Synthesis of (9R)-9-Dihydro-6,9-anhydroerythromycin A

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One of the major disadvantages of the antibiotic erythromycin A is the poor pharmacokinetic profile caused by its acid instability. Under low pH conditions, the formation of the relatively inactive 6,9-enol ether **1** and 6,9–9,12 spiroketal **2** occurs[1] (Scheme I), and many attempts at avoiding this degradative process through modification of the C-9 carbonyl center or blocking of the C-6 hydroxyl have been pursued.[2] In this paper we report our synthesis of 9(*R*)-dihydro-6,9-anhydroerythromycin A (**4**) and other 6,9-linked analogs which should have increased acid stability as compared to erythromycin A.

The 6,9-ether structure was targeted since modeling studies predicted conservation of the structural integrity of the lactone and pendant sugars. Our first approach involved the catalytic hydrogenation of erythromycin A enol ether and was initially carried out as per the reduction by Kurath[3] of the enol ether of erythronolide B. Under similar conditions (TFA, acetic acid, 50 psi of H_2), erythromycin A enol ether led to a complex mixture of products which, following a tedious isolation, afforded 6,9-ether **3a** as the major product in low yield. Compound **3a** was shown to have the 9(*R*) configuration and the unnatural 8(*S*) stereochemistry by X-ray crystallographic analysis[4] of the 2′-*O*-*p*-bromobenzoate derivative prepared in standard fashion (Figure 1). This stereochemical result indicates a cis addition of the reagent from the α face. By modifying the conditions[5] for the reduction, we could produce **3a** in an optimal 50% yield.

Since compound **3a** had the unnatural and normally less active configuration at C-8, other approaches toward the preparation of the natural 8(*R*) ring system were attempted. Diazotization of 9(*R*),9(*S*)-erythromycylamines,[6] deoxygenation of C-9 hemiketal intermediates,[7] and hydroboration of the enol ether all failed to produce the desired product. Since the conformation of the known 9(*S*)-dihydroerythromycin A placed the C-6 hydroxyl and the C-9 carbon within close proximity,[8] we studied the possibility of utilizing an intramolecular displacement of a C-9 leaving group.

In order to selectively modify the C-9 position, the protected derivative 2′-*O*-acetyl-4″-*O*-(benzyloxycarbonyl)-9-dihydroerythromycin A 11,12-carbonate (**6**) was required. This key intermediate was obtained through careful reduction of the triprotected analog **5** with sodium borohydride (Scheme II). No formation of the 9(*R*) epimer was observed with this substrate or other protected derivatives under these conditions. Treatment of **6** with methanesulfonic anhydride or methanesulfonyl or toluenesulfonyl chloride led only to recovery of the starting material; however, the use of thionyl chloride led to a new less polar product. Upon deacylation at C-2′ and C-4″, the product was isolated and characterized as the cyclic 6,9-sulfite **7**. Selective removal of the 11,12 carbonate was not possible without simultaneous hydrolysis of the sulfite. Although the stereochemistry of the sulfoxyl center is unknown, all data suggest that it was formed as only one epimer. The chemistry of this interesting intermediate is currently being explored.

Finally, treatment of **6** with trifluoromethanesulfonic anhydride cleanly led to a new product which was shown to be the 6,9(*R*)-ether **8a**. The triflate formed in situ is apparently displaced by the proximal C-6 hydroxyl group. Hydrogenolysis of the 4″-*O*-(benzyloxycarbonyl) group, followed by deacylation of the C-11,12-carbonate and the 2′-*O*-acetyl afforded **4** which, after X-ray crystallographic study, proved to have the desired 8(*R*),9(*R*) configuration (Figure 2).[4]

The hydrogenation of erythromycin A enol ether under our conditions has been previously reported by Omura et al. to afford a 6,9-ether shown as structure **4** although no details are available regarding its preparation or structure.[9] Our X-ray data fully establish the structure of this product to be **3** rather than **4**.[10] Even though different catalysts, solvents, and temperatures were used in our attempts to optimize this reduction, the major product in each case was **3a**.

Compound **4**, the corresponding 8(*S*),9(*R*) epimer **3a**, and cyclic sulfite **7c** all exhibit good to moderate in vitro antibacterial activity which will be described elsewhere. Further use of alcohol **6** for the preparation of other cyclic derivatives is being studied.

