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Attempts to produce the enantiomers of thioridazine

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Acknowledgments

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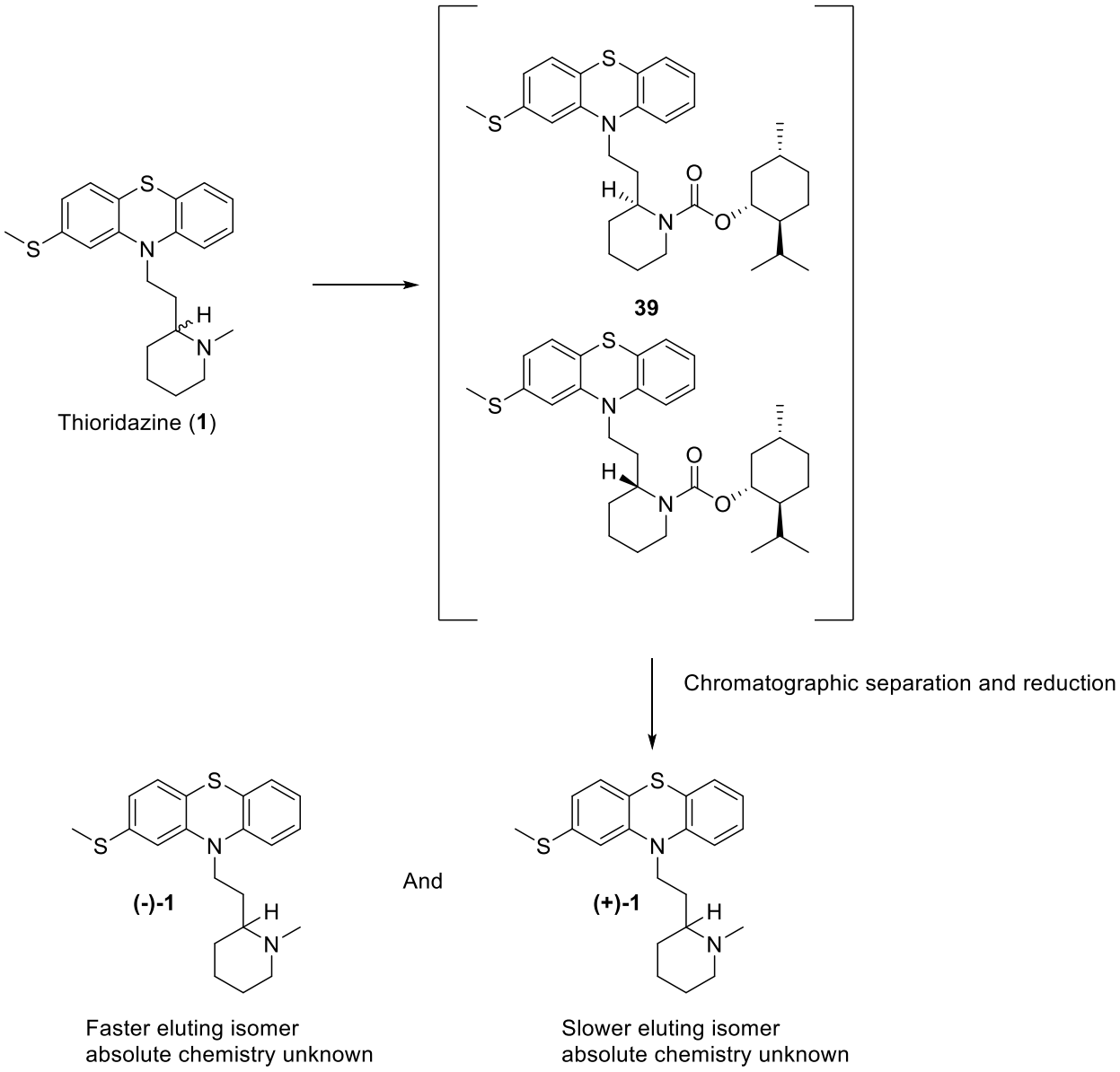
Ås, 13. Mai 2019

Erik Oscar Tylleskär

Graphical abstract

Racemate resolution



Attempt at synthesis

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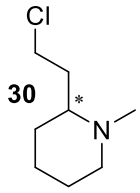
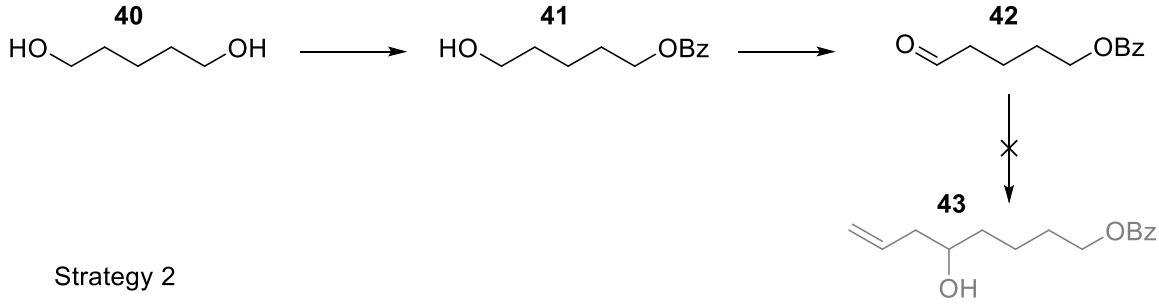


Strategy 1



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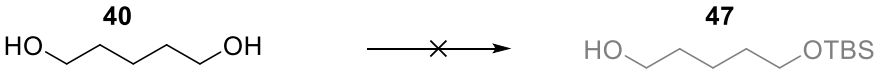

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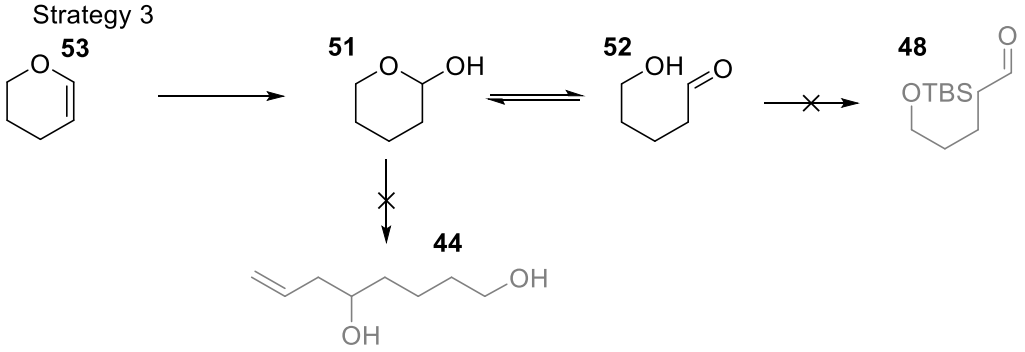
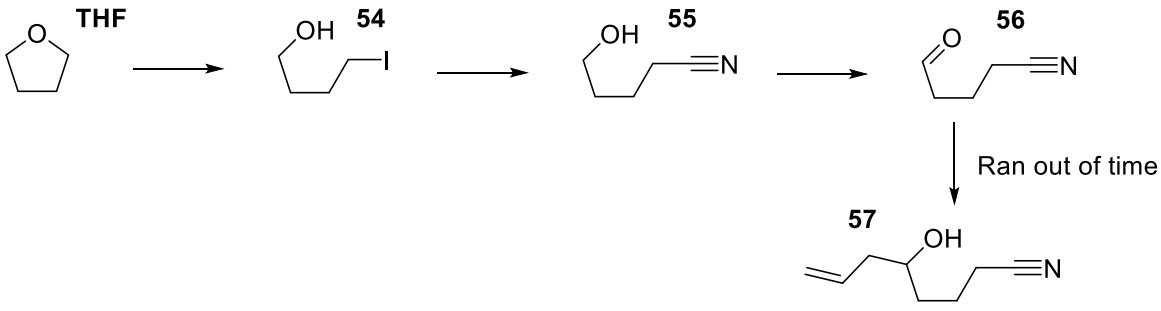


Strategy 4



Abstract

The aim of the project was to produce the enantiomers of thioridazine (**1**).

The enantiomers of **1** can be synthesized and isolated in multiple ways. The least efficient way is to first synthesize the molecule as a racemic mixture, and then use a chiral resolution to isolate the enantiomers. The first procedure describing this for **1** is from 1958. The procedure was copied step by step, but it was long and cumbersome. The process was based on reacting **1** with a tartaric acid derivative. The enantiomers reacted with different speeds and due to the different solubility of the diastereomers, they could be separated from each other. The main drawback with this procedure is that it has to be repeated several times to achieve sufficient purity. For this reason, a new procedure was tried.

This procedure used another chiral reagent, *R*-(-)-menthyl chloroformate (**38**). The resulting mixture of the two carbamates of **1** gave the diastereomers (**39-f and 39-s**) that were possible to separate on a silica column. Also, the procedure had no time-consuming reactions. This chiral resolution was a success and the yields were similar to the reported ones. However, the separation was not as good as the reported. A small portion co-eluted. The method was described as a "one-pot" procedure, but the method was also done with purification between each step. This gave the same purity, but lower yields. So there was no benefit to purifying between each step. Optical rotation was measured to -22° for **(-)-1**, and +19° for **(+)-1**, in accordance to reported values.

The next part focused on trying to do a total synthesis. There have been total syntheses reported earlier, but only of the racemic mixture. There is a reliable reaction between piperidine derivative **30** and the corresponding phenothiazine **31**, so the goal was to make **30** as a pure enantiomer. There is a reported synthesis of **30** from 2015 that we tried to copy, but replacing the toxic allyl tributyltin with the more benign allyltrimethylsilane. This did not work because the addition happened in the protection group. The strategy was changed to use a silyl-based protecting group. Multiple groups and conditions were tried but none worked, so this strategy was eventually abandoned. The last strategy that was tried started with THF that was opened with I_2 and TMS, then replacing iodine with CN to create a five-carbon skeleton. This was followed by oxidation of the alcohol to the corresponding aldehyde. This oxidation was tried with many different methods, but Dess-Martin seemed to work best. Finally, we tried to introduce an allyl group via a Grignard reaction. According to NMR spectral analysis the reaction did work, but running out of time caused the reaction to not be elaborated further.

Sammendrag

Målet med oppgaven var å lage de ulike enatiomerene av thioridazin (**1**).

Enantiomerene av **1** kan syntetiseres og isoleres på ulike måter. Den minst effektive er å lage stoffet som en rasemisk blanding, og deretter skille enantiomerene ved å utføre en rasematoppløsning. Den første prosedyren som beskriver dette er fra 1958. Den ble kopiert trinn for trinn, men den var lang og tungvint. Den gikk ut på å reagere **1** med et vinsyrederivat. De to enantiomerene reagerte med ulik hastighet, og diastereomerene hadde ulike løselighet i aceton. Resultatet var at de lot seg skille fra hverandre. Problemet var at dette krever mange steg for å få en høy renhet, derfor ble denne metoden forkastet og en nyere racematoppløsning ble forsøkt.

Den nyere fremgangsmåten brukte et annet kiralt reagens, *R*-(-)-mentyl kloroformat (**38**). Den resulterende blandingen av karbamater av **1** ga to diastereomere (**39-f og 39-s**) som var mulig å separere på vanlig silikakolonne og hadde ingen tidkrevende reaksjoner. Denne rasematoppløsningen ble en suksess og utbyttet ble likt som i litteraturen. Separasjonen gikk dog ikke like bra som i litteraturen, da vi opplevde co-elusjon. Metoden beskrevet var «one pot», og metoden ble også prøvd med rensing mellom hvert trinn. Dette ga samme renhet, men dårligere utbytte. Optisk rotasjon til **(-)-1** ble målt til -22° i EtOH, og **(+)-1** ble målt til +19°, noe som stemmer god overens med det som er rapportert.

Neste del av oppgaven gikk ut på å prøve og gjøre en totalsyntese. Det er gjort synteser tidligere der piperidinderivatet **30** blir reagert med den korresponderende fenotiazinen **31** i godt utbytte. Så planen først var å lage **30** asymetrisk for så å koble den sammen med resten av molekylet. I en publikasjon fra 2015 beskrives en asymmetrisk syntese av **30**. Denne ble forsøkt kopiert, men med erstatning det giftige reagenset allyltributyltin med allyltrimetylsilane. Dette fungerte ikke da addisjonen skjedde i beskyttelsesgruppen, så reaksjonsrekken skulle prøves på nytt med en sillylbasert beskyttelsesgruppe. Denne beskyttelsen fungerte ikke så denne reaksjonssekvensen ble oppgitt. Den siste strategien som ble prøvd gikk ut på å starte med THF som ble åpnet opp med jod. Jodidet ble reagert med cyanid for derved å gi den tilsvarende 5-karbonenheten. Så ble alkoholen oksidert til det tilsvarende aldehydet, som ble etterfulgt av en Grignardreaksjonen for å innføre en allylgruppe. Dette så ut til å være vellykket ifølge analyse av NMR-spekteret av råoljen. Grunnet tidsnød ble ikke denne reaksjonen fulgt opp videre.

Abbreviations

BiNOL	1,1'-Bi-2-naphthol
CDR	Chiral derivation reagent
DMP	Dess-Martin periodinane
DMSO	Dimethyl sulfoxide
ee	Enantiomeric excess
MDR-TB	Multidrug resistant tuberculosis
MIC	Minimum inhibitory concentration
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSSA	Methicillin-Sensitive *Staphylococcus aureus*
SPM	Specialized pro-resolving mediator
TB	Tuberculosis
TBS	Tert-butyldimethylsilyl
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TES	Triethylsilyl
TMS	Trimethylsilyl
XDR-TB	Extremely drug resistant tuberculosis

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1 Introduction

1.1 General background and aim

Thioridazine (**1**, Figure 1-1) is a phenothiazine derivative, that was discovered in 1951. Originally used to treat schizophrenia, it has also been shown to help make bacteria sensitive to antibiotics again.[1] These studies have revealed that the negative side effects and the effectiveness against bacteria vary between the two enantiomers of **1**.[2-4]

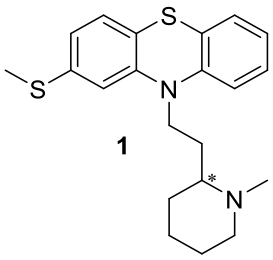


Figure 1-1. The structure of thioridazine (1), the unspecified stereocenter is marked with an asterisk.

The aim of this master project was to prepare the two enantiopure enantiomers of thioridazine. These two isomers and the racemate will be investigated against bacterial cells to investigate the antimicrobial effects.

This project is part of a collaboration with a group at the University of Southern Denmark, and further experiments will be carried out by our Danish colleagues.

For the previous experiments on enantiomers of thioridazine, the enantiomers have been prepared by chiral resolution of the racemate. As the published procedures have limitations, we wanted to develop an asymmetric strategy that can give both enantiomers in high yields.

1.2 Medicinal chemistry

Medicinal chemistry is the study of structure, synthesis, design and development of bioactive drugs. The first drugs ever used were natural products, and evidence suggests that this precedes recorded human history.[5] Natural products are still of interest because they have evolved over thousands of years by evolution, and fulfills some sort of useful purpose either defending against predators or helping the organism itself. Creating natural products in an organism uses a lot of energy, so if the product serves no useful purpose, that organism will have a disadvantage from an evolutionary perspective. A classic natural product used as a medicine is penicillin G (**2**), the first antibiotic discovered by Alexander Fleming.[6] With modern spectroscopic methods and synthesis knowledge a new category within medicinal chemistry

was created: natural product analogs. By modifying nature's own structure, chemists could modify the structure in order to suppress or enhance certain characteristics such as solubility, efficiency or stability in the human body.[7] An example of this is the painkiller aspirin (**3**), created by reacting acetic anhydride with salicylic acid found in willow bark. The last class of medicinal chemistry are the totally synthetic compounds, examples of this are phenothiazines and methylene blue (**4**).

1.3 Phenothiazines

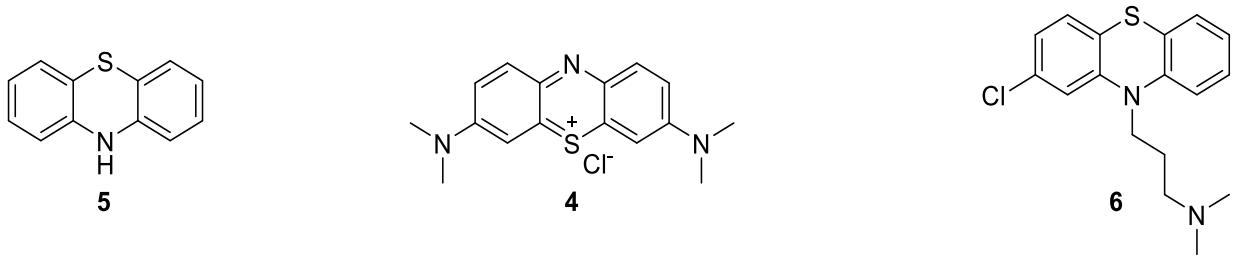


Figure 1-2. The structure of phenothiazine (left), methylene blue (middle) and chlorpromazine (right)

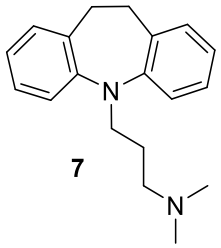
Phenothiazine (**5**, Figure 1-2) is an organic compound which has been used both as an insecticide and deworming medicine, starting in 1935. It is no longer used for any of those purposes. Phenothiazines is also a class of synthetic compounds, which contain the aromatic tricyclic motif. They have a long history as pharmaceutical drugs.[8] This is due to their antipsychotic, antiemetic and antibacterial properties among other things.

Methylene blue (**4**, Figure 1-2), was the first known phenothiazine, synthesized in 1876. It has seen wide use as a medication. It was one of the early effective treatments for malaria, because **4** inhibits the growth of *Plasmodium falciparum* (the parasite responsible for malaria). It has also been used as antidote for cyanide and carbon monoxide poisoning, because of the reductive capabilities. This help to reduce the damage to the electron transport chain that KCN and CO poisoning causes. To the larger public it is probably best known as a dye for biological staining. Bacteria are mostly transparent, so **4** and other stains are used to make visual analysis easier. Since **4** has a formal charge it is attracted to acids, and bacteria will have the deepest blue color where the DNA is. Another property **4** exbibits is that it colors urine blue/green, so double blind testing of the medicine is hard. On the other hand, it can be used to give a strong placebo effect, since the color change is very potent.

The drug chlorpromazine (**6**, Figure 1-2) was first synthesized in 1950. It was used for potential anesthetic effects during surgery.[9] Extending the use to psychiatric patients, as reported by Hamon et al., it was discovered that **6** has antipsychotic activity.[10] After initial reluctance psychiatrists agreed to test it, and the results were striking. The treatment improved the mood, thinking and behavior, and even in patients that had suffered relentlessly for many years. Compound **6** replaced earlier treatment methods in most cases, making electroconvulsive

therapy, hydrotherapy, psychosurgery and insulin shock therapy mostly obsolete. It also acts on histamine receptors 1 and 2 giving it some antiallergic effects. Compound **6** saw use from 1953, and because the wide use the antimicrobial effects soon became evident. However, since this was during the golden age of antibiotics, there was little interest in these antimicrobial properties of phenothiazines.[11] The antimicrobial effects were also produced in vitro at clinically irrelevant concentrations; they were therefore not seen as a potential source of new useful antibiotics.

The discovery of **6** paved the way for the era of psychopharmacology, and led to the discovery of the first antidepressants, imipramine (**7**). Similar to phenothiazines **7** also featured a tricyclic motif, Figure 1-3. Compound **7** was first tested in schizophrenic patients where it showed no results. However, the antidepressant effects were serendipitously discovered when a patient who suffered from extreme depression got better during treatment with **7**.



7

Figure 1-3. The structure of imipramine (7)

The success of **6** opened the door for similar compounds to be tested. This led to the discovery of thioridazine (**1**).

