# TETRACYCLINES

## 1. Introduction

The tetracyclines are a group of antibiotics having 4-ring carbocyclic structure as a basic skeleton and differing from each other chemically only by substitutuent variation. Figure 1 shows the absolute configuration of tetracycline (**1**) and the principal tetracycline derivatives now used commercially.

The first tetracycline discovered was produced by a soil organism, *Streptomyces aureofaciens*, and is now known as chlortetracycline [57-62-5] (**2**), $C_{22}H_{23}ClN_2O_8$ (1). This compound ushered in a new era in antibacterial chemotherapy because it was effective orally and against a broad range of gram-positive and gram-negative bacteria. Chlortetracycline was joined by second member of the family, oxytetracycline [79-57-2] (**3**), $C_{22}H_{24}N_2O_9$, produced by another actinomycete, *Streptomyces rimosus*, in 1950 (2). Tetracycline [60-54-8] (**1**), $C_{22}H_{24}N_2O_8$, discovered in 1953, lacks the 7-chloro of chlortetracycline (**2**) and the 5-hydroxyl group of oxytetracycline (**3**). Tetracycline was produced either by reductive dechlorination of (**2**), (3,4) or by direct fermentation (5). 6-Demethylchlortetracycline [127-33-3], $C_{21}H_{21}ClN_2O_8$, (**4**) was discovered as a metabolite of a mutant strain of the original *Streptomyces aureofaciens* and was introduced in 1958 (6).

The three tetracyclines most recently marketed were made by a semisynthetic pathway. The first of these were methacycline (6-methylene oxytetracycline) [914-00-1] (**5**), $C_{22}H_{22}N_2O_8$, (7), and its reduction product doxycycline [564-25-2] (**6**), $C_{22}H_{24}N_2O_8$ (7,8). The latter compound is a potent antibiotic which is well absorbed and slowly excreted, thus, allowing small and infrequent (once or twice a day) dosage schedules. Finally, the most recent addition to the commercial tetracyclines is minocycline [10110-90-8] (**7**), $C_{23}H_{27}N_3O_7$ (9), which is also well absorbed and slowly excreted. Minocycline protects mice against infection caused by certain staphylococcal strains resistant to most tetracyclines, pencillins, and many other antibiotics. In addition, it showed a marked superiority over other tetracyclines when tested against a large numbers of randomly occurring, hospital-isolated gram-positive bacteria at the time when it was discovered (10).

During the years from 1948 to 1952 considerable research was devoted to determining the structures of the tetracyclines. The gross structure (**3**) was first determined by a combination of degradation sequences and spectral studies (11) before the structure of (**2**) was also determined (12,13). The more subtle points of structure, such as the stereochemical configurations (Fig. 1), were later determined by x-ray methods (14) and the absolute configuration by degradation. Structures of the tetracyclines developed since 1952 are easily arrived by comparison to the structures of the earlier compounds.

## 2. Physical Properties

In general, the tetracyclines are yellow crystalline compounds that have amphoteric properties (Fig. 2) (15). They are soluble in both aqueous acid and aqueous

base. The acid salts tend to be soluble in organic solvents such as 1-butanol, dioxane, and 2-ethoxyethanol.

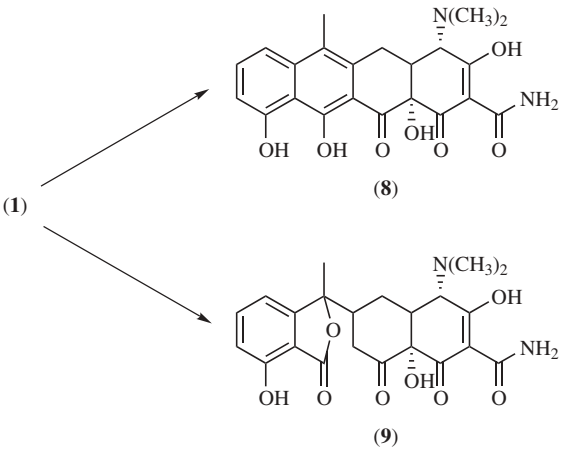
The tetracyclines are strong chelating agents. Both the A-ring and 11,12 β-diketone systems are active sites for chelation (16). This ability to chelate to metals, such as calcium, results in tooth discoloration when tetracycline is administered to children (17). Each tetracycline possesses a characteristic uv-absorption spectrum and this property is used extensively in structure elucidation (12,13). This spectrum results from the contribution of two chromophores: the BCD ring system gives a $\lambda_{max}$ at approximately 350 nm and the A-ring a $\lambda_{max}$ at approximately 265 nm.

Traditionally, paper chromatography was used to monitor chemical modification reactions to determine the composition of the reaction mixture (13,18–20). The tetracyclines were usually detected by uv fluorescence on the paper strip or by bioautography where the areas on the paper strip that inhibited the growth of microorganisms were determined. The transference of paper chromatography solvent systems to column chromatography has been accomplished and since 1978, high-pressure liquid chromatography (hplc) has been used with excellent success (21). Mass spectral analysis (22) and nmr (9) have become useful tools for tetracycline structure determinations.

## 3. Semisynthetic Modifications

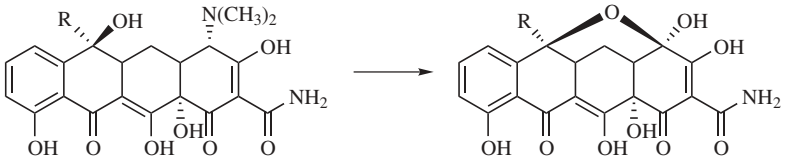
The tetracycline molecule (**1**) presents a special challenge with regard to generate new derivatives for the study of structure–activity relationships. The difficulty has been to devise chemical pathways that preserve the BCD ring chromophore and its antibacterial properties. The lability of the 6-hydroxy group to acid and base degradation (12,13), plus the ease of epimerization (23) at position 4, contributed to chemical instability under many reaction conditions.