Experimental Section

Unless otherwise noted, materials were obtained from commercial sources and used without further purification. Methylene chloride was distilled from calcium hydride, and tetrahydrofuran was distilled from sodium. Melting points are uncorrected, and optical rotations were measued at 26 °C. Mass spectral data were obtained via FAB-MS methods.

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(2) Oximes: Egan, R. S.; Freiberg, L. A.; Washburn, W. H. *J. Org. Chem.* **1974**, *39*, 2492. Amines: Massey, E. H.; Kitchell, B. S.; Martin, L. D.; Gerzon, K. *J. Med. Chem.* **1974**, *17*, 105. 6-*O*-methyl: Watanabe, Y.; Morimoto, S.; Omura, S. US Patent 4,331,803, 1982.

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(4) Cromer, D. T.; Waber, J. T.; *International Tables for X-ray Crystallography*, The Kynoch Press: Birmingham, England, 1974; Vol. IV, Table 2.2 A. Ibers, J. A.; Hamilton, W. C. *Acta Crystallogr.* **1964**, *17*, 781. Cromer, D. T. *International Tables for X-ray Crystallography*; The Kynoch Press: Birmingham, England, 1974; Vol. IV, Table 2.3.1. TEXSAN-TEXRAY Structure Analysis Package, Molecular Structure Corp., 1985.

(5) The use of difluoroacetic acid and a buffered workup (see Experimental Section) afforded optimal yields of **3a**. Other C8,C9 isomers were also isolated by HPLC and partially characterized.

(6) We appreciate discussions with Dr. J. Tadanier about this approach.

(7) Attempted thioacylation of erythromycin A 11,12-carbonate 6,9-hemiketal simply effected 10,11-elimination to the enone. Thioacylation of **6** with (thiocarbonyl)diimidazole gave the 9-*O*-thioimidazolide which upon treatment with tributyltin hydride gave 9-deoxoerythromycin A. Formation of the 6,9-thiocarbonate was also obtained in this work, and further attempts toward its deoxygenation are ongoing.

(8) Everett, J. R.; Tyler, J. W. *J. Chem. Soc., Chem. Commun.* **1987**, 815.

(9) Sunazuka, T.; Tsuzuki, K.; Marui, S.; Toyoda, H.; Omura, S.; Inatomi, N.; Itoh, Z. *Chem. Pharm. Bull.* **1989**, *37*, 2701–2709. Omura, S.; Tsuzuki, K.; Sunazuka, T.; Marui, S.; Toyoda, H.; Inatomi, N.; Itoh, Z. *J. Med. Chem.* **1987**, *30*, 1941.

(10) The author has deposited atomic coordinates for **3a** and **4** with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.

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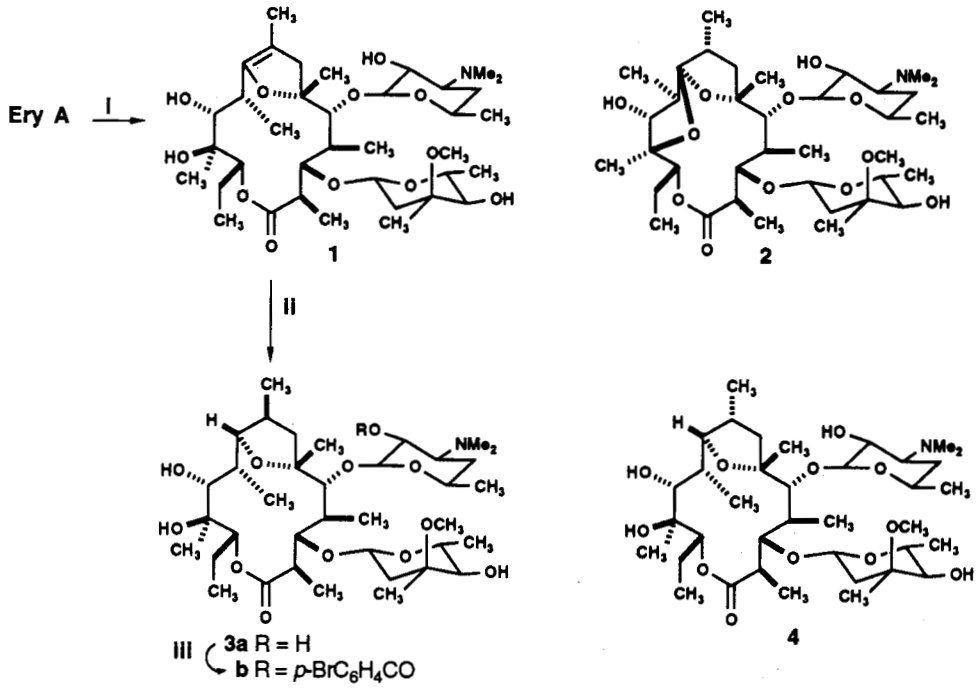
Scheme I[a]



[a] Key: (i) ref 1a; (ii) H_2, CHF_2CO_2H, HOAc; (iii) p-BrC_6H_4COCl, TEA, CH_3CN, 56%.

