J. Antibiot. 60(2): 85–122, 2007

REVIEW ARTICLE

THE JOURNAL OF ANTIBIOTICS

Azalides from Azithromycin to New Azalide Derivatives

Stjepan Mutak

Received: August 22, 2006 / Accepted: January 22, 2007

Abstract Azalides are semi-synthetic macrolides, in which a nitrogen atom is introduced into a macrolactone ring *via* a Beckmann rearrangement. Starting from erythromycin, oximes, depending on the reaction conditions lactams, or bicyclic-imino-ethers were formed, which were further reduced to aminolactones. The cyclic amine 9a- became the precursor for novel, significantly more active derivatives, especially for 9-dihydro-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A with the generic name azithromycin. It showed a broad spectrum of antibacterial activity covering all significant bacteria causing respiratory tract infections. The greatest advantages of azithromycin are its unusual pharmacokinetics (high tissue distribution), metabolic stability and high tolerability. These properties have led in recent years to the widespread use of the azalide scaffold for the synthesis of new compounds with advantageous pharmacokinetics.

The azalide scaffold possesses an amino and several hydroxyl groups, which could be substituted or transformed to obtain new compounds. Different derivatives were obtained by substitution on the nitrogen but a large variety of derivatives, such as ethers, esters and carbamates, were made by reactions with various hydroxyl groups. Substitutions on both nitrogen and hydroxyl or two hydroxyl groups yielded new, bridged compounds. The 4″-hydroxy group was oxidized to 4-oxo-, which was transformed *via* the oxime to 4-amino, or *via* epoxide to 4″-methylamino compounds. Cleavage of the cladinose sugar and further transformations gave 3-acyl or 3-oxo compounds, which were less active than 14-membered acylides or ketolides. Beckmann rearrangement of some 16-membered macrolide oximes yielded only 17-membered lactams, which were less active than starting macrolides, and could not be reduced to amines.

Intramolecular rearrangement of azalide imino-ethers yielded 13-membered azalides. Some new 11a-azalides were obtained after oxidative cleavage of some 16-membered macrolides and additional cyclisation.

Keywords azalides, azithromycin, 8a- and 9a-lactams, azalide-*N*,*O*-substituted derivatives, descladinosyl-azalides, ketoazalides, bicyclic-azalides

S. Mutak: Consultant for Medicinal Chemistry and Chemical Process Development, PLIVA Research Institute,† 10090 Zagreb, Croatia, E-mail: stjepan.mutak@zg.t-com.hr
† till March 2006

This article is dedicated to the memory of Professor Vladimir Prelog and celebrates the 100th anniversary of his birth. The period Prelog spent in Zagreb (1935～1941) marked the beginning of the development of organic synthetic chemistry in the Zagreb University and, at the same time, the start of 70 years of organized research within the pharmaceutical company PLIVA. A major consequence of Prelog's influence on organic synthetic chemistry in Zagreb was the discovery of azithromycin.

Twenty five years after the first patent application for the synthesis and discovery of azithromycin and 20 years from the first publication of its antibacterial activity, azalide chemistry has expanded significantly and many new derivatives have been developed for other indications.

Azithromycin, the first 15-membered macrolide antibiotic on the market, is characterized by a basic nitrogen atom inserted into the macrocyclic ring. It was synthesized in 1980 by a team of researchers at PLIVA laboratories: Gabrijela Kobrehel, Gorjana Radobolja-Lazarevski, Zrinka Tamburasev and Slobodan Djokic. For their great contribution to chemistry, on August 21, 2000, Gabrijela Kobrehel and Slobodan Djokic received the medal of "Heroes of Chemistry", the higher honor that is bestowed by the American Chemical Society.

Once the excellent properties of the new azalide scaffold had been noticed, researchers from many companies started to work on it, never forgetting that PLIVA was the originator.

Following the discovery of azithromycin, PLIVA chemists and engineers developed an excellent production process which made the company the largest producers of the active substance. This was the basis for the broader synthesis of new biologically active chemical entities, in which a new generation of PLIVA chemists have played an important role.

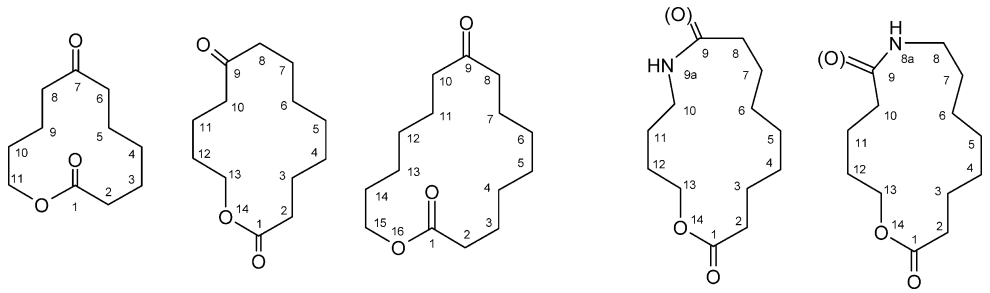


Fig. 1 Basic structures of macrolide lactones.

1. Introduction

Macrolides represent a well-known family of oral antibiotics. In 1952 the first and most widely used macrolide, erythromycin (**1**), was introduced to the market and medical practice. Medically important macrolide antibiotics were originally characterised by a 12-, 14- or 16-membered lactone to which amino-sugars and neutral sugars are attached. Macrolides express their antibacterial activity by binding to bacterial 50S ribosomal subunits and inhibiting protein synthesis.

As a broader term, macrolides, isolated from natural sources, encompasses all macrocyclic lactones (Fig. 1) with larger than 8-membered rings varying in size up to 62-membered rings [1]. They consist not only of simple carboxylic monolactones but also of more complex lactones such as macropolydes and macrocyclic lactones that contain amino nitrogen, amide nitrogen, an oxazole ring, or thiazole ring in their skeleton. Macrolide-producing organisms are actinomycetes, myxobacteria, fungi, algae, plants and insects. Many macrolides have different biological activities, but the starting point of this overview will be azalides as semi-synthetic macrolides based on classical antibacterials.

The first and most important 14-membered erythromycin A (**1**) is active against Gram-positive and certain Gram-negative microorganisms and is still used to treat infections of the respiratory tract, skin and soft tissues and genital tract. After confirmation of the precise structure of the fermentation products (from *Streptomyces erythreus*, later reclassified as *Saccharopolyspora erythrea*) worldwide efforts in medicinal chemistry were undertaken to improve the biological profile (better activity, higher stability, and improved bioavailability). Macrolides have low toxicity and are well tolerated, but they are unstable in acidic media. **1** is metabolized in the acidic environment of the stomach to its inactive 8,9-anhydroerythromycin-6,9-hemiketal and anhydroerythromycin-6,9:9,12-spiroketal (Fig. 2). This reaction is the result of intramolecular interreaction between of the hydroxyls and the keto group.

To improve acidic stability and oral bioavailability of **1**, the first generation of semisynthetic macrolides were prepared as 2′-esters and 11,12-cyclic carbonates and introduced to medical practice (as 2′-propionyl or erythromycin estolate, Ilosone®-Eli Lilly and 2′-acetyl octadecanoate or erythromycin acistrate, Erasis, Orion Pharma).

Further modification at the 9-keto group of **1** was performed, including oximation or further reduction to amino group, which finally resulted in the synthesis of roxithromycin and dirithromycin. Alternatively, methylation at position 6 yielded the stable and highly active clarithromycin, which became the market leader among macrolide antibiotics during the final decade of the last century.

1 is the most important starting substance for the preparation of 14-membered semisynthetic macrolide antibiotics. This approach has yielded various new chemical entities [2∼6]. The key derivative **1** for this group of new derivatives (Fig. 3) was 9(*E*)-erythromycin A oxime (**2**) prepared from **1** and hydroxylamine hydrochloride [7∼9] in the presence of weak base or buffer (Scheme 1).

Suitable reaction conditions had to be sought for this sterically hindered keto-group which is unstable in acidic media. Later, it was found that under the chosen reaction conditions isomeric 9(*Z*)-erythromycin A oxime (**3**) could be formed also [7, 8].

A qualitatively new group of macrolide antibiotics was discovered when a nitrogen atom was introduced into the aglycone ring, yielding a 15-membered imino-ether by Beckmann rearrangement of **2** [10∼13]. The compound was further reduced to an amino-lactone, thereby introducing a second amino group. This new class of 9-dihydro-9a-aza-9a-homoerythromycins was named

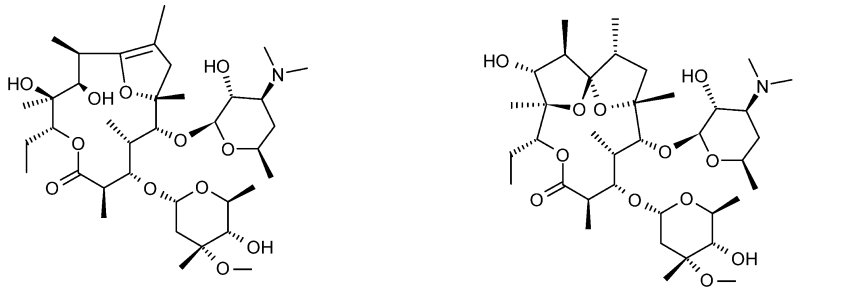


Fig. 2 Structures of erythromycin acid rearrangement products.

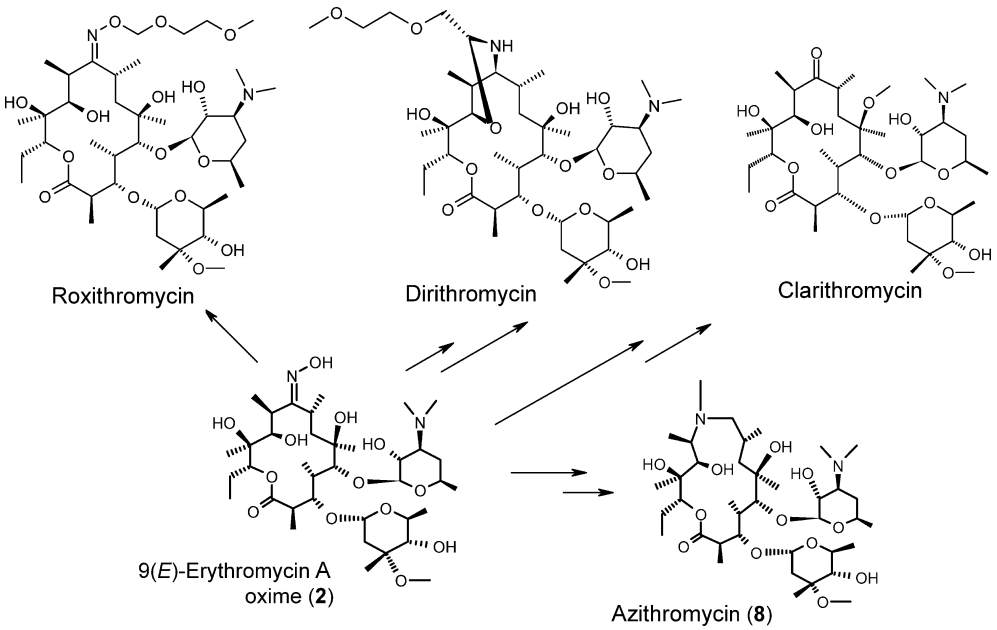


Fig. 3 Structures of semisynthetic macrolides obtained from erythromycin A and its oxime.

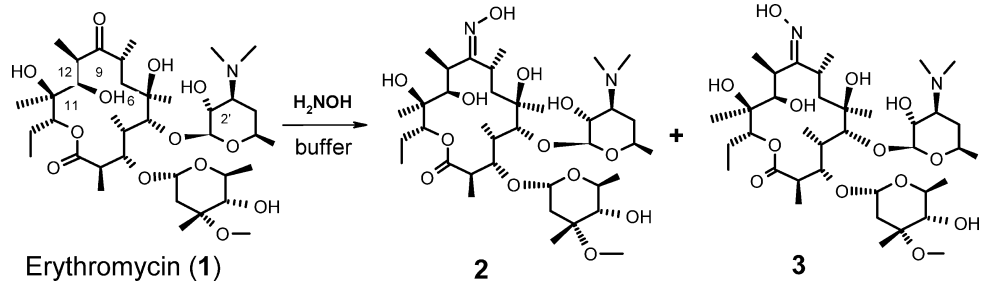
"azalides". The first azalide, azithromycin (**8**, 9a-methyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin), was discovered in 1980 by PLIVA [10~14]. Its broad spectrum of activity covers all relevant bacteria causing respiratory tract infections, including *Haemophilus influenzae* and *Moraxella catarrhalis*.

The greatest advantages of **8** compared to other macrolide antibiotics are its unusual pharmacokinetics (high tissue distribution), metabolic stability and high tolerability. These properties have led in recent years to the widespread use of the azalide scaffold for synthesis of new compounds with advantageous pharmacokinetics, not only as antibacterials, but also for other indications. It is known that macrolides generally have anti-inflammatory and immunomodulatory activity [15~18], but some have shown tuberculostatic, antimalarial, antiviral and antitumor activity [19]. To increase these activities, azalide scaffolds were combined with compounds with the desired actions to form new complex or hybrid molecules called "conjugates" and active compounds have been bonded to new compounds to generate improved bioavailability.

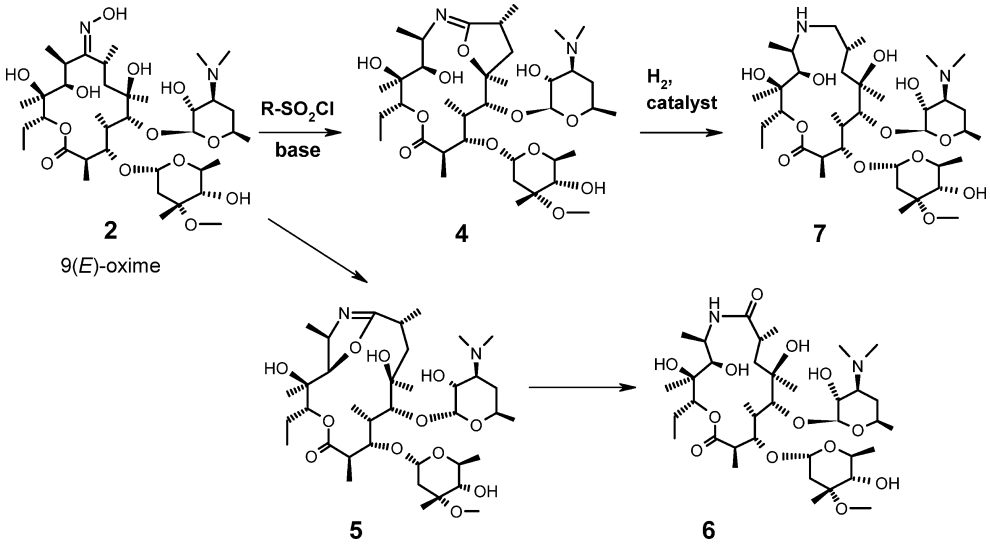
2. 15-Membered Azalides

2.1. 9a-Azalides

The key reaction in the formation of azalides was established during the synthesis of *O*-sulfonyl derivatives of oxime **2** [10~12]. It is well known that sulfonyl chlorides are catalysts for the Beckmann rearrangement to transform oximes to amides. However, this chemical derivatisation step was not applied previously to macrolactone scaffolds



Scheme 1 Synthesis of erythromycin A 9-oximes (**2** and **3**).



Scheme 2 Beckmann rearrangment of 9(*E*)-erythromycin A oxime.

[13]. Treatment of oxime **2** with benzenesulfonyl chloride in an acetone - water mixture with sodium bicarbonate yielded an unexpected product. Physico-chemical analysis indicated that in the assumed structure one water molecule was missing and that the compound exhibited a bicyclic structure. The presumed structure was erythromycin-6,9-imino-ether (**4**), which was later confirmed by X-ray structural analysis [14]. The product had no antibacterial activity and was further transformed in various subsequent reactions. Further investigations provided evidence that **4**, the main product of the first successful Beckmann rearrangement, was formed from readily available **2** [7, 14].

When the Beckmann rearrangement was performed in ether (Scheme 2) in the presence of pyridine at −45°C, the mixture of **4** and 9,11-iminoether **5** was formed [8].

The same conditions and temperature (0 to 5°C) yielded the lactam **6** [8]. The bicyclic compound **4** was further reduced to an amino-lactone **7**, thereby introducing a second amino group. Catalytic hydrogenation in the presence of Pt-catalyst gave the cyclic amine **7**, named 9-dihydro-9-deoxo-9a-aza-9a-homoerythromycin A, which was cited earlier as 10-dihydro-10-deoxo-11-azaerythromycin A according to the older nomenclature.

To this new class named azalides belongs also cyclic amide 9a-aza-9a-homoerythromycin (9a-lactam **6**), which showed lower antibacterial activity than its corresponding cyclic amine **7**. Lactam **6** could not be reduced to its amine **7** [13].

The structures of the imino-ether **4** and the 15-membered amine **7** were determined by mass spectroscopy and ^{1}H and ^{13}C NMR analysis. Finally, the structures were confirmed by X-ray analysis of the respective aglycone derivatives prepared by removal of both sugars [13].

The antibacterial activity of **7** was close to that of **2**: similar activity against Gram-positive bacteria and better activity against Gram-negative bacteria than **1**. **7** become the main precursor for novel, significantly more active derivatives, especially for 9-dihydro-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (**8**) with the generic name azithromycin (see section 2.3.).

The azalide scaffold possesses a number of functional groups, which could be substituted or changed.

Figure 4 highlights positions for possible derivatization of the azalide scaffold, which will be described in the following chapters.

The intention was to get the review structured according to the site and type of substitution or derivatization, but they can not be totally separated, because frequent combinations of the various changes have been made.

2.2. 8a-Azalides

Isomeric 9-dihydro-8a-aza-8a-homoerythromycins were prepared starting from **3**, along with various *N*- or *O*-alkyl or acyl substituted derivatives.

As mentioned above, **2** [7] is the main product of oximation of **1**. After isolation of **3** [20] and basic isomerisation of the *E* isomer into the *Z* isomer, researchers at Merck&Co applied the Beckmann rearrangement and synthesized isomeric 8a-homoerythromycin-6,9-iminoether (**9**), 8a-homoerythromycin-9,12-iminoether (**10**) and isomeric 8a-lactam **11** [21, 22]. Under aqueous conditions, imino-ether **9** and lactam **11** were formed.

When anhydrous conditions were used, imino-ethers **9** and **10** were isolated. **10** was in equilibrium with its 10-methyl epimer (Scheme 3). Conformational analysis and application of molecular modeling techniques to **4** and **9** were used to establish the predominant solution-state conformation [20, 23] and to explain chemical reactivity. **9** and **10** were further reduced with hydrogen in the presence of Pt or with $NaBH_4$ to the corresponding 9-dihydro-9-deoxo-8a-aza-8a-homoerythromycin (**12**). The reduction rate of 9a-aza- and 8a-aza-6,9-imino-ethers **4** and **9**, as well as 9a-aza-9,11-imino-ether **5**, have been studied using molecular modeling techniques [23].

