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Synthesis and Biological Evaluation of 9-Substituted Tetracycline Derivatives

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Abstract—The synthesis of 9-substituted tetracycline derivatives has been accomplished by the reaction of C9 diazonium tetrafluoroborate tetracycline salts with organotin reagents under modified Stille coupling conditions. Several of these unreported derivatives show promising in vitro biological activity against tetracycline resistant and antibiotic resistant bacteria.

Tetracyclines belong to a family of structurally related compounds that includes tetracycline, doxycycline, minocycline, chlortetracycline, sancycline, and methacycline and are widely used antibiotics.[1,2] Since their discovery in 1943, this class of antibiotics has found use in the clinical treatment of a wide variety of infectious microorganisms. Tetracyclines remain the drugs of choice for most rickettsial and chlamydial diseases as well as inhibiting a wide spectrum of Gram-negative bacteria. The importance of these derivatives in treating atypical pneumonia, syphilis, gonorrhea, pneumococcal pneumonia has recently been overshadowed by increasing resistance curtailing their current clinical use. The most common resistance mechanism against tetracyclines in Gram-negative bacteria is based on the active efflux of this broad spectrum antibiotic out of the bacterial cell before it can bind to the ribosome and inhibit protein synthesis.[3] However, it has been recently confirmed[4] that certain substituted tetracycline derivatives are not subject to this bacterial efflux. The resulting protein synthesis inhibition restores the efficacy of these antibiotics and allows even multi-antibiotic resistant (MAR) bacteria to be treated effectively with these derivatives. Efforts in our lab have focused on the synthesis of tetracycline derivatives that are not subject to bacterial efflux and restore the efficacy of this interesting class of antibiotics. Despite the numerous chemical modifications reported in this class of antibiotics, contemporary methods of carbon–carbon bond formation afford derivatives not available by traditional methods.

The majority of D-ring substitutions in the 9-position have been accomplished by diazotization followed by Sandmeyer reactions to incorporate a variety of nucleophiles.[5–7] Some acyloxy and fluoro analogues have also been prepared by photolysis of the diazonium salts in the appropriate acid.[8] However, these reactions are limited by the nucleophiles that can be incorporated under Sandmeyer conditions. The absence of further modifications in the 9-position is surprising in light of number of transition metal catalyzed reactions producing carbon–carbon bonds from diazonium chlorides and diazonium tetrafluoroborate salts.[9–11] The facile synthesis of the precursor (9-aminodoxycycline) and the characteristically high yields associated with Stille coupling provides a new class of tetracycline derivatives which are effective against antibiotic resistant bacteria.

Nitration of doxycycline **1** (Fig. 1) with potassium nitrate in concentrated sulfuric acid followed by reduction with H_2 and Pd/C affords 9-aminodoxycycline in 79% yield.[12] Diazotization of the amine with *n*-butylnitrite in 0.1 N methanolic HBF_4 affords a quantitative yield of the C9 diazonium salt **3**.[13] Since aryl diazonium tetrafluoroborates undergo oxidative addition to palla-

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dium(0) complexes at moderate temperatures[14] these compounds are suitable for coupling with organotin reagents under modified Stille coupling conditions.

The product yields are affected by the stability of the diazonium salts under the reaction conditions employed. Although we have had success coupling to diazonium chlorides under Stille and Suzuki conditions, the more stable tetrafluoroborate salts give superior yields with the majority of electrophilic tin species.[15] The choice solvent is also a key consideration in these transformations. It is well known that the use of protic solvents results in reduction of the diazonium salts. Thus, removal of the solvent (MeOH) from the in situ prepared diazonium salt is advantageous in these cases. The crude residue is dissolved in acetonitrile and the Stille coupling smoothly carried out at room temperature with very little reduction of the diazonium salt.[16]

