Bachelor's project

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Chemistry

Nicholas Alexander Janjua

The synthesis and appliance of natural antibiotic (+)-negamycin

Bachelor's project in BKJ

Supervisor: Odd Reidar Gautun

April 2020

NTNU
Norwegian University of
Science and Technology

Bachelor of Chemistry

The appliance and synthesis of natural antibiotic (+)-negamycin

Nicholas Alexander Janjua
nicholj@ntnu.stud.no
April 30, 2020

Supervisor
Odd Reidar Gautun

Institute of Chemistry
Norwegian University of Science and Technology

Abstract

In this paper the potential benefits and weaknesses of the natural antibiotic (+)-negamycin are highlighted and discussed. These comprise of its potency within the medical field, as well as its toxicity toward patients among other things. A comparison between (+)-negamycin's capabilities and that of other similar-purpose drugs is also discussed, along with summary of the antibiotics most promising analogues. Some of the compounds various syntethic approaches are also discussed in contrast to each other in order to find an efficient method for further development. (+)-Negamycin's multiple potential medicinal uses make it a sought after compound in medical research, and make it an attractive target for organic synthesis. After its isolation, many synthetic routes have been proposed and tried, with varying success.

Contents

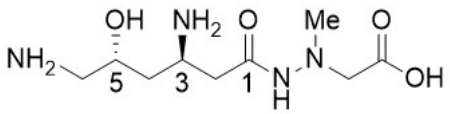
1 Introduction and Objective

1.1 A brief history of antibiotics

The discovery of modern antibiotics is one of, if not the biggest improvement to human health and longevity in modern history.[1] Researchers have found evidence of the use of antimicrobial materials as far back as 350-550 current era (CE). These include the use of tetracycline containing plants in their diets to combat certain diseases. Today, we associate the start of the modern "antibiotic era" with the discoveries of Paul Erlich and Alexander Fleming. In 1904 Erlich took inspiration from microbe-selective dyes, such as aniline, to study compounds that could specifically target disease-causing microbes. 20 years later, Fleming discovered penicillin and became one of the first to warn about the potential risks of antibiotic overuse and the dangers of antibiotic resistance.

Since the dawn of the antibiotic era, many new classes of antibiotics have been produced, both naturally isolated and synthetically produced.[1] These are now more important than ever with the aggressive uprising of antibiotic-resistance we see today.[2] Research shows that the mortality rate for patients with diseases caused by antibiotic resistant bacterium is almost twice that of a patient with its non-resistant counterpart.

1.2 (+)-Negamycin; a potential dual-purpose medicament



1

Scheme 1.1: Structure of (+)-Negamycin (**1**)

(+)-Negamycin (**1**) (See Scheme 1.1) is a naturally ocurring dipeptide-like compound that may be isolated from the *Streptomyces purpeofuscus* bacteria, originally found in japanese soil.[3] The compound **1** has shown considerable levels of biochemical activity against both gram positive and gram-negative bacteria, giving merit to further research in hopes of revealing a new class of antibiotics. Since it's discovery in 1970,[4] many syntheses have been proposed with varying success and effiency. The earliest of these approaches aimed to synthesise negamycin racemically. These do not afford the target product in satisfying yields with respects to the complexity of separation. Additionally, the biological activity of racemic negamycin is onyl 50% that of (+)-negamycin (**1**).[5] This paper will therefore focus on the asymmetric synthesis of (+)-negamycin (**1**).

In addition to its antibacterial properties, the compound **1** has also showed great potential against Duchenne's muscular dystrophy (DMD).[6] The disorder is a result of a rare mutation in the gene for the protein dystrophin, which is responsible for connecting the muscular tissue to the surrounding cells. This mutation causes the translation of the gene to end prematurely, by producing termination-codons where there should be none. The result is a loss in dystrophin production which in turn progressively damages muscle

tissue. The disease is ultimately fatal. (+)-Negamycin, along with other aminoglycoside-derived antibiotics make it possible to "read through" these codons, rendering them irrelevant. The advantage of negamycin compared to other aminoglycoside antibiotics (e.g. gentamicin) is its comparatively low toxicity and weaker side effects.

1.3 Objective

In this paper, (+)-negamycin's role and possible future appliance as an antibiotic and as a medicament against Duchenne's muscular dystrophy will be discussed and highlighted. Benefits and weaknesses of the compound will also be compared to other antibiotics and DMD treatments. Further, the compound's various syntheses will be compared and discussed in detail to investigate its viability as a commercial drug.

2 Theory

2.1 Antibiotic resistance and (+)-negamycin

Studies have shown an absolute correlation between the use of antibiotics and resistance against such medicine in bacteria.[7] Resistance within a colony of bacteria may be inherited upon cell division or by genetic transfer via plasmids. The latter is called horizontal gene transfer and enables resistance to spread to other species. In addition, bacteria may mutate to acquire resistance.

(+)-Negamycin's (**1**) antibiotic activity stems from its ability to bind to the ribosomes of the bacterial cell, inhibiting translocation, a vital step during the protein synthesis which enables the ribosome to move along the mRNA thread.[8] This behaviour is usually observed in aminoglycosidic antibiotics, which (+)-negamycin (**1**) does not share its structure with.[9] The antibiotic activity of (+)-negamycin (**1**) is therefore puzzling to many. Studies have been performed to further investigate its mechanism and importance of its stereocenters for these mechanisms.[10]

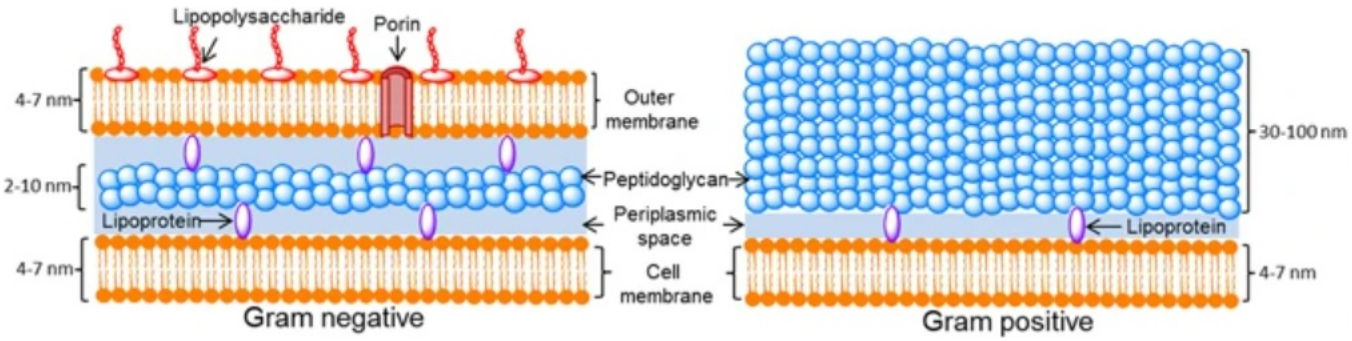


Figure 2.1: Structure of cell walls for gram-negative and positive bacteria. Reprinted from Berezin *et al.* (2017).[11] Licensed under CC BY 4.0, for more information see *https://creativecommons.org/licenses/by/4.0*

One of the key traits of **1** is its antibiotic activity against gram-negative bacteria (see Figure 2.1).[11][10][8] These bacteria have an additional outer membrane compared to

gram-positive. The outer membrane contains lippolysaccharides and serves as an extra precautionary measure for the bacteria, hindering certain antibiotics entering the cell. This gives the gram-negative bacteria an intrinsic resistance towards most antibiotics. Gram-negative bacteria can also acquire resistance against several antibiotic classes, making them increasingly dangerous. Such multidrug resistant bacteria may prove to be one of the biggest challenges the healthcare industry faces for years to come. The most severe of these gram-negative infections are usually caused by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter*.