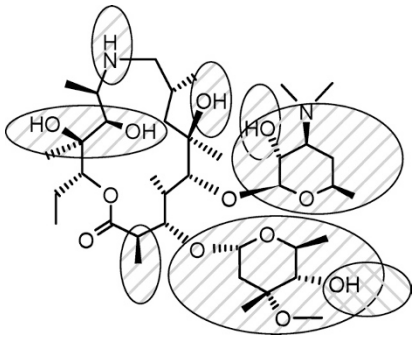
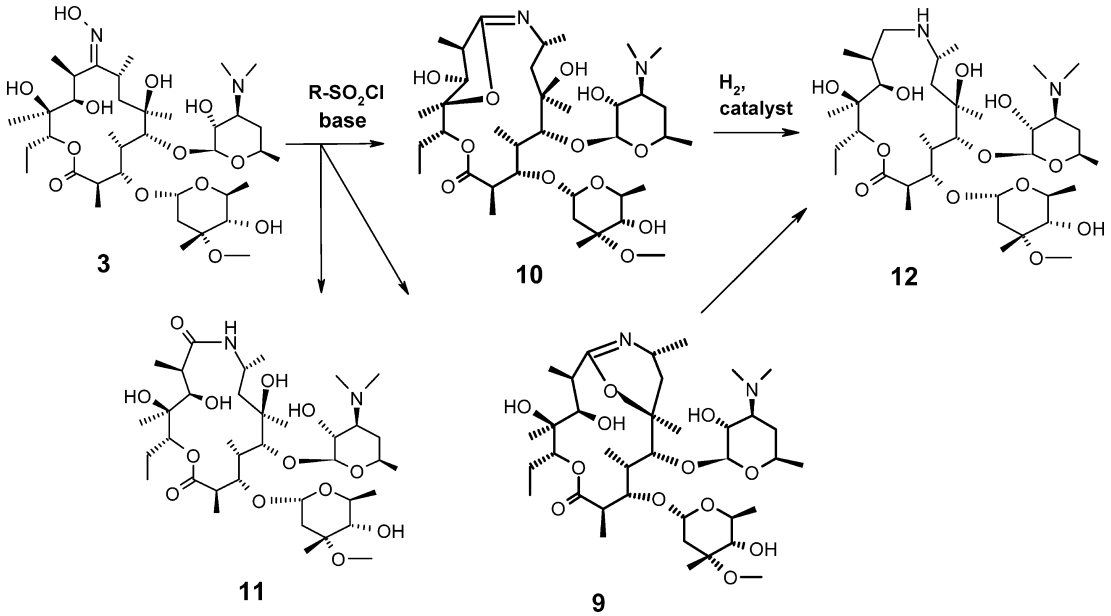


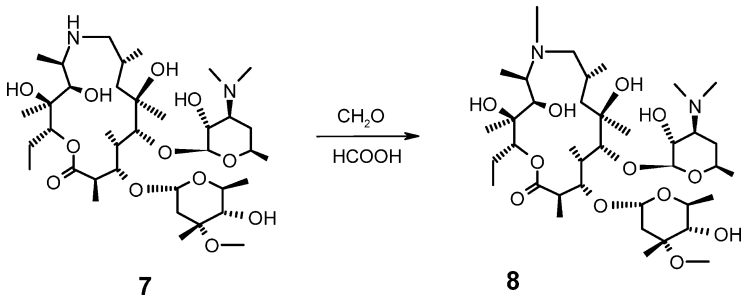
Fig. 4 Azalide structure modification and derivatisation.

2.3. *N*-Substituted Azalide Derivatives and Azithromycin

Starting from the first synthesized azalide, cyclic aminolacton **7**, a number of derivatives have been produced. The best antibacterial activity was achieved with **8**, which was prepared by reductive methylation (Scheme 4) with formaldehyde and formic acid [14]. The new product, **8**, named azithromycin, showed broad antibacterial activity and strongly improved acid stability. The full chemical name, following Chemical Abstracts is [2*R*-(2*R**,3*S**,4*R**,5*R**,8*R**,10*R**,11*R**,12*S**,13*S**,14*R**)]-13-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexapyra-



Scheme 3 Beckmann rearrangment of 9(*Z*)-erythromycin A (**3**).



7 **8**

Scheme 4 Synthesis of azithromycin (**8**).

nosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexapyranosyl]oxy]-1-oxa-6-aza-pentadekan-15-on.

8 is the first and presently the only 15-membered macrolide antibiotic on the market with a basic nitrogen atom inserted into the macrocyclic ring. It was synthesized in 1980 by the researchers at PLIVA laboratories [10, 11].

In 1986 Pfizer, whose scientists also worked on this compound class [24, 25], decided to licence-in azithromycin from PLIVA for worldwide markets. During the process development, **8** was first isolated as the anhydrous substance [14]. Later various crystal forms were prepared [24, 26~29], and the most stable was found to be its dihydrate [26]. For intravenous application, water-soluble salts were prepared [14, 29], and some stable divalent metal ion complexes were formed [30] as possible oral dosage forms for treatment of stomach ulcers.

Further investigation of the biological properties of **8** revealed its high activity against some important Gram-negative microorganisms [31] such as *Haemophilus influenzae*, *Neisseria gonorrhoeae* and *Moraxella catarrhalis*. Preclinical investigations very soon showed its promising and favourable profile, such as stability in acid environment [32], high oral bioavailability compared to erythromycin [33] longer half-life and high tissue concentration [34]. This profile allowed a short dosing regimen, one of the key advantages of **8** compared to other antibacterials and macrolides [35].

In 1988 **8** was introduced to the domestic (previous Yugoslavian) market in its dihydrate form by PLIVA under its brand name "Sumamed". Pfizer achieved market approval in its first markets in 1990 under the brand name "Zithromax". After 2000, **8** became the market leader of antibiotics for RTI.

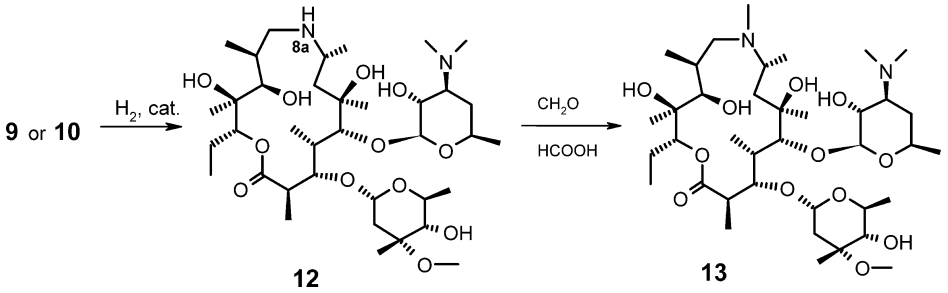
The conformation of **8** in solution was determined by NMR spectroscopy and molecular modeling, and compared with its crystal structure and some derivatives **1** [36]. With the determination of the crystal structure of the bacterial ribosome various scientists tried to prepare binding complexes of ribosome and macrolide antibiotics. Analysis of the crystal structure of the large ribosomal subunit (50S) from *Deinococcus radiodurans* complexed with azithromycin, showed that azithromycin exerts its antimicrobial activity by blocking the protein exit tunnel, but in contrast to other macrolides, a second binding site was also recognized [37]. Nitrogen inserted into the lactone ring does not directly contribute to the binding of **8** to the ribosome. Is seems, that this modification alters the conformation of the lactone ring sufficiently to induce novel contacts. One molecule of **8** interacts with domains IV and V of 23S rRNA, and second **8** interacts with ribosomal proteins L4 and L22 and domain II of 23S rRNA. Furthermore, AZI-2 makes direct contact to AZI-1 through a hydrogen bond between its desosamine sugar and O1 in the lactone ring of AZI-1.

The introduction of the Beckman rearrangement opened a completely new derivatisation line and various attempts were undertaken to improve the properties of **8**. Using the same method as for **8**, isomeric 9-dihydro-9-deoxo-8a-aza-8a-homoerythromycin (8a-azalide **12**), subjected to reductive methylation (Scheme 5), yielded the corresponding 9-dihydro-9-deoxo-8a-methyl-8a-aza-8a-homoerythromycin (**13**).

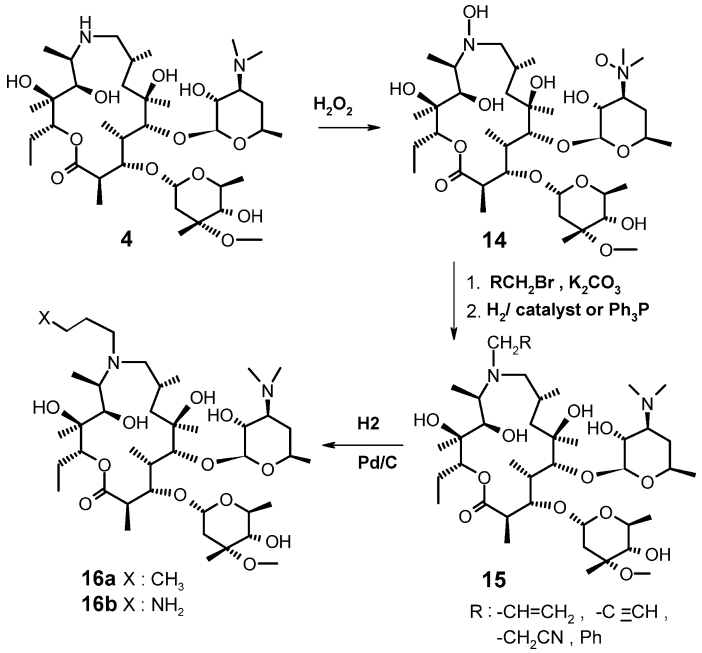
Antibacterial activities of 8a-azalide **12** and its *N*-methyl derivative **13** were similar to those of 9a-azalide **7** and azithromycin (**8**) respectively [21]. The 11-deoxy 8a-azalide retained the same *in vitro* antimicrobial potency [38].

2.4. Other *N*-Substituted Azalide Derivatives

The generation of the first azalide **7** permitted a derivatisation line at the 9a-nitrogen. Using reductive alkylation with formaldehyde or acetaldehyde, **8** or its 9a-ethyl analogue were prepared. To avoid the formation of quaternary ammonium salt, some *N*-alkyl substituted derivatives were prepared by reaction (Scheme 6) of the corresponding halides and the *N*-hydroxy derivative **14** was generated from amine **7** [25].



Scheme 5 Synthesis of 9-dihydro-9-deoxo-8a-methyl-8a-aza-8a-homoerythromycin (**13**).



Scheme 6 Synthesis of 9a(*N*)-substituted-9-dihydro-9a-aza-9a-homoerythromycins.

These new derivatives, *e.g.* **15**, were further transformed into their *N*-allyl derivatives, subsequently transformed by hydrogen and Pd catalyst to the *N*-propyl derivative **16**, which was confirmed by X-ray analysis of its aglycone backbone [39]. The application of the reaction on acrylonitrile yielded new cyano-alkyl derivatives which were reduced with hydrogen in the presence of Raney Ni to corresponding amino-alkyl compounds. However, none of these derivatives showed better biological activity than **8**. The *N*-aminopropyl derivative **16b** was later used as an intermediate for various new *N*-substituted derivatives. Similar, *N*-substituted derivatives on isomeric **12** have been prepared by chemists at Merck [21, 22].

The lactam **11** was *N*-alkylated at position 8a with alkyl halides and NaH with prior protection of the hydroxyl groups by TMS [21]. Another method for preparation of *N*-substituted lactams was based on the primary oxidation of imino-ethers **9** or **10** to the *N*-oxide of the sugar's dimethylamino group, followed by reaction with alkyl halides [21] and finally by hydrolysis to yield the lactam and deoxygenated product of the *N*-oxide.

It is known that some macrolides and **8** itself have anti-inflammatory properties [15～18]. Scientists from PLIVA working on the synthesis of new anti-inflammatory compounds have prepared a number of 9a-substituted azalides bound to known steroidal and non-steroidal anti-inflammatory agents (Fig. 5). Most derivatives were prepared starting from amine **16b** or some longer amino-methylene chain, using 1-hydroxybenzotriazole and carbodiimide as condensation agents. The idea was to use azalide properties for improving cellular targeting to inflammatory cells [40～43].

Another *N*-derivatisation line of azalides was performed in PLIVA. Starting from **7**, various 9a-carbamoyl and

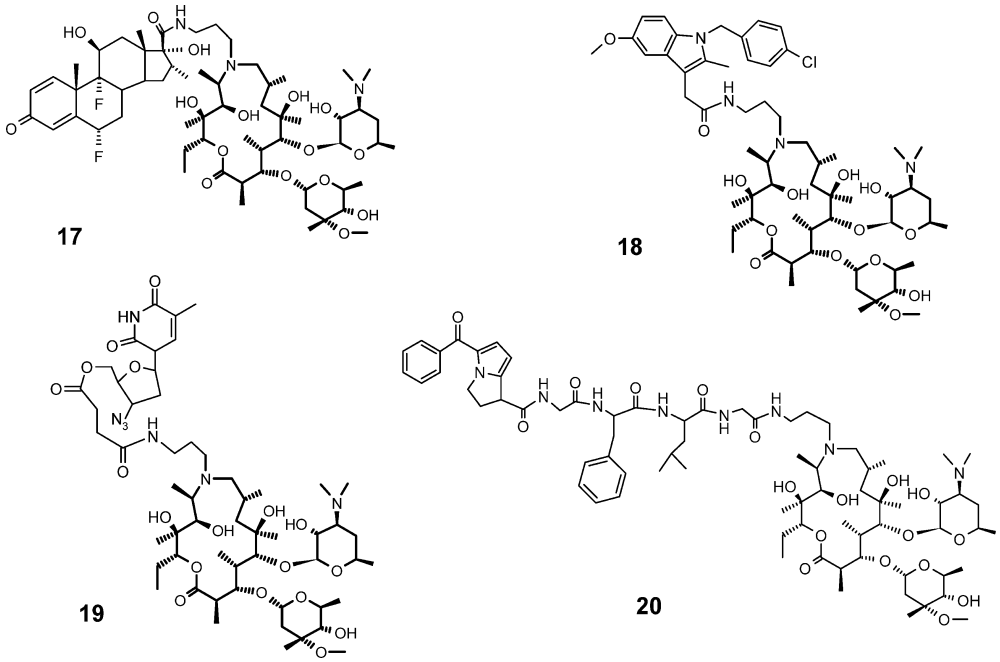
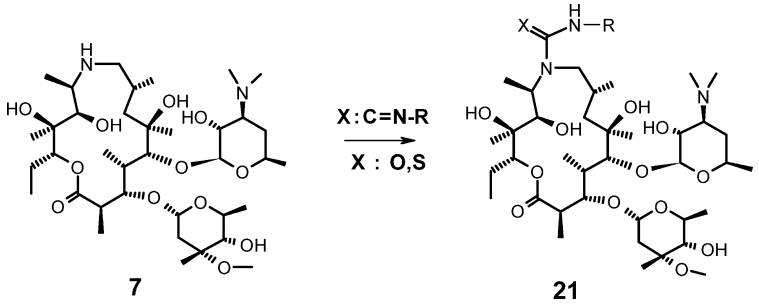


Fig. 5 Structures of 9a,*N*-substituted azalides with some biologically active scaffolds.



Scheme 7 Synthesis of 9a-carbamoyl- and thiocarbamoyl-9-dihydro-9a-aza-9a-homoerythromycins.

thiocarbamoyl derivatives **21** were prepared (Scheme 7) by reaction of **7** with corresponding isocyanates or isothiocyanates [44]. Reactions were usually conducted in toluene to achieve easily crystallisable *N′*-alkyl or *N′*-aryl substituted ureas. Structures of the *N′*-isopropyl and *N′*-(4-pyridyl) derivatives were confirmed by X-ray analysis [45].

Representatives of various ureas included isopropyl-, 4-methyl-5-oxazolyl-, 2-furyl-, 4-pyridyl-, phenyl-, benzyl-, and 1-naphthyl ureas, and *N′*-benzyl-thiourea. In biological testing, only a few derivatives **21** showed moderate antibacterial activity. Additional halogen-aryl derivatives of **21** have been synthesized showing activity against resistant strains [46]. Later 9a-(4-aminosulfonyl)phenyl-carbamoyl derivatives of 9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin **22** were prepared (Fig. 6) as compounds with antibacterial properties [47, 48]. Sulphonylureas **23**, bonded to the azalide scaffold with a propylenamino-linker [49] were prepared to obtain improvement in specific antibacterial activity.

Ethenyl derivatives **24** (Fig. 6) were prepared, with potential antibacterial activity, and with broad possibility for derivatisation [50].

2.5. *O*-Substituted Azalide Derivatives (Esters, Ethers and Carbamates)

8 and its 8a-aza isomer possess five hydroxyl groups, of which two are secondary hydroxyl groups on sugar moieties while one secondary and two tertiary hydroxyl groups are located on the aglycone ring itself. Their presence explains the difficulties in targeted derivatisation. The most reactive hydroxyl group is at the 2′-position on the amino sugar. Initial derivatives targeting the hydroxyl

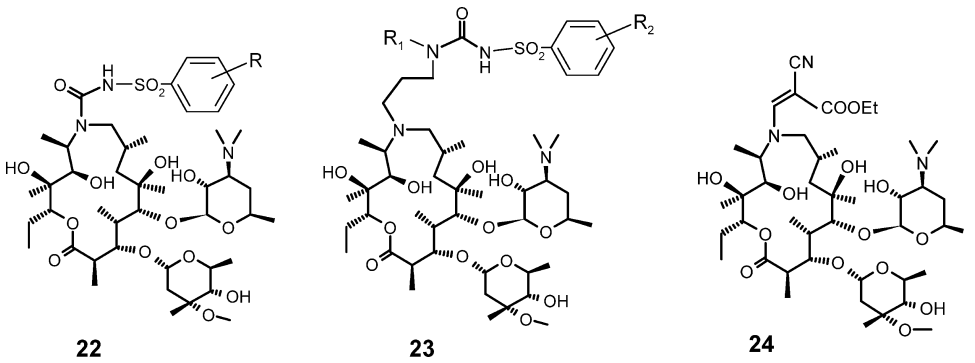


Fig. 6 Structures of 9a-sulfonyl-carbamoyl-9-dihydro-9a-aza-9a-homoerythromycins and 9a-ethenyl-9-dihydro-9a-aza-9a-homoerythromycins.

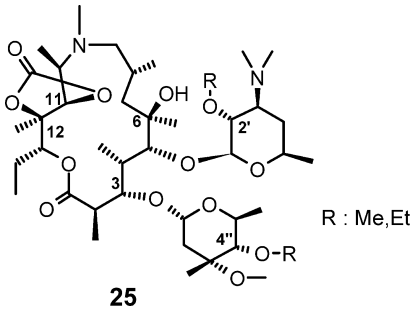


Fig. 7 Structure of azithromycin 11,12-carbonates.

