Synthesis of Antibiotics

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Abstract The synthesis of β-lactams, tetracyclines, and erythromycins as three of the major families of antibiotics will be described herein. We will describe why these antibiotics were the ultimate synthetic targets in the past and how modern synthetic organic chemistry has evolved to address these challenges with new, improved strategies and methods. An additional aspect we would like to highlight here is the fact that these first syntheses had to be particularly creative as most of the modern synthetic methods were not available at that time, or were developed in the course of these syntheses.

Contents

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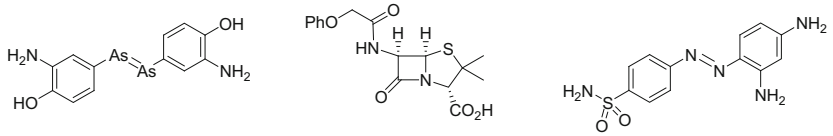
1 Introduction

The history of antibiotics goes hand-in-hand with the synthesis of natural and unnatural substances. The first synthetic antibiotic, arsphenamine (Fig. 1) was prepared in 1907 and used for the treatment of syphilis (Alt 1909). However, arsenic-based drugs had limited use and were toxic to the human hosts as well as to the bacteria. The compounds that truly revolutionized mankind's fight against pathological bacteria were penicillins, discovered in 1928 (Fleming 1929). It took some time though, for the world to realize their importance and meanwhile another class of antibiotics, sulfonamides, was identified and marketed (Domagk 1935). The Second World War brought penicillin and antibiotic research into focus, and during the following two decades a number of different classes of antibiotics had been discovered: Aminoglycosides (Schatz et al. 1944), tetracyclines (Duggar 1948), macrolides (McGuire et al. 1952), glycopeptides (McCormick et al. 1956), quinolones (Lesher et al. 1962) etc.

A comprehensive overview on the synthesis of antibiotics is not in the scope of this chapter and there are a number of reviews written on this subject matter (Lukacs and Ohno 1990; Lukacs 1993; Krohn et al. 1993; Bruggink 2001; Nicolaou et al. 2009). Here, we have chosen to cover some of the more prominent antibiotics and describe the challenges accompanying their synthesis as well as recent advances in that field.

2 Synthesis of β-Lactam Antibiotics

The β-lactams (Fig. 2) constitute the biggest and most important class of antibiotics. Their chemistry and biology has been thoroughly reviewed on many occasions (Morin and Gorman 1982; Page 1992; Georg 1993). They all have the



arsphenamine (**1**) penicillin V (**2**) Prontosil (**3**)

Fig. 1 First antibiotics

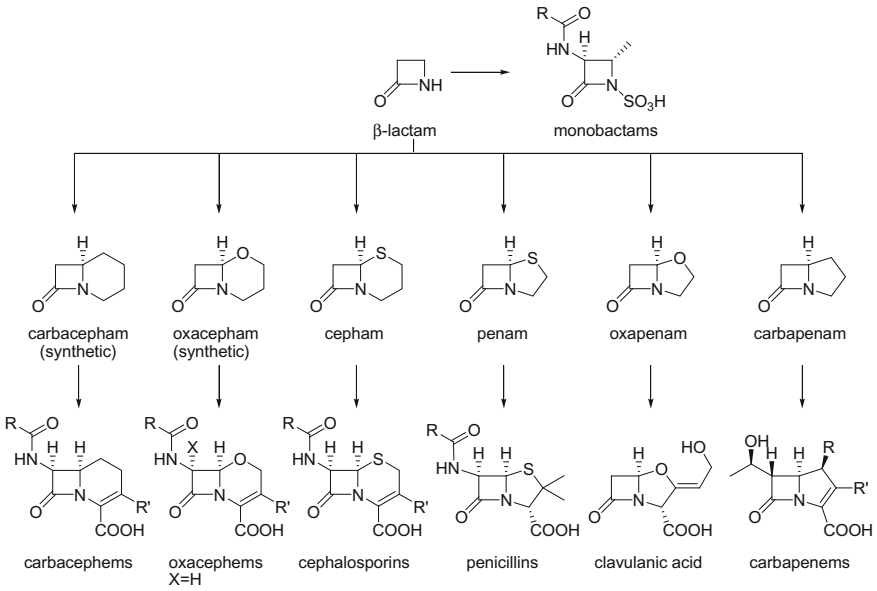


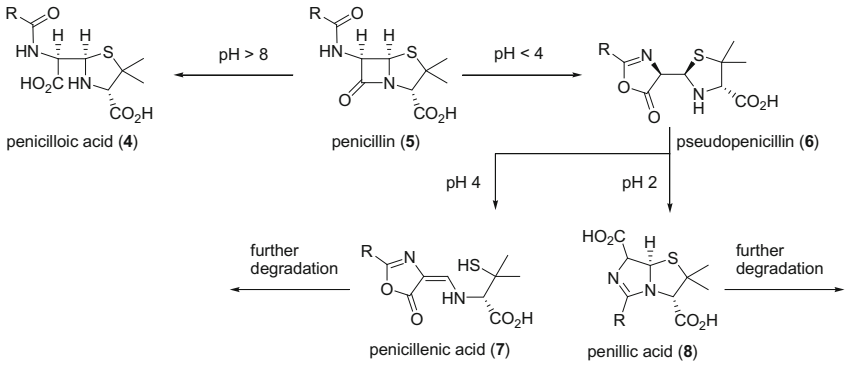
Fig. 2 β-lactam family

strained four-membered β-lactam ring in common which serves as the pharmacophoric group. The individual members of this family differ in their ring size, and substitution pattern as depicted (Fig. 2).

2.1 Synthesis of Penicillin V (Sheehan 1957)

Even though the penicillins were first discovered in the late 1920s (Fleming 1929), their structure remained a topic of controversy for more than 15 years. Finally, in 1945, the X-ray crystal structure of penicillin G was obtained by Dorothy Crowfoot Hodgkin and the existence of the characteristic β-lactam ring was unambiguously proven (Clarke 1949). However, the first total synthesis was not achieved until 1957, when Sheehan et al. completed a decade of work on this topic with the synthesis of the potassium salt of penicillin V (**2**) (Sheehan and Henery-Logan 1957, 1959, 1962; Sheehan 1982).

The most challenging feature of penicillin is its four-membered β-lactam ring fused to a thiazolidine ring. The fused ring system disrupts the conjugation of nitrogen's free electron-pair to the carbonyl group. That, together with high ring-strain makes the amide bond labile to both acidic and basic conditions. Penicillin rapidly opens at the β-lactam ring and degrades, e.g., into pseudopenicillin (**6**) (under acidic conditions), which can further undergo rearrangement to stable inactive compounds (Scheme 1) (Chain 1948; Deshpande et al. 2004). The



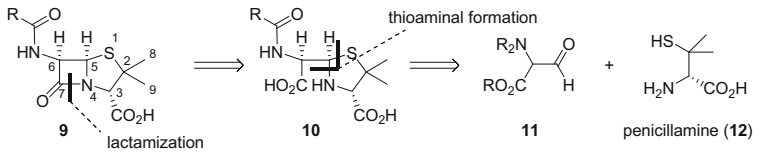
Scheme 1 Decomposition of the β-lactam ring

aforementioned difficulty required devising completely new mild amide coupling conditions to effect the synthesis of penicillin.

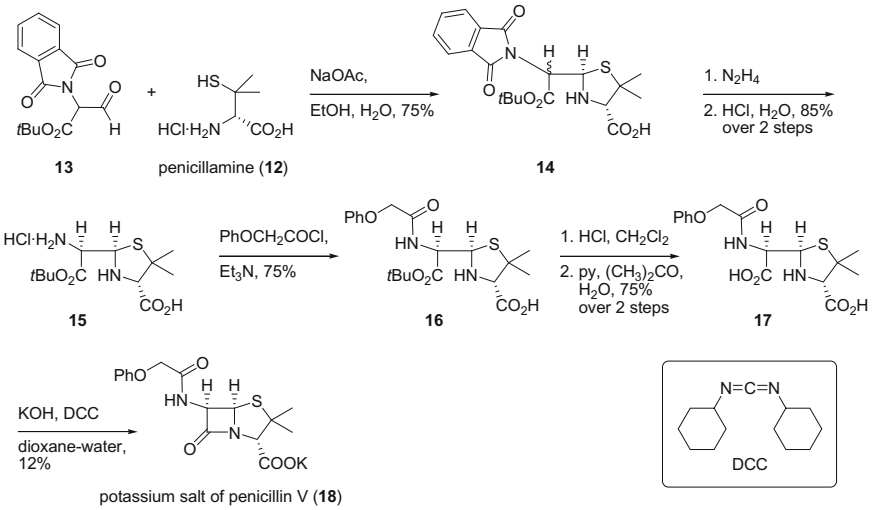
Sheehan's synthesis of penicillin V (**2**) utilized penicillamine **12** and aldehyde **11** as key intermediates (Scheme 2). Penicillamine was synthesized in eight steps from racemic valine and obtained in enantiopure form through crystallization as a brucine salt (Clarke et al. 1949). Aldehyde **11** was prepared in three steps from glycine.

