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Development of an improved psilocybin synthesis

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Abstract

Development of an improved psilocybin synthesis

Reham Shaba

Psilocybin is a hallucinogenic compound found in fungi and is currently evaluated in clinical trials for treatment of depression, anxiety and addiction. Psilocybin is a prodrug of the pharmacologically active metabolite, psilocin. The synthetic route to psilocybin relies on synthesizing psilocin from the starting material, 4-hydroxyindole and latter converting psilocin into psilocybin by phosphorylation. The synthesis of psilocybin has been challenging because of the labile nature of the phosphorylation dibenzyl ester reagent and related intermediates. Several attempts to optimize psilocybin synthesis have been published but there is still a need for further improvements.

The first aim of this project was to synthesize psilocybin using literature methods, while the second aim was to optimize the phosphorylation step with different reagents and conditions.

Initial studies focused on coupling the two-side chain carbon onto position three of the indole, this required protection of the hydroxyl group which was achieved by acylation in room temperature. With sufficient amount of dimethylamine solution, the amine addition reaction was investigated and resulted in a pure product. The following reduction with lithium aluminum hydride provided an unknown side-product instead of psilocin.

The first aim was successfully accomplished, up to psilocin, with pure intermediates but low yields. The second aim was not achieved due to lack of time and access to the laboratory during covid-19 crisis. However, a literature survey of reagents and conditions for phosphorylation was performed which enables continuation of the project.

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Popular Scientific Summary

Hallucinogenic drugs have long been produced through natural products, in year 1957 the hallucinogen psilocybin was identified in a fungal species called *Psilocibe Mexicana*. This specie produced the active substance, psilocybin, it was found to be a psychological active substance with effects similar to mescaline and lysergic acid diethylamide (LSD). The hallucinogenic effects occur because psilocybin and psilocin are agonists to receptors for serotonin, primarily 5HT_{2a}. The powerful psychoactive effects of psilocybin led to its inclusion in the United Nations Convention on Psychotropic Substances in 1971, leading to prohibition in all member countries. Illicit use of psilocybin in form of mushrooms continued despite the prohibition, at relatively low levels compared to other narcotics. In recent years clinical research on psilocybin has been initiated in a range of psychiatric disorders such as depression.

Psilocybin is a more stable compound compared to psilocin therefore psilocybin is today preferred as the Active Pharmaceutical Ingredient (API) in clinical trials. There has been significant work done on synthesis of psilocybin in previous published reports. There has been many improvements of the yield and purity of psilocybin however there are still important drawbacks with the published methods. Previous studies use the same phosphorylation reagent tetra benzyl pyrophosphate (TBPP), not favored for large scale production because it is expensive and produces toxic phosphate byproducts.

In this project, the literature synthesis was conducted as the first aim and the second aim was to examine different phosphorylation reagents. This to evaluate which were most favorable conditions for good yield and purity of psilocybin in the phosphorylation step. Furthermore, a benzylated psilocin was intended to be synthesized for the purpose of determining whether it would result into purer psilocybin by protecting the dimethylamine position.

Insights during synthesis for the first aim were firstly, to perform the dimethyl amine addition synthesis in dry conditions from the beginning to avoid occurrence of biproducts. Secondly, even though the salt of dimethylamine could be employed, it was necessary to use the commercially available solution of dimethylamine and consequently lead to an improved reaction time. Also, it was not optimal to use an impure product for reduction regardless of purification afterwards. As a conclusion, it was critical to confirm and purify every product in this synthesis.

A literature survey was conducted to identify potential phosphorylation reagents depending on reactivity, stability and availability however, due to lack of time the experiments of the chosen reagents was not performed. Thereby, the most suitable reagent and conditions leading to higher purity and yield of psilocybin could not be determined. Nevertheless, the selected reagents for the study are suitable for a large-scale synthesis since they are stable, available and inexpensive (unlike TBPP). The usage of benzylated psilocin would possibly lead to an easier phosphorylation. This could result into lower cost, higher yield and purity of psilocybin. However, this cannot be confirmed without experimental verification.

In conclusion, this project resulted in partially good accomplishment of the first aim, up until psilocin with pure intermediates however with a low yield. This report enables further research on selecting an appropriate phosphorylation reagent and also suggests impact of benzylated psilocin for psilocybin synthesis. This is certainly a synthesis with enormous potential for the pharmaceutical process development.

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List of abbreviations

LSD – Lysergic acid diethylamide

API – Active Pharmaceutical Ingredient

TBPP - Tetra benzyl pyrophosphate

NCE – New Chemical Entities

STS - Speeter-Anthony tryptamine synthesis

n-BuLi – Butyllithium

DCM – Dichloromethane

LCMS – Liquid chromatography – mass spectrometry

EtOAc – Ethyl acetate

NMR – Nuclear magnetic resonance

THF - Tetrahydrofuran

LAH - Lithium aluminum hydride

TLC - Thin layer chromatography

DMAP - 4-dimethylaminopyridine

DIPEA - N,N-diisopropylethylamine

1. Introduction

The pharmaceutical industry is growing every day because of natural products since it's the largest source leading to various drugs [1]. According to Harvey [1] almost 80 % of all medicine today originates from natural products. In fact, during 1981-2001 there were far more discoveries of new chemical entities (NCE) for therapeutic use than it was in previous years [2]. Natural products have made it possible for also developing the semi-synthetic as well as synthetic compounds by copying the nature. This kind of development has enabled modifications of different substances into becoming more potent. There are several types of natural products used today for making drugs from plants, animals and fungi. An example of an approved drug derived from plant source is elliptinium which is used in treatment of cancer. Further example is anti-fungal agent called caspofungin isolated from fungus, it is used for infections such as *Aspergillus* and *Candida* [1].

Fungi natural products have contributed to a large number of lead compounds in drug discovery by isolating bioactive compounds from different species. Thenceforth, these compounds can provide different biological activates. For instance, the fungus specie called *Ganoderma lucidum* has antihepatic and anticancer effects. Another example is the bioactive compound isolated from *Cordyceps* mushrooms prompted anticancer effect and is also utilized in treatment for disruptive immune system. Hence, the utility of mushrooms has proven to be successful for several treatments [3].

Since species of fungi such as mushrooms grow wild in nature it enables people to find and ingest it. There are fungi called magic mushrooms originated from Mexico 3000 years ago. These were eaten by Indians since they believed the mushrooms enabled them to speak with God. In fact, this mushroom could be eaten only by the pure individuals since it was assumed to be holy. This mushroom whose biological name is *Psilocibe mexicana* and *Stropharia cubensis*, was collected by a scientist called Gordon Wasson in Mexico in 1957 [4]. The colleague to Wasson, Albert Hofmann isolated successfully the bioactive compound called psilocybin (O-phosphoryl-4-hydroxy-N,N-dimeethyltryptamine) from the mushroom in 1958. Only a year later Hofmann synthesized psilocybin and it was utilized for experimental research at Sandoz. Psilocybin was found to be a psychological active substance with LSD effects since it's a serotonin agonist which results in hallucinated effects [5].