1.4 Thioridazine

Thioridazine (**1**) is a totally synthetic compound that was made for the first time in 1951. It was commercialized in 1959 by Novartis under the tradename Mellaril in the US and Melleril in Europe. Its main use was as an antipsychotic/neuroleptic for treatment of schizophrenia. [12] It was one of the most widely used antipsychotic in the UK for over 30 years, with over 50 million patient-years of use across the world.[13]

While being used as a neuroleptic, severe negative side effects of **1** were discovered. The most serious one is a disturbance of the cardiac rhythm, a prolongation of the QT interval in a dose dependent manner, even in low concentrations. The QT interval is the time from the initiation of a contraction of the ventricles of the heart until the end. Prolonged QT interval is associated with ventricular arrhythmia and sudden death.[14] The prolongation seems to be caused by a significantly higher inhibition of the rapid component of the delayed rectifier potassium current, IKr, by **(+)-1**.[15]

A study covering 1.23 million patients found 74 cases of probable sudden death, and 27 cases of confirmed sudden death. The patients in the former category were mostly old, 76% were older than 65 years, and only one patient was younger than 50 years old.[16]

These are similar findings to other studies on antipsychotics. Glassman and Bigger stated "Although sudden unexpected death occurs almost twice as often in populations treated with antipsychotics as in normal populations, there are still only 10–15 such events in 10,000 person-years of observation".[17] The same article goes on to state that thioridazine had the most severe and well documented risk among currently marketed antipsychotic drugs.

These and similar findings led Novartis to discontinue **1** across the world in 2005. The cardiotoxic properties was cited as well as the fact that other alternatives were available. Use of **1** was estimated to increase the risk of sudden death with 5-8 cases per 10,000 patient-years, compared to the healthy population.[18] This risk was not worth it, considering the lethality of the disease being treated, and the fact that other treatment options for schizophrenia are available.

While the days that **1** was used to treat schizophrenia are passed, maybe there is a new use for the drug. Tuberculosis (TB) started coming back in the 80's during the HIV/AIDS epidemic. During this epidemic more and more reports of resistant TB started showing up. World Health Organization states that "TB is one of the top 10 causes of death worldwide." In 2017, 10 million people fell ill with TB, and 1.6 million died from the disease (including 0.3 million among people with HIV). TB is a leading killer of HIV-positive people."[19]

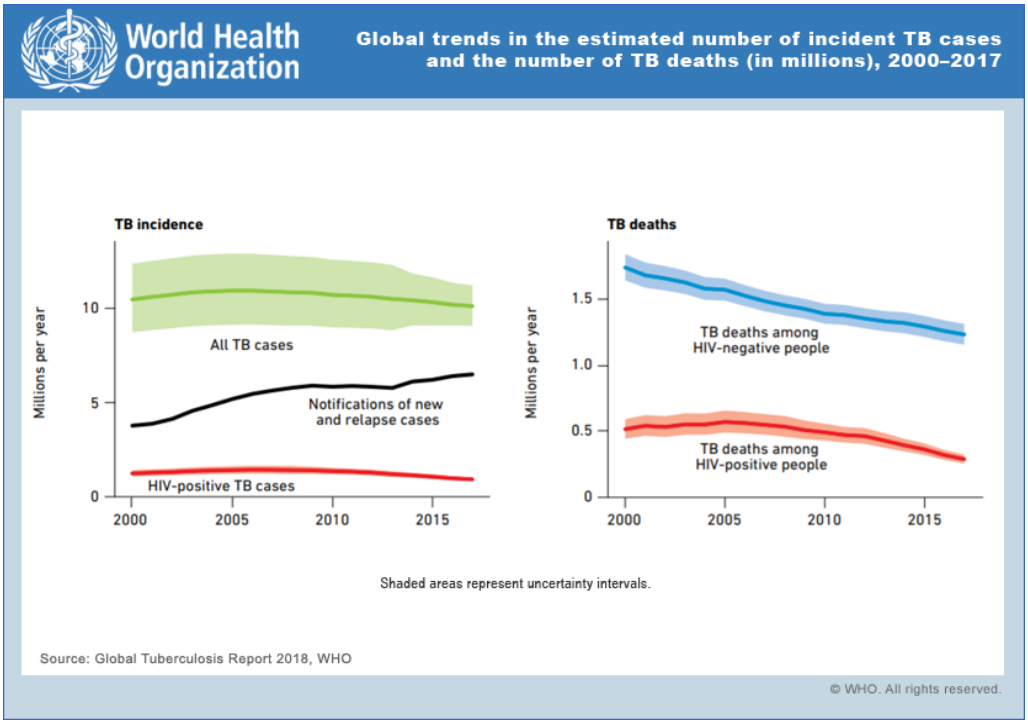


Figure 1-4. Global trends in incident TB and TB deaths from 2000-2017, notice the rise in new and relapse cases.

Since the late 1980's Amaral *et al.* have been collecting and studying severe multi-resistant tuberculosis, focusing and highlighting the antimicrobial effects of **1**.[20] The antimicrobial effects were independent of the level of resistance the bacteria strains showed. In 2016 it was estimated that 490,000 had become ill with multidrug resistant TB (MDR-TB), and around 6% of them had extremely drug resistant TB (XDR-TB). "MDR-TB is TB that does not respond to at least isoniazid and rifampicin, the two most powerful anti-TB drugs. XDR-TB, is a form of multidrug-resistant TB with additional resistance to more anti-TB drugs that therefore responds to even fewer available medicines."[21] Since the mortality rate for these patients are around 50% (240,000 died in 2016), a treatment for these patients is very much in demand.[19] In Figure 1-4 from World Health Organization, the black line on the left side of the graph shows and increase in new and relapse cases, most of the relapse cases are either MDR or XDR-TB.

In 1992 Bourlioux *et al.* performed a structure-activity relationship study *in vitro* of the 18 phenothiazines, **1** was found to have the strongest antimicrobial effect.[2] *In vivo* studies in mice shows that an injection of **1** can increase the survival rate from 13%, in the control group (receiving only *Salmonella enterica*), to 95% in the group receiving 200 µg **1**/ pr mouse. A study on 316 strains of both gram-positive and gram-negative bacteria found that at 400 µg/mL **1** inhibited a wide range of bacteria, both gram-positive and gram-negative. The bacteria that it worked the best against was *Staphylococcus aureus* and *Vibrio cholerae*. *Mycobacterium tuberculosis* was not tested in this study.

The initial *in vitro* testing determined that the minimum inhibitory concentration (MIC) for phenothiazines and **1** were at concentrations that would not be possible to get in the bloodstream. However, **1** and other phenothiazines are concentrated 100-fold in macrophages[22] and in lung tissue[23].

Bourlioux et al. also found that **1** eliminated antibiotic resistance in certain strains of *E. coli*, *Shigella flexneri* and *V. cholerae*, most likely due to elimination of R plasmids.[1]

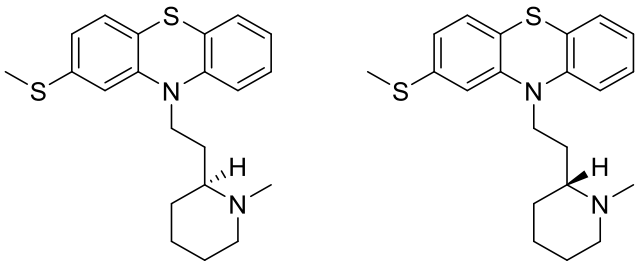
Compound **1** also eliminated antibiotic resistance in MDR-TB and XDR-TB. A mechanism for this has been proposed. First, there is accumulation of **1** in macrophages and lung homogenate that are equal to the MIC determined in *in vitro* studies, combined with a boost to the killing ability of the intracellular macrophages.[22,24-26] The second step is inhibition of the efflux pumps in the bacteria. These pumps are antibiotic-extruding that pump out the usual antibiotics from the cells. This prevents the antibiotics from reaching their targets, and are commonly overexpressed in MDR-TB infections.[27-29] In addition, it is probable that the cytotoxic properties of **1** contributes to the direct killing of *M. tuberculosis*.[30-32]

When treating resistant strains on *M. tuberculosis* the best treatment seems to be **1** combined with antibiotics. A study from Argentina showed 15 of 16 patients were cured of XDR-TB where the initial treatment was ineffective.[33]

Compound **1** can also be used on patients with no hope of being cured. Five patients with terminal XDR-TB were treated with only **1** and their quality of life was significantly improved,

and their lifespan were increased. Since **1** does not restore lost pulmonary tissue, the patients eventually died.[30]

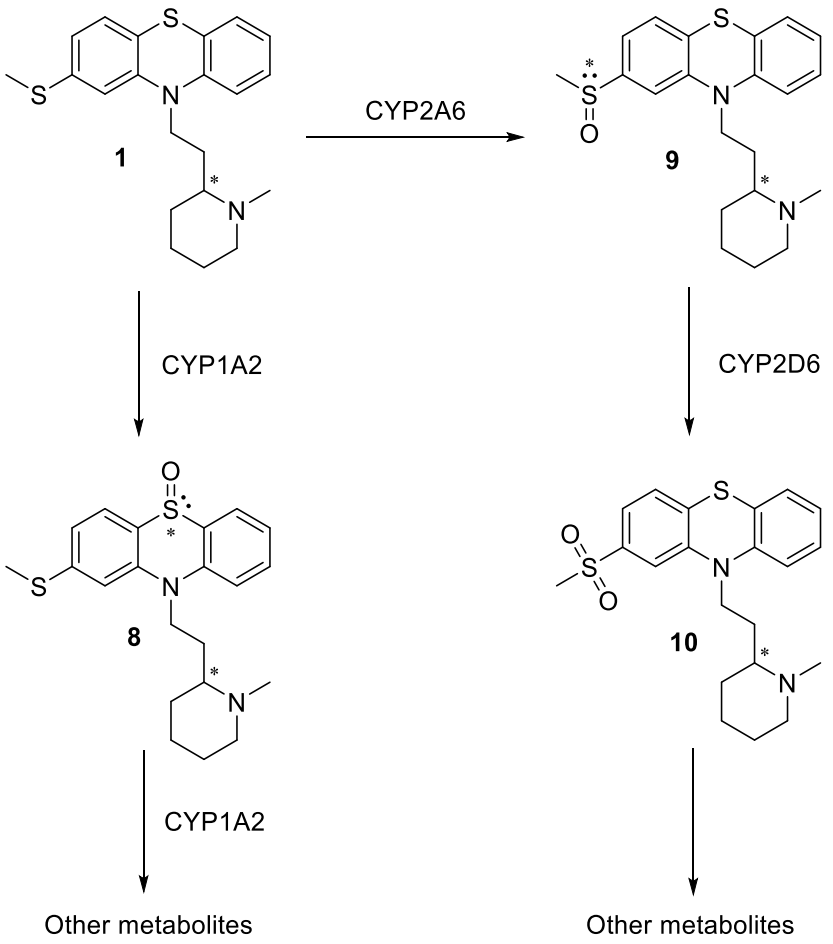
Compound **1** was withdrawn from the market because of it cardiotoxic side effects. A low probability of sudden death that increases with chronic use, was considered a too severe side effect to ignore. Especially when there were alternatives for schizophrenia treatment. In contrast, this side effect is considered insignificant compared to the 50% lethality of MDR-TB and XDR-TB. Given the rising tide of antibiotic resistance, what other drug resistant bacteria could **1** potentially be used against? Why does not **1** see more wide use? What if the cardiotoxic properties could be mitigated?



(-)-1 **(+)-1**

Figure 1-5. The structure of (-)-thioridazine [(-)-1] and (+)-thioridazine [(+)-1]

When **1** first was introduced to the market, there was no awareness that the different enantiomers could have different medical effect. However, after the thalidomide scandal it was clear that enantiomers could act very different in the body; one curing and the other giving severe side effects. The Kefauver-Harris Amendment was passed in USA and similar legislation in Europe, suddenly there was more testing necessary for racemic drugs to get approved.[34] However, **1** was approved prior to the requirement of research of the effects of the different enantiomers. Compound **1** has two enantiomers, one dextrorotary **(+)-1** and one levorotary **(-)-1**. The different properties have become a target for research in recent years. Hendricks *et al.* demonstrated that racemic **1** and **(-)-1** reversed resistance in vancomycin-resistant enterococci and in both Methicillin-Resistant and Methicillin-Sensitive *Staphylococcus aureus* strains (MRSA & MSSA).[35]



Scheme 1-1. The metabolism of thioridazine in the human body, stereocenters are marked with an asterisk. The stereocenter in the piperidine part is preserved during these first steps. The names of enzymes are taken from ref. 36.

Eight years later, racemic **1**, **(-)-1** and **(+)-1** were compared on 55 different strains of bacteria (both gram positive and negative). The results showed that **(-)-1** had a higher antibiotic effect, both *in vitro* and *in vivo*.[2] In addition, **(-)-1** cumulates to higher concentrations in human tissue than **(+)-1**.[37] And **(-)-1** has less effect on the central nervous system (e.g. weaker blocking of the dopamine D-2 receptor).[3,4]

Lastly, when looking at the cardiotoxicology of the isomers, a study on rabbits showed that **(-)-1** had negligible effect on the QT interval, while both the racemic and **(+)-1** showed prolongation of the QT interval.

1 is metabolized into thioridazine-2-sulphoxide (**8**) and mesoridazine (**9**). The latter is further broken down into sulforidazine (**10**). This metabolism is shown in Scheme 1-1, from Eap *et al.*[36]. Compound **8** has no antipsychotic effect, it mimics dopamine in its binding.[38] Compound **9** and **1** have similar prolongation if the QT interval,[39] but **9** also have stronger binding to many brain receptors. For example **9** binds about ten times as strong to the D-2 receptor compared to **1**, this is the receptor believed to be responsible for the therapeutic effects of antipsychotics.[40]

There is no reason to believe that the original stereocenter is affected by the metabolism, but in **9** there is a new stereocenter introduced.[41]

With all the research put down here, especially reduced side effects from the **(-)-1** while better antibacterial properties, shows that if **(-)-1** would be easily available it could be used to treat XDR-TB without the risk of *arrythmia*/sudden death.

1.5 Chemical background

1.5.1 Racemate resolution

Racemate resolution approach consist of synthesis of the target molecule as a racemate. Then to separate the two enantiomers, they are reacted with an optically pure chiral derivation reagent (CDR) to give diastereomers. These can now be separated with normal flash chromatography. One condition for this to work is that the target molecule has a functional group that can easily attach and detach the CDR. This reaction is shown in Figure 1-6.

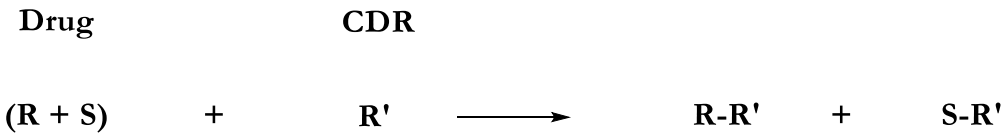


Figure 1-6. How a chiral derivation reagent creates one pair of diastereomers.

It is important that the CDR is optically pure, so only one pair of diastereomers is created. If the CDR is not pure, two pairs of diastereomers are created, Figure 1-7. The problem here is that the R-R' and S-S' would coelute, so would S-R' and R-S'. This would lead to impure product and analytical error.

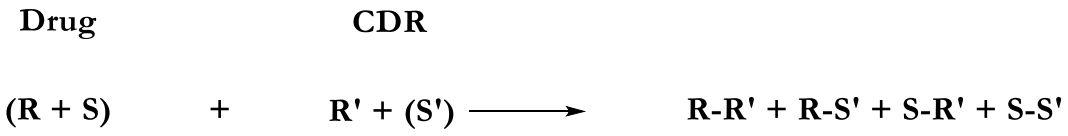


Figure 1-7. A reaction with an impure CDR creates two pairs of diastereomers.

The last step is removing the CDR to give a product that is optically pure. Even if this approach may reduce yields because of lost product in the addition and removal of a CDR, the method is useful since the separation can happen with normal flash column and all the flexibility that those columns offer.[42]

1.5.2 Asymmetric synthesis

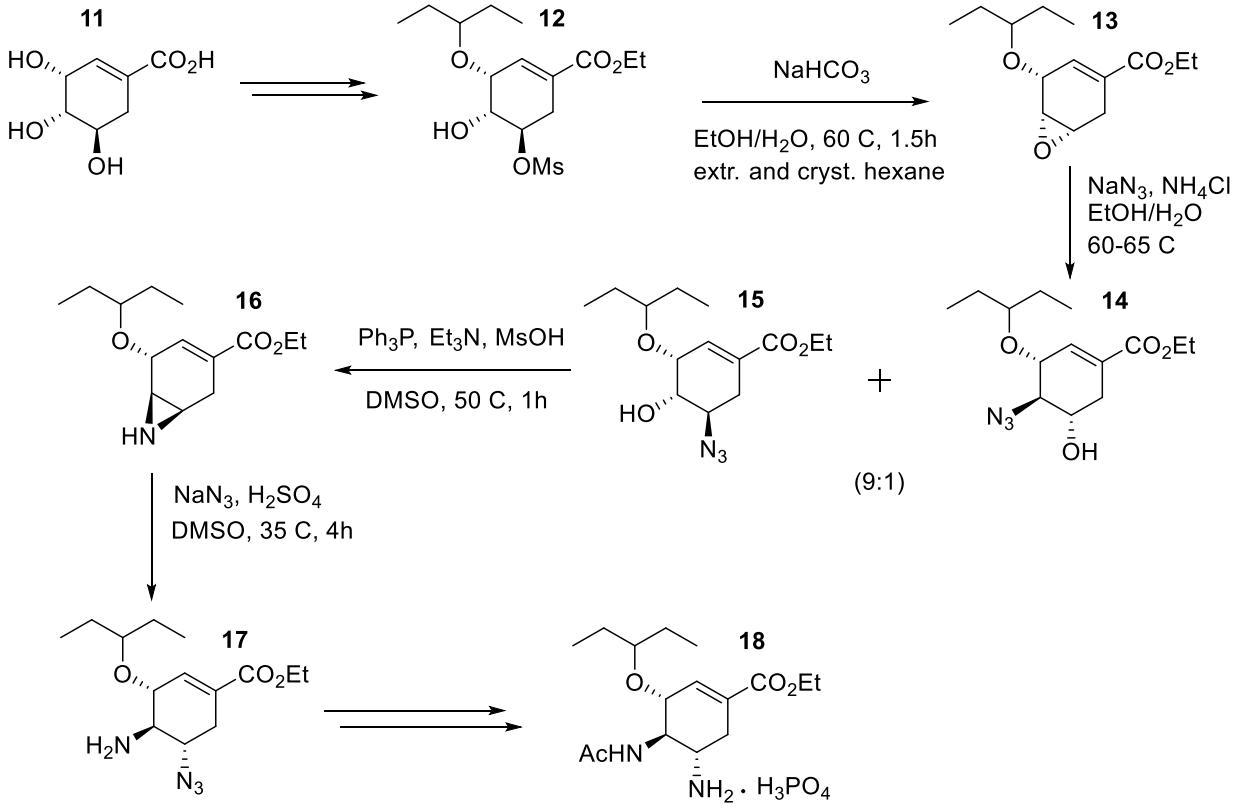
When doing a total synthesis of a chiral molecule, new stereocenters will be incorporated. When creating new stereocenters through asymmetric synthesis, one stereoisomer is produced

in excess. If no asymmetric reactions are used only chiral resolution can give an enantiomerically pure product. In a best-case scenario, the yield is lowered to a maximum of 50%. That is why asymmetric reactions are needed to make a pure product with good atom economy.

There are three main ways to get a stereoisomerically pure product: Chiral pool, catalytic approach and substrate controlled.

Chiral pool synthesis is a synthesis method that utilizes a chiral starting material that is available as a pure enantiomer. Examples of common starting molecules are amino acids, monosaccharides, terpenes and other molecules that available from nature in its pure form. With a chiral starting compound, the goal is to only use reaction that preserve the built-in chirality, that way reaching the desired compound with only the correct enantiomer.

If the desired compound is very similar to an existing compound chiral pool synthesis can be a very good strategy. Since the stereocenter is present during the whole synthesis, racemizing conditions need to be avoided.



Scheme 1-2. Tamiflu synthesis, showing only the reactions affecting the stereochemistry. Ref 43.