Sheehan's total synthesis of penicillin started with glycine derived aldehyde **13**, where the carboxylic acid moiety was protected as a *tert*-butyl ester and the primary amine was masked as a phthalimide. Thioaminal formation under basic conditions (Sheehan and Johnson 1954) with penicillamine (**12**) was followed by removal of phthalimide protecting group with hydrazine and recrystallization of the hydrochloride salt to give compound **15** in optically pure form (Scheme 3). Next, the acyl side chain was installed to C-6 nitrogen followed by removal of *tert*-butyl protecting group from the acid and crystallization of ammonium salt of acid **17** from aqueous acetone. The crucial lactamization was achieved by employing the mild coupling reagent *N,N′*-dicyclohexylcarbodiimide (DCC) developed by Sheehan a few years earlier for coupling of amino acids under pH neutral conditions (Sheehan and Hess 1955). With that, the first total synthesis of the potassium salt of penicillin V (**2**) was achieved.

Penicillins for pharmaceutical use are nowadays, as they were back in the 1950s, produced by fermentation and semi-synthesis. Nevertheless, the first total synthesis of penicillin V (**2**) serves as a benchmark in β-lactam chemistry and as a starting



Scheme 2 Sheehan's retrosynthesis of penicillin V (**2**)



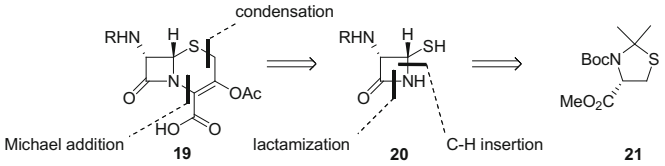
Scheme 3 Sheehan's synthesis of potassium salt of penicillin V (**18**)

point for the synthesis of other β-lactam derivatives. Sheehan utilized for the first time the *tert*-butoxy group as an acid labile protecting group for carboxylic acids and introduced the idea of using phthalimide as a protecting group for primary amines. Nevertheless, the most influential aspect of this synthesis would be the employment of the DCC reagent for coupling of carboxylic acids with amines. This reagent has enabled the solid-phase peptide coupling chemistry and is still in use nowadays—60 years after the initial introduction.

2.2 *Synthesis of Cephalosporin C (Woodward 1966)*

Cephalosporin C was first discovered in 1955 from a species of *Acremonium* (Newton and Abraham 1955), and its structure was determined in 1961 (Loder et al. 1961; Hodgkin and Maslen 1961). It belongs to a class of β-lactams called cephalosporins bearing a close resemblance to the penicillins but differing in the size of the ring connected to the β-lactam. It attracted close interest because of its antibacterial activity, which, though moderate, persisted through different classes of bacteria that had become resistant to penicillins (Chauvette et al. 1962).

The challenges in the cephalosporin C synthesis were somewhat different from the challenges of the synthesis of penicillin. Whereas the latter was usually prepared by a late stage β-lactam formation from parent β-amino acid (e.g., **10**), which was known to be a decomposition product of naturally occurring penicillin; the

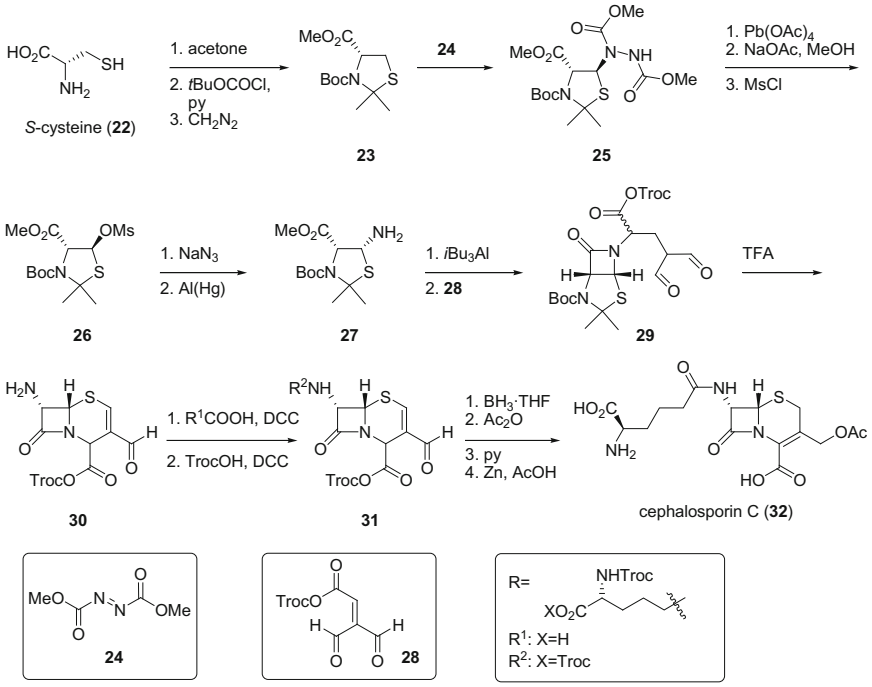


Scheme 4 Woodward's retrosynthesis of cephalosporin C (**32**)

corresponding β-amino acid for the former was not a known compound. Therefore, a different retrosynthetic approach was chosen by Woodward et al., in which they decided to form the β-lactam ring early in the synthesis and then generate the dihydrothiazine ring system at a later stage (Scheme 4) (Woodward et al. 1966; Woodward 1966). The β-lactam system was prepared from naturally occurring *S*-cysteine by stereoselective amination of its β-position.

Woodward's synthesis of cephalosporin C (**32**) (Scheme 5) started from *S*-cysteine (**22**), which was protected at different functionalities by first treating it with acetone, then *tert*-butanol and phosgene in the presence of pyridine, and finally the acid moiety was converted into methyl ester **23** by treatment with diazomethane. The key step of this synthetic strategy followed, which was the stereoselective functionalization of β-position of the parent amino acid. This was achieved by treatment with dimethyl azodicarboxylate (**24)** (DMAD). Supposedly, suphur adds to DMAD, which at the same time deprotonates the carbon next to the sulphur, and rearrangement of the sulfonium ylid leads to the formation of C–N bond. The reaction proceeded in a stereospecific manner, though unfortunately leading to the undesired stereoisomer. Therefore, an inversion of configuration at that centre was required, which was achieved by sequence of oxidation, mesylation, and azidation. The azide was reduced to the amine **27** in the presence of aluminum amalgam and a Lewis acid-induced lactamization gave the β-lactam, which was directly coupled with the Michael acceptor **28** to give **29**. Treating the latter with TFA removed the Boc-protecting group, which induced opening of the thiazolidine ring and liberated the sulphur for the attack of the carbonyl moiety of the side chain. Dehydration gave the cephem core **30**. Compound **31** was obtained after two DCC mediated couplings—the first functionalizing the amine nitrogen and the second protecting the free carboxylic acid moiety in the side chain. Reduction of the carbonyl group was followed by acetylation and double bond isomerization in anhydrous pyridine. Finally, the trichloroethyl (Troc) protecting groups were removed under reductive conditions with Zn in acetic acid giving cephalosporin C (**32**).

With this, the first total synthesis of cephalosporin derivatives from simple starting materials was achieved. The synthesis featured a novel stereoselective method for C–H functionalization of the β-position of the cysteine and one of the first uses of the Troc group as a protecting group for carboxylic acids.



Scheme 5 Woodward's synthesis of cephalosporin C (**32**)

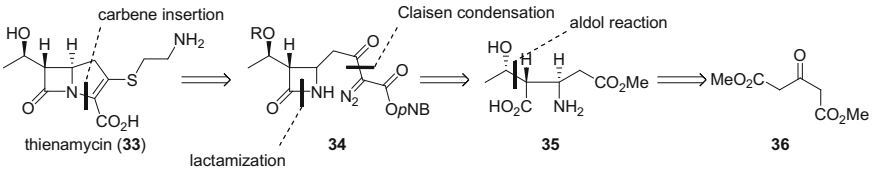
2.3 *The Merck Synthesis of Thienamycin (Mellio 1980, 1986)*

The first member belonging to the class of carbapenems was thienamycin (**33**), which was discovered by scientists at Merck in late 1970s (Albers-Schoenberg et al. 1978). It attracted immediate interest by displaying both—broad-spectrum antibacterial and β-lactamase inhibitory activity. As opposed to penicillins and cephalosporins, biosynthetic production of carbapenems was inefficient and therefore synthetic approach to these structures gained a great importance. A variety of different syntheses have been carried out (Hanessian et al. 1990; Ishibashi et al. 1995; Jacobi et al. 1996; Cabri and Di Fabio 2000).

