A ketolide was prepared from 14,15-dehydroerythromycin A by two different routes. The first approach involving oxidation of the 3-OH of 3-descladinosyl-14,15-dehydroerythromycin A 2'-O-acetate gave unexpectedly high levels of 3,11-double oxidation. This may be due to greater formation of the 9,12-hemiketal in 14,15-dehydroerythromycin A and concomitant exposure of the 11-OH group for oxidation. NMR studies of 14,15-dehydroerythromycin A support this hypothesis, revealing a 9 : 1 ratio of 9-ketone to 9,12-hemiketal in CDCl₃ and a 1 : 1 ratio in CD₃OD as contrasted with the corresponding tautomer ratios of 30 : 1 in CDCl₃, and 6 : 1 in CD₃OD with erythromycin A. Alteration of the 13-substituent on the erythronolide A ring from ethyl to vinyl thus favors formation of the 9,12-hemiketal. A second route to the ketolides was developed based on these findings, in which the 11-OH is eliminated prior to oxidation of the 3-OH.

Macrolide antibiotics such as erythromycin have long been used for treatment of upper and lower respiratory infections against Gram-positive bacteria. Such antibiotics have the advantage of a relatively small number of side effects. Degradation of erythromycin in the acidic environment of the stomach yields 8,9-anhydroerythromycin A 6,9-hemiketal, which causes gastrointestinal discomfort.¹ The analogs clarithromycin,² azithromycin³ and roxithromycin⁴ were developed to avoid formation of such by-products and improve pharmacokinetic properties of erythromycin.⁵

A more recent problem with erythromycin A and subsequent derivatives is the increasing incidence of resistance. A new class of macrolides known as ketolides show promising activity against erythromycin A-resistant microorganisms. Ketolides have been synthesized by replacing the 3-cladinosyl group with a carbonyl and addition of an N-alkylaromatic substituted C₁₁-C₁₂ cyclic carbamate which enhances ribosomal binding.⁶ Synthesis and biological evaluation of a number of different ketolides with various aromatic substituents has proven useful in optimizing the effectiveness of such compounds. However, the effects of substitution at the 13-position of the erythromycin A derivatives on antibacterial activity have not been thoroughly investigated.

Technology developed by Khosla and co-workers allows manipulation of the polyketide synthases that generate a number of natural products.⁷ This technique has been applied to make erythromycin analogues with different substituents at the 13 position such as 14,15-dehydroerythromycin.⁸ We report here the synthesis of a 14,15-dehydroketolide and describe unexpected effects of the 13-substitution on the tautomeric equilibria of erythromycin A.

Results and Discussion

The 14,15-dehydroerythromycin A was prepared by precursor-directed biosynthesis.⁹ A route previously reported for making erythromycin-based (13-ethyl) ketolides was initially used in an attempt to make the 14,15-dehydroketolide ⁷b. However, the conformational or
Fig. 1. Conversion of 6-O-methylerythromycin A (1a) and 6-O-methyl-14,15-dehydroerythromycin A (1b) to the corresponding ketolides by initial route.

In all cases (a) R=CH₂CH₃, (b) R=CH=CH₂. Reagents: (A) HCl, H₂O; (B) acetic anhydride, K₂CO₃, CH₂Cl₂; (C) L-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, DMSO, pyridinium trifluoroacetate; (D) methanesulfonyl chloride, pyridine; (E) 1,8-diazabicyclo[5.4.0]undec-7-ene, acetone.

Upon complete consumption of 3b, the products 4b and 5b were detected in approximately equal amounts. Mass spectrometric analysis of partial reactions indicated a 1:2:1 mixture of starting material 3b, product 4b, and the doubly-oxidized product 5b, suggesting that it would not be possible to control the formation of 5b and still achieve acceptable levels of conversion. An efficient separation of 4b and 5b could not be achieved, so the mixture was carried forward. Treatment of a mix of 4b and 5b with methanesulfonyl chloride and subsequently with diazabicycloundecene provided 7b and 8b, which could be separated and fully characterized. Structural determination...
of 8b corroborated the suspected structure of the doubly oxidized compound 5b.

