

THE SYNTHESIS OF RESVERATROL

By

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Synthesis of Resveratrol

ABSTRACT

The purpose of this research experiment was to perform and improve the synthesis of resveratrol (CAS: 501-36-0), (Fig. 1). Resveratrol (trans-3,4',5-trihydroxystilbene), a natural polyphenolic, non-flavonoid antioxidant, is a phytoalexin found in many plants including grapes, nuts and berries. Recent studies have documented that resveratrol has various health benefits, such as cardiovascular and cancer preventive properties. The Grignard reaction was first carried out to determine if it could be used as a model to synthesize a stilbene, both FTIR and NMR revealed that one had been created, but its purity and conformation was inconclusive. The Wittig and Perkin reactions were considered but aborted before any tests were done. The decarbonylative Heck reaction seemed to be the most favored reaction among other scientists. A copy of the experiment done by Jing Liu, Ph.D. using the Heck reaction was attained from Dr. Liu directly. A similar procedure to his experiment was then carried out and tested. Several improvements were made to the original. The four-step reaction generated poor yields but the spectra indicate that the overall resveratrol compound was obtained. Lois Ablin, Ph.D. was the supervising advisor; all NMR and IR spectrum were collected by me, Dustin Sprouse; and the overall experiment took nine months with approximately 430 hours of lab time logged.

INTRODUCTION

Background

For many past centuries man has been in search of an elixir. Of course, we know none exists, but we continue to search for ways to extend the length of our lives, such as eating right, not smoking, drinking more water, and keeping fit. The Georgians were some of the first people to make wine from grapes, and they believed wine was the drink of the gods and that it extended youth. Today we have discovered the chemical believed to be responsible for wine's health benefits. This chemical is called resveratrol (Figure 1) and is now known to be produced by several plants only when under the attack of pathogens.¹ This phytoalexin can be chemically synthesized and is sold as a nutritional supplement in some health food stores around the country. The compound was first tested on rats, mice² and fish;³ the results suggested that it is anti-carcinogenic, lowers blood glucose levels, anti-inflammatory, protects from ischemia and neurotoxicity, has beneficial cardiovascular effects,⁴ and diminished mitochondrial oxidative phosphorylation.

¹ Sinclair, D., S. Lavu, B. Rogina, K. Howitz, S. L. Helfand, and M. Tatar. *Nature*. 2004. 430.7000 686-89.

² Sinclair, D., R. De Cabo, D. K. Ingram, J. A. Baur, K. J. Pearson, N. L. Price, H. A. Jamieson, C. Lerin, A. Kalra, V. V. Prabhu, J. S. Allard, G. Lopez-Lluch, K. Lewis, P. J. Pistell, S. Poosala, K. G. Becker, O. Boss, D. Gwinn, M. Wang, S. Ramaswamy, K. W. Fishbein, R. G. Spencer, E. G. Lakatta, D. Le Couteur, R. J. Shaw, P. Navas, and P. Puigserver. *Nature*. 2006. 444.7117 337-42.

³ Sinclair, D., S. Lavu, B. Rogina, K. Howitz, S. L. Helfand, and M. Tatar. *Nature*. 2004. 430.7000 686-89.

⁴ Baur, J. A., and D. A. Sinclair. *Nat Rev Drug Discov* 2006. 5.6 (206): 493-506.

Resveratrol's effects were associated with an induction of genes for oxidative phosphorylation and mitochondrial biogenesis and were largely explained by a resveratrol-mediated decrease in PGC-1alpha acetylation and an increase in PGC-1alpha activity. This mechanism is consistent with resveratrol being a known activator of the protein deacetylase, SIRT1, and by the lack of effect of RSV in SIRT1(-/-) MEFs. Importantly, resveratrol treatment protected mice against diet-induced-obesity and insulin resistance.⁵

In some tests, resveratrol has been able to extend the life of *Saccharomyces cerevisiae*,⁶ *Caenorhabditis elegans*, *Drosophila melanogaster*,⁷ and the mammal *Nothobranchius furzeri*. Valenzano states that: "Resveratrol prolongs lifespan and retards the expression of age-dependent traits in short-lived vertebrates."⁸ In Sinclair's experiments of mice he says: "Gene expression analysis indicated the addition of resveratrol opposed the alteration of 144 out of 155 gene pathways changed by the high-fat diet."⁹ Sinclair suggests that insulin and glucose levels are

⁵ Lagouge, M., C. Argmann, Z. Gerhart-Hines, H. Meziane, C. Lerin, F. Daussin, N. Messadeq, J. Milne, P. Lambert, P. Elliot, B. Geny, M. Laakso, and J. Auwerx. *Cell*. 2006. 127.6 1091-093.

⁶ Sinclair, D., S. Lavu, K. T. Howitz, K. J. Bitterman, H. Y. Cohen, D. W. Lamming, J. G. Wood, R. E. Zipkin, A. Kisielewski, L. L. Zhang, B. Scherer, and P. Chung. *Nature*. 2003. 425.6954 191-96.

⁷ Bass, T. M., D. Weinkove, K. Houthoofd, D. Gems, and L. Partridge. *Mechanisms of Ageing and Development*. 2007. 128.10 546-52.

⁸ Valenzano, D. R., E. Terzibasi, T. Genade, A. Cattaneo, L. Domenici, and A. Cellierino. *Current Biology*. 2006. 16.3 296-300.

⁹ Sinclair, D., R. De Cabo, D. K. Ingram, J. A. Baur, K. J. Pearson, N. L. Price, H. A. Jamieson, C. Lerin, A. Kalra, V. V. Prabhu, J. S. Allard, G. Lopez-Lluch, K. Lewis, P. J. Pistell, S. Poosala, K. G.

more controlled in mice supplemented with resveratrol.¹⁰ There have been dozens of studies dealing with anticancer activity although no human trials have been performed yet. It is known that in vitro resveratrol interacts with multiple molecular targets and has positive effects on the cells of breast, skin, gastric, colon, esophageal, prostate, and pancreatic cancer, and leukemia.^{11,12}

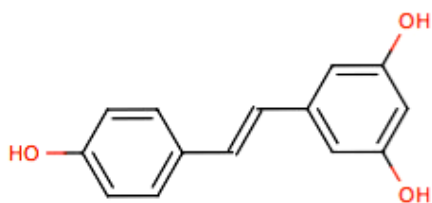


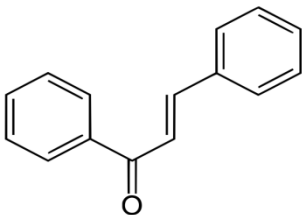
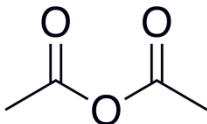
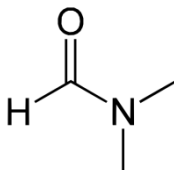
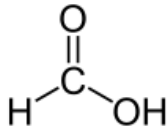
Figure 1: Resveratrol

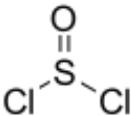
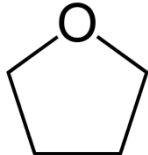
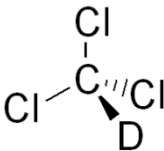
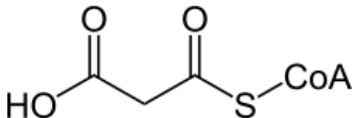
¹⁰ Sinclair, D., S. Lavu, B. Rogina, K. Howitz, S. L. Helfand, and M. Tatar. *Nature*. 2004. 430.7000 686-89.

¹¹ Baur, J. A., and D. A. Sinclair. *Nat Rev Drug Discov* 2006. 5.6 (206): 493-506.

¹² Sinclair, D., R. De Cabo, D. K. Ingram, J. A. Baur, K. J. Pearson, N. L. Price, H. A. Jamieson, C. Lerin, A. Kalra, V. V. Prabhu, J. S. Allard, G. Lopez-Lluch, K. Lewis, P. J. Pistell, S. Poosala, K. G. Becker, O. Boss, D. Gwinn, M. Wang, S. Ramaswamy, K. W. Fishbein, R. G. Spencer, E. G. Lakatta, D. Le Couteur, R. J. Shaw, P. Navas, and P. Puigserver. *Nature*. 2006. 444.7117 337-42.

Definitions

<i>Drosophila melanogaster</i> – Fruit fly.
<i>Caenorhabditis elegans</i> – Worm.
<i>Saccharomyces cerevisiae</i> – Yeast.
<i>Nothobranchius furzeri</i> – Killi Fish
SIRT1 – Sirtuin 1, Gene on Chromosome 10, q21.3. 69.31- 69.35 Mb
Chalcone – Phenyl styryl ketone 
Ac ₂ O – Acetic Anhydride 
DMF – Dimethylformamide 
Formic acid – Methanoic acid 

SOCl ₂ – Thionyl chloride, 118g/mol 
THF – Tetrahydrofuran 
R _f – distance product / distance solvent
CDCl ₃ – Deuterated chloroform 
Malonyl-CoA - C ₂₄ H ₃₈ N ₇ O ₁₉ P ₃ S 
R _f – distance product / distance solvent.

Reactions

The chemical synthesis of resveratrol with the decarbonylative Heck reaction is done in four major steps. The general diarylethene conformation is called a stilbene. Stilbenes are hydrocarbons consisting of a *trans* or *cis* ethene double bond substituted with a phenyl group on both carbon atoms of the double bond. Resveratrol is a 3,4',5-stilbenetriol. There are many reactions used to synthesize the chemical but the most commonly used synthesis is the decarbonylative Heck reaction (see fig. 7).

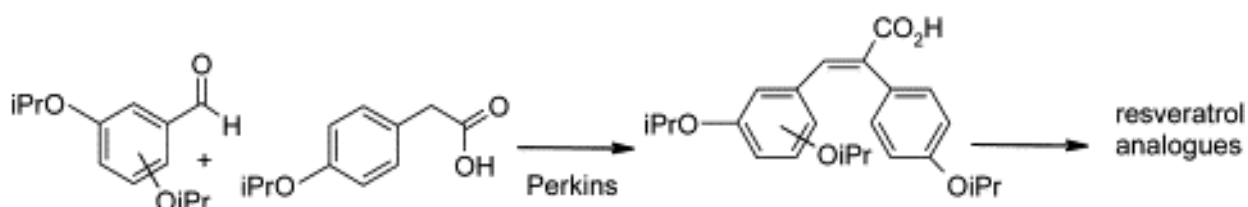


Figure 2. The Perkin Reaction.

The Heck reaction is the chemical reaction of an unsaturated halide with an alkene and strong base with a palladium catalyst to form a substituted alkene. Aside from the Heck reaction the Perkin reaction is another popular organic reaction used. The Perkin reaction (Fig. 2) is achieved via the aldol condensation of aromatic aldehydes and acid anhydrides in the presence of an alkali salt of the acid. It is considered to be greener and requires fewer steps with an average percent yield of 70%. Resveratrol can also be biosynthetically created with enzymes by starting with 4-Coumaroyl-CoA and adding Malonyl-CoA three times and looping the chain into a six-ring configuration (see fig. 3). All plants possess these two molecules and are used in a similar fashion to make chalcone, the precursor for ubiquitous flavonoids and anthocyanins (Schröder).

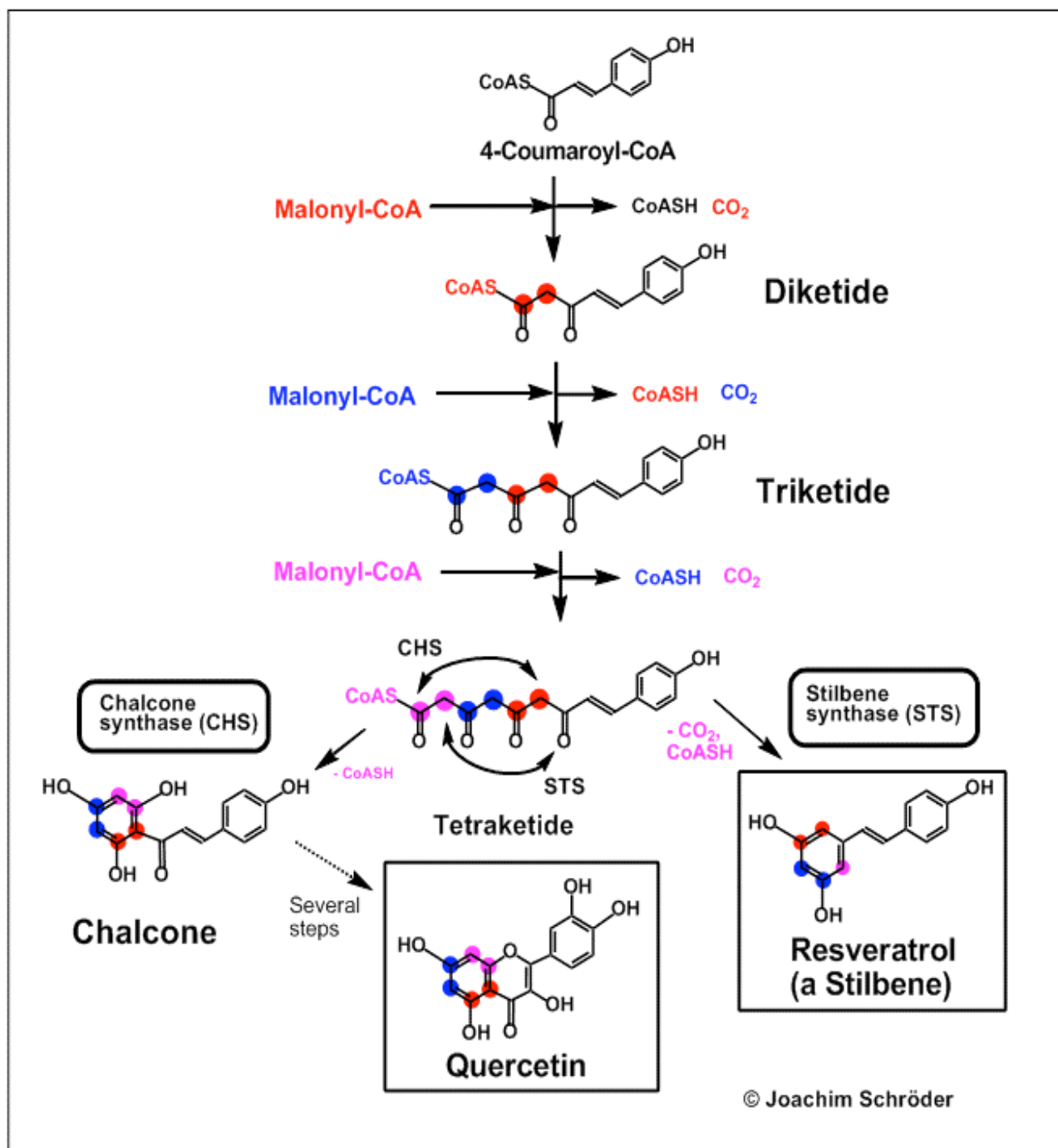


Figure 3: A biosynthesis of resveratrol, chalcone, and quercetin done naturally in plants.¹³

¹³ Schröder, G., J. Schröder, J. Fliegmann, L. Schröder, S. Raiber, S. Tropsch, and T. Lanz. *Websites of the Schröder Group*. 2007.

The Wittig reaction can also be used to synthesize resveratrol as seen in figure 4. Unfortunately, the Wittig couplings that give the mixtures of olefin isomers require 7–8 steps and are tedious.

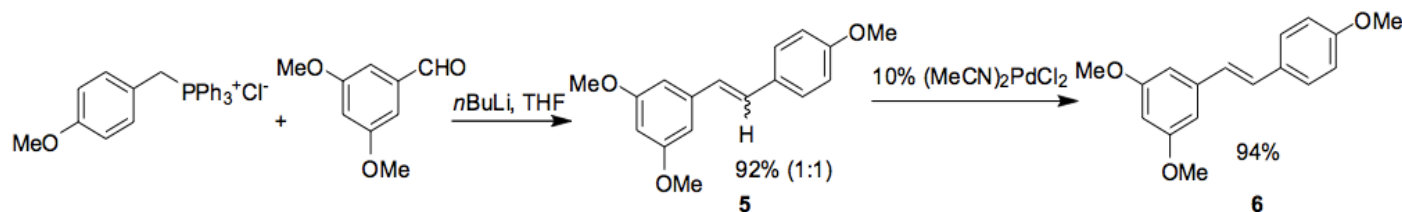


Figure 4: The Wittig reaction to synthesize resveratrol.

A vinylsilane Heck based reaction can be used by coupling vinyltrimethylsilane with 4-methoxyiodobenzene and using methyl ether protecting groups; this is shown in figure 5.

