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Synthesis of a Novel Macrolide: 9(*S*)-9-Dihydro-12-*O*-methylerythromycin A via Regioselective Methylation[§]

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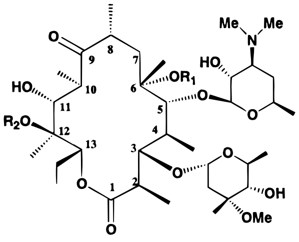
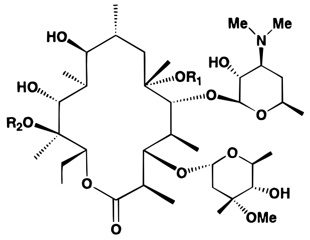
Erythromycin A (**1**) is one of the most widely used macrolide antibiotics, known for its effectiveness in treating a variety of bacterial infections.[1,2] The major drawback to erythromycin A is its acid instability. Erythromycin A easily undergoes acid degradation below pH 4,[3,4] in which an enol ether forms between the 9-carbonyl and the 6-hydroxyl groups, and a spiroketal forms via an internal ketalization between the 9-carbonyl and the 6- and 12-hydroxyl groups. To overcome this problem, numerous erythromycin derivatives,[5,6] including 9(*S*)-9-dihydroerythromycin A (**4**) and 9(*S*)-9-dihydroclarithromycin (**5**),[7] have been prepared. Although these 9-hydroxyl derivatives address the internal ketalization problem between the 9-carbonyl and the 6- and 12-hydroxyl groups, they have less antibacterial activity. However, they are important intermediates for further chemical modifications.

The reported 9(*S*)-9-dihydroerythromycin A and 9(*S*)-9-dihydroclarithromycin are obtained via reduction of the 9-carbonyl groups.[7] Analogous reduction of 12-*O*-methylerythromycin A (**3**), producing 9(*S*)-9-dihydro-12-*O*-methylerythromycin A (**6**) is unfeasible because 12-*O*-methylerythromycin A has never been reported. In erythromycin A, the 12-hydroxyl group on the aglycon ring is the least reactive of the hydroxyl groups. Conventional methylation leads only to a mixture of products, i.e., 6-*O*-methyl, 11-*O*-methyl, 6,11-bis(*O*-methyl) and 6,-12-bis(*O*-methyl) erythromycin A.[8,9]

We wish to report here the first synthesis of 9(*S*)-9-dihydro-12-*O*-methylerythromycin A (**6**). The synthesis involves a different approach compared to the chemistry used to produce 9(*S*)-9-dihydroerythromycin A and 9(*S*)-9-dihydroclarithromycin. Because erythromycin A possesses three different hydroxyl groups on the aglycon ring, it is impracticable to methylate one hydroxyl group selectively in the presence of other unprotected hydroxyl groups.[8,9] Selective 6-*O*-methylation was achieved only with erythromycin A oxime derivatives,[10] which may have a conformation of the aglycon ring different from that of erythromycin A.[8] In our synthesis, we have found that selective methylation of the 12-hydroxyl can be accomplished with the boron complex of 2′,4″-*O*-bis(trimethylsilyl)-9(*S*)-9-dihydroerythromycin A (**8**), the NaBH$_4$ reduction product that has unprotected 6-, 9-, and 11-hydroxyl groups.



1. R$_1$ = H, R$_2$ = H
2. R$_1$ = Me, R$_2$ = H
3. R$_1$ = H, R$_2$ = Me



4. R$_1$ = H, R$_2$ = H
5. R$_1$ = Me, R$_2$ = H
6. R$_1$ = H, R$_2$ = Me

Results and Discussion

In a separate project, we wished to reduce the 9-carbonyl group and then protect the 9-hydroxyl group. It is known that the 9-carbonyl group of erythromycin A can be easily reduced with NaBH$_4$ to give predominantly 9(*S*)-9-dihydroerythromycin A.[11,12] We found that reduction of 2′,4″-*O*-bis(trimethylsilyl) erythromycin A (**7**) with NaBH$_4$ went smoothly to give the 2′,4″-*O*-bis(trimethysilyl)-9(*S*)-9-dihydroerythromycin A in high yield. When this crude reduction product isolated by simple aqueous workup was reacted with MeI/NaH in DMSO/THF (1:1), only one methylation product was obtained. Another major product came from partial desilylation of the starting material and was more polar than the starting material.

