Tetracycline Antibiotics

DOI: 10.1002/anie.201102480

Isoxazole Functionalization Technologies Enable Construction of Tetracycline Derivatives**

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antimicrobial agents · bacterial resistance · isoxazole · seragakinone A · tetracycline

The tetracyclines (Figure 1) are naturally occurring (**1**, **2**, **7**) and semi-synthetic (**3**–**6**) antimicrobial agents.[1–3] The bacteriostatic mode of action[1,2,4] of the tetracyclines is characterized by inhibition of bacterial protein translation by binding reversibly to the prokaryotic 30S ribosomal subunit. This event allosterically blocks the interaction of the ribosome with aminoacyl-tRNA and protein synthesis ceases. Tetracycline antibiotics are effective against a broad spectrum of microorganisms including Gram-positive bacteria, Gram-negative bacteria as well as eukaryotic protozoan parasites.[1,2]

The tetracycline polyketide[5] carbon skeleton is comprised by four linearly fused carbocyclic rings (A–D) adorned with up to six contiguous stereocenters and a congested array of acid- and base-sensitive functionality.[3] The western D-ring is phenolic and electron-rich. The A-ring exhibits polar functionality including a dimethylamino group and a key pharmacophoric[6] β-keto carboxamide (vinylogous carbamic acid). The β-diketone keto-enol systems (Figure 1, C11–C12 and C1–C3) are known to chelate divalent cations such as magnesium.[1,2] This chelation ability influences binding and pharmacokinetic properties and facilitates diffusion of the molecule through biological membranes to gain access to the cytosolic ribosome.

Annual consumption levels on the order of 5000 metric tons attest to the extensive use of this class of antibiotics for clinical, veterinary and agricultural applications.[2,3] This has contributed to the emergence of widespread bacterial resistance due to genetic acquisition of *tet* genes whose protein products function to protect ribosomes within the cell from tetracyclines. Widespread efflux- and ribosome-based resistance to first- and second-generation tetracyclines is largely attributed to three distinct biochemical mechanisms:[1,2] 1) expression of tetracycline efflux proteins, 2) expression of ribosomal protection proteins, and 3) enzymatic inactivation of tetracycline by chemical modification.

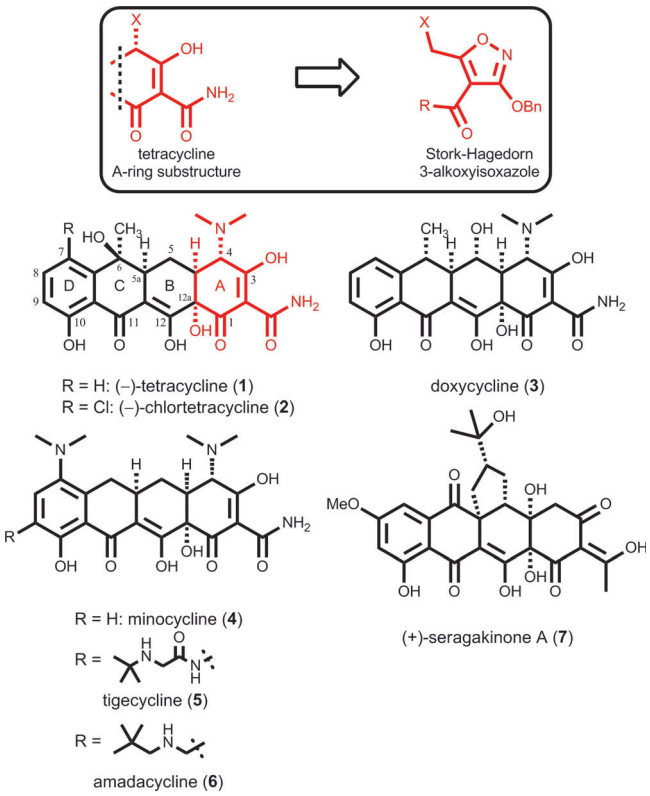

Figure 1. Representative tetracycline antibiotic natural products (**1**, **2**, **7**) and semi-synthetic second-generation (**3**, **4**) and third-generation (**5**, **6**) tetracyclines. Box: As established by Stork and Hagedorn in 1978,[7a] a 3-benzyloxyisoxazole serves effectively as a protected form of the densely functionalized tetracycline A-ring.