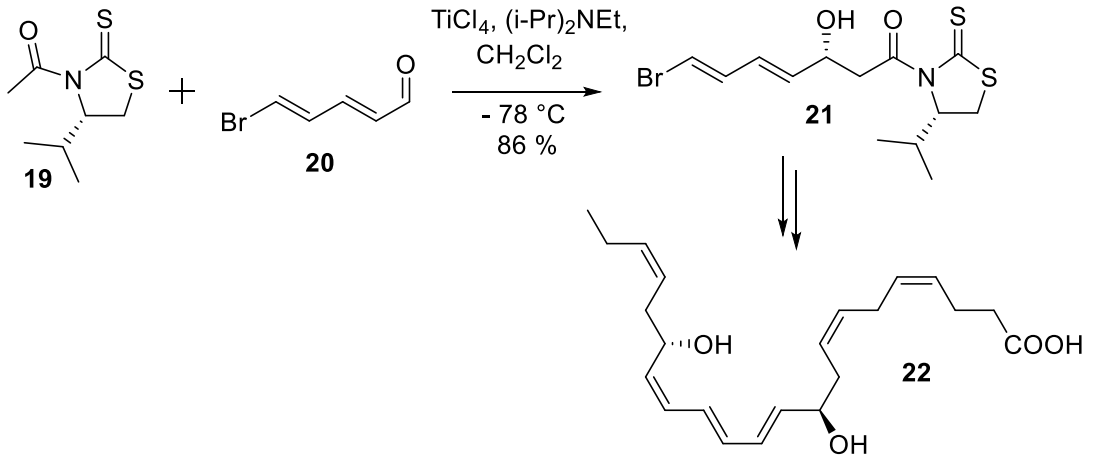
A good example is the synthesis of the flu medicine Tamiflu (**18**), Scheme 1-2. Starting with shikimic acid (**11**), with 3 stereocenters already defined. Since only one of the 8 stereoisomers are active as medicine, starting with them gives a purity very hard to match using other methods. However, chiral pool synthesis is by no means easy just because the stereocenters

are defined. The synthesis of **18** is a good example of this, since you are limited to reaction that either keep or invert the stereochemistry, or reactions that create diastereomers that can be separated.

Another method for asymmetric synthesis is the catalytic approach (asymmetric catalysis). With a catalyst present, a certain reaction happens with specific symmetry. A common reaction is asymmetric hydrogenation where a double bond is preferentially hydrogenated on one face of the molecule, induced by a chiral catalyst. The catalysts themselves can be enzymes, inorganic molecules or organic molecules with chirality/C2 symmetry. The first asymmetric catalysts discovered were enzymes, and for a long period of time it was thought that enzymes were the only molecules capable of creating enantiopure products (*i.e.* a high enantiomeric excess [*ee*]). Because enzymes are sensitive to high/low temperatures and pH this also limits their usage. However, W. S. Knowles reported that organic molecules could give high yields of a single enantiomer in hydrogenation reactions. For this, he was awarded the Nobel Prize in 2001.

Similar to asymmetric hydrogenation, a common reaction is to reduce a prochiral group (such as a keton) down to an alcohol in the presence of an organocatalyst, yielding one enantiomer with very good *ee*.

A substrate controlled asymmetric reaction is a reaction where the geometry of the molecule determines where the reaction will happen. Selectivities larger than 99% has been reported. There are two different versions of this method: one is designing the reaction sequence so that the reaction that need to be selective comes at a point in the synthesis where the geometry of the molecule will be correct. The other version uses an auxiliary that will either react with the molecule or coordinate with it, producing only the desired compound.



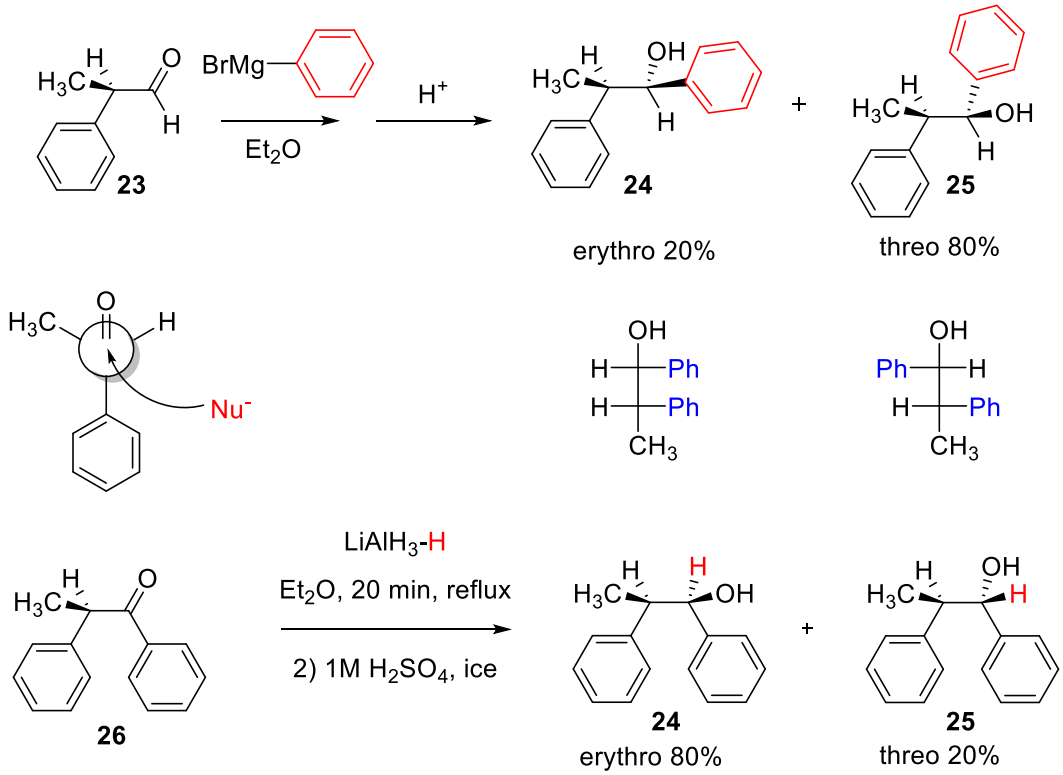
Scheme 1-3. The key asymmetric reaction from synthesis of Protectin D-1 (22). Ref. 44

Here is an example of and aldol reaction with a chiral auxiliary, Scheme 1-3. Hansen and coworkers used an Evans type auxiliary (**19**) in the synthesis of specialized pro-resolving

mediators (SPMs).[44] SPMs are basically specialized metabolites of polyunsaturated fatty acids. For this the group are reporting high stereochemical outcomes. First $TiCl_4$ coordinates with the carbonyl and sulfoxide group make the entire Crimmins thiazolidinethione part of the molecule rigid, since the isopropyl group is going inwards in the plane, the attack must come from above. N,N-Diisopropylethylamine deprotonates and giving an electrophilic attack from only one side of the molecule, the alcohol is pushed back and creates only the *R*-enantiomer.

1.5.2.1 Cram's rule

Two models can be used to predict the major configuration achieved from an enantioselective reaction, the first is Cram's rule of asymmetric addition, first stated in 1952. It simply observes the steric hindrance of the reactant and predicts what diastereomer will be generated most favorably in a reaction. For instance, in a Grignard reaction on an aldehyde, the most stable conformation is with the carbonyl opposite of the bulkiest substituent on the alfa carbon. Then the Grignard reagent will attack the carbonyl from the side of the molecule with the smallest substituent on the α-carbon, thereby favoring the one diastereomer. This is shown in Scheme 1-4 taken from Cram's 1952 publication.[45]



Scheme 1-4. A graphical representation of Cram's rule.

1.5.2.2 Felkin-Ahn model

Later the Felkin-Ahn model came and superseded the cram model, because Cram's model had certain weaknesses that it did not account for. First the transitional state has a staggered

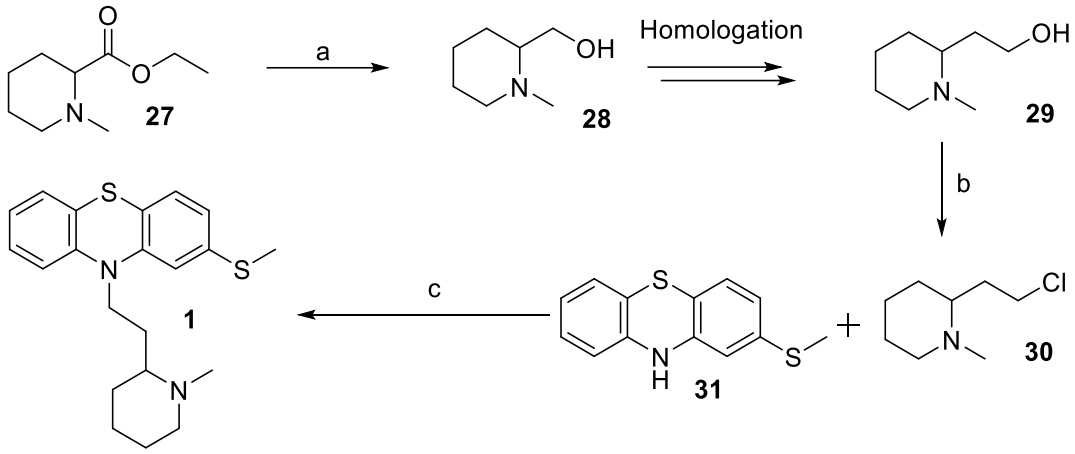
conformation in the Felkin-Ahn model, and it also accounts for polar interaction between groups.

There are also many more models that try to account for even more different parameters, such as chelating effects. In the end, doing it in the laboratory is the only sure way to know which isomer is favored.

These models are simple examples of substrate-controlled selectivity.

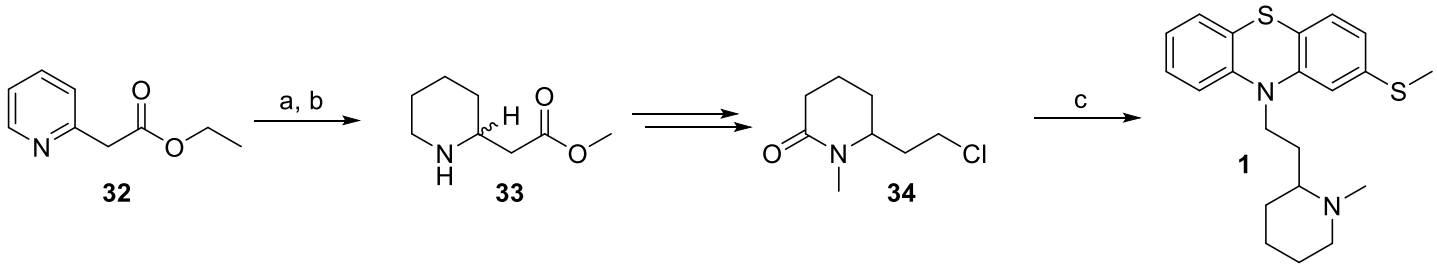
1.6 Retrosynthesis

Before planning the synthesis, previous syntheses were reviewed. In the 1980's T. Mohammad published two different synthesizes of **1**. The studies were of deuterium labeled analogs to conduct metabolic and pharmacokinetic studies.



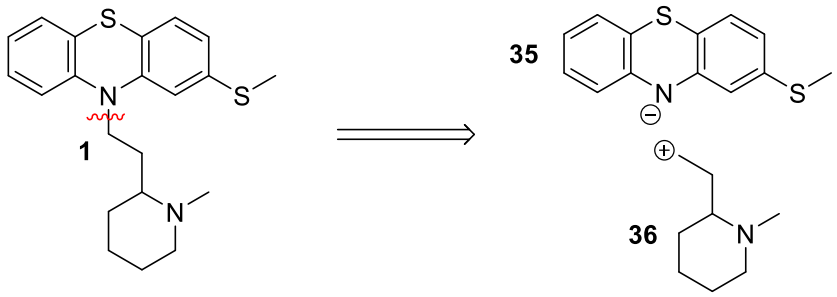
Scheme 1-5. T. Mohammad's synthesis of 1, published in 1986. a) LiAlH4, Et_2O; b) $SOCl_2$, CH_3Cl, Δ; c) NaOH, $PhCH_3$.

The synthesis from 1986 shown in Scheme 1-5, starts with a racemic stereocenter already defined.[46] The start compound is commercially available as a pure enantiomer, but very expensive. The alternative would be to synthesize the start compound, but then it would be better to synthesize it one carbon longer, to save steps.



Scheme 1-6. T. Mohammad's synthesis of 1, published in 1988. a) Ni-Al, KOH, H_2O; b) HCl gas, CH_3OH; c) NaOH, $PhCH_3$, Compound 31.

The second synthesis (Scheme 1-6), published two years later featured another route.[47] This synthesis starts off with a reduction of an pyridine ring, but this reduction is not asymmetric. This could potentially be achieved by coupling some sort of chiral auxiliary before reduction. This seems complex, so some easier strategy could be tried first. However, the last reactions, used to fuse the phenothiazine moiety **31** with the **30** are still good reaction. And the **31** is commercially available.



Scheme 1-7. Retrosynthetic analysis of a first disconnection on 1.

In the retrosynthetic analysis (Scheme 1-7), this seems like a good last reaction step. The tricky part is to synthesize piperidine **30** (Figure 1-8) as a pure enantiomer.

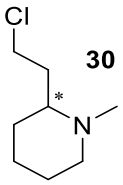


Figure 1-8. The structure of the goal molecule 30

2 Results and Discussion

2.1 Chiral resolution

As the hydrochloride of racemic thioridazine(**1**-HCl) is commercially available, the most obvious approach to get enantiopure thioridazine was chiral resolution.

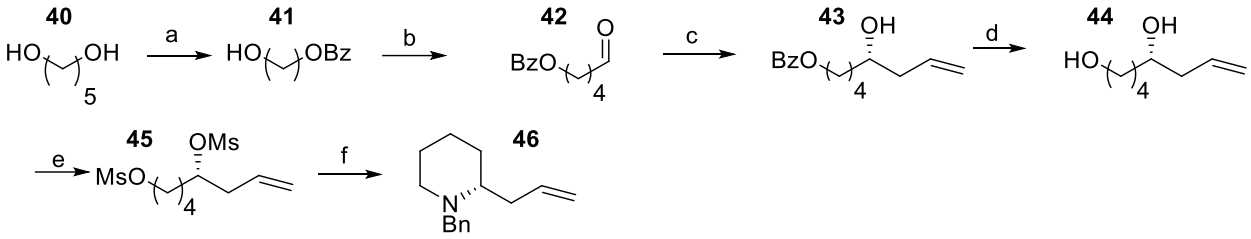
The first reported chiral resolution of thioridazine was by Bourquin *et al.* in 1958.[48] In this procedure, **1** is reacted with di-*p*-tolyl-*L*-tartaric acid (**37**). The diastereomers that are created have different solubility in acetone, and the reaction of **(+)-1** is faster, thus enriching the mother liquid with the diastereomer created from the (-)-enantiomer. However, we did not get sufficient optical purity. Several recrystallizations were found to be necessary.

However, another chiral resolution is reported by Schlauderer *et al*.[49] This relies on a chiral reagent, *R*-(-)-menthyl chloroformate (**38**). The procedure has fewer time-consuming steps, and the separation is done with flash chromatography.

First the commercially available **1**-HCl salt is turned into the free base, before being demethylated and reacted with the **38**. After creating the diastereomer pair **(39)**, the pair was separated with flash chromatography. The separation went well, a large portion of each diastereomer was separated, but a small portion coeluted. However, the absolute yields were similar to what was reported. 84 mg of **(-)-1** was obtained and 91 mg of **(+)-1** from 800 mg of racematic **1** (11% **(-)-1**, 11% **(+)-1**). The optical rotation measured was -21° for **(-)-1** yielded from the faster eluting diastereomer (**39-f**), and +19° for the enantiomer yielded from the slower eluting diastereomer(**39-s**). The same procedure was repeated, not as a one pot synthesis, but with purification after each step. The same purity was obtained, but the yields was lower. There was no benefit to purifying after each step.

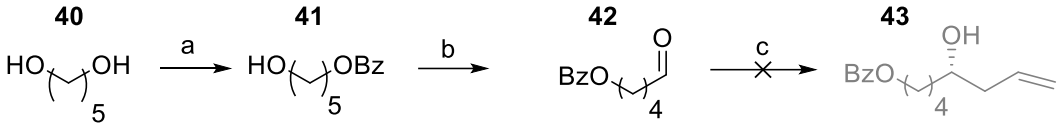
2.2 Attempt at synthesis

The first attempt at a synthesis started with trying to create **30**. Raju *et al*. reported the synthesis shown in Scheme 2-1.[50] We wanted to investigate whether allyltributyltin could be replaced by allyltrimethylsilane. This is because allyltributyltin is highly toxic.



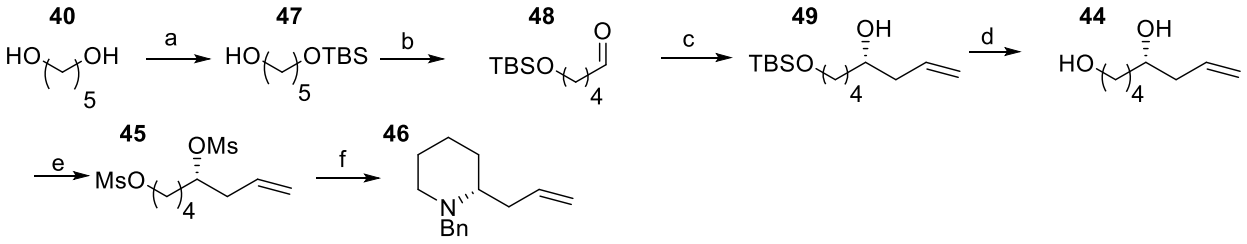
Scheme 2-1. Raju *et al.* reported synthesis of 46 precursor to compound 30. a) BzCl, anhyd CH_2Cl_2, 0 °C, 12 h, 85%; b) PCC, anhyd CH_2Cl_2, 0 °C to r.t., 4 h; c) (R)-BINOL, $Ti(Oi\text{-}Pr)_4$, allyltributyltin, anhyd CH_2Cl_2, –78 °C to –20 °C, 48 h, 72% (in 2 two steps) d) K_2CO_3, MeOH, 0 °C to r.t., 6 h, 75% e) Et_3N, MsCl, DMAP, CH_2Cl_2, 0 °C to r.t., 1 h, 91%; f) $BnNH_2$, 70 °C, 48 h, 92%.

Reaction 40→41 was successful, yielding 88% of the protected **41**. The oxidation of **41**, with Dess-Martin perodinanone (DMP) gave aldehyde **42** in 48% yield. The next step, which is the key step, is the planned asymmetric addition. However, the reaction was tried racemic first with allylmagnesium bromide. We expected that the ester group would react, but we assumed that it would be possible to obtain some of **43** by tuning the equivalents of the Grignard reagent.



Scheme 2-2. A summary of the first attempted strategy. a) NaH, THF, 0 °C to r.t., BzCl, overnight, 88%; b) DMP, CH_2Cl_2, r.t., 2 hours, 48%; c) THF, C_3H_5MgBr (2.2 equivalents), -78 °C, 50 min.

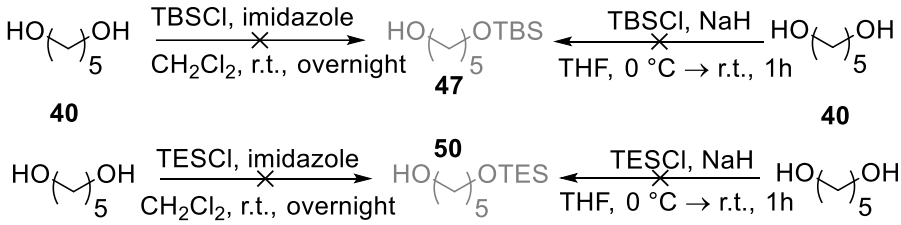
The reaction gave an inseparable mixture of products resulting from reactions on both the aldehyde and the ester. A summary of the first strategy is shown in Scheme 2-2. To avoid this problem, we altered the strategy to use silyl ethers for protection instead of a benzoyl group. This updated strategy is shown in Scheme 2-3.



Scheme 2-3. Our second strategy based on *Raju et al.*, but with a TBS-protected alcohol instead of Bz

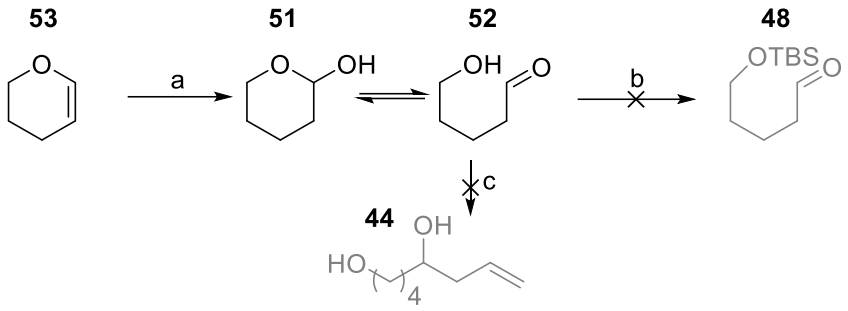
First, 1,5-pentadiol (**40**) was reacted with TBSCl, with imidazole as a base, unsuccessfully. The same reaction, but with NaH as base was attempted. These two reactions were tried again with TESCl instead of TBSCl. First with imidazole as base and then with NaH as base, neither worked.