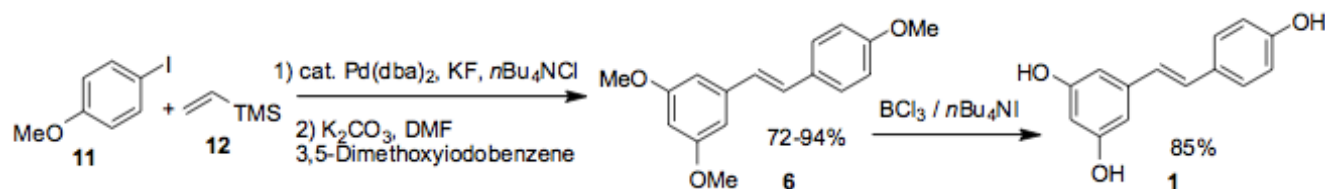


Figure 5: A vinylsilane Heck reaction to synthesize resveratrol.

One final way considered to synthesize resveratrol was the optimized Horner–Emmons approach. This reaction is also long, requiring seven steps with poor yields, and the starting materials are costly.

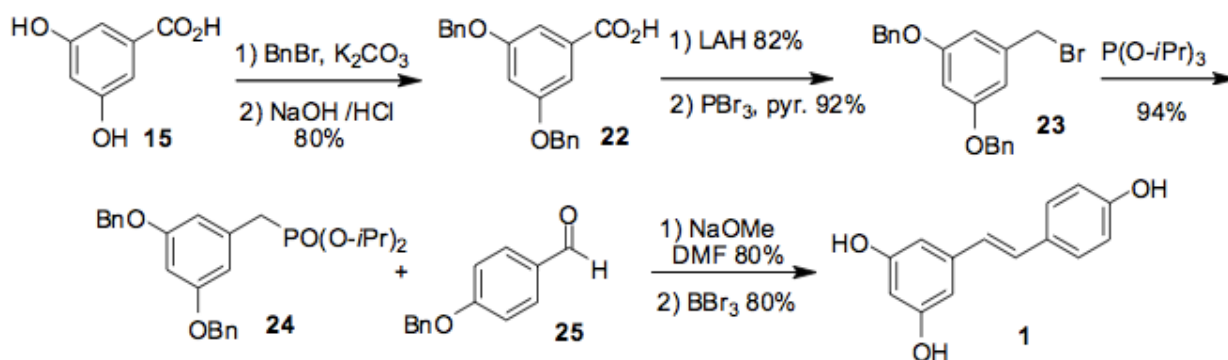


Figure 6: The optimized Horner–Emmons reaction to synthesize resveratrol.

Principles

The selected Heck reaction (see fig. 7) starts with the component 3,5-dihydroxybenzoic acid(2) which in turn is reacted with acetic anhydride in the presence of pyridine and then reacted with aqueous formic acid. To 3,5-diacetoxybenzoic acid, benzene, DMF, and thionyl chloride are added. Thionyl chloride (SOCl_2) is used to convert the protected acid to the acid chloride. This yields 3,5-diacetoxybenzoyl chloride(3) which is then reacted with 4-acetoxystyrene(4) in the presence of *N,N*-bis-(2,6-diisopropyl-phenyl)-4,5-dihydro imidazolium chloride(5) and palladium acetate $\text{Pd}(\text{OAc})_2$ catalyst in *p*-xylene. The Heck reaction involves the use of a palladium catalyst to form a substituted alkene. Resveratrol triacetate(6) is then mixed with THF (Tetrahydrofuran) and sodium hydroxide. The product is washed with water and brine and dried over sodium sulfate (Na_2SO_4) to yield resveratrol (1).

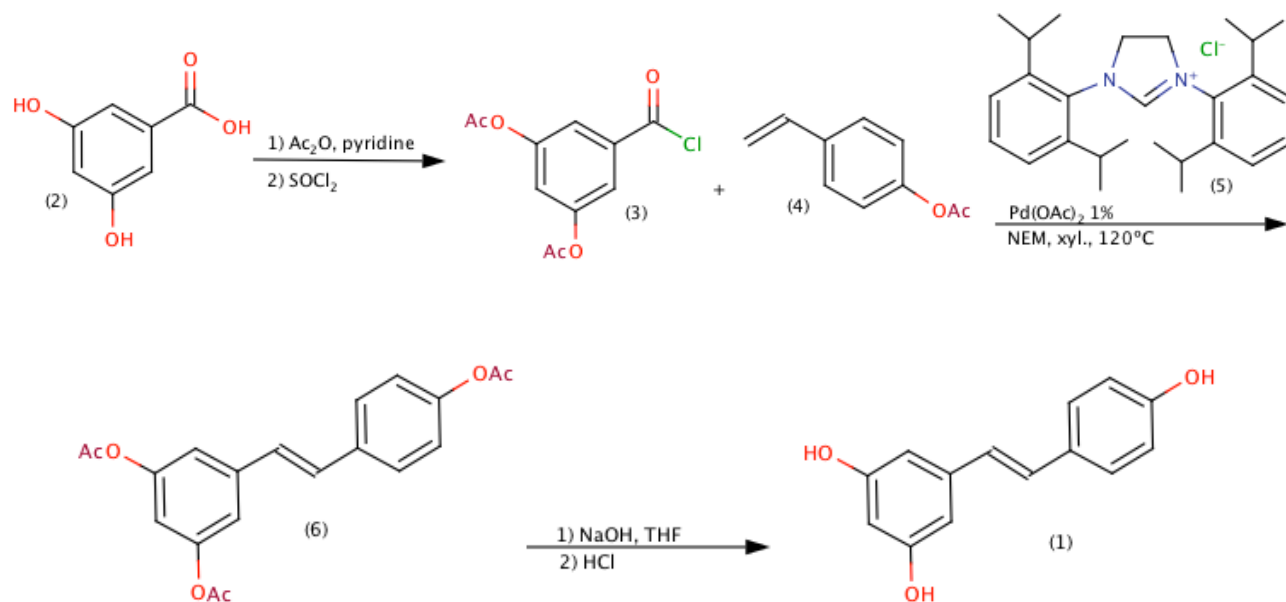


Figure 7: Synthesis of resveratrol using decarbonylative Heck reaction.

The original volumes and amounts of chemicals used in the reaction performed by Dr. Liu are as follows:

3,5-dihydroxybenzoic acid	7.71 g	Palladium acetate	62.86 mg
Acetic anhydride	12.25 mL	3,5-diacetoxybenzoyl chloride	7.19 g
Pyridine	8.08 mL	4-acetoxystyrene	5.35 mL
3,5-diacetoxybenzoic acid	8.00 g	<i>N</i> -ethyl morpholine	4.2 mL
Thionyl chloride	16 mL	Resveratrol triacetate	6.02 g
<i>p</i> -xylene	56 mL		

Table 1: The original volumes used by Dr. Liu in his experiments.

These are taken and considered to be 100% volume from which my amounts are based upon.

Chemicals and Equipment

The chemicals and supplies used throughout the reaction include:

3,5-dihydroxybenzoic acid, ethyl acetate, acetic anhydride, pyridine, formic acid, brine, sodium sulfate, *n*-heptane, *n*-hexane, benzene, dimethylformamide, thionyl chloride, toluene, *p*-xylene, *N,N*-bis-(2,6-diisopropylphenyl)-4,5-dihydro imidazolium chloride, 4-acetoxystyrene, *N*-ethyl morpholine, hexanes, sodium hydroxide, tetrahydrofuran, DMSO, chloroform, hydrochloric acid, potassium bromide, silica gel, sand, and cotton wool.

Equipment used: a fully charged nitrogen tank, 50-mL, 25-mL, and a 15-mL 3-necked round-bottomed flask with attachments, glass disposable pipettes, automatic pipette and tips, assorted Erlenmeyer flasks and beakers, side-arm Erlenmeyer flasks, stoppers, Hirsch funnel, rubber hosing, hot plate, bubble tubing, magnetic stir bars, ice machine, flash chromatography columns, pellet press, FTIR, and NMR.

RESULTS AND DISCUSSION

Grignard reaction

p-anisaldehyde + benzyl magnesium chloride → stilbene

A TLC done on the crude product with 50:50 ethyl acetate/hexane showed two spots. R_f 's were 0.714 and 0.952. This indicates that there is a major impurity, and is considered to be starting material. The IR spectra (see Fig. 8) showed characteristics of a stilbene; aromatic alkenes at 1600 cm^{-1} , carbonyl at 1700 cm^{-1} , a broad OH peak at 3350 cm^{-1} indicating OH stretch frequency, and alkene peaks at 2900 cm^{-1} , aromatic and aliphatic C-H from $2800 - 3100\text{ cm}^{-1}$. Because the product was unpurified there was starting materials found in the crude IR. At lower wavenumbers the IR fingerprint had many peaks. Once the crystals were purified the overall mass decreased significantly, indicating that the reaction was fairly unsuccessful. An NMR was run (see fig. 10) NMR ^1H (CDCl_3 , 300MHz) δ 9.85 singlet, 7.85 doublet, 7.28 multiplet, 7.05 doublet, 6.9 doublet, 4.85 triplet, 4.64 doublet, 3.8 singlet (solvent), 3.1 doublet, 3.0 singlet, 2.0 broad peak. The two IR spectra (Fig. 8 and Fig. 9) collected were almost identical, proving that the two trials were precise but not accurate. This part of the experiment could be improved if a bromine halide could be used in the Grignard reaction instead of a chlorine halide. The benzyl chloride was probably impure. It is important that the Grignard is done with pure reagents. If the *p*-anisaldehyde could be added more slowly and heated gently to prompt the reaction to flux it would not react so violently all at one time. Another change suggested would be to do the recrystallization with ligroin and ethyl acetate instead of isopropyl alcohol, toluene, or diethyl ether.

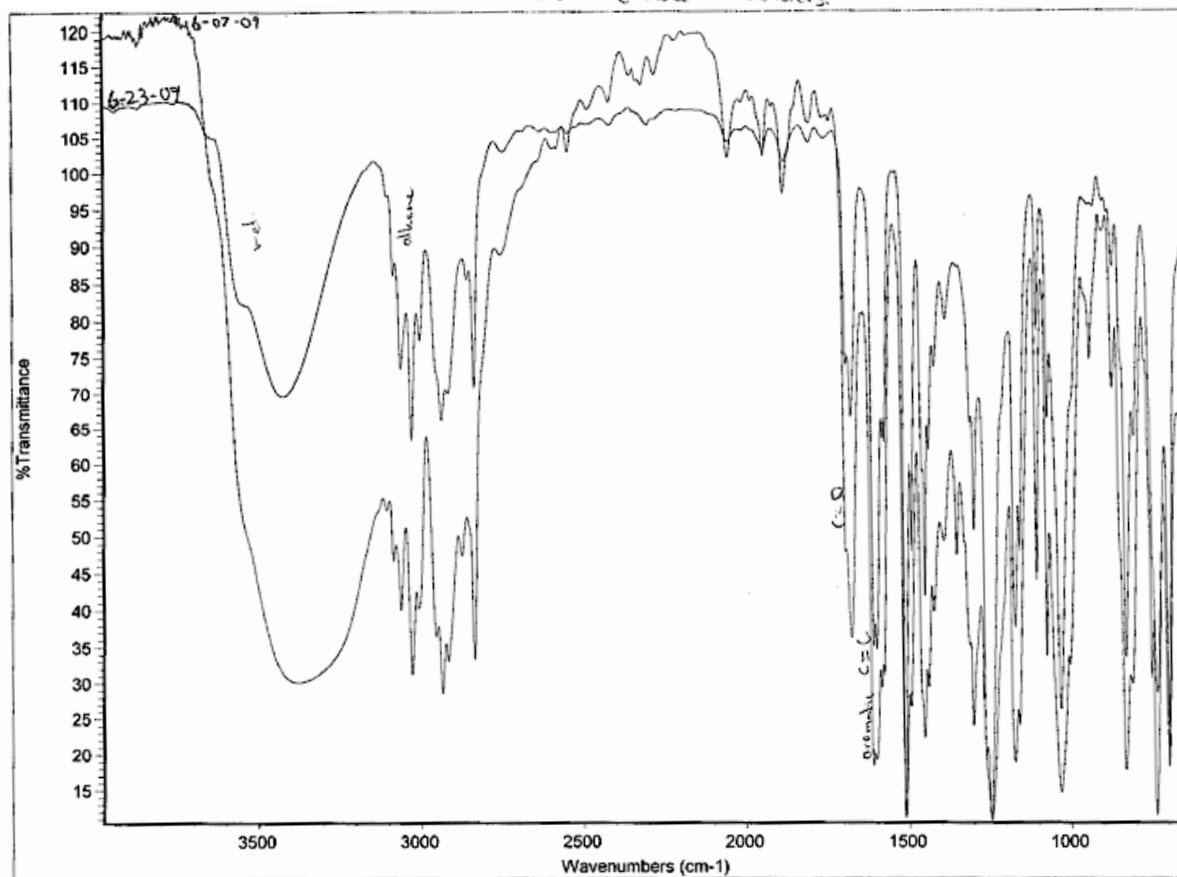


Figure 8: The IR spectra of trial 1 and 2, crude products from the Grignard reaction.

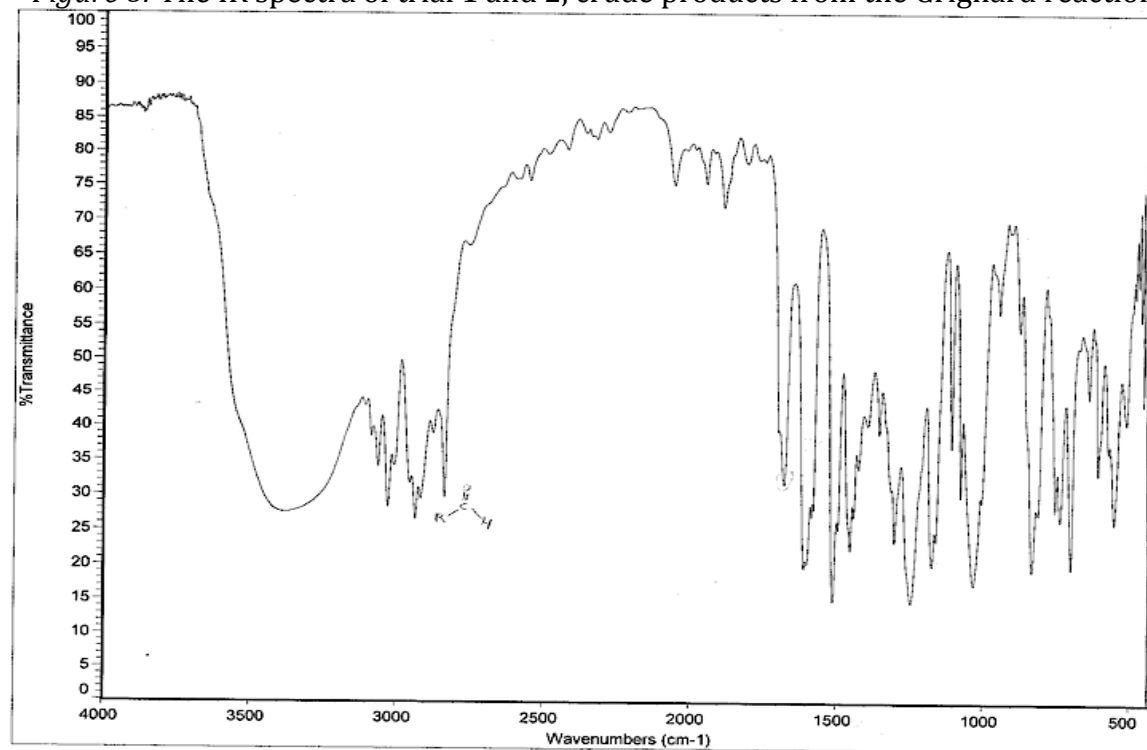


Figure 9: The IR of Trial 3 crude product of the Grignard reaction.

