Bachelor's thesis

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Potential natural antibiotic: Application of and comparison of synthetic routes to (-)-Hygromycin A

Bachelor's thesis in Bachelor i Kjemi
Supervisor: Odd-Reidar Gautun
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Kunnskap for en bedre verden

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Potential natural antibiotic: Application of and comparison of synthetic routes to (-)-Hygromycin A

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Abstract

The overuse of antibiotics has given a rise to an increasing antimicrobial resistance. This is one of the biggest threats against human health, and could possibly lead to high mortality rates if not dealt with. The overuse of antibiotics is also giving increasing health issues in the form of chronic diseases. The need for new narrow-spectrum antibiotics, that is kinder to the gut microbiome is getting bigger. Hygromycin A that was rediscovered in a selective screening against *Borrelia burgdorferi* that causes Lyme disease has potential to be one of these antibiotics. This thesis has evaluated the different published synthetic pathways to the compound, to find the best possible total synthesis.

Sammendrag

Overdrevent bruk av antibiotika har gitt en kraftig økning i antimikrobiell resistens. Dette er en av de største trusslene innen helse, og kan gi en kraftig økning i dødelighet hvis det ikke tas på alvor. Overforbruket har også ført til en økning av kroniske sykdommer. Behovet for nye smalspektede antibiotika som er snillere mot tarmmikrobiomen øker. Hygromycin A ble gjennoppdaget i en selektiv testing mot *Borrelia burgdorferi* som fører til borreliose, og har potensiale til å være en av disse nye antibiotikaene. I denne oppgaven har forskjellige publiserte synteseveier blitt sammenlignet, for å finne best mulig total syntese av forbindelsen.

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Abbreviation

B. *burgdorferi*	Borrelia burgdorferi
PTLDS	Post treatment Lyme disease
DNA	Deoxyribonucleic acid
ATP	Adenosine triphosphate
PTC	Peptidyl transferase center
THP	Tetrahydropropanyl
Boc_2O	Di-tert-butyl-dicarbonate
NMO	N-Methylmorpholine-N-oxide
DMF	Dimethylformamide
TFA	Trifluoroacetic acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
THF	Tetrahydrofuran
TMSOTf	Trimethyl trifluoromethanesulfonate
NBS	*N*-bromosuccinimine
CDI	1,1-carbonyldiimadazole
TMEDA	Tetramethylethylenediamine
TIPS	Triisopropylsilyl
DMSO	Dimethylsulfoxide
DMP	Dess-Martin periodane
DIBALH	Diisobutylaluminium hydride
MIC	Minimun inhibitory concentration

1 Introduction and objective

1.1 Brief history of antibiotics

The discovery of antibiotics is one of the biggest medical breakthrough in modern medicine, and has since its discovery made big improvements within health [1]. Before the discovery diseases like sepsis and tuberculosis was the cause of many deaths [2]. The introduction of antibiotics has lowered the mortality rate of diseases caused by bacteria and, extended the general life expectancy for humans. The years 1940-1960 is described as the golden age of antibiotic discovery, where new antibiotics where discovered frequently, it started with the discovery of penicillin. Most of the new compounds was natural products from microorganisms, many of the antibiotics in use today comes from natural products [3]. The great characteristics of antibiotics as a drug lead to an overconsumption that has resulted in a rise in antibiotic resistance. The importance of finding new, and improving already existing antibiotics is increasing with the increase of resistant bacteria. If not, diseases treated easily today will return as a health threat, and mortality will possibly increase [2]. The rise in antibacterial resistance has gotten the attention back to previously forgotten antibiotics in a hope that they can contribute to solving the problem.

1.2 (-)-Hygromycin A, a potential antibiotic

Hygromycin A (**1**, see figure 1) is a natural product that is produced by *Streptomyces hygroscopius* [4]. It was discovered in 1953 from a sample of soil, and has earlier been evaluated as a potential treatment for swine dysentery because of its efficiency against *Brachyspira*, the pathogen that causes the disease. But its low activity against other Gram positive and negative bacteria made the economical part of production not worth pursuing, as an antibiotic just used in animal health[5]. Recent discoveries shows that it is effective against lyme disease, this has sparked new interest in the compound. Flightpath bioscience, a biotech company are conducting clinical trials of Hygromycin A. The trials are scheduled to move into phase one in the spring of 2024 [6, 7].

The compound is built up of three parts, a furanose sugar **2**, a cinnamic acid **3** and an aminocyclitol **4** (see figure 1)[8, 9]. They can each be synthesised on their own, and then be coupled together. Since its discovery there has only been reported two total synthesis of **1** [4, 10], but there has been one formal synthesis [11], and a few unique synthesis of the aminocyclitol **4** the most promising one will be presented in this thesis[12].

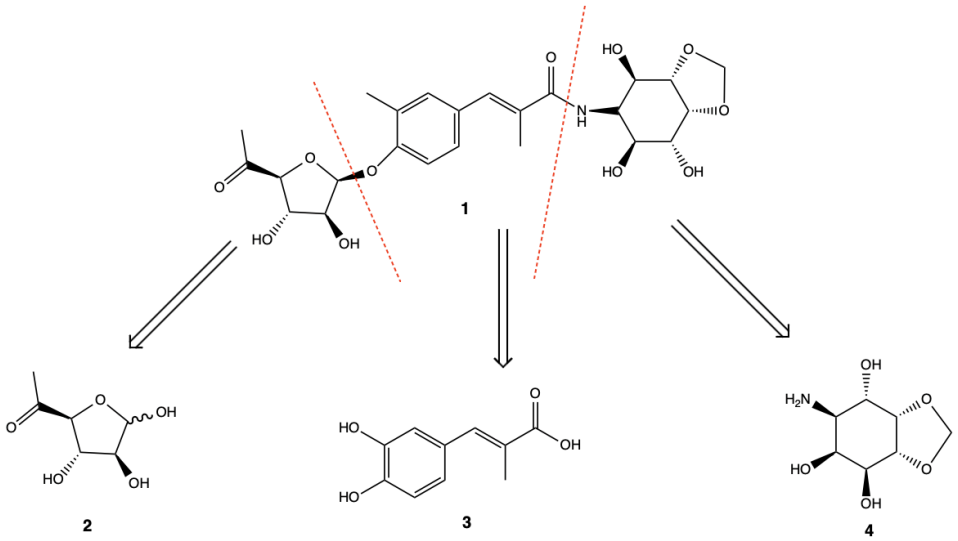

Figure 1: HygA (**1**) and its three parts

1.3 Objective

In this thesis Hygromycin A (**1**) potential as an antibiotic will be discussed, especially against Lyme disease. Syntheses of the aminocyclitol **4** and the furanose sugar **2** will be discussed separately, before discussing the best combination of the methods to build up a good total synthesis.

2 Theory

2.1 Potential of (-)-Hygromycin A as a drug - mechanism and resistance

In 2021 it was discovered that **1** has high efficiency and selectivity against spirochaetes trough a selective screening against *B. burgdorferi* which is the bacteria that causes lyme disease[13]. It is a disease caused by tick bite that gives flu like symptoms. In some cases, specially if not treated early it can give post treatment lyme disease syndrome (PTLDS), that gives long lasting joint and muscle pain, neurological complications and fatigue [14]. In the United States there is estimated 300 000 cases of lyme disease annually where between 10-20 % develop PTLDS [15]. The current treatment against Lyme disease is an extensive antibiotic cure lasting from 10 days up to a month depending on the symptoms [16]. Preventative antibiotic treatment is also used, in areas where Lyme disease is common. There has been studies that shows correlation between PTLDS and a change in the gut microbiome [15]. The poor activity of **1** against other bacteria makes it less harmful to the gut microbiome than the broad-spectre antibiotics that is used to treat lyme disease today [13]. The compound has also recently been found to be very effective against *Treponema pallidum*, the bacteria that causes syphilis [17]. Resistance against the antibiotics used to treat syphilis is an increasing problem, the specificity of **1** is thought to make resistance less likely. The high selectivity is explained due to the fact that spirochaetes does not have the enzyme that synthesizes purines. Purines includes adenine and guanine that are constitutes of some important biomolecules, such as deoxyribonucleic acid (DNA) and adenosine triphosphate (ATP) [18]. Spirocheates relies on the uptake of host nutrients to maintain their replicative capacity [19]. They need to get purines transported into the cell, and does so through a purine nucleoside transporter (see figure 2). **1** has a structure resembling a purine nucleoside, and therefore gets a free ride into the cell with the purine nucleoside transporter [13, 17] .

