SYNTHESIS AND BIOLOGICAL ACTIVITY OF THE LIPOSIDOMYCINS AND CAPRAZAMYCINS, MEMBERS OF A NOVEL CLASS OF DIAZEPANONE-CONTAINING NUCLEOSIDES

DOI: http://dx.medra.org/10.17374/targets.2016.19.239

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Abstract. *The liposidomycins and caprazamycins represent an important class of nucleoside-type antibiotics featuring the presence of a highly functionalized 1,4-diazepan-2-one system. Their prominent antibiotic properties against various Gram-positive and Gram-negative bacteria, including particularly threatening strains and Mycobacterium tuberculosis have elicited great biological and chemical interest. This chapter will focus on the important synthetic efforts directed towards their total syntheses and the design and synthesis of analogues that have impacted the further understanding of the chemistry and biology of these novel antibiotics.*

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1. Introduction

The liposidomycins (LPMs, **1**) and caprazamycins (CPZs, **2**) (Figure 1) represent unique classes of complex nucleosides,[1] structurally characterized by the presence of a 3,6,7-trisubstituted-1,4-dimethyldiazepan-2-one system, which is linked to a uridine moiety through a carbon bridge. Located at this bridging carbon, we find a hydroxyl group linked to a 5-deoxy-5-amino-ribofuranosyl residue by a glycosidic bond. These intriguing natural antibiotics were isolated from various culture broths of *Streptomyces*. Chronologically, liposidomycins A (**1a**), B (**1c**) and C (**1e**) were the first members discovered

in 1985 from *Streptomyces griseosporeus*, wherein they exhibited antibiotic activity against various bacterial strains, with the *Mycobacterium* genus exhibiting the greatest sensitivity to these nucleosides with a MIC value of 1.6 µg/mL.[2] Rapidly, the antibiotic properties of these compounds were attributed to their ability to block the biosynthesis of the bacterial cell wall via inhibition of phospho-*N*-acetylmuramoylpentapeptide transferase (MraY),[3] also known as translocase I, a key enzyme in the biosynthesis of the peptidoglycans (Figure 2A). Translocase I, an integral membrane protein, catalyzes the reaction of UDP-*N*-acetylmuramoyl pentapeptide (UDP-Mur*N*Ac-pentapeptide) with the lipidic chain undecaprenylphosphate to give undecaprenyl-P-P- Mur*N*Ac-pentapeptide (Lipid I), which is the first step of the lipidic cycle allowing the transport of this key molecule from the cytoplasm to the outer membrane where the cell wall is constructed (Figure 2B).[4] The inhibition displayed by the LPMs against translocase I of *E. coli* is particularly striking compared with other known translocase I inhibitors, such as those depicted in Figure 3, which will be discussed further later. For example, LPM B-1 (**1c**) displayed a K_i of 80 nM.[5] However, despite potent inhibitory activities, their antimicrobial activities were not as strong as expected, likely due to the presence of the hydrophilic sulfate groups that confer poor permeability through the cell membrane. With the discovery of new LPMs members in 1998 (**1g-1v**),[6] many of which do not possess the sulfate moiety, it was possible to confirm the importance of this group for a lack of antibiotic activity. Thus, the LPMs displayed approximately 30-500 times greater antimicrobial potency than other related nucleosides, such as the tunicamycins (**8**), and comparable antibiotic activity to other antibiotics, such as rifampicin and claritheromycin.[7] The unprecedented molecular structures of the LPMs was elucidated a few years later after their initial discoveries.[8] Although, the absolute stereochemistry of these nucleosides was not initially established, subsequent synthetic studies carried out by Knapp allowed a tentative proposal of the absolute configurations of the various chiral centers present in the molecule. Given the striking antibiotic properties, combined with the intriguing and unprecedented molecular complexity of the liposidomycins, a great deal of scientific interest in the biological and chemical arenas was generated. The discovery of these important molecules was followed by the isolation in 2003 of the caprazamycins (CPZs, **2**) from a culture broth of *Streptomyces* sp. MK730-62F2,[9] whose structure differs from the LPMs by the absence of a sulfate group and the presence of a permethylated L-rhamnose moiety linked to the lipidic residue. The absolute stereochemistry for the CPZs was conclusively established with the obtention of an X-ray crystal structure of caprazol,[10] the core structure of the caprazamycins, and further confirmed by total synthesis, as will be described in the following section. Biologically, the CPZs are notable because of their excellent antituberculosis (anti-TB) activity, specifically against multidrug-resistant and extensively multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB and XDR-TB, respectively), and no significant toxicity in mice. Among them, caprazamycin B (**2b**) has been shown to be the most potent anti-TB agent (MIC=3.13 µg/mL) according to the same mechanism of action as the LPMs, by inhibition of translocase I. Recently, other caprazamycin members (H, I, J, K, L, M) have been identified, in which it was proposed that their fatty acid units corresponded to the fatty acids of liposidomycins Y, Z, A, G, K and N, respectively, although complete structural elucidation was not possible.[11] On the other hand, during earlier investigations, it was assumed that the absence of a sulfate moiety at the aminoribosyl unit of the caprazamycins represented a differentiating feature with respect to the liposidomycins, until recently when it was demonstrated that sulfated caprazamycins can also be produced by the genuine producer *Streptomyces* sp.

MK730-62F2 and also by the heterologous host, *Streptomyces coelicolor* M512.[12] This interesting finding was later followed by the discovery of the sulfated caprazamycin-related compounds A-90289A (**2h**) and B (**2i**),[13] in which a sulfate moiety is present at the uridine fragment instead of the aminoribosyl unit. In addition, compounds closely related to the caprazamycins, termed muraminomicins, for example muraminomicin B (**2j**), have been isolated from *Streptosporangium amethystogenes* SANK 60709.[14]

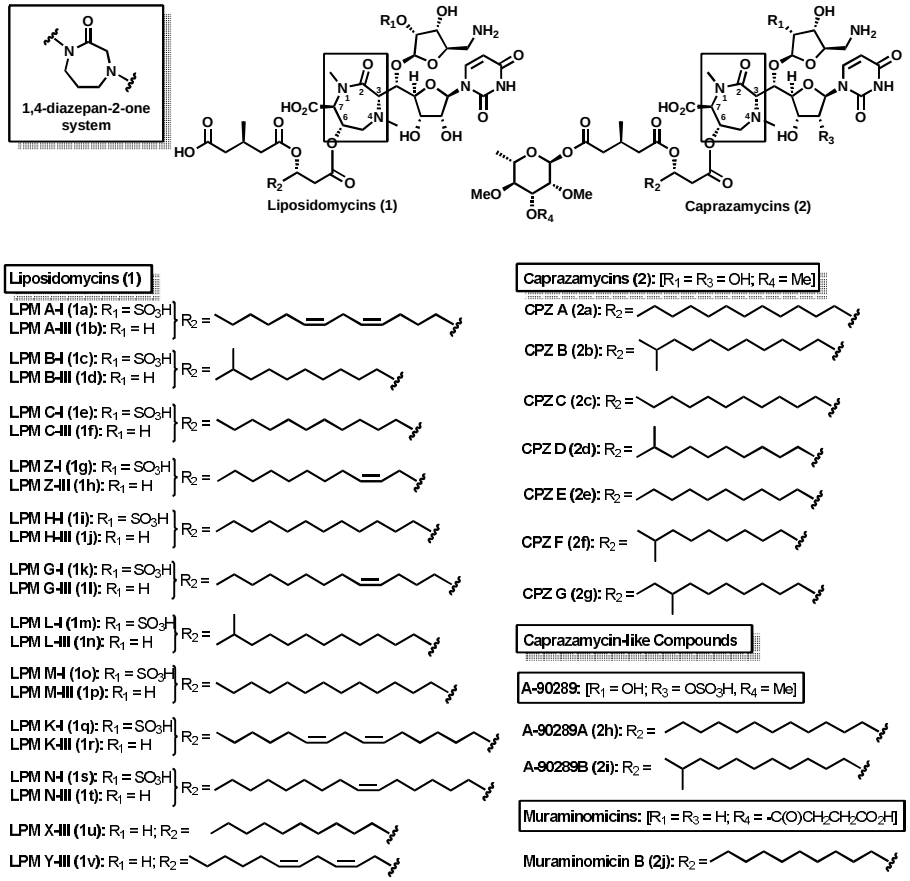


Figure 1. Molecular structures of the liposidomycins (**1**), the caprazamycins (**2**) and caprazamycin-like compounds.

The biosynthesis of this class of complex nucleosides has similarly drawn the attention of biochemists and prompted intense research activity, leading to the identification of the gene cluster responsible for their biosynthesis.[15] According to these studies, the construction of the caprazamycin core is initiated by an aldol-like reaction between uridine-5'-aldehyde and glycine to generate 5'-C-glycyluridine. The transfer of a 3-amino-3-carboxypropyl group to the 5'-amino group of the aforementioned glycyluridine arises from *S*-adenosylmethionine to generate the framework that will constitute the subsequent cyclic diazepanone. After the transfer of the aminoribosyl moiety, the subsequent biosynthetic steps, which entail cyclization to form

the diazepanone ring, *N*-methylation and attachment of the fatty acid remain undetermined and, at present, are highly speculative. At the molecular level, despite the fact that the three-dimensional structure of the complex substrate-translocase I is not yet known, extensive biological studies support the hypothesis that three strictly conserved aspartic acid residues (Asp115, Asp116, Asp267) are essential for the enzyme's catalytic activity.[16] In fact, replacement of each of these Asp residues by Asn results in a loss of enzyme activity.[17] In addition, it has been proposed that Asp115 and Asp116 interact with Mg^{2+}, which is part of the salt bridge with the diphosphate moiety of the UDP-Mur-*N*-Ac-pentapeptide, with Asp267 being the possible active site nucleophile. The recent publication of the crystal structure of MraY[18] has confirmed the importance of the aforementioned Asp residues, as well as the presence of a hydrophobic channel through the centre of the enzyme that could lead to a determination of the binding mode and the explanation of the slow-binding inhibition observed for the nucleosides LPMs and CPZs.[19] Based on these evidences, a binding model for the LPMs and CPZs has been proposed in which the ammonium group of the aminoribosyl fragment forms an ionic bond with essential Asp residues located at the active site (Figure 2C).

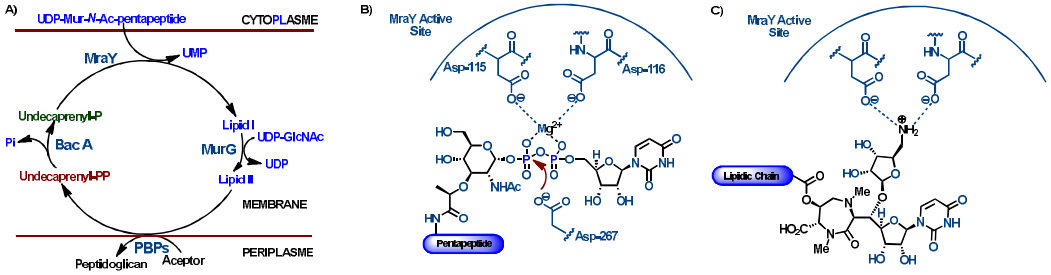


Figure 2. MraY in the biosynthesis of bacteria cell wall and proposed binding models.

As mentioned earlier, the LPMs and CPZs are not the only translocase I inhibitors identified thus far. Indeed, other related nucleosides, that contain a uridyl fragment as a common structural component, have similarly been characterized as potent translocase I inhibitors, for example the peptidyl nucleosides muraymycins (**3**), mureidomycins (**4**), pacidamycins (**5**) and napsamycins (**6**), together with FR-900493 (**7**), tunicamycins (**8**) and the capuramycins (**9**) (Figure 3).[20]

Due to the severe threat posed by the growing emergence of bacterial strains resistant to the current arsenal of antibiotics,[21] particularly vancomycin-resistant *Enterococcus* (VRE), methycillin-resistant *Staphylococcus aureus* (MRSA) and the aforementioned *Mycobacterium tuberculosis*, the discovery of new antibiotics with novel mechanisms of action has become a worldwide priority. For this reason, we have witnessed in the last few years increasing interest in this class of antibiotics by virtue of their prominent biological profiles, which has elicited a flurry of research activity in the biological and synthetic arenas. Herein, we wish to report the state of the art with regards to the chemistry and biology of the liposidomycins and caprazamycins, paying special attention to the synthetic efforts directed to the construction of the seven-membered 1,4-diazepan-2-one system, which represents an unprecedented scaffold in natural products and an unexplored building block in medicinal chemistry,[22] with the exception of the 1,4-benzodiazepin-2-ones which are precursors of the clinically important benzodiazepines.[23]

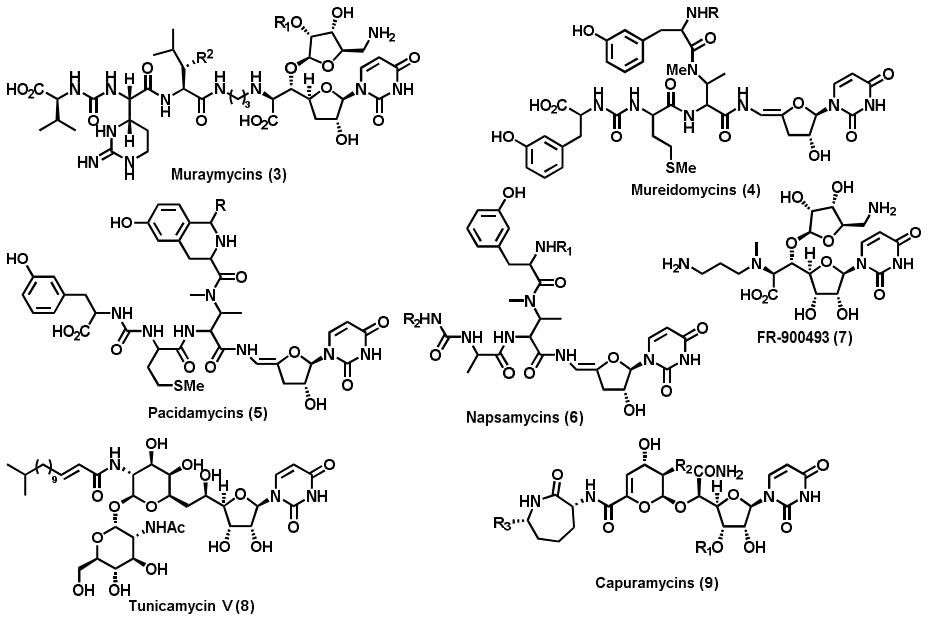
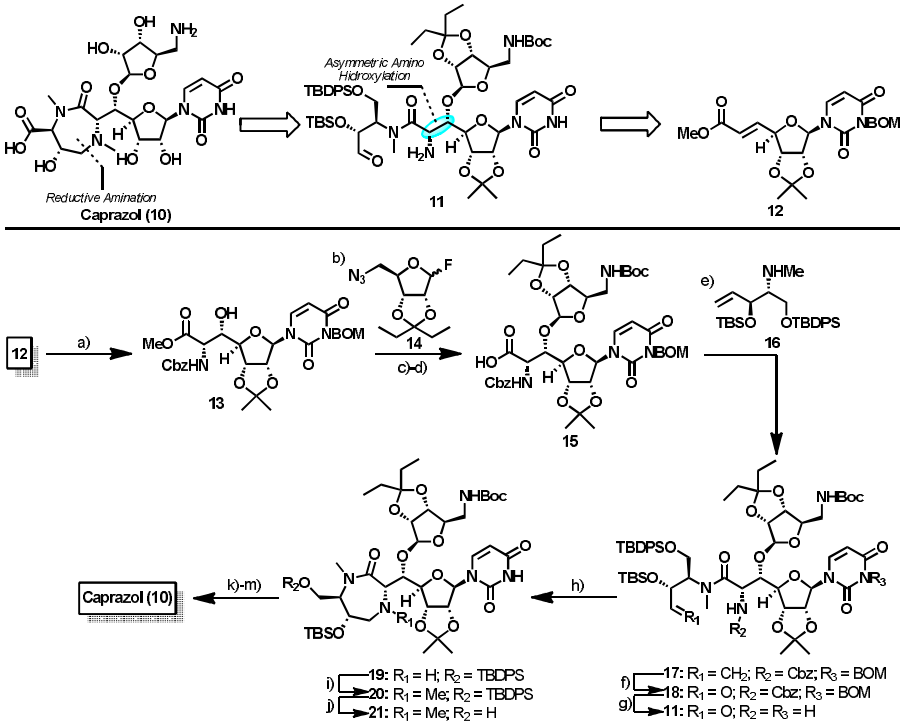


Figure 3. Molecular structures of other MraY inhibitors.

