Biomimetic Synthesis of Macrolide/Ketolide Metabolites through a Selective *N*-Demethylation Reaction

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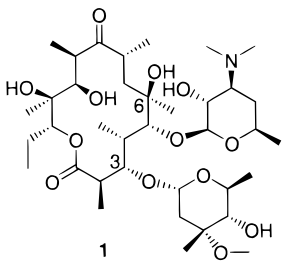
The prevalence of multiple drug resistant bacteria has renewed interest in the area of antibiotic research.[1] Structural modification of existing, naturally occurring antibiotics remains one of the most effective approaches for overcoming bacterial resistance. Erythromycin (**1**), a safe and effective macrolide antibiotic with well-defined mechanism of action and resistant mechanism, is one of the focal points for such modifications.[2] Recently, we have discovered a novel series of erythromycin derivatives, the 6-*O*-substituted ketolides, depicted by structure **2**, which



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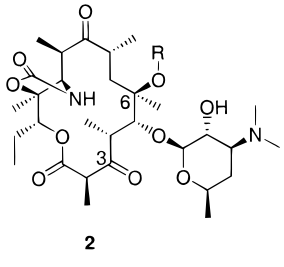

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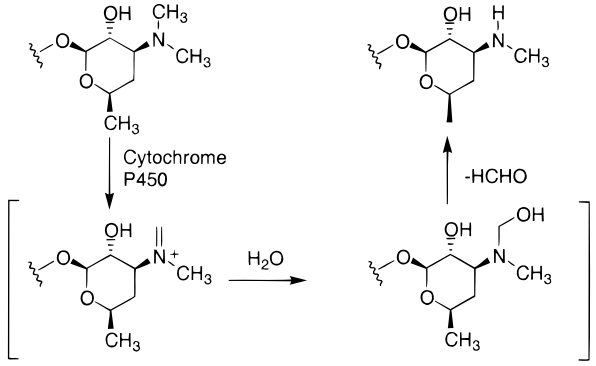
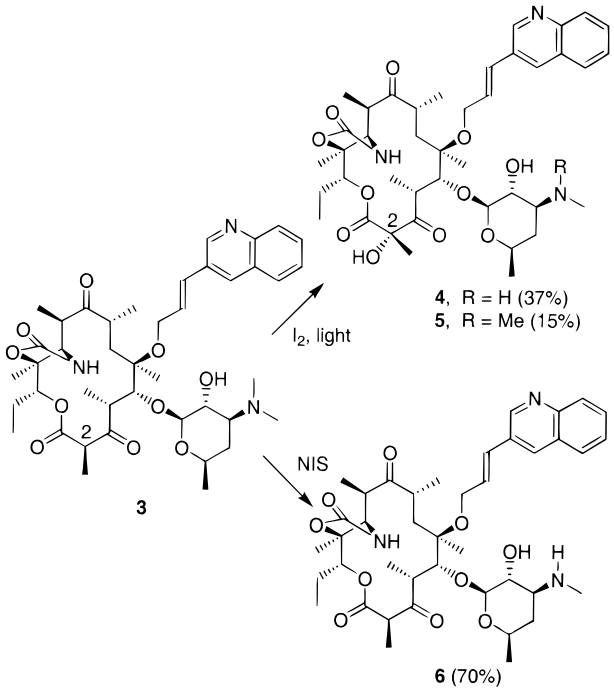
are highly active against multiple drug resistant bacteria.[3] Macrolides and ketolides share a common metabolic pathway mediated by human cytochrome P450 enzymes, resulting in the *N*-demethylation of the desosamine group (Scheme 1).[4] To properly characterize the activity/toxicity profiles and prepare derivatives of these metabolites, an efficient chemical synthesis of the *N*-desmethyl metabolites thus became necessary.

