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ASSESSMENT OF THE BIOLOGICAL VALUE OF THE 11-HYDROXY GROUP OF THE AZALIDES.

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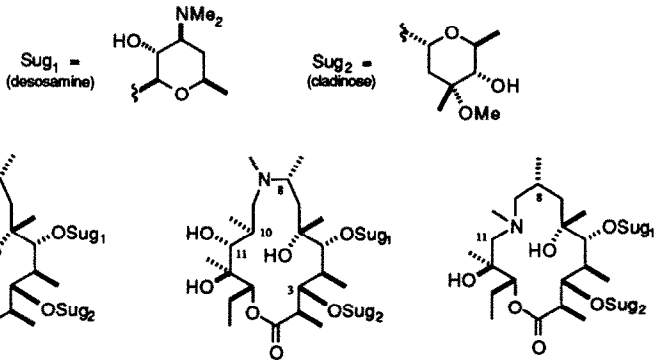
Abstract : *Two deoxy azalides, 11-deoxy L-701,677 (18) and 11-deoxy -10-epi L-701,677 (15), were prepared and retained much of the in vitro antimicrobial potency of the parent azalide.*

The azalides are a new class of macrolide antibiotic. The first of these, azithromycin (**1**), recently made its market debut (as Zithromax™) with indications for skin and repiratory tract infections and uncomplicated genital chlamydial infections.[1] Work from these laboratories has been reported recently describing the preparation of two new azalide types; L-701,677 (**2**)[2] and a 14-membered azalide, L-731,352 (**3**).[3] It is clearly desirable to evaluate the biological significance of various features of these molecules given the important changes in antimicrobial character that the azalides display (relative to erythromycin A). During work with a precursor to L-701,677 we observed a facile dehydration process. The subsequent follow up study revealed unexpected leniency in the substitution pattern of the C10-C11 region and it is this we now wish to report.

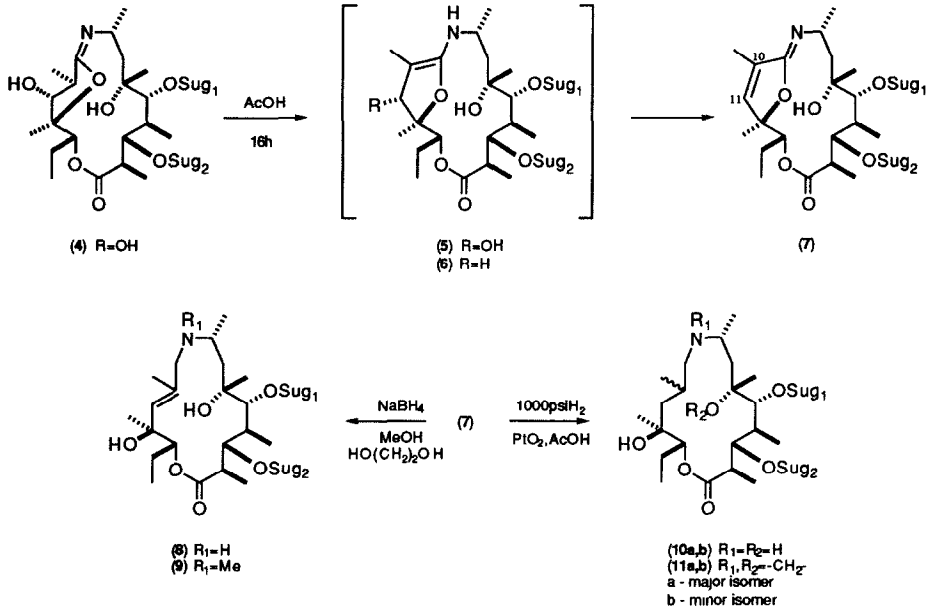


(**1**) Azithromycin (**2**) L-701,677 (**3**) L-731,352

Imino ether (**4**) is an intermediate in the preparation of L-701,677.[2] The relatively inaccessible nature of the 11-hydroxy group in the unfettered macrolide aglycone had prompted us to investigate this material in the hope that the residue would prove more promiscuous in this setting. In so doing we discovered that the anhydro derivative (**7**)[4] could be produced, in high yield, simply by dissolving **4** in acetic acid at room temperature.[5] Indeed it was difficult to avoid this dehydration. The net process is one of *syn*-elimination and suggested the intermediacy of ketene aminal (**5**).