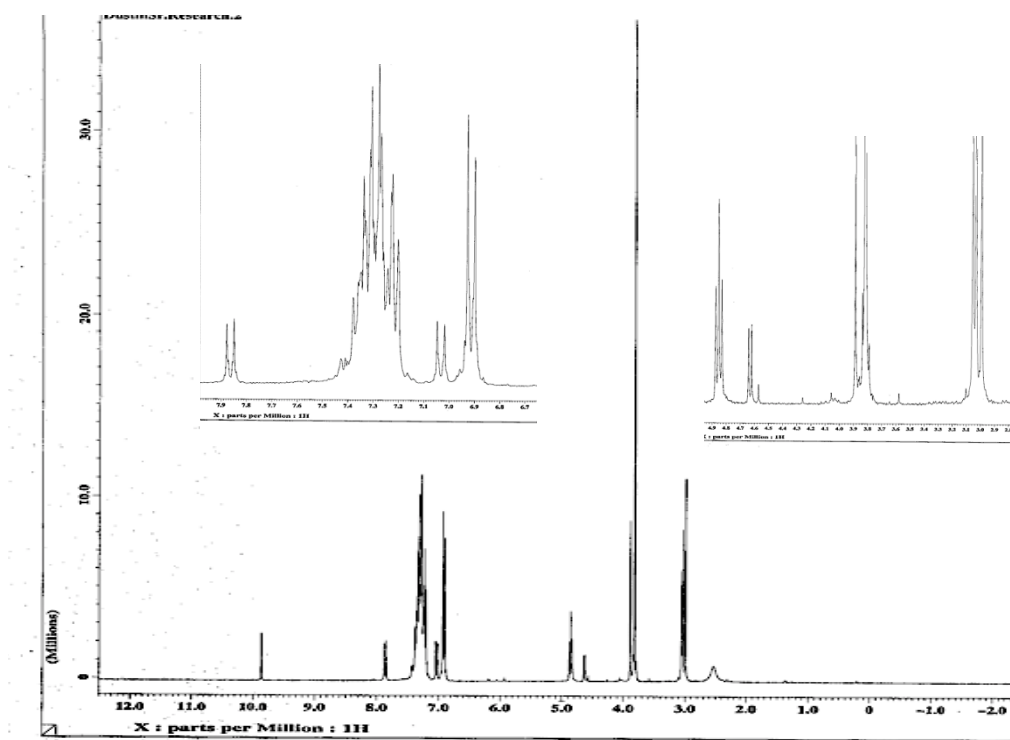


Figure 10: The NMR spectrum of the crude products from the Grignard reaction.

The Heck synthesis

Step one: 3,5-dihydroxybenzoic acid → 3,5-diacetoxybenzoic acid

The melting point obtained from the purified crystals was 161-162 °C, identical to that of 3,5-diacetoxybenzoic acid¹⁴. The IR(see fig. 11) showed para-substituted aromatic hydrocarbons at 1650 cm⁻¹, terminal vinyl olefins at 1600 cm⁻¹, an OH band in the 3400 cm⁻¹ area, and carbonyl peak at 1700 cm⁻¹. The desired compound was achieved in step one; however, only a 48% yield was attained with the first trial. After the fourth trial many improvements had been made and percent yield increased to 85%. Future improvement with this step could include: keep bubbling nitrogen into the reacting solution the entire time, finding a better way to seal the openings and making them airtight, and a more effective way to purify the crystals.

¹⁴ Liu, Jing. PhD. paper. 2007. Brigham Young University.

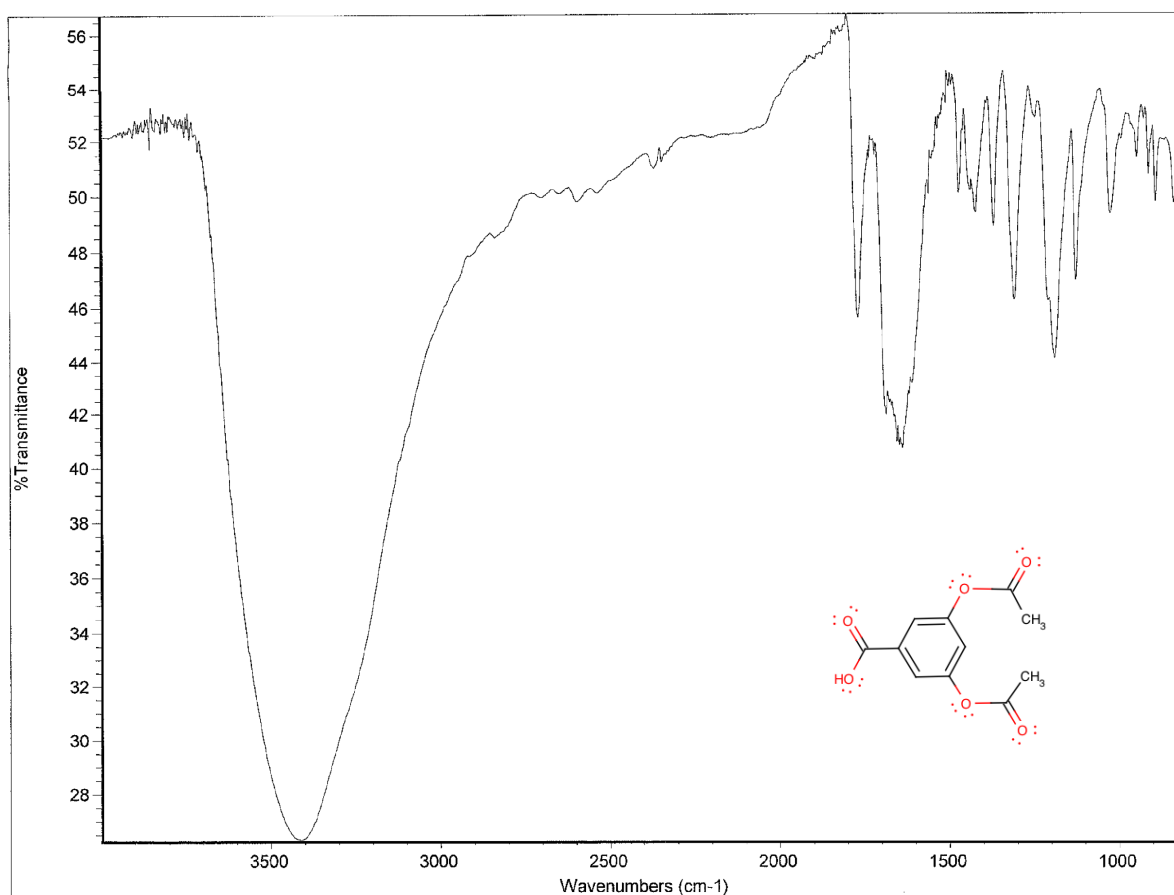


Figure 11: The IR spectrum of 3,5-diacetoxybenzoic acid (Step 1)

Step two: 3,5-diacetoxybenzoic acid → 3,5-diacetoxybenzoyl chloride

The NMR of the first trial did not compare very well to the known NMR spectrum of 3,5-diacetoxybenzoyl chloride. A TLC performed on the two different trials along with the original starting product revealed that the first trial did not react completely; however, the second trial did react. The R_f data: Starting product 0.277, first trial 0.238 and 0.481, second trial 0.530. The NMR of the second trial showed that the singlet, doublet and triplet were obtained that were in Dr. Liu's findings. Data: NMR ¹H (CDCl₃, 300MHz) δ 7.74 (d, J=2.19 Hz, 2H), 7.28 (t, J=2.82 Hz, 1H), 2.31 (s, 6H); ¹³C NMR (CDCl₃, 300MHz) δ 59.7. The TMS peak was very strong and had ringing sidebands. Unfortunately only one peak, besides the solvent peak, was found in the carbon 13 NMR. A possibility for this could be

that the 3,5-diacetoxybenzoyl chloride did not completely dissolve in the CDCl_3 . A flame test was done to test for the chlorine; the green colored flame proved that the chlorine halide had been acquired. The IR of the first trial had a large OH band in the $3000\text{--}3500\text{ cm}^{-1}$ region, meaning that the product was probably still wet. The IR of the second trial had a broad band in the $3000\text{--}3500\text{ cm}^{-1}$ region probably from H_2O , an ester peak at 1750 cm^{-1} and another strong peak at 1200 cm^{-1} . There were C-H aliphatic bonds at 2900 cm^{-1} but these peaks are from the diethyl ether solvent that was used (see fig. 13). Percent yield was calculated to be 45%. The melting point of the first trial ranged from $89\text{--}120\text{ }^\circ\text{C}$. The second trial had a more accurate melting point that ranged from $89\text{--}95\text{ }^\circ\text{C}$. Literature says it melts at $89.5\text{--}91\text{ }^\circ\text{C}$.

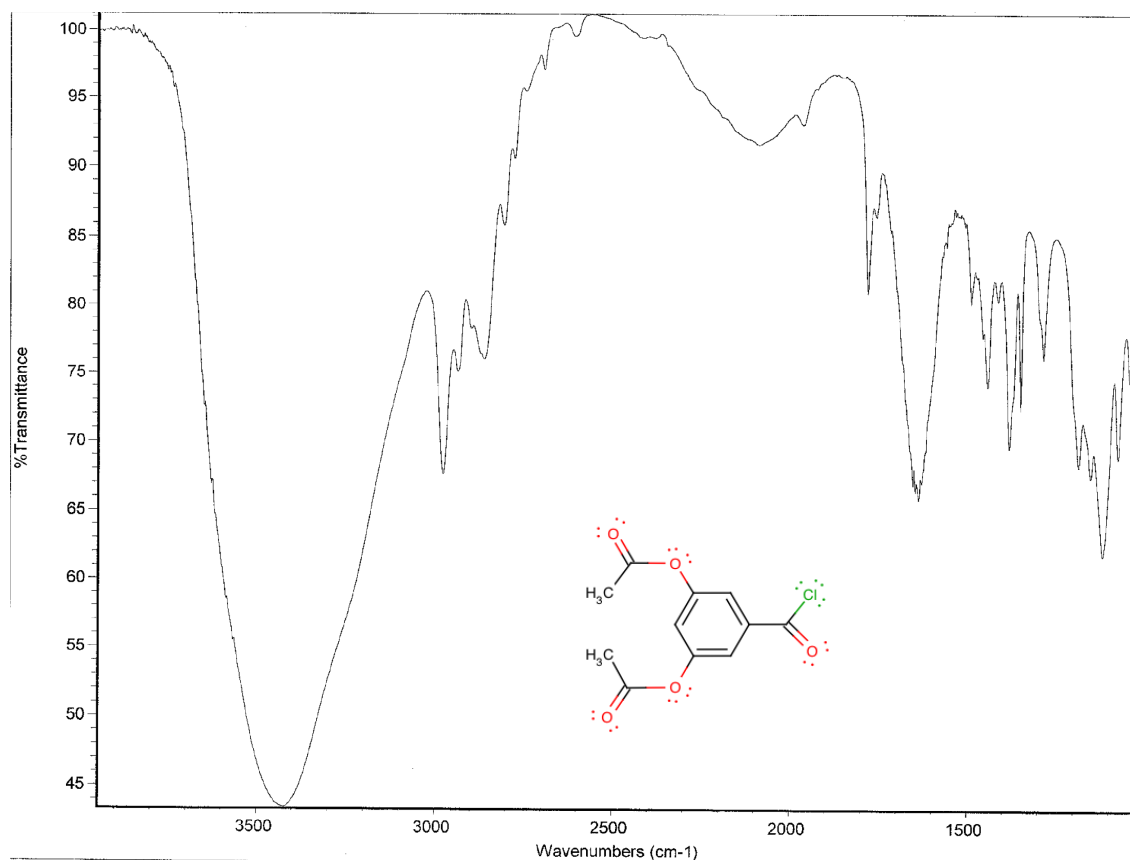


Figure 12: The IR of step two purified product, 3,5-diacetoxybenzoyl chloride

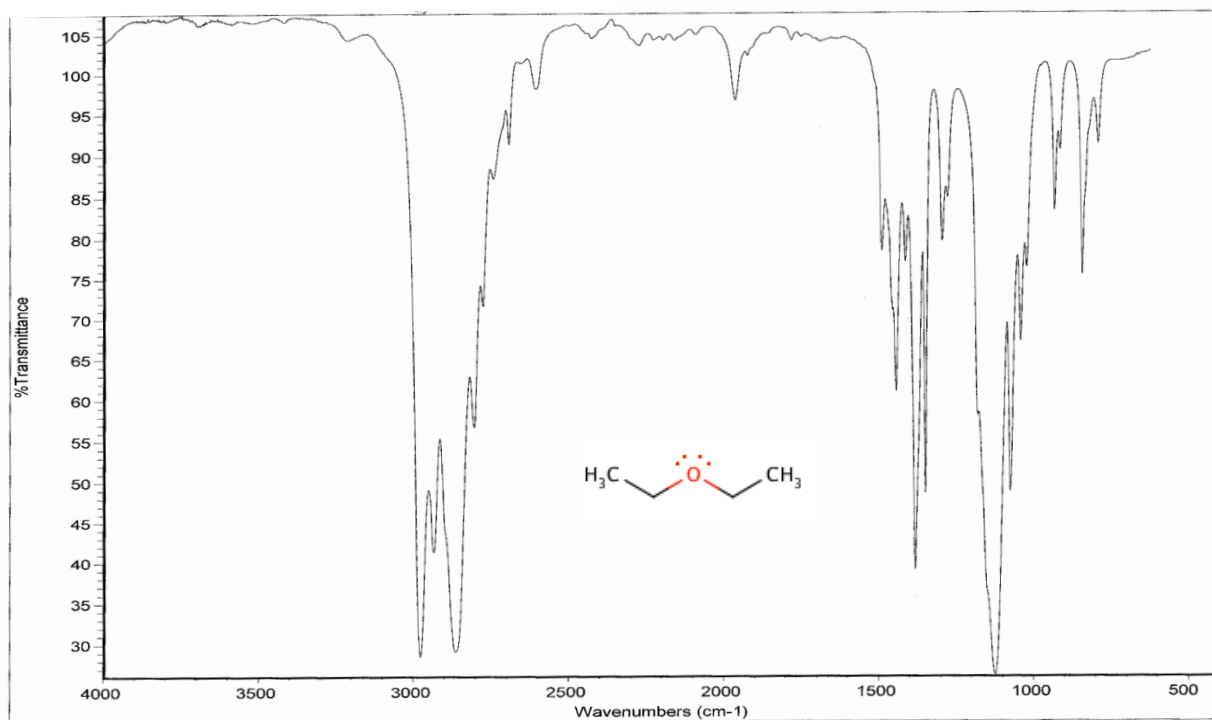


Figure 13: The IR of diethyl ether, solvent used to dissolve 3,5-diacetoxybenzoyl chloride, the product of step two.

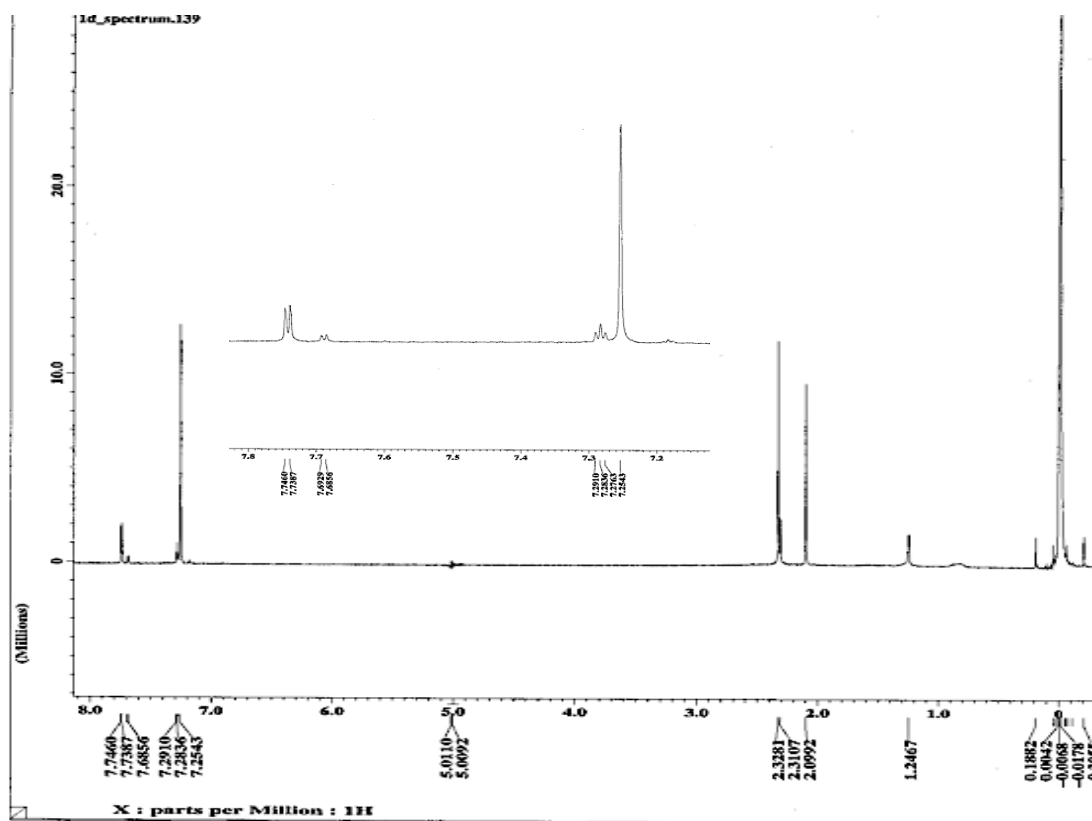


Figure 14: The NMR of purified step 2 product, 3,5-diacetoxybenzoyl chloride.

