THE UNIVERSITY OF HULL

Synthesis of Opiate Derivatives: Investigations into a Key Step From the Manufacturing of Buprenorphine and Definitive Preparation of Degradation Impurities Related to Naloxone

being a Thesis submitted for the Degree of

MSc Chemistry

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by

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Contents

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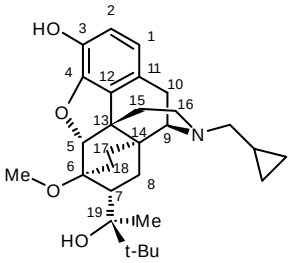
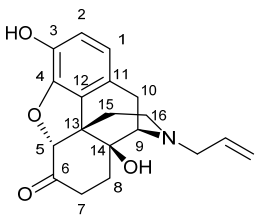
0 Introduction

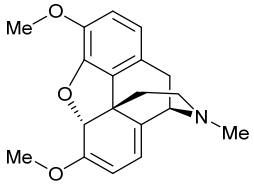
The subject of this thesis is the synthesis and impurity profiling of semi-synthetic opiates, namely buprenorphine and naloxone. Opiates are a class of naturally occurring narcotic analgesic drugs produced from the opium poppy *Papaver somniferum*, including morphine, codeine and thebaine. Semi-synthetic opiates are a class of drugs chemically derived from naturally occurring opiates; typically these are narcotic analgesics such as diacetylmorphine and buprenorphine. A final related class of drug, the opioids, are fully synthetic drugs such as fentanyl, designed to be structurally similar to opiates and elicit similar pharmacological effects. Whilst these drugs and a number of their analogues can be used as analgesics in a medical setting, a number of serious side effects such as addiction and respiratory depression limit their application and can lead to long term dependency for the patient. The narcotic effects of opium, morphine and diacetylmorphine (heroin) makes them attractive to recreational drug users, who often become addicted to and dependent upon them. These drugs are controlled through various legislations worldwide as potential drugs of abuse.[1]



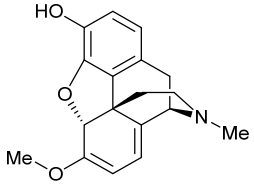
1: Buprenorphine



2: Naloxone



3: Thebaine



4: Oripavine

Buprenorphine (**1**) and naloxone (**2**) are both semi-synthetic opiates, derived from either thebaine (**3**) or oripavine (**4**) depending on the route of manufacture. Buprenorphine was first synthesised in the late 1960s during a search by Reckitt & Coleman to find an opiate analgesic which had a reduced addictive quality. Buprenorphine acts as a μ receptor partial agonist and a δ and κ opioid receptor antagonist,[2] offering pain and opiate addiction withdrawal relief whilst not eliciting a full reward reaction (or "high"). The partial agonist effect on the δ and κ are responsible for this partial reward effect, alongside a secondary beneficial property of the drug in producing a ceiling effect on reparatory depression, meaning that higher doses do not elicit dangerous levels of respiratory depression and potential suffocation.

Naloxone is a full receptor antagonist,[3] offering no analgesic effects. It is also competitive with other opiates, suppressing the reward and respiratory depression effects caused by intoxication with a full receptor agonist. The main application of naloxone is in the treatment for opiate intoxication and overdose in products such as Narcan, which can revive an intoxicated patient quickly and avert fatal overdose with the only side effect being withdrawal symptoms. Furthermore, naloxone injection can be used to diagnose opiate addiction as the drug causes fast acting withdrawal symptoms in an opiate addicted patient.

Buprenorphine combined with naloxone is used as a treatment for opiate dependence in the pharmaceutical product Suboxone, produced as sublingual tablets and film strips. Sublingual use of these products as withdrawal suppressants results in the absorption of buprenorphine *via* the sublingual mucosa into the blood stream, while the naloxone is dissolved in the saliva and carried into the gut where it is destroyed by first pass metabolism. Attempting to abuse or divert the drug, such as by dissolving and injecting a tablet, results in naloxone being released into the blood stream and blocking the effects of buprenorphine, precipitating withdrawal in the patient. In this case,

buprenorphine is being used as the active pharmaceutical to treat the disease of opiate addiction and naloxone is used as a chemical deterrent to abuse or diversion.

As both buprenorphine and naloxone are semi-synthetic opiates, both have the potential to contain impurities generated by the process by which they are derived from thebaine or oripavine. These impurities are classed as "synthetic impurities", so called as they are formed by side reactions in the manufacture. These can be created by undesired reactions between the drug molecule and the reagents added to it, reactions with impurities in the reagents used in the synthesis, reactions between drug molecules during synthesis, structural rearrangements caused by reaction conditions or from the incomplete conversion of a starting material in a synthetic step carrying through to either the end product or into the next step in the synthetic process. For example, both thebaine and oripavine have an *N*-methyl group that must be removed to allow for an alkylation. If this *N*-demethylation is inefficient, the resultant intermediate would not have the appropriate *N*-alkyl group and lead to an undesirable *N*-methyl product.

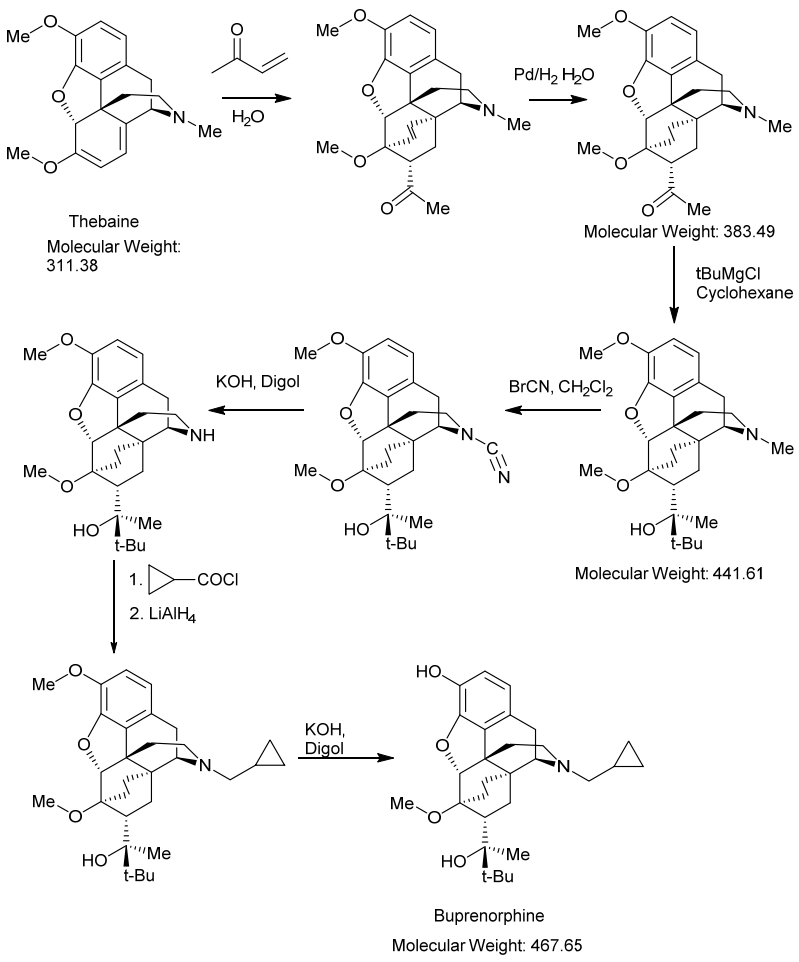
Once synthesised, these drugs need to undergo a manufacturing process whereby they are added to other components and processed to produce the finished tablet or film strip for the patient. This process can be highly stressful to the drug molecule, potentially including processes such as solvation to distribute the drug amongst bulking agents, drying under heat and high pressure in tabletting presses. These processes can cause further impurity formation from interactions with other tablet components, accelerated oxidation in air under heating or pressure. These processes can continue in the end product if the materials used to form the tablet are not compatible with the drug or form some sort of catalytic surface or environment for the drug to react with atmospheric oxygen or moisture. These impurities are classed as "degradation products" or "degradation impurities".

This thesis aims to look at the synthetic and degradation impurities in buprenorphine and naloxone respectively. Firstly, synthetic impurities formed in the manufacture of buprenorphine will be investigated by assessing the Grignard reaction used as an intermediate step in the synthetic process to convert thebaine into buprenorphine, with the aim of reducing their formation and improving the overall yield of the reaction. Secondly, degradation products of naloxone will be identified *via* their definitive synthesis under controlled conditions and characterisation by spectroscopic and analytical techniques.

Chapter 1 – Investigations into the Potential Impurity Products Formed by Grignard Reactions in the Synthesis of Buprenorphine

1 The First Synthesis of Buprenorphine

As previously discussed, the first reported synthesis of buprenorphine was as an eight step process from thebaine as shown in Scheme 1.[4] This was part of a drug discovery exercise by Reckitt and Coleman, working in the field of opiate analgesics with an overall aim of discovering new analgesics with reduced side effects which produced buprenorphine as part of a raft of substituted thebaine derivatives.[4] These thebaine derivatives all had similar structures and were based on the Diels-Alder addition of a dienophile across C^6-C^{14}. The significant differences between these potential pharmaceuticals were whether or not the C^6-C^{14} bridge was saturated, the substituent groups on the tertiary alcohol, the substituent on the tertiary amine and whether the phenolic oxygen was protonated or methylated. These variations produced a spectrum of different analgesic effects, ranging from altered level of analgesia, conversion from agonistic to antagonistic receptor interactions and altered levels of respiratory depression.[5]



Scheme 1: The First Reported Synthesis of Buprenorphine

Numerous improvements to the synthesis have been made since the first synthesis was reported (discussed through section 3, pages 20 to 24). Typically, the synthesis of buprenorphine now involves 6-8 synthetic steps using either thebaine (**3**) or oripavine (**4**) as a starting material. The key elements in the synthesis are:

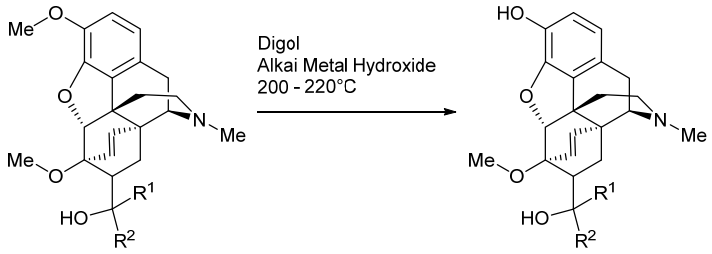
- The formation of an additional ring across the diene, *exo* to the original ring leaving a methyl ketone substituent with the *(R)* configuration
- Conversion of the ketone to the tertiary alcohol with methyl and tertiary butyl groups in the *(R)* configuration
- Conversion of the *N*–methyl group into a *N*–cyclopropyl methyl group
- Ensuring that an unsubstituted phenol ring is present.

These criteria are essential to maintaining buprenorphine's activity on the on the μ, δ and κ opioid receptors.[267]

As can be seen, the majority of the stages in the synthesis of buprenorphine from both oripavine and thebaine are common. Both raw materials require a Diels-Alder cycloaddition, saturation of the double bond left by the Diels-Alder addition, a *tert*-butyl Grignard addition to the ketone to form a tertiary alcohol, the removal of the *N*-methyl group, and the addition of an *N*-cyclopropyl methyl group. The only difference between using thebaine and oripavine is in the need for protection of the phenol on oripavine, and the demethylation of the methoxy at C_3 of thebaine. The stages of the synthesis of buprenorphine can occur in a number of different sequences and are essentially dependent on the starting material and processes used. For example, the use of oripavine as a starting material typically necessitates the addition of a protecting group to the phenol ring prior to the Grignard addition, whereas the use of thebaine negates this step, but instead means that an *O*-demethylation must be performed. *O*-Demethylation of thebaine can occur as a discrete synthetic stage or can be performed alongside the *N*-demethylation prior to the *N*-alkylation, but typically is performed after the Grignard addition to protect the phenolic oxygen. The hydrogenation and Grignard addition can occur in either order following the Diels-Alder addition, which can itself take place either prior to or subsequent the demethylation and alkylation. Similarly, the hydrogenation of the 6,14 *endo* alkene double bond can occur directly after the Diels-Alder addition or following any subsequent step without major interference to other synthetic steps.

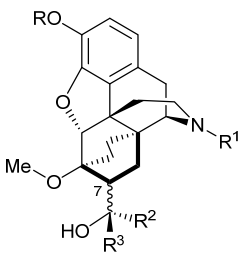
The subject of this chapter will focus on the Grignard reaction as it is a potential area for improvement in most synthetic routes currently explored in the manufacture of buprenorphine, as discussed later (Sections 2 and 3, pages 11 to 24).

Numerous patents currently cover the synthesis of buprenorphine and related substituted thebaine or oripavine derivatives as pharmaceutical grade materials.[4,8](see also section 3, page 20) The *O*-demethylation of functionalised thebaine derivatives to their phenol derivative[8] preceded the first synthesis of buprenorphine but show a number of the characteristics of the target molecule. The main focus of this patent was in the demethylation of the methyl ether to produce the preferable phenolic molecule, essential converting from a thebaine to an oripavine derivative.



Scheme 2: *O*-Demethylation of Functionalised Thebaine Derivatives Using High Temperature in the Presence of an Alkali Metal Hydroxide in Digol as Patented by Bentley in 1963[8]

Bentley's patent (1963) [8] declares R^1 to be either, a hydrogen atom, an alkyl group of 1 – 3 carbon atoms or an aryl group, and R^2 to be a hydrogen atom, an alkyl or alkenyl group of containing up to 8 carbon atoms with numerous potential substituent groups quoted. The target molecule can easily be considered an intermediate to the synthesis of buprenorphine, with dehydration of C^{17}-C^{18} and the substitution of the *N*-methyl group the only remaining synthetic steps to take. The synthesis of numerous *endo* oripavine and thebaine derivatives gave the following patented generic structure **5**.[4]



5: Generic Structure as per US Patent 3433791[4]

The declared substituents of **5** are R as hydrogen or methyl, R^1 is hydrogen, alkyl or alkynyl of up to 8 carbon atoms or cycloalkyl methyl of 4-6 carbon atoms, R^2 is alkyl or alkenyl of up to 3 carbon atoms, phenyl or tolyl and R^3 is cycloalkyl of 5-7 carbon atoms, or numerous substituted and non substituted alkyl or alkenyl groups. Included in this patent is a synthesis of buprenorphine, where in R is H, R^1 is cyclopropyl methyl (-CH_2-$\overline{CHCH_2CH_2}$), R^2 is methyl (-CH_3) and R^3 is *tert*-butyl (-C (CH_3)$_3$). Numerous patents have followed this work, with various refinements of numerous steps in the synthesis, which will be discussed later in this chapter. Before assessing the successes of any improvements, the intrinsic characteristics of buprenorphine and its synthesis must first be considered.

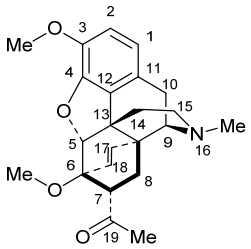
2 Effects of the Diels-Alder and Grignard Additions on the Stereochemistry of the Target Molecule

The stereochemistry surrounding the ethano bridge between C^6 to C^{14} and the order of rotation around C^7 and C^{19} are of particular interest to the investigation of the overall synthesis of buprenorphine. These chiral centres are not dictated by the starting material (thebaine), unlike the chiral centres at C ^{5}, C^9 and C^{13} which are. As thebaine is produced *via* an enzymatic process, its stereochemistry can be assumed to be set by that process. As can be seen in **1**, C^7 to C^{19} are in the *(R)* configuration, and the tertiary alcohol is in the *(S)* configuration. Intramolecular molecular hydrogen bonding between the alcohol at C^{19} and the oxygen at C^6 has been shown to lock the spatial conformation of the other substituents around C^{19} in position regardless of stereochemistry.[6] While it was initially suggested that this intermolecular hydrogen bond was critical to the specific pharmaceutical actions of buprenorphine by locking rotation,[6] later data suggested that the spatial conformation of the C^{19} *(S)* isomer allowed access to an inhibitory site of the κ receptor[9]. Regardless of which factor is most important to the activity of the molecule, the stereochemistry around this centre must be maintained to ensure consistent pharmaceutical activity and as such must be carefully controlled through whatever advancement is made to the overall synthesis of buprenorphine.

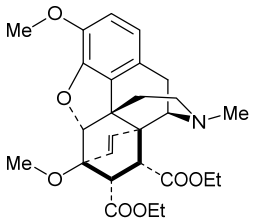
2.1 Stereochemistry of the Diels-Alder addition

The stereochemistry of the Diels-Alder addition, and subsequent functionalisation of the ketones produced, was the subject of extensive research during the discovery of the series of ketones derived from 6,14-*endo*-ethenotertahydrothebaine.[5,10,11,12,13,14,15] In the literature, Diels-Alder additions of numerous dienophiles across C^6 to C^{14} were performed to assess the steric and electronic driving forces of the reaction.[10] This was

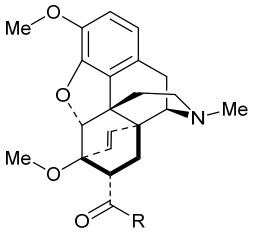
performed by reacting thebaine with a number of dienophiles and establishing the resultant products structure by NMR.[15] The overall conclusion of the series of additions carried out was that the dienophile can only add to the exposed face of thebaine, leaving the 6,14-etheno bridge *endo*. This was true of the addition of numerous functionalised dienophiles to give the structures 6 - 10:



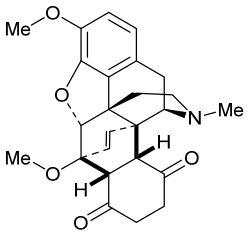
6: 7α-6,14-*endo*-ethanotetrahydrothebaine



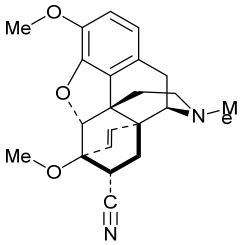
9: 7α-ethoxycarbonyl, 8α-ethoxycarbonyl-6,14-*endo*-ethanotetrahydrothebaine



7: 7α-Substituted-6,14-*endo*-ethanotetrahydrothebaine



10: Thebaine cyclohex-2-ene-1,4-dione adduct



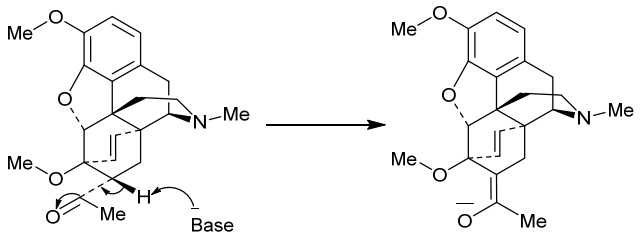
8: 7α-cyano-6,14-*endo*-ethanotetrahydrothebaine

The stereochemistry of **6** was examined in more detail. This examination was performed by using different dienophile to produce a series of 7α-Substituted-6,14-*endo*-ethanotetrahydrothebaines as per **7**, to give an aldehyde (R=H), a ketone (R=CH_3) and an ester (R=OEt) alongside the nitrile (8) to assess the steric hindrance of the eight

stereoisomers that could be formed through the addition across C^6 and C^{14}. NMR analysis confirmed that the respective reactions formed predominantly one stereoisomer of the potential arrangements. The dienophile will only add across the open face of the diene, locking C^6 to C^7 and C^{14} to C^8 in the conformation shown. C^7 was always the substituted atom, never C^8, so in this regard the addition can be considered fully regioselective. Of the two potential remaining stereoisomers, the *(R)* configuration at C^7 predominated[10,15] for all listed functional groups of the asymmetric dienophiles. Of the two epimers, the *(R)* configuration is described as 7α, and the reverse orientation 7β. This discussion concludes that the electronic control of the addition of asymmetrical dienophiles allows for only C^7 substituted products under electronic control. Furthermore, this addition, whilst having no great steric hindrance in either orientation for the substituents discussed (aldehyde, methyl ketone, ethyl ester and nitrile respectively), is driven to predominately give the 7*α* configuration, with the 7*β* being only a minor product (quoted as 0.5% in the mother liquors for the ketone).[10] This regioselectivity and stereoselectivity from the Diels-Alder reaction allows for great control of the subsequent products of the synthesis.

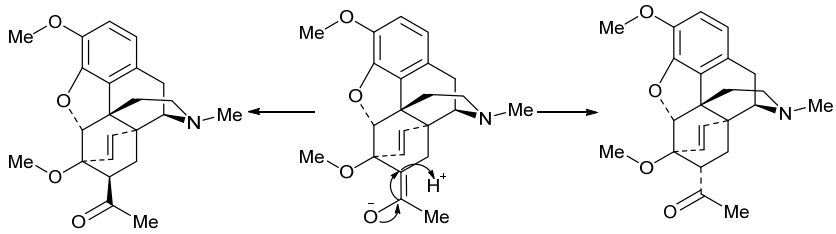
2.2 Stereoisomeration Around C^7 and C^{19} Following Grignard Additions

Following the investigation of the stereo chemistry of the Diels-Alder addition, basic rearrangement of **6** was considered.[11,14] This is of great interest with regard to the initial stereochemistry of C^7, the potential for epimerisation at this centre and with regard to potential interactions with Grignard reagents, essential in subsequent additions. Epimerisation under basic conditions would theoretically lead to the following general scheme:



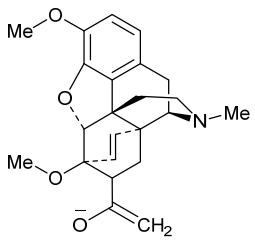
Scheme 3: Enolisation of 6 Under Influence of Base on the Proton at C7

This enolate should in theory be capable of being discharged to a mixture of both epimers of the original ketone as follows:

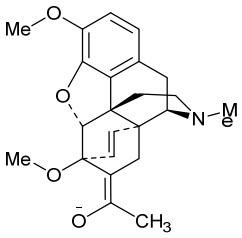


Scheme 4: Theoretical Discharge of Enolate to the Epimers of 6

In practice, the following was reported. Firstly, the kinetically favoured ion was formed in preference to the thermodynamically favoured as shown below.

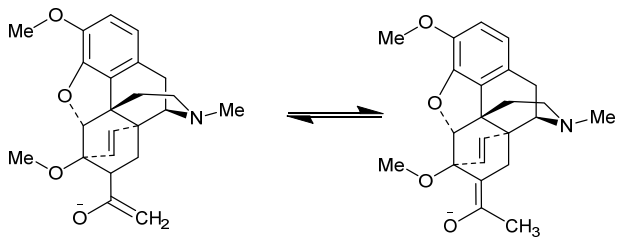


11: Kinetic Enolate ion of 6



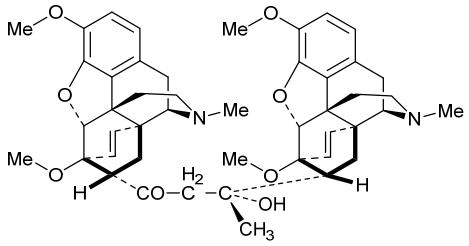
12: Thermodynamic Enolate ion of 6

Experimentally, **11** was shown to convert to the thermondynamically stable **12** under reversible enolisation conditions as per Scheme 5:



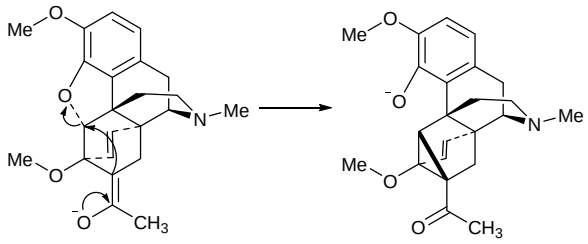
Scheme 5: Conversion between 11 and 12

Experimentally however, both enolate ions undergo a series of unfavourable reactions, with other available molecules in solution and within their own ring structures through electron transfer. The kinetic enolate **11** undergoes a series of other interactions with any remaining ketone in solution in a reversible dimerisation[10] to produce **13**.



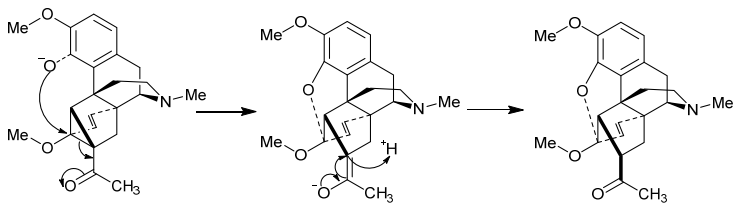
13: Dimeric Product of Attempted Epimerisation of 6

Of more interest, is the potential of enolate **11** to perform a series of electron transfers across its own ring structure and open the furan ring as follows:[10 14]



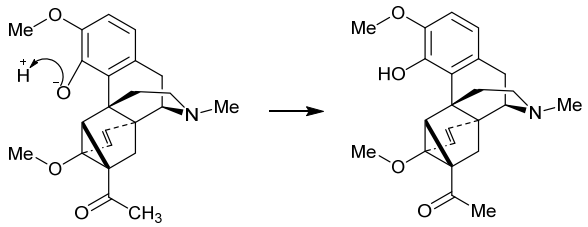
Scheme 6: Ring Opening of 12

The product of this ring opening is now open to further reactions dependent on the environment it is in. If a base is not available to react with the phenol following the opening of the furan ring, there is also the potential for further electron transfer within the molecule as per Scheme 7:[10,14]



Scheme 7: Intermolecular Collapse Following Ring Opening of 12

The above reaction in Scheme 6 means that the ketone is still present. This theoretically allows further reaction with Grignard reagents allowing a number of potential unwanted products, namely the associated tertiary alcohol created by the Grignard acting on the ketone. If the intramolecular rearrangement does not take place, the phenolate could be quenched with an available proton as follows:[14]

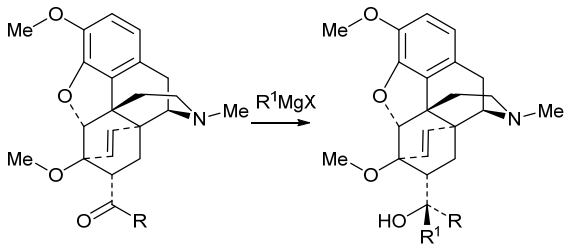


Scheme 8: Acidic Quench of Product from Scheme 6

The scheme above shows how a proton could add to the phenolate to create unfavourable side reactions and produce a new ketone, free to react with further Grignard reagent in solution, meaning that the ring opened ketone and the related tertiary alcohol of the Grignard addition are potential side products of the Grignard addition.[14] Once the ketone has been converted to a tertiary alcohol, the potential for this ring opening is removed, so it is favourable to prioritise the nucleophilic addition of Grignard reagent over the basic abstraction of the proton at C^7. As previously discussed, the Diels-Alder addition of asymmetric dienophiles produces the favourable 7α stereochemistry,[10] so these attempts to epimerise **6** are not essential to the production of a product of high steric purity.

2.3 Stereochemistry of the Grignard Addition at C^{19}

The Grignard addition to C^{19} creates a new chiral centre to the molecule. The Grignard addition is a highly stereoselective reaction leading to the configuration at C^{19} being easily controlled. The stereochemistry is dependent upon the ketone used in the Diels-Alder addition and the Grignard reagent used, as described below:



Scheme 9: Steric Arrangement of Grignard Additions to a Generic 6,14-*endo*-ethenotetrahyrdano-thebaine

The Grignard reagent predominantly adds into the ketone on the top face of the molecule, so much so that the epimer at C^{19} is only formed as a minor component of the Grignard reaction.[11,14,15] This was confirmed experimentally by a number of examples, but in most detail were the addition of a phenolic Grignard reagent to a methyl ketone and the addition of a methyl Grignard to a phenolic ketone.[10,15] This experiment and the resultant NMR analysis demonstrated that in each case, the predominant product of each reaction was for R^1 (the Grignard reagent) to be added on the top face of the molecule.

This series of experiments demonstrated that the stereochemistry at C^{19} is not controlled by steric hindrance. The stereoselection of this reaction is driven by proximity of the methoxy group to the ketone. The proximity of these two oxygen atoms allows them to co-ordinate to the magnesium of the Grignard reagent and therefore dictate the direction of approach of the Grignard reagent. This is demonstrated in Figure 1 and Figure 2 below:

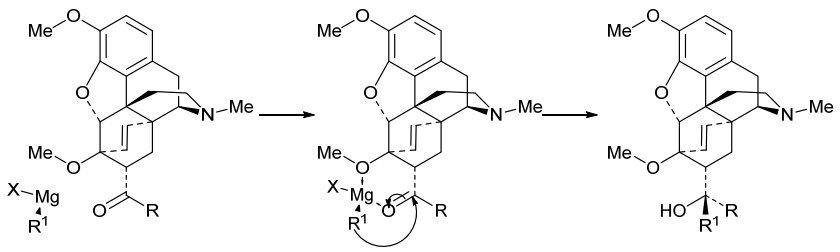


Figure 1: Co-ordination of the Grignard Magnesium Atom Leading to Stereoselective Addition of Grignard Reagents to C^{19} of a Generic 6,14-*endo*-ethenotetrahyrdano-thebaine ketone

This can be confirmed in the Newman projection of C^{19} below:

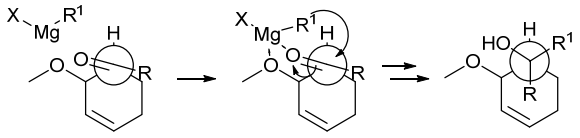


Figure 2: Newman Projection of the Co-ordination of the Grignard Magnesium Atom Leading to Stereoselective Addition of Grignard Reagents to C^{19} of a Generic 6,14-*endo*-ethenotetrahyrdano-thebaine ketone

Later work by Uff *et.al.* (1985)[16], used the stereochemical outcomes of the Diels-Alder and Grignard reactions to create compounds isomeric at C^7 and C^{19} in this series, in order to investigate the effects of this stereo isomerisation on their NMR spectra. This was achieved by using dienophiles and Grignard reagents with the appropriate R groups to create the desired stereoisomer, namely methyl vinyl ketone (dienophile) and *tert*-butyl chloride (Grignard) to create the *(S)* configuration and *tert*-butyl vinyl ketone (dienophile) and methyl magnesium iodide (Grignard) to produce the *(R)* configuration. This work confirmed that the compounds created from these differing stereoisomers can be predictable identified by NMR and that the stereochemistry around C^{19} can be reliably controlled through the choice of dienophile and organo magnesium halide used in synthesis.

Given that the C^{19} *(S)* configuration is favoured as a product through this mechanism and is also the favoured form from a pharmaceutical activity point of view, the addition of *tert*-butyl magnesium chloride to the methyl ketone is the obvious order of the

synthesis. Any subsequent alterations to the synthesis, especially around the Grignard reaction, must retain this stereochemistry to be considered successful.

3 Advancements Published in the Synthesis of Buprenorphine and Related Substances

Numerous patents and publications have followed the first synthesis of buprenorphine with suggested improvements to the synthesis by various routes. These improvements can be placed into different categories, from improving overall yields through reducing the number of synthetic steps between starting material and end product, removing harsh reaction conditions in favour of cleaner, milder or greener conditions, alternative *N*-alkylation reactions, the use of oripavine as a starting material, telescoped or one pot reactions, combined *N*- and *O*- demethylations and combinations of the above.

3.1 Reported Work in Optimising the *O*-Demethylation

The *O*-demethylation was reported in the first synthesis of buprenorphine as the terminal step of synthesis by "... heating to a temperature of above 200 °C, in the presence of an alkali metal hydroxide in solution in diethylene glycol".[8] This method of *O*-demethylation was initially reported to be performed on an *N*-methyl derivative of buprenorphine,[8] and was later applied to the synthesis of buprenorphine as the terminal stage of synthesis at an elevated temperature of 210-220°C following the *N*-alkylation.[4] As previously discussed, the *O*-methyl group at C^3 is favourable during synthesis as a protective group for the phenol, but is unfavourable for pharmaceutical activity.[9] These harsh conditions are not ideal, as they can lead to any number of side reactions and damage overall purity and yields. A large amount of work has been carried out to improve reaction conditions, yields, purities and generally create efficiencies around this stage of the synthesis.

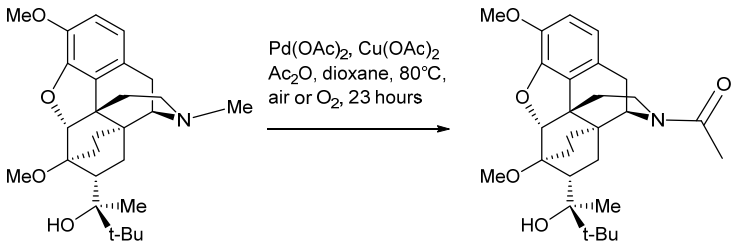
3.2 The use of Alternative Starting Materials to Negate *O*-Demethylation

A number of papers have discussed the use of oripavine as a starting material, which negates the need for this *O*-demethylation.[17,18,19,20] This does however potentially necessitate the need for protection of the phenol prior to the Diels-Alder reaction, the Grignard reaction or prior to the *N*-alkylation as the more reactive phenolic group could create a side reaction under any of these conditions. This would provide the potential for new synthetic impurities of unknown pharmacological activity and potentially damage overall yields due to cleanup. The use of oripavine does however allow for the use of more readily removed protective groups for the phenol and as such opens the possibility of milder, higher yielding methods of creating a phenolic group. Werner *et al.* (2011)[17] demonstrated the high potential for oripavine as a starting material. The reaction scheme followed resulted in the Grignard reaction as the terminal step, and demonstrated that an excess of *tert*-butyl magnesium chloride could be used to remove the phenolic protective group, this excess to product ratio was unfavourable and inevitably lead to de-protection being needed. Huang (2011)[20] opted to protect the phenol with a benzyl group directly after the Diels-Alder addition of methyl vinyl ketone, using benzyl bromide in toluene. This synthesis leaves the 6,14 etheno linkage unsaturated until the final stage, wherein the saturation and deprotection of the phenol occur in a one pot reaction, increasing the economy of synthetic stages by removing the need for additional deprotection.

3.3 Concomitant *O*- and *N*- Demethylation

Work has also been carried out to perform one pot *N*- and *O*- demethylation. The first synthesis of buprenorphine used two separate demethylation steps, both using potassium

hydroxide in diethylene glycol as shown in Scheme 1. It was later demonstrated that these reaction steps could be carried out in a one pot reaction leaving only the *N*-alkylation as a terminal reaction.[21] One such example of this one pot reaction by Allen *et al.* (2010)[22] demonstrated that once the *N*-methyl group had been substituted for the more readily removed cyano group, both the *N*-cyano and *O*-methyl groups could be removed with potassium hydroxide in diethylene glycol at 185°C over 4-5 hours. Furthermore, partially reacted product that had undergone *N*-demethylation but still retained the *O*-methyl group at C^3, can be recovered *via* a recrystallisation that precipitates this impurity and retains the desired product in solution. This recovered intermediate can then be fed back into the starting conditions of the reaction to give the desired end product. Another such example from Allen *et al.* on behalf of Mallinckrodt (2010)[22] eliminated the need for the use of cyano compounds in the *N*-demethylation, instead opting for the use of palladium catalysed acylation to produce an intermediate acetamide, as shown in Scheme 10.



Scheme 10: Demethylation *via* Palladium Catalysed Acylation [18]

The intermediate acetamide was then converted to buprenorphine *via* two different routes. Firstly through sequential stages to create the secondary amine, alkylate and finally demethylation of the phenol. The reported demethylation is of note, as it was achieved at a temperature of 145°C using sodium dodecanethiolate, which is milder than the first synthesis[4] and gave a yield of 80%,. This method of demethylation is also an improvement on a previously reported demethylation using propanethiol.[23] The

process was repeated through concomitant *O*- and *N*-demethylation, again with potassium hydroxide in ethylene glycol at 170°C for 7 hours.

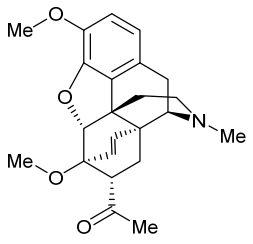
Another avenue explored was in the use of L-Selectride for *O*-demethylation and *N*-demethylation *via* an intermediate *N*-carbamate by Coop *et al.* (1998).[24] While the *O* demethylation was shown to be successful on a number of opiates, as was the *N* demethylation and concomitant *N*- and *O*- demethylation, these were not specifically applied to direct buprenorphine intermediates. An attempt was made to truncate demethylation and alkylation of the nitrogen of a naltrexone intermediate, *via* an intermediate cyclopropyl methyl ketone carbamate with the aim to leave the relevant *N*-cyclopropyl methyl group. In practice, this resulted in the secondary amine as the main product, but opened up the potential for the use of an intermediate carbamate for concomitant demethylation.

4 Advances in the Preparation and use of Organomagnesium Reagents

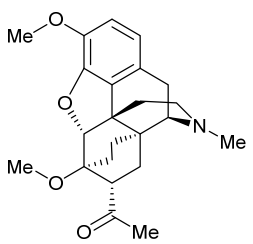
As the Grignard addition of a *tert*-butyl group to a methyl ketone (e.g. stage 3 of Scheme 1) is common reaction in synthesis, efforts have been made to improve the overall effectiveness of this transformation. Before discussion of potential advances to this stage of the synthesis, a simplified nomenclature used for the structures in question needs to be explained.

4.1 Nomenclature in the Discussion of Substituted Thebaine Derivatives

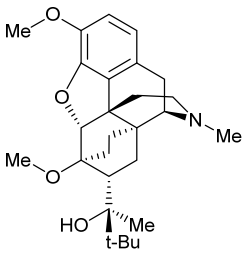
Bentley, Hardy and Meek (1967)[11] proposed a simplified nomenclature for the 6,14-etheneno and 6,14-ethano derivatives of thebaine based on a selection of syllables from the constituents used in synthesis; namely thebaine and methyl vinyl ketone. The trivial name opted for was "Thevinone" for the ketone adduct of thebaine and methyl vinyl ketone. This naming convention was then expanded to cover products formed from the hydrogenation of the 17-18 double bond (dihydrothevinone, see **1** for atomic numbering), the conversion to secondary alcohols (thevinol) and tertiary alcohols (alkyl-thevinol). Examples of this nomenclature that are key to the further discussion of the Grignard reaction are as follows:



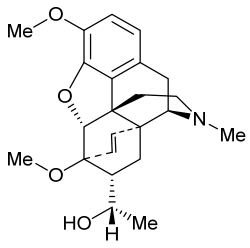
14: 7α-6,14-*endo*-ethenotetrahydrothebaine – Thevinone



15: 7α-6,14-*endo*-ethanotetrahydrothebaine – Dihydrothevinone



16: 6,14-*endo*-ethano-7α-(2-hydroxy-3,3-dimethyl-2-butyl) tetrahydrothebaine – *tert*butyl-dihydrothevinol



17: 6,14-*endo*-etheno-7α -(2-hydroxy-2-ethyl) tetrahydrothebaine – Thevinol

Where not explicitly stated, the α-epimer at C^7 is implied, with the β given an explicit prefix (e.g. β-dihydrothevinone). Likewise, substituents at C^{19} are assumed to be in the *(S)* configuration unless otherwise expressed explicitly in the trivial name assigned. This nomenclature can be extended to discuss further substitution of the related alcohols as shown in **16**. This nomenclature can be extended to discuss the products of further reactions with thevinone, for example thevinoic acid and its esters. Conversion of the methoxy at C^3 to the hydroxyl would then render the product an oripavine derivative, and as such the trivial name would be adjusted to being an orvinone, orvinol or alkyl orvinol.

4.2 Variations of the Grignard Reaction in the Synthesis of Buprenorphine

The first reported synthesis of buprenorphine, as per Scheme 1[4], quoted Grignard reaction conditions as follows:

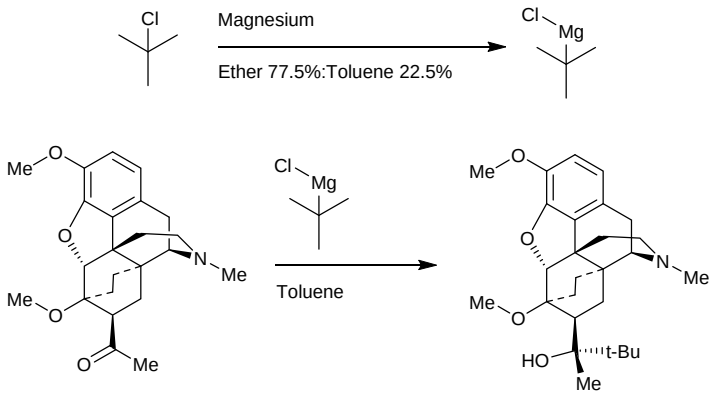
"A solution of *tert*-butyl magnesium chloride was prepared from magnesium (38.1 g) in ether (300 ml) and 2-chloro-2-methylpropane (*tert*-butyl chloride) (145 g) in ether (200 ml) and benzene (200 ml). The mixture was stirred overnight and titration of a sample indicated a 67 % conversion to the Grignard reagent. 7-acetyl-6,14-endoethanotetrahydrothebaine (100 g) in benzene (500 ml) was added over 1 hour to the stirred mixture. After standing overnight, the mixture was added to a saturated aqueous

solution of ammonium chloride (5 l), the organic layer was separated, the aqueous layer was further extracted from methanol to give 28.4 g. A sample further recrystallised from methanol had M.P. 188 °C. (Found: C, 73.2; H, 9.0; N, 2.9 % C H NO requires C, 73.4; H, 8.9; N, 3.2 %.)" Bentley (1969)[4]

This process needed refinement for a number of reasons. Using the values given in the text above and the molecular weights quoted in Scheme 1, the yield is calculated to be 25 %. Furthermore, using the weights and conversions quoted above, 6 molar equivalents of magnesium and *tert*-butyl chloride (*t*BuCl) were used to create 4 molar equivalents of *tert*-butyl magnesium chloride (*t*BuMgCl). This constitutes a substantial wastage of starting materials. The use of benzene is avoided in the modern laboratory and is prohibited in the synthesis of pharmaceutical ingredients to be used in products designated for human consumption due to its carcinogenicity.[25] As such, modern synthesis has moved away from the use of benzene to ensure the safety of the final product. The literature in reference to buprenorphine synthesis shows a fairly diverse range of solvents and conditions for this reaction to be performed under, each with varying yields, substrates and stages in the overall synthetic route.