2. Total synthesis of caprazol and the caprazamycins

Despite the discovery and structural determination of the first liposidomycins being achieved in the late eighties, the first total synthesis of caprazol (**10**), which represents the core of this class of nucleosides, was not reported until 2005 by the group of Matsuda,[24] while the first total synthesis of the complete structure of a caprazamycin (CPZ A, **1a**), including the lipidic fragment, was not reported until 2015 by the Takemoto group.[25] During these intervening years, many synthetic strategies have been reported and, with them, the preparation of simple analogues. However, none of them described the completion of a total synthesis of the natural products. This fact reflects the molecular complexity that the LPMs and CPZs possess, and the difficulty that such molecular complexity brings for their construction. The principle synthetic challenge lies with the construction of the highly functionalized diazepanone system and its linkage to the uridyl fragment. No less important is the introduction of the 5-aminoribose moiety whose introduction via a glycosidic bond could present two important hurdles: 1) the presence of a tertiary amine which could suppress the action of any Lewis acid utilized for the glycosylation reaction, and 2) the highly sterically hindered environment that is located near the 5'-hydroxyl group, which is the acceptor in the glycosilation reaction. These synthetic challenges were effectively solved by Matsuda et al. in their first synthesis for caprazol. The synthesis is based on the brief retrosynthetic analysis depicted in Scheme 1. Accordingly, the diazepanone system contained in caprazol was disconnected at the C-N bond, which would be formed though an intramolecular reductive amination in the synthetic direction, leading to **11** as the acyclic key precursor, which should contain the 5-aminoribose unit incorporated in the earlier steps of the synthesis. The required amino alcohol system could be introduced by means of a Sharpless aminohydroxylation from the α,β-unsaturated ester **12**, readily available from the corresponding aldehyde

uridyl derivative via a Wittig reaction. In practice, the asymmetric aminohydroxylation of **12** led to the amino alcohol derivative **13** in a 52% yield, together with its diastereoisomer in a 9% yield (Scheme 1).



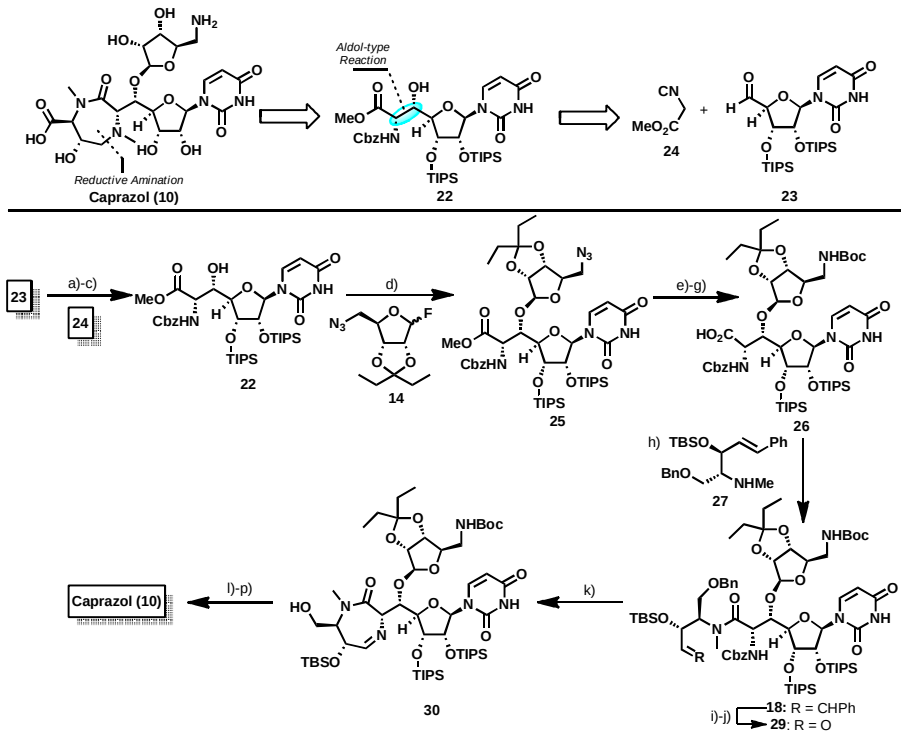
Reagents and Conditions: a) $CbzNH_2$, $K_2OsO_2(OH)_4$, $(DHQD)_2AQN$, NaOH, ^{t}BuOCl, *n*PrOH/H_2O, 5 ºC→25 ºC, 52%; b) **14**, $BF_3.OEt_2$, MS 4Å, CH_2Cl_2, -30 ºC, 80%; c) Ph_3P, H_2O, benzene/THF, 50 ºC; then $(Boc)_2O$, 95% over 2 steps; d) $Ba(OH)_2$, THF/H_2O, 25 ºC, 50%; e) **16**, DEPBT, $NaHCO_3$, THF, 0 ºC, 89%; f) OsO_4, NMO, ^{t}BuOH, acetone/H_2O; then $NaIO_4$, 60% over 2 steps; g) H_2, Pd/C, ^{i}PrOH, 25 ºC; h) $NaBH(OAc)_3$, AcOH, RT, 24% for **19** and 34% for **20** over 2 steps; i) $(CH_2O)_n$, $NaBH(OAc)_3$, AcOH, 25 ºC, 65%; j) NH_4F, MeOH, 25 ºC, 72%; k) Dess-Martin periodinane, CH_2Cl_2, 25 ºC; l) $NaClO_2$, NaH_2PO_4, ^{t}BuOH/H_2O, 56% over 2 steps; m) HF, THF/H_2O, 50%. BOM=benzyloxymethyl, DEPBT=3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one, $(DHQD)_2AQN$=1,4-bis(dihydroquinidinyl)anthraquinone.

Scheme 1. Synthesis of caprazol by Matsuda.

At this stage, the glycosylation was carried out by using the ribosyl fluoride **14** as the glycosyl donor, which was activated with $BF_3.Et_2O$ in the presence of **13** to furnish the corresponding riboside in an 80% yield as a 96:4 mixture in favor of the desired α-anomer. The use of **14** as the glycosyl donor, as well as the final reaction conditions, was the result of an extensive optimization study of this key glycosylation reaction.[26] With compound **14** in hand, several functional group interconversion reactions were carried out to provide the acid **15**, which was coupled with the secondary amine **16**, prepared from D-serine, by treatment with 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEBT) to provide the amide **17**. Subsequently, amide **17** was prepared for the key reductive amination reaction by oxidatively cleaving the olefin and removing the Cbz protecting group. The resulting amino aldehyde **11** was then subjected to the action of $NaBH(OAc)_3$ in the presence of acetic acid to afford the coveted diazepanone derivative **19** in a

24% yield over 2 steps, together with the *N*-methyl derivative **20** in a 34% yield. This unexpected *N*-methylation reaction was adscribed to the formation of formaldehyde during the cleavage of the BOM group in the previous step. Having constructed the caprazol precursor **19**, together with its *N*-methyl derivative **20**, the synthesis proceeded with the *N*-methylation of **19** for the full conversion to **20**, followed by desilylation and oxidation via a two-step sequence of the primary alcohol **21** to its corresponding acid. Finally, global deprotection with aqueous HF provided caprazol (**10**).

The second total synthesis of caprazol (**10**), recently published by the research group of Watanabe and Shibasaki,[27] is based on the same strategy used by Matsuda for the key processes, which are a) the construction of the diazepanone system via a reductive amination and b) installation of the ribose moiety by a glycosylation reaction (Scheme 2).



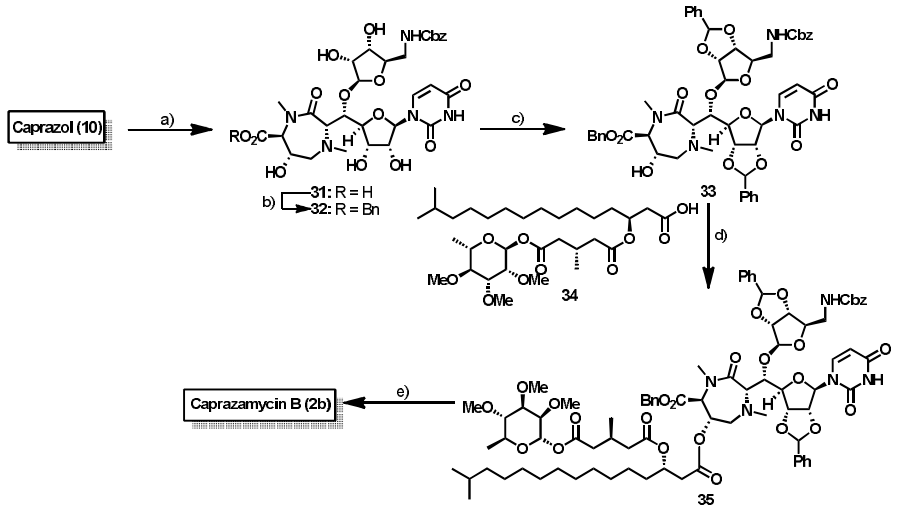
Reagents and Conditions: a) **24**, CuCl, PPh_3, DIPEA, THF, 25 ºC; b) 4 M HCl, THF, RT, 67% over 2 steps (dr=88:12); c) CbzCl, $NaHCO_3$, THF/H_2O, 0 ºC, 90%; d) **14**, $BF_3.OEt_2$, MS 4Å, CH_2Cl_2, -30 ºC, 72%; e) Ph_3P, H_2O, toluene/THF, 50 ºC; f) $(Boc)_2O$, $NaHCO_3$, 90% over 2 steps; g) LiI, EtOAc, 80 ºC, 64%; h) **27**, DEPBT, $NaHCO_3$, THF, 25 ºC, 90%; i) OsO_4, NMO, 1,4-dioxane/H_2O, 60 ºC; j) $NaIO_4$, acetone/phosphate buffer, 25 ºC, 71% over 2 steps; k) H_2, Pd/C, MeOH, 60 ºC; l) $NaBH_3CN$, AcOH, 25 ºC; m) $(CH_2O)_n$, $NaBH_3CN$, $NaBH(OAc)_3$, AcOH, 25 ºC, 51% over 3 steps; n) Dess-Martin periodinane, CH_2Cl_2, 25ºC; o) $NaClO_2$, NaH_2PO_4, 60% over 2 steps; p) HF, CH_3CN, 50 ºC, 45%.

Scheme 2. Synthesis of caprazol by Shibasaki.

A key difference between the two is the manner in which the amino alcohol system is constructed, being a stereoselective aldol-type reaction between aldehyde **23** and isocyanoacetate (**24**) the reaction

employed by Watanabe and Shibasaki. Thus, treatment of **23** with **24** in the presence of catalytic amounts of Cu (I), PPh_3 and DIPEA afforded the corresponding oxazoline adduct, which could not be isolated due to its instability on silica gel. Thus, the crude aldol product was hydrolyzed under acidic conditions to provide the desired amino alcohol as the major product (dr=88:12) in a combined 67% overall yield. The protection of the amino group as the Cbz carbamate **22** was followed by a glycosylation reaction with **14**, which was achieved under identical conditions to those reported by Matsuda, to furnish the compound **25** in a 72% yield as a single anomer. After manipulation of **25** in an identical manner as described previously for **15**, the resulting acid **26** was coupled with amine **27** by the action of the coupling reagent DEPBT. The resulting amide **28**, obtained in a 90% yield, was subjected to the oxidative action of $OsO_4/NaIO_4$ to give aldehyde **29**, which was treated with Pd/C in a H_2 atmosphere to furnish the imine intermediate **30**. This compound was carried towards caprazol following the same synthetic scheme as reported by Matsuda in Scheme 1. Remarkably, in this case, the imine reduction was accomplished with $NaBH_3CN$, followed by *N*-methylation to afford the corresponding protected caprazol in a 51% yield over 3 steps from **29**. Finally, oxidation of the primary alcohol to the acid and a deprotection step under acidic conditions provided caprazol without any issue.

Soon after their reported synthesis of caprazol, Shibasaki et al. reported the complete total synthesis of one member of the caprazamycins, caprazamycin B (**2b**),[28] which required the preparation of a rhamnose-containing lipidic acid **34**, found in the western zone of the molecule (Scheme 3).



Reagents and Conditions: a) CbzCl, $NaHCO_3$, $MeCN/H_2O$, 0 ºC, 79%; b) BnOH, BOP-Cl, Et_3N, DMF, 0 ºC, 63%; c) $PhCH(OMe)_2$, *p*-TsOH, DMF, 25 ºC, 74%; d) **34**, MNBA, DMAP, Et_3N, CH_2Cl_2, 25 ºC, 32%; e) Pd black, HCO_2H, EtOH, 25 ºC, 56%. BOP-Cl=bis(2-oxo-3-oxazolidinyl)phosphinic chloride, MNBA=2-methyl-6-nitrobenzoic anhydride.

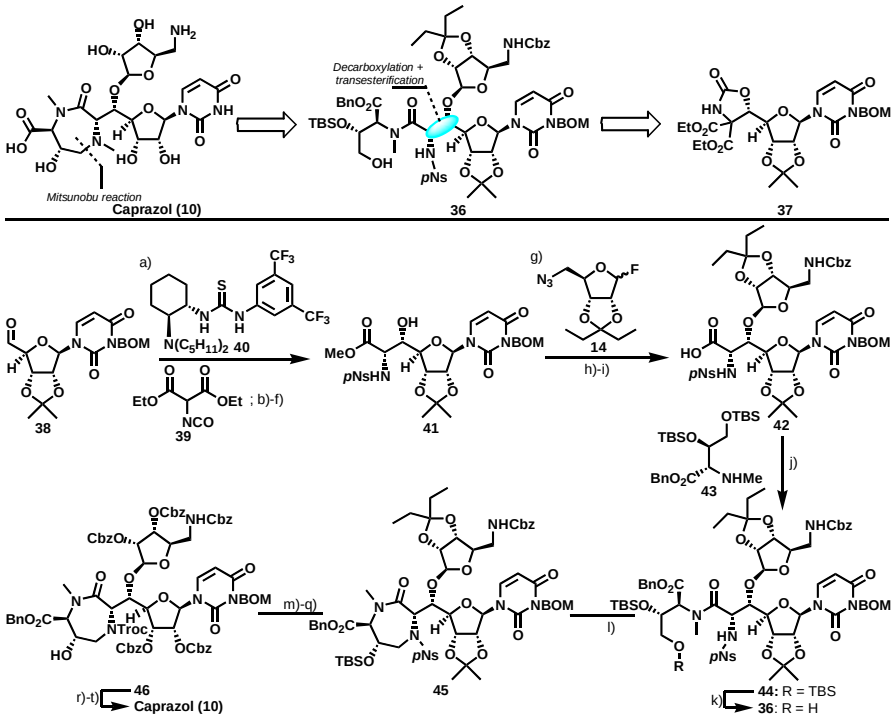
Scheme 3. Total synthesis of caprazamycin B.

Having prepared caprazol (**10**) and the lipidic fragment **34**,[29] Shibasaki and coworkers faced the challenge of connecting the both fragments. Initially, the authors attempted the direct esterification between acid **34** and a caprazol derivative obtained during the synthetic course described in Scheme 2.