Psilocybin and psilocin are structures of tryptamine hallucinogens. These substances are agonists to receptors of serotonin 5HT_{2A/C} and 5HT_{1A}. These receptors are responsible for the hallucinogenic effects from psilocin. Psilocybin is the prodrug of the dephosphorylated precursor called psilocin which is the active compound. Psilocybin have been shown to be more stable than psilocin and is therefore preferred in drug clinical research. Psilocybin is today in clinical trials since it showed promising results for treatment of such as depression, anxiety and addiction [5].

1.2 Illicit use

Recently, it has been shown *psilocybe* or magic mushrooms grow in the wild in Europe, North America and Taiwan. The spread of magic mushrooms all over the globe caused the adolescents to acknowledge the properties of psilocybin mushrooms and as a consequence the consumption of psilocybin increased [6]. Psilocybin is contained in sweets and sold for the purpose of

consuming the drug illicitly [7]. In a study made by Hallock et al [6] on psilocybin usage in a college in New York showed almost 92 % of the participant consumed psilocybin out of curiosity in order to obtain the hallucinogenic effects such as magical experience, improved creativity and increased socialization. This study also presented that student users of psilocybin is high but only a small group of students desires to frequently use the drug [6]. However in another study by Amsterdam et al [7] the usage of psilocybin was less than 10 % among students of age 15-16 years in Netherlands. Psilocybin is considered to have low abuse potential, with no addiction potential shown in animal or human studies [8].

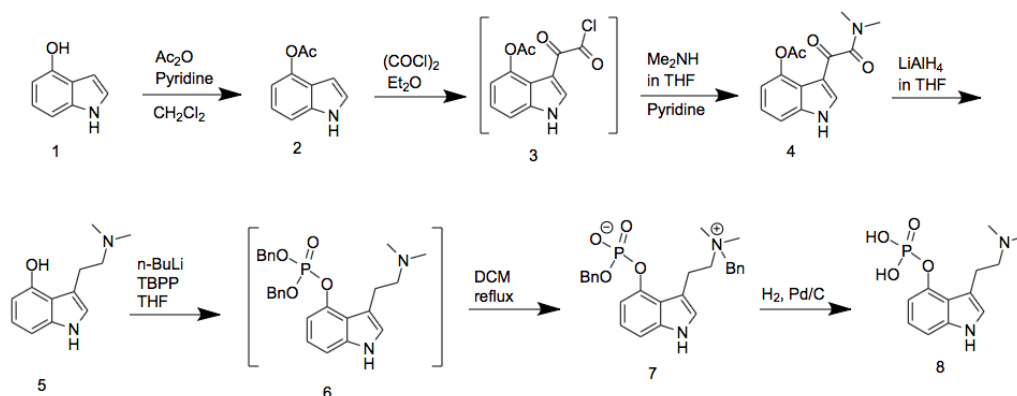
1.3 Clinical use

Since psilocybin is shown to be safe, have a long duration of action and high bioavailability, it became a good alternative for human experimental research for different treatments. Psilocybin has been in clinical studies for addiction of alcohol and nicotine where they received psilocybin doses depending on body weight for 12 or 15 weeks, respectively [5]. Alcohol dependence study showed the addiction decreased during the first month, whilst in nicotine addiction the results were remarkably well where 80 % of participants developed abstinence after six months [5]. Psilocybin has also been in phase II and III in clinical trials for treatments of anxiety and depression particularly for patients with cancer diagnosis. In a study by Johnson et al [9], the data from psilocybin showed with high doses of psilocybin almost 80 % of the patients resulted in an increased of positive effects such as optimism and improved anxiety symptoms. However, some side effects could be seen from this study for patients with high blood pressure, but this occurred only in high doses. In contrast to most psychiatric pharmaceutical treatments that are to be taken daily, the large potential of psilocybin lies in that a single or few treatments are sufficient for a durable effect. Hence, apart from the small side effects psilocybin verified to be a promising pharmaceutical for treatment of addiction, anxiety and depression [10].

1.4 Synthesis

Whilst the first synthesis of psilocybin made by Hoffman et al [11] was extremely useful for other scientist, it still needed some improvements of the yield and purity of psilocybin. An example of improvement from [11] synthesis, was the phosphorylation of psilocin to psilocybin where Hoffman used an impure dibenzyl phosphoryl chloride in carbon tetrachloride solution. In a study by Nichols and Fresca [12] demonstrated the negative properties of using dibenzyl phosphoryl chloride such as being unstable and used tetra benzyl pyro phosphate (TBPP) instead. Additional developments were needed in order to clinically use psilocybin, Shirota et al [13] and Sherwood et al [14] performed similar synthesis of psilocybin but with different purposes.

The general synthetic approach to psilocybin is presented in scheme 1, where Speeter-Anthony tryptamine synthesis (STS) is performed. STS is a synthetic route whereby a tryptamine is produced from a reaction with an indole and oxalyl chloride followed by a reduction [15]. In [13] an Acylation of 4-hydroxyindole **2** was synthesized, thereafter, STS was applied and resulted into psilocin **5** with 85% yield. The last step was the phosphorylation of psilocin to acquire psilocybin where Shirota et al [13] used TBPP with the strong base butyllithium (n-BuLi). This phosphorylation reagent was selected because it showed relatively good stability from [12]. Also, this experiment was successively made without utilizing any chromatographic purifications [13]



Scheme 1. Psilocybin synthesis acquired from Shirota et al [13].

In Sherwood et al [14], the synthesis was similar to Shirota et al [13] but with some developments and more practical synthesis for a large scale. The same phosphorylation reagent, TBPP, was used in this synthesis. The main purpose in this synthesis was to produce a relatively pure rather than high yield of psilocybin in order to be used in clinical study [14].

1.4.1 Drawbacks of literature methods

The phosphorylation of psilocin to synthesize psilocybin is still challenging. This is because a zwitterionic material **7** in scheme 1 occurs when phosphorylating agent is added. The intermediate, O,O-dibenzyl ester of psilocybin **6** seemed to be very labile because one O-benzyl group undergoes hydrolysis according to Nichols and Fresca [12]. While Shirota et al [13] presented that compound **6** in fact formed a benzyl migration resulting in compound **7**. Nevertheless, the zwitterionic compound happened to be complex to purify and thus resulted into a low yield of psilocybin. All the previous experiment done on psilocybin except Hoffmann's synthesis, used the same phosphorylating reagent, TBPP, this awakens questions such as the why is the expensive TBPP preferred? Can another phosphorylating agent with different base be utilized instead? How does different phosphorylating agent affect the purity and yield of psilocybin?