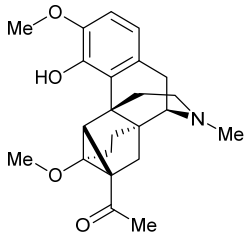
4.2.1 Synthesis of 7β 19-*Tert*-Butyl-Dihydrothevinol

The synthesis of 7β 19-*tert*-butyl-dihydrothevinol was described wherein 6 equivalents of Grignard reagent (*t*BuMgCl) were applied to the substrate in the presence of toluene and ether by Marton *et. al.* 1998,[21] as follows in Scheme 11:

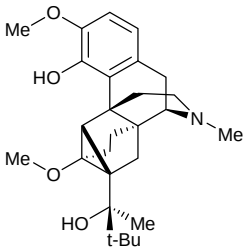


Scheme 11: Grignard Reaction of 7β Dihydrothevinone, Marton *et. al.* 1998[21]

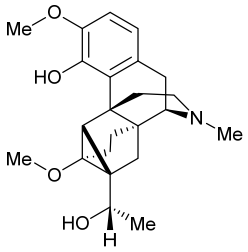
This reaction was shown to produce the desired product at a yield of 34%. Of note, a number of impurities created by the Grignard acting as a base were recovered at relatively high yields. These impurities were formed, as discussed previously in section 2.2, *via* the removal of the proton at C^7 of thevinone creating an unstable enolate that can be discharged *via* the intramolecular transfer of electrons as shown in Scheme 6 to yield **12**. As previously discussed, this product is open to further interaction with the Grignard reagent, and in this instance resulted initially in **18**. This can then react with *t*BuMgCl to give **19** as the tertiary alcohol, or can undergo β hydride transfer from the Grignard reagent to produce the tertiary alcohol **20**. In practice, Marton *et.al* (1998)[21] reported a yield of 7.8% **18**, 6.8% recovery **19** and 5.1% **20**, giving a total of 19.7% yield of isolable and identifiable impurities.



18: 5,7-*endo*-ethano-4-hydroxy dihydrothevinone

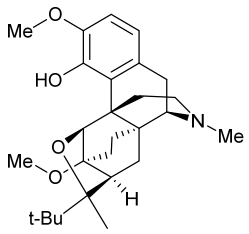


19: 5,7-*endo*-ethano-4-hydroxy-19 *tert*-butyl dihydrothevinol



20: 5,7-*endo*-ethano-4-hydroxy-thevinol

All three of these side products were found following the reaction carried out by Marton *et.al* (1998),[21] and could be due to two potential root causes. Firstly, the excess of Grignard reagent will increase the basic nature of the reagent and increase the rate at which the C^7 proton is removed from the substrate, making the basic rearrangement products more likely to form. The excess of Grignard reagent in solution will further allow the formation of the secondary and tertiary alcohols of 5,7-*endo*-ethano-4-hydroxy dihydrothevinone, as has been confirmed by their presence in the resultant product. Secondly, the proton at C^7 could potentially be more open to abstraction by the Grignard reagent when it is in the α configuration. Given that the Grignard reagent coordinates between the oxygen alpha to C^6 and the ketone oxygen, typically driving the stereoselectivity of the reaction, having the proton sit below the plane of the C^7-C^8 bond would make it more susceptible to attack from the bottom face as the Grignard co-ordinated to the neighbouring oxygen. These results differ from those found by Uff *et.al.*(1985)[16], wherein 7β dihydrothevinone was dissolved in 6.5 volumes of toluene and added to 5 equivalents of *t*BuMgCl in light petroleum (b.p. 100-120°C). The overall yield was poor (calculated to 31 % given the weights presented in Uff's experimental section), and under these conditions abstraction of the α-proton of C^7 occurred, however the only reported side product was **21**.



21: The 4 Phenol reported as a Grignard By-product by Uff et.al. (1985)[16]

When comparing the findings of the Grignard reaction of 7β dihydrothevinone to Uff's work, Marton *et.al.* (1998)[21] declared that they had not isolated **21** in the recovery. Both reactions took place with a similar level of excess *t*BuMgCl, however the differing solvent seems to be the key in the different outcomes. Firstly, Marton *et.al.* (1998)[21] reported a 34 % yield of isolated product and a separate recovery of three impurities totalling a yield of 20 %, while Uff *et.al.* (1985)[16] reported a yield of 31 % total product and impurities. From this, we can see that the use of ether and toluene by Marton *et.al.* (1998)[21] has significantly increased the reactivity of the *t*BuMgCl and substrate when compared to the use of light petroleum (Uff *et.al.* (1985)).[16] Furthermore, the use of 6 equivalents of magnesium and *t*BuCl can be compared directly to Bentley (1969)[4], as the same ratios are used. The main difference is the substitution of toluene for benzene in the creation of the Grignard reagent and as the solvent used to dissolve and add the substrate to the Grignard reagent. This substitution seems to have increased the reactivity of the Grignard reagent, potentially due to altering the basic *versus* nucleophilic nature of the Grignard or potentially due to altering the Schlenk equilibrium to give a more reactive solution. The presence of impurities generated *via* basic rearrangement indicate that the *t*BuMgCl is acting more as a base than a nucleophile in the ether solution.

While a trend in solvent effects can be seen, there is also the consideration that the above examples were employed to create the C^7 isomer product and as such the basic impurities could have originated from 7β dihydrothevinone being used as the substrate.

Whilst this may indeed have some part to play in the substrate being readily enolised and further converted into 4-phenolic compounds, the effects of the overall yield of converted product does show a solvent effect. This is further supported by an example disclosed by Saxena for Unichem Laboratories (1986),[26] who carried out the Grignard reaction on 7α dihydrothevinone in ether and benzene. In this example, 10 equivalents of Grignard are created in dry ether; without any co-solvent present in the generation of the Grignard slurry at a concentration of 3 M (with regard to the *t*BuCl in ether), roughly twice the concentration of that reported by Bentley (1969).[4] One equivalent of dihydrothevinone is then added in a 0.5 M solution in benzene, overall giving a 2 part ether to 1 part benzene solution in which the reaction takes place. One would assume that the increased concentration and increased molar equivalency of the Grignard reagent would increase the overall yield of the reaction, however the reported yield is calculated to be 14 %. The cause of this low yield was not disclosed, there was no discussion of the generation of side products or the amount of unreacted starting material remaining after the reaction, however given the above discussion on the effects of co-solvent, it seems that the elevated levels of ether compared to co-solvent could be the cause of the poor rate of conversion to product.

4.2.2 Solvent Effects on Yields and Side Products of Grignard Reaction

As described above in section 4.2.1, the use of diethyl ether as the main solvent leads to a numerous basic rearrangements of the substrate and low yields, so consideration should be given to the effects of other solvents.

As a general rule, alkylmagnesium chlorides in diethyl ether predominantly form a halogen bridged dimer in solution,[27] which in the case of *t*BuMgCl in ether would give a dimer as per Figure 3.

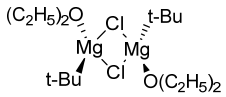
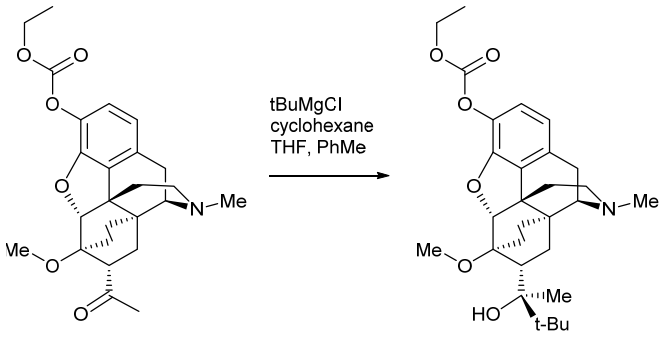


Figure 3: Co-ordination of *t*BuMgCl with Diethyl Ether in Solution.

The use of tetrahydrofuran (THF) as the primary solvent increases the amount of monomeric species in solution as the oxygen in the furan ring co-ordinates with the magnesium more strongly than the ethereal oxygen atom.[28] Furthermore, as THF is a stronger Lewis-base than diethyl ether, the nucleophilicity of the carbon centre and the electrophilicity of the magnesium atom in the *t*BuMgCl is increased, which theoretically should promote rates of reaction.[27] Given that the basic action has been demonstrated to create a number of undesirable side products, the use of THF as primary solvent offers the potential to improve purity and increase yields of the reaction.

A number of authors discuss the use of THF as the primary solvent in the formation of the Grignard reagent. One such THF-based Grignard reagent was discussed by Machara *et. al.* (2012)[18] in the application to dihydroorvinone ethyl carbonate (see Scheme 12). Under these conditions, 11 equivalents of *t*BuMgCl were made as a 1 M suspension in 3 parts cyclohexane to 1 part THF, and a 1 M solution of substrate in toluene was added drop wise at room temperature. After work up, recovery and purification *via* silica gel column chromatography, an overall yield of 71 % was reported.



Scheme 12: Grignard Addition of *t*BuMgCl to Dihydroorvinone Ethyl Carbonate[18]

The high molar amount of Grignard reagent compared to substrate does lead to low overall efficiency in terms of wastage, however the overall recovery stands well above the example in ether quoted previously. When compared directly to Saxena (1986)[26] wherein a similar level of excess Grignard reagent was used, the recovery in a THF based system affords over 5 times as much pure product. As Saxena did not disclose whether the low yields were due to low conversion rates or high levels of impurity formation, a solid conclusion as to whether the Grignard and substrate are more or less reactive in THF or ether cannot be drawn with these data alone, simply that THF appears to be a more suited solvent for the creation of the Grignard reagent.

Further evidence for THF being a preferred solvent can be seen in the work of Werner *et.al*.(2011),[17] in the application to *N*-cyclopropylmethyl dihydroorvinone ethyl carbonate. This is worth discussion for two reasons, firstly as it directly precedes the above example from Machara *et.al.* (2012),[18] and secondly as the authors demonstrate the effects of Grignard equivalence on the overall yield of the reaction in an attempt to use the Grignard to deprotect the product. The Grignard in this example was created in 1 part THF to 3.5 parts cyclohexane and used at a 1 M concentration, while substrate was dissolved to a 2.2 M solution in toluene and added drop wise at room temperature to 6, 10 and 17 equivalents of *t*BuMgCl. The results are summarised in Table 1.

Table 1: Effects of Amount of Grignard Vs. Product Yield Reported by Werner *et.al*.(2011)[17]

Molar Equivalents of tBuMgCl	Yield of main product (%)	Yield of Side products (%)	Yield of Buprenorphine (%)	Total Conversion of Substrate (%)
6	76	0	0	**76**
10	66	5	5	**76**
17	15	30	30	**75**

As can be seen, the total conversion of starting material under these conditions does not exceed 76 %, and below 10 equivalents of *t*BuMgCl, the protective carbonate is not affected by the reaction. Elevating the levels of *t*BuMgCl does work to aid in deprotection, however the recoveries do not warrant the extra wastage in staring

material. This was further compared against a direct conversion of unprotected *N*-cyclopropylmethyl dihydroorvinone to buprenorphine with excess of Grignard in the same conditions, and a yield of ≈ 30 % buprenorphine could not be exceeded regardless of the excess used, with both unreacted substrate and unidentified side products being declared as the yield limiting factors.

Comparing these examples to the previous discussion of the use of *t*BuMgCl in ether, it is clear to see that simply moving to THF as the Grignard solvent can provide over double the yield of product. Furthermore, a ceiling effect can be seen, in that some amount of starting material will remain regardless of the excess of Grignard used in the reaction. Work has been presented into the recycling this unreacted starting material present in the reaction product to conserve the amount of starting material needed.[22] This recycling has been demonstrated when converting dihydrothevinone (**15**) to 19-*tert*-butyl-dihydrothevinol (**16**), using 3 equivalents of *t*BuMgCl in an approximately 1 M slurry in 1 part THF to 2.45 toluene and 1 equivalent of dihydrothevinone in a 0.74 M solution in toluene. After crystallisation from heptane, these conditions afforded a yield of 55 % 19-*tert*-butyl-dihydrothevinol, with the crystallisation filtrate found to contain 4.1 % w/w product and 2.1 % w/w unreacted starting material. The filtrate is retained and added to approximately 1 volume of toluene and the mixture concentrated by heating under vacuum to drive off approximately 92 % of the solution, leaving an oil containing 16 % w/w substrate and 30 % w/w product. This is then added to the charge of toluene used for the dissolution of the substrate for the next reaction with a proportional reduction in the amount of substrate added to the subsequent reaction mixture. The yields and assay values for multiple recycling processes adapted from the original source[22] are given in Table 2.

Table 2: Effects of Recycling Recrystallisation Filtrate on the Purity and Yield of Product as Reported by Allen *et.al.* (2010)[22]

Run	Isolated Yield %	Assay wt%	Material recovery%
Virgin Batch	55.10	98.95	86.17
1st Recycle	77.24	94.88	93.73
2nd Recycle	76.78	97.78	91.68
3rd Recycle	78.06	88.13	83.84
4th Recycle	78.44	91.17	90.84
Total	72.58	NA	85.69

Data beyond the 4th recycling the filtrate are not given, with the levels of by-products said to increase to unacceptable levels beyond this point. Again, the overall yield does not exceed 80 %, but the recycling of the recrystallisation filtrate does have a clear benefit in improving the recoverable amount of product in subsequent stages. This increased recovery does come at a demonstrable loss in purity, which could potentially affect the products and by-products of subsequent intermediate reactions.

4.3 Advances in the Field of Organomagnesium Halides

Due to the versatility, wide applicability and utility in creating carbon – carbon bonds, a wealth of research has been performed into the field of Grignard reagents. Grignard reagents offer the potential of adding functionalised molecules together to form a complex target molecule with a degree of certainty that side reactions are minimised as long as the intermediates are selected correctly. It is also possible to create functionalised Grignard reagents, which allows for the synthesis of a complex molecule by bypassing numerous small addition steps and protection steps, overall reducing wastage and costs.

As discussed previously, the Grignard reactions utilised in the synthesis of buprenorphine intermediates or related substances can be low yielding due to low reactivity of the substrate or the generation of unwanted side products. Whilst refinements and advancements have been made to the Grignard additions employed in the production of buprenorphine, there is still potential room for improvement in this

synthetic step. As shown in some of the examples above, buprenorphine intermediates tend to need an excess of Grignard reagent to ensure that enough product is formed (as an extreme example, Saxena (1986)[26] used 10 equivalents of Grignard reagent to dihydrothevinone to yield roughly 14% of product). Typically, the steric control of the process is high, and the formation of side products, *via* enolisation, result in either dimeric tertiary alcohols (such as **13**) or in a discharge of the enolate *via* a phenolate resulting in 4 hydroxy products such as **18**, **19** and **20** discussed on page 15 and page 28. Whilst not a major product, reduced amounts of these products and increased overall yield would be a favourable as would an increased rate of conversion without loss of steric control.

Work in the field of organometallic halides over the decades has opened up a number of possibilities in relation to the addition of *tert*-butyl Grignard reagents to buprenorphine intermediates. Of particular interest is the use of lanthanide halides and lithium chloride to catalyse and mediate the addition of *t*BuMgCl to a buprenorphine intermediate substrate, such as thevinone or dihydrothevinone. The use of lanthanide group halides has been documented to activate carbonyl compounds and improve the yields of Grignard additions[29,30,31] and in some cases allow otherwise non-yielding addition reactions to progress to give favourable product.[32,33] Furthermore, the use of lanthanide halides have been demonstrated to reduce competitive side reactions, namely enolisation and β-hydride transfer reduction[33] which is of specific interest in the synthetic step under scrutiny.

5 Investigation into the Potential Improvement of the Grignard Reaction

A series of experiments will be carried out to explore the potential for improving the overall yield of the Grignard reaction used by Reckitt Benckiser in the synthesis of buprenorphine (described in more detail later). These investigations and potential improvements will begin with scaling the industrial process to the laboratory scale to assess the quality of the material produced, and then will follow a series of attempts to improve yields and purity as follows:

- Assessing the effect of the Grignard acting as a base on C^7 of the substrate
- How well the β substrate is converted by the Grignard reagent
- The effects of varying the equivalences of Grignard reagent to substrate
- The potential for performing the reaction without a co-solvent (in THF only)
- The potential to introduce additives to catalyse the reaction

Any changes to the synthesis must be considered against the following factors:

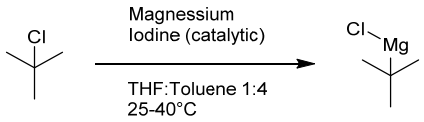
- Improvements in the yield of the reaction
- Improvement in the level of purity of the end product
- Reduced amount of Grignard reagent needed to facilitate conversion
- Reduction in preparation and/or reaction times

As discussed previously (in section 2.3, page 17), the Grignard reaction is a highly stereoselective process[11] and the stereochemistry at C^{19} must be retained to ensure pharmacological activity,[4,6,9] so any impairments to the stereoselectivity of the reaction will be considered of high consequence when assessing the success of any changes to the experimental protocol. Furthermore, dimerisation leading to **13** and ring opening

reactions such as Scheme 6 are known potential side reactions of the Grignard process. The impurities formed this way can be readily removed through work up and crystallisation, but increase of these impurities will also be measured to ensure that the overall quality of the reaction is maintained and their formation is not increasing at the cost of the overall yield.

5.1 Industrial Scale Formation of the *t*BuMgCl Grignard Reagent

The route of synthesis used as a starting point for comparison uses the Grignard reagent in the industrial process one currently used by Reckitt Benckiser. This is a two part process, beginning with the formation of the Grignard reagent as a slurry in 1 part tetrahydrofuran (THF) to 4 parts toluene on the day of reaction as per Scheme 13, followed by the addition of dihydrothevinone in toluene, as per Scheme 14 discussed later.



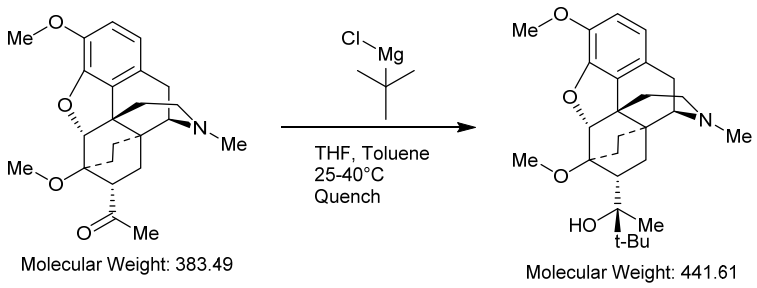
Scheme 13: Formation of *tert*-Butyl Magnesium Chloride(*t*BuMgCl) Grignard Reagent

The industrial scale operation proceeds as follows. The reactor vessel is dried by heating for 3 hours under vacuum, then purged with nitrogen and allowed to cool. Magnesium (23 Kg) is added to the vessel and stirred to even out. THF (90 l) added to the tank and heated to 55 – 60 °C, which is then transferred to a larger vessel followed by a rinse of THF (10 l, a total of 100 l of THF). Toluene (30 l) and *t*BuCl (10 l) are charged to a header tank and mixed. An initiating portion of the toluene *t*BuCl mixture is added (5-6 l), followed by iodine (50-150 g) and the vessel is monitored for exotherm and effervescence. The reaction temperature is allowed to stabilise, after which two further small aliquots of the toluene *t*BuCl mixture is added (1-2 l at a rate of 1 l/min) to confirm initiation and exotherm. Once initiation has been confirmed, the vessel is

stirred and allowed to cool to 25-30 °C. The remaining toluene *t*BuCl solution is added (at a rate of 60-100 l/hour), maintaining tank temperature to between 25-30 °C using the exotherm of initiation.

5.2 Industrial Scale Addition of Substrate to Grignard Reagent

The second part of the reaction is the addition of the substrate (Dihydrothevinone, **15**) to the Grignard reagent in toluene to give *tert*-butyl-dihydrothevinone (**16**), as outlined in Scheme 14 below.



Scheme 14: Grignard Addition of *t*BuMgCl to Dihydrothevinone

On the industrial scale being used as for comparison, this is achieved as follows. Toluene (315 l) is charged to a header tank, into which dihydrothevinone (92 Kg) is charged and stirred until dissolved. This solution is then added to the Grignard (over a period of 40 minutes) and the resultant mixture is allowed to stir for 30 minutes before being quenched by adding aqueous ammonium chloride (50 Kg in 170 l added over 2 hours, maintaining a temperature of 40-45 °C). Water (300 l) is added and the mixture is pH adjusted with concentrated hydrochloric acid to pH 7.5-8. The layers are separated and the aqueous portion discarded, and the remaining organic portion is distilled to dryness under vacuum. The resultant solid is then agitated in methanol (450 l) and DCM (45 l) and heated to reflux until fully dissolved. Solvent is distilled (120 l removed) and water added (100 l over 30 minutes) and cooled to 15 °C. The resultant precipitate is filtered and dried to give the desired product.

5.3 Lab Scale Application of the Comparison Grignard Reaction

A sample of dihydrothevinone, the second stage intermediate as per Scheme 1, was supplied by Onyx Scientific to use as substrate in the experimental work. The sample of dihydrothevinone was confirmed by routine release testing to be of a high purity, which was confirmed by ^{1}H NMR to confirm structure. The same bulk sample of this raw material was used in all experimental work to ensure that any impurities in the raw material were at the same levels in all experiments and allow for direct comparison of any impurities formed in the reactions.

5.3.1 Lab Scale Formation of the *t*BuMgCl Grignard Reagent

For the lab scale comparison, the following protocol was followed. All glassware was dried overnight in a drying oven then purged under 1 atmosphere of nitrogen prior to reaction. Magnesium (4.1 equivalents with regard to dihydrothevinone) was stirred in THF to produce a 9.9 M slurry under nitrogen which was heated to 55 °C. A premix of *tert*-butyl chloride (*t*BuCl) in toluene was made, comprising of 3.6 equivalents of *t*BuCl as a 2.2 molar solution in toluene. A catalytic trace of iodine was added to the magnesium slurry, followed by the addition of an initiating portion of the tBuCl in toluene solution. Initiation was confirmed by an exotherm and the slurry darkening from a clear solution with a dark sediment into a dray grey homogenous slurry. The mixture was allowed to cool to 25 °C and the remaining *t*BuCl solution is added drop wise, maintaining a temperature between 25-40 °C using the exotherm until the total solution had been added. The resultant slurry was allowed to stir for an hour and yielded *tert*-butyl magnesium chloride (*t*BuMgCl) in slurry, at roughly 3 equivalents to substrate.

5.3.2 Lab Scale Addition of Substrate to Grignard Reagent

For the lab scale experiments, the reaction was performed as follows. Dihydrothevinone (**15**) was added to toluene under nitrogen to create a 0.75 M solution and stirred until dissolved. This was added to the Grignard slurry *via* drop wise addition, again maintaining reaction temperature between 25-40 °C using the reaction's exotherm. This is allowed to stir for a further 30 minutes to allow completion. The reaction was quenched with drop wise addition of saturated aqueous ammonium chloride to yield an amorphous white precipitate and an exotherm. The resultant solution pH adjusted to between 7 and 8 by the addition of aqueous hydrochloric acid and the layers separated. The organic portion is retained and distilled at approximately 105 °C to remove roughly half of the solution, the remaining solution is distilled under vacuum to yield 19-*tert*-butyl-dihydrothevinol (**16** from page 25) as product (see section 7.2, page 66).

For the purposes of comparison, yields of materials are calculated on the assumption that the material recovered is the relevant tertiary alcohol product, using the following calculation:

$$\frac{W_{St}}{M_{St}} \times M_{\mathrm{Pr}} = Y_{Max}$$

$$\frac{W_{\mathrm{Pr}}}{Y_{Max}} \times 100 = Y_{Actual}$$

Where:

W_{St} is the weight of starting material (g)

M_{St} is the Molar mass of the starting material (Kg/mol)

M_{Pr} is the Molar mass of the product (Kg/mol)

W_{Pr} is the weight of product recovered (g)

Y_{Max} is the theoretical maximum yield of product (g)

Y_{Actual} is the actual yield of the experiment (%)

Purity assessments were performed either by ^{1}H NMR and TLC if the reaction is shown to have had little progress, or ^{1}H NMR and HPLC of the reaction has shown some progress or if investigation of the conversion and impurities would be of benefit.

5.3.3 Lab Scale Benchmarking of Routine Synthesis

This protocol was followed twice on a lab scale. The first lab scale synthesis following this protocol used 23.06 g (60 mmol) of dihydrothevinone as substrate in 80 ml of toluene. Following the addition of substrate to the Grignard reagent, the reaction was quenched after 30 minutes of stirring and recovered to give 17.70 g of product, an initially poor yield of around 70 % crude material. This poor recovery was found to be due to the pH of the aqueous layer following the quench to be at pH of 6.5. The residual aqueous layer was pH adjusted to pH7 and extracted with DCM to give an additional 4.82 g of crude material, giving a total combined yield of 85 % crude material. This material was recrystallised by dissolving in DCM methanol mixture and heated at reflux until fully dissolved. A portion of solution was distilled to allow a white precipitate to begin to form and cool water was added to lead to further precipitation. This solution was stirred over night, filtered and the resultant solid washed to give purified product. The material was analysed by ^{1}H NMR and identified as 19-*tert*-butyl-dihydrothevinol (**16** from page 25) by comparison to a known spectra.

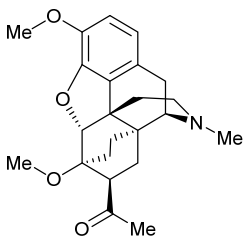
Under the HPLC conditions discussed in section 7.1.2, the purity of the material produced above was found to be 99.4 % by percentage area of the product peak in the chromatogram. No single impurity was found to be greater than 0.50% by area of the

chromatogram. This material was deemed suitable as a reference material for comparison against in further experimental work.

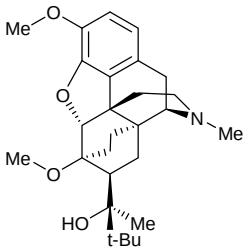
5.3.4 Impurity Profiling of the Routine Grignard Reaction

The second lab scale synthesis following this protocol used 23.20 g of dihydrothevinone in 80 ml of toluene as substrate. During the addition of substrate to Grignard reagent, the temperature of the slurry was noted to exceed 60 °C. The reaction was stirred for 30 minutes, and quenched to give 26.93 g of product, a yield of 85 % crude material. This recovery confirms that the previous poor recovery was due to the pH adjustment of the aqueous layer following the quench.

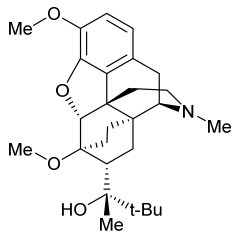
This material was produced to assess the quality of the crude material, assess the main impurities present and the overall conversion rate from substrate to product. This crude material was tested under the same HPLC conditions and found to be 84 % pure with the main single impurity being unreacted starting material at 7.0 %. Of the remaining impurities identified, 1.6 % was found to be 5,7-*endo*-ethano-4-hydroxy-19 *tert*-butyl dihydrothevinol (**19** from page 28), 1.0 % was found to be 7β dihydrothevinone (**22**), 0.5 % was found to be the diastereoisomer, 7β-19-*(R)*-*tert*-butyl thevinol (**25**) and 4.6 % was an unknown impurity which could not be further identified.



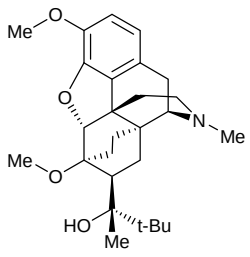
22: 7β dihydrothevinone



23: 7β-19-*tert*-butyl-dihydrothevinol



24: 19-*(R)-tert*-butyl thevinol

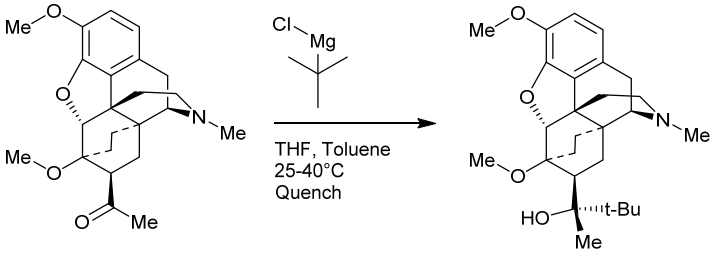


25: 7β-19-*(R)-tert*-butyl thevinol

Half of the crude material was recrystallised as before, giving a yield of 81 % (overall yield 69 %). The recrystallised material was found to be 97.0 % pure, with the main impurity to be 5,7-*endo*-ethano-4-hydroxy-19 *tert*-butyl dihydrothevinol (**19**) at 1.1 %, all others had been reduced to less that 1 %.

5.3.5 Application of the Routine Grignard Protocol to 7β-Dihydrothevinone to Investigate Impurity Formation

To assess whether the formation of these impurities in the lab scale Grignard was due to the Grignard acting as a base or due to traces of 7β dihydrothevinone (**22**) in the substrate, the Grignard reaction was carried out on a sample of 7β dihydrothevinone of high purity in line with Scheme 15.



Scheme 15: Grignard Addition to 7β -Dihydrothevinone (22) to Produce 7β-19*(R)-tert*-butyl-Dihydrothevinol (25)

The Grignard reagent was formed following the protocol described previously in section 5.3. 7β-dihydrothevinone was added to toluene under nitrogen. The first observation of note is that this compound was much less soluble in toluene than dihydrothevinone. 7β-dihydrothevinone failed to form a full solution at the nominal concentration, so further

toluene was added and heated to 90 °C until a solution was formed that remained clear once cooled. This was a 0.39 M solution of substrate in toluene, almost half the concentration of the typical substrate solution used in the routine Grignard reaction with dihydrothevinone.

Once cooled, the substrate solution was added drop wise *via* an addition funnel to a Grignard slurry. The reaction temperature was maintained between 25-40 °C *via* the rate of substrate addition and an ice bath. It was noted that less heat was produced than in the previous experiments, but this was attributed to the lower concentration of substrate. The reaction was quenched and material was recovered, giving a 91 % yield of crude material. The crude material produced was confirmed by ^{1}H NMR analysis to be 7β-19-*(R)*-*tert*-butyl thevinol (**25**). Purity assessment under the same HPLC conditions as before showed that the crude material was only 54.5 % **25** (the diastereoisomer of the routine product). The main impurities identified were unreacted 7β-dihydrothevinone at 17.1 %, 5,7-*endo*-ethano-4-hydroxy-19 *tert*-butyl dihydrothevinol (**19**) at 16.0 %, 7α-dihydrothevinone (**15**) at 3.3 %, *tert*-butyl-dihydrothevinol (**16**) at 0.5 % and two impurities that could not be further identified at levels of 2.2 % and 2.2 % respectively. The remainder of the peaks in the chromatogram were less than 0.5 % and did not elute in line with known peaks on the chromatogram.

5.3.6 Discussion of the C^{7} Isomer Impurity Profiling Experiments

Data presented above is displayed in Table 3 on page 46 for ease of discussion. The results of these syntheses and subsequent analysis show a number of interesting attributes to these routine conditions. Firstly, the rate of conversion shows a marked difference from the yield of the product of the standard reaction carried out using 7α-dihydrothevinone (an 85 % recovery of material with a 83.7 % conversion to product

compared to a 91 % recovery of material with a 54.5 % conversion). The lower conversion of the 7β-dihydrothevinone can be attributed primarily to the lower concentration of the solution due to the decreased solubility when compared to the C^7α-isomer. High levels of unreacted substrate show that this is a factor in the differing rates of conversion, but the elevated levels of impurities formed demonstrate this is not the only factor contributing to the low yield of product.

Assuming that both samples of raw material were stereochemically pure, it can be assumed that all stereo isomer and ring opened impurities are formed *via* the Grignard acting as a base on the proton at C^7, and that the total of these impurities can be directly compared to assess how basic the proton at C^7 is. This is a gross simplification for the purpose of this discussion, firstly as there is a thermodynamic enolate and a kinetic enolate formed by this process, both capable of discharging in a number of ways, as discussed previously in section 2.2 and by Bentley, Hardy and Meek (1967)[11] and Bentley *et. al.* (1967).[14] In effect, this means that some of the material classified as unreacted starting material would be due to re-protonation of the thermodynamic enolate and is not being accounted for here. This assumption does not account for any secondary alcohol formed by the Grignard acting as a base at C^{19}, which have not been identified by the HPLC analysis, nor does it account for unreacted starting material that is present due to the enolate discharging back to the original stereochemistry.

Table 3: Impurity Profiles for Grignard Addition to 7α and 7β Thevinone

Experiment	% Area of Chromatogram due to Primary Product (Retained C7 Stereochemistry)[1]	% Area of Chromatogram due to Peaks for Individual Impurities					
		Inverted Product (Inverted C7 Stereochemistry)[1]	7α-dihydrothevinione (15)	7β-dihydrothevinione (22)	5,7-endo-ethano-4-hydroxy-19 tert-butyl dihydrothevinol (19)	Unidentified Peak (RRT of 0.21)[2]	Unidentified Peak (RRT of 0.34)[2]
7α Grignard addition	83.65%	0.48%	6.95%	0.95%	1.59%	4.55%	Not Present
7β Grignard addition	54.45%	0.50%	3.31%	17.11%	16.03 %	2.16%	2.18%

Notes:
1 –Primary product of 7α Grignard addition is 7α-19 *(S) tert* butyl-thevinol (**16**) and the inverted product is 7β-19 *(R) tert* butyl-thevinol (**25**), the primary product for 7β Grignard addition is 7β-*19 (R) tert* butyl-thevinol (**25**) and the inverted product is 7α-19 *(S) tert* butyl-thevinol (**16**).
2 - RRT = Relative Retention Time calculated with regard to the retention time of the peak of 7α-19 *(S) tert* butyl-thevinol (**16**), the typical target product for the Grignard reaction.

Based on the above assumptions, the impurity profiles of both reactions demonstrate that the Grignard reagent does indeed act as a base and interact with the proton at C^7, but at different rates for the two substrates. The total impurities due to the loss of the C^7 proton are remarkably different for the two substrates, with the α substrate producing a total of 3.0 % of impurities *via* this interaction and the β substrate producing a total of 20.8 % of impurities in the product *via* this route. This difference of almost 8 times the level of impurities due to the removal of the acidic proton strongly suggests that the proton at C^7 is more easily removed by the Grignard reagent in the β-configuration.

This interaction could be due to either to the proton at C^7 being more readily removed by the Grignard reagent due to the β configuration placing it in a position more exposed to the Grignard once co-ordinated to the oxygen atoms at C^6 and C^{19} or due to the elevated levels of toluene in the reaction mixture promoting the Grignard to act as a base as opposed to acting as a nucleophile. The difference in these interactions cannot be assigned to difference in concentration as the lower conversion of the β-substrate could be, as this would result in a comparable lowering of these impurities. Regardless of cause, the elevated levels of 5,7-endo-ethano-4-hydroxy-19 *tert-butyl* dihydrothevinol (**19**) produced from the 7β isomer compared to the 7α isomer confirms that removal of this proton results primarily in the subsequent ring opening and not in reversible enolisation and discharge as either stereoisomer. Presence of 7α-dihydrothevinone at 3.3 % in the product of the reaction is evidence that the enolate formed by the removal of the proton at C^7 can discharge to either stereoisomer of the starting material, but this is less favourable to the formation of the 5,7-*endo*-ethano bridge discussed above.

5.3.7 Conclusions from the C^7 Isomer Impurity Profiling Experiments

From the above results, it has been demonstrated that the main action of the Grignard reagent under these conditions is the expected addition to C^{19} in a stereoconfiguration driven by the proximity of the ketone to the ether at C^6. The *(R)* configuration of the tertiary alcohol is formed from the β substrate and the *(S)* configuration from the α substrate, and this confirms that the stereoselectivity of the Grignard reaction driven by these neighbouring groups and is dependent upon the configuration at C^7. The co-ordination of the magnesium atom in the Grignard reagent between the oxygen atoms at C^6 and the ketone overcomes any steric hindrance and drives stereoselectivity of the addition. The secondary action of the Grignard is that of acting as a base upon the acidic proton at C^7 to primarily produce the 5,7-*endo*-ethano phenolic product and secondarily a stereoisomer product of either the inverted substrate or the inverted product. Conversion of dihydrothevinone to the corresponding tertiary alcohol occurs in a ratio of roughly 32:1 with regard to the competing basic interactions of the Grignard reagent, while this ratio is 2.6:1 for β-dihydrothevinone.

These experiments have shed much light on the products formed *via* basic interaction of the Grignard reagent with dihydrothevinone substrates. However, a question has been raised as to whether the recorded interactions are due to the configuration of C^7 affecting how well the Grignard reagent can act as a base upon the substrate or due to the increased levels of toluene in the reaction mixture of the β-dihydrothevinone experiment decreasing the nucleophilic nature of the Grignard reagent and increasing its ability to act as a base.

5.4 The Application of Tetrahydrofuran-only Grignard Reagents to Dihydrothevinone

One potential avenue for the improvement of this synthetic step, is the use of industrially produced Grignard reagents. These could decrease setup times for the reaction and constitute a significant time saving. Furthermore, as Sigma Aldrich produce a 1.0 M solution of *t*BuMgCl in THF, this can be used to assess the reaction in a THF only environment in a homogenous solution as a counterpoint to the above experimental work. This should allow for the investigation of the effects of toluene on the Grignard reagent and how it affects the basic versus nucleophilic nature of *t*BuMgCl.

A series of experiments were attempted using this 1.0 M *t*BuMgCl in THF solution on a roughly 2 g scale. Firstly using 1.05 equivalents of Grignard reagent with regard to substrate with the substrate dissolved in dry THF. TLC analysis of the reaction solution after overnight stirring at ambient temperature showed that no product had formed. The reaction was quenched to give a waxy solid that was confirmed by TLC and ^{1}H NMR to be unreacted starting material.

The failure of this reaction to yield product could be due to a number of issues. Firstly, the lower equivalents of *t*BuMgCl when compare to the standard Grignard reaction and the overall lower concentration of the reaction mixture. The lower exotherm could also be responsible for the failure of the reaction to yield the expected tertiary alcohol product. As no conversion has occurred, the action of the Grignard reagent as a base could not be assessed.

As low concentration and low equivalence of Grignard reagent were suspected to be the limiting factors of this initial failure to yield product, the reaction was repeated with a

more concentrated substrate solution and 2 equivalents of Grignard reagent in dry THF. Addition of the Grignard reagent was this time accompanied with an exotherm, with the solution heating to around 40 °C. The reaction was quenched and an off white solid recovered with a yield of 70 %. ^{1}H NMR analysis of the product proved inconclusive while HPLC analysis showed that the material contained 50 % starting material and 17 % target product by peak area of the chromatogram, with the remainder being a number of impurities that could not be fully identified.

As increased concentration and equivalence of *t*BuMgCl had shown some improvement in conversion, the experiment was repeated under reflux to assess if the poor conversion could be improved *via* heating and to attempt to move the reaction closer to completion. The reaction was carried out as before, but heated to reflux for 2 hours and quenched to again give a white solid. ^{1}H NMR analysis again proved inconclusive and HPLC analysis showed that the material contained 54.9 % starting material and 20.6 % target product. See Table 4 (page 51) for the impurity profile obtained by HPLC. Of interest, the ring opened product seen in the routine Grignard reaction and isomerism experiment, 5,7-*endo*-ethano-4-hydroxy-19 *tert*-butyl dihydrothevinol (**19**), only constituted 0.7 % of this product, the main impurities were found to be late retained peaks in an area of the chromatogram previously shown to be dimeric impurities by mass spec detection.

