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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF O-METHYL DERIVATIVES OF AZALIDE ANTIBIOTICS: I. 4", 11 AND 12-OMe DERIVATIVES VIA DIRECT METHYLATION

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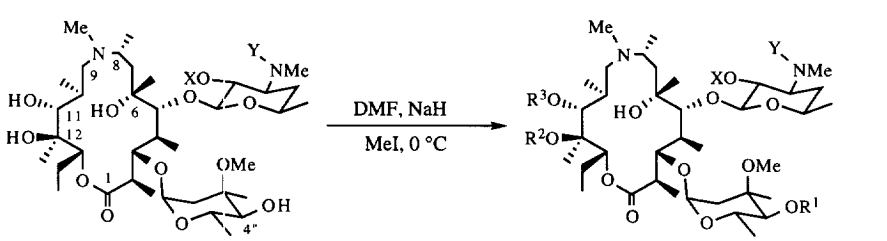
Abstract: A series of O-Me derivatives of 9-deoxo-8a-aza-8a-homoerythromycin has been prepared and evaluated for antibacterial activity. The relative rates of methylation of the four available hydroxyls (4", 6, 11 and 12) in 2',3'-bis-Cbz protected 9-deoxo-8a-aza-8a-homoerythromycin were compared to those given in a published report for the similarly protected 9a-azalide. An incongruity in the results prompted reinvestigation of the O-methylation of the 9a-azalide, and an error in structure assignment in the published report was discovered: the compound reported as the 6-OMe-9a-azalide has been determined to be the 12-OMe derivative.

Landmarks in the second generation of macrolide antibiotics include clarithromycin and azithromycin. The former is derived from erythromycin simply by methylation of the 6-OH group, and displays improved activity against a variety of gram-positive organisms.[1] The latter is the prototypical "azalide", derived from erythromycin by formal insertion of an N-Me function at the 9a-position (along with reduction of the the 9-keto function to a methylene) to create a 15 membered ring (structure **7b**), and introduces increased (and clinically useful) potency against many important gram negative species.[2] It was subsequently discovered that the isomeric azalide with the nitrogen in the 8a-position (**1b**) has similar potency to the 9a-aza prototype, and represents an important alternative platform.[3] We desired to investigate the effects of methylating the hydroxy groups of the 8a-azalide (**1b**), in hopes that a hybrid "clarithro-azalide" would show improved properties. With this aim, we were particularly interested in the 6-OMe compound. The 9a-azalide (**7b**) has been the previous subject of such an investigation, and the preparation of 6-OMe-9-deoxo-9a-aza-9a-homoerthromycin has been reported.[4] We have shown in the course of this work, however, that this structural assignment was incorrect.[5]

Equation 1: O-Methylation of the 8a-Azalide



1a

(1) H_2, Pd/C
(2) $HCHO,HCO_2H$

a X, Y = Cbz
b X = H, Y = Me

2a,b R^1 = Me, R^2 = H, R^3 = H
3a,b R^1 = H, R^2 = Me, R^3 = H
4a,b R^1 = Me, R^2 = H, R^3 = Me
5a,b R^1 = Me, R^2 = Me, R^3 = H
6a,b R^1 = Me, R^2 = Me, R^3 = Me

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The classical method for O-methylating macrolides proceeds by initial protection of the reactive sites on the desosamine, typically as 2'-OCbz-3'-NMeCbz. This protected derivative is then O-methylated in a dipolar aprotic solvent (e.g., DMSO/THF or DMF) using a base (e.g., KOH or NaH) and MeI. Removal of the Cbz's and Eschweiler-Clarke methylation of the 3'-nitrogen completes the sequence. It should be noted that there are four hydroxyls that can be methylated (4", 6, 11 and 12), and mixtures of various mono-, and di-, and tri-O-methylated derivatives are generally obtained. The relative rates of methylation of the four hydroxyls presumably depend on subtle conformational details, and are not predictable by a cursory inspection of the structure. In this regard, we were interested to see what differences would be observed in these relative rates between the 8a- and 9a-azalide platforms.

