Preparation and *In Vitro* Antibacterial Activity of 6-*O*-Methylerythromycin D

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The commercial success of 6-*O*-methylerythromycin A (clarithromycin)[1,2] has prompted further study of alkylation reactions in macrolide synthesis. While synthetic methods for 6-*O*-methylation of erythromycin A are well-known, appropriate processes for methylation of structurally simpler erythromycin A precursors have not been reported in the literature. Erythromycin D[3] is a biosynthetic precursor of erythromycin A, lacking the 12-hydroxy group and the 3″-methoxymethyl group of erythromycin A. We now report the synthesis and *in vitro* antibacterial activity of 6-*O*-methylerythromycin D.

Results and Discussion

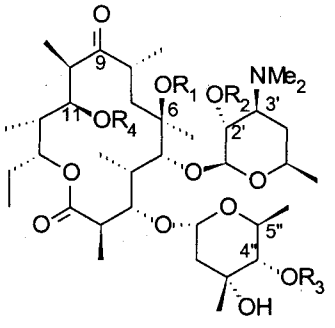
Chemistry

The compounds cited in this study are shown in Figure 1.

We have determined that the 2′ and 4″ hydroxy groups (R_2 and R_3 respectively) can be protected as the acetyl esters when erythromycin D is the starting material. By contrast, a 4″ acetate protecting group in erythromycin A is very difficult to cleave[4], possibly due to its neopentyl character. Forcing conditions are necessary to effect reaction, which frequently cleaves the macrocyclic ring as well[5]. Acetylation of erythromycin D (**1**) with acetic anhydride in pyridine/acetonitrile gave a mixture of products, from which the 2′,4″-bis-acetate **3** was isolated in 44% yield after chromatography. The acetylation sites were deduced by 2D NMR, and from the ^{13}C NMR shifts. Acetylation at the 2′ and 4″ positions force an upfield shift in the resonances for the neighboring carbons. For the erythromycin D series, C2′ acetylation affords a 3~4 ppm upfield chemical shift in C1′ and C3′, while C4″ acetylation gives a similar upfield shift only for the methyl-substituted C5″. A summary of ^{13}C NMR shifts for compounds **1**~**6** is given in Table 1. Exhaustive acetylation of erythromycin D in EtOAc/pyridine/acetic anhydride gave a 93% yield of 2′,4″,11-tris-acetylerythromycin D (**4**) after chromatography. The acylated hydroxyl sites were deduced from ^{13}C NMR spectra. While an excellent yield of **4** was realized, this material proved refractory to methylation in our hands. Bis-acetate **3** was methylated with methyl iodide/KOH in DMF, affording 6-*O*-methylbis-acetate **5** in 62% yield after chromatography. ^{13}C NMR analysis of **5** showed a 3 ppm downfield shift for C6, and a similar upfield shift for C5, (both shifts relative to starting material) indicating that methylation at C6 had occurred. The 6-OMe resonance was observed at 50.7 ppm in the 75 MHz carbon and 3.05 ppm in the 300 MHz proton spectra, respectively. These chemical shifts for the 6-*O*-methyl group are consistent with those previously reported for 6-*O*-methylerythromycin A[2]. Deacetylation of **5** was accomplished in aqueous MeOH using potassium carbonate to give 6-*O*-methyl-erythromycin D (**6**) in nearly quantitative yield. Removal of the acyl groups resulted in a downfield shift of the ^{13}C NMR resonances for C1′, C3′, and C5″. The 6-OMe resonance for **6** was observed at 50.9 ppm in the 75 MHz ^{13}C and 3.10 ppm in the 300 MHz ^{1}H NMR spectra. Structural assignments were corroborated by 2D NMR.

Compound **2**, 2′-acetyl-6-*O*-methylerythromycin D, was

Fig. 1. Structure of erythromycin D based compounds.



	R_1	R_2	R_3	R_4
1	H	H	H	H
2	Me	Ac	H	H
3	H	Ac	Ac	H
4	H	Ac	Ac	Ac
5	Me	Ac	Ac	H
6	Me	H	H	H

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Table 1. ^{13}C NMR chemical shifts for compounds **1**~**6**.

