatus.

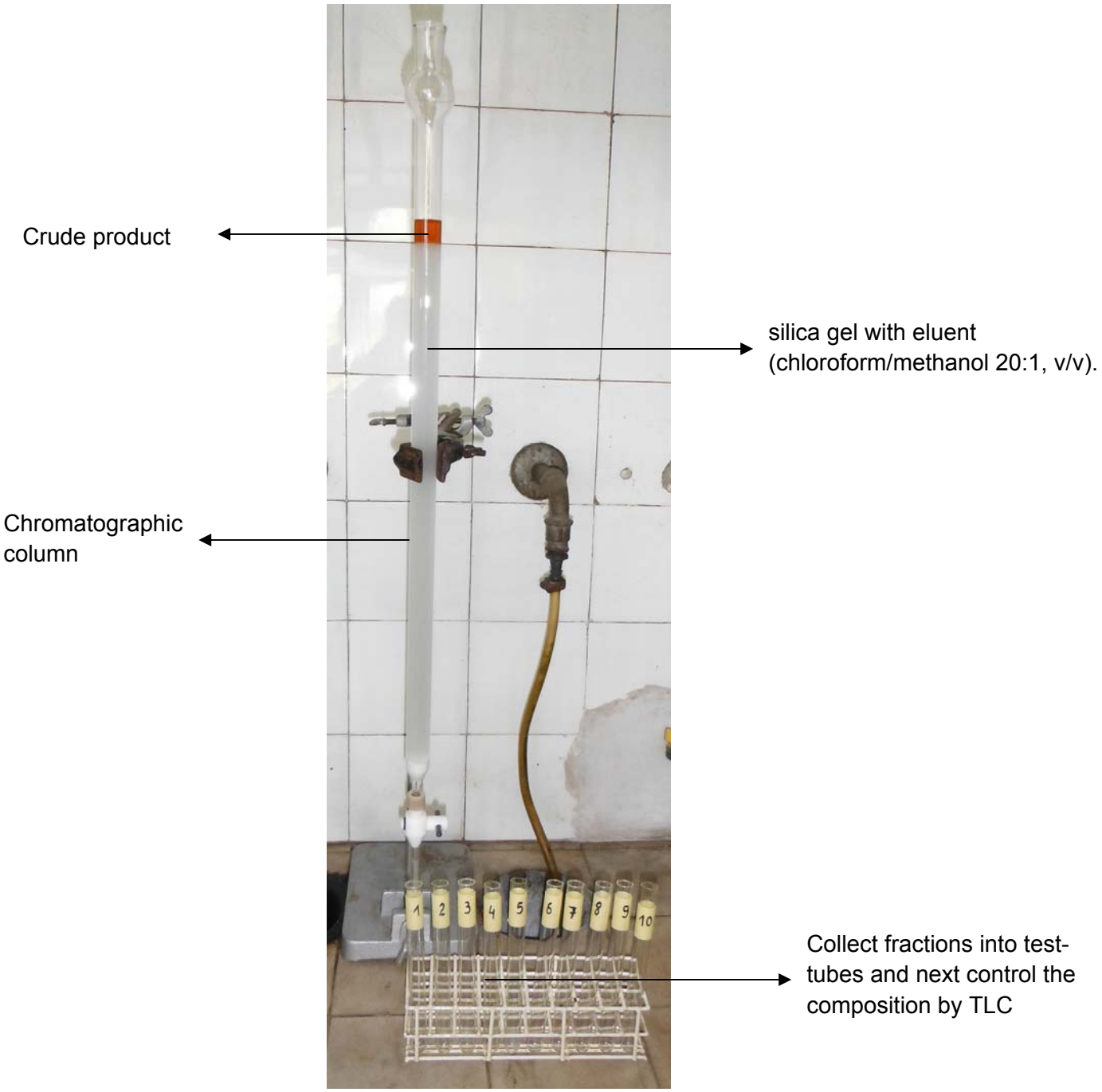


Figure SM 10.6.4 – Setup for separation and purification the mixture of *cis*/*trans* diastereoisomers of pyrano[2,3-*d*]pyrimidines **6** by column chromatography on silica gel.

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

4a

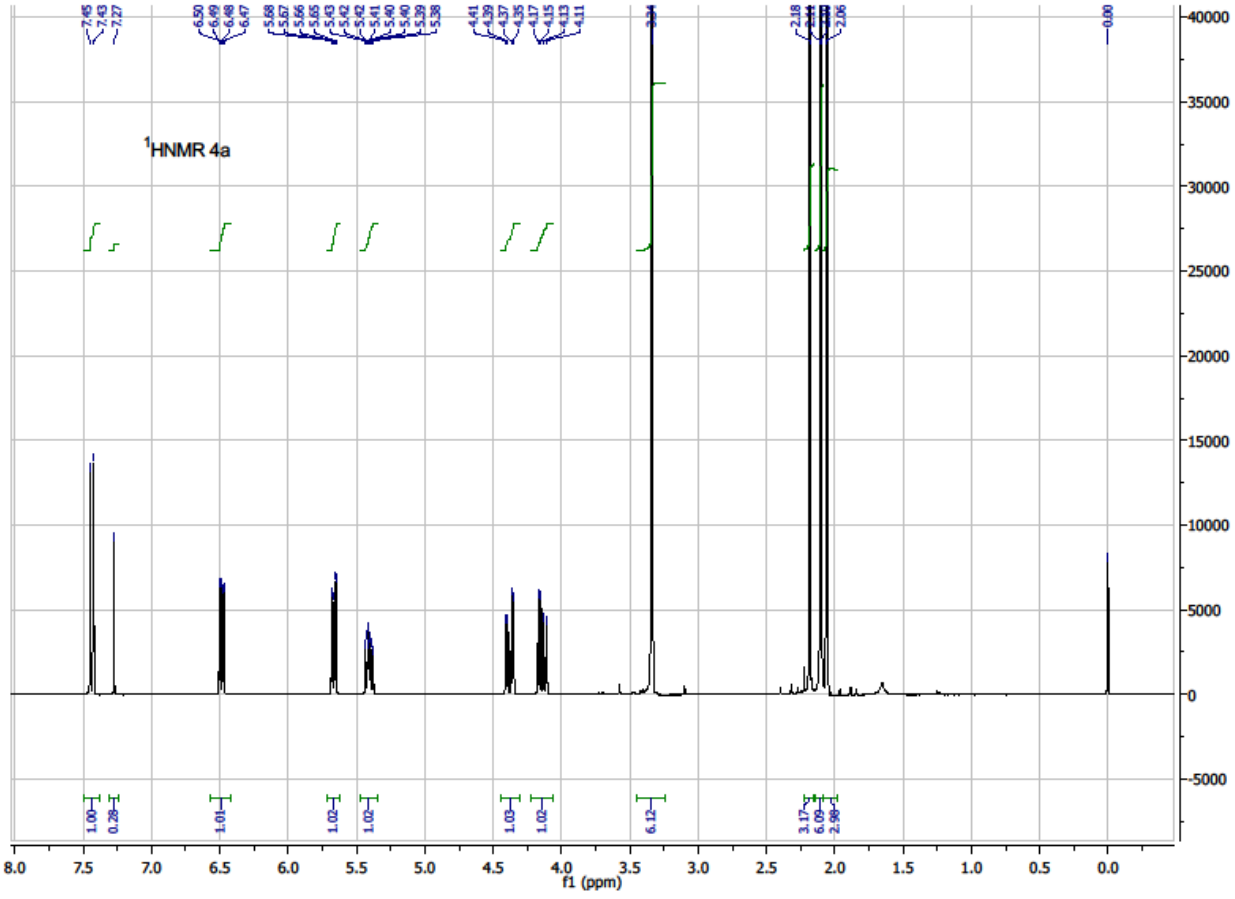


Figure SM 10.6.5 – ^{1}H NMR spectrum of 2,3,4,5-tetra-*O*-acetyl-1-deoxy-1-(1,3-dimethyl-2,4,6-trioxo-1*H*,3*H*,5*H*-pyrimidin-5-ylidene)-L-xylitol **4a**.[23]

^{1}H NMR **4a** (300 MHz, $CDCl_3$) δ 7.44 (1H, d, *J* 7.2 Hz, 1'-H), 6.49 (1H, dd, *J* 3.0, 7.2 Hz, 2'-H), 5.66 (1H, dd, *J* 3.0, 6.9 Hz, 3'-H), 5.41 (1H, ddd, *J* 3.9, 5.7, 6.9 Hz, 4'-H), 4.38 (1H, dd, *J* 3.9, 12.3 Hz, 5'-

H), 4.13 (1H, dd, *J* 5.7, 12.3 Hz, 5'-H), 3.34 (6H, s, N-CH_3), 2.18 (3H, s, $COCH_3$), 2.11 (3H, s, $COCH_3$), 2.10 (3H, s, $COCH_3$), 2.06 (3H, s, $COCH_3$).

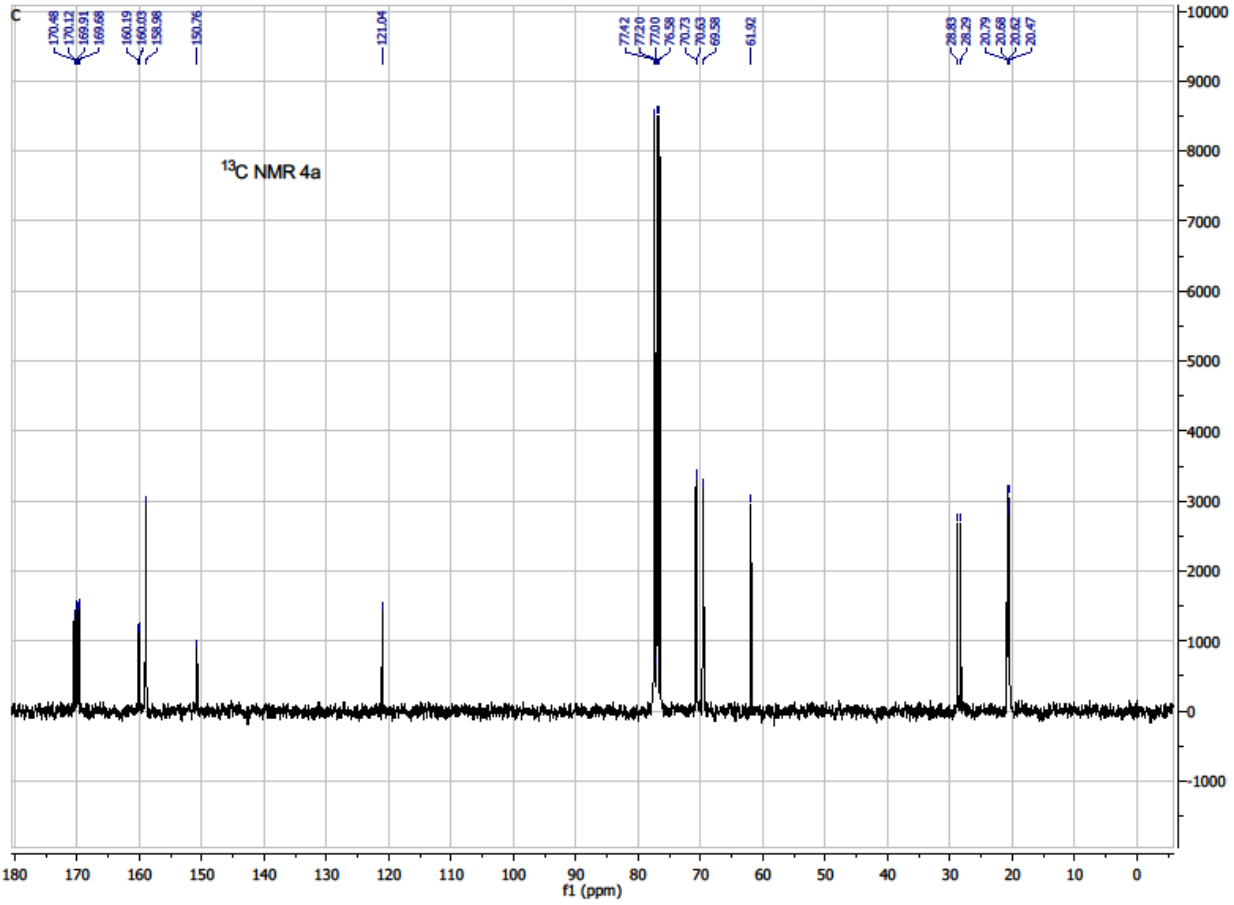


Figure SM 10.6.6 – ^{13}C NMR spectrum of 2,3,4,5-tetra-*O*-acetyl-1-deoxy-1-(1,3-dimethyl-2,4,6-trioxo-1*H*,3*H*,5*H*-pyrimidin-5-ylidene)-L-xylitol **4a**.[23]

^{13}C NMR **4a** (75.5 MHz, $CDCl_3$) δ 170.5 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 169.9 ($\underline{C}OCH_3$), 169.7 ($\underline{C}OCH_3$), 160.2(C=O), 160.0 (C=O), 159.0 (C=O), 150.8 (C=C), 121.0 (C=C), 70.7 (C-1'), 70.6 (C-2'), 69.6 (C-3'), 61.9 (C-4'), 28.8 (N-CH_3), 28.3 (N-CH_3), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$).

cis-**6a**

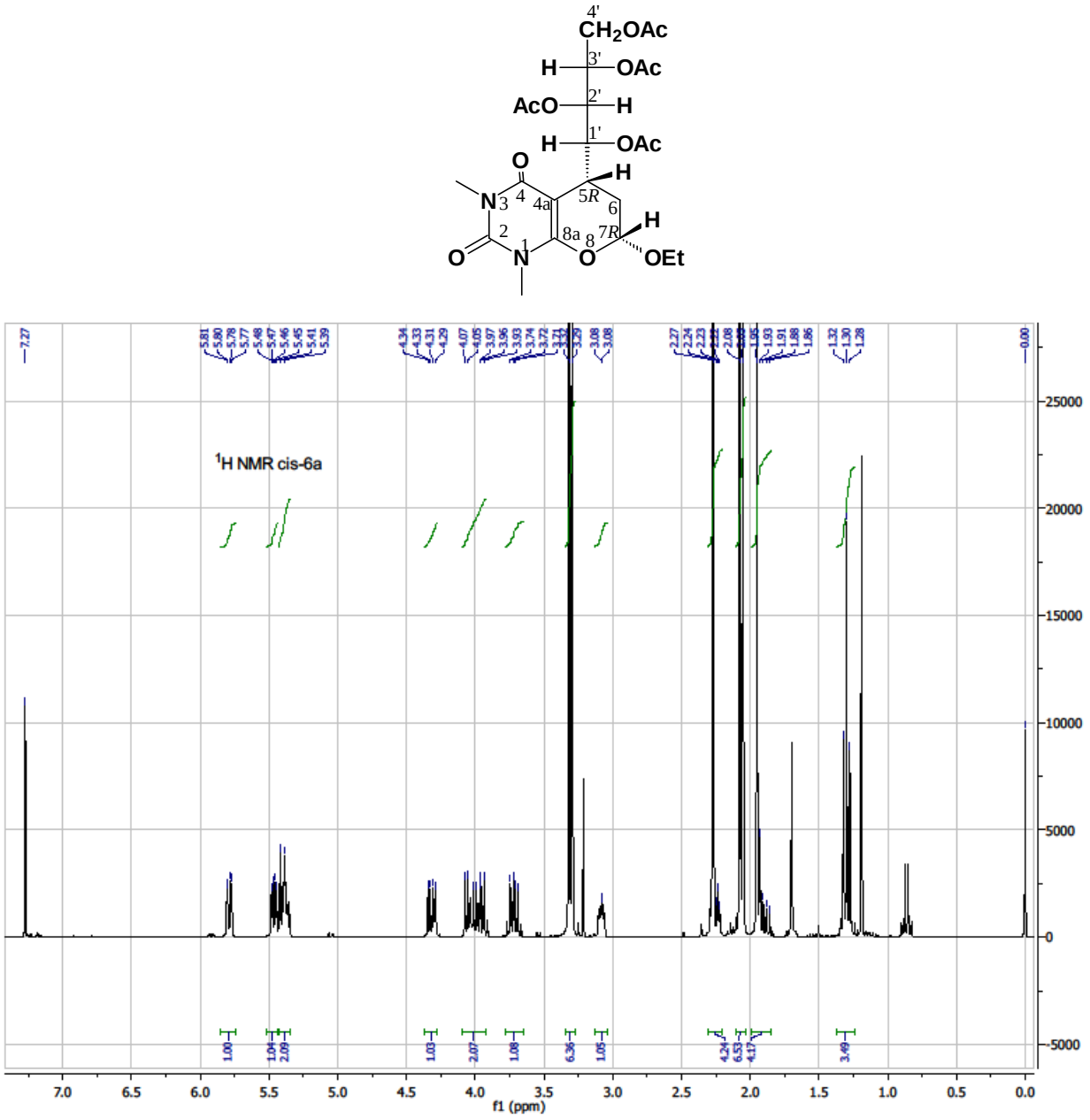


Figure SM 10.6.7 – ^{1}H NMR spectrum of major *cis* diastereoisomer of pyrano[2,3-*d*]pyrimidine -(5*R*,7*R*,1'*R*,2'*S*,3'*S*)-5-(1',2',3',4'-tetra-*O*-acetyl-butane-1',2',3',4'-tetraolyl)-7-ethoxy-1,3-dimethyl-1,5,6,7-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-2,4(3*H*)-dione *cis*-**6a**.[23]

^{1}H NMR *cis*-**6a** (300 MHz, $CDCl_3$) δ 5.78 (1H, dd, *J* 2.4, 8.1 Hz, 7-H), 5.46 (1H, dd, *J* 3.0, 8.1 Hz, 1'-H), 5.40 (1H, dd, *J* 3.0, 8.1 Hz, 2'-H), 5.37 (1H, ddd, *J* 3.0, 5.1, 6.9 Hz, 3'-H), 4.31 (1H, dd, *J* 5.1, 11.4 Hz, 4'-H), 4.04 (1H, dd, *J* 6.9, 11.4 Hz, 4'-H), 3.96 (1H, dq, *J* 7.2, 9.6 Hz, $OC\underline{H}_2CH_3$), 3.72 (1H, dq, *J*

7.2, 9.6 Hz, OC<u>H</u>$_2$CH$_3$), 3.32 (3H, s, N-Me), 3.29 (3H, s, N-Me), 3.08 (1H, ddd, *J* 3.0, 3.9, 7.2 Hz, 5-H), 2.27 (3H, s, COC<u>H</u>$_3$), 2.25 (1H, ddd, *J* 2.4, 3.9, 13.8 Hz, 6-H), 2.08 (3H, s, COC<u>H</u>$_3$), 2.05 (3H, s, COC<u>H</u>$_3$), 1.95 (3H, s, COC<u>H</u>$_3$), 1.93 (1H, ddd, *J* 7.2, 8.1, 13.8 Hz, 6-H), 1.30 (3H, t, *J* 7.2 Hz, OCH$_2$C<u>H</u>$_3$)

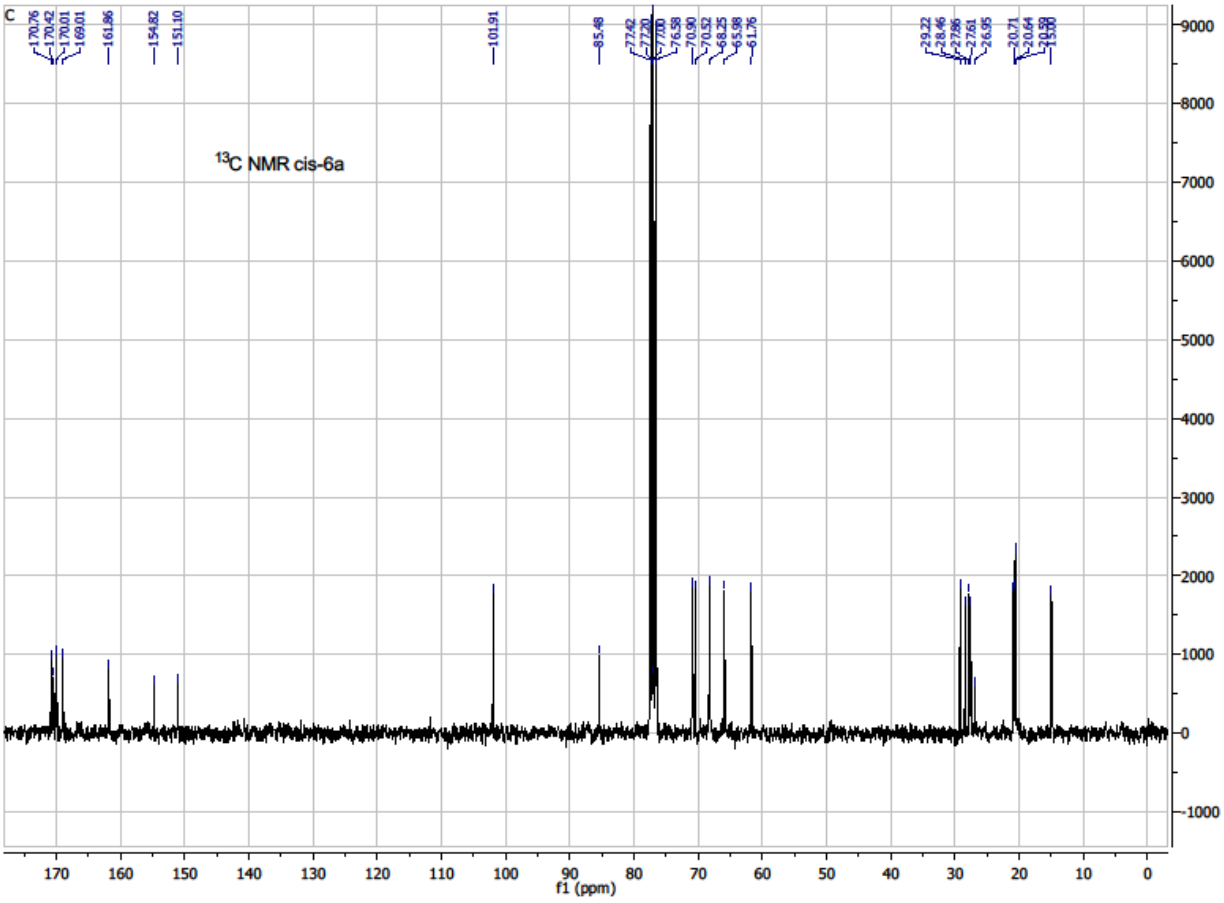
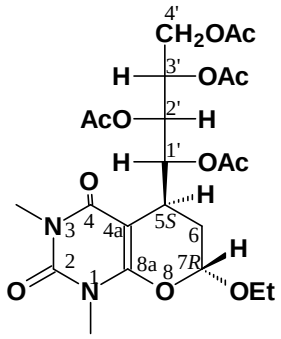


Figure SM 10.6.8 – ^{13}C NMR spectrum of major *cis* diastereoisomer of pyrano[2,3-*d*]pyrimidine -(5*R*,7*R*,1'*R*,2'*S*,3'*S*)-5-(1',2',3',4'-tetra-*O*-acetyl-butane-1',2',3',4'-tetraolyl)-7-ethoxy-1,3-dimethyl-1,5,6,7-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-2,4(3*H*)-dione *cis*-**6a**.[23]

^{13}C NMR *cis*-**6a** (75.5 MHz, $CDCl_3$) δ 170.8 (<u>C</u>OCH$_3$), 170.4 (<u>C</u>OCH$_3$), 170.0 (<u>C</u>OCH$_3$), 169.0 (<u>C</u>OCH$_3$), 161.9 (C=O), 154.8 (C=O), 151.1 (C-8a), 101.9 (C-7), 85.5 (C-4a), 70.9 (C-1'), 70.5 (C-2'), 68.3 (C-3'), 66.0 (OC<u>H</u>$_2$CH$_3$), 61.8 (C-4'), 29.2 (C-5), 28.5 (N-CH$_3$), 27.9 (N-CH$_3$), 27.6 (C-6), 21.0 (CO<u>C</u>H$_3$), 20.7 (CO<u>C</u>H$_3$), 20.7 (CO<u>C</u>H$_3$), 20.6 (CO<u>C</u>H$_3$), 15.0 (OCH$_2$C<u>H</u>$_3$).