Step three: 3,5-diacetoxybenzoyl chloride → Resveratrol triacetate.

The percent yield of this step was poor, and thus a recrystallization of the crude product was not done, which therefore made analysis of the yellow crystalline oil difficult. Because there was so little compound recovered in the crude form it was feared that by purifying it the amount would significantly decrease. The IR of the yellow crystalline oil from trial one and trial two were identical (spectra not included). Each had aromatic and aliphatic C-H bonds between 3100cm^{-1} and 2900cm^{-1} , an ester peak at 1750cm^{-1} , carbon double bonds C=C at 1600cm^{-1} , and an ester C-O-C peak at 1200cm^{-1} . Because the starting material, 3,5-diacetoxybenzoyl chloride, was from different previous trials it was surprising that the IR of each trial was so similar. The similarity was so close that it was thought that the product might still have been on the column, but because the IR matches that of what resveratrol triacetate presumingly would be, the yellow oily crystals were considered to be the desired product.

Trials	Rf value
Starting product	0.28
Trial 1	0.23 and 0.48
Trail 2	0.53
Trial 3	0.50

Table 2: The different R_f's from each trial of step 3.

All the remaining compound of 3,5-diacetoxybenzoyl chloride was used in the third trial, which was 8.5% original volume. The IR(Fig. 17) from trial one and two revealed that the same compound had been recovered. The ^1H NMR spectrum(Fig. 15) confirmed that the desired product was attained. Data: NMR ^1H (CDCl_3 , 300MHz) δ 7.72 (d, $J=2.19\text{ Hz}$, 3H), 7.25 (s, 1H), 7.19 (t, $J=2.19\text{ Hz}$, 3H), 2.29 (s). The ^{13}C NMR(see fig. 16) data: NMR ^{13}C (CDCl_3 , 300MHz) δ 170, 168, 151, 131, 121, 77, 21. Recovery was about 20%.

1d_13c_spectrum.47

Chemical shift (ppm): 220.0, 210.0, 200.0, 190.0, 180.0, 170.0, 160.0, 150.0, 140.0, 130.0, 120.0, 110.0, 100.0, 90.0, 80.0, 70.0, 60.0, 50.0, 40.0, 30.0, 20.0, 10.0, 0.0, -10.0, -20.0

Intensity (Arbitrary Units): 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4

Peak labels (ppm): 170.1722, 168.8973, 151.0947, 131.4523, 121.1158, 120.9249, 77.5330, 77.1131, 76.6856, 21.1021, 0.0779

Figure 16: The Carbon 13 NMR spectrum of Resveratrol Triacetate, product of step 3.

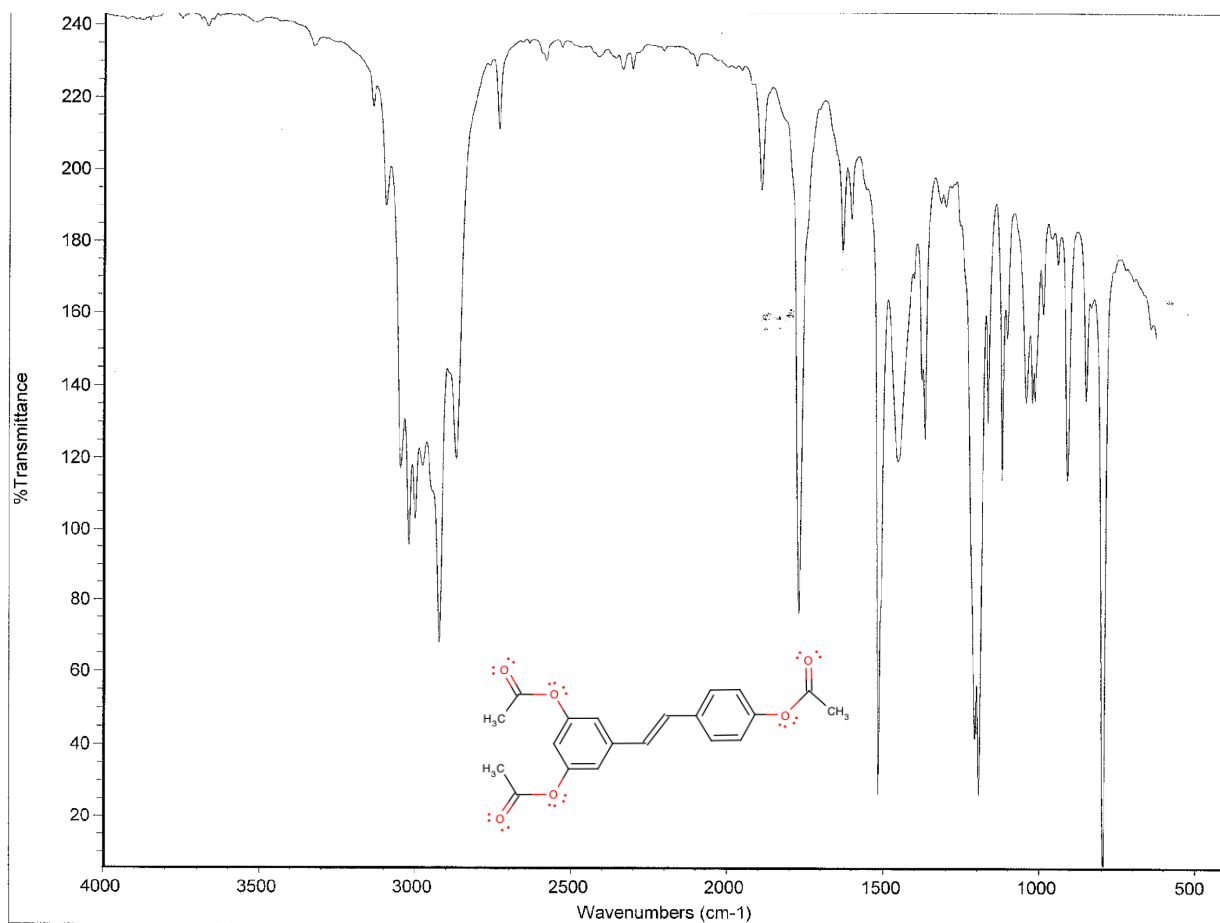


Figure 17: The IR of Resveratrol Triacetate, product of step 3.

Dr. Jing Liu's NMR data was: ^1H NMR (CDCl_3 , 300 MHz) δ 7.50 (d, $J = 9.0$ Hz, 2H), 7.12-6.94 (m, 6H), 6.82 (t, $J = 2.1$ Hz, 1H), 2.31 (s, 9H); ^{13}C NMR (CDCl_3 , 75 MHz) δ 169.6, 169.2, 151.5, 150.6, 139.7, 134.7, 129.9, 127.9, 127.4, 122.1, 117.1, 114.6, 21.4.¹⁵

During this step there was low recovery of the product with maybe only a 20% yield. Melting point was 115-130 °C. Literature says that resveratrol triacetate melts at 116-118 °C.¹⁵ This step could be greatly modified and many improvements would help yield more of the desired product. The first change suggested would be to try regular column chromatography instead of flash chromatography. Instead of just a single solvent

¹⁵ Liu, Jing. PhD. paper. 2007. Brigham Young University.

being used to push the product through the column, a varying polarity solvent could be used and the elute should be collected in fractions, preferably in small Erlenmeyer flasks. If flash chromatography is used it is vital that the column never gets dry, it is essential that while pushing the solvent through the column with pressurized nitrogen that the solvent does not go below the level of the sand. In the experiment, white crystals were seen on the inside of the round bottomed flask after it was removed from the sand bath and allowed to cool down to room temperature. In future experiments this should be noted and these crystals should be dissolved and put onto the column too. To help identify and collect the resveratrol triacetate coming off the chromatography column UV-Vis spectrometry could be used. This addition would mean that flash chromatography could be replaced with HPLC and a varying polarity solvent. Here it should be noted that resveratrol absorbs UV light at λ_{max} : 218, 235, 307, 321 nm.

Step four: Resveratrol triacetate → Resveratrol

Trial 1: A TLC was done with four different solvents, ranging from pure ethyl acetate to 25:75 ethyl acetate and hexanes. The R_f's and solvents are listed in Table 3. Unfortunately the spots had long tails and were streaked up the silica plates, even when the concentration was lowered the smears/tails still appeared.

Ethyl Acetate to Hexanes	R _f range
25:75	0.11
50:50	0.18→0.52
75:25	0.30→0.72
100% ethyl acetate.	0.40→0.63

Table 3: The Solvents used and R_f's associated with resveratrol.

The IR spectrum(see fig. 18) of trial 1 did not look promising; there was aliphatic C-H stretching at 2950 cm^{-1} , broad OH peak at 3300 cm^{-1} , carbonyl at 1730 cm^{-1} , and possibly an alcohol and/or ester C-O stretch at 1150 cm^{-1} . A possible solution why resveratrol was not obtained is because trial 1 was performed from the crude yield of step 3 and therefore had some impurities that could have altered the reaction mechanism. Another possibility was that not nearly enough NaOH was added to convert the acetate groups into alcohols.

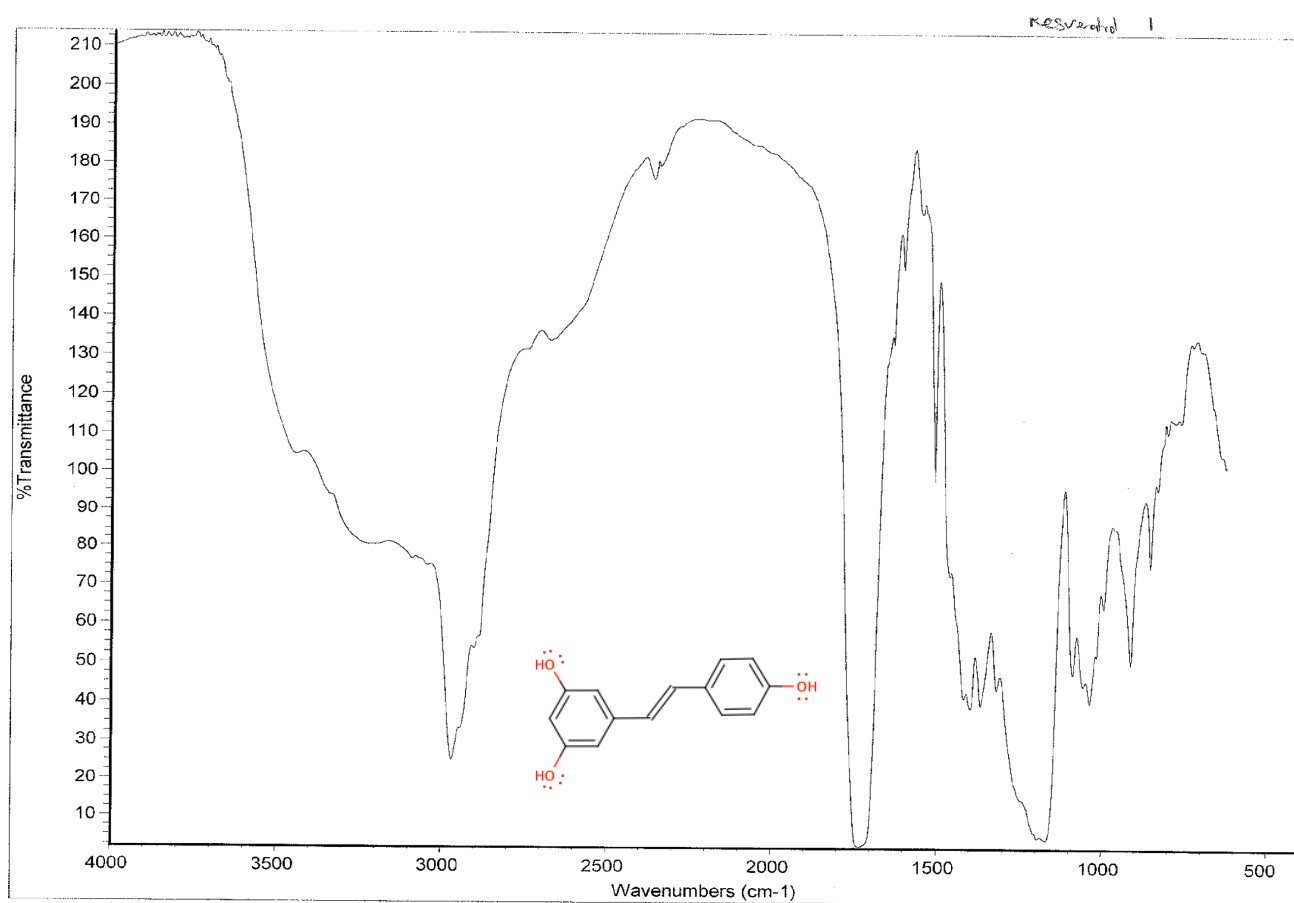


Figure 18: The crude IR spectrum of Resveratrol. Product of step four.

Trial 2 of the fourth step yielded slightly better results from the improvements made to trial 1. The IR (see fig. 19) had a larger OH band in the 3500 cm^{-1} region, the

aliphatic C-H stretching at 2950 cm^{-1} was still present, probably from some unreacted CH_3 on the resveratrol triacetate or the acetyl (protective) groups that were on resveratrol triacetate that were cleaved off. Unfortunately, there was still an ester $\text{O}-\text{C}=\text{O}$ peak at 1750 cm^{-1} most probably from the waste acetyl groups. The large OH band did indicate that some of the product was converted into resveratrol, but the ester peak and C-H stretching shows that either the entire product did not react, not all the ethyl acetate evaporated from the product, or the acetyl groups are still present with the crude product.

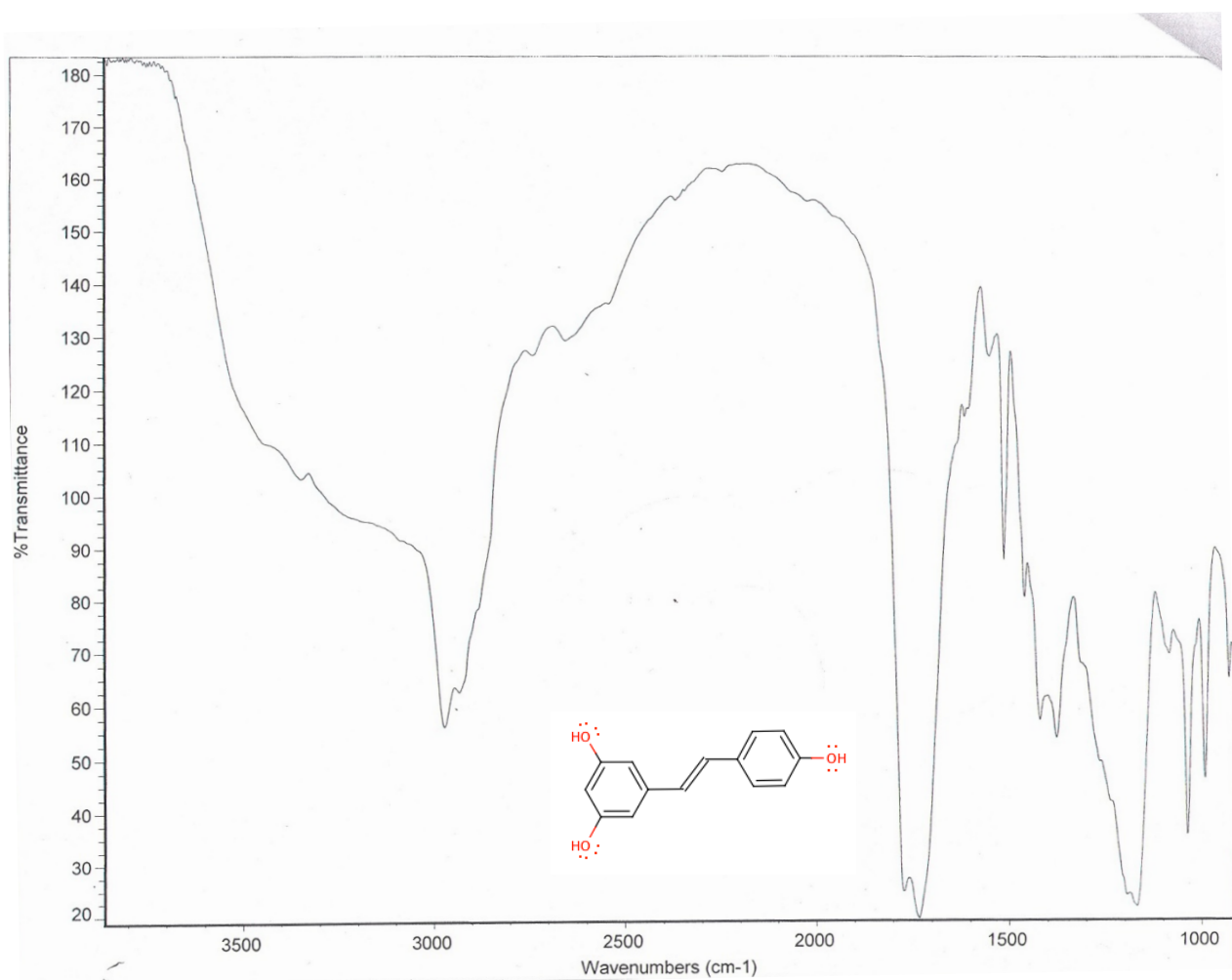


Figure 19: The IR spectrum of resveratrol, trial 2 of step 4.