The less polar product was isolated in 32% yield by preparative HPLC using a reverse-phase column. MS spectra indicated that a monomethylation product was obtained, i.e., MS(ESI) [M + H]$^+$ *m*/*z* 894 and the monomethylated descladinose derivative [M + H]$^+$ *m*/*z* 664. The chemical shifts in ^{13}C NMR spectra showed that the presence of the new methoxyl group causes downfield shifts on the C-12 (α-carbon) and upfield shifts on the C-13 (β-carbon). In the HMBC experiments, the proton and carbon long-range correlation observed between the pairs of H-13 and C-12, the OMe (at 3.43 ppm) and C-12, 11-OH and C-12, and 12-Me and C-12 all suggested that methylation occurred at the 12-OH position. In addition,

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[§] This paper is dedicated to Emeritus Professor Y. H. Ku of the University of Pennsylvania on the occasion of his 96th birthday.

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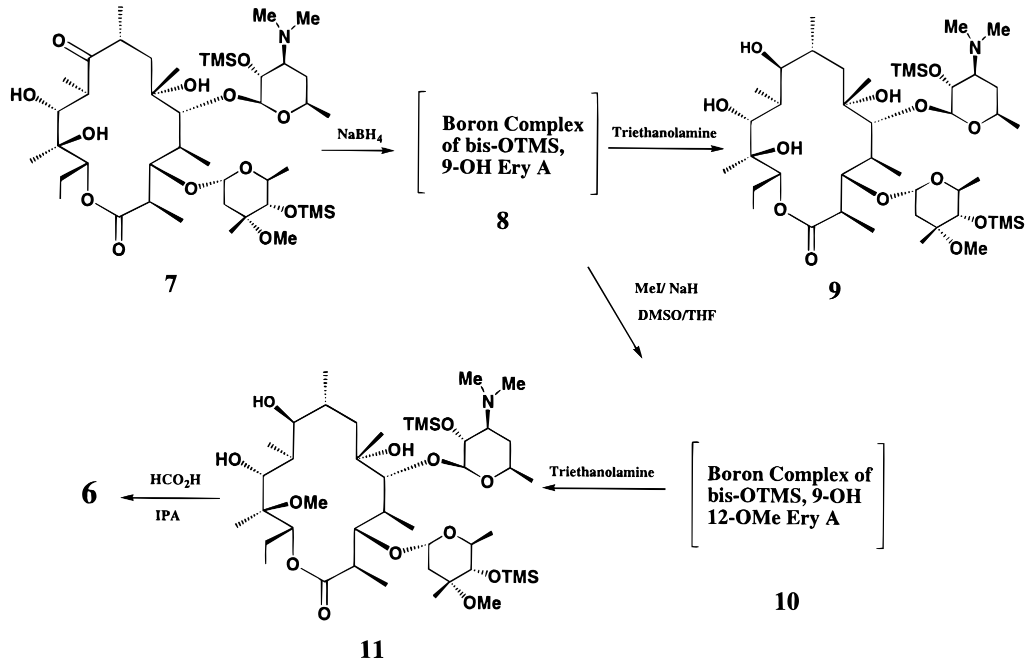
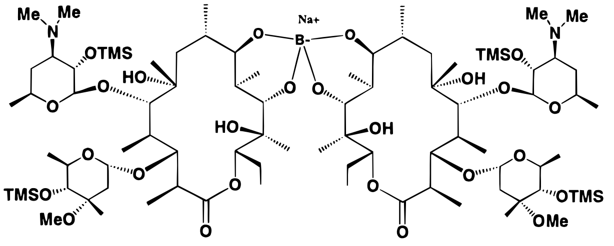
Scheme 1



the literature precedence[13,14] regarding the effects of O-methylation on NMR spectra suggested the same. We have assigned that the methoxyl group at 3.41 ppm (^{1}H NMR) and 52.5 ppm (^{13}C NMR) is attached to the 12-position. The structure confirmation was made on the basis of 2D NMR experiments (DQCOSY, HMQC, and HMBC). Thus, the structure of the methylation product has been elucidated as 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methyl-9(*S*)-9-dihydroerythromycin A (**11**) (Scheme 1). To our surprise, no 6-*O*-methyl, 9-*O*-methyl, or 11-*O*-methyl 9(*S*)-9-dihydroerythromycin A derivatives were detected.