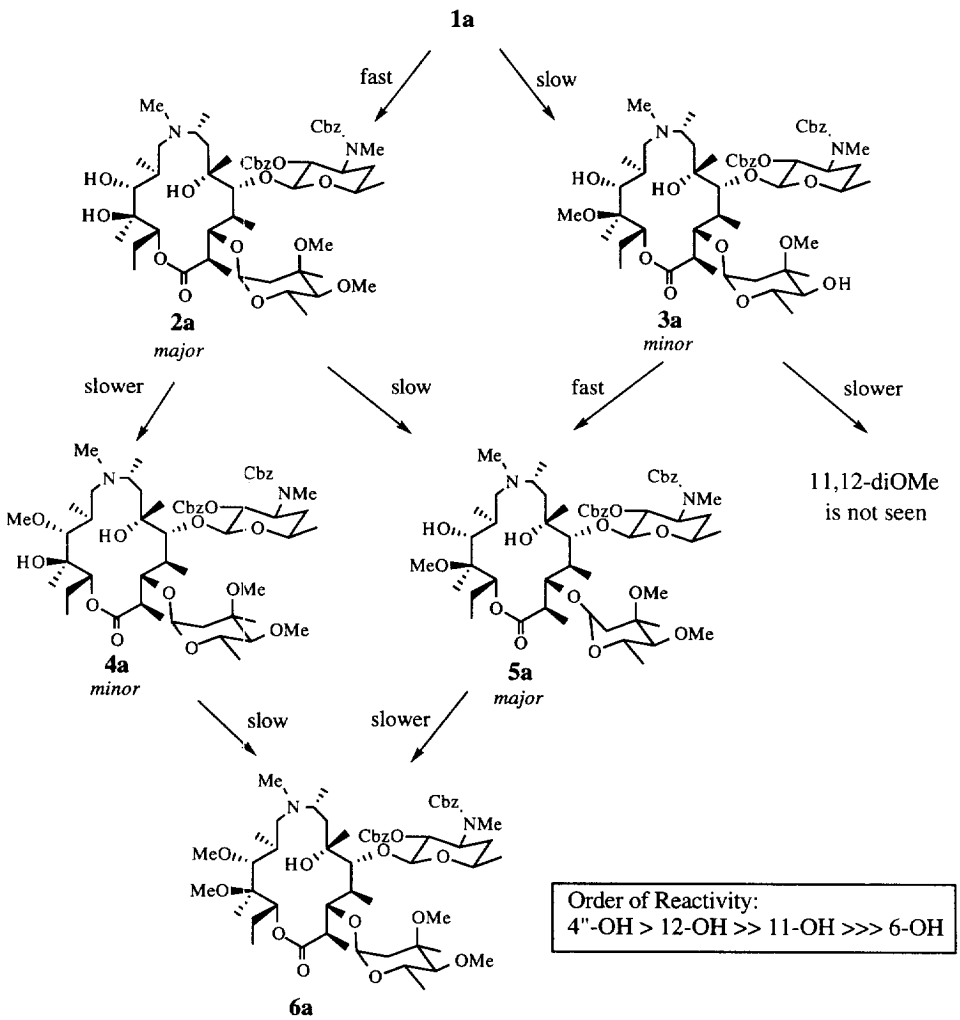
In our investigation, we principally employed DMF, MeI and NaH at 0 °C, at various concentrations in order to favor more or less O-methylation. Limited experimentation showed that conservative variation of the solvent (e.g., to DMSO/THF mixtures) or the base (e.g., to KH) gave results qualitatively similar to those obtained in the DMF/NaH system. These conditions are substantially similar to those used by Kobrehel, et al.[4] in their investigation of the 9a-aza platform (**7b**), but it should be noted that while the protected 9a-aza platform is stable to prolonged reaction at room temperature under these conditions, the analogous 8a-aza platform decomposes by loss of cladinose upon warming to room temperature, and so must be maintained at 0 °C for the duration of the reaction.

