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PREPARATION AND ACTIVITIES OF 4"-*EPI* AND 4"-DEOXY-4"-AMINO ANALOGS DERIVED FROM 9-DEOXO-8a-AZA-8a-HOMOERYTHROMYCIN A[1]

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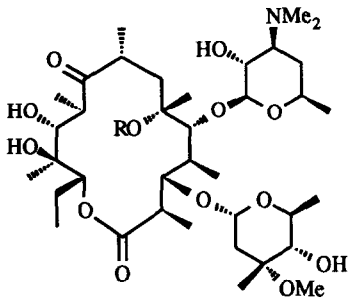
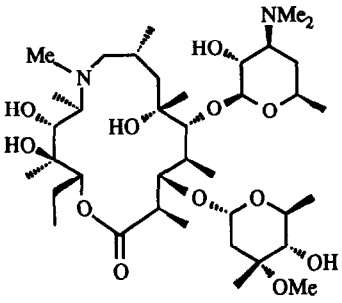
Abstract: The preparation and biological activity of the novel aza macrolides **6, 8, 11-13** and **15-20** are reported. These analogs display *in vitro* antibacterial properties that are superior to erythromycin A.

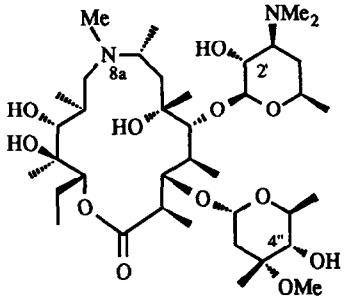
The last few decades have seen many reports in the literature that deal with the systematic chemical and structural modification of the macrolide antibiotic erythromycin A (**1**), all aiming to improve its potency and spectrum, oral bioavailability, acid stability, and to eliminate gastrointestinal side effects.[2,3] As a consequence of these studies, modified analogs such as clarithromycin (**2**)[4] and azithromycin (**3**)[5] have been recently marketed in the USA and Europe. Wilkening and coworkers recently reported the synthesis of the novel 15-membered aza macrolide **4** (L-701,677).[6] Analog **4**, a positional isomer of azithromycin, showed *in vitro* antibacterial properties similar to **3** and also displayed greater acid stability.[7] Encouraged by these results, we now report on derivatives of **4** modified at the 4"-and 8a-positions. Specifically, we report here on the preparation and biological activities of the 4"-*epi* analog **6** and 4"-amino analogs **8** and **11-13**. Further, we also report herein the efficient separation of the diastereomeric amines **15** and **16** and their subsequent derivatization.