Table 4: Impurity Profile for Grignard Addition to 7α Dihydrothevinone using 2 equivalents of Grignard with THF as the Only Solvent Under Reflux

Primary Product (% area)[a]	% Area of Chromatogram due to Peaks for Individual Impurities										
	24[a]	**25[a]**	**15**	**22[d]**	**19[c]**	**RRT of 0.21[b]**	**RRT of 0.27[b]**	**RRT of 1.41[b]**	**RRT of 1.77[b]**	**RRT of 2.26[b]**	**RRT of 2.76[b]**
20.57	0.50	0.02	54.90	0.77	0.66	0.82	1.57	3.70	3.47	10.49	0.42

Notes:
a –Primary product of 7α (**15**) Grignard addition is 7α-19*(S)tert* butyl-thevinol (**16**) and the inverted products are 7β-19*(R)tert* butyl-thevinol(**25**) and 7α-19*(R)tert* butyl-thevinol (**24**).
b - RRT = Relative Retention Time calculated with regard to the retention time of the peak of 7α-19(S)tert-butyl-thevinol, the typical target product for the Grignard reaction. The peaks with an RRT greater than 1.41 are assumed to be dimeric adducts of substrate based on mass spec work carried out on this HPLC method on other samples.
c - 5,7-endo-ethano-4-hydroxy-19 tert-butyl dihydrothevinol, a ring opening impurity.
d - 7β-dihydrothevinone, formed by enolisation

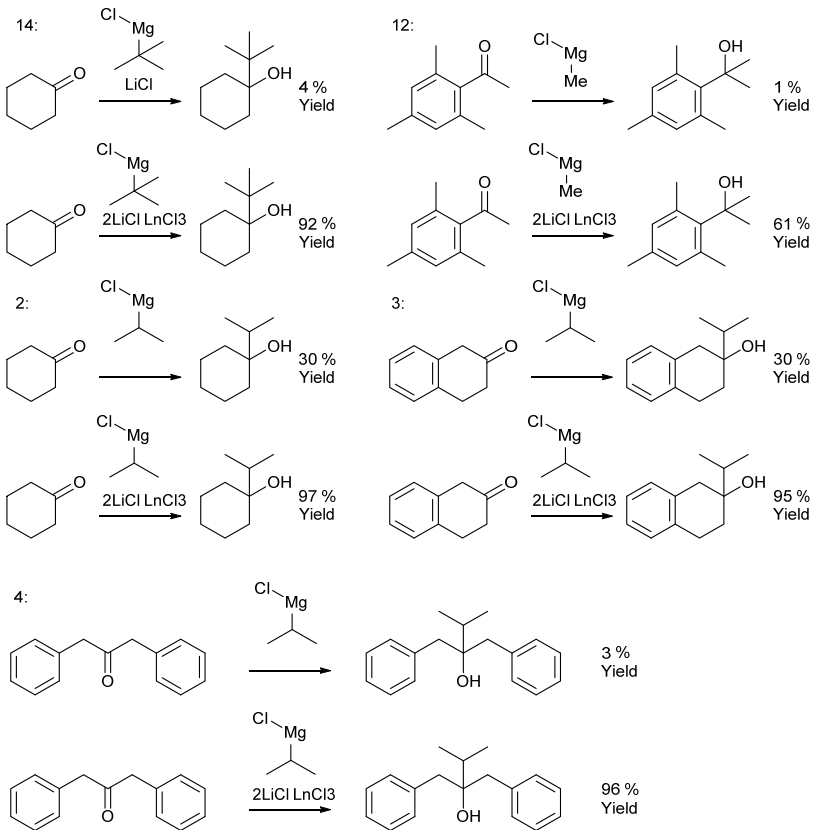
As reactions in THF only were showing limited conversion, the routine Grignard was repeated using lower equivalents of Grignard reagent, to allow better comparison between the activity of the Grignard in THF as sole solvent and in the routine THF/toluene mixture. This experiment was to assess if the poor yield without toluene was due to the lower level of *t*BuMgCl or the effects of the solvent on the reaction. Dihydrothevinone was reacted with 1.8 equivalents of Grignard reagent in THF/toluene, again using the exotherm of the reaction to maintain the temperature between 25-40 °C *via* the rate of addition. The reaction was quenched to give a yield of 33 % crude material. ^{1}H NMR analysis confirmed the presence of both starting material and target product in roughly equal amounts. Comparing this reaction in THF/toluene with the THF only experiments shows some promise in using THF as the sole solvent in the production of 19-*tert*butyl thevinol as a buprenorphine intermediate, but with work needed to improve the conversion rate. The data in Table 4 shows that products formed *via* the removal of the proton at C^{7} are reduced under these conditions, indicating that the Grignard reagent is acting more as a nucleophile and less as a base. This offers the potential to produce an intermediate with reduced impurity formation. The presence of large amounts of dimeric impurities offer cause for concern as they make up a total of 18.08 % of the sample produced. This is assumed to be due to the higher temperatures of the reflux experiment, which was demonstrated to be needed to initiate the conversion of the substrate to product.

5.5 The Application of Lanthanide Chloride and Lithium Chloride Salts to Grignard Reagents

The formation of dimeric impurities is most likely cause by heating the substrate in the presence of the Grignard reagent. As such, efforts were made to lower the reaction temperature and increase reactivity using lithium chloride and lanthanum chloride in

line with work presented by Krasovskiy, Kopp and Knochel (2006).[33] As discussed earlier (See section 4.3), the use of lanthanide chloride salts as catalysts have been demonstrated to improve the yields and viability of a number of Grignard reactions and reduce impurity formation in the reaction.[29,30,31,32,33] Krasovskiy, Kopp, and Knochel (2006)[33] presented a number of examples of the reactions between sterically hindered Grignard reagents and ketones being remarkably improved by the presence of $LnCl_3 \cdot 2LiCl$. Of these, a number of examples included highly functionalised ketones and organomagnesium halides which produced very poor yields under typical conditions, but could produce yields of over 90 % with the addition of lanthanide and lithium salts. A number of these are summarised in Scheme 16 for reference. Of particular interest is the reaction between *t*BuMgCl and cyclohexanone to produce 1-*tert*-butylcyclohexanol which gave a yield of 4% product without additives, while the addition of 1.0 equivalent $LnCl_3 \cdot 2LiCl$ gave a quoted yield of 92% (see example 14 given by Krasovskiy, Kopp, and Knochel (2006)[33], summarised in Scheme 16).

As these reagents are very similar to those under discussion here, a direct conversion of the protocol described was attempted following the typical process quoted in the experimental section. A solution of 0.6 M lanthanum (III) chloride bis lithium chloride complex solution in THF ($LnCl_3 \cdot 2LiCl$ in THF), was obtained from Sigma Aldrich to use as a catalyst for the Grignard reagent. The use of this reagent as a pre-bought solution by-passes the long preparation times described by Krasovskiy, Kopp, and Knochel (2006)[33], and ensures that long term safe storage can be maintained.



Scheme 16: Selected Examples from Krasovskiy, Kopp, and Knochel (2006)[33]. Example Numbers Given in-line with the Original Text.

An experiment was performed on the Grignard reaction of dihydrothevinone with *t*BuMgCl. The substrate was dissolved in the minimum dry THF needed to maintain solution under inert atmosphere in dry glassware and 1.0 equivalents of $LnCl_3 \cdot 2LiCl$ in THF was introduced and allow to stir for 1 hour. The solution was cooled to 0 °C and 1.05 equivalents of *t*BuMgCl in THF was added slowly to the flask which formed a golden yellow solution and which was left to stir. During sampling for TLC analysis, which showed some progress of the reaction, breaking the seal caused the solution to change to a dark olive green colour. The solution was then re sealed and allowed to stir over night to allow any reaction to progress.

Overnight the reaction mixture had changed to a light yellow solution with no sign of precipitation. The solution quenched, forming a white precipitate which was filtered and the solution retained. The resulting solution was dried under vacuum to give a

waxy residue which was analysed by ^{1}H NMR and TLC. The results of this analysis showed that the material contained neither starting material nor target product and the reaction was deemed to have been unsuccessful.

As the set up of the reaction could be compared directly to the literature and to the preceding work presented here, the outcomes of the failed experiment were assessed. The exotherm and colour changes confirm that interaction between the reagents has occurred, however the greater sensitivity to atmosphere causes great concern. An event such as this on the industrial scale would be highly costly, both in terms of consumed raw materials and damaged yields, but also in terms of decontamination, and unless the effects of the additives on the substrate can be fully understood, application to an industrial process could not be carried through. As the material produced could not be characterised, the interactions that occurred could not be assessed from this experiment.

A different approach was deemed necessary to assess if these additives could be applied to this process. As such, it was deemed prudent to assess the effects of the co-ordination of lithium chloride to the Grignard reagent, by adding lithium chloride to the routine reaction in line with the process described by Krasovskiy and Knochel (2004)[31]. This would allow the comparison of these additives directly to the bench mark and help identify the cause of the failure of the experiment using $LnCl_3 \cdot 2LiCl$.

This was performed experimentally by adding dry lithium chloride to the Grignard creation process to give a lithium chloride co-ordinated Grignard reagent, then reacting this slurry with the substrate in the routine THF/toluene solution. The lithium chloride was added to the magnesium as 1.0 equivalent to the *t*BuCl. This was the slurried in THF with a catalytic amount of iodine and a *t*BuCl toluene solution was added to initiate the reaction as per the routine protocol. This was found to require a larger initiating portion of the *t*BuCl solution and an elevated temperature to initiate and

maintain the reaction in the formation of the Grignard reagent. The exotherm required high levels of agitation from the stirrer to maintain the exotherm and maintain suspension of the slurry. The resultant slurry was allowed to stir for an hour. Once the solution had suitably cooled, a solution of dihydrothevinone dissolved in toluene was added to the Grignard slurry drop wise over 30 minutes and the resultant mixture stirred over 1 hour.

A number of observations were made during the addition of the substrate. Firstly, the addition of the substrate caused an instantaneous localised yellowing and clearing of the slurry not seen in the standard Grignard reaction. This quickly returned back to resembling the rest of the slurry mixture as agitation continued. Secondly, a higher temperature of 70 °C was needed to maintain the exotherm and ensure the reaction progressed. Temperatures below this lead to the solution cooling despite addition of further substrate.

After stirring for 1 hour, the reaction was quenched. The exotherm of the quench was much greater than seen in previous experiments and a portion of the reaction mixture escaped the vessel. HPLC analysis of the material recovered showed that the material contained 9.49 % starting material and 82.91 % target product. See Table 5 for the impurity profile obtained by HPLC.

As previously stated, this experiment was designed such that it could be compared directly to the literature and to the results of the routine protocol experiments presented earlier. The Grignard reagent in THF and toluene proved more difficult to form in the presence of lithium chloride in the flask, an issue assumed to be due to the bulk of material in the slurry overcome by increased temperature and stirring. This increased bulk then necessitated an elevated temperature to maintain the reaction during the addition of the substrate.

Despite this increased temperature of reaction and the addition of the lithium chloride, the conversion rate to product is roughly the same as when the reaction was carried out with the routine conditions. The only notable difference is in the increase in the amount of unreacted starting material from 6.95 % to 9.49 % and the slight increases in 5,7-*endo*-ethano-4-hydroxy-19 *tert*-butyl dihydrothevinol (**19**). The increased amounts of **19** could be due to the elevated reaction temperature or due to interactions of the LiCl with the Grignard reagent or the substrate. This could in principle create a more basic environment in which the proton at C^7 could be more readily removed and allow the ring opened product to form. As this impurity has been shown to remain after recrystallisation, elevated levels raise concerns regarding this material forming and being carried through to end product.

Table 5: Impurity Profile for Grignard Addition to 7α Dihydrothevinone Using the Routine Conditions with LiCl Additives

Experiment	% Area of Chromatogram due to Primary Product (Retained C7 Stereochemistry) (16)[1]	% Area of Chromatogram due to Peaks for Individual Impurities							
		7α-19(R)*tert*butyl-thevinol (Inverted C19 Stereo-chemistry) (24)[1]	7β-19(R)*tert*butyl-thevinol (Inverted C7 and C19 Stereo-chemistry) (25)[1]	7α-dihydro thevinone (15)	7β-dihydro thevinone (22)	5,7-endo-ethano-4-hydroxy-19 tert-butyl dihydroth evinol (19)	RRT of 0.21[2]	RRT of 0.27[2]	RRT of 2.26[2]
LiCl additives to Grignard Reagent	82.91%	0.11%	0.88%	9.49%	0.27%	1.90%	1.01%	0.48%	1.40%
Routine Crude from Section 5.3.4	83.65%	0.07%	0.48%	6.95%	0.95%	1.09%	4.55%	0.40%	0.41%

Notes:
1 – Primary product of 7α Grignard addition is 7α-19 *(S) tert* butyl-thevinol (**16**) and the inverted product is 7β-19 *(R) tert* butyl-thevinol (**25**) and 7α-19*(R) tert* butyl-thevinol (**24**).
2 - RRT = Relative Retention Time calculated with regard to the retention time of the peak of 7α-19*(S)tert* butyl-thevinol (**16**), the typical target product for the Grignard reaction. The peaks with an RRT greater than 1.41 are assumed to be dimeric adducts of substrate based on mass spectrometry analysis carried out on this HPLC method on other samples.

Elevated temperatures in the previous experiment to profile the impurities in the crude material (section 5.3.3) were assumed to be the root cause of impurity formation in the material, namely the unknown with a relative retention time of 0.21 with regard to the product peak. Direct comparison between the two profiles show that this impurity has been suppressed in the lithium chloride experiment; however impurity was readily removed by recrystallisation of the crude in previous work so is of minimal concern in carry through to end product. Conversely, there is a slight but noticeable increase in the levels of dimeric impurity with a relative retention time of 2.26 with regard to the product peak, suggesting that this has been in some way mediated by the presence of the LiCl in solution. Most interestingly, the LiCl experiment shows lowered levels of 7β dihydrothevinone than the comparison crude and a corresponding increase in the levels of 7β-19(R)*tert*-butyl-thevinol, suggesting that the LiCl has encouraged the conversion of 7β dihydrothevinone to the corresponding tertiary alcohol.

Overall, the addition of LiCl to the Grignard reagent appears to have had minimal positive impact. Conversion rates of starting material to product were marginally lowered and the amount of unreacted starting material was increased, suggesting that there is no acceleration of the addition process. Furthermore, the levels of isomeric impurities around C^7 and C^{19} were increased in the LiCl experiment, suggesting either some level of steric control is lost (such as in the formation of 7α-19(*R*) *tert*-butyl-thevinol, wherein the *tert* butyl group is added to the wrong side of the ketone) or that the rate of Grignard addition to traces of 7β dihydrothevinone is being increased under these conditions. Likewise, the elevated levels of 5,7-*endo*-ethano-4-hydroxy-19 *tert*-butyl dihydrothevinol suggest that removal of the C^7 proton is marginally promoted, either through the increased reaction temperature needed or changes in the activity of the *t*BuMgCl. Addition of LiCl under these conditions does not appear to produce any positive effects in the rate of reaction, conversion rate or significant impurity reduction.

6 Discussion and Conclusion:

As discussed in sections 2 and 3, the production of buprenorphine has been greatly researched and refined since the first reported synthesis in 1969.[4] The addition of a tertiary butyl group to a thevinone derivative is a key step in the manufacture of buprenorphine independent of the order of the synthetic steps in the process of converting thebaine or oripavine into buprenorphine. As such, a number of Grignard reactions related to this synthetic step have been presented across a range of buprenorphine intermediates in a variety of different solvent systems and a variety of different reaction conditions.

The experimental work presented here attempted to improve upon the addition of *t*BuMgCl to dihydrothevinone with 3.6 equivalents of Grignard to substrate in a THF-toluene solvent system. Benchmarking experiments of this reaction on a lab scale of roughly 23 g, showed that this reaction could yield roughly 85 % crude product of over 80 % purity. This material could be easily recrystallised in a DCM-methanol system to yield and 81 % recovery of material of 93 % purity. Overall, this gives a yield of 69 % for the reaction and recrystallisation, with the main impurity present in the crude being unreacted starting material. This overall yield is a significant improvement on that first reported by Bentley in the original patent,[4] which was shown to be a yield of roughly 25 % using 6 equivalents of magnesium and *t*BuCl in 1:1 diethyl ether-benzene to make 4 equivalents of *t*BuMgCl (Section 4.2, page 25).

As discussed in section 4.2.2, the solvent used in the Grignard formation and addition greatly effects the yield of the reaction, as such the use of toluene and THF as the solvent in the Grignard reaction can be attributed to the improved conversion rate when compared to the original synthesis. The change in yield can be explained by the solvent system shifting the Schlenk equilibrium towards the Grignard being more monomeric

and more nucleophilic in nature, thus making it much more reactive to the substrate. The presence of by-products formed *via* the action of a base on the substrate removing the proton at C^7 in this THF-toluene environment show that the Grignard reagent is still moderately basic in nature.

The basic action of the *t*BuMgCl in this reaction has been shown to be an effect of the toluene in the solution. Experiments presented above show that when the Grignard is performed solely in THF conversion is poor overall, with experiments using two equivalents of *t*BuMgCl at ambient conditions and under reflux giving roughly similar conversion rates of raw material to product. Using THF as the sole solvent showed some reduction in the amounts of impurities created *via* the removal of the proton at C^7, but a marked increase in the amount of dimeric impurities that were seen only at trace levels in the THF/toluene reaction. The presence of 19-*(R)-tert*-butyl thevinol (**24**), the C^{19} isomer of the desired product, in the material produced by the THF only reaction suggests that some of the steric control of the reaction has been lost with the removal of toluene from the reaction mixture. As such further alteration to increase the conversion rate of the reaction (such as increasing concentration of the Grignard reagent or higher equivalencies of reagent), would lead to higher levels of this impurity in the product.

When compared to the reaction using 1.8 equivalents of *t*BuMgCl in THF-toluene, the THF only reaction yields more material, however the lower conversion rate of the THF only reaction means that the lower yielding reaction has produced material containing more of the intended product. As such, these experiments show that a co-solvent is needed alongside THF to ensure suitable levels of conversion are achieved and to ensure that the reaction can progress at a concentration and temperature suitable to reduce the amounts of unwanted impurities produced. This is supported by the material presented throughout the preceding sections, especially in the examples given in section 4.2, as the conditions quoted for the reactions of *t*BuMgCl with the numerous

buprenorphine intermediates presented have all contained diethyl ether or THF and a co-solvent to provide a successful reaction.

Attempts presented to utilise $LnCl_3 \cdot 2LiCl$ as catalysts proved unsuccessful and difficult to handle in their practical application. The THF-toluene synthesis used as the benchmark was moderately robust to brief exposure to normal atmosphere during sampling for TLC analysis or for the opening of the seals to add addition funnels or similar. Momentary exposure of the reaction mixture containing the $LnCl_3 \cdot 2LiCl$ additives proved to be a critical issue, resulting in the reaction yielding an unidentifiable gum. As such, the reaction could not be sampled for testing to assess progress or applicability of the catalysts to the process so usable data could not be generated. Given this exposure constitutes a "mild quench", it is felt that quenching this reaction with the typical ammonium chloride solution would have similar effects. Given these findings, it is considered that the extra vigilance that would be needed to utilise these catalysts would outweigh the potential benefits in their use.

Attempts to improve the THF-toluene routine synthesis with the addition of LiCl to the Grignard reagent also proved to be unsuccessful. The reaction yielded comparable amounts of product, with a slightly lowered conversion rate. Whilst the material produced with these additives contained fewer impurities, a higher level of unreacted substrate was also found to be present. This suggests that the reported activity of the LiCl in reducing unwanted enolisation and β-hydride transfers in Grignard reactions is performed in this case, however the presence of 5,7-*endo*-ethano-4-hydroxy-thevinol (**20**) at slightly elevated levels undermines this finding. The overall reduction in impurities does not result in the promotion of the targeted interaction, so overall the recovery of the target material is the same. Furthermore, the increased bulk in the slurry required a substantial increase in heating and agitation to maintain a reaction, which would account for the higher levels of dimeric impurities and **20** in the final product.

Of concern is also the carry through of these lithium salts to the end product and their potential interactions with later stages of the synthesis. A significant improvement of this stage of the synthesis could negate these concerns, however the work presented here does not show the potential for such a change in the process.

Finally, the profiling of the synthesis of 7β-19-*(R)*-*tert*-butyl thevinol (**25**) has shown some interesting characteristics of the Grignard reaction utilised. The β-configuration of the substrate drastically alters the solubility in toluene, making the reaction mixture more dilute. This dilution has had a number of effects on the reaction. Firstly, the rate of conversion is much lower, which is to be expected. Secondly, the product of the Grignard reaction with 7β-19-*(R)*-*tert*-butyl thevinol yielded a large amount of impurities from the removal of the acidic proton at C^7. There are two potential causes for these by-products to form. Firstly the additional toluene used to dissolve the substrate could affect the nature of the Grignard reagent, making it more basic as the reaction progresses, thus opening the route for basic impurities to form. Secondly, it could be that the β-configuration makes the proton more susceptible to interaction with the Grignard reagent. The second hypothesis is felt to be the more likely as these impurities were seen in work presented by Marton *et.al*. (1998)[21] under different reaction conditions with the same substrate and the mechanism presented by Bentley and Hardy (1967)[10] shows that the Grignard reagent interacts with the C^6 oxygen atom in such a way that it is brought into closer proximity to whatever species lies below the ring (see Figure 1 and Figure 2 on page 18). As such, any β-substrate in the starting material, while low due to the stereoselectivity of the Diels-Alder addition,[10,15] is more likely to be removed as a basic rearrangement product than the α substrate, which in turn would lead to impurities that are more likely to be removed in the work up and recrystallisation. As such, control of the levels of impurities arising from the β-substrate in raw material are suitably maintained under the routine Grignard conditions.

6.1 Future Work

The level of detail in the impurity profiling of this reaction has shown that the products of these reactions can be critically assessed. This allows for unwanted by-products to be identified and their route of their formation can be assigned to various aspects of the reaction conditions. Further work with this impurity profiling can be used in balancing the ratios of solvents and open the potential for using lithium chloride as a co-ordinated additive to *t*BuMgCl. Whilst this work failed to utilise these catalytic additives, it could be beneficial to attempt an addition of *t*BuMgCl to dihydrothevinone in THF with LiCl present, to see if the poor yields of the reaction in THF only can be overcome with these additives.

Using the current THF-toluene solvent system, excellent steric control is achieved and most impurities are easily removed *via* a DCM-methanol recrystallisation. This work has shown that elevated reaction temperatures can lead to the formation of dimeric impurities as well as some materials that could be identified further if produced in a significant yield. Further work on finding the critical temperature for their formation and mechanism of synthesis could further refine the process and help further define how robust the reaction is.

7 Experimental:

7.1 Analytical Methods for Comparison of Experimental Results

The Grignard reaction utilised in the manufacture of buprenorphine is a well established industrial process. As such, characterised samples of the target product and the impurities discussed are available for comparative analysis by HPLC and ^{1}H NMR. Analysis of the materials produced in this experimental work was performed using the following equipment under the following conditions.

7.1.1 NMR Analysis

^{1}H NMR spectroscopy was carried out on a JEOL GSX270 Delta NMR, using samples dissolved in $CDCl_3$ at 270 MHz. The spectra generated were compared against spectra previously produced using characterised materials to assess purity.

Example ^{1}H NMR spectra are presented in Appendix A.

7.1.2 HPLC Analysis

Purity of the materials produced in the experimental work were assessed using the following HPLC conditions. A 1 mg/ml solution of the analyte was made in a solution of 25 parts 0.1% triethylamine aqueous solution and 75 parts acetonitrile. This was then analysed on an Agilent 1100 HPLC system with a gradient pump, auto injector, column thermostat and a UV detector. A solution of 0.1% triethylamine in water was used as mobile phase A and 100% acetonitrile was used as mobile phase B, with an isocratic elution set as 42% mobile phase A and 58% mobile phase B, with a flow rate of 1.5 ml/min. The column used was a 100 mm × 4.6 mm Phenomenex Gemini, with 3 μm C6-Phenyl 110 Å packing material, set to a temperature of 40 °C *via* column thermostat.

The UV detector was set to scan at 288 nm. A 40 µl injection of the sample was run under these conditions for 30 minutes and the peak areas recorded and assessed by percentage area of the chromatogram.

These chromatographic conditions were developed by Reckitt Benckiser Pharmaceuticals and validated using characterised materials to confirm the relative retention times of impurities with regard to the target product of the reaction in an internal report. Further work with HPLC/MS was performed to aid in the identification of peaks to which a known impurity could not be assigned.

7.1.3 Analysis by TLC

Some reactions were monitored by TLC analysis. This was performed using a mobile phase of 95% dichloromethane (DCM) and 5% methanol on Merck Kieselgel $60F_{254}$ 5 mm × 20 mm, using the starting material in DCM at a concentration of 30 mg/ml as a comparison to confirm reaction initiation and conversion.

7.2 Lab Scale Synthesis of 19-*tert*-butyl-dihydrothevinol (16) by the Routine Industrial Synthesis

All glassware was dried overnight in an oven then purged under nitrogen prior to reaction. Magnesium (5.75 g, 247 mmol, 4.1 equivalents with regard to dihydrothevinone) was stirred in THF (25 ml) to produce a slurry under nitrogen which was heated to 55 °C. A premix of *t*BuCl (24 ml, 215.5 mmol, 3.6 equivalents with regard to dihydrothevinone) in toluene (76 ml) was made in a separate flask and purged under nitrogen. A catalytic amount of iodine (25 mg) was added to the magnesium slurry, followed by the addition of an initiating portion of the *t*BuCl in toluene solution (5 ml) *via* a pressure-equalising addition funnel. Initiation was confirmed by an exotherm and the slurry darkening from a clear solution with a dark sediment into a

dray grey homogenous slurry. The mixture was allowed to cool to 25 °C and the remaining *t*BuCl solution was added drop wise, maintaining a temperature between 25-40 °C using the exotherm until the total solution had been added. The resultant slurry was allowed to stir for an hour and yielded *tert*-butyl magnesium chloride (*t*BuMgCl) in slurry.

Dihydrothevinone (**15**, 23.06 g, 60 mmol) was added to toluene (80 ml) in a separate flask under nitrogen and stirred until dissolved. This was added to the Grignard slurry *via* drop wise addition through an addition funnel, maintaining reaction temperature between 25-40 °C using the reaction's exotherm. The resulting slurry was stirred for a further 30 minutes. The vessel was placed over ice and the reaction was quenched with drop wise aqueous ammonium chloride (12.50 g in 42.5 ml of water) to yield an amorphous white precipitate. The resultant solution pH was adjusted to between 7 and 8 by the addition of aqueous hydrochloric acid and the layers separated. The organic portion was retained and distilled at approximately 105 °C to remove roughly half of the solvent, the remaining solution was distilled under vacuum to yield 19-*tert*-butyl-dihydrothevinol (**16**, 22.52 g, 85 % yield).

The product was purified by recrystallisation by first slurrying in a DCM methanol solution (22.52 g of product in 112.5 ml methanol and 33.5 ml DCM). The resultant slurry was heated at reflux until all material was fully dissolved. A portion of the solution was distilled (70 ml) to allow a white precipitate to form. Cool water (15 ml) was added to give further precipitation. This solution was stirred overnight, filtered and the resultant solid washed to yield 19-*tert*-butyl-dihydrothevinol (**16**, 18.97 g, 84 % yield, overall yield 71 %). This material was found to be of a purity of over 99 % by HPLC analysis.

7.3 Synthesis of 7β-19-*(R)-tert*-butyl thevinol (25)

A pre-mixed solution of *t*BuCl (24 ml, 215.5 mmol) was added to toluene (76 ml), purged under nitrogen in a round bottomed flask and allowed to stir. Finely divided magnesium (5.76 g, 237 mmol) was stirred in THF (25 ml) in a separate flask and heated to 55 °C under nitrogen. This was allowed to cool to below 30 °C and a catalytic amount of iodine (25 mg, 0.2 mmol) was added followed by an initiating amount of the *t*BuCl toluene mixture (approximately 5 ml) and initiation confirmed by exotherm. The remaining *t*BuCl toluene mixture was added drop wise *via* an addition funnel over the course of 1 hour, with the exotherm maintained between 25-40 °C. This was allowed to stir for 30 minutes.

7β-Dihydrothevinone (**22**) (23.01 g, 60 mmol) was added to 80 ml of toluene under nitrogen. Full dissolution was not achieved, additional toluene (75 ml, a total of 155 ml) was added and heated to 90 °C to aid dissolution and cooled to give a clear solution that remained a clear solution at room temperature.

The substrate solution was added drop wise *via* an addition funnel. The reaction temperature was maintained between 25-40 °C *via* the rate of substrate addition and an ice bath. Addition of substrate was completed over a period of 1 hour, and the mixture allowed to stir for 1 hour to ensure the reaction had gone to completion.

The reaction was quenched with ammonium chloride (12.50 g in 42.5 ml of water), added drop wise under nitrogen over an ice bath. The slurry formed a two layered system with a white precipitate. Water (70 ml) was added and the pH was adjusted to pH 7 with hydrochloric acid (conc.). The layers were separated and the organic layer retained, dried with magnesium sulphate, filtered and evaporated under vacuum to give

crude 7β-19-*(R)*-*tert*-butyl thevinol (**25**, 23.99 g, 90.6 % yield). The material was analysed by HPLC and found to be 54.5 % target material.

7.4 Attempted Synthesis of 15 using 1.0 M *tert*-Butyl Magnesium Chloride in Tetrahydrofuran Solution

A sample of **14** (2.30 g, 6 mmol) was dissolved in THF (36 ml) in a round bottomed flask attached to a distillation condenser and stirred under nitrogen to form a clear solution. After purging under nitrogen for 1 hour, the solution was heated to approximately 66 °C and 18 ml of solvent was evaporated to leave 18 ml of solution. The remaining solution was cooled in an ice bath to 0 °C. A pre-made solution of *t*BuMgCl in THF (6.3 ml of a 1.0 M solution, 6.3 mmol, 1.05 equivalents with regard to substrate) was added to the dihydrothevinone solution drop wise over 10 minutes *via* a needle through the seal septum to ensure that the inert atmosphere was not compromised.

Initial addition of the Grignard reagent formed a white precipitate and an exotherm was noted and maintained below 20 °C *via* addition rate of the Grignard reagent. After the addition of the *t*BuMgCl solution was compete, the precipitate dissolved back into solution under stirring to form a golden yellow solution. The ice bath was removed and the reaction mixture was allowed to stir at ambient temperature for 1 hour. Analysis of a sample after 1 hour by TLC showed no conversion of starting material to product. The solution was left to stir overnight. TLC analysis after overnight stirring at ambient showed that no product had formed.

An ice bath was again applied, and the reaction was quenched with aqueous ammonium chloride (5 ml of a 5.5 M solution). The exotherm of the quench was controlled *via* an ice bath and addition rate of the ammonium chloride solution over 10 minutes.

Aqueous hydrochloric acid was added (1 ml of a 1 M solution), resulting in the white precipitate readily dissolving and producing two clear layers. The layers were separate and the organic portion retained. The aqueous portion was washed with ethyl acetate (3 aliquots of 30 ml), which was again separated and the aqueous layer discarded. The organic portions were combined, dried with $MgSO_4$ and filtered to yield a pale yellow solution. The solvent was evaporated under vacuum to give a waxy solid (2.17 g, yield 68.5 %), which was confirmed by TLC and ^1H NMR to be unreacted starting material.

The above reaction was repeated with two equivalents of Grignard reagent. A sample of **14** (2.30g 6 mmol) was dissolved in THF (6ml). A premade solution of *t*BuMgCl in THF (12 ml of 1.0M solution, 2 equivalents) was added *via* needle through the seal septum and a colour change to golden yellow noted, along with an exotherm to approximately 45 °C. This was cooled to 30 °C, then reheated to 40 °C and stirred for 1 hour.

The reaction was quenched and recovered as before, to give an off-white solid (1.84 g, 69.5 %). ^1H NMR analysis of the material proved inconclusive. HPLC analysis showed that the material contained 50% starting material and 17% target product, with the remainder being a number of impurities as discussed in section 5.4.

This reaction was repeated under reflux as follows. A sample of **14** (2.32 g 6 mmol) was dissolved in THF (6 ml) in a three necked flask to form a clear solution. One neck of the flask was sealed, a thermometer was passed through the second and a reflux condenser was added to the central neck, to which a nitrogen purge was applied. The premade solution of *t*BuMgCl in THF (12 ml of 1.0 M, 2 equivalents) was added *via* needle through the seal septum and a colour change to golden yellow was noted, along with an exotherm to approximately 40 °C. The resultant solution was then heated to

reflux at 68 °C and stirred for 2 hours. It was noted that the golden yellow of the solution lightened to a pale yellow over the first 20 minutes of the reflux.

The reaction was quenched and recovered as before, to give an off white solid, 1.58 g, a yield of 59.2 %. ^{1}H NMR analysis proved inconclusive. HPLC analysis showed that the material contained 54.90 % starting material and 20.57 % target product. See Table 4, page 51 for the impurity profile obtained by HPLC.

7.5 Grignard Reaction Using 1.0 M *tert*-Butyl Magnesium Chloride in Tetrahydrofuran Solution in the Presence of Lanthanum (III) Chloride and Lithium Chloride

A sample of **14** (2.33 g, 6 mmol) was dissolved in THF (20 ml) in a three necked flask to form a clear solution. One neck of the flask was sealed, a thermometer was passed through the second and a distillation head was added to the central neck, to which a nitrogen purge was applied. Solvent (12 ml) was distilled at 66 – 68 °C to ensure residual water had been azeotropically removed, leaving 8 ml of the solution.

The solution was cooled to 30 °C and the condenser removed and replaced with a nitrogen line. The solution was allowed to stir under nitrogen to ensure an inert atmosphere was retained. The solution was cooled to 0 °C and 10 ml of the 0.6 M $LnCl_3 \cdot 2LiCl$ in THF added *via* needle. A clear golden brown solution formed with no precipitation noticed and the resultant solution was allowed to stir for 1 hour.

A solution of *t*BuMgCl in THF (6.5 ml of 1.0 M) was added *via* needle through the seal septum over a period of 10 minutes and a colour change to a darker reddish brown was noted, along with a mild exotherm. This was allowed to slowly warm to ambient and stirred for 1 hour.

The flask was sampled by passing a needle through the seal septum for TLC analysis. TLC showed some progress of the reaction, but it was difficult to interpret due to the concentration of the solution and streaking of the plate. The seal was removed to allow for better sampling, which caused the solution to change to a dark olive green in colour. The solution was then re sealed and allowed to stir out over night to allow any reaction to progress.

Overnight, the reaction mixture had changed to a light yellow solution with no sign of precipitation. The solution was sampled and analysed by TLC to give in conclusive results. Ammonium chloride solution (5 ml of a 5.5 M aqueous solution) was added to quench, forming a white precipitate. This was filtered through Celite 545 and the solution retained. The resulting solution was washed with brine, dried with $MgSO_4$, filtered and dried under vacuum to give 1.84 g of a waxy residue. ^1H NMR analysis and TLC analysis showed that the material contained neither starting material nor target product and the reaction was deemed to have been unsuccessful.

7.6 Routine Grignard Reaction in the Presence of Lithium Chloride

Magnesium (5.75 g, 247 mmol) and lithium chloride (9.15 g 215.5 mmol) were stirred in THF (25 ml) under nitrogen to produce a slurry. A premix of *t*BuCl (23.75 ml, 215.5 mmol) in toluene (75 ml) was made. A catalytic amount of iodine (25 mg) was added to the magnesium slurry, followed by the addition of an initiating portion of the *t*BuCl in toluene solution (5 ml). The solution was heated to 55 °C, but showed no signs of initiation. A further portion of *t*BuCl in toluene (10 ml) was added with no visible effect. The mixture was heated to 65 °C and initiation was confirmed by an exotherm and bubbling of the solution. The solution was allowed to cool to 25 °C and the remaining *t*BuCl solution was added drop wise, maintaining a temperature between 25-40 °C until the remaining solution was added. The solution required high levels of

agitation from the stirrer to maintain the exotherm and maintain suspension of the slurry. The resultant slurry was allowed to stir for an hour.

A sample of **14** (23.20 g, 60 mmol) was dissolved in toluene (80 ml) in a round bottomed flask to form a clear solution and stirred under. This substrate solution was added to the Grignard slurry *via* drop wise addition over 30 minutes. The reaction mixture was left to stir for 1 hour.

A number of observations were made during the addition of the substrate. Firstly, the addition of the substrate caused an instantaneous localised yellowing and clearing of the slurry not seen in the standard Grignard reaction. This quickly returned back to resembling the rest of the slurry mixture as agitation continues. Secondly, a higher temperature of 70 °C was needed to maintain the exotherm and ensure the reaction progressed. Temperatures below this lead to the solution cooling despite addition of further substrate.

The reaction was quenched with ammonium chloride solution (42.5 ml of a 5.5 M aqueous solution). The exotherm of the quench was much greater than seen in previous experiments and a portion of the reaction mixture escaped the vessel as an ice bath was being applied. The pH of the resultant solution was adjusted to 7.5 with 1 M HCl. The layers were separated and the aqueous was washed with ethyl acetate (3 aliquots of 30 mls). The organic portions were combined, dried with $MgSO_4$, filtered and dried under vacuum to give a white solid, 11.35 g, a crude yield of 42.5 %, low due to the escape of material during the quench. HPLC analysis showed that the material contained 9.49% starting material and 82.91 % target product. See Table 5 for the impurity profile obtained by HPLC.

Chapter 2 – The Synthesis of Naloxone Degradation Productions

8 Naloxone Impurity Formation in Suboxone Tablets

For a medicinal product to be accepted on the market, a sound testing regime to ensure safety must be established, presented to the market regulatory authority and maintained throughout the lifetime of that product in that market. These testing regimes are outlined in numerous documents controlled or agreed by the various marketing authorities such as the U.S. Food and Drug Administration (FDA), Medicines and Healthcare products Regulatory Agency (MHRA) in the UK, the European Medicines Agency (EMA) and the Therapeutic Goods Administration (TGA) in Australia. The main standard to which the industry is controlled is outlined by the International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH), which outlines the expected control procedures and documentation to be produced by the drug product marketer and ensures a degree of uniformity across all market submissions.

It is the responsibility of the company wishing to market a product to develop a suitable product specification in agreement with the guidelines and ensure that the product meets these specifications.[34] A sub sample of each batch produced must be analysed by suitable analytical methods to ensure that the batch is fit for release. This release testing is often performed on a number of samples from different parts of the batch produced, to demonstrate that the manufacturing process produces uniform tablets of equal dosage strength and quality. A further subsample of the product must then be stored under controlled conditions to mimic the market environment to demonstrate that the product maintains its quality, safety and efficacy for the duration of the assigned shelf life.[35] Together, these testing regimes ensure that all batches are consistent at release and that the product is of a suitable quality regardless of age. The testing performed typically involves assessments of the following criteria:

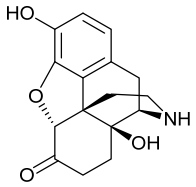
1. Physical characteristics of the product
2. Identification of the active pharmaceutical ingredient
3. Assay of mean active pharmaceutical ingredient content in the product
4. Assay of synthetic impurities and degradation products in the product
5. Release of active pharmaceutical ingredient on dissolution
6. Water content
7. Microbiological quality testing

Under the licensing agreements for marketed medicinal products, this testing must be performed to a pre defined specification that ensures product safety, quality and efficacy. Developmental batches of a medicinal product are tested against this specification over a set time frame under controlled environmental conditions to define the shelf life of the product.[35] This long term storage testing is repeated on an annual basis using a randomly selected batch of the product to confirm that the product meets these predefined criteria once on the market up to the defined shelf life (termed "stability testing"). Of the criteria listed above, the criteria found to trend towards the specification limit fastest or fall outside of the defined specification first is defined as the shelf life limiting factor.