A description of three experiments serves to illustrate the relative facility with which the various mono-, di-, and tri-O-methylated products are produced in the reaction (Eq. 1). Under the mildest conditions, treatment of a 0.1 M solution of **1a** in DMF at 0 °C (reactions were typically conducted using 100 mg to 1 g of **1**) with 6 equiv of MeI followed by 2.5 equiv of NaH (60% oil dispersion) for 20 min gave 24% (all yields given in this section are unoptimized, isolated yields) of the 4"-OMe product **2a** and 4% of the 12-OMe product **3a**, along with 25% recovered starting material.[6] In a somewhat more vigorous variation, treatment of a 0.1 M solution of **1** in DMF at 0 °C with 15 equiv of MeI followed by 5 equiv of 60% NaH (oil dispersion) for 1.5 h gave 42% of the 4",12-di-OMe product **5a** and 5% of the 4",11-di-OMe product **4a**, along with 17% of **2a** and 4% of **3a**. In a still more vigorous variation, treatment of a 0.5 M solution of **1** in DMF at 0 °C with 15 equiv of MeI followed by 10 equiv of 60% NaH (oil dispersion) for 5 h gave 55% of the 4",11,12-tri-OMe product **6a** and 18% of di-OMe product, mostly **5a**.

Although we did not do careful kinetics, these results clearly support the conclusion that the methylation of the 4"-OH is relatively fast, while that of the 12-OH is slower and that of the 11-OH is slower still. The consequences of this are illustrated in the reaction cascade scheme (Scheme 1). Fast 4"-O-methylation produces the major mono-O-methylation product **2a**, while slow 12-O-methylation produces the minor **3a**. The product of the slower 11-O-methylation is not observed as a mono-O-methylation product. The major di-O-methylation product **5a** (4",12-di-OMe) is produced both by slow 12-O-methylation of the major 4"-OMe and by fast 4"-O-methylation of the minor 12-OMe product, while the minor di-O-methylation product **4a** (4",11-diOMe) is produced only by slow 11-O-methylation of the major 4"-OMe product. The 11,12-di-OMe product, which would have to be produced via two consecutive slow steps, is not observed. Finally, the 4",11,12-tri-OMe product **6a** is produced principally by slow 11-O-methylation of the major 4",12-di-OMe product (the alternative route of production, 12-O-methylation of the 4",11-di-OMe isomer, is a negligible contributor on account of the low concentration of this di-OMe isomer.)

It can be seen that the 6-OH is not methylated under even the most vigorous reaction conditions (attempts to make the conditions still more vigorous, either by prolonged reaction at 0 °C or by allowing the

Scheme 1: Reaction Cascade



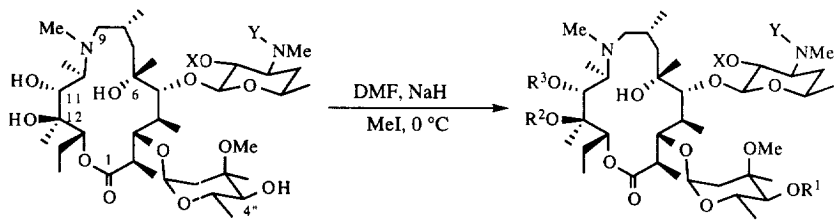
reaction to warm to room temperature, resulted in elimination of cladinose.) This is in constrast to the O-methylation of erythromycin (similarly protected as 2'-OCbz-3'-NMeCbz), in which system the 6-OH is easily methylated under conditions very similar to these.[1b] It is also in contrast to the literature report on the 2'-OCbz-3'-NMeCbz-9a-azalide (**7a**), which is reported to undergo O-methylation in the following order: 11-OH ≥ 6-OH > 4"-OH.[4] It seemed incongrouous that the pattern of O-methylation exhibited by the 9a-azalide should be more similar to that of erythromycin than to that of the 8a-azalide, and so we were prompted to reinvestigate the O-methylation of the 2'-OCbz-3'-NMeCbz-9a-azalide (**7a**). Using mild conditions, treatment of a 0.05 M solution of **7a** in DMF at 0 °C with 6.5 equiv of MeI followed by 2.5 equiv of NaH (60% oil dispersion) for 1 h gave 38% (unoptimized, isolated yields) of the 11-OMe product **8a** and 27% of the 12-OMe product **9a**, along with 13% recovered starting material. More vigorously, treatment of a 0.063 M solution of **7a** in DMF at 0 °C with