This unanticipated transformation revealed a convenient route to 10,11-anhydro and 11-deoxy azalides. Treatment of **7** with $NaBH_4$ in MeOH/ethylene glycol effected reduction of the iminoether clasp to generate the

Z-anhydro azalide (**8**)[6]. The yield for this process was, however, consistently low (10-15% conversion) and we have been unable to find conditions that allow efficient conversion. This has undermined the usefulness of **8** as a substrate for further modification. Nonetheless, reductive methylation of **8** (37% aq.HCHO, $NaCNBH_3$, MeOH) provided the N-methyl azalide (**9**) for comparison with the parents. In contrast, high pressure catalytic hydrogenation effected exhaustive reduction of **7** in high yield (90-95%). This produced a mixture of 11-deoxy azalides (**10a,b**) epimeric at C-10, in a ratio of ~10:1. Although we were able to obtain a pure sample of the major isomer (**10a**)[7], the minor isomer (**10b**) was never recovered unadulterated from this process. Inspection of molecular models of **7** suggested that, were the olefin reduction to precede iminoether reduction and occur while the 12,9-bridge was intact,[8] the 10-(*S*) configuration would be produced (i.e. the unnatural configuration (**13**)). Aminal (**11a**) could be generated cleanly and rapidly by simple exposure of **10a** to aqueous formaldehyde in EtOH. While we have been wary of the validity of NOE measurements when applied to conformationally mobile macrolides, the presence of a 6,8a-bridge in **11a** rigidifies the aglycone significantly and it seemed likely that such measurements would be useful in this case. In fact inspection of the NOESY spectrum of **11a** provided convincing evidence for the 10-(*S*) configuration (**14**) - Figure 1.



In searching for further support for this assignment we examined the hydrogenation of anhydro iminoether (**7**) under more mild conditions. Exposure to 1atm H_2 in the presence of 10%Pd/C produced dihydroiminoether (**12**). Inspection of the crude isolate from this reduction indicated that a single stereoisomer was produced. However, the 10-position proved to be stereochemically labile and readily equilibrated in solution to a 2:1 mixture of isomers,[9] albeit still favouring the initial product. That this was the locus of the isomerization was convincingly demonstrated by a deuterium exchange experiment. The initial isolate of **12** was dissolved in $CDCl_3$ containing D_2O and the ^1H NMR spectrum recorded at intervals. The gradual and ultimately complete erosion of the H-10 signal as deuterium became incorporated at that site and the simplification of the signals for the vicinal protons (H-11) was manifest. Exchange was complete after 19h. Clearly this process is mediated by ketene aminal (**6**). This finding proved to be a most helpful conduit to the 10-(*R*) 11-deoxy azalide.

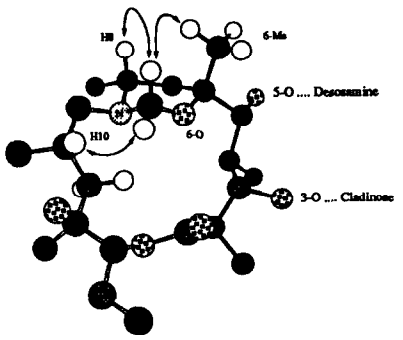


Figure 1

Representation of 10-epi-11-deoxy aminal (11a/14), based on the X-ray crystal structure of L-701,677, showing the most diagnostic NOE's. In the interest of clarity, carbohydrate residues and most protons are not shown.