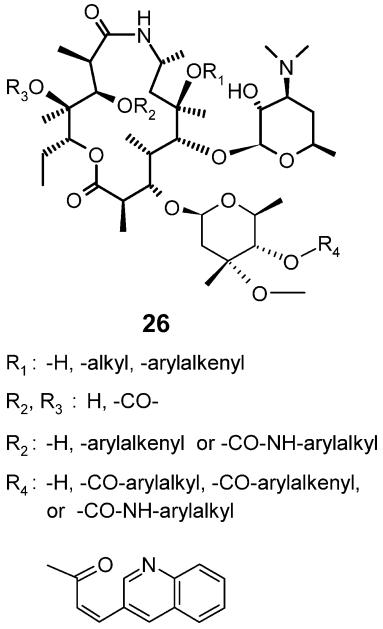


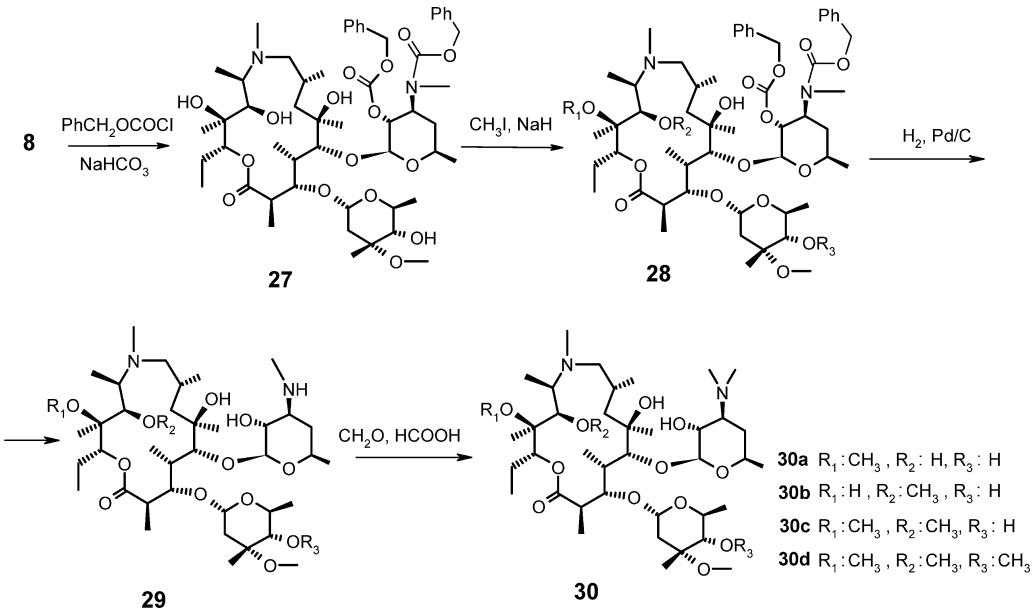
Fig. 8 Structure of 4″-arylacyl-8a-aza-8a-homoerythromycins (8a-lactams).

groups were esters: mono-, di- or tri-acetyl on the 2′-*O*- , 4″-*O*- and 11-*O*-hydroxyl groups, which were prepared using acetic anhydride with pyridine [32]. Reaction of **8** or its diacetyl derivative with ethylene carbonate and potassium carbonate yielded the corresponding 11,12-carbonates (**25**) or its diacetyl derivatives (Fig. 7). Acetyl groups could be easily removed by hydrolysis in methanol.

All esters and carbonates of free hydroxyl groups failed to show any improvement over **1** in antibacterial activity against Gram-positive bacteria, but they were found to exhibit higher activity against Gram-negative strains.

A number of 4″-arylalkyl esters or carbamates (**26**) have been synthesized on 8a-lactams (Fig. 8). Most esters were prepared using mixed anhydrides of carboxylic acids obtained with pivaloyl chloride. Some of them, such as 4″-quinolyl-acryloyl with 6-hydroxy- and 6-*O*-methyl showed antibacterial activity on azithromycin resistant *Streptococcus pneumoniae* strains [51, 52], but introduction of a second arylalkyl group, such as 6- or 11-quinolylallyl, decreased antibacterial activity. Corresponding derivatives of isomeric 9a-lactam (**6**) had little antibacterial activity.

Since esters most often are cleaved in biological fluids, formation of ether bonds was performed to generate stable *O*-substituted derivatives. The first efforts were focused on the synthesis of the 6-*O*-methyl derivative of **8**, similar to the synthesis of clarithromycin. Using benzyloxycarbonyl chloride in the presence of sodium bicarbonate, **8** was protected at the 2′-*O*- and 3′-*N*-positions to yield the di-benzyloxycarbonyl derivative **27**, which was methylated with methyl iodide and sodium hydride in DMSO-THF or DMF [53]. Unfortunately, only mixtures of 11-*O*- and 12-*O*-methyl derivatives with small amounts of di- or tri-methylated products (**28**) were generated (Scheme 8). These products were hydrogenated using palladium on carbon to yield **29** and then remethylated on position 3′-*N*- with formaldehyde and formic acid. The mixture of mono- and some di- or tri-



Scheme 8 Synthesis of *O*-methyl derivatives of **8**.

methylated azithromycins (**30**) was difficult to separate.

The structure of 12-*O*-methyl derivative **30a** was first described as 6-*O*-methyl azithromycin [53]. This error was corrected later by a publication from Merck [54] and confirmed by PLIVA using X-ray structural analysis of the trimethylated compound **30d** [55]. Finally, structure-activity studies using quantum mechanics of azithromycin, revealed that alkylation of the 6-hydroxy group is practically impossible to achieve.

Antibacterial testing of 12-*O*-methyl derivative **30a** indicated weaker activity than azithromycin, while 11-*O*-methyl **30b** was equally or slightly more active. The antibacterial activities decreased with increasing degree of methylation [53, 55].

Merck published the synthesis of mono-, di-, and trimethyl derivatives of 9-dihydro-9-deoxo-8a-methyl-8a-aza-homoerythromycin (**31**), and the authors established rates of *O*-methylation from 8a-aza- and 9a-aza-homoerythromycins: 11-OH(9a)≥12-OH(9a)>4″-OH(9a)≡4″-OH(8a)>12-OH(8a)>11-OH(8a). To methylate the 6-hydroxy group, they started with the 11,12-carbonate of 8a-azalide **13** and obtained the 12-*O*-methylated 3,6-ether accompanied by removal of cladinose. Direct methylation of the 6-hydroxy group of 8a-azalide was only achieved after removal of cladinose, which yielded the 3,6,11,12-tetra-*O*-methyl derivative. Furthermore, the 6-*O*-methyl-9a-azalide **31** and 6-*O*-methyl-8a-azalide **32**, starting from clarithromycin (6-*O*-methyl-erythromycin A), have been described [56]. The *E*- and *Z*-oximes were prepared and then converted using the reaction with TsCl in ether/pyridine to the corresponding 9a-aza-9,11-iminoether or 8a-aza-9,12-iminoether. Beckman reaction of *E*-oxime in aqueous acetone resulted in the 6-*O*-methyl derivative of 9a-lactam **7**. After reduction of imino-ethers and reductive methylation of 9a- and 8a-amines, the desired 6-*O*-methyl compounds **31** and **32** were generated (Fig. 9).

In the course of these experiments, PLIVA chemists discovered a new synthetic procedure for selective methylation of position 11- to obtain **30b** using diazomethane and catalyst [57, 58].

Authors from Hoechst-Marion-Roussel (now Sanofi-Aventis) published comparable results using the same reaction to synthesize the azalide **31**. The 6-*O*-methyl derivative **34** of 9a-lactam **7** was generated by reaction of 6-*O*-methyl-erythromycin-9(*E*)-oxime with TsCl in aqueous acetone [59].

Antibacterial evaluation of the 6-*O*-methyl derivatives **31** and **32** revealed lower activity compared to the 11- or 12-*O*-methyl analogues. Generally, *O*-methyl derivatives of 8a-azalides were less active than the corresponding 9a-azalides [56]. 6-*O*-Methyl derivatives **33**, **34** of lactams **6** and **11** were less active than the corresponding methylamines **31** and **32**.

Abbott's researchers reported the introduction of its telithromycin side chain 3-(3-quinolyl)allyloxy group at position 6-*O* of **8** [60]. Synthesis was carried out starting from protected erythromycin-9-oxime, the intermediate in the preparation of cethromycin, which was alkylated on the 6-hydroxyl with propargyl halide, treated with quinolyl bromide and partially reduced to 6-quinolylallyl-

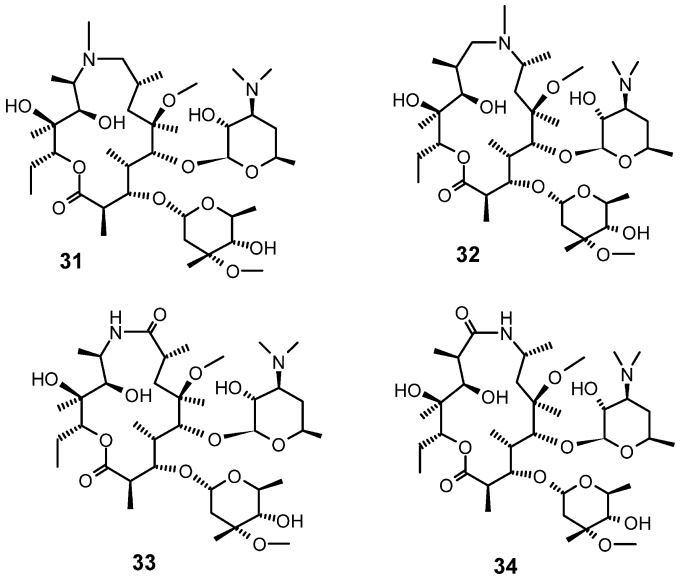


Fig. 9 Structures of 6-*O*-methyl- 8a- and 9a-aza-homoerythromycins.

erythromycil oxime. Another method for synthesis of the same oxime was given, applying the allyl intermediate under Heck conditions or using tert-butyl carbonate as the active intermediate. Deprotection yielded 6-substituted erythromycin A oxime, which was transformed, *via* a Beckmann rearrangement, to the imino-ether, then reduced to the azalide and finally methylated at position 9a (Scheme 9).

Some compounds, such as **42**, showed activity against azithromycin-resistant Gram positive bacteria strains.

Starting from 6-*O*-quinolylallyl-oxime **39** and its *Z*-isomer, scientists in PLIVA have prepared the corresponding 6-*O*-3-(3-quinolyl)allyl-9a- and 8a-lactam [51, 52].

Derivatization of macrolides including azalides at the 4″-position have been described by Pfizer chemists to obtain novel 4″-*O*-(aryl-methyl-amino)alkyl carbamates. These compounds, especially the 9-erythromycylamine analog, showed activity against resistant strains comparable to the ketolide telithromycin (HMR 3647, Ketek). The corresponding azalide derivative **43** was less active than the 14-membered compounds [61].

Subsequently, a number of carbamate derivatives (Fig. 10) of the 11-hydroxy group of 9a azalides (**44**) or 8a-azalides were prepared [62]. The family comprised carbamoyl substituents with various isomers of pyridyl or dimethoxybenzyl side chains.

Within the collaboration between PLIVA and GSK, a number of 4″-(*β*-ethylendiamino)propionyl esters and carbamates with an alkylendiamino chain and heteroaromatic *β*-keto-carboxylic acid as the side chain have been prepared on 8a-lactams, 9a-lactams and **8** [63, 64]. 4″-Substituted lactams were prepared by reaction of 2′-acetyl-protected lactams with 3-chloropropionyl-chloride in the presence of sodium carbonate, whereas 4″-acryloyl-azithromycin **49** was prepared indirectly from 2-acetyl-azithromycin **45** using 6-acryloyl-11,12-cyclic carbonate **48** (Scheme 10) as intermediate.

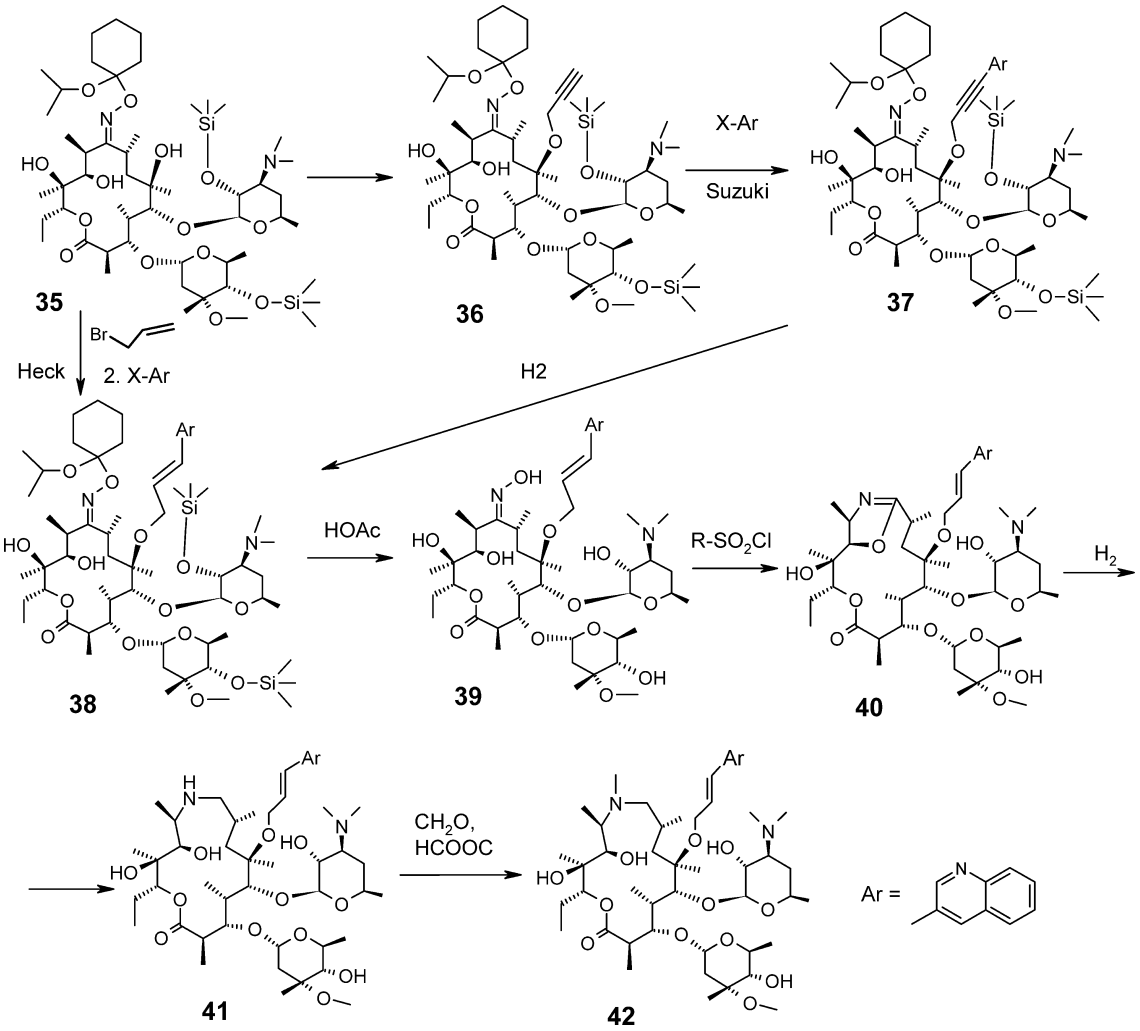
New *β*-amino esters **50**∼**53** (Fig. 11) were prepared applying Michael addition of substituted arylalkyl-amines to 4″-acryloyl precursors. One of the first compounds prepared on 8a lactam was a conjugate based on the fluoroquinolone, ciprofloxacin, which was linked *via* the piperazine moiety. The new compound **50** displayed lower antibacterial activity than 8a-lactam linked with an ethylendiamino group to oxoquinolonic acid.

Many of these alkylendiamino-quinolone derivatives (**51**, **52**, **53**) showed excellent activity against resistant *Streptococcus pneumoniae* and *Streptococcus pyogenes*, but 6- and 4″-disubstituted derivatives with the same side chain didn't show significant antibacterial activity. 4″-*O*-Carbamoyl derivatives were prepared using 4″-imidazolylcarbonyl-azithromycin as intermediate.

Later on, antibacterially active 4″-(*β*-ethylendiamino)-propionyl esters and carbamates (**54**) with another second heteroatom (Fig. 12) in linker have been prepared [65, 66], resulting in a profound increase in antibiotic activity.

In one of the latest patent applications, GSK chemists have described [67] 4″-arylheteroalkyl-sulphonates (**55**).

Using different ligands, 4″-(*β*-ethylendiamino)propionyl esters (**56**) were prepared by PLIVA (Fig. 13) as potential anti-inflammatory agents [68, 69].



Scheme 9 Synthesis of 6-*O*-(3-quinolyl)allyl derivative of **8**.

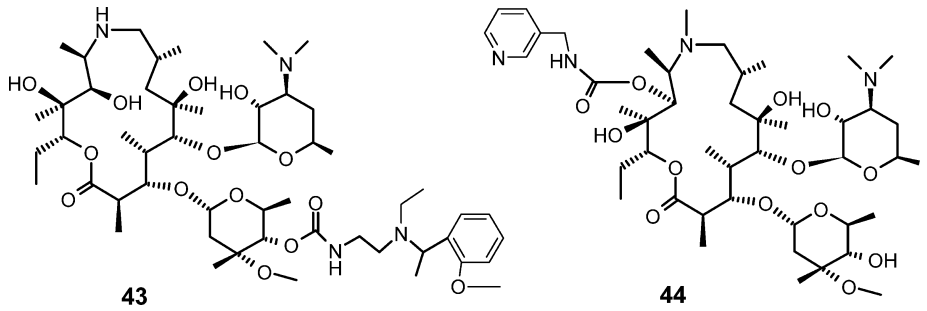
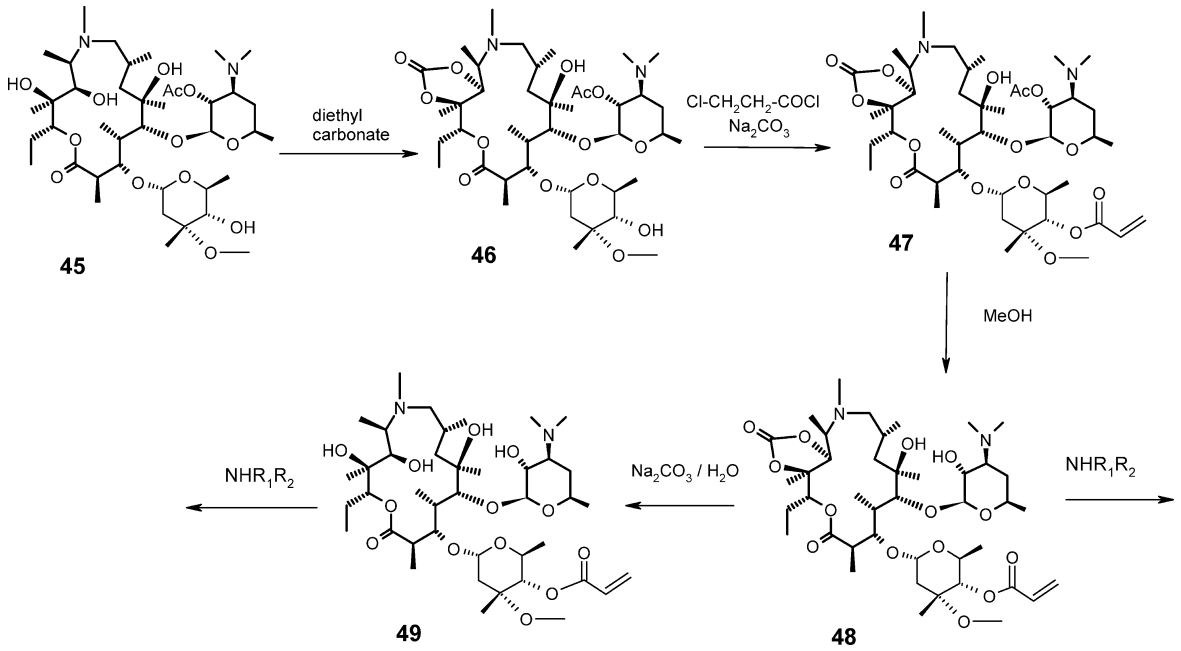


Fig. 10 Structures of *O*-carbamoyl derivatives of 9a-aza-9-dihydro-homoerythromycin and **8**.