Unfortunately, all attempts of esterification failed under a wide variety of coupling reagents and conditions. In light of these disappointing results, they decided to prepare the caprazol derivative **33** in three steps: 1) carbamate formation, 2) benzyl esterification and 3) diol protection as a benzylidene acetal. The coupling of **33** with **34** was then performed according to the Shiina´s protocol, by using of 2-methyl-6-nitrobenzoic anhydride (MNBA) and DMAP to provide the desired caprazamycin derivative **35** albeit in a poor 32% yield. The final removal of the protecting groups, all of them sensitive to reduction conditions, was not trivial and therefore required extensive experimental investigation. Finally, it was identified that treatment of **35** with Pd black in a mixture of ethanol and formic acid, in the absence of H_2, afforded caprazamycin B (**2b**) as the sole product in a 56% yield. Prior to the total synthesis of caprazamycin B (**2b**) by Shibasaki, Takemoto and coworkers published the first synthesis of a complete liponucleoside belonging to the CPZ family, caprazamycin A (**2a**).[25] Interestingly, this total synthesis utilized a novel strategy for the construction of the diazepanone-containing ring based on an intramolecular Mitsunobu-type reaction.

While the stereoselective synthesis of the *syn*-α-hydroxy amino acid moiety was conceived to proceed through a novel diastereoselective aldol reaction between aldehyde **38** and isocyanate **39** in the presence of a chiral organocatalyst that should provide compound **37** as a potential precursor of **36**. The authors tested the reaction of **38** with **39** and Et_3N in the absence of catalyst to afford a mixture of the aldol products with poor diastereoselectivity. However, the addition of the chiral thiourea **40** (7 mol%) in the reaction mixture provided the desired aldol carbamate **37** in 80% yield and high stereoselectivity (dr=5:1). The transformation of the aldol product **37** into the corresponding *syn* α-hydroxy amino acid was successfully accomplished by treating the dicarboxylate derivative with the zinc reagent $Zn_4(OCOCF_3)_6O$, resulting in a regioselective decarboxylation and transesterification that gave the more thermodynamically stable *trans*-oxazolidinone. Protection of the amino group and cleavage of the carbamate produced the derivative **41** (Scheme 4). At this point, the introductions of the aminoribosyl moiety and the α-amino ester **43**, via a glycosylation reaction and an amide coupling, respectively, were undertaken in a similar fashion as described in the Matsuda synthesis of caprazol, to provide intermediate **44**. Selective desilylation of **44** was then accomplished, setting up the key intramolecular Mitsunobu reaction of the resulting alcohol **36**. The cyclization was achieved by treatment of **36** with di-*tert*-butyl azodicarboxylate (DBAD) in the presence of Ph_3P to afford the desired diazepanone derivative **45** in a remarkable 73% yield. Protecting group manipulation was then required prior to the incorporation of the lipidic fragment. To this aim, **45** was converted into the protected caprazol **46**, whose structure was confirmed by transformation into the known caprazol (**10**) according to the steps r-t (Scheme 4).

For the completion of the synthesis of caprazamycin A (**2a**), the connection of the lipidic fragment to the caprazol framework was carried out in a sequential manner, first with the introduction of acid **47**, via conventional esterification with EDCI, followed by subsequent esterification of acid **49** with the resulting alcohol **48**, using Yamaguchi conditions, to give the protected caprazamycin **50** in a 64% yield. Caprazamycin A precursor **50** was then subjected to a global deprotection by hydrogenation with Pd black/formic acid, to provide caprazamycin A (**2a**) in essentially quantitative yield (Scheme 5).

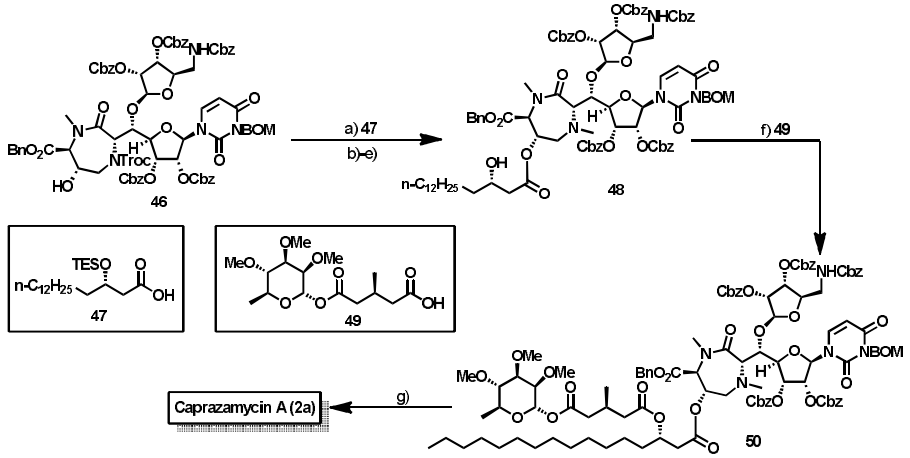


Reagents and Conditions: a) **39**, 7% **40**, toluene, 25 ºC, 80% (dr=5:1); b) aq. KOH, THF, 0 ºC; c) DBU, 70 ºC, 86%; d) $Zn_4(OCOCF_3)_6O$, MeOH, 50 ºC, quant.; e) NaH, *p*NsCl, DMF, 0 ºC; f) NaOMe, MeOH, 65% over 2 steps; g) **14**, $BF_3.OEt_2$, MS 4Å, CH_2Cl_2, -30 ºC, 71%; h) Ph_3P, H_2O, benzene/THF; then CbzCl, $NaHCO_3$, i) $Ba(OH)_2$, THF/H_2O, 0 ºC; j) **43**, Ghosez reagent, CH_2Cl_2, $NaHCO_3$, 0 ºC, 46% over 3 steps; k) CSA, MeOH/CH_2Cl_2, 0 ºC, 68%; l) PPh_3, DBAD, toluene, 0 ºC, 75%; m) K_2CO_3, PhSH, MeCN, 0 ºC, 73%; n) TrocCl, DMAP, pyridine, CH_2Cl_2, 0 ºC, 79%; o) *p*-TsOH, MeOH, 60 ºC, 41%; p) CbzCl, DMAP, CH_2Cl_2, 0 ºC; q) *p*-TsOH, MeOH, 60 ºC, 71% over 2 steps; r) Zn, AcOH, THF, 50 ºC; s) $(CH_2O)_n$, $NaBH(OAc)_3$, AcOH, 25 ºC, quant. for 2 steps; t) Pd black, EtOH/HCO_2H, 25 ºC, 46%. DBAD=di-*tert*-butyl azodicarboxylate, *p*Ns=*p*-nitrobenzenesulfonyl, TrocCl=2,2,2-trichloroethyl chloroformate, Ghosez reagent=1-chloro-*N*,*N*,2-trimethyl-1-propenylamine.

Scheme 4. Synthesis of caprazol by Takemoto.

2. Synthetic approaches towards the liposidomycins and the caprazamycins

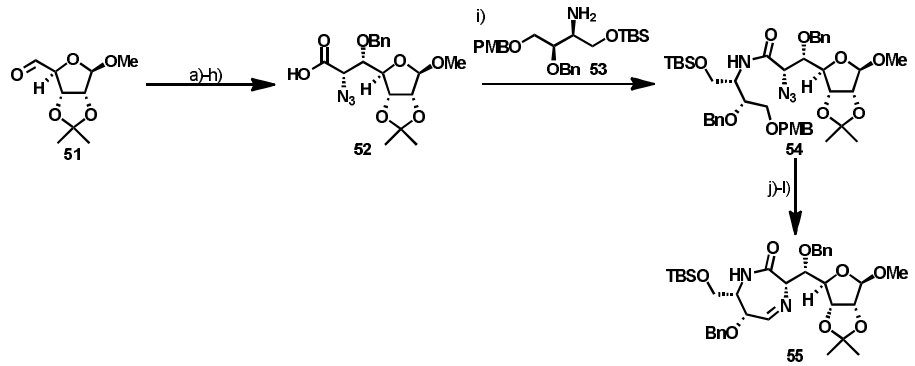
Since the discovery of the first liposidomycins and the recognition of their potential as novel antimicrobial agents, a great deal of excitement among chemists has been generated leading to a flurry of synthetic activity directed towards this class of molecules. Together with the total syntheses described in the previous section, diverse synthetic strategies have been developed towards the construction of the diazepanone ring system with the aim of applications to an eventual total synthesis. Ubukata and coworkers were one of the first authors who reported their initial synthetic efforts directed towards the liposidomycins, establishing the basis of an intramolecular reductive amination for the formation of the diazepanone ring system.[30] Thus, starting from the aldehyde **51**, derived from methyl-β-D-ribofuranoside, they prepared acid **52** and coupled it with amine **53** to obtain amide **54**. Both key products, **52** and **53**, were prepared utilizing epoxide chemistry, which were generated in a stereoselective manner by Sharpless asymmetric epoxidations.



Reagents and Conditions: a) **42**, EDCI, DMAP, CH_2Cl_2, 0 ºC; b) Zn, AcOH/THF, 25 ºC; c) AcOH, $ClCH_2CH_2Cl$, 25 ºC; d) $(CH_2O)_n$, $NaBH(OAc)_3$, AcOH, 25 ºC; e) HF.py, THF, 0 ºC, 43% over 5 steps; f) **49**, 2,4,6-trichlorobenzoyl chloride, DMAP, Et_3N, 0 ºC, 64%; g) Pd black, $EtOH/HCO_2H$, 25 ºC, 98%.

Scheme 5. Total synthesis of caprazamycin A.

The formation of the diazepanone ring system was accomplished from **54** via oxidation of the primary alcohol, obtained after PMB-ether group deprotection, and subsequent reduction of the azide group by catalytic hydrogenation to afford imine **55** (Scheme 6).

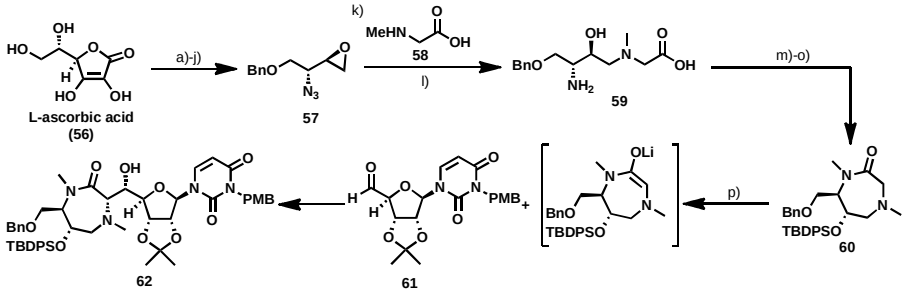


Reagents and Conditions: a) $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, KHMDS, 18-crown-6, toluene/THF, 80%; b) DIBAL, CH_2Cl_2, -20 ºC, 81%; c) L-(+)-DET, $Ti(O^iPr)_4$, 80% cumene hydroperoxide, 78%; d) NaN_3, NH_4Cl, $MeOCH_2CH_2OH/H_2O$, reflux; e) TBSCl, imidazole, DMF, 25 ºC, f) NaH, BnBr, THF, 25 ºC, 77% over 3 steps, g) TBAF, THF, 25 ºC, 95%; h) PDC, PTFA, DMF, 25 ºC; i) **53**, DCC, HOBt, DMF, 60%; j) DDQ, CH_2Cl_2/H_2O, 63%, k) CrO_3, pyridine, CH_2Cl_2, 25 ºC, 68%; l) H_2, Pd/C, EtOAc, 25 ºC, 36%.

Scheme 6. Synthesis of the diazepanone via imine formation.

Some years later, Kim et al. conducted extensive synthetic studies directed towards the preparation of the nucleoside core of the liposidomycins, in which they prepared the diazepanone ring system which subsequently could be linked to a uridine unit later. In addition, they designed the preparation of various isomers of the diazepanone fragment from a common starting material in order to address the absolute

configuration of these products, not yet established at the time. Scheme 7 describes the preparation of one of the stereoisomers of the diazepanone that they employed in the generation of the liposidomycin core. To this end, the synthesis commenced from L-ascorbic acid (**56**) which was converted into the epoxide **57** in a synthetic sequence of eleven steps that proceeded in overall good yields. The epoxide opening with sarcosine (**58**) was followed by azide reduction to give the amino acid **59** which was subjected to a lactamization reaction that led to diazepanone **60**, after some additional chemical manipulations.[31] The linkage between diazepanone **60** and the uridinyl fragment in the form of aldehyde **61** was devised to be done via an aldol reaction.[32] Thus, after formation of the corresponding enolate of **60**, the reaction with **61** provided aldol compound **62** as the major isomer together with the other three possible stereoisomers in a 70:21:5:4 ratio and a 61% combined yield. The major stereoisomer was isolated from the stereoisomeric mixture and its stereochemistry established via spectroscopic and chemical degradation studies (Scheme 7).

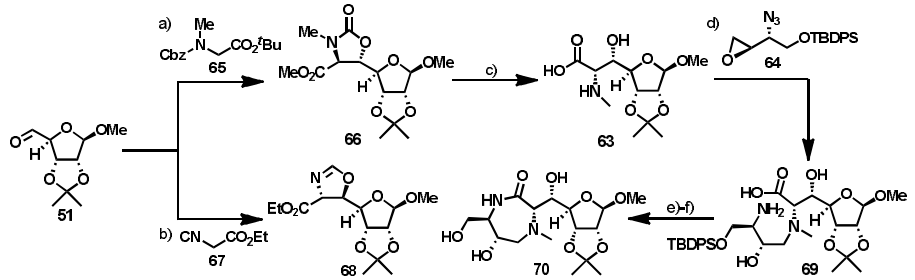


Reagents and Conditions: a) AcCl, acetone, 25 ºC; b) 35% H_2O_2, $CaCO_3$, H_2O, 0 ºC→ 25 ºC; c) MeI, $NaHCO_3$, $AcNMe_2$, 25 ºC, 65% over 3 steps; d) $NaBH_4$, EtOH, 0 ºC→ 25 ºC, 83%; e) (*n*-Bu)$_2$SnO, MeOH, reflux; then BnBr, DMF, 70-80 ºC, 90% over 2 steps; f) TsCl, pyridine, 0 ºC, 95%; g) NaN_3, DMF, 70-80 ºC, 90%; h) aq. HCl, MeCN, 87%; i) TsCl, pyridine, 0 ºC, 75%; j) K_2CO_3, MeOH, 25 ºC, 85%; k) $MeNHCH_2CO_2H$, Et_3N, MeOH, reflux, 83%; l) H_2, Pd/C, MeOH, 72%; m) DCC, CH_2Cl_2, 0 ºC, 71%; n) TBDPSCl, imidazole, DMF, 83%; o) MeI, NaH, DMF, 76%; p) *n*-BuLi, THF, -78 ºC, 30 min; then **61**, THF, -78 ºC, 61% (dr=70:21:5:4).

Scheme 7. Synthesis of the liposidomycins core via aldol reaction.