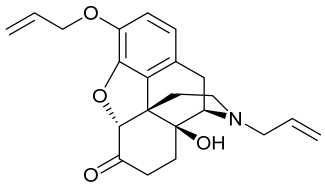
8.1 Known Naloxone Impurities and Nomenclature

As is the case with a wide number of marketed active pharmaceuticals, a monograph exists for naloxone in the European Pharmacopeia (Ph. Eur.). This monograph acts as an industry standard for the quality control, production and release of naloxone as a drug for use in humans. The monograph includes analytical testing methods to ensure

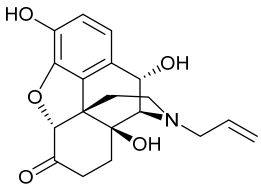
the quality and safety of the drug and to allow for the quantification of a number of synthetic impurities and degradation products, as outlined below:[36]



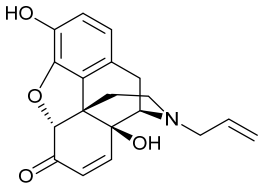
26: Ph. Eur. Impurity A - Noroxymorphone



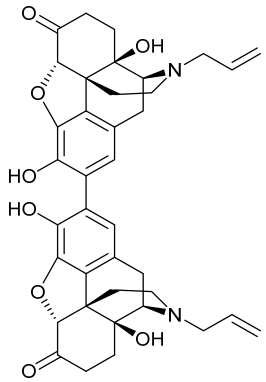
27: Ph. Eur. Impurity B – 3-*O*-Allylnaloxone



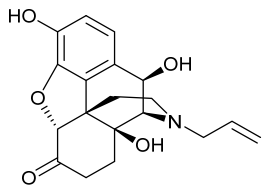
28: Ph. Eur. Impurity C - 10α Hydroxynaloxone



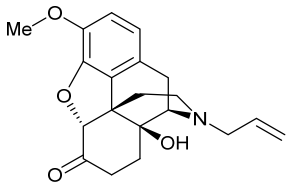
29: Ph. Eur. Impurity D – 7,8 Didehydronaloxone



30: Ph. Eur. Impurity E – 2,2'-binaloxone



31: Ph. Eur. Impurity F - 10β Hydroxynaloxone



32: Ph. Eur. Impurity G – 3-*O*-Methylnaloxone

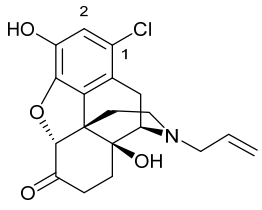
These listed impurities are the minimum that the material must be tested for and declared. Under the testing protocol outlined in the monograph, each of these impurities are given a maximum allowable level in the drug substance, levels in the end

product must be assessed against the dosage level of the product, the potential daily exposure to these impurities and their toxicity. Assessment of the structure of these impurities allows categorisation as being either a synthetic impurity, a degradation product or both. Impurity **26** for example could be formed during synthesis by incomplete *N* alkylation or could be formed in the product *via* oxidative de alkylation or a similar process, so this is classed as both a synthetic impurity and a degradation product. Conversely, **27** would form by concomitant *N* and *O* alkylation during synthesis if the intermediate is not a protected phenol, making it a synthetic only product. If the manufacturing process of the drug substance or potential degradation pathway in the product is such that other impurities may form, these must be identified, declared, monitored and controlled. Similarly, if these declared impurities are likely to themselves to further degrade in the product, these degradation products must also be identified.

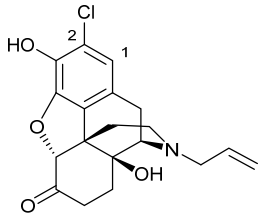
Identification criteria and specification limits for synthetic impurities and degradation products of the active pharmaceutical ingredients are set such that they control the safe exposure levels for the patient. This is performed on a threshold basis based on the daily dosage level and the toxicity or potency of the impurity in question, such that an impurity known to be toxic or harmful must be controlled to lower levels than an impurity known to have low toxicity or potency. When applying the guidelines set by ICH to Suboxone tablets, a naloxone related impurity representing 0.1 % of the amount of naloxone present in the product must be reported on testing and an impurity representing 0.2 % of the naloxone present in a must be fully identified and qualified for identification.[37] As such, an unknown impurity identified at 0.1 % with regard to the level of naloxone must be declared on testing, if the level rises to 0.2 % then efforts must be made to isolate and identify the impurity to assess the safety implications and declare its structure. Impurities known to be harmful must be controlled to below these

limits based on a level of safe daily exposure to the patient. In the case of naloxone, 7,8 didehydronaloxone (**29**) is controlled to a level of 75 ppm with regard to naloxone, indicative of a high level of toxicity.

A suitable testing regime and specification for buprenorphine, naloxone and their related impurities was developed by Reckitt Benckiser Pharmaceuticals for Suboxone tablets, resulting in the granting of numerous marketing authorisations and the successful launch of the product. Naloxone is known to degrade to a number of impurity products in Suboxone during stability testing which are suitably controlled and monitored by HPLC analysis. During routine analysis of Suboxone stability testing samples, an unknown impurity was identified by HPLC and found to be close to the identification threshold. This impurity was assessed by HPLC/MS and found to have a *m/z* and fragmentation pattern consistent with naloxone less one proton plus one atom of chlorine. Efforts were made to further identify and characterise this impurity, resulting in the speculation that the phenolic ring had undergone electrophilic substitution by an available chlorine atom source, resulting in chlorination in either the 1 or 2 position of the molecule (**33** and **34**).



33: 1-chloronaloxone

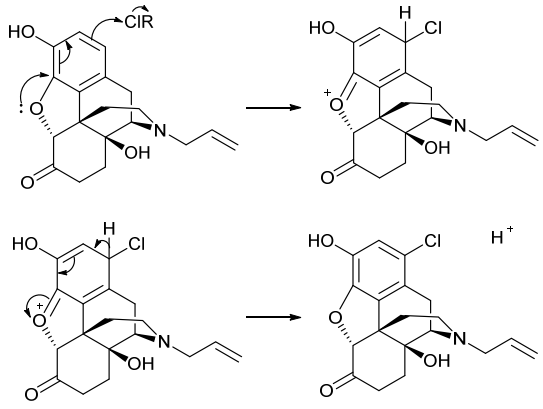


34: 2-chloronaloxone

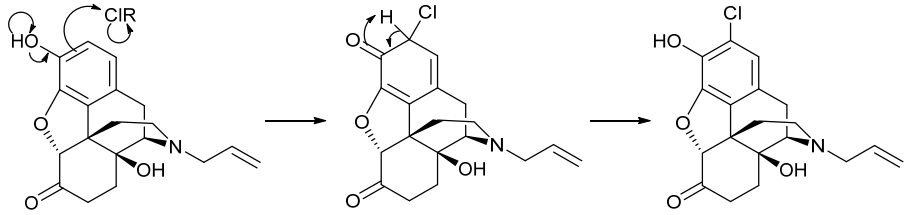
The route of formation is speculative, as it cannot be determined which of the other tablet components acts as the source of the chloride or if any of the other components are acting as a catalyst for the substitution. Both buprenorphine and naloxone are added to the tablet blend as the hydrochloride salt form, potentially offering the source of the chlorine atom to the reaction. If we assume some level of water enters the tablet, then

the chloride could be ion partially dissolved and then interact with other tablet components or materials in the immediate area to form a suitably electrophilic species. This could be *via* aluminium in the packaging, magnesium stearate, used as a tabletting lubricant, or through interactions with flavourings or organic dyes in the tablet. Once formed, this electrophile can react with the phenolic ring of naloxone in either the 1 or 2 position, as the oxygen atoms in the alcohol at 3 and ether at 4 can aid in resonance positions and act as a directing group, as shown in Scheme 17 and Scheme 18. It cannot be easily determined which is the most likely resonance, as we cannot easily determine what the pH of this environment could be, which could influence which directing group would be most influential.

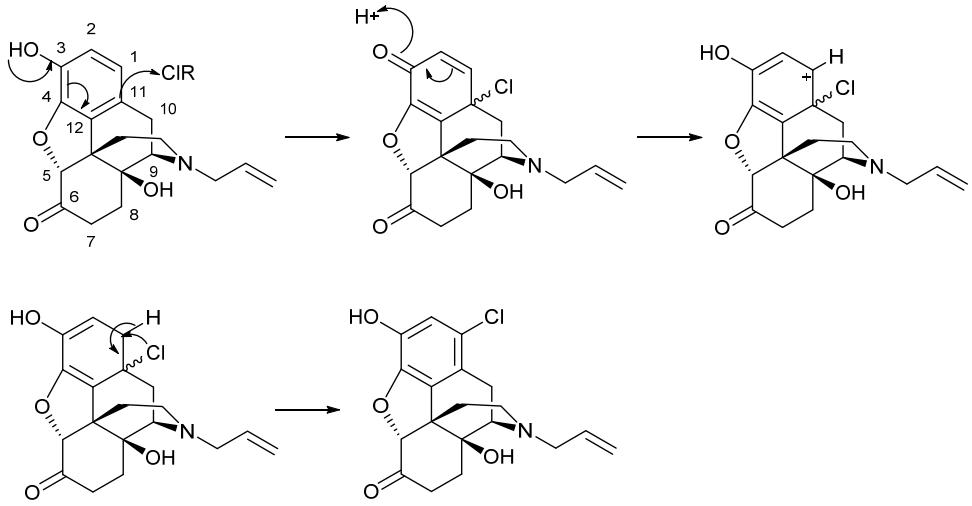
There is another potential route for the chlorination of the phenolic ring. This is *via* initial substitution at C^{11}, *para* to the phenolic alcohol, to give a dienone intermediate which then undergoes re-arrangement to restore aromaticity as outlined in Scheme 19. This is mechanistically slightly more complex, but has been suggested by Singh *et. al.* (1982), [38] in line with their findings when attempting directed chlorination of opiates. Again, this substitution requires a source of a positively charged chlorine ion, which attacks para to the phenolic alcohol at C^3 and reacts at C^{11}. This substitution is aided by the methyl bond to C^{10} acting as an *ipso* directing group, however, as there is no leaving group here, this cannot collapse to a substitution at this carbon. Instead, restoration of aromaticity can progress *via* transfer of the chloride ion to C^1 given an acidic catalyst.



Scheme 17: Chlorination of Naloxone at the 1 Position *via* an Ether Stabilised Resonance Structure



Scheme 18: Chlorination of Naloxone at the 2 Position *via* an Alcohol Stabilised Resonance Structure



Scheme 19: Chlorination of Naloxone at the 1 Position *via* Initial Attack at C^{11}

As stated, the exact environment that these substitutions take place in cannot be fully defined and identified. Whilst three mechanisms have been proposed here, it cannot be stated as a certainty which product would be the most likely to form. As the phenolic alcohol can lose its proton to maintain the intermediate resonance state, these mechanisms (Scheme 18 and Scheme 19) are more likely to be directing than the

resonance established through the ether (Scheme 17). With these mechanisms consideration, the potential still exists for chlorination at either C^1 or C^2, as substitution on either carbon can be stabilised *via* the phenolic alcohol.

9 Definitive Synthesis of 1-Chloronaloxone and 2-Chloronaloxone

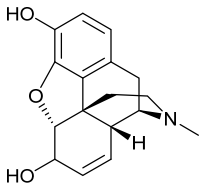
To confirm whether this unknown impurity is the 1-chloro or 2-chloro derivative, both must be synthesised *via* a definitive route and characterised. Chlorination of naloxone directly could lead to either product, as the phenolic ring is heavily substituted and open to a number of resonance states to stabilise the addition as discussed in Scheme 17, Scheme 18 and Scheme 19 previously. As such, reacting naloxone directly with a chlorinating electrophile would then require the separation of the two isomers and characterisational analysis, which would be less reliable than a definitive synthesis and characterisation. Nucleophilic substitution could produce a single product, however there is a high risk of side product formation *via* attack at the allyl group. A much more robust approach would be to direct the chlorination *via* functionalising the phenolic alcohol in a manner previously reported to direct additions to the ring of similar substances. This would provide a definitive and supported synthesis of the target compound, supported by data generated by characterising the products and any intermediates.

9.1 Previously Reported Substitution Reactions

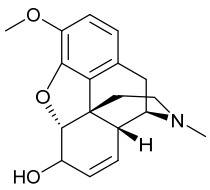
As naloxone is structurally very similar to a number of opiates, direct application of methods reported to chlorinate the phenolic ring of other opiates should be feasible. A number of papers have discussed substitution of the phenolic protons, either to form a definitive product substituted at the point of interest or to investigate the major directing groups of the ring and how they may be modified to influence their overall influence in the direction of substitutions. Similarly, the research presented in the field of directed

substitutions is near exhaustive with many methods being applicable to the problem at hand.

Singh *et. al.* (1982)[38] discussed their efforts to chlorinate morphine at the 1 and 2 position and the effects of the methyl ether on directionality. This was performed by assessing the products formed by chlorinating morphine (**35**) and comparing the product to the products of the reaction applied to codeine (**36**), which is the *O*-methyl derivative of morphine. Theoretically, the methyl ether will have a reduced directional effect to the free phenol ensuring that chlorination of the two drugs will produce substitution in different positions on the two molecules. Chlorination of morphine in concentrated hydrochloric acid, diluted in acetone at 70 – 75 °C to yielded a monochlorinated derivative. To assess whether this was the 1-chloro or 2-chloro product, it was methylated to convert it to a monochlorinated codeine derivative that could be compared to a known spectra of 1-chloro codeine produced by chlorinating codeine. Surprisingly, both were found to be chlorinated at the 1 position, signifying that the free phenol and methyl ether both produce the 1-chloro substituted product.



35: Morphine



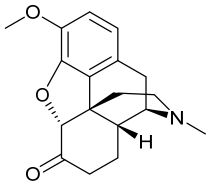
36: Codeine

Assessing this effect, it was concluded that the substitution was governed by the methyl bond at C^{11} (application of this finding to naloxone produced the mechanism presented in Scheme 19). This work was concluded *via* the comparison of nitration of the two drugs, followed by reduction to the amino derivative and finally halide substitution. Through this process, it was possible to direct the halogenations to the 1 or 2 position,

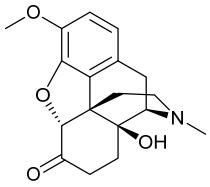
with the free phenol of morphine resulting in the C^2 substitution and the methyl ether resulting in the C^1 substitution.

Whilst this work gives great insight into the mechanisms of the synthesis of the target products, issues are presented in the differing functional groups of morphine and naloxone in the direct application of these methods. Firstly, the nitration reaction risks a potential side reaction with the allyl group of naloxone, creating unwanted intermediate products. Secondly, naloxone's tertiary alcohol at C^9 has the potential to be substituted by the halogenation process used by Singh *et. al.* (1982)[38], producing another unwanted product. As such, milder conditions are needed in this work.

Milder conditions for halogenation were presented by Wilson, Carroll and Dalton (2005)[39] and applied to hydrocodone (**37**) and oxycodone (**38**).



37: Hydrocodone

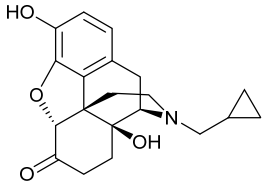


38: Oxycodone

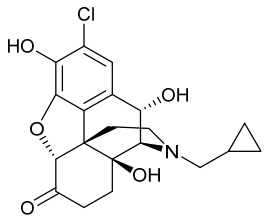
Halogenation of these two compounds was achieved using the relevant *N*-halosuccinimide in a variety of 0.1 N aqueous acids. Under these conditions the substitution occurred at C^1, with the exception of bromination using *N*-bromosuccinimide which produced the C^1 substituted product at 1.0 equivalence and the C^1, C^2 dibromide above this equivalence. This procedure supports the use of the C^3 phenolic methyl ether as a directing group for the halogenation in the C^1 position and is also mild enough to limit the production of side products from the reaction. Milder conditions have been presented as successful in the bromination of methoxybenzenes and napthalenes by Carreño *et. al.* (1995)[40], utilising *N*-bromosuccinimide to brominate a number of substituted rings in carbon tetrachloride and acetonitrile. Utilising

acetonitrile produced only ring substituted products and allowed the reaction to take place at lower temperatures than in carbon tetrachloride.

Similar work presented by Meredith *et. al.* (2003)[41], demonstrated definitive chlorination of naltrexone (**39**) in the 1 and 2 positions. The target synthesis was 2-chloro-10-α-hydroxynaltrexone (**40**), which was attempted *via* two different synthetic routes. This work is of particular interest as naltrexone and naloxone are very structurally similar, with the only difference between the two substances being that naltrexone has an *N* -cyclopropyl methyl group while naloxone has an *N*-allyl group.

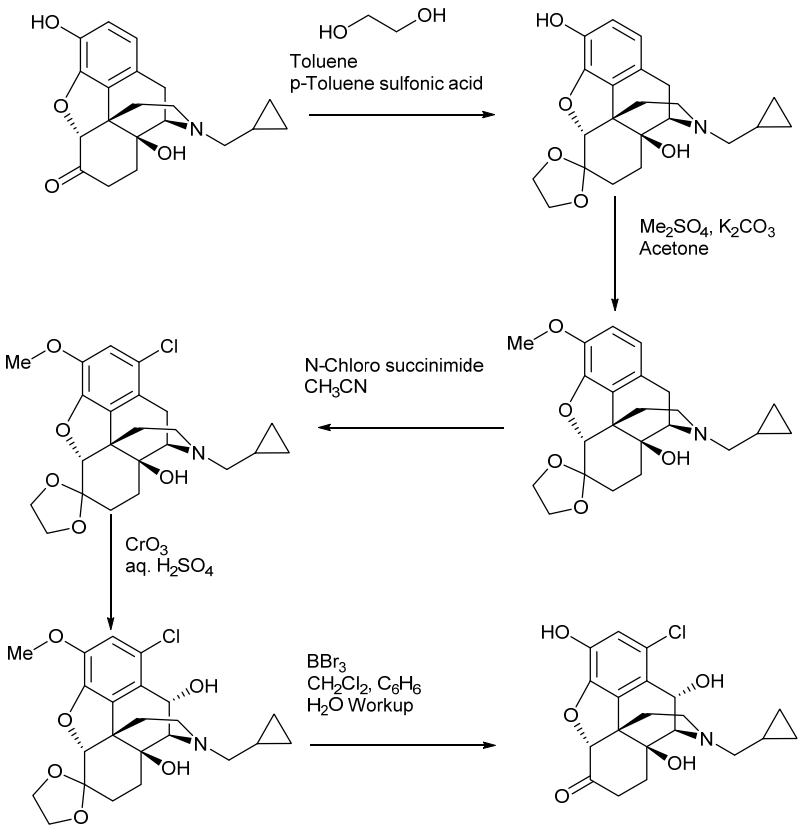


39: Naltrexone



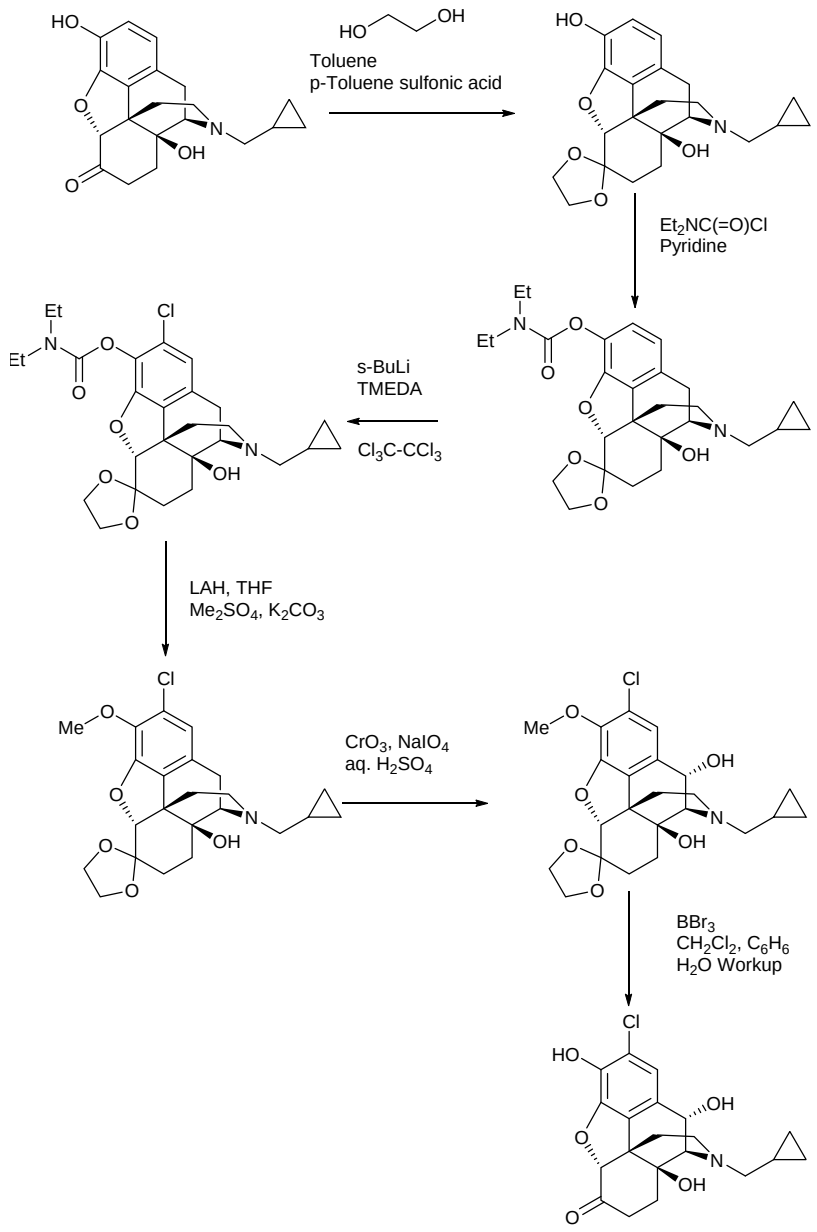
40: 2-Chloro-10-α-Hydroxynaltrexone

In the work presented by Meredith *et. al.* (2003)[41], the formation of the 1-chloro derivative is reported as a failed reaction, given that the overall target was the chlorination of C^2. This is however potentially directly applicable to naloxone. The synthesis of this 1-chloro derivative is presented in Scheme 20, is lead by directional control from the addition of the phenolic methyl ether. The protection of the ketone as the corresponding ethylene glycol ketal offers protection to limit the formation of unwanted side products, however it may not be essential in the application to naloxone as seen in earlier applications of halogenated succinimides to (**37**) and (**38**).[39] Furthermore, the phenolic methyl ether can be utilised for characterisation analysis *via* through space interactions with the proton in the 2 position in ^{1}H NMR analysis, offering the potential for NOE or NOESY NMR confirmation of the position of the chlorination.



Scheme 20: Synthesis of 1-Chloro-10-α-Hydroxynaltrexone as Reported by Meredith *et. al.* (2003)[41]

The second route presented by Meredith *et. al.* (2003)[41] (Scheme 21), utilises a diethyl carbamate to allow directed lithiation and subsequent chlorination of the carbon *meta* to the phenolic oxygen (C^2). Application of this synthesis to naloxone would again offer definitive synthesis of the desired 2-chloro product.



Scheme 21: Synthesis of 2-Chloro-10-α-Hydroxynaltrexone as Reported by Meredith *et. al.* (2003)[41]

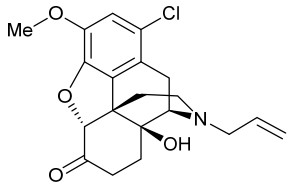
9.2 Proposed Synthesis of 1-Chloronaloxone and 2-Chloronaloxone

It was proposed that both target compounds **33** and **34** could be synthesised *via* the synthetic routes employed by Meredith *et. al.* (2003).[41] Both of these synthetic routes contain elements that are either not needed in the synthesis of **33** and **34**, or could be potentially removed to improve overall yields. The protection of the ketone at C^6 is potentially irrelevant in this synthesis of **33**, if this additional step could be omitted along with the subsequent deprotection, then an improved yield and time saving could

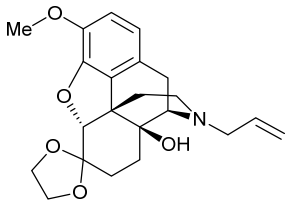
be made. With regard to the synthesis of **34**, the subsequent oxidation of C^{10} *via* chromium oxide can be disregarded for the purpose of this thesis, as this functionality is not of interest. Similarly the methylation of the 2-chloro intermediate to prevent side reactions in the oxidation is also irrelevant.

9.2.1 Proposed Synthesis of 1-Chloro Naloxone

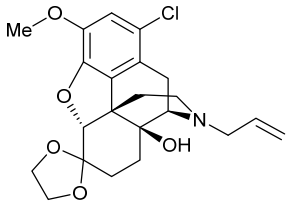
It was proposed that 3-*O*-methylnaloxone (**32**) could be chlorinated directly to give 1-chloro-3-*O*-methylnaloxone (**41**), as per Scheme 22. This was attempted under the assumption that if this proved unsuitable, then the ethylene glycol protection of the ketal could be performed to produce 3-*O*-methylnaloxone ketal (**42**), which will then be chlorinated to give 1-chloro-3-*O*-methylnaloxone ketal (**43**) as per Scheme 23.



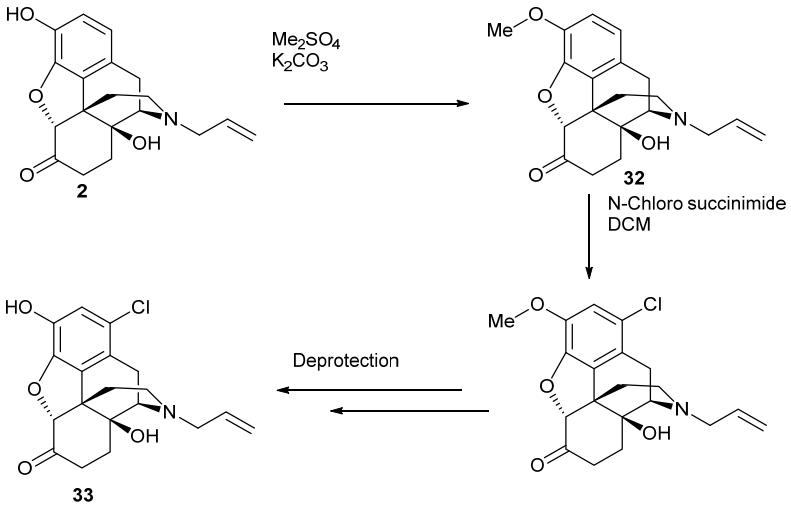
41: 1-chloro-3-*O*-methylnaloxone



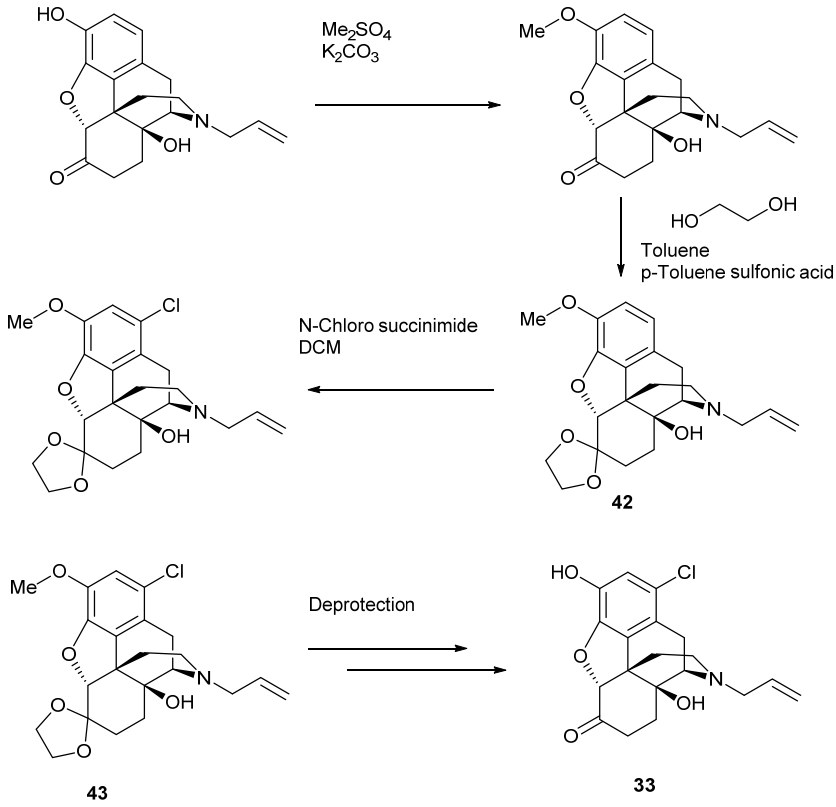
42: 3-*O*-methylnaloxone ketal



43: 1-chloro-3-*O*-methylnaloxone ketal



Scheme 22: Proposed Synthesis of 33 *via* 32



Scheme 23: Proposed Synthesis of 33 *via* 42

Both routes were attempted to different degrees of success. Direct chlorination of **32** proved unsuccessful due to a number of side reactions occurring. Due to this, the ketal protection was performed and found to give a much more suitable substrate for the chlorination to give **43**. As it was not certain that the deprotection of the methyl ether of **43** would result in the concomitant ketal deprotection and cleanly give **33**, so a

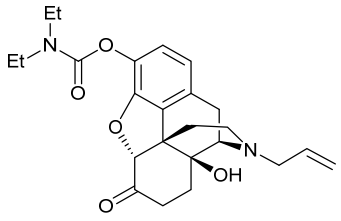
conservative approach was taken to employ separate deprotection steps by initially hydrolysing the ketal to give **41**, then demethylating the phenol to give the target compound **33**. Both of these proved successful and are discussed later (See section 9.3, page 96 for overview of planned deprotections and section 10.1.4 page 102 and section 10.1.5 page 105 for details of experimental findings).

9.2.2 Proposed Synthesis of 2-Chloronaloxone

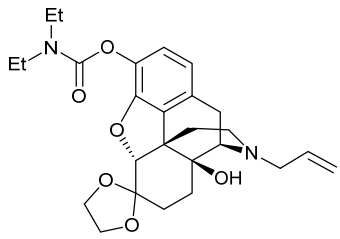
As previously stated, the *ortho* lithiation approach used by Meredith *et. al.* (2003)[41] would produce a definitive synthesis of 2-chloronaloxone *via* a mechanistic explanation of the addition. The addition of a suitable directing metalation group to the phenolic oxygen would result in definitive lithiation *ortho* to the phenolic oxygen once introduced to a suitable alkyllithium under favourable conditions. As the C^4 position is blocked by the ether bridge to C^5, the only available *ortho* position for the reaction is at C^2. This lithiate is then open to electrophilic attack, allowing direct substitution with chlorine from a suitable source. Meredith *et al.* (2003)[41] presented limited data on their reaction conditions (see Scheme 21), however numerous other examples the use of directing metalation groups exist, which can be used to provide additional information to ensure that suitable conditions could be established for the proposed synthetic steps in the application to naloxone.

The use of tertiary *O*-aryl carbamates as directing groups, as per Meredith *et al.* (2003)[41] appears to be a suitable choice of directing group. Tertiary *O*-aryl carbamates are relatively inert to nucleophilic attack by the lithiating species[42] and act as excellent directing groups for ortho metalation.[43,44,45] Furthermore, in most cases they can be readily formed from the addition of the relevant carbamyl chloride to the phenol of interest in the presence of an organic base[41,42,46] and any excess of carbamyl chloride can be safely destroyed by quenching with a suitable alkali metal carbonate.[47]

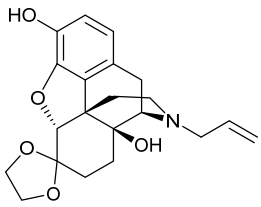
The synthesis of **34** by this route requires that 3-*O*-diethylcarbamoyl naloxone ketal (**46**) is synthesised as a key intermediate from naloxone (**2**). Two synthetic steps are needed for this synthesis, namely the ketalisation of the ketone at C^6 and the acylation of the phenol at C^3, which could be performed in either order presented in Scheme 24. Some considerations were made to the potential for the acidic reflux required by the ketalisation cleaving the carbamate releasing diethylamine and carbon dioxide. As the solubility and reactivity of these target intermediates was not known the preferred route of synthesis needed to be established through experimental work *via* both proposed routes of synthesis.



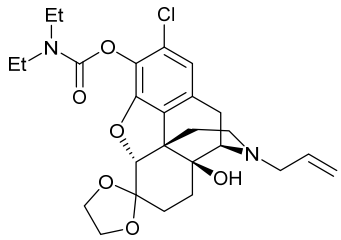
44: 3-*O*-diethylcarbamoyl-naloxone



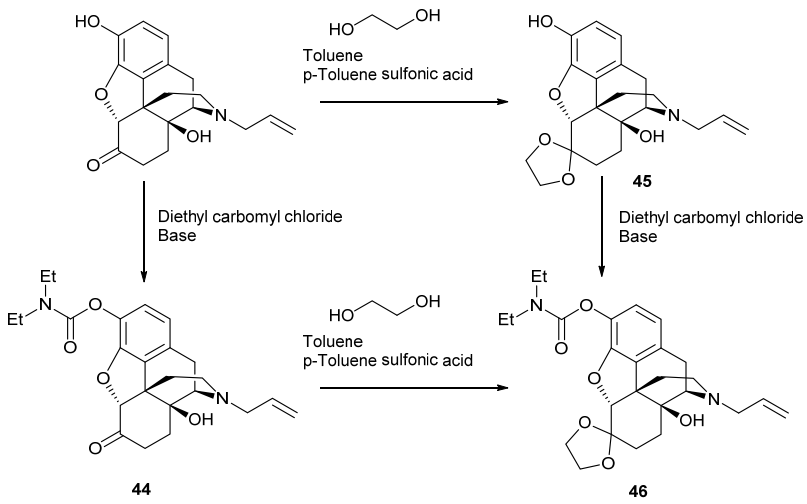
46: 3-*O*-diethylcarbamoyl naloxone ketal



45: naloxone ketal



47: 2-chloro, 3-*O*-diethylcarbamoyl naloxone ketal



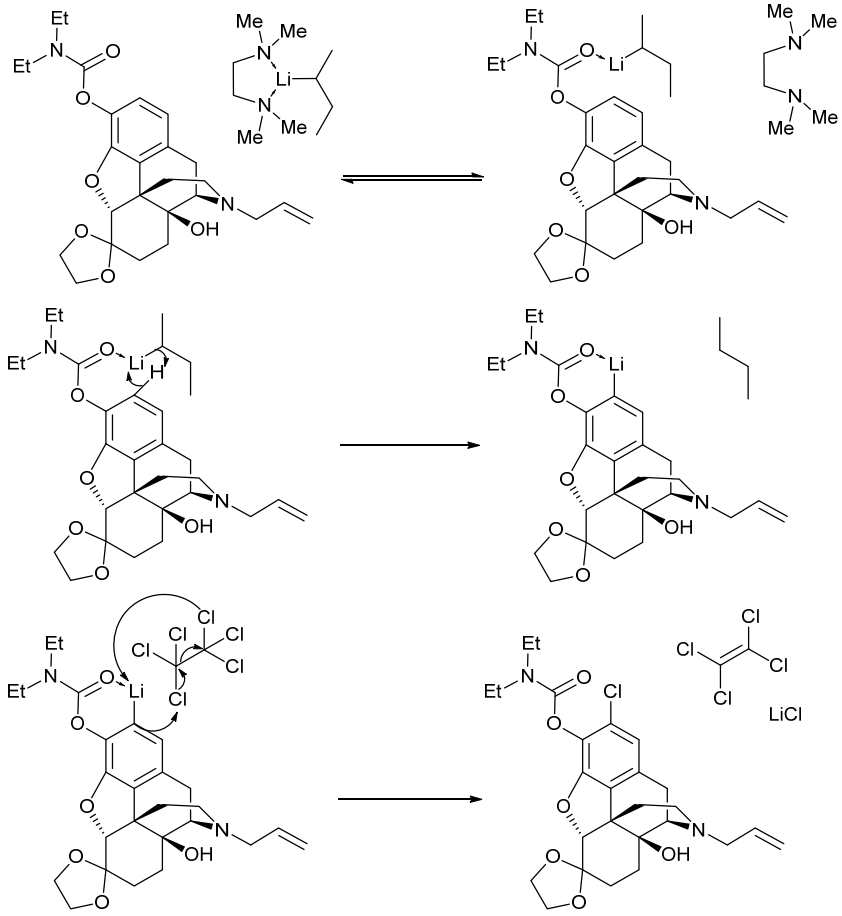
Scheme 24: The 2 Potential Routes of Synthesis of 3-*O*-diethylcarbamoyl naloxone ketal (46)

As discussed, it was established that both routes outlined in Scheme 24 would need to be attempted to assess their relative merits and feasibility. Once performed, the practical incompatibilities were compared and the overall yields assessed to establish whether it was more efficient to first acylate naloxone to give 3-*O*-diethylcarbamoyl-naloxone (**44**) or to perform the acylation of naloxone ketal (**45**).

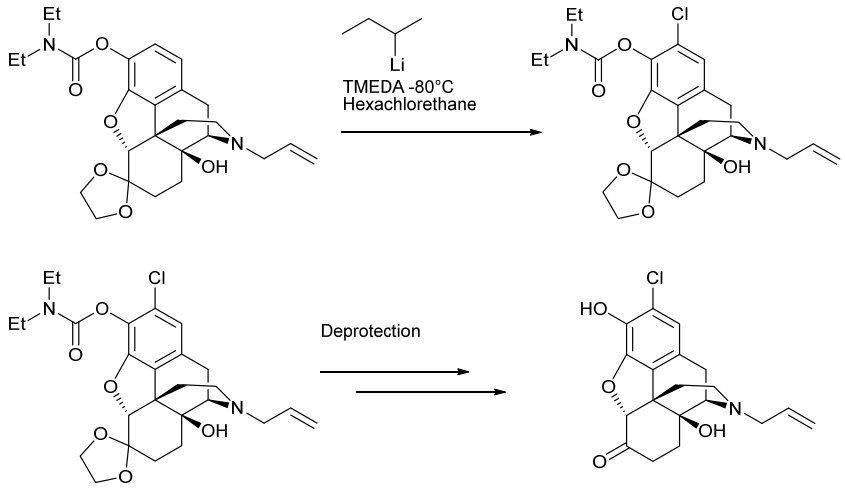
Given the precedent set by Meredith *et al.* (2003)[41], the *ortho* lithiation and subsequent substitution appeared to be highly feasible. As Meredith *et al.* (2003)[41] declared limited experimental detail for this process, some further research and experimental work was needed to assess this synthetic step and find optimum conditions. The use of *N,N,N′,N′*-tetramethylethane-1,2-diamine (TMEDA) as a catalyst has been shown to facilitate the lithiation of phenolic rings with alkyl lithium[41,42,43,44,45,48] by de-aggregating the alkyl lithium in solution thus facilitating aggregation to the target directing group.[43] Once the lithiating agent is co-ordinated to the directing group, it can either reversibly co-ordinate back to TMEDA in solution or irreversibly deprotonate the phenolic ring and substitute lithium (as per Scheme 25).

The literature precedents discussed above suggested *sec*-butyllithium (*s*-BuLi) would be a suitable lithiating agent in the presence of TMEDA at a temperature of -80 °C.

[41,42,43,44,45,48] With suitable reaction conditions identified to begin experimental investigations, a series of experimental assessments were planned starting with the addition of a slight excess of *s*-BuLi in the presence of an equivalent amount of TMEDA to a solution of **46** at a temperature of approximately -80 °C. It was anticipated that the addition of hexachloroethane as an electrophile[41,49] would then enable the substitution of the lithium for a chlorine atom, producing lithium chloride, tetrachloroethylene and **47** in a good yield as per Scheme 26.



Scheme 25: Mechanism of Directed Lithiation of 46 and Subsequent Electrophilic Substitution to Produce 34



Scheme 26: Proposed Synthesis of 34 from 46

This initial experiment proved to be lower yielding than expected, but did show some success. As this is a two part process (deprotonation followed by electrophilic substitution), a series of experiments were designed and performed to assess various reaction conditions and establish if the low yield was due to a poor deprotonation rate or a poor choice of electrophile. These experiments and their findings are discussed in detail later (see section 10.2.5, page 114), but in summary suitable conditions were found to successfully perform the directed lithiation and subsequent substitution using *tert* butyllithium (*t*-BuLi) in the presence of TMEDA at -80 °C using hexachloroethane as the electrophile for substitution to give **47** in a good yield.

Once **47** had been obtained, the hydrolysis of the two protective groups needed to be performed to give the final target compound **34**. This was proposed to be performed simultaneously under acidic conditions to give **34** in a one pot reaction to improve overall yields and reduce experimental time.

9.3 Planned Deprotection of Intermediates

9.3.1 Deketalisation *via* Acid Hydrolysis

Initial experimental plans showed that ketal protection would have to be performed and subsequent deprotection would be needed. This was established to be essential for the synthesis of both the 1-chloro product and 2-chloro product, despite attempts to bypass this protection in the synthesis of the 1-chloro product. It is reasonable to expect that both ketals can be hydrolysed by the same process, and as such a suitable method should be theoretically applicable to both intermediates. Meredith *et al.* (2003)[41] stated that the demethylation produced concomitant deketalisation due to the aqueous work up. This is likely due to the presence of hydrobromic acid as a side product from the reaction. As the ketalisation takes place under acidic conditions with the removal of water, the most feasible method of removal is under acidic conditions with an excess of water, preferably in a water miscible solvent to optimise reaction rates. Suitable examples of deketalisation were found utilising 30 % aqueous acetic acid at 90 °C[50] and an excess of 0.1 M hydrochloric acid in THF at room temperature.[51] Whilst these examples provided a good precedent, experimental assessment and optimisation was required to establish that these conditions were suitable. A series of experiments were performed under similar conditions with both substrates until the appropriate conditions for successful deprotection were found.

