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Palladium Catalyzed C−N Bond Formation in the Synthesis of 7-Amino-Substituted Tetracyclines

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Abstract: A facile synthesis of 7-alkylamino- and 7-cycloalkylaminotetracycline derivatives has been accomplished using an in situ generated aminostannane precursor. This procedure is advantageous in that it allows the concise synthesis of a number of unreported tetracycline derivatives that are cumbersome to prepare through traditional methods. These compounds are crucial to understanding structure activity relationships in the D-ring of tetracycline-type antibiotics and the acquired efflux resistance mechanism to this class of antibiotics.

Since 1944, when chlortetracycline was introduced to clinical use, tetracyclines have been important for the treatment of bacterial infections. The advantages of these compounds were that they inhibited a much wider spectrum of Gram-positive and gram negative microorganisms. In the subsequent years, lengthy programs directed at the chemical modification of tetracyclines have yielded few medically useful derivatives. Unfortunately, the overuse of this class of antibiotics has led to widespread bacterial resistance and replacement with more effective antibiotics such as fluoroquinolones and penicillins.[1]

Synthetic modification of tetracyclines to produce derivatives that are effective against resistant microorganisms has been the goal of several groups in recent years.[2] Tetracyclines have several distinct advantages over second- and third-generation fluoroquinolones and penicillins. Clinically, tetracyclines are much safer than other antibiotics, inhibit a wider spectrum of microorganisms, have a well-understood mechanism of action, and have relatively well-defined structure−activity relationships.[3,4] A number of modifications to the A, B, and C rings have been reported and mostly render tetracycline inactive against Gram-positive and Gram-negative microorganisms. Substituents in the aromatic D-ring of these antibiotics are tolerated and in a number of cases improve the biological activity against bacteria with acquired multi-antibiotic resistance. Although numerous D-ring modifications have been reported in the literature,[5,6] it is surprising that few derivatives are prepared by contemporary carbon−carbon and carbon−nitrogen bond formation.[7]

Research in our laboratory has focused on the preparation of D-ring-substituted compounds in hopes of reviving the efficacy of this interesting class of antibiotics against susceptible and resistant microorganisms. The ortho−para directing nature of the phenolic hydroxyl at C-10 allows for a variety of substitution reactions in the D-ring[8,9,10] in the 7 and 9 positions. Biological assessment of these derivatives has led to the general conclusion that C-7 substitution is more favorable for bioactivity than C-9 substitution. Since minocycline and 9-glycylaminominocycline show good activity against multi-antibiotic resistant (MAR) bacteria, we set out to prepare a number of 7-alkylamino and 7-arylamino derivatives using contemporary palladium-catalyzed carbon−nitrogen bond formation. The goal of this research is to provide a facile route to 7-aminotetracyclines compatible with combinatorial screening of the in vitro bioactivity of these compounds. Although a small number of these compounds have been prepared by the reaction of 7-aminosancycline with various electrophiles, this route has several disadvantages. 7-Aminosancycline is obtained by nitrating the C-9-protected sancycline followed by reduction and deprotection. The yields obtained through traditional methods are lower than the method reported herein. Further, these three steps are not easily carried out in a combinatorial fashion; rather, each derivative must be deprotected and purified individually before screening their biological activity. The method reported here has produced a 10 compound combinatorial library utilizing a single reaction from a readily available precursor. In addition, an expeditious route to the important semi-synthetic antibiotic minocycline by Stille coupling is reported using this method.