Related to the work of Kim et al. is the synthetic work developed by Le Merrer and coworkers[33] published in 2001, in which the diazepanone ring system was prepared by a lactamization reaction after the connection of both key fragments, the ribosylamino acid **63** and the epoxide **64**. Previously, epoxide **64** was prepared according to the procedure described by Kim from L-ascorbic acid (**56**), whereas ribosylamino acid **63** was synthesized via two different routes. The first route consisted of the aldol reaction between aldehyde **51** with the enolate of *tert*-butyl *N*-benzyloxysarcosinate (**65**) that afforded oxazolidinone **66** as the major isomer. The second route was based on the aldol-type reaction of **51** with ethyl isocyanoacetate **67**. This type of reaction was used later by Shibasaki for the total synthesis of caprazol, as described earlier (See Scheme 2). However, in this case, the reaction gave rise to the corresponding oxazolines with poor yield, in favour of the compound **68**. The difference in stereoselectivity observed in the two routes was adscribed to chelation effects in the first route and not in the second. Consequently, the synthesis continued with oxazolidinone **66** which was transformed into the ribosylamino acid **63**. The coupling of the fragments **63** and **64** proceeded efficiently to give the epoxide opened product **69**, which was smoothly cyclized by the

action of DCC and final desilylation to afford compound **70** (Scheme 8). Based on these synthetic studies, these authors published later an optimization study on the preparation of the diazepanones.[34]

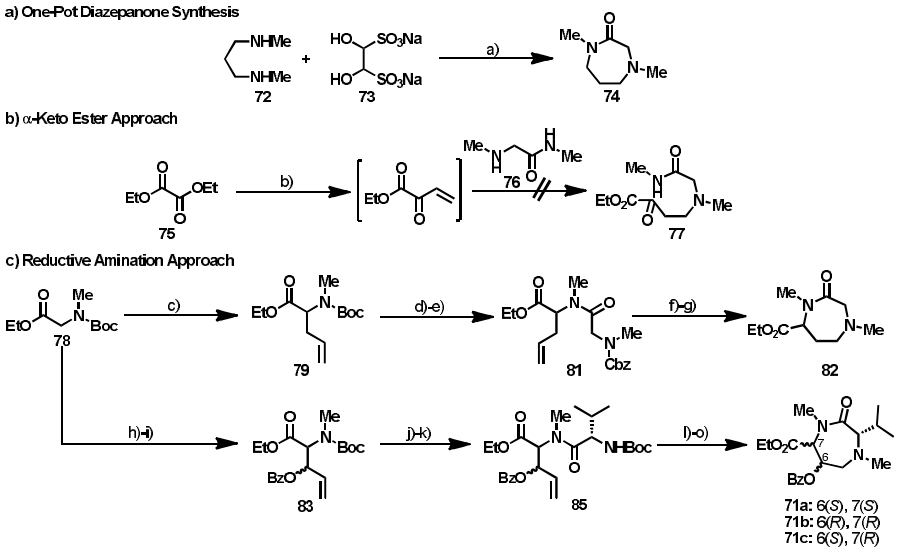


Reagents and Conditions: a) **65**, LDA, -78 ºC; then KOH/MeOH, 80 ºC, 47% (95:5); b) **67**, Et_3N, THF, 25 ºC, 76% (3:7); c) aq. KOH, 100 ºC, 72%; d) **64**, ^{t}BuONa, ^{t}BuOH, 100 ºC, 48 h; then H_2, Pd/charcoal, MeOH, 25 ºC, 65% over 2 steps; e) DCC, CH_2Cl_2, 0 ºC→25 ºC, 34%; f) TBAF, THF, 25 ºC, 74%.

Scheme 8. Synthetic approach to the liposidomycins via tandem epoxide opening/lactamization.

Of particular importance is the work by Knapp et al. whose contributions in this field focused on the assignment of the stereochemistry of the diazepanone contained in the liposidomycins. In this sense, the Knapp group prepared a set of diazepanone derivatives represented by the compounds **71a**-**71c** allowing them to correlate the absolute configurations of these diazepanones with the compound **86** (see Scheme 10), a degradation product obtained from natural liposidomycins. The preparation of the diazepanone derivatives was preceded by an extensive synthetic study directed to the establishment of a valid and efficient methodology for the synthesis of 1,4-diazepan-2-ones that it is summarized in Scheme 9.[35] Knapp initially explored various methodologies based upon synthetic precedents found in the literature. One of these procedures was the direct formation of simple diazepanones of the type represented by compound **74** via reaction of diamine **72** with the sodium bisulfite adduct of glyoxal **73**, which proved unsuitable for the preparation of more complex diazepanones. A second approach was based on α-keto ester amination as a method for closing the N-1/C-7 bond. In this case, ethyl glyoxylate (**75**) was reacted with vinylmagnesium bromide, to give an olefin keto ester intermediate, which was then treated with sarcosine *N*-methylamide (**76**) that should give compound **77**. However, this methodology was similarly unsuccessful as they did not obtain **77** in all cases. Finally, the reductive amination methodology by ring closure of the alternative N-4/C-5 bond proved to be more successful. This synthetic approach was validated starting from the sarcosine derivative **78**, which was alkylated with allyl iodide and then coupled with another sarcosine unit in the form of the derivative **80** to afford dipeptide derivative **81**. Subsequent oxidation of the double bond and hydrogenation provided the desired diazepanone **82** in good yield. The same synthetic sequence was then implemented towards the more complex diazepanones **71a**-**71c**, through derivatives **83** and **85**. The final reductive aminations of the different stereoisomers of **85** proceeded in good yields and the stereochemical assignment of the different diastereoisomers **71a**-**71c** was achieved by means of NMR spectroscopic analyses (Scheme 9). The stereochemical correlation between **71a**-**71c** and **86** was supported by extensive NMR spectroscopic studies and molecular modelling calculations of all these compounds. These studies led to a tentative assignment of the absolute configuration of the diazepanone ring system found in the

liposidomycins, which was found to be correct as demonstrated in future studies with the obtention of an X-ray structure of the natural products and supported by the total syntheses of some representative members. Closely related stereochemical assignments studies have also been carried out by Nakajima et al.[36]



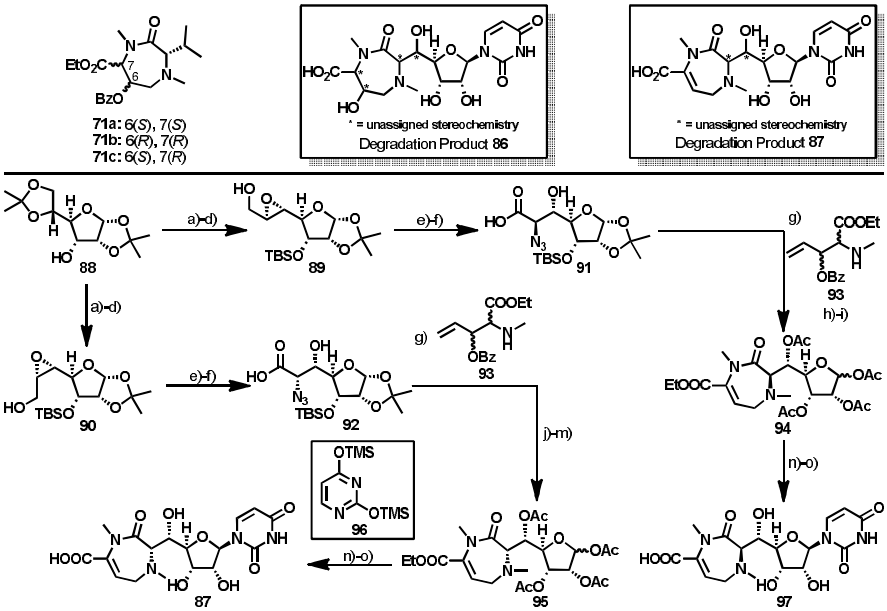
Reagents and Conditions: a) aq $NaHSO_3$, toluene, reflux, 40%; b) CH_2=$CHMgBr$, Et_2O, -100 ºC; then **76**, **77** not observed; c) LDA, THF, -78 ºC; then allyl iodide, 84%; d) TFA, CH_2Cl_2, 25 ºC; e) *N*-Cbz-sarcosine (**80**), DIPC, HOBt, CH_2Cl_2, 25 ºC, 78% over 2 steps; f) OsO_4, $NaIO_4$, aq. THF; g) H_2, Pd/C, MeOH, 25 ºC, 67% over 2 steps; h) LDA, THF, -78 ºC; then acrolein, -78 ºC→ -50 ºC; i) PhCOCl, -50 ºC, 81% over 3 steps (4:1 *anti*:*syn*); j) HCl, dioxane/CH_2Cl_2, 25 ºC; k) *N*-Boc-L-valine (**84**), 2-chloro-4,6-dimethoxy-1,3,5-triazine, *N*-methyl morpholine, CH_2Cl_2, 0 ºC, 72% over 2 steps; l) O_3, CH_2Cl_2, -78 ºC; then Me_2S, 23 ºC; m) HCl, dioxane/CH_2Cl_2, 0 ºC, n) $NaBH(OAc)_3$, DIPEA, CH_2Cl_2, 25 ºC, 42% combined yield o) CH_2=O, H_2, Pd/C, MeOH, 72% for **71a**, 70% for **71b**, 15% for **71c**.

Scheme 9. Synthetic approaches towards diazepanones by Knapp.

The reductive amination methodology was similarly extended to the synthesis of the diazepanone derivative **87**, which represented another degradation product obtained from the natural liposidomycins. With this synthesis, described in Scheme 10, it was possible to confirm the stereochemistry at C-5' and C-6' positions.[37] As described in the scheme, the synthesis was based on an intramolecular reductive amination of an amino aldehyde intermediate. In this case, the introduction of the amino alcohol system, represented by compounds **91** and **92**, was carried out via regioselective opening of the *trans*- and *cis*-epoxy alcohols **89** and **90** with sodium azide. Epoxy alcohols **89** and **90** were in turn obtained via Sharpless asymmetric epoxidation and *m*-CPBA mediated oxidation, respectively, from their corresponding allylic alcohols derived from the D-*allo*furanoside **88**. The couplings of **91** and **92** with the sarcosine derivative **93**, in the form of a mixture of their four possible diastereoisomers, was followed by the cyclization process, according to the reductive amination procedure described in Scheme 9, to deliver the corresponding diazepanone derivatives **94** and **95**, respectively. It is noteworthy to point out that an elimination reaction of the benzoate moiety occurred during the reduction of the presumed imine intermediate.

Also, it was surprising to find the presence of a methyl group at the *N*-4 in the resulting final cyclization product **94**, which can be rationalized by the reaction at some point of the primary amine with formaldehyde, produced during the ozonolysis of the terminal olefin. Interestingly, neither elimination nor *N*-methylation occurred for the *syn* isomer, which required additional steps (k-l) to obtain **95**.

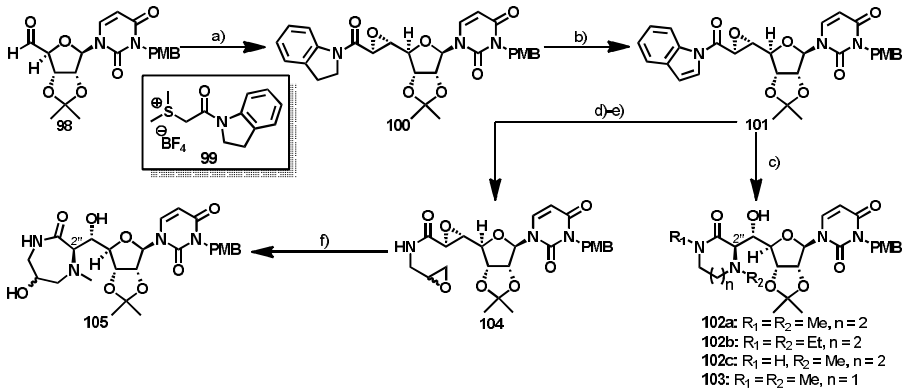
Finally, the introduction of the nucleoside residue was performed via glycosylation of the acetates **94** and **95** with [bis(trimethylsilyl)uracil] (**96**), albeit in poor yields, followed by base treatment of the resulting nucleoside derivatives to give **97** and **87**, respectively. Having prepared compounds **87** and **97**, a comparison of their NMR spectroscopic properties with the natural degradation product revealed a close match with **87** but not with **97**, thus allowing the unambiguous establishment of the stereochemistry at the chiral centres of the diazepanone system of the liposidomycins (Scheme 10).



Reagents and Conditions: a) TBSCl, imidazole, DMF, 25 ºC, 95%; b) 75% aq. AcOH, 25 ºC; then $NaIO_4$, H_2O, THF, 25 ºC; then $Ph_3P{=}CHCO_2Me$, CH_2Cl_2, 25 ºC, 69% over 3 steps for the *E*-α,β-unsaturated ester; $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, 18-crown-6, KMDS, CH_2Cl_2, -78 ºC, 60% for the *Z*-α,β-unsaturated ester; c) DIBAL-H, THF, -78 ºC→ 25 ºC, 67% for the *trans*-allylic alcohol, 55% for the *cis*-allylic alcohol; d) D-(-)-DET, $Ti(OiPr)_4$, 80% cumene hydroperoxide, 4 Å molecular sieves, -12 ºC→ 0 ºC, 79% for **89**; *m*-CPBA, CH_2Cl_2, 0 ºC→25 ºC, 67% for **90**; e) $NaIO_4$, CCl_4:CH_3CN:H_2O 1:1:1.5 volumen mixture, $RuCl_3$; f) NaN_3, NH_4Cl, MeOH, reflux, 91% over 2 steps for **91**, 66% over 2 steps for **92**; g) **93**, EEDQ, CH_2Cl_2, 25 ºC, 28% and 48% yields respectively; h) O_3, CH_2Cl_2, -78 ºC; then Ph_3P, THF, 25 ºC; then $NaBH(OAc)_3$, CH_2Cl_2, 25 ºC, 28%; i) Methanesulfonic acid, Ac_2O, 25 ºC, 72% for **94** as a 4:1 mixture of two anomers; j) O_3, CH_2Cl_2, -78 ºC; then EtOH, H_2, Pd/C; then $NaBH(OAc)_3$, 25 ºC, 8.6%; k) DBU, toluene, 25 ºC, 73%; l) $NaBH(OAc)_3$, formalin, AcOH:CH_3CN, 60 ºC, 83%; m) Ac_2O, methanesulfonic acid, 25 ºC, 52% for **95**; n) *O*,*O*-*bis*-(trimethylsilyl)uracil (**96**), CH_3CN:CH_2Cl_2, trimethylsilyl trifluoromethanesulfonate, reflux, 18% for the *anti* isomer, 15% for the *syn* isomer; o) LiOH, EtOH, 25 ºC, 64% for **97**, 24% for **87**. EEDQ=2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline.

Scheme 10. Synthesis of the diazepanone nucleosides by Knapp.

An alternative synthetic approach towards the diazepanone ring system contained in the liposidomycins has been developed by Sarabia et al. based on the synthesis of epoxy indole-amides, which are obtained via reaction of amide-stabilized sulfonium ylides with aldehydes, followed by their reactions with diamines to provide the corresponding diazepanones in a tandem transamidation/intramolecular oxirane-ring opening process.[38] This novel strategy represents a straightforward and expeditious approach towards the diazepanones that commenced with the reaction of aldehyde **98** with sulfonium salt **99** to afford epoxy amide **100** in good yield and excellent stereoselectivity. The oxidation of the indoline ring to its corresponding indole by treatment with DDQ provided a reactive epoxy indole-amide **101**, which was subjected to a set of reactions with various diamines under mild conditions. The result was the efficient formation of the corresponding diazepanone derivatives **102a-102c**, including the 2-oxopiperazine **103**. Having established a convergent and efficient methodology for the diazepanone systems, the subsequent steps required the preparation of functionalized diazepanone-containing nucleosides. Initial efforts have led them to the diepoxy derivative **104**, through reaction of **101** with allyl amine followed by epoxidation of the terminal olefin. Diepoxy amide **104** was efficiently converted into diazepanone **105**, as a 1:1 mixture of diastereoisomers, by reaction with *N*-methylamine in a double oxirane-ring opening process (Scheme 11). It is important to mention that due to the *trans* configuration of the epoxides obtained via the sulfonium ylides, the resulting liposidomycin analogues correspond to the 2"-*epi*-diazepanone derivatives.