Table 1: Complete NMR data for compounds **2b-6b**

	^{1}H NMR (500 MHz, 50 °C)						^{13}C NMR (500 MHz, 50 °C)				
proton	**2b**	**3b**	**4b**	**5b**	**6b**	**carbon**	**2b**	**3b**	**4b**	**5b**	**6b**
2	2.81	2.91	2.86	2.91	2.93	**1**	178.3	178.2	177.9	178.4	177.2
3	4.50	4.52	4.56	4.53	4.58	**2**	45.6	45.4	46.1	45.5	45.2
4	1.83	1.91	1.91	1.89	1.95	**3**	76.3	76.2	76.4	76.2	76.3
5	3.53	3.52	3.52	3.51	3.54	**4**	43.1	42.8	43.5	42.9	42.8
7a	1.90	1.91	1.96	1.93	2.00	**5**	84.8	85.0	84.7	84.8	84.2
7 b	1.12	1.12	1.12	1.08	1.14	**6**	74.7	74.5	74.1	74.7	74.1
8	3.00	3.02	3.00	2.98	3.02	**7**	36.8	36.9	36.9	37.0	38.0
9a	2.51	2.51	2.50	2.50	2.49	**8**	56.2	56.7	56.5	56.4	56.8
9 b	2.31	2.32	2.33	2.32	2.33	**9**	59.5	60.1	61.0	60.1	62.6
10	2.00	2.06	2.05	2.05	2.09	**10**	30.5	30.4	30.7	30.6	31.0
11	3.53	3.60	3.29	3.58	3.34	**11**	66.9	70.0	76.8	70.0	80.6
13	4.86	5.61	4.80	5.61	5.34	**12**	75.7	79.8	75.4	79.8	80.7
14a	1.91	1.80	1.94	1.78	1.76	**13**	76.9	73.0	77.2	73.0	74.9
14 b	1.49	1.53	1.46	1.51	1.53	**14**	21.6	21.5	22.0	21.7	22.1
15	0.91	0.96	0.91	0.96	0.92	**15**	10.8	10.4	11.0	10.7	12.1
1'	4.41	4.39	4.42	4.39	4.42	**1'**	103.0	103.1	102.6	102.9	102.6
2'	3.20	3.23	3.20	3.19	3.20	**2'**	70.9	70.7	70.8	70.8	70.7
3'	2.60	2.54	2.60	2.59	2.60	**3'**	65.0	65.6	nd	65.1	65.0
4'a	1.68	1.71	1.68	1.68	1.69	**4'**	28.8	29.0	nd	28.9	29.0
4'b	1.20	1.24	1.20	1.20	1.20	**5'**	68.6	68.9	68.2	68.7	68.5
5'	3.61	3.52	3.62	3.60	3.60	**6'**	20.9	21.0	20.9	21.0	20.8
6'	1.22	1.24	1.22	1.21	1.20	**1"**	94.5	94.5	94.6	94.5	94.9
1"	5.10	5.17	5.20	5.15	5.14	**2"**	35.2	34.8	35.3	35.4	35.2
2"a	2.31	2.30	2.32	2.31	2.33	**3"**	73.8	72.5	73.8	73.7	73.8
2" b	1.51	1.57	1.52	1.51	1.51	**4"**	89.2	78.2	89.2	89.4	89.1
4"	2.69	3.02	2.68	2.69	2.68	**5"**	64.2	65.1	64.1	64.2	64.1
5"	4.27	4.05	4.26	4.26	4.28	**6"**	18.0	17.9	17.9	18.0	17.8
6"	1.31	1.30	1.29	1.30	1.28	**2-Me**	14.1	13.8	14.1	14.0	18.5
2-Me	1.19	1.20	1.19	1.19	1.18	**4-Me**	10.8	11.4	11.2	11.8	11.8
4-Me	1.11	1.12	1.13	1.13	1.14	**6-Me**	27.3	27.1	27.8	27.2	27.2
6-Me	1.37	1.37	1.33	1.47	1.30	**8-Me**	12.2	12.3	12.0	12.6	10.5
8-Me	0.93	0.94	0.93	0.93	0.95	**10-Me**	11.5	11.1	12.0	11.3	12.3
10-Me	0.96	1.04	0.94	1.03	0.97	**12-Me**	15.9	17.1	16.9	17.3	16.7
12-Me	1.10	1.08	1.12	1.07	1.13	**3"-Me**	21.1	21.0	21.1	21.2	21.1
3"-Me	1.25	1.26	1.26	1.27	1.25	**NMe2**	39.9	40.0	40.1	40.1	40.0
NMe2	2.31	2.34	2.30	2.30	2.30	**NMe**	30.8	30.4	31.2	30.6	32.1
NMe	2.05	2.03	2.08	2.02	2.13	**3"OMe**	49.3	49.2	49.4	49.4	49.2
3"OMe	3.33	3.32	3.34	3.33	3.33	**4"OMe**	61.6	-	61.6	61.6	61.6
4"OMe	3.54	-	3.55	3.55	3.54	**11OMe**	-	-	62.4	-	61.6
11OMe	-	-	3.59	-	3.53	**12OMe**	-	52.4	-	52.5	51.9
12OMe	-	3.46	-	3.45	3.35						