The *tet* efflux genes, found in both Gram-positive and Gram-negative species, code for membrane-associated proteins which export tetracycline from the cell, thereby reducing intracellular drug concentration and protecting the ribosome. The efflux proteins exchange a proton for a tetracycline–cation complex against a concentration gradient and have sequence homology and structural similarities with other efflux proteins involved in multiple-drug resistance. Most of the efflux proteins confer resistance to **1** but not to subsequent generations of semi-synthetic tetracyclines. Ribo-

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[**] Alan Long, Hyoung Ik Lee and Bharathi Pandi are acknowledged for enriching discussions pertaining to organic and medicinal chemistry. Graphics: Michael J. Malaska. Background photo adapted from Public Health Image Library ID no. 9994 (CDC/Janice Haney Carr).

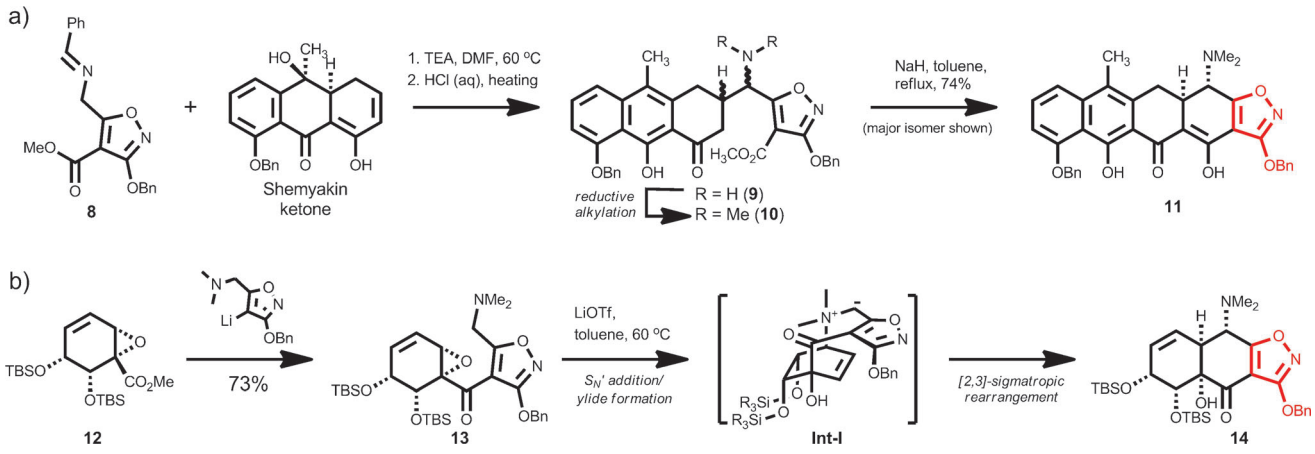
somal protection proteins confer a wider spectrum of resistance when compared with efflux-based mechanisms. Protection proteins bind to the ribosome inducing a negative allosteric modulation of the tetracycline binding site which decreases binding affinity of the ribosome for tetracycline. This binding interaction does not, however, inhibit or alter protein synthesis. The least common mode of resistance encountered amongst pathogenic and environmental bacteria is enzymatic inactivation. TetX, a 44 kDa flavin-dependent monooxygenase, is the single reported enzyme that confers resistance to tetracyclines by irreversible hydroxylation at C11a in the presence of NADPH and O_2.[3] The hydroxylated product is unable to form the Mg^{2+} complex which renders the drug inactive.