Carbon	Chemical shift (δ, ppm[a])					
	1	**2**	**3**	**4**	**5**	**6**
1	175.9	175.9	175.7	174.6	175.8	175.9
2	44.9	45.1	44.6	42.4	44.9	45.1
3	83.5	81.4	82.5	82.6	80.8	82.3
4	39.6	39.1	39.3	40.2	37.8	39.2
5	84.9	80.1	82.6	84.3	79.7	82.1
6	75.7	78.9	75.5	75.1	78.8	79.0
7	38.2	38.4	37.7	37.4	38.4	39.0
8	44.7	45.0	44.7	44.7	45.2	45.2
9	219.7	220.1	220.4	215.3	219.9	219.6
10	39.1	37.8	39.7	41.3	40.1	37.8
11	69.5	69.7	69.4	72.7	69.6	69.6
12	39.8	40.0	38.8	39.0	38.9	40.3
13	75.4	75.3	75.4	74.3	75.2	75.2
14	25.5	25.5	25.3	25.7	25.6	25.6
15	11.2	10.3	11.2	10.2	10.5	10.5
NMe2	40.2	40.4	40.5	40.7	40.6	40.2
1′	104.7	101.1	101.2	101.8	100.8	104.4
2′	70.8	71.7	71.6	71.6	71.7	70.9
3′	65.5	62.8	62.9	63.2	62.7	65.5
4′	28.5	30.3	30.7	30.8	31.0	28.4
5′	69.5	68.5	68.6	68.7	68.0	69.4
6′	21.3	21.2	20.8	21.2	20.9	21.4
1″	99.1	98.3	98.7	98.8	97.9	98.7
2″	40.5	40.3	41.0	41.2	40.9	40.4
3″	69.5	69.6	69.2	69.4	69.2	69.4
4″	76.4	76.3	77.1	77.5	77.3	76.4
5″	66.3	66.7	63.8	63.7	64.0	66.3
6″	18.4	18.5	18.3	18.1	18.3	18.6
2-Me	15.6	15.9	15.6	15.1	15.9	16.0
4-Me	9.4	9.1	9.1	9.3	9.2	9.1
6-Me	27.5	20.2	27.5	26.4	20.2	19.9
8-Me	18.5	18.0	18.0	20.5	18.2	18.4
10-Me	9.2	9.7	10.2	9.6	9.8	9.8
12-Me	9.1	9.1	9.1	9.6	9.0	9.0
3″-Me	25.5	25.4	25.4	25.6	25.6	25.5
6-OMe		50.6			50.7	50.9
11-Ac, C=O				170.8		
2′Ac, C=O		170.2	170.0	170.1	170.1	
4″Ac, C=O			170.5	170.4	170.3	
11Ac, Me				21.4		
2′Ac, Me		21.3	21.2	20.8	21.5	
4″Ac, Me			21.3	20.9	21.5	

[a] Chemical shifts are in ppm downfield of TMS. ^{13}C NMR spectra recorded in $CDCl_3$ at 75 MHz.

isolated by filtration as a solid from the saponification reaction at short reaction times. The deacetylated site is indicated by the downfield shift of C5″ in the carbon spectrum, and the structural assignment was corroborated by 2D NMR. In contrast to erythromycin A, a 4″ acetate is readily removed in erythromycin D.

In Vitro Antibacterial Activity

The *in vitro* antibacterial activity of 6-*O*-methylerythromycin D[6] is shown in Table 2, compared against erythromycin A, 6-*O*-methylerythromycin A (clarithromycin), and erythromycin D (compound **1**). The activity of 6-*O*-methylerythromycin D is somewhat less than that of clarithromycin.

Experimental

All reactions were performed under a nitrogen atmosphere. TLC experiments were performed using an eluent of 30:100:2 v:v:v acetone:hexane:NEt_3 on 250 μ silica gel plates, charred after immersion in ceric ammonium sulfate/molybdic acid in 10% aqueous sulfuric acid. HPLC analyses were run on a 250×4.6 mm YMC pack ODS-A column at 2 ml/minute flow rate, monitoring at 205 nm. The eluent was 50:50 v:v acetonitrile:0.25% KH_2PO_4 buffered at pH 7. Melting points are uncorrected. Carbon spectra were run at 75 MHz, and are given in Table 1 for all compounds. Proton data is reported for resolved peaks at or below 3 ppm. Peak assignments were deduced from 2D NMR experiments. Combustion analyses, mass spectra, and NMR spectra acquisition were performed by the Analytical Research Department of Abbott Laboratories.