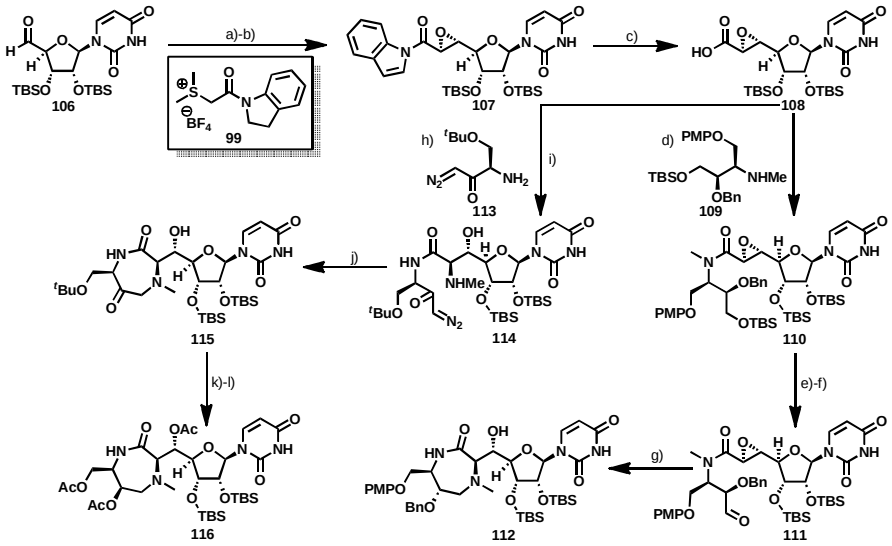
Reagents and Conditions: a) **99**, NaH or NaOH, 0 ºC, 78%; b) DDQ, benzene, reflux, 76%; c) $R_1NHCH_2(CH_2)_nNHR_2$, THF or DMF or MeOH, 25 ºC or reflux, 73% for **102a**, 72% for **102b**, 74% for **102c**, 83% for **103**; d) allyl amine, THF, 50 ºC, 59%; e) *m*-CPBA, CH_2Cl_2, 0 ºC, 62%; f) $MeNH_2$, MeOH, 25 ºC→ 65 ºC, 67%. DDQ=2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

Scheme 11. Synthesis of the diazepanone core of the liposidomycins by Sarabia.

As a continuation of these early studies and with the goal of extending the developed methodology to more highly functionalized diazepanone systems found in natural products, the authors attempted to install more complex amine residues via indole-amide displacement.[39] In this case, the authors prepared epoxy amide **107**, according to the sulfonium ylide methodology described before and rapidly, it was observed that more complex amines were incapable of direct nucleophilic substitution of the indole ring present in **107**. This disappointing result prompted the preparation of the acid **108** by treatment of **107** with aqueous LiOH, which can be coupled with the corresponding amine in an additional step. After numerous attempts of

diazepanone ring formation with the different coupled products, finally two synthetic strategies proved successful. One of them was based again on a reductive amination process in which acid **108** was coupled with amine **109** to afford epoxy amide **110**. Selective desilylation of the primary silyl ether and subsequent oxidation of the resulting primary alcohol furnished epoxy aldehyde **111**, which was finally treated under reductive amination conditions ($NaBH(OAc)_3$, AcOH) in the presence of *N*-Methylamine to provide diazepanone derivative **112** in a 40% yield over 2 steps.

The second approach represented a novel synthetic strategy with respect to all the reported methodologies delineated for the construction of the heterocyclic system of the liposidomycins and the caprazamycins. This new route, illustrated in Scheme 12, was based upon a carbene insertion reaction in which a diazo derivative was prepared as a suitable carbenoid precursor.



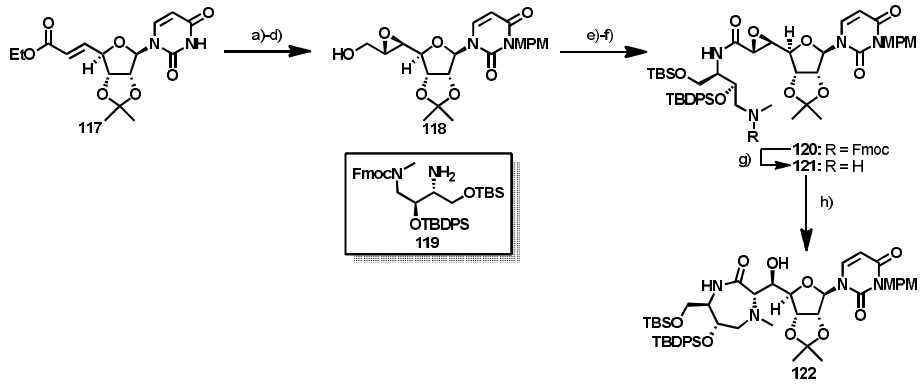
Reagents and Conditions: a) **99**, NaH or NaOH, 0 ºC, 61%; b) DDQ, benzene, reflux, 86%; c) LiOH, THF:H_2O, 0 ºC, 89%; d) **109**, BOP, DIPEA, 25 ºC, 65%; e) CSA, CH_2Cl_2:MeOH 1:1, 0 ºC→ 25 ºC, 88%; f) Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0 ºC; g) $NaBH(OAc)_3$, AcOH, $MeNH_2$, EtOAc, 25 ºC, 40% over 2 steps; h) **113**, HOBt, EDCI, CH_2Cl_2, 25 ºC, 49%; i) $MeNH_2$, MeOH, 60 ºC, 76%; j) $Rh_2(OAc)_4$, CH_2Cl_2, 25 ºC, 57%; k) H_2, Pd/C, MeOH, 25 ºC, 75%; l) Ac_2O, pyridine, 25 ºC, 44%. BOP=(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate.

Scheme 12. Synthesis of the functionalized diazepanones core by Sarabia.

Accordingly, epoxy diazo ketone was prepared by coupling acid **108** and diazo ketone **113**, which was further reacted with *N*-methylamine to give the amino diazo precursor **114**. When **114** was subjected to the action of a catalytic amount of $Rh_2(OAc)_4$, gratifyingly the expected diazepandione **115** was formed in a reasonable 57% yield as a consequence of the intramolecular NH trapping by the carbenoid species derived from the diazo group. Finally, reduction of the ketone group, cleavage of the *t*-butyl ether, and acetylation of the resulting triol provided the diazepanone derivative **116**.

In a very similar synthetic approach with respect to that developed by Sarabia et al., Miyaoka and coworkers recently reported the synthesis of the diazepanone core of the lipodisomycins based again on a

cyclization process mediated by an intramolecular oxirane-ring opening of the amino epoxy amide **121**.[40] The preparation of this acyclic precursor **121** was carried out by means of a Sharpless asymmetric epoxidation of the corresponding allylic alcohol, obtained from the α,β-unsaturated ester **117**, that provided epoxy alcohol **118**, followed by an oxidation to the epoxy acid and subsequent coupling with diamine **119**, which was prepared from the Garner aldehyde, in 9 total steps. The cyclization reaction was accomplished by heating of **121** in THF under reflux conditions to afford the diazepanone derivative **122**, which is closely related to compound **112** with the difference being the protecting groups utilized and the relative configuration at the 2" and 5' positions (Scheme 13).



Reagents and Conditions: a) DIBAL-H, CH_2Cl_2, -78 ºC; b) $NaBH_4$, MeOH, 0 ºC→25 ºC; c) MPMCl, DBU, MeCN, 25 ºC→60 ºC, 36% over 3 steps; d) TBHP, $Ti(O^iPr)_4$, L-(+)-DIPT, 4Å MS, CH_2Cl_2, -20 ºC→25 ºC, 87%; e) TEMPO, NaClO, KBr, 5% $NaHCO_3$ aq., acetone, 0 ºC→25 ºC; f) **119**, EDC, HOBt, THF, 25 ºC, 75%; g) Et_3N, CH_2Cl_2, 25 ºC, 90%; h) THF, reflux, 66%.

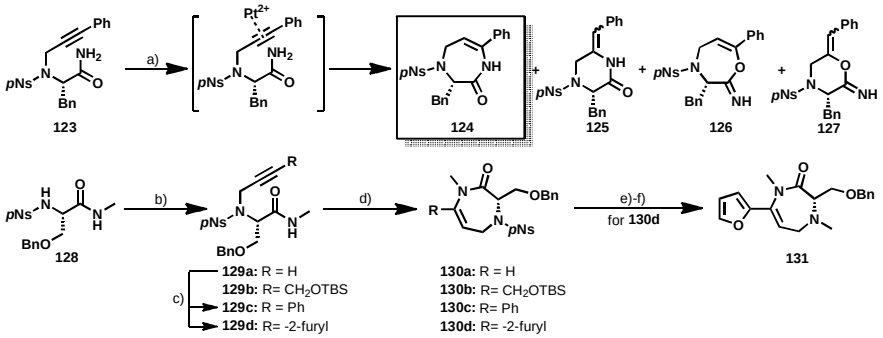
Scheme 13. Synthesis of the functionalized diazepanones core by Miyaoka.

To conclude this section, Takemoto and coworkers explored a transition metal-catalyzed cyclization of internal alkynyl amides as a novel route for the construction of the diazepanone ring.[41] With this objective in mind, they prepared alkynyl amide **123** as a model compound and, after a systematic study with various transition metals, they determined that platinum (II) was the transition metal of choice to promote a 7-*endo* cyclization process (compound **124**), in favour of other competitive cyclization processes (6-*exo* and the 7-*endo* and 6-*exo* by nucleophilic addition of the oxygen atom) to deliver compounds **125**-**127**, respectively. The application of this concise route for the synthesis of the caprazamycin core was achieved with the alkynyl amides **129a**-**129d**, readily prepared from the L-serine derivative **128**. The platinum catalyzed 7-*endo* cyclizations of these compounds provided the expected diazepanone derivatives in reasonable yields with the exception of **130b**, which was quite poor.

This result reflects the observation by the authors that aromatic substituted alkynyl amides provide much better yields compared with alkyl substituted derivatives, likely due to the stabilization that the aromatic ring exerts in the transition state of the 7-*endo* cyclization after the activation of the alkyne by the platinum (II) catalyst.

In light of these results, the authors focused their interest on the diazepanone **130d** since the furyl group could be converted into a carboxylate group by oxidation. Thus, **130d** was transformed into the *N*-

methyl derivative **131** via deprotection of the nosyl group followed by methylation of the resulting amine via reductive amination with formaldehyde (Scheme 14).



Reagents and Conditions: a) $PtCl_2$, $ClCH_2CH_2Cl$, 70 ºC, 99% for **124**; b) Propargyl bromide or ((4-bromobut-2-yn-1-yl)oxy)(*tert*-butyl)dimethylsilane, K_2CO_3, DMF, 0 ºC→25 ºC, 81% for **129a** and 57% for **129b**; c) PhI or 2-bromofuran, $Pd(PPh_3)_4$, CuI, Et_3N, THF, 50 ºC, 49% for **129c** and 71% for **129d**; d) $PtCl_2$, $ClCH_2CH_2Cl$, 70 ºC, 56% for **130a**, 7% for **130b**, 75% for **130c**, 58% (84% brsm) for **130d**; e) PhSH, K_2CO_3, CH_3CN, 96%; f) aq. HCHO, $NaBH_3CN$, AcOH, CH_3CN, MeOH, 79%.

Scheme 14. Synthesis of diazepanones *via* Pt (II)-mediated cyclizations.

4. Synthesis and antibiotic properties of liposidomycins and caprazamycins analogues

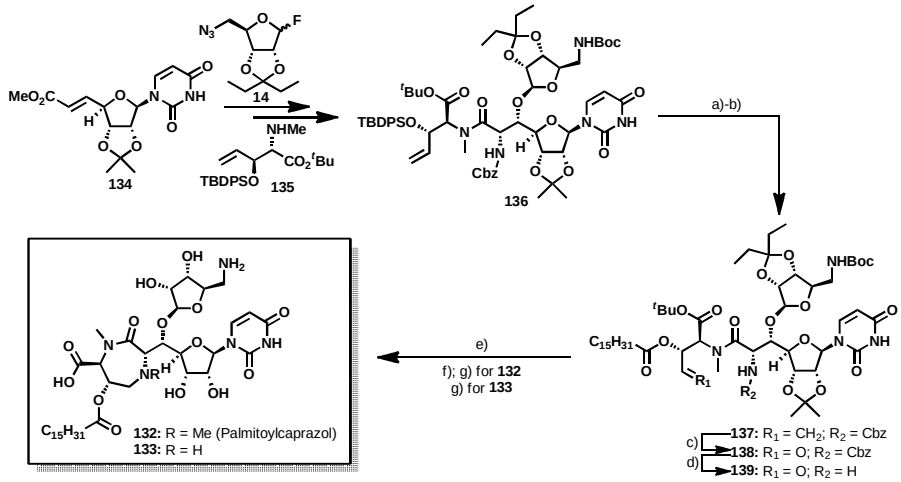
4.1. Synthesis of analogues

The successful campaigns for the total synthesis of the natural caprazamycins have paved the way for the chemical synthesis of a large number of designed analogues and their corresponding biological evaluations. The importance of these analogues lies with their biological evaluations which support the establishment of a pharmacophore for this class of nucleoside antibiotics, through extensive structure-activity relationships (SAR) studies. In addition, these analogues may facilitate the identification of new candidates for clinical use as novel antibiotics. Furthermore, the design of potent bioactive analogues could also address some of the important drawbacks associated with the natural congeners. For example, despite the outstanding biological profiles of some of the caprazamycins, in particular caprazamycin B (**2b**), its isolation from natural sources is difficult and its extremely poor solubility in water seriously limits its development as a new antibiotic. All these arguments justify the intense research activity related to the design and synthesis of analogues. In this field, we distinguish the two classes of analogues by virtue of their molecular structures. One class is comprised of simple analogues in which important fragments of the entire molecule have been removed (truncated analogues) and the second is constituted by structurally complex analogues, in which all the important sectors of the molecule are maintained (complex molecules) and, some of them have been modified (heterocyclic-modified analogues).

4.1.1. Complex analogues

This class of analogues emerged after the publication of the first total syntheses that opened the way to their preparations. In this sense, the group of Matsuda has been especially prolific in exploiting his successful synthetic strategy of caprazol towards the synthesis of caprazamycin analogues. One of the first analogues synthesized by his group were the palmitoyl caprazol derivatives **132** and **133**[42] (Scheme 15), in

which the relatively complex lipidic fragment, present in the natural caprazamycins, was replaced by a simple palmitoyl residue. For these analogues, Matsuda and coworkers applied their delineated synthesis for caprazol in the preparation of the advanced precursor **136** with some key modifications. Thus, starting from **134**, they connected the aminoribosyl unit **14** and amine fragment **135** according to the synthetic strategy detailed in Scheme 15.



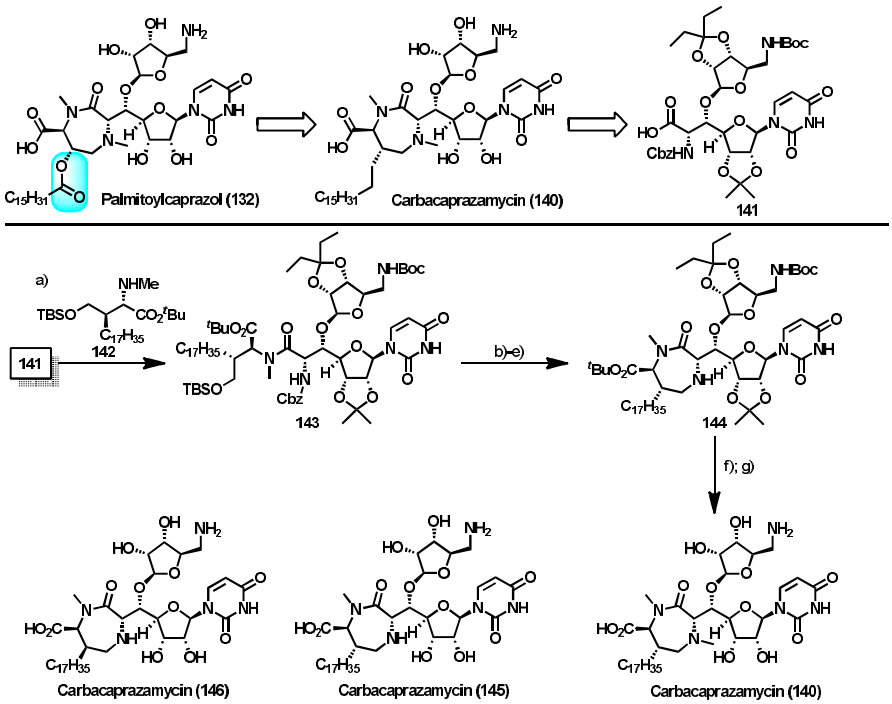
Reagents and Conditions: a) TBAF, AcOH, THF, 25 ºC, 78%; b) Palmitic acid, EDCI, DMAP, CH_2Cl_2, 25 ºC; c) OsO_4, NMO, acetone/H_2O; then $NaIO_4$, 83% over 3 steps; d) H_2, Pd black, ^{i}PrOH, 25 ºC; e) $NaBH(OAc)_3$, AcOH, EtOAc, 25 ºC, 96 % over 2 steps; f) $(CH_2O)_n$, $NaBH(OAc)_3$, AcOH, EtOAc, 77%; g) TFA, aq. THF, 25 ºC, quant. for **132** and **133**.