trans-**6a**



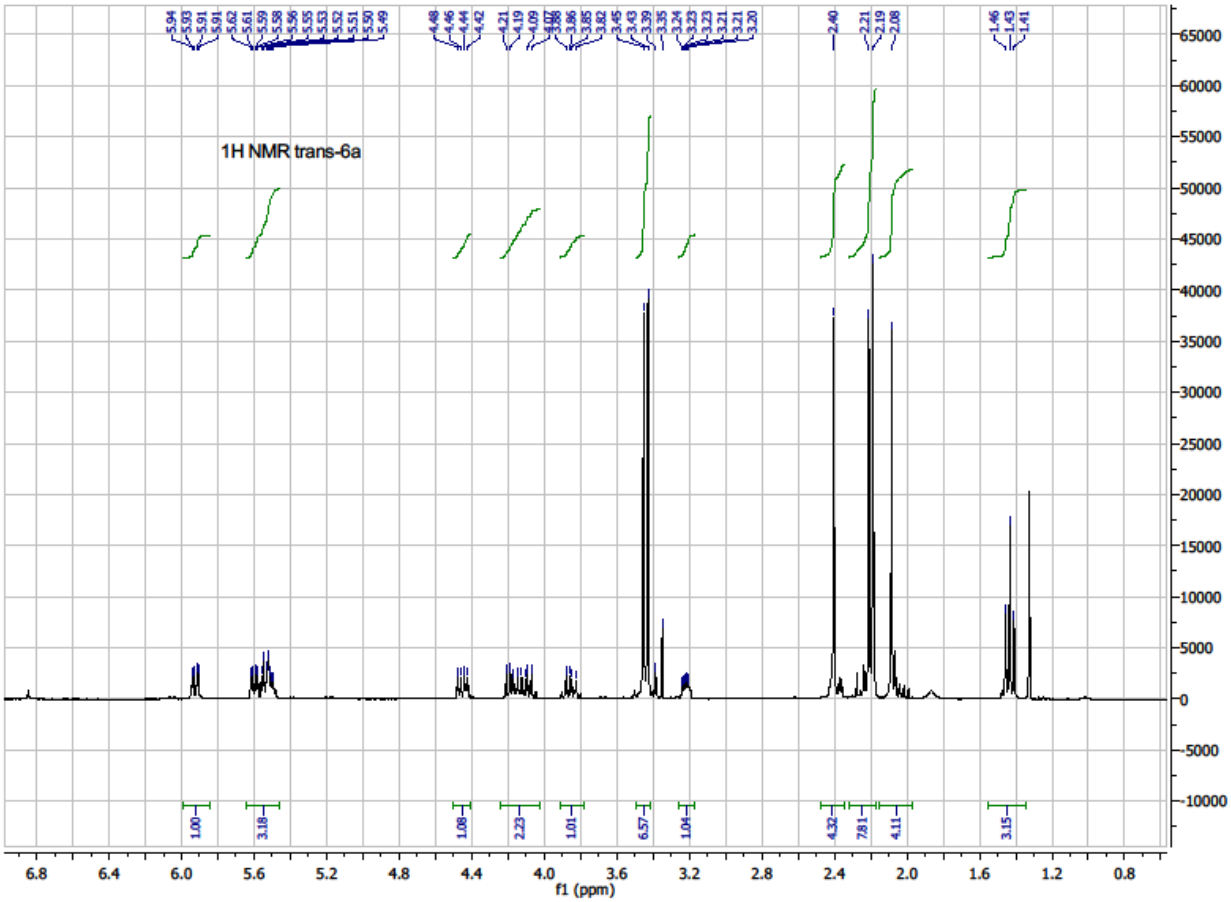


Figure SM 10.6.9 – ^{1}H NMR spectrum of minor *trans* diastereoisomer of pyrano[2,3-*d*]pyrimidine -(5*R*,7*R*,1'*R*,2'*S*,3'*S*)-5-(1',2',3',4'-tetra-*O*-acetyl-butane-1',2',3',4'-tetraolyl)-7-ethoxy-1,3-dimethyl-1,5,6,7-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-2,4(3*H*)-dione *trans*-**6a**.[23]

^{1}H NMR *trans*-**6a** (300 MHz, $CDCl_3$) δ 5.92 (1H, dd, *J* 2.4, 8.1 Hz, 7-H), 5.43 (1H, dd, *J* 8.4, 12.0 Hz, 1'-H), 5.42 (1H, dd, *J* 3.3, 12.0 Hz, 2'-H), 5.38 (1H, ddd, *J* 3.3, 4.8, 6.9 Hz, 3'-H), 4.28 (1H, dd, *J* 4.8, 11.4 Hz, 4'-H), 3.96 (1H, dd, *J* 6.9, 11.4 Hz, 4'-H), 3.95 (1H, dq, *J* 6.9, 9.6 Hz, $OC\underline{H}_2CH_3$), 3.71 (1H,

dq, *J* 6.9, 9.6 Hz, $OC\underline{H}_2CH_3$), 3.31 (3H, s, N-Me), 3.29 (3H, s, N-Me), 3.22 (1H, ddd, *J* 2.1, 8.4, 8.4 Hz, 5-H), 2.27 (3H, s, $COC\underline{H}_3$), 2.13 (1H, ddd, *J* 2.1, 8.1, 14.4 Hz, 6-H), 2.05 (3H, s, $COC\underline{H}_3$), 2.02 (1H, ddd, *J* 2.4, 8.4, 14.4 Hz, 6-H), 1.95 (3H, s, $COC\underline{H}_3$), 1.92 (3H, s, $COC\underline{H}_3$), 1.31 (3H, t, *J* 6.9 Hz, $OCH_2C\underline{H}_3$).

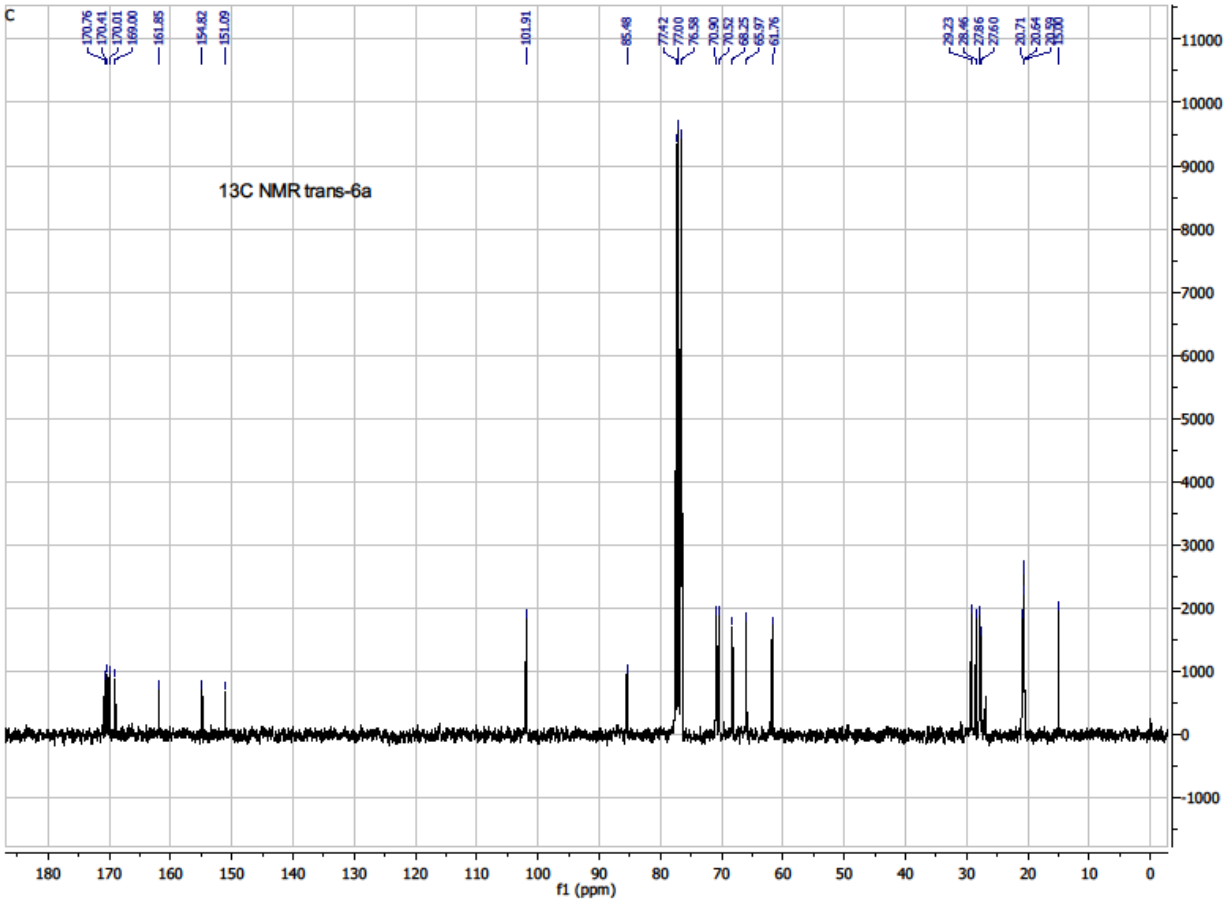


Figure SM 10.6.10 – ^{13}C NMR spectrum of minor *trans* diastereoisomer of pyrano[2,3-*d*]pyrimidine -(5*R*,7*R*,1'*R*,2'*S*,3'*S*)-5-(1',2',3',4'-tetra-*O*-acetyl-butane-1',2',3',4'-tetraolyl)-7-ethoxy-1,3-dimethyl-1,5,6,7-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-2,4(3*H*)-dione *trans*-**6a**.[23]

^{13}C NMR *trans*-**6a** (75.5 MHz, $CDCl_3$) δ 171.0 ($\underline{C}OCH_3$), 170.5 ($\underline{C}OCH_3$), 169.8 ($\underline{C}OCH_3$), 169.4 ($\underline{C}OCH_3$), 161.8 (C=O), 155.1 (C=O), 151.1 (C-8a), 101.8 (C-7), 85.3 (C-4a), 71.0 (C-1'), 69.7 (C-2'), 68.3 (C-3'), 66.3 ($OC\underline{H}_2CH_3$), 62.1 (C-4'), 29.2 (C-5), 28.4 ($N\text{-}CH_3$), 27.8 ($N\text{-}CH_3$), 27.6 (C-6), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.55 ($CO\underline{C}H_3$), 15.0 ($OCH_2C\underline{H}_3$).

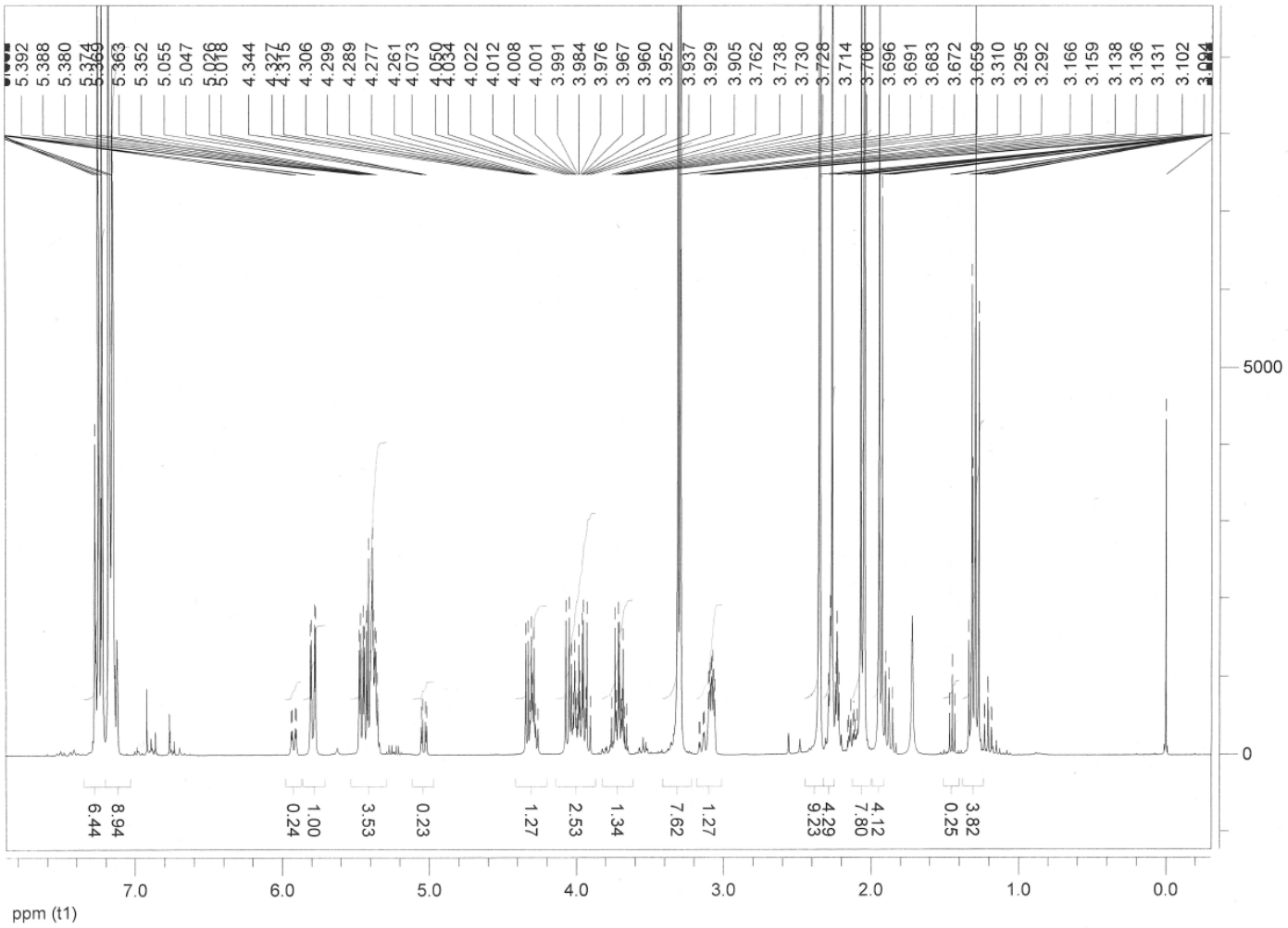


Figure SM 10.6.11 – ^{1}H NMR spectrum of crude product **6a**.

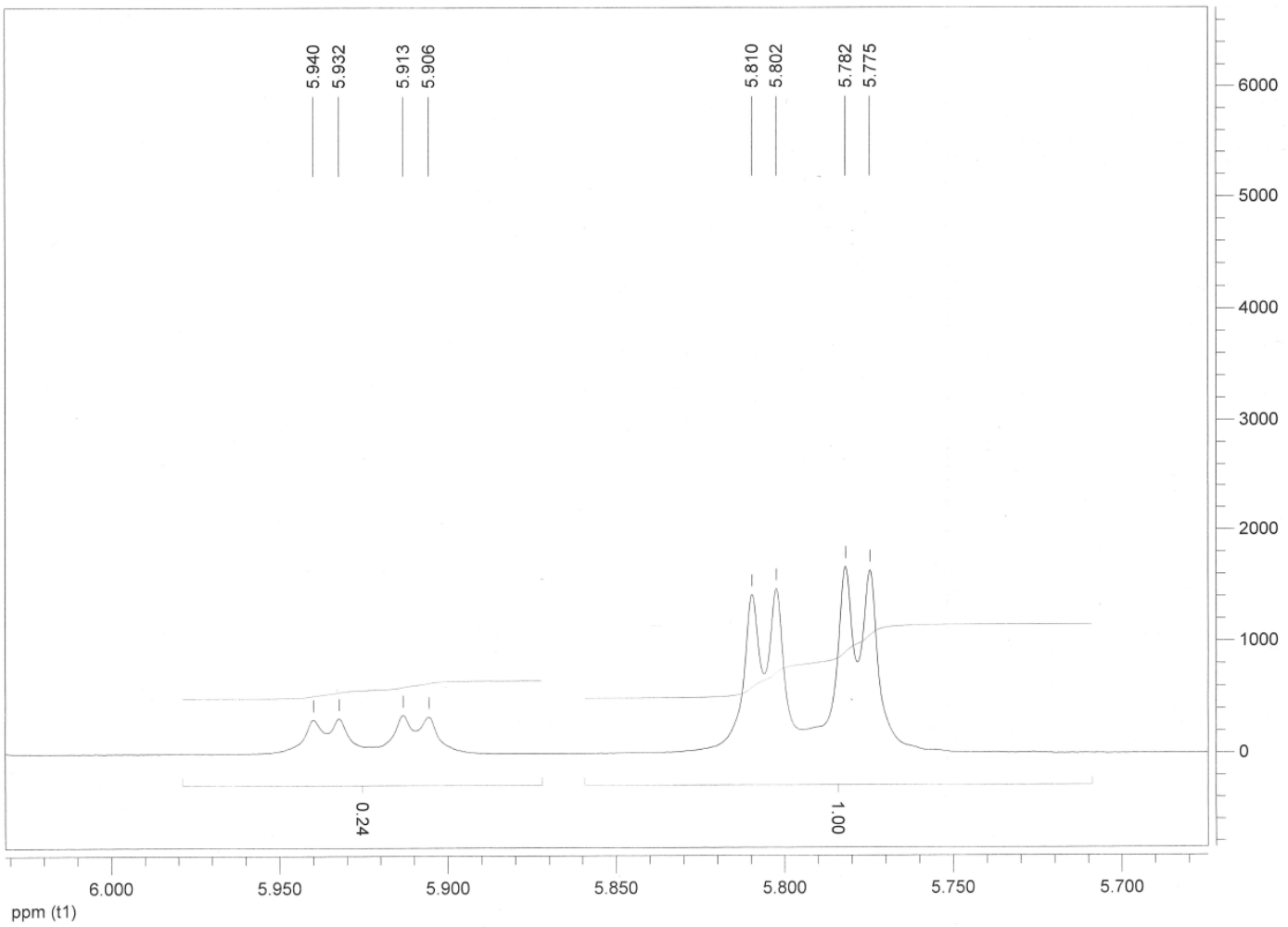


Figure SM 10.6.12 – ^{1}H NMR spectrum of crude product **6a** - the signal of proton 7-H: *cis*-**6a** *δ* 5.78 (dd, *J* 2.4, 8.1 Hz), *trans*-**6a** *δ* 5.92 (dd, *J* 2.4, 8.1 Hz), ratio of diastereoisomers major *cis* **6a**/minor *trans* **6a** 4:1.

Application of 2,4,6-trioxo-pyrimidin-5-ylidene alditol in the synthesis of pyrano[2,3-*d*]pyrimidine containing a sugar moiety by hetero-Diels-Alder reaction

Supplementary Material

Experiment Notes 1

An extensive background on the experiment topic…… … 1

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Isolation of *cis* and *trans* diastereoisomers of pyrano[2,3-*d*]pyrimidines by column chromatography… 5

Figures

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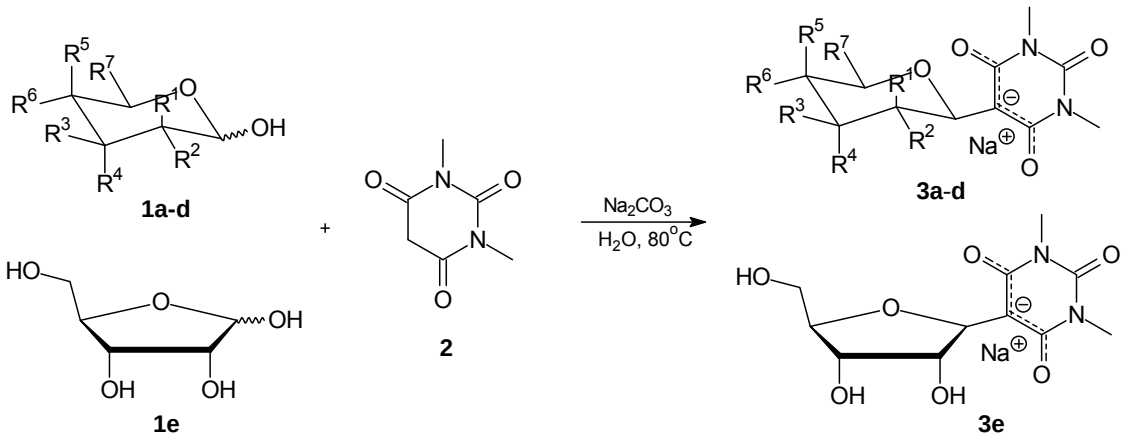
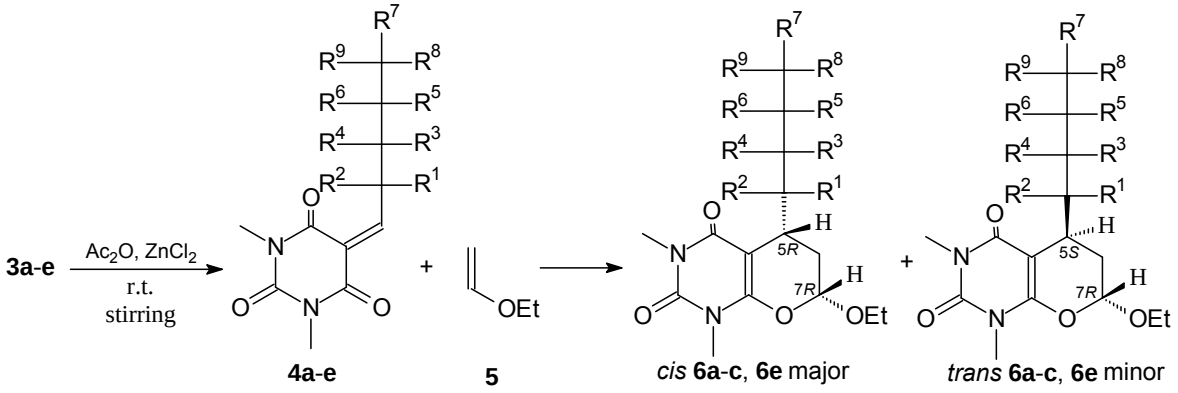
An extensive background on the experiment topic

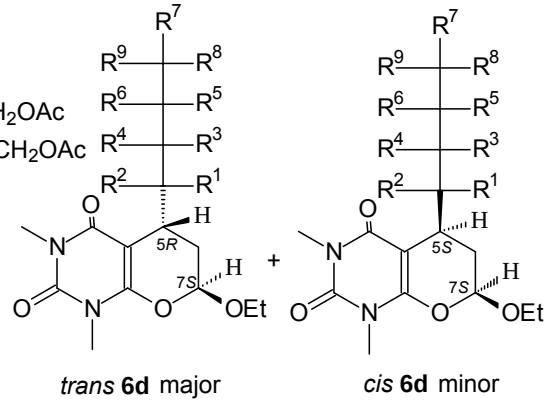
The aim of the experiment is to synthesize fused uracils such as pyrano[2,3-*d*]pyrimidines **6** (Scheme **SM 10.6.**1) containing a sugar moiety at carbon C(5) in the ring system as compounds with potential pharmacological activity. Students can investigate if 5-ylidene barbituric acids **4** with the carbohydrate substituent can act as active heterodienes in hetero-Diels-Alder reactions in synthesis of fused uracils **6**. First, potential heterodienes - 5-ylidene barbituric acids **4** bearing the sugar moiety are synthesized and in the second step their hetero-Diels-Alder reactions with enol ether **5** are conducted (Scheme **SM 10.6.**1).



1a: L-(-)-Xylose, $R^1=R^4=R^5=OH$, $R^2=R^3=R^6=R^7=H$
1b: L-(+)-Arabinose, $R^1=R^4=R^6=R^7=H$, $R^2=R^3=R^5=OH$
1c: D-(+)-Glucose, $R^1=R^4=R^5=H$, $R^2=R^3=R^6=OH$, $R^7=CH_2OH$
1d: D-(+)-Galactose, $R^1=R^4=R^6=H$, $R^2=R^3=R^5=OH$, $R^7=CH_2OH$
1e: D-(-)-Ribose

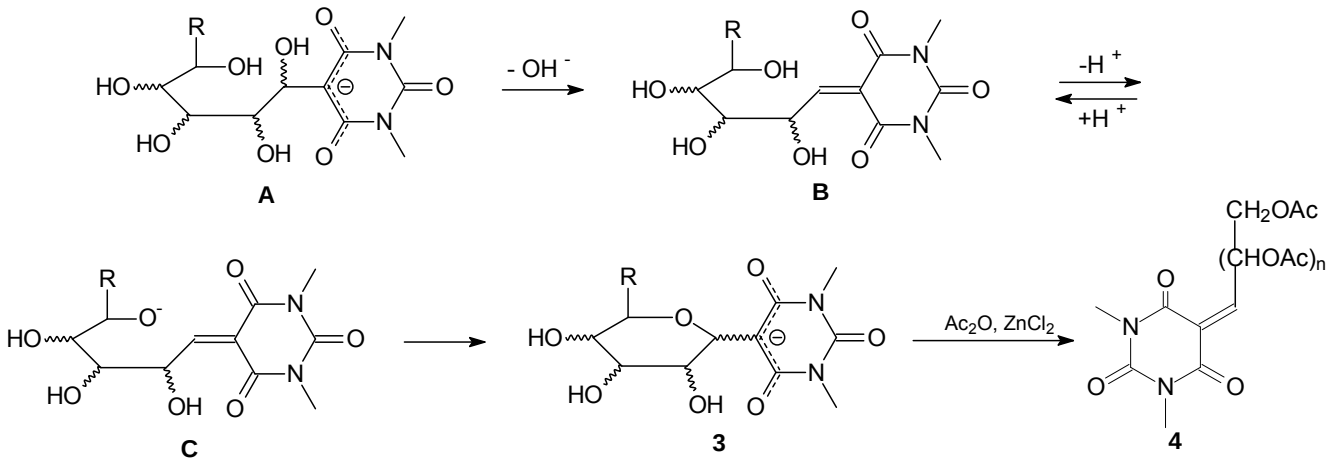


4a: L-Xylo, $R^1=R^4=R^5=R^8=OAc$, $R^2=R^3=R^6=R^7=R^9=H$
4b: L-Arabino, $R^1=R^4=R^6=R^7=R^9=H$, $R^2=R^3=R^5=R^8=OAc$
4c: D-Gluco, $R^1=R^4=R^5=R^8=H$, $R^2=R^3=R^6=R^9=OAc$, $R^7=CH_2OAc$
4d: D-Galacto, $R^1=R^4=R^6=R^8=H$, $R^2=R^3=R^5=R^9=OAc$, $R^7=CH_2OAc$
4e: D-Ribo, $R^1=R^3=R^5=R^7=R^9=H$, $R^2=R^4=R^6=R^8=OAc$



Scheme SM 10.6.1. Knoevenagel condensations of sugars **1a-e** with *N,N'*-dimethylbarbituric acid **2**. Acetylation of **3a**-**e** and hetero-Diels-Alder reactions of 5-ylidene derivatives **4a**-**e** with enol ether **5**.