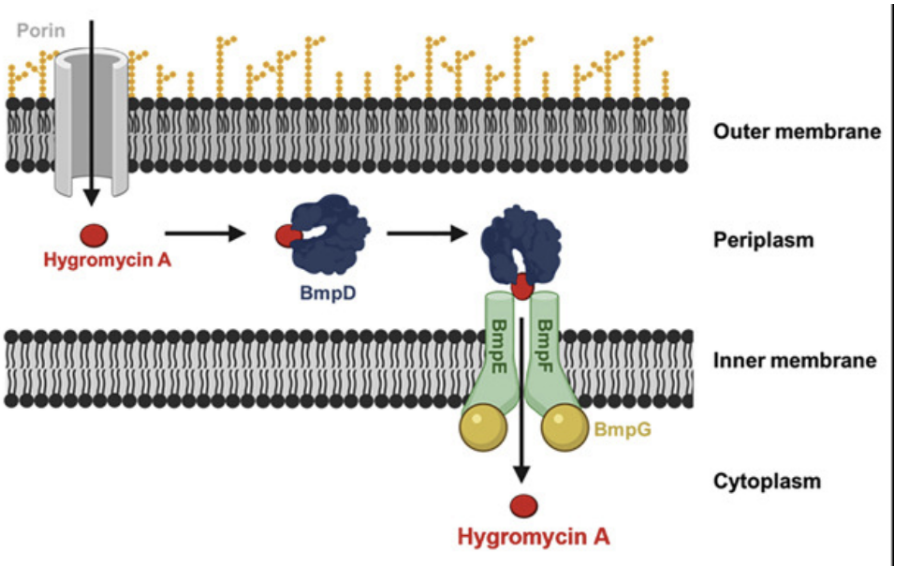

Figure 2: Transport of HygA into the cytoplasma of the cell with the help of a purine nucleoside transporter. Reprinted from Lewis et al.[13].

The antimicrobial mechanism of action for **1** involves inhibition of the protein synthesis [20]. Specifically the peptide bond formation step. Peptide bond formation requires that the aminoacyl-tRNA is properly positioned at the A site of the peptidyl transferase center (PTC) [21]. **1** binds to to the PTC and sterically prevents full accommodation of the aminoacyl-tRNA to the A site (see figure 3) [22]. This leads to a distortion of the acceptor arm and the CCA-end of the tRNA, which is the codon for the aminoacid proline and functions as the binding site for aminoacid attachment [23]. This distortion is what prevents the peptide bonds from forming. The inhibition occurs when the ribosome is on the start codon, so the elongation stage has not begun [22]. One resistance mechanism against **1** is phosphorylation of the ribose subunit by catalysed transfer of the phosphoryl group in ATP [22, 24]. When **1** is phosfhorylated it will not work as well as an inhibitor because it will be sterically hindered from binding well to the 23S rRNA that is a part of the peptidyl transferase center. This is allowing the ribosomes to initiate the elongation stage and move on from the start codon, making the drug inactive.

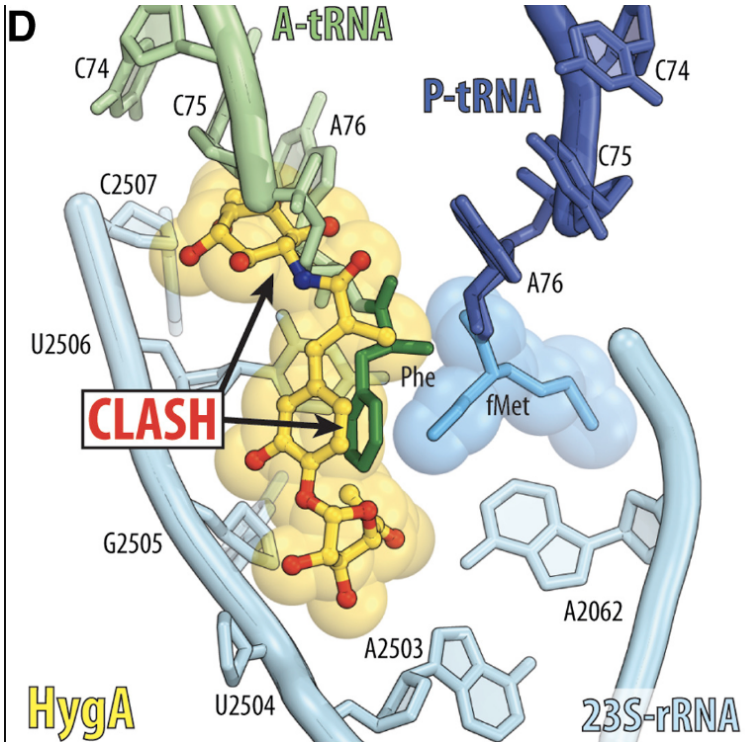

Figure 3: Hygromycin A (yellow) sterically hindering the full accomodation of the aminoacyl-tRNA (green) to the peptidyl transferase center (light blue). Reprinted from polikanov et al. [22]

2.2 Synthetic approaches

The approaches used to synthetisise Hygromycin A are asymmetric synthesis, and can be further divided into chiral pool and chemoenzymatic methods. An asymmetric synthesis is a reaction that gives one or more new chiral centers to the molecule [25]. It is a stereoselective reaction that produces the stereoisomeric products in unequal amounts, the conditions of the reaction can therefore be arranged to favor the desired isomer. The two total syntheses of **1** [4, 10], have long synthetic routes and use multiple approaches to reach the target.

The formal synthesis is based on the chiral pool method [11]. It is a method that uses a cheap chiral starting material, and build upon this to get to the target molecule [26]. The "chiral pool" referrs to a collection of these starting materials, such as amino acids, carbohydrates, carboxylic acids and terpents.

The chemoenzymatic method is based on combining enzymatic and synthetic trans-

formations in the synthesis [27]. It was used in the unique synthesis of the aminocyclitol **4** [12]. Combination of the methods is to make syntheses of complex natural products more easy. The use of enzymes as biocatalysts give high selectivity and is used to give optically pure chiral molecules. Nature has a few unique ways of doing reactions that does not have a chemical equivalent when it comes to efficiency [28]. Enzymatic dihydroxylation of arenes by microbial organisms is one of them. It is a highly sterioselective and enantioselective reaction.