1.4.2 Benzylated psilocin

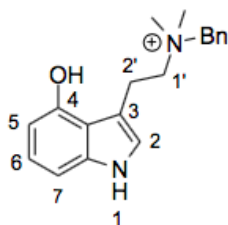
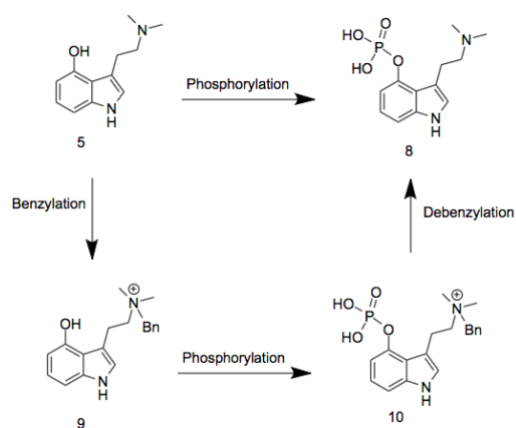


Figure 1. Structure of N-benzylated psilocin.

By synthesizing a benzylated psilocin with benzyl bromide it can minimize the possibilities of phosphorylation reagent reacting with nitrogen in position 1'. This would enable the

electrophilic phosphorylation reagent only reacting with hydroxyl group by obtaining a quaternary ammonium ion. Benzylated psilocin can increase the possibility of utilizing simple phosphorylating reagents which would normally not react with psilocin. This could examine whether benzylated product improves phosphorylation by reducing number of byproducts and obtain a pure psilocybin. Scheme 2 presents the suggested phosphorylation of psilocin and benzylated psilocin. The pH in benzylation is important to monitor in order to prevent reaction of hydroxyl group on position 4. The debenzilation of intermediate **10** to psilocybin **8** in scheme 2 is used in [14].



Scheme 2. Phosphorylation of psilocin and benzylated psilocin.

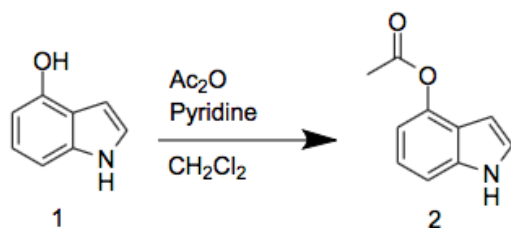
2. Aims and purposes

The aims of this project are to develop and optimize the synthesis of psilocybin through phosphorylation as compared to a reproduced literature synthesis of Shirota and Sherwood.

The purpose of the second aim is to find an optimal phosphorylation reagent and conditions for psilocin. Also, a question of issue in this project is if a benzylated psilocin make an easier phosphorylation?

3. Results and Discussion

3.1 Acylation



Scheme 3. Synthesis of 4-acetoxyindole.

The first step of synthesis was followed from Shirota et al [13] where the commercially available 4-hydroxyindole was acylated at room temperature in both open system and dry conditions. An open system is a method where a reaction exchanges energy with its environment. Acylation is necessary to avoid reaction between the alcohol and oxalyl chloride in the next step. This step was synthesized with acetic acid and pyridine as the base in dichloromethane (DCM) solvent, see *scheme 3*. As said, this step was conducted in both open system and in dry conditions with one gram-scale to compare the results. The reaction time did not differ between open system and dry condition therefore the first alternative was chosen for higher scale because of a more facile set up. Liquid chromatography – mass spectrometry (LCMS) showed completion of reaction, it differed slightly from the literature by two extra hours. Also, in the workup after evaporating the product, it was not necessary to collect the product by filtration and wash with H₂O/ethyl acetate (EtOAc) as stated in Shirota. However, a co-evaporation with DCM was enough in order to obtain a pure ivory/light black product with 78% yield, see *Figure 2*. A scale of 7g was done of 4-acetoxyindole with the exact procedure as previous. It occurred some problems during workup, due to spill of product in filtration of drying agent and it consequently led to low yield. However, the spilled product was collected by tissue extraction and dissolved in acetone in a beaker overnight. This was extracted with EtOAc, dried and co-evaporated once again which resulted into a 16% yield but nuclear magnetic resonance (NMR) indicated pure product, see appendix. Therefore, the extracted product was combined with the original product. The total yield of final intermediate **2** became 40.5%.

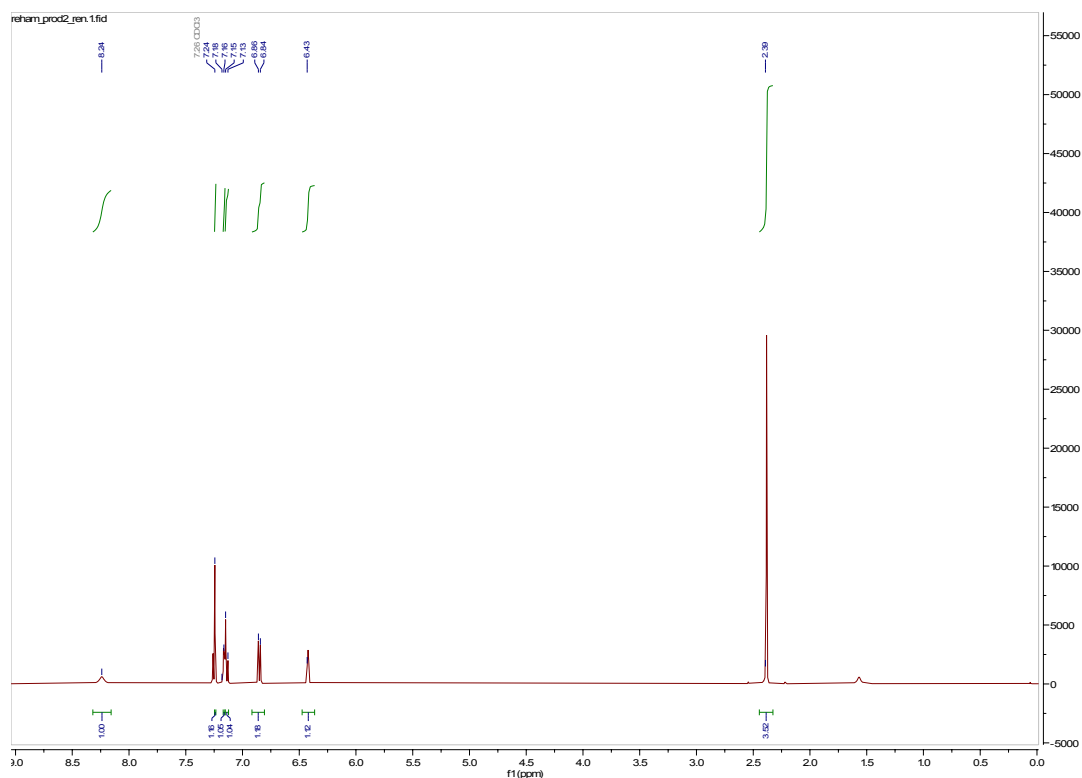
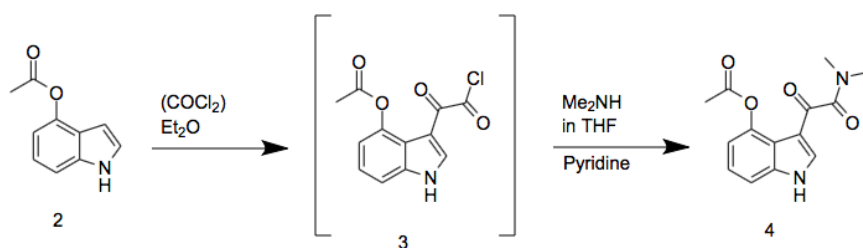