Scheme 15. Synthesis of palmitoyl caprazol.

Having prepared compound **136**, it was at this stage that they incorporated the palmitic acid moiety via an esterification reaction to obtain **137**. From this point onwards, the completion of the analogue syntheses proceeded in a similar manner as reported for the caprazol synthesis, utilizing a reductive amination protocol for the construction of the diazepanone rings from amino aldehyde intermediate **139**. With the synthesis of palmitoylcaprazol **132**, Matsuda improved significantly his first total synthesis of caprazol, obtaining this analogue in an impressive 10.5% overall yield in 16 steps from uridine and provided an efficient and streamlined route toward the preparation of new caprazamycin analogues, as will be discussed further.

The replacement of the ester linkage of palmitoyl caprazol **132** by C-C bonds was devised by Matsuda as a chemical change that could lead to greater chemical and biochemical stability for the new chemical entity. This new analogue, termed carbacaprazamycin (**140**),[43] potentially could represent a promising bioactive analogue possesing the excellent antibacterial activity displayed by palmitoyl caprazol, as will be discussed in section 4.2, with additional metabolic stability conferred by the C-C bond instead of the more labile ester group. The installation of this carbalipid fragment onto the nucleosidic core was accomplished from the advanced intermediate **141** by coupling with the amino ester **142**, prepared from L-aspartic acid γ-methyl ester in nine steps. The resulting product **143** was subjected to the reductive amination protocol for the construction of the diazepanone ring to give **144**, which carried the lipidic chain linked by a C-C bond. Finally, *N*-methylation and removal of the protecting groups afforded the targeted carbacaprazamycin **140**.

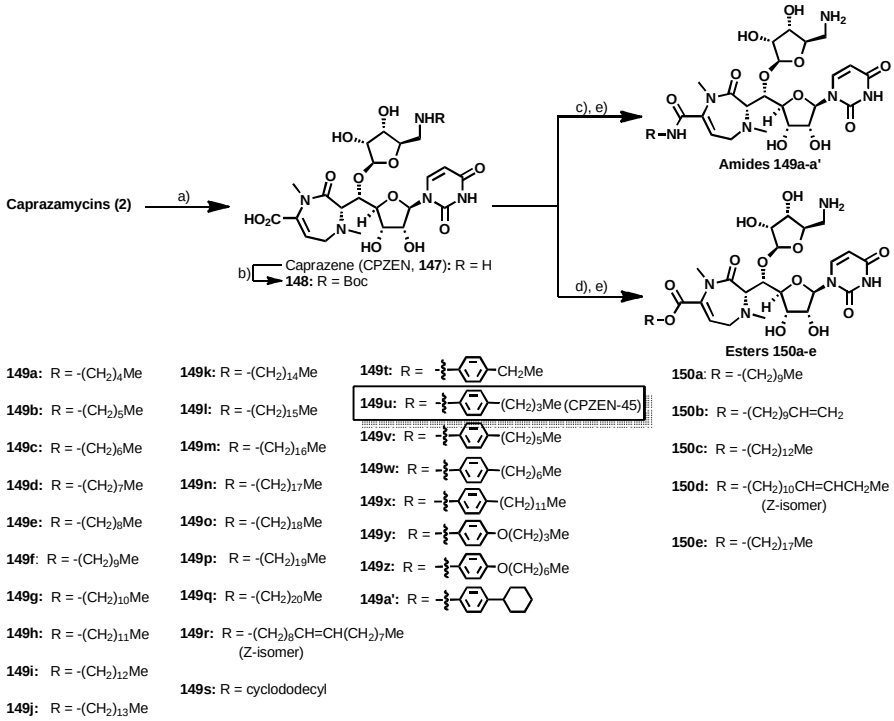
In a similar way, desmethylated derivative **145** and the 3"*S* diastereoisomer **146** were prepared for structure-activity relationships studies (see section 4.2) (Scheme 16).



Reagents and Conditions. a) **142**, DEPBT, $NaHCO_3$, CH_2Cl_2, 25 ºC, 51%; b) $3HF.NEt_3$, MeCN, 25 ºC, 87%; c) Dess-Martin periodinane, CH_2Cl_2, 25 ºC; d) H_2, Pd black, $^{i}PrOH$, 25 ºC; e) $NaBH(OAc)_3$, AcOH, CH_2Cl_2, 25 ºC, 73% over 4 steps; f) $(CH_2O)_n$, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, 25 ºC, 78%; g) aq. TFA, 25 ºC, quant.

Scheme 16. Synthesis of carbacaprazamycins.

In a completely different synthetic approach to the caprazamycin analogues, Takahashi et al., who were responsible for the discovery and isolation of the caprazamycins, were able to obtain caprazene (CPZEN, **147**) from a mixture of caprazamycins A-G, obtained from a fermentation broth, by acidic treatment and on a multigram scale.[44] Caprazene itself was inactive against various bacterial strains, however the introduction of a lipophilic side-chain could potentially restore the antibacterial activity found in the natural products. To this aim, the primary amine group of CPZEN was masked by protection as the Boc derivative and the resulting caprazene derivative **148** transformed into a library of amides **149** and esters **150** (Scheme 17), whose antibiotic properties were evaluated. The biological properties of all these analogues will be discussed in the following section, but it is important to highlight anilide **149u**, denoted as CPZEN-45 (Scheme 17), which was identified as the most active analogue, exhibiting an *in vitro* antibacterial activity superior to the natural caprazamycins, together with excellent solubility in water (>200 mg/mL) thus rendering it as a promising antibiotic agent.[45] In fact, CPZEN-45 is currently being investigated in *in vivo* assays against various mycobacterial strains.



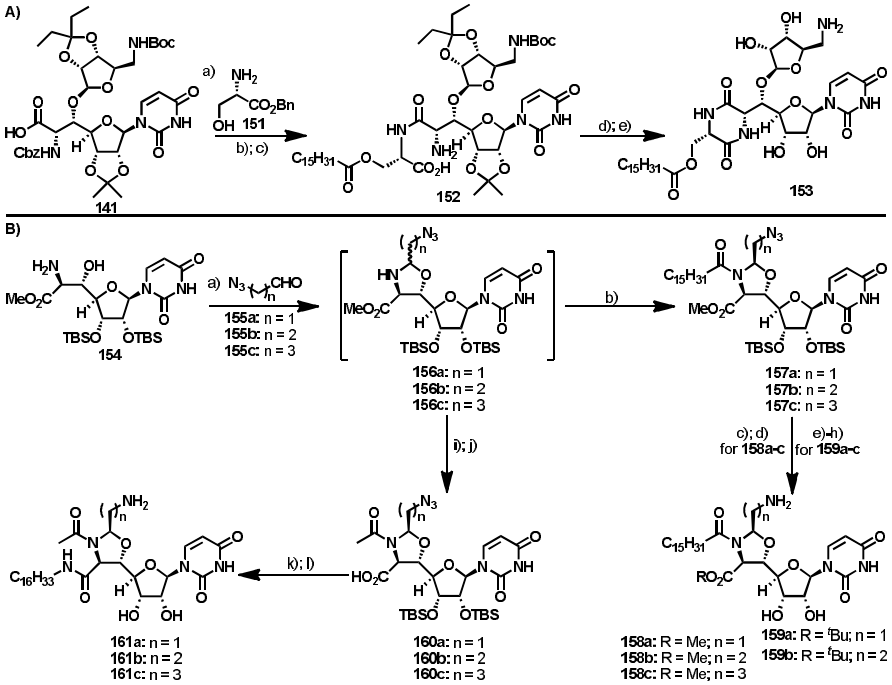
Reagents and Conditions: a) 80% aq. AcOH, 70 ºC, 64%; b) Boc_2O, Et_3N, aq. 1,4-dioxane, 25 ºC; c) RNH_2, BOP-Cl, Et_3N, THF, 25 ºC or DMT-MM, aq. 2-propanol, 25 ºC; d) ROH, BOP-Cl, pyridine, 25 ºC; e) TFA, MeOH, 25 ºC. DMT-MM=4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride.

Scheme 17. Semisynthesis of the caprazene-derived analogues.

4.1.2. Heterocyclic-modified analogues

Due to the synthetic difficulty encountered during the construction of the diazepanone ring system of the caprazamycins, relevant synthetic efforts have been directed towards the design of analogues in which the diazepanone ring is replaced by other types of heterocycles, which were more readily accessible. The designed heterocyclic surrogates should present structural features considered to be essential for antibiotic activity. On one hand, the new heterocyclic system should contain a suitable functional group for its assembly onto a lipidic fragment. On the other hand, the heterocyclic system also should possess a rigid platform to display a basic residue in the proper arrangement, replacing the aminoriboside unit linked to the nucleoside by a glycosidic bond. Using these preliminary considerations, Matsuda and coworkers have designed and synthesized a wide array of different heterocyclic analogues such as diketopiperazine, oxazolidine, lactam-fused isoxazolidine, isoxazolidine and piperidinyl analogues that are briefly discussed and summarized in the following schemes. For the diketopiperazine-type analogues,[46] Matsuda started from the aminoribosyl nucleoside **141**, which was coupled with a simple L-serine derivative **151**, instead of the complex amino acid fragment required for the diazepanone construction. In this case, once the coupled product was obtained, palmitic acid was appended via ester formation with the hydroxyl group of the L-serine residue to obtain **152**. Having prepared the compound, a simple lactamization provided the

corresponding ketopiperazine derivative **153** (Scheme 18, part A). The oxazolidine-containing uridine series proved to be more interesting from synthetic and biological standpoints.[47] In this case, for the analogues **158a-c**, **159a-c** and **161a-c**, the heterocycle not only replaces the diazepanone ring, but also the aminoribosyl residue is substituted by an aliphatic amine, which confers a basic character to the set of analogues that is likely required for effective binding of the molecule to the active site of translocase I. The spatial position of the amino group in all these analogues could be properly arranged at the enzyme active site, mimicking of this way the amino group of the amino ribose moiety, due to the conformational restriction that the oxazolidine ring imposes. For the synthesis of these heterocyclic-modified analogues, Matsuda et al. utilized amino alcohol **154**, which was condensed with azido aldehydes **155a-b** to give aminals **156** as a 2:1 mixture of diastereoisomers, which were reacted with palmitoyl chloride to give oxazolidine derivatives **157a-c** with the *R* configuration at the aminal center. From these oxazolidines, two series of ester derivatives were prepared, corresponding to the methyl and *tert*-butyl ester derivatives, **158** and **159**, respectively.

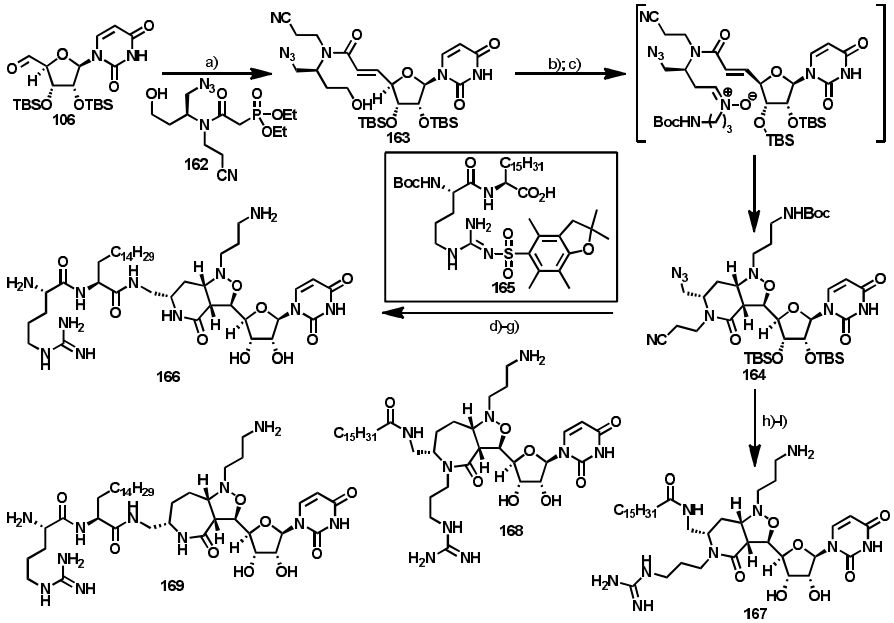


Reagents and Conditions: **Part A:** a) L-Ser(OBn) (**151**), EDCI, HOBt, DMF, 25 ºC; b) Palmitic acid, EDCI, DMAP, CH_2Cl_2, 25 ºC, 89% over 2 steps; c) H_2, $Pd(OH)_2$/C, MeOH, 25 ºC; d) EDCI, HOBt, CH_2Cl_2, 25 ºC; e) 80% aq. TFA, 25 ºC, 70% over 3 steps. **Part B:** a) **155a**, **155b** or **155c**, MS 4Å, CH_2Cl_2, 25 ºC; b) Palmitoyl chloride, Et_3N, 25 ºC, 76% for **157a**, 79% for **157b**, 87% for **157c**; c) 3HF.Et_3N, MeCN/CH_2Cl_2, 25 ºC; d) H_2, $Pd(OH)_2$/C, MeOH, 25 ºC, 49% for **158a**, 51% for **158b**, 52% for **158c** over 2 steps; e) LiI, EtOAc, reflux, 78% for n=1, 67% for n=2, 74% for n=3; f) *N,N'*-diisopropyl-*O*-*tert*-butylisourea, ^{t}BuOH/CH_2Cl_2, 0 ºC, 73% for n=1, 76% for n=2; g) 3HF.Et_3N, MeCN/CH_2Cl_2, 25 ºC; 79% for n=1, 80% for n=2; h) H_2, $Pd(OH)_2$/C, MeOH, 25 ºC, 54% for **159a**, 50% for **159b**; i) AcCl, Et_3N, 0 ºC; j) LiI, EtOAc, reflux, 63% for **160a**, 64% for **160b**, 71% for **160c** over 2 steps; k) Hexadecylamine, EDCI, HOBt, CH_2Cl_2, 25 ºC; l) H_2, $Pd(OH)_2$/C, HCl, MeOH, 25 ºC, 49% for **161a**, 50% for **161b**, 43% for **161c** over 2 steps.

Scheme 18. Synthesis of diketopiperazine and oxazolidine analogues.

The related analogues **161a-c** were prepared from the common aminal **156** by treatment with acetyl chloride, followed by ester hydrolysis and subsequent coupling of the resulting acids **160a-c** with hexadecylamine, followed by final desilylation (Scheme 18, part B).