The observed difference in reactivity between 3a and 3b strongly suggests underlying conformational or tautomeric differences between the two molecules. Evidence for this hypothesis was obtained through NMR studies of the corresponding erythromycins. Erythromycin A has been shown to exist as a mixture of 9-keto- (9A), 9,12-hemiketal- (9B), and 6,9-hemiketal- (9C) forms, with the ratio of tautomers depending strongly upon the solvent (Fig. 2). The ratio of 9A to 9B is approximately 30:1 in CDCl₃ but 6:1 in CD₃OD. Tautomer 9C is very minor (2~5%) in both media. Examination of the NMR spectra of 14,15-dehydroerythromycin A (10A) revealed similar tautomeric forms, although with markedly different ratios of the tautomers. The ratio of 10A to 10B is 9:1 in CDCl₃ and 1:1 in CD₃OD. As with erythromycin, IOC remains minor in both media. Tautomer 10B was clearly identified as the major hemiketal in CD₃OD by NMR. The 9-keto tautomer 10A shows resonances for C9, C12, and C6 at <5 219.9, 75.1, and 74.3, respectively, whereas the major hemiketal tautomer shows these signals at <5 107.0, 82.0, and 74.1, consistent with the 9,12-hemiketal 10B. Assignments were made using HSQC and HMBC experiments, and agree with values reported for the tautomers of 9.

Formation of the 9,12-hemiketal is thus more favored when the 13-group is vinyl than when it is ethyl. The cause of this is uncertain, although a reduced steric demand for the vinyl group in the 9,12-hemiketal is a likely contributing factor. Formation of 9B results in a syn-pentane-type interaction between the 14-CH₂ and 11-OH groups, which is lessened in 10B due to the planarity of the 14-CH. We hypothesize that formation of the 9,12-hemiketal and concomitant exposure of the normally unreactive 11-OH may account for the different reactivities of 3a and 3b towards oxidation.

To avoid these difficulties, we developed a synthetic sequence in which the 10,11-alkene is introduced prior to the oxidation step (Fig. 3). Following protection of the 2'-hydroxyl of 1, mesylation provides the 4",11-O-mesylate 12 and the 11-O-mesyl group is eliminated by treatment with base to yield 13. The 4-O-mesylcladinose is hydrolyzed, and the resulting 3-hydroxyl is oxidized to provide the ketolide 15. When applied to 1b, this sequence provided 15b in 28% overall yield, substantially improved over the 14% yield obtained using the sequence in Figure 1. Hydrolysis of the 4-O-mesylated-cladinose from 13 requires harsher conditions than observed with 1, yet provides 14 in good yield. This approach was also successful with R=CH₃CH₂, converting 1a into ketolide 15a in 35% overall yield compared with 25% yield obtained using the procedure of Figure 1.

These results demonstrate an unexpected effect of the 13-substituent on the tautomeric equilibria and consequent reactivity of erythromycin A. An alternate route for the preparation of the ketolides from clarithromycin and its analogs was developed to compensate for these effects which resulted in a higher overall yield and ease of
Materials and Methods

General Experimental Procedures

14,15-Dehydroerythromycin A was produced by fermentation by precursor-directed biosynthesis. All other materials were reagent grade and used as provided unless otherwise stated. All reactions were performed under N₂ atmosphere. Column chromatography was performed with Merck 60 230-400 mesh silica gel. Thin layer chromatography was performed on pre-coated glass with silica gel 60 and the spots were visualized by vanillin followed by heating. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded in CDCl₃ solution at 300 K (Bruker DRX-400 spectrometer). Mass spectra were obtained using electrospray ionization with a PE/Sciex API-100LC mass spectrometer equipped with a Turbo Ion Spray source.

6-O-Methyl-14,15-dehydroerythromycin A (1b)

(A) 14,15-Dehydroerythromycin A 9-oxime (IA)

A solution of 14,15-dehydroerythromycin A (102 mg, 0.139 mmol) in 0.310 ml of 2-propanol, 50% aq hydroxylamine (0.101 ml, 1.53 mmol), and AcOH (32 µl, 0.556 mmol) was heated at 50°C for 24 hours. The reaction mixture was cooled to ambient temperature and sat'd aq NaHCO₃ was added to pH 12. The product was extracted with CH₂Cl₂ (4×30 ml) and the organic layer was washed with brine and dried to give IA (90 mg, 87%) as a white solid. MS m/z 748 (M+H)+. The product was carried on without purification.