An equilibrium mixture of (*S,R*)-**12** (2:1) was reduced ($NaBH_4$, MeOH) to give a mixture of 11-deoxy azalides (2:1 **13/16**). It was apparent at this stage that the mild reduction procedure had expressed the same stereochemical preference as the exhaustive reduction (i.e. **13** corresponded to **10a**). Interestingly, while the initial isomer ratio of **12** is faithfully transcribed when the borohydride reduction is driven to completion, the minor isomer is reduced more rapidly and interruption of the reaction allows isolation of an azalide mixture enriched in **16**.[2] While a mixture of the N-methyl derivatives (**15** and **18**) could be prepared directly, the aminal derivatives (**14** and **17**) proved to be separable. Treatment of the mixture of **13** and **16** with aq.HCHO in ethanol, followed by silica gel chromatography[10] produced aminals **14** (which was identical to **11a**) and **17**. The separated aminals were then individually reduced ($NaCNBH_3$, MeOH) to give 11-deoxy-10-epi L-701,677 (**15**) and 11-deoxy L-701,677 (**18**).[11]

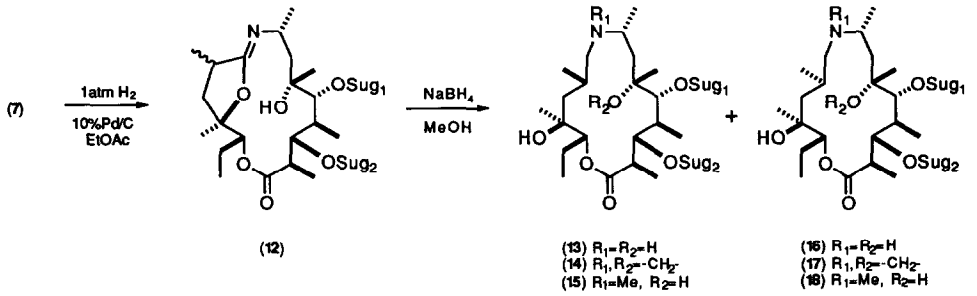


Table 1 contains the results of *in vitro* MIC determinations for the compounds of interest compared to erythromycin A and L-701,677 (**2**).[12] Anhydro iminoether (**7**) was predictably inactive. The MIC's for 11-deoxy L-701,677 (**18**) were only 0-2 fold higher than the parent azalide. Surprisingly, this was also the case for 11-deoxy-10-epi L-701,677 (**15**). Indeed even the anhydro azalide (**9**), which one might reasonably expect to be quite conformationally distinct, was not severely impaired.

The contribution of the individual hydroxy residues of the macrolides (excluding the desosaminyl 2'-OH) to *in vitro* bioactivity, insofar as it is fairly represented by the MIC's of the corresponding deoxy derivatives and may be compared from one aglycone to another, is comparable. 6-Deoxy erythromycin A has been reported to show a 2-4 fold loss of activity *in vitro*, relative to erythromycin A.[13] 12-Deoxy erythromycin A (erythromycin B) is comparably impaired.[14] 4"-Deoxy erythromycin A,[15] 4"-deoxy azithromycin[16] and 4"-deoxy L-701,677[16] show a 0-4 fold increase in MIC's. We are unaware of any similar evaluation of the 11-OH group[17] but this data indicates comparable significance for this residue. We found this information to be particularly interesting in the context of the evaluation of our recently disclosed 14-membered azalide (**3**)[3b] which lacks the 11-OH group.