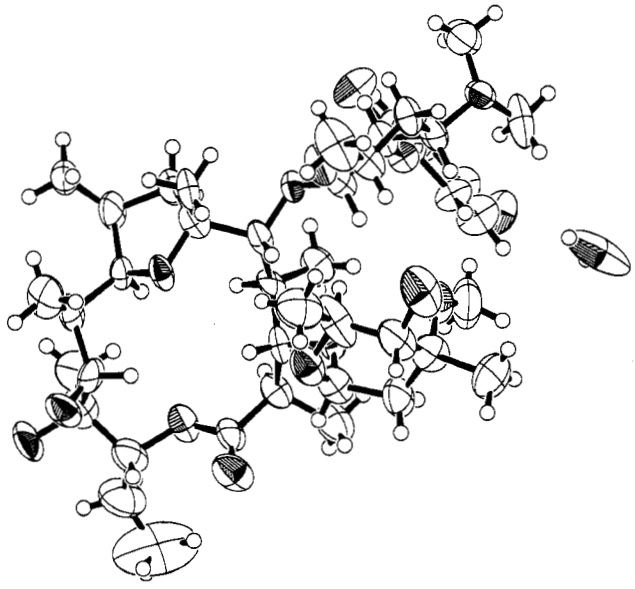


Figure 1. Molecular structure of compound **3b**.