1 R = H, Erythromycin A
2 R = Me, Clarithromycin



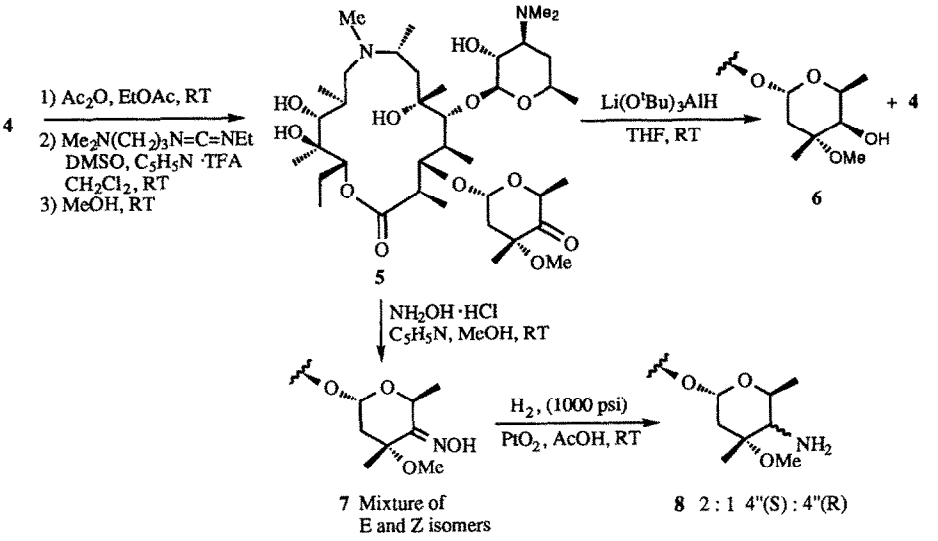
3 Azithromycin



4 L-701,677

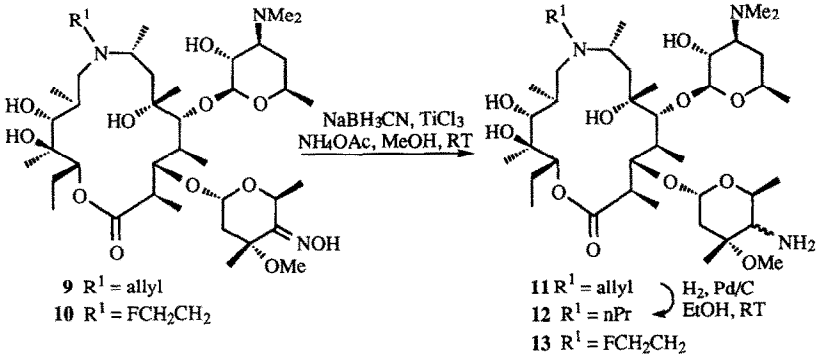
The syntheses of the 4"-*epi* analog and 4"-amino derivatives of 8a-azalide **4** parallel methods previously described[8] in the azithromycin series. Thus, as shown in Scheme I and starting from **4**, a three step sequence involving the protection of the C-2' hydroxyl by acetylation, selective oxidation at the 4"- hydroxyl by modified Moffat-Pfitzner conditions, and methanolysis of the 2'-acetate provided **5**.[9,10] The ketone **5** underwent smooth reduction with lithium tri-*tert*-butoxyaluminohydride to give a mixture of **4** and the desired 4"-epimer **6**. The more polar 4"-*epi* analog **6** was easily separated and the stereochemistry of the hydroxyl substituent at the 4"-position was established by means of high field NMR (400 MHz, 60°C, $CDCl_3$); wherein, the signal corresponding to the methine proton at C-5" of the cladinose ring appeared as a quartet at δ 4.52 (J = 6.9 Hz).

Scheme I