2.3 Total synthesis

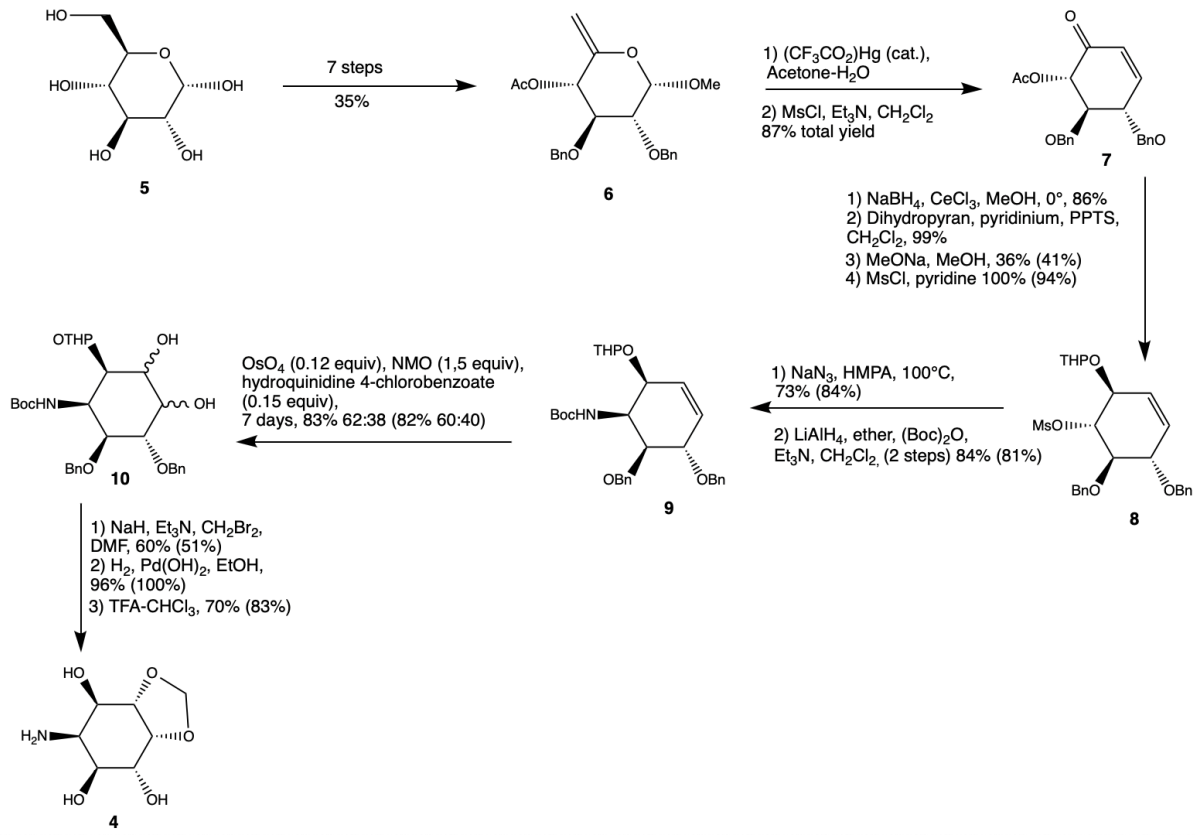

Figure 4: Chida et al. synthesis of the aminocyclitol part **4**. Drawing inspired by [4].

Chida et al. total synthesis

Although Hygromycin A(**1**) was discovered in 1953 the first synthesis was not reported until 38 years later in 1991, by Chida *et al.* [4]. Their synthesis is based on a coupling reaction of furanose **2** and cinnamic acid **3**, and then with aminocyclitol **4** (see figure 1).

The synthesis of the furanose sugar part is as follows (see figure 4); 5-enopyranoside **6** was used as starting material, prepared in 7 steps from D-glucose **5** in 0.35% yield [4, 29]. A Ferrier rearrangement with catalytic mercuric trifluoracetate in acetone [30], gave a mixture of α/β-alcohol isomers. The β-hydroxyl group was eliminated by methanesulfonyl chloride and triethylamine to give **7**. The ketone group was reduced with $NaBH_4$ in the presence of $CeCl_3$. The resulting hydroxyl group was then protected as a tetrahydropropanyl (THP) ether, before the O-acetyl group was removed with sodium methoxide in methanol. The THP group lead to the product being in a diastereoisomeric mixture which was separated before it was mesylated to give **8**. The two diastereomers had almost the same reactivities towards the reaction conditions in the following steps. The yield of the other diastereomer is showed in parentheses in figure 4. Sodium azide in hexamethylphosphoramide was used at 100 °C in an azidolysis. The azido group was then converted into the more bulky amino group, *tert*-butoxy-carbonyl. This was done with $LiAlH_4$ in ether, followed by di-tert-butyl-dicarbonate (Boc_2O). The product **9** was then oxidized by OsO_4 and *N*-Methylmorpholine *N*-oxide (NMO) in a Sharpless dihydroxylation with hydroquinine 4-chlorobenxoate as an additive over 7 days to give **10** [30]. To make compound **4**, a methylene acetal group was introduced by using methylene bromide and sodium hydride in *N,N*-dimethylformamide (DMF). Debenzylation was done with hydrogen and a palladium metal catalyst, and finally it was treated with trifluoroacetic acid (TFA) to afford the aminocyclitol part **4** in 10% yield over 13 steps from **5** [4].

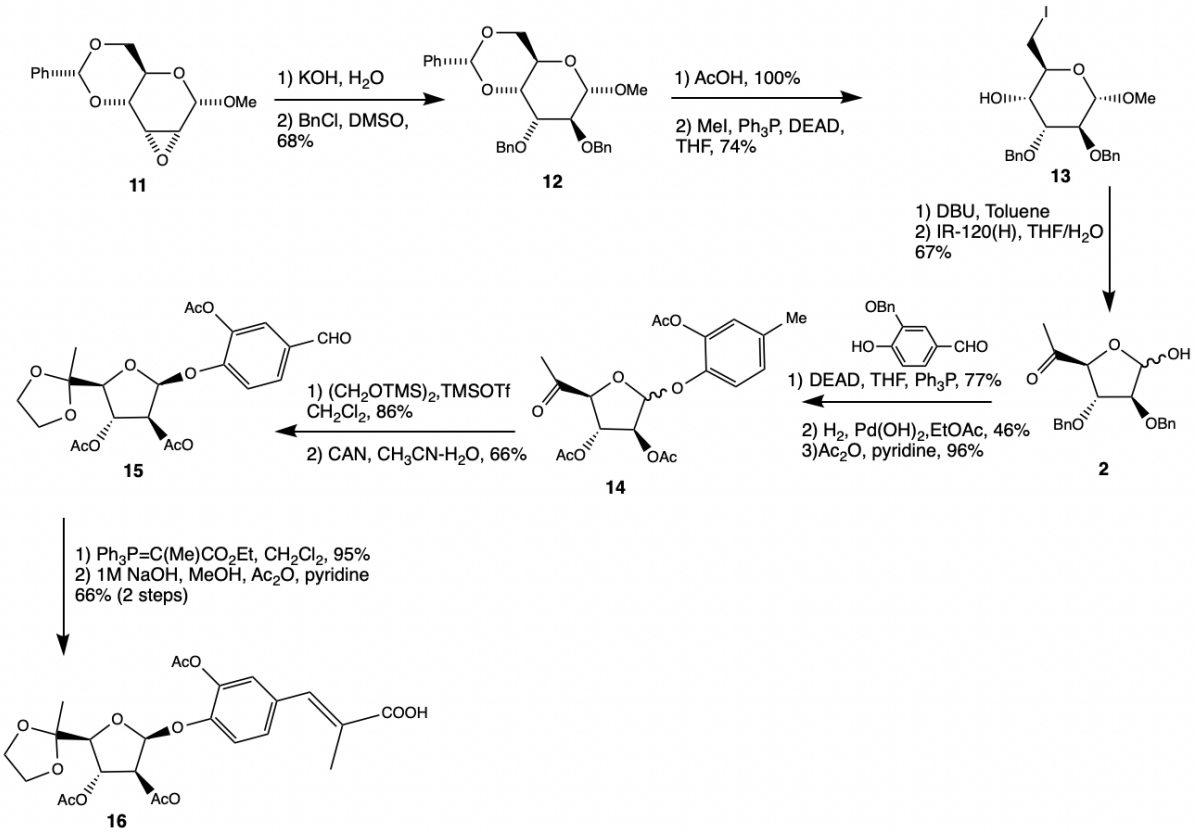

Figure 5: Chida et al. sythesis of the furanose **2**, and the coupling reaction with the cinnamic acid to get **16**. Drawing inspired by [4].

The synthesis of the furanose sugar **2** started with compound **11** that was treated with aqueous potassium hydroxide and a successive one pot benzylation with benzyl chloride to give **12** with benzyl as protecting groups (see figure 5). The bezylidene group in acetale **12** was then removed, and the resulting pimary hydroxyl was treated with iodomethane trough a Mitsunobu reaction to replace hydroxyl with iodide. The resulting product **13** was dehydroiodinated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to make 5-enopyranoside which was hydrolized with amberlite IR-120 resin in aqueous tetrahydrofuran (THF), to give the furanose sugar component **2** in 34% yield over 6 steps. Furanose **2** was then coupled to 3-hydroxy-4-phenoxybenzaldehyde trough a condensation with Mitsunobu conditions. The product was obtained as a inseparable diastereomeric mixture of O-furanoside aryl ethers, which were debenzylated towards **14** with hydrogen and a palladium catalyst. The latter reaction gave also an undesired reduction of the formyl group. The epimers were separated using chromatography, and the β epimer was acetylated to give

triacetate **14** as the product. Ketalization of the compound was done with trimethyl trifluoromethanesulfonate (TMSOTf) and $(CH_2OTMS)_2$ before it was oxidized with ceric ammonium nitrate in acetonitrile-water to regenerate the formyl group, and give **15**. Wittig olefination with $Ph_3P{=}C(Me)CO_2Et$ in toluene was performed to introduce ethyl isobuturate, that was converted to an acid with saponification with successive acetylation to give compound **16** in 4% yield over 13 steps from **11**.