(B) 14,15-Dehydroerythromycin A 9-(O-(1-isopropoxy-cyclohexyl)oxime (IB)

A mixture of IA (312 mg, 0.418 mmol) in 2.09 ml of CH₂Cl₂ and 1,1-diisopropoxycyclohexane (418 mg, 2.09 mmol) was stirred with 158 mg (0.627 mmol) of pyridinium
p-toluenesulfonate for 2 days. Addition of 1 N NaOH to pH 12 and extraction with CH₂Cl₂ (4×50 ml) yielded crude product. Column chromatography (99 : 1 toluene/Et₂N to 70 : 29 : 1 toluene/Me₂CO/Et₃N) gave **IB** (308 mg, 83%) as a white solid.

**H NMR** δ 6.06 (1H, ddd, J=17.3, 10.9, 4.6 Hz), 5.59 (1H, m), 5.23 (1H, dt, J=10.9, 1.6 Hz), 5.17 (1H, dt, J=17.3, 1.6 Hz), 4.88 (1H, d, J=4.6 Hz), 4.42−4.47 (1H, m), 4.14−4.00 (3H, m), 3.82−3.75 (2H, m), 3.59 (1H, d, J=7.3 Hz), 3.55−3.47 (1H, m), 3.33 (3H, s), 3.23 (1H, dd, J=10.1, 7.3 Hz), 3.07−2.98 (1H, m), 2.90 (1H, dq, J=7.5, 7.5 Hz), 2.70 (1H, q, J=7.0 Hz), 2.40−2.50 (1H, m), 2.37 (1H, d, J=15.4 Hz), 2.31 (6H, s), 2.09−2.01 (1H, m), 1.98−1.92 (1H, m), 1.90−1.81 (2H, m), 1.68−1.50 (9H, m), 1.49 (3H, s), 1.40−1.33 (2H, m), 1.32−1.10 (31H, m).

**MS** m/z 888 (M+H)+.

(C) 2',4'-bis-O-Trimethylsilyl-14,15-dehydroerythromycin A 9-[O-(1-isoproxyocyclohexyl)oxime] (IC)

A solution of **IB** (80 mg, 0.090 mmol) in CH₂Cl₂ (0.90 ml) was treated with 1-trimethylsilylimidazole (36 µl, 0.24 mmol) and chlorotrimethylsilane (20 µl, 0.153 mmol). The solution was stirred at ambient temperature for 10 minutes. The reaction mixture was diluted with 50 ml of CH₂Cl₂ and washed with 1 N NaOH solution. The aqueous layer was washed with CH₂Cl₂ (3×60 ml). The organic extracts were combined, dried, and evaporated to give the desired product as a white foam (90 mg, 97%). Analysis by LC-MS revealed the product to be more than 95% pure and was carried on without purification. **MS m/z** 1032 (M+H)+.

(D) 2',4'-bis-O-Trimethylsilyl-6-O-methyl-14,15-dehydroerythromycin A 9-[O-(1-isoproxyocyclohexyl)oxime] (ID)

A solution of **IC** (340 mg, 0.330 mmol) in 1.98 ml of 1 : 1 DMSO/THF was treated with 0.330 ml of a 2 M solution of methyl bromide in ether. The solution was cooled with an ice-water bath to 0°C. A mixture of 0.660 ml of a 1 M KO₂Bu in THF and 0.660 ml of DMSO was added dropwise over 4 hours until the reaction was completed as determined by TLC and MS. The reaction mixture was diluted with CH₂Cl₂ and washed with 1 N NaOH. The aqueous layer was washed with CH₂Cl₂ (4×50 ml). The organic extracts were combined, washed with brine, and evaporated to give **ID** as a white foam. **MS m/z** 1045.7 (M+H)+.