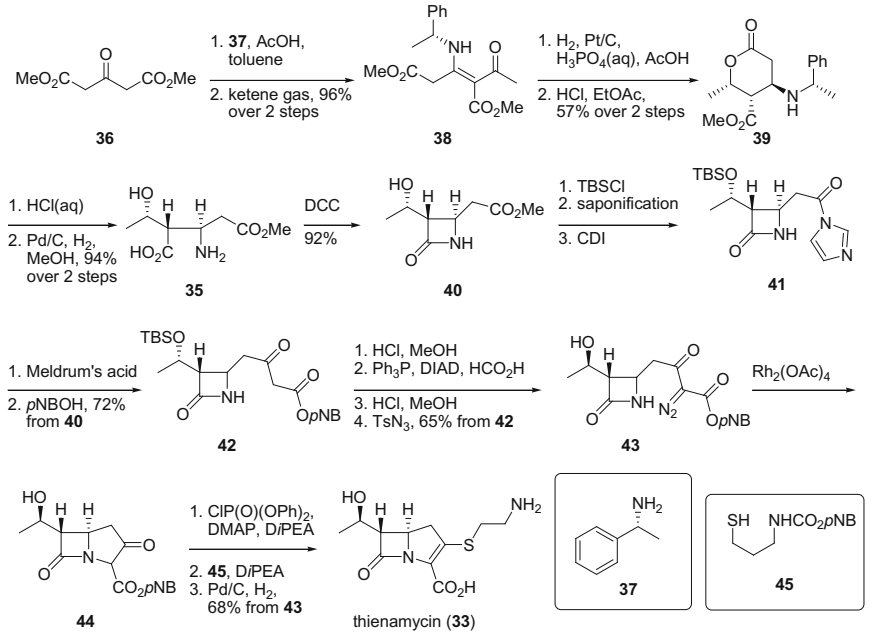
Carbapenems differ from aforementioned penicillins and cephalosporins in several distinct aspects. First of all, the ring connected to the β-lactam does not contain any additional heteroatoms. Secondly, varying appendage in the α-position to the amide group of the β-lactam ring, which plays great roll in the activity of penicillins and cephalosporins, does not significantly affect the activity of carbapenems. And third, the hydrogens attached to the β-lactam ring are *anti* to each other, whereas in the other two classes they have to be *syn* to display desired antibacterial activity.

Researchers from Merck were the first to achieve the total synthesis of thienamycin (**33**) (Johnston et al. 1978) as well as to make it amenable for industrial production (Melillo et al. 1980, 1986). Their optimized retrosynthetic approach (Scheme 6) relied on carbene chemistry for the creation of the pyrroline ring. Asymmetric induction was achieved by chiral auxiliary controlled stereoselective hydrogenation.

The Merck synthesis (Scheme 7) started from cheap and readily available dimethyl acetonediacarboxylate (**36**). Reaction with (*R*)-(+)-α-methylbenzylamine followed by treatment of the formed enamine with ketene gas gave the ketone **38**. Introduction of the chiral amine allowed a stereoselective reduction to take place upon hydrogenation with a platinum catalyst. Treatment with strong acid lead to lactonization and compound **39** could be obtained as optically pure crystals. The lactone opening in aqueous HCl was followed by hydrogenolysis, which exposed the β-amino and acid



Scheme 6 Merck's retrosynthesis of thienamycin (**33**)



Scheme 7 Merck's synthesis of thienamycin (**33**)

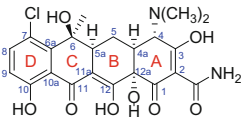
functionalities in **35**. A DCC mediated coupling gave the β-lactam core and a few functional group manipulations provided the activated amide **41**. Treatment with Meldrum's acid followed by opening the acyl-intermediate with *para*-nitrobenzyl alcohol, gave the β-keto ester **42**. The stereoselective hydrogenation of compound **38** had provided a single diastereomer but unfortunately the stereochemistry of the hydroxy group in the appendage adjacent to the carbonyl group of β-lactam was incorrect. Therefore, inversion of that stereocentre had to be undertaken by first deprotecting the compound **42**, and then performing the inversion under Mitsunobu conditions. Regitz diazotransfer yielded the diazo compound **43**. The pyrroline ring was closed through a rhodium catalyzed carbene insertion into the N–H bond giving compound **44**. Ketone **44** was converted to enol phosphate, then treated with N-protected cysteamine and after hydrogenation thienamycin (**33**) was obtained.

3 Synthesis of Tetracycline Antibiotics

Tetracyclines are antibiotics produced by strains of *Streptomyces*. The first compound of that class, chlortetracycline (aureomycin) (**46**), was isolated in 1948 by Duggard but the structure was first elucidated for another tetracycline—terramycin (**46**)—in 1950 by researchers from *Chas. Pfizer & Co.* together with Robert Burns Woodward (Muxfeldt 1962b). They found, that the structure of tetracyclines is based on polyoxygenated hydronaphthacene backbone, which can contain different other functionalities around the periphery (Fig. 3) (Korst et al. 1968). The unique structure of the tetracyclines in combination with their remarkable antibiotic activity attracted interest of several research groups. The synthetic challenges of preparing these compounds though were considerable—even Woodward stated in 1952 that it would hardly ever be possible to synthesize a molecule of such complexity (Der Spiegel 1968).

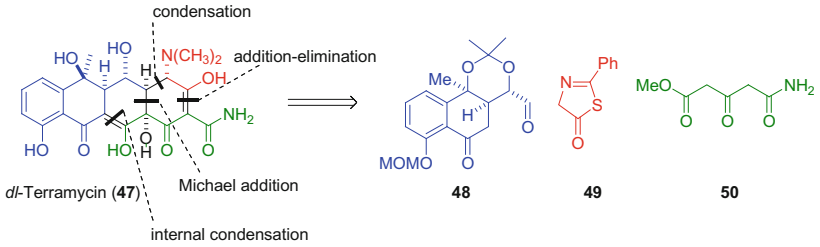
3.1 Racemic Total Synthesis of Terramycin (Muxfeldt 1968)

Muxfeldt et al. were the first to report the total synthesis of racemic terramycin in 1968. Retrosynthetically, they divided terramycin into three basic building blocks



chlorotetracycline (**46**)

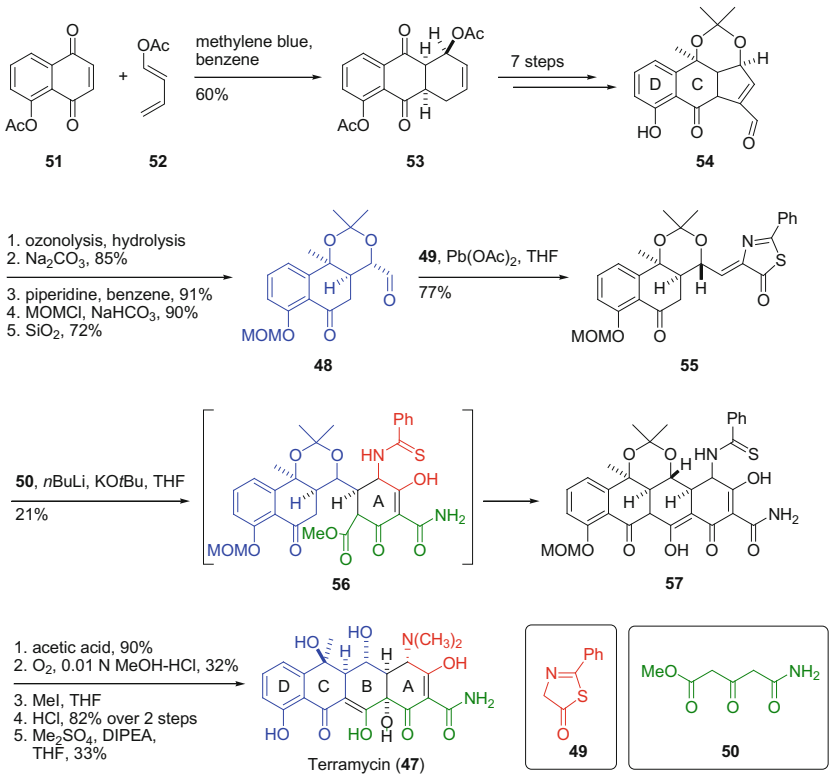
Fig. 3 Structure of chlorotetracycline (**46**)



Scheme 8 Muxfeldt's retrosynthetic analysis of terramycin (**47**)

using four retrosynthetic cuts (Scheme 8). These three building blocks were the aldehyde **48**, thiazolone **49,** and methyl-3-oxoglutarate **50** (Muxfeldt et al. 1968).

