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Treball Final de Grau

A bibliographic review on capuramycin, a nucleosidic natural product with antibiotic properties.

Una revisió bibliogràfica sobre la capuramicina, un nucleòsid natural amb propietats antibiòtiques.

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Universitat de Barcelona

B:KC Barcelona Knowledge Campus
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"Si sapigués què és el que estic fent, no l'anomenaria recerca, veritat?"

Albert Einstein

M'agradaria expressar el meu agraïment als meus pares, que m'han escoltat i animat en tot moment per tirar endavant; al piro, perquè a part de ser un bon fotògraf i pirotècnic, en el fons, és un bon germà. Als meus avis, que un cop per setmana em donen tots els ànims i abraçades des de la distància.

També m'agradaria agraïr de forma molt 'cuquirrina' a les de la colla, que m'han donat carinyo i suport en tot moment des de Vilafranca i les trobo moltíssim a faltar; al 'capsi', per ser el millor cuiner donostiarra en temps de quarantena (i no quarantena) i per poder desconnectar del treball coneixent nous llocs d'Euskal Herria; a la Kerima i l'Izas, per aguantar-me al pis durant la quarantena (i no quarantena) i endinsar-me, ara sí de veritat, al món de l'esport i fer-me sentir millor amb mi mateixa, i pels vostres pastissos de formatge.

Finalment, agraïr al meu tutor Dr. Jordi Robles Brau, que m'ha ajudat, en tot moment, en tot allò que ha fet falta per dur a terme aquest treball i que m'ha donat ànims des de bon inici.

REPORT

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1. SUMMARY

Antibacterial resistance has become an urgent issue for human being and animal's health. Among these resistant bacteria stand out important pathogens such as *M. tuberculosis*, *M. smegmatis* and *S. pneumoniae*. Notably, these pathogens were inhibited by natural uridine antibiotics such as capuramycin, a compound first isolated from *Streptomyces griseus*. This type of antibiotics acts by inhibiting MraY, an enzyme involved in the biosynthesis of peptydoglican component of the bacteria cell wall, but showed low antibacterial activity. Consequently, capuramycin analogues have been prepared by organic synthesis and biosynthesis to allow structure-activity studies as a prior step towards the development of new antibacterial drugs.

In this context, this present TFG report contains a bibliographic review on capuramycin, with a particular emphasis on the synthetic routes of the compound and its derivatives, the elucidation of the biosynthetic pathways and their applications, and studies of structure-antibacterial activity.

2. RESUM

La resistència bacteriana ha esdevingut un tema urgent per la salut dels éssers humans i dels animals. Entre les bactèries resistents destaquen patògens importants com *M. tuberculosis*, *M. smegmatis* i *S. pneumoniae*. En concret, aquests patògens resulten ser inhibits per antibiòtics naturals derivats d'uridina com la capuramicina, un compost aïllat inicialment del *Streptomyces griseus*. Aquests antibiòtics actuen inhibint l'MraY, un enzim involucrat en la biosíntesi del peptidoglicà, un component de la paret cel·lular bacteriana, però mostren una activitat antibacteriana baixa. Arran d'això, s'han preparat anàlegs de la capuramicina mitjançant la síntesi orgànica i la biosíntesi amb què realitzar estudis d'estructura-activitat com a primer pas cap al desenvolupament de nous fàrmacs antibacterians.

En aquest context, la present memòria de TFG conté una revisió bibliogràfica de capuramicina, fent èmfasi en les rutes sintètiques del compost i els seus derivats, l'elucidació de les vies biosintètiques i les seves aplicacions, i els estudis d'estructura-activitat antibacteriana.

3. INTRODUCTION

3.1. ANTIBACTERIAL RESISTANCE THREAT

Throughout the years, the extreme adaptability of the bacteria populations led to bacterial strains increasingly more resistant to antibacterial agents. The main features of resistant bacteria are: their intrinsic properties to become resistant to drugs (natural resistance), mechanisms of exchanging genetic material (horizontal resistance), great capacity of survival against environmental conditions, and exponential reproduction [1].

The apparition of transmission of antibiotic resistance genes among bacterial strains is due, mainly, to the excessive use of drugs. Moreover, the social and technological changes have promoted the transmission of these microorganisms and the appearance of super resistant genes. Consequently, new infectious diseases have come up which can provoke a huge sanitary problem for human beings and animals. With this challenge, new antibacterial products are urgently needed, preferently of natural origin, to fight against these new resistant microorganisms [2].

With the aim to obtain these new antimicrobials, we might pay attention to the mechanisms of antibacterial activity. The mechanisms of action of antibacterial agents mostly include the inhibition of the following elements: nucleotides and nucleic acids (DNA or RNA), proteins, peptidoglycan wall and the cellular membrane. Here, we will focus on the mechanism of action of the antibacterial agents that interferes the building of peptidoglycan wall [3].

To understand the mechanism of this antimicrobials is important to know the steps of biosynthetic pathway of peptidoglycan. All of these steps are catalyzed by different enzymes which have become a great target for antibacterial agents as their activity is essential since its inhibition affects to the biosynthesis of peptidoglycan wall [4].

3.2. BIOSYNTHESIS OF BACTERIAL PEPTIDOGLYCAN

The peptidoglycan is one of the main components of the bacterial cellular wall that is located in the external part of the cytoplasmic membrane of most bacteria. In these microorganisms, it is

also referred as murein. Gram-positive bacteria characteristically have a thicker peptidoglycan layer than in Gram-negative bacteria, which have an additional external membrane [5]. The main functions of peptidoglycan are to give form to the cell, to maintain rigidity offering osmotic resistance and to provide a surface for the anchoring of other components as proteins and teichoic acids which also participate in bacterial growing and cellular division. It constitutes a main target against bacteria as the inhibition of its biosynthesis or its specific degradation, for instance by lysosomal enzymes, will provoke the cell lysis [4].

The usual structure of peptidoglycan is an heteropolymer composed by peptide and disaccharides formed by the alternation of *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) residues which are united with a β (1→4) glycosidic bond. *N*-acetylmuramic acid is attached to a peptide chain formed by three to five amino acids. Peptide chains are additionally cross-linked by amide bonds to produce a tridimensional mesh-like layer [6].

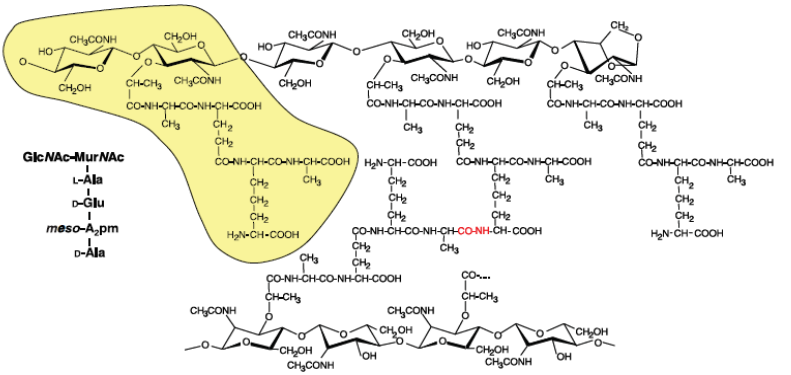
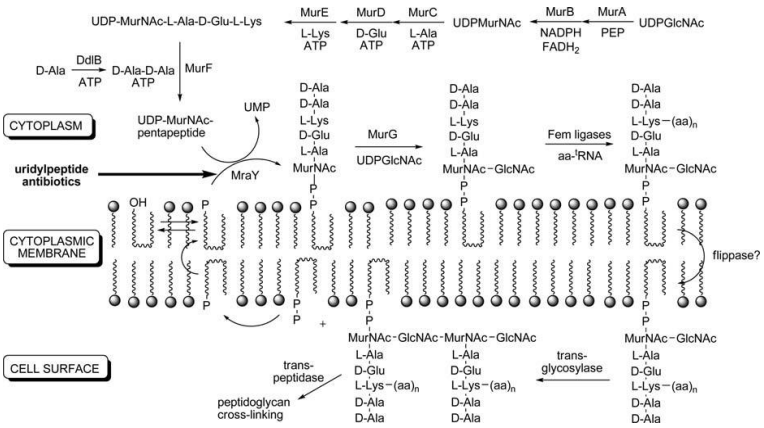


Fig. 1. Structure of the peptidoglycan of *Escherichia coli*. The encircled part in yellow represents the basic disaccharide tetrapeptide subunit (monomer), which is also written with the conventional amino acid and hexosamine abbreviations on the left-hand side. [The image was reproduced unaltered from reference no. 6].

The biosynthesis of peptidoglycan takes place in two different parts of the bacterial cell, the cytoplasm and the outer periplasmic space, as it is depicted in **fig. 2**.



Scheme 1. Peptidoglycan biosynthetic pathway, showing the site of action of the uridyl peptide antibiotics. [The image was reproduced unaltered from reference no. 7].

First, in the cytoplasm, UDP-MurNAc-pentapeptide is produced from UDP-GlcNAc by the catalytic activity of MurA-MurF enzymes (see **scheme 1**). Consequently, MraY enzyme transfers by phosphorylation the phospho-MurNAc-pentapeptide part to the undecaprenyl phosphate lipid obtaining lipid I which remains adhered to the plasmatic membrane. Finally, the MurG enzyme is in charge of forming a glycosidic bond (transglycosylation) between the monosaccharides GlcNAc and MurNAc to obtain lipid II. This lipid conjugate keeps adhered to the cytoplasmic membrane and it is driven to the periplasmic space by the catalytic activity of the flippase enzyme.