Table 1 : *In Vitro* Assessment of Azalide Derivatives - MIC's (μg/mL)[12]

Microorganism	ErA*	(2)**	(7)	(9)	(15)	(18)
E.faecalis MB 5407	1-2	2-4	64-128	4-8	2-4	4-8
S.aureus MB 2865	0.25-0.5	0.5-1	16-64	2-4	0.25-1	2
S.haemolyticus MB 5412	0.125	0.125-0.25	8-32	0.5-1	0.125-0.25	0.25-0.5
S.pyogenes MB 2874	0.03-0.06	0.03-0.06	1-4	0.03-0.06	0.03-0.06	0.03-0.06
S.pyogenes MB 5403†	>128	>128	>128	>128	>128	>128
S.pyogenes MB 5406††	32	8-16	>128	8-16	8-16	16-32
E.cloacae CL 4298	32-64	0.5-2	64->128	4-8	2-4	2
E.coli MB 2884	32-64	1-2	64->128	8-16	2-4	4
K.pneumoniae MB 4005	32-64	1-2	64->128	8	2-4	2-4
H.influenza MB 5363	4-8	1-4	32->128	4-8	2-4	4-8

* Erythromycin A ** L-701,677 † constitutively macrolide resistant †† inducibly macrolide resistant

The respectable performances of 11-deoxy-10-epi L-701,677 (**15**) and *Z*-10,11-anhydro L-701,677 (**9**) are equally interesting. In the case of **15**, it is possible to argue, on the admittedly limited basis of inspection of molecular models, that the inversion of configuration at C-10 and the loss of the 11-OH group are co-operative defects. That is, the distortion necessitated by relief of unfavourable interactions with the 10(*S*)-Me is cushioned by rotation of the 11-(*R*) site into the macrolide ring. Were the 11(*R*)-OH group present this would not be possible and the conformational changes would be more widespread, resulting in a more serious effect on bioactivity. Indeed 10-epi erythromycin B has been shown to be markedly less active than erythromycin B.[18] The valiant display of *Z*-10,11-anhyro L-701,677 (**9**) is less easy to rationalize,[19] but serves to demonstrate that this segment of the azalide framework can be relatively accomodating.

In summary then, we have been able to show that the 11-hydroxy group is not a major contributor to bioactivity of the azalides and that fairly significant structural alterations in the C10-C11 region of L-701,677 can be tolerated, suggesting that this might be a useful realm in which to incorporate bulk property modifiers (as opposed to specific biochemical modifiers) if effective chemical methods could be developed.[20]

Acknowledgment.

We are grateful to Mr. J. Leone and Mr. R. Wilkening for providing quantities of the azalide iminoether starting materials and to Dr. R. Ratcliffe for invaluable counsel regarding the chemistry and the manuscript preparation.

References and Notes.

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4. (**7**): ^{1}H NMR (400MHz, CD_3OD, 50°C); δ 6.65 (1H, d, *J* = 1.5 Hz, H-11), 5.08 (1H, dd, *J* = 10.6, 2.6 Hz, H-13), 4.75 (1H, d, *J* = 4.9 Hz, H-1"), 4.53 (1H, d, *J* = 7.1 Hz, H-1'), 4.23 (3H, m, H-3, H-8, H-5"), 3.67 (1H, m, H-5'), 3.59 (1H, d, *J* = 2.7 Hz, H-5), 3.28 (4H, m, H-2', OMe), 2.97 (1H, d, *J* = 9.5 Hz, H-4"), 2.68 (1H, ddd, *J* = 12.1, 10.3, 4.2 Hz, H-3'), 2.44 (1H, br m, H-2), 2.34 (6H, s, NMe_2), 2.32 (1H, m, H-2"), 1.87 (3H, d, *J* = 1.5 Hz, 10-Me), 1.81 (4H, m, H-4, H-7a, H-14a, H-4'eq), 1.53

(2H, m, H-14b, H-2"), 1.39 (3H, s, Me), 1.30-1.11 (20H, m, H-7b, H-4'ax, 2-Me, 4-Me, 5'-Me, 5"-Me, Me(s), Me(s)), 1.02 (3H, d, J = 6.4 Hz, 8-Me) and 0.92 (3H, t, J = 7.4 Hz, 3H-15) ppm.