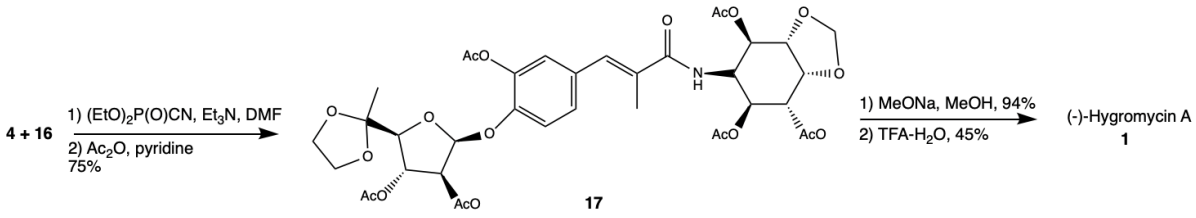

Figure 6: Chida et al. coupling of **4** and **16** finishing the total synthesis of (-)-Hygromycin A. Drawing inspired by [4].

Finally to complete the synthesis, the aminocyclitol **4** and the furanose sugar **16** needs to be coupled together (see figure 6). This was done with condensation with diethylphosphoryl cyanide, followed by an acetylation. The product **17** was deacylated with NaOMe in methanol before it was treated with aqueous TFA over 1 h to give (-)-Hygromycin A (**1**) in under 1% yield over 24 linear steps steps starting from **5**.

Since the first total synthesis was reported there has only been one other total synthesis of Hygromycin A, reported by Donohoe et al. in 2009 [10]. However there has been several reports on the synthesis of the aminocyclitol **4** and the furanose sugar **2**. The total synthesis and some of the other promising synthesis will be presented.

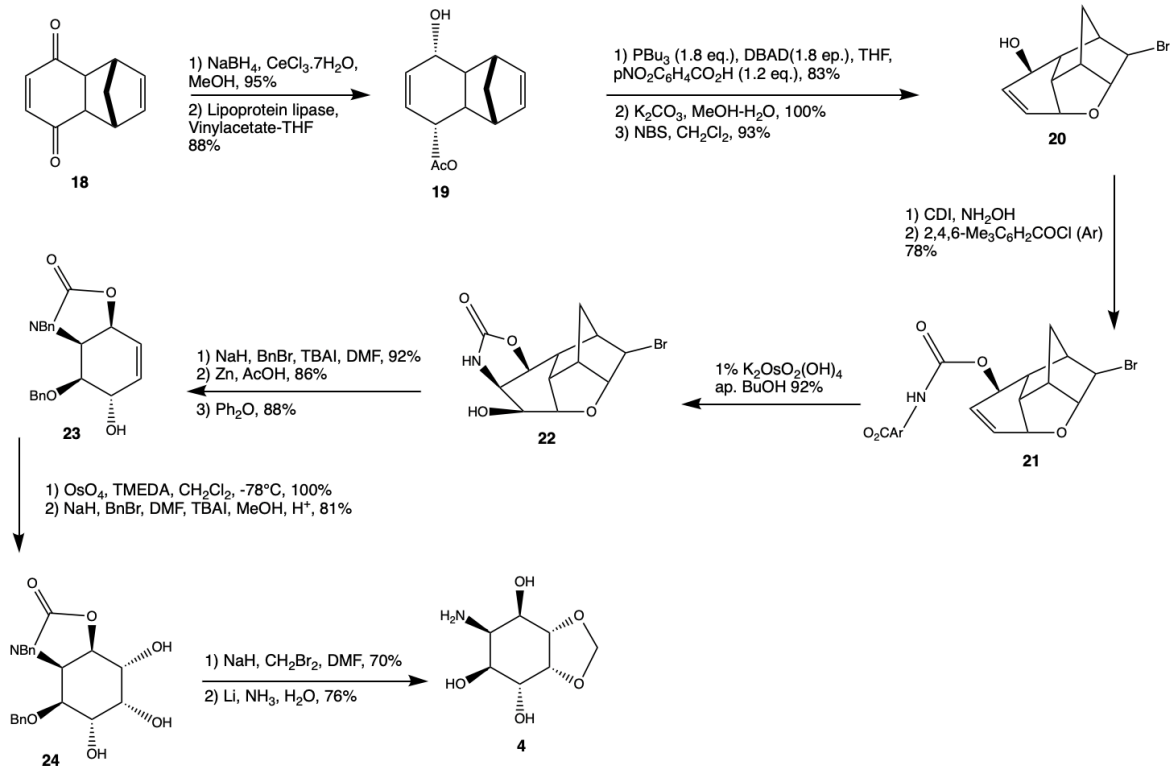

Figure 7: Donohoe et al. synthesis of the aminocyclitol **4**. Drawing inspired by [10].

Donohoe et al. total sythesis of (-)-Hygromycin A

The second total synthesis of Hygromycin A (**1**) was reported in 2009 by Donohoe et al. [10]. The preparation of the aminocyclitol moiety **4** started with dikenone **18** (see figure 7). **18** was reduced with $NaBH_4$ and then treated with vinylacetate in THF, in the presence of lipoprotein lipase to **19** [31]. Inversion of the alcohol in **19** was done under Mitsunobu conditions followed by ester hydrolysis with K_2CO_3, the resulting product was then treated with *N*-bromosuccinimine (NBS) to bromoether **20**. The alcohol on **20** was made into a carbamate with 1,1-carbonyldiimadazole (CDI) and hydroxylamine before it was esterified with mesitoyl chloride to give **21**[10]. This was done to get the N,O-acylated derivative that work as a good leaving group in the following aminohydroxylation reaction. Tethered aminohydroxilation gave **22** using 1 mol% of potassium osmate in aqueous butanol as catalyst [32]. The hydroxy and amino group in **22** was benzyl protected before the ether bridge was removed with zinc in acetic acid and then a retro Diels Alder reaction yielded alkene **23**[31]. Direct

dihydroxylation of **23** with a mixture of OsO_4 and Tetramethylethylenediamine (TMEDA)[33], yielded a osmate ester that was benzylated to give **24**. Introduction of a methylene acetal group was done with NaH in CH_2Br_2, finally the benzyl and oxazolidine protecting groups was removed to give the aminocyclitol **4** with 20% overall yield over 15 steps starting from **18** [10].

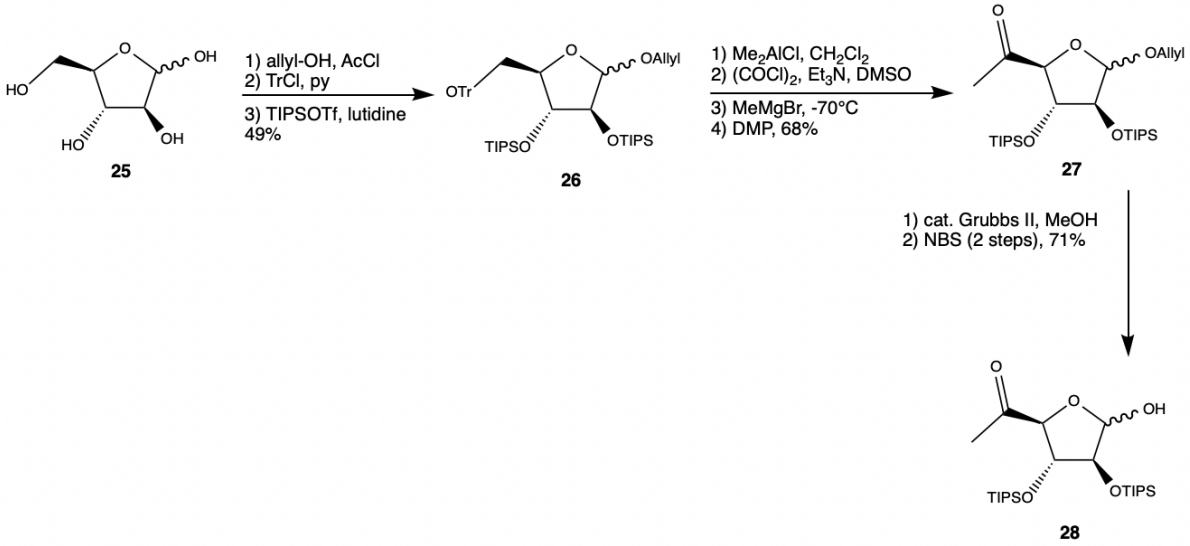

Figure 8: Donohoe et al. synthesis of the furanose sugar **24**. Draeing inspired by [10]