The preparation of thiazolone **49** began with thiobenzoylglycine, which was transformed to the corresponding hydrobromide and then converted to thiazolone **49** by treatment with sodium acetate (Muxfeldt et al. 1967). The synthetic benefits derived from this building block were threefold. First, it exhibited high acidity due to its cyclic unsaturated structure, leading to mild reaction conditions in the condensation step. Second, it served as an internal protecting group for the amino group; and third, the thioester did act as an activated carboxylate in the final condensation step. Methyl-3-oxoglutarate **50**, was readily available from dimethyl 3-oxoglutarate in two steps (Muxfeldt et al. 1968). The synthesis of aldehyde **48**, on the other hand, was a more complex endeavour. Its synthesis started with the cycloaddition reaction between 1-acetoxybutadiene (**52**) and juglone acetate (**51**), which provided **53** as a 3:1 mixture of regioisomers (Scheme 9). A series of protecting group manipulations, generation of the tertiary alcohol and an ozonolysis, followed by an aldol condensation, provided aldehyde **54** in seven steps. At this stage, both the D- and C-ring of terramycin (**47**) were already in place (Muxfeldt 1962a, b; Muxfeldt et al. 1979) but to obtain compound **48**, two carbon atoms had to be removed from the third ring of **54**. This task was achieved by another ozonolysis, followed by a basic hydrolysis, which induced a retro-aldol transformation. However, epimerization at C-5 under the aforementioned reaction conditions required a subsequent equilibration, which was performed via the formation of the corresponding enamine using piperidine. Then, the phenolic alcohol was protected with MOMCl at C-10 and enamine functionality was removed in the presence of deactivated silica providing **48**. These transformations set the stage for the condensation with thiazolone **49**, which could be achieved in the presence of basic lead acetate, building up thiazolone **55**. Finally, the A and B rings were formed through the Michael addition of methyl 3-oxoglutarate (**50**), followed by an intramolecular addition-elimination reaction giving intermediate **56** which then condensed to the tetracyclic compound **57**. This impressive transformation established the carbon skeleton. The remaining transformations which were required to complete the synthesis, were deprotections, oxidation of the carbon C-12a and alkylation of the amine nitrogen (Muxfeldt et al. 1968). The racemic synthesis of

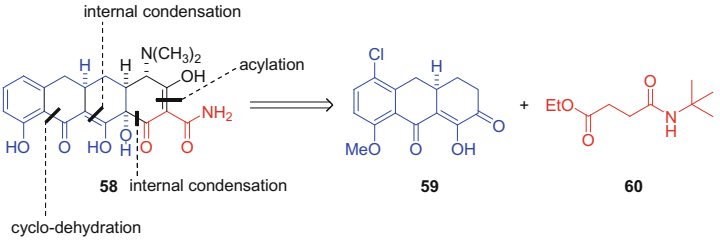


Scheme 9 Muxfeldt's racemic total synthesis of terramycin (**47**)

terramycin (**47**) was completed in 22 steps with an overall yield of 0.06 %. Regardless of the low overall yield, this synthesis is considered one of the milestones in organic synthesis as it showed for the first time that molecules of even that complexity can be generated by laboratory synthesis.

3.2 Racemic Total Synthesis of 6-Demethyl-6-Deoxytetracycline (Woodward 1968)

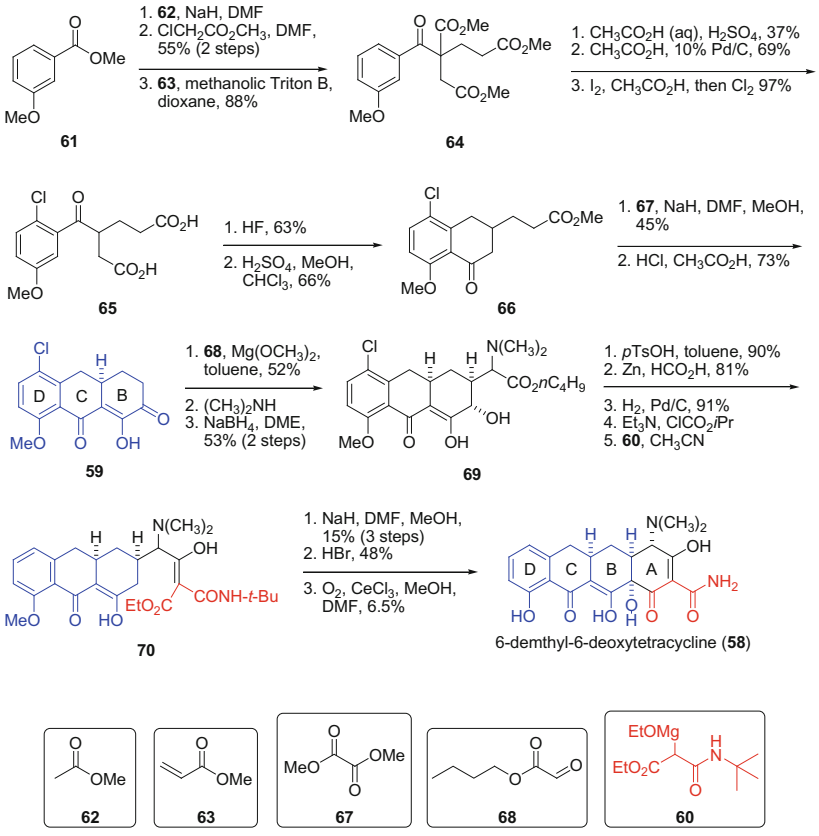
The racemic total synthesis of 6-demethyl-6-deoxytetracycline (**58**) by Woodward, which was also developed in 1968, followed a slightly different strategy than the one developed by Muxfeldt. Both syntheses started from the D-ring, but in contrast to Muxfeldt's approach (B ring was joined last), Woodward built the rest of the rings successively from C to A (Korst et al. 1968; Muxfeldt et al. 1968). Woodward's retrosynthetic approach featured four major disconnections (Scheme 10) and the A-ring, which contains three of the four stereogenic centres, was constructed late in



Scheme 10 Woodward's retrosynthetic analysis of 6–demethyl–6–deoxytetracycline (**58**)

the synthesis. Therefore, **58** can be reduced to tricyclic triketone **59** and ethyl *N-tert*-butylmalonate (**60**) (Korst et al. 1968).

Methyl *m*-methoxybenzoate (**61**) was used as the starting material for the tricyclic triketone **59**. It was first transformed in three steps into keto triester **64** (Scheme 11), then hydrolyzed and concomitantly decarboxylated to give the



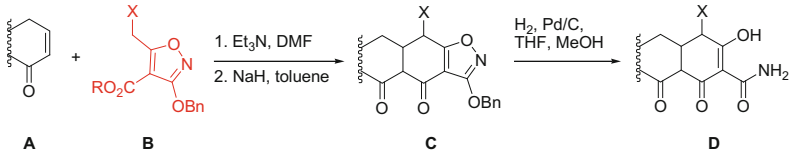
Scheme 11 Woodward's racemic total synthesis of 6-demethyl-6-deoxytetracycline (**58**)

corresponding diacid which in turn was re-esterified and the keto carbonyl group removed by hydrogenolysis. Then, chlorination was performed at C-7, (here and subsequently the chlortetracycline numbering is used, Fig. 3) giving **65**, to block cyclization in the *para*-position to the methoxy group. The cyclodehydration established the C-ring and esterification of the acid generated tetralone derivate **66**. Next, the B-ring was installed by an intermolecular condensation with dimethyl oxalate (**67**) giving the tricyclic ester, which could be decarboxylated to the key intermediate tricyclic triketone **59**. This key intermediate was reacted with aldehyde **68** to give aldol condensation product. This aldol product is an α,β-unsaturated 1,4-dicarbonyl compound, which could be attacked by nucleophiles in Michael manner on two different carbon atoms. In fact, due to steric reasons reaction with dimethylamine leads to only one regioisomeric product and at the same time also establishes *syn* relationship between hydrogens of the B-ring. Direct reduction of the intermediate carbonyl compound leads to **69**. Next, the hydroxy and chloro groups were removed: the former by lactonization-reduction sequence and the latter by catalytic hydrogenation. Thus, the liberated acid moiety was activated by forming the mixed anhydride, which was then acylated with the ethoxymagnesium derivate of ethyl *N-tert*-butyl-malonamate (**60**) giving the A-ring precursor **70**. The synthesis was completed by building up the A-ring by treatment of **70** with sodium hydride obtaining the tetracycline derivate, which was then deprotected with hydrobromic acid and oxygenated in the presence of cerium(III) chloride. This concluded the synthesis of 6-demethyl-6-deoxytetracycline (**58**) (Korst et al. 1968) in 25 steps with an overall yield of 0.02 %.