5. Iminoether (**4**) equilibrates to a 1:1 mixture with its 10-epi isomer (see ref. 2). Both the pure *R*-isomer (**4**) and the mixture dehydrated similarly. It was not known at the outset of this work that the iminoether components were 10-stereoisomers, but was revealed by the behaviour of the 11-deoxy analogues (see main text).
6. (**8**): ^{1}H NMR (400MHz, CD_3OD); δ 5.54 (1H, s, H-11), 5.08 (1H, d, J = 4.6 Hz, H-1"), 4.76 (1H, dd, J = 10.5, 2.4 Hz, H-13), 4.43 (1H, d, J = 7.3 Hz, H-1'), 4.23 (1H, dd, J = 3.4, 1.8 Hz, H-3), 4.17 (1H, dq, J = 9.4, 6.3 Hz, H-5"), 3.94 (1H, d, J = 11.6 Hz, H-9a), 3.66 (1H, m, H-5'), 3.56 (1H, d, J = 7.5 Hz, H-5), 3.34 (3H, s, OMe), 3.21 (1H, dd, J = 10.3, 7.3 Hz, H-2'), 3.17-3.08 (2H, m, H-8, H-9b), 3.02 (1H, d, J = 9.5 Hz, H-4"), 2.77 (1H, dq, J = 7.3, 3.6 Hz, H-2), 2.68 (1H, ddd, J = 12.1, 10.4, 4.0 Hz, H-3'), 2.42, (1H, d, J = 14.9 Hz, H-2"eq), 2.32 (6H, s, NMe_2), 2.01 (1H, m, H-4), 1.87 (3H, d, J = 1.3 Hz, 10-Me), 1.85 (1H, dd, J = 15.5, 9.0 Hz, H-7a), 1.77-1.66 (2H, m, H-14a, H-4'eq), 1.61-1.49 (2H, m, H-14b, H-2"ax), 1.56 (1H, dd, J = 15.7, 3.0 Hz, H-7b), 1.36 (3H, s, Me), 1.30-1.16 (19H, m, H-4'ax, 2-Me, 8-Me, 5'-Me, 5"-Me, Me(s), Me(s)), 1.11 (3H, d, J = 7.5 Hz, 4-Me) and 0.87 (3H, t, J = 7.3 Hz, 3H-15) ppm.
7. (**10a/13**): ^{1}H NMR (400MHz, $CDCl_3$, 60°C); δ 4.92 (1H, d, J = 4.2 Hz, H-1"), 4.72 (1H, dd, J = 10.1, 2.9 Hz, H-13), 4.35 (1H, d, J = 7.3 Hz, H-1'), 4.24 (1H, dd, J = 2.7, 1.9 Hz, H-3), 3.97 (1H, dq, J = 9.4, 6.3 Hz, H-5"), 3.56 (1H, d, J = 7.5 Hz, H-5), 3.48 (1H, m, H-5'), 3.32 (3H, s, OMe), 3.19 (1H, dd, J = 10.2, 7.3 Hz, H-2'), 3.17 (1H, br, OH), 2.96 (2H, m, H-8, H-4"), 2.78 (1H, dq, J = 7.2, 1.8 Hz, H-2), 2.60 (1H, dd, J = 12.8, 3.3 Hz, H-9a), 2.46 (1H, ddd, J = 12.3, 10.3, 1.6 Hz, H-3'), 2.37 (1H, dd, J = 15.3, 1.0 Hz, H-2"eq), 2.27 (6H, s, NMe_2), 2.24 (1H, dd, J = 12.5, 1.7 Hz, H-9b), 2.16-2.03 (3H, m, H-4, H-11a, 4"-OH), 1.99 (1H, m, H-10), 1.63 (1H, ddd, J 12.6, 3.8, 2.1 Hz, H-4'eq), 1.58-1.45 (3H, m, H-7a, H-14a, H-2"ax), 1.45 (1H, dd, J = 14.6, 3.3 Hz, H-7b), 1.38 (1H, m, H-14b), 1.34 (3H, s, Me), 1.23-1.15 (16H, m, H-4'ax, 2-Me, 5'-Me, 5"-Me, Me(s), Me(s)), 1.13 (3H, d, J = 6.3 Hz, 8-Me), 1.10 (3H, d, J = 7.5 Hz, 4-Me), 0.99 (1H, dd, J = 14.2, 7.2 Hz, H-11b), 0.93 (3H, d, J = 6.7 Hz, 10-Me) and 0.86 (3H, t, J = 7.4 Hz, 3H-15) ppm.