The starting compound to produce the furanose sugar **28** was D-arabinose **25** drawn in furanose form for clarity (see figure 8)[10]. An allyl group was introduced in the anomeric position in a substitution reaction using allyl-OH in AcCl. Then trityl was introduced as a protecting group on the primary hydroxy group, before triisopropylsilyl (TIPS) was introduced as protecting groups on the two remaining secondary hydroxy groups to give **26**. To get the ketone group in **27** a cleavage of the trityl group was done with Me_2AlCl in CH_2Cl_2 to give an alcohol which was oxidized to an aldehyde using Swern conditions [34]. The aldehyde was then reacted further with MeMgBr before it was oxidised to the ketone using Dess-Martin periodinane (DMP). The allyl protecting group was removed in two steps, starting with forming an enol ether with alkene isomerization using Grubbs II catalyst, before the enol ether was transformed to an alcohol with NBS to give **28** in 24% overall yield in 9 steps from **25**.

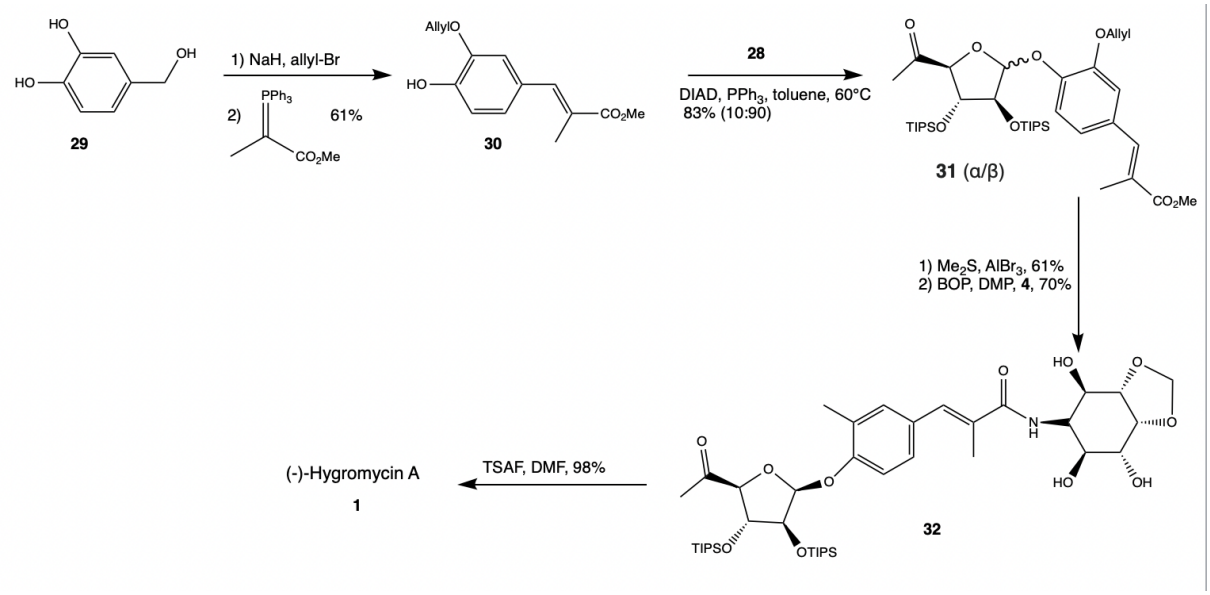

Figure 9: Donohoe et al. coupling of **30**, **28** and **31** completing the total synthesis of (-)-Hygromycin A. Drawing inspired by [10].

The final part of the synthesis is where the parts are connected. It started with converting **29** into **30** through an allylation with NaH and allyl-Br, followed by a Wittig olefinatition (see figure 9)[10, 35]. A selective Mitsunobu glycosilation of **30** with **28** was performed to get more of the β isomer [36]. The reaction was done with in toluene at 60 °C, which gave **31** with 90:10 selectivity for the β isomer. To get **32** the methyl ester protecting group in **31** was transformed to carboxylic acid, by reaction with Me_2S and $AlBr_3$, and then amide coupled with **4** using 1-benzotriazolyloxytris-(dimethylamino)phosphonium hexafluorphosphate in DMF. In the final step the protecting TIPS groups were removed from **32** to give **1** in 10% overall yield in 17 linear steps starting from **18**, **25** and **29** [10].

2.3.1 Chiral pool

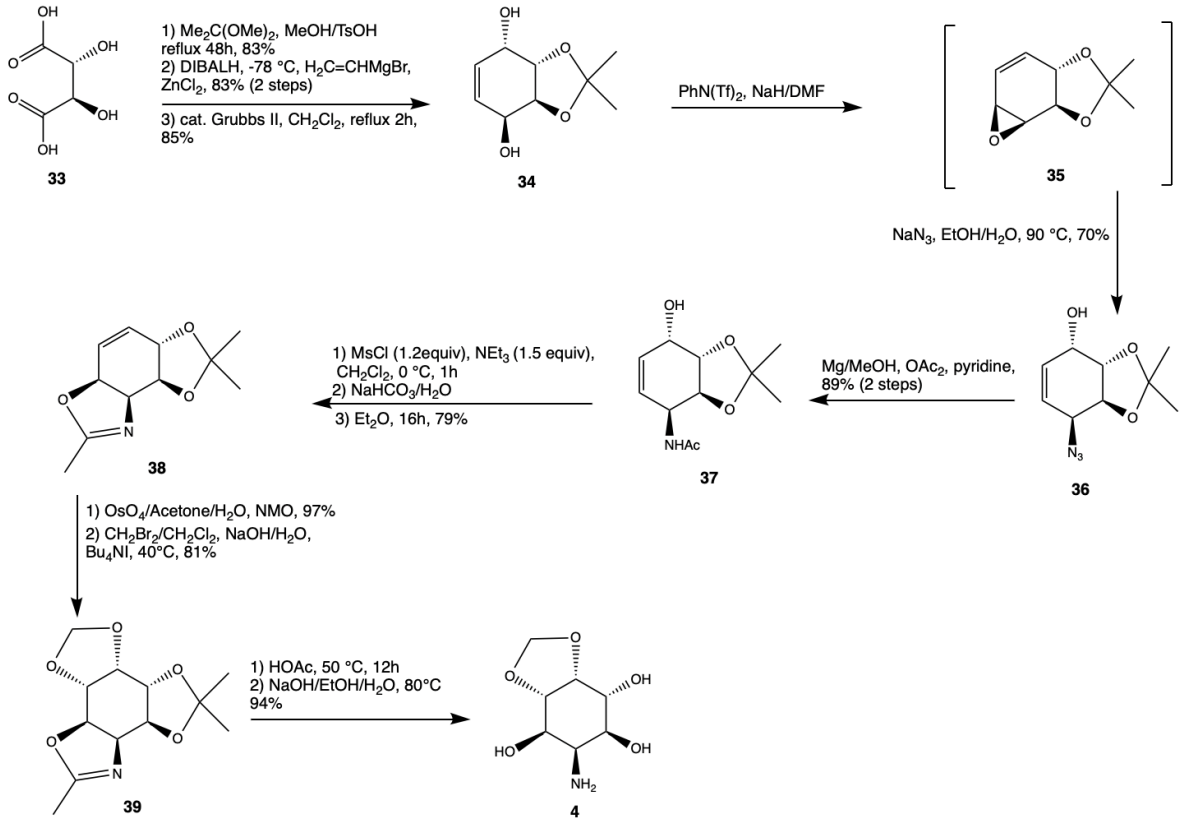

Figure 10: Lo et al. synthesis of the aminocyclitol **4**. Drawing inspired by [11, 37].

