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THE SYNTHESIS OF NOVEL 8a-AZA-8a-HOMOERYTHROMYCIN DERIVATIVES *VIA* THE BECKMANN REARRANGEMENT OF (9Z)-ERYTHROMYCIN A OXIME

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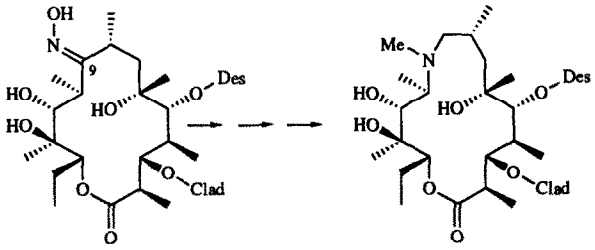
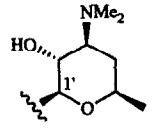
Abstract: The (9*E*)-oxime of erythromycin A (**1**) was isomerized to the (9Z)-isomer **2** in the presence of strong base. Stereospecific Beckmann rearrangement of the (9Z)-oxime led to a series of novel 8a-aza-8a-homoerythromycin A derivatives. *In vitro* data is provided that shows the 8a-methyl derivative **10** to be equally active with its positional isomer azithromycin.

The oxime of erythromycin A is an important intermediate in the synthesis of the clinically important macrolide antibiotics azithromycin,[1a,b] clarithromycin,[1a,c] dirithromycin[1a,d] and roxithromycin.[1a,e] Of these new compounds, azithromycin is unique in that it contains an aza substituted, ring expanded, 15-membered macrocyclic framework.[2] The ring nitrogen of azithromycin is introduced by a stereospecific Beckmann rearrangement[1b] of erythromycin A oxime (**1**), which is known to exist almost exclusively in the (9*E*) form.[3] In this paper we describe the isomerization of **1** to the (9Z)-erythromycin A oxime (**2**) followed by the stereospecific transformation of **2** into novel 8a-aza-8a-homoerythromycin analogs.

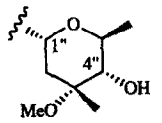


1

Azithromycin

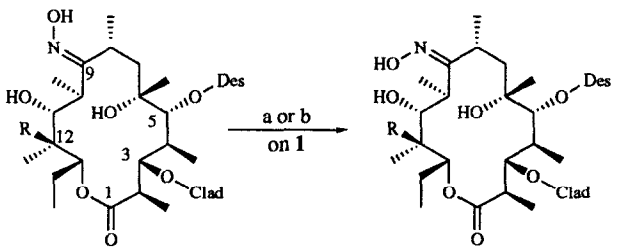


Des = Desosaminyl



Clad = Cladinosyl

The synthesis of the 8a-aza-8a-homoerythromycin series was initially attempted by both photolytic and oxidative rearrangements of the oxime **1**. Both approaches were unsuccessful and were abandoned after finding a successful route to the (9Z)-oxime **2**. The formation of **2** was discovered serendipitously during a Raney nickel reduction of **1** in which ethanolic sodium hydroxide was added to activate the catalyst.[4] The reaction failed to produce the desired (9*R*)-erythromycylamine,[5] giving instead a product that was isomeric with the starting oxime. Furthermore, it was determined that ethanolic sodium hydroxide alone was sufficient to facilitate the reaction.



1) R= OH
3) R= H

2) R= OH
4) R= H

(a) Raney-Ni, NaOH, EtOH; (b) LiOH, EtOH (74%)

Since it was not immediately obvious that the isomeric product was the (9Z)-oxime[6] **2** or a product resulting from base promoted epimerization at positions 2, 8 or 10, a detailed ^{1}H NMR analysis of the isomeric product was performed. A comparison of our data[7] with the published data for the (9*E*)- and (9*Z*)-oximes of erythromycin B[3] (**3** and **4** respectively) provided evidence that compound **2** was the (9*Z*)-oxime of erythromycin A and that no epimerization at positions 2, 8 or 10 had occurred. The H-10 and H-11 resonances of both (9*Z*)-oximes appeared at lower field than the corresponding resonances of the (9*E*)-oximes due to the deshielding effect of the proximal hydroxyimino group. Similarly, H-8 of **1** appeared at lower field than H-8 of **2**. Structural confirmation of **2** as the (9*Z*)-erythromycin A oxime was provided by an X-ray analysis[8] of a crystal grown in nitromethane.