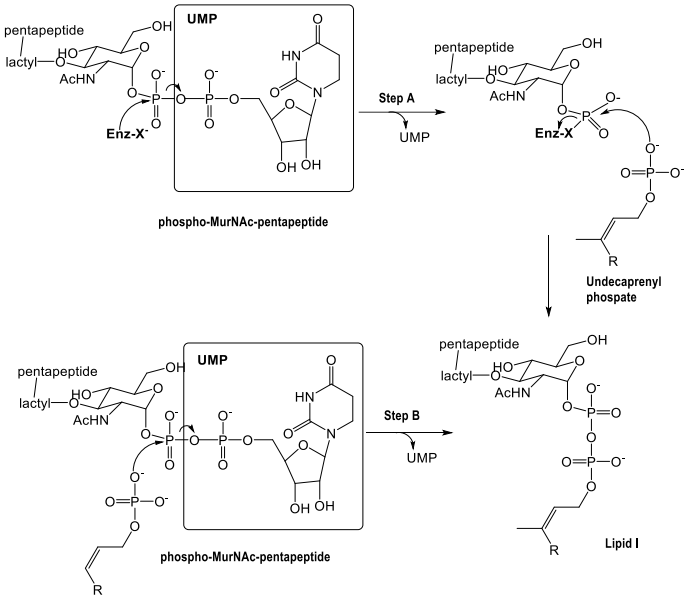
In the outer periplasmic space takes place the polymerization of lipid II. The first step consists on the transglycosylation between monosaccharides of different lipid II units by forming a glycosidic bond by the action of a glycosyltransferase enzyme. Finally, the union of these chains by peptide bonds (transpeptidation) with the help of transpeptidase enzyme completes the biosynthesis of peptidoglycan heteropolymer [4].

3.3. MRAY AS AN ANTIBIOTIC TARGET

MraY enzyme has been proposed as an interesting target for antibacterial drugs as it catalyzes the first phase of the lipid cycle (formation of lipid I) in the peptidoglycan biosynthesis, which is necessary for bacterial viability. Therefore, the inhibition of this enzyme is expected to

hamper the peptidoglycan production, a main component of the bacterial cell wall and, consequently, compromise the bacterial viability [8].

The MraY enzyme was observed to intervene by two possible alternative catalytic mechanisms of phosphotransfer reactions (see **scheme 2**).



Scheme 2. Possible two-step (step A) and one-step (step B) catalytic mechanisms for MraY.

The first mechanism consists in a two-step reaction: there is an initial transphosphorylation of phospho-MurNAc-pentapeptide to a nucleophilic center on the enzyme and, then, there is a second transphosphorylation with the undecaprenyl phosphate to produce lipid I. The second mechanism consists in a one-step reaction which involve a direct attack of the phosphate oxygen of undecaprenyl phosphate onto the β-phosphate of UDP MurNAc-pentapeptide [9].

To understand how the inhibition of this enzyme could take place was important to know the structure of Mray and its active site. The first MraY structure was determined from *Aquifex aeolicus* by X-ray crystallography in 2013 and it was named AaMraY. It was shown to

crystallize as a dimer and featured an oval shaped tunnel in the center of the dimer interface which is surrounded mostly by hydrophobic amino acids. This space was big enough for lipids to get stuck in there. Notably, three aspartic acid residues (Asp 115, Asp 116 and Asp 267) that were exposed on the cytoplasmic side of the lipid bilayer were present within all MraYs [10]. These three aspartic residues were close to the Mg^{2+} cofactor, in a way that Asp 115 and 116 might form a binding site while Asp 267 could be the active site residue. Also, it was found a triad of three histidine residues (His-324, His-325 and His-326, named HHH motif) which was located on the opposite site of the active site which were also conserved in bacterial sequences of MraYs [11]. The loss of Mg^{2+} was observed when *E. coli* MraY was inhibited with nucleoside analogues of mureidomycin A, which suggested that the antibiotic acted by binding partially to the same site occupied by the metal.

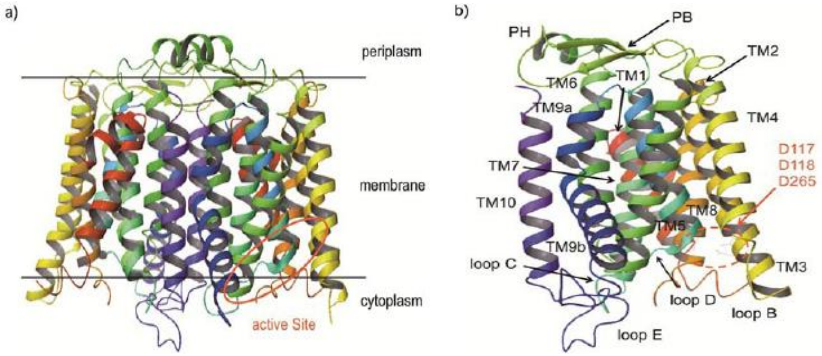


Fig. 2. Structure of MraY located in a) the membrane and b) with its components. [The image was reproduced unaltered from reference no. 12].

There are several different types of compounds that showed how to inhibit MraY activity: natural nucleoside antibiotics, bacteriophage ΦX174 lysis protein E, lipopeptides, cationic peptides and nucleoside antibiotics [13]. Within these natural nucleoside antibiotics, five structurally-related compounds showed high antibacterial activity (see **fig. 3**): tunicamycins, liposidomycins, mureidomycins, muraymycins and capuramycins. These compounds had in common the uridine as the nucleoside moiety. Herein, in this review, we focus on the capuramycin derivatives, which distinctly contained a caprolactam ring [10].

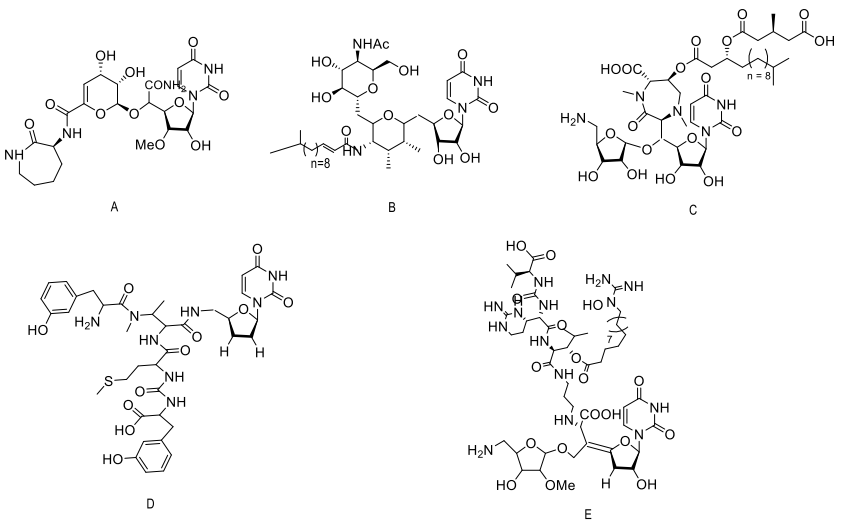


Fig. 3. Structures of capuramycin (A), tunicamycin (B), liposidomycin (C), mureidomycin (D) and muraymycin (E).

3.4. CAPURAMYCIN AS A NATURAL NUCLEOSIDE ANTIBIOTIC

Capuramycin was first isolated from *Streptomyces griseus* in Japan in 1982 [14]. This natural nucleoside has selective antimycobacterial activities against *Streptococcus pneumonia*, *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*, respectively. The antibacterial activity was clearly related to the inhibitory activity against MraY (K_i=10 ng/mL).

The structure of capuramycin, as it is depicted in **fig. 3**, contains three distinct moieties: uridine-5'-carboxamide (CarU), a rare unsaturated hexuronic acid and an amino-caprolactam ring (L-ACL). Biochemical studies showed that was biosynthesized from a multienzymatic system encoded by a gene cluster. Its first total synthesis was also accomplished in 1994 by Knapp's group and, later, it was totally synthesized in a concise way by Kurosu's group in 2009. [15].

Derivatives of capuramycin were also isolated from *S. griseus* cultures. These compounds were found to have different inhibitory activity against MraY and antibacterial activity against other well-known bacteria. On the basis of these results, capuramycin analogues were synthesized in order to improve antibacterial activity [16].

4. OBJECTIVES

In this context, the objective of this report was to gather the most relevant data on capuramycin towards the development of new antibacterial drugs.

Concretly, this report intended to focus on the following four main issues:

- Review of the organic synthetic methodologies leading to capuramycin and derivatives.
- Summary of the biosynthetic pathways of capuramycin in the producing bacteria.
- Potential use of biosynthetic procedures, in combination with chemical synthesis, in the production of capuramycin derivatives.
- Structure-antibacterial activity relationships of capuramycin and derivatives.

5. METHODS

In order to build this present bibliographic summary, searches were conducted by using databases accessible from CRAI-UB such as Scifinder, and Web of Science and, also, Google Scholar. The key words used for these searches were capuramycin, capuramycin activity, MraY, MraY inhibition, Bacterial Resistance, Peptidoglycan, Peptidoglycan Mycobacterium, Murein, Biosynthesis capuramycin, A-500359,Capuramycin analogs, Antimycobacterial activity, and CapW. Overall, these searches produced 35 references. Other references appearing in this work were selected from citations of the previous collection of articles.

Moreover, platforms such as Skype, BB Collaborate and Google Drive were used to keep regular contact with the tutor while the quarantine took place.

6. BIBLIOGRAPHIC REVIEW

According to the literature, capuramycin turned out to be a potential antibacterial drug for human health, notably against *Streptococcus pneumoniae* and *Mycobacterium smegmatis* bacteria. In the following sections, we will review on three main aspects focused at the development of capuramycin analogues as new antibacterials: Organic synthesis, biosynthetic pathways and structure-antibacterial activity studies.

6.1. ORGANIC SYNTHESIS OF CAPURAMYCIN

To date, only two scientific groups have reported a total organic synthesis of capuramycin: Knapp's group [17] and Kurosu's group [18]. This latter group reported an improvement of the synthesis in 2014 [19].