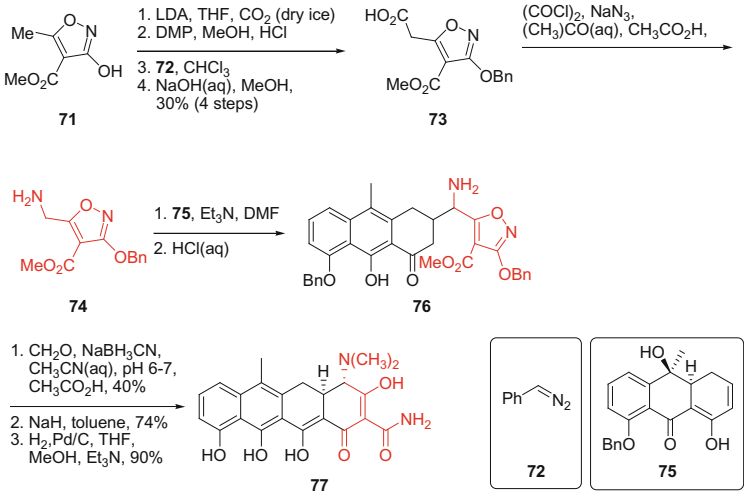
3.3 *Benzyloxyisoxazole Systems in Construction of Tetracyclines*

In the aforementioned syntheses of tetracyclines, a Claisen cyclisation was used to form a bond between C-1 and C-12a. This transformation could interfere with the sensitive functionalities at the A-ring (Korst et al. 1968). To circumvent this problem, Stork et al. used isoxazoles that served as internal protecting groups for the sensitive β-keto amide moiety (Scheme 12) (Stork and Hagedorn 1978).

The benefit of this strategy was that the tricyclic dienolone **75** was easily accessible, as demonstrated in the Muxfeldt's synthesis of terramycin (**47**)



Scheme 12 General method to protect the β-keto amide moiety as 3-benzoxy isoxazoles



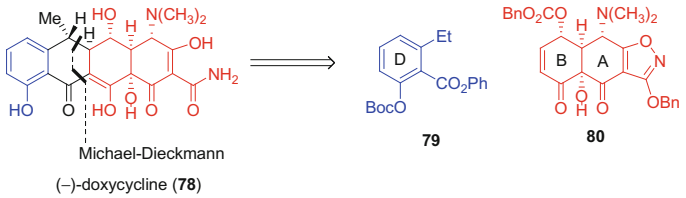
Scheme 13 Introduction of the benzyloxyisoxazole systems

(Muxfeldt 1962a), and the corresponding isoxazole derivate **74** could be obtained in five steps from 3-hydroxy-5-methylisoxazole-4-carboxylate (**71**) (Scheme 13). A Michael addition between **74** and **75** followed by dehydration gave **76** as a mixture of epimers at the amino group. The tertiary amine was obtained under reductive amination conditions and the A ring was closed by Claisen cyclization. Liberation of the β-keto amide functionality was achieved smoothly by hydrogenolysis giving the 12-deoxyanhydrotetracycline (**77**), which could then be transformed into tetracyclines (Stork and Hagedorn 1978).

3.4 Tetracycline Synthesis (Myers 2005)

During the next four decades, a number of novel tetracycline syntheses were developed. The common characteristic of those syntheses was the linear construction of the cyclic system from the D-ring to the A-ring. This was, however, not ideal for the synthesis of new active antibiotics, since substituents on the D-ring affected the antibiotic properties. Therefore, in 2005 Myers et al. reported a convergent three-step approach to tetracyclines, starting from building blocks **79** and **80** (Scheme 14). In this case, the C-ring is built up by a stereocontrolled Michael-Dieckmann cyclization, which forms two new C–C bonds and two stereogenic centres (Charest et al. 2005; Wright et al. 2014).

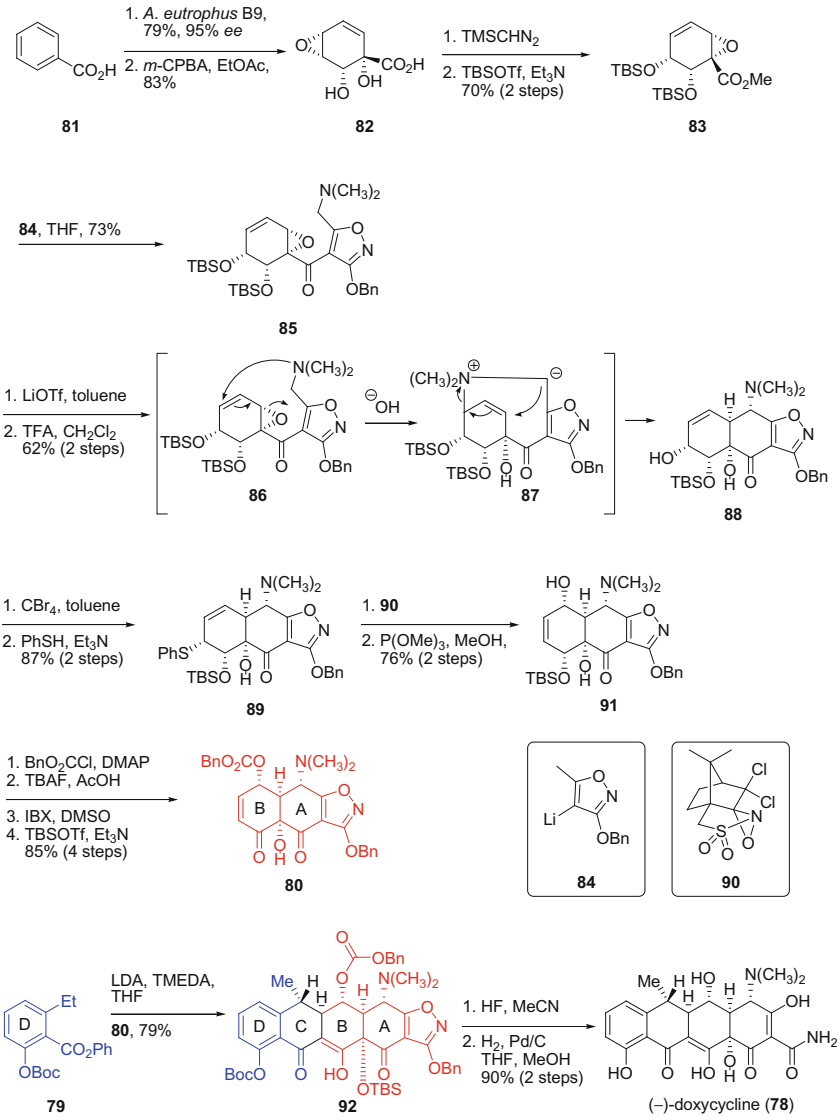
Myers' synthesis started from benzoic acid (**81**), which was dihydroxylated using a mutant strain of *Alcaligenes eutrophus*; subsequent epoxidation established the highly functionalized acid **82**. Esterification with trimethylsilyldiazomethane (Scheme 15) followed by double silylation and isomerization of this epoxide



Scheme 14 Myers' retrosynthetic analysis of (−)–doxycycline (**78**)

afforded the regioisomeric epoxy ester **83** (Charest et al. 2005), which was then attacked by organolithium reagent **84** (prepared in four steps from glyoxylic acid) giving desired ketone **85**. It is worth mentioning that here, similarly to Stork's synthesis, the carboxamide function was introduced as an internally protected 3-benzyloxyisoxazole (Stork and Hagedorn 1978). Ketone **85** provided the stage for the key transformation of this synthesis: the cyclization establishing the tricyclic AB-intermediate **88**. The cyclization is initiated by an S_N2' epoxide opening by the tertiary amine, which is followed by formation of ylide **87** and a [2,3]-sigmatropic Sommelet-Hauser-type rearrangement (Pine 2004). Next, the secondary hydroxy group of **88** was replaced by a thiophenyl group with retention of configuration. The allylic alcohol **91** was formed by a diastereoselective oxidation of the sulfide followed by a Mislow-Evans rearrangement and four further steps led to enone **80**. The D-ring building block **79** could be prepared in five steps from anisic acid (Myers et al. 2001). Michael-Dieckmann cyclization between **79** and **80** formed two carbon–carbon bonds and two stereogenic centres, therefore giving full tetracyclic core. It is worth mentioning, that remarkably high diastereoselectivities was observed in this transformation, even though up to this date no mechanistic rationale has been provided. Finally, the removal of protecting groups provided (−)–doxycycline (**78**) in 8.3 % yield and 18 steps (Charest et al. 2005; Wright et al. 2014).