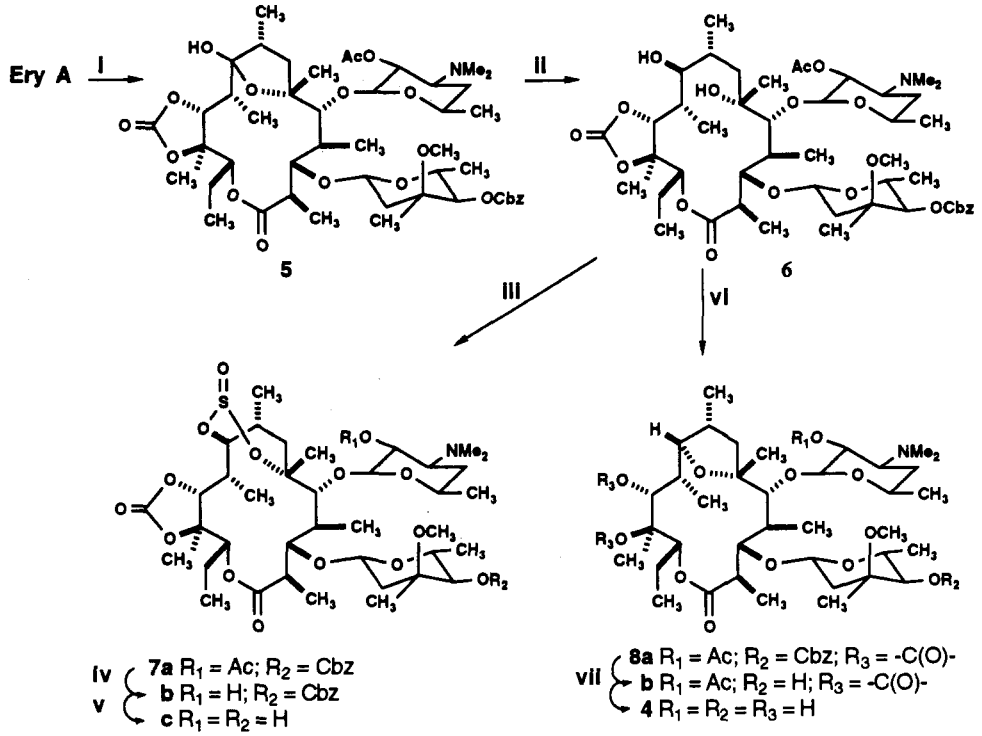
(9*R*),(8*S*)-9-Dihydro-6,9-anhydroerythromycin A (3a). To a sample of 1 (0.4 g, 0.56 mmol) dissolved in glacial acetic acid (40 mL) was added PtO_2 (0.4 g) and difluoroacetic acid (0.11 mL, 1.75 mmol). The mixture was shaken under 4 atm of H_2 at 25 °C in a Parr apparatus. The reduction was monitored by HPLC and was complete after 5 h. The reaction was quenched by the addition of ammonium acetate (0.28 g), and after 0.25 h the catalyst was removed by filtration. After concentration in vacuo (45 °C) the residue was dissolved in $CHCl_3$, 8% $NaHCO_3$, and concentrated NH_4OH (50 mL:80 mL:8 mL, respectively). The organic layer was separated and dried over sodium sulfate and the solvent removed to give crude product (0.352 g). The major product (0.206 g) was isolated by reversed-phase preparative HPLC (C-18, 7 μm, 20 × 250 mm) using H_2O/65% CH_3OH with an acetate buffer (10 g/L NaOAc·$3H_2O$, 2.5 mL/L glacial acetic acid): mp 132–138 °C; $[\alpha]_D = -55.2°$ ($c = 1.02$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.21 (d, 1H, H-1″), 4.78 (dd, 1H, H-13), 4.34 (d, 1H, H-1′), 4.22 (br t, 1H, H-3), 4.07 (dq, 1H, H-5″), 3.74 (d, 1H, H-5), 3.68 (br d, 1H, H-9), 3.49 (m, 1H, H-5′), 333 (s, 3H, OMe), 3.21 (d, 1H, H-11), 3.19 (dd, 1H, H-2′), 3.05 (d, 1H, H-4″), 2.73 (dq, 1H, H-2), 2.46 (m, 1H, H-3′), 2.37 (m, 1H, H-2″a), 2.3 (s, 6H, NMe_2), 2.24 (1H, H-8), 2.14 (m, 1H, H-10), 1.92 (m, 1H, H-14a), 1.88 (m, 1H, H-4), 1.69 (br d, 2H, H-4′), 1.57 (dd, 1H, H-2″b), 1.47 (m, 1H, H-14b), 1.46 (s, 3H, 6-Me), 1.31 (d, 3H, H-6″), 1.24 (s, 3H, 3″-Me), 1.21 (d, 3H, H-6′), 1.17 (d, 3H, 2-Me), 1.11 (s, 3H, 12-Me), 1.09 (d, 3H, 8-Me), 1.07 (d, 3H, 4-Me), 0.98 (d, 3H, 10-Me), 0.87 (t, 3H, H-15) H-7 overlapped: ^{13}C NMR ($CDCl_3$) δ 178.48 (C-1), 103.11 (C-1′), 94.47 (C-1″), 84.75 (C-6), 84.41 (C-5), 83.36 (C-9), 79.38 (C-13), 78.08 (C-4″), 76.56 (C-3), 74.65 (C-12), 72.97 (C-3″), 70.64 (C-2′), 68.91 (C-5′), 65.95 (C-5″), 65.63 (C-3′), 49.57 (C-OMe), 45.33 (C-2), 43.97 (C-4), 41.19 (C-7), 40.33 (C-NMe_2), 36.04 (C-2″), 34.69 (C-8), 33.6 (C-10), 29.41 (C-4′), 28.81 (6-Me), 21.56 (C-6′), 21.6 (3″-Me), 21.39 (C-14), 18.15 (12-Me), 18.09 (C-6″), 15.55 (C-15), 13.54 (2-Me), 11.01 (4-Me), 10.5 (10-Me), 9.84 (8-Me), C-11 overlapped: MS $[M + 1]^+ = 718$ m/z. Anal. Calcd for $C_{37}H_{67}NO_{12}·H_2O$: C, 60.39; H, 9.45; N, 1.7. Found: C, 60.59; H, 9.4; N, 1.8.

(9*R*),(8*S*)-2′-(*p*-Bromobenzoyl)-9-dihydro-6,9-anhydroerythromycin A (3b). To a solution of **3a** (30 mg) in acetonitrile (6 mL) and triethylamine (18 μL, 3 equiv) was added p-bromobenzoyl chloride (10 mg, 1 equiv). After 1 h concentrated NH_4OH (2 mL) was added and the solvent removed. The residue was purified by flash chromatography on silica gel using 5% CH_3OH in CH_2Cl_2 to give **3b** (21 mg) in 56% yield. This sample was used for X-ray analysis following recrystallization from acetonitrile, mp 165–168 °C.