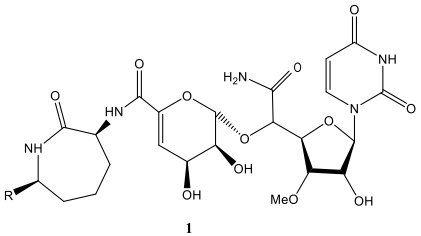


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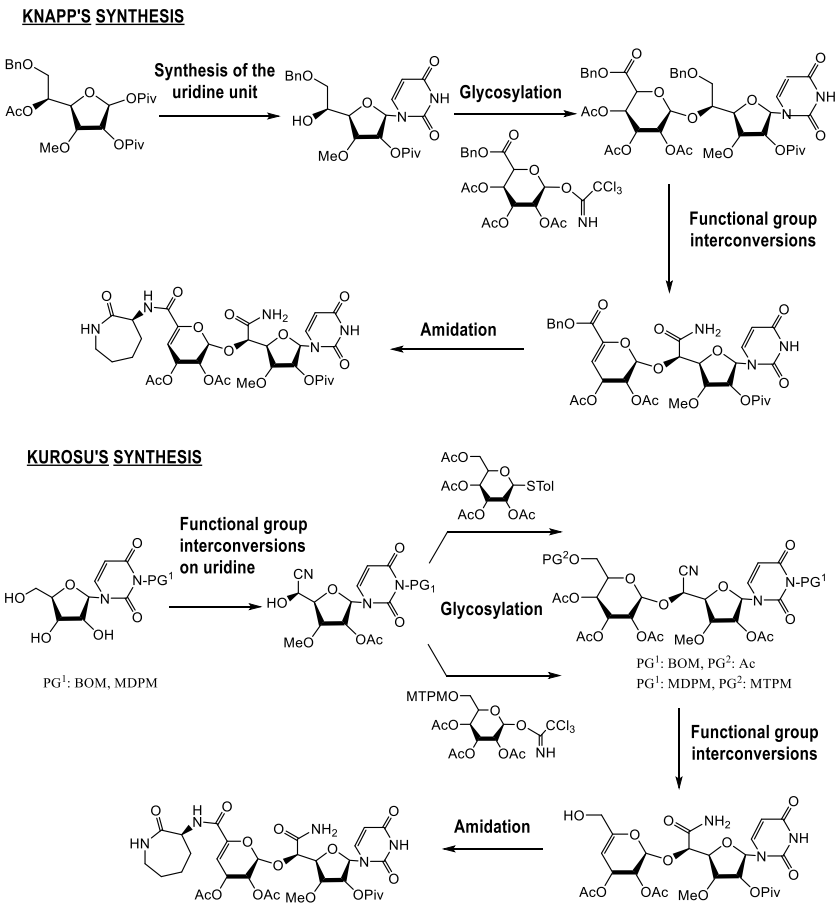
Fig. 4. Structure of capuramycin.

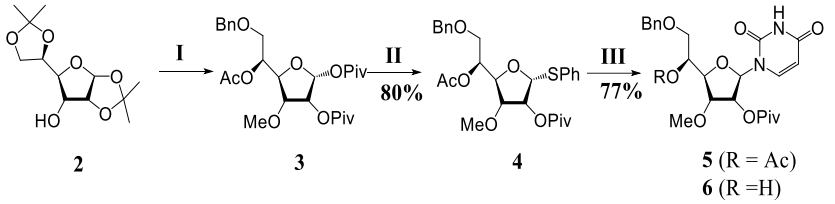
Overall, the same synthetic strategy was essentially used in all these reports, by performing a sequential nucleoside glycosylation followed by an amidation to construct the molecule. The two methodologies mostly differed in the type of acceptor and donor intermediates needed to carry out the glycosylation step.



Scheme 3. Summary of Knapp's and Kurosu's synthesis.

6.1.1. Knapp's group synthesis

This first synthesis of capuramycin was accomplished in 1994 by the Knapp's group [17]. As the following schemes illustrate, L-talo-uridine **6** as acceptor (See **scheme 4**) and D-manno-pyranuronate **11** as donor (see **scheme 5**) were assembled to build the central glycosidic bond (see **scheme 6**). The acceptor and the donor were first reacted to form the *O*-glycosidic linkage, and the capuramycin synthesis was completed by forming an amide bond with the caprolactam unit.

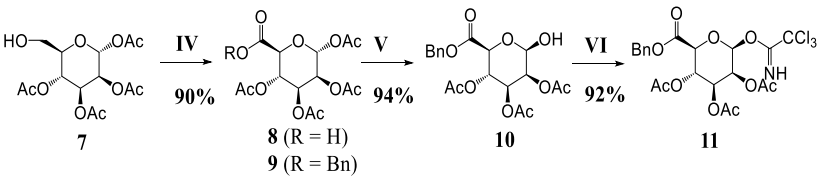


Scheme 4. Synthesis of acceptor L-talo-uridine.

Reaction conditions: I) Several deprotection and protection steps. II) TMS-OTf, TMS-SPh, CCl_4,reflux, 6h. III) *N*,*O*-bis(TMS)uracil,NIS, TfOH, CH_2Cl_2 followed by NaOMe, MeOH.

The diacetone glucose **2** was transformed into the L-talo-furanose derivative (dipivaloate) **3** in 80% yield, by several deprotection and protection steps previously reported. A substitution of the C-1' pivaloyloxy group (-OPiv) for the phenyl thio group (-SPh) with configuration retention led to the intermediate **4**, which was to be used as a donor for *N*-glycosylation to build uridine derivative **6**. A non-polar solvent was essential to get a high yield in step II. The conversion step III to obtain the L-talo-uridine **5** took place by *N*-glycosylation between the thioglycoside donor **4** and a silylated uracil. Finally, a selective deacetylation at C-5' led to uridine**6**.

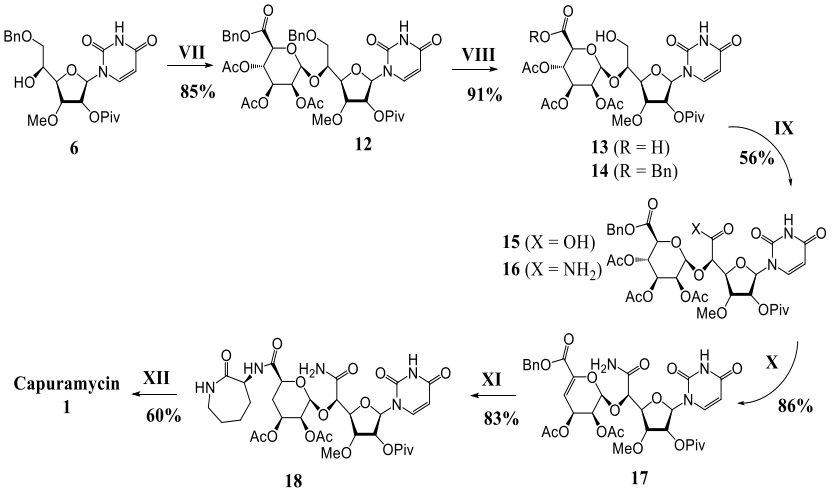
A mannopyranose**7** was selectively oxidized at the primary hydroxyl into acid **8** by reaction with $NaIO_4$ catalyzed by ruthenium(III). It was followed by esterification into benzyl ester **9** in 90% overall yield. Then, the selective removal of the anomeric acetate followed by activation of the anomeric center by reaction with trichloroacetonitrile produced the protected 1-*O*-trichloroimidoyl D-manno-pyranuronate **11**.



Scheme 5. Synthesis of D-manno-pyranuronate donor unit **11**.

Reaction conditions: IV) $RuCl_3 \cdot 3\ H_20$, $NaIO_4$, CCl_4, CH_3CN aq., followed by BnBr, $NaHCO_3$, DMF. V) 2-aminoethanol, EtOAc, DMSO. VI) Cl_3CCN, Na_2CO_3, CH_2Cl_2.

Mannopyranose **7** was selectively oxidized at the primary hydroxyl into acid **8** by reaction with $NaIO_4$ catalyzed by ruthenium(III). It was followed by esterification into benzyl ester **9** in 90% overall yield. Then, the selective removal of the anomeric acetate followed by activation of the anomeric center by reaction with trichloroacetonitrile produced the protected 1-*O*-trichloroimidoyl D-manno-pyranuronate **11**.



Scheme 6. Completion of the synthesis of capuramycin by *O*-glycosylation, followed by caprolactam amidation.

Reaction conditions: VII) compound **11** (8 eq.), TMS-OTf, CH_2Cl_2, -25ºC for 16h and, -5ºC for 6 h. VIII) H_2, 10% Pd-C, MeOH, followed by BnBr, $NaHCO_3$, DMF. IX) PDC, DMF, AcOH, followed by *N*-hydroxylsuccinimide·NH_3, DIPC, DMF. X) DBU, benzene, THF at 23 ºC for 23 min. XI)1,4-cyclohexadiene, 10% Pd-C, MeOH, followed by 2-(*S*)-aminocaprolactam, HOBt, DIPC, DMF. XII) NaOH, MeOH aq. for 2.5 h.

To perform the glycosilation, the D-manno-pyranuronate donor **11** was reacted in excess with the nucleoside acceptor **6** at low temperature to lead to disaccharide **12**. Hydrogenolytic

removal of the two benzyl groups produced the hydroxy acid **13**, which was subsequently esterified to hydroxy benzyl ester **14** in overall 91% yield. Then, a PDC oxidation of the primary hydroxyl furnished the carboxylic acid **15** which was then converted into primary amide **16**. Elimination of acetate at 4'-C with DBU led to **17** in 86% yield. Transfer hydrogenolysis with cyclohexadiene catalyzed by Pd led to the reduction of the easily-reduced C-4 olefin and selective elimination of benzyl ester into acid. This acid was then activated with HOBt and DIPC to react with 2-(*S*)-aminocaprolactam obtaining **18** in overall 83% yield. Finally, careful hydrolysis of the two acetate and the pivaloate protective groups for 2.5 h led to capuramycin **1** in 60% yield.