8. The selective reduction of the $\Delta_{10,11}$-olefin under mild hydrogenation conditions (*vide infra*) provides some support for this notion.
9. The facility for equilibration proved to be markedly solvent dependent. In $CDCl_3$ (stored over K_2CO_3) or AcOH the final 2:1 equilibrium position is reached after ~14-20h. In CD_3OD over this period little, if any, equilibration was apparent.
10. A certain amount of aminal hydrolysis was observed (i.e. back to **13** and **16**) during the course of the chromatography, even using a basic eluent (CH_2Cl_2/MeOH/NH_4OH).
11. (**18**): ^{1}H NMR (400MHz, $CDCl_3$, 60°C); δ 5.08 (1H, d, J = 4.5Hz, H-1"), 4.59 (1H, dd, J = 9.2, 2.9 Hz, H-13), 4.49 (1H, br s, H-3), 4.39 (1H, d, J = 7.4 Hz, H-1'), 4.02 (1H, dq, J = 9.4, 6.4 Hz, H-5"), 3.54 (1H, d, J = 7.1 Hz, H-5), 3.49 (1H, m, H-5'), 3.31 (3H, s, OMe), 3.19 (1H, dd, J = 10.1, 7.2 Hz, H-2'), 2.99 (1H, d, J = 9.4 Hz, H-4"), 2.96 (1H, br, H-8), 2.76 (1H, dq, J = 7.6, 2.9 Hz, H-2), 2.47 (2H, m, H-9a, H-3'), 2.33-2.27 (7H, m, H-2"eq, NMe_2), 2.15 (1H, t, J = 11.6 Hz, H-9b), 2.07 (3H, br s, NMe), 1.95 (1H, v.br, H-10), 1.90-1.78 (4H, m, H-4, H-7a, H-11a, H-14a), 1.66 (1H, ddd, J = 12.8, 3.8, 1.9 Hz, H-4'eq), 1.54 (1H, dd, J = 15.1, 5.0 Hz, H-2"ax), 1.46 (1H, m, H-14b), 1.35 (3H, s, Me), 1.27 (3H, d, J = 6.2 Hz, 5"-Me), 1.24-1.13 (14H, m, H-7b, H-4'ax, 2-Me, 5'-Me, Me(s), Me(s)), 1.09 (3H, d, J = 7.6 Hz, 4-Me), 1.01 (3H, d, J = 6.5 Hz, 10-Me), 0.97-0.91 (4H, br m, H-11b, 8-Me) and 0.88 (3H, t, J = 7.4 Hz, 3H-15)ppm.
12. MIC's were determined by a liquid turbidimetric microtiter assay. Macrolides were solubilized in ethanol and diluted to 4% ethanol in phosphate buffer. Concentrations in the range 128-0.00015μg/mL were tested. Stock cultures were stored at -80°C. Organisms were incubated at 35-37°C; *Haemophilus influenzae* was incubated in a 5% CO_2 atmosphere. 10^5cfu/well were added to microtiter plates containing the diluted macrolides. Plates were incubated at 35°C for 22-24h. MIC values of >8μg/mL indicate

microorganism resistance, whereas values of 1-4μg/mL indicate intermediate resistance. Mueller Hinton Broth was used for all strains with the following exceptions: *Haemophilus* were grown in Haemophilus Test Medium and*Streptococci* and *Enterococci* were grown in Mueller Hinton Broth supplemented with cations and 5% lysed horse blood.

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