Figure 2. ¹H NMR spectrum of pure product **2**.

3.2 Acid chloride formation/dimethylamine addition



Scheme 2. Synthesis of intermediate 4.

The second step of the synthesis was the one-pot where intermediate **3** is formed but not isolated, followed by dimethyl amine addition according to Sherwood et al [14]. There were many complications with this step because firstly the synthesis was not conducted under dry conditions as in Sherwood et al [14], it was performed in open system to observe the differences between the two conditions. The open system led to occurrence of a homogenous solution instead of a precipitation of intermediate **3** when oxalyl chloride was added. Also, the LCMS analysis showed another mass which indicated the occurrence of a hydroxyl group instead of chloride. The acid formed due to air, by adding more oxalyl chloride it removed the acid. When there was no acid remaining in the solution from LCMS, intermediate **3** was filtrated in order to remove excess of oxalyl chloride and dissolved in tetrahydrofuran (THF). This led to occurrence of the acid again in solution according to LCMS. So, in order to prevent appearance of the acid during filtration, the supernatant could be removed by pipette and dissolved the precipitate with THF.

Secondly there was no dimethylamine in THF solution available in the lab, only the salt of dimethylamine. The appropriate methods of using salt is either use it directly into the reaction with double equivalents of pyridine or extract dimethylamine with NaOH from the salt. These two reactions were both performed in small test tubes in order to determine the better method to use in gram-scale. Product **4** occurred in both of the samples according to LCMS, so the easier option for further experiments was the salt and double equivalents of pyridine. Pyridine in THF was added into the reaction to speed up the reaction, but the reaction lasted longer than in literature. However, this led to correct product **4** obtained according to LCMS but with impurities and longer reaction time compared to literature. This could depend on that the reaction was not conducted under dry conditions or that a salt of dimethylamine was used instead of a solution in THF. Since the reaction was done in open system it affected the results and provided a low yield. Since the reaction time was long, more dimethylamine salt was added with one equivalent of triethylamine base to speed up the reaction. When the reaction was finished, iso-hexane was added to dissolve the impurities and filtrate the product followed by addition of water to remove the pyridinium salt. The solution was filtrated once again and finally triturated to remove the remaining organic impurities, this resulted in 8.9 % yield which was very low. The loss of product could depend on the reaction performed in open system instead of dry conditions or that the salt of dimethylamine was used instead of a solution as in literature. The low yield indicated an enormous amount impurity of the intermediate.

This step was also conducted in dry conditions with scale of 500 mg compared to open system. The results from LCMS showed no evidence of the acid that occurred in first attempt, this indicates that it is crucial to use dry condition in this particular step. Furthermore, only 1.5

equivalent of oxalyl chloride was enough which meant there was no need to add more oxalyl chloride since the acid did not show. Intermediate **3** was obtained smoothly and quickly while in dry conditions. Also, since the solution was a suspension the supernatant could easily be removed by syringe and prevent loss of product. Intermediate **3** was dissolved in THF and the reaction could continue without filtrating off excess of oxalyl chloride. The use of syringe was essential since intermediate **3** could easily come in contact with air and convert into acid seen before. So, by removing supernatant and still under nitrogen was difficult however, the acid was not seen in LCMS throughout this process which made it a worthy step. Thereafter, in order to obtain a purer product **4**, dimethylamine solution in THF was commercially purchased for this step. When this solution was used in the reaction and still in dry condition product **4** was obtained in pure form and trituration resulted into a clear precipitation. When pyridine in THF was added the reaction was completed after 1.5 h according to LCMS almost the same time as in literature, a better result compared to first attempt. Since everything was done according to literature, it resulted into 32% yield which was a much higher yield than obtained previously. However, the yield is still low and can be improved by not filtrating intermediate **3** or when filtrating, aim to transfer the product for removal of pyridinium salt and trituration slowly with pipette so it doesn't cause loss of product.

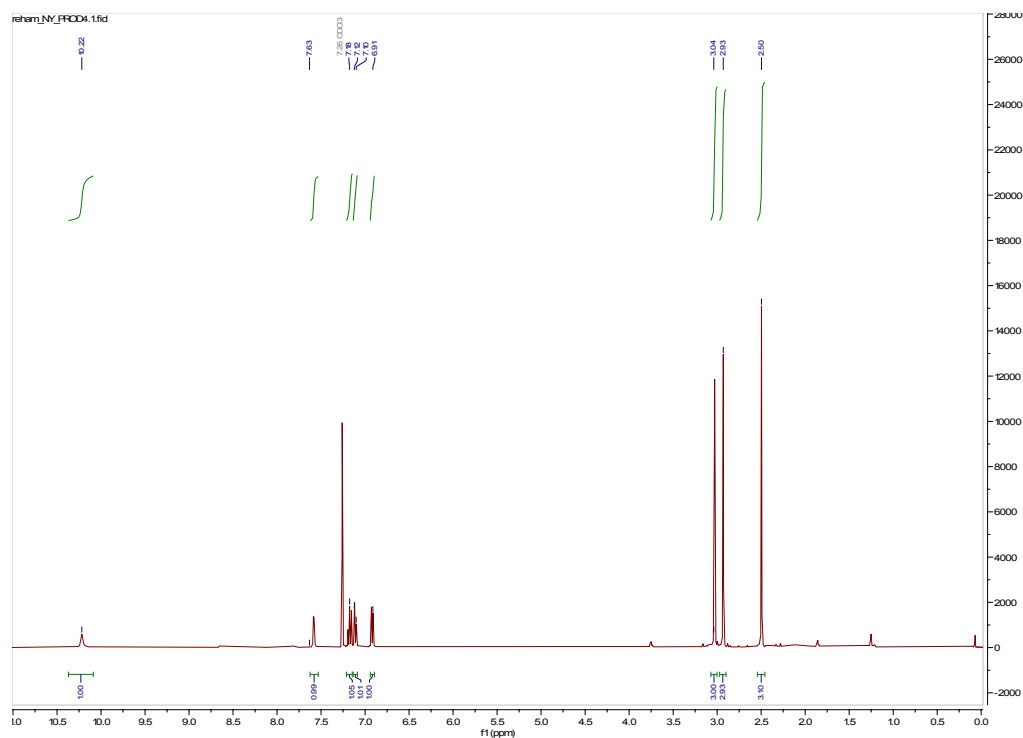


Figure 3. ^1H NMR spectrum of pure Product **4**.