In Vitro Antibacterial Activity

Antibiotic susceptibility was determined in two different media. For *Helicobacter pylori* strains, compounds were tested in serial twofold dilutions by broth microdilution. The medium was unbuffered brain heart infusion broth supplemented with 0.25% (w/v) yeast extract and 10% (v/v) horse serum, pH adjusted to 8.0. For all other bacterial strains, the medium consisted of brain heart infusion agar.

6-*O*-Methyl-2′-acetylerythromycin D (**2**)

A solution of 1.7 g of crude **5** in 40/10 ml MeOH/5% aqueous K_2CO_3 was stirred until solids appeared. The solids were collected by filtration and washed with MeOH to give 0.38 g (23.6%) of **2** after drying. ^1H NMR (300 MHz, $CDCl_3$) δ 5.29 (ddd, 1H, J=6, 3, 0.2 Hz, H13), 5.00 (d, 1H, J=2.5 Hz, H1″), 4.65 (dd, 1H, J=6.3, 4.2 Hz, H2′), 4.36 (d, 1H, J=4.2 Hz, H1′), 3.81 (d, 1H, J=5.7 Hz, H3), 3.73 (s, 1H, 3″-OH), 3.72 (dq, 1H, J=3.6, 1.8 Hz, H5″), 3.59 (dd, 1H, J=6.3, 0.4 Hz, H11), 3.55 (d, 1H, J=4.8 Hz, H5), 3.47 (m, 1H, H5′), 3.12 (d, 1H, J=1.4 Hz, 11-OH), 2.98 (s, 3H, 6-OMe), 2.18 (s, 6H, NMe_2), 1.99 (s,

Table 2. *In vitro* antibacterial activity of selected erythromycin derivatives.

Organisms	MIC (μg/ml)			
	Erythromycin A	Clarithromycin	Compound **6**	Compound **1**
Staphylococcus aureus 6538P	0.2	0.2	0.39	3.1
S. aureus A5177	6.2	3.1	6.2	>100
S. aureus CMX-642a	0.2	0.2	0.39	Not done
S. aureus CMX 553	0.2	0.2	0.39	Not done
S. aureus NCTC 10649M	0.2	0.2	0.39	1.56
S. epidermis 3519	0.2	0.2	0.39	1.56
Micrococcus luteus ATCC 9341	0.02	0.01	0.05	Not done
M. luteus ATCC 4698	0.39	0.78	0.39	0.78
Escherichia coli Juhl	100	50	>50	>100
Mycobacterium smegmatis ATCC 114	12.5	0.05	0.78	25
Nocardia asteroides ATCC 9970	0.1	0.02	0.1	0.78
Helicobacter pylori 2597	0.12	<0.03	0.25	Not done
H. pylori 4128	<0.03	<0.03	<0.03	Not done
H. pylori 5906	128	16	>128	Not done

Media: For all except *H. pylori*, brain heart infusion, inoculum size 10^4 cfu/spot. For *H. pylori*, Mueller-Hinton agar with horse blood, inoculum size 2×10^6 cfu/ml.

3H, AcMe); HR-MS Calcd for $C_{39}H_{70}NO_{13}$ (M+H): 760.4847. Found: 760.4836.