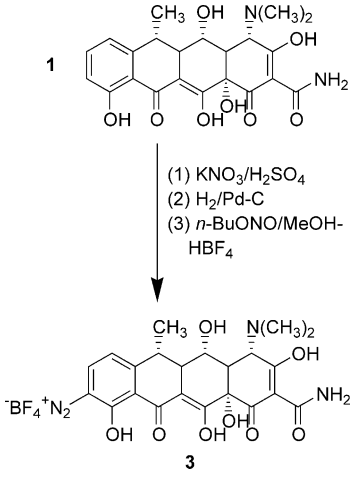
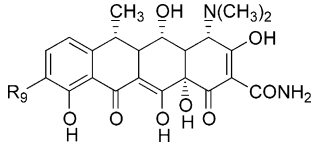


Figure 1. Synthesis of C9 diazonium tetracycline derivatives.



R_9		Yield
phenyl	4a	66%
4-nitrophenyl	4b	73%
4-aminophenyl	4c	59%
ethylenyl	4d	85%
ethynyl	4e	88%

Figure 2. Synthetic tetracycline derivatives.

Table 1. Minimum inhibitory concentrations for synthetic derivatives[a]

	S. aureus	*E. faecalis*	MRSA
Doxycycline	>50	6.25	6.25
Minocycline	3.12	<0.098	6.25
4a	3.12	1.56	0.78
4b	3.12	0.78	0.39
4c	3.12	3.12	1.56
4d	1.56	<0.098	<0.098
4e	1.56	0.78	0.39

[a]Reported in μg/mL.

The bioactivity of 9-aryl, 9-ethylenyl, and 9-ethynyl tetracycline derivatives show that it is possible to increase the in vitro antimicrobial activity with substituents in this position (Fig. 2). In fact, it has recently been reported that 9-position substituents are important in binding to methionine and arginine residues in the TetR protein.[17] Compounds **4a–e** were screened against methicillin resistant *Staphylococcus aureus*[18] and tetracycline resistant strains of *S. aureus* and *E. faecalis* to determine their in-vitro bioactivity (Table 1). All of the compounds show good activity against methicillin resistant *S. aureus* (MRSA). In addition, several derivatives show excellent activity against tetracycline resistant *S. aureus* and *E. faecalis* strains with most compounds showing more than a 5-fold increase in bioactivity versus minocycline. Further work in this position may lead to compounds with greatly enhanced antibiotic properties against multi-antibiotic resistant bacteria.

A simple and high yielding synthesis of 9-substituted tetracyclines has been described. A wide variety of substituents can be incorporated by this methodology utilizing the appropriate organotin reagent. With modifications this methodology could be applied to other tetracycline antibiotics providing completely new classes of antibiotic compounds.

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16. Typical coupling procedure: 25.0 mg of 9-aminodoxycycline (**2**) is dissolved in 4 mL of 0.1 N methanolic HBF_4 with stirring and 25 μL of *n*-butylnitrite is added to the yellow solution. After 30 min, the solution becomes a deep-red color (TLC indicates complete formation of the salt) and the solution is rotovapped to dryness. The residue is dissolved in 4 mL of acetonitrile and 2.5 mg of $Pd(OAc)_2$ is added with stirring. 8.0 μL of phenyl tri-butyltin is added and the solution allowed to stir for 4 h under positive pressure nitrogen. The solution is rotovapped to dryness and the crude residue purified by preparative thin-layer chromatography. Yield 66%. MS 520 amu. ^{1}H NMR (methanol, 400 MHz) δ 7.53 (d, $J = 6.72$ Hz, 2H), 7.47 (m, 3H), 7.24 (d, $J = 7.03$ Hz, 1H), 6.92 (d, $J = 7.04$ Hz, 1H), 4.48 (s, 1H), 3.51 (m, 3H), 2.94 (s, 7H), 1.54 (d, $J = 6.44$ Hz, 3H). IR (KBr) 3341, 3031, 2845, 2764, 1663, 1608, 1342, 1201, 1053, 708, 491 cm^{-1}. UV–vis $\lambda = 218$, 268, 365 nm.
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