Lo et al. formal synthesis of (-)-Hygromycin A

Lo et al. used a chiral pool strategy for their syntesis of the aminocyclitol **4** and the furanose sugar **28**. They refered to the work of the Donohoe group [10], for coupling **4** with **31** and the last step to **1** (see Figure 9). L-tartaric acid (**33**) was used as chiral pool the synthesis of the aminocyclitol part **4** (see figure 10) [11]. **33** was treated with 2,2-dimethoxypropane and TsOH in methanol to protect the hydroxy groups and prepare the carbolic acids for reduction [37]. Reduction is done with diisobutylaluminium hydride (DIBALH) to a bisaldehyde, followed by a stereoselective addition of divinylzinc, and finally a ring closing metatheses using Grubbs II catalyst was performed to gove diol **34**. The diol **34** was then converted to an allylic epoxide with $PhN(Tf)_2$ in the presence of NaH through a monotriflation of one of the hydroxyl leaving group s to give **35**. Followed by addition of sodium azide

in aqueous ethanol to get an azide conjugated ring opening of the allylic epoxide, which was heated at 90 °C for four hours to get regio- and stereo control to give **36**. The azide group in **36** was reduced to amine with magnesium in methanol and then acylated with acetic anhydride in pyridine to give **37** [11]. Cyclisation of **37** was done by mesylation of the hydroxyl group using MsCl and NEt_3, and then adding diluted sodium bicarbonate in ethanol over 16 hours to get **38**. Sharpless dihydroxylation of **38** with OsO_4 in aqueous acetone in the presence of NMO yielded a diol that was protected with a methylene group to **39**. In the last step the acetonide and oxazoline group in **39** were removed by treatment with aqueous acetic acid at 80 °C for 12 hours followed by addition of NaOH in ethanol to afford the aminocyclitol **4** in 21% overall yield over 9 steps from **29**[11].

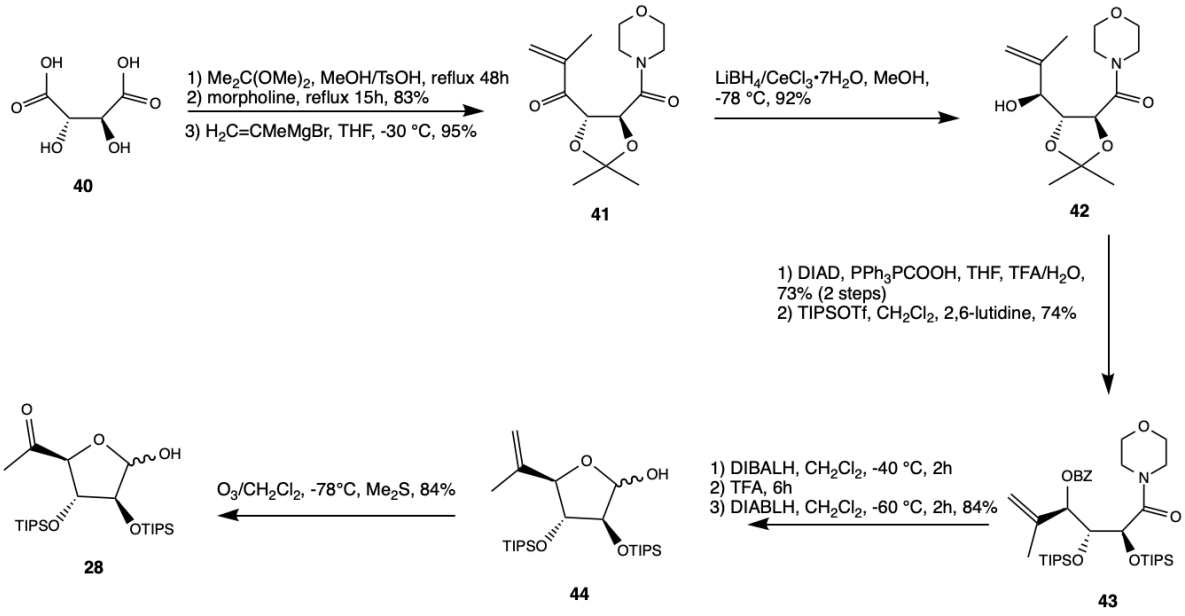

Figure 11: Lo et al. synthesis of the furanose sugar **28**. Drawing inspired by [11].

The synthesis of the furanose sugar **24** (see figure 11), started with treating D-tartaric acid (**40**) with acetone dimethyl acetal followed by addition of morpholine to introduce an acetonide protection group and a morpholine group [11]. Addition of 2-propenyl Grignard reagent to the latter compound yielded the γ-keto amide **41**. Reduction of the conjugated ketone group in **41** with a mixture of $LiBH_4$ and $CeCl_3$ in methanol at -78 °C yielded an epimeric mixture of **42** in a 16:1 ratio of S:R configuration. A Mitsunobu reaction of **42** inverted the hydroxyl group and

introduced a benzoyl group, and the resultant product was then TIPS protected to afford **43**. Compound **43** was then reduced with DIBALH followed by an TFA promoted γ-lactone formation and a reduction of the lactone with DIBALH to give **44**. Oxidative cleavage done by ozonolysis followed by workup with Me_2S afforded **28** in 28% overall yield over 10 steps starting from **40**.

2.3.2 Chemoenzymatic synthesis

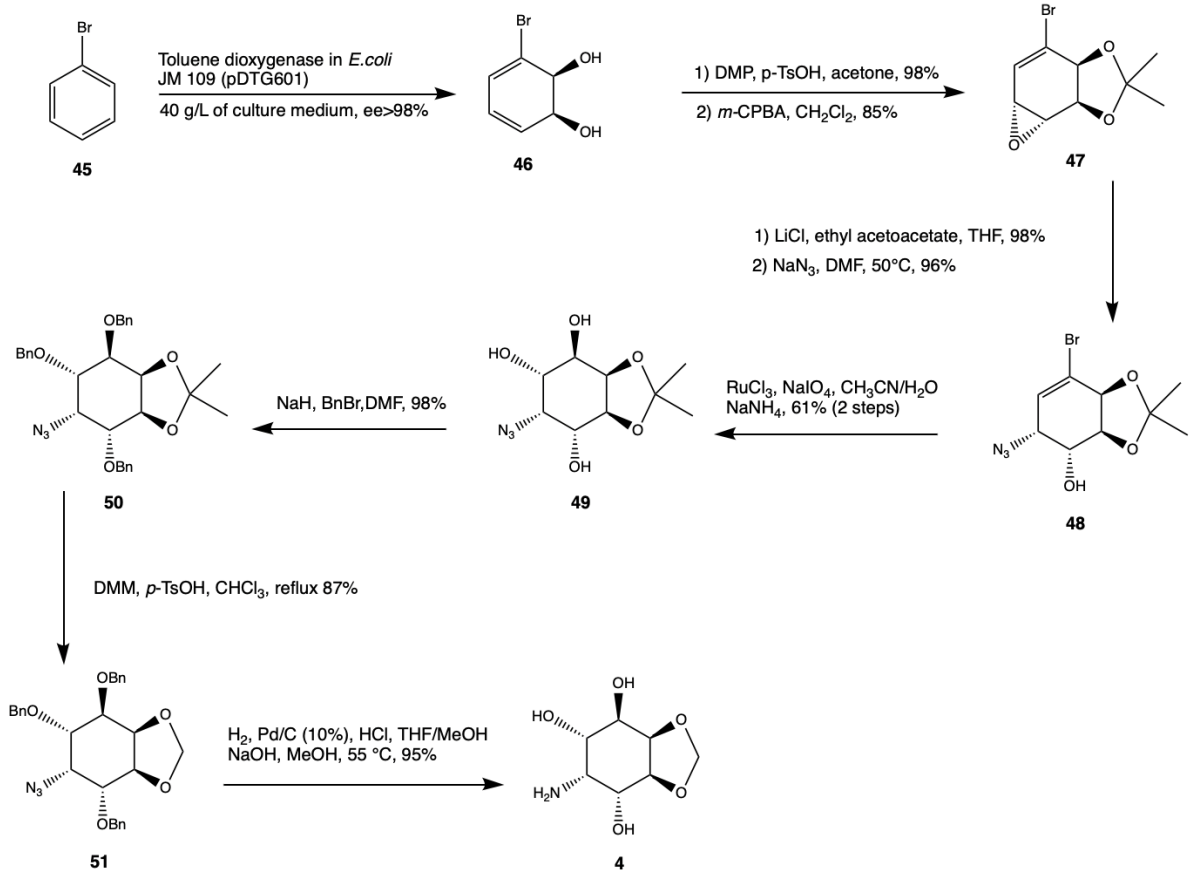

Figure 12: Carrau et al. synthesis of the aminocyclitol **4**. Drawing inspired by [12].