One possible explanation is that **40** was contaminated with water, and that this prevented the desired reaction. However, after drying all reagents, the reaction did still not work. The attempts are summarized in Scheme 2-4.



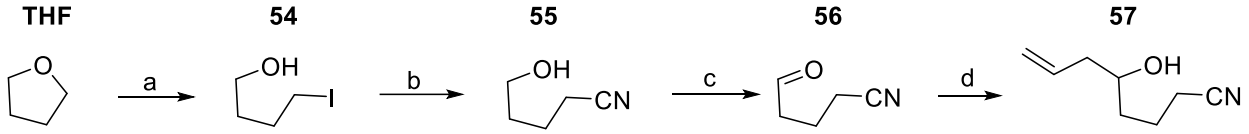
Scheme 2-4. Attempts at protecting compound 40 with a silyl protection group.

We also tried another way of getting to the protected aldehyde. Compound **51** is in an equilibrium with **52** as shown in Scheme 2-5. The hypothesis was that reacting TBSCl with this equilibrium would grant the protected aldehyde directly. We tried the protection reaction with NaH as a base, without success. Then with imidazole, however neither yielded the desired **48**. The second hypothesis was that doing a Grignard reaction directly on this combination of **51** and **52**, would give **44** directly potentially skipping two steps in the strategy. This did not work either. All these failures culminated in these strategies being abandoned in favor of something completely different.



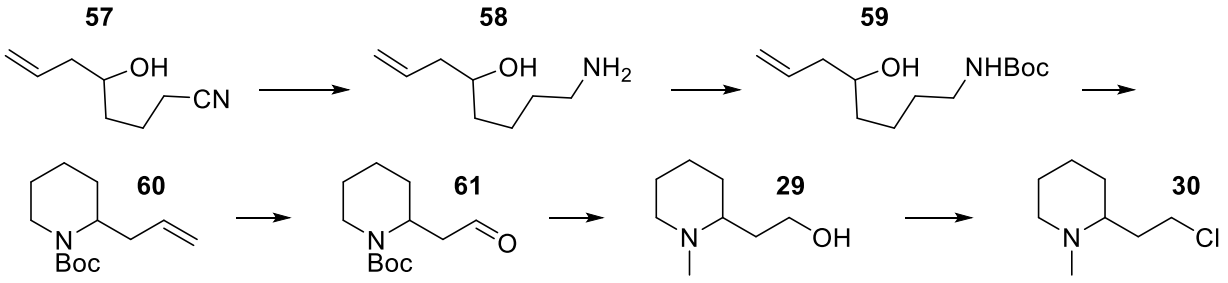
Scheme 2-5. Alternative strategy for synthesis of the protected aldehyde 48 or the allyl diol 44. a) 2M HCl, 0°C → r.t., 3 h, >95%[51]; b) TBSCl, NaH, THF, 0°C → r.t., overnight; c) THF, C_3H_5MgBr (1.1 equivalents), -78 °C, 1h.

This new strategy, Scheme 2-6, is a little different, does not focus as much around protecting groups.



Scheme 2-6. The last strategy tried. a) KI, TMSCl, THF, 60°C, overnight, 57%; b) NaCN, dry DMSO, 40°C, overnight, 46%; c) DMP, CH_2Cl_2, r.t., 2h, 25% d) C_3H_5MgBr (1.1 eqvivalent), THF, -78°C → r.t., 1h.

The first reaction used THF as both a solvent and a reagent to yield **54** in 57%. No time-consuming purification was needed. The second reaction was a nucleophilic substitution, where the iodine group was replaced with a nitrile group. Two different processes were tried, one with high temperature for two hours, and one with lower temperature overnight. Both gave similar yields, the highest was 46%. The next step was an oxidation to the aldehyde (**56**), which was tried with several methods. First a Swern-type oxidation was tried, which gave a yield of 1%. Then a Dess-Martin oxidation was attempted, giving a yield of 25%. A TEMPO-oxidation was also tried but, it did not work. The last reaction tried was an addition of allyl magnesium bromide on **56**, this reaction seemed to work from the crude spectra, but time ran out. This addition was also planned to be performed asymmetrically with a BiNOL catalyst. The rest of the planned strategy is shown in Scheme 2-7.



Scheme 2-7. The rest of the planned synthesis of compound 30.

2.3 Conclusion

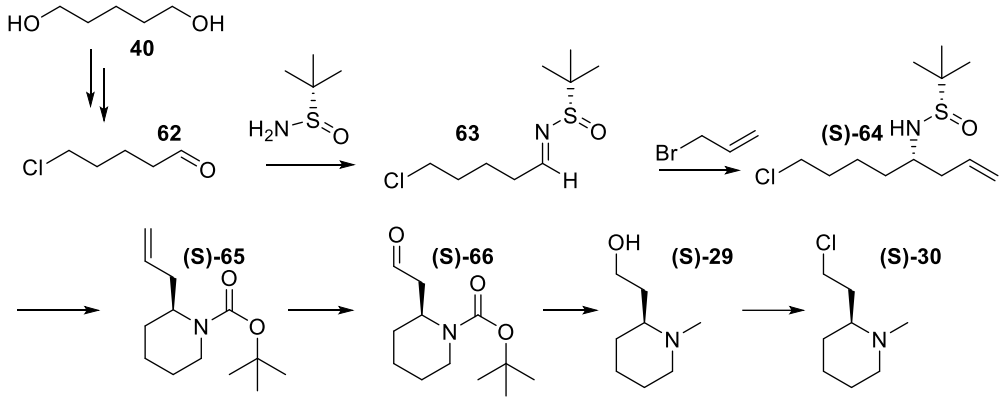
The first chiral resolution tested, based on Bourquin *et. al.*, was not successful. It did not yield sufficient purity. The second chiral resolution tried was based on Florian *et. al.* This chiral resolution used flash chromatograph for separation which gives more overview during the separation. The reactions gave similar yields to the literature, but the silica separation was not as complete as reported, a small portion co-eluted. The procedure was repeated with purification in between each reaction, but there was no increase in purity, only a decrease in yield.

The total synthesis was not successful. The first strategy was based on protecting **40** with benzoyl. The third step was a Grignard reaction, which was not successful because addition happened in the protection group. The next strategy was essentially the same strategy, but with a silly based protection group. The problem with this strategy was that none of the protection reactions worked. Even when we changed conditions and protecting group.

The last attempt started with a ring opening of THF. This reaction path had successful reactions, but unfortunately time ran out before we got to try the asymmetric addition. We only tried the racemic addition, which seems to work from preliminary NMR-spectra.

2.4 Future prospect

If more time was available, the rest of the synthesis strategy would be tested. Exceptionally interesting would be the asymmetric addition using a BiNOL-catalyst. Furthermore, the project is being carried on by another student trying a different synthesis strategy. This strategy also starts with **40** that is reacted with a chiral imine that help direct an organometallic addition to give different diastereomers, Scheme 2-8.



Scheme 2-8. The new strategy for synthesis of compound 30

Finally, there is biological testing of the enantiomers of **1**.

3 Experimental

3.1 General methods

NMR spectra were recorded on a Bruker AscendTM 400 at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR. All samples were dissolved in deuterated chloroform (CDCl3). Chemical shifts (δ) are given in ppm and the coupling constants (J) in Hz.

For TLC, Merck C-60 F254 silica gel plates were used and developed using UV-light and/or a $KMnO_4$ stain.

Flash chromatography was performed using a glass column packed with Merck 60 silica gel (40-63 µm particles) as stationary phase. All mobile phases are specified in the experimental procedures.

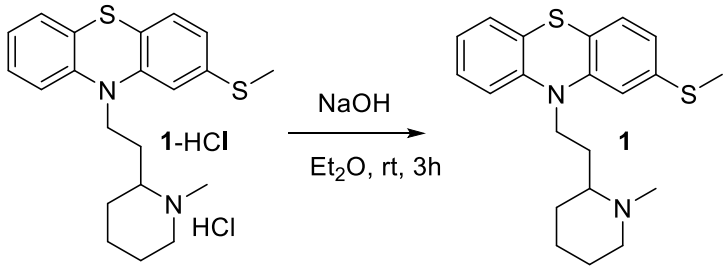
IR spectra (4000-600 cm^{-1}) were recorded on an Agilent Technologies 5500 series FTIR with and ATR-cell (diamond).

Optical rotations were measured using a mL cell with a 1.0 dm path length on a Perkin Elmer 341 polarimeter using EtOH.

All reactions were performed in degassed round-bottom flasks. All reactions were performed under a nitrogenous atmosphere. All reactions involving aldehydes were protected against light exposure.

3.2 Chiral resolution

3.2.1 Thioridazine as a free base



Yield: 95%

Citation: Burquin *et. al.* 1958 [48]

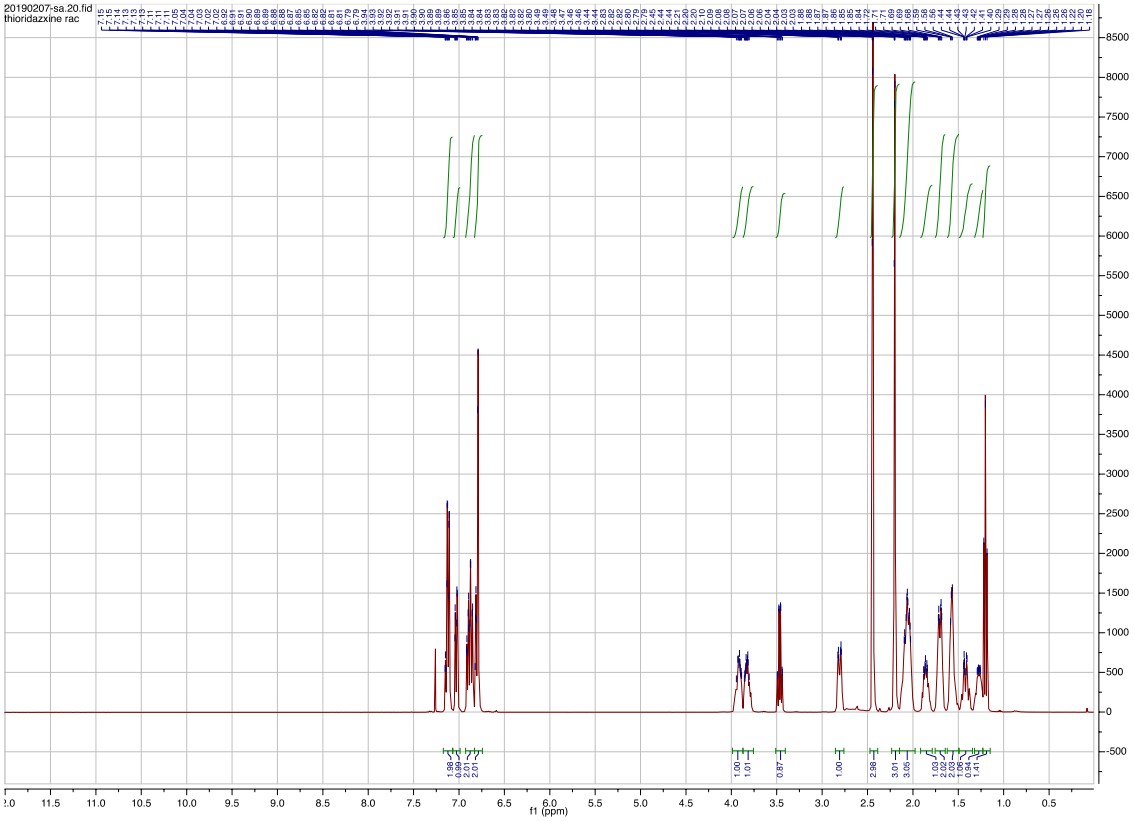
Method:

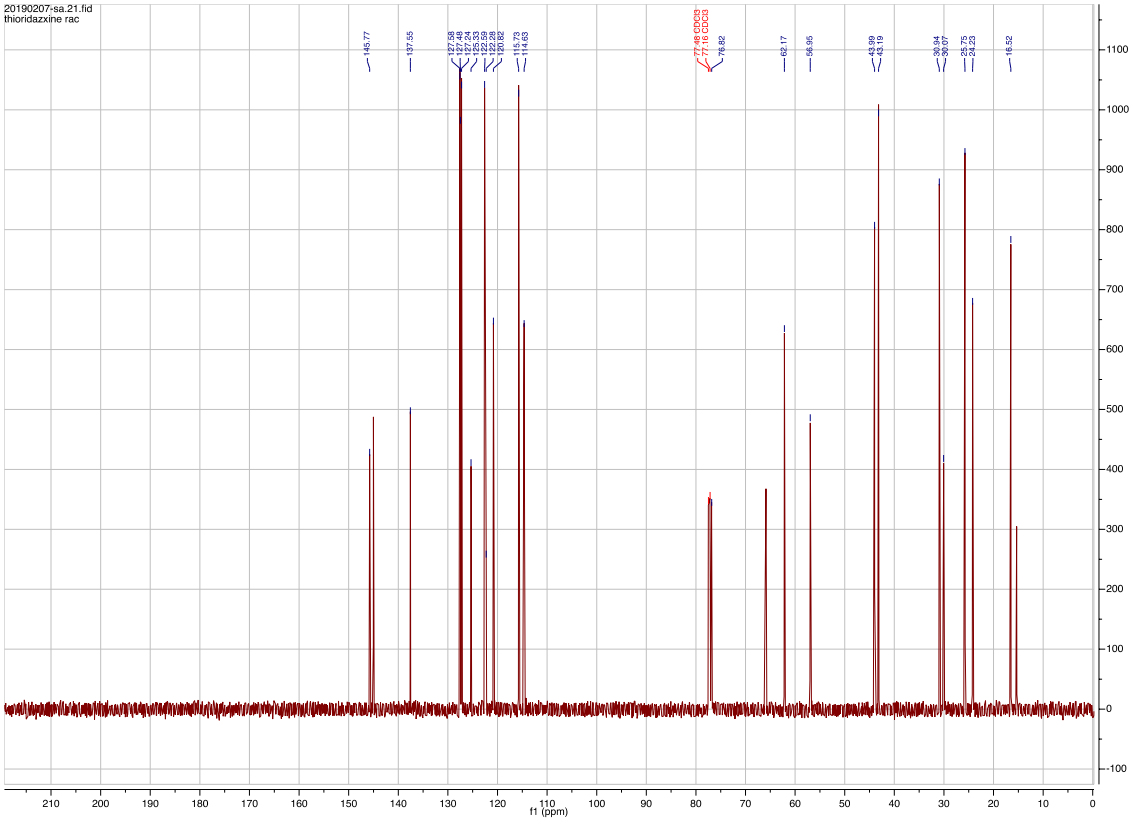
Thioridazine hydrochloride (**1**-HCl , 4.1g, 10mmol) was dissolved in a flask with Et_2O (25 mL). A solution of NaOH in water (33%, 25 mL) was added, and stirred at rt for 3 hours. The phases were separated, and the polar phase was extracted with Et_2O (3 x 25 mL). The combined organic phase was dried with Na_2SO_4 and concentrated under reduced pressure. This yielded the free base of thioridazine (**1**, 3.5 g, 9.5mmol, 95% yield).

Data:

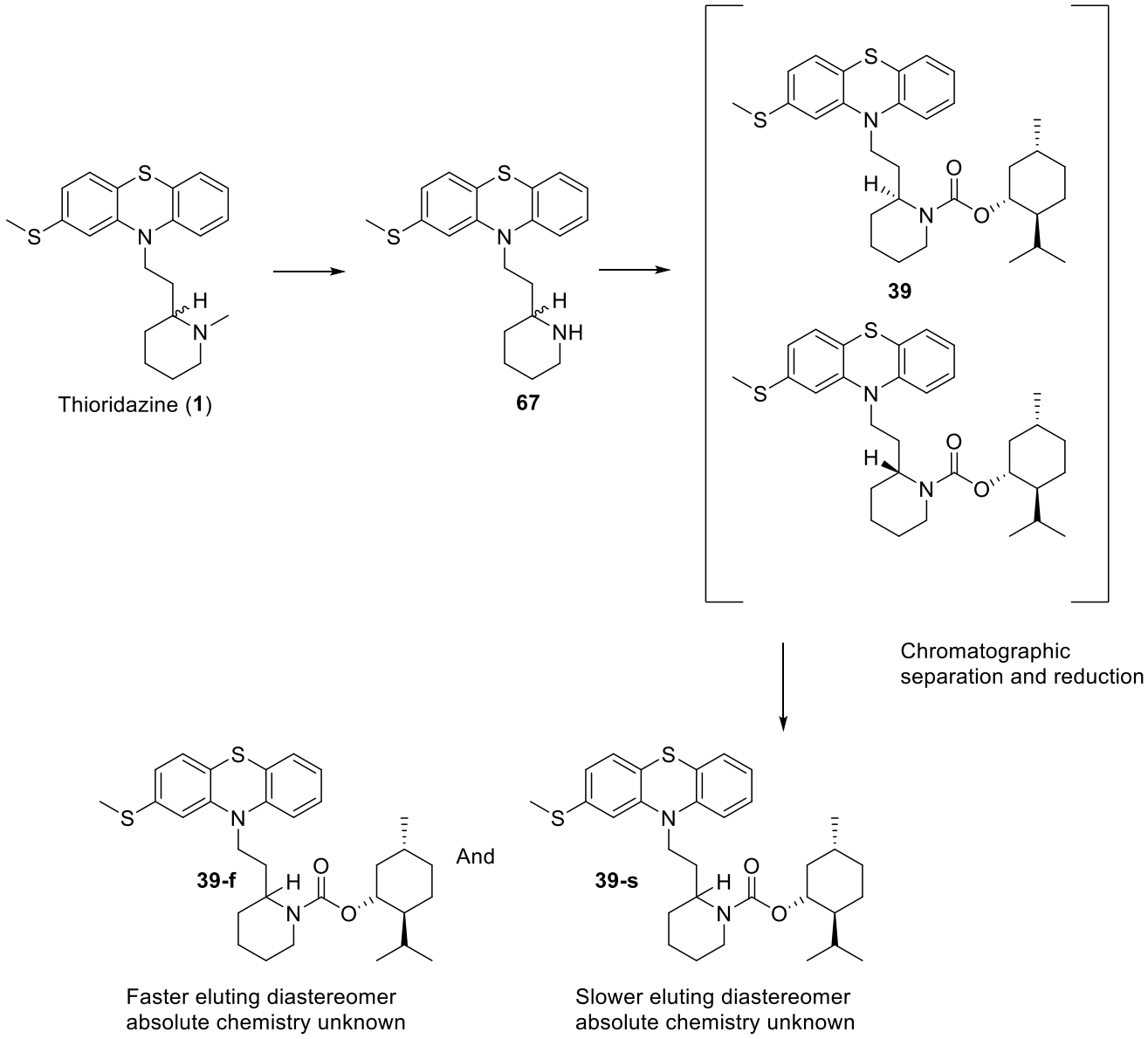
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (ddd, J = 9.2, 7.6, 1.6 Hz, 2H), 7.03 (dt, J = 7.8, 1.6 Hz, 1H), 6.93-6.83 (m, 2H), 6.80 (dd, J = 9.4, 1.7 Hz, 2H), 3.99-3.87 (m, 1H), 3.87-3.76 (m, 1H), 3.47 (qd, J = 7.0, 1.5 Hz, 1H), 2.85-2.76 (m, 1H), 2.47-2.39 (m, 3H), 2.23-2.15 (m, 3H), 2.15-1.98 (m, 3H), 1.86 (ddd, J = 8.5, 4.4, 2.0 Hz, 1H), 1.76-1.64 (m, 2H), 1.62-1.49 (m, 2H), 1.49-1.34 (m, 1H), 1.32-1.23 (m, 1H), 1.20 (t, J = 7.0 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 45.77, 145.02, 137.55, 127.58, 127.48, 127.24, 125.33, 122.59, 122.28, 120.82, 115.73, 114.63, 65.87, 62.17, 56.95, 43.99, 43.19, 30.94, 30.07, 25.75, 24.23, 16.52, 15.34.