Unremitting adaptation of pathogens has stimulated the systematic search for new tetracycline analogues that possess activity against organisms resistant to older members of the class. Medicinal chemists, over the last forty years, have sought to improve potency and evade resistance by chemical modification of the tetracycline scaffold.[8] These efforts have been largely enabled by the presence of the strongly activating C10 phenol group which facilitates chemoselective D-ring functionalization.[8a–c] This strategy has provided semi-synthetic tetracyclines (**3**–**6**) that show improved activity against resistant organisms. In recent years, new synthetic methods have emerged that provide access to a broad range of tetracycline analogues that are inaccessible by traditional semi-synthesis. Many of these synthetic advancements have exploited the use of an isoxazole N,O-heterocyclic nucleus to store 1,3-dicarbonyl functionality such as the β-keto amide system of the tetracycline A-ring.

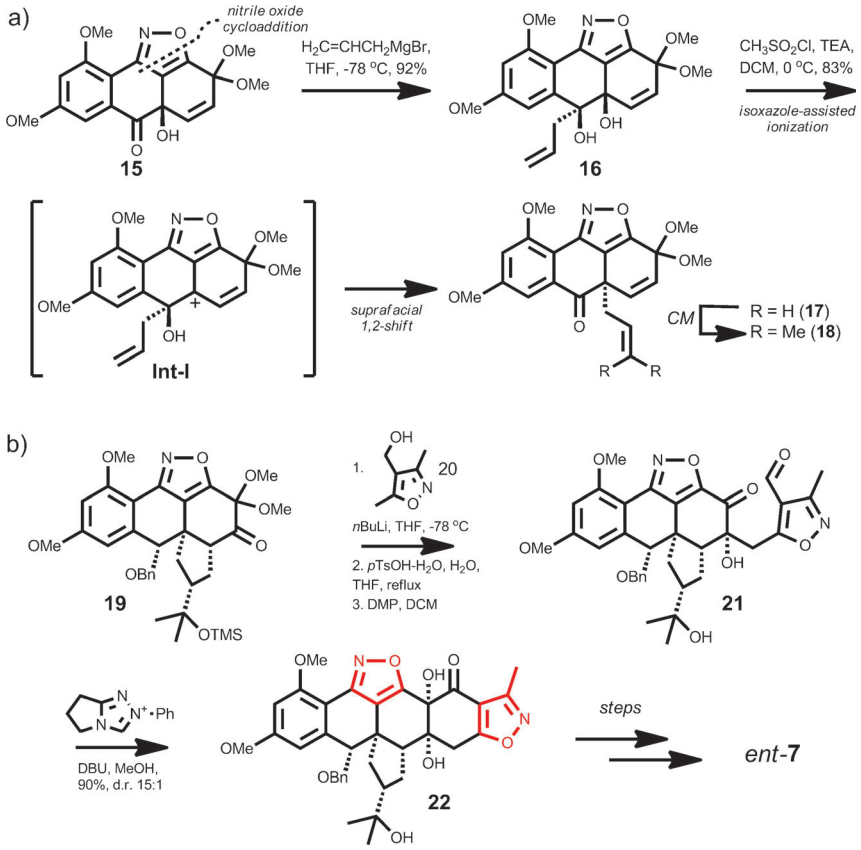
Thirty-three years ago, Stork and Hagedorn demonstrated that derivatives of 3-hydroxyisoxazoles could be employed as synthons for the polar and densely functionalized tetracycline A-ring (Figure 1, box).[7a] Significantly, deprotection of the vinylogous carbamic acid could be accomplished at a late synthetic stage under mild hydrogenolytic conditions. Arguably, the true value of this discovery was not realized until 2005 when Myers and co-workers completed the most concise synthesis to date of **3**,[9] a campaign which relied upon Stork's strategic precedent for protection of the tetracycline A-ring as a 3-benzyloxyisoxazole group. Stork's 1978 construction of the tetracycline system (Scheme 1 a)[7a] involved Michael addition of the isoxazole Schiff base **8** to Shemyakin's ketone[10] under mild conditions followed by C-ring dehydration and reductive methylation. Claisen cyclization then proceeded under basic conditions to afford **11** with control of the C4,4a-*trans* relative configuration. The isoxazole **11** could be subsequently advanced to (±)-12a-deoxyanhydrotetracycline by a single hydrogenolysis operation (five total linear steps from **8**).