9.3.2 Demethylation of 1-Chloronaloxone Intermediates

Regardless of the route followed for the synthesis of 1-chloronaloxone, the final intermediate is 1-chloro-3-*O*-methylnaloxone (**41**) (see Scheme 22 and Scheme 23). A number of demethylation approaches were discussed earlier in reference to buprenorphine and thebaine (see sections 3.1 and 3.3), such as heating to an excess of

200 °C in diethylene glycol in the presence of an alkali metal hydroxide[4,8] or the use of L-selectride.[24] Both of these examples could cause side reactions with the ketone of the final intermediate, and as such were avoided. The proposed method of demethylation for both the final intermediate (**41**) was *via* the addition of boron tribromide (BBr_3). This method was chosen primarily as it is the method used by Meredith *et al.* (2003)[41] in their work with naltrexone and as such can be assumed to be directly applicable. This method of dealkylation is fairly predictable, mild and unlikely to cause side reactions if used in a suitable manner.[52,53,54] To ensure deprotection of the intermediate product, one mole equivalent of BBr_3 is needed for the ether to be removed, plus one for each potentially basic *N* or *O* group present in the molecule.[55] In this instance, the tertiary amine is assumed to be basic so will require one equivalent. It was not known for certain whether the ether at C^4-C^5 or the ketone would interact, so the reaction was planned to be attempted at 2, 3 and 4 equivalents of BBr_3 until suitable results were obtained experimentally. Experimentally, this demethylation was found to be successful with 3.4 equivalents of BBr_3. The findings and discussions of this experimental work can be found in section 10.1.5, page 105.

9.3.3 Hydrolysis of the *O*-Carbamate-2-Chloro intermediate

Cleavage of aryl *O*-carbamates are reported to typically take place under basic conditions.[43,56] There are however reports of this being performed *via* acidic hydrolysis under reflux in ethanol with hydrochloric acid.[56,57] As the deprotection of the ketal was performed under acidic reflux, the initial plan was to assess whether this would perform a concomitant hydrolysis of the carbamate and allow for a one pot reaction. If this was found to not be the case, then a base catalysed hydrolysis would have been performed following the precedent set by Meredith *et al.* (2003)[41]. Experimentally, this

concomitant deprotection proved highly successful so basic conditions were not investigated practically.

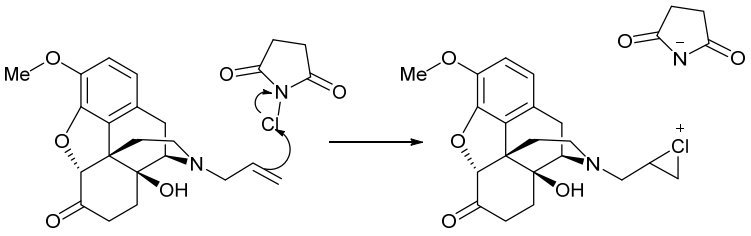
10 Results and Discussions

10.1 Synthesis of 1-Chloronaloxone

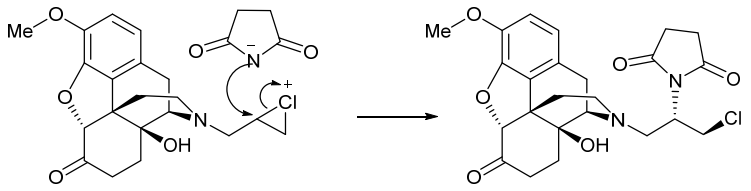
10.1.1 Methylation of 2 and Attempted Chlorination of 32

The methylation of naloxone (**2**) was achieved *via* reflux of a 0.1 M solution of naloxone in acetone in the presence of 1.9 equivalents each of potassium carbonate and dimethyl sulphate, as described in section 12.3, page 127, to give 3-*O*-methylnaloxone (**32**), in a yield of 91 %. Attempts to chlorinate **32** to give 1-chloro-3-*O*-methylnaloxone (**41**) were made in line with the proposed method in Scheme 22(Section 9.2.1, Page 90), but these proved unsuccessful due to poor conversion, unrecoverable products and side products evident by ^{1}H NMR. A series of experiments were performed, using between 1.5 and 5.0 equivalents of *N*-chlorosuccinimide (NCS) and 0.15 M solution of **32** in DCM at reflux for 1 hour and 3 equivalents of NCS at ambient for 24 hours followed by 5 hours of reflux. These reactions were found to give impure product, with substantial amounts of unreacted starting material still present or side products being formed, but established that the reaction was sensitive to heat and was time limited. A final attempt was made with a 0.15 M solution of **32** in DCM with 2 equivalents of NCS stirred at ambient for 92 hours. This showed the formation of a number of products by TLC, but the recovered material was found not to be suitable as it contained a large number of side products evident by the loss of allyl signals, signals from succinimide that could not be removed through purification and reduced signals from the proton at C^{7} α to the ketone by ^{1}H NMR. These signals could be rationalised through the NCS acting as a source of electrophilic chlorine in a number of undesired locations.

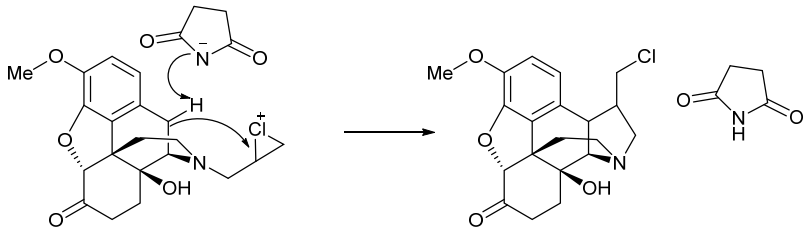
Firstly, electrophilic attack of the unsaturated allyl bond would chlorinate β to the nitrogen of the substrate, leaving a terminal carbocation (Scheme 27). This could then discharge by either accepting electrons from the succinimide (Scheme 28) or by bonding to the carbon at C^{10} to form a five membered ring, with the resultant proton being accepted by the succinimide (Scheme 29).



Scheme 27: Electrophilic Attack of the Allyl Group to Produce a Chloronium Intermediate

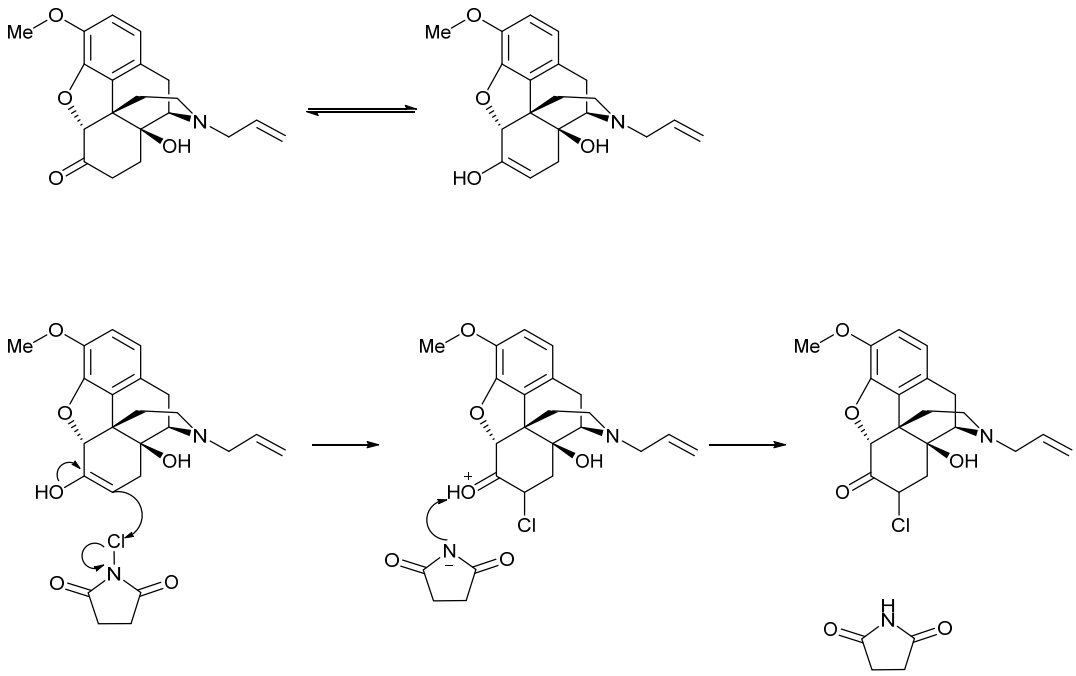


Scheme 28: Chloronium Discharge with Succinimide Ion



Scheme 29: Ring Closing of the Chloronium

The second evident site of undesired chlorination was at C^{7}, α to the ketone. This is easily rationalised as electrophilic substitution α to the ketone *via* the enol, as per Scheme 30.



Scheme 30: Chlorination of 32 by NCS at C^7 via the Enol

Evidence of both of these side reactions occurring was found in the ^{1}H NMR and was evident by multiple spots on the TLC plates run in monitoring the reaction and testing the purity of the recovered material. The in process TLC tests showed the formation of some products, but more spots appeared on the TLC plates following the work up of the reaction. The work up of these early experiments was to simply vacuum distil the reaction solution to recover solids. This clearly had an effect in promoting side reactions as the concentration of solution increased, evident by the increase in the number of TLC spots after recovery. From this data it was clear that the NCS was a suitable source of electrophilic chlorine, but that the reaction conditions needed some modifications. Firstly, the work up needed to remove or destroy any remaining NCS to prevent side reactions and secondly the substrate needed to be better protected from side reactions through the ketalisation as per Scheme 23 (Section 9.2.1 Page 90).

10.1.2 Ketalisation of 32

The ketalisation of **32** was performed using ethylene glycol as the alcohol and *para*-toluenesulfonic (TsOH) as a catalyst in toluene under reflux with a Dean Stark trap to remove water as it was eliminated from the condensation, to give 3-*O*-methylnaloxone ketal (**42**) in a yield of 67 %. Experimentally, it was found that the reaction would not progress without an excess of both ethylene glycol and TsOH, and the protection was successfully performed using 2.1 equivalents of TsOH and 2.9 equivalents of ethylene glycol (see section 12.4, page 127).

10.1.3 Chlorination of 42

During practical work, it was noted that 3-*O*-methylnaloxone ketal (**42**) was substantially less soluble in most solvents than **2** and **32**, and as such reactions had to take place at much lower concentrations. Successful chlorination of **42** was achieved in a 0.07 M solution in DCM with 2.3 equivalents of NCS stirred at room temperature over 4 days, to give 1-chloro-3-*O*-methylnaloxone ketal in a yield of 84 % (**43**, see section 12.5, page 128).

10.1.4 Deprotection of the ketal of 43

Deprotection of the ketal of **43** was achieved with an excess of aqueous hydrochloric acid under reflux to give 1-chloro-3-*O*-methylnaloxone (**41**). The method of deprotection was found *via* experimentation with different solvents and different equivalents of acid, as summarised in Table 6.

Table 6: Summary of Attempts at Deprotection of 43

Solvent	Equivalencies of HCl	Concentration of 43 (M)	Conditions	Quality/Comments
THF	4.2	0.12	Room temperature for 24 hours	Unconverted
THF	5.8	0.09	Reflux 6 hours	Unconverted
THF	24.9	0.12	Reflux 5 hours	Unconverted
Acetone	31.3	0.05	Reflux 4 hours	Partial Conversion
Acetone	51.4	0.05	100°C to drive off acetone, reflux	Converted Oil
Acetone	50.3	0.05	100°C to drive off acetone, reflux	Converted Solid

Deprotection of the ketal was attempted in THF with no success. Deprotection in THF as the main solvent with 1 M hydrochloric acid was unsuccessful at room temperature and under reflux, as was the subsequent attempt with 6 M acid. Acetone was substituted as the main solvent with 5 M hydrochloric acid under reflux at lower concentration, which provided some conversion of the starting material to the target compound.

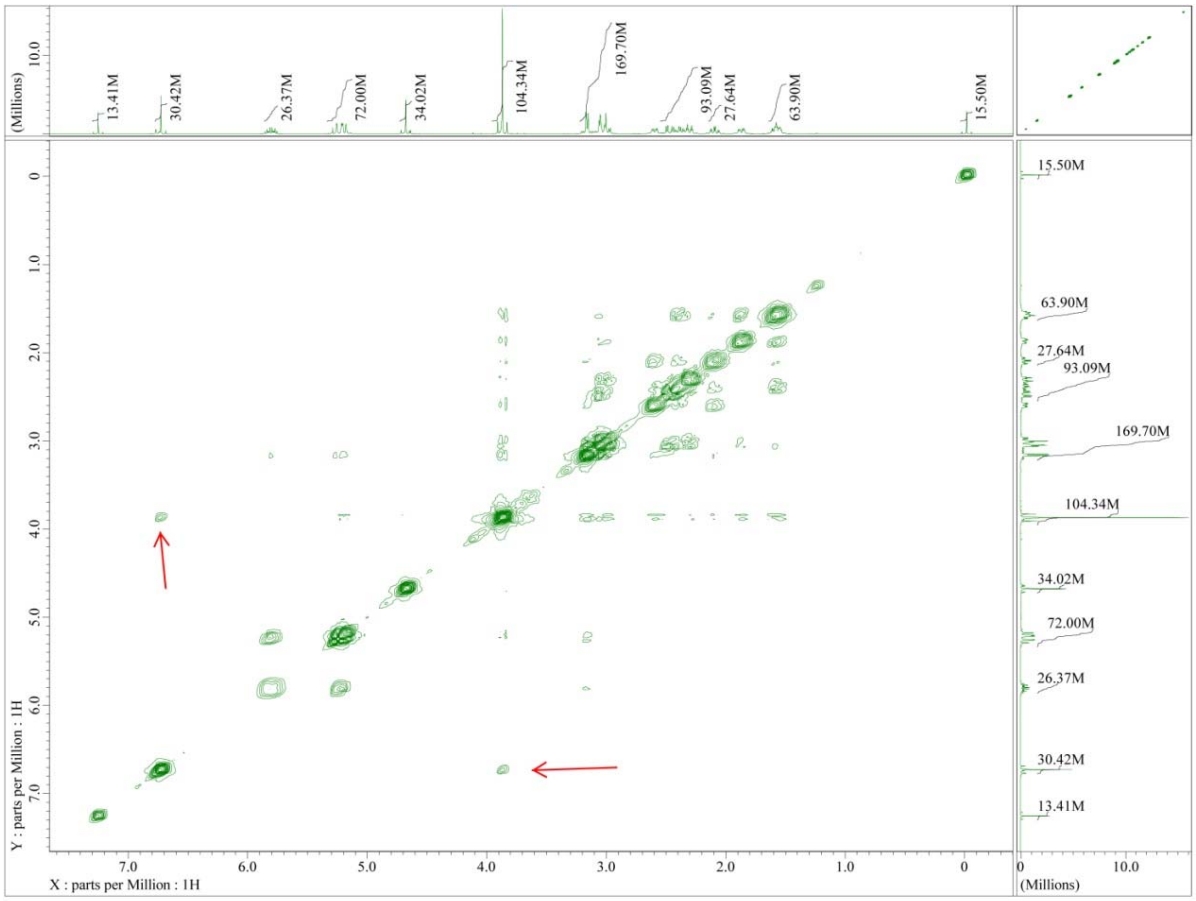
The true mechanism of this deprotection was not studied in detail but the use of acetone as a successful solvent for the process is of note. The failure of the reaction in THF and the success in acetone suggests that the reversible nature of the reaction may be a contributing factor to the speed and success of the reaction. Once the ketal has been hydrolysed to give the desired ketone, ethylene glycol is produced as a side product. As ethylene glycol has a high boiling point (197 °C), this will not be easily eliminated from solution and is free to react with the ketones in solution to give the corresponding ketal. Using THF as the solvent, the only ketone in solution is the desired product (**41**), which establishes an equilibrium reaction ketone back to the ketal. When acetone is used as the solvent, a second viable ketone is added to the reaction which can compete with **41** for any free ethylene glycol in solution to form 2,2-dimethyl-1,3-dioxolane, in short a transketalisation occurs. This by product is more volatile than ethylene glycol, with a boiling point of 92 – 93 °C, thus distilling the solvent at a temperature in excess of

100 °C will remove this product from solution. This removes the ethylene glycol from solution and prevents the desired product being re-protected.

Regardless of whether this favourable removal of ethylene glycol was the root cause of success, the experiment was repeated based on a number of observations made during the practical work. The solubility of the ketal was low in both THF and acetone, with initial dissolution proving difficult without the addition of some aqueous acid to the vessel. As the target reaction is an acid catalysed hydrolysis, and excess of water was needed, a larger amount of more dilute hydrochloric acid was used to ensure an excess of water was available for the hydrolysis and a greater starting equivalence of acid was available to catalyse the reaction. The reaction was attempted as a 0.05 M solution of **43** in one part acetone to four parts 3 M aqueous hydrochloric acid, which was distilled to remove the acetone and leave a more concentrate aqueous solution. This method was found to be successful in producing the desired product (1-chloro-3-*O*-methylnaloxone, **41**) at a good yield and was repeated on a larger scale to give solid product (see section 12.6 , page 129). Chlorination of the phenolic ring was confirmed by ^{1}H NMR data showing the loss of an aromatic proton and mass spectroscopy giving a mass ion of the expected mass with a characteristic chlorine isomer spectral pattern.

During characterisation, NOESY NMR was performed to confirm the location of chlorination by the through space interaction between the proton at C^2 and the three protons of the 3-*O*-methyl group. A positive NOE was demonstrated on the 2D spectra between these desired groups of protons, which satisfactorily confirmed the location of chlorination. This interaction has been highlighted on the spectra presented in Figure 4, where cross peaks can be seen between the protons of the C^3 *O*-methyl group at δ 3.9 and the aromatic proton at C^2 at δ 6.7. This confirmation that the remaining aromatic proton is in the C^2 position concludes that the proton at C^1 has been substituted during the reaction, as anticipated.

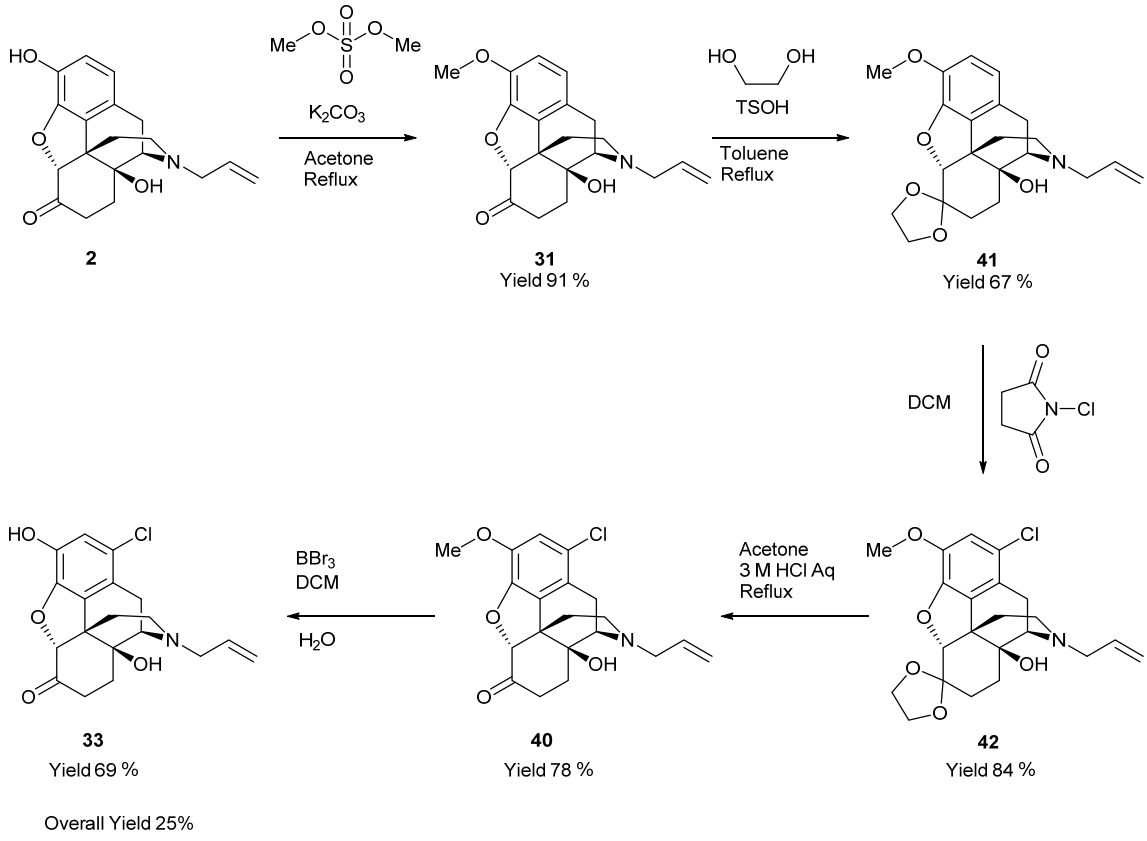
Figure 4: NOESY NMR 2D Spectra of 43 to Confirm that the Aromatic Proton found in the ^{1}H NMR is in the C^{2} Position



10.1.5 Demethylation of 41

With **41** in hand, the remaining step was the *O*-demethylation to give 1-chloronaloxone (**33**). As discussed previously in section 9.3.2, on page 96, the use of BBr_3 was identified as potentially mild and selective enough to remove the methyl ether without unwanted side reactions. The deprotection was initially attempted with two equivalents of BBr_3 in DCM under a nitrogen purge, with no conversion of starting material to the target product. This was repeated with four equivalents of BBr_3 in DCM under a nitrogen purge for 24 hours, which yielded **33** in an impure solid with side products evident by ^{1}H NMR. The partial success of this reaction confirmed that four equivalents of BBr_3 were needed for *O*-dealkylation but the side reaction needed to be addressed. It was noted that due to the small scale of the reaction, a substantial amount

of DCM had evaporated during the time over which the reaction had been left to stir. To address this, the reaction was repeated twice in a more dilute solution with 3.4 and 3.75 equivalents of BBr_3 respectively. Both experiments proved successful and gave **33**, which was then purified by recrystallisation (see section 12.7, page 130). The material was characterised by FTIR, ^1H NMR, ^{13}C NMR and MS and confirmed to be the target product. The overall synthesis of **33** is summarised in Scheme 31.



Scheme 31: Overall Route of Synthesis of 33 from 2

10.2 Synthesis of 2-Chlornaloxone

The first target of the synthesis of 2-chloronaloxone (**34**) was the production of 3-*O*-diethylcarbamoyl naloxone ketal (**46**) to furnish a protected product with a suitable directing metalation group on the phenolic ring to allow for definitive directed chlorination of the phenolic ring at C^2. As discussed in Scheme 24 (Page 93), the

production of **46** from naloxone (**2**) requires two synthetic steps (protection of the ketone at C^6 and acylation of the phenol), which could be theoretically be performed in either order. Both routes were attempted to assess which was the most viable and higher yielding.

10.2.1 Ketalisation of 2

Firstly, the ketalisation of **2** was performed, in line with the findings from the ketalisation of **32** as discussed in section 10.1. It was found that refluxing **2** in toluene with 1.6 equivalents of TsOH and 2.9 equivalents of ethylene glycol followed by recrystallising the product in DCM gave a good yield (86 %) of high purity naloxone ketal (**45**).

10.2.2 Attempted Chlorination of 45

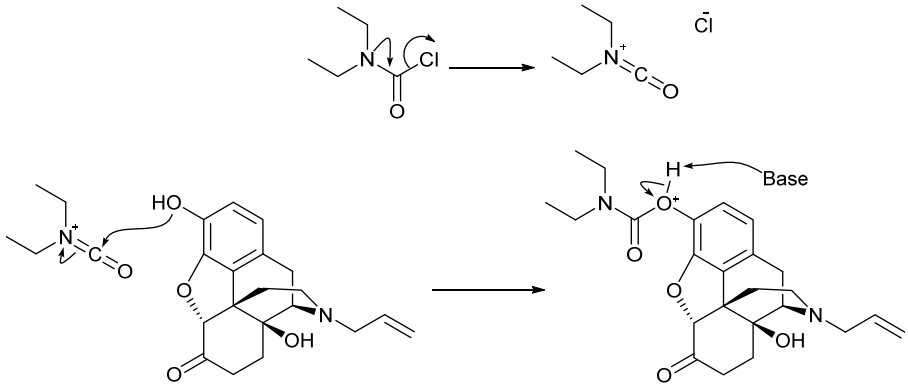
With **45** in hand, it was felt worthwhile to attempt the chlorination previously performed with **42** to establish the directing effect of the methyl ether *vs.* the phenolic alcohol. The same protocol was applied to **45**, namely stirring in DCM with 2.5 eq. of NCS for 4 days. The product of this reaction was tested by HPLC/MS and found to contain numerous compounds, each showing characteristic *m/z* patterns for chlorination. No one product predominated from this reaction and a number of the products showed a *m/z* pattern distinctive of poly chlorinated products. As such, this was felt unsuitable as a definitive chlorination method.

10.2.3 Acylation of 2

The solubility of **45** was found to be poor in most solvents used, as was expected from practical experience with **42**. Due to this poor solubility, it was anticipated that attempts to acylate this material would be difficult. An initial attempt was made to

acylate **45** with *N,N*-diethylcarbamoyl chloride (DCC) in DCM using pyridine as a base, but this proved unsuccessful as the material remained mostly undissolved and unreacted. A further attempt was made using THF as the solvent with triethylamine (TEA) as the base under reflux, but again the material was sparingly soluble and the reaction failed to yield the desired product.

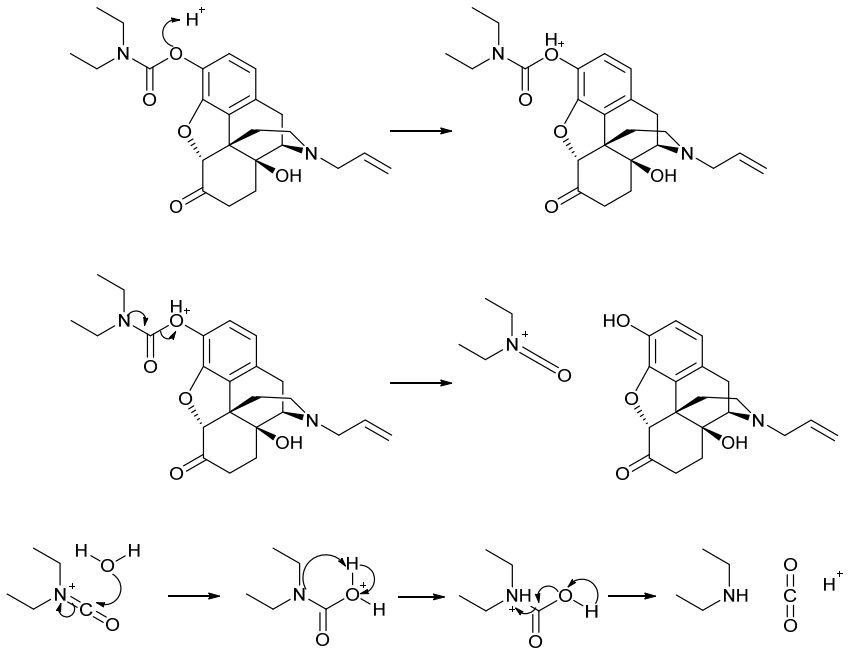
Due to the difficulties in working with **45** and the poor conversion encountered, experiments were performed with **2** to establish a suitable method of acylation to produce 3-*O*-diethylcarbamoyl-naloxone (**44**) which could then undergo the ketalisation. A series of solvents, conditions and bases were used to attempt this acylation, but most proved unsuccessful due to either low yield, difficult recoveries or the presence of side products in the recovered material. Each experiment used a slight excess of DCC (1.4 equivalents) and a slight excess of organic base (1.1 equivalents) to accept the proton produced by the reaction and buffer the pH of the solution (see Scheme 32).



Scheme 32: Theoretical Mechanism for Acylation of Naloxone (2) with *N,N*-diethylcarbamoyl chloride

These attempts at acylation did not yield product in the expected amounts, so investigations were carried out to assess whether the limiting factor was heat or ratios of the materials, as summarised in Table 7. Due to the low success rate, acylation was attempted with buprenorphine (**1**) to establish that the method was suitable for opiates,

that the reagents were of a suitable quality and that the techniques used were suitable. Testing of this reaction by TLC showed full conversion of the material, but as the product was not required it was not recovered. This experiment demonstrated that the reagents and conditions being used were suitable for acylation of an opiate, but demonstrated that the **2** and **45** were either less reactive than desired or their products less stable. It was felt that the product may be reacting with the proton liberated from the reaction (either before it could be removed by the base or by accepting it from the base in solution), and advantageous water to give starting material, diethylamine, carbon dioxide and regenerating the proton (as per Scheme 34). A number of organic bases were used to establish which was best suited and if this interaction could be limited, ultimately using pyridine as sole solvent to ensure an excess of base and reduce the reversal of the reaction, with limited success (see Table 7).

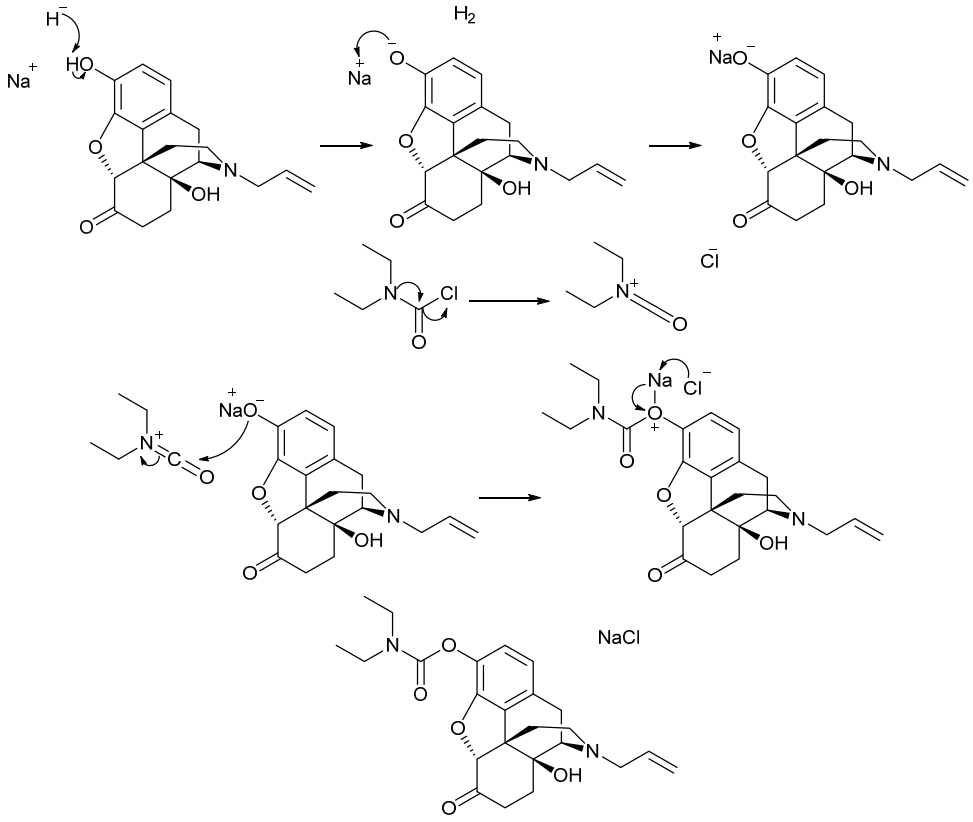


Scheme 33: Assumed Decomposition of 44

Table 7: Summary of Acylation Experiments

Substrate	Solvent	Base	Concentration of substrate (M)	Temperature	Yield	Quality/Comments
45	DCM	Pyridine	0.27	Room Temperature	Not Recovered	Unconverted by TLC
45	THF	TEA	0.15	Reflux (75 °C)	Not Recovered	Unconverted by TLC
2	DCM	TEA	0.31	Room temperature	Not Recovered	Unconverted by TLC
2	THF	TEA	0.31	Reflux (80 °C)	134%	Partial conversion, thick oil which could not be isolated as pure product
2	THF	TEA	0.61	Reflux (80 °C)	36%	Yellow solid
2	Pyridine	Pyridine	0.61	Reflux (120 °C)	35%	Solid
1	Pyridine	Pyridine	0.43	Reflux (120 °C)	Not Recovered	Full conversion by TLC
2	THF	Sodium Hydride	0.18	1 °C for hydride addition, room temperature for acylation	67%	Yellow wax, contained DCM from work up
2	THF	Sodium Hydride	0.18	1 °C for hydride addition, room temperature for acylation	84%	Cream solid
45	THF	Sodium Hydride	0.09	1 °C for hydride addition, room temperature for acylation	94%	Off white solid

To overcome this difficulty, the substrate was first converted to a more reactive phenolate using sodium hydride. This idea came from practical experience with **45**, which readily forms a phenolate with sodium hydroxide during work up making it much more soluble in an aqueous environment and difficult to recover. Furthermore, the use of sodium hydride to produce a phenoxide intermediate would remove the phenolic proton from solution as hydrogen gas; thus preventing the reversal of the reaction and preserving the product in solution (see Scheme 34). This approach was supported by a number of literature examples of phenoxides being used as reactive intermediates in reactions with carbamyl chlorides[5859] or chloromethyl ethers.[60]

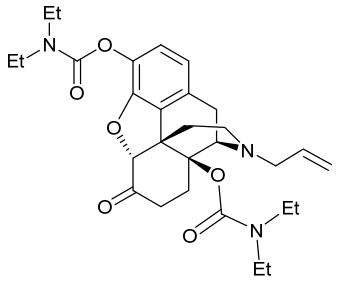


Scheme 34: Acylation of 2 *via* a Phenoxide Intermediate

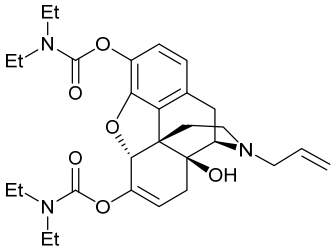
Careful addition of a THF solution of **2** to an ice cooled slurry of sodium hydride in THF formed a clear yellow solution to which DCC was added and stirred at room temperature overnight. Testing by TLC confirmed that these conditions gave almost full conversion with little starting material remaining, which was recovered as a thick

oil. Due to the success, this was repeated on a larger scale, and the oil produced was triturated with diethyl ether to give a solid which was confirmed to contain the target product **44** (see section 12.8, page 131).

Initial analysis of the crude product by ^{1}H NMR showed an over abundance of protons. This was initially assumed to be residual diethylamine formed as a by-product of unreacted DCC. Attempts were made to remove this by trituration and drying under vacuum with no change to the ^{1}H NMR spectra. It was felt that analysis of this material by ^{13}C NMR and HPLC/MS would be beneficial prior to any attempts at further purification to aid in identifying any impurities present in the material that had been synthesised. This analysis revealed the presence of a naloxone related side product in the material that had been recovered from the reaction. The HPLC chromatogram showed two main peaks, one which was assigned as the target product (**44**) by mass spectra. Assessment of the *m/z* spectra of the second peak established that the mass ion was a di-carbamate of naloxone and was tentatively established to be either 3-*O*, 14-*O*-di-diethylcarbamoyl naloxone (**48**) or 3-*O*, 6-*O*-di-diethylcarbamoyl naloxone (**49**). The presence of a peak in the mass spectrograph with a *m/*z equivalent to total mass minus a hydroxyl group indicated that **49** was the more likely structure.



48: 3-*O*, 14-*O*-di-diethylcarbamoyl naloxone



49: 3-*O*, 6-*O*-di-diethylcarbamoyl naloxone

Assessment of the ^{13}C NMR spectra showed a significant over abundance of signals, as expected from a mixture of two different compounds of similar structure. Of note, the

signals assigned to the carbamate group (at approximately δ ≈ 14, δ ≈ 45 and δ ≈ 150), all appeared to be duplicated, confirming that a di-diethylcarbamate was present. The location of the second carbamate was confirmed to be on the oxygen at C^6 (the unprotected ketone) by the presence of two signals at approximately δ ≈ 90, which was assigned to the ethereal carbon at C^5. The presence of two signals for this carbon confirms that the two compounds have significantly different environments near C^5, such as the difference in C^6 between **44** and **49**. The environment at C^5 in **44** and **48** is too similar to account for these signals, so **48** was discounted as the potential impurity.

With a mass and structure assigned to the impurity, the ^{1}H NMR spectra was consulted to quantify the levels of **49** in the product. This was calculated using the general formula for determining the molar ratio of two compounds (*X* and *Y*) in a mixture with the using the ratio of integrated area for the respective responses:[61]

$$\frac{n_x}{n_y} = \frac{I_x}{I_y} \times \frac{N_y}{N_x}$$

Where *n* is the relative number of moles, *I* is the integrated signal area of the response and *N* is the number of nuclei generating the response. Using the respective responses for the proton at C^5 for both compounds, this gives:

$$\frac{n_x}{n_y} = \frac{0.11}{0.20} \times \frac{1}{1}$$

This gives a ratio of 1.1 moles of **49**:2 moles of **44**. Adjusting for the molecular weights(426.51 for **44** and 525.65 for **49**), this gives an overall composition of 59.6 % **44** by weight. This high level of impurity was deemed to be highly detrimental to this route of synthesis. Furthermore, attempts to ketalise the crude **44** proved unsuccessful (discussed later, see 10.2.4 below), as such **44** was deemed a non-viable intermediate in

the target synthesis. Given that **49** formed readily and high levels as a by-product, this gave a clear indication that ketal protection was necessary for acylation to be successful.

10.2.4 Synthesis of 46

With both intermediates in hand, production of **46** was attempted with both **44** and **45**. Ketalisation of **44** under the same condition as the ketalisation of **2** proved to be unsuccessful as the acidic reflux deprotected the phenolic ring and resulted in a mixed product containing both the target compound **46** and **45** as a side product. The formation of this side product was not unexpected and confirmed that ketalisation of **44** was impractical and supported the findings from the failed acylation experiments that the intermediate can be readily deacylated in acidic conditions. The successful acylation method used on **2** was applied to **45**, in a more dilute solution to account for the reduced solubility of the ketal. Once the ketal was added to the sodium hydride slurry, a clear solution was again obtained. Addition of the DCC produced a slurry, which was stirred overnight at room temperature and produced the target **46** in a high yield (94 %) as an off-white crystalline solid once recrystallised (see section 12.10, page 135). This material showed no sign of containing a naloxone related dicarbamate by HPLC/MS, negating the concerns of this unwanted functional group affecting reactivity, yields or forming side products and confirming that the ketone was the site of the side reaction previously discussed in the formation of **49**.

10.2.5 Directed Chlorination of 46

With 3-*O*-diethylcarbamoyl naloxone ketal (46) in hand, attempts were made to chlorinate the material in line with the proposed synthesis. These experiments are summarised in Table 8.

Table 8: Summary of Deprotonation Experiments Performed with 46.

Concentration of substrate (mmol/ml)	Base	Eq. of base	Temperature	Quench	Quality/Comments
0.21	s-BuLi + TMEDA	1.5	-75°C - spike to -45°C	1.6 eq. C2Cl6	Some evidence of product by 1H NMR
0.12	s-BuLi	1.5	-75°C	1.7 eq. C_2Cl_6	Unconverted
0.25	s-BuLi + TMEDA	1.5	-72°C	10 eq. D_2O	Unconverted
0.25	s-BuLi	1.5	-72°C	10 eq. D_2O	Unconverted
0.25	s-BuLi + TMEDA	1.6	-10°C	20 eq. D_2O	Unconverted
0.25	s-BuLi	1.5	-10°C	20 eq. D_2O	Unconverted
0.26	s-BuLi	4.4	-5°C	20 eq. D_2O	Multiple products, none isolated or identified.
0.11	s-BuLi + TMEDA	6.5	-60°C to -50°C	6.8 eq. C_2Cl_6	Major substituent found by ^1H NMR is target with unknown impurity present alongside starting material
0.07	s-BuLi + TMEDA	5.4	-60°C to -50°C	4.6 eq. C_2Cl_6 as a reverse quench	Unidentified product, concentration lower due to extra solvent needed for reverse quench
0.11	t-BuLi + TMEDA	3.2	-60°C to -50°C	3.5 eq. C_2Cl_6	Converted to desired product, some starting material remains by ^1H NMR
0.12	t-BuLi + TMEDA	3.2	-60°C to -50°C	3.5 eq. C_2Cl_6	Converted to desired product, some starting material remains by ^1H NMR

Note: All experiments were carried out in dry THF. Where C_2Cl_6 was the quench it was introduced as a saturated solution in dry THF.

An initial attempt at the directed metalation was performed in THF at -75 °C, using a 1.4 M solution of *sec*-butyllithium in cyclohexanes (*s*-BuLi 1.6 eq.) as the base for deprotonation with *N,N,N',N'*-tetramethylethylenediamine (TMEDA, 1.6 eq.) as an additive to disaggregate the base, in line with the literature precendence.[43,48] The resulting anion was then quenched with an excess of hexachloroethane (2.0 eq.), to act as the chlorinating agent as shown in Scheme 25 (page 94).