6.1.2. Kurosu's group synthesis

This second synthesis of uramycin was accomplished in 2009 by the Kurosu's group [18]. As the following **scheme 7** illustrates, the strategy for the glycosilation step was different and consisted in using uridine cyanohydrin **22a** as an acceptor and mannose derivative **23** as a glycosyl donor. Scheme 4 depicts the linear synthesis of capuramycin that was devised.

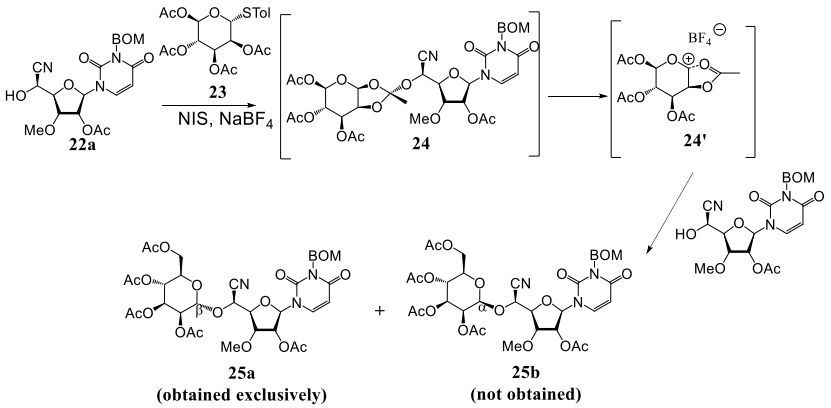
The first steps consisted in the conversion of uridine **19** into by selective 2'-*O*-methylation, 3'-*O*-acetylation and 5'-*O*-deprotection in an overall moderated 55% yield. The primary alcohol **20** obtained after detritylation (elimination of trityl protective group) was oxidized to aldehyde **21** under Pfitzner-Moffat conditions (DCC as oxidant, Cl_2CHCO_2H, DMSO) and quenched by passing through a SiO_2 plug.

Since the compound **21** was instable when it was exposed to bases, the transformation into nitrile derivatives by cyano addition to carbonyl was efficiently produced in neutral conditions by using $Ti(O^iPr)4$ as Lewis acid, to render a mixture of diastereomers **22a** and **22b** (see **scheme 8**). The major undesired cyanohydrin **22b** could be converted to the desired **22a** by invertion through Mitsunobu reaction (DIAD, TPP, $ClCH_2COH$, pyridine) and subsequent deacylation with thiourea in 90% overall yield.

The following step consisted in a glycosylation, by an α-selective mannosylation of **22a** by reaction with the glycosyl donor **23** (see **scheme 7**). This reaction was optimized after several assays to be best promoted by $NIS/AgBF_4$ for 16 h and produce **25a** exclusively in a nearly quantitative yield. It was observed that a shorter reaction time resulted in a mixture of orthoester **24** and a mixture of anomers **25a** and **25b**. In fact, intermediate **24** transformed to another

intermediate **24'** stabilized by the tetrafluoroborate anion which neutralized the positive charge of the oxonium carbocation. The fluoroboric acid (HBF_4) generated in the glycosylation eased the rearrangement of **24** into **25a** which were conveniently separated.

In the next step took place the hydrolysis of the cyano group at **25a** mediated by a Pt complex obtaining the primary amide **26**. It was followed by a selective deacetylation of the glycosidic primary hydroxyl furnishing **27**. Hydrogenolytic removal of BOM uracil group led to uridine **28**. The authors observed that deprotection of uracil was better performed at this stage before of the oxidation of primary alcohol.

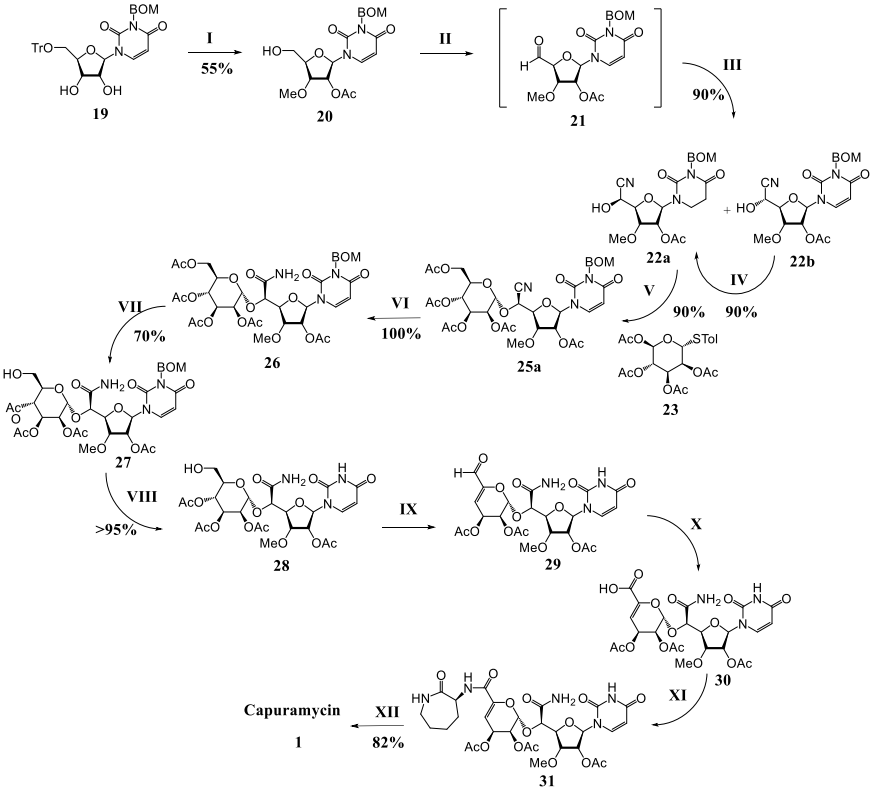


Scheme 7. α-Selective mannosylation of uridine cyanhydrin **22a** by reaction with mannose **23**.

In the next step took place the hydrolysis of the cyano group at **25a** mediated by a Pt complex obtaining the primary amide **26**. It was followed by a selective deacetylation of the glycosidic primary hydroxyl furnishing **27**. Hydrogenolytic removal of BOM protective group of uracil led to uridine **28**. The authors observed that deprotection of uracil was better performed at this stage before the oxidation of the primary alcohol.

The oxidation of primary alcohol into an α,β-unsaturated aldehyde **29** was carried out with Parikh-Doering conditions (SO_3·pyridine, DMSO/Et_3N in 3:1). After removing all volatiles, occurred the oxidation of the aldehyde to carboxylic acid **30** by treatment with NaClO generated *in situ* from $NaClO_2$ and 2-methyl-2-butene.

Finally, the acid carboxylic was coupled with 2-(*S*)-aminocaprolactam by using a typically peptide bond-forming reaction (HOAt, EDCl and NMM) obtaining **31** in an overall 82% yield from **28** to **31**. A saponification with LiOH eliminated all the acetate protective groups and led to capuramycin **1**.



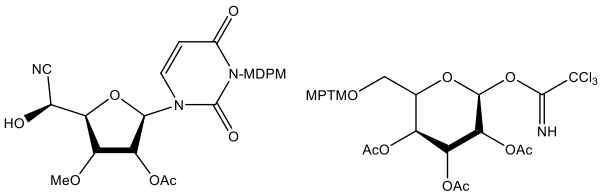
Scheme 8. Complete synthesis of capuramycin by Kurosu's group.

Reaction conditions: I) ^{n}BuSnO, toluene and MeI, ^{n}Bu$_4$I. Then, Ac$_2$O, pyridine followed by BF$_3$·OEt$_2$, TolSH, CH$_2$Cl$_2$. II) DCC, Cl$_2$CHCO$_2$H, DMSO, CH$_2$Cl$_2$ (Pfitzer-Moffat conditions). III) TMSCN, Ti(O^{i}Pr)$_4$, CH$_2$Cl$_2$-H$_2$O (1%) and, then, 80% AcOH. IV) DIAD, TPP, ClCH$_2$CO$_2$H, pyridine (1:1:1:1), toluene, followed by thiourea, MeOH, 50ºC. V) NIS/AgBF$_4$, CH$_2$Cl$_2$, 3Å MS, 0ºC for 16 h. VI) Parkins' [Pt] complex 5 mol %, EtOH aq., 70ºC. VII) [^{t}Bu$_2$SnCl(OH)]$_2$, MeOH. VIII) H$_2$/10% Pd-C, AcOH, ^{i}PrOH. IX) SO$_3$·pyridine, DMSO/Et$_3$N (3:1). X) NaClO$_2$, 2-methyl-2-butene, ^{t}BuOH-H$_2$O (1/1). XI) 2-(*S*)-aminocaprolactam, EDCl, HOAt, NMM. XII) LiOH, THF-H$_2$O (10/1).

6.1.3. Improvement of Kurosu's group synthesis

Kurosu's group devised an optimized route to synthesize capuramycin in 2014 [19]. The two main changes that were made were the use of a different protection scheme of glycosyl and uracil units, and the method of activation of the glycosylation by using the trichloroimidate chemistry as in Knapp's synthesis.

Two novel protecting groups were adopted, MDPM (monomethoxydiphenylmethoxymethyl) for the *N*-3 at uridine **32** and MTPM (monomethoxytetrachlorodiphenylmethyl) for primary alcohol of the glycosyl donor **33** (see **fig. 5**). These protective groups were stable in the presence of many different acids, were not susceptible to hydrogenation under standard conditions, and could be removed by 30% TFA at room temperature within 2 h without the addition of a cation scavenger. This improved significantly the synthesis since the hydrogenolytic cleavage of BOM group used in the previous Kurosu's synthesis often led to the over-reduction of the C_5-C_6 double bond of uracil.