As a continuation of their initial studies, Matsuda and coworkers conceived of a lactam-fused isoxazolidine as another scaffold that could satisfy the structural requisites required to replace the complex diazepanone and the synthetically complicated aminoribosyl unit.[48] Such a heterocyclic system could provide a suitable three dimensional orientation, which could be modulated by changing the stereochemistry, the ring size and functionalization. Additionally, its construction could be readily achieved via an intramolecular 1,3-dipolar cycloaddition of an alkenyl nitrone intermediate. Consequently, in order to check the validity of the lactam-fused isoxazolidine type analogues, the functionalized phosphonate **162** was prepared and reacted with aldehyde **106** to provide α,β-unsaturated amide **163** as a direct precursor of the coveted olefinic nitrone. To this aim, oxidation of the alcohol and reaction of the resulting aldehyde with a hydroxylamine derivative yielded, after heating, the corresponding *cis*-fused bicyclic isoxazolidine derivative as a mixture of diastereoisomers as a 1.2:1 mixture, with **164** being the slightly preferred isomer (Scheme 19).



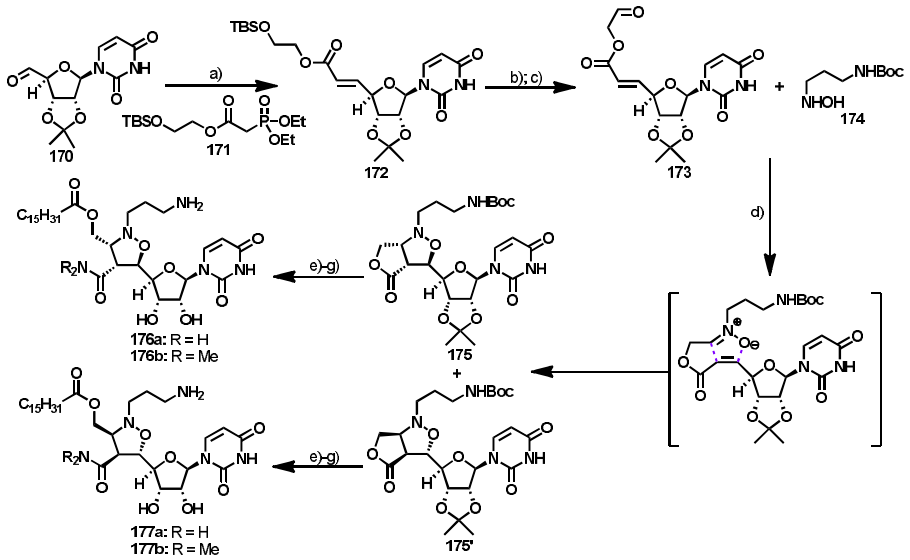
Reagents and Conditions: a) **162**, $Zn(OTf)_2$, TMEDA, Et_3N, THF, 25 ºC, 65%; b) IBX, MeCN/DMSO, 25 ºC; c) $BocNH(CH_2)_3NHOH$, MS 4Å, MeCN, 25 ºC; 68% (dr=1.2:1); d) ^tBuOK, THF, 25 ºC, 79%; e) H_2, Pd/C, MeOH, 25 ºC; f) **165**, EDCI, HOBt, CH_2Cl_2, 25 ºC; g) 80% aq. TFA, 25 ºC, 70% over 3 steps for **166**; h) H_2, Pd/C, MeOH, 25 ºC; i) Palmitoyl chloride, Et_3N, CH_2Cl_2, 25 ºC, 55% over 2 steps; j) H_2, PtO_2, MeOH, 25 ºC; k) BocHNC(=NBoc)NHTf, $NaHCO_3$, THF/H_2O, 25 ºC; l) 80% aq. TFA, 25 ºC, 69% over 3 steps for **167**. TMEDA=*N*,*N*,*N*',*N*'-tetra-methylethylenediamine.

Scheme 19. Synthesis of lactam-fused isoxazolidine-containing analogues.

Together with **164**, a small library of lactam-fused isoxazolidines was prepared by modification of the ring size of the lactam and the substituents of the respective phosphonates and hydroxylamines employed in the 1,3-dipolar cycloaddition. Among all the prepared analogues, the compounds derived from **164** were the most biologically interesting. Thus, from **164**, functional group manipulation and coupling with lipidic chains led to two different classes of analogues. One is characterized by compound **166** by assembly with **165**, and the other is a branched version with two substituents on the lactam ring such as **167**. In both cases, a guanidine residue was linked based on previous studies with muraymycin derivatives,[49] where in it was demonstrated that truncated muraymycin analogues with an arginine residue retained antibacterial activity comparable to their natural congeners. In a similar way, seven-membered lactam-fused isoxazolidines **168** and **169** were also synthesized (Scheme 19).

The biological activities of the previously described heterocyclic analogues, as will be discussed later, confirmed the validity of the Matsuda hypothesis, in which the role of the diazepanone ring was simply as a scaffold to link the apparently essential structural units for its biological activity, corresponding to the uridine, an amine group and a fatty acid moiety. As a result, the search for new scaffolds that could replace the role of the diazepanone system continued towards simpler heretocycles. In this continuing effort, simple isoxazolidine analogues were established as the next generation of heterocyclic-modified caprazamycin analogues.[50]

For the construction of such synthetic targets, an intramolecular 1,3-dipolar cycloaddition of a transient alkenyl nitrone was utilized, thus aldehyde **170** was reacted with the phosphonate **171** to provide the α,β-unsaturared ester **172**, properly functionalized for the nitrone formation (Scheme 20).



Reagents and Conditions: a) **171**, KHMDS, THF/toluene, 25 ºC, 92%; b) HF, aq. MeCN, 25 ºC, quant.; c) DMP, pyridine/CH_2Cl_2, 25 ºC, 80%; d) **174**, MS 4Å, CH_2Cl_2, 25 ºC, 16% for **175**, 4% for **175'**; e) NH_3, MeOH, 25 ºC or Me_2NH, CH_2Cl_2, 25 ºC; f) Palmitic acid, EDCI, DMAP, CH_2Cl_2, 25 ºC; g) aq. TFA, 25 ºC, 54% for **176a**, 72% for **176b**, 73% for **177a**, 59% for **177b** over 3 steps.

Scheme 20. Synthesis of isoxazolidine-containing analogues.

To this end, desilylation and oxidation led to the aldehyde intermediate **173**, which when exposed to the hydroxyl amine **174** generated the corresponding alkenyl nitrone. The nitrone intermediate gave rise to the cyclized compounds **175** and **175'** in poor 16 and 4% yields, respectively. The lactone-fused isoxazolidines were separately treated with either ammonia or dimethylamine to give the corresponding amides, followed by acylation of the alcohol with palmitic acid. Finally, TFA deprotection provided the isoxazolidine analogues **176a**-**176b** and **177a**-**177b**. It is worthy to highlight the degree of structural simplification reached with the latter analogues compared with the overwhelming molecular complexity of the parent caprazamycins thus allowing for rapid access to potential new antibiotics (Scheme 20).

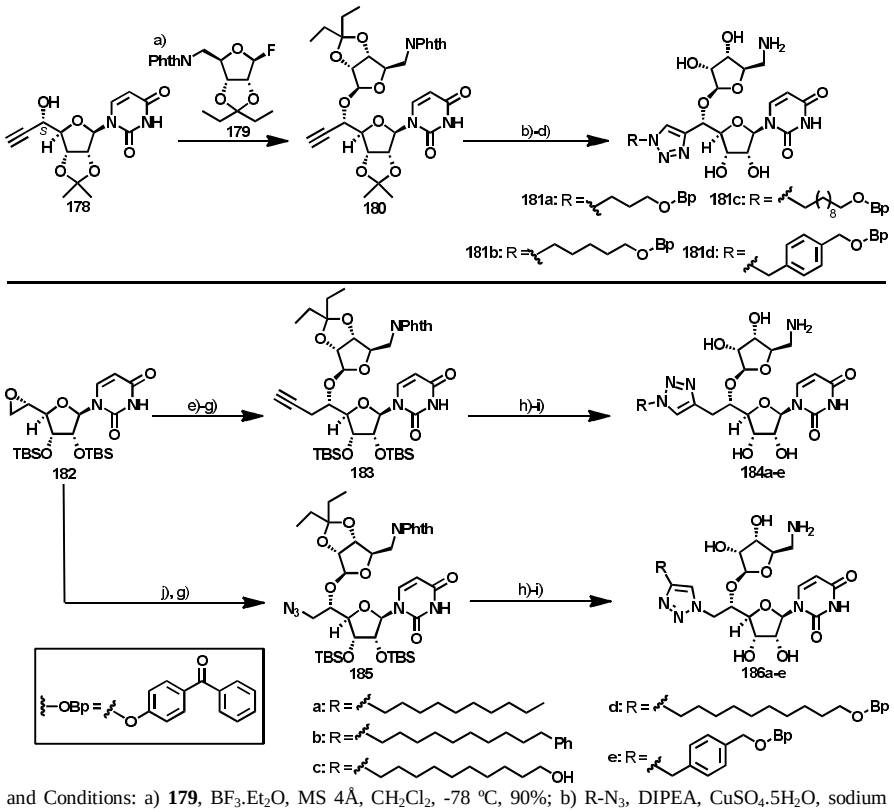
Another type of heterocyclic analogues of the caprazamycins were the designed by Gravier-Pelletier, in which the diazepanone ring system was replaced by a substituted-triazole heterocycle prepared via a Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC). The construction of the substituted triazoles, according to this strategy, allowed the introduction of various substituents, creating a small library of this type of analogues (Scheme 21).[51]

Within this class of triazole-modified analogues, they prepared three different subtypes, starting from alkyne **180** and derivatives **183** and **185**, in which a carbon bridge linked the triazole ring with the aminoribosyl uridine moiety.[52] The precursors **180**, **183** and **185**, in turn, were readily prepared from precursors **178** and **182**,[53] including a glycosylation reaction with donor **179** according to the Matsuda reaction conditions. The corresponding azido building blocks for the preparation of the **181**- and **184**-type analogues and the alkynes for the synthesis of the series **186** were properly selected in order to provide structural diversity with the introduction of either apolar (aliphatic and aromatic groups) as polar groups (hydroxyl, amino, etc..). The CuACC reaction was undertaken under similar conditions for all the cases to obtain the corresponding triazole derivatives in reasonable good yields, taking into account the bulkiness of the aminoribosyl uridinyl moiety. After deprotection of the phtalimido and the acetal groups, the final products (**181**, **184** and **186**) were obtained in form of ammonium trifluoroacetate salts. In Scheme 21, it is depicted those analogues that displayed better antibiotic activities as will be detailed in section 4.2.

Finally, the recognition of the importance of the basic nitrogen located at the 6"-position of the diazepanone ring for inhibitory activity against MraY prompted Matsuda and coworkers to design a new class of analogues wherein the seven-membered ring of the diazepanones is replaced by a simple piperidine ring (Scheme 22).[54] In this case, the new analogues contain the aminoribosyl residue, which is installed via a glycosylation reaction of the glycosyl donor **187** with the corresponding allylic alcohol, obtained from aldehyde **170** by treatment with vinylmagnesium bromide. With the resulting glycosylated nucleoside **188** in hand, the construction of the piperidinyl system was envisioned by an interesting aza-Prins-Ritter reaction with the intervention of the aldehyde **189**, *N*-protected homoallylamine **190** and acetonitrile. The combination of the three components proceeds, with formation of an iminium intermediate by condensation of **189** with **190**, followed by an intramolecular attack of the appending alkene that generates a piperidinyl cation, which finally is quenched by acetonitrile to form the final 4-acetamido piperidine adduct.

In practice, when aldehyde **189**, prepared from **188**, was treated with **190** in the presence of a catalytic amount of triflic acid in acetonitrile, a mixture of the diastereoisomers **191** and its 2*S*,4*S* isomer was obtained in 22 and 19% yields, respectively. Separation of the isomers provided pure **191**, which was then converted into **192** by the introduction of a palmitic acid moiety. Together with a series of different

stereoisomers of **192**, which did not show relevant antibiotic activities, the branched derivative **193** was similarly prepared according to a synthetic sequence used for **192**.

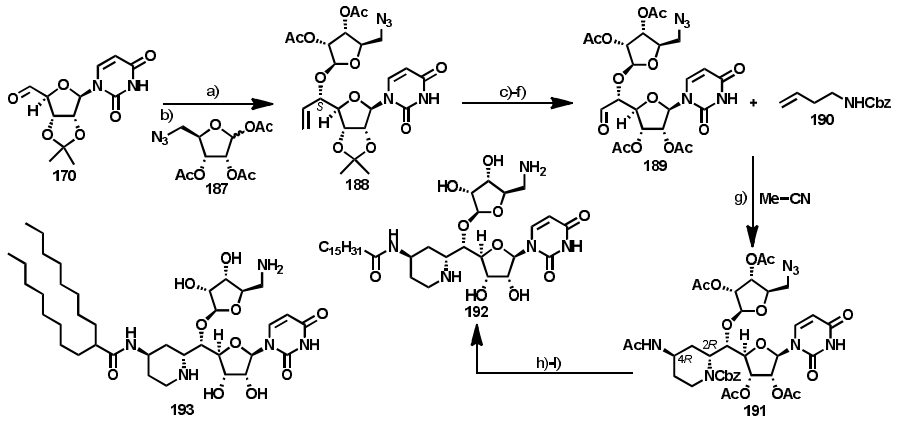


Reagents and Conditions: a) **179**, $BF_3.Et_2O$, MS 4Å, CH_2Cl_2, -78 ºC, 90%; b) R-N_3, DIPEA, $CuSO_4.5H_2O$, sodium ascorbate, ^{t}BuOH/H_2O, 25 ºC, 52% for **a**, 52% for **b**, 49% for **c**, 37% for **d**; c) $MeNH_2$, MeOH, 25 ºC, d) TFA/H_2O 4/1, 0 ºC→25 ºC, 46% for **181a**, 49% for **181b**, 51% for **181c**, 80% for **181d** over 2 steps; e) TMSC≡CLi, $BF_3.Et_2O$, THF, -78 ºC, 77%; f) K_2CO_3, MeOH, 92%; g) **179**, $BF_3.Et_2O$, MS 4Å, CH_2Cl_2, -78 ºC, 61% for **183**, 55% for **185**; h) R-N_3 or R-C≡CH, DIPEA, $CuSO_4.5H_2O$, sodium ascorbate, ^{t}BuOH/H_2O, 25 ºC, Series **184**: 58% for **a**, 57% for **b**, 59% for **c**, 57% for **d**, 67% for **e**; Series **186**: 55% for **a**, 60% for **b**, 66% for **c**, 58% for **d**, 67% for **e**; i) $MeNH_2$, MeOH, 25 ºC; then TFA/H_2O 3/1, 0 ºC→25 ºC, 62% for **184a**, 53% for **184b**, 65% for **184c**, 69% for **184d**, 64% for **184e**, 56% for **186a**, 52% for **186b**, 42% for **186c**, 64% for **186d**, 72% for **186e** over 2 steps. Bp=Benzophenone.

Scheme 21. Synthesis of triazole-containing analogues.