2.2 A new treatment for Duchenne's muscular dystrophy

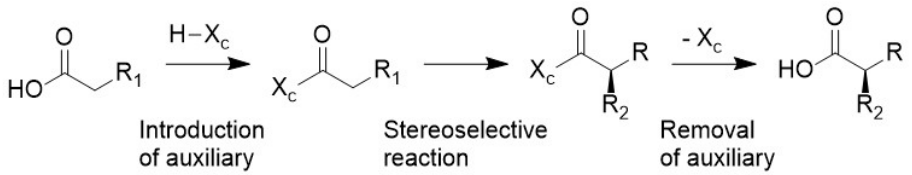
The compound **1** has also shown significant potential as a drug against Duchenne's muscular dystrophy.[8] Muscular dystrophies cause patients to progressively lose their strength ultimately ending with respiratory or cardiac failure.[12] Duchenne's is caused by a mutation in the gene responsible for the production of the protein dystrophin. This protein is required to maintain normal muscle breakdown and repair. Without it however, fatty tissue will progressively build up on muscle breakdown. The disease may be inherited, and is usually diagnosed within the first 3-4 years. There is no known cure for DMD, and research is mainly focused on the longevity of its patients.[6] Current treatment of the disease use glucocortoids therapeutically to prolong ambulation and minimise severe complications.

(+)-Negamycin's (**1**) potential against Duchenne's muscular dystrophy lies in its capability to "read through" the premature termination codons (PTCs) caused by nonsense mutations,[6] but not the normal ones. This way, the cell may produce full-length dystrophin proteins.

When tested on mice, studies found that the LD_{50} of negamycin (**1**) by intravenous injection was between 400-500 mg/kg.[4] Further, daily injections of 200 mg/kg showed no toxicity toward the mice, indicating few side effects of prolonged use.

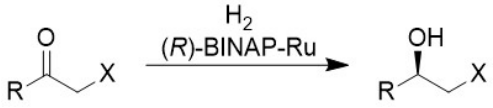
2.3 Synthetic approaches

The synthetic approaches may be divided into chiral pool, chiral auxiliary and catalytic methods.[13] The chiral pool method works by using a cheap chiral molecule which is commercially available as a pure enantiomer, and utilising it as a chiral "scaffold" for further synthesis. The method may be easily summarized as "building" upon a cheap naturally occuring chiral compound. The chiral "pool" refers to a selection of different naturally occuring molecules commonly used for synthesis, such as amino acids, carbohydrates, hydroxy acids and terpenes.



Scheme 2.1: General reaction utilizing an auxiliary $\mathbf{X}_c$ to control the stereochemical configuration of the product.[14]

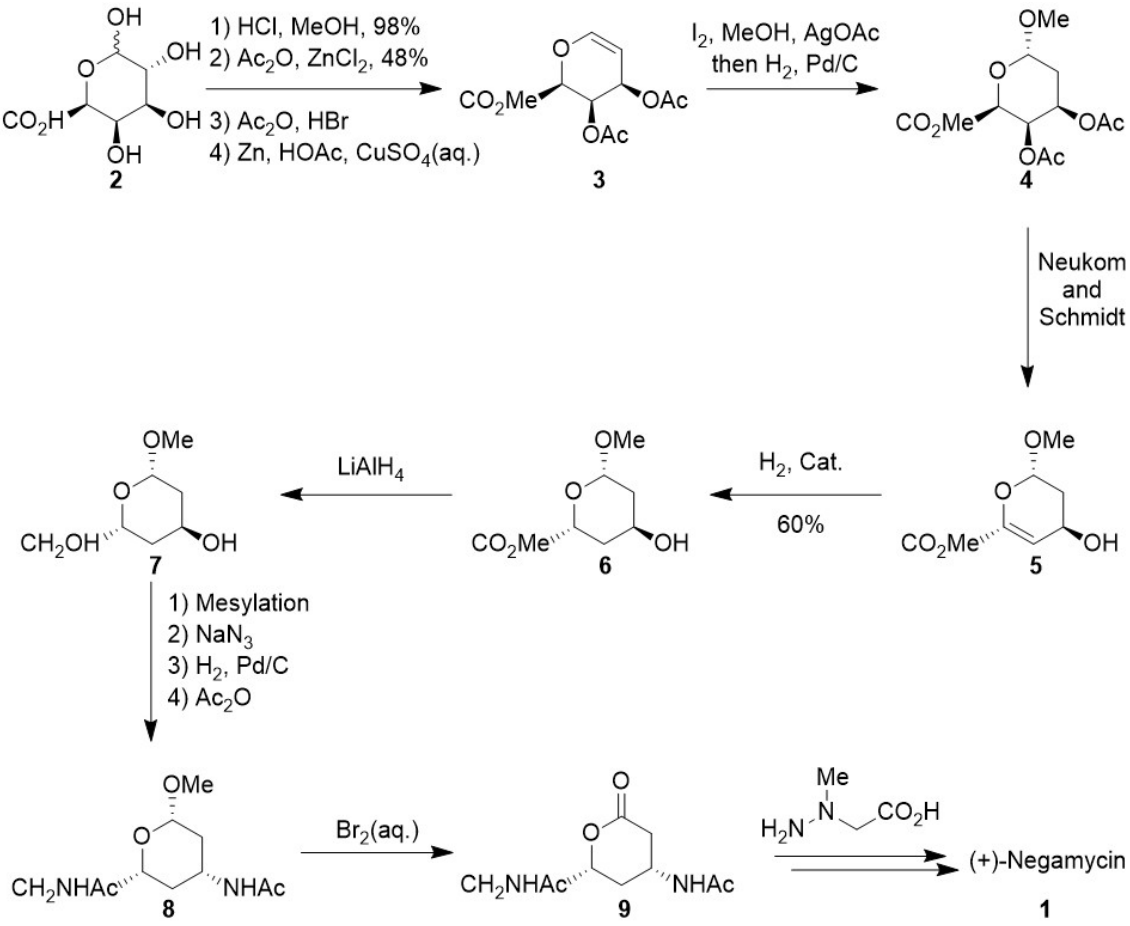
The chiral auxiliary method appends an auxiliary group $\mathbf{X}_c$ into the chiral molecule to direct the stereoselectivity of the reaction.[14] The group may be then be removed and reused in the future. A general example of this approach is illustrated in Scheme 2.1.