A summary of how to perform the synthesis of compound **4** is presented in *figure 4* to prevent unnecessary complications and obtain **4** successfully.

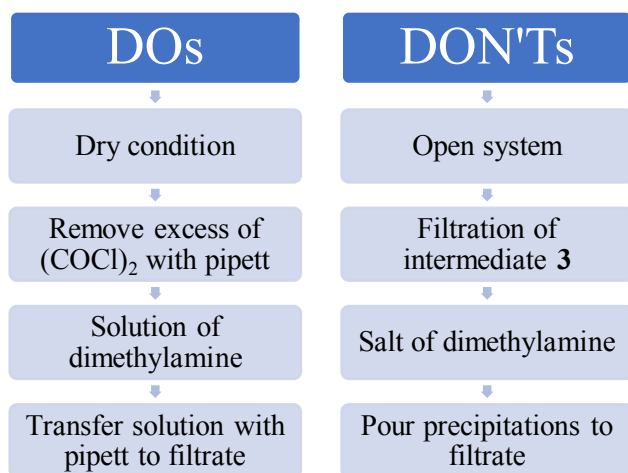
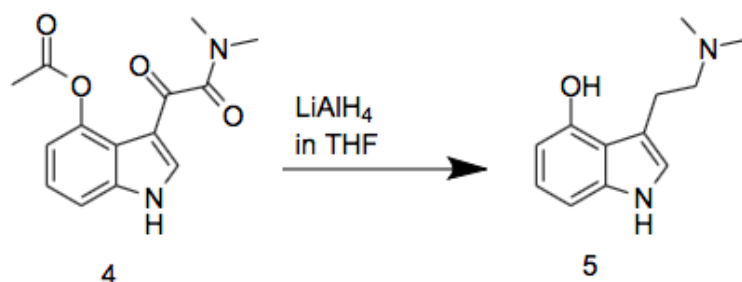


Figure 4. Process chart of do's and don'ts in synthesis of compound **4**.

3.3 Reduction



Scheme 5. Synthesis of psilocin.

Reduction step was done on the impure product **4** since the pure product was not synthesized at this point. The solvent available in the lab was only lithium aluminum hydride (LAH), LiAlH₄, in THF and not in 2-methyltetrahydrofuran solvent. The regular THF solvent extended the refluxing time due to poor stability as stated in [14] therefore if 2-methyltetrahydrofuran with higher boiling point was used it would possibly lead to a shorter reaction time as in literature. Another reason could be that product **4** was still impure so it required more time to react, therefore the reaction was left overnight at 66 °C to ensure complete production of psilocin. After reduction, psilocin showed on LCMS indicating correct product obtained however with some impurities and the desired product was the minor component. Unlike Sherwood et al [14], a column chromatography was used in order to purify psilocin in milligram-scale. Psilocin is very sensitive; it decomposes to either air or light. This made the synthesis more complex, however the experiment was conducted in dark fume hood as well as purification with column chromatography. The solvent was selected from numerous experimental analysis with thin layer chromatography (TLC) results, indicating DCM/MeOH/NH₄OH (100/5/1) to be favorable for this separation. This separation was monitored with both TLC and LCMS for assurance of right product in different fractions. LCMS showed certain fractions containing psilocin, these were latter collected and evaporated. After evaporation psilocin was obtained as an oil, not solid as expected.

¹H NMR spectrum for this product showed to be other than expected product and due to lack of time this product could not be identified. The reaction mixture was complex prior to column chromatography according to LCMS. However, since LCMS showed appearance of psilocin in the solution as minor component before purification, it could still lead to obtaining pure

psilocin. Furthermore, an ^1H NMR of the crude product before column chromatography was not conducted, this would have possibly prevented unnecessary purifications. The favorable method is to isolate exactly as in Sherwood et al [14] only silica and DCM were added followed by filtration and evaporated, however a drawback of this approach was the low yield despite of obtaining a pure product in literature. If this approach were to be used, it could have saved time and obtain the correct product, but possibly with low yield as in [14]. At the same time, product **4** used was still impure so this affected the results. Also, since psilocin is very sensitive, it could mean the obtained product is a decomposed psilocin, but this could not be confirmed.

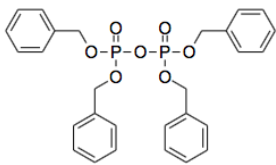
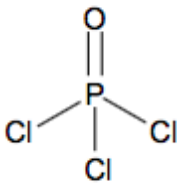
3.4 Phosphorylation

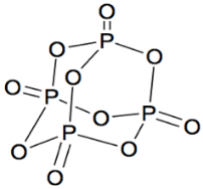
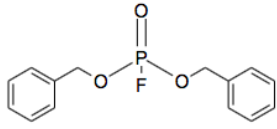
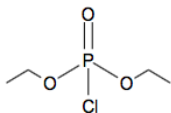
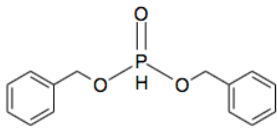
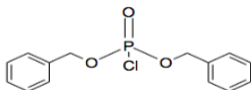
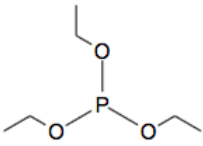
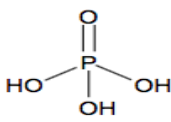
Since the published synthesis on psilocybin use the same phosphorylating agent from Nichols et al [12], TBPP. Due to lack of time, the experiments of selected phosphorylation reagents were not conducted therefore the results could not be compared to TBPP used in literature. Nevertheless, the study was to select several phosphorylating reagents and plan an efficient analytical procedure to determine yield and purity of tested conditions/reagents without the time-consuming isolation of psilocybin.