The above results indicate that the 12-hydroxyl group of the NaBH$_4$ reduction product is the most active toward methylation, and the 6-, 9-, and 11-hydroxyl groups are inert. This is completely contrary to the fact that methylation of erythromycin A derivatives gives a mixture of methylation products and the 12-hydroxyl group is the least active among the hydroxyl groups.[8,9] A possible explanation is the formation of a boron complex (**8**) during the NaBH$_4$ reduction. The boron complex (**8**) undergoes regioselective methylation at the 12-*O*-hydroxyl group. On the basis of the MS and NMR spectra data and elemental analysis of the NaBH$_4$ reduction product obtained by aqueous workup, the structure of boron complex **8** was proposed as follows:



The ESI and FAB MS spectra all indicate a Nominal Mass of 1766, which corresponds to a boron complex with one boron atom bonding to two molecules of 2′,4″-*O*-bis(trimethylsilyl)-9(*S*)-9-dihydroerythromycin A. The ^{11}B NMR spectrum of this reduction product shows a major signal at 1.8 ppm, which is in the expected range of ^{11}B chemical shift of a tetracoordinate boron bonded to four oxygen atoms.[15] The following observation further supports the above assumption. During the workup of the NaBH$_4$ reduction of 2′,4″-*O*-bis(trimethylsilyl)erythromycin A, the reaction mixture was treated with excess triethanolamine. Methylation of this triethanolamine-treated reduction product gave a mixture of products resulting from unselective methylation at 6-, 9-, 11-, and 12-hydroxyl groups. Triethanolamine is known to be a very efficient reagent for the removal of boron from a variety of compounds. Therefore, when the reaction mixture was treated with triethanolamine, the boron was removed from the molecule and the 6-, 9-, 11-, and 12-hydroxyl groups were methylated unselectively. In light of the literature precedence of using boron as a protecting group for 1,2-diols,[16,17] it is reasonable to propose that the formation of boron complex (**8**) protected the 6-, 9-, and 11-hydroxyl groups from methylation.

Desilylation of 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methyl-9(*S*)-9-dihydroerythromycin A (**11**) was carried out with HCO$_2$H/IPA at room temperature (Scheme 1). The desilylation product (**6**) was obtained as a colorless white solid: MS (FAB) $[M + H]^+$ *m/z* 750; ^{1}H NMR (CDCl$_3$) δ 2.31 (s, NMe$_2$), 3.34 (s, 3″-OMe), 3.43 (s, 12-OMe).

Thus, we have presented a novel approach to 12-*O*-methylerythromycin A derivatives. It is the first example of regioselective methylation of the 12-hydroxyl group of erythromycin A derivatives. The chemistry described here may open a new avenue of possible synthetic modification of the 12-hydroxyl group of erythromycin A derivatives, which may lead to a series of novel 12-*O*-substituted erythromycin A derivatives. The biological

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activity of (**6**) is under investigation. With 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methyl-9(*S*)-9-dihydroerythromycin A (**11**) in hand, we are currently attempting selective oxidation of the 9-hydroxyl group. Initial experiments were not successful. Efforts will continue to find the right reaction conditions to selectively oxidize the 9-hydroxyl group to give the novel 12-*O*-methylerythromycin A.