20190207-sa.20.fid
thioridazxine rac
f1 (ppm)


20190207-sa.21.fid
thioridazxine rac
145.77
137.55
127.58
127.48
127.24
125.53
122.59
120.88
120.62
115.73
114.63
77.46 CDCl3
77.16 CDCl3
76.82
62.17
56.95
43.39
43.19
39.94
39.07
25.75
24.23
16.52
f1 (ppm)

3.2.2 Creating and separating diastereomers



Yield: 15% (faster eluting), 7% (coelution) 17% (slower eluting)

Citation: Schlauderer *et. al.* 2013[49]

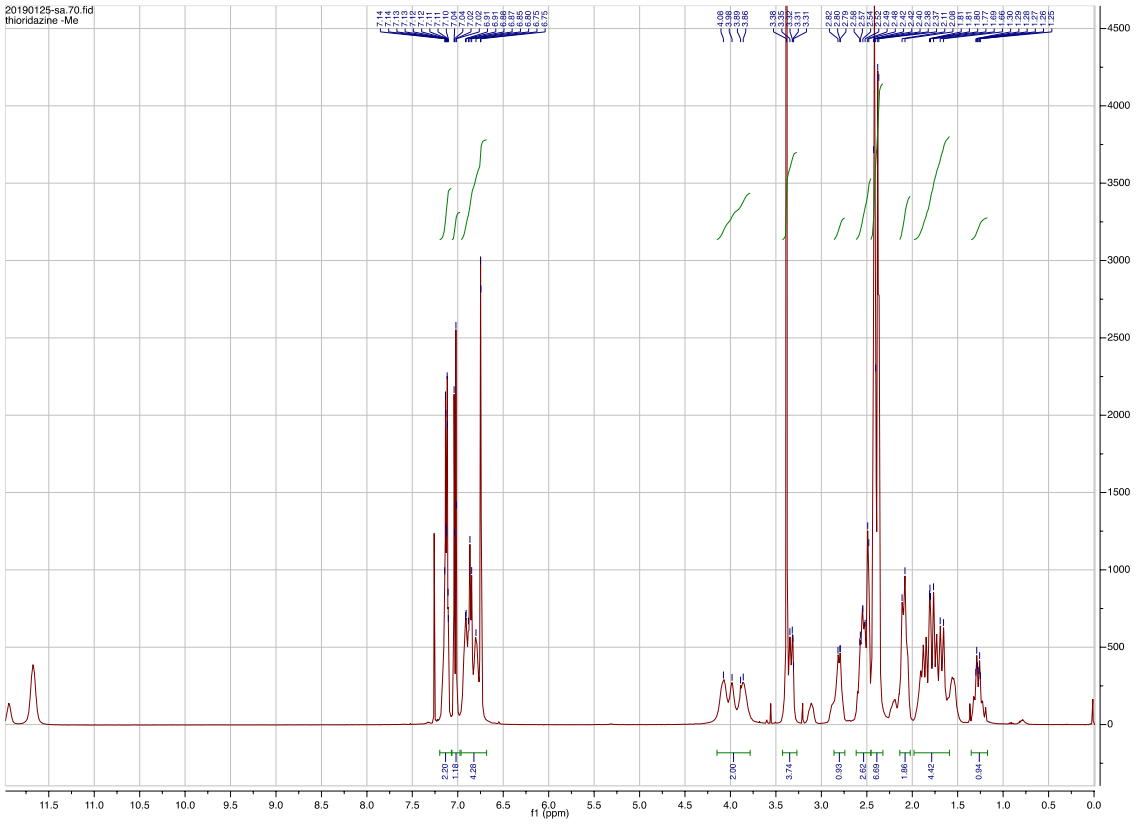
The racemate of **1** (800mg, 2.16mmol) was dissolved in 1,2-dichorethane at 0 °C, then 1-chloroethyl chloroformate (0.256 mL, 2.37) was added and the mixture was refluxed for 3 hours. It was cooled to r.t. and concentrated under reduced pressure. This mixture was dissolved in methanol (20 mL, 500mmol) and refluxed for 18 hours, after reflux the mix was concentrated under reduced pressure then dissolved in CH_2Cl_2. N,N-diisopropylethylamine (0.827 mL, 4.75 mmol) was added first, then R-(-)-menthyl chloroformate (0,555 mL, 2.59 mmol) was added and stirred for 18 hours at room temperature. The mixture was poured into a separatory funnel and diluted with CH_2Cl_2 (50 mL), it was washed three times with saturated

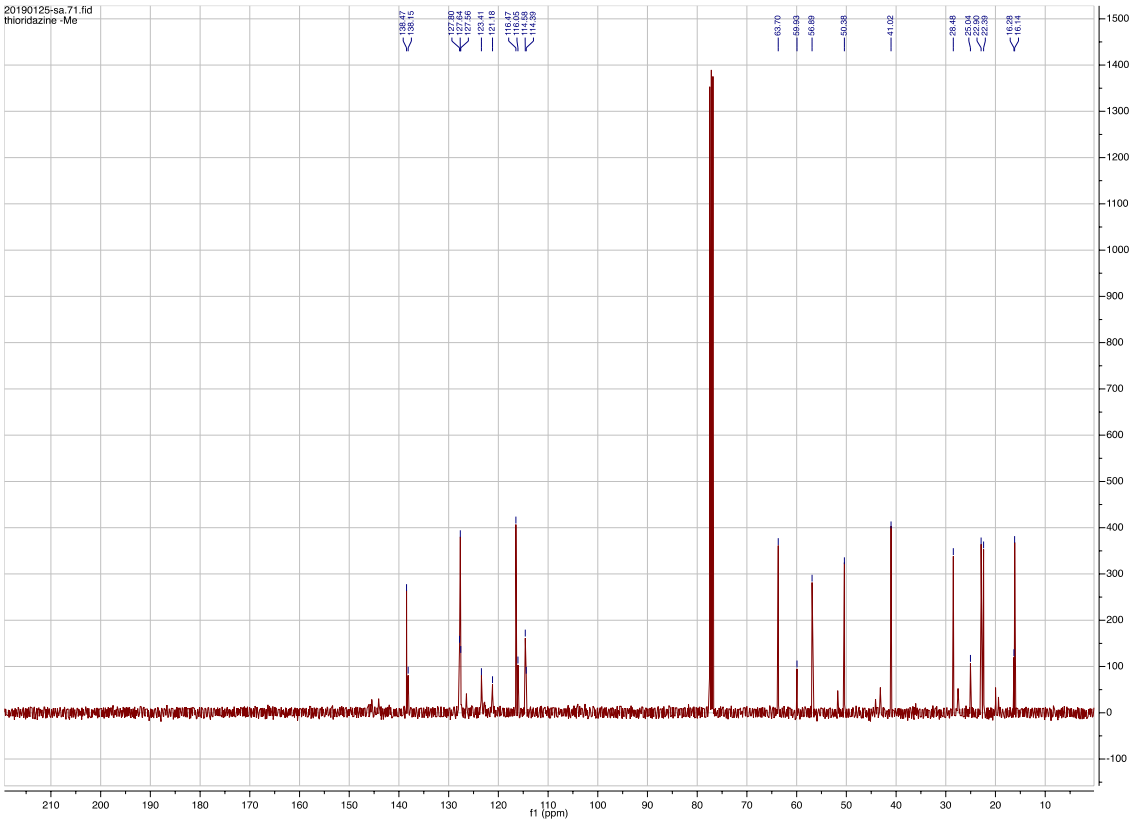
$NaHCO_3$, once with water and once with brine. The organic phase was dried with Na_2SO_4, filtered then concentrated under reduced pressure. The concentrate was purified with flash chromatography using 0-10% ethyl acetate in hexane to afford one faster eluting diastereomer (**39-f**, 175mg, 15%), and one slower eluting diastereomer (**39-s**, 190mg, 17%), there were also 100mg (7%) of coeluted diastereomers.

Data for **67** (after removing methyl group)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20-7.07 (m, 2H), 7.03 (dd, J = 8.0, 1.6 Hz, 1H), 6.96-6.68 (m, 4H), 4.15-3.78 (m, 2H), 3.38 (s, 4H), 2.86-2.74 (m, 1H), 2.62-2.45 (m, 3H), 2.40 (dd, J = 18.4, 4.2 Hz, 7H), 2.10 (d, J = 12.7 Hz, 2H), 1.98-1.59 (m, 4H), 1.27 (dt, J = 13.1, 3.6 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 138.47, 138.15, 127.80, 127.64, 127.56, 123.41, 121.18, 116.47, 116.05, 114.58, 114.39, 63.70, 59.93, 56.89, 50.38, 41.02, 28.48, 25.04, 22.90, 22.39, 16.28, 16.14.



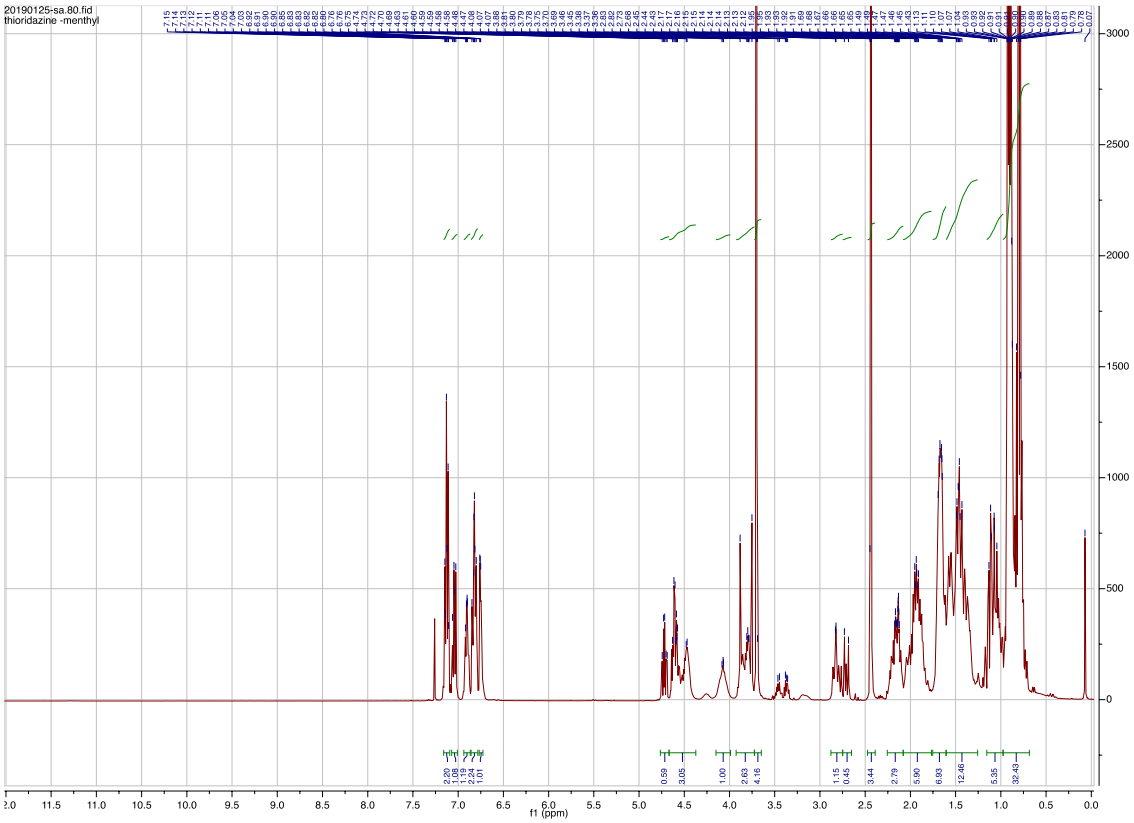


Data **39** (after adding menthol group)

Rf: **39-f** = 0.25 (7.5% EtOAc/Hexane) **39-s** = 0.13 (7.5% EtOAc/Hexane)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (dd, J = 8.2, 6.1 Hz, 2H), 7.04 (dd, J = 8.1, 5.9 Hz, 1H), 6.91 (t, J = 3.8 Hz, 1H), 6.86-6.78 (m, 2H), 6.77-6.73 (m, 1H), 4.76-4.67 (m, 1H), 4.67-4.37 (m, 3H), 4.08 (d, J = 4.7 Hz, 1H), 3.93-3.72 (m, 3H), 3.70 (s, 4H), 2.88-2.75 (m, 1H), 2.44 (d, J = 0.9 Hz, 3H), 2.26-2.08 (m, 3H), 2.08-1.77 (m, 6H), 1.75-1.60 (m, 7H), 1.60-1.26 (m, 12H), 1.16-0.97 (m, 5H), 0.97-0.68 (m, 32H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 155.59, 149.97, 148.31, 145.69, 145.18, 145.12, 137.75, 127.76, 127.67, 127.57, 127.34, 127.32, 122.73, 122.67, 120.86, 115.66, 115.53, 114.52, 114.41, 84.04, 80.91, 75.15, 75.11, 71.48, 50.17, 47.41, 46.88, 45.15, 44.84, 43.51, 41.60, 40.22, 40.18, 39.42, 34.43, 33.98, 33.86, 31.53, 31.45, 31.42, 29.15, 26.73, 26.36, 26.04, 25.60, 23.48, 23.28, 22.16, 22.09, 21.95, 21.89, 20.82, 20.71, 20.58, 19.33, 19.28, 16.43, 16.41, 16.35, 16.23.


20190125-sa.80.fid
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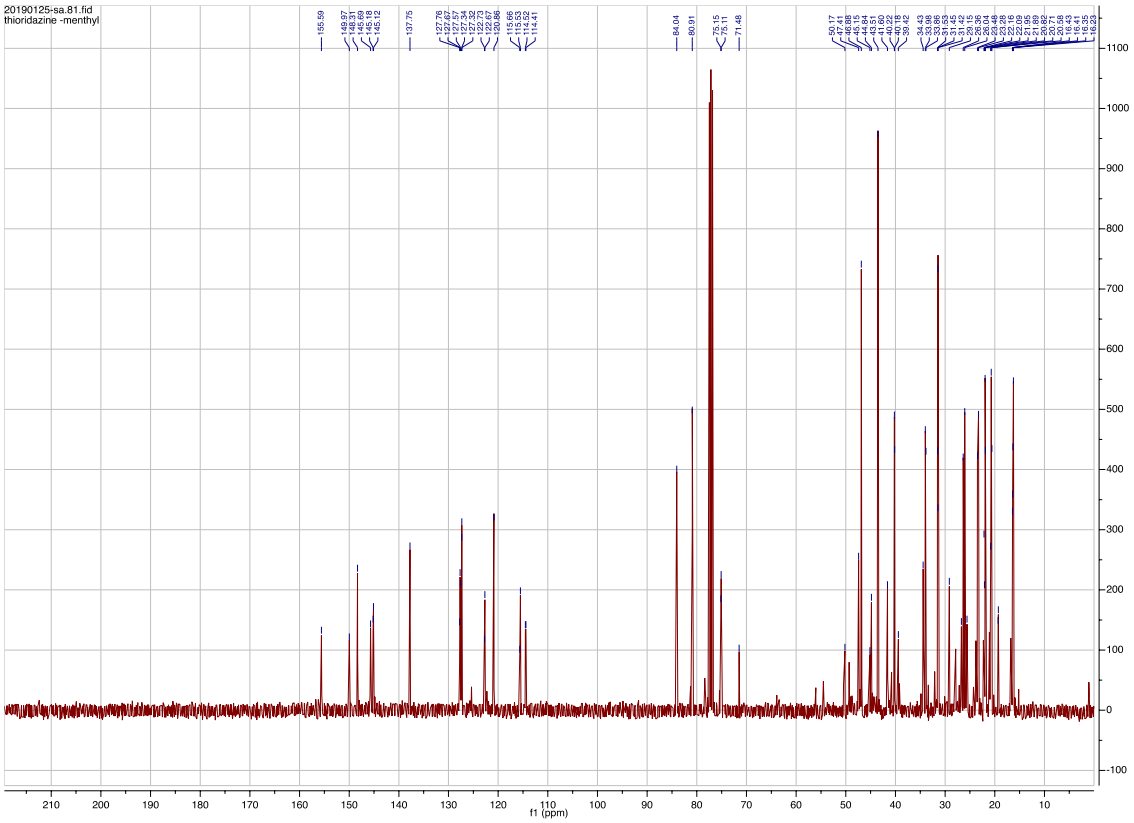
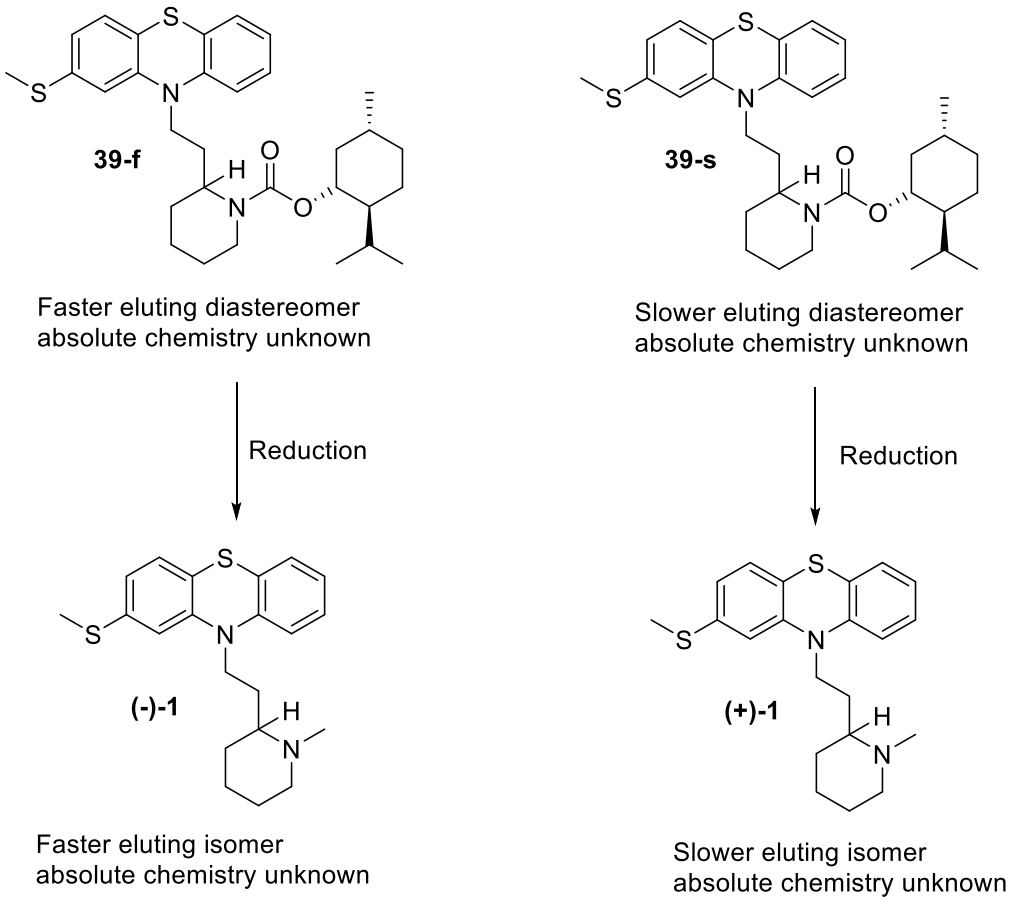
f1 (ppm)


20190125-sa.81.fid
thioridazine -menthyl
f1 (ppm)

3.2.3 Removing the chiral auxilary



The faster eluting diastereomer was dissolved in THF at 0 °C, to this mix 2.0 M lithium aluminumhydride in THF was added. The reaction was warmed to r.t., then heated at 50 °C for 18 hours. The reaction was cooled, then water was added, then 1 M NaOH, then water was added. This mixture was stirred overnight, and then filtered with celite, and the mix was concentrated under reduced pressure, then purified with a flash column. The faster eluting diastereomer was reduced to the levorotary isomer **(-)-1** (84mg, 70%).

Afterwards the exact same procedure was done on the slower eluting diastereomer. This was reduced to the dextrorotary isomer **(+)-1** (91mg, 72%).