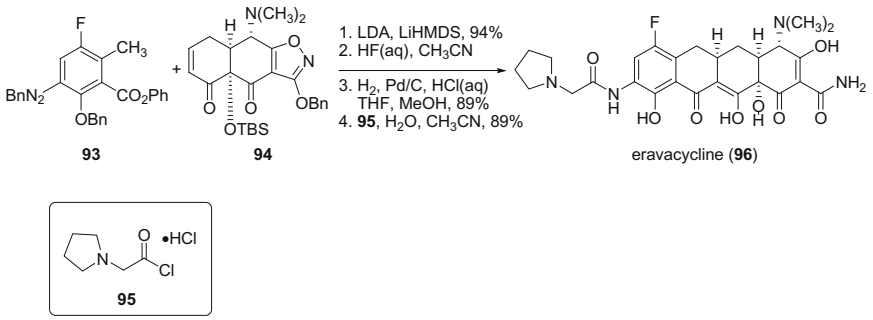
The Michael-Dieckmann reaction sequence for the synthesis of tetracyclines has proven to be robust and effective with a variety of different D-ring substitution patterns. This reaction can be performed on kilogram scale in >90 % yield and only deprotection steps are required to obtain the final product. That makes it a highly valuable method for the synthesis of new tetracyclines with different substitutions patterns which were not accessible before (Wright et al. 2014; Sun et al. 2008). Following this approach, more than 3000 new antibiotic candidates have been synthesized and tested. Currently, some of them are in different stages of clinical trials. The leading compound thereof is eravacycline (**96**) a broad-spectrum antibiotic, which is currently in phase III clinical trials (Wright et al. 2014). It can be synthesized in only four steps from **93** and **94** by the strategy of Myers et al. (Scheme 16) (Ronn et al. 2013).



Scheme 15 Myers' total synthesis of (−)–doxycycline (**78**)

4 Synthesis of Macrolide Antibiotics

Another very important class of antibiotic agents is the macrolides. The structure of these polyketidic natural products exhibits a lactone core (usually 14- to 16-membered) to which desoxy sugars may be attached. The biological activity within this class is extremely diverse and includes antibacterial, antifungal as well as



Scheme 16 Synthesis of eravacycline (**96**)

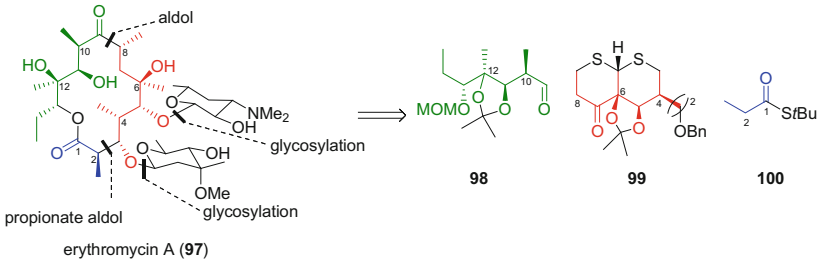
immunosuppressive properties. One of the most prominent and important representatives of macrolide antibiotics is erythromycin A, which itself as well as a variety of its derivatives are used as pharmaceutical drugs.

4.1 Synthesis of Erythromycin A (Woodward 1981)

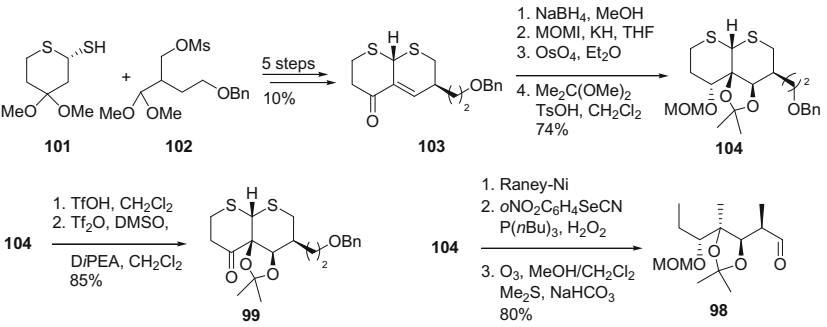
Erythromycin A (**97**) is the most important representative of the macrolide family of antibiotics. It was isolated over 60 years ago from *Saccharopolyspora erythraea* (formerly *Strepotmyces erythraeus*) and consists of a 14-membred macrocycle to which L-cladinose and D-desosamine are attached (Scheme 17). It is in clinical use as a drug to cure infections caused by Gram-negative as well as Gram-positive bacteria infecting the skin or the upper respiratory tract. When resistance to β-lactams occurs or if the patient is allergic to penicillins, erythromycin stays the medication of choice. The importance of erythromycin A as a drug is underlined by the World Health Organisation putting it on its List of Essential Medicines.

Even though erythromycin A is not produced by chemical synthesis, but by cultivation of erythromycin producing bacterial strains, the first total synthesis of this complex natural product accomplished by Woodward et al. in 1981a, b, c remains a milestone in organic chemistry (Woodward et al. 1981a, b, c).

The synthesis started with the connection and cyclization of building blocks **101** and **102** (Scheme 18), which were already literature known by that time (Gais 1977; Bennett and Hock 1927; Sudo et al. 1967). The so-gained α,β-unsaturated ketone **103** was stereoselectively reduced, MOM-protected, and dihydroxylated to give **104** after acetal protection. The bicyclic product was the key intermediate of the synthesis. Removal of the methoxymethyl protecting group and subsequent oxidation using "activated" DMSO lead to ketone **99**. On the other hand, hydrogenation of **104** with Raney–Ni simultaneously removed the thioethers and the benzyl protecting group. Selenoxide elimination of the alcohol followed by ozonolysis provided the aldehyde **98**.



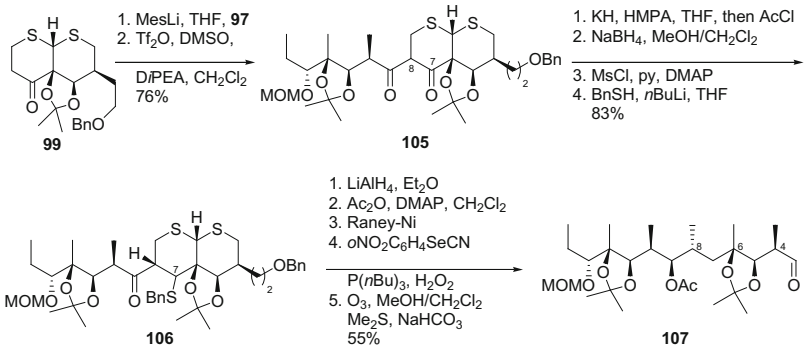
Scheme 17 Structure of erythromycin A (**97**) and retrosynthetic analysis by Woodward



Scheme 18 Synthesis of the key intermediates in Woodward's erythromycin A synthesis