Experimental Section

The NMR spectra were recorded on a Varain Unity 500 MHz instrument at 500.5 MHz for ^{1}H and 125.9 MHz for ^{13}C. All NMR spectra were collected at 30 °C in $CDCl_3$. The chemical shifts of the protons are measured relative to the residual $CHCl_3$ at 7.27 ppm, and the chemical shifts of carbon signals are referenced to 77.0 ppm of the $CDCl_3$. The 2D experiments, including DQCOSY, ROESY, HMQC, and HMBC, are acquired for all of the compounds, and their proton and carbon chemical shifts have been assigned completely. The electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) mass spectra were obtained using a Finnigan 7000 spectrometer, and fast atom bombardment (FAB) mass spectra were obtained using a JEOL SX102A spectrometer. 2′,4″-*O*-Bis(trimethylsilyl) erythromycin A was prepared following the literature procedure.[18,19] Flash chromatography was performed with silica gel (230−400 mesh) using 1% Et_3N and 2% MeOH in CH_2Cl_2 as the solvent system.

Boron Complex of 2′,4″-*O*-Bis(trimethylsilyl)-9(*S*)-9-dihydroerythromycin A (8). To a stirred solution of 2′,4″-*O*-bis(trimethylsilyl)erythromycin A **(7)** (10.0 g, 11.4 mmol) in ethanol (100 mL) was added sodium borohydride (0.9 g, 22.8 mmol) at ambient temperature under N_2. The mixture was stirred for 3 h before saturated NaCl solution was added. The resulting mixture was stirred for 3 min at room temperature. The mixture was partially concentrated to an aqueous suspension. The product was redissolved in EtOAc−H_2O. The organic layer was separated, washed with saturated NaCl solution, dried over anhydrous sodium sulfate, filtered, and concentrated to give the boron complex of 2′,4″-*O*-bis(trimethylsilyl)-9(*S*)-9-dihydroerythromycin A **(8)** (10.1 g 100%) as a crude product, which was used in the methylation reaction without any purification. Anal. Calcd for $C_{86}H_{166}BN_2O_{26}Si_4$: B, 0.61. Found: B, 0.58. MS: ESI(+) $(M + 2H)^+$ m/z 1768; ESI(−) M^- m/z 1766; FAB $(M + 2Na)^+$ m/z 1812. MW 1766. ^{11}B NMR: δ 1.8.

2′,4″-*O*-Bis(trimethylsilyl)-9(*S*)-9-dihydroerythromycin A (9). To a stirred solution of 2′,4″-*O*-bis(trimethylsilyl)-erythromycin A **(7)** (10.0 g, 11.4 mmol) in ethanol (100 mL) was added sodium borohydride (0.9 g, 22.8 mmol) at ambient temperature under N_2. The mixture was stirred for 3 h at room temperature. Saturated NaCl solution was added along with triethanolamine. The resulting mixture was stirred for 30 min. The product was extracted with EtOAc. The organic layer was separated, washed with saturated NaCl solution, dried over anhydrous sodium sulfate, filtered, and concentrated to give the crude product, which was further purified by flash column chromatography to give 2′,4″-*O*-bis(trimethylsilyl)-9(*S*)-9-dihydro erythromycin A **(9)** as a white solid (8.58 g, 85%). ^{1}H NMR ($CDCl_3$, ppm): δ 4.96 (d, 1H, J = 4.7), 4.85 (dd, 1H, J = 9.3, 2.7), 4.57 (d, 1H, J = 7.1), 4.24 (br. s, 1H), 4.19 (m, 1H), 4.13 (br. s, 1H), 3.70 (m, 2H), 3.67 (d, 1H, J = 7.2), 3.30 (s, 3H), 3.18 (dd, 1H, J = 9.6, 6.9), 3.15 (d, 1H, J = 9.3), 2.69 (m, 1H), 2.54 (m, 1H), 2.38 (d, 1H, J = 14.8), 2.21 (s, 6H), 2.11 (m, 1H), 1.96 (m, 1H), 1.91 (m, 1H), 1.79 (m, 1H), 1.62 (m, 2H), 1.48 (dd, 1H, J = 10.2, 4.7), 1.46 (m, 1H), 1.25 (m, 1H), 1.24 (s, 3H), 1.22 (d, 3H, J = 6.3), 1.22 (m, 1H), 1.16 (d, 3H, J = 7.1), 1.15 (d, 6H, J = 6.9), 1.14 (s, 3H), 1.11 (d, 3H, J = 6.9), 1.09 (s, 3H), 1.07 (d, 3H, J = 7.1), 0.88 (t, 3H, J = 7.4), 0.12 (s, 9H), 0.07 (s, 9H). ^{13}C NMR: δ 177.4, 102.1, 96.3, 83.2, 80.8, 80.7, 78.5, 77.2, 75.1, 75.0, 73.1, 73.0, 70.3, 68.0, 65.3, 65.0, 49.3, 44.6, 43.0, 40.9, 37.0, 35.3, 34.3, 32.3, 29.7, 26.0, 22.3, 21.7, 21.4, 19.6, 19.0, 16.4, 14.6, 13.9, 11.2, 9.9, 0.8, 0.8. FAB−HRMS: calcd for $C_{43}H_{85}NO_{13}Si_2$ $(M + H)^+$ 880.5679, found 880.5680. Anal. Calcd for $C_{43}H_{85}NO_{13}Si_2$: C, 58.67; H, 9.73, N, 1.59. Found: C, 58.87, H, 9.57, N, 1.30.