Under acidic conditions, dehydration to an anhydrotetracycline [20154-34-1] (**8**), $C_{22}H_{22}N_2O_7$, occurs; under basic conditions, ring C opens to an isotetracycline [3811-31-2] (9), $C_{22}H_{24}N_2O_8$. The anhydrotetracyclines, such as (**8**), appear to exhibit a mode of antibacterial action, but it is unlike that of tetracycline (24). Epimerization (23,25,26) at C-4 occurs in a variety of solvents within the pH range 2–6, particularly in acetic acid (25). A number of anions (27) facilitate this reaction. The reverse process, from 4-epitetracycline [79-85-6], $C_{22}H_{24}N_2O_8$, to tetracycline, is promoted by chelation with ions such as calcium and magnesium (28).




Conversion of the C-2 amide to a biologically inactive nitrile, which can be converted via a Ritter reaction (29) to the corresponding alkylated amide, has been accomplished. When the 6-hydroxyl derivatives are used, dehydration occurs at this step to give the anhydro derivative. Substituting an *N*-hydroxymethylimide for isobutylene in the Ritter reaction yields the acylaminomethyl derivative (30). Hydrolysis affords an aminomethyl compound. Numerous examples have been reported of the conversion of a C-2 amide to active Mannich adducts which are extremely labile and easily undergo hydrolysis to the parent tetracycline (31–35). This reverse reaction probably accounts for the antibacterial activity of these tetracyclines.

Reactions at the C-4 carbon atom have been studied. With the exception of (**3**), reaction with methyl iodide (36,37) converts the 4-amino group to a quaternary amine with a concomitant loss of antibacterial activity. Treatment of this quaternary derivative with zinc in acetic acid results in a selective removal of the 4-dimethylamino grouping (36). This deamination can also be accomplished by photolysis (38). A transformation (18) involving the C-4 position has resulted in the synthesis of 4-hydroxy-6-methylpretetramid [2011-31-6], $C_{20}H_{15}NO_7$, an important precursor (39) in the biosynthesis of tetracycline. Another unusual reaction (40,41) yielded the 4,6-hemiketals: reactions of (1) or 6- demethyltetracycline [987-02-0] (**10**), $C_{21}H_{22}N_2O_8$, with concentrated hydrochloric acid and sodium chlorate in acetic acid, or cupric or mercuric acetates yield the corresponding 4,6-hemiketals (**11**) and (**12**).

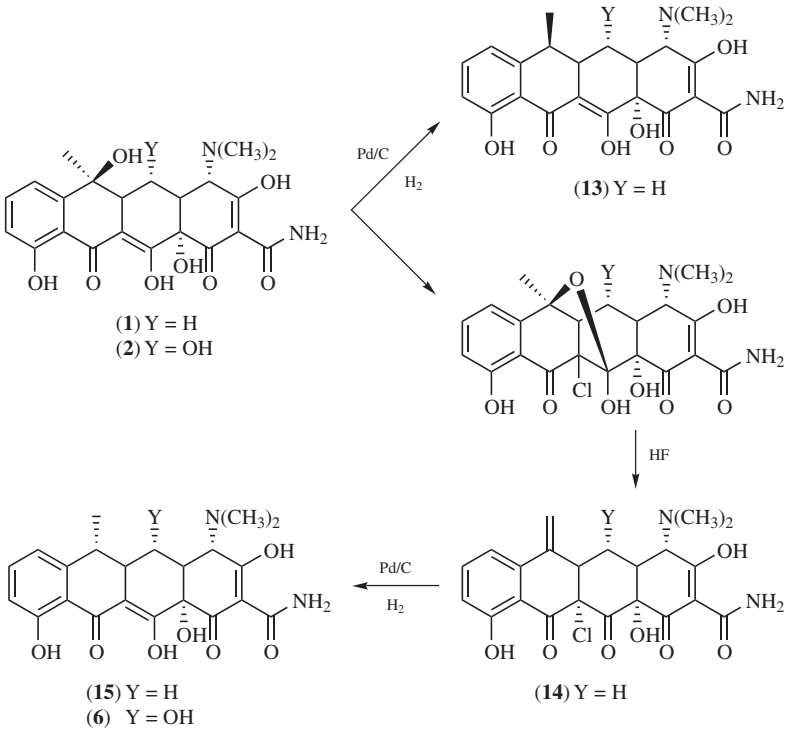



(**1**) R = $CH_3$
(**10**) R = H

(**11**) R = $CH_3$
(**12**) R = H

The hemiketal products (**11**) and (**12**) have been converted to the corresponding oximes, hydrazones, and substituted amines (40,41). Although many of these derivatives exhibit substantial antibacterial activity, they are generally less active than the parent tetracyclines. Reactions at the C-5 position of the tetracycline molecule have been limited to the introduction of an alkoxy group (42) and the acetylation of the hydroxy group (43) in 5-hydroxytetracycline. Neither of these modifications improved the biological activity of the molecule.

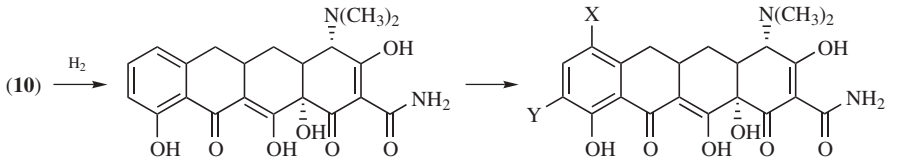
The isolation of the 6-deoxytetracyclines (44) led to other chemical modifications of (**1**). 6β-Deoxytetracycline [5614-03-9] (13), $C_{22}H_{24}N_2O_7$, was prepared by catalytic hydrogenolysis of tetracycline (**1**), resulting in an inversion (45) of the configuration at the C-6 position, but retention of antibacterial activity. Catalytic reduction (7,8) of the 6-methylene derivative (**14**) yields both the α-methyl (15) and 6 β-methyl compound (**13**). The 6 α-isomer (**15**) is reported (7,45) to be more active than the 6 β-isomer (**13**). The α-isomer, doxycycline (**6**), is an example of a semisynthetic tetracycline that has become commercially useful.




The 6-fluoro isomers, 6-deoxy-6-demethyl-6 α-fluorotetracycline [24333-20-8], $C_{21}H_{21}FN_2O_7$, and 6-deoxy-6-demethyl-6 β-fluorotetracycline [24333-21-9], $C_{21}H_{21}FN_2O_7$, have been prepared and showed relatively high *in vitro* and *in vivo* biological activities compared to the parent tetracyclines.