2.6. *N*,*O*-Disubstituted Bicyclic Azalides

It is clear from earlier examples, that most derivatives were made by combination of *N*- and *O*-substitutions, but in some cases bicyclic compounds were obtained.

In an effort to synthesize *O*-methyl azalides before introducing the 9a-*N*-methyl group, **7** was protected as its tris-carbobenzoxy derivative **57** and treated with methyl iodide in the presence of NaH in DMF [70]. The reaction resulted in a mixture of 2′-*O*-3′-*N*-bis-carbobenzoxy-9-dihydro-9-deoxo-9a-aza-9a-homoerythromycin-9a,11-cyclic carbamate and its 12-*O*-methyl derivative. The cyclic carbamates were deprotected using catalytic reduction and



Scheme 10 Synthesis of -4″-*O*-acryloyl derivatives of **8**.

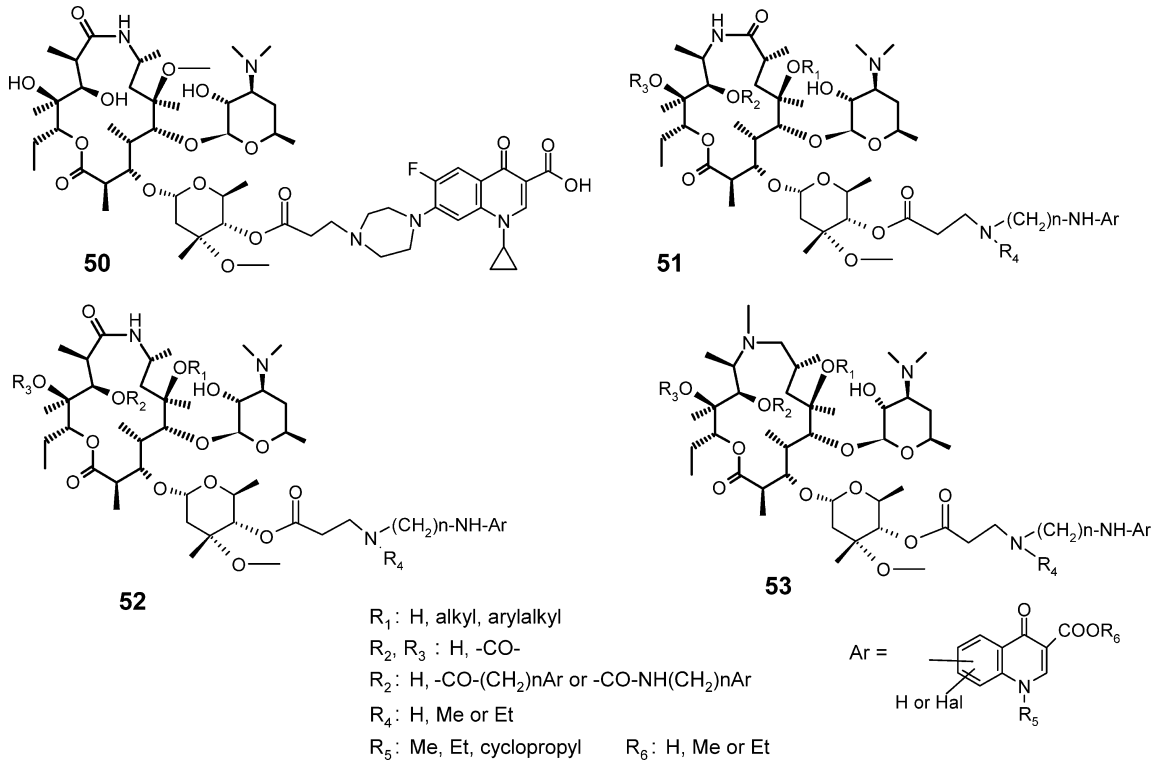


Fig. 11 Structures of heteroaryl substituted 4″-*O*-(*β*-amino)propionyl azalides.

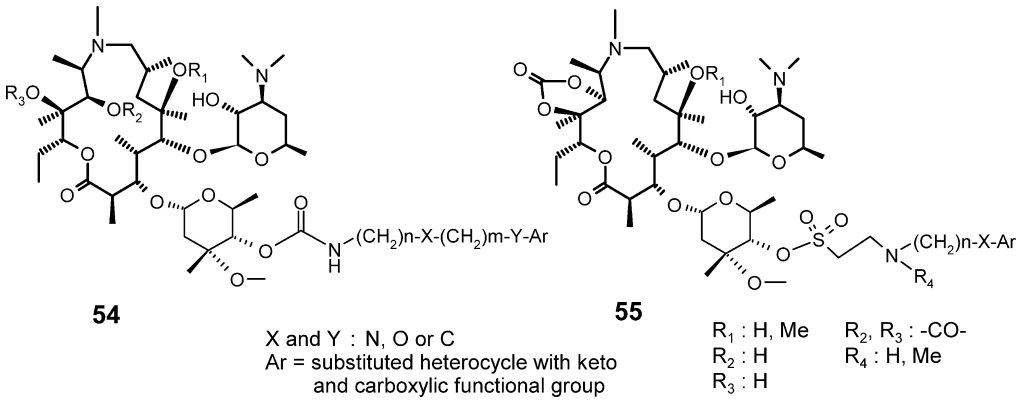


Fig. 12 Structures of heteroaryl substituted 4″-*O*-(*β*-amino)propyl-carbamate and 4″-*O*-(*β*-amino)propyl-sulfonate azalides.

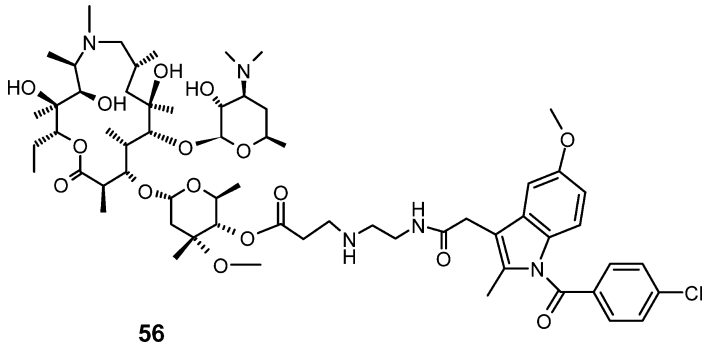
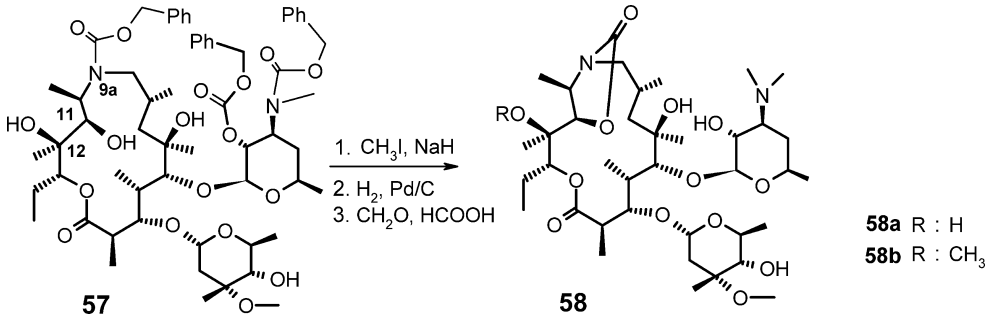


Fig. 13 Structure of potential anti-inflamatory 4″-*O*-(*β*-ethylendiamino)propionyl azalide.



Scheme 11 Synthesis of azithromycin-9a,11-cyclic carbamates.

3′-*N*-methylated with formaldehyde and formic acid to reintroduce the dimethylamino group (Scheme 11). Finally, 9-dihydro-9-deoxo-9a-aza-9a-homoerythromycin-9a,11-cyclic carbamate (**58a**) and its 12-*O*-methyl derivative (**58b**) were isolated by column chromatography.

In comparison to azithromycin, these novel bicyclic azalides **58a** and **58b** exhibited substantially decreased antibacterial activities *in vitro*.

Merck chemists have prepared 6,9a-bicyclic azalide carbamate and methylene derivatives [71, 72] as intermediates for ketolides (Fig. 14) to avoid formation of 3,6-hemiketals (chapter 2.9.2.).

During the last few years, ENANTA Pharm. Inc. entered into the area of macrolide antibiotics. They made many new analogues of 14, 15 and 16-membered macrolides. Starting from desmethyl-azithromycin or **7** and reacting the di-tert-butyl carbonate of the corresponding 2-methylene-1,3-propandiol with a palladium catalyst, 9a,11-3C-bicyclic 9a-azalide **62** was obtained (Scheme 12), which was later

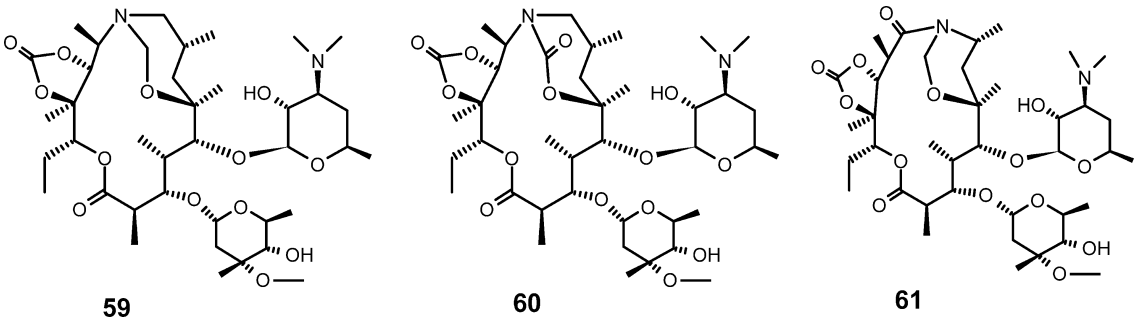
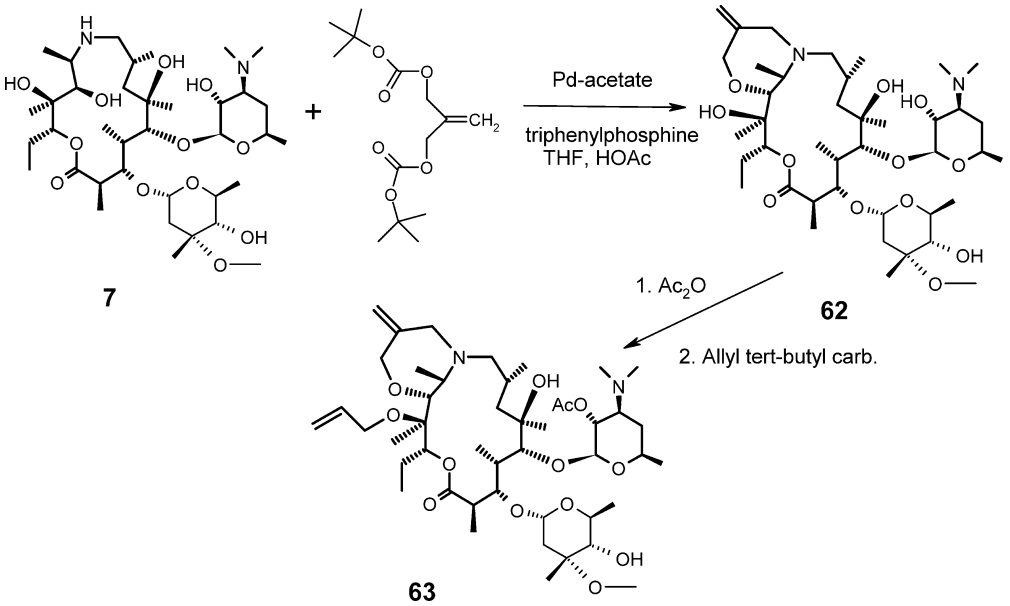


Fig. 14 Structures of 6,9a-bridged azalides.



Scheme 12 Synthesis of 9a,11-3C-bicyclic-9-dihydro-9a-aza-9a-homoerythromycins.

derivatized at various positions to as 11-allyl **63** [73].

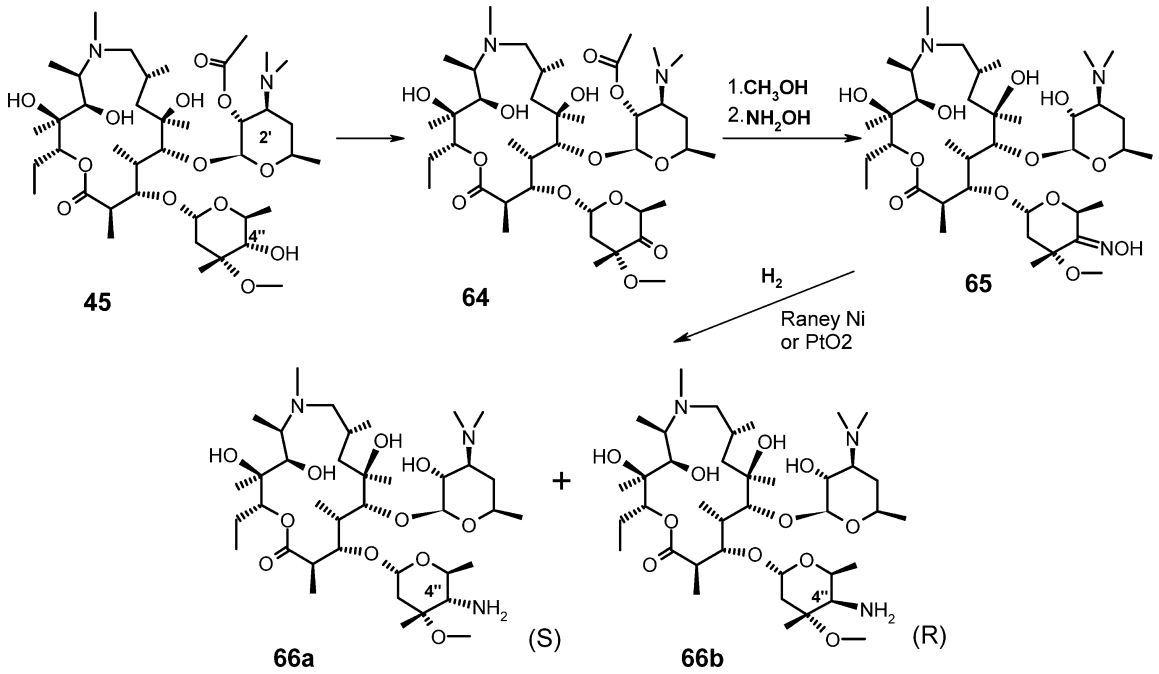
A bicyclic product was hydrogenated from methylene to give a methyl substituent, or acetylated at position 2′, derivatized and/or further hydrolysed to the descladinosyl analog and finally oxidised to the 3-keto derivative. (Chapter 2.9.2.). 9a,11-Bridged azalides didn't show satisfactory antibacterial activity but 3-keto derivatives were reported to be active against resistant strains [73].

2.7. Substitution on C-4″ Position

As a further line of derivatization of 9a-azalides [25], starting with **8**, another amino group was introduced on the cladinose sugar in place of the 4″-hydroxy group. After protection of the 2′-hydroxyl group as the acetate (**45**), the 4″-hydroxyl group was oxidized using Moffat-Pfitzer conditions (Scheme 13) to yield the respective 4″-oxo derivative **64**, which was transformed to **65** and then reduced to a mixture of epimers of the 4″-amine **66**.

In vitro and *in vivo* antibacterial activity of the amine **66a** was better than that of **1** and equaled that of **8**, especially against Gram-negative microorganisms. Similarly, Merck chemists have prepared 4″-oxo, 4″-oxime and epimers of 4″-amine from the corresponding 9-deoxo-9-dihydro-8a-aza-8a-homoerythromycin A [22, 74]. Further, a number of 4″-*N*-acyl derivatives of 8a-azalides were described [22, 75].

Starting from the 9a-aza scaffold (**8**), each of the epimers of the 4″-amino compound have been prepared by PLIVA chemists. Amides with heteroalkyl-linked quinolone derivatives were synthesized resulting in compounds with activity against resistant strains [75]. The derivative of the 4″-amine **67** with the natural (*S*) stereochemistry (Fig. 15) showed better biological activity than the corresponding (*R*) epimer.



Scheme 13 Synthesis of 4″-oximino- and 4″-amino-9a-methyl-9-dihydro-9a-aza-9a-homoerythromycins.

Chemists at Pfizer [76] have introduced a carbon atom at C-4″ applying Grignard reagents in DMF to the 4″-oxo derivative **64**. Starting from the 4″-ketone **64**, various reagents and reaction conditions were tried, and the epoxide **69** or its epimer were prepared (Scheme 14).

4″Hydroxy-4″carbon substituted compounds showed good antibacterial activity against bacteria in bovine and swine respiratory disease and one of them has been approved for the market as a veterinary drug under the generic name, tulathromycin (**70**) (Pfizer). Subsequently, the polymorph of the crystalline diphosphate salt was patented [77].

Scientists from PLIVA have attached a steroid moiety to 4″-C-substituted epoxy-azalides [68, 69] to obtain anti-inflammatory agents (Fig. 16) such as **71**, which acts predominantly at the inflamed site due to enrichment in inflammatory cells, such as granulocytes and macrophages.

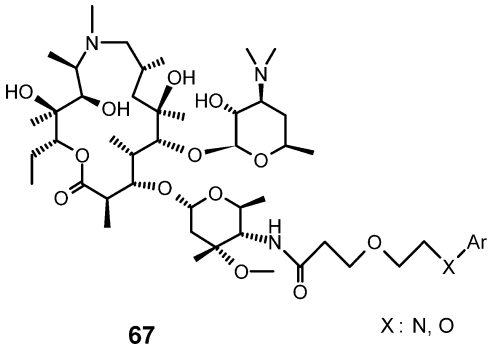
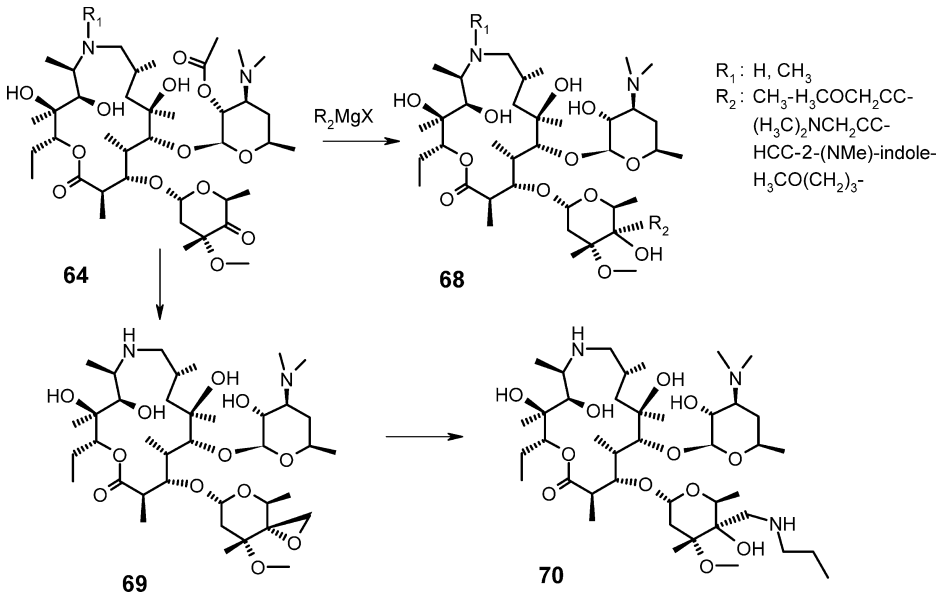


Fig. 15 Structure of 4″-deoxy-4″-acylamino derivatives of azithromycin.