Scheme 2.2: An enantioselective reaction using a 2,2-bis(diphenylphosphino)-1,1-binaphthyl ruthenium catalyst ((*R*)-BINAP-Ru).[15]

The catalytic method uses enantioselective catalysts, usually with chiral ligands, to produce the target compound.[16][15] Because it is possible to regenerate such catalysts, they may be used indefinitely to produce chiral products. An example of this type of reaction is illustrated in Scheme 2.2.

2.4 Total synthesis



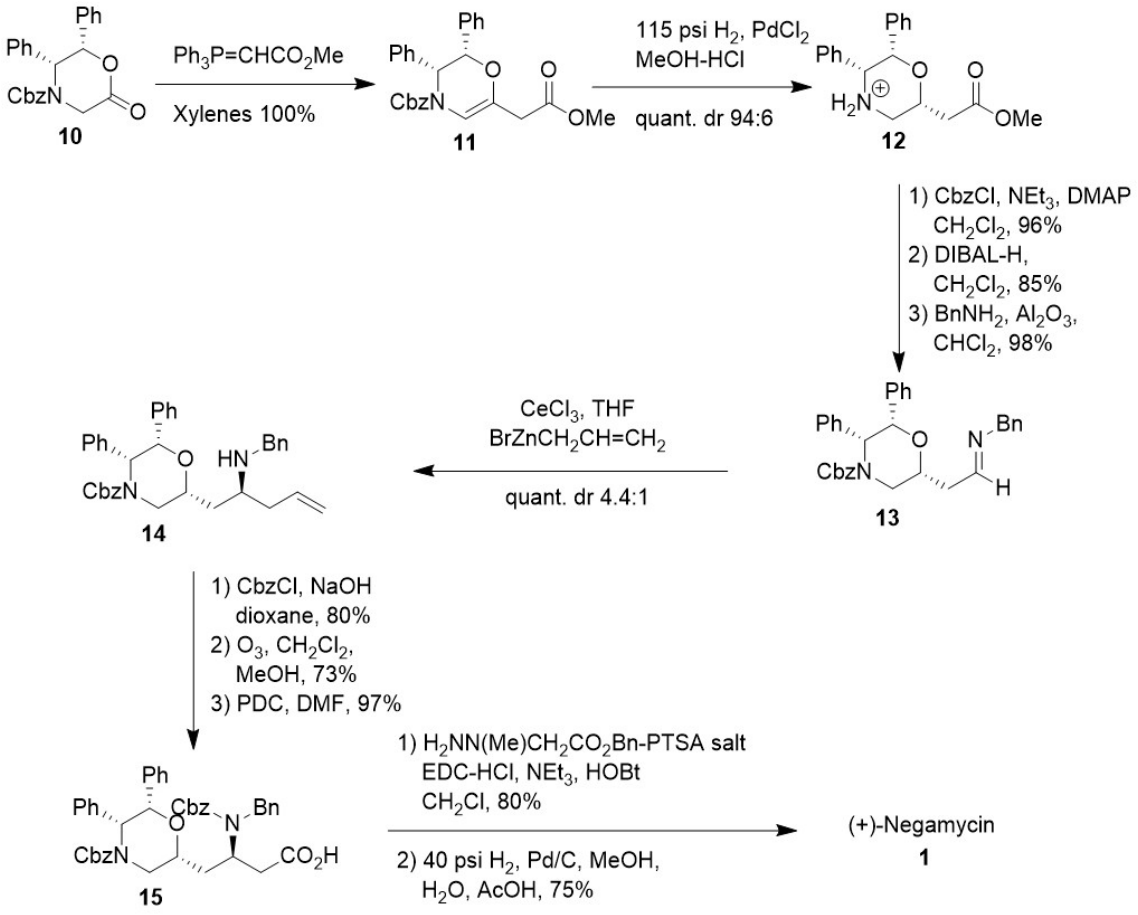
Scheme 2.3: Shibahara's total synthesis of (+)-negamycin (**1**).[17][18]

The first total synthesis of negamycin (**1**) was first reported by Shibahara *et al.*[17][18] in 1972 (see Scheme 2.3). Their synthesis is as follows; Starting with *D*-galacturonic

acid (**2**), glycal **3** was produced by methyl esterification, acylation of the triol, bromination of the anomeric carbon and finalized by zinc-promoted debrominative elimination. The glycal **3** was then treated with iodine dissolved in methanol with silver acetate, followed by catalytic hydrogenation with Pd/C to produce compound **4**. Neukom and Schmidt's method[19] was used to produce compound **5**. Further, catalytic hydrogenation yielded methyl(methyl 2,4-dideoxy-β-*L-erythro*-hexosid)uronate (**6**). A reduction of the carboxylic group by $LiAlH_4$ gave methyl 2,4-dideoxy-β-*L-erythro*-hexopyranoside (**7**). The two hydroxyl groups were then mesylated. The compound was then treated with sodium azide, hydrogenated with Pd/C and acetylated with Ac_2O to yield methyl 3,6-diacetamido-2,3,4,6-tetradeoxy-β-*L-threo*-hexapyranoside (**8**). The amino acid moiety of negamycin (**1**) was obtained by hydrolyzing and oxidizing **8** with aqueous bromine to afford **9**. By further treatment with *N*-methylhydrazinoacetic acid and deprotection with HBr-AcOH gave racemic negamycin in 40% yield.

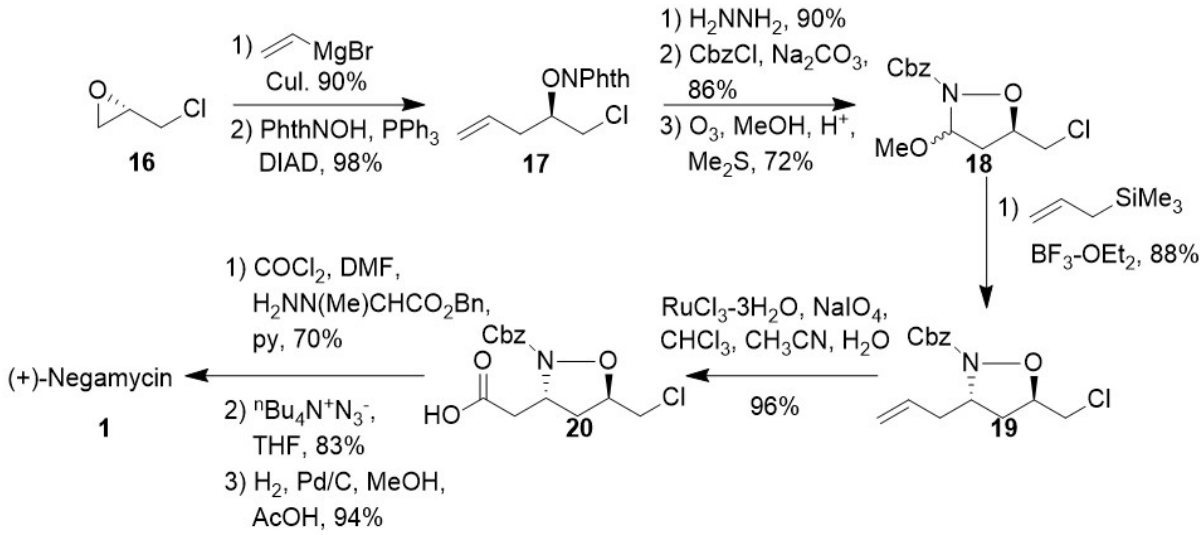
Since Shibahara and his team's discovery, many syntheses of **1** have been explored. The following syntheses are some of the more promising ones have been picked out for further discussion and comparison.