The increased chemical stability of the 6-deoxytetracyclines allows chemical modification with retention of biological activity: electrophilic substitutions have been carried out at C-7 and C-9 under strongly acidic conditions (46–53). Reac-

tions of 6-deoxy-6-demethyltetracycline [808-26-4] (**16**), $C_{21}H_{22}N_2O_7$, with electrophiles, such as nitronium ion (49), bromomium ion (46,47) (from *N*-bromosuccinimide), or *N* hydroxymethylphthalimide (53), yielded 7-substituted tetracyclines. In the case of the nitration reaction, both the 7- and 9-nitro isomers (**17**), X = $NO_2$, Y = H) and (**18**), X = H, Y = $NO_2$) were obtained.

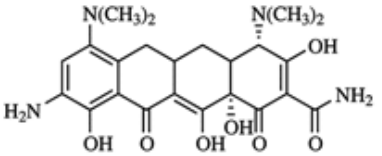



Oxidation (54) of tetracyclines using the Udenfriend reagent has yielded 9-hydroxytetracyclines and disubstituted (C-7 and C-9) products (48) can also be obtained. The 7- and 9-methyl tetracyclines have been prepared and reported to retain biological activity (55).

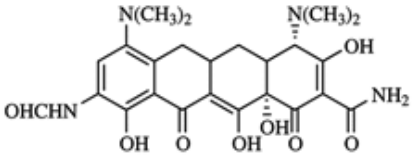
The 7- and 9-nitro isomers can be separated by crystallization and catalytic reduction affords 7-amino-6-demethyl-6-deoxytetracycline [5679005] (**19**), $C_{21}H_{23}N_3O_7$, and 9-amino-6-demethyl-6-deoxytetracycline [5874-95-3] (**20**), $C_{21}H_{23}N_3O_7$, respectively. Using these amino derivatives, scientists at Lederle Laboratories (presently Wyeth Research) reported the synthesis of a series of novel tetracyclines with modification at the D-ring (Fig. 3). An interesting reaction was described for the preparation of C-8 functionalized tetracyclines which were heretofore unknown among semisynthetic derivatives (56-57). Treatment of (**19**) or (**20**) with *n*-butyl nitrite gave the corresponding diazonium salts, and azide [61618-22-2] (**21**), $C_{21}H_{21}N_5O_7$, or [155819-15-1] (**22**), $C_{21}H_{21}N_5O_7$, was obtained by reacting the diazonium salts with potassium azide. Reaction of the azido compounds (**21**), or (**22**), with concentrated hydrochloric acid at room temperature gave the 7-amino-8-chloro-6-demethyl-6-deoxytetracycline [155819-14-0] (**23**), $C_{21}H_{22}ClN_3O_7$, and 9-amino-8-chloro-6-demethyl-6-deoxytetracycline [155819-18-4] (**24**), $C_{21}H_{22}ClN_3O_7$, respectively, in good yield. 8-Chloro-6-demethyl-6-deoxytetracycline [157579-03-8] (**25**), $C_{21}H_{21}ClN_2O_7$ was then prepared by heating the diazodium salt in methanol. 8-Bromo and 8-fluoro derivatives were also prepared in a similar fashion. These halogenated derivatives provided access to the synthesis of a series of different substitution at the 8-position via cross-coupling reaction (58). Numerous new tetracycline derivatives with modification at C-7 and C-9 have also been synthesized via either Heck, Suzuki or Stille cross-coupling reactions (59–61).

9-Amino-mino [149934-21-4] (**26**), $C_{23}H_{28}N_4O_7$, and 9-formamido-mino [153621-84-2] (**27**), $C_{24}H_{28}N_4O_8$, show notable improvement in activity against gram-positive bacteria carrying the *tet*(M) determinant, indicating the important effect of the 9-substituents. However, none of the above compounds have significant activity against Gram-negative bacteria expressing efflux determinants. The 9-glycyl-mino derivative [153621-75-1] (**28**), $C_{25}H_{31}N_5O_8$, designed with a peptidic attachment to enhance membrane permeation and ribosomal binding,

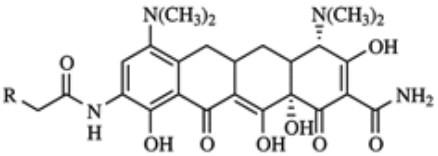
shows significant improvement in activity against Gram-negative bacteria containing the *tet*(B) determinant. Further structure–activity relationship studies led to the discovery of a series of novel tetracycline derivative referred to by Lederle scientists as 'glycylcyclines' (62,63). This group of compounds represents a significant advance in this class of antibiotics. Several of the glycylcyclines, eg, DMG-MINO [153621-76-2] (29), $C_{27}H_{35}N_5O_8$, DMG-DMDOT [153621-77-3] (30), $C_{25}H_{30}N_4O_8$, and TBG-MINO [220620-09-7] (**31**), $C_{29}H_{39}N_5O_8$ (also known as GAR-936, or tigecycline) has been studied extensively (64,65). Tigecycline has been selected for clinical development and it is now in phase III clinical trials. Tigecycline has potent broad spectrum of antibacterial activity and is able to overcome the two major tetracycline resistance mechanisms, ribosomal protection and efflux. Attachment of the 9-glycyl to either doxycycline or 8-chloro-6-demethyl-6-deoxytetracycline [157517-17-4] (**32**), $C_{25}H_{29Cl}N_4O_8$, produced compounds with similar antibacterial potency (66,67). Another new tetracycline derivative, BAY 73-6944/ PTK 0796 [389139-89-3] (**33**), $C_{29}H_{40}N_4O_7$, an aminomethylcycline, was reported to have *in vitro* activity against antibiotic-resistant Gram-positive and Gram-negative organisms (68).