Formation of the 5-arylidene derivatives of barbituric acids **4** can be explained as shown in Scheme **SM 10.6.**2 and have been proposed by Gonzalez *et al*.[12] In the first step, base-catalyzed addition of *N,N*-dimethyl barbituric acid **2** to appropriate sugar **1** provides initial intermediate adduct **A**, which undergoes β-elimination of water to produce the intermediate unsaturated compound **B**. Products **3** are formed by the intramolecular nucleophilic addition of the alkoxide **C** formed from intermediate **B** by loss of the HO-C(5') proton. Although the intermediate **B** has never been isolated, analogous structures **4** have been obtained by acetylation of the *C*-glycosylbarbiturates **3**. This result supports the participation of such olefinic structures in the formation of the *C*-glycosylbarbiturates **3**.[12]



Scheme SM 10.6.2 The mechanism of formation of *C*-glycosylbarbiturates **3**.

According to convenient procedure for Knoevenagel condensation of barbituric acids and unprotected sugars in water described by Gonzalez *et al*.[12] , the condensations of L-(-)-xylose **1a**, L-(+)-arabinose **1b**, D-(+)-glucose **1c**, D-(+)-galactose **1d** and D-(-)-ribose **1e** with 1,3-dimethylbarbituric acid **2** in water solution in the presence of sodium carbonate can be performed at 80 ^{o}C for 5 hours (Scheme **SM 10.6.**1, Figure **SM 10.6.**1). Sodium 5-glycopyranosyl-1,3-dimethylbarbiturates **3** as condensation products, do not represent a 1-oxa-1,3-diene system. Therefore, they do not react as active heterodienes in hetero-Diels-Alder reaction. Treatment of condensation products **3** with acetic anhydride and a large excess of zinc chloride give the *O*-acetylated 1,3-dimethyl-2,4,6-trioxo-

pyrimidin-5-ylidene derivatives **4**.[12] A large excess of zinc chloride is necessary. Treatment of Knoevenagel condensation products with equimolar amounts of acetic anhydride-zinc chloride gave compounds having cyclic pyranoid structures.[12] Acetylation products **4** possess a 1-oxa-1,3-diene system and additionally the introduction of the electron withdrawing acetyl groups, as protection of each of hydroxy groups, can enhance their reactivity in hetero-Diels-Alder reactions. The mixture of anhydrous zinc chloride in acetic anhydride and condensation product **3** should be stirred for 1h and next keep for 24 h at room temperature (Scheme **SM 10.6.**1, Figure **SM 10.6.**2). Acetylation products **4** can be obtained in good yields 78-89 %.[23] The cycloaddition reactions of *O*-acetylated 1,3-dimethyl-2,4,6-trioxo-pyrimidin-5-ylidene derivatives **4** with a ten-fold excess of ethyl-vinyl ether **5** are performed in the absence of solvent at room temperature for 3-5 min and pyrano[2,3-*d*]pyrimidines **6** can be obtained in good 82-88 % yields (Scheme **SM 10.6.**1, Table **SM 10.6.**1, Figure **SM 10.6.**3).[23] The progress of the reactions was monitored by TLC (20:1 CH_2Cl_2/MeOH). The excess of ether was evaporated and the mixture of diastereoisomers **6** was separated and purified by column chromatography on silica gel using dichloromethane/methanol 20:1 as an eluent, column length 60 cm, elution order: (1) minor *trans*-**6**, (2) major *cis*-**6**. (Figure **SM 10.6.**4). The students results reported were first obtained with chloroform instead dichloromethane but due to the known high toxicity of chloroform and considering that its use have been banned from most of the teaching institutions its use cannot be considered.

The reproducibility of the experiment was assessed by its repetitive execution, namely by 2nd year Chemistry M.Sc. students from Faculty of Chemistry of Jagiellonian University (Kraków, Poland).

Table of results of experiments for different sugars

Table SM 10.6.1. Synthesis of pyrano[2,3-*d*]pyrimidines **6** by solvent-free hetero-Diels-Alder reaction of alditols **4** with ethyl vinyl ether **5**.

Sugar derivative alditol **4**	Cycloaddition time (minutes)	**6**	Total yield % of cycloadducts **6**	Ratio of major/minor diastereoisomers[a] **6**	Melting Point (°C) of major diastereoisomer **6**
L-(-)-xylose derivative **4a**	3	**6a**	85-88	4:1	65-66
L-(+)-arabinose derivative **4b**	3	**6b**	82-86	4.8:1	oil
D-(+)-glucose derivative **4c**	5	**6c**	84-86	5.9:1	74-75
D-(+)-galactose derivative **4d**	3	**6d**	83-87	4.8:1	151-152
D-(-)-ribose derivative **4e**	3	**6e**	82-86	2.2:1	oil

[a]Ratio based on ^{1}H NMR (300 MHz) spectra of crude products **6**, analyzing the signal of proton 7-H.[23]

Isolation of *cis* and *trans* diastereoisomers of pyrano[2,3-*d*]pyrimidines by column chromatography

Isolation and purification of *cis* and *trans* diastereoisomers of pyrano[2,3-*d*]pyrimidines **6** by column chromatography can be realized using the setup presented on Figure **SM 10.6.**4. It is a column chromatography on silica gel using dichloromethane/methanol (20:1 v/v) as eluent. Students should collect fractions into test-tubes and control the composition of each fraction by TLC (CH_2Cl_2/CH_3OH 20:1). Next, they should place together the fractions with the same composition in round-bottomed flask and evaporate the solvent under reduced pressure using a rotary evaporator. Pure solid *cis* or *trans* diastereoisomers of pyrano[2,3-*d*]pyrimidines **6** can be isolated by crystallization from the appropriate mixture of *tert*-butyl methyl ether and petroleum ether. Products **6** are soluble in *tert*-butyl methyl ether and insoluble in petroleum ether.

Photos of the experiment

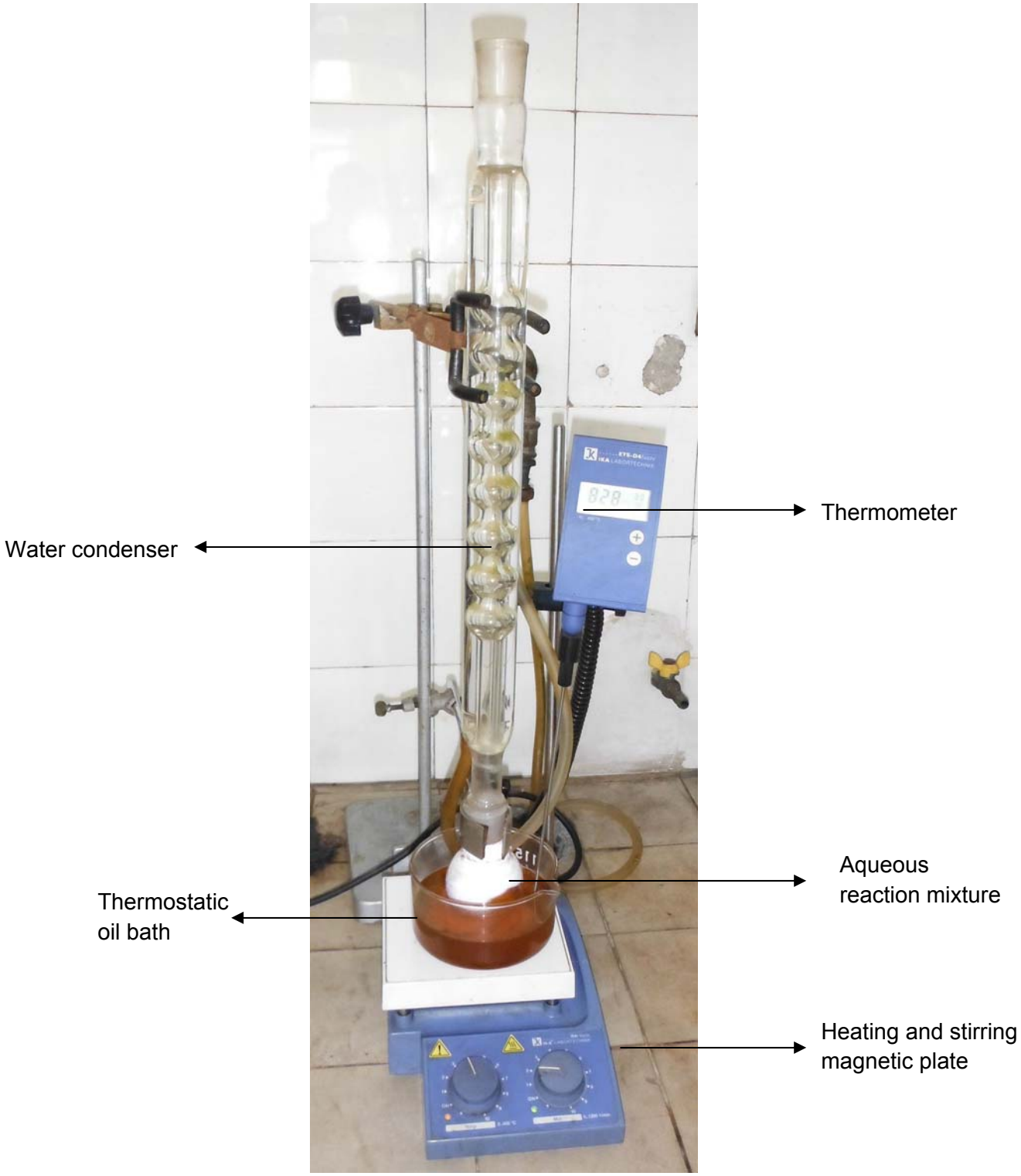


Figure SM 10.6.1 – Knoevenagel condensation setup apparatus with maintaining a temperature of 80 ^{o}C on a magnetic hot plate.

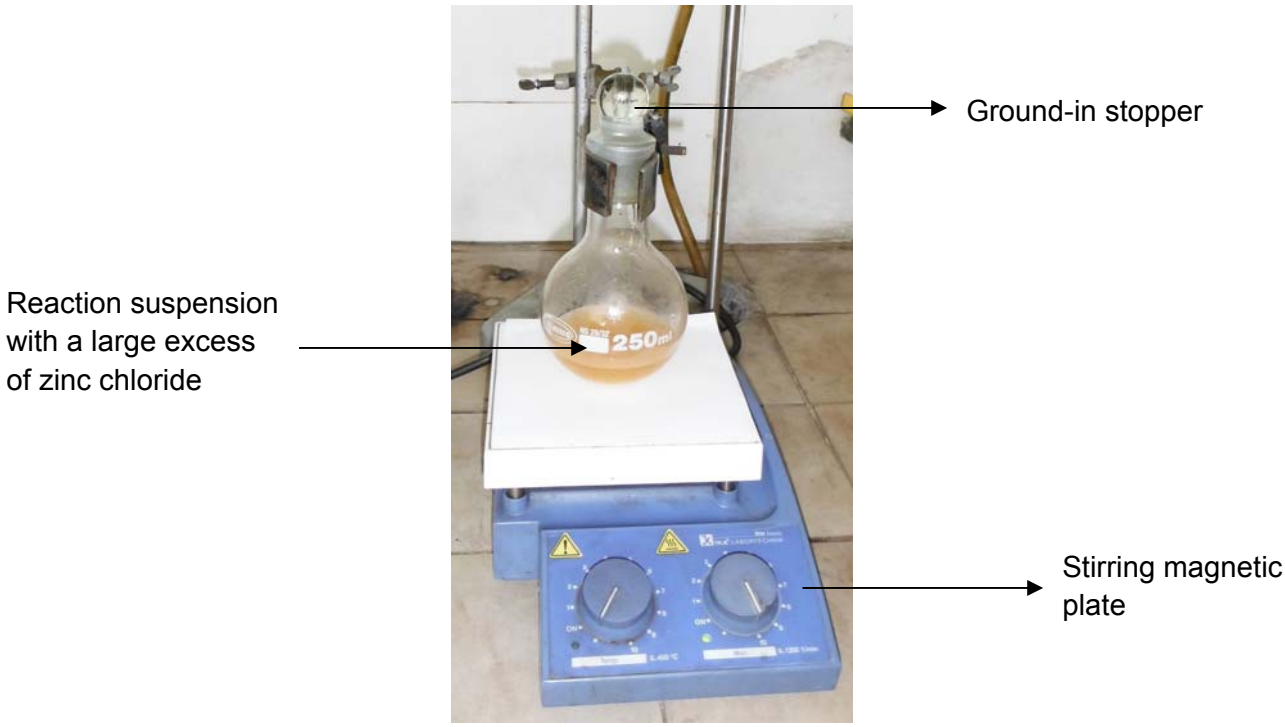


Figure SM 10.6.2 – Acetylation setup apparatus.

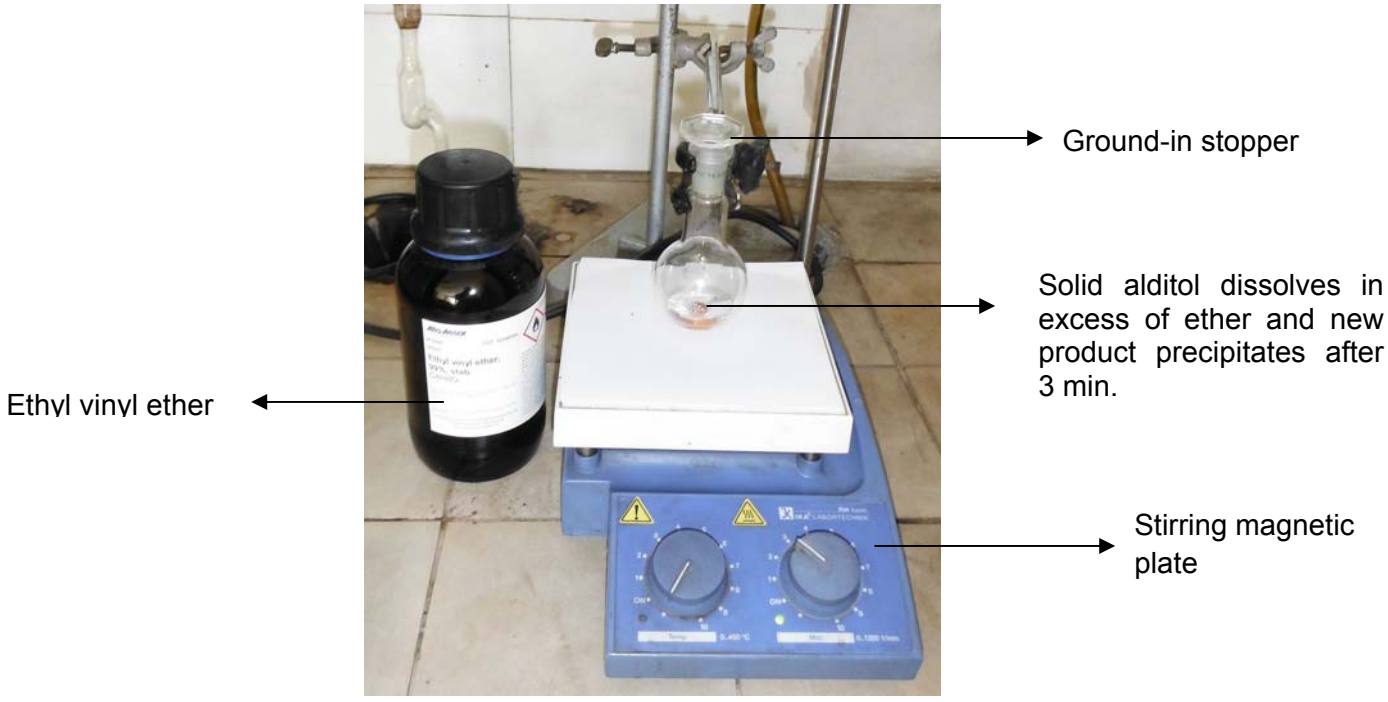


Figure SM 10.6.3 – Solvent-free hetero-Diels-Alder reaction setup apparatus.

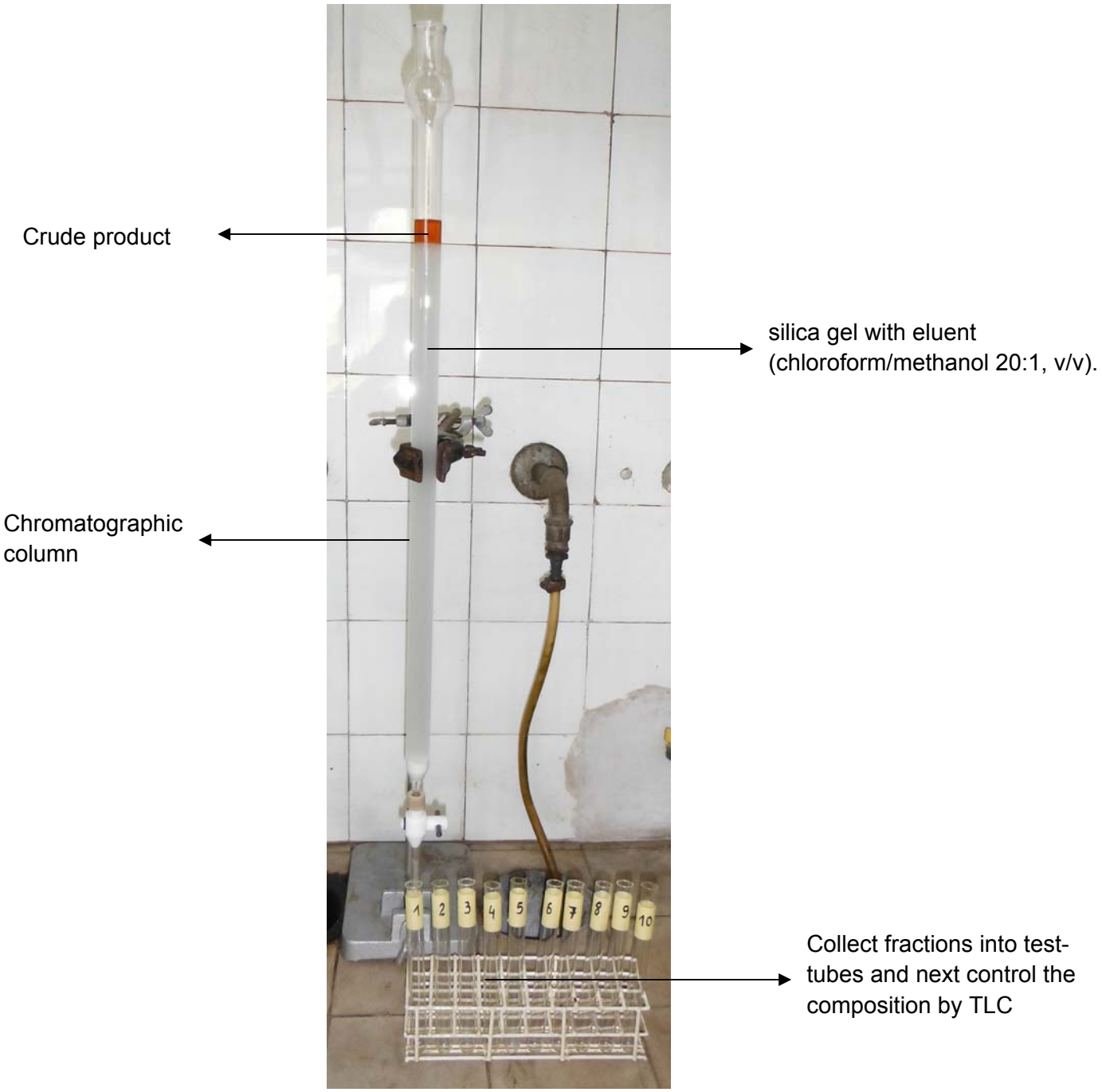


Figure SM 10.6.4 – Setup for separation and purification the mixture of *cis*/*trans* diastereoisomers of pyrano[2,3-*d*]pyrimidines **6** by column chromatography on silica gel.

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

4a

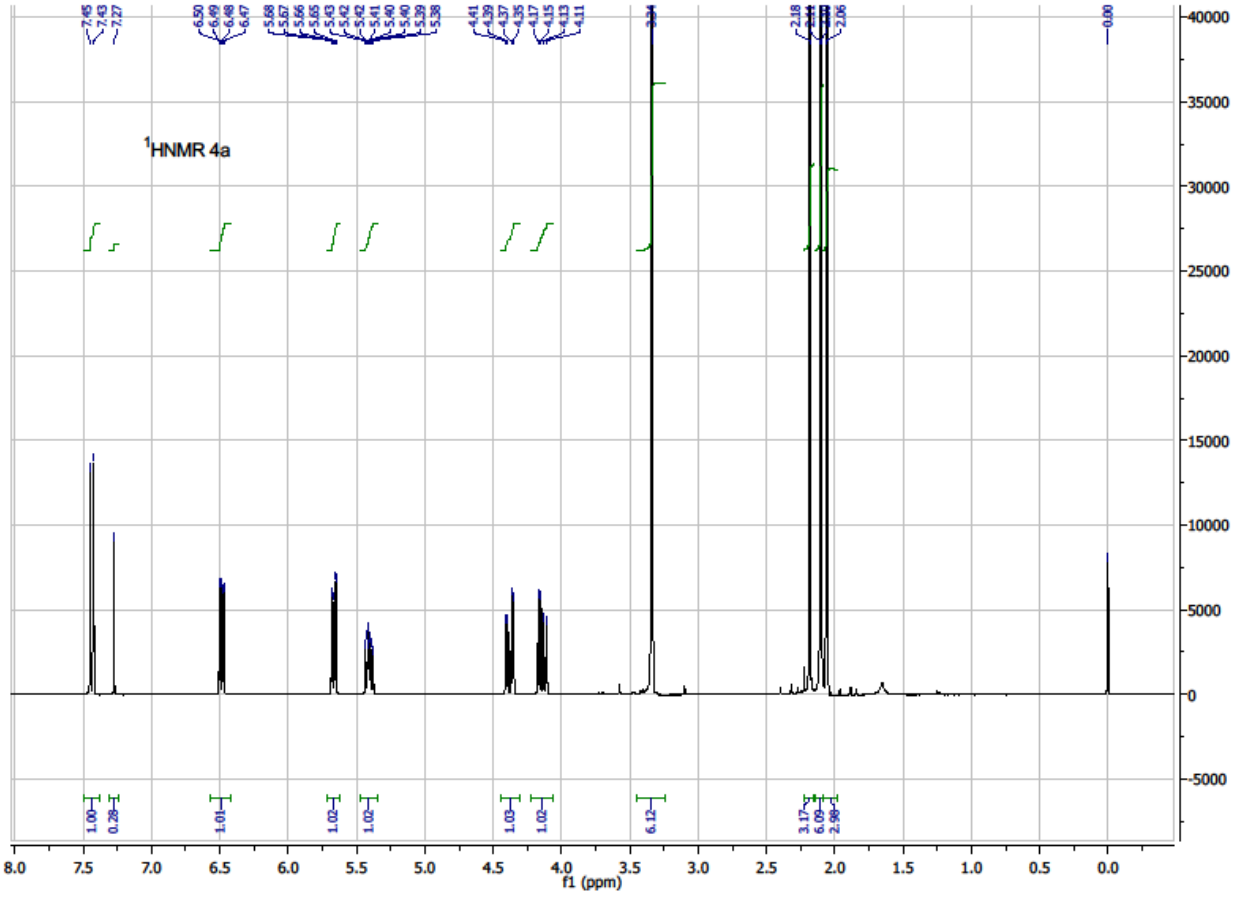


Figure SM 10.6.5 – ^{1}H NMR spectrum of 2,3,4,5-tetra-*O*-acetyl-1-deoxy-1-(1,3-dimethyl-2,4,6-trioxo-1*H*,3*H*,5*H*-pyrimidin-5-ylidene)-L-xylitol **4a**.[23]

^{1}H NMR **4a** (300 MHz, $CDCl_3$) δ 7.44 (1H, d, *J* 7.2 Hz, 1'-H), 6.49 (1H, dd, *J* 3.0, 7.2 Hz, 2'-H), 5.66 (1H, dd, *J* 3.0, 6.9 Hz, 3'-H), 5.41 (1H, ddd, *J* 3.9, 5.7, 6.9 Hz, 4'-H), 4.38 (1H, dd, *J* 3.9, 12.3 Hz, 5'-

H), 4.13 (1H, dd, *J* 5.7, 12.3 Hz, 5'-H), 3.34 (6H, s, N-CH_3), 2.18 (3H, s, $COCH_3$), 2.11 (3H, s, $COCH_3$), 2.10 (3H, s, $COCH_3$), 2.06 (3H, s, $COCH_3$).