32 **33**

Fig. 5. Novel protecting groups for *N*-3 uridine **32** and the glycosyl donor unit **33**.

Moreover, the previous capuramycin synthesis reported by Kurosu's group had high-yielding conversion when it was performed in small and medium scales, but several steps were not ideal for producing higher amounts of capuramycin and its analogues at a large scale. In order to improve the synthesis performance, some of the pivotal synthetic steps were further optimized. The results were as follows.

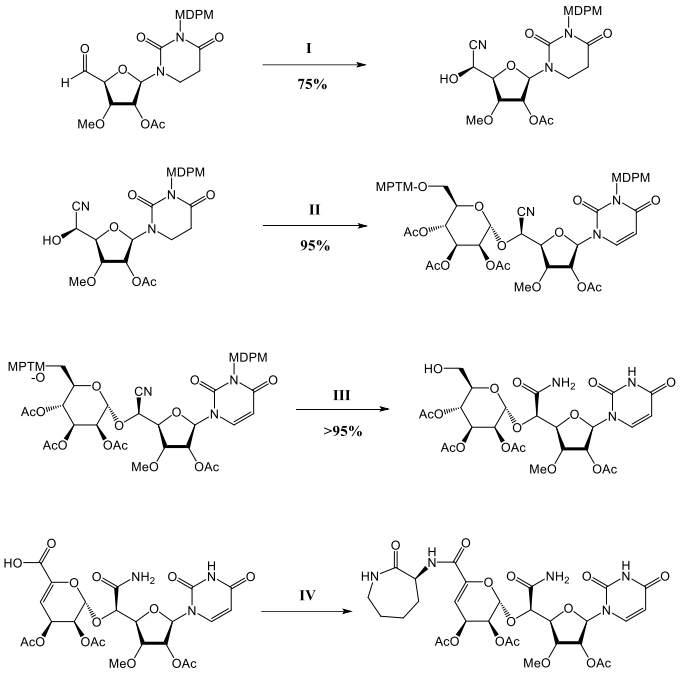
In the glycosylation step, $BF_3{\cdot}OEt_2$ catalyzed α-mannosylation of **32** with the imidate**33** in higher 75% yield. Notably, the glycosylation could be achieved at higher concentrations and in shorter reaction time, 3 h in this case, when compared to the reaction between **5** and **6** in the

previous Kurosu's synthesis, under NIS/$AgBF_4$ conditions during 12-16 h (see step V from **scheme 8**). This improvement in glycosylation was reproducible at any concentration between 0.1–0.5M (the concentration previously used was 0.05M) and could be applied to a gram-scale synthesis of the *O*-glycosylated product.

Besides, benzoyl cyanide (BzCN) in DMSO/H_2O was used advantageously for the cyanohydration of uridine instead of using TMSCN and [$Ti(OiPr)_4$] in CH_2Cl_2/H_2O (1%), affording a high-yield conversion (see step III from **scheme 8**).

The cyano group after *O*-glycosylation was hydrated by $InCl_3$–aldoxime in toluene instead of using Parkin's [Pt] complex (see step VI from **scheme 8**), furnishing the corresponding primary amide pure enough without purification to perform subsequently the removal of MDPM and MPTM groups with 30% TFA in CH_2Cl_2, affording **28** in > 95% overall yield for the two steps.

Finally, the last improvement was the use of glycerol acetonide-oxyme and EDCI to carry out the coupling between the carboxylic acid **30** and the aminocaprolactam (see step XI from **scheme 8**). Before, the use of EDCI, HOAt and NMM as amidation coupling reagents produced a mixture of subproducts difficult to be separated from the desired product **31** by column chromatography. The improved method also offered the advantage of only requiring purifications by chromatography for three of the total number of synthetic steps.



Scheme 9. Synthetic steps that were optimized after the second procedure reported by Kurosu's group.

Reaction conditions: I) **33**, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 3Å MS, -5 ºC for 3 h. II) BzCN, DMSO/H_2O. III) $InCl_3$-aldoxyme, toluene, 70 ºC followed by 30% TFA, CH_2Cl_2. IV) Glycerolacetonide-oxyme, EDCI, $NaHCO_3$, H_2O.

6.2. BIOSYNTHETIC PATHWAY FOR CAPURAMYCIN

6.2.1. Searching precursors

The complete biosynthetic pathway of A-500359B (see **fig. 6**), a close derivative of capuramycin, was first elucidated in 2003 by Ohnuki *et al*. by using feeding experiments with isotopically ^{13}C-labeled precursors to find the metabolic origin of the structural components [20]. On the basis of the experiments, the three main components 5'-carboxamide (CarU), D-manno-pyranuronate and L-aminocaprolactam (L-ACL) were proposed to be derived from uridine, D-mannose and L-Lys, respectively [21].

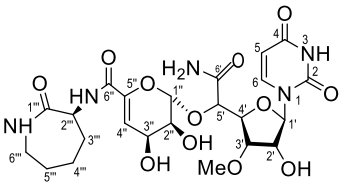


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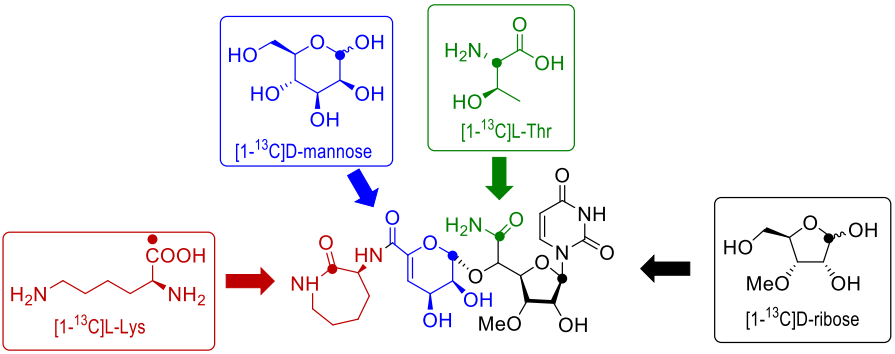
Fig.6. Structure of capuramycin-type nucleoside antibiotic A-500359Bs.

According to the ^{13}C-NMR analysis of the metabolite, feeding with isotopically labeled [1-^{13}C] D-ribose produced a 17-fold enrichment of the C-1' position, [1-^{13}C] D-mannose led to a 11-fold enrichment of the C-1'' position and [1-^{13}C] L-Lys yielded a 16-fold enrichment of the C-1''' position (see **scheme 10**).



Scheme 10. Metabolic origin of A-500359s as determined through feeding experiments using the indicated isotopically labeled precursors. Filled circles indicate the positions that were enriched with ^{13}C.

The precursor of the C-5' carboxamide of CarU was initially thought to come from phosphoenolpyruvate via pyruvate since this was the origin of the C-5' carboxylate of polyoxines. However, after feeding with [3-^{13}C]pyruvate only a 3-fold enrichment of the C-6' position was obtained. This low enrichment led to think of another precursor for carboxamide

which turned out to be L-Thr [20]. Finally, the origin of 3'-methoxy carbon on ribose was deduced after feeding with [methyl-^{13}C] L-Met, which was consistent with *S*-adenosyl-L-methionine (AdoMet) being the biosynthetic precursor of the methyl group.

6.2.2. Identification of the gene cluster

The biosynthetic gene cluster of A-500359s was reported in 2009 by Funabashi *et al.* [22]. It was identified by RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) by amplifying DNA fragments which encoded for putative nucleoside diphosphate (NDP)-glucose-4,6-dehydratases (NGDH). This gene for a putative NGDH was over-expressed during the production of A-500359s in a high producing strain and absent in a non-producing strain. Experiments showed a 65-kb DNA region that encoded for 38 open reading frames (ORFs) was probably involved in biosynthesis of A-500359s. A RT-PCR was used again to identify which ORFs are highly expressed. The results showed that almost all of the 38 ORFs were highly expressed during production of capuramycin.

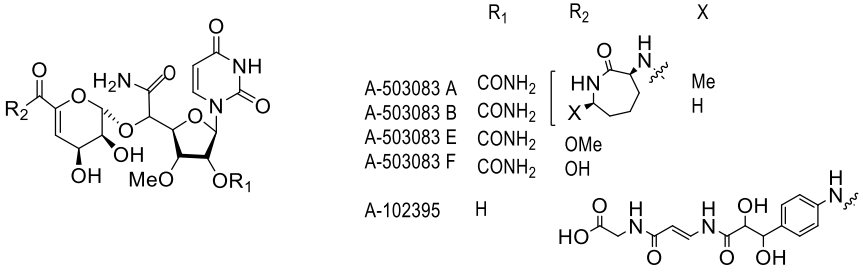


Fig.7. Structures of A-503083s and A-102395 capuramycin-type nucleoside antibiotics.

To provide enough evidence that the gene cluster for the capuramycin-type nucleoside antibiotics was identified, the gene cluster for the A-503083s and A-102395 (see **fig. 7**), other two capuramycin-type nucleoside antibiotics, were cloned and characterized to show that shared ORFs in their biosynthetic gene clusters with A-500359s, as it is summarized in the following **table 1**. The putative function of genes was deduced by sequence homology searching in genomic databases.