Before the NMR of the product was collected the NMR of just the solvent, *d*-acetone, was run to test purity of it. The ^1H NMR of *d*-acetone yielded two peaks; a doublet at 2.9 Hz and a multiplet at 2.1 Hz; this shows that the *d*-acetone was slightly impure and should be considered when looking at the products NMR spectrum. The product's ^1H NMR spectrum (Fig. 20) had many random peaks, probably caused from the impurities from the crude product. NMR ^1H (*d*-acetone, 300MHz) δ 7.5 (d, $J=8.8$ Hz, 3H), 7.2 (d, $J=8.8$ Hz, 3H), 5.8 (s, 1H), 5.7 (s, 1H), 4.3 (t, $J=12.6$ Hz), 4.0 (m, $J=25.2$ Hz), 2.55 (m, $J=29.3$ Hz), 0.9 (t, $J=7.41$ Hz). Several of the up-field peaks were identified as solvents and impurities. The carbon NMR was difficult to collect because the gain had to be manually set because of the weak concentration of the solute in acetone, $\text{OC}(\text{CH}_3)_2$. The carbon NMR(see fig. 21) data: NMR ^{13}C (*d*-acetone, 300MHz) δ 205, 127, 85, 70, 68, 65, 55, 30, 25, 10.

A new NMR spectrum was obtained by running the product in *d*-chloroform. The product did not dissolve easily in chloroform, but with a little heating and ultrasonication it did dissolve. The data was: NMR ^1H (*d*-chloroform, 300MHz) δ 7.4 (d, $J_{\text{trans}}=8.79$ Hz, 2H), 7.25 (s, 3H), 7.0 (d, $J_{\text{trans}}=8.79$, 2H), 6.7 (d, $J=17.58$ Hz, 1H), 6.6 (d, $J=17.58$ Hz, 1H), 5.6 (d, $J=17.58$ Hz, 2H), 5.2 (d, $J=10.44$ Hz, 2H), 4.3 (t). The peaks up-field are considered to be impurities and are not reported here but can be seen on the spectrum. A spectrum found in a journal online¹⁶ gives the following data for 3,5,4'-trihydroxystilbene: NMR ^1H (CD_3COCD_3 , 270MHz, Me_4Si) δ 8.53 (s, 2H), 7.42 (d, $J=8.6$ Hz, 2H), 7.06 (d, $J=16.4$ Hz, 2H), 6.89 (d, $J=16.4$ Hz, 1H), 6.89 (d, $J=8.6$ Hz, 2H), 6.57 (d, $J=2.2$ Hz, 2H), 6.30 (t, $J=2.2$ Hz, 1H). In a comparison between my NMR data and that found online, it can be seen that our

¹⁶ Jeffery, Tuyet, and Benoit Ferber. 2003. Tetrahedron Letters 44. 193-97.

peaks and coupling constants are similar, proposing that resveratrol was obtained in low yields and poor concentration in the NMR tube.

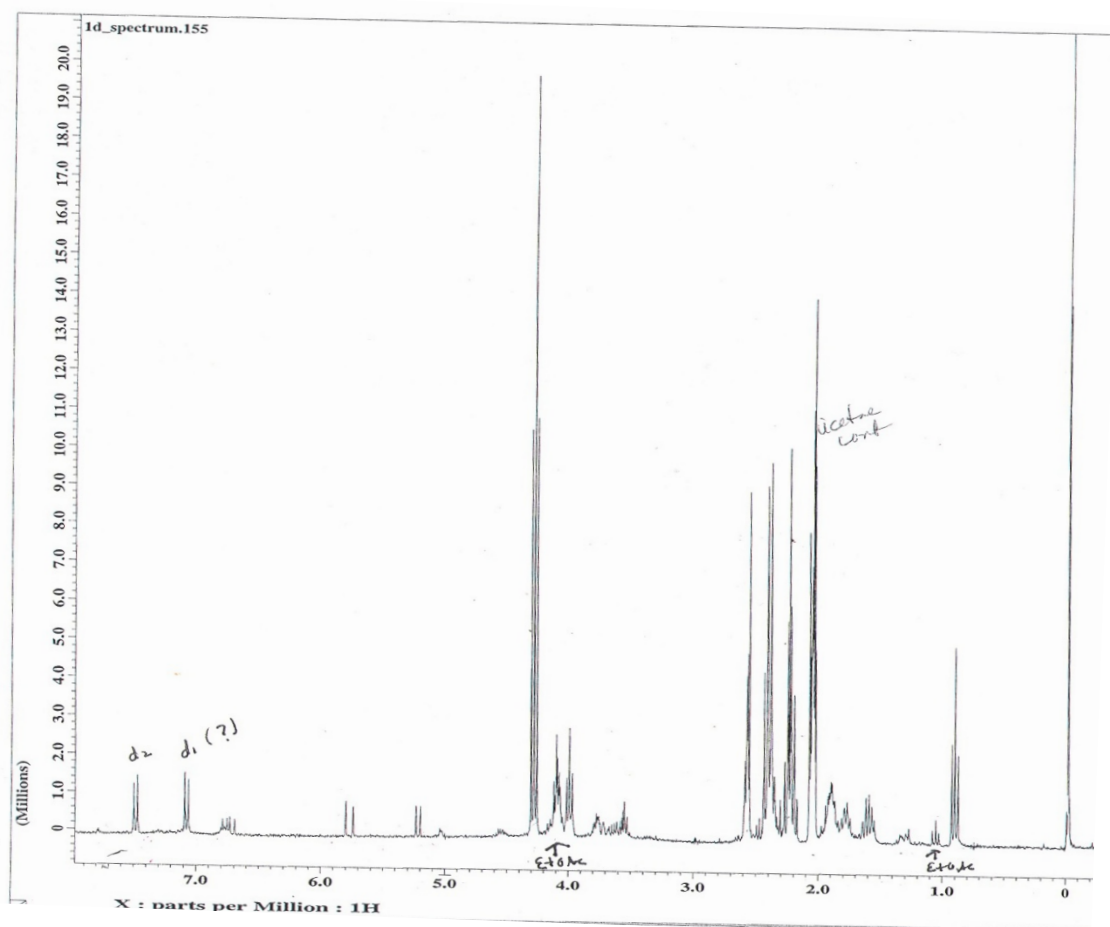


Figure 20: The ^1H NMR spectrum of resveratrol, trial 2 of step 4.

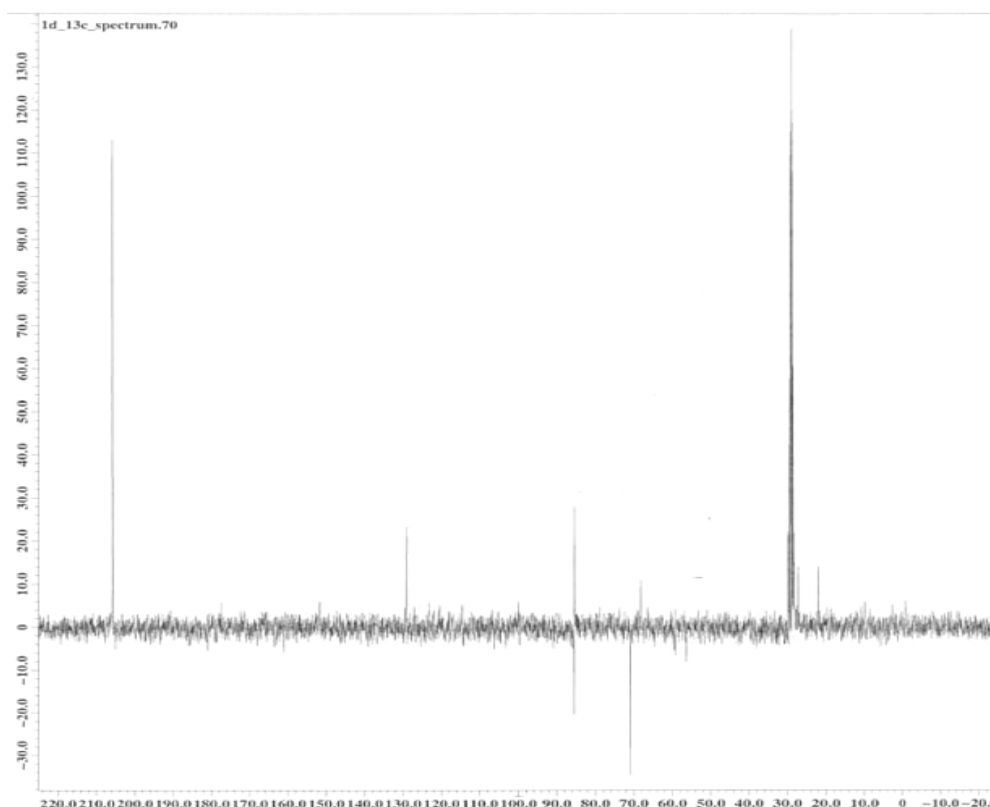


Figure 21: The ^{13}C NMR spectrum of resveratrol, trial 2 of step 4.

A small sample of resveratrol (98%, 100 mg) was purchased from VWR.

This sample was tested in the NMR, IR and its melting point was determined. The melting point was found to be 259 °C. The NMR sample was first run in *d*-chloroform, but resveratrol is insoluble in chloroform, so it was run again in $\text{DMSO-}d_6$. The spectrum of resveratrol in $\text{DMSO-}d_6$ can be seen in figure 22. The data was: NMR ^1H (*d*-DMSO, 300MHz) δ 9.55 (s, 1H), 9.19 (s, 2H), 7.37 (d, $J_{\text{trans}}=8.52$ Hz, 2H), 6.95 (d, $J=16.2$, 1H), 6.80 (d, $J=16.4$ Hz, 1H), 6.73 (d, $J_{\text{trans}}=8.52$ Hz, 2H), 6.36 (d, 2.2 Hz, 2H), 6.0 (t, $J=2.2$ Hz, 1H), 5.75 (s). The purchased resveratrol's IR spectrum can be seen in figure 23. Data is: large OH band at 3400 cm^{-1} , an arene 1600 cm^{-1} from C=C ring stretching.

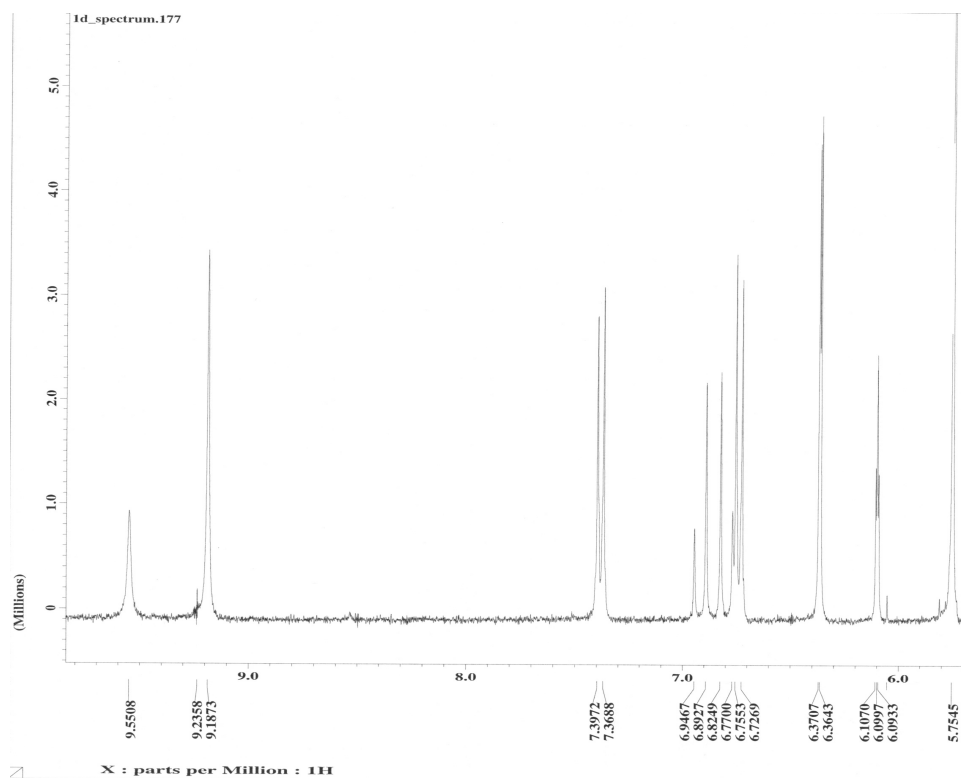


Figure 22: The purchased resveratrol 98% NMR spectrum.

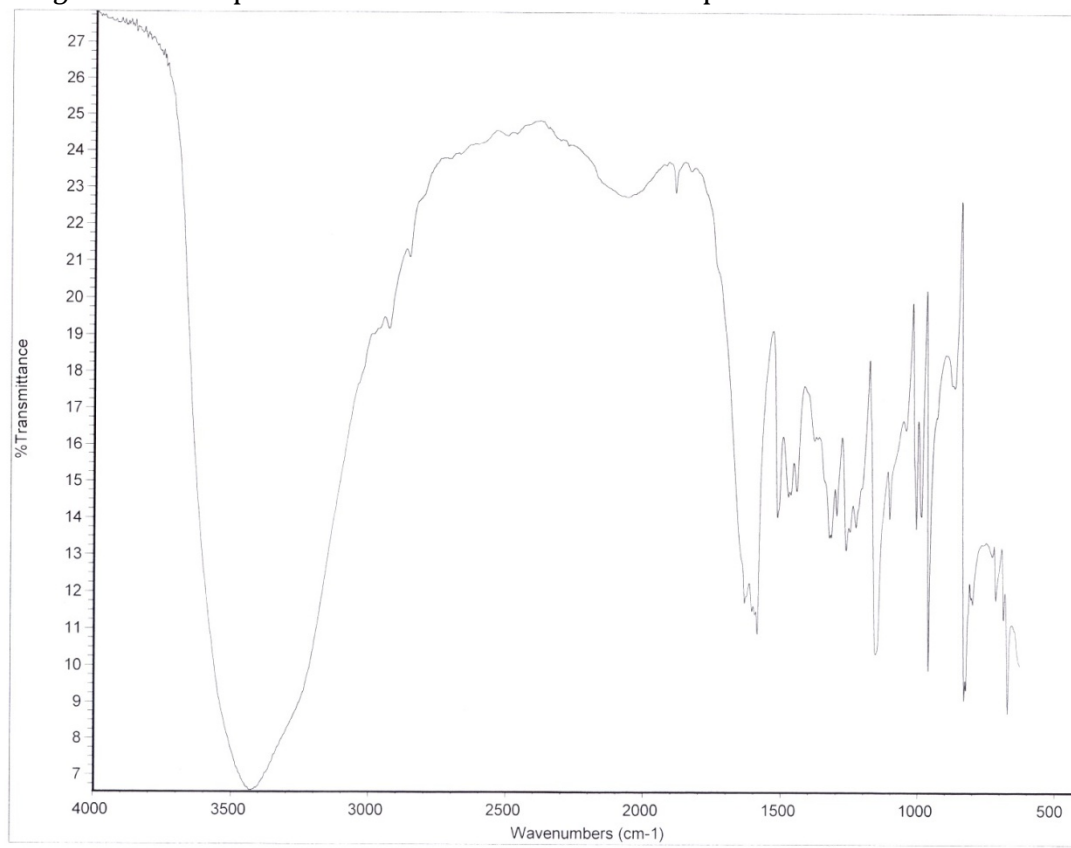


Figure 23: Purchased resveratrol 98% IR spectrum.