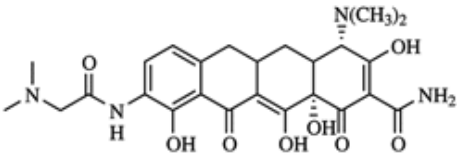
(**26**)



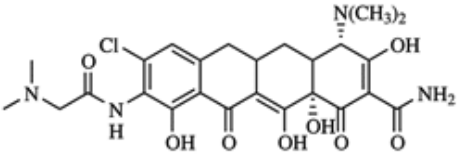
(**27**)



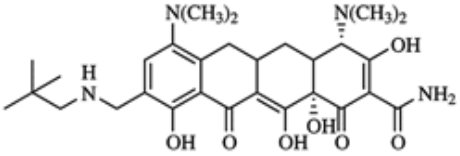
(**28**) R = $NH_2$
(**29**) R = $N(CH_3)_2$ (DMG-MINO)
(**31**) R = NHtBu (TBG-MINO/GAR-936/Tigecycline)



(**30**) DMG-DMDOT



(**32**) 8-Chloro-DMG-DMDOT



(**33**) BAY73/PTK0796

## 4. Structure–Activity Correlations

There are a number of tetracycline structural features that are prerequisites for biological activity. The linear arrangement of the rings, coupled with the pheno-

lic β-diketone system, is essential (69,70). Any structure variation at the 11a position results in loss of activity. The C-11 to C-12 β-diketone system has exceptional chelating qualities, and probably is involved in the binding of the tetracyclines to ribosomes (71), in the interactions with bacterial repressor proteins (71), and in transport of tetracyclines into the bacterial cell. The amide hydrogen can be replaced by a methyl group, but larger residues, if not rapidly cleaved in water, bring about a reduction in activity.

The configuration at the chiral centers C-4a, C-5a, and C-12a determine the conformation of the molecule. In order to retain optimum *in vitro* and *in vivo* activity, these centers must retain the natural configuration. The hydrophobic part of the molecule from C-5 to C-9 is open to modification in many ways without losing antibacterial activity. However, modification at C-9 may be critical because steric interactions or hydrogen bonding with the oxygen atom at C-10 may be detrimental to the activity.

X-ray crystallographic studies (72) have defined the conformations and hydrogen bonding of the tetracyclines under nonpolar and polar conditions. It is believed that the equilibrium between the zwitterionic and nonionized forms is of importance for the broad-spectrum antibacterial activity, membrane permeation, and pharmacokinetic properties.

Efforts have been made to correlate electronic structure and biological activity in the tetracycline series (73,74). In both cases, the predicted activities are of the same order as observed *in vitro* with some exceptions. The most serious drawback to these calculations is the lack of carryover to *in vivo* antibacterial activity. Attempts have also been made (75) to correlate partition coefficients and antibacterial activity. The stereochemical requirements are somewhat better defined. Thus 4-epitetracycline and 5a-epitetracycline [65517-29-5], $C_{22}H_{24}N_2O_8$, are inactive (76). The 6-epi compound [19369-52-9] is about one-half as active as the 6 α (or natural) configuration.

The unexpected biological activities of tetracyclines, such as 5a-epi-6-epitetracycline [19543-88-5], $C_{22}H_{24}N_2O_8$, and 7-chloro-5a,11a-dehydro-6-epitetracycline [22688-60-4], $C_{22}H_{21}ClN_2O_8$, make predicting structure–activity relationships difficult (77). In addition to the C-2 amide Mannich-base derivatives, variation at other centers in the molecule, ie, C-4, 4a, 5a, 12a, decreases the biological activity.

Modification of the 9-position has been by far the most fruitful. It has produced new class of antibiotics with significant advance in antibacterial activity. These compounds, especially glycylcyclines (including tigecycline, currently in phase III clinical trials), exhibit potent antimicrobial activity against a broad spectrum of both tetracycline-susceptible and tetracycline-resistant organisms. They are active against tetracycline-resistant strains carrying efflux resistance determinants [*tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(K), and *tet*(L)] and ribosome protection [*tet*(M), *tet*(O)] resistance determinants. Most important, they are active against multiply-resistant staphylococci, vancomycin-resistant enterococci, penicillin-resistant streptococci, many enteric bacteria and *Neisseria* (65,78).

Recent crystal structure detailed the binding pattern of tetracycline to the 30S ribosomal subunit (79,80), homology modeling based on the X-ray of the crystal structure should provide insight to designing new classes of antibiotics to combat future resistance problems.

## 5. Total Synthesis

The first synthesis of a tetracycline having full biological activity, 6-demethyl-6-deoxytetracycline (**16**) was reported in the early 1960s (81–84). This compound contained the important and difficult to obtain 4-dimethylamino group, but lacked the 6-hydroxyl. An alternative total synthesis of (**16**) was devised in 1965 (85) using methods that could lead to the synthesis of tetracyclines in large quantities and in good yields. The only total synthesis of a fermentation-produced tetracycline was carried out for oxytetracycline (**3**) which has six asymmetric centers (86). The following non-naturally tetracyclines have also been synthesized: 5a-methyltetracyclines (72), 6-oxatetracyclines (72), and 6-thiatetracyclines (72). In 1996, a stereospecific total synthesis of (±)-12a-deoxytetracycline which resolved the long-standing problem of establishing the proper relative stereochemistry of C5a and C4a centers was reported (87).

## 6. Biological Considerations

**6.1. Biosynthesis.** The overall biosynthetic pathway to the tetracyclines has been reviewed (91). Studies (81–83,92,) utilizing $^{13}C$ labeled acetate and malonate and nmr analysis of the isolated oxytetracycline, have demonstrated the exclusive malonate origin of the tetracycline carbon skeleton, the carboxamide substituent, and the folding mode of the polyketide chain. Feeding experiments using [1-$^{13}C$, $^{18}O_2$] acetate and analysis of the nmr isotope shift effects, led to the location of the [$^{18}O_2$] derived oxygen substituents in oxytetracycline: carbons 1, 3, 10, 11, and 12 were labeled. The remaining oxygen substituents, those at carbons 5, 6, and 12 in oxytetracycline, originate from subsequent oxidation of the 4-hydroxy-6-methylpretetramid [2011-31-6].

Feeding studies using deuterated [1-$^{13}C$] acetate and subsequent location of the deuterated sites by the nmr isotope shift effects showed labeling at carbons 7 and 9. The absence of a detectable β-$^{2}H$ isotope shift at carbon 4a indicates that only one of the carbon-5 hydrogens is acetate-derived and that this is stereospecifically eliminated by carbon-5α hydroxylation. 8-Methoxychlortetracyclines, with and without hydroxyl substitution at carbon-4a, have been isolated. These tetracyclines retain the original oxygen at C-8 from the polyketide chain (84,93,94).