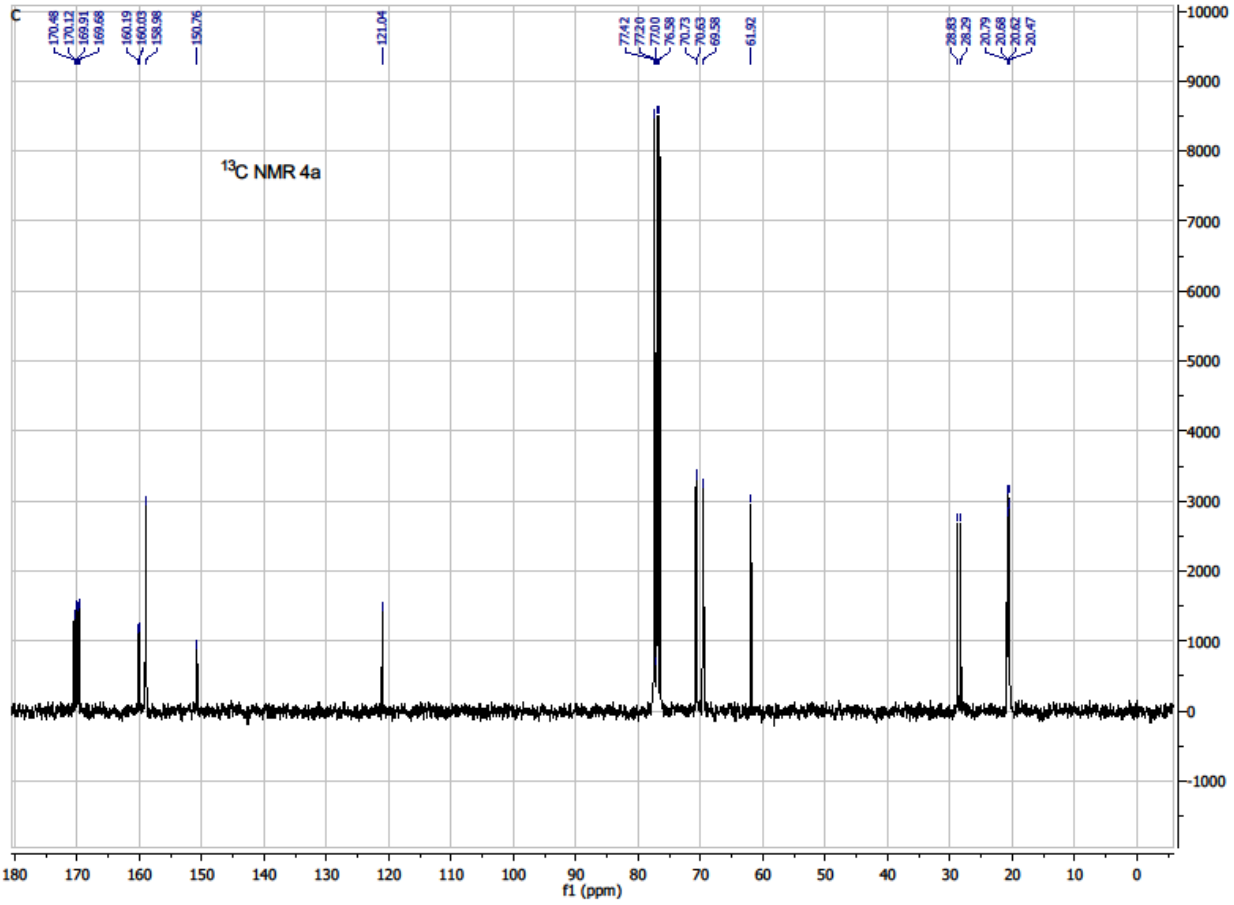


Figure SM 10.6.6 – ^{13}C NMR spectrum of 2,3,4,5-tetra-*O*-acetyl-1-deoxy-1-(1,3-dimethyl-2,4,6-trioxo-1*H*,3*H*,5*H*-pyrimidin-5-ylidene)-L-xylitol **4a**.[23]

^{13}C NMR **4a** (75.5 MHz, $CDCl_3$) δ 170.5 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 169.9 ($\underline{C}OCH_3$), 169.7 ($\underline{C}OCH_3$), 160.2(C=O), 160.0 (C=O), 159.0 (C=O), 150.8 (C=C), 121.0 (C=C), 70.7 (C-1'), 70.6 (C-2'), 69.6 (C-3'), 61.9 (C-4'), 28.8 (N-CH_3), 28.3 (N-CH_3), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$).

cis-**6a**

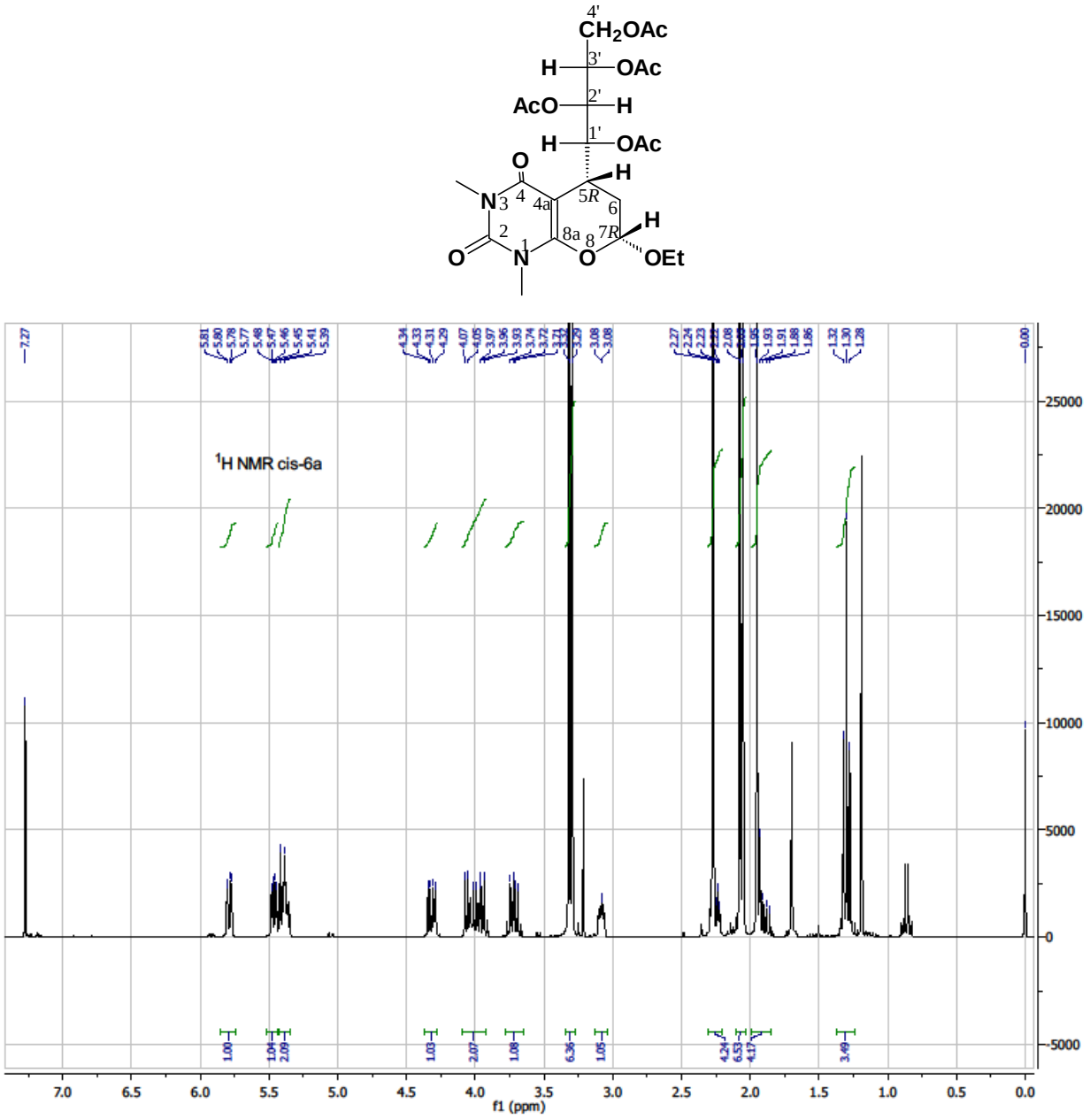


Figure SM 10.6.7 – ^{1}H NMR spectrum of major *cis* diastereoisomer of pyrano[2,3-*d*]pyrimidine -(5*R*,7*R*,1'*R*,2'*S*,3'*S*)-5-(1',2',3',4'-tetra-*O*-acetyl-butane-1',2',3',4'-tetraolyl)-7-ethoxy-1,3-dimethyl-1,5,6,7-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-2,4(3*H*)-dione *cis*-**6a**.[23]

^{1}H NMR *cis*-**6a** (300 MHz, $CDCl_3$) δ 5.78 (1H, dd, *J* 2.4, 8.1 Hz, 7-H), 5.46 (1H, dd, *J* 3.0, 8.1 Hz, 1'-H), 5.40 (1H, dd, *J* 3.0, 8.1 Hz, 2'-H), 5.37 (1H, ddd, *J* 3.0, 5.1, 6.9 Hz, 3'-H), 4.31 (1H, dd, *J* 5.1, 11.4 Hz, 4'-H), 4.04 (1H, dd, *J* 6.9, 11.4 Hz, 4'-H), 3.96 (1H, dq, *J* 7.2, 9.6 Hz, $OC\underline{H}_2CH_3$), 3.72 (1H, dq, *J*

7.2, 9.6 Hz, OC<u>H</u>$_2$CH$_3$), 3.32 (3H, s, N-Me), 3.29 (3H, s, N-Me), 3.08 (1H, ddd, *J* 3.0, 3.9, 7.2 Hz, 5-H), 2.27 (3H, s, COC<u>H</u>$_3$), 2.25 (1H, ddd, *J* 2.4, 3.9, 13.8 Hz, 6-H), 2.08 (3H, s, COC<u>H</u>$_3$), 2.05 (3H, s, COC<u>H</u>$_3$), 1.95 (3H, s, COC<u>H</u>$_3$), 1.93 (1H, ddd, *J* 7.2, 8.1, 13.8 Hz, 6-H), 1.30 (3H, t, *J* 7.2 Hz, OCH$_2$C<u>H</u>$_3$)

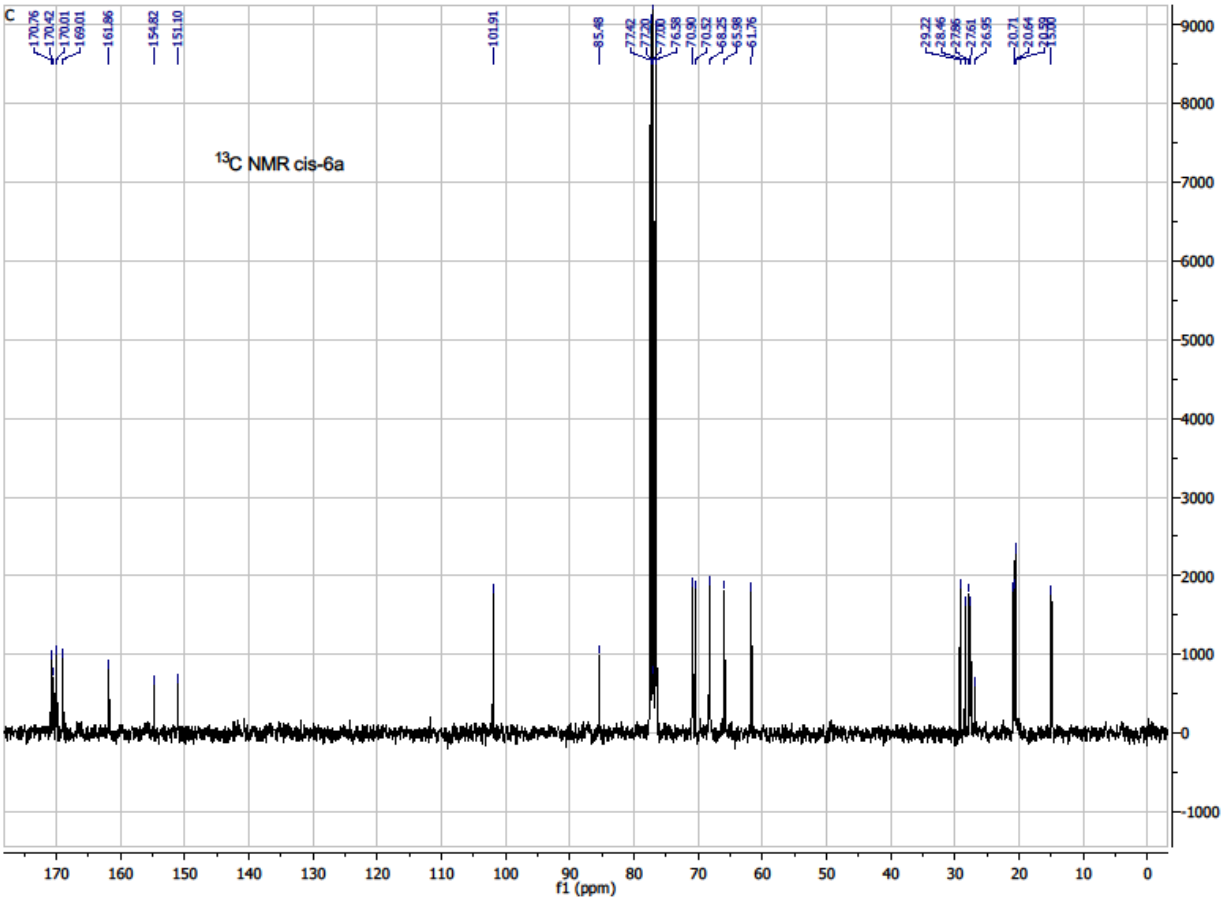
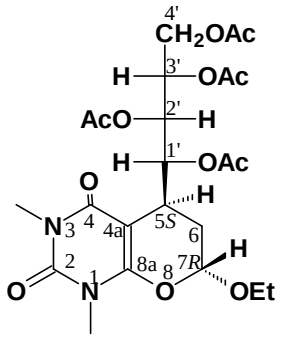


Figure SM 10.6.8 – ^{13}C NMR spectrum of major *cis* diastereoisomer of pyrano[2,3-*d*]pyrimidine -(5*R*,7*R*,1'*R*,2'*S*,3'*S*)-5-(1',2',3',4'-tetra-*O*-acetyl-butane-1',2',3',4'-tetraolyl)-7-ethoxy-1,3-dimethyl-1,5,6,7-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-2,4(3*H*)-dione *cis*-**6a**.[23]

^{13}C NMR *cis*-**6a** (75.5 MHz, $CDCl_3$) δ 170.8 (<u>C</u>OCH$_3$), 170.4 (<u>C</u>OCH$_3$), 170.0 (<u>C</u>OCH$_3$), 169.0 (<u>C</u>OCH$_3$), 161.9 (C=O), 154.8 (C=O), 151.1 (C-8a), 101.9 (C-7), 85.5 (C-4a), 70.9 (C-1'), 70.5 (C-2'), 68.3 (C-3'), 66.0 (OC<u>H</u>$_2$CH$_3$), 61.8 (C-4'), 29.2 (C-5), 28.5 (N-CH$_3$), 27.9 (N-CH$_3$), 27.6 (C-6), 21.0 (CO<u>C</u>H$_3$), 20.7 (CO<u>C</u>H$_3$), 20.7 (CO<u>C</u>H$_3$), 20.6 (CO<u>C</u>H$_3$), 15.0 (OCH$_2$C<u>H</u>$_3$).

trans-**6a**



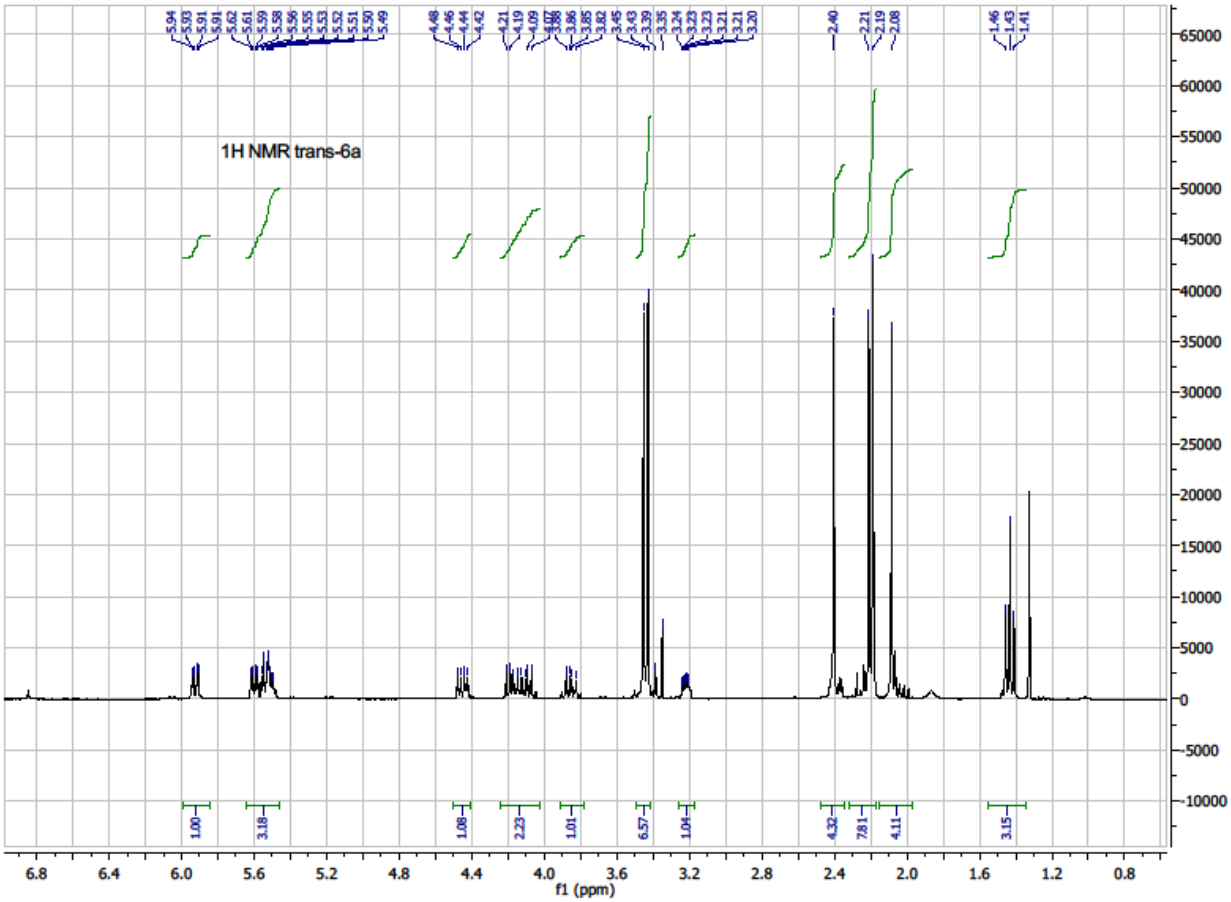


Figure SM 10.6.9 – ^{1}H NMR spectrum of minor *trans* diastereoisomer of pyrano[2,3-*d*]pyrimidine -(5*R*,7*R*,1'*R*,2'*S*,3'*S*)-5-(1',2',3',4'-tetra-*O*-acetyl-butane-1',2',3',4'-tetraolyl)-7-ethoxy-1,3-dimethyl-1,5,6,7-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-2,4(3*H*)-dione *trans*-**6a**.[23]

^{1}H NMR *trans*-**6a** (300 MHz, $CDCl_3$) δ 5.92 (1H, dd, *J* 2.4, 8.1 Hz, 7-H), 5.43 (1H, dd, *J* 8.4, 12.0 Hz, 1'-H), 5.42 (1H, dd, *J* 3.3, 12.0 Hz, 2'-H), 5.38 (1H, ddd, *J* 3.3, 4.8, 6.9 Hz, 3'-H), 4.28 (1H, dd, *J* 4.8, 11.4 Hz, 4'-H), 3.96 (1H, dd, *J* 6.9, 11.4 Hz, 4'-H), 3.95 (1H, dq, *J* 6.9, 9.6 Hz, $OC\underline{H}_2CH_3$), 3.71 (1H,

dq, *J* 6.9, 9.6 Hz, $OC\underline{H}_2CH_3$), 3.31 (3H, s, N-Me), 3.29 (3H, s, N-Me), 3.22 (1H, ddd, *J* 2.1, 8.4, 8.4 Hz, 5-H), 2.27 (3H, s, $COC\underline{H}_3$), 2.13 (1H, ddd, *J* 2.1, 8.1, 14.4 Hz, 6-H), 2.05 (3H, s, $COC\underline{H}_3$), 2.02 (1H, ddd, *J* 2.4, 8.4, 14.4 Hz, 6-H), 1.95 (3H, s, $COC\underline{H}_3$), 1.92 (3H, s, $COC\underline{H}_3$), 1.31 (3H, t, *J* 6.9 Hz, $OCH_2C\underline{H}_3$).

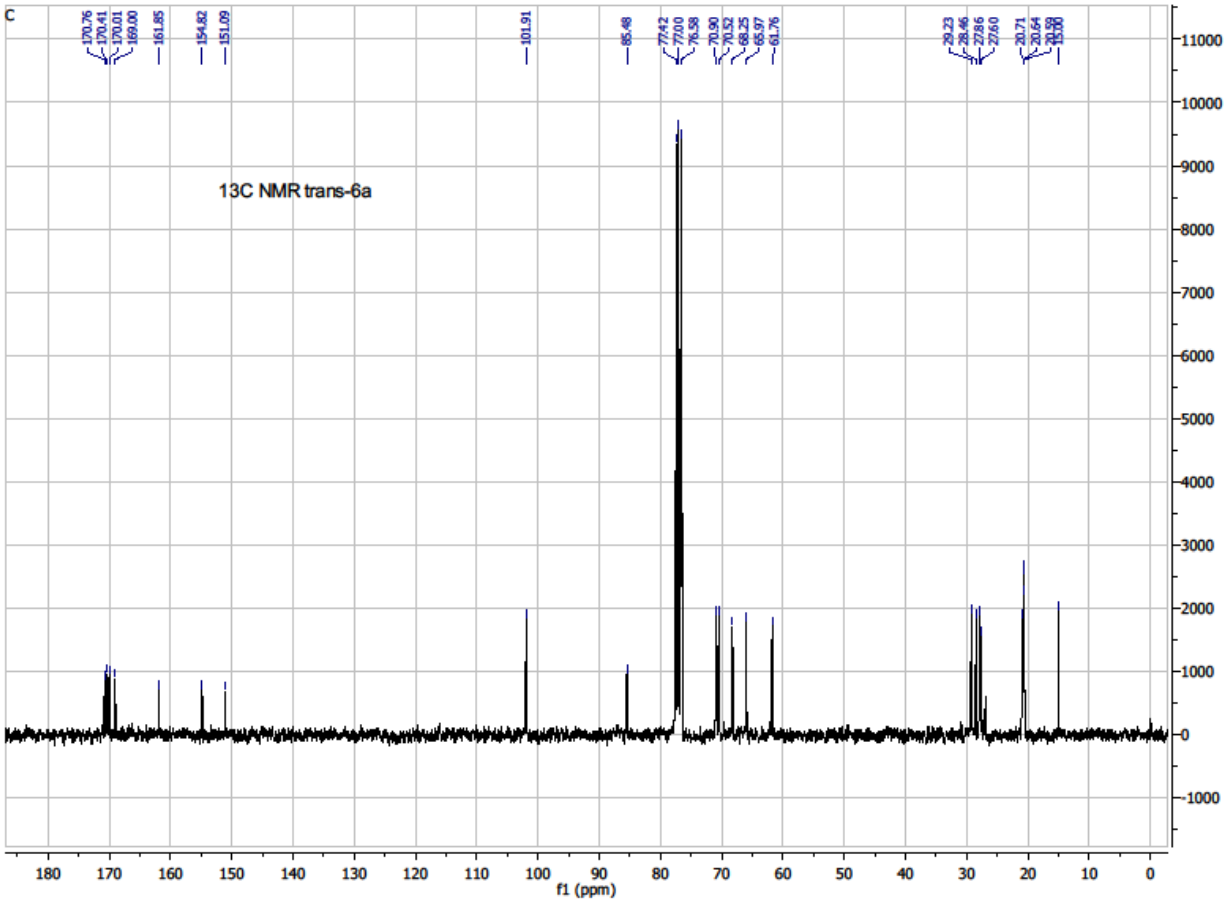


Figure SM 10.6.10 – ^{13}C NMR spectrum of minor *trans* diastereoisomer of pyrano[2,3-*d*]pyrimidine -(5*R*,7*R*,1'*R*,2'*S*,3'*S*)-5-(1',2',3',4'-tetra-*O*-acetyl-butane-1',2',3',4'-tetraolyl)-7-ethoxy-1,3-dimethyl-1,5,6,7-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidine-2,4(3*H*)-dione *trans*-**6a**.[23]

^{13}C NMR *trans*-**6a** (75.5 MHz, $CDCl_3$) δ 171.0 ($\underline{C}OCH_3$), 170.5 ($\underline{C}OCH_3$), 169.8 ($\underline{C}OCH_3$), 169.4 ($\underline{C}OCH_3$), 161.8 (C=O), 155.1 (C=O), 151.1 (C-8a), 101.8 (C-7), 85.3 (C-4a), 71.0 (C-1'), 69.7 (C-2'), 68.3 (C-3'), 66.3 ($OC\underline{H}_2CH_3$), 62.1 (C-4'), 29.2 (C-5), 28.4 ($N\text{-}CH_3$), 27.8 ($N\text{-}CH_3$), 27.6 (C-6), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.55 ($CO\underline{C}H_3$), 15.0 ($OCH_2C\underline{H}_3$).