Because the purchased pure resveratrol was tested in DMSO- d_6 it was decided that our crude product should also be run in the same solvent system for accurate results. Our two previous NMR tubes were poured into a watch glass and the solvents were allowed to evaporate overnight. In the morning there were yellow crystals on the watch glass and they were easily dissolved in DMSO- d_6 . The tube was run in the NMR and a spectrum was obtained (see figure 24). The data is: NMR ^1H (d -DMSO, 300MHz) δ 8.1 (s, 1H), 7.75 (s, 2H), 7.43 (d, $J_{\text{trans}}=8.49$ Hz, 2H), 7.02 (d, $J_{\text{trans}}=8.49$ Hz, 2H), 6.70 (d, $J=17.6$, 1H), 6.66 (d, $J=17.6$ Hz, 1H), 6.38 (d, 2.2 Hz, 2H), 6.1 (t, 1H), 5.77 (s), 5.71 (s).

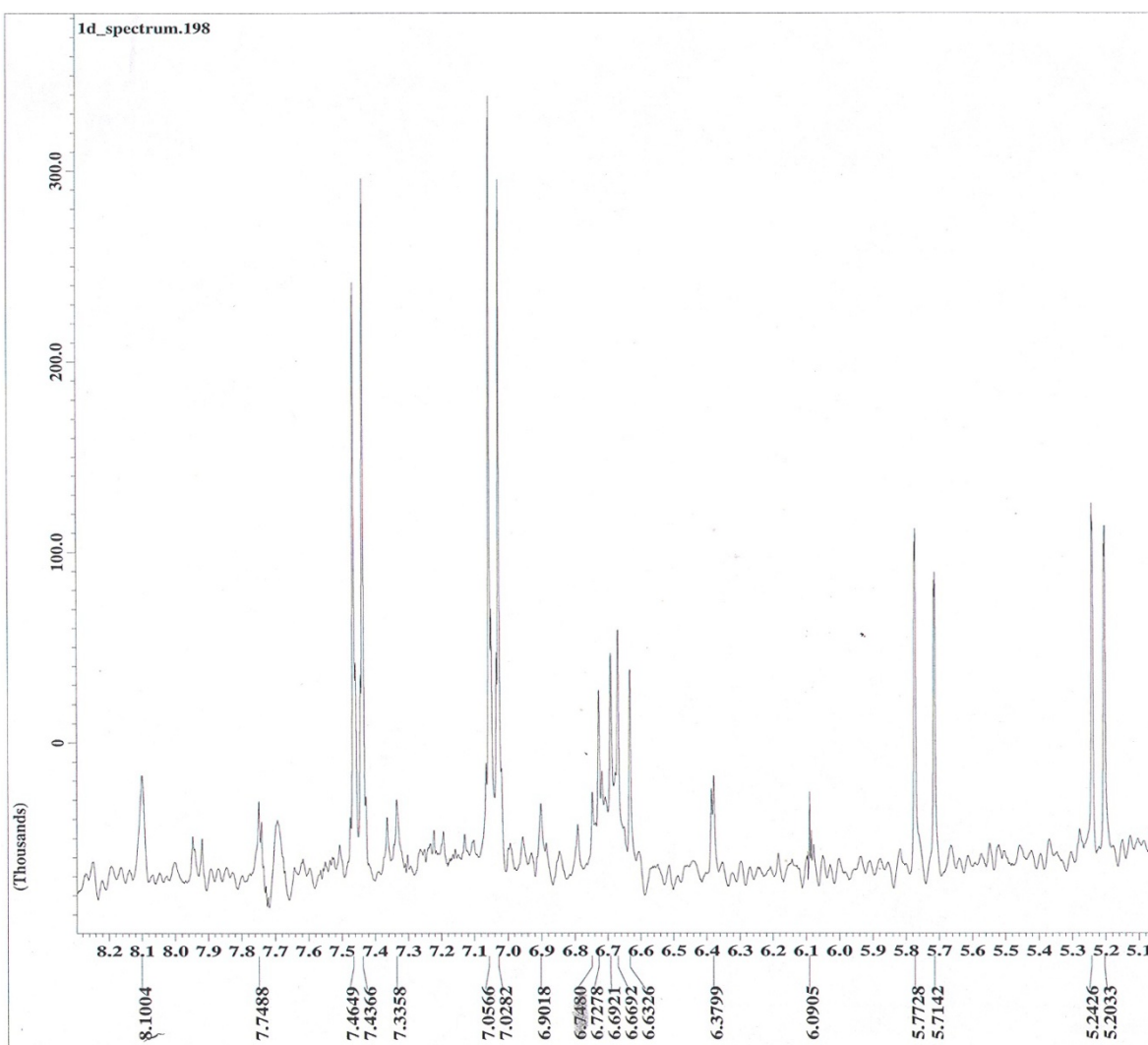


Figure 24: The NMR spectrum of trial 1 and 2 in solvent DMSO- d_6 .

<u>Ppm chemical shift(down→up)</u>	<u>Purchased resveratrol</u>	<u>T. Jeffery</u>	<u>Jing Liu</u>	<u>Our experiments results.</u>
Singlet	9.55 (1H)	8.53 (1H)	8.45 (1H)	8.10 (1H)
Singlet	9.19 (2H)	8.27 (2H)	8.18 (2H)	7.74 (2H)
Doublet	7.38 (8.52, 2H)	7.42 (8.6, 2H)		7.43 (8.5, 2H)
Doublet	6.92 (16.2, 1H)	7.06 (16.4, 1H)	7.41 (12.6, 3H)	6.70 (17.6, 1H)
Doublet	6.76 (16.4, 1H)	6.89 (16.4, 1H)		6.66 (17.6, 1H)
Doublet	6.73 (8.52, 2H)	6.86 (8.6, 2H)		7.02 (8.5, 2H)
Doublet	6.37 (2.2, 2H)	6.57 (2.2, 2H)	6.53 (3.3, 3H)	6.38 (2, 2H)
Triplet	6.10 (2.2, 1H)	6.30 (2.2, 1H)	6.27 (3.3, 1H)	6.09 (2, 1H)
Multiplet			7.05-6.82 (6H)	

Table 4: Shows the comparison between the pure purchased resveratrol, Jeffery's results, Jing Liu's results, and my experimental resveratrol's NMR coupling constants, chemical shifts, and hydrogen splitting.

Dr. Jing Liu's results do not look analogous to those collected by Jeffery or myself. My test results do match the purchased resveratrol and Jeffery's NMR in certain areas and aspects. The purchased product had unusually low downfield singlets. My doublet with 8.5Hz coupling constant at 7.02 ppm is shifted downfield by about 0.3 ppm. The triplet is also very weak and barely readable.

The two downfield singlets are considered to be the three OH groups on the molecule. It is known that OH groups tend to shift in NMR depending upon concentration. The electronegative ethyl acetate in the NMR tube probably also shifted the OH peaks.

CONCLUSION

The synthesis of resveratrol (CAS: 501-36-0) proved to be difficult and trying. Even though the resveratrol molecule was obtained at the end, it did not come without its struggles. In step one we saw the addition of the protecting groups on the alcohols on 3,5-dihydroxybenzoic acid and formed 3,5-diacetoxybenzoic acid. This step was easy and had a good yield of 85%. The IR showed positive results and the melting point was an exact match with that of 3,5-diacetoxybenzoic acid, 161 °C.

In step two we saw the synthesis of the acyl chloride. The procedure was simple and the product 3,5-diacetoxybenzoyl chloride was confirmed by the flame test, which was green, and the NMR spectrum, which had similar peaks to those of Dr. Liu's. The melting point was a little broad (89-95 °C) but the range covered the exact melting point of 3,5-diacetoxybenzoyl chloride. After the product was purified there was only a 45% recovery. This step could be improved greatly to yield better recovery rates.

Step three was by far the most challenging. This step is the Heck reaction between 3,5-diacetoxybenzoyl chloride and 4-acetoxystyrene with the aid of a palladium catalyst. This step is the most complex and requires constant visual watch over the reaction. The environment of the reagents has to be kept oxygen free (nitrogen gas was used) and precisely at 120 °C, which is difficult to monitor with a sand bath. Keeping the nitrogen bubbling into the round-bottomed flask was also difficult with the home made device I put together. The product is finally put through a column and filtered, but this technique greatly decreased recovery rate and did not filter out any impurities, it is thought that some of the desired product is still on the column. This technique should not be used with

micro-scale quantities. There was so little recovery that recrystallization was not done in fear that there would not be enough product left for step four; therefore, step four was run with crude product from step three.

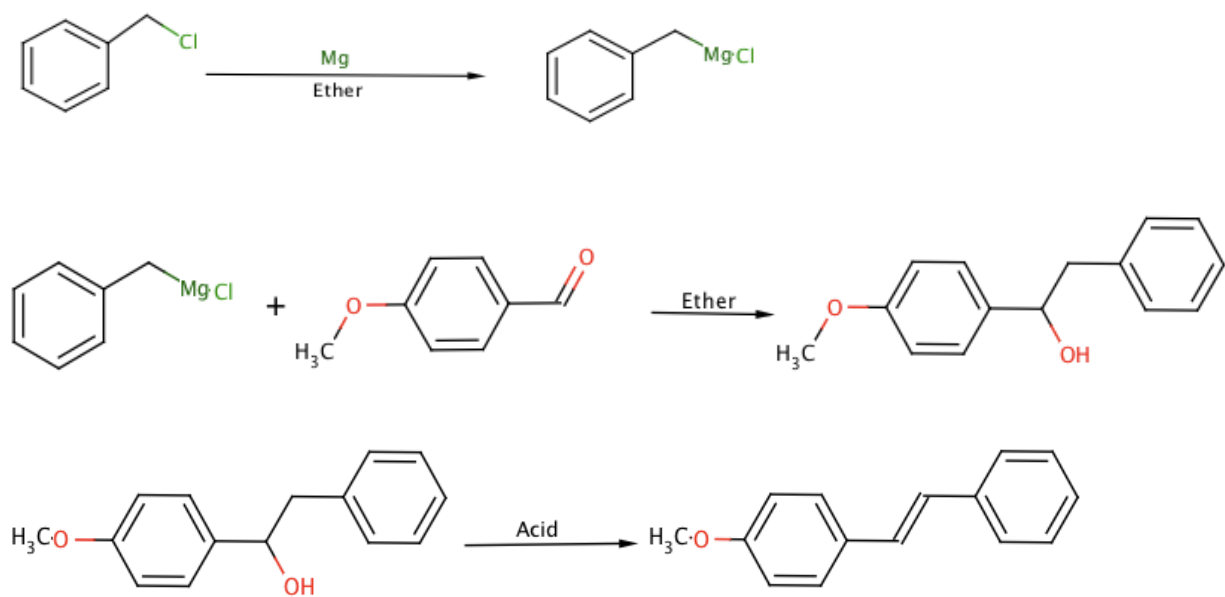
Step four was not as complicated as the previous step but it was still challenging with so little product to work with. The tiny amount of crude product of resveratrol triacetate was all used in two trials. The flash chromatography of this step was disregarded. The final IR and NMR spectra were difficult to analyze because of the impurities within the crude product. A sample of pure *trans*-resveratrol was purchased and compared with the spectra collected. After carefully comparing the spectra and noting the chemical shifts, J-J coupling constants and solvents used in the NMR tubes, it was decided that resveratrol was obtained.

EXPERIMENTAL

Grignard reaction

p-anisaldehyde + benzyl magnesium chloride → stilbene

Before the Heck reaction was tried for this synthesis, it was thought that the Grignard could work as a model to make a stilbene. The hypothesis was that *p*-anisaldehyde could be reacted with benzyl chloride in the presence of a magnesium catalyst. Bellow is the proposed mechanism for our Grignard reaction.



Reactions

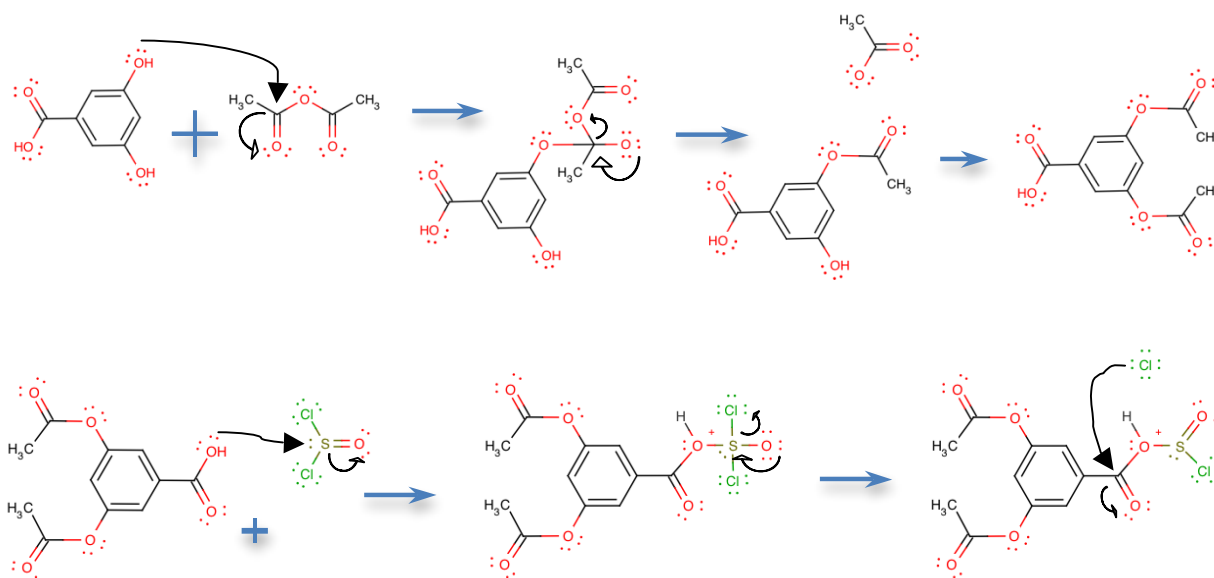
It is essential that with the Grignard reaction that all glassware is clean and dry and the reagents are pure. Glassware was dried in the oven for one hour prior to the start of the reaction. The diethyl ether was also dried over a drying agent. The anhydrous diethyl ether (3.5 mL) was placed in a screw-cap vial. Magnesium turnings (0.056 g) was placed in a 5 mL-glass vial and covered with 0.2 mL ether. The vial was capped and heated on a hot plate at no more than 35° C. To this vial a mixture of benzyl chloride (0.281 mL) and ether was added slowly (1 mL/minute) via a syringe. The reaction refluxed and the solution turned a gray color upon the addition of benzyl chloride. Here it should be noted that the benzyl chloride was probably not pure and contained some impurities. At this point the *p*-anisaldehyde (0.243 mL) was added slowly along with more ether. The solution was stirred for several minutes. Hydrochloric acid (3M, 5 drops) was added and two layers formed. The top ether layer was removed and placed into a dry vial. The aqueous polar layer was washed three times with ether and each time the ether was extracted and added to the nonpolar solution in the vial. Water was added to the polar layer to remove any unreacted acid. Once the water was removed the polar layer was dried over sodium sulfate crystals (0.5 g) overnight. The ether layer was removed the next morning and placed into a 10 mL-Erlenmyer flask and the solution was left to evaporate. Upon evaporation of the ether there was an oil left which was washed with 1 mL ligroin. Twenty minutes after removing the ligroin crystals started to appear. The crystals were a little sticky, possible still wet, indication of broad band on IR. A TLC was done on the crude product with 1:1 ethyl acetate/hexane. Two spots appeared. $R_f = 0.714$ and 0.952 . IR and NMR spectrum obtained showed the crude product may have been a stillbene. There was a singlet (δ 3.7 ppm) methoxy group and a strong multiplet (δ 7.3 ppm) from the two rings.