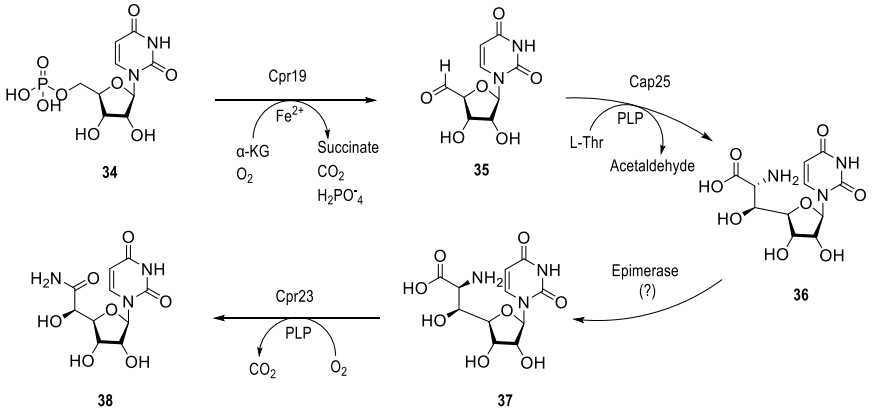
Proposed Function	A-500359	A-503083	A-102395
Fe(II)- and αKG-dependent dioxygenase	*orf7*	*capD*	*cpr18*
Capuramycin-2′-*O*-carbamoyltransferase	*orf8*	*capB*	*NA*
Putative 3-ketoreductase	*orf9*	*capC*	*cpr20*
Fe(II)-dependent, αKG:UMP dioxygenase	*orf10*	*capA*	*cpr19*
Putative 2,3-dehydratase	*orf11*	*capE*	*cpr21*
Putative 4-epimerase	*orf12*	*capF*	*cpr22*
PLP-dependent monooxygenase-decarboxylase	*orf12′*	*cap15*	*cpr23*
Putative glycosyl transferase	*orf13*	*capG*	*cpr24*
L-Thr:uridine-5′-aldehyde transaldolase	*orf14*	*capH*	*cpr25*
Putative pyrophosphatase	*orf15*	*capI*	*cpr26*
Putative CO dehydrogenase	*orf16*	*capJ*	*cpr28*
Putative O-methyltransferase	*orf16′*	*capK*	*cpr29*
Putative CO dehydrogenase	*orf17*	*capL*	*cpr30*
Putative CO dehydrogenase	*orf18*	*capM*	*cpr31*
Putative ABC transporter	*orf19*	*capN*	*NA*
Putative ABC transporter	*orf20*	*capO*	*NA*
Capuramycin 3′′-phosphotransferase	*orf21*	*capP*	*cpr17*
UDP-glucose-4,6-dehydratase	*orf22*	*capQ*	*NA*
Glucose-1-phosphate thymidylyltransferase	*orf23*	*capR*	*NA*
Putative carboxyl methyltransferase	*orf24*	*capS*	*cpr27*
Putative L-ACL C-methyltransferase	*orf25*	*capT*	*NA*
Nonribosomal peptide synthetase	*orf26*	*capU*	*NA*
Nonribosomal peptide synthetase	*orf27*	*capV*	*NA*
Transacylase	*orf28*	*capW*	*cpr51*

Table 1. Shared ORFs in the cloned biosynthetic gene clusters of three different capuramycin-type nucleoside antibiotics (A-500359s, A-503083s and A-102395) and their putative functions, according to the studies of Funabashi et al. [The table was reproduced unaltered from reference no. 22].

As the results showed, 16 ORFs were found to be shared among all three groups of capuramycin-type nucleoside antibiotics. Moreover, the A-500359s gene cluster consisted minimally of 26 ORFs, with 18 ORFs involved in biosynthesis, 6 ORFs involved in resistance, regulation and transport (ORFs 19–21, 30, 36 and 37), and 2 other ORFs of unclear function (ORFs 29 and 34).

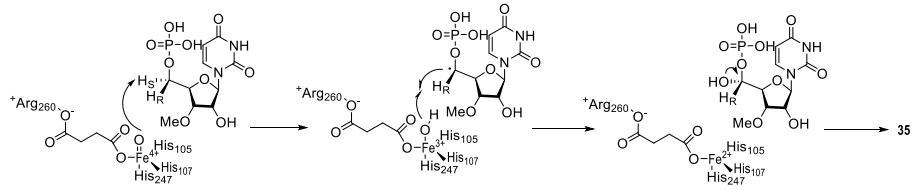
6.2.3. Biosynthesis of capuramycin

According to the proposal of Ohnuki *et al.* [20], the biosynthesis of the curamycin-type A-500359 consisted in the production of three building blocks [23], uridine-5'-carboxamide (CarU), 4,5-dehydro-D-manno-pyranuronate and L-aminocaprolactam (L-ACL), which were linearly coupled to complete the structure of the metabolite.



Scheme 11. Biosynthesis of uridine-5'-carboxamide (CarU) component.

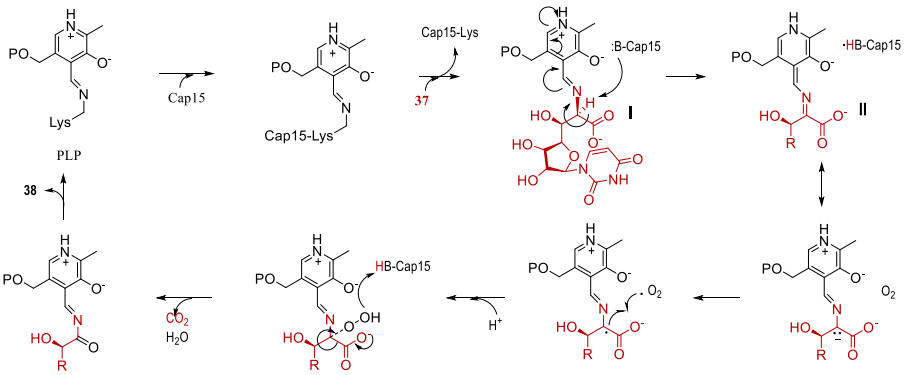
The biosynthesis of uridine-5'-carboxamide (CarU, **38**) started from UMP (**34**) [24]. The first reaction (see **scheme 11**) was catalyzed by Cpr19 (homologue to ORF10), a non-heme Fe(II) and α-ketoglutarate (αKG)-dependent dioxygenase (also known as UMP hydroxylase-phospholyases) which catalyze the O_2, Fe(II), and α-KG dependent conversion of **34** into uridine-5-aldehyde (UA, **35**). An oxidative decarboxylation of α-KG led to CO_2, succinate, and oxidation of Fe(II) into Fe(IV)-oxo species. The Fe(IV)-oxo acted as a strong oxidizing agent that reacted with **34**, abstracting an hydrogen followed by radical rebound hydroxylation. This led to a stereoselective hydroxylation at C-5' of **34** that led to an hemiacetal phosphate intermediate, which ultimately lost phosphate to be transformed into the first intermediate of the pathway, aldehyde **35** (**scheme 12**) [25].



Scheme 12. Proposal of the mechanism of hydroxylation and oxidative dephosphorylation reaction catalyzed by Cpr19.

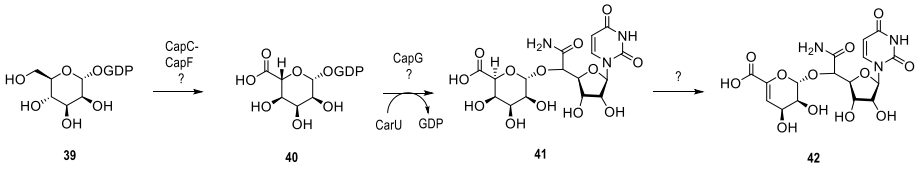
The next step that transformed **35** into (5'*S*,6'*S*)-GlyU (**36**) was carried out by Cpr25 (see **scheme 11**), which was predicted to be a pyridoxal-5'-phosphate (PLP)-dependent serine hydroxymethyl transferase (SHMT). This enzyme acted as a transaldolase by catalyzing the conversion of L-Thr and **35** into **36** and acetaldehyde by forming a C-C bond [26]. These enzymes typically catalyze an aldol-type reaction between amino acids and aldehydes to produce various β-hydroxy-α-amino acids. The Cpr25-catalyzed reaction was shown to be stereoselective both in the selection of the L-Thr as a substrate and the formation of **36**.

In the next step, the intervening enzyme still remained uncertain. Most probably, it was an epimerase which converted **36** to (5'*S*, 6'*R*)-GlyU (**37**) since the next enzyme in the pathway, Cpr23, was known to be stereoselective for the 6'*R*-isomer. Concretely, Cpr23 was an O_2- and PLP-dependent monooxygenase decarboxylase that converted an α-amino acid to a carboxamide. As it is shown in **scheme 13**, Cap15 enzyme was first bound to PLP oxygenase cofactor by an aldimine bond, intermediate which transiminated with **37** to give **I**. Imine **II** was obtained from **I** by isomerization, which reacted with activated dioxygen to give an unstable hydroperoxide intermediate, which ultimately transformed into the carboxamide **38** by an oxidative decarboxylation followed by hydrolysis and regeneration of PLP [27].



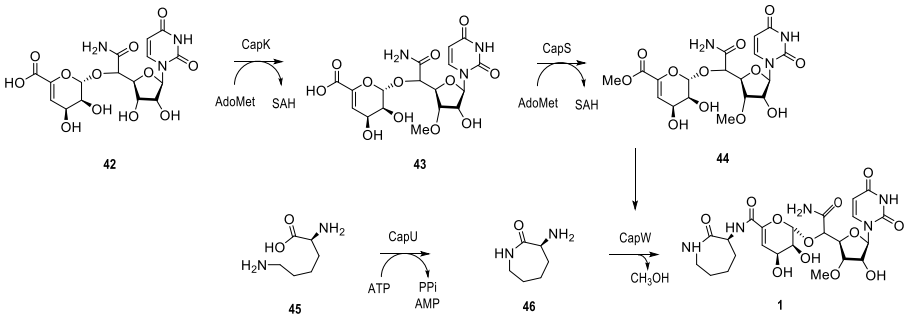
Scheme 13. Conversion of α-amino acid (5'S, 6'*R*)-GlyU to carboxamide CarU by Cap15 enzyme (one homologue of Cpr23).