Carrau et al. synthesis of (-)-Hygromycin A aminocyclitol part

The synthesis of the aminocyclitol **4** (see figure 12), reported by the Carrau group, is an chemoenzymatic synthesis where the first step is an enzymatical dihydroxylation of bromobenzene (**45**) by a recombinant strain of *E. coli* that harbors toluene dioxygenase genes, to achive **46** [12, 38]. Acetonide was introduced as a protecting

group using DMP and *p*-toluenesulfonic acid, before meta-chloroperoxybenzoic acid was used to introduce an epoxide group to give compound **47**. To get the hydroxy azide **48** the epoxide was opened by lithium chloride in the presence of ethyl acetoacetate, and the azide was then introduced using NaN_3. The hydroxy azide **48** was treated with $RuCl_3$ and $NaIO_4$ for dihydroxylation and then $NaBH_4$ for a reduction removing Br and inverting hydroxyl to give **49**. The three hydroxyl groups in **49** were benzylated with benzyl bromide in presence of NAH to **50**. The resulting compound **50** had a methylene acetal group introduced using dimetoxymethane and *p*-toluenesulfonic acid to give **51**. In the last step removal of the benzyl protecting groups and reduction of the azide was done by hydrogenolysis followed by workup with NaOH in methanol to give the aminocyclitol **4** in 39% overall yield over 8 steps starting from **45** [12].

3 Discussion

3.1 Antibiotic activity

Hygromycin A (**1**) has since its discovery in 1953 been neglected as a possible antibiotic because of its difficult synthetic pathway and only moderate activity against most bacteria. Before its rediscovery in 2021 it was found to be active against *Brachyspira* (that causes swine dysentery) with a minimal inhibitory concentration (MIC) of 1.56µg/mL [13]. It was considered as a possible drug against swine dysentery but the economical aspect of the production led to it not being pursued as a drug, only for use in animal health [5, 13]. Selective screening against *B. burgdorferi* done by Leimer et al. in 2021 got the attention back on Hygromycin A as a possible drug [13].

It works well against *B. burgdorferi* (the spirocheate that causes Lyme disease) because, the bacteria does not produce its own purine and needs it to be transported into the cell. Hygromycin A (**1**) and purine has a similar structure that allows **1** to be smuggled into the cell with the help of a purine nucleoside transporter (see figure 2). This special mechanism suggest that **1** will work as treatment against other diseases

caused by spirochaete bacteria, like syphilis and peridontol diasese, because they do not produce their own purine neither [7]. The Leimer research group found the MIC to be 0,25 µg/mL against B. *burgdorferi* and a MIC of 0,03 µg/mL against *treponema palladium* (that causes syphilis) [13]. When **1** was tested in mice it had little effect on the gut microbe and no detectable toxicity. The broad-spectrum antibiotics used to treat Lyme disease today causes damage to the gut microbe. Research indicates that microbial dyspiosis contributes to the development of chronic diseases [39]. Later stages of Lyme is treated with antibiotics for up to a month. The condition of microbial dyspiosis and post treatment Lyme disease syndrome (PTLDS) have many of the same symptoms and the heavy antibiotic treatment is suspected to be the cause of PTLDS [40]. This combined with the increasing antimicrobial resistance makes the need for new and narrow-spectre antibiotics high. Researchers hope that these antibiotics can have high efficiency without damaging the gut microbe, and that they are more resistant to antimicrobial resistance [13]. Development of more narrow-spectrum antibiotics will also take the pressure of the broad-spectrum antibiotics, that are the last resort to some life threatening diseases. Resistance against these can cause catastrophic health issues [7]. Hygromycin A (**1**) has not been approved for human use but was approved for clinical trials in December of 2023 and flightpath bioscience are planning on moving into phase one of the clinical trials in the spring of 2024 [7]. Research and findings done through these trials will give more knowledge on possible side effects and potential of the compound as a commercial drug.

3.2 Synthesis

Hygromycin A (**1**) has proven to be a difficult synthetic target with only two reported total synthesis since its discovery in 1953. Both synthesis used asymmetric synthesis methods where chiral centers was intoduced to make the compound. The first total synthesis was not reported before 1991 by the Chida research group [4]. The complex synthesis and the low overall yield (less than 1% yield over 24 steps) makes it not suited for large scale development. Although the synthesis is not suited for production, it laid the groundwork for further development and improvements of the

synthesis. Donohoe et al. synthesis is the second total synthesis of **1** and focused on improving the aspects of Chida's synthesis where there was room for it [10]. One of the largest improvements was the coupling of the phenol **30** and the furanose sugar **28** (see figure 9). The challenge with this formation is that the desired β-conformation is sterically hindered. Both of the synthesis used Mitsunobu conditions to perform the glycolasation (see figure 5 and 9). In Donohoe´s method toluene was used as a solvent instead of THF, and the reaction was done at 60 °C instead of room temperature as it was in Chida´s method. In addition to this optimisation of the reaction conditions the Donohoe research group used TIPS for protecting the hydroxyl groups at C2 and C3 (see compound **26** in figure 8) instead of benzyl (see compound **2** in figure 5). The bulky group at the C2 position was used to favor the α-anomer of the anomeric hydroxy group before the coupling, so that during the Mitsunobu inversion the β-conformation will be favoured (see figure 13).

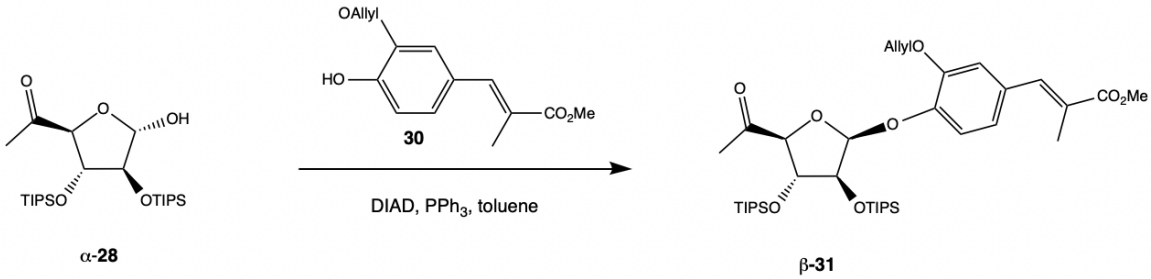

Figure 13: Mitsunobu glycosilation of α-**28** and **30** with an inversion of configuration to the desired β-**31** confirmation. Drawing inspired by [10, 36]

In Chida´s synthesis introduction of a ketal protecting group to the ketone in **14** (see figure 5) was done because the compound has proven to be sensitive to epimerization in the C4 position [4, 41]. The protecting TIPS group on the O-C3 carbon was used to prevent epimerization at the C4 carbon in compound **31** and **32** (see figure 9), without having to protect the ketone group and therefore reducing the amount of steps needed in Donohoe´s method [10]. There has not been found other coupling methods of the different parts after Donohoe et al. paper in 2009. However, there has been found more recent reports on the furanose sugar **2** [11], and the aminocyclitol **4** [11, 12]. Since the synthesis is divided into different parts there is a possibility of

combining different strategies to achieve a better total synthesis.