4.1.3. Truncated analogues

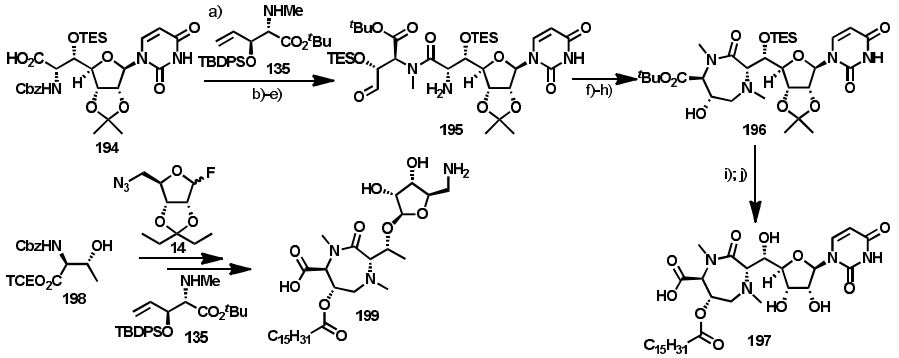
To complete this section, a group of several simple analogues featuring the absence of any of the regions found in the natural congeners were prepared for biological evaluation. This group of analogues, termed truncated analogues, includes compounds that lack the diazepanone ring, the amino riboside moiety and even the uridyl fragment. Initially, Matsuda wished to evaluate the impact upon antibacterial activity of the aminoriboside and the uridine units, while maintaining the complete functionalized diazepanone ring system.[55]



Reagents and Conditions. a) CH_2=CHMgBr, THF, -78 ºC, 78% of a mixture of the *R*- and *S*-isomers in a 5:1 ratio; b) **187**, $SnCl_4$, MS 4Å, CH_2Cl_2, 0 ºC, 75%; c) 80% aq. TFA, CH_2Cl_2, 25 ºC; d) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 25 ºC, 79% over 2 steps, e) OsO_4, NMO, acetone/H_2O; f) $NaIO_4$, aq. acetone, 86% over 2 steps; g) **190**, TfOH, MeCN, 0 ºC, 22% for **191**, 19% for its 2*S*,4*S* isomer; h) Boc_2O, DMAP, THF, 70 ºC; i) LiOH, THF/MeOH/H_2O, 0 ºC, 74% over 2 steps; j) TFA, CH_2Cl_2, 0 ºC; k) *N*-succinimidyl palmitate, DIPEA, THF, 25 ºC, 34% over 2 steps; l) H_2, Pd/C, TFA, MeOH, 25 ºC, 27%.

Scheme 22. Synthesis of piperidinyl analogues.

Thus, according to the methodology developed for the construction of the diazepanone unit, a reductive amination reaction, the aminoribosyl-truncated analogue **197** and uridine-truncated derivative **199** were prepared (Scheme 23).



Reagents and Conditions. a) **135**, DEPBT, $NaHCO_3$, THF, 25 ºC, 18% from **194**; b) TBAF, AcOH, THF, 25 ºC, 87%; c) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 ºC; d) OsO_4, NMO, ^{t}BuOH, acetone/H_2O; then $NaIO_4$, 70% over 2 steps; e) H_2, Pd black, ^{i}PrOH, 25 ºC; f) $NaBH(OAc)_3$, AcOH, EtOAc, 25 ºC, 94% over 2 steps; g) $(CH_2O)_n$, $NaBH(OAc)_3$, AcOH, EtOAc, 25 ºC, 84%; h) TBAF, AcOH, THF, 25 ºC, 27%; i) Palmitic acid, EDCI, DMAP, CH_2Cl_2, 25 ºC, 81%; j) TFA, aq. THF, 25 ºC, quant.

Scheme 23. Synthesis of truncated analogues.

Finally, Figure 4 depicts a collection of the most significant truncated analogues prepared thus far, which are characterized by the complete lack of the diazepanone ring system. All these analogues were prepared soon after the discovery of the liposidomycins and caprazamycins and their recognition as new promising new antibiotics. These simple analogues are comprised of a variety of glycosyl nucleosides

which, some of them, displayed interesting antibiotic activities. In this sense, the Dini research group was especially fruitful describing the preparation of compounds **200a/b** and **201**,[56] the **202** amine series,[57] the deoxy analogues **203**,[58] and the derivatives **204**-**207**.[59] Other types of simple analogues included the C-nucleoside-type derivatives **208**-**213**,[60,61] the *O*-serinyl aminoriboside derivative **214**,[62] and the *gem*-difluorinated nucleosides **215** and **216**[63] (Figure 4).

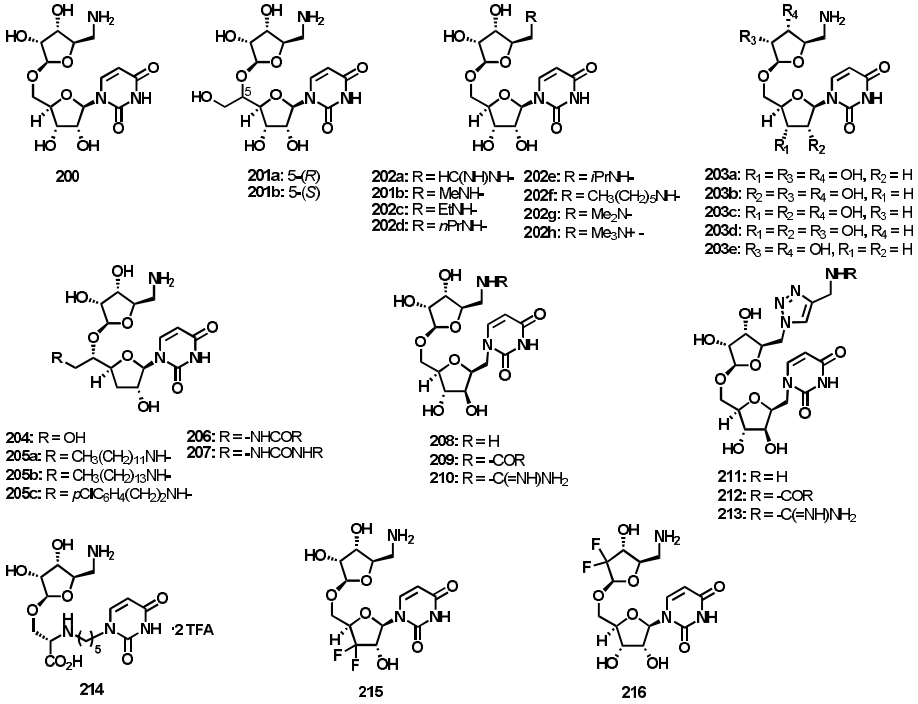


Figure 4. Molecular structures of truncated analogues.

4.2. Antibiotic properties of the liposidomycins, caprazamycins and analogues

The flurry of synthetic activity towards the preparation of liposidomycin and caprazamycin analogues described in the present review has been directed to the identification and discovery of new antimicrobial agents targeting MraY. As a consequence, extensive biological evaluations of all the synthetic compounds described in this review have been accomplished to assess their antibiotic activity. In addition, these biological evaluations have served to provide a comprehensive structure-activity relationship (SAR) that has allowed the determination of a pharmacophore map corresponding to this class of natural products for use in subsequent rational designs of new antibacterial leads. The described biological studies comprise the evaluation of the compounds against a range of bacterial strains, including *Mycobacterium sp*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Eschericia coli*, as well as the inhibitory activity against translocase I.

Of all the natural liposidomycin and caprazamycin members, especially striking was the anti-tuberculosis activity exhibited by caprazamycin B (**2b**) with a MIC of 3.13 μg/mL and a therapeutic dose of

1.5 mg $Kg^{-1}day^{-1}$ in a murine pulmonary tuberculosis model.[64] In a similar fashion, liposidomycin C-III (**1f**) displayed a potent antimicrobial activity against five kinds of *Mycobacterium avium* (MIC=6.3-25 μg/mL). An important first conclusion extracted from a general analysis of the biological data collected for all the synthesized analogues is the limited tolerance of the caprazamycins to structural modifications. Thus, simple analogues, such as those depicted in Figure 4, exhibit moderate, poor or completely no antibacterial activity. In contrast, the more complex analogues that possess the hydrophobic fatty acyl side, the diazepanone, the amino ribose and the uridine moieties exhibit similar potency to that of the natural caprazamycins.

In particular, simple analogues **200**-**207** displayed moderate to poor activity against translocase I. In this case, it was interesting to note that introduction of an additional CH_2OH group resulted in the discovery of the analog **201b** with enhanced inhibitory activity (IC_{50}=5.3 μM) compared to **200** (IC_{50}=50 μM).[56] This activity was then further improved with the introduction of an amino group, for example derivatives **205a**-**205c** with IC_{50} values of 0.70, 0.59 and 0.17 μM, respectively.[57] Related to these compounds, the urea derivatives (compounds of type **207**) were similarly potent with inhibitory activities in the range 0.16-0.14 μM.[59] On the contrary, for the simple analogues **208**-**216**,[60-63] no significant antibiotic activity was detected. In a similar manner, truncated analogues **197** and **199**, described by Matsuda, or caprazol (**10**), exhibited a complete loss of activity, which proved that the various regions of the caprazamycins are crucial for antibacterial activity.[55] For the more complex analogues prepared by Matsuda, the palmitoyl derivative **132** exhibited a potency similar to the caprazamycins with MIC values of 6.25, 3.13 and 12.5 μg/mL for *Mycobacterium smegmatis*, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) strains, respectively.[42] On the other hand, the inhibitory activity (IC_{50}) against MraY was a striking 1.2 nM. The removal of the methyl group at the *N*-position (**133**) resulted in a 4-fold reduction in potency against *Mycobacterium* (25 μg/mL), with retention of the antimicrobial activity against MRSA and VRE. The carbacaprazamycin analogues **140**, **145** and **146** similarly displayed potent inhibitory activities against MraY (6.9, 3.8 and 2.6 nM, respectively), and moderate antibacterial activity against a range of Gram-positive bacteria, including MRSA and VRE strains, with MIC values of 4-16 μg/mL, in contrast to **132** which exhibited MIC values between 0.5-1.0 μg/mL for the same bacterial strains.[43] Especially interesting was the antibiotic profile displayed by the caprazamycin analogue **149u** (CPZEN-45), an anilide derivative of caprazene, which was discovered to be the most potent member of a small-library of analogues.[44] The antibacterial activity of CPZEN-45 is particularly impressive against various *Mycobacterium* strains with MIC values in the range 0.20-3.13 μg/mL, being superior to natural caprazamycin B. In constrast, CPZEN-45, together with other members of the caprazene library, displayed reduced antibacterial activity against various Gram-positive and Gram-negative bacterial strains versus caprazamycin B. In addition, a comparative biological study involving CPZEN-45, CBZ-B, ethambutol and rifampicin in which their activities against drug-sensitive *M. tuberculosis* clinical isolates were evaluated, revealed CPZEN-45 as the most active with an MIC range, MIC_{50} and MIC_{90} of 0.78-12.5, 1.56 and 3.13 μg/mL, respectively. The differing antimicrobial profiles of caprazamycin B and CPZEN-45 led to the hypothesis that the mechanism of action of CPZEN-45 was different. This hypothesis encouraged further biological studies, wherein it was demonstrated that the anti-TB activity of CPZEN-45 was due to the inhibition of WecA,[45] an enzyme involved in the biosynthesis of mycolyl arabinogalactan, which is essential for *Mycobacterium tuberculosis*.[65] This novel mode of action, never identified before, together with its

outstanding anti-TB activity, negligible hemolysis and excellent water solubility (> 200 mg/mL) accelerated CPZEN-45 as a candidate for clinical trials.[66] With regard to the heterocyclic-based analogues, a variety of antibacterial properties have been identified. For example, the ketopiperazine-type compounds, such as **153**, did not exhibit any activity against *Mycobacterium smegmatis* and only moderate antibacterial potency against *Micrococcus* sp. or *Corynebacterium* sp. (MIC=12.5-50 µg/mL).[46] In the case of the oxazolidine-containing analogues, the ester derivatives **158** and **159** exhibited reasonable antibacterial activity against drug-susceptible *S. aureus*, *E. faecalis* and *E. faecium* with MICs in the range 2-16 µg/mL. In contrast, the corresponding acids showed no antibacterial activity with MICs greater than 64 µg/mL.[47] This contrast in biological activity between the acids and esters can be attributed to the difference in their permeability across the bacterial cell membrane. Despite the antibacterial properties displayed by compounds of the type **158** and **159**, their inhibitory activities against MraY were surprisingly weak with IC_{50} values in the range of 920-1200 µM. This observation has been attributed to an alternative mode of action, not associated with translocase I inhibition. For the most complex lactam-fused isoxazolidine analogues (**166-169** as representative examples), only moderate activity was observed against various Gram-positive bacteria (MRSA and VRE) with MIC values in the range of 8-64 µg/mL.[48] The isoxazolidine-containing analogues (compounds of the type **176** and **177**) exerted good activity against *H. influenzae* (MIC=0.25-0.5 µg/mL) and moderate activity against vancomycin-resistant *E. faecalis* (MIC=4-8 µg/mL), with **177a** and **177b**, which possess the same stereochemistry as the caprazamycins, being more potent than **176a** and **176b**.[50] More promising results were obtained for the piperidinyl analogues represented by the compounds **192** and **193**.[54] In this case, these compounds displayed good activity against a range of pathogens, including MRSA and VRE strains with MIC values of 2-32 µg/mL for **192** and 4-32 µg/mL for **193**. Importantly, **192** displayed potent activity against MraY with an IC_{50} value of 24 nM. Given the encouraging antibacterial properties of **192**, the compound was subjected to further biological evaluation. For example, its metabolic stability in human and mouse liver microsomes was determined, concluding that **192** was not affected after this treatment. In addition, CYP inhibitory activity and cytotoxicity against HepG2 cells for **193** was measured, and no cytotoxicity was observed at concentrations up to 100 µM. These biological results represent valid therapeutic parameters that support the progression of **192**, **193** or related compounds into potential clinical evaluations. Finally, for the triazole series (**181**, **184** and **186**), generally good antibacterial activity was observed against three Gram-positive bacteria strains (*S. aureus* and *E. faecium*) and Gram-negative *P. aeruginosa* with MIC values in the range 8-64 µg/mL, with the C-triazole containing compounds **184** being more potent than **181** and **186**.[52] Moreover, the inhibition of MraY revealed IC_{50} values ranging from 50 to 150 µM, with the best values exhibited by the benzophenone derivatives (**184d** and **184e**). The biological evaluation of the triazole-type analogues was coupled with molecular modelling studies which allowed correlations to be drawn between the observed activity of the most potent analogues and their interaction with the active site of MraY. The docking study revealed an interaction involving a leucine residue (Leu191) that favors a hydrophobic interaction of the lipidic side chain present in the most potent analogues with the hydrophobic groove of the enzyme.

5. Conclusions

The growing emergence of drug resistance by specific bacterial strains, such as MRSA, VRE and MDR-TB, to current antibiotics is becoming a serious problem for the worldwide health. Thus, the inevitable onset of resistant pathogens demands a continuing search for new antibiotics to deal with this real threat. In this search, natural products continue to be at the forefront of antibiotic research as history has demonstrated. With the challenge of developing a new class of clinically useful antibiotics, the complex-type nucleosides liposidomycins and caprazamycins, featuring a unique and rare molecular architecture, as well as striking antibiotic properties, have prompted intense research activity. The molecular complexity of this class of nucleosides has posed a considerable challenge to synthetic chemists and has offered new opportunities for the discovery and development of new synthetic methodologies and strategies. The recent total syntheses of these natural substances, together with the numerous synthetic approaches designed for these molecules, have paved the way for the rapid access to a large number of structurally diverse analogues. To this end, the synthetic contributions to this field have demonstrated the power of chemical synthesis in providing the natural products and analogues for further biological and biochemical studies. The corresponding biological evaluation of all the synthetic analogues has allowed the establishment of a complete structure-activity relationship that will provide insight into the design of future generations of analogues with greater potencies. Some key conclusions drawn from these investigations regarding the structural requirements for antibiotic activity within the liposidomycin and caprazamycin structures are that the uridine, the amine and the fatty acyl regions are crucial for activity. Additionally, the highly substituted diazepanone ring plays a role as a scaffold to link these critical pharmacophore elements and, consequently, can be replaced by simpler structural motifs in the form of other heterocycles. In summary, although much remains to be done for the entrance of these natural products or their analogues into clinical trials, the liposidomycins, caprazamycins and other related nucleosides are clearly promising new antibiotics and will likely contribute to the development of new leads for the treatment of serious infectious diseases caused by drug-resistant pathogens.

Acknowledgments

This chapter was financially supported by the Ministerio de Economía y Competitividad (ref. CTQ2014-60223-R). C. G.-R. thanks the Ministerio de Educación y Ciencia and Research Plan of the University of Málaga for predoctoral and postdoctoral fellowships, respectively. I. C.-S. thanks the Ministerio de Educación, Cultura y Deporte for a predoctoral fellowship (FPU programme).

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