Phosphorylation would be tested on both benzylated psilocin and psilocin in parallel. Benzylated psilocin would easily be synthesized according to Grove et al [16] by reacting psilocin with benzyl bromide in acetonitrile. This reaction is left under room temperature overnight which should be possible without decomposition of psilocin in the absence of light and air. Furthermore, the monitoring of pH in N-benylation synthesis is needed to avoid reaction of benzyl bromide with hydroxyl group. Therefore, in order to prevent the phenol from deprotonation at pH 11 is necessary to perform N-benylation in pH 9-10. This allows deprotonation of amine at pH 9 to obtain benzylated psilocin whilst avoiding reaction with phenol. So, the conditions of benzylated psilocin would possibly be optimized throughout this synthesis.

There is a disadvantage with utilizing TBPP, being an extremely expensive reagent, which is not attractive for large scale synthesis. There are many phosphorylating reagents that are cheaper alternatives. The planned phosphorylating reagents to be tested in this synthesis are presented in *table 1*.

Table 1. Summary of different phosphorylating reagent.

Phosphate name	Phosphate structure	Advantages	Disadvantages	Reference
Tetra benzyl pyrophosphate		Stable	Expensive	[14]
Phosphoryl chloride		Available and cheap	Hydrolytic unstable	[17]

Phosphorus pentoxide		Inexpensive and safe	Hydrolytic unstable	[18]
Dibenzyl phosphorofluoridate		Stable and reactive	Not available - must be synthesized	[19]
Diethyl Chlorophosphate		Available and reactive	Hydrolytic unstable	[20]
Dibenzyl Phosphite		Available and inexpensive	Low reactivity	[21]
Dibenzyl Phosphorochloridate		Reactive	Unstable	[22]
Triethyl Phosphite		Stable and inexpensive	Less electrophilic	[23]
Phosphoric acid		Available and cheap	Not as reactive as the above	[24]

The first planned reagent was going to be phosphoryl chloride, POCl_3 , which is widely used for phosphorylation and its commercially available, this makes it very attractive reagent. Conditions of Goryunov et al [17] was planned to be used, with excess of alcohol and magnesium as a catalyst, the reaction was successively heated from 140-200 °C which was later chromatographed on aluminum oxide, Al_2O_3 , with ether as eluent. This would result into a phosphate, its simple and straightforward reaction which makes it attractive. The exact conditions (temperature and reaction time), yield and purity could not be determined for psilocin and N-benzyl psilocin.

Phosphorous pentoxide, P_2O_5 , is another commonly available reagent for phosphorylation reactions. This has been used for phosphorylating chitin molecules where in acid conditions convert the alcohol substituent is converted into a phosphate [18]. The acid was used for esterification and enabled the P_2O_5 to react with the oxygen and resulted into O-phosphorylation. This resulted into a high yield which made this reagent an interesting candidate for psilocin. Another reference used P_2O_5 also, reacting triethyl phosphate onto an

aromatic alcohol and yielding 81% yield of a phosphate product [25]. The reaction was performed at 120 °C for only 24 h which resulted into a pure product.

An uncommon phosphorylating agent, dibenzyl phosphorofluoridate, was shown to be a good reagent when reacted with alcohols activated by cesium fluoride in acetonitrile by Watanbe et al [19]. This reaction was performed at room temperature which is very simple and straightforward. This type of reagent is unique because it uses fluoride as leaving group instead of the regular chloride, fluoride don't end to be a good leaving group but in [19] study it reacted smoothly with the alcohol and obtained the phosphate. Dibenzyl phosphorofluoridate is very stable compared to and by utilizing cesium fluoride as a base it facilitates the reaction and acquires higher yield. Cesium fluoride is a safer base compared to BuLi as used in previous literature studies which makes this synthesis attractive.

Diethyl chlorophosphite is electrophilic and under basic conditions it reacts with alcohol and provides O-phosphorylation. This reagent was used under dry conditions with pyridine as base and was shown to be effective and reactive providing a high yield of the phosphate. The usage of pyridine as a base makes this a convenient reaction compared to using TBPP [20].

Dibenzyl phosphite reagent is not as common as diethyl chlorophosphite even though the structure is similar. In Silverberg et al [21], it was shown to be efficient as phosphorylation reagent when used in acetonitrile, carbon tetra chloride, CCl₄, with the catalys 4-dimethylaminopyridine (DMAP) and N,N-diisopropylethylamine (DIPEA) as base at room temperature. This resulted into producing O-phosphorylation of different phenols rapidly with high yield. In this study they also stated that this strategy could enable scaleup. This is an advantage for this project aiming to optimize psilocybin synthesis and aiming to be applicable also in larger scale. Furthermore, in this process, DMAP and DIPEA were used which are more convenient reagents than BuLi.

The dibenzyl phosphorochloridate reagent is similar to one before but with chloride as leaving group instead, this has also showed to be a good phosphorylating agent for both alcohol and phenols. In Inage et al [22], the reagent was used with the strong base BuLi which resulted into dibenzyl phosphate. It gave a high yield, but the base is the same as in [14] which is in fact not favorable but since it showed pretty good results it is worth an attempt as a reagent.

Triethyl phosphate is in reality only used for phosphonates however in Stowell et al [23], where they dissolved it in dichloromethane and added iodine at 0°C it became a phosphorylating agent, which it was latter added into the alcohol with pyridine as base. This resulted into a phosphate with 98% yield and 97% purity after column chromatography. This is different and not so common comparing to other reagents for this reason it's a proper candidate for this reaction.

Phosphoric acid would be the last tested reagent since its indeed a very simple reagent to handle, not toxic and available however it's not as strong electrophile as the previous stated examples. However, it has been proven by Sakakura et al [24], that treating an alcohol with phosphoric acid in room temperature results in high yield of phosphates, the reaction was done in Soxhlet apparatus in order to collect the water forming in the reaction and the basic condition was chosen to be tributylamine. Tributylamine is used as a proton scavenger for the alcohol in order for the oxygen to perform a nucleophilic attack. The mild conditions make this an attractive alternative to TBPP and BuLi.

A summary of advantages and disadvantages of each selected phosphorylating reagent is presented in table 1 as stated previously. The most favorable reagent has to be reactive, stable and performed in mild conditions in order to provide decent results of psilocybin. Even though an experiment was not conducted, the most suitable reagent could be dibenzyl phosphorofluoridate because its unique, stable and reactive. Also, the conditions used are mild compared to published synthesis of psilocybin. Even though this reagent is not available, if it would provide high purity and yield of psilocybin it would be a worthy step.