(E) 6-O-methyl-14,15-dehydroerythromycin A 9-oxime (IE)

A solution of **ID** (344 mg, 0.329 mmol) in 2 : 1 CH₃CN/H₂O (5.06 ml) and AcOH (1.83 ml, 31.9 mmol) was stirred at ambient temperature for 8 hours. The reaction was adjusted to pH 12 with 1 N NaOH and extracted with CH₂Cl₂ (4×50 ml). The organic extracts were combined, washed with brine, and dried to give **IE** as a white solid (245 mg). **MS m/z** 761.5 (M+H)+.

(F) 6-O-Methyl-14,15-dehydroerythromycin A (1b)

A mixture of **IE** (245 mg, 0.322 mmol) in 3.22 ml of 1 : 1 EtOH/H₂O, sodium hydrosulfite (561 mg, 3.22 mmol) and 96% formic acid (121 µl, 3.22 mmol) was heated at 90°C for 2.5 hours. Partial evaporation of the solvent, addition of 1 N NaOH, extraction with CH₂Cl₂ (4×50 ml), and evaporation of the extract yielded **1b**. **MS m/z** 746.6 (M+H)+.

5-O-Desosaminyl-6-O-methyl-14,15-dehydroerythronolide A (2b)

A solution of **1b** (170 mg, 0.227 mmol) in 5 ml of 5% aqueous HCl was stirred for 2.5 hours, then 1 N NaOH was added and the mix was extracted with CH₂Cl₂ (4×50 ml). The organic extracts were combined, washed with brine and concentrated. The residue was purified by column chromatography (90 : 9 : 1 toluene/Me₂CO/Et₃N to 70 : 29 : 1) to give **2b** as a white solid (110 mg, 57% over 4 steps, from **ID**). **H NMR** δ 6.08 (1H, ddd, J=17.2, 10.8, 4.8 Hz), 5.70 (1H, m), 5.25 (1H, dt, J=10.8, 1.6 Hz), 5.20 (1H, dt, J=17.3, 1.6 Hz), 4.40 (1H, d, J=7.3 Hz), 3.94 (1H br s), 3.86 (1H, br s), 3.69 (1H, s), 3.54 (1H, d, J=9.7 Hz), 3.54−3.47 (1H, m), 3.44−3.35 (1H, m), 3.24 (1H, dd, J=10.2, 7.4 Hz), 3.01 (1H, q, J=7.3 Hz), 2.97 (3H, s), 2.71 (1H, dq, J=10.4, 6.6 Hz), 2.65−2.55 (1H, m), 2.49 (1H, ddd, J=12.3, 10.3, 3.7 Hz), 2.26 (6H, s), 2.25−2.18 (1H, m), 2.02−1.88 (1H, m), 1.67 (1H, d, J=12.9 Hz), 1.57 (1H, d, J=13.5 Hz), 1.59 (3H, s), 1.35−1.09 (20H, m). **13C NMR** δ 220.5, 174.0, 133.4, 116.8, 106.7, 88.5, 79.0, 78.1, 74.6, 74.2, 70.6, 70.2, 69.6, 65.7, 49.5, 45.5, 44.3, 40.2, 38.7, 37.7, 35.8, 28.1, 21.2, 18.8, 17.7, 16.4, 14.6, 12.4, 8.2. **MS: m/z** 588.4 ([M+H]+).

5-O-(2'-O-Acetyldesosaminyl)-6-O-methyl-14,15-dehydroerythronolide A (3b)

A mixture of **2b** (110 mg, 0.187 mmol) in 1.34 ml of CH₃Cl₂, potassium carbonate (77 mg, 0.561 mmol), and acetic anhydride (26 µl, 0.281 mmol) was stirred for 15 hours. The reaction was diluted with CH₂Cl₂ and washed with NaHCO₃. The aqueous layer was extracted with CH₂Cl₂ (5×50 ml) and the organic extracts were combined and dried to give **3b** as a white solid (100 mg, 85%). **MS m/z** 630.6 (M+H)+.