Because of the continued commercial importance of the tetracyclines, a study of the genetics of the oxytetracycline (OTC) producing organism has been undertaken to improve the efficiency of antibiotic production. The biosynthetic genes of *Streptomyces rimosus* have been cloned by taking advantage of the fact that the OTC genes are clustered around at least one OTC resistance gene. Using this resistance gene as a selectable marker to detect hybridizing clones, a 30–40 kilobase piece of DNA surrounding the resistance gene, and containing some of the biosynthetic genes, was identified (95).

Anhydrotetracycline oxygenase from *Streptomyces aureofaciens*, which catalyzes the conversion of anhydrotetracycline to dehydrotetracycline, has been isolated and characterized as a flavin-dependent oxygenase (96). It consists of

two subunits of mol wt = 57,500 based on SDS/polyacrylamide–gel electrophoresis. The cosynthetic factor 1 of *Streptomyces aureofaciens*, involved in the reduction of 5a,11a-dehydrochlortetracycline to chlortetracycline, has been identified as 7,8-didemethyl-8-hydroxy-5-deazariboflavin. This work was aided by comparison of spectral data with that of an authentic sample obtained from the hydrolysis of coenzyme F-420 (97).

**6.2. Biological Aspects.** It has been known for some time that tetracyclines are accumulated by bacteria and prevent bacterial protein synthesis. Furthermore, inhibition of protein synthesis is responsible for the bacteriostatic effect (98). Inhibition of protein synthesis results primarily from disruption of codon-anticodon interaction between tRNA and mRNA so that binding of aminoacyl-tRNA to the ribosomal acceptor (A) site is prevented (98). The precise mechanism is not understood. However, inhibition is likely to result from interaction of the tetracyclines with the 30S ribosomal subunit because these antibiotics are known to bind strongly to a single site on the 30S subunit (98).

Studies designed to characterize the nature of the tetracycline binding domain have revealed that when bound to ribosomes, tetracycline protects base A892 in 16sRNA from reactivity toward dimethyl sulfate and enhances reactivity towards bases U1052 and C1054 (99). These results suggest that the 892–1054 region of 16sRNA contributes together with 30S ribosomal proteins (100) to the antibiotic binding domain.

The crystal structure of complexes of *Thermus thermophilius* 30S ribosomal subunit with tetracycline has recently been determined (Fig. 4). In Ramakrishnan's group, they found two binding sites for tetracycline within the small ribosomal subunit (79). The better occupied site is located near the acceptor site (the A site) for aminoacylated tRNA between the head and the body of the 30S, and the less occupied site is at the interface between three RNA domains in the body of the subunit. In a separate report (by Franceschi's group), six binding sites for tetracycline were identified in the 30S subunit (80). However, both findings revealed that it was the physical blockage of the A-site tRNA binding by tetracycline bound at the primary binding site that can account for the inhibitory action of tetracycline.

The antibacterial activity of glycylcyclines is mediated by inhibition of bacteria protein synthesis, similar to the mode of action of most clinically used tetracyclines. It has been shown to inhibit the bacterial protein synthesis in both *in vitro* transcription and translation. Two representatives, DMG-MINO (**29**) and DMG-DMDOT (**30**) inhibit protein synthesis in a cell free preparation of either tetracycline-sensitive wild type ribosomes or tetracycline-resistant, *tet*(M) protected ribosomes (101). The exact mechanism by which glycylcyclines overcome the *tet*(M) mediated resistance has not been fully elucidated. It has been demonstrated that the binding of glycylcyclines to the prokaryotic ribosome is at least 5-fold stronger than that of tetracycline (**1**) or minocycline (**7**) (102). This enhanced ribosomal binding is likely the primary factor for the excellent activity of these compounds against the ribosomal protection mechanism of tetracycline resistance.

The mechanism by which glycylcyclines [eg, DMG-MINO (**29**), DMG-DMDOT (**30**) and tigecycline (**31**)] overcome the efflux-based tetracycline resistance has been investigated. Results from the induction assay and transport

experiments indicate that DMG-DMDOT still induces tetA(B) gene expression, however, the 9-glycylamido substituent appears to prevent recognition of the compound by TetA efflux protein (103,104). The crystal structure of DMG-DMDOT (**30**) in complex with Tet repressor class D, TetR(D) has been determined at 2.4 A resolution. Steric hindrance at the entrance of the tetracycline binding tunnel of TetR (Tet repressor) by the bulky and charged glycylamido substituent interferes with conformational changes required for the mechanism of induction, and leads to decreased induction efficiency (105).

An *E. coli* strain expressing the pump encoded by transposon Tn10, which is the most frequently encounted tetracycline resistance determinant among bacteria pathogens, has been tested for mutation resistance (106). The principal findings of these experiments are that mutations giving rise to strains with decreased susceptibility to glycylcyclines are difficult to obtain. When mutation does occur, only a 4- to 8-fold increase in resistance was observed for DMG-MINO (**29**) and DMG-DMDOT (30), with a concomitant loss in resistance to tetracycline.

## 7. Manufacture

Most of the fermentation and isolation processes for manufacture of the tetracyclines are described in patents (88,89). Manufacture begins with the cultivated growth of selected strains of *Streptomyces* in a medium chosen to produce optimum growth and maximum antibiotic production. Some clinically useful tetracyclines (2–4) are produced directly in these fermentations; others (5–7) are produced by subjecting the fermentation products to one or more chemical alterations. The purified antibiotic produced by fermentation is used as the starting material for a series of chemical transformations (72).

The choice of the strain of microorganism is one of the important variables in the process. The strains to be used in manufacture are mutants of the original producer, which are chosen as the result of a planned program of mutant selection. Sometimes a spontaneous mutation occurs; usually, it is induced by mutagenic agents or by irradiation of various sorts. The choice of the best strain depends on its ability to produce large amounts of the proper antibiotic in a reasonable time from ingredients that are economically feasible (90).

## 8. Economic Aspects

The total U.S. antibiotic market for 1990 was about $4.73 billion, $233 million of that was tetracyclines. In 2002, the global marketplace for anti-infective drugs has an estimated value of more than 25 billion per year and antibacterial drugs account for a significant proportion (107). The development of the semisynthetic β-lactam antibiotics (see CURBAPENEMS AND PENEMS) and emergence of resistance to the tetracyclines has steadily diminished the clinical usefulness of tetracyclines.