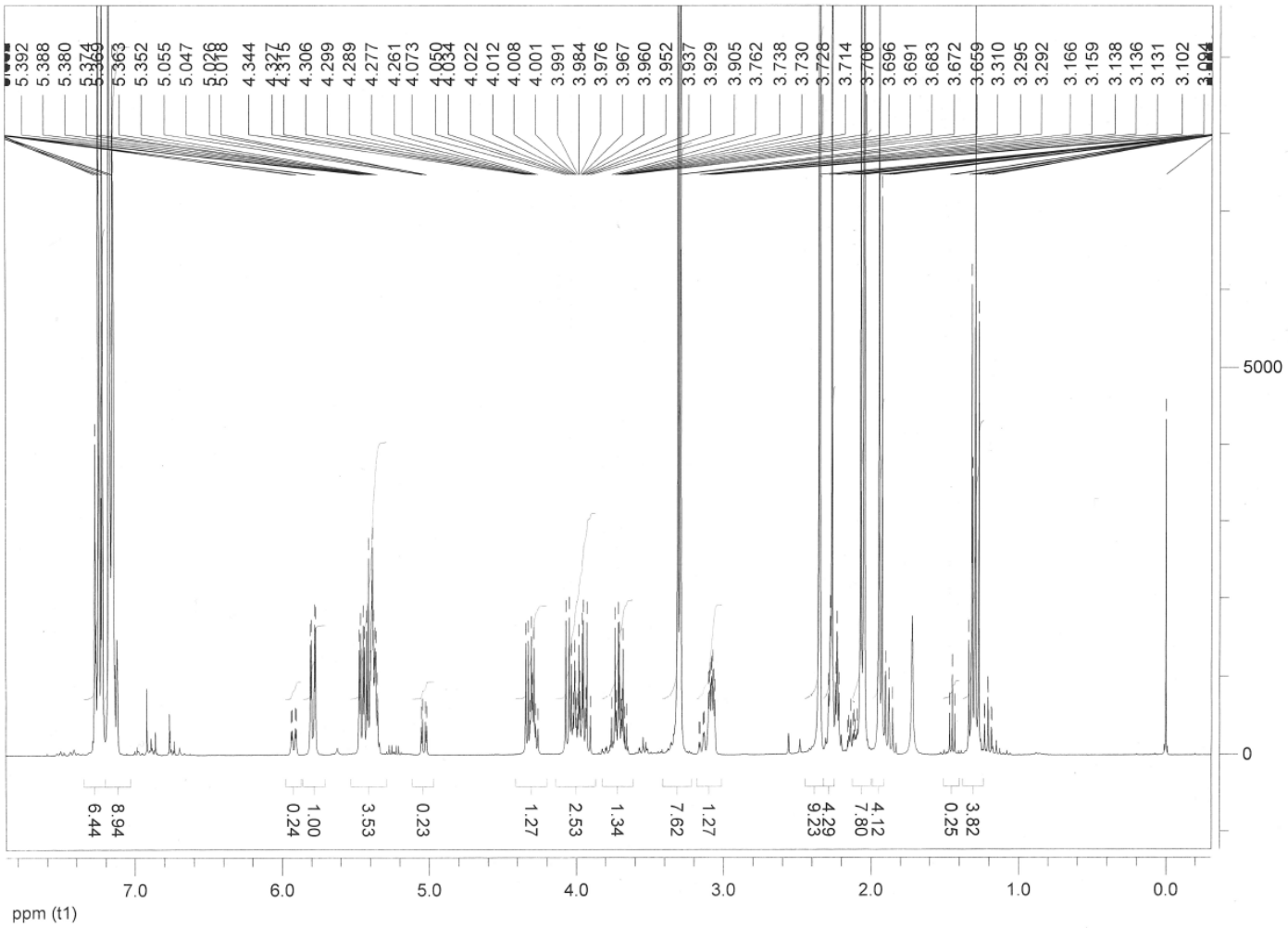


Figure SM 10.6.11 – ^{1}H NMR spectrum of crude product **6a**.

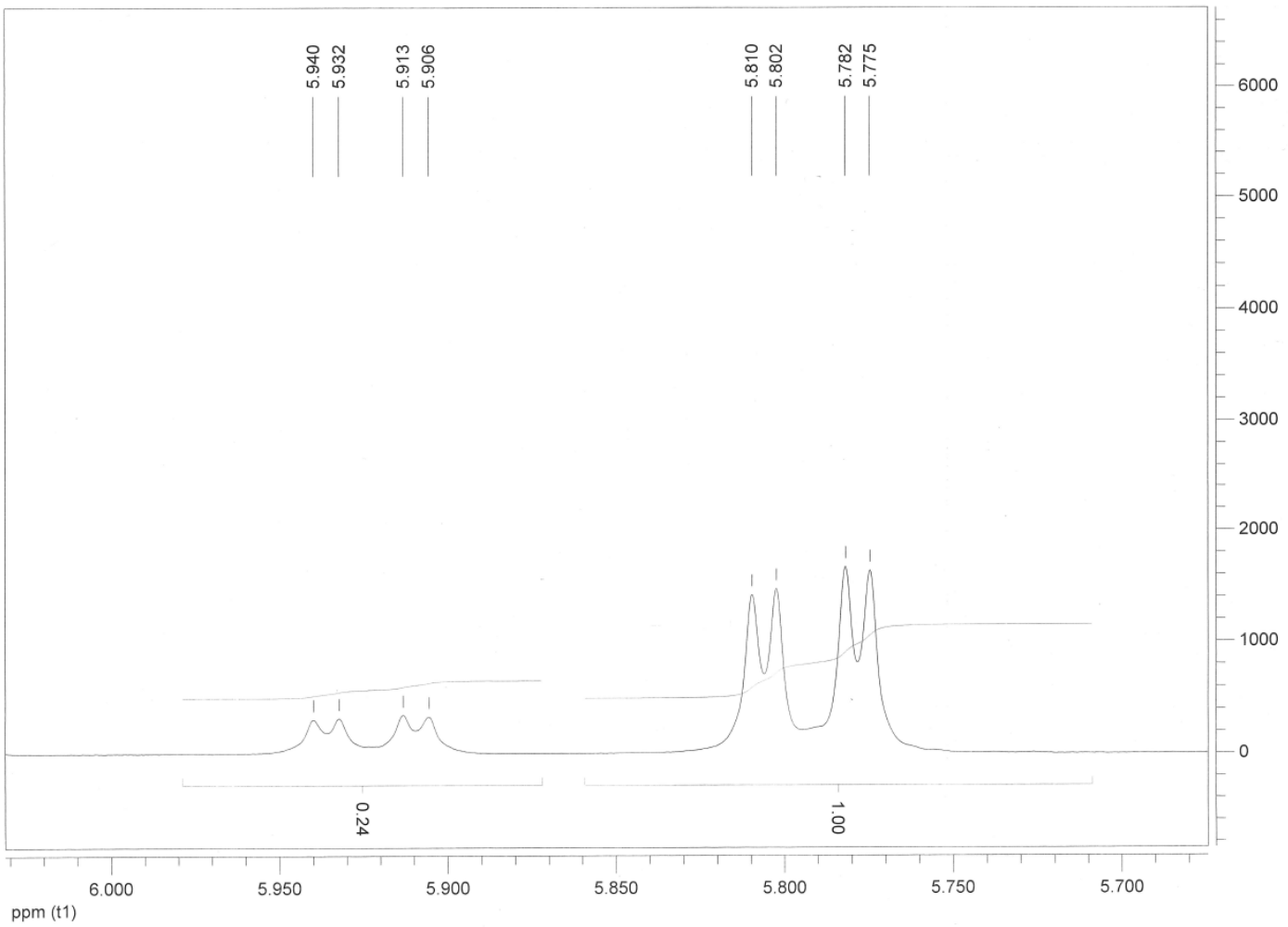


Figure SM 10.6.12 – ^{1}H NMR spectrum of crude product **6a** - the signal of proton 7-H: *cis*-**6a** *δ* 5.78 (dd, *J* 2.4, 8.1 Hz), *trans*-**6a** *δ* 5.92 (dd, *J* 2.4, 8.1 Hz), ratio of diastereoisomers major *cis* **6a**/minor *trans* **6a** 4:1.

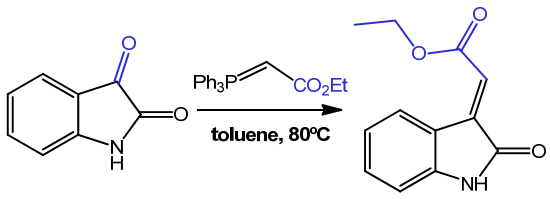
Synthesis of a spiroisoxazoline oxindole by 1,3-dipolar Cycloaddition

Supplementary Material

The reproducibility of the experiment was assessed by two master students and two PhD students with different backgrounds (biology, biochemistry, pharmaceutical sciences, and organic chemistry). Moreover, the four students display different chemistry laboratory skills, ranging from low to high experience.

This experiment is divided in two sessions, corresponding to two consecutive synthetic steps.

In the **first session** the 3-methylene indoline-2-one derivative, ((*E*)-ethyl 2-(2-oxoindolin-3-ylidene)acetate), will be synthesized (Scheme SM 10.7.1). This session encompass a 30 minutes reaction, liquid-liquid extraction, flash chromatography purification, and NMR and melting point characterization.



Scheme SM 10.7.1

General Information/ Troubleshooting:

- Isatin is not completely soluble in toluene at room temperature, therefore before heating, the reaction mixture is a suspension, but solubilize completely at 80ºC.

- An example of a TLC after 30 minutes of reaction is shown in Figure SM 10.7.1. Although the reaction is complete at 30 minutes, the product doesn't decompose in the reaction mixture even when left 24h at 80ºC. The spots corresponding to isatin and the product are yellow.

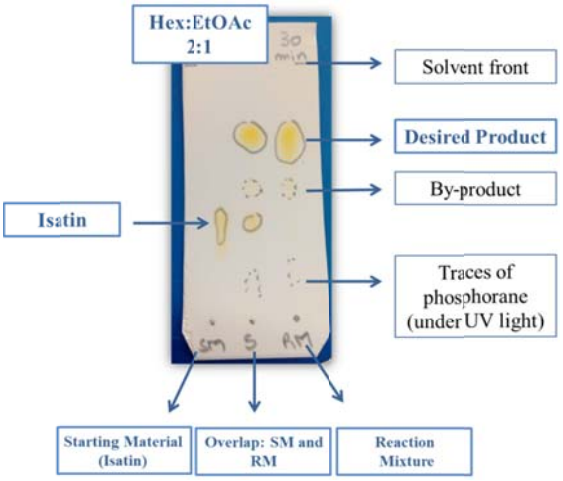


Figure SM 10.7.1

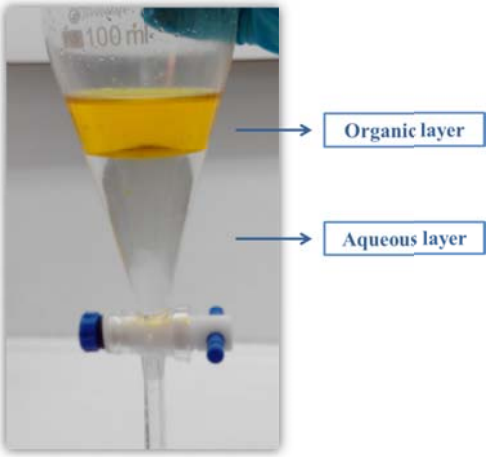


Figure SM 10.7.2

- The washing step of the organic layer with brine is represented in figure SM 10.7.2. Due to the fact that ethyl acetate is less dense that water, the organic phase with the desired compound (yellow) stays in the upper part of the separating funnel. If the aqueous layer stays yellowish, perform an extra extraction with ethyl acetate 25mL.

- The crude product is poorly soluble in the flash chromatography eluent, and also in a small amount of dichloromethane even when heated. Therefore it is recommended to first adsorb the crude (previous dissolved in ethyl acetate or dichloromethane) in silica gel.

- When using the recommended eluent gradient, and test tubes 1,5 x 10 cm, the desired product left the column around tube 25 – 43 (mean values) (Figure SM 10.7.3, SM 10.7.4).

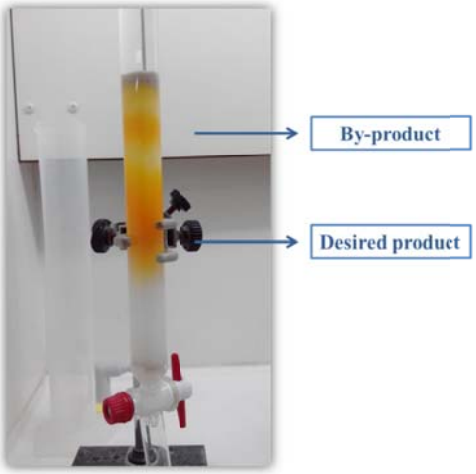


Figure SM 10.7.3

Figure SM 10.7.4

- The yields obtained for the four students were between 86-95% and the melting points (Lit [1]: 169-170ºC) between 168 -174ºC (in a range no wider than 3 degrees for each measure).

- ^{1}H-NMR and ^{13}C-APT spectra of 3-methylene indolin-2-one derivative are represented in Figure SM 10.7.5.

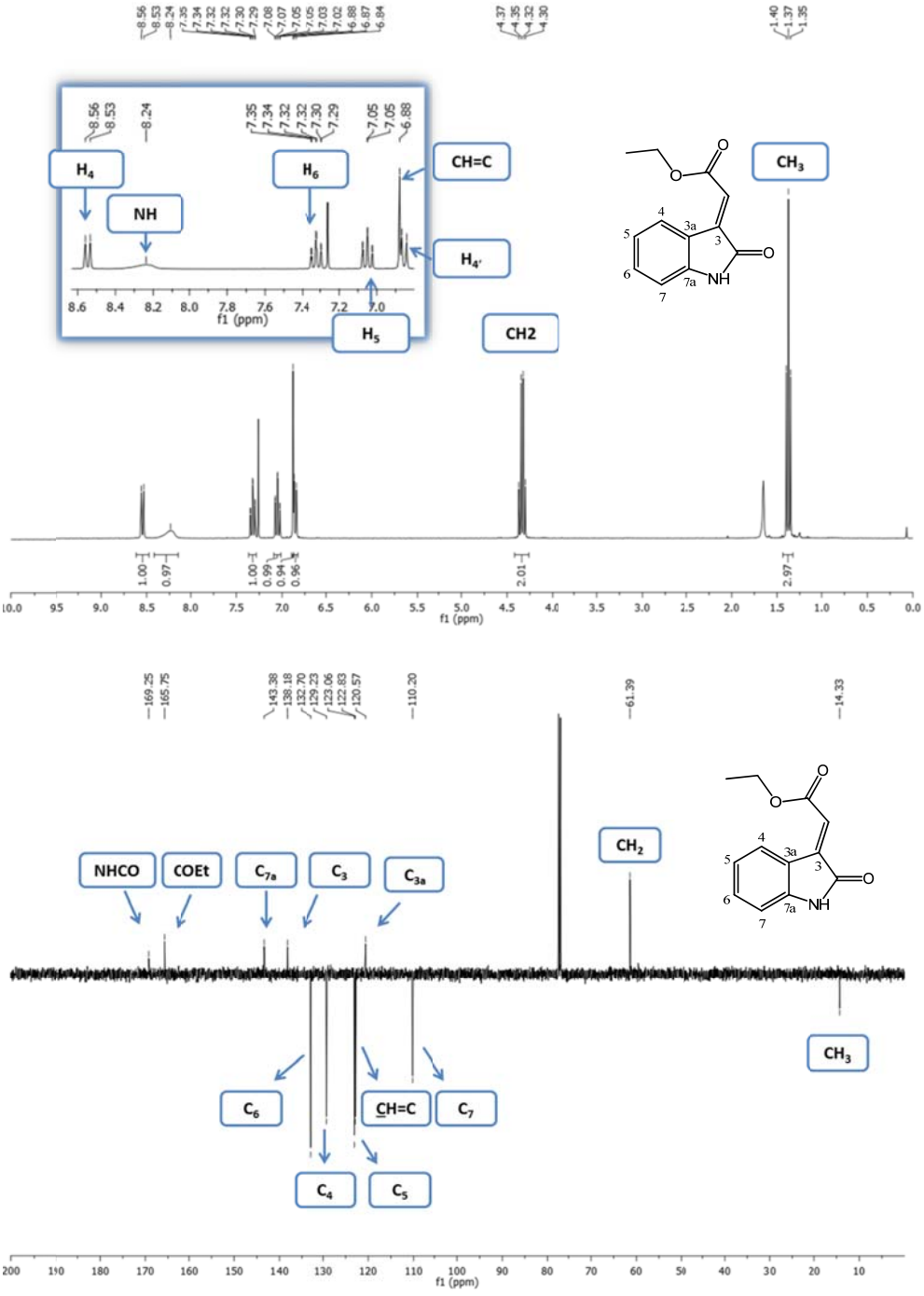
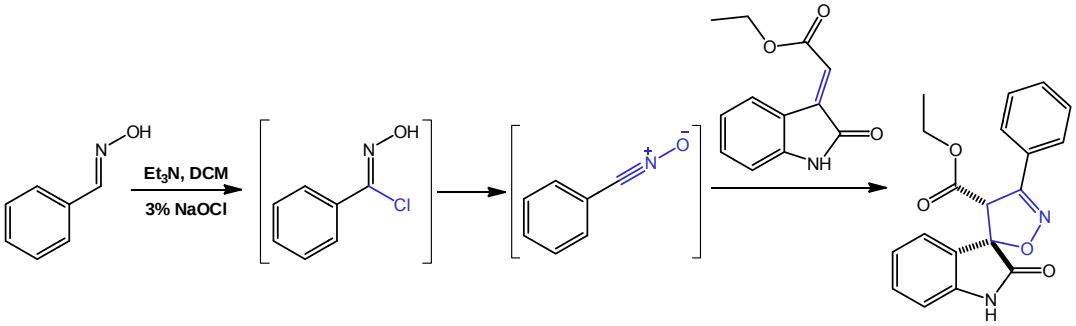


Figure SM 10.7.5

The **second reaction** corresponds to the 1,3-dipolar cycloaddition step to form ethyl 2-oxo-3'-phenyl-spiro[indoline-3,5'-isoxazoline]-4'-carboxylate (scheme SM 10.7.2). It is recommended to start this reaction at the end of session 1, due to its reaction time.



Scheme SM 10.7.2

General Information/ Troubleshooting:

- After adding sodium hypochlorite solution, two phases are observed in the round-bottom flask (Figure SM 10.7.6). The bottom yellow phase corresponds to the organic layer. It was used a common unscented household bleach, with approximately 3% of sodium hypochlorite, but higher percentages can be used.

- Example of a TLC after 18 hours of reaction (Figure SM 10.7.7). The spot corresponding to 3-methylene indolin-3-one is yellow. All the spots observed in the reaction mixture are only visible under UV light (254 nm). Therefore, although the by-product has a retention factor similar to the starting material, the lack of color indicates the end of reaction. In the benzaldehyde oxime spot it is observed the presence of both isomers, preferentially the *E*-isomer.

Figure SM 10.7.6

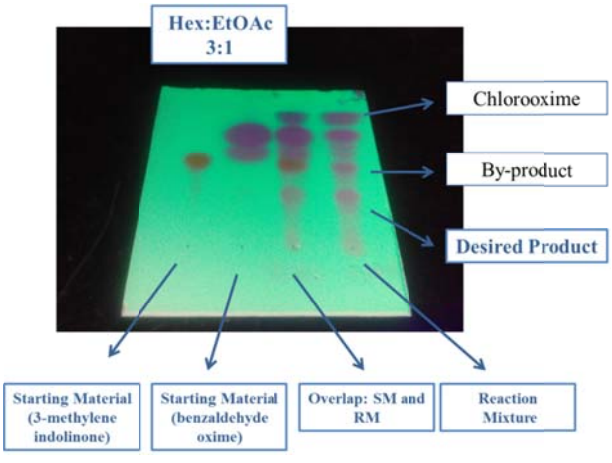


Figure SM 10.7.7

- Washing step of the organic layer with brine is represented in figure SM 10.7.8. Due to the fact that dichloromethane is denser that water, the organic phase with the

desired compound stays in the bottom part (yellow) of the separating funnel. If after washing with brine the aqueous layer stays yellowish, perform an extra extraction with dichloromethane 20mL.

- When using the recommended eluent gradient, and test tubes 1,5 x 10 cm, the desired product left the column around tube 37 – 50 (mean values) (Figure SM 10.7.9).

- After flash chromatography the solid obtained was yellowish. After washing with diethyl ether, remove the solvent (now yellow) by using a Büchner funnel or a Pasteur pipette with a small amount of cotton (Figure SM 10.7.10).

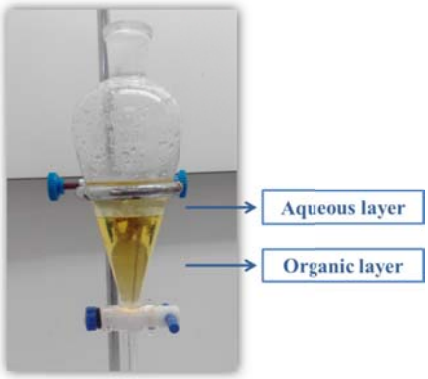


Figure SM 10.7.8

Figure SM 10.7.9

Figure SM 10.7.10

- After flash cromatography the yields obtained were between 66-90%, and after washing the mixture with diethyl ether they were 55-75%. The melting points were 195-199ºC (in a range no wider than 3 degrees for each measure). (Lit[2]: 198-199ºC)

- In the ^{1}H-NMR spectrum of the mixture, before washing with diethyl ether, it can be observed the duplication of certain signal, indicating the presence of diastereoisomers (Figure SM 10.7.11).

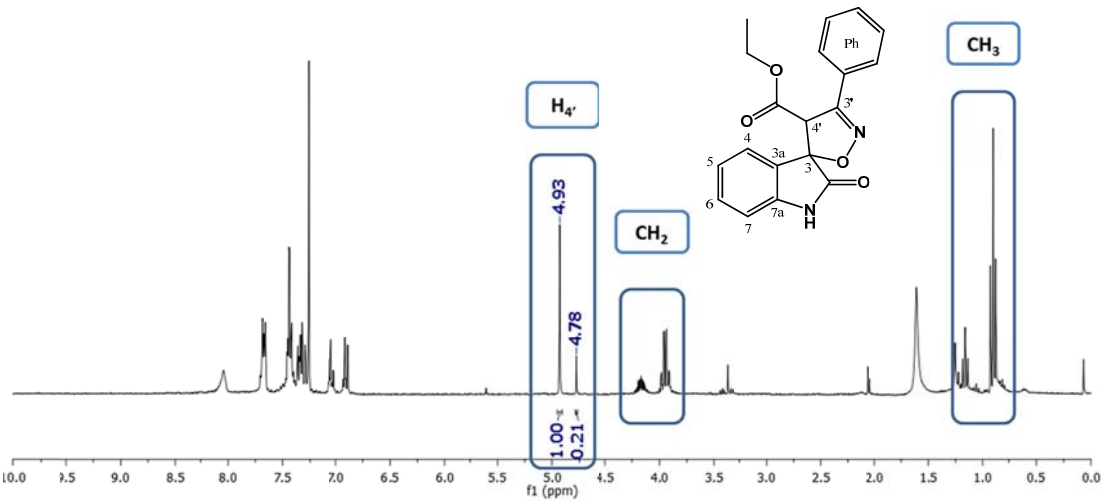
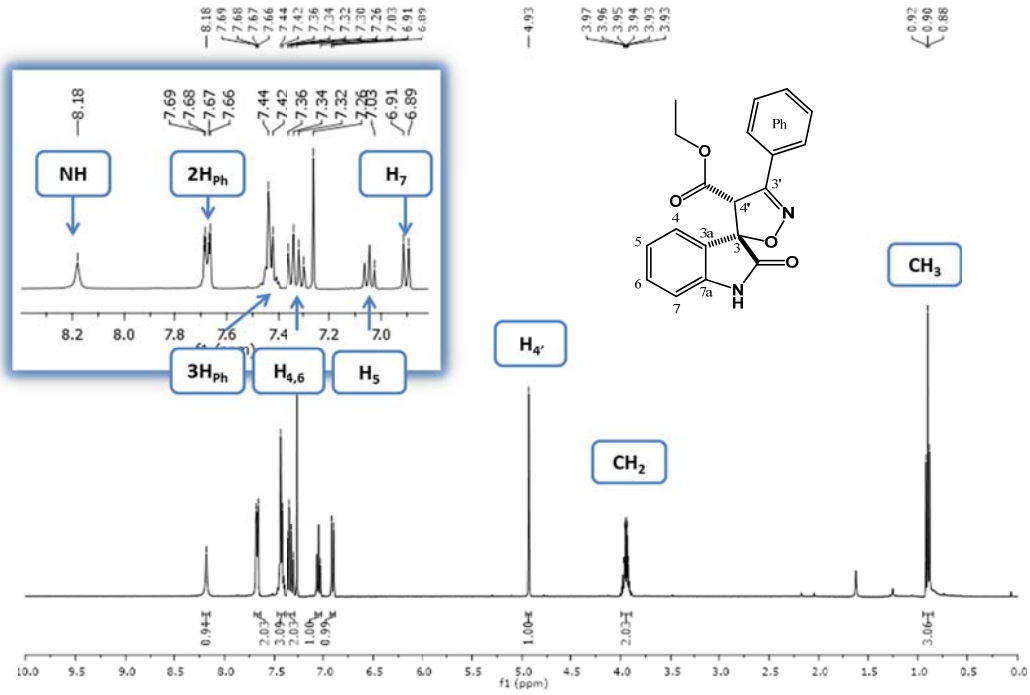


Figure SM 10.7.11

- ^{1}H-NMR and ^{13}C-APT spectra of the spiroisoxazoline oxindoles, after washing with diethyl ether, are represented in Figure SM 10.7.12.