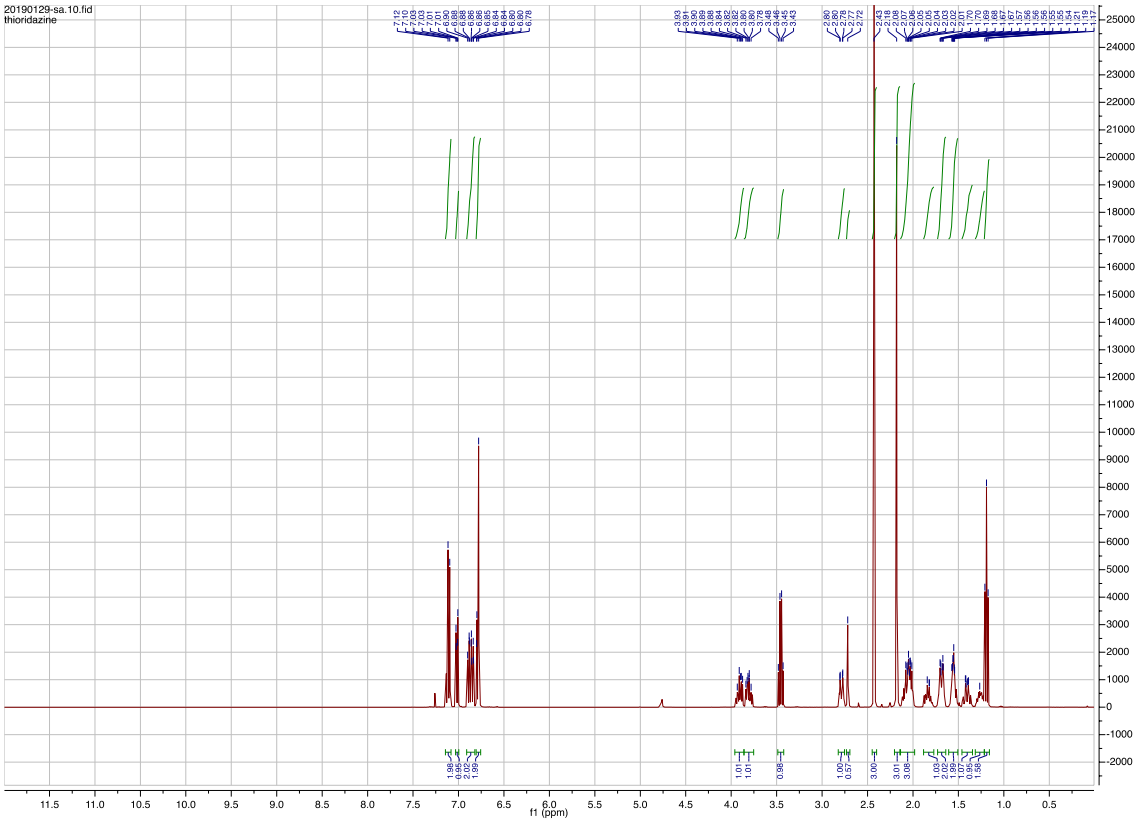
Data for **(-)-1**

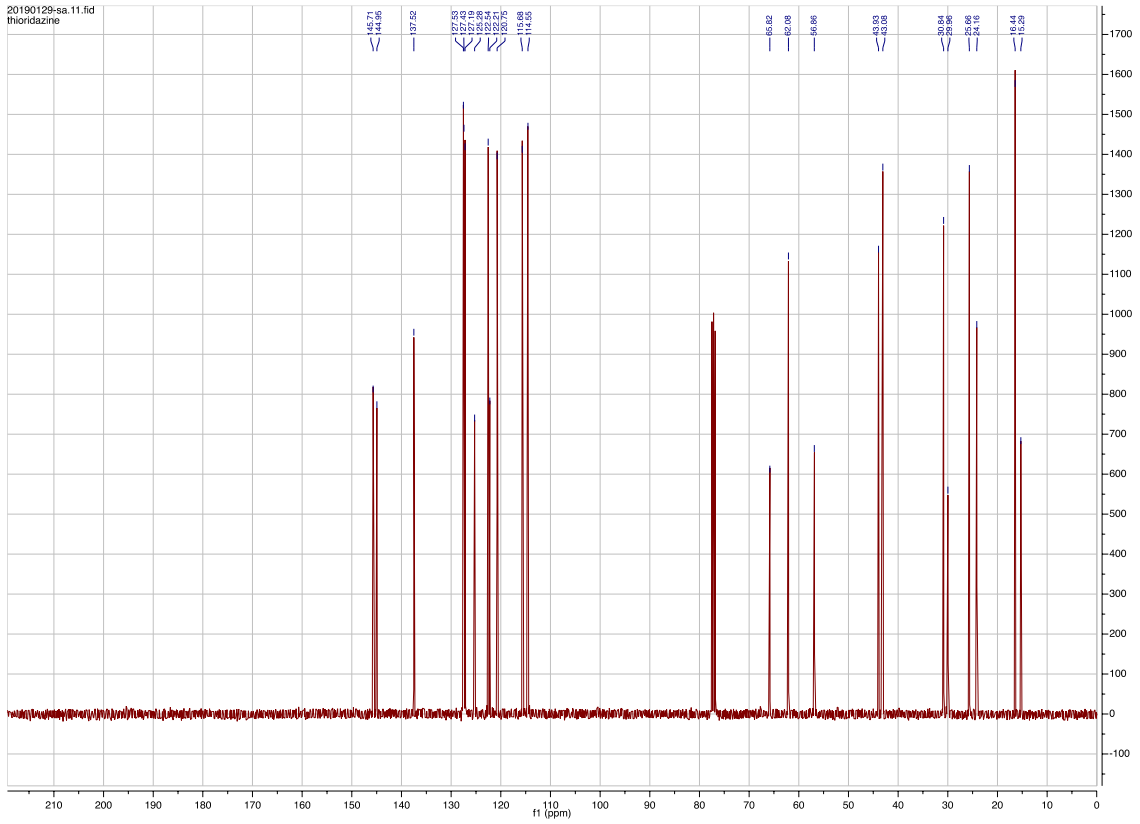
Rf: 0.5 (5% CH_3OH in CH_2Cl_2) 0 (CH_2Cl_2)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, J = 7.6 Hz, 2H), 7.03-7.00 (m, 1H), 6.91-6.82 (m, 2H), 6.79 (d, J = 7.4 Hz, 2H), 3.96-3.86 (m, 1H), 3.86-3.75 (m, 1H), 3.45 (q, J = 7.0 Hz, 1H), 2.79 (dd, J = 11.6, 1.4 Hz, 1H), 2.72 (s, 1H), 2.43 (s, 3H), 2.18 (s, 3H), 2.14-1.98 (m, 3H), 1.88-1.77 (m, 1H),

1.68 (dt, J = 9.8, 2.1 Hz, 2H), 1.61-1.51 (m, 2H), 1.46-1.35 (m, 1H), 1.27 (s, 1H), 1.19 (t, J = 7.0 Hz, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 145.71, 144.95, 137.52, 127.53, 127.43, 127.19, 125.28, 122.54, 122.21, 120.75, 115.68, 114.55, 65.82, 62.08, 56.86, 43.93, 43.08, 30.84, 29.96, 25.66, 24.16, 16.44, 15.29.



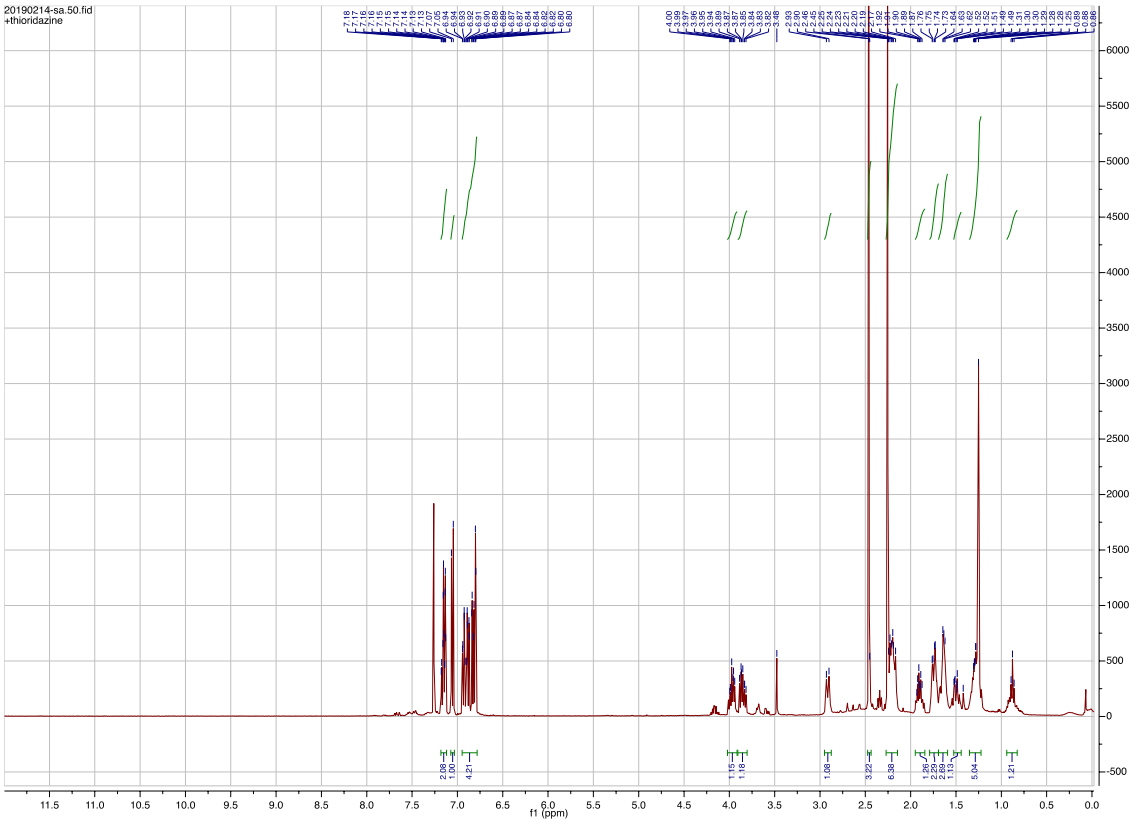


Data for **(+)-1**

Rf: 0.5 (5% CH_3OH in CH_2Cl_2) 0 (CH_2Cl_2)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18-7.12 (m, 2H), 7.06 (d, J = 8.0 Hz, 1H), 6.95-6.78 (m, 4H), 3.97 (td, J = 8.4, 4.2 Hz, 1H), 3.85 (ddd, J = 13.9, 8.3, 6.2 Hz, 1H), 2.92 (d, J = 11.6 Hz, 1H), 2.46 (s, 3H), 2.25 (s, 6H), 1.95-1.84 (m, 1H), 1.75 (dd, J = 10.0, 3.2 Hz, 2H), 1.69-1.59 (m, 3H), 1.53-1.44 (m, 1H), 1.25 (s, 5H), 0.88 (t, J = 6.7 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 145.86, 127.77, 127.69, 127.44, 122.88, 121.06, 115.98, 114.78, 62.46, 56.88, 44.01, 25.23, 23.91, 16.60.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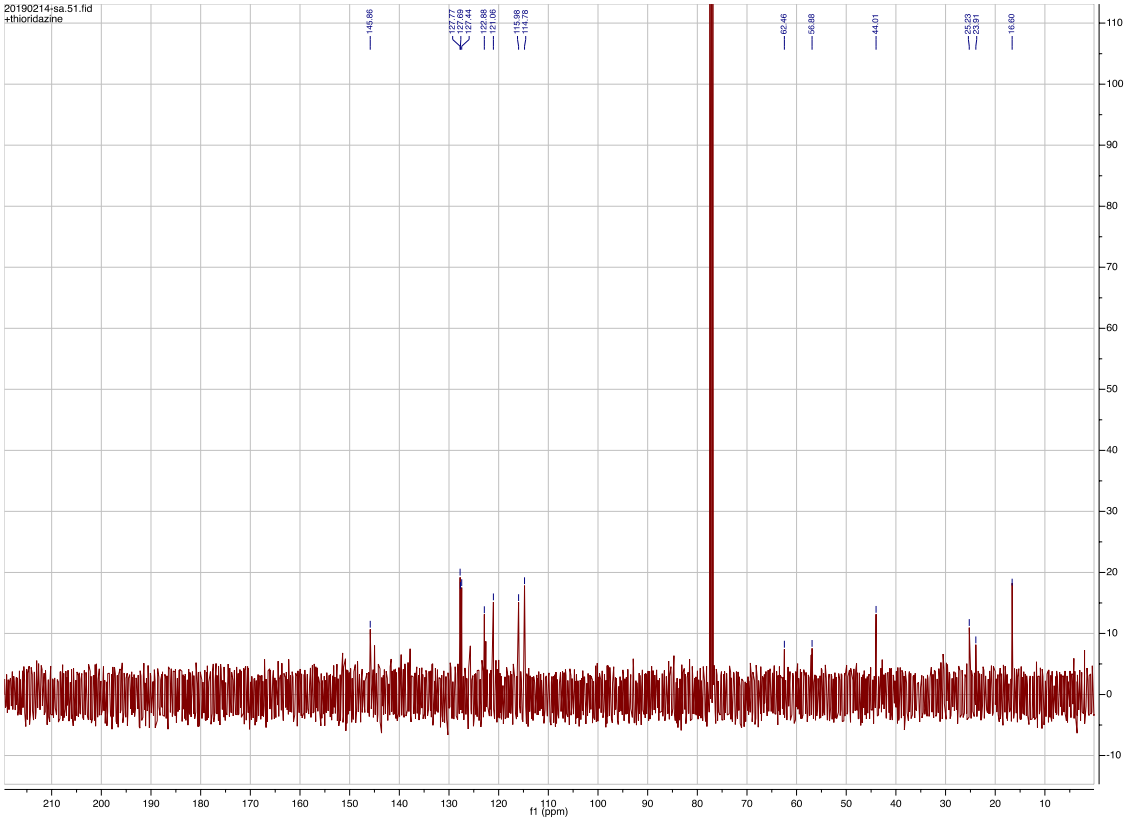
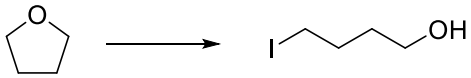
+thioridazine
f1 (ppm)


20190214-sa.51.fid
+thioridazine
f1 (ppm)

3.3 Attempt at synthesis

3.3.1 Synthesis of 4-iodobutan-1-ol (54)



THF **54**

Yield: 57%

Citation: Nyström *et. al.* 1988[52]

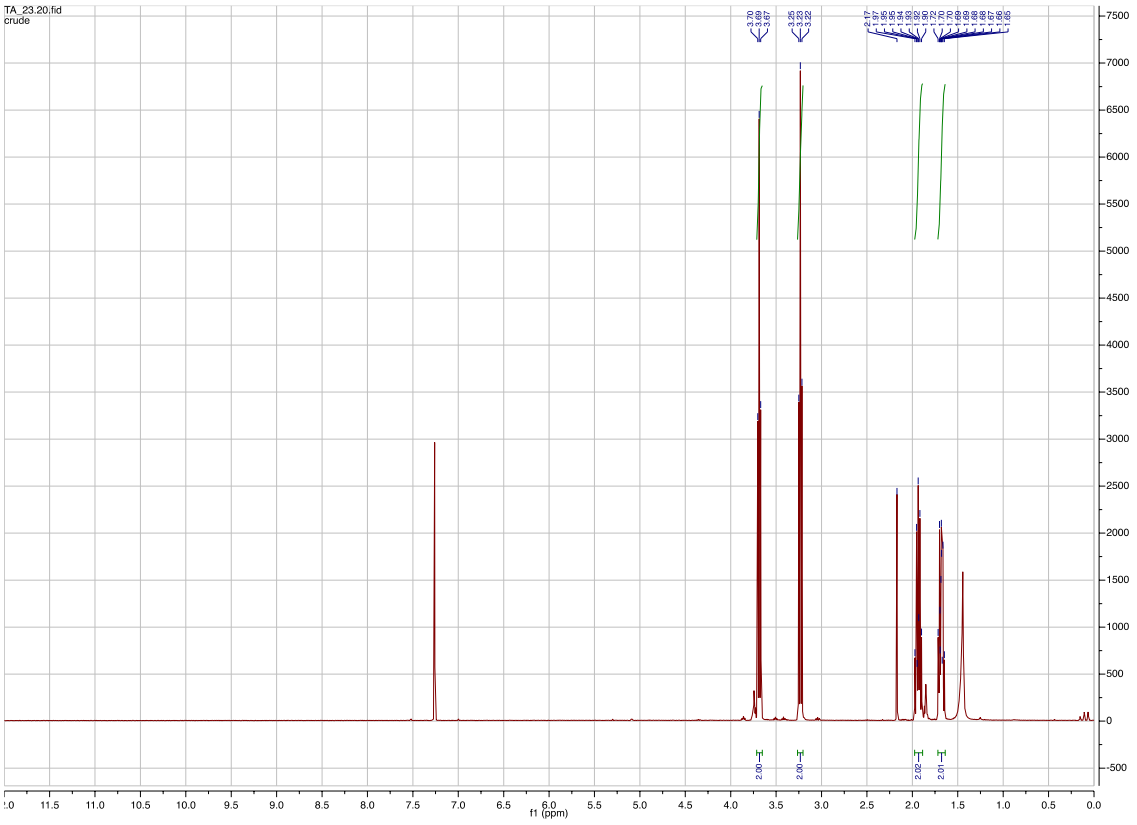
Method:

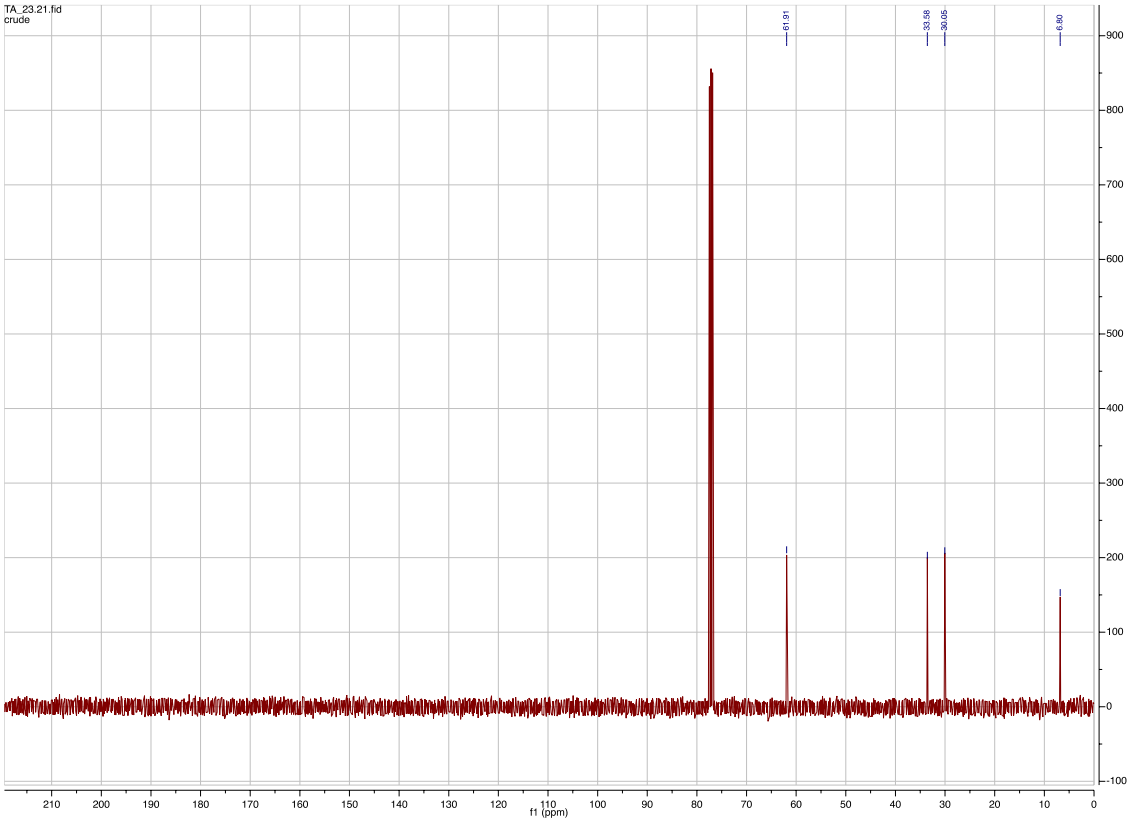
KI (24.3g, 160 mmol) and TMSCl (7.4 mL, 80mmol) was added to THF (100 mL). The mixture was stirred at 60 °C overnight. The mixture was quenched with brine (100 mL) and the product was extracted with ether (4 times). The extract was washed with brine twice, then dried with $MgSO_4$ (15 min). The solvent was removed under reduced pressure, giving the pure product **54** (9.1 g, 57%) as a brown oil.