In the United States, the manufacturers of fermentation-derived tetracyclines (**1**), (**2**), and (**3**) are the Lederle Laboratories, a division of American Cyanamid Co., Charles Pfizer Inc., Bristol Laboratories, and Rachelle Laboratories.

There are also several manufacturers abroad. Tetracycline is now sold generically by many companies. Pfizer's doxycycline (**6**) and Lederle's minocycline (**7**), both semisynthetic tetracyclines, are the only members of the group that have increasing sales. Table 1 lists the commercial tetracyclines and the corresponding trade names.

## 9. Uses

The commercially available tetracyclines, listed in Table 1, have fairly similar antibacterial activities, apart from doxycycline and minocycline, which show enhanced antibacterial activity compared to the others (72). Tetracyclines were the first prominent group of antimicrobial agents for which the term 'broad spectrum' was used. That is, they exhibit activity against a wide range of Gram-positive and Gram-negative bacteria, including obligate anaerobes (108,109). *Mycoplasmas, rickettsiae*, *chlamydia*, and protozoan parasites are also, in general, susceptible to tetracyclines (108–110) (see also ANTIPARASITIC AGENTS, ANTHELMINTICS).

**9.1. Clinical Uses.** The emergence of bacterial resistance to tetracyclines has limited the use of these agents as the drugs of first choice in the treatment of many infections for which they were previously effective. Nevertheless, they are still the treatment of first choice in the following cases: (*1*) for bacterial infections causing brucellosis, cholera, chancroid, granuloma inguinale, and Lyme disease (108,109,111); (*2*) for rickettsial infections, eg, typhus, scrubtyphus, and spotted fever (108,109); (*3*) for chlamydial infections, eg, psittacosis, lymphogranuloma venereum, trachoma, and inclusion conjunctivitis (108,109); (*4*) in the treatment of nonspecific urethritis because of *Chlamydia* or *Ureaplasmas* (108); and (*5*) in the treatment of acne vulgaris and rosacea (108,112).

Many sexually transmitted infections are polymicrobial in nature, ie, more than one type of microorganism is responsible for the infection (109,113,114). For example, pelvic inflammatory disease (PID) is usually caused by a concurrent infection with both *N. gonorrhoeae* and *C. trachomatis* (114). For these reasons many sexually transmitted diseases, including PID, are treated with mixtures of antibiotics to provide broad coverage for mixed, aerobic, and anaerobic infections (109). Tetracyclines play a role in such therapeutic regimens, eg, β-lactam antibiotics plus doxycycline are used in the therapy of PID (114).

It has been suggested that tetracyclines alone can be used for certain sexually transmitted syndromes, such as urethritis in which *N. gonorrhoeae*, *Chlamydia*, and *Ureaplasma* species are causative agents (109). Although β-lactam antibiotics remain the drugs of choice against *N. gonorrhoeae*, the tetracyclines are active are active against the associated pathogens. However, the increasing prevalence of tetracycline resistance in *N. gonorrhoeae* (115) is likely to seriously challenge this therapeutic strategy.

Tetracyclines are used as alternative drugs in a variety of circumstances when the patient is unable to take the drug of choice, eg, in patients allergic to penicillin (108,109). Tetracyclines are widely known to cause staining of teeth (and are therefore contra-indicated in children developing permanent teeth), photosensitivity, and, in the case of minocycline, vestibular toxicity. Details of

these adverse effects and others associated with administration of tetracyclines have been comprehensively reviewed (116–121).

**9.2. Veterinary Uses.** Tetracyclines are widely used for veterinary therapy. The types of pathogens encountered are frequently different from those for which tetracyclines are used in humans (122). Tetracyclines are also used in animal husbandry as growth promoters (108,122) (see GROWTH REGULATORS, ANIMAL). Tetracyclines, eg, chlortetracycline, used in this way are usually administered to the animals orally, at subtherapeutic doses, either in the feed or drinking water (see FEEDS AND FEED ADDITIVES, NONRUMINANT FEEDS). The procedure frequently improves the efficiency of feed conversion, or the rate of weight gain in poultry and other animals reared under commercial conditions (108,122). The mechanisms responsible for growth promotion are still obscure but, in the case of chlortetracycline, probably result from suppression of deleterious organisms in the animal's intestine (122). Various antibiotics, including tetracyclines, have demonstrable biochemical effects on microorganisms at drug concentrations which are below those required to achieve complete inhibition of microbial growth (123). These effects include suppression of bacterial adhesion and interference with the secretion of extracellular toxins, both of which may relate to the growth-promoting activity of tetracyclines. Some countries, eg, the United Kingdom, have introduced legislation forbidding the addition of tetracyclines to animal feed for growth promotion purposes. See reference (122) for a discussion.

**9.3. Resistance to Tetracyclines.** The tetracyclines still provide inexpensive and effective treatment for several microbial infections, but the emergence of acquired resistance to this class of antibiotic has limited their clinical usefulness. Studies to define the molecular basis of resistance are underway so that derivatives having improved antibacterial spectra and less susceptibility to bacterial resistance may be developed. The promising clinical results shown by one of the glycylcyclines, tigecycline, particularly in overcoming resistance, upon developed successfully, should provide much needed arsenals in combating bacterial resistance (62–65). Tetracyclines are antibiotics of choice for relatively few human infections encountered in daily clinical practice (124), largely as a result of the emergence of acquired tetracycline-resistance among clinically important bacteria (108,125,126). Acquired resistance occurs when resistant strains emerge from previously sensitive bacterial populations by acquisition of resistance genes that usually reside in plasmids and/or transposons (108,126,127). Furthermore, resistance determinants contained in transposons spread to, and become established in, diverse bacterial species (126).

*Mechanisms of Resistance.* Three distinct biochemical mechanisms of resistance to tetracyclines have been identified. The energy-dependent efflux of antibiotic mediated by resistance proteins located in the bacterial cytoplasmic membrane (98,108,125–129). The intracellular tetracycline concentration remains too low for effective binding to ribosomes. The complete sequence of the genes for several efflux proteins has been established and models for the organization of the proteins in the membrane have been proposed (129,130). Ribosomal protection is the second type of resistant mechanism, whereby tetracyclines no longer bind productively to the bacterial ribosome (108,131). In a tetracycline-resistant cell, tetracycline accumulation within the cell is similar to that in the sensitive cell, but the ribosome is modified so that tetracycline no

longer binds effectively to the ribosome. Although the molecular basis of this resistance mechanism is not fully understood, it may involve modification of ribosomal RNA or protein which affects binding of antibiotic to the 30S ribosomal subunit (131). The third type is chemical alteration of the tetracycline molecule by a reaction in the cytoplasm that requires oxygen. This renders the drug inactive as an inhibitor of protein synthesis (128). The altered tetracycline then diffuses out of the cell. This mechanism of resistance is poorly characterized and may not be expressed in the natural habitat of most pathogens especially because foci of infection in the human body are usually poorly aerated (128).