The conditions for the isomerization were investigated with the following conclusions: 1) the degree of *E* to *Z* conversion depends upon the extent to which the oxime is deprotonated and therefore requires a base of sufficient strength to substantially deprotonate the oxime (pK_a~12), 2) small counterions (Li^+, Na^+) give the highest conversion to the *Z*-oxime and 3) alcoholic solvents are more effective than non-protic solvents in promoting the isomerization. The reaction conditions which produced the most favorable ratio of isomers **2** to **1** involve the addition of 2 equivalents of lithium hydroxide monohydrate to a 10% ethanolic solution of the oxime **1**. After stirring overnight the ethanol was evaporated, the residue was partitioned between ethyl acetate and brine while adjusting the pH to 9.3, and the ethyl acetate was evaporated to give a 4:1 mixture of *Z*:*E* oximes in near quantitative yield. The *Z*-oxime **2** was purified by either silica chromatography or by crystallization from nitromethane. Compound **2** was found to be stable to isomerization as a crystalline solid. However, as a foam or in solution, **2** slowly equilibrated to the *E*-oxime **1** ($t_{1/2}$ of 7 days in $CDCl_3$ at room temperature).

The base mediated isomerization[9] of oximes is an interesting and unpredictable phenomenon. In this case, the *E* isomer of the neutral oxime is the thermodynamically preferred form whereas the *Z* isomer of the oxime anion is the thermodynamically preferred form. The factors involved in the reversal of the thermodynamic stability between the neutral and anionic forms of the oxime are unclear. However, our results suggest the following conclusions. Conformational changes associated with oxime deprotonation are probably not a primary factor since the ^{1}H NMR of both the *E*- and *Z*- oximes are not significantly different when determined in either CD_3OD or in CD_3OD containing 10 equivalents of CD_3ONa. Inspection of molecular models based on the X-ray structure of the *Z*-oxime suggest that the corresponding anion may be stabilized by

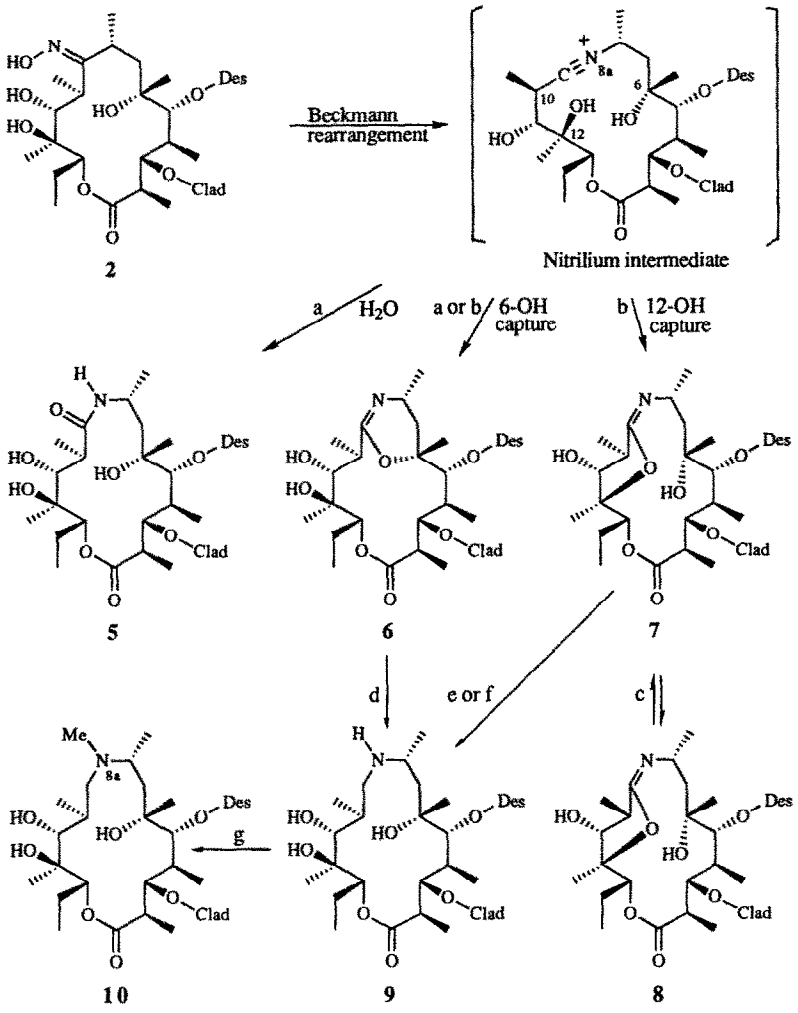
chelation of the metal counterion with the oxygen functions at C-1, C-6, C-11 and C-12, while in the *E*-oxime anion, such stabilization can occur primarily from the C-6 hydroxyl group. Finally, the observation that the addition of 15-crown-5 to a solution of the lithium oximinate had little effect upon the equilibrium provides evidence that the cation is highly coordinated to the oxygen functions of the macrolide.