The second building block in capuramyicn biosynthesis, D-manno-pyranunorate **42**, was obtained from GDP-mannose **39** although the enzymes involved in the transformation were not completely identified (see **scheme 14**). The first step, probably catalyzed by enzymes CapC through CapF, consisted in a four-electron oxidation at C-6 that transformed the primary alcohol into a carboxylic acid, and followed by an epimerization at 4-C to generate taluronic acid **40**. By sequence homology, CapC was identified as a putative 3-ketoreductase, CapE was a 2,3-dehydratase and CapF was probably an epimerase. Then, the putative enzyme CapG produced the glycosylation with GDP-taluronic acid into acetal **41**. Finally, a probably non-enzymatic and spontaneous 4,5-dehydration in the mannose ring led to **42**.



Scheme 14. Proposed biosynthesis of 4,5-dehydro-D-manno-pyranuronate A-500359H from GDP-mannose.

The last component needed to complete capuramycin biosynthesis was L-aminocaprolactam (L-ACL, **46**). It was produced form L-Lys by an ATP-dependent CapK, an amide-bond-forming catalyst or non-ribosomal peptide synthetase. The carboxyl group of L-Lys (**45**) was first activated by adenylation and followed by formation of a thioester by reaction with a sulfhydryl group of this enzyme. Then, an intramolecular aminolysis was followed to release the lactam **46** in a nonenzymatic step [28]. At the same time, an *O*-methylation of the C-3'-hydroxyl of **42** was catalyzed by CapK leading to **43** followed by an AdoMet-dependent carboxyl methylation mediated by CapS enzyme that generated the methyl ester **44**. This last methylation allowed to activate the carboxylate to undergo a transacylation catalyzed by CapW producing the L-ACL-containing product **1** [29].

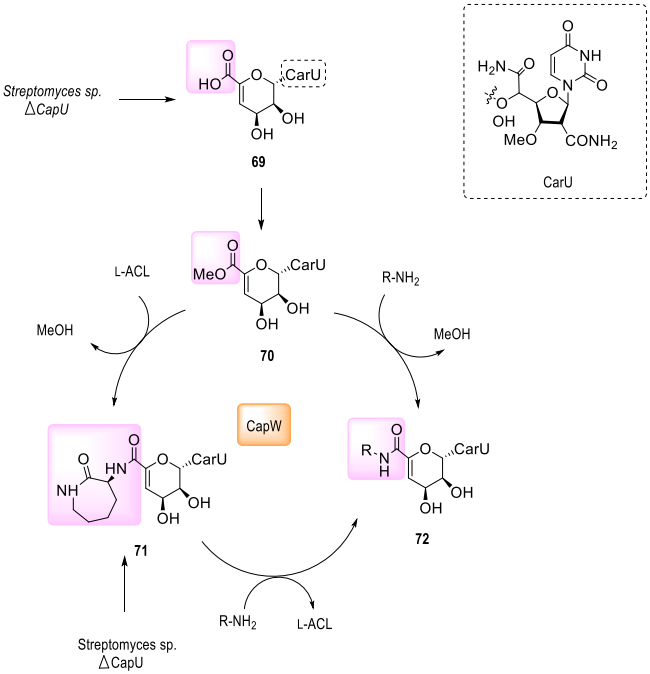


Scheme 15. Completion of the biosynthesis by attachment of L-aminocaprolactam (L-ACL) component.

6.2.4. Biocatalytic approach

Semisynthesis and total synthesis of bioactive compounds such as capuramycin derivatives are highly demanding and challenging processes. In order to facilitate and improve the production of these compounds, a biocatalytic route was reported by Xiaodong *et al.* in 2016 based on the previously elucidated biosynthesis of capuramcyin [30].

During the production of **1** and **47** by fermentation from a ΔCapU mutant strain of *Streptomyces sp.*, 2-aminoethyl-L-cysteine (AEC) was added as an inhibitor of aspartokinase which was required to biosynthesize L-Lys. That fact led to the isolation of carboxylic acid A-503083F (**69**) precursor which could be converted to the methylated ester A-503083E (**70**) analog. Then, the CapW enzyme was incubated in the same mutant strain along with **70** and an amine acceptor, obtaining **72** by amide-ester exchange. Amines with carboxylate functionalities (for instance, α-aminoacids) were not incorporated by CapW, but methyl esters or Boc-protected α-aminoacids were accepted. It was also reported that CapW could catalyze a direct transamidation reaction with **70** as the acyl donor and L-ACL as the acyl acceptor to obtain amide derivative **71**. By means of these two acyl exchanges mediated by CapW, a total of 43 analogs were synthesized using non-natives amines as acyl acceptors.



Scheme 17. Biocatalytic approach used to synthesize capuramycin analogs following the methodology devised by Xiaodong *et al.* [30].

6.3. ANTIBACTERIAL ACTIVITY OF CAPURAMYCIN ANALOGUES

In order to improve the translocase inhibition and hence, antimycobacterial activity, of capuramycin, semisynthetic strategies were developed to prepare new derivatives. Hotoda *et al.* from Sanyko Co. synthesized about 6769 analogues of **1** and its methylated derivative **47.** The methodology used to prepare all these compounds was patented [31]. Modifications were carried out by acylation or alkylation at the 2'-O position of the ribose, methylation at 3'-O position of the ribose, acylation at the 3''-O and 2''-O positions of the mannose, alkylation at the 3-*N* position of the uracil moiety, substitution of the caprolactam ring and derivatization at the primary amide site. These modifications did not extend the spectrum of activity of capuramycin but they turned out to be new compounds with better antimycobacterial activity, as outlined below [32].

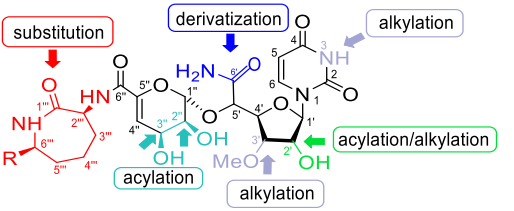
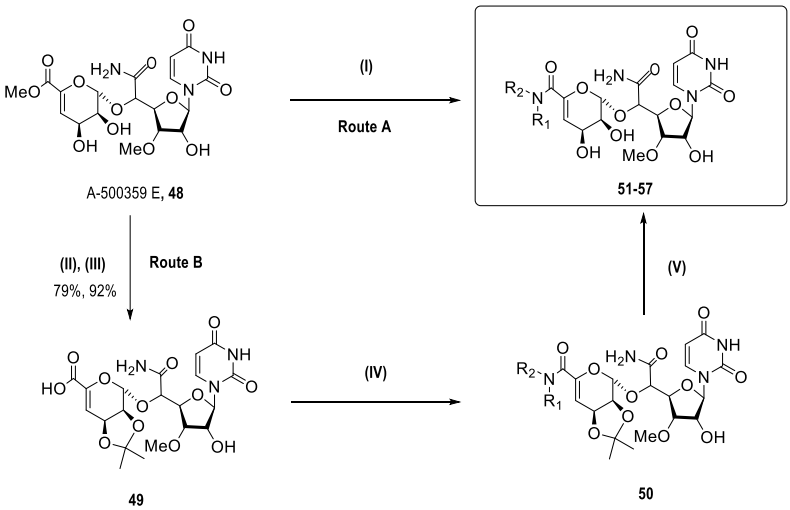


Fig. 8. Modifications in capuramycin **1** and its methylated derivative **47** to obtain analogues with improved activities as peptidoglycan translocase inhibitors, according to Sankyo company [34].

6.3.1. Semisynthetic analogues

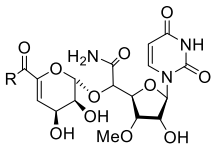
The synthesis of derivatives was initiated by carrying out the L-ACL substitution from **47**, prepared by a biosynthetic approach or an organic synthesis, by reaction with a variety of amines. The amines with enough nucleophilicity to react with the ester followed the route A (see **scheme 16**). On the other hand, the amines without enough nucleophilicity followed the route B, by multistep protection-deprotection via diolacetonization, saponification, and then amidation in the presence of condensing reagent (see **scheme 16**). These reactions led to the desired products **51-57** [33].



Scheme 16. Synthesis of capuramycin **51**-**57** analogues from capuramycin derivative **48**.

Reaction conditions: I) R_1R_2NH (excess), MeOH, 60ºC. II) $Me_2C(OMe)_2$, Amberlyst 15 (H^+), Me_2CO. III) 0,04N NaOH, MeOH-H_2O, followed by Dowex 50Wx8 (H^+). IV) R_1R_2NH, DIPC, HOBT, DMF. V) Amberlyst 15 (H^+), MeOH.

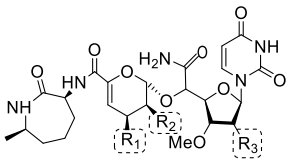
The amide derivatives included aliphatic, phenethyl, benzyl-type, and phenyl-type substituents on nitrogen. The inhibition of translocase I and antibacterial properties against *M. smegmatis* were subsequently studied. The results are summarized in **table 5**. In the aliphatic substituted derivatives, simple cyclohexyl and cycloheptyl moieties retained potent inhibitory activity against translocase I and *M. smegmatis* (MSMG) *in vitro.* However, long chain alkyl moiety and terminal modifications of the alkyl chain substituent with functional groups (EtO-, MeS-, HO-, H_2N-…) reduced both activities. The phenethyl-type substituents moderated the activity, but products were more potent than those with benzyl-type substituents. Finally, some of the phenyl-type substituted products showed to be more potent inhibitors than the natural capuramycin (above all **51**, **52** and **55**) as it is shown in **table 5**. The NH group has to be stand out as it appears in all of the analogues with improved activity. Antibacterial activities did not always correlate with the translocase I inhibitory activity. As translocase I is located in the lipophilic bacterial cell membrane, it was probably due to the differences in cell-permeability of the analogues [34].