2.4.1 Chiral pool



Scheme 2.4: Williams and Jain's total synthesis of (+)-negamycin (**1**)[20]

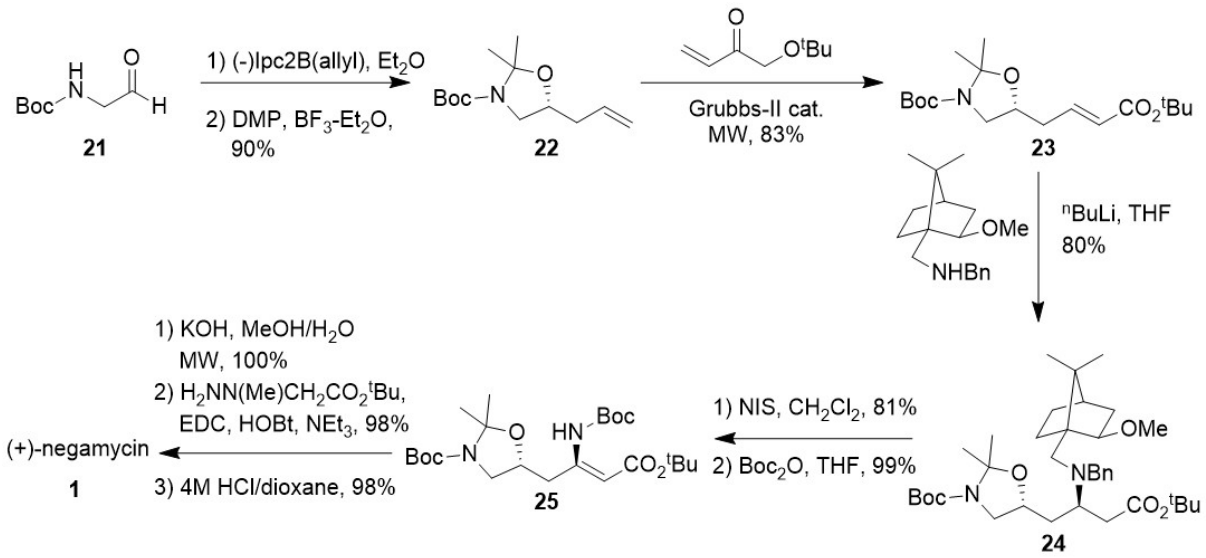
Williams and Jain's total synthesis of (+)-negamycin (1).[20]
By Wittig reaction with methyl (triphenylphosphoranylidene)acetate and isomerization, the lactone **10** (see Scheme 2.4) was converted into compound **11**, which was hydrogenated with $PdCl_2$ into all syn-substituted oxazine **12**. The secondary amine was benzyl chloroformate (Cbz) protected, the ester group reduced to aldehyde by diisobutylaluminium hydride (DIBAL-H) and treatment with benzylamine afforded imine **13**. Chelation controlled allylation of **13** was done by exposure to an allylzinc reagent in the presence of anhydrous cerium chloride. The resulting product **14** was protected using Cbz-Cl, and the terminal olefin was oxidatively cleaved to give **15** upon purification. Finally, **15** was condensated with a *p*-toluenesulfonic acid (PTSA) salt of benzyl (1-methylhydrazino)acetate and deprotected globally by hydrogenolysis to yield (+)-negamycin(**1**) in an overall yield of 25% over 11 steps.



Scheme 2.5: Bates' total synthesis of (+)-negamycin (**1**).[21]

Bates' *et al.* total synthesis of (+)-negamycin (1).[21]
Bates' *et al.* used isoxazolidine allylation to synthesise (+)-negamycin (**1**) in 2014 (see Scheme 2.5). The epoxide ring **16** was opened using vinyl magnesium bromide, catalysed by copper iodide. The resulting compound was treated with N-hydroxy-phtalamide through Mitsonobu reaction[22], and subsequently had its protecting groups exchanged to yield **18**. Compound **18** was created as a mixture of diastereomers using ozonolysis. Further, Sakurai reaction[23] converted it into **19** as a single stereoisomer. The terminal olefin was oxidated and cleaved under Sharpless conditions to generate **20** and subsequently treated with benzyl(1-methylhydrazino)acetate. The azide groups were introduced using *tetra*-n-butylammonium azide. Finally, the compound was hydrogenated to afford (+)-negamycin (**1**) in a 23% yield over 10 steps.