9(*S*)-Dihydro-2′-acetyl-4″-Cbz-erythromycin A 11,12-Carbonate (6). (i) A solution of 2′-acetylerythromycin A (41.01 g) and 4-(dimethylamino)pyridine (25.83 g) in methylene chloride (250 mL) was cooled to −78 °C and stirred under nitrogen. Carbobenzyloxy chloride (25 mL) was added dropwise and the mixture stirred for 2 days. The mixture was diluted with methylene chloride (250 mL) and extracted with ice-cold phosphate buffer (5% KH_2PO_4/1% K_2HPO_4 (1:1)) (250 mL × 3). The organic layers were washed with brine (200 mL × 3) and dried over sodium sulfate and the solvent removed to yield 56.94 g of crude residue. Recrystallization from acetonitrile (30 mL) produced 2′-acetyl-4″-Cbz-erythromycin A (31.22 g) in 65% yield.

(ii) A solution containing 2′-acetyl-4″-Cbz-erythromycin A (2 g), carbonyldiimidazole (1.44 g), and 4-(dimethylamino)pyridine (0.54 g) in toluene (10 mL) was heated to 80 °C for 1.5 h. The mixture was diluted with methylene chloride (200 mL) and extracted with ice-cold phosphate buffer (5% KH_2PO_4/1% K_2HPO_4 (1:1)) (100 mL × 3), 5% potassium carbonate (100 mL ×

Scheme II[a]



[a] Key: (i) Ac_2O, Et_3N, CH_2Cl_2; CBzCl, 65%; Im_2CO, DMAP, 80%; (ii) $NaBH_4$, i-PrOH, 66%; (iii) $SOCl_2$, pyr, 66%; (iv) MeOH, 50 °C; (v) H_2, 20% Pd/C, MeOH; 53% (iv and v); (vi) Tf_2O, pyr, 88%; (vii) H_2, 5%, Pd/C, MeOH; aq Na_2CO_3, MeOH, 43%.

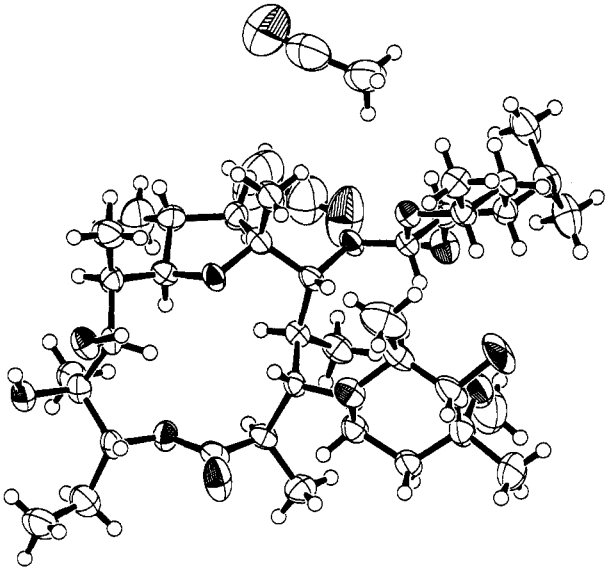


Figure 2. Molecular structure of compound 4.

3), and finally with brine (100 mL). After the organic layer was dried over sodium sulfate the solvent was removed and the crude residue was purified by flash chromatography on silica gel using 1% ammonium hydroxide in acetonitrile to give 2′-acetyl-4″-Cbz-erythromycin A 11,12-carbonate (1.65 g) in 80% yield, $[\alpha]_D$ = −55.6° (*c* = 1.12, $CHCl_3$). *Anal.* Calcd for $C_{48}H_{73}NO_{17}$: C, 61.58; H, 7.86; N, 1.5. Found: C, 61.97; H, 7.82; N, 1.5.