*Nomenclature of Tetracycline Resistance Determinants.* The majority of tetracycline resistance determinants are located on plasmids or transposons. These determinants have been grouped into classes defined by lack of cross-hybridization under stringent conditions (127,128). Letters of the English alphabet have been used to name tetracycline resistant determinants. All 26 letters have now been used to assign the known determinants. A nomenclature employing numerals has been recommended for future determinants and S. B. Levy's group has assumed the responsibility to coordinate the assignment (132). List of known tetracycline resistant determinants and the references tabulated by Levy and coworkers is shown in Table 2 (129,132).

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Table 1. **Tetracycline Used for the Therapy of Infectious Diseases**

| Generic name | Trade name | Year of discovery |
|---|---|---|
| chlortetracycline | Aureomycin | 1948 |
| oxytetracycline | Terramycin | 1948 |
| tetracycline | Achromycin | 1953 |
| demeclocycline | Declomycin | 1957 |
| methacycline | Rondomycin | 1965 |
| doxycycline | Vibramycin | 1967 |
| minocycline | Minocin | 1972 |

Table 2. **Known Tetracycline Resistance Determinants**

| Tet determinant or gene | Mechanism | GenBank accession no. | Reference |
|---|---|---|---|
| Tet A | efflux | X00006 | 133 |
| Tet B | efflux | J01830 | 134 |
| Tet C | efflux | J01749 | 135 |
| Tet D | efflux | X65876 | 136 |
| Tet E | efflux | L06940 | 137 |
| Tet F | efflux | unsequenced | 138,139 |
| Tet G | efflux | S52437 | 140 |
| Tet H | efflux | U00792 | 141 |
| Tet I | efflux | unsequenced | 142 |
| Tet J | efflux | AF038993 | 143 |
| Tet K | efflux | M16217 | 144 |
| Tet L (plasmid) | efflux | M11036 | 145 |
| Tet L (chromosomal) | efflux | X08034 | 146 |
| Tet M | ribosomal protection | X04388 | 147 |
| (Tet N) | (withdrawn) | | 148 |
| Tet O | ribosomal protection | M18896 | 149 |
| Tet P | efflux, ribosomal protection (two genes) | L20800 | 150 |
| Tet Q | ribosomal protection | X58717 | 151 |
| Tet S | ribosomal protection | L09756 | 152 |
| Tet T | ribosomal protection | L42544 | 153 |
| Tet U | unknown | U01917 | 154 |
| Tet V | efflux | AF030344 | 155 |
| Tet W | ribosomal protection | AJ222769 | 156 |
| Tet X | modification | M37699 | 157 |
| Tet Y | efflux | AF070999 | 158 |
| Tet Z | efflux | AF121000 | 159 |
| *otrA* | ribosomal protection | X53401 | 160 |
| *ortB* | efflux | AF079900 | 161 |
| *ortC* | unknown | Unsequnced | 142,162 |
| *Tcr3 (tcrC)* | efflux | D38215 | 163 |
| *Tet* | ribosomal protection | M74049 | 164 |
| Tet 30 [original unnamed determinant; protein is 46% identical to TetA(A)] | efflux | AF090987 (wild type) | 165 |

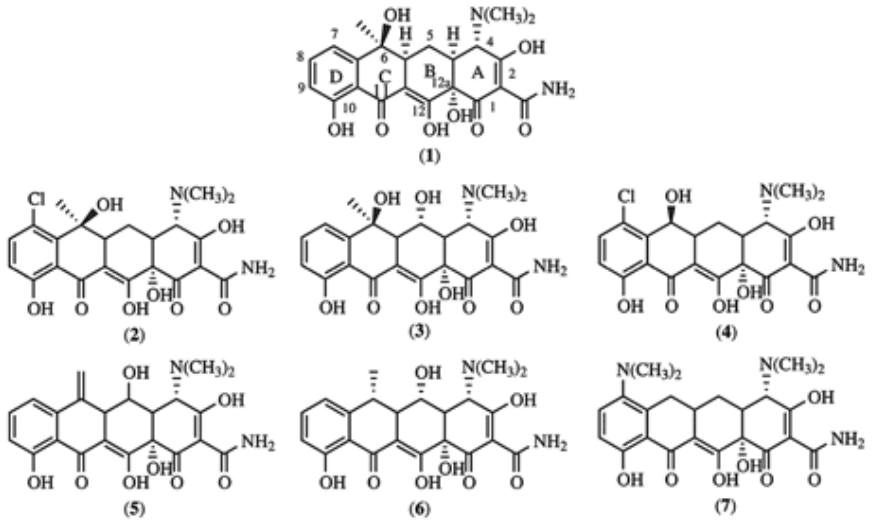


**Fig. 1. Tetracycline (1)** and its derivatives: (**2**) chlortetracycline (7-chlorotetracycline); (**3**) oxytetracycline (5-hydroxytetracycline); (**4**) demeclocycline (6-demethyl-7-chlorotetracycline); (**5**) methacycline (6-demethyl-6-deoxy-5-hydroxy-6-methylenetetracycline); (**6**) doxycycline (6-deoxy-5-hydroxytetracycline); and (**7**) minocycline (6-demethyl-6-deoxy-7-dimethylamino tetracycline).

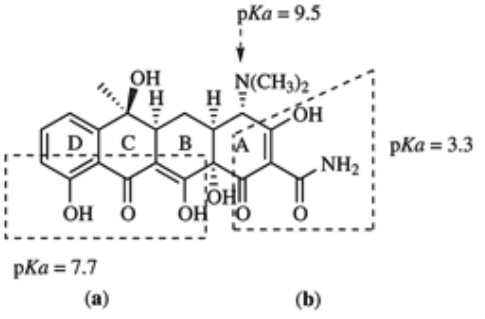


**Fig. 2. Tetracycline indicating the titratable hydrogens and showing (a)** the BCD-chromophore and (**b**) the-A-chromophore.