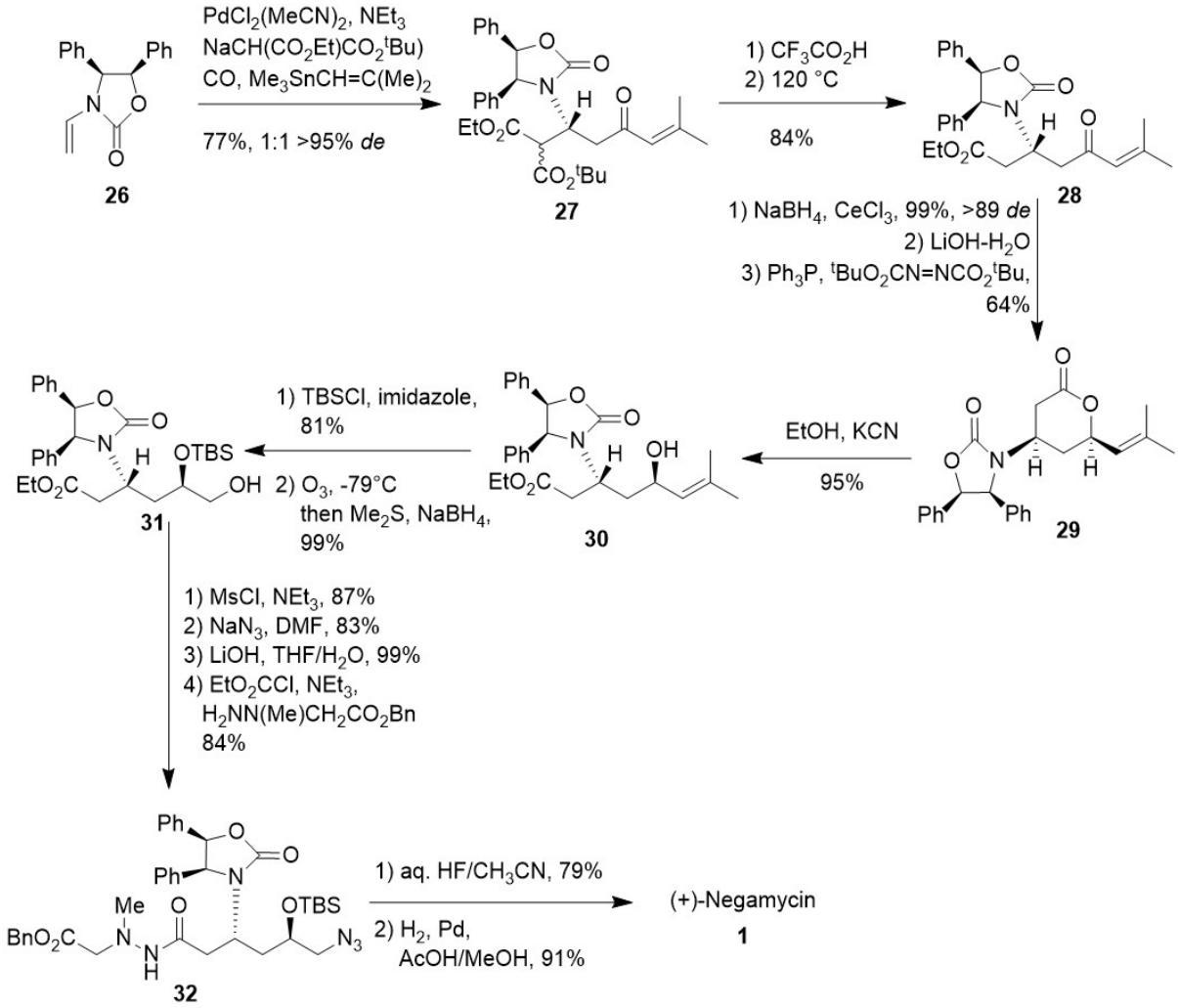
2.4.2 Chiral auxiliary



Scheme 2.6: Hayashi's total synthesis of (+)-negamycin (**1**).[24]

Hayashi's *et al.* total synthesis of (+)-negamycin (1).[24]
N-Boc-glycinal (**21**) (Boc = *tert*-butoxycarbonyl) was chosen as the starting compound and subjected to asymmetric allylation (see Scheme 2.6). This was followed by DMP-protection to give intermediate **22**. Cross-metathesis reaction of **22** and *tert*-butyl acrylate catalyzed by Grubbs second-generation [Ru-II]-catalysts yielded **23**. Asymmetric Michael addition with a chiral amine was used to produce a second chiral center into **24**. The auxiliary was removed by treatment with *N*-iodosuccinimide, and the amine Boc protected to produce **25**. The *tert*-butyl ester was hydrolyzed into its corresponding acid which was in turn coupled with the hydrazine moiety. The product was then deprotected to afford (+)-negamycin (**1**) in a 42% yield over 8 steps.

Hegedus' *et al.* total synthesis of (+)-negamycin (1).[25]
Starting compound **26** was treated with $PdCl_2(MeCN)$ (see Scheme 2.7), the sodium anion of *tert*-butyl ethyl malonate and isobutenyltrimethylstannane to produce **27** as a 1:1 mixture of diastereomers. This mixture was further hydrolyzed with trifluoroacetic acid and decarboxylyzed to yield **28**. Diastereoselective reduction using sodium borohydride and cerium trichloride, was used to converge the mixture into one of 18:1 (*S*) selectivity. Hydrolysis and subsequent Mitsonobu reaction[22] produced lactone **29**. Subsequent heating of **29** in the presence of catalytic amounts of KCN afforded allylic alcohol **30** in good yield. The hydroxyl group was protected using TBS, followed by ozonolysis and reduction of the C-C double bond to give **31**. The alcohol group was mesylated and treated with sodium azide, followed by mild saponification produced **32**. The compound was finally deprotected and hydrogenated to afford (+)-negamycin (**1**) in a 13% yield over 15 steps.

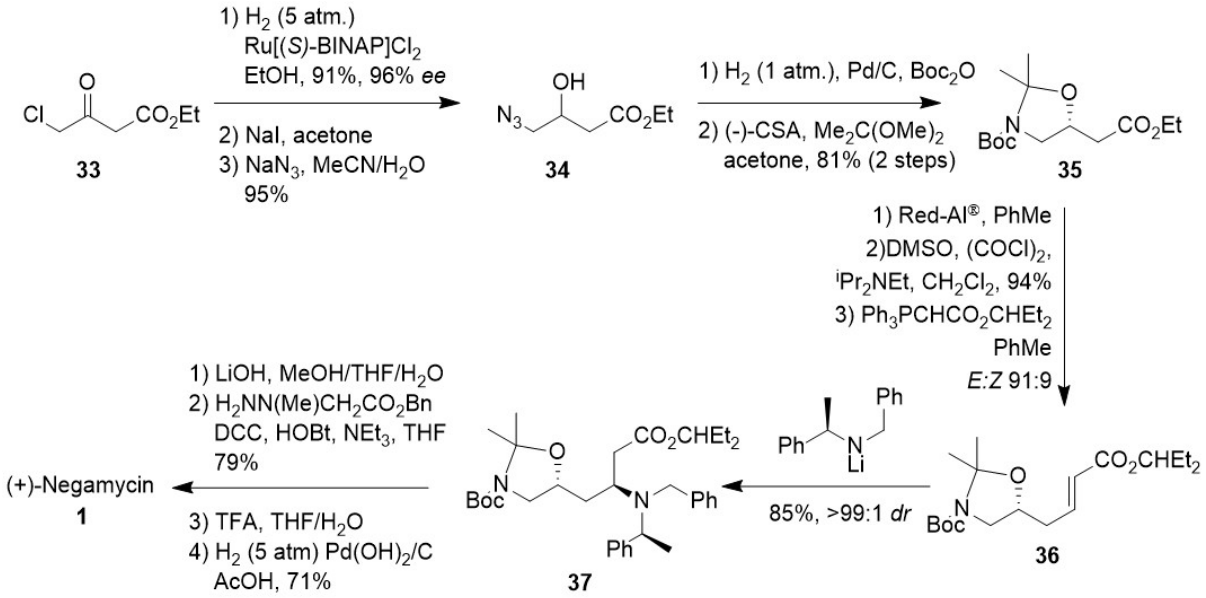


Scheme 2.7: Hegedus' total synthesis of (+)-negamycin **1**.[25]