Data:

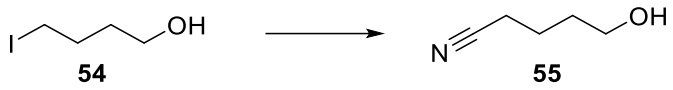
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (t, *J* = 6.3 Hz, 2H), 3.23 (t, *J* = 6.9 Hz, 2H), 1.98-1.83 (m, 2H), 1.73-1.62 (m, 2H), 1.44 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 61.91, 33.58, 30.05, 6.80.


TA_23.20.fid
crude
f1 (ppm)


TA_23.21.fid
crude
61.91
33.58
30.05
6.80
f1 (ppm)

3.3.2 Synthesis of 5-hydroxypentanenitrile (55)



Yield: 46%

Citation: Inoue *et. al.* 2006 [53]

Method:

54 (3.72g, 18.6 mmol) was dissolved in dry DMSO (30 mL), NaCN (1.82g, 37 mmol) was added. The mixture was allowed to react at 40 °C in a sealed vessel overnight. Then the mixture was cooled down to r.t. and quenched with an 1:1 mix of water and brine. The polar phase was extracted with EtOAC (5 times). More salt was added to the polar phase and extracted again with EtOAc. The extract was washed with brine twice, dried with $MgSO_4$ and lastly the solvent was removed under reduced pressure. The combined organic phases were purified with a 1 cm silica plug with EtOAc as eluent.

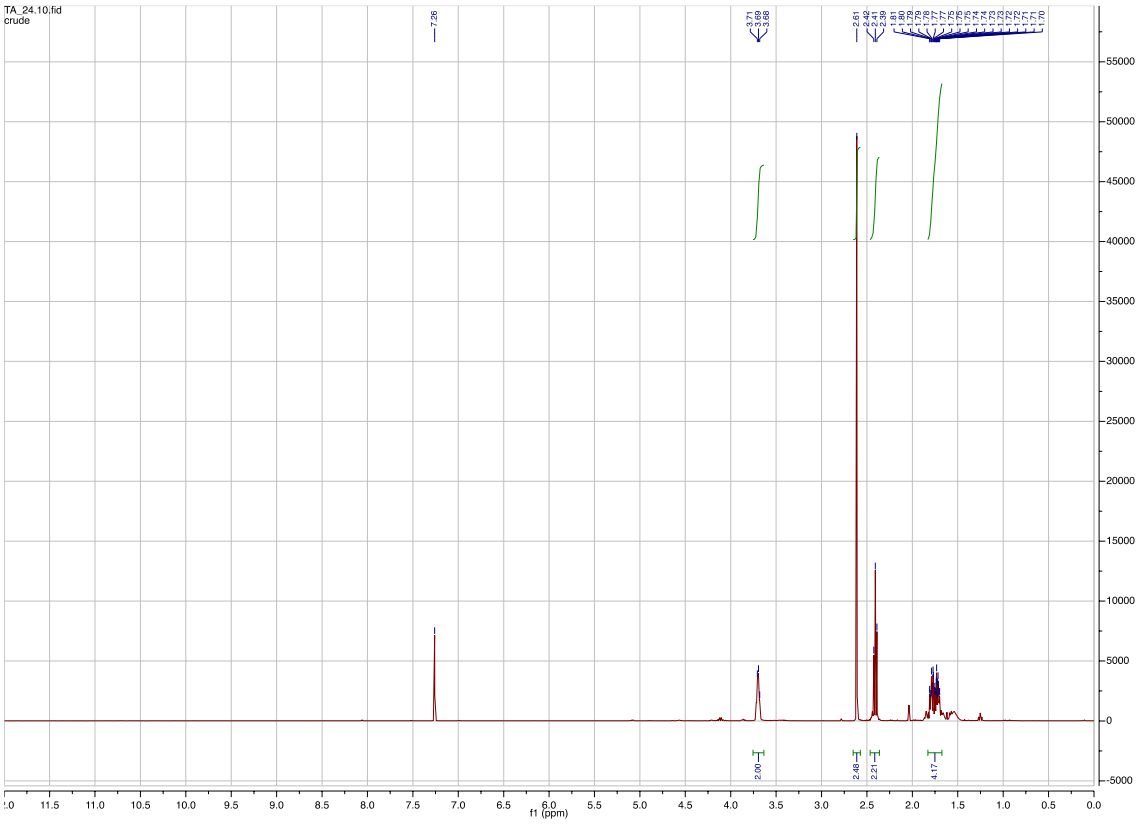
The combined phases gave 0.85 g of **55** as an yellowish oil. (46% yield).

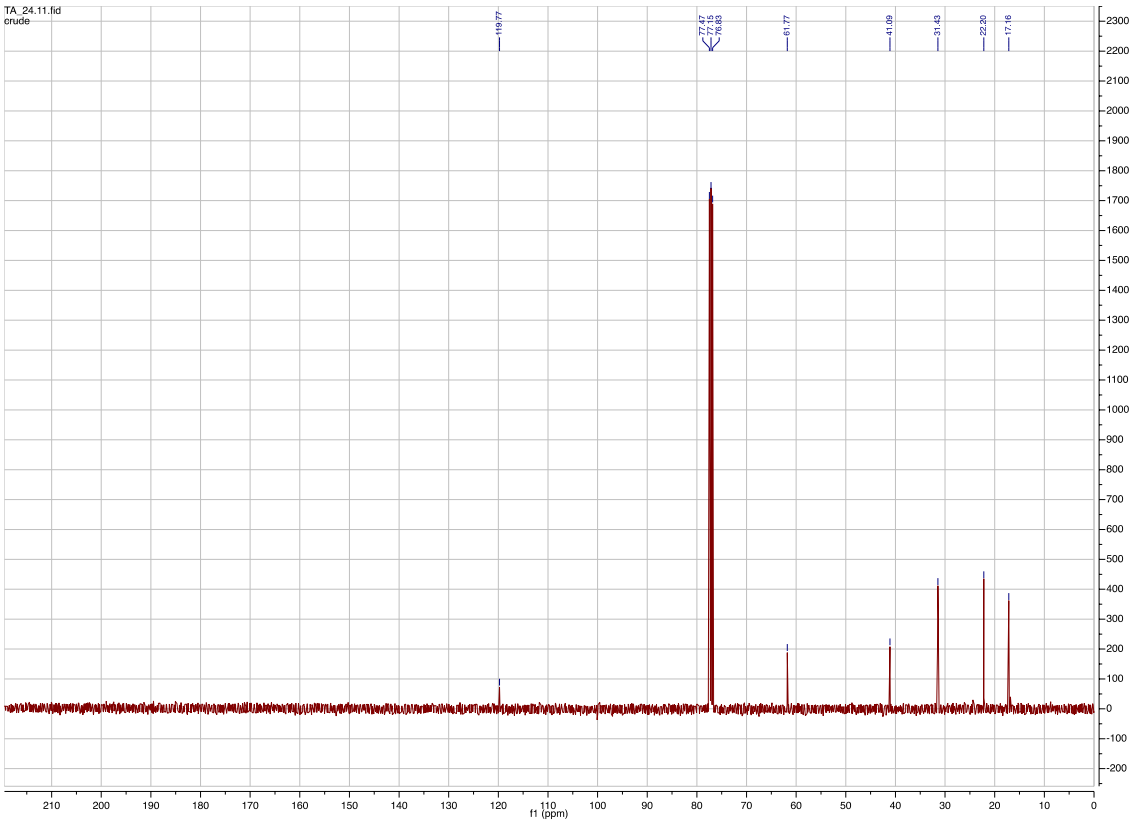
Data:

R_F: 0.33 in EtOAc/hexane (75/25)

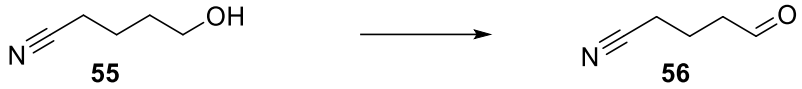
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70 (d, *J* = 5.0 Hz, 2H), 2.41 (t, *J* = 6.9 Hz, 2H), 1.82-1.68 (m, 4H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 119.77, 61.77, (41,09=DMSO), 31.43, 22.20, 17.16


TA_24.10.fid
crude
7.26
3.71
3.69
3.68
2.61
2.42
2.41
2.39
1.81
1.80
1.79
1.78
1.77
1.77
1.76
1.75
1.74
1.73
1.73
1.72
1.72
1.71
1.70
2.00
2.48
2.21
4.17
f1 (ppm)


TA_24.11.fid
crude
119.77
77.47
77.15
76.83
61.77
41.09
31.43
22.20
17.16
f1 (ppm)

3.3.3 Synthesis of 5-oxopentanenitrile (56)



Yield: 25%

Citation: Nair *et. al.* 2010[54]

Method:

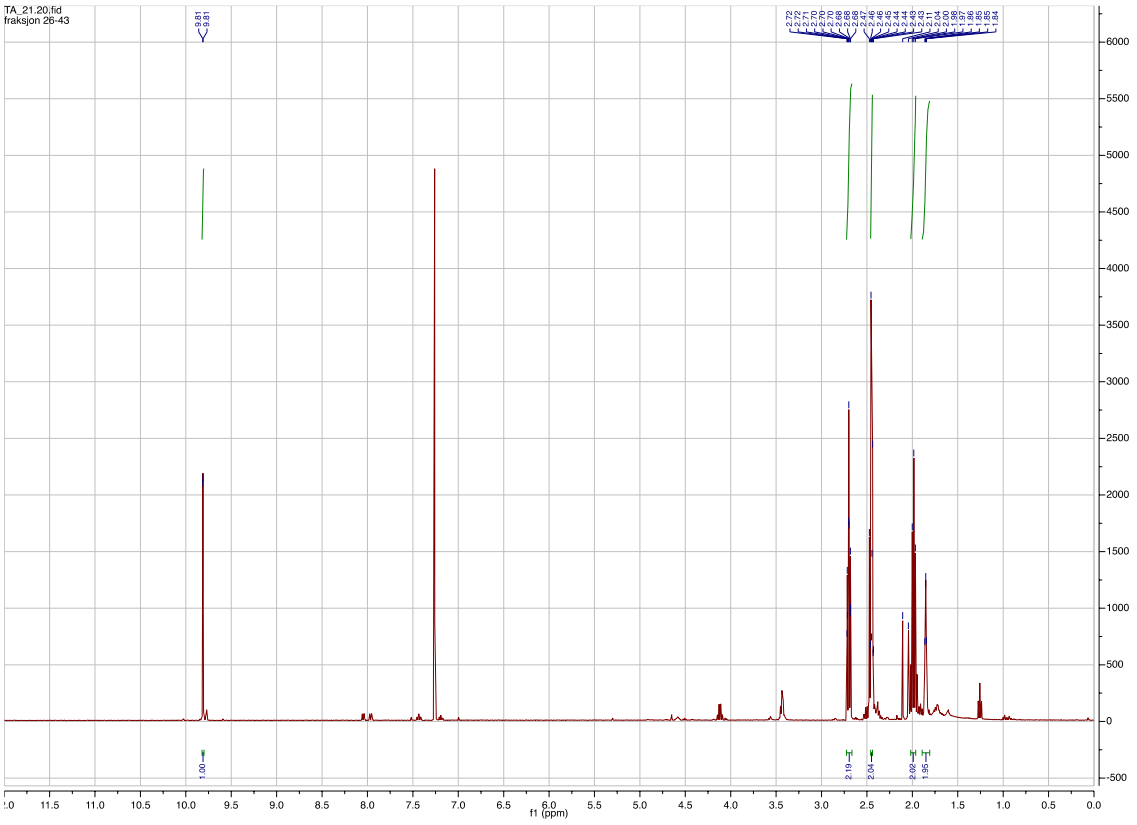
Dess–Martin periodinane (3.5 g, 8.256 mmol) was dissolved in 60 mL of CH_2Cl_2. **55** (0.774g, 7,51 mmol) was dissolved in 45 mL of DCM and then added dropwise to the first solution. After stirring at RT for two hours, the reaction was quenched. First with saturated $Na_2S_2O_3$ (60 mL), then with 60 mL of saturated $NaHCO_3$. The polar phase was extracted with Et_2O (6x 30 mL). The organic phase was washed twice with brine, then dried with Na_2SO_4. This product was purified using flash chromatography, where it was eluted using a 2:3 ratio of EtOAc:Hexane. The yield was 0.185g, 25%.

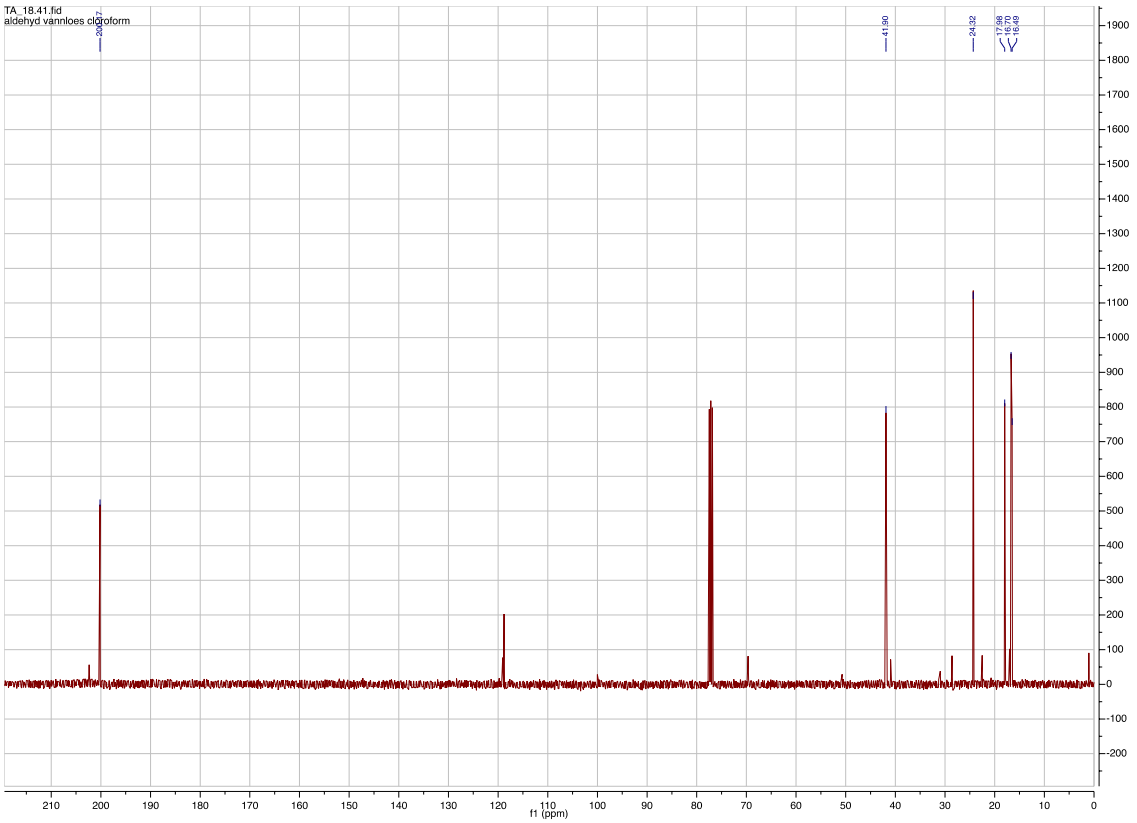
Data:

R_F: 0.2 in EtOAc/hexane (40/60)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (bs, 1H), 2.79 – 2.62 (m, 2H), 2.46 (t, *J* = 7.0 Hz, 2H), 1.98 (t, *J* = 7.0 Hz, 2H), 1.90 – 1.81 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 200.16, 118.80, (41.89=DMSO) 24.31, 22.52, 16.69.


TA_21.20.fid
fraksjon 26-43
f1 (ppm)


TA_18.41.fid
aldehyd vannloes chloroform
f1 (ppm)

3.3.4 Synthesis of 5-hydroxypentyl benzoate (41)

40 **41**

Yield: 88%

Method:

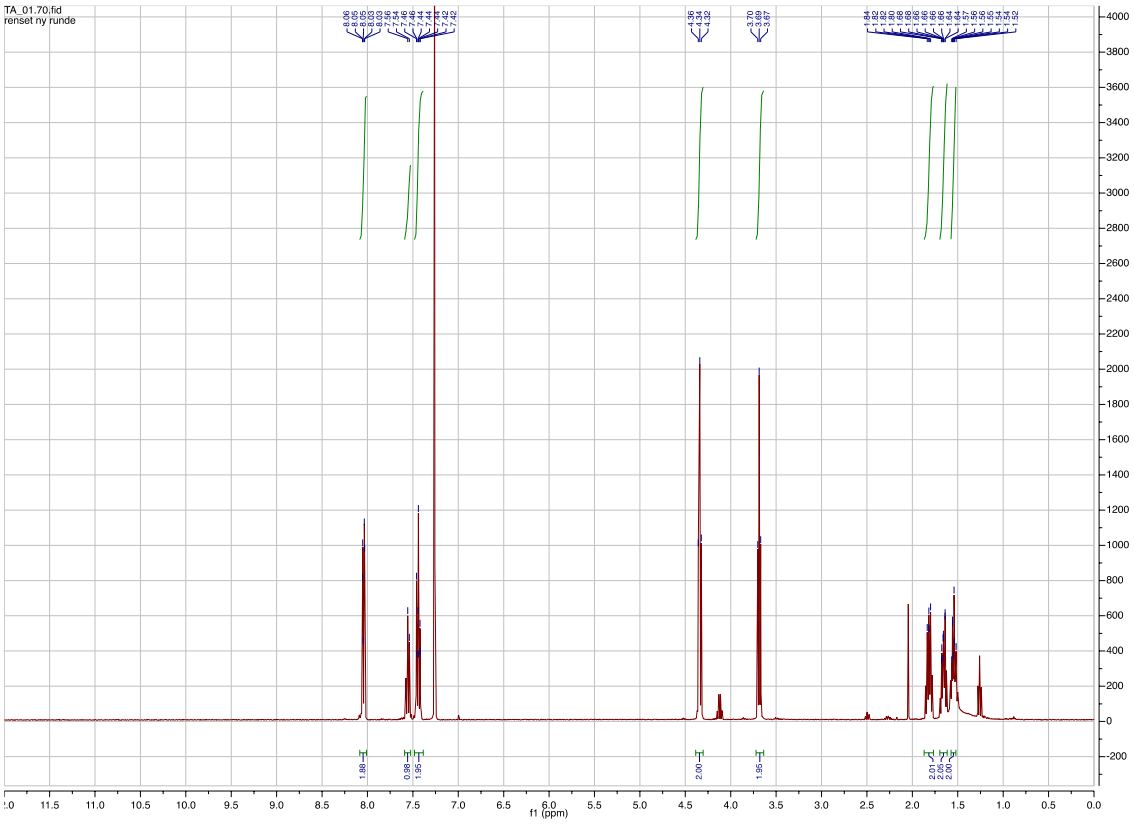
NaH (60%wt, 4.8g, 120 mmol) was dissolved in THF (36 mL) at r.t. The solution was cooled to 0°C. 1,5-pentadiol (74 mL, 700 mmol) was added in a dropwise manner, before benzoyl chloride (12 mL, 100 mmol) also was added dropwise. The reaction was allowed to warm up to r.t. while being stirred overnight. The next day the reaction was quenched with saturated NH_4Cl. The mixture was diluted with Et_2O and the extracted with EtOAc. The salt was filtered away and the liquid was dried with $MgSO_4$. The solvent was removed under reduced pressure, and this product was purified using flash chromatography, where it was eluted using a 1:9 ratio of EtOAc:Hexane. The yield was 88%.