This initial experiment produced mixed results with only partial conversion to a deprotonated product which was not confirmed to be a chlorination or rearrangement product. The metalation was repeated without TMEDA at a lower concentration to ensure all substrate was dissolved, but this yielded unreacted starting material. Work was performed to assess if the failure to chlorinate the material was due to the *s*-BuLi failing to deprotonate the substrate or in the chlorinating agent not being suitable. This was performed by repeating both experiments (once with 1.6 eq. of *s*-BuLi and 1.6 eq. of TMEDA and once with 1.6 eq. of *s*-BuLi only) with a quench of an excess of D_2O (10 eq.) in an effort to remove the proton at C^2 deutarate the aromatic ring, which could be confirmed by the loss of an aromatic proton by ^1H NMR.

Initial experiments at -78 °C with D_2O quench failed to deprotonate the substrate, yielding unreacted starting material with no evidence of proton substitution. Review of the initial partial successful experiment indicated that the partial conversion may have been due to the exotherm of the reaction causing the vessel to heat to -45 °C during the *s*-BuLi addition. Following this assessment, deprotonation and D_2O quench was again attempted at -15 °C with the same equivalences of reagents (both with and without TMEDA). This again failed to produce a substitution of the phenolic ring, so was repeated at -5 °C, producing numerous products, none of which were found to be the target substitution of the proton at C^2.

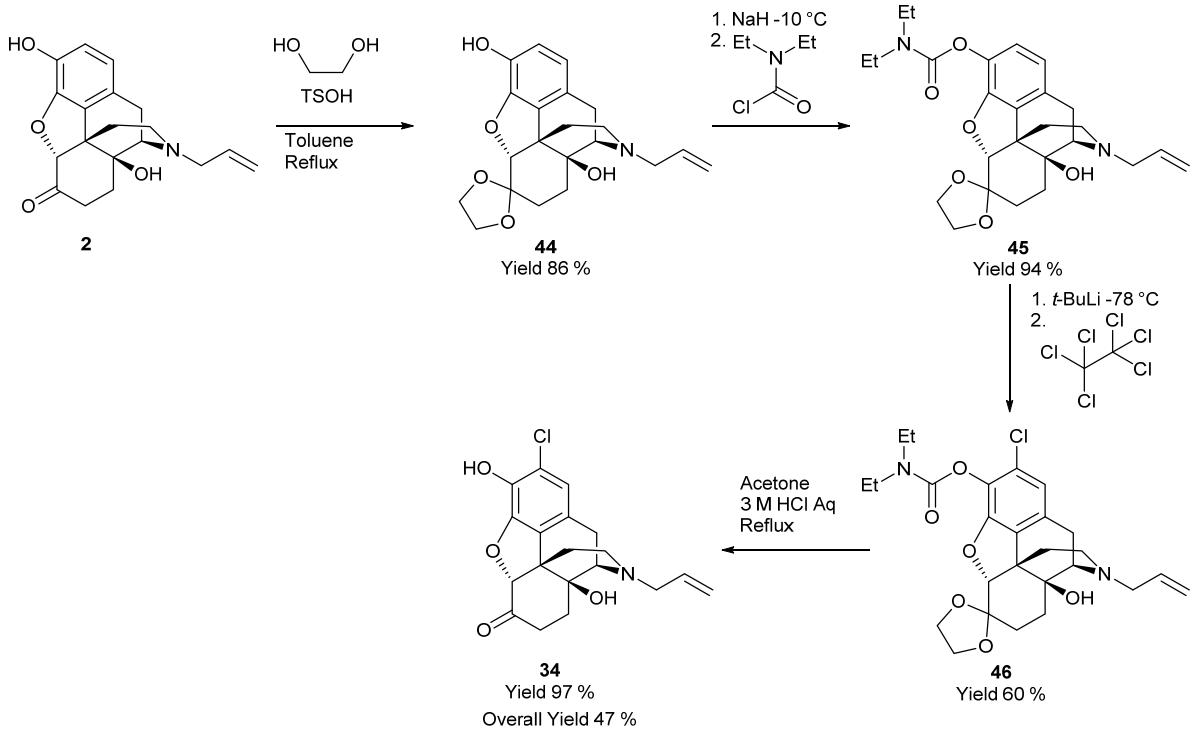
From this it was established that higher temperatures showed little improvement in conversion and that TMEDA would be needed in the reaction. The initial experiment was repeated with higher equivalences of *s*-BuLi (6.5 eq.) and TMEDA (4.2 eq.), followed by hexachloroethane quench (6.8 eq.). The deprotonation followed by quench produced a monochlorinated product in a good rate of conversion, with the presence of starting material confirmed by ^{1}H NMR, along with at least one other unknown product. This was performed simultaneously alongside a similar experiment with a reverse quench of hexachloroethane (4.6 eq.) in solution with the substrate, followed by the addition of TMEDA (4.2 eq.) then *s*-BuLi (5.4 eq.). The reverse quench experiment produced a yellow solid, which ^{1}H NMR confirmed was not the target product.

Given the partial success seen by using higher levels of *s*-BuLi, the reaction was attempted again at a temperature of -78 °C using a solution of 1.7 M *tert*-butyllithium in pentane (*t*-BuLi, 3.2 eq.) and TMEDA (3.2 eq.), followed by a quench of hexachloroethane in THF (3.5 eq.). Once worked up, this yielded a thick brown oil which was found to contain a deprotonated product as the main constituent alongside starting material and no presence of side reactions. This was purified by flash chromatography to give a white crystalline solid in a yield of 60 %. The material was tested by FTIR, ^{1}H NMR, ^{13}C NMR and HPLC/MS to confirm it was the desired product by confirming the loss of one aromatic proton and a *m/z* close to the calculated value with the characteristic pattern expected of a monochlorinated compound (see section 12.11, page 136).

It is of note that the synthesis presented by Meredith *et al.* (2003)[41] declared a yield of 30 % for this stage in their synthesis. As the full details of their protocol are not presented in the paper, it is difficult to ascertain if this is due to the use of a different butyllithium base or some other factor.

10.2.6 Deprotection of 46

With **46** in hand, only the deprotection of the ketal and the carbamate remained to furnish 2-chloronaloxone (**34**). This was achieved as a concomitant one pot reaction along the same lines as the deketalisation previously discussed in section 10.1.4, page 102. A solution of **46** in one part acetone and three parts 3 M aqueous HCl was heated to reflux. The acetone was distilled off, and the remaining aqueous solution was left at reflux over night forming a black solution. This was adjusted to pH 8 with aqueous sodium hydroxide and extracted into DCM. The solvent was removed under vacuum to give 2-chloronaloxone (**34**). The material was characterised by FTIR, ^{1}H NMR, ^{13}C NMR and MS and confirmed to be the target product (see section 12.12, page 138). A mixture of 1 part **33** to 3 parts **34** in $CDCl_3$ was analysed by ^{1}H NMR, which confirmed that the aromatic protons of each compound could be resolved and identified as separate compounds. The overall synthesis of **34** is given in Scheme 35.



Scheme 35: The Overall Route of Synthesis of 34 from 2

11 Conclusion

Both target molecules 1-chloronaloxone (**33**) and 2-chloronaloxone (**34**) have been synthesised *via* a definitive route of directed aromatic substitution. The synthesis of **33** was completed through a five stage synthetic route with an overall yield of 25 %. This could potentially be improved through concomitant deprotection of the final intermediate, however this was not investigated. The synthesis of **34** was completed through a 4 stage process with an overall yield of 47 %.

The position of chlorination for **33** has been confirmed through NOESY NMR through space interactions in a sample of the final intermediate in the synthesis (1-chloro-3-*O*-methylnaloxone, **41**) showing a through space interaction between the remaining aromatic proton at C^2 and the three protons of the 3-*O*-methyl group. The synthesis of **34** by a directed metalation followed by electrophilic substitution gives mechanistic confirmation that substitution has occurred at the desired position on the aromatic ring (in this case C^2, *ortho* to the directing group). Comparison of the ^{1}H NMR spectra of **33** and **34** confirm that both compounds have one remaining aromatic proton, each with a different chemical shift confirming that the substitution has occurred in different positions in each synthesis. Analysis of a 3:1 mixture of **33** and **34** by ^{1}H NMR demonstrated that the two compounds can be differentiated by their aromatic protons, again confirming that chlorination has taken place in different locations in both molecules.

Attempts to reduce the number of synthetic steps to produce both products proved un-successful. The attempted chlorination of 3-*O*-methylnaloxone (**32**) did not provide the desired target product in a suitable yield (as per Scheme 22) and ketalisation was required to produce the desired 1-chloro intermediate (as per Scheme 23). Furthermore, attempts to chlorinate naloxone ketal (**45**) in an attempt to produce a 2-chloro

intermediate were unsuccessful. The deprotection of **47** was however successfully performed as a simultaneous hydrolysis of the carbamate and the ketal to give the desired product.

Some differences were noted between this synthesis and the data reported in the literature, especially in the synthesis of 2-chloronaloxone. Firstly, attempts to acylate both naloxone and naloxone ketal in pyridine with *N,N,*-diethylcarbamoyl chloride under reflux failed to produce the result reported by Meredith *et. al.* (2003)[41] in their work with naltrexone (reported as a yield of 90 % for this stage of their synthesis). As this paper did not cite the full experimental parameters, attempts to duplicate this reaction were undertaken with different solvents and bases, but failed to yield similar results with naloxone. It was felt that the lower solubility of naloxone and naloxone ketal may be the inhibiting factor in this reaction. This difficulty was successfully over come with the use of a sodium hydride to form a more reactive phenolate.

The optimum route to **34** was identified experimentally. The key intermediate in the synthesis, **46** (3-*O*-diethylcarbamoyl naloxone ketal) had two proposed routes of synthesis. Through experimentation and analysis of the products of both routes, it was found that the optimum route was to first protect the ketone and then acylate this product. This was due to the carbamate being readily removed by the conditions used in ketalisation and acylation of naloxone producing an unwanted side product in a high level. This by-product was not isolated, but a structure was assigned based on data generated by UPLC/MS, ^{1}H NMR and ^{13}C NMR which indicated that the unprotected ketone had been converted to a carbamate. The high levels of the di-carbamate could have affected overall yields or interacted with the deprotonating agent. This order of synthetic steps is in agreement with the synthesis reported by Meredith *et. al.* (2003)[41], and supplies some information as to why they may have opted for this route.

Directed chlorination to the C^2 position by directed metalation required some investigation to produce favourable results. Deprotonation using *s*-BuLi proved to be low yielding and required a substantial excess of alkyl lithium to achieve any results. The successful chlorination in the C^2 position using *t*-BuLi as the deprotonation reagent produced a greater yield than that quoted in the literature for naltrexone (Meredith *et. al.* (2003)[41] gave a yield of 40 % for this stage). Again, experimental details were not cited, but using the attempted directed chlorination reactions using *s*-BuLi presented here as a direct comparison, it appears that the use of *t*-BuLi is responsible for this improved yield.

12 Experimental

12.1 Analytical Methods for Comparison of Experimental Results

The samples produced during the synthesis of the target compounds were analysed by TLC and ^{1}H NMR to confirm reactions had proceeded and produced the target compound. Once isolated and purified, the materials were characterised by ^{1}H NMR, ^{13}C NMR, FTIR, and MS.

12.1.1 NMR Analysis

^{1}H NMR spectroscopy was carried out on a JEOL GSX270 Delta NMR, using samples dissolved in $CDCl_3$ at 270 MHz. ^{13}C NMR spectroscopy was carried out on a JEOL GSX270 Delta NMR, using samples dissolved in $CDCl_3$ at 60 MHz. The ^{1}H NMR and ^{13}C NMR spectra for all intermediates are presented in Appendix B.

12.1.2 FTIR Analysis

FTIR spectroscopy was carried out on an Agilent Cary 630 FTIR with KBr internal windows and a single bounce diamond ATR. Samples were prepared by grinding and applying directly to the ATR.

12.1.3 MS Analysis

MS analysis of **2**, **32**, **42**, **43**, and **41** was kindly performed by staff at the University of Hull. Samples of these compounds were dissolved in methanol: water (50:50) at a concentration of 0.1 mg/Ml. These solutions were analysed as follows:

Instrument: Bruker HCT Ultra ion trap MSMS instrument connected to an Agilent 1100 LC system with G1312A binary pump.

Ionisation Source: Standard electrospray ion source in positive ion mode

Solvent : Methanol / Water 50:50 with 0.1% formic acid

Flow rate: 0.3 mls min^{-1}

Direct loop injection into the solvent stream: 5uL sample injection

Scan range: 75-2500 amu in ultrascan mode (26,000 amu s^{-1})

Acquisition time: 200ms

Nebuliser: 44 psi

Drying gas: nitrogen at 12 L min^{-1}

Drying Temp: 250 ° C

The MS analysis of **33**, **44**, **45**, **46**, **47** and **34** was performed *via* UPLC/MS. The samples were dissolved in a solution of acetonitrile/0.1 % trifluoroacetic acid in water (50:50) at a concentration of 1 mg/mls The solutions were analysed as follows:

Instrument: Waters Acquity H-Class UPLC with an Acquity QDa quadrapole MS detector and a photodiode array detector.

MS detector settings:

UPLC column outlet split 50:50 between photodiode array and MS detector

Ionisation Source: Standard electrospray ion source in positive ion mode

Capillary Voltage: 2.5KV

Cone Voltage: 72V

Extractor Voltage: 3V

RF lens: 0.0

Solvent: UPLC system eluent

Flow rate: 0.75 mls min^{-1}

Scan range: 120.00-1500.00 Da at 1500 Da s^{-1}

Acquisition time: Continuous

Nebuliser: 100 psi

Drying gas: Nitrogen at 8.85 L min^{-1}

Cone gas: Nitrogen 1.67 L min^{-1}

Source Temperature: 150 °C

Drying Temp: 250 °C

UPLC Conditions:

A solution of 0.1% trifluoroacetic acid in water was used as mobile phase A and 100% acetonitrile was used as mobile phase B. A linear gradient from 90 % mobile phase A and 10 % mobile phase B at initial to 5 % mobile phase A and 95 % mobile phase B at 30 minutes, with a flow rate of 1.5 ml/min. The column used was a 50 mm × 4.6 mm Phenomenex Kinetix, with 2.6 µm C18 100 Å packing material, set to a temperature of 40 °C *via* column thermostat. The UV detector was set to scan at 288 nm. A 10 µl injection of the sample was run under these conditions for 30 minutes and the peak areas recorded and assessed against their corresponding *m/z* spectra.

12.1.4 Analysis by TLC

Some reactions were monitored by TLC analysis. This was performed using an eluent of 95% dichloromethane (DCM) and 5% methanol on Merck Kieselgel $60F_{254}$ 5 mm × 20 mm, using the starting material in DCM at a concentration of 30 mg/ml as a comparison to confirm reaction initiation and conversion.

12.2 Purification of Naloxone Free Base (2)

A round bottomed flask was charged with naloxone hydrochloride dihydrate (181.74 g, 555.2 mmol) and dichloromethane (DCM, 1.8 L). An aqueous ammonia solution (175 ml of 6.2 % solution, 563.6 mmol) was added to the slurry. Further water was added to the slurry (180 mL). The slurry was stirred for 48 hours. The mixture was filtered through a pad of Celite to remove insoluble matter and the layers were separated. The aqueous layer was extracted with 800 mL DCM, the organic layers were combined, dried over $MgSO_4$ and filtered. The solvent was then removed by evaporation under vacuum to give an off white solid. The solid was broken up and dried at 60°C under vacuum overnight to give naloxone as a cream to off white solid, 123.79g, yield 83%. ^{1}H NMR (270 MHz $CDCl_3$) δ 6.73 (d, J = 8.3 Hz, 1 H), 6.60 (d, J = 8.0 Hz, 1 H), 5.89 – 5.75 (m, 2 H), 5.25 – 5.16 (m, 2 H), 4.75 (s, 1 H), 3.17 – 2.98 (m, 5 H), 2.63 - 2.37, (m, 3 H), 2.32 (dt, J = 14.6 Hz, 3.0 Hz 1 H), 2.16 (td, J = 12.1 Hz, 3.9 Hz, 1 H), 1.91 – 1.85 (m, 1 H), 1.70 – 1.55 (m, 2 H), the multiplet at 5.82 – 5.75 coincides with the broad peak for the phenolic proton; ^{13}C NMR (68 MHz $CDCl_3$), δ 209.8, 143.5, 138.8, 135.1, 129.0, 124.1, 119.8, 118.1, 117.9, 90.5, 70.5, 62.2, 57.6, 50.9, 43.3, 36.1, 31.2, 30.4, 22.7; MS calculated mass: 327.15, found *m/z* 328.1 (M+H); FTIR; cm^{-1} 3351 (broad peak), 3233 (Broad peak), 2952, 2907, 2809, 1730, 1618, 1511, 1455.

12.3 Synthesis of 3-*O*-Methylnaloxone (32)

Naloxone (10.1 g, 30.9 mmol) and potassium carbonate (8.1 g, 58.8 mmol) were stirred in acetone (300 ml). Dimethyl sulfate was added (5.5 ml, 7.3 g, 57.8 mmol) *via* syringe and the solution was heated to reflux. The solution was stirred under reflux for 24 hours, after which TLC sampling showed no spot for starting material. The solution was cooled to room temperature and the acetone evaporated under vacuum. The resultant pale yellow solid was dissolved in DCM (300 ml) and to which water (200 ml) was added. The layers were separated and the aqueous layer was extracted twice with DCM (200 ml, followed by 100 ml). The organic portions were combined, washed with brine (100 ml), dried with sodium sulphate and filtered. The DCM was evaporated under vacuum and the resultant solid dried overnight under vacuum at 50 °C to give 32 as a slightly yellow crystalline solid, 9.57 g, yield 91 %. ^{1}H NMR (270 MHz $CDCl_3$) δ 6.71 (d, J = 8.2 Hz, 1 H), 6.64 (d, J = 8.2 Hz, 1 H), 5.90 – 5.75 (m, 1 H), 5.31 - 5.16 (m, 2 H), 4.67 (s, 1 H), 3.89 (s, 3 H), 3.17 – 2.96 (m, 5 H), 2.62 – 2.53 (m, 2 H), 2.45 – 2.35 (m, 1 H), 2.29 (dt J = 14.1 Hz, 3.5 Hz, 1H), 2.14 (td, J = 12.1 Hz, 3.7 Hz, 1 H), 1.91 – 1.83 (m, 1 H), 1.68 – 1.59 (m, 2 H), the loss of the broad peak for the phenolic proton beneath the multiplet at 5.90 – 5.75 and the presence of the 3 proton singlet at 3.89 confirm successful conversion to the phenolic methyl ether; ^{13}C NMR (68MHz $CDCl_3$), δ 208.5, 144.9, 142.9, 135.1, 129.4, 124.8, 119.4, 118.1, 114.8, 90.3, 70.2, 62.2, 57.6, 56.7, 50.7, 43.3, 36.1, 31.4, 30.5, 22.6; MS calculated mass 341.16, found *m/z* 342.1 (M+H); FTIR; cm^{-1} 3337, 2954, 2928, 2844, 1726, 1605, 1497.

12.4 Synthesis of 3-*O*-methylnaloxone ketal (42)

3-*O*-Methylnaloxone (4.24 g, 12.43 mmol) was added to a three necked flask and dissolved in toluene (50 ml). Ethylene glycol (2 ml, 2.23 g, 35.86 mmol, 2.9 eq.) and *p*-toluene sulfonic acid (5.045 g, 26.52 mmol, 2.1 eq.) were added and the solution heated

to reflux for 16 hours under a Dean-Stark trap. The reaction was worked up by the addition of DCM (10 ml) and sodium hydroxide (1 M aqueous, 60 ml) to form a two-layered system. The layers were separated, the aqueous portion extracted with DCM (2 × 40 ml) and the organic portions combined, dried over $MgSO_4$, filtered and evaporated under vacuum to give 3-*O*-methylnaloxone ketal as an off white solid (3.82 g, yield 67 %). ^{1}H NMR (270 MHz $CDCl_3$) δ 6.75 (d, J = 8.4 Hz, 1 H), 6.62 (d, J = 8.4 Hz, 1 H), 5.83 – 5.72 (m, 1 H), 5.23 – 5.12 (m, 2 H), 5.00 (s, broad peak, 1 H), 4.57 (s, 1 H), 4.24 – 4.16 (m, 1 H), 4.03 (q, J = 6.16 Hz, 1 H), 3.94 – 3.85 (m, 4 H), 3.78 (q, J = 6.4 Hz, 1 H), 3.15 – 3.03 (m, 3 H), 2.91 (d, J = 5.7 Hz), 2.64 – 2.48 (m, 2 H), 2.36 – 2.07 (m, 3 H), 1.59 – 1.42 (m, 4 H). Conversion to the ketal was confirmed by the 4 signals in the region of 4.24 – 3.75 ppm containing 4 distinct protons showing splitting patterns consistent with that expected in their environment. The multiplet at 4.24 – 4.16 is quartet with further splitting to the central two peaks and has been assigned as one of the ketal protons undergoing through space interactions with the proton at C^5 or one of the protons at C^7. The multiplet at 3.94 – 3.85 is caused by one of the ketal protons and the methyl ether protons being coincidental, as such the J coupling constant for that signal could not be calculated accurately and is not reported. ^{13}C NMR (68MHz $CDCl_3$), δ 146.2, 142.3, 135.5, 130.9, 124.9, 118.1, 117.8, 113.7, 108.9, 93.8, 69.9, 66.5, 65.0, 62.6, 57.6, 56.5, 48.0, 43.6, 31.1, 29.2, 28.8, 22.7; The absence of the signal at ≈ 208 ppm confirms the loss of the ketone and further confirms conversion. MS calculated mass 385.45, found *m/z* 386.2 ($M+H^+$); FTIR; cm^{-1} 3391, 2941, 2905, 2818, 1726, 1637, 1613.

12.5 Synthesis of 1 Chloro, 3-*O*-Methylnaloxone Ketal (43)

A round bottomed flask was charged with *O*-methylnaloxone ketal (2.522 g, 6.54 mmol) and DCM (100 ml) and stirred until all solid was dissolved. Once the substrate

was fully dissolved, *N*-chlorosuccinimide (2.025 g, 15.14 mmol, 2.3 equiv.) was added and the resultant solution was left to stir for 96 hours. The solution was extracted with 1M aqueous hydrochloric acid (2 × 100 ml) and the combined aqueous portions were adjusted to pH 10 with 1M aqueous sodium hydroxide. The resultant aqueous solution was extracted with DCM (2 × 100 ml), the organic portions combined, dried with $MgSO_4$ and the solvent evaporated under vacuum to give 1-chloro naloxone methyl ether ketal as a cream solid (1.65 g). Poor recovery was addressed by distilling 50 ml from the retained organic solvent from the reaction and further extracting with 1 M aqueous hydrochloric acid (2 × 50 ml). The combined aqueous portions were adjusted to pH 10 with 1 M aqueous sodium hydroxide and extracted with DCM (2 × 100 ml). The combined organic portions were combined, dried over $MgSO_4$ and the solvent evaporated off under vacuum to give 1-chloro naloxone methyl ether ketal as a cream solid (0.67 g). Total recovery 2.32 g, yield 84 %. ^{1}H NMR (270 MHz $CDCl_3$) δ 6.77 (s, 1 H), 5.96 – 5.72 (m, 1 H), 5.30 – 5.15 (m, 2 H), 4.58 (s, 1 H), 4.21 – 4.10 (m, 1 H), 4.03 – 3.60 (m, 7 H), 3.15 – 2.96 (m, 4 H), 2.56 – 2.43 (m, 2 H), 2.30 – 2.03 (m, 3 H), 1.61 – 1.42 (m 4 H). Conversion confirmed by the loss of a phenolic proton ≈ 6.6 ppm. ^{13}C NMR (68MHz $CDCl_3$), δ 145.2, 143.0, 135.3, 132.1, 122.9, 122.3, 118.1, 113.8, 108.9, 94.3, 93.6, 69.8, 66.5, 65.0, 62.2, 57.6, 56.7, 48.3, 43.4, 31.1, 29.5, 29.2, 28.8, 21.9; MS *m/z* 420.2 ($M+H^+$); FTIR; cm^{-1}; 3363, 2941, 2905, 2866, 2821, 1633, 1607.

12.6 Synthesis of 1 Chloro-3-*O*-Methylnaloxone (41)

1-Chloro-3-*O*-methylnaloxone ketal (0.501 g, 1.19 mmol) was added to a three necked flask and slurried in acetone (5 ml). Hydrochloric acid (20 ml, 3 M, 60 mmol, 50.4 eq.) was added and full dissolution achieved. The solution was stirred and heated to 70 °C under a distillation head. The temperature was gradually increased to 105 °C and a total of 7 ml of solvent was distilled off. The solution was refluxed overnight and conversion

confirmed by TLC. The reaction was worked up with the addition of DCM (50 ml) and the resultant solution was pH adjusted to pH 11 with sodium hydroxide (1 M aqueous solution). The layers were separated and the aqueous extracted with DCM (3 × 50 ml). The organic layers were combined, dried over $MgSO_4$, filtered and evaporated under vacuum to give **41** as an off white solid (0.35 g, yield 78 %). ^{1}H NMR (270 MHz $CDCl_3$) δ 6.74 (s, 1 H), 5.87 – 5.75 (m, 1 H), 5.31 – 5.19 (m, 2 H), 4.69 (s, 1 H), 3.89 (s, 3 H), 3.19 – 2.96 (m, 5 H), 2.64 – 2.28 (m, 4 H), 2.16 – 2.06 (td, J = 12.1 Hz, 3.6 1 H), 1.91 – 1.85 (m 1 H), 1.65 – 1.53 (m 2 H). A broad peak under the multiplet at 5.31 – 5.19 and the singlet at 4.69 was found to be equivalent to 1 proton, but could not be integrated due to shape and co-incidence with other signals. ^{13}C NMR (68MHz $CDCl_3$), δ 208.0, 144.0, 134.9, 130.7, 123.7, 122.6, 118.4, 114.7, 90.6, 70.0, 61.8, 57.6, 56.8, 51.0, 43.0, 36.0, 31.3, 31.1, 30.5, 21.8: Conversion confirmed by the ketone signal at 208.0 ppm. MS calculated mass 375.12, found *m/z* 376.1 (M+H); FTIR; cm^-, 3382, 3078, 2928, 2823, 1726, 1687, 1635, 1601.

12.7 Synthesis of 1 Chloronaloxone (33)

A three-necked flask was charged with 1-chloro-3-*O*-methylnaloxone (0.501 g, 1.3 mmol), sealed and purged with nitrogen. DCM (10 ml) was added, the solution was chilled to 0 °C and stirred until fully dissolved. Boron tribromide in DCM (1 M solution, 5 ml, 5 mmol, 3.75 eq.), was added slowly, maintaining the temperature below 5 °C, forming an orange precipitate. The solution was allowed to warm to room temperature and stirred for 24 hours. The reaction as cooled to 0 °C and quenched with water (10 ml) and adjusted to pH 9 with sodium hydroxide (1 M aqueous solution). The layers were separated and the flask washed with DCM (30 ml) and water (10 ml), then rinsed with sodium hydroxide (1 M, 5 ml). The solutions were combined and the layers separated. The aqueous layer was extracted with DCM (2 × 30 ml) and the organic

portions combined. The organic portions were washed with brine (20 ml), dried over $NaSO_4$, filtered and evaporated under vacuum to give a red solid containing some product (0.417 g). ^{1}H NMR confirmed the product contained unwanted material alongside the product. The aqueous layer was pH adjusted to pH 8.5 with hydrochloric acid (1 M) and the resultant precipitate extracted into DCM (2 × 30 ml). The red solid was dissolved in DCM (10 ml) and washed with the aqueous layer. The aqueous layer was extracted with DCM (2 × 20 ml), all organic portions were combined and dried under vacuum to give a brown solid (0.54 g). This was further purified by being dissolved in diethyl ether (25 ml) and DCM (25 ml) to form a coloured suspension. This was washed with water (25 ml), and the layers separated. The aqueous layer was washed with DCM (2 × 15 ml). The organic portions were combined and dried under vacuum to give **33** as an off white solid (0.332 g, Yield 69 %). ^{1}H NMR (270 MHz $CDCl_3$) δ 6.79 (s, 1 H), 5.88 – 5.74 (m, 1 H), 5.28 – 5.19 (m, 2 H), 4.71 (s, 1 H), 3.19 – 2.99 (m, 6 H), 2.65 – 2.31 (m, 4 H), 2.18 – 2.08 (td, J = 11.9 Hz, 3.6 1 H), 1.93 – 1.86 (m 1 H), 1.69 – 1.52 (m 2 H). A broad peak under the multiplet at 5.31 – 5.19 and the singlet at 4.70 was found to be equivalent to 1 proton, but could not be integrated due to shape and co-incidence with other signals. ^{13}C NMR (68MHz $CDCl_3$), δ 210.7, 142.4, 140.7, 135.1, 134.7, 124.1, 118.2, 117.7, 90.4, 69.8, 61.5, 57.3, 52.8, 50.9, 42.8, 35.8, 31.0, 30.2, 21.6; MS calculated mass 361.11 (^{35}Cl isotope), 363.11 (^{35}Cl isotope), found *m/z* 362.2 (^{35}Cl isotope M+H), 364.2 (^{37}Cl isotope M+H)

12.8 Synthesis of Naloxone Ketal (45):

Naloxone (15.05 g, 46.0 mmol) was added to a three necked flask, dissolved in toluene (200 ml) and stirred. Once full dissolution was achieved, *p*-toluene sulfonic acid (13.72 g, 72.1 mmol, 1.6 eq.), a Dean-Stark trap was applied and the solution was heated to reflux. Once at reflux, ethylene glycol (7.5 ml, 8.35 g, 134.49 mmol, 2.9 eq.) was

added and stirring continued. After 16 hours at reflux, the level of water collected in the Dean-Stark trap was noted to be approximately 4.5 ml. The solution was cooled, causing a waxy solid to precipitate. The organic layer was removed and evaporated under vacuum to give no product. The solid was worked up by the addition of DCM (150 ml) and aqueous sodium hydroxide (40 ml, 2 M solution). Solid remained in the flask, and was broken up and dissolved by returning the aqueous portion to the reaction flask with additional DCM (50 ml) to break up and recover the remaining solid (repeated four times). The organic layers were combined, and dried under vacuum to give a brown solid, which was recrystallised by dissolving in DCM (100 ml) and driving off the excess solvent (70 ml). The resultant beige solid was washed with DCM and dried at 50°C under vacuum overnight (11.62 g recovered). Further solid was found suspended below the aqueous portion, which was recovered by vacuum filtration, washed with DCM and dried at 50 °C under vacuum overnight (3.02 g recovered). Both solids were confirmed to be naloxone ketal by TLC and ^{1}H NMR (14.64 g total, overall yield 86 %); ^{1}H NMR (270 MHz $CDCl_3$) δ 6.70 (d, J = 8.3 Hz, 1 H), 6.56 (d, J = 8.0 Hz, 1 H), 5.86 – 5.72 (m, 1 H), 5.30 - 5.13 (m, 2 H), 4.97 (broad peak, 1 H), 4.59 (s, 1 H), 4.21 – 4.09 (m 1H), 3.98 – 3.81 (m, 3 H), 3.14 – 2.95 (m, 4 H), 2.63 – 2.50 (m, 2 H), 2.31 – 2.08 (m, 3 H),1.62 – 1.41 (m 4 H). Conversion to the ketal is confirmed by the multiplet signals in the region of 4.21 – 3.81 ppm showing complex splitting patterns consistent with that expected in their environment. The multiplet at 4.21 – 4.09 is a quartet with further splitting to the central two peaks and has been assigned as one of the ketal protons undergoing through space interactions with the proton at C^5 or one of the protons at C^7. The multiplet at 3.98 – 3.81 is assigned to the remaining 3 protons of the ketal being coincidental, as such the J coupling constant for these signals could not be calculated accurately and is not reported. ^{13}C NMR (68MHz $CDCl_3$), δ 144.3, 137.5, 135.2, 130.6, 124.1, 118.2, 117.6, 116.5, 108.7, 93.5, 69.8, 66.4, 64.6, 62.3, 57.4,

48.0, 43.3, 30.8, 28.9, 28.4, 22.4 The absence of a signal ~ 208 confirms the conversion of the ketone to a ketal. MS calculated mass 371.17, found *m/z* 372.2 (M+H); *m/*z 354.2 (M-OH) FTIR; cm^{-}; 3246 (Broad peak), 3080, 2980, 2956, 2931, 2903, 2848, 2818, 1642, 1628.

12.9 Synthesis of 3-*O*-diethylcarbamoyl-Naloxone (44)

All glassware was dried overnight in an over prior to use. Naloxone (5.00 g, 15.28 mmol) was added to a flask which was then sealed and purged with nitrogen. Dry THF was added until full dissolution of the naloxone was achieved (72 ml total) and the resultant solution stirred under nitrogen. Sodium hydride (0.74 g of a 60 % suspension in mineral oil, 0.44 g adjusted weight, 18.33 mmol, 1.2 equivalents) was added to a three necked flask which was fitted with a thermometer, an addition funnel, sealed and purged with nitrogen. The flask was cooled over an ice bath, dry THF was added drop wise (15 ml) and the resultant slurry was stirred and allowed to cool to 1 °C. The naloxone solution was transferred to the addition funnel and added to the sodium hydride slurry over 10 minutes, maintaining reaction temperature below 6 °C by rate of addition and ice bath. The resultant solution was stirred for 1 hour at room temperature. *N,N*- diethylcarbamoyl chloride (DCC) (3 ml, 3.2 g, 23.6 mmol, 1.5 eq.) was added and the resultant solution stirred overnight. After 24 hours of stirring, the solution was tested by TLC to confirm that minimal starting material was present. Aqueous potassium bicarbonate (2.8 g in 28 ml water) was added to quench, the layers separated and the organic portion evaporated under vacuum to a manageable volume. The organic liquor was added to a separation funnel, the drying flask rinsed with DCM (20 ml) which was added to the liquor and the layers separated. The aqueous layers were combined and extracted with DCM (20 ml). All organic portions were combined, washed with citric acid (10 ml, 5 % solution), washed with brine (10 ml), dried over

$NaSO_4$, filtered and evaporated under vacuum to give a yellow wax which was tested by ^{1}H NMR and found to contain the product and a large amount of DCM. The wax was triturated with diethyl ether (20 ml) and the resultant slurry stirred to give an off white solid in a yellow solution. The slurry was filtered, the solid retained and dried (50 °C under vacuum overnight) to give **44** as an off white impure solid (5.46 g, yield 84 %). Analysis of this solid found that it contained a large amount of an unwanted by product that had not been removed by trituration. The sample analysed by ^{1}H NMR found an over abundance of proton signals. Closer inspection of the spectra found that the extra signals were in the region of δ 3.5 – 3.0 which had been assigned to the carbamate, which was initially assumed to be residual diethylamine formed from unreacted DCC. Analysis by HPLC/MS found the sample to contain two main constituents which disproved this hypothesis. The main component was the target compound **44**. The second major component was found to have a *m/z* consistent with naloxone plus two diethylcarbamate groups, minus two protons. Analysis by ^{13}C NMR confirmed the presence of two sets of signals for the carbamate group, and confirmed the position to be the oxygen at C^6, confirming 3-*O*, 6-*O*-di-diethylcarbamoyl naloxone (**49**) had been formed as a by-product. As **44** was subsequently found to be unsuitable for ketalisation, no attempts were made to further purify this material or scale the reaction up. The following experimental data reflects the data generated with the impure material used to assign the structure of the by-product.

^{1}H NMR (270 MHz $CDCl_3$); δ 6.89 (dd, J = 8.3Hz, 1.6 Hz, 1 H), 6.66 (septuplet, J = 8.0 Hz, 1 H), 5.99 – 5.78 (broad peak, 1 H), 5.57 (dd, J = 5.8 Hz, 2.2 Hz, 1/3 H), 5.30 – 5.19(m, 3 H), 4.72, (s, 1/3 H), 3.52 – 2.97 (m, 10 H), 2.78 – 1.88, (broad overlapping multiplets, 5 H), 1.76 – 1.43 (m, 2 H), 1.34 – 1.08 (m, 10 H); The signals at 5.57 and 4.75 are assigned to the signal from C^5 in the two different compounds. These responses were present in a ratio of 2:1, hence the assignment of 1/3 H and 2/3H

respectively, the doublet of doublets at 5.57 is assigned to **49** and the singlet at 4.72 is assigned to **44**. Using this response ratio and the molecular weights of the two compounds, the sample was calculated to be 59.6 % **44** by weight;^{13}C NMR (68MHz $CDCl_3$), δ 207.8, 153.5, 143.9, 134.4, 134.1, 133.6, 129.6, 123.5, 123.2, 119.2, 118.5, 116.2, 90.3, 86.9, 70.4, 70.2, 62.0, 57.8, 57.5, 50.3, 46.6, 43.6, 43.3, 42.3, 42.1, 41.8, 35.9, 31.5, 31.0, 30.3, 30.1, 23.4, 23.0, 14.1, 13.3, as discussed in section 10.2.3, page 107, this overabundance of signals is assigned to the material being impure and containing **49** as a side product; MS calculated mass 426.22, two major peaks were found in the chromatogram, the first peak at 4.9 minutes had the following MS trace; *m/z* 449.3 (M+Na), *m/z* 427.3 (M+H) *m/z* 409.3 (M-OH), this is consistent with that expected for the target compound; the second major peak found at 8.8 minutes had the following MS trace; *m/z* 548.3 (M+Na), *m/z* 526.3 (M+H) *m/z* 508.3 (M-OH), as UV response factors for these compounds is unknown, a formal quantification by UPLC/UV could not be performed; FTIR; cm^{-1}; 3425 (Broad peak), 2972, 2935, 2853, 1723, 1655, 1639, 1620.

12.10 Synthesis of 3-*O*-diethylcarbamoyl Naloxone Ketal (46)

All glassware was dried overnight in an over prior to use. Naloxone ketal (5.00 g, 13.46 mmol) was added to a flask which was then sealed and purged with nitrogen. Dry THF was added until full dissolution of the naloxone ketal was achieved (150 ml total) and the resultant solution stirred under nitrogen. Sodium hydride (0.68 g of a 60 % suspension in mineral oil, 0.41 g adjusted weight, 16.95 mmol, 1.3 equivalents) was added to a three necked flask which was fitted with a thermometer, sealed and purged with nitrogen. The flask was cooled over an ice bath, dry THF (2.5 ml) was added drop wise and the resultant slurry was stirred and allowed to cool to 1 °C. The naloxone ketal solution was transferred portion wise to the sodium hydride slurry over 30

minutes, maintaining temperature below 10 °C by rate of addition and ice bath. The resultant solution was stirred for 45 minutes over ice and 45 minutes at room temperature. Diethyl carbamyl chloride (2.8 ml, 3.0 g, 22.10 mmol, 1.6 eq.) was added and the resultant solution stirred overnight. After 16 hours of stirring, the solution was tested by TLC to confirm that minimal starting material was present. Aqueous potassium bicarbonate (2.94 g in 30 ml water) was added to quench, the layers separated and the organic portion retained. The organic portion was washed with brine (2 × 20 ml), dried over $NaSO_4$, filtered and evaporated under vacuum to give a brown oil. Diethyl ether (30 ml) was added to the oil, causing a white precipitate to form. To this precipitate, further diethyl ether was added (50 ml) to ensure all residue was washed with ether. The diethyl ether was evaporated under vacuum the solid retained and dried (50 °C under vacuum overnight) to give **46** as an off white solid (5.93 g, yield 94 %) ^{1}H NMR (270 MHz $CDCl_3$) δ 6.89 (d, J = 8.3 Hz, 1 H), 6.64 (d, J = 8.3 Hz, 1 H), 5.87 – 5.72 (m, 1 H), 5.24 - 5.14 (m, 2 H), 4.61 (s, 1 H), 4.18 – 4.09 (m 1 H), 3.97 – 3.72 (m, 3 H), 3.53 - 3.22 (m, 4 H), 3.15 – 2.92 (m, 4 H), 2.65 – 2.50 (m, 2 H), 2.29 – 2.08 (m, 3 H), 1.57 – 1.43 (m, 4 H), 1.26 – 1.18 (m, 6 H);^{13}C NMR (68MHz $CDCl_3$), δ153.7, 149.2, 135.3, 132.6, 131.4, 129.1, 122.9, 118.0, 117.8, 108.8, 94.2, 69.9, 66.3, 65.0, 62.4, 57.5, 48.0, 43.4, 42.3, 42.0, 30.8, 29.2, 28.7, 23.0, 14.1, 13.4; MS calculated mass 470.24, found *m/z* 471.3 (M+H), *m/z* 453.3 (M-OH); FTIR; cm^{-1}; 3403, 2957, 2928, 2902, 2823, 1715, 1678, 1624.