2.8. Substitution on C-3′ Position

The amino group on C-3′ is important for antibacterial activity and all described changes on the dimethylamino group resulted in a decrease in antibacterial activity. When *O*-methyl derivatives of azithromycin were synthesized [53], using a benzoyloxycarbonyl group for protection, the 3′-*N*-demethyl derivative **29** was obtained, which, after removing protecting groups, had to be remethylated with formaldehyde and formic acid. 3′-*N*-Demethyl-azithromycin is known as one of the impurities in **8** synthesis. Scientists from TEVA have studied the degradation products of **8** during manufacturing processes, processes that include formulation of the pharmaceutical dosage forms [78]. They have reported structures (Fig. 17) of azithromycin-3′-*N*-oxide **72**, 3′-*N*-didemethyl-3′-*N*-formyl **73** and 3′-de-dimethylamino-3′-oxo derivatives **74**.

Scientists from Sandoz [79] have reported a process for preparing azithromycin-3′-*N*-oxide, 3′-*N*-didemethyl-3′-*N*-formyl-azithromycin, then 3′-*N*-demethyl-3′-*N*-formyl (**75**), and 3′-*N*-didemethyl-azithromycin (3′-aminoazithromycin). From this line, 3′-de(dimethylamino)-3′-oxo- and 3′-de(dimethylamino)-3′,4′-didehydro-azithromycin (**76**) derivative (Fig. 17) could be prepared.



Scheme 14 Synthesis of 4″-C-substituted-9a-methyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycins.

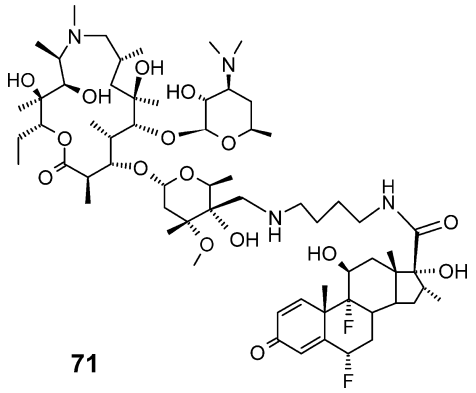


Fig. 16 Structure of potential anti-inflamatory 4″-*C*-tetramethylendiamino-steroido-substituted azalide.

Scientists working on anti-inflammatory agents at PLIVA have described in their patent applications the binding of a steroid moiety to 3′-demethyl azithromycin with some suitable linkers [40, 41].

2.9. 3-Descladinosyl-3-substituted Azalides—Acylides, Ketolides

2.9.1. Acylides

During the last few years resistance to macrolides has increased worldwide. One of the main mechanisms results from a base-specific mono- and dimethylation of 23S ribosomal RNA near or within the macrolide binding site. This decreases significantly the binding of macrolides, lincosamide or streptogramin [80, 81].

Macrolides lose antibacterial activity almost completely if the cladinose sugar at position 3 is hydrolyzed to form the 3-hydroxy analogs. However, further conversion to acylides, anhydrolides and ketolides not only restores the activity but overcomes typical macrolide resistance. The synthesis of various 3-*O*-esters was described by Taisho for different erythromycin derivatives. Especially 3-decladinosyl-arylacetyl macrolides demonstrated activity against inducible resistant strains. The 15-membered macrolides included 3-descladinosyl-3,6-di(*p*-nitrophenyl)-acetyl-azithromycin and its 11,12-carbonate **77** [82]. However, biological activity of aza-acylides was not mentioned. A similar 3-*O*-(3-pyridyl)-acetyl derivative (**78**) (Fig. 18) of **8** was prepared [83], but antibacterial activity against *Staphylococcus aureus* and *Streptococcus pneumoniae* was lower than with the corresponding 14-membered derivative; only activity against *H. influaenzae* was improved.

Synthesis of 3-descladinosyl-3-arylalkyl-8a-lactams, starting from 8a-lactam **12**, has been described by PLIVA chemists [84]. Some of these lactams, such as 3-*O*-(*p*-nitrophenyl)-acetyl-8a-lactam **79**, possess antibacterial activity similar to that of azithromycin but no improvement in activity towards resistant pathogens was observed. 9a-Lactam derivatives **80** were antibacterial inactive (Fig. 19).

In a further attempt to improve antibacterial activity of aza-acylides, corresponding 3-*O*-arylacyl derivatives of azithromycin-9a,11-cyclic carbamate **81** were prepared [85, 86], but with lower antibacterial activity (Scheme 15).

Chemists at Pfizer reported [87] the synthesis of aryl and

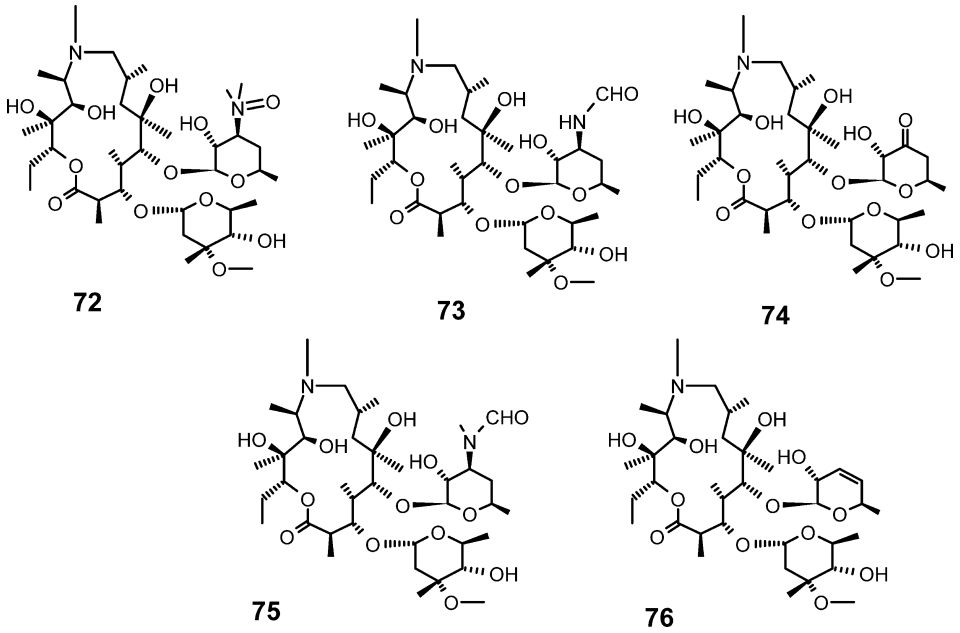


Fig. 17 Structures of 3′-substituted derivatives of azithromycin.

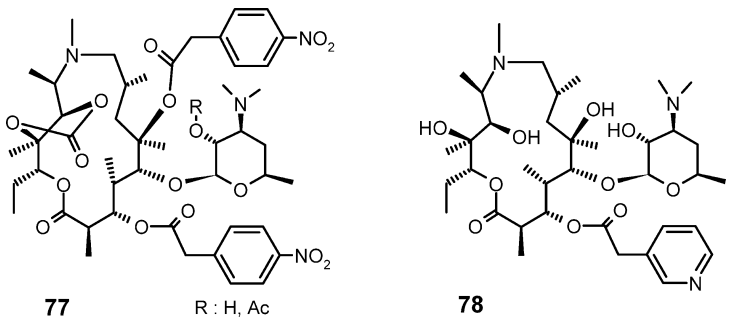


Fig. 18 Structures of various 3-acyl substituted 3-descladinosyl-azalides.

heteroaryl substituted 3,6-ketals of 15-membered azalide analogues **84** (Fig. 20) with *in vitro* antibacterial activity against veterinary pathogens, including *Staphylococcus aureus* and *Pasteurella multocida*.

3-Descladinosyl-3-acyl- derivatives of 9a,11-azalides prepared by ENANTA [73] have shown some antibacterial activity against resistant strains.

At PLIVA, chemists have prepared new descladinosyl compounds **86** with 9a-carbamoyl and thio-carbamoyl groups [88], which were acylated at position 3 to rich antibacterial activity (Fig. 21).

Other descladinosyl derivatives prepared in PLIVA were 9a-sulfonylureas **87** and 9a-propylamino-sulfonyl-ureas **88** [48, 49].

Scientists at ZAMBON Group s.p.a. have prepared new descladinosyl-azalides **89**, **90** (Fig. 22) macrolides with anti-inflammatory activity [89].

Macrolides and especially azalides possess some anti-inflammatory effects [15～18], and PLIVA chemists have prepared new active compounds (Fig. 23) with a steroid linked at positions 2′- and 3- of 3-descladinosyl-azithromycin [43]. Another group of azalide conjugates were compounds **91** and **92**, comprising binding of the aglycone to a steroid moiety [40, 42, 69]. Both sugars were removed probably to avoid antibacterial activity.

In the same group of patent applications, claims were also made for steroid ester-bonded conjugates at position 2′- and position 3- of descladinosyl-azithromycin **93** [42, 43].

2.9.2. Ketoazalides

Early reports on the 3-keto macrolides, narbomycin [90]

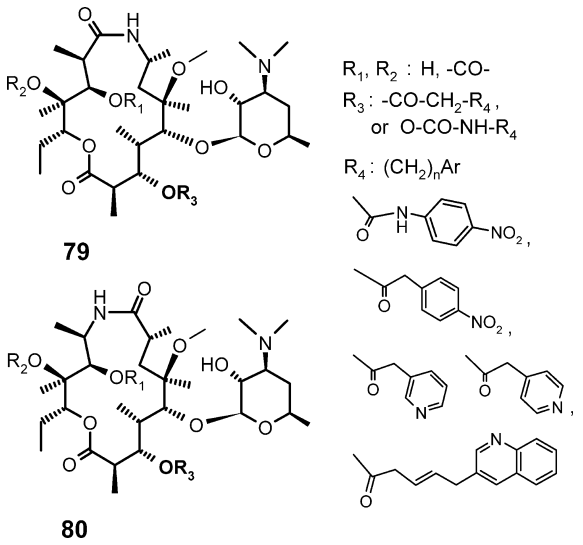
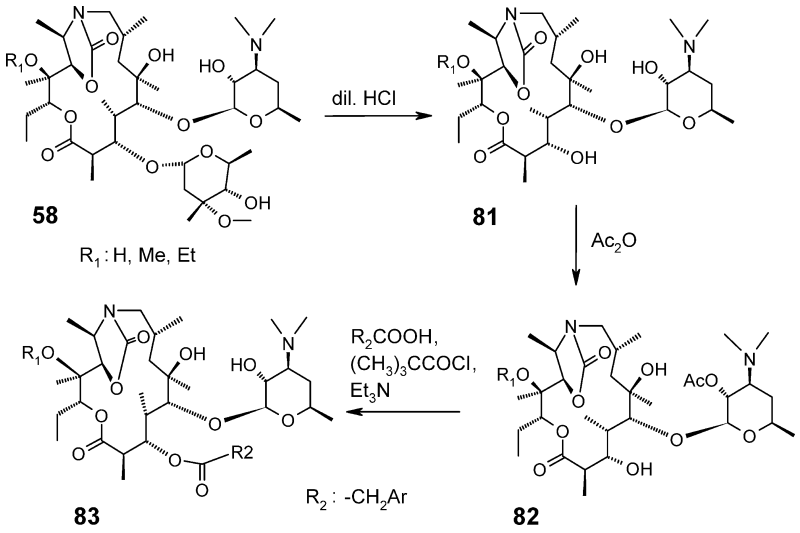


Fig. 19 Structures of various acyl substituted 3-descladinosyl 8a- and 9a-lactams.



Scheme 15 Synthesis of 3-acyl-9-dihydro-9a-aza-9a-homoerythromycin-9a,11-cyclic carbamates.

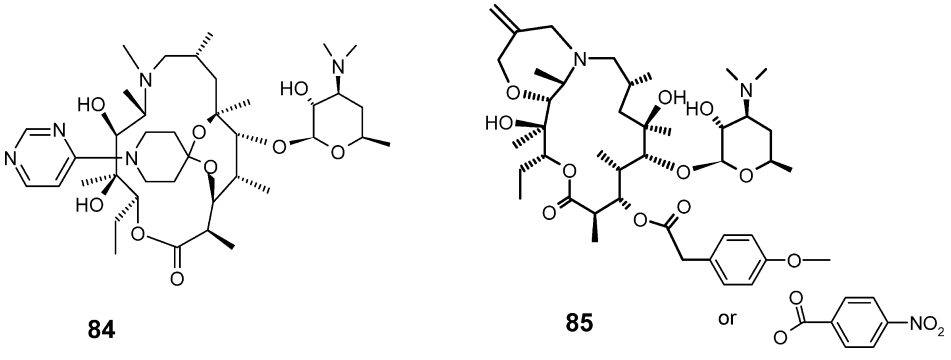
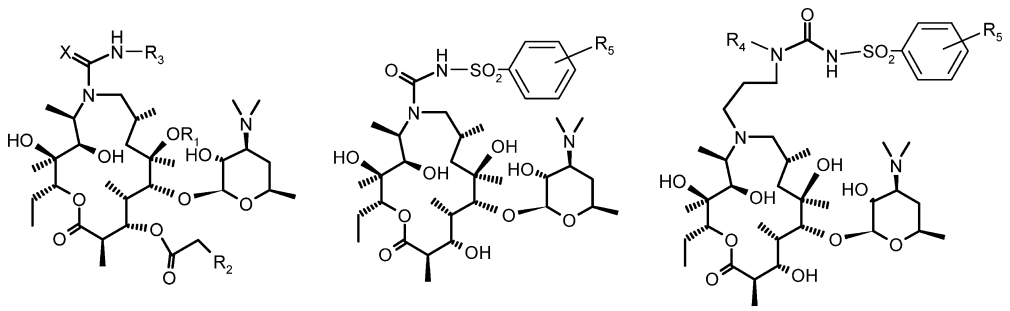


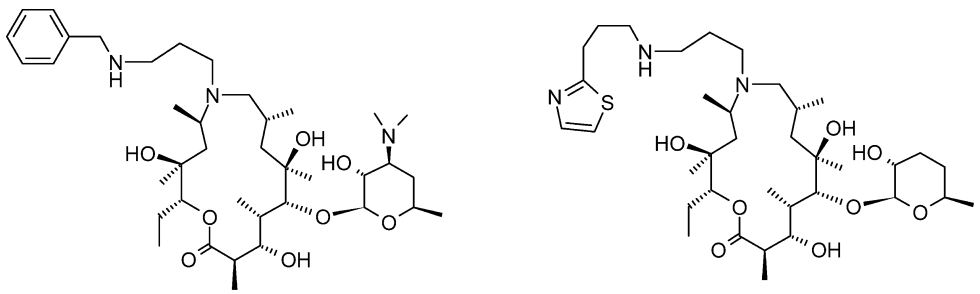
Fig. 20 Structures of 9a,11- and 3,6-bridged-3-descladinosyl-azalide derivatives.



86 **87** **88**

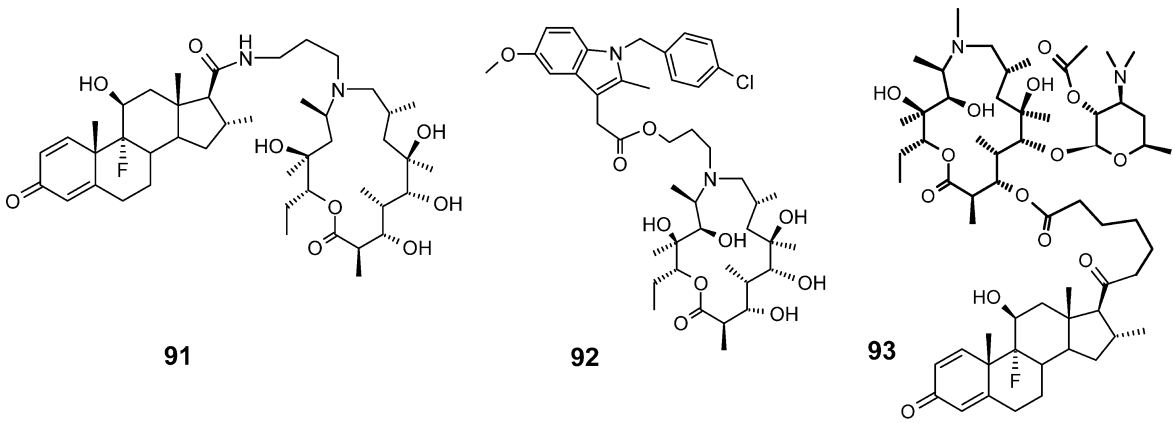
X : O, S
R_1 : H,-Me
R_2 : arylalkyl or arylalkenyl
R_3 : alkyl or Substituted aryl
R_4 : H, alkyl
R_5 : H, *p*-Me, *o*-Me, *p*-Cl, *o*-Cl, *p*-F

Fig. 21 Structures of 3-descladinosyl-*N*-carbamoyl derivatives of 9-deoxo-9-dihydro-9a-aza-homoerythromycin.



89 **90**

Fig. 22 Structures of different 9a-methylenamino-substituted 3-descladinosyl azalides.



91 **92** **93**

Fig. 23 Structures of complex 9a,*N*-substituted-aglycone and 3-substituted descladinosyl azalides.

and picromycin [91], which have only weak intrinsic antibacterial properties, indicated the absence of any potential to induce antibacterial resistance, in contrast to other macrolides [92]. This observation was the rationale for transformation of 3-descladinosyl-erythromycin-oxime derivatives to 3-oxo compounds [93]. However, the reaction resulted in the formation of 3,6-hemiketals, if the hydroxyl group at position 6 was not protected *e.g.* by prior methylation. A comparable hemiketal formation is observed if azalides are used [94].