Instead of time-consuming isolation of psilocybin from each tested reaction conditions, it was planned to use LCMS-based yield and purity determination. Standard solutions with pure psilocybin dissolved in ethanol and an internal standard (e.g. toluene) would allow preparation of a calibration curve. An internal standard is needed to correct unavoidable uncertainties in injection volume. The standard solutions would span several concentrations to prepare a calibration curve covering all theoretically achievable yields. The LCMS analysis would also result in a purity assessment and determine unknown products in the different phosphorylation conditions, without isolation of psilocybin. By preparing a calibration curve also with psilocybin by standard solutions and analysis in LCMS, the conversion of psilocin starting material to the desired product psilocybin could be determined. So, this could easily provide the conversion of starting material and yield of psilocybin without any isolation. This analytical procedure could enable us to test even more reagents than the selected ones in case those in *table 1* don't provide decent results of the yield and purity of psilocybin.

4. Conclusion

Insights during synthesis for the first aim were firstly, to perform the dimethyl amine addition synthesis in dry conditions from the beginning to avoid occurrence of biproducts. Secondly, even though the salt of dimethylamine could be employed, it was necessary to use the commercially available solution of dimethylamine and consequently lead to an improved reaction time. Also, it was not optimal to use an impure product for reduction regardless of purification afterwards. As a conclusion, it was critical to confirm and purify every product in this synthesis.

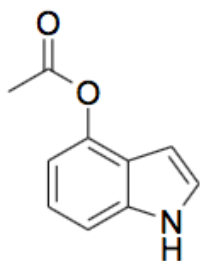
A literature survey was conducted to identify potential phosphorylation reagents depending on reactivity, stability and availability however, due to lack of time the experiments of the chosen reagents was not performed. Thereby, the most suitable reagent and conditions leading to higher purity and yield of psilocybin could not be determined. Nevertheless, the selected reagents for the study are suitable for a large-scale synthesis since they are stable, available and inexpensive (unlike TBPP). The usage of benzylated psilocin would possibly lead to an easier phosphorylation. This could result into lower cost, higher yield and purity of psilocybin. However, this cannot be confirmed without experimental verification.

In conclusion, this project resulted in partially good accomplishment of the first aim, with pure intermediates up until psilocin however with a low yield. This report enables further research on selecting an appropriate phosphorylation reagent and also suggests impact of benzylated psilocin for psilocybin synthesis. This is certainly a synthesis with enormous potential for the pharmaceutical process development.

5. Experimental

General. The starting material, reagents and solvents were commercially available and acquired from sigma Aldrich. Thin layer chromatography was performed on silica gel 60 F₂₅₄ plates and visualized with UV light at 254nm. An LCMS instrument was coupled to Agilent 1100 series HPLC and C18 Atlantis T3 column (3.0x50.0mm, 5mikrom) and the utilized mobile phase acetonitrile/deionized water with flow rate 0.75ml/min over 7.5min, This was coupled to waters micromass ZQ mass spectrometer with electrospray ionization a detector. Column chromatography was prepared with packed silica gel 60 (40-63 mikrom). Nuclear magnetic resonance spectra were recorded on Varian Mercury spectrometer at 400 MHz for proton spectrum. The chemical shift is reported in ppm with solvent reference CDCl₃ (δ =7.26 ppm). The coupling constant are reported in Hz.

4-acetoxyindole (Intermediate 2).

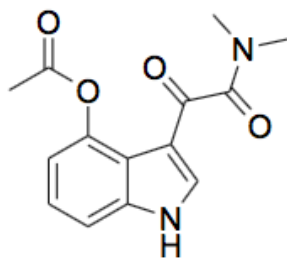


To a solution of 4-hydroxyindole (6.62 g, 50 mmol) in CH₂Cl₂ (60 mL) in a round bottomed flask was pyridine (5.35 mL, 66.5 mmol) and acetic anhydride (5.52 mL, 58.5 mmol) added while stirring in an ice bath. The mixture was stirred for 5 h in room temperature. After this time, H₂O (10 mL) was added and the mixture was evaporated in vacuo. The concentrate was dissolved in EtOAc and washed with (2 x H₂O) and once with brine. The organic phase was combined, dried over MgSO₄ and filtrated. The organic phase was evaporated in vacuo. The product was co-evaporated in CH₂Cl₂ and dried under vacuum. The solid was light black colored.

Yield (3.48 g, 40.5 %)

¹H NMR (400 MHz, CDCl₃) δ 8.18 (s, 1H), 7.20 (dd, J = 1, 7.6 Hz, 1H), 7.11 (t, J = 1 Hz, 1H), 7.09 (dt, J = 7.8, 16 Hz, 1H), 6.85 (dd, J = 1, 7.6 Hz, 1H), 6.42 (m, 1H), 2.32 (s, 3H).

3-dimethylaminoxylal-4-acetylindole (Intermediate 4).



Intermediate **2** (0.5 g, 2.7 mmol) was weighed into a two necked round bottomed flask with anhydrous Et₂O (6.5 mL) in dry conditions. The solution was stirred for 10 min in rt and then

cooled to 0°C in ice bath for 30 min. (COCl)₂ (0.34 mL, 4 mmol) in Et₂O (0.56 mL) was dropwise added with syringe, the temperature was kept under 5° C to prevent byproducts. The mixture was later stirred for 4h in ice bath. The reaction was monitored with LCMS. Intermediate **3** was completed after 4h. Iso-hexane (5 mL) was added and stirred for 30 min in ice-bath. The supernatant was collected with pipette and the Intermediate **3** was dissolved in dry THF (5 mL) and cooled to 0° C. The 2M solution of dimethylamine in THF (1.64 mL) was quickly added dropwise. Pyridine (0.43 mL, 5.35 mmol) in THF (0.94 mL) was added and stirred for 1h. Iso-hexane (6 mL) was added into the light-yellow solution and filtered with Büchner funnel. The filtered residue was transferred into a round bottomed flask and H₂O (40 mL) was added, it was stirred for 30 min. The solution was later filtered with Buchner funnel and triturated the off white solid with EtOAc/Hexane (6:4) for 40 min. The slurry was filtered once again with Büchner funnel and the solid was dried under vacuum.

Yield (0.23 g, 32%)

¹H NMR (400 MHz, Chloroform-*d*) δ 10.15 (s, 1H), 7.52-7.44 (m, 1H), 7.18-7.08 (m, 1H), 7.04 (dd, *J* = 1.1, 8.2 Hz, 1H), 6.85 (dd, *J* = 1.1, 8.2 Hz, 1H), 2.96 (s, 3H), 2.86 (s, 3H), 2.43 (s, 3H).

Unknown side-product. In dry conditions, **4** (0.14 g, 0.51 mmol) was dissolved in THF (1.2 mL). The solution was put in ice bath and charged dropwise with 1M LiAlH₄ in THF (2.5 mL, 5eq). Then the ice bath was removed and stirred for 30 min and then heated to reflux overnight. The reaction was monitored with LCMS. Thereafter, the reaction was quenched with THF/H₂O (1:3.7), Na₂SO₄ (0.4 g, 5.2 eq) and celite (0.2 g, 6.2 eq). This was filtrated and washed with DCM/MeOH (9:1). Column chromatography was used for purification with eluent DCM/MeOH/NH₄OH (100:5:1). The product obtained was yellow oil.