12.11 Synthesis of 2 Chloro, 3-*O*-Diethylcarbamoyl Naloxone Ketal (47)

3-*O*-Diethylcarbamoyl naloxone ketal (2.00 g, 4.3 mmol), was added to a round bottomed flask, a thermometer was added, the flask sealed and purged with nitrogen. The flask was cooled to -75 °C and dry THF (35 ml) was added. To this solution,

N,N,N',N',-tetramethylethylenediamine (TMEDA, 2.1 ml, 1.63 g, 14.0 mmol, 3.3 eq.) was added, forming a red solution which was stirred for 30 minutes. To this solution, *tert*-butyllithium (*t*-BuLi, 1.7 M in pentane, 8 ml, 13.6 mmol, 3.2 eq.) was added drop wise. The temperature of the solution was maintained below -65 °C during the *t*BuLi addition by the rate of addition and a yellow solution was formed, which was allowed to stir for 1.5 hours. The reaction was quenched with a solution of hexachloroethane (3.56 g, 3.5 mmol), dissolved in dry THF (10 ml), added drop wise maintaining a temperature below -50 °C. The remaining solution was allowed to slowly warm to room temperature and stir over night, forming a red solution. Aqueous ammonium chloride (7.50 g in 50 ml), and DCM (20 ml) were added and the layers separated. The aqueous layer was extracted into DCM (2 × 50 ml), the organic portions combined, washed with brine (50 ml), dried over $MgSO_4$ and evaporated under vacuum to give a thick brown oil. The oil was tested by TLC and ^{1}H NMR to confirm it contained two components, the minor constituent was the starting material and the major component showed deprotonation of the phenolic ring by NMR. The mixture was separated by column chromatography with an eluent system of ethyl acetate: hexane 4:6. The collected fractions were tested by TLC and those identified as containing the higher running spot were combined and evaporated under vacuum to give as a **47** white solid (1.3 g , 59.6 % yield); ^{1}H NMR (270 MHz $CDCl_3$) δ 6.75 (1 H), 5.82 – 5.70 (m, 1 H), 5.23 - 5.14 (m, 2 H), 4.63 (s, 1 H), 4.16 – 4.08 (m 1 H), 3.96 – 3.73 (m, 3 H), 3.56 - 3.26 (m, 4 H), 3.17 – 2.90 (m, 4 H), 2.62 – 2.50 (m, 2 H), 2.29 – 2.04 (m, 4 H), 1.61 – 1.46 (m, 4 H), 1.30 – 1.16 (m, 6 H), conversion to the target compound is partially confirmed by the loss of one aromatic proton confirming deprotonation in the correct place; ^{13}C NMR (68MHz $CDCl_3$), δ152.6, 151.0, 135.2, 130.2, 129.7, 129.4, 127.6, 119.0, 118.0, 108.6, 94.7, 69.7, 66.5, 65.0, 62.2, 57.5, 47.8, 43.3, 42.5, 42.1, 30.8, 29.2, 28.7, 23.0, 14.1, 13.4; MS calculated mass 504.20 (^{35}Cl isotope), 506.20 (^{35}Cl isotope), found *m/z* 505.2 (^{35}Cl

isotope M+H), *m/z* 507.2 (^{37}Cl isotope M+H), conversion to the target compound confirmed by M+H of the expected value in the ratio expected for chlorine isomers; FTIR; cm^{-1}; 2969, 2933, 2827, 1726, 1620, 1471, 1411, 1419.

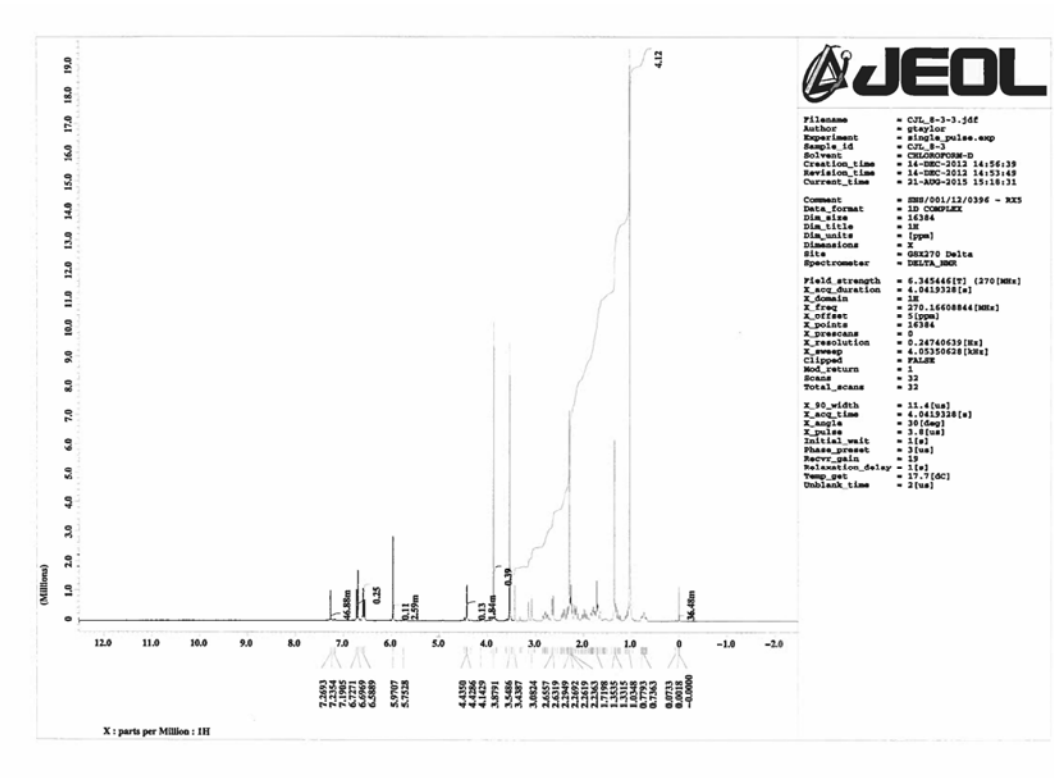
12.12 Synthesis of 2 – Chloronaloxone (34)

2-Chloro,3-*O*-diethylcarbamoyl naloxone ketal (0.75 g, 1.48 mmol) was added to a three necked flask and slurried in acetone (10 ml). Hydrochloric acid (30 ml, 3 M, 90 mmol, 60.8 eq.) was added and full dissolution achieved. The solution was stirred and heated to 90 °C under a distillation head. The temperature was gradually increased to 115 °C and a total of 15 ml of solvent was distilled. The solution was refluxed overnight to form a yellow solution, testing by TLC confirmed that no starting material remained. The reaction was worked up by the addition of sodium hydroxide (4 M aqueous solution) to adjust to pH 8. A white precipitate was formed, to which DC (30 mL) was added. The layers were separated and the aqueous extracted with DCM (2 × 30 ml). The organic layers were combined, washed with water (15 mL), washed with brine (15 mL), dried over $MgSO_4$, filtered and evaporated under vacuum to give **34** as an off white solid (0.52 g, yield 97 %). ^{1}H NMR (270 MHz $CDCl_3$) δ 6.73 (s, 1 H), 5.88 – 5.74 (m, 1 H), 5.63 – 5.17 (m, broad peak, 5 H), 4.72 (s, 1 H), 3.22 – 2.95 (m, 5 H), 2.64 – 2.30 (m, 3 H), 2.15 (td, *J* = 12.1 Hz, 3.9 Hz, 1 H), 1.93 – 1.85 (m, 1 H), 1.68 – 1.54 (m, 2 H), 1.35 – 1.20 (m, 1 H);^{13}C NMR (68MHz $CDCl_3$), δ209.0, 136.3, 135.0, 128.0, 123.8, 122.1, 120.0, 118.3, 90.9, 70.2, 62.0, 57.6, 50.9, 43.2, 42.3, 36.1, 31.3, 30.4, 22.5; MS calculated mass 361.11 (^{35}Cl isotope), 363.11 (^{37}Cl isotope), found *m/z* 362.2 (^{35}Cl isotope M+H), *m/z* 364.2 (^{37}Cl isotope M+H), *m/z* 344.2 (^{35}Cl isotope M-OH), *m/z* 346.2 (^{37}Cl isotope M-OH); FTIR; cm^{-1}; 3446 (broad peak), 3086 (broad peak), 3010, 2983, 2957, 2942, 2924, 2909, 2829, 1732, 1646, 1615.

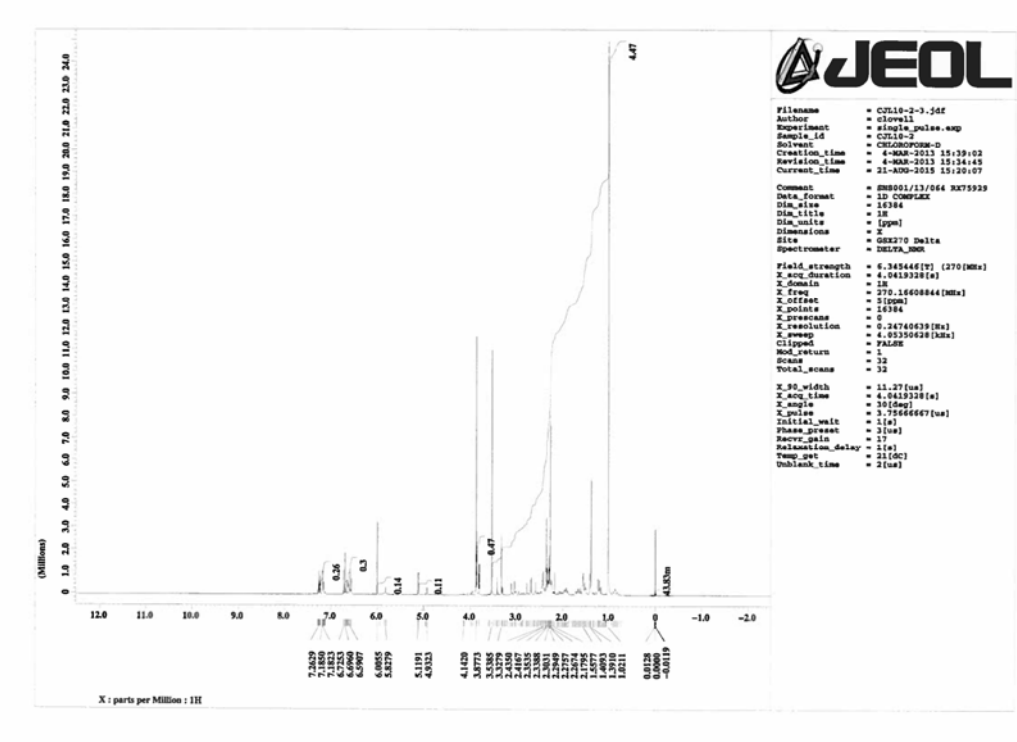
Appendices

Appendix A – Supplementary Data for Chapter 1

A 1: Example ^{1}H NMR Spectra for 16 (*Tert B*utyl-Dihydrothevinol)

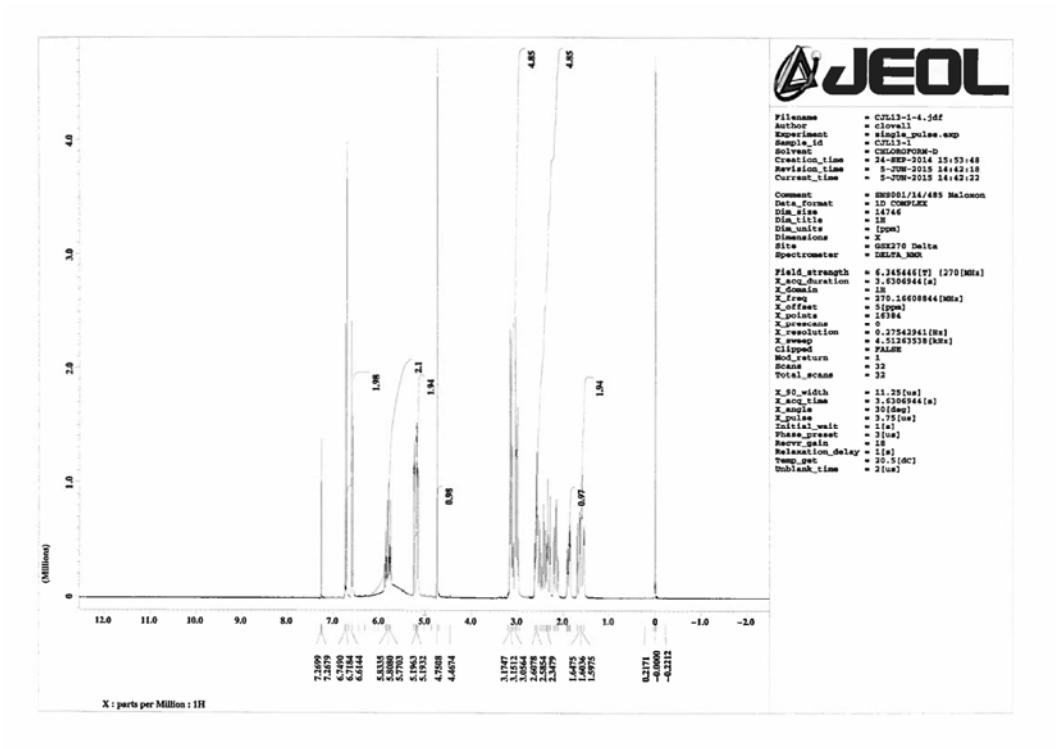


A 2: Example ^{1}H NMR Spectra for 23 (7β-19-*tert*-butyl-dihydrothevinol)

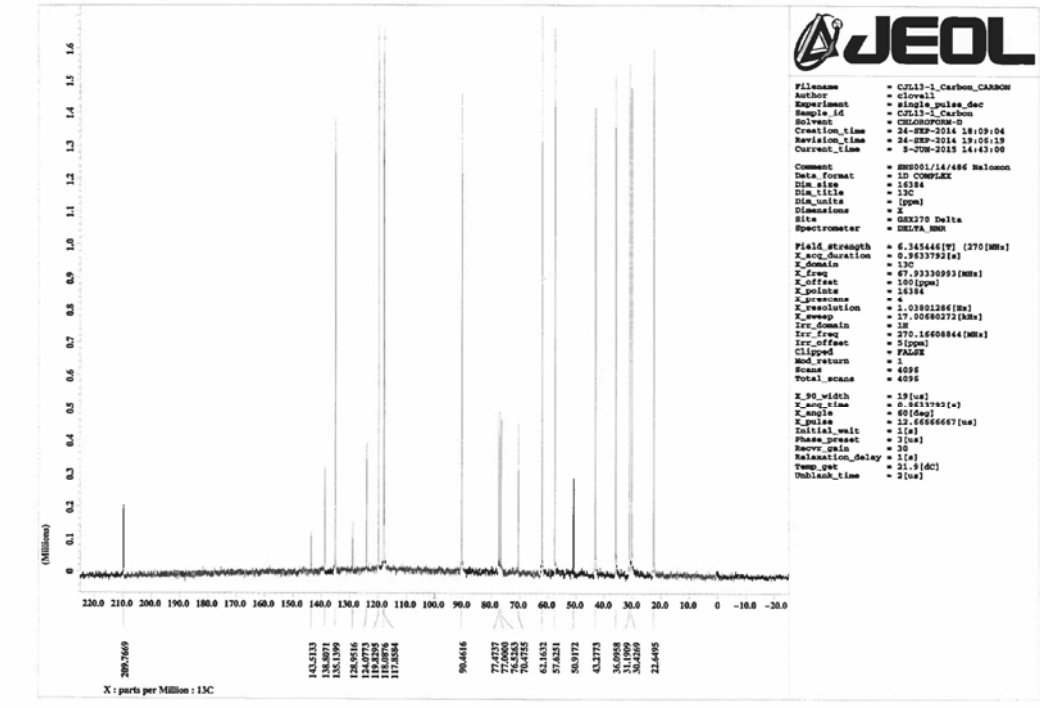


Appendix B – Supplementary Data for Chapter 2

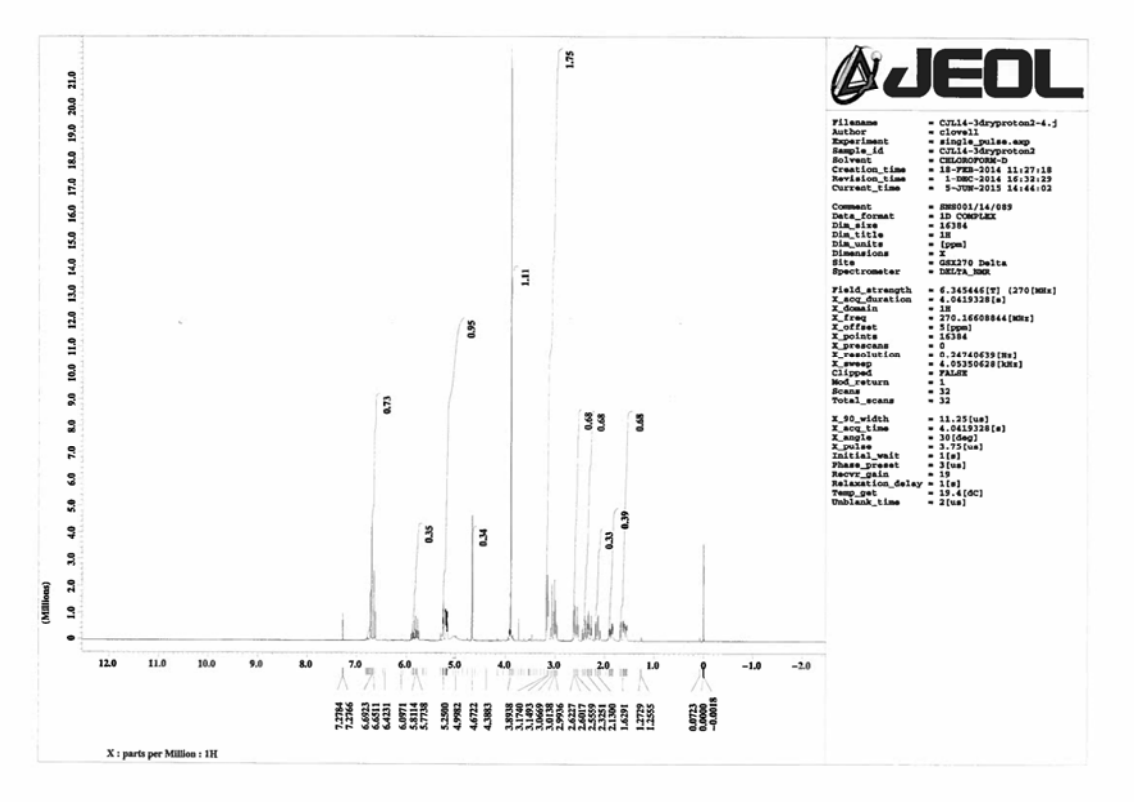
B 1: ^{1}H NMR Spectra for Naloxone



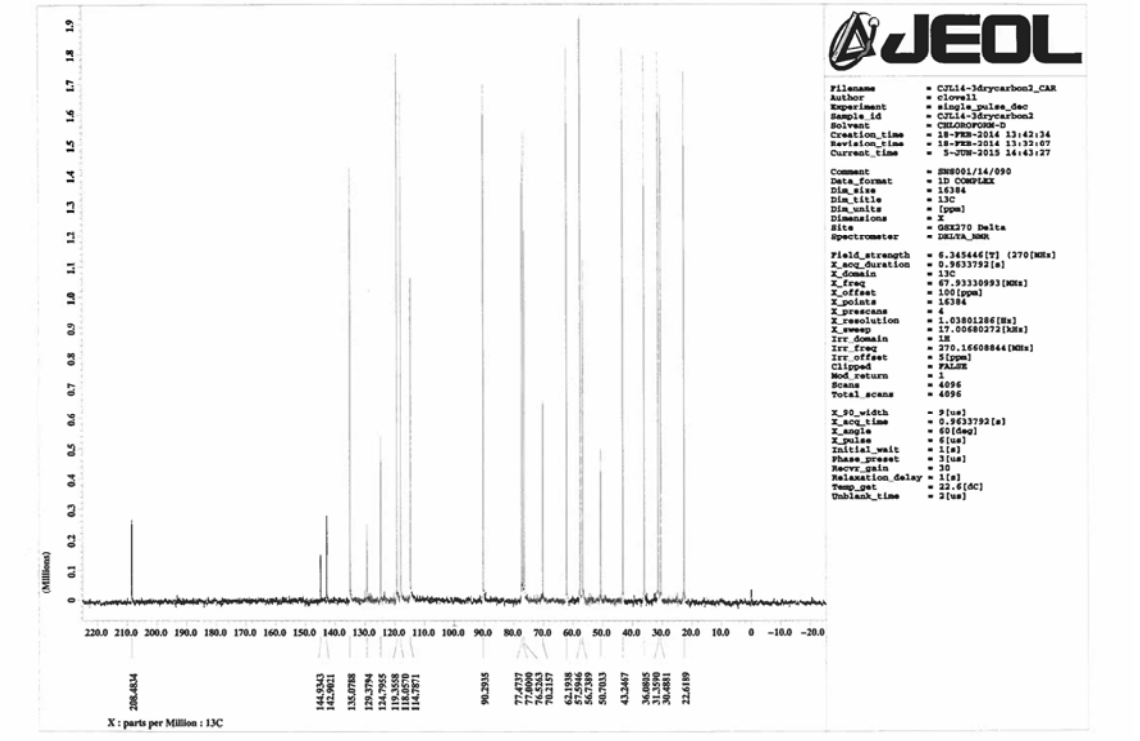
B 2: ^{13}C NMR Spectra for Naloxone



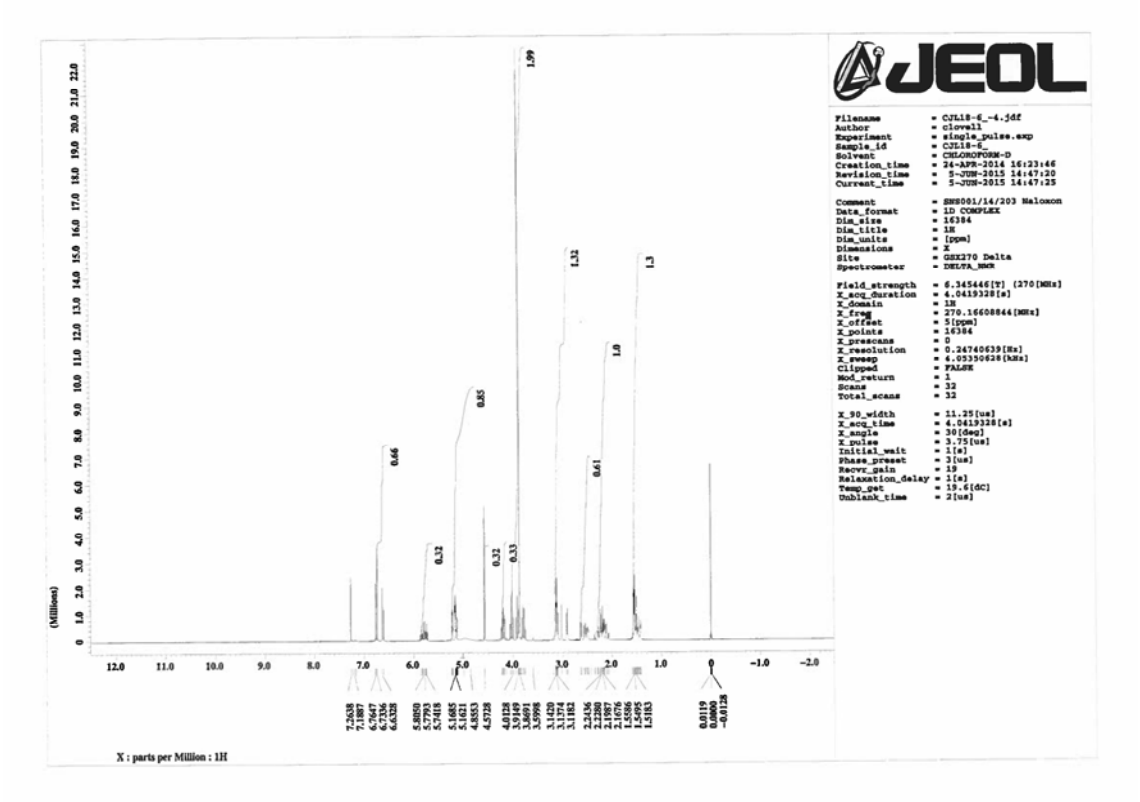
B 3: ^{1}H NMR Spectra for 3-*O*-Methylnaloxone



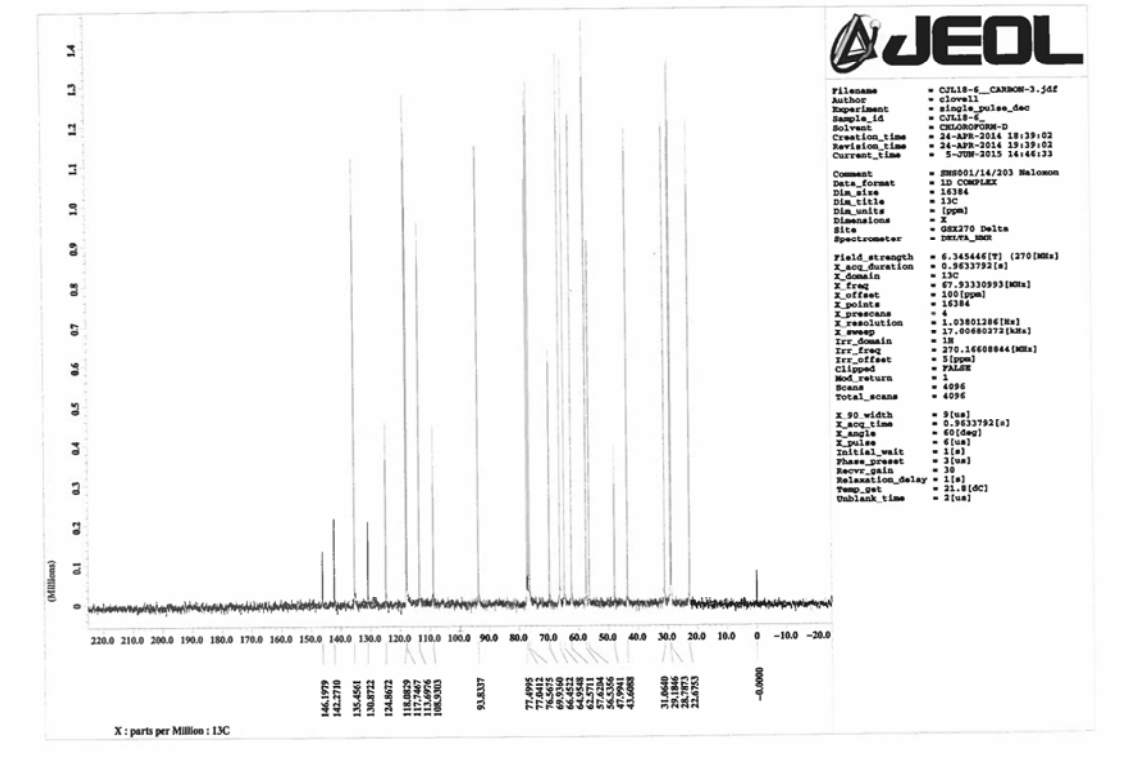
B 4: ^{13}C NMR Spectra for 3-*O*-Methylnaloxone



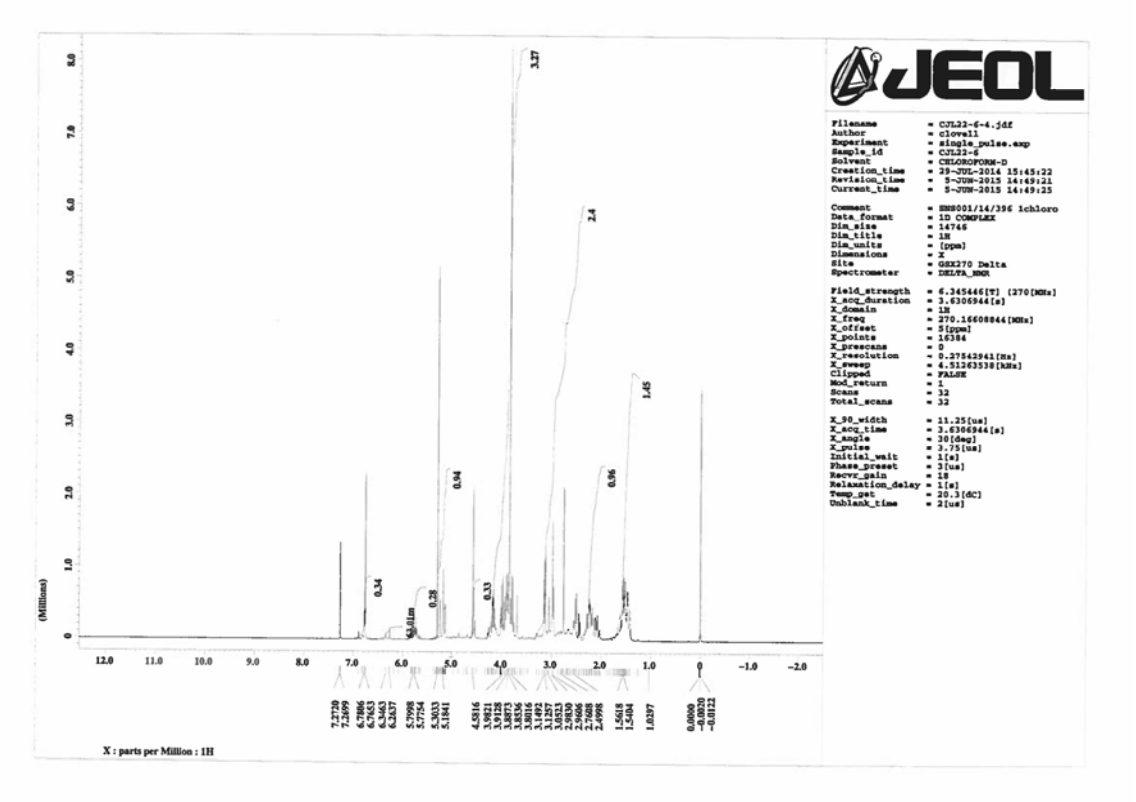
B 5: ^{1}H NMR Spectra for 3-*O*-Methylnaloxone Ketal



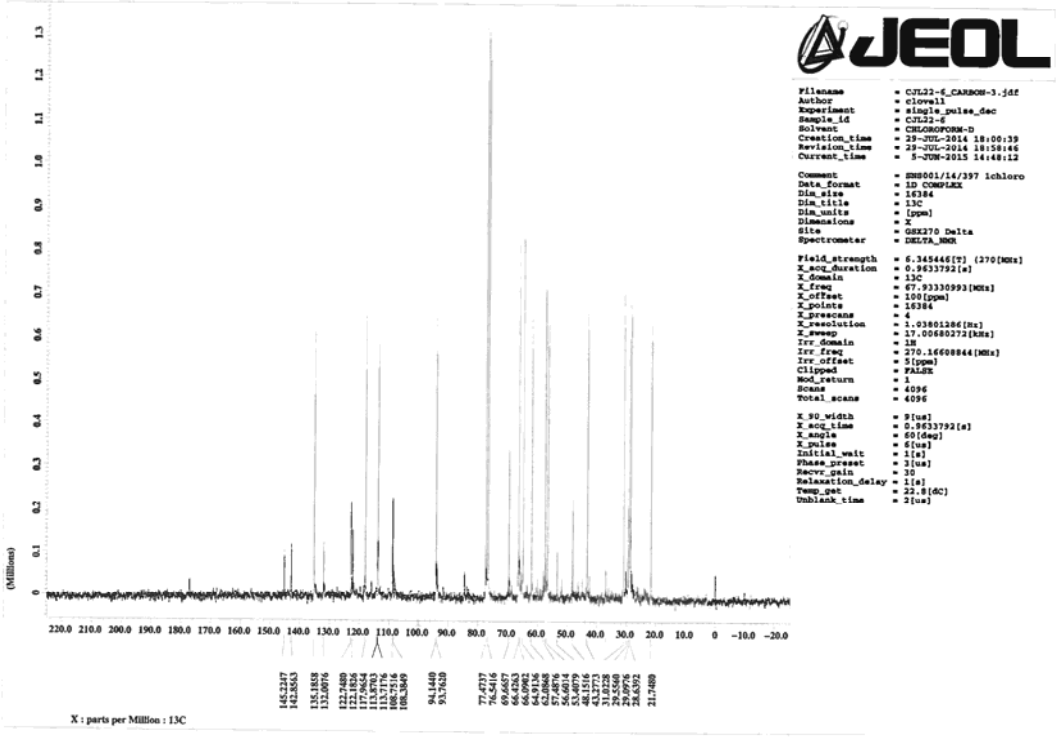
B 6: ^{13}C NMR Spectra for 3-*O*-Methylnaloxone Ketal



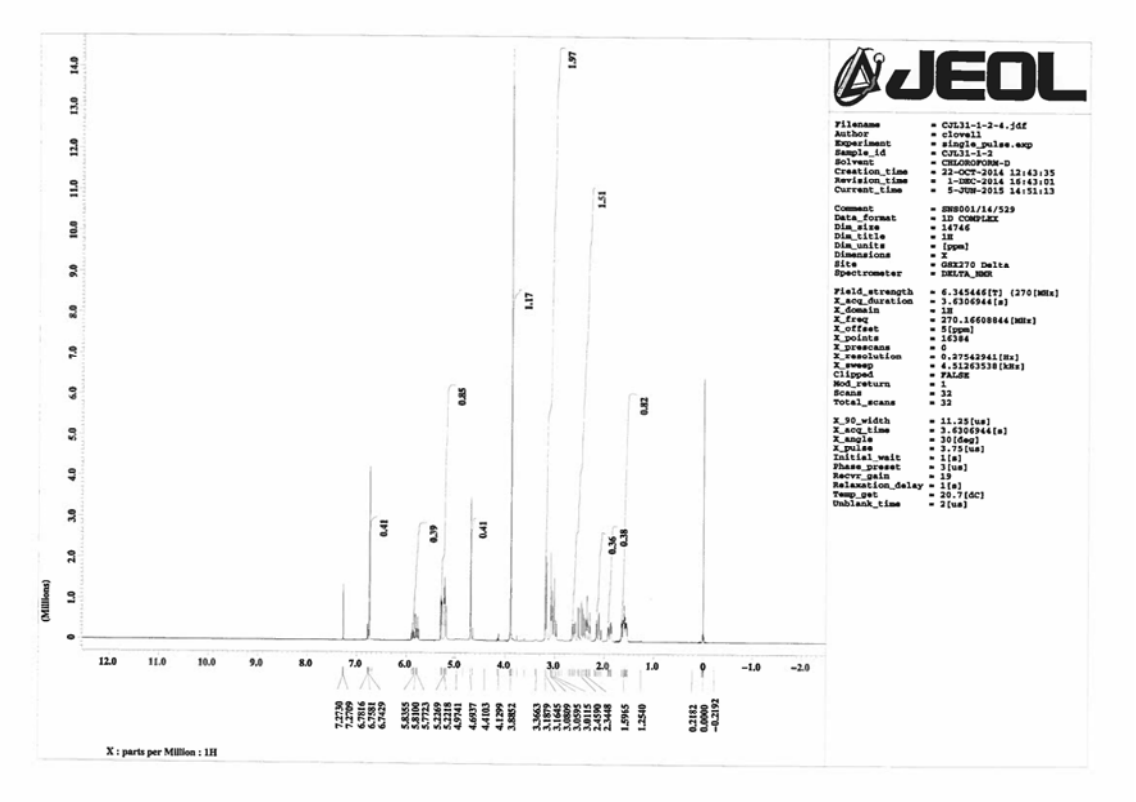
B 7: ^{1}H NMR Spectra for 1-Chloro, 3-*O*-Methylnaloxone Ketal



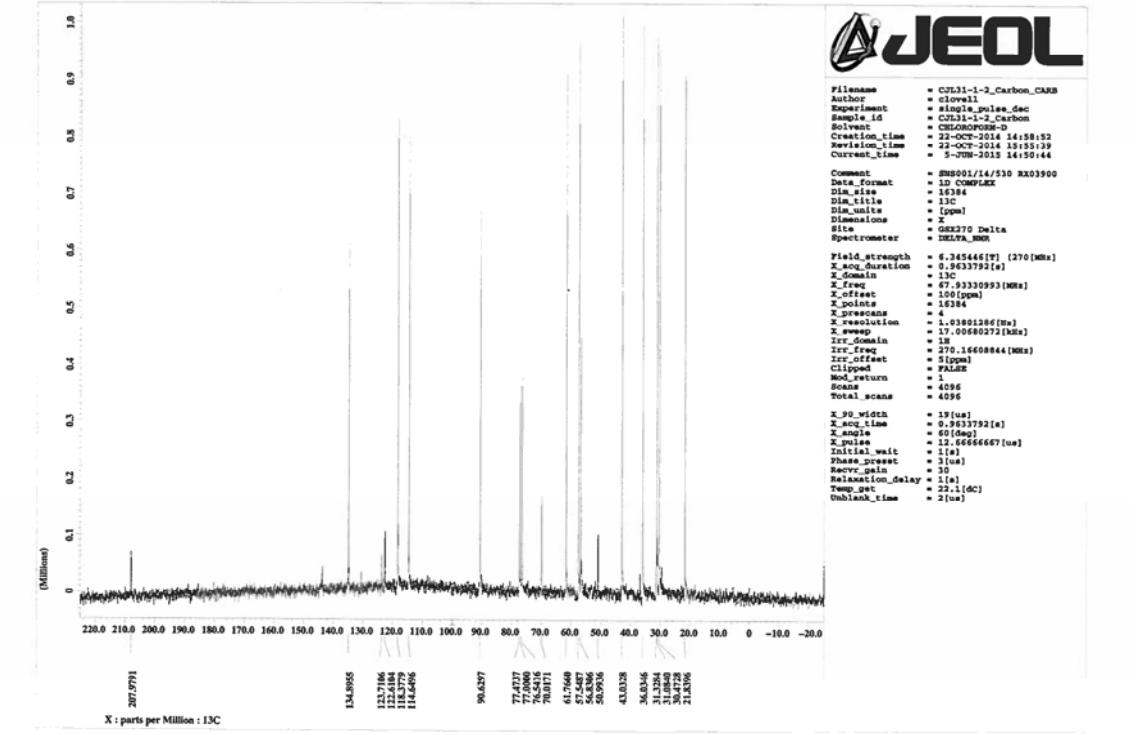
B 8: ^{13}C NMR Spectra for 1-Chloro, 3-*O*-Methylnaloxone Ketal



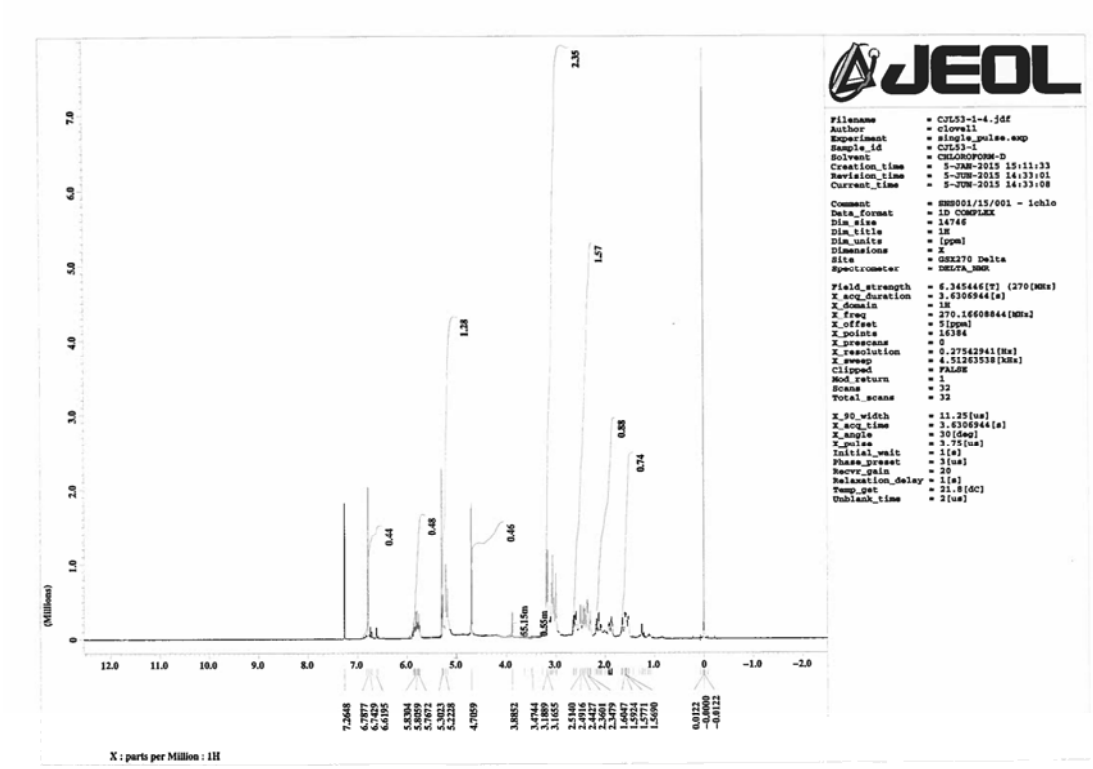
B 9: ^{1}H NMR Spectra for 1-Chloro, 3-*O*-Methylnaloxone