The Beckmann rearrangement of **2** gave 8a-aza-8a-homoerythromycin products without evidence of products[1b] derived from the (9*E*)-oxime. When the rearrangement was carried out under aqueous conditions, the lactam **5** and the 6,9-iminoether **6** were isolated. When anhydrous conditions were used, the 6,9-iminoether **6** and the 9,12-iminoethers **7** and **8** were isolated. The ratio of 6,9- to 9,12-iminoethers from the anhydrous Beckmann rearrangement could be adjusted by varying the reaction conditions. Generally, decreasing the polarity of the solvent, increasing the concentration of the reaction, and using stoichiometric quantities of pyridine all favor the formation of the 6,9-iminoether **6**.

The ^{1}H NMR of the crude Beckmann product produced under anhydrous conditions showed a mixture of 6,9-iminoether **6**, a major 9,12-iminoether **7**, and a minor 9,12-iminoether **8**. The 9,12-iminoethers were purified by silica chromatography or by crystallization from nitromethane. The silica purification gave a 1:1 mixture of **7** and **8**, while crystallization from nitromethane gave a disproportionately high recovery of the 9,12-iminoether **7**. This implied that in solution a dynamic equilibrium existed between **7** and **8** and that the removal of **7** from the reaction by crystallization drove the conversion to **7**. It was subsequently observed that a $CDCl_3$ solution of the crystalline isomer **7** equilibrated to the same 1:1 mixture of 9,12-iminoethers that was isolated by silica chromatography. Addition of CD_3OD to a $CDCl_3$ solution of **7** resulted in equilibration accompanied by slow deuterium exchange at C-10, thereby demonstrating that the locus of the isomerization involved C-10. The epimerization at C-10 presumably proceeds *via* a 9,10-didehydro enamine tautomer.[10] The crystalline 9,12-iminoether **7** was determined to be the major isomer in the crude Beckmann product and was shown by an NOE experiment and single crystal X-ray analysis[8] to have the same configuration at the C-10 position as that of erythromycin A.

The 6,9-iminoether **6** was unreactive towards sodium borohydride in methanol but was reduced to 9-deoxo-8a-aza-8a-homoerythromycin A (**9**) by high pressure hydrogenation using reagent quantities of platinum oxide in acetic acid. In contrast, sodium borohydride reduction of the crystalline 9,12-iminoether **7** in methanol gave the amine **9** in good yield. When a 1:1 isomeric mixture of the 9,12-iminoethers (**7** and **8**) was reduced[11] with sodium borohydride under the same conditions, the amine **9** and recovered 9,12-iminoether **8** were the major products. Apparently, the reduction of the 10-epi-9,12-iminoether **8** is significantly slower than the natural isomer **7**, which suggests that the hydride is delivered from the α-face. The borohydride reduction was greatly improved by using ethylene glycol as solvent. Under these conditions both the 6,9-iminoether **6** and the 9,12-iminoether **7** are reduced to **9**. In addition to activating the reducing agent, ethylene glycol serves to solvolyze the borate complexes formed during the reduction, thereby avoiding the necessity of an acidic work-up.

The amine **9** was methylated under Eschweiler-Clarke conditions to give the 8a-methyl derivative **10**.[12] The structure of **10** was determined by ^{1}H NMR and ^{13}C NMR spectroscopy and ultimately confirmed by an X-ray analysis[8] of a crystal grown in ethanol.