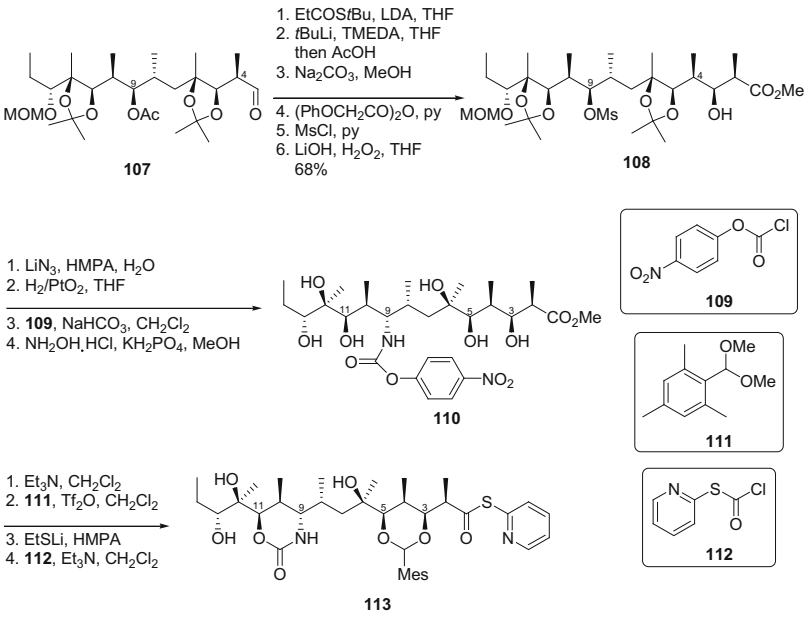
Ketone **99** and the aldehyde **98** were connected in the next step via an aldol reaction (Scheme 19) followed by oxidation of the resulting alcohol to diketone **105**. The stereocentre at C-8 (all atom numbers as in the erythromycin A) between the two keto groups of **105** was introduced by a substrate controlled sodium borohydride reduction of the enol acetate, which was generated by treatment of **105** with a base and subsequent quench with acetic anhydride. The hydroxy group at C-7 was then converted to the corresponding thioether **106** in two steps. Reduction of the remaining ketone functionality at C-9 with lithium aluminum hydride was followed by protection of that alcohol as an acetate. Hydrogenation with Raney-Ni resulted in (I) deprotection of the benzyl protecting group, (II) defunctionalization at C-7 and (III) liberation of the C-4, C-6 and C-8 methyl groups by cleavage of the cyclic thioethers. A sequence of selenoxide elimination and ozonolysis finished the synthesis of aldehyde **107**.

Introduction of the two missing carbon atoms of erythromycin's skeleton was accomplished by an aldol reaction between aldehyde **107** and the deprotonated propionyl thiolate (Scheme 20). This aldol reaction favoured the undesired *anti*-configured product. This could be corrected by a kinetically controlled inversion upon deprotonation/protonation at −100 °C giving the desired *syn*-product. Transformation of the C-9 acetate to the mesylate was carried out with interim selective protection of the C-3 alcohol as its phenyloxymethyl carbonate to afford



Scheme 19 Connection of the two key building blocks

the linear erythromycin skeleton **108** with all stereocentres in place. Next, the mesylate was converted to the azide with inversion of configuration at that centre (vide infra for the importance of the configuration there). The azide was reduced to the amine and directly protected as a carbamate, before the acetonides were cleaved and hexaol **110** was obtained. Upon treatment with trimethylamine, the cyclic carbamate protecting the functional groups at C-9 and C-11 respectively was introduced. The hydroxy groups at C-3 and C-5 were protected as the acetal of mesitylaldehyde, and the methyl ester transferred in two steps to its 2-pyridyl thio

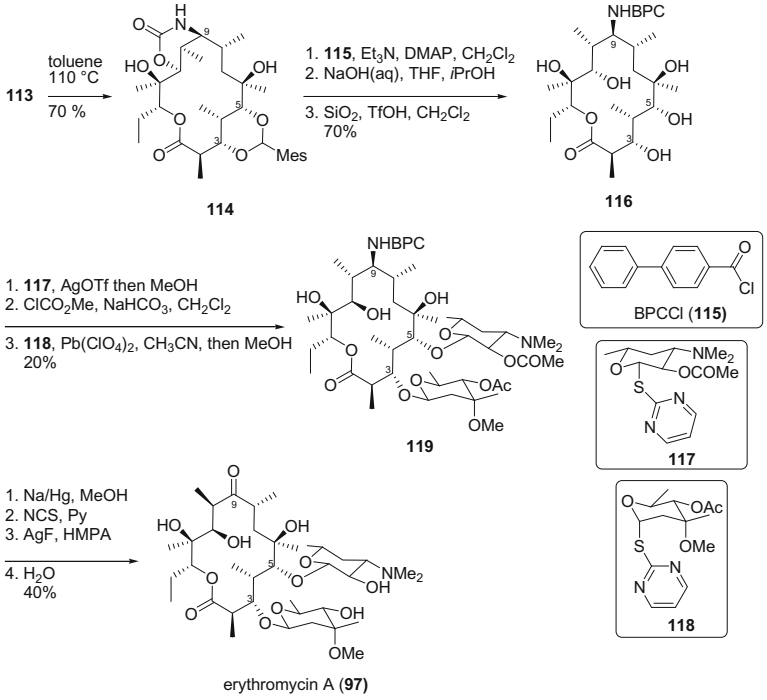


Scheme 20 Synthesis of the cyclisation precursor of erythromycin A (**97**)

analogue **113**. The necessity of a cyclic protecting group linking C-3 and C-5 was shown by a large screening of substrates for their ability to undergo cyclisation under the conditions developed by Corey (Corey and Nicolaou 1974). This screening also showed that the (*S*)-configuration at C-9 is crucial.

Cyclisation of **113** (Scheme 21) proceeded smoothly to yield **114** in 70 %. The carbamate was then *N*-acylated with phenyl benzoylchloride and subsequently cleaved under basic conditions. Deprotection of C-3 and C-5 gave **116**, the precursor for the remaining two glycosylations. It was anticipated that the C-3 OH would undergo glycosylation more rapid than C-5 OH. As the opposite was observed, C-5 was first glycosylated with D-desosamine **117** by a modified Koenigs-Knorr procedure affording the expected β-configuration (Hanessian and Banoub 1977). As methanolysis after the reaction removed the C-4″ carbonate it needed to be reattached prior to glycosylation with L-cladinoside **118**. The use of acetonitrile as solvent for this second glycosylation resulted in double inversion of configuration and gave the desired diglycosylated **119**.

The end game started with the removal of the protecting groups at the C-9 nitrogen and at the C-4″ oxygen with Na–Hg. Transformation to the amine



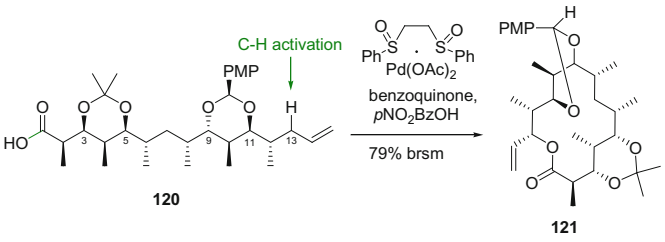
Scheme 21 Glycosylation and endgame of Woodward's erythromycin A (**97**) synthesis

hydrochloride (with *N*-Chlorosuccinimide) was followed by dehydrochlorination with silver fluoride to give erythromycinimine. Treatment of this imine with water liberated the keto group and finished the total synthesis of erythromycin A (**97**).

4.2 *Recent Strategies for the Erythromycin Core*

After the synthesis of erythromycin by Woodward a variety of different approaches were undertaken to synthesize other members of the erythromycin family as well as their aglycons (erythronolides). A very elegant and efficient approach, especially regarding the ring closure, was presented in 2009 by White (Stang and White 2009). The cyclisation precursor **120** was prepared in 18 steps from propionylated pseudoephedrine using standard transformations for the construction of polyketidic structures. The key step (Scheme 22) was the one-pot allylic oxidation of **120** at C-13 and macrolactonization catalyzed by a Pd(II)/bis(sulfoxide) catalyst. This transformation is highly atom economic as well as stereoselective, producing the desired stereoisomer at C-13 with a diastereomeric ratio of >40:1. From **120**, the synthesis of 6-desoxyerythronolide B was finished in three further steps.

A novel approach for the synthesis of polyketidic structures by nitrile oxide cycloaddition to double bonds of allylic alcohols was presented by Carreira et al. in 2001 and applied in the synthesis of erythronolide A in 2005 (Bode et al. 2001; Muri et al. 2005). Generation of the nitrile oxides was accomplished by treatment of an oxime with *tert*-butyl hypochlorite at low temperatures. Isoxazolines are the products of the cycloadditions and their N–O bond can be cleaved reductively to yield β-hydroxyketones. As the reaction proceeds via a transition state involving coordination of Mg(II) to the allylic alcohol, the newly generated hydroxy group is on the same face as the OH of the allylic alcohol. The configuration of the double bond defines the stereochemistry in α-position of the carbonyl group. Thus, using different double bond configurations as well as different configurations of the allylic alcohol, all possible combinations of stereochemical arrangements at the new stereocenters are accessible, making this a very versatile method.