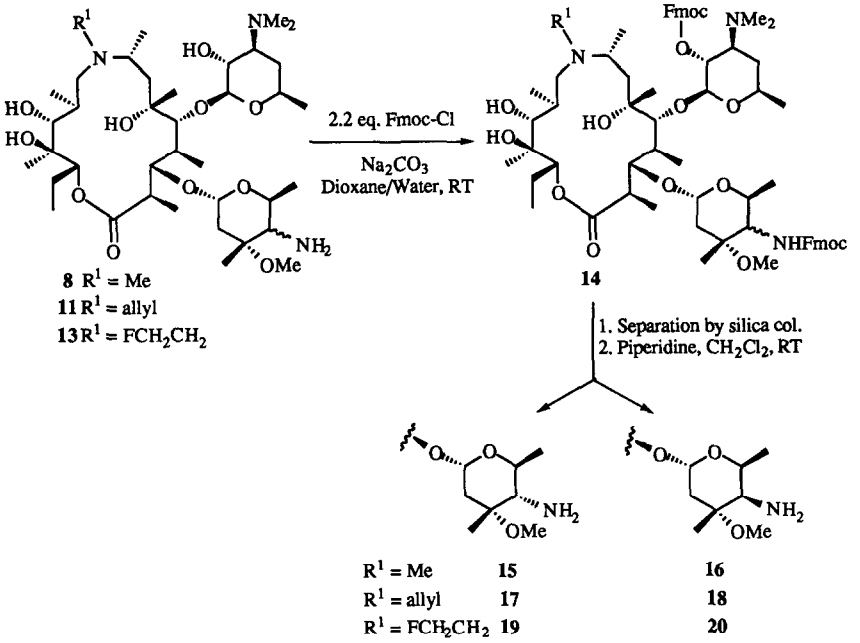
Preparation of the 4"-amino analogs began with the reaction of ketone **5** with hydroxylamine to give a mixture of the E and Z oximes **7** as shown in Scheme I. Reduction of the oxime mixture to a mixture of amines **8** (2 : 1 4"(S) : 4"(R)) was accomplished by hydrogenation at 1000 psi over PtO_2 in acetic acid. The 8a-allyl and 8a-fluoroethyl oximes **9** and **10** were prepared in an analogous manner starting from the corresponding 8a-substituted 8a-azalides.[1, 6b] Preparation of amines **11-13** from the corresponding oximes required alternative conditions as shown in Scheme II. The presence of the allyl residue in **9** precluded the use of catalytic hydrogenation, so we resorted to reduction conditions developed by Lilly workers.[11] This involved addition of $TiCl_3$ to a buffered solution of the oxime and $NaBH_3CN$ and was used to prepare the amines **11** and **13**. A 1 : 1 diasteromeric mixture of amines was obtained in each case. The amine **12** was easily obtained by Pd/C hydrogenation of **11**.