Data:

R_F: 0.29 in EtOAc/hexane (10/90)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 8.3, 1.4 Hz, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 4.34 (t, *J* = 6.6 Hz, 2H), 3.69 (t, *J* = 6.4 Hz, 2H), 1.86-1.77 (m, 2H), 1.70-1.62 (m, 2H), 1.58-1.51 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 132.85, 129.54, 128.34, 64.88, 62.77, 32.35, 28.57, 22.38.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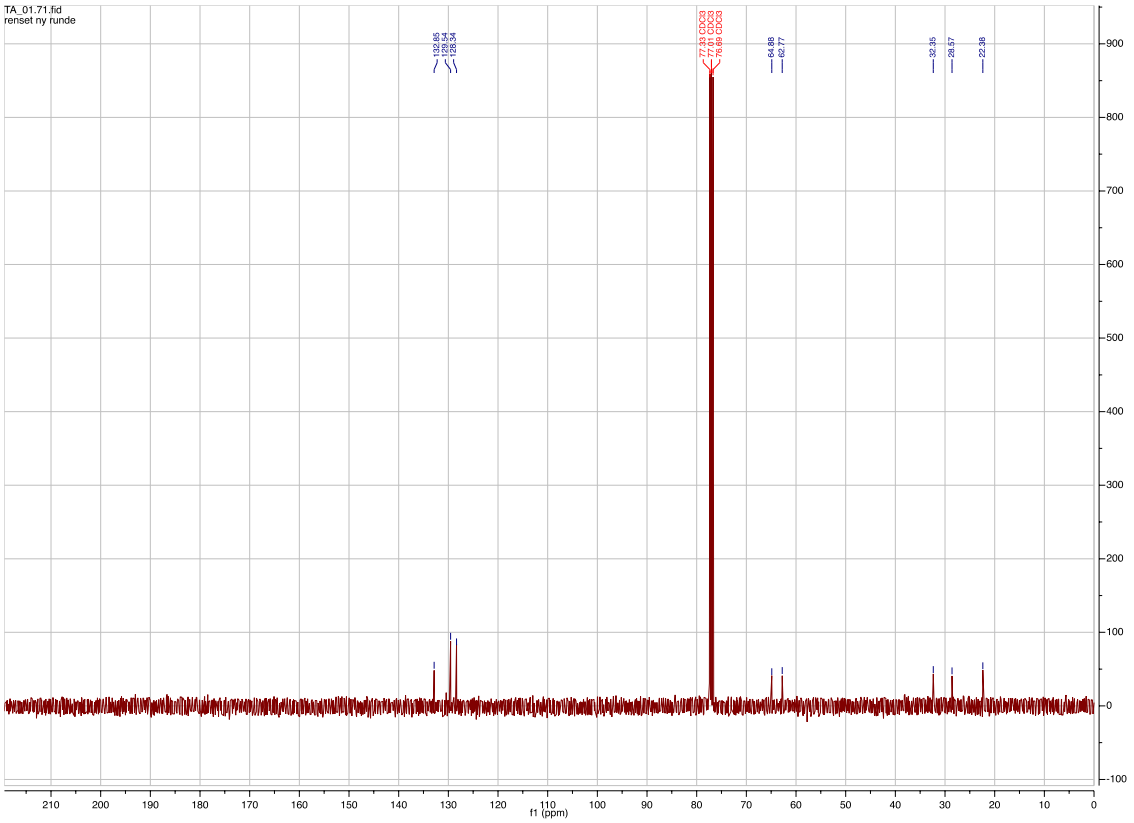
f1 (ppm)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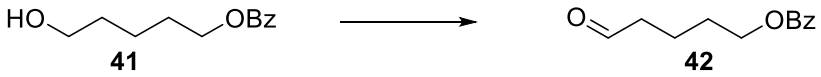
renset ny runde
f1 (ppm)

3.3.5 Synthesis of 5-oxopentyl benzoate (42)



41 **42**

Yield: 48%

Citation: Nair *et. al.* 2010[54]

Method:

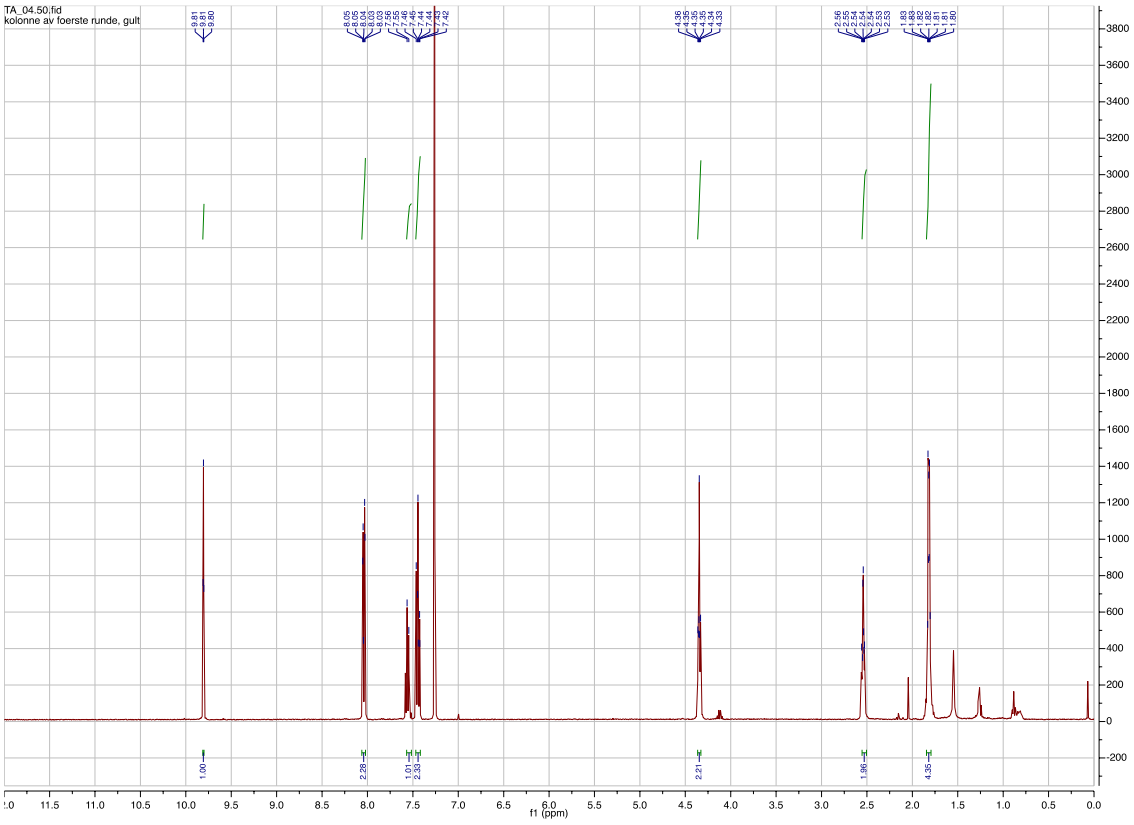
Dess–Martin periodinane(1.12 g, 2.6 mmol) was dissolved in 20 mL of CH_2Cl_2. **41** (0.500 g, 2,4 mmol) was dissolved in 15 mL of DCM and then added dropwise to the first solution. After stirring at r.t. for two hours, the reaction was quenched. First with saturated $Na_2S_2O_3$ (35 mL), then with saturated $NaHCO_3$ (35 mL). The polar phase was extracted with Et_2O. The organic phase was washed with brine, then dried with $MgSO_4$. This product was purified using flash chromatography, where it was eluted using a 2:3 ratio of EtOAc:Hexane. The yield was 0.238 g of **42**, 48%.

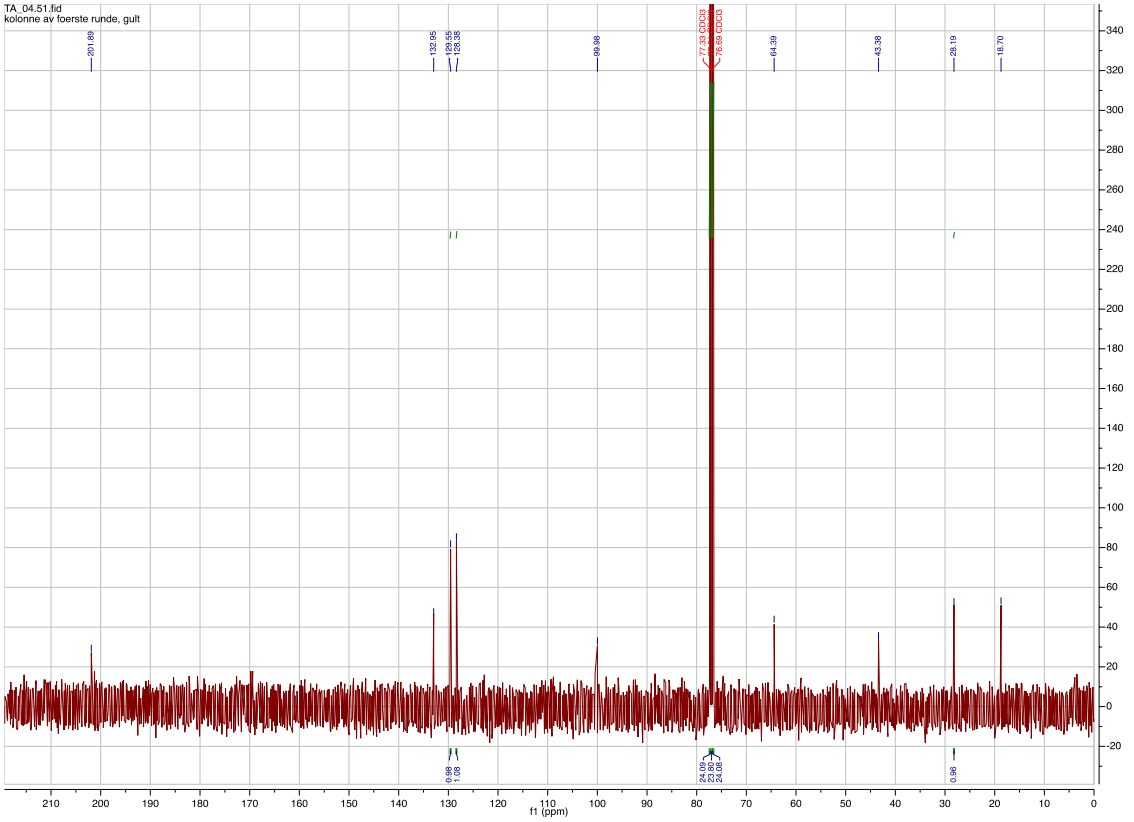
Data:

R_F: 0.5 in EtOAc/hexane (50/50)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (t, *J* = 1.5 Hz, 1H), 8.09-7.98 (m, 2H), 7.55 (d, *J* = 7.4 Hz, 1H), 7.44 (dd, *J* = 8.2, 7.0 Hz, 2H), 4.39-4.29 (m, 2H), 2.54 (q, 2H), 1.81 (m, 4H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 201.89, 169.25, 132.95, 129.55, 128.38, 99.98, 64.39, 43.39, 28.19, 18.70.

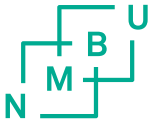

TA_04.50.fid
kolonne av foerste runde, gult
f1 (ppm)


TA_04.51.fid
kolonne av foerste runde, gult
f1 (ppm)

4 References

1. Radhakrishnan, V.; Ganguly, K.; Ganguly, M.; Dastidar, S. G.; Chakrabarty, A. N. *Indian J Exp Biol* **1999,** *37*, 671-5.
2. Christensen, J. B.; Hendricks, O.; Chaki, S.; Mukherjee, S.; Das, A.; Pal, T. K.; Dastidar, S. G.; Kristiansen, J. E. *PLoS One* **2013,** *8*, e57493.
3. Svendsen, C. N.; Froimowitz, M.; Hrbek, C.; Campbell, A.; Kula, N.; Baldessarini, R. J.; Cohen, B. M.; Babb, S.; Teicher, M. H.; Bird, E. D. *Neuropharmacology* **1988,** *27*, 1117-1124.
4. Getinet, A. *Research & Reviews: Journal of Chemistry* **2016**.
5. Ji, H.-F.; Li, X.-J.; Zhang, H.-Y. *EMBO reports* **2009,** *10*, 194-200.
6. Fleming, A. *British journal of experimental pathology* **1929,** *10*, 226-236.
7. Newman, D. J. *J Med Chem* **2008,** *51*, 2589-99.
8. Lopez-Munoz, F.; Alamo, C.; Cuenca, E.; Shen, W. W.; Clervoy, P.; Rubio, G. *Ann Clin Psychiatry* **2005,** *17*, 113-35.
9. Shen, W. W. *Compr Psychiatry* **1999,** *40*, 407-14.
10. Chin-Yee, B. H.; Hektoen Institute of Medicine: Hektoen International Journal, 2016.
11. Amaral, L.; Viveiros, M.; Molnar, J. *Antimicrobial activity of phenothiazines*, 2004; Vol. 18.
12. Fenton, M.; Rathbone, J.; Reilly, J.; Sultana, A. *Cochrane Database Syst Rev* **2007**, Cd001944.
13. Bisset, A. F. *BMJ (Clinical research ed.)* **2002,** *325*, 967-967.
14. Panoulas, V. F.; Toms, T. E.; Douglas, K. M. J.; Sandoo, A.; Metsios, G. S.; Stavropoulos-Kalinoglou, A.; Kitas, G. D. *Rheumatology* **2014,** *53*, 131-137.
15. Yap, Y. G.; Camm, A. J. *Heart (British Cardiac Society)* **2003,** *89*, 1363-1372.
16. Reilly, J. G.; Ayis, S. A.; Ferrier, I. N.; Jones, S. J.; Thomas, S. H. L. *British Journal of Psychiatry* **2002,** *180*, 515-522.
17. Glassman, A. H.; Bigger, J. T., Jr. *Am J Psychiatry* **2001,** *158*, 1774-82.
18. Poulsen, M. Ø.; Klitgaard, J. K.; Christensen, J. B.; Kallipolitis, B. H.; Kaatz, G. W.; Plenge, P.; Fey, S. J.; Kristiansen, J. E. *American Journal of Bioavailability & Bioequivalence* **2018**.
19. WHO, I., 2018.
20. Amaral, L.; Kristiansen, J. E.; Abebe, L. S.; Millett, W. *Journal of Antimicrobial Chemotherapy* **1996,** *38*, 1049-1053.
21. WHO. 2018.
22. Crowle, A. J.; Douvas, G. S.; May, M. H. *Chemotherapy* **1992,** *38*, 410-9.
23. Forrest, F. M.; Forrest, I. S.; Roizin, L. *Agressologie* **1963,** *4*, 259-65.
24. Dutta, N. K.; Pinn, M. L.; Karakousis, P. C. *Antimicrob Agents Chemother* **2014,** *58*, 4048-53.
25. Ordway, D.; Viveiros, M.; Leandro, C.; Bettencourt, R.; Almeida, J.; Martins, M.; Kristiansen, J. E.; Molnar, J.; Amaral, L. *Antimicrob Agents Chemother* **2003,** *47*, 917-22.
26. Martins, M.; Viveiros, M.; Ramos, J.; Couto, I.; Molnar, J.; Boeree, M.; Amaral, L. *Int J Antimicrob Agents* **2009,** *33*, 479-82.
27. Viveiros, M.; Leandro, C.; Amaral, L. *Int J Antimicrob Agents* **2003,** *22*, 274-8.
28. Machado, D.; Couto, I.; Perdigao, J.; Rodrigues, L.; Portugal, I.; Baptista, P.; Veigas, B.; Amaral, L.; Viveiros, M. *PLoS One* **2012,** *7*, e34538.
29. Rodrigues, L.; Ainsa, J. A.; Amaral, L.; Viveiros, M. *Recent Patents on Anti-Infective Drug Discovery* **2011**.
30. Amaral, L.; Viveiros, M. *Antibiotics (Basel, Switzerland)* **2017,** *6*, 3.
31. Dutta, N. K.; Mehra, S.; Kaushal, D. *PLoS One* **2010,** *5*, e10069.
32. van Ingen, J.; van der Laan, T.; Amaral, L.; Dekhuijzen, R.; Boeree, M. J.; van Soolingen, D. *Int J Antimicrob Agents* **2009,** *34*, 190-1.

33. Abbate, E.; Vescovo, M.; Natiello, M.; Cufré, M.; García, A.; Gonzalez Montaner, P.; Ambroggi, M.; Ritacco, V.; van Soolingen, D. *Journal of Antimicrobial Chemotherapy* **2012,** *67*, 473-477.
34. Tantibanchachai, C.: The Embryo Project Encyclopedia, 2014.
35. Hendricks, O.; Molnar, A.; Butterworth, T. S.; Butaye, P.; Kolmos, H. J.; Christensen, J. B.; Kristiansen, J. E. *Basic & Clinical Pharmacology & Toxicology* **2005,** *96*, 33-36.
36. Eap, C. B.; Guentert, T. W.; Schäublin-Loidl, M.; Stabl, M.; Koeb, L.; Powell, K.; Baumann, P. *Clinical Pharmacology & Therapeutics* **1996,** *59*, 322-331.
37. Jortani, S. A.; Valentour, J. C.; Poklis, A. *Forensic Science International* **1994,** *64*, 165-170.
38. Thanacoody, H. K. R. *British journal of clinical pharmacology* **2007,** *64*, 566-574.
39. S M Salih, I.; Thanacoody, H.; McKay, G.; H L Thomas, S. *Comparison of the Effects of Thioridazine and Mesoridazine on the QT Interval in Healthy Adults After Single Oral Doses*, 2007; Vol. 82.
40. Roth, B.; Driscol, J.; University of North Carolina at Chapel Hill and the United States National Institute of Mental Health., 2011.
41. Choi, S.; Haggart, D.; Toll, L.; Cuny, G. D. *Bioorganic & Medicinal Chemistry Letters* **2004,** *14*, 4379-4382.
42. Porter, W. H. *Pure and applied chemistry* **1991,** *63*, 1119-1122.
43. Rohloff, J. C.; Kent, K. M.; Postich, M. J.; Becker, M. W.; Chapman, H. H.; Kelly, D. E.; Lew, W.; Louie, M. S.; McGee, L. R.; Prisbe, E. J.; Schultze, L. M.; Yu, R. H.; Zhang, L. *The Journal of Organic Chemistry* **1998,** *63*, 4545-4550.
44. Tungen, J. E.; Aursnes, M.; Vik, A.; Ramon, S.; Colas, R. A.; Dalli, J.; Serhan, C. N.; Hansen, T. V. *Journal of Natural Products* **2014,** *77*, 2241-2247.
45. Cram, D. J.; Elhafez, F. A. A. *Journal of the American Chemical Society* **1952,** *74*, 5828-5835.
46. Mohammad, T.; Midha, K. K.; Hawes, E. M. *Journal of Labelled Compounds and Radiopharmaceuticals* **1986,** *23*, 749-760.
47. Mohammad, T.; Midha, K. K.; Hawes, E. M. *Journal of Labelled Compounds and Radiopharmaceuticals* **1988,** *25*, 415-427.
48. Bourquin, J. P.; Schwarb, G.; Gamboni, G.; Fischer, R.; Ruesch, L.; Guldimann, S.; Theus, V.; Schenker, E.; Renz, J. *Synthesen auf dem Phenothiazin-Gebiet. 2. Mitteilung. N-substituierte Mercaptophenothiazin-Derivate*, 1958; Vol. 41.
49. Schlauderer, F.; Lammens, K.; Nagel, D.; Vincendeau, M.; Eitelhuber, A. C.; Verhelst, S. H. L.; Kling, D.; Chrusciel, A.; Ruland, J.; Krappmann, D.; Hopfner, K.-P. *Angewandte Chemie International Edition* **2013,** *52*, 10384-10387.
50. Raju, G.; Anitha, K.; Palakodety, R. *Bisnucleophilic Substitution as a Synthetic Tool for Ready Access to the Piperidine Alkaloids (+)-Connine, (+)-ϐ-Conhydrine, (+)-8-Ethylnorlobelol, and (–)-Halosaline*, 2015; Vol. 26.
51. Ramos, J. A. F.; Nagem, T. J.; Taylor, J. G. *South African Journal of Chemistry* **2011,** *64*, 225-230.
52. Nyström, J.-E.; McCanna, T. D.; Helquist, P.; Amouroux, R. *Synthesis* **1988,** *1988*, 56-58.
53. Inoue, M.; Yamashita, S.; Ishihara, Y.; Hirama, M. *Organic Letters* **2006,** *8*, 5805-5808.
54. Nair, L. G.; Bogen, S.; Bennett, F.; Chen, K.; Vibulbhan, B.; Huang, Y.; Yang, W.; Doll, R. J.; Shih, N. Y.; Njoroge, F. G. *Tetrahedron Letters* **2010,** *51*, 3057-3061.



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