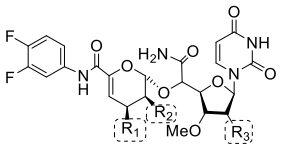


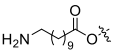
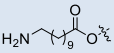
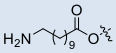
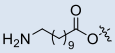
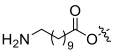
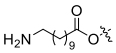
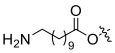
Compound	R	IC_{50} (ng/mL) (Translocase I)	MIC (µg/mL) (*M. smegmatis*)
1		10	12,5
47		10	6,25
48	MeO-	27	>100
51	PhNH-	6,5	6,25
52	3-Me-PhNH-	7,6	12,5
53	3-F-PhNH-	10	6,25
54	4-F-PhNH-	37	6,25
55	3,4-F_2-PhNH-	9	6,25
56	4-Cl-PhNH-	18	6,25
57	4-Br-PhNH-	20	6,25

Table 5. Translocase I inhibitory activity and antibacterial activity (*M. smegmatis*) of the best capuramycin phenyl-type analogues generated through semisynthesis.

The modification of the 2'-hydroxyl of **47** and **55** was performed by introducing different acyl or alkoxycarbonyl groups (see **table 6**, **58**-**65**) [35]. A few of these analogs showed improvements on their activities. Notably, **58** obtained by adding an acid decanoic (DEC) in the 2'-*O* position led to a high MIC although showed a low IC_{50} for inhibiting translocase I. The improved antibacterial activity of **58** was probably due to the higher lipophilicity of **58**, which allowed the compound to penetrate advantageously the *Mycobacterium* cell wall [36].





Compound	R_1	R_2	R_3
47	HO-	HO-	HO-
58	HO-	HO-	
59		HO-	
60			
61			
62	HO-	HO-	
63			

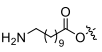
Compound	R_1	R_2	R_3
55	HO-	HO-	HO-
64	HO-	HO-	
65	HO-	HO-	

Table 6. Structures of capuramycin analogs generated through semisynthesis from **47** and **55** by 2'-acylation with decanoic acid (DEC), undecanoic acid (UA), and/or aminoundecanoic acid (AUA).

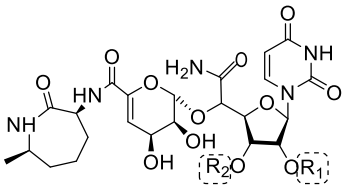
The result of **58** led to assay long chain ester modifications with decanoic acid (DEC), undecanoic acid (UA), and/or aminoundecanoic acid (AUA) at 2', 2'', and/or 3'' positions of **47**, **55** and **58**, to show that some of them improved their inhibitory activity against *Mtb* and *MSMG*. Substituting 2''-*O* and 3''-*O* with UA in **58** led to complete loss of activity in both bacteria but esterification with AUA, above all in 3''-*O* position, improved antibacterial activity. In the **55** analog, the addition of AUA improved the activity in *MSMG* but not in *Mtb*. The esterification with DEC did not improve the antimycobacterial activity in any of them. Finally, in the **47** analog, AUA conjugation improved the antibacterial activity in both bacteria. Although the antimycobacterial activity in *Mtb* is still higher than in *MSMG* for compounds **58**-**65**, the esterifications improved the activity against *Mtb* compared with the initial **47**, **55**, and **58**

analogues. The results of activity against *Mtb* and *MSMG* summarized in **table 7** suggested that the additional amino groups on the AUA moieties probably improved the permeability of capuramycin and/or affected drug efflux [33].

Compound	Conjugation	MIC (mg/mL) (*M. tuberculosis*)	MIC (mg/mL) (*M. smegmatis*)
47		8	16
55		2	2
58		0,5	2
59	58-AUA	1	1
60	58-2AUA	2	4
61	58-2UA	>32	>32
62	47-AUA	1	2
63	47-3AUA	2	4
64	55-DEC	4	16
65	55-AUA	8	1

Table 7. Antimycobacterial activity of capuramycin analogues **58**-**65** against *M. tuberculosis* and *M. smegmatis*.

The modification of the 3'-*O* methylated from the ribose led to some interesting results (see **table 8**. On one hand, when 3'-*O*-methyl group was eliminated turned the compound into an inactive analog. On the other hand, if the 3'-*O*-methyl was exchanged for the 2'-*OH*, the resulting analog had similar TL1 Inhibitory activity and antimycobacterial activity compared to **1**. However, neither **1** nor **67** were effective against dormant *Mtb.* Finally, the 2'-*O*-methyl and the 3'-*O*-methyl analogues (**68**, UT01320) could kill replicating but also nonreplicating *Mtb*. Although **68** could not inhibit TL1, it acted by stopping on *MSMG* RNA polymerase [37].



Compound	R_1	R_2	Activity
1	H	Me	Antimycobacterial TL1 inhibitor
66	H	H	Inactive
67	Me	H	Antimycobacterial TL1 inhibitor
68	Me	Me	RNA polymerase inhibitor

Table 8. Activity of 2'/3'-*O*-methylatedcapuramycin analogs.

These results showed that, IC_{50} and MIC values were not completely correlated in mycobacteria. The compound **47**, turned out to be a good inhibitor of TL1 but without enough antimycobacterial activity. **55**, the acylated derivative of the previous compound, despite its high IC_{50}, improved *in vitro* and intracellular activity. Reserpine, an efflux pump inhibitor, improved the activity of **55** but not of **47** suggesting that its reduced activity is due not to drug efflux, but to low permeability. Also, the esterification in the 3''-*O* position improved the activity of most of the compounds which would led to think that it is critical for antibiotic activity and self-resistance in gram-positive bacteria.

7. CONCLUSIONS

Capuramycin (**1**) is a novel antibacterial drug against S. *pneumoniae*, *M. smegmatis* and *M. tuberculosis*. *In vitro* studies showed that capuramycin inhibited MraY enzyme which is in charge of synthesizing the peptidoglycan, the main component of the bacterial cell wall.

Capuramycin and their derivatives could be produced either by organic synthesis or via biosynthesis.

Two different organic synthesis were reported to date: by Knapp's group in 1994 and by Kurosu's group in 2009, this one with an improvement in 2014. The synthetic routes differed mainly in the glycosylation step between the mannopyranose and uridine units. Also, the improvement by Kurosu's group included a better choice of protective groups for the intermediates and some pivotal synthetic steps to obtain higher yields at a large scale.

The biosynthetic pathway of capuramycin was elucidated by Ohnuki's group in 2003 by characterization of the gene cluster and ^{13}C-labelling experiments to as certain the biosynthetic origin of the main building blocks. The results permitted to devise an efficient biosynthetic route for producing capuramycin and derivatives, thereby providing a good alternative to organic synthesis.

A semisynthetic methodology that combines the biosynthetic production of a precursor and chemical synthesis permitted to obtain new capuramycin analogues, which were used to study inhibition and antibacterial activity. The **55** was improved by adding an AUA at the 2'-*O* position just for *MSMG* bacteria. The modifications that conferred better antibacterial activity were those that, in addition to inhibiting MraY, increase lipophilicity, most probably allowing the compound to penetrate better into the bacterial cell wall.

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APPENDICES

APPENDIX 1: ABBREVIATIONS AND ACRONYMS

AaMraY: *Aquifex aeolicus* MraY
AdoMet: S-adenosyl-L-methionine
AEC: 2-aminoethyl-L-cysteine
α -KG: α-ketoglutarate
Arg: arginine
Asp: aspartic acid
AUA: aminoundecanoic acid
BOM: benzyloxymethyl acetal
Cap15: PLP-dependent monooxygenase-decarboxylase
CapC: putative 3-ketoreductase
CapE: putative 2,3-dehydratase
CapF: putative 4-epimerase
CapG: putative glycosyl transferase
CapK: putative *O*-methyltransferase
CapS: putative carboxyl methyltransferase
CapU: non ribosomal peptide synthetase
CapW: transacylase
CarU: uridine-5'-carboxamide
Cpr19: Fe(II)-dependent, α-KG:UMP dioxygenase
Cpr23: PLP-dependent monooxygenase-decarboxylase
Cpr25: L-Thr:uridine-5'-aldehyde transaldolase
DBU: diazabicycloundecene
DCC: *N*,*N*′-dicyclohexylcarbodiimide
DEC: decanoic acid
DIAD: diisopropyl azodicarboxylate
DIPC: *N*,*N*′-diisopropylcarbodiimide
DMF: *N*,*N*-dimetilformamida
DMSO: dimethyl sulfoxide
EDCI: *N*′-ethylcarbodiimide hydrochloride
GDP: guanosine diphosphate
GlyU: 5'-C -glycyluridine
His: histidine
HOAt: 1-hydroxy-7-azabenzotriazole
HOBt: hidroxybenzotriazole
IC_{50}: inhibitory concentration at 50%
L-ACL: L-aminocaprolactam
L-Lys: L-Lysine
L-Thr: LTthrionine
MDPM: monomethoxydiphenylmethoxymethyl
MIC: minimum inhibitory concentration
MraY: phospho-*N*-acetylmuramoyl-pentapeptide transferase.
MS: molecular sieve
MSMG: *Mycobacterium smegmatis*
Mtb: *Mycobacterium tuberculosis*
MTPM: monomethoxytetrachlorodiphenylmethyl
NGDH: nucleoside diphosphate (NDP)-glucose-4,6-dehydratase
NIS: *N*-iodosuccinimide
NMM: *N*-methylmorpholine
OPiv: pivaloyl
ORF: open reading frame
PDC: pyridinium dichromate
PLP: pyridoxal-5'-phosphate
PP_i: pyrophosphate
RT-PCR: reverse transcriptase-polymerase chain reaction
SAH: *S*-adenosyl homocysteine
SHMT: serine hydroxymethyl transferase
STol: thiotoluenyl

TfOH: trifluoromethanesulfonic acid
TL1: translocase I
TMSCN: trimethylsilyl cyanide
TMS-OTf: trimethylsilyl trifluoromethanesulfonate
TMS-SPh: (phenylthio)trimethylsilane
TPP: thiamine pyrophosphate
UA: undecanoic acid
UDP: uridine diphosphate
UMP: uridine monophosphate