(a) TsCl, $NaHCO_3$, H_2O, Me_2CO (48% **5** and 17% **6**); (b) TsCl, C_5H_5N (25% **6** and 70% **7** + **8**); (c) the equilibration is fast in $CDCl_3$ and slow in CD_3OD; (d) H_2, PtO_2, HOAc (42%); (e) $NaBH_4$, CH_3OH [27% cryst. starting from a 3:1 mixture of (**7**+**8**): **6**]; (f) $NaBH_4$, ethylene glycol [45% cryst. starting from a 3:1 mixture of (**7**+**8**): **6**]; (g) HCHO, HCO_2H, $CHCl_3$ (85% cryst.)

Table 1 shows a comparison of the *in vitro* antibacterial activities[13] of several 8a-aza-8a-homoerythromycin derivatives with both azithromycin and erythromycin A against a panel of Gram positive and Gram negative bacteria. The oxygen bridged derivatives **6** and **7** were found to be the least effective antibiotics tested. The lactam **5** displayed Gram positive activity which was comparable to that of **9**, but lacked the Gram negative activity of **9** presumably because it does not contain a basic ring nitrogen.[14] Compound **10** was determined to be approximately 2-4 times more active than its non-methylated precursor **9** and exhibited *in*

vitro activity equal to that of azithromycin. Derivatization of **9** at both the 8a- and 4"- positions have led to a number of compounds with improved properties over **10**. The chemistry and biological data of these derivatives will be the subject of forthcoming publications.

Table 1. Minimum Inhibitory Concentration (MIC) μg/ mL

Microorganism		**5**	**6**	**7**	**9**	**10**	**azith**	**eryth**
E. faecalis	MB 5407	8	16	>128	4	2-4	4-8	1-2
S. aureus	MB 2865	0.5	32-64	16	0.5-1	0.25-1	0.5-1	0.125-0.25
S. epidermidis	MB 5414	0.5	16-128	16	0.5	0.25-0.5	0.25-0.5	0.125
S. pneumoniae	CL 2883	≤0.06	2	4	≤0.06	≤0.06	≤0.06	≤0.06
S. pyogenes	MB 2874	≤0.06	0.5	2	≤0.06	≤0.06	≤0.06	≤0.06
E. cloacae	CL 4298	NT	128	128	4	1	1	32-64
E. coli	MB 2884	16	128	128	2-8	1-2	1-2	16-64
H. influenzae	MB 5363	32	64	64	4	0.5-2	0.5-2	2-4
H. influenzae	CL 1830	NT	>128	>128	32	4-16	8-16	64-128
K. pneumoniae	MB 4005	32	>128	>128	4-8	1-2	1-2	32-64

NT= not tested

Acknowledgement: We would like to thank Drs. Karst Hoogsteen and Richard Ball for performing the X-ray analyses of compounds **2**, **7** and **10**; and Dr. L. Colwell and Ms. A. Bernick for mass spectral determinations.

References and Notes

1. a) Kirst, H. A. *Recent Progress in the Chemical Synthesis of Antibiotics*; Springer-Verlag, 1990; pp. 39-63. b) Djokic', S.; Kobrehel, G.; Lazarevski, G.; Loppotar, N.; Tamburasev, Z.; Kamenar, B.; Nagl, A.; Vickovic', I. *J. Chem. Soc. Perkin Trans. I* **1986**, 1881. c) Watanabe, Y.; Adachi, T.; Asaka, T.; Kashimura, M.; Morimoto, S. *Heterocycles* **1990**, *31*, 2121. d) Counter, F. T.; Ensminger, P. W.; Preston, D. A.; Wu, C. E.; Greene, J. M.; Felty-Duckworth, A. M.; Paschal, J. W.; Kirst, H. A. *Antimicrob. Agents Chemother.* **1991**, *35*, 1116. e) Gasc, J. C.; D'Ambrieres, A. L.; Lutz, A.; Chantot, J. F. *J. Antibiotics*, **1991**, *44*, 313.

2. Pfizer workers have coined the term "azalide" to describe the 9-deoxo-9a-aza-9a-homoerythromycin framework of azithromycin. Bright, G. M.; *et al. J.Antibiotics* **1988**, *41*, 1029.