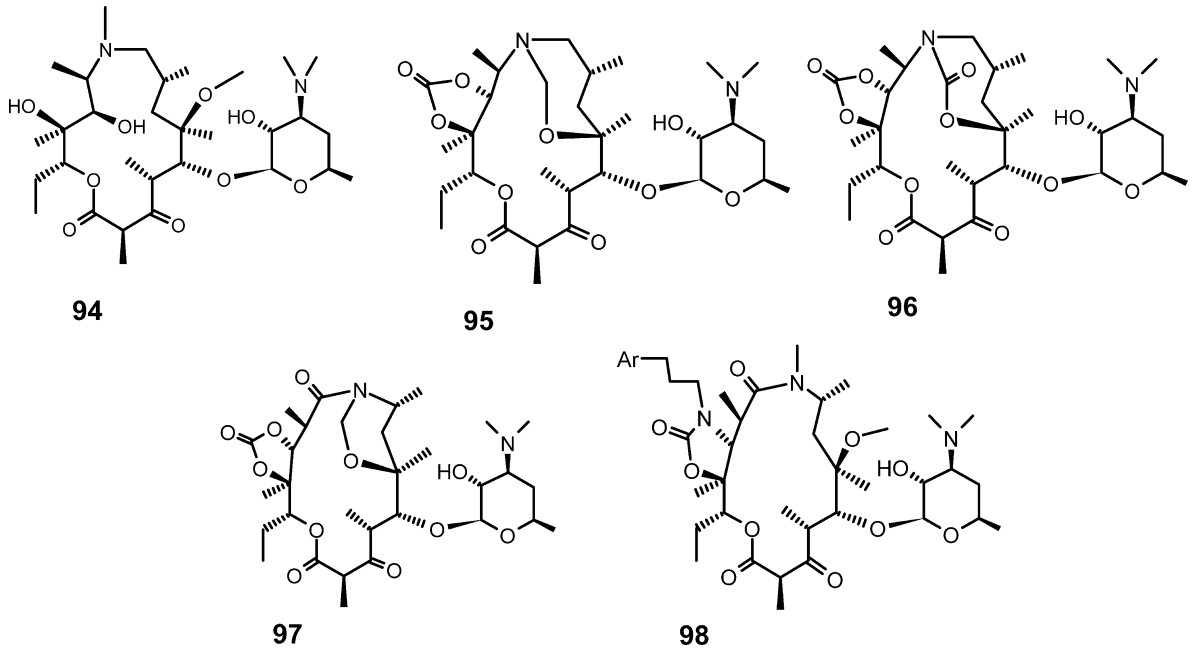


Fig. 24 Structures of azaketolides of 9a-aza-9-dihydro-homoerythromycin-11,12-carbonate-6,9a-methylene, bridged carbonyl derivative or 11,12-carbamate.

Recently, 3-decladinosyl-3-oxo-6-*O*-alkyl-erythromycin derivatives were reported to show significantly enhanced activity against resistant bacteria. Preparation of 3-oxo azalides (amines and lactams) requires protection of the 6-hydroxyl group, since otherwise inactive 3,6-hemiketals are formed. Starting from 6-*O*-methyl-azithromycin, however, the corresponding 3-keto-azalide was synthesized. 3-Keto-8a-and 9a-aza-lactams showed some limited antibacterial activity that warrants further derivatization.

3-Keto-9a-azalide was prepared, starting from 6-*O*-methyl-erythromycin-9(*E*)-oxime, which was converted to the 6-*O*-methyl derivative of 9,11-imino-ether (**5**) or, under aqueous conditions, to the corresponding lactam, **34** [59]. The imino-ether **6** was transformed to 6-*O*-methyl-azithromycin **31**, which after hydrolysis of cladinose, subsequent protection, oxidation and deprotection, yielded the ketoazalide **94**.

The antibacterial activity of azalides compared with azithromycin and the ketolide HMR-3647 (Ketek, telithromycin) [95] was decreased, while **94** was significantly less active.

94 was mentioned in a patent applied for by Merck [71], in which the inventors described the synthesis of various 9-dihydro-9a-aza-homoerythromycins and their 3-keto-derivatives (Fig. 24), such as 3-decladinosyl-3-oxo-9a-aza 9a-*N*, 6-*O*-methylene-9a-homo-erythromycin-11,12-carbonate (**95**) and 3-decladinosyl-3-oxo-9a-aza-9a-homoerythromycin-11,12-carbonate-9a,6-carbamate (**96**). Also various 11,12-cyclic-carbamates were mentioned, but without any antimicrobial data.

In another patent, chemists at Merck have described broad derivatisation of 8a-lactam **11** [72]. They have prepared 8a-methyl or 8a-*N*, 6-*O*-methylene-8a-aza-8a-homoerythromycin-11,12-carbonate (**61**), which was transformed in four steps to its 3-decladinosyl-3-keto derivative (**97**).

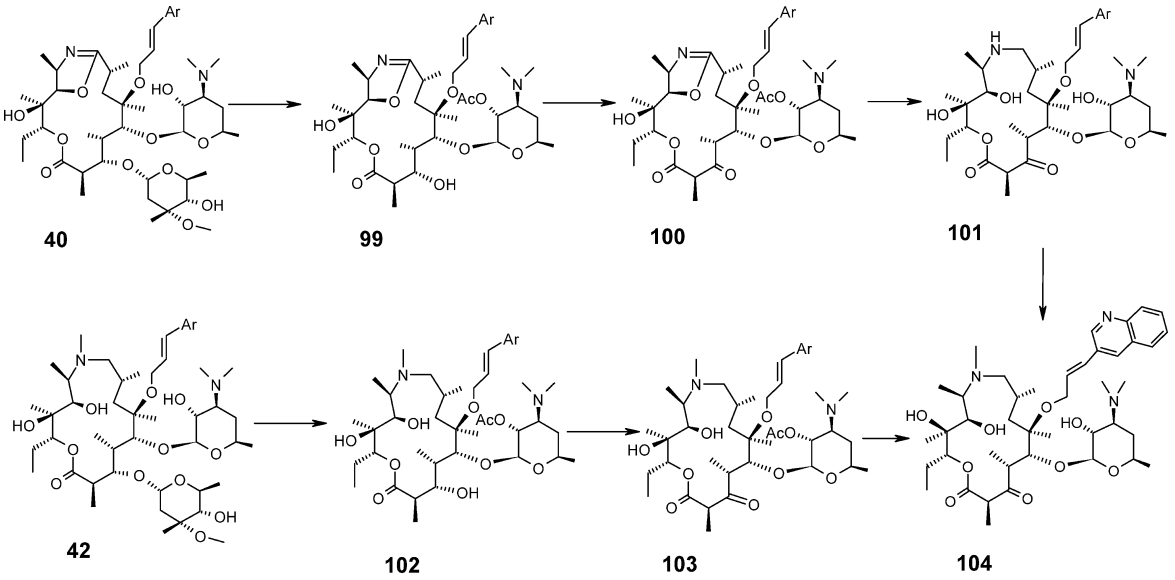
Various combinations of 3-hydroxy-substituted or 3-deoxy compounds have been prepared, but none of them showed antibacterial activity.

In the same patent application [72], various 3-oxo-arylalkyl-substituted 11,12-cyclic carbamates (**98**) were claimed (Fig. 24); aryl groups comprised phenyl-, naphthyl-, 4-quinolyl- and pyridyl-imidazolyl group.

Scientists at Abbott have prepared ketoazalides **104** containing 6-*O*-(3-quinolyl)allyl-azithromycin, with an analogous side chain to that of its ketolide, cethromycin [60, 96]. The cladinose sugar was removed from 2′-acetyl protected 6-*O*-(3-quinolyl)allyl-azithromycin or its 9,11-iminoether intermediate and oxidized (Scheme 16). Iminoether was reduced with sodium borohydride and methylated at position 9a.

Ketoazalide **104** was active against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Nocardia asteroides*.

Starting from 6-*O*-methyl-erythromycin 9(*E*)- or (*Z*)-oxime, PLIVA researchers prepared 8a-lactam **33** and 9a-



Scheme 16 Synthesis of 6-*O*-quinolylallyl-substituted-azaketolides.

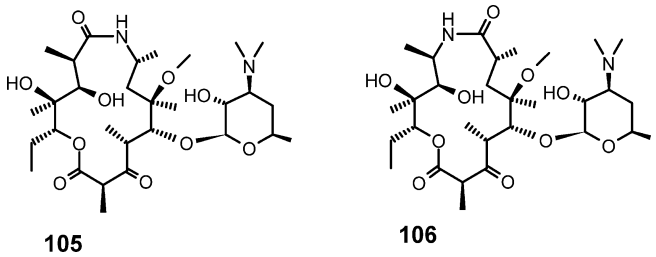


Fig. 25 Structures of 3-oxo-6-*O*-methyl-8a-aza-8a-homoerythromycin (ketolactam) and its 9a-aza-izomer.

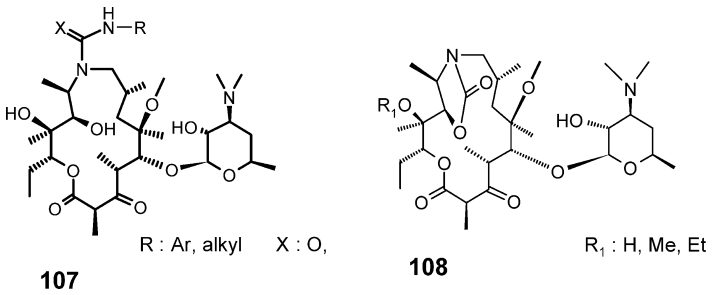
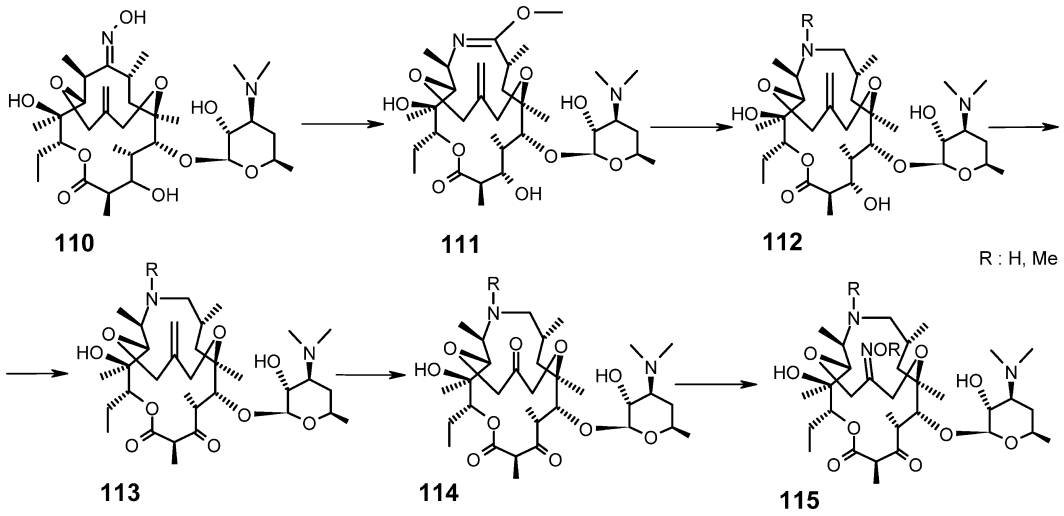


Fig. 26 Structures of 9a-carbamoyl-3-oxo-6-*O*-methyl-9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin- and 3-oxo-9a,11-cyclic carbamates.

lactam **34** by Beckmann rearrangement, which were converted, *via* the 3-descladinosyl derivatives, to the corresponding (Fig. 25) 8a- and 9a-3-ketolactams **105** and **106** [97, 84].

Antibacterial activity of 8a-lactam **34** was similar to that of **8**, while its 9a-isomer **33** was less active. Ketolactam **105** was less active than **5** and more active than **106**, but showed improved activity against inducible resistant *S. aureus*.

Within PLIVA, a group of descladinosyl compounds was prepared with 9a- carbamoyl and thio-carbamoyl 6-*O*-methyl-9a-aza-9-dihydro-9-homoerythromycin [88], which were oxidised on position 3 (**107**). This resulted in a 3,6-hemiketal if the hydroxyl group at position 6 was not protected. Similar results were obtained in the case of



Scheme 17 Synthesis of 3-oxo-6,11-3C-methylene-bridged 9a-aza-9a-homoerythromycin and structure of 3-oxo-6,11-4C-bridged 9a-aza-9a-homoerythromycin.

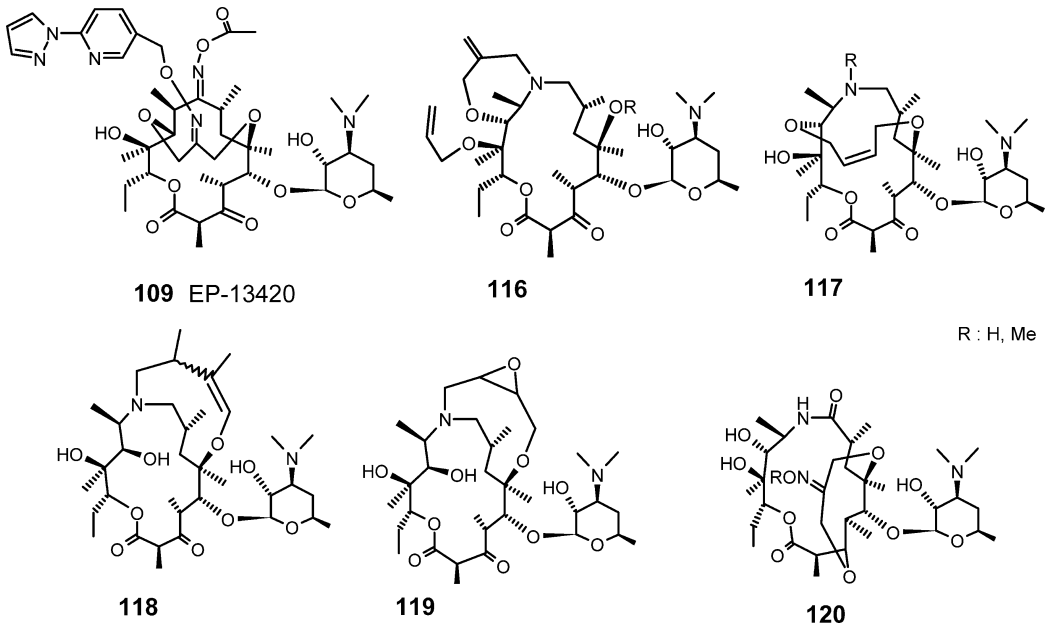


Fig. 27 Structures of 6,11-4C-bridged erythromycin-9-oxyme-ketolide and various 3-oxo-3C- and 4C-bicyclic- 9-dihydro-9a-aza-9a-homoerythromycins.

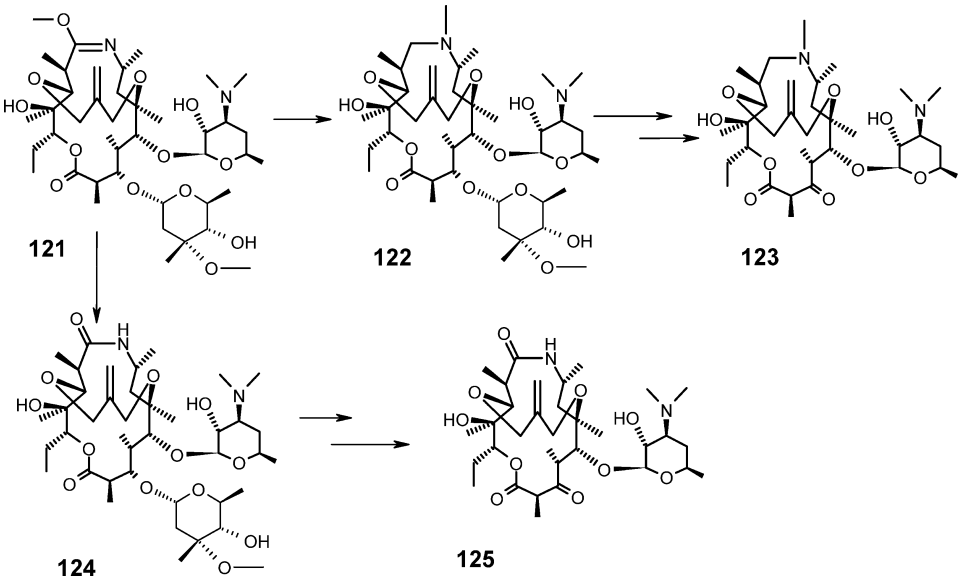
9a,11-cyclic carbamates **108** [85], starting with the 6-*O*-methyl precursor (Fig. 26).

ENANTA Pharm. Inc. has entered the area of macrolide antibiotics with bridged macrolides. The main focus is on ketolides derived from **1**. Major line of this research comprises 6,11-ether bridged erythromycin derivatives. 6,9-Bridged-3-oxo-erythromycin-9-oxyme (**109**) EP-13420 [96] was reported as a candidate antibacterial (Fig. 27).

Starting from bridged erythromycin 9-oxime (**110**), the new 6,11-3C-bicyclic-3-descladinosyl-9a-azalide iminoether (**111**), its 9a-amino **112** and finally ketolide **113** were synthesized [98, 99] (Scheme 17).

Applying the reaction ozonolysis, egzo-methylene group was transformed to keto **114**, which could be further derivatised by amination or oximation to **115**.

New 3-oxo derivatives of -9a,11-3C-bicyclic azalides **116** have been prepared starting from corresponding bicyclic azalide [73]. Other similar new 6,11-4C-bicyclic 9a-azalide derivatives and the 3-oxo analogs **117** have been prepared [100]. The double bond was again transformed to



Scheme 18 Synthesis of 3-oxo-6,11-3C-methylene-bridged 8a-aza-8a-homoerythromycins and corresponding 8a-lactams.

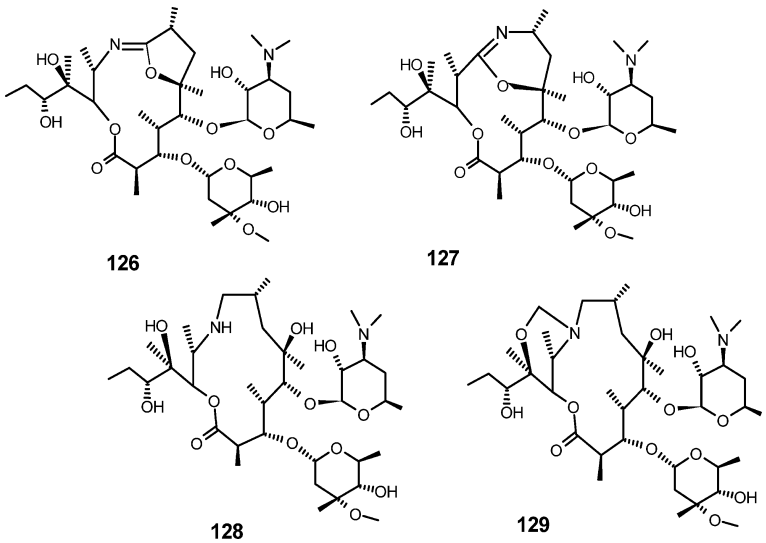


Fig. 28 Structures of 13-membered azalides.

other suitable compounds (Fig. 27).

Later, the same authors [101] have prepared various 3-oxo 6,9a-4C-bicyclic-9-dihydro-9a-aza-9a-homoerythromycins (**118** and **119**). In the patent application 3,6-bycyclic ethers **120** have been protected [102].