Oxidation of **3b** (4b+5b)

A solution of pyridinium trifluoroacetate (107 mg, 0.553
mmol) in 0.245 ml of CH$_2$Cl$_2$ was added dropwise to a suspension of 3b (52 mg, 0.083 mmol), 1-[(3-dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (106 mg, 0.553 mmol), and DMSO (106 µl) in 0.550 ml of CH$_2$Cl$_2$ at 0°C over 6 hours. The reaction was stopped by addition of H$_2$O and stirring for 5 minutes. The product was extracted with CH$_2$Cl$_2$ (5 x 50 ml) and purified by column chromatography (90: 9: 1 toluene/Me$_2$CO/Et$_3$N) to give 42 mg of a white solid. LC/MS shows a 3: 2 ratio of m/z 628.5 (4b (M+H)$^+$) and m/z 626.5 (5b (M+H)$^+$) at the end of the reaction.

5-O-(2′-O-Acetyldesosaminyl)-10,11-anhydro-3-deoxy-3-oxo-6-O-methyl-14,15-dehydroerythronolide A (7b) and 5-O-(2′-O-Acetyldesosaminyl)-9,10-anhydro-3,11-dideoxy-3,11-dioxo-6-O-methyl-14,15-dehydroerythronolide A (8b)

Methanesulfonyl chloride (26 µl, 0.337 mmol) is added to a solution of the mixture of 4b and 5b from above (42 mg, 0.067 mmol) in 0.25 ml of pyridine, and the solution was stirred at ambient temperature for 22 hours. The mixture was diluted with CH$_2$Cl$_2$ and washed sequentially with satd aq NaHCO$_3$ (4 x 30 ml) and brine. Column chromatography (90: 9: 1 toluene/Me$_2$CO/Et$_3$N) gave 32 mg (68%) of the mesylate as a white film. MS: m/z = 707 [M+H]$^+$.

A solution of this product (32 mg) in 0.16 ml of acetone and 1,8-diazabicyclo[5,4,0]undec-7-ene (35 µl, 0.23 mmol) was stirred for 20 hours. Column chromatography (90: 9: 1 toluene/Me$_2$CO/Et$_3$N) gave 20 mg (70%) of a 3: 2 mixture of 7b and 8b as a white solid. The products were purified by HPLC (C$_{18}$ 40–60% CH$_3$CN in H$_2$O) to obtain 11 mg of 7b. ^1H NMR δ 6.74 (1H, s), 5.91 (1H, ddd, J = 17.2, 10.8, 6.4 Hz), 5.41 (1H, dt, J = 10.8, 1.2 Hz), 5.32 (1H, dt, J = 17.2, 1.2 Hz), 5.27 (1H, d, J = 6.4 Hz), 4.72 (1H, dd, J = 10.8, 7.6 Hz), 4.34 (1H, d, J = 7.6 Hz), 4.15 (1H, d, J = 8.4 Hz), 3.79 (1H, q, J = 6.8 Hz), 3.57–3.47 (1H, m), 3.15 (1H, d, J = 7.6, 1.2 Hz), 2.85 (3H, s), 2.64 (1H, ddd, J = 12.4, 10.4, 4.4 Hz), 2.33 (6H, s), 2.02 (6H, s), 1.80 (1H, d, J = 15.2, 4.4 Hz), 1.75–1.68 (1H, m), 1.64–1.52 (1H, m), 1.48 (3H, s), 1.44–1.35 (1H, m), 1.35–1.18 (10H, m), 1.16 (3H, d, J = 6.8 Hz), 1.10 (3H, d, J = 7.6 Hz). ^13C NMR δ 206.8, 203.1, 194.7, 168.0, 141.4, 139.6, 131.5, 120.2, 101.9, 80.7, 80.4, 78.7, 72.6, 71.5, 69.1, 63.4, 51.2, 50.5, 47.1, 40.6, 39.9, 30.4, 29.3, 22.7, 22.2, 21.3, 21.0, 18.6, 14.4, 14.0, 13.6. MS m/z 611 (M+H)$^+$. The 2′-O-acetate was lost from 8b during purification.

2′-O-Benzoyl-6-O-methyl-14,15-dehydroerythromycin A (11b)

A solution of 1b (668 mg, 0.895 mmol), benzoic anhydride (385 mg, 1.70 mmol) and Et$_3$N (0.250 ml, 1.79 mmol) was stirred in 3.6 ml of CH$_2$Cl$_2$ for 2 days. Satd aq NaHCO$_3$ was added and the product was extracted with CH$_2$Cl$_2$ (3 x 50 ml). The solvent was removed in vacuo and the residue was purified by column chromatography (90: 9: 1 toluene/Me$_2$CO/Et$_3$N) to give 477 mg (58% over 4 steps from 1b) of 11b as a white solid. MS m/z 850.6 (M+H)$^+$. The 2′-O-acetate was lost from 11b during purification.