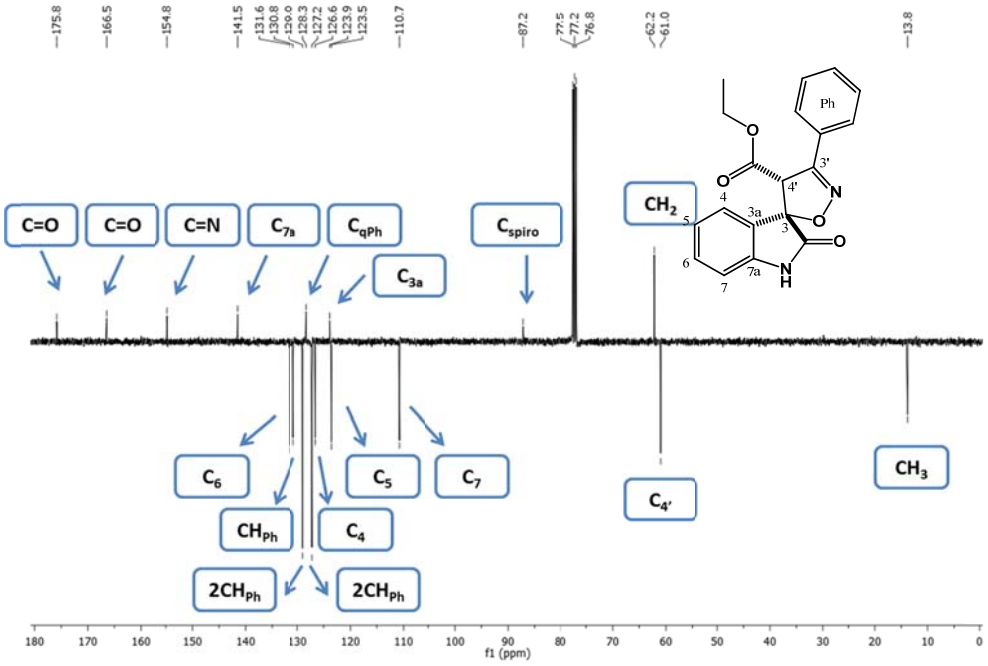
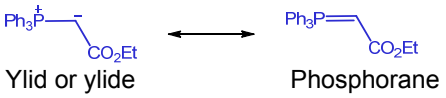
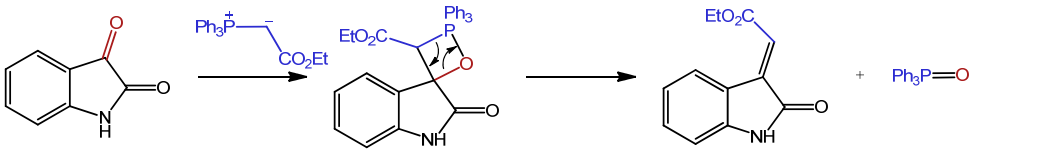


Figure SM 10.7.12

Results interpretation and additional questions

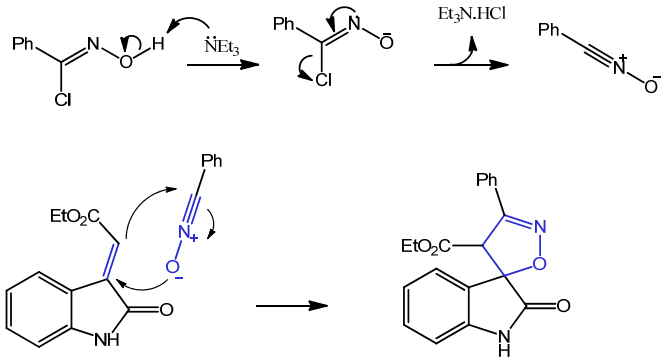
*2. Propose the reaction mechanism to obtain compound **3**.*





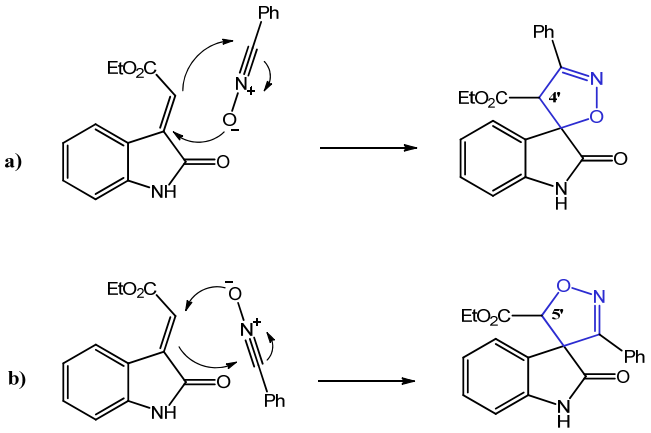
*3. Propose the reaction mechanism to obtain final compound **1**, starting from compounds **3** and **6**.*

Treatment of chlorooxime with triethylamine leads directly to the nitrile oxide formation with loss of HCl by a γ-elimination process.



5. Based on the NMR spectra of the pure spiroisoxazoline oxindole compound, discuss regioselectivity, and stereoselectivity of the 1,3-dipolar cycloaddition.

Possible regioisomers formed by 1,3-dipolar cycloaddition:



In the NMR the most relevant signals that contribute to enlighten the structure of the regiomer formed are from the proton of the isoxazoline ring and its carbon and from the spiro carbon. For both cases having vicinal oxygen makes the signal appear downfield in comparison to having an adjacent carbon. The C_{spiro} signal obtained for the compound synthesized is 87.2 ppm. This value is in the normal range obtained for spiro[indoline-3,5'-isoxazoline]-2-ones (a) or other spirooxindoles in which the spiro carbon is linked to three other carbons and one oxygen.[2-6] For spirooxindoles in which the spiro carbon is linked to four carbons (b) the value appears much more shielded (e. g. 66-69 ppm for spiro[indoline-3,4'-isoxazolidine]-2-ones.[6] The same can be rationalized for $C_{4'}/C_{5'}$ and $H_{4'}/H_{5'}$. $C_{4'}$ signal appears at 61.0 ppm versus around 80 ppm[6], and $H_{4'}$ at 4.93 ppm versus around 6 ppm.[7, 8]

In pericyclic reactions the relationship between substituents in the starting materials are preserved in the final product. The starting 3-methylene indoline-2-one derivative was synthetized as *E*-isomer (confirmed in the ^{1}H-NMR spectrum: H_4 signal is deshielded when compared to the one of the starting isatin (8.40 vs. 7.63 ppm) due to the presence of the spatially nearby CO_2Et chain (Figure SM 10.7.5 and SM 10.7.13). Therefore in the final compound the oxindole phenyl group points in the same direction as CO_2Et and consequently $H_{4'}$ and oxindole carbonyl will be projected to the opposite side (as seen by X-ray crystallography for a similar spiro[indoline-3,5'-isoxazoline]-2-one derivative[2], Figure SM 10.7.14). The formation of a minor diastereoisomer probably occurs due to isomerization of the 3-methylene indoline-2-one derivative in the presence of thiethylamine (prior to cycloaddition).[3, 9] The slightly more shielded $H_{4'}$ signal observed for the minor diastereoisomer (Figure SM 10.7.11) can be attributed to the fact that it is closer to the oxindole phenyl and away from its carbonyl group.

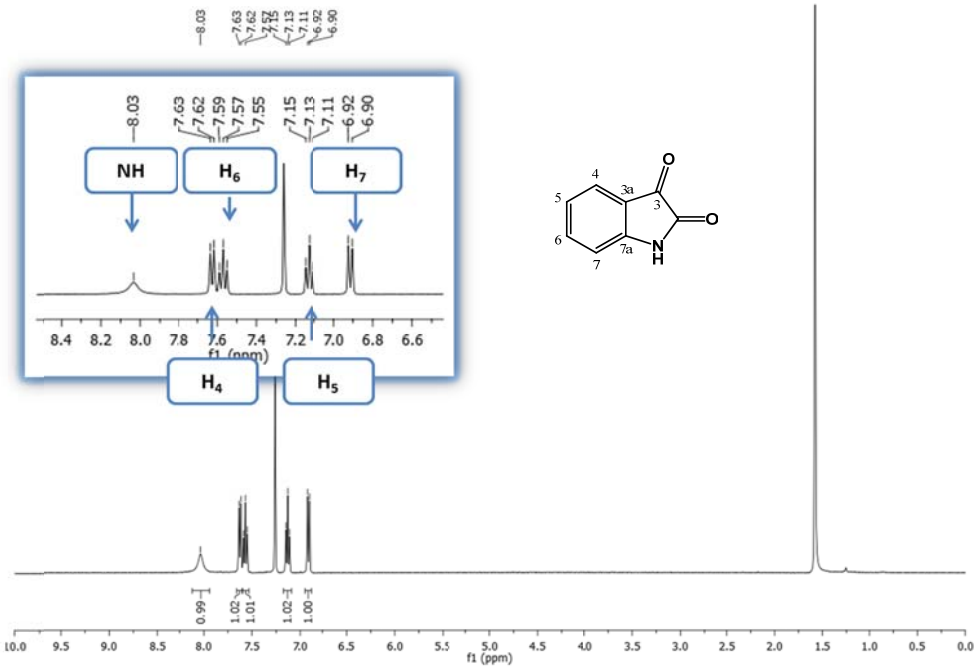


Figure SM 10.7.13

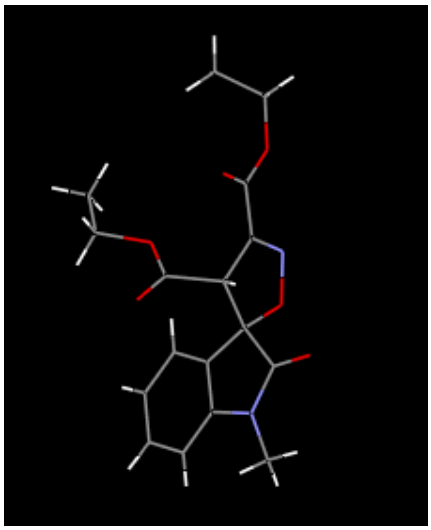


Figure SM 10.7.14

1. H. B. Rasmussen and J. K. MacLeod, *J. Nat. Prod.*, 1997, **60**, 1152.
2. C. J. A. Ribeiro, S. P. Kumar, R. Moreira and M. M. M. Santos, *Tetrahedron Lett.*, 2012, **53**, 281.
3. F. Risitano, G. Grassi, F. Foti, G. Bruno and A. Rotondo, *Heterocycles*, 2003, **60**, 857.
4. X. Lian, S. Guo, G. Wang, L. Lin, X. Liu and X. Feng, *J. Org. Chem.*, 2014, **79**, 7703.
5. C. J. A. Ribeiro, J. D. Amaral, C. M. P. Rodrigues, R. Moreira and M. M. M. Santos, *Bioorg. Med. Chem.*, 2014, **22**, 577.
6. Raunak, V. Kumar, S. Mukherjee, Poonam, A. K. Prasad, C. E. Olsen, S. J. C. Schaffer, S. K. Sharma, A. C. Watterson, W. Errington and V. S. Parmar, *Tetrahedron*, 2005, **61**, 5687.
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8. A. A. S. El-Ahl, *Pol. J. Chem.*, 1997, **71**, 27.
9. L. Sun, N. Tran, F. Tang, H. App, P. Hirth, G. McMahon and C Tang, *J. Med. Chem.,* 1998, **41***,* 2588.

Oxonitriles: Four-step Ozonolysis, Aldol, Conjugate Addition, and Enolate Acylation Sequence

Supplementary Material

Experiment Notes…….. 1
Analytical data…….. 6
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The experiment was tested over four years with cohorts of approximately 20 students each year. The experiment was performed over three consecutive laboratory sessions beginning with the ozonolysis, reduction, and cyclization of 2-(1-cyclopentenyl)acetonitrile **1**. In the first 3 hour session, the synthesis of the oxonitrile **4** was performed by passing gaseous ozone through a -78 °C, dichloromethane solution of 2-(1-cyclopentenyl)acetonitrile.[1] The key to processing multiple student samples was to develop a multi-port delivery system capable of simultaneously delivering ozone to four reactions (Figure **SM 10.8.**1, left).[2]

[1] 2-(1-Cyclopentenyl)acetonitrile, CAS: 22734-04-9; 10 g costs $63 (Sigma-Aldrich) / $60.90 (Acros Organics).

[2] A Welsbach ozonizator was used with the voltage set at 100V, a feed pressure of 5LPIn^2 and a flow of 2.5 SLPM.

Figure SM 10.8.1. Ozonolysis delivery system, left, and delivery tip, right.

The entire set-up, contained within one hood, is able to process four experiments in less than 15 minutes. Ozone is passed through a trap containing concentrated sulfuric acid to remove moisture and then through two empty traps before entering a glass delivery manifold. A flexible plastic tube from the manifold connects to the delivery tip (Figure **SM 10.8.**1, right) made from a 1 mL plastic syringe barrel which is inserted into a hole bored into 14/20 polyethylene cap (Aldrich cat. No. Z105813) and adjusted to place the syringe tip about ¼" beneath the surface of the solution. The cap is fitted with a syringe needle vent made from a 1 ½", 18 or 20 gauge hypodermic needle with the needle tip adjusted at least ½" above the tip of the syringe (Figure **SM 10.8.**2, left). The syringe vent can be used to direct the excess ozone from the needle to a saturated solution of potassium iodide and provides a modest back pressure that ensures equal delivery of ozone to each reaction.

The ozonolysis is performed at -78 °C using a dry ice/acetone cooling bath. Completion of the ozonolysis is easily observed by the formation of a blue solution indicative of excess ozone (Figure **SM 10.8.**2, right). Excess ozone is displaced by purging with oxygen, Me_2S is added, and the solution allowed to stand for at least 36 h. HPLC grade dichloromethane is sufficient for the ozonolysis and facilitates azeotropic removal of water upon completion of the aldol cyclization-dehydration sequence. Removal of the solvent affords pure oxonitrile **4** that can be used without purification in the conjugate addition experiment.

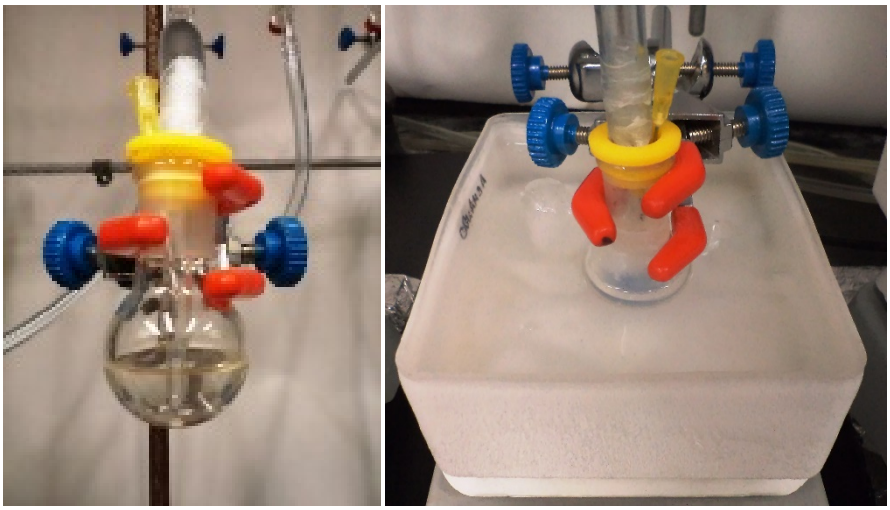


Figure SM 10.8.2. Charged reaction flask before, left, and after ozonolysis, right.

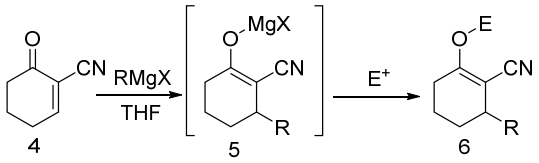
A series of conjugate addition reactions to oxonitrile **4** were performed with different Grignard reagents and trapping with various electrophiles to identify an inexpensive, moisture tolerant reagent combination that affords an easily isolated solid product **6** (Table **SM 10.8.**1).[3] Sequential addition of commercially available phenylmagnesium bromide (entry 1), 2-naphthylmagnesium bromide (entry 2), or *p*-methoxyphenylmagnesium bromide (entry 3), and trapping with *t*-butyldimethylsilylchloride was efficient but afforded enol silyl ethers **6a-6c** that were liquids.[4] Phenyl and vinylmagnesium bromide were evaluated for the conjugate addition with a series of acid chlorides for the enolate acylation: *p*-nitrobenzoyl chloride (entry 4), *p*-bromobenzoyl chloride (entries 5 and 6), and 3,4,5-trimethoxybenzoyl chloride.[5] The combination of phenylmagnesium bromide and *p*-bromobenzoyl chloride[6] proved optimal (Table **SM 10.8.**1, entry 5).

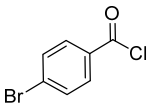
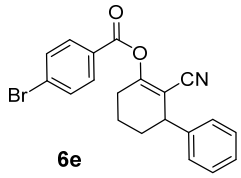
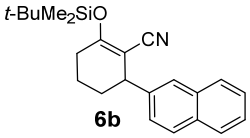
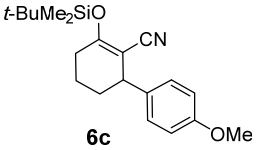
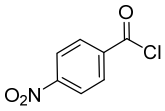
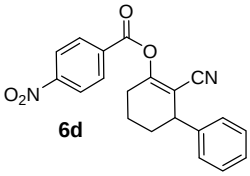
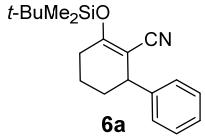
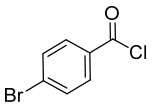
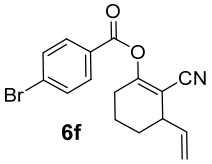
[3] Detailed procedures are provided later in the supporting information.

[4] Phenylmagnesium bromide 1.0 M in THF, CAS: 100-58-3; 100 mL $38.30 (Sigma-Aldrich). Phenylmagnesium chloride can be used without a detrimental influence on the yield or quality of the final product. A 100 mL bottle is useful for the execution of 80 essays.

[5] Attempts to employ vinylmagnesium bromide or phenylmagnesium bromide followed by trapping with 3,4,5-trimethoxybenzoyl chloride afforded unstable products.

[6] *p*-Bromobenzoyl chloride, CAS: 586-75-4; 25 g costs $38 (Oakwood Chemical). A 25 g bottle allows for the execution of 90 assays on the scale described.



	Reagents	Alkoxynitrile 6	Yield[a]
1	PhMgBr; 	 **6e**	37-46%[e]
2	2-NaphthMgBr; *t*-BuMe$_2$SiCl	 **6b**	33%[c]
3	*p*-MeOPhMgBr; *t*-BuMe$_2$SiCl	 **6c**	27%[b]
4	PhMgBr; 	 **6d**	52%[d]
5	PhMgBr; *t*-BuMe$_2$SiCl	 **6a**	39%[b]
6	VinylMgBr; 	 **6f**	33%[f]

a. Represents the overall four-step yield. b. Liquid. c. Slowly crystallizes on standing. d. Semisolid
e. Mp 133-133.5 °C. f. Liquid with an intense odor.

Table SM 10.8.1

The second 3 h lab session involved isolation of the oxonitrile, conjugate addition, and enolate trapping. Concentration of the crude oxonitrile, dissolution in ethyl acetate, and washing with $NaHCO_3$ affords material of greater than 90% purity that can be used directly in the conjugate addition. ^{1}H-NMR spectral analysis of select reactions provides a particularly good method for tracking the reaction

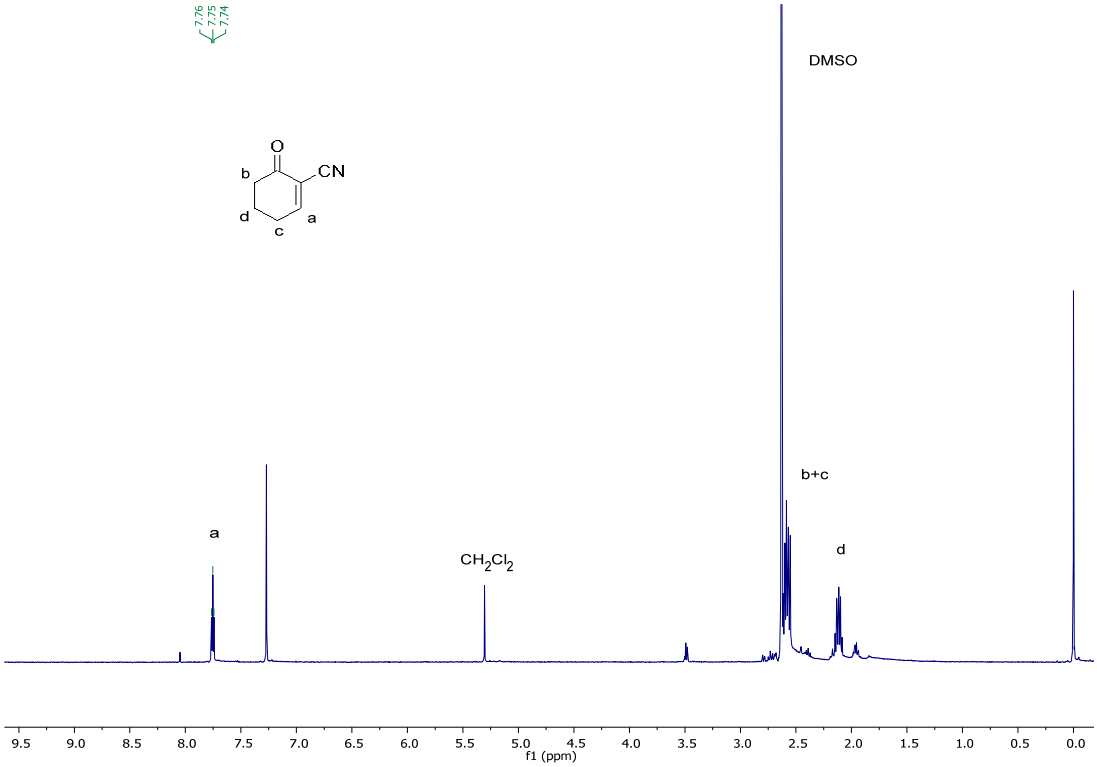
because the olefinic signal appears as a triplet at 7.75 ppm, well separated from the other signals and the diagnostic singlet at 9.78 ppm for the intermediate aldehyde.[7]

After the ethyl acetate solution of oxonitrile is dried (Na_2SO_4), the solvent is removed under reduced pressure, the flask purged with nitrogen and capped, and then commercially available, dry THF is added. The reaction flask was immersed in an ice bath prior to the addition of phenylmagnesium bromide or chloride, although no difference was observed for reactions performed at room temperature. After 5 min solid *p*-bromobenzoyl chloride was added and after a further 10 min the acylation is complete. At this stage the reaction can be worked up or stored for the final lab session.

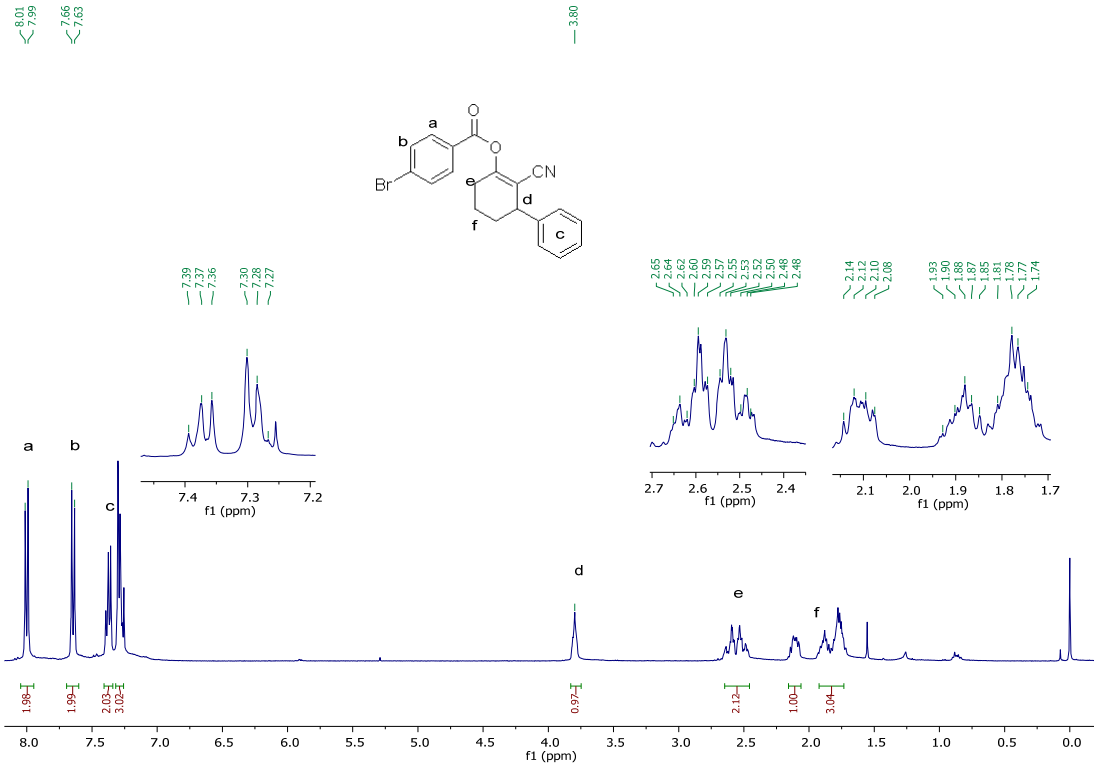
The last lab session was dedicated to the isolation and purification of the enol ester nitrile **6e**. ^{1}H-NMR spectral analysis of the crude mixture has a diagnostic broad singlet at 3.8 ppm corresponding to the benzylic methine that can be used to analyze the students' crude reaction mixtures. Column chromatography readily separates the enol ester nitrile because the impurities have a significantly different polarity. The overall yields are typically 35-45% for the four steps. Students regarded the experiment as "intensive" and "requiring good laboratory skills."