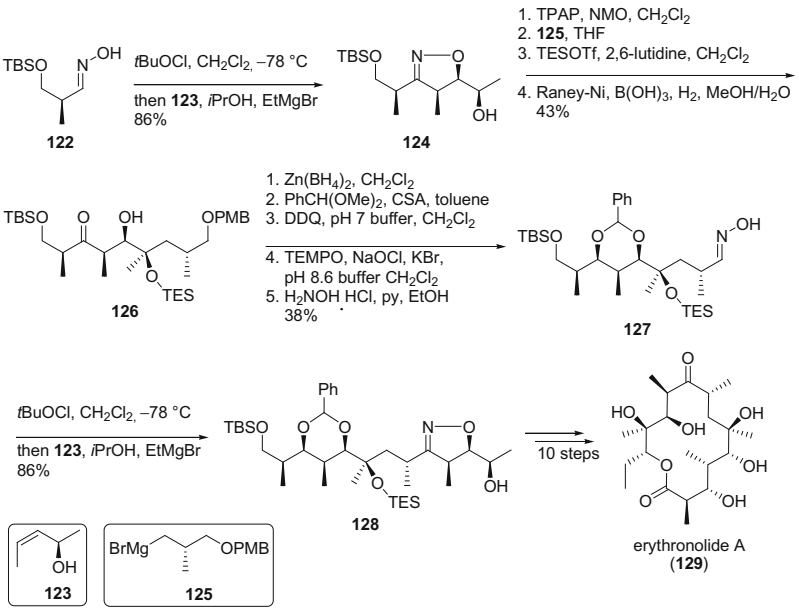


Scheme 22 Catalytic allylic oxidation/macrolactamization as key step in White's synthesis of 6-desoxyerythronolide B

Carreira's synthesis of erythronolide A (Scheme 23) started with the cycloaddition of readily available oxime **122** to allylic alcohol **123**. As expected, the stereogenic centres of the product were in an all-*syn* relationship (*d.r.* >98:2). The carbon chain was then extended by a sequence of oxidation and Grignard addition, and the resulting tertiary alcohol was protected as its triethylsilyl ether. The isoxazoline was subsequently cleaved using Raney–Ni to give the β-hydroxyketone **126**. Reduction of the ketone with zinc borohydride resulted in the *syn*-configuration of the diol, which was subsequently protected as the acetal of benzaldehyde. Deprotection and oxidation of the primary alcohol to the aldehyde, followed by treatment with hydroxylamine hydrochloride gave oxime **127**, which served as the precursor for another nitrile oxide cycloaddition to give **128**. Ten more steps finished the synthesis of erythronolide A (**129**).

4.3 Semisynthetic Antibiotics Derived from Erythromycin

For erythromycins the chemical synthesis was never a competitive way in order to provide the world supply. There are mainly two reasons for that: On the one hand, erythromycin is a complex natural product containing ten stereogenic centres in its 14-membered lactone. On the other hand, cultivation of the erythromycin producing bacterial strains and extraction of the desired product has always been comparably



Scheme 23 Nitrile oxide cycloaddition in Carreira's synthesis of erythronolide A (**129**)

easy and inexpensive. This rapid access to such a privileged structure, in contrast, makes erythromycin an interesting starting point for semisynthetic approaches to obtain derivatives that are superior either in terms of metabolic stability or exhibit a broader range of antibacterial activity. The antibiotics azithromycin, clarithromycin, roxithromycin, dirithromycin as well as flurithromycin, the last not being on the market, are important examples of structures semisynthetically derived from erythromycin.

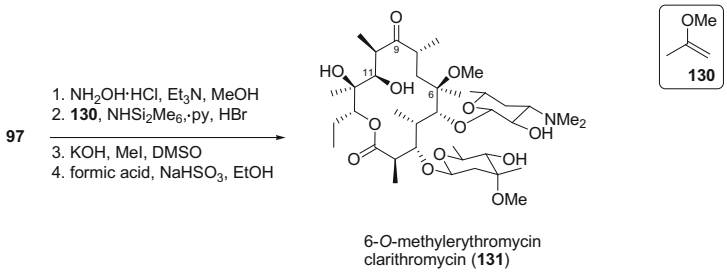
Clarithromycin (trade name Biaxin) for example is chemically one of the simplest derivatives of erythromycin as it differs only by methylation at the C-6 hydroxy group. It was first synthesized by Taisho Pharmaceuticals in 1980 (Watanabe et al. 1981), launched to the Japanese market in 1991 and FDA approved in 1995. Due to its improved bioavailability, compared to the rather acid labile erythromycin, it can be administered orally. It is considered a broad-spectrum antibiotic due to its activity against Gram-positive and Gram-negative bacteria (Hamilton 2014).

From erythromycin A (**97**), the synthesis starts with the introduction of two protecting groups (Scheme 24). The C-9 keto group is first protected as its oxime, followed by the transformation of the C-11 and C-12 hydroxy groups to an acetonide using 2-methoxypropene. Methylation is then carried out with iodomethane using potassium hydroxide as a base. Global deprotection with formic acid and sodium bisulfite gives then clarithromycin (**131**).

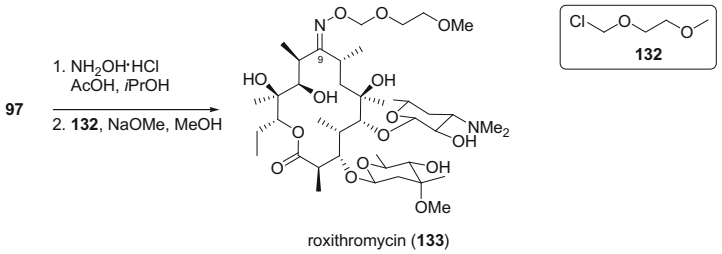
An agent that is similar in its pharmacological behaviour to clarithromycin is roxithromycin (**133**). The drug, which is sold under the trade name Rudil (or Rudile in Germany, Austria and Switzerland) is easily accessible from erythromycin in two steps (Krishna et al. 1998).

As the first step of the synthesis (Scheme 25), the C-9 ketone of erythromycin is converted to the oxime. The 2-methoxyethoxymethyl side chain is subsequently introduced via S_N2 displacement.

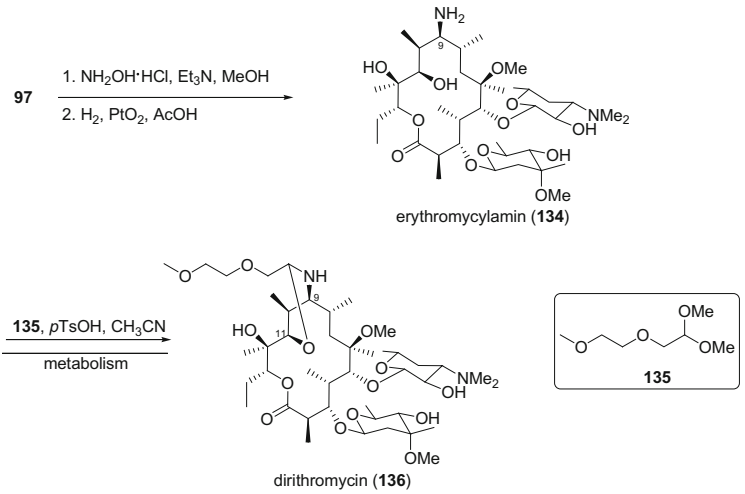
Erythromycylamine (**134**), an erythromycin derivative in which the C-9 keto group is replaced by an amine (Scheme 26), has very similar antibacterial activity to erythromycin itself, but can be administered orally. Dirithromycin (**136**) is the commercially available prodrug that is metabolized in the body to erythromycylamine (**134**).



Scheme 24 Synthesis of clarithromycin (**131**) from erythromycin A (**97**)



Scheme 25 Synthesis of roxithromycin (**133**) from erythromycin A (**97**)



Scheme 26 Synthesis of dirithromycin (**136**) via erythromycylamine (**134**) from erythromycin A (**97**)

Dirithromycin (**136**) is synthesized from erythromycin (**97**) in three steps (Mcgill 1992; Massey et al. 1970). The sequence starts with the formation of the hydroxylimine from the C-9 keto group (Scheme 26). After reduction of the amine with hydrogen and platinum(IV) oxide as a catalyst, the hemiaminal ether of **136** is formed under acidic conditions from the corresponding dimethyl acetal **135**.

5 Summary

As the aforementioned antibiotics are still the pivotal pillars of anti-infectives therapy and resistant strains are evolving worldwide, the synthetic contributions described above provide the essential background for today's anti-infectives

research. As described for the tetracyclines, new derivatives are still being synthesized today in order to fight emerging and resistant pathogens. The advent of new synthetic strategies might allow us to provide more rapid and efficient access to new and improved antibiotics. As one of the prerequisites of antibiotics research, synthetic chemistry will always play a key role in this area.

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