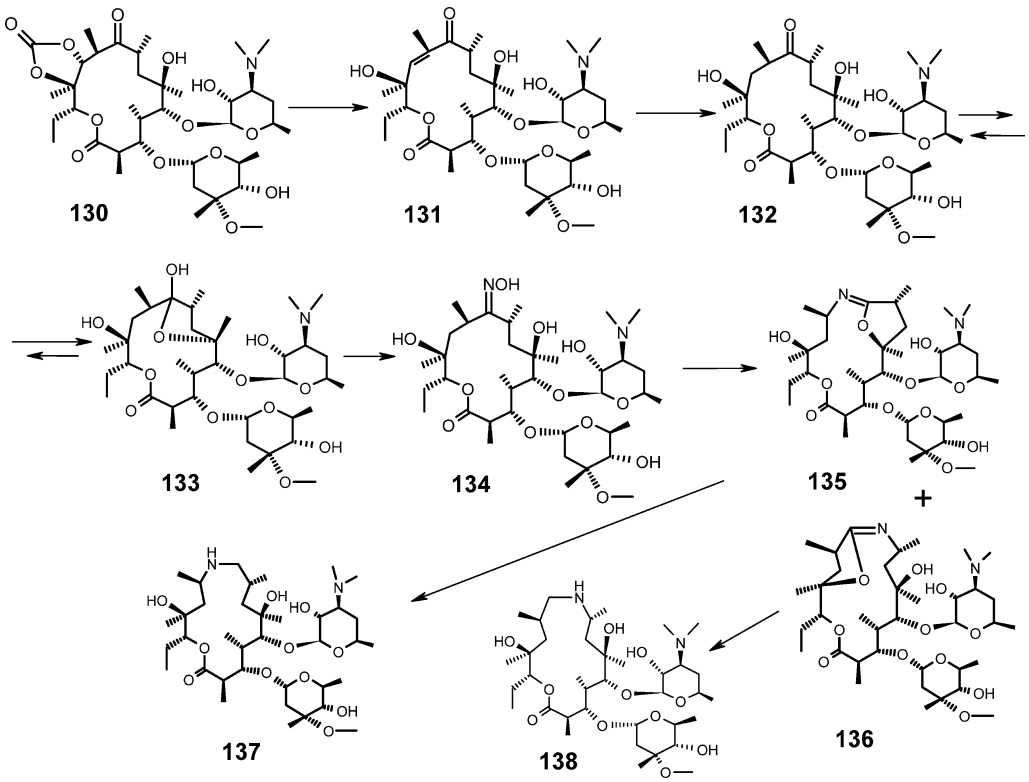
Starting from erythromycin *Z*-oxime, scientists at Merck [103] have prepared the analogous 6,11-bridged oxime, which was transformed into the corresponding 8a-iminoether **121** and further to the 8a-azalide **122**, finally yielding the ketolide **123** (Scheme 18).

6,11-Bridged 8a-lactams **124** and their ketolides **125** were also prepared, but no data about antibacterial were reported.

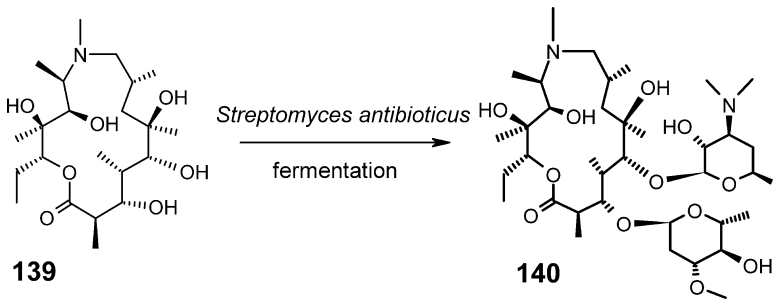
3. Other Azalides

3.1. 13-Membered Azalides

Intramolecular rearrangement of azalide imino-ethers yielded 13-membered azalides, as described by Merck [20]. The trans-annular reactions between the aglycone hydroxyl groups and imino-ether and lactone groups of the 9a- (**3**) and 8a-azalide (**9**) were investigated. Trans-lactonisation of **4** and **9**, which included the 11-hydroxy group, resulted in



Scheme 19 Synthesis of 11-deoxy-azalides.



Scheme 20 Microbial transformation of 3-oleandrosyl-azalide.

13-membered imino-ethers **126** and **127** (Fig. 28).

Reductive methylation of 13-membered azalide **128** (R=H) with formaldehyde and formic acid resulted in the formation of tetrahydro-oxazine **129** (Fig. 28).

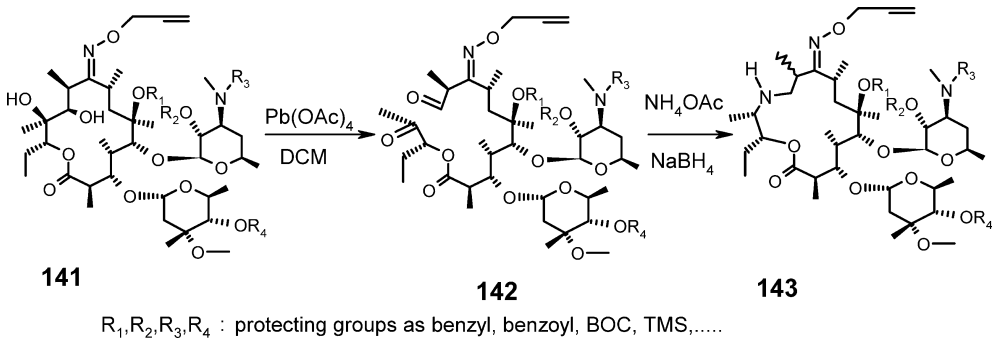
Thermal rearrangement of 9a-aza-6,9-imino-ether **4** produced an isomeric mixture of 9a-aza-9,11-imino-ether **6** and 8a-aza-9,11-imino-ether **10**, which were further transformed to azithromycin and its 8a-methyl isomer. Starting from imino-ether **5**, in the reaction with acetic acid, a dilactone was formed, which was then transformed to 9a-aza-lactam **6** by warming in methanol.

13-Membered azalides **128** were prepared at Pfizer by intramolecular translactonisation of 9-dihydro-9-deoxo-9a-aza-9a-homoerythromycin (**7**). Various azalides were described by derivatisation at positions 13- and 4″ [103]. Later, azalide **128** was mentioned as a potential impurity in azithromycin and prepared by chemists at Sandoz [79].

3.2. Other 15-Membered Azalides

3.2.1. 11-Deoxy-9a-Aza 15-Membered Azalides

Chemists at Abbott have prepared 9a- and 8a-11-deoxy-homoerythromycins **137** and **138** [104]. Starting from erythromycin-11,12-carbonate (**130**), 10,11-anhydro-erythromycin **131** was made at elevated temperature and after reduction of the double bond to **132**, 6,9-hemiketal **133** was formed (Scheme 19). Reaction with hydroxylamine



Scheme 21 Synthesis of 11a-azalides.

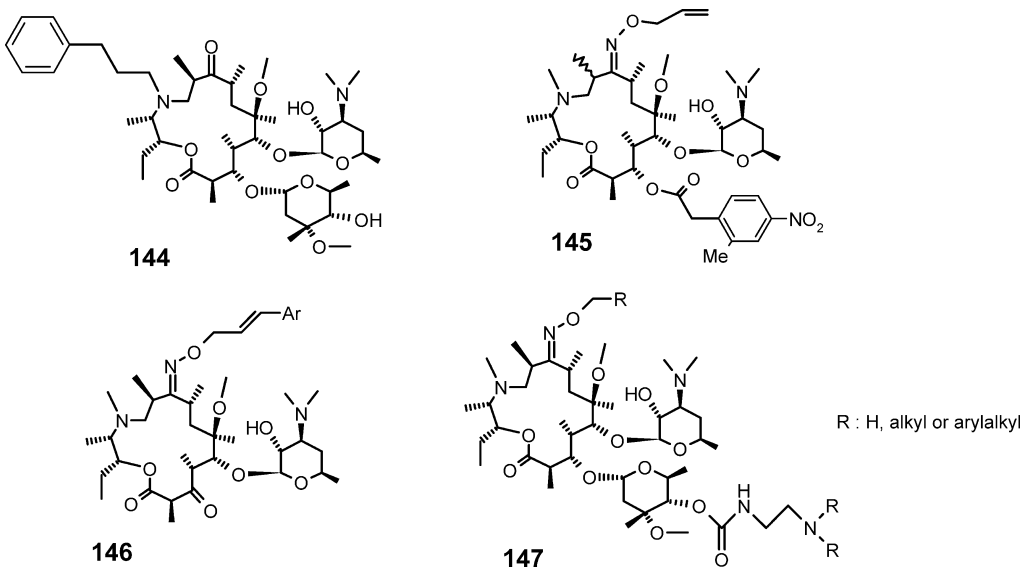


Fig. 29 Examples of various 11a-aza-11a-homoerythromycin derivatives.

resulted in 9-oxime **134**, which in the presence of sulphochloride gave 6,9-iminoether **135** and 9,12-iminoether **136**.

In addition its descladinosyl, 3-ethers, 3-carbonates and 3-carbamoyl derivatives were prepared. Compounds were described as being useful for prophylaxis or treatment of bacterial infections in fish or mammals.

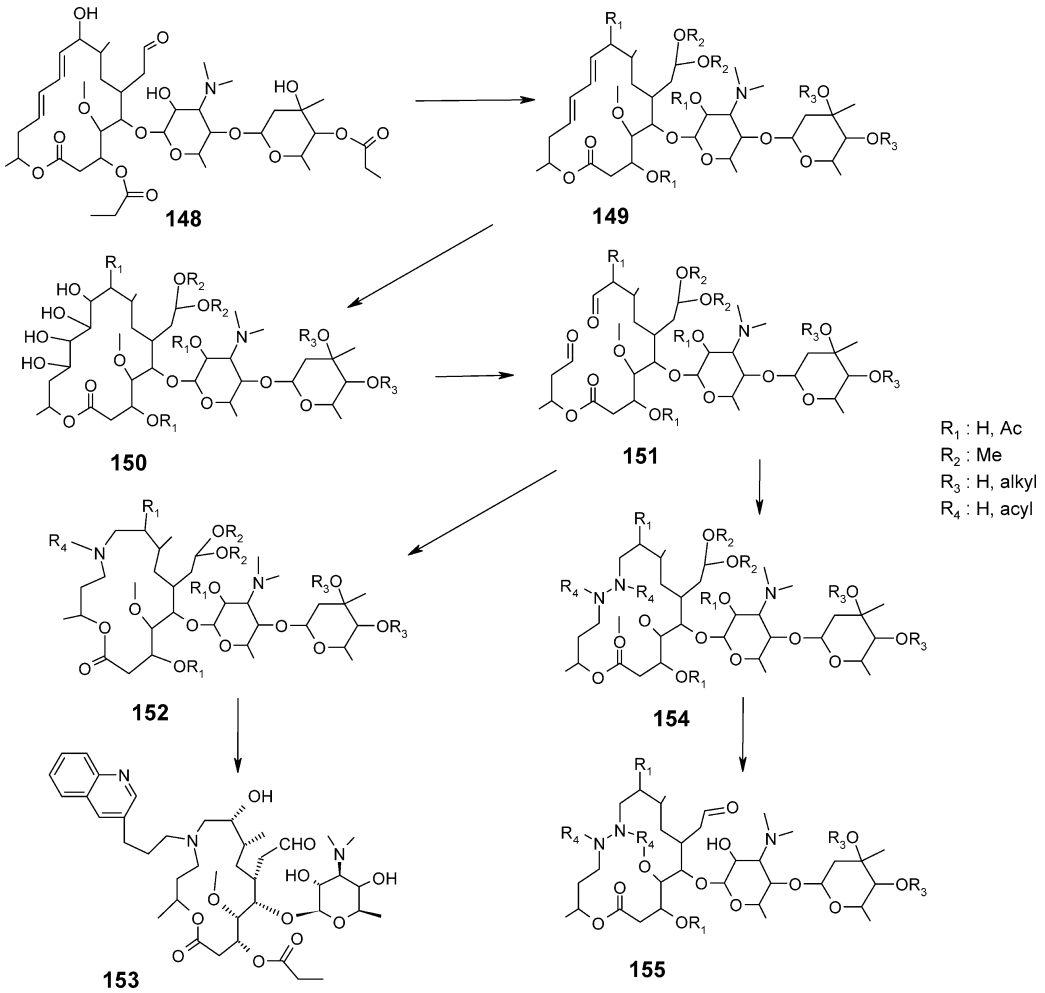
An unusual derivatisation of the azalide scaffold was made at Pfizer. 3-*O*-Oleandrosyl-5-*O*-desosaminyl-azithromycin was prepared from azithromycin aglycone **139** by microbial transformation using culture with *Streptomyces antibioticus* ATCC 202189 (Scheme 20). The oleandrose derivatives **140** are more stable under acidic conditions [105].

New compounds have been prepared as potential anticancer and antibacterial agents.

3.2.2. New 11a-Aza 15-Membered Azalides

In a new patent application, authors at Wockhard Research Centre have synthesized a new class of 15-membered azalide at position 11 of the aglycone ring [106]. They oxidized protected erythromycin A oxime (**141**) with lead tetraacetate to diketone **142**. Reductive amination in ammonia or ammonium acetate with sodium borohydride, sodium cyano-borohydride or with hydrogen in the presence of Pd/C and ammonia, led to ring closure to form 11a-aza-11,12-didehydro-9-oximino-11a-homoerythromycins (**143**). The reaction (Scheme 21) resulted in a mixture of diastereoisomers, which were separated on chiral columns.

In a further step, nitrogen was alkylated with aldehydes under reductive conditions. Further derivatisation resulted in a number of analogs (Fig. 29) similar to the most active macrolides of the second and third generation, such as **144**,



Scheme 22 Transformation of 16-membered macrolides to 15-membered 11a-azalides and 16-membered diazalides.

3-acyl **145**, 3-keto **146** and 4″-carbamoyl derivatives (**147**).

The resulting compounds were reported to be active against common strains of respiratory tract infections including resistant strains, such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, with MIC's from 0.5 to 32 μg/ml.

Another group of 11a-azalides have been prepared by authors at Meiji Seika, starting from 16-membered leucomycin A_3 **148** [107] (Scheme 22), which was protected and further oxidised to dialdehyde **151** and cyclised to 15-membered 11a-azalides **152** and **153** or 16-membered diazalides **154** and **155**.

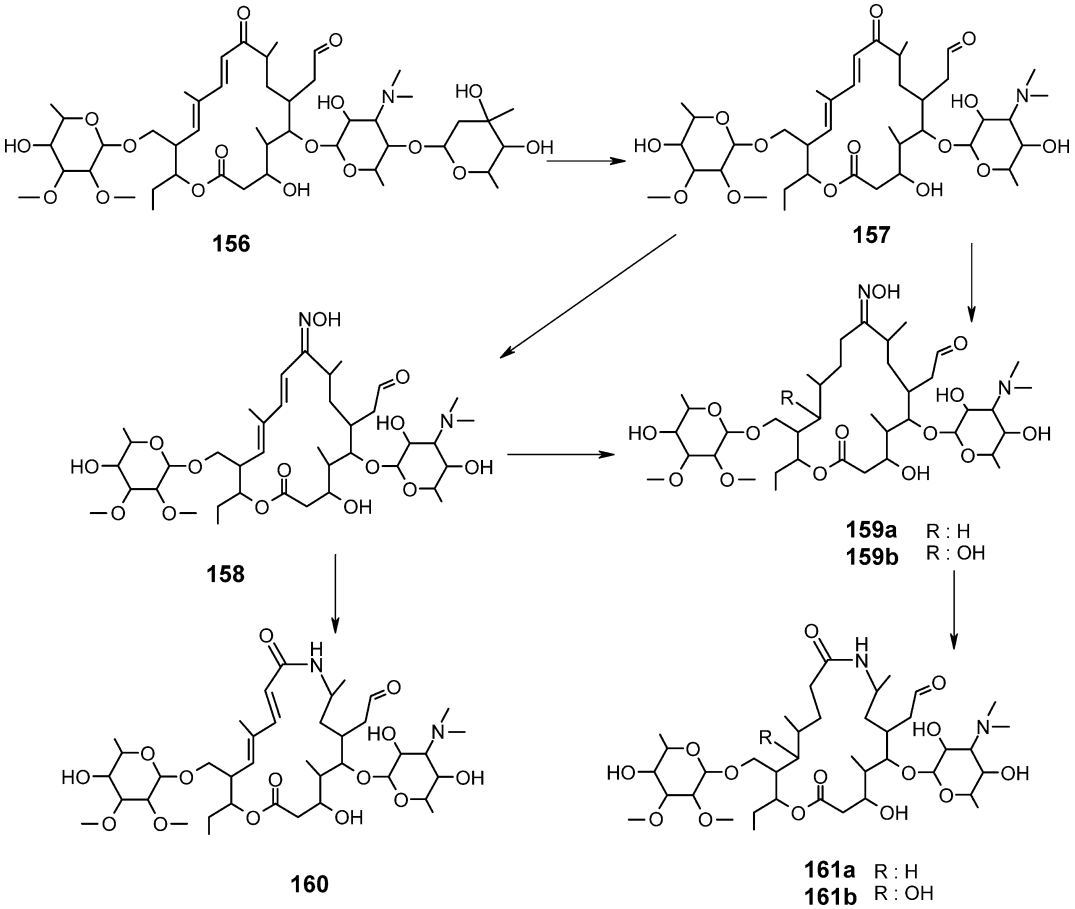
The new synthesized compounds were active against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae*.

3.3. 17-Membered Azalides

Various attempts have been undertaken to adapt the key chemical derivatisation step (Beckmann rearrangement) to other macrolide classes or to modify the azalide skeleton further to enhance its activity.

The Beckmann rearrangement was applied to oximes of the 16-membered macrolides tylosin or desmycosin, and corresponding 17-membered 8a- and 9a-lactams were formed.

The 17-membered ring macrolides have been prepared from natural 16-membered macrolides, another large and important family of macrolide antibiotics. These are traditionally divided into sub-families based upon the substitution patterns of their aglycones [2, 3]. The principal prototypes of this family are represented by leucomycin, spiramycin and tylosin. Tylosin (**156**) is a representative of the 16-membered macrolides and possesses a highly substituted aglycone with two double bonds (tylonolide) and a third saccharide substituent (β-D-mycinose) in addition to the disaccharide attached to the 5-hydroxyl group. Hydrolytic cleavage of mycarose from the disaccharide yielded desmycarosyl-tylosin (desmycosin,



Scheme 23 Synthesis of 8a-aza-homodesmycosins and its di- or tetrahydro derivatives.

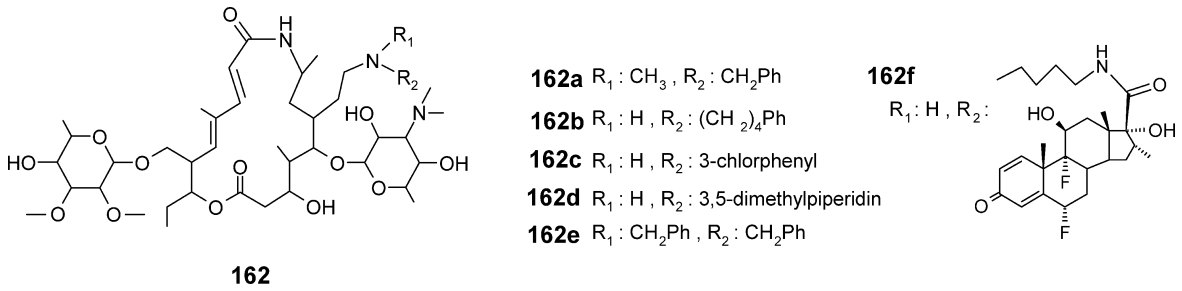


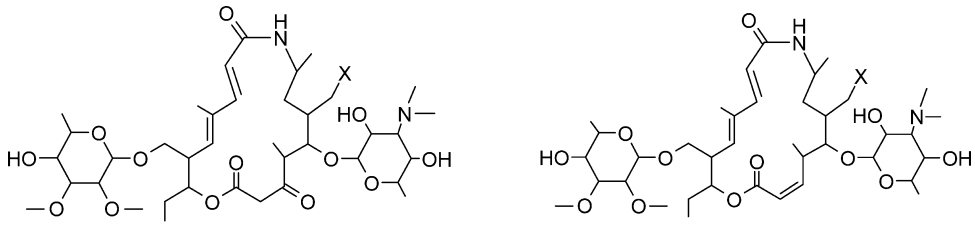
Fig. 30 Structures of 20-deoxo-20-amino-8a-aza-homodesmycosins.