3. Egan, R. S.; Freiberg, L. A.; Washburn W. H. *J. Org. Chem.* **1974**, *17*, 2492.

4. Bergeron, R. J.; Garlich, J. R. *Synthesis* **1984**, 782.

5. The synthesis and utility of (9*R*)-erythromycylamine have been reported: a) Massey, E. H.; Kitchell, B. S.; Martin, L. D.; Gerzon, K. *J. Med. Chem.* **1974**, *17*, 105. b) Lartey, P.A.; DeNinno, S. L.; Faghih, R.; Hardy, D. J.; Clement, J. J.; Plattner, J. J.; Stephens, R. L. *J. Med. Chem.* **1991**, *34*, 3390.

6. After completing our studies on the oxime isomerization, we observed a report using sodium hydroxide in aqueous methanol to effect the (9*E*)- to (9*Z*)-erythromycin oxime conversion: ref. 1e. Our NMR data (see ref. 7) is in excellent agreement with the limited data reported by Gasc *et al.* in ref 1e.

7. Physical data for **2**: mp 157-164°C; IR($CHCl_3$) 3680, 3435, 2970, 2940, 1725, 1455, 1375, 1345, 1165, 1105, 1085, 1045, 1005 and 950 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.00 (dd, H13), 4.90 (d, H1"), 4.45 (d, H1'), 4.04 (d, H3), 4.00 (dq, H5"), 3.83 (br s, H11), 3.56 (d, H5), 3.52 (m, H5'), 3.31 (s, OMe), 3.26 (dd, H2'), 3.02 (dd, H4"), 2.93 (m, H8), 2.84 (dq, H2), 2.79 (m, H10), 2.52 (m, H3'), 2.35 (d, H2"eq.), 2.33 (s, NMe_2), 2.32 (br s, 4"OH), 1.97 (m, H4), 1.91 (m, H14a), 1.72 (br d, H4'eq.), 1.56 (m, H7a +7b), 1.56 (dd, H2"ax.), 1.47 (m, H14b), 1.42 (s, 6Me), 1.33 (d, 10Me), 1.28 (d, 5"Me), 1.24 (m, H4'ax.), 1.24 (s, 3"Me), 1.22 (d, 5'Me), 1.17 (d, 2Me), 1.15 (s, 12Me), 1.12 (d, 8Me), 1.09 (d, 4Me), 0.84 (t, 14Me); ^{13}C NMR ($CDCl_3$) δ 176.0 (C-1), 168.1 (C-9), 102.8 (C-1') , 95.9 (C-1"), 83.5 (C-5), 79.4 (C-3), 77.9 (C-4"), 77.3 (C-13), 75.3 (C-6), 75.0 (C-12), 72.7 (C-3"), 71.0 (C-2'), 70.9 (C-11), 68.8 (C-5'), 65.4 (C-5"), 65.2 (C-3'), 49.4 (OMe), 44.6 (C-2), 40.2 (NMe_2), 39.7 (C-4), 37.8 (C-7), 35.7 (C-8), 34.9 (C-2"), 34.1 (C-10), 28.9 (C-4'), 26.1 (C-6Me), 21.6 (C-3"Me), 21.4 (C-14), 21.2 (C-5'Me), 19.8 (8Me), 18.4 (5"Me), 16.7 (12Me), 15.3 (2Me), 10.8 (10Me), 10.8 (C-15), 9.20 (4Me); FAB mass spectrum *m/z* 749 (M + H), 591, 416, 398, 174, 159, 158, and 116. Anal. Calcd for $C_{37}H_{68}N_2O_{13}$: C, 59.34; H, 9.15; N, 3.74 . Found: C, 59.12; H, 8.80; N, 3.82.

8 The details of the X-ray analyses of compounds **2**, **8** and **10** will be reported in a forthcoming publication.

9. The isomerization of oxime anions to variable, substrate dependent, *E*:*Z*-mixtures has been observed by others: a) Clack, D. W.; Khan, N.; Wilson, D. A. *J. Chem. Soc. Perkin II* **1981**, 860. b) Grubbs, E. J.; Parker, D. R.; Jones, W. D. *Tetrahedron Lett.* **1973**, 3279. c) MacConaill, R. J.; Scott, F. L. *Tetrahedron Lett.* **1970**, 2993. d) Barnish, I. T.; Hauser, C. R. *J. Org. Chem.* **1968**, *33*, 1372. e) Montgomery, R. S.; Dougherty, G. *J. Org. Chem.* **1952**, *17*, 823.