Scheme II



The separation of the amines **15** and **16** by silica chromatography proved very difficult, so we undertook the task of derivatizing the mixture of amines **8** in hope that the derivatives could be separated more easily and then transformed back to the isomers. Treatment of **8** with 2.2 eq. of Fmoc-chloride furnished a mixture of 2',4"-bis-Fmoc derivatives **14**.[12] After an efficient column separation of diastereomeric derivatives, the Fmoc group was deblocked with piperidine at room temperature to furnish amines **15** and **16**. The identity of amines **15** and **16** was fully established by means of high field NMR (400 MHz, 60°C, $CDCl_3$). As would be expected, the overall NMR spectrum of **15** was very similar to that of **4**, e.g. the methine proton at C-5" for **15** appeared as a doublet of quartets at δ 3.97 (the same proton for **4** appeared at δ 4.04). Likewise the C-5" protons in compounds **16** and **6** appear as quartets at δ 4.59 (J= 6.3 Hz) and δ 4.52 (J= 6.9 Hz) respectively. This protocol was extended for the separation of amine mixtures **11** (giving **17** and **18**) and **13** (giving **19** and **20**).

Scheme III



8 R^1 = Me
11 R^1 = allyl
13 R^1 = FCH_2CH_2

14

R^1 = Me	**15**	**16**
R^1 = allyl	**17**	**18**
R^1 = FCH_2CH_2	**19**	**20**

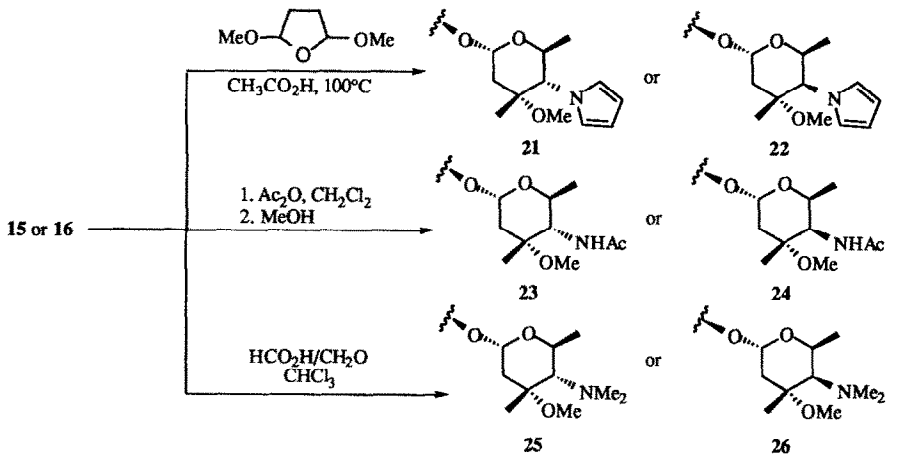
The *in vitro* potencies of **4**, **6**, **8**, **11**, **12** and **15-20** against three Gram-positive and three Gram-negative pathogens are shown in Table I along with that of erythromycin A (**1**) and azithromycin (**3**) for comparative purposes. It is evident from these results that the 4"-*epi* analog **6** does not offer any significant advantage over **4**; although, its Gram-negative potency is far better than erythromycin A itself. In general, the Gram-negative potency for the 4"-amino compounds **8**, **11**, **12** and **15-20** is better than that of azalides **3** and **4** and far better than that of erythromycin A. The 8a-methyl analog **8**, 8a-allyl analog **11** and 8a-propyl analog **12** showed gains in Gram-negative potency at the expense of Gram-positive potency. The 8a-fluoroethyl analogs **19** and **20** are more balanced in that there is now a restoration of Gram-positive activity. Further, the 4"-(S) and 4"-(R) amino compounds **15** and **16** are almost equipotent and this trend is more or less reflected in the compounds **17/18** and **19/20**. Overall, the compounds **19** and **20** display the best biological profile. Our results on the comparison of **4** to **6** and **8** generally parallel the findings reported by the Pfizer group in their analogous work on azithromycin.[8]

Table I : Minimum Inhibitory Concentration (MIC, μg/ml)[13]

Compound	*E. faec*[14]	*S. aur*	*S. pneu*	*E. coli*	*H. flu*	*K. pneu*
Erythromycin A (**1**)	1	0.25	0.02	32	2	32
Azithromycin (**3**)	4	1	0.03	1	0.5	2
4	2	0.5	0.03	1	1	1
6	1	0.5	0.06	1	1	4
8	4	2	0.03	0.25	1	0.13
11	4	2	≤0.06	0.5	0.5	0.25
12	2	1	≤0.06	0.5	0.5	0.5
15	2	1	≤0.06	0.13	0.25	0.13
16	2	1	≤0.06	0.25	0.5	0.25
17	4	1	0.03	0.25	1	0.13
18	2	1	0.03	0.5	0.5	0.25
19	2	0.5	0.03	0.25	1	0.13
20	1	0.25	0.03	0.25	0.5	0.13

Scheme IV delineates selected reactions for the amines **15** or **16** that seek to alter the basicity of the amino group at the 4" position of the cladinose moiety. These reactions are : 1) Clauson-Kass reaction[15] to give pyrroles **21** or **22**, 2) acylation of the amine to give **23** or **24**, and 3) Eschweiler-Clarke alkylation to afford **25** or **26**.

Scheme IV



The biological assay results shown in Table II for the samples **21-26** reveal a net loss of activity for the various pathogens by a factor ranging from 2-8 when compared to either **15** or **16**. These results suggests that both the basicity and steric effects play an important role in determining the overall potency of 4"-amino compounds.

Table II : Minimum Inhibitory Concentration (MIC, μg/ml)[13]

Compound	*E. faec*[14]	*S. aur*	*S. pneu*	*E. coli*	*H. flu*	*K. pneu*
21	2	4	0.13	4	4	4
22	4	8	0.25	4	16	8
23	8	2	0.25	2	8	2
24	8	16	0.5	1	4	4
25	8	8	0.5	1	2	2
26	16	32	0.5	0.5	2	2

In summary, this communication describes the synthesis of compounds **6, 8, 11-13** and **15-20**, all of which have an overall antibacterial profile exceeding that of erythromycin A. In contrast the C-4" derivatives **21-26** displayed unexceptional activity.

Acknowledgement : We wish to thank Ms. Amy Bernick for assistance in the mass spectral determinations.

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