There have been numerous reports of palladium-catalyzed carbon−nitrogen bond formation in recent years.[11] Since we have found 7-iodosancycline to be a useful coupling partner in Stille and Suzuki reactions, the application of recent efforts in the amination of aryl iodides[12] has allowed the preparation of a number of 7-alkylamino, 7-arylamino, and 7-cycloalkylamino derivatives not available from 7-aminosancycline. The majority of the aminations reported by Buchwald's group employ sodium *tert*-butoxide; however, it is known that

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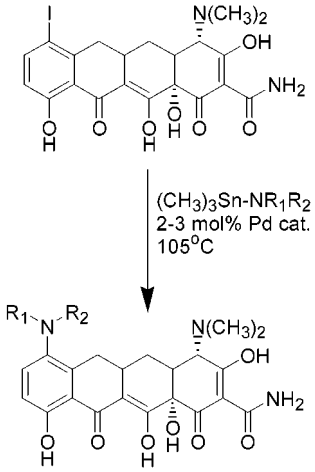
SCHEME 1. Synthesis of 7-Aminotetracyclines



tetracyclines are not tolerant of strongly basic conditions. We have found that under these conditions the tetracycline precursors decompose readily. However, aminations with in situ generated aminostannanes[11a] provide a mild alternative to amination of aryl bromides and iodides. This transamination reaction was originally reported by Jones and Lappert[13] and applied to the synthesis of primary and secondary aryl aminostannanes. The reaction conditions are sufficiently mild and allow the facile incorporation of a variety of amines in the C-7 position.

The reaction of 7-iodosancycline with (*N*,*N*-dimethylamino)trimethyltin in toluene in the presence of 2.5 mol % $PdCl_2(P(o\text{-tolyl})_3)_2$ affords 7-(*N*,*N*-dimethylamino)sancycline **2a** (minocycline) after 8 h in 68% yield. (Scheme 1) Alkyl-, aryl-, and cycloalkylamines (Figure 1) are incorporated by the transamination reaction with (*N*,*N*-dimethylamino)trimethyltin. Although no efforts were made to optimize the yields in these reactions, it is readily apparent that this methodology affords good yields of the desired product within 24 h. *N*,*N*-Dimethylaminosancycline was observed as a side product in several of the reactions and is most likely due to incomplete transamination of the (*N*,*N*-dimethylamino)trimethyltin species. In some cases (**2e**, **2h**, and **2i**), the yield of this side product approached 10%, significantly reducing the yield. Others have concluded that in order to achieve high yields it was necessary to distill the (*N*,*N*-dimethylamino)trimethyltin and handle it under anhydrous conditions. However, in this case, no effort was made to further purify the aminostannane, which may have also affected the reported yields due to the sensitivity of the aminostannane to water. The in situ generation of aminostannanes is a mild alternative to other aminations and applied to tetracyclines affords good yields of the desired products.

There are many significant advantages to this method for preparing 7-aminotetracyclines. It is experimentally simple, operates under mild conditions, and is tolerant of the polyhydroxylated substrate. A wide variety of amines may be incorporated via transamination with an aminostannane that is commercially available at a

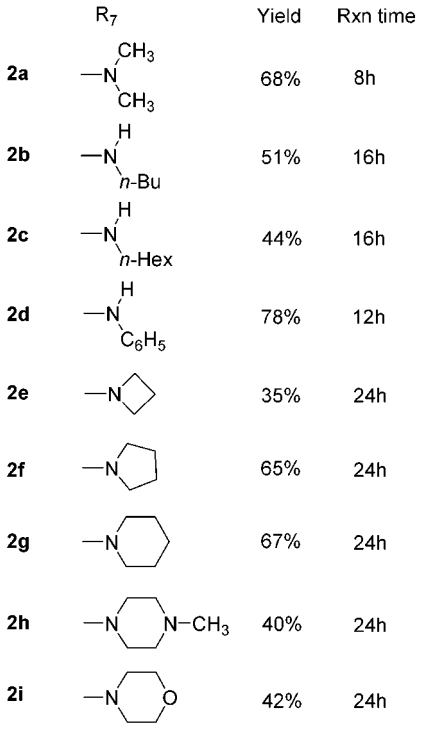


FIGURE 1. Isolated yields of 7-amino derivatives.

reasonable cost and does not require excessive amounts of catalyst to carry out. This method is also advantageous in that a commercially important antibiotic, minocycline, can be synthesized in two steps starting from sancycline. Industrially, minocycline is prepared by blocking the reactive C-9 position with a *tert*-butyl followed by nitration to afford 9-*tert*-butyl-7-nitrosancycline. The nitro group is then reduced in the presence of formaldehyde and the *tert*-butyl protecting group removed to afford minocycline. The methodology reported herein allows minocycline to be synthesized in 68% (unoptimized) yield from a readily available precursor.