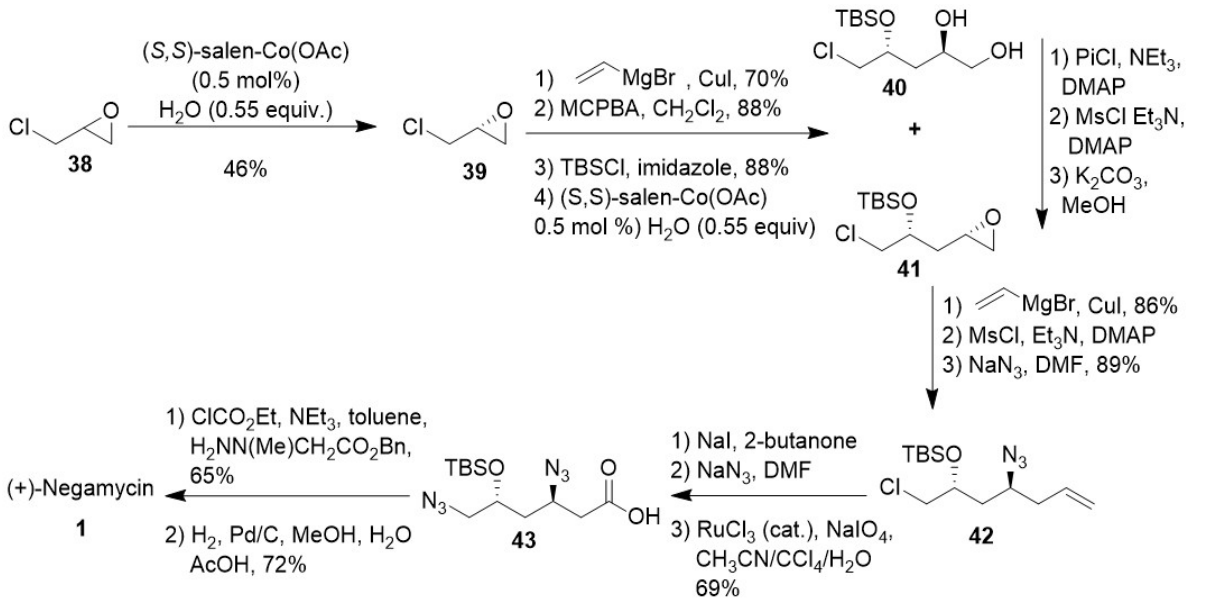
2.4.3 Enantioselective catalysis

Davies' and Ichihara's total synthesis of (+)-negamycin (1).[26]
Ethyl 4-chloroacetoacetate (**33**) was asymmetrically hydrogenated and iodinated (see Scheme 2.8). The iodo ester was displaced by sodium azide to produce **34**. Hydrogenation of the azide, Boc protection and acetonide formation gave **35**, which was reduced, Swern oxidated and Wittig olifinated to give (*E*)-**36**. This was treated with chiral lithium amide by Michael addition to give **37**. The ester was hydrolysed to produce its corresponding acid, coupled with benzyl(1-methyl hydrazino)acetate, deprotected and finally hydrogenated to produce (+)-negamycin (**1**) in a 27% yield over 13 steps.

Kumar's *et al.* total synthesis of (+)-negamycin (1).[27] Racemic epichlorohydrin **38** was subjected to Jacobsen's hydrolytic kinetic resolution to give **39** (see Scheme 2.9. The epoxide was reacted with vinylmagnesium bromide and the resulting alcohol, TBS protected and epoxidized. This produced a diastereomeric mixture of epoxides which was run through Jacobsen's hydrolytic kinetic resolution again to diastereomerically purify the epoxides into **40** and **41**.[28] **40** was converted into **41** in 3 steps. The ring was opened



Scheme 2.8: Davies' total synthesis of (+)-negamycin **1**.[26]



Scheme 2.9: Kumar's total synthesis of (+)-negamycin **1**[27]

using vinylmagnesium bromide, and the alcohol converted into azide **42**. The cloride was replaced by azide and activated by iodonation. The acid was formed by oxidatively cleaving the terminal olefin, and was treated with benzyl(1-methylhydrazino)acetate. The compound was finally hydrogenated to yield (+)-negamycin (**1**) in a yield of 4.6% over 13 steps.

All of the above mentioned approaches use chromatography on Amberlite CG50 to purify their product, with the exception of Kumar *et al.* which has not reported their means of separation.

3 Discussion

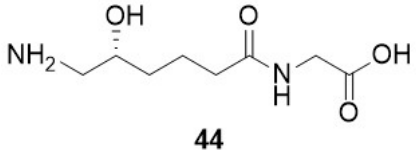
3.1 Biochemical activity

(+)-Negamycin **1** and aminoglycosides enter the bacteria without the need of a membrane carrier.[10] Research has therefore been focused on keeping this key trait, as well as modifying the compound to enhance its antibiotic effect.

Research has shown that inversions of both C3 and C5 stereocenters (see Scheme 1.1) impair its biological activity,[10] and that the (3*R*, 5*R*) configuration is the optimal configuration for such activity, showing an approximate ten-fold loss in activity for the inversion of C3, and a 25-fold loss in activity for the inversion of C5. By modifying the functional groups of the (+)-negamycin **1** through structure-activity relationship analysis (SAR), researchers have been able to identify which groups are vital to its activity and which are not. SAR showed that the removal of either terminal end, amino group and/or acid group, resulted in significant loss of biochemical activity.[8] Whereas the removal of the alcohol group did not impair its activity. Modifications to the functional groups on C5 and C6 have shown promising results in relation to increasing the biological activity, giving reason to further research into negamycin analogues such as deoxynegamycin and its derivatives.

Unlike aminoglycosides however, **1** has several alternate binding sites on the bacterial ribosomes.[8] This could potentially hinder the onset of ototoxicity, a common side effect of aminoglycoside antibiotics which causes damage to the ears and hearing, for patients in need of anitbiotic treatment. These alternate binding sites could also make **1** a tougher challenge for the bacteria when it comes to developing resistance.

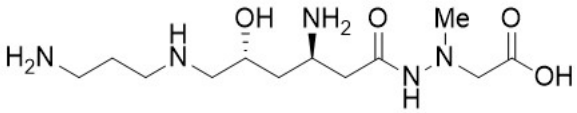
(+)-Negamycin's (**1**) potential as a Duchenne's muscular dystrophy medicament is promising in terms of alleviating symptoms and prolonging a patient's life by[29] Along with aminoglycosidic antibiotics, **1** has shown read-through activity, causing the mutation in the dystrophin gene to be suppressed and to restore the genes function back to normal. This could potentially slow the progression of the disease to a minimum. Medicaments used today in the fight against genetic diseases, such as DMD, have serious side effects that may outweigh the benefits for the patient over time. (+)-Negamycin (**1**) does not show these same side effects when tested in mice. In addition, comparing the median lethal dosage for an intravenous one-time dose of **1** (LD_{50} = 400 mg/kg)[4] in mice to those of gentamicin (LD_{50} = 70 mg/kg)[30] and kanamycin (LD_{50} = 200 mg/kg)[30] evidently shows a much lower toxicity for (+)-negamycin (**1**). These compounds were chosen as suitable comparisons due to their similarity in terms of purpose, both as antibiotics and DMD-medicament. Further research is required to see what potential side effects (+)-negamycin (**1**) may have. The compound **1** has not yet been approved for use in humans.