157), which possesses the same antibacterial activity as tylosin.

Other possibilities for tylosin derivatisation comprise transformations of double bonds by catalytic reduction or epoxidation. Researchers at PLIVA have prepared many polyhydro-derivatives of tylosin and desmycosin [108], among them 4′-deoxy-10,11,12,13-tetrahydro-desmycosin, which showed better antibacterial activity than tylosin [109]. Oximes of tylosin, especially desmycosin (**158**) and their polyhydro-derivatives (**159**) were prepared [110]. Most of the chemical transformations were made on the desmycosin scaffold (Scheme 23), but without achieving significant improvement in biological activities.

Beckmann rearrangement of these compounds resulted in the corresponding 8a-aza-8a-homodesmycosins (**160**, **161a**) with small amounts of 9a-aza isomers.

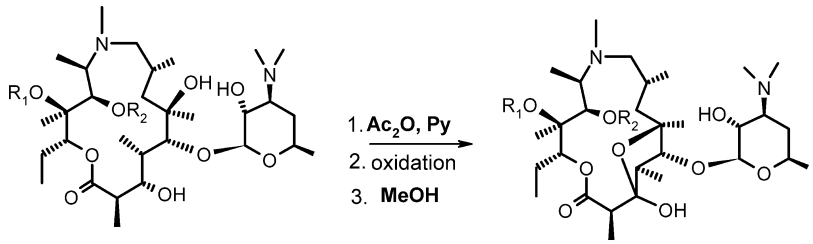
In an attempt to prepare 17-membered cyclic amines instead of lactams, 12,13-dihydro-13-hydroxy-desmycosin-



163a X : CH=O
163b X : $CH_2N(CH_2Ph)_2$

164a X : CH=O
164b X : $CH_2N(CH_2\text{-}Ph)_2$

Fig. 31 Structures of 3-oxo- and 2,3-anhydro-20-dibenzylamino-8a-aza-homodesmycosins.



165 **166**

Scheme 24 Oxidation of descladinosyl-azithromycin derivatives and formation of 3,6-hemiketals.

oxime and 10,11,12,13-tetrahydro-13-hydroxy-desmycosin-oxime (**159b**) have been synthesized [111]. Beckmann rearrangement did not result in the desired imino-ethers, but in 12,13-dihydro-13-hydroxy-8a-aza-8a-homodesmycosin, 10,11,12,13-tetrahydro-13-hydroxy-8a-aza-8a-homodesmycosin (**161b**) and its 9a-aza-9a-homo regioisomer [112].

Most 17-membered lactams showed low antibacterial activity. To improve activity, a series of 20-deoxo-20-amino derivatives of 8a-aza-8a-homodesmycosin (**161**) were prepared by reductive amination of the C-20 aldehyde (Fig. 30).

Only a few newer compounds (**162a**∼**e**) showed slight improvement in activity compared to other 20-deoxo-20-amino derivatives.

The activity of the 17-membered azalide, 4′-demycarosyl-20-deoxo-20-(*N*,*N*-dibenzylamino)-8a-aza-8a-homotylosin (**162e**), was examined *in vitro* for effects on the proliferation of five different human cell lines [113]. At a concentration of 10^{-4} M, these azalides completely inhibited the growth of all cell lines examined and induced morphological changes such as cell shrinkage, condensation and DNA fragmentation. This is typically observed in cells undergoing apoptosis.

Subsequently, new 20-aminosubstituted compounds, such as **162f**, were prepared as potential antinflammatory agents [42, 69].

In further synthetic work, 3-oxo-derivatives (**163**) and 2,3-anhydro-derivatives (**164**) of 8a-aza-8a-homodesmycosin or its 20-dibenzylamino analogues (Fig. 31) were synthesized [114] as compounds with antibacterial activity.

4. Intramolecular Rearrangements of Azalides

4.1. Hemiketals, Spiroketals and Iminoethers

Erythromycin has good efficacy, but low oral bioavailability and gastrointestinal side effects. As mentioned earlier, acidic conditions result in the formation of the inactive degradation products, 8,6-anhydroerytromycin-6,9-hemiketal and 6,9;9,12-spiroketal, by intramolecular rearrangement of hydroxy and keto groups.

In a first attempt to prepare a ketolide from protected erythromycin 9-oxime [115], a hemiketal was formed. After oxidation of its 3-decladinosyl derivative, the free 6-hydroxy group reacted with the 3-oxo group. 14-Membered erythromycin 3,6-hemiketals didn't show antibacterial activity [93].

Attempts to prepare ketoazalides by oxidation of 3-decladinosyl-3-hydroxy-azithromycin, 3-decladinosyl-

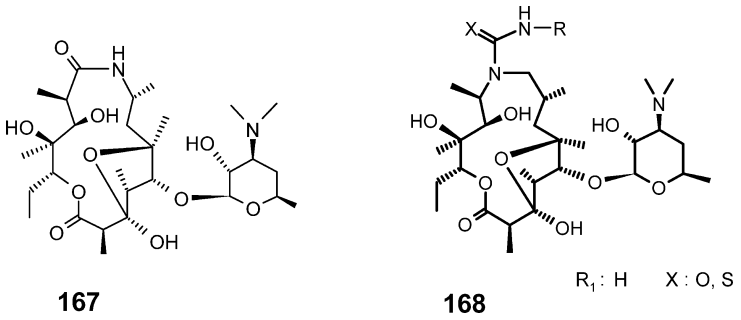


Fig. 32 Structures of 8a-lactam and 9a-carbamoyl-azalide 3,6-hemiketals.

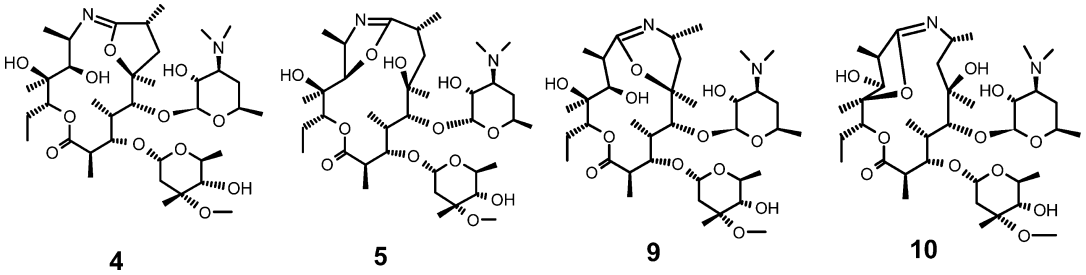
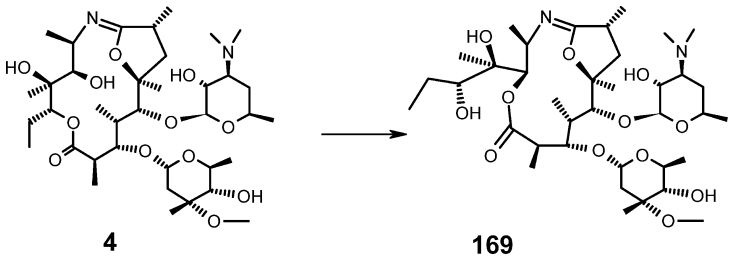


Fig. 33 Structures of azalide iminoethers.



Scheme 25 Base induced translactonisation of 9a-aza-9a-homoerythromycin-6,9-imino-ethers.

3-hydroxy-8a-lactam or 3-decladinosyl-3-hydroxy-azithromycin-9a,11-cyclic carbamate with 3-oxo groups resulted in the immediate formation of hemiketals with a 6-hydroxy group [85, 94].

Azithromycin (**8**) and its 11- or 12-*O*-methyl derivatives (**30b** or **30a**) yielded the corresponding 3-decladinosyl derivatives **165** by acid hydrolysis, which were protected by acylation of the 2′-hydroxyl group, oxidized and deprotected (Scheme 24) to 3-descladinosyl-3-oxo-9a-methyl-9-dihydro-9a-aza-9a-homoerythromycin-3,6-hemiketals (**166**).

Similar 3,6-hemiketals (Fig. 32) were formed after oxidation of 3-descladinosyl-8a lactam (**167**) and 9a-carbamoyl- or thiocarbamoyl-9a-aza-9-dihydro-9a-homoerythromycins (**168**) [88].

New azalide analogs formed by ring reorganization include the group of azalide precursors, such as the iminoethers (Fig. 33) mentioned above.

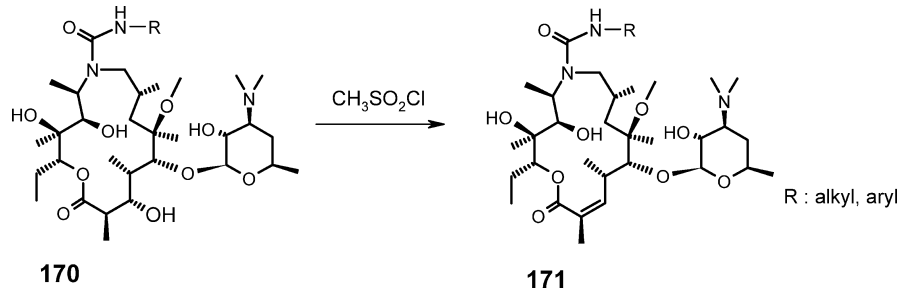
These compounds have no antibacterial activity.

Researchers at Merck studied transanular rearrangements of azalide iminoethers and obtained 13-membered iminoether (**169**) by translactonisation of 6,9-iminoether **4** with lithium hydroxide in ethanol [20, 21] (Scheme 25).

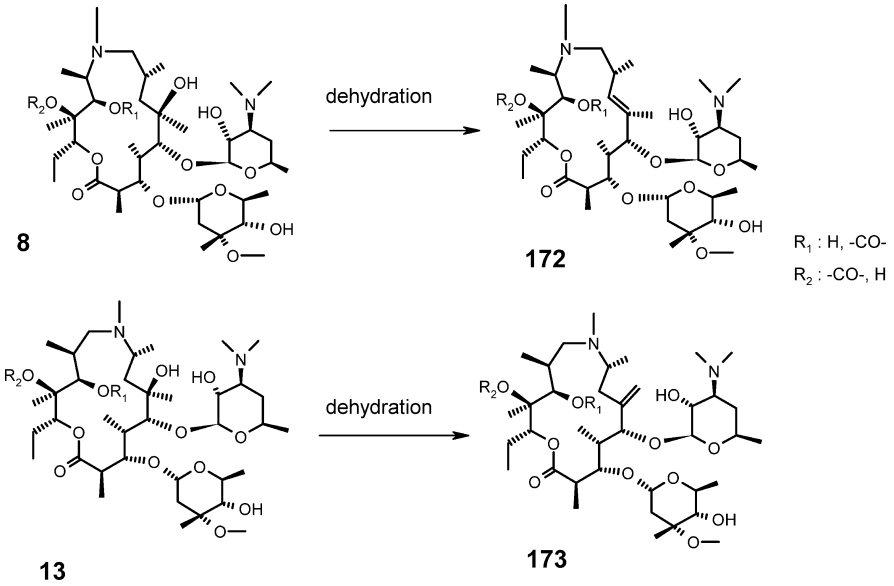
4.2. Anhydrolides

3-Descladinosyl-9a-carbamoyl-6-*O*-methyl-2,3-anhydro-9a-aza-9-dihydro-9-homoerythromycins **171** have been obtained, when 3-descladinosyl-9a-carbamoyl-6-*O*-methyl-9a-aza-9-dihydro-9-homoerythromycins **170** [88] were treated with mesyl-chloride (Scheme 26).

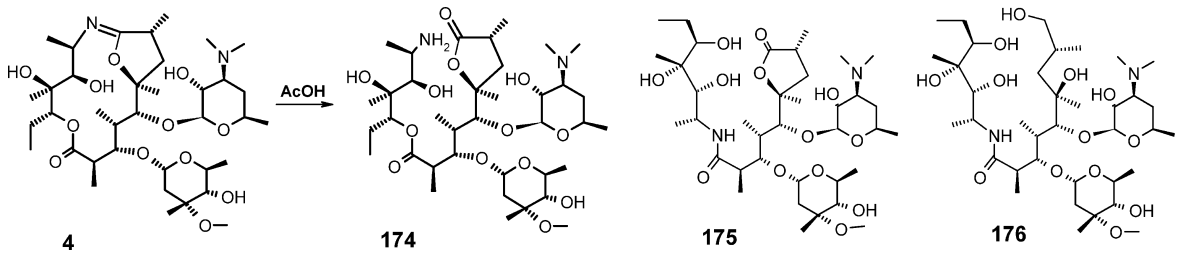
In the case of 3-descladinosyl-9a,11-cyclic carbamates, leaving the hydroxyl group at position 6 unprotected in the



Scheme 26 Synthesis of 3-descladinosyl-9a-carbamoyl-2,3-anhydro-6-*O*-methyl-9a-aza-9-dihydro-9a-homoerythromycin.



Scheme 27 Dehydration of isomeric 15-membered 9a- and 8a-azalides.



Scheme 28 Acid induced degradation of 9a-aza-9a-homoerythromycin-6,9-imino-ethers and formation of secomacrolides and secoazalides.

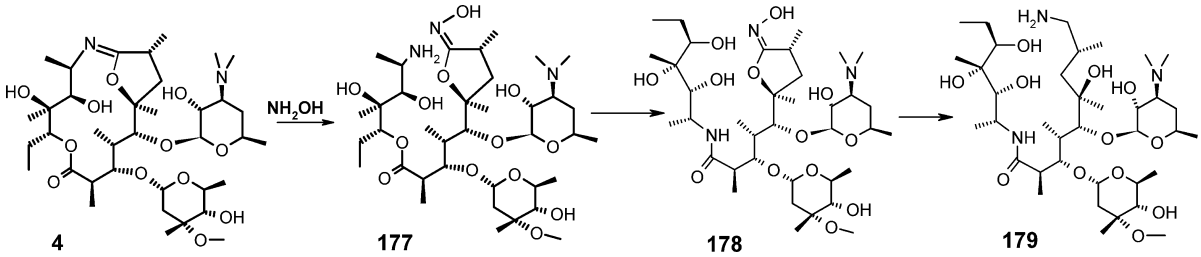
reaction with mesyl-chloride resulted in the 3,6-ether derivative [85].

Selectivity in the dehydration of 15-membered azalides was found between 8a- and 9a-isomers (Scheme 27). It shows opposing regioselectivity [116]. Azithromycin (**8**) favours the *E*-$\Delta_{6,7}$-isomer **172** while 8a-isomer **13** generates the -$\Delta_{6,18}$-isomer **173**.

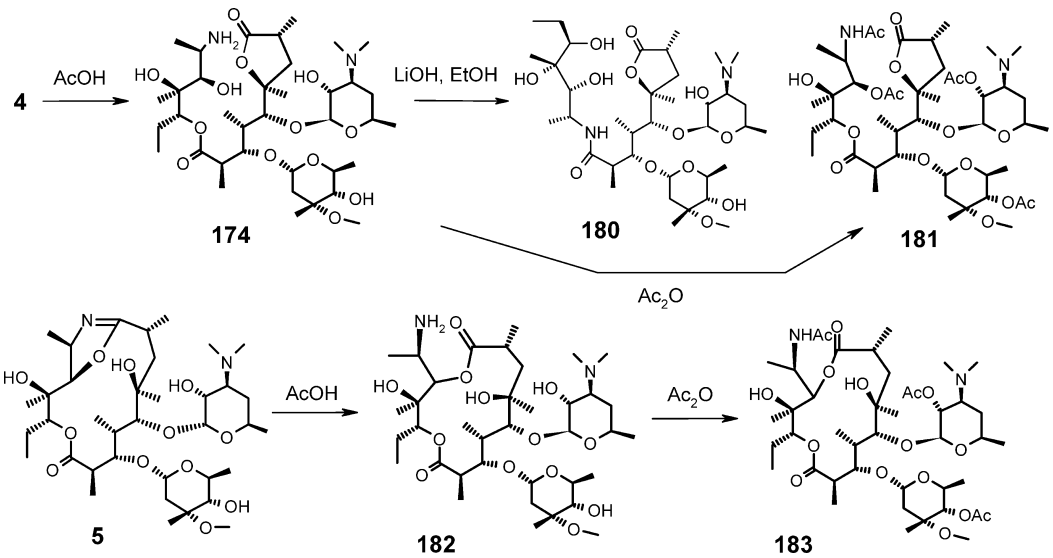
4.3. Open Chain Analogs

Intramolecular rearrangements by breaking the aglycone ring of some 15-membered azalides yielded corresponding secoazalides or 13-membered azalides.

In recent years, several reports have evidenced a growing interest in ring opening reactions of macrolide antibiotics, which has led to the synthesis of chimeric 9a- and 8a-



Scheme 29 Reaction of 9a-aza-9a-homoerythromycin-6,9-imino-ether with hydroxylamine and formation of secomacrolides and secoazalides.



Scheme 30 Reaction of 9a-aza-9a-homoerythromycin-6,9-imino-ether with acetic acid.

azalides [117]. Two different types of base-catalyzed ring-opening reactions of azalides have been reported [118, 119].

Compounds containing carbon–nitrogen double bonds are easily hydrolyzed to the corresponding aldehydes or ketones. The key intermediate in the synthesis of azithromycin 6,9-iminoether **4** has a C-9/9a –C=N (or carbon–nitrogen) double bond, which through acid-catalyzed hydrolysis (AcOH) yielded the amino-lactone **174** [120]. The amino group was acylated or methylated to its corresponding *N*-acetyl or *N*,*N*-dimethylamino derivative (Scheme 28). If the amino group was not protected during this reaction, amine **174** in acetone/water solution reacted by intermolecular transacylation to yield the amide **175**, which was further reduced to its amido-alcohol **176**. Various *O*-acetyl derivatives have been prepared. Compounds **174**~**176** and their acetyl derivatives, named secomacrolides or secoazalides, did not show any antibacterial activity, but are useful as intermediates for novel macrolides or azalides. Another ring-opening reaction was observed if the imino-ether **4** was treated with hydroxylamine hydrochloride [121]. During preparation of the lactam oxime, a simultaneous cleavage of the double bond led to formation of the seco-oxime **177**. The amino-oxime **177** was acetylated to *N*- and *O*-acetyl derivatives, or trans-acylated to seco-amide **178**. Catalytic reduction of the imino-ether group of amide **178** yielded the amine **179**, which was converted to the corresponding *N*,*N*-dimethylamine (Scheme 29).

Compounds **177**~**179** and their acetyl or *N*,*N*-dimethyl derivatives did not show any antibacterial activity.

Similar rearrangements and translactonisation were observed at Merck [20, 21], with the formation of novel dilactones **182** and **183** (Scheme 30).

No data on biological activity were provided.

These groups of compounds have the potential to be used as intermediates for new groups of derivatives, a general conclusion that can be applied to all the compound classes described here, due to the unlimited possibilities resulting from imaginative chemistry.

Acknowledgements Thanks for many suggestions and corrections to Wolfgang Schoenfeld and Gorjana Lazarevski, and for English corrections to Michael Parnham.

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