Scheme 1. Metabolism of Macrolides Mediated by Cytochrome P450 Enzymes



Scheme 2. *N*-Demethylation of Ketolides



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Although several *N*-demethylation methods are available, they are not applicable to macrolides due to the existence of many sensitive functionalities.[5] In the past, chemical *N*-demethylation of macrolides was typically performed in a stepwise manner by reacting macrolides with an alkyl chloroformate followed by removal of the acyl group.[6] Freiberg developed a one-step procedure for the *N*-demethylation of clarithromycin (**9**) by using iodine in methanol under light.[7] However, when we applied these conditions to ketolide **3**, a mixture of 2-hydroxy-*N*-desmethyl ketolide **4** and 2-hydroxy ketolide **5** was obtained (Scheme 2). None of the desired *N*-demethylation product **6** was observed. This unexpected preferential C-2 hydroxylation over the *N*-demethylation is apparently due to the introduction of the 3-keto group, which activates the C-2 position. Under Freiberg's conditions, the C-2 position appeared to be more reactive than the dimethylamino group, which resulted in the formation

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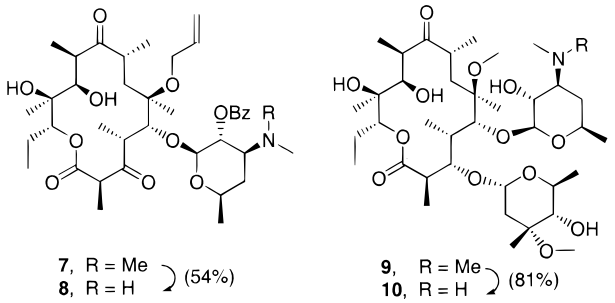
of 2-iodo derivatives through a radical process. The unstable 2-iodo compounds were then hydrolyzed upon aqueous work up to give **4** and **5**.[8]

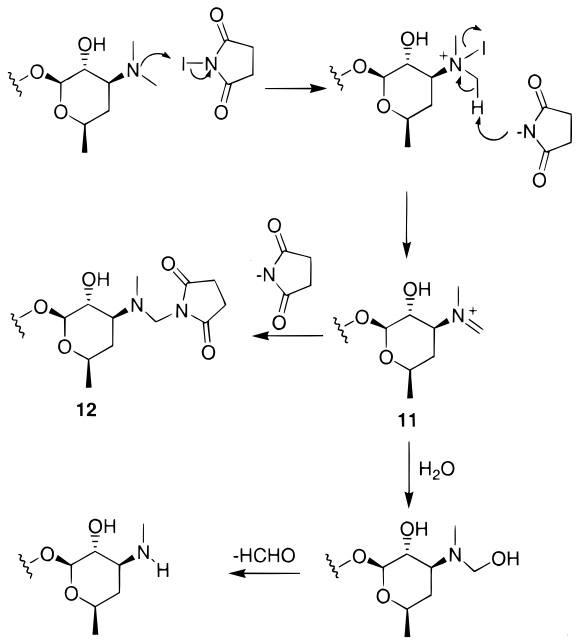
To reverse the selectivity between the C-2 position and the dimethylamino group, we examined *N*-iodosuccinimide (NIS), a more electrophilic iodonium source which is more likely to react with the nucleophilic dimethylamino group. Also the polarized N−I bond in NIS is a better mimic of the Fe−O species of the cytochrome P450 enzymes, which gives only the *N*-demethylation metabolites.[9] We found that NIS indeed selectively removes one methyl group from the desosamine nitrogen without interfering with the C-2 position. When ketolide **3** was treated with 1.2 equiv of NIS in anhydrous acetonitrile at 0−20 °C for 12 h, the desired *N*-desmethyl compound **6** was obtained in 70% yield after aqueous workup, with no 2-hydroxy side products observed (Scheme 2). Under the same conditions, 6-*O*-allyl ketolide **7** was converted to the desmethyl metabolite **8** in 54% yield. These conditions could also be applied to macrolides other than ketolides. For example, clarithromycin (**9**), upon treatment with NIS, gave the *N*-desmethyl metabolite **10** in 81% yield. When *N*-bromosuccinimide (NBS) was used in place of NIS for the demethylation of **7**, a mixture of undesired products was obtained.[10]



We postulated that the NIS demethylation proceeds in a fashion similar to the enzymatic process, which involves the formation of an iminium cation and carbinolamine intermediate (Scheme 1).[11] As illustrated by Scheme 3, the dimethylamino group attacks NIS to form an *N*-iodoammonium intermediate which eliminates to give the iminium cation **11**. Aqueous hydrolysis of **11** provides the carbinolamine, which eliminates formaldehyde to form the desired *N*-desmethyl metabolite. The formation of iminium intermediate **11** has been supported by the isolation of a minor product **12**, an addition product of the succinimide anion to the iminium cation **11**.