6. Acknowledgements

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Appendix

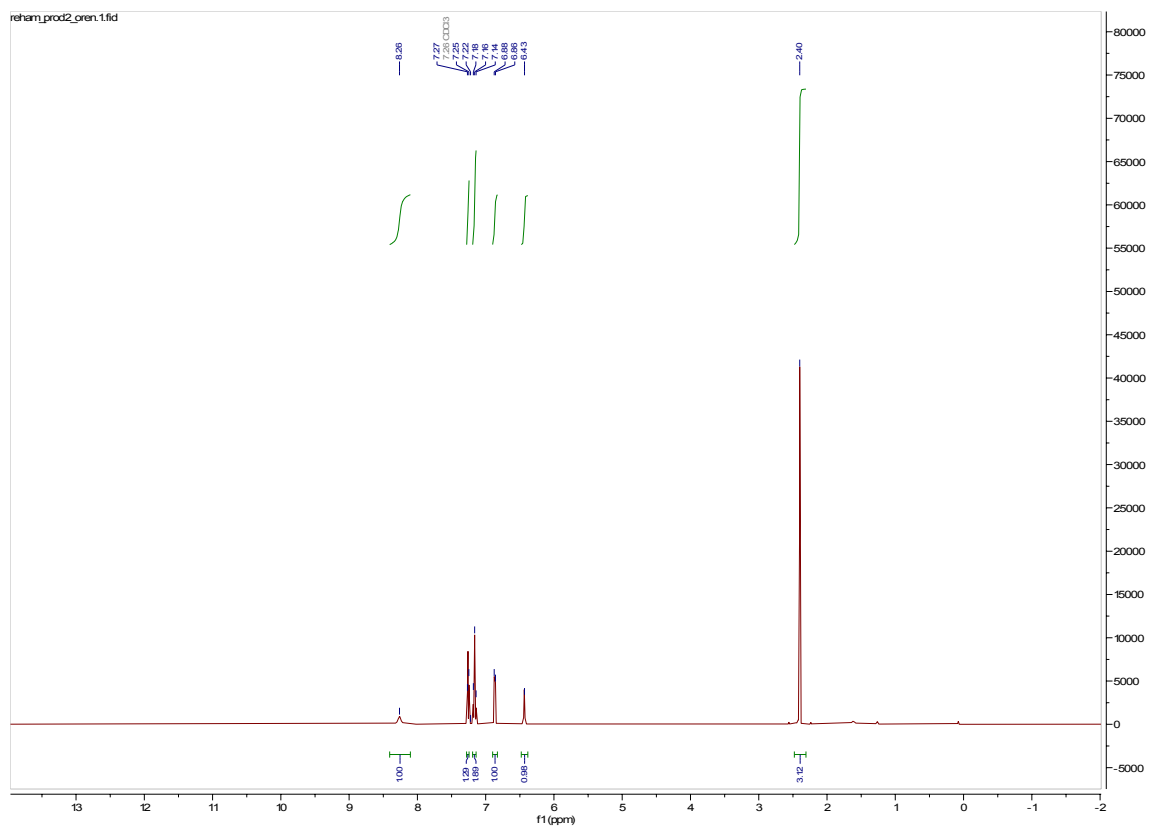


Figure 4. ^1H NMR spectrum for impure Intermediate 2.

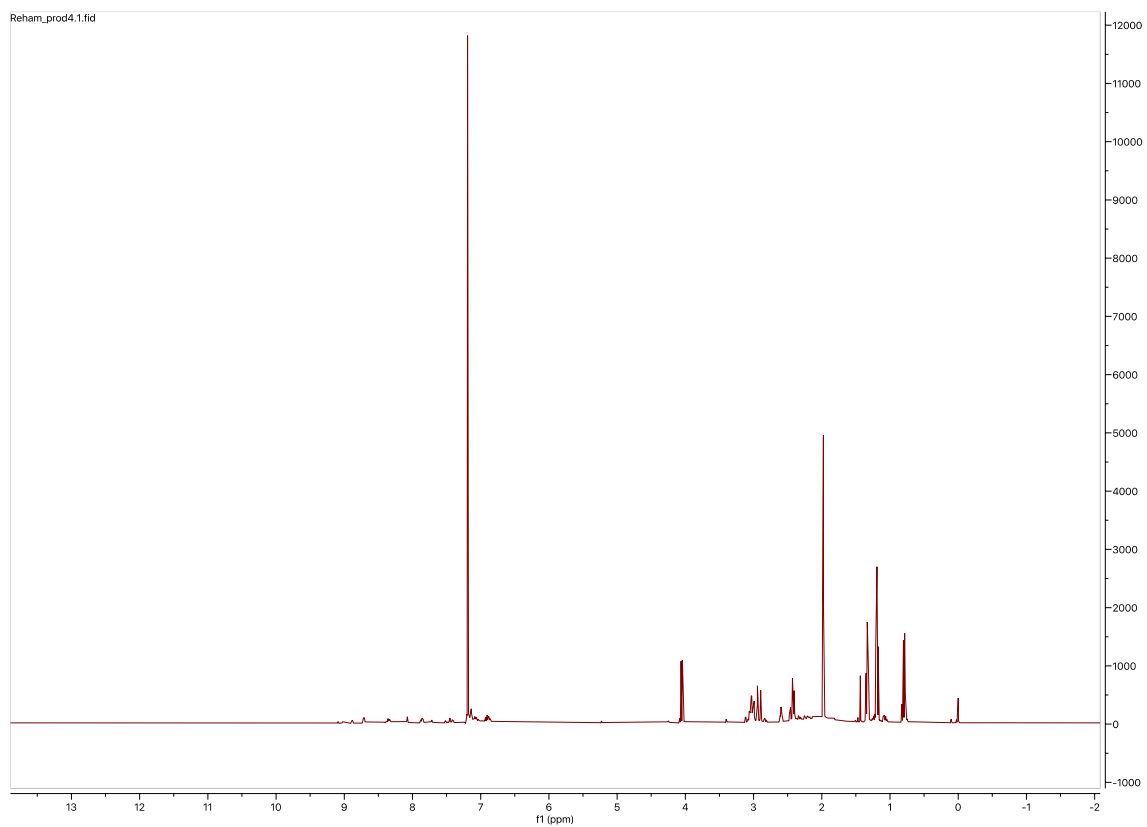


Figure 5. ^1H NMR spectrum of impure intermediate 4.