33 equiv of MeI followed by 5 equiv of 60% NaH (oil dispersion) for 1.5 h gave 26% of the 11,12-di-OMe product **10a** and 57% of the 4",11,12-tri-OMe product **11a**. These results are entirely in line with those reported by Kobrehel, et al.,[4] except that we discovered that all of their 6-OMe assignments are actually 12-OMe.[6] It can thus be seen that, as expected, the patterns of O-methylation of the two azalides are grossly similar, in that neither is O-methylated on the 6-OH, while both are O-methylated on the other three hydroxy groups, although at different rates.

Equation 2: O-Methylation of the 9a-Azalide



7a

1) H_2, Pd/C
2) $HCHO,HCO_2H$

a X, Y = Cbz
b X = H, Y = Me

8a,b R^1 = H, R^2 = H, R^3 = Me
9a,b R^1 = H, R^2 = Me, R^3 = H
10a,b R^1 = H, R^2 = Me, R^3 = Me
11a,b R^1 = Me, R^2 = Me, R^3 = Me

While the measurement of absolute rate constants was outside the scope of our study, it seems reasonable to assume that the absolute rate of O-methylation of the remote 4"-OH will be approximately the same in both the 8a- and 9a-aza platforms. This assumption allows us to qualitatively rank relative rates of O-methylation from both platforms on a single scale: 11-OH (9a) ≥ 12-OH (9a) > 4"-OH (9a) ≅ 4"-OH (8a) > 12-OH (8a) > 11-OH (8a). This ranking suggests that the 9a-aza platform is O-methylated more rapidly overall under these conditions than is the 8a-aza platform.

Examination of the NMR data in Table 1 reveals several key spectral changes upon O-methylation. The anticipated effects are seen for O-methylation at 4" and 11: specifically, an upfield shift of 0.2-0.3 ppm for a proton geminal to the methoxy, and a downfield shift on the order of 10 ppm for the carbon bearing the methoxy. It can be seen that O-methylation of the 12-OH produces more complicated and unexpected effects: here there is no proton geminal to the 12-OH, but it can be seen that the 13-H shifts downfield by as much as 0.75 ppm in compounds bearing a 12-OMe. In the carbon spectrum, we see that the 12-C is shifted downfield by a modest 4 ppm, but the 11-C shifts downfield by almost as much, and the 13-C is actually shifted *upfield* by about 4 ppm. Generally the chemical shift changes upon O-methylation are quite local, and so we may conclude that methylation of the 12-OH introduces a rather substantial alteration in the conformation. Our use of molecular modeling techniques to explore this issue will be the subject of a future report.