2′,4″-Bis-acetylerythromycin D (**3**)

To a suspension of 1.25 g (1.77 mmol) of erythromycin D and 1 ml of pyridine in 13 ml acetonitrile at 4°C was added 1.17 ml (12.4 mmol) of acetic anhydride. The reaction was warmed to ambient temperature for 45 hours. HPLC analysis showed that reaction was complete after this time. The reaction was quenched with 100 ml of 0.4 N NaOH, and product was extracted into 2×50 ml EtOAc. The pooled organic phases were serially washed with 50 ml each 0.4 N NaOH and distilled water, then stripped to dryness *in vacuo* and vacuum dried. The residue was flash chromatographed using 100/50/2 v/v/v hexane/acetone/triethylamine on a 38×170 mm silica gel column to give 622.4 mg (44%) of **3**, mp 118~119°C (heptane). IR ($CDCl_3$) cm^{-1} 1735 (s), 1695 (sh), 1458 (m); HR-MS Calcd for $C_{40}H_{70}NO_{14}$ (M+H): 788.4796. Found: 788.4800; ^1H NMR (300 MHz, $CDCl_3$) δ 5.33 (ddd, 1H, J=9, 5, 0.8 Hz, H13), 5.07 (br d, 1H, J=3.1 Hz, H1″), 4.75 (dd, 1H, J=11.1, 7.5 Hz, H2′), 4.63 (d, 1H, J=10.5 Hz, H4″), 4.47 (d, 1H, J=7.5 Hz, H1′), 4.25 (dd, 1H, J=9, 0.8 Hz, H3), 4.16 (m, 1H, H5″), 3.75 (br d, 1H, J=9.2 Hz, H11), 2.27 (s, 6H, NMe_2), 2.13 (s, 3H, 2′OAcMe), 2.07 (s, 3H, 4″OAcMe), 1.46 (s, 3H, C6Me); *Anal* Calcd for $C_{40}H_{69}NO_{14}\cdot(0.2\ NEt_3)$: C 61.23, H 8.98, N 2.08. Found: C 60.83, H 9.17, N 2.46.

2′,4″,11-Tris-acetylerythromycin D (**4**)

A mixture of 1.408 g (2 mmol) of erythromycin D in 4.8 ml of pyridine and 20 ml of EtOAc was cooled to 4°C, and 20 mg of 4,4-dimethylaminopyridine and 2.8 ml (29.7 mmol) of acetic anhydride were added. After 24, the reaction was quenched by addition of 20 g ice, and the emulsion was diluted by addition of 15 ml 4 N NaOH. The organic phase was serially washed with 10 ml each of 2 N NaOH and distilled water. The solvents were removed *in vacuo*, and the residue was flash chromatographed on a 38×152 mm column using 100/50/2 v/v/v hexane/acetone/triethylamine to give 1.55 g (93%) of **4** as a glassy foam after vacuum drying. IR ($CDCl_3$) cm^{-1} 1735 (vs), 1700 (m), 1468 (m); MS (DCl/NH_3) m/z $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) 5.15 (dd, 1H, J=9, 0.7 Hz, H11), 5.11 (d, 1H, J=2.5 Hz, H1″), 4.84 (dd, 1H, J=5.9, 3.2 Hz, H13), 4.79 (dd, 1H, J=6.1, 4.6 Hz, H2′), 4.64 (d, 1H, J=6.1 Hz, H4″), 4.50 (d, 1H, J=4.6 Hz, H1′), 4.41 (br d, 1H, J=5.8 Hz, H3), 4.19 (dq, 1H, J=6.1, 4 Hz, H5″), 3.60 (m, 1H, H5′), 3.58 (d, 1H, J=4.6 Hz, H5), 2.27 (s, 6H, NMe_2), 2.13 (s, 3H, 2′OAcMe), 2.09 (s, 3H, 11OAcMe), 2.06 (s, 3H, 4″OAcMe), 1.35 (s, 3H, C6Me).

6-*O*-Methyl-2′,4″-bis-acetylerythromycin D (**5**)