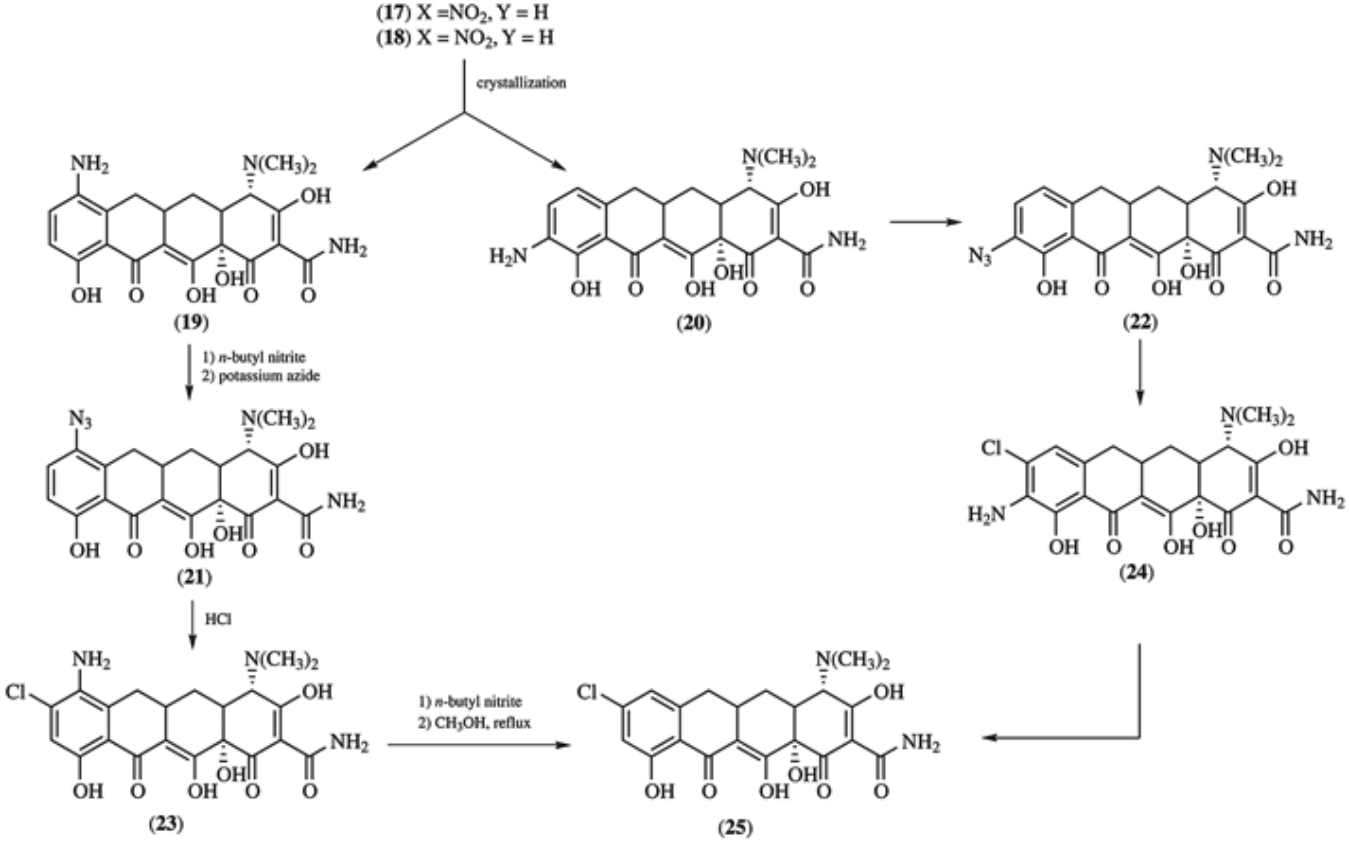


**Fig. 3.** Synthesis of novel tetracyclines with modification of D ring.

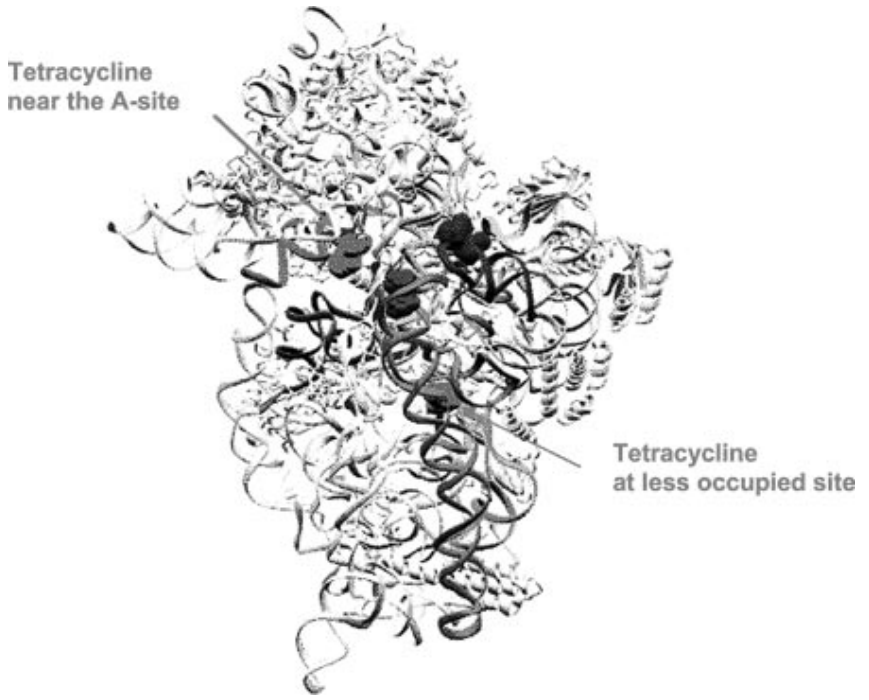


**Fig. 4. Crystal structure of complexes of** *Thermus thermophilius* 30S ribosomal subunit with tetracycline. Reproduced with permission from reference 128.