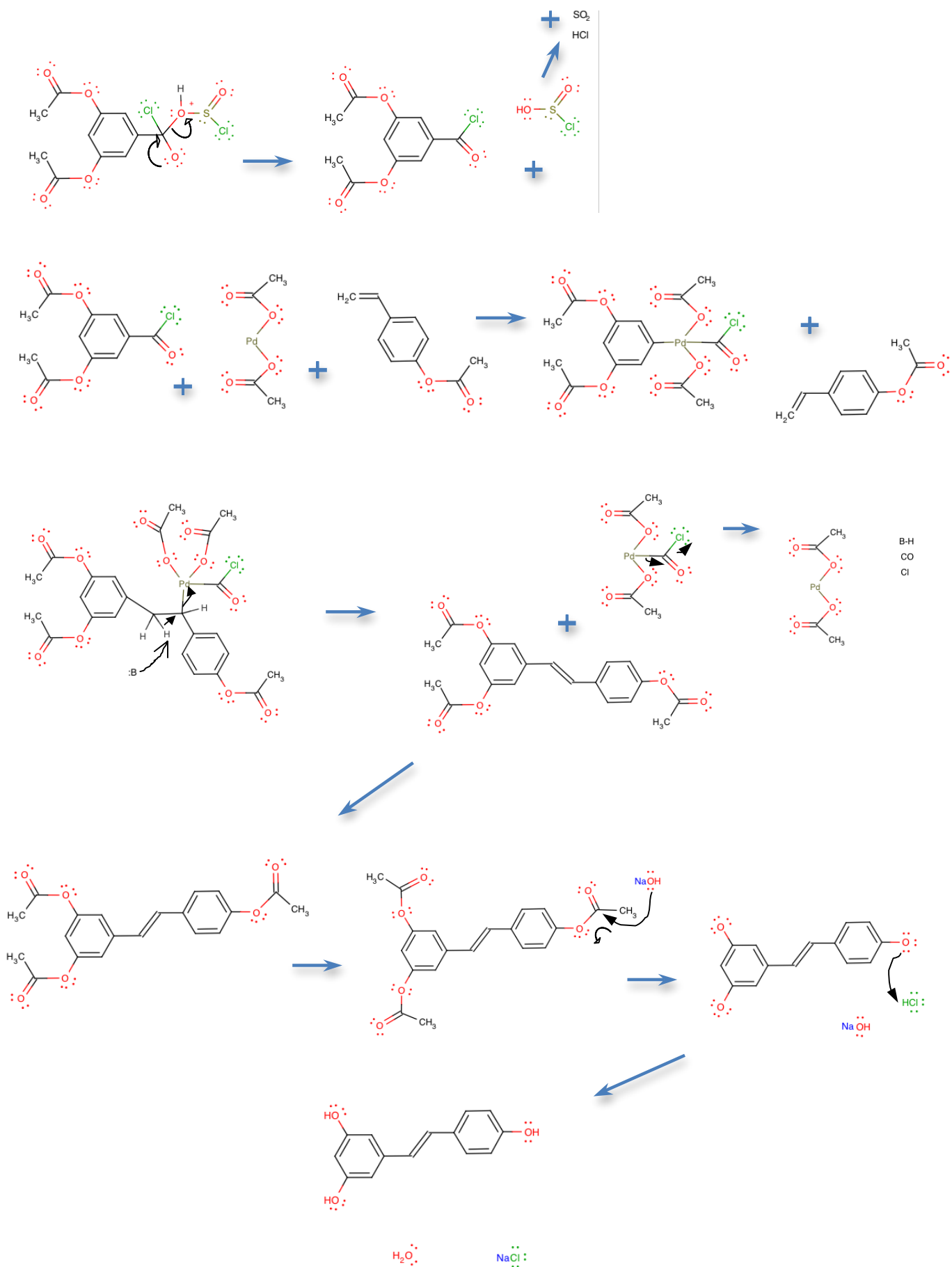
The two doublets at 7.9 and 7.1 have the same J-J coupling constant. The IR showed the aromatic alkenes at 1600 cm^{-1} , carbonyl at 1700 cm^{-1} , a H_2O peak at 3350 cm^{-1} , alcohol band at 3500 cm^{-1} , and alkene peaks at 2900 cm^{-1} . The crude product was recrystallized with a bisystem of 90:10 ligroin/ethyl acetate. This reaction was easy but it does not give good results. Many improvements could be made to this reaction but overall it is considered best to disregard the Grignard reaction for the synthesis of stilbenes.

Decarbonylative Heck reaction

The Heck reaction (see fig. 7) was divided into four stages; the first is the synthesis of 3,5-diacetoxybenzoic acid from 3,5-dihydroxybenzoic acid, the second is the addition of the chlorine halide to form 3,5-diacetoxybenzoyl chloride, the third was the synthesis of resveratrol triacetate, and the last step was the hydrolysis of the triacetate to form resveratrol.

The Mechanism





Step one: 3,5-dihydroxybenzoic acid → 3,5-diacetoxybenzoic acid

The 15-mL round-bottomed flask with 14/20 joints, was held securely in an ice bath over a hot plate with a magnetic stir bar inside. All joints throughout the experiment were sealed lightly with petroleum jelly. Ethyl acetate (11.5 mL) was added to the round-bottomed flask and nitrogen was bubbled in slowly through a glass pipette to remove all oxygen and carbon dioxide. 3,5-dihydroxybenzoic acid (0.771 g, 5 mmol) was added to the ethyl acetate along with the acetic anhydride (1.23 mL, 13 mmol) and 0.808 mL of pyridine (10 mmol). This solution was stirred at 0 °C for 40 minutes and then removed from the ice bath and stirred at room temperature for four hours. After four hours of stirring 99% formic acid (0.236 mL, 6 mmol) was added and then stirring continued for another hour. The solution was a pale yellow color. The solution was poured into a 30-mL separatory funnel and 5 mL DI water was added to remove the excess pyridine; this was done two more times with 2 mL water. The solution was then washed with brine two times to remove excess acid. The water and brine were drained off each time. The nonpolar organic layer was always left in the separatory funnel. The organic layer was then poured into a 25-mL Erlenmeyer flask and 1 g of sodium sulfate was added to dry the liquid. After 15 minutes the liquid was removed and the Na₂SO₄ crystals were washed with ethyl acetate and the liquid was left in a 10-mL Erlenmeyer flask over night for the ethyl acetate to evaporate and crystals to form. Crystals were slightly yellow but after washing them with cold ethyl acetate in a Hirsch funnel they were almost white. Purified yield weighed 0.529 g, a 69% yield. To further purify the crystals recrystallization was done; hot ethyl acetate was added to dissolve the 3,5-diacetoxybenzoic acid and the solution was heated to 95 °C. Heptane (10 drops) was added till a cloudy solution appeared and then

just enough ethyl acetate was added till it turned clear. The 10-mL Erlenmeyer flask was corked and left undisturbed for crystals to form, after one day the flask was put in the refrigerator to further induce crystal formation. Final purified yield was 62%. Melting point was 161-162 °C. *Step one* was repeated four times to increase percent yield by improving technique; this was done by changing a few methods.

First, to improve the reaction the nitrogen was allowed to bubble through the ethyl acetate for longer before adding the other chemicals to ensure all oxygen and carbon dioxide were removed. The three-necked round-bottomed flask was replaced by a 25-mL Erlenmeyer flask and the reaction amounts were doubled. Less water was used in the rinses in the 30-mL separatory funnel but more rinses were done with both DI water and brine. Washing the crystals in cold ethyl acetate was not done again for greatest loss of product was during this step, and improved technique of heating the crystals in ethyl acetate to recrystallize was done in a smaller amount of ethyl acetate. The amount of heptane added was increased so as more impurities could be left in solution. Placing the crystals under the house vacuum increased the rate of evaporation of ethyl acetate. The percent yield increased with these changes and the greatest recovery rate was recorded at 85% (1.3 grams). The IR spectrum was collected by making a pellet press of the 3,5-diacetoxybenzoic acid with KBr.

Step two: 3,5-diacetoxybenzoic acid → 3,5-diacetoxybenzoyl chloride

The second step of the Heck reaction is the formation of the acyl halide from the acid. For the first trial the experiment was run at 5% original volume aforementioned. In a 15-mL round-bottomed flask benzene (3 mL), new thionyl chloride (0.8 mL) and one drop of dimethylformamide were mixed. Nitrogen was bubbled through the mixture for five

minutes prior to adding the 3,5-diacetoxybenzoic acid; nitrogen was bubbled into liquid layer for the first hour of the experiment. 3,5-diacetoxybenzoic acid (0.4 g, 1.68 mmol) was weighed out and the crystals were crushed to reduce the size of the clumps of solid. Once the 3,5-diacetoxybenzoic acid was added it took five minutes to dissolve and the liquid turned slightly yellow. After mixing at room temperature for 20 minutes the round-bottomed flask was placed in a hot water bath at 80 °C. This mixture was stirred for two hours at a temperature range from 80-90 °C. Because of the high temperature and lack in seal the benzene evaporated quickly and more had to be added several times throughout the experiment. After two hours the walls had a dark brown oily substance on them, the liquid turned a yellow color. The liquid was allowed to evaporate and a yellow solid was left. Toluene was added and the vial was heated until the toluene was at a boil, the toluene was removed and placed in a clean, dry, preweighed flask. There was a dark oil left at the bottom of the first flask which was discarded. Once the toluene was evaporated yellow crystals were left. Melting point ranged from 89-101 °C.

The second trial was done similarly to the first; however, a few necessary changes were made. The reaction was run with twice the volume/amount of chemicals than the first trial. The setup was redone so that oxygen and the outside air could not enter the round-bottomed flask and benzene could not evaporate through any joints. At the opening of the condenser a drying tube was attached containing calcium chloride granules. Nitrogen was bubbled into the liquid throughout the two hours of heating. A 20% excess of benzene (3.6 mL) was added at first so that benzene would not have to be added later that had not previously been bubbled with nitrogen. Formic acid was added after 1 hour and then again after four hours, but only half the amount was added at the four-hour

mark. The water and brine washes were done more thoroughly and the washes were dried overnight on sodium sulfate crystals. The temperature of the water beaker was kept between 80-85 °C. Literature says 3,5-diacetoxybenzoyl chloride melts at 89.5-91 °C¹⁷. Once the liquid evaporated, hot toluene was added and additional heating was done to dissolve all the 3,5-diacetoxybenzoyl chloride. The hot toluene with the dissolved 3,5-diacetoxybenzoyl chloride dissolved in it was then pushed through a silica column with high pressured nitrogen. Once the toluene was evaporated off pale yellow crystals were left. Melting point revealed that the product 3,5-diacetoxybenzoyl chloride was attained, but probably included some impurities. Melting point was 89-96 °C, which is closer to the literature value. FTIR and NMR was performed on the crystals. The dry solid did not dissolve completely in CDCl₃ and thus was run in the NMR in *d*-benzene. This did not prove to be helpful and so CDCl₃ was used again but less amount of 3,5-diacetoxybenzoyl chloride was placed in more CDCl₃. Percent yield was calculated and found to be 45%.

¹⁷ Liu, Jing. PhD. paper. 2007. Brigham Young University.

Step three: 3,5-diacetoxybenzoyl chloride → Resveratrol triacetate.

In the third step the 3,5-diacetoxybenzoyl chloride is coupled with the 4-acetoxystyrene with the aid of two catalysts. Because the 3,5-diacetoxybenzoyl chloride is an unsaturated halide it reacts with the alkene in the presence of a strong base with a palladium and *N,N*-bis-(2,6-diisopropylphenyl)-4,5-dihydro imidazolium chloride catalyst to form a substituted alkene, and in our case resveratrol triacetate. This coupling with the two catalysts is the fundamental key in the Heck reaction. A dry 15 mL-three-necked round-bottom flask was charged with *p*-xylene (3 mL), Pd(OAc)₂ (0.005 g), *N,N*-bis-(2,6-diisopropylphenyl)-4,5-dihydro imidazolium chloride (0.021 g), 3,5-diacetoxybenzoyl chloride (0.354 g, 1.4 mmol), 4-acetoxystyrene (0.27 mL, 1.7 mmol), and *N*-ethyl morpholine (0.21 mL, 1.7 mmol). The liquid components were added first and nitrogen was bubbled through the liquid for five minutes prior to the addition of the solids. Once all the chemicals were in the round-bottomed flask a magnetic stir bar was added and the whole apparatus was lowered so that the lower half of the round-bottomed flask was sitting in a sand bath. The sand bath was monitored closely and the temperature was kept between 115 °C and 140 °C. Nitrogen was continuously bubbled in at a slow rate throughout the four hour reaction. While the reaction sat in the sand bath for four hours the flash chromatography apparatus was set up. The column was made by using glass tubing with a flat head filter at the end. The column was packed with silica gel and sand was placed on top. Rubber bubble tubing was used to connect the nitrogen gas tank to the top of the column. A T-valve was used along with a stopcock to be able to control the pressure of the gas on top of the column. The liquid in the round-bottomed flask started off a slightly yellow color and remained a pale yellow. On the sides of the glassware a

thick, black, sticky oil had formed. The column was wet with 20% ethyl acetate/ hexanes. The product was poured on top of the column and pressure was applied to push the solvent through; however, not much pressure was needed to push the mobile phase through the column. The mobile phase was tested by placing a drop of eluent on a TLC plate to detect if any product was still coming through the column. Once all the product had passed through the column and collected in several 10-mL Erlenmeyer flasks, the Erlenmeyer flasks were left for the solvent/mobile phase to evaporate overnight. About 50 mL of ethyl acetate/hexane was used to push the entire product through the column. Once the ethyl acetate and hexane had evaporated, very few crystals could be seen on the inside of each individual Erlenmeyer flask. The flasks were washed with ethyl acetate and the washes were combined into one Erlenmeyer flask; this was left for the ethyl acetate to evaporate. Once it had evaporated, wet yellow crystals were seen on the inside of the flask. Although the solution did not completely crystallize, the viscosity did increase.

Step three was run again at 5% of original volume. Amounts of chemicals used were:

Amount	Chemical
3 mL	<i>p</i> -Xylene
0.3 mL	4-acetoxystyrene
0.21 mL	<i>N</i> -ethyl morpholine
0.3585 g	3,5-diacetoxybenzoyl chloride
0.005 g	Palladium acetate Pd(OAc) ₂
0.006 g	<i>N,N</i> -bis-(2,6-diisopropylphenyl)-4,5-dihydro imidazolium chloride

Table 5: List of amounts of chemicals used in the second trial of resveratrol triacetate

The experiment was conducted similarly to the first trial; however, a few changes were made. The sand bath was allowed to heat up to 120 °C for an hour before the round-bottomed flask was placed in it with the chemicals. Similar to the previous experiment, the

liquids were added first and nitrogen was allowed to bubble through before the solid powders were added. The heat was controlled more precisely and it was kept between 120-125 °C. The reaction was heated at this temperature for 4 hours. A new silica column was made and the contents of the round-bottom flask was poured into the wet column and push through with 20:80 ethyl acetate and hexane. Pressurized nitrogen gas was used to push the solvent through the column. The round-bottomed flask was rinsed with the ethyl acetate and hexane to increase percent yield. About the same amount of solvent was used to push the product through the column. The five 10-mL Erlenmeyer flasks were left overnight for the solvent to evaporate. Once the solvent evaporated a yellow crystalline oil was left behind. An IR was run on the yellow oil as well as a TLC. Upon observing the IR and TLC for trials one and two it was thought that perhaps the resveratrol triacetate was still on the column. The column was washed with pure methanol to push any remaining compounds through the column. The mobile phase was collected in fractions and once the methanol evaporated yellow crystals were obtained. Determining the melting point was challenging because the crystals appeared wet. Melting point was 115-130 °C. Literature says that resveratrol triacetate melts at 116-118 °C.

Trial three was done similarly to trial two with the following amounts of chemicals.

Amount	Chemical
5.4 mL	<i>p</i> -Xylene
0.54 mL	4-acetoxystyrene
0.41 mL	<i>N</i> -ethyl morpholine
0.617 g	3,5-diacetoxybenzoyl chloride
0.009 g	Palladium acetate Pd(OAc) ₂
0.011 g	<i>N,N</i> -bis-(2,6-diisopropylphenyl)-4,5-dihydro imidazolium chloride

Table 6: Amount of chemicals used in trial three of step three.

A change made to trial three was that the mobile phase was gradually changed from 20:80 ethyl acetate:hexane to pure methanol. This was done to ensure that all the compounds came off the column. The mobile phase was collected in several 10-mL Erlenmeyer flasks so as to not mix all the compounds together. The Erlenmeyer flasks were left overnight for the solvents to evaporate. Again there was so little product that the tiny amounts from the first few Erylmeyer flasks were combined into one flask. The solvent range for the first few flask were from 20:80 ethyl acetate:hexane to 50:50 ethyl acetate:hexane. The percent recovery of this step was about 25%.