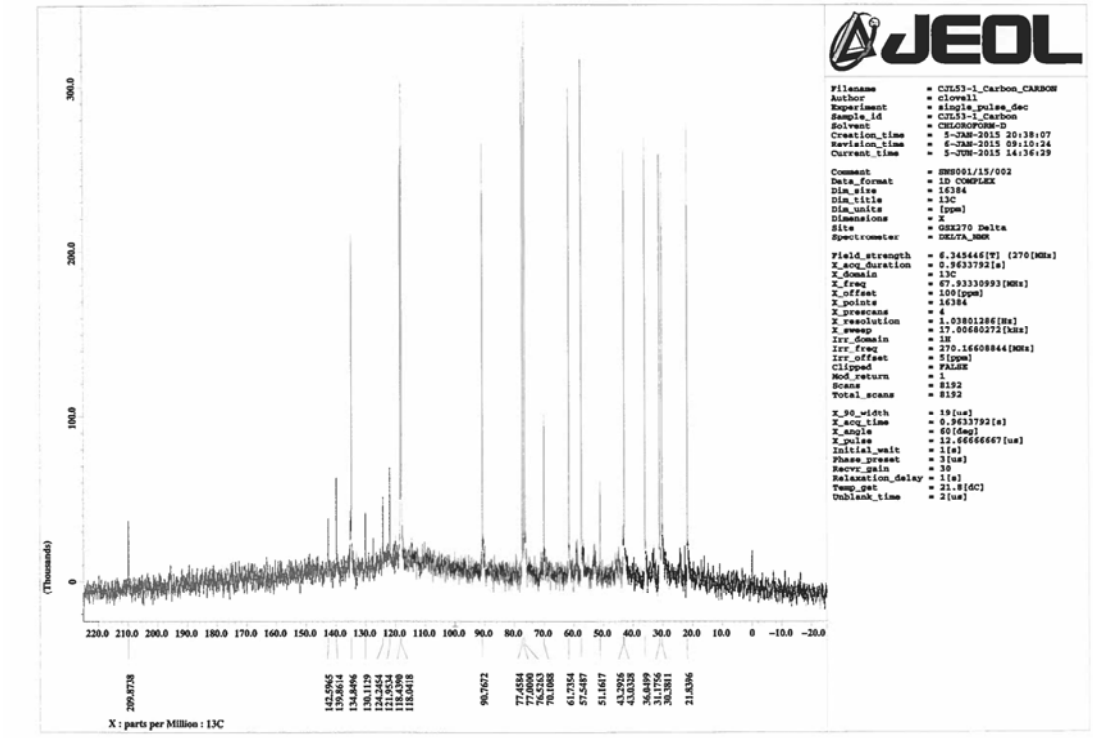
B 10: ^{13}C NMR Spectra for 1-Chloro, 3-*O*-Methylnaloxone



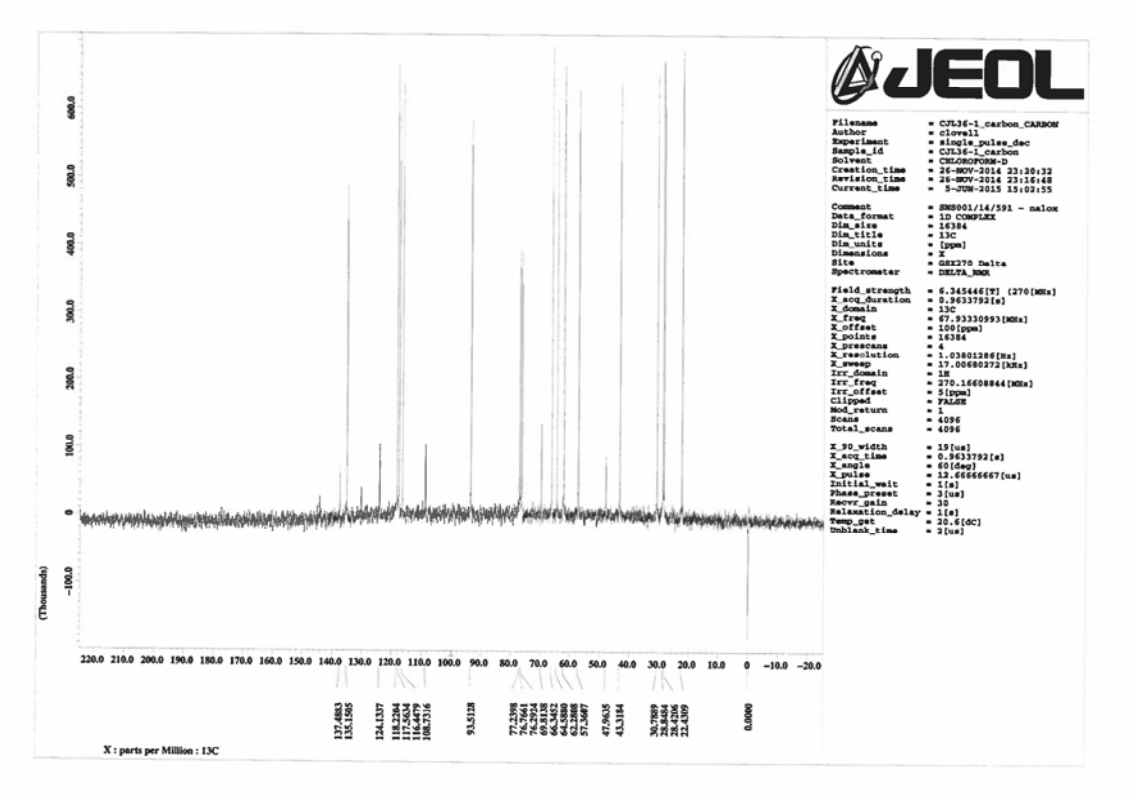
B 11: ^{1}H NMR Spectra for 1-Chloro Naloxone



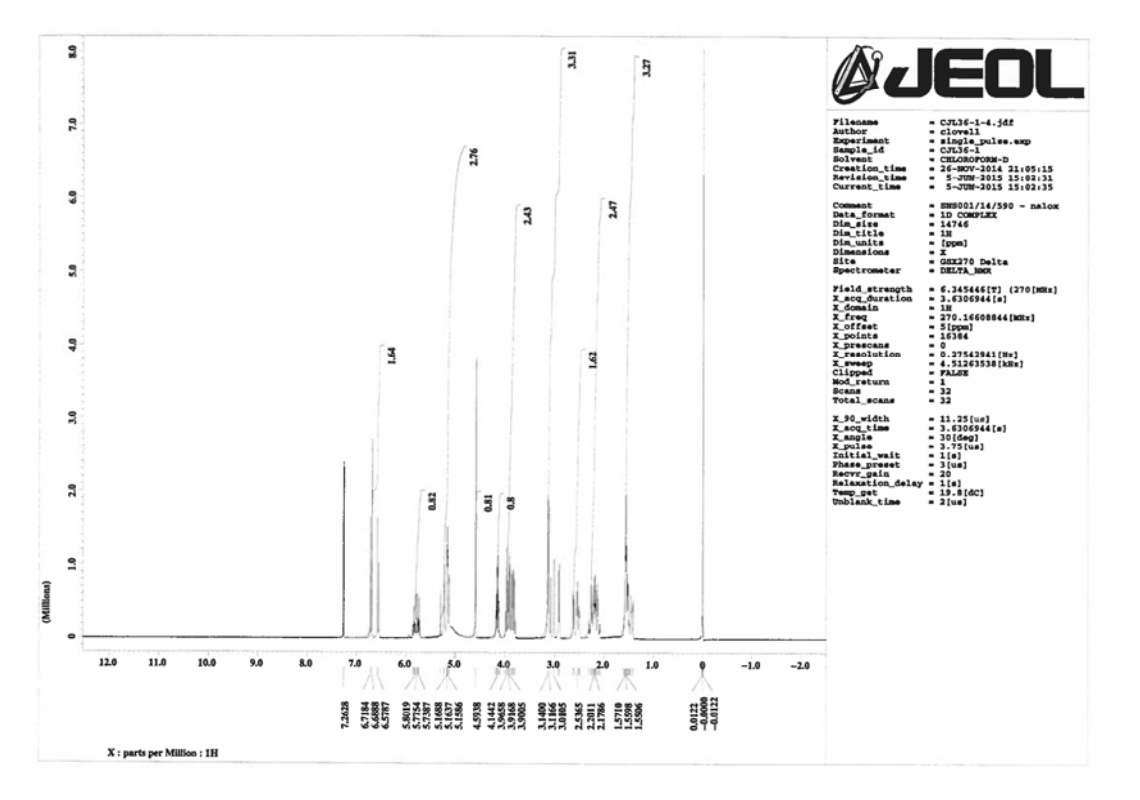
B 12: ^{13}C NMR Spectra for 1-Chloro Naloxone



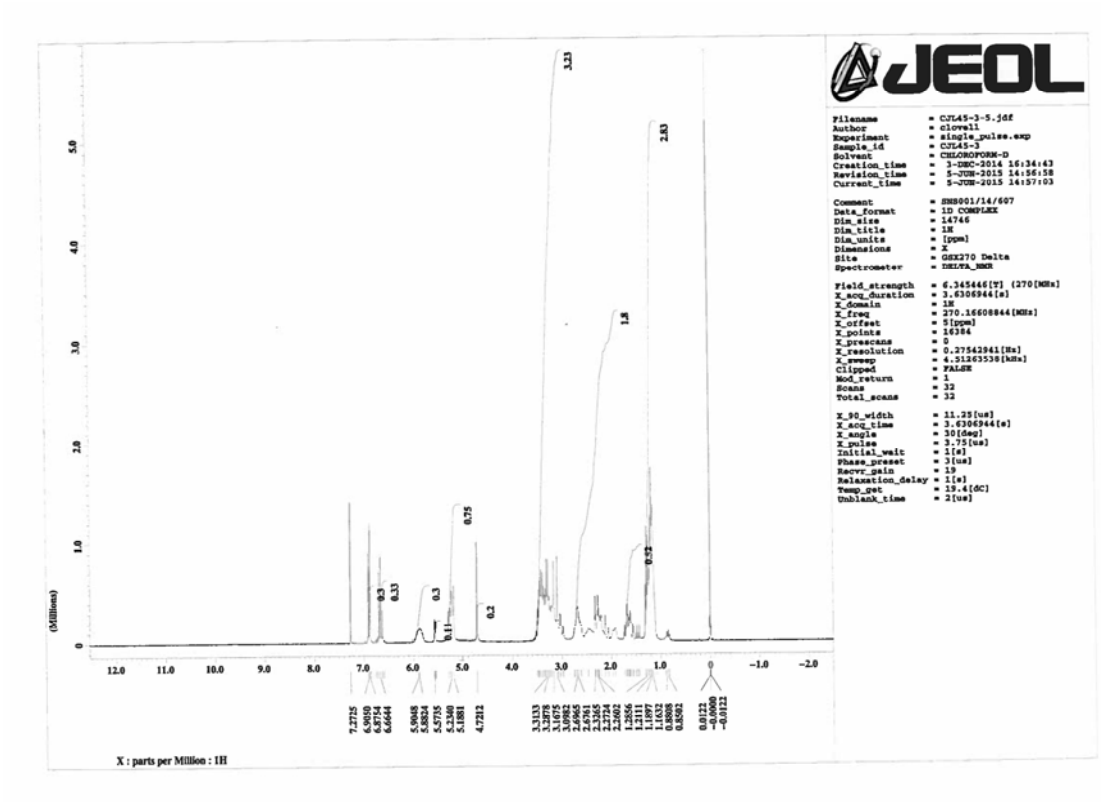
B 13: ^{1}H NMR Spectra for Naloxone Ketal



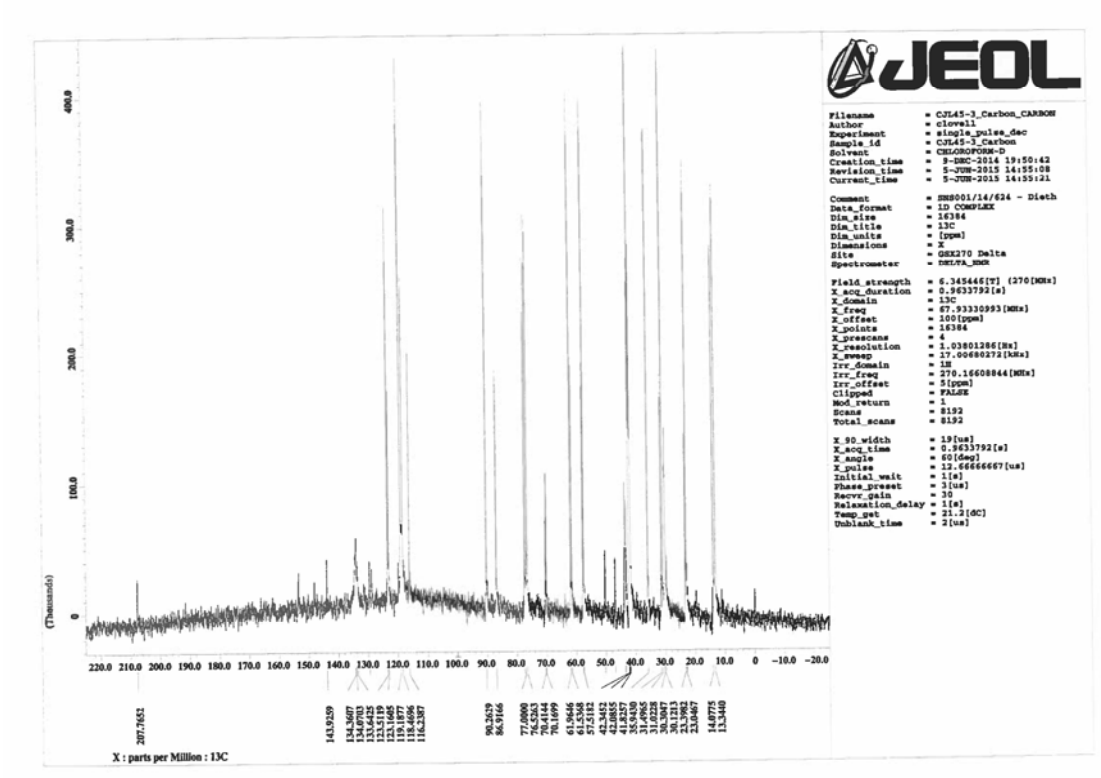
B 14: ^{13}C NMR Spectra for Naloxone Ketal



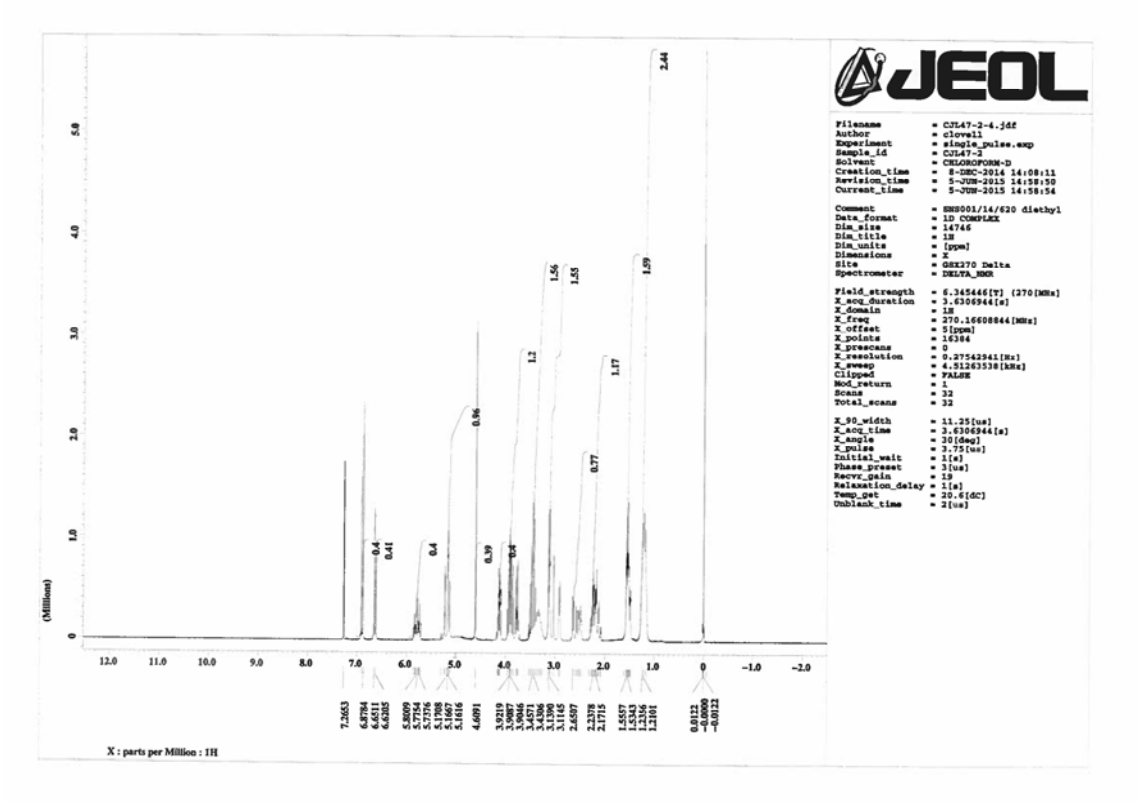
B 15: ^{1}H NMR Spectra for Crude 3-*O*-Diethylcarbamoyl Naloxone Containing 3-*O*-, 6-*O*- Di-Diethylcarbamoyl Naloxone



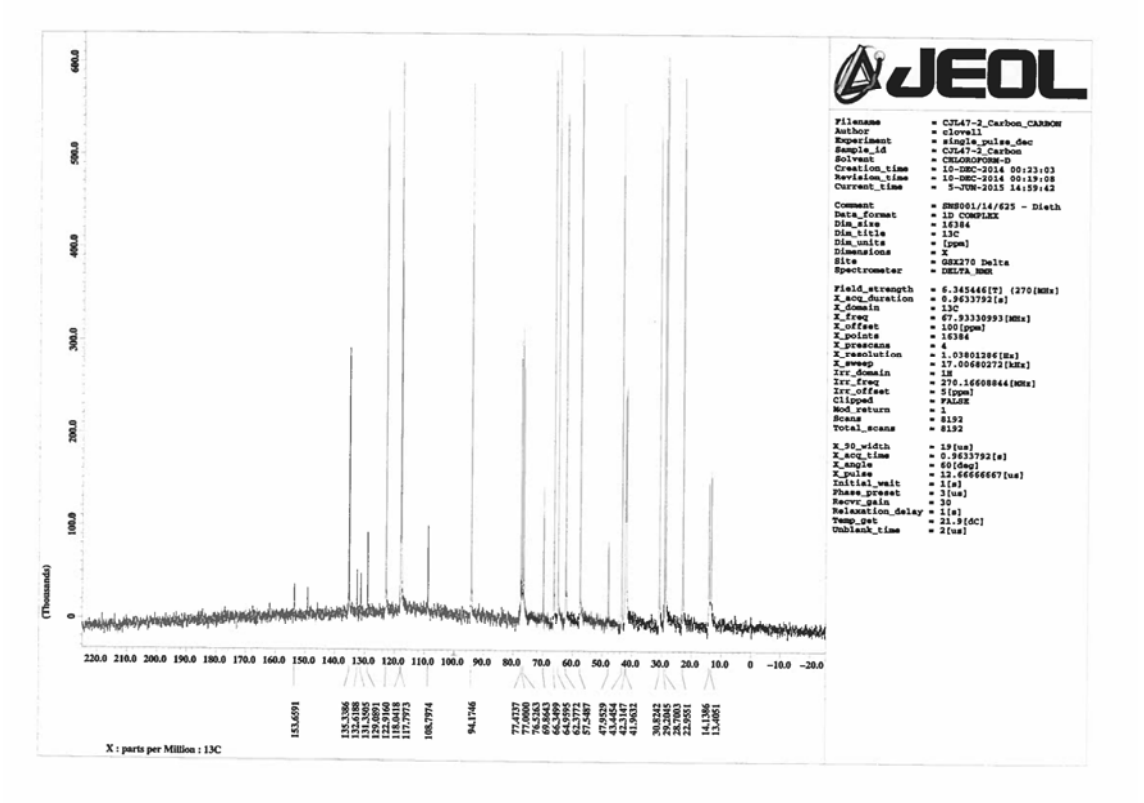
B 16: ^{13}C NMR Spectra for Crude 3-*O*-Diethylcarbamoyl Naloxone Containing 3-*O*-, 6-*O*- Di-Diethylcarbamoyl Naloxone



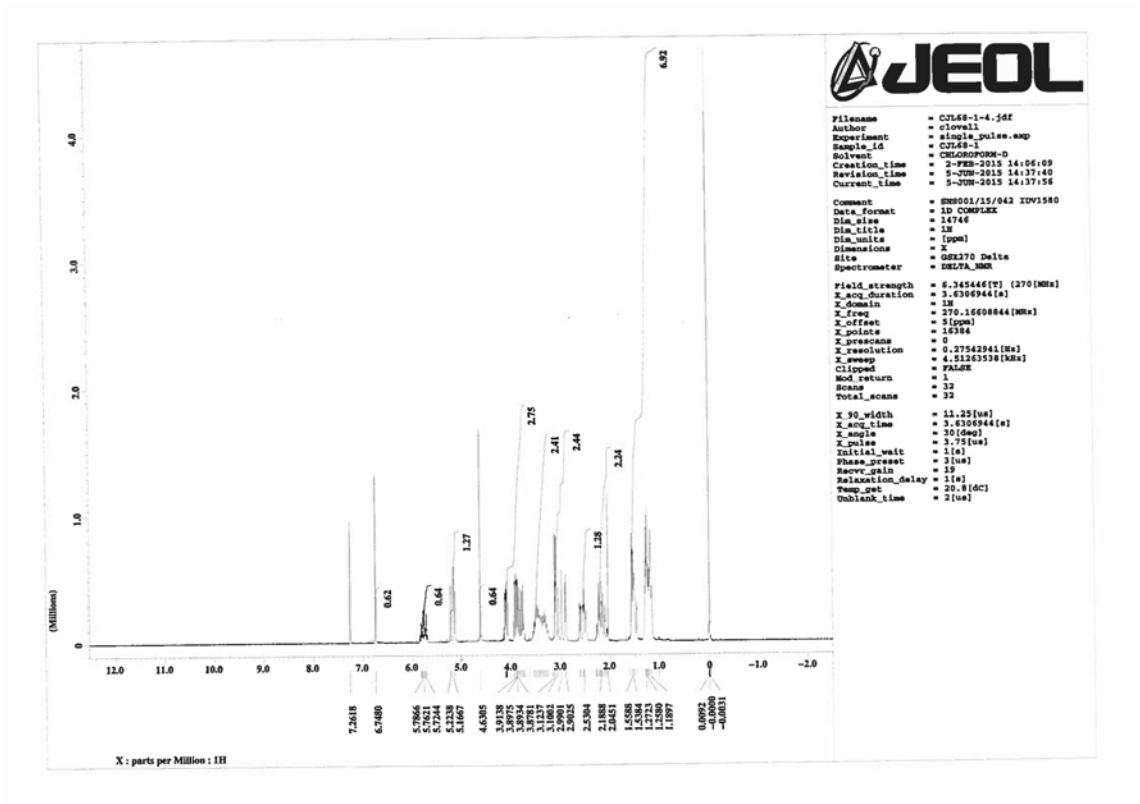
B 17: ^{1}H NMR Spectra for 3-*O*-Diethylcarbamoyl Naloxone Ketal



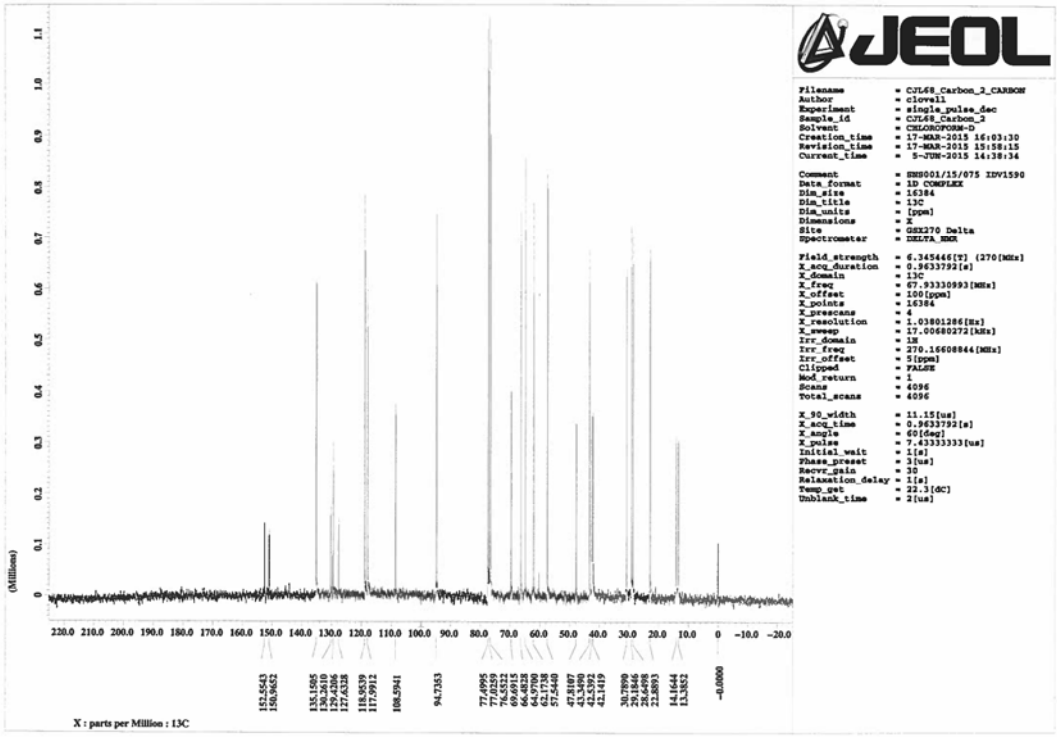
B 18: ^{13}C NMR Spectra for 3-*O*-Diethylcarbamoyl Naloxone Ketal



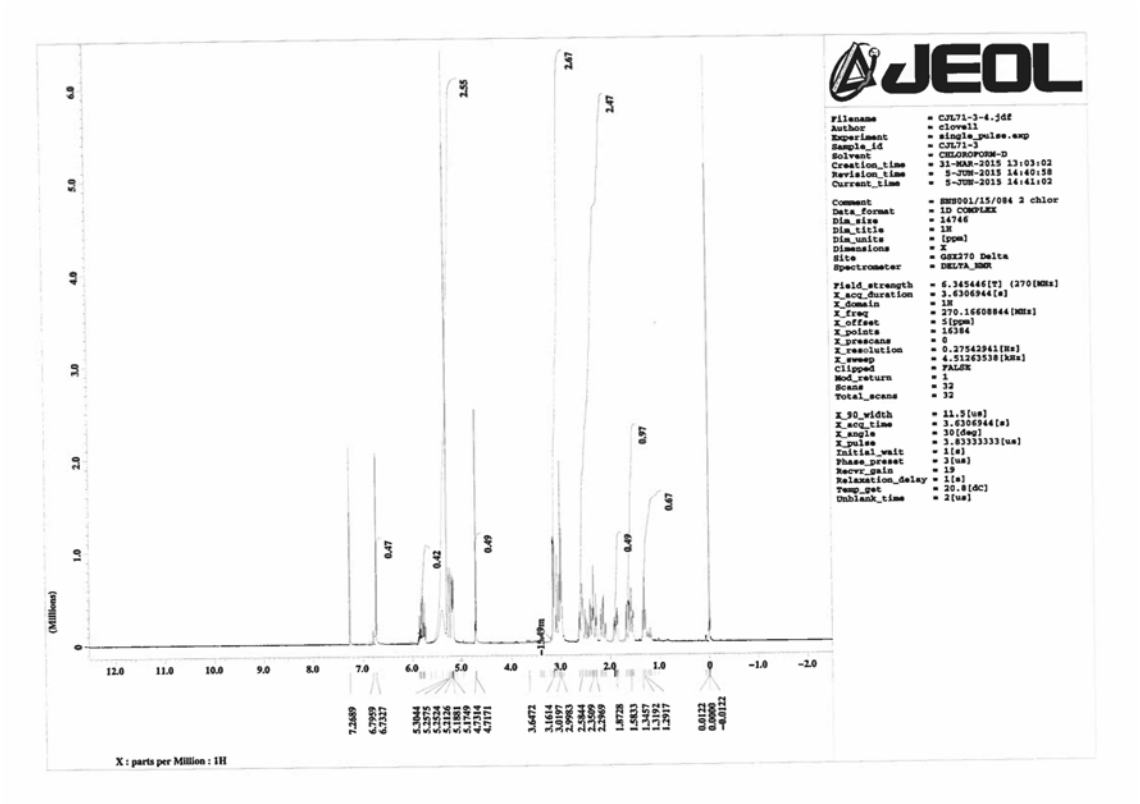
B 19: ^{1}H NMR Spectra for 2-Chloro, 3-*O*-Diethylcarbamoyl Naloxone Ketal



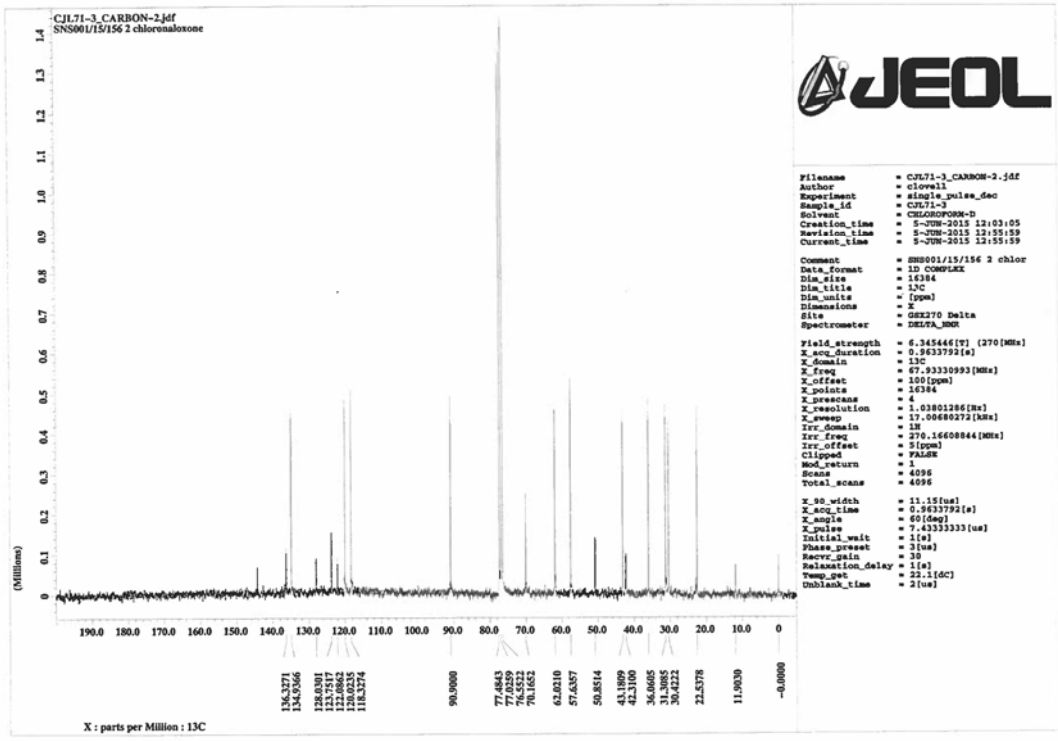
B 20: ^{13}C NMR Spectra for 2-Chloro, 3-*O*-Diethylcarbamoyl Naloxone Ketal



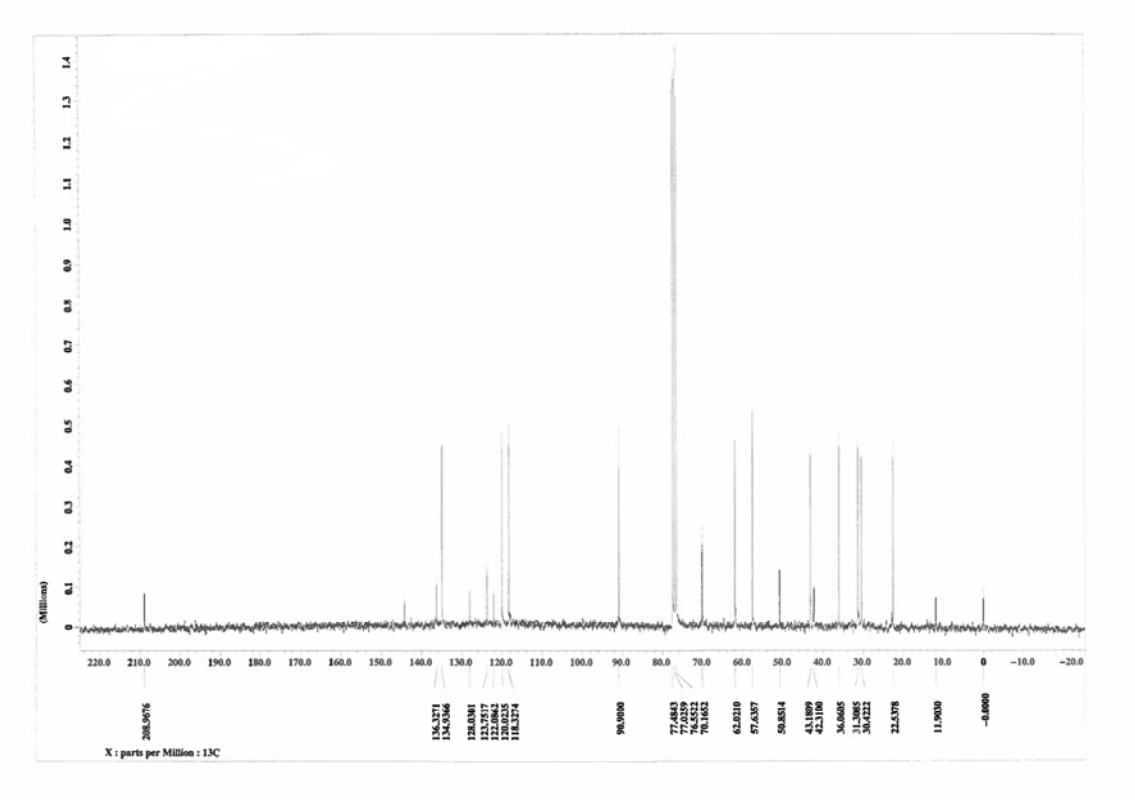
B 21: ^{1}H NMR Spectra for 2-Chloro Naloxone



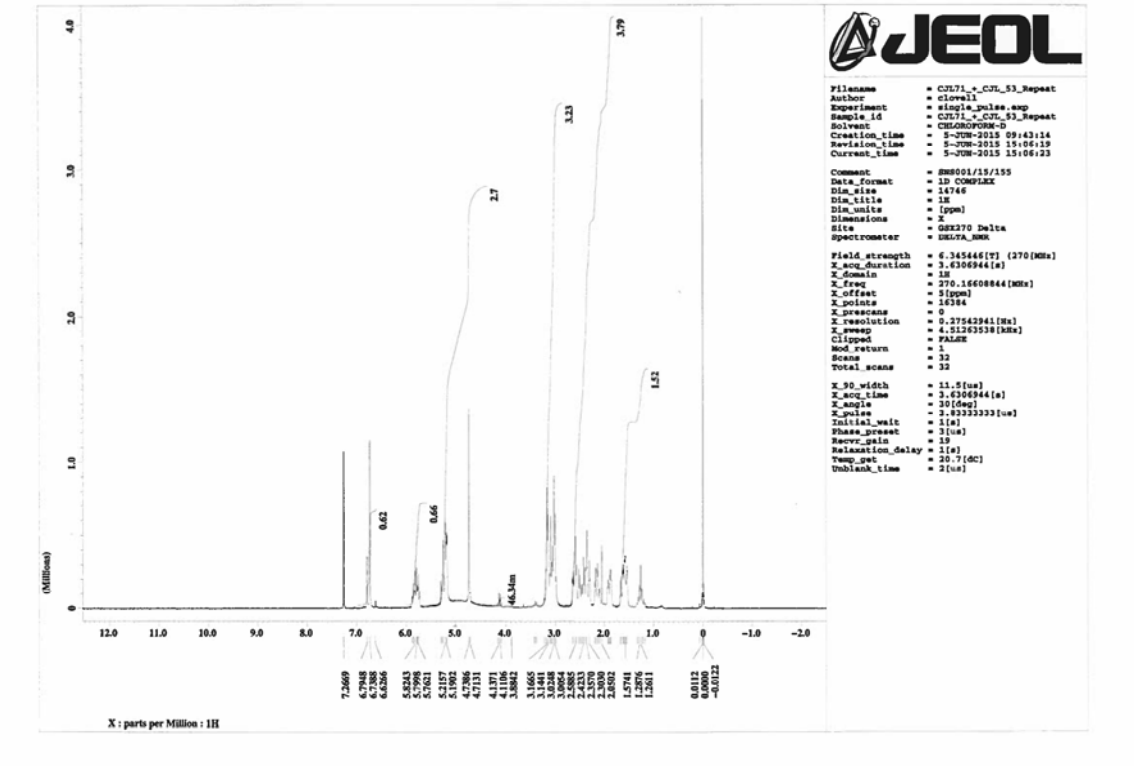
B 22: ^{13}C NMR Spectra for 2-Chloro Naloxone



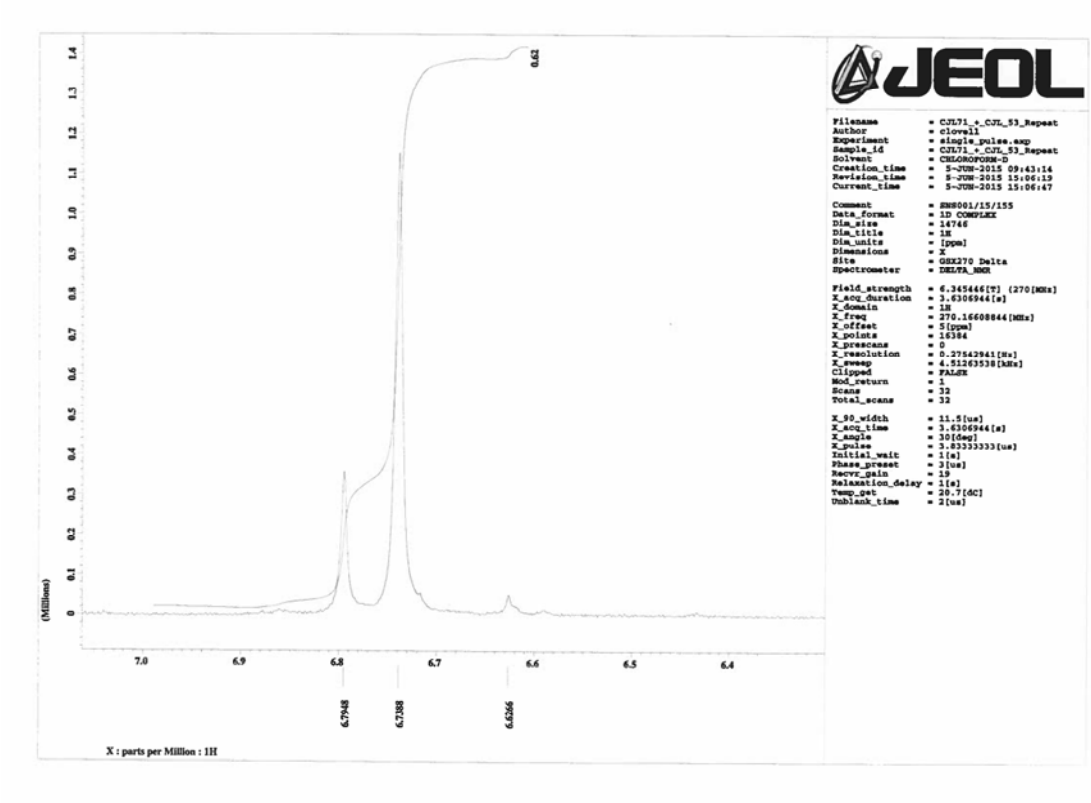
B 23: Expanded ^{13}C NMR Spectra for 2-Chloro Naloxone for C^6 Signal at $\delta \approx 210$



B 24: ^1H NMR Spectra for a Mixture of 1-Chloro Naloxone and 2-Chloro Naloxone (1:3 ratio)



B 25: Magnified View of the Aromatic Region of the [1]NMR Spectra for a Mixture of 1-Chloro Naloxone and 2-Chloro Naloxone (1:3 ratio).



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