[7] The main impurity consisted in uncyclized ketonitrile **4**. The crude reaction mixture must be extracted with aqueous $NaHCO_3$ and concentrated at 40 °C which will ensure complete cyclization. Any uncylized ketonitrile can be resubjected to aqueous $NaHCO_3$ and concentrated at 40 °C to obtain ketonitrile **4**.

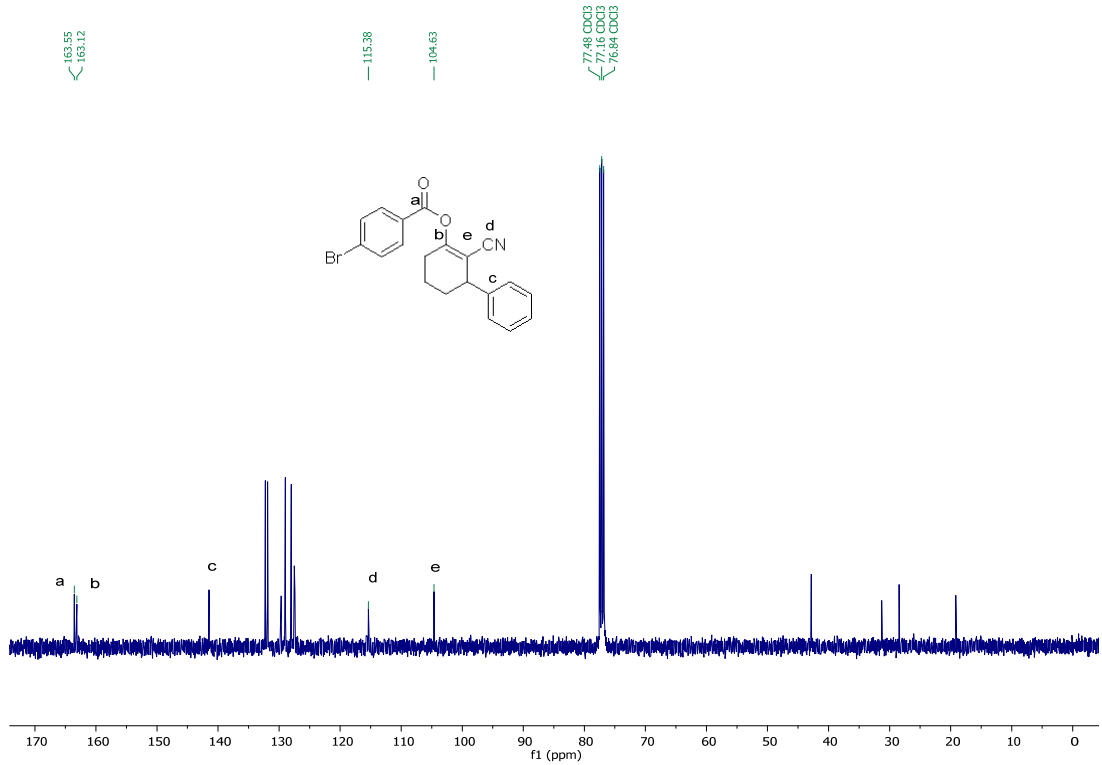
Representative ^{1}H-NMR of a random student sample of 6-oxocyclohex-1-ene-1-carbonitrile (4)



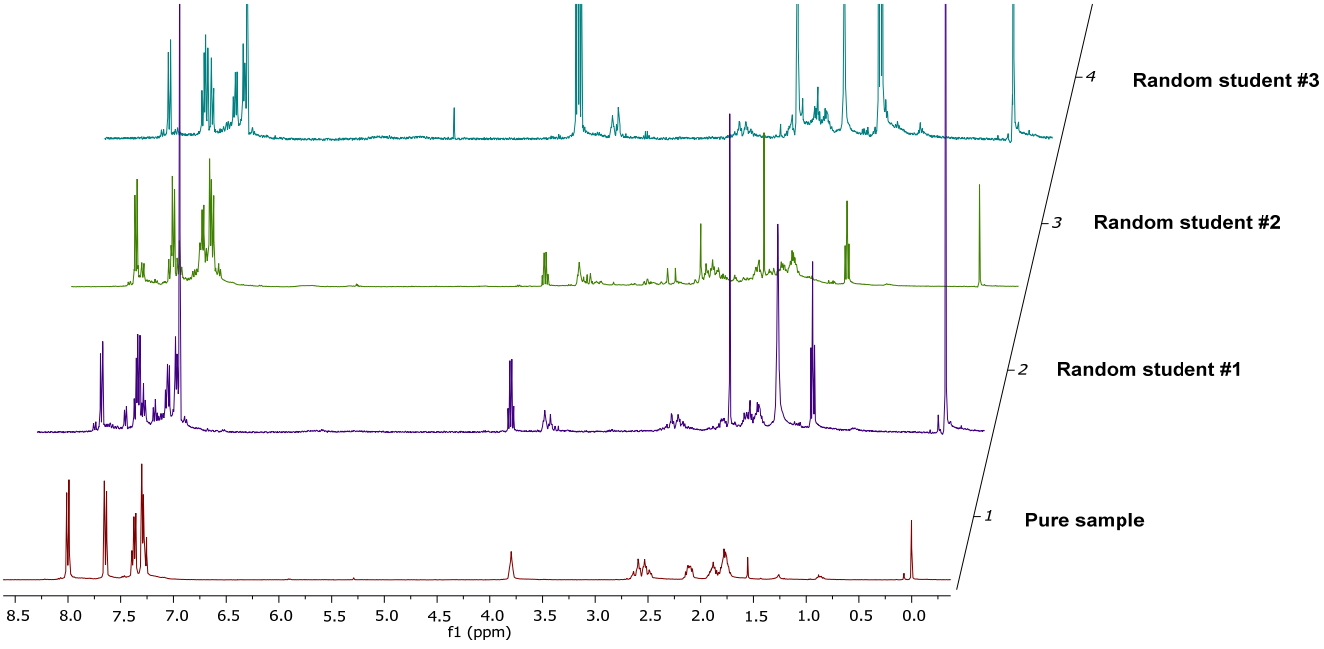
^{1}H-NMR of pure 2-cyano-1,4,5,6-tetrahydro-[1,1'-biphenyl]-3-yl 4-bromobenzoate (6a)



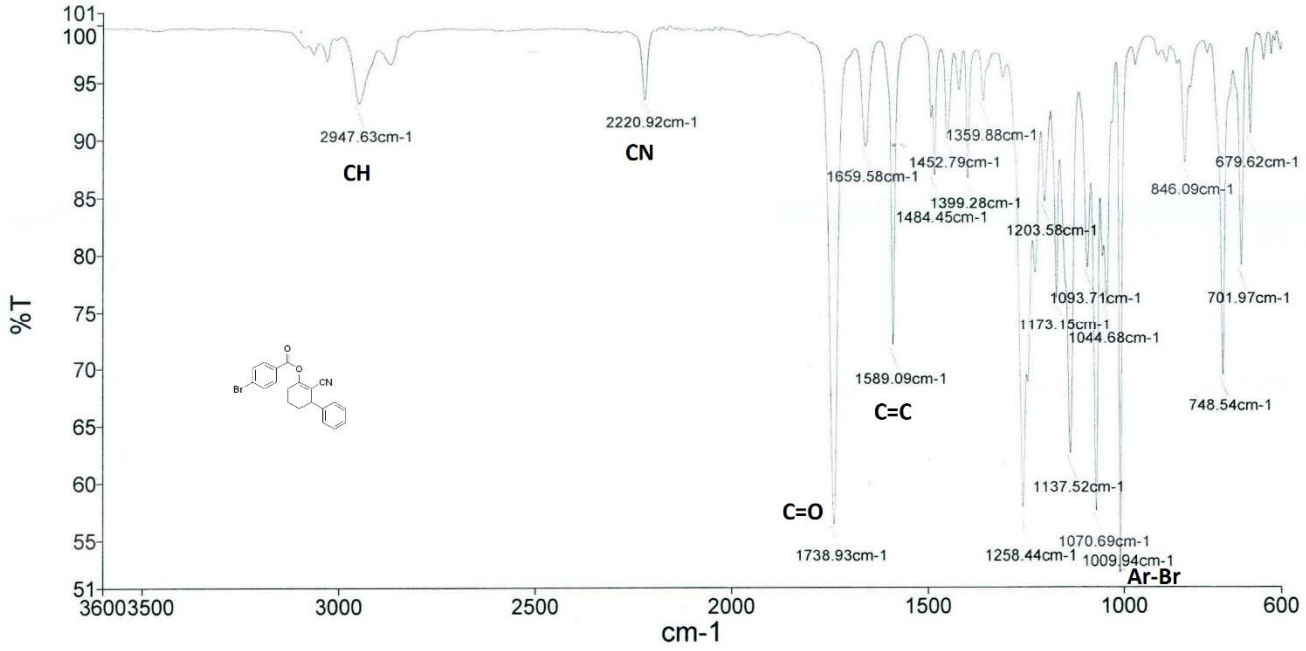
^{13}C-NMR of pure 2-cyano-1,4,5,6-tetrahydro-[1,1'-biphenyl]-3-yl 4-bromobenzoate (6a)



Representative ^{1}H-NMR random student's spectra of enol ester nitrile (6a) before chromatography



IR of pure 2-cyano-1,4,5,6-tetrahydro-[1,1'-biphenyl]-3-yl 4-bromobenzoate (6a)



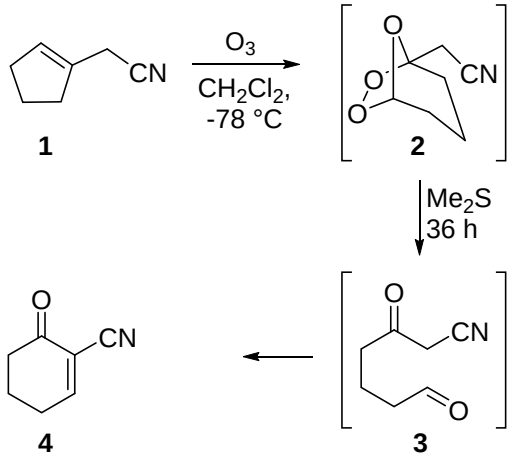
Students' guide

Oxonitriles: Four-step Ozonolysis, Aldol, Conjugate Addition, and Enolate Acylation Sequence

Alkenes bearing two strongly electron withdrawing groups are valuable reactants for conjugate addition reactions. The versatility of highly reactive, electron deficient olefins has led to several syntheses of cycloalkenones containing an additional electron-withdrawing group on the α-carbon. Oxoalkenenitriles are particularly attractive substrates for conjugate addition reactions because the two electron withdrawing groups are an ideal compromise between high reactivity and stability toward storage and chromatography.

In this experiment, an activated oxonitrile **4** will be synthesized through an ozonolysis-aldol sequence and then used in a conjugate addition-acylation reaction. The unsaturated 2-(1-Cyclopentenyl)acetonitrile (**1**) will be treated with ozone to cleave the olefin and unmask the ketonitrile **3**. The ketonitrile **3** will not be isolated but will be directly cyclized to 6-oxocyclohex-1-ene-1-carbonitrile (**4**) in a one-pot, domino ozonolysis-aldol sequence (Scheme **SM 10.8.**1)

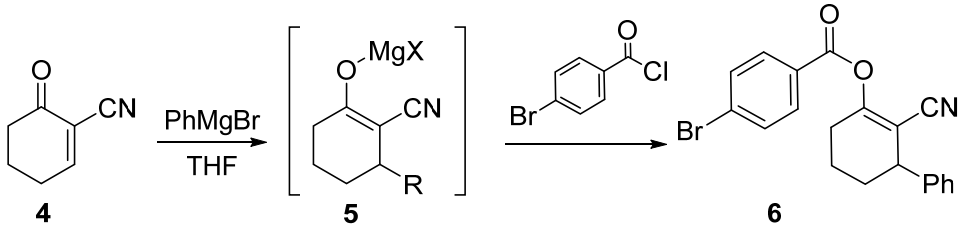
Ozonolysis, is a powerful organic reaction that uses gaseous ozone to cleave alkenes. The cleavage proceeds through a series of steps resulting in the formation of an ozonide **2** that can be reduced with a reagent such as zinc, triphenylphosphine or dimethylsulfide to give carbonyl compounds.



Scheme SM 10.8.1: Synthesis of 6-oxocyclohex-1-ene-1-carbonitrile through a domino sequence

- ***Provide the mechanism for the conversion of 2-(1-cyclopentenyl)acetonitrile (1) to ketonitrile (3) and for the conversion of 2 to 6-oxocyclohex-1-ene-1-carbonitrile (4).***

6-oxocyclohex-1-ene-1-carbonitrile (**4**) is an exceptional Michael acceptor that reacts with Grignard reagents in a conjugate addition without the need for a catalyst (Scheme **SM 10.8.**2). In this lab, the Grignard reagent adds to the oxonitrile **4** to form the enolate **5** that is trapped as the enol ester nitrile **6**. ^{1}H NMR and ^{13}C NMR analysis in $CDCl_3$ can be used to identify the product. IR spectra can be used to confirm the presence of the ester, nitrile, and olefin functionalities.



Scheme SM 10.8.2. 1,4-Grignard addition and enolate trapping of oxonitrile **4**.

Safety data

Eye protection and nitrile gloves must be worn while performing this experiment. Dispense reagents in a hood to avoid inhalation of vapors.

2-(1-Cyclopentenyl)acetonitrile (CAS 22734-04-9) Causes irritation, is harmful by inhalation, by contact with the skin, and if swallowed.

Dichloromethane (CAS 75-09-2) Vapors are narcotic in high concentrations. Can cause nausea and irritation by inhalation.

Dimethyl sulfide (CAS 75-18-3) Irritant to eyes and skin. Unpleasant odor even at low concentration. Harmful if swallowed.

Ozone (CAS 10028-15-6) Strong irritant to eyes, upper respiratory tract and lungs. Forms explosive mixtures with olefins. Has to be generated and used INSIDE A WELL-VENTILATED FUME HOOD!

Tetrahydrofuran (CAS 109-99-9) Highly flammable! Harmful by contact or inhalation.

Phenylmagnesium chloride THF solution (CAS 100-59-4) Very reactive and corrosive. Reacts violently with water. Harmful by contact and inhalation.

p-Bromobenzoyl chloride (CAS 586-75-4) Very corrosive. Harmful by contact.

Sodium bicarbonate (CAS 144-55-8) Causes serious eye irritation by contact.

Ammonium chloride (CAS 12125-02-9) Irritant.

Lab I

Synthesis of the oxonitrile 4: Weigh out neat 2-(1-cyclopentenyl)acetonitrile (0.2 g, 1.86 mmol) into a 10 mL, round-bottomed flask containing a magnetic stirring bar and then add 5 mL of dichloromethane. Attach the ozone delivery device (Figure **SM 10.8.**1, note 1) and cool the solution to -78 ^{o}C by immersing the flask into an acetone/dry ice bath.

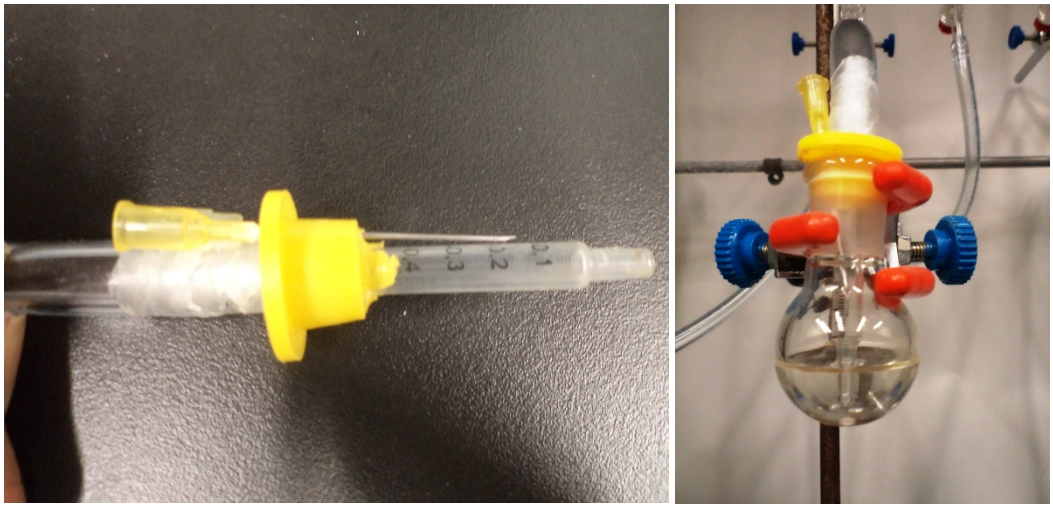


Figure SM 10.8.1. Detail of the ozone delivery device (left). Reaction flask charged with 2-(1-cyclopentenyl)acetonitrile, dichloromethane, and the ozone delivery device (right).

Carefully, open the manifold port to allow a gentle stream of dry ozone to pass through the solution [Welsbach ozonator setup: 5 psi O_2 feed, 2 Standard Liters Per Minute (SLPM) of ozone enriched oxygen, 1 SLPM for each two ports]. Continue the ozonolysis until the distinctive blue color of excess ozone is first

observed (usually 5-10 min) and then close the ozone delivery port (Figure **SM 10.8.**2). Leave the set up attached until all of the reaction mixtures turn blue (within 10 min).

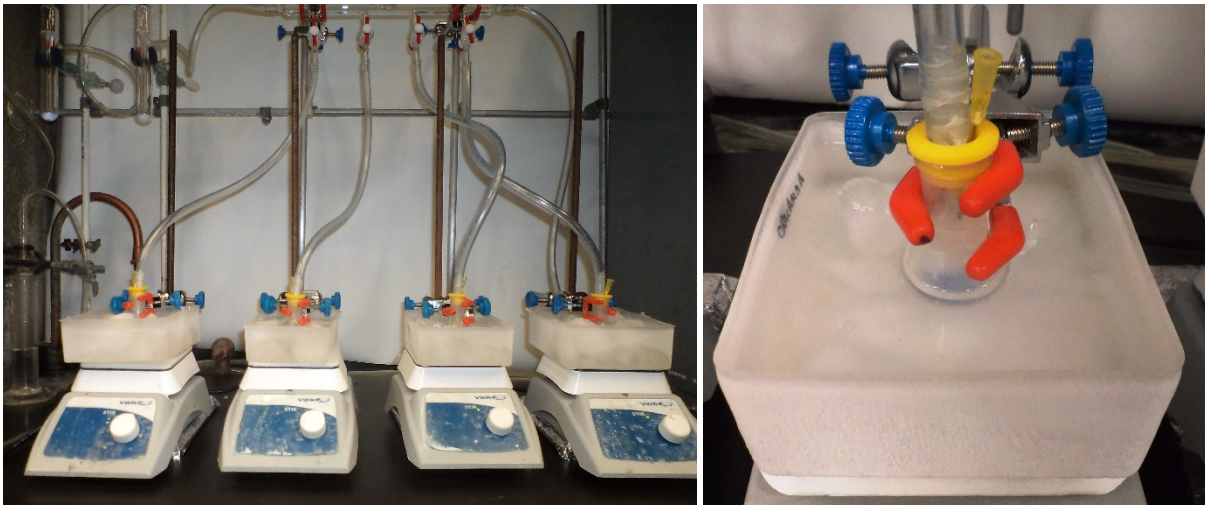


Figure SM 10.8.2. Ozonolysis in progress (left). Endpoint after ozonolysis showing the distinctive blue color of ozone (right).

Remove the excess ozone by lowering the ozonator voltage to zero and purging the -78 °C solution with a stream of oxygen (takes about 5-10 min). After the solution becomes colorless, remove the cooling bath and replace the ozonolysis adapter with a rubber septum. Add neat dimethyl sulfide (0.2 mL, 2.73 mmol) via syringe to the flask (this addition must be performed in a fumehood!). Allow the mixture to stir at room temperature for 5 min and then store the solution in the dark until the next lab period.

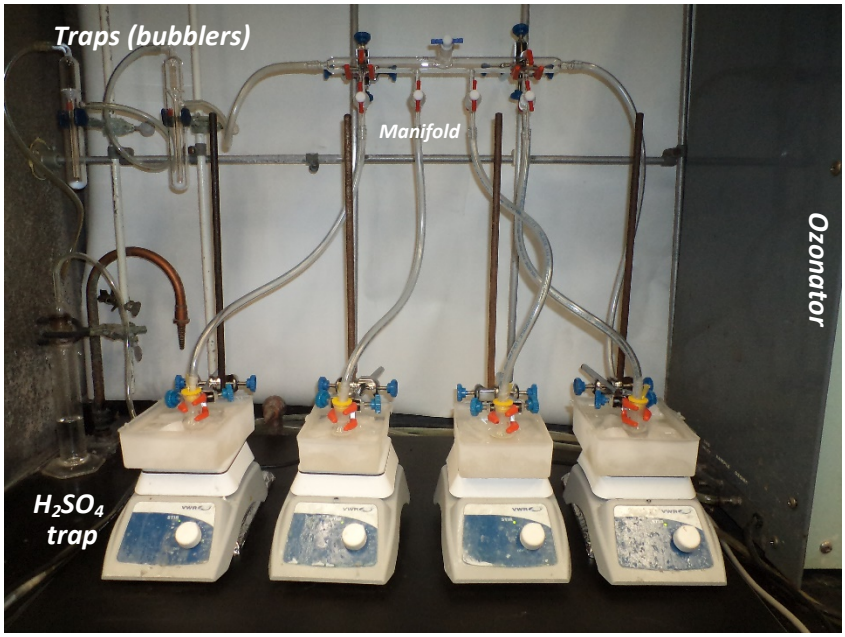
Concentrate the resulting solution under reduced pressure using a rotary evaporator keeping the water bath temperature at 40 °C or slightly below. Dilute the resulting thick syrup with 4 mL of ethyl acetate. Wash the resulting solution sequentially with saturated sodium bicarbonate (1 mL), water (1 mL), and brine (1 mL) in a small separation funnel or test tube. Dry by adding solid Na_2SO_4 and analyze by TLC using 1:1 EtOAc/hexanes as the eluent (if desired, a small sample of ~10 mg can be removed and analyzed by NMR). Filter the reaction mixture through a filter paper or cotton plug, and then concentrate the solution using a rotary evaporator. The

yield of 6-oxocyclohex-1-ene-1-carbonitrile (**4**) is essentially quantitative. The residual red oil contains a trace impurity that does not affect the conjugate addition. *From this stage onward, care must be taken to ensure the absence moisture.*

NOTES:

1. The ozonolysis adapter consists of a 1 mL plastic syringe with the barrel cut just below the finger supports to allow the barrel to be connected to ¼" plastic tubing. The tubing is sealed to the syringe by wrapping the joint with parafilm. The syringe barrel is inserted into a suitable hole bored into a polyethylene cap (Aldrich cat. No. Z105813) designed to fit a 14/20 ground glass joint. A 1 ½", 18 or 20 gauge hypodermic needle is inserted through the polyethylene cap so that the needle tip sits at least ½" above the tip of the syringe. The tip of the syringe is then submerged about ¼" beneath the surface of the solution with the venting syringe needle at least ¼" above the solution). A modified syringe connected to plastic tubing can be used to direct the excess ozone from the needle to a saturated solution of potassium iodide (optional).

2. The ozone is dried by passing the gas through a trap containing concentrated sulfuric acid.



3. Formation of the oxonitrile is accompanied by a trace contaminant that has a distinctive red color. A reaction that fails to go red, should be checked by TLC against a red-coloured sample.

4. The ozone can be purged with oxygen, air, or nitrogen. The recommended procedure uses oxygen to avoid exposure to the displaced ozone. In addition, the procedure minimizes the possibility of letting moisture enter the reaction.

5. The oxonitrile is highly UV active and the TLC is easily visualized under UV light. The red impurity has an identical Rf and is often seen on TLC without development.