44

Scheme 3.1: Analogue of (+)-negamycin showing potent read-through activity, as reported by Taguchi *et al.*[6]

In 2012, Taguchi *et al.* reported the syntheses of a series of (+)-negamycin (**1**) analogues.[6] These were evaluated based on their read-through activity in relation to their effect on Duchenne's muscular dystrophy. Their activity was expressed as a ratio compared to gentamicin, another drug promoting read-through activity. Taguchi and his team found that while (+)-negamycin had approximately the same activity as gentamicin, an analogue (see Scheme 3.1) showed much better read-through activity, with 1.34 that of gentamicin. The analogue was comprised of (+)-negamycin (**1**) which had its amino group and N-methyl group omitted. It is important to note that while **44** showed excellent read-through activity, it showed no antimicrobial activity, meaning its potential lies solely in DMD-treatment.



45

Scheme 3.2: Analogue of (+)-negamycin with improved antibacterial activity, as reported by Mckinney *et al.*[10]

Three years later, in 2015, McKinney and his team reported the findings and testing of several new (+)-negamycin analogues (**1**).[10] Their most promising analogue is illustrated in Scheme 3.2. **45** was developed by thourough SAR investigation. While most of their reported analogues showed minimum inhibitory concentrations (MICs) of between 50-300 μg/mL, **45** displayed a MIC of only 4-16 μg/mL for all tested bacterial strains, e.g. *E. Coli*, *K. Pneumoniae.* This makes it the most potent reported antibacterial analogue of (+)-negamycin (**1**) to date. Additionally, the terminal amine of **45** showed an additional binding interaction, not seen before in **1**.

3.2 Total synthesis

Since it was first discovered in 1970 by Hamada and his team of researchers,[4] (+)-negamycin (**1**) has proven to be a challenging synthetic target. Shibahara *et al.* (see Scheme 2.3) lay the ground work for further experimentation by reporting the first total synthesis of **1** in 1972,[17] and by being the first to reveal a distinct difference in biological activity based on changes of its absolute configuration. However, the relatively low yield coupled with its racemic end-product make it an ill-suited procedure for large scale development.

Chiral pool approaches have been efficient in producing **1** in satisfying quantities while keeping the amount of steps to a minimum. This has been exemplified by Williams and Jain (see Scheme 2.4).[20] Their total synthesis used (5*R*,6*S*)-4-(benzyloxycarbonyl)-5,6-diphenyl-2,3,5,6-tetrahydro-4H-1,4-oxazin-2-one (**10**) as starting compound, a commercially available lactone. The synthesis differs from earlier reported ones in that it does not rely on the pre-existing stereogenic centers in the starting compound. Further research into whether this technique is applicable for other amino acid-derived products may prove useful for future research. Williams' high yield and few steps, together with the low price of precursor compounds inherent to chiral pool approaches, make it one of the more effective syntheses of **1**.The same is true for Bates and his teams approach through the

same chiral pool strategy (see Scheme 2.5).[21] They both present similar yields (25% for Williams and 23% for Bates) with a similar amount of steps (10-11), and both reported specific optical rotations similar to that of the pure enantiomer (lit; $[\alpha]_D^{20} \sim 2.7$(c 1.6, H_2O))[31]

The auxiliary approaches come at a certain cost compared to the other strategies.[14] Among these is the additional compounds necessary in the form of auxiliaries. The addition and removal of the auxiliary also introduce additional steps to the synthesis. Despite this, the auxiliary method is often necessary to synthesise bigger and more complicated molecules. Of all approaches discussed in this paper, Hayashi's *et al.* (see Scheme 2.6) chiral auxiliary approach outshines the rest when it comes to yield and reaction steps,[24] exhibiting an astounding 42% yield over 8 steps compared to the more modest 13% yield over 15 steps for Hegedus' (see Scheme 2.7) synthesis.[25] This difference correlates well with the difference in steps for the two reactions.

Enantioselective catalysts are exemplary in that they are often cheaper in the long run, due to the catalysts regenerative properties, and their high enantiomeric excess compared to chiral pool and auxiliary approaches.[16] Both Davies' (see Scheme 2.8) and Kumar's (see Scheme 2.9) syntheses use 13 steps to afford the product **1**.[26][27] However, Davies' showed a much higher yield of 27% compared to the 4.6% yield of Kumar's synthesis. The comparatively low yield of Kumar's synthesis may be partly because of its first step involving Jacobsen's hydrolytic kinetic resolution and the choice of catalyst therein. The 46% yield of this reaction compared to Davies' catalytic step with 96% shows a considerable weakness in the synthesis efficiency.

Hayashi and his team's approach clearly outperforms the rest, both in yield and steps. To better understand how **1** could be produced for commercial use, their synthesis (see Scheme 2.6) will be discussed in further detail alone. One of their key steps is the preparation of **23** using the Grubbs second generation catalyst to induce a cross-metathesis (CM) reaction between the *tert*-butyl acrylate and **22**. Reactions involving such CM are prone to low yields because of the multiple possible side reactions. Hayashi *et al.* therefore optimalized their reaction to minimize these alternate reactions. However, the catalyst is very expensive and significantly lowers the synthesis' total yield even after optimalization, and could therefore jeopardize future efforts to commercialize the synthesis. The steps where the chiral auxiliary is introduced and removed also see yields around 80% leading to a considerable drop in efficiency overall. Further optimalization of these reaction steps would benefit the commercialization of the (+)-negamycin (**1**).

4 Conclusion

(+)-Negamycin shows great promise in treating diseases caused by gram-positive and gram-negative bacteria, as well as alleviating symptoms and slowing the progression of Duchennes muscular dystrophy. Several analogues of **1** have been developed. Some of these have displayed increased read-through and antibacterial activity. Hopefully, further development of such derivatives and analogues can contribute to the discoveries of multiple new DMD-treatments and antibiotics. Compared to aminoglycoside antibiotics, which are used for similiar purposes, (+)-negamycin shows lower toxicity and fewer side effects. It is important to emphasize that (+)-negamycin, as of April 2020, has not been approved for human testing, and that further research is required to guarantee its safety and viability.

Hayashi and his team's route of synthesis is much more efficient in terms of steps, while affording (+)-negamycin in excellent yield compared to its competition. The synthesis' utilization of a Grubbs' catalysator together with the introduction and removal of the chiral auxiliary are its main weak points, and should therefore be the focus of future procedural development. It is the most efficient synthesis to date and the approach could be used in future research to discover derivatives with greater antibiotic and read-through potency. By continuing optimalization efforts, a new class of antibiotics may be on the horizon.

Trondheim April 30, 2020,

Nicholas A. Janjua

References

[1] Aminov, R. I. A Brief History of the Antibiotic Era: Lessons Learned and Challenges for the Future. *Front. Microbio.* **2010**, *1*, 134.

[2] Exner, M. et al. Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? *GMS Hygiene and Infection Control; 12:Doc05* **2017**,

[3] Reimer, L.; Sarda Carbasse, J.; Podstawka, A.; Overmann, J. Streptomyces purpeofuscus Yamaguchi and Saburi 1955. 2019; doi:10.13145/bacdive15498.20191129.4.1.