2′,4″-*O*-Bis(trimethylsilyl)-12-*O*-methyl-9(*S*)-9-dihydroerythromycin A (11). A solution of the boron complex of 2′,4″-*O*-bis(trimethylsilyl)-9(*S*)-9-dihydroerythromycin A **(8)** (10.1 g, 5.7 mmol) in THF (75 mL) and DMSO (75 mL) was cooled to approximately 0 °C in an ice bath. To the cooled mixture was added iodomethane (2.8 mL, 45.7 mmol), followed by sodium hydride (60% pure) (1.8 g, 45.7 mmol). The mixture was stirred for 3 h at 0 °C before saturated NaCl solution was added along with triethanolamine. The resulting mixture was stirred at room temperature for 30 min, and the product was extracted with *i*-PrOAc. The organic layer was washed with saturated NaCl solution, dried over anhydrous sodium sulfate, filtered, and concentrated to give a crude product of 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methyl-9(*S*)-9-dihydroerythromycin A **(11)** (9.59 g, 93.4%). A sample (86 mg) of the crude product was isolated by preparatory HPLC to give 32 mg (37.2% yield) of pure **(11).** The rest of the material was purified by flash column chromatography. ^{1}H NMR ($CDCl_3$, ppm): δ 5.27 (dd, 1H, J = 10.2, 2.2), 4.96 (d, 1H, J = 4.7), 4.55 (d, 1H, J = 7.1), 4.28 (d, 1H, J = 3.8), 4.19 (m, 1H), 3.84 (d, 1H, J = 4.1), 3.70 (m, 1H), 3.66 (d, 1H, J = 7.1), 3.41 (s, 3H), 3.37 (m, 1H), 3.30 (s, 3H), 3.20 (dd, 1H, J = 9.9, 7.1), 3.16 (d, 1H, 9.1), 2.73 (m, 1H), 2.55 (m, 1H), 2.36 (d, 1H, J = 15.0), 2.21 (s, 6H), 2.16 (m, 1H), 2.06 (m, 1H), 1.79 (m, 1H), 1.76 (m, 1H), 1.69 (dd, 1H, J = 14.2, 5.5), 1.63 (m, 1H), 1.55 (m, 1H), 1.48 (dd, 1H, J = 15.0, 5.0), 1.29 (dd, 1H, J = 14.2, 7.7), 1.23 (s, 3H), 1.22 (d, 6H, J = 5.5), 1.19 (d, 3H, J = 7.1), 1.16 (d, 3H, J = 6.0), 1.14 (s, 3H), 1.10 (d, 3H, J = 5.2), 1.09 (s, 3H), 1.08 (d, 3H, J = 7.1), 0.93 (t, 3H, J = 7.4), 0.14 (s, 9H), 0.11 (s, 9H). ^{13}C NMR: δ 177.4, 102.2, 96.1, 83.2, 81.5, 80.8, 79.6, 78.2, 74.9, 74.6, 73.1, 73.1, 73.0, 67.9, 65.2, 65.1, 52.5, 49.3, 44.6, 42.6, 40.9, 37.7, 35.5, 34.1, 31.9, 29.6, 25.5, 22.3, 21.8, 21.5, 19.6, 19.0, 16.2, 14.0, 13.9,11.0, 10.0, 0.9, 0.8. FAB−HRMS: calcd for $C_{44}H_{87}NO_{13}Si_2$ $(M + H)^+$ 893.5780, found 893.5778. Anal. Calcd for $C_{44}H_{87}NO_{13}Si_2$: C, 59.09; H, 9.81, N, 1.57. Found: C, 59.11; H, 9.84, H, 1.59.