A solution of 468 mg (0.59 mmol) of compound **3** in 6 ml of DMF was cooled to 4°C, and 77.6 μl (1.25 mmol) of methyl iodide was added, followed by 70 mg (1.25 mmol) of powdered KOH. After stirring at 4°C for 20 minutes, the reaction was quenched by addition of 20/10 ml 2 N NaOH/EtOAc. The organic phase was washed with 10 ml distilled water, stripped to dryness *in vacuo*, and vacuum dried. The residue was flash chromatographed on a 38×170 mm silica gel column with 100/50/2 v/v/v hexane/acetone/triethylamine eluent to give 294.5 mg (61.8%) of **5**, mp 220~221°C (heptane). IR ($CDCl_3$) cm^{-1} 1735 (vs), 1690 (m), 1459 (m); FAB-MS (Nitrobenzyl alcohol) *m/z* 802 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 5.38 (ddd, 1H, J= 10.5, 4.8, 0.8 Hz, H13), 5.13 (d, 1H, J=3.8 Hz, H1″), 4.72 (dd, 1H, J=10.8, 7.5 Hz, H2′), 4.63 (d, 1H, J=10.5 Hz, H4″), 4.49 (d, 1H, J=7.5 Hz, H1′), 4.16 (m, 1H, H5″), 3.90 (br d, 1H, J=9.2 Hz, H3), 3.55 (br d, 1H, J=9.5 Hz, H11), 3.05 (s, 3H, 6-OMe), 2.26 (s, 6H, NMe_2), 2.12 (s, 3H, 2′OAcMe), 2.04 (s, 3H, 4″OAcMe), 1.39 (s, 3H, C6Me); *Anal* Calcd for $C_{41}H_{71}NO_{14}$: C 61.40, H 8.92, N 1.75. Found: C 61.41, H 8.74, N 1.79.

6-*O*-Methylerythromycin D (**6**)

A solution of 425 mg (0.53 mmol) of bis-acetate 5 and 168 mg (1.22 mmol) K_2CO_3 in 10/2.5 ml of MeOH/distilled water was stirred at ambient temperature for 19 hours. The reaction mixture was diluted with 10 ml distilled water, and the MeOH was removed *in vacuo*. The organic residue was extracted into 2×25 ml EtOAc, and the pooled organic extracts were stripped to dryness *in vacuo* to give 374.8 mg (98.5%) of crude **6**, mp 137°C (aqueous EtOH). IR ($CDCl_3$) cm^{-1} 2970 (vs), 1721 (s), 1688 (m), 1454 (s); HR-MS Calcd for $C_{37}H_{68}NO_{12}$ (M+H): 718.4741. Found: 718.4737; ^1H NMR (300 MHz, $CDCl_3$) δ 5.38 (dd, 1H, J=10.6, 3.5 Hz, H13), 5.06 (d, 1H, J=3.1 Hz, H1″), 4.27 (d, 1H, J=7.2 Hz, H1′), 3.88 (d, 1H, J=9.1 Hz, H3), 3.10 (s, 3H, 6-OMe), 2.27 (s, 6H, NMe_2), 1.46 (s, 3H, C6Me); *Anal* Calcd for $C_{37}H_{67}NO_{12}$: C 61.90, H 9.41, N 1.95. Found: C 61.71, H 9.64, N 1.93.

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References

1) MORIMOTO, S.; Y. TAKAHASHI, Y. WATANABE & S. OMURA: Chemical modification of erythromycins. I. Synthesis and antibacterial activity of 6-*O*-methylerythromycins A. J. Antibiotics 37: 187~189, 1984
2) MORIMOTO, S.; Y. MISAWA, T. ADACHI, T. NAGATE, Y. WATANABE & S. OMURA: Chemical modification of erythromycins. II. Synthesis and antibacterial activity of *O*-alkyl derivatives of erythromycin A. J. Antibiotics 43: 286~294, 1990
3) MAJER, J.; J. R. MARTIN, R. S. EGAN & J. W. CORCORAN: Antibiotic glycosides. 8. Erythromycin D, a new antibiotic. J. Am. Chem. Soc. 99: 1620~1622, 1977
4) HAUSKE, J. R.; M. GUADLIANA, G. KOSTEK & G. SCHULTE: Aglycon modifications of erythromycin A: Regiospecific and stereospecific elaboration of the C-12 position. J. Org. Chem. 52: 4622~4625, 1987
5) WADDELL, S. T. & T. A. BLIZZARD: Base catalyzed ring opening reactions of erythromycin A. Tetrahedron Letters 33: 7827~7830, 1992
6) LIU, J.-H. & J. E. CELEBUSKI: 6-*O*-Methylerythromycin D and process for making same. U. S. Patent 5,756,473, May 26, 1998