10. Epimerization at C-10 of bridged 9,12-iminoethers was first observed in these laboratories by A. B. Jones in a related 11-deoxy analog; A. B. Jones and C. A. Herbert, submitted for publication.

11. The conditions used in the catalytic reduction of **6** were unsuitable for the reduction of **7** or **8** due to dehydration to the 10,11-anhydro derivative on exposure to acetic acid. (see ref. 10)

12. Physical data for **10**: mp 187-188°C; IR($CHCl_3$) 3540, 3330, 2970, 2940, 2880, 2830, 1725, 1455, 1375, 1350, 1325, 1275, 1160, 1125, 1085, 1065, 1045, 995, 975, 955 cm^{-1}; ^1H NMR ($CDCl_3$, 55°C) δ 7.66 (brs, OH), 5.10 (d, H1"), 4.86 (dd, H13), 4.51 (t, H3), 4.38 (d, H1'), 4.04 (dq, H5"), 3.53 (br s, H11), 3.52 (d, H5), 3.51 (m, H5'), 3.32 (s, OMe), 3.23 (dd, H2'), 3.01 (dd, H4"), 2.99 (m, H8), 2.81 (dq, H2), 2.52 (m, H9a), 2.40 (m, H3'), 2.34 (s, NMe_2), 2.30 (m, H9b), 2.30 (d, H2"eq.), 2.04 (s, NMe), 1.99 (m, H10), 1.92 (m, H14a), 1.88 (m, H7a), 1.85 (m, H4), 1.72 (br d, H4'eq.), 1.55 (dd, H2"ax.), 1.48 (m, H14b), 1.37 (s, 6Me), 1.30 (d, 5"Me), 1.24 (d, 5'Me), 1.23 (m, H4'ax.), 1.23 (s, 3"Me), 1.19 (d, 2Me), 1.12 (m, H7b), 1.10 (d, 4Me), 1.10 (s,12Me), 0.96 (d, 10Me), 0.94 (d, 8Me), 0.92 (t, 14Me); ^{13}C NMR ($CDCl_3$, 55°C) δ 178.3 (C-1), 103.6 (C-1') , 94.7 (C-1"), 85.5 (C-5), 78.4 (C-4"), 77.2 (C-13), 76.7 (C-3), 75.9 (C-12), 74.9 (C-6), 73.1 (C-3"), 71.0 (C-2'), 69.1 (C-5'), 67.1 (C-11), 65.8 (C-3'), 65.4 (C-5"), 60.0 (C-9), 56.7 (C-8), 49.4 (OMe), 45.8 (C-2), 43.5 (C-4), 40.4 (NMe_2), 37.1 (C-7), 35.1 (C-2"), 30.9 (C-10), 30.9 (NMe), 29.3 (C-4'), 27.8 (C-6Me), 22.1 (CH_2CH_3), 21.7 and 21.3 (5'Me and 3"Me), 18.3 (5"Me), 16.4 (12Me), 14.3 (2Me), 12.7 (8Me), 12.0 (10Me), 11.4 (4Me), 11.3 (CH_2CH_3); FAB mass spectrum *m/z* 749 (M + H), 591, 573, 158, 116. Anal. Calcd for $C_{38}H_{72}N_2O_{12}$: C, 60.94; H, 9.69; N, 3.74 . Found: C, 60.87; H, 9.39; N, 3.70.

13. Antibacterial activities were determined by a standard broth microdilution assay. MIC ranges are given for compounds tested in two or more assays. Azithromycin (azith) and erythromycin A (eryth) were used as standards in the assay.

14. Azithromycin and dirithromycin exhibit increased activity against Gram negative bacteria when compared with erythromycin A. This may be due to the incorporation of a second basic nitrogen group in the molecules: Fernandes, P. B. *Antimicrob. Newsl.* **1987**, *4*, 25.