Lab II

Conjugate Addition Procedure: Place a magnetic stirring bar into the flask containing the dry, crude oxonitile **4**, and then cap the flask with a septum. Remove any oxygen in the flask by briefly purging with a stream of nitrogen from a tank or a nitrogen filled balloon, or using Schlenk techniques with a vacuum manifold. Add 2 mL of dry THF via syringe, connect a nitrogen-filled balloon fitted with a syringe by forcing the needle through the septum on the reaction flask, and then cool the flask to 0 ˚C by immersion in ice/water bath. Add the PhMgCl solution by syringe (1.1 equiv based on the crude weight of oxonitrile). After 5 min, remove the cooling bath, remove the septum and add solid *p*-bromobenzoyl chloride (1.2 equiv.) in one portion. Replace the septum and then stir the mixture for 10 min. The reaction mixture changes from an orange suspension to a translucent solution.

After 10 min, add saturated, aqueous ammonium chloride (5 mL) and stir the reaction mixture for 1 min. Add EtOAc (3 mL), separate the two phases, and extract the aqueous phase with EtOAc (2 x 3 mL) using a small separatory funnel or a test tube. Combine the organic extracts and sequentially wash the combined organic phase with aqueous sodium bicarbonate, water, and brine (3 mL each). Dry the organic phase over anhydrous

sodium sulfate, concentrate the solution, and analyze by IR and/or ^{1}H NMR. The enol ester nitrile is a solid that can be easily purified.

Lab III

Purify the crude ester nitrile by column chromatography using a 15 mm x 30 cm column, 10 g of silica gel, and a 90:5:5 Hexanes/dichloromethane/ether solvent mixture. Collect the eluent in small test tubes (5 mL) and follow the progress of the separation by TLC (use Hexanes/EtOAc 85/15 as the developing solvent). At the end of the column chromatography, combine the test tubes containing the pure enol ester nitrile **6a** and then concentrate the combined material. Weigh the pure enol ester nitrile **6a** for a yield and obtain a melting point, IR, and ^{1}H NMR spectrum as time permits.

(2-cyano-1,4,5,6-tetrahydro-[1,1'-biphenyl]-3-yl)methyl 4-bromobenzoate (**6a**): white, crystalline solid (mp 133-133.5 ˚C): IR (neat): 2948, 2221, 1739 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 8.6 Hz, 2H), 7.41 – 7.35 (m, 2H), 7.32 – 7.26 (m, 3H), 3.80 (bs, 1H), 2.65 – 2.46 (m, 2H), 2.18 – 2.05 (m, 1H), 1.96 – 1.82 (m, 1H), 1.85 – 1.70 (m, 2H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.55, 163.12, 141.47, 132.25, 131.89, 129.65, 128.97, 128.01, 127.52, 127.41, 115.38, 104.63, 42.84, 31.29, 28.45, 19.16.

Instructor's guide

Oxonitriles: Four-step Ozonolysis, Aldol, Conjugate Addition, and Enolate Acylation Sequence

General guidelines

a. The experiment requires three, 3h laboratory sessions. The first session is the alkene ozonolysis and reduction of the resulting ozonide with dimethylsulfide. The second consists in the isolation of the oxonitrile (which requires 36 h between the two lab sessions), the conjugate addition with PhMgBr or PhMgCl, and the O-benzoylation of the enolate with *p*-bromobenzoyl chloride. The third session is for the isolation and purification of the enol ester nitrile.

b. Every lab session requires an introductory explanation of the techniques and a description of the chemistry involved in the experiment:

   i. Session 1: A brief discussion of multistep syntheses, the synthetic advantages of convergent syntheses over linear sequences, and the increased efficiency of cascade sequences. Provide background to ozonolysis and aldol condensation.

   ii. Session 2: Provide a background to conjugate addition and nucleophilic acyl substitution. Students must be familiar with liquid-liquid extraction and thin layer chromatography.

   iii. Session 3: Thin layer chromatography; column chromatography.

c. Solutions of the following will be required for the experiment:

   Session 2:

   i. Saturated $NaHCO_3$

   ii. Hexane/EtOAc (40/60)

   Session 3:

   iii. Saturated, aqueous NH_4Cl

iv. Saturated NaCl (brine)

d. Central to the success of the ozonolysis is the use of the manifold to simultaneously provide ozone to four reaction vessels. As each reaction becomes blue, due to excess ozone, the gas outlet to the flask should be closed to maximize the ozone flow to the remaining vessels and shorten the overall time. The ozonolysis takes no more than 15 min to complete all four reactions using the parameters provided (a flow rate of 2.5 SLPM). Typically the first reaction is complete within 5-8 min. For a class of 24 students, between 90 and 120 min is required to complete all the ozonolyses.

e. The ozonator power is set to 120 V. Lowering the voltage to 100 V will require more time for the ozonolysis.

f. PhMgCl and PhMgBr can be used interchangeably. Both Grignard reagents are commercially available and can be used as received. Previously opened bottles are best titrated to determine the active molarity: Accurately weigh 1 mmol of menthol (0.156 g, CAS 2216-51-5) into a dry 10 mL round bottomed flask. Add phenanthroline indicator (1-2 mg, CAS 2216-51-5) and 2 mL of dry THF. Accurately fill a 2 mL syringe with PhMgX solution if the stated molarity is 1.0 M or a 1 mL syringe if the stated molarity is 2M. Add the solution dropwise to start the titration. The endpoint is reached when a pink-purple color is observed and maintained for 1 min. The titer is obtained from the following formula:

$$Conc.\ M = \frac{(1\ mmol)}{(x\ mL)}$$

g. Dry THF is required for the conjugate addition procedure. Dry THF obtained by distillation from sodium benzophenone ketyl was found to have a similar performance to THF obtained from a solvent system equipped with activated alumina columns (Innovative Technology solvent purification system). Commercial, dry THF is expected to have a similar performance (for example, Sigma-Aldrich Cat. No. 401757).

h. Sometimes the crude oxonitrile mixture did not show the characteristic red color indicative of complete conversion. Washing the reaction mixture with sodium bicarbonate and concentrating the organic fractions on a rotary evaporator with a water bath temperature of at least 40 °C is forces the cyclization to completion.

i. The Grignard reaction and capture of the enolate are fast. However, the experimental setup requires several manipulations that require a 3 hour time period.

j. Unless the students possess advanced skills in organic syntheses, the instructor is advised to dispense the Grignard reagent for the students.

k. Four rotary evaporators with vacuum pumps rated at 30 mmHg were used with 24 students.

l. The polarity of the enol ester nitrile is significantly different from that of the minor components, such that the purification is particularly easy.

m. Small aliquots from some reactions were removed and were analyzed by ^{1}H NMR (Bruker Avance 400 MHz). The spectrum was distributed to each student to include their analysis into the reports. The purity of the crude oxonitrile was ~95% while a ^{1}H NMR analysis of the crude ester-nitrile indicated a purity of between 60% and 80%. In both cases the diagnostic signals are well resolved without interference of impurities.

n. IR spectra of two samples were obtained for the final products.

o. Yields reported ranged from 5% to 60%. The students attributed problems to maintaining a positive pressure during the Grignard reagent addition, confusion during the liquid-liquid extraction, and during the chromatographic separation.

Flash vacuum pyrolysis of *o*-phenylene sulfite: formation and purification of cyclopentadienone dimer

Supplementary Material

Context of Experiment

This experiment has been performed by 3rd year students during the organic chemistry lab component of the BSc (Hons) degree at the University of St Andrews over a period of 5–6 years (30 students per year). With careful organisation 3–4 students can perform the pyrolysis step on one set of apparatus in a single 3 h session.

Preparation of the starting compound *o*-phenylene sulfite

This is best prepared each year in advance of the class by the laboratory technician. The published method[9] using carbon disulfide and pyridine was felt to be unduly hazardous and was not used. Instead the material was prepared using an improved method as follows:

Catechol (20.0 g, 182 mmol) was placed in a 500 cm^3 round-bottomed flask and toluene (200 cm^3) was added followed by thionyl chloride (13.4 cm^3, 21.8 g, 183 mmol). The mixture was heated under reflux in a fume hood for 3 h. The solvent and any unreacted thionyl chloride were removed by evaporation on a rotary evaporator and the residual liquid was distilled from a Vigreux flask under vacuum to give the product (21.7 g, 77%) as a colourless liquid, bp 34 °C / 0.1 Torr or 96–98 °C / 16 Torr. The compound is mildly lacrymatory and is best stored in the refrigerator. The quantity obtained in this preparation is sufficient for 35–40 students.

Description of FVP Apparatus (see Figure 10.9.1 and also **Figure SM 10.9.**1 and **10.9.**2)

This consists of a horizontal fused quartz furnace tube (30 x 2.5 cm with B24 sockets at each end) situated in a laboratory tube furnace. Use of fused quartz allows pyrolysis experiments up to 1000 °C without softening of the tube under vacuum but for use at 750 °C a borosilicate glass tube would suffice. The inlet tube is a test tube (10 x 2.5 cm) with B24 cone and a ridge half way up one side to act as a "dam" to prevent liquid materials running into the furnace. This is heated by a glass Kugelrohr oven or similar electrical heating device. The special design of U-shaped cold trap allows convenient dissolution of products in solvent for NMR analysis while still cold by adding the solvent through the removable top cap. For this experiment, where the product is a stable solid at RT, it is not required and a simple U-shaped trap could be substituted. Vacuum in the range 10^{-1}–10^{-3} Torr is provided by a high capacity rotary oil pump and monitored using a Pirani gauge.

Preparative TLC

This was carried out using glass plates spread in house with a 1.0 mm layer of silica gel containing $CaSO_4$ as binder and 0.5% fluorescent green indicator (254 nm). Pre-prepared plates are commercially available but are rather expensive. The template shown in Figure 10.9.2 is readily made by cementing two narrow strips of perspex (0.5 x 0.5 x 20 cm) along the short sides of a 22 x 20 x 0.5 cm perspex square to form a bridge shape. This can be laid across the plate without touching it and allows even application of the compound by leaning the dropper against the straight edge. The tank for development has dimensions 30 cm long x 10 cm wide x 22 cm deep and can be used to run two plates at a time. It must be covered during the development time and vapour saturation is assisted by inserting a large sheet of filter paper on one side of the tank to soak up the solvent.

Average results obtained

Yield of pure product **4** : 60–120 mg (23–47%)

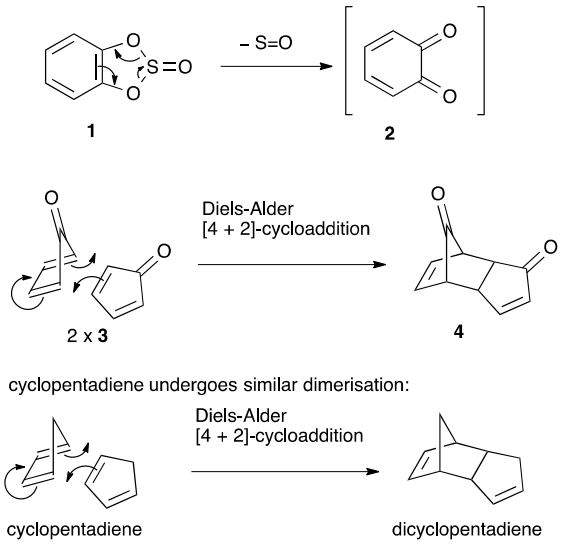
Melting Point: 95–100 °C (Lit.[9] 102–102.5 °C

IR spectrum (Nujol): ν_{max} /cm^{-1} 1780 (unconjugated C=O) and 1700 (conjugated C=O) (see SM Figure 3)

^{1}H NMR spectrum: δ ($CDCl_3$) 7.45–7.40 (1 H, m, CH=CH–CO), 6.40–6.35, 1 H, m, =CH–CO), 6.35–6.30 (1 H, m, =CH–CO), 6.20–6.15 (1 H, m, =CH–CO), 3.57–3.50 (1 H, m, CH–CO), 3.43–3.35 (1 H, m, CH–CO), 3.25–3.20 (1 H, m, CH–CO) and 2.95–2.88 (1 H, m, CH) (see SM Figure 4).

Answers to questions

1. Give arrow-pushing mechanisms both for formation of intermediate **2** from **1** and for formation of the product **4** from diene **3**. What other diene forms a dimer of similar structure?



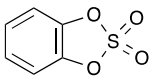
2. When the product trap was warming up you might have seen a yellow solid in the coldest region of the trap. What is this and why did it not collect in the region corresponding to its boiling point?

This is elemental sulfur formed by the disproportionation of SO into (eventually) SO_2 and S_8. The key point however is that the sulfur is not formed in the furnace but only in the cold trap when the SO is condensed from the gas phase. Therefore although it is a non-volatile solid at RT (bp ~440 °C) it condenses in the region corresponding to the volatility of the gas from which it was formed.

3. Interpret the IR and ^{1}H NMR spectra of the product **4** and, in particular, explain why there are two separate IR stretches in the C=O region and why the NMR signals in both the alkene (δ > 6.0) and alkane (δ 2.0–3.5) regions occur in a 3:1 ratio.

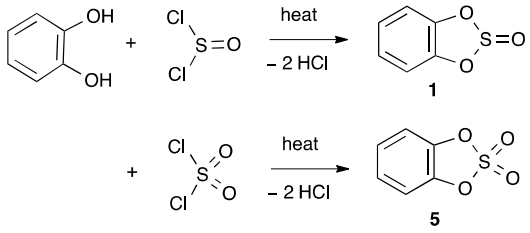
See spectroscopic listing under Results above. The significant features of the IR spectrum are strong C=O stretch peaks at 1700 and 1780 cm^{-1} corresponding respectively to the conjugated and non-conjugated ketone functions. In the ^{1}H NMR spectrum we have the alkene H beta to C=O which comes at much higher chemical shift (7.4) than the remaining three alkene H's not in this situation (6.2–6.4). Likewise for the saturated CH's, the three adjacent to C=O come at higher chemical shift (3.2–3.6) than the one which is not (2.9). The patterns of H–H coupling are rather complex and uninformative.

4. Write an equation for the formation of the substrate **1** from benzene-1,2-diol (catechol) and thionyl chloride. How might the corresponding cyclic sulfate **5** be prepared and how might it behave upon FVP?



5

The reaction involves loss of 2 equivalents of HCl gas. Formation of **5** is just the same using sulfuryl chloride.



Compound **5** loses SO_2 to generate the same intermediate **2** upon FVP. Thereafter the sequence is the same so the final product is also dimer **4**.

Figures

Figure SM 10.9.1: Photograph of FVP apparatus showing product collection trap

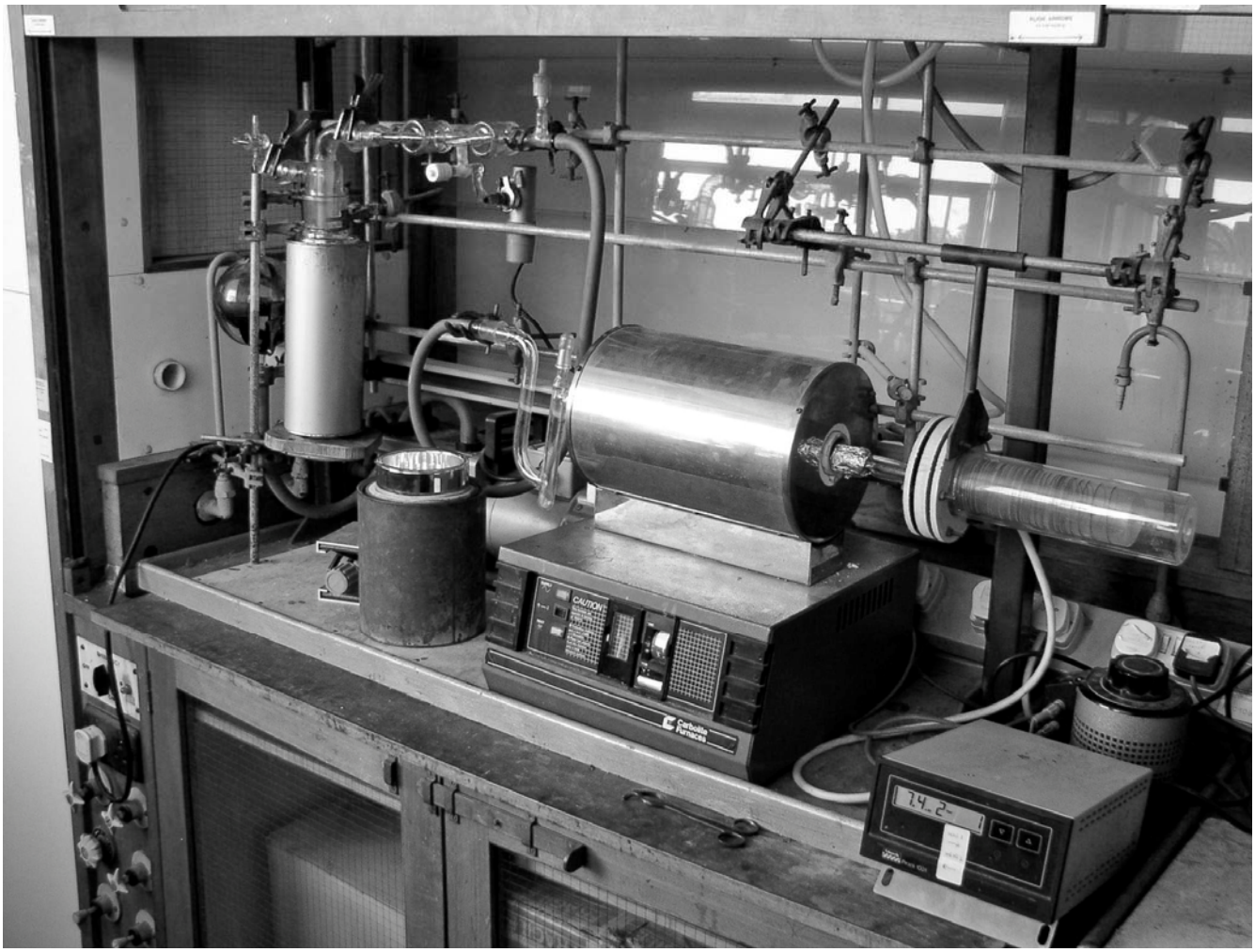


Figure SM 10.9.2: Photograph of FVP apparatus showing inlet tube

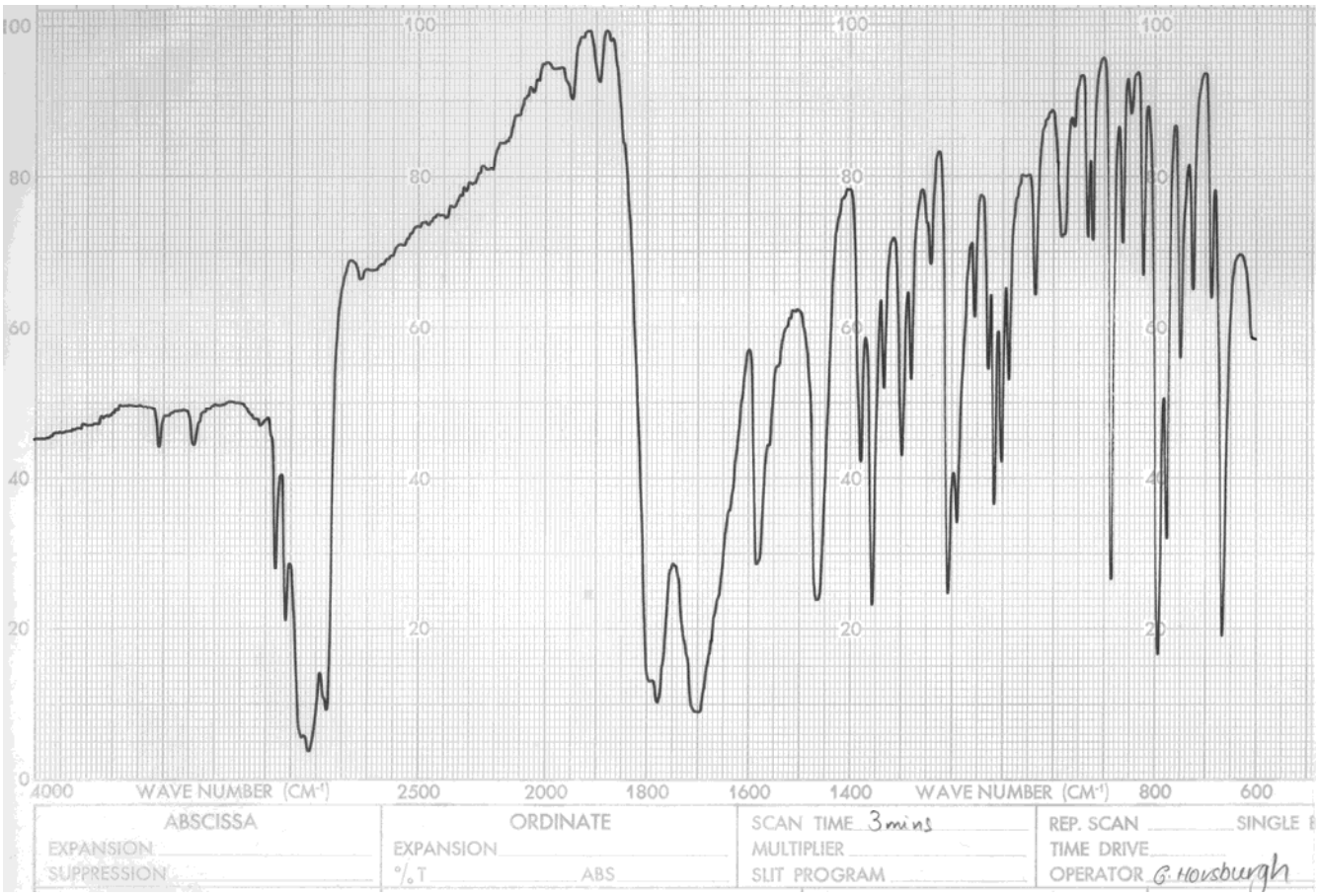


Figure SM 10.9.3: IR spectrum of product **4**

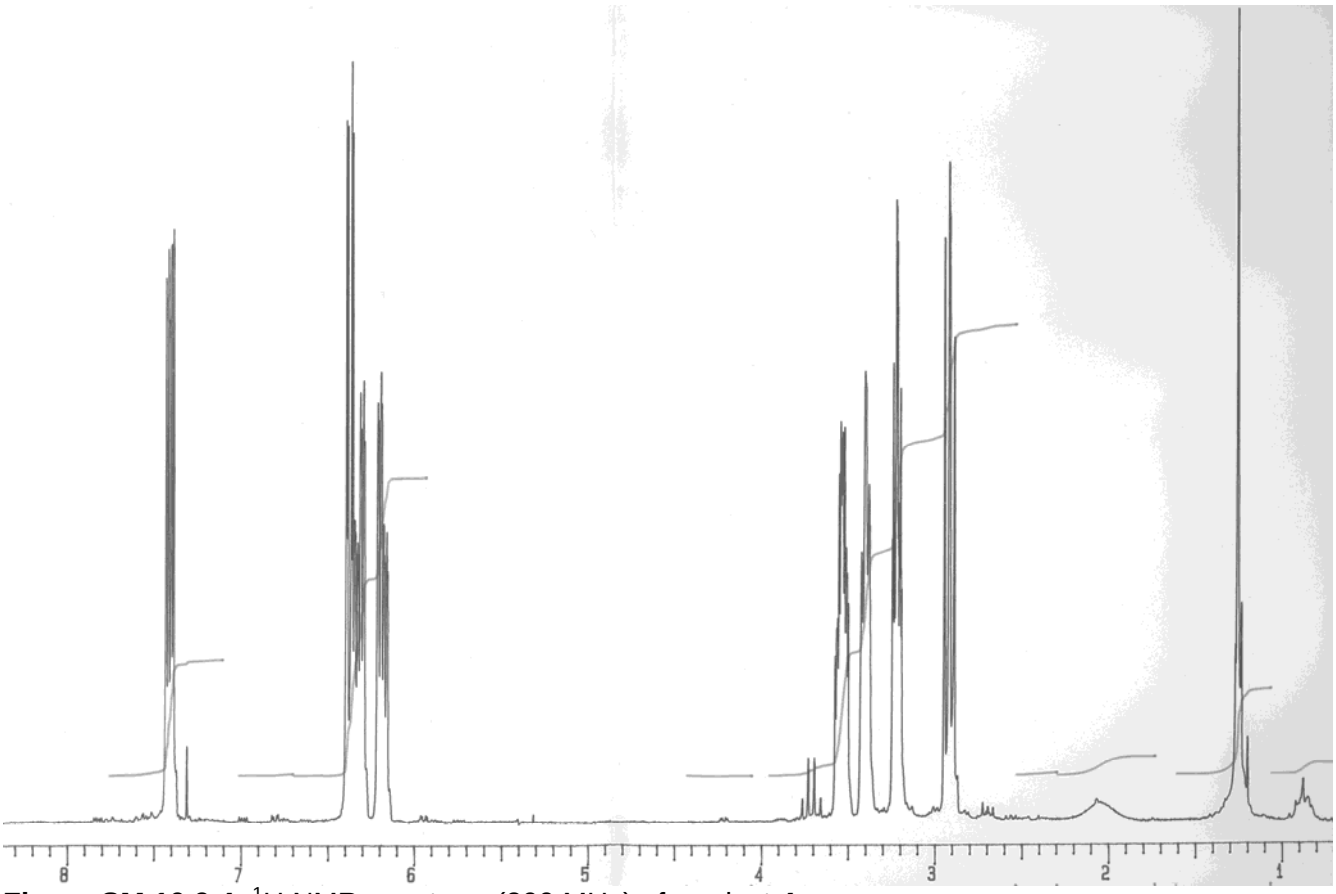


Figure SM 10.9.4: ^{1}H NMR spectrum (200 MHz) of product **4**