For the aminocyclitol **4**, the chiral pool method developed by Lo et al. [11], was a large improvement compared to the earlier reported methods, considering the reactions steps (10% yield over 13 steps for Chida and 20% yield over 15 steps for Donohoe). The chemoenzymatic method reported by Carrau et al.[12] had a similar number of reaction steps (9 steps for Lo and 8 steps for Carrau), but outperforms the other syntheses when looking at the yield. With an astonishing 39% yield, that is almost the double of Lo et al. 21% yield. The fact that the yield is almost doubled and the number of reaction steps is almost the same indicates that the chemoenzymatic method works better to make a simpler synthetic pathway to complicated natural compounds. In this synthesis an arene was asymmetric dihydroxylated. A reaction that to our knowledge is only possible to perform by using enzymes as catalyst [38]. The reaction proceeds with high enantioselectivity (98% ee) in an efficient way [9, 28].

For the synthesis of the furanose sugar **2** the preparations for the following coupling reaction is just as important. Chida´s synthesis of **2** has the highest yield and fewest steps with 34% in 6 steps (see figure 5). The issue with the Chida´s group methods is the following steps in the total synthesis. As mentioned before, one of the largest improvements from Chida´s method to Donohoe´s method is the coupling reaction where the TIPS protecting groups contributes in favouring the desired configuration. The amount of reaction steps used in connecting the parts substantiates this point. Chidas method used 11 steps (see figure 5 and 6) compared to Donohoes 6 steps (see figure 9). The importance of this can be seen in the overall yield of the total syntheses (less than 1% for Chida and 10% for Donohoe). Because of this, it is not the method with the highest yield of **2** that is the best approach when looking at the total synthesis of **1**. Lo has based their synthesis of **2** on Donohoes approach of the coupling (see figure 11), so their synthesis used TIPS protecting groups, that has proved to be important. They also reported similar yields and reaction steps (24% over 9 steps for Donohoe and 28% over 10 steps for Lo). With regard to the possible commercialisation of Hygromycin A (**1**) as a drug, economic sustainability is an important aspect. The starting material of Lo´s synthesis is D-tartaric acid

(**40**), and it is approximately half of the price to D-arabinose **25** that is the starting material in Donohoe´s synthesis. (1140 NOK for 100 g D-tartaric acid and 2340 NOK for D-arabinose)[42, 43]. When considering the price of the reagents there is one reagent used in Donohoes method that stands out as an especially expensive one. The Grubbs II catalyst with a price of 6950 NOK for 2 g [44], makes it not suited for large scale production even as a catalyst.

Looking at the different synthetic strategies that have been discussed in this thesis, a possible way of optimising the total synthesis of Hygromycin A (**1**) is to combine the different methods for the different parts of the molecule. For the coupling and for the synthesis of the aminocyclitol **4** there is a method that clearly outshines the others. Donohoe´s method for the coupling of the furanose sugar **28**, compound **30** and the aminocyclitol **4** (see figure 9). Carrau´s method for the synthesis of **4** (see figure 12). However, for the synthesis of the furanose sugar **2**, none of the presented synthesis clearly sticks out when looking at the overall yield. When considering which method to use here, the availability and price of the chemicals is an important aspect. Lo´s method uses a cheap starting material and does not use Grubbs II catalyst for removing the protecting group at the anomeric position as Donohoe does. Even though these synthetic strategies has an acceptable yield alone, the overall yield will be low when combining them together. Futher development of the methods will be beneficial for the possibility of commercialising Hygromycin A (**1**) as a drug.

4 Conclusion

(-)-Hygromycin A (**1**) has shown a great potential as a drug against Lyme disease and other diseases caused by spirochaetes. The similarity in structure to a purine nucleoside and therefore the possibility to get a free ride into the cell makes it a great selective antibiotic against these bacterias. It hasn't been approved as an antibiotic for human use yet, but human clinical trials are planned to start in 2024. The research done in the clinical trials is needed to guarantee its safety. Hopefully they will give a positive result.

The best syntheses reported in relation to yield. steps and cost is a combination of Carrau et al. synthesis of the aminocyclitol **4**, Lo et al. synthesis of the furanose sugar **2** and Donohoe et al method for coupling the parts together.

The increasing need for new antibiotics will not be solved by one new drug alone. The hope that introducing more specific antibiotics will work better against antibiotic resistance, be better for the gut microbe and take pressure of the overused broad spectre antibiotics makes Hygromycin A a good candidate to be one of the antibiotics that contributions towards solving the problem.

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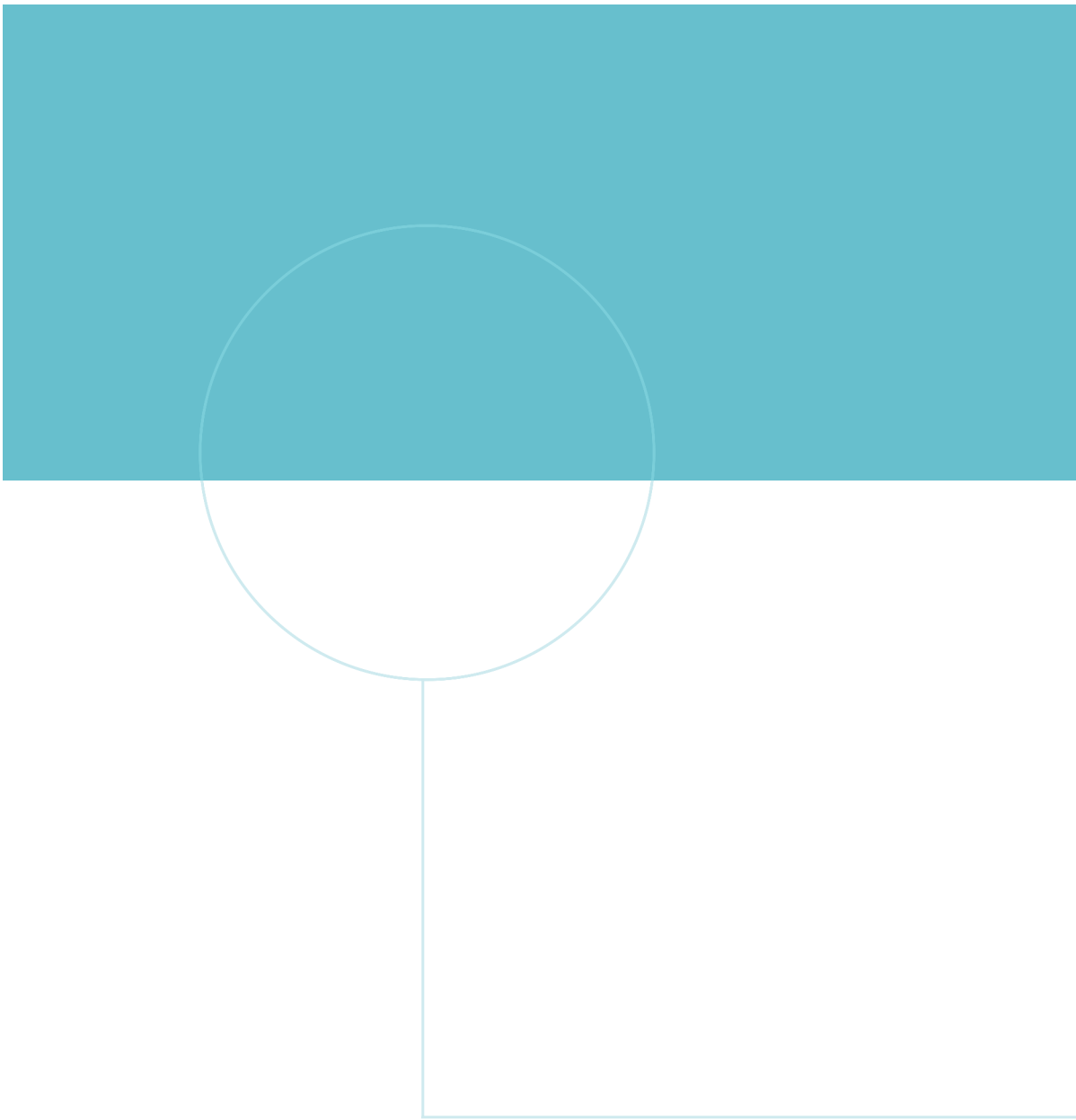

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