Table 2 shows the MIC's of the O-methylated derivatives of the 8a- and 9a-azalides versus selected macrolide susceptible microorganisms. It can be seen that the effect of O-methylation is uniformly deleterious, with the general trend being that a greater number of methoxy groups results in a less active compound. Although the 12-OMe compound **3b** was not tested (samples of this compound were never isolated free from significant contamination by **2b**), comparison of the di-O-methylated derivatives **4b** and **5b** suggests that

methylation of the 12-OH is better tolerated than methylation of the 11-OH. This is perhaps surprising in light of NMR evidence for a more substantial conformational reorganization upon 12-O-methylation than upon 11-O-methylation (*vide supra*). In general, it seems that the 9a-azalide platform tolerates a given degree of methylation somewhat better than the 8a-azalide platform, but only in the 4",11,12-tri-OMe case (**6b** vs. **11b**) is a direct comparison possible.

Table 2: MIC's versus selected organisms (susceptible strains)

		MIC's (mg/mL)									
strain		**1 b**	**2 b**	**4 b**	**5 b**	**6 b**	**7 b**	**8 b**	**9 b**	**10 b**	**11 b**
S. pneumo	(MB3957)	<0.06	0.5	2	1	4	<0.06	0.03	0.03	1	1
S. pyogenes	(MB2874)	<0.06	0.03	0.5	0.25	0.5	0.03	0.03	0.03	0.12	0.25
S. aureus	(MB2865)	0.3	8	32	8	32	0.5	1	1	8	8
E. faecalis	(MB5407)	2	8	32	16	32	4	8	4	16	16
E. faecium	(MB5516)	0.125	2	16	4	8	0.25	0.5	0.25	2	4
B. subtilis	(MB5586)	0.3	4	16	4	2	1	1	-	4	8
M. smegmatis	(MC2155)	4	-	8	-	64	4	2	2	8	16

In conclusion, we have shown that patterns of O-methylation of the 8a- and 9a-azalides, while resembling each other, differ from that exhibited by erythromycin. Specifically, the azalides undergo facile methylation of the 12-OH while methylation of the 6-OH is not observed, whereas with erythromycin, the 6-OH (along with the 11-OH) is first to be methylated.[1b] Critical to reaching this conclusion was the unifying discovery that the compound reported in the literature as 6-OMe-azithromycin[5] is actually 12-OMe-azithromycin.

References and Notes

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5. The synthesis of 6-OMe-9-deoxo-9a-aza-9a-homoerythromycin will be the subject of a subsequent report.
6. Structural determination was performed on the deprotected and remethylated final products **2b-10b**. By using a combination of two-dimensional NMR techniques, the complete carbon and proton spectra could be assigned unambiguously. COSY (proton-proton correlation) and HMQC (one bond proton-carbon correlation) together allowed for an almost complete assignment, with only the quaternary carbons (3", 6 & 12), protons on methyl groups attached to quaternary carbons (3"-Me, 6-Me & 12-Me), and methoxy protons (3"-OMe plus any introduced methoxys) remaining unassigned. The long-range HMBC experiment, which correlates protons to carbons over 2 and 3 bonds, eliminated all remaining ambiguities. First, it allowed carbons 6 and 12 to be unambiguously distinguished in the ^{13}C NMR spectrum. Next, we found that in every case a very strong coupling is seen from the protons on a methoxy group to the carbon bearing that methoxy group. With a complete carbon assignment, this makes it a simple matter to determine the locations of the methoxy groups.