In summary, we have demonstrated a concise approach to the synthesis of novel 7-amino substituted tetracycline derivatives. This method is allows a large number of derivatives to be synthesized in a combinatorial fashion which are critical for structure activity relationships. Extension of this methodology to other tetracyclines such as methacycline may be easily accomplished under similar conditions. Efforts to modify the reaction conditions and apply this methodology to other halogenated substrates such as chlortetracycline are ongoing. Presently, biological assessment of these compounds is being undertaken and the synthesis of derivatives with unique amino pharmacophores may shed light on the importance of functionalization in this position.

Experimental Section

General Procedures. All reagents were of reagent-grade quality and used without further purification. 7-Iodosancycline was prepared using a previously reported method.[15] Melting points were measured with a Mel-Temp apparatus and were uncorrected. Elemental analysis was performed by MWH Laboratories, Phoenix, AZ. Analytical thin-layer chromatography was

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performed using 0.25 mm Merck reversed-phase C18 silica gel 60 plates developed with 0.1% trifluoroacetic acid in acetonitrile. Components were visualized by illumination with ultraviolet light (254 nm) after exposure to ammonia vapor and by staining with Fast Blue B salt in methanol. IR spectra were run at room temperature as KBr pellets between 4000 and 400 cm^{-1}. HPLC analysis was carried out using a binary pump, polymeric stationary phase column, and UV detection at 330 nm using 50/50 mixture of glacial acetic acid and water as the mobile phase. High-resolution mass spectral (HRMS) data was obtained by flow injection analysis. ^1H NMR spectra were recorded at 300 MHz and referenced to internal TMS or residual protio solvent.

7-Phenylaminosancycline 2d (Representative Procedure). A 20.0 mg (0.01 mmol) sample of (*N*,*N*-dimethylamino)-trimethyl tin was added to 10.0 mg (0.01 mmol) of aniline in 2.0 mL of toluene. The resulting light brown solution was heated to reflux for 1 h under argon purge. A solution of 50.0 mg of 7-iodosancycline and 2 mg of $PdCl_2(P(o\text{-tolyl})_3)_2$ in 3.0 mL of toluene was added to the initial solution and the resulting dark brown solution allowed to reflux under nitrogen for 12 h or until the reaction appeared complete by thin-layer chromatography. The solution was cooled to room temperature, treated with 20% aqueous KF, and extracted (3×) with ethyl acetate. The combined ethyl acetate fractions were dried over sodium sulfate, and the solvent was removed in vacuo yielding 36 mg (78%) of the title compound as dark yellow crystals: ^1H NMR (300 MHz, DMSO-d_6) δ 7.61 (m, 5H), 7.10 (m, 2H), 4.08 (s, 1H), 3.2−3.4 (br m, 12H), 2.63 (m, 2H), 1.61 (m, 4H), 0.98 (m, 2H); IR (KBr) 3346, 3031, 2869, 1665, 1613, 1575, 1453, 1359, 1296, 1203, 1054, 709, 497 cm^{-1}; HRMS calcd for $C_{27}H_{27}N_3O_7$ 505.5193 amu, found 505.5195 amu. Anal. Calcd for $C_{27}H_{27}N_3O_7$: C, 64.15; H, 5.38; N, 8.31. Found: C, 64.10; H, 5.41; N, 8.32. The crystalline compound obtained was sufficiently pure for preliminary biological analysis. Analytical samples were purified by preparative thin-layer chromatography and converted to the corresponding hydrochloride salt by dissolving the compound in methanol and adding concentrated $HCl_{(aq)}$.

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