2′-O-Benzoyl-6-O-methyl-4″-O-methanesulfonyl-14,15-dehydroerythromycin A (12b)

A solution of 12b (59 mg, 0.059 mmol) in 0.195 ml of acetone was stirred with 18 µl (0.12 mmol) of DBU for 1 day. The residue was dried in vacuo and purified by column chromatography (90: 9: 1 toluene/Me$_2$CO/Et$_3$N) to give 50 mg (75%) of 13b as a white foam. MS m/z 910.5 (M+H)$^+$. The 2′-O-acetate was lost from 12b during purification.

5-O-(2′-O-Benzoyldesosaminyl)-10,11-anhydro-6-O-methyl-14,15-dehydroerythronolide A (14b)

A mixture of 13b (337 mg, 0.370 mmol) in 1.5 ml of CH$_3$CN and 6.9 ml of a 3 N aq HCl was stirred for 22 hours. The CH$_3$CN was removed in vacuo and 1 N NaOH was added to pH 12. The product was extracted with CH$_2$Cl$_2$ (4 x 40 ml), and the combined organic extracts were concentrated to dryness. Column chromatography using a gradient (96: 4 CH$_3$Cl$_2$/MeOH to 95: 4: 1 CH$_3$Cl$_2$/MeOH/ Et$_3$N) gave 197 mg (79%) of 14b as a white foam. MS m/z 674.4 (M+H)$^+$. The 2′-O-acetate was lost from 14b during purification.
5-O-(2'-O-Benzoyldesosaminyl)-10,11-anhydro-3-deoxy-3-oxo-6-O-methyl-14,15-dehydroerythronolide A (15b)

A suspension of 14b (226 mg, 0.335 mmol) in CH₂Cl₂ (14.6 ml) was stirred with the Dess-Martin periodinane (427 mg) for 1 hour. The mixture was diluted with CH₂Cl₂, satd aq NaHCO₃ was added, and the product was extracted with CH₂Cl₂ (4×40 ml). The combined extract was dried in vacuo. Column chromatography (90:9:1 toluene/Me₂CO/Et₃N) followed by a second column (CH₂Cl₂ to CH₂Cl₂+1% Et₃N) gave 168 mg (75%) of 15b as a white foam. ¹H-NMR: δ 8.01 (2H, d, J=7.2 Hz), 7.54 (1H, t, J=7.2 Hz), 7.42 (2H, t, J=7.2 Hz), 6.69 (1H, s), 5.87 (1H, ddd, J=17.5, 10.8, 6.0 Hz), 5.38 (1H, d, J=10.8 Hz), 5.26 (1H, d, J=17.5 Hz), 5.23 (1H, d, J=6.0 Hz), 5.01 (1H, dd, J=10.4, 7.5 Hz), 4.51 (1H, d, J=7.5 Hz), 4.17 (1H, d, J=8.4 Hz), 3.68 (1H, q, J=6.8 Hz), 3.65-3.55 (1H, m), 3.18-3.08 (1H, m), 3.03 (1H, dq, J=8.0, 8.0 Hz), 2.83 (3H, s), 2.62 (6H, s), 2.00 (3H, s), 1.90-1.70 (2H, m), 1.60-1.42 (2H, m), 1.38 (3H, s), 1.29-1.23 (7H, m), 1.16 (3H, d, J=6.8 Hz), 0.92 (3H, d, J=7.6 Hz).

¹³C-NMR: δ 206.8, 203.1, 168.1, 165.1, 141.4, 139.6, 132.7, 131.5, 130.5, 129.8, 128.3, 120.2, 102.1, 80.8, 80.4, 78.7, 72.5, 71.9, 69.2, 63.8, 51.1, 50.5, 47.1, 40.7, 39.9, 37.8, 31.2, 22.2, 22.1, 21.0, 18.5, 14.3, 14.2, 13.6. MS m/z 672.4 (M+H)⁺.

References