Step four: Resveratrol triacetate → Resveratrol

It should be noted that for the first trial of step four the previously obtained yellow crystalline crude products from step three were used; therefore, the starting material was not pure to begin with. In a 15-mL round-bottomed flask resveratrol triacetate (0.01 g, 0.03 mmol) was dissolved in 1 mL tetrahydrofuran (THF). Sodium hydroxide (1.5 mL, 0.687M) was added to this and the whole mixture was stirred for two hours at room temperature. The flask was not sealed and nitrogen was not bubbled through the mixture. After two hours hydrochloric acid (3M) was added dropwise until the pH was 4. This required about 10 drops of acid. The pH was tested on standard universal indicator paper. The flask was then sealed and connected to the house vacuum and left for the tetrahydrofuran to evaporate. The desired resveratrol product was then extracted with ethyl acetate and placed into a small 10-mL beaker. The ethyl acetate was washed with water and brine to remove any excess acid and THF. The ethyl acetate was then pipetted off the water and brine and placed over sodium sulfate in a clean dry 10-mL beaker and left for one hour to dry the solution. After an hour the ethyl acetate was pulled off the

crystals and placed in a clean dry 10-mL beaker and was left for the ethyl acetate to evaporate and yield crystals. Once the ethyl acetate evaporated, oily yellow crystals were left. The oil was run in the IR and the spectrum collected did not look comparable to that of resveratrol.

$$\begin{aligned} \text{NaOH Molarity Calculation: } \quad & \frac{4.67 \text{ g NaOH}}{170 \text{ ml H}_2\text{O}} = \frac{0.55 \text{ g NaOH}}{20 \text{ ml H}_2\text{O}} \\ & \frac{0.55 \text{ g} \times 50}{40 \text{ g/mol}} = 0.687M \text{ NaOH} \end{aligned}$$

The second trial of step 4 was done in a similar manner as the first but two important things were done first. Firstly, I tried to figure out the mechanism for this step and secondly the IR gave us important details as to what might have happened in the reaction. The starting amount of resveratrol triacetate was approximately 0.05 g. This small amount was dissolved in tetrahydrofuran (2 mL), it was placed in the small 15-mL round-bottomed flask and sodium hydroxide (2 mL, 0.8M) was added. After two hours of stirring at ambient temperature, the pH tested was not basic, so more NaOH was added till the pH was basic, this was done because it is the base that donates the electrons to convert the acetate to phenols. Finally, sodium hydroxide (10 mL) was added before the solution turned basic, this was left to stir for another 30 minutes before the acid was added. Hydrochloric acid (5 mL, 6M) was added before the pH was 4. The round-bottomed flask was left under vacuum over-night to evaporate the THF and some of the water. By morning there was about only 2 mL of liquid left along with a white solid on the bottom. The products were extracted with cold ethyl acetate; three washes were done. The ethyl acetate and product was placed into a large test tube and washed twice with water and brine solution. The nonpolar layer was then poured into a 10-mL Erlenmeyer flask with sodium sulfate crystals and left for 30 minutes to dry. The nonpolar liquid was extracted

with a glass disposable pipette and placed into a preweighed 10-mL Erlenmeyer flask and left over the weekend for the ethyl acetate to evaporate.

A heterogeneous mixture of yellow oil with crystals was left in the 10-mL Erlenmeyer. The yellow oil and crystals were analyzed with IR and NMR spectroscopy. Both the IR and NMR showed positive results. The product was dissolved in *d*-acetone, which made the collection of the C^{13} NMR difficult because of the three carbon atoms in acetone; the gain had to be manually set for the instrument to run the single pulse decoupled experiment. A new NMR spectrum was obtained by running the product in *d*-chloroform. The product did not dissolve easily in *d*-chloroform, but with gentle heating and ultrasonication it partially dissolved.

Resveratrol 98% was purchased from VWR in order to run an IR and NMR spectra of the pure substance. The first NMR spectra run did not turn out well, the resveratrol was dissolved in deuterated chloroform, which as it happens to be, not able to dissolve resveratrol. A new NMR tube was used and resveratrol was dissolved in deuterated DMSO. Resveratrol is only soluble in a few solvents, such as: ethanol, DMSO, and dimethyl formamide. The solubility of resveratrol in these solvents is approximately 65 mg/mL. The solubility of *trans*-resveratrol in PBS (pH 7.2) (phosphate buffered saline) is approximately 100 $\mu\text{g/mL}$. The NMR spectrum of the *trans*-resveratrol (98%) looked comparable to the previous spectrum of resveratrol obtained from trial 1 and 2.

Now that a good NMR spectrum had been obtained our results could be analyzed; however, the NMR of the purchased resveratrol was done in a $\text{DMSO-}d_6$ solvent whereas our crude NMR spectrum was run in *d*-chloroform solvent. The previous NMR tube that had the resveratrol dissolved in *d*-chloroform was used by evaporating off the solvent and

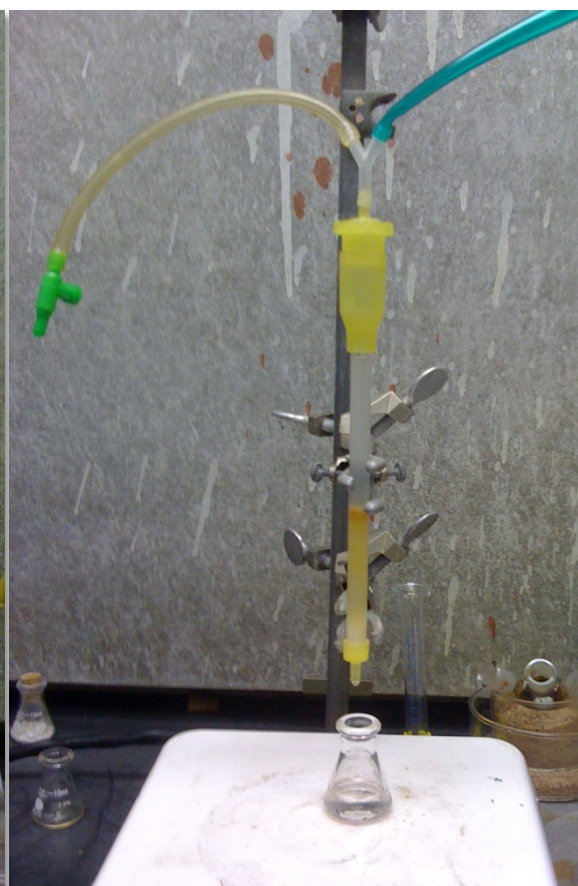
then redissolving the crystals in DMSO-*d*₆. The NMR spectrum looked good and the coupling constants were similar to before.

The purchased resveratrol (98%) was also analyzed in the FTIR. A small amount of the resveratrol was dissolved in ethanol and placed on the salt plate. The ethanol was allowed to evaporate before the spectrum was run. The IR showed a large OH band and arene C=C stretching at 1600 cm⁻¹ from the two C₆ rings.

APPENDIX

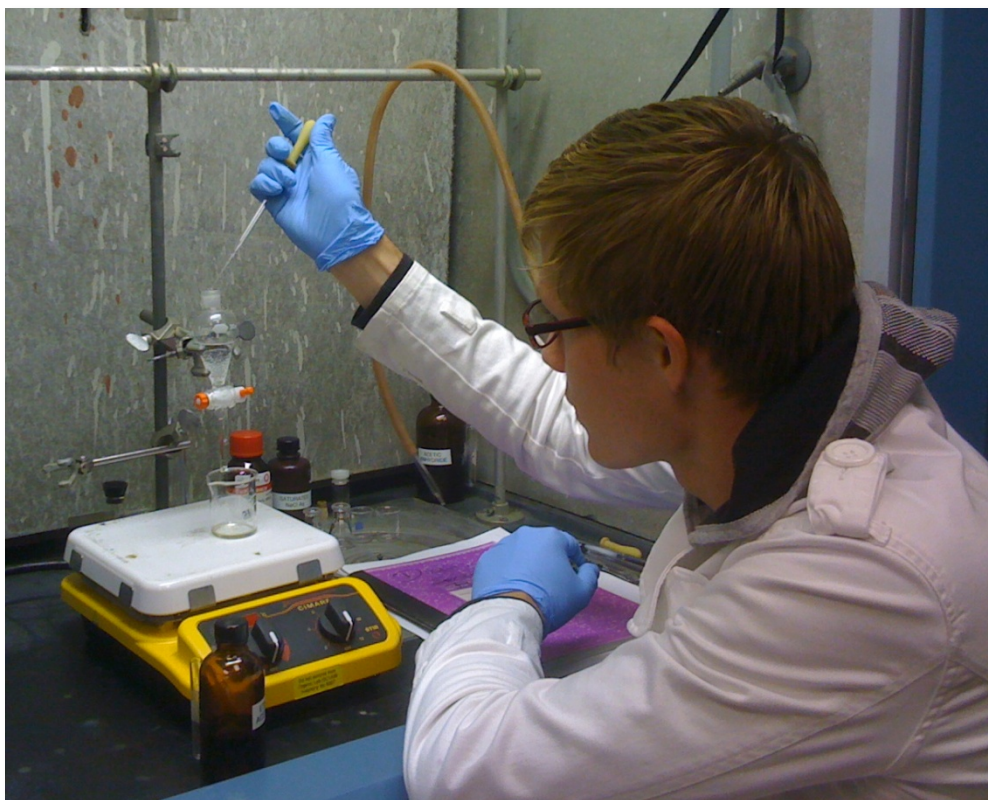


The setup with the round-bottomed flask in A hot water bath with condensation tube.

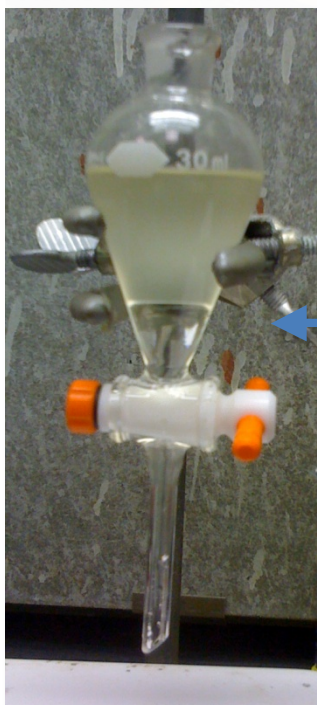


The apparatus I made to push the solvent through the column with pressurized Nitrogen.





Washing the product with water and brine in a separatory funnel.



A view at the two layers in the separatory funnel



The TLC of resveratrol

REFERENCES

- "Anti-Inflammatory Responses of Resveratrol." *Inflammation & Allergy - Drug Targets* 6.3 (2007): 168-173. *E-Journals*. Web. 22 Mar. 2010
- Bass, T. M., D. Weinkove, K. Houthoofd, D. Gems, and L. Partridge. "Effects of resveratrol on lifespan in *Drosophila melanogaster* and *Caenorhabditis elegans*." *Mechanisms of Ageing and Development* 128.10 (Oct 2007): 546-52. *PubMed*. 14 Aug. 2007. Web. 6 Sept. 2009. <<http://www.ncbi.nlm.nih.gov/pubmed/17875315>>.
- Baur, J. A., and D. A. Sinclair. "Therapeutic potential of resveratrol: the in vivo evidence." *Nat Rev Drug Discov* 5.6 (2006): 493-506. *PubMed*. ACS, 26 May 2006. Web. 05 Oct. 2009. <<http://www.ncbi.nlm.nih.gov/pubmed/16732220>>.
- Do, Gyeong-Min, et al. "Long-term effects of resveratrol supplementation on suppression of atherogenic lesion formation and cholesterol synthesis in apo E-deficient mice." *Biochemical and Biophysical Research Communications* 374.1 (2008): 55-59. *E-Journals*. Web. 22 Mar. 2010.
- Jeffery, Tuyet, and Benoit Ferber. "One-pot palladium-catalyzed highly chemo-, regio-, and stereoselective synthesis of trans-stilbene derivatives. A concise and convenient synthesis of resveratrol." *Tetrahedron Letters* 44 (2003): 193-97. Print.
- Lagouge, M., C. Argmann, Z. Gerhart-Hines, H. Meziane, C. Lerin, F. Daussin, N. Messadeq, J. Milne, P. Lambert, P. Elliot, B. Geny, M. Laakso, and J. Auwerx. "Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 α ." *Cell* 127.6 (Dec 2006): 1091-093. *PubMed*. U.S. National Library of Medicine, Nov. 2006. Web. 4 Sept. 2009. <http://www.ncbi.nlm.nih.gov/pubmed/17112576?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.>.
- Liu, Jing. Synthesis of Resveratrol and its analogs, phase-transfer catalyzed asymmetric glycolate aldol reactions, and total synthesis of 8,9-methylamido-geldanamycin. PhD. Thesis, 2007. Brigham Young University
- Resveratrol; 501-36-; Cayman Chemical Company: Ann Arbor, MI., 8/17/2005. 2/15/2010.
- Schröder, G., J. Schröder, J. Fliegmann, L. Schröder, S. Raiber, S. Tropf, and T. Lanz. "Resveratrol: Basic Aspects and Biosynthesis." *Websites of the Schröder Group*. 09 Mar. 2007. Web. 22 Sept. 2009. <<http://www.biologie.uni-freiburg.de/data/bio2/schroeder/Resveratrol.html>>.
- Sinclair, D., R. De Cabo, D. K. Ingram, J. A. Baur, K. J. Pearson, N. L. Price, H. A. Jamieson, C. Lerin, A. Kalra, V. V. Prabhu, J. S. Allard, G. Lopez-Lluch, K. Lewis, P. J. Pistell, S. Poosala, K. G. Becker, O. Boss, D. Gwinn, M. Wang, S. Ramaswamy, K. W. Fishbein, R.

G. Spencer, E. G. Lakatta, D. Le Couteur, R. J. Shaw, P. Navas, and P. Puigserver.
 "Resveratrol improves health and survival of mice on a high-calorie diet." *Nature*
 444.7117 (Nov 2006): 337-42. *PubMed*. 1 Nov. 2006. Web. 7 Sept. 2009.
 <<http://www.ncbi.nlm.nih.gov/pubmed/17086191>>.

Sinclair, D., S. Lavu, B. Rogina, K. Howitz, S. L. Helfand, and M. Tatar. "Sirtuin activators
 mimic caloric restriction and delay ageing in metazoans." *Nature* 430.7000 (Aug
 2004): 686-89. *PubMed*. 14 July 2004. Web. 7 Sept. 2009.
 <<http://www.ncbi.nlm.nih.gov/pubmed/15254550>>.

Sinclair, D., S. Lavu, K. T. Howitz, K. J. Bitterman, H. Y. Cohen, D. W. Lamming, J. G. Wood, R.
 E. Zipkin, A. Kisieleski, L. L. Zhang, B. Scherer, and P. Chung. "Small molecule
 activators of sirtuins extend *Saccharomyces cerevisiae* lifespan." *Nature* 425.6954
 (Sep 2003): 191-96. *PubMed*. 24 Aug. 2003. Web. 7 Sept. 2009.
 <<http://www.ncbi.nlm.nih.gov/pubmed/12939617>>.

Valenzano, D. R., E. Terzibasi, T. Genade, A. Cattaneo, L. Domenici, and A. Cellerino.
 "Resveratrol prolongs lifespan and retards the onset of age-related markers in a
 short-lived vertebrate." *Current Biology* 16.3 (Feb 2006): 296-300. *PubMed*. Web. 7
 Sept. 2009. <<http://www.ncbi.nlm.nih.gov/pubmed/16461283>>.

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SYNTHESIS OF CHEMISTRY AND CHRISTIANITY

All who are born into this afflicted world know that one day we will be taken out of it. Along the way we create our journey, a journey is more than just a path, it is our story and our history. But upon reaching our destination we will be judged, judged on our journey and the choices we made along the way.

Like our earthly journey, a synthesis is not about the final product, it is about the steps taken to achieve it. It is important that during any synthesis that no shortcuts or compromises be taken. Life is like a synthesis; there are slow reactions, fast ones, catalysts to help us, and impurities to hinder us. Similarly to a synthesis, where we need to check the intermediate steps' purity before we may proceed, life has its check points before we can move on, or else we move forward with unwanted baggage. Throughout my synthesis, I encountered difficulties, frustration, and hardship. Equally, in our walk in Christianity we encounter snares, trials, and temptations along the way. But it is the destination at the end, encouragement from others, and dedication that helps us finish the journey and achieve our goals.

Resveratrol is a chemical believed to extend life, which is great, but eternal life is only possible through the acceptance of Jesus Christ. Anyone may accept Him, but it is a life lived for him that seals the deal.