(iii) To a solution of 2′-acetyl-4″-Cbz-erythromycin A 11,12-carbonate (1.84 g) in 2-propanol (72 mL) was added sodium borohydride (0.48 g) at 25 °C. After being stirred overnight the mixture was cooled to 0 °C and quenched with phosphate buffer (5% KH_2PO_4/1% K_2HPO_4 (1:1)) (75 mL). The mixture was then extracted with methylene chloride (450 mL × 2), and the combined extracts were washed again with cold phosphate buffer (250 mL), cold 5% potassium carbonate (250 mL), and brine (200 mL × 3) before being dried over sodium sulfate and evaporated. Purification of the crude residue (1.84 g) via flash chromatography on silica gel was performed using 1% ammonium hydroxide in acetonitrile to give **6** (1.04 g) in 66% yield: mp 106–112 °C; $[\alpha]_D$ = −45.5° (*c* = 1.04, $CHCl_3$); MS [M + 1]$^+$ = 938 *m/z*. Anal. Calcd for $C_{48}H_{75}NO_{17}$: C, 61.46; H, 8.06; N, 1.49. Found: C, 61.39; H, 8.13; N, 1.43.

2′-Acetyl-4″-Cbz-9(*R*)-dihydroerythromycin A 6,9-Sulfite 11,12-Carbonate (7a). To a solution of alcohol **6** (120 mg) in pyridine (5 mL) stirred under nitrogen at 0 °C was added thionyl chloride (23 mg) dropwise causing coloration of the mixture. After 5 min, saturated ammonium chloride (1 mL) was added and the reaction mixture was partitioned between water and ethyl acetate. The separated organic layer was washed with dilute ammonium hydroxide and brine and dried over sodium sulfate before removal of solvent. The crude residue was purified by flash chromatography on silica gel using 5% methanol in methylene chloride to give 83 mg of **7a** as a solid (66%).

9(*R*)-Dihydroerythromycin A 6,9-Sulfite 11,12-Carbonate (7c). The crude **7a** from above was dissolved in methanol (10 mL) and heated under nitrogen to 50 °C. After 4 h the solvent was removed and the crude residue was transferred into a hydrogenation apparatus in methanol (50 mL) using 20% Pd/C (15 mg) and 4 atm of hydrogen at 25 °C. After 8 h the mixture was filtered and purified by flash chromatography on silica gel using 5% methanol–methylene chloride–2% ammonium hydroxide to give 36 mg of **7c** (53%): mp 162–164 °C; $[\alpha]_D$ = −18.3° (*c* = 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.33 (1H, H-11), 5.12 (1H, H-13), 4.94 (1H, H-1″), 4.49 (1H, H-1′), 4.03 (1H, H-3), 4.24 (1H, H-9), 3.96 (1H, H-5″), 3.54 (1H, H-5′), 3.31 (3H, OMe), 3.19 (1H, H-2′), 3.04 (1H, H-4″), 2.97 (1H, H-2), 2.88 (1H, H-8), 2.43 (1H, H-3′), 2.34 (1H, H-2″a), 2.28 (6H, NMe_2), 2.18 (1H, H-10), 2.04 (1H, H-7a), 1.87 (1H, H-14a), 1.8 (1H, H-4), 1.73 (3H, 6-Me), 1.68 (2H, H-4′), 1.61 (1H, H-2″b), 1.59 (1H, H-14b), 1.44 (3H, 12-Me), 1.28 (3H, H-6″), 1.27 (3H, 2-Me), 1.25 (3H, 3″-Me), 1.23 (3H, H-6′), 1.2 (3H, 4-Me), 1.12 (3H, 10-Me), 1.11 (3H, 8-Me), 0.89 (3H, H-15); ^{13}C NMR ($CDCl_3$) δ 174.2 (C-1), 102.6 (C-1′), 96.4 (C-1″), 84 (C-6), 84.5 (C-12), 83.7 (C-9), 81.2 (C-11), 81 (C-5), 79.5 (C-3), 77.8 (C-4″), 76.7 (C-13), 72.7 (C-3″), 70.8 (C-2′), 69 (C-5′), 65.8 (C-5″), 65.6 (C-3′), 49.4 (C-OMe), 44.8 (C-2), 39.1 (C-4), 36.5 (C-7), 40.2 (C-NMe_2), 35.1 (C-2″), 33.9 (C-10), 33.5 (C-8), 28.3 (C-4′), 24.7 (6-Me), 21.7 (C-14), 21.4 (C-6′), 21.4 (3″-Me), 21.1 (8-Me)18.6 (C-6″), 14.8 (10-Me), 6.3 (2-Me), 13.4 (12-Me), 10.1

(C-15), 9.3 (4-Me); MS $[M + 1]^+ = 808$ m/z. Anal. Calcd for $C_{38}H_{65}NO_{15}S \cdot H_2O$: C, 55.26; H, 8.18; N, 1.7. Found: C, 55.44; H, 7.79; N, 1.58.