In conclusion, we have demonstrated that NIS can be used as a selective *N*-demethylating agent under mild conditions. These conditions were successfully applied to the synthesis of metabolites of potent antibacterial agents within the macrolide and ketolide classes. The conditions developed here should be applicable to other synthesis where selective *N*-demethylation is required.

Scheme 3. Mechanism of *N*-Demethylation Mediated by NIS



Experimental Section

Clarithromycin (**9**) was provided by the Abbott Process Research Department. 6-*O*-Allyl ketolide **7** and 6-*O*-(3-quinolyl)-allyl ketolide **3** were synthesized according to reported procedures.[12] All solvents and reagents were obtained from commercial sources and used without further purification. Flash column chromatography was performed on Merck silica gel 60 (230−400 mesh). Melting points were recorded on a Fisher-Johns apparatus and are uncorrected. All elemental analyses were performed by Robertson Microlite Laboratories, and the data for carbon, hydrogen and nitrogen reported are within 0.4% of theoretical values.

General Procedure. ***N*-Demethylation of 6-*O*-(3-Quinolyl)-allyl Ketolide 3**. To a solution of 6-*O*-(3-quinolyl)allyl ketolide **3** (382 mg, 0.500 mmol) in 20 mL of anhydrous acetonitrile was added *N*-iodosuccinimide (135 mg, 0.600 mmol) in small portions at 0 °C under N_2. The reaction mixture was warmed to room temperature and stirred for 12 h to give a brown solution. The reaction mixture was diluted with ethyl acetate and washed sequentially with 5% $NaHSO_3$, 5% Na_2CO_3, and brine. The resulting organic solution was dried over Na_2SO_4 and evaporated. Flash chromatography eluting with 95:5:0.5 CH_2Cl_2:MeOH:NH_3·H_2O gave *N*-desmethyl-6-*O*-(3-quinolyl)allyl ketolide **6** (261 mg, 0.348 mmol, 70%) as white foam: MS (ESI) *m/e* 752 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 9.02 (1H, d, $J = 2.4$ Hz), 8.19 (1H, d, $J = 2.4$ Hz), 8.06 (1H, d, $J = 8.4$ Hz), 7.84 (1H, d, $J = 8.4$ Hz), 7.64 (1H, m), 7.52 (1H, m), 6.57 (1H, d, $J = 16.5$), 6.19 (1H, dt, $J = 16.5$, 6.0 Hz), 5.51 (1H, br s), 4.96 (1H, dd, $J = 9.0$, 3.6 Hz), 4.44 (1H, d, $J = 4.5$ Hz), 4.38 (1H, d, $J = 7.5$ Hz), 3.97 (1H, q, $J = 6.6$ Hz), 3.92 (1H, br s), 3.85 (1H, m), 3.72 (1H, m), 3.61 (1H, m), 3.23 (1H, m), 3.14 (1H, dd, $J = 9.0$, 7.5 Hz), 2.98 (1H, m), 2.65 (1H, m), 2.48 (1H, m), 2.42 (3H, s), 1.50−2.10 (3H, m), 1.50 (3H, s), 1.45 (3H, s), 1.42 (3H, d, $J = 6.9$ Hz), 1.39 (3H, $J = 7.8$ Hz), 1.10−1.19 (9H, m), 0.80 (3H, t, $J = 7.5$ Hz). Anal. Calcd for $C_{41}H_{57}N_3O_{10}$·H_2O: C 63.96, H 7.72, N 5.46. Found: C 64.14, H 7.73, N 5.34.

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Supporting Information Available: Synthetic procedure for **4** and **5**; analytical data for **4**, **5**, **8**, and **10.** This material is available free of charge via the Internet at http://pubs.acs.org.

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