12-*O*-Methyl-9(*S*)-9-dihydroerythromycin A (6). A stirred solution of 2′,4″-*O*-bis(trimethylsilyl)-12-*O*-methyl-9(*S*)-9-dihydroerythromycin A **(11)** (100 mg, 0.1 mmol) in isopropyl alcohol (4 mL) was acidified to pH 3 with formic acid. The mixture was stirred for 30 min at ambient temperature under N_2. The mixture was then basified to pH > 9 with potassium carbonate (20% solution). Saturated NaCl solution was added, and the product was extracted with EtOAc. The organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated to yield crude product (90 mg, 107%). A pure product was isolated by preparatory HPLC to yield 12-*O*-methyl-9(*S*)-9-dihydroerythromycin A **(6)** (60 mg, 65.7%). ^{1}H NMR ($CDCl_3$, ppm): δ 5.25 (dd, 1H, J = 10.4, 2.1), 5.00 (d, 1H, J = 4.1), 4.53 (d, 1H, J = 7.3), 4.22 (dd, 1H, J = 6.4, 1.5), 4.03 (m, 1H), 3.88 (s, 1H), 3.71 (d, 1H, J = 7.0), 3.56 (m, 1H), 3.43 (dd, 1H, J = 5.8, 1.5), 3.43 (s, 3H), 3.34 (s, 3H), 3.28 (dd, 1H, J = 10.1, 7.2), 3.05 (d, 1H, J = 9.3), 2.82 (m, 1H), 2.49 (m, 1H), 2.37 (dd, 1H, J = 15.1, 1.2), 2.31 (s, 6H), 2.21 (m, 1H), 2.10 (m, 1H), 1.91 (m, 1H), 1.79 (m, 1H), 1.67 (m, 2H), 1.60 (dd, 1H, J = 15.1, 4.9), 1.57 (m, 1H), 1.40 (dd, 1H, J = 14.5, 6.1), 1.32 (d, 3H, J = 6.4), 1.29 (d, 3H, J = 7.0), 1.28 (s, 3H), 1.27 (m, 1H), 1.26 (s, 3H), 1.25 (d, 3H, J = 6.4), 1.22 (d, 3H, J = 7.0), 1.12 (d, 3H, J = 6.1), 1.11 (s, 3H), 1.08 (d, 3H, J = 7.0), 0.94 (t, 3H, J = 7.4). ^{13}C NMR: δ 177.3, 102.9, 95.8, 83.6, 83.4, 79.5, 78.8, 77.9, 75.3, 74.4, 73.1, 72.8, 70.9, 69.2, 65.9, 65.4, 52.6, 49.3, 44.7, 41.4, 40.3, 37.1, 34.9, 34.0, 31.4, 28.8, 25.4, 21.7, 21.6, 21.3,19.8, 18.3, 16.2, 14.5, 14.5, 10.9, 9.3. FAB−HRMS: calcd for $C_{38}H_{72}NO_{13}$ $(M + H)^+$ 750.5004, found 750.4990.

Acknowledgment. Authors would like to thank Dr. Paul West of the Structure Chemistry Department of the Pharmaceutical Products Division, Abbott Labs. for obtaining and interpretation of the MS date for compound **8**.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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