In order to install the hydroxy group at a fusion point of the A and B rings (C12a) at an early stage, Myers et al. utilized a new synthetic sequence[9] (Scheme 1 b) wherein the vinylogous carbamic acid was masked using Stork's[7a] protection strategy. An organolithium isoxazole reagent was first condensed with epoxy ester **12** to arrive at isoxazole ketone **13**. Next, putative intramolecular S_N' epoxide opening is followed by ylide formation (**13**→**Int-I**). A 2,3-sigmatropic rearrangement then closes the A-ring with excellent control of the C4 amine-bearing stereogenic position. The isoxazole **14** is converted in five additional synthetic steps to a key AB enone intermediate (not shown) that has also been produced on >40 gram scale by an alternate route which relies on an *endo*-selective intramolecular furan Diels–Alder cycloaddition.[11] By implementation of a Michael-Claisen C-ring cyclocondensation onto the AB precursor,[9,12] in conjunction with Stork's A-ring hydrogenolytic deprotection method,[7a] natural tetracyclines and new tetracycline analogues were prepared in a highly convergent fashion. The recognition by the Myers group of the tetracycline C-ring as a viable retrosynthetic disconnection point has led to concise syntheses of **1**,[13] 6-deoxytetracyclines including **3**,[9] 6-aryltetracyclines,[12] pentacyclines[8e,9,12] and 8-azatetracyclines,[8d] to name a few.

Suzuki and co-workers have recently completed a total synthesis of (−)-seragakinone A (*ent*-**7**),[16] an antifungal and antibacterial polyketide natural product[15] bearing structural similarities to the tetracycline family. Suzuki's synthetic strategy (Scheme 2) employs the isoxazole ring substructure



Scheme 1. a) Highlights from Stork's synthesis[7a] of (±)-12a-deoxyanhydrotetracycline from a 3-benzyloxyisoxazole precursor (**8**). b) Myers' synthesis[9] of a key intermediate (**14**) en route to concise total syntheses of tetracycline derivatives.



Scheme 2. a) Isoxazole-directed pinacol rearrangement[14] for stereospecific installation of an angular prenyl substituent onto seragakinone A[15] (**7**). b) Stereoselective benzoin condensation (**21**→**22**) and isoxazole protection (**22**, shown in red) of two β-diketone moieties.

both as a protected 1,3-diketone equivalent[7a] as well as a directing group to facilitate the stereocontrolled construction of a quaternary carbon center.[14] For example, treatment of allylated dervative **16** with sulfene induces ionization of the bridgehead tertiary alcohol wherein the electron-releasing nature of the embedded isoxazole stabilizes the adjacent cation (**Int-I**), thereby accomplishing regioselective activation of the pinacol (**16**). A rapid 1,2-shift ($t < 10$ min) of the allyl group then gives rise to ketone **17** bearing an angular allyl substituent as a single stereoisomer.[14,16] A second isoxazole unit is installed at a later stage in the synthesis (Scheme 2b) by nucleophilic addition of the lithiated O,C-dianion derived from isoxazole **20** to the ketone **19** to generate an alcohol intermediate that was advanced in two additional steps to the ketoaldehyde **21**. A stereocontrolled benzoin cyclization employing a modified Rovis triazolium salt[17] then delivers cyclic ketol **22** with excellent diastereoselectivity. In the next synthetic operation, hydrogenation induces reductive cleavage of two isoxazoles to reveal latent 1,3-diketone functionality and the ensuing intermediate was subsequently advanced to (−)-seragakinone A.[16]

In 2011 isoxazoles continue[8d,e,16,18] to "*admirably serve the purpose of storing the β-keto amide system of the A ring of tetracyclines*" as predicted by Stork in 1978.[7a] In addition, isoxazoles have been proven uniquely capable of directing regio- and stereoselective 1,2-migrations by facilitating formation of an adjacent carbenium ion. This has provided a general method for installing quaternary stereogenic centers at angular positions,[14] an outcome not easily achieved by conventional strategies. These important technologies have the potential to deliver new clinically useful antibiotics as a means to overcome the inevitable progression of bacterial resistance.

Received: April 10, 2011
Published online: June 29, 2011

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