[4] Hamada, M.; Tadeuchi, T.; Kondo, S.; Ikeda, Y.; Naganawa, H.; Maeda, K.; Okami, Y.; Umezawa, H. A new antibiotic, negamycin. *J. Antibiot.* **1970**, *23*, 170–171.

[5] Streicher, W.; Einshagen, H.; Rnowsky, F. Total synthesis of rac. negamycin and of negamycin analogs. *J. Antibiot.* **1978**, *31*, 725–728.

[6] Taguchi, A.; Nishiguchi, S.; Shiozuka, M.; Nomoto, T.; Ina, M.; Nojima, S.; Matsuda, R.; Nonomura, Y.; Kiso, Y.; Yamazaki, Y.; Yakushiji, F.; Hayashi, Y. Negamycin Analogue with Readthrough-Promoting Activity as a Potential Drug Candidate for Duchenne Muscular Dystrophy. *ACS Med. Chem. Lett.* **2012**, *3*, 118–122.

[7] Ventola, C. L. The antibiotic resistance crisis: part 1: causes and threats. *Phys. Ther.* **2015**, *40*, 277–283.

[8] Zhu, L.; Hong, R. Pursuing effective Gram-negative antibiotics: The chemical synthesis of negamycin. *Tetrahedron Lett.* **2018**, *59*, 2112–2127.

[9] Uehara, Y.; Kondo, S.; Umezawa, H.; Suzukake, K.; Hori, M. NEGAMYGIN, A MISCODING ANTIBIOTIC WITH A UNIQUE STRUCTURE. *J. Antibiot.* **1972**, *25*, 685–688.

[10] McKinney, D. C.; Basarab, G. S.; Cocozaki, A. I.; Foulk, M. A.; Miller, M. D.; Ruvinsky, A. M.; Scott, C. W.; Thakur, K.; Zhao, L.; Buurman, E. T.; Narayan, S. Structural Insights Lead to a Negamycin Analogue with Improved Antimicrobial

Activity against Gram-Negative Pathogens. *ACS Med. Chem. Lett.* **2015**, *6*, 930–935.

[11] Berezin, S.; Aviv, Y.; Aviv, H.; Goldberg, E.; Tischler, Y. R. Replacing a Century Old Technique – Modern Spectroscopy Can Supplant Gram Staining. *Sci. Rep.* **2017**, *7*, 3810.

[12] Sinha, R.; Sarkar, S.; Khaitan, T.; Dutta, S. Duchenne muscular dystrophy: Case report and review. *J. Family Med. Prim. Care* **2017**, *6*, 654.

[13] Hanessian, S. Reflections on the total synthesis of natural products: Art, craft, logic, and the chiron approach. *Pure Appl. Chem.* **1993**, *65*, 1189–1204.

[14] Gnas, Y.; Glorius, F. Chiral Auxiliaries - Principles and Recent Applications. *Synthesis* **2006**, *2006*, 1899–1930.

[15] Kitamura, M.; Ohkuma, T.; Inoue, S.; Sayo, N.; Kumobayashi, H.; Akutagawa, S.; Ohta, T.; Takaya, H.; Noyori, R. Homogeneous asymmetric hydrogenation of functionalized ketones. *J. Am. Chem. Soc.* **1988**, *110*, 629–631.

[16] Halpern, J.; Trost, B. M. Asymmetric Catalysis Special Feature Part I: Asymmetric Catalysis. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5347–5347.

[17] Shibahara, S.; Kondo, S.; Maeda, K.; Umezawa, H.; Ohno, M. Total syntheses of negamycin and the antipode. *J. Am. Chem. Soc.* **1972**, *94*, 4353–4354.

[18] Kondo, S.; Shibahara, S.; Takahashi, S.; Maeda, K.; Umezawa, H.; Ohno, M. Negamycin, a novel hydrazide antibiotic. *J. Am. Chem. Soc.* **1971**, *93*, 6305–6306.

[19] Schmidt, H.; Neukom, H. 4-Deoxy uronic acids. *Carbohydr. Res.* **1969**, *10*, 361–369.

[20] Jain, R. P.; Williams, R. M. Asymmetric Synthesis of (+)-Negamycin. *J. Org. Chem.* **2002**, *67*, 6361–6365.

[21] Bates, R. W.; Khanizeman, R. N.; Hirao, H.; Tay, Y. S.; Sae-Lao, P. A total synthesis of (+)-negamycin through isoxazolidine allylation. *Org. Biomol. Chem.* **2014**, *12*, 4879.

[22] Mitsunobu, O.; Yamada, M. Preparation of Esters of Carboxylic and Phosphoric Acid *via* Quaternary Phosphonium Salts. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380–2382.

[23] Hosomi, A.; Sakurai, H. Syntheses of ,-unsaturated alcohols from allylsilanes and carbonyl compounds in the presence of titanium tetrachloride. *Tetrahedron Lett.* **1976**, *17*, 1295–1298.

[24] Hayashi, Y.; Regnier, T.; Nishiguchi, S.; Sydnes, M. O.; Hashimoto, D.; Hasegawa, J.; Katoh, T.; Kajimoto, T.; Shiozuka, M.; Matsuda, R.; Node, M.; Kiso, Y. Efficient total synthesis of (+)-negamycin, a potential chemotherapeutic agent for genetic diseases. *Chem. Commun.* **2008**, 2379.

[25] Masters, J. J.; Hegedus, L. S. Palladium(II)-assisted difunctionalization of monoolefins: total synthesis of (+)-negamycin and (-)-5-epi-negamycin. *J. Org. Chem.* **1993**, *58*, 4547–4554.

[26] Davies, S. G.; Ichihara, O. Asymmetric synthesis of (+)-negamycin. *Tetrahedron Asymmetry* **1996**, *7*, 1919–1922.

[27] Naidu, S. V.; Kumar, P. A simple and efficient approach to 1,3-aminoalcohols: application to the synthesis of (+)-negamycin. *Tetrahedron Lett.* **2007**, *48*, 3793–3796.

[28] Tokunaga, M. Asymmetric Catalysis with Water: Efficient Kinetic Resolution of Terminal Epoxides by Means of Catalytic Hydrolysis. *Science* **1997**, *277*, 936–938.

[29] Arakawa, M. Negamycin Restores Dystrophin Expression in Skeletal and Cardiac Muscles of mdx Mice. *J. Biochem.* **2003**, *134*, 751–758.

[30] Fujisawa, K.-I.; Hoshiya, T.; Kawaguchi, H. AMINOGLYCOSIDE ANTIBIOTICS. VII. *J. Antibiot.* **1974**, *27*, 677–681.

[31] Davies, S. G.; Ichihara, O.; Roberts, P. M.; Thomson, J. E. Asymmetric syntheses of (+)-negamycin, (+)-3-epi-negamycin and sperabillin C via lithium amide conjugate addition. *Tetrahedron* **2011**, *67*, 216–227.

NTNU

Norwegian University of
Science and Technology