9(*R*)-Dihydro-2′-acetyl-4″-Cbz-6,9-anhydroerythromycin A 11,12-Carbonate (8a). To 2′-acetyl-4″-Cbz-9(S)-9-dihydroerythromycin A-11,12-carbonate (**6**) (140 mg, 0.15 mmol) in methylene chloride (5 mL) at 0 °C was added pyridine (37 mg, 0.45 mmol) followed by triflic anhydride (84 mg, 0.3 mmol) creating an orange solution. After 0.5 h at 0 °C TLC analysis showed the reaction to be complete. Saturated sodium bicarbonate (5 mL) was added, and the organic layer was separated. The solvent was removed, and the residue was purified by flash chromatography on silica gel using 4% methanol in methylene chloride to yield 120 mg **8a** as an oil (88%).

(9*R*)-9-Dihydro-6,9-anhydroerythromycin A (4). To (9*R*)-9-deoxo-2′-acetyl-4″-Cbz-6,9-anhydroerythromycin A 11,12-carbonate (**8a**) (120 mg, 0.13 mmol) in methanol (5 mL) was added 10 mol % of 5% Pd/C. To this flask was attached a hydrogen balloon, and the mixture was stirred vigorously for 1 h at which time TLC analysis showed the reaction to be complete. The catalyst was filtered and solvent removed. The crude product was stirred with methanol (2 mL) and saturated potassium carbonate (2 mL) for 24 h at which time it was partitioned between methylene chloride and brine. The organic layer was separated, and the solvent was removed. The crude residue was purified by flash chromatography on silica gel using 5% methanol/2% ammonium hydroxide in methylene chloride to yield 40 mg of **4** as a white solid (43%): mp 142–146 °C; $[\alpha]_D = -63.9°$ ($c = 0.64$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.23 (d, 1H, H-1″), 4.79 (dd, 1H, H-13), 4.36 (d, 1H, H-1′), 4.18 (br t, 1H, H-3), 4.06 (m, 1H, H-5″), 3.75 (d, 1H, H-5), 3.5 (m, 1H, H-5′), 3.35 (s, 3H, OMe), 3.34 (1H, H-11), 3.2 (1H, H-2′), 3.2 (m, 1H, H-9), 3.03 (t, 1H, H-4″), 2.71 (dq, 1H, H-2), 2.43 (m, 1H, H-3′), 2.4 (m, 1H, H-2″a), 2.28 (s, 6H, NMe_2), 2.11 (m, 1H, H-10), 2.05 (m, 1H, H-8), 1.95 (m, 1H, H-14a), 1.84 (M, 1H, H-4), 1.66 (m, 2H, H-4′), 1.6 (m, 2H, H-7), 1.59 (m, 1H, H-2″b), 1.47 (m, 1H, H-14b), 1.32 (d, 3H, 6″-Me), 1.32 (s, 3H, 6-Me), 1.26 (s, 3H, 3″-Me), 1.22 (d, 3H, 6′-Me), 1.18 (d, 3H, 2-Me), 1.15 (s, 3H, 12-Me), 1.09 (d, 3H, 4-Me), 0.93 (d, 3H, 10-Me), 0.89 (t, 3H, 15-Me), 0.88 (d, 3H, 8-Me); ^{13}C NMR ($CDCl_3$) δ 178.75 (C-1), 103.26 (C-1′), 94.28 (C-1″), 89.2 (C-9), 83.35 (C-6), 82.19 (C-5), 79.19 (C-13), 78.03 (C-4″), 76.64 (C-3), 74.95 (C-12), 72.87 (C-3″), 70.7 (C-11), 70.62 (C-2′), 68.99 (C-5′), 65.96 (C-5″), 65.69 (C-3′), 49.57 (C-OMe), 45.63 (C-2), 43.75 (C-4), 42.28 (C-7), 40.31 (C-NMe_2), 34.6 (C-2″), 32.54 (C-10), 32.05 (C-8), 28.61 (C-4′), 28 (6-Me), 21.56 (C-6′), 21.31 (3″-Me), 21.26 (C-14), 18.23 (C-6″), 16.71 (12-Me), 14.71 (10-Me), 13.85 (2-Me), 11.05 (15-Me), 8.69 (4-Me), 8.22 (8-Me); MS $[M + 1]^+ = 718$ m/z; X-ray structure from acetonitrile recrystallization.